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Prokaryotic expression of the truncated duck enteritis virus UL27 gene and characteristics of UL27 gene and its truncated product

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Summary. – Duck enteritis virus (DEV) is an acute and contagious herpesvirus infection in waterfowls, which leads to heavy economic losses in the commercial duck industry due to its high mortality rate and decreasing duck egg production. In order to understand this virus better, truncated UL27 gene (tUL27) was used to construct pET-28a-tUL27 recombinant plasmid. The tUL27 gene product, truncated gB protein (tgB), was used to prepare rabbit anti-tgB protein polyclonal antibody. The polyclonal antibody was used for characteristics of DEV glycoprotein B (gB) intracellular localization. Protein gB was firstly detected in nuclear membrane of the infected DEF, and later during the infection it appeared as the punctiform fluorescence in cytoplasm. We also proved that DEV gB is a late protein. Localization of DEV gB in nuclear membrane, suggested a possible role in virus assembly, while appearance in cytoplasm suggests its role in viral egress. All these data provide a basal and useful knowledge about the functions of DEV UL27 gene and its product gB.

Keywords: duck enteritis virus; UL27 gene; gB protein

Introduction

Duck enteritis virus (DEV), also known as duck plague, belongs to the family *Herpesviridae*. It is an acute and contagious herpesvirus infection in waterfowls such as ducks, geese, and swans, which leads to high morbidity and mortality (Sandhu and Metwally, 2008; Montali *et al*., 1979; Davison *et al*., 1993). DEV infection causes heavy economic losses to the commercial duck industry due to its high mortality rate and decreasing egg production (Sandhu and Metwally, 2008).

Only little is known about this disease, despite its seriousness and its early discovery in 1923. Recently, with the development of molecular biology, more information about the DEV genes and proteins, such as gC (Lian *et al*., 2010; Zou *et al*., 2010), gE (Chang *et al*., 2009), UL38 (Xiang *et al*., 2010), gK (Zhang *et al*., 2011), and TK gene (Wen *et al*., 2010) are being reported. From our knowledge, little information about the DEV UL27 and its product gB has been reported. In this article, we focused on the gB glycoprotein localized in the virion envelope. Glycoproteins are key determinants in membrane fusion during the virus life cycle. The DEV possesses at least twelve glycoproteins, for instance gB, gC, gD, gE, gG, gH, gI, gK (Wang *et al*., 2011), which are expressed in infected cells. These glycoproteins have several important roles in virus entry via fusion of the viral envelope with cellular membranes, virus-induced cell fusion, cell-to-cell spread, and egress of infectious virion particles (Mettenleiter, 2000).

Glycoproteins are the major antigens to the immune response. They are important for the antibody response as they are the major targets for both major histocompatibility complex class I and class II restricted T lymphocytes (Glorioso

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Abbreviations: DEF = duck embryo fibroblast; DEV = duck enteritis virus; gB = glycoprotein B; p.i. = post infection; tUL27 = truncated UL27 gene; tgB = truncated gB protein

et al., 1985; Carter *et al*., 1981; Schrier *et al*., 1983; Lawman *et al*., 1980). Therefore, glycoproteins have an important biological activity in virus replication. Since the prokaryotic expression of whole UL27 gene was not successful, we have constructed recombinant plasmid with truncated UL27 gene. Protein tgB was used in the characteristics of DEV gB functions and provided more data to elucidate the pathogenic mechanism of DEV.

