Bovine Immunodeficiency-Like Virus Encodes Factors Which *trans* Activate the Long Terminal Repeat

LUKE A. PALLANSCH, CAROL S. LACKMAN-SMITH, AND MATTHEW A. GONDA*

Laboratory of Cell and Molecular Structure, Program Resources, Inc./DynCorp, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland 21702-1201

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Lentiviruses are known to encode factors which *trans* activate expression from the viral long terminal repeat (LTR); the primary *trans* activator is the *tat* gene product. One of the putative accessory genes (*tat*) of the bovine immunodeficiency-like virus (BIV) bears sequence similarity to other lentivirus *tat* genes. This finding suggests that BIV may encode a *trans*-activating protein capable of stimulating LTR-directed gene expression. To test this hypothesis in vitro, BIV LTR-chloramphenicol acetyltransferase (CAT) reporter gene plasmids were constructed and transfected into three cell lines established from canine, bovine, or lapine tissues that are susceptible to BIV infection. The level of BIV LTR-directed CAT gene expression was significantly elevated in BIV-infected cells compared with uninfected cells. The relatively high basal-level expression of BIV LTR-CAT in uninfected canine and bovine cell lines suggests that cellular factors play a role in regulating BIV LTR-directed gene expression. Additionally, by using a clonal canine cell line in which the BIV LTR-CAT plasmid is stably expressed, BIV LTR-directed CAT expression is elevated 15- to 80-fold by cocultivation with BIV-infected cells, supporting the notion that BIV encodes a *trans* activator. The relative specificity of this viral activation was assessed by coculturing the clonal BIV LTR-CAT cell line with bovine leukemia virus- or bovine syncytial virus-infected cells; these bovine retroviruses increased expression from the BIV LTR only two- to threefold. Thus, BIV LTR regulatory elements in infected cells, like those of human immunodeficiency virus type 1 and other lentiviruses, are *trans* activated, presumably through the action of a Tat-like protein and cellular factors.

Recent characterization of bovine immunodeficiency-like virus (BIV) allows its classification as a member of the lentivirus subfamily of retroviruses (19). Nucleotide sequencing of functional proviral molecular clones of BIV has demonstrated that BIV is an organizationally complex retrovirus (6, 16). In addition to *gag*, *pol*, and *env* structural genes, which are flanked on the 5' and 3' ends by the long terminal repeats (LTRs), there are five additional short open reading frames in, or overlapping, the *pol* and *env* open reading frames. Three of these short open reading frames have been identified by location and structural similarity to the nonstructural/regulatory or accessory genes (*vif*, *tat*, and *rev*) of other lentiviruses; two of these open reading frames, *W* and *Y*, are unique to BIV. The transcriptional pattern of BIV is also intricate (16, 30, 31). There are at least five size classes of viral RNA in BIV-infected cells; mRNAs of 1.4 and 1.7 kb for the *tat* and *rev* accessory genes, respectively, have been unambiguously identified, thereby supporting their presence and importance in the genome (31).

The functions of the LTR and accessory genes of human immunodeficiency virus type 1 (HIV-1) have been studied extensively; they are believed to play an important role in HIV-1's replication, infectivity, and pathogenesis (reviewed in references 8, 9, 21, 26, and 47). For example, the *tat* gene product (Tat) of HIV-1 activates transcription from the viral LTR and increases the steady-state levels of viral RNA in infected cells (7, 17, 33). Tat also appears to enhance translation of viral transcripts through interaction with viral RNA at the TAR (*trans*-activation region) site, a region of conserved secondary structure near the 5' end of all viral RNAs (5, 13, 37, 42). While structural maps have been

derived for the BIV LTR and accessory genes (16, 31), little is known of their function.

As a first step toward understanding the molecular mechanisms controlling BIV gene expression, we have constructed BIV LTR-chloramphenicol acetyltransferase (CAT) gene reporter plasmids and cell lines which stably express these plasmids to study *trans* activation of the BIV LTR by cellular and viral factors. We have used these reporter plasmids to characterize the responsiveness of the BIV LTR to endogenous cellular factors in several cell lines susceptible to BIV infection and the ability of BIV to *trans* activate its LTR in vitro. The relative specificity of this *trans* activation was also tested with two other distantly related bovine retroviruses, bovine syncytial virus (BSV) and bovine leukemia virus (BLV).

