HIV-1 Tat Protein-mediated Transactivation of the HIV-1 Long Terminal Repeat Promoter Is Potentiated by a Novel Nuclear Tat-interacting Protein of 110 kDa, Tip110*

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Human immunodeficiency virus type 1 (HIV-1) gene expression and replication is highly dependent on and modulated by interactions between viral and host cellular factors. Tat protein, encoded by one of the HIV-1 regulatory genes, tat, is essential for HIV-1 gene expression. A number of host cellular factors have been shown to interact with Tat in this process. During our attempts to determine the molecular mechanisms of Tat interaction with brain cells, we isolated a cDNA clone that encodes a novel Tat-interacting protein of 110 kDa or Tip110 from a human fetal brain cDNA library. GenBank® BLAST search revealed that Tip110 was almost identical to a previously cloned KIAA0156 gene with unknown functions. In vivo binding of Tip110 with Tat was confirmed by immunoprecipitation and Western blotting, in combination with mutagenesis. The yeast three-hybrid RNA-protein interaction assay indicated no direct interaction of Tip110 with Tat transactivating response element RNA. Nevertheless, Tip110 strongly synergized with Tat on Tat-mediated chloramphenicol acetyltransferase reporter gene expression and HIV-1 virus production, whereas down-modulation of constitutive Tip110 expression inhibited HIV-1 virus production. Northern blot analysis showed that Tip110 mRNA was expressed in a variety of human tissues and cells. Moreover, digital fluorescence microscopic imaging revealed that Tip110 was expressed exclusively in the nucleus, and within a nuclear speckle structure that has recently been described for human cyclin T and CDK9, two critical components for Tat transactivation function on HIV-1 long terminal repeat promoter. Taken together, these data demonstrate that Tip110 regulates Tat transactivation activity through direct interaction, and suggest that Tip110 is an important cellular factor for HIV-1 gene expression and viral replication.

Unlike simple retroviruses, the complex retrovirus human immunodeficiency virus type 1 (HIV-1)† encodes additional regulatory proteins that are essential for HIV-1 gene expression and viral replication (for reviews, see Refs. 1 and 2). One of them is HIV-1 Tat, which transactivates HIV-1 transcription from the viral long terminal repeat (LTR) promoter (for reviews, see Refs. 1 and 3). Tat distinguishes itself from other classic transcriptional activators by functioning at the stage of transcription elongation (4–9) and interacting with a cis-activating stem-loop RNA structure called transactivating response element (TAR), which is located immediately 3′ of the LTR transcription start site (6, 10–13).

A number of host cellular factors have been demonstrated to play a role in mediating the transactivating properties of Tat through direct or indirect interaction with Tat or TAR (for review, see Ref. 14). Identification of human cyclin T interaction with Tat has proved to be most critical and significant (6, 15). Evidence has accumulated to support a model, i.e. human cyclin T forms a complex with Tat and a cyclin-dependent kinase CDK9, a component of positive transcription elongation factor b (P-TEFb). Subsequently, the Tat-cyclin T-containing complex is recruited to TAR by specific interactions between Tat and the bulge region of TAR, and between cyclin T and the loop region of TAR. As a result, the cyclin T-associated CDK9 kinase induces phosphorylation of the C-terminal domain of RNA polymerase II, thus resulting in efficient and progressive HIV-1 transcription. Besides cyclin T, other Tat-interacting proteins and/or cofactors include Tip60, HT2A, CA150, TFIIID, Tat-SF1, Tip30, p300, and CBP (7, 16–24). Many of these factors regulate Tat transactivation function through directly regulating Tat interaction with TAR, and/or Tat interaction with other transcription factors.

In addition to Tat protein, a number of cellular transcription factors can also regulate the HIV-1 LTR promoter activity through targeting the regions or elements other than TAR within the LTR promoter (for review, see Ref. 14). These regions are located upstream of TAR, and include the basal promoter, the core enhancer, and the modulatory region (25). Transcription activators, such as SP1, NF-κB, and TBP, bind within the core promoter and stimulate HIV-1 transcription (26, 27), whereas others including NF-AT, USF, and COUP regulate the HIV-1 LTR promoter through interaction with the response element; P-TEFb, positive transcription elongation factor b; LTR, long terminal repeat; CAT, chloramphenicol acetyltransferase; HAT, half-a-tetratricopeptide repeat; TPR, tetratricopeptide repeat; RRM, RNA recognition motif; NLS, nuclear localization signal; SD, synthetic dropout medium; LRP, low density lipoprotein receptor-related protein; AD, Gal4 activation domain-containing yeast vector; BD, LexA DNA binding domain-containing yeast vector; β-gal, β-galactosidase; RTase, reverse transcriptase; GFP, green fluorescence protein; DAPI, 4,6-diamidino-2-phenylindole; aa, amino acid(s); GST, glutathione S-transferase; ORF, open reading frame.

