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Identification of the Simian Foamy Virus Transcriptional Transactivator Gene (*taf*)

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Simian foamy virus type 1 (SFV-1), a member of the spumavirus subfamily of retroviruses, encodes a transcriptional transactivator that functions to strongly augment gene expression directed by the viral long terminal repeat (LTR). The objective of this study was to identify the viral gene responsible for transactivation. Nucleotide sequences between the *env* gene and the LTR of SFV-1 were determined. The predicted amino acid sequence revealed two large open reading frames (ORFs), designated ORF-1 (311 amino acids) and ORF-2 (422 amino acids). In the corresponding region of the human foamy virus, three ORFs (bel-1, bel-2, and bel-3) have been identified (R. M. Flugel, A. Rethwilm, B. Maurer, and G. Darai, EMBO J. 6:2077–2084, 1987). Pairwise comparisons of the ORF-1 and ORF-2 with bel-1 and bel-2 show small clusters of homology; less than 39% overall homology of conserved amino acids is observed. A counterpart for human foamy virus bel-3 is not present in the SFV-1 sequence. Three species of viral RNA have been identified in cells infected with SFV-1; an 11.5-kb RNA representing full-length transcripts, a 6.5-kb RNA representing the *env* message, and a 2.8-kb RNA from the ORF region. Analysis of a cDNA clone encoding the ORF region of SFV-1 reveals that the 2.8-kb message is generated by complex splicing events involving the 3′ end of the *env* gene. In transient expression assays in cell lines representing several species, ORF-1 was shown to be necessary and sufficient for transactivating viral gene expression directed by the SFV-1 LTR. The target for transactivation is located in the U3 domain of the LTR, upstream from position −125 (+1 represents the transcription initiation site). We propose that ORF-1 of SFV-1 be designated the transcriptional transactivator of foamy virus (*taf*).

Foamy viruses, also designated syncytium-forming viruses, are members of the *Spumavirinae* subfamily of retroviruses (reviewed in references 11, 26, and 34). These viruses, found in many mammalian species, appear to be nonpathogenic in their natural hosts even though they have a wide tissue range and induce extensive cytopathic effects in cell culture. A human foamy virus (HFV) has been molecularly cloned, and its genome has been completely sequenced (8, 17). HFV contains four open reading frames (ORFs), designated S-1, bel-1, bel-2, and bel-3, in addition to genes for virion structural proteins, *gag*, *pol*, and *env*. The ORF S1 is located at the intergenic region of *pol* and *env*, and bel-1, bel-2, and bel-3 are found in the region beyond the *env* gene extending into the 3′ long terminal repeat (LTR). We have recently cloned the genome of simian foamy virus type 1 (SFV-1), an isolate from a rhesus macaque (13), and determined the DNA sequence of the LTR, the endonuclease domain of the *pol* gene, and the entire *env* gene (18, 19). The SFV-1 *pol* gene overlaps the *env* gene; thus, the ORF S1 reported for HFV is not present in SFV-1 (18). Amino acid sequence comparisons of HFV and SFV-1 revealed that the endonuclease domains of the *pol* and the *env* genes are about 84 and 70% related, respectively (18). Comparison of the R-U5 domains of HFV (346 bp) and SFV (343 bp) reveals 84% nucleotide homology (19). In contrast, the U3 regions of HFV (777 bp) and SFV-1 (916 bp) show only 24% homology (19). Thus, HFV and SFV-1 are clearly related; however, some regions of their genomes are highly divergent.

We have previously demonstrated that SFV-1 encodes a transcriptional transactivator which functions to strongly augment gene expression directed by the viral LTR in cell lines from different species (19); HFV has also been shown to encode a transcriptional transactivator (31). To characterize the potential transactivator, the nucleotide sequence between the *env* gene and the LTR of SFV-1 was determined; the predicted amino acid sequence for this region showed two large ORFs which partially overlap each other. Analysis of viral transcripts revealed structures of multiply spliced mRNA for the ORF region. In transient expression assays in tissue culture cells, ORF-1 transactivated viral gene expression directed by the SFV-1 LTR. In addition, the target for the transactivator was localized to the U3 domain of the LTR. We propose that ORF-1 of SFV-1 be designated the transcriptional transactivator for foamy virus (*taf*).

MATERIALS AND METHODS

Virus and cell cultures. Dog thymus Cf2Th (canine fibroblast), L929 (murine fibroblast), and COS-7 (simian fibroblast) cell lines were obtained from the American Type Culture Collection (Rockville, Md.). Cells were grown in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum. SFV-1 was propagated in Cf2Th cell lines and titers were determined as described previously (18).

