*Proc. Natl. Acad. Sci. USA*
Vol. 83, pp. 9734–9738, December 1986
Medical Sciences

# Elevated levels of mRNA can account for the trans-activation of human immunodeficiency virus

**(acquired immunodeficiency syndrome/transcription)**

B. MATIJA PETERLIN*, PAUL A. LUCIW†, PHILIP J. BARR†, AND MICHAEL D. WALKER‡

*Howard Hughes Medical Institute, Department of Medicine and Microbiology, and the ‡Hormone Research Institute, University of California, San Francisco, CA 94143; and †Chiron Corp., Emeryville, CA 94608

*Communicated by William J. Rutter, August 26, 1986*

ABSTRACT **The genome of human immunodeficiency virus encodes a protein that dramatically elevates amounts of viral proteins. The precise mechanism of this trans-activation remains to be established. It has been reported that trans-activation can occur without major changes in the levels of mRNA. We constructed recombinant plasmids containing those viral sequences required in cis for trans-activation linked to the chloramphenicol acetyltransferase gene. These plasmids were introduced into cultured cells in either the presence or absence of a second plasmid that directed expression of the viral trans-activator protein. Expression of the chloramphenicol acetyltransferase gene was measured at the level of protein (by enzymatic assay) and RNA (by ribonuclease protection and primer extension). Our results demonstrate that trans-activation is accompanied by large increases in mRNA levels; these increases may be sufficient to explain the elevated levels of trans-activated protein.**

Human immunodeficiency virus (HIV), a lymphocytopathic retrovirus, is the cause of acquired immunodeficiency syndrome (AIDS) and AIDS-related complex (ARC) (1–3). By morphological and genetic criteria, HIV is a member of the lentivirus family (1, 4). The HIV genome encodes genes for virion structural proteins, including the *gag* gene (specifying the virion core proteins), the *pol* (polymerase) gene (specifying the viral protease, reverse transcriptase, and endonuclease), and the envelope (*env*) gene (specifying the glycoprotein present in the virion membrane) (5–8). Additional open reading frames (ORFs) encode polypeptides of unknown function that are found in infected cells (9–12) (see Fig. 1*B*). Viral gene expression from the HIV long terminal repeat (LTR) was shown to be activated in infected cells (13). Subsequently a trans-activator (TAT) gene was identified by analyses of cDNA clones made from mRNA of infected cells (14, 15). Mutational studies have shown that TAT is required for viral replication (16–18). The viral message encoding TAT arises from multiple splicing events that join an ORF (72 codons) located on the 5′ side of the envelope gene to an ORF (28 codons for ARV-2) within the 3′ end of the *env* gene (14, 15). TAT acts upon sequences in the HIV LTR called the trans-acting responsive element (TAR) (19); the interaction between TAT and TAR leads to increased expression of viral genes (13, 19). An additional gene that overlaps both coding exons of TAT has been described. This gene, designated ART (anti-repression trans-activator), may either stabilize viral transcripts or permit efficient translation of gag and envelope messages (20).

Functions of the TAT gene have been analyzed in HIV-infected cells (13, 19, 21) and in transfected cells in which TAT gene expression is directed by a transfected plasmid (13, 21) or by a stably integrated plasmid (22). TAR has been mapped to nucleotides −17 to +80 with respect to the transcription initiation site in the HIV LTR (19); thus, the cap site is located within TAR. Previous studies by Haseltine and colleagues suggested that trans-activation has a significant transcriptional component (19). These experiments were performed by transiently transfecting plasmids containing TAR into HIV-infected or uninfected cell lines and measuring gene expression. More recently, these workers have produced cell lines that stably express the TAT gene (22). When TAR constructs were introduced into these lines and into their parent cells, which do not express TAT, trans-activation was observed without significant elevation of RNA levels (22). These experiments raise the possibility that translational mechanisms play a dominant role in TAT-mediated trans-activation.

With the goal of understanding the molecular basis of trans-activation in HIV gene expression, we have constructed a series of recombinant plasmids containing TAR together with well-defined viral transcriptional control elements linked to the gene coding for chloramphenicol acetyltransferase (CAT; EC 2.3.1.28) (23). Human and rodent cells were cotransfected with such plasmids in the absence and presence of a second plasmid containing a simian virus 40 (SV40) early promoter driving expression of the TAT gene. Transient expression of the CAT gene was measured at the level of RNA and protein. Our experiments show that TAR sequences influence gene expression in a fashion distinct from enhancers and promoters. Further, a striking correlation between steady-state RNA levels and protein levels was seen, suggesting a dominant role for control of transcription initiation or mRNA stabilization in trans-activation.

## MATERIALS AND METHODS

**Cell Lines.** HUT 78 (24) is a human T-cell line permissive for HIV replication (25). HeLa is a human cervical carcinoma cell line. HIT cells are hamster endocrine pancreas cells (26) that are presumably not permissive for HIV.

