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NUCLEOTIDE SEQUENCES OF TWO cDNA CLONES OF INFECTIOUS PANCREATIC NECROSIS VIRUS (IPNV)—Ab TYPE[1]

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Jen-Leih Wu, Sheau-Wen Yih, Go-Mo Her, Chwan-Chiun Tung, Chiou-Yueh Lee and Ya-Li Hsu (1992) Nucleotide sequences of some cDNA clones of infectious pancreatic necrosis virus (IPNV)—Ab type. *Bull. Inst. Zool., Academia Sinica* **31**(1): 15-22. The E1S virus, isolated from the spleen of a diseased Japanese eel (*Anguilla japonica*), was identified as being the Ab type of infectious pancreatic necrosis virus (IPNV). It belongs to the Birnaviridae viruses which contain bi-segmented dsRNA genomes. The E1S viral genome consists of 3.2 Kb (A) and 2.9 Kb (B) segments. We sequenced two cDNA clones (pA28, pB21) by using the Sanger dideoxy method, and then showed that the pB21 has a 56.9% homologous with the B segment of the infectious bursal disease virus (IBDV); we further found that the pA28 has a 74.6% homologous with A segment of the Jasper strain of IPNV. The sequence similarities indicate close evolutionary relationships between birnaviruses. Analysis of the locations of homologies indicate that the pB21 and pA28 clones reside in the 3′-end nearby region of the E1S genome.

Key words: Birnaviridae, Infectious pancreatic necrosis virus, Sanger dideoxy sequencing, Infectious bursal disease virus.

The infectious pancreatic necrosis virus (IPNV) is an important viral pathogen in salmonid and some non-salmonid fishes; belongs to the newly-established family Birnaviridae (Brown, 1986; Dobos *et al.*, 1979). Birnaviruses possess bi-segmented, double-stranded RNA genomes, and are contained with in a medium-sized, unenveloped, icosahedral capsid (Moss and Gravell, 1969; Dobos and Roberts, 1983; Kell and Loh, 1972).

Birnavirus gene expression involves the production of four unrelated gene products which either undergo post-translational, cleavages or independent translation initiation at internal sites to produce three to produce three to five viral structural proteins (Nagy *et al.*, 1987; Hudson *et al.*, 1986; Mertens and Dobos, 1982; Duncan *et al.*, 1987; Manning *et al.*, 1990; Manning and Leong, 1990). The

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largest protein (VP1) —encoded in B segment genomic RNA— is thought to have RNA transcriptase activity; the three remaining major gene products—encoded by segment A RNA—are a major structural capsid protein (VP2), a minor structural protein (VP3), and a nonstructural protein (NS) (Azad *et al.*, 1985; Mertens and Dobos, 1982; Nagy *et el.*, 1987; Dobos, 1977; Dobos and Rowe, (1977).

Three major serological groups of IPNV have been identified (Okamoto *et al.*, 1983; MacDonald and Gower, 1981; Hedrick and Okamoto, 1982). Two of these serotypes, SP and Ab, are the predominant strains found in Europe; the third serotype, VR299, is prevalent in North America. Hill and Way (1983) have uncovered six additional serotypes be sites original which serotypes constitute a major serogroup. However, a number of isolated strains isolated found in Asian fish are closely related to the Ab strain. These can be differentiated by variation in RNA genome size, and by viral polypeptide size (Hedrick *et al.*, 1983; Lipipun *et al.*, 1989; Wu *et al*, 1987; Hsu *et al.*, 1989).

The eel virus strain (E1S), which belongs to the Ab serotype of IPNV, contains a bi-segmented double-stranded RNA genome consisting of a 3.2 Kb segment A, and a 2.9 Kb segment B (Wu *et al.*, 1987; Chang, 1983). Previously, we purified its dsRNA genome and constructed a cDNA library (Her, 1989). For this report, we determined some partial sequences in the two clones (pB21 and pA28) by using the Sanger dideoxy method; we then compared their sequences with published sequences of other birnaviruses. Sequence homologies were calculated, and approximate locations of these sequences in the genome of the E1S virus were deduced.

