Virus Research 273 (2019) 197758

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Major capsid protein of *Autographa californica* multiple nucleopolyhedrovirus contributes to the promoter activity of the very late viral genes

Huimin Bai[a,1], Yangyang Hu[b,1], Xue Hu[c], Jing Li[d], Jingfang Mu[c], Yuan Zhou[c], Xinwen Chen[c], Yun Wang[c,*]

[a] Department of Basic Medicine and Forensic Medicine, Baotou Medical College, Baotou, China
[b] Shanghai Public Health Clinical Center, Fudan University, Shanghai, China
[c] State Key Lab of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China
[d] College of Pharmacy, Nankai University, Tianjin, China

ARTICLE INFO

Keywords:
AcMNPV
VP39
Polyhedrin
P10
BEVS
Very late viral gene promoter

ABSTRACT

The baculovirus expression vector system (BEVS) is one of the most powerful eukaryotic expression systems. Recombinant protein expression is usually controlled by promoters of the baculovirus very late genes (i.e., *polyhedrin* and *p10*); therefore, identifying novel regulatory factors for these promoters is key to increasing BEVS productivity. *Autographa californica* multiple *nucleopolyhedrovirus* (AcMNPV) is the viral vector most frequently used in BEVS. VP39 is the major nucleocapsid protein of AcMNPV and plays a pivotal role in nucleocapsid assembly in the nucleus. In this study, we found that knocking out *vp39* from the AcMNPV genome resulted in decreased protein abundance of polyhedrin and P10. Further assays revealed that the mRNA transcripts and the promoter activities of *polyhedrin* and *p10* were decreased in the absence of *vp39*, suggesting that VP39 contributes to the activity of the very late viral gene promoters and may represent a means of optimizing the current BEVS.

1. Introduction

Baculoviridae is a family of rod-shaped viruses that specifically infect insects. As large DNA viruses, baculovirus genes are divided into four groups according to their temporal expression patterns: immediate early, delayed early, late, and very late genes. Immediate early and delayed early genes are transcribed by host RNA polymerase II, while late and very late gene transcription is dependent on viral genome replication and virus-encoded RNA polymerase (Rohrmann, 2019a). A unique transcriptional cascade organizes the four groups of viral genes into a pyramid of biological magnification. The immediate early genes are located on the top of the pyramid and serve as the general transactivators for the downstream genes. The very late genes are located at the bottom of the pyramid (Rohrmann, 2019c). Products of the immediate early genes (e.g., IE-1) and some late genes (e.g., VLF-1, FP25, PK-1, P6.9) work synergistically or independently to enhance the very late gene transcription (Fan et al., 1996; Harrison et al., 1996; Li et al., 2015; Mistretta and Guarino, 2005), which leads to overexpression of the very late gene products (i.e., P10 and Polyhedrin (Polh)). Taking advantage of this unique transcriptional cascade, the baculovirus expression vector system (BEVS) uses very late gene promoters to control exogenous gene expression and is one of the most frequently used eukaryotic expression systems (Chambers et al., 2018; Felberbaum, 2015).

As the prototype of baculoviruses, *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is the most heavily researched baculovirus, and several commercially available BEVS have been developed based on AcMNPV (Luckow et al., 1993).

VP39, one of the most abundant late viral gene products, is distributed evenly on the viral nucleocapsid surface and is therefore designated the major capsid protein of AcMNPV (Thiem and Miller, 1989). As a structural protein, VP39 reportedly interacts with several viral and cellular proteins, including FP25 (Braunagel et al., 1999), 38 K (Wu et al., 2008), IE-2 (Wu et al., 2013), Kinesin-1 (Danquah et al., 2012), and actin (Lanier and Volkman, 1998). The contribution of VP39 to AcMNPV replication includes but is not limited to directing nucleocapsid assembly in the nucleus and transporting the nucleocapsid from

* Corresponding author at: State Key Lab of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, China.
E-mail address: wangyun@wh.iov.cn (Y. Wang).
[1] Authors contributed equally to this work.

https://doi.org/10.1016/j.virusres.2019.197758
Received 27 August 2019; Received in revised form 12 September 2019; Accepted 13 September 2019
Available online 18 September 2019
0168-1702/

the nucleus to the cell membrane for budding (Danquah et al., 2012).

In this report, we found that knocking out *vp39* from the AcMNPV genome resulted in a drastic reduction of the very late gene viral products. Further assays revealed that *vp39* knockout decreases the promoter activity of very late viral genes. This finding demonstrates that VP39 plays a novel role in modulating very late gene expression and may represent a means of optimizing the current BEVS.

2. Materials and methods

2.1. Construction of plasmids and recombinant bacmids

To knock out *vp39* from AcMNPV bacmid (bMON14272, Invitrogen), the λ red combination system was employed as previously described (Wang et al., 2008). A chloramphenicol acetyl transferase (CAT) expression cassette was cloned by polymerase chain reaction (PCR) using primer set vp39-cat-F/vp39-cat-R (Supplementary table 1) and inserted into *vp39*'s open reading frame (ORF) to disrupt *vp39*. The resulting bacmid was designated bAc^{vp39ko}. Recombinant bacmids were constructed by transposing the indicated gene cassettes into bacmid using the Bac-to-Bac System (Invitrogen). All the bacmid constructs were verified by PCR and the right constructs were extracted using the NucleoBond Xtra Midi-prep kit (Macherey-Nagel) according to the manufacturer's protocol.

