馬雷克病毒之 UL9 基因為一 origin-binding 蛋白質

Marek's disease virus UL9 gene encodes an origin-binding protein

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主持人：吳定峰　嘉南藥理學院藥學系

中文摘要

在先前研究裏，經由電腦分析結果顯示，馬雷克病毒 UL9 基因與第一型疱疹病毒 UL9 基因有許多相似之構造單位。所以馬雷克病毒之 UL9 基因也許擁有與包疹病毒之 UL9 基因相類似之生化功能，特別是複製起始點結合的功能。

在此計劃中,利用膠體位移實驗法證明馬雷克病毒之 UL9 基因會與馬雷克病毒之複製起始點結合，而且證實它辨識的鹼基序列為 TTCGCACC。另外,一系列具有刪除突變之馬雷克 UL9 蛋白質已構築完成,將利用來決定 DNA-binding 的區域。

關鍵詞：馬雷克病毒,馬雷克 UL9 蛋白質,origin binding 之蛋白質

Abstract

In previous studies, from the computer analyses, MDV (Marek's disease virus) UL9 shares several structural motifs with HSV-1 (Herpes simplex virus type 1) UL9. It is likely that MDV UL9 possesses the biochemical activities similar to those of HSV-1 UL9, especially the origin-binding protein.

In this project, using the electrophoretic mobility gel shift assay, we demonstrated that MDV UL9 could bind to the origin of DNA replication and its recognition site was TTCGCACC. We also constructed a series of deletion mutants that can be used in the future to determine the DNA-binding domain of MDVUL9 protein.

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

Cause and goal

HSV-1 UL9 protein, one of seven essential genes for HSV-1 viral DNA replication, encodes an origin binding protein (OBP) (Olivo et al., 1988). It cooperatively binds as a dimer to two high-affinity sites (sites I and II) within the HSV-1 DNA replication origin (ori_S), and exhibits DNA-helicase (Boehmer et al., 1993) and ATPase activities (Dodson et al., 1993). The biochemical activities of HSV-1 UL9 suggests that HSV-1 UL9 may play a role in initiation of viral DNA replication.

Marek's disease virus (MDV) is a highly cell-associated avian herpesvirus. In chickens, MDV is the etiologic agent of Marek's disease (MD), characterized by malignant lymphoma of T cells. Functional MDV origins of DNA replication have been identified in serotype 2 MDV defective virus (Camp *et. al.*,1991) and in serotype 3 MDV (Smith *et. al.*, 1995). Within the serotype 2 MDV origin of viral DNA replication, two presumed MDV UL9 recognition sequences

(MDV UL9 recognition site I, CGTTCGCACCG and MDV UL9 recognition site II, CGTTCGCACCT) have been identified which are highly homologous to HSV-1 UL9 site I (CGTTCGCACTT). In previous studies, a MDV UL9 gene has been identified which lies between the *BamHI* C and *BamHI* G fragments of the serotype 1 MDV strain GA genome. Computer analysis of predicted amino acid sequences of MDV and HSV-1 UL9s revealed that MDV UL9 shares numerous structural motifs with HSV-1 UL9. MDV has been shown to synthesize the MDV UL9 protein by northern and western blot analyses (Wu *et. al.*, 1996). This project is intended to test the hypothesis that MDV UL9 protein has the domain structures and biochemical activities similar to HSV-1 UL9 protein, especially the origin-binding activity.

Results and Discussion

1. Cloning of MDV UL9 gene

MDV UL9 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. The pBKCMVHistag was constructed by cloning the Kozak sequence-$(his)_6$ oligonucleotide into the pBKCMV (Stratagene, La Jolla, CA). The resulting clone was designated by pBKhistagMDVUL9 in which histidine tag-MDV UL9 fusion protein was driven by T3 promoter.

2. Coupled *In vitro* transcription and translation

^{35}S-labled MDVUL9 protein was synthesized by TNT®coupled *in vitro* transcription and translation kit (Promega, Madison, WI) with T3 polymerase using pBKHistagMDVUL9 as the template. The MDVUL9 protein produced in the lysate was confirmed by immunoprecipitation by anti-penta histidine monoclonal antibody (Qiagene, Velnecia, CA). A 95 kd protein was precipitated by the monoclonal antibody.

