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# Reciprocal modulations between p53 and Tat of human immunodeficiency virus type 1

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**ABSTRACT** **Infection by human immunodeficiency virus type 1 (HIV-1) causes acquired immunodeficiency syndrome (AIDS) after a long clinical latency. This disease is associated with a spectrum of cancers. Here we report that wild-type p53 is a potent suppressor of Tat, a major transactivator of HIV-1. Reciprocally, Tat inhibits the transcription of p53. Downregulation of p53 by upregulated tat may be important for the establishment of productive viral infection in a cell and also may be involved in the development of AIDS-related malignancies.**

Tat protein is the most potent transactivator of the human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) (1, 2). It is actively released from HIV-infected cells as a biologically active protein (3, 4). Expression of the *tat* gene in transgenic mice induces hyperplasia in lymphoid tissues and tumors of different histotypes, including lymphoma, suggesting that Tat could play a direct role in causing cancer (5–7). Extracellular Tat and basic fibroblast growth factor synergize in inducing Kaposi sarcoma (8).

The existence of host suppressors for activation of HIV-1 is indicated because HIV-1 infection has an AIDS latency varying from several months to as long as decades. Wild-type p53 may be such a suppressor since it has been shown to suppress the basal activity of viral gene promoters, including HIV-1 LTR (9–12). Conversely, several DNA oncovirus-encoded proteins, such as large T antigen of simian virus 40 (13), E1B of adenovirus 5 (14), X protein of hepatitis B virus (15), and E6 of human papilloma virus types 16 and 18 (16), cause dysfunction of p53 (9). The expression of E6 in cervical cancers, for example, results in ubiquitin-mediated degradation of p53 protein (17). Interestingly, transgenic mice defective in the p53 tumor-suppressor gene show phenotypes similar to those reported for transgenic mice that overexpress Tat (6, 7, 18).

In this study we found that wild-type p53 is a potent suppressor of HIV-1 activation by Tat. This suppression was partially antagonized by tumor necrosis factor $\alpha$ (TNF-$\alpha$), an exogenous activator of HIV-1. Conversely, accumulation of Tat protein inhibited the promoter activity of p53.

## MATERIALS AND METHODS

**Materials.** TNF-$\alpha$ was purchased from Genzyme. Plasmid 469 is an HIV-1 LTR-driven chloramphenicol acetyltransferase (CAT) construct (19). pCMV53-SN, an expression construct for wild-type p53, has been described (20). pSVL-tat is a tat expression vector driven by the simian virus 40 late promoter (a generous gift from J. Sodroski, Dana–Farber Cancer Institute). p5DU$\varepsilon$ 2400 is a recombinant plasmid of the human p53 promoter–leader region fused to a CAT plasmid (21).

**Cell Cultures.** Jurkat cells were cultured in RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (FCS; Sigma). Jurkat-tat cells were derived from Jurkat cells that were stably transfected with the HIV-1 *tat* gene driven by the promoter of human BK virus (22). The expression of Tat was confirmed by immunoblotting and by transactivation of the CAT gene driven by HIV-1 LTR (23). Jurkat-control cells are Jurkat cells transfected stably with the vector pRPneo-c (22). Jurkat-control and Jurkat-tat cells were cultured in RPMI 1640 medium supplemented with 10% FCS and 400 $\mu$g of G418 (Life Technologies) per ml. HeLa and HeLa-tat-III (AIDS Research and Reference Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health; donated by W. Haseltine and E. Terwillinger) cell lines were cultured in Dulbecco's modified Eagle's medium (Life Technologies) supplemented with 10% FCS, 2 mM L-glutamine, and 800 $\mu$g of G418 per ml.

**Transfections.** Jurkat cells and HeLa cells were transfected with plasmid DNA by a modified electroporation method (23, 24). The amount of plasmid used for one transfection was as follows: 2 $\mu$g for 469, 5 $\mu$g for proliferating cell nuclear antigen (PCNA) promoter–CAT, 5 $\mu$g of p5DU$\varepsilon$2400, various amounts of pCMV53-SN or pSVL-tat as indicated in figure legends. Cells were harvested and assayed for CAT activity (23) 40 h after transfection. In some experiments, transfected cells were treated with TNF-$\alpha$ (2 ng/ml) 16 h before being harvested.

**CAT Assay.** Cell extracts were prepared and incubated at 37°C with [$^{14}$C]chloramphenicol in the presence of acetyl coenzyme A, and CAT activity was determined as described previously (23). CAT activity was quantified with an Ultroscan XL enhanced laser densitometer (LKB Bromma, Pharmacia LKB).

**Northern Blot Analysis.** Preparation of total RNA and Northern blot analysis using 20 $\mu$g of RNA per lane were carried out as described (25). The 1.8-kb fragment of pCMV53-SN [kindly supplied by Bert Vogelstein (20)], including the entire p53 coding region, was used as a probe.

