JOURNAL OF VIROLOGY, Mar. 1991, p. 1392–1399
0022-538X/91/031392-08$02.00/0

Vol. 65, No. 3

Cellular Factors Regulate Transactivation of Human Immunodeficiency Virus Type 1

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Received 12 October 1990/Accepted 13 December 1990

It is hypothesized that the immediate-early (IE) gene products of human cytomegalovirus (CMV) and the transactivator (TAT) of human immunodeficiency virus type 1 (HIV-1) regulate HIV-1 gene expression through mechanisms involving host cell factors. By using transient transfection assays with the gene for chloramphenicol acetyltransferase (CAT) under the transcriptional control of the HIV-1 long terminal repeat (LTR), we examined transactivation of the LTR by plasmids that express either the HIV-1 gene for TAT or human CMV IE. The ratio of the level of transactivation by CMV IE to the level of transactivation by TAT varied up to 1,000-fold between cell types. The difference in the activities of these transactivators in various cell types was not a consequence of differential expression of the transactivator gene. Analysis of RNA species initiated in the HIV-1 LTR supports the conclusion that cellular factors regulate the level of elongation of the transcription complex on the LTR. Furthermore, evidence that in some cell types the predominant mechanism of transactivation by HIV-1 TAT involves posttranscriptional processes is presented.

Several mammalian DNA viruses (e.g., herpesviruses and adenoviruses) and certain retroviruses (e.g., human T-lymphotropic virus types I and II, spumaviruses, and lentiviruses) encode proteins which regulate viral transcription and provide temporal control of viral gene expression and replication (8, 20, 32, 42). Mechanisms by which these viral regulatory genes, designated transactivators, stimulate gene expression are poorly understood. Viral transactivator proteins may not bind directly to the promoters whose expression they stimulate (for a review, see reference 15). Thus, the presence or absence of specific cellular proteins appears to govern the ability of viral transactivators to function. Other reports have noted the effects of host cells on the abilities of viral transactivators to stimulate expression from the human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) (17, 54). This has important clinical implications, because the level of required cellular proteins may determine whether a viral infection is productive or abortive in specific cell types and whether a virus is maintained in a latent state.

The virally encoded TAT protein of HIV-1 stimulates HIV-1 gene expression and is essential for viral replication (13, 14). TAT functions through the *trans*-acting responsive element (TAR) located downstream of the start site of transcription between +19 and +42 (relative to the cap site at +1 in the HIV-1 LTR) (33, 46, 48). Despite extensive studies, the mechanism by which TAT increases gene expression still requires resolution. TAT appears to increase the frequency of transcription initiation (28, 43) by binding to TAR RNA and interacting with components of the transcription complex (5, 49). By a mechanism that may be intrinsically related to transcription initiation, TAT eliminates a block to transcription elongation on the HIV-1 LTR (25, 28, 48). Its effects may also be mediated at a posttranscriptional level (6, 7, 11, 39, 45, 54). Whatever the precise mechanism, TAT regulates HIV-1 gene expression by interacting with a target element in the HIV-1 LTR; this interaction may involve cellular proteins (35, 54).

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The immediate-early (IE) gene of cytomegalovirus (CMV) encodes multiple proteins which are translation products of differentially spliced mRNAs (22, 52). Although the functions of IE are not fully understood, these regulatory gene products are capable of stimulating expression from other CMV promoters, as well as autoregulating their expression (9, 41). The most abundant IE protein (IE1) increases expression from the major IE promoter (9), while the IE2 gene product both negatively regulates IE expression and positively regulates heterologous promoters (12, 22, 41). IE1 by itself does not transactivate heterologous promoters but appears to have small synergistic effects on the transactivation capacities of IE2 (22). The mechanism by which CMV IE transactivates promoters is not known. For the HIV-1 promoter, CMV IE probably functions by increasing the frequency of transcription initiation through interactions within a region of the HIV-1 LTR encompassing the cap site; sequences in the LTR between −6 and +20 (relative to the start site of transcription at +1) have been shown to mediate responsiveness to CMV IE (3). Like TAT, CMV IE most likely requires cellular proteins as intermediates for activation of gene expression.

This report examines transactivation of the HIV-1 LTR by HIV-1 TAT and CMV IE in cultured mammalian cells representing different tissue types. The activities of these transactivators were found to be dependent on the specific cell type. Thus, levels of different cellular factors regulate transactivation by TAT and CMV IE. These findings have implications for viral replication and cell tropism in tissue culture systems and infected hosts.

