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The Tumor Suppressor Protein p53 Strongly Alters Human Immunodeficiency Virus Type 1 Replication

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The p53 tumor suppressor gene product, a sequence-specific DNA-binding protein, has been shown to act as a transcriptional activator and repressor both in vitro and in vivo. Consistent with its role in regulating transcription are recent observations that the N-terminal acidic domain of p53 binds directly to the TATA box-binding protein subunit of the general transcription factor, TF IID. It is now demonstrated that wild-type p53 (wt-p53) inhibits human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR)-directed chloramphenicol acetyltransferase activity in a cotransfection assay system. Importantly, this effect of wt-p53 on the HIV-1 LTR was also demonstrated by in vitro transcription assays. In addition, the Sp1 sites and the TATA box of the HIV-1 LTR are demonstrated to be the primary sites involved with p53-induced effects on this viral promoter. The upstream elements of the HIV-1 LTR, including the nuclear factor kappa B (NF-κB) binding sites, decrease the p53-induced inhibitory effects on viral transcription. In the presence of the HIV-1 TAR sequence and Tat protein, the HIV-1 LTR also becomes less sensitive to wt-p53-induced inhibition. By using a retroviral vector delivery system, mutant forms of p53 genes were expressed in two HIV-1 latently infected cell lines, ACH-2 and U1. In the ACH-2 cell line, which is now demonstrated to contain an endogenous mutant form of p53 (amino acid 248, Arg to Gln), additional mutant p53 proteins did not alter HIV-1 replication. In U1 cells, which completely lack endogenous p53, overexpression of mutant p53 led to an increase in HIV-1 replication. Thus, these data indicate a possible functional role for wt-p53 and mutant p53 proteins in the control of HIV-1 replication patterns and proviral latency.

Somatic mutations in the p53 gene are some of the most common genetic alterations found in human neoplasia (26). Germ line mutations in the p53 gene are associated with inherited susceptibility to a wide range of tumor types (35). The p53 gene product, a nuclear phosphoprotein, is a sequence-specific DNA-binding protein that is active as a transcription factor and appears to be involved in the regulation of cellular proliferation of many types of cells (30, 61). p53 has been shown to activate transcription of constructs bearing p53 binding sites both in vitro and in vivo (67). However, transcription of a number of other promoters that lack p53 binding sites is inhibited by wild-type p53 (wt-p53) (68). These include several viral promoters, such as the Simian virus 40 (SV40) promoter, human cytomegalovirus immediate-early promoter, human T-cell leukemia virus type I long terminal repeat (LTR), Rous sarcoma virus LTR, and others (68). Over the past few years, it has been established that p53 forms a complex with the TATA box-binding protein (TBP) in vitro, and further work has confirmed that wt-p53 can directly bind the general transcription factor, TF IID (8). wt-p53 may also interact with the Sp1 protein (5). Each of these transcription factors is involved in human immunodeficiency virus type 1 (HIV-1) LTR-directed transcription through Sp1 binding sites and the TATA box in the HIV-1 LTR (23, 49).

Mutant p53 proteins that are transforming or are found in tumor cells have properties that are different from those of the wild-type protein (61). Transforming mutant p53 proteins do not bind to the SV40 T antigen but form a stable complex with a heat shock protein (hsc70) (42). Sequence-specific DNA binding by p53 has also been reported. Wild-type but not mutant p53 binds to the 10-bp motif 5′-PuPuPuC(A/T)(A/T)GPyPyPy-3′. Typically, there are two copies of this motif which are separated by up to 13 bp of random sequence (14). These motif repeats are present in the human ribosomal gene cluster (14), muscle creatinine kinase gene (64), GADD45 gene (28), and an element in certain retroviral LTRs (1). wt-p53 inhibits SV40 DNA replication in vitro and in vivo by forming complexes with viral T antigen and inhibiting the unwinding capability of T antigen, whereas the transforming mutants do not perform these functions (63). The p53 protein has recently been shown to inhibit c-*fos* and human interleukin 6 transcription (50) and to repress transcription from several cellular and viral promoters (19, 54). Nevertheless, wt-p53 has also been demonstrated to activate a promoter with p53 binding sites both in vitro and in vivo (67). It has been shown that the mouse muscle creatinine kinase enhancer can be activated by wt-p53 (64). The p21 protein, which is involved in regulating cyclin-dependent kinases (Cdks), was also up-regulated by wt-p53 (22). wt-p53 has been reported to activate mdm-2 expression, as well as to enhance its own promoter's activity (3). Interestingly, wt-p53 inhibited the multiple drug resistance (MDR1) promoter, whereas one p53 mutant (amino acid 175, Arg to His) activated the MDR1 promoter (9). Mutant p53 proteins may also activate the human proliferating cell nuclear antigen (PCNA) promoter (12).

The HIV-1 LTR contains a typical, albeit compact, inducible RNA polymerase II promoter that can be activated by certain

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physiological agents (mitogenic stimulation, UV light, and others), as well as by viral regulatory proteins (18, 60). Notably, the initiation stage of messenger RNA synthesis is a major site for regulation of gene expression. In eukaryotes, initiation is governed by DNA sequence elements comprising several functional classes. These include a core promoter element, which contains the binding site for RNA polymerase II and controls the location of the site of transcription initiation, and upstream promoter elements and enhancers which regulate the rate at which RNA polymerase II initiates new rounds of transcription from the core promoter. These sequence elements direct the action of two classes of transcription factors: general initiation factors, which are essential for initiation and which are sufficient to direct a basal level of transcription from many core promoters, and regulatory factors, which are not required for initiation but which mediate the action of upstream promoter elements and enhancers (11). Transcription initiation by RNA polymerase II involves a stepwise assembly of general transcription factors on the promoter to form a preinitiation complex (PIC) (10). Several studies have indicated that transcription activator proteins function, at least in part, by increasing PIC assembly (10). This implies that one or more steps in PIC assembly are normally limited, by rate or extent, and are increased by the activator. Recent evidence suggests that TF IIB can be a limiting step in complex assembly and this step can be enhanced by an acidic activator through the direct interaction between TF IIB and the acidic activator domain (10). Direct interaction between the acidic activator and the TBP has also been reported (31). The general transcription factor TF IID is composed of TBP and a set of tightly bound polypeptides, designated TBP-associated factors. These have coactivator activity in that they are required for activator function but do not affect the low level of basal transcription observed in the absence of an activator (10). Therefore, the TBP-associated factors somehow enable an activator to increase PIC assembly. Biochemical data from p53 experiments have shown that its N-terminal acidic domain (1 to 75 amino acids) can directly bind TF IID, and binding between p53 and TF IID may be involved in the regulation of the TBP assembly process (58).

On the basis of these data, it was hypothesized that wt-p53 or mutant p53 proteins, with mutations affecting specific DNA-binding abilities but not protein-protein interactions, might be involved in altering basal HIV-1 LTR-directed transcription through the interaction of the acidic activator N-terminal domain of p53 with TBP-associated factors on the Sp1 site (GC-rich region) and the TATA box in the HIV-1 LTR. It has been demonstrated that, in normal cells, wt-p53 levels are extremely low, because of the very short half-life of the protein, and in some cases wt-p53 is barely detectable (47). By contrast, for reasons not yet entirely understood, transformed cells with the SV40 virus T antigen, or human tumors, often contain relatively high levels of mutant p53 with a much longer half-life (21). In certain cells exposed to ionizing radiation, UV light, or mitomycin, p53 accumulates to high levels (32). In the case of HIV-1-infected cells, superinfection with other DNA viruses or cellular mutant forms of p53 may change the activity of wt-p53. Those changes may alter the level of HIV-1 LTR basal transcription and may account for some cases of HIV-1 expression pattern shifts from latency to a productive replicative state.

MATERIALS AND METHODS

Plasmids. Wild-type and mutant human p53 expression plasmids utilized in these studies contained the human cytomegalovirus major immediate-early promoter-enhancer from the vector pCMV-Neo-Bam (24). pCMV-wt-p53 contains wt-p53 1.8-kb *Bam*HI cDNA. pC53-SCX3 (Val to Ala at amino acid 143) and pC53-273H (Arg to His at amino acid 273) are mutant p53 expression plasmids which were constructed in same pCMV-Neo-Bam vector. The plasmid, pG13-CAT, which contains a wt-p53-specific binding DNA sequence (5′-CCTGC CTGGACTTGCCTGG-3′) was constructed by inserting an oligonucleotide of this p53 binding site upstream of the polyomavirus early promoter, which drives expression of the chloramphenicol acetyltransferase (CAT) gene. These plasmids were kindly provided by B. Vogelstein (Johns Hopkins Oncology Center) (24).

