Characterization of a Late Expression Gene of *Bombyx mori* Nucleopolyhedrovirus

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Bombyx mori nucleopolyhedrovirus (BmNPV) ORF5 (*Bm5*) is a gene present in many lepidopteran nucleopolyhedroviruses (NPVs), but its function is unknown. In this study, *Bm5* was characterized. The transcript of *Bm5* was detected 12 – 72 h post infection (p.i.). Polyclonal antiserum raised to a His-BM5 fusion protein recognized BM5 in infected cell lysates from 24 to 72 h p.i., suggesting that *Bm5* is a late gene. Immunofluorescence analysis by confocal microscopy showed that the BM5 protein is localized primarily in the cytoplasm. Localization of BM5 in budded virion (BV) and occlusion-derived virion (ODV) by Western analyses demonstrated that BM5 is not a structural protein associated with BV or ODV.

Key words: BmORF5, Transcription, Subcellular Location

Introduction

Nucleopolyhedrovirus (NPV) of *Bombyx mori* (BmNPV) is a member of the Baculoviridae, which are large, enveloped, rodshaped viruses with a double-stranded, circular, closed, and supercoiled DNA genome of 128 kb that encodes 136 genes (Herniou *et al.*, 2003; Jakubowska *et al.*, 2006). The baculovirus life cycle typically involves the production of two virion phenotypes, budded virions (BVs) and occlusion-derived virions (ODVs). Although these two types of virions are similar in their nucleocapsid structure, they differ in the origin and composition of their envelopes and their roles in the virus life cycle. ODVs are responsible for horizontal transmission between insect hosts, whereas BVs are responsible for the systemic spread through the insect host and propagation in tissue culture (Williams and Faulkner, 1997). In the early stage of infection, viral DNA replication occurs within a virus-induced specific nuclear region, called the virogenic stroma (VS). The newly replicated viral genome is condensed and packaged into rod-shaped capsids to form nucleocapsids. These nucleocapsids egress from the nucleus, bud through the plasma membrane, and acquire envelopes to form BVs. Later in infection, nucleocapsids retain within the peristromal space, called the ring zone, bundle together, and are enveloped in intranuclear membrane profiles, to form ODVs. The ODVs are then embedded in a paracrystalline matrix consisting mainly of virus-encoded polyhedrin protein (Williams and Faulkner, 1997). An intranuclear viral replication structure (VS) generally was thought to be the active site for viral DNA replication, late gene transcription, condensation, and packaging into capsids, ODV assembly. Mature nucleocapsids then migrate into a peristromal compartment (ring zone) (Young *et al.*, 1993; Williams and Faulkner, 1997). Nucleocapsids egress from the nucleus, and then move to the plasma membrane from which they bud forming BVs (Williams and Faulkner, 1997). Later in infection, nucleocapsids acquire an envelope to form preoccluded virions (POVs) in the nucleus, and the resulting virions are subsequently embedded into a paracrystalline matrix consisting mainly of the polyhedrin protein to form ODVs (Williams and Faulkner, 1997).

China has a long history of over 5,000 years in raising silkworms (*Bombyx mori* L.). At present, over 30 million farmer households are involved in sericultural production in China's over 10 provinces. Silkworm viral diseases are major diseases

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causing great loss in sericulture, among which the nucleopolyhedrosis caused by BmNPV infection is one of the most disastrous (Chen *et al.*, 2007). Since the first baculovirus is completely sequenced (Ayres *et al.*, 1994), 44 other baculovirus genomes have been reported so far (http://athena.bioc.uvic.ca/database.php?item=listGenomes&db=Baculoviridae). Based on the comparative analysis of 29 baculoviruses, 62 ORFs have been identified in common and designated as baculovirus core genes, suggesting their importance in the viral life cycle (Jehle *et al.*, 2006). At present, the baculovirus gene function is focused on 62 core genes, such as *vp39*, *gp41*, and especially *gp64.* In order to reduce the disease outbreaks and minimize the losses, many scientists have made extensive research on BmNPV. Analysing these core genes, scientists hoped to understand the characteristics of BmNPV towards its hosts, especially fast infection and high lethality. *Bm5* (4,607–5,600 nt), which is one of 62 baculovirus core genes and a homologue of *orf1*3 of AcMNPV (Gomi *et al.*, 1999), encodes a putative protein of 331 amino acids with a predicted molecular mass of 39.3 kDa. Sequence-based queries performed with the InterProScan program showed that BM5 is a protein of unknown function.

Therefore, we studied the transcription, characterized the structural, subcellular localization, and demonstrated the expression pattern of BM5 protein.

Material and Methods

Cells, virus, bacterial strains, and antibiotics

BmNPV (Z J strain) was propagated in BmN (BmN-4) cells. The BmN cell line was cultured at 27 °C in TC-100 insect medium (Gibco, Tulsa, OK, USA) supplemented with 10% (v/v) fetal bovine serum (Gibco) using standard techniques.

Computer analysis

The protein sequence was analyzed using the ExPASy (Swiss Institute of Bioinformatics, Lausanne, Switzerland) server (www.expasy.ch) for prediction of motifs, domains, transmembrane regions, and signal peptides. Homologues were explored using the BLASTP searching tool in the updated GenBank/EMBL and SWISS-PROT databases. Sequence alignment was performed with the software ClustalX, and homology shading was done using GeneDoc software. The functional domains and motifs of BM5 were predicted by the software ExPASy.

Expression of Bm5 and preparation of antibody

The complete *Bm5* (993 nt) was amplified by PCR using the primers 5'-AGGATCCATGCTATCCTGGTTATGG-3' (containing the *Bam*HI site) and 5'-CCGCTCGAGTTACAATACTTCTTGTAT-3' (containing the *Xho*I site) from the BmNPV genomic DNA. The amplified fragment was digested with *Bam*HI and *Xho*I and fused in frame with a hexa-histidine tag (His-tag) in pET30a(+) expression vector (Novagen, Madison, WI, USA). The recombinant plasmid, pET-*Bm5*, was verified by PCR and restriction analysis. The recombinant plasmid was transformed into *Escherichia coli* BL21 cells for expression. The recombinant BM5 protein, purified by an Ni^{2+}-NTA column (Novagen), was used to raise polyclonal antibodies in rabbits.

