A Human Immunodeficiency Virus Type 1 (HIV-1) Tat Cofactor Absent in Rodent Cells is a TAR-associated Factor

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ABSTRACT

Background: Although Tat plays a role as a potent transactivator in the viral gene expression from the Human Immunodeficiency Virus type 1 long terminal repeat (HIV-1 LTR), it does not function efficiently in rodent cells implying the absence of a human specific factor essential for Tat-medicated transactivation in rodent cells. In previous experiments, we demonstrated that one of chimeric forms of TAR (transacting responsive element) of HIV-1 LTR compensated the restriction in rodent cells. **Methods:** To characterize the nature of the compensation, we tested the effects of several upstream binding factors of HIV-1 LTR by simple substitution, and also examined the role of the configuration of the upstream binding factor(s) indirectly by constructing spacing mutants that contained insertions between Sp1 and TATA box on Tat-mediated transactivation. **Results:** Human Sp1 had no effect whereas its associated factors displayed differential effects in human and rodent cells. In addition, none of the spacing mutants tested overcame the restriction in rodent cells. Rather, when the secondary structure of the chimeric HIV-1 TAR construct was destroyed, the compensation in rodent cells was disappeared. Interestingly, the proper interaction between Sp1 and TATA box binding proteins, which is essential for Tat-dependent transcription, was dispensable in rodent cells. **Conclusion:** This result suggests that the human-specific Tat cofactor acts to allow Tat to interact effectively in a ribonucleoprotein complex that includes Tat, cellular factors, and TAR RNA, rather than be associated with the HIV-1 LTR upstream DNA binding factors. **(Immune Network 2002;2(3):150-157)**

Key Words: Tat, transactivation, HIV-1-LTR, TAR

Introduction

Human Immunodeficiency Virus type 1 (HIV-1), the causative agent of Acquired Immunodeficiency Syndrome (AIDS), is associated with many opportunistic infections, malignancies, and a profound immunosuppression (1), which pathogenesis is species-specific so that infection leads to AIDS only in humans. Even though HIV-1 is capable of infecting chimpanzees, it is not pathogenic (2). Moreover, rodents are nonpermissive to HIV-1 infection and at least four blocks to viral replication have been identified, among which one barrier is associated with a viral encoded regulatory protein, Tat. Tat is essential for HIV-1 replication, but do not function efficiently in rodent cells (3,4). These observations imply the importance of human-specific cellular factors in HIV-1 pathogenesis and gene expression.

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Transactivation of the HIV-LTR by the Tat functions by a novel mechanism that involves the direct interaction of the Tat and cellular factors with nascently transcribed viral RNA encoding the Tat responsive element (TAR). So far, a number of cellular regulatory factors have been reported to be associated with Tat-mediated transactivation, and species-specific differences in some of those factors, including some yet unidentified factors, may contribute to the reduced expression of HIV-1 LTR in rodent cells. Indeed, it was found that human chromosome 12 encodes a species-specific factor(s) functionally absent in rodent cells (4,5).

Transcription factors typically contain separate binding and activation domains. To analyze the mechanism of Tat-mediated transactivation, fusion proteins of Tat with heterologous RNA- or DNA-binding proteins have been utilized by many groups (6-9). We also described previously the construction of chimeric TAR sequences with the heterologous RNA sequence derived from the bacteriophage R17 genome that binds to the bacteriophage MS2 coat protein (9). These

chimeric TAR constructs were cotransfected into rodent and human cells with a plasmid encoding a chimeric Tat protein with the RNA binding domain of the MS2 coat protein. Interestingly, TAR constructs containing the MS2 coat protein binding region inserted into the three nucleotide bulge region of Tat displayed a high level of transactivation by Tat-MS2 coat protein chimeras in both human and rodent cells.

The chimera TAR structure might provide a binding site for TAR associated factor(s) essential for Tat-mediated gene expression in rodent cells, suggesting that the defect in rodent cells is due to lack of a TAR-associated factor(s) required for an optimal Tat-mediated transactivation. On the other hand, the chimeric construct might be transactivated at a high level in rodent cells because the heterologous binding site of the TAR region of the chimeric construct provides the proper spacing required for efficient interaction between Tat and the promoter elements, suggesting that the restriction of Tat-mediated gene expression in rodent cells may be due to lack of interaction with a upstream binding factor(s) required for an optimal Tat-mediated transactivation. In fact, numerous cellular proteins have been reported to be involved in HIV-1 basal expression and Tat-mediated transactivation, among which upstream DNA binding factors such as Sp1 and TBP are known to be crucial for Tat-mediated transactivation since mutations in their binding sites cause severe down-regulation of Tat function (10,11). Thus, in this study, to investigate whether or not the human cellular factor(s) missing in rodent cells is related to upstream binding proteins or to TAR-associated proteins, various mutant HIV-1 LTR-driven CAT constructs were transiently transfected into cell lines from two different species, one human (HeLa) and the other rodent (A9), and the effects of upstream binding factors and TAR-associated factors were examined.