Donor plasmids encoding dual-luciferase reporter were constructed with standard molecular cloning protocols. Briefly, the renilla luciferase (R-Luc) expression cassette (Fig. 5A) was cloned and inserted into Bst1107I site of donor plasmid pFastBac-dual (Invitrogen). Gene encoding firefly luciferase (F-Luc) was inserted into downstream region of indicated viral gene promoters. Promoters of *p10* (P*p10*) and *polh* (P*polh*) are provided in pFastBac-dual. For promoters of *p6.9* (P*p6.9*) and *ie1* (P*ie1*), Primer sets P*p6.9*-F/R and P*ie1*-F/R (Supplementary table 1) were used to cloned the two viral gene promoters from AcMNPV. The resulting promoters were then inserted into pFastBac-dual between SmaI and NheI.

2.2. Cell culture and viral infectivity assay

Sf9 cells were maintained in Grace's medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) at 27 °C.

Sf9 cells were seeded on a 6-well plate one day prior to transfection. Plasmid or bacmid transfection was performed using Cellfectin II (Invitrogen) according to the manufacturer's instructions. For the infectivity assay, the viral supernatants were collected at 72 h post-transfection (hpt) and added to fresh Sf9 cells to initiate secondary infection. After a 1-h incubation at 4 °C, viral supernatants were removed, and fresh complete medium was added to the cells.

2.3. Confocal microscopy

Cells were seeded on coverslips and incubated overnight. After transfection and incubation, cells were washed and fixed using 3.7% paraformaldehyde for 10 min at room temperature followed by incubation with Hoechst 33258 (Invitrogen) for 5 min. The coverslips were then observed with a Leica SP2000 laser confocal microscope and the attached camera was set to "auto-mode" to capture pictures.

2.4. Transmission electron microscopy (TEM) assay

Sf9 cells (5×10^5) transfected with the indicated bacmids were harvested at 72 hpt. The cells were fixed in 2.5% glutaraldehyde for 3 h at 4 °C and treated with 1% osmic acid for 2 h. The samples were then dehydrated, embedded in Epon 812, cut into ultrathin sections, and stained with uranyl acetate and lead citrate. All the samples were observed with a Tecnai G2 TEM at 75 kV.

2.5. Western blot assay

Cell lysates containing 30 μg of total protein from recombinant bacmids transfected cells were subjected to Western blot analysis. The membrane with transferred proteins was blocked in 5% bovine serum albumin (Beyotime) overnight at 4 °C and incubated with the anti-Polh antibody (a kind gift from Prof. Kai Yang of Sun Yat-sen University) for 1 h at room temperature followed by washing the membrane 3 times with a generous amount of TBST (50 mM Tris. HCl, pH = 7.4, 150 mM NaCl, 0.1% Tween 20). The membrane was then incubated with the HRP-conjugated goat anti-rabbit secondary antibody (1:5000 dilution, Abcam) for 1 h at room temperature before development with West-Pico ECL reagent (Pierce). The housekeeping gene product ERK was used as an endogenous control.

2.6. Quantitative PCR (qPCR)

Total DNA and RNA were extracted from bacmid-transfected cells at 12, 24, 48, 72, and 96 hpt using Trizol reagent (Invitrogen) according to the manufacturer's protocols. The viral DNA was digested with DpnI (NEB) overnight to remove the bacmid template before qPCR was performed. Primer set Q-65972 F/Q-66072R (Supplementary table 1) was used to quantify the viral genomic DNA. The viral total RNA was quantified and subjected to a one-step qRT-PCR assay using the QuantiTect SYBR green RT-PCR kit (Qiagen). Cellular 28 s rRNA transcripts were used as endogenous reference. The primer sets (Q-*ie0*-F/R, Q-*ie2*-F/R, Q-*ac9*-F/R, Q-*p10*-F/R, Q-*polh*-F/R, Q-*28 s*-F/R) used in qRT-PCR were listed in Supplementary table 1. A two-tailed *t*-test was used to compare the statistical difference between bAc^{vp39ko}- and bAc^{c42ko}-derived viral DNA and gene transcripts.

2.7. Luciferase assay

Bacmids encoding dual-luciferase reporter were transfected into Sf9 cells. The luciferase assay was performed 24 hpt using the Dual Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions. A two-tailed *t*-test was used to compare the promoter activities of different viral genes between bAc^{vp39ko} and bAc^{c42ko}.

3. Results

3.1. Construction of recombinant bacmids

To disrupt *vp39* without affecting the regulatory sequences of neighboring genes, a CAT expression cassette was inserted into the AcMNPV genome (GenBank accession number NC_001623.1) between nucleotide (nt) 76187 and 75928, which substitutes the ORF of *vp39* from nt 391 to 650 (Fig. 1A). A PCR was employed to verify the substitution. As shown in Fig. 1B, PCR with the primer sets (Supplementary table 1) cat-F/vp39-R generated a 0.9-kb DNA band, vp39-F/cat-R generated a 1.0-kb band, and vp39-F/vp39-R generated a 1.9-kb band, which demonstrated that the CAT expression cassette successfully disrupted the coding sequence of VP39. The resulting *vp39*-nulled bacmid bearing transposed expression cassettes of enhanced green florescent protein (EGFP) and Polh was designated bAc^{vp39ko} (Fig. 1C).

To test whether the neighboring genes were affected by *vp39* disruption, a rescue bacmid was constructed by transposing a VP39 expression cassette controlled by its native promoter to the *polh* locus of bAc^{vp39ko}. The resulting rescue bacmid was designated $bAc^{vp39res}$ (Fig. 1C). To test the viral infectivity, bAc^{vp39ko}, $bAc^{vp39res}$, and bAc^{wt} (a wild-type AcMNPV bacmid bearing expression cassettes of EGFP and Polh) were transfected into Sf9 cells. EGFP was limited to some isolated cells in bAc^{vp39ko} transfection, even at 72 hpt, as shown by the fluorescence microscopic assay (Fig. 2A). In contrast, both $bAc^{vp39res}$ and bAc^{wt} transfection generated apparently more dispersed EGFP

