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Biochemistry

HIV-1 Tat transactivator recruits p300 and CREB-binding protein histone acetyltransferases to the viral promoter

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ABSTRACT **In cells infected with HIV type 1 (HIV-1), the integrated viral promoter is present in a chromatin-bound conformation and is transcriptionally silent in the absence of stimulation. The HIV-1 Tat protein binds to a stem-loop structure at the 5′ end of viral mRNA and relieves this inhibition by inducing a remodeling of the nucleosome arrangement downstream of the transcription-initiation site. Here we show that Tat performs this activity by recruiting to the viral long terminal repeat (LTR) the transcriptional coactivator p300 and the closely related CREB-binding protein (CBP), having histone acetyltransferase (HAT) activity. Tat associates with HAT activity in human nuclear extracts and binds to p300 and CBP both *in vitro* and *in vivo*. Integrity of the basic domain of Tat is essential for this interaction. By a quantitative chromatin immunoprecipitation assay we show that the delivery of recombinant Tat induces the association of p300 and CBP with the chromosomally integrated LTR promoter. Expression of human p300 in both human and rodent cells increases the levels of Tat transactivation of the integrated LTR. These results reinforce the evidence that p300 and CBP have a pivotal function at both cellular and viral promoters and demonstrate that they also can be recruited by an RNA-targeted activator. Additionally, these findings have important implications for the understanding of the mechanisms of HIV-1 latency and reactivation.**

In the cell nucleus, the eukaryotic genome is packaged into a highly condensed chromatin structure. The fundamental subunit of chromatin is the nucleosome core, formed by the wrapping of DNA around an octamer of core histone proteins. Nucleosomes negatively regulate gene expression by restricting access to DNA-binding factors and by impeding elongation by RNA polymerase II (1–4).

In cells infected with HIV type 1 (HIV-1), the integrated proviral genome is also tightly packaged into chromatin. In particular, the viral long terminal repeat (LTR), which acts as a very strong promoter when analyzed as naked DNA *in vitro* (see, for example, ref. 5), is almost silent when integrated into the cellular genome in the absence of stimulation (6, 7). Nuclease accessibility studies of the proviral chromatin structure indicate that the LTR, independently of the integration site, is incorporated into two distinct nucleosomes, separated by a nuclease-hypersensitivity region containing the enhancer and basal promoter elements (8–10). Through genomic footprinting studies, we have shown that in the silent LTR, several critical protein–DNA interactions are still preserved in this region (11, 12).

The Tat protein of HIV-1 is a powerful activator of viral gene expression from the integrated LTR. The protein binds to TAR, a highly structured RNA element located at the 5′ end of all viral transcripts (13), and is active at the level of transcriptional initiation by augmenting the rate at which the cellular RNA polymerase II starts transcription, and at the level of transcriptional elongation by increasing the processivity of the polymerase (for reviews, see refs. 14 and 15). In an exciting crescendo of findings, a converging number of observations recently have indicated that the role of Tat in transcriptional elongation can be ascribed to the specific interaction of the factor with protein complexes possessing protein kinase activity and being able to phosphorylate the carboxyl-terminal domain of the larger subunit of RNA polymerase II (5, 16–19). This is an essential step for the recruitment of processive transcriptional complexes on the LTR promoter.

While these data contribute to the elucidation of the functions of Tat in transcriptional processivity, some important questions still are unanswered. In fact, it remains to be explained how Tat relieves the block in transcriptional initiation imposed on the LTR by chromatin. When transcription is activated, the chromatin associated with sequences immediately downstream of the transcription start site becomes accessible to nucleases (9). In particular, remodeling of the chromatin structure can be induced by Tat, but not by other stimuli acting through the upstream enhancer sequence (20). Chromatin remodeling associated with activation of transcription generally is accomplished by reversible acetylation of lysine residues in the amino-terminal domains of core histones H2A, H2B, H3, and H4. This modification, induced by proteins having histone acetyltransferase (HAT) activity, weakens histone—DNA interactions, thereby relieving the repressive effects of the chromatin scaffold (for reviews, see refs. 21 and 22). Consistently, the silent, integrated LTR also can be strongly activated by drugs inducing sustainedly high levels of histone acetylation in latently infected cell lines (23–25).

Altogether, these observations strongly suggest that histone acetylation at the LTR promoter plays a key role in the activation of HIV transcription. We therefore have explored the possibility that the function of Tat in transcriptional initiation could be ascribed to the recruitment of HAT proteins to the viral promoter. Our results demonstrate that Tat associates with p300 and with the closely related CREB-binding protein (CBP) HATs both *in vitro* and *in vivo* and that it targets these proteins to the integrated LTR promoter. Overexpression of p300 both in human and in rodent cells increases Tat-mediated transactivation of the integrated LTR promoter.

