Functional Comparison of the Basic Domains of the Tat Proteins of Human Immunodeficiency Virus Types 1 and 2 in *trans* Activation

B. ELANGO VAN, T. SUBRAMANIAN, AND G. CHINNADURAI*

*Institute for Molecular Virology, St. Louis University School of Medicine,
3681 Park Avenue, St. Louis, Missouri 63110*

Received 28 October 1991/Accepted 2 January 1992

The *trans*-activator Tat proteins coded by human immunodeficiency virus type 1 (HIV-1) and HIV-2 appear to be similar in structure and function. However, the Tat protein of HIV-2 (Tat2) activates the HIV-1 long terminal repeat (LTR) less efficiently than Tat1 (M. Emerman, M. Guyader, L. Montagnier, D. Baltimore, and M. A. Muesing, EMBO J. 6:3755–3760, 1987). To determine the functional domain of Tat2 which contributes to this incomplete reciprocity, we have carried out domain substitution between Tat1 and Tat2 by exchanging the basic domains involved in Tat interaction with its target *trans*-activation-response (TAR) RNA structure. Our results indicate that Tat1 proteins containing substitutions of either 8 or 14 amino acids of the basic domain of Tat2 exhibited reduced *trans* activation of the HIV-1 LTR by about 1/20 or one-fourth the level induced by *wt* Tat1. In contrast, Tat2 containing a substitution of the 9-amino-acid basic domain of Tat1 *trans* activated HIV-1 LTR like native Tat1. A substitution of the highly conserved core domain of Tat2 with that of Tat1 did not have any significant effect on *trans* activation of the HIV-1 LTR. These results indicate that the basic domain of Tat2 contributes to its inefficient *trans* activation of the HIV-1 LTR. Mutation of an acidic residue (Glu) located between the core domain and the Arg-rich basic domain of Tat2 at position 77 to a Gly residue increased the activity of Tat2 substantially. These results further suggest that the presence of an acidic residue (Glu) adjacent to Arg-rich sequences may at least partially contribute to the reduced activity of the Tat2 basic domain.

The human immunodeficiency viruses (HIVs) have been etiologically linked with AIDS. These pathogenic human retroviruses have been grouped under two types. HIV type 1 (HIV-1) is the predominant type isolated from most patients with AIDS. HIV-2, which was isolated from patients with AIDS in western Africa (9, 20), is less prevalent. Further, HIV-2 also appears to be less virulent than HIV-1. The primary sequences of HIV-1 and HIV-2 vary substantially (25). HIV-2 is more closely related to simian immunodeficiency viruses (SIVs) than to various strains of HIV-1. In spite of the variations in the primary sequences of their genomes, HIV-1 and HIV-2 are similar in genetic organization. Particularly, the structure and function of various regulatory genes are conserved among the various primate immunodeficiency viruses (HIV-1, HIV-2, and SIV). For example, both HIV-1 and HIV-2 code for a strong *trans* activator, Tat, which activates the expression of genes linked to the viral long terminal repeat (LTR). Although the precise mechanism of Tat-mediated *trans* activation still remains unclear, a number of recent studies with the Tat protein of HIV-1 (referred to here as Tat1) indicate that Tat may play an important role at the level of transcriptional initiation and elongation (reviewed in reference 11). Unlike most of the eukaryotic transcriptional activators, Tat functions by direct interaction with an RNA target termed TAR (4, 17, 19, 28). In addition, Tat may also play a role in translational utilization of viral mRNA under certain conditions (5). Although the biochemical properties of the Tat protein of HIV-2 (referred to here as Tat2) have not been extensively studied, it appears that Tat2 may function in a manner similar to that of Tat1. Both Tat1 and Tat2 have an obligatory requirement for TAR in most of the cell types (1,

3, 15, 19, 28) for *trans* activation of the LTR. In addition, both Tat1 and Tat2 contain protein domains that are conserved (25) (see Fig. 1). Further, both Tat1 and Tat2 exhibit reciprocal *trans*-activation effects on the HIV-1 and HIV-2 LTRs.