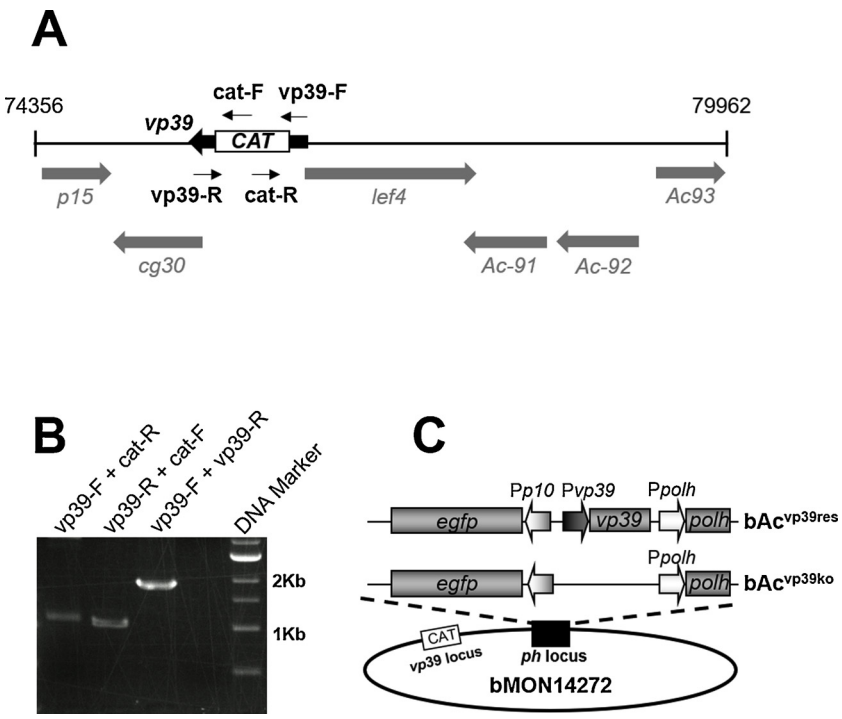


Fig. 1. Construction of recombinant bacmids. (A). Construction of a *vp39* knockout bacmid. A CAT expression cassette (white box) was inserted into the *vp39* locus (black box) of bMON14272 using λ-red recombineering. Arrows indicate the primers used in validation of *vp39* knockout. (B). Validation of *vp39* knockout by PCR. PCR with the primer set CAT-F/VP39-R generated a 0.9 kb DNA band, VP39-F/CAT-R generated a 1.0 kb band, and VP39-F/VP39-R generated a 1.9 kb band. (C). A diagram of recombinant bacmids prepared in the study.

fluorescence at 72 hpt (Fig. 2A). Viral supernatants were collected at 72 hpt and incubated with fresh Sf9 cells. At 72 h post-infection (hpi), no EGFP-positive cells were present in vAc^{vp39ko} (bAc^{vp39ko}-derived virus) supernatant infection (Fig. 2A), indicating that *vp39* disruption from the viral genome is lethal to AcMNPV replication. As expected, both vAc^{vp39res} and vAc^{wt} (bAc^{vp39res}- and bAc^{wt}-derived virus) supernatant infection resulted in EGFP expression in almost all cells within 72 hpi (Fig. 2A). A one-step virus growth curve assay demonstrated that vAc^{vp39res} and vAc^{wt} showed similar virus propagation kinetics (Fig. 2B), showing that a VP39 expression cassette restored to the *polh* locus successfully rescued vAc^{vp39ko} infectivity, and *vp39* disruption had no impact on its neighboring genes.

3.2. Knockout of vp39 resulted in a few polyhedra phenotype

When the recombinant bacmids transfected cells were observed under a light microscope, we noticed that few polyhedra (FP) were present in the bAc^{vp39ko}-transfected cells, even at 96 hpt, presenting a sharp contrast to the cells transfected with bAc^{wt}, which showed abundant polyhedra (Fig. 3A). Since VP39 is a capsid protein and is replication-essential, we selected bAc^{c42ko}, a replication-defective virus lacking the capsid protein C42 (Vanarsdall et al., 2007; Wang et al., 2008), to serve as the control. Compared to bAc^{vp39ko}, bAc^{c42ko} apparently generated more polyhedra (Fig. 3A), indicating that FP was not due to the loss of infectivity of vAc^{vp39ko}. To further verify the FP phenotype, a TEM assay was performed. In both bacmid-transfected cells, the electron-dense virogenic stroma was observed in an enlarged nuclear region (Fig. 3B), suggesting that viral DNA is under replication in the nucleus. In accordance with the light microscopic assay, FP was present in the bAc^{vp39ko}-transfected cells, and abundant polyhedra were observed in the bAc^{wt}-transfected cells (Fig. 3B). In addition to polyhedra, P10 was also seen to sharply decrease in the bAc^{vp39ko}-transfected cells (Fig. 3B), suggesting that *vp39* knockout resulted in a reduction of the very late viral gene products. As supporting evidence, a western blot analysis showed a reduced protein abundance of Polh in the bAc^{vp39ko}-transfected cells, which was in contrast to the results obtained for bAc^{c42ko}-transfected cells (Fig. 3C).

3.3. VP39 modulates the very late viral gene expression at the transcriptional level

To investigate how VP39 modulates very late viral gene expression, quantitative reverse transcription PCR (qRT-PCR) was performed to test the influence of *vp39* knockout on viral gene transcription. Compared to bAc^{c42ko}, bAc^{vp39ko} generated similar kinetics of *ie2* and *ie0* transcripts from 12 hpt to 72 hpt (Fig. 4A, B), indicating that *vp39* has no impact on early viral gene transcription. Similar transcriptional kinetics also occurred for *ac9 (orf1629)* (Fig. 4C), a late viral gene that transcribed by the virus-encoded RNA polymerase (Pham and Sivasubramanian, 1992), suggesting that viral RNA polymerase could normally transcribe viral late gene. However, the very late viral gene transcripts, including *p10* and *polh*, were decreased sharply from 24 hpt to 48 hpt (Fig. 4D, E), showing that *vp39* knockout impaired very late viral gene transcription.

