Mol Biol Rep
DOI 10.1007/s11033-009-9546-y

Molecular cloning and characterization of the UL31 gene from Duck enteritis virus

Wei Xie · Anchun Cheng · Mingshu Wang · Hua Chang · Dekang Zhu · Qihui Luo

Received: 21 November 2008 / Accepted: 30 April 2009

Abstract Using a combination of bioinformation analysis and Dot blot technique, a gene, designated hereafter as the duck enteritis virus (DEV) UL31 gene (GenBank accession number EF643559), was identified from the DEV CHv genomic library. Then, the UL31 gene was cloned and sequenced, which was composed of 933 nucleotides encoding 310 amino acids. Multiple sequence alignment suggested that the UL31 gene was highly conserved in *Alphaherpesvirinae* and similar to the other herpesviral UL31. Phylogenetic analysis showed that the gene had a close evolutionary relationship with the avian herpervi-ruses, and DEV should be placed into a single cluster within the subfamily *Alphaherpesvirinae*. Antigen prediction indicated that several potential B-cell epitopes sites located in the UL31 protein. To further study, the UL31 gene was cloned into a pET prokaryotic expression vector and transformed into *Escherichia coli* BL21 (DE3). A 55 kDa fusion protein was induced by the further culture at 37°C after addition of 0.8 mM IPTG. Polyclonal antibody raised against the recombinant UL31 from rabbit was prepared. A protein about 55 kDa that reacted with the antibody was detected in immunoblots of bacterial proteins, suggesting that the 55 kDa protein was the product of the UL31 gene. Immunofluorescence analysis revealed that the protein was localized in very fine punctate forms in the nuclei of infected cells**.** Our results may provide some insight for further research about the gene and also enrich the database of herpesvirus.

Keywords Duck enteritis virus (DEV) · UL31 gene · Characterization · Expression

Abbreviations

DEV	Duck enteritis virus
HSV-1	Herpes simplex virus 1
HCMV	Human cytomegalovirus
EBV	Epstein-barr virus
DVE	Duck viral enteritis
HHV-3	Human herpesvirus 3
MDV-1	Marek's disease virus type 1
CeHV-9	Cercopithecine herpesvirus 9
MeHV-1	Meleagrid herpesvirus 1
EHV-1	Equid herpesvirus 1
BoHV-5	Bovine herpesvirus 5
MDV-2	Marek's disease virus type 2
ILTV	Infection laryngotracheitis virus
CeHV-16	Cercopithecine herpesvirus 16
HHV-2	Human herpesvirus 2
BoHV-1	Bovine herpesvirus 1
CeHV-1	Cercopithecine herpesvirus 1
SuHV-1	Suid herpesvirus 1
HHV-1	Human herpesvirus 1
HHV-7	Human herpesvirus 7
PCMV	Porcine cytomegalovirus
MCMV	Murine cytomegalovirus
HHV-6	Human herpesvirus 6
HHV-5	Human herpesvirus 5
HHV-8	Human herpesvirus 8

W. Xie · A. Cheng (✉)
Avian Diseases Research Center, College of Veterinary Medicine, Sichuan Agricultural University, Ya'an, Sichuan 625014, China
e-mail: chenganchun@vip.163.com

W. Xie
e-mail: xiew007@sohu.com

A. Cheng · M. Wang · H. Chang · D. Zhu · Q. Luo
Key Laboratory of Animal Diseases and Human Health of Sichuan Province, Ya'an, Sichuan 625014, China

Published online: 23 May 2009

BoHV-4	Bovine herpesvirus 4
SaHV-2	Saimiriine herpesvirus 2
EHV-2	Equid herpesvirus 2
MDPV	Muscovy duck parvovirus
Prv	Pseudorabies virus
Gpv	Goose parvovirus

Introduction

Several gene clusters are conserved throughout mammalian and avian *alpha-, beta-,* and *gammaherpesviruses*, which encoding proteins are required for fundamental steps of the viral life cycle [1]. One of these clusters include the UL31 gene of the *alphaherpesvirus* Herpes Simplex Virus 1 (HSV-1) [2–4], whose homologues were named UL53 in the *betaherpesvirus* human cytomegalovirus (HCMV) [5] and BFLF2 in the *gammaherpesvirus* Epstein-Barr virus (EBV) [6, 7]. Previous researches have indicate that the UL31 gene is a core gene, and the gene product is a large insoluble, evenly dispersed nuclear phosphoprotein that co-fractionates with the nuclear matrix and is required for optimal processing and packaging of viral DNA into pre-formed capsid [4, 8–10]. The UL31 protein also has been found to depend on physical interaction with UL34 protein to form a complex colocalized at the nuclear rim of infected cells, and become incorporated into virions during envelopment at the inner nuclear membrane [6, 8, 9]. With many similarities and a few differences, accumulating evidence indicates that the UL31 protein and its homology play similar roles in nuclear egress of *Alpha-*, *Beta-*, and *Grammherpesviruses* [11–14].

Duck viral enteritis (DVE), an acute and contagious disease, is highly lethal in all ages of birds from the order *Anseriformes* (ducks, geese, and swans) [15–17]. Since the first reported of DVE in domestic ducks in 1923 [18], more outbreaks were reported in the North America [19], Canada [20], France [21] and China [22] et al. DEV is the causative agent for DVE and is currently classified to the *Alpha-herpesvirinae* subfamily of the *Herpesviridae*, but has not been grouped into any genus yet [23]. DEV is composed of a linear, double-stranded DNA genome with 64.3% G + C content, higher than any other reported avian herpesvirus in the subfamily *Alphaherpesvirinae* [24].

To date, although the DEV genomic library was successfully constructed, only limited sequences of DEV were published [25]. Here, we report the identification, cloning and molecular characterization analysis of the DEV UL31 gene and its prokaryotic expression. These works may provide some insights for further research about the gene and a platform for further study on the diseases caused by DEV.

