J. Biochem. **129**, 635–641 (2001)

Tip60 Is a Cell-Type–Specific Transcriptional Regulator[1]

Falk Hlubek, Christian Löhberg, Johannes Meiler, Andreas Jung, Thomas Kirchner, and Thomas Brabletz[2]

Department of Pathology, University of Erlangen-Nürnberg, Krankenhausstr. 8-10, 91054 Erlangen, Germany

Received July 27, 2000; accepted January 31, 2001

Tip60 was originally identified as cellular HIV-Tat interacting protein and has been shown to augment Tat-dependent transcription. It has also been shown to interact with various cellular transcription factors and to belong to the nuclear histone acetyltransferase (HAT) family. To further elucidate the function of Tip60 and its HAT domain in transcription regulation, we compared Tip60 activity in HeLa and Jurkat T lymphoma cells. Here we show that Tip60 augments the HIV-1 Tat activity at the HIV-LTR promoter in HeLa but inhibits it in Jurkat cells. Moreover, we isolated two new variants of the Tip60 protein (Tip60Δ1, Tip60Δ2) from Jurkat cells. The Tip60Δ2 variant lacks the entire HAT domain but modulates HIV-1 Tat activity like full-length Tip60. In addition, Tip60 and the transcriptional repressor ZEB (zinc finger E box binding protein) interact specifically in the yeast two-hybrid system and additively inhibit the CD4 enhancer/promoter activity in Jurkat cells. Thus, Tip60 may function as corepressor of the ZEB protein. In summary, these data show that Tip60 functions as a cell-type–specific transcriptional regulator and that the HAT domain is not required for either transcriptional activation or inhibition. This indicates that Tip60 may function by recruiting additional cell-type–specific cofactors.

Key words: CD4, celltype, HIV-1 Tat, Tip60, transcriptional regulation.

Tip60 was originally isolated as a cellular HIV-1 Tat interacting protein and has been shown to modestly augment Tat-dependent transcriptional activation at the HIV-LTR promoter in HeLa cells (*1*). The activity of the viral Tat protein is also enhanced by interaction with the coactivators PCAF and p300 (*2–4*). The Tip60 protein belongs to the growing family of proteins containing a histone acetyltransferase (HAT) domain. In addition, Tip60 contains a zinc finger and a chromo-like domain of unknown function (*5*). It has been suggested that both the zinc finger motif and the HAT domain are required for HAT function (*6*). Accumulating literature has connected acetylation and deacetylation of (histone or non-histone) proteins with positive and negative regulation of gene transcription (*7–12*). Histone acetyltransferases (HATs) and deacetylases (HDACs) are the enzymes mediating gene regulation *via* protein acetylation *in vivo*. The human protein Tip60 [HIV Tat interactive protein of 60 kDa (*1*)] contains an evolutionary conserved region that includes the HAT domain (*13*). This region is shared by a growing number of Tip60 homologue proteins, the yeast SAS2 and YBF2/SAS3 (*14*), *Drosophila* MOF (*5*) and the human MOZ (*15*), HBO1 (*16*), and MORF protein (*17*). All proteins belong to the MYST family (called after its founding members), which is a subgroup of the nuclear HAT proteins. Most members of this family have been associated with transcriptional activation. However, the SAS proteins and the recently identified human MORF protein are activators as well as repressors of transcription. Furthermore, it has been shown that Tip60 enhances transcriptional activation by nuclear hormone receptors but inhibits CREB activity (*18*, *19*).

Tip60 has been shown to have acetyltransferase activity on free histones and to exhibit a similar substrate specificity to SAS3 and Esa1 (*6*, *20*). Even though recombinant Tip60 failed to acetylate histones in nucleosomal context *in vitro* (*20*), the isolated Tip60 complex was able to acytelate this substrate (*21*). Recently Ikura *et al.* have shown that the Tip60 HAT activity is important for DNA repair and induction of apoptosis (*21*). However, the function of the Tip60 complex in transcriptional regulation is not known. Moreover, Tip60 has no intrinsic transactivation activity when brought to DNA by heterologous DNA binding domain in african green monkey kidney cells Cos-1 (*19*), but activates transcription in the same system in yeast by a HAT-dependent mechanism (*18*). Thus Tip60 may function as transcriptional coactivator or corepressor, depending on the cellular context. We therefore studied the cell-type–specific differences of Tip60 function on transcriptional regulation. In this report we show that the function of Tip60 on HIV-LTR transcriptional activity is cell-type dependent. Furthermore, Tip60 inhibits the CD4-enhancer/promoter activity in concert with the transcriptional repressor ZEB

[1] This work was supported by funds from the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 466).

[2] To whom correspondence should be addressed. Phone: +49-9131-8522856, Fax: +49-9131-8524745, E-mail: thomas.brabletz@patho.imed.uni-erlangen.de

Abbreviations: CREB, cAMP response element binding protein; HAT, histone acetyltransferase; HBO1, histone acetyltransferase binding to origin recognition complex; HIV-1, human immunodeficiency virus-1; MOF, males absent on the first; MORF, MOZ related factor; MOZ, monocytic leukemia zinc finger protein; PCAF, p300/CBP-associated factor; SAS, something about silencing; Tip60, HIV-1 Tat interactive protein 60 kDa; ZEB, zinc finger E box binding protein; 3-AT, 3-amino-1,2,4-triazole.

