

## Molecular Cloning and Nucleotide Sequences of the *Perina nuda* Nucleopolyhedrovirus (PenuNPV) p10 Gene

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Molecular cloning and nucleotide sequences of the *Perina nuda* nucleopolyhedrovirus (PenuNPV) p10 gene. *Zoological Studies* 36(1): 48-57. The *Perina nuda* nucleopolyhedrovirus (PenuNPV) p10 gene was located in a *Pst* I 4.5 kilo-base pair (kb) fragment of the viral genome. A portion of 696 nucleotides (nt) containing this gene was sequenced. An open reading frame (ORF) of 276 nt encoded the p10 protein of 92 amino acid residues. The conserved TAAG motif, which is associated with transcription of most baculovirus late genes, was identified 53 nt upstream of the translational start codon of the PenuNPV p10 gene. Northern blot analysis using a DIG-labeled PenuNPV p10 gene probe detected 2 major transcripts, 0.62 kb and 3.08 kb mRNA in PenuNPV-infected cells at 96 to 120 h postinfection. The PenuNPV p10 gene was expressed as a 0.62 kb transcript. The PenuNPV p10 protein showed 38%, 44%, 44%, 22%, and 96% amino acid identity, respectively, with the p10 proteins of *Autographa californica* MNPV (AcMNPV), *Bombyx mori* NPV (BmNPV), *Choristoneura fumiferana* MNPV (CfMNPV), *Spodoptera exigua* MNPV (SeMNPV), and *Orgyia pseudotsugata* MNPV (OpMNPV). This implies that PenuNPV p10 protein is most closely related to OpMNPV p10 protein.

**Key words:** p10 Gene, *Perina nuda* nucleopolyhedrovirus, Baculovirus.

In the late phase of nucleopolyhedrovirus (NPV, Murphy et al. 1995) infection, there are 2 main polypeptides, polyhedrin and p10, produced in the infected-insect cells. These 2 polypeptides are synthesized in large amounts representing up to half of the stainable proteins in cells 48 h post-infection (Smith et al. 1982). Polyhedrin is the major protein of the viral occlusion bodies that are found exclusively in the nuclei of baculovirus-infected insect cells (Vlak and Rohrmann 1985, Rohrmann 1986), whereas p10 protein is associated with fibrillar structures in both nuclei and cytoplasm of virus-infected cells. In the nucleus, the fibrillar structures are often found to associate with electron-dense spacers which suggests that p10 protein may play an important role in polyhedron mor-

phogenesis (van der Wilk et al. 1987, Williams et al. 1989, van Lent et al. 1990, Quant-Russel et al. 1991). Moreover, in deletion and mutagenesis studies, p10 gene-deleted mutants of *Autographa californica* multiple nucleocapsid NPV (AcMNPV) failed to liberate their polyhedra from infected cells, which suggests that p10 protein is also involved in cell lysis (Williams et al. 1989, van Oers et al. 1993).

So far, 5 p10 genes from baculoviruses have been sequenced, but the only 3 which have been studied in detail are AcMNPV (Kuzio et al. 1984), *Orgyia pseudotsugata* MNPV (OpMNPV, Leisy et al. 1986), and *Spodoptera exigua* MNPV (SeMNPV, Zuidema et al. 1993). The polyhedrin genes of baculoviruses are highly conserved, with more than 75% identity in nucleotide sequence and 85% in

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amino acid sequence between species and isolates (Rohrmann 1986). On the other hand, p10 genes are found to have less than 50% identity in both nucleotide and amino acid sequences. Rohrmann (1992) has therefore suggested that p10 genes are more divergent than polyhedrin genes. In order to understand the structure and function of p10 protein, comparative studies of the p10 gene from other baculoviruses are necessary.

The *Perina nuda* NPV (PenuNPV) is a baculovirus with a narrow host range, which in Taiwan is limited to the ficus transparent wing moth, *P. nuda* (Lo et al. 1990, Wang and Tsai 1995). The characteristics of PenuNPV, including its host ranges in vitro and in vivo, the protein profiles of its polyhedra and virions, the restriction enzyme profiles of its DNA, the location of the polyhedrin gene in the genome, and the nucleotide sequence of the polyhedrin gene have all been studied previously (Wang and Tsai 1995, Chou et al. 1996). The in vitro replication system of PenuNPV in NTU PN-HH cells has also been established (Wang et al. 1996). PenuNPV is distinct from other NPVs, such as AcMNPV, *Spodoptera litura* NPV (SINPV), SeMNPV, *Heliothis zea* NPV (HzNPV), and *Bombyx mori* NPV (BmNPV), but it may be closely related to OpMNPV (Wang et al. 1996). Other characteristics of PenuNPV especially at the molecular level are needed to establish a suitable taxonomic position.

The polymerase chain reaction (PCR, Mullis and Faloona 1987) technique provides a very sensitive tool to identify genes. The same strategy was employed here as in a previous study on the polyhedrin gene (Chou et al. 1996). Subsequently, the PCR product was used as a probe to localize and isolate the p10 gene from the PenuNPV genome. In this report we describe the identification, nucleotide sequence, and transcriptional analysis of the PenuNPV p10 gene. Our data indicate that PenuNPV p10 is very similar at the amino acid level to that of OpMNPV.

