Induction of NF-κB DNA-Binding Activity during the G_0-to-G_1 Transition in Mouse Fibroblasts

ALBERT S. BALDWIN, JR.,[1,2,3]* JANE CLIFFORD AZIZKHAN,[1,3,4,5] DAVID E. JENSEN,[1] AMER A. BEG,[1,2]
AND LAVANYA R. COODLY[1]

Lineberger Comprehensive Cancer Center,[1] Departments of Biology,[2] Pediatrics,[4] and Pharmacology,[5] and Curriculum in Genetics,[3] University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7295

A DNA-binding factor with properties of NF-κB and another similar activity are rapidly induced when growth-arrested BALB/c 3T3 cells are stimulated with serum growth factors. Induction of these DNA-binding activities is not inhibited by pretreatment of quiescent cells with the protein synthesis inhibitor cycloheximide. Interestingly, the major NF-κB-like activity is not detected in nuclear extracts of proliferating cells, and thus its expression appears to be limited to the G_0-to-G_1 transition in 3T3 cells. These DNA-binding activities bind many of the expected NF-κB target sequences, including elements in the class I major histocompatibility complex and human immunodeficiency virus enhancers, as well as a recently identified NF-κB binding site upstream of the c-*myc* gene. Furthermore, both the class I major histocompatibility complex and c-*myc* NF-κB binding sites confer inducibility on a minimal promoter in 3T3 cells stimulated with serum growth factors. The results demonstrate that NF-κB-like activities are immediate-early response proteins in 3T3 cells and suggest a role for these factors in the G_0-to-G_1 transition.

Much of our knowledge concerning the signals that are activated following the interaction of serum growth factors with their receptors and that are involved in the stimulation of cell growth has been generated by using serum-deprived BALB/c 3T3 fibroblasts. In the absence of serum growth factors, 3T3 cells enter a state called G_0 in which growth and cell division stop. The addition of growth factors initiates a complex series of events which causes cells to enter G_1 and which culminates in the synthesis of DNA and cell division (1, 13, 15, 16, 18, 29, 41). During the G_0-to-G_1 transition, the expression of several transcription factors, including those encoded by certain proto-oncogenes, is activated, and these gene products play a critical role in initiating and controlling cell growth and DNA synthesis (13, 15–18, 28, 32, 38, 41, 48, 50, 58, 64). Aberrant expression of these proto-oncogenes, such as c-*fos* or c-*myc*, is implicated in the pathogenesis of a variety of neoplasms (21, 39).

NF-κB is a DNA-binding protein originally identified as a B-cell-specific factor involved in the control of expression of immunoglobulin kappa genes (59). Subsequently, regulatory elements in or upstream of various genes involved in immune function or in inflammation and elements in certain viruses such as the human immunodeficiency virus (HIV) have been shown to interact with NF-κB and to be critical for appropriate regulated or cell-type-specific gene expression (7, 11, 20, 23, 30, 34, 42, 43, 45, 49, 53, 55, 67). NF-κB DNA-binding activity can be induced in certain cell types with the cytokines tumor necrosis factor alpha and interleukin-1 (34, 42, 46, 52, 61), with the phorbol ester phorbol myristate acetate (PMA) (60), by virus infection, and by double-stranded RNA treatment (23, 42, 43, 67). Furthermore, NF-κB DNA-binding activity is activated by treatment of pre-B cells and Jurkat T cells with mitogens (11, 20, 49, 60). Thus NF-κB acts as an intracellular messenger affecting specific gene expression. NF-κB is cytoplasmically localized in many cells, where it is associated with the

inhibitor IκB (2, 3, 69). Purified NF-κB consists of two proteins, one of 50 kDa and another of 65 kDa. It is the 65-kDa species that interacts with the inhibitor IκB (3, 66). Phosphorylation of IκB releases NF-κB from the complex, allowing its movement to the nucleus and the detection of its DNA-binding activity (26, 62). Recently both the NF-κB p50 and p65 cDNAs have been cloned, and the deduced proteins have been shown to be related to the products of the dorsal maternal effect gene of *Drosophila melanogaster* and to the c-*rel* proto-oncogene (27, 40, 51, 56). Furthermore, NF-κB and the DNA-binding protein KBF1 (33), which bind highly related sites, have apparently identical 50-kDa DNA-binding subunits (27, 40).

We demonstrate here that NF-κB nuclear DNA-binding activity, or that of a highly related factor, is induced when quiescent BALB/c 3T3 cells are stimulated with serum growth factors. Interestingly, we do not detect any NF-κB binding activity in nuclear extracts of proliferating cells, suggesting that the relatively high levels of expression of NF-κB DNA-binding activity is specific for the G_0-to-G_1 transition in 3T3 cells. In addition, we demonstrate that a second NF-κB-like DNA-binding activity is also induced in the G_0-to-G_1 transition that, in contrast with NF-κB, is detected in nuclear extracts of proliferating 3T3 cells. The inducible NF-κB-like activities have the binding specificity expected of authentic NF-κB and also bind a recently described (22) NF-κB site found upstream of the c-*myc* gene. Furthermore, the NF-κB binding sites found in the class I major histocompatibility complex (MHC) enhancer and upstream of the c-*myc* gene function as inducible gene expression elements when quiescent 3T3 cells are stimulated with serum growth factors. These results suggest a role for NF-κB in the G_0-to-G_1 transition in 3T3 cells.

MATERIALS AND METHODS

Cells and nuclear extracts. Low-passage BALB/c 3T3 cells were maintained in Dulbecco modified Eagle medium (DMEM) plus 10% Colorado calf serum. For generation of

---

* Corresponding author.

nuclear extracts, cells were grown to an approximate density of 50 to 70% and then maintained in DMEM plus 0.5% Colorado calf serum for approximately 48 h. Colorado calf serum was added to 20% final volume, and the cells were maintained for various periods of time before nuclear extracts were prepared. For the protein synthesis inhibition experiments, cycloheximide was added to a final concentration of 10 μg/ml for 1 h prior to the addition of serum to the medium and remained in the medium during the serum stimulation. Jurkat T cells were grown in RPMI 1640 medium and 10% fetal calf serum. For stimulation of Jurkat T cells, phytohemagglutinin (PHA) and PMA were added to final concentrations of 1 μg/ml and 50 ng/ml, respectively. Nuclear extracts were prepared as previously described (65).

DNA binding assays and methylation interference. To detect DNA-protein interaction, the gel mobility shift assay (4, 24) was used. Nuclear extracts (approximately 6 μg for each extract) were incubated with a radiolabeled DNA probe (10,000 cpm/0.2 ng) in a buffer containing 10 mM Tris-HCl (pH 7.9), 50 mM NaCl, 0.5 mM EDTA, 10% glycerol, 1 mM dithiothreitol, and 2 μg of poly(dI-dC) · (dI-dC) (Pharmacia) in a final volume of 15 μl. Reactions were incubated at room temperature for approximately 20 min and then loaded onto 5% polyacrylamide gels and electrophoresed in a Tris-glycine-EDTA buffer (4). For the inhibition studies, IκB/MAD-3 mRNA was translated in reticulocyte lysates as described previously (31). Reticulocyte lysates that were either programmed with IκB/MAD-3 mRNA or mock translated were preincubated with nuclear extracts from 3T3 cells stimulated with serum for 2 h. Subsequently poly(dI-dC) · (dI-dC) and class I MHC enhancer probe were added. The reactions were analyzed as described above. Gels were dried and exposed for autoradiography.

