Control of the Histone-Acetyltransferase Activity of Tip60 by the HIV-1 Transactivator Protein, Tat†

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ABSTRACT: Tip60, a cellular histone-acetyltransferase, is known to interact with the HIV-1-encoded transactivator protein, Tat. In this work, we show that the interaction of Tat with Tip60 efficiently inhibits the Tip60 histone-acetyltransferase activity. Besides its histone-acetyltransferase activity, Tip60 can undergo an autoacetylation which is not affected by Tat interaction. Our data show that Tip60 does not significantly influence Tat-dependent transcriptional activation of the 5′-LTR of HIV, suggesting that its interaction with Tat affects some intrinsic cellular process. We were then able to identify a cellular gene, Mn-dependent superoxide dismutase (Mn-SOD), that has a Tip60-dependent transcriptional activity. Interestingly, the simultaneous expression of Tat and Tip60 abolishes the effect of Tip60 on the activity of the Mn-SOD promoter. We postulate that the HIV-1 transactivator, Tat, in targeting Tip60 hinders the expression of cellular genes (such as Mn-SOD) which normally interfere with the efficient replication and propagation of the virus.

The acetylation and deacetylation of cellular proteins, histones and nonhistones, appear to be part of a complex signaling process involved in the control of gene expression (*1*−*5*). Acetyltransferases and deacetylases are the actors in this signaling process, and it is now well established that they are important regulators of transcription (*1*, *2*). It is therefore expected that viral proteins target these enzymes to enhance transcription from the viral genome and to affect the expression of cellular genes. Indeed, the adenovirus-encoded protein, E1A, targets two different histone-acetyltransferases, PCAF and p300/CBP, and modulates the enzymatic activity of these proteins (*6*−*9*).

Another viral protein that interacts with histone-acetyltransferases is the HIV-1-encoded transactivator protein, Tat. Tat is able to form a ternary complex with PCAF and p300 which has been shown to stimulate the ability of Tat to activate LTR-dependent transcription (*10*−*12*). Moreover, the formation of such a complex containing Tat and p300 targets these proteins to the viral promoter (*12*). Interestingly, Tat is also capable of forming a complex with a component of the general transcription factor TFIID, the $TAF_{II}250$ (*13*). In this case, the Tat−$TAF_{II}250$ complex does not affect transcription from the LTR, but interferes with the transcriptional activity of cellular genes. Indeed, the Tat-mediated inhibition of major histocompatibility class 1 gene expression appears to be due to the interaction of Tat with this molecule and the inhibition of its HAT activity (*13*). This targeting of HATs by Tat seems therefore to have different functional consequences. In the case of PCAF and p300, the interaction with Tat stimulates the ability of this protein to transactivate LTR-dependent transcription (*10*−*12*), while $TAF_{II}250$−Tat interaction controls the transcriptional activity of cellular genes (*13*).

In addition to these three HATs, another cellular protein, Tip60 (*14*), a member of the nuclear HAT family (*15*), is also capable of interacting with Tat. The significance of the interaction between Tat and Tip60 is not clear. This is essentially because the function of Tip60 is unknown.

In this work, we have shown that Tat can modulate the HAT activity of Tip60. Furthermore, we have identified, for the first time, a cellular gene that could be regulated by Tip60, thus demonstrating a putative role for the Tat−Tip60 interaction in modulating the activity of cellular genes which potentially interfere with the efficient replication and propagation of the virus.

EXPERIMENTAL PROCEDURES

Production of Tip60 and the Tat and HAT Assay. His-tagged bacterially expressed Tip60 (amino acids 212−513) was purified exactly as previously described (*15*). An

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expression vector (pcDNA3.1, Invitrogen) containing the entire open reading frame of Tip60 and an additional Flag sequence at its N-terminal end was used to express the protein in vivo, after the transfection of HeLa cells, or in vitro, in a TNT-coupled reticulocyte lysate system (Promega). HAT assays were performed using purified histones as described by Yamamoto et al. (*15*). In the case of the HAT assay performed in the presence of GST and GST−Tat fusion proteins, after the preincubation of Tip60 with the ^{3}H-labeled acetyl-CoA (7.7 Ci/mmol, ICN) for 5 min at 30 °C, histones and different amounts of Tat fusion proteins were added and the incubation was carried out for a further 30 min at 30 °C. The HAT activity was estimated either by gel electrophoresis and autoradiography or by a filter binding assay as described previously (*15*).

