Analysis of the Transcription Pattern and Mapping of the Putative *rev* and *env* Splice Junctions of Bovine Immunodeficiency-Like Virus

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The bovine immunodeficiency-like virus (BIV) genome contains the obligatory structural genes of all retroviruses and, in addition, the complex central region of lentiviruses; this novel region may code for at least five nonstructural/regulatory genes in BIV (K. J. Garvey, M. S. Oberste, J. E. Elser, M. J. Braun, and M. A. Gonda, *Virology* 175:391–409, 1990). As a prelude to determining the function of these novel open reading frames, the transcriptional pattern of BIV was studied by Northern analysis of RNA from BIV-infected cells. Five size classes of BIV-specific RNAs of 8.5, 4.1, 3.8, 1.7, and 1.4 kb were detected. The 8.5-kb RNA contains sequences from all regions of the genome; it is the virion RNA and probably serves as the *gag-pol* transcript as well. By using gene-specific probes, subgenomic viral RNAs of 3.8, 1.7, and 1.4 kb were tentatively identified as the *env*, *tat*, and *rev* spliced messages, respectively. The 4.1-kb RNA could not be unambiguously identified but may encode *vif*. The hybridization patterns of the putative *tat* and *rev* mRNAs suggest that they are the products of multiple splicing events. Discrete transcripts for the BIV W and Y central region open reading frames were not defined. The characterization of partial cDNA clones has permitted the mapping of the *env* and putative *rev* splice junctions.

Members of the lentivirus subfamily of retroviruses are responsible for a number of chronic, multisystemic diseases of humans and other mammals (21, 23). This family includes the agents of AIDS, human immunodeficiency virus type 1 (HIV-1) and HIV-2 (2, 5, 19), as well as the simian immunodeficiency viruses (10, 26), visna virus (41), ovine progressive pneumonia virus (9, 27), caprine arthritis encephalitis virus (7), feline immunodeficiency virus (33), equine infectious anemia virus (30), and bovine immunodeficiency-like virus (BIV) (3, 22, 42).

BIV resembles other lentiviruses in certain aspects of its pathogenesis, ultrastructure, genome organization, and infectious cycle in culture (3, 20, 22). The complete nucleotide sequence of BIV has been determined; the BIV genome contains the usual retrovirus structural genes, *gag*, *pol*, and *env*, as well as open reading frames (ORFs) similar to the nonstructural/regulatory genes *vif*, *tat*, and *rev* found in the lentivirus central region (20). BIV lacks the *nef* gene seen in the primate lentiviruses (25) but contains two unique ORFs, W and Y, in a genome location analogous to that of the R, U, and X ORFs of HIVs and SIVs (20). The organization of the BIV genome suggests that multiple transcripts are expressed during the replicative cycle of the virus, as seen with HIV and other lentiviruses (12, 25, 31, 32, 34, 35, 38, 44). To determine whether BIV has a complex transcriptional pattern, we have analyzed viral mRNAs related to BIV infection in bovine cells, and we also report mapping data on the splice junctions of putative *rev* and *env* transcripts.

The molecular cloning of biologically active proviruses (BIV 106 and BIV 127) from the genomic DNA of BIV-infected bovine cells has been reported (3). BIV 106, BIV 127, and parental BIV stocks were propagated in bovine leukocyte adherent cells (BLAC-20 cells) as described pre-

viously (20). BIV-infected cultures were monitored for maximum syncytium induction before being split in a 1:2 ratio (infected:uninfected) with uninfected BLAC-20 cells. Total cellular RNA was extracted from BIV-infected and uninfected BLAC-20 cells 24 to 48 h after passage by the method of Chomczynski and Sacchi (4). RNA (5 µg per lane) was fractionated on 1% agarose-formaldehyde gels (13) and visualized by ethidium bromide staining prior to transfer to GeneScreen Plus (Du Pont NEN).