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modulatory region (28–30). Thus, it has become increasingly evident that interaction factors between Tat, host cellular factors, and transcription factors forms a complex regulatory network, through which regulation of HIV-1 gene expression is achieved in a diverse range of host cells and under a variety of extracellular stimuli.

During our attempts to characterize the roles of Tat protein in HIV-1-induced neuropathogenesis, we isolated a cDNA clone that encodes a novel nuclear Tat-interacting protein of 110 kDa, or Tip110. We show that Tip110 directly transactivates the HIV-1 LTR promoter, but also interacts and synergizes with Tat to argument Tat-mediated transactivation activity. Moreover, we show that down-modulation of constitutive Tip110 expression inhibits HIV-1 viral gene expression. In corroboration with the observed activities, we show that Tip110 is a nuclear protein and exclusively localized in a nuclear structure termed nuclear speckles. These data together suggest that Tip110 plays an important role in regulating HIV-1 gene expression.

MATERIALS AND METHODS

Cell Lines and Cell Transfections—293T, BEBM17, U138.MG, Jurkat, U937, KG-1, and HeLa cells were purchased from American Tissue Culture Collection (ATCC) and maintained in either Dulbecco's modified Eagle's medium (293T, U138.MG, and HeLa) or RPMI 1640 medium (BEBM17, Jurkat, U937, and KG-1), supplemented with 10% fetal bovine serum at 37 °C with 5% CO₂. Human oligodendroglioma T620 cells were a gift from Dr. E. Schaeffer and were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Cell transfections were performed by the standard calcium phosphate precipitation method. pcDNA3 was used to equalize the amounts of DNA transfected for all transfections throughout the studies unless stated otherwise. pTPK/Gal was included to normalize variations in transfection efficiency for all transfections unless stated otherwise. The chloramphenicol acetyltransferase (CAT) reporter gene assay was performed as previously described (31, 32).

Plasmids—pTat.Myc, pAD-LR, pBD-Tat68, pBD-Tat72, pBD-Tat57, pBD-Tat48, pBD-Tat83, and pBD-Tat21 have been described elsewhere (33). pBD-M2S, pMS2, pMS2-IRE, pMS2-TAR, pAD-IRP, and pAD-Tat were generously provided by Dr. M. Wenkins. pZeo was a generous gift from Dr. E. Schaeffer and was maintained in Escherichia coli (Invitrogen), and the transformed bacteria containing the recombinant plasmid were induced with 1 mM isopropyl-D-thiogalactopyranoside for 4 h. For pAs-Tip110 plasmid, the Tip110 ORF was amplified by PCR using primers with 5′-GCC GTC ATG GCC GCG GGC GAA-3′ and 5′-CCC CCT CGA TTC AAT gat gat gat gat gat gct TTC TCA GAA ACA GTC TGG CGG-3′ sequences (reverse strand), pDNAAs, pAD-Tip110, and 5′-GGGA ATT CCT GTC GGG GGC GAA-3′ and 5′-CCC CCT CGA TTC AAT gat gat gat gat gct TTC TCA GAA ACA GTC TGG CGG-3′ sequences (reverse strand), pDNAAs, pAD-Tip110, and 5′-GGGA ATT CCT GTC GGG GGC GAA-3′ and 5′-CCC CCT CGA TTC AAT gat gat gat gct TTC TCA GAA ACA GTC TGG CGG-3′ sequences (reverse strand), pDNAAs, pAD-Tip110. All recombinant plasmids and deletion mutants were verified by sequencing.

Preparation of Recombinant Tip110 and Anti-Tip110 Sera—pGST-Tip110 plasmid was transformed into Escherichia coli BL21 cells (Invitrogen), and the transformed bacteria containing the recombinant plasmid were induced with 1 mM isopropyl-D-thiogalactopyranoside for 2 h at 37 °C. The glutathione S-transferase (GST)-Tip110 fusion protein was purified using a GST fusion protein purification kit (Pierce). GST was removed by treating the eluted protein with thrombin protease (10 units) (Invitrogen) at room temperature for 18 h. The digested protein solution was dialyzed overnight in 4 liters of phosphate-buffered saline and cleared of GST protein by additional incubations with fresh glutathione beads. The purified Tip110 was electrophoresed on a 5% SDS-polyacrylamide gel and stained with Gold-Blue (Pierce) to ensure the complete removal of GST protein as well as undigested fusion protein. To remove residual lipopolysaccharide, the recombinant Tip110 protein was incubated with polymyxin beads for 1 h and then filter-sterilized. The adjuvant slurry containing an estimated 10⁵ µg of protein was injected into mice. Two booster doses were given to each animal to generate the anti-Tip110 sera.

