Characterization of the Internal Promoter of Simian Foamy Viruses

MEL CAMPBELL, LISA RENSHAW-GEGG, ROLF RENNE, AND PAUL A. LUCIW*

Department of Pathology, School of Medicine, University of California, Davis, Davis, California 95616

Received 22 February 1994/Accepted 6 May 1994

Simian and human foamy viruses (HFV and SFV), genetically related members of the spumavirus genus of retroviruses, have complex genome structures which encode the *gag*, *pol*, and *env* genes for virion proteins as well as additional open reading frames. One of these open reading frames is a viral transactivator, encoded by genes designated *taf* for SFV and *bel-1* for HFV, which augments transcription directed by the long terminal repeat (LTR) through *cis*-acting targets in the U3 domain of the LTR. Recently, an internal transcriptional promoter has been identified in sequences within the 3' end of the HFV *env* gene (M. Lochelt, W. Muranyi, and R. M. Flugel, Proc. Natl. Acad. Sci. 90:7317–7321, 1993). We have demonstrated by using transient expression assays in several tissue culture cell lines and by analyzing viral transcripts in infected cells that SFV-1 from a rhesus macaque and SFV-3 from an African green monkey also encode an internal promoter in the *env* gene. Transcription directed by the internal promoters of SFV-1 and SFV-3 is activated by the *taf-1* and *taf-3* gene products, respectively, in several cell types. The importance of a TATA box for the SFV-1 internal promoter was established by site-specific mutagenesis, and the 5' ends of transcripts initiating in the internal promoter have been determined. *cis*-acting sequences in the SFV-1 *env* gene required for the response to *taf-1* are contained within a 121-bp element located 5' to the TATA box in the internal promoter. This *taf-1*-responsive element in the internal promoter functions in a position- and orientation-independent fashion in a heterologous promoter and thus has the properties of an enhancer which depends on *taf-1* activity. Alignments reveal that the SFV-1 internal promoter and the SFV-1 LTR have little sequence relatedness. Cross-transactivation studies show that the transactivators of SFV-1 and HFV function on the internal promoter and LTR of the homologous virus but not on the heterologous virus. In summary, the genomes of simian and human foamy viruses direct viral transcription through both the promoter in the LTR and an internal promoter within the *env* gene, and each promoter contains unique enhancer-like elements regulated by the viral transactivator.

Spumaviruses, also designated foamy viruses, constitute a genus of retroviruses with complex genomes and have been isolated from several mammalian species, including humans and nonhuman primates (reviewed in reference 11). Although foamy viruses establish chronic latent and/or persistent infections in the host, the pathogenic potential of spumaviruses has not been clearly established (1, 36). These viruses encode *gag*, *pol*, and *env* as well as additional open reading frames (ORFs). The human foamy virus (HFV) has three ORFs in the region between *env* and the 3' long terminal repeat (LTR); these are designated *bel-1*, *bel-2*, and *bel-3* (reviewed in reference 6). Unique simian foamy viruses (SFVs) from rhesus macaques (SFV-1) and African green monkeys (SFV-3) possess two ORFs (ORF-1 and ORF-2) that exhibit sequence homology with *bel-1* and *bel-2* (20, 29). Several investigators have demonstrated that *bel-1* of HFV and ORF-1 of both SFV-1 and SFV-3 encode transcriptional transactivators which act through sequences in the U3 portion in the LTR of the homologous virus (13, 23, 24, 30, 31, 33). Accordingly, ORF-1 of the simian viruses has been designated the transactivator of foamy viruses (*taf-1* for SFV-1 and *taf-3* for SFV-3). The functions of *bel-2* and *bel-3* of HFV and ORF-2 of SFV-1 and SFV-3 remain to be defined.

To elucidate the mechanism of foamy virus transactivation, properties of both the target element in the LTRs and the transactivator proteins (i.e., products of *bel-1* and *taf-1*) have been studied in transient expression assays in tissue culture

cells. The target for *bel-1* and *taf-1* involves unique sequences dispersed in the U3 portion of the 5' LTR of HFV and SFV-1, respectively; these target elements are upstream from the TATA box in the LTR and have properties of transcriptional enhancer elements which are dependent on the viral transactivator (5, 12, 21, 30, 33). Foamy virus transactivators, like many other transcriptional regulatory factors (7), have a modular structure composed of multiple domains which function to localize the transactivator to the cell nucleus, influence selection of the viral DNA target element(s), and activate transcription (8, 10, 22, 34). Because the primate foamy viruses display no homology with LTRs and regulatory genes of other complex retroviruses (i.e., human and simian immunodeficiency viruses and human and simian T-cell leukemia viruses), the mechanisms which mediate foamy virus gene expression may be unique (6, 20). Whether the foamy virus transactivators interact directly or indirectly (via cellular factors) with target DNA sequences in the homologous LTR is not established. Accordingly, the precise mechanism of foamy virus transactivation and the potential cellular factors involved in this process remain to be elucidated.

Although the 5' LTR of the primate foamy viruses contains a transcriptional promoter for synthesis of full-length and spliced viral transcripts, recent studies have identified an internal promoter located in nucleotide sequences within the 3' end of *env* of HFV (17). This internal promoter is transactivated by Bel-1 and directs expression of unspliced and spliced viral RNAs that direct synthesis of Bel-1 as well as other viral proteins (16). The target element for Bel-1 in the HFV internal promoter has not been identified, and the transcriptional properties of this element have not been investigated. SFV-1

* Corresponding author. Phone: (916) 752-3430. Fax: (916) 752-4548.