Materials and Methods

Cell culture. Cell lines were maintained in 1X Dulbecco's Modified Eagle's Media (DMEM) supplemented with 2 mM glutamine, penicillin (100 U per ml), and 10% calf serum at 37°C in a humidified cell culture incubator maintaining 5% of CO_2.

Construction of plasmids. Construction of HIV-1 LTR-CAT plasmids containing mutations in the TAR region; the chimeric transactivation system was originally developed by Selby and Peterlin (7), and some of the parental plasmids which were utilized in this research were kindly provided by them. Briefly, pHIVSCAT contains the wild-type HIV-1 LTR linked to the chloramphenicol acetyl transferase gene (Fig. 1). In pHIVSRCAT, the TAR region of the HIV-1 LTR is replaced by the operator region of bacteriophage R17 which provides the binding site for the bacteriophage MS2 coat protein. Two effector plasmids, pSVTat (1-67) and pSVTat (1-67)CP, produce a truncated form of Tat containing 67 amino acids and a chimeric form of Tat containing 2-129 amino acid residues of MS2 coat protein in addition to the truncated Tat, respectively. In pHIVTRCAT, +25 to +28 of TAR (the "bulge region", known to be the Tat recognition site) of pHIVSCAT is replaced by a 17 nucleotide R17 operator that is recognized by MS2 coat protein. The pHIVRCAT was constructed from pHIVTRCAT with a 2 base mutation in the loop region of TR-RNA by end-filling and ligation after double-digestion with Nco I and Afl II. This plasmid does not have the sequence behind the loop of the TAR structure of pHIVTRCAT, resulting in the absence of the typical stem-loop structure of the TAR secondary structure which is known to provide binding sites for cellular factors.

All the SR CAT insertion plasmids were constructed by inserting either a Bgl II linker (10 mer or 12 mer) or a Sal I linker into the Xba site of pHIVSRCAT, which is located between the Sp1 binding site and the TATA box.

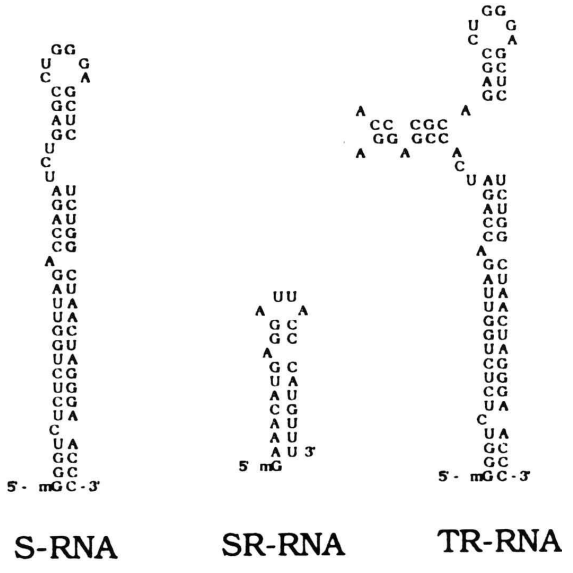


Figure 1. Sequences and secondary structures of wild-type and mutant TAR RNA. The sequences of the TAR regions (S-RNA, SR-RNA, and TR-RNA) in pHIVSCAT, pHIVSRCAT, and pHIVTRCAT, are shown. pHIVSCAT contains the wild-type HIV-1 LTR including a wild-type TAR region (S-RNA) linked to the chloramphenicol acetyl transferase gene. In pHIVSRCAT the TAR region of the HIV-1 LTR is replaced by the operator region of bacteriophage R17 which provides the binding site for the bacteriophage MS2 coat protein (SR-RNA). pHIVTRCAT contains S-RNA with the R17 coat-protein recognition sequences in place of the bulge (TR-RNA).