DNA probes used in the experiments were HindIII-EcoRI fragments derived from pUC polylinkers with oligonucleotides for the various binding sites cloned into the BamHI site. Following restriction enzyme digestions of the plasmids, the restriction fragments were labeled with the Klenow fragment of DNA polymerase I and [α-³²P]dATP as described previously (6). Radiolabeled probes were isolated on 1.5% low-melting-temperature agarose gels. The adenovirus major late transcription factor (MLTF) binding site is ACCCGGTCACGTGGCCTACA (14). The various NF-κB binding sites are as follows: class I MHC, TGGGGATTCC CCA; β₂-microglobulin, AAGGGACTTTCCC; kappa immunoglobulin light chain, AGGGGACTTTCCG; HIV (4G), TGGGGACTTTCCA; HIV (3G), AAGGGACTTTCCG; and c-myc, CCGGGTTTTCCCC. The MHC double-point mutant is TGCGGCTTCCCGA.

Methylation interference was performed as described (7), using a DNA-binding probe from the class I MHC enhancer that was labeled at a single end and that was methylated with dimethyl sulfate. To generate the single-end-labeled probe, the class I MHC enhancer-containing plasmid was cut and labeled at either the HindIII or the EcoRI site and then digested with the other enzyme. Following recovery of the free probe and the bound DNA-protein complexes obtained from gel shift separation, the methylated DNA probe was cleaved with piperidine, electrophoresed on a 6% polyacrylamide–7.5 M urea gel, and exposed for autoradiography.

Transient expression analysis. BALB/c 3T3 cells were transfected by either calcium phosphate precipitation or electroporation procedures. Reporter plasmids were the following: Δ56 CAT, a minimal fos promoter extending to −56 fused to the bacterial chloramphenicol acetyltransferase (CAT) gene (28); MHC-NF-κB CAT, with three copies of the

class I MHC enhancer element TGGGGATTCCCCA inserted in the SalI site of Δ56CAT; MHCmut-NF-κB CAT, with three copies of the mutated enhancer sequence TGCG GATTCCCGA inserted in the SalI site of Δ56CAT; and myc-NF-κB CAT, with either two copies (2×) or one copy (1×) of the c-myc NF-κB binding site CCGGGTTTTCCCC inserted at the SalI site of Δ56 CAT (see Fig. 5C for diagrams of the constructs). Cells transfected by the calcium phosphate procedure were seeded 24 h prior to transfection at 8 × 10⁵ cells per 10-cm dish. A precipitate containing 10 μg of DNA was left on the cells overnight, followed by glycerol (12.5% volume) shock. For electroporation, 4 × 10⁶ cells were transfected with 10 μg of DNA in a volume of 0.5 ml of DMEM plus 10% fetal calf serum at 300 V and 960 μF. Electroporated cells were left overnight to reattach. For both procedures, the cell culture medium was changed to 0.5% serum 16 to 18 h after transfection and cells were maintained for 24 h. Half of the cells received 20% serum (final volume), and both the serum-starved and serum-stimulated cells were maintained for an additional 24 h. Extracts were prepared for CAT analysis as described previously (65). Equal volumes of extracts of starved or stimulated cells, representing an equivalent numbers of cells that were initially transfected, were assayed for CAT activity by thin-layer chromatography. The thin-layer chromatography plates were exposed for autoradiography for approximately 24 h. The results presented are from a typical experiment; virtually identical results were obtained in six different transfections using either calcium phosphate precipitation or electroporation.

RESULTS

Identification of serum growth factor-inducible DNA-binding activities. Since NF-κB has been shown to be inducible by mitogen treatment of pre-B and Jurkat T cells and by PMA treatment of a variety of cell types (11, 49, 60), we chose to analyze whether NF-κB could be induced by serum growth factor treatment of quiescent 3T3 cells. BALB/c 3T3 cells were grown to an approximate density of 50 to 70% and were serum starved for 48 h. Serum was added to a final concentration of 20% for some of the tissue culture plates at different time points, while others remained in 0.5% serum. Cells were collected at the various time points, and nuclear extracts were prepared. The DNA-binding properties of the nuclear extracts were assayed by the gel mobility shift procedure using a radiolabeled class I MHC enhancer element probe, containing the sequence TGGGGATTCCCCA, previously shown to bind to NF-κB (7). Equal amounts of protein from the different time points were incubated with the MHC probe, and the reactions were analyzed on nondenaturing polyacrylamide gels. Figure 1A (lanes 1 and 2) demonstrates that nuclear extracts of quiescent 3T3 cells are relatively devoid of DNA-binding activity and that a major DNA-protein complex (complex I) was detected following only 30 min of serum growth factor stimulation. Analysis of the nuclear extracts from the different time points revealed that this major complex was detected at relatively high levels at time points 30 min, 2 h, and 6 h (lanes 2 to 4) following addition of serum growth factors. The 12- and 24-h time points demonstrated significantly reduced levels of this DNA-protein complex (lanes 5 and 6). Also detected were two faster-migrating complexes (complexes II and III; Fig. 1A). Complex II is present in quiescent cells and appeared to gradually increase during the time course of stimulation (Fig. 1A). Complex III was shown to be induced by growth factor
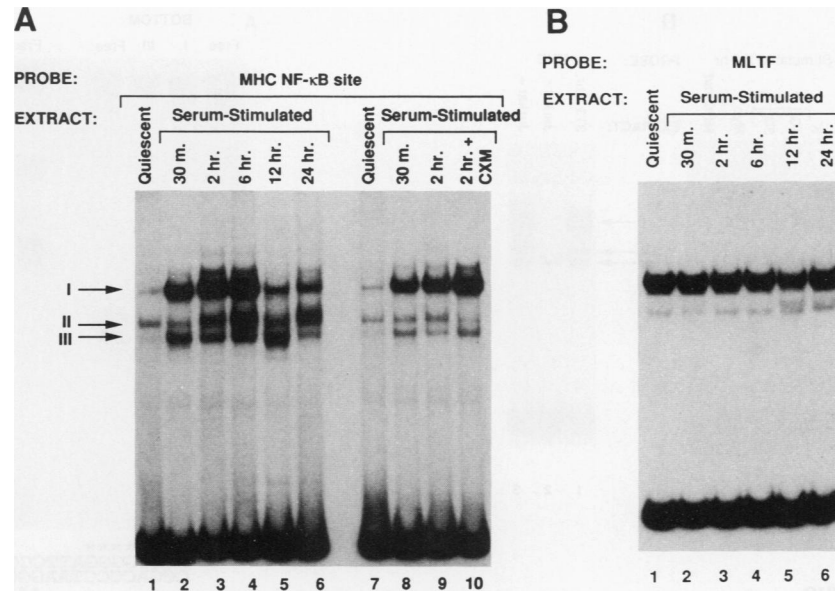
FIG. 1. Analysis of serum growth factor-inducible DNA-binding proteins. (A) Gel mobility shift analysis of nuclear extracts from quiescent and serum-stimulated 3T3 cells, using a class I MHC enhancer element probe (see Materials and Methods for the sequence of the probe). Equivalent amounts of the following extracts were used: quiescent cells (lane 1) and cells stimulated with serum for 30 min (lane 2), 2 h (lane 3), 6 h (lane 4), 12 h (lane 5), and 24 h (lane 6). Lanes 7 to 10 exhibit a separate experiment analyzing the effects of cycloheximide on the inducibility of the DNA-binding proteins. Proteins were from extracts of quiescent 3T3 cells (lane 7), cells stimulated with serum for 30 min (lane 8), or 2 h (lane 9), or cells that were pretreated with cycloheximide for 1 h and then given serum for an additional 2 h (lane 10). Complexes I, II, and III are indicated. (B) Normalization of extracts using an MLTF binding site probe (see Materials and Methods). Lanes correspond to lanes 1 to 6 in panel A.

stimulation and remained relatively high through the 12-h time point but was significantly reduced by the 24-h time point (Fig. 1A, lanes 1 to 6). Virtually identical DNA-binding results were obtained for extracts of BALB/c 3T3 cells stimulated with sis-conditioned medium (5), suggesting that platelet-derived growth factor is capable of inducing these DNA-binding activities.

