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# A Proliferative p53-Responsive Element Mediates Tumor Necrosis Factor Alpha Induction of the Human Immunodeficiency Virus Type 1 Long Terminal Repeat

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**Transforming mutants of the p53 tumor suppressor gene can positively regulate transcription from several promoters that do not contain known p53 binding sites. Here, we report the identification of a novel p53 binding site in the human immunodeficiency virus long terminal repeat that specifically mediates mutant p53 transactivation. This DNA element was bound by endogenous Jurkat p53 when these cells were stimulated by tumor necrosis factor. Mutation of this sequence inhibited p53 transactivation and tumor necrosis factor inducibility of the human immunodeficiency virus type 1 long terminal repeat. In addition, this DNA element was found to be sufficient to confer mutant p53 responsiveness on a heterologous minimal promoter. It has been hypothesized that transforming mutants of p53 represent a proliferative conformational stage that can be adopted by the native protein under stimulation by growth factors. The data presented suggest that proliferative and antiproliferative p53 conformations recognize different DNA binding sites in order to mediate distinct biological functions. Thus, transforming mutants of p53 that fold into the proliferative conformation would favor proliferative over antiproliferative functions.**

The p53 protein plays a critical role in the control of cellular proliferation, and inactivation of p53 is a key event in the induction of malignant transformation (28, 36). This inactivation may occur by several mechanisms, including viral insertion, gene rearrangements or deletions, overexpression of p53-associated proteins, and binding to viral proteins (54). More than 60% of human tumors contain elevated levels of mutant p53 protein (28, 36). These mutations are mostly single missense mutations within the coding sequence (46, 54).

Although the mechanism of action of p53 is not fully understood, many studies have implicated p53 in the regulation of transcription. Wild-type p53 is known to bind specific DNA sequences (3, 24, 33, 51), and a consensus p53 binding site has been identified (18). p53 activates transcription of reporter genes that contain multiple copies of the wild-type p53 binding site (20, 24, 33). Importantly, it has been shown that p53 also regulates the expression of several cellular promoters (1–3, 31, 68, 69, 71, 72). Mutated forms of p53 can transactivate certain promoters, but no DNA binding site for p53 has been shown to be responsible for this effect (12, 16). The amino terminus of p53 is highly acidic and functions as a transcriptional activation domain (21). MDM2, a p53-associated protein, has been shown to inhibit p53 transcriptional activity by binding to this activation domain (42). Evidence indicating that the wild-type p53 binding activity resides in the highly conserved central region of the protein has been recently reported (3, 10, 13, 49, 67). Interestingly, the vast majority of the transforming mutations are located in this region (see reference 46 and references therein).

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Most point mutations inactivate p53 tumor suppressor function, and some may also induce p53 to acquire oncogenic properties and promote proliferation (10, 51). The localization of the mutations may reflect a selective pressure in tumors to activate the cell's proliferative mechanisms or to inactivate tumor suppressor functions (46). The latter hypothesis is consistent with the generalization that the mechanism of action of transforming mutants of p53 is to block the site-specific DNA binding activity of wild-type p53. Indeed, the p53 mutant forms inhibit wild-type p53-regulated expression of genes containing p53 consensus binding sites (33). However, it has been shown that expression of mutated p53 in human cell lines that completely lacked endogenous p53 expression conferred a growth advantage to the cells (10). This study indicates that mechanisms other than a blockage of wild-type p53 binding activity may be implicated in the proliferative effects of mutant p53 protein forms. In support of this point, most tumors involving p53 have one deleted p53 allele and one mutated p53 allele (46).

Point mutations can alter the tertiary structure of p53, thus affecting its reactivity to specific anti-p53 monoclonal antibodies. Changes in antibody specificity correlate with changes in presumed p53 functions. For example, p53 forms that express the PAb 421 or PAb 122 epitope are associated with cell growth and p53 from growth-arrested cells displays the PAb 246 and PAb 1620 epitopes (62, 63). In cells that express wild-type and mutant p53 simultaneously, wild-type p53 associated with mutant p53 adopts the PAb 240 mutant conformation and is no longer able to induce growth arrest (62). To explain how p53 may both enhance and inhibit cell proliferation, it has been postulated that p53 exists in two alternative protein conformations, the so-called antiproliferative and proliferative forms (see references 39, 41, and 62 and references therein). In response to growth stimuli, wild-type p53 appar-

ently undergoes a conformational change during the $G_1$ phase of the cell cycle and adopts a proliferative or mutant-like conformation. The forces that may drive p53 from a proliferative to a nonproliferative form and vice versa are unclear, although it has been suggested that phosphorylation plays an important role in stabilizing the antiproliferative conformation (62, 65). The identification of p53 in a mutant-like conformation in acute myeloid leukemia cells was interpreted as involving mutations in the p53 gene. However, a survey of p53 variants in acute myeloid leukemia patients revealed that 75% of the p53 samples immunoprecipitated by PAb 240 (mutant-like conformation) contained no mutations (73). Thus, alteration of p53 conformation, rather than acquisition of point mutations, may be the mechanism underlying an altered p53 tumor suppressor function in most cases of acute myeloid leukemia or blast crisis of chronic myeloid leukemia. In addition, this PAb 240 epitope has been demonstrated in normal lymphocytes induced to proliferate (73) and in p53 coimmunoprecipitated with Sp1 in erythroleukemia cells (6).

Here, we report the identification of a p53-specific binding site in the human immunodeficiency virus (HIV) long terminal repeat (LTR) which mediates tumor necrosis factor (TNF) induction of gene expression. This DNA element was bound by endogenous Jurkat p53 (antibody 240 positive) when cells were stimulated by TNF. Transfection studies demonstrate that mutant p53 specifically transactivates gene expression through this novel DNA element. These data suggest that p53 plays an important role in the transcriptional activation of the HIV LTR by TNF. In addition, the data support a model in which proliferative and antiproliferative forms of p53 bind to different DNA elements and therefore differentially regulate gene expression.