The antibody was prepared using standard techniques. Purified 6×His-BM5 protein (about 2 mg) in complete Freund's adjuvant was injected subcutaneously to immunize New Zealand white rabbits, followed by two booster injections in incomplete Freund's adjuvant within a gap of 2 weeks before exsanguinations. The polyclonal rabbit antibody against His-BM5 was used for the immunoassay.

Mass spectrometry analysis and database searching

Protein spots were manually excised from the gels. Spots from Coomassie gels were washed with 100 ml of 50% acetonitrile/50 mM ammonium hydrocarbonate, pH 8, while spots from silver gels were washed with 50 ml of 15 mM potassium hexacyanoferrate/50 mM sodium thiosulfate. Gel pieces were then dehydrated with acetonitrile and vacuum-dried. After rehydration in 10 ml of 50 mM ammonium hydrocarbonate, pH 8, containing 0.5 mg of porcine trypsin, samples were incubated overnight (16–18 h) at 37 °C. Peptide fragments from digested proteins were then crystallized with α-cyano-4-hydroxycinnamic acid as a matrix and subjected to a MALDI-TOF (Bruker Daltonics, Bremen, Germany) spectrometer for peptide mass fingerprinting. This instrument was equipped with an N_2 laser (337 nm, laser of 20 Hz). Samples were acquired in the positive reflection mode with a delay of extraction time of 130 ns. The trypsin autodigestion peaks at 842.509 and 2211.104 were used for internal calibration.

Transcription of Bm5 in infected BmN cells

Total RNA was extracted from BmNPV-infected BmN cells at a multiplicity of infection (MOI) of 10 with Trizol (Invitrogen, Carlsbad, CA, USA) 0, 6, 12, 24, 48, and 72 h post infection (h p.i.). For cDNA synthesis, the extracted RNA was treated with RNase-free Dnase I (Takara, Dalian, China) to eliminate any potential genomic DNA contamination. RT-PCR was performed using the RNA PCR kit Ver. 3.0 (Takara) with 2 μg RNA as the template per time point. First-strand cDNA was synthesized with AMV reverse transcriptase (Takara) and an oligo(dT) primer (Takara). Subsequently, cDNA was PCR-amplified by the gene-specific primers 5'-GATGTGGGCAACAGGTTTG-3' and 5'-CACCGTCATAGAGCACTTCCA-3' within 40 cycles of 94 °C for 20 s, 58 °C for 30 s, and 72 °C for 15 s. BmNPV *ie-1* gene and *p10* gene were used as the control for the early gene and late gene, respectively.

A quantitative real-time PCR (Q-PCR) assay was performed with SYBR Premix ExTaq (Takara) under the following conditions: 40 cycles of 94 °C for 20 s, 58 °C for 30 s, and 72 °C for 15 s with the gene-specific primers 5'-ACTGAAGGCGAGCGTGAT-3' and 5'-AAATGCTGGTGTTTGGTAAT-3'.

Temporal expression of Bm5 in infected BmN cells

For time course analysis, BmN cells were infected with BmNPV at an MOI of 10, and harvested at designated time points (0, 6, 12, 24, 48, 72 h p.i.), pelleted at 4,000 × *g*, resuspended in PBS, lysed in SDS-PAGE loading buffer by boiling for 10 min, and harvested 0, 6, 12, 24, 48, and 72 h p.i.

Protein samples were separated by 12% SDS-PAGE and transferred onto a PVDF membrane (Millipore, Billerica, MA, USA). The membrane was blocked in 3% skimmed milk powder in PBST (1×PBS, 0.1% Tween-20) for 1 h followed by incubation with the anti-BM5 polyclonal antiserum diluted 1:5,000 for 1 h at room temperature. After incubation with a 1:2,000 dilution of horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibody, signals were detected using diaminobenzidine (DAB) (Sigma, Ronkonkoma, NY, USA).

BVs and ODVs purification

Hemolymph-derived BVs were purified from BmNPV-infected larvae as described previously (Chen *et al.*, 2007). Larvae of the silkworm *B. mori* were infected with BmNPV as described by Iwanaga *et al.* (2000). ODVs were purified from polyhedra as described previously (Braunagel and Summers, 1994; Xu *et al.*, 2006).

For analysis of structural proteins, BV and ODV fractions were analyzed by Western blotting. BmNPV-infected BmN cells were used as positive control.

