JOURNAL OF VIROLOGY, June 1993, p. 3441–3445
0022-538X/93/063441-05$02.00/0

Vol. 67, No. 6

Juxtaposition between Activation and Basic Domains of Human Immunodeficiency Virus Type 1 Tat Is Required for Optimal Interactions between Tat and TAR

YING LUO AND B. MATIJA PETERLIN*

Departments of Medicine, Microbiology, and Immunology, Howard Hughes Medical Institute, University of California, San Francisco, California 94143

Received 21 January 1993/Accepted 17 March 1993

trans **activation of the human immunodeficiency virus type 1 long terminal repeat requires that the viral *trans* activator Tat interact with the *trans*-acting responsive region (TAR) RNA. Although the N-terminal 47 amino acids represent an independent activation domain that functions via heterologous nucleic acid-binding proteins, sequences of Tat that are required for interactions between Tat and TAR in cells have not been defined. Although in vitro binding studies suggested that the nine basic amino acids from positions 48 to 57 in Tat bind efficiently to the 5′ bulge in the TAR RNA stem-loop, by creating several mutants of Tat and new hybrid proteins between Tat and the coat protein of bacteriophage R17, we determined that this arginine-rich domain is not sufficient for interactions between Tat and TAR in vivo. Rather, the activation domain is also required and must be juxtaposed to the basic domain. Thus, in vitro TAR RNA binding does not translate to function in vivo, which suggests that other proteins are important for specific and productive interactions between Tat and TAR.**

The human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) is *trans* activated by interactions between the virus-encoded *trans* activator Tat and its cognate TAR RNA stem-loop, which is located at the 5′ end of all viral transcripts. This interaction is essential for high levels of viral gene expression, replication, and cellular cytopathology (15). By comparing sequences between Tats of different lentiviruses, Tat of HIV-1 was divided into five structural domains, which are called N-terminal (proline-rich), cysteine-rich, core, basic (arginine-rich), and C-terminal domains (4). Whereas core and basic sequences are most conserved between Tats, greater differences are observed in their N- and C-terminal extensions (7). Since the N-terminal 47 amino acids, which contain N-terminal, cysteine-rich, and core domains, when fused to heterologous RNA- and DNA-binding proteins increase expression from hybrid HIV-1 LTRs with appropriate target sequences, they represent the activation domain of Tat (20, 22). However, domains of Tat that are required for interactions with TAR RNA in vivo have not been defined.

In vitro studies demonstrated that a nonapeptide, which contains only the arginine-rich amino acids from positions 48 to 57 in Tat, binds to the 5′ bulge in TAR RNA stem-loop (3, 8, 19). Surprisingly, binding coefficients of these short peptides were similar to those of the full-length protein (26). This basic domain is also absolutely required for *trans* activation in vivo (2). However, the precise sequence of arginines is unimportant, since only one arginine in the context of eight basic amino acids supported *trans* activation (2). Furthermore, basic sequences from Rev and bacteriophage λ N protein could functionally replace the basic domain of Tat (23). Although these results indicate that the arginine-rich domain is important for RNA binding, they also suggest that the basic domain by itself is not sufficient for specific interactions between Tat and TAR in vivo. Otherwise, any nuclear protein with one arginine in the context of other basic amino acids should be able to bind to TAR and inhibit the function of Tat.

To investigate whether the arginine-rich domain can independently associate with TAR in vivo, a series of mutants designed to separate activation and binding domains of Tat was constructed. Our results indicate that the basic domain of Tat is not sufficient by itself and cannot be separated from the activation domain for productive interactions between Tat and TAR. Thus, the activation domain of Tat plays an important role in physical interactions between Tat and TAR in vivo.

