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MINIREVIEW

The Remarkable Coding Strategy of Borna Disease Virus: A New Member of the Nonsegmented Negative Strand RNA Viruses

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Viruses use many different methods for control of gene expression and nowhere is this more apparent than with the nonsegmented and segmented negative-strand RNA viruses. Different methods for controlling expression of negative-strand RNA virus genomes include spliced and alternatively spliced RNAs, bicistronic mRNAs, ambisense RNAs, coupled translation of tandem cistrons, the use of non-AUG initiation codons and RNA editing (Shaw *et al.* 1983; Curran and Kolakofsky, 1988; Thomas *et al.*, 1988; Cattaneo, 1989; Krug, 1989; Lamb, 1989; Paterson *et al.*, 1989; Bishop, 1990; Horvath *et al.*, 1990; Schmaljohn and Patterson, 1990; Kolakofsky *et al.* 1991; Lamb and Horvath, 1991; Lamb and Paterson, 1991; Giorgi *et al.*, 1983; Spiropoulou and Nichol, 1993; Lamb and Kolakofsky, 1995; Lamb and Krug, 1995). These different coding strategies not only create diversity by increasing the number of proteins that a virus can encode from a compact genome but also provide a means by which to regulate expression of individual proteins.

Borna disease virus (BDV) is a neurotropic enveloped nonsegmented, negative-strand RNA virus (Briese *et al.*, 1992, 1994; Cubitt and de la Torre, 1994; Cubitt *et al.*, 1994a; Compans *et al.*, 1994; Zimmermann *et al.*, 1994) that appears to be a member of the Order *Mononegavirales*. The *Mononegavirales* include *Filoviridae* (e.g., Marburg virus), *Paramyxoviridae* (e.g., Sendai virus, mumps virus, SV5, measles virus), and *Rhabdoviridae* (e.g., vesicular stomatitis virus (VSV) and rabies virus). Originally thought to be a natural pathogen only for horses and sheep in Southeastern Germany, BDV is now known to infect species ranging from birds to primates across the world. Recently, virus particles have been isolated (Briese *et al.*, 1992; Richt *et al.*, 1993) and molecular cloning (Lipkin *et al.*, 1990) and nucleotide sequencing of the BDV genome have been completed. The nucleotide sequence of BDV subgenomic RNAs disclosed complex mechanisms for control of replication and transcription that were not anticipated. As discussed below, BDV employs a number of strategies to direct expression of its compact genome including RNA splicing, overlap of transcription units and transcription signals, readthrough of transcription initiation sites, and possibly differential use of translational initiation codons. While there is precedent for use of each of these strategies by the *Mononegavirales*, BDV is thus far unique among them in the diversity of its repertoire.

BDV GENOME ORGANIZATION

The BDV RNA was molecularly cloned from ribonucleoproteins (strain He-80) (Cubitt *et al.*, 1994a) and virus particles (strain V) (Briese *et al.*, 1994) to reveal a genome composed of approximately 8900 nucleotides with complementary termini, encoding five major open reading frames (ORFs) (Fig. 1). Although similar in organization to other *Mononegavirales*, the BDV genome is smaller than those of either rhabdoviruses (e.g., VSV, 11,091 nucleotides; Rose and Schubert, 1987) or paramyxoviruses (e.g., SV5, 15,246 nucleotides; Parks *et al.*, 1992). Small differences between the two published sequences of BDV have led to differences in the placement of ORFs (compare diagrams in review by de la Torre (1994) and this review). ORFs *I, II, III, IV,* and *V* described by Cubitt and co-workers (1994a) are similar to ORFs *p40, p23, gp18, p57,* and *p180 (pol)* (Fig. 1) (Briese *et al.*, 1994), with the following differences: (1) ORFs *I, II, III, IV,* and *V* are in frame with one another, whereas *p57* is in the +1 frame relative to the other ORFs in Briese and coworkers (1994) sequence; (2) ORF *IV* predicts a protein of 40 kDa vs the 57-kDa protein predicted by ORF *p57*. The differences seem most likely due to cloning and/or sequencing errors rather than natural differences between the nucleotide sequences of different strains.