© 2001 by The Japanese Biochemical Society.

(zinc finger E box binding protein) (*22*), thus acting as a potential corepressor. Moreover, we isolated a HAT-less variant from Jurkat cells, which behaves similarly to the full-length Tip60, suggesting that the HAT domain of Tip60 is not essential for either transcriptional activation or inhibition. The potential function of Tip60 as transcriptional cofactor is discussed.

MATERIALS AND METHODS

Plasmids—Tip60 full-length cDNA (provided by Dr. J. Kamine) was cloned into pCIneo (Promega, Medison, USA) as *Eco*RI–*Xba*I insert. Tip60Δ1 and Tip60Δ2 were retrieved by PCR amplification of cDNA from total RNA of TPA/ionomycin-activated Jurkat cells. The primers used correspond to the Tip60 sequence at nucleotide 237–254 (5′ primer) and 1801–1783 (3′ primer, Gene Bank accession No. U74667). The PCR products were cloned into pCIneo (Promega, Medison, USA) or pACT2 (Clontech, Palo Alto) for expression in yeast. The pACT2-Tip60 deletion mutants were cloned by PCR with Tip60 full length or Tip60Δ2 as template using primers corresponding to the nucleotide sequences of the indicated fragments. For two-hybrid interaction assays the N terminal (nt 1–1692), central (nt 1033–2252), or C terminal (nt 2170–3379) regions of ZEB cDNA (provided by Dr. A. Postigo) were in-frame cloned as blunted *Xba*I–*Pst*I, *Hin*cII, or *Pvu*II–*Xba*I fragments into pAS2 (Clontech, Palo Alto, USA).

Transient Transfections and Reporter Assays—CsCl gradient-purified plasmid DNAs were transfected into 2×10^5 HeLa (human cervix carcinoma) or 2×10^6 Jurkat (human T lymphoma) cells by electroporation (280 V, 1050 μF) using a gene pulser (EquiBio, Angleur, Belgium). Cells were harvested 24 h after transfection and lysed by sonification. Cell extracts were assayed for luciferase and β-galactosidase activity following the manufacturer's protocol (Dual-Light system; Tropix, Bedford, MA). Transfection efficiencies were normalized using pSV-β-Gal as transfection control. All transfections were performed at least three times.

Yeast Two-Hybrid Interaction Assays—For the yeast two-hybrid interaction assays the MATCHMAKER Two-Hybrid System 2 (Clontech, Palo Alto, USA) was used. The yeast strain Y190 was transformed by the LiAc method with the pAS2-ZEB plasmids according to the manufacturer's protocol. After reconfirmation of ZEB expression by Western blotting, these yeast clones (and as control one expressing human lamin C or none) were transformed with the indicated pACT2-Tip60 clones. The protein–protein interaction was measured by a quantitative (liquid) or qualitative (colony lift) LacZ assay following the manufacturer's protocol.

RESULTS

Tip60 Augments in HeLa and Inhibits in Jurkat Cells the HIV-1 Tat Transcriptional Activity—To characterize Tip60 function in a different cellular context, we transfected HeLa and Jurkat cells with the HIV-LTR-luc reporter construct. Upon cotransfection with a HIV-1 Tat expression construct, the activity of the reporter gene was increased by 62-fold in HeLa cells (Fig. 1A) and 3.2-fold in Jurkat respectively (Fig. 1B). Cotransfection of the Tip60 expression plasmid pCIneo-Tip60 enhanced the promoter activity dose-dependently in HeLa cells (Fig. 1A). By contrast, overexpression of Tip60 in Jurkat T cells resulted in a dose-dependant inhibition of reporter activity up to 4.3-fold (Fig. 1B). The coactivator p300 had only a minor additive effect on the reporter activity in both cell lines. This prompted us to further investigate Tip60 function in Jurkat T cells on a T cell–specific promoter.

Tip60 and ZEB Have an Additional Inhibitory Effect on CD4-Enhancer/Promoter Activity—We decided to test Tip60 function in Jurkat T cells on the CD4-enhancer/promoter, since it is a well characterized T cell–specific gene regulatory element. We have reported earlier that the CD4-enhancer/promoter is a target of the zinc finger E box binding protein ZEB (*22*). ZEB belongs to the zinc finger homeodomain protein family and is known to be an active repressor of transcription (*23*). Therefore we tested whether Tip60 would have an additional effect on the inhibitory function of ZEB.