In spite of the structural and functional relatedness of Tat1 and Tat2, it has been reported that Tat1 and Tat2 efficiently *trans* activate the HIV-2 LTR, while Tat2 is less efficient than Tat1 in activation of the HIV-1 LTR (15). These results suggest that Tat1 and Tat2 may have some functional differences with regard to their action on the HIV-1 LTR. On the basis of secondary structure analysis and mutational studies, it has been suggested that the TAR structure of HIV-2 (TAR2) contains two TAR elements (1, 3, 18, 30). These studies imply that Tat2 may function more efficiently on a duplicated stem-loop structure, as in TAR2, than on a single stem-loop, as in the TAR of HIV-1 (TAR1). In support of this view, it has been reported that the addition of a second stem-loop to TAR1 increased the responsiveness to Tat2 (3). However, it is not known whether any specific protein region of Tat2 is responsible for the differential activity. A number of recent studies have revealed that an arginine-rich basic domain of Tat1 specifically binds to the pyrimidine bulge region of the TAR1 structure (10, 29, 34). These binding studies have further established a direct correlation between Tat-binding to TAR and *trans* activation (13, 29). Since Tat appears to function by direct interaction with TAR RNA, it is possible that the basic domain of Tat2 may be inefficient in recognizing TAR1. Here we report the construction of chimeric Tat1 and Tat2 substitution mutants expressing the basic domains of Tat2 and Tat1, respectively, and show that the basic domain of Tat2 contributes to the inefficient *trans* activation of the HIV-1 LTR.

* Corresponding author.

MATERIALS AND METHODS

Plasmids and mutants. Plasmid pLTR-1 CAT, which expresses the bacterial chloramphenicol acetyltransferase (CAT) gene under control of the HIV-1 (SF2) LTR, has been described previously (27). Plasmid pTat1 expressing the *tat* gene of HIV-1 (SF2) under transcriptional control of the cytomegalovirus immediate-early (CMV-IE) promoter in the vector pBC12 (24) was constructed by transferring the Tat-coding sequences from a previously described plasmid, pTat (27). Plasmid pTat2 was constructed by cloning the Tat2-coding genomic sequences (5783 to 8569) from pROD214 (15) into a pBC12-based vector.

Mutants Tat1/2BD-A and Tat1/2BD-B were constructed by cloning the corresponding double-stranded oligonucleotides coding for the Tat2 basic domain sequences between the unique *Sac*II and *Sac*I sites of Tat57-A (22) and subsequently inserting the chimeric gene into the CMV-IE (BC12) expression vector. Mutant Tat2(99) was constructed by deleting the second exon region (sequences located between three *Pvu*II sites) of Tat2. Mutations Tat2(99)/1BD, Tat2(99)₇₇, and Tat2(99)/1CD were introduced by a triple-primer polynucleotide chain reaction method (22). The DNA fragments obtained from the polynucleotide chain reaction were digested with *Sac*I and were cloned between a *Sac*I and a unique *Pvu*II site in plasmid pTat2(99). Tat2(99)/1BD contains a substitution of the basic domain of Tat1 (residues 48 to 57) for the corresponding Tat2 domain, located between residues 76 and 91. Mutant Tat2(99)₇₇ contains a substitution of a glycine residue for a glutamic acid residue at position 77 of Tat2. Tat2(99)/1CD contains a substitution of the core domain of Tat1 (residues 39 to 46) for the corresponding Tat2 domain, located between residues 67 and 76.