We next determined whether the induction of these factors required protein synthesis. We pretreated serum-starved 3T3 cells with cycloheximide for 1 h and then stimulated the cells, in the presence of cycloheximide, with serum for 2 h. As controls, cells stimulated with serum growth factors for 30 min and 2 h in the absence of cycloheximide were analyzed. As shown in Fig. 1A (lanes 7 to 9), the pattern of induction for the quiescent, 30-min, and 2-h extracts was virtually identical to that presented in lanes 1 to 3. Furthermore, cycloheximide did not prevent and may actually have stimulated expression of the major DNA-binding factor (complex I) and the complex III factor as well, but it reduced complex II (lane 10). That the cycloheximide functioned to block protein synthesis is demonstrated by the observation that the induction of another serum growth factor-inducible DNA-binding protein was inhibited (70).

To demonstrate that the various extracts contained the normal complement of DNA-binding proteins, the 3T3 nuclear extracts from the different time points were incubated with a radiolabeled probe for the MLTF (14) (also known as USF) binding site in the adenovirus major late promoter (Fig. 1B, lanes 1 to 6). The DNA-binding activity of this factor is approximately the same for the different time points, demonstrating that the extracts are not degraded and that similar amounts of protein were used in the different binding reactions.

Properties of the major inducible factor are virtually identical to those of NF-κB. The major inducible factor (associated with complex I) binds the class I MHC element and is inducible in the presence of cycloheximide (Fig. 1A, lanes 7 to 10). Since these properties are characteristic of NF-κB (7, 60), we next analyzed the binding specificities of the serum growth factor-induced DNA-binding factors. As shown in Fig. 2A (lanes 1 to 5), the major inducible factor binds with approximately equal affinity to related regulatory elements found upstream of the class I MHC, β_2-microglobulin, and HIV genes and in the enhancer of the immunoglobulin kappa light-chain gene. This result is consistent with the growth-inducible factor being similar or identical to NF-κB (42). While this work was in progress, it was reported that NF-κB-like factors found in WEHI 231 B cells bind to an element located approximately 1,100 bp upstream of the c-myc gene (22). Since c-myc transcription is activated in the G_0-to-G_1-transition in 3T3 cells (29, 38), we tested whether the inducible NF-κB activity binds to this sequence. The major inducible 3T3 factor binds to the CCGGGTTTTCCCC element upstream of the c-myc gene with an affinity similar to, although possibly lower than, that of the other identified NF-κB sites (Fig. 2A, lane 6). The factors associated with the constitutive complex II and with the inducible complex III bind to these various sites with an affinity similar to that of the slower, more prominent factor (complex I), although the complex II factor appears weaker for the immunoglobulin kappa probe (lane 3). All three factors are specific in their interaction with the various regulatory elements since they do not bind a double-point-mutated class I MHC enhancer probe (lane 7).

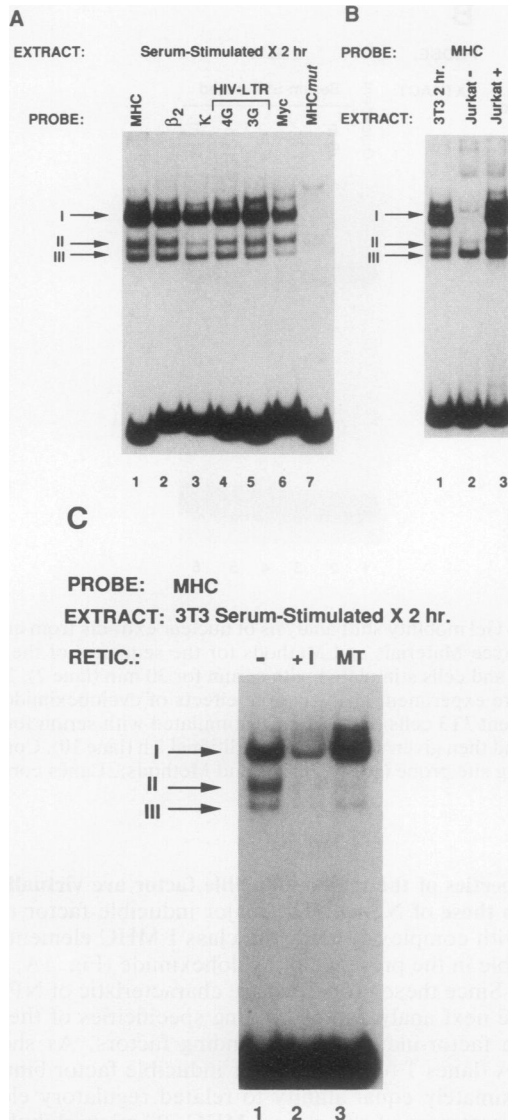We next compared the mobility of the inducible 3T3 complexes with that of NF-κB induced in the Jurkat T-cell

FIG. 2. The serum-inducible factors have properties of NF-κB. (A) Gel mobility shift analysis using nuclear extracts from 3T3 cells stimulated for 2 h with serum. Probes are MHC (lane 1), β₂-microglobulin (lane 2), immunoglobulin kappa (lane 3), HIV 4G (lane 4), HIV 3G (lane 5), c-myc (lane 6), and MHC double-point mutant (lane 7). See Materials and Methods for the sequences of the various probes. (B) Comparison of serum growth factor-induced 3T3 complexes with factors induced by mitogen and phorbol ester treatment of Jurkat T cells. All lanes have the class I MHC enhancer probe. Extracts are 2-h serum-stimulated 3T3 cells (lane 1), uninduced Jurkat T cells (lane 2), and Jurkat T cells stimulated for 2 h with PHA and PMA (lane 3). (C) Inhibition of DNA-binding activity by IκB, as determined by gel mobility shift analysis using nuclear extracts from 3T3 cells stimulated for 2 h with serum and using the class I MHC probe. Lanes: 1, no reticulocyte lysate added; 2, reticulocyte lysate programmed with MAD-3/IκB mRNA (31); 3, mock-translated lysates. Complexes I, II, and III are indicated.

