The Presence of Tat Protein or Tumor Necrosis Factor Alpha Is Critical for Herpes Simplex Virus Type 1-Induced Expression of Human Immunodeficiency Virus Type 1

WALDEMAR POPIK¹ AND PAULA M. PITHA^{1,2*}

Oncology Center¹ and Department of Molecular Biology and Genetics,² The Johns Hopkins University School of Medicine, Baltimore, Maryland 21231

Received 27 August 1993/Accepted 15 November 1993

Tat-independent transcription of human immunodeficiency virus type 1 (HIV-1) plays an important role in virus life cycle before biologically significant levels of Tat protein have been accumulated. Using a latently infected T-cell line containing an integrated Tat-defective HIV-1 provirus, we examined whether factors known to up-regulate the HIV-1 expression in vitro can replace the requirement for a functional Tat protein and induce the expression of the Tat-defective HIV-1 provirus. Both tumor necrosis factor alpha (TNF- α) and herpes simplex virus type 1 (HSV-1) infection stimulated transcription of the Tat-defective HIV-1 provirus to comparable levels, but in HSV-1-infected cells, the cytoplasmic HIV-1 transcripts were not efficiently translated in the absence of Tat protein and were excluded from the large polysomes. However, HSV-1 infection did not affect the distribution of cellular γ -actin RNA or 28S RNA in the polysomal fractions. The translational block of HIV-1 RNA was not mediated by the virion-associated host cell shutoff protein (vhs); dissociation of HIV-1 transcripts from the polysomes and inefficient translation was also observed in cells infected with the vhs-defective mutant of HSV-1 (*vhs-1*). Overexpression of Rev protein did not rescue the synthesis of HIV-1 proteins in these cells; however, the observed inhibition of HIV-1 RNA translation was efficiently overcome in the presence of Tat protein or TNF- α . These findings suggest that, in contrast to TNF- α , HSV-1 infection is not able to induce a full cycle of HIV-1 replication and that cytokines and Tat have a critical role in the activation of HIV-1 provirus by HSV-1.

Replication of human immunodeficiency virus type 1 (HIV-1) provirus depends on two virus-encoded nonstructural RNA-binding proteins, Tat and Rev (17, 62). While the effect of Tat is primary at the level of transcription initiation and processing (16, 30, 35, 37, 38), the effect of Rev is at the posttranscriptional level (2, 3, 10, 11, 36, 56). In the absence of the functional Tat protein (12, 19) or the Tat binding site (TAR element), replication of the HIV-1 provirus is significantly impaired (27). In contrast, the Rev protein acts post-transcriptionally to increase the transport of the unspliced and singly spliced viral transcripts from the nucleus to the cytoplasm (15, 18, 25, 26, 37) and translation of the *gag-pol* and *vpu-env* mRNAs (2, 6, 25, 61).

The transcriptional activity of the HIV-1 long terminal repeat (LTR) can be, however, also enhanced by a Tat-independent mechanism. A large number of cellular stimuli, such as mitogens, stress, cytokines, and viruses, were shown to transactivate HIV-1 LTR and stimulate expression of the endogenous HIV-1 provirus (13, 29, 40, 41, 50). The signal transduction pathway by which these stimuli influence the transcriptional activity of HIV-1 LTR generally includes the activation of binding of a heterodimeric NF- κ B complex to the *cis*-acting DNA enhancer element in the HIV-1 LTR (13, 22, 23, 49, 66). The activation of HIV-1 provirus by the NF- κ B involves release of the I κ B inhibitor from p65 and transport of the p50-p65 dimer into the nucleus (21). It has been recently shown that several closely related transcriptional factors can form homo- or heterodimer complexes and up-regulate or down-regulate the κ B site-dependent transcription (4, 28, 32,

42). Interestingly, the κ B-regulated transcription of HIV-1 LTR is independent of the virus-encoded Tat transactivation, and we have recently shown that the tumor necrosis factor alpha (TNF- α) not only is able to activate the expression of the Tat-defective provirus in T-cells but also allows the Tat-defective virus to establish infection and spread throughout the culture (50). Analysis of the transcriptional rate of the Tat-defective HIV-1 indicated that TNF- α stimulated the initiation of the transcription but, unlike Tat, did not reduce effectively the transcriptional polarity. These data suggest that cytokines may not only complement but also replace the requirement for Tat transactivation, although in a much less effective manner.

The transcriptional activity of HIV-1 LTR was found also to be enhanced in cells infected with the family of herpesviruses (1, 40, 43, 46) that often accompany HIV-1 infection in AIDS patients (52). Many of these viruses contain the NF- κ B-specific enhancer sequences in the promoter regions of the early regulatory genes and use the NF- κ B factors to induce their own transcription (33). We have previously analyzed the molecular mechanism by which herpes simplex virus type 1 (HSV-1) infection transactivates the HIV-1 LTR (41) and induces expression of the endogenous HIV-1 provirus in a T-lymphocytic cell line (ACH-2) containing a single copy of an integrated HIV-1 provirus (65). Infection of these cells with HSV-1 which led to activation of a full cycle of HIV-1 replication was associated with binding of two nuclear proteins (55 and 85 kDa) to the NF- κ B sites and a novel 50-kDa protein (named HLP-1) to the LBP-1 binding site in the leader sequence of HIV-1 LTR. In transient-expression assays, both the NF- κ B and the LBP-1 sequences, either alone or in combination, were able to confer inducibility by HSV-1, suggesting that HSV-1 activates HIV-1 provirus by both the NF- κ B-dependent and the NF- κ B-independent mechanism.

* Corresponding author. Mailing address: The Johns Hopkins University, Oncology Center, 418 N. Bond St., Baltimore, MD 21231-1001. Phone: (410) 955-8871. Fax: (410) 955-0840.

The mechanism by which HSV-1 virus induces binding of NF- κ B proteins is not clear at present, and neither is it clear whether HLP-1 is a viral or a cellular protein. We have, however, recently shown that the activation of HIV-1 provirus in T-cells does not require HSV-1 replication and the expression of the immediate-early (IE) and delayed-early genes is sufficient. Among these, the ICP0 gene seems to play an essential role in the activation of HIV-1 LTR (66).

The aim of this study was to determine whether infection with HSV-1 can activate expression of the Tat-defective HIV-1 provirus in T cells. Using a T-cell line (CEMR7/neo) that contains an integrated Tat-defective HIV-1 provirus (50) which is expressed constitutively only at very low levels, we have shown that HSV-1 stimulates efficiently HIV-1 transcription, but no induction of viral protein synthesis and production of HIV-1 virions could be detected. In HSV-1-infected cells, both the unspliced and spliced HIV-1 mRNAs were present in the cytoplasm, but they were excluded from the polysomes. Transfection with the Rev-expressing plasmid did not rescue synthesis of HIV-1 proteins in these cells. The inhibition of translation was specific for HIV-1 RNA, and no inhibition of cellular protein synthesis was detected under the conditions of the experiments. The observed block of the translation of HIV-1 RNAs was not a consequence of HSV-1-induced shutoff of host protein synthesis and could be seen only in the absence of the functional Tat protein. The transfection of the HSV-1-infected cells with the Tat-expressing plasmid or treatment with TNF- α reversed the inhibition and led to a complete virus expression. These data indicate that, in contrast to TNF- α , HSV-1 infection can induce transcription of Tat-defective HIV-1 but not the synthesis of the HIV-1 proteins and that Tat is required for the effective translation of HIV-1 transcripts.

