嘉南藥理科技大學教師專題研究計劃成果報告

計劃名稱：The UL9 homolog of marek's disease virus possesses the helicase activity

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Abstract

In previous studies (Wu et al., Virus Genes13: 143-157, 1996), we have identified a MDV gene that is homologous to herpes simplex virus type 1 (HSV-1) UL9 gene that encodes an origin-binding protein. It lies between the *BamH*I C and G fragments of the serotype 1 MDV strain GA genome and named as MDV origin-binding protein (MDV OBP). In this study, a MDV OBP of 95 kDa was detected in coupled *in vitro* transcription-translation reactions by immunoprecipitation with penta-histidine specific monoclonal antibody. Further characterization of MDV OBP was accomplished by the use of electrophoretic mobility shift assays (EMSA) using *in vitro* expressed MDV OBP. The results of EMSAs indicated that MDV OBP could bind to the putative MDV OBP binding sites within the serotype 1 and 2 MDV replication origins. Furthermore, a series of two-base-pair substitutions across the MDV OBP binding site within serotype 2 MDV replication origin were used in competitive EMSAs. Results demonstrated that the recognition sequence of MDV OBP was the TTCGCACC that is a subset of a 9-bp element (CGTTCGCAC) conserved in the replication origins of alphaherpesviruses. Taken together, our results suggested that MDV OBP encodes an origin-binding protein of MDV.

Key words : Marek's disease virus, MDV UL9, origin-binding activity

Introduction

Marek's disease virus (MDV) is a highly cell-associated avian oncogenic herpesvirus that causes a highly contagious Marek's disease (MD). Marek's disease is characterized by malignant T-cell lymphomas, neurological disorder and immunodeficiency (Calnek et al., 1997). Replication origins of the serotypes 1, 2 MDV and HVT have structures similar to those of other alphaherpesviruses such as herpes simplex type 1 (HSV-1) as well as varicella-zoster virus (VZV) (Camp et al., 1991). Each replication origin consists of a core stretch of AT-rich palindromic sequence and three repeats of a 9- bp element (CGTTCGCAC) that is highly conserved in the replication origins of alphaherpesviruses. The 9-bp element functions as the recognition sequence for HSV-1 UL9 protein (Deb and Deb, 1989) that encodes an origin-binding protein (OBP) and is one of seven genes required for viral DNA synthesis (Wu et al., 1988). It possesses the origin-binding (Arbuckle et al., 1993), DNA-dependent helicase (Boehmer et al., 1993) and DNA-dependent ATPase (Dodsojn et al., 1993) activities. It has been suggested that HSV-1 UL9 gene is the initiator protein for HSV-1 DNA replication (see reviewed by Boehmer et al., 1997).

A MDV gene, homologous to HSV-1 UL9 and thus named as MDV OBP, lies between the *BamH*I C and *BamH*I G fragments of the MDV serotype 1 strain GA genome (Wu et al., 1996). Computer analysis of predicted amino acid sequence of MDV OBP reveals that it shares numerous structural

motifs with both HSV-1 UL9. It is plausible that MDV OBP has the similar biochemical activities to those of HSV-1 UL9 protein

The objective of this study was to functionally identify MDV origin-binding protein. A radiolabeled 26-mer oligonucleotide containing the putative recognition sequence for MDV OBP and *in vitro* translated MDV OBP were used in electrophoretic mobility gel shift assay (EMSA). Results showed that MDV OBP bound to a conserved binding site similar to that recognized by HSV-1 UL9 protein.

Results and discussion

Coupled *In vitro* transcription and translation of MDV OBP gene

MDV OBP gene was amplified from the cellular DNA isolated from the MDV GA-infected CEF cells and cloned in frame with the (histidine)$_6$ tag of pBKCMVHistag (Fig. 1). L-[^{35}S]methionine-labeled *in vitro* translated product of MDV OBP gene was analyzed by SDS-PAGE and immunoprecipitation. The least migrating protein band corresponding to molecular weight of 95 kDa was synthesized in the *in vitro* translation and immunoprecipitated by the penta-histidine and tetra-histidine specific monoclonal antibodies (Qiagene, Velnecia, CA) (Fig. 2, lanes 2-4). The apparent molecular size of this protein was consistent with the predicted molecular size of 94,987.

Origin-binding activity of *in vitro* synthesized MDV OBP

In order to detect the origin-binding activity of MDV OBP, *in vitro* synthesized MDV OBP was utilized in the EMSAs using ds MDVUL9-II (Fig. 3) as the probe. The results of EMSAs demonstrated that in the presence of wild-type MDV OBP, three complexes (C, M and M') were formed. Among them, complex C ran at the same mobility rate as the complex formed in EMSAs with the mock programmed lysate (Fig. 3, lane 8). The complex C might be resulted from the binding of cellular protein(s) to the oligonucleotide probe. However, complexes M and M' ran at the different mobility rates. (Fig. 3, lanes 2 and 3). Complexes M and M' was MDV OBP-specific because 1 to 100 -fold unlabeled ds MDVUL9-II could gradually outcompete the signal but not the 100- fold GATA-1 oligonucleotide (nonspecific competitor) (Fig. 3, lanes 3-7).