Tat (SF2 isolate) and deletion mutants of Tat (generated by PCR) were cloned in the pGEX-5X-3 vector (Pharmacia), and GST fusion proteins were purified using glutathione Sepharose 4B beads.

GST Pull-Down Assays. GST pull-down assays were performed essentially as described previously (*16*). Equal amounts of various GST fusion proteins coupled to glutathione Sepharose beads were incubated with in vitro-translated, ^{35}S-radiolabeled Tip60 for 30 min in Z′ binding buffer [25 mM Hepes (pH 7.6), 12.5 mM $MgCl_2$, 150 mM KCl, 0.1% NP-40, and 20% glycerol]. After washes in NETN buffer [100 mM NaCl, 1 mM EDTA, 0.5% NP-40, and 20 mM Tris (pH 8.0)], proteins were analyzed on SDS−polyacrylamide gels.

Transfection and Reporter Gene Assay. HeLa cells were transfected with a plasmid containing the HIV 5′-LTR-CAT or the Mn-SOD promoter luciferase reporter gene alone or in combination with different amounts of Tat or Tip60 expression vectors, using either lipofectin (BRL)- or standard calcium phosphate-based transfection protocols. The reporter gene activity was then measured 24−48 h after transfection. CAT assays were performed according to the protocol published by Nordeen et al. (*17*), and luciferase activity was measured using a luciferase-based assay system (Promega).

RESULTS

Inhibition of the Histone-Acetyltransferse Activity of Tip60 by Tat. To evaluate the functional significance of the Tat−Tip60 interaction, we first investigated the influence of Tat on the HAT activity of Tip60. Bacterially expressed Tip60 was used to perform an in vitro HAT assay both in the absence and in the presence of increasing amounts of GST−Tat and GST alone using total chicken erythrocyte histones as the substrate. Histones were then resolved on a 15% polyacrylamide gel, and the HAT activity was revealed after autoradiography of the dried gel. Figure 1A shows that, as reported previously (*15*), Tip60 can efficiently acetylate histones H3, H4, and H2A. We have also observed an efficient acetylation of histone H5 compared to those of H1s. The addition of an increasing amount of GST−Tat inhibited efficiently the HAT activity of Tip60, while the addition of a comparable amount of GST had no effect on this activity (Figure 1A, autorad panel). In an attempt to evaluate whether Tat can be acetylated by Tip60, we incubated Tip60 with radiolabeled acetyl-CoA in the presence or absence of GST−Tat. Interestingly, we could not detect acetylation of Tat but instead found Tip60 to be acetylated under the same conditions (Figure 1B, autorad panel). Surprisingly, the autoacetylation of Tip60 was not affected by Tat, suggesting that Tat does not interfere either with the binding of acetyl-CoA or with the transfer of the acetate group to the enzyme. The interaction of Tat with Tip60 seems therefore to block either the binding of the substrate or the transfer of the acetate group to the substrate.

