Identification of the Marek's Disease Virus Serotype 2 Genes Homologous to the Glycoprotein B (UL27), ICP18.5 (UL28) and Major DNA-Binding Protein (UL29) Genes of Herpes Simplex Virus Type 1

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ABSTRACT. We determined the nucleotide sequence of non-pathogenic Marek's disease virus serotype 2 (MDV2) strain HPRS24 glycoprotein B (gB) (UL27), ICP18.5 (UL28) and major DNA-binding protein (MDBP) (UL29) genes homologous to herpes simplex virus type 1 (HSV-1). The sequence data revealed that important motives in the proteins are conserved in MDV2 ICP18.5 and MDBP, however the sequence of viral DNA replication origin which exists in the regions between the UL29 and UL30 genes of other alphaherpesviruses was not found in the regions of the MDV2 genome. By northern blot analyses, we also demonstrated that 8.9, 5.0 and 2.6 kb transcripts were actually transcribed from the sequenced region in MDV2-infected cells. The MDV2 UL28 and UL29 genes have not been reported in other serotypes of MDV.—**KEY WORDS:** Marek's disease virus serotype 2, transcript, UL27-UL29.

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Marek's disease virus (MDV) is a cell-associated alphaherpesvirus of poultry and causes a naturally occurring malignant T cell lymphoma in chickens. The virus is divided into three serotypes. They belong to alphaherpesviruses, based on their genomic structures and arrangements [4, 11, 21]. The disease can be prevented by vaccination with attenuated MDV serotype 1 (MDV1), non-pathogenic MDV serotype 2 (MDV2) or herpesvirus of turkeys (HVT) known as a MDV serotype 3 (MDV3). The MDV2 genome differs in restriction endonuclease digestion patterns from those of either MDV1 or HVT [10, 25, 30]. However, information about the genomic analysis of MDV2 is limited and only the genes encoding for glycoproteins gB (SB-1 strain) [34], gC [17], gD [15], gE [13], gH [28], gI [14], and thymidine kinase, UL24 [29], UL25, UL26, UL26.5 [16] homologous to HSV-1, and MDV unique phosphoprotein pp38 [22] have been reported. It is conceivable that the comparison of biological and molecular biological aspects of a virulent MDV1 with those of non-pathogenic MDV strains would provide insight into the mechanisms by which MDV causes tumors in chickens. To pursue this objective, our group has been trying to determine the full sequence of non-pathogenic MDV2 strain, HPRS24. In this study, we report the MDV2 genome sequence including UL27, UL28 and UL29 homologous genes encoding for gB, ICP18.5 and MDBP, respectively. This is the first report dealing with UL28 and UL29 homologues of HSV-1 in MDV serotypes.

A DNA library of MDV2 strain HPRS24 constructed previously [21] was used (Fig. 1A). MDV2 *Bam*HI-N and -

T2, and *Eco*RI-V fragments were cloned into pBluescript SK⁺ (Stratagene, La Jolla, CA). A *Bam*HI-W1 fragment was made using pMOSBlueTvector kit (Amersham, Buckinghamshire, England). Moreover 3.0 kb *Sma*I-*Pst*I and 3.8 kb *Sma*I-*Eco*RI subfragments from the *Eco*RI-H fragment (Fig. 1B) were then deleted by exonuclease III to construct deletion clones. After that DNA sequences were determined on both strands using an autosequencer system (Applied Biosystems, Foster City, CA). DNA and amino acid sequences were analyzed with the computer program GENETYX-MAC (version 8.0). Homology searches and multiple sequence alignments from the GenBank and EMBL Data Libraries were performed using the UWGCG programs BESTFIT and PILEUP, respectively.

The nucleotide sequences of the MDV2 UL27, UL28 and UL29 homologues have been submitted to the DDBJ, EMBL and GenBank Databanks with the Accession No. AB024711.

The location of the MDV2 (HPRS24 strain) UL27, UL28 and UL29 homologues in virus genome, pertinent restriction endonuclease sites are illustrated in Fig. 1. We determined the nucleotide sequence of a total of 9,650 bp containing the putative sequences of MDV2 UL27, UL28 and UL29 homologues of the HSV-1 gene (Fig. 1C).

