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

Yang Zhou^a, Keping Chen^{a,*}, Qin Yao^a, Hongxing Shen^b, Guiting Liang^a, Xiaogang Li^a, Nan Wang^a, and Yijia Li^c

- ^a Institute of Life Sciences, Jiangsu University, No. 301 Xuefu Road, Zhenjiang 212013, P. R. China. Fax: +86-5 11-88 79 19 23. E-mail: kpchen@ujs.edu.cn
- School of Medical and Laboratory Medicine, Jiangsu University, No. 301 Xuefu Road, Zhenjiang 212013, P. R. China
- ^c Zhejiang Province Yuyao No. 2 Middle School, Weiba Road, Yuyao 315400, P. R. China
- * Author for correspondence and reprint requests
- Z. Naturforsch. 65 c, 508-518 (2010); received January 29/March 3, 2010

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

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 orf13 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'-AGGATCCATGCTATC-CTGGTTATGG-3' (containing the BamHI site) and 5'-CCGCTCGAGTTACAATACTTCTTG-TAT-3' (containing the XhoI site) from the Bm-NPV genomic DNA. The amplified fragment was digested with BamHI and XhoI 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 Ni2+-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 N₂ 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 μ g 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 PCRamplified by the gene-specific primers 5'-GAT-GTGGGCAACAGGTTTG-3' and 5'-CACCGT-CATAGAGCACTTCCA-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, respec-

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'-ACTGAAG-GCGAGCGTGAT-3' and 5'-AAATGCTGGT-GTTTGGTAAT-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 $4{,}000 \times g$, 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-

cgcgctcacgttgcagtattggccggacgtggacagggatatttttttgtaacgttaacaa acaaatacgcgcaccgtacagctataattataagggaactaaattttctcgttgtataac aaaggaattgctagacaaattaaagcaatgctatcctggttatggaattggtggatgtgg M L S W L W N W W W tccqqtqacaacqacqacqacqacqccqccatcqccqccqaaqatcqqttcqat S G D N D D D D N D A A I A A E D R F D ccagacgactacaaaaagtaccacataaacgtccaacaatggtcgcacatcgttaaatgg PDDYKKYHINVQQW S H I V gattcattcaaatgcaacacgcacagtttcaagtacagatacgtgcacaacgacacgaac D S F K C N T H S F K Y R Y V H N D gcaaaattctacaatgtaatagatttttgcaaaggtcttgaaattgcgcacgacgacata AKFYNVIDFCKGLEIAHDDI cttgattgcaattgggacagcgatcaagtttaccatttaaacgaaattatttttcacaag LDCNWDSDQVYHLNEIIF cagaaatccaaacgcgatctcaactcgttgggcgcattgttcgcgaccaagcaggggttg Q K S K R D L N S L G A L F A T K Q G L ttgaaaattttgatgcggttaaattttgacaacaaaagcaacgcgttgctgcaccttcaa LKILMRLNFDNKSNALLHL Q actgaaggcgagcgtgatgatttgcgcgacaaaattgaatctgttttaaaacatgtaaaa TEGERDDLR D K I E S V L K H aaactgaatacaaacagcgaaaaatttatggtcacccacgaaacgttcaagaacgatgtg K L N T N S E K F M V T H E T F K N D V G N R F E Q F E L R L N E L D A K L N M ctgcagtcggccgaaaaattgaaaaccgccatcgtaacggaaagcaaaaatggcacggtg 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 gaccgcatcaaactcgcttttgtttttgggccaagagcgacattttcgcaagcgaaaaatg L A F VLGQERHF cgctttgaagacgacatggaagtgctctatgacggtgtgcacccaaatcccttgttggca R F E D D M E V L Y D G V H P N P I, I, A attcaatgtattaacgaaaactctacgataaacattacaaaattagaaaaatagctaaa I Q C I N E K L Y D K H Y K I R K I A K cgtgtaatcgacgtggattgtactcataatgtagttaaagaggttatacaagaagtattg RVIDVDCTHNVVKEVIQEVL aaatagtttgattgtacattttgttttttttttaaatatttacacaacgaaacataaata attgcagtaatcagatgacaatcttgtcaaataattcttgaggcatatttacaatgacga cgcttcgtggttgaggctgcca

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 pro-

tein 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 Bm-NPV *Bm5* (Fig. 2).

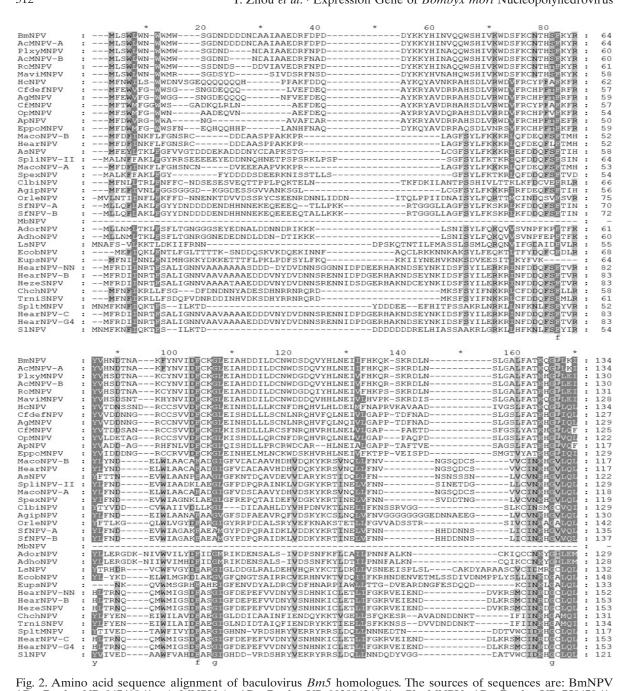


Fig. 2. Amino acid sequence alignment of baculovirus *Bm5* homologues. The sources of sequences are: BmNPV (GenBank, NP_047418.1), AcMNPV-A (GenBank, YP_002884244.1), PlxyMNPV (GenBank, YP_758479.1), AcMNPV-B (GenBank, NP_054042.1), RoMNPV (GenBank, NP_703003), MaviMNPV (GenBank, YP_950735.1), HcNPV (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), SpexN-PV (GenBank, NP_037773.1), ClbiNPV (GenBank, YP_717660.1), AgipNPV (GenBank, YP_002268051.1), OrleNPV