Transient transfection. The calcium phosphate transfection method was used to introduce plasmid DNA into cells (12). 20 hours before transfection, cells were plated in 6 cm tissue culture dishes at 3×10^5 cells per dish in complete medium. 2 hours prior to transfection, cells were fed with fresh medium. Ca PO4/DNA mixture was prepared by combining, in order: H_2O, plasmid DNA, 31 ul of 2 M $CaCl_2$, and 250 ul of 2×HBSP (1.5 mM Na_2PO4, 10 mM KCl, 280 mM NaCl, 12 mM glucose, 50 mM HEPES adjusted to pH 7). The final volume of the mixture was 500 ul per transfection. The amount and the ratio of reporter/effector plasmids varied in each experiment and are indicated in each figure legend. Either pCMV A+ or pSV40 A+ was added to avoid the variation in total promoter amount between transfection if needed, and sheared salmon sperm DNA was used as carrier DNA to keep total DNA amount to 5 ug per transfection. A precipitate was allowed to form for 30 minutes in a cell culture hood at room temperature, and then added slowly into the plate. After 5 hours, cells were treated with 1 ml of 15% glycerol in 1× HBSP for 1 minute at room temperature to increase transfection efficiency (12). After the glycerol shock, cells were incubated for 48 hours in the complete growth medium, and then harvested.

Protein quantitation. Bicinchoninic acid (BCA) protein assay was performed for protein quantitation to normalize plate-to-plate protein variation. 5 ul of the cell extract from each transfection was placed in a microtiter plate well in duplicate and then mixed with 200 ul of the BCA reagent. The absorbance was measured at 560 nm with a microtiter plate reader.

Chloramphenicol acetyl transferase assay. Reaction mixture for each assay contained 70 ul of 1 M Tris (pH 7.8), 1.5 ul of ^{14}C labeled chloramphenicol (60 mCi/mmole), 20 ul of acetyl coenzyme A (3.5 mg/ml in H_2O), cell extract (up to 50 ul), and H_2O to bring the total reaction volume to 150 ul. The amount of cell extract used for the assay varied depending on several factors such as the transfection efficiency, protein concentration, and the presence or the absence of Tat to obtain % acetylation in the linear range (between 1 to 50%). Normally, 25 ug of total protein in the absence of Tat expressing plasmid and either 5 ug or 2.5 ug of total protein from A9 or HeLa, respectively, in the presence of Tat expressing plasmid were used. The reaction mixture was incubated for one

Effect of Human and Rodent Sp1 on Tat-mediated *Trans*activation in A9 cells.

	% acetylation		
	no Tat	Tat	Fold activation
no Sp1	10.9	**22.8**	**2.1**
rSp1 (1ug)	10.2	**18.9**	**2.0**
hSp1 (1ug)	12.4	**27.2**	**2.2**
Effect of rSp1	6% Decrease	17% Decrease	
Effect of hSp1	13% Increase	18% Increase	

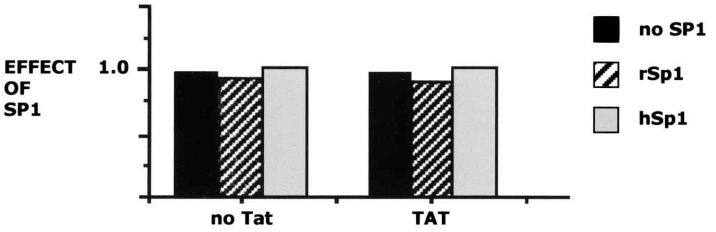


Figure 2. Effects of human and rat Sp1 in A9 cells. pHIVSCAT (2 ug) was cotransfected to A9 with pCMVhSp1 or pCMVrSp1 (1 ug) in the presence (pSV40 Tat or pCMV7fd/Tat) or absence (pSV A+) of Tat (0.5 ug). CAT activity was shown as % acetylation was normalized to internal pXGH (for A9 cells) or pCMVhGH (for HeLa cells) transfection control to account for different transfection efficiency. Fold activation was determined as the ratio of % acetylation from the cotransfections of target plasmids with the mock plasmid. The data represent the averages of two experiments. The effects of Sp1 on basal and Tat-mediated transactivation in A9 cells are illustrated in the graph below the table. The values in the graph were obtained by dividing the % acetylation in the presence of Sp1 by those in the absence of Sp1. The % acetylation of basal and Tat-mediated transactivation in the absence of Sp1 were set as 1.

hour. The % acetylation was corrected by subtracting the % acetylation of mock transfection (transfection with salmon sperm DNA) afterwards. The actual % acetylation was obtained by normalizing the transfection efficiency (using the hGH assay system).