## MATERIALS AND METHODS

### Viruses, insects, and cells

*Perina nuda* nucleopolyhedrovirus (PenuNPV) was propagated and multiplied in NTU PN-HH cells or larvae of the moth *P. nuda* as described previously (Lo et al. 1990, Wang et al. 1996). AcMNPV strain E2 (Smith and Summers 1978) was kindly supplied by Dr. M. J. Fraser of the Department of Biological Sciences, University of Notre Dame,

USA., and propagated in IPLB SF-21 AE cells. Both NTU PN-HH and IPLB SF-21 AE cells were maintained in TNM-FH medium (Hink 1970) supplemented with 100 IU/ml penicillin, 100  $\mu$ g/ml streptomycin, 1.25  $\mu$ g/ml fungizone, and 10% fetal calf serum at 28 °C. The ficus transparent wing moth, *P. nuda* Fabricius, was reared with leaves of panya (*Ficus* sp.). Fourth instar larvae or 1-d-old pupae were used for mass production of polyhedra by oral-feeding or by viral-suspension injection, respectively. The polyhedra and occluded virus were purified by sucrose gradient centrifugation, and genomic DNA was extracted from the gradient-purified virions as described in a previous paper (Chou et al. 1996).

### PCR amplification

The amino acid sequences of the p10 genes from AcMNPV (Kuzio et al. 1984) and OpMNPV (Leisy et al. 1986) were compared. Two conserved regions, located at amino acids 6-14 and 54-62, were chosen to design primers for PCR. The primer sequences were: primer 10, 5'-TTT TGA CGC AAA T(T/C)(T/C) TAG ACG CCG T-3'; and primer 11, 5'-CG(A/C) (T/C)(A/G)(G/C) T(T/C)A (A/G)(T/C)A T(T/G) G ATT G(A/G)A T(T/A)T -3'. PCR was performed in a 100- $\mu$ l reaction mixture containing 2.5 units of Taq polymerase (Promega, Madison, WI, USA), 100 ng of viral DNA, 0.5  $\mu$ g of each primer, 200  $\mu$ M of 4 dNTPs and 1X reaction buffer (10 mM Tris-HCl, pH 9, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.1% Triton X-100). Amplifications were performed over 30 cycles in an MJR PTC-100 Thermocycler (Watertown, MA, USA), with each cycle consisting of denaturing at 94 °C for 1 min, followed by annealing at 50 °C for 1 min, and elongating at 72 °C for 3 min. There was a final extension step of 5 min at 72 °C. Aliquots 10  $\mu$ l of amplified products were analyzed in 1.5% agarose gels in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) containing 0.5  $\mu$ g/ml ethidium bromide and visualized with short-wave ultraviolet light. The PCR products were then subcloned into the *Sma* I site of plasmid pUC18 (Yanisch-Perron et al. 1985) and sequenced by the dideoxy chain termination method (Sanger et al. 1977) using a sequenase kit (United States Biochemical Corp., Cleveland, OH, USA) with universal primers. Oligonucleotides were synthesized commercially by an Applied Biosystems DNA synthesizer.

### Southern blot, cloning, and sequence analysis

PenuNPV DNA was digested with various re-

striction enzymes (*Bam* HI, *Eco* RI, *Eco* RV, *Hind* III, *Kpn* I, *Pst* I, *Sal* I, *Sma* I, *Xba* I, and *Xho* I) and then electrophoretically separated in 0.8% agarose gels. The gel was denatured and neutralized according to standard molecular cloning procedures (Sambrook et al. 1989), and transferred under 20X SSC (0.3 M sodium citrate, 3 M NaCl) to a Hybond-N nylon membrane (Amersham, Little Chalfont, Buckinghamshire, England) by using a Hoefer TE-80 vacuum transfer unit (Hoefer, San Francisco, CA, USA) for 60 min. The blots were hybridized at 37 °C for 16 h with DIG-labeled probes. The PenuNPV p10 gene PCR fragment was used to prepare the DIG-labeled probe using the random primer method (Boehringer Mannheim Co., Germany). After hybridization, membranes were washed and detection of the DIG probe was performed following the manufacturer instructions (Boehringer Mannheim Co., Germany) as described in Chou et al. (1996). Membranes were exposed to Kodak XAR-5 film at 37 °C for 15-60 min, and then the film was developed. Cloning of positive restriction enzyme fragments of PenuNPV DNA was achieved by isolating the DNA fragment from the agarose gel and subcloning it into the plasmid pUC18 (Yanisch-Perron et al. 1985). DNA sequence analysis was performed using the sequenase kit as described above. The NPV p10 genes were obtained by searching the nucleic acid database using the FastDB program (Intelligenties). The various NPV p10 genes were analysed using the following alignment program: PC/GENE 6.8 (Intelligenties), NALIGN, CLUSTAL, and MULTALIGN program (GeneWorks Ver. 2.45. Intelligenties). The phylogenies of NPV p10 genes were constructed by MULTALIGN using the protein data set. The outputs were used in phylogenetic estimations and for the determination of compositional biases.

### RNA isolation

For Northern blot hybridization and primer extension analysis, total RNA was isolated from PenuNPV-infected NTU PN-HH cells at 0, 12, 24, 48, 72, 96, 120, and 144 h postinfection. NTU PN-HH cells were seeded in 25-cm<sup>2</sup> tissue culture flasks (Nunc, Inc., Naperville, IL, USA) at a density of  $3 \times 10^6$  cells/flask and infected with PenuNPV. Total cellular RNA was isolated according to BIOTECHX RNAzol B RNA isolation protocol (BIOTECHX Laboratories, Inc., Houston, TX, USA) as described in Chou et al. (1996). The RNA concentration was determined by measuring UV absorbance at 260 nm.

