Duck plague virus Glycoprotein J is functional but slightly impaired in viral replication and cell-to-cell spread

Yu You1,2,3, Tian Liu1,2,3, Mingshu Wang1,2,3, Anchun Cheng1,2,3, Renyong Jia1,2,3, Qiao Yang1,2,3, Ying Wu1,2,3, Dekang Zhu1,2,3, Shun Chen1,2,3, Mafeng Liu1,2,3, XinXin Zhao1,2,3, Shaqiu Zhang1,2,3, Yunya Liu1,2,3, Yanling Yu1,2,3 & Ling Zhang1,2,3

To analyse the function of the duck plague virus (DPV) glycoprotein J homologue (gJ), two different mutated viruses, a gJ deleted mutant ΔgJ and a gJR rescue mutant gJR with US5 restored were generated. All recombinant viruses were constructed by using two-step of RED recombination system implemented on the duck plague virus Chinese virulent strain (DPV CHv) genome cloned into a bacterial artificial chromosome. DPV-mutants were characterized on non-complementing DEF cells compared with parental virus. Viral replication kinetics of intracellular and extracellular viruses revealed that the ΔgJ virus produce a 10-fold reduction of viral titers than the gJR and parental virus, which especially the production of extracellular infectivity was affected. In addition, the ΔgJ virus produced viral plaques on DEF cells that was on average approximately 11% smaller than those produced by the gJR and parental viruses. Electron microscopy confirmed that although DPV CHv without gJ could efficiently carry out viral replication, virion assembly and envelopment within infected cells, the ΔgJ virus produced and accumulated high levels of anuclear particles in the nuclear and cytoplasm. These results show that the gJ slightly impaired in viral replication, virion assembly and cell-to-cell spread, and is not essential in virion envelopment.

Duck plague (DP), also called duck virus enteritis (DVE), is one of the major acute, fatal and contagious diseases of duck, geese, and swans, characterized by vascular damage, tissue hemorrhages, digestive mucosal eruptions, and lesions of lymphoid organs. Due to high mortality, morbidity as well as decreased egg production and hatchability, DP caused significant economic losses around the world1–4. DPV, which belong to the genus Mardivirus, subfamily Alpha-herpesvirinae, and family Herpesviridae, is the pathogen of DP disease 3,4. The entire DNA sequence of the DPV has been determined few years ago5. However, not too many genes and gene products have been characterized in term of relevance or functional cooperation in viral lifecycle6–8.

Herpesvirus glycoproteins play important roles in the different stages of viral lifecycle, such as enter the target cells, direct cell-to-cell spread, and the egress of virions from infected cells9,10, and 12 glycoproteins have been identified and designated gB, gC, gD, gE, gG, gH, gl, gK, gL, gM and gN in herpes simplex virus type 1 (HSV-1)11. DPV glycoproteins are named in accordance with the nomenclature used for the HSV-1 glycoproteins. However, previously studies about DPV focused on the epidemiology and prevention, instead of the studying of molecular biology and the function of glycoproteins5. To date only a few gens such as gC and gE from DPV have been addressed by studying mutant viruses12,13. gC involved in alphaherpesvirus adsorption were present in the DPV genome and relatively conserved, but the function of DPV gC also makes a difference with other alphaherpesvirus, which is independent of interaction with heparin sulfate of cell surface12,14. Therefore, although
alphaherpesvirus share many common strategies for their replication cycle\textsuperscript{15}, they also keep some individually patterns to invade and infect target cells. The US5 gene of alpha-herpesvirus encodes glycoprotein J (gJ), which shares lowly nucleotide and amino acid similarity among alpha-herpesvirus sub-family. Exploring the functions of gJ is far behind other glycoproteins. The only previously reported function of alpha-herpesvirus gJ was its ability to inhibit apoptosis and viral egress, and the mechanisms of these functions have not been fully revealed\textsuperscript{16–20}. DPV glycoprotein J (gJ) is encoded by US5 in the viral genome which is positional homologous in the HSV-1 genome. Comparisons of nucleotide and deduced amino acid sequences uncovered that gJ is lowly conserved throughout the alpha-herpesvirus subfamily, but highly conserved among the different strain of DPV.