**PCR and Primers.** cDNA was prepared from total RNA by using 200 units of murine leukemia virus reverse transcriptase (Bethesda Research Laboratories) with oligo(dT) as a primer. A 1.3-kb fragment, including the entire p53 coding region, was generated from the cDNA by PCR amplification using the oligonucleotides 5′-AAGCTTCCACGACGGTGACACGCTTC (upstream primer) and 5′-GAATTCCGCACACCTATTGCAAGCAAGG (downstream primer).

## RESULTS

**Wild-Type p53 Suppressed Transactivation of HIV-1 LTR by Tat.** To test the possibility that p53 and Tat reciprocally

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Abbreviations: HIV-1, human immunodeficiency virus type 1; LTR, long terminal repeat; CAT, chloramphenicol acetyltransferase.

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negatively interact (see Fig. 1), we first asked whether Tat activity is suppressed by p53, thereby blocking HIV-1 LTR activation. We cotransfected Jurkat cells with an HIV-1 LTR-driven CAT reporter construct, a *tat* expression vector, and an expression construct for human wild-type p53. Tat-mediated activation of HIV-1 LTR was suppressed by wild-type p53 (Fig. 2*A*). Similar results were observed in other cell lines (data not shown). To test whether this inhibition is a secondary consequence of growth arrest by p53, we assayed CAT expression driven by the PCNA promoter; wild-type p53 did not inhibit PCNA–CAT activity (Fig. 2*A*). Wild-type p53, in cells that constitutively express Tat protein (22, 26), inhibited transactivation of HIV-1 LTR by Tat (Fig. 2*B*), suggesting that p53 inhibits Tat activity after Tat protein is made. This possibility is particularly attractive since both Tat and wild-type p53 interact with the TATA-binding protein (TBP; refs. 27 and 28). Therefore, TBP could mediate an antagonism of p53 against Tat.

TNF-$\alpha$ activated HIV-1 LTR which had been suppressed by expression of wild-type p53 (Fig. 2*C*), suggesting that external activators of HIV-1 could antagonize the suppressing activity of wild-type p53.

**HIV-1 Tat Downregulated p53 Promoter Activity.** We then looked for the reciprocal effect of HIV-1 Tat on p53 expression. We cotransfected Jurkat cells with a human p53 promoter–CAT reporter construct (p5DU$\varepsilon$ 2400) (21) and a Tat expression vector (pSVL-tat). Tat inhibited p53 promoter activity (Fig. 3 *A* and *B*). When an equal amount of pSVL-tat was cotransfected with p5DU$\varepsilon$ 2400, p53 promoter activity was reduced by 90% (Fig. 3*B*), but when p5DU$\varepsilon$ 2400 was cotransfected with 1/100th the amount of pSVL-tat plasmid, p53 promoter activity was enhanced; the magnitude of this enhancement varied from 1- to 10-fold in different experiments (data not shown).

We asked whether constitutive expression of Tat inhibits p53 promoter activity. We used Tat-expressing Jurkat cells (22) pooled from different stable *tat* transfectants, in which constitutive expression of functional Tat was confirmed by transiently introducing HIV-1 LTR–CAT (23). We transfected the p53 promoter–CAT reporter construct into Jurkat and Jurkat-tat cells. Expression of CAT driven by p53 promoter was suppressed in the Jurkat-tat cells (Fig. 3*C*).

We studied changes in p53 mRNA when cells constitutively express Tat in those Jurkat-tat cell lines (22). p53 mRNA appeared as a faint band in Jurkat cells and was undetectable in the three Jurkat-tat cell lines (Fig. 4*A*, lanes 1–4). We next

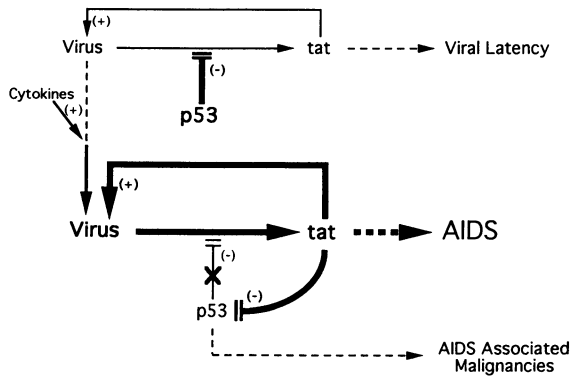


FIG. 1. Proposed interactions between Tat and wild-type p53 underlie a switching mechanism. Initially, inhibition of Tat by wild-type p53 could dominate and contribute to prolonged survival of an HIV-infected cell. Eventually, stimulation by external factors would elevate Tat expression, which would downregulate p53. This in turn leads to even higher Tat levels and viral replication. Downregulation of p53 may also be involved in the development of AIDS-related malignancies.

