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A capsid protein termed p87 of the Orgyia pseudotsugata multicapsid nuclear polyhedrosis virus (OpMNPV) was located to the HindIII-G fragment of the viral genome by Southern hybridization. A 3.6 kb segment of this region was sequenced and an 1872-nt open reading frame encoding the p 87 protein was identified and transcriptionally mapped by Northern blot and 3' S1 analysis. The p87 is a baculovirus late gene and transcription is initiated from the late gene consensus GTAAG sequence. A p87 gene fusion was constructed by fusing 1116 nucleotides of the p87 gene (encoding the C-terminal for 367 amino acids with a predicted molecular weight of 42 kDa) to the bacterial trpE gene. Polyclonal antibodies were produced and the p87 protein was examined by Western blot analysis and high levels of expression was found to occur at 36 hours post infection. Further analysis indicated, p87 is a component of purified OpMNPV PDV, BV virions and isolated capsids. The trpE-p87 antibody did not cross react with any AcMNPV specific proteins in AcMNPV infected Spodoptera frugiperda cells.

OpMNPV p87, a Baculovirus Capsid Protein: Genetic Location, Nucleotide Sequence, Transcriptional Mapping and Immunochemical Characterization

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INTRODUCTION

The Baculoviridae is a virus family pathogenic to arthropods, primarily holometabolic insects. Baculoviruses can be used as biological pesticides and they have the advantage of being very host-specific thereby providing very selective pest control without disturbing the beneficial insects and other organisms (Ignoffo, 1968). It has been suggested that approximately 30% of the insect pest problems in agricultural crops in the Western Hemisphere could be controlled by Baculovirus (Falcon, 1976). Baculovirus have been used successfully in field crops to control Heliothis spp. (Chapman and Ignoffo, 1972) and in plantations and orchards to regulate Cydia pomonella (Jaques et al., 1981). For the protection of forest, various baculoviruses were used in large areas against Lymantria dispar (Franz and Huber, 1979), Orgyia pseudotsugata (Stelzer et al., 1975) and sawfly species such as Neodiprion sertifer (Franz and Huber, 1979). Baculoviruses also can be used as a powerful protein expression system by fusing foreign genes under the control of the polyhedrin promoter (Fraser et al., 1983; Pennock et al., 1984). Many different polypeptides have been expressed using the baculovirus expression system (For review, Maeda, 1989).

LITERATURE REVIEW

Classification

Baculoviruses infect more than 500 different species of insects in the holometabolous orders Trichoptera, Diptera, Hymenoptera, Coleoptera and Lepidoptera. Two crustacean species (Couch, 1974) and one mite species (Reed and Hall, 1972) also are reportedly infected. The order Lepidoptera shows the highest number of susceptible species.

All baculoviruses have double-stranded, supercoiled DNA genome varying from 88 to 153 kilobases (kb) (Burgess, 1977). The DNA is packaged inside of a rod-shaped protein capsid. This structure termed a nucleocapsid is surrounded by a lipoprotein envelope (Tinsley and Harrap, 1978) forming the complete virion.

The genus baculovirus is divided into the three subgroups (A, B and C), which are distinguished by the nature of the occlusion body. Subgroup A, the nuclear polyhedrosis virus (NPV) and Subgroup B, the granulosis virus (GV), both form occlusion bodies (OBs) which are protein crystals around their virions. The OBs of the NPVs are formed by a protein called polyhedrin and the OBs of the GVs by a protein termed granulin. The NPVs are divided into two morphological types, the single enveloped nucleocapsids (SNPVs) which contain many single enveloped nucleocapsids within one occlusion body, and the multiple nuclear polyhedrosis virus (MNPV, where one or more nucleocapsids within one envelope are occluded by the protein crystal. The GVs contain commonly only one single virion inside of one OB. Subgroup C are non occluded baculovirus.

Life cycle of occluded Baculovirus

Most research has been done on A. californica MNPV (AcMNPV) and its life cycle is described below. The occluded baculovirus can be found in the soil or on leaf surfaces. The larvae ingest the virus during feeding which allows the polyhedra to enter the midgut, where the high pH dissolves the polyhedra (Fig. 1(1)) This dissolution step results in the release of the infectious virions known as polyhedra derived virus (PDV). The virion envelope fuses with the microvilli of the midgut epithelial cells (Fig. 1(2)). The nucleocapsid enters the cytoplasm and is transported into the nucleus of the midgut cells. After one hour post infection (hr p.i.) the nucleocapsid is uncoated and the viral replication begins(Fig. 1(3)). During replication the nucleus enlarges and a virogenic stroma which is a large fibrous structure is detectable in the nucleus and cytoplasm of baculovirus infected cells. At 8 hr p.i. a newly synthesized nucleocapsid can be observed. At 12 hr p.i. the de novo synthesized nucleocapsid leaves the nucleus and buds through the cytoplasmic membrane of the midgut epithelial cell and acquires an envelope derived from the plasma membrane modified by at least one virally encoded protein (Blissard and Rohrmann, 1989) (Fig. 1(4)). These virions are called budded virus (BV). Within the hemocoel many different cell types are infected by the BV (Fig. 1(5)). In infected cells the newly synthesized nucleocapsids can develop to become BV which continue to infect other cells inside the larvae, or the nucleocapsids can stay inside the nucleus to develop into PDV and become embedded within an occlusion body (Fig. 1(6)). The occluded virions can be detected at 24 hr p.i. and remain inside the cell until the insect dies. The cell degrades and the occluded virions are freed (Granados and Lawler, 1981). The occluded virions are stable in the environment because of their occlusion body and remain infectious almost indefinitely.

The PDV is highly infectious at the midgut level but much less infectious to cells in the hemocoel, whereas the BV is highly infectious

during the secondary infection (Volkmann, 1986) and in insect cell cultures (Keddie and Volkmann, 1985). The two phenotypes enter the host cell in distinct ways. The PDV envelope fuses with the midgut cell and the nucleocapsid enters the cytoplasm. The budded virus enters the cell through adsorptive endocytosis (Fig. 1(5)). The BV becomes invaginated into an endosome which allows the encapsulated virion to enter the cytoplasm and fuse with acidic vesicles thus lowering the pH inside the endosome. The BV envelope fuses with the endosomal membrane and the nucleocapsid is released into the cytoplasm (Volkmann and Goldsmith, 1985).

The BV and PDV envelopes are obtained in different ways. The PDV envelope protein is synthesized during replication and assembles around the nucleocapsid in the nucleus (Fig. 1(2)). The BV acquires its envelope by budding through the cytoplasmic membrane where a Baculovirus specific protein is located (Blissard and Rohrmann, 1989). BV and PDV are phenotypically different but they are genetically identical (Smith and Summers, 1978).

Gene expression

Baculoviral gene expression is divided into three temporally distinct phases and is believed to be regulated in a cascade-like system. Early expressed genes regulate the expression of later expressed genes (Friesen and Miller, 1985). The early genes are divided into immediate early (IE) genes and delayed early genes (DE). IE genes use the insect cell system for their transcription. By using cyclo-hexamide to block protein synthesis of the virus, only the IE genes are transcribed. The DE genes require the trans acting IE gene products for their expression (Guarino and Summers, 1987; Carson et al., 1988). It was shown that the DE transcription of 39K in A. californica MNPV (AcMNPV) is dependent on the gene product of the immediate early gene IE-1. This gene acts as a transactivator for the 39K gene (Carson et al., 1988). The host RNA polymerase recognize the immediate early

promoters and can transcribe the genes. The transcription of early-expressed genes like IE-1 is regulated by two different promoter sequences from the point at which transcription is initiated at different times. An early promoter is used for transcription between 0 and 2 hr p.i. and its activity slows down after two hours and a downstream promoter takes over as a transcriptional start site. The transcriptional initiation site for early genes such as IE-1 or gp64 is a consensus sequence (CAGT) which is 21 to 24 nucleotides downstream of the putative TATA box. In AcMNPV homologous repeat DNA sequences were found which are cis acting enhancer elements to increase the rate of transcription from specific promoter areas. These regions increase transcription of delayed early genes in the absence of immediate early genes by 12 to 20 fold. In the presence of the IE genes the transcription increases 1000 to 3000 fold (Guarino and Summers, 1986). Late genes are not transcribed if the host cells are previously treated with aphidicoline. Aphidicoline inhibits viral and host DNA polymerase activity, indicating that late genes are transcribed after or during viral DNA replication.

Hyperexpressed late genes are expressed after DNA replication and stay highly expressed while normal expressed late genes decline in their expression rate after DNA replication. All late genes have a common promoter sequence; their transcription starts at the A at the third position or the T of the consensus sequence A/GTAAG (Rohrmann, 1986). In AcMNPV a RNA polymerase was discovered which could be distinguished from the host RNA polymerase by its resistance to alpha amanitin (host RNA polymeraseII is inactivated by alpha amanitin) (Fuchs et al., 1983; Grula et al., 1981). These results indicate that late genes are transcribed from a viral encoded or a viral modified host RNA polymerase which recognizes the consensus promoter sequence. In the genome, early and late genes of baculovirus are not distributed according to their temporal expression. Transcriptional units are often organized as overlapping RNA's which terminate at a common 3' end. Longer transcripts are in general transcribed later during infection and the

later transcripts can repress transcription of earlier genes by overlapping their promotor sequence (Friesen and Miller, 1985). In later occlusion, specific genes like p10 and polyhedrin, different size transcripts were detected.

Structural genes and proteins

Using two dimensional gel electrophoresis more than 80 distinguished polypeptides were detected in the occluded virus AcMNPV and in Lymantria dispar MNPV (LdMNPV) (Singh et al., 1983). It is not known if all of these polypeptides are coded by specific genes or if some of these proteins appear to be a different size because of modifications such as glycosylation or phosphorylation (Vlak and Rohrmann, 1985). Some baculovirus structural proteins have been characterized on the genomic, protein and functional level (Fig. 2).

Proteins common to both PDV and BV

The two baculovius phenotypes PDV and BV share the structural proteins of the nucleocapsid. Inside the nucleocapsid, the viral DNA is associated with a very basic DNA binding protein. This protein is called p6.9 (Fig. 2) and is arginine rich (40%) (Wilson et al., 1987) and appears to be associated with DNA in a nucleosome-like structure. P6.9 may be involved in the condensation of the viral DNA prior to their packaging inside the nucleocapsid (Wilson et al., 1985). It is thought that p6.9 is phosphorylated during early stages of infection and this fascilitates the release of the DNA from the nucleocapsid. The nucleocapsid assembles inside the nucleus of the infected cell during replication. The major nucleocapsid protein in both AcMNPV and OpMNPV is p39 (Fig. 2) (Blissard et al., 1989; Thiem and Miller, 1989)

In additon to these proteins which are common to both PDV and BV, there also are phenotype-specific proteins.

BV specific proteins

Gp64 is an envelope glycoprotein which is BV specific and is present in the cytoplasm of the host cell early during infection, subsequently the protein moves to periphery of the cytoplasm and becomes integrated in the plasma membrane (Blissard and Rohrmann, 1989). Nucleocapsids bud through the cytoplasmic membrane acquire the envelope containing the gp64 protein. This protein is glycosylated and phosphorylated (Volkmann et al., 1984). Binding of monoclonal antibodies (MAb) against gp64 causes a three to four fold decline in infectivity (Volkmann and Goldsmith, 1985; Keddie and Volkmann, 1985). These results show that the gp64 is very important for the infectivity of the budded virus. It seems possible that the gp64 protein of the viral envelope fuses with the endosomal membrane to release the nucleocapsid into the cytoplasm of the cell (Volkmann and Goldsmith, 1985). The gp64 protein has a short hydrophobic N-terminus which appears to be the signal sequence of the protein. The hydrophobic C-terminus appears to anchor the protein to the cellular membrane (Blissard and Rohrmann, 1989).