MATERIALS AND METHODS

Bacterial strain, plasmid, enzymes, chemicals

E. coli JM101 (supE thi Δ (lac-proAB) F' [traD36 proA proB lacI lacZΔ M15]), an ampicillin-sensitive derivative of *E. coli*, was used as the host of transformation for cDNA cloning. Plasmid pUC18 was purchased from Bethesda Research Laboratories, Inc.. Plasmids PB13, pB21, pA28 and pA87 are pUC18 derivatives containing viral cDNA inserts. All restriction enzymes were purchased from the same laboratory. T7 DNA Polymerase Sequencing Kit was from the Promega Co.., The forward pirmer was a 24 mer (5'-d (CGCCAGGGTTTTCCCAGTCACGAC)-3'), and the reverse primer was a 22 mer (5'-d(TCACACAGGAAACAGCTATGAC)-3'). Both were derived from the pUC/M13 polylinker region. Other chemicals were obtained from either the Sigma Co. or Merck Co.

Isolation of plasmid DNA

Small-scale plasmid preparations were performed by using a modification of the alkaline lysis procedure (Birnboim and Doly, 1979; Maniatis *et al.*, 1982). The plasmid DNA which was isolated on a large scale was further purified by CsCl-ethidium bromide centrifugation (13,000×g, 36 hr at 25°C in a Beckman Type 65 rotor). The purity of the DNA was checked by its absorbance ratio (O.D. 260/O.D. 280).

Nucleotide sequence determination and computer analysis

The nucleotide sequence was determined by use of the modified T7 DNA polymerase according to the technical manual supplied by the Promega Co. Nucleotide sequence data were aligned, overlapped, and analyzed using GCG Genetic Analysis Pairway programs on

a VAX computer. Restriction mapping, open reading frame analyses, and homology comparisons were done using the same software.

RESULTS AND DISCUSSION

The determined nucleotide sequences of clones pA28 and pB21 were 430 bp and 310 bp in length, respectively. They are shown in Figs. 1 and 2. Computer-assisted analysis revealed possible restriction enzyme sites; in addition, open reading frames were deduced and used for recombinant DNA manipulation by using the same analytical tool.