DNA Isolation and Northern Blot Analysis—Total cellular RNAs were isolated using an In VitroGen TRIzol RNA isolation kit according to the manufacturer's instruction. Human fetal brain RNA was purchased from Invitrogen. 20 µg of total RNA was fractionated on a 1.2% formamide-containing agarose gel, transferred to the Hybond-N membrane (Amersham Biosciences), and hybridized at 42 °C overnight with the probe.

HIV-1 Reverse Transcriptase (RTase) Activity Assay—Cell culture supernatants were collected 48 h after medium change, filtered, and assayed for RTase activity, as previously described (41). Briefly, viral pellets were recovered by centrifugation of the cell culture supernatants at 18,000 × g for 4 h at 4 °C and then solubilized in the dissociation buffer (0.1% sodium dodecyl sulfate, 0.5 mg/ml of poly(A)-dT 15 (Roche Molecular Biochemicals) and 2 µCi [³H]dGTP (3,000 Ci/mmol) for 5 min at 37 °C). The reaction mixture was then spotted onto DE81 membrane disks (Whatman) and washed three times with 2 × SSC (0.3 M NaCl, 30 µM sodium citrate). The membrane disks were then counted on a scintillation counter for the RTase activity.

Preparation of Recombinant Tip110 Protein and Anti-Tip110 Sera—pGST-Tip110 plasmid was transformed into Escherichia coli BL21 cells (Invitrogen), and the transformed bacteria containing the recombinant plasmid were induced with 1 mM isopropyl-D-thiogalactopyranoside for 2 h at 37 °C. The glutathione S-transferase (GST)-Tip110 fusion protein was purified using a GST fusion protein purification kit (Pierce). GST was removed by treating the eluted protein with thrombin protease (10 units) (Invitrogen) at room temperature for 18 h. The digested protein solution was dialyzed overnight in 4 liters of phosphate-buffered saline and cleared of GST protein by additional incubations with fresh glutathione beads. The purified Tip110 was electrophoresed on a 5% SDS-polyacrylamide gel and stained with Gold-Blue (Pierce) to ensure the complete removal of GST protein as well as undigested fusion protein. To remove residual lipopolysaccharide, the recombinant Tip110 protein was incubated with polymyxin beads for 1 h and then filter-sterilized. The adjuvant slurry containing an estimated 10 µg of protein was injected into mice. Two booster doses were given to each animal to generate the anti-Tip110 sera.
The nucleotide and deduced amino acid sequence of the Tip110 gene. Predicted functional domains of Tip110 are seven HAT domains (underlined), two RRM domains (double underlined), and NLS domain (dotted). The pretruncated variant contains an 11-bp deletion between nucleotides 1051 and 1062 (strike-through), and thus encodes a Tip110 isoform of 364 amino acids with 14 different amino acid residues at its C terminus, i.e. RSTTESKGFGFICT. The GenBank/H23042 accession number for the full-length Tip110 and the isoform is AF387506.


Tip110 Interaction with Tat and the HIV-1 LTR Promoter

RESULTS

Isolation of the Full-length and 11-bp Deletion-containing Tip110 cDNAs—During our attempts to identify the endocytic receptor for Tat on neurons, we used Tat as a bait protein and performed the yeast two-hybrid cDNA screening of a human fetal brain cDNA library. In addition to low density lipoprotein receptor-related protein (LRP), the Tat receptor (33), we also obtained several Tat-interacting cDNA clones in these studies. One of them was Clone 60. When co-transformed with Tat bait expression plasmid in the yeast two-hybrid assay, Clone 60 reproducibly exhibited a strong transactivation of the β-gal reporter gene, determined by the filter/galactosidase (β-gal) assay (data not shown). We recovered and sequenced the cDNA insert from Clone 60, which was determined to be ~2.0 kb in length. GenBank® BLAST search revealed that the cDNA completely aligned to the N-terminal ORF of KIAA0156, one of the genes previously cloned from a human cell line KG-1 cDNA library (42). To ensure that the cDNA from Clone 60 is the original Clone 60, which was determined to be ~2.0 kb in length, GenBank® BLAST search revealed that the cDNA completely aligned to the N-terminal ORF of KIAA0156, one of the genes previously cloned from a human cell line KG-1 cDNA library (42). To ensure that the cDNA from Clone 60 is the reported KIAA0156 gene, we decided to clone the full-length cDNA. We performed 5'- and 3'-rapid amplification of cDNA ends using the Marathon Ready human fetal brain cDNA (CLONTECH) as a template, and primers 5'-CTC GTC ATG CAG CCA CCT CAC CAG GA-3' and 5'-ATG ACA CTT CTC TTC CAC CCT GCA CAG C-3' based on the partial cDNA sequence obtained. We then cloned both rapid amplification of cDNA ends DNA products into pCR-TOPO (Invitrogen) and sequenced both strands of each DNA insert using appropriate primers. We confirmed that the full-length nucleotide and deduced amino acid sequence (Fig. 1) was identical to the Tip110/GAL1 reporter gene expression, which turned blue on the filter membrane (Fig. 2). In contrast, co-transformation of AD-LRPII or Clone 60 with BD-Tat transactivated the β-gal reporter gene expression, which turned blue on the filter membrane (Fig. 2). Co-transformation of the full-length Tip110 (AD-Tip110) with BD-Tat exhibited a blue color on the filter membrane, whereas no color was developed on the membrane when co-transformed with BTM116 (Fig. 2). These results confirmed that, like Clone 60, the full-length Tip110 indeed bound to Tat.