CAT assays. The functional activity of the various chimeric *tat* genes was tested by cotransfection of the pLTR-1 CAT plasmid and the various Tat-expressing plasmids in HeLa or COS-7 cells. Cells (1.5×10^6 to 2×10^6 /60-mm² dish) were transfected with 1 μ g of pLTR-1 CAT and various concentrations of the Tat-expressing plasmids by the calcium phosphate method. All transfections included 1 μ g of pRSV β -Gal, which expresses the *Escherichia coli lacZ* gene under the transcriptional control of the Rous sarcoma virus LTR as an internal control for monitoring transfection efficiency. CAT activities were quantified by scraping the acetylated chloramphenicol spots and counting the radioactivity by scintillation counting. CAT activities are expressed relative to the basal level of LTR-CAT expression.

Immunoprecipitation and indirect immunofluorescence. COS-7 cells (1.5×10^6 to 2.0×10^6 /60-mm² dish) were transfected with 1 to 2 μ g of various Tat-expressing plasmids. A total of 48 h after transfection, cells were labelled with 200 μ Ci of [³⁵S]cysteine for 1 h. Immunoprecipitation of Tat1 proteins was carried out with polyclonal rat antibodies directed against Tat1 (2) or with rabbit anti-peptide antibody raised against a synthetic peptide corresponding to N-terminal 17 amino acids (26). Immunoprecipitation of Tat2 proteins was carried out with rabbit polyclonal antibodies raised against a synthetic peptide corresponding to amino acids 76 to 99 of Tat2 (18). Indirect immunofluorescence analyses of Tat1 and Tat2 proteins in COS-7 cells transfected with various plasmids were carried out essentially as described previously (33). The Tat antibodies were used at a dilution of 1:200, while the second antibody (fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G) was used at a dilution of 1:50.

FIG. 1. (A) Organization of Tat1 and Tat1/Tat2 chimeric proteins. Domain map of Tat1 (strain SF2) is based on previous mutational analysis (23). Tat1/2BD-A and Tat1/2BD-B contain substitutions of the indicated residues of the basic domain from Tat2 (ROD214). Mutants *tat1*/2BD-A and *tat1*/2BD-B were constructed by cloning the corresponding double-stranded oligonucleotides between the unique *Sac*II and *Sac*I sites of mutant *tat*57-A as described previously (32). (B) *trans* activation of HIV-1 LTR by Tat1/2BD mutants. HeLa cells were transfected with pLTR-1 CAT alone or along with various Tat-expressing plasmids. CAT activities were determined 48 h after transfection as described in Materials and Methods and are expressed relative to the basal level of LTR-CAT expression.

RESULTS

Effect of Tat2 basic domain on *trans* activation by Tat1. To determine whether the basic domain of Tat2 contributes to the lower level of *trans* activation of the HIV-1 LTR, we constructed two different substitution mutants (Fig. 1A) of the *tat1* gene by substituting the Tat1 basic domain (referred to here as 1BD) with the basic domain of Tat2 (2BD). Mutant *tat1*/2BD-A contains a substitution of an 8-amino-acid region (ERKGRRRR) of Tat2 in place of the 1BD (RKRRRQRRR). Mutant *tat1*/2BD-B expresses an additional 6-amino-acid region of the 2BD (ERKGRRRRTPKKTK). The ability of these mutants to *trans* activate the expression of the reporter bacterial CAT gene linked to HIV-1 LTR (pLTR-1 CAT) was determined by DNA transfection on HeLa cells (Fig. 1B). As expected from previous studies (3, 15, 18), Tat2 (wild type [*wt*]) *trans* activated pLTR-1 CAT expression at about one-half the efficiency of Tat1. In contrast, mutant *tat1*/2BD-A, which expresses the arginine-rich half of the 2BD, *trans* activated pLTR-1 CAT poorly (6% of the Tat1-induced