MATERIALS AND METHODS

Plasmids. Plasmid pCMV-Tat101 was constructed by cloning the cDNA of wild-type 101-aa Tat in pcDNA3 (Invitrogen). Plasmids pBS-KS+hTAF32, containing the cDNA of human TBP-associated factor 32 (TAF32), was kindly provided by R.

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Abbreviations: HAT, histone acetyltransferase; CBP, CREB-binding protein; HIV-1, HIV type 1; LTR, long terminal repeat; GST, glutathione *S*-transferase; CAT, chloramphenicol acetyltransferase.

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Tjian (University of California, Berkeley). Plasmid pcDNA3-p300 was constructed by cloning the cDNA of p300 (obtained from plasmid pCMVβp300, a gift from D. M. Livingston, Dana–Farber Cancer Institute, Boston) in pcDNA3. Plasmid pULBLTR-CAT contains the chloramphenicol acetyltransferase (CAT) gene downstream of the LTR promoter (26).

Recombinant Proteins. Glutathione *S*-transferase (GST), GST-Tat, and GST-Tat mutants were prepared as already described (11). Plasmids pBS-KS+hTAF32 and pcDNA3-p300 were used as templates to produce the *in vitro* ^{35}S-labeled hTAF32 and p300 proteins, respectively, by using the TNT Reticulocyte Lysate System (Promega) according to the manufacturer's protocol.

***In Vitro* Binding Assays.** To remove contaminant bacterial nucleic acids, recombinant proteins were pretreated with nucleases (0.25 unit/μl DNase I and 0.2 μg/μl RNase) for 1 hr at 25°C in 50 mM Tris·HCl, pH 8/5 mM $MgCl_2$/2.5 mM $CaCl_2$/100 mM NaCl/5% glycerol/1 mM DTT. Subsequently, GST fusion proteins immobilized on agarose beads were washed and resuspended in NETN buffer (20 mM Tris·HCl, pH 7.5/100 mM NaCl/1 mM EDTA/0.5% Nonidet P-40/1 mM DTT/1 mM phenylmethylsulfonyl fluoride) supplemented with 0.2 mg/ml ethidium bromide to impede the possible formation of nonspecific interactions between residual DNA and proteins. Six hundred cpm of ^{35}S-labeled p300 or hTAF32 proteins was added and incubated at 4°C on a rotating wheel. After 1 hr, bound proteins were washed five times with 1 ml of NETN buffer and separated by electrophoresis in an SDS/7% polyacrylamide gel. Dried gels were quantitated by INSTANT IMAGER (Packard).

GST Pull-Down Assays for HAT Activity. Two micrograms of glutathione–agarose-immobilized proteins in a final volume of 1 ml of IPH buffer (50 mM Tris·HCl, pH 8/150 mM NaCl/5 mM EDTA/1 mM DTT/0.5% Nonidet P-40/1 mM phenylmethylsulfonyl fluoride) with the addition of 0.2 mg/ml ethidium bromide was incubated with 200 μg of Dignam nuclear extract at 4°C for 2 hr. Bound proteins were washed five times with 1 vol of IPH buffer and subsequently assayed for HAT activity.

HAT Assays. Protein samples obtained by immunoprecipitation or GST pull-down assay were analyzed as described (27). Acetylated histones were detected by autoradiography after separation by SDS/PAGE. Alternatively, incorporated [^{14}C]acetyl groups were measured by scintillation counting after spotting the samples on p-81 filters (Whatman) and extensive washing with 0.5 M $NaHCO_3$, pH 9.2.

Cells and Transfections. A hamster cell line containing the integrated LTR-CAT (CHO/LTR-CAT) was obtained by calcium phosphate transfection of plasmid pULBLTR-CAT and pcDNA3 in CHO-K1 cells, followed by selection for neomycin-resistant clones with 500 μg/ml G418 (GIBCO/BRL). HL3T1 cells, kindly donated by B. Felber (28), are a HeLa derivative cell line containing an integrated LTR-CAT construct. CHO and HL3T1 cells were grown in DMEM and Ham's F10 medium, respectively, supplemented with 10% fetal calf serum/2 mM glutamine/50 μg/ml gentamicin. Cells were transfected by the standard calcium phosphate procedure (29). All transfections were adjusted to the same content of transfected plasmid [and of cytomegalovirus (CMV) promoter sequences] by addition of the appropriate amounts of pcDNA3. CAT assays were performed 48 hr after transfections; the results shown in Fig. 5 represent the average values obtained in several (at least three) independent transfections.

Treatment with Recombinant Proteins. HL3T1 cells were grown overnight to reach about 80% confluence. Cells then were treated with 1 μg/ml GST or GST-Tat proteins and 100 μM chloroquine or Lipofectin (GIBCO/BRL) according to a published procedure (11). After a 5-hr incubation, cells were washed four times with ice-cold PBS, scraped off the plates, and lysed in 1 ml RIPA lysis buffer 50 (50 mM Tris·HCl, pH 7.5/50 mM NaCl/1% Nonidet P-40/1% sodium deoxycholate/0.1% SDS/2 mM EDTA) with protease inhibitors (500 μM phenylmethylsulfonyl fluoride/1 μM leupeptin/1 μM pepstatin). The cell lysate was passed through a 24-gauge needle and centrifuged for 10 min at 14,000 rpm in an Eppendorf bench centrifuge at 4°C. The cleared supernatant was used for immunoprecipitation.