		180	*		200	*	220	*	240	# ####################################		
BmNPV	:	MRIN-DONE	SNALLHIQTEG	;	ERD	DLR	DKII	ESVIKHVI	KKINTNSEK	FMVTHETFKND	:	191
AcMNPV-A	:	IMRIN-FDNK	SNALLHIQTEG	;	ERD	DLR	DKI	ESVI KHVI	KKINTNSEK	FMVTHETFKND	:	191
PlxyMNPV	:	MRIN-FANK	SNALLHIQTEG	;	ERD	DLR	DKII	ESVIKHVI	KKINANSEK	FMVTHETFKNEV	:	187
AcMNPV-B	:	IMRIN-FANK	SNALLHIQTEG	i	ERD	DLR	DKII	ESVIKHVI	KKINANSEK	FMVTHETFKNEV	:	187
RoMNPV	:	MOUN-FANS	SNALLHIQTEG	;	ERD	DLR	DKII	ESVI KHVI	KKINANSEK	FMVTHETFKNEV	:	188
MaviMNPV	:	MRIIN-PANE	SAALLHIQTEG		ERD	DIR	DKII	ESVI KHVI	KKINTNSEN	FMVTHETFKNEV	:	185
HcNPV	:	P QUI P - ITVNIS	DDVLLARTDK	G	YDRD	DVR	DKII	EAVERH	KTTANSDK	FISAHKSFKLEV		193
CfdefNPV	•	P P INNE	TDVIMA KTDK	G	YDHD	DVR	DKII	ETVIKH	KAHNANSDK	FISAHKSFKLDV	1 :	186
AgMNPV	•	COLD I LA	DDVIMALKTUK	G	INHD	DVR	DKII	ETW KH	MANSOK	FISANKSFKLEV	:	188
CfMNPV OpMNPV	:	OOFS-EANE	PDVELA KIDK	.G	IDRD	NIT T	DK	E T WE NIE	THANSDA	TISANKSFKNEN	:	184 181
ApNPV	:	COLD-DINK	EOVI I A BADR		ERD	DMR	FK	ESW KHWI	CT SANSDK	EINSHKSEKI DI	:	174
EppoMNPV	:	COLS-DEVE	DDVIJAKTOK	G	YDCD	DVR	DN	KTW KH	TUNNSDK	FINAHKLEENOV	:	188
MacoNPV-B	:	DNII D-II VINIS	AEFTAW LDNV	F	VELEAK	FVP	SPLDEK	TKWICA	COHNSE	MACTNEOFKLOV	1	182
HearNPV		DNID-RVNK	AEFTAW LDNV	F	VELEAK	FVP	SPLDEK	TKVI ČA	COHNSE	MACTNEOFKLOV		182
AsNPV	:	DHID-PPNK	AEFTAW IENV	F	TELEGK	LVTT	SSIDEK	GKMI KAVI	GKQHNDE	MARTSEQFKSQV	:	188
SpliNPV-II	:	IDHI D-FKNK	AEFTAWHIEEV	Y	VELENK	FLP	SPLDDK	NKVIAAVI	TEKQHNNE	ASRTNDQFKNQV	:	194
MacoNPV-A	:	DNID-DINK	AEFTAW LDNV	F	VELEGK	FVP	SPLDEK	TKVIÇA	OG QQHNSE	MAQTNEQFKLQV	:	183
SpexNPV	:	IDRID-EKNE	AEFTAWNIEEV	Y	VELENK	FLP	SPIDDK	NKVIAAVI	TEKQHNNE	ALRTDDQFKDQV	:	184
ClbiNPV	:	KSCN-KLEL	ANDLICAINSL	KP	NTNKVDVA	VEKRQNSNE-	SIE	QKLATMU	EYEKCNKT	LVDTNQCFKNE	:	199
AgipNPV	:	DOND-DONE	AEFMAWITENV	Y	AELENK	FEP	SPIDEK	NKMITAVI	DDIKQHNDE	LARTNEHFKSOV		195
OrleNPV SfNPV-A	•	DNIE-CGNE	ALAAARWULKL	N	ELAA	TORDUDELH-	CDIDDIN	DKI CALL	OVEGTNNAV.	LLSYHDVLKNER	. :	207
SINPV-A SINPV-B	- :	DNI D-RKNE	AEFITWHIEDV	1	LELENK	FIP	SPIDEK	SKW AAV	TEREBUSE	VVKINDEFKIQ	1 :	200
MbNPV	:	EDMED-MAKE	ADDITO	1	LELENK	FVP	SPIDER	TKWICA	COHNSE	VACINDEFKIO	:	42
AdorNPV	:	BESWC-DENK	AVENSKULELO	N	EIEGN	DNSNNV	GKETENKE	EKTYKI	KNSNES	MSOSIKTOV	:	194
AdhoNPV	- :	ESWC-DENK	AVFVSK LEME	N	EIEGN	NNHNVNSNNV	GKETELNE	EKYKI	S KHSNKS	MSQSLKTQ MSQSLKTQ NAKLNEQFKFKA LLSGADNLNNQF LNVCYAQLINK	:	197
LsNPV	- :	ERWR-PDG	TEFTVW MSNK	TFAS	SSSSSSEAS	EOSKDNS	Vii	ON DHW	RRDNET	NAKLNEOFKFKA	:	203
EcobNPV	:	EHUD-EDNE	LEFVSC RETF	N	DLELG	FNDGNKVVAP	PPSPTLEHKF	NOLICA	SVTNET	LLSGADNLNNOF		222
EupsNPV	:	IDNIE-IDNE	AKFIIC LETI	N	MLESA	NEKEQRIED-	EKI	SKIINAVI	CLANENNTN	LNVCYAQLINK	:	199
HearNPV-NN	:	INHIE-FANK	SEFIAW VTYA	FDKLYS	HMPSSSTVAP	KTSSSSSSS	LSQLCVEKS	ÇMIYEKI	DERADHHS.	ANENMHKS ANENMHKS ANENMHKS	:	233
HearNPV-B	:	INHIE-FANK	SEFIAW VTYA	FDKLYS	HMPSSSTVAP	KTSSSSS	LSQLCVEKS	ÇMIFEKI	D RADHHS	ANENMHKS	:	231
HezeSNPV	:	INHTE-FANK	SEFIAW VTYA	FDKLYS	HMPSSSTVAP	KTSSSSSSSS	LSQLCVEKS	ÇMIYEKI	DERADHHS.	ANENMHKS	:	234
ChchNPV	:	DNVN-PKNK	IEFTTWVIENV	F	DAMKEE	HLSTKKKPLP	LASLPIEEK	TEMBRVF	CAFVRNNET	ANENMHKS NTIETLTKNYCA FTLDTLTKNYCA VAFRDTV ANENMHKS ANENMHKS	:	206
TrniSNPV	:	I DNWN-IIKNE	IEFTTW IETV	F	DGMKEE	HLTSKKKPLP	IVSLPIEEK	TEM RVF	CAFVKNSET	FTLDTLTKNYCA	:	209
SpltMNPV	:	DIFFERENCE	SNETVWELN		DIVAR	LEKKDGE		NRIIIDSVI	SQUKRDD	VAFRDTV		171
HearNPV-C	:	NH E-EANS	SEFIAW VIYA	FDKLYS	HMPSSSTVAP	KTSSSSS	LSQLCVEKS	CMIFER	SD RADHHS	ANENMHKS	1 :	231
HearNPV-G4 S1NPV	:	DA EDISER	SELIMMENTIN	FUKLIS	DTUAD	VISSSSS	POÓPCAEVO	ND TEL	DIKADING.	AREFRNTY		175
SINFV	•	f k	SDEIVWIEN		DIVAN	LEKKDGE	755	63	ZEILLOD-	ALEE KIVI		1/5
								OT				
								61				
								61				
		260	*	280	*	300	*	320)	* 340		007
BmNPV	:	260 GNRFEQFELR	* NE	280 DAKLNM	LQSAEKLKTA	300 IVTESK	*	320)	* 340	:	227
AcMNPV-A	: :	260 GNRFEQFELR GNRFEQFELR	* INEI	280 DAKINM DAKINM	* LQSAEKĪKTA LQSAEKĪKTA	300 IVTESK IVTESK	*	320)	* 340	: :	227
AcMNPV-A PlxyMNPV	: : :	260 GNRFEQFELR GNRFEQFELR GNRFEQFELR	* INEI	280 DAKUNM DAKUNM DAKUNM	* LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA	300 IVTESK IVTESK VVAESK	*	320)	* 340	: : :	227 227 223
AcMNPV-A PlxyMNPV AcMNPV-B	: : : : :	260 GNRFEQFELR GNRFEQFELR GNRFEQFELR GNRFEQFELR	* INEI INEI IHEI IHEI	280 LAKINM LAKINM LAKINM LAKINM	* LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA	300 IVTESK IVTESK VVAESK VVTESG	*	320)	* 340	: : : : :	227 227 223 223 224
AcMNPV-A PlxyMNPV AcMNPV-B RoMNPV	: : : : : :	260 GNRFEQFELR GNRFEQFELR GNRFEQFELR GNRFEQFELR GNRFEQFELR	* INEI INEI IHEI IHEI	280 LAKINM LAKINM LAKINM LAKINM	* LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKL	300 IVTESK IVTESK VVAESK VVTESQ VASENN	*	320)	* 340	: : : : : : : : : : : : : : : : : : : :	227 227 223 223 224 219
AcMNPV-A PlxyMNPV AcMNPV-B	: : : : : : :	260 GNRFEQFELR GNRFEQFELR GNRFEQFELR GNRFEQFELR GNRFEQFELR GNRFEQFESR GRRFEQFESR	* INEI INEI IHEI IHEI IHE	280 DAKLNM DAKLNM DAKLNM DAKLNM DAKLNM	* LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSVETAV LCAPTOTTE-	300 IVTESK IVTESK VVAESK VVAESK VVTESQ APVS	*	320)	* 340		227 227 223 223 224 219 226
AcMNPV-A PlxyMNPV AcMNPV-B RoMNPV MaviMNPV HcNPV	: : : : : : : :	260 GNRFEQFELR GNRFEQFELR GNRFEQFELR GNRFEQFELR GNRFEQFESR GARFEQFESR GARFEQFEQR	* INEI INEI IHEI IHEI IHEI IFFI	280 DAKINM DAKINM DAKINM DAKINM DAKINM DAKINM DAKINT NTKVNA	* LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSVET AV LQAPTQTTE- ISHERTTPS I	300 IVTESK IVTESK VVAESK VVAESK VASENN APVS	*	320)	* 340		227 227 223 223 224 219 226 220
AcMNPV-A PlxyMNPV AcMNPV-B RoMNPV MaviMNPV	: : : : : : : : : : : : : : : : : : : :	260 GNR FEQFELR GNR FEQFELR GNR FEQFELR GNR FEQFELR GNR FEQFELR GNR FEQFELR GNR FEQFER GAR FEQFER NAR FRÇMEQ	* INE I INE I IHE I IHE I IHE I IES I FEN I	280 LAKLNM LAKLNM LAKLNM LAKLNM LAKLNM LAKLNM LAKLNT LKVNA LINKLNS	LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSVET - AV LÇAPTQTTE- ISHERTTPSI IPHERTTSSI	300 IVTESK IVTESK VVAESK VVAESK VVAESK APVS APVS AIPG	*	320		* 340		227 227 223 223 224 219 226 220 222