## MATERIALS AND METHODS

**Electrophoretic mobility shift assays (EMSAs).** The LTR A and B sequences used were (double-stranded) tcgacGGGACTTTCCAGGGAGGCGTGGCCTG (LTR A) and CAGGGAGGCGTGGCCTGGGCGGGACTGGGG (LTR B). *Sal*I sites were added to the LTR A sequence for subcloning purposes. Other oligonucleotide sequences used were as follows (duplexes): the p53 site, CAGG GAGGGCGTGGCCTGGGTTTTACTGGGG; the Sp1 site, CAGGGAGGCT TTTCCTGGGCGGGACTGGGG; LTR B GGGAGGm, CATTTTGGCGTG GCCTGGGCGGGACTGGGG; p53kB, cgacGGATTGGGGTTTTCCCCTCC CATGTGCTCAG, containing the κB site in the human p53 promoter (61); IC, TGGGCGGAGTGGCCTTCTGTGGACGAAT, containing the Sp1 site in the HLA-DRA promoter (7); RGC, tcgacCTTGCCTGGACT TGCCTGG, and MCK, TGGCCGGGGCCTGCCTCTCTCTGCCTCTGA, with the p53 binding site of the muscle creatine kinase (MCK) promoter (71); MgBH, tcgacGACAC TGGTCACACTTGGCTGCTTAGGAATG (23); and SRE and MRE, which were described previously (66). DNA oligonucleotides were prepared with an Applied Biosystems 391EP DNA synthesizer by the phosphoramidite method and were purified by denaturing polyacrylamide gel electrophoresis (PAGE) and Sep-Pak C18 cartridges (Waters Associates). Gel shift mobility assays were prepared as follows. In a 10-μl reaction volume containing the binding reaction buffer (20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 7.5], 400 mM NaCl, 1 μM $ZnCl_2$, 1 mM EDTA, 1 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 5% glycerol), 5 μg of poly(dIdC) · (dIdC) and 1 μg of nuclear extract protein per reaction were used, with an incubation time of 20 min. The reaction mixtures were loaded into a 3.8% polyacrylamide gel previously prerun for 15 min at 200 V (65). Electrophoresis was performed at 20 V/cm in 22 mM Tris borate buffer with 0.5 mM EDTA. The gels were dried and exposed to film overnight at −70°C with an intensifying screen. The probes used in the binding assays were labeled with polynucleotide kinase and [γ-$^{32}$P]ATP (>4,500 Ci/mmol; Amersham). Typically, the labeled probes were purified with Elutip-D columns (Schleicher and Schuell) according to the directions of the manufacturer. Competition experiments were carried out with a fixed concentration of the probe and a 200- to 500-fold excess of the nonlabeled competitor.

**Methylation interference footprinting.** Probes for methylation interference footprinting were prepared as follows. Single-stranded oligonucleotides containing the sequence 5′-tcgaGGGACTTTCCAGGGAGGCGTGGCCTG-3′ and its complementary sequence were phosphorylated independently and radiolabeled as indicated below. Nonradiolabeled complementary strands were added and annealed, and duplex oligonucleotides were methylated in 200 ml of a 1:100 dilution of dimethylsulfate for 15 min. Preparative gels for EMSA were prepared by scaling up by 10-fold gel shift assay reactions with probes partially methylated with dimethylsulfate. After preparative gel shift, bands containing free and bound probes were excised and eluted overnight in 2 ml of Tris-HCl (100 mM, pH 7.5)–EDTA (1 mM)–200 mM NaCl. DNA was purified by Elutip-D columns (Schleicher & Schuell), ethanol precipitated, and cleaved by incubation in 100 μl of 10% piperidine at 90°C. Piperidine was eliminated by three rounds of evaporation in a speed vacuum and resuspension in 100 μl of deionized water. Cleaved oligonucleotides were resuspended in DNA sequencing sample buffer and resolved in a 20% denaturing polyacrylamide gel. Gels were covered with plastic wrap and exposed to film at −80°C.

**UV cross-linking.** LTR A-UV, the template for UV cross-linking, was an LTR A probe with a bromodeoxyuridine (BrdU)-for-T substitution at position 25. For cross-linking experiments with the LTR B probe, a BrdU-for-T substitution was introduced at position 11. Tenfold scale-up EMSA reaction mixtures were prepared as indicated above and irradiated for 20 min at room temperature with 312-nm UV light. The reaction mixtures were loaded onto 3.8% polyacrylamide gels and run as indicated. Gels were covered with plastic wrap and exposed to film. DNA-protein complexes were excised and loaded onto a sodium dodecyl sulfate (SDS)–10% polyacrylamide gel. Gels were run with a Bio-Rad electrophoresis system, dried, and exposed to film. In cross-linking–immunoprecipitation studies, 250 μl of immunoprecipitation buffer was added and the immunoprecipitation was carried out as indicated below.

**Biotinylated DNA affinity protein purification.** Nuclear extracts from TNF-treated cells (100 μg) were incubated with 100 ng of a biotinylated probe in the presence of 100 μg of poly (dIdC) · (dIdC), for 20 min at room temperature. Streptavidin MagneSphere beads (Promega; 150 μl) were added, and the mixture was incubated for an additional 15 min before precipitation through a magnetic field. The pellet was resuspended in 250 μl of phosphate-buffered saline (PBS) with 10 μg of poly(dIdC) · (dIdC) before being reprecipitated. The second pellet was then boiled in SDS-PAGE loading buffer, and the presence of p53 was determined by Western (immunoblot) analysis. The oligonucleotide sequences used were (duplexes) biotin-CGGGACTTTCCAGGGAGGCGTG GCCTG (biotin-LTR A), biotin-CAGGGAGGCGTGGCCTGGGCGGGACT GGGG (biotin-LTR B), and biotin-CAGGGAGGCGTAAACTGGGCGGGA CTGGGG (biotin-LTR B p53m).

**Antibodies, immunoprecipitations, and Western blotting.** Mouse monoclonal anti-p53 PAb 240 and PAb 1801 and rabbit polyclonal anti-Sp1 antibodies were purchased from Santa Cruz. Mouse monoclonal anti-p53 1620 was obtained from Oncogene Sciences. In immunoprecipitation studies, $2 \times 10^7$ Jurkat cells were washed twice for 10 min in 10 ml of methionine-free RPMI 1640 medium and incubated for 1 h at 37°C in 5 ml of new medium with 2.5 mCi of [$^{35}$S]methionine (1,175 Ci/mmol; NEN). Cells were collected by centrifugation and lysed in 1 ml of immunoprecipitation buffer (PBS containing 1% Triton X-100, 0.1% SDS, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride) prior to centrifugation at $1,500 \times g$ for 10 min. Cell lysates were incubated in the presence of 10 μg of the appropriate antibody at 4°C for 1 h before a further 4-h incubation with protein A/G-agarose (Promega). The immunoprecipitates were collected by centrifugation at $5,000 \times g$ for 10 min, washed twice with 250 μl of immunoprecipitation buffer, and resuspended in SDS-PAGE sample buffer which was boiled for 5 min and electrophoresed. In Western blotting assays, the electrophoresis was carried out in 10% polyacrylamide minigels (Bio-Rad). Equal amounts of proteins were assayed under all conditions, as determined by the Bradford protein assay (Bio-Rad). Proteins were transferred to Immobilon-P membranes (Millipore) and probed with anti-p53 PAb 1801 (1:500) by the manufacturers' recommended method.

**Plasmids.** HIV type 1 (HIV-1) LTR chloramphenicol acetyltransferase (CAT) reporter plasmid constructs were a gift from B. Stein (56). Site-directed mutated HIV LTR 53Δ CAT plasmid and minimal enhancer elements were constructed as described previously (50). HIV LTR 53Δ contains the mutation ACTCGA for GGCGTG at the HIV p53 site. 53-E1B TATA and 53Δ-E1B TATA contain the HIV LTR sequences (duplex) CGCTGGGGACTTTCCAGGGAGGCGTGGC CTGA, and CGCTGGGGACTTTCCAGGGAGACTCGAGCTGA upstream of an E1B TATA box CAT reporter plasmid (the underlined sequence represents the wild-type or mutant p53 site). The cytomegalovirus (CMV) enhancer/promoter-driven expression vectors (containing wild-type p53 and the tumor-promoting p53 143ala form) and the reporter plasmid PG13 (containing 13 copies of a wild-type p53 consensus binding site) were a gift from B. Vogelstein (33). Expression vector 53D was prepared by digestion of the CMV-p53 vector with *Stu*I and *Acc*I and religation. The murine sarcoma virus LTR-driven p53 expression vectors were gifts from C. Finley (wt, 175his, 248trp, and 273his) and G. Lozano (135val). All plasmids were prepared with Qiagen columns according to the directions of the manufacturers.