Immunoprecipitation and Immunoblotting. Cleared cell lysates were incubated with the appropriate antibodies overnight at 4°C. After incubation, 40 μl of a 50% suspension of protein-A-Sepharose CL-4B beads (Pharmacia) in RIPA buffer was added. After a 2-hr incubation at 4°C, beads were washed three times with 1 ml of RIPA buffer 150 (RIPA lysis buffer with 150 mM NaCl). Samples then were assayed for HAT activity or analyzed by Western blotting using the indicated antibodies. The membrane was developed by the enhanced chemiluminescence kit (Amersham). All antibodies were obtained from Santa Cruz Biotechnology, with the exception of the anti-Tat antiserum [National Institutes of Health (NIH) AIDS Research and Reference Reagent Program of the NIH, contributed by B. Cullen] and of the anti-Tau antibody (kind gift of M. Novak, International School for Advanced Studies at Trieste, Italy).

Chromatin Immunoprecipitation. HL3T1 cells were treated with GST or GST-Tat as described above. After a 5-hr incubation, protein–DNA complexes were fixed by formaldehyde and treated as described in ref. 30. Chromatin pellets were resuspended in 500 μl of RIPA lysis buffer 50 with protease inhibitors (500 μM phenylmethylsulfonyl fluoride/1 μM leupeptin/1 μM pepstatin; Sigma) and subjected to 20 cycles of 10-sec sonication on ice. Sonicated samples were centrifuged to spin down cell debris and immunoprecipitated as described above. Protein-bound immunoprecipitated DNA was resuspended in 100 μl of TE buffer (10 mM Tris·HCl, pH 8.0/1 mM EDTA) and digested with 5 units of DNase-free RNase (Boehringer Mannheim) for 30 min at 37°C. The samples were treated successively for 3 hr at 56°C with 300 μg/ml proteinase K (Sigma) in 0.5% SDS/100 mM NaCl and for 6 hr at 65°C to revert cross-links. DNA was extracted with phenol/chloroform/isoamyl alcohol, precipitated with ethanol, and resuspended in 100 μl of water for quantification.

DNA Quantification by Competitive PCR. Primer sequences and amplification conditions already have been described for LTR-CAT (11), B48, and B13 (31) and β-globin (32). The multicompetitor DNA fragment was constructed by a recombinant PCR procedure as already described (33) and outlined in Fig. 4. Competitive PCR experiments were carried out by mixing a fixed amount of immunoprecipitated DNA with increasing amounts of competitor, followed by amplification with each of the four primer pairs. A detailed outline of the competitive PCR protocol was published elsewhere (33, 34).

RESULTS

Tat Associates with a HAT Activity *in Vivo* and *in Vitro*. We investigated the ability of HIV-1 Tat protein to interact with HAT enzymes. HL3T1 cells, a HeLa-derivative cell line carrying an integrated HIV-1 LTR-CAT construct, were treated with recombinant wild-type Tat (Fig. 1*a Left*), by exploiting the property of the protein to enter intact cultured cells (11, 35). Five hours after protein delivery, cell lysates were obtained, immunoprecipitated with an anti-Tat antibody, and analyzed for the ability to acetylate purified histones in the presence of radiolabeled acetyl-CoA. Using this procedure, we recovered HAT activity from the lysates of cells treated with wild-type Tat but not from cells treated with GST. To confirm the specificity of the interaction between Tat and HAT protein(s), we also expressed wild-type Tat in HL3T1 cells by transfection of an eukaryotic expression vector containing the wild-type Tat cDNA (Fig. 1*a Right*). Consistently, immunoprecipitation with an anti-Tat antibody specifically recovered HAT activity, whereas only background activity was detected in the immunoprecipitate obtained by the use of an anti-GST antibody. It must be noted that the HAT activity coimmunopre-