MATERIALS AND METHODS

Plasmid constructions. pHIVSCAT and pSVTAT were described previously (20). pSVTATRI was constructed by inserting four amino acids containing an *Eco*RI site (SFEG; New England Biolabs) into the *Stu*I site of pSVTAT. pSVTATRV was constructed by inserting four amino acids containing an *Eco*RV site (GGYP) upstream of the arginine-rich domain of pSVTAT. pSVTATRVΔ was constructed by deleting sequences from *Eco*RV to *Stu*I sites of pSVTATRV. pSVTATRVΔM was constructed by replacing the *Pst*I-*Mlu*I fragment of pSVTATRVΔ with the *Pst*I-*Xma*I fragment of pSVTATRV. This created an insertion of 11 amino acids (GGSRVSQEKDP) between basic and core domains. pSVTATRVΔA was constructed by replacing the *Pst*I-*Avr*II fragment of pSVATRVΔ with the *Pst*I-*Sma*I fragment of pSVTATRV. This created an insertion of 30 amino acids (GGYPLGLLVTIAIVKSVAFIATRVSQEKDP) between basic and core domains. pSVTAT2XCS was constructed by replacing the *Pst*I-*Stu*I fragment of pSVTAT with the *Pst*I-*Eco*RV fragment of pSVTATRV. This created an insertion of 10 amino acids between core and basic domains, which included GGYP and amino acids from positions 42 to 47 of Tat. pTat2XCM was constructed by replacing the *Pst*I-*Mlu*I fragment of pSVTAT with the *Pst*I-

* Corresponding author.

*Xma*I fragment of pSVTATRV. This created an insertion of 17 amino acids which included GGYPG and amino acids from positions 36 to 47 of Tat. pTat2XCA was constructed by replacing the *Pst*I-*Avr*II fragment of pTat1StuI with the *Pst*I-*Sma*I fragment of pSVTATRV. This created an insertion of 32 amino acids which included GS plus amino acids from positions 19 to 47 of Tat. pSVTATCP was described previously (20). pSVTATRVΔMCP was constructed by ligating the *Pst*I-*Sac*I fragment of pSVTATRVΔM into the *Pst*I-*Sac*I sites of pSVTATCP. In this construction, R17 coat protein was fused to the C-terminus Tat containing an insertion of 11 amino acids. All constructions resulted in proteins that maintained correct reading frames. pHβ/TAR-12 and pHβ/ASTAR-12 were as previously described (12).

Transient expression and CAT enzymatic assays. HeLa cells were transfected with 10 μg of total DNA and 250 μg of DEAE-dextran per ml as described previously (20). Cells were harvested 40 to 48 h after transfection, and chloramphenicol acetyltransferase (CAT) enzymatic assays were performed on cellular lysates. Experiments were repeated at least three times. Protein concentrations were used to normalize CAT data. Standard errors of the mean were less than 25%.

RESULTS

Insertions between activation and basic domains of Tat block *trans* activation of the HIV-1 LTR. Previous reports suggested that Tat interacts with TAR via the binding of its basic or arginine-rich domain to the 5′ bulge in the TAR RNA stem-loop (2, 3, 6, 26). If these basic residues also represent an independent TAR-interactive domain in vivo, they should function when separated from the N-terminal activation domain of Tat. In this scenario, the boundary between functional activation and binding domains should be between structural core and basic domains or somewhere in the core domain of Tat. To test this hypothesis, we constructed several mutants of Tat (Fig. 1). In pSVTATRV and pSVTATRI, four amino acids were inserted between core and basic domains and five amino acids were inserted into the core domain, respectively. Inserted amino acids were chosen at random, and introduced sequences created convenient restriction endonuclease sites (Fig. 1). These two plasmids were then cotransfected with pHIVSCAT, which contains the HIV-1 LTR linked to the CAT reporter gene, into HeLa cells.