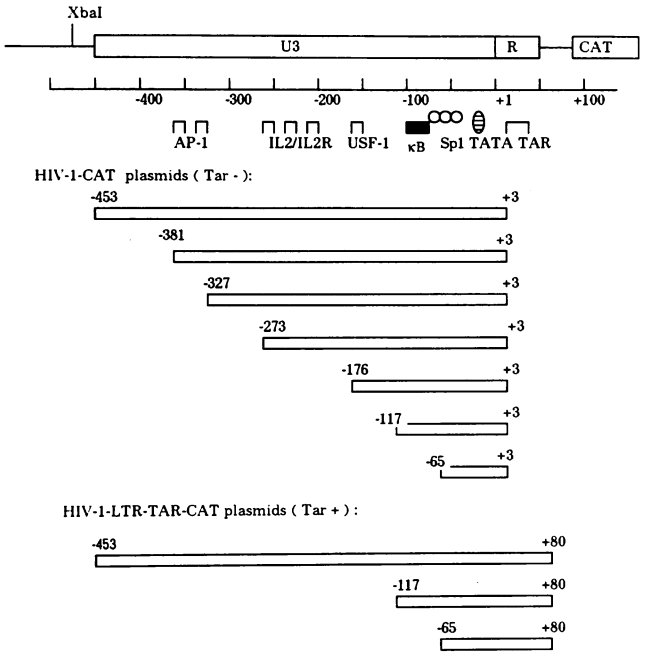

FIG. 1. Schematic diagram of HIV-1 LTR constructs. The numbering system is based on the +1 notation representing the viral RNA initiation site. This diagram illustrates only certain key binding motifs in the HIV-1 LTR and does not contain all known or putative protein binding sites. IL, interleukin; κB, NF-κB.

pActin-Tat which expresses HIV-1 Tat under control of the human β-actin promoter has been described previously (57). pActin-Tat was constructed by inserting a PCR-amplified fragment containing the coding sequence for the first 86 amino acids of Tat downstream of the human β-actin promoter in the plasmid pHFβA-1 (20). The pSV-β-Gal reporter plasmid, which was purchased from Promega, Inc., contains an SV40 early promoter driving the *Escherichia coli* β-galactosidase (β-gal) gene.

The CAT expression plasmids utilized in these experiments all contained the *E. coli* CAT gene under the transcriptional control of the HIV-1 LTR. All cloning procedures followed standard protocols (36). Different deleted HIV-1 LTRs linked to the reporter gene, CAT, with or without the HIV-1 TAR sequence were generated by oligonucleotide-derived PCR amplifications and then inserted into the backbone of the pBLCAT-2 plasmid (33, 57), as shown in Fig. 1. The numbering system used for these plasmids utilizes +1 as the transcription initiation site of HIV-1. The HIV-1 TAR-deleted plasmids include p−435/+3, p−381/+3, p−327/+3, p−273/+3, p−176/

+3, p−117/+3, and p−65/+3. The plasmids which contain the TAR sequence include p−453/+80 (full length), p−117/+80, and p−65/+80.

The pET-wt-p53 construct, which expresses wt-p53 protein in *E. coli*, under control of the T7 promoter, was created via PCR amplification by a standard PCR procedure (36, 53), with the plasmid pCMV-wt-p53 as a DNA template, using two primers: Oligo 1, 5′-CCACATATGGAGGAGCCGCAGTC AGAT-3′; and Oligo 2, 5′-ATGGATCCTCAGTCTGAGTCA GGCCCTTC-3′.

A 1,195-bp PCR product was gel purified and digested with *Nde*I and *Bam*HI and then inserted into the pET19b vector (*Nde*I-*Bam*HI sites) to construct plasmid pET-wt-p53, which was confirmed by DNA sequencing (69).

Purification of recombinant p53 and hepatitis B X (HBX) proteins. *E. coli* BL21(DE3) cells were transformed with the pET-wt-p53 plasmid, incubated in Luria broth medium to an optical density at 600 nm of 0.4 at 37°C, and induced with 0.5 mM IPTG (isopropyl-β-D-galactopyranoside) for 3 h at 30°C. Induced cells were collected by centrifugation at 5,000 × *g* for 5 min, the supernatant was discarded, and the cell pellet was washed with ice-cold phosphate-buffered saline (PBS) and then resuspended in a 1/10 culture volume of binding buffer (40 mM imidazole, 4 M NaCl, 160 mM Tris-HCl, pH 7.9). The preparation was then sonicated and centrifuged at 20,000 × *g* for 15 min to collect the inclusion bodies. The pellet was resuspended in 5 ml of binding buffer which contained 6 M urea and was incubated on ice with stirring for 1 h to completely dissolve the protein. The insoluble material was removed by centrifugation at 39,000 × *g* for 20 min. The supernatant was filtered through a 0.45-μm-pore-size membrane before being loaded on a column. Ni^{2+} column purification was performed per the manufacturer's (Novagen, Inc.) suggested protocol. The washed fraction containing purified p53 protein was slowly dialyzed against different concentrations of urea in PBS to renature p53 protein at 4°C. The purified p53 protein was stored at −70°C until use. The purity of the p53 preparation was greater than 90%, as suggested by sodium dodecyl sulfate-polyacrylamide gel electrophoresis silver staining.

The pET-HBX expression vector was constructed via PCR amplification with Oligo-1, 5′-AGT CAT ATG GCT GCT AGG CTG TGC-3′, and Oligo-2, 5′-GAA GAT CTT CAG ATG ATT AGG CAG AGG-3′, using the pTKHH2 plasmid as a DNA template (65). A 487-bp PCR product was gel purified and digested with *Nde*I-*Bgl*II and then inserted into the pET19b vector's *Nde*I-*Bam*HI sites to create the *E. coli*-derived HBX expression plasmid, pET-HBX. HBX protein expression, driven by the T7 promoter, was induced with IPTG and purified with an Ni^{2+} column and renatured by the same procedure as for purification of the recombinant p53 protein from *E. coli*.

Retroviral expression vectors. The murine retroviral vector, pLXSN (40), carrying mutant p53-143 (Val to Ala at amino acid 143) and p53-273 (Arg to His at amino acid 273) genes, was used for insertion of *Bam*HI p53 fragments (1.8 kb) from pC53-SCX3 and pC53-273 into the pLXSN *Bam*HI site to create plasmids pLXp53-143 and pLXp53-273. Plasmids pLX-CAT and pLX-GAL were constructed by inserting *E. coli* CAT and β-gal genes into the pLXSN vector, as previously described (13). Construction of the N-terminally deleted p53-expressing retroviral vector was performed by deletion of a 477-bp *Nco*I fragment from a 1.8-kb p53 cDNA fragment. This *Nco*I-deleted p53 *Bam*HI fragment was inserted into the pLXSN *Bam*HI site to create plasmid pLX-Nd-p53. The genes of interest in each retroviral shuttle vector were driven by the 5′ murine leukemia virus (MLV) LTR.

Cell cultures and transfections. U1 and ACH-2 cells were propagated in RPMI-1640 media supplemented with 10% fetal calf serum. The U1 and ACH-2 cells are monocytic and T-lymphocytic lines latently infected with HIV-1 which can be stimulated by various exogenous agents to dramatically increase HIV-1 expression (44). Human hepatocyte carcinoma cell lines Hep3B and HepG2 (45), plus NIH/3T3 and PA317 cells (40), were obtained from the American Type Culture Collection and propagated in Dulbecco's modified Eagle's media supplemented with 10% fetal calf serum. For CAT transient transfection assays, subconfluent cells were placed in 100-mm-diameter plates 1 day prior to transfection. Four hours prior to transfection, the medium was changed to 10 ml of fresh, prewarmed 10% fetal calf serum-Dulbecco's modified Eagle's medium. Ten to twenty micrograms of total plasmids was used in each transfection, which was performed by a calcium phosphate-DNA coprecipitation method (41). In a typical experiment, 1×10^6 cells were cotransfected with different quantities of CAT reporter and p53 expression plasmids (or the expression plasmid's backbone without the p53 gene). In Tat expression experiments, 1 μg of the pActin-Tat construct was also cotransfected. All transfection experiments were repeated two to six times in duplicate. A 10 to 30% variation in activities between experiments was observed.