In previous studies, we searched for special concensus sequences between E1S

```
pA28    GAATTCCCTGGACATAAGGACAGTCTGGAAGACTGAGGAGTACAAGGAGCGGACAAGAGTC  61
          :    :::::::: :: ::  ::::::: ::::::::: ::::: ::  :::::::::
Jasper  AGAGGAGCTGGACATTAGAACCGTCTGGAGGACTGAGGAATACAAAGAAAGGACAAGAGCA  1397

pA28    TTCAACGAGATCACCGACTTCTCCAGTGACCTGCCCACGTCAAGGGCATGGGGTTGGAGG  121
        ::::: :::::::: :::::: : :::::::: :: :: :::: ::::::::: ::::::
Jasper  TTCAAAGAGATCACTGACTTCACAAGTGACCTACCAACCTCAAAGGCATGGGGATGGAGG  1457

                        ----- HpaII             ------- HincII
             BamHI  -------               ------- ScaI
pA28    GACATAGTCAAAGGGATCCGGAAAGTCGCCGCCCCAGTACTGTCAACGCTGTTTCCGATG  181
        ::: : :::: ::: ::: : ::::: :::::::: :: ::::::::::: :: :: :::
Jasper  GACCTGGTCAGAGGCATCAGAAAAGTGGCCGCCCCCGTGCTGTCAACGCTCTTCCCAATG  1517

pA28    GCAGCACCACTCATTGGAATGGCAGACCAACTCATCGGAGATCTCACCAACACCAACGCA  241
        :: :: :: :: :: :::   :: :::::: :::: :: :: :::::::: :::::: ::
Jasper  GCGGCTCCCCTTATAGGAGCTGCCGACCAATTCATTGGGGACCTCACCAAGACCAACTCA  1577

                   KpnI
                  ------
pA28    GCAGGCGGAAGGTACCGCTCCATGGCCGCAGGAGGCCGCTACAAAGACGTAATGGACTCC  301
        :: :: ::: : ::::  ::    :: :: :::::::::::: : :: :: ::::::::
Jasper  GCCGGGGGACGCTACCTGTCACACGCAGCCGGAGGCCGCTACCATGATGTCATGGACTCA  1637
                       *********                  *********

pA28    TGGGCCAGCGGCGGACCCGACGGGAAGTTCTCCCAGGCTCTAAAGAACAGGCTGGAGTCT  361
        :::::::::::       :  :: :  :  ::  ::   :: :::: : :::: :::::
Jasper  TGGGCCAGCGGGTCCGAGGCAGGAAGCTATTCAAAGCACCTCAAGACCCGGCTTGAGTCC  1697

                           AluI
                           ----
pA28    GCCAACTACGAGGAAGTCGAGCTTCCTCCCCCTTCAAAAGGAGTCATTGTCCCTGTGGTG  421
           ::::: :::::::: ::::::::    ::  ::::  ::::::::  : :::::::::
Jasper  AATAACTATGAGGAAGTGGAGCTTCCAAAGCCAACAAAGGGAGTCATCTTTCCTGTGGTG  1757

pA28    CACACCGTC                                                     430
        ::::::::
Jasper  CACACCGTT                                                     1766
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Fig. 1. An alignment of the DNA sequence of the pA28 (upper) and A segment genome of the IPNV Jasper strain (lower). The nucleotides of the Jasper strain are numbered beginning with the first n.t. at the 5′-untranslated region. Nucleotides identical to both pA28 and the Jasper strain are indicated by a colon (:). The asterisks indicate that the precise boundaries of the pVP2 and NS coding regions have not been determined (Duncan *et al.*, 1987). Enzymes used in cloning or in hybridization studies are indicated.

clones and other birnaviruses (unpublished data). In this report, a comparison of sequence homologies between pA28, pB21, and known nucleotide sequences of other birnaviruses was made using University of Wisconsin Genetics Computer Group Sequence Analysis Software. The only sequence of aquatic birnaviruses published was that of the complete segment A genome of the IPNV Jasper strain (Duncan *et al.*, 1987; Duncan and Dobos, 1986). The best alignment between the PA28 sequence and the A segment genome of the IPNV Jasper strain (Fig. 1) shows a 76.7% homology. The region which is homologous to pA28 covers the boundary between the pVP2 and NS coding regions (Duncan *et al.*, 1987; Duncan and Dobos, 1986). Following the reading frame assignment of the IPNV, the pA28 sequence was translated into an amino acid sequence (reading frame 2 in Fig. 3); it showed a 81.8% homologous with the IPNV coding sequence (Fig. 3). This high degree of sequence homology indicates a close evolutionary relationship between the two strains of fish birnaviruses.

To date, no segment B sequence data for the IPNV or other aquatic birnaviruses are available for comparison; only information on segment B of the infectious bursal disease virus (IBDV) is available for comparison (Morgan *et al.*, 1988). The pB21 DNA sequence we obtained showed a 56.9% homology with IBDV (Fig. 2). Following the reading frame assignment of IBDV, the pB21

```
                HpaII                                   ClaI
pB21   GAATTCCGGAATTCCGCGAGAGCTGAAAGACCTGAGATCGATCATAATGGAGGCTGTGGA   60
IBDV   CAACTTCAAGATTGAGAGGTCTATTGATGACATCAGGGGCAAGCTAAGACAGCTTGT..C  1635

pB21   CACAGCCCCCATGACGGCTACCTAGCAGATGGGTCAGACCTTCCACCACGCGTGCCTGGC   120
IBDV   CCCCTTGCACAACCAGGGTACCTGAGTGGGGGG.....GTTGAACCAGAACAATCCAGC   1695

                                                        XhoI ———
                                                  AluI  ——
pB21   AAAGCAGTGGAACTCGACCTACTCGGCTGGTCAGCCGTTTACAGCCGACAGCTCGAGATG   180
IBDV   CCAACTGTCGAGCTCGACCTACTAGGCTGGTCAGCAACGTACAGCAAAGATCTCGGGATC  1755

pB21   TTCGTTCCCGTTCTTGAGAACGAGAGACTAATTGCATCAGCAGCCTACCCAAAAGGGCTA   240
IBDV   TACGTGCCAGTACTTGACAAGGAACGCTTGTTCTGCTCAGCAGCATATCCCAAAGGGGTT  1815