Nucleotide sequencing. The cloning and characterization of a 5.7-kb SFV-1 DNA that contains the endonuclease domain of the *pol* gene, the entire *env* gene, and a portion of the 3′ LTR region have been described elsewhere (18, 19). For this report, the nucleotide sequence of the region between the *env* gene and the 3′ LTR was determined. DNA fragments of about 500 bp in size were subcloned into pUC118 plasmid vectors for nucleotide sequence analysis. The sequences were determined by the dideoxy chain termination

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method (32) using double-stranded DNA template [α-^{35}S] dATP and Sequenase polymerase (U.S. Biochemical, Cleveland, Ohio). Plasmids with SFV-1 inserts were denatured and annealed with M13 primers for DNA sequencing. Additional primers were prepared by using the Pharmacia Gene Assembler (Pharmacia, Inc., Piscataway, N.J.) for automated oligonucleotide synthesis. Both strands of SFV-1 DNA were sequenced.

RNA blot hybridization. Monolayer cultures of Cf2Th cells were infected at a high multiplicity of infection with SFV-1; at 72 h postinfection, total RNA was prepared by the guanidine thiocyanate method (3). Poly(A)$^+$ RNA was prepared by oligo(dT)-cellulose column chromatography, and a 2-μg portion was fractionated on a 1% agarose gel containing 2.2 M formaldehyde and transferred to nitrocellulose membranes for hybridization. These membranes were hybridized to [α-^{32}P]dATP-labeled DNA fragments representing the U5, *gag*, *pol*, *env*, and ORF regions of SFV-1 as previously described (19).

Isolation of cDNA clones. The Amersham cDNA kit (Arlington Heights, Ill.) was used to synthesize double-stranded cDNA from 2 μg of poly(A)-selected RNA from SFV-1-infected cells, and the cDNA was cloned via *Eco*RI linkers into bacteriophage λgt10 vector (12). The recombinant library was screened (2) with a 400-base DNA fragment from the ORF region. DNA inserts representing SFV-1 sequences were subsequently cloned into plasmid vectors (i.e., pUC118).

Plasmid constructions. DNA inserts representing SFV-1 sequences were cloned into a mammalian cell expression vector which contains the simian virus 40 early promoter. pORFs15A (spliced) and pORFs16A (unspliced) contained sequences from position 27 to the 3′ end of the SFV-1 message (Fig. 1). Deletions in the ORF region were generated by taking advantage of restriction enzyme sites. The structure of each plasmid was confirmed by extensive restriction enzyme analysis. pSVORF1 and pSVORF2 contained nucleotide sequences from positions 27 to 1517 and position 651 to the 3′ end of the message, respectively. pORF5A had a deletion in both ORF-1 and ORF-2 (positions 532 to 826, Fig. 1). The plasmid pSFV-1LTR/CAT contains the SFV-1 LTR placed upstream from the CAT gene and has been described previously (19). Deletion mutants of the SFV-1 LTR were also generated by taking advantage of unique restriction enzyme sites. Deleted LTRs were obtained by cleavage of plasmid DNAs with appropriate restriction enzymes; staggered ends were filled with DNA polymerase I (Klenow fragment) and subcloned after gel purification into the CAT expression plasmid. pSFV-1LTR/CAT-41 (see Fig. 5) contains nucleotide sequences from −1192 (5′ end of the LTR; +1 represents the cap site) to +6 (*Sac*I). pSFV-1LTR/CAT-46 contains nucleotide sequences from −125 (*Eco*RI site) to +334 (5 bases downstream from the 3′ end of the LTR; *Nar*I site).

DNA transfections. Transfections were performed by the DEAE-dextran method on L929, Cf2Th, and COS-7 cells as described previously (1). For each experiment, duplicate cell cultures were transfected with 2 μg of CAT expression plasmid (pSFV-1LTR/CAT or pSFV-1LTR/CAT deletion mutants) and 3 μg of plasmid DNA of the pSVORF constructs or carrier DNA (pSP65). CAT assays, performed on whole-cell extracts prepared 48 h after transfection, measured the conversion of ^{3}H-acetyl coenzyme A to ^{3}H-acetylated chloramphenicol (24).

