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Biochemistry

Transcriptional activation *in vitro* by the human immunodeficiency virus type 1 Tat protein: Evidence for specific interaction with a coactivator(s)

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ABSTRACT **The Tat protein encoded by human immunodeficiency virus type 1 is a strong transcriptional activator of gene expression from the viral long terminal repeat and is essential for virus replication. We have investigated the molecular mechanism of Tat trans-activation by using a cell-free transcription system. We find that the trans-activation domain of Tat, amino acid residues 1–48 [Tat-(1–48)], can inhibit specifically—i.e., "squelch," transcriptional activation by full-length Tat [Tat-(1–86)]. Squelching depends upon the functional integrity of the Tat trans-activation domain because the mutant [Ala^{41}]Tat-(1–48), which is defective in Tat trans-activation *in vivo* and *in vitro*, does not squelch *in vitro* Tat trans-activation. Inhibition is selective because Tat-activated transcription, but not Tat-independent transcription, is squelched. Preincubation experiments with Tat or Tat-(1–48) and nuclear extracts show that the trans-activation region of Tat can interact with cellular coactivator(s) required for Tat trans-activation and that this interaction can occur in the absence of the human immunodeficiency virus long terminal repeat promoter. Furthermore, the putative coactivator(s) mediating trans-activation by Tat differ from those mediating trans-activation by the acidic activator VP16, as shown by reciprocal squelching experiments *in vitro*. Our results suggest that specific cellular coactivator(s) are required for mediating activated transcription by human immunodeficiency virus type 1 Tat.**

Human immunodeficiency virus type 1 (HIV-1) Tat strongly activates gene expression from the HIV-1 long terminal repeat (LTR) through the trans-activation response region (TAR) located immediately downstream of the initiation site (for review, see ref. 1). Based on genetic analysis, a cofactor, presumably an unidentified transcription factor, has been proposed to be required for Tat trans-activation (refs. 2 and 3; for review, see ref. 4). Additionally, human-specific factors encoded by chromosome 12 were required for Tat-mediated trans-activation in rodent cells (5, 6). Candidate cellular proteins have been identified. These proteins include TAP (Tat-associated protein) (7) and transcription factor Sp1 (8), which have been shown to bind Tat in solution assays, as well as Tat-binding protein 1 (9) and the mammalian suppressor of sgv1 (MSS1) protein (10), for which direct binding to Tat has not been demonstrated. The functions of these candidate Tat-binding proteins in Tat-mediated transcriptional activation remain to be established.

Transcriptional activators such as Tat are thought to function by interacting with components of the basic transcription machinery (for review, see ref. 11). Because initiation of transcription by RNA polymerase II requires the ordered assembly of general factors (TFII-A, -B, -D, -E, -F, and -H) and RNA polymerase II, any one of these factors could be the target of an activator. Different classes of trans-activators may interact with the general transcription apparatus by different mechanisms. (*i*) They may directly contact general transcription factors (12–18). (*ii*) A new class of factor, termed coactivator, adaptor, or mediator, may bridge the activator to the general transcriptional apparatus (19–29). (*iii*) The transcriptional activation process may be more complex and involve multiple contacts between activator, coactivator, and general transcriptional factors (30).

Because of our interest in host-cell factors involved in Tat-mediated trans-activation, we have developed an *in vitro* "squelching" assay that responds to the Tat trans-activation region Tat-(1–48) (31, 32). Our results provide direct biochemical evidence that a cellular factor, or factors, are a direct target of the Tat activation domain. The factor appears to function as a coactivator because it is required for Tat-activated but not for Tat-independent transcription. Further, the factor can interact with the Tat activation domain [Tat-(1–48)] in the absence of HIV LTR DNA but cannot interact with the trans-activation-deficient mutant, [Ala^{41}]Tat-(1–48)]. Finally, we show that the Tat-interacting coactivator is not required for function of the acidic activator VP16.