MATERIALS AND METHODS

Cell lines. The cell types used in this work were as follows (American Type Culture Collection [Rockville, Md.] reference numbers are in parentheses unless otherwise indicated): NIH 3T3 mouse fibroblasts (CRL1658), MRC-5 human fetal lung cells (CCL171), Hs68 human foreskin fibroblasts (CRL1635), BC-1 nontransformed mouse mammary epithelium cells (52a), GR3A mouse mammary tumor virus-transformed murine mammary tumor cells (44) (kindly provided

by D. Morris, University of California, Davis), Y79 human retinoblastoma cells (HTB18), L929 murine fibroblasts (CCL1), SW480 human colon carcinoma cells (CCL228), GCT human histiocytoma cells (TIB223), HuT78 human $CD4^+$ T-lymphoid cells (TIB161), Jurkat human $CD4^+$ T-lymphoid cells (47), HTB-17 (also known as U-373MG) human glioblastoma cells (HTB-17), HuPBL human peripheral blood lymphocytes (PBL) isolated from a CMV-seronegative donor, Cf2Th canine thymus cells (CRL1430), RhPBL rhesus macaque PBL isolated from a CMV-seropositive donor, NC37 Epstein-Barr virus-positive human lymphoblasts (CCL214), Cos simian virus 40 (SV40)-transformed African green monkey kidney fibroblasts (CRL1650), and HeLaS3 human cervical carcinoma cells (CCL2).

HuPBL and RhPBL were prepared by a Ficoll-Hypaque gradient and cultured for 72 h in *Staphylococcus* enterotoxin A protein (0.5 μg/ml; Toxin Technology Inc., Madison, Wis.) and phytohemagglutinin (0.25%; Difco, Detroit, Mich.) and then in interleukin 2 (20 U/ml; Genzyme, Boston, Mass.) for 24 h, and finally in phorbol 12-myristate 13-acetate (50 ng/ml; Sigma, St. Louis, Mo.) for 24 h. After electroporation, the cells were cultured in phorbol 12-myristate 13-acetate for 24 h and then harvested for CAT activity. Other cells were cultured according to recommended specifications.

Transfection and CAT assays. Cell cultures were transfected by DEAE-dextran (3, 29), calcium phosphate ($CaPO_4$) (19, 53), or electroporation (2). CAT enzymatic assays utilizing whole-cell extracts were performed by measuring conversion of [^{3}H]acetyl coenzyme A to [^{3}H]acetyl chloramphenicol (38).

Plasmids. pUCHIVLTR/CAT contains the HIV-1 LTR (positions −515 to +185) of the SF2 isolate (40) upstream of the CAT-encoding sequences, the SV40 small *t* intron, and the SV40 polyadenylation signal (18) in a pUC12 plasmid background. pUCSVTAT contains exons 1 and 2 of the HIV-1 SF2 gene for TAT as an unspliced construct (40) under transcriptional control of the SV40 early promoter and polyadenylation signal in a pUC12 plasmid vector. pUCSVIE contains CMV IE regions 1 and 2 as a *Sac*II-*Sal* restriction fragment from the *Xba*E fragment of the Towne strain of human CMV. It was constructed by removing the TAT-encoding sequences from pUCSVTAT and substituting the CMV IE coding region.

Plasmids pGEM2/WT (3) was used for synthesis of antisense RNA probes and contains positions −138 to +82 of the HIV-1 LTR.

Radioimmunoprecipitation. Radioimmunoprecipitation of IE proteins in cells transfected with pUCSVIE used murine monoclonal antibody (MAb) 810 (Chemicon, Temecula, Calif.), which recognizes an epitope in either exon 2 or 3 (27). Cells were placed into 2 ml of leucine-free medium supplemented with 100 μCi of L-[4,5-^{3}H]leucine per ml (185 MBq/ml; Amersham Corp., Clearbrook Ill.) at 4 h posttransfection. Cells were labeled for 24 to 48 h, and then the cultures were lysed in 0.1% sodium dodecyl sulfate–1.0% Triton X-100–0.2 mM phenylmethylsulfonyl fluoride and subjected to three freeze-thaw cycles. Cell extracts were precleared by incubation for 1 h with rabbit anti-mouse immunoglobulin G (IgG; Sigma) and protein A-Sepharose. Supernatant was then incubated overnight with MAb 810 (diluted 1:50), followed by 4 h of incubation with the secondary antibody and protein A-Sepharose. The protein-antibody conjugate was washed seven times in lysis buffer. Protein was eluted by boiling in 80 μl of electrophoresis buffer.

RNase protection. Total cellular RNA was isolated as described by Chomczynski and Sacchi (10) and treated with RNase-free DNase (Promega, Madison, Wis.) in the presence of RNasin (Promega) (24). We used 10 to 40 μg of total RNA for RNase protection. [^{32}P]-labeled RNA probes were synthesized as described by Melton et al. (31). The protocol for RNase protection has already been described (3, 48).