3.4. VP39 modulation of very late gene transcription is viral genome replication independent

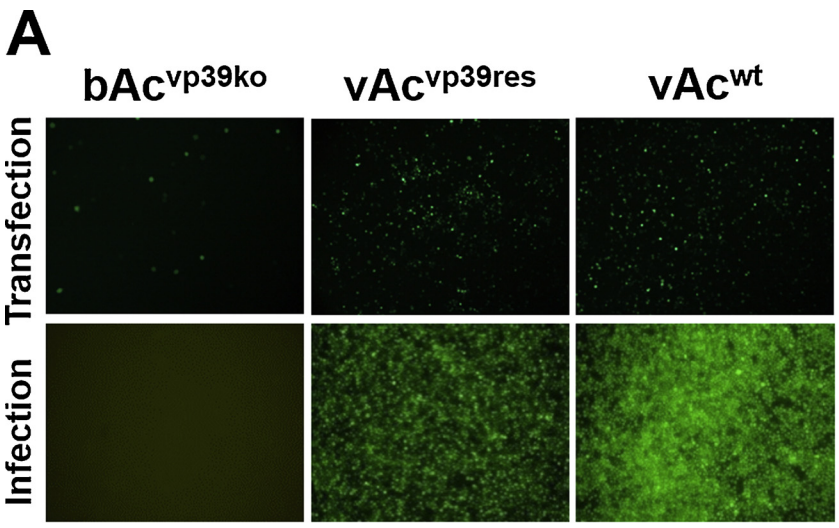
Viral genome replication is required for AcMNPV late and very late gene transcription (Rohrmann, 2019a). Deletion of *vp39* impaired very late gene transcription, prompting us to exclude the possibility that *vp39* modulates viral genome replication.

Sf9 cells were transfected with bAc^{vp39ko} and bAc^{c42ko}, respectively. qPCR assays showed that the copy number of bAc^{vp39ko} genomic DNA increased at a similar speed compared to bAc^{c42ko} (Fig. 4F), implying that *vp39* knockout has no influence on viral genome replication.

3.5. Very late viral gene promoter activity is impaired by vp39 knockout

Since viral genome replication was not impaired and virus-encoded RNA polymerase still normally transcribed late viral gene upon *vp39* knockout, the most possible pathway for *vp39* modulation of very late gene transcription is through regulating its promoter activity.

To assess the impact of *vp39* knockout on viral gene promoter activities, a series of dual-luciferase reporter vectors were constructed (Fig. 5A). In these vectors, a F-Luc expression cassette is controlled by a viral gene promoter (P*polh*, P*p10*, P*p6.9*, or P*ie1*), and an EGFP-fused R-



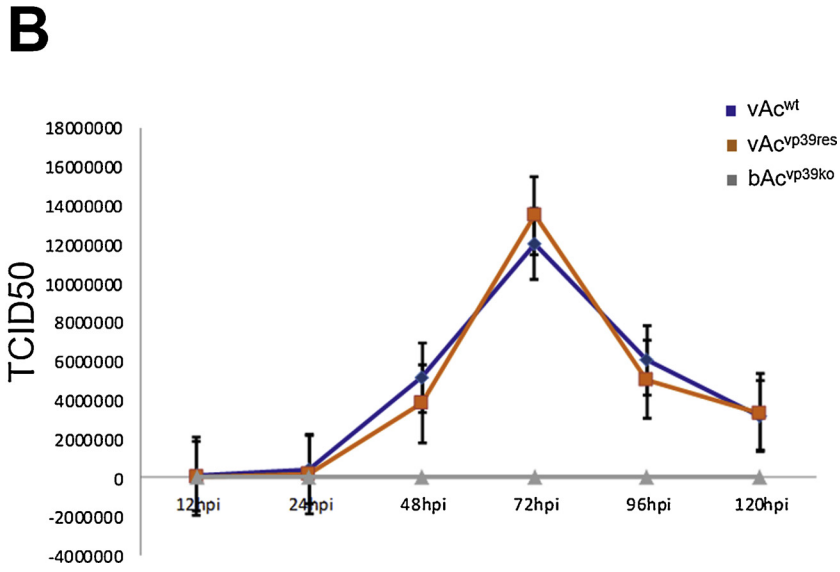


Fig. 2. Impact of *vp39* knockout on viral replication. (A). Viral infectivity assay. Sf9 cells were transfected with the indicated bacmids. At 72 hpt, viral supernatants were collected and added to fresh SF9 cells to initiate secondary infection. (B). One-step viral growth curve. Viral stock of vAc^{wt} and $vAc^{vp39res}$ (multiplicity of infection = 5), and viral supernatant of bAc^{vp39ko} were used to infect Sf9 cells and generated the one-step growth curve. The viral titers were determined by monitoring EGFP expression at the indicated times. The data points indicate the averages of triplicate transfections, and the error bars represent standard deviations. TCID50, 50% tissue culture infective dose.

Luc expression cassette controlled by an hsp70 promoter (P*hsp70*) serves as the control. Both luciferase expression cassettes were inserted into pFastBac-dual, and the resulting donor vectors were then transposed to either bAc^{vp39ko} or bAc^{c42ko}.

Sf9 cells were transfected with the indicated bacmids bearing dual-luciferase reporter. At 24 hpt, cells were lysed and subjected to luciferase activity assay. Viral gene promoter activity was normalized to the P*hsp70*. Among the tested viral gene promoters, P*ie1* and P*p6.9* showed no significant difference in the presence or absence of *vp39* (Fig. 5B). However, P*polh* and P*p10* activities showed a significant reduction in the *vp39*-knockout bacmids compared to the *c42*-knockout bacmids. This phenotype suggested that *vp39* plays an active role in upregulating very late viral gene transcription, and the FP phenotype induced by *vp39* knockout is due to reduced P*polh* activity and not a mutant virus-encoded RNA polymerase.