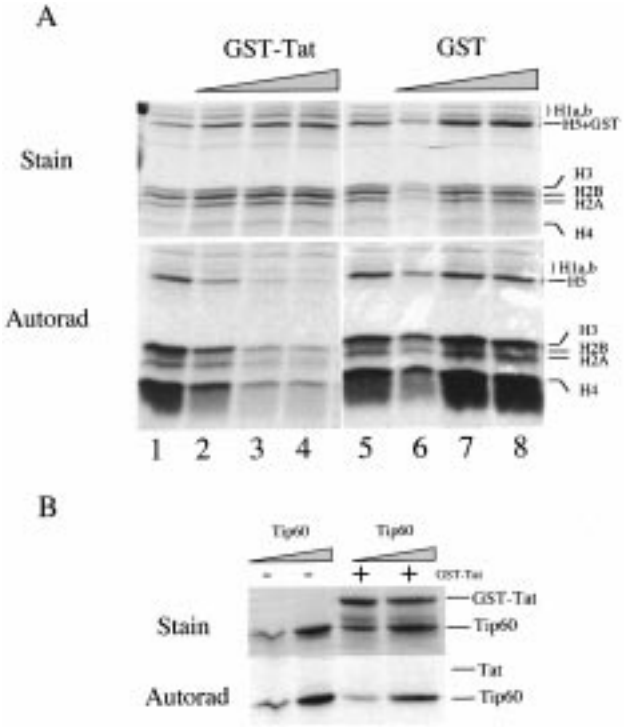

FIGURE 1: HIV-1 transactivator, Tat, inhibits the histone-acetyltransferase activity of Tip60. (A) Tip60 (5 pmol) was incubated with 5 μg of total chicken erythrocyte histones with GST−Tat (left panel) and GST (right panel) in either equimolar (lanes 2 and 6) or 2- and 3-fold molar excess amounts (lanes 3 and 7, and 4 and 8, respectively) in the presence of ^{3}H-labeled acetyl-CoA. After 30 min at 30 °C, proteins were resolved on a 15% polyacrylamide gel, stained (stain panel), dried, and revealed by autoradiography (autorad panel). (B) Tip60 can undergo an autoacetylation reaction that is not inhibited by Tat. Tip60 (5 and 10 pmol) was incubated with ^{3}H-labeled acetyl-CoA in the absence (−) or in the presence of 10 pmol of GST−Tat (+). After the incubation period, proteins were analyzed as described above.

Tip60−Tat Interaction Is Necessary for the Modulation of Tip60 Enzymatic Activity. To elucidate the relationship between the Tat−Tip60 interaction and the inhibition of Tip60 HAT activity, we produced a number of Tat deletion mutants (Figure 2A). Addition of an equimolar amount of GST−Tat resulted in a 50% inhibition of Tip60 HAT activity. Mutants 1 and 2 missing 15 and 41 C-terminal amino acids, respectively, were almost as efficient as the wild-type Tat in inhibiting Tip60 HAT activity. However, a Tat protein containing the first 48 N-terminal amino acids (missing the essential K/R rich domain, Figure 2A) cannot interfere with the HAT activity (Figure 2B, Mut3). Similarly, another Tat mutant containing only 22 N-terminal amino acids has no effect on the Tip60 HAT activity (Figure 2B, Mut4). These data show that the 12 amino acids encompass-

