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Activation of the Human Immunodeficiency Virus Type 1 Long Terminal Repeat by Transforming Mutants of Human p53

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We have studied the effects of human wild-type and mutant p53s on the long terminal repeat (LTR) promoter of human immunodeficiency virus type 1 (HIV). HeLa cells were cotransfected with a wild-type or mutant p53 expression plasmid and a plasmid containing a chloramphenicol acetyltransferase reporter gene under HIV LTR promoter control. As expected, expression of wild-type p53 inhibited promoter function. Expression of a p53 mutated at any one of the four amino acid positions 175, 248, 273, and 281 correlated with a significant increase of the HIV promoter activity. The HIV LTR was also significantly activated in Saos-2 cells that do not express endogenous p53. This finding suggests a gain-of-transactivation function by mutation of the p53 gene. Cotransfection of wild-type and mutant p53-281G expression plasmids indicated that either the wild type or the mutant was dominant in inhibiting or enhancing promoter activity, respectively, when transfected in excess of the other. Transfection experiments showed transactivation even when the Sp1, NF-κB, and TATA sites in the LTR were individually mutated. Synthetic minimal promoter constructs containing two Sp1 sites or two NF-κB sites or an ATF site are also significantly activated by the mutant p53-281G. Thus, the mutant protein may activate transcription through interaction with either a general transcription factor or a common factor that bridges the basal transcription machinery and the transcription factors Sp1, NF-κB, and ATF.

The gene encoding p53 is highly conserved between vertebrate species and is involved in the regulation of cellular proliferation (34, 39, 42, 88). Wild-type p53 can suppress cell proliferation and oncogene-mediated cell transformation and eliminate the tumorigenic potential of tumor-derived cell lines (2, 12, 16, 23). Mutation of p53 often results in the loss of its tumor suppressor function and has been associated with tumor progression (17, 19, 42, 52, 54, 61).

p53 interacts with the transforming proteins of three separate DNA tumor viruses. Simian virus 40 T antigen (40, 44), adenovirus type 5 E1B (73), and E6 of human papillomavirus (75, 92) all bind to wild-type p53. Recently it has been shown to interact with the hepatitis B virus X protein (20) and EBNA-5 of Epstein-Barr virus (84). Cellular proteins have also been found to be associated with p53. Among these are the heat shock protein hsc70 (31, 64), two protein kinases, $p34^{cdc2}$ and casein kinase II (5, 53, 55, 82), the product of the *mdm-2* oncogene (56, 62), Sp1 (6), CCAAT-binding factor (1), and the TATA-binding protein (TBP) (45, 47, 48, 67, 76, 85). p53 has also been shown to interact with the Wilms' tumor suppressor gene product (46).

Mutant p53 proteins that are transforming or are found in tumor cells have properties that are different from those of the wild-type protein. Wild-type (but not mutant) p53 is a sequence-specific DNA-binding protein (4, 15, 25, 35). Wild-type p53 inhibits simian virus 40 DNA replication in vivo and in vitro, while the transforming mutants do not (7, 24, 27, 89). Wild-type (but not mutant) p53 has been shown to inhibit a number of cellular and viral promoters, including the human immunodeficiency virus type 1 (HIV) long terminal repeat (LTR) (8, 29, 72, 83). Interestingly, mutants of p53 activated the human proliferating cell nuclear antigen (PCNA) and the multiple drug resistance gene (*MDR-1*) promoters significantly (8, 10). Wild-type p53 (but not a mutant) activated a promoter with p53-binding sites both in vivo and in vitro (18, 25, 36, 93). Weintraub et al. (91) first showed that the mouse muscle creatine kinase enhancer can be activated by wild-type p53. Recently Zambetti et al. (93) detailed binding of wild-type p53 to the muscle creatine kinase enhancer region and showed a relationship between p53-mediated activation and p53 binding. Wild-type p53 has also been reported to activate *mdm-2* expression (3) as well as enhance its own promoter activity (11).