A near-genome-length DNA probe representing nucleotides (nt) 695 to 8716 of the BIV 106 provirus was generated by digesting a cloned 9.6-kb *Sma*I fragment derived from BIV 106 (3) with *Sma*I and *Bgl*II and isolating the 4.6- and 3.4-kb fragments on a gel (Fig. 1A). Equimolar amounts of these fragments were ³²P labeled by nick translation. A *gag*-specific 1.0 kb *Hind*III-*Bam*HI restriction fragment and a BIV long terminal repeat (LTR)-specific probe (*Ava*II-*Nar*I) were gel purified and ³²P labeled by random priming using the Klenow fragment of *Escherichia coli* DNA polymerase I (Pharmacia). Probes specific for other regions of the genome were synthesized by polymerase chain reaction (PCR) using Amplitaq (Perkin-Elmer Cetus) and primers specific for the target gene (Table 1; Fig. 1A). The synthesis reaction for PCR probes contained 10 mM Tris-HCl (pH 8.3), 3.0 mM MgCl₂, 50 mM KCl, 0.01% gelatin, 200 µM each deoxynucleoside triphosphate, 0.3 µM each oligonucleotide primer, 20 ng of BIV 106 *Sma*I fragment, and 0.5 U of Amplitaq in a total volume of 100 µl. The reaction mixture was overlaid with mineral oil, and synthesis was carried out in a DNA Thermal Cycler (Perkin-Elmer Cetus) by using 30 cycles of 94°C for 1 min, 55°C for 2.0 min, and 72°C for 2.0 min. The PCR products were extracted with chloroform-isoamyl alcohol (24:1) and purified by low-melting-point agarose gel electrophoresis. Probe fragments were excised from the gel and labeled with ³²P by the random priming method. Probes were hybridized to the immobilized RNA at

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FIG. 1. Northern analysis of BIV-specific transcripts in infected cells. (A) Genetic map of the BIV genome, with major ORFs indicated. Potential splice donors (filled triangles) and acceptors (open triangles) are marked above the ORF map. Restriction sites: A, *AvaII*; B, *BglII*; N, *NarI*; S, *SmaI*. The following probes (shown as stippled rectangles) were used for Northern blot hybridization: LTR, *AvaII-NarI*; 1, full length (*SmaI-BglII* plus *BglII-BglII*); 2, *gag*; 3, *pol*; 4, *env*; 5, *vif*; 6, *vifW*; 7, *vifY*; 8, *Y/tat* exon 1; 9, *env/rev* exon 2/*tat* exon 2. (B) Proposed BIV transcription pattern. The splicing pattern was deduced from Northern analysis (Fig. 1C; Table 2) and is compared with the transcription patterns of HIV-1, visna virus, and equine infectious anemia virus (20, 25, 31, 32, 35, 38, 44). The sizes (in kilobases) of the predicted spliced mRNAs are shown on the right. (C) Northern blot hybridization using full-length and subgenomic probes. Lanes 1a through d; BLAC-20 cells infected with BIV 106, BIV 127, or parental virus and uninfected control, respectively, with probe 1; lanes 2 through 9; BIV 106 with the indicated probe. Arrows indicate the positions of 28S (upper arrow) and 18S (lower arrow) rRNAs, as visualized by ethidium bromide staining prior to blotting.

42°C in 50% formamide–5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.0])–1× Denhardt's solution (0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% acetylated bovine serum albumin)–0.02 M sodium phosphate (pH 6.8)–0.2% sodium dodecyl sulfate (SDS)–0.005 M EDTA–10 µg of yeast tRNA per ml–10 µg of sheared salmon sperm DNA (5'→3') per ml. Following hybridization, the filter was washed at 55°C in 0.1× SSC–0.5% SDS and exposed to X-ray film. For hybridization with gene-specific probes (probes 2 to 9 in Fig. 1A), 50 µg of total RNA extracted from BIV 106-infected BLAC-20 cells was loaded into a 7-cm-wide well in a 1% agarose–formaldehyde gel.