Human growth hormone (hGH) assay. To minimize transfection variation within experiments (from plate to plate) and between experiments, the hGH assay system (Nicholes Institute) was used. Either pXGH5 or pCMV hGH (100 ng per transfection) was cotransfected as an internal control, containing the mouse metallothionein-1 promoter (mMT-1) or the cytomegalovirus immediate early gene promoter, respectively. For the assay, 100 ul aliquots of media were taken from each dish at the end of transfection since hGH is secreted. To obtain transfection efficiency coefficients, each hGH concentration was divided by the highest hGH concentration among a set of experiments which is presumably transfected the most efficiently.

Results

Substitution of human sp1 and its associated factors into rodent cells. Two cDNA clones encoding human and rat Sp1 were isolated separately elsewhere (13,14). The primary amino acid sequence of rat Sp1 is highly similar (98%) to that of human Sp1, and different amino acids between the two sequences are clustered in potential transcriptional domains (15). Since Sp1 is one of the most important regulatory elements in HIV-1 gene expression (11,18), the effect of human Sp1 on the rodent restriction in Tat-mediated transactivation was examined by substitution. In addition, the effect of rat Sp1 was also examined. Unlike the SV40 early (SV40E) promoter, which is activated by Sp1, no distinct GC boxes have been identified in the CMVIE promoter (17). Thus, CMVIE-driven Tat (pCMV7fd/Tat) was cotransfected as an effector.

Upon transfection of A9 cells with the human Sp1 and the HIV-1 LTR-driven CAT construct, pHIVSCAT, in the presence or absence of Tat, no detectable effects on either the basal or the Tat-mediated expression was observed (Fig. 2). The transfection of the rat Sp1 to A9 cells showed similar results to those obtained with the human Sp1. Even though the Sp1 gene is localized in human chromosome 12 (18), it is unlikely that Sp1 is the Tat-cofactor encoded in human chromosome 12 since no effect of human Sp1 on Tat-mediated transactivation was detected in our studies.

BTEB is another GC box-binding transcription

Effect of Rat BTEB on Tat-mediated *Trans*activation in A9 and HeLa cells.

	A9			HeLa		
	% acetylation			%acetylation		
	no Tat	Tat	Fold-A	no Tat	Tat	Fold-A
no BTEB	12.2	54.0	5.1	5.5	440.3	85.6
BTEB (2 ug)	9.7	51.0	5.3	1.7	151.6	86.2
Effect of BTEB	20 % Decrease	6 % Decrease		69 % Decrease	66 % Decrease	

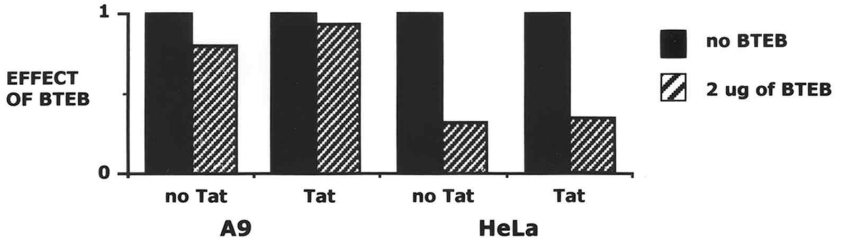


Figure 3. Effects of rat BTEB on the basal and Tat-induced expression. pHIVSCAT (2 ug) was cotransfected to A9 or HeLa cells with pRSVBTEB (2 ug) in the presence (pSV40 Tat) or absence (pSV A+) of Tat (0.1 ug). Fold-A indicates fold activation. The graphic representation of the effects of BTEB on the basal and Tat-mediated transactivation from the HIV-1 LTR is shown. As described in Fig. 2, the values in the graph were obtained by dividing the % acetylation in the presence of BTEB by those in its absence. The % acetylation of basal and Tat-mediated transactivation in the absence of BTEB were set as 1.

factor cloned from a rat cDNA library (19). Both BTEB and Sp1 have a DNA binding zinc finger domain. Although these proteins showed a high sequence similarity in the zinc finger domain, they are distinct in other areas. Therefore, we tested whether BTEB contributes the species-specific expression of the HIV-1 LTR in the presence of Tat. BTEB showed down regulation of the HIV-1 LTR in HeLa cells, but had no effect in A9 cells (Fig. 3).