Whereas cotransfections of pHIVSCAT with pSVTAT resulted in 272-fold *trans* activation, cotransfections of pHIVSCAT with pTATRV and pTATRI yielded 29% of wild-type levels and no *trans* activation, respectively (Fig. 1). This initial result suggested that the basic domain can interact independently with TAR but that core sequences form part of the activation domain. However, if the arginine-rich domain is indeed independent and separable from the activation domain, then Tat should still function when more amino acids are inserted between core and basic domains. To test this hypothesis, two more mutants of Tat were constructed, pSVTATRVΔM and pSVTATRVΔA, which contained insertions of 11 and 30 amino acids between core and basic domains, respectively (Fig. 2). However, cotransfections of pSVTATRVΔM and pSVTATRVΔA with pHIVSCAT resulted in only 2.1 and 0.01% of wild-type levels of *trans* activation (Fig. 2). Since in these two mutants the N-terminal activation domain was intact (20, 22), this

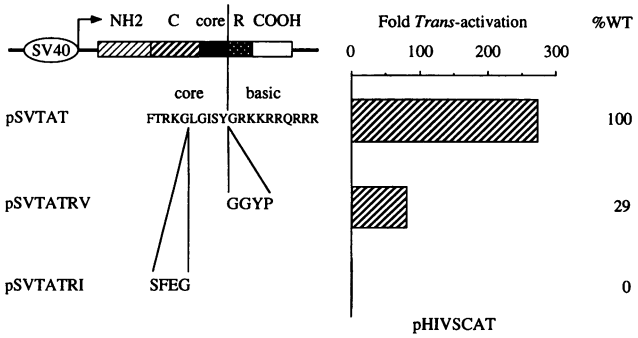

FIG. 1. Insertions of four amino acids between activation and basic and six amino acids into the core domain of Tat. Levels of *trans* activation of the HIV-1 LTR target (pHIVSCAT) are given as fold *trans* activation and percent wild-type (WT) levels at the right; the latter was calculated by dividing values obtained with pSVTA TRV or pSVTATRI and pHIVSCAT by those obtained with pS VTAT and pHIVSCAT. On the left are depicted three Tat effectors, pSVTAT, pSVTATRV, and pSVTATRI. Whereas in pSVTATRV, GGYP residues were inserted between the core and basic domains, in pSVTATRI, SFEG residues were inserted between the leucine and glycine five amino acids (aa) into the core domain. Above these effectors is a diagram of the structural domain of Tat, which consist of N-terminal (NH2), cysteine-rich (C), core, basic or arginine-rich (R), and C-terminal (COOH) amino acids. Core and basic sequences of Tat are given below the bar diagram. All effectors were transcribed from the simian virus 40 (SV40) early promoter. These results are representative of two experiments done in triplicate. Standard error of the mean was less than 25%.

result suggested that separation of basic and activation domains of Tat prevents interactions between Tat and TAR.

However, to exclude the possibility that inserted amino acids in pSVTATRVΔM and pSVTATRVΔA interfered with the function of the activation domain of Tat, three additional mutants of Tat were constructed. Although pSVTAT2XCS, pSVTAT2XCM, and pSVTAT2XCA contained insertions of 10 to 32 amino acids between basic and core domains, these insertions represented duplications from positions 19 to 47 of Tat (Fig. 3). Moreover, in these three mutants of Tat, the

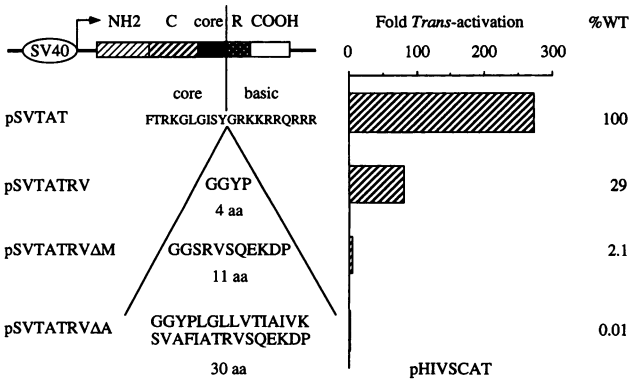

FIG. 2. Insertions of more than four amino acids between activation and basic domains of Tat. Data are presented as in Fig. 1. Below the diagram of structural domains of Tat are depicted four effectors: wild-type Tat (pSVTAT) and three mutant Tats with progressively larger insertions of random amino acids. pSVTATRV, pSVTATRVΔM, and pSVTATRVΔA contain 4, 11, and 30 additional residues between core and basic domains. These results are representative of three experiments done in triplicate.