FIG. 2. Immunoprecipitation of Tat1 and Tat2 proteins. COS-7 cells ($1.5 \times 10^5/60\text{-mm}^2$ dish) were transfected with 1 to 2 μg of various Tat-expressing plasmids. A total of 48 h after transfection, cells were labelled for 1 h with 200 μCi of [^{35}S]cysteine. Immunoprecipitations of Tat1 proteins were carried out with polyclonal rat antibodies directed against Tat1 (2) or with rabbit antipeptide antiserum raised against a synthetic peptide corresponding to the N-terminal 17 amino acids (26). Immunoprecipitations of Tat2 proteins were carried out with rabbit polyclonal antiserum raised against a synthetic peptide corresponding to amino acids 76 to 99 of Tat2 (18). (A) Tat1 and Tat1/2BD; (B) Tat2 and Tat2/1BD. M indicates protein molecular weight markers. In panel B, Tat2(99)/1BD was precipitated with a mixture of Tat1 and Tat2 antibodies. The bracketed regions contain Tat-specific bands. It appears that both antibody preparations do not have significant reactivity against epitopes of the Tat2/1BD chimeric protein.

level). Inclusion of the Lys-rich half of the 2BD (*tat1/2BD-B*) further increased the level of *trans* activation to about 25% of the level observed with Tat1. Similar relative effects on *trans* activation were also observed in COS-7 cells (results not shown). These low levels of *trans* activation by the chimeric *tat* genes do not appear to be due to any defect in the level of accumulation of the chimeric proteins, since COS-7 cells transfected with mutants *tat1/2BD-A* and *tat1/2BD-B* expressed Tat protein at levels comparable to Tat1 (Fig. 2A). Further, the low level of *trans* activation appears to be primarily contributed to by the Tat2 basic domain, since we have previously shown that a heterologous basic domain from HIV-1 Rev can efficiently substitute for the Tat1 basic domain when inserted at the same site within the Tat1 coding region (33).

Effect of Tat1 basic domain on *trans* activation by Tat2. To further confirm the partial functional reciprocity of the Tat2 basic domain, we constructed a substitution mutant of Tat2 containing a substitution of the 1BD (GRKKRRQRRR) (Fig. 3A) and determined its activity on LTR-1 CAT expression. The sequences coding for the 1BD were inserted in frame into the Tat2-coding sequences [exon 1; Tat2(99)] by polynucleotide chain reaction (22). For this chimeric gene construction, we used a *tat2* gene expressing only the first exon [*tat2*(99)] as the backbone. The *trans*-activation potential of Tat2(99)/1BD was compared with those of the parental Tat2(99), Tat2 (*wt*), and Tat1 (Fig. 3B). The parental Tat2(99) activated LTR-1 CAT at about one-fourth the efficiency of Tat1 and about one-half the efficiency of Tat2 (*wt*). However, mutant *tat2*(99)/1BD *trans* activated LTR-1 CAT to a level similar to that by *wt* Tat1, suggesting that the basic domain is the primary determinant for the differential activity of Tat2 on HIV-1 LTR. Since this Tat2 substitution mutant exhibits near *wt* Tat1 activity, all the protein regions of Tat2 other than the basic domain may be functionally equivalent to the corresponding Tat1 regions.

To ascertain that the enhanced *trans*-activation potential observed with mutant Tat2(99)/1BD is indeed contributed to

FIG. 3. (A) Organization of Tat2 and Tat2/Tat1 chimeric proteins. Domain map of Tat2 is based on sequence similarity to Tat1, shown in Fig. 1A. Mutant *tat2*(99) was constructed by deleting the second exon region (sequences located between three *PvuII* sites) of Tat2. Mutant *tat2*(99)/1BD contains a substitution of the basic domain of Tat1 (residues 48 to 57) for the corresponding Tat2 domain, located between residues 76 and 91. All Tat1 and Tat2 sequences are expressed from a CMV-IE-based expression vector, pBC12 (24). (B) *trans* activation (CAT assay) of HIV-1 LTR by Tat1/2BD mutants. HeLa cells were transfected with 1 μg of pLTR-1 CAT (27) alone or along with 0.1 μg of various Tat-expressing plasmids. CAT activities are as described in the legend to Fig. 1.