PDV specific proteins

The most characteristic feature of NPVs is their occlusion body. All proteins related to this structure are PDV specific. The occlusion body is mainly composed of a protein called polyhedrin (Vlak and Rohrmann, 1985). Polyhedrin is a 29 kd protein (Fig. 2) which is hyperexpressed late during infection (Rohrmann, 1986) and it eventually comprises up to 20% of the total alkali soluble proteins present in the infected cell (Maeda, 1989). NPVs and GVs show 50% homology in their

occlusion protein structure (Rohrmann, 1986). Polyhedrins from different virus demonstrates 80% nucleotide sequence homology making it the most conserved proteins detected so far in Baculoviruses. In addition to polyhedrin other proteins also are involved in the occlusion body structure. The hyperexpressed late gene coding for the p10 (Fig. 2) protein also is associated with the occlusion of the virions. A fibrillar structure in infected cells is formed by p10 which is associated with the cytoskeleton (Quant-Russel et al., 1987). Polyhedra are surrounded by a polyhedral envelope. The proper association to polyhedrin appears to be dependent on the p10 protein (Williams et al., 1989). Baculovirus with the p10 gene deleted are more fragile, have a reduced stability in the environment (Vlak et al., 1988) and lack the proper attachment of the polyhedral envelope. A major component of the polyhedral envelope is the viral protein p32 (Fig. 2) in OpMNPV and p36 in AcMNPV. The two proteins show 58% homology (Gombart et al., 1989b). Early studies indicated that the polyhedral envelope is composed mainly by carbohydrates (Minion et al., 1987).

MATERIALS AND METHODS

Cells, virus, capsid purification

Lymantria dispar cells (IPLB-LD-652Y) were propagated in TC-100 medium as previous described (Quant-Russell et al., 1987). OpMNPV virus were propagated, isolated and purified as previous described (Quant-Russell et al., 1987). Purified PDV and BV for Western blot analysis were purified as described by Blissard et al. (1989). The capsid of the OpMNPV was purified using the method described by Thiem and Miller (1989).

Enzymes, Isotopes

Restriction enzymes and DNA modifying enzymes were purchased from Bethesda Research Laboratories, New England Biolabs, United States Biochemical and used according to the manufacturer's instructions. All isotopes were purchased from New England Nuclear.

Restriction endonuclease mapping

Cosmid 39 DNA (Leisy et al., 1984) was isolated using standard procedures (Maniatis et al., 1982) and digested in single and double digestions. The restriction fragments were separated on 0.8-1.0% TBE or TAE agarose gels (Maniatis et al., 1982). As a standard for sizing the DNA fragments a 1 kilobase ladder (BRL) was used. Restriction fragments were isolated and purified from the agarose gel using the Gene Clean procedure (Bio101,Inc.).

Cosmid 39 DNA was digested in single digests with HindIII, PstI, SstI and double digests with PstI/EcoRI, PstI/XhoI, PstI/BamHI, PstI/ClaI. The HindIII fragments of cosmid 39 were isolated and digested with PstI. The HindIII 8.4 kbp fragment also was digested with PstI and ClaI, the HindIII 6.5 kbp fragment with PstI and XhoI, the HindIII 5.2 kbp fragment with PstI/SstI, the HindIII 4.4 kbp fragment with PstI and XhoI and the HindIII 3.95 kbp fragment with PstI and SstI. By analysis of the fragment patterns from a combination of different digests, a complete PstI restriction map was generated for the cosmid 39.

Southern blot analysis and DNA labeling

Insert DNAs were isolated from λgtll recombinants which were immunoreactive to polyclonal antiserum (Quant-Russell et al., 1987) produced against the purified PDV. The λ DNA was digested with EcoRI the insert was and gel purified, hexamer labeled with [α^{32}P]dATP (specific activity 2x10^{6}cpm/μg) (Feinberg et al., 1984), and used as a probe for Southern hybridization (Maniatis et al.,1982). Agarose gels (0.8%) containing appropriate DNA digests were blotted to Gene Screen (Dupont,Inc.) according to the manufactures instructions. The blots were prehybridized at 42°C in prehybridization buffer (6x SSC, 5x Denhardts, 0,5% SDS, 100 μg/ml denatured salmon testis DNA, 50% formamide). After four hours, this solution was replaced and labeled probe (1x10^{6}cpm) was added and incubated overnight at 42°C. The blots were washed as follows: five minutes in 2x SSC-0.5% SDS at room temperature (RT), 15 min in 2x SSC-0.1% SDS at RT, and 2.5 hours at 42°C in 0.1x SSC-0.5% SDS. The third wash was repeated for 0.5 h in fresh solutions.

Cloning vectors, host cells

Escherichia coli host strains HB101, JM83, JM103 and DH5α were used for plasmid production. The inserts from the reactive λgt11 recombinants were cloned into bacteriophage M13 strains mp18. Isolated restriction fragments from cosmid 39 were cloned into pUC18, pUC19 and bluescribe [pBS(-)] (Stratagene cloning systems, Inc.) which was modified to contain a BglII restriction site. The recombinant plasmid and M13 were transformed into competent Escherichia coli cells (Maniatis et al., 1982).

DNA sequencing

For λ sequencing one μg of DNA was added to 2 μl 5x sequencing buffer and 1 μg of primer (complementary to the Lac Z sequences adjacent to the EcoRI site in the λ gt11) and the sequencing reaction was performed by following the procedure of the manufacturer (U.S. Biochemicals Corp.). For M13, single stranded DNA from the bacteriophage M13 was isolated and used as a template for sequencing (Sanger et al., 1977). Double stranded plasmid DNA was sequenced using Sequenase (U.S. Biochemicals Corp.) and [α^{35}S]dATP by the modified dideoxy chain termination method of Toneguzzo et al. (1988). The DNA was sequenced in both directions.

Nucleotide sequence analysis

The nucleotide sequence and the predicted protein were analyzed with the bionette IntelliGenetics sequence analysis programs. The Protein Identification Resource of the National Biomedical Research Foundation (PIR) protein data base was searched for sequence homology of the predicted proteins. The hydropathy and protein chain flexibility graphic plots were computed on the PC-Gene program from IntelliGenetics.

Northern blot hybridization analysis

Total RNA isolated from OpMNPV infected L. dispar cells (Blissard et al., 1989) was electrophoresed on 1% agarose, 6% formaldehyde gel in a buffer of 20 mM MOPS (3[N-morpolino] propanesulfonic acid, pH 8.0); 5 mM Sodium acetate, 1 mM EDTA. The RNA was transferred to Gene Screen plus (Dupont, Inc.) following the manufacturers instructions. Prehybridization was performed at 65°C for 9 hours in hybridization buffer [50% formamide, 5x Denhardts, 0.1% SDS, 150 μg/ml Salmon Sperm DNA, 5x SSPE (20x SSPE: 3.6 M NaCl, 20 mM EDTA, 0.2 M NaPO4, pH 7.7)]. The RNA probe complementary to part of the p87 gene (Fig. 9) was derived by transcribing a restriction fragment subcloned in pBS(-) with T3 RNA polymerase (Boehringer) and [^{32}P]-UTP according to the instruction of the manufacturer. The blots were hybridized with 1x10^6 cpm/ml at 65°C for 12 hours. The blots were washed in 3 changes of 2x SSC-0.1% SDS (10 minutes/wash) followed by 3 changes of 0.1x SSC-0.1% SDS at 60°C (20 minutes/wash).

3'mapping by S1-Nuclease protection

A restriction fragment containing a portion of the 3' end of p87 and a downstream region (Fig. 11) was end-labeled by filling in with Klenow and [α^{32}P]dATP (Maniatis et al., 1982). The end-labeled DNA probe and 10 μg total RNA from OpMNPV infected cells were suspended in 20 μl S1 hybridization buffer [80% formamide, 40 mM PIPES (Piperazine N,N'-bis (2 ethanesulfonic acid) pH 6.4, 1 mM EDTA, 0.4 M NaCl. The DNA-RNA mixture was denatured at 85°C for 15 minutes and reannealed at 51°C for 3 hours. 300 μl S1-nuclease digestion buffer [0.28 M NaCl, 50 mM Sodium acetate (pH 4.4), 4.45 mM $ZnCl_2$,20 μg/ml denatured salmon sperm and 600 U/ml S1-nuclease] was added to the hybridization mixture and incubated for 30 min at 37°C. The S1 nuclease was inactivated by adding 75 μl S1 termination buffer (2.5 M amonium acetate, 50 mM EDTA) and the nucleic acid was precipitated with 2 volumes of ethanol. The DNA

(2000cpm) was resuspended in loading buffer (DNA, 95% formamide, 20 mM EDTA, 0.05% Bromophenol Blue, 0.05% Xylene Cyanol FF) loaded on a 8% polyacrylamide/7 M UREA denaturing gel (Favaloro et al., 1980; Blissard et al., 1989).

Construction of the trpE-p87 gene fusion

The 2.65 kbp PstI fragment from cosmid 39 was cloned into the PstI restriction site of pBS(-) modified with an additonal BglII site (Fig. 15.). This recombinant was digested with HindIII and PstI and the 1200bp PstI/HindIII restriction fragment containing 1116 nt of the 3' region of the p87 ORF was isolated and subcloned into the pUC8 digested with PstI/HindIII. The insert was removed and subcloned into pATH3 (trpE) expression vector [similar to pATH2 (Dieckmann et al., 1985)]. A primer was made to the pATH vector and plasmid sequencing was performed to confirm that the p87 ORF was in frame with trpE ORF of the vector.

Expression of trpE-p87 fusion protein

The pATH3 trpE-p87 recombinants were transformed into HB101 cells and grown overnight in M9 media (Maniatis et al., 1982) plus 1% casamino acids, 100 μg of ampicillin per ml, and 20 μg of tryptophan (Trp) per ml and then diluted 1:10 in the same media lacking tryptophan and incubated at 30°C for 60 min. Expression of the trpE-p87 gene fusion was induced by adding 20 μg/ml ß-indoleacrylic acid (Sigma). The culture was incubated overnight at 30°C (Tollefson et al., 1988; Spindler et al., 1984). Total cell lysates were prepared by resuspending the cells in urea-SDS buffer (6 M urea, 1% SDS, 1% 2-mercaptoethanol, 0.01 M sodium phosphate buffer, pH 7.2) (Kleid et al., 1981), an equal volume of 2x reducing sample buffer was added and the samples were boiled for 5 min. The total cell lysates were electrophoresed on 6 and 9% SDS-polyacrylamide gels (Laemmli, 1970). The proteins were visualized by

staining with Coomassie brilliant blue (Fig. 16).