RESULTS

Viral transcripts in cells infected with SFV-1. To determine the pattern of SFV-1 transcripts, RNA from infected cells was characterized by Northern (RNA) blot hybridization analysis with probes representing various subgenomic regions of the viral genome. At 72 h postinfection, three species of viral transcripts were detected at 11.5, 6.5, and 2.8 kb (Fig. 2). Probes representing the *gag* and *pol* regions detected only full-length transcripts at 11.5 kb (Fig. 2). Thus, this mRNA species probably functions as a *gag-pol* message as well as genomic RNA. The 6.5-kb mRNA hybridized with a probe from the *env* region; this result suggests that the 6.5-kb transcript is the *env* message (Fig. 2). The probe for the ORF region hybridized to transcripts at 2.8 kb as well as to the 11.5- and 6.5-kb transcripts (Fig. 2). Therefore, transcripts at 2.8 kb represent a message(s) from the ORF region. The 11.5-, 6.5-, and 2.8-kb transcripts were also detected with a probe representing the U5 region of the LTR (Fig. 2). Thus, as for other retroviruses, subgenomic SFV-1 transcripts share 5′ and 3′ ends. These observations support the notion that subgenomic SFV-1 transcripts are generated by splicing from the full-length transcript.

Nucleotide sequence of SFV-1. Nucleotide sequences of the endonuclease domain of the *pol* gene, the entire *env* gene, and the LTR were determined previously (18, 19). The DNA sequence between the *env* gene and the LTR is shown in Fig. 1. Predicted amino acid sequences revealed two large ORFs (Fig. 1). The first ORF (designated ORF-1) is 311 amino acids long; 12 amino acids at the N terminus of ORF-1 overlap the end of the *env* gene (Fig. 1). A second ORF (designated ORF-2), 422 amino acids long, overlaps ORF-1 at the N terminus by 145 amino acids and extends into the 3′ LTR (Fig. 1). Amino acid comparisons of ORF-1 with bel-1 of HFV and ORF-2 with bel-2 of HFV show clusters of homology (39 and 38%, respectively) (Fig. 3). No significant similarity was found between bel-3 of HFV and ORF-1, ORF-2, or other potential translation frames in SFV-1. Relative to the *pol* and *env* regions of SFV-1 and HFV, which show about 85 and 70% similarity in predicted amino acids, respectively, these two viruses appear to be greatly diverged when the ORF and bel regions are compared.

Structures of transcripts for viral ORFs. Structures of viral transcripts representing the ORF region were determined by preparing and characterizing a cDNA library constructed in the bacteriophage vector λgt10 by using poly(A)-containing RNA from Cf2Th cells infected with SFV-1. The library was screened with probe from the ORF region (*Bgl*II-*Bam*HI fragment, positions 323 to 1061 in Fig. 1). Five cDNA clones greater than 2.5 kb in size were characterized by sequence analysis. None of the five clones contained the R and U5 regions; the 5′ ends of the clones ranged between nucleotide sequence positions 27 and 39 (Fig. 1). Three clones were missing the sequence between 121 and 243; this observation suggested that a splicing event had occurred. To obtain a complete picture of the splice patterns of ORF transcripts, it will be necessary to perform RNase protection experiments and to clone the 5′ end of the viral messages. Since all transcripts of SFV-1 have the same 3′ end, the two unspliced cDNA clones could represent the *env* message or the full-length transcript of SFV-1. The spliced message potentially encodes 22 amino acids in frame with the predicted N terminus of ORF-1 (Fig. 4b); this spliced sequence does not specify an initiation codon for methionine. The first ATG codon in ORF-1 is located at position 252 (Fig. 1) and matches the translational start consensus sequence A/GXX