by the 1BD sequences, we also constructed a different substitution mutant [Tat2(99)/1CD] in which the core domain (domain C) of Tat1 was substituted for the corresponding Tat2 domain (Fig. 4A). Domain C is not only conserved among the Tat proteins encoded by the various primate immunodeficiency viruses, but it is also conserved in the Tat protein encoded by a more distantly related equine infectious anemia virus (8, 14). Comparison of the *trans*-activation potential of mutant Tat2(99)/1CD with that of the parental Tat2(99) indicates that the core domain of Tat1 does not enhance the activity of Tat2 (Fig. 4B).

The above results strongly suggest that the differential activities of Tat1 and Tat2 are primarily contributed to by the basic domains of the respective Tat proteins. However, comparison of the sequences of the 1BD and the 2BD indicate that these domains share a substantial homology (Fig. 5, boxed areas). Further, the 1BD and the 2BD have a similar net charge density (+8). However, an acidic residue

FIG. 4. (A) Organization of Tat2(99) and mutants; (B) *trans* activation (CAT assay) of HIV-1 LTR by Tat2(99)/1CD or Tat2(99)₇₇. CAT activities are as described in the legend to Fig. 1.

(Glu) is present at the junction of the core Tat2 domain (domain C in Fig. 4A) and the basic domain (domain D in Fig. 4A). To determine whether this acidic residue has an inhibitory role in the activity of the 2BD, we constructed a mutant, Tat2(99)₇₇, in which this Glu residue (position 77) was converted to a Gly residue. A Gly residue is also present at the corresponding position in Tat1. The assay for the *trans*-activation potential of this mutant (Fig. 4B) indicates that it *trans* activated HIV-1 LTR CAT expression to a level more or less similar to that of Tat1. This result further strengthens the data presented above, which show that domain D of Tat2 contributes at least partially to the relatively inefficient *trans* activation of the HIV-1 LTR by Tat2.

Effect of 1BD and 2BD on subcellular localization of Tat. A number of previous studies (21, 31, 33) have shown that the basic domain is required for efficient nuclear-nucleolar local-

FIG. 5. Comparison of the basic domains of Tat1 and Tat2.

ization of Tat1 protein. To determine whether the basic domain of Tat1 or Tat2 has any differential effect on subcellular localization of Tat proteins, COS-7 cells were transfected with the various Tat-expressing plasmids described above [Tat1, Tat2, Tat2(99), Tat(99)/1BD, Tat(99)₇₇, Tat(99)/1CD] and expression of the various Tat proteins was determined by indirect immunofluorescence with antibodies specific for Tat1 or Tat2 (Fig. 6). These studies indicate that both the *wt* Tat1 and the *wt* Tat2 as well as the various mutant Tat proteins efficiently localized in the nuclear and nucleolar regions. No apparent differences among the subcellular localization phenotypes of the various mutant proteins were observed.

DISCUSSION

Our results demonstrate that the differential activities of Tat1 and Tat2 on the HIV-1 LTR are primarily contributed to by the differences in the individual basic domains. This conclusion is based on our results obtained with the Tat2 substitution mutants expressing the 1BD as well as with Tat1 substitution mutants expressing the 2BD. In these studies, the increase or decrease in *trans* activation of the HIV-1 LTR by various mutants clearly reflected the basic domain contained. In contrast to the results obtained with the BD mutants, swapping of the conserved core domain (domain C) between Tat1 and Tat2 did not alter the *trans*-activation potential significantly. However, we cannot fully rule out any potential effects of other regions, such as the Cys-rich domain, in the differential *trans*-activation property, since we have not been able to construct a functional Tat1/Tat2 chimera by exchanging the Cys-rich domain. Our conclusion that the 2BD contributes to inefficient *trans* activation of the HIV-1 LTR by Tat2 is strongly supported by the single-amino-acid substitution mutant Tat(99)₇₇, which exhibits enhanced activity compared with Tat(99). It appears that the differential effects observed with various mutants may not be attributed to other characteristics of mutant proteins, such as subcellular accumulation and transport to the nuclear-nucleolar locations.