MATERIALS AND METHODS

Plasmids. The adenovirus type 2 major late promoter (MLP)-chloramphenicol acetyltransferase (CAT) (33), pCD23 (HIV-LTR-CAT), which contains the HIV LTR sequence from −117 to +80 upstream of CAT gene (34), pHIV-TAR(+), and pHIV-TAR(+35/+38) (35) were used as templates for *in vitro* transcription. pQTat-6His, which expresses a Tat-(1–86) fusion protein containing six histidine residues at its C terminus, was constructed as described (Li Yu, P.M.L., Zhenhua Zhang, and M.G., unpublished data). pGEX-KG-Tat-(1–48) and pGEX-KG-[Ala^{41}]Tat-(1–48) were constructed by PCR amplification, using as templates pGEX-KG-Tat or pGEX-KG-[Ala^{41}]Tat (constructed by Q. Tang, Saint Louis University Health Sciences Center); as upstream sense-primer GCATGGCCTTTGCAGGGC, which anneals to a pGEX-KG sequence, and as downstream antisense-primer GCTAAGCTTGCCATAGGAGATGCC, which anneals with the Tat sequence encoding amino acid residues 44–48. The PCR DNA products were digested with *Bam*HI and *Hin*dIII and inserted between the *Bam*HI and *Hin*dIII sites of pGEX-KG (36). Both constructs were confirmed by DNA sequencing. pG5E1bCAT contains 5 GAL4-binding sites upstream of the adenovirus E1b promoter (37).

Preparation of Recombinant Tat Protein Derivatives. Full-length Tat-6His was prepared by expression of pQTat-6His in *Escherichia coli* M15 and purified using Ni-NTA affinity chromatography in 6 M guanidine·HCl followed by stepwise

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Abbreviations: HIV-1, human immunodeficiency virus type 1; LTR, long terminal repeat; TAR, trans-activation response; GST, glutathione *S*-transferase; MLP, major late promoter; CAT, chloramphenicol acetyltransferase.

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renaturation as recommended by Qiagen (Chatsworth, CA). Cultures of *E. coli* XA90 transformed with pGEX-KG, pGEX-KG-Tat-(1–48), and pGEX-KG-[Ala^{41}]Tat-(1–48) were grown to an absorbance of 0.6 at 600 nm and induced with 0.5 mM isopropyl β-D-thiogalactoside. After 3 hr, cells were harvested by centrifugation, resuspended in 1/40th the culture volume of RS buffer (20 mM Hepes, pH 7.9/0.2 mM EDTA/1 M NaCl/1 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride), and lysed by sonication in an ice bath. Cell debris was removed by centrifugation, and the supernatant was incubated with preswollen glutathione-agarose beads at 4°C for 1 hr. Beads were collected by centrifugation at 1000 × *g* for 1 min, washed with RS buffer, and stored at 4°C. The glutathione *S*-transferase (GST)–Tat fusion proteins were eluted from the beads with 50 mM reduced glutathione, pH 8.0. For some applications, Tat proteins were cleaved from the beads with thrombin (36). Purified GAL4–VP16 protein used in transcription reactions was provided by S. Roberts and M. R. Green, University of Massachusetts Medical Center. The purity and concentration of the fusion proteins were determined by SDS/PAGE and Coomassie blue staining using bovine serum albumin as a standard.