**Cell culture, cell extracts, transfections, and CAT assays.** Jurkat T-leukemia cells from the American Type Culture Collection were cultured at densities of $0.1 \times 10^6$ to $0.5 \times 10^6$ cells per ml in RPMI 1640 medium with glutamine and 2% fetal calf serum (Irvine) and penicillin-streptomycin. When TNF incubations were performed, cells were centrifuged, resuspended in new media at $1 \times 10^6$ cells per ml in the absence or presence of 10 ng of recombinant TNF-α (rTNF-α) (Promega) per ml, and incubated for 30 to 45 min. The incubations were stopped by centrifugation, and the cells were processed for immunoprecipitations or preparation of nuclear extracts. Nuclear cell extracts were prepared as described previously (4), but a

final phenylmethylsulfonyl fluoride concentration of 1 mM was used. Extracts were divided into aliquots and stored at −80°C. For transfection experiments, Jurkat and Akata cells were cultured in RPMI 1640 medium plus glutamine, penicillin-streptomycin, and 10% fetal calf serum. Cells were transfected by electroporation (300 V, 960 μF; Bio-Rad Electroporator) at 5 million cells per 0.5 ml of PBS and resuspended in 10 ml of medium. Incubations were carried out for 24 to 48 h. Cells were lysed by 3-s ultrasonic vibrations at 4°C with a Branson Sonifier at setting 3. Equal amounts of protein, as determined by the Bradford assay (Bio-Rad), were then analyzed for CAT activity by fluor diffusion assay (44).

## RESULTS

**Identification of a TNF-responsive region and TNF-inducible complexes (TICs) on the HIV LTR.** The ability of HIV-1 to replicate in T lymphocytes and mononuclear phagocytes is strongly influenced by several immunoregulatory cytokines. However, only TNF-α or TNF-β has been shown thus far to trigger viral expression in both T cells and mononuclear phagocytes (5). As a step forward in elucidating the mechanism of HIV-1 induction by TNF, we attempted to define the DNA-protein interactions that mediate the transactivation of the HIV-1 LTR by this cytokine. Jurkat T cells were transfected by electroporation with the full-length and stepwise-deleted fragments of the HIV-1 LTR subcloned upstream of a CAT reporter gene (56). After a 24-h incubation, cells were induced with 10 ng of TNF per ml for 16 h, harvested, and assayed for CAT activity. The results of these experiments (Fig. 1A) define a promoter fragment between nucleotides −91 and −65 that is necessary for the response to TNF. This fragment contains the 3′ κB site and the most 5′ Sp1 site of the LTR. This result was in agreement with a recent report that also pointed out the necessity of this promoter region for TNF induction of HIV-1 LTR-mediated transcription (50). The deletion of promoter sequences between positions −121 and −91 (including the 5′ κB site) resulted in reductions of basal and TNF inducibility.

Using oligonucleotide probe LTR A from the TNF-responsive sequence in the HIV LTR, we investigated whether specific protein complexes would bind this DNA element. Jurkat T cells, which carry mutant p53 (11), were incubated for 30 min in the presence (10 ng/ml) or absence of TNF, and then nuclear extracts were prepared and assayed by EMSA. Two TICs (TIC 1 and TIC 2) were observed (Fig. 1B). Addition of the protein synthesis inhibitor cycloheximide did not inhibit the formation of these complexes, indicating that TNF-induced posttranslational modifications on preformed factors were responsible for their formation. Similar results were obtained following treatment of Jurkat cells with a mitogen (phytohemagglutinin) (data not shown). Here, we focused our attention on the TNF induction of Jurkat cells.

To determine the specificities of the TICs, we performed oligonucleotide competition EMSAs. The competition assays demonstrated that only probes LTR A (Fig. 1C, lane 3) and LTR B (Fig. 1C, lane 4) were able to specifically compete for TIC 1 and 2. A probe containing a κB site (lane 2) had no effect, indicating that NF-κB is not present in the TICs. The absence of NF-κB complexes can be explained by the lack of a 5′ nucleotide necessary for this factor to bind the LTR A probe. Distinct complexes containing immunoreactive p65 NF-κB were observed with probes containing additional upstream sequences (data not shown). Interestingly, both the LTR A and B probes overlap in the sequence CAGGGAGG CGTGGCCTG that contains a nonconsensus Sp1 binding site (underlined), suggesting that Sp1 is the transcription factor responsible for the TICs. In order to address this point, we performed methylation interference footprinting and UV cross-linking on the major TIC, TIC 1.

The methylation interference data shown in Fig. 2A and B identified a protein binding site (GCGTGGCCT) that partially overlapped the right flank of the Sp1-like binding site. A detailed examination of this DNA binding site shows that it does not resemble the footprinting of the Sp1 family of transcription factors, but it shows significant homology to the p53 RGC consensus element (Fig. 2B). Further evidence for the identification of a novel TNF-responsive element was elucidated through UV cross-linking experiments. In these experiments, a thymidine at position 11 in the LTR B probe was replaced by BrdU (probe LTR B-UV1). The probe was allowed to interact with nuclear extracts of TNF-incubated Jurkat cells, UV cross-linked for 15 min at 312 nm, and resolved by EMSA as described above. The upper TIC band was then isolated, and the DNA-protein complex was electrophoresed in an SDS–10% acrylamide gel (Fig. 2C). This protein-DNA complex presented a mobility of 60 kDa. Since probe LTR B-UV1 exhibited an apparent molecular mass of 5 to 10 kDa (data not shown), this experiment indicated that TIC 1 contains a pro-