MATERIALS AND METHODS

Cells and viruses. A T-cell line, CEMR7/neo, containing the integrated Tat-defective HIV-1 provirus, was described recently (16). The cells were grown in RPMI medium supplemented with 10% fetal calf serum, gentamicin (50 μ g/ml), and 2 mM L-glutamine. Stocks of wild-type HSV-1 strain MPcl-20 and the mutant *vhs-1* (54) which exhibits a defective virion host shutoff function were prepared by infection of Vero cells.

Expression plasmids and transfections. Cytomegalovirus (CMV) Rev contains a cDNA fragment of the *rev* gene under the control of the promoter-enhancer region from the CMV IE94 gene, as previously described (59). pGAGPOL-RRE-r contains the *Bgl*II-*Bam*HI fragment from HIV-1 joined to a DNA fragment that contains the open reading frames for the *gag*, *pol*, and *vif* genes (59). CEMR7/neo cells were transfected by using the DEAE-dextran method as previously described (57). The cells were harvested 24 h after transfection.

Preparation and analysis of RNA. Total, nuclear, and cytoplasmic cellular RNAs were prepared as previously described (49). Briefly, nuclei and cytoplasm were prepared by lysis of the cells with 0.5% Nonidet P-40 buffer, and isolated nuclei and cytoplasmic fractions were diluted in guanidine isothiocyanate buffer and processed as previously described (9). For Northern (RNA) analysis, RNAs (15 μ g/lane) were separated in 0.8% agarose-2.2 M formaldehyde gels and transferred to nitrocellulose filters. For detection of HSV-1 IE110 and late virion protein VP16 transcripts, filters were hybridized with DNA probes encompassing the *Bam*HI-to-*Sal*I fragment of pIGA-15 and the *Bam*HI-to-*Kpn*I fragment of pGR212, respectively (40, 48). HSV-1 thymidine kinase (TK) transcripts were detected by using a riboprobe, pBK87, encompassing the *Bam*HI-to-*Eco*RV fragment from the HSV-1 TK gene. The

HIV-1 riboprobe, pJM105, encodes the antisense sequence of the *Xba*I-*Pst*I fragment of the pHXBc2 clone (5). Human γ -actin riboprobe was described previously (24).

Polysomal RNA analysis. Polysomes were prepared by the method described by Katze et al. (31), with small modifications. CEMR7/neo cells were collected by centrifugation and washed twice with ice-cold phosphate-buffered saline containing 100 μ g of cycloheximide per ml. The cell pellet was suspended in ice-cold lysis buffer containing 10 mM Tris hydrochloride (pH 7.4), 1.5 mM KCl, 2.5 mM magnesium acetate, 0.2% Tween 20, 0.5% Triton X-100, 0.5% sodium deoxycholate, cycloheximide (100 μ g/ml), RNasin (100 U/ml), and 2 mM dithiothreitol. After 5 min on ice, nuclei and cell debris were removed by two centrifugations at 13,000 rpm for 6 and 10 min, respectively. Equal optical density at 260 nm units of each cytoplasmic lysate were layered over 20 to 50% sucrose gradients in 10 mM Tris hydrochloride (pH 7.4)-5 mM magnesium acetate-2 mM dithiothreitol-500 mM KCl and centrifuged at 30,000 rpm in an SW60 rotor at 4°C for 2.5 h. Gradient fractions (125 μ l) were collected on ice and UV (260 nm) monitored. The fractions were immediately frozen on dry ice and stored at -70°C. Four consecutive fractions (500 μ l) were pooled, and RNA was prepared as previously described (9). RNA pellets were resuspended in water, and aliquots were analyzed by Northern blotting for the presence of viral and cellular transcripts.

Western immunoblot analysis. Cells were lysed in a triple-detergent lysis buffer (50 mM Tris, 150 mM NaCl, 0.1% sodium dodecyl sulfate [SDS], 1% Nonidet P-40, 0.5% sodium deoxycholate, 2 mM phenylmethylsulfonyl fluoride [pH 8.0]) as previously described (49). One hundred fifty micrograms of protein extracts was separated on SDS-polyacrylamide gels and electrotransferred to a nitrocellulose filter. Filters were then blocked for 1 h in TN buffer (10 mM Tris, 150 mM NaCl [pH 7.4]) with 5% nonfat milk, washed with TN-TN buffer (TN buffer with 0.3% Tween 20 and 0.5% Nonidet P-40), and incubated overnight with a 1:1,000 dilution of serum from an HIV-1-seropositive patient, a 1:500 dilution of serum from an HSV-1-seropositive patient, or a 1:100 dilution of rabbit anti-HIV Gag serum in TN-T buffer (TN buffer with 0.3% Tween 20). After washing with TN-TN and TN-T buffers, membranes were incubated with ¹²⁵I-labeled protein A in TN-T buffer with 3% bovine serum albumin for 90 min. Finally, filters were washed in TN-TN buffer with 10 mM EDTA and TN buffer, dried, and exposed to an X-ray film.

RESULTS

HSV-1 infection induces expression of the Tat-defective HIV-1 genome in CEMR7/neo cells. We have previously shown that HSV-1 infection of HeLa cells carrying the integrated Tat-mutated HIV-1 genome induces transcription of the Tat-defective provirus (48). Since HSV-1 infection has been shown to activate transcription from the HIV-1 LTR and may be a cofactor in AIDS pathogenesis, we wanted to determine whether HSV-1 infection of T cells containing the Tat-defective provirus could activate production of an infectious virus. We used for these experiments a T-cell line, CEMR7/neo, carrying an integrated Tat-defective HIV-1 genome (16), which expresses low but detectable levels of both unspliced (9.2-kb) and spliced (4.2- and 2.0-kb) HIV-1 RNAs (Fig. 1A). Infection of these cells with HSV-1 (MOI of 3) resulted in an increase in the relative levels of the HIV-1 transcripts as early as 4 h postinfection, with maximal accumulation at 24 h after infection followed by a decrease at 48 h. In contrast to CEMR7/neo cells transfected with the Tat-expressing plasmid

FIG. 1. Analysis of the expression of the Tat-defective HIV-1 provirus in CEMR7/neo cells infected with HSV-1. (A) Time course of the HIV-1 induction by HSV-1. Total RNA was prepared at the indicated times (hours) from HSV-1-infected cells and uninfected controls (lane 0) and analyzed by Northern blot hybridization with an HIV-1 riboprobe (pJM105) (5). As a positive size control, RNA prepared from both chronically infected promyelocytic OM10.1 cells and CEMR7/neo cells transfected with the Tat expression SV₂ Tat plasmid (lane TAT) was used. Three major HIV-1 RNA species of 9.2, 4.2, and 2.0 kb are marked. (B) Analysis of HIV-1-specific proteins in CEMR7/neo cells infected with HSV-1 (3 MOI), induced with TNF- α or TPA, or transfected with the SV₂ Tat plasmid. Cells were harvested at indicated times after HSV-1 infection, cellular proteins (100 μ g per lane) were resolved on an SDS-8% polyacrylamide gel, and HIV-1-specific proteins were detected by Western blot hybridization with a polyclonal serum from an HIV-1-seropositive patient. In TNF- α - and TPA-treated cells or Tat-transfected cells, HIV-1 proteins were analyzed 24 h after induction or transfection, respectively. Molecular mass markers (in kilodaltons) are shown on the left, and HIV-1-specific proteins are indicated on the right.

that show a presence of the three major viral transcripts (9.2, 4.2, and 2.0 kb) in HSV-1-infected cells, we can detect a 5.0-kb *vif* transcript and two additional transcripts of about 8.0 and 3.0 kb. The presence of these HIV-1 transcripts is not unique to the HSV-1-infected cells, since they can also be detected in OM10.1 cells and in TNF- α -induced cells (see Fig. 3). We cannot determine presently whether these aberrant transcripts are a result of an abnormal splicing (3.0 kb) or an incorrect termination (8.0 kb). A number of additional transcripts can be also seen in chronically infected OM10.1 cells. While in acutely infected T cells only three major HIV-1 transcripts can be detected (59), chronically infected T cells show generally a more complex profile (data not shown). These results show that the HSV-1 infection of T cells can stimulate transcription of the Tat-defective HIV-1 provirus.