Materials and Methods

pMD18-tUL27 plasmid construction. Primers for DEV truncated UL27 gene (GenBank Acc. No. EF608147 of whole UL27 gene) were designed by Primer5.0 software. The forward primer (P1): 5′-GAAT TCGAGGACAAATTCTATGTCTGCC-3′ and the reverse primer (P2): 5′-AAGCTTCCTTATTAGACCGTCAGGCCCT-3′ contained the restriction sites for *Eco*RI and *Hind*III (underlined) and gained 1200 bp product. DEV DNA was extracted from the duck embryo fibroblasts (DEF) infected with DEV (Cheng *et al*., 2006). For the 50 μl PCR reaction we used 25 μl of PrimeSTAR^{TM}HS (Premix) DNA polymerase (TaKaRa, Japan), 0.5 μl of DEV DNA, and 1.0 μl of each 20 pmol primer (TaKaRa). Whole PCR reaction was subjected to agarose gel electrophoresis and analyzed by gel imaging system. The amplified product of tUL27 was excised and purified by TIANgel Midi Purification Kit (TianGen, Germany) according to the manufacturer's instructions. The purified product was cloned into pMD18-T vector (TaKaRa), followed by transformation into *Escherichia coli* DH5α competent cells. The recombinant clone plasmid pMD18-tUL27 was verified by *Eco*RI and *Hind*III digestion and sequencing (TaKaRa).

pET-28a-tUL27 plasmid construction. Construction of the recombinant expression plasmid pET-28a-tUL27 was done according to the previous article (Zhang *et al*., 2010a). Recombinant plasmid pMD18-tUL27 and pET-28a (+) (Novagen, Germany) vector were digested by *Eco*RI and *Hind*III. After gel extraction and purification, tUL27 was inserted into pET-28a (+) vector. The recombinat pET-28a-tUL27 plasmids were verified by the colony PCR, digestion by *Eco*RI and *Hind*III and sequencing.

Protein tgB expression. Single *E. coli* BL21 bacterial colony transformed with pET-28a-tUL27 plasmid was cultured in 5 ml of LB medium with kanamycine (100 μg/ml) for 2 hrs at 37°C. When the A_{600} reached approximately 0.5, it was diluted in 1:100 in new LB medium with kanamycine and induced by IPTG with final concentration 0.4 mmol/l. As negative controls, we used induced and uninduced *E. coli* BL21 transformed by pET-28a (+) vector and uninduced *E. coli* BL21 transformed by pET-28a-tUL27 plasmid. Two ml of samples were collected, centrifuged and the pellets were dissolved in 80 μl of PBS and 20 μl of 5×SDS buffer, boiled for 10 min and centrifuged at 10000 rpm for 10 min. Samples were separated by SDS-PAGE (Xie *et al*., 2010). Gel was stained with Coomassie brilliant blue for 40 min and then destained for 40 min. Another gel was subjected to Western blot analysis with anti-DEV polyclonal antibodies (Shen *et al*., 2009a).

Rabbit anti-tgB polyclonal antibody. Because the tgB in *E. coli* BL21 bacteria formed inclusion bodies, we used ultrasonic waves for disruption. The pellets were resuspended and washed in 20 ml of lysis buffer (3 mol/l urea, 1 mmol/l EDTA, 50 mmol/l pH8.0 Tris buffer, 0.1% Triton X-100, and 150 mmol/l NaCl). This procedure was repeated five times. The sample was centrifuged at 10000 rpm, the pellets were dissolved in 8 mol/l urea, and dialyzed in water pH 10. Purified tgB was analyzed by SDS-PAGE and used to prepare the rabbit polyclonal antibody according to the previous article (Zhang *et al*., 2010b). Briefly, rabbits were immunized with 1 mg of purified tgB with complete