Immunofluorescence microscopy

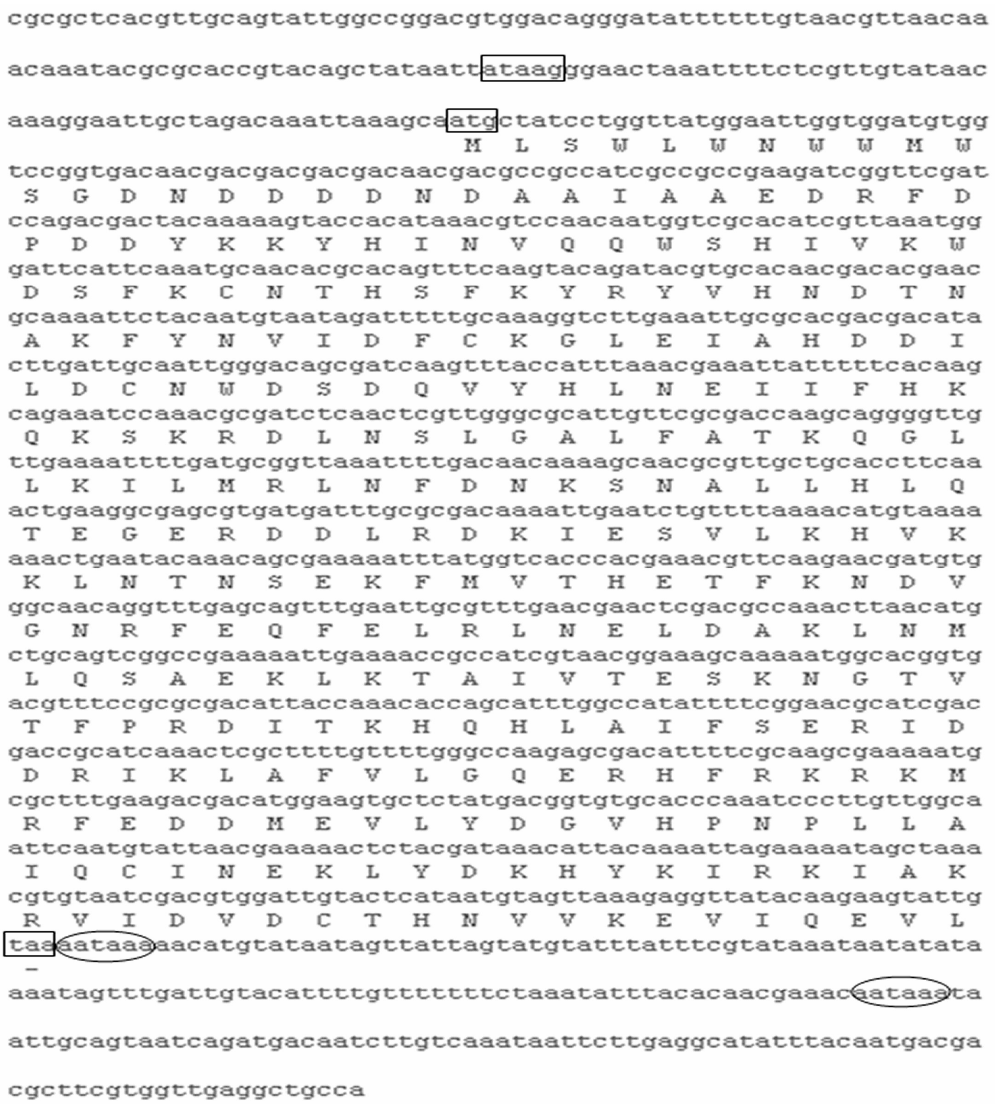
BmN cells were infected with BmNPV at an MOI of 10 and incubated until 24, 48 and 72 h p.i. BmN cells were washed three times with cold PBS and fixed with 2 ml of 4% paraformaldehyde for 15 min. Cells were then washed three times with PBS and permeabilized with 0.1% Triton X-100 in PBS for 15 min. After washing three times with cold PBS, cells were incubated with anti-BM5 polyclonal antibody (1:1,000 dilution) as primary antibodies in 1×PBS for 1 h at room temperature. After washing three times with 1×PBS, cells were incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG for 1 h and examined with a Leica laser confocal microscope. Background staining was removed by washing with PBS three times.

Results

Sequence analysis of Bm5 and its homologues

The coding region of *Bm5* is 993 bp in length, which could encode a 331-aa peptide with a predicted molecular weight of 39.3 kDa. It is transcribed in the reverse direction as the *polyhedrin* gene. A putative late transcription motif, ATAAG, was found at 58 nt, upstream of the start codon ATG, suggesting that *Bm5* might be a late transcriptional gene. Two polyadenylation signal sequences (AATAAA) were located at 1 and 115 nt downstream of the translational stop codon TAA.

Searches in the protein databases GenBank and SWISS-PROT showed that the aa motif anal-



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cgcgctcacgttgcagtattggccggacgtggacagggatatttttgtaacgttaacaa

acaaatacgcgcaccgtacagctataattataagggaactaaattttctcgttgtataac

aaaggaattgctagacaaattaaagcaatgctatcctggttatggaattggtggatgtgg
                            M  L  S  W  L  W  N  W  W  M  W
tccggtgacaacgacgacgacgacaacgacgccgccatcgccgccgaagatcggttcgat
 S  G  D  N  D  D  D  D  N  D  A  A  I  A  A  E  D  R  F  D
ccagacgactacaaaaagtaccacataaacgtccaacaatggtcgcacatcgttaaatgg
 P  D  D  Y  K  K  Y  H  I  N  V  Q  Q  W  S  H  I  V  K  W
gattcattcaaatgcaacacgcacagtttcaagtacagatacgtgcacaacgacacgaac
 D  S  F  K  C  N  T  H  S  F  K  Y  R  Y  V  H  N  D  T  N
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 A  K  F  Y  N  V  I  D  F  C  K  G  L  E  I  A  H  D  D  I
cttgattgcaattgggacagcgatcaagtttaccatttaaacgaaattattttcacaag
 L  D  C  N  W  D  S  D  Q  V  Y  H  L  N  E  I  I  F  H  K
cagaaatccaaacgcgatctcaactcgttgggcgcattgttcgcgaccaagcaggggttg
 Q  K  S  K  R  D  L  N  S  L  G  A  L  F  A  T  K  Q  G  L
ttgaaaattttgatgcggttaaattttgacaacaaaagcaacgcgttgctgcaccttcaa
 L  K  I  L  M  R  L  N  F  D  N  K  S  N  A  L  L  H  L  Q
actgaaggcgagcgtgatgatttgcgcgacaaaattgaatctgttttaaaacatgtaaaa
 T  E  G  E  R  D  D  L  R  D  K  I  E  S  V  L  K  H  V  K
aaactgaatacaaacagcgaaaaatttatggtcacccacgaaacgttcaagaacgatgtg
 K  L  N  T  N  S  E  K  F  M  V  T  H  E  T  F  K  N  D  V
ggcaacaggtttgagcagtttgaattgcgtttgaacgaactcgacgccaaacttaacatg
 G  N  R  F  E  Q  F  E  L  R  L  N  E  L  D  A  K  L  N  M
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 L  Q  S  A  E  K  L  K  T  A  I  V  T  E  S  K  N  G  T  V
acgtttccgcgcgacattaccaaacaccagcatttggccatattttcggaacgcatcgac
 T  F  P  R  D  I  T  K  H  Q  H  L  A  I  F  S  E  R  I  D
gaccgcatcaaactcgcttttgttttgggccaagagcgacattttcgcaagcgaaaaatg
 D  R  I  K  L  A  F  V  L  G  Q  E  R  H  F  R  K  R  K  M
cgctttgaagacgacatggaagtgctctatgacggtgtgcacccaaatcccttgttggca
 R  F  E  D  D  M  E  V  L  Y  D  G  V  H  P  N  P  L  L  A
attcaatgtattaacgaaaaactctacgataaacattacaaaattagaaaaatagctaaa
 I  Q  C  I  N  E  K  L  Y  D  K  H  Y  K  I  R  K  I  A  K
cgtgtaatcgacgtggattgtactcataatgtagttaaagaggttatacaagaagtattg
 R  V  I  D  V  D  C  T  H  N  V  V  K  E  V  I  Q  E  V  L
taaaataaaaacatgtataatagttattagtatgtatttatttcgtataaataatatata
 -
aaatagtttgattgtacatttgtttttttctaaatatttacacaacgaaacaataaata