CAT and β-gal assays. Transfected cells were washed with 10 ml of PBS, harvested at 48 h posttransfection, and lysed with 0.9 ml of Promega CAT lysis buffer at room temperature for 15 min. Extracts were normalized for protein concentrations and assayed for CAT enzyme activity (2). CAT activity was detected by thin-layer chromatographic separation of [^{14}C]chloramphenicol from its acetylated derivatives and was quantitated by using an image-scanning system (Molecular Dynamics Inc.; with a volume integration program). Fifty to one hundred microliters of the lysed extracts (20 to 100 μg of total protein) was used in each CAT assay, with a fixed incubation time of 2 h. Each baseline CAT assay was prerun to determine the proper protein extract concentration to be used to allow the basal expression to be in the linear range of the CAT assay. The baseline percent conversions of chloramphenicol were maintained between 15 and 30% in these experiments. As such, alterations in relative CAT activities by p53 represented effects on a strong basal signal. β-gal activity was assayed with the β-Galactosidase Enzyme Assay System (Promega, Inc.) using purified β-gal as a standard control per the manufacturer's suggested procedure (41).

In vitro transcription experiments with p53 and HBX. Whole-cell extracts were prepared from HeLa cells grown in suspension culture in minimal essential medium (Quality Biochemical) supplemented with 5% horse serum, as described by Manley et al. (37). Template DNAs were prepared by *Eco*RI digestion of pHIV−435/+80 (pCd 12-CAT) and a plasmid expressing CAT via the myelin basic protein promoter (pMBP-CAT) to generate runoff transcripts of 356 and 298 nucleotides, respectively. Transcription reaction mixtures of 15 μl containing 0.1 μg of template DNA, 5 μl of HeLa whole-cell extract, 20 μCi of [α^{32}P]UTP (400 Ci/mmol; Amersham), 1.3 mM ATP, 1.3 mM CTP, 1.3 mM GTP, and 33 μg of poly(dI-dC) per ml were carried out in a buffer containing 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 7.9), 100 mM KCl, 12.5 mM $MgCl_2$, 0.1 mM EDTA, 2 mM dithiothreitol, and 17% glycerol (vol/vol). Runoff transcripts were analyzed on a denaturing 6% polyacrylamide gel and detected by autoradiography.

DNA sequence of endogenous p53 in ACH-2 cells. Total

cellular RNA was purified from ACH-2 and U1 cells by a single-step isolation technique (2). Five micrograms of purified total cellular RNA were reverse transcribed with antisense p53 oligonucleotides 2, 4, 6, and 8 (see the sequences below) and with the avian myeloblastosis virus reverse transcriptase (RT) enzyme (Bethesda Research Laboratories) in a 10-μl reaction mixture at 37°C for 1 h. Two and one-half microliters of the RT mixture was amplified by PCR, with each of the four p53 primer pairs from the Novagen-p54 cDNA assay system: Oligo-1, 5′-ATGGAGGAGC CGCAGTCAGATCC-3′, and Oligo-2, 5′-CCCAGAATGCAA GAAGCCCAGACG-3′; Oligo-3, 5′-CTGTCATCTTCTGTC CCTTCCCA-3′, and Oligo-4, 5′-TCTGTCATCCAAATACT CCACACG-3′; Oligo-5, 5′-CTGGCCCCTCCTCAGCATCTT AT-3′, and Oligo-6, 5′-CTCGTGGTGAGGCTCCCCTTTC TT-3′; and Oligo-7, 5′-GAGAATCTCCGCAAGAAAGGGG A-3′, and Oligo-8, 5′-TCAGTCTGAGTCAGGCCCTTCTG T-3′. The PCR amplification cycle consisted of 1.5 min at 94°C, 50°C for 1.5 min, and 72°C for 2 min. After 35 cycles, an additional 10 min at 72°C for the final extension reaction was added. As a control, cDNA for human β-actin was amplified in each reaction by using specific sense and antisense oligonucleotides (Genemad, Inc.): BAC101, 5′-ATGGATGATGATATCG CCGCG-3′, and BAC102, 5′-CTAGAAGCATTGCGGTCGAC GATGGAGGGGCC-3′. This PCR yielded a 1,120 bp amplified β-actin cDNA product. Ten microliters of each PCR mixture was separated, postamplification, on a 1.2% agarose gel. Each p53 cDNA PCR product was also cloned into a pT7 Blue (R) vector, and DNA sequencing was performed with T7 and U19 primers (Novagen, Inc.) (69) per the manufacturer's suggested procedure.

Production of MLV retroviral vectors and transduction into U1 and ACH-2 cell lines. Retroviral vector constructs, pLXp53-143, pLXp53-273, pLX-Nd-p53, pLX-CAT, and pLX-β-GAL, were transfected into the amphotropic packaging cell line, PA317, by a calcium phosphate precipitation method (20 μg of plasmid per 5×10^5 cells) as previously described (40). Stable packaging cell lines were produced by G418 selection, and the titers of MLV vector virions were measured on NIH/3T3 cells, as previously described (40). Typically, supernatants from each of the packaging cell lines demonstrated 2×10^5 CFU/ml. U1 and ACH-2 cells (1×10^6) were superinfected with 10 ml of packaging-cell supernatant (2×10^5 CFU/ml), including Polybrene at a final concentration of 4 μg/ml. Supernatants from transduced cells were collected for HIV-1 p24 antigen assays, and pelleted cells were used for nucleus extraction for the evaluation of activated nuclear factor kappa B (NF-κB) levels. pLX-CAT and pLX-β-GAL expression controls were performed, as previously described (13). p53 expression in transduced U1 cells was also confirmed by indirect immunostaining with a murine monoclonal antibody to p53 (AB-1; Oncogene, Inc.) (39).

Assay of activated NF-κB expression after transduction of U1 and ACH-2 cells. Forty-eight hours after superinfection of U1 and ACH-2 cells with various MLV-derived retroviral virions, nuclei were extracted and electrophoretic mobility shift assays (EMSAs) were performed to assess the activation of NF-κB, as previously described (43). Eight micrograms of total nuclear protein was used in each EMSA reaction. The probe consisted of a ^{32}P-labelled oligonucleotide containing a single NF-κB binding site, and unlabelled wild-type and mutant competitor oligonucleotides were added to certain reaction mixtures (43).

HIV-1 p24 antigen assays. Forty-eight hours after transduction of different MLV-derived virions into U1 and ACH-2 cells, the HIV-1 p24 antigen levels were measured in the supernatants of these cell populations by a sensitive enzyme-linked immunosorbent assay (ELISA; Dupont, Inc., Wilmington, Del.).

RESULTS

Inhibition of HIV-1 LTR promoter activity by wt-p53. The 5′ HIV-1 LTR is a key regulatory sequence in the HIV-1 proviral genome and controls viral transcription. HIV-1 LTR-directed transcription is not only affected by its integrated flanking sites' DNA sequences but is also regulated by viral regulatory proteins such as Tat and nonviral factors such as NF-κB and Sp1 proteins (18, 29). Thus, activation of the latent provirus can be initiated by cellular factors. Notably, NF-κB has been demonstrated to be activated by various exogenous stimuli, including cytokines such as tumor necrosis factor alpha (TNF-α) (18). We and others have previously observed that HBX protein can stimulate HIV-1 replication through NF-κB-dependent and -independent mechanisms (reference 59 and unpublished results), and the HBX protein has been shown to form in vitro and in vivo complexes with cellular p53 protein (17). In order to understand those complex interactions, it is important to further elucidate the functions of p53 in the regulation of HIV-1 LTR-directed transcription. To examine the role of wt-p53 in HIV-1 LTR-directed transcriptional regulation, full-length HIV-1 LTR-CAT plasmids were cotransfected with different quantities of the p53 expression vector, pCMV-wt-p53, in Hep3B cells, which completely delete endogenous p53 gene expression (17, 45). It is critical to investigate p53-induced effects on HIV-1 LTR-directed transcription in cells which do not express wild-type or mutant endogenous p53, since background p53 moieties might confound the analyses of these molecular interactions. As demonstrated in Fig. 2A, the HIV-1 LTR is clearly suppressed by overexpression of the wt-p53 protein. Importantly though, relatively high levels of wt-p53 were necessary to fully inhibit the full-length HIV-1 LTR (Fig. 2A). Notably, the basal level of percent conversions of chloramphenicol in these CAT assays was always maintained between 15 and 30%, within the linear range of CAT activity. Therefore, our interpretation of the effects of p53 is based on changes of only strong signals in the CAT assays (not illustrated).

