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The Interferon-Inducible p202 Protein as a Modulator of Transcription: Inhibition of NF-κB, c-Fos, and c-Jun Activities

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The antimicrobial, immunomodulatory, and cell growth-regulatory activities of the interferons are mediated by interferon-inducible proteins. One of these is p202, a nuclear protein that is encoded by the Ifi 202 gene from the interferon-activatable gene 200 cluster. Overexpression of p202 in transfected cells slows down cell proliferation. As shown earlier, p202 binds to the hypophosphorylated form of the retinoblastoma susceptibility protein. Here we report that p202 inhibits the activities of the NF-κB and the AP-1 enhancers both in transiently transfected cells and in transfected stable cell lines overexpressing p202. Furthermore, p202 binds the NF-κB p50 and p65 and the AP-1 c-Fos and c-Jun transcription factors in vitro and in vivo. NF-κB, c-Fos, and c-Jun participate in the transcription of various cellular and viral genes, and thus p202 can modulate the expression of these genes in response to interferons.

The interferons (IFNs) are cytokines with antiviral, antibacterial, antiprotozoal, immunomodulatory, and cell growth-regulatory functions. They exert these functions by controlling the expression of numerous genes encoding effector proteins (16, 41, 55, 65). One set of effector proteins, including p202, p203, p204, and D3, is encoded by six or more structurally related and IFN-activatable murine genes at the q21-q23 region of chromosome 1 (gene 200 cluster) (13, 36, 42, 48, 59). Two homologous human proteins (myeloid cell nuclear differentiation antigen [MNDA] and IFI16) have also been described (7, 64). The proteins encoded by all these genes have homologous C-terminal 200-amino-acid segments (42).

At least four of the proteins (p202, p204, MNDA, and IFI16) are nuclear (7, 11, 12, 15, 42). Upon treatment of cells with IFN, the induced p202 first appears in the cytoplasm, where it is attached to a fast-sedimenting subcellular component, and then accumulates in the nucleus after a delay (12). In metaphase cells, p202 appears to be associated with chromatin. Although p202 binds DNA in vitro, the cleavage of DNA in isolated nuclei does not result in the release of p202. This and other results suggest that the binding of p202 in the nucleus is at least in part due to protein-protein interactions.

The proteins found to bind to p202 in vitro and in vivo include the retinoblastoma susceptibility protein pRb (10), which has a central role in controlling cell cycling (68). It is the hypophosphorylated, growth-inhibitory form of pRb that binds to p202. Overexpression of p202 in transfected cells slows down cell proliferation. The contribution of the interaction between p202 and pRb to the cell growth-inhibitory activity of p202 remains to be explored. However, the finding that p202 interacts with pRb prompted us to test whether p202 also interacts with other proteins controlling transcription. Here we describe studies concerning the effect of p202 on the NF-κB p50 and p65 and the AP-1 c-Jun and c-Fos transcription factors.

NF-κB controls the expression of numerous genes of the immune and inflammatory responses (including IFN-β) and of various viral genes (including those of human immunodeficiency virus [HIV]) (5, 56, 57, 60). The NF-κB protein is a heterodimer consisting of two proteins, p50 (also designated NF-κB_1) and p65 (also designated Rel A). p50 and p65 are members of the Rel/NF-κB family of proteins. These proteins serve as inducible eukaryotic transcription factors that form various homo- and heterodimers. NF-κB (i.e., the p50-p65 complex) is present in essentially all cells and is the most abundant of the Rel/NF-κB family heterodimers. NF-κB is retained in an inactive form in the cytoplasm as a consequence of the binding of particular proteins of the IκB family (6, 24) (i.e., IκB-α and IκB-β) (62). The activation of NF-κB and its movement to the nucleus can be correlated with the proteolytic degradation of one or both of the IκB proteins (8, 46, 49, 60, 62, 63). This can be triggered by the phosphorylation of these proteins. The long list of agents activating NF-κB includes various cytokines (e.g., interleukin-1 and tumor necrosis factor alpha), phorbol esters (e.g., phorbol myristate acetate [PMA] and tetradecanoyl phorbol acetate [TPA]), bacterial lipopolysaccharide, and double-stranded RNA (5, 6, 24, 44, 57, 60). Of these agents, tumor necrosis factor alpha and PMA elicit the inactivation and degradation of only IκB-α and the transient activation of NF-κB (62). Interleukin-1 and lipopolysaccharide, however, elicit the inactivation and degradation of both IκB-α and IκB-β and a more persistent activation of NF-κB.

The activity of the AP-1 transcription factors is rapidly induced in response to a vast array of extracellular stimuli, including growth factors and UV irradiation. The set of AP-1 transcription factors includes proteins of the Fos family (e.g., c-Fos) and the Jun family (e.g., c-Jun) (14, 34). These proteins have leucine zippers (38), allowing the formation of various homo- and heterodimers (25). The c-Jun–c-Fos heterodimer binds to TPA-responsive elements in DNA with higher affinity than the c-Jun homodimer, whereas the c-Fos homodimer does not bind appreciably. AP-1 function is modulated by control-

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ling the synthesis of the proteins as well as their activities in general by phosphorylation and dephosphorylation at several sites (4, 17, 31, 32). AP-1 function was shown to be enhanced through several signaling cascades: some result primarily in the induction of c-Fos, and others (involving Jun kinases) result primarily in the activation of c-Jun by phosphorylation (27, 43, 45).

Here we report that p202 inhibits the expression of various endogenous and reporter genes that depend on the NF-κB p50 and p65 or the AP-1 c-Fos and c-Jun transcription factors. Such inhibition is seen in both transiently transfected cells and transfected stable cell lines which overexpress p202. Furthermore, p202 binds to the NF-κB p50 and p65 proteins and the AP-1 c-Jun and c-Fos proteins both in vitro and in vivo.

MATERIALS AND METHODS

Construction of plasmids. p202 cDNA (13), p50 cDNA (23), and p65 cDNA (47) cloned in pBluescript-SK$^+$ were used for plasmid constructions. To obtain mammalian expression plasmids, the appropriate cDNA segments containing the entire coding regions of p202, p50, or p65 were cloned into pcDNA3 (Invitrogen). A p202 antisense expression plasmid was generated by inserting the entire coding region in the antisense orientation into pcDNA3. To obtain bacterial expression plasmids for fusions with glutathione *S*-transferase (GST), GST-p202, GST-p50, and GST-p65, appropriate cDNA segments were cloned in frame into the pGEX-kg vector. The correctness of the constructs was confirmed by sequencing.

Preparation of GST-p202, GST-p50, and GST-p65 fusion proteins and of antiserum to p202. Fusion proteins expressed in *Escherichia coli* XL-1 Blue (Gibco) were affinity purified on glutathione-Sepharose beads (Pharmacia) (58) and dialyzed extensively against binding buffer (50 mM KCl, 25 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, pH 7.5], 1 mM dithiothreitol, 20 μM $ZnSO_4$, 10% glycerol, 0.1% Nonidet P-40). Protein concentrations were determined with a Bio-Rad kit (Bio-Rad Laboratories, Cambridge, Mass.). The entire p202 protein was used for immunization of a rabbit at the Animal Facility of Yale University. The anti-p204 antiserum kindly provided by D. Choubey was prepared as described earlier (12).

Construction of cell lines ov1, ov2, and ov3, overexpressing p202. Cell culture reagents were from Gibco. L929 cells (ATCC CCL1) were grown in monolayers in 100-mm dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic and antimycotic agents (complete DMEM). When the culture reached 40 to 60% confluency, the cells were transfected with the p202 expression construct in pcDNA3 or with the pcDNA3 vector by the calcium phosphate method (9). Following G418 (800 μg ml^{-1}) selection, 10 to 15 G418-resistant colonies were obtained. Three of these (ov1, ov2, and ov3) were found to express p202 at a level approximately threefold higher than that in the control cell lines (L929 or L929 carrying the pcDNA3 vector). The ov1, ov2, and ov3 lines were grown and maintained in complete DMEM containing 800 μg of G418 per ml.