TABLE 1. Synthetic oligonucleotides for constructing plasmids and mutagenesis

Insert	Region amplified	Sense primer/antisense primer ^a
S ¹ IP-1	SFV-1 bp 8051–8502	5' GCGGGTACCGCGTGTGAAATGCAACAG 3'/ 5' CGGGATCCCGGCTGCAGCAGGCCAAACG 3'
S ¹ IP-2	SFV-1 bp 8183–8502	5' GCGGGTACCGCGGCATATGTACCTAGTG 3'/ 5' CGGGATCCCGGCTGCAGCAGGCCAAACG 3'
S ¹ IP-3	SFV-1 bp 8333–8502	5' GCGGGTACCGCGGCCAGCTTGAATCAC 3'/ 5' CGGGATCCCGGCTGCAGCAGGCCAAACG 3'
S ¹ IP-4	SFV-1 bp 8249–8502	5' GCGGGTACCGCGCCACTTTATGCTGAAAC 3'/ 5' CGGGATCCCGGCTGCAGCAGGCCAAACG 3'
S ¹ IP-5	SFV-1 bp 8249–8420	5' GCGGGTACCGCGCCACTTTATGCTGAAAC 3'/ 5' CGGGATCCCGGAGAAAGCTGTGCTTTGG 3'
S ¹ IP-6	SFV-1 bp 8183–8370	5' GCGGGTACCGCGGCATATGTACCTAGTG 3'/ 5' CGGGATCCCGGTAGAAGTAACCTTCTATTTC 3'
S ¹ IP-5 mutant M1 ^b	SFV-1 bp 8249–8420	5' GCGGGTACCGCGCCACTTTATGCTGAAAC 3'/ 5' CGGGATCCCGGAGAAAGCTGTGCTTTGGCCCT TTCGATCTGGTCTTTTGGGTTCTCTGTGTAG 3'
S ³ IP-2	SFV-3 bp 9534–9820	5' GGTACCGCATATGTTCTAGTATTGTAAC 3'/ 5' CGGGATCCGCAAGTTGTTGAATCCAAGCAGG 3'
HFV internal promoter	HFV bp 8921–9332	5' GCGGGTACCGCGTCTGGGCTGAAGC 3'/ 5' CGGGATCCCGGCTGGCCAGACGTC 3'
HFV LTR	HFV bp 53–834	5' GGTACCGTGGTGAATGCCACTAG 3'/ 5' CGGGATCCGAGCTCCCGTACAATCTAG 3'
HFV <i>bel-1</i>	HFV bp 9447–10420	5' GAAGATCTGGTCATTCTCTTGGTTATTC 3'/ 5' TGCGGTCGACCAGCCTGCAGCAAGTTAG 3'

^a *Asp* 718 and *Bam*HI sites used for cloning purposes are underlined.

^b The 3-nt mutation in the TATA box of pS¹IP-5 to produce pS¹IP-5/CAT-M1 is in boldface.

and SFV-3 show some homology to HFV throughout their genomes; however, sequence alignments are not a reliable means to identify *cis*-acting regulatory elements. Accordingly, we performed several functional analyses to demonstrate that both SFV-1 and SFV-3 encode internal promoters which are regulated by the respective viral transactivators, encoded by *taf-1* and *taf-3*. In addition, we identified and characterized the *cis*-acting target element in the SFV-1 internal promoter that responds to *taf-1*. These and other observations in this report support a model in which different mechanisms regulate transcriptional transactivation of the LTR and internal promoter of the primate foamy viruses. Differential regulation of viral transcription through two viral promoters (i.e., the LTR and the internal promoter) may play an important role in viral latency and/or persistent infection in primates.

MATERIALS AND METHODS

Cell culture and transfections. L929 (transformed murine fibroblast), COS-7 (simian virus 40 [SV40]-transformed African green monkey fibroblast), Vero (African green monkey kidney cell), and Cf2Th (canine thymus fibroblast) cell lines were obtained from the American Type Culture Collection (Rockville, Md.). Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. For transfections in all four cell lines, 2 µg of reporter plasmid expressing chloramphenicol acetyltransferase (CAT) and 2 µg of either pSP65 (control) or effector plasmid (expressing a foamy virus transactivator; see below) were mixed with DEAE-dextran and applied to 3×10^5 cells in a 35-mm-diameter plate (21). At 48 h after transfection, cell extracts were prepared and assayed for CAT activity by measuring conversion of ³H-acetyl coenzyme A to ³H-acetyl chloramphenicol (21, 27). All transfections were performed in triplicate, and variation was less than 15%.

Plasmid constructions. Plasmids p3'SFV-1, p3'SFV-3, and pHSRV (i.e., proviral form of HFV) served as the sources of DNA for all recombinant constructs used in this study (18, 21,

29). SFV-1 plasmids used in this study are derived from cloned viral DNA which yielded infectious virus in transfected cells (22a).

Various segments in the 3' end of the *env* gene in each of the foamy virus clones listed above were amplified by PCR, using the primer pairs in Table 1. Oligonucleotides were synthesized by using a Gene Assembler (Pharmacia, Piscataway, N.J.). One hundred nanograms of template was amplified by 30 cycles of PCR consisting of 1 min of denaturation at 95°C, 1 min of annealing at 55°C, and 1 min of extension at 72°C (32). Following amplification, the viral DNA fragments were purified by electrophoresis through agarose gels, digested with appropriate restriction enzymes, and inserted into reporter plasmids encoding *cat*. Viral DNA fragments to be tested for promoter activity were inserted upstream of *cat* in plasmid pCAT-22A2s (32). We have designated these reporter plasmids pS¹IP-*n*/CAT and pS³IP-*n*/CAT for SFV internal promoter inserts derived from SFV-1 and SFV-3, respectively; *n* is assigned a number to identify plasmids with unique viral inserts. (See Fig. 2 for structures of reporter plasmids with inserts from the internal promoter region of SFV-1.) Plasmid pS¹IP-1/CAT/A/H was constructed by digesting pS¹IP-1/CAT with *Asp* 718 and *Hind*III, followed by fill-in and religation. Plasmid pS¹IP-1/CAT/H/B was similarly constructed by digesting pS¹IP-1/CAT with *Hind*III and *Bam*HI, followed by fill-in and religation. The mutation designed to change the putative TATA box in the internal promoter of SFV-1 (bp 8380 to 8385) was constructed by PCR mutagenesis. This mutation produces in a 3-bp change (bp 8380 to 8382) from TAT to CCC (5' TATAAA 3' to 5' CCCAAA 3') and was introduced through the antisense primer (Table 1). To test for enhancer activity, a fragment from the SFV-1 internal promoter region (bp 8183 to 8370), upstream from the TATA box, was cloned either upstream or downstream of *cat* in plasmid pCAT-Promoter (Promega, Madison, Wis.), which contains the SV40 basal promoter but lacks the SV40 enhancer elements. Viral DNA inserts in all plasmids in this report were verified by

DNA sequencing (data not shown). The reporter plasmid containing the SFV-1 LTR is pS¹L/CAT-41 (formerly designated pLTR/CAT-41 [21]), and the reporter plasmid containing the SFV-3 LTR is pS³L/CAT-41 (formerly designated pSFV-3/ Δ R-U5/CAT [30]). Each LTR in these two reporter plasmids is truncated immediately downstream of +1 in R (i.e., at the *Sac*I site in the LTR), and thus the plasmids lack all of R and U5 (21, 30). The *Taf*-1 and *Taf*-3 expression plasmids (pSV-*taf*-1 and pSV-*taf*-3, respectively) have been previously described (24, 30).