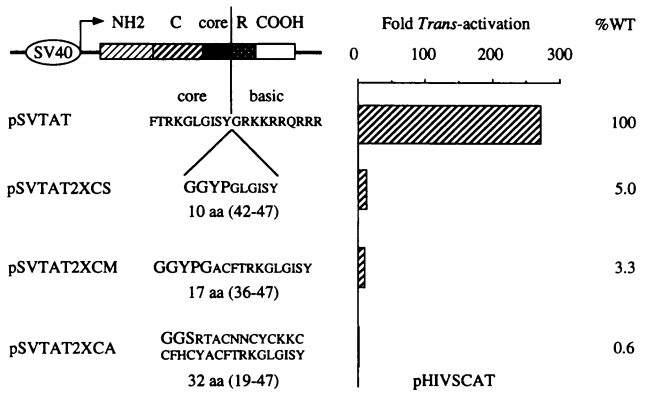

FIG. 3. Insertions of duplicated residues of Tat between its activation and basic domains. Data are presented as in Fig. 1. Below the diagram of structural domains of Tat are depicted four effectors: wild-type Tat (pSVTAT) and three mutant Tats with progressively larger insertions of amino acids from Tat. pSVTAT2XS, pSVTAT2 XCM, and pSVTAT2XCA contain 10 (4 random and 6 from Tat), 17 (5 random and 12 from Tat), and 32 (3 random and 29 from Tat) additional residues between core and basic domains, respectively. While random amino acids are depicted as larger letters, residues from Tat are given in the smaller script. Next to the numbers of additional amino acids are given their positions in Tat. These results are representative of three experiments done in triplicate.

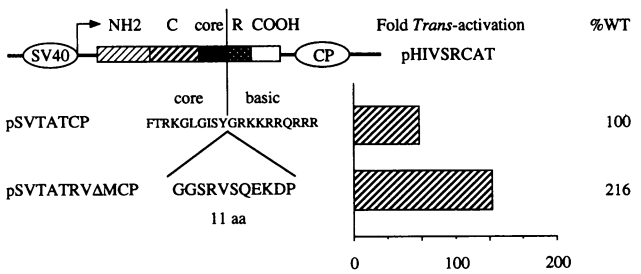

FIG. 4. Insertion of 11 amino acids between activation and basic domains of the hybrid Tat-coat protein. Data are presented as in Fig. 1. Below the diagram of structural domains of Tat fused to the N terminus of the coat protein of bacteriophage R17 at position 67 of Tat are depicted two effectors. pSVTATCP and pSVTATRVΔMCP contain 67 residues of Tat and an insertion of 11 amino acids between core and basic domains of Tat, respectively, fused to the coat protein. The parental plasmid for pSVTATRVΔMCP is pSVTATRVΔM in Fig. 2. These results are representative of three experiments done in triplicate.

activation domain was intact (20, 22). Since increases in CAT activities were less than 5% of wild-type levels, pSVTAT2XCS, pSVTAT2XCM, and pSVTAT2XCA also could not *trans* activate the HIV-1 LTR (Fig. 3). Actually, levels of *trans* activation declined with larger insertions. It should be noted that although core sequences were duplicated in pSVTAT2XCM, and core and cysteine-rich domains were duplicated in pSVTAT2XCA, these sequences surrounding the basic domain were still not sufficient for interactions between Tat and TAR. In other words, the entire N-terminal activation domain must be placed next to the basic domain for interactions between Tat and TAR in vivo.

Insertions between activation and basic domains of Tat do not affect the function of the activation domain. To demonstrate conclusively that the N-terminal activation domain was not influenced by these insertions and that their defects were due solely to their inability to interact with TAR, a mutant Tat with an insertion of 11 amino acids (pSVTAT RVΔM) was also fused to the coat protein of bacteriophage R17 (pTATRVΔMCP; Fig. 4). Since this hybrid protein contained a heterologous RNA-binding domain, effects of insertions in Tat could be evaluated independently of interactions between Tat and TAR. pHIVSRCAT, in which the operator of bacteriophage R17, which is the RNA-binding site of the coat protein, replaced TAR in pHIVSCAT, was used as the substituted target.