Following electrophoresis and transfer of the RNA to Gene-Screen Plus, the membrane was cut into 10 strips of equal width. Each strip was hybridized with one of the subgenomic probes. The results were virtually identical when BIV 127 RNA was used (data not shown).

To map splice junctions, cDNA clones were synthesized according to procedures described elsewhere (32a, 39). Briefly, BIV cDNAs were synthesized from total cellular RNA extracted from BIV 106-infected BLAC-20 cells. Negative-strand cDNA synthesis was initiated with the phosphorylated primer GATAGGCGTCGTTCTTGCTT (nt 7818 to 7799) or ACTGCCAACTATCCTTGCTT (nt 5712 to

TABLE 1. Synthetic oligonucleotides used as PCR primers for probe synthesis^a

ORF (strand)	Sequence (5' to 3')	Position in BIV 127 RNA (nt)	Length (nt)
<i>pol</i> (+)	GATGGAGGATGCTAATGG	2248–2265	351
<i>pol</i> (–)	CCATCCTTGTGGTAGAAC	2598–2581	
<i>env</i> (+)	AACTCTCACTATGGCAGGAA	5747–5766	138 ^b
<i>env</i> (–)	CACCAATCTATGGTATCTCT	5971–5952	
<i>vif</i> (+)	CCACTGAAGAAATAGGTCACTTAG	4887–4911	174
<i>vif</i> (–)	AGCGTTGGAGGGAGTGTCTAGGGG	5060–5037	
<i>vif</i> W (+)	AGTTCGTGAGGCAGACAGAA	4734–4753	327
<i>vif</i> W (–)	AGCGTTGGAGGGAGTGTCTAGGGG	5060–5037	
<i>Y/tat1</i> (+)	GATAATGTTGCCACAGCCCA	5251–5270	114
<i>Y/tat1</i> (–)	CATATGGGACAACAACATGTGGACAG	5364–5338	
<i>vif</i> Y (+)	GTCTCTGTTGGTACCGGTTA	5091–5110	134
<i>vif</i> Y (–)	GCCACAATCTATGAATGTTACGACGAG	5224–5198	
<i>env/rev2/tat2</i> (+)	AGTGGACTGGCTCAATAGCT	7481–7500	338
<i>env/rev2/tat2</i> (–)	GATAGGCGTCGTTCTTGCTT	7818–7799	

^a BIV 106 DNA was used as the PCR template.^b The *env* probe spans the 87-bp deletion in BIV 106 relative to BIV 127. With a BIV 127 template DNA, the PCR product would be 225 bp long.

5693), which corresponds to the *env/rev* or *env* region of the BIV genome, respectively (20). BIV-specific cDNAs were PCR amplified with the phosphorylated primers GATAG GCGTCGTTCTTGCTT (nt 7818 to 7799) or ACTGCCAAC TATCCTTGCTT (nt 5712 to 5693) and TAAGAGAGAC TCGGCTCGAG (nt 231 to 250; common 5'-leader sequence [20]) and were cloned into the phage vector M13mp10 for further analysis. DNA sequencing and analysis were carried out as described previously (20, 22).

BIV-specific RNAs were mapped by hybridization with the whole-genome probe and a series of gene-specific sub-genomic probes. The whole-genome probe detected five BIV-specific RNAs of 8.5, 4.1, 3.8, 1.7, and 1.4 kb (Fig. 1C; Table 2). The BIV LTR-specific probe, which was expected to hybridize with all viral mRNAs, recognized the same transcripts as the full-length probe, demonstrating that each mRNA contained a common 5'-leader sequence (data not shown).