We further examined Sp1-associated factors, such as human p53 and human retinoblastoma tumor suppressor, RB. Two regulatory factors, human p53 and RB, have been reported to bind to and cooperate with Sp1 (20,21). On examination of these factors, the substitutions did not rescue the restriction in rodent cells. Although we hoped to identify a human-pecific Tat-cofactor by complementing the Tat defect in rodent cells by the substitution experiments with Sp1 and its associated factors, none of the upstream binding factors tested in this study function as a activator in Tat induced expression in A9 cells. This result indicates that at least the factors examined in this study are not likely involved in the restriction of Tat-mediated transactivation in rodent cells.

Space adjustment between Tat and upstream promoter elements by insertion did not rescue the restriction in rodent cells. To investigate the effects of spacing between upstream elements and Tat on the restriction of Tat-ediated transactivation in rodent cells, various HIV-1 LTR insertion mutants were constructed and transfected. The mutants contain multiples of approximately 5 base pairs inserted at position -40 (between Sp1 and TATA box) in pHIVSRCAT.

Upon transfecting these SR insertion mutants, the restriction in Tat-mediated transactivation was still observed in A9 cells; all constructs displayed low levels of Tat-mediated transactivation (between 1.2 and 3.7 fold-activation) (Fig. 4). This result indicates that the spacing between Tat and upstream promoter elements did not rescue the restriction in rodent cells and that the differences between pHIVSRCAT and pHIVTRCAT are not due to spacing, considering that pHIVTRCAT is normally transactivated by the chimeric Tat construct more than 8-fold as previously shown in reference 9. Interestingly, however, SR insertion mutants in HeLa cells displayed various levels of Tat-mediated transactivation depending on the size of insertion. When the number of base pairs in the insertion was multiples of a single turn of the DNA helix (10 bp), relatively high levels of Tat-ediated transactivation were observed when compared to pHIVSRCAT. The levels of transactivation were inversely proportional to the lengths of the insertions in case that the insertions were multiples of 10 bases. The gradual loss of transactivation suggests that Tat becomes increasingly less able to function as Sp1 was

Effect of Insertions Between Sp1 and TATA on Tat-mediated *Trans*activation

	A9					HeLa				
	pSVA+	pSVTat		pSVTatCP		pSVA+	pSVTat		pSVTatCP	
TARGET	CAT%	CAT%	Fold-A	CAT%	Fold-A	CAT%	CAT%	Fold-A	CAT%	Fold-A
pHIVSRCAT	5.5	4.4	0.8	20.4	3.7	15.6	18.7	1.2	204.3	13.1
p4bSRCAT	2.5	1.8	0.7	5.1	2.0	10.1	9.1	0.9	37.7	3.8
p11bSRCAT	9.8	6.9	0.7	21.1	2.1	12.5	16.3	1.3	123.6	9.9
p15bSRCAT	4.8	4.3	0.9	9.1	1.9	13.0	14.4	1.1	33.8	2.6
p20bSRCAT	9.3	7.5	0.8	14.0	1.5	7.8	9.4	1.2	61.2	7.7
p25bSRCAT	4.9	3.9	0.8	7.4	1.5	6.4	7.7	1.2	16.3	2.5
p31bSRCAT	6.1	3.7	0.6	7.3	1.2	7.5	7.6	1.0	31.5	4.2

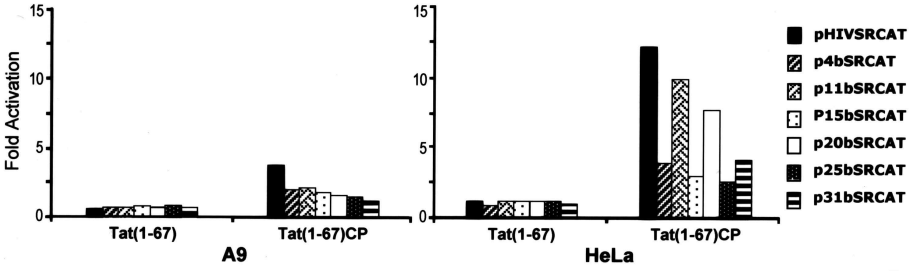


Figure 4. Effects of Insertions between Sp1 and TATA on the expression of HIV-1 LTR each insertion mutant construct (2 ug) was cotransfected into A9 and HeLa cells in the presence of pSV A+, pSV Tat (1-67), or pSV Tat (1-67) CP (0.5 ug). The data represent the averages of two experiments. Graphic representation of the fold activation is shown below the table.

moved distal to the promoter. On the other hand, when the number of base pairs in the insertion was not a multiple of 10bp, little, if any, activation by Tat was observed. Nonetheless, CAT activities in the absence of the chimeric Tat-coat protein did not change substantially with these insertion mutants, suggesting that basal transcription was not affected.

