Virus Research 138 (2008) 81–88

Contents lists available at ScienceDirect

Virus Research

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

Characterization of a *Bombyx mori* nucleopolyhedrovirus with *Bmvp80* disruption

Xu-Dong Tang, Yi-Peng Xu, Lin-lin Yu, Guo-Jun Lang, Cai-Hong Tian, Jin-Fang Zhao, Chuan-Xi Zhang*

Institute of Insect Science, Zhejiang University, 268 Kaixuan Road, Hangzhou 310029, Zhejiang, China

ARTICLE INFO

Article history:
Received 19 June 2008
Received in revised form 19 August 2008
Accepted 21 August 2008
Available online 5 October 2008

Keywords:
Bombyx mori
Nucleopolyhedrovirus
Bmvp80
Ac104
Gene disruption
BV production

ABSTRACT

A BmNPV Bacmid with the *Bmvp80* gene disrupted was constructed using the ET-recombination system in *Escherichia coli* to investigate the role of *Bmvp80* during the baculovirus life cycle. Disruption of *Bmvp80* resulted in single cell infection phenotype, whereas a rescue BmBacmid restored budded virus titers to wild type levels; however, the homologous gene Ac104 (*Acvp80*) from AcMNPV could not complement the BmBacmid lacking a functional *Bmvp80* gene. Electron microscopy of cells transfected with BmNPV lacking functional *Bmvp80* revealed that the number of nucleocapsids was markedly lower. These results suggest that *Bmvp80* is essential for normal budded virus production and nucleocapsid maturation, and is functionally divergent between baculovirus species.

1. Introduction

Bombyx mori nucleopolyhedrovirus (BmNPV), a member of the family *Baculoviridae*, has a double-stranded DNA genome of 128 kb. Two distinct virion forms, an occlusion-derived virus (ODV) and a budded virus (BV), are produced during its infection cycle. The ODV, which appears in the very late stages of infection, is responsible for transmission between insects and is adapted for infection of the insect midgut. The BV buds from cell membranes during the late phase of infection and can cause systemic infection throughout the insect body. To date, many proteins, including P87, P91 (Russell et al., 1997), P39 (Blissard et al., 1989), P24 (Wolgamot et al., 1993), PP83 (Nie et al., 2006), FP25K (Braunagel et al., 1999), BV/ODV-C42 (Braunagel et al., 2001), VP1054 (Olszewski and Miller, 1997a), Vlf-1 (Yang and Miller, 1998) and AC142 (McCarthy et al., 2008), have been identified as structural components of both ODV and BV forms in different baculoviruses, and play important roles in the baculovirus life cycle.

The 2079 nucleotide ORF88 of BmNPV (*Bmvp80*) encodes a 692 amino acid polypeptide with a predicted molecular mass of 80 kDa. Homologues of *Bmvp80* are present in all lepidopteran NPVs sequenced to date, indicating that this gene might play an important role in the life cycle of lepidopteran NPVs. Amino acid sequence comparisons reveal that *Bmvp80* has the highest identity (96.4%) with p82 (Ac104, *Acvp80*) of *Autographa californica* MNPV (AcMNPV) among Group I NPVs and with P87 of *Spodoptera frugiperda* MNPV (31.3% identity) among group II NPVs. The *Bmvp80* homologue P87 from *Orgyia pseudotsugata* nucleopolyhedrovirus (OpMNPV) was reported to be a component of both budded and occlusion-derived virus (Muller et al., 1990). A homologue of P87 has been reported in AcMNPV, and its organization and temporal expression are very similar to those of the OpMNPV *P87* gene (Lu and Carstens, 1992). Another P87 homologue, P82 of the *Choristoneura fumiferana* multicapsid nucleopolyhedrovirus (CfMNPV), has been characterized by immunoblot analysis (Li et al., 1997). The proteomics analysis of *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus (HearNPV) also showed that vp80 was a component of the occluded virus virion (Deng et al., 2007).

Although some features of *P87*, such as its transcriptional start site, temporal expression and subcellular localization, have been studied in different NPVs, its function during the infection cycle remains unknown. In this report, we constructed a BmNPV Bacmid with a disruption in the *Bmvp80* gene to characterize the functions of its protein product in the baculovirus life cycle.

