The atypical strategies used for gene expression of Borna disease virus, a nonsegmented, negative-strand RNA virus.

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Abstract

Borna disease virus (BDV) is a neurotropic agent that causes disturbances in movement and behavior in vertebrate host species ranging from birds to primates. Although the virus has not been isolated from human subjects, there is indirect evidence to suggest that humans with neuropsychiatric disorders may be infected with BDV. Recently, virus particles have been isolated and the viral genomic RNA has been cloned. This analysis revealed that BDV is a nonsegmented, negative-strand RNA virus. Unusual features such as RNA splicing, overlap of transcription units and transcription signals, as well as sequence dissimilarity for four of five major open reading frames to genes of other nonsegmented, negative-strand RNA viruses suggest that BDV is likely to represent a new taxon within the order *Mononegavirales*.

Introduction

The neurologic syndrome known as Borna disease (BD) was first described approximately 200 years ago (Abildgaard, 1785). The agent responsible for the disease, Borna disease virus (BDV), remained elusive for many years due to its low level of replication in infected hosts. The identification of BDV cDNA clones by subtractive hybridization (Lipkin et al., 1990) and more recently the advent of a method for isolation of virus particles (Briese et al., 1992), has opened the fields of BDV biology and pathogenesis for rigorous investigation. BDV has now been defined as an enveloped, nonsegmented negative-strand RNA virus (Briese et al., 1992 ; Compans et al., 1994 ; Zimmermann et al., 1994) and its sequence has been determined independently in two laboratories (Briese et al., 1994 ; Cubitt et al., 1994b). Although aspects of gene organization and deduced protein sequence suggest relatedness to members of the order *Mononegavirales* (paramyxoviruses, rhabdoviruses and filoviruses), BDV shows unusual features such as RNA splicing (Schneider et al., 1994 ; Cubitt et al. 1994c) and overlap of transcription units and transcription signals (Schneemann et al., 1994). These features indicate that BDV is likely to represent a previously unrecognized genus or family within this order.

Originally considered to be a natural infection of horses and sheep in Southeastern Germany, infection has now been reported in cats, cattle and birds in Europe, North America and Africa. (Lundgren and Ludwig, 1993 ; Kao et al., 1993 ; Malkinson et al., 1993 ; Caplazi et al., 1994). It is not known whether this apparent extension in host and geographic range represents improved survey methods or spread of BDV to new species and locations. The potential host range is still larger. In addition to the natural hosts already described, rodents, rabbits and primates are readily infected experimentally (Ludwig et al., 1988). Whether BDV naturally