Growth comparison of transfected cell lines constitutively overexpressing p202 and the parental cell line. Cells (0.18×10^6) from the parental L929 line carrying a pcDNA3 vector and from the transfected cell lines ov1, ov2, and ov3, constitutively overexpressing p202, were plated in 10-cm dishes in 10 ml of complete DMEM supplemented with G418 (800 μg ml^{-1}). Sets of dishes were treated with trypsin at 0, 24, 48, and 72 h, and the cells were counted in a Coulter counter. The control L929 culture reached confluency at 72 h.

RNA detection by Northern (RNA) blotting. Cells were cultured in 100-mm dishes in 10 ml of medium to 40 to 60% confluency. Poly(rI) · poly(rC) (0.1 mg ml^{-1}) (Sigma) or PMA (50 ng ml^{-1}) was added to induce IFN-β or collagenase I mRNA, respectively. Total RNA was extracted (9), and Northern blotting was performed (53) with a mouse IFN-β probe (26) (a gift from P. Pitha-Rowe) or a rat collagenase I probe (50) (kindly provided by J. Jeffrey). After hybridization with the probe, the membrane was stripped and rehybridized with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe (20).

Transient transfection. EL4 (murine T) cells (ATCC TIB181) were cultured in suspension in RPMI medium supplemented with 10% FBS and 1% antibiotic and antimycotic agents (Gibco) and transfected with the indicated constructs by the DEAE-dextran procedure (Promega) according to the manufacturer's instructions. After transfection, the cultures were incubated for 40 h and split into two aliquots. One aliquot was incubated with concanavalin A (2 μg ml^{-1}) and PMA (20 ng ml^{-1}) for 14 h before harvesting. The other aliquot was incubated without further supplementation.

L929 (murine connective tissue) cells and AKR-2B (cloned murine embryo) cells (22) (from H. Moses, Vanderbilt University, Nashville, Tenn.) were grown in monolayers (unless otherwise indicated) in 100-mm dishes. These were seeded with 0.8×10^6 to 2.4×10^6 cells in 10 ml of complete DMEM and incubated at 37°C in a CO_2 incubator for 18 to 24 h, when the cells reached 50 to 80% confluency. Transfections with the indicated constructs were performed with the lipofectamine reagent (Gibco) following the manufacturer's instructions. The transfected cultures were incubated for 36 to 40 h and (if so indicated) divided into two sets. One set was incubated with either poly(rI) · poly(rC) (0.1 μg ml^{-1}) or PMA (50 ng ml^{-1}) for 14 h before harvesting. The other aliquot was incubated without further supplementation.

Reporter assays. In the −110 huIFNβ-CAT plasmid (18) from D. Thanos and T. Maniatis, the human IFN-β promoter (from nucleotide −110 upstream of the start site of transcription) drives the expression of the chloramphenicol acetyltransferase (CAT) gene. In the PRDII-CAT plasmid (33) from D. Thanos and T. Maniatis, four copies of the PRDII elements linked to the IFN-β gene TATA box drive the expression of the CAT gene. The PRDII sequence is a binding site for the NF-κB and the HMGI(Y) proteins. In the HIV-LUC plasmid (37) from M. Feinberg, luciferase expression is driven by the wild-type HIV 5′ long terminal repeat (LTR) *Xho*I-*Hin*dIII fragment cloned into the pGL2 plasmid (Promega). The bulk of PMA-induced activity is due to the activation of 2 κB sites in the LTR. In the AP-1-LUC plasmid (52) from R. Flavell, luciferase expression is driven by four TPA-responsive elements (from human collagenase) in the context of the minimal rat prolactin promoter. In the pBIIXLUC plasmid (37), luciferase expression is driven by two κB sites from the immunoglobulin kappa enhancer. In the A2BCAT plasmid (72) from T. Williams, CAT expression is driven by three copies of the AP-2 site in the BCAT reporter construct. In the 4OCT-LUC plasmid from K. Prakash, luciferase expression is driven by four copies of the OCT1 consensus oligodeoxynucleotide (Promega) in the PGL2 promoter vector (Promega). In the CAT-HSI plasmid (71) from T. Williams, CAT expression is driven by the human metallothionein gene (hMetIIa) segment from −764 to +69 in the BLCAT3 plasmid. pSVgal, serving as an internal standard for transfection, was purchased from Promega.

The transfected cells were harvested and lysed in 1 ml of lysis buffer (Promega reporter assay system), and the assays were performed according to the protocols from Promega. The measured luciferase and CAT activities were normalized to the β-galactosidase activity for each dish.

Immunoprecipitation and immunoblotting. AKR-2B cells were grown in monolayers in 100-mm dishes in complete DMEM. When the cultures reached 40 to 60% confluency, IFN (1,000 U ml^{-1}) and/or PMA (50 ng ml^{-1}) was added for 24 h. The recombinant human IFN-α_2/α_1-83 (specific activity, 8.1×10^7 U ml^{-1}) (67) used, which is highly active in murine cells, was a generous gift from H. Weber and C. Weissmann.

For preparing total cell extracts, cells from one dish were lysed with 500 μl of IP buffer (150 mM NaCl, 10 mM Tris-HCl [pH 7.4], 0.1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 50 μg of leupeptin per ml) and briefly sonicated, and the cell debris was pelleted. Cytoplasmic and nuclear fractions were prepared (11).

Immunoprecipitation was performed with 200 μl of cell extract and a 1,000-fold dilution of the preimmune serum or the anti-p50 (Santa Cruz), anti-Jun, or anti-Fos antiserum in IP buffer at 4°C overnight. The reaction mixtures were incubated with protein A-Sepharose beads (Sigma), 30 to 50 μl in a 50% suspension in IP buffer, at 4°C for 90 min on a rotator, and the beads were sedimented by centrifugation and washed with IP buffer five times. The washed beads were incubated with 50 μl of a 0.05% solution of the NF-κB p50 control peptide (Santa Cruz) or c-Jun or c-Fos protein (generously provided by T. Curran) at 4°C. The released proteins were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western immunoblotting with the anti-p202 antiserum and the ECL detection system (Amersham) following the instructions of the manufacturer. The anti-Fos and anti-Jun antisera were kindly provided by S. Xanthoudakis and T. Curran. These antisera, designated anti-Fos 2.2 and anti-Jun 2.2, were raised in rabbits immunized with full-length rat Fos and Jun proteins.

Labeled p202 protein was obtained by transcription and translation from a 202 cDNA insert in pBluescript (13) in vitro (TNT T7 coupled reticulocyte lysate system; Promega) in the presence of [^{35}S]methionine. Protein binding in vitro was assayed as described before (35). Equal aliquots (20 μl) of labeled p202 protein were incubated in 400 μl of NETN buffer (100 mM NaCl, 20 mM Tris-HCl [pH 8.0], 1 mM EDTA, 0.5% Nonidet P-40) with 20 μl of Sepharose beads loaded, if so indicated, with 50 ng of GST fusion proteins (GST, GST-p50, or GST-p65) or with 20 μl of Ni-nitrilotriacetic acid-agarose (Qiagen) loaded (if so indicated) with 50 ng of fusion protein His_6-c-Jun, His_6-c-Fos, His_6-wbJun (a Jun segment extending from amino acid residues 206 to 315), or His_6-wbFos (a Fos segment extending from amino acid residues 116 to 211), each carrying a six-histidine tag (2). The reaction mixtures were rotated at room temperature for 1 h, the beads were washed five times in NETN buffer containing 0.005% SDS, and the proteins were released by SDS-PAGE buffer and analyzed by SDS-PAGE. The gel was soaked in an enhancer (Resolution; E.M. Corporation) and processed for fluorography.