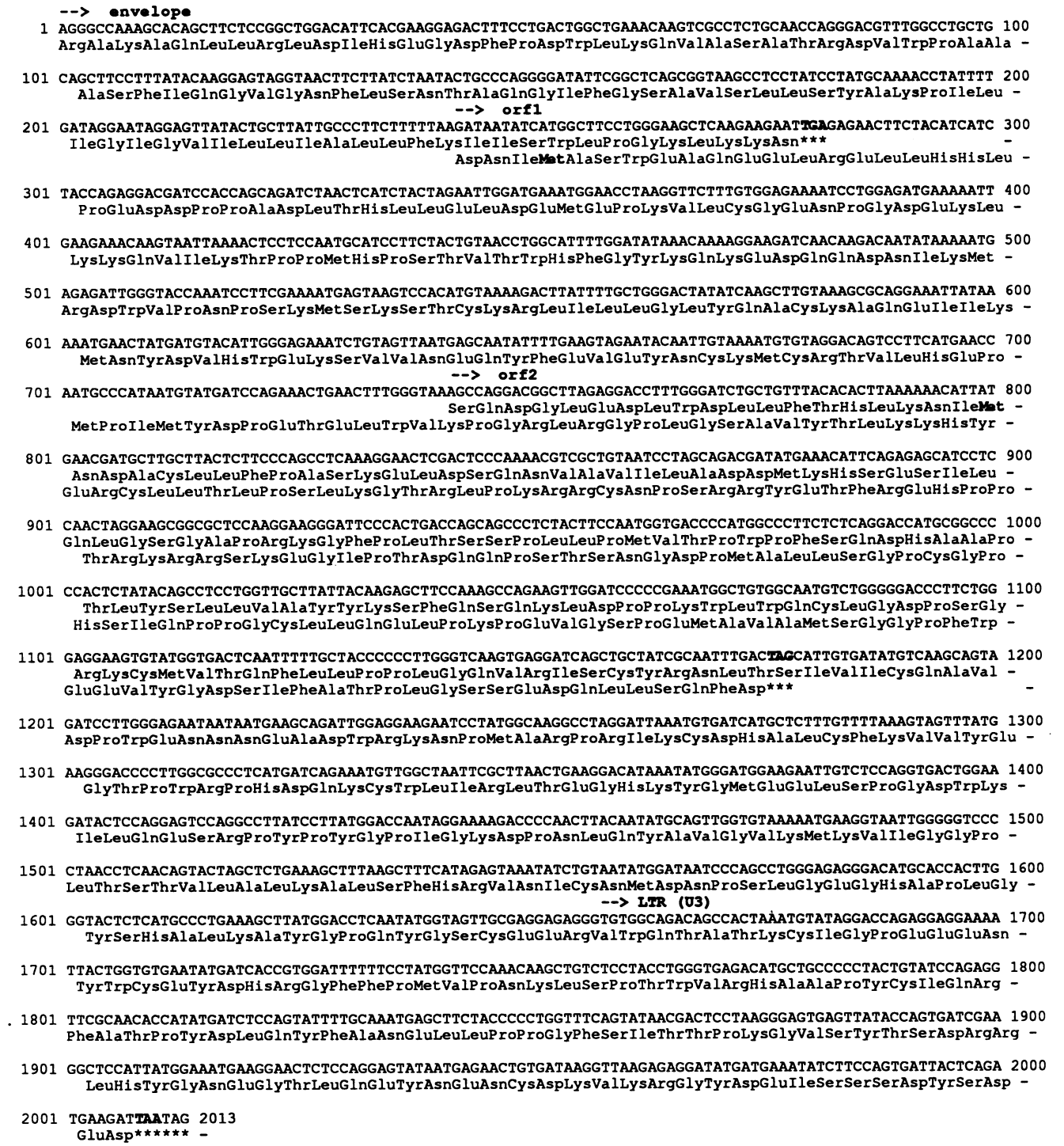

FIG. 1. Nucleotide sequence of the ORF region of SFV-1. The DNA sequence has been numbered from the 3′ end of the *env* gene to the end of the ORF region. The predicted amino acid sequences for the ORFs are shown below the DNA sequence.

ATGG (14). These results suggest that the predicted translation frames in the ORF region presented in Fig. 1 may be sufficient to generate functional protein(s).

Identification of the SFV-1 transcriptional transactivator. Previous studies involving transient expression assays in cells infected with SFV-1 demonstrated that the viral genome encodes a transcriptional transactivator that functions through sequences in the LTR (19). To determine whether the ORF region encodes a functional transactivator, both spliced and unspliced cDNA clones were examined for the ability to activate gene expression directed by the SFV-1 LTR (Fig. 4). Expression of ORF gene products in these cDNA plasmid clones was directed by the early promoter of simian virus 40 (21). Plasmid constructs containing the

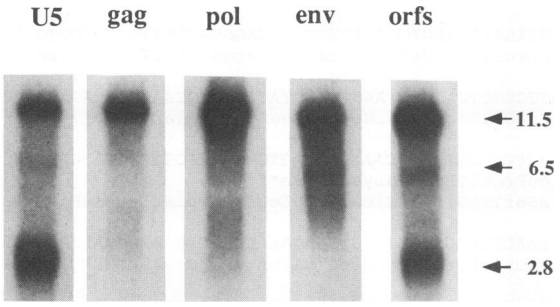

FIG. 2. Northern analysis of SFV-1 transcripts from infected Cf2Th cells. Subgenomic probes of SFV-1 used for identification of viral messages are shown at the top. Conditions for infection, isolation, electrophoresis, and Northern blotting are described in Materials and Methods.