RESULTS

Transactivation of the HIV-1 LTR by CMV IE and HIV-1 TAT. In an earlier study, we examined the mechanisms by which the CMV IE gene and the gene HIV-1 for TAT transactivate the HIV-1 LTR (3). Transient expression assays were performed in MRC-5 cells which are nontransformed cells derived from human fetal lungs. We have addressed the question of whether the mechanisms of transactivation of the HIV-1 LTR by CMV IE and TAT are the same in different cell types. For this analysis, we cotransfected a plasmid containing the gene for chloramphenicol acetyltransferase (CAT) under transcriptional control of the HIV-1 LTR (pUCHIVLTR/CAT) in the presence or absence of plasmids expressing either the HIV-1 gene for TAT or the CMV IE gene. TAT function was provided by pUCSVTAT, which contains the HIV-1 gene for TAT (40) under transcriptional control of the SV40 early promoter and polyadenylation signal in a pUC12 plasmid vector (see Materials and Methods). pUCSVIE expresses both regions IE1 and IE2 of human CMV (22) and was constructed by substituting the CMV IE coding region for the TAT-encoding region of pUCSVTAT (see Materials and Methods). pUCHIVLTR/CAT was cotransfected with either pUCSVTAT or pUCS VIE into MRC-5, L929 (transformed murine fibroblast), HTB-17 (human glioblastoma, also known as U-373 MG), and HeLaS3 (human cervical carcinoma) cell cultures which were harvested at 44 h for CAT activity. The results are presented in Table 1.

Both pUCSVTAT and pUCSVIE increased expression from pUCHIVLTR/CAT in all four cell types, compared with transfection with pUCHIVLTR/CAT alone. However, the relative abilities of pUCSVTAT and pUCSVIE to transactivate the HIV-1 LTR varied dramatically among these cell types. In MRC-5 cell cultures, pUCSVIE increased CAT expression over 66-fold, compared with an 11.5-fold increase in CAT activity with pUCSVTAT (Table 1). A basis of standardization is required to compare transfections in different cell types. We used as the standard the ratio of the level of transactivation of the HIV-1 LTR by pUCSVIE to the level of transactivation by pUCSVTAT. In MRC-5 cultures, this ratio was 5.8, indicating that in this cell type CMV IE transactivated the HIV-1 LTR almost sixfold better than did HIV-1 TAT. In comparison, in HeLa cell cultures, pUCSVIE increased CAT activity 2.5-fold, while pUCS VTAT stimulated CAT expression 207-fold; the ratio of transactivation by pUCSVIE to transactivation by pUCS VTAT was 0.01, almost 600-fold lower than the ratio in MRC-5 cultures. In L929 and HTB-17 cultures, pUCSVIE transactivated the HIV-1 LTR 134- and 28.5-fold, respectively, while pUCSVTAT transactivated the LTR 186- and 169-fold, respectively. The ratio of pUCSVIE to pUCS VTAT transactivation in L929 cells was 0.7, while that in HTB-17 cells was 0.2.

Transactivation of the HIV-1 LTR in different cell types. The transfections described in Table 1, in which identical experiments were performed with four cell types, produced different results. We extended this analysis to 18 cell types representing five mammalian species. Results from repre-

TABLE 1. Transactivation of the HIV-1 LTR by CMV IE and HIV-1 TAT[a]

Cell type	Cpm of CAT activity[b] (relative activity)[c] after transfection with:			IE/TAT ratio[d]
	pUCHIVLTR/CAT	pUCHIVLTR/CAT + pUCSVIE	pUCHIVLTR/CAT + pUCSVTAT	
MRC-5	9460 (1)	628,937 (66.5)	109,026 (11.5)	5.8
L929	12,224 (1)	1,639,270 (134.1)	2,278,710 (186.4)	0.7
HTB-17	9250 (1)	263,866 (28.5)	1,565,620 (169.3)	0.2
HeLa	6406 (1)	16,207 (2.5)	1,326,870 (207.1)	0.01

[a] Transfections into MRC-5, L929, HTB-17, and HeLa cell cultures were performed in parallel with the indicated plasmids. Descriptions of cell types are given in Materials and Methods.
[b] Values represent averages of two transfections. Variation was less than 15%.
[c] Relative level of stimulation of CAT activity by pUCSVIE and pUCSVTAT compared with transfection with pUCHIVLTR/CAT alone (arbitrarily set at 1).
[d] Ratio of the level of transactivation of pUCHIVLTR/CAT by pUCSVIE to the level of transactivation by pUCSVTAT.

sentative experiments with each cell type are presented in Table 2, and several features were noted. There was a very wide range in the abilities of pUCSVIE and pUCSVTAT to transactivate the HIV-1 LTR. In NIH 3T3 cell cultures (immortal but nontransformed murine fibroblast), pUCSVIE increased CAT expression from pUCHIVLTR/CAT over 95-fold, while pUCSVTAT increased expression less than 4-fold. The ratio of transactivation of pUCSVIE to pUCS VTAT in NIH 3T3 cells was 25 to 1. In HeLa cell cultures transfected by DEAE dextran (in experiments independent of those described in Table 1), pUCSVIE transactivated pUCHIVLTR/CAT only 2-fold while pUCSVTAT transactivated the LTR over 60-fold; the ratio of pUCSVIE-to-pUCSVTAT transactivation in HeLa cell cultures was 0.03, almost 1,000-fold less than the ratio in NIH 3T3 cells. These two cell types represented the extremes we observed in the ratios of pUCSVIE-to-pUCSVTAT transactivation. Since expressions of HIV-1 TAT and CMV IE were both under transcriptional control of the SV40 early promoter, differences in the ratio were not a function of differential promoter activity of the two transactivator expression plasmids.

