Identification and Sequence Analysis of the Marek's Disease Virus Serotype 2 Gene Homologous to the Herpes Simplex Virus Type 1 UL52 Protein

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ABSTRACT. The gene of Marek's disease virus serotype 2 (MDV2) homologous to the UL52 gene of herpes simplex virus type 1 (HSV-1) was identified and characterized. The MDV2 UL52 homologous gene encodes 1,071 amino acids with a molecular weight of 118.7 kDa, which includes putative metal-binding site and overlapping region with the UL53 homologous gene. Although a putative polyadenylation signal sequence was found in the downstream of the MDV2 UL52 gene, a MDV2 UL52 DNA probe reacted only with the polycistronic 6.3 kb transcript, representing the UL52 and the downstream genes of UL53 and UL54. Transcriptional pattern of this region of MDV2 was somewhat different from corresponding regions of HSV-1 and infectious laryngotracheitis virus. —KEY WORDS: Marek's disease virus serotype 2, transcript, UL52.

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Marek's disease virus (MDV) causes a lymphoproliferative disease known as Marek's disease (MD) and serious economic problems in poultry industry. The disease is caused by pathogenic MDV serotype 1 (MDV1). MD has been prevented by vaccination with attenuated MDV1, naturally nonpathogenic MDV serotype 2 (MDV2) isolated from chickens and other birds belonging to the genus *Gallus* [3, 14, 20], or herpesvirus of turkeys (HVT) which is antigenically related to MDVs and is also termed MDV serotype 3.

All three serotypes of MDV genome structures are similar to those of other alphaherpesviruses [4, 9, 10, 16] and numerous genes are homologous to and collinear with the prototype sequence of herpes simplex virus type 1 (HSV-1) [1]. Recently we constructed a restriction endonuclease map of MDV2 and demonstrated a collinear relationship among genomes of the three MDV serotypes [16]. Since MDV2 is naturally occurring nonpathogenic and/or nononcogenic strain of MDV in chickens, comparative studies on the virus genome and its gene products with those of oncogenic MDV1 might be a crucial for understanding viral oncogenicity and natural immunity. Especially studies on DNA replication mechanism for MDV2 may lead to understand the pathogenic differences among MDVs. The genome of HSV-1 was extensively studied and contained seven essential DNA replication-specific genes (UL5, UL8, UL9, UL29, UL30, UL42 and UL52) [17]. Further, it was recently reported that the UL5, UL8 and UL52 gene products constitute a heterotrimeric helicase-primase complex, and the UL5 and UL52 products have been shown to form a stable subassembly of HSV-1 [2, 7]. However, gene products and genomes relating DNA replication of MDV have not been studied sufficiently.

Except the UL42 homologous gene [11], all of these genes have not been sequenced in MDV2 and especially the UL52 homologous gene has not yet been identified among the genomes of three MDV serotypes. In the present study, we report the nucleotide and amino acid sequences of MDV2 which turned out to be UL52 homologue encoding an essential component for the viral DNA helicase-primase complex, and the transcript of this gene.

The genomic libraries of restriction enzyme fragments (*Bam*HI and *Eco*RI) of MDV2 strain HPRS24 [16] were used in this study. The genomic *Bam*HI fragment P (2.8 kb) was inserted into a plasmid pBluescript SK(+) vector (Stratagene, La Jolla, CA) by standard procedures [18] and then deleted by exonuclease III (Toyobo, Osaka, Japan) to construct deletion clones as described previously [12]. DNA sequences were determined on both strands using the Dye primer cycle sequencing kit supplied by Applied Biosystems (ABI, Foster city, CA) and then analyzed with a Model 377 ABI autosequencer as suggested by the manufacturer. The junction sequences among the fragments (*Bam*HI-T1 and -P or -P and M2) were confirmed by the dideoxy-chain termination method with the Dyedeoxy terminator cycle sequencing kit (ABI, Foster city, CA). DNA sequences were assembled and analysed with the UWGCG program BESTFIT as described previously [12]. The GenBank accession numbers included in this study were as follows: HSV-1 (D10879), varicella-zoster virus (VZV; X04370), bovine herpesvirus type 1 (BHV-1; AJ004801), equine herpesvirus type 1 (EHV-1; M86664), pseudorabies virus (PRV; X87246), Epstein-Barr virus (EBV; V01555), and human cytomegalovirus (HCMV; X17403).