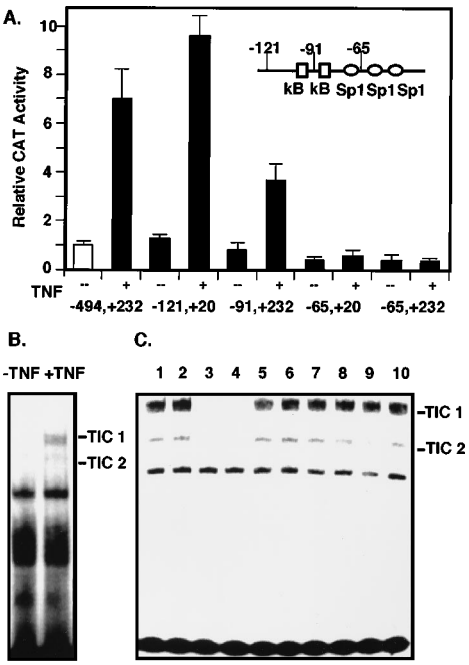


FIG. 1. (A) Identification of HIV LTR promoter region responsive to TNF. Jurkat T cells ($5 \times 10^6$ cells per condition) were transfected by electroporation with 20 μg of the indicated stepwise-deleted fragments of the HIV-1 LTR upstream of a CAT reporter gene plasmid. A map of the region is inset. After a 24-h incubation, samples were divided in two and incubated in the absence of or presence of 10 ng of rTNF-α per ml for 16 h. After incubation, the cells were harvested and assayed for CAT activity. CAT activity was normalized to the basal activity of the −494,+232 HIV LTR CAT construct (2 to 5% CAT conversion; open bar). Data represent the means and standard deviations for three independent experiments. (B) Gel shift mobility assay showing complexes bound to the LTR A sequence with nuclear extracts of TNF-induced Jurkat cells. Jurkat T-leukemia cells cultured in RPMI 1640 medium with glutamine, 2% fetal calf serum and penicillin-streptomycin were resuspended in new media at $1 \times 10^6$ cells per ml in the absence (−TNF) or presence (+TNF) of 10 ng of rTNF-α per ml and incubated for 30 min. Cells were then centrifuged and processed for the preparation of nuclear extracts. Five-microgram aliquots of extracts were assayed by EMSA, with 0.2 ng of the LTR A sequence used as a probe and 1 μg of poly(dIdC) · (dIdC). (C) EMSA competition assay with TNF-induced nuclear cell extracts and the LTR A probe. Two-microgram aliquots of 30-min 10-ng/ml TNF-induced nuclear extracts were assayed by EMSA with 0.2 ng of LTR A probe and 5 μg of poly(dIdC) · (dIdC). Competition for binding of nuclear factors to the LTR A probe was performed with 40 ng of the following probes: p53kB (lane 2), LTR A (lane 3), LTR B (lane 4), IC (lane 5), RGC (lane 6), MCK (lane 7), MgBH (lane 8), SRE (lane 9), and MRE (lane 10). For a description of these oligonucleotides, see Materials and Methods.

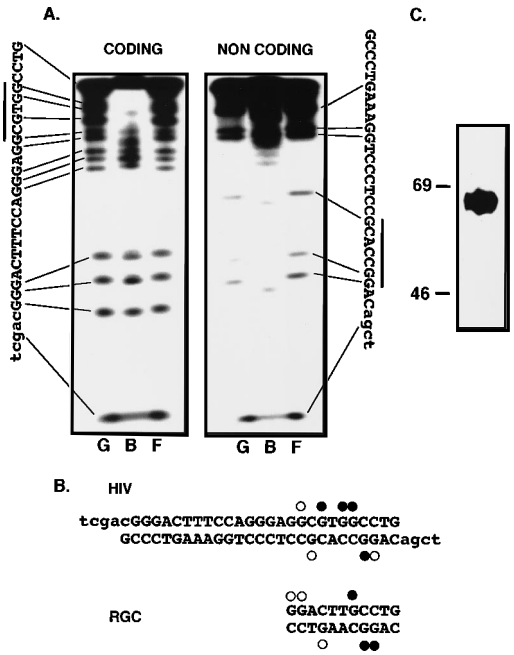


FIG. 2. (A) Methylation interference footprinting of TIC 1 with LTR A probe. LTR A probes were labeled with [$\gamma$-$^{32}$P]ATP on the coding or noncoding strands, annealed with their respective nonlabeled complementary strands, partially methylated, and used in a 10-fold scale-up EMSA with 45-min 10-ng/ml TNF-induced Jurkat nuclear extracts. TIC 1 (lanes B) and free DNA (lanes F) were excised and treated as indicated in Materials and Methods. The unused methylated probes were utilized for the G ladders (lanes G). Binding sites are indicated by lines above the nucleotide sequences. (B) Comparison of the methylation interference footprints of TIC 1 in LTR A (HIV) and wild-type p53 in the RGC sequence (RGC) (32). Partial interference (open circles) and complete interference (closed circles) are indicated. (C) Cross-linking of TIC 1. A 10-fold scale-up EMSA was prepared with TNF-induced nuclear extract and the LTR A-UV probe. DNA-protein complexes were UV irradiated, and the reaction mixtures were loaded onto 3.9% polyacrylamide gels and run as indicated in Materials and Methods. TIC 1 was excised and loaded onto an SDS–10% PAGE gel. Molecular size markers (in kilodaltons) are shown on the left.

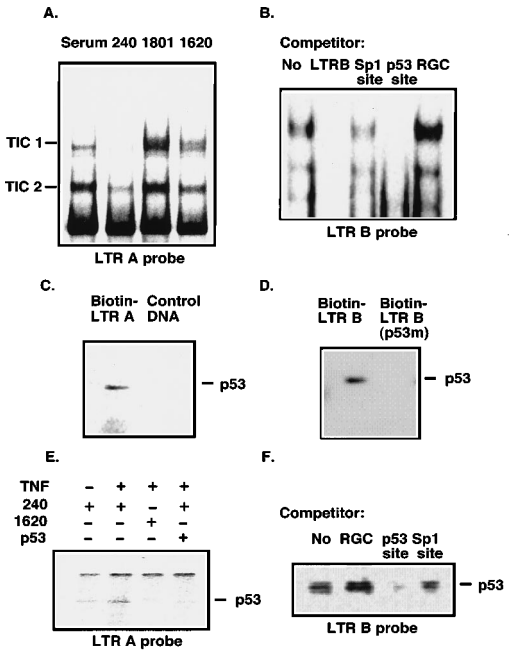


FIG. 3. (A) EMSA-antibody blocking experiment characterizing proteins binding LTR A probe in TNF-induced nuclear extracts. Gel shift reactions were prepared as described in the legend for Fig. 1B, the anti-p53 antibodies indicated above the lanes were added, and the mixtures were incubated for 45 min before loading. (B) EMSA competition experiment with TNF-induced extracts and 0.2 ng of LTR B probe. Extracts were incubated with a 500-fold excess of the oligonucleotide competitors indicated above the lanes. The Sp1 site and p53 site oligonucleotides are LTR B sequences with mutations in the p53 and Sp1 sites, respectively (see Materials and Methods). (C) Western blot indicating the presence of p53 in biotin-LTR A affinity-purified Jurkat nuclear extracts. TNF-induced nuclear extracts (100 $\mu$g) were incubated with 100 ng of a biotinylated LTR A probe in the presence of 100 $\mu$g of poly(dIdC) · (dIdC) for 20 min at room temperature. Streptavidin MagneSphere beads (150 $\mu$l) were added, and the mixture was incubated for an additional 15 min prior to precipitation through a magnetic field. The pellet was resuspended in 250 $\mu$l of PBS with 10 $\mu$g of poly(dIdC) · (dIdC) and reprecipitated. The second pellet was then boiled in SDS-PAGE loading buffer, and the presence of p53 was determined by Western analysis with PAb 1801 at a 1:500 dilution. (D) Western blot of biotin-LTR B affinity-purified p53 from TNF-induced Jurkat nuclear extracts. Biotin-LTR B (p53m) is a biotinylated LTR B oligonucleotide with a mutated p53 site. The purification and Western blot were carried out as described for panel C. (E) UV cross-linking–immunoprecipitation experiment showing p53 interaction with the LTR A probe. Tenfold scale-up EMSA reaction mixtures were prepared as described above with probe LTR A-UV and were irradiated for 20 min at room temperature with 312-nm UV light. Immunoprecipitation buffer (250 $\mu$l) was added, and immunoprecipitations were carried out as described in Materials and Methods. Several antibodies were employed, as indicated above the gel. (F) Oligonucleotide competition and UV cross-linking–immunoprecipitation experiment showing specific p53 interaction with the LTR B probe. Nuclear extracts from TNF-induced Jurkat cells were preincubated with a 500-fold excess of the oligonucleotides indicated above the lanes before a final incubation with an LTR B probe. The p53 site and Sp1 site oligonucleotides are as described for panel B. The reactions were carried out as described for panel E.

tein of a molecular mass of 50 to 55 kDa. Since Sp1 has a molecular mass of 95 to 105 kDa, this result proved that Sp1 is not the protein binding to the site in these assays. Also probe IC (Fig. 1C, lane 5) containing the Sp1 site in the major histocompatibility complex class II invariant chain promoter did not compete for factor binding.