Determination of the recognition sequence of MDV OBP

To determine the recognition sequence for MDV OBP, ten consecutive mutants each containing a 2-bp point mutation within or outside the conserved 9-bp element (Fig. 4) were tested for their competition abilities in the competitive EMSAs. The *in vitro* translated product of MDV OBP gene was incubated with the radiolabeled MDVUL9-II in the presence or absence of an excess of mutant MDVUL9-IIs. The competition ability of each mutant MDVUL9-II was compared to that of wild-type MDVUL9-II. Results showed that the mutants 3, 4, 5 and 6 lost the competition abilities even at the 100-fold excess (Fig. 4, lanes 9-16), suggesting that the recognition sequence for MDV OBP was TTCGCACC.

In this report, the expression and functional identification of the product of MDV OBP gene were described. The wild-type MDV OBP gene product, expressed in the reticulocyte lystae *in vitro* transcription-translation system, had a molecular weight of 95,000 which was consistent with the predicted molecular weight of 94,987. EMSAs results indicated that the wild-type MDV OBP as well as cellular proteins could bind to the specific sequence within the MDV serotype 1 and 2 replication origins.

The recognition sequence (TTCGCACC) of MDV OBP was a subset of a 9-bp element (CGTTCGCAC) that is conserved in the replication origins of alphaherpesviruses. In addition, consistent with HSV-1 UL9 results of Hazuda *et al.* (17), a CGC triplet within a 9-bp element was critical to the interaction between MDV OBP and the origin. Although the homology of the origin-binding domains of HSV-1 and MDV OBPs is less (35% identity) than the overall homology of two molecules (49% identity)(31), the origin binding proteins of HSV-1, MDV, Equine herpesvirus type 1 (EHV-1) (23), VZV (11), and Human herpesvirus 6B (HHV-6B, a betaherpesvirus) (18) appear to have virtually identical recognition sequence, suggesting that the functions of origin-binding proteins are highly similar within alphaherpesviruses, even within betaherpesviruses. Moreover, the recognition sequence of the DNA-binding domain of MDV OBP was the same as that of wild-type MDV OBP, suggesting that the wild-type MDV OBP bound to the origin via the C-terminal domain *per se*.

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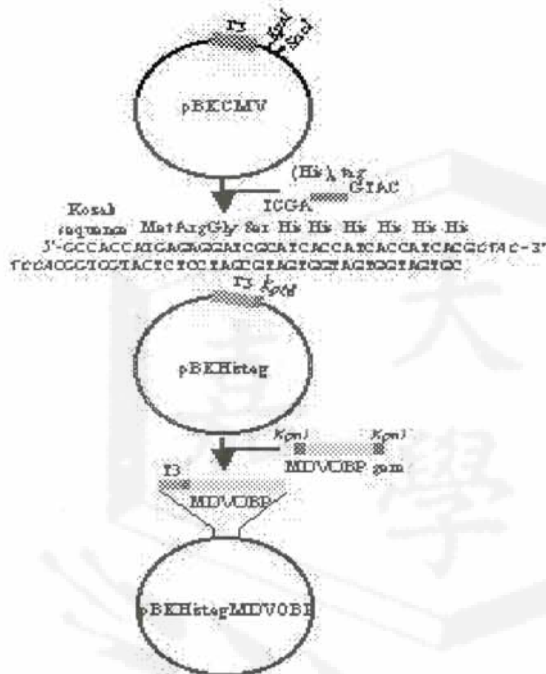

Figure 1. Cloning of MDV OBP gene.

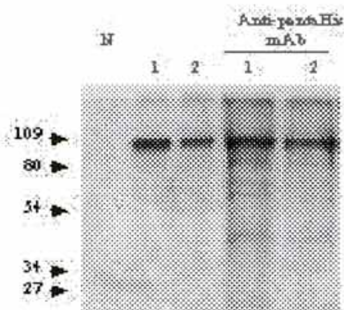

Figure 2. SDS-polyacrylamide gel analysis and immunoprecipitation of L-[35S]methionine-labeled MDV OBP gene product synthesized *in vitro* by using a reticulocyte lysate.