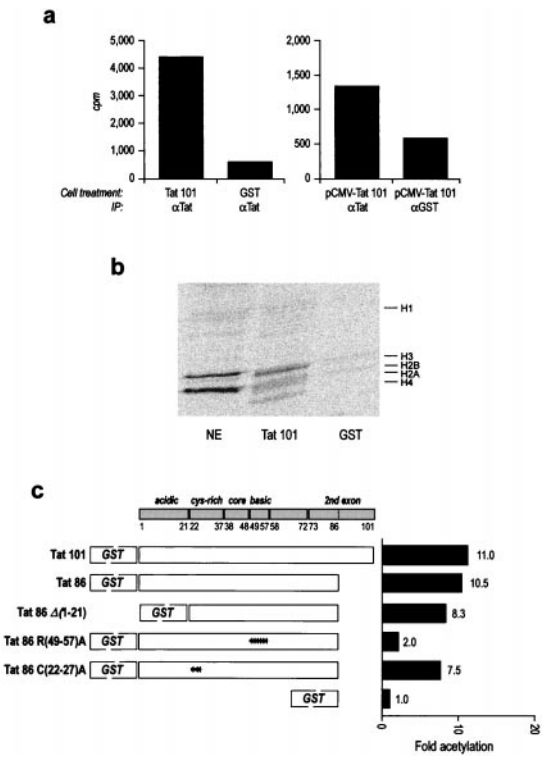

FIG. 1. Association of Tat with HAT activity. (*a*) Tat coimmunoprecipitates with a HAT activity. Whole-cell extracts from HL3T1 cells treated with GST-Tat 101 or GST (*Left*) or transfected with a Tat-expressing vector (*Right*) were immunoprecipitated with the indicated antibodies. Immunoprecipitates were analyzed for HAT activity by liquid scintillation counting. (*b*) GST-Tat pulls down a HAT activity specific for all four core histones. A Dignam nuclear extract from HeLa cells was incubated with GST-Tat 101 or GST on agarose beads. Bound proteins were assayed for HAT activity; acetylated histones were resolved by SDS/PAGE and detected by autoradiography. NE, pattern of histone acetylation by the nuclear extract before pull-down. (*c*) The arginine-rich domain of Tat is involved in the association with HAT activity. GST pull-down experiments were performed as in *b* with the indicated proteins. The results are expressed as fold acetylation with respect to GST.

cipitated with recombinant Tat is severalfold stronger than that associated with plasmid-expressed Tat. As verified by Western blotting (not shown), this is most likely because of the higher concentration of Tat in the cell nucleus resulting from direct protein delivery as opposed to transfection of the expression vector.

We next determined the specific pattern of histone acetylation of the Tat-associated HAT. Agarose bead-immobilized wild-type (wt) Tat or GST was used in pull-down experiments on nuclear extract (Fig. 1*b*). We assayed the HAT activity of the proteins thus recovered and that of an aliquot of nuclear extract before treatment. All four core histones, H3, H2B, H2A, and H4, were acetylated by the Tat-associated HAT, the band of H3 being the most intense. Interestingly, the pattern of histone acetylation observed in total nuclear extracts is substantially different from that associated with Tat, suggesting that the latter HAT activity is not the predominant one present in the nuclear extract.

To determine the domains of Tat that are important for association with HAT activity, GST pull-down experiments were performed by using wt Tat 101 (present in several primary HIV isolates), wt Tat 86 (HXB2 clone), and some mutated derivatives of the latter [Tat 86 Δ(1–21), lacking the amino-terminal acidic domain; Tat 86R(49–57)A, with six arginines in the basic domain mutated to alanines; and Tat 86C(22–27)A, with three cysteines mutated to alanines in the cysteine-rich domain]. As shown in Fig. 1*c*, wild-type 101 and 86 Tat proteins bound the HAT activity present in the nuclear extract with similar efficiencies. The affinity was only slightly decreased in mutants Tat 86 Δ(1–21) and Tat 86C(22–27)A. On the contrary, amino acid substitutions of the arginines in the arginine-rich domain almost abolished association of Tat with the HAT activity.

Tat Binds p300 and CBP *in Vivo*. The results reported above demonstrate that Tat associates with a HAT activity capable of acetylating all four core histones. Several HAT enzymes so far have been identified and characterized in terms of molecular structure and substrate specificity (for review, see refs. 36 and 37). Among the known nuclear HATs, only p300 and CBP are capable of acetylating all four core histones (38–40). Therefore, we assessed the association of Tat with p300 and CBP *in vivo* by coimmunoprecipitation studies using lysates of cells treated with Tat (Fig. 2). Immunoprecipitation with an anti-CBP or an anti-p300 antibody resulted in the coimmunoprecipitation of CBP or p300 as well as Tat in Tat- but not GST-treated cells. Accordingly, immunoprecipitation with an anti-Tat antibody recovered Tat as well as CBP and p300. None of the three proteins was immunoprecipitated by an unrelated control antibody. These results prove that the interaction between Tat and p300/CBP occurs also within the cell.

Tat Binds p300 *in Vitro*. Does Tat directly interact with p300/CBP or is the binding mediated by another cellular component present in the complex? To answer this question, we assayed the ability of immobilized GST-Tat to bind to *in vitro*-translated [^{35}S]p300. We found that p300 but not another protein such as hTAF32 (Fig. 3*a*) or luciferase (not shown) specifically binds to Tat. Thus, there is probably a direct interaction between the two proteins, although we cannot exclude the possibility that an unknown component of the translation lysate mediates indirect binding.