Although cotransfections of pHIVSCAT and pSVTATR VΔM resulted in only 2.1% of wild-type levels of *trans* activation, cotransfections of pTATRVΔMCP and pHIVSR-CAT resulted in 132-fold *trans* activation, 2-fold higher than that observed with our previous hybrid Tat-coat protein (pSVTATCP) and this substituted target (pHIVSRCAT; Fig. 4) (20). Moreover, unlike pSVTATCP, pTATRVΔMCP could not *trans* activate the wild-type HIV-1 LTR (pHIVS CAT; data not presented). These results demonstrate that the activation domain not only functions in these insertional mutants of Tat but also functions better when separated from the basic domain. A similar phenotype has been observed previously with a hybrid Tat-coat protein which lacked the basic domain of Tat (20). This experiment also rules out the possibility that the separation of basic and activation domains lowers the stabilities of mRNA or protein of Tat mutants. Thus, the separation of basic and activation domains must be preventing the interactions between Tat and TAR and has no negative effect on the activation domain.

Insertions between activation and basic domains of Tat block interactions between Tat and TAR. To prove directly that these insertions in Tat interfered with interactions between Tat and TAR, we performed competition studies using TAR decoys. It has been reported that high levels of single or multiple TAR RNAs in cells compete for the binding of Tat to nascent TAR during transcription from the HIV-1 LTR (12, 24). Further mapping of sequences in TAR decoys revealed that both the 5′ bulge and the loop of the stem-loop in TAR are required for this competition. Moreover, these TAR decoys are thought to function by sequestering either Tat, cellular TAR RNA-binding proteins, which might be required for interactions between Tat and TAR, or both (12, 24).

If separation between core and basic domains of Tat affected interactions between Tat and TAR, then the ability of pTATRVΔMCP to *trans* activate the HIV-1 LTR via interactions between the coat protein and operator of bacteriophage R17 should not be inhibited by TAR decoys (Fig. 5). To test this hypothesis, we performed experiments in which hybrid effectors and targets were cotransfected with plasmids that directed the synthesis of 12 contiguous TAR transcripts in either the sense or antisense orientation (pHβ/TAR-12 or pHβ/ASTAR-12; Fig. 5) (12). Whereas cotransfections of pHβ/TAR-12, but not pHβ/ASTAR-12, resulted in 17% of levels of *trans* activation of pSVTATCP, which contains Tat sequences from positions 1 to 67 fused to the coat protein (20), neither plasmid reduced the activity of pTATRVΔMCP, which contains the insertion of 11 amino acids between activation and basic domains of Tat. Thus, our insertions between core and basic domains of Tat affected only interactions between Tat and TAR. We conclude that the basic domain of Tat does not interact with TAR even in the context of the entire cysteine-rich, core, and C-terminal domains and that the entire N-terminal activation domain must be juxtaposed to the basic domain of Tat for these interactions.

