Identification of Transactivation-Response Sequences in the Long Terminal Repeat of Bovine Immunodeficiency-Like Virus

SUSAN CARPENTER,^{1*} SUSAN A. NADIN-DAVIS,² YVONNE WANNEMUEHLER,¹
AND JAMES A. ROTH¹

*Department of Microbiology, Immunology, and Preventive Medicine, College of Veterinary Medicine,
Iowa State University, Ames, Iowa 50011,¹ and Animal Diseases Research Institute
NEPEAN, Agriculture Canada, Nepean, Ontario K2H 8P9, Canada²*

Received 19 January 1993/Accepted 31 March 1993

Transient expression assays using the reporter gene that encodes chloramphenicol acetyltransferase were used to identify *cis*-acting sequences necessary for bovine immunodeficiency-like virus (BIV) transactivation. Computer analyses identified two RNA stem-loop structures located immediately downstream of the transcription start site in the long terminal repeat. Deletion analysis of the long terminal repeat indicated that sequences containing the proximal stem-loop structure located between +4 and +31 are required for virus-specific transactivation. Therefore, BIV likely utilizes a mechanism of transactivation similar to that of the human and simian lentiviruses.

Bovine immunodeficiency-like virus (BIV) is a naturally occurring lentivirus of cattle which is antigenically and genetically related to other members of the lentivirus subfamily of retroviruses, including human immunodeficiency virus (HIV) and equine infectious anemia virus (EIAV) (23, 49). Lentiviruses are associated with a lifelong, persistent infection and a chronic, variable disease course. The clinical outcome of infection may depend, in part, on levels of viral replication within cells of the immune system. Therefore, understanding of the mechanisms by which various lentiviruses are able to regulate their own replication is of interest for identification of factors which contribute to clinical disease and viral pathogenesis.

Lentiviruses encode a number of auxiliary proteins shown to be important in the regulation of viral gene expression. All of the lentiviruses characterized have been shown to encode a viral transactivating protein, Tat, which increases the rate of transcription of genes linked to the viral long terminal repeat (LTR) (2–4, 16, 31, 35, 37, 43, 44, 46, 47). Interestingly, the mechanisms of transactivation differ among various members of the lentivirus subfamily. In HIV, EIAV, and simian immunodeficiency virus, transactivation requires the presence of a highly structured RNA sequence, termed the transactivation-response region (TAR), located downstream of the transcription initiation site (1, 3, 7, 12, 18–20, 28, 33). In most cases, the LTRs exhibit relatively low levels of basal activity yet are highly activated in the presence of homologous Tat (14, 16, 42). In contrast, LTR sequences required for transactivation by visna virus and feline immunodeficiency virus (FIV) are located in the U3 region of the LTR and include AP-1 and AP-4 enhancer sequences (32, 47). Both visna virus and FIV LTRs exhibit a high level of basal activity in most cell types but are weakly transactivated (15, 31, 47). At the protein level, the HIV and simian immunodeficiency virus Tat proteins contain highly conserved functional domains, which include a cysteine-rich domain, a highly basic domain, and a conserved core region (14, 21, 26, 27, 29, 41). EIAV Tat lacks the cysteine-rich domain but retains a conserved core region as well as a highly basic

domain (17, 48). Similar, functionally analogous domains are absent in the transactivating protein of visna virus (15, 25), as well as in the putative FIV Tat protein (36, 47).

The regulatory mechanisms of BIV are less well characterized. BIV has been found to encode a transacting protein derived from an open reading frame located in the *pol-env* intragenic region (35, 37). However, the *cis*-acting sequences in the LTR required for transactivation have not been identified. The BIV LTR is a relatively strong promoter in some cell types and is poorly transactivated in the presence of virus in these cells (37). This indicates that transactivation by BIV may be mechanistically similar to that of visna virus and FIV. However, the Tat protein of BIV is structurally more analogous to primate Tat proteins and contains the cysteine, core, and basic functional domains (22, 35). Moreover, computer analysis of the BIV RNA for the presence of TAR-like secondary structures in the 5' end identified two potential stem-loop structures between nucleotides +4 and +69 (Fig. 1B). The structure of BIV Tat and the presence of a TAR-like element in the extreme 5' end of the BIV RNA are highly suggestive that viral transactivation is mediated through an RNA enhancer similar to those of EIAV and primate lentiviruses. To test this hypothesis, we created a series of deletion and substitution mutations in the BIV LTR and used transient expression assays to examine the effects of these mutations on viral transactivation.