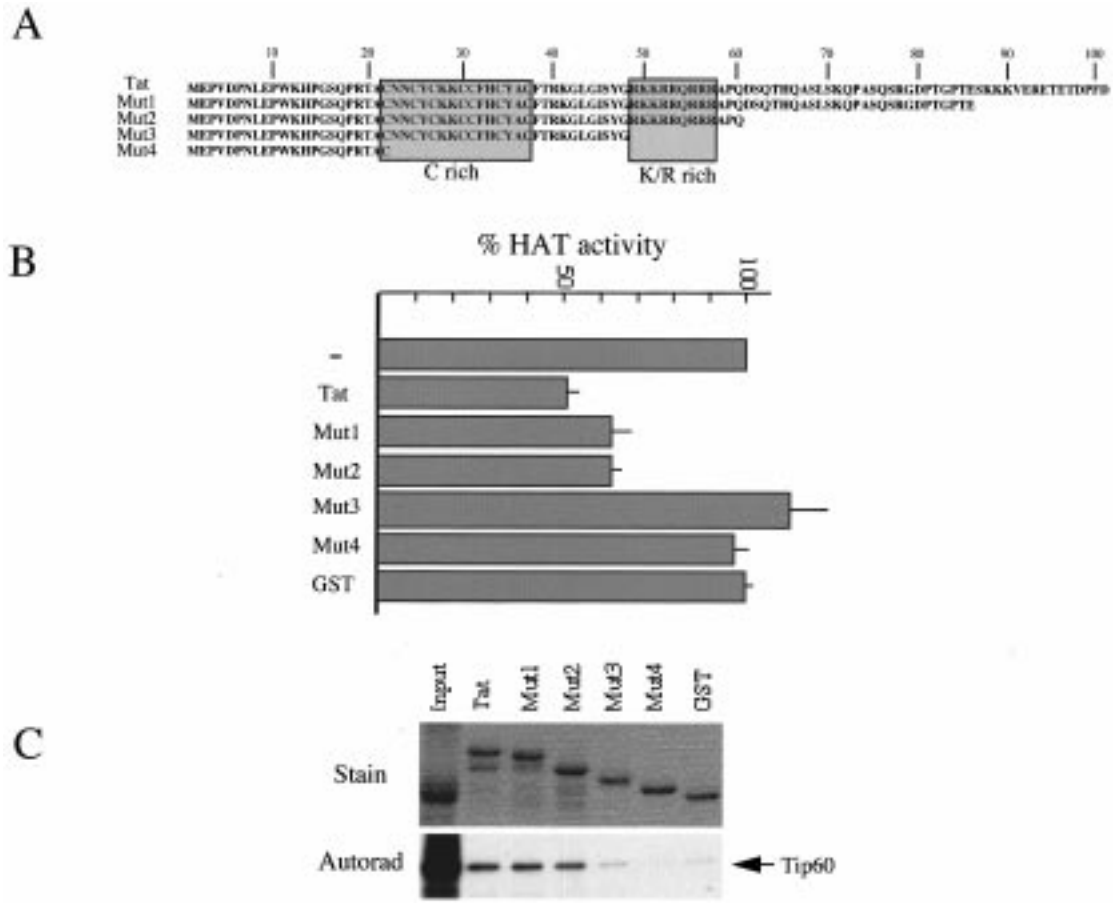

FIGURE 2: Tip60−Tat interaction modulates the HAT activity of Tip60. (A) Wild-type Tat−GST (Tat) and different deletion mutants of Tat fused to GST (Mut1−Mut4) were produced and used to determine their ability to inhibit the HAT activity of Tip60. (B) Tip60 was incubated with histones and ^{3}H-labeled acetyl-CoA either alone (−) or in the presence of an equimolar amount of wild-type GST−Tat (Tat) or similarly for different mutants (Mut1−Mut4) or GST alone (GST). The HAT activity was measured by counting labeled histones (filter binding). Values represent a percentage of the HAT activity of Tip60 alone, and histograms represent the mean values of five independent experiments. Bars represent the standard deviation. (C) The ability of Tip60 to bind wild-type Tat−GST and the different deletion mutants was examined via GST pull-down assays. Tip60 was translated in vitro in the presence of [^{35}S]methionine, and the labeled protein was used in a pull-down assay with either the wild-type Tat−GST (Tat), the indicated mutants, or GST alone. The stain panel shows different GST fusion proteins used for the pull-down assay, and the autorad panel shows the autoradiography of the gel.

ing the lysine and arginine rich domain of Tat play an important role in interfering with the HAT activity of Tip60.

GST pull-down assays in which full-length Tat or these mutants were used shows that there is a very good relationship between the ability of Tat to interact with Tip60 and the inhibition of its HAT activity (Figure 2C). Indeed, Tat mutants that are not capable of binding Tip60 are not inhibitors of the HAT activity of this protein.

Tip60−Tat Interaction Does Not Stimulate the HIV 5′-LTR Transactivation by Tat. The effect of Tat on Tip60 therefore appears to be very similar to the reported effect of this protein on $TAF_{II}250$ (*13*), in that the effect is mediated through the modulation of enzymatic activity. Previous work has shown that the interaction of Tat and HATs such as p300 and PCAF enhanced the efficiency of Tat in transactivating the 5′-LTR of HIV (*10*−*12*). We therefore tried to show how the expression of Tip60 can influence the activity of Tat on the 5′-LTR of HIV. HeLa cells were transfected with a plasmid containing the CAT gene under the control of the 5′-LTR of HIV, and both Tat and Tip60 were coexpressed. Figure 3 shows that the expression of Tip60 had almost no effect on the ability of Tat to transactivate the transcription from the 5′-LTR. These data suggest that the targeting of Tip60 by Tat and the modulation of its HAT activity do not concern the regulation of HIV 5′-LTR transcription.

Tip60 Is Capable of Stimulating the Activity of the Mn-Dependent Superoxide Dismutase (Mn-SOD) Promoter. Because of the similarity of our data with those reported on the effect of Tat on the HAT activity of $TAF_{II}250$ (*13*), we sought a role for Tat targeting of Tip60 in modulating the activity of cellular genes controlled by Tip60. To identify genes potentially regulated by Tip60, we considered cellular genes that are known to be regulated by Tat. Besides the major class 1 histocompatibilty genes, the expression of a gene involved in the control of the cellular redox state, Mn-dependent superoxide dismutase (Mn-SOD), is known to be affected by Tat (*18*). We therefore tested whether the expression of Mn-SOD can be affected by Tip60. HeLa cells were transfected with a plasmid containing the luciferase reporter gene under the control of the Mn-SOD promoter. Figure 4A shows that the expression of an increasing amount of Tip60 enhances the activity of the Mn-SOD promoter. This enhancement of the Mn-SOD transcriptional activity by Tip60 can be suppressed when Tat is simultaneously coexpressed (Figure 4B).

