MINIREVIEW

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

ANETTE SCHNEEMANN,* PATRICK A. SCHNEIDER,*+ ROBERT A. LAMB,† and W. IAN LIPKIN*†§

*Laboratory for Neurovirology, Department of Neurology, ‡Department of Anatomy and Neurobiology, †Department of Microbiology and Molecular Genetics, University of California at Irvine, Irvine, California 92717, and §Howard Hughes Medical Institute and Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, Illinois 60208-3500

Received January 17, 1995; accepted April 3, 1995

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, antisense 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; Spiropoulos 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., 1994; 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 borna 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-30) (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 co-workers (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.
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 VI) are represented by arrows pointing downstream. Transcription termination sites and splice sites are indicated by downward vertical lines. Dashed lines indicate that transcriptionally 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. p40 could be also 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 and 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 1.8-kb RNA or the 1.4-kb RNA serve as messengers 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 (pI ~ 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 guanylyltransferase 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 (Schneemann 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, 3.6, 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
the transcriptional map established by Briese and co-workers (1994). Specifically, the sites of transcription initiation mapped to nucleotides (nt) 1, nt 43 (S1), nt 1175 (S2), and nt 1886 (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 reminiscent 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 aberrant 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, 1980; 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 1993, has been shown to serve as the translation 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 1993) 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, 1988; Lamb and Krug, 1995). Thus, a comparison to influenza virus is worthwhile. Two of the influenza A viral mRNAs, NS1 and M1, are spliced to form mRNAs coding for two other proteins, NS2 and M2, respectively (Lamb and Lai, 1980, 1982; Lamb et al., 1981). The M1 mRNA is also spliced to form another mRNA, mRNA3, 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 NS1 and M1 genes were expressed using DNA vectors (Lamb and Lai, 1982, 1984). Consequently, it has been concluded that splicing of the NS1 and M1 mRNAs is catalyzed by host-cell nuclear enzymes.

As both the unspliced (NS1 and M1) and spliced (NS2 and M2) 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 (Hadzioptoulo-Ciadaras et al., 1989; Malim et al., 1989a,b), the NS1 protein regulates the nuclear export of mRNA (Alonso-Caplen et al., 1992; Fortes et al., 1994; Qi 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 NS1 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

---

**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 (Priese et al., 1984) 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 6′ 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.

ACKNOWLEDGMENTS

Work in the authors’ laboratories is supported by Public Health Service Research Grants NS-39425 (W.L.), AI-23172 (R.A.L.), and the Wayne and Gladys Valley Foundation (W.L.). W.L. is a recipient of a Pew Scholars Award from the Pew Charitable Trusts and R.A.L. is an Investigator of the Howard Hughes Medical Institute. We thank A. D. Jackson and A. Lewis for helpful comments.

REFERENCES


rupted mRNAs and cloned DNA coding for the two overlapping non-

RNAs synthesized from cloned influenza virus M DNA in an SV40
vector: expression of the influenza virus membrane protein (M1).
*Virology* 123, 237–256.

spliced NS2 mRNA, and a spliced chimera mRNA from cloned influ-


derived from genome RNA segment 7 of influenza virus: Colinear
Acad. Sci. USA* 78, 4170–4174.

paramyxoviruses. In “The Paramyxoviruses” (D. W. Kingsbury, Ed.)


Lipkin, W. I., Travis, G., Carbone, K., and Wilson, M. (1990). Iso-
lation and characterization of Borna disease agent cDNA clones.

1828.

Functional dissection of the HIV-1 Rev trans-activator—Derivation of a

The HIV-1 rev trans-activator acts through a structural target se-
quence to activate nuclear export of unspliced viral mRNA. *Nature*
338, 254–257.

Sequence similarity between Borna disease virus p40 and a duplica-
ted domain within the paramyxovirus and rhabdovirus polymerase

the NP and L genes of simian virus 5: Identification of highly con-
served domains of the paramyxovirus NP and L proteins. *Virology*
126, 259–279.

templated nucleotide addition to a simian virus 5 mRNA: Prediction
of a common mechanism by which unrecognized hybrid P-cysteine-
rich proteins are encoded by paramyxovirus “P” genes. In “Genetics
and Pathogenicity of Negative Strand Viruses” (D. Kolakofsky and

domains of the influenza virus NS1 protein are required for regulation
of nuclear export of mRNA. *J. Virol.* 68, 2433–2441.

Qiu, Y., and Krug, R. M. (1994). The influenza virus NS1 protein is a
poly A-binding protein that inhibits the nuclear export of mRNAs

Analysis of virus-specific RNA species and proteins in Freon-113
271–280.

products. In “The Rhabdoviruses” (R. Wagner, Ed.) pp. 129–166. Pla-
num, New York.

terization of a 14,500 molecular weight protein from brains and tissue
66, 2479–2484.

replication. Part II: Replication of Bunyaviridae. In “Virology” (B. N.
Fields and D. M. Knipe, Eds.), 2nd ed., pp. 1175–1194. Raven Press,
New York.

Identification of signal sequences that control transcription of borna
68, 6514–6522.

in Borna disease virus, a nonsegmented, negative-strand RNA virus.
*J. Virol.* 68, 5007–5012.

unrecognized influenza B virus glycoprotein from a bicistronic mRNA
that also encodes the viral neuraminidase. *Proc. Natl. Acad. Sci. USA*
80, 4879–4883.

is encoded in overlapping reading frame within the P gene of vesicu-

Thierer, J., Riehle, H., Grebenstein, O., Binz, T., Herzog, S., Thiedemann,

that differ by two non-templated nucleotides encode the amino co-
terminal proteins P and V of the paramyxovirus SV5. *Cell* 54, 891–
902.


(B. N. Fields and D. M. Knipe, Eds.), 2nd ed., pp. 867–881. Raven
Press, New York.

disease virus: Immunoelectronmicroscopic characterization of cell-
free virus and further information about the genome. *J. Virol.* 68,
6755–6758.