Previous studies have demonstrated that in certain cell types, the BIV LTR is a relatively strong promoter but is weakly transactivated in the presence of virus or BIV Tat (35, 37). Therefore, we chose to use MDBK cells, in which the basal promoter activity is low. All assays were done with uninfected MDBK cells (ATCC CCL22) and MDBK/BIV26, a clonal cell line derived from MDBK cells chronically infected with the R29 isolate of BIV (49, 51). To establish the clonal cell line, persistently infected MDBK cells were seeded in 100-mm-diameter tissue culture dishes at 10 to 100 cells per dish. After 10 to 14 days in culture, individual cell clones were removed and expanded in 24-well tissue culture plates. Following expansion, culture medium was tested for the presence of reverse transcriptase activity as previously described (8). In addition, the cells were screened for the presence of BIV and bovine viral diarrhea virus by using a

* Corresponding author.

FIG. 1. (A) Nucleotide sequence of the BIV LTR cloned from MDBK cells persistently infected with the R29 isolate of BIV. The U3, R, and U5 borders are based on a report by Garvey et al. (22). The sequence is numbered with reference to the transcription initiation site (+1) (22). The locations of potential promoter and enhancer sequences are indicated by open boxes (CAAT and TATAA) or by underlines. The positions of restriction enzyme sites used in subcloning are shown. (B) Secondary-structure model of predicted TAR sequence of BIV. The structure was generated by using the RNA Fold program and PC Gene Sequence Analysis software with a maximum distance of 100 nucleotides between base pairs. The free energy of the structure is -88.7 kcal/mol (1 cal = 4.184 J).

focal immunoassay similar to those previously described (9, 10, 13, 45). A bovine viral diarrhea virus-free clonal cell line, designated MDBK/BIV26, was found to be BIV positive by both immunofluorescence and reverse transcriptase activity and was used for transient expression studies. All cells were propagated in Dulbecco's minimum essential medium supplemented with antibiotics, 10% fetal calf serum, and 1% newborn bovine serum.

The complete BIV LTR (11) was inserted at the *Hind*III site into expression plasmid pCATBASIC (Promega Biotec, Madison, Wis.), upstream of the gene for bacterial chloramphenicol acetyltransferase (CAT). To generate 3' deletions (p3D series), pBIVLTRCAT was digested with *Xho*I and *Sph*I, removing 63 nucleotides from the 3' end of the LTR (Fig. 1A). A series of deletions in which progressively more of the sequence was removed from the 3' end of the LTR was then generated by the method of Henikoff (30). For construction of 5' deletions, pBIVLTRCAT was cut with *Hind*III to remove the entire LTR. The vector DNA was treated with Klenow to create blunt ends and then cut with *Sal*I. To create a nested set of U3 deletions, the LTR insert was digested with selected restriction enzymes which cut in the U3 region of the BIV LTR (Fig. 1A), including *Bam*HI (-319), *Acc*I (-207), *Nsi*I (-115), and *Acl*I (-74). The ends were filled in with Klenow, and the DNAs were digested with *Xho*I (+140) and inserted into the *Sal*I-digested expression vector. In each case, the *Xho*I-*Sal*I cohesive ends ensured insertion of the LTR fragment in the correct orientation. All clones were verified by DNA sequencing.