We mapped the sites of interaction of Tat with p300 by using a series of mutated Tat derivatives (Fig. 3 *b* and *c*). The transcriptionally active proteins (Tat 101, Tat 86, and Tat 72), as well as the protein mutated in the cysteine-rich domain efficiently bound to p300 (Fig. 3*b*). On the contrary and in remarkable agreement with the results obtained studying the association of Tat with HAT activity (Fig. 1*c*), the interaction of Tat with p300 strongly depended on the integrity of the arginine-rich domain (Fig. 3*c*). Deletion of the N-terminal 21 aa (one of the regions that are essential for the interaction of Tat with cellular cofactors)

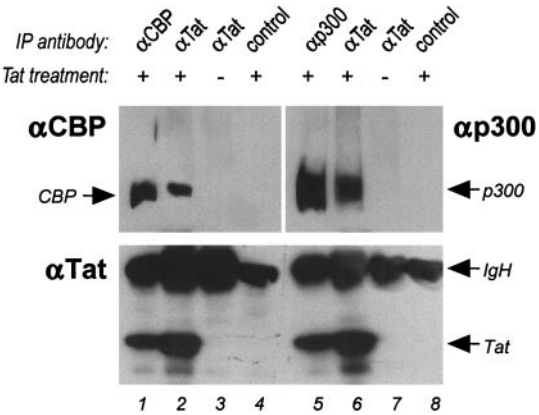

FIG. 2. Association of Tat with p300 and CBP *in vivo*. Whole-cell lysates prepared from HL3T1 cells either untreated (lanes 3 and 7) or treated with GST-Tat added to the culture medium in the presence of chloroquine (lanes 1, 2, 4–6, and 8) were immunoprecipitated with the indicated antibodies. Bound proteins were resolved by SDS/PAGE (5% acrylamide, *Upper*; 10%, *Lower*) and transferred to a nitrocellulose membrane, which subsequently was cut into three parts. The lower portion of the filter was reacted with an anti-Tat antibody, while the upper two parts were reacted with anti-CBP (*Upper Left*) or anti-p300 (*Upper Right*) antibodies. The positions of the p300, Tat, and CBP proteins are indicated. IgH, Ig heavy chain.

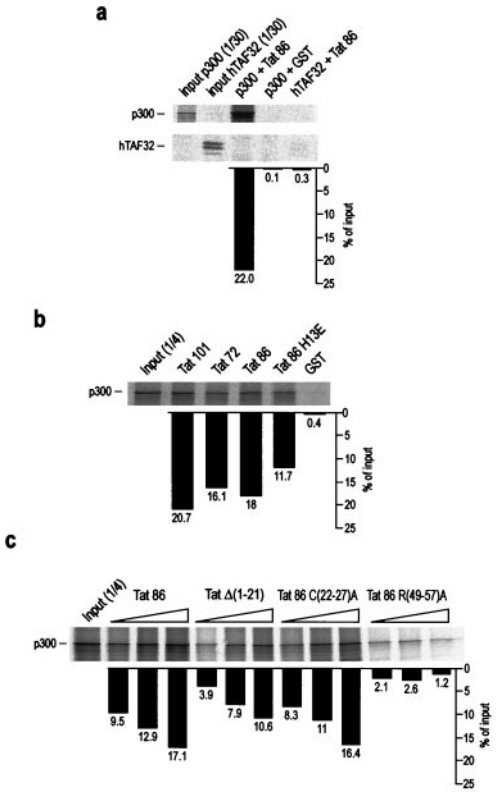

FIG. 3. Interaction of Tat with p300 *in vitro*. The indicated GST fusion proteins (5 μg) on agarose beads were incubated with ^{35}S-labeled p300 or hTAF32 (negative control). Bound proteins are expressed as percentages of the input radiolabeled protein. (*a*) GST-Tat binds specifically to p300. (*b*) Effects of truncations at the C terminus of Tat. (*c*) Binding of p300 to Tat mutants. Immobilized GST fusion proteins (1.5, 3, and 6 μg) were incubated with ^{35}S-labeled p300. Samples were processed as in *a*.

lowered the affinity of Tat for p300 (Fig. 3*c*). The same was observed in a Tat protein bearing a single point mutation in this domain (histidine at position 13 to glutamine; Fig. 3*b*). Consistently, this mutant is a very poor transactivator of the LTR (less than 5% of wild type; not shown). Mutation at this residue recently was detected in the defective integrated provirus of the latently infected U1 monocytic cell line (41).

The suppression of Tat–p300 interaction *in vitro*, resulting from mutations of the six arginines in the basic domain, suggests a direct role of the basic domain in the complex formation. Nevertheless, it cannot be excluded that such a modification could drastically alter the tertiary structure of the protein.