FIG. 3. (A) Methylation interference of the serum-inducible 3T3 complexes. The class I MHC enhancer probe was labeled at either the HindIII or EcoRI end and methylated as described in Materials and Methods. This probe was used in gel mobility shift analysis with extracts of 3T3 cells that were stimulated with serum for 2 h. Complexes I and III and the free probe were cut out of the gel. DNA was recovered, cleaved with piperidine, and electrophoresed on polyacrylamide sequencing gels. The bottom and the top stands are shown, each with cleaved free probe and cleaved complexes I and III. (B) Sequence of the binding site. Guanines that interfere with binding are indicated with asterisks.

line by phorbol ester (PMA) and mitogen (PHA) stimulation. PMA and PHA treatment of Jurkat T cells has been shown to induce NF-κB binding activity (11, 49). As shown in Fig. 2B (lanes 1 and 3), the pattern of binding of the extracts of induced Jurkat cells is virtually identical to that of the extracts of serum growth factor-stimulated 3T3 cells. In both
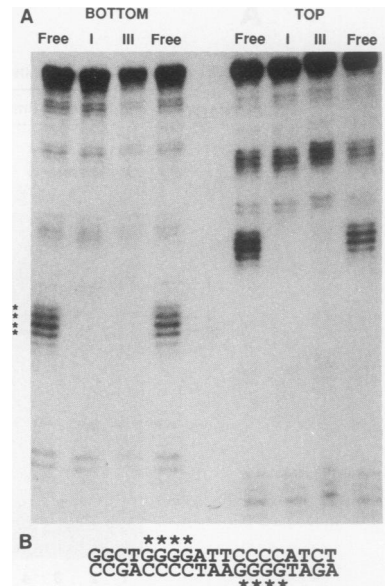
cases, nuclear extracts were used from cells that had been stimulated for 2 h with either serum growth factors (3T3 cells) or PMA and PHA (Jurkat cells). Similarly, binding specificities of the induced Jurkat and 3T3 factors are also identical (5). The factor associated with the inducible 3T3 complex III is specific, has many properties in common with NF-κB (Fig. 1A and 2A), and has a gel shift mobility similar to that of purified KBF1 (5).

To further relate the inducible 3T3 factors with NF-κB, we determined whether a recently identified IκB activity (31) could inhibit their DNA-binding activities. This IκB-like activity, MAD-3, inhibits the DNA-binding activity of NF-κB but not that of KBF1, MLTF, Oct-1, or H2TF1 (31). In vitro-translated MAD-3 specifically inhibited the DNA-binding activity of the inducible factors associated with complexes I and III but not that associated with complex II (Fig. 2C, lanes 1 to 3). Mock-translated reticulocyte lysates nonspecifically inhibited the formation of complex II (lane 3) but did not block formation of complexes I and III. These results demonstrate that the serum-inducible factors associated with complexes I and III are NF-κB-like.

To determine which nucleotides in the class I MHC enhancer element are capable of close interaction with the induced 3T3 factors, methylation interference analysis was performed on the complexes I and III. The methylated class I MHC enhancer probe was used in a gel shift reaction with extracts of 3T3 cells that had been serum stimulated for 2 h. Piperidine cleavage of DNA isolated from bands I and III in comparison with that of the free DNA probe revealed (Fig. 3) that methylation of all guanines in the symmetrical class I MHC enhancer element interfered with the binding of the
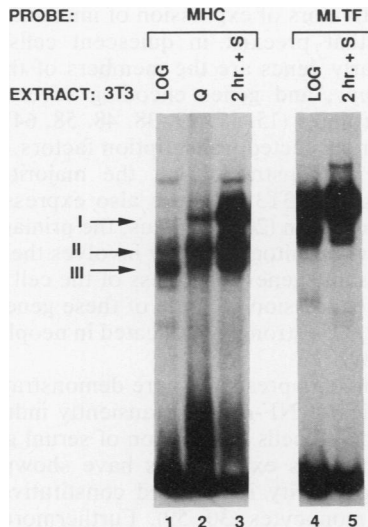
FIG. 4. Comparison of nuclear extracts of proliferating, quiescent, and serum-stimulated 3T3 cells, using gel mobility shift analysis. Lanes 1 to 3, class I MHC enhancer probe; lanes 4 and 5, MLTF probe. Equivalent amounts of the following extracts were analyzed: proliferating 3T3 cells (lanes 1 and 4), quiescent 3T3 cells (lane 2), and 3T3 cells serum stimulated for 2 h (lanes 3 and 5). The mobilities of complexes I, II, and III are indicated.
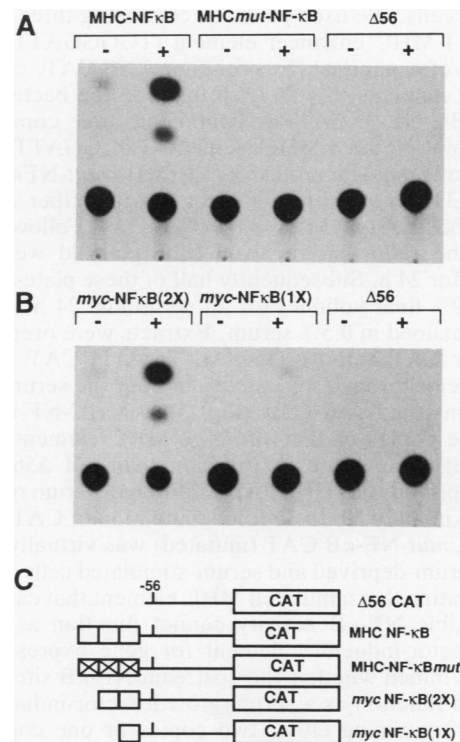

FIG. 5. Transient expression analysis in quiescent and serum-stimulated 3T3 cells transfected with CAT reporter plasmids. The CAT analysis presented is from a typical calcium phosphate coprecipitation experiment that has been reproduced in six experiments. (A) Expression analysis using plasmids MHC-NF-κB CAT, MHC-*mut*-NF-κB CAT, and Δ56 CAT, as indicated, in the absence of serum ($-$) and after serum stimulation ($+$). (B) Expression analysis using *myc*-NF-κB CAT (2×) or (1×) or Δ56 CAT, as indicated, in the absence ($-$) or presence ($+$) of serum. (C) Diagrams of the plasmid constructs used in the transfections. Open boxes represent wild-type MHC or c-*myc* NF-κB binding sites; an X within a box represents a double-point mutant. For transfection procedures, details of cell maintenance, and descriptions of plasmids, see Materials and Methods.

factors. Thus, the methylation interference patterns for complexes I and III are identical. These results are consistent with our previously published methylation interference pattern of NF-κB from lipopolysaccharide (LPS)-treated pre-B cells on the MHC element (7), although no partial interference is seen with the 3T3 complexes on the external guanines (Fig. 3).

Expression of the major NF-κB-like DNA-binding activity is specific for the G_0-to-G_1 transition in 3T3 cells. To determine whether NF-κB binding activity could be detected in proliferating 3T3 cells and whether its expression is independent of G_0, a nuclear extract was prepared from BALB/c 3T3 cells that were at an approximate density of 50 to 70% and were growing in fully supplemented medium. This extract was used in a gel mobility shift experiment in comparison with extracts of quiescent cells and 3T3 cells stimulated with serum for 2 h. As shown in Fig. 4 (lane 1), no NF-κB DNA-binding activity (complex I) is detected in the proliferating cells. However, complexes II and III are readily detected in these extracts. Levels of the MLTF binding activity are similar between proliferating 3T3 cells and cells stimulated with serum for 2 h (lanes 4 and 5), serving to normalize the extracts. The quiescent cells demonstrate a small amount of the NF-κB activity, and the serum-induced extracts contain a relatively large amount of the activity (lanes 2 and 3), as previously demonstrated. Lanes 1 to 3 were autoradiographed for 48 h in order to determine whether NF-κB DNA-binding activity could be detected. We reproducibly detect a small amount of the NF-κB like activity in the quiescent cell extracts (Fig. 1A, lanes 1 and 7; Fig. 4, lane 2), and this may represent a G_0-specific form of the binding activity or leakiness of some of the cells to enter G_1 once they have been maintained in G_0. The demonstration that nuclear extracts of proliferating 3T3 cells contain no detectable NF-κB binding activity is consistent with the study of Sen and Baltimore (59). Cytoplasmic extracts of

proliferating 3T3 cells contain a detergent-activatable NF-κB that gives a complex identical in mobility to complex I (5). Thus, proliferating 3T3 cells appear to have NF-κB DNA-binding activity, but virtually none of the DNA-binding activity is localized in the nucleus. The extracts of proliferating 3T3 cells contain a factor with properties of complex III (Fig. 4, lane 1). These results demonstrate that the levels of the NF-κB-like activity induced in the G_0-to-G_1 transition are not reproduced in cycling 3T3 cells. We cannot rule out the possibility that NF-κB binding activity is expressed at low levels in these cells. Furthermore, the DNA-binding activity associated with complex III is inducible in the G_0-to-G_1 transition (Fig. 1A), falls 12 to 24 h following serum stimulation of quiescent cells (Fig. 1A, lanes 5 and 6), is detected in the proliferating 3T3 cells (Fig. 4, lane 1), and may therefore be cell cycle regulated.