3. Origin-binding activity of MDV UL9 protein

In order to detect the origin binding activity, *In vitro* synthesized MDV UL9 protein was utilized in the electrophoretic mobility gel shift assay (EMSA) using a 26-mer oligonucleotide (MDV UL9 site II DNA) designed from the presumed MDV UL9 site II (CGTTCGAACCT) as the probe. The results demonstrated that one complex (C) of three complexes formed in EMSAs with the *in vitro* synthesized MDV UL9 protein run at same mobility rate as one of two complexes (C and C') formed in EMSAs with the lysate programmed without the plasmid while the other two complexes (M and M') run at the different mobility rate. (Figure 1, lane 2 and 8). Consistent with this result, Schaffer *et. al.* (1998) reported that cellular proteins can bind to the HSV-1 DNA origin. Complexes M and M' was specific because 1 to 100 fold unlabeled oligonucleotide could outcompete the signal but not the 100 fold nonspecific competitor (Figure 1, lane 3-7). Complex M' might result from the OBPC-like protein. Schaffer *et. al.* (1996) showed that an OBPC protein can translated from the internal AUG codon of HSV-1 UL9 gene. Super-EMSAs with anti-pentahistidine monoclonal antibody demonstrated that complexes M and M'

conatined the MDV UL9 protein.

4. Determination of MDV UL9 recognition sequence

In order to determine the recognition sequence of MDV UL9 protein, a series of mutant MDV UL9 site II DNA was used as the competitor in the competition EMSAs. The results suggested that the recognition sequence was TTCGCACC (Table 1). Consistent with this results, the consensus recognition sequence of UL9 protein has been reported to be YGYTCGCACTT (where Y is a pyrimidine) .

5. Construction of deletion mutants of MDV UL9 protein

A series of deletion mutants from the N-terminal domain was constructed in order to determine the boundary of DNA-binding domain. Suitable restriction enzymes were used to construct the deletion mutants. The constructed deletion mutants were shown in Figure 2. The deletion mutants will be utilized in EMSAs to measure the binding activity on the MDV UL9 site II DNA to determine the boundary of DNA-binding domain.

Self evaluation

The progress of this project is consistent with the expectation except that the deletion mutants have not used in EMSAs to determine the boundary of DNA-binding domain. The results demonstrated that MDV UL9 protein possesses the origin-binding activity. It is likely that mutant MDV UL9 protein will inhibit the replication of MDV in the cells. Therefore, a replication-deficient serotype 1 MDV vaccine can be constructed by deletion of MDV UL9 gene or insertion of a mutant MDV UL9 gene into the U_s region which is not essential for the viral DNA replication. The results of this project are suitable for publication. The paper is in preparation.

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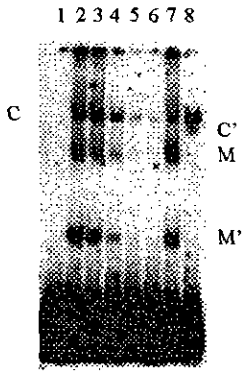

Figure 1. The results of EMSAs with in vitro synthesized MDV UL9 protein using MDV UL9 site II DNA as the probe. Lane 1, The probe only. Lane 2, EMSAs with in vitro synthesized MDV UL9 protein. Lane 3-6, EMSAs in the presence of 1X, 10X, 50X and 100X unlabeled MDV UL9 site II DNA, respectively. Lane 7, EMSAs in the presence of 100X nonspecific competitor. Lane 8, EMSAs with the lysate programmed without the plasmid.

Table 1. The results of competition EMSAs

Oligonucleotide	Competition ability
GGACGGCGTTCGCACCTTGCGCCAAT	++[a]
GGAC**TT**CGTTCGCACCTTGCGCCAAT	++
GGACGG**AT**TTCGCACCTTGCGCCAAT	++
GGACGGCG**GG**CGCACCTTGCGCCAAT	-[b]
GGACGGCGTT**AT**CACCTTGCGCCAAT	-
GGACGGCGTTCG**AC**CTTGCGCCAAT	-
GGACGGCGTTCGCA**AA**TTGCGCCAAT	-
GGACGGCGTTCGCACC**G**TGCGCCAAT	++
GGACGGCGTTCGCACCT**GT**CGCCAAT	++
GGACGGCGTTCGCACCTTG**AT**CCAAT	++
GGACGGCGTTCGCACCTTGCG**AA**AAT	++

a: ++ means the strong competition

b : - means no competition

Figure 2. The map for deletion mutants of MDV UL9 protein.