EMSA. The gel electrophoretic mobility shift assay (EMSA) was performed in binding buffer (50 mM KCl, 25 mM HEPES [pH 7.5], 5 mM dithiothreitol, 20 μM $ZnSO_4$, 0.2 mg of bovine serum albumin per ml, 10% glycerol, Nonidet 0.1% P-40) containing 0.1 μg of poly(dI) · poly(dC) (Sigma). Each of the proteins specified was added (50 ng) to binding buffer (final volume, 20 μl) and incubated at 37°C for 10 min. Then 1 μl of ^{32}P-labeled AP-1 oligodeoxynucleotide probe (Promega) (1×10^4 to 2×10^4 cpm) was added, and the incubation was continued for 15 to 20 min prior to electrophoresis on a 5% native gel in 0.5× TBE buffer (53) in the cold room at 105 V for 1 h in the Bio-Rad minigel system.

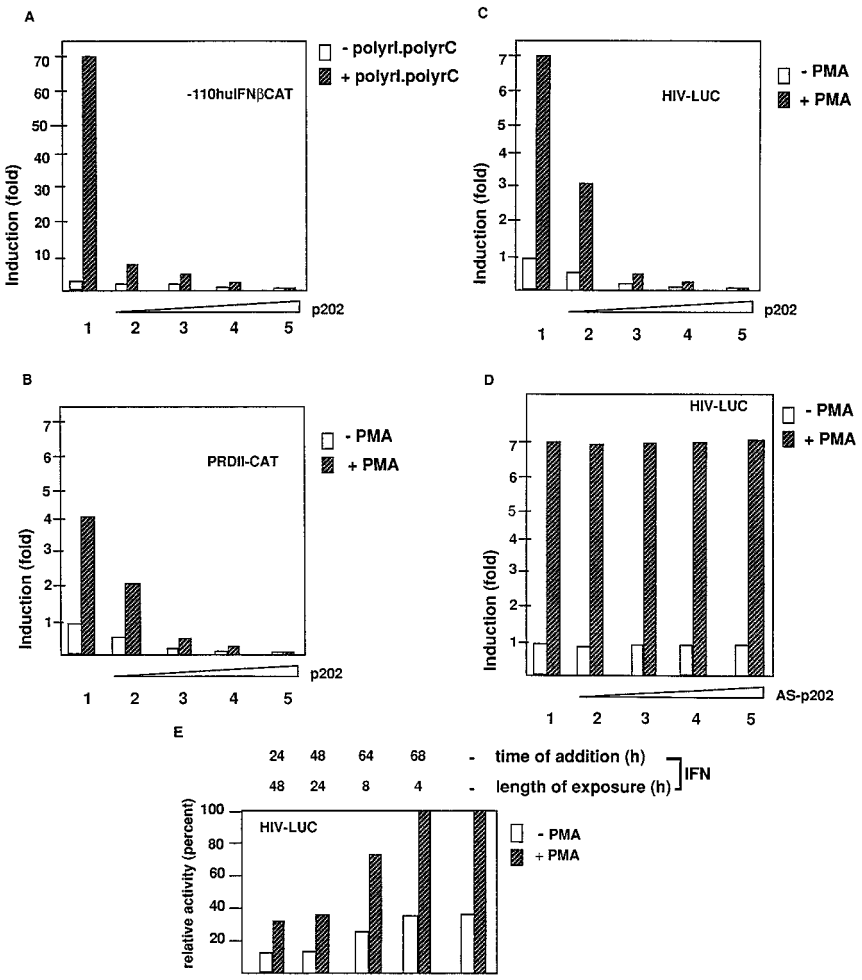

FIG. 1. Inhibition of the activity of NF-κB enhancer-driven reporter genes by increasing amounts of p202 expression plasmid or by IFN. Transient-transfection assays were performed as follows. L929 cells in monolayers (in 100-mm dishes) (A) or EL4 cells (in suspension in 10 ml of medium in 50-ml flasks) (B, C, and D) were transfected with 5 μg of the indicated reporter plasmid together with various amounts (0, 0.1, 0.5, 2, or 5 μg) of DNA (lanes 1 to 5, respectively) of either p202 expression plasmid (A, B, and C) or p202 antisense expression plasmid (AS-p202) (D). Two micrograms of pSVgal (internal standard) was cotransfected with pcDNA3 vector DNA (without insert) in an amount so that the total amount of DNA transfected per dish was 12 μg. At 36 h after transfection, the cultures were divided into two sets. One set was supplemented with poly(rI) · poly(rC) (A) or PMA (B, C, and D) and further incubated for 14 h. The second set was not supplemented and served as a control. The reporter gene activities measured were normalized to the β-galactosidase activities for each dish. The relative activities were calculated by taking the transactivations in the dishes with no transfected p202 plasmid and no poly(rI) · poly(rC) (A) or no pMA treatment (B, C, and D) as 1 (control transfection). [Note: In the control transfections, luciferase activity was approximately 104 light units per μg of total cell protein, and CAT activities resulted in 0.5 to 5% conversion. β-Galactosidase activities per microgram of total cell protein from the transfected dishes were not affected by cotransfection with p202 or by treatment with poly(rI) · poly(rC) (A) or PMA (B, C, and D), and variations between dishes were less than 15%.] (A) Inhibition of the IFN-β enhancer-driven CAT reporter (−110huIFNβ-CAT) activity by increasing amounts of p202 expression plasmid. (B) Inhibition of the PRDII element (NF-κB site)-driven CAT reporter activity by increasing amounts of p202 expression plasmid. (C) Inhibition of the HIV enhancer-driven HIV-LUC reporter activity by increasing amounts of p202 expression plasmid. (D) Lack of inhibition of the HIV enhancer-driven HIV-LUC reporter activity by increasing amounts of p202 antisense expression plasmid. (E) Delayed inhibition of the HIV NF-κB enhancer-driven LUC reporter gene (HIV-LUC) activity by IFN. L929 cells in monolayers in ten 100-mm dishes were transfected (at 0 h) with 5 μg of HIV-LUC reporter plasmid and 5 μg of pSVgal plasmid (serving as an internal control). Pairs of dishes were supplemented with IFN (1,000 U ml^{-1}) at 24, 48, 64, and 68 h as indicated. One dish from every pair was further supplemented with PMA (50 ng ml^{-1}) at 66 h (shaded bars). The other dish from the pair served as a control (open bars). The dishes were incubated for 72 h. The luciferase activities measured were normalized to the β-galactosidase activity for each dish. Relative activities were calculated by taking the activity in the dish supplemented with PMA (but no IFN) as 100%. For further details, see Materials and Methods.

The wet gels were transferred onto Whatman paper and exposed to X-ray film at −70°C for the appropriate times.

RESULTS

Transfected p202 inhibits the NF-κB- or AP-1-dependent transcription of various transfected reporter genes: transient-transfection assays. Poly(rI) · poly(rC) can induce IFN-β synthesis in various cultured mammalian cells (16). It also strongly activated a reporter gene (18), −110 huIFNβ-CAT, in which the IFN-β enhancer-promoter segment was driving CAT expression (Fig. 1A, lane 1). p202 inhibited both basal and poly(rI) · poly(rC)-activated expression of this reporter gene in a transient-transfection assay: the extent of inhibition of CAT activity increased with the amount of p202 expression plasmid in the transfection mixture (Fig. 1A, lanes 2 to 5).