4. Discussion

Harboring two robust very late viral gene promoters, AcMNPV-based BEVS is one of the most frequently used eukaryotic expression systems. Identifying more factors involved in modulating the very late viral gene promoter activity is therefore the key to optimizing BEVS. In this report, we identified that VP39, the major capsid protein of AcMNPV, contributes to the activity of very late viral gene promoter activity.

To assess the impact of VP39 on viral gene expression, qRT-PCR and luciferase assay were employed. Although both approaches let to the same conclusion that *vp39* knockout down-regulates very late viral genes expression, it appeared that the difference between very late viral gene promoter activity of bAc^{vp39ko} and bAc^{c42ko} revealed by luciferase assay (Fig. 5B) is smaller compared to the difference between very late viral gene transcripts of bAc^{vp39ko} and bAc^{c42ko} quantified by qRT-PCR assay (Fig. 4D, E). As generally known, the amount of gene transcripts is determined by both gene promoter activity and mRNA stability, meanwhile, the luciferase activity is determined only by gene promoters as they controlled the same F-luc. Therefore, different mRNA stabilities of *p10* and *polh* could be responsible for the "discrepancy" between Figs. 4D, E and 5 B. In our opinion, such a "discrepancy" is acceptable between qRT-PCR and luciferase assay and supports the conclusion that FP phenotype induced by *vp39* knockout is attributed to a compromised very late viral gene promoter activity.

The template of very late viral gene transcription is supposed to be derived from the viral DNA that is not incorporated into the nucleocapsid (Rohrmann, 2019b). A recent study performed by Katsuma S