All probes hybridized with the 8.5-kb RNA, indicating that it encompasses all regions of the genome and is most likely the full-length viral RNA. In retroviruses, the genome-length RNA acts as the mRNA for both *gag* and *pol* (43). All probes also detected an RNA of about 5.0 kb which migrates at the leading edge of the 28S rRNA band. We believe this to be an artifact due to the large amount of 28S rRNA. The BIV *gag*

and *pol* probes (probes 2 and 3, respectively) hybridized exclusively with the 8.5-kb RNA, confirming its identity as the only putative *gag-pol* message (Fig. 1C; Table 2). In the human lentiviruses (25, 31, 34), visna virus (12, 38, 44), and equine infectious anemia virus (32, 35), the mature *env* transcript is derived from the genome-length RNA by a single splice which generates a 4- to 5-kb subgenomic message. These *env* mRNAs are processed from the primary transcript by using a splice donor between the 5' LTR and the *gag* ORF and a splice acceptor immediately 5' of the *env* ORF. Probe 4 (*env*) hybridized to the 8.5-, 4.1-, and 3.8-kb RNAs (Fig. 1C; Table 2), suggesting that the BIV *env* message is a 3.8- or 4.1-kb spliced mRNA containing the untranslated 5' leader fused to the 3' portion of the genome (Fig. 2A). Probe 9 (*env/rev2/tat2*) also hybridized to the 3.8-kb RNA; we expected this result, since the *env* transcript contains untranslated sequences for the *rev* and *tat* ORFs (Fig. 1C; Table 2).

We previously predicted the location of a potential splice acceptor site, immediately 5' of the *env* ORF at nt 5396, that would be used by both *env* and *rev* transcripts (20). cDNA cloning of BIV transcripts was undertaken to derive experimental data to support this assignment. We selected two oligonucleotide primers (one from sequences of the common 5' leader and the other from the middle of the overlapping *rev* exon 2 and *env* ORFs) to synthesize and amplify cDNAs encompassing this region. Our preliminary characterization of putative *env*- and *rev*-specific partial cDNAs indicates that nt 5396 is an authentic splice acceptor for these transcripts (Fig. 2A and B, respectively). The use of the splice acceptor at nt 5396 (Fig. 2A), immediately upstream of the *env* ORF, would result in a mature transcript of 3376 nt (without polyadenylation); the first AUG 3' of this splice acceptor site is the first AUG of the *env* ORF. Since *env* and the first *rev* coding exon coincide in the same reading frame, it is possible that Rev translation begins at the first AUG while Env translation initiates at a downstream AUG. Baculovirus expression of an *env/rev* construct whose first AUG is that of *rev* fails to produce detectable Env protein (34a). A synthetic *env* construct which begins with an AUG created at pre-

TABLE 2. Summary of probe hybridization in Northern blot analysis^a

RNA (kb)	Hybridization with probe:								
	1	2	3	4	5	6	7	8	9
8.5	+	+	+	+	+	+	+	+	+
4.1	+			+	+	+	+	+	+
3.8	+			+					+
1.7	+						+	+	+
1.4	+								+

^a Results from Northern blots (Fig. 1) using a single lot of RNA, except as noted.^b Results from separate experiments with other lots of RNA.

A

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1 TAAGAGAGACTCGGCTCGAGTAAAGAAAGACCCAGCTCGAACGAGAAGACTCCGGACAGAAAAATCTAGGAATCAACTATGGATCAGGACCTAGACCCGG 100
start env → M D Q D L D R A
101 CGGAACCGCGGGGAAAGGGGAGGAGATCCGAAGAACTGCTTCAGGAGGAGATCAACGAAGGGAGGCTGACAGCCAGAGAAGCTTTACAAAATGGATCAA 200
E R G E R G G G S E E L L Q E E I N E G R L T A R E A L Q T W I N
201 TAACGGTGAGATCCACCCTTGGCTCCTGGCAGGAATGCTGTCCATGGGAGTAGGAATGCTACTAGGAGTATATTGTCACTTACCAGACACTGATTGG 300
N G E I H P W V L A G M L S M G V G M L L G V Y C Q L P D T L I W
301 ATACTAATGTTTCAATTATGCCCTTATTGGGGTTTGGGTGAAACATCTAGAGAATTAGACAAGGATAGTTGGCAGT 376
I L M F Q L C L Y W G L G E T S R E L D K D S W Q