**Plasmid Constructions.** The HIV genome was subcloned from a molecularly cloned, biologically active, HIV-SF2 isolate (27, 28). TAR is a *Pvu* II–*Hin*dIII (−17 to +80) fragment from the HIV LTR (7). HIV LTR is an *Xho* I–*Nar* I (−633 to +185) fragment from the HIV genome (7). Rous sarcoma virus (RSV) enhancer and promoter and SV40 enhancer and early promoter fragments are from pRSVcat and pSV2cat, respectively (29). SV40 early promoter is from pSV1cat (23), and herpes simplex thymidine kinase (TK)

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HIV, human immunodeficiency virus; LTR, long terminal repeat; TAT, trans-activator; ORF, open reading frame; TAR, trans-acting responsive element; ART, anti-repression trans-activator; CAT, chloramphenicol acetyltransferase; AIDS, acquired immunodeficiency syndrome; SV40, simian virus 40; bp, base pair(s); TK, thymidine kinase; RSV, Rous sarcoma virus; $\beta_2$m, $\beta_2$-microglobulin.

promoter is from pBR.TK.CAT (30). A schematic representation of the HIV genome and plasmid constructions containing TAR and TAT are shown in Fig. 1. The structure of plasmids used to prepare RNA probes for RNase protection studies is shown in Fig. 2. The $\beta_2$-microglobulin ($\beta_2$m) RNA protection probe was constructed by subcloning a *Pst* I–*Hin*cII (146 bp) fragment of $\beta_2$m cDNA (31) into gemini 1 vector (Promega Biotec, Madison, WI). pSV2-HIV is a cloned provirus of HIV-SF2, which has the leftward LTR replaced with the SV40 early promoter. TAT-1 is a plasmid that contains the HIV-SF2 TAT gene (300 bp) positioned between SV40 early promoter and poly(A) addition site. To construct the TAT gene, a 150-bp fragment (*Xba* I to *Sac* I), encoding amino acids 9–58 (in the first coding exon), was ligated to a chemically synthesized 24-bp oligonucleotide encoding amino acids 1–8. Subsequently, chemically synthesized oligonucleotides corresponding to the 126 bp encoding amino acids 58–100 were ligated to the 3′ end. All plasmids were purified by two cycles of equilibrium sedimentation in CsCl. Details of all plasmid constructions are available from the authors on request.

**Transient Transfection Assays.** Human cells were transfected by using a modification of the DEAE-dextran procedure (32). Briefly, $10^7$ cells in logarithmic growth phase were extensively washed in TS [(in g/liter) 8 NaCl; 0.38 KCl; 0.1 $Na_2HPO_4 \cdot 7H_2O$; 3.0 Tris·HCl; 0.1 $MgCl_2$; 0.1 $CaCl_2$, pH 7.4] (33) and resuspended in 1 ml of TS containing 500 μg of DEAE-dextran and 5 μg of supercoiled plasmid DNA. After 30 min at room temperature, 5 vol of RPMI 1640 medium containing 200 μg of chloroquine phosphate were added and incubated for an additional 1 hr (HUT 78) or 3 hr (HeLa). Cells were then spun down and resuspended in RPMI 1640 medium containing 10% fetal calf serum and penicillin/streptomycin. Two days later the cells were harvested and extracts were prepared for CAT assays. HIT cells were transfected as described (30).

**CAT Assays.** CAT assays were performed as described (23). To ensure accurate quantitation of CAT activities, samples of each extract were assayed for 10 min and 1 hr. Values for chloramphenicol conversion falling below 0.2% and above 60% were discarded. Activities were normalized with respect to incubation time and cell number.

**RNA Analysis.** RNA was isolated from $10^8$ cells by homogenization of cells in guanidinium thiocyanate followed by centrifugation through CsCl (34). T-7 or SP6 polymerase was used to synthesize [$^{32}$P]UTP-labeled radioactive probes complementary to mRNA transcribed from transfected plasmids. The full-length probe was excised from 5% urea/polyacrylamide gels, eluted, and precipitated with EtOH. Ten to 50 μg of total cellular RNA or 1–10 μg of poly(A) RNA was used in hybridization reactions with $5 \times 10^5$ cpm of specific probe. After an overnight hybridization in 80% formamide/40 mM Pipes, pH 7.4/0.4 M NaCl/1 mM EDTA at 37°C, the reaction mixture was diluted 1:20 in 0.3 M NaCl/10 mM Tris·HCl, pH 7.5/5 mM EDTA, treated with RNase A (10 μg/ml) and RNase T1 (1 μg/ml) at room temperature for 1 hr, and analyzed by electrophoresis on 5% polyacrylamide/urea sequencing gels (35). The gels were dried and exposed to x-ray film. The intensity of protected bands was measured using a Zaineh densitometer.

**Primer Extension.** This was performed as described (30). The oligonucleotide primer (Fig. 2) was synthesized on an Applied Biosystems 380A DNA synthesizer.