***In Vitro* Transcription Reactions.** HeLa nuclear extracts were prepared by the procedure of Dignam *et al.* (38) as described (39). *In vitro* Tat trans-activation reactions (25 μl) analyzed by run-off assay contained 12 mM Hepes (pH 7.9); 4 mM creatine phosphate; 0.1 mM EDTA; 10 mM $MgCl_2$; 0.7 mM dithiothreitol; 60 mM KCl; 8% (vol/vol) glycerol; 500 μM ATP, CTP, and GTP; 25 μM UTP; 5 mCi of [α-^{32}P]UTP (1 Ci = 37 GBq); 6 mM sodium citrate (40); 500 ng of template DNA; 20 units of placental RNase inhibitor (Boehringer Mannheim); and 10 μl of HeLa nuclear extract. As templates, pHIV-TAR(+) was linearized with *Eco*RI or *Bam*HI to yield run-off transcripts of ≈1000 and 800 nt, respectively. Transcripts of uncut MLP-CAT, HIV-LTR-CAT, and pG5E1bCAT templates were analyzed by primer extension. The reaction mixture described for run-off assay was modified to contain 500 μM of the four NTPs, no labeled precursor, and no sodium citrate. RNA transcripts were isolated by phenol-chloroform extraction, ethanol precipitation, and hybridized at 65°C for 20 min with 50 fmol of the 5′-end-labeled 31-mer CAT primer (41) in 11 μl of a buffer containing 50 mM Tris (pH 8.3), 50 mM KCl, 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM spermidine, and 1 mM each of the four dNTPs. The reverse transcription reaction was initiated by the addition of 9 μl of solution containing 50 mM Tris (pH 8.3), 50 mM KCl, 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM of each dNTP, 1 mM spermidine, 6 mM sodium pyrophosphate, 2.5 units of avian myeloblastosis virus reverse transcriptase (Promega) followed by incubation at 42°C for 30 min. The primer-extension products and run-off transcripts were analyzed by denaturing PAGE and visualized by autoradiography.

RESULTS

The Tat Trans-Activation Domain Tat-(1–48) But Not the Trans-Activation-Defective Mutant [Ala^{41}]Tat-(1–48), Can Squelch Specifically Tat Trans-Activation *in Vitro*. To investigate the molecular mechanism of Tat transactivation, we have used an *in vitro* trans-activation system containing 6 mM sodium citrate, which suppresses basal level transcription (40). The addition of 100 ng of recombinant Tat-(1–86) purified to near homogeneity (data not shown) strongly stimulated transcription from the HIV-1 LTR promoter. Correct initiation of transcription was established by run-off analysis using as templates pHIV-TAR(+) linearized with either *Eco*RI or *Bam*HI, which produces transcripts of ≈1000 and 800 nt, respectively (Fig. 1). Tat trans-activation is authentically TAR-dependent because the TAR-defective mutant pHIV-TAR(+35/+38) (35), which contains a deletion

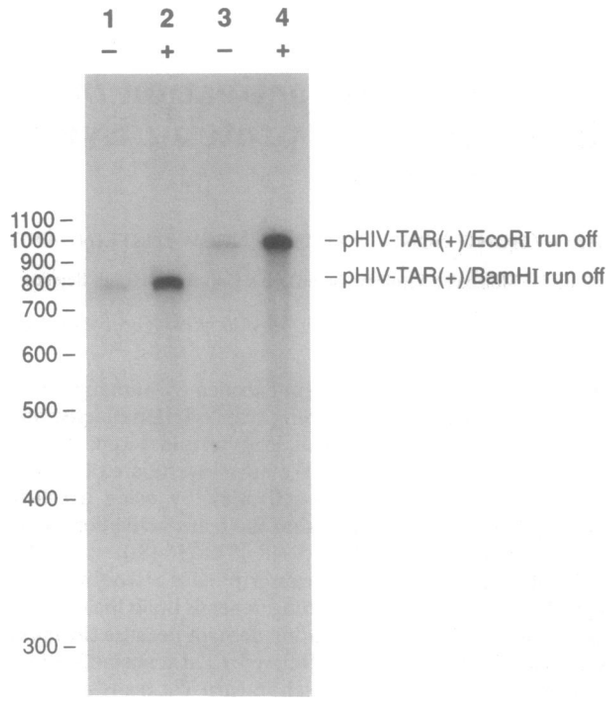

FIG. 1. Tat-(1–86) trans-activation of the HIV-1 LTR promoter in a nuclear transcription extract by purified recombinant Tat-(1–86). Run-off transcription assays were done by using reaction mixtures containing no Tat (–) or 100 ng of the Tat protein (+) and 500 ng of DNA templates, HIV-TAR(+) cut with *Bam*HI (lanes 1 and 2) or with *Eco*RI (lanes 3 and 4).

of nt +35 to +38 in the TAR region, was not activated by Tat (data not shown).