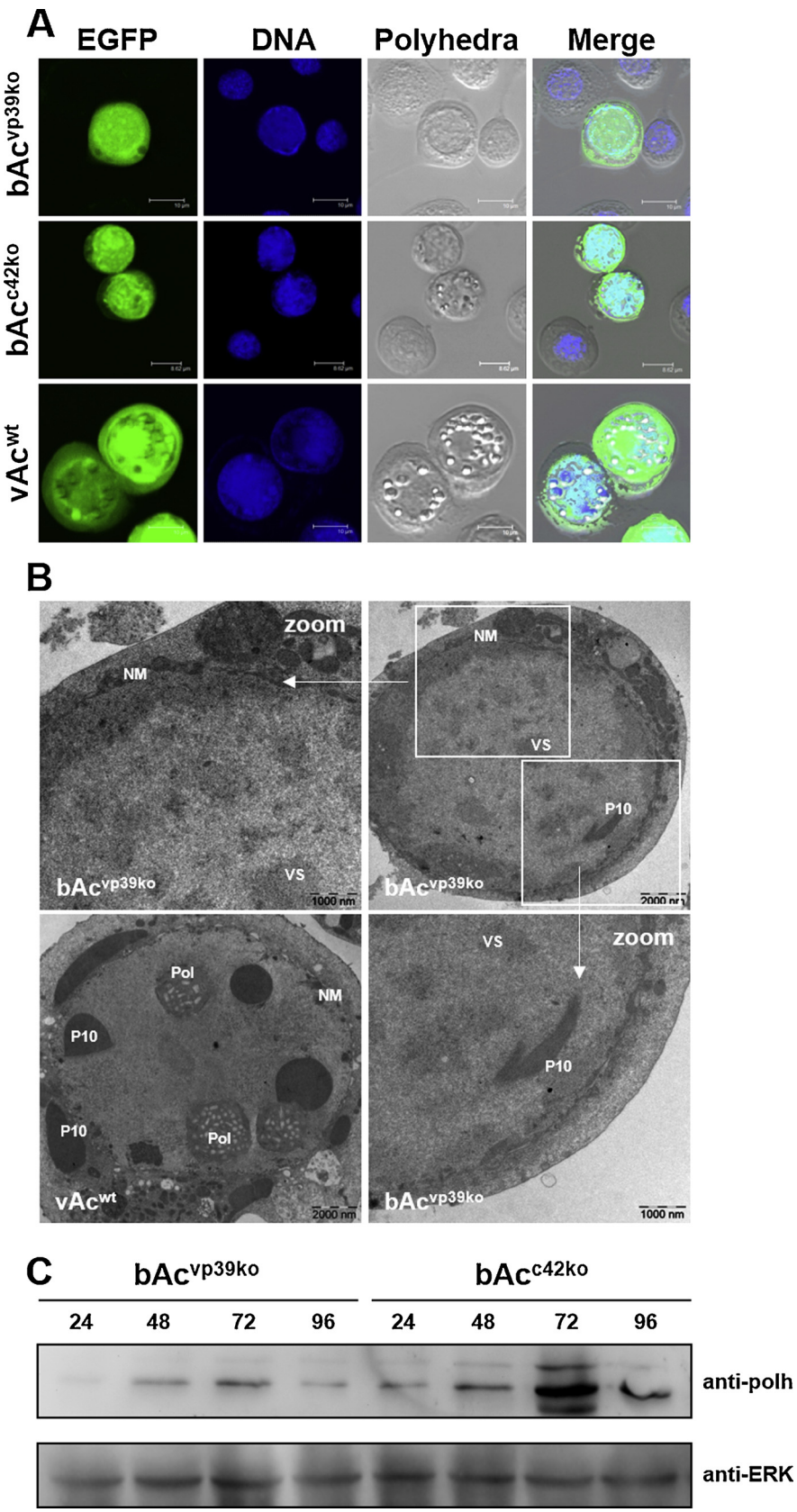


Fig. 3. FP phenotype induced by *vp39* knockout. (A). Confocal microscopic assay of bacmid-transfected cells. Sf9 cells were transfected with bAc^{wt}, bAc^{vp39ko} and bAc^{c42ko}. At 72 hpt, cells were fixed and observed with a Leica SP2000 Laser Confocal Microscope. (B). TEM assay of bacmid-transfected cells. Sf9 cells were transfected with bAc^{vp39ko} or bAc^{wt}. At 96 hpt, cells were collected and subjected to TEM assay with a Tecnai G2 transmission electron microscope. Pol represents the polyhedra, P10 represents the P10 protein, and VS represents the virogenic stroma. (C). Abundance of Polh. Sf9 cells were transfected with two replication-defective bacmids, bAv^{vp39ko} and bAc^{c42ko}. At the indicated time points, cells were lysed and subjected to Western blot analysis using anti-Polh and anti-ERK (endogenous control).

et al. showed that a point mutation at glycine 276 (G276S) of VP39 of Bombyx mori nuclear polyhedrosis virus (BmNPV) resulted in reduced transcription and expression of the two very late viral gene products (Katsuma and Kokusho, 2017). They also showed that the $VP39^{G276S}$ mutation induced electron-dense aggregates in the virological stroma, and they proposed that these aggregates could incorporate viral DNA, which could serve as the template for the very late viral gene transcription, thereby leaving less “free” viral DNA for the virus-encoded

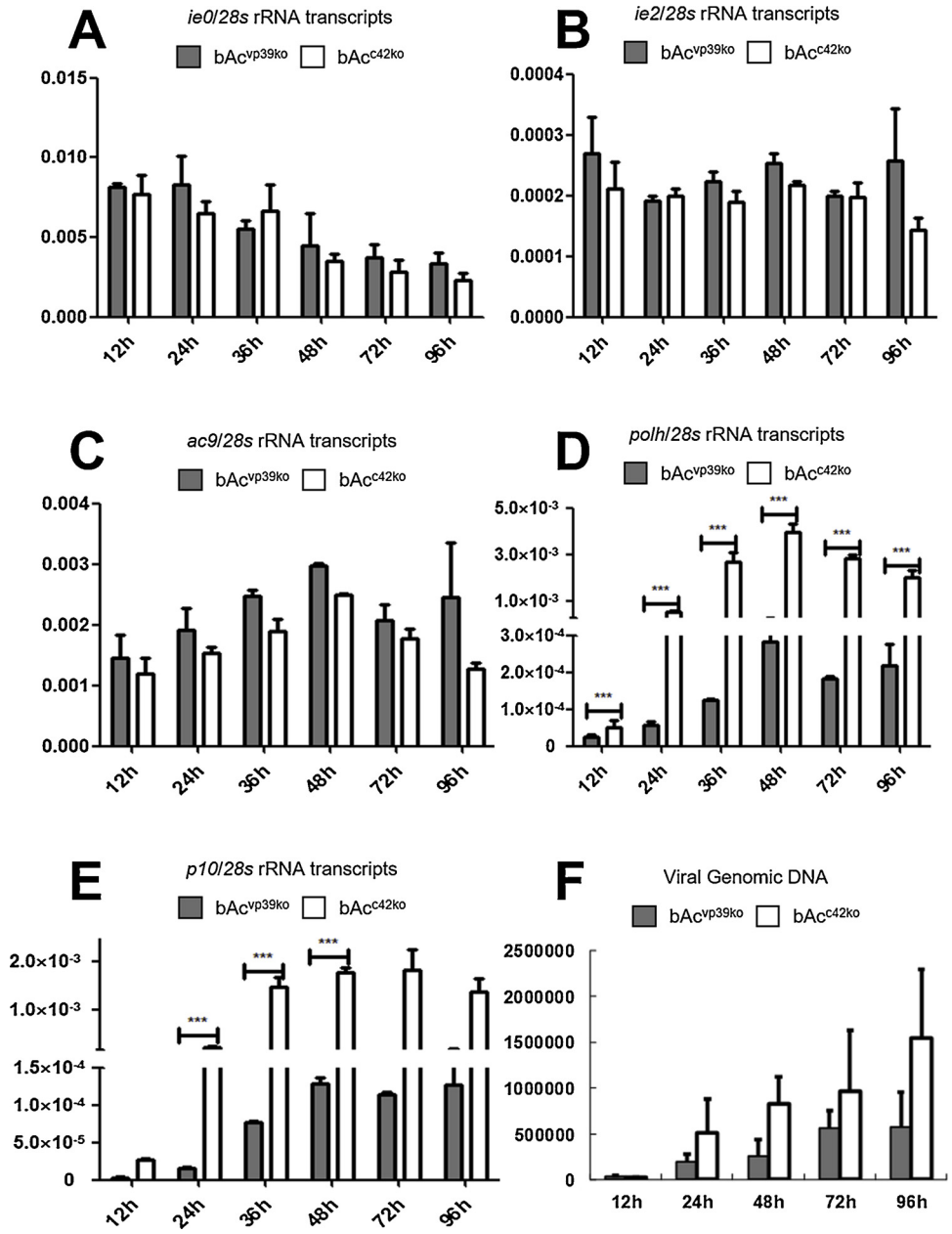


Fig. 4. Impact of *vp39* knockout on viral genome replication and gene transcription. Total DNA and RNA were extracted from the cells transfected with recombinant bacmids (bAc^{c42ko} and bAc^{vp39ko}) at the indicated time points. qRT-PCR (A–E) or qPCR (F) was performed to quantify immediate early (A, B), late (C), very late viral gene (D, E) expression, and viral genomic DNA replication (F). Values are displayed as averages of indicated viral gene transcripts compared to cellular 28 s rRNA transcript levels (endogenous reference) from three independent experiments, with error bars indicating standard deviations. *** ($P < 0.001$).