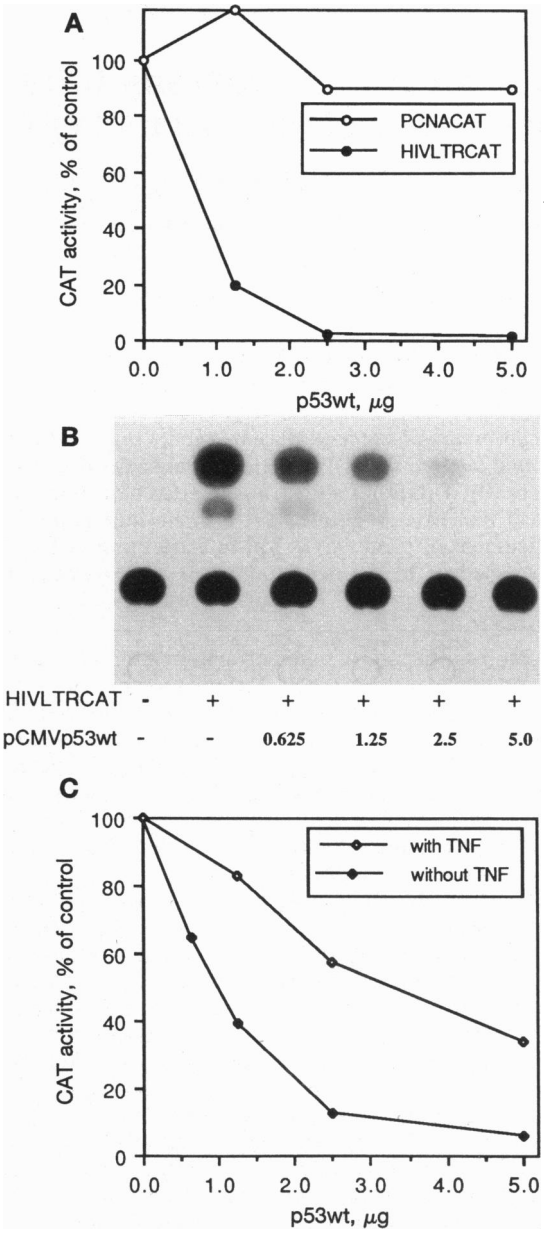


FIG. 2. (*A*) Tat activity was suppressed by wild-type p53 when the expression constructs for Tat and wild-type p53 were cotransfected transiently. Jurkat cells, a human lymphoma cell line, were transiently cotransfected with HIV-1 LTR–CAT reporter construct and a Tat expression vector, together with increasing amounts of an expression construct encoding human wild-type p53 protein. In a control experiment, Jurkat cells were transfected with a PCNA–CAT reporter construct, together with increasing amounts of the wild-type p53 expression vector. The ordinate is the percent change in CAT activity due to cotransfection of the p53 expression construct at the amount indicated on the abscissa. The results shown are from one of three independent experiments. Similar results were also observed when using U38 (human monocytic cell line) and HeLa (human epithelial cell line) cells (data not shown). (*B*) Activity of Tat was suppressed by wild-type p53 in cells containing Tat protein. Jurkat-tat cells that constitutively express Tat (22) were transiently transfected with HIV-1 LTR–CAT reporter construct, together with different amounts of an expression vector encoding wild-type p53. Autoradiogram of CAT assay data is shown. Similar results were obtained with HeLa-tat-III, another Tat-expressing cell line (data not shown). (*C*) TNF-$\alpha$ antagonized the inhibition of Tat activity by wild-type p53. Jurkat-tat cells were transiently transfected with HIV-1 LTR–CAT reporter construct and increasing amounts of expression vector for wild-type p53. TNF-$\alpha$ was added to transfected cells 16 h before they were harvested.