Comparison of transactivation among the deletion mutants indicated that sequences between -76 and +54 are required for transactivation (Fig. 2). This region encompasses the TATAA box at -25 and the 5'-proximal stem-loop at +4 to

+31 and extends into the distal stem-loop at +32 to +69. Transactivation was completely abolished in constructs p3D+12, p3D+19, and p3D+26. These constructs retain the TATAA box and sequences upstream of the transcription start site but lack sequences required to form the proximal stem-loop structure. These results indicated that BIV transactivation requires the presence of sequences downstream of the transcription initiation site, including the presence of the potential RNA stem-loop structure located at +4 to +31.

Transactivation was reduced to 60% of wild-type levels in p3D+54 (Fig. 2), suggesting that the second stem-loop structure between +32 and +69 contributes to transactivation. The predicted secondary structure of the BIV LTR is most similar to those of HIV type 2 (HIV-2) and simian immunodeficiency virus insofar as there are multiple stem-loops (1, 3, 19, 20). The roles of additional stem-loop structures in transactivation are not well characterized. Deletion analysis of the HIV-2 LTR has shown that the proximal loop is sufficient for transactivation (20). Mutational analysis of HIV-2 TAR indicated that distal loop 2 does play a role in viral transactivation; however, the contribution of loop 2 was masked in the presence of an intact wild-type loop 1 (20). Although our data indicate that BIV loop 1 is sufficient for optimal CAT activity, a role for loop 2 in transactivation is suggested by a reported three- to sixfold reduction in BIV transactivation when the LTR is deleted downstream of +64 (35). Interestingly, disruption of the predicted structure of loop 2 in p3D+54 resulted in an increase in CAT activity in both MDBK and MDBK/BIV26 cells. This suggests that the secondary structure of the distal stem-loop plays a role in translational regulation, perhaps by making the RNA less accessible to ribosome binding. Previous studies have found that disruption of the secondary

FIG. 2. Deletion analysis of the BIV LTR showing CAT activity in uninfected MDBK cells and in MDBK/BIV26, a clonal cell line persistently infected with the R29 isolate of BIV. The structure of the full-length LTR is shown at the top, and the locations of the U3, R, and U5 regions (22) and CAAT and TATAA motifs are indicated. Sequences retained in the truncated plasmids are represented by solid lines. The 5'- or 3'-terminal nucleotide is indicated for each truncation and is numbered with respect to the transcription start site (+1). MDBK and MDBK/BIV26 were transfected with 2 μ g of reporter plasmid DNA by calcium phosphate-mediated transfection (24). At 48 h following transfection, cells were harvested and lysed by three rounds of freeze-thawing, and the protein concentration of each lysate was determined by the bicinchoninic acid method (Pierce). CAT activity was determined by using 20 μ g of lysate and previously described procedures (24). Results are given as percentages of [14 C]chloramphenicol converted to its acetylated form and are means of three transfections $\pm$ the standard errors of the means. Fold transactivation was determined by using the mean percent conversion in MDBK/BIV compared with the mean value in uninfected MDBK cells.

structure of HIV-1 increases binding to initiation factor eIF-4B (38). Similarly, mRNAs containing a spliced TAR of simian immunodeficiency virus, with simpler and less stable secondary structures, are more efficiently translated than are mRNAs containing a highly structured, unspliced TAR (50). A more precise delineation of the contribution of loops 1 and 2 in BIV transactivation requires a detailed mutational analysis of the stem-loop structures.

The BIV LTR contains several enhancer sites in the U3 region (22) (Fig. 1A), and a series of 5' deletion plasmids was generated to determine the role of U3 elements in Tat-mediated transactivation. The most significant change was an approximately 40% decrease in transactivation in p5D-74 (Fig. 2), indicating that sequences between -115 and -74 are required for maximum virus transactivation. p5D-74 retains three potential SP-1 sites (Fig. 1A) but lacks the CAAT enhancer motif (Fig. 1A). Restoration of the CAAT motif in p5D-115 resulted in levels of transactivation comparable to that of the wild type. Thus, optimum BIV transactivation required the presence of upstream enhancer motifs, similar to HIV-1 Tat (6). Deletion of enhancer elements further upstream, including the NF- κ B and AP-1 sites, did not greatly affect BIV transactivation (Fig. 2). However, one deletion construct, p5D-207, did show an increase in transactivation. Negative regulatory elements have been found previously in a number of lentivirus LTRs, including EIAV (18) and HIV-1 (39), and it is possible that the BIV LTR contains a similar negative regulatory element located between -207 and -319.