The class I MHC enhancer and c-*myc* NF-κB binding sites function as serum growth factor-inducible gene expression elements. To determine whether the inducible factors are capable of regulating gene expression mediated through the class I MHC NF-κB binding site, we performed transient expression experiments in serum-starved and serum-stimu-

lated 3T3 cells. We used plasmids containing three copies of the class I MHC enhancer element (TGGGGATTCCCCA) upstream of a minimal *fos* promoter (Δ56 CAT, containing promoter elements to −56 [28]) fused to the bacterial CAT gene (MHC-NFκB-CAT) or containing three copies of the double-point-mutated MHC element (TGCGGATTCCCGA) in the identical position of Δ56CAT (MHC*mut*-NFκB-CAT). BALB/c 3T3 cells were transfected with either Δ56CAT, MHC-NFκB-CAT, or MHC*mut*-NFκB-CAT. Following transfection, the cells that received each plasmid were serum deprived for 24 h. Subsequently half of these plates received serum (20% final volume) for an additional 24 h, while the others remained in 0.5% serum. Extracts were prepared and tested for CAT activity (Fig. 5). The Δ56 CAT construct gave extremely low CAT activity in both the serum-starved and serum-stimulated cells (Fig. 5A). MHC-NF-κB CAT, with three copies of the wild-type MHC element, demonstrated higher levels of expression than did Δ56 CAT in serum-deprived cells (Fig. 5A). Addition of serum resulted in an approximately 20- to 30-fold stimulation of CAT activity. The MHC*mut*-NF-κB CAT (mutated) was virtually inactive in both serum-deprived and serum-stimulated cells (Fig. 5A), demonstrating that a mutated MHC element that cannot bind the inducible NF-κB activity cannot function as a serum growth factor-inducible element for gene expression. We next determined whether the upstream NF-κB site in c-*myc* (22) could function as a serum growth factor-inducible site. Plasmids containing either two copies or one copy of the CCGGGTTTTCCCC element were tested in serum-deprived and serum-stimulated 3T3 cells as described above. Both the CAT plasmid containing two copies (*myc*-NF-κB 2×) and the plasmid containing one copy (*myc*-NF-κB 1×) of the *myc* site were induced by serum growth factor treatment (Fig. 5B). The 2× plasmid demonstrated higher basal activity than did the 1× plasmid. These results demonstrate that the c-*myc* NF-κB binding site functions as a serum growth factor-inducible element when combined with a heterologous promoter. Virtually identical gene expression results were obtained with use of either calcium phosphate precipitation or electroporation procedures (5). The results shown are those of a typical experiment; virtually identical results have been obtained in six different experiments. Since there are only two nucleotide differences in the wild-type and mutated regulatory elements cloned upstream of the *fos* promoter, it seems likely that the enhanced expression detected with the class I MHC enhancer is mediated by transcriptional stimulation. The slightly elevated levels of basal expression of MHC-NF-κB CAT and *myc*-NF-κB CAT in the quiescent cells (Fig. 5A and B) are consistent with the presence of specific transcriptional regulatory proteins in growth-arrested cells. As shown in Fig. 1A, there are such candidate proteins in extracts of quiescent cells, and the potential for undetected regulatory factors exists as well. The data demonstrate that the class I MHC enhancer and c-*myc* NF-κB binding sites function as inducible elements in the G_0-to-G_1 transition when linked to a minimal promoter.

DISCUSSION

Serum growth factors regulate a variety of cellular processes, including growth and differentiation (13, 18). In growth-arrested 3T3 cells, one of the first cellular responses to the addition of serum growth factors is the rapid transcriptional activation of a set of specific genes (1, 15, 16, 18, 41, 64). These immediate-early genes are characterized by their inducibility in the presence of protein synthesis inhibitors.

Thus, the activators of expression of immediate-early genes are factors that preexist in quiescent cells. Among the immediate-early genes are the members of the *fos* and *jun* families, c-*myc*, and genes encoding several zinc finger-containing proteins (15, 17, 29, 38, 48, 58, 64), all of which are known or suspected transcription factors. Furthermore, it has been demonstrated that the majority of primary response genes in 3T3 cells are also expressed in T cells treated with mitogen (20, 71). Thus, the primary response to growth factors or mitogens likely involves the expression of many of the same genes regardless of the cell type induced. Deregulated expression of some of these genes, such c-*fos*, c-*jun*, or c-*myc*, is strongly implicated in neoplastic transformation (21, 39).

The experiments presented here demonstrate that factors with properties of NF-κB are transiently induced in quiescent BALB/c 3T3 cells by addition of serum growth factors (Fig. 1A). Previous experiments have shown that NF-κB DNA-binding activity is detected constitutively only in B cells and in monocytes (30, 59). Furthermore, it has been shown that NF-κB is induced upon mitogenic activation of T and pre-B cells (11, 20, 49, 60). The demonstration that NF-κB exists in a wide range of cells in a cytoplasmically inactive form (2) and the identification of functional NF-κB binding sites in genes not expressed specifically in B or T cells or in monocytes (7, 22, 23, 42) is consistent with a diversified role for this factor. Our demonstration that serum growth factor treatment of quiescent 3T3 fibroblasts induced NF-κB-like activities suggests a role for these factors in the growth response of 3T3 cells. Data presented here are consistent with this role being in the transition from G_0 to G_1. Furthermore, these DNA-binding activities are rapidly induced by serum growth factors and are induced in the presence of cycloheximide (Fig. 1A). This result demonstrates that the NF-κB-like activities are immediate-early response factors in 3T3 cells. Interestingly, preliminary data suggest that NF-κB mRNA levels are also induced by serum growth factor treatment of quiescent 3T3 cells, but with kinetics slower than for the induction of the DNA-binding activity (35). It has recently been demonstrated that mitogenic activation of T cells results in significant increases of p50 NF-κB mRNA (12).