### Northern blot

Aliquots of 20 µg of total RNA prepared from different time intervals were denatured, electrophoresed, and blotted to nylon membranes as described by Sambrook et al. (1989). To identify PenuNPV p10-specific transcripts, the blot was hybridized for 16 h at 37 °C in hybridization buffer containing DIG-labeled PenuNPV p10 gene probe (prepared as above). After hybridization, the blot was washed and exposed to film following the procedure described above in the Southern blot section.

### Primer extension

An 18-base oligonucleotide (5'-CGC CTC TAG AGT TTT GGA-3') complementary to PenuNPV p10 mRNA was synthesized and used to identify the transcriptional start site of the p10 gene. The oligonucleotide (approximately 10 pmol) was 5'-end-labeled with [ $\gamma$ -<sup>32</sup>P]ATP (3000 Ci/mmol, Amersham) using T4 polynucleotide kinase (8-10 units, Promega) in a forward exchange buffer (50 mM Tris-HCl, pH 7.5, 10 mM MgCl<sub>2</sub>, 5 mM DTT, 0.1 mM spermidine) for 30 min at 37 °C. The sample was heat inactivated at 90 °C for 10 min, and after 90 µl DEPC-treated water had been added, it was stored at -20 °C. Two microliters of labeled primer was added to 20 µg total RNA in AMV primer extension buffer (50 mM Tris-HCl, pH 8.3, 50 mM KCl, 10 mM MgCl<sub>2</sub>, 10 mM DTT, 1 mM each dNTP, 0.5 mM spermidine). The mixture was denatured at 58 °C for 20 min, and then annealed at 37 °C for 10 min. After annealing, 5 µl of 2X primer extension buffer, 1.4 µl of 40 mM sodium pyrophosphate, 1.6 µl of DEPC-treated water, and 1 unit of AMV reverse transcriptase (Promega) were added to the mixture. The reverse transcriptase reaction proceeded for 30 min at 42 °C. The reaction was stopped by the addition of 20 µl loading buffer (98% formamide, 10 mM EDTA, pH 8.0, 0.1% xylene cyanol FF, 0.1% bromophenol blue). Five microliters of this reaction mixture was analyzed in a 6% polyacrylamide sequencing gel. After drying, the gel was subjected to autoradiography at -70 °C for 24 h.

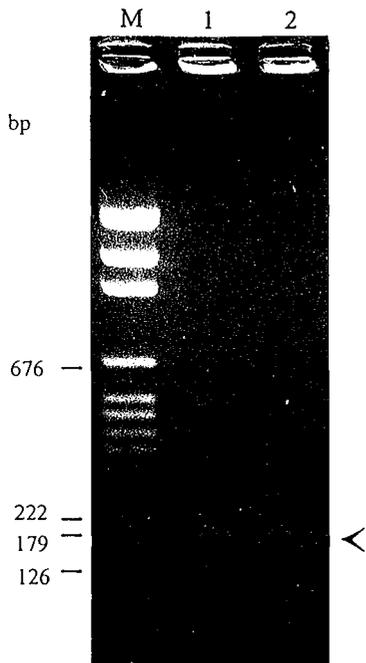
## RESULTS

### Localization, cloning, and sequencing of PenuNPV p10 gene

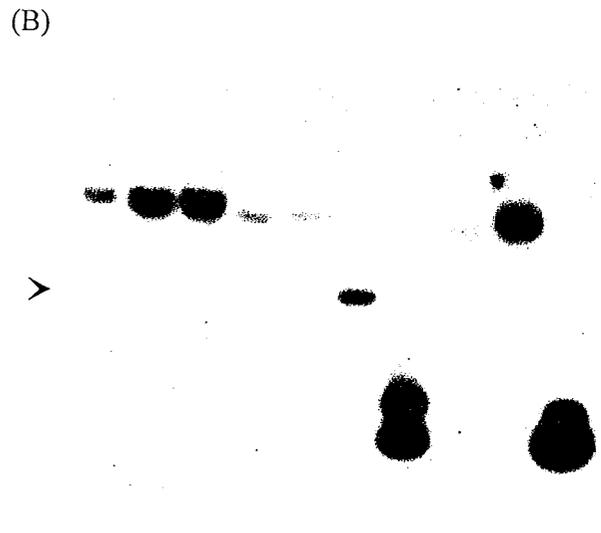
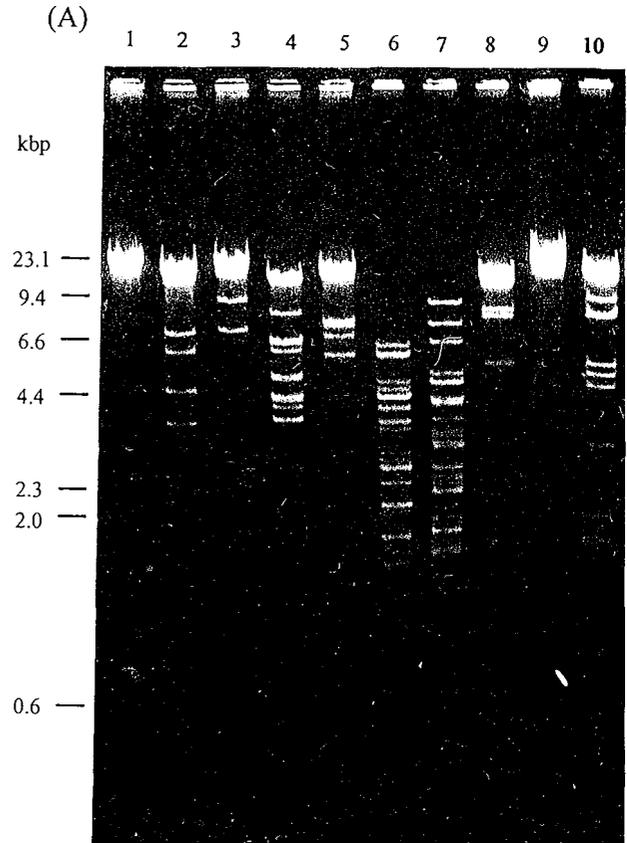
With the set of primers, we were able to amplify