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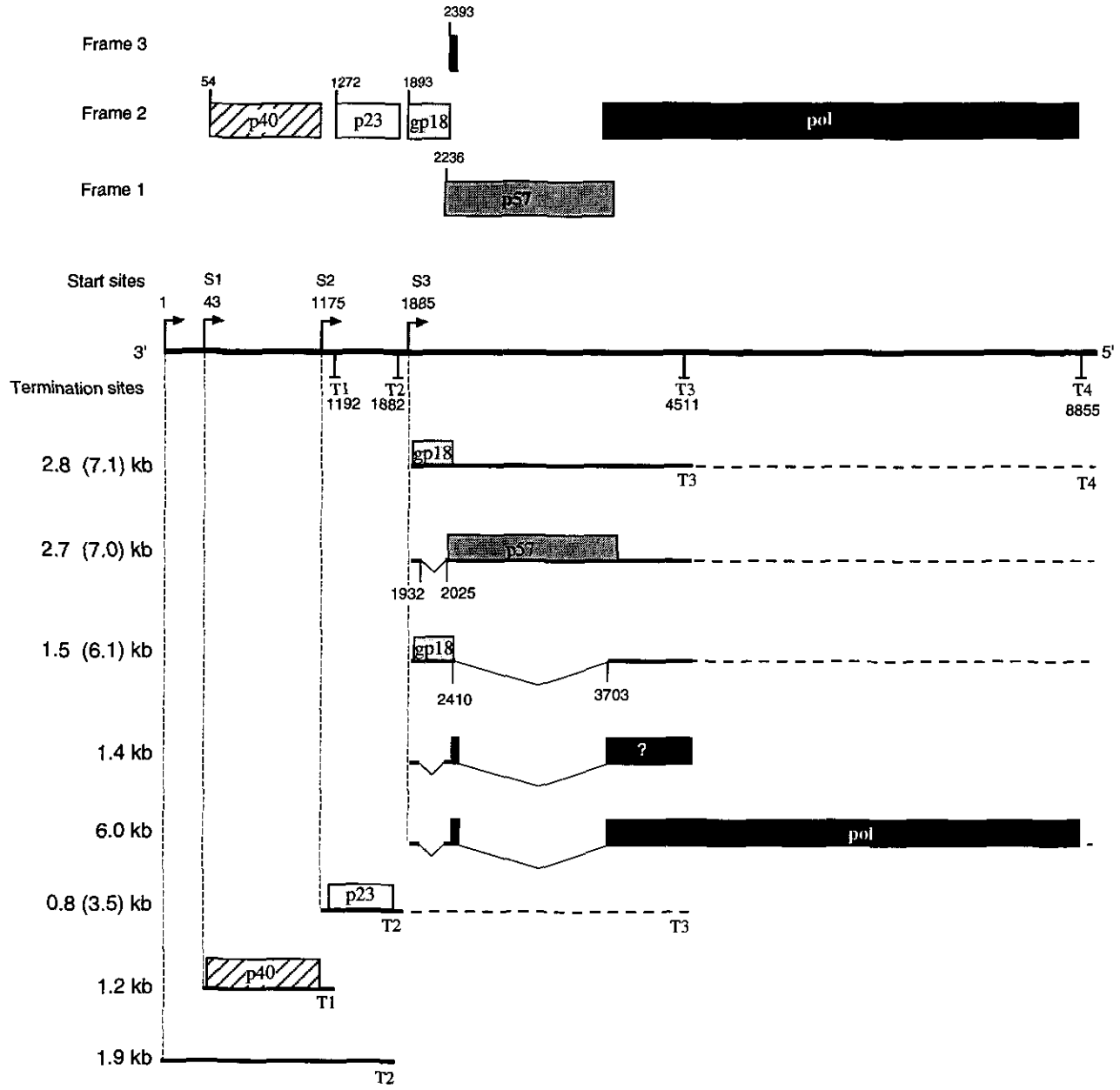

FIG. 1. Genomic organization and transcriptional map of BDV. The BDV genome is shown as a solid line in 3′ to 5′ direction. Coding regions and their respective reading frames are represented as boxes at the top; the number above each upward vertical line indicates the nucleotide position of the first AUG codon in the respective ORF. Transcription initiation sites and their nucleotide positions on the viral genome (strain V) are represented by arrows pointing downstream. Transcription termination sites and splice sites are indicated by downward vertical lines. Dashed lines indicate that readthrough at termination sites T2 and T3 results in synthesis of longer RNAs terminating at T3 and T4, respectively. The 1.2- and 0.8-kb RNA have been shown to represent the mRNAs for p40 and p23, respectively. p23 could also be translated from the 3.5-kb RNA. Transcripts that are likely to represent mRNAs for gp18, p57, and pol are indicated. Note that gp18 can only be translated from RNAs containing intron 1. Splicing of intron 1 preserves the gp18 initiation codon but introduces a stop codon such that only the first 13 amino acids could be translated from the 2.7 (7.0)-kb transcripts and the 1.4 (6.0)-kb transcripts. It is not known whether the 1.9-kb RNA or the 1.4-kb RNA serve as messages for the translation of BDV proteins.