Tat Recruits p300 and CBP to the LTR. An essential issue of our research study was to understand whether the interaction of Tat with p300/CBP takes place specifically on the integrated promoter. We analyzed the chromosomal events involved in Tat transactivation by a procedure used for quantitative chromatin immunoprecipitation (Fig. 4). This technique is based on the crosslinking of protein—DNA and protein—protein complexes within the cell by formaldehyde treatment (30), followed by chromatin sonication, immunoprecipitation with specific antibodies, and precise quantification of the immunoprecipitated DNA segments by competitive PCR (Fig. 4*d*). This procedure quantitatively assesses the *in vivo* direct or indirect binding of a given protein to a defined chromosomal region. Four different genomic sites were investigated in HL3T1 cells: HIV-1 LTR, two regions of the lamin B2 gene domain [B48, close to a human origin of DNA replication (33), and B13, ≈7 kb away from the origin], and one region in the β-globin gene (Fig. 4*a*). We used as a competitor a single DNA fragment containing all four primer pairs arranged to generate PCR products of different lengths from the ones obtained from genomic DNA (Fig. 4*b*). The competitive PCR quantifications were carried out by the addition of increasing amounts of the multicompetitor to a fixed volume of immunoprecipitated DNA, followed by PCR amplification of aliquots of the mixture with the appropriate primer pairs.

Analysis of protein interactions at the selected regions was performed in HL3T1 cells after treatment with Tat or GST. In both cases, immunoprecipitation with an antibody against cellular transcription factor USF resulted in the enrichment for the DNA segments encompassing the LTR and the B48 region (Fig. 4*e*; the actual competitive PCR results for the quantification of anti-USF immunoprecipitates in GST-treated cells are shown in Fig. 4*c*). This finding is consistent with our previous results showing that the LTR and B48 regions are targets for USF and that both sequences actually are bound by the protein *in vivo* (42, 43). Strikingly, in the absence of Tat both the anti-CBP and the anti-p300 antibodies failed to immunoprecipitate the LTR DNA segment as well as the other segments. After Tat treatment, a remarkable enrichment for this genomic region (10-fold for p300 and 33-fold for CBP) was observed (Fig. 4*e*). These data demonstrate that Tat-mediated activation of the integrated LTR *in vivo* is concomitant with the recruitment of p300 and CBP specifically to the promoter region.

Overexpression of p300 Enhances Tat Transactivation. Expression of p300 and CBP in human HeLa cells is constitutive and relatively high, as detected by Western blotting on total cell lysates. Consequently, cotransfection of p300 in HL3T1 cells had only a modest, although reproducible, positive effect on Tat-mediated transactivation of the LTR (≈2-fold increase over Tat alone; data not shown). Conversely, the synergistic effect of p300 and Tat could be better observed in a hamster CHO cell line. This cell line was obtained by transfection of an LTR-CAT cassette and selection for stable integration. As in other rodent cells, Tat activity in these cells is poor when suboptimal amounts of Tat are transfected (Fig. 5, 50 ng of Tat per plate). This is in agreement with the well established idea that rodent cells do not support efficient Tat transactivation (44, 45). In these conditions, transfection of pCMVβp300 significantly enhanced Tat-mediated activation of the LTR, whereas p300 alone had a very modest effect on the basal level of transcription from the integrated LTR. When Tat concentration was increased (500 ng of transfected expression plasmid), the potentiation effects of p300 still could be observed although in a less pronounced manner.

DISCUSSION

The results described in this work suggest that an additional function of HIV-1 Tat is to relieve chromatin inhibition on transcription by recruiting p300 and CBP HAT to the LTR promoter. This conclusion is in agreement with several observations showing that inhibitors of histone deacetylation, such as sodium butyrate (23, 24), trapoxin, and trichostatin (25), cause a remarkable activation of viral gene expression.

p300 and CBP are two evolutionarily conserved and highly homologous proteins, acting as molecular bridges between transcription factors and components of the basal transcriptional machinery (38, 39). In the last few years, a growing number of cellular transcription factors have been identified for their capacity to interact with p300/CBP, including, among others, nuclear hormone receptors, CREB, c-Jun/v-Jun, Sap 1a, MyoD, c-Fos, and NF-κB p65 (for a recent review, see ref. 46). Given the pivotal role of p300/CBP in the control of gene expression, it is not surprising that several viruses encode proteins targeting the two factors. The adenovirus E1A, the HTLV-I Tax, and the simian virus 40 large T proteins (39, 47, 48) are among these viral products.