The secondary structure of pHIVTRCAT is essential to bypass the restriction in Tat-mediated transactivation. To examine whether the secondary structure of the TR-RNA region of pHIVTRCAT is necessary for its maximal activation in rodent cells, we tested the transactivation level of pHIVRCAT, in which RNA the stem region of TR-RNA has been destroyed (Fig. 5). On cotransfecting with the chimeric Tat-coat protein, the pHIVRCAT showed the restriction in A9 cells (3.4 fold-activation). In addition, it was observed that the transactivation level in HeLa cells is also severely impaired (25.4 fold-activation in Fig. 5) compared to the level of pHIVSCAT (85.6 fold-ctivation in Fig. 3) confirming importance roles of the stem binding factors in Tat-mediated transactivation. Since R-RNA does not retain the secondary structure of the HIV-1 TAR, this observation suggests that a TAR-associated factor that recognizes the intact secondary structure of the TAR stem is not only important for the maximal TAR activation, but also may be responsible for the rescue of the restriction in the Tat-mediated transactivation of TR-NA in rodent cells.

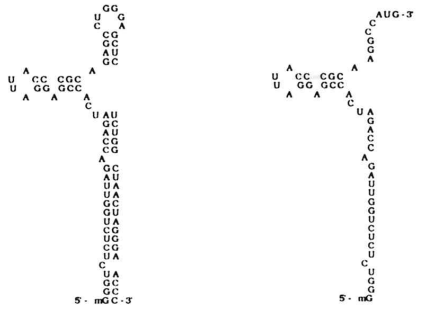
Discussion

To more understand the action of HIV-1 Tat, based on our previous data shown that a chimeric HIV-1 TAR construct (pHIVTRCAT) is transactivated efficiently in rodent cells, we analyzed the role and the level of action of a human-specific Tat-ofactor(s) absent in rodent cells in this study.

Since the interactions on HIV-1 LTR may be different between rodent cells and human cells, either by the absence of specific protein components or by proteins with different tertiary structures in the rodent cells, we first investigated the possibility that a human specific cofactor(s) is involved in the proper spacing between Tat and the upstream promoter elements, so that important human factors which may differ in rodent cells were transfected to examine whether the substitution of these factors rescues the restriction of Tat-mediated transactivation in rodent cells. Initial work focused on Sp1 which is a key factor for Tat-mediated transactivation since its gene has been mapped to human chromosome 12, which

*Trans*activation of pHIVRCAT by Chimeric Tat-Coat Protein Construct in A9 and HeLa Cells.

			A9		HeLa	
TARGET	EFFECTOR	Expressed Tat	CAT%	Fold-A	CAT%	Fold-A
pHIVRCAT	pSVA+	no Tat	3.2	1.0	10.4	1.0
	pSVTat	Tat(1-67)	2.6	0.8	14.2	1.4
	pSVTatCP	Tat(1-67)CP	11.2	3.4	260.2	25.4



TR-RNA R-RNA

Figure 5. Transactivation of pHIVRCAT by chimeric Tat-coat protein in A9 and HeLa cells. pHIVRCAT (2 ug) was cotransfected to A9 or HeLa cells in the presence of pSV A+, pSV Tat (1-67), or pSV Tat (1-67)CP (0.5 ug). CAT activity was normalized to account for different transfection efficiency. CAT % and Fold-A represent the normalized % acetylation and the fold activation as described previously. The secondary structure of the TAR-region (R-RNA) of pHIVRCAT is shown in comparison to that (TR-RNA) of pHIVTRCAT.