                                        ——— SmaI
                          HpaI  ——
pB21   GAAAACAAAACCCTAGCCCGAAAACCCGGGGCAGAGATCGCGTACCAGATAGTTCGGTAC   300
IBDV   GAGAATAAGAGTCTCAAGTCAAAAGTTGGGATCGAGCAAGCATACAAGGTGGTCAGGTAC  1875

pB21   GAGGGAATTC                                                    310
IBDV   GAGGCGTTGA                                                   1885
```

Fig. 2. An alignment of the DNA sequence of the pB21 (upper) and B segment genome of IBDV (lower). Dots represent the gaps created to align the two sequences. Other statements are the same as Fig. 1.

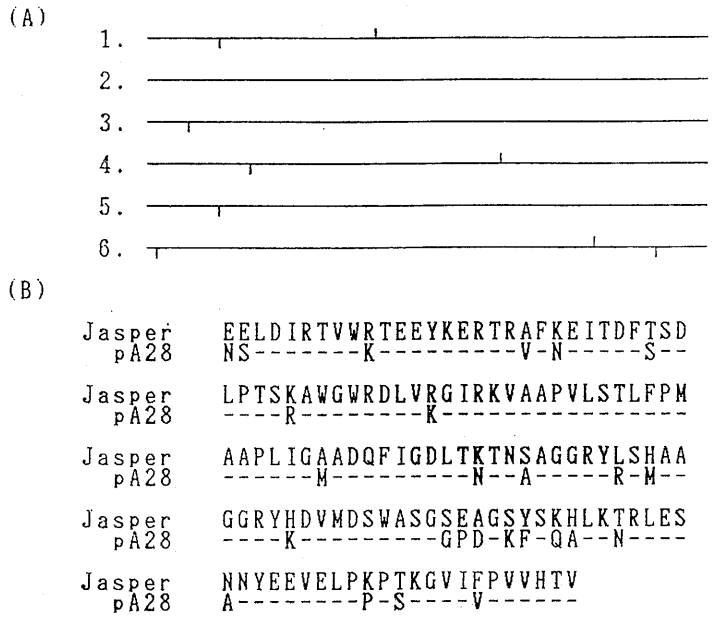

Fig. 3. (a) Schematic illustration of all possible six-way open reading frames of pA28. There were several possible methionine codons (⊥) and termination codons (⊤) over the reading frames.
(b) Comparison of the predicted amino acid sequence of pA28 with that of the IPNV Jasper strain. Identical amino acid sequences are represented by dashed lines.

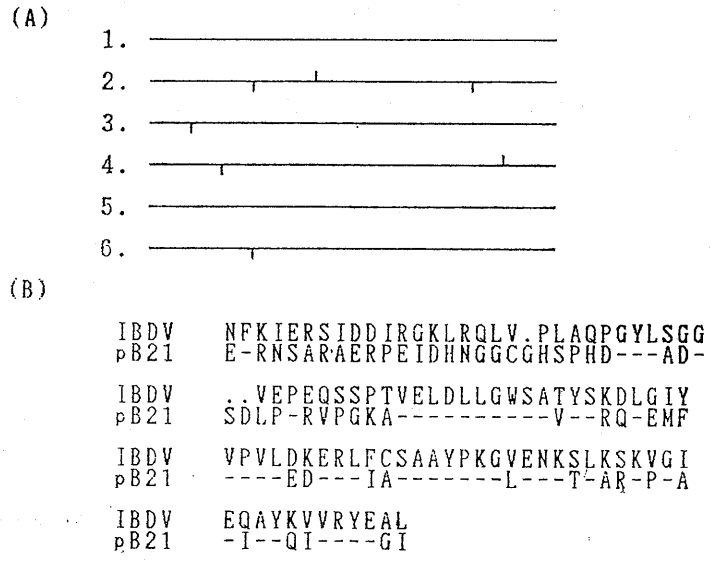

Fig. 4. (a) Schematic illustration of all possible six-way open reading frames of pB21.
(b) Comparison of the predicted amino acid sequence of pB21 with that of IBDV. Dots indicate gaps inserted to improve the alignment. Other legends are the same as Fig. 3.

sequence was translated into an amino acid sequence (reading frame 1 in Fig. 4); it shows an overall 44.7% homologous with the pVP1 sequence (Fig. 4). However, in a span of 76 amino acid residues, the two sequences share a 59.2% homologous,

including several long stretches that match perfectly. This high level of homology clearly indicates that the RNA transcriptase (pVP1) genes of the two viruses are homologous, and that the pB21 clone represents part of the E1S pVP1 gene. The conserved regions may possibly represent important functional sites in the RNA transcriptase, sites which play an essential role in specific RNA synthesis during viral replication.

In summary, we determined the DNA sequences of two cDNA clones of the E1S viral genome. Sequence comparisons with other birnaviruses show close evolutionary relationships. Sequence comparisons also allowed us to place the pA28 clone in the region covering the boundaries between pVP2 and NS in the A segment, and the pB21 clone to an internal region in the B segment (Figs. 1 and 2). Information described here contains important implications for future vaccine design and construction of anti-sense viral RNA for antiviral regulation.

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REFERENCES

Azad, A.A., S.A. Barrett and K.J. Fahey (1985) The characterization and molecular cloning of the double-stranded RNA genome of an Australian strain of infectious bursal disease virus. *Virology* **143**: 35-44.

