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Trans-activation of human immunodeficiency virus gene expression is mediated by nuclear events

(*tat* gene/transcription/transfection)

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ABSTRACT **Human immunodeficiency virus encodes a gene product termed tat that is able to activate viral gene expression when present in trans. The mechanism of action of the *tat* gene product appears to be bimodal, resulting in both an increase in the steady-state level of viral mRNA and the enhanced translation of that RNA. In this report we have examined the mechanism by which tat elevates viral mRNA levels. Data are presented demonstrating that tat acts by increasing the rate of viral transcription, rather than by modulating the stability of viral mRNA. Indirect immunofluorescence was used to show that tat is predominantly localized in the nucleus of expressing cells, a location consistent with a role in the regulation of viral transcription. These results suggest that tat could play a role in human immunodeficiency virus replication essentially similar to that proposed for the trans-acting nuclear gene products described for several other virus species.**

The human pathogen human immunodeficiency virus (HIV), previously termed human T-lymphotropic virus III (HTLV-III) or lymphadenopathy-associated virus (LAV), is a retrovirus with an unusually complex genetic structure. The HIV genome encompasses not only the three structural genes (*gag, pol, env*) common to other known retroviruses, but also at least four nonstructural gene products of, as yet, uncertain function (1). The functional expression in trans of two of these nonstructural genes, termed *tat* and *art/trs*, appears to be essential for viral replication in culture (2–5). Thus, efficient expression of the viral structural genes *gag* and *env* requires the coexpression of the viral *art/trs* gene product. It remains uncertain whether *art/trs* exerts its effect by enhancing the efficiency of translation of viral mRNAs (4) or by modulating the splicing pattern of the complex family of HIV subgenomic mRNAs (5).

The mechanism of action of the other essential viral trans-acting gene product, tat, is also not fully resolved. A number of investigators have demonstrated that *tat* coexpression causes a large increase in protein synthesis from genes controlled by the HIV long terminal repeat (LTR) (5–10). Initial reports suggested that this enhancement occurred independently of any marked increase in HIV-specific mRNA levels, and it was therefore proposed that tat acts post-transcriptionally by enhancing the efficiency of translation of HIV-specific mRNAs (5, 11). This hypothesis was consistent with data suggesting that the viral target sequence required for tat action was located within a region of the HIV LTR that contributes the nontranslated leader for all the HIV mRNAs (12). More recently, it has been shown that tat coexpression can also result in a significant enhancement of the steady-state level of HIV-specific mRNAs (7–10), thus suggesting a bimodal mechanism of action. This dual effect of tat on HIV LTR-specific gene expression could, nevertheless, still occur at an entirely post-transcriptional level. The tat protein might, for example, interact directly with the 5′ termini of the viral mRNAs and thereby both enhance mRNA stability and facilitate the translation of these mRNAs. The alternative hypothesis, that tat acts in part by enhancing the rate of transcription from the HIV LTR, is suggested by the observation that HIV LTR sequences 5′ to the site of transcription can play a significant role in determining the level of *tat*-mediated trans-activation (7, 12).

In this report we have directly examined the effect of tat protein on the rate of transcription from the HIV LTR and on the stability of the resultant viral transcripts. We demonstrate that *tat* coexpression has no detectable effect on the stability of HIV-specific mRNAs but instead results in an enhancement of the HIV LTR transcription rate. We further present data demonstrating that the tat protein is primarily located within the nucleus in expressing cells. These results are discussed with reference to a possible nuclear site of action for the *tat* gene.

MATERIALS AND METHODS

Construction of Molecular Clones. All molecular clones used in this work are derived from the vector pXF3/ori, which contains a simian virus 40 origin of replication inserted into a poison sequence-minus derivative of pBR322 (7). All the plasmids used have been described (7), with the exception of pBC12/RSV/γIFN. pBC12/RSV/γIFN was derived from the Rous sarcoma virus (RSV) LTR-based expression vector pBC12MI (7) by insertion of a 964 base-pair (bp) interferon γ (IFN-γ) gene cDNA (*Hin*dIII–*Bst*EII) fragment between the vector *Hin*dIII and *Bst*EII sites. This IFN-γ gene fragment was obtained from pHIT3709 (13) and consists of the 498-bp IFN-γ-coding sequence flanked by 30 bp of 5′ noncoding and 436 bp of 3′ noncoding sequences.