Purification and isolation of trpE-p87 fusion protein

Bacterial cells (100ml) containing the recombinant plasmid were expressed as described above and resuspended in 2 ml of 50 mM Tris HCl (pH 7.5), 5 mM EDTA and 3 mg/ml lysozyme and the cells were lysed on ice for 30 min. NaCl and Nonidet P-40 (NP-40) was added to a final concentration of 0.3 M and 0.5% respectively. The incubation on ice was extended for 30 min. The solution was then adjusted to a final concentration of 2 μg DNaseI and 5 mM $MgCl_2$ per ml and incubated for 60 min. on ice. The soluble and insoluble protein was fractionated by centrifugation in a microfuge at 10 K for 30 min and both the supernatant and pellet portions were collected. The pellet was resuspended in 2 ml of 10 mM Tris (pH 7.5) and both fractions were stored at -80°C. 100 μl of the pellet was mixed with an equal volume of 2x PAGE gel sample buffer [Urea-SDS buffer (6 M urea, 1% SDS, 1% 2-mercaptoethanol, 0.01 M sodium phosphate buffer, pH 7.2)], boiled for 5-10 min, and electrophoresed on a 9% SDS polyacrylamide gel (gel size 15 cm x 20 cm x 1.5 mm; lane 1 cm x 20 cm x 1.5 mm) (Laemmli, 1970). A strip of the gel was removed and stained with Coomassie blue to determine the location of the trpE-p87 fusion band. The fusion protein band was then excised from the nonstained part of the SDS-PAGE gel. The protein was isolated from the SDS-PAGE gel by electroelution at 200 V for 4-6 hours using a Elutrap (Schleicher and Schuell, Inc.). The eluate was stored at -80°C. The amount of protein in the eluate was determined by comparison with bovine serum albumin of known concentration.

Antiserum production against the trpE-p87 fusion protein

A New Zealand White rabbit was initially injected with 300 μg of isolated fusion protein emulsified in complete Freund's adjuvant. The rabbit was boosted twice with 100 μg and 50 μg fusion protein emulsified in incomplete Freund's adjuvant. Immune serum was collected 7 days after each boost. Antiserum from the second boost was used to study the p87 protein.

Western blots

Infected cell proteins from different time points post infection were derived from Lymantria dispar cell lysates ($4x10^6$ cells/well) which were infected with OpMNPV (Quant-Russell et al., 1987). PMSF (final concentration 0.5 mM) was added to the cell lysates to inhibit protease activity. Polyhedra derived virus and purified OpMNPV capsids were provided by Dr. Margot Pearson. The purified BV and anti gp64 MAb (AcV_5) where a gift from Dr. Gary Blissard. The monoclonal antibody specific for p39 (MAb-236) (Pearson et al., 1988) was provided by Dr. Margot Pearson. Tissue culture fluid containing the MAb AcV_5 and MAb-236 were used undiluted for Western blot analysis. Anti-trpE-p87 antiserum was used on the Western blots at a dilution of 1:500 in TBS-Tween. The protein samples for Western blot analysis were electrophoresed on 6 or 9% SDS polyacrylamide gels (Laemmli, 1970). Proteins from the gels were electroblotted onto nitrocellulose membranes (Schleicher and Schuell) for 2 hours and incubated with the p87 antibody for 2 hours. The blots were washed 3 times in TBS-Tween (20 mM Tris, pH 7.5, 500 mM NaCl, 0.05% Tween 20) and incubated for one hour to bind a second anti-rabbit IgG antibody with conjugated alkaline phosphatase as a detection system (BRL) (Quant-Russell et al., 1987).

RESULTS

Localization of the p87 gene

To localize the genes coding for structural proteins of the OpMNPV, a λgtll expression library was constructed (Quant-Russell et al., 1987) and monoclonal antibodies (Quant-Russell et al., 1987; Pearson et al., 1987) and polyclonal antiserum (Gombart et al., 1989a), against purified OpMNPV polyhedra derived virion proteins were produced. Screening the expression library with polyclonal antiserum identified two immunoreactive clones (λ11 and λ18) which did not react to antibodies characterized in earlier studies (polyhedrin, p10, p39 and p32), personal communicaton with M. Pearson. The inserts were isolated from the λgtll clones and radioactively labeled and used to probe blots of OpMNPV and cosmid DNA (Leisy et al., 1984). Both inserts hybridized to the Hind-G region located in cosmid 39 (personal communication with Dr. M.Pearson). A detailed restriction map of cosmid 39 was produced (Fig 3). Both inserts hybridized to the 1.0 kbp EcoRI/PstI and 2.65 kbp PstI restriction fragment (Dr. M. Pearson personal communications see Fig. 5). To determine the complete sequence of the immunoreactive reading frame present in the λgtll inserts, 3555 nucleotides of DNA from this region were sequenced (Fig. 6). An open reading frame of 1875 nt encoding a predicted protein of 70.6 kDa was found. This reading frame was in the same orientation and contained the ORF portions of which were found in the λ11 and λ18 clones. The translational start site (ATG) (nt 216 Fig. 6) follows the Kozak rules of an efficient translational start (ATAATGG) with a purine bases at the -3 position and a +4 positions (Kozak, 1986). A single polyadenylation signal (AATAAA) was found 103 nucleotides downstream of the translational termination site of p87 at nt 2090 (Fig.6). The predicted protein sequence contains 7 possible N-glycosylations sites (Asn-X-Ser/Thr). A consensus sequence (GTAAG) for transcriptional initiation of baculovirus late genes (Rohrmann, 1986)

was found to be located 34 nucleotides upstream of the translational start site at nt 182 (Fig. 6). Through primer extension analysis the transcriptional start site of the p87 gene is situated at the T of the GTAAG (nt 182 Fig. 6) (Russell personal communication).

The p87 gene is located upstream and in the opposite orientation of p6.5 DNA binding protein (Fig. 14), which is homologous to the p6.9 of AcMNPV (Wilson et al., 1987). Three open reading frames p48, p12 and p40 were found between ORF p87 and ORF p6.5. The open reading frames were all oriented in the same direction as p6.5 and their transcription was initiated from ATAAG's (Russel and Rohrmann, submitted).

No significant homology was found by searching the PIR protein data base between the predicted p87 protein and other proteins.

Repeated regions

In the central region of the p87 gene two sets of repeated sequences were observed (Fig. 6). The first set of repeats (39 nt long) (nt 981-1019 and 1020-1055 Fig.6) had 81% and 85% identity at the nt and amino acid level respectively. A second sequence of (33 nt) is repeated three times. The three repeats (nt 1062-1094, nt 1101-1133 and 1155-1187 Fig. 6) are identical. The first set of repeats is related to the second set and other permutations of the two types of repeats are present. Homology search of the PIR protein data base for the repeated area did not show any significant similarities to other polypeptides in the data base.

Hydropathy of p87

Hydrophilicity profiles (Fig. 7) of the protein were predicted using the method of Hopp and Woods (1981). Their calculation was based

on charged and polar amino acid residues which had hydrophilic values whereas uncharged amino acids had negative values. The p87 protein is hydrophilic over much of its sequence. The area of highest hydrophilicity is at amino acid 400 in the polypeptide chain. Two hydrophobic regions are predicted, one at amino acid 280-320 and another close to the amino terminal. The hydrophobic area around amino acid 300 corresponds to the region of the second repeat. Hydrophilicity studies are commonly used to predict the antigenicity of polypeptides. Hydrophilic regions of the proteins are mainly found on the protein surface which are exposed to a greater extent to solvents and show a higher immunogenic response.

Northern blot analysis

The transcription of p87 was studied by Northern blot analysis (Fig. 9 and 10). A cRNA probe was hybridized to total RNA isolated from OpMNPV infected L. dispar cells at different time points post infection. The 2.2 kbp PstI fragment from cosmid 39 was subcloned into pBS(-). The clone was digested with BglII which linearized the plasmid. From the T3 promotor of the linear plasmid a labeled cRNA probe was synthesized using radioactive ^{32}P UTP and T3 RNA polymerase (Fig. 9). The cRNA complementary to parts of the p87 ORF (nt 984-259 Fig. 9) was used as a probe for hybridization to Northern blots of RNA's isolated from OpMNPV infected L. dispar cells at different times post infection. The probe hybridized to a RNA of approximately 2100 nt at 36 hr p. i.

Mapping of the 3' terminus of the p87 specific transcript

To determine the 3' end of the p87 mRNA a restriction fragment containing parts of the p87 ORF was subcloned into pBS(-) and the construct was digested with HinfI (Fig. 11). The 945 bp HinfI restriction fragment is composed of 37 bp from the 3' end of the p87

gene, 673 bp of OpMNPV DNA and 235 bp of vector DNA. This restriction fragment was endlabeled, denatured and hybridized to total RNA isolated from infected (36 hr p.i.) and uninfected cells. The RNA-DNA hybrids were treated with S1 nuclease which is specific for the digestion of single stranded DNA. Two protected fragments were detected (Fig. 12) which are specific for the infected L. dispar cells. One fragment with the size of 232 nucleotides indicated a termination of one transcript 192 nucleotide downstream of the p87 translational stop codon (Fig. 6) and a second protected fragment of about 700 nucleotides corresponds to the end of the viral DNA (SalI virus/plasmid junction) on the labelled DNA fragment. This indicates a second larger transcript runs through this region. A single polyadenylation site (AATAAA) is present about 90 bp upstream of the end of the mRNA at nt 2194 (Fig. 6).

Investigation of the p87 protein expression and function

A 1214 nt segment from the PstI site at nt 984 and extending to the HindIII site at nt 2198 of the p87 open reading frame was fused in frame to the truncated trpE gene of the pATH3 E. coli plasmid expression vector (Fig. 15) (Dieckmann and Tzagoloff, 1985). The trpE-p87 fusion protein contained 37 kDa trpE component and 1101 nucleotide of the open reading frame of p87 which encoded 367 amino acids with a predicted molecular weight of 42kDa resulting in a fusion protein with the predicted size of 79 kDa (Fig. 6). This segment of the p87 molecule contains a highly hydrophilic region (Fig. 7) and therefore is predicted to be highly antigenic.

Western blot analysis

The antibody reacted to a protein with a molecular weight of 87 kDa in PDV virions, BV virions and purified capsid (Fig. 17). In addition p87 antibodies reacted to a protein of 87 kDa on Western blots

of extracts from OpMNPV infected but not uninfected Tussock moth Orgyia pseudotsugata larvae (data not shown). To compare the presence of p87 to other structural proteins, purified PDV virions, BV virions and purified capsid were Western blotted and reacted with a mixture of antibodies against the capsid protein (p39), the BV specific envelope protein (gp64) and p87 (Fig. 19). Both p87 and p39 capsid protein was present in PDV, BV and the purified viral capsid while the gp64 protein was only present in the BV preparation. This suggests p87 is also a component of the capsid.

The synthesis of the p87 protein during the infectious cycle of OpMNPV in infected L. dispar cells was investigated by Western blots of cell lysates from various time points post infection using p87 polyclonal antiserum. A major protein band of approximately 87 kDa was detectable in cells 24 hr p.i. (Fig. 17). The intensity of the protein band increased at later time points through 72 hr p.i.. Several other bands with lower molecular weight could be seen from 24 hr p.i. until 72 hr p.i., particularly a band of ca. 50kDa. In order to determine the earliest time p87 could be detected, eight times the number of cells were added per lane and were reacted with p87 antibodies (Fig. 18.). The p87 protein was visible at 18 hr p.i.. A strongly staining peptide of ca. 50 kDa is also present starting at 18 hr p.i.

DISCUSSION

A capsid protein of 39 kDa present in OpMNPV (Pearson, 1988; Blissard, 1989) as well as in AcMNPV (Thiem, 1989) has been shown to be the major component of the baculovirus capsid. Results of my studies indicate, that in addition to p39, a second capsid protein is present. It was termed p87 according to its apparent size determined on a SDS-polyacrylamide gel. A protein of similar size was described in previous studies as a possible structural component of the baculovirus virions (Smith and Summers, 1981). The gene encoding the p87 capsid protein was mapped to the OpMNPV genome by Southern blotting, its nucleotide sequence was determined, its expression was transcriptionally mapped and characterized by Northern blot analysis, its location on virus structural components was investigated.