Whereas wild-type p53 acts as a tumor suppressor, tumor-derived p53 mutants can cooperate with a mutated *ras* gene to transform cells. These transforming mutants of p53 may gain a function, for example, transactivation of certain promoters (13). Thus, it is important to investigate how versatile this transactivation is and what the potential mechanism could be. Mutated p53 proteins may interfere with wild-type p53 function in a dominant-negative manner (34, 39, 42, 88). Therefore, it needs to be determined whether wild-type or mutant p53 will be dominant in its negative or positive effect on promoter function when both forms are present.

In this communication, we report activation of the HIV LTR by tumor-derived mutants of human p53. Cotransfection experiments demonstrate that inhibition or activation of the HIV LTR promoter is dependent on the relative amounts of wild-type or mutant p53 expression plasmids used. Transactivation by p53 mutants was observed even when the Sp1, NF-κB, and TATA sites in the LTR were individually mutated. Synthetic minimal promoter constructs containing two Sp1 sites, two NF-κB sites, or a single ATF site are significantly activated by the mutant p53-281G. Thus, it is possible that the mutant protein interacts with a general transcription factor or a common factor that bridges the basal transcription machinery and the transcription factor Sp1, NF-κB, or ATF to enhance transcription efficiency.

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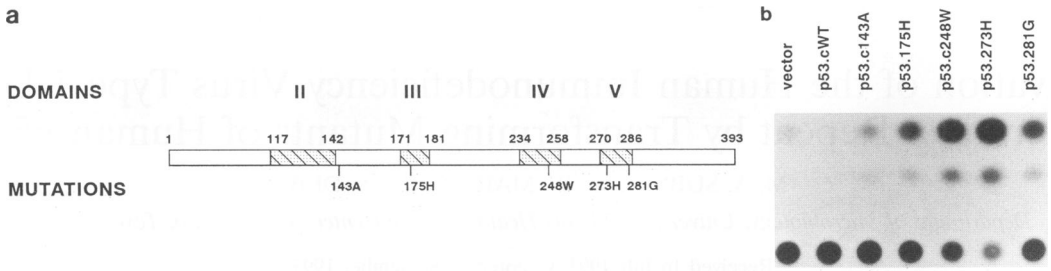

FIG. 1. (a) Schematic representation of the p53 gene product. Conserved domains II to V are indicated by stippled areas. Positions of amino acid substitutions in the mutants that were used in this study are indicated below. (b) Effects of expression of wild-type and mutant human p53s on HIV LTR promoter activity. HeLa cells were cotransfected with HIV-CAT and pCMV-Bam (vector alone) or pCMV-Bam expressing either wild-type p53 (p53.cWT) or one of the mutant p53s (143A [V to A at amino acid 143], 175H [R to H at amino acid 175], 248W [R to W at amino acid 248], 273H [R to H at amino acid 273], and 281G [D to G at amino acid 281]) as described in Materials and Methods.

MATERIALS AND METHODS

DNA plasmids. Wild-type and mutant human p53 expression plasmids use the human cytomegalovirus major immediate-early promoter-enhancer (−671 to +73) from the vector pCMV-Neo-Bam (33). p53-cWT contains a wild-type p53 cDNA, while p53-c143A (Val to Ala at amino acid 143) and p53-c248W (Arg to Trp at amino acid 248) contain mutant p53 cDNAs (33). p53-175H (Arg to His at amino acid 175), p53-273H (Arg to His at amino acid 273), and p53-281G (Asp to Gly at amino acid 281) are mutant p53 cDNA-genomic DNA chimeras; all contain introns 2 through 4 (33). "c" indicates cDNA clones, and capital letters indicate mutant amino acids. The neomycin resistance gene was removed from all plasmids by treatment with *Hin*dIII and *Xba*I.