spliced (pORFs15a) or unspliced (pORFs16A) cDNA clones were cotransfected with pSFV-1LTR/CAT into mouse L929, canine Cf2Th, and simian COS-7 cells. Cell lysates were prepared 48 h after transfection and assayed for CAT activity. The basal promoter activity of the SFV-1 LTR in the transient expression assays was very low (less than 2,000 cpm in all three cell types), whereas expression of CAT directed by the LTR was greatly increased in cells cotransfected with plasmids encoding the spliced or unspliced ORF region cDNA (Table 1). Cells cotransfected with the plasmid containing the spliced form of the ORF region (i.e., pORF15A) had CAT levels 38-, 49-, and 380-fold over basal levels in Cf2Th, COS-7, and L929 cells, respectively (Table 1). The plasmid containing the unspliced form of the ORF region (pORF16A) yielded transactivation levels 26-, 58-, and 275-fold over basal levels in Cf2Th, COS-7, and L929 cells, respectively (Table 1). These results show that the ORF region, encompassing both ORF-1 and ORF-2, encodes the transcriptional transactivator.

To define the transactivator gene of SFV-1, deletion

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SFV-1   1 MASWEAQEELRELLHHLPEDDPPADLTHLLEL....DEMEPKVLCGENPG 46
          | | |  |                 ||  | ||       |       | |
HFV-1   1 MDSYEKEES.......VASTSGIQDLQTLSELVGPENAGEGELTIAEEPE 43
                                    .       .       .       .
         47 DEKLKKQVIKTPPMHPSTVTWHFGYKQKEDQQ.DNIKMRDWVPNPSKMSK 95
                           |  |  ||  ||     ||  || | |  |||
         44 EN..PRRPRRYTKREVKCVSYH.AYKEIEDKHPQHIKLQDWIPTPEEMSK 90
                                    .       .       .       .
         96 STCKRLILLGLYQACKAQEIIKMNYDVHWEKSVVNEQYFEVEYNCKMCRT 145
            | |||||| ||| | || ||  |   | || |      | | | |  |
         91 SLCKRLILCGLYSAEKASEILRMPFTVSWEQSDTDPDCFIVSYTCIFCDA 140
                                    .       .       .       .
        146 VLHEPMPIMYDPETELWVKPGRLRGPLGSAVYTLKKHYERCLLTLPSLEG 195
            | | ||||  |||   |||   |||  ||||    ||   | |  ||
        141 VIHDPMPIRWDPEVGIWVKYKPLRGIVGSAVFIMHKHQRNCSLVKPSTSC 190
                                    .       .       .       .
        196 TRLPKRRC..NPSRRYETFREHPPTRKRRSKEGIPTDQQPSTSNGDPMAL 243
               || |    |  |   |  |   |  |       |     |  | ||
        191 SEGPKPRPRHDPVLRCDMFEKHHKPROKRPRR.RSIDNESCASSSDTMAN 239
                                    .       .       .       .
        244 LSGPCGPHSIQPPGCLLQELPKPEVGSPEMAVAMSGGPFWEEVYGDSIFA 293
              |          || ||  |       |   | ||||||||||||||
        240 EPGSLCTNPLWNPGPLLSGLLEESSNLPNLEVHMSGGPFWEEVYGDSILG 289
                                    .
        294 TPLGSSEDQLL 304
             | || |   |
        290 PPSGSGEHSVL 300
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FIG. 3. Alignment of the predicted amino acid sequences of the ORF-1 of SFV-1 with bel-1 of HFV. Identical amino acids are indicated by vertical lines. Acidic regions (potential activation domains) and basic regions (putative nuclear localization domains) are underlined. Alignment was determined by using the Gap program, version 6.1, provided by the Genetics Computer Group (University of Wisconsin).