The p87 protein was localized to the capsid structure of both PDV and BV and its temporal expression was characterized by Western blot analysis. The gene encoding the p87 protein had a reading frame of 1872 nucleotides encoding a predicted protein of 70.6 kDa, classified as a late baculoviral protein because temporal transcription analyzed by Northern blotting determined that the p87 gene was expressed at 36 hr p.i.. This corresponds to the results of the Western blot analysis where the p87 protein is present in OpMNPV infected insect cells at a high level at 36 hr p.i. and the p87 protein can be detected at very low amounts as early as 18 hr p.i.. The results from the immunofluorescence correspond to the results determined by Western and Northern blotting (M.Pearson personal communication). The p87 protein appears at a low level at 24 hr p.i. and is present at high level in the nucleus at 36 hr p.i.. The intensity of staining increases at later time points may be due to increase of concentration protein in the nucleus or through increased concentration of the protein to a smaller area after assembly of the virions. A peptide of 50 kDa also stains at earlier time point (18 hr p.i.) indicating a cross hybridization of the p87 antibodies to

an second viral protein or that the p87 is either incompletely synthesized or partially degraded.

The expression of p87 gene seems to be tightly regulated. The transcription of the p39 capsid protein is also temporally restricted, but the mRNA can be detected from 24 to 48 hr p.i. with a decreasing intensity after 36 hr p.i. (Blissard, 1989). Both, the p87 and p39 proteins seem to have a transcriptional control mechanism which differs from the regulation of the hyperexpressed polyhedrin gene which is expressed at a high level from 24 hr p.i. until very late in infection without a decrease in intensity (Leisy, 1986). All baculovirus late genes have a consensus promotor sequence (A/GTAAG) (Rohrmann, 1986; Wilson et. al., 1987; Thiem and Miller, 1989; Gombart et al., 1989a). A GTAAG sequence was found 33 nucleotides upstream of the translational start size of the p87 gene, and the transcription initiation site was mapped to this sequence (Russell personal communication)

By S1-protection analysis, a 3′ end of the p87 specific mRNA was located 192 nucleotides downstream of the translational stop codon which indicates a mRNA transcript of 2097 nucleotides. This corresponds to the results of the Northern blot, where the probe hybridized to an approximately 2100 nt mRNA transcripts. A second strong transcript of about 700 nt also was detected by S1-protection. The exact termination site of this long message was not determined however it corresponds to the junction of the viral inserted plasmid DNA and indicates there is a second, larger transcript transcribed in this region.

Repeated sequences have been observed in baculovirus proteins previously characterized (Gombart et al., 1989a; Blissard, 1989; Rohrmann, 1986). Repeated regions were also found in p87. One sequence of 13 amino acids is repeated once (repeat 1) followed by a 11 amino acid sequence repeated three times (repeat 2), both of these repeated sequences are proline rich. The significance of these repeats is unclear. Another unusual feature of p87 is the hydrophilic amino acid

composition. The hydropathy of a protein has an important influence on the conformation of a protein (Creighton, 1984). The hydrophilicity of p87 may be important for the location of the protein in the viral capsid and its interaction with other capsid proteins. The hydrophilicity of p87 can account for the difference between the predicted protein size (70.6 kDa) and the appearance of this protein on SDS-polyacrylamide gels (87 kDa). The p87 protein has a high concentration of charged amino acids with arginine, glutamic acid, aspartic acid and lysine representing 28.1% of the molecular weight. It is possible that the charged molecules of the protein interact with the SDS of the polyacrylamide gel such that the mobility of the protein in the gel is retarded. The same artefact was observed for other polypeptides with unusual high hydrophiliciy (Gracella et al., 1988). This difference in size also may be caused by O- or N- linked glycosylation or other post translational modifications which can occur during baculovirus development (Maeda, 1989). Seven possible N-glycosylation sites are present in the p87 polypeptide sequence. Treatment of purified PDV virions with N-glycosidase did not show a size shift of the p87 protein which indicates that the p87 protein is not N-glycosylated (M. Pearson personal communication)

The PDV and BV phenotypes have different properties which are related to their functions in the life cycle of the virus. The two phenotypes have different protein composition but they share the structural proteins associated with the nucleocapsid. The p6.9 DNA binding protein in AcMNPV (Wilson et al., 1986), its homolog p6.5 in OpMNPV as well as the capsid protein p39 (Pearson et al., 1988; Blissard, 1989; Thiem and Miller, 1989) occur in the PDV as well as in the BV phenotype. The p87 antiserum reacted strongly with the purified PDV virion, BV virion and the purified viral capsid. The preparations were also reacted with antibodies against the p39 and gp64. The p39 antibody reacted in the same manner as the p87 antiserum. Both p39 and p87 antibodies reacted similarly indicating they are both associated with the viral capsid. In contrast, the gp64 reacted only with the BV

preparation.

The p87 gene was not detected in the AcMNPV genome through Southern hybridization under conditions of low stringency using a OpMNPV probe containing approximately 1200 (Fig. 6 BglII/SstI nt 260-1688) nt of the p87 gene (M. Pearson personal communication) and the OpMNPV p87 poloyclonal antiserum did not cross react to any protein specific to AcMNPV in AcMNPV infected Spodoptera frugiperda cells. These negative results are surprising because the two virus are shown to be closely related (Leisy, 1984). Gombart et al. (1989b) has shown, that not all genes are shared between baculovirus and some proteins diverged during evolution at a higher speed than other proteins. In the future, the role of the p87 protein as a structural component in the viral capsid will be examined in depth by using the antiserum for immunoelectron microscopy studies.

CONCLUSION

The discovery of the p87 gene, protein, and its functional analysis indicates that the capsid of the OpMNPV is composed of at least two structural proteins, the p39 and the p87 capsid proteins. These studies on the p87 gene and protein have modified the present model of the OpMNPV baculoviral structure from a single protein capsid to a viral capsid, composed of two major structural proteins. The p87 gene is a late gene transcribed from a baculovirus late promotor (GTAAG) and the protein is detectable at 36 hr p.i., late in the infection cycle, concentrated in the nucleus of the viral infected insect cells.

The present and future use of baculoviruses in agriculture, medicine, and research is based on the understanding of the viral biology. Few baculovirus genes have been clearly characterized in structure and function. The knowledge of one additional gene with its location in the genome, complete nucleotide sequence, expression, and function of the gene product contributes to the progress in the field of baculovirology.

Figure 1. Life cycle of occluded baculovirus.

The numbers refer to the numbers in the introduction (life cycle of occluded baculovirus).

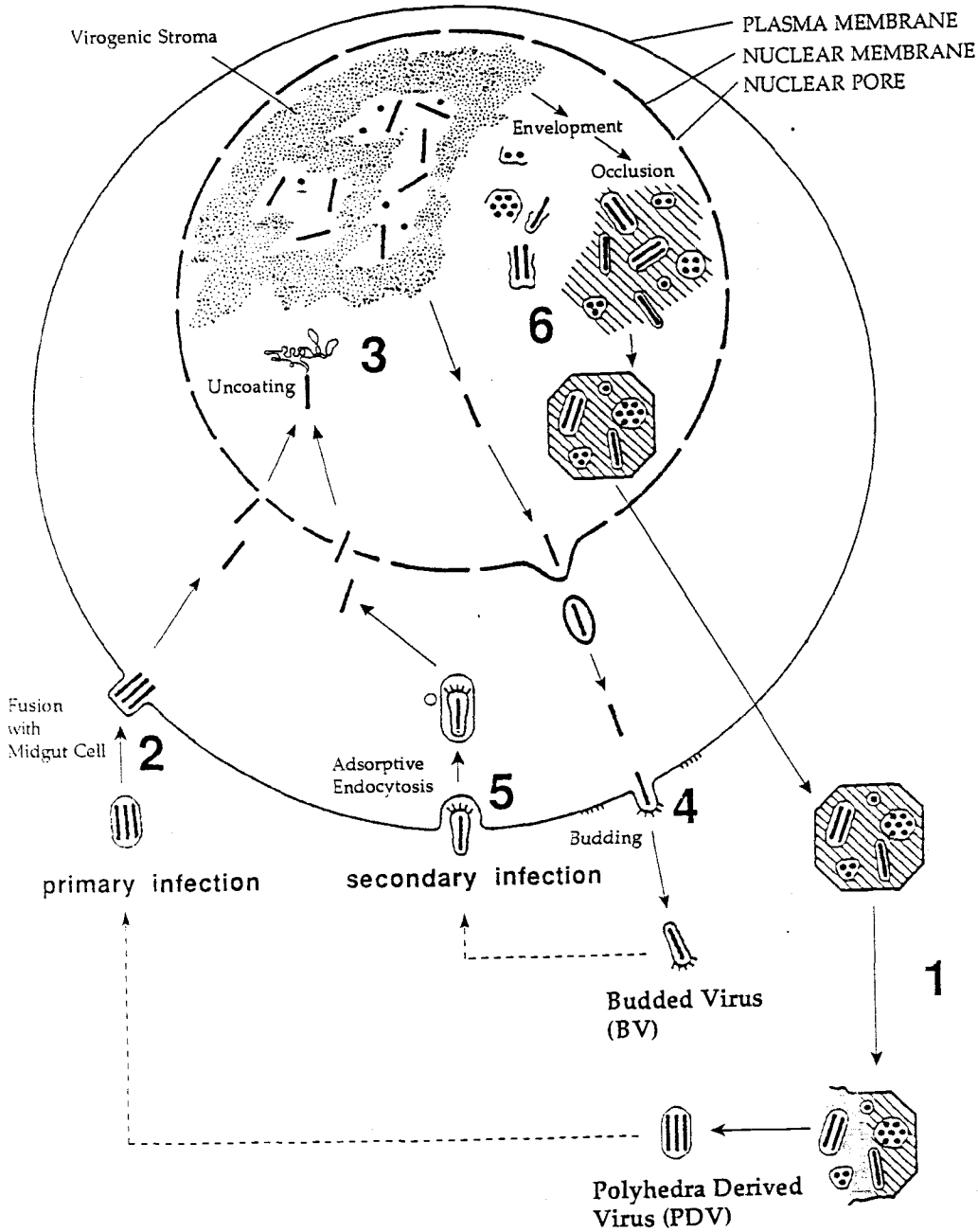

Virogenic Stroma
PLASMA MEMBRANE
NUCLEAR MEMBRANE
NUCLEAR PORE
Envelopment
Occlusion
6
3
Uncoating
Fusion
with
Midgut Cell
2
Adsorptive
Endocytosis
5
4
Budding
primary infection
secondary infection
Budded Virus
(BV)
1
Polyhedra Derived
Virus (PDV)

Figure 2. Structural proteins of baculovirus phenotypes.

The structural components specific to PDV and,or BV as well as the structures shared by both phenotypes. The name of the protein related to the structural component is indicated in parentheses.