Lack of HIV-1 protein synthesis in HSV-1-infected CEMR7/neo cells. We have shown earlier that the Tat-defective HIV-1 provirus can be effectively induced in CEMR7/neo cells by both transfection with the Tat-expressing plasmid or treatment with TNF- α (50). Both of these inducers were able to activate a full cycle of replication and production of viral particles. To determine whether infection with HSV-1 also leads to the induction of viral progeny, we analyzed the levels of HIV-1 proteins accumulated in CEMR7/neo cells infected with HSV-1. Western blot analysis showed that uninfected cells produced constitutively low levels of p55^{Gag} and gp160 precursors (Fig. 1B). No increase in the levels of p55^{Gag} and gp160 in HSV-1-infected cells was detected. In contrast, viral proteins could be detected in tetradecanoyl phorbol acetate (TPA)- or TNF- α -treated cells and cells transfected with Tat plasmid. We have previously shown that the processing of HIV-1 proteins in TNF- α -treated cells is slowed down (50). Protein with a

molecular mass of about 110 kDa which was detected in HSV-1-infected but not in TNF- α - or TPA-treated cells represents an HSV-1-specific protein and was also detected by human anti-HSV-1 serum (Fig. 2B). These results indicate that the human anti-HIV-1 serum used contains antibodies against HSV-1.

The lack of accumulation of HIV-1 proteins in HSV-1-infected cells was unexpected in view of the high levels of HIV-1 transcripts detected in these cells. The CEMR7/neo cells are fully permissive to HSV-1 replication, and the HSV-1-specific transcripts of IE110 and TK genes can be detected in infected cells as early as 4 h postinfection (Fig. 2A). At 8 h after infection, late HSV-1 transcripts such as virion protein VP16 mRNA start to accumulate in the infected cells, reaching maximum levels at 24 h postinfection. The increase in the relative levels of HSV-1 transcripts correlates with the accumulation of HSV-1-specific proteins, as determined by Western blot (Fig. 2B).

Subcellular distribution of HIV-1 transcripts in HSV-1-infected cells. It has been previously suggested (16) that little or no Rev protein is synthesized in the absence of Tat, and we have observed that, in HeLa cells, the TPA-mediated expression of the Tat-defective HIV-1 provirus had characteristics of a *rev*-defective virus (49). Since in the absence of Rev the unspliced genomic RNA encoding Gag and Gag-Pol proteins is localized predominantly in the nucleus (15, 18), we examined and compared the subcellular distribution of HIV-1 transcripts in HSV-1-infected and TNF- α -treated cells. However, the results (Fig. 3A) show that, in HSV-1-infected cells, as in cells stimulated with TNF- α , both the unspliced and the spliced HIV-1 transcripts were present in the cytoplasm. These results suggest that the block in HIV-1 protein synthesis in HSV-1-

FIG. 2. Relative levels of HSV-1 mRNAs and viral proteins in CEMR7/neo cells infected with HSV-1. (A) Total RNA, isolated at the indicated times from infected cells, was analyzed by Northern blot hybridization with probes detecting IE110 (ICP0), TK, and virion protein VP-16 transcripts. (B) HSV-1-specific proteins were detected by Western blot analysis with serum from HSV-1-infected patient. Molecular mass markers (in kilodaltons) are shown on the right.

infected cells is not due to the absence of genomic and envelope HIV-1 RNAs in the cytoplasm. To exclude the possibility that the cytoplasmic HIV-1 RNAs detected represent a leak from the nucleus during the isolation procedure, we analyzed both cytoplasmic and nuclear RNAs for the presence of rRNA (Fig. 3B). The results show that the nucleus-specific 47S rRNA precursor and its 32S rRNA-processed intermediate were detected only in the nuclear fractions. In contrast, 28S rRNA was present predominantly in the cytoplasm. These results demonstrate that the contamination of the cytoplasmic fractions with nuclear components was very low, if any, and

FIG. 3. Nuclear and cytoplasmic distribution of HIV-1 transcripts in CEMR7/neo cells infected with HSV-1 or treated with TNF- α . (A) Nuclear (N) or cytoplasmic (C) RNAs (15 μ g) from untreated cells (control), or cells treated for 6 h with TNF- α (100 U/ml) or infected for 8 h with HSV-1 (MOI of 3) were analyzed by Northern blot hybridization with the HIV-1-specific pJM105 riboprobe. (B) Detection of rRNAs in cell fractionations. Nuclear (N) and cytoplasmic (C) RNAs analyzed in panel A were stained with ethidium bromide before the transfer to nitrocellulose filters. Positions of rRNA precursors (47S and 32S) and mature rRNA (28S and 18S) are indicated on the right.

FIG. 4. Sucrose gradient analysis of polysomes from HSV-1-infected or TNF- α -treated cells. Cytoplasmic extracts (equal optical density at 260 nm [OD 260] units) from HSV-1-infected or TNF- α -treated cells (24 h postinfection or TNF- α treatment) were centrifuged through a 3.5-ml 20 to 50% sucrose gradient for 2.5 h at 30,000 rpm at 4°C. The OD 260 of a 1:10 dilution of each fraction is shown. Four consecutive fractions (500 μ l) were pooled into A to H samples. The pelleted fraction I (not shown) was dissolved in 500 μ l of H₂O.

suggest that HIV-1 transcripts present in the cytoplasm of the HSV-1-infected cells were not effectively translated.

Differential distribution of HIV-1 RNA on polysomes from TNF- α -treated and HSV-1-infected cells. To determine whether the block of HIV-1 mRNA translation in HSV-1-infected cells was at the level of ribosomal loading, we analyzed the distribution of the cytoplasmic HIV-1 mRNAs on the polysomes isolated from CEMR7/neo cells infected with HSV-1 and compared it with the distribution of HIV-1 mRNAs on polysomes isolated from TNF- α -induced cells. Cytoplasmic extracts were prepared from CEMR7/neo cells 24 h after infection with HSV-1 or TNF- α treatment and applied directly to a 20 to 50% sucrose gradients containing 500 mM KCl to minimize artifactual sticking of RNA to polysomes (31). Figure 4 shows the absorbance profile of the sucrose gradient. Because of the presence of 500 mM KCl, most of the 80S monosomes dissociated into 60S and 40S ribosomal subunits and were not present as a separate peak. However, no major differences between the proportions of polysomes and ribosomal subunits or the sizes of polysomes in TNF- α -induced and HSV-1-infected cells were seen. This polysome pattern indicates that HSV-1 infection has not resulted in a general polysome degradation.