Materials and methods

Viruses and cells

DEV CHv strain was a high-virulence field strain isolated from China, obtained from Key Laboratory of Animal Disease and Human Health of Sichuan Province. Duck embryo fibroblasts (DEF) was cultured in MEM medium (Gibco-BRL) supplemented with 10% fetal bovine serum (FBS) (Gibco-BRL) at 37°C. For virus infection, MEM medium supplemented with 2–3% FBS was used.

Isolation of DEV UL31 gene

The coding region of DEV UL31 gene was amplified using one pair of primer. Forward primer: P1(+) 5′-GGATCCATGTCTGACTACGATACCACA-3; reverse primer: P2(-) 5′-AAGCTTTCAGCGAGGAGGAGGA-3′, containing the *BamH I* and *Hind III* restriction sites (underlined), respectively. PCR was carried out in a 25 μl reaction mixture containing 1.0 μl of each primer (20 pmol each), 1.0 μl DNA template(4 ng), 12.5 μl PrimeSTAR HS (Premix) DNA polymerase (0.625 Units) and 9.5 μl water (all reagents were purchased from TaKaRa).

Dot blot analysis

For dot blot analysis, one digoxigenin-labeled DNA probe was designed as follows: Dig-5′-CAAGGATCAAAGTTGAGCGATCTATGCGATTCAAACGAGG-3′.The sample mixture (200 ng/μl sample DNA, 10 mM EDTA, 0.4 N NaOH) was spotted two times onto nitrocellulose membranes, which prewetted in 20 × SSC(3 M NaCl, 0.3 M sodium citrate, PH 7.0). Nucleic acid was fixed to the membrane by baking it in an oven at 80°C for 2 h. The membrane was pre-hybridized at 42°C for 3 h. Hybridization was carried out overnight at the same temperature with Digoxigenin-labeled DNA probe and detected by 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) [26].

To test species specificity, the nucleic acid of Duck hepatitis virus-1 (DHV-1), Infectious laryngotracheitis virus (ILTV), Porcine Parvovirus (PPv), Marek's disease virus (MDV), Muscovy duck parvovirus (MDPV), Pseudorabies virus (Prv) and Goose parvovirus (Gpv) were extracted and analyzed by the dot blot assay. Non-infected DEF served as the blank control.

Bioinformatics analysis

Firstly, amino acid sequence comparisons and homology searches were performed using the computer-assisted NCBI BLASTP [27]. Predicted signal peptide sequences were determined using Signal IP 3.0 [28]. Multiple

sequence alignment and phylogenetic analysis were obtained on the bases of 24 UL31 gene sequences (Table 1) by using CLUSTAL-V [29, 30]. The phylogenetic tree was showed by TreeView [31]. Finally, the B-cell epitopes prediction of DEV UL31 protein was performed by DNAstar6.0 programs [32, 33].

Construction of bacterial expression vector

The amplified UL31 gene was digested with two restriction enzymes (*BamH I* and *Hind III*) and then cloned into *BamH I* + *Hind III* digested pET 32a(+) (Novagen), generating the pET-UL31. Then, the recombinant DNA was transformed into competent *E. coli* DH5α cells, which were used for propagation of plasmid constructs. The transformants were selected in Luria-Bertani (LB) plates containing 100 ug/ml ampicillin. After mini-scale isolation of plasmid DNA using plasmid DNA QIAprep Spin Mini Kit (Qiagen), the recombinant pET-UL31 was confirmed by restriction analysis and by sequencing. DNA sequencing were performed in Dalian TaKaRa Co. Ltd [34].

Prokaryotic expression and antibody preparation

Once the sequence of the pET-UL31 was verified by sequencing, the construct was transformed into *E. coli* BL21(DE3) host cells. Transformants were grown on LB plates with 100 ug/ml ampicillin at 37°C for 24 h. A single colony from the culture was grown in LB medium with ampicillin to an optical density of 0.6. Protein expression was induced by the addition of IPTG (final concentration 0.8 mM) with further growth at 37°C for 3 h. The recombinant His-tagged proteins were purified by nickel affinity chromatography according to the manufacturer's protocol (Bio-Rad). Antibodies against the purified His-tagged UL31 were raised in rabbits [34].

Western blotting

To identify the UL31 protein, the recombinant fusion protein was resolved on 12% (w/v) SDS-PAGE and electro-blotted onto polyvinylidene difluoride PVDF membrane using wet transfer method according to a standard procedure [34, 35]. Membrane was subsequently incubated with anti-UL31

Table 1 Abbreviations and accession no. of 25 UL31 protein from different species

Species	Virus name (Abbreviation)	GenBank accession no.
Alphaherpesvirinae	Duck entritis virus (DEV)	EF643559
	Human herpesvirus 3 (HHV-3)	P09283
	Marek's disease virus type 1 (MDV-1)	AAF66766
	Cercopithecine herpesvirus 9 (CeHV-9)	NP_077442
	Meleagrid herpesvirus 1 (MeHV-1)	NP_073325
	Equid herpesvirus 1 (EHV-1)	YP_053074
	Bovine herpesvirus 5 (BoHV-5)	NP_954916
	Marek's disease virus type 2 (MDV-2)	NP_066863
	Infection laryngotracheitis virus (ILTV)	NC_006623
	Cercopithecine herpesvirus 16 (CeHV-16)	YP_443878
	Human herpesvirus 2 (HHV-2)	NP_044501
	Bovine herpesvirus 1 (BoHV-1)	NP_045327
	Cercopithecine herpesvirus 1 (CeHV-1)	NP_851891
	Suid herpesvirus 1 (SuHV-1)	AAP82011
	Human herpesvirus 1 (HHV-1)	NP_044633
Betaherpesvirinae	Human herpesvirus 7 (HHV-7)	YP_073777
	Porcine cytomegalovirus (PCMV)	AAF80112
	Murine cytomegalovirus (MCMV)	CAP08102
	Human herpesvirus 6 (HHV-6)	NP_050218
	Human herpesvirus 5 (HHV-5)	AAF91256
Gammaherpesvirinae	Human herpesvirus 8 (HHV-8)	YP_001129427
	Bovine herpesvirus 4 (BoHV-4)	NP_076562
	Saimiriine herpesvirus 2 (SaHV-2)	CAC84367
	Equid herpesvirus 2 (EHV-2)	NP_042668
	Epstein-Barr virus (EBV-1)	YP_401647

antibodies (1:200 dilutions) for overnight at 4°C. After three washes with PBS-T (0.2% Tween 20 in PBS, PH 7.4), the membranes were incubated with horseradish peroxidase-linked goat anti-rabbit immunoglobulin G (IgG) (Amersham) and specific band was detected using DAB Enhancer Reagent Solution (Beijing Zhong Shan-Golden Bridge Biological Technology Co., Ltd., China).