To find out whether transcripts are really transcribed from the domain of MDV2 genome containing the identified genes of MDV2 UL27, UL28, and UL29, northern blot analyses were performed using the different probes. The maintenance of primary chicken embryo fibroblasts (CEF) and infection of CEF with MDV2 strain HPRS24 were performed as described previously [14]. To isolate total RNA, monolayers were harvested at 96 hr postinfection and treated with 0.05% ethylenediaminetetraacetic acid in phosphate-buffered saline. Total RNAs were extracted from these lysates by ISOGEN (Nippon Gene, Tokyo, Japan). Total RNA was separated on an agarose formaldehyde-gel

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Fig. 1. Diagram of the MDV2 genome. (A) The genome is organized into the unique long (UL) and short (US), internal repeat long (IR_L) and short (IR_S), and terminal repeat long (TR_L) and short (TR_S) regions. (B) *Bam*HI-W1 (0.9 kb), -N (3.4 kb) and -T2 (2.0 kb) fragments, *Sma*I-*Pst*II (3.0 kb) and *Sma*I-*Eco*RI (2.0 kb) sites of *Eco*RI-H fragment, and *Eco*RI-V (1.2 kb) fragment were cloned and the nucleotide sequence of a total of 9,650 bp containing MDV2 UL27, UL28 and UL29 homologues of the HSV-1 gene was determined. (C) The locations and directions of the MDV2 UL27, UL28 and UL29 ORFs are indicated by bold arrows. (D) The subclones used as probes in the northern blot analysis are shown as bold lines. The approximate sizes of subclones are as follows: I, 1.7 kb; II, 1.3 kb; III, 1.0 kb.

Fig. 2. Northern blot analysis of the transcripts encoded by MDV2 UL27, UL28 and UL29 homologues. Cells either mock-infected (M) or infected with MDV2 (V) were processed at 96 hr post-infection. Total cell RNAs were extracted, separated by electrophoresis on agarose formaldehyde-gel, transferred to a nylon membrane filter and hybridized with the probes I, II, III, as indicated in Fig. 1 (D). The RNA markers (Gibco BRL, Grand Island, NY) are used as molecular size standards. Open arrowheads denote location of 28S and 18S rRNA. Molecular sizes of the transcripts are shown in kilobases.

and transferred to a nylon membrane filter (Biodyne, Pall BioSupport, NY). DNA probes (Fig. 1D) were radiolabeled with (α - 32 P)dCTP by Nick Translation Kit (Boehringer Mannheim GmbH, Germany), and hybridized [9]. The RNA markers (Gibco BRL, Grand Island, NY) are used as molecular size standards. The probe I which is located in ORF of MDV2 UL27 hybridized to transcripts of abundant RNAs of approximately 8.9, 5.0, 4.2, 2.6 and 1.9 kb transcripts (Fig. 2-I). The probe II which is located in MDV2 UL28 ORF hybridized to transcripts of approximately 8.9, 5.0, 4.2 and 1.9 kb transcripts (Fig. 2-II), whereas the probe III in MDV2 UL29 ORF hybridized to 8.9, 4.2 and 1.9 kb transcripts (Fig. 2-III). The 4.2 and 1.9 kb transcripts are most likely caused by non-specific reaction of degraded viral RNAs co-migrated with the cellular 28S and 18S rRNA as previously reported [12]. The results of northern blot analysis would suggest that the 2.6 kb transcript is specific for UL27, that the 5.0 kb transcript covers UL27 and UL28, and that the 8.9 kb transcript covers the cluster of genes from UL27 to UL29. We have not determined the directions and fine mapping of the transcripts and we can not exclude the possibility that some of these transcripts are not specific for the genes. Further experiments using riboprobes will be required to identify the specific transcripts of the genes exactly. However, we would clearly conclude that a set of transcripts is actually transcribed from the domain of MDV2 genome sequenced in this study.