Cell Culture and DNA Transfection. COS cells were maintained as previously described (7) and were transfected with 2.5 μg of plasmid DNA per 100-mm culture dish using DEAE-dextran and chloroquine (14). Cell culture medium and transfected cells were harvested at 72 hr after transfection.

RNA Analysis. Total RNA was prepared from transfected cultures as previously described (15). Cytoplasmic and nuclear RNA were fractionated as described by Greenberg and Ziff (16), and RNA was isolated as described for whole cells (15). RNA aliquots, as indicated in the text, were then used to quantitate the levels of IL-2 and insulin gene-specific mRNA using S1 nuclease protection analysis (7, 15, 17).

Nuclei to be used for nuclear run-off analysis were isolated by Nonidet P-40 lysis (16), washed, and then stored at −70°C

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Abbreviations: tat, protein encoded by *tat* gene; HIV, human immunodeficiency virus; LTR, long terminal repeat; RSV, Rous sarcoma virus; IL-2, interleukin 2; IFN-γ, interferon γ.

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in glycerol buffer (50 mM Tris·HCl, pH 8.3/40% glycerol/5 mM $MgCl_2$/0.1 mM EDTA) until used. The nuclear run-off experiments and the hybridization of the labeled RNA to nitrocellulose membranes were done as described (16). The hybridization membranes were obtained by immobilizing both interleukin 2 (IL-2) and IFN-γ DNA on nitrocellulose (Schleicher & Schuell, BA 85). For each experiment, 400 ng of DNA from each test gene, dissolved in 0.18 ml of 10 mM Tris·HCl/1 mM EDTA, pH 7.4, was denatured by adding 0.02 ml of 3 M NaOH and incubating for 40 min at 65°C. After mixing with 0.2 ml of 2 M ammonium acetate, pH 7.0, the DNA was directly spotted onto nitrocellulose using a Schleicher & Schuell Minifold II apparatus. The filters were then baked at 80°C for 2 hr in a vacuum oven. The IL-2 and IFN-γ DNA sequences used were directly excised from pBC12/HIV/IL-2 (775 bp *Pvu* II–*Bam*HI fragment) and pBC12/RSV/γIFN (964-bp *Hin*dIII–*Bst*EII fragment), respectively, and represent transcribed sequences.

Interleukin 2 and Immune Interferon Assay. Medium harvested from transfected COS cultures at 72 hr after transfection was used directly for measurement of secreted IL-2 or IFN-γ levels. IL-2 levels were measured using the IL-2-dependent murine T-cell line CTLL (7). Immune interferon levels were quantitated using an assay that measures the reduction in cytopathic effect of vesicular stomatitis virus on the sensitive WISH cell line (18).

Indirect Immunofluorescence. A synthetic peptide consisting of the first 61 amino acids encoded by the HIV *tat* open reading frame was prepared by the solid-phase peptide synthesis procedure and purified by HPLC (19). The peptide was then used directly to immunize a New Zealand White rabbit after mixing with Freund's complete adjuvant. The rabbit response was boosted by the injection of peptide in Freund's incomplete adjuvant and rapidly demonstrated a high level of specific anti-tat antibody as measured by ELISA using the purified peptide and by immunoblotting extracts of an *Escherichia coli* strain that had been engineered to express high levels of the tat protein (M. Graves and B.R.C., unpublished results). This polyclonal antibody was also able to specifically immunoprecipitate the ≈15-kDa tat protein from extracts of COS cells transfected with *tat* expression vectors (20). The antibody used for indirect immunofluorescence was obtained from bleeding the rabbit 40 days after initial injection. Fixation and indirect immunofluorescence protocol used on the transfected COS cells has been described (14). The primary anti-*tat* polyclonal antibody was used at a 1:500 dilution, whereas the second antibody (rhodamine-conjugated goat anti-rabbit IgG, Boehringer Mannheim) was used at a 1:50 dilution.