The resulting DNA sequence of the complete *Bam*HI-P fragment was comprised 2,837 nucleotides (nt) and encoded an incomplete open reading frame (ORF) homologous to the HSV-1 UL52 gene. In the MDV2 genomic DNA library containing *Bam*HI fragments, the *Bam*HI-P fragment was

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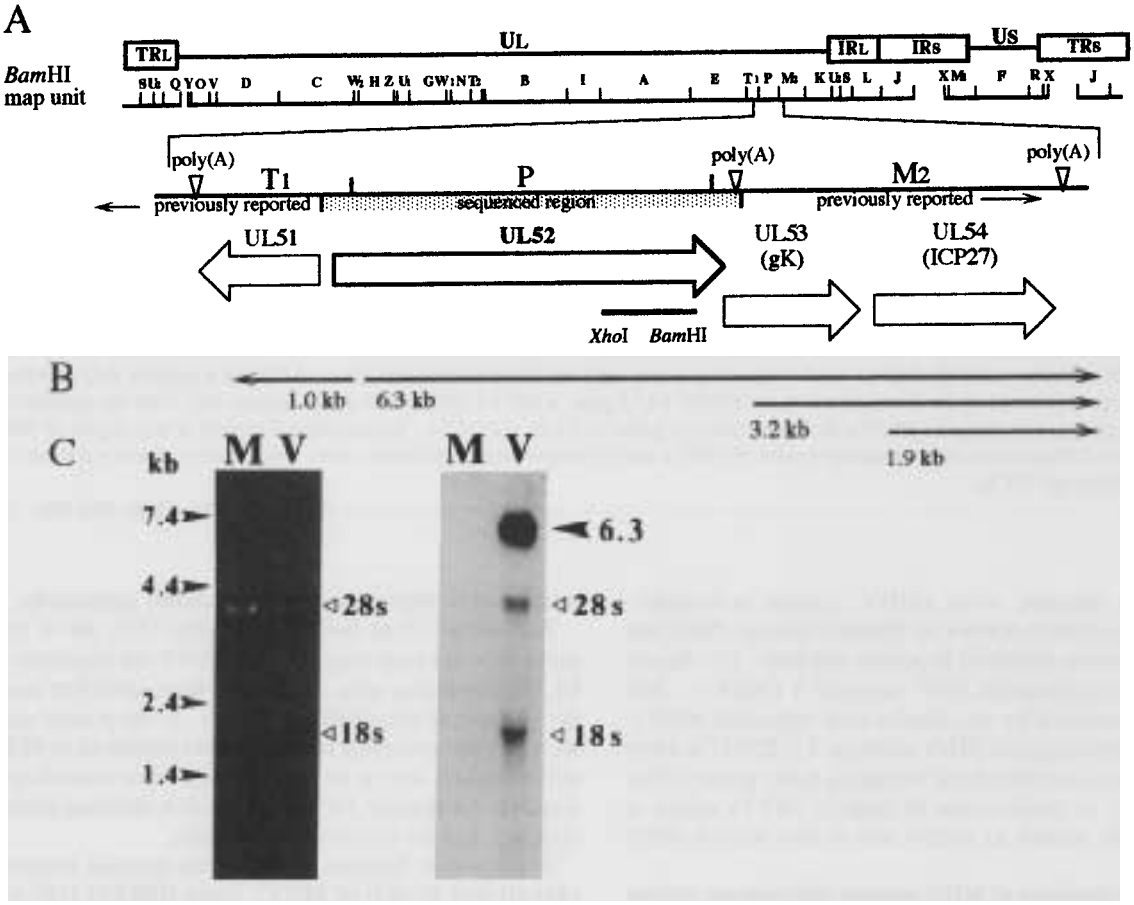