It has been recently demonstrated that wt-p53 function is involved in different signal transduction pathways and is dependent upon phosphorylation of the wt-p53 protein (38). As noted above, Hep3B cells lack endogenous wt-p53 expression, but by evaluating cotransfection of pG13-CAT, which contains wt-p53 DNA binding sites, with the pCMV-wt-p53 plasmid in these cells, it is demonstrated that Hep3B cells can still support wt-p53 function (Fig. 2B). Thus, the pG13-CAT-specific response to wt-p53 expression in Hep3B cells indicates that inhibition of HIV-1 LTR activity by wt-p53, in our transfection system, is not due to nonspecific cytotoxic effects from overexpression of wt-p53.

It has been demonstrated that promoters, which carry either a TATA box or initiator elements, have differing responses to wt-p53 protein (34). To compare the HIV-1 LTR activity with a known p53-sensitive promoter, such as the SV40 early basic promoter, pSV-β-Gal and full-length HIV-1 LTR-CAT plasmids were cotransfected with different quantities of the pCMV-wt-p53 plasmid into Hep3B cells. As shown in Fig. 3, the full-length HIV-1 LTR revealed more resistance to p53-induced inhibition than the SV40 promoter.

The profound complexity of the HIV-1 LTR has been demonstrated by several upstream sequence elements, which are involved with the regulation of HIV-1 LTR activity (18). In order to further identify which motifs are the major regions

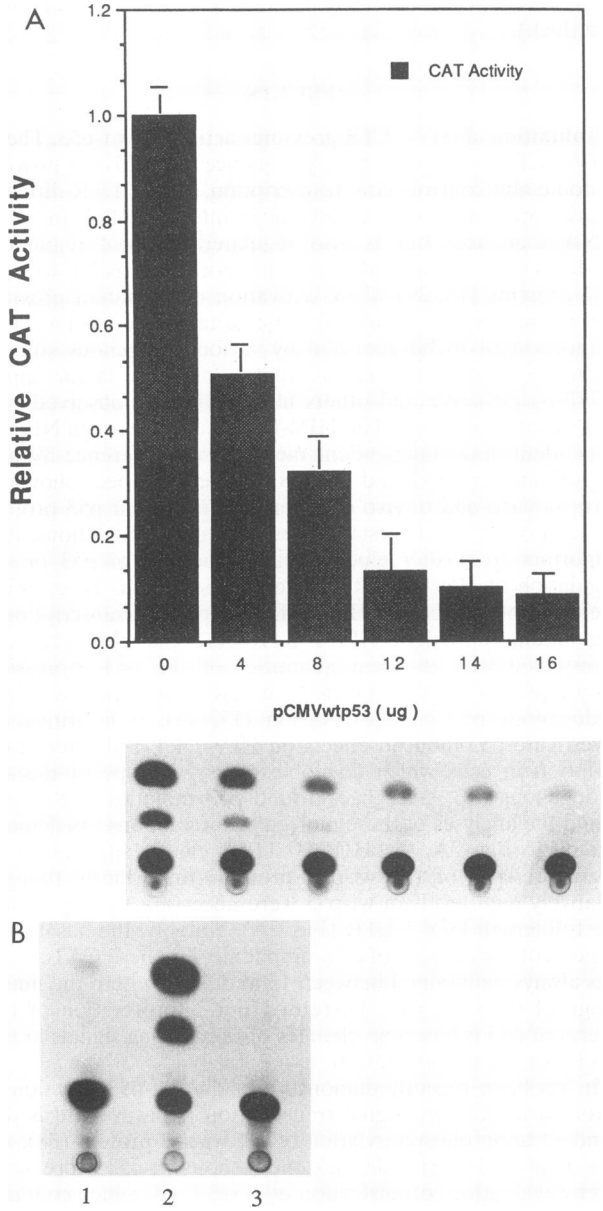

FIG. 2. Inhibition of HIV-1 LTR-directed CAT expression by wt-p53. (A) Increasing quantities of the wt-p53 expression vector (pCMV-wt-p53) cotransfected with the full-length HIV-1 LTR-CAT plasmid (5 μg) in Hep3B cells. These data are representative of at least three independent experiments performed in duplicate (± standard deviations). CAT activity is expressed as fold multiples of the baseline level. (B) Transfection of a plasmid containing wt-p53 binding sites driving CAT gene expression (pG13-CAT) with the wt-p53-expressing plasmid (pCMV-wt-p53) in Hep3B cells. Lane 1, pG13-CAT alone (5 μg); lane 2, pG13-CAT (5 μg) plus pCMV-wt-p53 (5 μg); lane 3, pG13-CAT (5 μg) plus pCMV (5 μg of plasmid backbone lacking the wt-p53 gene). This figure is representative of at least two experiments performed in duplicate.

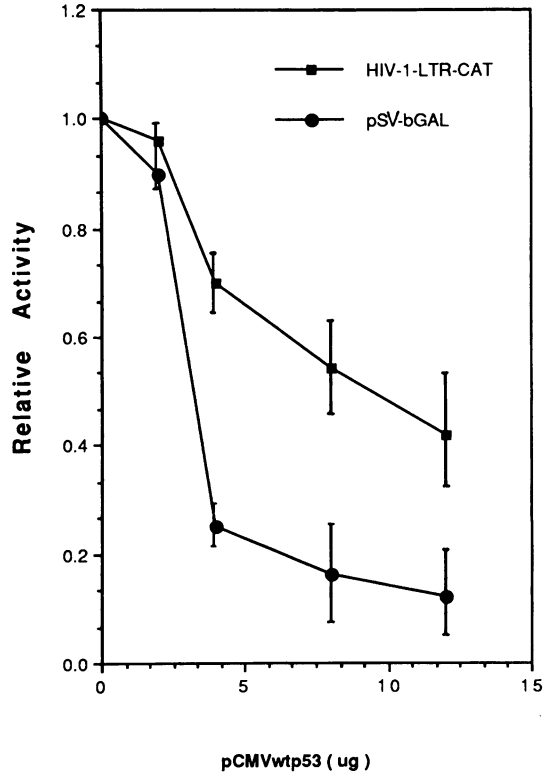

FIG. 3. Cotransfections of various quantities of the wt-p53-expressing plasmid with either the full-length HIV-1 LTR-CAT construct or an SV40-driven β-gal construct (pSV-β-Gal) in Hep3B cells. Various quantities of pCMV-wt-p53 (0 to 12 μg) were cotransfected into Hep3B cells with either HIV-1 LTR-CAT (5 μg) or pSV-β-Gal (5 μg), and CAT or β-gal activities were measured 48 h posttransfection. Total levels of transfected plasmids were normalized (20 μg) by using the pCMV plasmid backbone lacking the wt-p53 gene. This figure is illustrative of at least two independent experiments performed in duplicate (± standard deviations).

which interact with wt-p53 and which elements may convert the HIV-1 (TATA box-directed) promoter towards relative resistance to wt-p53 in these transfection CAT assays, we created several different 5′ deletions of the HIV-1 LTR (Fig. 1). These deletion HIV-1 LTR-CAT plasmids were then cotransfected with pCMV-wt-p53 into Hep3B cells. As shown in Fig. 4A and B, 5′ deletion of the HIV-1 LTR sequence downstream of the second NF-κB site, leaving only the Sp1 sites and TATA box intact, allowed the HIV-1 LTR to become extremely sensitive to wt-p53-induced inhibition. These data demonstrated that wt-p53 may alter the interactions with transcription factors, which are mainly involved in either, or both, the Sp1 or TATA box regions of the HIV-1 LTR. Interestingly, if two NF-κB sites are maintained on the HIV-1 LTR, the HIV-1 LTR activity can be restored to a full-length HIV-1 LTR activity level in terms of its relative resistance to wt-p53 inhibition in transfection assays (Fig. 4). Notably, the transfections illustrated in Fig. 4A utilized relatively low quantities of pCMV-wt-p53, to illustrate the increased sensitivity of the HIV-1 LTR to p53-induced inhibition upon deletion of the NF-κB binding sites. The quantity of wt-p53-expressing plasmid necessary to inhibit the CAT activity by 95% in the NF-κB-deleted HIV-1 LTR-CAT construct was utilized in these studies. This allowed precise evaluation of the sensitivity of the other HIV-1 LTR constructs to wt-p53 expression (Fig. 4A). Notably, TAR-deleted constructs were utilized in several of these studies, since the basal level of CAT expression was higher than that in TAR-positive constructs. Although basal levels of CAT activity were relatively low (5 to 10% conversion) in Hep3B cells with TAR-positive constructs, the same trend of greater wt-p53-