The major serum-inducible factor that we detect is identical to NF-κB in binding specificity and comigrated in the gel mobility shift assay with NF-κB induced in Jurkat T cells and in 70Z pre-B cells (Fig. 2A and B and data not shown). Consistent with the major inducible activity being NF-κB is the observation that an IκB-like activity (31) inhibited its DNA-binding activity (Fig. 2C). IκB has been previously shown to inhibit the binding of NF-κB through interaction with the p65 subunit (3, 66). Methylation interference of the major inducible factor is similar to that published for NF-κB binding to the class I MHC enhancer element, although we have noted previously that the external guanines of the class I MHC enhancer element give only partial interference in this assay with NF-κB induced in pre-B cells (7) and the 3T3 factor yields complete interference at all four guanines (Fig. 3). Thus, we cannot say conclusively whether the major inducible 3T3 factor is identical to that of NF-κB in other cell types but, as we noted above, 3T3 cells contain mRNA that is recognized by authentic NF-κB probe, and this mRNA is induced following serum growth factor stimulation. Furthermore, a second inducible factor (complex III) is detected that generates a DNA-protein complex similar in mobility to purified KBF1 (5) and has binding properties similar to or identical those of the inducible NF-κB like activity. The

identity of this factor is unknown, but it is likely to be related to NF-κB since its DNA-binding activity is inhibited by IκB (Fig. 2C).

Experiments demonstrate that the NF-κB DNA-binding activity is transiently induced in quiescent 3T3 cells following treatment with serum; the activity is at or near maximal levels following only 30 min of treatment and remains relatively high until at least 6 h of treatment. By 12 h, the DNA-binding activity has returned to much lower levels. Interestingly, we cannot detect the NF-κB-like activity in nuclear extracts of proliferating 3T3 cells (Fig. 4), although NF-κB activity is detected in deoxycholate-treated cytoplasmic extracts (data not shown). However, we detect elevated levels of this DNA-binding activity at least 6 h following serum stimulation, which is well into the initial G_1 phase. The cell cycle of the 3T3 cells used in this study is approximately 24 h, with a G_1 period of approximately 12 to 14 h (70). Thus, if NF-κB were expressed during an equivalent period of the G_1 period in cycling cells, we should be able to detect its activity in nuclear extracts of proliferating cells. Thus, it may be that NF-κB is induced only as cells leave the quiescent state (G_0) and would not be detected in cycling cells. Since we (Fig. 4) and others (59) have not detected any nuclear DNA-binding activity in proliferating 3T3 cells, we favor the view that NF-κB is expressed only in the G_0-to-G_1 transition. It is also possible that NF-κB is expressed in cycling cells, but in such small quantities or in such a narrow time frame that we cannot detect it in our gel mobility shift assays.

The inducibility of the NF-κB-like activities during the G_0-to-G_1 transition suggests that these factors play a role in control of expression of genes not strictly involved in immune function. A role for NF-κB in cell growth is suggested by several observations. One observation suggesting that NF-κB plays a role in T-cell growth is that the human T-cell lymphotropic virus type I *tax* gene product induces NF-κB DNA-binding activity (8, 44, 57). This virus is strongly implicated in T-cell leukemia, and thus *tax* activation may contribute to leukemogenesis by the activation of NF-κB, with the subsequent transcriptional stimulation of the interleukin-2 receptor gene. Recently, it has been demonstrated that tumor necrosis factor alpha has growth factor-like effects on quiescent 3T3 cells (19). Since this cytokine has been shown to induce NF-κB (46, 52), it may be that NF-κB is responsible for its growth stimulatory effects. NF-κB is also implicated in cell growth by the observation that the v-*rel* oncogene product, which is related to NF-κB, can bind a functional NF-κB target site and inhibit its function (9). The assumption here is that the transforming potential of v-*rel* is based on its ability to block normal NF-κB function. Finally, NF-κB binds and regulates expression of the β_1-interferon gene (23, 43, 67), and interferons have been shown to have negative growth effects on certain cells (references 25 and 54 and references within). The latter two observations would be consistent with a role for NF-κB in negative growth control, while the first two would be consistent with a positive growth role. Thus, in addition to a role in the transcriptional regulation of genes involved in immune function (42), NF-κB may regulate the expression of certain genes involved in growth control.

Interestingly, both of the inducible NF-κB-like factors bind to an element found some 1,100 bp upstream of the P1 transcription start site of the c-*myc* gene (Fig. 2A). This site was initially identified as a sequence capable of interacting with NF-κB-like factors found in WEHI 231 cells (22). Changes in NF-κB interactions with this sequence were noted upon growth arrest of these cells with anti-immunoglobulin treatment. c-*myc* expression is inducible by serum growth factor treatment of quiescent 3T3 cells (29). Although c-*myc* gene expression is complex (10, 37, 47, 63), it is tempting to speculate that NF-κB may play a role in the control of *myc* gene expression during the G_0-to-G_1 transition. It has been demonstrated that c-*myc* expression is inducible by LPS, cycloheximide, platelet-derived growth factor, and phorbol ester treatment of cells (38), and each of these reagents has been shown to induce NF-κB (5, 60). Experiments presented in Fig. 5B demonstrate that the c-*myc* NF-κB binding site confers serum growth factor inducibility on a minimal heterologous promoter. Thus, NF-κB may directly regulate c-*myc* gene expression following serum growth factor stimulation of quiescent 3T3 cells. Mudryj et al. (47) have demonstrated that another candidate regulator of serum growth factor inducibility of the c-*myc* gene is the factor E2F. E2F is inducible approximately fourfold by serum treatment of quiescent cells but binds much closer to the c-*myc* P1 promoter than does NF-κB.

The sequence TGGGGATTCCCCA found in the class I MHC enhancer confers growth inducibility on a minimal *fos* promoter (Fig. 5). Since we detect only the NF-κB activities as being serum inducible, we believe that these factors are in fact transcriptionally activating gene expression through the MHC enhancer element. Interestingly, we find that a construct that contains two copies of the highly related NF-κB site found in the immunoglobulin kappa enhancer is not inducible in serum-stimulated 3T3 cells (36). This construct is inducible in a pre-B-cell line when these cells are stimulated with LPS (53). Thus, it is not clear whether the NF-κB-like activity that we have detected in 3T3 cells is incapable of transcriptionally activating the kappa enhancer construct and is therefore a different NF-κB from that in B cells, whether some factor is missing in 3T3 cells that is required for expression mediated by the kappa element, or whether it is actually some other undetected factor (not NF-κB) that activates expression through the class I MHC enhancer and c-*myc* elements in 3T3 cells.

NF-κB is found in the cytoplasm of a wide variety of cell and tissue types, where it is associated with an inhibitor protein, IκB. Activation of NF-κB DNA-binding activity is associated with the phosphorylation of IκB (26, 62). NF-κB binding activity can be induced by interleukin-1, tumor necrosis factor alpha, and double-stranded RNA, by mitogenic stimulation of pre-B and Jurkat T cells, and by phorbol ester (11, 20, 23, 34, 42, 46, 49, 52, 60, 61). Most of these stimuli are known to activate protein kinases, consistent with the activation of nuclear NF-κB DNA-binding activity upon phosphorylation of IκB (26). Serum growth factor treatment of quiescent cells results in the activation of tyrosine kinases, hydrolysis of phosphatidylinositol, alterations in pH, increases in cytosolic calcium levels, and elevation of cyclic AMP, which results in activation of protein kinase C and cyclic AMP-activated kinases (68). Thus, activation of protein kinases by serum growth factor treatment of quiescent cells may lead to phosphorylation of IκB and the subsequent relocalization of NF-κB DNA-binding activity to the nucleus. The localization of NF-κB in the cytoplasm of serum-arrested cells makes it an ideal candidate for an immediate-early response factor in the growth response. Our demonstration that induction of NF-κB nuclear DNA-binding activity is near maximal following only 30 min of serum stimulation and does not require protein synthesis is consistent with this idea. These results establish a potential role for NF-κB in signal transduction,

linking engagement of growth factor receptors by specific protein ligands with regulation of specific gene expression. The induction of expression of an NF-κB DNA-binding activity by serum growth factor treatment of growth-arrested 3T3 cells and the lack of similar expression in proliferating cells suggest a direct role for this transcription factor in the G_0-to-G_1 transition.