Fig. 1. A: Diagram of the MDV2 genome and restriction map units. The genome is organized into the unique long (U_L) and short (U_S), internal repeat long (IR_L) and short (IR_S) terminal repeat long (TR_L) and short (TR_S) regions. Polyadenylation signals are indicated by open triangles. The locations and directions of the MDV2 UL51, UL52, UL53 (gK) and UL54 (ICP27) homologous genes are indicated by open arrows. The UL53 and UL54 homologous genes and those of transcripts were determined by Tsushima *et al.* [22]. The subfragment of *Xho*I-*Bam*HI (847 bp) used as probe in the northern blot analysis is shown as bold line. B: Schematic diagram of the transcriptional products in the MDV2 sequenced region. The possible locations and directions of the viral transcripts indicated by bold arrows. C: Panel shows the hybridization result between total RNA with MDV2- or mock-infected chick embryo fibroblasts (each panel of lane 'V' or 'M'). Molecular sizes (kb) of the RNA markers (Gibco BRL, Grand Island, NY) are indicated at the left. Open arrowheads denote location of 28S and 18S rRNA.

located in the right part of MDV2 unique long region and positioned between *Bam*HI-T1 and -M2 fragments [16] (Fig. 1A). The 3'- and 5'-end parts of *Bam*HI-T1 and -M2 fragments included 138 bp of 5'- and 238 bp of 3'-end homologous sequences of the HSV-1 UL52 gene, respectively (Fig. 2). Further, the junction sites of *Bam*HI-T1 and -P, and -P and -M2 fragments were respectively confirmed by dye terminator sequence method. From these results, a complete ORF of 3,213 bp (nt 210 to 3,422) encoding 1,071 amino acids was identified, which is similar in length to the UL52 homologues of HSV-1 (1,058 amino acids) [15], VZV (1,083 amino acids) [6], BHV-1 (1,074 amino acids) [19], and EHV-1 (1,081 amino acids) [21]. The MDV2 UL52 ORF was encoded by forward strand same as the UL53 ORF, which started 19 nt before the end of the UL52 ORF, creating an out-of-frame overlap (Fig. 2). An equivalent overlaps exist in BHV-1 (23 nt) [19], and EHV-1 (11 nt) [21], and HSV-1 (43 nt) [15]. When analyzed putative transcriptional regulatory sites of the MDV2 UL52 homologous gene, no TATA-like sequences [5] for transcription initiation was evident. However, a putative polyadenylation signal sequence (AATAAA) [24] was found at 192 bp downstream of the termination codon of the MDV2 UL52 ORF (Fig. 2). This feature was similar to that of infectious laryngotracheitis virus (ILTV) [13] but different from other alphaherpesviruses, BHV-1, EHV-1, HSV-1 and VZV [6, 15, 19, 21].

As shown in Fig. 1C, Northern blot analysis of the UL52 gene was performed using total cellular RNAs from MDV2- or mock-infected chick embryo fibroblasts as described previously [12] (Fig. 1C each panel of lane 'V' or 'M'). The probe of the MDV2 UL52 ORF, hybridized with 6.3,