Distribution of viral and cellular mRNAs on polysomes in pooled gradient fractions (samples A to I) was analyzed. Sample A contained the material sedimenting more slowly than ribosomal subunits, samples B through D represent ribosomal subunits and residual monosomes, samples E through H represent polysome fractions, and fraction I (Fig. 5) represents the largest pelleted polysomes. RNA, isolated from gradient fractions, was analyzed by Northern hybridization for the presence of viral and cellular transcripts (Fig. 5). No major differences in the distribution of cellular γ -actin mRNA, HSV-1 TK mRNA, and 28S rRNA in the gradients prepared from TNF- α -treated or HSV-1-infected cells were observed. Both γ -actin and HSV-1 TK mRNAs predominantly accumulated in E/F, G, and I fractions, but the levels of these

FIG. 5. The HIV-1 RNA is dissociated from polysomal fraction in HSV-1-infected cells but not in TNF- α -treated cells. RNA, prepared from pooled sucrose gradient fractions A to I, was analyzed by Northern blot hybridization with riboprobes specific for HIV-1 (pJM105), γ -actin, and HSV-1 TK mRNAs. The locations of 28S and 18S rRNAs are indicated. The levels of RNA in each fraction that were analyzed are shown by ethidium bromide staining of the gel before transfer to nitrocellulose filters.

transcripts were found to be highest in fraction I compared with those in the more slowly sedimenting polysomal fractions F and G. These results, together with the similarity in the absorbance profile (Fig. 4), suggest that, within the first 24 h, HSV-1 infection of CEMR7/neo cells did not lead to overall disruption of cellular protein synthesis.

The analysis of the HIV transcripts (Fig. 5) has shown, however, a significant difference in the polysomal distribution of HIV-1 mRNAs in TNF- α -treated and HSV-1-infected cells. In TNF- α -induced cells, HIV-1 mRNAs were evenly distributed through the fractions C to F, with the highest accumulation in polysomal fraction I. In contrast, in HSV-1-infected cells, most of the HIV-1 RNAs were found in fractions representing ribosomal subunits and monosomes, and relatively little HIV-1 transcript could be detected in polysomal fraction I. The HIV-1 transcripts were also associated with polysomes (fraction I) in Tat-transfected cells, and in the presence of Tat, HSV-1 infection was not able to dissociate the HIV-1 transcripts from the polysomes (Fig. 6).

To determine whether the dissociation of the HIV-1 RNA from the polysome in HSV-1-infected cells is a result of inhibition of cellular protein synthesis mediated by the HSV-1-mediated shutoff host cell protein synthesis (Vhs function), we analyzed the distribution of HIV-1 RNA on polysomes in cells infected with the *vhs* mutant of HSV-1 (described later). However, cells infected with the *vhs* mutant also showed very few HIV-1 transcripts on the polysomes. To confirm that the viral and cellular mRNAs detected on the largest polysomes (fraction I) were indeed associated with polysomes and not with some other subcellular structures, cytoplasmic extracts were treated before centrifugation with EDTA to dissociate

FIG. 6. HSV-1 does not dissociate HIV-1 RNA from polysomes in Tat-transfected CEMR7/neo cells. CEMR7/neo cells were transfected with SV₂ Tat (5 μ g) and 4 h later split into three parts. One part was infected with HSV-1, the second was infected with *vhs-1* mutant of HSV-1, and the third served as a control. Cytoplasmic fractions from infected cells and uninfected controls were prepared 24 h later, and sucrose density analysis was done as described in the legend to Fig. 4. RNA prepared from pooled sucrose gradient fractions was analyzed by Northern blot hybridization with a HIV-1-specific riboprobe, JM105. The locations of 28S and 18S rRNAs are indicated.

polysomes into ribosomal subunits. The Northern blot analysis of the polysome-associated mRNAs has shown that EDTA treatment dissociated effectively polysomes and released about 95% of the γ -actin and HIV-1 RNAs to the supernatant (data not shown).

Transfection with Rev-expressing plasmid does not rescue HIV-1 mRNAs translation in HSV-1-infected cells. Several reports have suggested that expression of the Rev protein is required for the efficient translation of the *gag-pol* and the *env* mRNAs (2, 6, 11, 25, 26). To determine whether the absence of translation of HIV-1 mRNAs in CEMR7/neo cells infected with HSV-1 is due to the inefficient expression of the Rev protein, cells were transfected with the plasmid containing the Rev cDNA under the direction of the CMV early regulatory region (60), and 24 h later cells were infected with HSV-1 (Fig. 7). The results showed that the expression of the exogenous *rev* gene was not able to promote synthesis of HIV-1 proteins in HSV-1-infected cells. However, the levels of the p55^{Gag} precursor in TNF- α -treated cells were increased in the presence of exogenous Rev (compare TNF- α alone and TNF plus Rev). The expression of Rev protein in the transfected cells was analyzed by a functional assay of the expression of Gag proteins encoded by the plasmid pGAGPOL-RRE-r (59) (expression of the *gag* gene in this plasmid is strictly dependent on the presence of functioning Rev). High levels of p55^{Gag} could be detected in cells transfected with the Rev expression plasmid but not in the untransfected controls (data not shown). These results indicate that the transfected Rev plasmid is expressed in these cells and that the poor translatability of HIV-1 transcripts in HSV-1-infected cells is not a consequence of low levels of Rev protein.

HSV-1 infection does not inhibit synthesis of HIV-1 proteins in TNF- α -induced CEMR7/neo cells. The absence of HIV-1

FIG. 7. Effects of Rev protein on the expression of p55^{Gag} precursor protein in CEMR7/neo cells. Cells were transfected with 10 μ g of Rev-expressing plasmid CMV Rev, and 24 h later both transfected (Rev) and untransfected cells (control) were stimulated with TNF- α (100 U/ml) or infected with HSV-1 (3 MOI). Cellular proteins isolated 24 h later were resolved on a SDS-8% polyacrylamide gel and analyzed by Western blot hybridization with the human anti-HIV-1 serum. This serum detects also some HSV-1-specific proteins (e.g., 100, 50, and 40 kDa). Molecular mass markers (in kilodaltons) are on the right.

mRNA on polysomes in HSV-1-infected cells could be a consequence of HSV-1-induced degradation of HIV-1 transcripts or their replacement by HSV-1 transcripts. To determine whether HSV-1 infection interferes also with the translation of TNF- α -induced HIV-1 mRNAs, CEMR7/neo cells

were either first treated with TNF- α and 8 h later infected with HSV-1 or first infected with HSV-1 and after 8 h treated with TNF- α . The levels of HIV-1-specific proteins were determined at 24 h after the start of the induction. The results show that, while only low levels of p55^{Gag} precursor (Fig. 8A) were present in cells infected with HSV-1, both p55^{Gag} and gp160 could be easily detected in cells that were treated with TNF- α and infected with HSV-1. It should be noted that the gp160 precursor can be repeatedly observed in TNF- α -induced CEMR7/neo cells (50), while it is not easily detectable in Tat-transfected cells. The levels of p55^{Gag} in TNF- α - and HSV-1-treated cells were slightly higher than those observed in cells stimulated with TNF- α . These data suggest (i) that HSV-1 infection did not prevent translation of the TNF- α -induced HIV-1 transcripts and (ii) that the presence of TNF- α facilitates translation of HSV-1-induced HIV-1 transcripts. It should be noted that, in these experiments, the levels of p55^{Gag} were also slightly higher in cells infected with HSV-1 than in the uninfected controls. This enhancement is partially due to the cross-reactivity of HSV-1-encoded p55 protein with the human anti-HIV-1 serum; however, we cannot exclude that, in addition, low levels of p55^{Gag} were synthesized in HSV-1-infected cells. The important point is, however, that the translation of HIV-1 transcripts in HSV-1-infected cells is very inefficient in the absence of Tat protein or TNF- α . To verify that expression of HSV-1 proteins in infected cells was not inhibited by treatment with TNF- α , the protein extracts from TNF- α -treated and HSV-1-infected cells were analyzed for the presence of HSV-1 proteins. As shown in Fig. 8B, the levels of HSV-1 proteins were not significantly altered in the presence of TNF- α . Similarly, when cells were transfected with the plasmid that expressed the *tat* gene under the control of HIV-1 LTR and then infected with HSV-1, HIV-1 mRNAs were