**Identification of a proliferative (antibody 240-positive) p53 binding site in the HIV LTR.** The presence of p53 in the TICs was demonstrated in a number of experiments. In an EMSA (Fig. 3A), formation of TIC 1 was blocked by incubation of the extracts with a monoclonal antibody (PAb 240) against p53. PAb 240 recognizes the proliferative (mutant-like) p53 conformation. In addition, PAb 240 partially blocked the formation of TIC 2. As a control, we found that PAb 240 had no effect on NF-$\kappa$B binding in an EMSA using a $\kappa$B probe (data not shown). Antibodies PAb 1801 (recognizing denatured p53) and PAb 1620 (recognizing the antiproliferative conformation) were not able to block p53 binding to the LTR A probe. An anti-Sp1 antibody showed no effect on the LTR A TICs in these assays (data not shown). However, Sp1 complexes were optimally detected when the binding reaction mixture contained 5 mM $MgCl_2$, no EDTA, and low NaCl concentrations and when extracts were prepared from cells cultured at high density ($0.5 \times 10^6$ to $1 \times 10^6$ cells per ml [data not shown]). An EMSA competition experiment using nuclear extracts from TNF-induced Jurkat cells, an LTR B probe, and a 500-fold excess of oligonucleotides LTR B, LTR B with a mutation in the GCGTGGCCT p53 site (Sp1 site), LTR B with a mutation in the GGGCGG Sp1 site (p53 site), and RGC is shown in Fig. 3B. The LTR B and p53 site, but not the Sp1 site and RGC, oligonucleotides were able to compete with the LTR B probe for TIC formation. This experiment indicated that the complexes identified by anti-p53 PAb 240 bind specifically to the GCGTGGCCT site. Competition with an LTR B oligonucleotide with a mutation in the GGGAGG Sp1 site did not show any difference from the native sequence in the TIC competition (data not shown). These experiments further confirmed that the TIC contained a factor that does not belong to the Sp1 family.

To obtain further evidence of the presence of p53 in the TICs, two different approaches were taken. First, nuclear extracts from TNF-treated Jurkat cells were incubated with an LTR A (Fig. 3C) or an LTR B (Fig. 3D) probe linked to biotin. Following washing, the probe and potentially associated proteins were precipitated with streptavidin-magnetic beads. The presence of p53 in the precipitates was demonstrated by SDS-PAGE and Western analysis with antibody 1801. A nonbiotinylated DNA control probe demonstrated the specificity of the reaction with the LTR A probe (Fig. 3C). In another experiment (Fig. 3D), a second LTR B biotinylated probe with a mutation in the GCGTGGCCT site (biotin-LTR B 53m) was used. This probe failed to precipitate p53. A second approach utilized UV cross-linking of TNF-induced Jurkat cell nuclear extracts to a radiolabeled LTR A probe with a BrdU-for-T substitution at position 25 followed by precipitation of the protein-DNA complex with monoclonal antibodies against p53 (Fig. 3E). Antibody PAb 240, but not PAb 1620, was able to specifically recognize p53 in these complexes. Incubation of the cells with TNF increased the amount of PAb 240-positive p53-DNA complex. Addition to the nuclear extracts of 5 μg of recombinant baculovirus-expressed p53 overcame PAb 240 interaction with endogenous p53. These results and those shown in Fig. 2 indicated that p53 is present in the TICs and that interaction of this protein with the HIV LTR element is accompanied by the presentation of the PAb 240-positive (proliferative) epitope of the protein. Also, the presence of p53 and the specificity of its interaction to the LTR probes were demonstrated in an experiment where nuclear extracts from TNF-induced Jurkat cells were cross-linked to an LTR B probe with a BrdU-for-T substitution at position 11. Prior to the addition of the probe, the extracts were incubated with a 500-fold excess of the LTR B native or mutant oligonucleotides used for the experiment shown in Fig. 3B. The result, which is shown in Fig. 3F, indicated that p53 is specifically bound to the GCGTG GCCT site in these extracts. In several competition experiments, the addition of a 200- to 500-fold molar excess of Sp1 containing oligonucleotides (Fig. 1C, lane 5; Fig. 3B, Sp1 site; Fig. 3F, Sp1 site) produced a decrease in p53-LTR probe interaction. Also, in experiments with purified proteins, p53 was unable by itself to bind to the LTR probes unless transcription factor Sp1 was provided in the binding reaction (data not shown) (see Discussion).

Experiments of oligonucleotide competition EMSA using the LTR A probe showed that the p53 protein present in the TICs does not recognize reported p53 binding sites. Figure 1 shows that probes RGC (lane 6) (32); MCK, containing the p53 binding site in the muscle-specific creatine kinase gene (71) (lane 7); and MgBH, another consensus p53 binding site (23) (lane 8), were unable to compete for binding of the TICs on the LTR A probe. Thus the TNF-induced p53 complex does not recognize previously identified p53 DNA binding sites. TNF induction did not modify the amount of Jurkat p53 capable of binding to an RGC probe (data not shown). These results indicate that two different pools of p53 may be present in Jurkat cells. One of these pools is represented by the anti-proliferative p53 that may not be responsive to growth factors or $G_0/G_1$-acting cytokines, as would be expected of a transcription factor which functions at the $G_1/S$ boundary.

**TNF induces the antibody 240-positive p53 epitope.** The detection of PAb 240-reactive p53 in the TICs suggested that the appearance of this epitope can be induced by incubation with TNF. To directly address this question, Jurkat T cells were labeled with [$^{35}$S]methionine and were stimulated with TNF. Interestingly, the PAb 240-positive p53 form was induced by incubation with TNF, as indicated by immunoprecipitation experiments (Fig. 4). Little or no change was observed when antibody PAb 1620 was used. This result was in agreement with our observation that no change in binding to p53 consensus sites is induced by TNF and further suggests the existence of two different pools of p53. Induction of the PAb 240 epitope has been demonstrated previously in normal lymphocytes incubated in the presence of the T-cell receptor activator phytohemagglutinin (73).

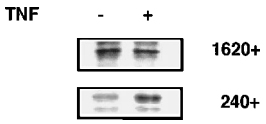


FIG. 4. Immunoprecipitation of PAb 1620+ and 240+ p53 after TNF induction. Jurkat cells ($2 \times 10^7$) were incubated for 1 h in methionine-free RPMI 1640 medium with 0.5 mCi of [$^{35}$S]methionine per ml and 5 μg of cycloheximide per ml and in the presence (10 ng/ml) or absence of rTNF-α. Extracts were prepared for immunoprecipitations with anti-p53 antibodies and analyzed as indicated in Materials and Methods.