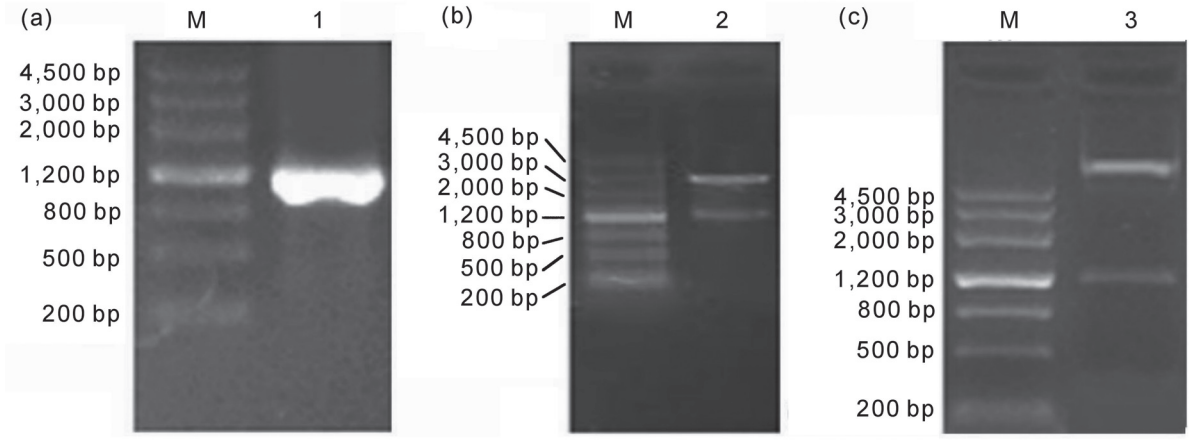

Fig. 1

Amplification of the tUL27 gene and verification of pMD18-tUL27 and pET-28a-tUL27 plasmids

PCR of tUL27 gene with P1 and P2 primers with 1200 bp product (a). pMD18-tUL27 plasmid verification with *Eco*RI and *Hind*III (b). pET-28a-tUL27 plasmid verification with *Eco*RI and *Hind*III (c). M: DNA marker.

Freud´s adjuvans (Promega, USA) and two boosters of 2 mg of tgB in one-week intervals were added. After the fourth immunization, the antiserum was collected from the carotid artery and stored at -20°C. The rabbit anti-tgB polyclonal antibody was initially purified by saturated ammonium sulfate, and then by DEAE-Sepharose column (Bio-Rad, USA) according to the manufacturer´s instructions. The purified IgGs were determined by 12% SDS-PAGE and stored at -20°C.

Indirect immunofluorescence assay. DEFs were seeded in six-well plates with cover slips and infected with DEV. The cover slips were collected at different time points (4, 8, 12, 24, and 36 hrs post infection (p.i.)) and fixed with 4% paraformaldehyde for 12 hrs at 4°C. Cells were washed and saturated with 3% BSA at 37°C for 1 hr. Incubation with rabbit anti-tgB polyclonal serum (1:100) for 2 hrs at 37°C followed. Secondary antibody, FITC conjugated goat anti-rabbit antibody (Sino-American Biotechnology, China), was incubated at 37°C for 1 hr. The 4, 6-diamidino-2-phenylindole (DAPI; Sigma, Japan) staining was used to visualize the cell nuclei (Shen *et al*., 2009b; Zhang *et al*., 2011). Immunofluorescence was viewed by the Bio-Rad MRC 1024 imaging system.

Results

Construction of the recombinant plasmid pMD18-tU-L27and expression plasmid pET-28a-tUL27

The tUL27 was amplified by the primers P1 and P2. A band with approximately 1200 bp was amplified, correlating with the theoretical nucleic acid length of tUL27 (Fig. 1a). The amplified product was cloned into pMD18-T vector and transformed into *E. coli* DH5α competent cells. Recombinant plasmid pMD18-tUL27 was verified by the *Eco*RI and *Hind*III digestion, yielding two bands of approximately 2600 bp and 1200 bp (Fig. 1b). They were consistent with the theoretical nucleic acid length of pMD18-T vector and tUL27.

The expression plasmid pET-28a-tUL27 was verified by *Eco*RI and *Hind*III restriction analysis, and two bands of approximately 5300bp and 1200bp were consistent with the theoretical nucleic acid length of pET-28a (+) vector and tUL27 (Fig. 1c).

Both recombinant plasmids pMD18-tUL27 and pET-28a-tUL27 were verified by sequencing.