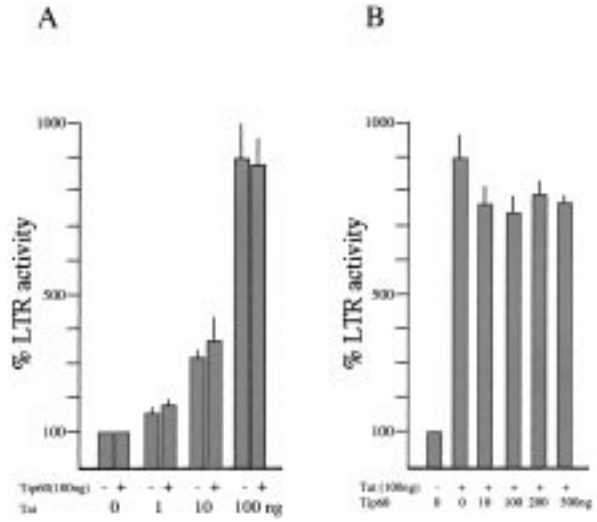

FIGURE 3: Tip60−Tat interaction does not stimulate the transactivator capacity of Tat. (A) HeLa cells were transfected with 1 μg of LTR-CAT construct with (+) or without (−) 100 ng of a Tip60 expression vector. The indicated amount of Tat expression vector was used in combination with the described plasmids. (B) HeLa cells were transfected with LTR-CAT as described above without (−) or with (+) 100 ng of the Tat expression vector and the indicated amount of the Tip60 expression vector. Values represent a percentage of the CAT activity of LTR-CAT alone. Bars represent the standard deviation of six independent experiments for panel A and two for panel B. For each transfection, the total amount of expression plasmid was kept constant (200 ng for panel A and 600 ng for panel B) using geneless CMV promoter-containing plasmids.

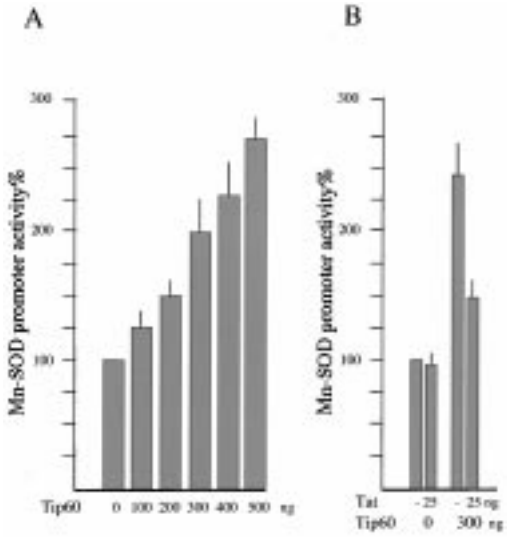

FIGURE 4: Tip60 stimulates the activity of a Mn-dependent superoxide dismutase promoter. (A) HeLa cells were transfected with 500 ng of a construct containing a luciferase gene under the control of the Mn-SOD promoter and the indicated amount of a Tip60 expression vector. Values represent a percentage of the activity of the Mn-SOD promoter in the absence of Tip60. (B) The expression of Tat suppresses the Tip60-dependent transactivation of the Mn-SOD promoter. HeLa cells were transfected with 500 ng of the Mn-SOD luciferase gene and the indicated amount of a Tip60 expression vector in the absence (−) or presence of 25 ng of the Tat expression vector. Data represent the average of two or three independent experiments. Bars represent the standard deviation. For each transfection, the total amount of expression plasmid was kept constant (500 ng for panel A and 325 ng for panel B) using geneless CMV promoter-containing plasmids (as described in the legend of Figure 3).

These observations show therefore that Tip60 is capable of controling the transcriptional activity of cellular genes and that Tat expression can abolish this Tip60-dependent transcriptional regulation.