2. Materials and methods

2.1. Cells, virus and bacterial strains

BmN cells were maintained in TC100 with 10% fetal bovine serum (Gibco-BRL). *E. coli* strains BW25113, harboring plasmid pKD46 encoding the λ red recombination system and DH10B (Datsenko and Wanner, 2000) were kindly provided by Mary Berlyn (Yale University, CT, USA). BmDH10Bac used to isolate BmBacmid

* Corresponding author. Tel.: +86 571 86971697; fax: +86 571 86971697.
E-mail address: chxzhang@zju.edu.cn (C.-X. Zhang).

0168-1702/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2008.08.013

Table 1
Primers used in this report

Name	Sequence	Position in BmNPV genome
CATF	5′-AAGCTTTATCCTTGACCGGACCCA-3′	
CATR	5′-AAGCTTTTAAAAAAATTACGCCCC-3′	
BmVP80F	5′-GGATCCATGGATGCTGGAGAATT-3′	84140–84153
BmVP80R	5′-CTCGAGTTACAGCTCGTCGATT-3′	85126–85138
BmVP80ReF	5′-GCGGCCGCGTCGGATTGTAATATG-3′	82865–82880
BmVP80ReR	5′-CTGCAGACATTCGCCACAACAT-3′	85449–85461
Bm80PROF	5′-GAATTCGTCGGATTGTAATATG-3′	82865–82880
Bm80PROR	5′-GTCGACATTATAAGGTTATATT-3′	83224–83239
Ac80F	5′-GGATCCATGAACGATTCCAATT-3′	
Ac80ReR	5′-CTGCAGTTATATAACATTGTAG-3′	

The restriction sites are underlined.

DNA and a helper plasmid pMON7124 encoding a transposase were kindly provided by Park EY (Shizuoka University, Japan). The transfer plasmid pFBI-ph-gfp was kindly provided by Pang Y (Zhongshan University, China).

2.2. Construction of a BmBacmid lacking Bmvp80

A 999 bp fragment of the *Bmvp80* gene containing a HindIII site 1483 nt downstream from the start codon was amplified by PCR using the primers BmVP80F and BmVP80R (Table 1) and cloned into the plasmid vector pGEX-4T-2 (Invitrogen); the resulting plasmid was named pGEX-vp80. The plasmid pRADZ3 was used as a template to amplify the *cat* gene using the primers CATF and CATR (Table 1). The amplified *cat* gene was ligated into pGEX-vp80 to generate the plasmid pGEX-vp80-cat, which contains portions of the *Bmvp80* gene on both sides of the *cat* gene. A fragment containing the *cat* gene cassette and *Bmvp80* regions used for homologous recombination was amplified with primers BmVP80F and BmVP80R. The 1947 bp PCR product was digested with BamHI and XhoI to eliminate template DNA, and purified using a DNA Fragment Quick Purification Kit (DingGuo).

The BmNPV *Bmvp80* mutant virus, Bm-vp80-D, was constructed by electroporating the fragment containing the *cat* gene cassette and *Bmvp80* regions into *E. coli* BW25113 competent cells, which harbor the BmBacmid (Ge et al., 2008). Recombined clones were identified by PCR with primer sets BmVP80F + BmVP80R, BmVP80F + CATR and BmVP80R + CATF (Table 1).

2.3. Generation of a Bmvp80 rescue plasmid and a wt BmBacmid with the gfp marker

To generate a *Bmvp80* rescue plasmid, the entire *Bmvp80* gene with its native promoter and poly (A) tail was amplified from BmNPV genomic DNA using primers BmVP80ReF and BmVP80ReR, then cloned into the multiple cloning site of the transfer vector pFBI-ph-gfp, which contains a *gfp* reporter gene under the control of the AcMNPV *ie-1* promoter as well as a *polyhedrin* gene. The resulting plasmid was named pFBI-80Re. A helper plasmid encoding a transposase (pMON7124) and the BmBacmid with *Bmvp80* disrupted were transformed into DH10B cells. The resulting cells were transformed with transfer plasmid pFBI-ph-gfp and pFBI-80Re to generate a BmBacmid with *Bmvp80* disrupted ($vBm^{vp80\text{-}D\text{-}gfp}$) and a rescued BmBacmid ($vBm^{vp80\text{-}Re\text{-}gfp}$), respectively (Fig. 1A). A control BmBacmid (vBm^{wt}) was constructed by transforming pFBI-ph-gfp into DH10B cells harboring the wt BmBacmid. All of the BmBacmids thus contained a *gfp* reporter gene under the control of the *ie-1* promoter and a *polyhedrin* gene under the control of its own promoter. BmBacmid DNA was extracted and purified with a QIAGEN large-construct kit and quantified by spectrometry.