The chloramphenicol acetyltransferase (CAT) plasmids described here all contain the *Escherichia coli* CAT gene under the transcriptional control of the HIV LTR (HIV-CAT). HIV-CAT (mutant NF-κB), HIV-CAT (mutant Sp1), and HIV-CAT (mutant TATA) are derivatives of HIV-CAT in which NF-κB, Sp1, and TATA sites are mutated as described previously (59) (Fig. 3). These three plasmids and the parental HIV-CAT are generous gifts from Gary Nabel, Howard Hughes Medical Institute, University of Michigan. TATA-CAT, 2×Sp1-CAT, and ATF-CAT (Fig. 5) were generously provided by J. D. Gralla (90). TATA-CAT contains the adenovirus major late promoter TATA box as the sole promoter element, 2×Sp1-CAT contains two Sp1 sites and a TATA box, and ATF-CAT contains one ATF-binding site and a TATA box. 2×NF-κB-CAT was constructed by oligonucleotide insertion upstream of the TATA box in TATA-CAT. This oligonucleotide represents the two NF-κB sites found in the HIV LTR sequence (5′-AGGGACTTTCCGCTGGGGA CTTTCC-3′).

Cell culture and transfection. Human cervical carcinoma (HeLa) and human osteosarcoma (Saos-2) cells were obtained from the American Type Culture Collection and propagated in minimum essential medium containing 10% fetal calf serum and Dulbecco's minimum essential medium containing 5% calf serum and 10% fetal calf serum, respectively. Subconfluent cells were transfected by the calcium phosphate-DNA coprecipitation method with dimethyl sulfoxide shock 4 h posttransfection (10, 83). In a typical experiment, 5×10^6 cells were cotransfected with 2.5 μg of a CAT construct and 5 μg of a p53 expression plasmid (or 5 μg of the expression vector without p53 sequences as a negative control). All transfection experiments were repeated several times. Variation in activities of 20 to 40% was observed from one experiment to another. Thus, an increase or a decrease of activity less than twofold may not be considered significant.

CAT assay. Cells were harvested 36 to 40 h posttransfection and lysed by three successive cycles of freezing and thawing. Extracts were normalized for protein concentration and assayed for CAT enzyme activity (30). CAT activity was detected by thin-layer chromatographic separation of [^{14}C]chloramphenicol from its acetylated derivatives and was quantitated by cutting out radioactive spots from the thin-layer chromatography plate following autoradiography or by using a Betascope by Betagen.

RESULTS

Activation of the HIV LTR promoter by mutants of human p53 in HeLa cells. The HIV LTR is a key regulatory sequence in the proviral genome which controls transcription. Activation of the latent virus may be initiated by nonviral factors (e.g., by cellular factors or extracellular stimuli) that may affect LTR function (28, 52, 57, 59, 60, 71, 77). We had previously observed inhibition of LTR function by wild-type human p53 (83) and wished to examine the LTR response to five point mutants of p53 (143A, 175H, 248W, 273H, and 281G). These mutant residues fall in or near four domains (II to V) which are highly conserved in vertebrate species (Fig. 1a) (78). HIV-CAT was cotransfected into HeLa cells by the calcium phosphate precipitation technique as described in Materials and Methods with the pCMV-Bam expression vector or with a plasmid expressing either wild-type p53 or one of the mutant forms. CAT activity was assayed in these cells after 36 to 40 h as described in Materials and Methods. Wild-type p53 inhibited HIV-CAT activity by fivefold (Fig. 1b; Table 1). However, four of the mutants (175H, 248W, 273H, and 281G) enhanced HIV-CAT activity significantly (7- to 22-fold). The data in Fig. 1b show a representative example of assays.