Indirect immunofluorescence

Indirect immunofluorescence tests of the DEV UL31 subcellular localization was performed according to previously described [34]. DEF cells were grown on coverslips and were mock or infected with DEV. At 36 h postinfection, the cells were fixed with acetone for 20 min at −20°C, and subsequently incubated with rabbit anti-UL31 serum (diluted 1:200) for overnight at 4°C. Then, the cells were reacted with FITC-conjugated goat anti-rabbit immunoglobulin (diluted 1:100) for 1 h at 37°C. The cell nuclei were visualized by DAPI counter-staining (5 μg/ml, Beyotime). Fluorescent images were viewed and recorded with the Bio-Rad MRC 1024 imaging system.

Result

Identification and molecular characteristics of DEV UL31 gene

To isolate the UL31 gene, PCR was carried out on total DNA from DEV. A predicted 933 bp product was amplified. After cloning and sequencing, the identical nucleotide sequence was obtained and analyzed by BLASTN. The results indicated that the sequence displayed homologies of 58.781, 59.140 and 55.063% to the UL31 gene from Marek's disease virus type 1 (MDV-1), Meleagrid herpesvirus 1 (MeHV-1) and Bovine herpesvirus 1 (BoHV-1), respectively, and suggested that the DEV UL31 gene has been amplified.

The isolated DEV UL31 gene consisted of 933 nucleotides encoding 310 amino acids (Mr35.76 kDa), which had a typical open reading frame (ORF) structures, including core promoters, TATA motifs and polyadenylation sites. No signal peptide or any transmembrane helix was present and the subcellular localization was nucleolus. The motif structure prediction shown it has six casein kinase II, three cAMP-dependent protein kinase, four protein kinase C phosphorylation sites and one potential N-linked myristoylation site at 142–147 a (Fig. 1). In addition, the UL31 protein was highly conserved among the *Alphaherpesviruses* and four conserved regions were predicted by multiple alignments on the 11 reference strains (Fig. 2).

Dot blot analysis

Amount of nucleic acid extracted from different viruses was approximately 1.0 ug. To avoid misinterpretation of the results caused by the detection of false positive signals, we blotted two dots for each nucleic acid sample. The result show in Fig. 3, the DEV DNA gave a very distinct color signal, whereas the non-infection DEF and others showed no signals. It implied that the UL31 gene existed only in DEV.