The set of transcription factors participating in IFN-β gene expression, as induced by poly(rI) · poly(rC), includes the NF-κB p50 and p65 proteins that bind to the PRDII domain of the enhancer/promoter (61). Transfected p202 inhibited the basal and PMA-activated expression of a PRDII-CAT reporter gene in a transient-transfection assay (Fig. 1B). This suggested that p202 can impair the functioning of one or both of the p50

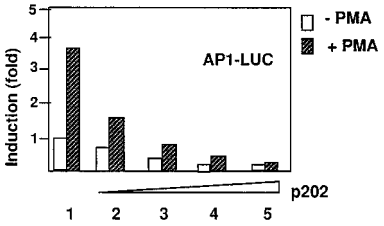

FIG. 2. Inhibition of the activity of an AP-1 enhancer-driven LUC reporter gene by increasing amounts of p202 expression plasmid. For the transient-transfection assays, L929 cells in monolayers were transfected with p202 expression plasmid, treated with PMA, if so indicated, and analyzed as described in the legends to Fig. 1B, C, and D except that the reporter plasmid was AP-1-LUC.

and p65 transcription factors. It is in line with this conclusion that the basal and PMA-activated expression of another reporter gene (HIV-LUC) (37) driven by the NF-κB domain of the HIV enhancer/promoter was also strongly impaired in cells transfected by a p202 expression plasmid in a concentration-dependent manner (Fig. 1C). In contrast, the transfection of an expression plasmid encoding p202 antisense RNA did not inhibit either the basal or PMA-activated expression of the HIV-LUC reporter gene (Fig. 1D).

Since p202 can be induced in L929 cells by IFN, it was expected that IFN treatment should diminish the basal and PMA-activated expression of the HIV-LUC reporter gene. The data in Fig. 1E reveal that this was the case. The extent of this inhibition increased with the time of exposure of the culture to IFN: thus, in the case of PMA-activated expression it was about 30% at 8 h and increased to more than 60% by 24 h and to about 70% by 48 h. This slow increase in the extent of inhibition by IFN treatment is in line with the time course of the accumulation of the IFN-induced p202 in the nucleus (12).

The basal and PMA-activated expressions of the AP-1-LUC reporter gene were also inhibited by p202 in transient-transfection assays (Fig. 2). The extent of inhibition increased with the amount of p202 expression plasmid in the transfection mixture.

p202 constitutively overexpressed in stable lines inhibits the NF-κB- or AP-1-dependent transcription of various endogenous genes and transfected reporter genes. The level of p202 expression might be unphysiologically high in transient-transfection assays. Thus, to explore the physiological relevance of the p202 effects described so far, we generated stable cell lines in which the constitutive level of p202 was elevated but still remained much below the level induced by IFN treatment. For this purpose, we transfected L929 cells with a p202 expression plasmid driven by a strong enhancer (pcDNA3) and thereby generated three independent cell lines (ov1, ov2, and ov3). In these lines, the constitutive level of p202 was approximately threefold higher than in L929 cells not exposed to IFN but still much lower than in L929 cells exposed to IFN for 24 h (Fig. 3A). The growth rate of these three lines was slower than that of the parental L929 line: the number of cells (per 10-cm dish) of the parental line increased from 0.18×10^6 to 0.31×10^6 in 24 h, to 0.52×10^6 in 48 h, and to 1.15×10^6 in 72 h, whereas the corresponding numbers for the ov1, ov2, and ov3 lines were only 0.18×10^6, 0.30×10^6, 0.42×10^6, and 0.53×10^6, respectively.

The overexpression of p202 in ov1, ov2, and ov3 did not diminish the induction of p204 by IFN (Fig. 3B, compare lane 2 with lanes 4, 6, and 8). The p204 protein is encoded by Ifi204, one of the genes from the gene 200 cluster, of which the gene encoding p202 is also a member (11). The induction of endo-

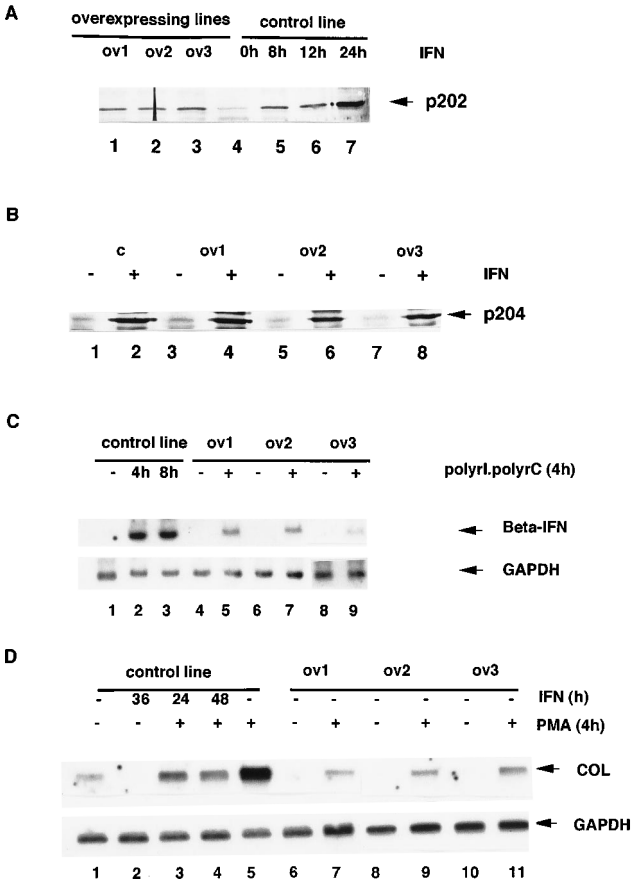

FIG. 3. Overexpression of p202 in stable cell lines inhibits the induction of endogenous IFN-β and collagenase I. (A) p202 is overexpressed in cell lines ov1, ov2, and ov3 generated by transfecting an L929 culture with a p202 expression plasmid. The ov1, ov2, and ov3 lines and the control L929 line (obtained by transfecting an L929 culture with the pcDNA3 vector) were cultured in 100-mm dishes in 10 ml of complete DMEM supplemented with 800 μg of G418 per ml. When the cultures reached 40 to 60% confluency, some of the L929 cultures were treated with 1,000 U of IFN per ml for the lengths of time indicated (lanes 5 to 7). Other cultures of L929 cells (lane 4) and the ov1, ov2, and ov3 cultures (lanes 1 to 3) were incubated without supplementation for 24 h. Total cell protein was extracted, and 10 μg of protein from each sample was analyzed for p202 by Western blotting with the anti-p202 antiserum. The p202 band is indicated. (B) Overexpression of p202 does not inhibit the induction of p204 protein by IFN. The control L929 line and the ov1, ov2, and ov3 lines were cultured in two sets of 100-mm dishes in complete DMEM supplemented with G418 (800 μg ml^{-1}). When the cultures reached 40 to 60% confluency, one set of dishes was incubated with IFN (1,000 U ml^{-1}) for 24 h. The other set of dishes was further incubated for 24 h without supplementation. Total cell protein (10 μg) from each sample was used to detect p204 expression (lanes 1 to 8) by Western blotting with the anti-p204 antiserum. The p204 band is indicated. (C) Overexpression of p202 inhibits the induction of endogenous IFN-β mRNA by poly(rI) · poly(rC). The control L929 line and the ov1, ov2, and ov3 lines were cultured as described for panel A. One set of ov1, ov2, and ov3 dishes were treated with 0.1 mg of poly(rI) · poly(rC) for 4 h (lanes 2, 5, 7, and 9) or 8 h (lane 3). The other set of ov1, ov2, and ov3 dishes were incubated without further supplementation (lanes 1, 4, 6, and 8). Total RNA (20 μg) from each dish was used for Northern blotting with an IFN-β probe. The same blot was stripped and rehybridized with a GAPDH probe. The IFN-β RNA and GAPDH RNA bands are indicated. (D) Overexpression of p202 or treatment with IFN decreases the endogenous level of collagenase I mRNA and inhibits the induction of this mRNA by PMA. Control L929 cultures (lanes 1 to 5) and ov1, ov2, and ov3 cultures (lanes 6 to 11) were grown as described for panel A. When the cultures reached 40 to 60% confluency (0 h), they were serum starved in serum-free OPTI-MEM medium (with L-glutamine) (Gibco) for 24 h. Some of the L929 cultures were treated with IFN for 36, 24, or 48 h (lanes 2 to 4). For the last 4 h of the incubation, some of the cultures were supplemented with PMA (50 ng ml^{-1}) (lanes 3 to 5, 7, 9, and 11). All cultures were harvested at 72 h. Total RNA (20 μg) from each culture was used for Northern blotting with a rat collagenase I probe; after stripping, the blot was rehybridized with a GAPDH probe. The collagenase I (COL) and GAPDH bands are indicated. For further details, see Materials and Methods.