Tat specifically associates with p300 and CBP *in vitro* and within the cells. Moreover, by *in vivo* quantitative chromatin crosslinking experiments, we also show that the interactions

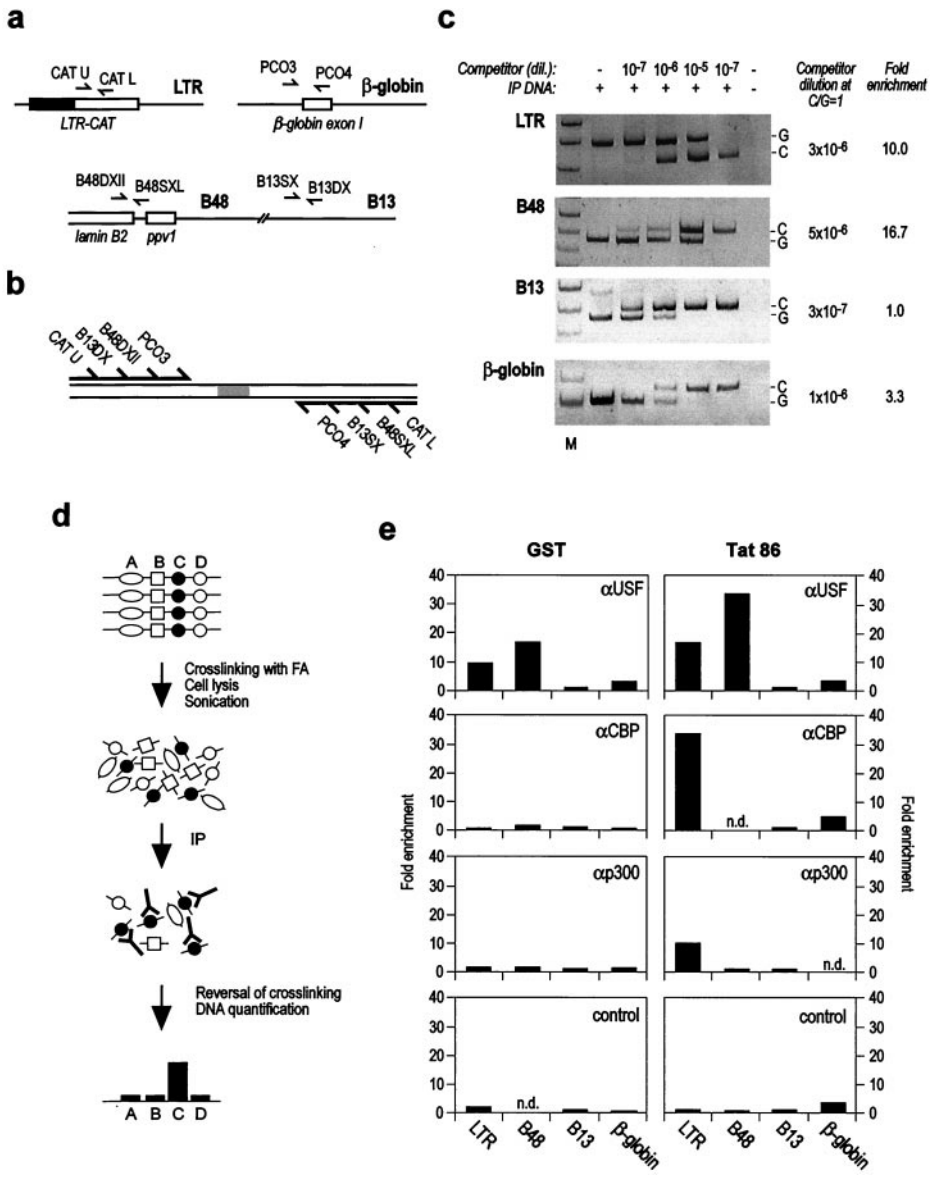

FIG. 4. Recruitment of p300 and CBP to the LTR upon Tat-mediated transcriptional activation *in vivo*. (*a*) Human chromosomal regions analyzed by quantitative chromatin immunoprecipitation. LTR-CAT, β-globin gene exon I, and lamin B2 gene B13 and B48 DNA segments were studied. For each of these regions, two primers were selected (small, converging arrows). The boxes schematically indicate the location of relevant genomic elements (LTR-CAT cassette, β-globin exon I, lamin B2 gene 3′ end, and *ppv1* gene) with respect to primer localization. (*b*) Multicompetitor DNA for competitive PCR. The multicompetitor DNA fragment contains all primer recognition sites arranged to generate PCR products of size different from but comparable to those obtained from amplification of genomic DNA. (*c*) Quantification of the sample obtained from GST-treated HL3T1 cells immunoprecipitated with anti-USF antibody (*e Top Left*). Quantification of immunoprecipitated DNA was obtained by mixing a fixed amount of immunoprecipitated DNA with the indicated scalar amounts of competitor DNA, followed by PCR amplification with each primer pair. DNA quantification was obtained from the ratio between the amplification products for genomic (G) and competitor (C) DNAs. M, molecular weight markers. (*d*) Flow chart of the quantitative chromatin immunoprecipitation assay. A, B, C, and D indicate four genomic DNA segments that directly or indirectly are crosslinked to different proteins *in vivo* by treatment with formaldehyde (FA). When immunoprecipitation (IP) of sonicated chromatin is performed with an antibody reacting with the protein crosslinked to C, the immunoprecipitate will be enriched for this DNA segment. (*e*) Results of quantitative chromatin immunoprecipitations of the four analyzed regions after treatment of HL3T1 cells with GST (*Left*) or GST-Tat 86 (*Right*) and in the presence of chloroquine, using the indicated antibodies (control, antibody against the HA epitope). Results are expressed as fold enrichment with respect to B13 region. Antibody against USF immunoprecipitates crosslinked B48 and LTR-CAT regions but not B13 and β-globin; the effect is augmented by Tat treatment. Antibodies against CBP and p300 immunoprecipitate only crosslinked LTR-CAT DNA after Tat treatment. Control antibody failed to immunoprecipitate any of the four DNA regions after GST as well as Tat treatment. The graph reports the results obtained in a representative experiment. At least three independent experiments have been performed for each antibody and each DNA region, and consistent results were obtained. n.d., not done.