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B

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1 ttccagctcgtgtagctcattagctccgagctcccaaacctacagcctgagaggcactggctcggttgggttagccagccttccgggtaataaaggcttgtt 100
ggcatttcggcatctacccgtgctcctgcttctgtcttactcgagcgaaccacaaactccgtcctgctgagctcagagctcggggcggtgaagaacacc 200
201 caacagttggcgcccaactggggctcgagtaAGAGAGACTCGGCTCGAGTAAAGAAAGACCCAGCTCGAACGAGAAGACTCCGGACAGAAAAATCTAGG 300
301 AATCAACTATGGATCAGGACCTAGACCCGCGGAAACCGGGGAAAGGGGAGGAGATCCGAAGAACTGCTTCAGGAGGAGATCAACGAAGGGAGGCTGAC 400
start rev → M D Q D L D R A E R G E R G G G S E E L L Q E E I N E G R L T
401 AGCCAGAGAAGCTTTACAAATCGATCAATATGATTCTCCTAGGTATGTTAAGAAAGCTGCGCCAAAGTCAGCCAGAATTACCAACATCTCCCGCGGA 500
A R E A L Q T W I N N D S P R Y V K K L R Q G Q P E L P T S P G G
501 GGAGGAGGACCGGGACACAGAGCCAGAAAGCTCCCGCGGAGAGAGACCCGGCTTCTGGAAGTCTCTACGAGAATTGGTTGAACAAAATAGGAGAAAGC 600
G G G R G H R A R K L P G E R R P G F W K S L R E L V E Q M R R K Q
E E D G D T E P E S S P A R C D P A S F W S L Y E N W L N K I G E S
601 AAGAACGAGcctatcggtctggacagaagaatacaacagcttgaggatctgttgcacatgctcggtggatctcctgaccctcaactccttcagc 700
E R R L S G L D R R I Q Q L E D L V R H M S L G S P D P S T P S A
K N D A Y R V W T E E Y N S L R I L F A T C R W D L L T P Q L L Q L
701 ttccgttctttctgttaacccctcctgctcaaacctccttgggacatcttcgccacgctcctattttaaacctaaagggtggactgtggggcagggtgg 800
S V L S V M P P A Q T P L G H L P P R S Y F K L K R V D C G A G W
P F F L L T L L K L L W D I F R H A P I L M L K G W T V G Q G G
801 gacctcaggacacagcagcccccggacttcccatatgtgaattggactggatccagggaacaaaataaccagaaggggattagactctggggcttgg 900
D L R T T A A P G L P I C E L D W I Q G T K *
T S G Q Q Q P P D F P Y V N W T G S R E Q N N P E G G L D S G A W
901 tatgaaggcctgagaggttctcagtagattgtaagtcttcggcgagactgcatgtctgcacgtagacaggaaatgtttatctctcagctgattgtggtt 1000
Y E G L R G S Q *
1001 agggcgattactgtgaaactagacaacctgattcattagtggttaagattatgcataagtctcgcaatgatgtagctgcttactcgcttactccgccc 1100
tgaacgcctacctaacacgcaacacgcccacctgtaagaatataaacatattcttactctgtacttcagctcgtgtagctcattagctccgagct 1200
1201 ccccaacctacagcctgagaggcactggctcggttgggttagccagccttcgggtaataaaggcttgttggcattcgcca 1280

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FIG. 2. Sequence analysis of BIV cDNAs. (A) Partial sequence of a BIV cDNA containing the *env* coding exon. The *env* cDNA was amplified with primers TAAGAGAGACTCGGCTCGAG (nt 231 to 250) and ACTGCCAACTATCCTTGCT (nt 5712 to 5693) (see text). (B) Sequence of a BIV cDNA containing putative *rev* exons. The *rev* cDNA was amplified with primers TAAGAGAGACTCGGCTCGAG (nt 231 to 250) and GATAGGCGTCTGTTCTTGCTT (nt 7818 to 7799) (see text). The region sequenced is shown in uppercase letters; the region inferred from the BIV nucleotide sequence is shown in lowercase letters (20). The amino acid translation of the major ORFs is shown below the sequence. Vertical arrows indicate splice junctions. Where the cDNA sequence in panel B differs from the previously published BIV 127 sequence (20), the nucleotide from the previously published sequence is shown above the cDNA sequence.

dicted *env* amino acid 50 (CTG→ATG; Leu→Met) produces immunologically detectable BIV Env. This artificial start codon is just upstream of the second *env* AUG, near the amino terminus of the putative Env signal sequence (20).