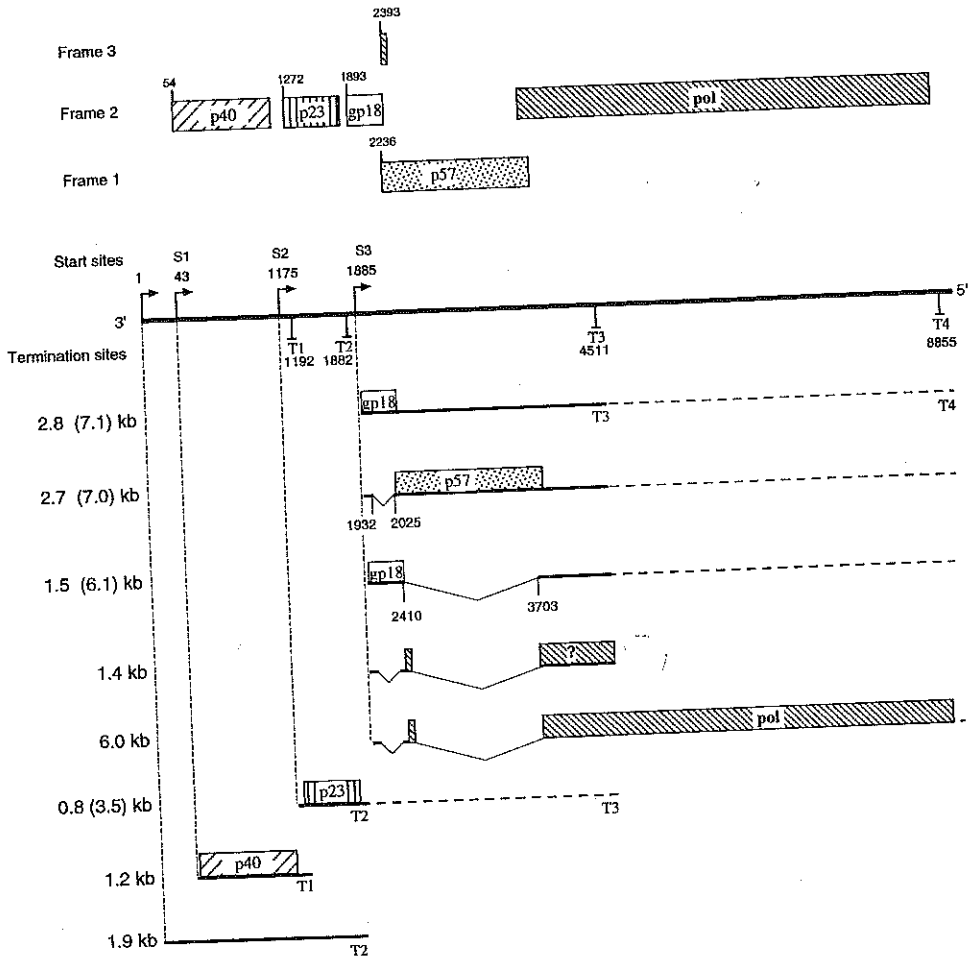

Figure 1. Genomic organization and transcriptional map of BDV.
The BDV genome is shown as a solid line in 3' to 5' direction. Coding regions for BDV proteins and their respective frames on the antigenome are shown as boxes at the top. The numbers above the boxes indicate the position of the first AUG codon of each open reading frame using the sequence of BDV strainV as a reference. The positions of RNA transcription initiation sites on the genome of BDV strainV are shown as arrows pointing downstream. Transcription termination sites and splice sites are shown as downward vertical lines. Stippled lines indicate that readthhrough at termination sites T2 and T3 results in longer RNAs terminating at T3 and T4, respectively. The 1.2 kb RNA and the 0.8 kb RNA have been shown to be used as mRNAs for the translation of p40 and p23, respectively. p23 could also be translated from the 3.5 kb RNA. Transcripts that might be used for the translation of gp18, p57 and pol are indicated. It is not known whether the 1.9 kb RNA and the 1.4 kb RNA are used for the translation of BDV proteins.

different sites than those proposed for transcription initiation by Cubitt et al. (1994b). 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 strain V (figure 1). A semiconserved, U-rich motif that is partially copied into the respective transcripts was identified at sites S1, S2 and S3 (figure 2). This motif is present in all BDV strains for which genomic sequence is available (strain He80, strain He80-1

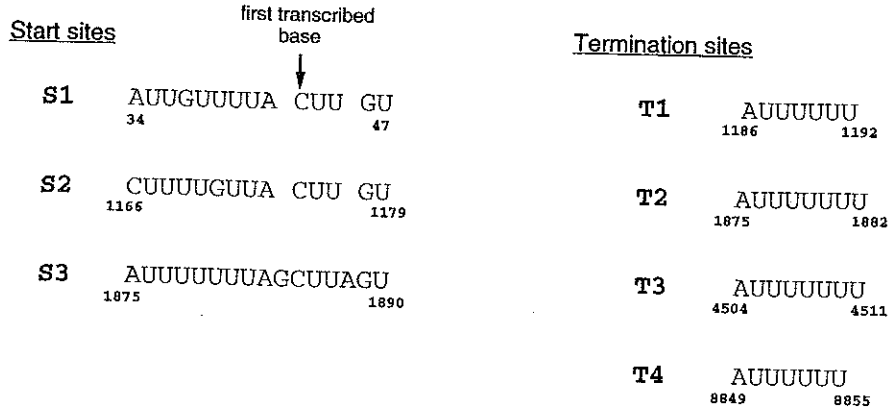

Figure 2. Comparison of nucleotide sequences at transcription initiation- and termination sites on the BDV genome.
The sequences, shown in 3' to 5' direction, are aligned for maximal similarity. The arrow indicates the position at which cRNA synthesis begins within the transcriptional start signal. The nucleotide sequences and the numerical assignations are from the complete sequence of BDV strainV (Briese et al., 1994). Data for start and stop sites are taken from Schneemann and co-workers (1994).