Since it appears that the basic domain specifically binds to TAR, it is possible that the 1BD and the 2BD may have varied affinity for the TAR1 structure, thus contributing to the partial reciprocal activity. By functional substitution with a number of heterologous basic domains, we have postulated that an Arg-rich motif, R/KXXRRXRR, which is also conserved in all HIV-1 isolates, is required for efficient *trans* activation of the HIV-1 LTR (32). The left half of the 2BD has a substantial resemblance to the 1BD (Fig. 5; boxed areas). However, substitution of the Arg-rich half of the 2BD for the 1BD substantially reduces the *trans*-activation potential of Tat1 (6% of that of *wt* Tat1). This level could be further increased by inclusion of the Lys-rich half of the 2BD. This observation suggests that the overall charge density may play a role in efficient *trans* activation. The importance of the overall charge density, in addition to the specific sequence requirements for efficient recognition of TAR by the Tat BD, has also been suggested by other studies (6, 7, 12, 16). Examination of 2BD sequences indicates that the 2BD consists of an Arg-rich half (indicated by the boxed areas in Fig. 5) and a Lys-rich half (indicated by a double underline in Fig. 5). It would be interesting to determine whether the 2BD is a bipartite BD evolved for interaction with a more complex TAR2 structure. It should be noted that the Tat2 mutant [Tat2(99)] lacking the second exon *trans* activates the HIV-2 LTR at about one-half the

FIG. 6. Subcellular localization of Tat1, Tat2, and various mutant Tat proteins. COS-7 cells were transfected with various Tat-expressing plasmids and analyzed by indirect immunofluorescence. Panels: a, Tat1; b, Tat2; c, Tat2(99); d, Tat(99)1BD; e, Tat(99)₇₇; f, Tat(99)1CD.

level of *wt* Tat2 (Fig. 3B). It remains to be determined whether the protein region encoded by exon 2 has any role in Tat2-TAR2 interaction. Since exon 2 clearly is not required for *trans* activation of LTR-1, while it is required for efficient *trans* activation of LTR-2, it may be possible that the exon 2-encoded protein region cooperates with the basic domain or confers a favorable structural feature for efficient recognition of TAR2.

ACKNOWLEDGMENTS

This work was supported by research grants AI-29541 and AI-29200.

We thank J. Nelson, K.-T. Jeang, and the AIDS Research and Reference Program for Tat1 and Tat2 antibodies, M. Emerman for pROD214, and B. R. Cullen for pBC12. We also thank L. K. Venkatesh for advice and discussions.