MATERIALS AND METHODS

BIV proviral molecular clones and virus stocks. The functional proviral molecular clones of BIV used in these studies are BIV106 and BIV127. The molecular cloning, characterization, and nucleotide sequencing of the functional BIV106 and BIV127 proviral clones have been reported previously (6, 16). BIV^{par} refers to the parental virus produced by cells infected with progeny obtained from the original isolate of BIV (48). BIV106 and BIV127 virus stocks are strains of BIV^{par} obtained from the replication of progeny of functional proviral molecular clones following microinjection into bovine embryonic spleen cells (BESp).

Virus and cell culture. The canine thymus (Cf2Th) and embryonic rabbit epidermis (EREp) cells used in these studies were obtained from the American Type Culture Collection (Rockville, Md.); their cell repository line numbers are CRL 1430 and CRL 6498, respectively. Bovine

* Corresponding author.

leukocyte adherent cells (BLAC-20) were derived in this laboratory from the extended cultivation of phytohemagglutinin- and interleukin-2-stimulated primary bovine leukocytes as described before (34). All cells used in the reported experiments were maintained at 37°C in Dulbecco's modified Eagle's medium (DMEM; GIBCO BRL) supplemented with 7.5% fetal bovine serum, 2 mM glutamine, and antibiotics, in a humidified atmosphere of 5% CO₂.

The bovine retroviruses used in *trans*-activation experiments were BIV, BSV, and BLV. BSV was isolated in this laboratory from a BSV-infected calf and propagated in BLAC-20 and Cf2Th cells. BLV was grown in FLK cells (10) supplemented with BLAC-20 cells to enhance virus expression. For these experiments, cells persistently infected with BIV were obtained by placing cell-free supernatants from either BIV106- or BIV127-producing BEsp cells onto Cf2Th, EREp, or BLAC-20 cells. The successful infection of these cells with BIV was confirmed by observation of syncytium formation, positive reverse transcriptase assay, and/or the observation of virus particles by electron microscopy (6, 19). All retrovirus cultures were periodically monitored for virus production by the reverse transcriptase assay, and prior to use in *trans*-activation assays, all infected cells were actively producing their respective virus.

Construction of recombinant BIV LTR-CAT reporter plasmids. The 5' LTRs from the two distinct BIV proviral molecular clones BIV106 and BIV127 were subcloned into the promoterless CAT expression vector pBLCAT3 (27) to produce the recombinant CAT reporter plasmids p106LTR-CAT and p127LTR-CAT, respectively (Fig. 1). Briefly, each LTR region was excised from the proviral molecular clones with appropriate restriction enzymes, and when necessary, the LTR ends were filled in with the Klenow fragment of DNA polymerase I. The modified LTRs were cloned into the pBLCAT3 vector, at a Klenow fragment-filled-in *SalI* site, in both the 5' → 3' and 3' → 5' orientations, to place the LTR upstream of the CAT reporter gene in the correct and incorrect orientations for expression, respectively. Following subcloning of the BIV LTRs into pBLCAT3, fragment orientation and junction sequences were confirmed by sequencing of the double-stranded DNA plasmid constructs shown in Fig. 1 by using Sequenase II (USB), according to the manufacturer's protocol. Recombinant plasmids were amplified in *Escherichia coli* DH5α cells, and purified plasmids were obtained by banding the plasmid DNA twice in cesium chloride gradients by standard recombinant DNA methods (40).

Derivation of clonal cell lines which stably express BIV127 LTR-CAT. To obtain cell lines which stably express BIV127 LTR-CAT, p127LTR-CAT was cotransfected with pMC1 neopolyA (Stratagene), 10 µg of each, into Cf2Th cells; 48 h later, the transfected cells were placed under antibiotic drug selection with G418 (GIBCO BRL), at a final concentration of 1 mg/ml. After 3 weeks of G418 drug selection, p127LTR-CAT clonal cell lines were prepared by limiting dilution in 96-well plates and expanded by subculture for initial characterization by polymerase chain reaction, Southern analysis, and LTR *trans*-activation assays. One (B4C) of several clonal Cf2Th cell lines which exhibited strong *trans* activation of the integrated p127LTR-CAT gene following coculture of the reporter cell line with BIV-infected cells was subsequently expanded for the experiments described here.

***trans*-activation assay in LTR-transfected and stably expressing cells.** For transient LTR expression assays, uninfected and BIV-infected cells were transfected with LTR-reporter plasmids and lipofectin (GIBCO BRL). Briefly, 2 ×

10⁵ infected or uninfected cells were plated in 60-mm dishes and cultured overnight. Cells were thoroughly rinsed with serum-free OptiMem (GIBCO BRL) and then transfected with lipofectin at 20 µg of plasmid DNA. After incubation at 37°C for 16 h, the transfected cells were aspirated and cultured with DMEM containing 7.5% fetal bovine serum and incubated at 37°C for an additional 48 h.