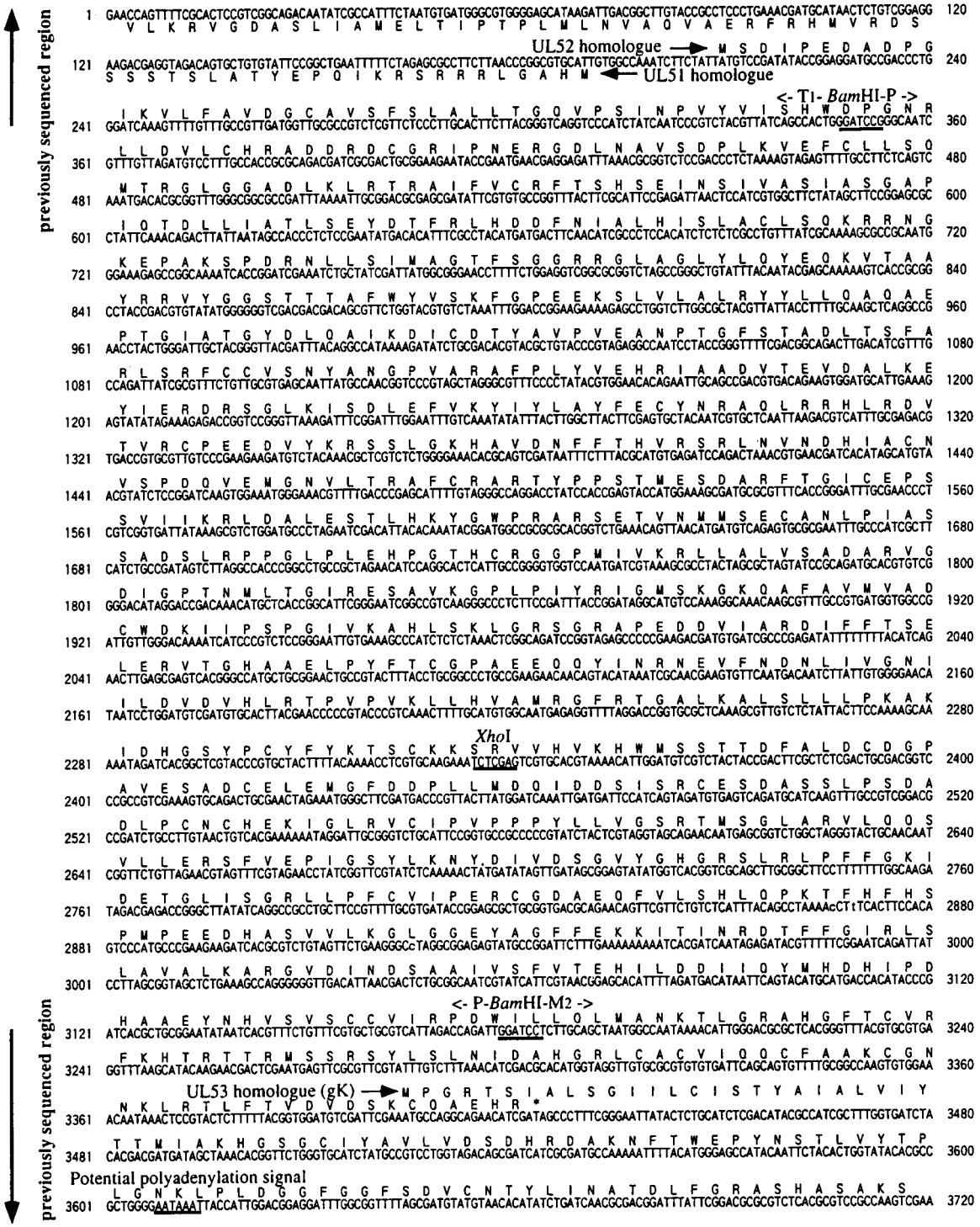

Fig. 2. Nucleotide sequence and predicted amino acid sequence of a portion of UL51 (nt < 190), UL52 (nt 210 to 3422) and UL53 (nt > 3404) homologues. The predicted amino acid sequence is given in the single-letter code above the DNA sequence and the stop codon is represented by asterisk. The previously sequenced regions are shown with bold arrows on the left. The restriction and the potential polyadenylation signal sites are bold underlined.

4.2 and 1.9 kb transcripts, but the signals obtained at 4.2 and 1.9 kb are most likely caused by non-specific reactions of degraded viral RNA within the 28S and 18S cellular rRNAs (Fig. 1C open arrows) as observed previous studies [11, 22]. Therefore we considered only 6.3 kb mRNA as a viral specific transcript. In other viral genomes of HSV-1

Table 1. Character and homology of predicted MDV2 UL52 gene product to other herpesviruses

Virus	Protein			Identity and similarity[b)]
	Amino acids	Mol. mass (kDa)[a)]	Isoelectric point[a)]	
MDV2 (UL52)	1071	118.7	6.87	–
PRV (UL52)	962	103.3	6.56	41.8/50.7
BHV-1 (UL52)	1074	113.9	8.46	38.4/45.6
EHV-1 (Gene7)	1081	119.0	6.44	39.8/49.6
VZV (ORF6)	1083	122.5	6.82	39.4/48.7
HSV-1 (UL52)	1058	114.4	6.42	41.3/50.6
EBV (BSLF1)	874	98.2	8.42	32.8/39.7
HCMV (UL70)	1062	120.9	8.16	28.6/36.8

a) Values are percentage similarity/identity, generated using the UWGCG program BESTFIT.
b) Molecular masses and isoelectric points of the predicted proteins were calculated from the predicted primary translation products.