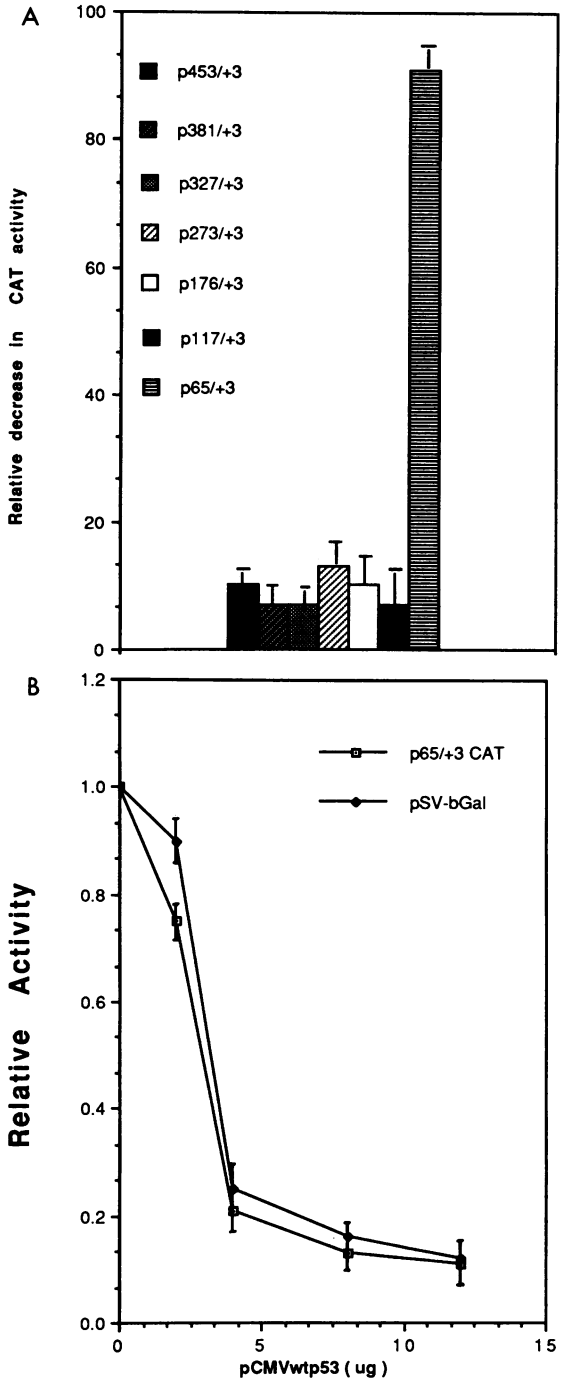

FIG. 4. Deletion mutants of the HIV-1 LTR, driving CAT gene expression, cotransfected with the wt-p53-expressing vector (pCMV-wt-p53) in Hep3B cells. (A) Various deletion mutants of the HIV-1 LTR driving CAT expression (8 μg) were cotransfected with pCMV-wt-p53 (4 μg) into Hep3B cells, and CAT activities were measured 48 h posttransfection. This figure is representative of three independent experiments performed in duplicate (± standard deviations). The *y* axis represents the fold decrease of the percent conversion decrease. The decrease in CAT activity with wt-p53 cotransfections with each HIV-1 LTR construct was compared with the particular HIV-1 LTR-CAT construct's basal CAT activity without wt-p53. (B) Cotransfections of various quantities of pCMV-wt-p53 with either an HIV-1 LTR-CAT construct deleted after the downstream NF-κB binding site or an SV40-driven β-gal construct (pSV-β-Gal) in Hep3B cells. Various quantities of pCMV-wt-p53 (0 to 12 μg) were cotransfected into Hep3B cells with either the HIV-1 LTR-CAT construct deleted just downstream of the NF-κB binding sites (5 μg) or pSV-β-Gal (5 μg), and CAT or β-gal activities were measured 48 h posttransfection. Total levels of transfected plasmids (20 μg) were normalized by using the pCMV backbone without wt-p53. This figure illustrates data from two independent experiments performed in duplicate (± standard deviations).

induced transcriptional expression in LTR constructs with a deletion just upstream of the Sp1 sites was noted (not illustrated).

Effects of mutant p53 proteins on the HIV-1 LTR. Although the interference of certain mutant p53 proteins with wt-p53 function through *trans*-dominant negative inhibition has been described, several groups have reported that certain mutant p53 proteins themselves can directly transactivate cellular promoters, without background wt-p53 (46). To examine mechanisms by which mutant p53 proteins may affect HIV-1 LTR activity in these transfection assays, the Hep3B cell line, which completely deletes endogenous p53, and the HepG2 cell line, which has endogenous wt-p53 expression, were utilized (45). Two mutant p53 expression plasmids, pC53-SCX3 (mutation at amino acid 143) and pC53-237, were cotransfected with the full-length HIV-1 LTR-CAT plasmid and plasmids containing the HIV-1 LTR-CAT with various LTR deletions demonstrated in Fig. 1. In Hep3B cells, these two mutant p53 proteins did not significantly affect HIV-1 LTR-CAT activity in this transfection assay system (Fig. 5). Notably, only mildly enhanced HIV-1 LTR-CAT activities of one-half- to twofold increases in various independent experiments were observed in the HepG2 cell line. Thus, very modest alterations in HIV-1 LTR-directed activities by these mutant p53 proteins were only demonstrable in cells harboring endogenous wt-p53.

Tat can increase the resistance of the HIV-1 LTR to wt-p53-induced inhibition. As a viral regulatory protein, the HIV-1 Tat protein can dramatically alter HIV-1 LTR activity through the Tat-binding sequence, TAR (51). To compare the degrees of p53-induced inhibition of the HIV-1 LTR in the presence or absence of Tat protein expression, cotransfections of various HIV-1 LTR-CAT plasmids, containing TAR, with the Tat expression plasmid and the wt-p53 expression plasmid were performed. As the data presented in Fig. 6 (lanes 1 and 2) indicate, in the presence of the TAR sequence, Tat expression can enhance resistance of the HIV-1 LTR to wt-p53 inhibition in this transfection assay system. Nevertheless, when the NF-κB binding sites are deleted from the HIV-1 LTR, Tat cannot potently alter the p53-induced inhibitory effects on expression from this LTR construct (Fig. 6, lanes 3 and 4). When the TAR-deleted LTR plasmids were used, Tat had no effect on CAT expression in Hep3B cells (not illustrated). The data from these experiments indicated that the binding of the HIV-1 Tat protein to the TAR sequence could alter the inhibitory effects of wt-p53 in the regulation of HIV-1 transcription. The argument for an effect on Tat-Sp1 interactions by wt-p53 may be supported by these data (5). Nevertheless, the interactions of Tat with wt-p53-induced effects on the HIV-1 LTR may also be due to competition between Tat and wt-p53 binding to TF IID (8).

***E. coli*-derived wt-p53 can down-regulate HIV-1 LTR-directed transcription in vitro.** In order to directly demonstrate the interactions between wt-p53 protein and HIV-1 LTR-directed transcription, wt-p53 was expressed and purified from *E. coli*. The renatured soluble fraction was directly tested in vitro for its ability to inhibit HIV-1 transcription. As shown in

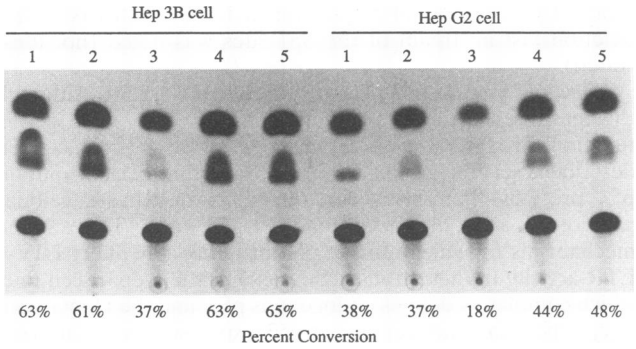

FIG. 5. Effects of mutant p53 proteins on HIV-1 LTR-directed CAT expression in Hep3B and HepG2 cells. Five micrograms of the full-length HIV-1 LTR-CAT construct was cotransfected with p53-mutant-expressing plasmids (5 μg) or the wt-p53-expressing plasmid (5 μg) into Hep3B and HepG2 cells. The pCMV backbone without p53 genes was utilized in controls. Lanes 1, HIV-1 LTR-CAT alone (5 μg); lanes 2, pCMV plasmid backbone, without wild-type or mutant p53; lanes 3, pCMV-wt-p53; lanes 4, pCMV-p53-143; lanes 5, pCMV-p53-273. This figure is representative of three independent studies performed in duplicate.