attgcagtaatcagatgacaatcttgtcaaataattcttgaggcatatttacaatgacga

cgcttcgtggttgaggctgcca
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Fig. 1. Nucleotide sequence and deduced amino acid sequence of *Bm5*. The baculovirus consensus late transcriptional start motif ATAAG is shown in a box, ATG and TAA are shown in boxes, two typical polyadenylation signals (AATAAA) are shown in ellipses.

ysis did not reveal any signal peptide sequence, transmembrane region, nuclear localization signal, or membrane retention signal, but two segments of low compositional complexity (aa 1–12, aa 14–28), a coiled coil (aa 151–215), a tyrosine kinase phosphorylation site (aa 298–304), three putative *N*-glycosylation sites (aa 68–71, aa 142–145, aa 228–231), two *N*-myristoylation sites (aa 83–88, aa 122–127), three putative casein kinase II phosphorylation sites (aa 144–147, aa 152–155, aa 175–178), and six putative protein kinase C phosphorylation sites (aa 53–55, aa 60–62, aa 114–116, aa 177–179, aa 186–188, aa 247–249) (Fig. 1).

They also showed that *Bm5* was conserved among baculoviruses and was shared by all baculoviruses whose complete genomes have been sequenced so far. Comparison analysis showed that *Bm5* had the highest identity (100%) with ORF13 of AcMNPV. The homologues from the other NPVs shared 23–93% identity with BmNPV *Bm5* (Fig. 2).