part of the p10 gene fragments (approximately, 168 bp) from the genomic DNA of AcMNPV and PenuNPV (Fig. 1). To identify the location of the p10 gene in the PenuNPV genome, Southern blot analysis was performed. When an AcMNPV p10 gene fragment was used as a probe, it failed to show any signal on the PenuNPV genome, whereas the probe from the PenuNPV p10 gene PCR fragment hybridized with the PenuNPV p10 gene in various restricted fragments from the PenuNPV genome (Fig. 2B). A *Pst* I-F fragment of 4.5 kb was chosen for further analysis. (Fig. 2B).

The restriction endonuclease sites of the *Pst* I-F fragment of PenuNPV are shown in Fig. 3A. The location of the PenuNPV p10 gene was determined precisely in the *Pst* I-F fragment, and fragments containing portions of the PenuNPV p10 gene were subcloned into the plasmid pUC18. The strategy of DNA sequence analysis is shown in the lower part of Fig. 3A. The relative ORFs (open reading frames) of p10 and the contiguous genes (p26 and p74 genes) are shown in Fig. 3B. An ORF of 276 nt with the potential to encode the 92 amino acid residues of the p10 protein is shown in Fig. 4.



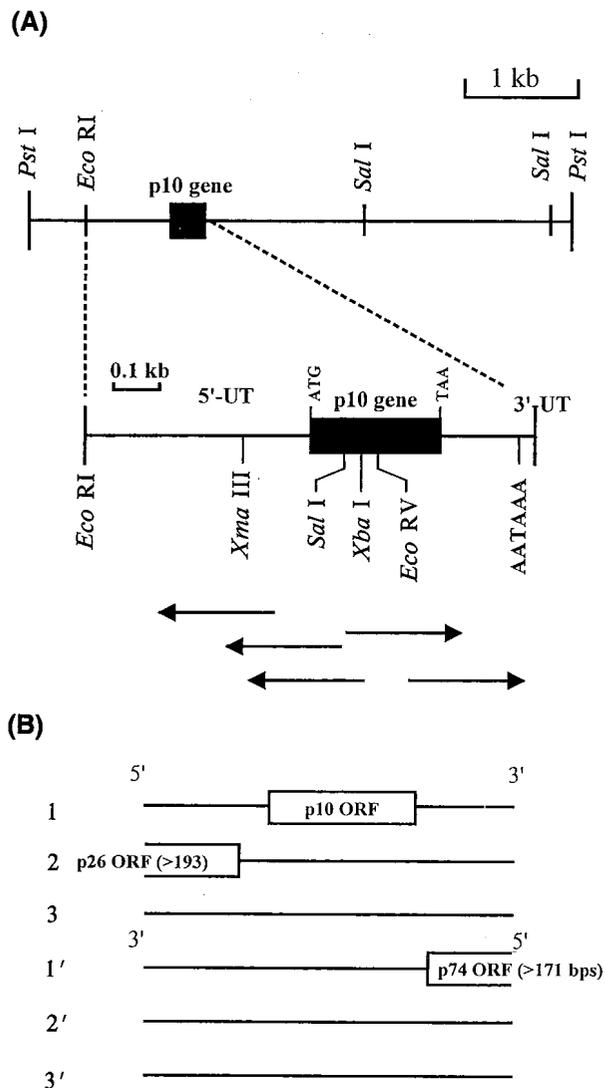
**Fig. 1.** Analysis of PCR products on 1.5% agarose gel. Lanes 1 and 2 are PCR products of AcMNPV and PenuNPV p10 gene fragments, respectively. The bp scale on the left is derived from pGEM DNA size marker.



**Fig. 2.** Localization of the p10 gene on PenuNPV DNA. (A) PenuNPV DNA was digested with *Bam* HI, *Eco* RI, *Eco* RV, *Hind* III, *Kpn* I, *Pst* I, *Sal* I, *Sma* I, *Xba* I, and *Xho* I (lanes 1 to 10, respectively). The fragments were separated by agarose gel electrophoresis, stained with ethidium bromide, and the gels were photographed under UV light. (B) The same gel was transferred to a nylon membrane and probed with a PenuNPV p10 gene fragment. The arrowhead indicates a *Pst* I-F fragment (4.5 kb) which was subcloned further in this study. The kb scale on the left is derived from DNA size markers from *Hind* III-digested lambda DNA.