ACMNPV-A P1xyMNPV ACMNPV-B ROMNPV MaviMNPV HCNPV CfdefNPV AgMNPV CfMNPV		260 GN: FECFELR GN: FECFELR GN: FECFELR GN: FECFELR GN: FECFELR GN: FECFELR GN: FECFELR NA: FKCMECR NA: FKCMECR NA: FKCMECR CV: FECFECR	* INE - I I I I I I I I I I I I I I I I I I	280 IAKINM IAKINM IAKINM IAKINM IAKINM IAKINM IAKINT IAKINT ITKVNA INTINS	LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSVETAV LQAPTQTTE- ISHERTTPSI IPHERTTSSI IPFASTQG	300 IVTESK IVTESK VVAESK VVAESK VTESQ ASENN APVS AIPG APV	*	32(* 340		227 227 223 223 224 219 226 220 222 214
ACMNPV-A P1xyMNPV ACMNPV-B ROMNPV MaviMNPV HCNPV CfdefNPV AGMNPV CfMNPV OPMNPV		260 GNT FECTELE GNT FECTELE GNT FECTELE GNT FECTELE GNT FECTELS GNT FECTELS GNT FECTELS GNT FECTELS GNT FECTELS CAT FECTELS CAT FECTELS CAT FECTELS CAT FECTELS CAT FECTELS	*	280 IAKLNM IAKLNM IAKLNM IAKLNM IAKLNM ITKUNA ITKUNA INRINS INRINS ITKINA	LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSVETAV LQAPTQTTE- ISHERTTPSI IPHERTTSSI IPHASTQG LQCAAPTRT	300 IVTESK IVTESK VVAESK VVAESK VAESK VATESQ ASENN APVS AIPG APV APV	*	32(* 340		227 227 223 223 224 219 226 220 222 214 213
ACMNPV-A P1xyMNPV ACMNPV-B ROMNPV MaviMNPV HCNPV CfdefNPV AgMNPV CfMNPV OPMNPV APMPV		250 GNTFECFELE GNTFECFELE GNTFECFELE GNTFECFELE GNTFECFECE GNTFECFECE CNTFECFECE CTTFECFECE GATFECFECE CTTFECFECE GATFECFECE	*	280 ITAKINM ITAKINM ITAKINM ITAKINM ITAKINM ITAKINT ITAKINT ITAKINS ITAKINS ITKINS ITKINS ITKINS	LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSVET - AV LQAPTQTTE- ISHERTTPSI IPHERTTSSI PFASTQG LQCAAPTRT- PS-ERD	300 IVTESK IVTESK VVAESK VVAESK VVTESQ VASENN APVS AIPG AIPG AIPG APV APG AAPG AAPG	*	320		* 340		227 227 223 223 224 219 226 220 222 214 213 203
Acmnev-A Plxymmev Acmnev-B Romnev Mavimmev Hcnev Cfdefmev Agmnev Cfmnev Opmnev Apnev Eppomnev		260 GN: FECFELR NA: FKCMECR GN: FECFELR GN: FECFELR GN: FECFELR GN: FECFELR GN: FECFELR	* INE	280 IAKI NM INMI NM IAKI NM IA	LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSVET AV LQAPTQTTE ISHERTTPSI IPHERTTSSI IPHERTTSSI LQCAAPTRT PS-ERD	300 IVTESK VVAESK VVAESK VVAESK VASENN APVS AIPG APV APPG APPG APPG APPG APPG APPG APPG	*	320		* 340		227 227 223 223 224 219 226 220 222 214 213 203 213
ACMNPV-A P1xyMNPV ACMNPV-B ROMNPV MaviMNPV HCNPV CfdefNPV AGMNPV CPMNPV APNPV EPPOMNPV MacONPV-B		260 GNT FECTELE CVT FECTELE CVT FECTELE NAT FECTELE NAT FECTELE NAT FECTELE CVT FECTELE NAT FECTELE NAT FECTELE NAT FECTELE THE FEATOR	* INE	280 IAKINM IAKINM IAKINM IAKINM IAKINM IAKINM IAKINT IX INRINS INRINS INKINA ITKINA ITKINA ITKINA	LQSAEK KTA LQSAEK KTA LQSAEK KTA LQSAEK KTA LQSAEK KTA LQSVET - AV LQSVET - AV LGAPTQTTE- ISHERTTFSI IPHERTTSSI PFASTQG LQCAAPTR- PS-ERD PS-ERD PT VENVDDLYRR	300 IVTESK IVTESK IVTESK VVAESK VVAESK VVTESQ ASENN APVS AIPG APV APG APG REHHR	*	32()	* 340		227 227 223 223 224 219 226 220 222 214 213 203 213 239
AcMNPV-A PlxyMNPV AcMNPV-B ROMNPV HCNPV CfdefMPV AgMNPV CfMNPV OpMNPV ApNPV EppoMNPV MacoNPV-B HearNPV		250 GNTFECFELE GNTFECFELE GNTFECFELE GNTFECFELE GNTFECFECE GNTFECFECE GNTFECFECE GNTFECFECE GNTFECFECE CATFECFECE GATFECFECE GATFECFECE CATFECFECE GATFECFECE GATFECFECE GATFECFECE GATFECFECE GATFECFECE GATFECFECE LEFEAFDRT	*	280 DAKLIMM DAKLIMM DAKLIMM DAKLIMM DAKLIMM DAKLIMM DAKLIMM DAKLIMM DIKLIMM	LQSAEKLETA LQSAEKLETA LQSAEKLETA LQSAEKLETA LQSAEKLETA LQSAEKLETA LQSVET AV LQAFTQTT- ISHERTTPSI ISHERTTPSI IPHERTTSSI PFASTQG PT PT PT PT PT	300 IVTESK IVTESK VVAESK VVAESK VASENN APVS APVS APVS APPS APG APG	-TLERPTLERPNI ADV	32(SFLSSSNT SFLSSNT SFLSSSNT SFLSSNT SFLSSSNT SFLSSSNT SFLSSSNT SFLSSSNT SFLSSSNT SFLSSSNT SFLSSNT SFLSSSNT SFLSSNT SFL	* 340		227 227 223 223 224 219 220 222 214 213 203 213 239 239
AcMNPV-A PlxyMNPV AcMNPV-B RoMNPV MaviMMPV HcNPV CfdefNPV AgMNPV CpMNPV ApNPV EppoMMPV MacoNPV-B HearNPV AsNPV	:	IERFELFDRR	*	280 IDAKINM	LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSVETAV LQAPTQTTE ISHERTTFSI IPHERTTSSI IPHERTTSSI PFASTQG LQCAAPTRT PFASTQG YENVDDLYRR FDNVDDLYRR FDNVDDLYRR	300 IVTESK IVTESK VVAESK VVAESK VAESK VASENN APVS AIPG AIPG APY APG IREHHR IREHHR IREHHR IREHHR IREHHR IREHHR	-TLERPTLERPNLAPNTLAPN	32(ISFLSSNT ISFLSSNTSFLSSVCS	* 340		227 223 223 224 226 220 222 214 213 203 213 239 239 255
ACMNPV-A PlxyMNPV ACMNPV-B ROMNPV MaviMNPV HCNPV CfdefNPV AGMNPV CFMNPV EppoMNPV EppoMNPV MacoNPV-B HearNPV ASNPV SpliNPV-II	:	IERFELFDRR IERFEWFNVQ	* INE	280 ITAKI NM	LQSAEK KTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSAEKLKTA LQSVET - AV LQSAEKTTS-I ISHERTTS-I ISHERTTS-I ISHERTTS-I IPFASTQG LQCAAPTRT PS-ERD PT YENVDDLYRR YENVDDLYRR LNNVDELYKR LNNVDELYKR	300 IVTESK IVTESK VVAESK VVAESK VVTESQ VASENN APVS AIPG AIPG APG	TLERP TLERP NLAPN STTTS TLERP	320 320 320 320 320 320 320 320 320 320	SFLSSSNT SFLSSSNT SFLSSSNT SFLSSVCS: SIFSTEGN	* 340 INDDH		227 227 223 223 224 219 220 222 213 203 213 2239 2255 256
ACMNPV-A PlxyMNPV ACMNPV-B ROMNPV MaviMMPV HCNPV CfdefMPV AGMNPV CFMNPV APNPV EPPCMMPV MacONPV-B HearNPV ASNPV SpliMPV-II MacONPV-II MacONPV-II	:	IERFELFDRE IERFEWFNVQ IERFEAFDRE	TAEI TSEI VAEI	NEKMTM NKKMTM NEKMNM	FDNVDDLYRR LNNVDELYKR YENVDDLYRR	RDHHR QDYHK REHHR	NLAPN STTTS TLERP	QNLRRI	SFLSSVCS: SIFSTEGNO	SPSDPÇANAVDR GGSGGGDDE INDDH	:	255 256 240
ACMNPV-A P1xyMNPV ACMNPV-B RCMNPV MaviMNPV HCNPV CfdefNPV AGMNPV CfMNPV CPMNPV APNPV EPPOMNPV ADNPV SPLINPV SPLINPV-II MACONPV-A SPEXNPV SPLINPV-II MACONPV-II	: : : :	IERFELFDRR IERFEWFNVQ IEKFCAFDRR IERFEWFNVQ	IAEL ISEL VAEL ISEL	NEKMTM NKKMTM NEKMNM NKKMST	FDNVDDLYRR LNNVDELYKR YENVDDLYRR LNNVDELYRR	RDHHR REHHR	NLAPN STTTS TLERP SNNIN	QNLRRI RTRI QHI	SFLSSVCS: LSIFSTEGNO LSFLSSSNT: SNNASSST	SPSDPÇANAVDR GGSGGGDDE INDDH ALSSSSSYENNI	:	255 256 240 249