The HFV reporter plasmid, pHIP/CAT, was constructed by inserting a PCR-amplified fragment encompassing the HFV internal promoter region (HFV bp 8921 to 9332) upstream of *cat* in pCAT-22A2s (17, 32). A plasmid for expressing HFV Bel-1 was constructed by cloning a PCR fragment corresponding to the *bel*-1 ORF (HFV bp 9447 to 10420) into the SV40 expression plasmid pSV7d (18a). To produce a reporter plasmid with the HFV LTR, a PCR fragment corresponding to the HFV U3 region (HFV bp 53 to 834) was inserted into pCAT-22A2s to produce pHIL/CAT (18, 32).

RNA extraction and primer extension. Total RNA was harvested from L929 cells 48 h following transfection with CAT-based plasmids or from Cf2Th cells 72 h postinfection with a stock of SFV-1. RNA was isolated by the single-step method (3) using Tri-Reagent (Molecular Research Center, Inc., Cincinnati, Ohio) followed by DNase I treatment and reprecipitation. The primer extension reaction mixture contained 10 μ g of RNA and an oligonucleotide primer labeled at the 5' end in a kinase reaction with [³²P]ATP. Antisense primer 387 (5' GTTCTTTACGATGCCATTGGG 3') is complementary to the 5' part of the *cat* gene, and antisense primer 396 (5' TATAAAGGAAGCTGCAGCAGGCCAAACG 3') is complementary to SFV-1 bp 8484 to 8512. Primer extension reactions were performed under conditions recommended by the manufacturer, using a commercially available kit (Promega). Radiolabeled cDNA products from the extension reactions were electrophoresed under denaturing conditions on a 6% acrylamide gel.

In vitro transcription. Transcription reactions were done with 100 ng of template DNA and a HeLa cell nuclear extract and were carried out as recommended by the manufacturer (Promega); the radioactive label was [³²P]GTP for the in vitro reactions. To prepare templates for transcription, plasmids containing candidate promoter sequences were digested with enzymes that bracketed the putative internal promoter element and the LTR. DNA fragments containing the candidate promoter were purified by electrophoresis on agarose gels and then used as templates for in vitro transcription. Radiolabeled RNA made in vitro with [³²P]GTP was electrophoresed under denaturing conditions on a 6% acrylamide gel.

Nucleotide sequence accession numbers. The following are GenBank accession numbers for the primate foamy viruses: M55729 for the SFV-1 LTR, M33561 for the SFV-1 *env* gene, M74895 for the SFV-3 genome, and X58484 for the HFV genome.

RESULTS

SFV-1 contains an internal promoter within the envelope coding region. The 3' end of the *env* gene of HFV encodes a transcriptional promoter which is activated by Bel-1 (17). Nucleotide sequence comparisons reveal that the 3' end of the *env* gene of SFV-1 shows clustered sequence homology with HFV and SFV-3; in addition, a putative TATA box is conserved in this region for all three viruses (Fig. 1). Accordingly, we tested whether the 3' end of the *env* gene of SFV-1 also

encodes a promoter in transient expression assays in several cell lines. A DNA fragment containing bp 8051 to 8502 of SFV-1 was inserted upstream of *cat* in the promoterless reporter plasmid pCAT-22A2s to produce the reporter plasmid pS¹IP-1/CAT (Fig. 2). This reporter plasmid was transfected into primate (COS-7 and Vero), murine (L929), and canine (Cf2Th) cell lines in the presence and absence of a plasmid expressing the *taf*-1 gene (i.e., pSV-*taf*-1). After 48 h of incubation, extracts were prepared from the transfected cells and assayed for CAT activity. As shown in Table 2, pS¹IP-1/CAT yielded very low basal levels of CAT activity; however, in cells cotransfected with pS¹IP-1/CAT and pSV-*taf*-1, high levels of CAT activity were produced in all four cell lines. Several promoter plasmids with deletions in the SFV-1 insert in pS¹IP-1/CAT were tested to map the internal promoter more precisely (Fig. 2). The promoterless reporter plasmid pCAT-22A2s exhibited no response to pSV-*taf*-1 in transient expression assays (data not shown). These studies revealed that efficient expression of CAT in response to *Taf*-1 required the SFV-1 sequence between bp 8249 to 8420 (pS¹IP-5/CAT) (Table 2 and Fig. 2). Because the basal levels of CAT activity (i.e., transfection of reporter plasmid and pSP65) were similar for all plasmids tested in Fig. 2, the 3' portion of SFV-1 *env* does not encode a negative regulatory element for the internal promoter.

The TATA box in the internal promoter is necessary for full *taf* responsiveness. To provide additional evidence that the sequence within the 3' end of SFV-1 *env* functions as a promoter element, PCR mutagenesis was used to alter the sequence of the putative TATA box (bp 8380 to 8385) from 5' TATAAA 3' to 5' CCCAAA 3'. The oligonucleotide containing this mutation (Table 1) was used to generate a PCR fragment (bp 8249 to 8420) which corresponds to the SFV-1 insert in pS¹IP-5/CAT (Fig. 2). This PCR product was cloned upstream of the *cat* gene in pCAT-22A2s to generate pS¹IP-5/CAT-M1. Cells transfected with this TATA mutant plasmid showed levels of transactivation that were reduced 7-fold (Cf2Th cells) to 15-fold (Vero cells) relative to the levels of transactivation with the wild-type plasmid in these cell lines (Table 2). The level of transactivation of the TATA mutant plasmid was only two- to fourfold above the basal level. Thus, the TATA box is a critical component of the internal promoter, and this finding supports the claim that the internal promoter functions to initiate transcription by the host cell RNA polymerase II complex. This conclusion is consistent with the results obtained with the reporter plasmids pS¹IP-6/CAT and pS¹IP-1/CAT-H/B; both of these reporter plasmids lack the TATA box at bp 8380 to 8385 and do not respond efficiently to *Taf*-1 (Table 2).

SFV-3 contains an internal promoter. SFV-3, a foamy virus isolate from an African green monkey, exhibits sequence homology with SFV-1 (Fig. 1). To determine whether SFV-3 also encodes an internal promoter, a DNA fragment representing sequences in the 3' portion of SFV-3 *env* (bp 9534 to 9820) (Fig. 1) was cloned upstream of the *cat* gene in pCAT-22A2s to produce the reporter plasmid pS³IP-2/CAT. In COS-7, Cf2Th, and Vero cells, low basal promoter activity is observed after transfection with pS³IP-2/CAT; however, high levels of CAT activity are observed in each cell line cotransfected with pS³IP-2/CAT and a plasmid expressing the homologous transactivator (i.e., pSV-*taf*-3) (Table 3). Thus, the 3' end of SFV-3 *env* also encodes a promoter element which is transactivated by *taf*-3.