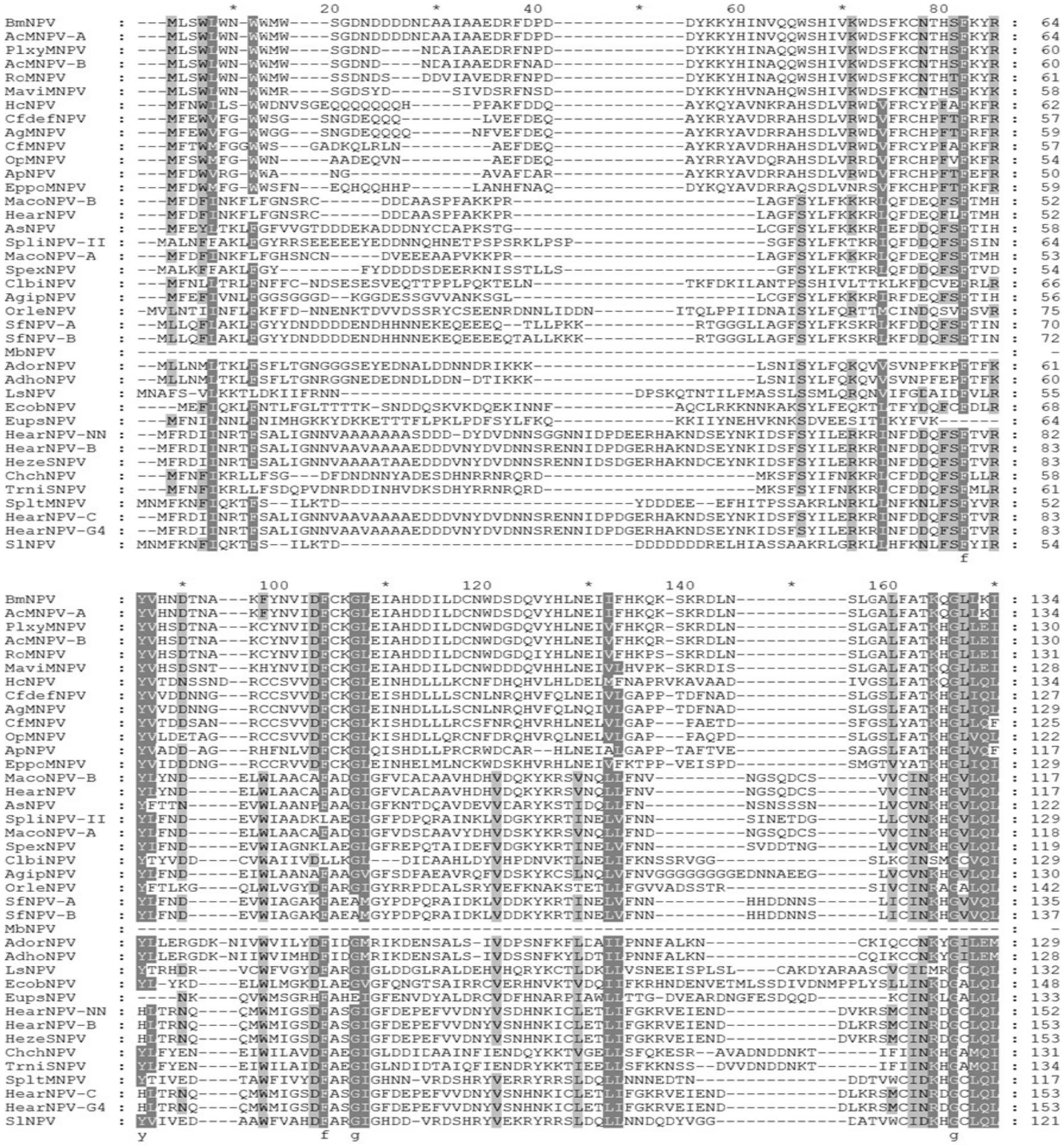
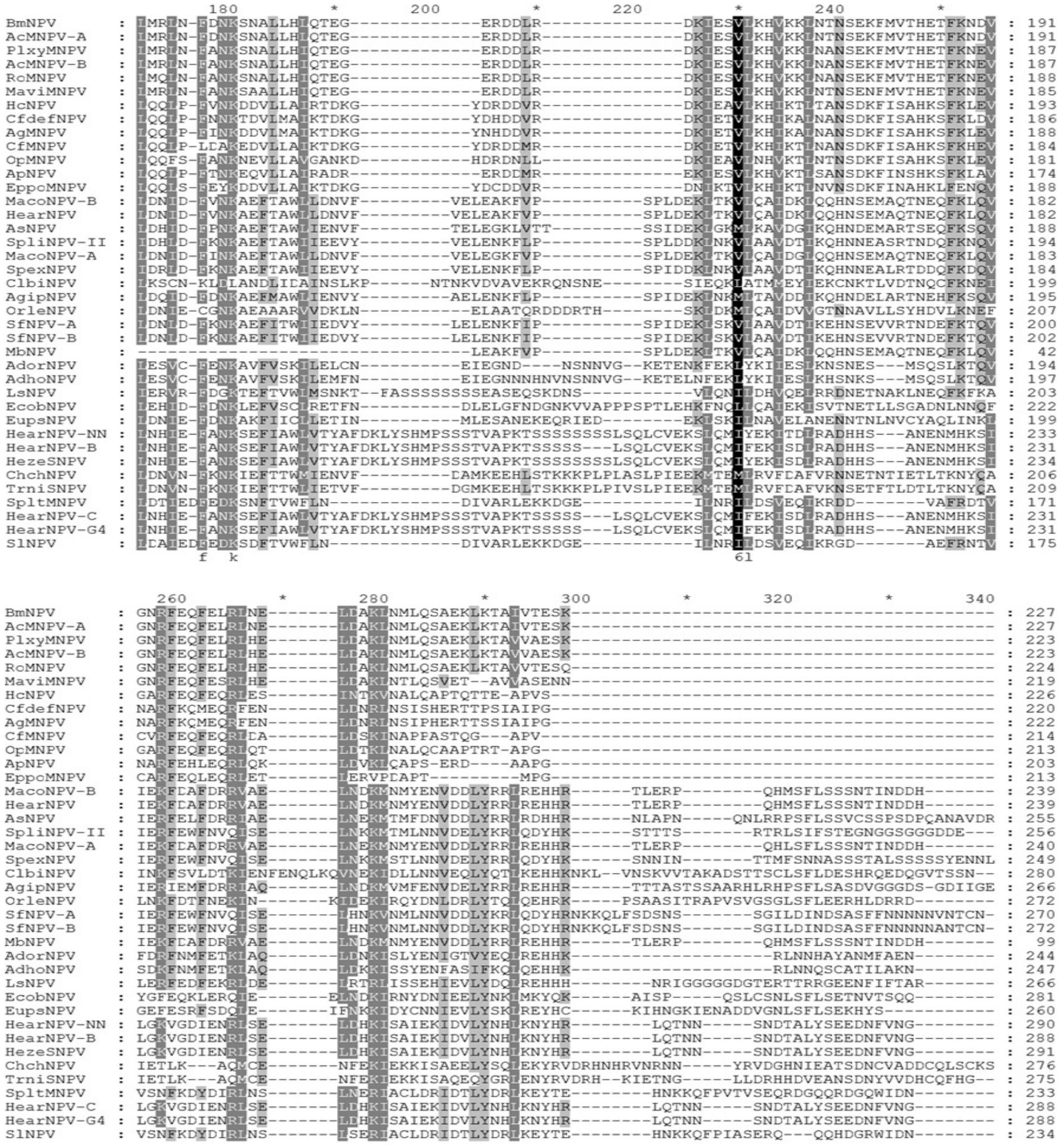


Fig. 2. Amino acid sequence alignment of baculovirus *Bm5* homologues. The sources of sequences are: BmNPV (GenBank, NP_047418.1), AcMNPV-A (GenBank, YP_002884244.1), PlxyMNPV (GenBank, YP_758479.1), AcMNPV-B (GenBank, NP_054042.1), RoMNPV (GenBank, NP_703003), MaviMNPV (GenBank, YP_950735.1), HcNPV (GenBank, YP_473328.1), CfdefNPV (GenBank, NP_932627.1), AgMNPV (GenBank, YP_803414.1), CfMNPV (GenBank, NP_848324.1), OpMNPV (GenBank, NP_046168.1), ApNPV (GenBank, YP_611101.1), EppoMNPV (GenBank, NP_203179.1), MacoNPV-B (GenBank, NP_689206.1), HearNPV (GenBank, YP_002332566.1), AsNPV (GenBank, YP_529686.1), SpliNPV-II (GenBank, YP_002332713.1), MacoNPV-A (GenBank, NP_689206.1), SpexNPV (GenBank, NP_037773.1), ClbiNPV (GenBank, YP_717660.1), AgipNPV (GenBank, YP_002268051.1), OrleNPV