Effect of p53 expression on HIV LTR promoter activity in the Saos-2 cell line. The effects of wild-type and mutant human p53s on HIV LTR promoter activity in a cell line lacking endogenous p53 were determined by using Saos-2 cells to observe the effect in a p53-null background (12, 51). As shown in Fig. 2, HIV LTR activity was inhibited by wild-type p53. The HIV LTR was activated significantly by the mutants of p53 as observed previously in HeLa cells. The mutants enhanced the LTR activity relative to vector alone as follows: 143A, 2.4-fold; 175H, 3.0-fold; 248W, 3.5-fold; 273H, 5.2-fold; and 281G, 8.5-fold. Differences in transactivation potential of mutants of p53 in HeLa and Saos-2 cells possibly reflect cell type depen-

TABLE 1. Effects of wild-type and mutant p53 expression on promoter activities of different HIV LTR constructs in HeLa cells

Plasmid	Activity relative to vector alone (%)					
	Wild-type p53	143A	175H	248W	273H	281G
HIV-CAT (wild type)	20.9	202.5	724.2	1,659.3	2,213.7	717.0
HIV-CAT (mutant NF-κB)	23.2	143.0	381.7	983.8	1,786.6	2,088.0
HIV-CAT (mutant Sp1)	1.6	96.4	181.5	330.0	524.0	823.6
HIV-CAT (mutant TATA)	11.8	305.9	973.5	600.0	623.5	3,170.0

dence. In our experience, most of the promoters have less activity in Saos-2 cells (data not shown).

Effects of wild-type and mutant p53s on promoter activities of HIV LTR mutants. The NF-κB sites, Sp1 sites, and the TATA box have been found to be important for regulation of HIV LTR-mediated transcription (58, 70, 80). To determine whether these HIV LTR sequences are required for activation by p53 mutants, we used three different HIV LTR-CAT constructs with mutations affecting the NF-κB sites, Sp1 sites, and the TATA box, respectively (Fig. 3). These mutants have also been used previously to determine the mechanism of LTR transactivation by the ICP0 gene product of herpes simplex virus type 1 (59). Cotransfection experiments followed by CAT assays were carried out with use of individual HIV LTR-CAT constructs and either wild-type or mutant p53 (143A, 175H, 248W, 273H, and 281G) or vector alone. Data presented in Fig. 4 show that all three of the HIV LTR mutants were activated by the p53 mutants, although to different extents (Table 1). That individual mutations in Sp1 and NF-κB sites could not knock out mutant p53-mediated transactivation may suggest that mutant p53 can interact with one or more generalized transcription factors to activate transcription, and this interaction can be enhanced by Sp1 or NF-κB.

Minimal promoter elements required for transactivation by a mutant p53. Since the promoters that are activated by a mutant p53 have specialized transcription factor-binding sites, we tested whether the mutant p53-281G can activate a minimal promoter with a TATA box alone, a TATA box and two Sp1 sites, or a TATA box and two NF-κB sites by performing transfection experiments in Saos-2 cells. As a control, we used a minimal promoter with one ATF site and a TATA box (10, 90). This promoter was previously found to be activated by p53-281G (10). All of the synthetic promoters were cloned upstream of a CAT gene as described in Materials and Methods (90) (Fig. 5a). Figure 5b shows the results of a transfection assay with these constructs in Saos-2 cells. Promoter activities were determined in the presence of vector alone or vector expressing wild-type p53 or mutant p53-281G. p53-281G activated all of the promoter constructs studied. Similar results were also found with HeLa cells (data not shown). That TATA-CAT was activated suggests that the mutant p53 may interact with a general transcription factor favoring transcription. This inference agrees with the recent observation that wild-type p53 and mutant p53-281G bind to TBP (47, 48). However, the presence of two Sp1 or NF-κB sites or an ATF site enhanced transcription efficiency to some extent. This finding suggests possible direct interaction of the mutant p53 with these factors. Alternatively, mutant p53 may activate transcription from promoters containing Sp1, NF-κB, or ATF sites by interacting with a common factor(s) that interacts with each of these proteins. Recent observation of p53-Sp1 interaction in DNA binding also suggests an interaction between p53 and Sp1 (6). To investigate further, we tested the effect of p53-281G on the promoter activity (as measured by CAT activity) of promoter-CAT constructs 2×Sp1-CAT (TATA-less), ATF-CAT (TATA-less), and NFκB-CAT (TATA-less), in which the TATA element was eliminated by deletion. This was done by transient transfection assays with Saos-2 cells. Scanning of the CAT assay thin-layer chromatography plates revealed activation by p53-281G as follows: 2×Sp1-CAT (TATA-less), 9-fold; 2×ATF-CAT, 26-fold; and 2×NFκB-CAT, 4-fold. Thus, even in the absence of a TATA box, the synthetic promoters were activated significantly, and presumably the specialized transcription factors could nucleate the transcription machinery well, even in the absence of a TATA box when the mutant p53-281G was present.