Identification of the *taf*-1-responsive element in the SFV-1 internal promoter. Plasmid pCAT-Promoter (Promega) contains the basal SV40 early promoter upstream of *cat* but lacks

FIG. 1. Sequence alignment of the region of *env* encompassing the internal promoters of HFV (top), SFV-1 (middle), and SFV-3 (bottom). Alignments were established with the Geneworks program, version 2.2.1, provided by the Genetics Computer Group, University of Wisconsin. The regions aligned for each virus are bp 8847 to 9347 for HFV, bp 8011 to 8511 for SFV-1, and bp 9362 to 9862 for SFV-3. GenBank accession numbers for these three primate foamy viruses are given in Materials and Methods. The TATA box for cellular RNA polymerase II is boxed. The arrow labeled H designates the 5' end of transcripts initiated in the HFV internal promoter (17), and the arrow labeled S designates the 5' end of transcripts initiated in the SFV-1 internal promoter as deduced from experiments in this study (see Fig. 4). Nucleotide sequences conserved for all three viruses are shaded. Pairwise homologies for the sequences shown are as follows: SFV-1 versus SFV-3, 77%; SFV-3 versus HFV, 69%; and SFV-1 versus HFV, 66%.

the SV40 enhancer elements; thus, this vector is useful for identifying and characterizing transcriptional enhancers. Hybrid promoters were constructed in which the SFV-1 sequence upstream of the TATA box in the internal promoter (bp 8183 to 8370) was cloned upstream and downstream of *cat* in both

orientations in pCAT-Promoter (Fig. 3). Transient expression assays in Cf2Th and Vero cells with these hybrid promoter plasmids revealed that CAT activity directed by the basal SV40 early promoter was not significantly altered by the SFV-1 insert (Fig. 3). However, in cotransfection experiments with each of

FIG. 2. Schematic presentation of the SFV-1 plasmids encompassing the internal promoter in the *env* gene. The hatched box indicates the TATA box (bp 8380 to 8385), and the arrow indicates the 5' end of viral transcripts (bp 8408; see Fig. 1). The *Hind*III site used for construction of pS¹IP-1/CAT/A/H and pS¹IP-1/CAT/H/B is shown. *Asp* 718 and *Bam*HI sites were present in the sense and antisense PCR primers, respectively (Table 1). The solid lines represent SFV-1 sequences remaining in the reporter plasmids.

these hybrid promoter plasmids and the effector plasmid expressing *taf-1* (pSV-*taf-1*), levels of CAT in both cell lines were enhanced from 10- to 30-fold (Fig. 3). Thus, the region from bp 8183 to 8370 functions as a transcriptional enhancer which is dependent on the viral transactivator. Additional transient expression assays have demonstrated that the region between bp 7112 and 8182 does not contain an enhancer element (data not shown). These experiments with reporter plasmids containing hybrid promoters, taken together with the deletion mapping experiment shown in Table 2, localize the *taf-1*-responsive element in the SFV-1 internal promoter (TRE^{IP}) to bp 8249 (5' end of the SFV-1 insert in pS¹IP-5/CAT; Table 2 and Fig. 2) to 8370 (pCAT5'SIP-6S; Fig. 3). Additional studies will be necessary to more precisely define the minimal sequence element within this TRE^{IP} that is required for transactivation.

Identification of the transcription start site in the SFV-1 internal promoter. To determine the start site of transcription for the SFV-1 internal promoter, DNA fragments encompass-

ing the SFV-1 internal promoter were tested in an in vitro transcription reaction. When used as templates in a HeLa cell nuclear extract, both the *Eco*RI and *Eco*RI-*Pvu*II fragments of pS¹IP-2/CAT produced a low level of runoff transcript of the expected size (Fig. 4A, lanes 2 and 3); this size estimate is based on the assumption that the 5' ends of transcripts initiate about 25 bp 3' of the TATA box in the internal promoter (Fig. 1) (9). Accordingly, the in vitro transcription analysis maps the start site for the internal promoter to bp 8408 to 8410. Levels of transcripts produced by the SFV-1 internal promoter in this in vitro transcription system were low relative to those produced by the cytomegalovirus immediate-early promoter fragment (Fig. 4A, lane 1). Under the conditions used, a runoff transcript was not detected from an *Eco*RI fragment template representing the promoter region of the SFV-1 LTR (Fig. 4A, lane 4). Attempts to demonstrate transactivation of the SFV-1 internal promoter and LTR templates by adding purified recombinant Taf-1 to the in vitro transcription reactions have been unsuccessful (37).

To precisely map the initiation site for transcription directed by pS¹IP-2/CAT, RNA from cells transfected with this plasmid was analyzed in primer extension reactions. Total RNA was isolated from L929 cells transfected with a mixture of pSV-*taf-1* and SP65 or a mixture of pS¹IP-2/CAT and pSV-*taf-1*. Antisense oligonucleotide 387 was used in a primer extension reaction with RNA isolated from cotransfected cell cultures. Products of the primer extension reaction were electrophoresed on an acrylamide gel adjacent to the reaction products of a dideoxynucleotide sequencing ladder of pS¹IP-2/CAT primed with oligonucleotide 387. When aligned with the sequencing ladder, the 219-nucleotide (nt) cDNA produced in the primer extension reaction identifies the cytosine at bp 8408 in the antisense strand (marked by an asterisk in Fig. 4B). This maps the 5' end of SFV-1 transcripts initiated in the internal promoter to the guanine at bp 8408, which is 23 bp 3' of the TATA box at bp 8380 to 8385 (Fig. 1).

To determine if the internal promoter is active in SFV-1-infected cells, total RNA harvested from Cf2Th cells infected with SFV-1 was analyzed in primer extension reactions using primer oligonucleotide 396. This oligonucleotide is 5' of the small intron (123 nt) just upstream of the *taf-1* ORF (24). A predominant cDNA of 105 nt was produced by priming with oligonucleotide 396 (Fig. 4C, lane 3). Thus, the 5' end of transcripts produced by the SFV-1 internal promoter in in-

TABLE 2. Deletion analysis to identify the SFV-1 internal promoter in transient expression assays^a