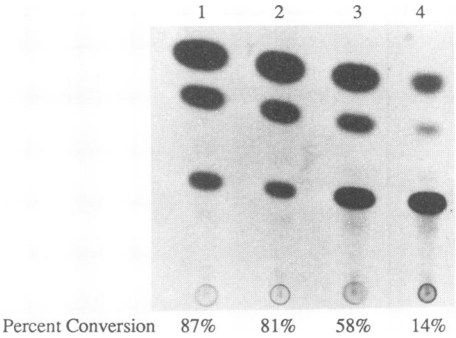

FIG. 6. HIV-1 Tat decreases the inhibitory effects of wt-p53 on HIV-1 LTR-directed expression. Hep3B cells were cotransfected with HIV-1 LTR-CAT constructs which maintain the NF-κB binding sites (pHIV−117/+80) (5 μg) or HIV-1 LTR-CAT plasmids deleted just downstream of the second NF-κB binding site in the HIV-1 LTR (pHIV−65/+80) and a Tat-expressing plasmid (pActin-Tat; 1 μg). pCMV-wt-p53 (4 μg) was also cotransfected in certain experiments. The total plasmid quantity (20 μg) utilized in each experiment was normalized with the pCMV backbone. Lane 1, pHIV−117/+80 plus pActin-Tat; lane 2, pHIV−117/+80 plus pActin-Tat plus pCMV-wt-p53; lane 3, pHIV−65/+80 plus pActin-Tat; lane 4, pHIV−65/+80 plus pActin-Tat plus pCMV-wt-p53. This figure illustrates data from three independent experiments performed in duplicate. No appreciable differences in CAT activities, between transfections demonstrated in lanes 1 and 2, were noted with reduced extract concentrations in assays performed with lower percent conversions of chloramphenicol (not illustrated).

Fig. 7A (lanes 1, 2, and 3), this *E. coli*-derived wt-p53 protein specifically inhibited HIV-1 LTR-directed transcription in a dose-dependent manner. When the *E. coli*-derived p53-binding protein, HBX, was added to the transcription reaction mixture, the inhibition of wt-p53 on HIV-1 transcription was specifically reversed (Fig. 7A, lanes 4 and 5). In parallel, as a control for promoter specificity, transcriptional activity of the human myelin basic protein (MBP) promoter was examined under similar conditions. Purified p53 protein showed no inhibitory effect on the MBP promoter (Fig. 7B). This control demonstrates that the ability of p53 to reduce transcription may not be a general effect on all polymerase II transcription units. These data strongly demonstrate the direct effects of wt-p53 on transcription directed by the HIV-1 LTR. Also, these studies confirm findings of our previous work (17), that the HBX protein can bind to p53 and alter p53 function.

Sequence of endogenous p53 in ACH-2 and U1 cells. Although the HIV-1 LTR activity was affected by wt-p53 expression in the transfection assays, it was demonstrated (see the description above) that the most sensitive HIV-1 LTR elements involved with wt-p53 inhibition were near the Sp1 and TATA box regions. Addition of the NF-κB binding sites was shown to interfere with the p53 inhibitory function. Those data indicated that wt-p53 may alter basal level transcription of the HIV-1 LTR mainly in a state in which only low levels of active NF-κB exist (i.e., proviral latent state). In order to develop a system for studying p53 function in the HIV-1 latent state, the endogenous p53 moieties for several common cell lines which are often used to evaluate HIV-1 replication must be analyzed. Total cellular RNAs were extracted from U1, ACH-2, and HepG2 cells for RT-PCR, and Northern (RNA) blotting with a p53 cDNA probe. HepG2 cells contain wt-p53 mRNA and were used as positive controls (Fig. 8, lane 1). In these experiments, it was revealed that U1 cells harbor no endogenous p53 mRNA (Fig. 8, lanes 2). ACH-2 cells were shown to have a normal pattern of p53 mRNA on Northern blots, but [^{35}S]methionine labelling-chase experiments for p53 in ACH-2 cells demonstrated a slightly longer half-life than for wt-p53 (data not shown). After RT-PCR amplification of full-length p53 cDNA from ACH-2 cells (Fig. 8, lanes 3), this p53 moiety was confirmed, by DNA sequencing, to be a mutant protein with a change at amino acid 248 of wt-p53 from CGG to CAG (Arg to Gln).

Superinfection of U1 and ACH-2 cells with retroviral vectors expressing mutant forms of p53 leads to dramatic increases in HIV-1 expression in U1 but not ACH-2 cells. HIV-1 latently infected U1 and ACH-2 cells have been utilized frequently to study HIV-1 control mechanisms (16, 43, 44). Importantly, transfection procedures nonspecifically activate HIV-1 replication in these lines (13, 43). Hence, retroviral shuttle vectors must be used to introduce exogenous proteins into U1 and ACH-2 cells (13). The p53 DNA sequence data, described above, demonstrate that these two cell lines also provide us with model systems to study p53 functions in different endogenous p53 backgrounds. It is instructive to evaluate how mutant p53 proteins affect basal levels of viral transcription activity in these two profoundly different cell lines. Transductions of two mutant p53 MLV virions, pLXp53-143 and pLXp53-273, into U1 cells were performed. As shown in Fig. 9, both mutant p53 moieties stimulated HIV-1 replication more than 1,000-fold, but transduction of those two mutant p53 MLV virions into ACH-2 cells did not alter HIV-1 replication (data not illustrated). The fact that p53 N-terminal deletion-mutant MLV virions (pLX-Nd-p53) did not stimulate U1 cells demonstrates that the p53 N-terminal acidic transactivation domain is important for function (Fig. 9). Notably, β-gal-expressing and CAT-expressing retroviral vectors were used as negative controls and to evaluate transduction efficiencies in U1 and ACH-2 cells (Fig. 9). Staining for β-gal expression demonstrated similar levels (60 to 65%) in both U1 and ACH-2 cells, and CAT levels were similar in both cell lines (not illustrated). As noted above, mutant p53 moieties did not alter HIV-1 replication in ACH-2 cells. It is difficult to distinguish whether this is because the endogenous mutant p53 already maintains a basal level of viral transcription at a specifically higher level in ACH-2 cells or because of the