We examined whether the methods of transfection might influence the abilities of pUCSVIE and pUCSVTAT to transactivate the HIV LTR and thus alter the ratio of transactivation. In L929 cultures transfected with DEAE dextran, the ratio of pUCSVIE-to-pUCSVTAT transactiva-

TABLE 2. Transactivation of the HIV-1 LTR by CMV IE and HIV-1 TAT in different cell types

Cell type[a]	Transfection method[b]	Cpm of CAT activity[c] (relative activity)[d] after transfection with:			IE/TAT ratio[e]
		pUCHIVLTR/CAT	pUCHIVLTR/CAT + pUCSVIE	pUCHIVLTR/CAT + pUCSVTAT	
NIH 3T3 (M)	DEAE	746	71,177 (95.4)	2,865 (3.8)	25.1
MRC-5 (H)	DEAE	10,628	279,600 (26.3)	31,602 (3.0)	8.8
Hs68 (H)	DEAE	7,766	132,460 (17.1)	31,168 (4.0)	4.3
Bc-1 (M)	DEAE	8,534	60,552 (7.1)	20,790 (2.4)	3.0
GR3A (M)	DEAE	6,504	48,056 (7.4)	39,268 (6.0)	1.2
Y-79 (H)	EPN	21,790	674,780 (31.0)	569,340 (26.1)	1.2
L929 (M)	DEAE	5,398	1,056,180 (195.7)	1,442,900 (267.3)	0.7
L929 (M)	$CaPO_4$	4,756	20,286 (4.3)	45,944 (9.7)	0.4
SW480 (H)	$CaPO_4$	5,355	69,787 (13.0)	118,867 (22.2)	0.6
GCT (H)	DEAE	11,842	228,180 (19.3)	681,420 (57.5)	0.3
HuT78 (H)	EPN	30,009	90,757 (3.0)	347,030 (11.6)	0.3
Jurkat (H)	EPN	275,820	542,040 (2.0)	2,167,900 (7.9)	0.2
HTB-17 (H)	DEAE	12,403	162,280 (13.1)	769,530 (62.0)	0.2
HuPBL (H)	EPN	21,920	83,013 (3.8)	532,340 (24.3)	0.2
Cf2Th (C)	DEAE	890	12,082 (13.6)	92,607 (104.0)	0.1
RhPBL (Rh)	EPN	8364	19,040 (2.3)	252,273 (30.2)	0.08
NC-37 (H)	EPN	13,554	48,056 (2.0)	426,130 (31.4)	0.06
Cos (AGM)	DEAE	4,045	36,000 (8.9)	765,450 (189.2)	0.05
HeLa (H)	DEAE	5,066	10,679 (2.1)	307,230 (60.6)	0.03
HeLa (H)	$CaPO_4$	9,110	9,134 (1.0)	741,180 (81.4)	0.01

[a] pUCHIVLTR/CAT was cotransfected in the presence or absence of pUCSVIE and pUCSVTAT into 18 different cell types, representing five mammalian species, including human (H), murine (M), canine (C), rhesus monkey (Rh), and African green monkey (AGM) cells. Cell types are ranked in descending order of CMV IE-to-TAT transactivation. Descriptions of the cell types are given in the Materials and Methods.
[b] The methods of transfection used were electroporation (EPN), DEAE-dextran (DEAE), and calcium phosphate-mediated DNA transfection ($CaPO_4$).
[c] Values represent averages of two transfections. Variation was less than 15%.
[d] See Table 1, footnote *c*.
[e] See Table 1, footnote *d*.

tion was 0.7, while in cultures transfected by $CaPO_4$, the ratio was 0.4. Similarly, in HeLa cultures transfected by DEAE dextran, the ratio was 0.03, while in $CaPO_4$ transfected cultures, the ratio was 0.01. Similar ratios for pUCS VIE-to-pUCSVTAT transactivation were observed with two methods of transfection; thus, the results (Tables 1 and 2) were not a reflection of the method used to facilitate entry of transfecting DNA into cells. Furthermore, the ratios were independent of the time when the cells were harvested posttransfection and the amount of DNA used in the transfection (data not shown).

The relatively high level of transactivation of the HIV-1 LTR by TAT in murine L929 cells is at variance with reports indicating that rodent cells are insufficient in the ability to support TAT-mediated transactivation of the HIV-1 LTR, including subclones of mouse L cells (21, 37). In our study, DEAE-mediated transfection of L929 cells resulted in higher levels of CAT activity than did $CaPO_4$-mediated transfection (Table 2). We do not know whether DEAE-mediated transfection was responsible for the higher levels of transactivation by TAT than those previously reported (21, 37). To avoid the possibility of contamination of our L929 cells with human cells, we assayed a second lot of L929 cells obtained directly from the American Type Culture Collection. These cells were just as fully capable as our older stock of L929 cells of supporting a high level of transactivation of the HIV-1 LTR; the ratio of transactivation by CMV IE relative to TAT was 0.4 (data not shown).