Brown, F. (1986) The classification and nomenclature of viruses: summary of results of meetings of the International Committee on Taxonomy of Viruses in Sendai. September, 1984. *Intervirology* **25**: 140-143.

Birnboim, H.C. and J. Doly (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acid Res.* **7**: 1513-1523.

Chang, Chi-Yao (1983) Characteristics of a birnavirus isolated from Japanese eel (*Anguilla japonica*). Master thesis. National Taiwan University, Taipei, Taiwan, R.O.C.

Dobos, P., R. Hallett, D.T.C. Kells, O. Sorensen and D. Rowe (1977) Biophysical studies of infectious pancreatic necrosis virus. *J. Virol.* **22**: 150-159.

Dobos, P. and D. Rowe (1977) Peptide map comparison of infectious pancreatic necrosis virus-specific polypeptides. *J. Virol.* **24**: 805-820.

Dobos, P. (1977) Virus-specific protein synthesis in cells infected by infectious pancreatic necrosis virus. *J. Virol.* **21**: 242-258.

Dobos, P. and T.E. Roberts (1983) The molecular biology of infectious pancreatic necrosis virus: A review. *Can. J. Microbiol.* **29**: 377-384.

Dobos, P., B.J. Hill, R. Hallet, D.T.C. Kells, H. Becht and D. Teninges (1979) Biophysical and biochemical characterization of five animal viruses with bi-segmented double-stranded RNA genomes. *J. Virol.* **32**: 593-605.

Duncan, R. and P. Dobos (1986) The nucleotide sequence of infectious pancreatic necrosis virus (IPNV) dsRNA segment A reveals one large ORF encoding a precursor polyprotein. *Nucleic Acids Res.* **14**: 5934.

Duncan, R., E. Nagy, P.J. Kreel and P. Dobos (1987) Synthesis of the infectious pancreatic necrosis virus polyprotein, detection of a virus-encoded protease, and fine structure mapping of genome segment A coding regions. *J Virol.* **61**: 3655-3664.

Hedrick, R.P. and N. Okamoto (1982) *Biochemical and antigenic characteristics of eel virus European compared to selected strains of infectious pancreatic necrosis virus. In*: Double Stranded RNA Viruses. (R.W. Compans and D.H.L. Bishop eds.). Amsterdam: Elsevier Biomedical. pp. 245-252.

Hedrick, R.P., J.L. Fryer, S.N. Chen and G.H. Kou (1983a) Characteristics of four birnaviruses isolated from fish in Taiwan. *Fish Pathology* **18**: 91-97.

Hedrick, R.P., N. Okamoto, T. Sano and J.L. Fryer (1983b) Biochemical characterization of eel virus European. *J. Gen. Virol.* **64**: 1421-1426.

Her, Go Mo (1989) Molecular cloning and structure study of infectious pancreatic necrosis virus (IPNV) RNA genome. Master thesis, National Taiwan Ocean University, Keelung, Taiwan, R.O.C.

Hill, B.J. and K. Way (1983) Serological classification of fish and shellfish birnaviruses. *Abstracts of Fish International Conference of the European Association of Fish Pathologists*, 10.

Hsu, Y.L., B.S. Chen and J.L. Wu (1989) Comparison of RNA and polypeptides of infectious pancreatic necrosis virus isolated from eel and rainbow trout. *J. Gen. Virol.* **70**: 2233-2239.