Expression and identification of recombinant protein tgB

Recombinant expression plasmid pET-28a-tUL27 was transformed into expression bacteria *E. coli* BL21. Culture with appropriate optical density was induced with IPTG. The production of recombinant protein tgB was analyzed by SDS-PAGE and revealed a specific band, compared to pET-28a (+) vector transformed bacteria (Fig. 2a, lane 2), of approximately 45 kDa (Fig. 2a, lane 1). In order to identify whether the recombinant tgB really was a DEV protein, the Western blot analyzes was used. Polyclonal anti-DEV antibody reacted with the recombinant protein tgB revealing a faint band with molecular weigh of 45 kDa (Fig. 2b).

Preparation and purification of the rabbit anti-tgB polyclonal antibody

The quality of protein tgB purification was analyzed by SDS-PAGE (Fig. 2c). Rabbit anti-tgB polyclonal antibody purified through saturated ammonium sulfate, which revealed two bands of heavy and light chain (Fig. 2d, lane 1), was followed by DEAE-Sepharose column purification (Fig. 2d, lane 2). The purified polyclonal antibody was used for further characterization of gB.

Characteristics of intracellular localization of DEV gB

In comparison to mock (Fig. 3, mock), specific fluorescence was observed in DEV infected DEF cells at 8 hrs p.i. (Fig. 3, 8 h). More pronounced fluorescence could be seen in nuclear membrane of infected DEF cells after 12 hrs p.i. (Fig. 3, 12 h) and still increased during the infection. At 24 hrs p.i., the specific fluorescence could be seen in vicinity of nuclear membrane and later on it appeared as punctiform signal in the cytoplasm (Fig. 3, 24 h). At the end of 36 hrs p.i., gB protein could be seen at the membrane of the infected DEF cells (Fig. 3, 36 h).

Discussion

DEV, a threat to the commercial duck industry, was first isolated in 1923. Although there are many reports considering DEV, there is only little information about the UL27 gene. In this article, DEV tUL27 gene was firstly amplified and cloned to gain recombinant plasmid pMD18-tUL27 and then subcloned to obtain expression plasmid pET-28a-tUL27. By induction of pET-28a-tUL27 transformed bacteria we obtained tgB protein, which was purified and used for the rabbit anti-tgB polyclonal antibody preparation. The recombinant tUL27 gene product, tgB, was recognized by the rabbit anti-DEV polyclonal antibody and identified as one of the DEV proteins.

From the results of DEV gB intracellular localization, two important findings were provided. According to the reported literatures DEV late proteins UL31 protein and UL51 protein were detected at 6 hrs p.i. and 8 hrs p.i. (Xie *et al*., 2009; Shen *et al*., 2009a). The gB is firstly detected at 8 hrs p.i. in infected DEFs, therefore DEV gB was identified as a late protein. This is also in agreement with the theory, that most of the structural proteins like viral nucleocapsid proteins and envelope glyco-