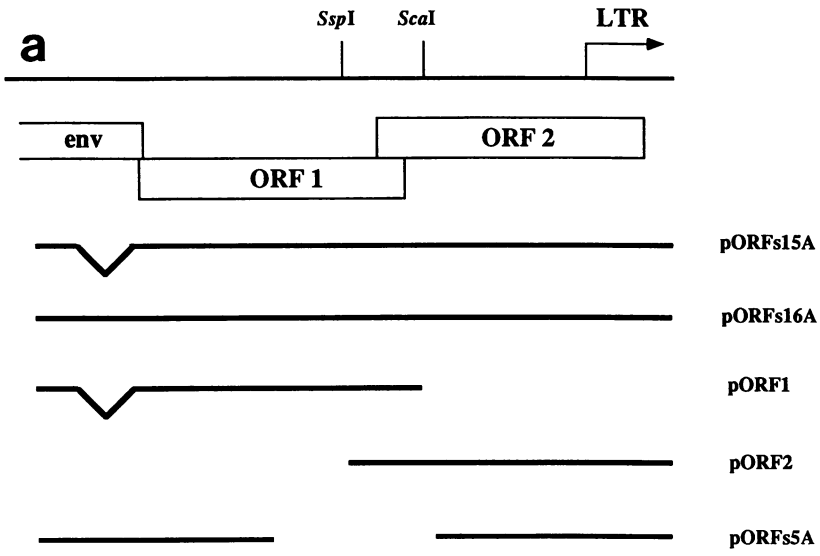
mutations of the cDNA clone representing the ORF region were constructed and placed downstream from the simian virus 40 early promoter in a sense orientation (Fig. 4a). The plasmid expressing ORF-1 (pORF-1) had transactivator activity; CAT activity was stimulated 16-, 15-, and 617-fold in Cf2Th, COS-7, and L929 cells, respectively (Table 1). A plasmid with a deletion in ORF-1 and ORF-2 (pORFs5A) did not transactivate (Table 1). In addition, a plasmid containing only ORF-2 (pORF-2) did not transactivate the SFV-1 LTR (Table 1). Thus, ORF-1 was necessary and sufficient for transcriptional transactivation of the SFV-1 LTR.

Target for the transcriptional transactivator in the SFV-1 LTR. To identify *cis*-acting elements of the SFV-1 LTR involved in transactivation, deletion mutations in the LTR were constructed and placed upstream from the CAT gene in an expression vector (Fig. 5). Plasmids containing mutations in the SFV-1 LTR as well as the wild-type LTR were cotransfected with a plasmid (pORFs15A) expressing the transactivator into COS-7 cells. The wild-type LTR and the deletion mutation LTRs showed low basal levels of CAT activity (less than 1,900 cpm) (Fig. 5). The plasmid expressing ORF-1 increased CAT activity from the wild-type LTR 40-fold. Deletion of the R and U5 domains, from +6 to +334 (+1 represents the cap site for initiation of transcription), yielded transactivation levels 71-fold over the basal level (Fig. 5). Removal of most of the upstream U3 region (−125 to −1296), maintaining the putative TATA box, abolished transactivation (Fig. 5). Thus, the target for transactivation is located in the U3 domain of the LTR upstream from position −125. To define precisely the *cis*-acting regulatory element(s), it will be necessary to analyze additional deletion mutations in transient expression assays and to carry out binding studies aimed at identifying sites in the LTR which are recognized by protein factors in infected cells.

DISCUSSION

This report investigates the structure and function of the region of SFV-1 between the *env* gene and the rightward LTR. The predicted amino acid sequence revealed two large partially overlapping ORFs, designated ORF-1 and ORF-2. In the corresponding region of HFV, three ORFs (bel-1, bel-2, and bel-3) have been identified (8). Pairwise comparisons of the predicted amino acid sequences of ORF-1 and ORF-2 with those of bel-1 and bel-2, respectively, showed less than 39% relatedness; only small clusters of homology were noted. The role of the ORF region in the regulation of SFV-1 gene expression was also investigated. Previous studies revealed that the SFV-1 genome encodes a transcriptional transactivator which augments gene expression directed by the viral LTR (19). Experiments in this report revealed that ORF-1 is the transcriptional transactivator of SFV and the target is in the U3 region of the LTR upstream from position −125.

Lentiviruses (e.g., human immunodeficiency virus types 1 and 2 [HIV-1 and HIV-2], simian immunodeficiency virus, and visna maedi virus) and certain oncoviruses (e.g., human T-lymphotropic virus types I and II [HTLV-I and HTLV-II] and bovine leukemia virus) encode transactivators which require target elements in the LTR (6, 9, 23, 27, 33). These transactivator proteins are specified by messages that involve multiple splicing events (6, 9, 23, 25, 27). Analysis of transcripts and cDNA clones representing the bel region of HFV showed that viral mRNA is generated by multiple splicing events (22). Northern blot and cDNA clone analysis of the SFV-1 ORF region presented in this study indicate



b

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--> envelope
1 AGGGCCAAAGCACAGCTTCTCCGGCTGGACATTCACGAAGGAGACTTTCCTGACTGGCTGAAACAAGTCGCCTCTGCAACCAGGGACGTTTGGCCTGCTG 100
   GlyGlnSerThrAlaSerProAlaGlyHisSerArgArgArgLeuSer***LeuAlaGluThrSerArgLeuCysAsnGlnGlyArgLeuAlaCysCys-