has been reported to contain a human specific Tat cofactor (16,18,22). Although we additionally tested other factors that have binding affinity to, or cooperate with Sp1, such as p53 and RB as well as BTEB that has a strong binding affinity to Sp1 binding sites, the substitutions were not able to bypass the restriction in rodent cells. Sp1-associated factors played different roles in rodent and human cells. Human p53 appeared to function differently in rodent and human cells in Tat-mediated transactivation. In rodent cells, p53 acts as a strong repressor in both basal and Tat-induced expression, whereas in human cells it acts as strong repressor only in the basal expression from the HIV-1 LTR. RB also appeared to function differently in the basal expression from the HIV-1 LTR between the two species. RB acts as a mild repressor in both basal and Tat induced expression in A9 cells whereas it acts as a strong repressor only in the basal expression in HeLa cells. BTEB showed downregulation of the expression from the HIV-1 LTR in HeLa cells, but not in A9 cells. Thus, in all cases, the substituted upstream factors functioned as repressors of HIV-1 expression in rodent cells, and these observations indicate that, at least the factors examined in this study, none are involved in the restriction of Tat-mediated transactivation in rodent cells. Interestingly, the levels of inhibition by these factors were not significantly altered by Tat in A9 cells, whereas Tat acts as a strong derepressor of the inhibition by RB and p53 in HeLa cells.

It has been reported that BTEB, like Sp1, activated the HIV-1 LTR both in the presence and absence of Tat when tested in various T cell and macrophage/monocyte cell lines (19). Interestingly, the results were different in our experiments (Fig. 3). The differences in results obtained from two laboratories could be due to differential effects of BTEB on the two promoters of the Tat expression vectors used, SV40E-driven Tat (pSVTat) vs. CMVIE-driven Tat (pCMV7fd/Tat). BTEB is a basic transcriptional factor so that it could respond to the two promoters differentially. The difference in cell lines used also can not be excluded.

However, since lack of an upstream binding factor(s) may be compensated by the altered secondary structure of pHIVTRCAT in rodent cells, we examined the effect of a upstream binding factor(s) on Tat-mediated transactivation indirectly by altering the distance between Sp1 and TATA box.

Although none of the insertion mutations between the Sp1 binding site and the TATA box was able to overcome the restriction to Tat action in rodent cells, it is very interesting that the insertion mutations revealed a strict requirement for preservation of the phase of the DNA helix to which factors bind in human cells. Thus, insertions of 4, 15, and 25 bp abolished Tat-mediated transactivation in human cells, without significantly affecting basal transcription. This observation suggests two important aspects in correct spacing between Sp1- and TATA-associated factors for optimal Tat-induced expression. First, distance appears to be critical; under conditions in which basal promoter activity was unperturbed, an increase in the Sp1-TATA distance decreased Tat-induced expression as has been observed by others (16). Second, the correct three-dimensional arrangement among Tat, Sp1, and TATA-binding factors appears to be required in Tat-induced expression. The transcriptional components, such as Sp1 and TATA-binding factors must be in the same phase for optimal Tat-induced expression, whereas the components do not need to be in phase for basal expression. Considering that two different types of transcription complexes exist on the HIV-1 LTR (one is less processive which governs Tat-induced expression and the other is more processive which governs basal expression (23, 24), the results in this experiment suggest that a spatially-correct interaction between Sp1- and TATA-associated factors is required for the transcriptional complex which governs Tat-induced expression, but not for the transcriptional complex which governs basal expression.

In this study, it was observed that the human-specific Tat-cofactor recognizes the secondary structure of the stem region of TAR RNA and aids Tat to efficiently associate with TAR RNA in human cells. Recently, we found evidence that the restriction to Tat-mediated transactivation in rodent cells might be associated with a factor binding to the lower TAR stem binding protein termed SBP (unpublished data). SBP binding ability was correlated with the level of transactivation in rodent cells. Although both human and rodent cells have SBP activity, it is likely that rodent cells express a different form of SBP from that found in human cells. This difference may contribute to the differential gene expression from the HIV-1 LTR between human and rodent cells.

Since the discovery of HIV-1 in 1983, significant progress has been made toward the discovery and development of anti-HIV drugs. In vitro screens against specific viral targets have resulted in the development of candidate drugs acting at several stages of the viral life cycle (25-27). Despite these advances, none of the existing therapies have been successful in fully blocking clinical progression. In addition, the emergence of resistant viruses occurs rapidly during chemotherapy (28,29). In the face of such obstacles, deeper insights into the regulation of viral gene expression and pathogenicity of HIV-1 are needed. In this work we hoped that the analysis of the

species-specific restrictions of HIV-1 gene expression in rodent cells may provide new targets for antiviral therapy.

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