We then investigated whether Tip110 would bind to Tat in vivo. We transfected 293T cells with expression plasmids pTip110.His and pTat.Myc, which were tagged with 6-his epitope and Myc epitope, respectively, for immunodetection. Western blot analysis showed that both Tip110 (Fig. 3, panel WB:His) and Tat (Fig. 3, panel WB:Myc) were expressed in 293T cells at the expected molecular weights. We then performed immunoprecipitation of cell lysates for Tip110 followed by Western blotting for Tat. The results showed that Tip110 was detected in the immunoprecipitation complex of Tip110 only when Tat and Tip110 were co-expressed (Fig. 3, panel IP:His/WB:Myc). Similar results were obtained by immunoprecipitation for Tat followed by Western blotting for Tip110 (data not shown).

In corroboration with the previous results from the yeast two-hybrid assay, these results demonstrated that Tip110 can form a complex with Tat in vivo.
interaction, we constructed a series of Tip110 mutants that were deleted for the N-terminal HAT-rich domain, RRM domains, and/or NLS domain (Fig. 4c). We transfected 293T cells with Tat expression plasmid pTat.Myc, along with each of Tip110 mutants, and determined the complex formation between Tip110 and Tat, as described above. Tip110 mutants (Fig. 4b, panel WB:His) as well as Tat protein (Fig. 2b, panel WB:Myc) were expressed in 293T cells at the expected molecular weights. Transfection of the Tip110 mutants into 293T cells showed that deletion of the NLS domain (ΔNLS), RRM domain (ΔRRM), and both NLS and RRM domains (ΔCT) had no effects on Tip110-Tat complex formation (Fig. 2b, panel IP:Myc/WB:His). However, deletion of the HAT-rich domain (ΔNT) abolished Tip110 binding to Tat.

Similarly, we determined the domain(s) of Tat that are directly involved in interaction with Tip110. Structurally, HIV-1 Tat protein is divided into five distinct functional domains, i.e. the N-terminal domain (aa 1–21), the cysteine-rich domain (aa 22–37), the core domain (aa 38–48), the basic domain (aa 49–57), a C-terminal region (aa 58–72), and the second exon of variable lengths (45, 46) (Fig. 5a). We took advantage of a series of Tat deletion mutants in the context of pBTM116 that we previously constructed (33) and determined Tip110 interaction with each of Tat deletion mutants using the yeast two-hybrid assay. We transfected the yeast strain L40 with pAD-Tip110 and each of the Tat deletion mutants, as indicated, and plated out the transformants on the SD plates lacking tryptophan and leucine. The binding between Tip110 and Tat deletion mutants was determined for the β-gal gene expression by the filter β-gal assay. The results showed that, similar to pBD-Tat66 expressing the full-length Tat, deletion of the second exon (pBD-Tat72), the C-terminal region (pBD-Tat57), and the basic domain (pBD-Tat48) exhibited blue color on the membrane and thus, the β-gal gene expression (Fig. 5b). However, deletion of the core domain (pBD-Tat37) showed no color change on the membrane and, thus, no β-gal gene expression, nor did the deletion of the cysteine-rich domain (pBD-Tat21). These results were further confirmed by a GST “pull-down” assay using recombinant GST-Tat and Tip110 proteins and the mutants (data not shown). Based on these results, we concluded that the N-terminal HAT-rich domain of Tip110 and the core domain of Tat is directly involved in interaction between Tip110 and Tat.