DISCUSSION

Treatment of cell lines latently infected with HIV-1 with the two specific inhibitors of histone deacetylase, trapoxin (TPX) and trichostatin A (TSA), causes a global hyperacetylation of cellular core histones (*19*). Treatment with both drugs also results in the transcriptional activation of the HIV-1 promoter and in a marked increase in the level of virus production (*20*). Chromatin analysis of the HIV-1 genome showed that nuc-1 (a nucleosome precisely positioned immediately after the transcriptional start site) is selectively disrupted after the treatment of cells with TPX and TSA (*20*). These observations further support the crucial role played by nuc-1 in the suppression of HIV-1 transcription and demonstrate that transcriptional activation of HIV-1 can proceed through chromatin modification. Namely, histone acetylation appears to play a major role in the control of the HIV-1 5′-LTR activity (*21*). Interestingly, the interaction of the HIV-1 transactivator, Tat, with four different cellular acetyltransferases has been reported (*10*−*14*). These findings establish a direct link between the ability of Tat to transactivate transcription from the 5′-LTR and histone acetylation. However, although at least two Tat-interacting cellular HATs (PCAF and p300) help Tat in transactivating the 5′-LTR-dependent transcription (*10*−*12*), a third HAT, $TAF_{II}250$, does not influence this process (*13*). Indeed, this HAT controls instead the transcriptional activity of cellular genes (*13*). The fourth histone-acetyltrasferase that interacts with Tat is Tip60 (*14*). Our data show that Tip60 like other nuclear HATs plays a role in the control of transcription of cellular genes. This HAT is member of a specific group of nuclear histone-acetyltransferases, the MYST family (*22*). Members of this family are found from yeast to humans, suggesting an important role for these proteins, presumably in the modulation of transcription. Such a role is documented for MOF (a member of the MYST family in *Drosophila*) by its involvement in the enhancement of X-linked gene transcription in males (*23*). In yeast, the involvement of two members of the MYST family (Sas1p and Sas2p) in transcriptional regulation has also been demonstrated. Interestingly, these proteins have been found to be involved in yeast silencing (*24*). Our results show clearly that Tip60 is involved in the control of the transcriptional activity of cellular genes. Moreover, the fact that Tat targets this protein and inhibits its enzymatic activity suggests that Tip60 is involved in the control of the transcriptional activity of a specific group of genes encoding products that interfere with the efficient replication and propagation of HIV-1.

These data also highlight the dual role of Tat: its well-known role in the activation of transcription of the viral promoter (*25*) and a poorly understood role in the control of the activity of cellular promoters. Indeed, although the molecular basis of the activation of transcription of the HIV-1 5′-LTR has been relatively well established, the action of Tat on the control of the activity of cellular genes remains unclear. Our finding is the second report demonstrating a role for Tat in interfering with the expression of cellular genes through the modulation of the enzymatic activity of

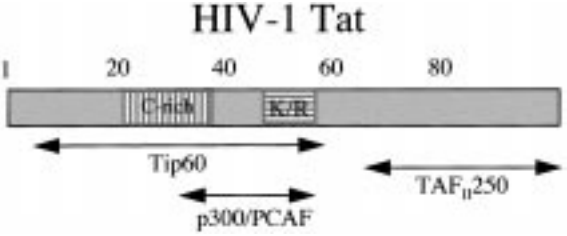

FIGURE 5: Different regions of the HIV-1-encoded transactivator Tat are involved in physical interaction with histone-acetyltransferases. Four histone-acetyltransferases are known to interact with HIV-1 Tat. The C-terminal domain (amino acids 67−101) of Tat was found to interact with TAF$_{II}$250 (*13*). p300/PCAF acetyltransferases seem to interact with amino acids 30−57 of Tat (*10*−*12*), while a region of this protein encompassing amino acids 6−57 can direct an efficient binding to Tip60 (*14*; this work).

cellular HATs. These studies show that the targeting of nuclear HATs (Tip60 and TAF$_{II}$250) and the inhibition of their enzymatic activity is one of the strategies adopted by HIV-1 to control the activity of cellular genes. Since very recently the inhibition of the HAT activity of p300 and PCAF by another viral protein, E1A, has been reported (*8*, *9*), we propose that the targeting of cellular HATs could be part of a general strategy adopted by different types of viruses to dominate the control of cellular functions.

These findings also suggest that Tat has structurally evolved to target cellular HATs. Indeed, different regions of Tat appear to be involved in the interaction with HATs (Figure 5). The C-terminal domain of Tat was found to be necessary for its interaction with TAF$_{II}$250 (*13*), while the lysine and arginine rich domain and the region of amino acids 30−45 were important for its interaction with CBP/p300 and PCAF (*10*−*12*). In the case of Tip60, besides the 31-amino acid N-terminal portion of the protein (*14*), we found that the lysine and arginine rich domain is also necessary for the binding of Tip60 and the inhibition of its HAT activity. These observations show that one important function of Tat is based on the ability of this viral protein to modulate nuclear acetyltransferase activity. The fact that different regions of Tat are involved in the interaction with HATs (Figure 5) shows that this targeting of cellular HATs is crucial to the function of Tat. The need to modify different types of cellular HAT activities probably created a selective pressure to shape different regions of Tat to interact with structurally different HATs.

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