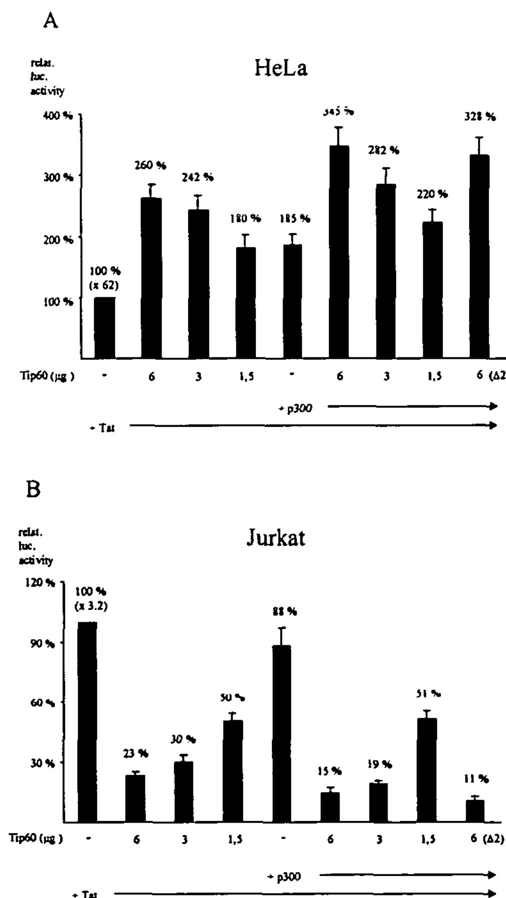

Fig. 1. **Effect of Tip60 on the HIV-LTR promoter in HeLa and Jurkat cells.** Tip60 augments HIV-LTR transcriptional activity in HeLa but inhibits it in Jurkat cells. Tip60Δ2 shows no functional differences at the HIV-LTR as compared to Tip60. (A) HeLa cells were transfected with 2 μg of pHIV-LTR-Luc and 1 μg of pSV40-Tat (+Tat), 3 μg of pCMV-p300 (+p300) and 1.5, 3, 6 μg of pCIneo-Tip60 (+Tip60) or 6 μg pCIneo-Tip60Δ2 (Δ2) as indicated. Cells were harvested 24 h posttransfection and assayed for relative luciferase activity. (B) Jurkat cells were transfected by the same method and with the same amounts of plasmid DNA as in (A). The percentage of luciferase activity is shown relative to the reporter activity enhanced by cotransfection of pSV40-Tat in HeLa (62-fold induction), and in Jurkat (3.2-fold increase), which is set to 100%.

The Tip60 expression plasmid was cotransfected with a CD4-enhancer/promoter luciferase (CD4-E/P) reporter in Jurkat cells. As shown in Fig. 2A, Tip60 had only a minor inhibitory effect on the CD4-E/P activity. Overexpression of ZEB alone resulted in a repression to 52% of the promoter activity. However, Tip60 and ZEB together inhibited the CD4-E/P activity to 23% (Fig. 2A). To further test the hypothesis of a ZEB-dependent inhibitory function of Tip60, we mutated the ZEB recognition site of the CD4-E/P, converting it into an E-box not recognized by ZEB as previously described (*22*). As shown in Fig. 2B, the Tip60 protein had no inhibitory effect on the activity of the mutated CD4-E/P (CD4-E/P M) when overexpressed alone or with ZEB. This indicates that the inhibitory function of Tip60 at the CD4-E/P is ZEB-dependent. Taken together, these results suggest that Tip60 may function as corepressor of ZEB at the CD4-enhancer/promoter in Jurkat cells.

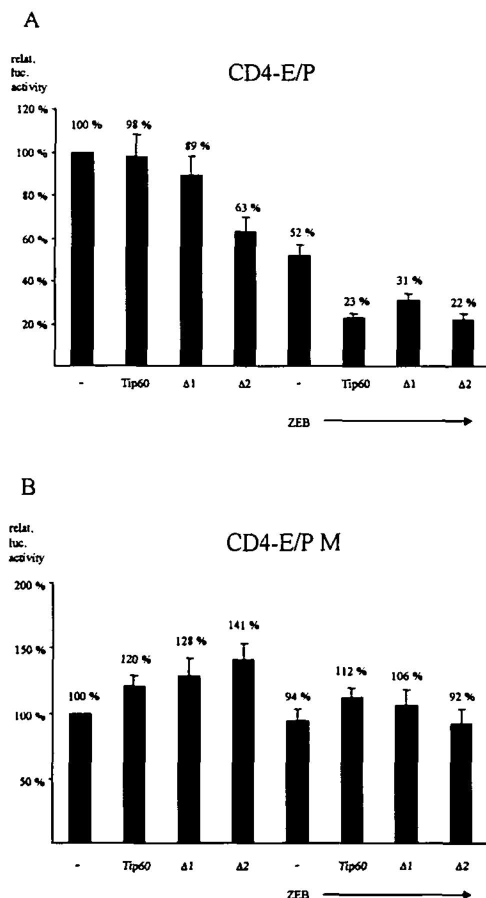

Fig. 2. **Effect of Tip60 on the transcriptional repression of the CD4-enhancer/promoter by ZEB.** (A) Tip60 and ZEB have an additional inhibitory effect on CD4-enhancer/promoter activity. Both Tip60 variants augment ZEB repressor function at the CD4-enhancer/promoter in Jurkat cells. Jurkat cells were transfected with 2 μg of pGLB-CD4E/P-Luc and 3 μg of pCIneo-flag-ZEB (ZEB), 3 μg of pCIneo-Tip60 (Tip60), or pCIneo-Tip60Δ1 (Δ1) or pCIneo-Tip60Δ2 (Δ2) as indicated. The percentage of luciferase activity is shown relative to the reporter activity when cotransfected with the control plasmid pCIneo (−), set to 100%. (B) To test for ZEB specificity of the transcriptional repression, the cotransfection experiment was performed as descibed in (A) with a mutated ZEB recognition site in the reporter construct (pGLB-CD4E/PM-Luc). The transcriptional activity of the mutant CD4-E/P reporter construct could not be inhibited by cotransfection of ZEB, Tip60, or both. The basal activity of CD4-E/PM was increased 1.55-fold compared to the wild-type enhancer.