**TNF-responsive element in HIV LTR is a target for mutant (proliferative) p53.** To define the potential role of the proliferative p53 binding site in the HIV LTR, a series of deletion mutants of the HIV LTR were tested in cotransfection studies with expression vectors encoding either the wild type or a tumor-promoting mutant, p53 143ala, under the transcriptional control of a CMV promoter. The p53 143ala mutant was used because it has been shown that this p53 form is able to transactivate the HIV LTR and other promoters such as the proliferating cell nuclear antigen (PCNA) promoter that do not contain consensus p53 binding sites (16). These experiments were done with Jurkat cells and with Akata B cells, which do not contain the p53 gene. In Akata cells, overexpression of wild-type p53 had a minor repressive effect on all the promoter fragments of the HIV LTR (Fig. 5A). However, p53 143ala was able to strongly transactivate HIV LTR expression. A similar result was obtained with the p53 248trp transforming mutant form (data not shown). Deletion of the promoter se-

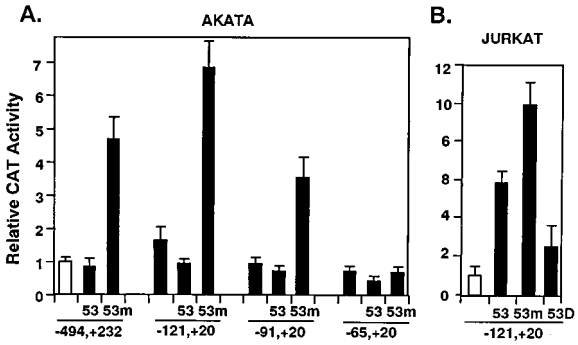


FIG. 5. (A) Transient transfection assay showing the effects of p53 and mutant p53 form (143ala) on a CAT reporter plasmid containing HIV LTR deletion mutations. Akata cells were cotransfected by electroporation with 10 μg of the indicated stepwise-deleted fragments of the HIV-1 LTR and 3 μg of a CMV-driven expression vector containing wild-type p53 sequences (53), 53 143ala (53m), or no insertion. Cells were incubated for 48 h, and CAT activity was measured as indicated in Materials and Methods. CAT activity was normalized to the basal expression level of the −494,+232 HIV LTR CAT construct (open bar). The data represent the means and standard deviations for three independent experiments. (B) Effects of p53 on the transcriptional activity driven by a −121,+20 HIV LTR CAT reporter plasmid (−121,+20) in Jurkat cells. Cells were cotransfected, and CAT activity was assayed as described above. The basal expression level of the −121,+20 HIV LTR construct is shown as an open bar. The data represent the means and standard deviations for three independent experiments.

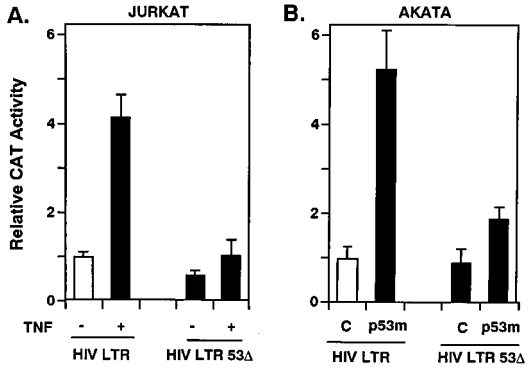


FIG. 6. Effects of the mutation of the p53 binding site on the transactivation of the HIV LTR by TNF (A) and p53 143ala (p53m) (B). Jurkat (A) or Akata (B) cells were transfected with 10 μg of a native −494,+232 HIV LTR CAT reporter (HIV LTR) or a site-directed mutated reporter (HIV LTR 53Δ). Transfections, incubations, and CAT assays were performed as described for Fig. 5. Jurkat cells were transfected with 5 μg of HIV LTR CAT reporter vectors, incubated for 24 h, and then induced with 10 ng of rTNF-α per ml for an additional 16 h. CAT activity was measured as indicated in Materials and Methods. Activity was related to basal expression of the wild-type vector (open bar). Akata cells were cotransfected with 5 μg of the native and mutated HIV LTR CAT reporters and 3 μg of CMV-p53 143ala expression vector. Cells were incubated for 48 h, harvested, and assayed for CAT activity as indicated in Materials and Methods. The data in panel A and B represent the means and standard deviations for three independent experiments.

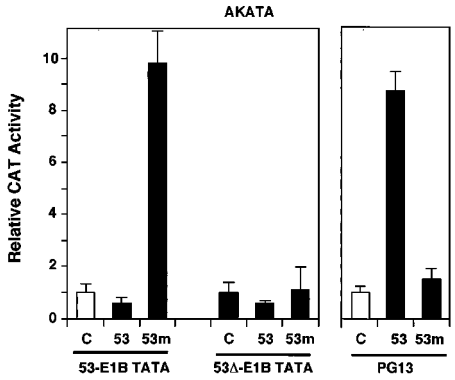


FIG. 7. Transient transfection assay showing the effects of p53 and p53 143ala (53m) on minimal transcriptional elements containing the p53 binding site in the HIV LTR (53-E1B TATA), a mutated HIV p53 site (53Δ-E1B TATA), and a consensus wild-type p53 binding site (PG13) in Akata cells. Expression vectors (3 μg) and 10 μg of reporters were used. Transfections, incubation, and CAT assays were carried out as described for Fig. 5. CAT activity was normalized to the basal expression levels of the 53-E1B TATA and PG13 vectors (open bars; 1 to 4% CAT conversion). These data represent the means and standard deviations for three independent experiments.

quences that contain the p53 site (from nucleotide −91 to −65) drastically decreased transactivation by p53 143ala. The deletion mutations of the HIV LTR were mapped with the p53-responsive fragment in the same area where we initially identified the HIV LTR TNF responsiveness. Intriguingly, in Jurkat cells, a positive effect was obtained with both the wild-type and mutant p53 forms (Fig. 5B). 53D, a p53 expression vector that contains a 30-amino-acid deletion (position 347 to 377) in the carboxy-terminal domain, did not effectively transactivate the HIV LTR reporter construct. This deletion is located outside the core binding domain of p53 (49) in a region that contains the nuclear localization sequence and that has been implicated in the ability of p53 to oligomerize and to interact with other proteins (51). Transfection of Sp1 increased HIV LTR CAT expression by sixfold, indicating that under our conditions the HIV LTR Sp1 sites were not saturated. However, only an additive effect was observed with cotransfections of Sp1 with p53 expression vectors in Jurkat cells (data not shown).

To obtain independent evidence of the importance of the HIV p53 site, cotransfection experiments with a site-directed HIV LTR mutant construct were performed. CAT reporter vector HIV LTR 53Δ contains point mutations in the p53 site (see Materials and Methods). These mutations substantially reduced TNF induction of the HIV LTR (Fig. 6A). Also, the results shown in Fig. 6B revealed that these mutations decreased the ability of mutant p53 143ala to transactivate the HIV LTR. However, they could not completely block all the transactivation by p53, indicating that other p53-responsive elements may be present in upstream sequences. While we were preparing our manuscript, a report indicating that mutant p53 forms are able to transactivate the HIV LTR was published (57). No sequence-specific element was identified in that study, and its authors hypothesized that mutant p53 transactivation was mediated by interaction with the basic transcription machinery. In contrast, our results show the absence of activation by mutant p53 of the −65,+20 HIV LTR construct, which lacks the p53 binding site but contains two consensus Sp1 binding sites and the HIV LTR TATA box (Fig. 5A). Also, the results obtained with HIV LTR 53Δ (Fig. 6B) indicate that the p53 site is necessary for full activation of the HIV LTR by the proliferative p53 forms. Importantly, Duan et al. (17) have recently shown that overexpression of wild-type p53 inhibited HIV-1 LTR-mediated transcription and that the mechanism of that repression is mediated by LTR sequences from positions −117 to −65. Additionally, it was shown that mutant forms of p53 strongly stimulated HIV replication (17).