(GenBank, YP_001651043.), SfNPV-A (GenBank, YP_001036309.1), SfNPV-B (GenBank, YP_001036309.1), MbNPV (GenBank, AF108960_1), AdorNPV (GenBank, YP_002300639.1), AdhoNPV (GenBank, NP_818772.1), LsNPV (GenBank, YP_758446.1), EcobNPV (GenBank, YP_874318.1), EupsNPV (GenBank, YP_002854737.1), HearNPV-NN (GenBank, NP_203680.1), HearNPV-B (GenBank, NP_203680.1), HezeSNPV (GenBank, NP_542750.1), ChchNPV (GenBank, YP_249741.1), TrniSNPV (GenBank, YP_309018.1), SpltMNPV (GenBank, NP_258396.1), HearNPV-C (GenBank, YP_002274053.1), HearNPV-G4 (GenBank, NP_075192.1), and SlNPV (GenBank, AF527603.8).

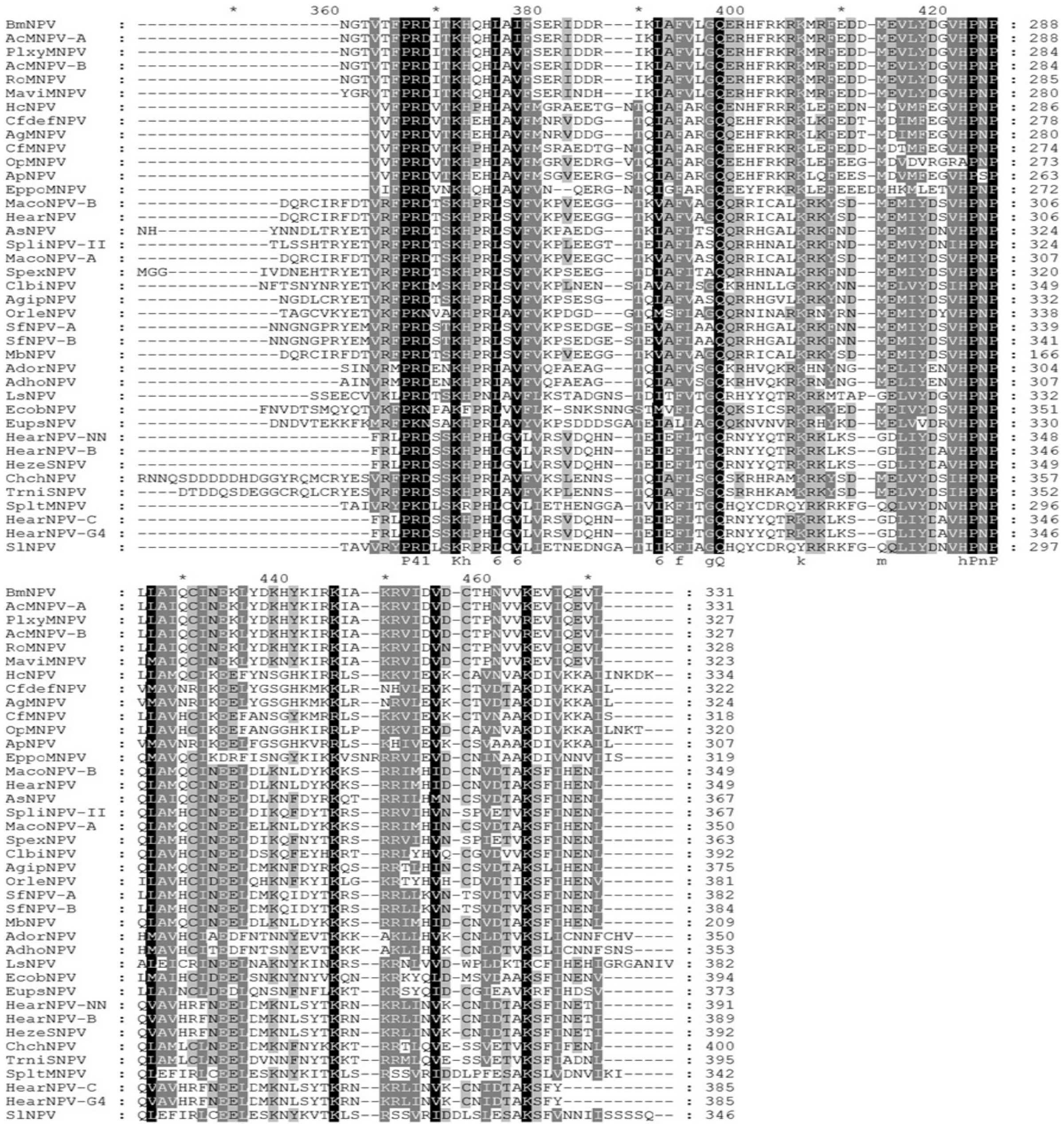


Fig. 2 continued.

Transcriptional analysis of Bm5 and Q-PCR analysis

To determine the temporal expression of the *Bm5* transcript, RT-PCR was performed at different time points using total RNA isolated from BmNPV-infected BmN cells as template. We took advantage of BmNPV *ie-1* gene and *p10* gene as the controls for the early gene and very late gene, respectively. As expected, a 283-bp *Bm5* fragment was amplified by *Bm5*-specific primers from 12 h p.i. to 72 h p.i. indicating that the *Bm5* gene is a late gene. By contrast, the 315-bp *ie-1* fragment was detectable from 6 h p.i. to 72 h p.i., when amplified by the *ie-1*-specific primers IE-1-F (5'-GAAGGAGGACGGCAGCAT-3') and IE-1-R (5'-TCGGACAACGGAACCAGA-3'). The 193-bp *p10* fragment was detectable from 24 h p.i. to 96 h p.i., when amplified by the *p10*-specific primers P10-F (5'-GACACGAATTTTA-GACGCCATTGCG-3') and P10-R (5'-TTAG-GAGTCTGGAGGATCCGGAGC-3') (Fig. 3). None of the *ie-1, Bm5* or *p10* fragments was detected in the control experiments in which no reverse transcriptase was added prior to the PCR step (data not shown), indicating no possible contamination of BmNPV DNA.