In the present study, to clarify roles of gJ in DPV lifecycle, we describe the construction of a DPV mutant virus, based on the infectious BAC clone by using two-step of RED recombination for generation of US5 deletion and revertant mutant in \textit{E. coli}. Viral particles lacking gJ in the envelope were produced in non-complementing DEF cells, which demonstrated that US5 gene of DPV is nonessential for virus replication. We determined kinetics of virus growth, relative plague morphology, and conducted ultrastructural visualization of gJ-deficient mutant and parental virions in the same DPV genetic background to gain an understanding of the glycoprotein J contribution in infectious virion replication cycle. The results firstly show that gJ is nonessential for DPV virion replication and slightly impaired in viral replication, virion assembly and cell-to-cell spread.

Results

Construction and molecular analysis of recombinant virus. The DPV genome has been cloned into a BAC by our laboratory, which enables the rapid and efficient genetic manipulation of the DPV genome in \textit{E. Coli DH10B}. To investigate of the relative role of DPV gJ, the gJ deletion mutant and its revertant was generated using the two-step Red recombination mutagenesis system implemented on the pBeloBAC11 bacterial artificial chromosome carrying the DPV genome, as described in Materials and Methods. The DPV CH\textit{v}-BAC-ΔgJ virus is the deletion of the entire gJ open reading frame (ORF) (Fig. 1). Meanwhile, to eliminate the small possibility that this deletion causes indirect epigenetic effects on foreign DNA insertion, we constructed the DPV CH\textit{v}-BAC-gJR virus which is the revertant of the entire gJ ORF. The engineered mutations were confirmed via diagnostic PCR, restriction fragment length polymorphism (RFLP) analysis and DNA sequencing (data not shown). Specifically, the BAC DNAs of DPV CH\textit{v}-BAC-ΔgJ and DPV CH\textit{v}-BAC-gJR were extracted, and then were identified by primer UL48 (2555 bp), gJ (1820 bp), sopB (966 bp) and repA (681 bp), among which UL48 is a DPV conserved gene to identify the existence of DPV genome and sopB as well as repA are two important genes to identify BAC-Mini-F sequence (Fig. 2A). As a result, we found repA, sopB and UL48 can be detected in all recombinant viruses, which indicated the presence of BAC component and DPV genome in recombinant viruses. What’s more, the whole fragment of gJ gene can be detected in the parental virus of DPV CH\textit{v}-BAC and derived recombinant DPV CH\textit{v}-BAC-ΔgJ, but cannot be found in the DPV CH\textit{v}-BAC-ΔgJ, which showed that the construction of gJ-deleted DPV virus was successful.

Furthermore, the BAC DNA of recombinant virus extracted from the PCR identified clones was digested with BamHI for RFLP analysis. As a result, the restriction patterns of BamHI digestion products of DPV CH\textit{v}-BAC, DPV CH\textit{v}-BAC-ΔgJ and DPV CH\textit{v}-BAC-gJR were as same as we prediction, respectively (Fig. 2B).

Rescue and confirm of the recombinant virus. To generate virus stocks from the mutant BAC genomic constructs, individual BAC DNAs were transfected into DEF cells. Passage of these viruses up to three times in DEF cells eliminated the effects of transient transfection of plasmid containing EGFP and did not led to any phenotypic revertants, which also purified and enriched the recombinant viruses (Fig. 3A).