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 other nonsegmented, negative-strand RNA viruses. Direct terminal sequencing confirmed the four transcription termination sites proposed by Briese et al. (1994) and Cubitt et al. (1994b) : nt 1192 (T1), nt 1882 (T2), nt 4511 (T3) and nt 8855 (T4) (figure 1). Each termination site consists of 6 to 7 U residues preceded by a single A residue (figure 2). This consensus sequence is reminiscent of the transcription termination signals in rhabdoviruses and paramyxoviruses. In these virus families, the stretch of U-residues is believed to cause polymerase stuttering, which results in the synthesis of a poly(A) tail at the 3' end of the viral transcripts (Banerjee, 1991). It is likely that the U residues in BDV have an analogous function.

One of the more striking features of the BDV genome organization is the unusual constellation of transcription termination and initiation signals at the gene junctions (Schneemann et al., 1994). The transcription initiation site for the 0.8 kb RNA is located 18 nt upstream of the termination site of the 1.2 kb RNA. Thus the two genes overlap instead of showing the intergenic region typically observed in nonsegmented, negative-strand RNA viruses. Gene overlap has been observed previously in respiratory syncitial virus, a paramyxovirus. In this virus, the start site for the polymerase gene is located 68 nt upstream of the termination signal for the 22K gene (Collins, 1991) and it has been proposed that this arrangement serves as a mechanism to attenuate expression of the polymerase protein. However, in BDV, the 1.2 kb and the 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 overlap 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 noticeable decrease in transcription of the 0.8 kb RNA. However, until the mechanistic details of polymerase function are better understood, this remains speculative.

The second and third transcription units (0.8 kb and 2.8 kb/7.1 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 (figure 2). This

arrangement contrasts with the organization of the rhabdovirus and paramyxovirus gene junction, which can usually be divided into three separate domains : transcription termination signal, intergenic region, and transcription initiation signal (Banerjee, 1991). 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 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 since it promises new insights into the mechanisms for regulation of gene transcription in nonsegmented, negative-strand RNA viruses.

The transcriptional map of BDV contains several polycistronic RNAs that arise from readthrough at the various termination sites. Transcriptional readthrough is not uncommon in nonsegmented, negative-strand RNA viruses, however, it is usually considered to be aberrant and the biological significance of the readthrough products is unknown (Banerjee, 1991). In contrast, the ability of the BDV polymerase to continue transcription beyond a termination signal is critical for viability of the virus. The 7.1 kb RNA and its splice products are the only RNAs that contain the pol ORF and they are generated by readthrough at termination site T3. 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 protein, 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. It is possible, however, 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 8 to 9 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 a leader-containing RNA similar to those observed for rhabdoviruses and paramyxoviruses. In these virus families transcription of the sequence at the 3' end of the genome usually results in formation of a short leader RNA that terminates upstream of the first gene at a sequence similar to a transcription termination signal (Banerjee, 1991). Although no such short leader RNAs have yet been found in BDV-infected cells, analysis of the genomic sequence upstream of the start of the first transcription unit revealed a motif located within start signal S1 that is very similar to the consensus termination signal 3' AUUGUUUU (nt 34-42). It is possible that this signal is a weak terminator due to the exchange of a G residue for a U residue at the fourth position. RNA synthesis initiating at the extreme 3' end of the genome might therefore proceed into the adjacent gene to result in the synthesis of a leader-containing RNA. There is precedent for such leader-containing RNAs in measles virus, a paramyxovirus. Measles virus leader-containing transcripts are functionally distinct from their leaderless counterparts in that they are not translated but preferentially encapsidated into ribonucleoprotein complexes (Castaneda and Wong, 1990).

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 nonsegmented, negative-strand RNA viruses, 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.

The BDV genome is extremely compact : 99.4% of its nucleotides are transcribed into subgenomic RNAs (Schneemann et al., 1994). Only 55 out 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.

Posttranscriptional modification of BDV transcripts

Several observations suggested that BDV transcripts might be modified by RNA splicing. These included (1) the nuclear localization of BDV transcription, (2) the apparent absence of mRNAs initiating and terminating close to the start and stop sites of the p57 and pol ORFs and (3) the existence of transcripts that are not collinear with the BDV genome. That splicing does indeed occur was subsequently confirmed through RT-PCR and Northern hybridization analysis of RNAs from infected tissues and cultured cells (Schneider et al., 1994 ; Cubitt et al., 1994c). Two BDV transcripts, the 7.1 kb RNA and the 2.8 kb RNA, were each found to contain two introns that span nt positions 1932-2025 (intron-1, 94 nt) and 2410-3703 (intron-2, 1.3kb) (figure 1). Sequence analysis revealed that 78% of the positions in the 5' and 3' splice sites of both introns matched the mammalian splice site consensus sequence. In addition, similar to mammalian RNAs, branchpoint nucleotides are present 18 nt (intron-1) and 30 nt (intron-2) from the splice site junctions (Schneider et al., 1994). To determine whether these viral RNAs could serve as substrates for host spliceosomes, a truncated cDNA clone of the 2.8 kb RNA was transiently transfected into COS-7 cells. This construct did not encode the two viral proteins already known to localize to the nucleus, p40 and p23. Nonetheless, the transfected cells synthesized the predicted splice products suggesting that p40 and p23 are not essential to splicing of the 2.8 kb RNA.