Transcriptional activators at high levels have been shown to inhibit—i.e., squelch transcription by sequestering a limiting cellular cofactor (refs. 23 and 42 and the references therein). To study the nature of the cellular target for Tat, we determined whether the Tat activation region alone, Tat-(1–48) (31, 32), would squelch trans-activation *in vitro* mediated by full-length Tat. One would expect the trans-activation and squelching functions to be encoded within the same Tat sequence. We prepared Tat-(1–48) and [Ala^{41}]Tat-(1–48) by proteolytic removal of GST from the fusion proteins GST–Tat-(1–48) and GST–[Ala^{41}]Tat-(1–48). [Ala^{41}]Tat-(1–48) contains a single amino acid substitution at position 41 (Lys → Ala). This amino acid substitution in full-length Tat inactivates trans-activation *in vivo* and *in vitro* (35, 43, 44). When increased amounts of Tat-(1–48) were included in the *in vitro* transcription reaction containing Tat-(1–86) (100 ng), trans-activation was strongly squelched (Fig. 2*A*). In contrast, the same concentrations of the mutant protein, [Ala^{41}]Tat-(1–48), did not affect Tat trans-activation (Fig. 2*B*). Similar results were obtained when the experiments of Fig. 2 *A* and *B* were repeated using GST–Tat-(1–48) and GST–[Ala^{41}]Tat-(1–48) (data not shown). The GST–Tat-(1–48) fusion protein was found as effective as Tat-(1–48) on a molar level and used in subsequent experiments.

We next determined the minimum concentration of GST–Tat-(1–48) needed for squelching. Fig. 2*C* shows that Tat trans-activation was significantly squelched by 50 ng of Tat-(1–48) and completely squelched by 100–400 ng. These results suggest a functional correlation between trans-activation and squelching and support the hypothesis that high levels of the Tat activation region sequester cofactor(s) required for Tat trans-activation of the HIV LTR promoter.

Tat-Activated Transcription, But Not Tat-Independent Transcription, Is Specifically Squelched by Tat-(1–48). The