REFERENCES

1. Arya, S. K., and R. C. Gallo. 1988. Human immunodeficiency virus type 2 long terminal repeat: analysis of regulatory elements. *Proc. Natl. Acad. Sci. USA* **85**:9753-9757.
2. Berkhout, B., A. Gatignol, A. B. Robson, and K.-T. Jeang. 1990. TAR-independent activation of the HIV-1 LTR: evidence that Tat requires specific regions of the promoter. *Cell* **62**:757-767.
3. Berkhout, B., A. Gatignol, J. Silver, and K.-T. Jeang. 1990. Efficient *trans*-activation by the HIV-2 Tat protein requires a duplicated TAR RNA structure. *Nucleic Acids Res.* **18**:1839-1846.
4. Berkhout, B., R. Silverman, and K.-T. Jeang. 1989. TAT *trans*-activates the human immunodeficiency virus through a nascent RNA target. *Cell* **59**:273-282.
5. Braddock, M., A. Chambers, W. Wilson, M. P. Esnouf, S. E. Adams, A. J. Kingsman, and S. M. Kingsman. 1989. HIV-1 Tat "activates" presynthesized RNA in the nucleus. *Cell* **58**:269-279.
6. Calnan, B. J., S. Biancalana, D. Hudson, and A. D. Frankel. 1991. Analysis of arginine-rich peptides from the HIV tat protein reveals unusual features of RNA-protein recognition. *Genes Dev.* **5**:201-210.
7. Calnan, B. J., B. Tidor, S. Biancalana, D. Hudson, and A. D. Frankel. 1991. Arginine-mediated RNA recognition: the arginine fork. *Science* **252**:1167-1171.
8. Carroll, R., L. Martarano, and D. Derse. 1991. Identification of lentivirus Tat functional domains through generation of equine infectious anemia virus/human immunodeficiency virus type 1 *tat* gene chimeras. *J. Virol.* **65**:3460-3467.
9. Clavel, F., M. Guyader, D. Guetard, M. Salle, L. Montagnier, and M. Alizon. 1986. Molecular cloning and polymorphism of the human immune deficiency virus type 2. *Nature (London)* **324**:691-695.
10. Cordingley, M. G., R. L. LaFemina, P. L. Callahan, J. H. Condra, V. V. Sardana, D. J. Graham, T. M. Nguyen, K. LeGrow, L. Gotlib, A. J. Schlabach, and R. J. Colonno. 1990. Sequence-specific interaction of Tat protein and Tat peptides with the transactivation-responsive sequence element of human immunodeficiency virus type 1 *in vitro*. *Proc. Natl. Acad. Sci. USA* **87**:8985-8989.
11. Cullen, B. R. 1990. The HIV-1 tat protein: an RNA sequence-specific processivity factor? *Cell* **63**:655-657.
12. Delling, U., S. Roy, M. Sumner-Smith, R. Barnett, L. Reid, C. A. Rosen, and N. Sonenberg. 1991. The number of positively charged amino acids in the basic domain of tat is critical for *trans*-activation and complex formation with TAR RNA. *Proc. Natl. Acad. Sci. USA* **88**:6234-6238.
13. Dingwall, C., I. Ernberg, M. J. Gait, S. M. Green, S. Heaphy, J. Karn, A. D. Lowe, M. Singh, and M. A. Skinner. 1990. HIV-1 *tat* protein stimulates transcription by binding to U-rich bulge in the stem of the TAR RNA structure. *EMBO J.* **9**:4145-4153.
14. Dorn, P., L. DaSilva, L. Martarano, and D. Derse. 1990. Equine infectious anemia virus *tat*: insights into the structure, function, and evolution of lentivirus *trans*-activator proteins. *J. Virol.* **64**:1616-1624.
15. Emerman, M., M. Guyader, L. Montagnier, D. Baltimore, and M. A. Muesing. 1987. The specificity of the human immunodeficiency virus type 2 transactivator is different from that of human immunodeficiency virus type 1. *EMBO J.* **6**:3755-3760.
16. Endo, S.-I., S. Kubota, H. Siomi, A. Adachi, S. Oroszlan, M. Maki, and M. Hatanaka. 1989. A region of basic amino-acid cluster in HIV-1 tat protein is essential for *trans*-acting activity and nucleolar localization. *Virus Genes* **3**:99-110.
17. Feng, S., and E. C. Holland. 1988. HIV-1 *tat trans*-activation requires the loop sequence within *tar*. *Nature (London)* **334**:165-167.
18. Fenrick, R., M. H. Malim, J. Hauber, S.-Y. Le, J. Maizel, and B. R. Cullen. 1989. Functional analysis of the Tat *trans* activator of human immunodeficiency virus type 2. *J. Virol.* **63**:5006-5012.
19. Garcia, J. A., D. Harrich, E. Soultanakis, F. Wu, R. Mitsuyasu,