Reporter	CF2TH			Vero			COS-7			L929		
	cpm		Fold ^b	cpm		Fold	cpm		Fold	cpm		Fold
	-Taf-1	+Taf-1		-Taf-1	+Taf-1		-Taf-1	+Taf-1		-Taf-1	+Taf-1	
pS ¹ IP-1/CAT	4,475	167,535	37	4,580	90,708	20	4,332	170,537	39	5,328	1,327,171	249
pS ¹ IP-2/CAT	3,341	169,397	51	5,508	417,203	76	4,255	264,895	62	5,374	1,474,432	274
pS ¹ IP-3/CAT	3,151	27,239	9	5,771	64,926	11	7,224	55,859	8	ND ^c	ND	ND
pS ¹ IP-4/CAT	3,774	178,136	47	5,525	298,260	54						
pS ¹ IP-5/CAT	4,258	120,400	28	5,126	148,102	29						
pS ¹ IP-5/CAT-M1	4,325	18,324	4	2,322	4,676	2						
pS ¹ IP-6/CAT	3,257	9,468	3	5,464	11,408	2						
pS ¹ A/H/CAT	3,715	29,240	8	5,879	22,605	4	4,359	57,768	13	ND	ND	ND
pS ¹ H/B/CAT	4,385	5,420	1	4,534	7,295	2	3,875	9,660	2	4,282	43,846	10
pS ¹ L/CAT-41	3,501	185,733	53	5,414	39,902	7	4,163	222,155	53	3,961	1,249,189	315

^a Values in the -Taf-1 (cotransfection of reporter plasmid and pSP65) and +Taf-1 (cotransfection of reporter plasmid and pSV-Taf-1) columns represent the means from triplicate transfected cultures.

^b Relative activity in the presence of Taf-1.

^c ND, not done.

TABLE 3. Internal promoter in the *env* gene of SFV-3^a

Cell type	Reporter	cpm		Fold ^b
		-Taf-3	+Taf-3	
COS-7	pS ³ IP-2/CAT	5,240	18,377	4
	pS ³ L/CAT-41	2,426	382,975	158
Cf2Th	pS ³ IP-2/CAT	4,192	133,948	32
	pS ³ L/CAT-41	4,964	276,867	56
Vero	pS ³ IP-2/CAT	7,057	349,459	50
	pS ³ L/CAT-41	7,982	2,099,482	263

^a Values in the -Taf-3 (cotransfection of reporter plasmid and pSP65) and +Taf-3 (cotransfection of reporter plasmid and pSV-Taf-3) columns represent means from triplicate transfected cultures.

^b Relative activity in the presence of Taf-3.

fect cells maps to the guanine at bp 8408. A larger cDNA, about 185 nt, was also observed; although the exact identity of this product was not determined, this cDNA product likely represents viral mRNA initiated in the 5' LTR. In control experiments, no primer extension products were detected when SFV-1 oligonucleotide 396 was used to prime cDNA synthesis on RNA from uninfected Cf2Th cells (Fig. 4C, lane 2).

Specificity of *taf-1* and *bel-1* for heterologous foamy virus promoters. The internal promoters and LTRs of the primate foamy viruses exhibit patterns of clustered sequence homology (Fig. 1 and 5). In addition, the transactivators Taf-1 and Bel-1 have similar functional domain structures (8, 10, 22, 34). Accordingly, the LTR and internal promoter of both SFV-1 and HFV were tested for responses to the heterologous transactivator as well as the homologous transactivator. Taf-1 demonstrated a small transactivation effect on the HFV internal promoter (twofold) and had no effect on the HFV LTR (Table 4). HFV Bel-1 was active only on the HFV internal promoter and LTR; Bel-1 did not act on either the internal promoter or LTR of SFV-1 (Table 4). These results suggest that the *taf-1* and *bel-1* gene products function either through different sequences or through different cellular factors to transactivate promoters in SFV-1 and HFV, respectively (see Discussion).

DISCUSSION

This report identifies and characterizes the internal promoter encoded in the genomes of simian foamy viruses (i.e., SFV-1 and SFV-3), and transcriptional initiation by this promoter is shown to be regulated by the viral transactivators (i.e., Taf-1 and Taf-3, respectively). In addition, comparisons are made between transactivation of the SFV-1 LTR and the internal promoter to provide a basis for further investigations

FIG. 3. Hybrid promoter constructs to evaluate *taf-1*-responsive-ness and enhancer activity in the SFV-1 internal promoter. (A) Schematic representation of pCAT-Promoter (Promega). S and AS designates sense and antisense orientations of the SFV-1 insert (bp 8183 to 8370) with respect to the *cat* gene. (B) Results of transfection experiments. Cf2Th and Vero cells were transfected with the indicated reporter plasmid and either pSP65 or pSV-Taf-1. At 48 h following transfection, cells were harvested and assayed for CAT activity. The numbers in columns labeled -Taf-1 (cotransfected with pSP65) and +Taf-1 (cotransfected with pSV-taf) are counts per minute for CAT activity, and Fold indicates the relative CAT activity in the presence of Taf-1. ND, not done.

on mechanisms regulating foamy virus transcription. Transient expression assays with various reporter plasmids in several cell lines, representing different species, were used to demonstrate that the *taf-1*-responsive element (i.e., TRE^{IP}) is contained within a 121-bp sequence (bp 8249 to 8370) located immediately 5' to a TATA box in the SFV-1 internal promoter (Fig. 1). The 5' ends of viral transcripts initiated within the internal promoter were identified both in cells transfected with a reporter plasmid containing the SFV-1 internal promoter and in cells productively infected with SFV-1. The initiation site in these experiments in transfected cells and infected cells is about 25 bp downstream from the TATA box (bp 8380 to 8385) (Fig. 1). Mutation of this TATA box in the internal promoter reduced the response level to *taf-1*. Although very little sequence homology is noted in the regions immediately adjacent to the TATA box in the internal promoter and the LTR (Fig. 5A), it is presumed that both promoters interact with TATA-binding factor TFIID, which is required for initiation by the host cell RNA polymerase II complex (9). A search for consensus sequences for transcription factors (TFD

TABLE 4. SFV-1 and HFV LTR and internal promoter responses to Taf-1 and Bel-1^a

Reporter	COS-7					Vero				
	cpm			Fold ^b		cpm			Fold	
	Basal	+Taf-1	+Bel-1	Taf-1	Bel-1	Basal	+Taf-1	+Bel-1	Taf-1	Bel-1
pS ¹ IP-2/CAT	7,630	84,965	3,251	11	1	7,899	669,732	6,812	85	1
pS ¹ L/CAT-41	2,129	47,301	2,312	22	1	7,997	167,377	6,779	21	1
pHIP/CAT	3,575	8,286	46,602	2	13	5,696	9,177	194,187	2	34
pHL/CAT-41	1,872	1,791	106,588	1	57	8,775	10,061	71,005	1	8

^a Values in the basal (cotransfection of reporter plasmid and pSP65), +Taf-1 (cotransfection of reporter plasmid and pSV-Taf-1), and +Bel-1 (cotransfection of reporter plasmid and pSV-Bel-1) columns represent means from triplicate transfected cultures.

^b Relative activity in the presence of the indicated transactivator.