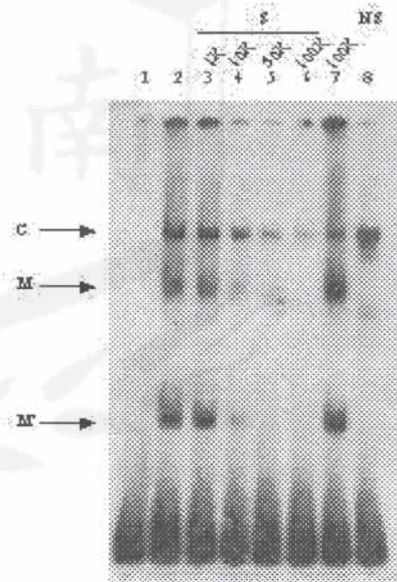

Figure 3. Binding of *in vitro* synthesized MDV OBP gene product to ds MDVUL9-II oilgonucleotide.

		Competition
WT	GGACGGCGTTCGCACCTTGCGCCAAT	+
M-1	GGACTTCGTTCGCACCTTGCGCCAAT	+
M-2	GGACGGATTTCGCACCTTGCGCCAAT	+
M-3	GGACGGCGGGCGCACCTTGCGCCAAT	-
M-4	GGACGGCGTTATCACCTTGCGCCAAT	-
M-5	GGACGGCGTTCGACCCTTGCGCCAAT	-
M-6	GGACGGCGTTCGCAAATTGCGCCAAT	-
M-7	GGACGGCGTTCGCACCGTGCGCCAAT	+
M-8	GGACGGCGTTCGCACCTGTCGCCAAT	+
M-9	GGACGGCGTTCGCACCTTGATCCAAT	+
M-10	GGACGGCGTTCGCACCTTGCGAAAAT	+

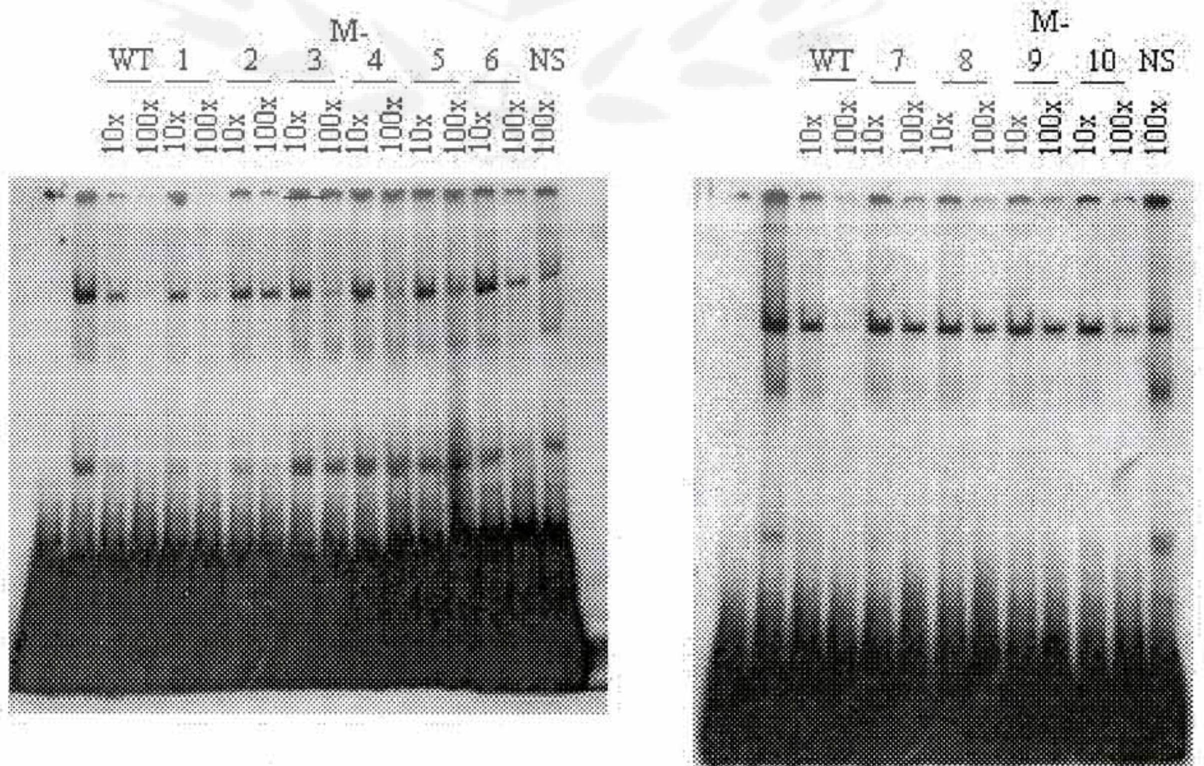

Figure 4. Mutation analysis of ds MDVUL9-II oilgonucleotide with wild-type MDV OBP.