BDV PROTEINS

Gene products have only been identified for ORFs *p40, p23,* and *gp18* (McClure *et al.,* 1992; Thierer *et al.,* 1992; Kliche *et al.,* 1994; Schädler *et al.,* 1985). The position of ORFs *p40* and *p23* on the genome and features of the polypeptides found in virus-infected cells such as molecular weight, charge, abundance in infected cells and particles, as well as post-translational modifications, suggest that *p40* encodes the viral nucleoprotein (N) and *p23* encodes a phosphoprotein (Thierer *et al.,* 1992); the latter is a likely candidate for the phosphoprotein (P) found in *Mononegavirales.* The third ORF of the genome of most *Mononegavirales* directs synthesis of a nongly-

cosylated matrix protein (M) (Kingsbury, 1990; Wagner, 1990), whereas the BDV third ORF *gp18* encodes an 18-kDa glycoprotein (Kliche *et al.,* 1994). Although glycosylated M proteins that resemble gp18 in size and isoelectric point (p*I*~10) have been found with other enveloped viruses, such as the E1 protein of coronaviruses (Armstrong *et al.,* 1984), preliminary evidence suggests that gp18 is present in the virus envelope and that it may serve as a viral attachment protein (Kliche *et al., 1994*). Computer analysis of ORF *p57* revealed multiple potential N-glycosylation sites as well as N-terminal and C-terminal hydrophobic anchor domains (Briese *et al.,* 1994), suggesting that this ORF directs expression of a BDV glycoprotein (G). The most 5′ ORF on the viral genome, *pol,* encompasses more than half of the genome and contains motifs considered critical to viral RNA polymerase activity (Briese *et al.,* 1994; Cubitt *et al.,* 1994a). Cubitt and co-workers (1994a) predicted a protein of 170 kDa from ORF *V.* However, the presence of conserved sequences between strain V and strain He/80 upstream of the AUG codon of ORF *V,* as well as analysis of viral transcripts (see below), suggests that pol is expressed from two separate exons to yield a protein of 190 kDa. Additional smaller ORFs with coding capacities of less than 16 kDa have been identified on both the positive (antigenomic) and negative (genomic) RNA strands (Briese *et al.,* 1994; Cubitt *et al.,* 1994a). Whether any of these ORFs is expressed is unknown.

TRANSCRIPTION OF THE BDV GENOME

Transcription of rhabdovirus and paramyxovirus genomes by the endogenous nucleocapsid-associated viral RNA polymerase from the 3′ end of the genome is sequential and polar following the gene order. As a result of this transcription strategy, the molar abundance of each mRNA is regulated by the location of its gene, i.e., transcription attenuates in a 3′ to 5′ direction. Although transcription occurs sequentially, it is not known for certain whether the polymerase enters the template only at a single site at the 3′ terminus of the genome or also at the internal gene start sites but with transcription of a gene dependent on transcription of the 3′ proximal upstream gene (reviewed in Banerjee, 1987). In addition to faithful initiation and elongation of transcription, the RNA polymerase complex of rhabdoviruses and paramyxoviruses has guanyltransferase and methylating activity and polyadenylates the mRNAs.

In contrast to the vast amount of information known about rhabdovirus and paramyxovirus transcription, studies of BDV transcription are still in the early stages. The BDV genome is transcribed in the nucleus of infected cells (Briese *et al.,* 1992). While this is a unique feature among nonsegmented, negative-strand animal RNA viruses, it is not unusual for negative-strand RNA viruses in general. Some plant rhabdoviruses (Heaton *et al.,* 1987) as well as the segmented influenza viruses (reviewed in Krug, 1989) are known to use the nucleus for transcription and replication. Although it is not known what aspects of transcription and/or replication dictate the nuclear localization for the plant rhabdoviruses — the plant rhabdovirus sonchus yellow net virus does not splice RNAs or use host cell mRNAs to prime transcription (J. D. Wagner and A. O. Jackson, personal communication) — influenza viruses have been shown to engage in both activities. BDV does not appear to employ influenza virus-like "cap stealing" to initiate gene transcription (Schneemann *et al.,* 1994); however, it does require the cellular splicing machinery to process some of its primary RNA transcripts (Schneider *et al.,* 1994).

