Virus Research 135 (2008) 220–229

Contents lists available at ScienceDirect

Virus Research

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

Characterization of *Bombyx mori* nucleopolyhedrovirus with a deletion of *bm118*

Zhang-Nv Yang[a,1], Hai-Jun Xu[a,1], Enoch Y. Park[b], Chuan-Xi Zhang[a,*]

[a] *Institute of Insect Science, Zhejiang University, 268 Kaixuan Road, Hangzhou 310029, Zhejiang, China*
[b] *Integrated Bioscience Section, Graduate School of Science and Technology, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan*

ARTICLE INFO

Article history:
Received 29 February 2008
Received in revised form 25 March 2008
Accepted 25 March 2008
Available online 7 May 2008

Keywords:
Bombyx mori
Nucleopolyhedrovirus
ORF118
BV production
Nucleocapsids maturation

ABSTRACT

Bombyx mori nucleopolyhedrovirus (BmNPV) ORF118 (*bm118*) is homologous to *Autographa californica* nucleopolyhedrovirus (AcMNPV) ORF142, one of the core genes existing in all baculovirus genomes sequenced to date, suggesting that Bm118 plays a critical role in viral infection. In this study, the primary role of Bm118 was investigated by using homologous recombination in *Escherichia coli* to generate a *bm118* knockout bacmid containing the BmNPV genome. In addition, the *bm118* rescue bacmid was constructed by transposing a *bm118* gene cassette into the *polh* locus of the *bm118* knockout bacmid. Transfection assays demonstrated that the *bm118* knockout bacmid was incapable of producing budded virion (BV). Nevertheless, this defect could be partially recovered by a rescue bacmid. Electron microscopy analysis revealed that the *bm118* knockout produced aberrant capsids characterized by translucent, elongated nucleocapsids present as bundles within the nuclei. This construct also produced polyhedra lacking virions. These results reveal that Bm118 is essential for BV production and nucelocapsid maturation.

1. Introduction

Baculoviruses are pathogenic to most arthropods, particularly insects of the order Lepidoptera. Nucleopolyhedroviruses (NPVs), a genus of *Baculoviridae*, typically produce two virion phenotypes during their unique biphasic life cycle: occlusion derived virion (ODV) and budded virion (BV). The ODV transmits infection through an oral route, whereas the BV is responsible for causing systemic infection (Keddie et al., 1989). After occlusion bodies are ingested by susceptible insects, the ODVs are liberated in the highly alkaline digestive fluids of the midgut (Granados, 1978) and enter the midgut cells through the fusion of the virion envelope with the brush (Blissard and Rohrmann, 1990; Horton and Burand, 1993). They subsequently move into the nucleus, where viral transcription, DNA replication, and assembly of progeny nucleocapsids (NCs) occur. Within the nucleus, DNA replication associated with capsid assembly occurs on virogenic stroma, which are induced by virus infection and involved in progeny virion assembly (Young et al., 1993). Newly replicated viral DNA is condensed and thought to be packaged into a preformed capsid sheath to form NCs (Lu et al., 1997). During the early phase of infection, NCs are exported from the nucleus to the cytoplasm, and they acquire a loosely adhering envelope when budding through a modified plasma membrane to produce BVs (Volkman, 1986, 1997; Monsma et al., 1996). BVs initiate systemic infection among the host tissues. As infection proceeds to very late phase, NCs are exported to form ODVs. After cells disintegrate, they are released into the environment to cause secondary infection. The two viral forms are efficient for the natural propagation of the occluded baculoviruses.