No Direct Interaction between Tip110 and TAR—Interaction of Tip110 with Tat and the HIV-1 LTR Promoter

Specific Binding of Tip110 to Tat—To further determine the specificity of Tip110 interaction with Tat and also to identify which domain(s) of Tip110 are directly involved in Tip110-Tat
As expected (38), co-transformation of AD-IRP and MS2-IRP, or of AD-Tat and MS2-TAR, resulted in strong activation of the reporter gene H9252-gal expression by 146.8/110 and 7.5 units and 39.4/110 and 3.3 units, respectively, whereas transformation of hybrid RNA plasmid alone, hybrid protein plasmid alone, and/or their backbone control plasmids only exhibited a basal level of H9252-gal reporter gene expression (Table I, upper rows). However, compared with the H9252-gal gene expression, i.e. 1.2/110 and 0.4 units by co-transformation of AD-Tip110 and MS2, co-transformation of AD-Tip110 with MS2-TAR only resulted in a very marginal increase in the H9252-gal expression (5.5/110 and 1.1 units), which appeared to be nonspecific, as co-transformation of AD-Tip110 and MS2-RAT showed the H9252-gal expression by 3.8/110 and 0.9 units (Table I, middle rows). These results together suggest that there was no direct interaction between Tip110 and TAR.

Next, we determined whether Tip110 expression would have any effects on Tat-TAR interaction using the yeast three-hybrid assay. We transfected the yeast strain L40-coat with the MS2-TAR hybrid RNA plasmid, AD-Tat plasmid, as well as AD-Tip110 plasmid. Then, we compared the β-gal reporter gene expression between transfections with and without Tip110 plasmid, and with different amounts of AD-Tip110 plasmid. The AD backbone (pACT2) DNA was added to equalize the total amounts of DNA transfected. The results showed that Tip110 expression exhibited little or no change on the β-gal gene expression resulting from Tat-TAR interaction, as the β-gal expression in the transformation of AD-Tat and MS2-TAR was 42.6/110 and 4.2 units when no pAD-Tip110 was added, 39.4/110 and 3.3 units when 0.1/110 g of pAD-Tip110 was added, and 41.8/110 and 4.4 when 10-fold more pAD-Tip110 (1/110 g) was added (Table I, lower rows). Similar results were obtained using gel mobility retardation assay with Tip110 and Tat recombinant proteins and 32P-radiolabeled TAR probe (data not shown). These results further demonstrated that Tip110 did not bind to TAR, and suggest that Tip110 binding to Tat may occur independently of Tat-TAR interaction.

**Potentiation of Tat-mediated Gene Expression by Tip110**—Because the main function of HIV-1 Tat protein is to transactivate the HIV-1 LTR promoter in HIV-1 gene expression, we examined whether Tip110 interaction with Tat would affect Tat transactivation activity on the HIV-1 LTR promoter. First, we determined whether Tip110 expression alone has any effects on the HIV-1 LTR promoter. We transfected 293T cells with HIV-1 LTR promoter-driven CAT reporter plasmid pLTR-CAT and increasing amounts of Tip110 expression plasmid pTip110.His. We also included Tat expression plasmid pTat.Myc as a control. The vector backbone pcDNA3 was added to equalize the total amounts of DNA transfected, and pTKβGal was co-transfected to normalize for variations in transfection efficiency. As expected, Tat expression strongly transactivated the CAT gene in a dose-dependent manner (Fig.
All of the yeast three-hybrid components except pAD-Tip110 plasmid were obtained from Dr. M. Wickens and described elsewhere (38). The yeast strain L40-coat was transformed with 0.1 μg of each hybrid RNA plasmid and hybrid protein 2 plasmid, as indicated. The transformants were grown on an SD plate lacking tryptophan, leucine, and uracil for 48 h. Five random colonies from each transformation were inoculated into the SD medium, and determined for the β-gal gene expression using the liquid culture β-gal assay.* 1 μg of pAD-Tip110 was transfected. Data represent means ± S.E. from triplicate samples.

### Table I: Tip110 Interaction with Tat and the HIV-1 LTR Promoter

| Hybrid protein 1 | Hybrid RNA | Hybrid protein 2 | β-Gal activity |
|------------------|------------|------------------|----------------|
| MS2-coat         | MS2        | AD               | 0.5 ± 0.3      |
| MS2-IRE          | MS2-IRE    | AD               | 1.4 ± 0.8      |
| MS2              | MS2-IRE    | AD-IRP           | 2.1 ± 0.7      |
| MS2-IRE          | MS2-IRE    | AD-IRP           | 146.8 ± 7.5    |
| MS2              | MS2        | AD               | 0.7 ± 0.2      |
| MS2-IRE          | MS2-IRE    | AD-Tat           | 1.8 ± 0.5      |
| MS2-IRE          | MS2-IRE    | AD-Tat           | 39.4 ± 3.3     |
| MS2-IRE          | MS2-IRE    | AD-Tip110        | 0.4 ± 0.2      |
| MS2              | MS2        | AD-Tip110        | 1.2 ± 0.4      |
| MS2-IRE          | MS2-IRE    | AD-Tip110        | 5.5 ± 1.1      |
| MS2-IRE          | MS2-IRE    | AD-Tip110        | 3.8 ± 0.9      |
| MS2-IRE          | MS2-IRE    | AD-Tip110 + AD-Tip110 | 1.4 ± 0.6 |
| MS2-IRE          | MS2-IRE    | AD-Tip110 + AD-Tip110 | 42.6 ± 4.2 |
| MS2-IRE          | MS2-IRE    | AD-Tip110 + AD-Tip110 | 41.8 ± 4.4 |