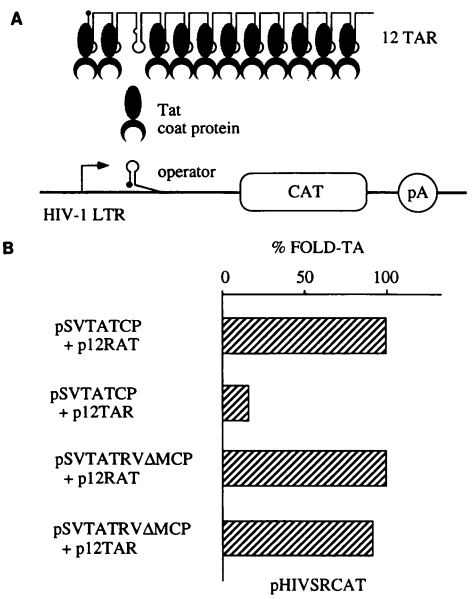

FIG. 5. Competition between TAR decoys and the operator of bacteriophage R17 for the activation of HIV-1 LTR by the R17 coat protein. (A) Diagram of TAR decoy, which consists of 12 contiguous TAR RNA sequences in the sense orientation, the hybrid Tat-coat protein, and the target HIV-1 LTR substituted with the operator of bacteriophage R17. TAR decoys are thought to remove Tat from nascent TAR RNA. In these experiments, they should remove the hybrid Tat-coat protein from nascent operator RNA. (B) Results of cotransfections with two effectors, pSVTATCP and pSVTATRVΔMCP, which contain wild-type Tat and an insertion of 11 amino acids between core and basic domains of Tat, respectively (see also Fig. 2 and 4), TAR decoys in the sense (pHβ/TAR-12) and antisense (pHβ/ASTAR-12) orientations, and the target pHIVSR CAT. Values are presented as percent fold *trans* activation (% FOLD-TA) of cotransfections with wild-type effector and target (pSVTATCP and pHIVSRCAT) in the presence of TAR decoy in the antisense orientation (pHβ/ASTAR-12). These results are representative of two experiments done in triplicate.

DISCUSSION

This study demonstrates that the separation of activation and basic domains of Tat prevents interactions between Tat and TAR. Thus, from the viewpoint of associations between Tat and TAR, Tat contains a binding domain that overlaps the activation domain. These results are consistent with the observation that mutations in the activation domain of Tat not only abolish *trans* activation but also fail to exhibit a *trans*-dominant negative phenotype (13, 18). Thus, these mutants are unable to associate with TAR to competitively inhibit interactions between wild-type Tat and TAR. Furthermore, this study leads to a reassessment of previous mutants of Tat, since mutations in the activation domain could have also affected RNA-binding properties of Tat.

Our findings extend in vitro TAR RNA-binding studies. With recombinant Tat, or with nonapeptides which contain either nine basic amino acids of Tat or a single arginine in the context of eight lysines, efficient binding to the 5′ bulge in the TAR RNA stem-loop was observed (2, 3, 8, 14, 19, 25). In these studies, the central loop of TAR was completely dispensable for binding. Although basic regions from Rev and bacteriophage λ N protein were not assayed in a similar in vitro binding assay, they also can replace the basic domain of Tat and retain wild-type levels of *trans* activation in cells (23). These findings suggest that although the basic domain of Tat binds efficiently to TAR RNA in vitro, it lacks binding specificity in vivo. This view was confirmed by studies that revealed that the central loop of TAR is also essential for interactions between Tat and TAR in vivo (1, 5, 9, 10). Either directly or with TAR decoys, no interactions between Tat and TAR could occur without this central loop in cells. Thus, the 5′ bulge and the central loop in the TAR RNA stem-loop contribute to productive interactions between Tat and TAR in vivo.

Since the basic domain of Tat is involved in the binding of the bulge of TAR RNA, interactions between the central loop of TAR and Tat must be different. Our study suggests that these could represent associations between cellular proteins that bind to the central loop of TAR and the activation domain of Tat. Since the activation domain itself contains only 48 amino acids, this cellular protein could also be the transcriptional effector of Tat. Such a scenario has been observed with bacteriophage λ; in this case, bacterial NusA protein not only is required for productive interactions between N and the *nutB* stem-loop, which is the RNA target of the N protein, but also forms the link between N and core RNA polymerase (16, 17). The end result of these interactions is antitermination of bacteriophage λ transcription, which is similar to effects of Tat on processivity or transcriptional elongation in HIV-1. Alternatively, two to several proteins interact with the activation domain of Tat; one increases interactions between Tat and TAR, and the other affects transcription. That increased distance between activation and basic domains of Tat interferes more with interactions between Tat and TAR than with the activation of transcription would tend to favor the latter hypothesis. However, it is also possible that interactions between this cellular protein that associates with Tat are stronger with RNA polymerase II than with the central loop of TAR or cellular proteins that interact with it. Moreover, it is unlikely that these cellular proteins are either TRP-185/TRP-1 or TRP-2, which are complexes of proteins that bind to the central loop or the 5′ bulge of TAR, respectively (11, 21). Although both of these proteins can increase rates of transcription from the HIV-1 LTR, both also displace the binding of Tat to TAR in vitro (11, 21). Further binding studies with Tats of different lentiviruses and the isolation and characterization of cellular proteins that bind to Tat may discriminate between these possibilities.