In order to determine more accurately the site specificity of p53 mutant transactivation, we used minimal elements containing the LTR A sequence subcloned upstream of a heterologous promoter, E1B TATA (50). As shown by Fig. 7, this minimal promoter construct (53-E1B TATA) was activated 10-fold by p53 143ala, yet no effect was observed with a construct containing a mutated p53 site (53Δ-E1B TATA). This result demonstrated that transactivation by the p53 mutant form is site dependent. As expected, a small repressive effect was found with the cotransfection of wild-type p53 and 53-E1B TATA in Akata cells (Fig. 7). Somewhat surprisingly, wild-type p53 was able to achieve fourfold transactivation of the 53-E1B TATA construct in Jurkat cells (see Discussion). Cotransfection of a multimer p53 consensus site (PG13) in Akata cells with the expression construct containing wild-type p53 clearly resulted in high levels of CAT activity, while mutant form 143ala had no effect (Fig. 7). Also, murine sarcoma virus LTR-driven expression vectors containing wild-type p53 and a number of mutant p53 proteins (including Val-135, His-175, Trp-248, and His-273 sequences) were able to transactivate this minimal promoter element in Jurkat cells (data not shown). These results correlate with those of the in vitro binding experiments and establish a transcriptional base for describing the functional difference between the proliferative and antiproliferative forms of p53.

## DISCUSSION

This paper focuses on two major findings: the identification of a novel binding site in the HIV LTR for the proliferative form of p53 and the role of this site in the induction of HIV gene expression by TNF. Evidence that the TNF-inducible binding activity contains p53 is based on methylation interfer-

ence patterns and UV cross-linking (Fig. 2 and 3), EMSAs (Fig. 3), and transfection experiments (Fig. 5 to 7). Evidence that the p53 protein is present in the proliferative conformation is based on the results of multiple experiments (as discussed below).

TNF is a multifunctional cytokine secreted primarily by mitogen-activated macrophages that has a wide range of biological activities. TNF can induce both apoptotic and necrotic forms of cell death (15, 34). The expression of this cytokine is also associated with activation of the immune system (29). It has recently been shown that TNF can enhance the proliferation of thymocytes and T cells (58). Other reports have shown that TNF may have a bidirectional effect, with the increase or inhibition of T-cell proliferation depending on the concentration of mitogenic costimulators (27). These studies show that TNF has unique regulatory effects on both T-cell growth and differentiation processes. It has been demonstrated that the two different receptors of TNF, TNF-R1 and TNF-R2, mediate distinct cellular responses (58, 59). TNF-R2 initiates signals for the proliferation of T cells, whereas TNF-R1 initiates signals for cytotoxicity. However, it is still unclear how the two receptors initiate distinct signaling pathways since both receptor types are expressed simultaneously in multiple cell lines (59). Thus, our studies suggest that one mechanism whereby TNF can control cell growth is through the modulation of p53 activity.

Although the TNF-responsive element in the HIV LTR is bound by endogenous p53 when Jurkat cells are induced by TNF, we did not see binding to this element with recombinant wild-type or mutant p53 unless the transcription factor Sp1 was provided (25). Thus, it is possible that p53 is converted to a proliferative conformation through an interaction with Sp1. Interestingly, it has been reported that a physical interaction between p53 and Sp1 can occur (6). Cooperativity between p53 and Sp1 for DNA binding was suggested by EMSA and UV-cross-linking oligonucleotide competition experiments (Fig. 3B and F). Current experiments in our laboratory are focused on the potential role of ternary complexes involving p53, Sp1, and NF-κB family members in the regulation of HIV LTR-mediated expression. As described above, our data strongly suggest that the form of p53 that binds to the HIV LTR is the so-called proliferative form. Consistent with the proposal that proliferative p53 binds to this element are the observations that antibodies that recognize proliferative forms of p53 recognize the p53-DNA complex (Fig. 3) and that oncogenic forms of p53 are able to specifically activate gene expression through the HIV LTR p53 site (Fig. 5 to 7). Furthermore, our data demonstrate that this inducible form of p53 binds to DNA elements different from, although related to, those to which recombinant p53 binds (Fig. 1 and data not shown).

The identification of this novel p53 site may have implications for the understanding of the mechanism of replication of HIV. Leonard et al. (35) demonstrated that deletion of either the TAR element or the region containing the p53 and Sp1 sites, but not of the κB sites, blocks the ability of HIV-1 to replicate in peripheral blood lymphocytes. In addition, TNF has been shown to activate HIV replication in both T and mononuclear cells (5). Parrott et al. (48) and Ross et al. (53) demonstrated that deletion of the p53 and Sp1 binding region of the HIV LTR had no effect on HIV-1 replication in blood lymphocytes. However, this deletion markedly delayed basal replication and TNF-induced replication in T-cell lines. Thus, it is possible that a transcription factor like p53 which has been shown to be highly expressed in replicating normal human lymphocytes (73) has an important role in HIV replication in vivo. Specifically, it appears that the virus utilizes the so-called proliferative form of p53. p53 may be also important for the replication of the virus in other cells, such as macrophages. In addition, p53 may play a more-critical role in HIV biology in the context of infection within the host and its role may not be fully apparent in experiments performed in tissue culture. Also, the variability of the status of the p53 gene may help to explain the diverse levels of HIV-1 expression obtained in different lymphocyte cell lines. Obviously, an analysis of mutations of the HIV LTR p53 site is necessary to elucidate the role of this protein in the replication of the virus. Interestingly, a recent publication (17) suggested a role for p53 in the regulation of HIV gene expression and viral replication. A region encompassing the p53 site identified in our work was shown to mediate repression of HIV LTR-directed gene expression by wild-type p53. Consistent with our data, a mutated form of p53 was shown to strongly activate viral replication. We propose that mutant forms of p53 activate viral gene expression through some type of direct interaction with the p53 site identified in our study. In addition, it is possible that other as-yet-unidentified p53 sites exist on the HIV LTR. Furthermore, we suggest that normal p53 plays an important role in the activation of HIV gene expression through the activation of the so-called proliferative form.