CAT assay. Transfected or cocultured cells were harvested, and cell extracts were prepared in 100 mM Tris-HCl, pH 7.8, by three freeze-thaw cycles. After centrifugation for 5 min (14,000 × *g*) to remove cellular debris, the cell extracts were heated at 70°C for 10 min to inactivate endogenous acylases. The supernatants were then analyzed for CAT activity by the fluor diffusion method (12, 29) with [³H]acetyl coenzyme A (CoA; New England Nuclear) to monitor acetylation of the chloramphenicol substrate. CAT activity is expressed in terms of the rate of radioactive acetylated chloramphenicol formed as a function of time; the initial slope of this reaction is proportional to the concentration of CAT present in the cellular extract. Protein concentration in the cell extracts was determined by the bicinchoninic acid method (BCA; Pierce), and CAT activities were normalized to the protein content of the cell extract. Additionally, in some experiments the BIV LTR-CAT constructs were cotransfected with a cytomegalovirus-β-galactosidase construct (CMV-β-gal), and the CAT activities were normalized to β-galactosidase activity to control for variations in transfection efficiencies (32). In either case, normalizing the reported CAT activities to cell extract protein content or to β-galactosidase activity did not yield significantly different results. All *trans* activation experiments were performed in triplicate, and the results were reported as an average.

RESULTS

Cellular factors influence *trans* activation of the BIV LTR in vitro. To examine the effects of host cellular factors on BIV LTR *trans* activation, transient-expression assays were performed with BIV LTR-CAT constructs in which the BIV106 and BIV127 LTRs were subcloned, in both the 5' → 3' and 3' → 5' orientations, in front of the CAT reporter gene (Fig. 1). Cloning of the BIV LTR in the 3' → 5' orientation yields a functionally defective BIV LTR-CAT construct; this construct serves as a control for endogenous CAT activity in the assay and the possible presence of cryptic upstream promoters in the reporter plasmids. CAT expression from the correctly oriented 5'→3' LTR-CAT construct, compared with that from the nonfunctional 3'→5' LTR-CAT construct, directly reflects the contribution of endogenous cellular factors to the BIV LTR *trans* activation observed in the three BIV replication-permissive cell lines examined. As shown in Table 1, uninfected Cf2Th and BLAC-20 cells displayed appreciable CAT activity when transfected with the 5'→3' LTR-CAT constructs, whereas uninfected EREp cells are essentially devoid of endogenous cellular factor-mediated CAT expression; there were no significant differences in CAT activity obtained with either the BIV106 or BIV127 LTR (Table 1; 5'→3' versus 3'→5'). These results demonstrate the regulation of LTR-directed CAT expression by cellular factors and the potential for differential regulation of BIV *trans* activation by cellular factors from different species or cell types.

Viral factors in BIV-infected cells *trans* activate the BIV LTR in vitro. Having demonstrated that cellular factors may influence *trans* activation of the BIV LTR in transfected cells, we wished to determine the possible influence of viral

FIG. 1. Construction of BIV LTR-CAT reporter plasmids. p106LTR-CAT consists of an 835-bp *HindIII*-*SmaI* fragment from BIV106, which includes 141 bp of 5'-cellular-flanking sequences, the entire 5' LTR (587 bp), and 107 bp of untranslated leader sequence prior to the first *gag* AUG codon. Similarly, p127LTR-CAT consists of a 1,230-bp *SmaI*-*SmaI* fragment from BIV127, which includes 536 bp of 5'-cellular-flanking sequence, the entire 5' LTR, and 107 bp of untranslated leader sequence prior to the first *gag* AUG codon. The BIV106 and BIV127 LTRs were also cloned in the opposite orientation to produce functionally deficient BIV LTR-CAT constructs which were used as negative controls during transient-expression assays. S, *SalI*; Sm, *SmaI*; MCS, multiple cloning site; H, *HindIII*; CAT, chloramphenicol acetyltransferase gene; SV40, simian virus 40 polyadenylation signal; Amp^r, ampicillin resistance gene.

factors, such as the putative *tat* gene (16), in BIV-infected cells in mediating gene expression from the BIV LTR. With the 5'→3' and 3'→5' BIV LTR-CAT reporter plasmids in transient-expression assays, a further increase in BIV LTR *trans* activation was observed in BIV^{par}-infected cells (Table 1; 5'→3' versus 3'→5').