RESULTS

Tat Does Not Modulate the Stability of HIV-Specific mRNAs. Because it has previously been proposed that tat acts at the post-transcriptional level (5, 10, 11), we initially examined the effect of *tat* on the stability of an indicator gene mRNA transcribed from the HIV LTR. The IL-2 indicator gene plasmid pBC12/HIV/IL-2 (7) was introduced into two sets of COS cell culture plates by transfection either with or without the cotransfected *tat* expression construction pBC12/CMV/t2 (7). Additionally, each culture was transfected with a constant level of the reference plasmid pBC12ΔI that contains the rat insulin II gene under the control of the RSV LTR. The RSV LTR is unaffected by *tat* coexpression (7, 11), and this construction therefore acts as an internal reference standard. At 72 hr after transfection the cell culture media was sampled for IL-2 assay, and one culture from each set was removed for RNA preparation. The other cultures were each supplemented with 5 μg of actinomycin D per ml and were then harvested in groups of two over a period of 6 hr. This level of actinomycin D can inhibit ≥95% of *de novo* mRNA synthesis in treated COS cells as determined by incorporation of [^{3}H]uridine (data not shown). The level of indicator (IL-2) gene mRNA and reference (insulin) gene mRNA in the COS cells at different times after addition of actinomycin D was determined using a quantitative S1 nuclease protection assay (Fig. 1) (7). Because the level of the HIV LTR-specific IL-2 mRNA is modulated by tat, whereas the RSV LTR-specific insulin mRNA is not (7), any tat-mediated mRNA stabilization would result in a shift in the ratio of the intensities of the two bands with time. Quantitation of the level of each of the two mRNAs (Table 1) revealed that the *tat*$^+$ culture exhibited a significantly (≈8-fold) higher steady-state level of HIV LTR-specific mRNA than did the *tat*$^-$ culture, but this comparison did not indicate any stabilization of the indicator gene mRNA relative to the reference insulin mRNA. Indeed, the RNAs all appeared quite stable with similar half-lives of ≥6 hr. The similar stability of these two mRNA species was not unexpected, because the IL-2 mRNA expressed by pBC12/HIV/IL-2 consists primarily of rat insulin gene noncoding sequences flanking the short human IL-2 gene-coding region (7). Overall, the data derived in Table 1 confirmed the previous observation that tat results in a significant enhancement of the steady-state level of HIV-specific IL-2 mRNA but suggested that this phenomenon was not the result of enhanced mRNA stability. However, this observation does not preclude the possibility that tat might affect the stability of a labile mRNA under HIV LTR control.

Tat Enhances the Rate of Transcription from the HIV LTR. To determine if there is a difference in the rate of transcription from the HIV LTR with and without *tat*, nuclear run-off

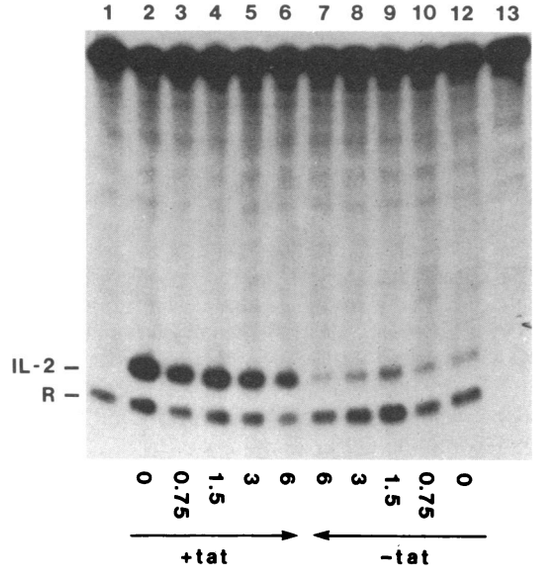

FIG. 1. Effect of *tat* expression on the steady-state level and stability of HIV LTR-specific mRNA. COS cells were transfected with equal amounts of pBC12/HIV/IL-2 DNA in the presence (lanes 2–6) and absence (lanes 7–12) of the *tat* gene expression vector pBC12/CMV/t2. All cultures (except lane 13) were also transfected with equal amounts of the reference plasmid pBC12ΔI. At 72 hr after transfection the media was supplemented with 5 μg of actinomycin D per ml. Total cellular RNA was harvested from the cultures at different times after antibiotic addition (indicated in hr below the lanes), and 2-μg aliquots were used in the quantitative S1 nuclease protection assay visualized here. Levels of the indicator IL-2 mRNA (175-nt protected probe fragment) and the reference (R) mRNA (156 nt) in the culture are proportional to the level of rescued probe (7). Lane 1 represents a culture transfected with pBC12ΔI and pBC12/CMV/t2 only, whereas the culture of lane 13 was transfected with pBC12/CMV/t2 alone.