Initial mapping of the BDV subgenomic RNAs to the antigenome was done by Northern blot hybridization (Briese *et al.,* 1994; Cubitt *et al.,* 1994a). This analysis revealed a complex pattern of overlapping transcripts that included several polycistronic RNAs (Fig. 1). Transcription of the BDV genome results in the synthesis of at least six primary, polyadenylated RNAs with apparent chain lengths of 0.8, 1.2, 1.9, 2.8, 3.5, and 7.1 kb (Briese *et al.,* 1994; Cubitt *et al.,* 1994a). An additional RNA of 4.7 kb has been reported by Cubitt and co-workers (1994a). The abundance of these RNAs in infected cells and tissues (Briese *et al.,* 1994; Cubitt *et al.,* 1994a) is consistent with the 3′-to-5′ transcriptional gradient found for the *Mononegavirales.* In addition, Briese and co-workers (1994) found 1.5- and 6.1-kb RNAs that initiated and terminated at the same positions as the 2.8- and 7.1-kb RNAs, respectively, but contained 1.3-kb internal deletions not present in the two larger RNAs (see below). The results obtained by the Lipkin and de la Torre groups differed with respect to the location of the 2.8-kb RNA, the 1.5-kb RNA, and the 6.1-kb RNA (compare Fig. 1 in this Minireview and Fig. 4 in Briese *et al.,* 1994 with Fig. 5B in Cubitt *et al.,* 1994a).

TRANSCRIPTION INITIATION AND TERMINATION SITES

The genomes of rhabdoviruses and paramyxoviruses typically have blocks of nucleotides at the gene junctions specifying transcriptional termination/polyadenylation, the nontranscribed intergenic region and the gene transcriptional start sites: for VSV these blocks of sequence are invariant. The transcriptional termination–polyadenylation signal consists in part of 4–5 U residues and it is thought that transcription terminates with the polymerase stuttering to create the poly(A) tail (reviewed in Banerjee, 1987; Kingsbury, 1990).

The precise transcription initiation and termination sites for BDV were determined by purification of viral subgenomic RNAs and examination of the terminal sequences by RNA circularization and RT-PCR over the ligated ends (Schneemann *et al.,* 1994). This analysis indicated that the genome contains three transcription initiation sites and four termination sites and confirmed

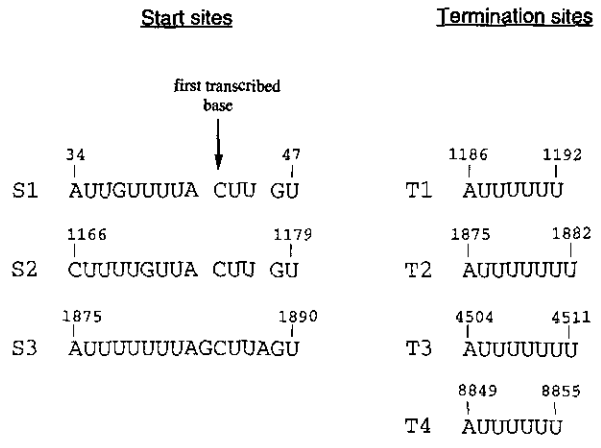

FIG. 2. Nucleotide sequences around the BDV transcription initiation and transcription stop sites. The nucleotide sequences and the numerical assignations are from the complete sequence of BDV strain V (Briese *et al.*, 1994). Data for the start and stop sites taken from Schneemann and co-workers (1994).