The viral contribution (CAT index) to *trans* activation of the 5'→3' BIV LTR-CAT constructs in BIV-infected versus uninfected cells was determined (Table 1). In EREp cells, which have a relatively low level of endogenous factor-mediated LTR-directed CAT expression, we observed a four- to sevenfold elevation of CAT activity when the EREp cells were infected with BIV^{par}. Similarly, we observed a two- to sixfold increase in BIV LTR-directed CAT expression in the BIV^{par}-infected Cf2Th and BLAC-20 cell lines. Although CAT expression in the BIV^{par}-infected Cf2Th and BLAC-20 cells is significantly greater than CAT expression in the BIV-infected EREp cells (Table 1), high basal-level expression of the CAT reporter gene in the uninfected Cf2Th and BLAC-20 cells reduces the degree of *trans* activation reported for these cell types in transient-expression assays. It remains to be determined whether the observed *trans* activation reflects direct interactions of the putative BIV Tat with the LTR or whether the *trans* activation is indirectly mediated by Tat through unidentified cellular factors.

To better quantify the extent of *trans* activation occurring in BIV-infected cells, we used a clonal "reporter" or "indicator" cell line (B4C), in which the BIV127 LTR-CAT construct was stably integrated into the Cf2Th cell genome. The BIV127 LTR-CAT construct was used for preparing the reporter cell lines because previous characterization of the BIV LTRs demonstrated structural similarity between the BIV^{par} and BIV127 LTRs (16). We envisioned that the use of such a reporter cell line would simplify quantitative mea-

TABLE 1. CAT activity and *trans* activation of BIV LTR-CAT constructs in BIV^{par}-infected and uninfected cells^a

Cell line	p106LTR-CAT			p127LTR-CAT		
	3'→5'	5'→3'	CAT index ^b	3'→5'	5'→3'	CAT index
EREp						
Uninfected	0.8	1.8		1.3	1.7	
BIV ^{par}	0.4	13.1	7.3	0.3	6.8	4.0
Cf2Th						
Uninfected	0.7	16.2		1.0	16.7	
BIV ^{par}	0.9	40.4	2.5	1.6	33.6	2.0
BLAC-20						
Uninfected	2.9	8.2		1.5	6.3	
BIV ^{par}	5.2	25.6	3.1	4.2	40.0	6.3

^a CAT activity is determined from measuring the rate of conversion of [³H]chloramphenicol to acetylated-[³H]chloramphenicol. BIV LTR-directed CAT activity is expressed as the maximal rate (V_{max}) of the CAT enzyme reaction (change in counts per minute per minute) per milligram of protein of the prepared cell extract (12, 29).

^b CAT index reflects the level of viral *trans* activation of the BIV LTR and is expressed as the CAT activity of the 5'→3' BIV LTR-CAT construct in BIV^{par}-infected cells divided by that in uninfected cells.

surements of *trans* activation because variability in reporter gene transfection efficiency would no longer be an issue. Moreover, coculture of BIV-infected with uninfected cells is the most rapid and efficient mechanism of transmitting the BIV infection (19). *trans* activation of the BIV LTR, during coculture of BIV-infected cells with the B4C reporter cells, is demonstrated by increased BIV LTR-directed CAT expression. BIV106 and BIV127 cause a 10- to 15-fold activation of CAT expression, while the BIV^{par} strain induces an 80-fold *trans* activation of the BIV127 LTR (Fig. 2).

BSV and BLV *trans* activate the BIV LTR at low levels. Two bovine retroviruses, BSV and BLV, were also tested for their ability to cross-*trans* activate the BIV LTR. Although these viruses belong to different retrovirus subfamilies than BIV, they are highly prevalent in cattle populations and could serve as important regulatory cofactors for BIV infec-

FIG. 2. *trans* activation of the clonal, stably expressed BIV127 LTR-CAT Cf2Th reporter cell line (B4C) cocultured with retrovirus-infected cells. 127, 106, Par, and BSV represent coculture experiments with BIV127-infected, BIV106-infected, BIV^{par}-infected, and BSV-infected Cf2Th cells, respectively. BLV indicates coculture experiments with BLV-infected FLK cells. Control indicates coculture of B4C cells with uninfected Cf2Th cells.