Northern blot analysis of RNA isolated from BDV-infected rat brain tissues shows that splicing is not 100% efficient. Roughly equal proportions of spliced and unspliced messages are present in infected cells. This low splicing efficiency is likely to be critical to BDV biology as both spliced and unspliced transcripts are predicted to function as mRNAs in their own right and to encode distinct proteins (see below). The low splicing efficiency may be due to decreased recognition of the 3' splice sites where BDV RNAs deviate slightly from the mammalian consensus sequence. Fifty-five percent of all mammalian 3' exons start with a G residue (Krawczak et al., 1992) while BDV 3' exons start with either a U residue (exon-2) or a C residue (exon-3). A decrease in the efficiency of splicing, presumably due to inaccessibility of the 3' splice site to components of the splicing machinery, has also been reported in influenza virus (Plotch and Krug, 1986).

Regulation of BDV protein expression

Only two primary monocistronic mRNAs have been identified among BDV transcripts : the 1.2 kb RNA, which represents the mRNA for p40 (McClure et al., 1992), and the 0.8 kb RNA, which represents the mRNA for p23 (Thierer et al., 1992). The lack of primary monocistronic RNAs for the gp18, p57 and pol ORFs and the synthesis of transcripts that contain all three ORFs suggests that posttranscriptional modifications are necessary for the expression of the downstream ORFs, p57 and pol. The most reasonable interpretation of the data obtained to date, considering the position of the coding sequences on the genome, the splicing of the mRNAs and the "context" of the various possible AUG initation codons (Kozak, 1989), is shown in figure 1. For gp18, the translation initiation codon has been identified ; it is located 8 nt downstream of the 5' end of the 2.8 kb/7.1 kb RNAs. This short untranslated region is likely to result in inefficient translation initiation by ribosomal preinitiation complexes and might serve as a mechanism to

downregulate gp18 expression. Splicing of intron-1 from either the 2.8 kb or 7.1 kb RNA effectively eliminates the gp18 ORF by removing 23% of its coding capacity and juxtaposing the 13th amino acid codon with a stop codon (Schneider et al., 1994). Following translation of such a minicistron, ribosomes might continue to scan along the RNA and reinitiate at a downstream AUG codon for the translation of p57 (from transcripts containing intron-2) or pol (from transcripts lacking intron-2). Removal of intron-2 from the 7.1 kb RNA extends the pol ORF by 459 nt, increasing the size of the putative polymerase protein to 190 kDa.

Translation of the 2.7 kb or 7.0 kb RNA to synthesize p57 would require that initiation of protein synthesis begins at the 4th or 5th AUG codon from the 5' end. The first AUG codon is known to be used for translation of gp18 while the second and third AUG codons would only result in the synthesis of small peptides, 25 and 42 amino acids in length, respectively. Similarly, the sixth AUG codon in the doubly spliced 6.0 kb mRNA (an AUG codon in a strong context) would be used to initiate the synthesis of pol.

Summary

The molecular characteristics of BDV suggest that it represents a new taxon within the order Mononegavirales. In contrast to other members of this order, BDV uses an unprecedented variety of mechanisms to regulate its gene expression, including the use of overlapping reading frames, overlapping transcription units, alternate RNA splicing and possibly leaky scanning of ribosomes during protein translation. Further insights into the mechanistic details of gene expression in this multilevel system will be necessary for understanding BDV biology, including its propensity to persist in cells of the central nervous system.