ACMNPV-A PlxyMNPV ACMNPV-B ROMNPV MaviMMPV HCNPV CfdefMPV AGMNPV ADNPV EPPCMMPV MacONPV-B HearNPV ASNPV SpliNPV-II MacONPV-II MacONPV-A SPEXNPV ClbiNPV	: : : : : :	IERFELFDRE IERFEWFNVQ IERFEWFNVQ IERFEWFNVQ INKFSVLDT	IAEI ISEI VAEI ISEI IENFENQLKQV	NEKMTM NKKMTM NEKMNM NKKMST NEKIDL	FDNVDDLYRR LNNVDELYKR YENVDDLYRR LNNVDELYRR LNNVEQLYQT	RDHHR QDYHK REHHR QDYHK KEHHKNKL-	NLAPN STTTS TLERP SNNIN -VNSKVVTAK	QNLRRI RTRI QHI TTMI	SFLSSVCS: LSIFSTEGN LSFLSSSNT: SNNASSST LSFLDESHR	SPSDPÇANAVDR GGSGGGDDE INDDHALSSSSSYENNI QEDQGVTSSN		255 256 240 249 280
ACMNPV-A PlxyMNPV ACMNPV-B ROMNPV MaviMNPV HCNPV CfdefNPV AGMNPV CfMNPV OPMNPV APNPV EPPOMNPV ADONPV HearNPV SpliNPV-II MacONPV-A SPEXNPV ClbiNPV ClbiNPV AgipNPV AgipNPV	: : : : : :	IERFELFDRE IERFEWFNVQ IERFEWFNVQ IERFEWFNVQ INKFSVLDT	IAEI ISEI VAEI ISEI IENFENQLKQV	NEKMTM NKKMTM NEKMNM NKKMST NEKIDL	FDNVDDLYRR LNNVDELYKR YENVDDLYRR LNNVDELYRR LNNVEQLYQT	RDHHR QDYHK REHHR QDYHK KEHHKNKL-	NLAPN STTTS TLERP SNNIN -VNSKVVTAK	QNLRRI RTRI QHI TTMI	SFLSSVCS: LSIFSTEGN LSFLSSSNT: SNNASSST LSFLDESHR	SPSDPÇANAVDR GGSGGGDDE INDDHALSSSSSYENNI QEDQGVTSSN		255 256 240 249 280
ACMNPV-A PlxyMNPV ACMNPV-B ROMNPV MaviMMPV HCNPV CfdefNPV AGMNPV CfMNPV APNPV APNPV APNPV MACONPV-B HEARNPV SplinPV-II MACONPV-A SPEXNPV ClbiNPV AgipNPV OrleNPV OrleNPV OrleNPV SfNPV-A	: : : : : :	IERFELFDRE IERFEWFNVQ IERFEWFNVQ IERFEWFNVQ INKFSVLDT	IAEI ISEI VAEI ISEI IENFENQLKQV	NEKMTM NKKMTM NEKMNM NKKMST NEKIDL	FDNVDDLYRR LNNVDELYKR YENVDDLYRR LNNVDELYRR LNNVEQLYQT	RDHHR QDYHK REHHR QDYHK KEHHKNKL-	NLAPN STTTS TLERP SNNIN -VNSKVVTAK	QNLRRI RTRI QHI TTMI	SFLSSVCS: LSIFSTEGN LSFLSSSNT: SNNASSST LSFLDESHR	SPSDPÇANAVDR GGSGGGDDE INDDHALSSSSSYENNI QEDQGVTSSN		255 256 240 249 280
ACMNPV-A PlxyMNPV ACMNPV-B ROMNPV MaviMNPV HCNPV CfdefMPV AGMNPV OPMNPV APNPV EPPOMNPV MacONPV-B HearNPV ASNPV SplinPV-II MacONPV-A SpexNPV ClbiNPV ClbiNPV AGIPNPV SfNPV-B	: : : : : :	IERFELFDRE IERFEWFNVQ IERFEWFNVQ IERFEWFNVQ INKFSVLDT	IAEI ISEI VAEI ISEI IENFENQLKQV	NEKMTM NKKMTM NEKMNM NKKMST NEKIDL	FDNVDDLYRR LNNVDELYKR YENVDDLYRR LNNVDELYRR LNNVEQLYQT	RDHHR QDYHK REHHR QDYHK KEHHKNKL-	NLAPN STTTS TLERP SNNIN -VNSKVVTAK	QNLRRI RTRI QHI TTMI	SFLSSVCS: LSIFSTEGN LSFLSSSNT: SNNASSST LSFLDESHR	SPSDPÇANAVDR GGSGGGDDE INDDHALSSSSSYENNI QEDQGVTSSN		255 256 240 249 280
ACMNPV-A PlxyMNPV AcMNPV-B ROMNPV MaviMMPV HCNPV CfdefMPV AgMNPV CfMNPV ApMNPV ADNPV EPPOMMPV AGONPV-B HeanNPV ASNPV SpliNPV-II MacONPV-A SpexNPV AgipNPV AgipNPV AgipNPV AgipNPV AgipNPV AgipNPV AgipNPV SfNPV-B SfNPV-B SfNPV-B SfNPV-B MbNPV	: : : : : :	IERFELFDRE IERFEWFNVQ IERFEWFNVQ IERFEWFNVQ INKFSVLDT	IAEI ISEI VAEI ISEI IENFENQLKQV	NEKMTM NKKMTM NEKMNM NKKMST NEKIDL	FDNVDDLYRR LNNVDELYKR YENVDDLYRR LNNVDELYRR LNNVEQLYQT	RDHHR QDYHK REHHR QDYHK KEHHKNKL-	NLAPN STTTS TLERP SNNIN -VNSKVVTAK	QNLRRI RTRI QHI TTMI	SFLSSVCS: LSIFSTEGN LSFLSSSNT: SNNASSST LSFLDESHR	SPSDPÇANAVDR GGSGGGDDE INDDHALSSSSSYENNI QEDQGVTSSN		255 256 240 249 280
ACMNPV-A PlxyMNPV ACMNPV-B ROMNPV MaviMNPV HCNPV CfdefNPV AGMNPV CfMNPV OPMNPV APNPV EPPOMNPV ADNPV EPPOMNPV ASNPV SpliNPV-II MaconPV-A SpexNPV ClbiNPV OrleNPV OrleNPV SfNPV-A SfNPV-B MbNPV ASNPV ASNPV AGIPNPV AGIPNPV AGIPNPV AADONPV-A AGIPNPV AADONPV-B MbNPV AdorNPV AdorNPV	: : : : : :	IERFELFDRE IERFEWFNVQ IERFEWFNVQ IERFEWFNVQ INKFSVLDT	IAEI ISEI VAEI ISEI IENFENQLKQV	NEKMTM NKKMTM NEKMNM NKKMST NEKIDL	FDNVDDLYRR LNNVDELYKR YENVDDLYRR LNNVDELYRR LNNVEQLYQT	RDHHR QDYHK REHHR QDYHK KEHHKNKL-	NLAPN STTTS TLERP SNNIN -VNSKVVTAK	QNLRRI RTRI QHI TTMI	SFLSSVCS: LSIFSTEGN LSFLSSSNT: SNNASSST LSFLDESHR	SPSDPÇANAVDR GGSGGGDDE INDDHALSSSSSYENNI QEDQGVTSSN		255 256 240 249 280
ACMNPV-A PlxyMNPV ACMNPV-B ROMNPV MaviMMPV HCNPV CfdefMPV AGMNPV CfMNPV ADNPV EPPOMMPV ACONPV-B HearNPV ASNPV SpliNPV-II MacOMPV-A SpexMPV ClbiNPV AGIONPV AGIONPV AGIONPV AGIONPV AGIONPV AGIONPV AGONPV-B MDNPV AddorNPV AddorNPV AddorNPV	: : : : : :	IERFELFDRE IERFEWFNVQ IERFEWFNVQ IERFEWFNVQ INKFSVLDT	IAEI ISEI VAEI ISEI IENFENQLKQV	NEKMTM NKKMTM NEKMNM NKKMST NEKIDL	FDNVDDLYRR LNNVDELYKR YENVDDLYRR LNNVDELYRR LNNVEQLYQT	RDHHR QDYHK REHHR QDYHK KEHHKNKL-	NLAPN STTTS TLERP SNNIN -VNSKVVTAK	QNLRRI RTRI QHI TTMI	SFLSSVCS: LSIFSTEGN LSFLSSSNT: SNNASSST LSFLDESHR	SPSDPÇANAVDR GGSGGGDDE INDDHALSSSSSYENNI QEDQGVTSSN		255 256 240 249 280
ACMNPV-A PlxyMNPV AcMNPV-B ROMNPV MaviMMPV HcNPV CfdefMPV AgMNPV CfMNPV ApMNPV ApNPV EppoMMPV MacoNPV-B HearMPV JShinPV-II MacoMPV-A SpexNPV ClbiNPV OrleNPV OrleNPV AfipNPV AdorNPV-B SfNPV-B MDNPV AdorNPV AdorNPV AdorNPV AdorNPV AdorNPV ALSNPV LSNPV	: : : : : :	IERFELFDRE IERFEWFNVQ IERFEWFNVQ IERFEWFNVQ INKFSVLDT	IAEI ISEI VAEI ISEI IENFENQLKQV	NEKMTM NKKMTM NEKMNM NKKMST NEKIDL	FDNVDDLYRR LNNVDELYKR YENVDDLYRR LNNVDELYRR LNNVEQLYQT	RDHHR QDYHK REHHR QDYHK KEHHKNKL-	NLAPN STTTS TLERP SNNIN -VNSKVVTAK	QNLRRI RTRI QHI TTMI	SFLSSVCS: LSIFSTEGN LSFLSSSNT: SNNASSST LSFLDESHR	SPSDPÇANAVDR GGSGGGDDE INDDHALSSSSSYENNI QEDQGVTSSN		255 256 240 249 280
ACMNPV-A PlxyMNPV ACMNPV-B ROMNPV MaviMMPV HCNPV CfdefMPV AGMNPV CfMNPV ADNPV EPPOMNPV MacOMPV-B HearNPV ASNPV SplinPV-II MacoNPV-A SpexNPV OrleMPV AGIDNPV AGINPV AGINPV AGINPV AGINPV ACONPV ACONPV ACONPV ACONPV ACONPV ACONPV ACONPV ACONPV LSNPV LSNPV ECODNPV	: : : : : :	IERFELFDRE IERFEWFNVQ IERFEWFNVQ IERFEWFNVQ INKFSVLDT	IAEI ISEI VAEI ISEI IENFENQLKQV	NEKMTM NKKMTM NEKMNM NKKMST NEKIDL	FDNVDDLYRR LNNVDELYKR YENVDDLYRR LNNVDELYRR LNNVEQLYQT	RDHHR QDYHK REHHR QDYHK KEHHKNKL-	NLAPN STTTS TLERP SNNIN -VNSKVVTAK	QNLRRI RTRI QHI TTMI	SFLSSVCS: LSIFSTEGN LSFLSSSNT: SNNASSST LSFLDESHR	SPSDPÇANAVDR GGSGGGDDE INDDHALSSSSSYENNI QEDQGVTSSN		255 256 240 249 280