tion and/or replication if they infect the same subsets of cells as BIV. Both of these viruses can replicate in Cf2Th cells and induce syncytia. When BSV-infected Cf2Th cells or BLV-infected FLK/BLAC-20 cells were cocultured with the B4C reporter cell line (Fig. 2), low levels of BIV LTR-CAT *trans* activation were observed relative to the *trans* activation produced by BIV106, BIV127, and BIV^{par} (2- to 3-fold versus 10- to 80-fold). Polyethylene glycol-mediated fusion of BLV-infected FLK cells to the B4C cells did not produce higher levels of BIV-LTR *trans* activation (data not shown) than observed in the coculture experiments described above. These results imply that incompatible cell-cell or virus-receptor interactions do not account for the low *trans* activation of the BIV LTR by BLV-infected cells. It remains to be determined whether the levels of BIV LTR *trans* activation produced by BSV and BLV are important in BIV infection or whether BIV expression influences BSV and BLV infections.

DISCUSSION

Lentiviral gene expression is influenced by regulatory sequences found in the proviral LTR, proteins encoded by the viral genome, and host cellular factors (4, 8, 9, 21, 26, 38, 47). In addition to *cis*-acting regulatory elements found upstream of the retroviral 5' LTR promoter (46, 49), most lentiviruses possess a unique posttranscriptional regulatory element, TAR, which has been mapped downstream of the mRNA cap site (15, 22, 25, 38, 41). Several lentiviruses, such as HIV-1 (2, 43, 45), HIV-2 (20), simian immunodeficiency virus (1), equine infectious anemia virus (11), and visna virus (23), are known to *trans* activate their LTRs during viral replication. In many of these viruses, the *tat* gene product, Tat, has been shown to mediate this *trans* activation by interacting with the specific TAR element found in the viral LTR. In addition to the Tat/TAR-mediated *trans*-activation mechanism of primate lentiviruses, another mechanism for LTR activation involving recognition sites for transcription factors AP-1 and AP-4 has been proposed for visna virus (24).

In the present report, we demonstrate that BIV, like most other lentiviruses, *trans* activates its LTR during in vitro viral replication. Using transient-expression assays in BIV replication-permissive Cf2Th, EREp, and BLAC-20 cells, we observed a two- to sevenfold viral *trans* activation of BIV LTR-CAT constructs in BIV^{par}-infected cells compared with uninfected cells (Table 1). By analogy to other lentiviruses, these results imply that BIV contains a *trans*-acting factor which activates expression of BIV LTR-linked genes. Presumably, virus-mediated *trans* activation is further facilitated by the interaction of the putative BIV Tat protein with a TAR present in the BIV LTR mRNA sequences to enhance translation.

To complement the inherently variable results in transient-expression assays, we also developed a clonal indicator-reporter cell line (B4C) in which the BIV LTR-CAT construct was stably integrated into the host cell genome and used it to quantify the LTR *trans* activation in BIV-infected cells. As demonstrated in coculture experiments with our B4C indicator cells (Fig. 2), BIV106 and BIV127 induce a 10- to 15-fold activation of CAT expression while, interestingly, the parental BIV strain causes an 80-fold *trans* activation of the BIV127 LTR. These data suggest that either the BIV^{par}-infected cells contain a higher virus titer or the BIV^{par} isolate contains several genetic variants of BIV, some of which are stronger *trans* activators of the BIV127 LTR promoter than

either BIV106 or BIV127. If the first interpretation were true, a higher virus titer for BIV^{par} would be reflected in increased levels of BIV viral RNA found in BIV^{par} than in BIV127- and BIV106-infected cells. Data from our laboratory do not support this, since it was found that there are insignificant differences in the levels of total RNA in these BIV-infected cells overall (32). Also, the level of *tat* mRNA is not significantly higher in BIV^{par}-infected cells than in BIV127- and BIV106-infected cells, as determined by Northern analysis (31). The absence of increased levels of total viral RNA and/or increased levels of specific viral RNA transcripts (e.g., *tat*) implies the existence of *tat* genes encoding more potent *trans*-activator proteins and suggests that the high BIV^{par} *trans* activation results from altered Tat function rather than from increased viral transcription. It will be important to isolate and characterize these BIV^{par} *tat* genes to gain insight into the factor(s) involved in regulating viral transcription and replication. In addition to the *trans*-activation studies reported here, these indicator-reporter cell lines will be useful for monitoring virus recovery or infection in potentially BIV-infected cells and tissues, for testing the role of other putative BIV regulatory proteins in the LTR *trans* activation, and for examining the effectiveness of various antiviral protocols in preventing virus replication.