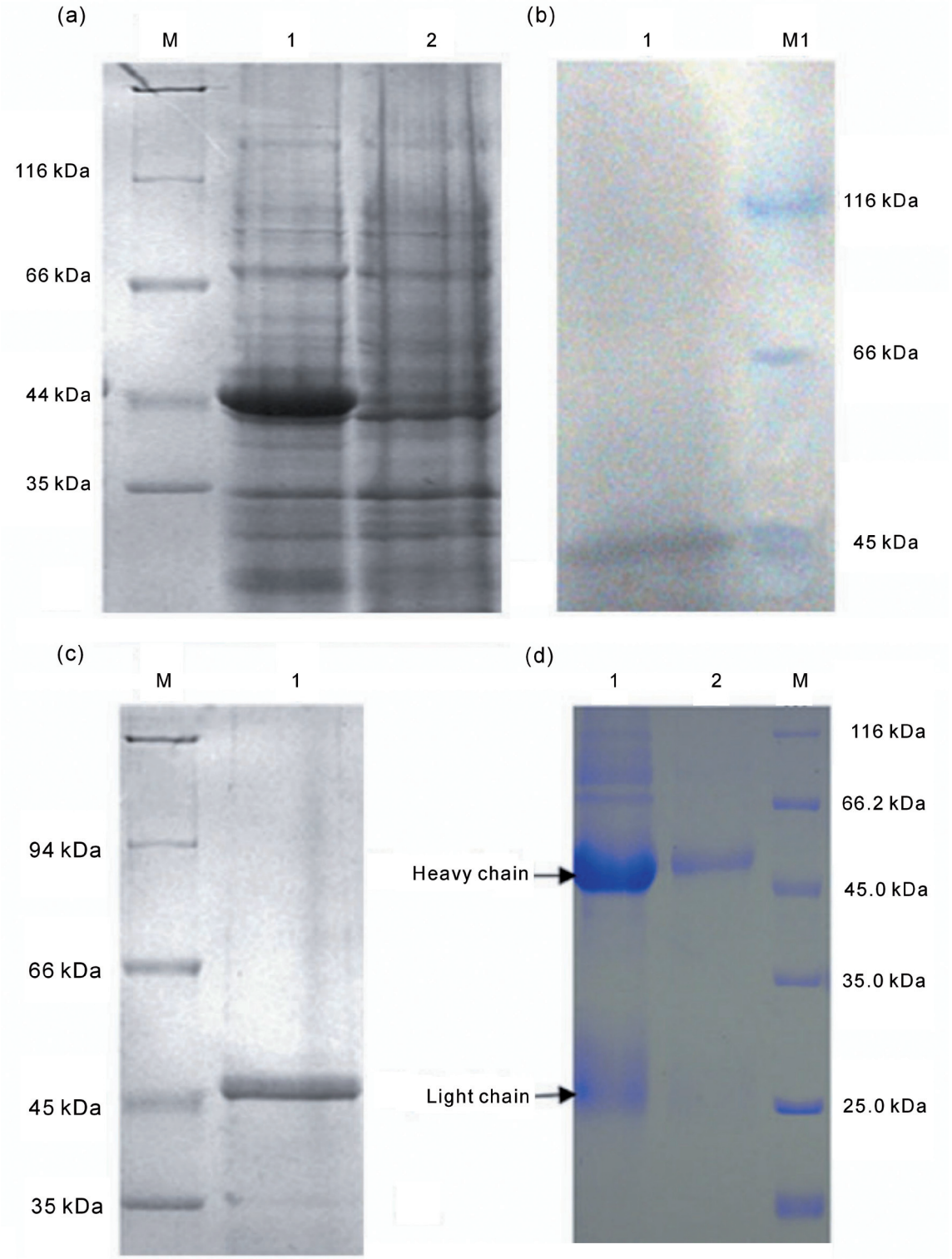

Fig. 2

Expression and purification of the tgB and rabbit anti-tgB polyclonal antibody

Expression of tgB protein from pET-28a-tUL27 plasmid (lane 1) and pET-28a (+) vector as negative control (lane 2) (a). Western blot analysis of tgB protein with anti-DEV polyclonal antibodies (b). SDS-PAGE of purified tgB protein (c). Purification of the rabbit anti-tgB polyclonal antibodies (d). Rabbit anti-tgB polyclonal antibody purified with saturated ammonium sulfate (lane 1). Second purification of anti-tgB polyclonal antibody on DEAE-Sepharose column (lane 2). M: protein marker.