The mutant viruses of DPV CH\textit{v}-BAC-ΔgJ and DPV CH\textit{v}-BAC-gJR were identified indirect immunofluorescence assay (IFA) and western blotting. The expression of gJ protein in parental virus of DPV CH\textit{v}-BAC and derived recombinant DPV CH\textit{v}-BAC-ΔgJ and DPV CH\textit{v}-BAC-gJR infected host cells were recognized by polyclonal rabbit anti-gJ in IFA and WB (Fig. 3B,C).
unessential to DPV, the deletion of this gene could influence viral replication at late stage of infection. The conclusion is that although gJ gene is more efficient than DPV CHv-BAC-gJR and DPV CHv-BAC. The replication of viruses kept quiescence at the first 12 h after infection, then significant increases were observed during the whole observation time in supernatants while it stopped increasing at 72 hpi and slightly dropped after that in cells except DPV CHv-BAC-ΔgJ mutant virus. It was worthy to mention that viral titers in cells were higher than titers in medium at 24 hpi. However, on the contrary, at 48 hpi, titers of mature viral particles in supernatant was more than virus in cells. Moreover, DPV CHv-BAC-ΔgJ presented with a marked growth defect. Within these experiments, the replication of viruses kept quiescence at the first 12 h after infection, then significant increases were observed during the whole observation time in supernatants while it stopped increasing at 72 hpi and slightly dropped after that in cells except DPV CHv-BAC-ΔgJ mutant virus. It was worthy to mention that viral titers in cells were higher than titers in medium at 24 hpi. However, on the contrary, at 48 hpi, titers of mature viral particles in supernatant was more than virus in cells. Moreover, DPV CHv-BAC-ΔgJ mutant viruses were approximately 11% smaller than the parental virus plaques. To better assess the virus plaque sizes produced by individual mutant viruses, 30 randomly chosen viral plaques were selected and statistically analyzed as described in Materials and Methods. This analysis confirmed that the gJ-deleted mutant virus slightly reduced plaque size in comparison to that of the parental virus. Meanwhile, these data showed a statistically significant difference in mean plaque areas of the parental and recombinant viruses (t-test, p < 0.05) (Fig. 5).

Plaque morphology of mutant viruses and relative plaque area measurements. Envelop proteins of alpha-herpesvirus play an important role on viral cell-to-cell spreading or plaque forming. We performed plaque morphology assays to explore whether the gJ-deleted viruses had some effects on the transmission of viruses between adjacent cells. The plaque morphologies of the ΔgJ and ΔgJR mutant viruses were examined in DEF cells. As expected, the DPV CHv-BAC parental virus and the DPV CHv-BAC-gJR mutant viruses produced plaques that were similar in size to each other and, on average. The plaques produced by DPV CHv-BAC-ΔgJ mutant viruses were approximately 11% smaller than the parental virus plaques. To better assess the virus plaque sizes produced by individual mutant viruses, 30 randomly chosen viral plaques were selected and statistically analyzed as described in Materials and Methods. This analysis confirmed that the gJ-deleted mutant virus slightly reduced plaque size in comparison to that of the parental virus. Meanwhile, these data showed a statistically significant difference in mean plaque areas of the parental and recombinant viruses (t-test, p < 0.05) (Fig. 5).

Ultrastructural characterization of parental and mutant viruses. The herpesvirus viral life cycle contains the following major steps: entry into the host cell, expression and replication of viral genes, virion assembly, and egress of the new generation of viral particles. The entire process takes approximately 18 to 20 hours in permissive cells. Thus, there was a higher opportunity to observe viral ultrastructural phenotypes and life process stages at 36 hpi. Furthermore, according to the growth curve of the recombinant virus, at 36 hpi, its intracellular titer was similar to the parental virus, but its extracellular titer was significantly lower. Meanwhile, the virus extracellular titer gap narrowed at 48 hpi. Moreover, the cells did not show significant rupture and lesions at 36 hpi, but observed at 48 hpi (not shown). In the state of cell fragmentation, it is not conducive to observe the life process of recombinant virus in cells via transmission electron microscopy.

To test whether the deletion of DPV gJ caused a defect in viral assembly and maturation. The ultrastructural phenotypes of recombination viruses relative to the parental virus were investigated at 36 hpi utilizing transmission electron microscopy, visually examining more than 30 individual cells. As expected, the parental virus did not exhibit any apparent defects in nuclear virion assembly or cytoplasmic virion envelopment, as evidenced by the presence of fully enveloped virions intracellularly. Although the empty capsids could be observed on DPV CHv-BAC infected cells, a large amount of nucleic acids could also be found next to these particles (Fig. 6A,B and C). On the contrary, ultrastructural visualization of DEF cells infected with the gJ-deleted mutant viruses revealed nuclear and cytoplasmic defects in virion assembly and envelopment. The most-pronounced effects, produced by the ΔgJ mutant virus, were that numerous unenveloped and enveloped empty capsids were found in nuclear and cytoplasm of infected cells, which may cause the formation of immature of non-infectious virions (Fig. 6D,E and F).
The alpha-herpesvirus share some strategies for the lytic replication cycle, which mainly includes the viral DNA replication in nucleus, capsid assembly and egress from the nucleus, the envelopment of viral particles in the cytoplasm and the exocytosis of mature virions. During the viral replication cycle, the viral glycoproteins mainly played important roles on viral envelopment, which interact with the viral tegument protein to drive this budding. Ultrastructural visualization of DEF cells infected with the mutant viruses without the expression of gJ revealed that the process of viral replication could work. As the result showed, the gJ-deleted viral nucleocapsids in the nuclear (Fig. 7A) bound onto the surface of inner nuclear membranes (Fig. 7B), and then these perinuclear enveloped particles fused with outer nuclear membranes (Fig. 7C) to enter the cytoplasm, coated-tegument capsids in the cytoplasm bound onto the surfaces of TNG membranes that contained glycoproteins to produce envelopment virions (Fig. 7D,E and F), and the envelopment particles were released from cells (Fig. 7G,H and I).