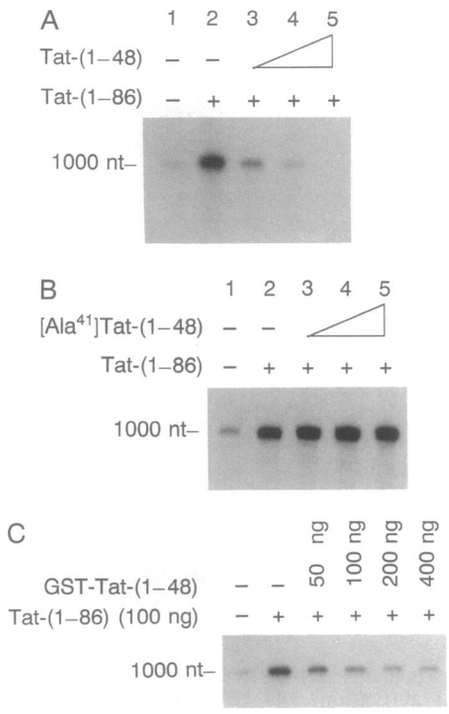

FIG. 2. The Tat trans-activation domain peptide, Tat-(1–48), but not mutant [Ala[41]]Tat-(1–48), can squelch trans-activation by full-length Tat. (*A*) Tat-(1–48) inhibits transcriptional activation by Tat. Tat-(1–48) was cleaved with thrombin from GST–Tat-(1–48), and thrombin was removed by filtration through a Centricon-30 membrane (Amicon) with a 30-kDa cut-off. The filtrate containing Tat-(1–48) was concentrated and buffer changed to phosphate-buffered saline using a Centricon-3 concentrator. Lanes 3–5 contain 400, 800, and 1600 ng of Tat-(1–48), respectively, and 100 ng of Tat-(1–86). (*B*) A single-amino acid substitution in the Tat trans-activation domain abrogates its ability to inhibit Tat-activated transcription. Transcription reactions are the same as *A*, except [Ala[41]]Tat-(1–48) was used instead of Tat-(1–48). [Ala[41]]Tat-(1–48) was purified as described above for Tat-(1–48). (*C*) Titration of the minimum amount of GST–Tat-(1–48) required for significant inhibition of Tat trans-activation. Lanes 3–6 contain 50, 100, 200, and 400 ng of Tat-(1–48), respectively.

HIV-1 LTR promoter is actively transcribed under standard *in vitro* conditions in the absence of Tat. To determine whether Tat-(1–48) sequesters a factor(s) also required for Tat-independent transcription, the transcription reaction was done with the HIV-1 LTR and MLP promoters in the same reaction mixture in the absence of Tat-(1–86) and sodium citrate and analyzed by primer extension. The addition of Tat-(1–48) or [Ala[41]]Tat-(1–48) did not alter transcription of the HIV-1 LTR or MLP promoters (Fig. 3). The highest concentration of Tat-(1–48) used in these reactions (1600 ng, Fig. 3) was >30-fold higher than needed to squelch significantly Tat-(1–86)-activated transcription (see Fig. 2*C*). In addition, run-off experiments with HIV TAR(+) as template in the absence of Tat-(1–86) and sodium citrate also showed no inhibition of Tat-independent transcription by addition of 1600 ng of Tat-(1–48) (data not shown). On the other hand, full-length Tat-(1–86) inhibited basal LTR and MLP transcription when present at levels of 600 ng or more per reaction (data not shown), in agreement with the report by Marciniak *et al.* (35). It should be noted, however, that the 100 ng of Tat-(1–86) per reaction used for Tat transactivation of the LTR promoter in our squelching studies does not show any effect on transcription of the MLP. In conclusion, our results strongly suggest that Tat-(1–48) specifically interacts with a factor(s) required for Tat-activated transcription but not for Tat-independent transcription.

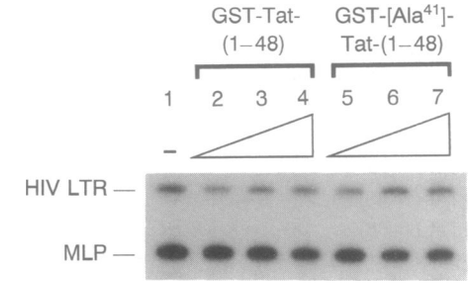

FIG. 3. Tat-(1–48) does not affect Tat-independent transcription *in vitro*. MLP-CAT and HIV-LTR CAT were used as templates for *in vitro* transcription reactions, and transcripts were analyzed by primer extension. For these experiments on Tat-independent transcription, sodium citrate was not included in the transcription extracts. Lanes 2–4 and 5–7 contain 400, 800, and 1600 ng of GST–Tat-(1–48) or GST–[Ala[41]]Tat-(1–48), respectively, as indicated.

Evidence That Coactivator(s) Involved in Tat Trans-Activation Can Interact with the Tat Trans-Activation Domain in the Presence or Absence of the HIV-LTR Promoter. We reasoned that if the trans-activation domain present in Tat-(1–48) is competing with full-length Tat-(1–86) for a limiting coactivator(s), then preincubation of Tat-(1–48) with the transcription extract should render the extract incapable of supporting Tat-mediated trans-activation. To test this hypothesis, we preincubated the transcription extract with either full-length Tat or Tat-(1–48), followed by addition of ribonucleoside triphosphate precursors. Fig. 4 shows that preincubation of Tat-(1–86) with extract for 30 min rendered Tat trans-activation resistant to squelching by subsequent addition of Tat-(1–48) (Fig. 4, lanes 4). On the other hand, preincubation of the extract with Tat-(1–48) blocked trans-activation by subsequent addition of Tat-(1–86) (Fig. 4, lanes 3). Fig. 4*A* also shows that the HIV-LTR promoter is dispensable for the interaction between Tat and its putative coactivator(s), although inclusion of HIV-1 LTR promoter in the preincubation reaction somewhat increased the overall transcription level. Most important, preincubation of [Ala[41]]Tat-(1–48), in lieu of Tat-(1–48), with the nuclear extract showed no effect on Tat-(1–86) trans-activation (Fig. 4*B*, compare lanes 3 and 5). These results lend further support to the conclusion that the Tat activation domain interacts with a transcriptional coactivator(s) required for Tat-mediated trans-activation.