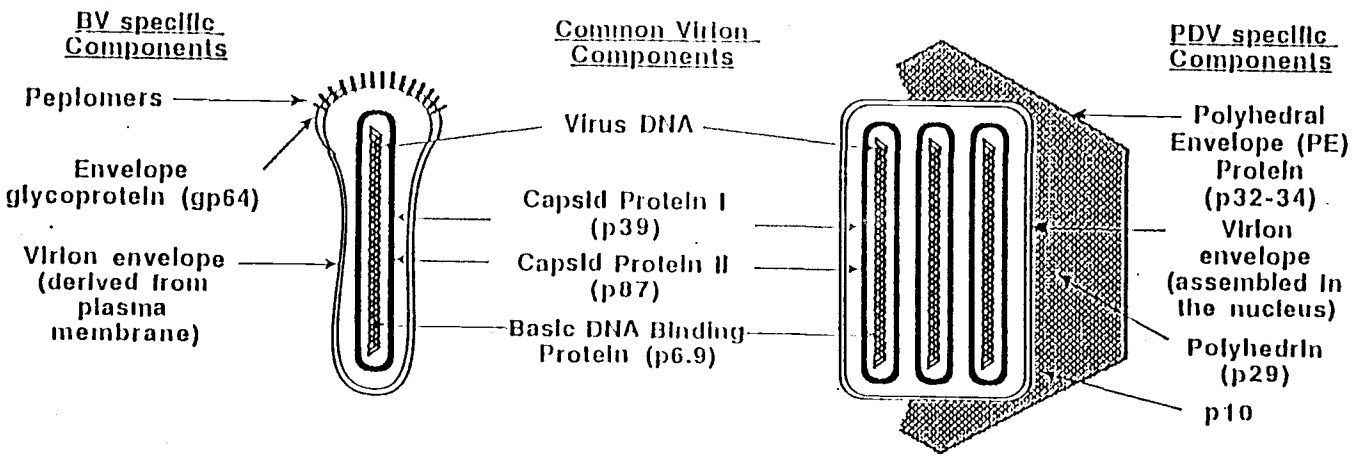

BV specific Components
Peplomers
Envelope glycoprotein (gp64)
Virion envelope (derived from plasma membrane)
Common Virion Components
Virus DNA
Capsid Protein I (p39)
Capsid Protein II (p87)
Basic DNA Binding Protein (p6.9)
PDV specific Components
Polyhedral Envelope (PE) Protein (p32-34)
Virion envelope (assembled in the nucleus)
Polyhedrin (p29)
p10
Budded Virus (BV)
Polyhedra Derived Virus (PDV)

Figure 3. Restriction map of cosmid 39.

(a) HindIII restriction map of OpMNPV indicating the position of the cosmid clones 47, 1, 54, 58, 39 which represent the total OpMNPV genome (Leisy et al., 1984). The arrows below the map show the direction and location of some OpMNPV genes. PH (polyhedrin), Capsid (39K capsid protein), DNA pol (DNA polymerase), p6.5 (DNA binding protein), gp64 (envelope glycoprotein), PE (32kDa polyhedral envelope protein), p10

(b) Map of cosmid 39 indicating restriction sites for HindIII (H), XhoI (X), SstI (Ss), BglII (Bg), EcoRI (E), BamHI (B) and ClaI (C). Pertinent restriction sites of the cosmid vector are marked on the right side. The lower map shows the location of the PstI restriction sites in cosmid 39. The location of each PstI restriction site is indicated in kilobasepairs (kbp) from the left end of the cosmid insert. The HindIII site on the left is given the value zero.

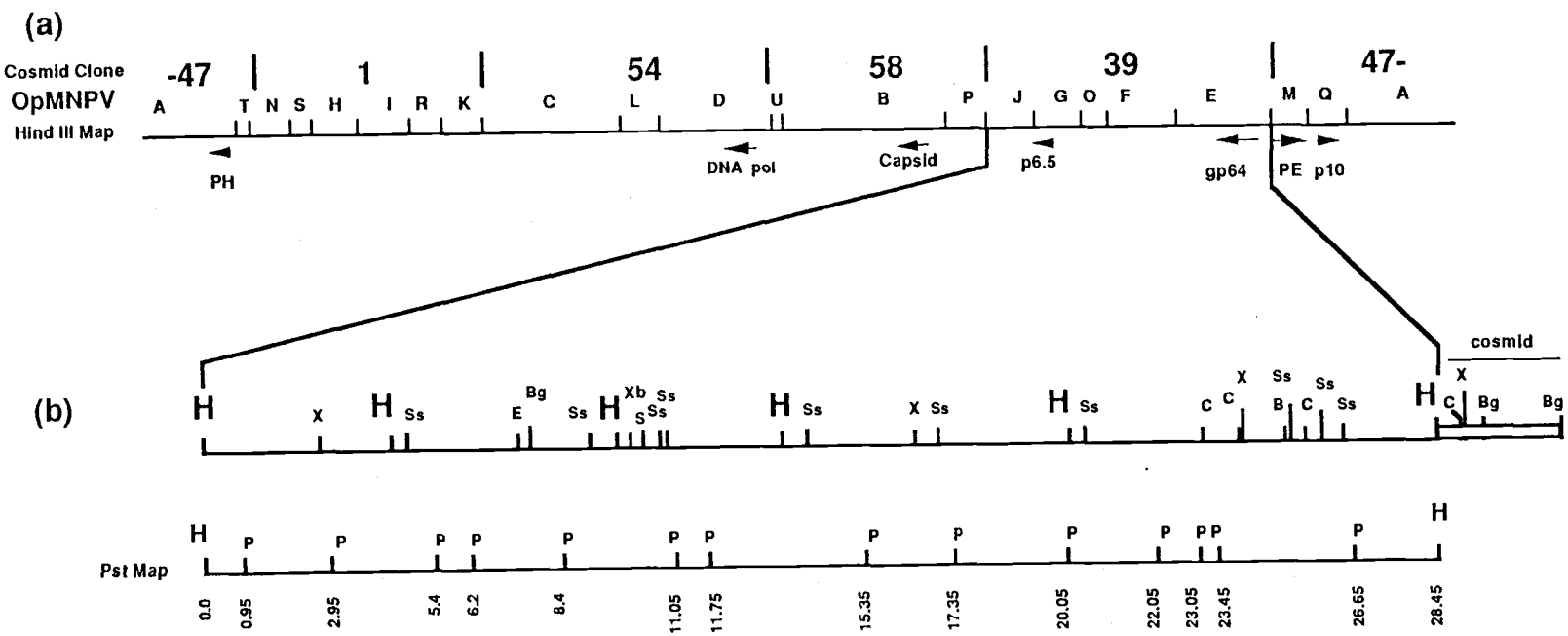

(a)
Cosmid Clone
OpMNPV
Hind III Map
-47
1
54
58
39
47-
A T N S H I R K C L D U B P J G O F E M Q A
PH
DNA pol
Capsid
p6.5
gp64
PE p10
cosmid
(b)
Pst Map
0.0
0.95
2.95
5.4
6.2
8.4
11.05
11.75
15.35
17.35
20.05
22.05
23.05
23.45
26.65
28.45

Figure 4. Main clones constructed for the sequencing project.

The upper line represents a HindIII map of the OpMNPV genome. The HindIII-G and HindIII-O region of cosmid 39 is expanded. The main clones constructed for this project are shown below. The double line represents the inserted OpMNPV DNA. The vector DNA (pBS(-)) is indicated by a single line. The size of the inserted DNA is indicated above the double line.

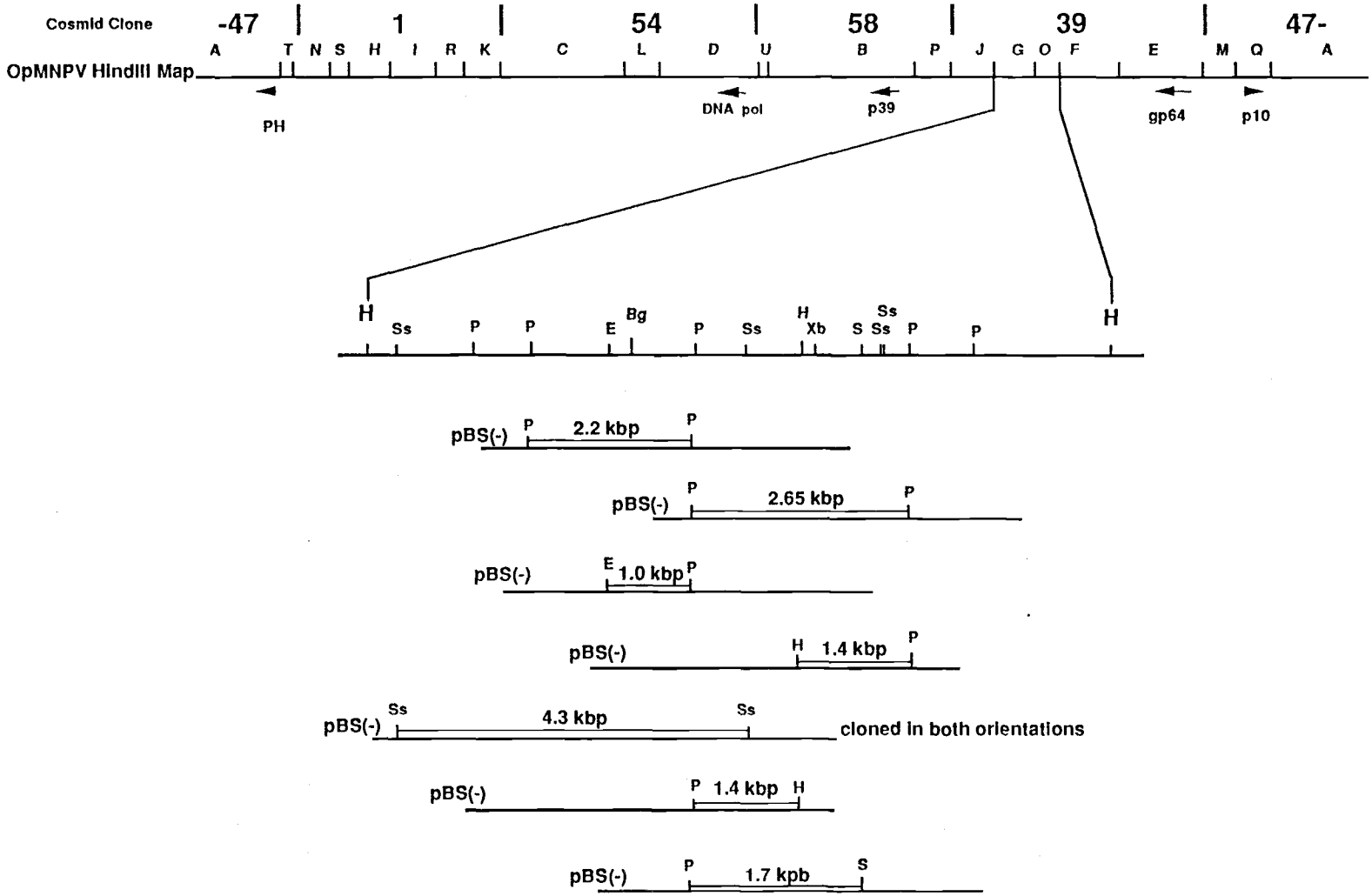

Cosmid Clone
-47
1
54
58
39
47-
A T N S H I R K C L D U B P J G O F E M Q A
OpMNPV HindIII Map
PH
DNA pol
p39
gp64
p10
H
Ss P P E Bg P Ss H Xb S Ss Ss P P
H
pBS(-) P 2.2 kbp P
pBS(-) P 2.65 kbp P
pBS(-) E 1.0 kbp P
pBS(-) H 1.4 kbp P
pBS(-) Ss 4.3 kbp Ss cloned in both orientations
pBS(-) P 1.4 kbp H
pBS(-) P 1.7 kpb S

Figure 5. Genomic location of p87 gene, location of insert DNA from immunoreactive λ clones and sequencing strategy of the region.

The upper line represents the HindIII-G and HindIII-O region of cosmid 39. The sequenced area is expanded. The location of the λgtll and λgt18 and the open reading frame of p87 and other ORF's found in this region are indicated below the expanded area. The sequencing strategy of the 3.6 kbp EcoRI/PstI region is shown by arrows.