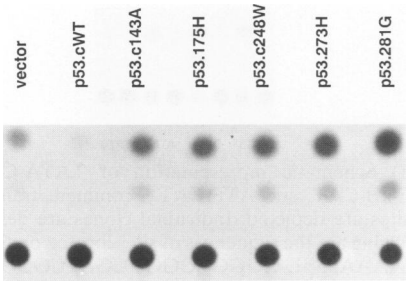

FIG. 2. Effects of expression of wild-type p53 (p53.cWT) and mutant human p53s on the promoter activity of HIV-CAT in Saos-2 cells. Experiments were performed as described in the legend to Fig. 1 and in Materials and Methods.

Transdominance of wild-type p53 or p53-281G on HIV LTR promoter activity. In a large number of tumors, a reduction to homozygosity is observed at the p53 locus such that in a majority of cases there is a loss of both wild-type p53 alleles, one through a deletion and the other through a point mutation (32–34, 42). This finding suggests that the wild-type protein may be dominant. Occasionally, however, heterozygosity persists (42), suggesting that the mutant protein gains a function. Perhaps one such gain of function, at least in p53 mutations, is transcriptional activation (8, 10, 13). Thus, whether mutant p53 proteins could act as dominant positive factors to disrupt wild-type p53-mediated inhibition of promoter function is an important unanswered question. Therefore, we wished to determine whether the transcriptional activation of the HIV LTR by p53-281G can be eliminated by wild-type p53 and, conversely, whether the transcriptional inhibition of the HIV LTR by wild-type p53 could be eliminated by mutant p53-281G. Two series of experiments were performed. In one set, HIV-CAT was cotransfected into HeLa cells with a fixed amount (5 μg) of wild-type p53 expression plasmid along with zero or increasing amounts of the mutant p53-281G. In the second set, 5 μg of p53-281G was used along with zero or increasing amounts of the wild-type expression plasmid. As evident from the data shown in Fig. 6, wild-type p53 inhibited HIV LTR activity when transfected in excess of p53-281G,