RNA polymerase to transcribe *polh* and *p10*. However, such electron-dense aggregates were not present in our TEM assay, indicating that the FP phenotype induced by *vp39* knocking out from AcMNPV may be due to other reasons. Actually, knocking out *vp39* is supposed to completely abolish the viral DNA encapsidation process, which was supported by our TEM observation that nucleocapsids were absent in the cell harboring vAc^{vp39ko} (Fig. 3B), and thus leave more "free" viral DNA for the virus-encoded RNA polymerase to transcribe very late viral genes. As our evidence indicated that the activity of virus-encoded RNA polymerase remained intact upon *vp39* knockout, the most plausible scenario is that VP39 serves as a transcriptional activator specifically for the promoters of very late viral genes.

In conclusion, our work demonstrated that VP39 is involved in modulating very late baculoviral gene expression. Further research is necessary to elucidate how VP39 manipulates the promoter activity of very late viral genes.

Compliance

This article does not contain any studies with human or animal subjects performed by any of the authors. The authors declare no conflict of interest.

Author contributions

Conceptualization, Xinwen Chen and Yun Wang; Formal analysis, Huimin Bai, Jingfang Mu and Yun Wang; Funding acquisition, Huimin Bai, Jingfang Mu and Yun Wang; Investigation, Huimin Bai, Yangyang Hu, Xue Hu and Jing Li; Methodology, Huimin Bai, Yangyang Hu, Xue Hu and Yun Wang; Resources, Yuan Zhou; Supervision, Xinwen Chen

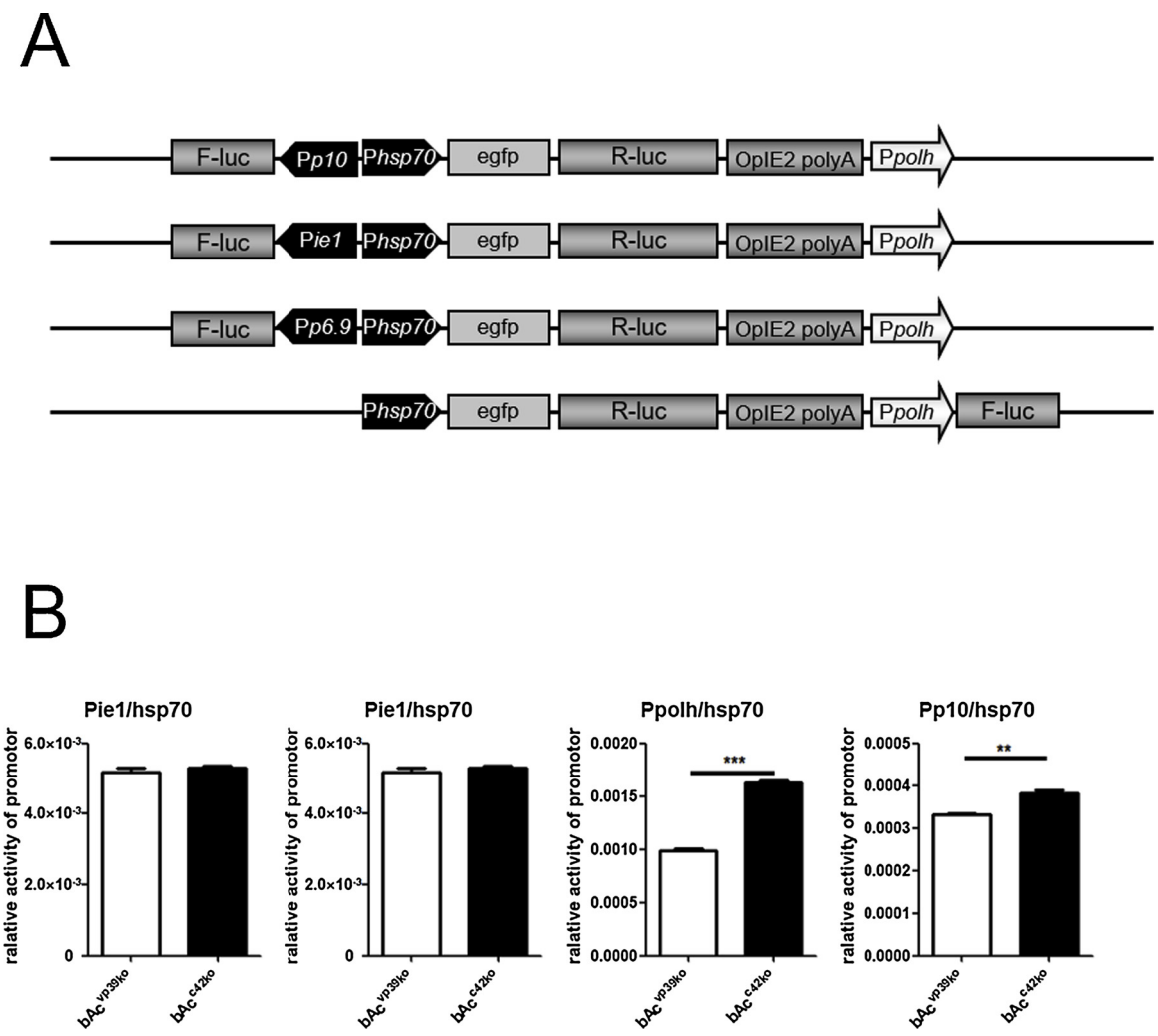


Fig. 5. Impact of *vp39* knockout on viral gene promoter activity. (A). Diagram of donor plasmids encoding the dual-luciferase reporter. Different viral gene promoters were cloned upstream of F-Luc, and a *hsp70* promoter-controlled R-Luc expression cassette served as an endogenous control. The donor plasmids encoding the dual-luciferase reporter were transposed to bAc^{c42ko} and bAc^{vp39ko}, respectively. (B). Luciferase assay of different viral gene promoters. Sf9 cells were transfected with bAc^{vp39ko} or bAc^{c42ko} bearing a transposed dual-luciferase reporter. At 24 hpt, cells were lysed and subjected to luciferase assay. The value indicated the averages of triplicate transfections, and the error bars represent standard deviations. ** ($P < 0.01$),*** ($P < 0.001$).

and Yun Wang; Validation, Huimin Bai; Writing – original draft, Huimin Bai and Yangyang Hu; Writing – review & editing, Yun Wang.