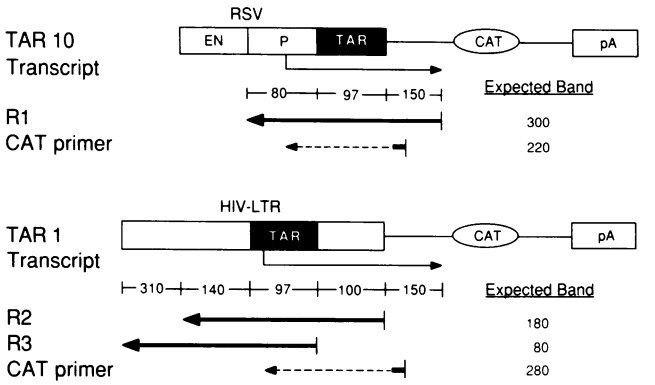


FIG. 2. Schematic representation of RNase protection probes (R1–R3) and of the oligonucleotide used for primer extension. When cells are transfected with TAR-10 (Fig. 1*A*), probe prepared from construction R1 should protect 300 nucleotides of RNA corresponding to initiation directed by the RSV promoter. Extension of the CAT primer (complementary to positions +50 to +70 in the CAT gene) should lead to a protected sequence 220 nucleotides long. When cells are transfected with TAR-1, probes derived from constructions R2 and R3 should lead to protected fragments of 180 and 80 nucleotides, respectively, corresponding to transcription directed by the HIV promoter. Primer extension using the CAT primer should lead to a 280-nucleotide product.

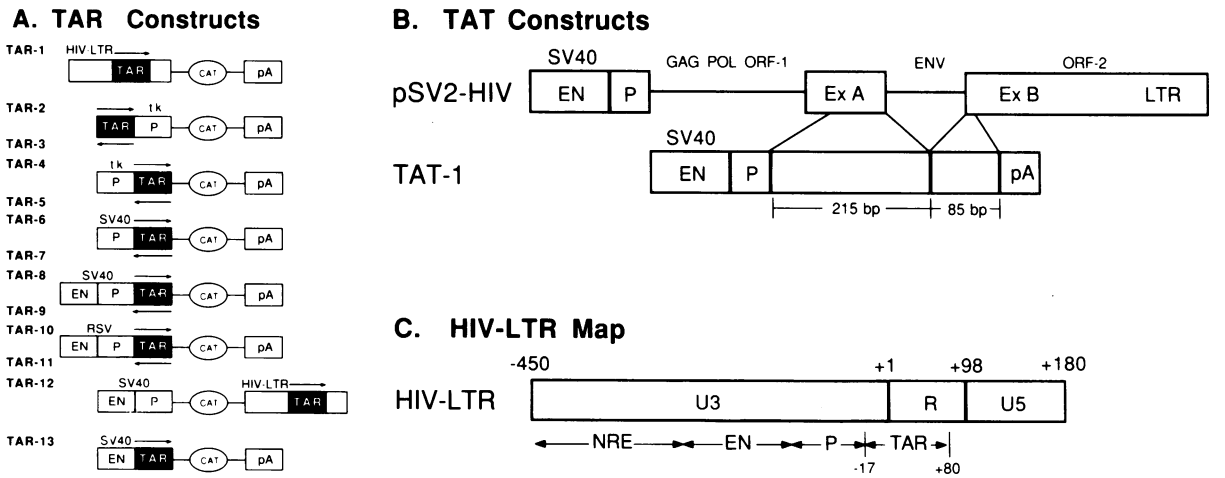


FIG. 1. Schematic representation of TAR and TAT constructions and HIV LTR. (*A*) Structure of TAR-1 through TAR-13, with TAR sequences in black. The CAT gene is flanked on the 3′ end by the SV40 early poly(A) signal. (*B*) Structure of pSV2-HIV and TAT-1 constructions. pSV2-HIV and TAT-1 are both driven by the SV40 enhancer and promoter sequences. The former construction contains the HIV genome lacking the 5′ LTR, whereas the latter construction contains a synthetic 300-base-pair (bp) TAT gene. (*C*) HIV LTR is composed of U3, R, and U5. Also shown are the negative regulatory signals (NRE) (19), enhancer (EN) (19), and promoter (P) sequences (U3 region) and TAR (R region) (19). Map distances are not drawn to scale.

## RESULTS

**TAT Is Sufficient for Trans-Activation.** Two plasmids were used to direct expression of TAT in transfected cells. In pSV2-HIV (Fig. 1*B*), the SV40 early promoter was used to drive expression from an intact HIV genome missing the LTR sequences. In TAT-1 (Fig. 1*B*) the SV40 promoter was placed directly upstream of the TAT coding DNA. These two plasmids produced equivalent trans-activation (Table 1). Since TAT-1 is capable of expressing only TAT and no other HIV gene products, including ART (20), TAT is sufficient to induce trans-activation. Furthermore, since other gene products can be expressed by pSV2-HIV, these products seem to have no significant effect on the degree of trans-activation.