The UL27 homologous genes encode gB which is an essential envelope proteins required for viral entry [26]. The MDV2 UL27 gene contains a 2,598 bp ORF encoding 865 amino acids. Only three nucleotides (CCC: 648–650 bp) of the UL27 gene of MDV2 HPRS24 strain are different from those of the MDV2 SB-1 strain (GGG: 648–650 bp) [34]. A putative TATA box lies -256 to -253 bp upstream of the translational start codon of MDV2 UL27. A potential Poly (A) signal sequence, AATAAA, is located at 21 to 26 bp downstream of the stop codon of MDV2 UL27. The complete MDV2 UL27 ORF encoded an expected polypeptide of 97 kDa. The comparison of the amino acid of MDV2 UL27 homologue with other herpesviruses showed a high identity with MDV2 (SB-1 strain), MDV1 and MDV3, a moderate identity with HSV-1, varicella-zoster virus (VZV), bovine herpesvirus type-1 (BHV-1), equine herpesvirus type-1 (EHV-1), pseudorabies virus (PRV), and a low identity with human cytomegalovirus (HCMV) and Epstein-Barr virus (EBV) (Table 1). Further, potential N-linked glycosylation and proteolytic cleavage sites are conserved among MDV strains.

The MDV2 UL28 gene includes 2,364 bp ORF encoding 787 amino acids. Putative TATA and CCAAT boxes lie at positions -73 to -70 and -38 to -34 upstream of the translational start codon of MDV2 UL28, respectively. The complete MDV2 UL28 ORF encoded an expected polypeptide of 92 kDa. The comparison of the amino acids of MDV2 UL28 homologue with other herpesviruses indicated that UL28 proteins of other alphaherpesviruses

Table 1. Comparison of the amino acid sequences of deduced proteins of the MDV2 ORFs with other herpesviruses*

MDV2 ORFs	Herpesvirus homologues	Homology	
		Identity (%)	Similarity (%)
UL27	MDV2** gB	99.8	99.8
	MDV1 gB	82.3	87.6
	HVT gB	76.8	82.9
	HSV-1 gB	50.0	59.3
	VZV gB	52.4	62.2
	BHV-1 gB	50.8	60.1
	EHV-1 gB	50.9	61.7
	PRV gB	53.2	63.2
	HCMV gB	28.9	39.2
	EBV gB	28.6	37.0
UL28	HSV-1 ICP18.5	48.0	58.2
	VZV ICP18.5	45.2	56.7
	BHV-1 ICP18.5	45.6	54.9
	EHV-1 ICP18.5	47.7	56.8
	PRV ICP18.5	48.8	58.7
	HCMV ICP18.5	24.3	42.7
	EBV ICP18.5	26.7	39.0
UL29	HSV-1 MDBP	56.3	45.2
	VZV MDBP	46.8	57.6
	BHV-1 MDBP	47.8	59.3
	EHV-1 MDBP	59.4	48.5
	EHV-1 MDBP	59.4	48.5
	HCMV MDBP	22.3	28.6
	EBV MDBP	25.7	33.9

* Homology searches from the GenBank and EMBL Data Libraries were performed using the UWGCG program BESTFIT. The entire UL27, UL28 and UL29 amino acid sequences were used. References: MDV1 [26], MDV2 (SB-1 strain) and HVT [34], HSV-1 [19], VZV [6], BHV-1 [20, 27], EHV-1 [31], PRV [23], HCMV [5] and EBV [3]. ** SB-1 strain.

MDV2 UL28	
amino acid position	500 - 503
MDV2	LVRASADVA RRRK MYAERL SKRSLASLGR
HSV-1	IATQAAADV ERRR LYADRL TKRSLASLGR
VZV	LLSKAKIDAA ERRR VYLEHL SKRSLTSLGR
EHV-1	LAARARADAD VRRQR YAEERL SKRSMDSLGR
EHV-1	LLERATADAS QRRR MYAERL SKRSLASLGR
PRV	LPAAALRDAE RRRK LYADRL SRRSAASLAQ
HCMV	RIHNVTREVN VRRR AYLRQV SEVGYGKQIR
EBV	GPSGALSDEL KRK EQYLRQV ATEGLAKLQS

Fig. 3. Multiple alignment of UL28 homologues at the nuclear localization signal (bold characters). Alphaherpesviruses (MDV2, HSV-1 [19], VZV [6], BHV-1 [20], EHV-1 [31] and PRV [23]), betaherpesvirus (HCMV [5]) and gammaherpesvirus (EBV [3]) were aligned together. The putative nuclear localization signal (MDV2 a.a. 500–503) is well conserved among other herpesviruses.