the transcriptional map established by Briese and coworkers (1994). Specifically, the sites of transcription initiation mapped to nucleotides (nt) 1, nt 43 (S1), nt 1175 (S2), and nt 1885 (S3), using nucleotide positions of BDV strain V (Fig. 1). A semiconserved, U-rich motif that is partially copied into the respective transcripts was identified at sites S1, S2, and S3. This motif is present in all BDV strains for which genomic sequence is available (strain He80, strain He80-1, and strain V), suggesting that it has a functional role, presumably as a transcription initiation signal. The motif appears to be specific for BDV in that similar sequences are not present at the gene start sites of previously described *Mononegavirales*. Direct terminal sequencing confirmed the four transcription termination sites proposed by Briese *et al.* (1994) and Cubitt *et al.* (1994a): nt 1192 (T1), nt 1882 (T2), nt 4511 (T3), and nt 8855 (T4) (Fig. 1). Each termination site consists of 6 or 7 U residues preceded by a single A residue. This consensus sequence is reminiscient of the transcription termination–polyadenylation signals in known *Mononegavirales* described above, and it seems likely that polyadenylation for BDV also occurs by polymerase stuttering on these U residues.

One of the more striking features of the BDV genome organization is the unusual positioning of transcription termination and initiation signals at the gene junctions (Schneemann *et al.*, 1994) (see Figs. 1 and 2). Unlike the rhabdoviruses and paramyxoviruses where the gene junctions can usually be divided into the transcription termination–polyadenylation region, intergenic region, and the transcription initiation region, the BDV transcription initiation site for the 0.8-kb RNA (S2) is located 18 nt upstream of the termination site of the 1.2-kb RNA (T1) (Fig. 1). An exception to this pattern has been observed in the paramyxovirus respiratory syncytial virus where the start site for the polymerase (L) gene is located 68 nt upstream of the 22K gene (reviewed in Collins, 1991). It has been proposed that this arrangement serves as a mechanism to attenuate expression of the L gene. However, in BDV the 1.2- and 0.8-kb RNAs are the most abundant RNAs in infected cells, implying that the overlap does not significantly affect transcription of the respective genes. It is possible that the degree of attenuation is a function of the length by which the two transcription units overlap. If so, a stretch of 18 nt may not be sufficient to cause a noticable decrease in transcription of the 0.8-kb RNA.

The second and third transcription units (0.8- and 2.8-kb RNAs, respectively) are separated by only 2 nt. Interestingly, the transcription initiation signal for the 2.8-kb RNA (S3) extends upstream across the intergenic region into the termination signal of the 0.8-kb RNA (T2) such that T2 is completely contained within S3 (Figs. 1 and 2). The overlap of these domains in BDV does not appear to interfere with their recognition by the BDV polymerase since termination and initiation occur efficiently at this gene junction. It is not clear how the polymerase recognizes the overlapping domains as separate functional entities, but it is possible that there are additional sequences upstream of the termination site that prepare actively transcribing polymerase for termination. It will be of considerable interest to elucidate the mechanistic details of RNA synthesis at this gene junction.

The transcriptional map of BDV indicates that several polycistronic RNAs arise by readthrough at the various termination sites. Transcriptional readthrough is not uncommon in *Mononegavirales;* however, it is usually considered to be abberrant and the biological significance of the readthrough products is unknown (reviewed in Banerjee, 1987; Kingsbury, 1990). In contrast, for BDV transcriptional readthrough of the T3 site is vital. The 7.1-kb RNA transcript resulting from readthrough at termination site T3 is the only RNA transcript that contains the *pol* ORF. Transcriptional readthrough may provide a mechanism for regulating BDV gene expression. For example, low level readthrough at T3 would lead to a decreased level of the BDV polymerase, which should be needed only in catalytic amounts. Support for this hypothesis comes from the observation that the levels of the 7.1-kb RNA and its splice products are indeed lower than those of the 2.8-kb RNA and its splice products. However, it cannot be currently ruled out that this observation merely reflects a difference in the stabilities of the respective RNAs.

The 1.9-kb RNA was found to be fundamentally different from the other subgenomic BDV RNAs. First, in contrast to the 1.2-kb RNA, the 1.9-kb RNA initiates at the extreme 3′ end of the BDV genome, which does not contain the consensus sequence observed at S1, S2, and S3 (Schneemann *et al.*, 1994). In addition, the 1.9-kb RNA is not capped and not fully polyadenylated: it contains only eight to nine adenylate residues at the 3′ end, seven of which are encoded by the termination signal (Schneemann *et al.*, 1994). These observations suggest that the 1.9-kb RNA represents an analog of leader-containing subgenomic

RNAs found in other nonsegmented negative-strand RNA viruses such as measles virus and Sendai virus (Castaneda and Wong, 1990; Vidal and Kolakofsky, 1989). It is intriguing that the 1.9-kb RNA is not polyadenylated to the same extent as the other subgenomic RNAs (Schneemann *et al.*, 1994). From the perspective of the transcription–replication model of *Mononegavirales*, the 1.9-kb transcript could also be considered a replication intermediate that was aborted at termination site T2. If this model is correct, the polymerase would not be expected to synthesize a poly(A) tail at the 3′ end of the newly synthesized RNA strand.