Table 1. *tat* increases the steady-state level but not the stability of HIV LTR-specific mRNAs

	Time after actinomycin D addition, hr	Ratio of indicator (IL-2) mRNA* to reference mRNA
+*tat*	0	7.3
	0.75	9.2
	1.5	8.9
	3.0	8.6
	6.0	8.3
	Average	8.5
−*tat*	0	0.90
	0.75	1.13
	1.5	1.22
	3.0	0.96
	6.0	0.81
	Average	1.0

*Ratio of the HIV LTR-driven IL-2 indicator mRNA to the RSV LTR-driven reference mRNA over the time course after actinomycin D addition is a measure of the effect of tat protein on the relative stability of the two mRNAs; this ratio also measures the effect of tat protein on the steady-state level of the HIV LTR-specific mRNA. Ratios are derived from the autoradiograph of Fig. 1 by gel scanning using a Beckman DU-8 spectrophotometer and are given in arbitrary units. The cotransfection of *tat* resulted in a 32-fold increase in the expression of the HIV LTR-driven IL-2 indicator gene measured at the protein level.

experiments were done on the transfected COS cultures. These experiments allow the *in vitro* elongation of mRNAs initiated *in vivo* in the presence of [α-^{32}P]UTP followed by quantitative hybridization of the labeled RNA to the appropriate DNA immobilized on nitrocellulose filters (16, 21–23). The level of mRNA synthesized *in vitro* is not affected by differences in the *in vivo* stability of the mRNA species (23), and this assay is therefore a direct measure of differences in the rate of transcription. Transcription of a human IFN-γ gene under the control of the RSV LTR (pBC12/RSV/γIFN) served as an internal control in these experiments. A representative example of five similar experiments is shown in Fig. 2 and demonstrates that *tat* coexpression results in a significant enhancement in the transcription of the HIV LTR-driven IL-2 gene when compared to the control IFN-γ gene. Quantitation of the levels of IL-2 and IFN-γ transcription and protein expression in the transfected COS cells (Table 2) reveals that the increase in the transcriptional activity of the HIV LTR is fully sufficient to explain the observed enhancement in the steady-state level of HIV-specific mRNA induced by *tat* coexpression (Table 1).

The Tat Protein Is Localized in the Nucleus. The data presented in Tables 1 and 2 reveal that *tat* acts to increase the level of HIV LTR-specific transcription. These results, however, also confirm the previous observation that the

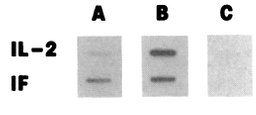

FIG. 2. Analysis of the effect of *tat* on the HIV LTR transcription rate using a nuclear run-off transcription assay. RNAs were elongated *in vitro* in the presence of [α-^{32}P]UTP using nuclei isolated from transfected COS cultures. The labeled RNAs were then isolated and hybridized to filters containing a large excess of immobilized IL-2 (row 1) and IFN-γ (row 2) DNAs. (*A*) Nuclei derived from cells transfected with pBC12/HIV/IL-2 and pBC12/RSV/γIFN DNA as well as a negative control plasmid, pXF3/ori. (*B*) Cells transfected with pBC12/HIV/IL-2, pBC12/RSV/γIFN, and the *tat* expression vector pBC12/CMV/t2. (*C*) Cells transfected with pBC12/CMV/t2 only.

Table 2. *tat* enhances HIV LTR-specific mRNA steady-state levels by increasing the transcription rate

	IL-2* expression, units per ml		IFN-γ* expression, units per ml	
Transfected DNA	RNA	Protein	RNA	Protein
pBC12/HIV/IL-2 + pBC12/RSV/γIFN + pXF3/ori	0.9	8	3.6	380
pBC12/HIV/IL-2 +pBC12/RSV/γIFN +pBC12/CMV/t2	12.2	384	6.7	480
pBC12/CMV/t2 only	0.2	<1	0.1	<4
Induction†	9×	48×	–	–

*Relative RNA levels (transcription rates) were determined by nuclear run-off analysis and were derived by scanning of the autoradiograph shown in Fig. 2. The level of secreted IL-2 and IFN-γ in the transfected COS cultures is given in units per ml of supernatant medium.