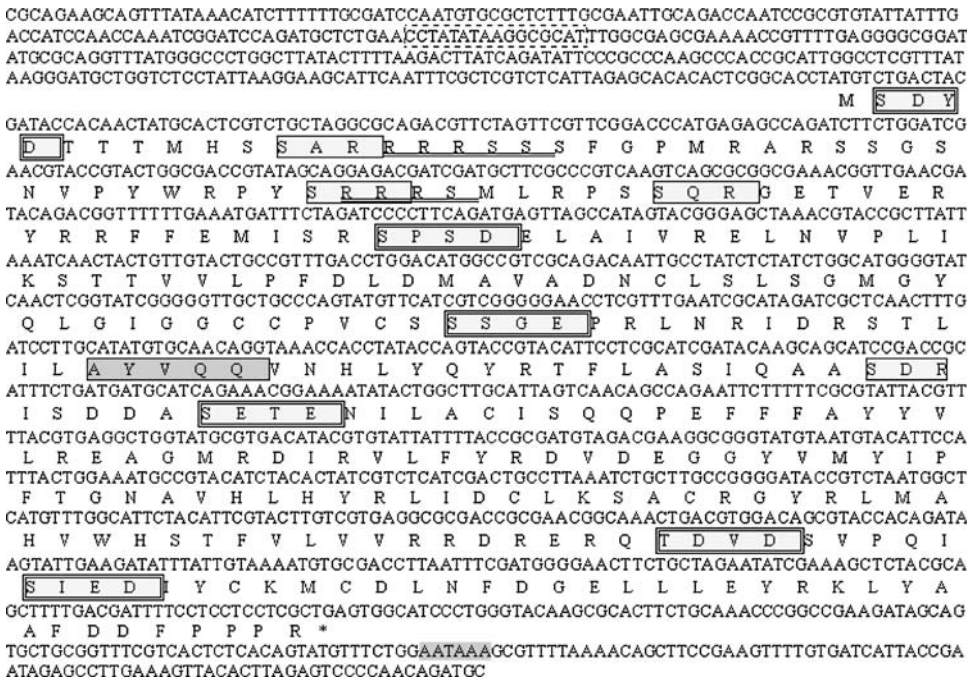
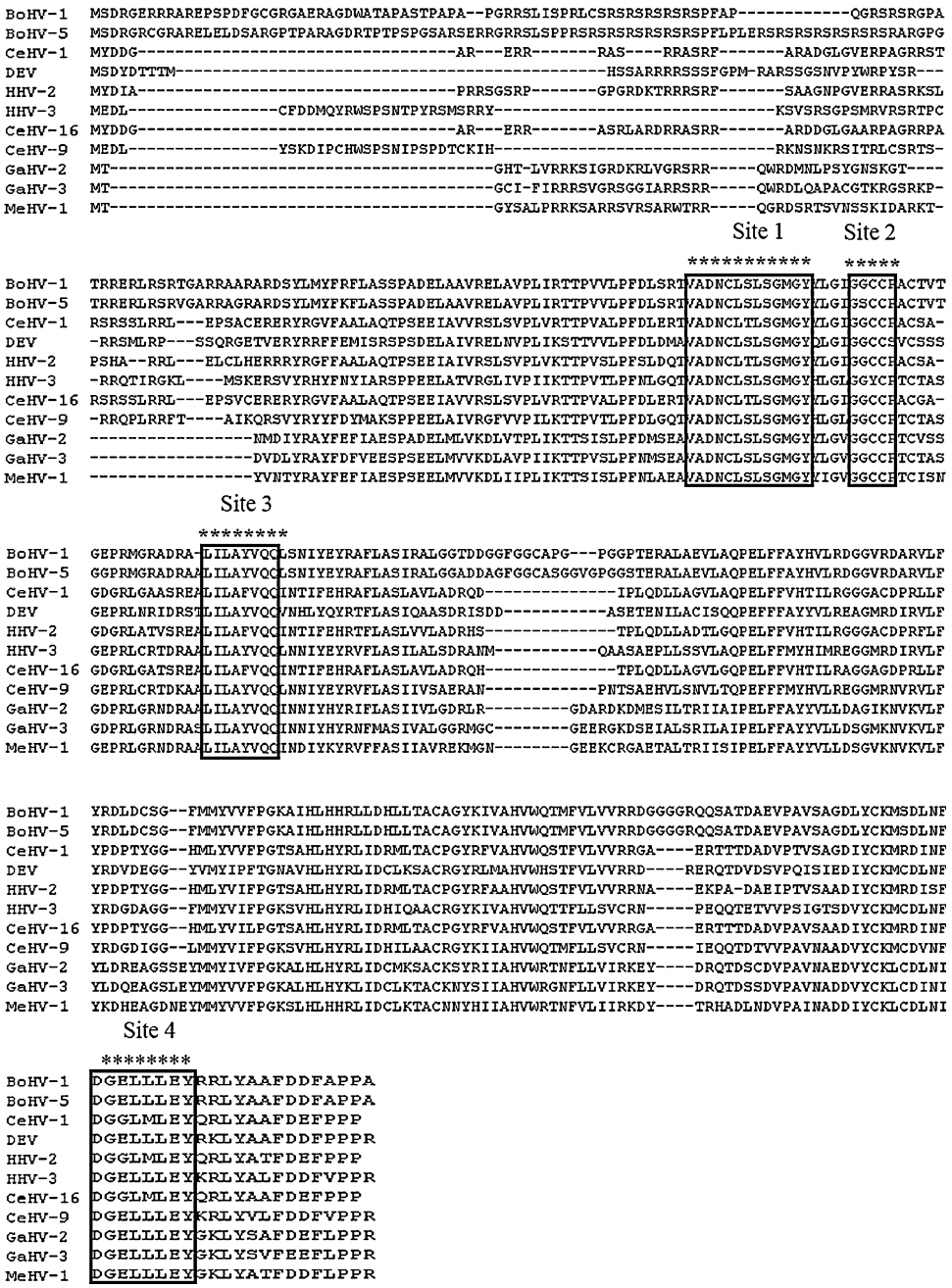


Fig. 1 Nucleotide sequence of DEV UL31 gene and its deduced amino acid sequence. In the nucleotide sequence, *the boxed letters* in *dotted line* indicate TATA box; a putative poly(A) signal (AATAAA) are identified by the *letters* highlighted in *gray*; the *asterisk* indicates the stop codon; In the deduced protein sequence, *the boxed letters* indicate a putative protein kinase C phosphorylation sites; the *double-boxed letters* indicate putative casein kinase II phosphorylation sites; the *double-lined letters* indicate cAMP-dependent protein kinase sites; the *boxed letter* highlighted in *gray* indicate the putative N-myristoylation site

Fig. 2 Multiple sequence alignment of the deduced amino acid sequences of DEV UL31 and selected members of the UL31 family. Four highly conserved sites are named as 'site 1 to 4' above the alignment and boxed



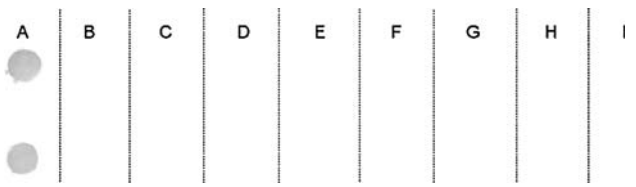


Fig. 3 Specificity of DIG-labeled oligonucleotide probe which was complementary to the 5'end (nt 384–424) of UL31 gene in detecting purified DNA. Samples were analyzed in duplicate in the same lane. Nucleic acid extracts were prepared from DEV(A), DHV(B), ILTV(C), PPv (D), MDV(E), MDPV(F), Prv(G), Gpv(H), and DEF(I) and subjected to dot blot analysis

Evolutionary relationship of the putative proteins in the herpesviruses

Phylogenetic analysis of DEV and other herpesviruses were performed based on the amino acid sequences of UL31 proteins. Phylogenetic analysis clustered herpesviruses into three major groups (*alpha*-, *beta*-, and *gammaherpesvirinae*) irrespective of the gene used for analysis (Fig. 4). Furthermore, the results also showed that DEV UL31 had a close evolutionary relationship with some fowl herpesviruses such as MeHV-1, MDV-1 and MDV-2, which were