FIG. 4. Mapping of 5' ends of transcripts initiated in the SFV-1 internal promoter. (A) In vitro transcription analysis of restriction enzyme fragments encoding the SFV-1 internal promoter. In vitro transcription reactions were performed with a HeLa cell nuclear extract and 100 ng of purified restriction fragment DNA. The MgCl_2 concentration was 3 mM, and the radiolabeled nucleotide triphosphate was [^{32}P]GTP (3,000 Ci/mmol, 10 mCi/ml; New England Nuclear). Reaction products were phenol extracted, ethanol precipitated, dried, and resuspended in 10 μl of loading dye. One half of the reaction was electrophoresed under denaturing conditions on a 6% acrylamide gel. Templates used for the in vitro transcription reaction and the predicted sizes for the runoff transcripts are as follows: lane 1, positive control with the cytomegalovirus immediate-early gene promoter, 363 nt; lane 2, *EcoRI* fragment from pS¹IP-2/CAT, 369 nt; lane 3, *EcoRI*-*PvuII* fragment from pS¹IP-2/CAT, 266 nt; lane 4, pS¹LCAT-41 *EcoRI* fragment, 259 nt. The arrows indicate the runoff transcripts from these internal promoter templates. A dideoxy sequencing ladder of pS¹IP-2/CAT served as a marker for size in nucleotides (not shown). The autoradiogram shown is a 7-day exposure. (B) Identification of the 5' ends of transcripts initiated in the SFV-1 internal promoter in transfected cells. Primer extension reactions were performed with ^{32}P -labeled oligonucleotide 387 and 10 μg of total RNA isolated from L929 cells transfected with pSP65 and pSV-taf-1 (lane 2) or pS¹IP-2/CAT and pSV-taf-1 (lane 3). Lane 1 is product from a control reaction with no template RNA. The sequencing ladder is pS¹IP-2/CAT primed with oligonucleotide 387. The sequence of the antisense strand is shown, and the cytosine at bp 8408 corresponding to the 5' end of the transcript initiated in the SFV-1 internal promoter is designated with an asterisk. The autoradiogram is a 7-day exposure. (C) Identification of the 5' ends of transcripts initiated in the SFV-1 internal promoter in infected Cf2Th cells. Primer extension reactions were performed with ^{32}P -labeled oligonucleotide 396 and 10 μg of total RNA isolated from uninfected Cf2Th cells (lane 2) or acutely infected Cf2Th cells 3 days postinfection (lane 3). Lane 1 is a control with no RNA template. The sequencing ladder is p3'SFV-1 primed with oligonucleotide 396. The lower arrow designates the 105-nt cDNA that initiates at position 8408 within the internal promoter. The upper arrow shows the cDNA at about 185 nt which is presumed to represent a viral transcript initiated in the 5' LTR. The autoradiogram is a 7-day exposure.

release 3.0, National Center for Biotechnology Information) in the SFV-1 internal promoter region revealed a potential binding site for nuclear factor 1 in the TRE^{IP} at nt 8323 to 8336, upstream from the TATA box (Fig. 1). In addition, a consensus binding site for the glucocorticoid response element is located upstream from the SFV-1 TRE^{IP} at bp 8171 to 8176 (Fig. 1). Consensus sequences for nuclear factor 1 and the glucocorticoid response element are missing in the HFV internal promoter region. Whether these sites play a role in transcription from the SFV-1 internal promoter is not known, and the potential remains that this promoter of primate foamy viruses encodes *cis*-acting sequences which bind novel transcription factors.

SFV-1 TRE^{IP} has the properties of an enhancer element which is dependent on the viral transactivator (2). In addition, the SFV-1 LTR contains promoter-proximal (bp -403 to -125) and promoter-distal (bp -1196 to -880) elements which respond to the viral transactivator and behave like enhancer elements (21; also see references 5 and 14). Accordingly, in the context of an integrated provirus, Taf-1 has the capacity to regulate both viral promoters and may also influence transcription of cellular promoters located near the site of proviral integration (15).

Although the LTR and internal promoter of the primate foamy viruses contain enhancer elements which respond to the transactivator of the respective virus, a critical issue is whether

FIG. 5. Sequence relationships of the SFV-1 internal promoter and the SFV-1 LTR. All alignments were performed with the Geneworks program, version 2.2.1, as described in the legend to Fig. 1. (A) Relationship of the region encompassing the TATA box of the internal promoter and the SFV-1 LTR. A region in the SFV-1 internal promoter (bp 8371 to 8458) encompassing the TATA box was selected; this region, designated IP (internal promoter), demonstrated alignment with bp -37 to +50 in the LTR as shown. (B) Relationship of the SFV-1 TRE^{IP} (bp 8249 to 8370) and the promoter-distal *taf*-responsive element in the LTR (bp -1196 to -880) (24). Sequences in the TRE^{IP} demonstrated alignment with a portion of the promoter-distal *taf*-responsive element in the LTR (bp -996 to -880); the remaining sequences in the promoter-distal *taf*-responsive element exhibited no capacity for alignment by the Geneworks program and are not shown. (C) Sequence alignment of the SFV-1 TRE^{IP} (bp 8249 to 8370) and the promoter-proximal *taf*-responsive element in the SFV-1 LTR (bp -403 to -125) (21). Sequences in the TRE^{IP} demonstrated alignment with a portion of the promoter-proximal *taf*-responsive element in the LTR (bp -253 to -125); the remaining sequences in the promoter-proximal *taf*-responsive element displayed no capacity for alignment by the Geneworks program and are not shown.

both promoters in each virus are regulated through identical mechanisms. Insight into this issue is provided by analysis of sequence homologies in the target elements and the results of transactivation experiments in different cell types. Alignment of the sequences of the SFV-1 TRE^{IP} (bp 8249 to 8370) with the promoter-distal (bp -1196 to -880) and promoter-proximal (bp -403 to -125) *taf*-responsive sequences in the LTR reveal only very short stretches of homology (Fig. 5B and C). In addition, very low homologies are noted when the putative TRE^{IP} and sequences in the LTR of either SFV-3 or HFV are

aligned in the respective viruses (2a). Thus, the low homology between TRE^{IP} and LTR sequences within each virus supports a model in which transactivation through these two elements may not involve the same factor(s).

The pattern of transactivation displayed in different cell types also suggests that the mechanism of transactivation through the TRE^{IP} and the LTR of each virus may differ because the ratio of transactivation for the internal promoter relative to the LTR is not constant for different cell types. For example, in Vero cells, the SFV-1 internal promoter shows a

greater response to Taf-1 than the SFV-1 LTR does, whereas both the internal promoter and the LTR of SFV-1 show similar levels of transactivation in COS-7, Cf2Th, and L929 cells (Tables 2 and 4). Studies on SFV-3 and HFV also demonstrate that cell type has a large effect on the magnitude of transactivation of the internal promoter relative to the LTR by the transactivator of the homologous virus (Tables 3 and 4). These patterns of cell-type-specific transactivation on the internal promoter and LTR of each virus may be due to differences in levels of a putative cellular factor(s) which mediates transactivation in the cell lines tested.