Several structural and spatial features are required for optimal transactivation of the HIV-1 TAR, including the presence of a base-paired stem structure (7, 28, 40), as well as specific nucleotides within both the bulge and the loop (5, 19, 40). The latter includes a CUG sequence which is conserved in primate lentiviruses, as well as in EIAV (5, 12, 19). Examination of specific nucleotide sequences within the bulge and loop of BIV identified several differences between

BIV and primate lentiviruses which might affect overall levels of transactivation. These included potential base pairing within the BIV bulge, as well as the absence of a CUG sequence in loop 1. A limited number of BIV TAR mutations were analyzed to determine whether levels of BIV transactivation could be increased over that observed in the wild-type LTR.

The trinucleotide bulge sequence in each of the predicted stem-loops of the BIV LTR contains the requisite uridine in the correct position. However, the structure of the bulge in the proximal stem-loop may be compromised by potential base pairing of the middle guanine in the UGU bulge with a cytidine at +25. To determine whether elimination of the base pairing would increase BIV transactivation, the G at +11 was mutated to a C (Fig. 3). Rather than increasing transactivation, this single nucleotide change reduced transactivation by 60%. These results suggest that the secondary structure in the UGU bulge is sufficient for Tat-TAR interaction and, moreover, that specific nucleotides within the TAR bulge are required for optimal transactivation. To explore the role of specific sequences within the BIV TAR loop, we generated a BIV construct containing a CUG sequence in loop 1. This change resulted in only a slight increase in levels of transactivation (Fig. 3). Other studies have found that transactivation by HIV-1 Tat can be highly sensitive to point mutations in the loop region (5, 19), and the absence of either a significant increase, or decrease, in transactivation mediated by the altered BIV loop sequence was somewhat surprising. More extensive mutational analyses of the BIV TAR are required to fully delineate the role of specific nucleotides in BIV transactivation.

In the present study, we showed that transactivation by BIV requires the presence of sequences downstream of the transcription initiation site predicted to contain an RNA stem-loop structure highly analogous to the TAR structure of primate lentiviruses. Although the data did not establish whether the BIV TAR structure acts as an RNA or a DNA

FIG. 3. Effects of point mutations within the bulge-and-loop sequence of TAR on BIV transactivation. A *Bgl*II site was introduced into p3D+140 just upstream of the TAR sequence by mutation of bases -8 to -10 by using standard oligonucleotide-directed mutagenesis methods (34). The resulting plasmid, pBIVLTRBgICAT, has a TAR sequence flanked by *Bgl*II and *Sac*I restriction sites. Thus, this TAR cassette can be replaced readily with synthetic oligonucleotides, enabling introduction of specific mutations (open box). Oligonucleotides containing specific mutations in the TAR loop (loop CUG) and bulge (bulge C) were synthesized and inserted into pBIVLTRBgICAT. Bulge C contains a G-to-C point mutation at +11, while loop CUG contains an AU-to-UG substitution at +18 and +19. Plasmid DNA was isolated from selected colonies and sequenced to verify the point mutations. MDBK and MDBK/BIV26 cells were transfected, and CAT activity was determined as described in legend to Fig. 2. Results are expressed as mean values of three transfections ± the standard errors of the means.

element, taken together with the structural similarities among the BIV and primate lentivirus Tat proteins, these findings indicate that the mechanism of BIV transactivation is similar to that of primate lentiviruses. Interestingly, there does not appear to be a correlation between the clinical-pathological signs of lentivirus infection and the mechanism of viral transactivation. Primate and feline lentiviruses replicate in CD4⁺ cells and are associated with severe immunosuppression and AIDS. The pathogenesis of BIV is less well characterized, although there is some evidence of early lymphoid changes characteristic of immunodeficiency viruses (11). In contrast, visna virus and EIAV replicate solely in cells of the monocyte-macrophage lineage and are not associated with an immunodeficiency syndrome. In terms of transactivation, however, FIV is most similar to visna virus and caprine arthritis-encephalitis virus, whereas BIV and EIAV are mechanistically similar to the primate immunodeficiency viruses. A thorough understanding of both the similarities and the differences among lentiviruses in the mechanisms that control viral replication may help to identify key events that contribute to the alternate disease courses.