REFERENCES

1. Almendral, J., D. Sommer, H. MacDonald-Bravo, J. Burckhardt, J. Perera, and R. Bravo. 1988. Complexity of the early genetic response to growth factors in mouse fibroblasts. Mol. Cell. Biol. 8:2140–2148.
2. Baeuerle, P., and D. Baltimore. 1988. A specific inhibitor of the NF-κB transcription factor. Science 242:540–546.
3. Baeuerle, P., and D. Baltimore. 1989. A 65-kD subunit of active NF-κB is required for inhibition of NF-κB by IκB. Genes Dev. 3:1689–1698.
4. Baldwin, A. 1990. Analysis of sequence-specific DNA-binding proteins by the gel shift mobility shift assay. DNA Protein Eng. Tech. 2:73–76.
5. Baldwin, A., J. Azizkhan, and L. Coodly. Unpublished data.
6. Baldwin, A., and P. Sharp. 1987. Binding of a nuclear factor to a regulatory sequence of the mouse H-2K^b class I major histocompatibility gene. Mol. Cell. Biol. 7:305–313.
7. Baldwin, A., and P. Sharp. 1988. Two transcription factors, NF-κB and H2TF1, interact with a single regulatory sequence in the class I major histocompatibility complex promoter. Proc. Natl. Acad. Sci. USA 85:723–727.
8. Ballard, D., E. Bohnlein, J. Lowenthal, Y. Wano, B. R. Franza, and W. Greene. 1988. HTLV-1 tax induces cellular proteins that activate the κB element in the IL-2 receptor α gene. Science 241:1652–1655.
9. Ballard, D., W. Walker, S. Doerre, P. Sista, J. Molitor, E. Dixon, N. Peffer, M. Hannink, and W. Greene. 1990. The v-rel oncogene encodes a κB enhancer binding protein that inhibits NF-κB function. Cell 63:803–814.
10. Bentley, D., and M. Groudine. 1986. A block to elongation is largely responsible for decreased myc transcription in differentiated HL60 cells. Nature (London) 321:702–706.
11. Bohnlein, E., J. Lowenthal, M. Siekevitz, D. Ballard, B. R. Franza, and W. Greene. 1989. The same inducible protein(s) regulates mitogen activation of both the IL-2 receptor α gene and type I HIV. Cell 53:827–836.
12. Bours, V., J. Villalobos, P. Burd, K. Kelly, and U. Siebenlist. 1990. Cloning of a mitogen-inducible gene encoding a κB DNA-binding protein with homology to the rel oncogene and to cell-cycle motifs. Nature (London) 348:76–80.
13. Bravo, R. 1990. Growth factor-responsive genes in fibroblasts. Cell Growth Differ. 1:305–309.
14. Carthew, R., L. Chodosh, and P. Sharp. 1985. An RNA polymerase II transcription factor binds to an upstream element in the adenovirus promoter. Cell 43:439–448.
15. Chavrier, P., M. Lerial, P. Lemaire, J. Almendral, R. Bravo, and P. Charnay. 1988. A gene encoding a protein with zinc fingers is activated during G_0/G_1 transition in cultured cells. EMBO J. 7:29–35.
16. Cochran, B., A. Reffel, and C. Stiles. 1983. Molecular cloning of gene sequences regulated by platelet-derived growth factor. Cell 32:939–947.
17. Cochran, B., J. Zullo, I. Verma, and C. Stiles. 1984. Expression of the c-fos gene and of a fos-related gene is stimulated by platelet-derived growth factor. Science 226:1080–1082.
18. Collins, B., and C. Stiles. 1989. Serum-inducible genes. Adv. Cancer Res. 53:1–32.
19. Cornelius, P., M. Marlowe, M. Lee, and P. Pekala. 1990. Growth factor-like effects of tumor necrosis factor α. J. Biol. Chem. 265:20506–20516.
20. Crabtree, G. 1989. Contingent genetic regulatory events in T lymphocyte activation. Science 243:355–361.
21. Curran, T., and B. R. Franza. 1988. Fos and jun: the AP-1 connection. Cell 55:395–397.
22. Duyao, M., A. Buckler, and G. Sonenshein. 1990. Interaction of an NF-κB-like factor with a site upstream of the c-myc promoter. Proc. Natl. Acad. Sci. USA 87:4727–4732.
23. Fan, C.-M., and T. Maniatis. 1989. Two different virus-inducible elements are required for human β-interferon gene regulation. EMBO J. 8:101–110.
24. Fried, M., and D. Crothers. 1981. Equilibria and kinetics of lac repressor-operator interactions by polyacrylamide gel electrophoresis. Nucleic Acids Res. 9:6505–6525.
25. Garcia-Blanco, M., J. Porter, E. Morrison, and C. Stiles. 1990. The beta-interferon-mediated antimitogenic state resembles the anti-viral state. Oncogene Res. 5:323–328.
26. Ghosh, S., and D. Baltimore. 1990. Activation in vitro of NF-κB by phosphorylation of its inhibitor, IκB. Nature (London) 344:678–682.
27. Ghosh, S., A. Gifford, L. Riviere, P. Tempst, G. Nolan, and D. Baltimore. 1990. Cloning of the p50 DNA-binding subunit of NF-κB: homology to rel and dorsal. Cell 62:1019–1029.
28. Gilman, M., R. Wilson, and R. Weinberg. 1986. Multiple protein-binding sites in the 5' flanking region regulate c-fos expression. Mol. Cell. Biol. 6:4305–4316.
29. Greenberg, M., and E. Ziff. 1984. Stimulation of 3T3 cells induces transcription of the c-fos protooncogene. Nature 311:433–438.
30. Griffin, G., K. Leung, T. Folks, S. Kunkel, and G. Nabel. 1989. Activation of HIV gene expression during monocyte differentiation by induction of NF-κB. Nature (London) 339:70–73.
31. Haskill, S., A. Beg, M. Tompkins, J. Morris, A. Yurochko, A. Sampson-Johannes, K. Mondal, P. Ralph, and A. Baldwin. 1991. Characterization of an immediate-early gene induced in adherent monocytes that encodes IκB-like activity. Cell 65:1281–1289.
32. Heikkila, R., G. Schwab, E. Wickstrom, S. Loke, D. Pluznik, R. Watt, and L. Neckers. 1987. A c-myc antisense oligodeoxynucleotide inhibits entry into S phase but not progress from G_0 to G_1. Nature (London) 328:445–449.
33. Israel, A., A. Kimura, M. Kieran, O. Yano, J. Kanellopoulos, O. LeBail, and P. Kourilsky. 1987. A common positive trans-acting factor binds to enhancer sequences in the promoters of mouse H-2 and β$_2$-microglobulin genes. Proc. Natl. Acad. Sci. USA 84:2653–2657.
34. Israel, A., O. LeBail, D. Hatat, J. Piette, M. Kieran, F. Logeat, D. Wallach, M. Fellous, and P. Kourilsky. 1989. TNF stimulates expression of MHC class I genes by inducing an NF-κB-like enhancer binding activity which displaces constitutive factors. EMBO J. 8:3793–3800.
35. Ito, C., J. Azizkhan, and A. Baldwin. Unpublished data.
36. Jensen, D., A. Baldwin, and J. Azizkhan. Unpublished data.
37. Kakkis, E., K. Riggs, W. Gillespie, and K. Calame. 1989. A

transcriptional repressor of c-myc. Nature (London) 339:718–721.