Studies on cross-transactivation reveal that Bel-1 is inactive on the SFV-1 internal promoter and LTR; in addition, Taf-1 is inactive on the HFV internal promoter and LTR (Table 4). This lack of cross-transactivation is consistent with a model in which the foamy virus transactivators function by direct binding to sequence elements unique to the internal promoter and LTR of the respective virus. On the other hand, it is possible that Bel-1 and Taf-1 function through indirect mechanisms involving different cellular factors which recognize unique viral target elements. To resolve these alternatives, it will be necessary to (i) examine binding of Bel-1 and Taf-1 to targets in the homologous and the heterologous virus and (ii) identify a putative cellular factor(s) which may, in concert with the viral transactivator, bind to target elements and thereby augment transcription.

Knowledge of the complex domain structure of the foamy virus transactivators also provides a basis for considering mechanisms that control transcription in both the LTR and internal promoter. The domain structures of Taf-1 and Bel-1 have been elucidated through studies on transcription regulated by the LTR: (i) an N-terminal domain influences the efficiency of transactivation, (ii) a central domain contains a nuclear localization signal and is presumed to govern target specificity, and (iii) a C-terminal domain is essential for transactivation (8, 10, 22, 34). Whether this domain structure for Taf-1 and Bel-1, defined by studies on transactivation of the LTR of the homologous virus, will hold for transactivation through the internal promoter has yet to be established. It is possible that different transactivator domains are involved in regulating the LTR and internal promoter of the homologous virus; additional studies with site-specific mutants in the viral transactivators will be essential to address this intriguing possibility.

The significance of two promoters in the genomes of primate foamy viruses remains to be determined. Full-length viral transcripts as well as spliced subgenomic viral transcripts are produced by both promoters (16, 26), and genetic studies with site-specific mutations in the proviral clone of HFV reveal that the internal promoter as well as *bel-1* are required for efficient viral replication in tissue culture cells (14a). With respect to other retroviruses, the presence of an internal promoter in the foamy viruses is not unique. Mouse mammary tumor virus and human T-cell leukemia virus type I produce transcripts initiated within the *env* gene as well as the LTR (4, 25, 28); however, the internal promoters of these viruses appear not to be transactivated by a gene in the respective virus. Several DNA viruses encode two or more promoters to provide a means for temporal regulation of viral transcription (35). Although studies on HFV and SFV-1 in tissue culture cell lines show that both the LTR and internal promoter are expressed at both early and late times after infection (2a, 16), it is possible that the balance of initiation in the LTR versus the internal promoter plays a role in vivo in a unique cell type(s). This differential transcription may be mediated by the relative activities of the enhancer elements in each viral promoter.

Accordingly, the transactivation experiments in Tables 2 to 4 support the notion that the cell type influences the relative levels of transcription directed by the LTRs and internal promoters of SFV-1, SFV-3, and HFV. It is also tenable that cell activation signals and/or cell proliferation conditions may elicit differential transcription from the LTR and internal promoter. Although the HFV LTR appears to respond to cell activation signals in transfected cells (19), in similar transfection experiments we have observed that neither the SFV-1 LTR nor internal promoter responds to agents which activate cells by the protein kinase A and protein kinase C pathways (2b). Nonetheless, in an infected host, differential regulation of foamy virus gene expression through these two promoters may be important for establishing a state of viral latency and/or persistence (6, 11). Therefore, to discover the biological significance of two promoters in the foamy virus genome, it will be necessary to obtain greater knowledge about these viruses in primates (i.e., cell and tissue distribution of virus and levels of viral gene expression in various tissues).

ACKNOWLEDGMENTS

We gratefully acknowledge the expert technical assistance of Karen Shaw, Heather Kerr, Elissa Pratt-Lowe, and Mike Stout. We thank Rolf Flugel (University of Heidelberg) and Axel Rethwilm (University of Wurtzburg) for providing HFV cloned DNA. We thank Barbara L. Shacklett (University of California, Davis) and Karen Shaw for expert assistance with computer programs.

This research was supported by National Institute of Allergy and Infectious Diseases grant AI30525 to P.A.L. R.R. is a recipient of a fellowship from Landes Graduierten Forderung, Baden-Wurttemberg, Germany. In addition, resources from the California Regional Primate Research Center were used for tissue culture experiments on SFV-1 (NIH National Center for Research Resources, base grant RR00169).

ADDENDUM IN PROOF

A recent publication by A. Mergia (Virology 199:219–222, 1994) reports that the 5' end of an SFV-1 transcript that initiates in the internal promoter maps to position 8425; this result was obtained by sequence analysis of cDNA clones made from RNA from infected cells. We utilized two independent methods, i.e., in vitro transcription (Fig. 4A) and primer-extension analysis of transcripts in transfected (Fig. 4B) and infected (Fig. 4C) cells, to show that the 5' end of a transcript that initiates in the SFV-1 internal promoter maps to position 8408. Presumably, Mergia analyzed cDNA clones which were not complete.

REFERENCES

1. Aguzzi, A., K. Bothe, E. F. Wagner, A. Rethwilm, and I. Horak. 1992. Human foamy virus: an underestimated neuropathogen? *Brain Res.* 2:61–69.
2. Atchison, M. L. 1988. Enhancers: mechanisms of action and cell specificity. *Annu. Rev. Cell Biol.* 4:127–153.
- 2a. Campbell, M., and P. Luciw. Unpublished data.
- 2b. Campbell, M., L. Renshaw-Gegg, and P. A. Luciw. Unpublished data.
3. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156–159.
4. Elliot, J. F., B. Pohajdak, D. J. Talbot, J. Shaw, and V. Paetkau. 1988. Phorbol diester-inducible, cyclosporine-suppressible transcription from a novel promoter within the mouse mammary tumor virus *env* gene. *J. Virol.* 62:1373–1380.
5. Erlwein, O., and A. Rethwilm. 1993. BEL-1 transactivator responsive sequences in the long terminal repeat of human foamy virus. *Virology* 196:256–268.
6. Flugel, R. M. 1993. The molecular biology of the human spuma-