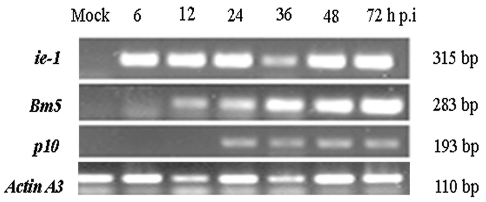


Fig. 3. RT-PCR analysis of *Bm5* transcription. Total RNA was extracted from BmNPV-infected cells different times post infection (p.i.). PCR products of different genes are indicated on the left-hand side. The sizes (in bp) of different PCR products are indicated on the right-hand side. *ie-1* and *p10* are positive controls for early and late genes. The actin A3 RT-PCR product was used as the control.

To further analyse the temporal expression of the *Bm5* transcript, Q-PCR was also performed at different time points using total RNA isolated from BmNPV-infected BmN cells as templates. Q-PCR analysis showed that the *Bm5* transcript was detected from 24 to 72 h p.i. and reached a maximal level 72 h p.i. (data not shown).

Expression of BM5 and immunodetection of BM5 protein in infected cells

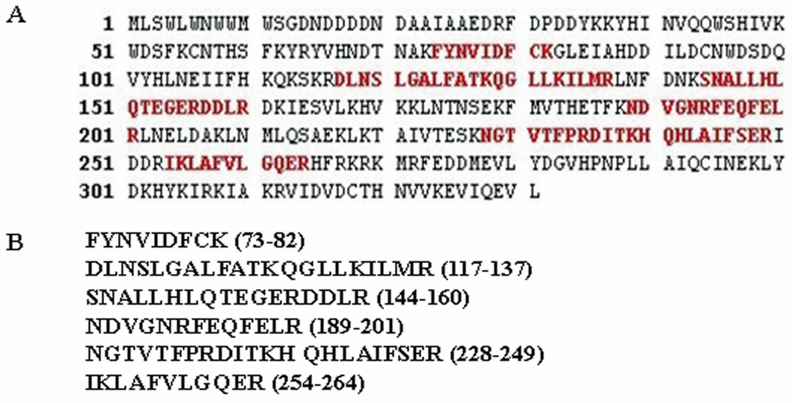
Expression of 6×His-*Bm5* gene fusion in *E. coli* resulted in the production of a 46-kDa protein. Western blot analysis using specific anti-His antiserum confirmed that the 46-kDa protein was the fusion protein (data not shown). The purified fusion protein was used to immunize rabbits to produce the specific antiserum against BM5.

To determine the time course of BM5 protein expression, a time course of BmNPV-infected BmN cells was analysed by Western blotting using anti-*Bm5* antiserum. The results revealed a specific immunoreactive band with approx. 39 kDa, which was first detected 24 h p.i. and could be detected until 72 h p.i. (data not shown). This is consistent with the results from RT-PCR analysis of transcript synthesis (Fig. 3).

No immunoreactive band was detected in the mock-infected control. The protein size of 39 kDa was in agreement with the predicted molecular weight of 39 kDa, suggesting that no major post-translational modification of the BM5 protein occurred.

Mass spectrometry analysis and database searching

The MASCOT (Matrix Science, London, UK) search was performed with carbamidomethyl as the fixed modification of cysteine and variable *N*-



A

1 MLSWLWNWWM WSGDNDDDDN DAAIAAEDRF DPDDYKKYHI NVQQWSHIVK
51 WDSFKCNTHS FKYRYVHNDT NAK**FYNVIDF CK**GLEIAHDD ILDCNWDSDQ
101 VYHLNEIIFH KQKSKR**DLNS LGALFATKQG LLKILMR**LNF DNK**SNALLHL**
151 **QTEGERDDLR** DKIESVLKHV KKLNTNSEKF MVTHETFK**ND VGNRFEQFEL**
201 **R**LNELDAKLN MLQSAEKLKT AIVTESK**NGT VTFPRDITKH QHLAIFSER**I
251 DDR**IKLAFVL GQER**HFRKRK MRFEDDMEVL YDGVHPNPLL AIQCINEKLY
301 DKHYKIRKIA KRVIDVDCTH NVVKEVIQEV L

B

FYNVIDFCK (73-82)
DLNSLGALFATKQGLLKILMR (117-137)
SNALLHLQTEGERDDLR (144-160)
NDVGNRFEQFELR (189-201)
NGTVTFPRDITKH QHLAIFSER (228-249)
IKLAFVLGQER (254-264)

Fig. 4. Identification of BM5 protein by MALDI-TOF analysis. (A) Amino acid sequences of BM5 protein. Matched peptide sequences are shown as red characters. (B) Peptide sequences identified by mass spectrometry.

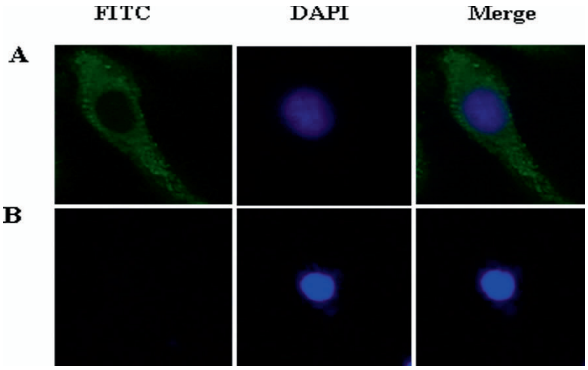


Fig. 5. (A) Subcellular localization of BM5 in infected cells 24, 48, and 72 h p.i. BmNPV-infected BmN cells were treated with anti-BM5 antibody, followed by treatment with FITC-conjugated goat anti-rabbit IgG. From left to right: green fluorescence for BM5, DAPI and the merge images. Nuclei were stained with DAPI (blue). (B) For a control, preimmune serum was used as the primary antibody. Samples were observed under a confocal laser scanning microscope.

terminal Gln-pyroGlu. The protein was confirmed to be AcMNPV ORF13 by a MASCOT score of 91, with 6 peptides matched and 28% amino acid coverage (Fig. 4). The result indicated that the protein was BmNPV ORF5 (*Bm5*).