**HIV TAT Trans-Activates TAR in a Position-, Orientation-, and Enhancer-Dependent Manner.** In TAR-1, the HIV LTR containing the HIV enhancer, Sp-1 binding sites (36), "TATA box," and TAR sequences was placed upstream of the CAT gene (Fig. 1*A*). When introduced into HUT 78, HeLa, and HIT cells, low but detectable levels of CAT activity were observed. When cotransfected with TAT-1, CAT levels were increased ≈100-fold in HUT 78 and HeLa cells but only 5-fold in HIT cells (Table 1). The weak trans-activation in HIT cells may be due to low intracellular TAT levels; the SV40 early promoter used to drive the TAT gene has low activity in these cells (M.D.W., unpublished observations).

Table 1. Trans-activation of CAT gene measured by enzymatic assay and analysis of RNA levels

| Construction | % CAT conversion* With TAT | % CAT conversion* Without TAT | Trans-activation,† fold CAT | Trans-activation,† fold RNA |
|---|---|---|---|---|
| HUT 78 cells | | | | |
| TAR-1‡ | 25 | 0.3 | 83 | |
| TAR-1 | 39 | 0.4 | 98§ | 40 |
| TAR-2 | 0.7 | 0.4 | 1.8 | |
| TAR-3 | 0.6 | 0.4 | 1.5 | |
| TAR-4 | 1.3 | 0.6 | 2.1 | |
| TAR-5 | 0.5 | 0.4 | 1.2 | |
| TAR-6 | 1.8 | 0.6 | 3.0 | |
| TAR-7 | 0.5 | 0.6 | 0.8 | |
| TAR-8‡ | 27 | 1.9 | 14 | |
| TAR-8 | 19 | 1.5 | 13 | |
| TAR-9‡ | 1.4 | 1.4 | 1.0 | |
| TAR-9 | 2.0 | 1.3 | 1.3 | |
| TAR-10‡ | 40 | 2.8 | 14 | |
| TAR-10 | 44 | 2.4 | 18§ | 12 |
| TAR-11 | 2.9 | 2.3 | 1.3 | |
| TAR-12 | 1.7 | 1.5 | 1.1 | |
| TAR-13 | 29 | 2.9 | 10 | |
| HeLa cells | | | | |
| TAR-1 | 54 | 0.4 | 135§ | 80 |
| TAR-1a | 44 | 0.4 | 110 | |
| TAR-1b | 1.4 | 1.2 | 1.2 | |
| TAR-2 | 0.5 | 0.5 | 1.0 | |
| TAR-3 | 0.5 | 0.4 | 1.3 | |
| TAR-8 | 19 | 1.5 | 13 | |
| TAR-9 | 1.5 | 1.6 | 0.9 | |
| TAR-10 | 39 | 1.2 | 32§ | 38 |
| TAR-11 | 1.8 | 1.6 | 1.1 | |
| HIT cells | | | | |
| TAR-1 | 4.1 | 0.8 | 5.1 | 5 |
| TAR-10 | 8.9 | 1.3 | 6.8 | 5 |

*Conversion of chloramphenicol by HUT cells (extract from 2.5 × $10^6$ cells, 10-min assay), HeLa cells (0.5 × $10^6$ cells, 10 min), and HIT cells (10 μg of protein, 10 min). The "With TAT" column contains data from cells cotransfected with equal amounts of TAR- and TAT-containing plasmids. The "Without TAT" column contains data from cells cotransfected with equal amounts of TAR-containing plasmids and pUC18.

†The "CAT" column refers to data from enzymatic assay. The "RNA" column is based on densitometry of autoradiograms shown in Figs. 3–5.

‡TAR-containing plasmids were cotransfected with pSV2-HIV; in all other experiments, TAR-containing plasmids were cotransfected with TAT-1 (Fig. 1*B*).

§Values of trans-activation present the mean of three or more independent transfections. Standard errors were <35% of the mean value.

In subsequent constructions, TAR was placed downstream of a strong heterologous transcription unit including enhancer and promoter elements derived from SV40 (TAR-8 and TAR-9) and RSV (TAR-10 and TAR-11) (Fig. 1*A*). TAT was found to trans-activate TAR-8 and TAR-10 constructs (TAR in the normal orientation) but not TAR-9 and TAR-11 (TAR in the inverted orientation) (Table 1). In HUT 78 and HeLa cells, this trans-activation was between 10- and 30-fold, whereas in HIT cells, a 5-fold trans-activation was observed (Table 1). The fact that TAR functions when placed downstream of the promoter distinguishes it from previously characterized promoter elements for genes transcribed by RNA polymerase II. A 10-fold trans-activation was observed in HUT 78 cells even after removal of the SV40 early promoter from the TAR-8 construct (compare TAR-8 and TAR-13, Table 1). This suggests that a TATA box is not essential for trans-activation.