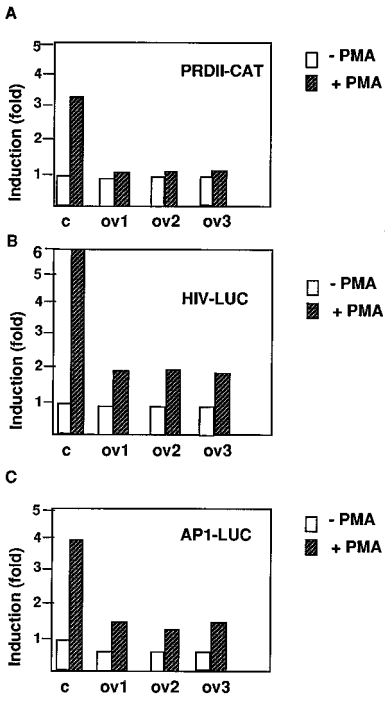

FIG. 4. Overexpression of p202 in stable cell lines inhibits the activities of NF-κB enhancer-driven reporter genes. The control L929 line and the ov1, ov2, and ov3 lines were grown in 100-mm dishes in complete DMEM supplemented with 800 ng of G418 ml^{-1} to 40 to 60% confluency. Cells were transfected with 5 μg of reporter construct as indicated and 5 μg of pSVgal (internal control plasmid). The cultures were incubated for 36 h and split into two sets. One set was supplemented with PMA (50 ng ml^{-1}) (shaded bars). The second set served as a control (open bars). The cultures were further incubated for 14 h before harvesting and assaying for luciferase, CAT, and β-galactosidase activities. For each culture, the luciferase or CAT activity was normalized to the β-galactosidase activity. Relative activities were calculated by taking the activities in the parental line with no PMA treatment as 1. (A) Inhibition of PRDII-CAT reporter activity in cell lines overexpressing p202. (B) Inhibition of HIV-LUC reporter activity in cell lines overexpressing p202. (C) Inhibition of AP-1-LUC reporter activity in cell lines overexpressing p202. For further details, see Materials and Methods.

genous IFN-β mRNA by poly(rI) · poly(rC) was, however, strongly diminished in the ov1, ov2, and ov3 lines compared with that in the control line (Fig. 3C, compare lane 2 with lanes 5, 7, and 9). There was a similarly strong decrease in these three lines compared with the control line in the constitutive level (Fig. 3D, compare lane 1 with lanes 6, 8, and 10) and the PMA-induced level (compare lane 5 with lanes 7, 9, and 11) of endogenous collagenase I mRNA. Since p202 can be induced by IFN, it is expected that the levels of constitutive and PMA-induced endogenous collagenase I mRNA (3) should also be diminished in L929 cells upon treatment with IFN, and as shown in Fig. 3D, this was found to be the case (compare lane 1 with lane 2 and lane 5 with lanes 3 and 4).

We also tested the effect of the constitutive overexpression of p202 in the ov1, ov2, and ov3 lines on the activation by PMA of three reporter genes driven by NF-κB or AP-1 enhancers (i.e., PRDII-CAT, HIV-LUC, and AP-1-LUC). The activation of these genes by PMA was strongly impaired in the lines overexpressing p202 (Fig. 4). However, the constitutive overexpression of p202 diminished appreciably only the basal expression of AP-1-LUC, not of PRDII-CAT or of HIV-LUC. As shown in Fig. 1 and 2, transfected p202 did diminish the activated as well as the basal expression of the PRDII-CAT and HIV-LUC reporter plasmids in transient-transfection assays. The lack of effect on the basal expression in the three cell lines constitutively overexpressing p202 might be the consequence of a lower level of p202 in these cell lines than in the transiently transfected cells. It is conceivable that p202 inhibits the activation of NF-κB by PMA and the action of (activated) NF-κB by two distinct mechanisms and that the two mechanisms require different levels of p202.

The results presented indicate that p202, when constitutively overexpressed in the ov1, ov2, and ov3 lines, can impair the activation by poly(rI) · poly(rC) or PMA of various endogenous and reporter genes whose transcription depends on the NF-κB (p50 or p65) or AP-1 proteins. Therefore, to further explore the mechanisms of the inhibition of p50, p65, c-Jun, and c-Fos activities by p202, we tested whether p202 can physically interact with any of these transcription factors.

p202 binds p50 and p65 in vitro and p50 in vivo. Labeled p202 translated in vitro (Fig. 5A, lane 1) was retained on immobilized p50 (GST-p50) and p65 (GST-p65) but not on GST alone (compare lanes 3 and 4 with 2). This demonstrates that p202 can physically interact with p50 and p65 in vitro.

As expected, the level of p202 in AKR-2B cells was greatly increased by treatment with IFN (Fig. 5B, compare the anti-p202 immunoblots in lanes 2 and 1). Immunoprecipitation from an extract of IFN-treated cells with antibodies to p50 resulted in a precipitate which was analyzed by SDS-PAGE and immunoblotting with antibodies to p50 and p202. This analysis revealed the presence of both p50 (Fig. 5B, lane 6) and p202 (lane 4) in the immunoprecipitate. Immunoprecipitation with anti-p50 antibodies from an extract of cells not treated with IFN resulted in an amount of p50 similar to that in the immunoprecipitate of an extract from cells treated with IFN. (Although in the experiment shown in Fig. 5B there was less p50 in the immunoprecipitate from the extract of IFN-treated cells than in that from control cells, no such difference was found in the amounts of p50 between the two types of extracts in three further experiments.) Furthermore, the immunoprecipitate obtained with anti-p50 antibodies and an extract of cells not treated with IFN contained only a faint p202 band (not shown), with much less p202 than the corresponding immunoprecipitate from an extract of cells treated with IFN. In view of the very low level of p202 in cells not treated with IFN (lane 1), this is not unexpected. Preimmune serum did not precipitate either p50 or p202 (lane 3). These results indicate that p202 binds p50 in vivo.

Treatment with PMA increases the amount of p202 bound to p50 in vivo. Treatment of cells with PMA, an activator of the NF-κB/Rel system, is known to result in the phosphorylation and proteolytic inactivation of IκB-α, a protein binding to and retaining p50/p65 in the cytoplasm. The p50/p65 released then translocates to the nucleus (57).