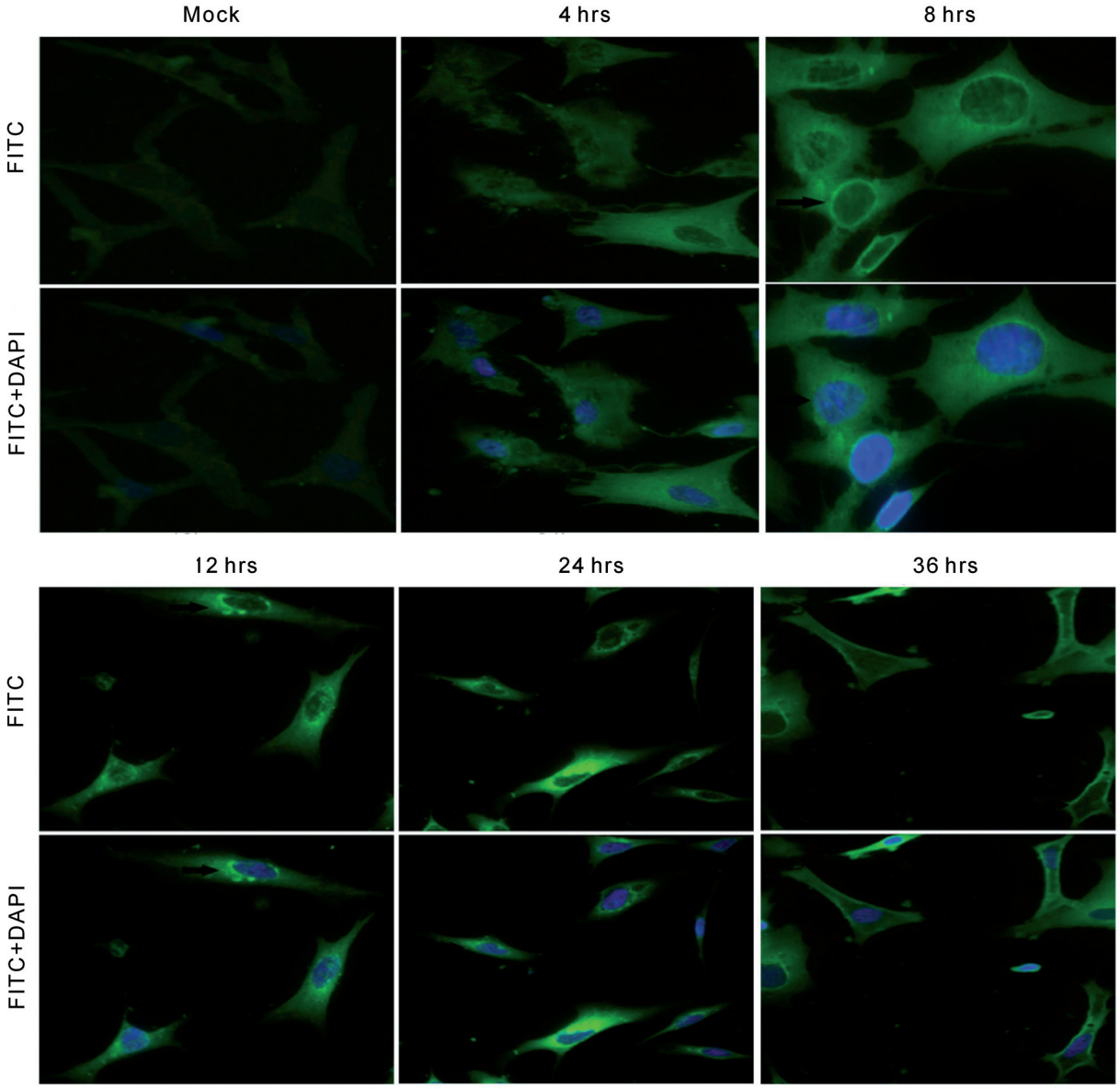

Fig. 3

Immunofluorescence of DEV gB at different infection time points

Uninfected DEF cells (mock). DEV infected DEF cells collected at different time points (4, 8, 12, 24, and 36 hrs p.i.) stained with the rabbit anti-tgB polyclonal antibody.

proteins belong to late proteins family. The second important fact is that DEV gB firstly appeared in nuclear membrane of the infected DEFs, then diffused into the vicinity of nuclear membrane, followed by punctiform appearance in cytoplasm and finally migrated to the cellular membrane. This was in agreement with earlier reports, that the gB glycoprotein has been localized at the nuclear envelope, in the endoplasmatic reticulum, in the Golgi complex and finally at the cell surface (Torrisi *et al.*, 1992; Gilbert and Ghosh, 1993; Gilbert *et al.*, 1994). The intracellular localization of DEV gB demonstrated that DEV gB localization at nuclear membrane shows a possible role in virus assembly; meanwhile the DEV gB localization in the cytoplasm describes its role in viral egress.