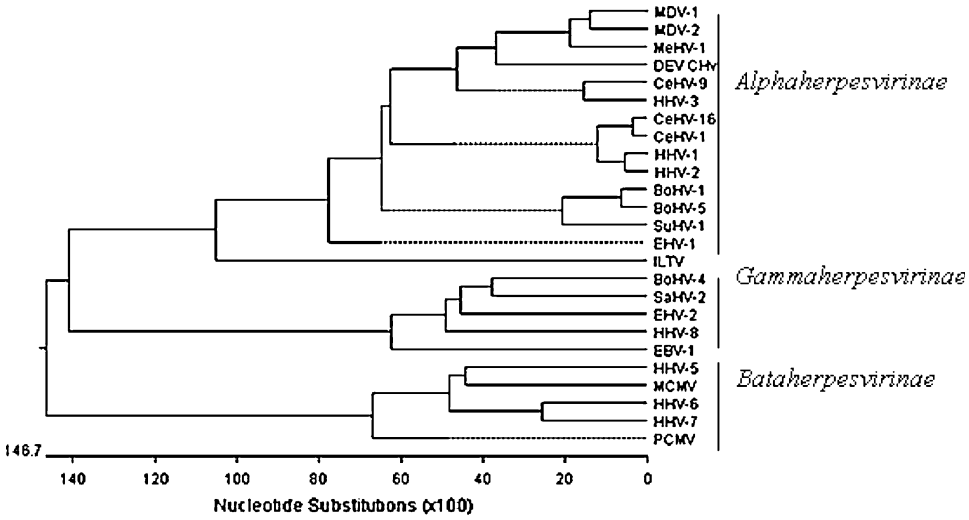


Fig. 4 Phylogenetic tree of the UL31 gene based on 25 UL31 amino acid sequences from different species (Table 1) by using the MEGALIGN program in LASERGENE (DNAStar) with Clustal V Method multiple alignment and Sequence distance indicated by the scale was calculated using weight matrix PAM250

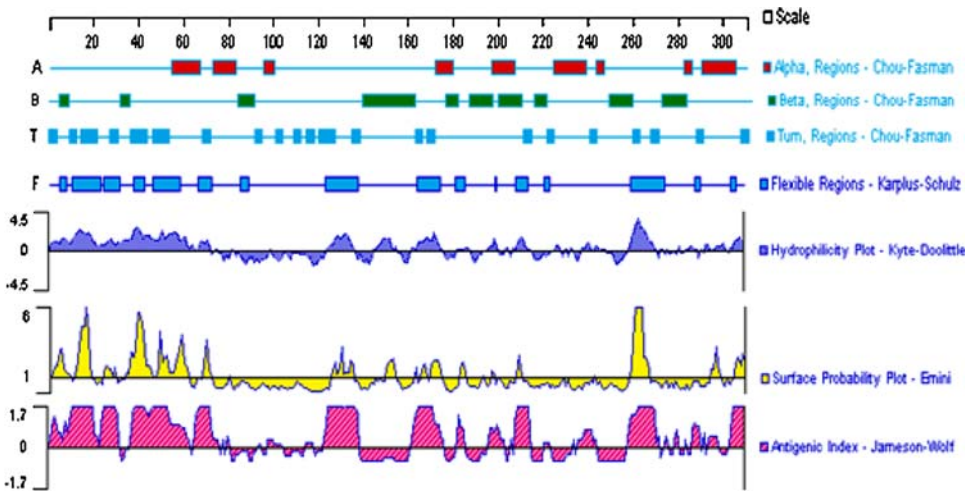


Fig. 5 Antigenic analysis of the deduced amino acid sequence of DEV UL31 was carried out by PROTEAN software of DNAStar based on it hydrophilicity, flexibility, antigenic index, surface probability and β-turn in the secondary structure by the determination of its primary structure

clustered within a monophyletic clade, indicating that the DEV should be placed into a single cluster within the subfamily *Alphaherpesvirinae*.

Antigen prediction

The deduced amino acid sequence of DEV UL31 was analyzed for the prediction of potential B-cell epitopes determinants by using the PROTEAN software of DNAStar (Fig. 5.). This analysis predicted several potential B-cell epitopes sites which located in or adjacent to amino acids 1–65, 123–140, 162–178, 207–217, 258–274, and 304–310.

Construction of bacterial expression vector

The result of PCR reaction was shown in Fig. 6a, which was similar to the size of DEV UL31 gene as expected. Then, the UL31 gene was cloned into pET 32a(+) to construct the recombinant prokaryotic expression vector pET-UL31, which was confirmed by restriction enzyme analysis (Fig. 6b) and by DNA sequencing.

Expression and identification of the DEV UL31

A fusion protein of expected molecular mass 55 kDa, which consisted of 20 kDa tag protein and 35 kDa UL31 proteins, was observed by analysis of total cell protein on a 12% SDS-polyacrylamide gel. A time course expression study was performed and aliquots taken at 0, 1, 2, 3 and 4 h after IPTG induction (Fig. 7a).

Western blotting was performed to identify the UL31 product. As shown in Fig. 7b, the UL31 antiserum reacted with a band in the IPTG induced cell lysates with an apparent molecular mass of 55 kDa (lane 7). However, The UL31 antiserum did not react with any proteins present in uninduced cell lysates (lane 6), indicating that the 55 kDa protein is the product of the DEV UL31 gene.

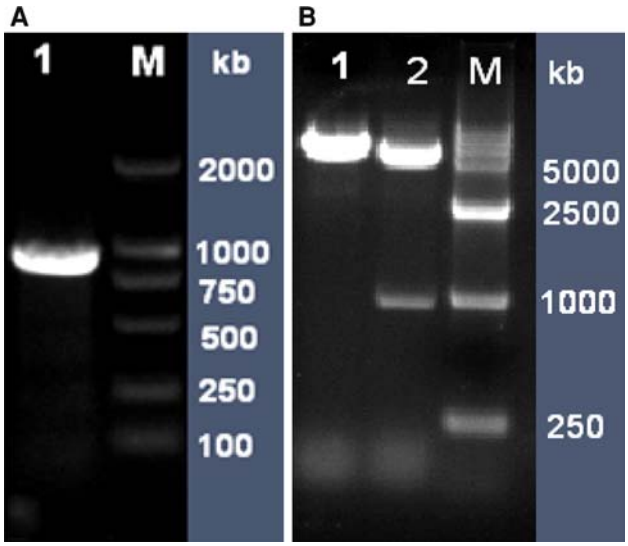


Fig. 6 DEV UL31 gene encoding DNA sequence was amplified and cloned into pET 32a(+) prokaryotic expression vector as described in materials and methods. **a** Result of PCR amplification for DEV UL31 gene. M: DNA marker; *Lane* 1, amplified product of DEV UL31. **b** The construct was digested with two restriction enzymes. M, DNA marker; *Lane* 1, *Bam*HI generating one restriction fragment; *Lane* 2, *Bam*HI and *Hind*III generating two restriction fragments