The experiment shown in Fig. 5C reveals that PMA treatment did not induce p202 and did not affect the induction of p202 by IFN, as tested in a total cell lysate (compare lane 1 with lane 2 and lane 3 with lane 4). However, the PMA treatment resulted in an increase in the amount of p202 coimmunoprecipitable with p50 from both the nuclear and the cytoplasmic fractions (compare lane 5 with lane 6 and lane 7 with lane 8). The increase was much more pronounced in the nuclear fraction (10-fold) than in the cytoplasmic fraction (less than 2-fold). This increase is most likely due to the increase in p50 concentration (slight in the cytoplasmic fraction, large in the nuclear fraction) resulting from the PMA treatment. The finding that p202 is bound to p50 in both compartments raises the possibility that PMA, which triggers the translocation of p50 (and of p65) from the cytoplasm to the nucleus (57), may also accelerate the relatively slow translocation of IFN-induced p202 from the cytoplasm to the nucleus (12).

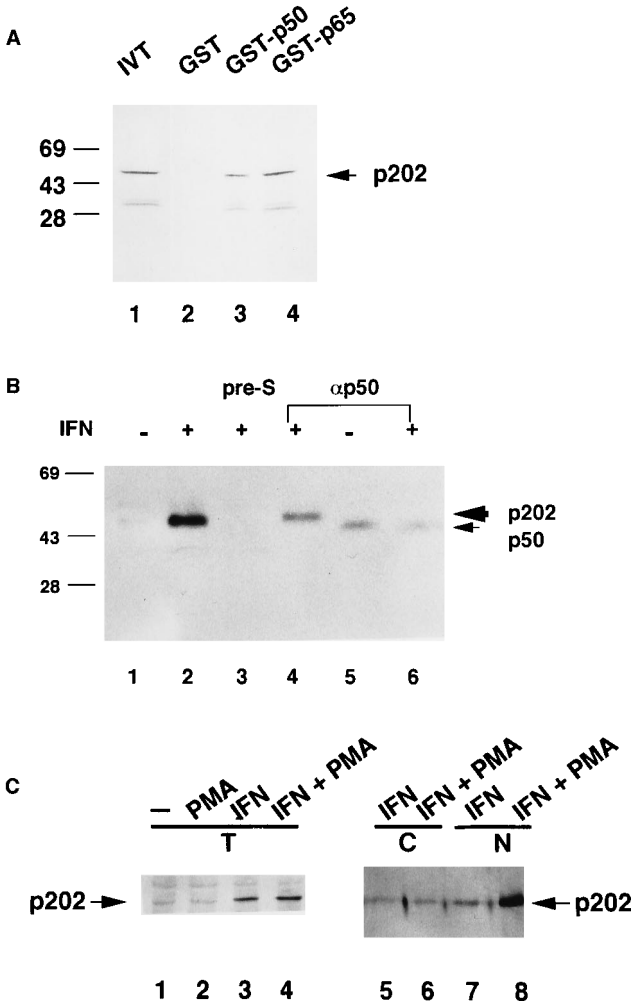

FIG. 5. p202 binds NF-κB proteins in vitro and in vivo. (A) p202 binds p50 and p65. [^{35}S]methionine-labeled p202 protein translated in vitro was analyzed by SDS-PAGE and fluorography (lane IVT). The p202 preparation was incubated with immobilized GST (lane 2), GST-p50 (lane 3), or GST-p65 (lane 4). The bound protein was eluted, analyzed by SDS-PAGE, and visualized by fluorography. The positions of size markers are indicated (in kilodaltons). The p202 protein band is indicated by an arrow. The selectivity of the binding of proteins to p202 was shown earlier (10). (B) Association of p202 and p50 in vivo, assayed by immunoprecipitation followed by immunoblotting. AKR-2B cells not treated (−) or treated (+) with IFN as indicated were lysed. An aliquot from the lysate was analyzed by SDS-PAGE and immunoblotting with anti-p202 antiserum (lanes 1 and 2). A second aliquot was precipitated with preimmune serum (pre-S; lane 3), and other aliquots were precipitated with anti-p50 antibodies (αp50; lanes 4 to 6). The precipitates were extracted with a solution including the p50 peptide (against which the antibody had been raised), and the solubilized proteins were analyzed by SDS-PAGE followed by immunoblotting with anti-p202 antiserum (lane 3 and 4) or anti-p50 antibodies (lanes 5 and 6). The ECL system (Amersham) was used for visualization. The p202 protein band and a p50 band are indicated. The positions of size markers are indicated. (C) PMA treatment increases the proportion of p202 bound to p50 in both nuclear and cytoplasmic fractions, as assayed by immunoprecipitation and immunoblotting. AKR-2B cells not treated (—) (lane 1) or treated with IFN (lanes 3, 5, and 7), PMA (lane 2), or both IFN and PMA (lanes 4, 6, and 8) were lysed, and the lysates were processed into cytoplasmic and nuclear fractions. An aliquot from the total lysate (indicated as T) was tested for p202 by SDS-PAGE and immunoblotting with the anti-p202 antiserum (lanes 1 to 4). Aliquots from the cytoplasmic fractions (lanes 5 and 6, indicated as C) and nuclear fractions (lanes 7 and 8, indicated as N) from the lysate were first immunoprecipitated with anti-p50 antibodies, and the immunoprecipitates were further processed as described for panel A for immunoblotting with the anti-p202 antiserum. The p202 bands are indicated by arrows. For further details, see Materials and Methods.

p202 binds c-Jun, c-Fos, and truncated derivatives of them in vitro. The binding of p202 to c-Jun and to c-Fos in vitro is shown in Fig. 6A. Labeled p202 translated in vitro (lane 1) was retained on immobilized c-Jun (His_6-c-Jun) and c-Fos (His_6-c-Fos) but not on the immobilizing matrix (nitrilotriacetic acid beads) (compare lanes 3 and 5 with 2). p202 was also retained on immobilized truncated c-Jun (His_6-wbJun) and truncated c-Fos (His_6-wbFos) (lanes 4 and 6).

p202 binds c-Jun and c-Fos in vivo. p202 could be coimmunoprecipitated with c-Jun and also with c-Fos from an extract of IFN-treated AKR-2B cells with polyclonal antibodies to c-Jun and c-Fos, respectively (Fig. 6B and C, lanes 2). No p202 was detected in an immunoprecipitate from cells not treated with IFN (lanes 1), and no p202 was immunoprecipitated by a preimmune serum even from extracts of IFN-treated cells (lanes 4). Treatment with IFN and PMA had the same effect on the coimmunoprecipitability of p202 as treatment with only IFN (lanes 3 and 6). These results indicate an association of p202 with c-Jun and also with c-Fos in cells in which the level of p202 was increased by treatment with IFN.

p202 inhibits the specific DNA binding of complete and truncated c-Jun and c-Fos proteins in vitro. As noted earlier, p202 could bind to the truncated c-Jun and c-Fos proteins, at least in vitro. These truncated proteins contain the dimerization and DNA-binding domains but lack, in the case of c-Jun, the only transactivating domain and, in the case of c-Fos, one of two transactivating domains (1). These findings are consistent with the possibility that the inhibition of the transcriptional activity of these proteins by p202 is at least partly a consequence of an inhibition of specific DNA binding. This possibility was supported by the finding that p202 did not bind in vitro to a truncated c-Jun derivative (wbjun/ΔB) (28) consisting of the dimerization and DNA-binding domains but lacking a 7-amino-acid segment (amino acids 260 to 266) from the DNA-binding domain (not shown). This finding indicated that the DNA-binding domain of c-Jun is directly or indirectly involved in the binding of p202.