## Sequence analysis

Comparison of the p10 amino acid sequence of PenuNPV and 5 other NPVs is shown in Fig. 5. The overall sequence identities between PenuNPV p10 and those of AcMNPV (Kuzio et al. 1984),



**Fig. 3.** Restriction map of the *Pst* I-F fragment containing the PenuNPV p10 gene. (A) Restriction enzyme map of the 4.5 kb *Pst* I-F fragment which was cloned into pUC18. The p10 gene is indicated by the solid bar. The start codons (ATG), stop codons (TAA) and the polyadenylation signal (AATAAA) of the p10 gene are indicated. The flanking untranslated regions (5'-UT and 3'-UT) are also shown. Subcloning and sequencing strategy are shown in the lower portion of the restriction map. Arrows indicate the sequencing orientation and length of the sequenced DNA. (B) The distribution of open reading frames greater than 50 amino acids predicted from the nucleotide sequence is shown for all 6 reading frames. The locations of the p10 ORF, partial p26, and p74 ORFs are indicated.

BmNPV (Hu et al. 1994), SeMNPV (Zuidema et al. 1993), OpMNPV (Leisy et al. 1986), and CfMNPV (Wilson et al. 1992) are 38%, 44%, 22%, 96%, and 44%, respectively. The phylogenetic tree constructed with data from Fig. 5 using MULTALIGN is shown in Fig. 7. From this phylogenetic tree, the 6 species are clustered into 2 distinct groups: 1 group includes AcMNPV, BmNPV, CfMNPV, OpMNPV, and PenuNPV; the other includes only SeMNPV. Within the phylogenetic tree, PenuNPV is closest to OpMNPV, followed by CfMNPV, BmNPV, AcMNPV, and then SeMNPV.

In the 5'-untranslated region of the PenuNPV p10 gene, there is a TAAG motif that is the consensus motif of the transcriptional start site in baculovirus late and very late gene expression (Blissard and Rohrmann 1990). The TAAG motif is located at positions -3 to -50 with respect to the translational start codon of the PenuNPV p10 ORF (Fig. 4). Three putative polyadenylation signal sequences (AATAAA) were found in the 3'-untranslated region of the PenuNPV p10 gene, located approximately 54, 105, and 142 nt downstream of the translational stop codon (Fig. 4).

As shown in Fig. 3B, 2 putative ORFs are located upstream and downstream of the PenuNPV p10 gene. The partially identified p26 ORF (Fig. 3B, reading frame 2) had a high amino acid sequence similarity to p26 gene of AcMNPV and OpMNPV (50% and 97%, respectively). Likewise, the p74 ORF (Fig. 3B, reading frame 1') had a high amino acid sequence similarity to the p74 gene of AcMNPV and OpMNPV (64% and 96%, respectively). The sequence analysis also demonstrated that the location of the PenuNPV p10 gene is colinear with the p26 and p74 genes, as is the case with the p10 gene flanking regions of AcMNPV, SeMNPV, and OpMNPV.

## Transcriptional analysis of the PenuNPV p10 gene

Using the primer extension assay, the PenuNPV p10 gene transcriptional start site was determined to be 52 nt upstream of the translational start codon of the p10 gene (Fig. 6A). Other minor signals also indicated alternative start sites at positions -51 and -53.

PenuNPV p10-specific transcripts were identified by Northern blot hybridization. The results of the Northern blot analysis are shown in Fig. 6B. Two major transcripts, 0.62 kb and 3.08 kb in length, were identified from PenuNPV-infected insect cells at 96 through 144 h postinfection

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-241
GCTCGT
partial p26 ORF --> L V
-181

GTACGGGCCG GTGCAGCTGC CGTACAAGCA GCTAAAAGCG CACGCGTTTC GCAAACAACG
Y G P V Q L P Y K Q L K A H A F R K Q R

XmaIII -121
CCGCGACGCG GCCGAGTCTT GCGCGCTGTT TTACAACGAC TCCGAGGTGC GCATCACTTT
R D A A E S C A L F Y N D S E V R I T F
-61
CAACAAGGGC GAGTTTGAAC TAATGCATTG GCGGCTGCCG GGCCCTTTTG TGAGCCACGG
N K G E F E L M H W R L P G P F V S H G
-52
transcription start -1
TTTTTAATAA GCATTATCAT AAAATATTAG TATATTTACC AAATTGATTG CAATATCATC
F ***

ATGTCCAAGC CCAGCATTTT GACGCAAATC TTAGACGCCG TAAGGGCCGT TGACAGCAAG
M S K P S I L T Q I L D A V R A V D S K

Sall XbaI 120
GTCACTGCCG TGCAAACCCA AGTCGACCAG CTGGGCGAGG ATTCCAAAAC TCTAGAGGGC
V T A L Q T Q V D Q L G E D S K T L E A

EcoRV 180
CTCACCGATC AACTCGGTGA CGTTGACAAT AAAGTCAGCG ATATCCAATC AATGCTAAGC
L T D Q L G D V D N K V S D I Q S M L S

240
ATCGAGGAGG AACTGCCCGA GCGCCAGCA CCGGGCGCCG AGCCTGAGCT CCCCAGATC
I E E E L P E P P A P A P E P E L P E I

300
CCCACGTTT CCGGCCTTCG CCGCAGTCGC AAGCAGTAAT CAAAACAGCA AATCGACGGT
P D V P G L R R S R K Q ***A GTTTTGTCGT TTAGCTGCCA
* ** F L L D V T

360
TTTGAATAC TCGTACGGCT TTTGATTAG GTAATAAAAT GCAAGCATCA AAAATATTGC
AAACTTTATG AGCATGCCGA AAAACTAATC CATTATTTTA CGTTCGTAGT TTTTATAACG
K F Y E Y P K K I L Y Y F A L M L F I A