Interestingly, Tip110 expression also exhibited a slight increase on CAT gene expression when more pTat.Myc was co-expressed with pAD-Tip110 (2.7 ± 0.4-fold). These results demonstrated that direct binding of Tip110 to Tat was required for the synergistic effects of Tip110 with Tat on the HIV-1 LTR promoter.

### Effects of Tip110 Expression on HIV-1 Viral Gene Expression

—We next investigated whether Tip110 effects on the HIV-1 LTR promoter could also be obtained in the context of HIV-1 genome. We transfected 293T cells with a recombinant HIV-1 proviral DNA plasmid pNL4–3 (47) and Tip110 expression plasmid pTip110.His. We also included the Tip110ΔNT mutant in the transfections. pcDNA3 was added to equalize the total amounts of the DNA transfected, whereas pEGFP was transfected to ensure a comparable level of transfection efficiency among the transfections. HIV-1 viral gene expression in 293T cells was monitored by measuring the RTase activity of the cell culture supernatants. At day 3 after transfection, Tip110 expression increased HIV-1 virus production by more than 3.5-fold (Fig. 7a). However, co-expression of Tip110ΔNT mutant showed fewer effects. These data further support the notion that Tip110 can transactivate the HIV-1 LTR in cooperation with Tat through direct interaction.

To determine the significance of Tip110 protein in Tat-mediated HIV-1 gene expression, we down-modulated constitutive Tip110 expression and determined its effects on HIV-1 gene expression. We took advantage of the antisense RNA expression vector-based strategy, which has successfully and widely been used to study the function of a number of genes (for review, see Ref. 48). We made an anti-Tip110 antisense RNA expression plasmid pAs-Tip110 that expressed the Tip110 cDNA in a reverse orientation in the context of pcDNA3 backbone. We transfected 293T cells with pNL4–3 HIV-1 proviral DNA and different amounts of pAs-Tip110 plasmid DNA, and we determined HIV-1 viral gene expression. Western blot analysis showed that transient expression of the anti-Tip110 antisense RNA down-modulated constitutive Tip110 protein expression in a dose-dependent manner (Fig. 7b), whereas the housekeeping gene actin exhibited no detectable changes in expression. Corresponding with Tip110 down-regulation, a parallel inhibition of HIV-1 viral gene expression, measured by the RTase activity, was also noted in 293T cells (Fig. 7c, open bar). In contrast, expression of anti-human β-globin antisense RNA (34), which we included as a control, had no detectable effects on HIV-1 virus production (Fig. 7c, closed bar). Taken together, these results suggest that Tip110 plays an important role in HIV-1 gene expression through interaction with Tat.

### Tip110 mRNA Expression and Protein Intracellular Localization

—We next determined Tip110 expression at the mRNA level by Northern blotting analysis. We used a human multiple tissue Northern blot from CLONTECH. Consistent with the size of the Tip110 cDNA published by others (42) and determined by ourselves, the results showed a band of 3.6 kb in all of the human tissues tested, including brain, heart, skeletal muscle, colon, thymus, spleen, kidney, liver, small intestine, placenta, lung, and peripheral blood lymphocytes (Fig. 8a). There were some variations of the Tip110 mRNA expression among the tissues. A higher level of expression was detected in placenta, lung, and peripheral blood lymphocytes (Fig. 8a).
293T cells were transfected with 0.1 \( \mu \)g of pLTR-CAT expression plasmid, along with various amounts of pTat.Myc (open bar) or pTip110.His DNA (closed bar), as indicated. Transfected cells were harvested 48 h after transfection for the CAT assay. b, TAR-dependent synergistic effects of Tip110 and Tat on the HIV-1 LTR promoter. 293T cells were transfection with 0.1 \( \mu \)g of pLTR-CAT (shaded bar) or pLTR\_TAR-CAT (dotted bar), 1 ng of pTat.Myc, and various amounts of pTip110.His DNA, as indicated. c, direct binding of Top110 to Tat is required for the synergistic effects of Tip110 and Tat on the HIV-1 LTR promoter. 293T cells were transfection with 0.1 \( \mu \)g of pLTR-CAT, 1 ng of pTat.Myc, and 1 ng of each Tip110 mutant expression plasmid. In all of these transfections, pcDNA3 backbone DNA was added to equalize the total amounts of DNA transfected and pTK\_Gal (CLONTECH) was also included to normalize transfection variations among transfections. Data represent means \( \pm \) S.E. from triplicate samples.