Hudson, P.J., N.M. Mckern, B.E. Power and A.A. Azad (1986) Genomic structure of the large RNA segment of infectious bursal disease virus. *Nucleic Acid Res.* **14**: 5001-5012.

Kelly, R.K. and P.C. Loh (1972) Electron microscopical and biochemical characterization of infectious pancreatic necrosis virus. *J. Virol.* **10**: 824-832.

Lipipun, V., P. Caswell-Reno, Y.L. Hsu, J.L. Wu, M.C. Tung, P.W. Reno, W. Wattanavijarn and B.L. Nicholson (1989) Antigenic analysis of Asian aquatic birnavirus isolates using monoclonal antibodies. *Fish Pathology* **24**(3): 155-160.

Macdonald, R.D. and D.A. Gower (1981) Genomic and phenotypic divergence among three serotypes of aquatic birnaviruses (infectious pancreatic necrosis virus). *Virology* **114**: 187-195.

Maniatis, T., E.F. Fritsch and J. Sambrook (1982) *Molecular cloning: A laboratory manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Manning, D.S. and J.C. Leong (1990) Expression in *Escherichia coli* of the large genomic segment of infectious pancreatic necrosis virus. *Virology* **179**: 16-25.

Manning D.S., C.L. Mason and J.C. Leong (1990) Cell-free translational analysis of the processing of infectious pancreatic necrosis virus polyprotein. *Virology* **179**: 9-15.

Mertens, P.P.C. and P. Dobos (1982) Messenger RNA of infectious pancreatic necrosis virus is polycistronic. *Nature* **297**: 243-246.

Moss, L.H. and M. Gravell (1969) Ultrastructure and sequential development of infectious pancreatic necrosis virus. *J. Virol.* **3**: 52-58.

Morgan, M.M., I.G. Macreadie, V.R. Harley, P.J. Hadson and A.A. Azad (1988) Sequence of the small double-stranded RNA genomic segment of infectious bursal disease virus and its deduced 90-KDa product. *Virology* **163**: 240-242.

Nagy, E., R. Duncan, P. Krell and P. Dobos (1987) Mapping of the large RNA genome segment of infectious pancreatic necrosis virus by hybrid arrested translation. *Virology* **158**: 211-217.

Okamoto, N., T. Sano, R.P. Hedrick and J.L. Fryer (1983) Antigenic relationships of selected strains of infectious pancreatic necrosis virus and eel virus European. *J. Fish Diseases* **6**: 19-25.

Wu, J.L., C.Y. Chang and Y.L. Hsu (1987) Characteristics of an infectious pancreatic necrosis-like virus isolated from Japanese eel (*Anguilla japonica*). *Bull. Inst. Zool., Academia Sinica* **26**: 201-214.

魚類感染性胰臟潰瘍病毒(Ab型) cDNA選殖株的核苷酸序列

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E1S 病毒乃自日本鰻 (*Anguilla japonica*) 之病變脾臟所分離得到的一種具二段雙股 RNA 病毒，在分類上屬於 Birnaviridae 之感染性胰臟潰瘍病毒 (infectious pancreatic necrosis virus，簡稱 IPN 病毒)，屬 Ab 型。其基因體大小分別是，A段爲 3.2 Kb 及B段爲 2.9 Kb。經由 Sanger dideoxy 之方法已定出二 cDNA 選植株 (pB21 及 pA28) 的序列。分析結果顯示 pB21 序列與 IBDV 的B段基因體有 56.9%的相似性；pA28 則與 IPNV (Jasper type) 的A段基因體有 74.6%的類似。由序列的同源性，說明了存在於雙股 RNA 病毒間的進化關係。另外，也預期 pB21 及 pA28 所含病毒蛋白的部分可能是靠近病毒基因體 3′-端的位置。