2.4. Construction of Acvp80 rescue BmBacmids

To investigate whether the *Bmvp80* gene displays species specificity, rescue of the *Bmvp80* disrupted virus $vBm^{vp80\text{-}D}$ was attempted using the homologous gene *Acvp80* from AcMNPV under the control of the *ie-1*, *Bmvp80* and *polyhedrin* promoters. The *Acvp80* coding sequence was amplified from AcMNPV genomic DNA with primers Ac80F and Ac80ReR, and the *Bmvp80* promoter was amplified with primers Bm80PROF and Bm80PROR (Table 1). The EcoRI/SalI digested *ie-1* promoter of AcMNPV fragment and Not I/Pst I digested *Acvp80* fragment were inserted into the transfer plasmid pFBI-ph-gfp to generate the transfer plasmid containing the *Acvp80* ORF under the control of the AcMNPV *ie-1* promoter. The *ie-1* promoter of AcMNPV could drive green fluorescence protein (gfp) gene expression in BmN cells as shown in Fig. 2A. As the same, the EcoRI/SalI digested *Bmvp80* promoter and NotI/PstI digested *Acvp80* fragment were inserted into the transfer plasmid pFBI-ph-gfp to generate the transfer plasmid containing the *Acvp80* ORF under the control of the *Bmvp80* promoter. The *Acvp80* ORF under the control of the AcMNPV *polyhedrin* promoter was constructed with the transfer plasmid pFastBac1 using the Bac-to-Bac system. The *polyhedrin* promoter of AcMNPV could drive foreign gene expression in BmN cells (Yang et al., 2008). All the above *Acvp80* recombination bacmids were confirmed by PCR with M13+ and M13− primers, and the primer sets of Ac80F+ M13− and Ac80ReR+ M13+. Transformation and screening were performed as described above. To confirm if the *Acvp80* was transcribed in the cells transfected with the repair Bacmids of *Acvp80* promoted under *ie-1*, *Bmvp80* and *polyhedrin* prompters, RT-PCR analysis was performed. All the RNA samples were collected at 96 h post transfection. Total RNA was extracted and purified by incubating with Deoxyribonuclease I (DNase I) (Takara) to remove contaminating genomic DNA. RT-PCR was performed using RevertAidTM First Strand cDNA Synthesis Kit (Takara) with 1 μg purified RNA as the template. First strand of cDNA was synthesized with M-MLV reverse transcriptase and oligo-*p* (dT)18 primer. Subsequently, the nested PCR was amplified by the *Acvp80* specific primer Ac80F and Ac80ReR (Table 1). The PCR products were analyzed on a 1.0% agarose gel. To further eliminate the possibility of viral DNA contaminant, RNase-treated total RNA sample was used as a control template for each PCR analysis.

2.5. Virus growth curves and confocal microscopy

To generate virus growth curves, BmN cells were transfected with each BmBacmid ($vBm^{vp80\text{-}D\text{-}gfp}$, $vBm^{vp80\text{-}Re\text{-}gfp}$ and vBm^{wt}). Supernatants were collected at selected time points and the titers were determined by $TCID_{50}$ on BmN cells.

2.6. Transmission electron microscopy

BmN cells were transfected with $vBm^{vp80\text{-}D\text{-}gfp}$. After 96 h the cells, which fluoresced, were suspended in 10% BSA and fixed in 4% glutaraldehyde overnight at 4 °C, then further fixed with 1% osmium tetroxide for 1 h at room temperature. The fixed cells were dehydrated in a graded ethanol series (50–100%) and acetone for 20 min each. Infiltration was performed in 50, 75 and 100% (spurr (Sigma, USA): acetone, v/v). Samples in 100% spurr were maintained at 70 °C for 16 h. Ultrathin sections were stained with uranyl acetate and lead citrate. Samples were observed using a 10 JEM-1230 transmission electron microscope (JEOL).

2.7. Confocal laser-scanning microscopy

BmN cells were infected with BmNPV and collected at 24, 48 and 72 h post infection. The cells were rinsed three times with 1×