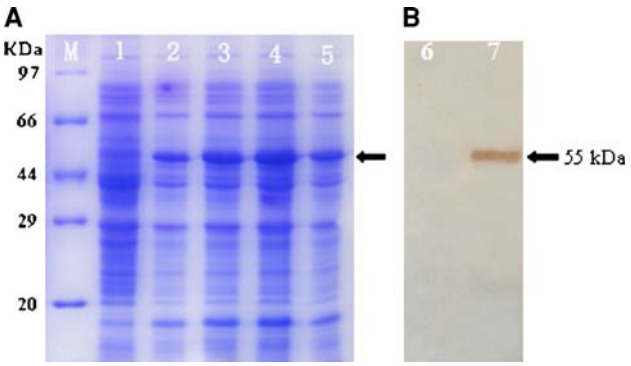


Fig. 7 **a** The SDS-PAGE analysis of recombinant UL31 produced by *E.coil*. *Lane* 1: uninduced; *lanes* 2–5: after IPTG induced (1–4 h). M: molecular markers. **b** Identifiction of the UL31 protein by Western blot. *Lane* 6: uninduced; *lanes* 7: IPTG induced. The *arrows* indicate the fusion protein of pET-UL31

Sub-cellular localization of UL31 protein

Intracellular localization of the UL31 protein in DEV-infected DEF cells was analyzed by immunofluorescence assay. The UL31 gene product of DEV localized in very fine punctate forms dispersed throughout the nucleus of infected cells (Fig. 8, F2). In contrast, no significant fluorescence was observed in mock-infected cells (Fig. 8, F1).

Discussion

Although there has been little information about the molecular characteristics of DEV since the disease was report in 1923, the DEV genomic library was successfully constructed in our laboratory [25]. In this study, we reported the cloning and characterization of the UL31 gene from DEV CHv strain. Sequence analysis indicated that the protein, encoding by UL31 gene, have no transmembrane helix structure and signal peptide sequence, which provide some information for its expression. In addition, the location and phosphorylation sites of the UL31 protein from the DEV CHv have similar to the UL31 protein of HSV-1 [4]. It suggests that the UL31 protein may serve the same role as homologous in HSV-1.

Dot-blot hybridization has been applied widely since it was developed in 1983 [36]. Here, we used this method to demonstrate the UL31 gene existing only in DEV. The genomic DNA isolated from DEV showed only signals in the dot blot analysis, although the same amounts of DNA were blotted as in the analysis of other viruses DNA (Fig. 3). It implied clearly that the UL31 gene existed only in DEV. Subsequently, DEV UL31 gene had been expressed successfully in E. coil strain BL21(DE3), and polyclonal antibody raised against the recombinant UL31 from rabbit was prepared. Using this antibody, we found that the UL31 protein was approximate 55 kDa and localized in very fine punctate forms dispersed throughout the nucleus of infected cells. The homologous PRV and HSV-2 proteins exhibit similar nuclear locations, correlating with important functions during egress of viral nucleocapsids from the nucleus [37–40].

The origin of herpesviruses is a topic of great interest, and currently of many unverifiable speculations. The similarities in the sequence structure of herpesviruses suggest that they have arisen from a common ancestor [30, 41]. To clarify the taxonomic position of DEV, phylogenetic analysis was performed on the putative proteins of UL31 (Fig. 4). Firstly, sequence comparison of DEV and 22 other herpesvirus (Table 1) UL31 genes showed that the DEV CHv strain was more similar to *alpha-herpervirus* than *beta-herpervirus* or *gamma-herpervirus*. Consequently, we focused on the members of subfamily *Alphaherpesvirinae* in order to limit DEV to a genus. The DEV CHv formed a solitary branch and was closed to the *Mardivirus* (MDV-1, MDV-2 and MeHV-1). Previously studies indicated that the UL31 gene conserved among the whole family *Herpesviridae*, especially in *Alpha-herpesvirinae* [4, 42–44]. Therefore, the phylogenetic tree of UL31 may be used as a basis for DEV classification.

As more information becomes available on protein antigens, it should be possible to use this information to predict the locations of antigenic determinants before any