We thank W. Allen Miller for critical review of the manuscript and Soren Alexandersen for construction of pBIVLTRCAT. Mary Jane Long, Dawne Buhrow, Kurtimis Plagge, and Shirley Chang are acknowledged for excellent technical assistance.

This work was supported in part by PHS grant CA505159 and Iowa State University biotechnology grant 480-23-22.

REFERENCES

1. Arya, S. K. 1988. Human and simian immunodeficiency retroviruses: activation and differential transactivation of gene expression. *AIDS Res. Hum. Retroviruses* 4:175-186.
2. Arya, S. K., B. Beaver, L. Jagodzinski, B. Ensoli, P. J. Kanki, J. Albert, E. M. Fenyo, G. Biberfeld, J. F. Zagury, F. Laure, M. Essex, E. Norrby, F. Wong-Staal, and R. C. Gallo. 1987. New human and simian HIV-related retroviruses possess functional transactivator (Tat) gene. *Nature (London)* 328:548-550.
3. 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.
4. Arya, S. K., C. Guo, S. F. Josephs, and F. Wong-Staal. 1985. *trans*-activator gene of human T-lymphotropic virus type III (HTLV-III). *Science* 229:69-73.
5. Berkhout, B., and K.-T. Jeang. 1989. *trans* activation of human immunodeficiency virus type 1 is sequence specific for both the single-stranded bulge and loop of the *trans*-acting-responsive hairpin: a quantitative analysis. *J. Virol.* 63:5501-5504.
6. Berkhout, B., and K.-T. Jeang. 1992. Functional roles for the TATA promoter and enhancers in basal and Tat-induced expression of the human immunodeficiency virus type 1 long terminal repeat. *J. Virol.* 66:139-149.
7. Berkhout, B., R. H. Silverman, and K.-T. Jeang. 1989. Tat *trans*-activates the human immunodeficiency virus through a nascent RNA target. *Cell* 59:273-282.
8. Carpenter, S., and B. Chesebro. 1989. Change in host cell tropism associated with in vitro replication of equine infectious anemia virus. *J. Virol.* 63:2492-2496.
9. Carpenter, S., L. H. Evans, M. Sevoian, and B. Chesebro. 1987. Role of the host immune response in selection of equine infectious anemia virus variants. *J. Virol.* 61:3783-3789.
10. Carpenter, S., L. H. Evans, M. Sevoian, and B. Chesebro. 1990. In vivo and in vitro selection of equine infectious anemia virus variants, p. 99-115. *In* E. Kurstak, R. G. Marusyk, F. A. Murphy, and M. H. V. Van Regenmortel (ed.), *Applied virology research II: virus variation, epidemiology, and control*. Plenum Publishing, New York.
11. Carpenter, S., L. D. Miller, S. Alexandersen, C. A. Whetstone, M. J. VanDerMaaten, B. Viuff, Y. Wannemuehler, J. M. Miller, and J. A. Roth. 1992. Characterization of early pathogenic effects after experimental infection of calves with bovine immunodeficiency-like virus. *J. Virol.* 66:1074-1083.
12. Carvalho, M., and D. Derse. 1991. Mutational analysis of the equine infectious anemia virus Tat-responsive element. *J. Virol.* 65:3468-3474.
13. Chesebro, B., and K. Wehrly. 1988. Development of a sensitive quantitative focal assay for human immunodeficiency virus infectivity. *J. Virol.* 62:3779-3788.
14. Cullen, B. R. 1992. Mechanism of action of regulatory proteins encoded by complex retroviruses. *Microbiol. Rev.* 56:375-394.
15. Davis, J. L., and J. E. Clements. 1989. Characterization of a cDNA clone encoding the visna virus transactivating protein. *Proc. Natl. Acad. Sci. USA* 86:414-418.
16. Derse, D., P. L. Dorn, L. Levy, R. M. Stephens, N. R. Rice, and J. W. Casey. 1987. Characterization of equine infectious anemia virus long terminal repeat. *J. Virol.* 61:743-747.
17. 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.
18. Dorn, P. L., and D. Derse. 1988. *cis*- and *trans*-acting regulation of gene expression of equine infectious anemia virus. *J. Virol.* 62:3522-3626.
19. Feng, S., and E. C. Holland. 1988. HIV-1 Tat *trans*-activation requires the loop sequence within TAR. *Nature (London)* 334:165.
20. 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.
21. Garcia, J. A., D. Harrich, L. Pearson, R. Mitsuyasu, and R. B. Gaynor. 1988. Functional domains required for Tat-induced