FIG. 8. Effects of HSV-1 infection on the expression of HIV-1 proteins in CEMR7/neo cells induced by TNF- α . (A) Cells were untreated (control) or either infected with HSV-1 (3 MOI) (lane HSV) or treated with TNF- α (100 U/ml) (lane TNF). Half of the HSV-1-infected cells was simultaneously induced with TNF- α (HSV + TNF) or treated with TNF- α 8 h later (HSV TNF). Half of the TNF- α -treated cells (8 h) was then infected with HSV-1 (TNF HSV). Twenty-four hours after induction and/or infection, cells were collected and the presence of viral proteins was detected by Western hybridization with a human anti-HIV-1 serum. HIV-1-specific proteins are marked on the left. Some HSV-1-specific proteins were also detected by this serum. (B) The proteins analyzed in panel A were reacted with human anti-HSV-1 polyclonal serum. Molecular mass markers (in kilodaltons) are shown on the right.

efficiently translated. The levels of viral proteins detected in the cells transfected with Tat-expressing plasmid in the presence and absence of HSV-1 were comparable, indicating that HSV-1 infection did not lead to the general degradation of the HIV-1 mRNAs (data not shown).

Inhibition of translation of HIV-1 transcripts is not mediated by the virion-associated host shutoff (vhs) function. Infection of permissive cells with HSV-1 at a high MOI (>5 to 10) leads to a rapid cessation of cellular protein synthesis which can be mediated by the virion-associated host cell shutoff protein (vhs) (34, 54, 62). Although we have not seen an impairment in the host protein synthesis or significant degradation of cellular and viral RNAs up to 24 h postinfection, we could not eliminate that the block in translation of HIV-1 transcripts is caused by the HSV-1-encoded vhs protein. To determine unambiguously whether vhs is involved in the inhibition of HIV-1 mRNA translation, we infected the CEMR7/neo cells with the vhs-defective mutant of HSV-1 virus (*vhs-1* mutant). Northern analysis (Fig. 9A) has shown that the infection of CEMR7/neo cells with the *vhs-1* mutant virus increased efficiently the relative levels of HIV-1 transcripts in these cells. The kinetics of induction of HIV-1 RNA was slower than with the wild type HSV-1, since little HIV-1 transcript could be detected at 8 h postinfection (cf. Fig. 1A). The slower induction of HIV-1 transcripts is probably due to the slower replication rate of the *vhs-1* mutant virus, since *vhs-1* virus-encoded mRNA (TK) and proteins were detected later (8 and 24 h, respectively) than they were after infection with wild-type HSV-1 (Fig. 9B and C).

When the levels of HIV-1 proteins were analyzed (Fig. 9D), the HIV-1 p55^{Gag} precursor was efficiently expressed in TNF- α -treated cells, but only at a very low level in the cells infected for 24 h with *vhs-1* mutant virus. Cells treated with TNF- α and infected with the *vhs-1* mutant show slightly higher levels of p55^{Gag} precursor than the cells treated with TNF- α alone, showing a cooperative effect between TNF- α and *vhs-1*. These data, together with the polysomal distribution of HIV-1 RNA (Fig. 6), indicate that even in the absence of HSV-1-encoded vhs function, the translation of the HIV-1 transcripts was much less efficient than in TNF- α -treated cells, suggesting that the block in the translation of the HIV-1 transcripts in HSV-1-infected cells is not a consequence of vhs phenotype.

DISCUSSION

We have shown in this study that infection of CEMR7/neo cells with HSV-1 can activate transcription of the integrated Tat-defective HIV-1 provirus; however, synthesis of HIV-1 proteins was very inefficient even though high levels of unspliced and spliced HIV-1 mRNAs were present in the cytoplasm of HSV-1-infected cells. The inability of HSV-1-induced HIV-1 transcripts to be translated occurred only in the absence of functional Tat protein. No inhibition of HIV-1 protein synthesis in CEMR7/neo cells that were both transfected with the Tat-expressing plasmid and infected with HSV-1 was observed. Similarly, we have previously shown that HSV-1 infection of ACH-2 cells, which contain the integrated, latent HIV-1 provirus, induced a full cycle of HIV-1 replication and production of virions (65). Several studies, which used either an in vitro translational system (21, 59) or *Xenopus* oocytes (7, 8), have indicated that Tat can affect the translational efficiency of HIV-1 RNAs. However, HIV-1 transcripts could be efficiently translated in the absence of functional Tat protein (50) in TNF- α -treated CEMR7/neo cells (50). The presence of virus-encoded Rev protein was shown to be required for the efficient loading of HIV-1 transcripts on polysomes and trans-

FIG. 9. Induction of HIV-1 provirus in CEMR7/neo cells infected with a *vhs-1* mutant of HSV-1. Cells were infected with the *vhs-1* mutant at a MOI of 3, and total RNA was isolated at the indicated times postinfection and analyzed by Northern blot hybridization with the HIV-1-specific riboprobe (pJM105) (A) or with the HIV-1 TK-specific probe (B). HIV-1-specific (D) and HSV-1-specific (C) proteins were detected by Western blot hybridization with human anti-HIV-1 or HSV-1 serum. Cells treated with TNF- α for 24 h were used as a positive control. Molecular mass markers (in kilodaltons) are shown on the right.

lation of Rev-responsive element-containing viral RNAs (2, 6, 11, 25, 26). However, the block of HIV-1 mRNA translation in HSV-1-infected cells was not rescued by the overexpression of Rev protein, implying that the observed effect was not a consequence of low levels of Rev protein in HSV-1-infected CEMR7/neo cells.

Infection of permissive cells with a high multiplicity of HSV-1 results in a rapid cessation of the host protein synthesis. The initial shutoff of the host protein synthesis appears to be determined by a structural component of HSV-1 virions (Vhs) which facilitates nonspecific degradation of cellular mRNAs and polysome disaggregation (34, 54). The second viral activity which affects selectively stability of cellular and viral transcripts and their processing (55, 63) is encoded by the product of the IE gene ICP27. To understand the mechanism by which HSV-1 blocks the translation of HIV-1 transcripts, we compared the polysomal distribution of the HIV-1 transcripts in HSV-1-infected, TNF- α -treated, and Tat-transfected CEMR7/neo cells. We have not found any major difference between overall polysomal profile in Tat-transfected or TNF- α -treated cells; the association of HIV-1 transcripts with polysomes in cells infected with HSV-1 was very ineffective. While, in TNF- α -treated cells, the HIV-1 transcripts were associated with polysomes, and in HSV-1-infected cells, HIV-1 transcripts were mostly excluded from the polysomal fractions.