![Figure 6](image-url)  
**Fig. 6.** Synergistic effects of Tip110 with Tat on the HIV-1 LTR promoter. a, Tip110 as a weak transactivator of the HIV-1 LTR promoter. b, TAR-dependent synergistic effects of Tip110 and Tat on the HIV-1 LTR promoter. c, direct binding of Top110 to Tat is required for the synergistic effects of Tip110 and Tat on the HIV-1 LTR promoter.

**Fig. 7.** Tip110 is required for HIV-1 gene expression. a, enhancement of HIV-1 gene expression by Tip110. 293T cells were transfected with 0.1 \( \mu \)g of pNL4-3 proviral DNA alone (open bar), with 5 ng of pTip110.His (closed bar), or with 5 ng of pTip110\_ANT.His DNA (shaded bar). Cell culture supernatants were collected at the time points as indicated and determined for HIV-1 virus production using the RTase activity assay. b, and c, inhibition of HIV-1 gene expression by down-modulation of constitutive Tip110 expression. 293T cells were transfected with 0.1 \( \mu \)g of pNL4-3 proviral DNA, and the anti-Tip110 antisense RNA expression vector pAs-Tip110 at the amounts as indicated. Cells were harvested 3 days after transfection for whole cell lysates (b), whereas the cell culture supernatants were collected for RTase activity assay (c). Tip110 expression was determined by Western blot using the anti-Tip110 serum (1:200) as described under "Materials and Methods." The housekeeping gene actin expression was also analyzed by Western blot using anti-actin antibody (Santa Cruz). The relative expression level of Tip110 protein (Rel.) was determined by densitometry scanning of the blots and expressed as a fraction of the housekeeping gene actin. The one without pAs-Tip110 expression (lane 1) was set as 1.00. As-DNA, pAs-Tip110 (open bar) or pZeo\_AS (closed bar). Data represent means \( \pm \) S.E. from triplicate samples (a and c).

![Figure 7](image-url)  
**Fig. 7.** Tip110 is required for HIV-1 gene expression. a, enhancement of HIV-1 gene expression by Tip110. b, and c, inhibition of HIV-1 gene expression by down-modulation of constitutive Tip110 expression.

In this study, we report a novel HIV-1 Tat-interacting protein and its potentiating activity on Tat-mediated gene expression and HIV-1 virus production. We initially isolated the partial cDNA during the yeast two-hybrid cloning. Sequence analysis of the partial and full-length cDNA revealed that the cDNA was almost identical to the KIAA0156 gene. In vitro coupled transcription/translation analysis demonstrated exclusive nuclear localization of GFP, in a distinct nuclear speckled pattern. However, GFP when fused with Tip110 resulted in exclusive nuclear localization of GFP, in a distinct nuclear speckled pattern.

**Discussion**

In this study, we report a novel HIV-1 Tat-interacting protein and its potentiating activity on Tat-mediated gene expression and HIV-1 virus production. We initially isolated the partial cDNA during the yeast two-hybrid cloning. Sequence analysis of the partial and full-length cDNA revealed that the cDNA was almost identical to the KIAA0156 gene.
strated that the full-length cDNA encoded a protein of 110 kDa (data not shown). Thus, we re-named the gene as a Tat-interacting protein of 110 kDa, or Tip110. The KIAA0156 gene was earlier cloned from a human myeloid cell line KG-1 cDNA library with unknown functions (42). Subsequent studies have implicated this gene in RNA metabolism (49) and in tumor antigen presentation (50). Our studies demonstrate that KIAA0156, or Tip110 as we termed it, exhibits strong synergistic effects with Tat through direct interaction. In addition, we have also identified a 11-bp deletion isoform of Tip110, which encodes a protein containing only the N-terminal 350 amino acids of Tip110 (Fig. 1), suggesting that alternate splicing may be involved in the regulation of Tip110 expression level and function.