ACMNPV-A PlxyMNPV AcMNPV-B ROMNPV MaviMMPV HCNPV CfdefMPV AgMNPV CfMNPV ApNPV EPPOMMPV MacoNPV-B HearNPV SpliNPV-II MacoMPV-A SpexNPV ClbiNPV AgipNPV ClbiNPV AGipNPV AGipNPV AGipNPV ClbiNPV AGipNPV LSNPV LSNPV LSNPV LSNPV ECODNPV ECODNPV	: : : : : :	IERFELFDRE IERFEWFNVQ IERFEWFNVQ IERFEWFNVQ INKFSVLDT	IAEI ISEI VAEI ISEI IENFENQLKQV	NEKMTM NKKMTM NEKMNM NKKMST NEKIDL	FDNVDDLYRR LNNVDELYKR YENVDDLYRR LNNVDELYRR LNNVEQLYQT	RDHHR QDYHK REHHR QDYHK KEHHKNKL-	NLAPN STTTS TLERP SNNIN -VNSKVVTAK	QNLRRI RTRI QHI TTMI	SFLSSVCS: LSIFSTEGN LSFLSSSNT: SNNASSST LSFLDESHR	SPSDPÇANAVDR GGSGGGDDE INDDHALSSSSSYENNI QEDQGVTSSN		255 256 240 249 280
ACMNPV-A PlxyMNPV ACMNPV-B ROMNPV MaviMNPV HCNPV CfdefMPV AGMNPV CfMNPV OPMNPV APNPV EPPOMNPV MacONPV-B HearNPV ASNPV SPLINEV-II MacONPV-A SPEXNPV ClbiNPV AGIPNPV AGIPNPV AGIPNPV AGIPNPV LSNPV LSNPV LSNPV LSNPV LSNPV LSNPV LSNPV LSNPV LSNPV EUDSNPV HearNPV HearN	: : : : : :	IERFELFDRE IERFEWFNVQ IERFEWFNVQ IERFEWFNVQ INKFSVLDT	IAEI ISEI VAEI ISEI IENFENQLKQV	NEKMTM NKKMTM NEKMNM NKKMST NEKIDL	FDNVDDLYRR LNNVDELYKR YENVDDLYRR LNNVDELYRR LNNVEQLYQT	RDHHR QDYHK REHHR QDYHK KEHHKNKL-	NLAPN STTTS TLERP SNNIN -VNSKVVTAK	QNLRRI RTRI QHI TTMI	SFLSSVCS: LSIFSTEGN LSFLSSSNT: SNNASSSTA LSFLDESHR	SPSDPÇANAVDR GGSGGGDDE INDDHALSSSSSYENNI QEDQGVTSSN		255 256 240 249 280
ACMNPV-A PlxyMNPV AcMNPV-B ROMNPV MaviMMPV HCNPV CfdefMPV AgMNPV CfMNPV ApNPV EDPOMMPV AGNPV-B HeanPV ASNPV SpliNPV-II MacoNPV-B HeanPV ASNPV ClbiNPV AGIDNPV AGIDNPV AGIDNPV AGIDNPV AGIDNPV CLbiNPV AGONPV-B WNPV ACONPV-B WNPV ACONPV LSNPV LSNPV LSNPV LSNPV LSNPV LSNPV HearNPV-NN HearNPV-NN HearNPV-NN HearNPV-NB	: : : : : :	IE FELFORE IE FEWENVO IE FEAFORE IE FEWENVO IE SEWENVO IE SEWENVO IE FEWENVO	AE	NERMIM NERMINM	FDNVDDLYER LINNVDELYER YENVDDLYER LINNVDELYER LINNVDELYER YDNLDRLYTQ LINNVDDLYER LINNVDDLYER YENVDDLYER YENVDDLYER YENVDDLYER YENVDDLYER YENVDLYER YENIGTYEQ SEHIEVLYD LYNNELDE LYNNELDE LYNNELDE LYNNLE LYNNLE LYNNLE LYNNLE LYNLE LYNLE LYNLE LEKLDVLYNH LEKLDVLYNH	RDHHR- QDYHK- REHHR- QDYHK- REHHRNKL- REHHR- QDYHRNKKQ QDHRNKKQ REHHR- REHHK- REHKREHK- REHKREHREHREHREHREHREHREHREHREHREHREHREHREHR	-NLAPNSTITSTLERPSNNINVNSKVVTAK -TTTASTSS -PSAASITRA LFSDSNSTLERPNRIGGG -AISPKIHNGKIE -LQTNN- LQTNN-	QNLRRIRTRIIQH	SFLSSVCS. SIFSTEGN SFLSSSNT SFLSSSNT SFLSSSNT SFLESHR SFLESHR JDINDSASF JDINDSASF ISFLSSSNT MNHAYANM MNQSCATI TRRGEENF SFLSEKHV SFLSEKHV ALYSEEDN ALYSEEDN	SFSDPÇANAVDR GGSGGGDE INDDH- LISSSSYENNI LISSSSYENNI EDDQGVTSSN- GGDS-GDIIGE PRNDN- FNNNNNTCN- FNNNNNANTCN- INDDH- INDDH- IFTAR- IFTAR- IFTAR- IFSQQ- S- FVNG- FVNG-		255 256 240 280 266 272 272 299 244 247 266 290 288
ACMNPV-A PlxyMNPV ACMNPV-B ROMNPV MaviMNPV HCNPV CfdefMPV AGMNPV CfdefMPV AGMNPV OPMNPV ADNPV EPPOMNPV MacoNPV-B HearNPV ASNPV SplinPV-II MacoNPV-A SpexnPV ClbinPV ClbinPV AGipNPV ClbinPV AGipNPV SfNPV-B MbNPV AdorNPV AdorNPV AdorNPV EcobNPV EcobNPV EcobNPV HearNPV-B HearNPV-N HearNPV-N HearNPV-N HearNPV-N HearNPV-N HearNPV-N	: : : : : :	IE FELFORE IE FEWENVO IE FEAFORE IE FEWENVO IE SEWENVO IE SEWENVO IE FEWENVO	AE	NERMIM NERMINM	FDNVDDLYER LINNVDELYER YENVDDLYER LINNVDELYER LINNVDELYER YDNLDRLYTQ LINNVDDLYER LINNVDDLYER YENVDDLYER YENVDDLYER YENVDDLYER YENVDDLYER YENVDLYER YENIGTYEQ SEHIEVLYD LYNNELDE LYNNELDE LYNNELDE LYNNLE LYNNLE LYNNLE LYNNLE LYNLE LYNLE LYNLE LEKLDVLYNH LEKLDVLYNH	RDHHR- QDYHK- REHHR- QDYHK- REHHRNKL- REHHR- QDYHRNKKQ QDHRNKKQ REHHR- REHHK- REHKREHK- REHKREHREHREHREHREHREHREHREHREHREHREHREHREHR	-NLAPNSTITSTLERPSNNINVNSKVVTAK -TTTASTSS -PSAASITRA LFSDSNSTLERPNRIGGG -AISPKIHNGKIE -LQTNN- LQTNN-	QNLRRIRTRIIQH	SFLSSVCS. SIFSTEGN SFLSSSNT SFLSSSNT SFLSSSNT SFLESHR SFLESHR JDINDSASF JDINDSASF ISFLSSSNT MNHAYANM MNQSCATI TRRGEENF SFLSEKHV SFLSEKHV ALYSEEDN ALYSEEDN	SFSDPÇANAVDR GGSGGGDE INDDH- LISSSSYENNI LISSSSYENNI EDDQGVTSSN- GGDS-GDIIGE PRNDN- FNNNNNTCN- FNNNNNANTCN- INDDH- INDDH- IFTAR- IFTAR- IFTAR- IFSQQ- S- FVNG- FVNG-		255 256 240 280 266 272 272 299 244 247 266 290 288
ACMNPV-A PlxyMNPV ACMNPV-B ROMNPV MaviMMPV HCNPV CfdefMPV AGMNPV CfdefMPV AGMNPV ADNPV EPPOMMPV MacoNPV-B HearNPV ASNPV SpliNPV-II MacoNPV-A SpexMPV ClbinPv AGjnPV OrleNPV SfNPV-A SfNPV-A SFNPV-B MbNPV AddorNPV LSNPV EUpsNPV EupsN	: : : : : :	IE FELFORE IE FEWENVO IE FEAFORE IE FEWENVO IE SEWENVO IE SEWENVO IE FEWENVO	AE	NERMIM NERMINM	FDNVDDLYER LINNVDELYER YENVDDLYER LINNVDELYER LINNVDELYER YDNLDRLYTQ LINNVDDLYER LINNVDDLYER YENVDDLYER YENVDDLYER YENVDDLYER YENVDDLYER YENVDLYER YENIGTYEQ SEHIEVLYD LYNNELDE LYNNELDE LYNNELDE LYNNLE LYNNLE LYNNLE LYNNLE LYNLE LYNLE LYNLE LEKLDVLYNH LEKLDVLYNH	RDHHR- QDYHK- REHHR- QDYHK- REHHRNKL- REHHR- QDYHRNKKQ QDHRNKKQ REHHR- REHHK- REHKREHK- REHKREHREHREHREHREHREHREHREHREHREHREHREHREHR	-NLAPNSTITSTLERPSNNINVNSKVVTAK -TTTASTSS -PSAASITRA LFSDSNSTLERPNRIGGG -AISPKIHNGKIE -LQTNN- LQTNN-	QNLRRIRTRIIQH	SFLSSVCS. SIFSTEGN SFLSSSNT SFLSSSNT SFLSSSNT SFLESHR SFLESHR JDINDSASF JDINDSASF ISFLSSSNT MNHAYANM MNQSCATI TRRGEENF SFLSEKHV SFLSEKHV ALYSEEDN ALYSEEDN	SFSDPÇANAVDR GGSGGGDE INDDH- LISSSSYENNI LISSSSYENNI EDDQGVTSSN- GGDS-GDIIGE PRNDN- FNNNNNTCN- FNNNNNANTCN- INDDH- INDDH- IFTAR- IFTAR- IFTAR- IFSQQ- S- FVNG- FVNG-		255 256 240 280 266 272 272 299 244 247 266 290 288
ACMNPV-A PlxyMNPV ACMNPV-B ROMNPV MaviMNPV HCNPV CfdefMPV AGMNPV CfdefMPV AGMNPV OPMNPV ADNPV EPPOMNPV MacoNPV-B HearNPV ASNPV SplinPV-II MacoNPV-A SpexnPV ClbinPV ClbinPV AGipNPV ClbinPV AGipNPV SfNPV-B MbNPV AdorNPV AdorNPV AdorNPV EcobNPV EcobNPV EcobNPV HearNPV-B HearNPV-N HearNPV-N HearNPV-N HearNPV-N HearNPV-N HearNPV-N	: : : : : :	IE FELFORE IE FEWENVO IE FEAFORE IE FEWENVO IE SEWENVO IE SEWENVO IE FEWENVO	AE	NERMIM NERMINM	FDNVDDLYER LINNVDELYER YENVDDLYER LINNVDELYER LINNVDELYER YDNLDRLYTQ LINNVDDLYER LINNVDDLYER YENVDDLYER YENVDDLYER YENVDDLYER YENVDDLYER YENVDLYER YENIGTYEQ SEHIEVLYD LYNNELDE LYNNELDE LYNNELDE LYNNLE LYNNLE LYNNLE LYNNLE LYNLE LYNLE LYNLE LEKLDVLYNH LEKLDVLYNH	RDHHR- QDYHK- REHHR- QDYHK- REHHRNKL- REHHR- QDYHRNKKQ QDHRNKKQ REHHR- REHHK- REHKREHK- REHKREHREHREHREHREHREHREHREHREHREHREHREHREHR	-NLAPNSTITSTLERPSNNINVNSKVVTAK -TTTASTSS -PSAASITRA LFSDSNSTLERPNRIGGG -AISPKIHNGKIE -LQTNN- LQTNN-	QNLRRIRTRIIQH	SFLSSVCS. SIFSTEGN SFLSSSNT SFLSSSNT SFLSSSNT SFLESHR SFLESHR JDINDSASF JDINDSASF ISFLSSSNT MNHAYANM MNQSCATI TRRGEENF SFLSEKHV SFLSEKHV ALYSEEDN ALYSEEDN	SFSDPÇANAVDR GGSGGGDE INDDH- LISSSSYENNI LISSSSYENNI EDDQGVTSSN- GGDS-GDIIGE PRNDN- FNNNNNTCN- FNNNNNANTCN- INDDH- INDDH- IFTAR- IFTAR- IFTAR- IFSQQ- S- FVNG- FVNG-		255 256 240 280 266 272 272 299 244 247 266 290 288