Thus, the BDV genome is extremely compact: 99.4% of its nucleotides are transcribed into subgenomic RNAs (Schneemann *et al.*, 1994). Only 55 of 8910 bases (strain V) are not found in the primary viral transcripts. These bases represent the trailer region at the 5′ end of the genome. The region between the 3′ end of the genome and the first base of the first transcription unit is 42 nt long and probably corresponds to the leader sequence found in other nonsegmented negative-strand RNA viruses. Two other bases located in the intergenic region between T2 and S3 are found only in the rare polycistronic 3.5-kb RNA.

SPLICING OF BDV mRNAs

Several observations suggested that some of the BDV primary transcripts might be processed by the cellular splicing machinery: (1) the apparent absence of mRNAs initiating and terminating close to the translational start sites of the *p57* and *pol* ORFs; (2) the detection in Northern blots of transcripts that were not colinear with the BDV genome (discussed above).

The precise sites of the interrupted regions in the processed RNAs were analyzed by RT-PCR and Northern blot hybridization using RNAs isolated from either virus-infected cells or from infected tissue (Schneider *et al.*, 1994). Primary transcripts initiating at S3 (the 7.1- and 2.8-kb RNAs) were found to contain introns that spanned nt 1932–2025 (intron-1, 94 nt) and nt 2410–3703 (intron-2, 1.3 kb) (Fig. 1). The nucleotide sequences around the 5′ and 3′ junctions of the interrupted regions showed 78% identity with the mammalian splice junction consensus sequences. In addition, sequences expected for splicing branchpoints were found in the introns 18 nt (intron-1) and 30 nt (intron-2) from the 3′ splice junction, respectively (Schneider *et al.*, 1994). By using an analogous approach, Cubitt and co-workers (1994b) confirmed the presence of RNA transcripts containing single- or double-interrupted regions at the exact same sites. To demonstrate that a truncated BDV 2.8-kb RNA transcript could serve as a substrate for the cellular splicing machinery, a cDNA clone of the truncated 2.8-kb RNA was transiently expressed in the nucleus of COS-7 cells and both colinear 2.8-kb and interrupted 1.5-kb RNAs were recovered. Northern blot analysis of RNA isolated from