- transcriptional activation of the HIV-1 long terminal repeat. *EMBO J.* 6:3761-3770.
22. Garvey, K. J., M. S. Oberste, J. E. Elser, M. J. Braun, and M. A. Gonda. 1990. Nucleotide sequence and genome organization of biologically active proviruses of the bovine immunodeficiency-like virus. *Virology* 175:391-409.
 23. Gonda, M. A., M. J. Braun, S. G. Carter, T. A. Kost, J. W. Bess, Jr., L. O. Arthur, and M. J. VanDerMaaten. 1987. Characterization and molecular cloning of a bovine lentivirus related to human immunodeficiency virus. *Nature (London)* 330:388-391.
 24. Gorman, C. M., G. T. Merlino, M. C. Willingham, I. Pastan, and B. H. Howard. 1982. The Rous sarcoma virus long terminal repeat is a strong promoter when introduced into a variety of eukaryotic cells by DNA-mediated transfection. *Proc. Natl. Acad. Sci. USA* 79:6777-6781.
 25. Gourdou, I., V. Mazarin, G. Quéral, N. Sauze, and R. Vigne. 1989. The open reading frame S of visna virus genome is a *trans*-activating gene. *Virology* 171:170-178.
 26. Green, M., and P. M. Loewenstein. 1988. Autonomous functional domains of chemically synthesized human immunodeficiency virus Tat *trans*-activator protein. *Cell* 55:1179-1188.
 27. 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.
 28. Hauber, J., and B. R. Cullen. 1988. Mutational analysis of the *trans*-activation-responsive region of the human immunodeficiency virus type 1 long terminal repeat. *J. Virol.* 62:673-679.
 29. 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.
 30. Henikoff, A. 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. *Gene* 28:351-359.
 31. Hess, J. L., J. E. Clements, and O. Narayan. 1985. *cis*- and *trans*-acting transcriptional regulation of visna virus. *Science* 229:482-485.
 32. Hess, J. L., J. A. Small, and J. E. Clements. 1989. Sequences in the visna virus long terminal repeat that control transcriptional activity and respond to viral *trans*-activation: involvement of AP-1 sites in basal activity and *trans*-activation. *J. Virol.* 63:3001-3015.
 33. Jakobovits, A., D. H. Smith, E. B. Jakobovits, and D. J. Capon. 1988. A discrete element 3' of human immunodeficiency virus 1 (HIV-1) and HIV-2 mRNA initiation sites mediates transcriptional activation by an HIV *trans* activator. *Mol. Cell. Biol.* 8:2555-2561.
 34. Kunkel, T. A. 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Proc. Natl. Acad. Sci. USA* 82:488-492.
 35. Liu, Z.-Q., D. Sheridan, and C. Wood. 1992. Identification and characterization of the bovine immunodeficiency-like virus *tat* gene. *J. Virol.* 66:5137-5140.
 36. Olmsted, R. A., A. K. Barnes, J. K. Yamamoto, V. M. Hirsch, R. H. Purcell, and P. R. Johnson. 1989. Molecular cloning of feline immunodeficiency virus. *Proc. Natl. Acad. Sci. USA* 86:2448-2452.
 37. Pallansch, L. A., C. S. Lackman-Smith, and M. A. Gonda. 1992. Bovine immunodeficiency-like virus encodes factors which *trans* activate the long terminal repeat. *J. Virol.* 66:2647-2652.