Identification of Two Variants of Tip60 Expressed in Jurkat T Cells—To further investigate functional differences of Tip60 in HeLa and Jurkat cells, we tried to isolate the endogenous expressed Tip60 from Jurkat cells. Tip60 cDNA was retrieved by PCR amplification with Tip60-specific primers of the published cDNA sequence. We identified two new variants of Tip60 (Fig. 3). Both cDNA clones, Tip60Δ1 and Tip60Δ2, contain an in-frame deletion of nucleotides 527 to 682 (corresponding to amino acids 96–146) between the chromo and the zinc finger domains. No function is yet known for this region of the protein. The second clone, Tip60Δ2, contains an additional second in-frame deletion of nucleotides 1299 to 1676 (amino acids 353–477), thereby losing the entire histone actyltransferase domain (HAT). Since HAT domains are known for their transactivation function, the two variants might possess different functions in Jurkat cells.

Tip60Δ2 Shows No Functional Differences at the HIV-LTR Enhancer as Compared to Tip60—Because Tip60 showed the opposite activity at the HIV-LTR enhancer in HeLa and Jurkat cells, we asked whether the activity of the HAT domain could cause these differences. Therefore we examined the activity of the HAT deletion variant Tip60Δ2 at the HIV-LTR enhancer in both cell lines. As shown in Fig. 1, A and B, Tip60Δ2 augmented the HIV-LTR activity in HeLa and repressed it in Jurkat cells to the same extent as Tip60. Thus, the Tip60Δ2 variant showed no functional differences compared to Tip60, demonstrating that the HAT domain is not required for either activation or repression of the HIV-LTR activity by Tip60.

Both Tip60 Variants Augment ZEB Repressor Function at the CD4-Enhancer/Promoter in Jurkat Cells—To test the variants of Tip60 for functional differences on a T cell–specific promoter, Tip60Δ1 and Tip60Δ2 were each cotransfected again with the CD4-E/P reporter plasmid in Jurkat cells. Like Tip60, Tip60Δ1 had only a minor inhibitory effect on the CD4-E/P activity (Fig. 2A). Tip60Δ2 repressed the reporter activity to 63% compared to the reporter alone. Furthermore, overexpression of one of the Tip60 variant proteins with ZEB augmented its inhibitory activity about twofold, which is similar to the effect of full-length Tip60. Again the inhibitory effects of the Tip60 variants were ZEB-dependent, since they did not influence the reporter activity of CD4-E/P M (Fig. 2B). Because Jurkat cells pre-

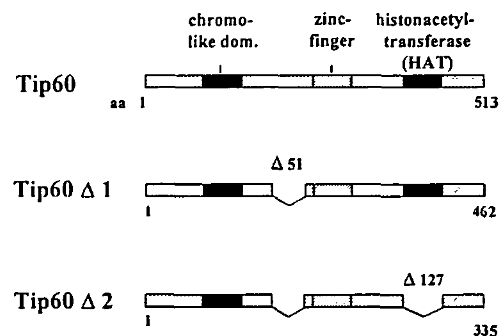

Fig. 3. **Structure of the full-length and the variant Tip60 proteins.**

sumably express endogenous Tip60 and ZEB as determined by mRNA expression (*1*, *24*), the twofold augmentation of ZEB repression seems to be a significant effect of transcriptional repressor function at the CD4-enhancer/promoter. Therefore these data further support the idea that Tip60 is a potential cofactor of ZEB-repression in Jurkat cells.

Tip60 Interacts with ZEB in the Yeast Two Hybrid Interaction Assay, Which Is Correlated with Its Repressor Function—Since the Tip60 protein contains no functional DNA-binding domain (*19*) and its inhibition of the CD4-E/P activity is ZEB-dependent, we tested the possibility that Tip60 interacts with the ZEB protein. Thereby, Tip60 would function as a potential corepressor protein in these cells. We therefore transformed the yeast strain Y190 with the vectors coding for the N terminal (nt 1–1692), the central (nt 1033–2252), or the C terminal (nt 2170–3379) portion of the ZEB protein as a Gal4DBD fusion. After reconfirmation of protein expression by Western blotting, we cotransformed the plasmid encoding a Tip60Δ1-Gal4AD fusion protein in each of these yeast clones. The protein–protein interaction was measured by the promoter activity of the lacZ reporter gene in β-Galactosidase units. This quantitative two-hybrid interaction assay shows that the N terminal portion of ZEB interacts with the Tip60Δ1 protein *in vivo* (Fig. 4). No interaction could be detected in a control assay with the Gal4DBD or the unrelated protein human Lamin C (data not shown). Furthermore, we found interaction of Tip60Δ1 with the corresponding N terminal region of the chicken homolog of ZEB (δEF1), but not with the C terminal half of either the human ZEB protein or δEF1 (data not shown). Next, by testing various deletion mutants of Tip60, the interaction domain was determined to be the amino acids 255–484 of the Tip60 protein (Fig. 5A). This domain has been reported to be highly conserved within the MYST family proteins (*6*, *20*) and includes the zinc finger domain, possibly involved in substrate binding, and the HAT domain (*6*). Next we tested the Tip60 deletion constructs for functional activity. The entire Tip60, which interacted with the ZEB protein in the yeast assay, and Tip60Δ2, but no mutated Tip60 construct, was able to repress the CD4-E/P in concert with ZEB (Fig. 5B). The failure of the C terminal portion of Tip60 to inhibit CD4-E/P activity even though it interacts with ZEB in the yeast assay could be explained by the absence of the entire N terminal portion of the protein, which includes the chromo domain. Thus, the protein-protein interaction and the repressor function correlate well in these assays. Surprisingly, the Tip60Δ2 variant did not interact with ZEB in the yeast interaction assay, but had a functional activity at the