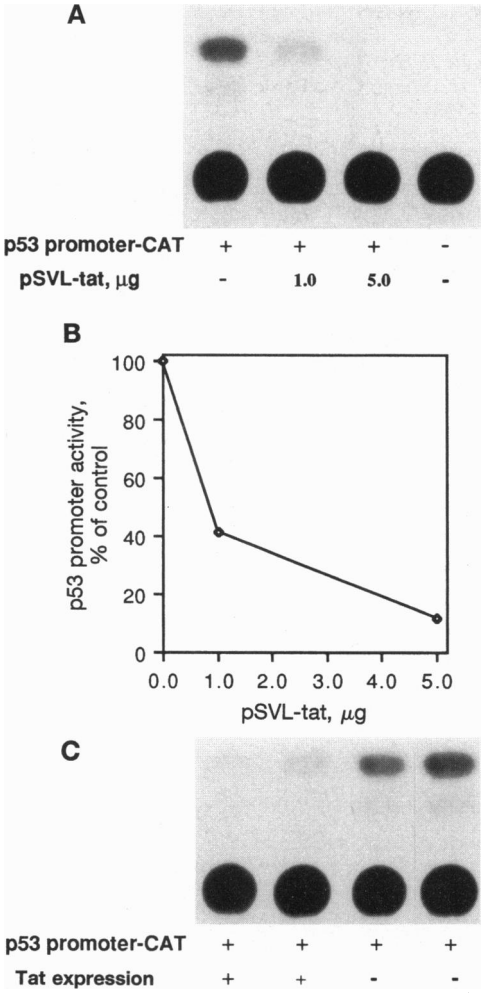


FIG. 3. (*A*) HIV-1 Tat-inhibited promoter activity of human p53. Jurkat cells were transfected with human p53 promoter–CAT reporter construct p5DUε 2400, together with different amounts of an expression vector for Tat, PSVL-tat. Cells were harvested 48 h after transfection. Autoradiogram of CAT assay data is shown. (*B*) Quantitative analysis of CAT activities of three independent experiments as described in *A*. (*C*) Human p53 promoter-driven gene expression was suppressed in cells that constitutively express Tat. Jurkat and Jurkat-tat cells were transfected with human p53 promoter–CAT reporter construct. Autoradiogram of CAT assay data is shown.

used the more sensitive reverse transcription–polymerase chain reaction method to detect p53 mRNA in Jurkat-tat cell lines. p53 mRNA was detected in Jurkat cells (Fig. 4*B*, lane 2), but it was barely detectable in the Jurkat-tat cell lines (Fig. 4*B*, lanes 3–5). Thus, Tat appeared to decrease p53 transcription or mRNA stability. In HeLa cells, constitutive expression of Tat decreased p53 mRNA without altering its half-life (data not shown).

## DISCUSSION

We have shown that wild-type p53 is a potent endogenous inhibitor of Tat-mediated transactivation of HIV-1 LTR and that Tat modulates p53 transcription. We propose that reciprocal interactions between p53 and Tat are important in the establishment of productive viral infection and also may underlie the pathogenesis of AIDS-related malignancies and other syndromes. Our results suggest a switching mechanism. Wild-type p53 might keep the virus and Tat in check in latently infected cells. Occasionally, cytokines and bacterial infections stimulate HIV-1 replication in individual infected cells and,

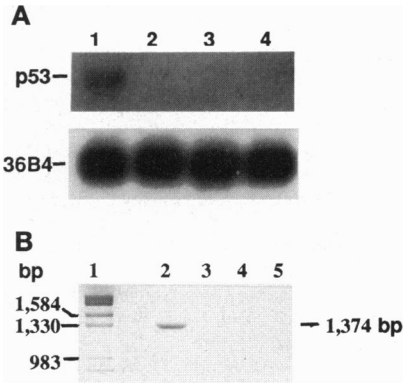


FIG. 4. (*A*) p53 mRNA level is decreased in Jurkat-tat cells. Total RNA was prepared from exponentially growing Jurkat cells and three different Jurkat-tat cell lines, Jurkat-34, Jurkat-42, and Jurkat-44. Endogenous level of p53 mRNA was analyzed by Northern blot assay. As a loading control, the blot was stripped and then hybridized with 36B4, a cDNA probe used as a loading control (30). Lane 1, Jurkat; lane 2, Jurkat-34; lane 3, Jurkat-42; and lane 4, Jurkat-44. (*B*) Detection of p53 mRNA in Jurkat and Jurkat-tat cells by reverse transcription–PCR assay. Lane 1, molecular size marker, lane 2, Jurkat; lane 3, Jurkat-34; lane 4, Jurkat-42; and lane 5, Jurkat-44.

thus, would lead to accumulation of Tat. When the Tat level becomes high enough, it would decrease p53, causing more Tat to be produced, resulting in disruption of viral latency. Furthermore, this loss of p53 in infected and uninfected cells could relax the $G_1$ checkpoint (29), permitting accumulations of chromosomal aberrations and lead to AIDS-related malignancies.

Five mechanisms have been described for inactivation of p53 (9): missense mutation, nonsense mutation, deletion, enhanced protein degradation by E6 of human papilloma virus, and inhibition by binding to MDM2. Here, we show that HIV-1 Tat inhibits p53 at the mRNA level, which is different from any of the above mechanisms (9). To our knowledge, Tat is the first viral factor shown to modulate transcription of p53, and p53 is the first host suppressor of the transactivational activity of Tat. Although Tat could have a direct negative effect on the p53 promoter, the effect could alternatively be mediated by an indirect mechanism, such as increasing a cellular negative regulator of p53, because Tat is a positive transcriptional factor for HIV-1 and some cellular genes (2).

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