ACMNPV-A PlxyMNPV AcMNPV-B ROMNPV MaviMMPV HCNPV CfdefMPV AgMNPV CfMNPV ApMNPV ApMNPV ApNPV EppoMMPV MacoNPV-B HearNPV ASNFV SplinPV-II MacoMPV-A SpexNPV ClbinPV AgipNPV OrleNPV AgipNPV AdonPV-A SfNPV-B MbNPV AdonPV EcobNPV EcobNPV EcobNPV EcobNPV EcobNPV EcobNPV EcobNPV EcobNPV TSNPV-B HearNPV-NN HearNPV-NN HearNPV-NN HearNPV-NN HearNPV-NN HearNPV-NN SpltMNPV SpltMNPV SpltMNPV SpltMNPV SpltMNPV SpltMNPV HearNPV-C		IE FELFORE IE FEWENVO IE FOAFDRE IE FEWENVO IE FEWENVO IE FEWENVO IE FEWENVO IE FEAFDRE FOAFWEFE EVENVO EVEN EVEN	AE	NERMTM NEKMTM NEKMTM NEKMST NEKMS NEKMST NEKMS NEKMS NEKMST NEKMST NEKMST NEKMST NEKMST NEKMST NEKMST NEKMST NEKMS	FDNVDDLYER LNNVDELYER YENVDDLYER LNNVDELYER LNNVDELYER YDNLDRLYTQ LNNVDLYER YDNLDRLYTQ LNNVDDLYER YDNLDRLYTQ LNNVDDLYER YENVDDLYER YENVDDLYER YENIGTVYEQ CONIEVLYEL LELULYDQ KISHEVLYDQ KISHEVLYDQ KISHEVLYNH LIEKLDVLYNH	RDHHR QDYHK REHHR REHHR QDYHKNKQ QDYHRNKKQ QDYHRNKKQ QDYHRNKKQ QDHRNKKQ REHHR REHHK REHHK REHHK REHHK REHHK REYNC KNYHR KNYHR KNYHR KNYHR KNYHR KNYHR KNYHR	- NIAPN - STTTS - TIERP - SNNIN - VNSKVVTAK - TTTASTSS - PSAASITRA LFSDSNS - TLERP NRIGGG - AISP - KIHNGKIE - LQTNN - LQTNN - LQTNN - LQTNN - KIETNG - KIETNG - KIETNG - LOTNN	-QNLRR -RTR, -QHI -QHI -QHI -ADSTTSC -AARHLRH -PVSVGSGI -SGIIQHRI -RISGIIQHRIR	SFLSSVCS. SFISTSGNASSSTITSFUSSHRESSNT SFLESSNT SFLESSNT SFLESSNT SFLESSNT JFLESSNT JFLESSNT JFLESSNT JFLESSNT JFLSSSNT JFLSSSNT JFLSSSNT JFLSSSNT JFLSSSNT JFLSSSNT JFLSSSNT JFLSSSNT JFLSSSNT JRAGEENF JFLSESTNV JRAGEENF JFLSESTNV JRAGEEDN JRAGEN JRAGEEN	SPSDPÇANAUDR GGSGGGDDE INDDH- LSSSSSYBN LSDQGVTSSN- GGDS-GDIIGE DRRD- FNNNNVNTCN- FNNNNNANTCN- FNNNNNANTCN- FAEN- LFTAR- LFTAR- LFTAR- FVNG- FVN		255 256 249 280 262 270 247 247 247 2266 2290 281 275 2273 2273 2273 2273 2273 2273 2273
ACMNPV-A PlxyMNPV ACMNPV-B ROMNPV MaviMNPV HCNPV CfdefMPV AGMNPV CfdefMPV AGMNPV CfMNPV AGMNPV EMPOMNPV ADNPV EMPOMNPV ASNPV SPLINPV-II MACONPV-A SPEXNPV ClbiMPV AGIDNPV AGIDNPV AGIDNPV LSNPV EMPOMNPV AddonPV LSNFV EMPOMPV LSNFV EUDSNPV HearNPV-B HezeSNPV ChchNPV TrniSNPV SPLENNPV HearNPV-B HearNPV-C		IE FELFORE IE FEWENVO IE FOAFDRE IE FEWENVO IE FEWENVO IE FEWENVO IE FEWENVO IE FEAFDRE FOAFWEFE EVENVO EVEN EVEN	AE	NERMTM NEKMTM NEKMTM NEKMST NEKMS NEKMST NEKMS NEKMS NEKMST NEKMST NEKMST NEKMST NEKMST NEKMST NEKMST NEKMST NEKMS	FDNVDDLYER LNNVDELYER YENVDDLYER LNNVDELYER LNNVDELYER YDNLDRLYTQ LNNVDLYER YDNLDRLYTQ LNNVDDLYER YDNLDRLYTQ LNNVDDLYER YENVDDLYER YENVDDLYER YENIGTVYEQ CONIEVLYEL LELULYDQ KISHEVLYDQ KISHEVLYDQ KISHEVLYNH LIEKLDVLYNH	RDHHR QDYHK REHHR REHHR QDYHKNKQ QDYHRNKKQ QDYHRNKKQ QDYHRNKKQ QDHRNKKQ REHHR REHHK REHHK REHHK REHHK REHHK REYNC KNYHR KNYHR KNYHR KNYHR KNYHR KNYHR KNYHR	- NIAPN - STTTS - TIERP - SNNIN - VNSKVVTAK - TTTASTSS - PSAASITRA LFSDSNS - TLERP NRIGGG - AISP - KIHNGKIE - LQTNN - LQTNN - LQTNN - LQTNN - KIETNG - KIETNG - KIETNG - LOTNN	-QNLRR -RTR, -QHI -QHI -QHI -ADSTTSC -AARHLRH -PVSVGSGI -SGIIQHRI -RISGIIQHRIR	SFLSSVCS. SFISTSGNASSSTITSFUSSHRESSNT SFLESSNT SFLESSNT SFLESSNT SFLESSNT JFLESSNT JFLESSNT JFLESSNT JFLESSNT JFLSSSNT JFLSSSNT JFLSSSNT JFLSSSNT JFLSSSNT JFLSSSNT JFLSSSNT JFLSSSNT JFLSSSNT JRAGEENF JFLSESTNV JRAGEENF JFLSESTNV JRAGEEDN JRAGEN JRAGEEN	SPSDPÇANAUDR GGSGGGDDE INDDH- LSSSSSYBN LSDQGVTSSN- GGDS-GDIIGE DRRD- FNNNNVNTCN- FNNNNNANTCN- FNNNNNANTCN- FAEN- LFTAR- LFTAR- LFTAR- FVNG- FVN		255 256 249 280 262 270 247 247 247 2266 2290 281 275 2273 2273 2273 2273 2273 2273 2273
ACMNPV-A PlxyMNPV AcMNPV-B ROMNPV MaviMMPV HCNPV CfdefMPV AgMNPV CfMNPV ApMNPV ApMNPV ApNPV EppoMMPV MacoNPV-B HearNPV ASNFV SplinPV-II MacoMPV-A SpexNPV ClbinPV AgipNPV OrleNPV AgipNPV AdonPV-A SfNPV-B MbNPV AdonPV EcobNPV EcobNPV EcobNPV EcobNPV EcobNPV EcobNPV EcobNPV EcobNPV TSNPV-B HearNPV-NN HearNPV-NN HearNPV-NN HearNPV-NN HearNPV-NN HearNPV-NN SpltMNPV SpltMNPV SpltMNPV SpltMNPV SpltMNPV SpltMNPV HearNPV-C		IE FELFORE IE FEWENVO IE FOAFDRE IE FEWENVO IE FEWENVO IE FEWENVO IE FEWENVO IE FEAFDRE FOAFWEFE EVENVO EVEN EVEN	AE	NERMTM NEKMTM NEKMTM NEKMST NEKMS NEKMST NEKMS NEKMS NEKMST NEKMST NEKMST NEKMST NEKMST NEKMST NEKMST NEKMST NEKMS	FDNVDDLYER LNNVDELYER YENVDDLYER LNNVDELYER LNNVDELYER YDNLDRLYTQ LNNVDLYER YDNLDRLYTQ LNNVDDLYER YDNLDRLYTQ LNNVDDLYER YENVDDLYER YENVDDLYER YENIGTVYEQ CONIEVLYEL LELULYDQ KISHEVLYDQ KISHEVLYDQ KISHEVLYNH LIEKLDVLYNH	RDHHR QDYHK REHHR REHHR QDYHKNKQ QDYHRNKKQ QDYHRNKKQ QDYHRNKKQ QDHRNKKQ REHHR REHHK REHHK REHHK REHHK REHHK REYNC KNYHR KNYHR KNYHR KNYHR KNYHR KNYHR KNYHR	- NIAPN - STTTS - TIERP - SNNIN - VNSKVVTAK - TTTASTSS - PSAASITRA LFSDSNS - TLERP NRIGGG - AISP - KIHNGKIE - LQTNN - LQTNN - LQTNN - LQTNN - KIETNG - KIETNG - KIETNG - LOTNN	-QNLRR -RTR, -QHI -QHI -QHI -ADSTTSC -AARHLRH -PVSVGSGI -SGIIQHRI -RISGIIQHRIR	SFLSSVCS. SFISTSGNASSSTITSFUSSHRESSNT SFLESSNT SFLESSNT SFLESSNT SFLESSNT JFLESSNT JFLESSNT JFLESSNT JFLESSNT JFLSSSNT JFLSSSNT JFLSSSNT JFLSSSNT JFLSSSNT JFLSSSNT JFLSSSNT JFLSSSNT JFLSSSNT JRAGEENF JFLSESTNV JRAGEENF JFLSESTNV JRAGEEDN JRAGEN JRAGEEN	SFSDPÇANAVDR GGSGGGDE INDDH- LISSSSYENNI LISSSSYENNI EDDQGVTSSN- GGDS-GDIIGE PRNDN- FNNNNNTCN- FNNNNNANTCN- INDDH- INDDH- IFTAR- IFTAR- IFTAR- IFSQQ- S- FVNG- FVNG-		255 256 249 280 262 270 247 247 247 2266 2290 281 275 2273 2273 2273 2273 2273 2273 2273