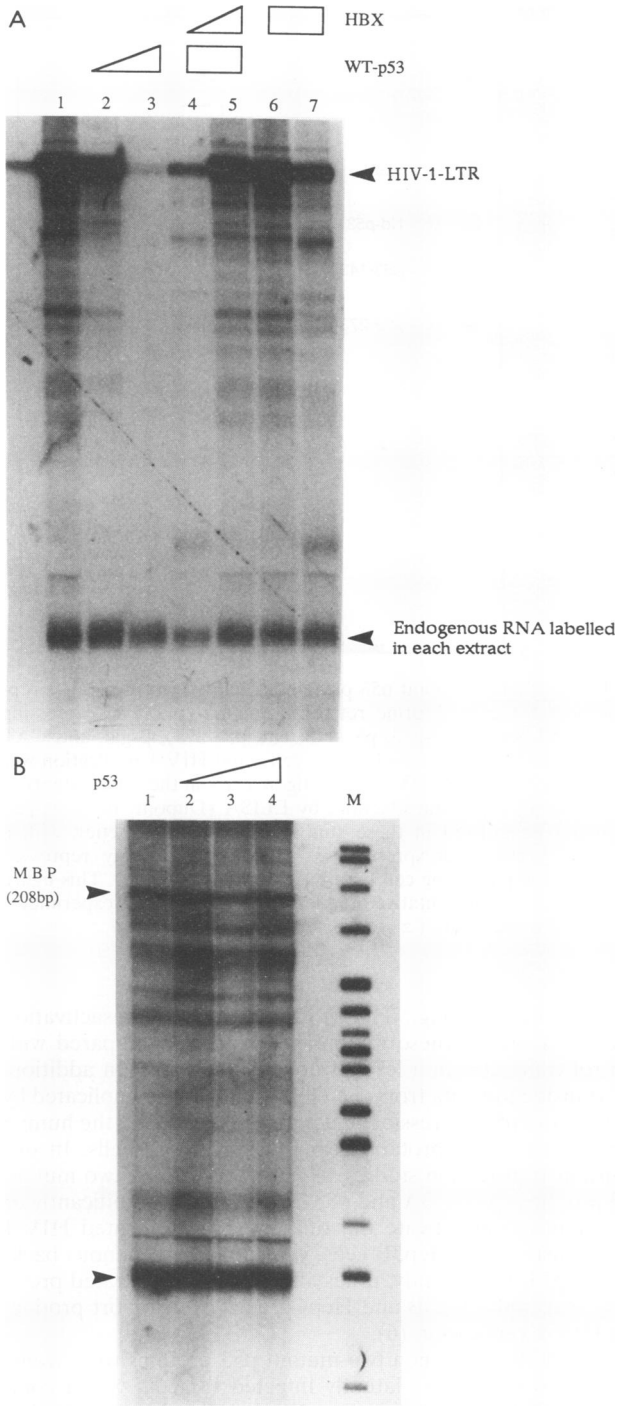

FIG. 7. Effects of wt-p53 protein on in vitro HIV-1 LTR-directed transcription. (A) Various quantities of *E. coli*-derived p53 protein were added to HIV-1 LTR in vitro transcription assays, and the HIV-1 LTR-directed [^{32}P]labelled transcripts were assessed by gel electrophoresis, as described in Materials and Methods. In addition, increasing quantities of *E. coli*-derived HBX were added in certain experiments. Linearized HIV-1 LTR template DNA (100 ng) was transcribed in cellular extract alone (lane 1) or was preincubated with 1 (lane 2) and 3 (lane 3) μg of bacterially expressed p53, 3 μg of p53 plus 1 (lane 4) and 2 (lane 5) μg of purified HBX protein, or 1 (lane 6) and 2 (lane 7) μg of HBX protein alone. The LTR-directed transcripts and the endogenous cellular RNA labelled in each extract are indicated. This autoradiograph is representative of at least three independent experiments. (B) The MBP promoter-driven construct (pMBP-CAT) (100 ng) was transcribed in cell extract (lane 1) or was preincubated with 1, 3, and 6 μg of purified p53 (lanes 2 to 4, respectively.) A 208-bp product was expressed by the MBP promoter (upper arrowhead). The lower arrowhead designates the endogenous cellular RNA labelled in these extracts. Lane M, standard markers.

differing proximal control mechanisms of restricted replication between U1 and ACH-2 cells, as has been previously demonstrated (7, 13, 66). Unfortunately, wt-p53 could not be evaluated in these cells because transduction of retroviral packaging cells with a vector expressing wt-p53 led to dramatic decreases in cellular proliferation (not illustrated).

Transduction of retroviral vectors expressing mutant p53 proteins into U1 cells does not activate NF-κB expression. Any alterations of NF-κB in either U1 or ACH-2 cells (such as by phorbol esters or TNF-α) can dramatically increase HIV-1 replication through increases in nuclear NF-κB activity (43). In order to rule out the possibility that retrovirally transduced mutant p53 proteins in U1 cells increased HIV-1 replication by nonspecific increases in NF-κB activity, EMSAs were performed, as previously described (43), after U1 cells were transduced with MLV mutant p53 virions. As shown in Fig. 10, mutant p53 stimulation of HIV-1 replication in U1 cells is through an NF-κB-independent pathway, as NF-κB was not activated by retroviral transduction of U1 cells with these proteins.

DISCUSSION

There is a growing body of evidence that the diversity of responses to HIV-1 during infection is due to a plethora of factors contributing to the pathogenesis of the disease states (16, 48). The predominant cellular targets for HIV-1 are the CD4-positive T lymphocytes and mononuclear phagocytes, such as blood monocytes and tissue-bound macrophages (25, 48). Since HIV-1 specifically targets CD4-positive cells, there is a gradual alteration in the host's immune defense system in the later stages of the disease (48). The development of human neoplasia in HIV-1-infected patients is also common in the later stages of infection (16, 48). In addition, it is a common feature of HIV-1-infected patients to have coinfection with other viruses such as Epstein-Barr virus, hepatitis B virus, human cytomegalovirus, human papillomavirus, and others (48). Proteins from Epstein-Barr virus, hepatitis B virus, and human papillomavirus have been shown to form complexes with cellular p53 proteins, such as HBX of hepatitis B virus (17), E6 of human papillomavirus (62), and EBNA-5 of Epstein-Barr virus (56). Notably, the inactivation of wt-p53 and mutations of p53 are also the most common mutational events in neoplastic cells (26).

HIV-1 is dependent on its host cell for the synthesis and processing of viral RNA. Steady-state levels of viral RNA can be regulated by several mechanisms, including the rate of transcription initiation, elongation, processing, export, and packaging. Various regulatory mechanisms have, therefore, evolved to control transcription of viral RNA during HIV-1 replication. These factors play a critical role in the regulation of viral replication. *cis*-acting regulatory elements control retroviral gene expression and are thought to reside in the 5′ LTR of the virus, particularly within the U3 region. A variety of different cellular transcription factors appear to contribute to the constitutive expression of HIV-1 (18). Among these factors, Sp1 appears to play an important role, and deletion of the three Sp1 sites reduces enhancer activity by approximately

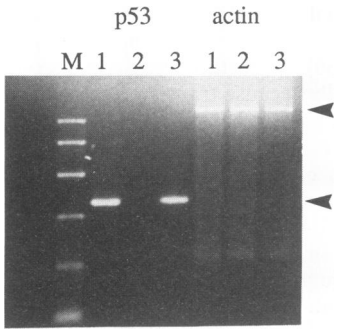

FIG. 8. RT-PCR for endogenous p53 mRNA in U1 and ACH-2 cells. Total cellular RNA was isolated from U1, ACH-2, and HepG2 cells, and RT-PCR was performed with four pairs of p53-specific oligonucleotides (Novagen, Inc.) (see Materials and Methods). With oligonucleotide primers 1 and 2 for p53 cDNA, a 351-bp product would be generated by PCR, as shown here. Lanes 1, HepG2 cells; lanes 2, U1 cells; lanes 3, ACH-2 cells; lane M, standard markers. Notably, no specific bands for p53 cDNA were detected by RT-PCR using any of the other three primer pairs for p53 cDNA, in U1 cells (not illustrated). The β-actin (upper arrowhead) and p53 (lower arrowhead) cDNA bands are indicated. This figure is representative of three independent experiments.

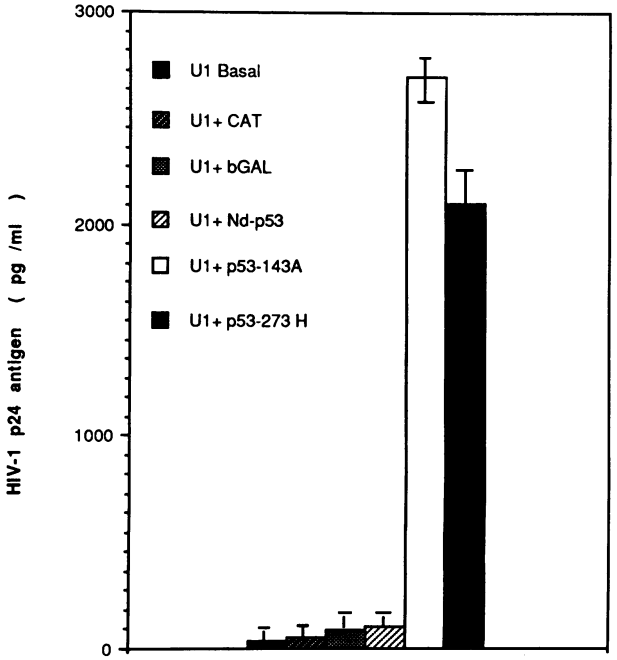

FIG. 9. Certain mutant p53 proteins potently increase HIV-1 replication in U1 cells. Murine retroviral vectors (pLXSN) expressing mutant p53 genes (Nd-p53, p53-143, and p53-273), β-gal, and CAT genes were utilized to transduce U1 cells, and HIV-1 replication was assessed by measuring HIV-1 p24 antigen levels in the supernatants of these cells 48 h posttransduction, by ELISA (Dupont, Inc.). wt-p53 could not be utilized in these studies because transduction with a retroviral vector overexpressing wt-p53 led to severely repressed growth of the packaging cell line, PA317 (not illustrated). This figure illustrates data representative of at least two independent experiments performed in duplicate (± standard deviations).

5- to 10-fold (27). More recently, the possibility has been raised that Sp1 is required not only for basal transcription but also for inducible gene expression through interactions with NF-κB (4). Several other *cis*-acting regulatory elements are found near the TATA box and the transcription initiation site. Binding to the promoter by the TATA factor TF IID is not absolutely required for NF-κB-mediated inducibility of the HIV-1 enhancer, but mutation of the TATA box results in a substantial loss of basal activity and a marked reduction in Tat-induced transactivation (4). Another important function of the TATA element is its role in facilitating the assembly of other members of the transcription initiation complex. An essential component in this process is the site of transcription initiation. Several studies have suggested that a specific DNA-binding protein is required for efficient transcription initiation at this position (52).