- virus, p. 193–211. In B. R. Cullen (ed.), Human retroviruses. Oxford University Press, Oxford.
7. Frankel, A. D., and P. S. Kim. 1991. Modular structure of transcription factors: implications for gene regulation. *Cell* **65**: 717–719.
 8. Garrett, E. D., F. He, H. P. Bogerd, and B. R. Cullen. 1993. Transcriptional transactivators of human and simian foamy viruses contain a small, highly conserved activation domain. *J. Virol.* **67**:6824–6827.
 9. Greenblatt, J. 1991. Roles of TFIID in transcriptional initiation by RNA polymerase II. *Cell* **66**:1067–1070.
 10. He, F., J. D. Sun, E. D. Garrett, and B. R. Cullen. 1993. Functional organization of the Bel-1 *trans* activator of human foamy virus. *J. Virol.* **67**:1896–1904.
 11. Hooks, J. J., and C. J. Gibbs. 1975. The foamy viruses. *Bacteriol. Rev.* **39**:169–185.
 12. Keller, A., E. D. Garrett, and B. R. Cullen. 1992. The Bel-1 protein of human foamy virus activates human immunodeficiency virus type 1 gene expression via a novel target site. *J. Virol.* **66**:3946–3949.
 13. Keller, A., K. M. Partin, M. Lochelt, H. Bannert, R. M. Flugel, and B. R. Cullen. 1991. Characterization of the *trans*-activator of human foamy retrovirus. *J. Virol.* **65**:2589–2594.
 14. Lee, K. J., A. H. Lee, and Y. C. Sung. 1993. Multiple positive and negative *cis*-acting elements that mediate transactivation by Bel1 in the long terminal repeat of human foamy virus. *J. Virol.* **67**:2317–2326.
 - 14a. Lochelt, M. Personal communication.
 15. Lochelt, M., M. Aboud, and R. M. Flugel. 1993. Increase in the basal transcriptional activity of the human foamy virus internal promoter by the homologous long terminal repeat promoter in *cis*. *Nucleic Acids Res.* **21**:4226–4230.
 16. Lochelt, M., R. M. Flugel, and M. Aboud. 1994. The human foamy virus internal promoter directs the expression of the functional Bel1 transactivator and Bet protein early after infection. *J. Virol.* **68**:638–645.
 17. Lochelt, M., W. Muranyi, and R. M. Flugel. 1993. Human foamy virus genome possesses an internal, Bel-1-dependent and functional promoter. *Proc. Natl. Acad. Sci. USA* **90**:7317–7321.
 18. Lochelt, M., H. Zentgraf, and R. M. Flugel. 1991. Construction of an infectious DNA clone of the full-length human spumaretrovirus genome and mutagenesis of the bel1 gene. *Virology* **184**:43–54.
 - 18a. Luciw, P. Unpublished data.
 19. Maurer, B., E. Serfling, V. ter Meulen, and A. Rethwilm. 1991. Transcription factor AP-1 modulates the activity of the human foamy virus long terminal repeat. *J. Virol.* **65**:6353–6357.
 20. Mergia, A., and P. A. Luciw. 1991. Replication and regulation of primate foamy viruses. *Virology* **184**:475–482.
 21. Mergia, A., E. Pratt-Lowe, K. E. S. Shaw, L. W. Renshaw-Gegg, and P. A. Luciw. 1992. *cis*-acting regulatory regions in the long terminal repeat of simian foamy virus type 1. *J. Virol.* **66**:251–257.
 22. Mergia, A., L. W. Renshaw-Gegg, M. W. Stout, R. Renne, and O. Herchenroeder. 1993. Functional domains of the simian foamy virus type 1 transcriptional transactivator (*taf*). *J. Virol.* **67**:4598–4604.
 - 22a. Mergia, A., and L. W. Renshaw-Gegg. Unpublished data.
 23. Mergia, A., K. E. S. Shaw, E. Pratt-Lowe, P. Barry, and P. A. Luciw. 1990. Simian foamy virus type 1 is a retrovirus which encodes a transcriptional transactivator. *J. Virol.* **64**:3598–3604.
 24. Mergia, A., K. E. S. Shaw, E. Pratt-Lowe, P. Barry, and P. A. Luciw. 1991. Identification of the simian foamy virus transcriptional transactivator gene (*taf*). *J. Virol.* **65**:2903–2909.
 25. Miller, C. L., R. Garner, and V. Paetkau. 1992. An activation-dependent, T-lymphocyte-specific transcriptional activator in the mouse mammary tumor virus *env* gene. *Mol. Cell. Biol.* **12**:3262–3272.
 26. Muranyi, W., and R. M. Flugel. 1991. Analysis of splicing patterns of human spumaretrovirus by polymerase chain reaction reveals complex RNA structures. *J. Virol.* **65**:727–735.
 27. Nordeen, S. K., P. I. Green, and D. M. Fowlkes. 1987. A rapid, sensitive, and inexpensive assay for chloramphenicol acetyltransferase. *DNA* **6**:173–178.
 28. Nosaka, T., Y. Ariumi, M. Sakurai, R. Takeuchi, and M. Hatanaka. 1993. Novel internal promoter/enhancer of HTLV-I for tax expression. *Nucleic Acids Res.* **21**:5124–5129.
 29. Renne, R., E. Friedl, M. Schweizer, U. Fleps, R. Turek, and D. Neumann-Haefelin. 1992. Genomic organization and expression of simian foamy virus type 3 (SFV-3). *Virology* **186**:597–608.
 30. Renne, R., A. Mergia, L. W. Renshaw-Gegg, D. Neumann-Haefelin, and P. A. Luciw. 1993. Regulatory elements in the long terminal repeat (LTR) of simian foamy virus type 3 (SFV-3). *Virology* **192**:365–369.
 31. Rethwilm, A., O. Erlwein, G. Baunach, B. Maurer, and V. ter Meulen. 1991. The transcriptional transactivator of human foamy virus maps to the *bel 1* genomic region. *Proc. Natl. Acad. Sci. USA* **88**:941–945.
 32. 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.
 33. Venkatesh, L. K., P. A. Theodorakis, and G. Chinnadurai. 1991. Distinct *cis*-acting regions in U3 regulate trans-activation of the human spumaretrovirus long terminal repeat by the viral bel 1 gene product. *Nucleic Acids Res.* **19**:3661–3666.
 34. Venkatesh, L. K., C. Yang, P. A. Theodorakis, and G. Chinnadurai. 1993. Functional dissection of the human spumaretrovirus transactivator identifies distinct classes of dominant negative mutants. *J. Virol.* **67**:161–169.
 35. Voyles, B. A. 1993. Expression and replication of the viral genome in eucaryotic cells: the DNA viruses, p. 165–196. In *The biology of viruses*. The C.V. Mosby Co., St. Louis.
 36. Weiss, R. A. 1988. A virus in search of a disease. *Nature (London)* **333**:497–498.
 37. Zou, J., M. Campbell, and P. Luciw. Unpublished data.