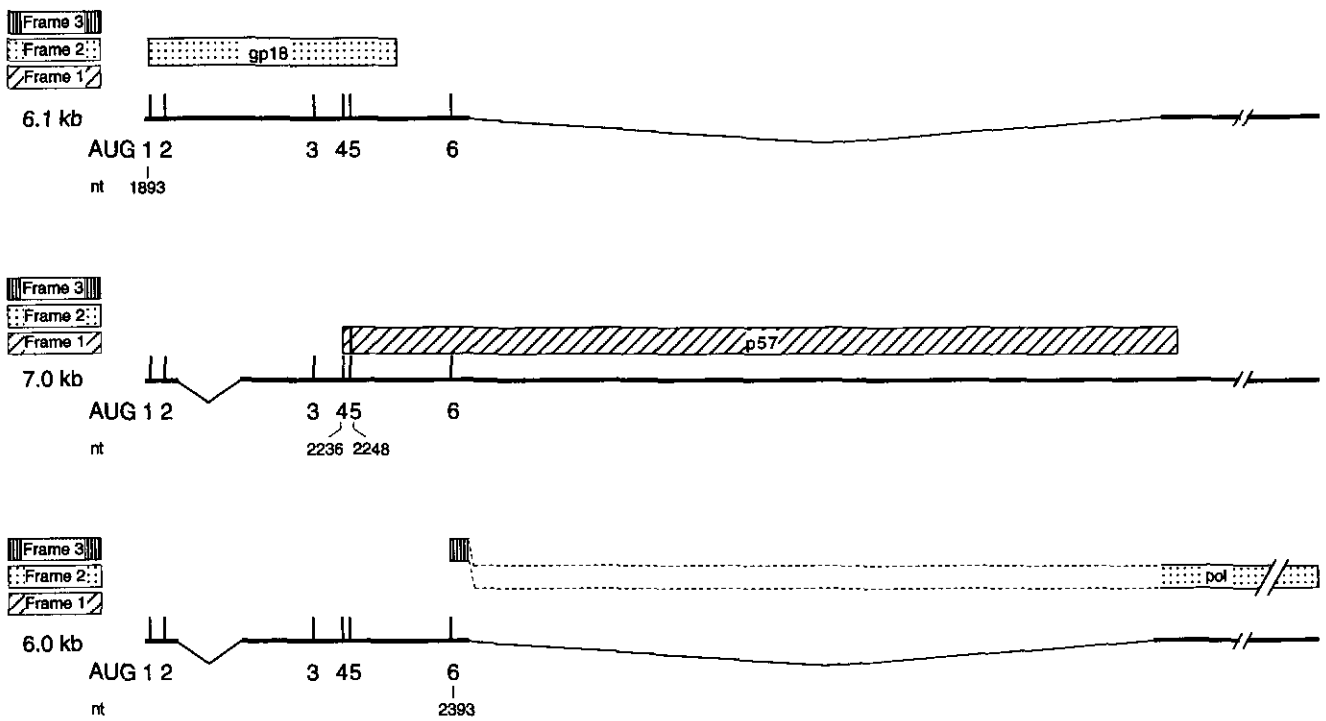

FIG. 3. Location of AUG codons in the 5′ terminal segment of transcripts initiating at start site S3. AUG codon 1, located at nt position 1893, has been shown to serve as the initiation codon for gp18 (Kliche *et al.*, 1994). Initiation at AUG codon 2 would result in the synthesis of small peptides, 7 amino acids (6.1 kb RNA), or 25 amino acids (6.0- and 7.0-kb RNAs) in length. Initiation at AUG codon 3 would result in the synthesis of a 42-amino-acid peptide. Both AUG codon 4 (nt 2236) and 5 (nt 2248) could serve as initiation sites for translation of p57, while AUG codon 6 (nt 1393) is likely to serve as initiation codon for translation of pol. Note that gp18 and p57 are translated in a single reading frame, whereas pol translation begins in frame 3 but shifts to frame 2 after splicing of intron 2.

BDV-infected rat brain tissue showed that splicing is not 100% efficient. Roughly equal proportions of spliced and unspliced mRNAs were found (Schneider *et al.*, 1994). However, the inefficiency of splicing is likely to be critical for the successful replication of BDV as the unspliced RNA transcripts are expected to function as mRNAs in their own right and to encode proteins different from the spliced RNAs (see Fig. 1).

BDV mRNA SPLICING IN COMPARISON TO INFLUENZA VIRUS mRNA SPLICING

Previously, the only other known RNA animal virus (which does not have a DNA intermediate) to replicate in the nucleus and to have spliced mRNAs was influenza virus (Lamb and Lai, 1980; reviewed in Lamb, 1989; Lamb and Krug, 1995). Thus, a comparison to influenza virus is worthwhile. Two of the influenza A viral mRNAs, NS_1 and M_1, are spliced to form mRNAs coding for two other proteins, NS_2 and M_2, respectively (Lamb and Lai, 1980, 1982; Lamb *et al.*, 1981). The M_1 mRNA is also spliced to form another mRNA, $mRNA_3$, which has a coding potential for only nine amino acids. The splice junctions (like those of BDV) are similar to those found in polymerase II transcripts, and most of these splice junctions were used when the NS_1 and M_1 genes were expressed using DNA vectors (Lamb and Lai, 1982, 1984). Consequently, it has been concluded that splicing of the NS_1 and M_1 mRNAs is catalyzed by host-cell nuclear enzymes.