                                          ---->ORF-1
101 CAGCTTCCTTTATACAAGGAATAATATCATGGCTTCCTGGGAAGCTCAAGAAGAATTGAGAGAACTTCTACATCAT
    SerPheLeuTyrThrArgAsnAsnIleMetAlaSerTrpGluAlaGlnGluGluLeuArgGluLeuLeuHisHis
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FIG. 4. (a) Schematic representations of ORF plasmids. Shown are the end of the *env* gene, ORF-1 and ORF-2, and the beginning of the 3′ LTR. Details of the plasmid constructions are presented in Materials and Methods. (b) Nucleotide sequence of the 5′ end of the spliced cDNA clone (pORFs15A). The predicted amino acid sequence in frame with the N terminus of ORF-1 is shown below the DNA sequence. The position of the splice junction is underlined. The first methionine of ORF-1 is shown in boldface type.

that the ORF transcript is also processed by multiple splicing events; the last exon contains sequences that encode ORF-1 and expresses functional transactivator protein (Fig. 4 and Table 1). Basal levels of transcription directed by the LTRs of primate lentiviruses (i.e., HIV-1 and simian immunodeficiency virus) and primate oncoviruses (i.e., HTLV-I and HTLV-II) are also very low when assessed in similar transient expression systems (5, 10, 27). Mutational analyses have revealed that the HIV-1 *tat* gene and the HTLV-I *tax* gene are required for viral replication (5, 10, 27). Similar genetic studies on the role of the SFV-1 transactivator remain to be performed; however, the very low basal levels of expression directed by the SFV-1 LTR and the large transactivation responses are observations which support the notion that ORF-1 encodes a gene product which is required for SFV-1 replication.

TABLE 1. Transient expression assays with pSFV-1LTR/CAT and ORF plasmids

Cotransfecting plasmid[a]	cpm (relative activity) in[b]:		
	L929 (murine) cells	Cf2Th (canine) cells	COS-7 (primate) cells
pSP65	1,820	1,670	1,770
pORFs15A	691,000 (380)	63,400 (38)	85,900 (49)
pORFs16A	501,000 (275)	42,700 (26)	102,000 (58)
pORF-1	1,200,000 (617)	26,200 (16)	26,200 (15)
pORF-2	1,740 (1)	2,130 (1.3)	1,010 (0.6)
pORFs5A	1,680 (0.9)	ND (ND)	ND (ND)

[a] Structures of the ORF plasmids are shown in Fig. 4a. The relative activity was obtained by comparaing the level of CAT activity of each transfection with basal levels observed with the control pSP65. ND, not determined.

[b] Cells were transfected with the indicated plasmids, and CAT assays were performed 48 h later as described in Materials and Methods. The conversion of ^{3}H-acetyl coenzyme A to ^{3}H-acetylated chloramphenicol is shown as counts per minute. Less than 15% variation in replicate samples was observed.

Diverse mechanisms account for transactivation by different retroviruses. Studies in the HIV system suggest that *tat* may affect the initiation of transcription as well as a posttranscriptional event (5, 15, 27, 33). Efficient transactivation by HIV-1 *tat* depends on specific sequences, secondary structure, and orientation of the *tat* responsive element (TAR), which is a sequence mapped to a region (+19 to +42) downstream from the cap site (+1) in the HIV-1 LTR (5, 27, 33). Transactivation by the HTLV-I *tax* gene requires a specific sequence element in the U3 portion of the HTLV-I LTR immediately upstream from the cap site; host cell proteins act through a triple 21-bp sequence to mediate transcriptional transactivation by *tax* (10). For SFV-1, the *cis*-acting element for transactivation is in the U3 region of the LTR. Additional investigations are required to define more precisely the *cis*-acting element(s) for the SFV-1 transactivator. It is not known whether the SFV-1 transactivator functions by direct interaction with the *cis*-acting element(s) in the viral LTR or indirectly through cellular factors. The nucleotide sequences of both SFV-1 and HFV have no significant similarity with primate oncoviruses or primate lentiviruses (8, 17–19) (Fig. 1). Therefore, it is likely that the mechanism of transactivation for primate foamy viruses is different from that of the other retroviruses. For these reasons, ORF-1 of SFV-1 is identified as the transcriptional