The block in the translation of HIV-1 transcripts in HSV-1-infected cells was not a consequence of ICP27-mediated impairment in processing or splicing of HIV-1 mRNA, since no major difference in size and distribution of unspliced and

spliced HIV-1 mRNAs could be detected between TNF- α - and HSV-1-induced cells. The inefficient translation of HIV-1 mRNA was also not a result of the *vhs* function and consequent shutoff of cellular protein synthesis. At the MOI used, HSV-1 infection did not lead to a general inhibition of cellular protein synthesis, and the absence of HIV-1 transcripts on polysomes and their inefficient translation were also observed in cells infected with the *vhs-1* mutant of HSV-1 that fails to shut off host protein synthesis.

Unexpectedly, the HSV-1 infection could neither impose restriction on the translation of HIV-1 mRNAs nor displace the HIV-1 transcripts from polysomes of the TNF- α -treated or Tat-transfected cells, indicating that TNF- α , as is Tat, is able to overcome the translational block and allow translation of HIV transcripts. It was shown that the first 100 nucleotides of HIV-1 mRNAs, containing the TAR sequence, have a strong inhibitory effect on translation in vitro and in *Xenopus* oocytes (20, 45, 58). The mutation that destroyed the secondary structure relieved the translational inhibition and increased efficiency of binding of the 28-kDa cap binding protein eIF-4B, while the Tat protein was not able to relieve the inhibition of HIV-1 mRNA translation in vitro (data not shown). It is likely that in vivo Tat may enhance the translatability either directly or with the help of a cellular cofactor(s). It is not without interest that TNF- α may also enhance the translatability of the HIV-1 mRNAs, since it was shown that TNF- α enhances phosphorylation of eIF-4E (39), which facilitates the corecruitment of the mRNA to ribosomes (for a review, see reference 64). On the basis of the results of this study, we suggest that both Tat and TNF- α not only activate the transcription of HIV-1 provirus but also facilitate the translation of the HIV-1 transcripts. In contrast, HSV-1 infection activates the transcriptional step; however, the translation of the HIV-1 mRNA is very inefficient, most likely because HSV-1 does not encode a function(s) that would facilitate the translation of poorly translatable HIV-1 mRNAs. The suppression by the virion-associated Vhs function of the HSV-1 seems to play only a marginal role in this translation block. The poor translatability of the HSV-1-induced HIV-1 transcripts can be then overcome in the presence of Tat or TNF- α .

A question concerning the contribution of HSV-1 infection, if any, to the pathogenicity of HIV-1 infection remains. It has been recently shown that, during the clinical latency, a large amount of the HIV-1 virus is present in secondary lymphoid organs (44) and that a majority of the infection in lymph nodes is of the latent nature (14). It is expected that some of these HIV-1 proviruses may be replication defective as a consequence of high levels of errors in retroviral replication (51). Since the *tat* gene product plays a key role in the viral replication, it is important to determine whether any of the cellular and extracellular factors that can transcriptionally activate HIV-1 LTR can effectively replace its function, rescue the expression of the nonreplicative Tat-defective HIV-1 provirus, and support its spread (50). The results of this study indicate that HSV-1 infection is not able to rescue the full cycle of HIV-1 replication because of the translational suppression of HIV-1 transcripts. Rescue of HIV-1 provirus by HSV-1 can occur only in the presence of Tat, TNF- α , and possibly other cytokines that were shown to up-regulate HIV-1 expression at the posttranscriptional level (47). However, the observation that the HIV-1 transcripts may be translationally repressed in the absence of Tat or TNF- α may have to be taken into consideration in the evaluation of HIV-1 replication in clinical samples.

ACKNOWLEDGMENTS

We thank G. Sullivan Read for the *vhs-1* mutant, Marie-Louise Hammarskjöld for the pGAGPOL-RRE-r and CMV Rev plasmids, and Kent Wilcox for his helpful suggestions during the course of this work. We also thank Barbara Schneider for typing the manuscript.

This work was supported by Public Health Service grant AI26123 from the National Institute of Allergy and Infectious Diseases and grant 001684 from the American Foundation for AIDS Research.

REFERENCES

1. Albrecht, M. A., N. A. DeLuca, R. A. Byrn, P. A. Schaffer, and S. M. Hammer. 1989. The herpes simplex virus immediate-early protein, ICP4, is required to potentiate replication of human immunodeficiency virus in CD4⁺ lymphocytes. *J. Virol.* **63**:1861-1868.
2. Arrigo, S. J., and I. S. Y. Chen. 1991. Rev is necessary for translation but not cytoplasmic accumulation of HIV-1 vif, vpr, and env/vpu 2 RNAs. *Genes Dev.* **5**:808-819.
3. Arrigo, S. J., S. Heaphy, and J. K. Haines. 1992. In vivo binding of wild-type and mutant human immunodeficiency virus type 1 Rev proteins: implications for function. *J. Virol.* **66**:5569-5575.
4. Ballard, D. W., W. H. Walker, S. Doerre, P. Sista, J. A. Molitor, E. P. Dixon, N. J. Pepper, M. Hannink, and W. C. Greene. 1990. The *v-rel* oncogene encodes a κ B enhancer binding protein that inhibits NF- κ B function. *Cell* **63**:803-814.
5. Bednarik, D. P., J. D. Mosca, N. B. K. Raj, and P. M. Pitha. 1989. Inhibition of human immunodeficiency virus (HIV) replication by HIV-trans-activated α_2 -interferon. *Proc. Natl. Acad. Sci. USA* **86**:4958-4962.
6. Benko, D. M., R. Robinson, L. Solomin, M. Mellini, B. K. Felber, and G. N. Pavlakis. 1990. Binding of trans-dominant mutant rev protein of human immunodeficiency virus type 1 to the cis-acting rev responsive element does not affect the fate of viral mRNA. *New Biol.* **2**:1111-1122.
7. Braddock, M., A. Chambers, W. Wilson, M. P. Esnouf, S. E. Adams, A. J. Kingsman, and S. M. Kingsman. 1989. HIV-1 TAT "activates" presynthesized RNA in the nucleus. *Cell* **58**:269-279.
8. Braddock, M., A. M. Thorburn, A. Chambers, G. D. Elliott, G. J. Anderson, A. J. Kingsman, and S. M. Kingsman. 1990. A nuclear translational block imposed by the HIV-1 U3 region is relieved by the Tat-TAR interaction. *Cell* **62**:1123-1133.
9. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**:5294-5299.
10. Cochrane, A. W., K. S. Jones, S. Beidas, P. J. Dillon, A. M. Skalka, and C. A. Rosen. 1991. Identification and characterization of intragenic sequences which repress human immunodeficiency virus structural gene expression. *J. Virol.* **65**:5305-5313.
11. D'Agostino, D. M., B. K. Felber, J. E. Harrison, and G. N. Pavlakis. 1992. The Rev protein of human immunodeficiency virus type 1 promotes polysomal association and translation of *gag/pol* and *vpu/env* mRNAs. *Mol. Cell. Biol.* **12**:1375-1386.
12. Dayton, A. I., J. G. Sodroski, C. A. Rosen, W. C. Goh, and W. A. Haseltine. 1986. The *trans*-activation gene of the human T-cell lymphotropic virus type III is required for replication. *Cell* **44**:941-947.
13. Duh, E. J., W. J. Maury, T. M. Folks, A. S. Fauci, and A. B. Rabson. 1989. Tumor necrosis factor α activates human immunodeficiency virus type 1 through induction of nuclear factor binding to the NF- κ B sites in the long terminal repeat. *Proc. Natl. Acad. Sci. USA* **86**:5974-5978.
14. Embretson, J., M. Zupancic, J. L. Ribas, A. Burke, P. Racz, K. Tenner-Racz, and A. T. Haase. 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature (London)* **362**:359-362.
15. Emerman, M., R. Vazeux, and K. Peden. 1989. The rev gene product of the human immunodeficiency virus affects envelope-specific RNA localization. *Cell* **57**:1155-1165.
16. Feinberg, M. B., D. Baltimore, and A. D. Frankel. 1991. The role of Tat in the human immunodeficiency virus life cycle indicates a primary effect on transcriptional elongation. *Proc. Natl. Acad. Sci. USA* **88**:4045-4049.
17. Feinberg, M. B., R. F. Jarret, A. Aldovini, R. C. Gallo, and F. Wong-Staal. 1986. HTLV-III expression and production involve