TAR sequences from position −17 to +80 have been identified as the genetic element responsive to TAT gene action (19). To test the ability of TAR to function as a TAT-dependent enhancer, TAR sequences were placed in normal and inverted orientation either 5′ or 3′ of the TK promoter. These sequences were placed 5′ to the CAT gene (Fig. 1*A*, TAR-2 to TAR-5). Similar constructions, TAR-6 and TAR-7, were made with the SV40 early promoter. Upon introduction into HUT 78 cells, these plasmids were not trans-activated by TAT (Table 1). The inability to activate a heterologous promoter in a position- and orientation-independent fashion indicates that TAR does not function as an enhancer.

In infected cells, viral transcripts retain some TAR sequences at the 5′ and 3′ ends. To determine if trans-activation could occur when TAR was near the 3′ end of the CAT gene, the SV40 poly(A) site in the pSV2cat was replaced with the whole HIV LTR (TAR-12, Fig. 1). Such a construction should generate a transcript containing TAR sequences 3′ of the CAT sequences. No trans-activation by TAT was observed in this case. Thus, the presence of TAR sequences at the 3′ end of a transcript is insufficient to permit trans-activation. In addition, this result emphasizes the dissimilarity between TAR and enhancer elements (see above).

TAT-mediated trans-activation of TAR sequences appears to involve activation rather than derepression. This can be seen by comparing the activity of TAR-10, which contains RSV LTR and TAR, with the activity of RSV CAT, which contains only RSV LTR (data not shown). These constructs express equivalent levels of CAT in the absence of TAT. However, in the presence of TAT, only TAR-10 is significantly trans-activated (Table 1).

**HIV TAT Trans-Activation of TAR Leads to Increases in Steady-State mRNA Levels.** Two independent methods of RNA analysis, RNase protection and primer extension, were used to determine the effects of TAR on transcript levels and start sites. RNA from HUT 78 cells transfected with TAR-1 and from HUT 78 cells cotransfected with TAR-1 and TAT-1 was found to initiate predominantly from the appropriate HIV LTR transcription start site (Fig. 3). RNA from similarly transfected HeLa and HIT cells (Figs. 4 and 5) was also found to initiate from the HIV LTR transcription initiation site. On the other hand, RNA from HUT 78 (Fig. 3), HeLa (Fig. 4), and HIT (Fig. 5) cells, transfected singly with TAR-10 or

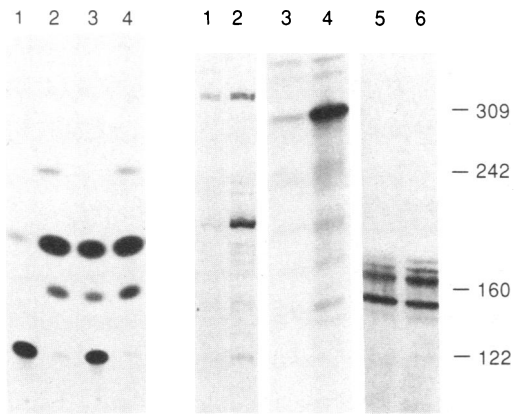


FIG. 3. Trans-activation in HUT 78 cells as measured by CAT assay (*Left*) and RNase protection (*Right*). Cells were transfected and harvested for assay of CAT activity and RNA analysis. CAT signals following 1 hr of incubation are shown. For quantitation, 10-min CAT assays were performed, where chloramphenicol conversion is linear. HUT 78 cells were transfected with TAR-1 (lanes 1), TAR-1 and TAT-1 (lanes 2), TAR-10 (lanes 3), and TAR-10 and TAT-1 (lanes 4). Probe R2 was used in lanes 1 and 2 (protecting 180 nucleotides), probe R1 was used in lanes 3 and 4 (protecting 300 nucleotides), and $\beta_2$m probe was used in lanes 5 and 6 (protecting 150 nucleotides). The same RNA was used in lanes 3 and 5 and in lanes 4 and 6. The $\beta_2$m probe was used in this experiment (lanes 5 and 6) and all subsequent RNase protection experiments (data not shown) as an internal control for the amount of RNA present in each sample.

cotransfected with TAR-10 and TAT-1, initiated from the RSV transcription start site. Densitometry of the autoradiograms shown in Fig. 3 revealed that in HUT 78 cells, TAT-1 trans-activated TAR-1 transcripts 40-fold and TAR-10 transcripts 12-fold. In that experiment, TAR-1 CAT signals were trans-activated 90-fold and TAR-10 CAT signals 12-fold. In HeLa cells, TAR-1 transcripts were trans-activated 75- to 80-fold while protein levels were increased 135- to 170-fold (Fig. 4). TAR-10 was trans-activated 36-fold at the RNA level and 32-fold at the protein level (Fig. 4*B*). In HIT cells, TAR-1 was trans-activated 5-fold at the RNA level and 5.1-fold at the protein level, and TAR-10 was trans-activated 5-fold at the RNA level and 6.8-fold at the protein level. In all of the above cases, trans-activation was accompanied by substantial increases in mRNA; these increases were similar in magnitude to the increases in protein level as determined by CAT assays (Table 1).