Acknowledgements

We are grateful to the core facility of the Wuhan Institute of Virology for providing technical support for TEM work and confocal analysis. This work was supported by grants from the National Natural Science Foundation of China (31770170, 31800140), the Natural Science Foundation of Inner Mongolia (2015BS0301), and the Doctoral Research Work Initializing Funding of Baotou Medical College (BSJJ201624).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.virusres.2019.197758.

References

Braunagel, S.C., Burks, J.K., Rosas-Acosta, G., Harrison, R.L., Ma, H., Summers, M.D., 1999. Mutations within the Autographa californica nucleopolyhedrovirus FP25K gene decrease the accumulation of ODV-E66 and alter its intranuclear transport. J. Virol. 73 (10), 8559–8570.

Chambers, A.C., Aksular, M., Graves, L.P., Irons, S.L., Possee, R.D., King, L.A., 2018. Overview of the baculovirus expression system. Curr. Protoc. Protein Sci. 91, 541–546.

Danquah, J.O., Botchway, S., Jeshtadi, A., King, L.A., 2012. Direct interaction of baculovirus capsid proteins VP39 and EXON0 with kinesin-1 in insect cells determined by fluorescence resonance energy transfer-fluorescence lifetime imaging microscopy. J. Virol. 86 (2), 844–853.

Fan, X., Thirunavukkarasu, K., Weaver, R.F., 1996. Temperature-sensitive mutations in the protein kinase-1 (pk-1) gene of the Autographa californica nuclear polyhedrosis virus that block very late gene expression. Virology 224 (1), 1–9.

Felberbaum, R.S., 2015. The baculovirus expression vector system: a commercial manufacturing platform for viral vaccines and gene therapy vectors. Biotechnol. J. 10 (5), 702–714.

Harrison, R.L., Jarvis, D.L., Summers, M.D., 1996. The role of the AcMNPV 25K gene, "FP25," in baculovirus polh and p10 expression. Virology 226 (1), 34–46.

Katsuma, S., Kokusho, R., 2017. A conserved glycine residue is required for proper functioning of a baculovirus VP39 protein. J. Virol. 91 (6).

Lanier, L.M., Volkman, L.E., 1998. Actin binding and nucleation by Autographa california M nucleopolyhedrovirus. Virology 243 (1), 167–177.

Li, A., Zhao, H., Lai, Q., Huang, Z., Yuan, M., Yang, K., 2015. Posttranslational modifications of baculovirus protamine-like protein P6.9 and the significance of its hyperphosphorylation for viral very late gene hyperexpression. J. Virol. 89 (15), 7646–7659.

Luckow, V.A., Lee, S.C., Barry, G.F., Olins, P.O., 1993. Efficient generation of infectious recombinant baculoviruses by site-specific transposon-mediated insertion of foreign genes into a baculovirus genome propagated in Escherichia coli. J. Virol. 67 (8), 4566–4579.

Mistretta, T.A., Guarino, L.A., 2005. Transcriptional activity of baculovirus very late

factor 1. J. Virol. 79 (3), 1958–1960.
Pham, D.Q., Sivasubramanian, N., 1992. Sequence and in vitro translational analysis of a 1629-nucleotide ORF in Autographa californica nuclear polyhedrosis virus strain E2. Gene 122 (2), 345–348.
Rohrmann, G.F., 2019a. Baculovirus late transcription. In: Rohrmann, G.F. (Ed.), Baculovirus Molecular Biology, Bethesda (MD).
Rohrmann, G.F., 2019b. DNA replication and genome processing. In: Rohrmann, G.F. (Ed.), Baculovirus Molecular Biology, Bethesda (MD).
Rohrmann, G.F., 2019c. Early events in infection: virus transcription. In: Rohrmann, G.F. (Ed.), Baculovirus Molecular Biology. National Center for Biotechnology Information (US), Bethesda (MD).
Thiem, S.M., Miller, L.K., 1989. Identification, sequence, and transcriptional mapping of the major capsid protein gene of the baculovirus Autographa californica nuclear polyhedrosis virus. J. Virol. 63 (5), 2008–2018.
Vanarsdall, A.L., Pearson, M.N., Rohrmann, G.F., 2007. Characterization of baculovirus constructs lacking either the Ac 101, Ac 142, or the Ac 144 open reading frame. Virology 367 (1), 187–195.
Wang, Y., Wang, Q., Liang, C., Song, J., Li, N., Shi, H., Chen, X., 2008. Autographa californica multiple nucleopolyhedrovirus nucleocapsid protein BV/ODV-C42 mediates the nuclear entry of P78/83. J. Virol. 82 (9), 4554–4561.
Wu, W., Liang, H., Kan, J., Liu, C., Yuan, M., Liang, C., Yang, K., Pang, Y., 2008. Autographa californica multiple nucleopolyhedrovirus 38K is a novel nucleocapsid protein that interacts with VP1054, VP39, VP80, and itself. J. Virol. 82 (24), 12356–12364.
Wu, Y., Wu, Y., Wu, Y., Tang, H., Wu, H., Zhang, G., Wang, W., 2013. Screening of candidate proteins interacting with IE-2 of Bombyx mori nucleopolyhedrovirus. Mol. Biol. Rep. 40 (10), 5797–5804.