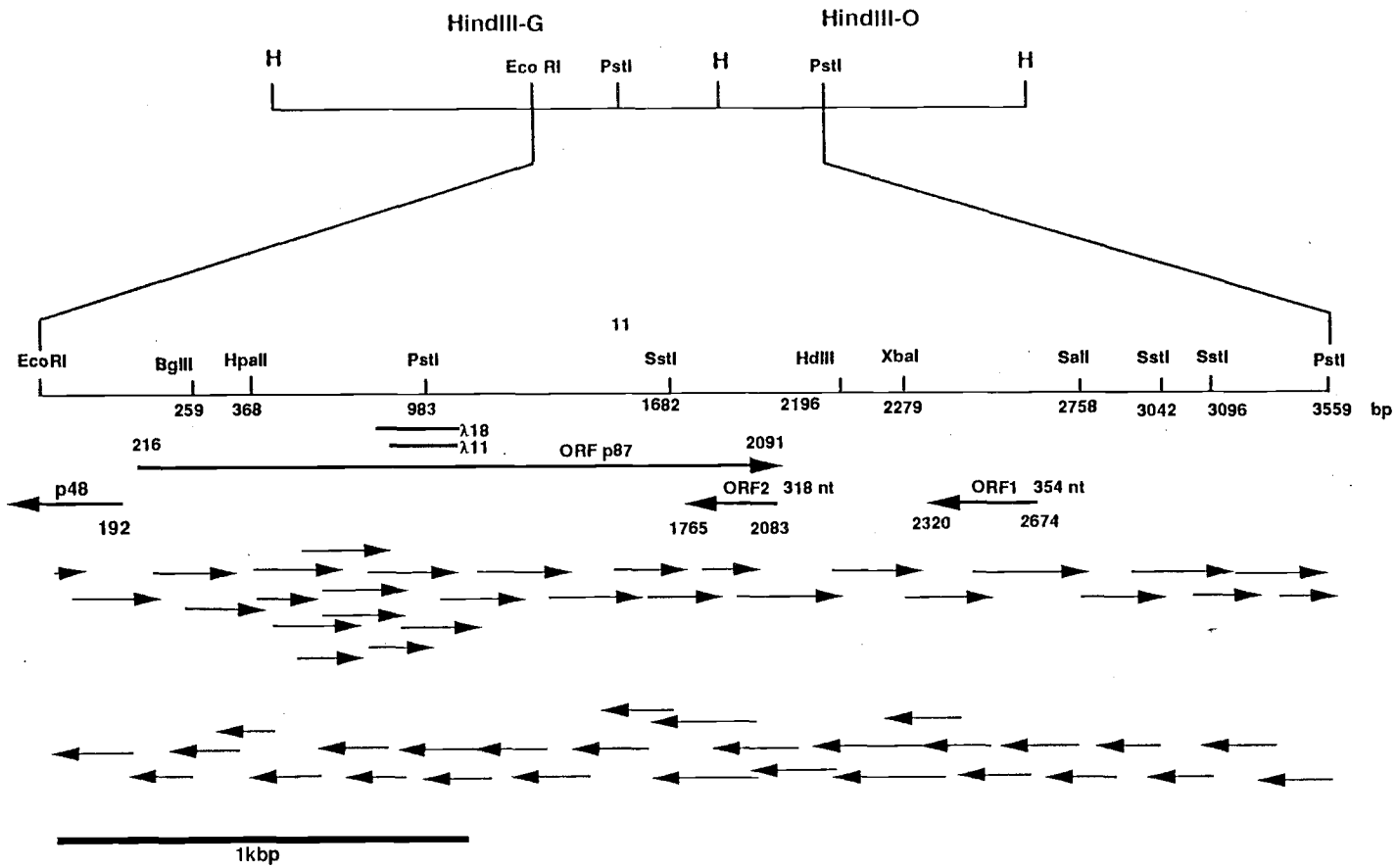
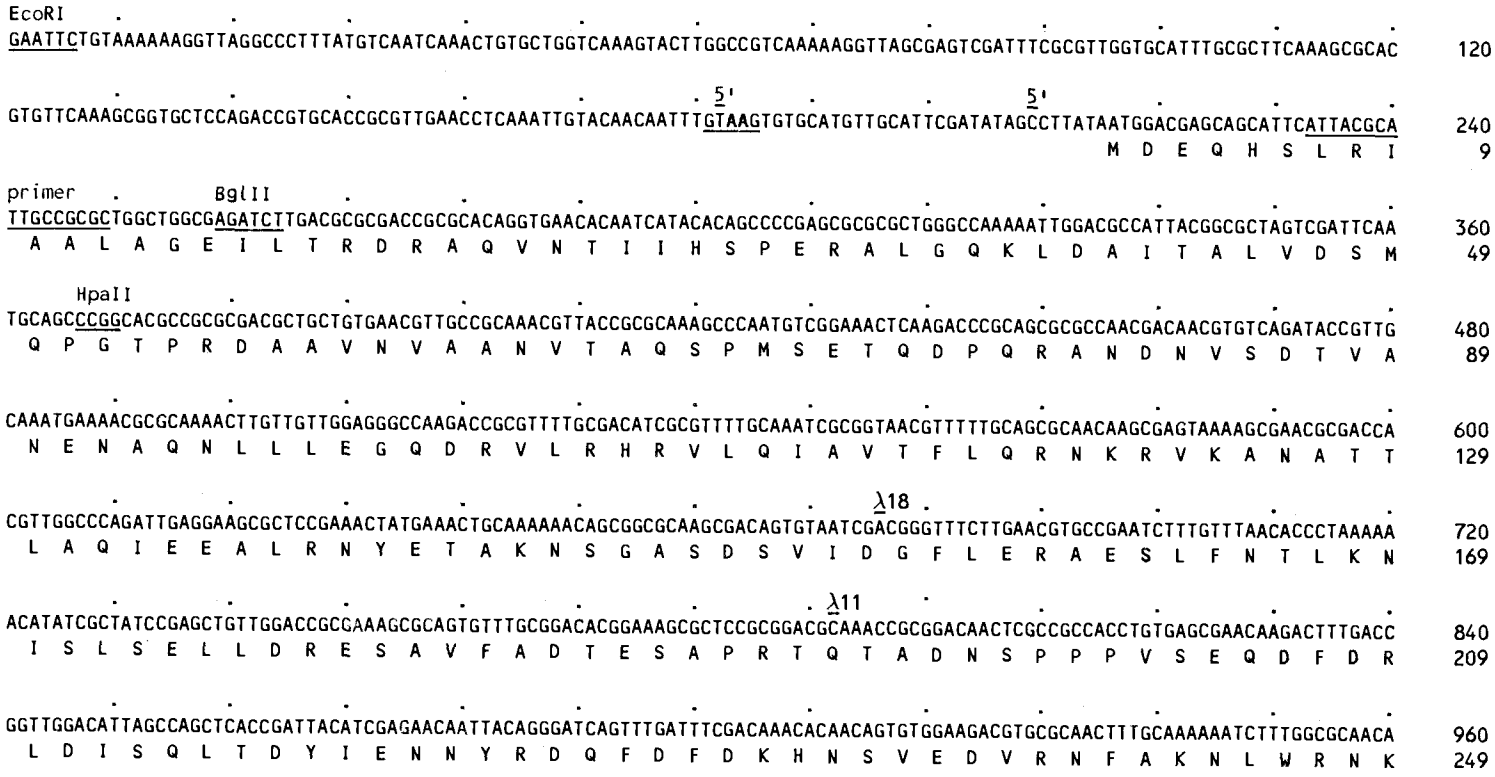

HindIII-G
HindIII-O
H
Eco RI
PstI
H
PstI
H
11
EcoRI
BglII
HpaII
PstI
SstI
HdIII
XbaI
SalI
SstI
SstI
PstI
259
368
983
1682
2196
2279
2758
3042
3096
3559
bp
λ18
λ11
216
ORF p87
2091
p48
192
ORF2 318 nt
1765
2083
ORF1 354 nt
2320
2674
1kbp

Figure 6. Nucleotide sequence of the p87 gene region.