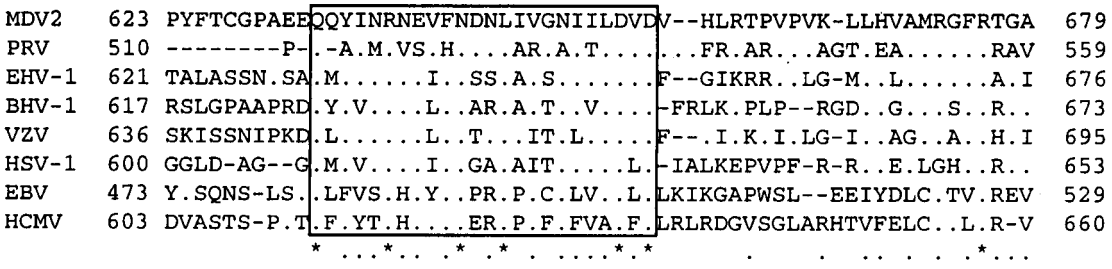

Fig. 3. Alignment of the predicted an essential component of helicase-primase complex proteins of MDV2, PRV, EHV-1, BHV-1, VZV, HSV-1, EBV and HCMV. Identical amino acids and conservative substitution are indicated by asterisks (*) and dots (.), respectively. The most conserved regions among the UL52 homologous proteins containing the herpesviral primase DXD motif indicated by box.

and ILTV, the UL52 homologous genes were transcribed into three different species of transcripts, 6.8, 4.5 and 2.8 kb for HSV-1 [23] and 5.8, 3.7 and 1.8 kb for ILTV [13], respectively. Our recent study showed that the 6.3 kb transcript was continuously recognized with 3.2 and 1.9 kb transcripts using a number of probes of the downstream genes from the MDV2 UL52 and possibly represent 3'-coterminal with the above two transcripts [22] (Fig. 1B). Although there is a potential polyadenylation signal downstream of the MDV2 UL52 gene, we did not find any UL52 gene-specific transcript, corresponding to those of HSV-1 and ILTV. These result suggested that the MDV2 UL52 homologous gene is transcribed as a polycistronic 6.3 kb transcript corresponding to 6.8 kb transcript of HSV-1 or 5.8 kb transcript of ILTV.

The size and property of the identified MDV2 UL52 gene are summarized in Table 1. The predicted protein of MDV2 UL52 exhibits homology to other herpesvirus helicase-primase proteins (Table 1). In performing multiple sequence alignments of the herpesvirus UL52 homologues, we confirmed several conserved regions in the MDV2 UL52 homologue. A conserved region of variable hydrophobic and hydrophilic characters containing within the UL52 homologue was particularly noticeable (data not shown). One-third of the carboxyl-terminal region (Fig. 3 box) which was one of the highest conserved region of the protein contained two conserved aspartic acid (Asp) in common (MDV2 residues 654 and 656). These Asp residues (DXD motif) were separated by a variable hydrophobic amino acid and were analogous to the bivalent metal-binding site of DNA polymerase and several DNA primase [8]. These residues were also conserved in beta- and gamma-herpesviruses (Fig. 3). The role of the DXD motif was studied in HSV-1 by introducing site-directed changes of the UL52 gene [8]. The helicase activity of the HSV-1 holoenzymes was unaffected by any of the introduced changes but the primase activity of the holoenzymes *in vitro* was significantly reduced, suggesting that the HSV-1 UL52 gene product was an essential component for the viral DNA helicase-primase complex. Although the viral helicase-primase protein of MDV2 has not yet been isolated and functionally tested, based on amino acid sequence homology and existing the DXD motif, it was suggested that the MDV2 UL52 homologous gene encodes a putative viral helicase-primase component. The results obtained from the present study would provide a framework for further studies on the basic molecular biology of MDVs.