The ratios of pUCSVIE-to-pUCSVTAT transactivation were consistent between experiments. This is illustrated for MRC-5, L929, HTB-17, and HeLa cell cultures by comparing the results of both Tables 1 and 2, which represent independent experiments with each cell type. Although the actual levels of CAT enzymatic activity with each transactivator varied between the two experiments represented (in Tables 1 and 2), the ratios of pUCSVIE-to-pUCSVTAT transactivation remained relatively constant (5.8 and 8.8 for MRC-5; 0.7 and 0.7 for L929; 0.2 and 0.2 for HTB-17; 0.01 and 0.03 for HeLa). Thus, the ratio of pUCSVIE-to-pUCS VTAT transactivation was characteristic for each cell type.

Differences in the ratios were not related to cell tropism for CMV infection and replication. MRC-5 and Hs68 cells (human foreskin fibroblast) were productively infected with CMV (data not shown) and showed ratios of 8.8 and 4.3, respectively (Table 2). Similarly, HTB-17 cells were also infected by CMV (data not shown) yet had a 10-fold lower ratio of 0.2. CMV does not productively infect NIH 3T3, L929, or HeLa cell cultures (ratios of 25.1, 0.7, and 0.03, respectively) (26 and data not shown). Thus, permissiveness for CMV infection was not related to the abilities of pUCS VIE and pUCSVTAT to transactivate the HIV-1 LTR.

A general correlation noted from the results in Tables 1 and 2 was a higher ratio of CMV IE-to-TAT transactivation in cell types that had a finite life span in culture, including MRC-5, Hs68, and Bc-1 (murine mammary epithelium) cells, and in nontransformed cells (e.g., NIH 3T3 cells). MRC-5, Hs68, and Bc-1 cell strains, derived from primary cells, grew in monolayers, and all had CMV IE-to-TAT ratios of approximately 5 to 1. NIH 3T3 cells, immortal but nontransformed, had the highest ratio at 25 to 1. With two exceptions, the other cell lines assayed were transformed lines; each had a CMV IE-to-TAT ratio less than or equal to approximately 1. Transfections into primary PBL are an interesting exception to the correlation described above. The ratios of CMV IE-to-TAT transactivation in HuPBL and RhPBL were 0.2 and 0.08, respectively. After isolation from the donor, the PBL were cultured for 5 days under mitogenic and T-cell activation conditions, which were optimal for growth stimulation. Preliminary evidence indicates that the ratio of transactivation may change under different growth conditions for PBL, particularly when the cells are cultured under less stimulatory conditions (data not shown).

CMV IE proteins in transfected cell cultures. The preceding data indicate that the abilities of CMV IE and HIV-1 TAT to transactivate the HIV-1 LTR varied between different cell types. Although expression of both TAT and CMV IE was driven by the SV40 early promoter, we examined whether the CMV IE-to-TAT transactivation ratio differences between cell types were due to differences in the synthesis and localization of the transactivator proteins. We focused our attention on CMV IE.

The CMV IE gene encodes multiple proteins from a differentially spliced precursor transcript (22, 52). We examined whether there were differences in the presence or relative levels of specific CMV IE proteins in various transfected cell cultures. Cultures of MRC-5, L929, HTB-17, and HeLa cells were cotransfected with pUCHIVLTR/CAT plus pUCSVIE and labeled with [^{3}H]leucine. Radiolabeled CMV IE proteins were immunoprecipitated with MAb 810, which recognizes an epitope in exon 2 or 3 (27). Radioimmunoprecipitation of CMV IE proteins from MRC-5 cells infected with the Towne strain of human CMV under IE conditions (Materials and Methods) served as a positive control, and cells transfected with pUCHIVLTR/CAT alone served as a negative control.

In MRC-5 cell cultures infected with CMV (Fig. 1, lane 11), proteins with apparent molecular masses of 77, 66, 59, 47, 43, and 38 kDa were observed; the 66- and 43-kDa proteins were predominant. The 66-kDa protein most likely represents the major IE1 protein, and the 59-kDa protein represents the major species of IE2 (22, 50, 51). We noted an abundant 43-kDa protein in cultures which appeared within 3 h of reversal of the cycloheximide block. It is not certain whether this represents the previously characterized 38-kDa IE1 protein (50), albeit in much greater levels than reported, or another, uncharacterized, CMV IE protein.