420
AAAATACACA AAAAAGTAA GTTCTTTGTG CGCAATAAAA GCCGCAAGGG CCACCGCTGT
TTTTATGTGT TTTTGCATT CAAGAAACAC GCGTTATTTT CGGCGTTCCT GGTGGCGACA
F Y V F F T L E K H A I F A A L A V A T

450
ATTTGTCAA AATAAACCCG CTATTACCCC
TAAACAGTTT TTATTTGGGC GATAATGGGG
N T L F L G A I V G <-- partial p74 ORF

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**Fig. 4.** Nucleotide sequence of PenuNPV p10 gene and its flanking regions. The + strand is shown. The first nucleotide of the translational start signal ATG is given number +1. The predicted amino acid sequence is indicated by a 1-letter code and displayed below the nucleotide sequence. The sequence at nt -52 initiates the 5' end of the mRNA, and the arrow indicates the direction of transcription. The putative transcription initiation motif (TAAG) is underlined. The potential polyadenylation signal (AATAAA) is indicated by double underlining. The primer used in the primer extension assay is also shown and the arrow indicates the direction of extension. The deduced amino acid sequences of parts of the p26 and p74 genes, upstream and downstream of the p10 gene, are also shown. The translation stop codon (TAA or TGA) is indicated with 3 asterisks (\*\*\*). The nucleotide sequence has been assigned GenBank accession no. U50411.

					50
PenuNPV	MSKPSILTQILDVAVRAVDSKVTALQTQVDQLGEDSKTLEALTDQLGDDVDN				
OpMNPV	.....V.....BL..				
AcMNPV	...NV.....TEINT...DSV...LNG.E.SFQL.DG.PA..T.LNT				
BmNPV	...NV...R...IAEINT...DSV...LNG.E.SFQP.DG.PA..T.FNT				
SeMNPV	..Q-N...LL.RADIK...E...D...QA.NDVSAN---.PDTSELSAKL.A				
CfMNPV	.....Q...T...QD...T...D...A.LTE.DGKVQP.DG.SE..TAL.T				
					90
PenuNPV	KVSDIQSMLS--IEBELPEPPAPAPELPEIPDVPGLRRSRKQ				
OpMNPV	.....-V.....				96%
AcMNPV	.I.E...I.TGD.VPD...DSLK.KLKSQAF.LDSDARRGKRSSK				38%
BmNPV	.I.E...I.T--GDTA.D...DS				44%
SeMNPV	QATTLDTIVT--QVNNINDVLN.DLPDVPGNLQKQQQKK.N.K				22%
CfMNPV	..TT...DI.G-GA.VPDV.....L..N.LNKTRSRSK				44%

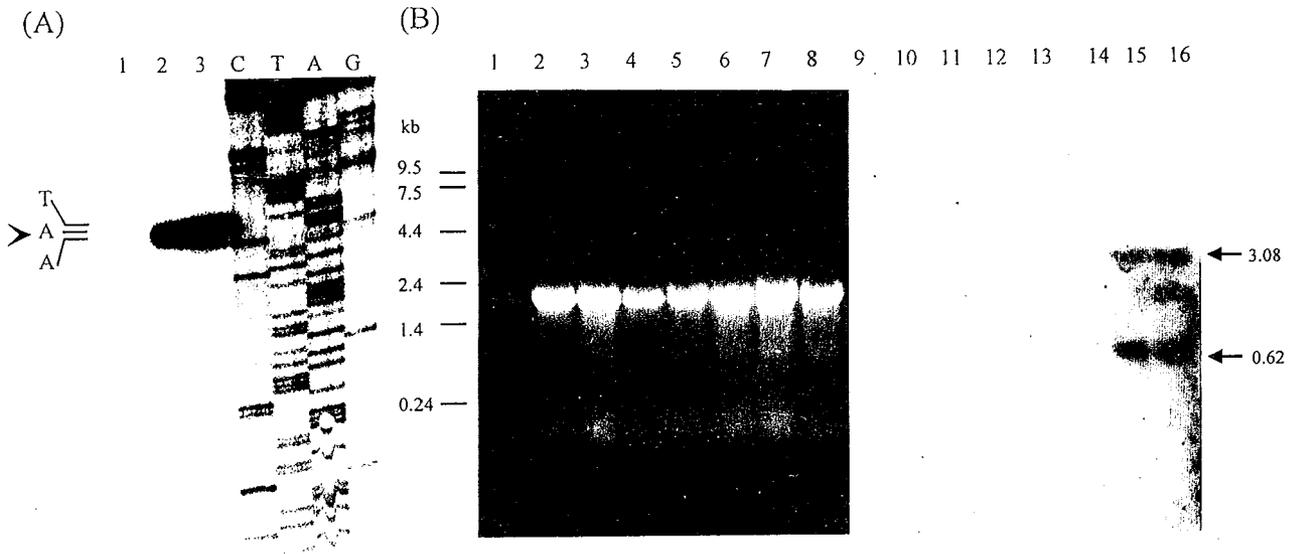
**Fig. 5.** Comparison of the amino acid sequences of 6 NPV p10 genes. To maximize the alignment, gaps are introduced and indicated as dashes. Dots indicate identical amino acid residues. Amino acid sequences of p10 are from the following baculoviruses: AcMNPV (Kuzio et al. 1984), BmNPV (Hu et al. 1994), SeMNPV (Zuidema et al. 1993), CfMNPV (Wilson et al. 1992), and OpMNPV (Leisy et al. 1986).