The 4.1-kb RNA hybridized with probes 1, 4, and 7 through 9 but not with probe 2 or 3 (Fig. 1C; Table 2), suggesting that it arises from a splicing event which deletes the *gag-pol* region and joins the central region to the 5' untranslated leader. In some experiments, with other preparations of RNA, probes 5 and 6 hybridized weakly with the 4.1-kb RNA (Table 2 and data not shown). Because of this ambiguity, the identity of the 4.1-kb RNA cannot be unequivocally determined. If hybridization with probe 5 (*vif*) and probe 6 (*vifW*) does occur, the 4.1-kb RNA may represent the *vif* mRNA, but it could also direct synthesis of a protein from BIV ORF W (Fig. 1A and B; Table 2). The 4.1-kb band is probably not the mRNA for any other central region ORF, because the large number of potential AUG

start codons upstream of the authentic translation start site would result in very inefficient translation of these proteins. Although the 4.1-kb RNA could contain the entire *env* coding sequence, we believe that it does not code for the *env* ORF products, since it has nine potential start codons upstream of the *env* ORF (20).

With the exception of *vif*, *vpr*, and *vpu* (1, 40) (and possibly W and Y), the nonstructural/regulatory genes of the lentivirus central region are encoded by multiply spliced mRNAs, usually of less than 2 kb (12, 25, 31, 32, 34, 35, 38, 44). The 1.7-kb RNA is recognized by probes 7 (*Ytat1*), 8 (*vifY*), and 9 (*env/rev2/tat2*) (Fig. 1C; Table 2). The broadness of this band suggests that it may encompass more than one RNA species ranging from 1.7 to 1.8 kb. These mRNAs probably represent the *tat* or *Ytat* transcript(s) because probes from the *tat* and *Y* ORFs hybridized to the 1.7-kb RNA but not to the 1.4-kb RNA (Fig. 1C; Table 2). The proposed *Y* splice acceptor is 54 nt upstream of the predicted

tat splice acceptor, suggesting that a 1.8-kb RNA might encode *Y*, while the 1.7-kb RNA is believed to be the *tat* message (20). The *tat* message of primate lentiviruses often contains a short noncoding exon derived from the 3' end of the *pol* region (25), but none of the probes used in this study would have detected such an exon.

The 1.4-kb RNA hybridized only with probes 1 and 9, indicating that its 5'-most splice acceptor is 3' to nt 5364 (the 3' end of probe 7). In light of this finding and the data discussed below, we believe that the 1.4-kb RNA is the *rev* message. Coding exon 1 of BIV *rev* (Fig. 1) was identified in the BIV sequence by its location (overlapping and in the same reading frame as *env*) and by the similarity (in character, if not identity) of its deduced amino acid sequence to those of other lentivirus Rev proteins (20). The location of BIV *rev* coding exon 1 is not without parallel; the first coding exon of visna virus *rev* occupies a similar position at the 5' end of and in the same reading frame as *env* (11, 29). The second coding exon of visna virus *rev* is near the 3' end of the genome, overlapping *env*, but in a different reading frame. In the cDNA sequencing studies reported here, we have identified a doubly spliced BIV transcript (Fig. 2B) with the same structure as the visna virus *rev* mRNA (11, 29). The boundaries of the first intron of BIV *rev* are identical to those of the single intron in the *env* transcript, since they share the same splice donor and acceptor for this region (Fig. 2). The *rev* transcript is spliced a second time (post-*rev* coding exon 1), deleting sequences (*rev* intron 2) found in the 3' end of the genome. We had previously predicted the splice donor and acceptor for the second intron of *rev* to reside at nt 5395 and nt 7698, respectively (20). However, our present data indicate that the splice donor and acceptor for the second *rev* intron are at nt 5540 and 7636, respectively (Fig. 2B).