(GenBank, YP_001651043.), SfNPV-A (GenBank, YP_001036309.1), SfNPV-B (GenBank, YP_001036309.1), MbN-PV (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), Chch-NPV (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).

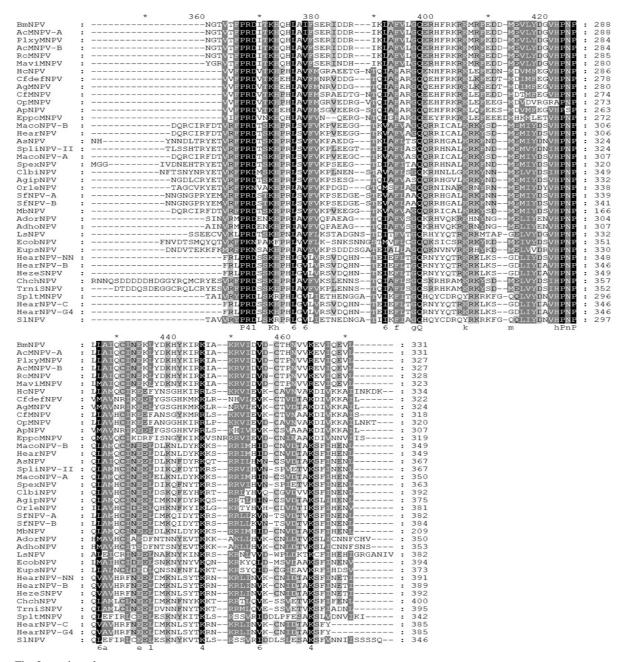


Fig. 2 continued.