†The level of *tat*-mediated trans-activation at the RNA and protein levels is shown. The RNA induction level is corrected for the background observed in row 3 and is also corrected for the experimental variation observed for the IFN-γ control, which is known not to respond to tat protein (7, 11).

increase in mRNA level appears insufficient to fully explain the enhancement in indicator gene expression at the protein level. This dual action has previously led to the suggestion that the *tat* gene product acts both at the transcriptional and at the translational level (7, 8)—i.e., in both the nuclear and cytoplasmic compartments of expressing cells.

To elucidate the subcellular location of tat protein we raised a polyclonal rabbit anti-serum directed against a synthetic peptide containing the first 61 amino acids of the *tat* open reading frame. This antiserum was then used to localize the tat protein within COS cells transfected with *tat* expression plasmids by indirect immunofluorescence microscopy. The results obtained (Fig. 3) clearly demonstrate that tat protein is predominantly located within the nucleus of expressing cells. The nuclear localization of tat was not affected by the absence (Fig. 3 *A–D*) or presence (Fig. 3 *E–F*) of the proposed target sequence for *tat* action (12) within the *tat* expression vector used. No fluorescence was observed when COS cells were transfected with a vector lacking *tat* sequences or if preimmune serum was used (data not shown). The tat protein was not randomly distributed within the nucleus, but instead was concentrated in subnuclear bodies that were refractory under phase contrast microscopy (Fig. 3), a property normally associated with nucleoli. It is of interest to compare this subcellular distribution with that reported for other viral transacting transcriptional activators such as the pX protein encoded by human T-cell leukemia virus I or the ICP4 protein encoded by herpes simplex virus (24, 25). These proteins were also localized within the nucleus of transfected expressing cells, but they appeared to be specifically excluded from nucleoli. Our inability to detect any significant cytoplasmic tat protein, as would be predicted if tat protein exerted a direct effect on HIV mRNA translation, is especially notable when one considers that COS cells transfected with *tat* expression vectors have been reported to express considerably higher levels of tat protein than are normally observed in HIV-infected cells (5). However, it is possible that a low but significant level of tat protein in the cytoplasm could elude assay detection.

The demonstration that tat acts to enhance HIV transcription and is predominantly located within the nucleus raises the question of whether tat could appear to modulate HIV mRNA translational efficiency from a nuclear location. We have previously (7) presented evidence suggesting that HIV-