- complex regulation at the levels of splicing and translation of viral RNA. *Cell* **46**:807–817.
18. Felber, B. K., M. Hadzopoulou-Cladaras, C. Cladaras, T. Copeland, and G. N. Pavlakis. 1989. *rev* protein of human immunodeficiency virus type 1 affects the stability and transport of the viral mRNA. *Proc. Natl. Acad. Sci. USA* **86**:1495–1499.
 19. Fisher, A. G., M. B. Feinberg, S. F. Josephs, M. E. Harper, L. M. Marselle, G. Reyes, M. A. Gonda, A. Aldovini, D. Debouk, R. C. Gallo, and F. Wong-Staal. 1986. The trans-activator gene of HTLV-III is essential for virus replication. *Nature (London)* **320**:367–371.
 20. Geballe, A. P., and M. K. Gray. 1992. Variable inhibition of cell-free translation by HIV-1 transcript leader sequences. *Nucleic Acids Res.* **20**:4291–4297.
 21. Ghosh, S., and D. Baltimore. 1990. Activation *in vitro* of NF- κ B by phosphorylation of its inhibitor I κ B. *Nature (London)* **344**:678–682.
 22. Gimble, J. E., E. Duh, J. M. Ostrove, H. E. Gendelman, E. E. Max, and A. B. Rabson. 1988. Activation of the human immunodeficiency virus long terminal repeat by herpes simplex virus type 1 is associated with induction of a nuclear factor that binds to the NF- κ B/core enhancer sequences. *J. Virol.* **62**:4104–4112.
 23. Griffin, G. E., K. Leung, T. M. Folks, S. Kunkel, and G. J. Nabel. 1989. Activation of HIV gene expression during monocyte differentiation by induction of NF- κ B. *Nature (London)* **339**:70–73.
 24. Gunning, P., P. Ponte, H. Okayama, J. Engel, H. Blau, and L. Kedes. 1983. Isolation and characterization of full-length cDNA clones for human α -, β -, and γ -actin mRNAs: skeletal but not cytoplasmic actins have an amino-terminal cysteine that is subsequently removed. *Mol. Cell. Biol.* **3**:787–795.
 25. Hadzopoulou-Cladaras, M., B. K. Felber, C. Cladaras, A. Athanassopoulou, A. Tse, and G. N. Pavlakis. 1989. The *rev* (*trs/art*) protein of human immunodeficiency virus type 1 affects viral mRNA and protein expression via a *cis*-acting sequence in the *env* region. *J. Virol.* **63**:1265–1274.
 26. Hammariskjold, M. L., J. Heimer, B. Hammariskjold, I. Sangwan, L. Albert, and D. Rekosh. 1989. Regulation of human immunodeficiency virus *env* expression by the *rev* gene product. *J. Virol.* **63**:1959–1966.
 27. Harrich, D., J. Garcia, R. Mitsuyasu, and R. Gaynor. 1990. TAR independent activation of the human immunodeficiency virus in phorbol ester stimulated T lymphocytes. *EMBO J.* **9**:4417–4423.
 28. Inoue, J. I., L. D. Kerr, L. J. Ransone, E. Bengel, T. Hunter, and I. M. Verma. 1991. *c-rel* activates but *v-rel* suppresses transcription from κ B sites. *Proc. Natl. Acad. Sci. USA* **88**:3715–3719.
 29. Israel, N., U. Hazan, J. Alcamí, A. Munier, F. Arenzana-Seisdedos, F. Bachelierie, A. Israel, and J.-L. Virelizier. 1989. Tumor necrosis factor stimulates transcription of HIV-1 in human T lymphocytes, independently and synergistically with mitogens. *J. Immunol.* **143**:3956–3960.
 30. Kato, H., H. Sumimoto, P. Pognonee, C.-H. Chen, C. A. Rosen, and R. G. Roeder. 1992. HIV-1 Tat acts as a processivity factor *in vitro* in conjunction with cellular elongation factors. *Genes Dev.* **6**:655–666.
 31. Katze, M. G., D. DeCorato, and R. M. Krug. 1986. Cellular mRNA translation is blocked at both initiation and elongation after infection by influenza virus or adenovirus. *J. Virol.* **60**:1027–1039.
 32. Kieran, M., V. Blank, F. Logeat, J. Vandekerckhove, F. Lottspeich, O. Le Bail, M. B. Urban, P. Kourilsky, P. A. Baeuerle, and A. Israel. 1990. The DNA binding subunit of NF- κ B is identical to factor KBF-1 and homologous to the *rel* oncogene product. *Cell* **62**:1007–1018.
 33. Kowalik, T. F., B. Wing, J. S. Haskill, J. C. Azizkhan, A. S. Baldwin Jr., and E.-S. Huang. 1993. Multiple mechanisms are implicated in the regulation of NF- κ B activity during human cytomegalovirus infection. *Proc. Natl. Acad. Sci. USA* **90**:1107–1111.
 34. Kwong, A. D., and N. Frenkel. 1989. The herpes simplex virus virion host shutoff function. *J. Virol.* **63**:4834–4839.
 35. Laspia, M. F., A. P. Rice, and M. B. Mathews. 1989. HIV-1 *tat* protein increases transcriptional initiation and stabilizes elongation. *Cell* **59**:283–292.
 36. Maldarelli, F., M. A. Martin, and K. Strebel. 1991. Identification of posttranscriptionally active inhibitory sequences in human immunodeficiency virus type 1 RNA: novel level of gene regulation. *J. Virol.* **65**:5732–5743.
 37. Malim, M. H., J. Hauber, S.-Y. Le, J. V. Maizel, and B. R. Cullen. 1989. The HIV-1 *rev* transactivator acts through a structured target sequence to activate nuclear export of unspliced viral mRNA. *Nature (London)* **338**:254–257.
 38. Marciniak, R. A., and P. A. Sharp. 1991. HIV-1 Tar protein promotes formation of more-processive elongation complexes. *EMBO J.* **10**:4189–4196.
 39. Marino, M. W., L. J. Feld, E. A. Jaffe, L. M. Pfeffer, H.-M. Han, and D. B. Donner. 1991. Phosphorylation of the proto-oncogene product eukaryotic initiation factor 4E is a common cellular response to tumor necrosis factor. *J. Biol. Chem.* **266**:2685–2688.
 40. Mosca, J. D., D. P. Bednarik, N. B. K. Raj, C. A. Rosen, J. G. Sodroski, W. A. Haseltine, G. S. Hayward, and P. M. Pitha. 1987. Activation of human immunodeficiency virus by herpesvirus infection: identification of a region within the long terminal repeat that responds to a trans-acting factor encoded by herpes simplex virus 1. *Proc. Natl. Acad. Sci. USA* **84**:7408–7412.
 41. Mosca, J. D., D. P. Bednarik, N. B. K. Raj, C. A. Rosen, J. G. Sodroski, W. A. Haseltine, and P. M. Pitha. 1987. Herpes simplex virus type-1 can reactivate transcription of latent human immunodeficiency virus. *Nature (London)* **325**:67–70.
 42. Muchardt, C., J.-S. Seeler, A. Nirula, D.-L. Shurland, and R. B. Gaynor. 1992. Regulation of human immunodeficiency virus enhancer function by PRDII-BF1 and *c-rel* gene products. *J. Virol.* **66**:244–250.
 43. Ostrove, J. M., J. Leonard, K. E. Weck, R. A. Rabson, and H. E. Gendelman. 1987. Activation of the human immunodeficiency virus by herpes simplex virus type 1. *J. Virol.* **61**:3726–3732.
 44. Pantaleo, G., C. Graziosi, J. F. Demarest, L. Butini, M. Montroni, C. H. Fox, J. M. Orenstein, D. P. Kotler, and A. S. Fauci. 1993. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature (London)* **362**:355–358.
 45. Parkin, N. T., E. A. Cohen, A. Darveau, C. Rosen, W. Haseltine, and N. Sonnenberg. 1988. Mutational analysis of the 5' non-coding region of human immunodeficiency virus type 1: effects of secondary structure on translation. *EMBO J.* **7**:2831–2837.
 46. Pitha, P. M., and D. P. Bednarik. 1990. Transactivation of the human immunodeficiency virus (HIV) promoter by heterologous virus infection, p. 290–308. *In* L. Aurelian (ed.), *Herpesviruses, the immune system, and AIDS*, vol. 6. Kluwer, Boston.
 47. Poli, G., P. Bressler, A. Kinter, E. Duh, W. C. Timmer, A. Rabson, J. S. Justement, S. Stanely, and A. S. Fauci. 1990. Interleukin 6 induces human immunodeficiency virus expression in infected monocytic cells alone and in synergy with tumor necrosis factor α by transcriptional and post-transcriptional mechanisms. *J. Exp. Med.* **172**:151–158.
 48. Popik, W., and P. M. Pitha. 1991. Inhibition by interferon of herpes simplex virus type 1-activated transcription of *tat*-defective provirus. *Proc. Natl. Acad. Sci. USA* **88**:9573–9577.
 49. Popik, W., and P. M. Pitha. 1992. Transcriptional activation of the Tat-defective human immunodeficiency virus type-1 provirus: effect of interferon. *Virology* **189**:435–447.
 50. Popik, W., and P. M. Pitha. 1993. Role of tumor necrosis factor- α in activation and replication of *tat*-defective human immunodeficiency virus type 1. *J. Virol.* **67**:1094–1099.
 51. Preston, B. D., B. J. Poiesz, and L. A. Loeb. 1988. Fidelity of HIV-1 reverse transcriptase. *Science* **242**:1168–1171.
 52. Quinn, T. C., P. Piot, J. B. McCormick, F. M. Feinsod, H. Taalman, B. Kapita, W. Stevens, and A. S. Fauci. 1987. Serologic and immunologic studies in patients with AIDS in North America and Africa. The potential role of infectious agents as cofactors in human immunodeficiency virus infection. *JAMA* **257**:2617–2621.
 53. Raj, N. B. K., and P. M. Pitha. 1981. Analysis of interferon messenger RNA in human fibroblast cells induced to produce interferon. *Proc. Natl. Acad. Sci. USA* **78**:7426–7430.
 54. Read, G. S., and N. Frenkel. 1983. Herpes simplex virus mutants defective in the virion-associated shutoff of host polypeptide synthesis and exhibiting abnormal synthesis of α (immediate early) viral polypeptides. *J. Virol.* **29**:677–697.