(HSV-1, VZV, BHV-1, EHV-1 and PRV) showed a moderate degree of identity with MDV2 UL28, whereas those of beta (HCMV) and gammaherpesvirus (EBV) showed a lower identity (Table 1). The alignment analysis indicates that the predicted nuclear localization sequence [24] in MDV2 ICP18.5 (500-RRRK-503) is conserved like other herpesviruses (Fig. 3). In HSV-1, it has been shown

MDV2 UL29		amino acid position	
		497-----510	800-----846
MDV2	DVPCILCDKA	SRIYCAHTTI	KRLEYRLPKF-----YQREAKA.MH
HSV-1	DVPCNLCTFD	TRHACVHTIL	MRLRARHPKF-----YQKEDK.RVD
VZV	EIPCSLCEKH	TRFVCAHTTV	HRLRQRMRF-----YQREK.RVD
BHV-1	DVPCGLCSR	DRHACHTIL	HRLRHLRF-----YQKPEGGVN
EHV-1	DVPCGLCNA	TRPACHTIL	HRLRQLRF-----YRKPESGGVN
HCMV	AGLCEACG	CGTCTCYQTAF	VRVTRLFPV-----FRRDHVPFRN
EBV	SEMCSLCEGR	APAVCIATLF	FRLDRFPFV-----IKSTIRENY
			INGPYMKFL
			NTYHKLFPD
			TKLSS.....
			LYLWHSR

Fig. 4. Multiple alignment of UL29 homologues at the DNA-binding motifs (bold characters). Alphaherpesviruses (MDV2, HSV-1 [19], VZV [6], BHV-1 [20], EHV-1 [31]), betaherpesvirus (HCMV [5]) and gammaherpesvirus (EBV [3]) were aligned together. The first alignment (MDV2 a.a. 497–510) which represents the putative zinc finger motif is conserved among other herpesviruses. The second alignment (MDV2 amino acid 800–846) is well fitted to the DNA-binding motif consensus sequence.

that products of the UL28 gene, together with those of UL6, UL25, UL32 and UL33 genes, are required for formation of full capsids, for packaging of viral DNA and for expression of viral glycoproteins on the surface of virus-infected cells [1, 2, 32].

The MDV2 putative UL29 gene contains 3,573 bp ORF encoding 1,190 amino acids. A putative TATA box lies at -210 to upstream of the start codon of MDV2 UL29. The complete MDV2 UL29 ORF encoded an expected polypeptide of 130 kDa. The degree of homology of MDV UL29 with those of other alphaherpesviruses is similar to that of MDV UL28 (Table 1). In MDV2 UL29, there are two domains which are highly conserved among UL29 homologues of herpesviruses. The first (a.a. 497–510) domain contains a putative zinc finger motif which appears to be well conserved only for alphaherpesviruses. It fits the consensus sequence of zinc finger motif C-X₂₋₅-C-X₂₋₁₅-C/H-X₂₋₄-C/H (X is any amino acid residue.) [7]. This motif has been shown to be nonessential for DNA binding but might correlate with modulation of the DNA-binding activity of HSV-1 major DNA-binding protein (MDBP). The second conserved domain (a.a. 800–846) contains aromatic and basic residues. These types of residues are directly associated with DNA-binding by several single-stranded DNA-binding proteins [33]. The MDV2 UL29 domain fits the consensus DNA-binding motif, K/R-X₂₋₃-K/R-X₉₋₁₀-F-X₄-F-X₁₋₃-K/R-X₁₀₋₁₂-W-X₆-R (Fig. 4). Therefore, this consensus motif may serve as a core for the DNA-binding site of MDV2 MDBP with the putative zinc finger motif. The UL29 gene product (MDBP, ICP8) of HSV-1 is required for viral DNA replication and regulation of viral transcription [18]. MDBP contains several regions which may be important for DNA-binding based on sequence similarities in single-stranded DNA-binding proteins of other herpesviruses [33].

Finally, we would note that putative sequence of origin for viral DNA replication, OriL, was not found in the region between UL29 and UL30 of the MDV2 genome where OriL of other alphaherpesviruses except BHV-1 exists [8, 27].

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