**Discussion**

Alpha-herpesvirus encodes at least 12 glycoproteins which play important roles in the virus lifecycle, including virus-induced cell fusion, virion assembly and viral egress. Over the years, a number of gene-deleted mutant
virus based on infectious BAC clone have been constructed and characterized. Previously studies showed that the lack of expression of gB, gH, or gL alone has drastically effect on viral maturation, which is necessary for viral replication, while HSV-1, EHV-1 or other alpha-herpesvirus could replicate and produce infection progeny virus without the expression of other glycoproteins, such as such as gE, gI, gK and so on. As a non-conserved gene among the alpha-herpesvirus, the specific characteristic and function of glycoprotein J are just a little known. Previously, studies of HSV-1, ILTV, EHV-1 and EHV-4 gJ suggested that the viruses without expression of gJ could replicate and proliferate, but it had different decrease on virus titer except HSV-1.

In this study, based on the construction of infectious BAC clone containing the genome of DPV, experiments were conducted to elucidate the function of the deletion of gene US5, which leads to the absence of gJ from the DPV. The salient findings of these experiments were that the DPV gJ is important for virus growth in vitro, and that gJ is slightly involved in direct cell-to-cell spread and in virion maturation. These above conclusions were drawn based on the finding that the gJ-deleted DPV gJ-deleted BAC mutant, DPV CHv-BAC-ΔgJ, replicated to significantly decreased titers in DEF cells, which exhibited a 10-fold reduction of total titers when compared to parental virus and a 4-fold reduction of endocellular titers, and that especially the production of extracellular infectivity was affected. This means that the DPV gJ did not only have some negatively influence on viral replication, but also slightly mediate viral egress. What’s more, the gJ-deleted mutant virus exhibited an approximately 11% reduction in mean plaque diameters when compared to parental or gJ-revertant virus. Furthermore, more immature virions, non-nuclear particles in nuclear and cytoplasm, were observed by electron microscope, and lower cytoplasmic virion envelopment level. Although numerous immature particles were found, the replication cycle of gJ-deleted mutant virus have still worked.
Ultrastructural examination of cells infected with gJ-deleted mutant viruses revealed that the recombination virus exhibited no appreciable defects in cytoplasmic virion envelopment, as also evidenced by the presence of fully enveloped virions in extracellular spaces and secondary-enveloped process in the cytoplasm of infected cells. Surprisingly, the ΔgJ mutant virus exhibited appreciable defect in viral nuclear assembly. Previously studies showed that glycoproteins of herpesvirus mainly played roles in cytoplasmic virion envelopment. What's more, gB, gD and the heterodimer gH-gL, also found in the nuclear members, were confirmed that these glycoproteins could participate in de-envelopment. However, there is no evidence that glycoproteins of herpesvirus have effects on viral nuclear assembly. This study is the first to show the result that the absence of DPV gJ exhibited a certain degree of effects in viral nuclear assembly, but the mechanism has not been uncovered and still needs to explore. In summary, a marked replication defect was shown after deleting DPV CHv gJ, slightly influencing the efficiency in cell-to-cell spread and virus egress. In combination with analyses of section of infected cells by electron microscopy, the mechanisms and functions of DPV gJ are complex and still need to be exposed.

Materials and Methods

Cells and Viruses. The duck embryo fibroblast (DEF) monolayer was incubated at 37 °C with 5% CO₂ in Minimal Essential Medium (MEM, Gibco, Grand Island, NY) supplemented with 10% newborn calf serum (NBS, Gibco, Grand Island, NY), 100 U/ml penicillin and 100 μl/mg streptomycin. For virus infection, MEM supplemented with 3% NBS was used. DPV CHv strain was separated and preserved in the laboratory.