Two features were observed for radiolabeled CMV IE proteins in cell cultures transfected with pUCSVIE. The first was that the same CMV IE proteins were observed in all four cell types as in CMV-infected MRC-5 cells (Fig. 1, lanes 1, 4, 7, 8, 10, and 11). These proteins were not detected in the negative controls (Fig. 1, lanes 2, 3, 5, 6, and 9). The two predominant bands in the transfected cell cultures were the 66- and 43-kDa proteins. The second feature was the variation between cultures in the relative levels of the different proteins, particularly the 66- and 43-kDa species. In MRC-5 cells (Fig. 1, lane 10), there was a much greater amount of the 66-kDa protein than the 43-kDa protein. In HeLa cells (Fig. 1, lane 2), there was a greater level of the 66-kDa protein, although the difference from that of the 43-kDa protein was not as great as in MRC-5 cells. In L929 (Fig. 1, lane 5) and HTB-17 (Fig. 1, lanes 7 and 8) cells, the 43-kDa species predominated over the 66-kDa protein. The differences between the relative levels of these two proteins were most likely a function of the time of harvesting after transfection. This is illustrated for HTB-17 cells which were harvested at 24 (Fig. 1, lane 7) and at 48 (Fig. 1, lane 8) h posttransfection. At 24 h posttransfection, there was a large excess of the 43-kDa protein, while at 48 h posttransfection, there were essentially equimolar amounts of the 66- and 43-kDa proteins. To achieve a high level of CMV IE synthesis, the four cell cultures were transfected by electropora-

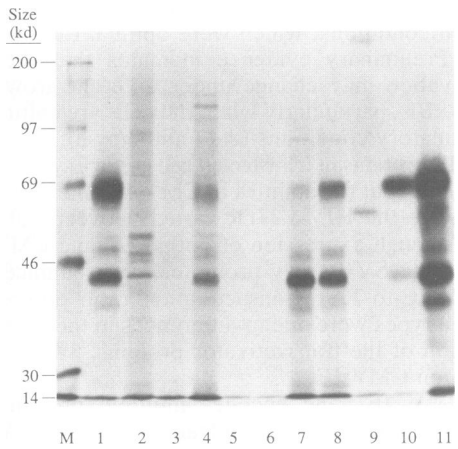

FIG. 1. Expression of CMV IE in transfected cell cultures. Cultures of HeLa (lanes 1 and 2), L929 (lanes 3 and 4), HTB-17 (lanes 5 to 8), and MRC-5 cells (lanes 9 and 10) were transfected with pUCHIVLTR/CAT (lanes 2, 3, 5, 6, and 9) or pUCHIVLTR/CAT plus pUCSVIE (lanes 1, 4, 7, 8, and 10). In addition, MRC-5 cells were infected with the Towne strain of CMV (lane 11). Cells were labeled with [^{3}H]leucine and harvested at 48 or 24 (lanes 5 and 7 only) h posttransfection. CMV IE antigens were immunoprecipitated with MAb 810 and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The molecular masses of the protein standards in lane M (in kilodaltons [kd]) are given on the left. The calculated sizes of the CMV IE proteins are given on the right.

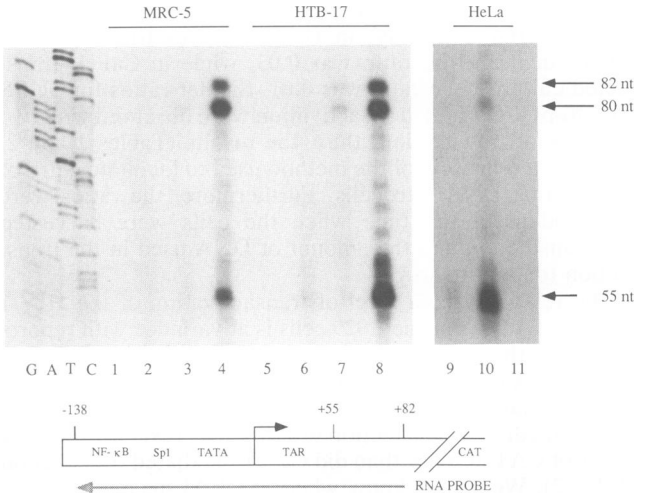

FIG. 2. HIV-1 transcripts in transfected cell cultures. Cultures of MRC-5 (lanes 1 to 4), HTB-17 (lanes 5 to 8), and HeLa cells (lanes 9 to 11), were transfected with no DNA (lanes 1, 5, and 11), pUCHIVLTR/CAT (lanes 2, 6, and 9), pUCHIVLTR/CAT plus pUCSVTAT (lanes 3 and 7), or pUCHIVLTR/CAT plus pUCSVIE (lanes 4, 8, and 10). Total RNA was isolated at 44 to 48 h posttransfecton, and steady-state levels of RNA were analyzed by RNase protection with an antisense RNA probe of the HIV-1 LTR (depicted at the bottom). Full-length transcripts (corresponding to 82 and 80 nt due to secondary structure of the RNA) are indicated. Incompletely elongated transcripts of 55 nt are also indicated. DNA sequencing reactions of the HIV-1 LTR (GATC) were used as size markers.

tion. While the cultures were harvested at 48 h posttransfection, different cell types responded differently to electroporation in terms of resumption of cell growth (data not shown). We believe that any differences in the levels of the different CMV IE proteins were a reflection of the electroporation process and not due to fundamental differences between cell types in the expression of the multiple CMV IE proteins. In addition, the pattern of temporal expression of the 43-kDa protein, relative to the 66-kDa species, indicates that the 43-kDa protein was not a degradation product.