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'-GACACGAATTTA-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.

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

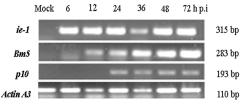


Fig. 3. RT-PCR analysis of *Bm5* transcription. Total RNA was extracted from BmNPV-infected cells different times post infection (p.i.). PCR products of different genes are indicated on the left-hand side. The sizes (in bp) of different PCR products are indicated on the right-hand side. *ie-1* and *p10*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

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*-

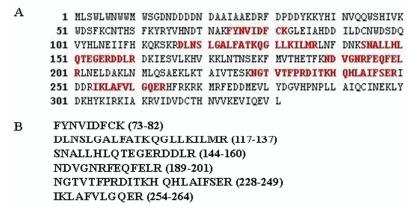


Fig. 4. Identification of BM5 protein by MALDI-TOF analysis. (A) Amino acid sequences of BM5 protein. Matched peptide sequences are shown as red characters. (B) Peptide sequences identified by mass spectrometry.

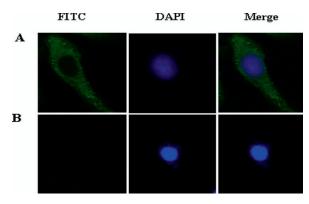


Fig. 5. (A) Subcellular localization of BM5 in infected cells 24, 48, and 72 h p.i. BmNPV-infected BmN cells were treated with anti-BM5 antibody, followed by treatment with FITC-conjugated goat anti-rabbit IgG. From left to right: green fluorescence for BM5, DAPI and the merge images. Nuclei were stained 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.

Acknowledgements

This work was supported by the National Program of High-tech Research and Development (863 High-Tech Program, No. 2008AA10Z145), grants from Jiangsu Sci-Tech Support Project – Agriculture (No. BE2008379), and National Natural Science Foundation (No. 30871826).

- Ayres M. D., Howard S. C., Kuzio J., Lopez-Ferber M., and Possee R. D. (1994), The complete DNA sequence of *Autographa californica* nuclear polyhedrosis virus. Virology **202**, 586–605.
- Braunagel S. C. and Summers M. D. (1994), *Autogra-pha californica* nuclear polyhedrosis virus, PDV, and ECV viral envelopes and nucleocapsids: structural proteins, antigens, lipid and fatty acid profiles. Virology **202**, 315–328.
- Chen H. Q., Chen K. P., Yao Q., Guo Z. J., and Wang L. L. (2007), Characterization of a late gene, ORF67 from *Bombyx mori* nucleopolyhedrovirus. FEBS Lett. **581**, 5836–5842.
- Du M. F., Yin X. M., Guo Z. J., and Zhu L. J. (2006), Characterization of a late gene, ORF60 from *Bombyx mori* nucleopolyhedrovirus. J. Biochem. Mol. Biol. **39**, 737–742.
- Fang M., Dai X., and Theilmann D. A. (2007), *Autogra-pha californica* multiple nucleopolyhedrovirus EXON0 (ORF141) is required for efficient egress of nucleocapsids from the nucleus. J. Virol. **81**, 9859–9869.

- Friesen P. D. and Miller L. K. (1986), The regulation of baculovirus gene expression. Curr. Top Microbiol. Immunol. **131**, 31–49.
- Ge J. Q., Yang Z. N., Tang X. D., Xu H. J., Hong J., Chen J. G., and Zhang C. X. (2008), Characterization of a nucleopolyhedrovirus with a deletion of the baculovirus core gene Bm67. J. Gen. Virol. **89**, 766–774.
- Ge J. Q., Zhao J. F., Shao Y. M., Tian C. H., and Zhang C. X. (2009), Characterization of an early gene *orf122* from *Bombyx mori* nucleopolyhedrovirus. Mol. Biol. Rep. **36**, 543–548.
- Gomi S., Majima K., and Maeda S. (1999), Sequence analysis of the genome of *Bombyx mori* nucleopolyhedrovirus. J. Gen. Virol. **80**, 1323–1337.
- Herniou E. A., Olszewski J. A., Cory J. S., and O'Reilly D. R. (2003), The genome sequence and evolution of baculoviruses. Annu. Rev. Entomol. 48, 211–234.
- Hooft van Iddekinge B. J., Smith G. E., and Summers M. D. (1983), Nucleotide sequence of the polyhedrin gene of *Autographa californica* nuclear polyhedrosis virus. Virology **131**, 561–565.

- Huang J., Hao B., Deng F., Sun X., Wang H., and Hu Z. (2008), Open reading frame Bm21 of *Bombyx mori* nucleopolyhedrovirus is not essential for virus replication *in vitro*, but its deletion extends the median survival time of infected larvae. J. Gen. Virol. **89**, 922–930.
- Imai N., Kurihara M., Matsumoto S., and Kang W. K. (2004), Bombyx mori nucleopolyhedrovirus orf8 encodes a nucleic acid binding protein that colocalizes with IE1 during infection. Arch. Virol. 149, 1581–1594.
- Iwanaga M., Kang W. K., Kobayashi M., and Maeda S. (2000), Baculovirus infection blocks the progression of fat body degradation during metamorphosis in *Bombyx mori*. Arch. Virol. 145, 1763–1771.
- Jakubowska A. K., Peters S. A., Ziemnicka J., Vlak J. M., and van Oers M. M. (2006), Genome sequence of an enhancin gene-rich nucleopolyhedrovirus (NPV) from Agrotis segetum: collinearity with Spodoptera exigua multiple NPV. J. Gen. Virol. 87, 537–551.
- Jehle J. A., Blissard G. W., Bonning B. C., Cory J. S., Herniou E. A., Rohrmann G. F., Theilmann D. A., Thiem S. M., and Vlak J. M. (2006), On the classification and nomenclature of baculoviruses: a proposal for revision. Arch. Virol. 151, 1257–1266.
- Long G., Chen X., Peters D., Vlak J. M., and Hu Z. (2003), Open reading frame 122 of *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus encodes a novel structural protein of occlusion-derived virions. J. Gen. Virol. **84**, 115–121.
- Nie Z. M., Zhang Z. F., Wang D., He P. A., Jiang C. Y., Song L., Chen F., Xu J., Yang L., Yu L. L., Chen J., Lv Z. B., Lu J. J., Wu X. F., and Zhang Y. Z. (2007), Complete sequence and organization of *Antheraea*

- pernyi nucleopolyhedrovirus, a dr-rich baculovirus. BMC Genomics 8, 248.
- Rankin C., Ooi B. G., and Miller L. K. (1988), Eight base pairs encompassing the transcriptional start point are the major determinant for baculovirus polyhedrin gene expression. Gene **70**, 39–49.
- Shen H., Chen K., Yao Q., and Zhou Y. (2009), Characterization of the Bm61 of the *Bombyx mori* nucleopolyhedrovirus. Curr. Microbiol. **59**, 65–70.
- Tang X. D., Xu Y. P., Yu L. L., Lang G. J., Tian C. H., Zhao J. F., and Zhang C. X. (2008), Characterization of a *Bombyx mori* nucleopolyhedrovirus with Bmvp80 disruption. Virus Res. 138, 81–88.
- Tian C. H., Zhao J. F., Xu Y. P., Xue J., Zhang B. Q., Cui Y. J., Zhang M. J., Bao Y. Y., and Zhang C. X. (2009), Involvement of *Bombyx mori* nucleopolyhedrovirus ORF41 (Bm41) in BV production and ODV envelopment. Virology 387, 184–192.
- Wang D. and Zhang C. X. (2006), HearSNPV *orf83* encodes a late, nonstructural protein with an active chitin-binding domain. Virus Res. **117**, 237–243.
- Williams G. V. and Faulkner P. (1997), Cytological changes and viral morphogenesis during baculovirus infection. In: The Baculoviruses (Miller L. K., ed.). Plenum Press, New York, USA, pp. 61–107.
 Xu H. J., Yang Z. N., Wang F., and Zhang C. X. (2006),
- Xu H. J., Yang Z. N., Wang F., and Zhang C. X. (2006), *Bombyx mori* nucleopolyhedrovirus ORF79 encodes a 28-kDa structural protein of the ODV envelope. Arch. Virol. **151**, 681–695.
- Young J. C., MacKinnon E. A., and Faulkner P. (1993), The architecture of the virogenic stroma in isolated nuclei of *Spodoptera frugiperda* cells *in vitro* infected by *Autographa californica* nuclear polyhedrosis virus. J. Struct. Biol. **110**, 141–153.