Construction of DPV mutant viruses. Mutagenesis was constructed in E. coli DH10B by using the Red recombination mutagenesis system with synthetic oligonucleotides implemented on the bacterial artificial chromosome (BAC) plasmid pBeloBAC11 carrying the DPV CHv genome. The DPV CHv-BAC-ΔgJ virus was constructed by deleting the whole US5 open reading frame (ORF). Moreover, the ΔgJ recombinant virus was used as the backbone for construction of the DPV CHv-BAC-gJR reverse mutant by restoring the US5 ORF. Synthetic oligonucleotides used to mutagenize each targeted gene are shown in Table 1. Specifically, the 5′ end of the forward primer for each mutagenesis contains 56 bp of homologous sequence upstream of the site of mutation, and 20 or 21 bp at the 3′ ends correspond to the kanamycin resistance (KanR) gene (Table 1). A 1590 bp PCR fragment containing the KanR gene flanked on both sides by gJ sequence was amplified from the pKD4 vector by the primers.

Maintenance and mutagenesis of the BAC constructs were performed in E. coli strain DH10B. Firstly, the recombineering plasmid, pKD46, containing a λ prophage encoding recombination enzymes Exo, Beta, and Gam under a heat-inducible promoter was transformed into E.coli strain DH10B. Secondly, Bacteria carrying the target BAC and pKD46 were grown in 50 ml LB cultures with chloramphenicol (25 μl/ml), ampicillin (100 μl/ml), and L-arabinose (100 μl/ml, Sigma) at 30 °C to an OD600 of 0.5–0.6. Electrocompetent bacteria were transformed with 800 ng of the PRC products. Chloramphenicol-resistant (CmrR)/KanR transformants were confirmed sufficient resistance, and then screened by PCR using the identification of primer (Table 1). A 1590 bp PCR fragment containing the KanR gene flanked on both sides by gJ sequence was amplified from the pKD4 vector by the primers.

Figure 6. Ultrastructural morphologies of mutant viruses. Electron micrographs of DEF cells infected at an MOI of 2 with different viruses and processed for electron microscopy at 36 hpi are shown. (A,B,C) Showed the DEF cells infected DPV CHv-BAC. (D,E,F) Showed the DEF cells infected DPV CHv-BAC-ΔUS5. Nucleus (n) and cytoplasm (c) are marked.
selected again in Cm plates at 42 °C and then tested for kanamycin sensitivity and loss of ampicillin resistance. The primarily confirmed colonies were ulteriorly screened by PCR using the identification of primer, and then the PCR products were sequenced to confirm the desired targeting.

**Confirmation of the targeted mutations and recovery of infectious virus.** DPV CHv-BAC DNAs (plasmids pΔgJ and pgJR) were purified from 100 ml of BAC cultures with a Qiagen Plasmid Midi Kit (Qiagen, Valencia CA). The plasmids pΔgJ and pgJR were confirmed by PCR using the identification of primers designed to lie outside of the target mutation site.

Viruses were recovered from cells transfected with BACs as follows: DEF cells were grown to 70–90% confluent in 6-well or 12-well plates. Cells were transfected with BAC DNAs mixed with Lipofectamine 3000 in Opti-MEM medium recommended by the manufacturer (Invitrogen). After 6 h of incubation at 37 °C, the medium was removed from the transfected cells, and the cells were washed with phosphate-buffered saline (PBS).
| No. | Primers | Sequence (5′-3′) | Product |
|-----|---------|-----------------|---------|
| 1   | sopB-for | atgtatgagaggcgcattggag | sopB    |
|     | sopB-rev | atgtatgagaggcgcattggag | sopB    |
| 2   | repA-for | cgctgagtatttattcatttcct | repA    |
|     | repA-rev | cgctgagtatttattcatttcct | repA    |
| 3   | ΔgJ/ΔgJR-for | ttatitgacgggaagtgtt | ΔgJ/ΔgJR identification product |
|     | ΔgJ/ΔgJR-rev | cgctgagtatttattcatttcct | ΔgJ/ΔgJR identification product |
| 4   | ΔgJ-Kana-for | ggaagcagctccagcctacactcatacaaaggcat | gJ gene flanked by homology arms of gJ |
|     | ΔgJ-Kana-rev | aacaacaacagaactgtaatgggtacattaaacatacgcgcatatacatattgccgatgggaattagccatggtcc | gJ gene flanked by homology arms of gJ |
| 5   | gJ/gJR-for | gjaatatttataacgagggataggctctcctgtagtcgccatattcttcatcatgccaggtgtaggtgccgctct | gJ fragment with left homology arm of gJ |
|     | gJ/gJR-rev | gjaatatttataacgagggataggctctcctgtagtcgccatattcttcatcatgccaggtgtaggtgccgctct | gJ fragment with left homology arm of gJ |
| 6   | gJ/Kana-for | atgcctttgtatggtatgagtgtaggctggagctgcttc | Kana fragment with right homology arm of gJ |
|     | gJ/Kana-rev | atgcctttgtatggtatgagtgtaggctggagctgcttc | Kana fragment with right homology arm of gJ |