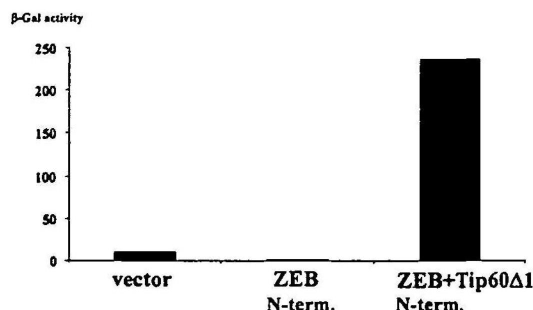

Fig. 4. **Tip60 and ZEB interact in the yeast two hybrid interaction assay.** Yeast strain Y190 was transformed with plasmids encoding the N terminal (aa 1–564) portion of the ZEB protein as Gal4DBD fusion and the Tip60Δ1-Gal4AD fusion protein or the control vector as indicated. After reconfirmation of ZEB expression by Western blotting, the protein-protein interaction was assayed by the β-Galactosidase activity in a liquid LacZ assay. β-Galactosidase activity, reported in Miller units, was measured in triplicate using a least three independent colonies. This quantitative two-hybrid interaction assay shows that in yeast the N terminal portion of ZEB interacts with the Tip60Δ1 protein *in vivo.*

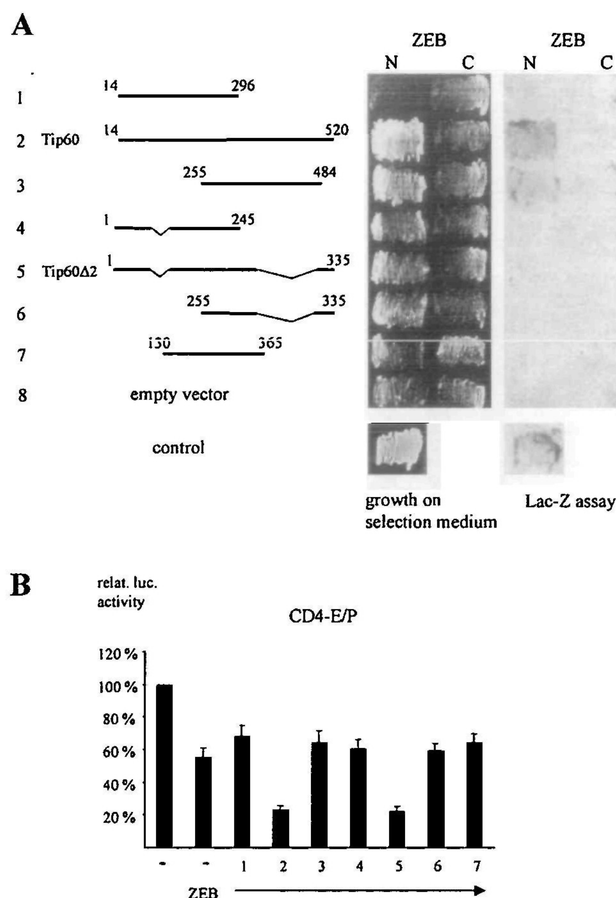

Fig. 5. **Interaction of different Tip60 deletion constructs and their effect on the repressor activity of ZEB.** (A) Interaction of Tip60 deletion mutants detected by the yeast two-hybrid assay. The yeast strain Y190 transformed with the Gal4DBD fusion of the N terminal ZEB (N) or the Gal4DBD fusion of the C terminal ZEB (C) was cotransformed separately with either the Gal4AD fusion of the Tip60 or Tip60Δ2 or the corresponding deletion mutants (amino acids are indicated) and plated on selective medium. The protein interaction was determined by qualitative colony lift LacZ-assay of at least 90 colonies per transformation. Left panel: Representative yeast clones of each transformation plated on Trp/Leu/His + 3-AT (25 mM) drop-out medium are shown. Right panel: The corresponding qualitative Lac Z colony lift assay is shown. True positive protein-protein interaction is indicated by blue coloration of yeast colonies in the LacZ assay. Note that only the yeast clones No. 2 and No. 3 show positive interaction of the ZEB N terminus and not the ZEB C terminus, narrowing down the interaction domain to the conserved region of the MYST proteins in the C terminal part of Tip60. Positive control: Gal4DBD p53 fusion cotransformed with Gal4AD fusion of the SV40 large T-antigen. (B) Effect of the same Tip60 construct as in (A) on the repressor activity of ZEB at the CD4E/P. Numbers of Tip60 clones correspond to numbers in (A).