These characteristics of the gB provided basal data for understanding the functions of DEV UL27 gene and protein gB. Since the glycoproteins are the major targets of the immune response, our studies will focus on the immunologic mechanism of DEV gB in future.

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References

Carter VC, Schaffer PA, Tevethia SS (1981): The involvement of herpes simplex virus type 1 glycoproteins in cell-mediated immunity. J. Immunol. 126, 1655–1660.

Chang H, Cheng AC, Wang MS, Guo YF, Xie W, Lou KP (2009): Complete nucleotide sequence of the duck plague virus gE gene. Arch. Virol. 154, 163–165. http://dx.doi.org/10.1007/s00705-008-0284-6

Cheng AC, Wang MS, Wen M, Zhou WG, Guo YF, Jia RY, Xu C, Yuan GP, Liu YC (2006): Construction of duck enteritis virus gene libraries and discovery, cloning and identification of viral nucleocapsid protein gene. High Technol. Lett. 16, 948–953.

Davison S, Converse KA, Hamir AN, Eckroade RJ (1993): Duck viral enteritis in domestic muscovy ducks in Pennsylvania. Avian Dis. 37, 1142–1146. http://dx.doi.org/10.2307/1591927

Gilbert R, Ghosh HP (1993): Immunoelectron microscopic localization of herpes simplex virus glycoprotein gB in the nuclear envelope of infected cells. Virus Res. 28, 217–231. http://dx.doi.org/10.1016/0168-1702(93)90023-G

Gilbert R, Ghosh K, Rasile L, Ghosh HP (1994): Membrane anchoring domain of herpes simplex virus glycoprotein gB is sufficient for nuclear envelope localization. J. Virol. 68, 2272–285.

Glorioso J, Kees U, Kümel G, Kirchner H, Krammer PH (1985): Identification of herpes simplex virus type 1 (HSV-1) glycoprotein gC as the immunodominant antigen for HSV-1-specific memory cytotoxic T lymphocytes. J. Immunol. 135, 575–582.

Lawman MJ, Courtney RJ, Eberle R, Schaffer PA, O´Hara MK, Rouse BT (1980): Cell-mediated immunity to herpes simplex virus: specificity of cytotoxic T cells. Infect. Immun. 30, 451–461.

Lian B, Xu C, Cheng AC, Wang MS, Zhu DK, Luo QH, Jia RY, Bi FJ, Chen ZL, Zhou Y, Yang ZX, Chen XY (2010): Identification and characterization of duck plague virus glycoprotein C gene and gene product. Virol. J. 7, 349. http://dx.doi.org/10.1186/1743-422X-7-349

Mettenleiter TC (2000): Aujeszky's disease (pseudorabies) virus: the virus and molecular pathogenesis state of the art, June 1999. Vet. Res. 31, 99–115. http://dx.doi.org/10.1051/vetres:2000110

Montali RJ, Bush M, Greenwell GA (1979): An epornitic of duck viral enteritis in a zoological park. J. Am. Vet. Med. Assoc. 169, 954–958.

Sandhu TS, Metwally SA (2008): Duck virus enteritis(Duck Plague). In Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK, Swayne DE: Diseases of Poultry. 12th ed. Oxford, Blackwell Publishing, pp. 384–393.

Schrier RD, Pizer LI, Moorhead JW (1983): Type-specific delayed hypersensitivity and protective immunity induced by isolated herpes simplex virus glycoprotein. J. Immunol. 130, 1413–1418.