The Coactivator(s) Required for Tat Trans-Activation Differ from Those Required for Function of an Acidic Activator. To address the question of whether coactivator(s) required for Tat trans-activation are common to other trans-activators, we examined the well-studied GAL4–VP16-dependent *in vitro* transcription system. The chimeric trans-activator GAL4–VP16, which contains the DNA-binding domain of GAL4 and the potent acidic activating domain of VP16, strongly stimulated transcription from pG5E1bCAT, which contains a core promoter (adenovirus E1b TATA box) and five upstream GAL4-binding sites (Fig. 5*A*, lanes 1 and 2). In agreement with previous studies (21, 23, 25), high levels of GAL4–VP16 decreased transcriptional activation due to self-squelching (compare Fig. 5*A*, lanes 2 with lanes 3 and 4). If the acidic activating domain of VP16 and the activation domain of Tat use the same coactivator(s), one would anticipate reciprocal inhibition of trans-activation by high concentrations of either trans-activator. Fig. 5*B* shows that Tat-(1–48) did not inhibit trans-activation by GAL4–VP16, even when 2400 ng was added to the reaction mixture, 50-fold higher than that needed for significant inhibition of Tat-(1–86) trans-activation (see Fig. 2*C*). To exclude the possibility that the lack of squelching may be due to a much higher affinity

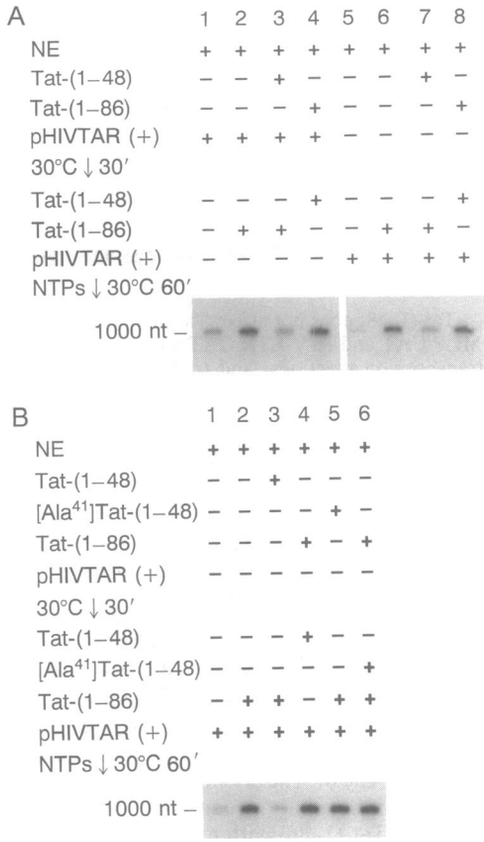

FIG. 4. Preincubation of transcription extracts with Tat-(1–48) sequesters a coactivator(s) required for Tat trans-activation *in vitro*. Preincubation with the indicated components consisting of nuclear extract (NE), Tat-(1–86) (100 ng), Tat-(1–48) (100 ng), [Ala^{41}]Tat-(1–48) (100 ng), and/or pHIV-TAR(+) was done at 30°C for 30 min (′). Tat-(1–86) or Tat-(1–48), with or without pHIV-TAR(+), was then added as indicated together with ribonucleoside triphosphates (NTPs) followed by incubation at 30°C for 60 min (′), and run-off transcription assays were done. In *A*, lanes 1–4 were exposed for 6 hr, and lanes 5–8 were exposed for 12 hr. In *B*, exposure was for 12 hr.