55. **Sandri-Goldin, R. M., and G. E. Mendoza.** 1992. A herpesvirus regulatory protein appears to act post-transcriptionally by affecting mRNA processing. *Genes Dev.* **6**:848–863.
56. **Schwartz, S., B. K. Felber, and G. N. Pavlakis.** 1992. Distinct RNA sequences in the *gag* region of human immunodeficiency virus type 1 decrease RNA stability and inhibit expression in the absence of Rev protein. *J. Virol.* **66**:150–159.
57. **Selby, M. J., E. A. Bain, P. A. Luciw, and B. M. Peterlin.** 1989. Structure, sequence, and position of the stem-loop in *tar* determine transcriptional elongation by *tat* through the HIV-1 long terminal repeat. *Genes Dev.* **3**:547–558.
58. **SenGupta, D. N., B. Berkhout, A. Gatignol, A. Zhou, and R. H. Silverman.** 1990. Direct evidence for translational regulation by leader RNA and Tat protein of human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA* **87**:7492–7496.
59. **Shirazi, Y., and P. M. Pitha.** 1992. Alpha interferon inhibits early stages of human immunodeficiency virus type 1 replication cycle. *J. Virol.* **66**:1321–1328.
60. **Smith, A. J., M.-I. Cho, M.-L. Hammarskjold, and D. Rekosh.** 1990. Human immunodeficiency virus type I Pr55^{gag} and Pr160^{gag-pol} expressed from a simian virus 40 late replacement vector are efficiently processed and assembled into viruslike particles. *J. Virol.* **64**:2743–2750.
61. **Sodroski, J., W.-C. Goh, C. Rosen, A. Dayton, E. Terwilliger, and W. Haseltine.** 1986. A second post-transcriptional *trans*-activator gene required for HTLV-III replication. *Nature (London)* **321**:412–417.
62. **Sodroski, J., C. Rosen, F. Wong-Staal, S. A. Salahuddin, M. Popovic, S. Arya, R. C. Gallo, and W. A. Haseltine.** 1985. Trans-acting transcriptional regulation of human T-cell leukemia virus type III long terminal repeat. *Science* **227**:171–173.
63. **Sorenson, C. M., P. A. Hart, and J. Ross.** 1991. Analysis of herpes simplex virus-induced mRNA destabilizing activity using an *in vitro* mRNA decay system. *Nucleic Acids Res.* **19**:4459–4465.
64. **Thach, R. E.** 1992. Cap recap: the involvement of eIF-4F in regulating gene expression. *Cell* **68**:177–180.
65. **Vlach, J., and P. M. Pitha.** 1992. Herpes simplex virus type 1-mediated induction of human immunodeficiency virus type 1 provirus correlates with binding of nuclear proteins to the NF- κ B enhancer and leader sequence. *J. Virol.* **66**:3616–3623.
66. **Vlach, J., and P. M. Pitha.** 1993. Differential contribution of herpes simplex virus type-1 gene products and cellular factors to the activation of human immunodeficiency virus type-1 provirus. *J. Virol.* **67**:4427–4431.