Table 1. Primers used in this paper. *Complementary sequence for overlap PCR.

and then fresh MEM with 3% NBS was added. After incubating cells for 2–4 days at 37 °C, virus stocks were collected and designated as passage 0 (P₀). To remove the transient expression of EGFP, the virus of P₀ was blindly passaged. Passage P₁, viruses were used for all experiment described in the manuscript.

**One-step viral growth kinetics.** One-step growth curves were performed as follows. Sub-confluent (85 to 90%) DEF cell monolayers grown in 24-well cell culture dish were infected with each virus at 37 °C at 1000 50% Tissue Culture Infection Does (TCID₅₀). After adsorption at 37 °C for 2h, the cell monolayers were rinsed with PBS overlaid with 2 ml MEM supplemented with 3% NBS (set as 0 h on the time scale), and then returned to 37 °C humidified incubator (5% CO₂). At selected time points (12, 24, 36, 48, 72, and 96 h post-infection [h.p.i.]), supernatants and cell pellets were separated at different times post-infection and stored at −20 °C. The samples were frozen and thawed three times, and virus titers were determined by TCID₅₀ on DEF cells. All experiments repeated three times.

**Plague morphology of mutant viruses and relative plaque area measurements.** Near-confluent DEF cell monolayers in 12-well plates were infected with the virus at 100 TCID₅₀. After 2h at 37 °C, medium was discarded and cells were washed with PBS twice. And then 0.5% methylcellulose was added to cover the cells. After 48h post-infection, cells were washed three times with PBS to remove methyl cellulose medium and fixed with ice-cold 4% paraformaldehyde for 30 min. Photographs of viral plaques were taken at ×200 magnification on microscope. Thirty randomly selected plaques were imaged in this manner for each of the parental and recombination viruses under consideration.

**Electron Microscopy.** The ultrastructural morphology of virions within infected cells was examined by transmission electron microscopy essentially as described previously36,37. Cell monolayers were infected with the indicated virus at a multiplicity of infection (MOI) of 2. All samples were prepared for transmission electron microscopy examination according to previously reports38. Briefly, DEF cells were washed with PBS at 36 hpi and fixed with 2.5% glutaraldehyde at 4 °C for 2h. After that, the fixed adherent cells were collected by scraping from the plates and then centrifuged at 10,000 rpm/min for 1 h. Then the pellets were mixed with 2% low melting-temperature agarose at 37 °C, and centrifuged at 6000 rpm/min for 10 min. Samples were post-fixed in 1.0% osmium tetroxide. After a stepwise dehydration in acetone, samples were embedded in epoxy resin 618 and polymerized at 80 °C for 72 h. Then, 50 nm ultra-thin sections were prepared, collected on grids and stained with uranyl acetate and lead citrate for subsequent examination with the Tecnai G² F20 transmission electron microscope.