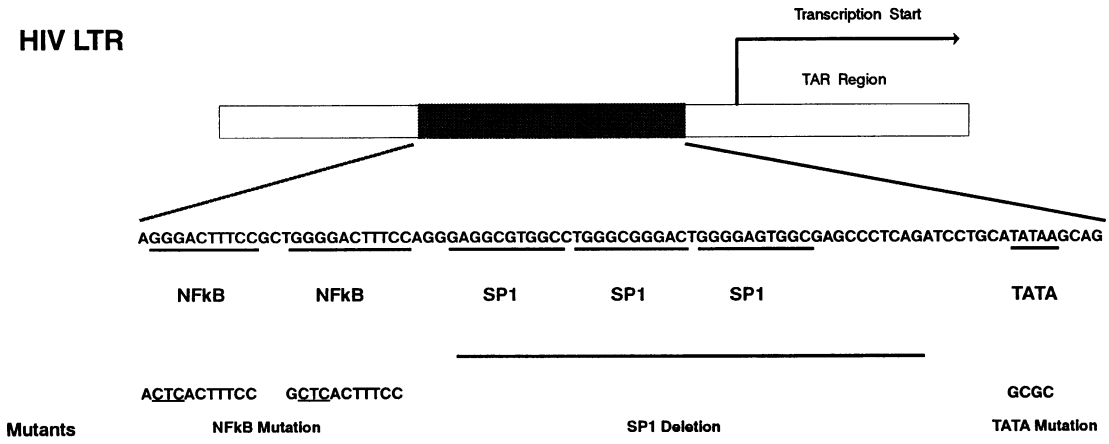

FIG. 3. Schematic representation of HIV LTR and its mutants (42). Mutant plasmids contained either alterations in both NF-κB sites as shown (mutant NF-κB), deletion of all Sp1 sites (mutant Sp1), or alteration of the TATA box (mutant TATA). The mutations in the NF-κB mutant and the extent of deletion in the Sp1 deletion mutant are underlined. In the TATA mutant, TATA has been changed to GCGC.

while p53-281G activated the LTR when transfected in excess of wild-type p53. Thus, it is clear from the data that mutant p53-281G could block the inhibitory effect of wild-type p53, possibly through its own activating effects. Similar results were also found with the human PCNA promoter (9a). Although we checked expression of wild-type and mutant p53 after individual transfection experiments (data not shown), p53 amounts expressed from wild-type and mutant p53 could not be differentiated in the cotransfection experiments described above. The data presented above, therefore, represent a qualitative picture.

DISCUSSION

The diversity of responses to HIV infection suggests that many factors contribute to the pathogenesis of the disease. Human cytomegalovirus is a critical cofactor in AIDS, associated with more rapid progression to disease (21, 59, 63); other DNA viruses have also been implicated (37, 57, 66, 81, 86). Glucocorticoids (26), morphine (79), tumor necrosis factor alpha (14), and *myb* oncogene expression (9) have been reported to activate the HIV LTR. Our observation that tumor-derived p53 mutants can activate the HIV LTR may be significant. Cellular p53 mutations, either before or after latent HIV infection, may increase the rate of proviral activation.

There is a growing body of evidence that strongly suggests involvement of the tumor suppressor p53 in transcriptional regulation of various cellular and viral promoters (1, 4, 8, 10, 15, 22, 25, 29, 35, 56, 67, 68, 72, 74, 83, 87). One group of

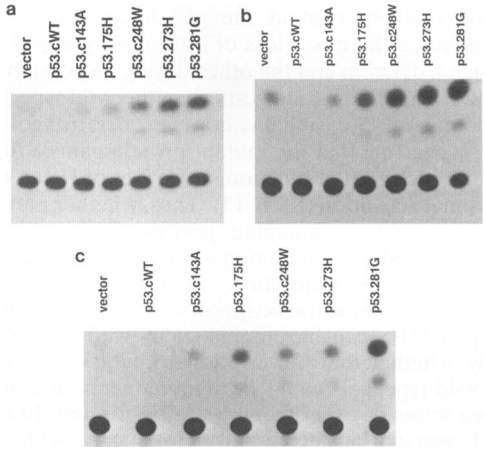

FIG. 4. Effect of expression of mutant p53s on the promoter activity of HIV LTR mutants (as CAT constructs) in HeLa cells. Three different mutants of HIV LTR were tested for their promoter activities. (a) NF-κB mutant; (b) Sp1 mutant; (c) TATA mutant. Mutants are described in the legend to Fig. 3. Assays were performed as detailed in the legend to Fig. 1 and in the text. p53.cWT, wild-type p53.