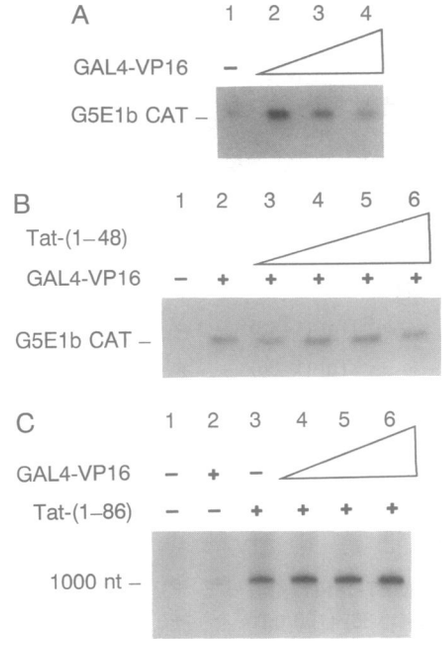

FIG. 5. Tat and GAL4–VP16 use different coactivators for transcriptional activation. (*A*) GAL4–VP16 trans-activation of pG5E1bCAT is squelched by high levels of GAL4–VP16. Lanes 2–4 contained 1, 3, and 6 μl of GAL4–VP16. (*B*) Tat-(1–48) does not squelch GAL4–VP16-dependent transcription of pG5E1bCAT. Lanes 2–6 contained 1 μl of GAL4–VP16. Lanes 3–6 contain 400, 800, 1600, and 2400 ng of Tat-(1–48), respectively. Transcripts were analyzed by primer extension in *A* and *B*. (*C*) GAL4–VP16 does not squelch Tat trans-activation. Run-off transcription assays were done. GAL4–VP16 does not stimulate transcription of HIV-LTR promoter (lane 2). Fixed amounts of Tat-(1–86) (100 ng) were added in lanes 3–6. Lanes 4–6 contain 1, 3, and 6 μl of GAL4–VP16, respectively.

of VP16 than Tat-(1–48) for the coactivator(s), we determined whether GAL4–VP16 can inhibit Tat trans-activation. As shown in Fig. 5*C*, GAL4–VP16, at a concentration that significantly exhibited self-squelching, did not inhibit transcriptional activation by Tat. These results support the conclusion that distinct coactivators mediate trans-activation by Tat and the acidic activator VP16.

DISCUSSION

We have developed an *in vitro* squelching assay that responds to the Tat activation region, Tat-(1–48). Tat-(1–48) is unable to function as an activator because it lacks the Tat basic domain, amino acids 49–57, which is necessary for binding to the TAR RNA element. The use of Tat-(1–48) instead of full-length Tat avoids complications due to binding to TAR RNA in the squelching assay, as well as nonspecific binding of other factors to the Tat basic region. Indeed, we find that high levels of Tat-(1–86) or transcriptionally inactive [Ala^{41}]Tat-(1–86) (600 ng or more per reaction), inhibit Tat trans-activation as well as transcription of the adenovirus type 2 MLP, in agreement with findings of Marciniak *et al.* (35). However, the Tat concentration used for trans-activation in our studies is 100 ng, a level that activates transcription of the LTR but does not affect the adenovirus type 2 MLP (data not shown). But most important, the activation domain, Tat-(1–48), which is used to squelch Tat trans-activation, does not inhibit basal transcription of the LTR or MLP at 1600 ng per reaction. Thus, the transcriptional squelching measured in our assays is not the nonspecific inhibition seen with high levels of full-length Tat-(1–86).