References

1) Abildgaard, P. C. (1785). Pferde- und Vieharzt in einem kleinen Auszuge ; oder, Handbuch von den gewönlichsten Krankheiten der Pferde, des Hornviehes, der Schafe und Schweine, sammt der bequemsten und wohlfeilesten Art sie zu heilen. Zum Gebrauch des Landmanns. (J. T. Edlen von Trattnern, Ed.), Wien.
2) Armstrong, J., Niemann, H., Smeekens, S., Rottier, P., and Warren, G. (1984). Sequence and topology of a model intracellular membrane protein, E1 glycoprotein, from a coronavirus. *Nature* **308**, 751-752.
3) Banerjee, A. K. (1991). Gene expression of nonsegmented negative strand RNA viruses. *Pharmac. Ther.* **51**, 47-70.
4) Bode, L., Riegel, S., Ludwig, H., Amsterdam, J., Lange, W. and Koprowski, H. (1988). Borna disease virus-specific antibodies in patients with HIV infection and with mental disorders. *Lancet* ii, 689.
5) Briese, T., De La Torre, J. C., Lewis, A., Ludwig, H., and Lipkin, W. I. (1992). Borna disease virus, a negative strand RNA virus, transcribes in the nucleus of infected cells. *Proc. Natl. Acad. Sci. USA* **89**, 11486-11489.
6) Briese, T., Schneemann, A., Lewis, A., Park, Y., Kim, S., Ludwig, H., and Lipkin, W. I. (1994). Genomic organization of Borna disease virus. *Proc. Natl. Acad. Sci. USA* **91**, 4362-4366.
7) Carbone, K., Duchala, C., Griffin, J., Kincaid, A., and Narayan, O. (1987). Pathogenesis of Borna disease in rats. Evidence that intraaxonal spread is the major route for virus dissemination and the determination for disease incubation. *J. Virol.* **61**, 3431-3440.
8) Carbone, K., Park, S. W., Rubin, S. A., Waltrip, R. W., and Vogelsang, G. B. (1991). Borna disease : association with a maturation defect in the cellular immune response. *J. Virol.* **65**, 6154-6164.
9) Caplazi, P., Waldvogel, L., Stitz, L., Braun, U., and Ehrensperger, F. (1994). Borna disease in naturally infected cattle. *J. Comp. Path.* **111**, 62-72.
10) Castaneda, S. J., and Wong, T. C. (1990). Leader sequence distinguishes between translatable and encapsidated Measles virus RNAs. *J. Virol.* **64**, 222-230.
11) Collins, P. L. (1991). The molecular biology of human respiratory syncitial virus (RSV) of the genus *Pneumoviruses*. In "The Paramyxoviruses" (D. W. Kingsbury, Ed.), pp. 103-162. Plenum Press, New York and London.
12) Compans, R. W., Melsen, L. R., and De La Torre, J. C. (1994). Virus-like particles in MDCK cells persistently infected with Borna disease virus. *Virus Res.* **33**, 261-268.