Using a series of deletion mutants of the HIV LTR, we mapped the location of a TNF-responsive element in the LTR (Fig. 1). This promoter fragment contains the binding sites of several transcription factors, including NF-κB and Sp1. Numerous reports have indicated the role of NF-κB and Sp1 in the TNF induction of LTR-mediated expression. The κB elements are required for HIV LTR-inducible expression in activated T cells and mature monocytes (43), while Sp1 is thought to regulate the basal level of transcription (30). Interestingly, of the three HIV Sp1 sites described by Jones et al. (30), the most 5′ site was not necessary for Sp1 transactivation of the HIV LTR. These authors concluded that the more 3′ Sp1 sites were primarily responsible for promoter strength, whereas binding to the most 5′ Sp1 site was unimportant for stimulation of RNA synthesis. It has been shown previously that an interaction between NF-κB and Sp1 is critical for activation of gene expression directed by the HIV LTR (50), and a cooperative interaction between NF-κB and Sp1 was observed. This functional interaction occurs in the region identified as controlling the p53 response. Thus, it is possible that the p53-mediated pathway reported here involves a pathway for HIV LTR-directed gene expression that is distinct from that utilized by NF-κB and Sp1. Alternatively, complex protein-protein interactions that involve NF-κB, p53, and Sp1 may form on the LTR. It is also likely that some cellular promoters contain Sp1-p53 composite sites. This hypothesis is most interesting when promoters that have been shown to be transactivated by transforming mutant forms of p53 and yet do not contain known p53 binding sites are considered. Some examples of these genes are the *PCNA* (16) and *MDR1* genes (12). The case of the *MDR1* gene is particularly important because MDR1 expression is induced by TNF (8) and the promoter sequences do not contain κB sites but possess a functional Sp1 site (14).

Several studies have shown that p53 gene expression is strongly induced in lymphocytes after stimulation by mitogens (38, 40, 60). Also, experiments of microinjection of monoclonal anti-p53 antibodies (37) and antisense p53 (55) demonstrated that p53 is required for the induction of cell proliferation by growth factors. The data reported here suggest that p53 may play a role as a positive regulator of the $G_0/G_1$ transition, as suggested by previous observations (reference 62 and references therein). It has been proposed that p53 can exist in two

distinct functional forms, the so-called antiproliferative form and the proliferative form (39, 62). Some of the antibodies that recognize the proliferative form of p53 also recognize mutant (oncogenic) forms of p53. Thus, it is possible that a conformational change in p53 is associated with the proliferative functions of p53 and that certain mutant forms of p53 are unable to adopt the antiproliferative conformation and thus may function exclusively in a proliferative role (51, 62). Presumably then, proliferative p53 functions as a transcription factor by directing gene expression through a different DNA binding site than exists for nonproliferative p53. The site within the HIV LTR site likely represents a new class of p53 binding sites that functions in a proliferative manner. Genes that contain sites functionally related to the HIV LTR p53 site would be regulated in a proliferative manner by p53 (as discussed below), and those that contain sites for nonproliferative p53 would function to inhibit cell proliferation. Recently, it was shown that the *p21/Waf1/Cip1* gene is regulated by p53 (19, 26, 70). The protein encoded by *p21/Waf1/Cip1* negatively regulates cyclin-cdk activity, and thus the loss of wild-type p53 function and the loss of expression of this protein may explain the growth suppressor function of p53. Thus, deletion of wild-type (nonproliferative) p53 in tumor cells would lead to the loss of the growth suppressor function of p53. Mutations that generate oncogenic forms of p53 (46) may lead to gene-specific transcription mediated by sites similar to the one identified in the HIV LTR. An important question is whether mutant forms of p53 can bind to DNA in a specific fashion. Several of the mutant forms of p53 exhibit mutations in amino acids that are known to critically contact residues in the p53 consensus site (13). Thus, the ability of mutant p53 to bind to nonconsensus DNA sequences may be explained by several possibilities. A mitogen-triggered posttranslational modification and/or interaction with other proteins (potentially Sp1) may restructure the mutant form of p53 such that it can make critical DNA contacts. Alternatively, certain DNA sites (such as the site in the HIV LTR) that are not identical to a p53 consensus site may bind to mutant p53 forms because an amino acid-nucleotide contact essential for p53-consensus DNA interaction is not found in the mutant p53-nonconsensus DNA complex. A combination of these two possibilities may allow for potential high-affinity interaction between mutated forms of p53 and certain DNA elements.

What functions to convert p53 from the nonproliferative to the proliferative form? p53 has a short half-life, 6 to 10 min, in proliferating T cells (52). This extraordinarily rapid turnover indicates that p53 has a tightly regulated function in T cells. Recently, Appella and coworkers indicated that phosphorylation at specific sites regulates p53 ability to induce growth arrest and to associate with the simian virus 40 T antigen (22, 64). It is feasible that posttranslational modifications are necessary to regulate p53 transcriptional functions. Consistent with this idea is our observation that the inducible form of p53 can be activated in the presence of protein synthesis inhibitors. It is also possible that signal transduction events lead to the differential association of p53 with modulatory proteins. For example, MDM2 is a p53-associated protein that functions to inhibit presumably the nonproliferative functions of p53. Since our data demonstrate a role for Sp1 in the proliferative functions of p53, a signal transduction event may lead to the modification of p53 with its subsequent dissociation from MDM2 and its association with Sp1. The p53-MDM2 interaction domains have been recently mapped to the N-terminal domains of both proteins (9), and the p53 N-terminal domain has been shown to contain its activation domain (45). Furthermore, studies on *MDM2* gene expression demonstrate that it is regulated by the levels of p53 protein (69). These results indicate that p53 regulates MDM2 expression and that MDM2 regulates p53 transcriptional activity, creating an autoregulatory feedback loop (69).

Since mutant forms of p53 have lost the ability to bind to consensus p53 elements, these mutations may represent an important gain-of-function phenotype that is operative in the oncogenic roles of these proteins. Intriguingly, in our transfection experiments with Akata cells, mutant p53, but not wild-type p53, was able to transactivate the HIV LTR; however, in Jurkat cells, a positive effect was observed with both wild-type and mutant p53 forms (Fig. 7). It is unclear why the wild-type p53 form can activate the HIV LTR in Jurkat cells, although several explanations can be proposed. It is known that mutant forms of p53 can oligomerize with wild-type p53 and alter its conformation (39). Thus, the presence of the endogenous p53 mutant in Jurkat cells (11) may alter the effect of the transfected wild-type p53, leading to activation of gene expression. Consistent with this point is the observation that only mutated p53 can stimulate gene expression in Akata cells, which do not express endogenous p53. Another explanation of why wild-type p53 can activate HIV LTR-directed gene expression is that Jurkat T cells may have an activated signal transduction pathway that leads to the conversion of wild-type (nonproliferative) p53 to a proliferative form.

In summary, the interpretation of the data presented here is that proliferative conformations of wild-type p53 exhibit growth-regulatory properties by interacting with sites specific for the proliferative forms. It is possible that the gain-of-growth properties of tumor-promoting p53 forms are mediated by their transcriptional activities. p53 mutant forms would then be expected to be transcriptionally active because they mimic specific functions of a wild-type p53 proliferative conformation. We hypothesize that proliferative p53 and antiproliferative p53 recognize different DNA binding sites which can mediate proliferative and antiproliferative functions, respectively. Wild-type p53 may shift from an antiproliferative to a proliferative conformation by TNF or growth factor induction. The effect of the TNF signal pathway may allow p53 to interact with other transcription factors, such as Sp1, and facilitate the binding of these proteins to composite p53-Sp1-responsive DNA elements. The data also present further evidence that HIV-1 utilizes a pathway for replication that is directly modulated by the growth status of the cell. It would be interesting to determine if regulatory proteins of HIV, such as nef or tat, have effects on this unique transcriptional activation pathway.

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