We also used MAb 810 to determine the intracellular location of CMV IE. In all four cell types (MRC-5, L929, HTB-17, and HeLa), IE proteins recognized by MAb 810 were localized by immunohistochemistry to the nuclei of transfected cells (data not shown).

HIV-1 transcripts in transfected cell cultures. The experiments described above measured transactivation of the HIV-1 LTR by CMV IE and TAT at the protein level (i.e., increases in CAT activity). We assessed the response of the HIV-1 LTR to these transactivators at the RNA level by measuring steady-state levels of LTR transcripts. Cultures of MRC-5, HTB-17, and HeLa cells were transfected with no DNA, pUCHIVLTR/CAT, pUCHIVLTR/CAT plus pUCS VTAT (MRC-5 and HTB-17 only), or pUCHIVLTR/CAT plus pUCSVIE. Total RNA was isolated and analyzed by RNase protection by using an RNA probe complementary to nucleotides −138 to +82 of the antisense strand of the HIV-1 LTR. In cells transfected with no DNA, there were no protected transcripts (Fig. 2, lanes 1, 5, and 11). Transfection with pUCHIVLTR/CAT resulted in low levels of transcripts predominantly 55 nucleotides (nt) long and little full-length transcript, which in this experiment corresponded to 82 nt (Fig. 2, lanes 2, 6, and 9). The short transcripts represent incompletely elongated transcripts (25, 48). Cotransfection with pUCSVTAT (Fig. 2, lanes 3 and 7) resulted in transcriptional elongation in both MRC-5 and HTB-17 cells, resulting in predominantly full-length transcripts. This was also observed in HeLa cells (data not shown). The full-length transcripts appeared as a doublet, most likely due to secondary structure. Cotransfection of pUCHIVLTR/CAT with pUCSVIE resulted in dramatically different patterns of protected transcripts. In MRC-5 cells (Fig. 2, lane 4), there were large increases in steady-state levels of both full-length and short transcripts, with greater levels of the former. In HTB-17 cells (Fig. 2, lane 8), there were also large increases in steady-state levels of short and full-length species, with essentially equimolar amounts of each. The results of MRC-5 and HTB-17 were similar to those previously reported (3, 4). Strikingly, in HeLa cells (Fig. 2, lane 10), cotransfection with pUCSVIE resulted in large increases in short transcripts only, with only a small increase in the full-length transcript. We found that equal amounts of RNA from the different HeLa transfectants were utilized for RNase protection by analyzing steady-state levels of the cellular γ-actin transcript (data not shown). In HeLa cells, the inability of pUCSVIE to increase CAT expression from pUCHIVLTR/CAT occurred at the level of transcription elongation.

We noted a curious aspect to transactivation by pUCS VTAT in these analyses. In some cases, there was no correlation between the level of CAT activity upon cotransfection with pUCSVTAT and the steady-state level of the full-length transcript. This is illustrated for transfections of HTB-17 cell cultures (Fig. 2, lanes 5 to 8). Without exception, cotransfections with TAT into HTB-17 cells resulted in larger increases in CAT activity than did cotransfection with CMV IE (Tables 1 and 2) (4). However, with respect to

steady-state levels of RNA, there were greater levels of the full-length transcript upon cotransfection with CMV IE than with TAT (Fig. 2) (4). Although we did not investigate this further, such a result is consistent with a posttranscriptional role for TAT (6, 7, 11, 39, 45), in addition to a role in transcription initiation and elongation (5, 25, 28, 43, 48, 49).

DISCUSSION

Productive viral infection and replication are dependent on several cellular processes, including cell surface receptors, as well as the transcriptional and translational machinery. The success or failure of viral replication, or the maintenance and abrogation of latency, hinges on the presence of critical cellular factors. Differential expression of these cellular proteins could occur in different cell types and, perhaps, at specific stages in the cell growth cycle. We have identified a point in the life cycle of HIV-1, elongation of transcription, which requires host cell factors and proceeds with various efficiencies, depending on the cell type.

This report analyzes the responses of the HIV-1 LTR to its autologous transactivator, TAT, and a heterologous transactivator, CMV IE. Expression of both TAT and CMV IE was under transcriptional control of the same promoter, the SV40 early promoter, to eliminate variability that might occur in different cell types. When transient expression assays were performed with multiple cell types, dramatic variations in the abilities of the two transactivators to increase expression directed by the HIV-1 LTR were observed (Tables 1 and 2). The variation in transactivation between cell types, measured as changes in the level of transactivation of the HIV-1 LTR by CMV IE relative to the level of transactivation by TAT, was almost 1,000-fold (Table 2).