As both the unspliced (NS_1 and M_1) and spliced (NS_2 and M_2) mRNAs code for proteins, the extent of splicing is regulated (like that of BDV) such that some of the unspliced precursor is preserved at the same time that a sufficient amount of the spliced product is produced. In influenza virus-infected cells, this regulation results in a steady-state amount of the spliced mRNAs that is only about 10% of that of the unspliced mRNAs (Lamb *et al.*, 1980, 1981). This type of splicing regulation also occurs with retroviruses, both "simple" retroviruses (such as avian and murine leukemia viruses) and "complex" retroviruses (such as *Lentiviridae*) (Katz and Skalka, 1990; Malim *et al.*, 1989a,b). In these systems, the cytoplasmic concentrations of unspliced and spliced mRNAs are regulated at the levels of splicing efficiency and nuclear export. Usually pre-mRNAs are retained in the nucleus through binding of splicing factors to the 5′ splice site, 3′ splice site and/or branchpoint, thereby committing the pre-mRNA to spliceosome formation (Legrain and Rosbash, 1989). Like the Rev and Rex proteins of lentiviruses (Hadzopoulou-Cladaras *et al.*, 1989; Malim *et al.*, 1989a,b), the NS_1 protein regulates the nuclear export of mRNA (Alsonso-Caplen *et al.*, 1992; Fortes *et al.*, 1994; Qiu and Krug, 1994; Qian *et al.*, 1994) and inhibits pre-mRNA splicing (Lu *et al.*, 1994). However, whereas the HIV-1 Rev protein facilitates the nuclear export of unspliced viral pre-mRNAs, the NS_1 protein inhibits the nuclear export of spliced viral and cellular mRNAs (Lu *et al.*, 1994). It will be of great interest to determine whether BDV proteins are involved in regulation of splicing or transport of mRNAs from the nucleus.

TRANSLATION OF UNSPLICED AND SPLICED BDV mRNAs

Figures 1 and 3 show the most reasonable interpretation of the BDV coding sequences when taking into consideration the positions of the known AUG codons, the splicing of the mRNAs, and the "context" of the initiation AUG codons (Fig. 4) using Kozak's rules (Kozak, 1989) and the known ability of ribosome initiation complexes to scan mRNAs. Whereas BDV p40 and p23 are translated from monocistronic, unspliced mRNAs, the other viral proteins are predicted to be translated from polycistronic and/or spliced RNAs (gp18, polycistronic; p57, singly spliced; pol, doubly spliced). Of these three proteins, a translation initiation site (nt 1893, AUG 1, Fig. 3) has only been identified for gp18 (Kliche *et al.*, 1994). Other, so far unrecognized translation products, may also be synthesized (see Fig. 1). Translation of the 7.0-kb mRNA

	-3 +4	ORF	context
Consensus	**C RCC AUG G**		**++++**
AUG 1	C ACC AUG AAU	gp18	+++
AUG 2	U CCU AUG UGG	?	+
AUG 3	A UAC AUG UUC	?	+
AUG 4	A UCA AUG CAG	p57-1	+
AUG 5	U UCA AUG UCU	p57-2	+
AUG 6	G AAA AUG UCA	pol	+++

FIG. 4. Potential translational initiation codons for p57 and pol and their fit to the Kozak consensus sequence. The nucleotide sequences around the AUG codons 1–6 that lie between nt 1893 and 2393 of BDV strain V (Briese *et al.*, 1994) are shown below the Kozak consensus sequence (boldface type) (Kozak, 1989). AUG codon 1, located at nt position 1893, has been shown to serve as the initiation codon for gp18 (Kliche *et al.*, 1994). The suggested start sites for p57 and pol are indicated. R represents purines.

to synthesize p57 would require that initiation of protein synthesis begins at the 4th or 5th AUG codon from the 5′ end of the mRNA (see Fig. 3). Although AUG 1 is known to be used to translate gp18, in the spliced 7.0-kb mRNA, this AUG is followed 13 residues later by a stop codon. Utilization of AUGs 2 and 3 in the spliced 7.0-kb RNA would only yield small products, 25 and 42 amino acids, respectively, whereas AUG 4 or 5 could initiate synthesis of the p57 polypeptide. Similarly, initiation at AUG 6 in the doubly spliced mRNA (an AUG codon in a strong context) is predicted to initiate pol. However, confirmation of this scheme will require meticulous experimental determination.

SUMMARY

BDV uses a remarkably broad range of mechanisms to direct expression of its 8.9-kb genome. Although much remains to be elucidated, it is clear that BDV genome expression is modulated by the use of multiple strategies, including differential gene transcription, post-transcriptional modification, and translational efficiency. Further insights into the details of this multilevel system will be essential to understanding BDV biology, pathogenesis, and neurotropism.

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