- and R. B. Gaynor. 1989. Human immunodeficiency virus type 1: LTR TATA and TAR region sequences required for transcriptional regulation. *EMBO J.* **8**:765–778.
20. Guyader, M., M. Emerman, P. Sonigo, F. Clavel, L. Montagnier, and M. Alizon. 1987. Genome organization and transactivation of the human immunodeficiency virus type 2. *Nature (London)* **326**:662–669.
 21. Hauber, J., M. H. Malim, and B. R. Cullen. 1989. Mutational analysis of the conserved basic domain of human immunodeficiency virus *tat* protein. *J. Virol.* **63**:1181–1187.
 22. Kammann, M., J. Laufs, J. Schell, and B. Gronenborn. 1989. Rapid insertional mutagenesis of DNA by polymerase chain reaction (PCR). *Nucleic Acids Res.* **17**:5404.
 23. Kuppuswamy, M., T. Subramanian, A. Srinivasan, and G. Chinnadurai. 1989. Multiple functional domains of Tat, the *trans*-activator of HIV-1, defined by mutational analysis. *Nucleic Acids Res.* **17**:3551–3561.
 24. Malim, M. H., J. Hauber, R. Fenrick, and B. R. Cullen. 1988. Immunodeficiency virus *rev* *trans*-activator modulates the expression of the viral regulatory genes. *Nature (London)* **335**:181–183.
 25. Meyers, G., S. F. Josephs, J. A. Berzofsky, A. B. Rabson, T. F. Smith, and F. Wong-Staal. 1990. Human retroviruses and AIDS. Los Alamos National Laboratory, Los Alamos, N.Mex.
 26. Pearson, L., J. Garcia, F. Wu, N. Modesti, J. Nelson, and R. Gaynor. 1990. A transdominant *tat* mutant that inhibits *tat*-induced gene expression from the human immunodeficiency virus long terminal repeat. *Proc. Natl. Acad. Sci. USA* **87**:5079–5083.
 27. Peterlin, B. M., P. A. Luciw, P. J. Barr, and M. D. Walker. 1986. Elevated levels of mRNA can account for the *trans*-activation of human immunodeficiency virus (HIV). *Proc. Natl. Acad. Sci. USA* **83**:9734–9738.
 28. Rosen, C. A., J. G. Sodroski, and W. A. Haseltine. 1985. The location of cis-acting regulatory sequences in the human T cell lymphotropic virus type III (HTLV-III/LAV) long terminal repeat. *Cell* **41**:813–823.
 29. Roy, S., U. Delling, C.-H. Chen, C. A. Rosen, and N. Sonenberg. 1990. A bulge structure in HIV-1 TAR RNA is required for Tat binding and Tat-mediated *trans*-activation. *Genes Dev.* **4**:1365–1373.
 30. Selby, M. J., E. S. Bain, P. A. Luciw, and B. M. Peterlin. 1989. Structure, sequence, and position of the stem-loop in *tar* determine transcriptional elongation by *tat* through the HIV-1 long terminal repeat. *Genes Dev.* **3**:547–558.
 31. Siomi, H., H. Shida, M. Maki, and M. Hatanaka. 1990. Effects of a highly basic region of human immunodeficiency virus *tat* protein on nucleolar localization. *J. Virol.* **64**:1803–1807.
 32. Subramanian, T., R. Govindarajan, and G. Chinnadurai. 1991. Heterologous basic domain substitutions in the HIV-1 Tat protein reveal an arginine-rich motif required for *trans*-activation. *EMBO J.* **10**:2311–2318.
 33. Subramanian, T., M. Kuppuswamy, L. Venkatesh, A. Srinivasan, and G. Chinnadurai. 1990. Functional substitution of the basic domain of the HIV-1 *trans*-activator, Tat, with the basic domain of the functionally heterologous Rev. *Virology* **176**:178–183.
 34. Weeks, K. M., C. Ampe, S. C. Schultz, T. A. Steitz, and D. M. Crothers. 1990. Fragments of the HIV-1 Tat protein specifically bind TAR RNA. *Science* **249**:1281–1285.