The ratio of CMV IE-to-TAT transactivation of the HIV-1 LTR has been remarkably constant between experiments, despite the observation that the absolute CAT activities used to calculate the ratio varied (Tables 1 and 2) (1, 3, 4). Even though the SV40 promoter was used to drive expression of both CMV IE and HIV-1 TAT, potential posttranscriptional mechanisms could lead to differential expression of CMV IE in various cell types. After transfection of pUCSVIE into MRC-5, L929, HTB-17, and HeLa cell cultures, we detected CMV IE expression by immunohistochemistry in nuclei of all four cell types (data not shown). Thus, the localizations of CMV IE were similar in all of the cell types. By radioimmunoprecipitation of CMV IE, we detected no overt differences among these four cell types in the pattern of expression of the multiple IE proteins (Fig. 1). It is possible that there are critical minor proteins that either were not recognized by the antibody to CMV IE or were below the level of detection. In addition, there may be cell-specific posttranslational modifications (e.g., phosphorylation) that we would not have detected.

The region of the HIV-1 LTR between −6 and +20 (relative to the cap site at +1) is involved in mediating responsiveness to transactivation by CMV IE in both MRC-5 cells (3) and L929 cells (4). Thus, CMV IE transactivates the HIV-1 LTR through the same *cis*-acting element in different cell types from several species. Data on patterns of RNA and CAT transactivation are consistent with a model in which CMV IE functions to increase the frequency of transcription initiation. If CMV IE functions to increase the frequency of transcription initiation (an assumption that requires direct examination), the mechanism of the block to elongation is independent of the mechanism of initiation. In the presence of CMV IE in transfected HeLa cell cultures, there was a large increase in the steady-state levels of the short 55-nucleotide transcript (Fig. 2, lane 10) compared with cells transfected with HIVLTR/CAT alone (Fig. 2, lane 9). The block to elongation is dominant over the increase in the frequency of initiation, such that in the absence of TAT function in HeLa cells, most of the transcription complexes on the HIV-1 LTR fail to elongate fully and produce a full-length message. In MRC-5 cells, the block to elongation is not as efficient as in HeLa cells; thus, most initiated transcription complexes are capable of producing full-length transcripts. In HTB-17, elongation is blocked at a level intermediate between those observed in HeLa and MRC-5 cells. The ratios of CMV IE-to-TAT transactivation correlate inversely with the efficiency of the block in elongation; the greater the ratio, the less efficient the block to transcription initiation (MRC-5 cells), and the lower the ratio, the more efficient the block to elongation (HeLa cells).

Recent reports have shown that human chromosome 12 encodes a factor necessary for efficient transactivation by TAT in human cell cultures (21, 37). Furthermore, rodent (hamster and mouse) cells are apparently deficient in this factor and do not support efficient transactivation by TAT. Our results suggest that inability of TAT to transactivate the HIV-1 LTR is not a function of all rodent cells. In the experiments reported here, TAT was a very strong transactivator in mouse L929 cells (Tables 1 and 2). Notably, this was the only rodent cell line in which we observed a high level of transactivation of the HIV-1 LTR by TAT.

One issue that requires consideration is whether TAT transactivates the HIV-1 LTR by a bimodal mechanism (5, 6, 7, 11, 25, 28, 39, 43, 45, 49, 54) and whether the transcriptional and posttranscriptional components could be cell type specific. In HTB-17 cells, TAT transactivated the HIV-1 LTR to a level fivefold higher than did CMV IE (i.e., the CMV IE/TAT ratio was 0.2). However, there were greater steady-state levels of HIV-1 transcripts with cotransfection with CMV IE than with TAT (Fig. 2) (4). This is strong support for the notion that in HTB-17 cells the predominant mode of transactivation of the HIV-1 LTR TAT is posttranscriptional versus a primary role in initiation and elongation. Depending on the cell type, one mode of transactivation by TAT may be predominant over the other, and thus, this difference may affect the ratio of CMV IE-to-TAT transactivation (Tables 1 and 2).

In summary, there are fundamental differences between cell types in the state of the transcriptional machinery, manifested in this case as differential transactivation of the HIV-1 LTR by two viral transactivators. Complete elucidation of specific mechanisms and the role of cellular factors (16, 23, 24, 30, 34, 55, 56) will require reconstitution experiments in vitro. This report provides a basis for identification of cellular factors which provide the transcriptional selectivity we have observed. Such information is vitally important for understanding of the mechanisms that regulate viral latency and pathogenesis for both HIV and CMV. The studies reported here are also relevant for investigation of interactions of heterologous viruses, since it has been demonstrated that HIV and CMV can coinfect the same cell within a patient with AIDS (36). Given the great variation in the abilities of these two viral transactivators (HIV-1 TAT and CMV IE) to function within different cell types in tissue culture, the biological consequences of coinfection of a host would depend on the cell type.

ACKNOWLEDGMENTS

We thank Ayalew Mergia, Mark Selby, Matija Peterlin, Adam Geballe, Richard Spaete, and Kathy Jones for insightful discussions and comments.

This work was supported in part by a grant from the National Heart, Lung, and Blood Institute. P.A.B. is a recipient of a National Research Service Award. P.A.L. is a recipient of an investigator award from the California Universitywide Task Force on AIDS.

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