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REFERENCES

1. Buckmaster, A. E., Scott, S. D., Sanderson, M. J., Boursnell, M. E. G., Ross ,L. J. N. and Binns, M. M. 1988. *J. Gen. Virol.* 69: 2033–2042.
2. Calder, J. M. and Stow, N. D. 1990. *Nucleic. Acids Res.* 18: 3573–3578.
3. Calnek, B. W., Schat, K. A., Peckham, M. C. and Fabricant, J. 1983. *Avian Dis.* 27: 844–849.
4. Cebrian, J., Kaschka-Dierich, C., Berthelot, N. and Sheldrick, P. 1982. *Proc. Natl. Acad. Sci. U.S.A.* 79: 555–558.
5. Corden, J., Wasylyk, B., Buchwalder, A., Sassone-Corsi, P., Kedinger, C. and Chambon, P. 1980. *Science* 209: 1406–1414.
6. Davison, A. J. and Scott, J. E. 1986. *J. Gen. Virol.* 67: 1759–1816.
7. Dodson, M. S. and Lehman, I. R. 1991. *Proc. Natl. Acad. Sci. U.S.A.* 88: 1105–1109.
8. Dracheva, S., Koonin, E. V. and Crute, J. J. 1995. *Proc. Natl. Acad. Sci. U.S.A.* 270: 14148–14153.
9. Fukuchi, K., Sudo, M., Lee, Y. S., Tanaka, A. and Nonoyama, M. 1984. *J. Virol.* 51: 102–109.
10. Igarashi, T., Takahashi, M., Donovan, J., Jessip, J., Smith, M., Hirai, K., Tanaka, A. and Nonoyama, M. 1987. *Virology* 157: 351–358.
11. Izumiya, Y., Jang, H. K., Kashiwase, H., Cai, J. S., Nishimura, Y., Tsushima, Y., Kato, K., Miyazawa, T., Kai, C. and Mikami, T. 1998. *J. Gen. Virol.* 79: 1997–2001.
12. Jang, H. K., Ono, M., Kim, T. J., Cai, J. S., Tsushima, Y., Niikura, M. and Mikami T. 1996. *J. Vet. Med. Sci.* 58: 1057–1066.
13. Johnson, M. A., Prideaux, C. T., Kongsuwan, K., Tyack, S. G. and Sheppard, M. 1995. *Arch. Virol.* 140: 623–634.
14. Lin, J., Kitagawa, H., Ono, M., Iwanaga, R., Kodama, H. and Mikami, T. 1990. *Avian Dis.* 34: 336–344.
15. McGeoch, D. J., Dalrymple, M. A., Davison, A. J., Dolan, A., Frame, M. C., McNab, D., Perry, L. J., Scott, J. E. and Taylor, P. 1988. *J. Gen. Virol.* 69: 1531–1574.
16. Ono, M., Katsuragi-Iwanaga, R., Kitazawa, T., Kamiya, N., Horimoto, T., Niikura, M., Kai, C., Hirai, K. and Mikami, T. 1992. *Virology* 191: 459–463.
17. Roizman, B. and Sears, A. E. 1996. pp. 2231–2295. *In*: Fields Virology, 3rd ed. (Field, B. N. Knipe, D. M. and Howley, P. M. eds.), Lippincott-Raven Publishers, Philadelphia, New York (in English).
18. Sambrook, J., Fritsch, E. F., Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, New York.
19. Schwyzer, M., Styger, D., Vogt, B., Lowery, D. E., Simard, C., LaBoissière, S., Misra, V., Vlcek, C. and Paces, V. 1996. *Vet. Microbiol.* 53: 67–77.
20. Shat, K. A. and Calnek, B. W. 1978. *J. Natl. Cancer. Inst.* 60: 1075–1082.
21. Telford, E. A. R., Watson, M. S., McBride, K. E. and Davison, A. J. 1992. *Virology* 189: 304–316.
22. Tsushima, Y., Jang, H. K., Izumiya, Y., Cai, J. S., Kato, K., Miyazawa, T., Kai, C. and Mikami, T. 1999. *Virus Res.* (in press).
23. Uprichard, S. L. and Knipe, D. M. 1996. *J. Virol.* 70: 1969–1980.
24. Wickens, M. 1990. *Trends Biochem. Sci.* 15: 277–281.