That p202 indeed inhibited the specific DNA binding of purified c-Jun and also of the c-Jun/c-Fos heterodimer is shown in the EMSA in Fig. 7 (compare lanes 1 and 2 and also lanes 5 and 6). As expected, c-Fos alone did not appreciably bind DNA specifically even in the absence of p202 (lanes 3 and 4).

p202 also inhibited the specific DNA binding of truncated c-Jun (wbJun) (compare lanes 7 and 8). Actually, p202 inhibited the binding of the wbJun homodimer the most (lanes 7 and 8, mobility marker 4), that of the Jun-wbJun heterodimer to an intermediate extent (mobility marker 3), and that of the Jun homodimer the least (mobility marker 1). The DNA binding of the Jun homodimer in lane 8 was inhibited less by p202 than in lane 2, since the amount of p202 was the same in the two reaction mixtures, whereas the total Jun protein concentration (i.e., Jun plus wbJun) was twice as high in lane 8 as in lane 2.

p202 affects the activities of different enhancers nonuniformly. Having found that transfected p202 inhibited the functioning of the NF-κB and AP-1 (i.e., c-Jun and c-Fos) enhancers, we tested the effects of p202 on the activity of other enhancers. Experiments involving transient-transfection assays revealed that p202 inhibited the activity of the AP-2 enhancer (72) to a similar extent as it did the NF-κB and AP-1 enhancers (Table 1). However, p202 did not impair the activity of all enhancers uniformly: the activities of SP1 (30) and OCT1 (19) were not significantly altered (Table 1).

Furthermore, the expression of a reporter driven by the 5′-terminal and flanking segment (extending from nucleotides

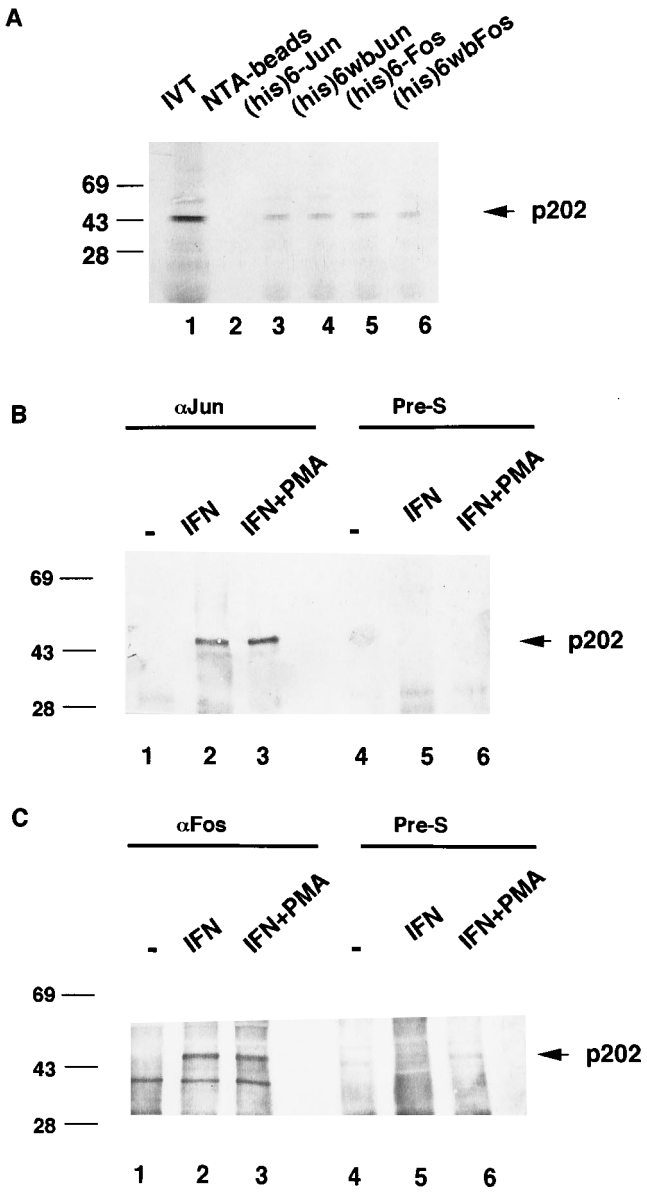

FIG. 6. p202 binds the AP-1 proteins c-Jun and c-Fos in vitro and in vivo. (A) p202 binds c-Jun and c-Fos or their DNA-binding domains in vitro, as assayed by binding to immobilized His_6-c-Jun, His_6-wbJun (minimum DNA-binding domain of c-Jun), His_6-c-Fos, and His_6-wbFos (minimum DNA-binding domain of c-Fos). A 10-μl amount of [^{35}S]methionine-labeled p202 translated in vitro (IVT) was analyzed by SDS-PAGE and fluorography (lane 1). Then 10 μl of this preparation was incubated with Ni-nitrilotriacetic acid beads alone or immobilized His_6-c-Jun, His_6-wbJun, His_6-c-Fos, and His_6-wbFos (lanes 2 to 6). The bound protein was eluted, analyzed by SDS-PAGE, and visualized by fluorography. The positions of size markers are indicated (in kilodaltons). The p202 protein band is indicated by an arrow. (B and C) Association of p202 with c-Jun (B) and c-Fos (C) in vivo, assayed by immunoprecipitation followed by immunoblotting. AKR-2B cells were cultured in complete DMEM to 40 to 60% confluency, followed by 24 h of serum starvation. Cells not treated (—) (lanes 1 and 4) or treated with 1,000 U of IFN per ml (lanes 2 and 5) or 1,000 U of IFN per ml and 50 ng of PMA per ml (lanes 3 and 6) for 24 h during serum starvation were lysed, and the total cell lysates were divided into aliquots. One aliquot was immunoprecipitated with anti-c-Jun antiserum (lanes 1 to 3 in panel B) or anti-c-Fos antiserum (lanes 1 to 3 in panel C), and the second aliquot was precipitated with preimmune serum (lanes 4 to 6 in panels B and C). The precipitates were extracted with a solution including c-Jun protein (B) or c-Fos protein (C), and the solubilized proteins were analyzed by SDS-PAGE followed by immunoblotting with anti-p202 antiserum. The ECL system (Amersham) was used for visualization. A p202 protein band is indicated by an arrow. For further details, see Materials and Methods.

TABLE 1. Effects of p202 on the activities of different enhancers[a]

Reporter	Enhancer	Effect	Activity (p202/vector)
pBII×LUC	NF-κB	Inhibition	0.14, 0.22, 0.33
AP-1-LUC	AP-1	Inhibition	0.14, 0.18, 0.33
A2BCAT	AP-2	Inhibition	0.16, 0.17, 0.32
pSV-Gal	SP1	None	0.92, 1.12, 1.10
4OCT-LUC	OCT1	None	0.94, 1.10, 1.13
CAT-HSI	hMtIIa	Stimulation	2.5, 2.0, 3.3

[a] L929 cells grown in monolayers in 100-mm dishes to 40 to 60% confluency were transfected with 5 μg of the indicated reporter plasmid, 0.5 μg of p202 expression plasmid or vector (pcDNA3), and 2 μg of pSVgal (serving as an internal standard). CAT and luciferase reporter gene activities measured 48 h after transfection were normalized to the β-galactosidase activities for each dish. Relative activities were calculated by dividing the normalized activity of the reporter gene indicated in a dish transfected with the p202 expression plasmid by the normalized activity of the same reporter gene in a dish transfected with the vector. In the case of experiments with pSVgal, the ratios of the specific activities (i.e., β-galactosidase activity per optical density unit in a dish transfected with the p202 expression plasmid divided by β-galactosidase activity per optical density unit in a dish transfected with the vector) are shown. The data for three independent experiments are shown. For further details, see Materials and Methods.

−764 to +69) of the human metallothionein IIa gene (71) was actually increased by transfected p202 (Table 1). The identities of the enhancers in this long regulatory segment activated by p202 remain to be established.