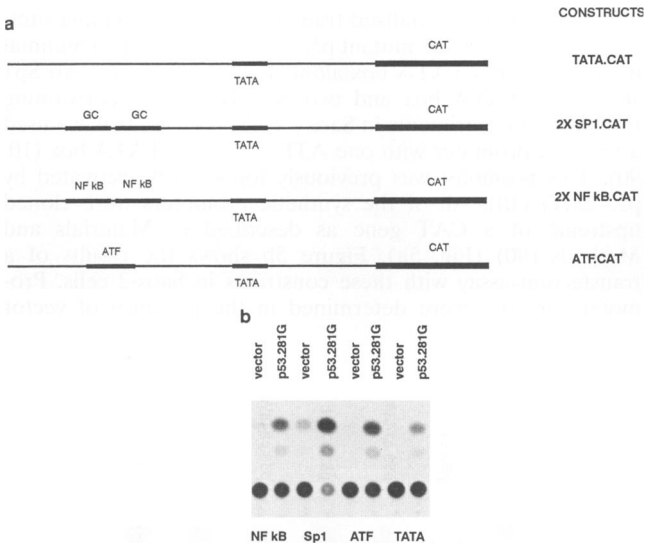

FIG. 5. (a) Schematic representation of TATA-CAT, 2×Sp1-CAT, 2×NF-κB-CAT, and ATF-CAT. Prominent transcription factor-binding sites are depicted. Individual clones are described in the text. The following are the sequences of sites located on the promoters: TATA, TATAAAA; Sp1, GGGGCGGGGCGATCGGGGCGGGGC; NF-κB, GGAAAGTCCCCAGCGGAAAGTCCCT; ATF, TCGTCA. (b) Effects of expression of mutant p53s on the promoter activity of synthetic minimal promoters (as CAT constructs) in Saos-2 cells. Mutants are those described above; assays were performed as detailed in the legend to Fig. 1 and in the text.

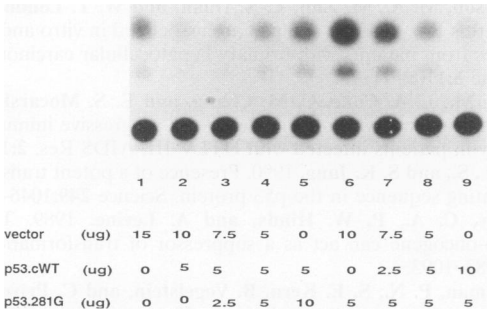

FIG. 6. Transdominance of wild-type p53 or p53-281G on HIV LTR promoter activity in HeLa cells. HeLa cells were cotransfected with HIV-CAT and vector and/or wild-type p53 (p53.cWT), p53-281G, or a mixture of wild-type p53 and p53-281G expression plasmids as indicated. CAT activities were determined after 36 to 40 h of transfection as described in Materials and Methods.

reports shows that wild-type p53 inhibits several promoters in a sequence-independent manner (8, 29, 72, 83). On the other hand, wild-type p53 activates promoters with p53-binding sites both in vivo and in vitro, while tumor-derived p53 mutants do not (4, 15, 25, 35, 56, 69). However, tumor-derived p53 mutants transactivate the *MDR-1* and PCNA promoters (8, 10, 94). The results discussed in the present report show that overexpression of wild-type human p53 can, as expected, exert an inhibitory effect on HIV LTR promoter activity, while p53 mutants significantly upregulate the promoter. Thus, the HIV LTR serves as a useful model promoter with which to analyze the mechanism of mutant p53-mediated transactivation.