Several groups recently reported an inhibitory activity of wt-p53 on different cellular and viral promoters. Chin et al. demonstrated that wt-p53 inhibited the MDR1 gene but a mutant (at amino acid H-175) significantly activated this gene (9). Subler et al. have shown that various mutant p53 proteins may functionally interact with the transcription factor ATE/CREB, resulting in an increase of PCNA promoter activity (54). This suggests that wt-p53 is an inhibitor, whereas certain mutant p53 proteins are activators, of gene expression. The results described in our study demonstrate that overexpression of human wt-p53 can, as expected, exert an inhibitory effect on the HIV-1 LTR in CAT transfection assays. The inhibitory effects of wt-p53 on HIV-1 LTR-directed gene expression were also dramatically demonstrated in an in vitro HIV-1 LTR transcription assay system. Importantly, mutations of p53 have very modest transactivator function in the transfection assays, in cells which have background wt-p53, such as HepG2 cells, but not in the Hep3B cell line, which completely deletes endogenous p53 gene expression.

A recent report by Subler et al. (55) has demonstrated, by transient transfection assays, that certain mutant p53 proteins may augment HIV-1 LTR-directed transcription in cells which lack endogenous p53 (e.g., Saos-2) and in cells which express endogenous p53 (e.g., HeLa). Notably, the transactivation levels of many of these mutant p53 moieties compared with control transactivation levels were relatively low. In addition, any transfection data from the HeLa cell line is complicated by background p53 expression and p53 complexes with the human papillomavirus E6 protein, also found in these cells. In our transient transfection studies, as noted above, the two mutant p53 proteins (p53-143A and p53-273H) did not significantly or consistently transactivate the full-length or truncated HIV-1 LTR constructs in Hep3B cells, which lack endogenous background p53. Importantly, it has also been demonstrated previously that both Hep3B and HepG2 cells can support productive HIV-1 replication (6).

Notably though, when two mutant p53 proteins were transduced into the HIV-1 latently infected U1 cell, which completely deletes p53 gene expression, via an MLV retroviral vector system, a potent increase in HIV-1 replication was observed. These data strongly suggest that mutant p53 alone can directly activate HIV-1 LTR activity in certain cellular milieus without interactions with wt-p53. Thus, these data further dramatically demonstrate that the effects of cofactors on HIV-1 transcription may differ when assayed in transient subgenomic transfections or when the full viral genome is used. These observations also indicate that with U1 cells lacking endogenous p53 background, the U1 cell line may be a good model system for the study of the interactions of p53 in the regulation of HIV-1 replication.

A recent study from A. Haase's laboratory demonstrated

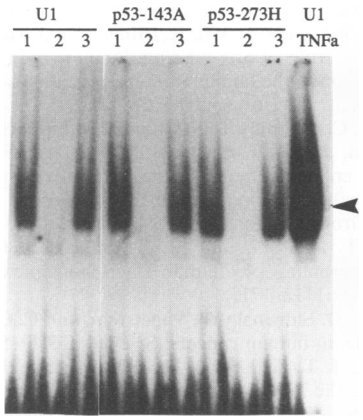

FIG. 10. EMSAs for NF-κB in U1 cells transduced with retroviral vectors expressing mutant p53 genes. Nuclear extracts were obtained 48 h posttransduction of U1 cells with retroviral vectors expressing mutant p53 genes, as well as from nontransduced U1 cells, and assessed for activated NF-κB by EMSA (see Materials and Methods). As a positive control, U1 cells stimulated with TNF-α (100 U/ml for 48 h) were used. Lanes 1, U1 nuclear extracts; lanes 2, U1 nuclear extracts with unlabelled wild-type oligonucleotide competitor (50 ng); lanes 3, U1 nuclear extracts with unlabelled mutant competitor (50 ng). The activated NF-κB-specific bands (arrowhead) are indicated. This autoradiograph is illustrative of two independent experiments.

remarkably high levels of HIV-1 replication in metastatic colonic carcinoma cells, in an HIV-1-infected individual (15). The data from the present studies suggest that mutations in p53, found in the majority of colonic carcinomas (26), may account for the dramatically high levels of HIV-1-specific RNA demonstrated in these carcinoma cells in vivo compared with viral RNA levels in most monocytes and T lymphocytes within infected individuals.

In cotransfection experiments, it was demonstrated that the full-length HIV-1 LTR was not as sensitive to wt-p53 inhibition as other TATA box-associated promoters. As reported by Mack et al., TATA box promoters were, as a class, sensitive to wt-p53-induced inhibition (34). In the experiments using different 5′ deletions of the HIV-1 LTR, cotransfections with pCMV-wt-p53 clearly demonstrated that the basic Sp1-TATA element of HIV-1 LTR is very sensitive to wt-p53 inhibition. This completely agrees with the reports showing that p53 directly interacts with the TBP and Sp1 factors (8, 58). It is interesting that when the two NF-κB sites are restored to the truncated Sp1-TATA HIV-1 LTR, this promoter became more resistant to wt-p53 inhibition in the transfection assays. These data provide some understanding of NF-κB interactions with TBP and Sp1 factors. NF-κB binding sites appear to make the TATA box promoters significantly more functionally resistant to wt-p53 inhibition, as is noted for the promoters using the initiator element (34). In this manner, when T lymphocytes are stimulated by phorbol esters, mitogens, or cytokines, intracellular increases in activated NF-κB will make the HIV-1 LTR function more like a housekeeping gene promoter, avoiding the inhibition by cellular factors such as wt-p53. As such, the truncated HIV-1 LTR, lacking NF-κB binding sites, may represent the effects of p53 on HIV-1 LTR-directed transcription in nonproliferating cells, since the direct study of such cells in vitro is problematic.

On the basis of the data presented in these studies, a transcriptional regulation model for the HIV-1 LTR can be proposed (Fig. 11). In the resting or quiescent HIV-1-infected

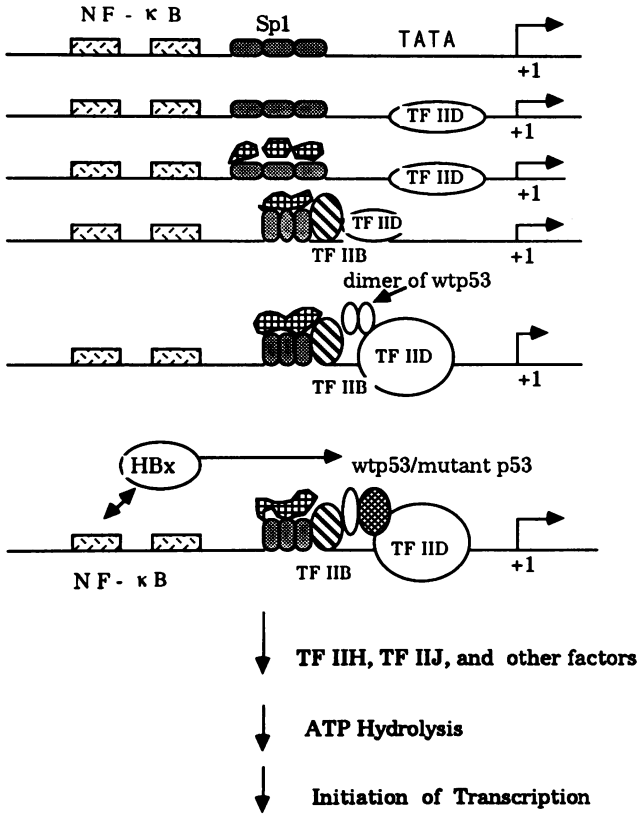

FIG. 11. Schematic diagram of the molecular model for activator-mediated assembly of PICs on the HIV-1 LTR.

cell, low levels of active NF-κB will render HIV-1 LTR-directed expression dependent primarily on the baseline level of assembly of PICs for determination of the basal level of HIV-1 transcription. Thus, the regulation of this basal transcription of HIV-1 may involve the expression of p53. If p53 is involved in this regulation, any change of p53 function will alter the inhibitory balance. Mutant p53 or other viral proteins which bind cellular p53 and inhibit p53 function, such as the HBX protein, may alter HIV-1 transcription (45). Also, when HIV-1 latently infected cells are stimulated, by overexpression of TNF-α or other cytokines, activation of NF-κB will alter the HIV-1 promoter into a housekeeping gene-like promoter model of regulation. NF-κB binding to its cognate binding site motifs will efficiently increase the assembly of general transcription factors on the HIV-1 LTR to form preinitiation complexes. At this "transcription turn-on" stage, wt-p53 would not be a prime regulator of viral expression. HIV-1 will, thus, shift replication from the proviral latent state to an active productive level.

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