of Tat with p300 and CBP actually occur at the LTR. This finding reinforces the idea that the two HAT proteins become components of the protein complex inducing promoter activation during transcription initiation. In this respect, this demonstrates that p300/CBP also can be recruited to a promoter by an RNA-targeted activator. Given the

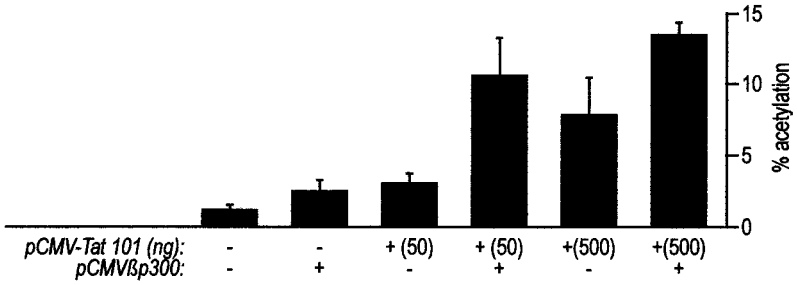

FIG. 5. Effects of p300 expression on Tat activity. Hamster CHO cells were transfected with a plasmid containing an HIV-1 LTR-CAT cassette and the neomycin-resistance gene, and stable transfectants were obtained by G418 selection. CAT assays were performed in the absence or 48 hr after transfection of the indicated amounts of pCMV-Tat 101 and of pCMVβp300 (10 μg, where indicated) expression vectors. The results represent the average of at least three independent transfections.

numerous factors demonstrated to be bound by these large adapter proteins, it remains to be formally elucidated whether Tat contacts and associates with p300/CBP directly or via another cellular component.

Tat acts as an extremely powerful transcriptional activator targeting the LTR promoter through various and distinct pathways. Besides associating with HAT proteins, Tat interacts with transcription factors binding to the LTR [e.g., Sp1 (7)], associates with components of the basal transcriptional machinery [e.g., TBP (49)], induces NF-κB, which, in turn, binds to the LTR (11), and recruits protein kinases phosphorylating the carboxyl terminus of RNA polymerase II (50). Furthermore, Tat also affects LTR promoter activity through several indirect pathways deriving from its nontranscriptional functions within the cell and at the cell membrane, ranging from the regulation of apoptosis to the induction of cytokine gene expression. Given these pleiotropic functions, it is not surprising that the synergistic effects of Tat and p300 in transient transfection experiments could be observed only in conditions in which the levels of Tat protein were limiting. Most likely, these are the same conditions that physiologically occur at the integrated LTR in nonactivated cells.

Our findings provide a molecular explanation for the long-standing observation that, in addition to its function in promoting the recruitment of processive RNA polymerase II complexes, Tat has a role in increasing the rate of transcriptional initiation at the LTR (14). It appears plausible that chromatin remodeling and the increase in polymerase processivity are two highly coordinated processes occurring at both cellular and viral promoters. Not surprisingly, additional nonhistone targets of acetylation by p300 are other components of the basal transcription machinery, including TFIIE and TFIIF (51). The former factor also can stimulate the TFIIH-dependent phosphorylation of the carboxyl-terminal domain of RNA polymerase II (52, 53). This provides another possible indirect link between Tat-mediated promoter activation and the increase in efficiency of transcriptional elongation.

Our findings, besides explaining the function of Tat in transcriptional initiation from the LTR promoter, also have some implications for the understanding of the pathogenetic mechanisms of HIV disease. In fact, in HIV-infected patients a large number of cells harbor proviral DNA molecules that are transcriptionally inactive (54–56). The Tat-mediated recruitment of HATs to the viral promoter in these latently infected cells is likely to represent a critical step in viral reactivation.

During the preparation of this manuscript, it was brought to our attention that similar results about the interaction of Tat with cellular HAT proteins also had been obtained by an independent study by K. T. Jeang and coworkers (57).

Note Added in Proof. While this manuscript was in proof, similar results on the interaction of Tat with p300 and CBP were reported also by Hottiger *et al*. (58).

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