13) Cubitt, B., and De La Torre, J. C. (1994a). Borna disease virus (BDV), a nonsegmented RNA virus, replicates in the nuclei of infected cells where infectious BDV ribonucleoproteins are present. *J. Virol.* **68**, 1371-1381.
14) Cubitt, B., Oldstone, C. and De La Torre, J. C. (1994b). Sequence and genomic organization of Borna disease virus. *J. Virol.* **68**, 1382-1396.
15) Cubitt, B., Oldstone, C., Valcarel, V., and De La Torre, J. C. (1994c). RNA splicing contributes to the generation of mature mRNAs of Borna disease virus, a non-segmented negative strand RNA virus. *Virus Res.* **34**, 69-79.
16) Dittrich, W., Bode, L., Ludwig, H., Kao, M., and Schneider, K. (1989). Learning deficiencies in Borna disease virus-infected but clinically healthy rats. *Biol. psychiatry* **26**, 818-828.
17) Fu, Z. F., Amsterdam, J. D., Kao, M., Shankar, V., Koprowski, H., and Dietzschold, B. (1993). Detection of Borna disease virus-reactive antibodies from patients with affective disorders by western immunoblot technique. J. Affective Disord. **27**, 61-68.
18) Jackson, A. O., Francki, R. I. B., and Zuidema, D. (1987). Biology, structure, and replication of plant rhabdoviruses. *In "The rhabdoviruses"* (R. R. Wagner, Ed.), pp. 427-508.
19) Kao, M., Hamir, A. N., Rupprecht, C. E., Fu, Z. F., Shankar, V., Koprowski, H., and Dietzschold, B. (1993). Detection of antibodies against Borna disease virus in sera and cerebrospinal fluid of horses in the USA. *Veterinary Record* **132**, 241-244.
20) Kingsbury, D. W. (1991). Paramyxoviridae and their replication. *In* "Virology" (B.N. Fields and D.M. Knipe, Eds.), 2nd ed., pp. 945-962. Raven Press, New York.
21) Kliche, S., Briese, T., Henschen. A., Stitz. L., and Lipkin, W. I. (1994). Characterization of a Borna disease glycoprotein, gp18. *J. Virol.* **68**, 6918-6923.
22) Kozak, M. (1989). The scanning model for translation : an update. *J. Cell. Biol.* **108**, 229-241.
23) Krawczak, M., Reiss, J., and Cooper, D. N. (1992). The mutational spectrum of single base -pair substitutions in mRNA splice junctions of human genes : causes and consequences. *Hum. Genet.* **90**, 41-54.
24) Lipkin, W. I., Travis, G., Carbone, K., and Wilson, M. (1990). Isolation and characterization of Borna disease agent cDNA clones. *Proc. Natl. Acad. Sci. USA* **87**, 4184-4188.
25) Ludwig, H., Bode, L., and Gosztony, G. (1988). Borna disease : a persistent virus infection of the central nervous system. *Prog. Med. Virol.* **35**, 107-151.
26) Lundgren, A. L., and Ludwig, H. (1993). Clinically diseased cats with non-supportive meningoencephalomyelitis have Borna disease virus-specific antibodies. *Acta vet. Scand.* **34**, 101-103.
27) Malkinson, M., Weisman, Y., Ashash, L., Bode, L., and Ludwig, H. (1993). Borna disease in ostriches. *Vet. Rec.* **133**, 304.
28) McClure, M. A., Thibault, K. J., Hatalski, C. G., and Lipkin, W. I. (1992). Sequence similarity between Borna disease virus p40 and a duplicated domain within the paramyxovirus and rhabdovirus polymerase proteins. *J. Virol.* **66**, 6572-6577.
29) Narayan, O., Herzog, S., Frese, K., Scheefers, H., and Rott, R. (1983). Behavioral disease in rats caused by immunopathological responses to persistent Borna virus in the brain. *Science* **220**, 1401-1403.
30) Plotch, S., J., and Krug, R., M. (1986). *In vitro* splicing of influenza viral NS1 mRNA and NS1-β-globin chimeras : possible mechanisms for the control of viral mRNA splicing. *Proc. Natl. Acad. Sci. USA* **83**, 5444-5448.
31) Rott, R., Herzog, S., Fleischer, B., Winokur, A., Amsterdam, J., Dyson, W., and Koprowski, H. (1985). Detection of serum antibodies to Borna disease virus in patients with psychiatric disorders. *Science* **228**, 755-756.
32) Schädler, R., Diringer, H., and Ludwig, H. (1985). Isolation and characterization of a 14,500 molecular weight protein from brains and tissue cultures persistently infected with Borna disease virus. *J. Gen. Virol* **66**, 2479-2484.
33) Schneemann, A., Schneider, P. A., Kim, S., and Lipkin, W. I. (1994). Identification of signal sequences that control transcription of Borna disease virus, a nonsegmented, negative-strand RNA virus. *J. Virol.* **68**, 6514-6522.
34) Schneider, P. A., Schneemann, A., and Lipkin, W. I. (1994). RNA splicing in Borna disease virus, a nonsegmented, negative-strand RNA virus. *J. Virol.* **68**, 5007-5012.
35) Solbrig, M. V., J. H. Fallon, and W. I. Lipkin. (1994). Behavioral disturbances and pharmacology of Borna disease. *In* "Borna Disease Virus" (W. I. Lipkin and H. Koprowski, Eds.), in press, Springer Verlag, Heidelberg.

36) Solbrig, M. V., Koob, G. F., Fallon, J. H., and Lipkin, W. I. (1995). Tardive dyskinetic syndrome in rats infected with Borna disease virus. Neurobiol. Dis. (in press).

37) Thierer, J., Riehle, H., Grebenstein, O., Binz, T., Herzog, S., Thiedemann, N., Stitz, L., Rott, R., Lottspeich, F., and Niemann, H. (1992). The 24 K protein of Borna disease virus. *J. Gen. Virol.* **73**, 413-416.

38) Thiedemann, N., Presek, P., Rott, R. and Stitz, L. 1992. Antigenic relationship and further characterization of two major Borna disease virus -specific proteins. *J. Gen. Virol.* **73**, 1057-1064.

39) Zimmermann, W., Breter, H., Rudolph, M., and Ludwig, H. (1994). Borna disease virus : immunoelectronmicroscopic characterization of cell-free virus and further information about the genome. *J. Virol.* **68**, 6577-6758.