 38. Parkin, N. T., E. A. Cohen, A. Darveau, C. Rosen, W. Haseltine, and N. Sonenberg. 1988. Mutational analysis of the 5' non-coding region of human immunodeficiency virus type 1: effects of secondary structure on translation. *EMBO J.* 7:2831-2837.
 39. 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.
 40. Roy, S., N. T. Parkin, C. Rosen, J. Itovitch, and N. Sonenberg. 1990. Structural requirements for *trans* activation of human immunodeficiency virus type 1 long terminal repeat-directed gene expression by *tat*: importance of base pairing, loop sequence, and bulges in the *tat*-responsive sequence. *J. Virol.* 64:1402-1406.
 41. Ruben, S., A. Perkins, R. Purcell, K. Joung, R. Sia, R. Burghoff, W. A. Haseltine, and C. A. Rosen. 1989. Structural and functional characterization of human immunodeficiency virus Tat protein. *J. Virol.* 63:1-8.
 42. Sakuragi, J., M. Fukasawa, R. Shibata, H. Sakai, M. Kawamura, H. Akari, T. Kiyomasu, A. Ishimoto, M. Hayami, and A. Adachi. 1991. Functional analysis of long terminal repeats derived from four strains of simian immunodeficiency virus SIV_{AGM} in relation to other primate lentiviruses. *Virology* 185:455-459.
 43. Sherman, L., A. Gazit, A. Yaniv, T. Kawakami, J. E. Dahlberg, and S. R. Tronick. 1988. Localization of sequences responsible for *trans*-activation of the equine infectious anemia virus long terminal repeat. *J. Virol.* 62:120-126.
 44. Sherman, L., A. Yaniv, H. Lichtman-Pleban, S. R. Tronick, and A. Gazit. 1989. Analysis of regulatory elements of the equine infectious anemia virus and caprine arthritis-encephalitis virus long terminal repeats. *J. Virol.* 63:4925-4931.
 45. Sitbon, M., J. Nishio, K. Wehrly, D. Lodmell, and B. Chesebro. 1985. Use of a focal immunofluorescence assay on live cells for quantitation of retroviruses: distinction of host range classes in virus mixtures and biological cloning of dual-tropic murine leukemia viruses. *Virology* 141:110-118.
 46. Sodroski, J. G., C. A. Rosen, and W. A. Haseltine. 1984. *trans*-acting transcriptional activation of the long terminal repeat of human T-lymphotropic viruses in infected cells. *Science* 225:381-385.
 47. Sparger, E. E., B. L. Shacklett, L. Renshaw-Gegg, P. A. Barry, N. C. Pedersen, J. H. Elder, and P. A. Luciw. 1992. Regulation of gene expression directed by the long terminal repeat of the feline immunodeficiency virus. *Virology* 187:165-177.
 48. Stephens, R. M., D. Derse, and N. R. Rice. 1990. Cloning and characterization of cDNAs encoding equine infectious anemia Tat and putative Rev proteins. *J. Virol.* 64:3716-3725.
 49. VanDerMaaten, M. J., A. D. Boothe, and C. L. Seger. 1972. Isolation of a virus from cattle with persistent lymphocytosis. *J. Natl. Cancer. Inst.* 49:1649-1657.
 50. Viglianti, G. A., E. P. Rubinstein, and K. L. Graves. 1992. Role of TAR RNA splicing in translational regulation of simian immunodeficiency virus from rhesus macaques. *J. Virol.* 66:4824-4833.
 51. Whetstone, C. A., M. J. VanDerMaaten, and J. W. Black. 1990. Humoral immune response to the bovine immunodeficiency-like virus in experimentally and naturally infected cattle. *J. Virol.* 64:3557-3561.