The promoter mRNA start site consensus sequence (GTAAG, nt 181), the messenger RNA termination site (nt 2282), the polyadenylation site (AATAAA nt 2194) and selected restriction sites are underlined. The mRNA start sites (nt 181 and 209) are indicated by 5′ symbol. The translational start site (ATG nt 216) is indicated and the predicted amino acid sequence is shown below the nucleotide sequence. Major restriction sites and the primer (nt 233-249) used for 5′ mapping are underlined. The positions of the immunoreactive λgt11 clones λ11 (from nt 676 to nt 1171) and λ18 (from nt 792 to nt 1188) are indicated by λ's above the nucleotide sequence. A series of repeated amino acid sequences found in the open reading frame are underlined. The numbers below the repeat corresponds to the type of repeat.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5'
5'
GTGTTCAAAGCGGTGCTCCAGACCGTGCACCGCGTTGAACCTCAAATTGTACAACAATTTGTAAGTGTGCATGTTGCATTCGATATAGCCTTATAATGGACGAGCAGCATTCATTACGCA 240
M D E Q H S L R I 9
primer
BglII
TTGCCGCGCTGGCTGGCGAGATCTTGACGCGCGACCGCGCACAGGTGAACACAATCATACACAGCCCCGAGCGCGCGCTGGGCCAAAAATTGGACGCCATTACGGCGCTAGTCGATTCAA 360
A A L A G E I L T R D R A Q V N T I I H S P E R A L G Q K L D A I T A L V D S M 49
HpaII
TGCAGCCCGGGCACGCCGCGCGACGCTGCTGTGAACGTTGCCGCAAACGTTACCGCGCAAAGCCCAATGTCGGAAACTCAAGACCCGCAGCGCGCCAACGACAACGTGTCAGATACCGTTG 480
Q P G T P R D A A V N V A A N V T A Q S P M S E T Q D P Q R A N D N V S D T V A 89
CAAATGAAAACGCGCAAAACTTGTTGTTGGAGGGCCAAGACCGCGTTTTGCGACATCGCGTTTTGCAAATCGCGGTAACGTTTTTGCAGCGCAACAAGCGAGTAAAAGCGAACGCGACCA 600
N E N A Q N L L L E G Q D R V L R H R V L Q I A V T F L Q R N K R V K A N A T T 129
λ18
CGTTGGCCCAGATTGAGGAAGCGCTCCGAAACTATGAAACTGCAAAAAACAGCGGCGCAAGCGACAGTGTAATCGACGGGTTTCTTGAACGTGCCGAATCTTTGTTTAACACCCTAAAAA 720
L A Q I E E A L R N Y E T A K N S G A S D S V I D G F L E R A E S L F N T L K N 169
λ11
ACATATCGCTATCCGAGCTGTTGGACCGCGAAAGCGCAGTGTTTGCGGACACGGAAAGCGCTCCGCGGACGCAAACCGCGGACAACTCGCCGCCACCTGTGAGCGAACAAGACTTTGACC 840
I S L S E L L D R E S A V F A D T E S A P R T Q T A D N S P P P V S E Q D F D R 209
GGTTGGACATTAGCCAGCTCACCGATTACATCGAGAACAATTACAGGGATCAGTTTGATTTCGACAAACACAACAGTGTGGAAGACGTGCGCAACTTTGCAAAAAATCTTTGGCGCAACA 960
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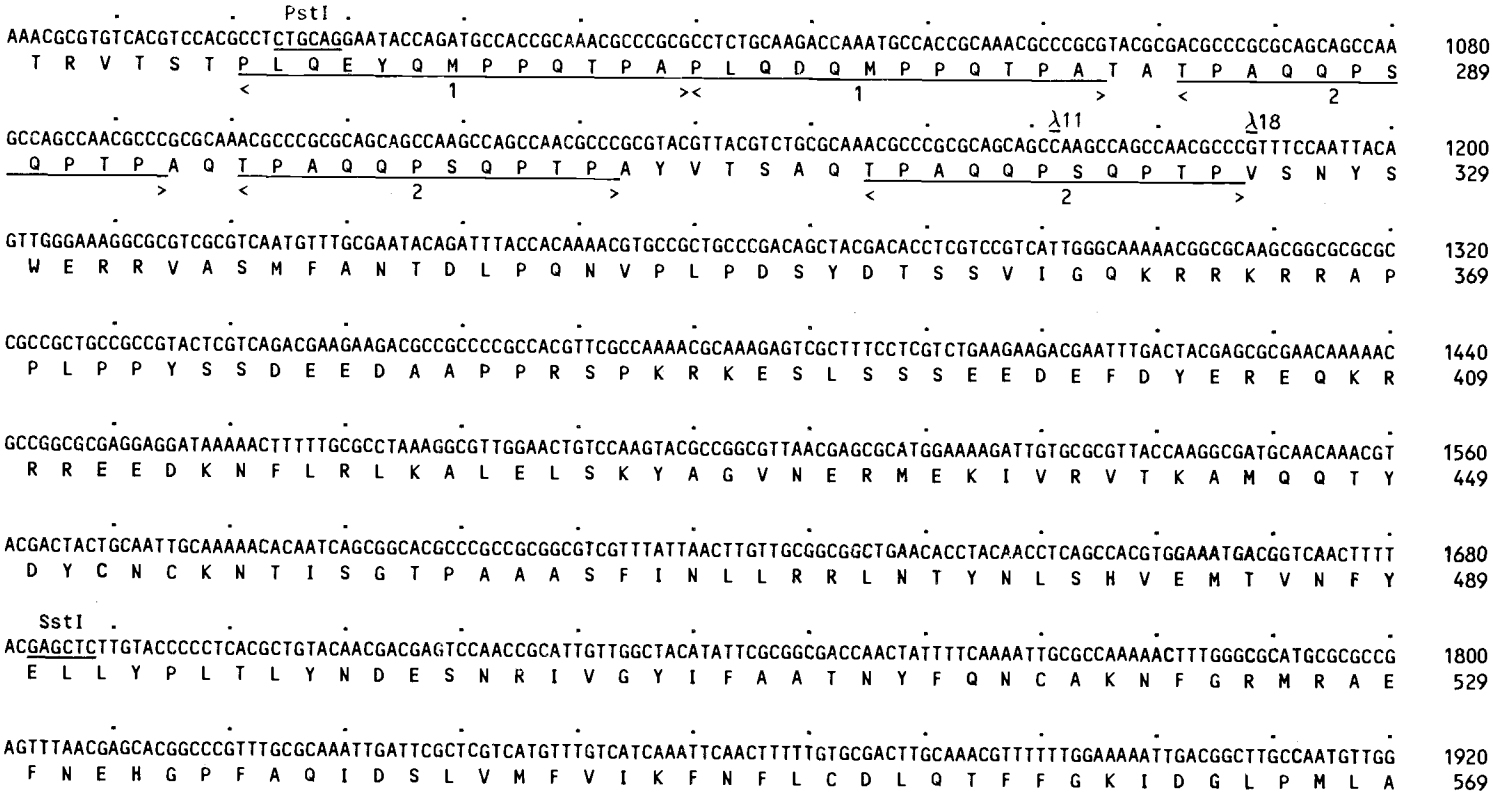
PstI
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T R V T S T P L Q E Y Q M P P Q T P A P L Q D Q M P P Q T P A T A T P A Q Q P S 289
< 1 >< 1 > < 2
λ11
λ18
GCCAGCCAACGCCCGCGCAAACGCCCGCGCAGCAGCCAAGCCAGCCAACGCCCGCGTACGTTACGTCTGCGCAAACGCCCGCGCAGCAGCCAAGCCAGCCAACGCCCGTTTCCAATTACA 1200
Q P T P A Q T P A Q Q P S Q P T P A Y V T S A Q T P A Q Q P S Q P T P V S N Y S 329
> < 2 > < 2 >
GTTGGGAAAGGCGCGTCGCGTCAATGTTTGCGAATACAGATTTACCACAAAACGTGCCGCTGCCCGACAGCTACGACACCTCGTCCGTCATTGGGCAAAAACGGCGCAAGCGGCGCGCGC 1320
W E R R V A S M F A N T D L P Q N V P L P D S Y D T S S V I G Q K R R K R R A P 369
CGCCGCTGCCGCCGTACTCGTCAGACGAAGAAGACGCCGCCCCGCCACGTTCGCCAAAACGCAAAGAGTCGCTTTCCTCGTCTGAAGAAGACGAATTTGACTACGAGCGCGAACAAAAAC 1440
P L P P Y S S D E E D A A P P R S P K R K E S L S S S E E D E F D Y E R E Q K R 409
GCCGGCGCGAGGAGGATAAAAACTTTTTGCGCCTAAAGGCGTTGGAACTGTCCAAGTACGCCGGCGTTAACGAGCGCATGGAAAAGATTGTGCGCGTTACCAAGGCGATGCAACAAACGT 1560
R R E E D K N F L R L K A L E L S K Y A G V N E R M E K I V R V T K A M Q Q T Y 449
ACGACTACTGCAATTGCAAAAACACAATCAGCGGCACGCCCGCCGCGGCGTCGTTTATTAACTTGTTGCGGCGGCTGAACACCTACAACCTCAGCCACGTGGAAATGACGGTCAACTTTT 1680
D Y C N C K N T I S G T P A A A S F I N L L R R L N T Y N L S H V E M T V N F Y 489
SstI
ACGAGCTCTTGTACCCCCTCACGCTGTACAACGACGAGTCCAACCGCATTGTTGGCTACATATTCGCGGCGACCAACTATTTTCAAAATTGCGCCAAAAACTTTGGGCGCATGCGCGCCG 1800
E L L Y P L T L Y N D E S N R I V G Y I F A A T N Y F Q N C A K N F G R M R A E 529
AGTTTAACGAGCACGGCCCGTTTGCGCAAATTGATTCGCTCGTCATGTTTGTCATCAAATTCAACTTTTTGTGCGACTTGCAAACGTTTTTTGGAAAAATTGACGGCTTGCCAATGTTGG 1920
F N E H G P F A Q I D S L V M F V I K F N F L C D L Q T F F G K I D G L P M L A 569


CCCAGCCCAACATCAAGACGCACACCGTGCTGGTGATGCGCGACAAGATCGTCAAGCTGGCCTTTGGCGCGCTGCAATACGACACCAGTCTGAAAACGGACAATCGGCGGGACCCCAAAC 2040
Q P N I K T H T V L V M R D K I V K L A F G A L Q Y D T S L K T D N R R D P K H 609
HinfI
ACCTGCAACGACTCATCCAGTTGATGAACGCCGATTTTAACATAATGTAAACAAATGTGTTAGCAATTTATTTATTACAAACAGAGTTACGATATTCGGTGTACATGGTTTTATAATCTT 2160
L Q R L I Q L M N A D F N I M > 624
polyA Hind3
XbaI
TGTGATCAAAGTTAAAACGTTTTCTATACAAATAATAAAGCTTGTTAAACAGCTGTTTTCGTTTTTTTTTGTGTCGCAAACAAATTTTTTGTGGCAATGGCGTAGCACATCTAGAAAATT 2280
3'
AAATTTTTCGCCGACCGCACACTCTTCATTAAGCTCGTTACGGCTGGCCGCCTATGCGATAACTGCACGTGACACACAGGGTGTAGTGGCCGCATGGCGCCGCCACCATGCGCTTCGGCT 2400
CGCTCATGCAAACAATGCACTCGTCGTTGGCTTCGTCGGTAAACACGCGCTCAACAGTAATTTTACAAACGGTAACGGCGTCTGGTTTTATGTAATAATAGCATTTGGAGCGGCAAATTA 2520
AGGAACTGTCTTTGGGCGCCACGGCAAAAGTGGCGCCCTCGCGCCGCAACCGCTTGGGAAAGGTGCAGTTGGCGTCGCGCCGATAGGCGGGATCGTCTATACGCACGCGCAGCCTGCCGT 2640
TCACCTTTACACAAATCAACTTAACGTGCGCCATTGCACGGTCGAATGTAGGACCAGTGTCAATTATTTAAGACACCGTTAGCATAACCGCTGGCCCAATGGACGCCAAATACAAGGTGG 2760
SalI
TCGACGTGGACACCTTTGCCCGCCAGCTAATCACGGACAAGTGCAGCGAGCTGATTGAAACCGAAAATCTATTGCCTGCAAACATTTTGCATGTTGTTAAACAAGCAAGAGACAAATACT 2880
TTGAAGACCCCTCGGTCAAAAACTACGAATATGTCAAAAACCTGTTTTTGCGCACAAAATACATGGACGACTCAATTGACTACAAAAATTTTAATCGGCGCGTGTTGCTGATTGTGTTCA 3000
SstI
SstI
AATTTGCGCTCAACCGAGGCTCCGGCTACTTTCCCTCGTACAGGGAGCTCATTGAAGTGGCCGTCAAGCGCCTCAACAAAATCAACCCCGACCTCAAGAGCTCGCCGCGCGCCATGTTGC 3120
AGCACTACAACGAGTGCCTGGAAAACCTGGACAACCCGGTGACCGACGAGCACCACCTGTTAACGTTTGGAAAAGAAGTGGCCACCAAAATGTTTATCGAGGCGTTCGAGTTTAGCTACG 3240
TaqI
CCAGCAACAACGAAATCAACTTAACTACCAACAAGCGCGGCTCGGACCTGTTCGACCCGATACCGATGCCCGCGCCCGCGCCCGCGCCTTCGGCGTCTTTGTTGGACAACGTCATGAACG 3360
AGCGCAAGCGCAAGTTGCAGGCGTCGGTCACGACAACGCCGCCCAAACGATGCAAATTGGCCGACCGGCCGGCGCAGACCACACAAGACACGCCGCGCGCGCCGCAACCGGCGCCTGTGC 3480
PstI
GCGCGCAGCGGCCCCTGTTTACGCTGTAGACGCGCGCGCAGAGGCGTCGGTGGGCGGCGCGTAATTTCGCTGCAG 3555

Figure 7. Hydropathy profile of the OpMNPV p87 protein.

The hydropathy profile was computed with a window size of 21 amino acids using the method of Hopp and Woods (1981). The regions above the line indicate hydrophilic groups; regions below the line indicate hydrophobic areas. The area of the repeated region is located in the hydrophobic area at position 256 to 325, the immunoreactive λgt11 clones (λ11 and λ18) and the portion of the p87 gene used in the trpE fusion are indicated by bars.