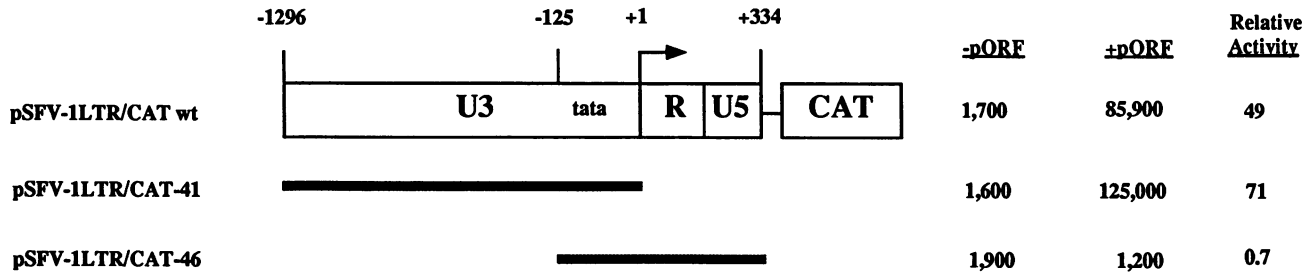

FIG. 5. Transient expression assays of deletion mutants of SFV-1 LTR in COS-7 cells. Lines represent SFV-1 LTR regions remaining after deletion. Cells were transfected with the indicated plasmids, and CAT assays were performed 48 h later as described in Materials and Methods. The values shown are from reactions measuring the conversion of ^{3}H-acetyl coenzyme A to ^{3}H-acetyl chloramphenicol. Generally, less than 15% variation in replicate samples was observed. −pORF represents cotransfection of the reporter plasmid (pSFV-1LTR/CAT) with pSP65; this value represents basal promoter activity. +pORF refers to cotransfection of the reporter plasmid (pSFV-1LTR/CAT) and the transactivator plasmid (pORF-1).

transactivator of foamy virus (*taf*); the bel-1 region of HFV also encodes a transcriptional transactivator (30).

Mechanisms of other viral and cellular transcriptional activators offer useful paradigms for investigations on SFV-1 *taf* (reviewed in references 20, 28, and 29). One class of activators is functional in many cell types; these activators have a domain for DNA binding and an activation domain on one protein (e.g., the immediate-early gene of pseudorabies virus) (4, 16). The second class of activators has only one of these domains and depends on a cellular factor(s) to provide the missing function. An example of the latter class of activators is the VP-16 gene product of herpes simplex virus; VP-16 has an activation domain and a separate region which interacts with the cellular DNA-binding protein Oct-1 (4, 16). The activation domains of both the immediate-early gene of pseudorabies virus and VP-16 have a large proportion of acidic amino acids; these acidic domains are proposed to interact with a component(s) of the transcription initiation complex attached to promoters containing TATA boxes (16, 20). It is interesting to note that the N termini of both SFV-1 *taf* and HFV bel-1 have high concentrations of glutamates and aspartates; 15 of the first 44 predicted amino acids of *taf* are acidic (Fig. 3). Several transactivator proteins contain stretches of basic amino acids which may be essential for nuclear localization (7); similarly, the predicted amino acid sequence of SFV-1 *taf* (and HFV bel-1) shows clusters of basic amino acids which may be essential for nuclear localization (Fig. 3). The predicted amino acid sequence of *taf* contains eight cysteine and five histidine residues (Fig. 3); the arrangement of these residues does not conform to the metal-binding domains of several known DNA-binding proteins (e.g., Sp1) (20). Mutational analysis of ORF-1 coupled with in vitro binding studies and analysis of in vitro transcription systems will be required to identify an activation domain and to determine whether the SFV-1 transactivator binds to target DNA sequences.

In infected hosts, foamy viruses appear to establish latency or a low-level persistent infection (11, 34). Primate lentiviruses (e.g., HIV-1, HIV-2, and simian immunodeficiency virus) and oncoviruses (e.g., HTLV-I, HTLV-II, and simian T-lymphotropic virus) can also establish either a latent or a low-level persistent infection. Cell activation events regulate viral transcription mediated by the LTRs of primate lentiviruses and oncoviruses (10). Temporal regulation of viral gene expression has been observed in tissue culture systems for HTLV-I, HIV-1, and other lentiviruses (5, 10). These findings support a model for viral replication in the animal in which a cell activation event stimulates viral transcription from a quiescent provirus (5, 10, 27). Subsequently, messages for viral transactivator genes are produced; these further enhance expression of virion protein genes through transcriptional and posttranscriptional mechanisms. It is not known whether SFV-1 expression is affected by cell activation signals and whether viral RNA synthesis is temporally regulated. In summary, foamy viruses as well as lentiviruses and oncoviruses offer opportunities both to investigate basic mechanisms which regulate viral gene expression and to determine the biological significance of transcriptional transactivation with respect to latency and persistent infection in the host.

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