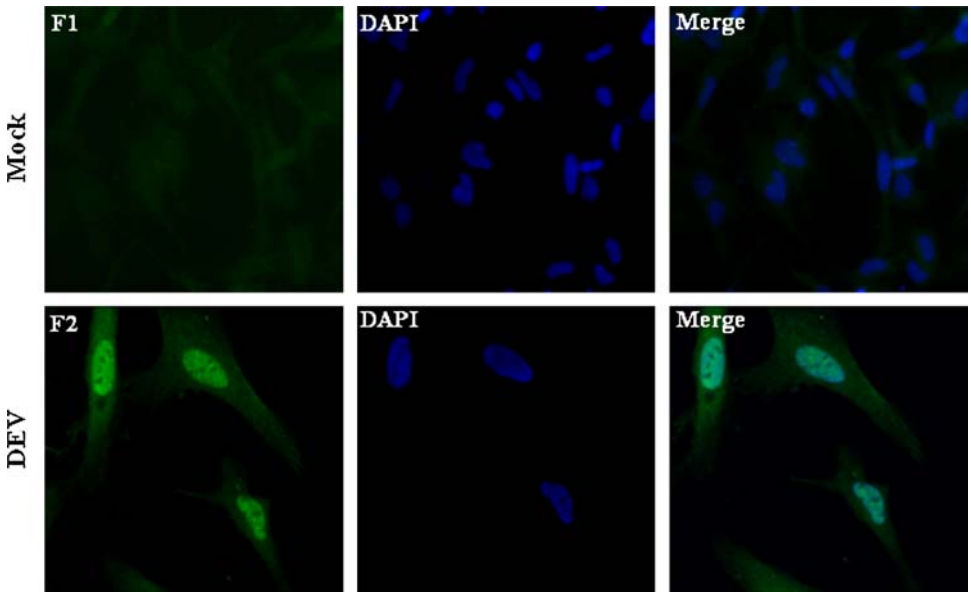


Fig. 8 Localization of the UL31 protein in DEV-infected cells. Mock-infected (F1) and DEV-infected (F2) cells were fixed with acetone at 36 postinfection and incubated with UL31 antiserum, and were then stained with FITC-conjugated goat anti-rabbit immunoglobulin and DAPI as described in Materials and Methods. (Images were acquired by using 40× objective)

immunological testing has been carried out. However, the elucidation of protein antigenic structures is presently a difficult, uncertain, and time-consuming task. Earlier methods are based on the assumption that antigenic regions are primarily hydrophilic regions at the surface of the protein molecule [45, 46]. There have some inaccuracy and limitation about it. To improve the accuracy, the B-cell epitopes of DEV UL31 have been predicted using DNA-Star PROTEAN programs based on it hydrophilicity, flexibility, antigenic index, surface probability and β-turn in the secondary structure by the determination of its primary structure. The results suggested that the improved knowledge of the antigenic and structural properties of DEV UL31 protein resulting from this study might yield methods for developing new antibodies and immunoassays for use in the clinical diagnosis of DEV.

In conclusion, in this work we present the cloning and characterization of DEV UL31 gene. Elucidating the relationship between molecular characterization and genetic evolution of DEV UL31 gene will contribute to us understanding of this virus at the molecular level and also enrich the database of herpesvirus.

Acknowledgments The research was supported by the National Natural Science Foundation of China (30771598), Changjiang Scholars and Innovative Research Team in University(IRT0848), the earmarked fund for Modern Agro-industry Technology Research System (2009–2013), New Century Excellent Talents Program in University (NCET-06-0818), the Cultivation Fund of the Key Scientific and Technical Innovation Project, Ministry of Education of China (706050), the Cultivation Fund of the Key Scientific and Technical Innovation Project, department of Education of Sichuan Province (07ZZ028), Sichuan Province Outstanding Youths Fund (07ZQ026-132), and Sichuan Province Basic Research Program (07JY029-016/07JY029-017).

References

1. Roizman B, Pellett PE (2001) The family herpesviridae: a brief introduction. Field Virol 2:2381–2397
2. Park R, Baines JD (2006) Herpes simplex virus type 1 infection induces activation and recruitment of protein kinase C to the nuclear membrane and increased phosphorylation of lamin B. J Virol 80:494–504. doi:10.1128/JVI.80.1.494-504.2006
3. Simpson-Holley M, Colgrove RC, Nalepa G et al (2005) Identification and functional evaluation of cellular and viral factors involved in the alteration of nuclear architecture during herpes simplex virus 1 infection. J Virol 79:12840–12851. doi:10.1128/JVI.79.20.12840-12851.2005
4. Chang PC, Hsieh ML, Shien JH et al (2001) Complete nucleotide sequence of avian paramyxovirus type 6 isolated from ducks. J Gen Virol 82:2157–2168
5. Dal Monte P, Pignatelli S, Zini N (2002) Analysis of intracellular and intraviral localization of the human cytomegalovirus UL53 protein. J Gen Virol 83:1005–1012
6. Gonnella R, Farina A, Santarelli R et al (2005) Characterization and intracellular localization of the epstein-barr virus protein BFLF2: interactions with BFRF1 and with the nuclear lamina. J Virol 79:3713–3727. doi:10.1128/JVI.79.6.3713-3727.2005
7. Lake CM, Hutt-Fletcher LM (2004) The epstein-barr virus BFRF1 and BFLF2 proteins interact and coexpression alters their cellular localization. Virology 320:99–106. doi:10.1016/j.virol.2003.11.018
8. Simpson-Holley M, Baines J, Roller R et al (2004) Herpes simplex virus 1 U(L)31 and U(L)34 gene products promote the late maturation of viral replication compartments to the nuclear periphery. J Virol 78:5591–5600. doi:10.1128/JVI.78.11.5591-5600.2004
9. Reynolds AE, Liang L, Baines JD (2004) Conformational changes in the nuclear lamina induced by herpes simplex virus type 1 require genes U(L)31 and U(L)34. J Virol 78:5564–5575. doi:10.1128/JVI.78.11.5564-5575.2004
10. Zhu HY, Yamada H, Jiang YM et al (1999) Intracellular localization of the UL31 protein of herpes simplex virus type 2. Arch Virol 144:1923–1935. doi:10.1007/s007050050715