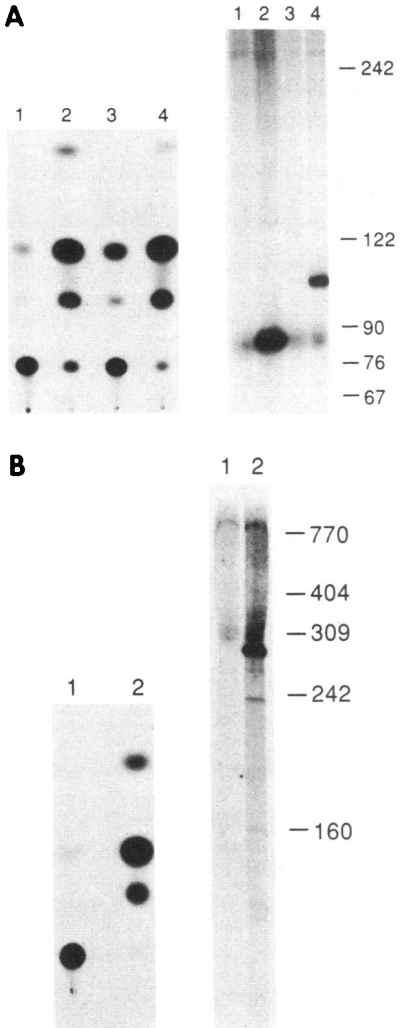


FIG. 4. Trans-activation in HeLa cells. (*A*) CAT levels for TAR-1 (lanes 1), TAR-1 and TAT-1 (lanes 2), TAR-10 (lanes 3), and TAR-10 and TAT-1 (lanes 4) are shown alongside RNase protection experiments using probe R3. An 80-nucleotide-long protected fragment is seen in lanes 1 and 2, corresponding to transcription initiation within TAR. In lanes 3 and 4, the entire TAR is protected since transcription initiates off the RSV promoter. (*B*) CAT activity (*Left*) and primer extension analysis (*Right*) of cells transfected with TAR-1 (lanes 1) and TAR-1 with TAT-1 (lanes 2). Utilization of the HIV promoter results in a 280-nucleotide extension product.

## DISCUSSION

Trans-activation of viral gene expression has been demonstrated for several retroviruses, including human T-cell leukemia virus type I (37) and type II (38), bovine leukemia virus (39), and Visna (40). Haseltine and colleagues have shown that the HIV LTR is dramatically trans-activated in HIV-infected cells (13). This effect is mediated through the action of a virally encoded trans-activator gene (TAT) (14, 15) on a target sequence (TAR) in the HIV LTR (19).

As an approach to understanding the role of TAR in mediation of trans-activation, we determined in what context TAR is optimally active, by varying its orientation and position relative to heterologous transcription units. In the absence of enhancer elements, TAR was unable to activate either the TK promoter (TAR-2 to TAR-5) or the SV40 promoter (TAR-6 and TAR-7). Thus, TAR does not function as an enhancer element (41). In the presence of an enhancer element, with TAR located downstream of a promoter, effective trans-activation was observed (TAR-8 and TAR-10). Thus, TAR functions optimally when located downstream of a transcription unit containing a promoter and an enhancer. Analysis of transcripts from TAR-10 shows that TAR sequences are located within mRNA. The position dependence of TAR distinguishes it from previously characterized promoter elements. The presence of TAR at any position within the transcript is insufficient for activity (see TAR-12, Table 1). The experiments confirm and extend previous observations suggesting that TAR represents a member of a distinct class of cis-acting regulatory elements (19, 42, 43).

In parallel with CAT activity measurements, we have determined the levels and start sites of RNA transcripts in three different cell types. Our experiments, measuring RNA by primer extension and RNase protection, show a good correlation between levels of mRNA and the corresponding protein. The magnitude of trans-activation depended on the recipient cell used: the effects ranged from 5-fold (HIT cells) to 100- to 150-fold (HUT and HeLa cells) (Table 1). The data are consistent with earlier results showing that transfection of plasmids containing TAR into HIV-infected cells leads to increased levels of mRNA (19). However, when similar plasmids were introduced into cell lines stably expressing the

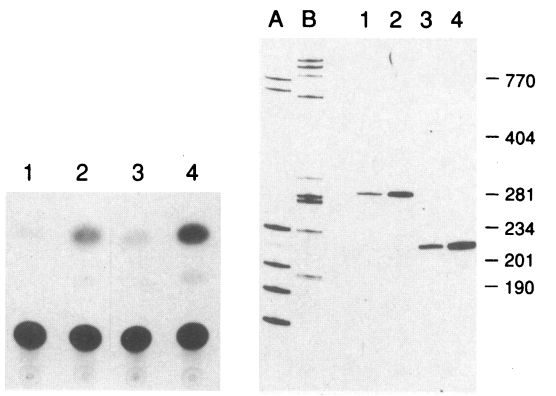


FIG. 5. Transfections in HIT cells. CAT levels (*Left*) and primer extension analysis (*Right*) for TAR-1 (lanes 1), TAR-1 and TAT-1 (lanes 2), TAR-10 (lanes 3), and TAR-10 and TAT-1 (lanes 4). Extension of the CAT primer produces 280- and 220-nucleotide-long products with RNA derived from cells transfected with TAR-1 and TAR-10, respectively.