Using an *in vitro* trans-activation assay driven by noninhibitory levels of Tat-(1–86), we first showed that the region necessary for inhibition of activated transcription resides in Tat amino acid residues 1–48. By using the mutant [Ala^{41}]Tat-(1–48), we further demonstrated that the ability of the Tat activation domain to trans-activate correlates with its ability to squelch trans-activation. To further confirm and establish specificity and correlation between trans-activation and squelching, two additional mutants, [Ala^{33}]Tat-(1–48) and [Ala^{38}]Tat-(1–48), which are defective in Tat transactivation *in vivo* (31), were prepared and tested for their ability to squelch Tat trans-activation *in vitro*. Like [Ala^{41}]Tat-(1–48), both of these mutants were unable to squelch (data not shown). By monitoring Tat-independent transcription from both HIV-1 LTR and adenovirus type 2 MLP promoters, we find that squelching exerted by high levels of the Tat activation domain is specific for Tat-activated transcription and that Tat-independent transcription is unaffected. Based on these combined results we suggest that the Tat activation region, when present at high concentration, sequesters a cellular target(s) required for Tat trans-activation and thereby squelches transcriptional activation by full-length Tat.

We next used a preincubation strategy to obtain more direct evidence for the existence of a coactivator(s) required for Tat transcriptional activation. We preincubated the activator Tat-(1–86) or the competitor Tat-(1–48) with the nuclear transcription extract to permit prior formation of an activator–coactivator or a competitor–coactivator complex. After pre-

incubation the template, ribonucleoside triphosphates, and either Tat-(1–48) or Tat-(1–86) were added, and the reaction was permitted to proceed. If the trans-activating domain of Tat is indeed interacting with its coactivator required for Tat function, then we should be able to observe an anti-squelching or anti-trans-activation effect, depending upon whether Tat-(1–86) or Tat-(1–48) was precomplexed with the coactivator. Preincubation of Tat-(1–48) with the nuclear extract was shown to eliminate the trans-activation by Tat-(1–86). Conversely, by initially forming a complex between Tat-(1–86) and its putative cellular target, subsequent addition Tat-(1–48) could not squelch Tat trans-activation (Fig. 4). The interaction between Tat and its coactivator can occur in the absence of a DNA template.

To characterize further the putative Tat-responsive coactivator(s), we asked whether it was also required for trans-activation by an acidic activator, VP16. If Tat and VP16 use a common coactivator for trans-activation, we should observe reciprocal squelching. In agreement with previous reports, high concentration of GAL4–VP16 squelched GAL4–VP16-dependent trans-activation. However, no squelching of GAL4–VP16 trans-activation was observed by the Tat trans-activating domain and vice versa. These results indicate that coactivators required for mediating trans-activation by Tat are distinct from those required for the acidic activator VP16.

On the basis of the biochemical results presented here and previous genetic studies on Tat trans-activation (2, 3), we propose that Tat-specific coactivator(s) are required for Tat-activated transcription. Elucidation of the exact roles played by coactivator(s) in Tat transcriptional activation—e.g., initiation, elongation, or facilitation of Tat–TAR interaction—will provide further insight into the molecular mechanisms governing the regulation of HIV-1 gene expression. Our data that the interaction between Tat and its coactivator(s) can occur in solution and the assay system we have developed should facilitate biochemical approaches to the purification and cloning of the Tat-specific cellular coactivator(s). We have recently obtained evidence that the ultimate cellular target of transcriptional squelching by Tat-(1–48) is the basal transcription factor TFIIB. This is of great interest because TFIIB has been shown to be recruited to the transcription initiation complex by three classes of transcriptional activators (30). However, TFIIB does not bind directly to Tat-(1–48) (our unpublished data), thus implicating an intermediate between Tat and TFIIB. Workers in our laboratory have identified a candidate cellular protein, TAP (Tat-associated protein) which strongly and specifically binds to both Tat and TFIIB (ref. 7; Li Xu, P.M.L., Zhenhua Zhang, and M.G., unpublished work). We speculate that TAP may serve as an adaptor/coactivator that links Tat to the transcription machinery via TFIIB.

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