(Fig. 6B, lanes 14 to 16).

## DISCUSSION

In this study, we isolated and sequenced the PenuNPV p10 gene. From the nucleotide sequence (Fig. 4), it is interesting to note that this gene is flanked by 2 ORFs which are homologous to baculovirus p26 and p74 genes. That is, the partial amino acid sequences corresponding to p26 ORF and p74 ORF show high amino acid identity to those of AcMNPV and OpMNPV p26 and p74 genes, respectively, and, as with the location of the p10 gene in AcMNPV and OpMNPV (Bicknell et al. 1987), this organization is colinear.

The AcMNPV, SeMNPV, and OpMNPV p10-specific transcript sizes were 0.63 kb (Kuzio et al. 1984), 0.45 kb (Zuidema et al. 1993), and 0.63 kb (Leisy et al. 1986), respectively. The 5' terminus of



**Fig. 6.** (A) Primer extension analysis of the 5' end of p10 mRNA. A radiolabeled oligonucleotide (see Materials and Methods) was hybridized to total RNAs from non-infected NTU PN-HH cells (lane 1) or PenuNPV-infected NTU PN-HH cells at 48 h and 120 h p.i. (lanes 2 and 3), and reverse transcription was then conducted. The DNA ladder was generated from a plasmid containing a PenuNPV *Pst* I-F fragment using the same primer in the presence of dideoxyribonucleotides ddCTP (lane G), ddTTP (lane A), ddATP (lane T), or ddGTP (lane C). The transcriptional start site is indicated with an arrowhead in a region specified by the nucleotide sequence of the DNA strand in the same sense as p10 mRNA. The size of the extended product was determined against the sequence ladder and the sequence of the mRNA transcription start site is indicated by the letters at the side of photograph. (B) Northern blot analysis of PenuNPV p10 gene transcripts. Total RNA was isolated from uninfected NTU PN-HH cells (lane 1) and PenuNPV-infected NTU PN-HH cells collected at 12, 24, 48, 72, 96, 120, and 144 h p.i. (lanes 2 to 8, respectively). The samples (20  $\mu$ g RNA per lane) were electrophoretically separated on 1% agarose gel (containing formaldehyde), stained with ethidium bromide, and photographed under UV light. The same gel was transferred to a nylon membrane (lanes 9 to 16, respectively), and hybridized to a DIG-labeled PCR fragment containing the PenuNPV p10 gene. The 0.62-kb and 3.08-kb transcripts are indicated. The PenuNPV p10 gene is expressed as a 0.62-kb transcript. The size of the transcripts are indicated on the right. The kilo-base scale on the left is derived from the RNA size marker (Life Technologies, Inc.).

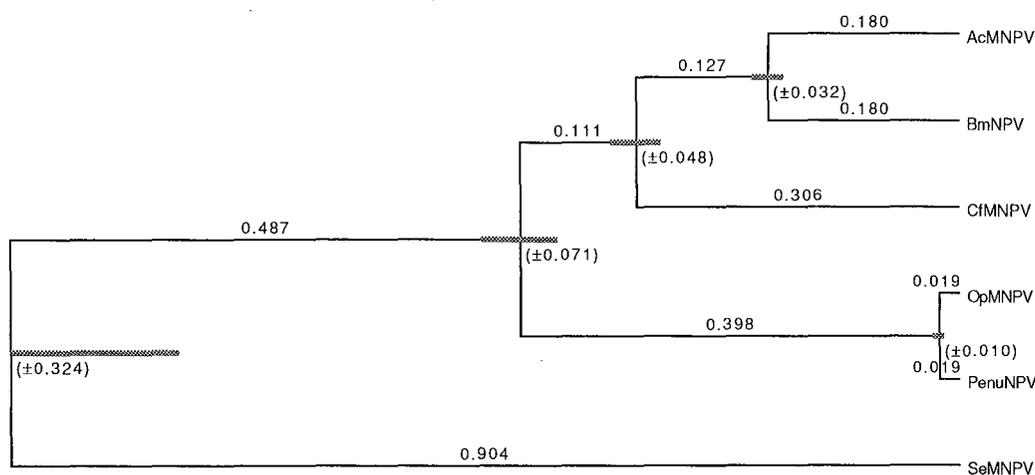
the PenuNPV p10 mRNA was mapped by primer extension analysis and was located at position -52 within the sequenced region (Figs. 4, 6A). The sequence predicts polyadenylation signals at positions +54, +105, and +142, i.e., at 385, 446, and 483 bases downstream from the transcription start site. From the Northern blot analysis (Fig. 6B), it is suggested that the PenuNPV p10 gene was expressed as a 0.62 kb transcript in length. This implies that the PenuNPV p10 ranscript contains a poly(A) tail of about 150 bp. In a transcriptional analysis of the OpMNPV p10 gene (Leisy et al. 1986), a large 3.5 kb mRNA was detected that is transcribed in the opposite direction as the p10 gene. The large 3.5 kb mRNA may represent the message for the open reading frame (p74 ORF) lying downstream from the p10 gene.