DISCUSSION

As reported earlier, the IFN-inducible p202 protein is primarily nuclear, is chromatin-associated, and binds DNA nonspecifically in vitro (12). Efforts to identify specific oligodeoxynucleotide sequences recognized by purified p202 were not successful. However, p202 binds several proteins, including pRb and p107, in vitro and in vivo (10).

The experiments reported here revealed that transfected p202 inhibited the basal and activated functioning of the NF-κB transcription factors in response to enhancers in the IFN-β gene regulatory segment (i.e., −110huIFNβ and its

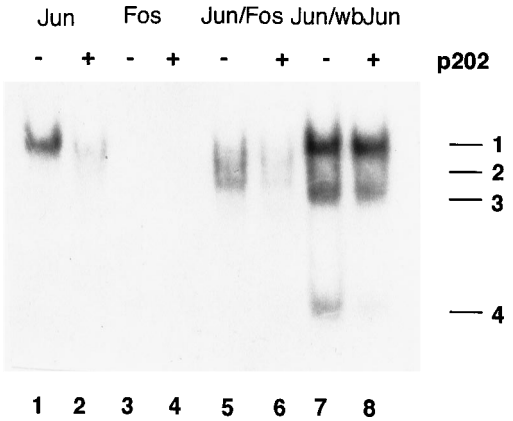

FIG. 7. Inhibition of the specific DNA-binding activity of complete and truncated Jun and Fos proteins by p202 in vitro. His_6-c-Jun (lanes 1 and 2), His_6-c-Fos (lanes 3 and 4), His_6-c-Jun and His_6-c-Fos (lanes 5 and 6), and His_6-c-Jun and His_6-wbJun (lanes 7 and 8) were incubated without (−) (lanes 1, 3, 5, and 7) or with (+) 50 ng of GST-p202 (lanes 2, 4, 6, and 8). Subsequently, labeled AP-1 oligodeoxynucleotide probe was added, and the reaction mixtures were further incubated prior to loading on a gel for EMSA. The positions of mobility markers 1 to 4 are indicated. For further details, see Materials and Methods.

PRDII sequence) and also in the HIV LTR. p202 antisense expression had no such effect. Treatment with IFN, resulting in p202 induction, also inhibited the basal and PMA-activated and the NF-κB-dependent expression of the HIV-LUC reporter plasmid. The extent of inhibition increased with the length of exposure of the cells to IFN (up to 48 h). This finding is in line with the fact that transfer of IFN-induced p202 to the nucleus is a slow process (12).

The inhibition of the NF-κB-dependent expression of genes by transfected p202 and also by IFN treatment makes it conceivable that the induction of p202 by IFN contributes to the transiency of the induction of IFN-β by poly(rI) · poly(rC) (69). In mice of the C57BL/6 inbred strain (unlike in mice from several other strains), p202 is not induced by IFN (21). At the same time, infection by some viruses or treatment with poly(rI) · poly(rC) was reported to induce much more IFN-α/β in C57BL/6 mice than in mice from several other strains (16). Whether these characteristics of C57BL/6 mice are causally related, i.e., whether p202 is contributing to the attenuation of the induced synthesis of IFN-β, will have to be established in mice from which the 202 gene has been knocked out. It should be noted that a negative regulatory element in the IFN-β regulatory segment that is required for the inhibition of IFN-β synthesis by the DSP1 protein (40) is not required for the inhibitory activity of p202.

Transfected p202 also impaired both basal and PMA-activated expression of the AP-1-LUC reporter gene. This suggests that p202 can inhibit the activity of at least some of the AP-1 set of transcription factors.

To enable us to examine the effects of the constitutive overexpression of endogenous p202, we generated three independent stable cell lines (ov1, ov2, and ov3) in which p202 was overexpressed two- to threefold. The level of p202 protein in these lines was at most one-fifth of that in cells treated with IFN. It is in line with earlier observations that even this low level of constitutive p202 overexpression slowed down cell proliferation (12). In spite of using a powerful enhancer to drive p202 expression in the transfected cells, we did not obtain cell lines overexpressing p202 constitutively at a higher level. This makes it likely that higher p202 levels may impair cell proliferation.

Experiments with the ov1, ov2, and ov3 lines revealed that p202 can inhibit the expression not only of transfected reporter genes but also of endogenous genes involving NF-κB activity (i.e., IFN-β) (61) or AP-1 activity (i.e., collagenase I) (3). The PMA-activated expression of three reporter genes involving NF-κB or AP-1 action was also inhibited in these three cell lines. The selectivity of the inhibition of gene activity by overexpressed p202 was demonstrated by showing that the induction of p204 by IFN-β was unaffected.

As revealed by experiments involving binding to immobilized proteins and immunocoprecipitation, p202 bound to the NF-κB p50 and p65 and the AP-1 c-fos and c-jun proteins both in vitro and in vivo. The direct interaction between p202 and these transcription factors most likely contributes to the inhibition of the activity of these factors. It is possible, however, that p202 also interacts with other proteins of the transcriptional machinery and that this also contributes to the inhibition of the transcriptional activity of these factors by p202.

p202 diminished the specific DNA binding of the c-Jun homodimer and of the c-Jun/c-Fos heterodimer. This is in line with the fact that p202 could bind, at least in vitro, not only to the intact but also to a truncated c-Jun protein retaining only the DNA-binding and dimerization domains but did not bind to a truncated c-Jun protein from whose DNA-binding domain a 7-amino-acid segment had been deleted. The inhibition of DNA binding might be a consequence of an impairment by p202 of the interaction between the protein dimer and DNA or of dimer formation or both. Several other transcription-regulatory proteins (e.g., adenovirus E1A and human T-lymphotropic virus type 1 Tax) have been reported to target DNA-binding domains of transcription factors (66). In contrast, p202 did not seem to decrease the specific DNA binding of NF-κB, as tested in an EMSA (not shown). This suggests that p202 might impair the activity of various transcription factors by different mechanisms.

The experiments reported here established that p202 could impair the activity of several enhancer-specific transcription factors, including NF-κB p50 and p65 and AP-1 c-Jun and c-Fos. The data in Table 1 reveal that this inhibition was selective. Of three other enhancers tested, p202 inhibited the activity of only one, AP-2 (55). The activity of two other enhancers, SP-1 (30) and OCT-1 (19), was not appreciably affected by p202. Furthermore, a gene (hMetIIa) (39) whose expression was actually enhanced by p202 was found. The mechanism of this enhancement and the enhancers affected remain to be explored.

The data presented reveal that transfected or constitutively overexpressed p202 could selectively inhibit the activity of some enhancers and of the corresponding transcription factors. Thus, p202 may serve as a modulator of transcription which can alter the pattern of gene expression in response to IFN (or conceivably other stimuli affecting the level of p202). It appears to affect gene expression by inhibiting the activity of sequence-specific transcription factors, apparently without directly binding to specific oligodeoxynucleotide sequences. In this characteristic, p202 resembles, e.g., the adenoviral E1B protein and the cellular MDM2 protein, as they impair the activity of the p53 transcription factor (75, 76). Furthermore, in inhibiting the transcriptional activities of NF-κB, c-Jun, and c-Fos proteins by binding to them, p202 resembles, e.g., the glucocorticoid receptor, which also impairs the activity of these transcription factors by forming complexes with them, apparently without having to bind directly to DNA (29, 51, 54, 73, 74).

It is conceivable that the inhibition of cell proliferation by p202 is at least partly the consequence of the alteration in the pattern of gene expression elicited by p202. It remains to be established whether the increased level of p202, e.g., in cells treated with IFN, inhibits the replication of particular viruses. Such an inhibition might be the consequence of direct impairment of the activity of some transcription factors (e.g., NF-κB) and also of the slowing down of cell proliferation. The latter effect might also contribute to antitumor activity. The mechanisms by which p202 modulates the activities of the various transcription factors remain to be explored.

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