TAT gene, large increases in the levels of protein were accompanied by minor changes in the levels of corresponding mRNA, as measured primarily by slot blot analysis (22). These latter data raised the possibility that trans-activation is mediated by translational mechanisms (22). Our experiments emphasize that the effects on steady-state levels of mRNA can be the dominant factor in trans-activation. It is possible that trans-activation by HIV is mediated at transcriptional and posttranscriptional levels, and the relative contributions of these effects are influenced by the experimental conditions. The most likely stages at which alterations in mRNA level may be mediated are transcription initiation and RNA processing or stabilization. The latter possibility is intriguing in view of the fact that TAR sequences are present on the mRNA molecule. Direct measurement of the rate of initiation of RNA synthesis will help to distinguish between these alternate mechanisms.

The mechanism of HIV pathogenesis is incompletely understood (2, 3, 44, 45). Although cytopathic effects on lymphoid cells are a characteristic feature of HIV infection (46–49), the molecular basis for cell killing is unknown. Our results show that HIV trans-activation can lead to increases in viral mRNA levels. It is unknown whether this effect is limited to viral transcripts or can result in changes in host mRNA. Further investigation of the mechanisms underlying HIV trans-activation may contribute to a better understanding of the interaction between the virus and permissive cells.

We thank Dr. William J. Rutter for his support and valuable discussions. We are grateful to Ms. Anne S. Larsen for expert technical assistance and to Mr. Michael Armanini for preparation of the manuscript. This work was supported in part by grants from the State of California Universitywide Task Force on AIDS (86SF023) to B.M.P. and from the National Institutes of Health to William J. Rutter (GM 28520). M.D.W. is supported by a Career Development Award from the Juvenile Diabetes Foundation.