We confirmed in vivo interaction between the full-length Tip110 and Tat using the yeast two-hybrid assay (Fig. 2) and Western blot with combined with immunoprecipitation (Fig. 3). The direct and specific binding was further supported by the data obtained from mutagenesis analysis (Figs. 4 and 5) and a GST pull-down assay (data not shown). The protein structure analysis predicted that Tip110 contained seven motifs of HAT, a well conserved protein-protein interaction module, structurally consisting of two antiparallel alpha helices (for review, see Ref. 51). Consistent with the role of TPRs, our studies identified the N-terminal HAT-rich domain of Tip110 that was responsible for interaction of Tip110 and Tat (Fig. 4). In addition, our data suggest that multiple copies of HATs may be able to form functional TPR(s) and thus, determine the specificity of protein-protein interaction.

Like many Tat-interacting proteins/co-factors, Tip110 was found to interact with the core domain of Tat (Fig. 5), which is essential for Tat transactivation function. Although Tip110 contained two RNA recognition motifs (Fig. 1) and has been shown to bind to RNA (49), Tip110 did not exhibit the TAR binding activity, or any effects on Tat-TAR interaction, determined by the yeast three-hybrid RNA-protein interaction assay (Table I and the gel mobility retardation assay (data not shown). Transient expression of Tip110 in 293T cells showed transactivation activity on the HIV-1 LTR promoter (Fig. 6a), and also on the TAR-deleted LTR promoter (Fig. 6b). Importantly, co-expression of Tip110 and Tat greatly potentiated Tat transactivation function on the LTR-driven reporter gene assay (Fig. 6b). Interestingly, although Tip110 did not bind to TAR (Table 1), the synergistic effects were TAR-dependent, as these effects were attenuated by deletion of the TAR sequence from the HIV-1 LTR promoter (Fig. 6b). Furthermore, the synergistic effects were also dependent on the Tat binding activity, as deletion of the Tat-binding HAT-rich domain of Tip110 (Tip110ΔNT) resulted in no changes on Tat-mediated reporter gene expression (Fig. 6c). These data together demonstrate that Tip110 is capable of targeting TAR through interaction with Tat, as well as interacting with other regulatory regions or elements other than TAR within the HIV-1 LTR promoter.

Consistent with the LTR-driven CAT reporter gene assay, our results also showed that Tip110 expression also enhanced HIV-1 viral gene expression (Fig. 7a). The enhancement effects were correlated with Tat binding activity of Tip110, as Tip110ΔNT mutant exhibited no effects on HIV-1 gene expression (Fig. 7a). Surprisingly, down-modulation of constitutive Tip110 expression inhibited HIV-1 viral gene expression in a dose-dependent manner (Fig. 7c). These results support an essential role of Tip110 in the process of Tat-mediated HIV-1 gene expression. Because TPR-containing proteins have been shown to be often associated with multiprotein complexes and involved in functioning of chaperone, cell-cycle, transcription, and protein transport (for review, see Ref. 4), we speculate that Tip110 may be part of the transcription elongation complex P-TEFb. This is further supported by our findings that Tip110 was localized exclusively in a nuclear structure called nuclear speckles (Fig. 9), in which cyclin T and CDK9, two critical components of the P-TEFb complex, have recently been localized (52). Nevertheless, we were not able to detect any direct interaction between Tip110 and cyclin T and/or CDK9 (data not shown). In addition, many cellular splicing factors are localized in the nuclear speckled structure, where the post-transcriptional mRNA processing is believed to occur (53–56). Our studies did not rule out the possibility that Tip110 binding to Tat may play a role in the splicing process of HIV-1 viral mRNA transcripts and/or other Tat-mediated processes, such as HIV-1 reverse transcription (57). Therefore, additional studies are needed to address these questions.

![Fig. 8. Ubiquitous expression of Tip110 mRNA in human tissues (a) and cell lines (b).](image)

Northern blots containing RNAs from multiple human tissues (CLONTECH) and RNAs from various cell lines (human fetal brain RNA obtained from Invitrogen) were probed with 32P-radiolabeled Tip110 cDNA as described above. The probes on the blots were stripped in 0.1% SDS at 100 °C for 10 min and then re-probed with a human actin probe (CLONTECH) as a RNA loading control. The relative expression level of Tip110 mRNA was determined by densitometry scanning of the blots and expressed as a fraction of the housekeeping gene actin mRNA.

![Fig. 9. Subcellular localization of Tip110 protein.](image)

293T cells were transfected with 5 μg each of pEGFP (upper row), pTip110.GFP, or pTip110.GFPΔNLS expression plasmid, and cultured for 48 h. The transfected cells were then fixed in 2% paraformaldehyde and stained in 1 ng/ml DAPI (Sigma). Images of the cells (bright field), GFP (green) localization, and the nucleus (blue) were captured using the digital fluorescence microscope (Zeiss). Subcellular localization was determined by overlaying images of GFP and the nucleus, shown as GFP/DAPI.
needed to define the molecular mechanisms of Tip110 function in Tat-mediated HIV-1 gene expression.

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