HIV-LTR (Fig. 1, A/B) and the CD4-E/P (Fig. 2A, see "DISCUSSION").

Taken together, these results suggest that Tip60 may function as a corepressor of ZEB in Jurkat cells. However, we were not able to carry out coimmunoprecipitations due to the inability to overexpress the full-length or the N terminal portion of ZEB (which is required for Tip60 interaction as determined by two-hybrid interaction assays) either in bacteria, in reticulocyte lysates or in cultured cells in appropriate amounts.

DISCUSSION

In this report, we have compared the function of the HAT-protein Tip60 in HeLa and Jurkat human T lymphoma cells. Tip60 weakly enhances transcriptional activity of the Tat protein at the HIV-LTR in HeLa cells. In contrast, the HIV-1 Tat activity is inhibited by Tip60 in Jurkat T cells. Both effects of Tip60 are dose-dependent. Furthermore, the Tip60 protein has an additive inhibitory effect when overexpressed with the zinc finger E box binding protein ZEB, which is a transcriptional repressor of the CD4 gene (*22*). This function of Tip60 is ZEB-dependent, because it could be abolished by a point-mutation introduced in the ZEB recognition site of the CD4-E/P. In addition, we isolated two new variants of the Tip60 protein (Tip60Δ1, Tip60Δ2) from Jurkat cells. The Tip60Δ2 protein lacks the entire HAT domain, and both variants contain a deletion in the N-terminal region of the protein. Like Tip60, the variant Tip60Δ2 was able to enhance Tat activity at the HIV-LTR in HeLa cells and to inhibit it in Jurkat T cells to the same extent, suggesting that the HAT domain is not necessary for transcriptional activation and repression by Tip60. Moreover, we showed that ZEB interacts specifically with Tip60 or Tip60Δ1 but not the Tip60Δ2 variant in the yeast two-hybrid system.

Tip60 is a member of the growing family of proteins containing a histone acetyltransferase (HAT) domain. Its HAT domain has been shown to be functional *in vitro* (*20*). Tip60 has been identified as coactivator of the HIV-1 Tat protein (*1*) and the nuclear hormone receptors (*19*). However, Tip60 itself contains no intrinsic transcriptional activity when tethered to a promoter (*19*). Furthermore, Tip60 inhibits the activation of CREB (cAMP response element binding protein) by PKA (protein kinase A), thereby negatively regulating gene expression (*18*). Therefore, Tip60 may function as both a coactivator and a coinhibitor of transcription factors. However, the mechanism of either activity of Tip60 is not known. Our data showing a coactivation of HIV-1 Tat activity by Tip60 in HeLa and an inhibition in Jurkat cells confirm these findings and support a cell-type-specific regulatory mechanism. There are mainly two possible mechanisms to consider. One would be the regulation of Tip60 activity by cell-type-specific postranslational modification. The second possibility is the recruitment of cell-type–specific proteins to the transcription factor complex by Tip60. The observation that, in addition to the conserved MYST domain, Tip60 contains less conserved and unique sequences suggests a regulatory or protein–protein interaction function for these regions (*18*, *20*). As mentioned before, Tip60 contains no intrinsic transcriptional activity when tethered to a promoter (*19*). Therefore Tip60 needs additional partners to function as part of a protein complex. In this feature Tip60 is similar to the coactivator PCAF, which is known to enhance transcription only in the presence of its cognate transcription factor (*19*). Brady *et al.* have hypothesized that Tip60 may act in a similar way to PCAF by recruiting factors necessary for the action of a particular transcription factor. We postulate that Tip60 recruits cell-type–specific cofactors to the promoter, which lead to a Tip60 dependent transcriptional activation in HeLa and repression in Jurkat cells at the HIV-LTR promoter. Thus, Tip60 may be an effective modulator of HIV replication. However, we can not exclude the possibility that Tip60 activity is additionally regulated by cell-type–specific modification.