1. Wong-Staal, F. & Gallo, R. C. (1985) *Nature (London)* **317**, 395–402.
2. Lane, H. C. & Fauci, A. S. (1985) *Annu. Rev. Immunol.* **3**, 477–500.
3. Gluckman, J. C., Klatzmann, D. & Montagnier, L. (1986) *Annu. Rev. Immunol.* **4**, 97–117.
4. Haase, A. T. (1986) *Nature (London)* **322**, 130–136.
5. Ratner, L., Haseltine, W., Patarca, R., Livak, K. J., Starcich, B., Josephs, S. F., Doran, E. R., Rafiaski, J. A., Whitehorn, E. A., Baumeister, K., Ivanoff, L., Petteway, Jr., S. R., Pearson, M. L., Lautenberger, J. A., Papas, T. S., Ghrayeb, J., Chang, N. T., Gallo, R. C. & Wong-Staal, F. (1985) *Nature (London)* **313**, 277–284.
6. Wain-Hobson, S., Sonigo, P., Danos, O., Cole, S. & Alizon, M. (1985) *Cell* **40**, 9–17.
7. Sanchez-Pescador, R., Power, M. D., Barr, P. J., Steimer, K. S., Stempien, M. M., Brown-Shimer, S. L., Gee, W. W., Renard, A., Randolph, A., Levy, J. A., Dina, D. & Luciw, P. A. (1985) *Science* **227**, 484–492.
8. Muesing, M., Smith, D., Cabradilla, C., Benton, C., Laskey, L. & Capon, D. (1985) *Nature (London)* **313**, 450–458.
9. Allan, J. S., Coligan, J. E., Lee, T. H., McLane, M. F., Kanki, P. J., Groopman, J. E. & Essex, M. (1985) *Science* **230**, 810–813.
10. Lee, T. H., Coligan, J. E., Allan, J. S., McLane, M. F., Groopman, J. E. & Essex, M. (1986) *Science* **231**, 1546–1549.
11. Kan, N. C., Franchini, G., Wong-Staal, F., DuBois, G. C., Robey, W. G., Lutenberger, J. A. & Papas, T. S. (1986) *Science* **231**, 1553–1555.
12. Arya, S. K. & Gallo, R. C. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2209–2213.
13. Sodroski, J. G., Rosen, C. A., Wong-Staal, F., Salahuddin, S. Z., Popovic, M., Arya, S. & Gallo, R. C. (1985) *Science* **227**, 171–173.
14. Sodroski, J., Patarca, R. & Rosen, C. (1985) *Science* **229**, 74–77.
15. Arya, S. K., Guo, C. & Wong-Staal, F. (1985) *Science* **229**, 69–73.
16. Dayton, A. I., Sodroski, J. G., Rosen, C. A., Goh, W. C. & Haseltine, W. A. (1986) *Cell* **44**, 941–947.
17. Fisher, A. G., Feinberg, M. B., Josephs, S. F., Harper, M. E., Marselle, L. M., Reyes, G., Gonda, M. A., Aldovini, A., Debouk, C., Gallo, R. C. & Wong-Staal, F. (1986) *Nature (London)* **320**, 367–371.
18. Luciw, P. A., Cheng-Mayer, C. & Levy, J. A. (1987) *Proc. Natl. Acad. Sci. USA*, in press.
19. Rosen, C. A., Sodroski, J. G. & Haseltine, W. A. (1985) *Cell* **44**, 813–823.
20. Sodroski, J., Goh, W. C., Rosen, C., Dayton, A., Tervilliger, E. & Haseltine, W. (1986) *Nature (London)* **321**, 412–417.
21. Seigel, L. J., Ratner, L., Josephs, S. F., Derse, D., Feinberg, M. B., Reyes, G. R., O'Brien, S. J. & Wong-Staal, F. (1986) *Virology* **148**, 226–231.
22. Rosen, C. A., Sodroski, J. G., Goh, W. C., Dayton, A. I., Lippke, J. & Haseltine, W. A. (1986) *Nature (London)* **319**, 555–559.
23. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2**, 1044–1051.
24. Gazdar, A. F., Carney, D. N., Bunn, P. A., Russell, E. K., Jaffe, E. S., Schechter, G. P. & Guccion, J. G. (1980) *Blood* **55**, 409–417.
25. Levy, J. A., Hoffman, A. D., Kramer, S. M., Landis, J. A., Shimabukuro, J. M. & Oshiro, L. S. (1984) *Science* **225**, 840–842.
26. Santerre, R. F., Cook, R. A., Crisel, R. M. D., Sharp, J. D., Schmidt, R. J., Williams, D. C. & Wilson, C. P. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4339–4343.
27. Luciw, P. A., Potter, S. J., Steimer, K., Dina, D. & Levy, J. A. (1984) *Nature (London)* **312**, 760–763.
28. Levy, J. A., Cheng-Mayer, C., Dina, D. & Luciw, P. A. (1986) *Science* **232**, 998–1001.
29. Gorman, C. M., Merlino, G. T., Willingham, M. C., Pastan, I. & Howard, B. H. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6777–6781.
30. Walker, M. D., Edlund, T., Boulet, A. M. & Rutter, W. J. (1983) *Nature (London)* **306**, 557–561.
31. Suggs, S. V., Wallace, R. B., Hirose, T., Kawashima, E. H. & Itakura, K. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6613–6617.
32. Sompayrac, G. M. & Danna, K. J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7575–7578.
33. Grosschedl, R. & Baltimore, D. (1985) *Cell* **41**, 885–897.
34. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
35. Krieg, P. A. & Melton, D. A. (1984) *Nucleic Acids Res.* **12**, 7057–7070.
36. Jones, K. A., Kadonaga, J. T., Luciw, P. A. & Tjian, R. (1986) *Science* **232**, 755–759.
37. Sodroski, J. G., Rosen, C. A. & Haseltine, W. A. (1984) *Science* **225**, 381–384.
38. Slamon, D. J., Shimotohno, K., Cline, M. J., Golde, D. W. & Chen, I. S. Y. (1985) *Science* **226**, 61–64.
39. Derse, D., Caradonna, S. J. & Casey, J. W. (1985) *Science* **227**, 317–320.
40. Hess, J. L., Clements, J. E. & Narayan, O. (1985) *Science* **229**, 482–485.
41. Khoury, G. & Gruss, P. (1983) *Cell* **33**, 313–314.
42. Klemenz, R., Hultmark, D. & Gehring, W. J. (1985) *EMBO J.* **4**, 2053–2060.
43. Lindquist, S. (1986) *Annu. Rev. Biochem.* **55**, 1151–1191.
44. Sodroski, J., Goh, W. C., Rosen, C., Campbell, K. & Haseltine, W. A. (1986) *Nature (London)* **322**, 470–473.
45. Fisher, A. G., Ratner, L., Mitsuya, H., Marselle, L. M., Harper, M. E., Broder, S., Gallo, R. C. & Wong-Staal, F. (1986) *Science* **233**, 655–659.
46. Gallo, R. C., Salahuddin, S. Z., Popovic, M., Sheraer, G. M., Kaplan, M., Haynes, B. F., Palker, T. J., Redfield, R., Oleske, J., Safai, B., White, G., Foster, P. & Markham, P. D. (1984) *Science* **224**, 500–502.
47. Folks, T., Penn, S., Rabson, A., Theodore, T., Hoggan, M. D., Martin, M., Lightfoote, M. & Sell, K. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4539–4543.
48. Klatzman, D., Barre-Sinoussi, F., Nugeyre, M. T., Dauget, C., Vilmer, E., Griscelli, C., Brun-Vezinet, F., Rouzioux, C., Gluckman, J. C., Chermann, J. C. & Montagnier, L. (1984) *Science* **225**, 59–62.
49. Levy, J. A., Kaminsky, L. S., Morrow, W. J. W., Steimer, K., Luciw, P. A., Dina, D., Hoxie, J. & Oshiro, L. (1985) *Ann. Intern. Med.* **103**, 694–703.