Since the complete genome of baculovirus BmNPV (T3 strain) was determined (Gomi et al., 1999), extensive studies have focused on understanding the function of individual genes. BmNPV orf118 (*bm118*) is homologous to orf142 of *Autographa californica* nucleopolyhedrovirus (AcMNPV), one of the baculovirus core genes (Herniou et al., 2003) conserved among all sequenced baculovirus genome available to date, strongly suggesting that the gene plays a critical role in the infection cycle of baculovirus. Its counterparts, orf142 from AcMNPV and orf30 from *Culex nigripalpus* nucleopolyhedrovirus (CuniNPV), have been identified as components of ODVs using GeLC–MS/MS and MALDI-TOF MS (Braunagel et al., 2003; Perera et al., 2007). Recently, the Ac142 gene was further identified to be structural protein of BVs in two reports. In one report, however, cells transfected with an *ac142* knockout bacmid produced normal-appearing nucleocapsids that appeared to be unable to bud out of the cells (McCarthy et al., 2007), while another report

* Corresponding author. Tel.: +86 571 86971697; fax: +86 571 86971697.
E-mail address: chxzhang@zju.edu.cn (C.-X. Zhang).
[1] These authors contributed equally to this work.

0168-1702/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2008.03.016

(Vanarsdall et al., 2007) showed that defective-appearing nucleocapsids were produced.

The BmNPV genome is over 90% identical to about three-quarters of the genome of AcMNPV (Gomi et al., 1999), and the *bm118* gene shares 98% identity in amino acid sequence with Ac142. To examine the phenotype caused by deletion of this gene in BmNPV, we generated and characterized a *bm118* knockout mutant. Transfection of cells with the *bm118* knockout bacmid led to cytopathogenic effects in single cells, and this defect was repaired by a *bm118* rescue bacmid although the titer of BV did not fully reach wild-type levels. Additionally, electron microscopy revealed that occlusion bodies without ODV and containing aberrant nucleocapsids were formed in cells transfected by the *bm118* knockout.

2. Materials and methods

2.1. Cells, viruses and antibodies

BmNPV (ZJ strain) and BmNPV bacmid viruses were propagated in BmN (BmN-4) cells, maintained at 27 °C in TC-100 insect medium supplemented with 10% (v/v) fetal bovine serum (Invitrogen). The titration of virus and other routine manipulations were performed according to standard protocols (O'Reilly et al., 1992). The monoclonal antibody against AcMNPV GP64 was purchased from Invitrogen Life Technology. The polyclonal antibody against AcMNPV polyhedrin was kindly provided by Prof. Y. Pang (Sun Yat-sen University, China).

2.1.1. Bacterial strains, Bacmid DNA and plasmids

The *E. coli* strain DH10B and pFastBac1 were purchased from Invitrogen Life Technology (USA). The *E. coli* strain BW25113 (pKD46) was kindly provided by Dr. Mary Berlyn (Yale University, USA), and the plasmid pKD46 contains the phage λ Red system under the control of arabinose promoter. The *E. coli* strain DH10Bac (Invitrogen Life Technology) was used to isolate the helper plasmid (pMON7124), which encodes a transposase. The *E. coli* strain BmDH10B, containing BmNPV Bacmid (BmBac) DNA, was provided by Dr. E.Y. Park (Shizuoka University, Japan). The pRADZ3 plasmid, containing the chloramphenicol resistance gene (Cm^R), was kindly provided by Dr. Y.J. Hua (Zhejiang University, China). All strains were cultured in LB medium with appropriate antibiotics. The plasmid pFastBacHte, containing the enhanced green fluorescence protein gene (*egfp*) under the AcMNPV *polh* promoter, was constructed using standard protocols.

2.2. RT-PCR and qRT-PCR analysis

Monolayers of BmN cells were infected with BmNPV at a multiplicity of infection (M.O.I.) of 5. Total RNA was extracted from mock- or BmNPV-infected cells at various time intervals (3, 6, 12, 18, 24, 36, 48, 72, and 96 h p.i.). Total RNA was purified by incubation with Deoxyribonuclease I (DNase I) (Worthington, Canada) to remove contaminating genomic DNA. Purified RNA without DNA was checked by PCR with primers ORF118F and ORF118R (Table 1). RT-PCR was performed using the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Canada) with 1 μg purified RNA as the template. First strand cDNA was synthesized with AMV reverse transcriptase and oligo-p$(dT)_{18}$ primers. Subsequently, RT products were amplified by the *bm118* specific primer ORF118F and De118 UR (Table 1). The PCR products were analyzed on a 1.0% agarose gel.