ACKNOWLEDGMENTS

We thank Michael Armanini for expert secretarial assistance and members of the laboratory for helpful discussions and comments on the manuscript.

Y. Luo is supported by a fellowship from the UC Taskforce on AIDS.

REFERENCES

1. **Berkhout, B., and K. T. Jeang.** 1991. Detailed mutational analysis of TAR RNA: critical spacing between the bulge and loop recognition domains. Nucleic Acids Res. **19**:6169–6176.
2. **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.
3. **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.
4. **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.
5. **Carvalho, M., and D. Derse.** 1991. Mutational analysis of the equine infectious anemia virus Tat-responsive element. J. Virol. **65**:3468–3474.
6. **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.
7. **Derse, D., M. Carvalho, R. Carroll, and B. M. Peterlin.** 1991. A minimal lentivirus Tat. J. Virol. **65**:7012–7015.
8. **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 a U-rich bulge in the stem of the TAR RNA structure. EMBO J. **9**:4145–4153.
9. **Feng, S., and E. C. Holland.** 1988. HIV-1 tat trans-activation requires the loop sequence within tar. Nature (London) **334**: 165–167.
10. **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.
11. **Gaynor, R., E. Soultanakis, M. Kuwabara, J. Garcia, and D. S. Sigman.** 1989. Specific binding of a HeLa cell nuclear protein to RNA sequences in the human immunodeficiency virus transactivating region. Proc. Natl. Acad. Sci. USA **86**:4858–4862.
12. **Graham, G. J., and J. J. Maio.** 1990. RNA transcripts of the human immunodeficiency virus transactivation response element can inhibit action of the viral transactivator. Proc. Natl. Acad. Sci. USA **87**:5817–5821.
13. **Green, M., M. Ishino, and P. M. Loewenstein.** 1989. Mutational analysis of HIV-1 Tat minimal domain peptides: identification of trans-dominant mutants that suppress HIV-LTR-driven gene expression. Cell **58**:215–223.
14. **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.
15. **Karn, J., and Graeble, M. A.** 1992. New insights into the mechanism of HIV-1 *trans*-activation. Trends Genet. **8**:365–368.
16. **Mason, S., and J. Greenblatt.** 1991. Assembly of transcription elongation complexes containing the N protein of phage λ and the *Escherichia coli* elongation factors NusA, NusB, NusG, and S10. Genes Dev. **5**:1504–1512.
17. **Nodwell, J. R., and J. Greenblatt.** 1991. The *nut* site of bacteriophage λ is made of RNA and is bound by transcription antitermination factors on the surface of RNA polymerase. Genes Dev. **5**:2141–2151.
18. **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.
19. **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.
20. **Selby, M. J., and B. M. Peterlin.** 1990. Trans-activation by HIV-1 Tat via a heterologous RNA binding protein. Cell **62**:769–776.
21. **Sheline, C. T., L. H. Milocco, and K. A. Jones.** 1991. Two distinct nuclear transcription factors recognize loop and bulge residues of the HIV-1 TAR RNA hairpin. Genes Dev. **5**:2508–2520.
22. **Southgate, C., M. L. Zapp, and M. R. Green.** 1990. Activation of transcription by HIV-1 Tat protein tethered to nascent RNA through another protein. Nature (London) **345**:640–642.
23. **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 transactivation. EMBO J. **10**:2311–2318.
24. **Sullenger, B. A., H. F. Gallardo, G. E. Ungers, and E. Gilboa.** 1991. Analysis of *trans*-acting response decoy RNA-mediated inhibition of human immunodeficiency virus type 1 transactivation. J. Virol. **65**:6811–6816.
25. **Tao, J., and A. D. Frankel.** 1992. Specific binding of arginine to TAR RNA. Proc. Natl. Acad. Sci. USA **89**:2723–2726.
26. **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.