Figure 1B shows the putative BIV transcription pattern, deduced from Northern (RNA) analyses (Fig. 1C; Table 2), preliminary cDNA sequencing (Fig. 2), and a comparison between BIV and other lentiviruses. The pattern of BIV transcription shows a complexity characteristic of the lentivirus subfamily of retroviruses. All transcripts presumably initiate at the same site at the 5' end of the R region of the left LTR and terminate at the 3' end of the R region of the right LTR (20). The genome-length RNA encodes the virion RNA and the *gag-pol* polypeptide, which in lentiviruses is believed to be translated through a -1-nt frameshift, since these two ORFs are in different but overlapping reading frames (20, 43). The remaining proteins appear to be translated from spliced messages.

BIV has recently been shown to transactivate its LTR (32c), presumably through the action of the *tat* gene product. The protein potentially encoded by sequences in the BIV 1.7-kb mRNA contains a Cys-rich region that is highly conserved among lentivirus Tat proteins (20). This conserved motif has been shown to be required for the activity of HIV-1 Tat (16, 36, 37). This region has also been implicated in the formation of metal-linked Tat homodimers (17, 18). The HIV-1 Rev protein has been shown to regulate the translation of the *gag-pol* and *env* transcripts by specifically controlling the transport of these mRNAs from the nucleus to the cytoplasm (14, 15, 24, 28). The putative *rev* gene product of BIV does not align well with other Rev proteins, but its position in the genome and splicing pattern (1.4-kb doubly spliced message) are analogous to those of visna virus *rev* (11, 20, 29). The predicted BIV Vif protein also bears little similarity to its counterparts in other lentiviruses, except in its genome location (20) and the presence of a short

amino acid motif which is highly conserved among all lentiviruses (32b).

The transcriptional map of the putative BIV nonstructural/regulatory genes (*vif*, *tat*, and *rev*) derived here closely corresponds to those of the lentiviruses HIV-1 and visna virus, supporting the tentative identification of each of these ORFs (8, 11, 38, 44). Transcripts specific to the BIV *W* and *Y* ORFs could not be identified in the present study because of the significant overlap in central region ORFs. However, the location of the BIV *W* and *Y* ORFs in the central region of the genome is similar to the location of the *R*, *U*, and *X* genes of primate lentiviruses (20). In HIV-1, the *R* and *U* genes are singly spliced messages of about 4 kb, slightly smaller than the *vif* mRNA (1, 40). The BIV *W* and *Y* ORFs may be somewhat analogous to the HIV-1 *R* and *U* ORFs, respectively. This hypothesis is based on several features of these putative ORFs. First, predicted splice acceptor sites are located immediately upstream of both *W* and *Y* at nt 4714 and 5057, respectively (20). Second, a singly spliced *W* message, using a splice donor at nt 289 and a splice acceptor at nt 4714, would be slightly smaller than a singly spliced BIV *vif* transcript. Two splicing events, removing introns on either side of the BIV *Y* ORF, would result in a *Y* mRNA of approximately 1.4 to 1.7 kb; the length of this message would depend upon the exact location of the borders of the second intron. Finally, the predicted BIV *W* and *Y* products have a number of basic amino acids, suggesting a nucleic acid- or nucleotide-binding domain and thus a possible interaction with nucleic acids. Interestingly, the HIV-1 *R* gene product has recently been shown to be a virion-associated protein and to interact in *trans* with HIV-1 promoter sequences in the LTR to enhance expression of viral proteins (6). Direct identification of BIV regulatory proteins must await the development of specific immunologic reagents, as well as the characterization of complete viral cDNAs and their functional study.

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