11. Klupp BG, Granzow H, Fuchs W et al (2007) Vesicle formation from the nuclear membrane is induced by coexpression of two conserved herpesvirus proteins. Proc Natl Acad Sci USA 104:7241–7246. doi:10.1073/pnas.0701757104
12. Helferich D, Veits J, Mettenleiter TC et al (2007) Identification of transcripts and protein products of the UL31, UL37, UL46, UL47, UL48, UL49 and US4 gene homologues of avian infectious laryngotracheitis virus. J Gen Virol 88:719–731. doi:10.1099/vir.0.82532-0
13. Mou F, Forest T, Baines JD (2007) US3 of herpes simplex virus type 1 encodes a promiscuous protein kinase that phosphorylates and alters localization of lamin A/C in infected cells. J Virol 81:6459–6470. doi:10.1128/JVI.00380-07
14. Schnee M, Ruzsics Z, Bubeck A et al (2006) Common and specific properties of herpesvirus UL34/UL31 protein family members revealed by protein complementation assay. J Virol 80:11658–11666. doi:10.1128/JVI.01662-06
15. Davison S, Converse KA, Hamir AN et al (1993) Duck viral enteritis in Muscovy ducks in Pennsylvania. Avian Dis 37:1142–1146. doi:10.2307/1591927
16. Montali RJ, Bush M, Greenwell GA (1976) An epornitic of duck viral enteritis in a zoological park. J Am Vet Med Assoc 169:954–958
17. Proctor SJ (1976) Pathogenesis of duck plague in the bursa of fabricius, thymus, and spleen. Am J Vet Res 37:427–431
18. Baudet AE (1923) Mortality in ducks in the Netherlands caused by a filterable virus: fowl plague. Tijdschr Diergeneeskd 55:455–459
19. Leibovitz L, Hwang J (1968) Duck plague on the American continent. Avian Dis 12:361–378. doi:10.2307/1588237
20. Jansen J, Kunst H (1964) The reported incidence of duck plague in Europe and Asia. Tijdschr Diergeneeskd 89:765–769
21. Lucam F (1949) La peste aviaire en France. Repoc 14th Int Vet Congr 2:380–382
22. Saif YM, Barnes HJ, Glisson JR et al (2003) Diseases of poultry, 11th edn. Lowa State University Press, Ames
23. Fauquet CM, Mayo MA, Maniloff J (2005) Virus taxonomy: eighth report of the international committee on taxonomy of viruses. Elsevier Academic Press, California
24. Gardner R, Wilkerson J, Johnson JC (1993) Molecular characterization of the DNA of Anatid herpesvirus 1. Intervirology 36:99–112
25. Cheng AC, Wang MS, Wen M (2006) Construction of duck enteritis virus gene libraries and discovery, cloning and identification of viral nucleocapsid protein gene. High Technol Lett 16:948–953
26. Oraveerakul K, Choi CS, Molitor TW (1990) Detection of porcine parvovirus using nonradioactive nucleic acid hybridization. J Vet Diagn Invest 2:85–91
27. Altschul SF, Madden TL, Schaffer AA et al (1997) A new generation of protein database search programs. Nucleic Acids Res 25:3389–3402. doi:10.1093/nar/25.17.3389
28. Bendtsen JD, Nielsen H, von Heijne G et al (2004) Improved prediction of signal peptides: signalP 3.0. J Mol Biol 340:783–795. doi:10.1016/j.jmb.2004.05.028
29. Higgins DG, Bleasby AJ, Fuchs R (2002) CLUSTAL V: improved software for multiple sequence alignment. Oxford University Press, Oxford
30. Higgins DG (1994) CLUSTAL V: multiple alignment of DNA and protein sequences. Methods Mol Biol 25:307–318. doi:10.1385/0-89603-276-0:307
31. Page RDM (1996) Tree view: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358
32. Burland TG (2000) DNASTAR's Lasergene sequence analysis software. Methods Mol Biol 132:71–91
33. Van Regenmortel MH (1992) Protein antigenicity. Mol Biol Rep 16:133–138. doi:10.1007/BF00464700
34. Xie W, Cheng AC, Wang MS, Chang H et al (2009) Expression and characterization of the UL31 protein from Duck enteritis virus. Virol J 6:19. doi:10.1186/1743-422x-6-9
35. Towbin H, Staehelin T, Gordin J (1976) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354. doi:10.1073/pnas.76.9.4350
36. Leary JJ, Brigati DJ, Ward DC (1983) Rapid and sensitive colorimetric method for visualizing biotin-labeled DNA probes hybridized to DNA or RNA immobilized on nitrocellulose: bioblots. Proc Natl Acad Sci USA 80:4045–4049. doi:10.1073/pnas.80.13.4045
37. Chang YE, Van Sant C, Krug PW (1997) The null mutant of the U(L)31 gene of herpes simplex virus 1: construction and phenotype in infected cells. J Virol 11:8307–8315
38. Klupp BG, Granzow H, Fuchs W (2004) Pseudorabies virus UL3 gene codes for a nuclear protein which is dispensable for viral replication. J Virol 1:464–4672
39. Fuchs W, Klupp BG, Mettenleiter TC (2002) The interacting UL31 and UL34 gene products of Pseudorabies virus are involved in egress from the host-cell nucleus and represent components of primary enveloped but not mature virions. Virology 76:364–378. doi:10.1128/JVI.76.1.364-378.2002
40. Mark L, Zsolt R, Ulrich HK (2006) Functional domains of murine cytomegalovirus nuclear egress protein M53/p38. J Virol 80:73–84. doi:10.1128/JVI.80.1.73-84.2006
41. McGeoch DJ, Cook S (1994) Molecular phylogeny of the alphaherpesvirinae subfamily and a proposed evolutionary timescal. J Mol Biol 238:9–22. doi:10.1006/jmbi.1994.1264
42. Bjerke SL, Roller RJ (2006) Roles for herpes simplex virus type 1 UL34 and US3 proteins in disrupting the nuclear lamina during herpes simplex virus type 1 egress. Virology 347:261–276. doi:10.1016/j.virol.2005.11.053
43. Reynolds AE, Ryckman BJ, Baines JD (2001) U(L)31 and U(L)34 proteins of herpes simplex virus type 1 form a complex that accumulates at the nuclear rim and is required for envelopment of nucleocapsids. J Virol 75:8803–8817. doi:10.1128/JVI.75.18.8803-8817.2001
44. Yamauchi Y, Shiba C, Goshima F, Nawa A (2001) Herpes simplex virus type 2 UL34 protein requires UL31 protein for its relocation to the internal nuclear membrane in transfected cells. J Gen Virol 82:1423–1428
45. Hopp TP, Woods KR (1981) Prediction of protein antigenic determinants from amino acid sequences. Proc Natl Acad Sci USA 78:3824–3828. doi:10.1073/pnas.78.6.3824
46. Welling GW, Weijer WJ (1985) Prediction of sequential antigenic regions in proteins. FEBS Lett 188:215–218. doi:10.1016/0014-5793(85)80374-4