38. Kelly, K., B. Cochran, C. Stiles, and P. Leder. 1983. Cell-specific regulation of the c-myc gene by lymphocyte mitogens and platelet-derived growth factor. Cell 35:603–610.

39. Kelly, K., and U. Siebenlist. 1986. The regulation and expression of c-myc in normal and malignant cells. Annu. Rev. Immunol. 4:317–438.

40. Kieran, M., V. Blank, F. Logeat, J. Vondekerckhove, F. Lottspeich, O. LeBail, M. Urban, P. Kourilsky, P. Baeurele, and A. Israel. 1990. The DNA-binding subunit of NF-κB is identical to factor KBF1 and homologous to the rel oncogene product. Cell 62:1007–1018.

41. Lau, L., and D. Nathans. 1985. Identification of a set of genes expressed during the G0/G1 transition of cultured mouse cells. EMBO J. 4:3145–3151.

42. Lenardo, M., and D. Baltimore. 1989. NF-κB: a pleiotropic mediator of inducible and tissue-specific gene control. Cell 58:227–229.

43. Lenardo, M., C.-M. Fan, T. Maniatis, and D. Baltimore. 1989. The involvement of NF-κB in β-interferon gene regulation reveals its role as a widely inducible mediator of signal transduction. Cell 57:287–294.

44. Leung, K., and G. Nabel. 1988. HTLV-I transactivator induces IL-2 receptor expression through an NF-κB-like factor. Nature (London) 333:776–778.

45. Liebermann, T., and D. Baltimore. 1990. Activation of IL-6 gene expression through the NF-κB transcription factor. Mol. Cell. Biol. 10:2317–2324.

46. Lowenthal, J., D. Ballard, N. Bogerd, E. Bohnlein, and W. Greene. 1989. Tumor necrosis factor-alpha activation of the IL-2 receptor-alpha gene involves the induction of κB-specific DNA binding proteins. J. Immunol. 142:3121–3128.

47. Mudryj, M., S. Hiebert, and J. Nevins. 1990. A role for the adenovirus inducible E2F transcription factor in a proliferation dependent signal transduction pathway. EMBO J. 9:2179–2184.

48. Muller, R., R. Bravo, J. Burckhardt, and T. Curran. 1984. Induction of c-fos gene and protein by growth factors precedes activation of c-myc. Nature (London) 312:716–720.

49. Nabel, G., and D. Baltimore. 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature (London) 326:711–713.

50. Nishikura, K., and J. Murray. 1987. Antisense DNA of protooncogene c-fos blocks renewed growth of quiescent 3T3 cells. Mol. Cell. Biol. 7:639–649.

51. Nolan, G., S. Ghosh, H.-C. Liou, P. Tempst, and D. Baltimore. 1991. DNA binding and IκB inhibition of the cloned p65 subunit of NF-κB, a rel-related polypeptide. Cell 64:961–969.

52. Osborn, L., S. Kunkel, and G. Nabel. 1989. Tumor necrosis factor α and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor κB. Proc. Natl. Acad. Sci. USA 86:2236–2340.

53. Pierce, J., M. Lenardo, and D. Baltimore. 1988. An oligonucleotide that binds nuclear factor NF-κB acts as a lymphoid-specific and inducible enhancer element. Proc. Natl. Acad. Sci. USA 85:1482–1486.

54. Resnitzky, D., and A. Kimchi. 1991. Deregulated c-myc expression abrogates the interferon and interleukin 6-mediated G_0/G_1 cell cycle arrest but not other inhibitory responses in M1

myeloblastic cells. Cell Growth Differ. 2:33–41.

55. Ron, D., A. Brasier, K. Wright, J. Tate, and J. Habener. 1990. An inducible 50-kilodalton NF-κB-like protein and a constitutive protein both bind the acute-phase response element of the angiotensinogen gene. Mol. Cell. Biol. 10:1023–1032.

56. Ruben, S., P. Dillon, R. Schreck, T. Henkel, C.-H. Chen, M. Maher, P. Baeuerle, and C. Rosen. 1991. Isolation of a rel-related human cDNA that potentially encodes the 65 kD subunit of NF-κB. Science 251:1490–1493.

57. Ruben, S., H. Poteat, T. Tan, K. Kawakami, R. Roeder, W. Haseltine, and C. Rosen. 1988. Cellular transcription factors and regulation of IL-2 receptor gene expression by HTLV-I tax gene product. Science 241:89–91.

58. Ryseck, R., S. Hirai, M. Yaniv, and R. Bravo. 1988. Transcriptional activation of c-jun during the G_0/G_1 transition in mouse fibroblasts. Nature (London) 334:535–537.

59. Sen, R., and D. Baltimore. 1986. Multiple nuclear factors interact with the immunoglobulin enhancer sequence. Cell 46:705–716.

60. Sen, R., and D. Baltimore. 1986. Inducibility of κ immunoglobulin enhancer-binding protein NF-κB by a post-translational mechanism. Cell 47:921–928.

61. Shirakawa, F., M. Chedid, J. Suttles, B. Pollock, and S. Mizel. 1989. Interleukin 1 and cAMP induce κ immunoglobulin light-chain expression via activation of an NF-κB-like DNA-binding protein. Mol. Cell. Biol. 9:959–964.

62. Shirakawa, F., and S. Mizel. 1989. In vitro activation and nuclear translocation of NF-κB catalyzed by cyclic AMP-dependent protein kinase and protein kinase C. Mol. Cell. Biol. 9:2424–2430.

63. Siebenlist, U., P. Bressler, and K. Kelly. 1988. Two distinct mechanisms of transcriptional control operate on c-myc during differentiation of HL60 cells. Mol. Cell. Biol. 8:867–874.

64. Sukhatme, V., X. Cao, L. Chang, C. Tsai-Morris, D. Stamenovich, P. Ferreira, D. Cohen, S. Edwards, T. Shows, T. Curran, M. LeBeau, and E. Adamson. 1988. A zinc finger-encoding gene coregulated with c-fos during growth and differentiation and after cellular depolarization. Cell 53:37–43.

65. Swick, A., M. Blake, J. Kahn, and J. Azizkhan. 1989. Functional analysis of GC element binding and transcription in the hamster dihydrofolate reductase gene promoter. Nucleic Acids Res. 17:9291–9304.

66. Urban, M., and P. Baeuerle. 1990. The 65 kD subunit of NF-κB is a receptor for IκB and a modulator of DNA-binding specificity. Genes Dev. 4:1975–1984.

67. Visvanathan, K., and S. Goodbourn. 1989. Double-stranded RNA activates binding of NF-κB to an inducible promoter element in the human β-interferon promoter. EMBO J. 8:1129–1138.

68. Williams, L. 1989. Signal transduction by the platelet-derived growth factor receptor. Science 243:1564–1570.

69. Zabel, U., and P. Baeuerle. 1990. IκB can rapidly dissociate the complex of NF-κB transcription factor with its cognate DNA. Cell 61:255–270.

70. Zeleznik-Le, N., J. Azizkhan, and J. Ting. Unpublished data.

71. Zipfel, P., S. Irving, K. Kelly, and U. Siebenlist. 1989. Complexity of the primary genetic response to mitogenic activation of human T cells. Mol. Cell. Biol. 9:1041–1048.