The putative upstream p26 ORF is terminated 3 nt before the TAAG transcription initiation motif of the PenuNPV p10 gene and transcribed in the same direction as the PenuNPV p10 gene (Fig. 3B). The putative downstream p74 ORF is transcribed in the opposite direction to the PenuNPV p10 gene. The stop codon of the p74 ORF is very close to the TAA stop codon of the PenuNPV p10 gene (Fig. 4).

Although p10 protein is highly conserved among baculoviruses, its precise function is still uncertain. P10 protein is an abundant viral protein in baculovirus-infected cells and is associated with fibrillar structures in the nucleus and cytoplasm of these cells (van der Wilk et al. 1987, Quant-Russell et al. 1987); it is also involved in cellular or nuclear lysis (Williams et al. 1989), and in bundling

or cross-linking microtubules (Volkman and Zaal 1990), as well as polyhedron envelope morphogenesis (Vlak et al. 1988, Quant-Russell et al. 1991). However, the functions of p10 protein are still under investigation. Recently, in a study of deletion mutagenesis of the AcMNPV p10 gene, van Oers et al. (1993) found that there are at least 2 functional domains present in the p10 molecule. One domain at the carboxy terminus is required for the formation of fibrillar structures, but not for liberation of polyhedra from the nuclei. The other domain at the amino-terminal half of p10 is involved in the supramolecular organization of p10 molecules. In a BmNPV p10 gene study, Hu et al. (1994) showed that a single-base deletion within the coding region caused a frame shift, which resulted in the production of a truncated form of p10 protein which lacked the carboxy terminal 24 amino acid residues. Hu et al. (1994) further demonstrated that infected Bm-N cells could not form fibrillar structures, although the polyhedra were still released from the infected cells.

Although AcMNPV and BmNPV have a high degree of homology (around 95%) in their genomic DNA, they exhibit different host specificity (Kamita et al. 1993, Majima et al. 1993). In the case of PenuNPV and OpMNPV, not only are their p10 genes very similar, but their polyhedrin genes are also almost the same (Chou et al. 1996). Both of them have a narrow host range, limited to *P. nuda* and *O. pseudotsugata*, respectively, although in an in vitro study, PenuNPV also could replicate in IPLB LD-652Y cells (Wang et al. 1996). Moreover, OpMNPV and its host insect, *O. pseudotsugata*,



**Fig. 7.** Phylogeny of NPV p10 genes as determined by the MULTALIGN program (GeneWorks Ver. 2.45, Intelligenties). Relatedness is proportional to the length of the branches. Standard errors are given in parentheses.

are distributed in North America and have not been recorded in Taiwan, while *P. nuda* is distributed mainly in Southeast Asia (Wang and Tsai 1995). Thus both insects differ in morphology, host plants, and distribution area; they are distinct species. Nonetheless their pathogens, OpMNPV and PenuNPV, contain 2 important genes with high homology. When Rohrmann reviewed NPVs in 1992, he found the amino acid and nucleotide sequences of p10 genes from different baculoviruses to be divergent. We have shown here, however, that the AcMNPV p10 gene is highly homologous to the BmNPV p10 gene at the level of amino acid and nucleotide sequences (89% and 94% identity, respectively). In addition, PenuNPV and OpMNPV p10 genes are very similar at the level of amino acid and nucleotide sequences (96% and 95% identity, respectively). Fig. 7 shows an amino acid sequence phylogeny comparison for the p10 gene and illustrates the relatedness of the NPVs under discussion. The present study thus supports the suggestion recently made by Wang et al. (1996) and Chou et al. (1996) that PenuNPV and OpMNPV are closely related, but not identical.

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## 榕樹透翅毒蛾核多角體病毒 p10 基因之選殖和核苷酸之定序

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榕樹透翅毒蛾核多角體病毒 p10 基因，位於病毒基因體之 *Pst* I 4.5 kbp 片段內。在此 DNA 片段中已經有 696 核苷酸完成定序。分析此 DNA 序列，發現有一個 276 核苷酸序列的可譯讀區(open reading frame)，可轉譯出具有 92 個胺基酸(amino acids)片段的 p10 基因產物，p10 蛋白質。在榕樹透翅毒蛾核多角體病毒 p10 基因，轉譯起始點(translation start codon)上游 53 核苷酸的距離，亦發現與大多數桿狀病毒後期表現基因轉錄(transcription)有關的保守序列 TAAG。在北方轉印法(Northern blot)分析中，發現有兩個主要的轉錄產物分別為 0.62 kb 和 3.08 kb 會與 PenuNPV p10 基因的探針反應。其中 0.62 kb 之轉錄產物即為 PenuNPV p10 基因的 mRNA。榕樹透翅毒蛾核多角體病毒 p10 蛋白質的胺基酸序列分別為 *Autographa californica* MNPV (AcMNPV), *Bombyx mori* NPV (BmNPV), *Choristoneura fumiferana* MNPV (CfMNPV), *Spodoptera exigua* MNPV (SeMNPV) 和 *Orgyia pseudotsugata* MNPV (OpMNPV) 有 38%, 44%, 44%, 22% 和 96% 的同源性。桿狀病毒 p10 基因是一個保守的基因(conserved gene)，根據文獻顯示在不同種桿狀病毒間，此基因的歧異性(diversity)較高，因此可利用此基因建立桿狀病毒之間的分類。根據本論文所比較的桿狀病毒 p10 蛋白質之相似程度推測，榕樹透翅毒蛾核多角體病毒與 OpMNPV 之親源較近。

關鍵詞：p10 基因，榕樹透翅毒蛾核多角體病毒，桿狀病毒。

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