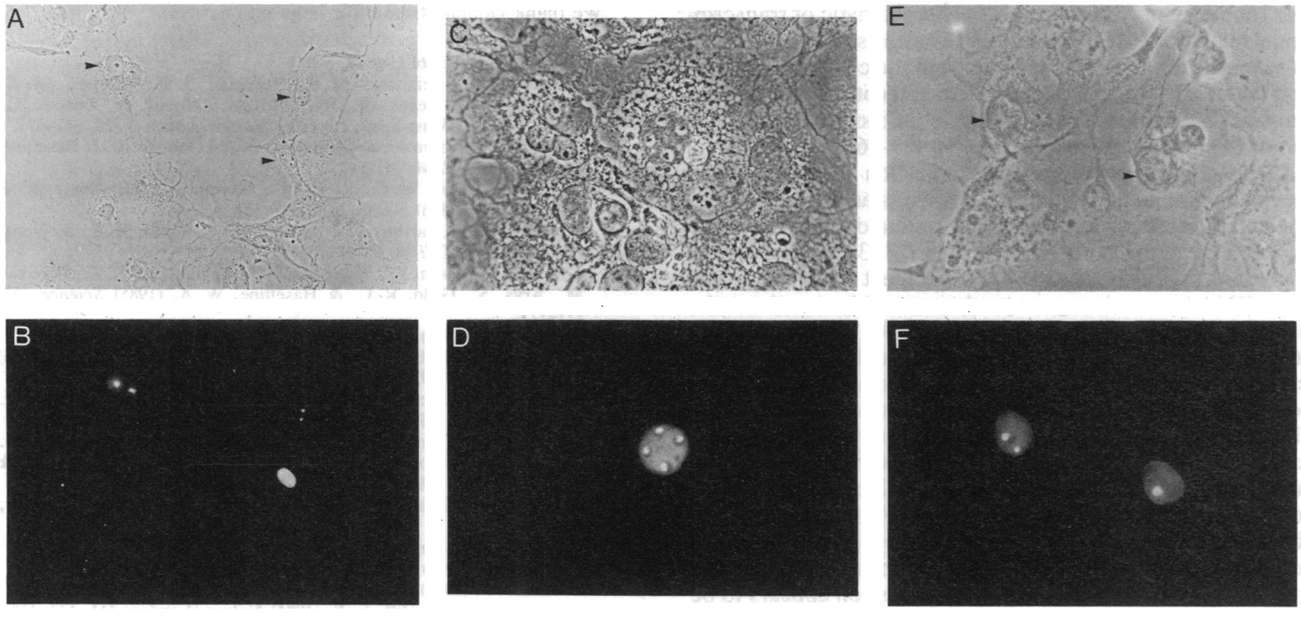

FIG. 3. Subcellular localization of tat protein within transfected COS cells by indirect immunofluorescence. Phase-contrast and corresponding immunofluorescence photographs of fixed transfected COS cell cultures. Cells were treated with rabbit polyclonal tat peptide antiserum followed by rhodamine-conjugated goat anti-rabbit IgG. (*A–D*) Cells transfected with pBC12/CMV/t2, which expresses the *tat* gene under the control of the human cytomegalovirus immediate early promoter (7). Similar results were obtained with vectors that express *tat* under the control of the homologous HIV LTR (pBC12/HIV/t2—*E* and *F*) or the RSV LTR (pBC12/RSV/t23; data not shown). *tat* gene expression could be detected in 5–10% of the transfected cells (*A* and *B*), the percentage expected to have incorporated plasmid DNA as a result of our transfection procedure (14). Note the intense nuclear staining and the presence of brightly staining subnuclear structures. Magnification, ×85 (*A* and *B*); ×210 (*C–F*).

specific mRNAs are poorly utilized by the translational apparatus in the absence of *tat* coexpression. One possible explanation for this observation is that the HIV LTR-specific mRNA is normally sequestered within the nucleus, away from the cytoplasmic translational machinery, and that tat acts to specifically enhance the nuclear export of HIV mRNAs. A similar mechanism of action has been proposed for the adenovirus trans-acting gene product E1B (26, 27). This hypothesis was directly tested by measuring the steady-state level of indicator and reference gene mRNAs within the nuclear and cytoplasmic compartment after transfection of COS cells with the test plasmids pBC12/HIV/IL-2 and pBC12ΔI with and without the *tat* expression vector pBC12/CMV/t2. Results (Table 3) do not provide evidence for a role for tat in modulating the nuclear export of HIV-specific mRNAs.

DISCUSSION

Previously, we and others have provided evidence that the HIV trans-acting *tat* gene product can enhance HIV-specific gene expression by increasing the steady-state level of HIV-specific transcripts (7–10). Here, we have extended these earlier results by directly demonstrating that tat exerts its effect on HIV mRNA levels by increasing the rate of HIV transcription rather than by stabilizing HIV-specific mRNA molecules. In addition, we also provide evidence that the HIV *tat* gene product is localized predominantly within the nucleus of expressing cells. This subcellular location is fully consistent with a role for tat protein as a viral trans-acting transcriptional activator—the origin of the designation *tat* (6). For technical reasons, these experiments were done using the simian virus 40-transformed monkey cell line COS, rather than the $T4^+$ primary human lymphocytes that are the normal host for HIV replication. COS cells are, however, permissive for HIV replication *in vitro* and have been shown to produce and process HIV transcripts in a manner similar to infected T lymphocytes (5, 28). We therefore believe that COS cells form a valid model for the study of *tat*-mediated trans-activation of HIV gene expression.

The data reported here support the recent observation that HIV-infected cells contain a nuclear factor(s) able to stimulate *in vitro* transcription from the HIV LTR (29). While our results clearly demonstrate a role for tat protein in this transcriptional activation, it remains unclear whether tat protein acts directly or instead by modulating the activity of a cellular transcription factor, as has been proposed for the adenovirus trans-acting transcriptional activator E1A (30). Inspection of the proposed primary sequence of the 86-amino acid *tat* gene product (31, 32) does however reveal features consistent with a role for tat protein as a nuclear transcriptional regulatory protein. Particularly notable is the presence of a remarkable four copies of the canonical sequence Cys-Xaa-Xaa-Cys within the short tat protein. This sequence is believed to form the core of the metal-binding domains that have been postulated to be important for function in a wide range of nucleic acid binding proteins (33). The occurrence of