Shen CJ, Guo YF, Cheng AC, Wang MS, Zhou Y, Lin D, Xin HY, Zhang N (2009a): Identification and characterization of the duck enteritis virus UL51 gene. Arch. Virol. 154, 1061–1069. http://dx.doi.org/10.1007/s00705-009-0407-8

Shen CJ, Guo YF, Cheng AC, Wang MS, Zhou Y, Lin D, Xin HY, Zhang N (2009b): Characterization of subcellular localization of duck enteritis virus UL51 protein. Virol. J. 6, 92. http://dx.doi.org/10.1186/1743-422X-6-92

Torrisi MR, Di Lazzaro C, Pavan A, Pereira L, Campadelli-Fiume G (1992): Herpes simplex virus envelopment and maturation studied by fracture label. J. Virol. 66, 554–561.

Wang J, Höper D, Beer M, Osterrieder N (2011): Complete genome sequence of virulent duck enteritis virus (DEV) strain 2085 and comparison with genome sequences of virulent and attenuated DEV strains. Virus Res. 160, 316–325. http://dx.doi.org/10.1016/j.virusres.2011.07.004

Wen YP, Cheng AC, Wang MS, Ge H, Shen CJ, Xiang J, Jia RY, Zhu DK, Chen XY, Lian B, Chang H, Zhou Y (2010): A TK recombinant protein-based ELISA for detecting antibodies to Duck Plague Virus. Virol. J. 7, 77. http://dx.doi.org/10.1186/1743-422X-7-77

Xiang J, Ma GP, Zhang SC, Cheng AC, Wang MS, Zhu DK, Jia RY, Luo QH, Chen ZL, Chen XY (2010): Expression and intracellular localization of duck enteritis virus pUL38 protein. Virol. J. 7, 162. http://dx.doi.org/10.1186/1743-422X-7-162

Xie W, Cheng AC, Wang MS, Chang H, Zhu DK, Luo QH, Jia RY, Chen XY (2009): Expression and characterization of the UL31 protein from Duck enteritis virus. Virol. J. 6, 19. http://dx.doi.org/10.1186/1743-422X-6-19

Xie W, Cheng AC, Wang MS, Chang H, Zhu DK, Luo QH (2010): Molecular cloning and characterization of the UL31 gene from Duck enteritis virus. Mol. Biol. Rep. 37, 1495–1503. http://dx.doi.org/10.1007/s11033-009-9546-y

Zhang SC, Ma GP, Xiang J, Cheng AC, Wang MS, Zhu DK, Jia RY, Luo QH, Chen ZL, Chen XY (2010a): Expressing gK gene of duck enteritis virus guided by bioinformatics and its applied prospect in diagnosis. Virol. J. 7, 168. http://dx.doi.org/10.1186/1743-422X-7-168

Zhang SC, Xiang J, Cheng AC, Wang MS, Li X, Li LJ, Chen XW, Zhu DK, Luo QH, Chen XY (2010b): Production, purification and characterization of polyclonal antibody against the truncated gK of the duck enteritis virus. Virol. J. 7, 241. http://dx.doi.org/10.1186/1743-422X-7-241

Zhang SC, Xiang J, Cheng AC, Wang MS, Wu Y, Yang XY, Zhu DK, Jia RY, Luo QH, Chen ZL, Chen XY (2011): Characterization of duck enteritis virus UL53 gene and glycoprotein K. Virol. J. 8, 235. http://dx.doi.org/10.1186/1743-422X-8-235

Zou Q, Sun KF, Cheng AC, Wang MS, Xu C, Zhu DK, Jia RY, Luo QH, Zhou Y, Chen ZL, Chen XY (2010): Detection of anatid herpesvirus 1 gC gene by TaqManTM fluorescent quantitative real-time PCR with specific primers and probe. Virol. J. 7, 37. http://dx.doi.org/10.1186/1743-422X-7-37