**References**

1. Cheng, A. Duck plague (ed. Cheng A.) 1–4 (Beijing, 2015).
2. Kaleta, E. F. et al. Outbreak of duck plague (duck herpesvirus enteritis) in numerous species of captive ducks and geese in temporal conjunction with enforced biosecurity (in-house keeping) due to the threat of avian influenza A virus of the subtype Asia H5N1. Dtw Deutsche Tierarztliche Wochenschrift 114, 3–11 (2007).
3. Dhama, K. et al. Duck virus enteritis (duckerplague) - a comprehensive update. Veterinary Quarterly 37, 57 (2017).
4. Liu, T. et al. RNA-seq comparative analysis of Peking ducks spleen gene expression 24h post-infection with duck plague virulent or attenuated virus. Veterinary Research 48, 47 (2017).
5. Wu, Y. et al. Complete genomic sequence of Chinese virulent duck enteritis virus. Journal of virology 86, 5965 (2012).
6. Liu, C. et al. Regulation of viral gene expression by duck enteritis virus UL54. Scientific reports 7, 1076 (2017).
7. Hu, X. et al. The duck enteritis virus early protein, UL13, found in both nucleus and cytoplasm, influences viral replication in cell culture. Poult. Sci. 96, 2899–2907 (2017).
8. He, Q. et al. Replication kinetics of duck enteritis virus UL16 gene in vitro. Virol J. 9, 281 (2012).
9. Owen, D. J. & Crump, C. M. & Graham, S. C. Tegument Assembly and Secondary Envelopment of Alphaherpesviruses. Viruses 7, 5084–5114 (2015).
10. Johnson, D. C. & Baines, J. D. Herpesviruses remodel host membranes for virus egress. Nature Reviews Microbiology 9, 382–394 (2011).
11. Nishiya, Y. Herpes simplex virus gene products: the accessories reflect her lifestyle well. Nature Immunology 13, 33–46 (2004).
12. Jang et al. Role of duck plague virus glycoprotein C in viral adsorption: Absence of specific interactions with cell surface heparan sulfate. Journal of Integrative Agriculture 16, 1145–1152 (2017).
13. Sun, K. Distribution characteristics of DNA vaccine encoded with glycoprotein C from duck plague virus in ducks and preliminary study of the biological characteristics of C-deleted DPV and gE-deleted DPV. Sichuan Agricultural University (2013).
14. Trybala, E. & Lilleqvist, J., Bo, S. & Bergström, T. Herpes Simplex Virus Types I and 2 Differ in Their Interaction with Heparan Sulfate. Journal of virology 74, 9106–9114 (2000).
15. Zeevbenmordehai, T., Hagen, C. & Grünewald, K. A cool hybrid approach to the herpesvirus ‘life’ cycle. Current Opinion in Virology 5, 42–49 (2014).
16. Mundt, A., Mundt, E., Hogan, R. J. & Garcia, M. Glycoprotein J of infectious laryngotracheitis virus is required for efficient egress of infectious virions from cells. *Journal of general virology* **92**, 2586–2589, https://doi.org/10.1099/vir.0.031443-0 (2011).

17. Aubert, M. et al. The antiapoptotic herpes simplex virus glycoprotein J localizes to multiple cellular organelles and induces reactive oxygen species formation. *Journal of virology* **82**, 617–629, https://doi.org/10.1128/jvi.01341-07 (2008).

18. Aubert, M., Kranz, E. M. & Jerome, K. R. Herpes simplex virus genes Us3, Us5, and Us12 differentially regulate cytotoxic T lymphocyte-induced cytotoxicity. *Viral immunology* **19**, 391–408, https://doi.org/10.1089/vim.2006.19.391 (2006).

19. Fuchs, W., Wiessner, D., Veits, J., Teifke, J. P. & Mettenleiter, T. C. In vitro and in vivo relevance of infectious laryngotracheitis virus gJ proteins that are expressed from spliced and nonspliced mRNAs. *Journal of virology* **79**, 705–716, https://doi.org/10.1128/jvi.79.2.705-716.2005 (2005).

20. You, Y. et al. The suppression of apoptosis by alpha-herpesvirus. *Cell death & disease* **8**, e2749, https://doi.org/10.1038/cddis.2017.139 (2017).

21. Kukhanova, M. K., Korovina, A. N. & Kochetkov, S. N. Human herpes simplex virus: Life cycle and development of inhibitors. *Biochemistry Biokhimia* **79**, 1635–1652 (2014).

22. Chouljenko, D. et al. Functional hierarchy of herpes simplex virus 1 viral glycoproteins in cytoplasmic virion envelopment and egress. *J. Virol.* **86**, 4262–4270 (2012).