Table 3. Tat protein has no effect on the relative distribution of HIV-specific mRNA between the nucleus and cytoplasm of transfected cells

	− *tat**		+ *tat**	
	Nuclear	Cytoplasmic	Nuclear	Cytoplasmic
IL-2 mRNA, %	1.1	98.9	2.5	97.5
Insulin mRNA, %	1.3	98.7	1.5	98.5

*COS cell cultures were transfected with pBC12/HIV/IL-2 and pBC12ΔI in the presence or absence of the *tat* expression vector pBC12/CMV/t2. At 72 hr after transfection, total RNA was isolated from the nuclear and cytoplasmic cellular compartments of each culture as described, and 4-μg aliquots were used for quantitative S1 nuclease protection analysis. The level of IL-2 and insulin mRNA was then determined by scanning of the resultant autoradiograph. The relative level of each mRNA within the nucleus and cytoplasm of the transfected cells is expressed as a percentage of the total signal after correction for RNA yield.

this sequence may therefore be a characteristic of transcriptional regulatory proteins (33), and examples have been noted in several such proteins, ranging from the cellular transcription factor TFIIIA and glucocorticoid receptor to the nuclear trans-acting gene products E1A of adenovirus and large tumor (T) antigen of simian virus 40 (33–36).

Clearly, our results raise the question of whether the mechanism of action of tat protein is similar to that of other nuclear trans-acting transcriptional activators such as those encoded by some other retroviruses (24, 37, 38) or by the DNA tumor viruses (25, 39, 40). The recent observation that some DNA tumor virus gene products are able to activate the HIV LTR at the transcriptional level (41, 42) may indeed provide evidence for such a functional similarity. Nevertheless, it should be emphasized that *tat*-mediated trans-activation of the HIV LTR may be unusual in several respects. Thus, the target nucleic acid sequence required for tat protein action is located, at least partly, 3′ to the start of transcription initiation (7, 8, 12). This target sequence location appears to be unusual, although not unique (43). A second potential difference is that tat protein has also been shown to act at the post-transcriptional level (7, 8). Indeed, post-transcriptional enhancement of HIV-specific gene expression appears to be the major effect of the *tat* gene product in some cases (5, 11). Although the primary control level of gene expression in many viruses appears to be transcriptional (39, 44, 45), interesting and possibly relevant examples of post-transcriptional gene regulation do exist. Thus, cytomegalovirus (CMV) early gene transcripts appear to contain a cis-dominant sequence within the noncoding mRNA leader that prevents utilization of these transcripts, either by blocking transport to the cytoplasm or by inhibiting their translation (46). This repression is reversed later in the CMV replication cycle by the action of an as yet unidentified viral trans-acting protein. This example has some similarity to the HIV case, where evidence has been presented suggesting that HIV-specific mRNAs are inefficiently translated in the absence of the viral *tat* gene product (5, 7, 8, 11). Another interesting example is the adenovirus trans-acting protein E1B. E1B appears to affect adenovirus gene expression, at least partly, at the post-transcriptional level and may modulate the efficiency of nuclear export of adenovirus and host cell mRNAs (26, 27). This phenomenon, by altering the availability of mRNAs to the cytoplasmic translational machinery, could easily mimic the effect of a shift in translational efficiency. Although our initial results do not provide any evidence for a role for tat protein in modulating HIV mRNA export from the nucleus (Table 3), this does raise the possibility that tat could affect the efficiency of translational utilization of HIV mRNA from a nuclear location. Potential mechanisms range from effects on the processing of HIV mRNA (capping, RNA methylation, etc.) to the possibility that tat might target HIV mRNAs to a translationally optimal subcytoplasmic compartment (47, 48). The alternative possibility remains that tat protein acts directly or indirectly to relieve the inhibition of translation of HIV mRNAs. While our inability to observe any detectable tat protein within the cytoplasm by immunofluorescence (Fig. 3) might favor an indirect mechanism, either would presumably occur via the recognition of an mRNA sequence, as opposed to the DNA sequence recognition required for transcriptional activation of the HIV LTR. This dual mechanism hypothesis therefore suggests that it should be possible to segregate the translational from the transcriptional effects of tat protein.

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