The tumor-derived mutants of p53 activated the HIV LTR in the Saos-2 cell line, which does not have a detectable p53 gene (12, 51). This experimental result suggests that the mutant p53 directly activates the HIV LTR, and this activation is not the result of a loss in inhibition by endogenous wild-type p53. However, one cannot rule out a general activation of cellular growth as the cause of the HIV LTR promoter activation. It is interesting that p53 mutants can activate LTR promoters with mutations in Sp1 or NF-κB sites. This finding suggests that activation may not depend on a particular factor. The generality of the mutant p53-mediated transactivation becomes more apparent when we examine the results obtained with synthetic promoters containing either a lone TATA box, two NF-κB sites and a TATA box, two Sp1 sites and a TATA box, or a single ATF site and a TATA box. In all cases, we observed significant activation, although activation was slightly higher when the specialized transcription factor-binding sites were present. Perhaps the mutant p53 protein interacts with a general transcription factor(s) and activates initiation of transcription, and transcription factors bound to Sp1-, NF-κB-, or ATF/CREB-binding sites cooperate in this activation (10; this report). Significantly, the presence of Sp1-p53 DNA-binding heterocomplexes has been shown in a human erythroleukemia cell line, indicating interaction between Sp1 and p53 (6). Thus, p53 may interact with these transcription factors more directly, although no association with ATF or NF-κB has yet been shown. Our results with TATA-less constructs suggest further that the factors ATF, NF-κB, and Sp1 can efficiently nucleate the transcription machinery when the mutant p53-281G is present even in the absence of a recognizable TATA box.

In E1a-mediated activation of promoters containing ATF sites, E1a has been proposed to bridge TFIID and ATF (43, 49, 50). E1a has been shown to interact with TBP (40). A tethering factor, bridging Sp1 and TFIID, has also been suggested to explain Sp1-mediated transcription activation (65), since Sp1 is not known to bind TBP directly (4a). One can speculate that the mutant p53 interacts with this tethering factor and activates transcription. It is conceivable that NF-κB and ATF/CREB interact with TFIID by a similar mechanism, and the mutant p53 may also positively interact with their bridging factors. It is also possible that all three factors, Sp1, NF-κB, and ATF, function through the same tethering factor with which the mutant p53 interacts. This factor may be similar to that described by Kretzschmar et al. (38), who showed that for efficient transcription by HIV LTR, in addition to NF-κB, a cofactor fraction was required. The exact transcriptional role of mutant p53 remains to be determined. Activation of synthetic promoters with binding sites for specialized transcription factors like Sp1, NF-κB, and ATF suggests that the transforming mutant p53-281G could potentially activate a number of cellular genes that depend on these factors for transcription.

It is highly significant that the mutant p53 protein can activate a promoter with a single TATA box as the lone promoter element. This finding strongly suggests that activation by the mutant protein may be mediated via a component of the general transcription complex. Similar results have been obtained recently by Zastawny et al. (94). They have analyzed the *MDR-1* promoter and observed that the basal promoter element was enough for transcriptional modulation by p53. Since tumor-derived p53 mutants are generally defective in both specific and nonspecific DNA binding (35), transcriptional activation by mutants may be through protein-protein interaction. Studies with p53/GAL4 fusion constructs indicate that mutants 248W and 273H are as effective as wild-type p53 in transactivating promoters bearing GAL4-binding sites (22, 68). Mutants p53-281G, p53-248W, and p53-273H have been shown to directly bind to TBP (47, 85). This interaction may represent part of their activation scheme. Transactivating mutants may also interact at the level of the TFIIA-TFIID-TATA complex (DA complex) formation. Recently Ragimov et al. (67) reported that wild-type p53 interferes at the DA complex level to inhibit transcription. They observed some enhancement in DA complex formation in the presence of a mutant p53. Enhancement of DA complex formation may suggest a possible mechanism for activation by p53 mutants. One can also imagine that DA complex formation is enhanced in the presence of factors like Sp1, ATF, and NF-κB or that enhancement of DA complex formation in the presence of mutant p53 results in a more pronounced effect on gene expression when the specialized factors are present. Taken together, these observations suggest that tumor-derived p53 mutants can activate a variety of promoters, albeit to different extents, by interacting with the basal transcription unit and specialized transcription factors. We should not, however, neglect to add that it is entirely possible that the effect of mutant p53 on CAT activities result from enhanced transcriptional activity generated by increased rate of cell proliferation or from a factor induced by mutant p53. Elucidation of the exact mechanism of transactivation by mutant p53 will require analysis of the individual steps involved in transcription. In the future, demonstration of in vitro transactivation by the mutant p53 will help accomplish this task.

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