The RNA was extracted from BmN cells infected with $BmBac^{WT}$ and $BmBac^{Re}$ at an M.O.I. of 5 and harvested at 16 h p.i. Five micrograms of RNA without DNA contamination were used to synthesize cDNA. The genes, *odv-e18*, *exon0*, *ie0*, and *bm118* were analyzed using the qRT-PCR assay. Quantitative PCR was performed with SYBR Premix ExTaq (Takara) using the iCycler iQ Muticolor Real-Time PCR Detection System (Bio-Rad) under the following conditions: 95 °C for 10 s, 40 cycles of 95 °C 5 s and 55 °C 30 s, with a 1.65-ng/μl concentration of each primer listed in Table 1. The *egt* gene, whose locus is far from the *exon0-ie1* region, was selected as a control.

2.3. Preparation of a linear fragment for homologous recombination

To generate *bm118* knockout viruses by recombination in *E. coli* (Fig. 1A), we constructed a transfer vector (pET-ufs/egfp/Cm^R/dfs) in which 200 bp of the *bm118* encoding region (corresponding to 114,241–114,440 in the genome) was removed, and 845 bp of the 5′-end and 386 bp of the 3′-end were retained so that the deletion would not affect transcription of the adjacent genes *ie-0* and *e18*. The transfer vector was constructed as follows. First, an 861 bp *egfp* sequence under the control of the AcMNPV *polh* promoter was PCR amplified from pFastBacHte plasmid DNA with primers PphF and egfpR (Table 1) and then cloned into pETblue-2 (Novagen, Germany) to create vector pET-egfp. Next, a 1126-bp (113,115–114,240 bp in the genome) upstream flanking sequence (ufs) was PCR amplified from BmBac genomic DNA with primers De118UF and De118UR (Table 1) and cloned into pET-egfp to generate pET-ufs/egfp. Afterwards, a 1272-bp (114,440–115,711 bp in the genome) downstream flanking sequence (dfs) was amplified from BmBac genomic DNA with primers De118DF and De118DR (Table 1) and cloned into pET-ufs/egfp to generate pET-ufs/egfp/dfs. Finally, using the pRADZ3 plasmid as the template, a 948-bp Cm^R sequence was amplified with primers Cm^RF and Cm^RR (Table 1) and cloned into pET-ufs/egfp/dfs to generate pET-ufs/egfp/Cm^R/dfs. The reconstructed vector was verified by sequencing.

The pET-ufs/egfp/Cm^R/dfs vector was then cleaved with restriction enzymes BamH I and Xho I to generate a linear donor fragment (ufs/egfp/Cm^R/dfs). The linear donor fragment was used to electrotransform competent cells.

2.4. Generating a Bacmid with the bm118 deletion

BW25113/pKD46 competent cells were made according to the method described by Datsenko and Wanner (2000). The BmNPV bacmid DNA was electro-transformed into BW25113/pKD46 competent cells to generate bacterial strain BW25113 containing pKD46 and BmNPV Bacmid, designated BW25113/pKD46/BmBac.

The Red system-induced BW25113/pKD46/BmBac electrocompetent cells were made as described previously (Pijlman et al., 2002). The electroporation was performed according to the manufacturer's instructions by use of a Bio-Rad Gene Pulser II (2.5 kV, 25 Ω and 25 μF) and a 2-mm diameter cuvette. The genomic DNA of the recombinant ($BmBac\Delta118$) was extracted and identified by PCR with primers of iF/egfpR, iR/PphF and iF/iR (Fig. 1B).

The extracted $BmBac\Delta118$ DNA was then electro-transformed into *E. coli* strain DH10B, designated DH10B/$BmBac\Delta118$. Then, the helper plasmid (pMON7124) was chemically transformed into DH10B harboring $BmBac\Delta118$, designated DH10B/BmBac $\Delta118$/helper.

2.5. Construction of donor plasmids

The phenotype of the BmNPV bacmid is *polh* gene negative. To introduce the *polh* gene cassette into the wild-type BmBac and the *bm118* knockout BmBac, a donor plasmid of pFastBac-Bmph containing the BmNPV *polh* promoter was constructed as previously reported (Ge et al., 2008).