We found that the HAT activity of Tip60 is necessary for neither its activator nor its inhibitor function on the HIV-LTR enhancer. These data agree with the report by Creaven *et al.* (*25*) that the Tip60 HAT activity is efficiently inhibited by HIV-1 Tat protein interaction, even though autoacetylation of Tip60 is not affected. Thus, the HAT activity is dispensable for both the activation and inhibition function of Tip60. This is further supported by the report that the HAT activity of Tip60 is not required for inhibition of CREB activity (*18*). Even though Tip60 failed to acetylate nucleosomal histones (*20*), the recent report by Ikura *et al.* shows that the Tip60 complex acetylates this substrate (*21*). However, no function of the Tip60 HAT domain was shown in transcriptional regulation, but instead, evidence for its role in DNA damage–induced apoptosis was presented (*21*). Thus, a possible differential expression of the naturally occurring HAT-less Tip60 variant (Tip60Δ2), described here, may be of special interest in respect of apoptotic processes, *e.g.*, during T cell development. Because our observations show, that Tip60 activity is cell-type–dependent and the HAT activity is not primarily required for inhibition or activation of transcription, one could envision the following hypothetical model for Tip60 function in analogy to the function of p300/CBP at nuclear hormone receptors. Chen *et al.* (*26*) reported that the nuclear hormone receptor coactivator ACTR as part of a cofactor complex is acetylated by the recruited coactivator p300/CBP. Acetylation of ACTR attenuates hormone-induced activation and promotes dissociation of ATCR from the nuclear receptor in gel-shift assays. A similar mechanism is known for CBP at the enhanceosome of the interferon-β promoter (*27*). Similarly, gene regulation by Tip60 may first involve recruitment of cell-type– and transcription-factor–specific cofactors to enhance or repress transcription (see above). Subsequent acetylation of cofactors by Tip60 HAT activity would decrease cofactor activity and lead to disruption of the complex, thereby creating a negative feedback loop. This model aggrees with two of our observations. First, the HAT-less variant Tip60Δ2 has the same cell-type–specific activity as Tip60 when overexpressed with the HIV-1 Tat protein at the HIV-LTR (Fig. 1), due to the inhibition of Tip60 HAT activity by Tat protein (*25*). This supports the idea that the HAT activity is not primarily necessary for transcriptional activation or inhibition function of Tip60, which may be accomplished by cofactor recruitment. Second, the HAT-less variant Tip60Δ2 has an increased repressor activity at the CD4-E/P in Jurkat cells, because the lack of HAT activity would prevent the downregulation of corepressor activity by the generation of a negative feedback loop. However, this hypothesis needs to be tested by experimental proce-

dures. We are aware that full-length Tip60 and Tip60Δ2 exhibit differences in repressor activity only on the endogeneous ZEB protein, but not in the presence of exogeneously expressed ZEB, which could be due to the overexpression of the ZEB protein. An alternative explanation would be that repression of the CD4-E/P activity to approximately 20% is already the maximum repression of this promoter that can be achieved in our cell culture model.

Mutation of the ZEB recognition site of the CD4-E/P, which has been shown to abolish ZEB binding (*22*), blocks the inhibitory effect of Tip60 (Fig. 2B). Therefore the corepressive function of Tip60 was associated with its ability to bind ZEB-N terminus, which has been identified as a repressor domain in hematopoetic cells (*23, 28*). However, there was one exception indicating an additional functional interaction between both proteins without direct binding: Tip60Δ2, albeit not binding to ZEB in the yeast two-hybrid assay, also demonstrated ZEB-specific repression on the CD4-E/P. We thus speculate that both a direct binding of Tip60 to ZEB and an indirect corepressive effect, also depending on ZEB, are combined to exert the corepressive activity of Tip60. The nature of this repressive mechanism is part of ongoing investigations.

In conclusion, we have shown, that the function of Tip60 is cell-type–dependent. Tip60 inhibits transcription controlled by the HIV-LTR in Jurkat cells, but activates it in HeLa. This supports the hypothesis that Tip60 functions by recruiting cell-type–specific cofactors to the promoter. Furthermore, Tip60 inhibits the CD4-enhancer/promoter activity in concert with ZEB, possibly acting as corepressor. The Tip60 variant without HAT domain, Tip60Δ2, shows no difference in activity as compared with the Tip60 protein at the HIV-LTR. This suggests that the HAT domain is not essential for either direct activation or inhibition by Tip60, but may be important for modulating the activity of the cofactor complex. Further work, including a characterization of the differential expression pattern of the here described variants, will also investigate the role of Tip60 in T cell development.

We thank Dr. J. Kamine, Dr. A. Postigo, Dr. A. Bauer, Dr. M. Elbl, Dr. S. Lang, and Dr. Schüller for generously providing reagents and various plasmids. For expert technical assistance we thank U. Suchy.