While levels of *trans* activation observed in BIV-infected cells are lower than the levels reported for some other lentiviruses (11, 23, 44, 50, 51), host cellular cofactors, transfection efficiency, and LTR structure could all dramatically affect the reported results. The dissimilar experimental controls used in previously reported studies will strongly influence the levels of *trans* activation reported for the various lentiviruses. It is also noted that *trans*-activation studies with HIV-1 LTR-CAT constructs have routinely been done with HIV-1 LTRs in which the U5 region is absent (2, 44). Using a similar BIV LTR structure, we observed that deletion of the U5 region from our BIV LTR-CAT construct (BIV LTRΔU5-CAT) produces an additional two- to fourfold amplification in BIV LTR-CAT *trans* activation above that reported in Fig. 2 in the present study. Deletion of the U5 region appears to increase the translational efficiency of the LTR-CAT mRNA rather than to affect transcriptional rates or mRNA stability, since the levels of CAT mRNA found in cells transfected with BIV LTR-CAT or BIV LTRΔU5-CAT were the same (32).

In studies with HIV-1, several unrelated viruses have been reported to *trans* activate the HIV-1 LTR (3, 17, 35). Since many of these viruses are found in HIV-1-seropositive individuals, it has been suggested that they play a role as cofactors in the activation of HIV-1 expression and replication or in the progression of AIDS. In a similar vein, we note that in the limited number of bovine herds surveyed in the United States, from 5 to 80% of the animals in these herds are found to be infected with BIV, and many of these are also infected with BLV and/or BSV (18). Because of the nearly ubiquitous nature of these three bovine retroviruses, we wished to examine whether BLV, which is known to encode a *trans*-activating factor (10, 39), or BSV, which has recently been cloned (36) and is related to the primate spumavirus that *trans* activates its LTR (28), can *trans* activate the BIV LTR and potentially serve as cofactors that influence BIV expression or replication. When BSV- or BLV-infected cells were cocultured with the B4C indicator cell line, very low levels of BIV LTR-CAT *trans* activation were observed compared with the level in the BIV-infected cells (2- to 3-fold versus 10- to 80-fold; Fig. 2). A similar lack of cross-*trans* activation between a lentivirus (HIV-1) and an

oncovirus (human T-cell lymphotropic virus type I) has been observed previously (14, 44). For the bovine retroviruses studied, these results demonstrate the relative specificity of BIV LTR *trans* activation, presumably by virtue of site-specific interactions between the putative BIV Tat and TAR sequence. The specificity of this Tat-TAR interaction is important, since the ability of these and other bovine viruses to activate BIV gene expression, and vice versa, may be critical in the regulation of replication and disease potential.

From our present studies, it is not discernable whether *trans* activation of the BIV LTR results from direct interaction of BIV Tat with the LTR or from indirect effects of BIV Tat (or other regulatory proteins) on unidentified host cell factors that interact with the LTR. Based on the similarities between BIV and other lentiviruses (16), we speculate that *trans* activation of the BIV LTR is mediated by direct interaction of Tat with TAR as well as the interaction of cellular factors with the BIV LTR TAR region. From published BIV LTR sequences (16), an RNA-folding algorithm predicts the presence of a double stem-loop structure 3' to the BIV RNA cap site (32) at a position comparable to that of the TAR sequence found in HIV-1 (38). The importance of this stem-loop structure and its function as a putative TAR element is presently being investigated. In addition to Tat-TAR interactions, recent studies show that cellular factors dramatically regulate the *trans* activation of HIV-1 in a variety of different cell types (4). By analogy, unidentified cellular factors could strongly influence the degree of *trans* activation observed with BIV in different cell types. In fact, the lack of proper host cellular factors might account for the relatively low level of *trans* activation observed compared with HIV-1, simian immunodeficiency virus, or equine infectious anemia virus. Once the actual bovine target cells of BIV infection are identified and propagated, we may find a significantly greater *trans* activation of the BIV LTR. We are isolating distinct populations of bovine leukocytes to study BIV cell tropisms within the natural host. In addition, we are presently attempting to identify cells which support BIV expression by creating transgenic mice which express the BIV127 LTR-CAT construct. The BIV LTR-CAT-carrying mice will be instrumental in determining both tissue-specific expression and temporal regulation of BIV LTR-directed genes. Future characterization of the *cis*-regulatory elements in the BIV LTR will help determine which LTR regions are critical for BIV gene expression in general and which LTR regions influence the specificity of BIV expression. Clearly, a better understanding of the molecular mechanisms of BIV *trans* activation is important for the development and advancement of BIV-animal models.

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