Localization of the BM5 protein in cell, BV and ODV

Purified ODVs and BVs were subjected to Western blot analysis to determine whether the BM5 protein was associated with ODV and BV, but no predominant band was detected with antiserum against BM5 (data not shown). The above results suggested that BM5 was not a structural protein associated with ODV or BV.

Cellular localization of BM5 in BmN cells

Confocal laser scanning fluorescence microscopy was utilized to determine the cellular localization of BM5 protein in host cells. Since the BM5 protein was first detected 24 h p.i., the time points 24, 48, and 72 h p.i. were chosen for observation. The results showed that the BM5 protein was primarily in the cytoplasm and was scarcely detectable in the nucleus from 24 to 72 h p.i. (Fig. 5). As control experiment, no obvious fluorescence signal was observed in infected cells reacted with FITC-conjugated goat anti-rabbit IgG.

Discussion

The preliminary characteristics of *Bm5* were explored. Homologues of *Bm5* have been identified in genomes of all lepidopteran baculoviruses (Nie *et al.*, 2007). Based on the phylogenetic analysis, 62 genes, including *Bm5*, were conserved among all sequenced lepidopteran NPVs. These genes were considered as core genes for all lepidopteran NPVs (Jehle *et al.*, 2006). It is suggested that *Bm5* and its homologues might play an important role in baculoviridae infection cycles. Here we presented the transcription, expressional and cellular localization, and structure localization analysis of the *Bm5* gene. It was expressed as a late gene, primarily localized in the cytoplasm, and it was a non-structurally functional protein.

The transcription analysis of *Bm5* by RT-PCR showed that it started to transcribe 12 h p.i. and remained until at least 72 h p.i. This result suggested that *Bm5* might be a late gene. It has been estimated that the AcMNPV genome codes for 70–100 protein products. The synthesis of these products is regulated in cascade fashion in four separate classes. Products of the first class, or immediate-early genes, require only host cell factors for their expression. The delayed-early genes precede and are necessary for replication of the viral genome, which begins at about 6 h p.i. Expression of later genes, many of which code for viral structural proteins of both forms of AcMNPV, commences only after viral DNA replication has begun. The very late genes are primarily involved in occlusion of the late form of the virus protective polyhedral matrices of protein (Friesen and Miller, 1986). Baculovirus late genes were transcribed using a conventional RNA polymerase II promoter (Hooft van Iddekinge *et al.*, 1983). Baculoviruses encode a novel RNA polymerase composed of four subunits that transcribe late and very late genes and that recognize the unique promoter consensus sequence. It is not clear why a virus that replicates in the nucleus would encode its own RNA polymerase, since many such viruses depend on exploiting the host enzyme for transcribing all their genes. It was demonstrated experimentally (Rankin *et al.*, 1988), and eventually it was determined, that the core sequence is normally ATAAG, GTAAG, or TTAAG, and that CTAAG is apparently not used. By analyzing the sequence of *Bm5*, we found the motif ATAAG, which was at 58 nt upstream of the start codon

ATG. The result showed that the forecast corresponded to the experiments. Western blot analysis showed that the translational product of BM5 was about 39 kDa, which is consistent with the predicted protein size. It further confirmed that *Bm5* was a late gene which was detected from 24 to 72 h p.i. by BM5 polyclonal antiserum.

Our Western blot analysis demonstrated that BM5 is not a structural component of BV or ODV. A number of proteins were the structural proteins of BV or ODV or both BV and ODV. Little proteins were non-structural proteins associated with ODV or BV, such as *Bm41* (Tian *et al.*, 2009), *Bm67* (Chen *et al.*, 2007), and *Ha83* (Wang and Zhang, 2006). *Bm67* and *Ha83* were also late genes. The subcellular location revealed that BM5 localized primarily in the cytoplasm from 24 h p.i. to 72 h p.i. Motif analyses did not reveal any signal peptide sequence, transmembrane region, nuclear localization signal, or membrane retention signal, consistent with the subcellular localization of BM5 in the cytoplasm of infected cells. A number of important structural proteins were found that localized in the nucleus (Fang *et al.*, 2007; Ge *et al.*, 2009; Huang *et al.*, 2008; Imai *et al.*, 2004; Shen *et al.*, 2009; Tang *et al.*, 2008; Xu *et al.*, 2006) or in the cytoplasm during early stage and in the nucleus during late stage of infection (Du *et al.*, 2006; Long *et al.*, 2003). There were only little structural proteins that localized in the cytoplasm from 24 h p.i. to 72 h p.i. Bm67 was also found in the cytoplasm and required for the production of infectious budded viruses and for the assembly of envelope and nucleocapsids (Chen *et al.*, 2007; Ge *et al.*, 2008). The product of BM67 was also a non-structural functional protein (Chen *et al.*, 2007). It is suggested that BM5 may have some same functions compared to BM67.

In summary, the results of sequence, Western blot and cellular localization analysis implicated that *Bm5* plays an important role in the replication of lepidopteran NPVs. Although some basic characteristics of *Bm5* were studied, the exact role of *Bm5* in the virus infection cycle remained unknown. To further understand the functions of *Bm5*, a *Bm5*-knockout virus should be constructed and analyzed.

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