23. Lee, H., Chouljenko, V., Chouljenko, D., Boudreaux, M. & Kousoulas, K. The herpes simplex virus type 1 glycoprotein D (gD) cytoplasmic terminus and full-length gD are not essential and do not function in a redundant manner for cytoplasmic virion envelopment and egress. *J. Virol.* **83**, 6115–6124 (2009).

24. Neubauer, A. & Osterrieder, N. Equine herpesvirus type 1 (EHV-1) glycoprotein K is required for efficient cell-to-cell spread and virus egress. *Virology* **329**, 18–32 (2004).

25. Mashchenko, A., Riblet, S. M., Zavala, G. & Garcia, M. In ovo vaccination of commercial broilers with a glycoprotein J gene-deleted strain of infectious laryngotracheitis virus. *Avian diseases* **57**, 523–531 (2013).

26. von Einem, J. et al. The truncated form of glycoprotein gp2 of equine herpesvirus 1 (EHV-1) vaccine strain KyA is not functionally equivalent to full-length gp2 encoded by EHV-1 wild-type strain Racl11. *Journal of virology* **78**, 3003–3013 (2004).

27. Rudolph, J. & Osterrieder, N. Equine herpesvirus type 1 devoid of gM and gp2 is severely impaired in virus egress but not direct cell-to-cell spread. *Virology* **293**, 356–367, https://doi.org/10.1006/viro.2001.1277 (2002).

28. Zhou, G., Galvan, V., Campadelli-Fiume, G. & Kotzman, B. Glycoprotein D or J delivered in trans blocks apoptosis in SK-N-SH cells induced by a herpes simplex virus 1 mutant lacking intact genes expressing both gD and gJ. *Journal of virology* **74**, 11782–11791 (2000).

29. Farnsworth, A. et al. Herpes simplex virus glycoproteins gB and gH function in fusion between the virion envelope and the outer nuclear membrane. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 10187 (2007).

30. Mettenleiter, T. C., Müller, F., Granzow, H. & Klupp, B. G. The way out: what we know and do not know about herpesvirus nuclear egress. *Cellular Microbiology* **15**, 170 (2013).

31. Cardone, G., Heymann, J. B., Cheng, N., Trus, B. L. & Steven, A. C. Procapsid Assembly, Maturation, Nuclear Exit: Dynamic Steps in the Production of Infectious Herpesviruses. *Advances in Experimental Medicine & Biology* **726**, 423 (2012).

32. Wu, Y. et al. Establishment of real-time quantitative reverse transcription polymerase chain reaction assay for transcriptional analysis of duck enteritis virus UL55 gene. *Virology journal* **8**, 1–13 (2011).

33. Datsenko, K. A. & Wanner, B. L. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 6640–6645, https://doi.org/10.1073/pnas.120163297 (2000).

34. Wu, Y. et al. Preliminary study of the UL55 gene based on infectious Chinese virulent duck enteritis virus bacterial artificial chromosome clone. *Virology journal* **14**, 78 (2017).

35. Cotta-de-Almeida, V., Schonhoff, S., Shibata, T., Leiter, A. & Snapper, S. B. A new method for rapidly generating gene-targeting vectors by engineering BACs through homologous recombination in bacteria. *Genome research* **13**, 2190–2194 (2003).

36. Guo, Y. et al. Anadit herpesvirus 1 CH virulent strain induces syncytium and apoptosis in duck embryo fibroblast cultures. *Vet. Microbiol.* **138**, 258–265 (2009).

37. Guiping, Y. et al. Preliminary study on duck enteritis virus-induced lymphocyte apoptosis in vivo. *Avian Dis.* **51**, 546–549 (2007).

38. Yuan, G. et al. Electron microscopic studies of the morphogenesis of duck enteritis virus. *Avian Dis.* **49**, 50–55 (2005).

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Author Contributions

YY, conceived, designed and carried out the experiments, analyzed the data and drafted the manuscript; TL, M.W. and A.C. conceived and designed the experiments, and revised the manuscript; D.Z., S.C., R.J., M.L., Q.Y., Y.W., X.Z., S.Z. and X.C. analyzed and interpreted the data. Y.L, Y.Y, and L.Z provided cells and reagents. All the authors reviewed the manuscript.

Additional Information

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