REFERENCES

1. Kamine, J., Elangovan, B., Subramanian, T., Coleman, D., and Chinnadurai, G. (1996) Identification of a cellular protein that specifically interacts with the essential cysteine region of the HIV-1 Tat transactivator. *Virology* **216**, 357–366
2. Benkirane, M., Chun, R.F., Xiao, H., Ogryzko, V.V., Howard, B.H., Nakatani, Y., and Jeang, K.T. (1998) Activation of integrated provirus requires histone acetyltransferase. p300 and P/CAF are coactivators for HIV-1 Tat. *J. Biol. Chem.* **273**, 24898–24905
3. Hottiger, M.O. and Nabel, G.J. (1998) Interaction of human immunodeficiency virus type 1 Tat with the transcriptional coactivators p300 and CREB binding protein. *J Virol.* **72**, 8252–8256
4. Marzio, G., Tyagi, M., Gutierrez, M.I., and Giacca, M. (1998) HIV-1 tat transactivator recruits p300 and CREB-binding protein histone acetyltransferases to the viral promoter. *Proc. Natl. Acad. Sci. USA* **95**, 13519–13524
5. Hilfiker, A., Hilfiker-Kleiner, D., Pannuti, A., and Lucchesi, J.C. (1997) mof, a putative acetyl transferase gene related to the Tip60 and MOZ human genes and to the SAS genes of yeast, is required for dosage compensation in *Drosophila*. *EMBO J.* **16**, 2054–2060
6. Takechi, S. and Nakayama, T. (1999) Sas3 is a histone acetyltransferase and requires a zinc finger motif. *Biochem. Biophys. Res. Commun.* **266**, 405–410
7. Kuo, M.H. and Allis, C.D. (1998) Roles of histone acetyltransferases and deacetylases in gene regulation. *Bioessays* **20**, 615–626
8. Struhl, K. (1998) Histone acetylation and transcriptional regulatory mechanisms. *Genes Dev.* **12**, 599–606
9. Imhof, A., Yang, X.J., Ogryzko, V.V., Nakatani, Y., Wolffe, A.P., and Ge, H. (1997) Acetylation of general transcription factors by histone acetyltransferases. *Curr. Biol.* **7**, 689–692
10. Grant, P.A., Sterner, D.E., Duggan, L.J., Workman, J.L., and Berger, S.L. (1998) The SAGA unfolds: convergence of transcription regulators in chromatin-modifying complexes. *Trends Cell. Biol.* **8**, 193–197
11. Boyes, J., Byfield, P., Nakatani, Y., and Ogryzko, V. (1998) Regulation of activity of the transcription factor GATA-1 by acetylation. *Nature* **396**, 594–598
12. Kouzarides, T. (1999) Histone acetylases and deacetylases in cell proliferation. *Curr. Opin. Genet. Dev.* **9**, 40–48
13. Brownell, J.E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D.G., Roth, S.Y., and Allis, C.D. (1996) Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. *Cell* **84**, 843–851
14. Reifsnyder, C., Lowell, J., Clarke, A., and Pillus, L. (1996) Yeast SAS silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases. *Nat. Genet.* **14**, 42–49
15. Borrow, J., Stanton, V.P., Jr., Andresen, J.M., Becher, R., Behm, F.G., Chaganti, R.S., Civin, C.I., Disteche, C., Dube, I., Frischauf, A.M., *et al.* (1996) The translocation t(8;16)(p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein. *Nat. Genet.* **14**, 33–41
16. Iizuka, M. and Stillman, B. (1999) Histone acetyltransferase HBO1 interacts with the ORC1 subunit of the human initiator protein. *J. Biol. Chem.* **274**, 23027–23034
17. Champagne, N., Bertos, N.R., Pelletier, N., Wang, A.H., Vezmar, M., Yang, Y., Heng, H.H., and Yang, X.J. (1999) Identification of a human histone acetyltransferase related to monocytic leukemia zinc finger protein. *J. Biol. Chem.* **274**, 28528–28536
18. Gavaravarapu, S. and Kamine, J. (2000) Tip60 inhibits activation of CREB protein by protein kinase A. *Biochem. Biophys. Res. Commun.* **269**, 758–766
19. Brady, M.E., Ozanne, D.M., Gaughan, L., Waite, I., Cook, S., Neal, D.E., and Robson, C.N. (1999) Tip60 is a nuclear hormone receptor coactivator. *J. Biol. Chem.* **274**, 17599–17604
20. Yamamoto, T. and Horikoshi, M. (1997) Novel substrate specificity of the histone acetyltransferase activity of HIV-1-Tat interactive protein Tip60. *J. Biol Chem.* **272**, 30595–30598
21. Ikura, T., Ogryzko, V.V., Grigoriev, M., Groisman, R., Wang, J., Horikoshi, M., Scully, R., Qin, J., and Nakatani, Y. (2000) Involvement of the TIP60 histone acetylase complex in DNA repair and apoptosis. *Cell* **102**, 463–473
22. Brabletz, T., Jung, A., Hlubek, F., Lohberg, C., Meiler, J., Suchy, U., and Kirchner, T. (1999) Negative regulation of CD4 expression in T cells by the transcriptional repressor ZEB. *Int. Immunol.* **11**, 1701–1708
23. Postigo, A.A. and Dean, D.C. (1997) ZEB, a vertebrate homolog of *Drosophila* Zfh-1, is a negative regulator of muscle differentiation. *EMBO J.* **16**, 3935–3943
24. Postigo, A.A., Sheppard, A.M., Mucenski, M.L., and Dean, D.C. (1997) c-Myb and Ets proteins synergize to overcome transcriptional repression by ZEB. *EMBO J.* **16**, 3924–3934
25. Creaven, M., Hans, F., Mutskov, V., Col, E., Caron, C., Dimitrov, S., and Khochbin, S. (1999) Control of the histone-acetyltransferase activity of Tip60 by the HIV-1 transactivator protein, Tat. *Biochemistry* **38**, 8826–8830
26. Chen, H., Lin, R.J., Xie, W., Wilpitz, D., and Evans, R.M. (1999) Regulation of hormone-induced histone hyperacetylation and

gene activation via acetylation of an acetylase. *Cell* **98**, 675–686
27. Munshi, N., Merika, M., Yie, J., Senger, K., Chen, G., and Thanos, D. (1998) Acetylation of HMG I(Y) by CBP turns off IFN beta expression by disrupting the enhanceosome. *Mol. Cell* **2**, 457–467
28. Postigo, A.A. and Dean, D.C. (1999) Independent repressor domains in ZEB regulate muscle and T-cell differentiation. *Mol. Cell. Biol.* **19**, 7961–7971