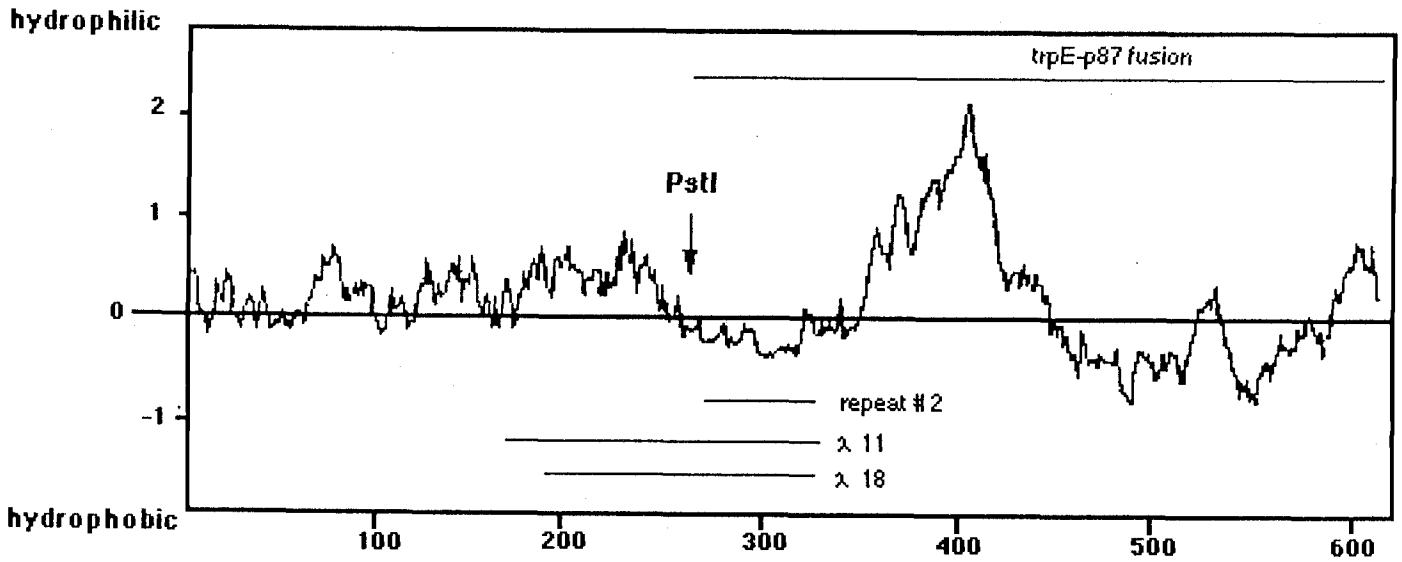
hydrophilic
hydrophobic
2
1
0
-1
trpE-p87 fusion
PstI
repeat #2
λ 11
λ 18
100
200
300
400
500
600

Figure 8. Preparation of a cRNA probe for Northern blot analysis.

The location and direction of p87 is symbolized by an arrow (ORF p87). The T3 promotor region of pBS(-) is presented by a box. The Pst 2.2 kb clone has been digested with BglII and was gel purified. The cDNA to parts of the ORF p87, generated by in vivo transcription from the T3 promotor is shown on the bottom part.

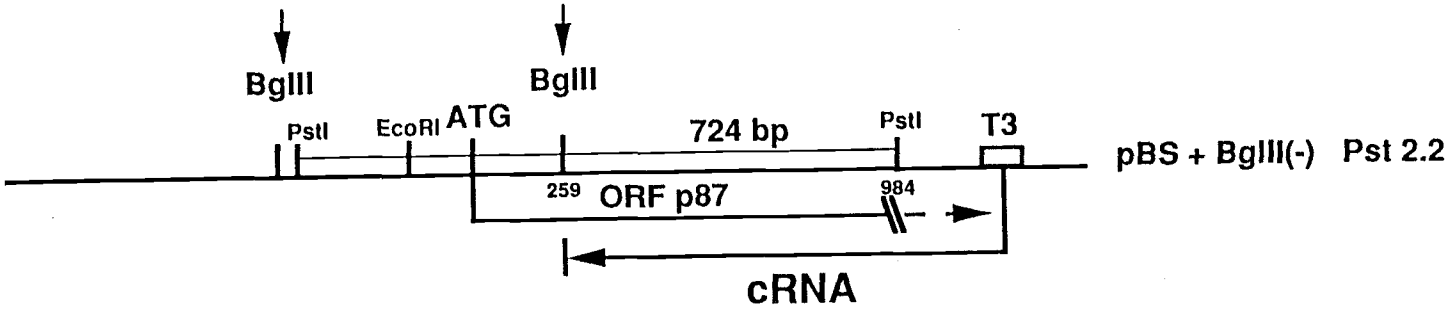
BglII
BglII
PstI
EcoRI
ATG
724 bp
PstI
T3
pBS + BglII(-) Pst 2.2
259
ORF p87
984
cRNA

Figure 9. Northern blot using a p87 gene-specific probe.

The probe was hybridized to total RNA (20 μg/lane) isolated from OpMNPV infected L. dispar cells at various time points post infection. The numbers at the top indicate hours post infection. The RNA was isolated and the numbers on the left show the sizes of labeled marker (nucleotides). The p87-specific mRNA is indicated by an arrow and the number on the right indicates the approximate size of the transcript (nucleotides).

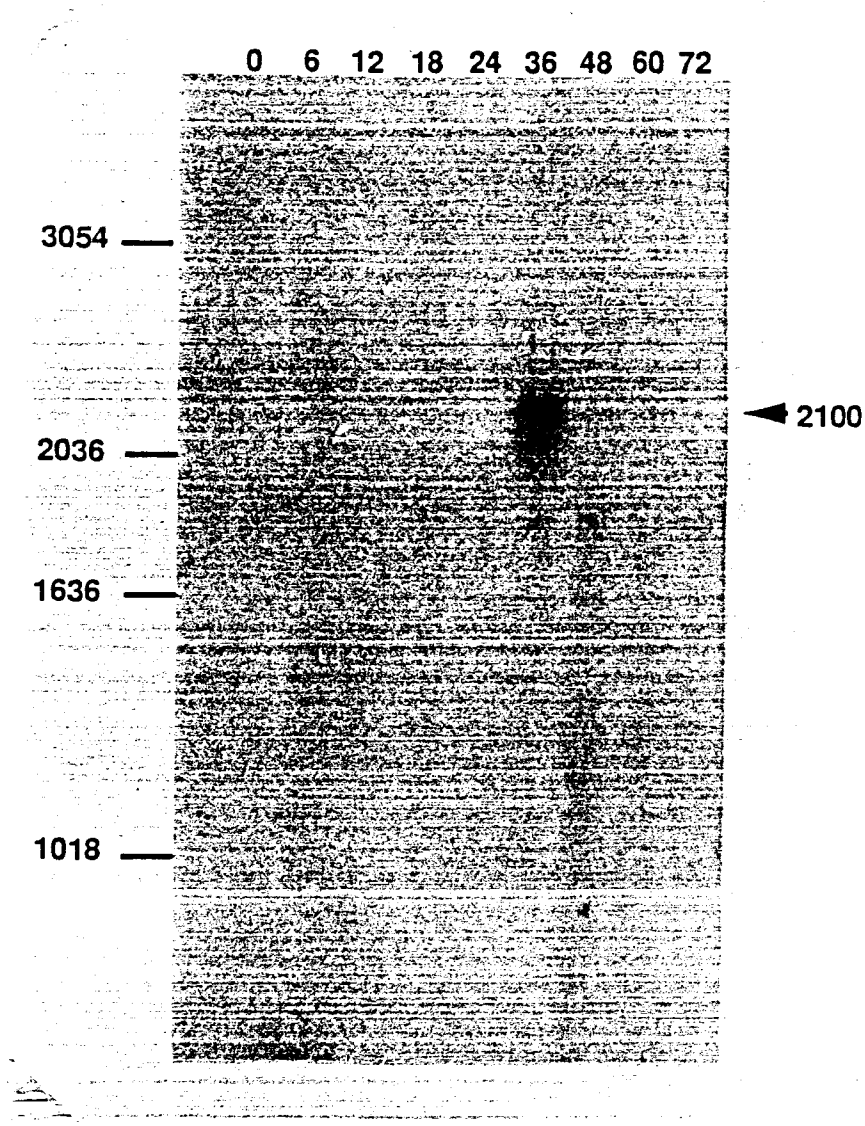

Figure 9.

Figure 10. Strategy and results of the 3′ S1 nuclease mapping of p87.

The clone used in this experiment was constructed by digestion of the pBS(-) 2.65 kbp clone with a deleted PstI site on the 5′end (PstI), with PstI and SalI (SalI/PstI) followed by digestion of the 5′ overhangs with S1 nuclease and blunt end religation. The 945 nt HinfI fragment containing 37 nt of the p87 ORF, 672 nt of 3′ flanking DNA and 235 nt of pBS(-) vector DNA was isolated from an agarose gel and endlabeled. The black bar represents the undigested probe. The star (*) indicates the radioactive labeled end. The 945 nt labeled probe was hybridized to 20 μg total RNA isolated from L. dispar cells 36 hr p.i. with OpMNPV. The RNA-DNA hybrid was treated with S1-nuclease and electrophoresed on a polyacrylamide sequencing gel. The mottled lines represent the protected fragments.

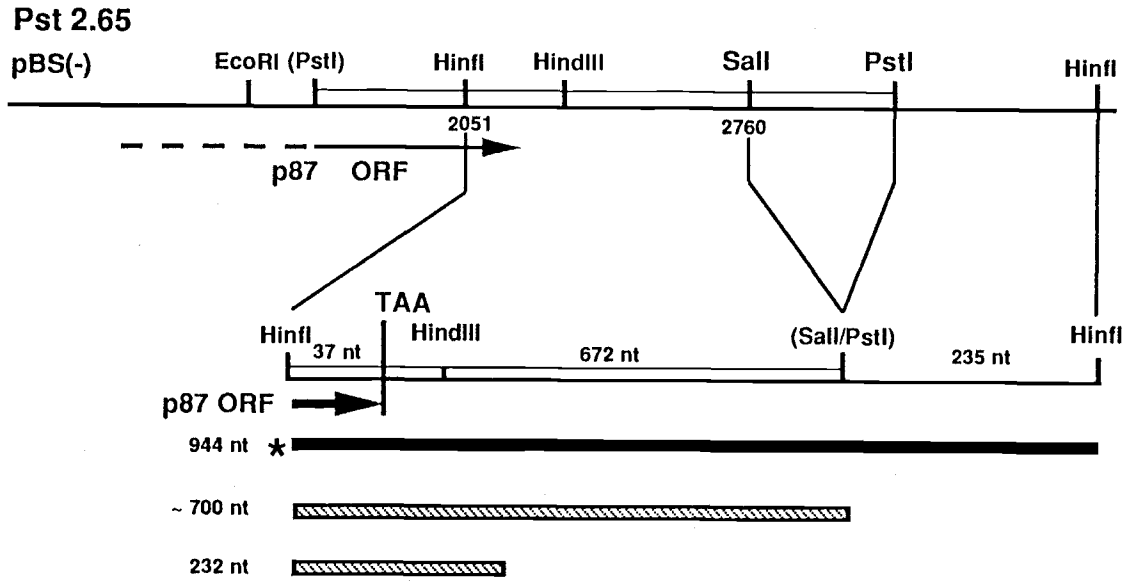
Pst 2.65
pBS(-)
EcoRI (PstI)
HinfI
HindIII
SalI
PstI
HinfI
2051
2760
p87 ORF
TAA
HinfI
37 nt
HindIII
672 nt
(SalI/PstI)
235 nt
HinfI
p87 ORF
944 nt
~ 700 nt
232 nt

Figure 11. Results of the 3′ mapping by S1 protection assay.

Total RNA (20 μg) isolated from OpMNPV infected cells at 36 hours post infection (36), RNA (20μg) from mock infected cells (M) hybridized to the radioactive probe and S1 nuclease treated and undigested probe (P) (944 nt) electrophoresed on a 8% polyacrylamide-urea gel. Lanes labeled with pBS(-) represent a DNA sequencing ladder of pBS(-) (modified with an additional BglII restriction site) used to size the protected fragments. Numbers with arrows on the left margins indicate the size of the protected fragments and the size of the undigested probe (size in nucleotides, nt)

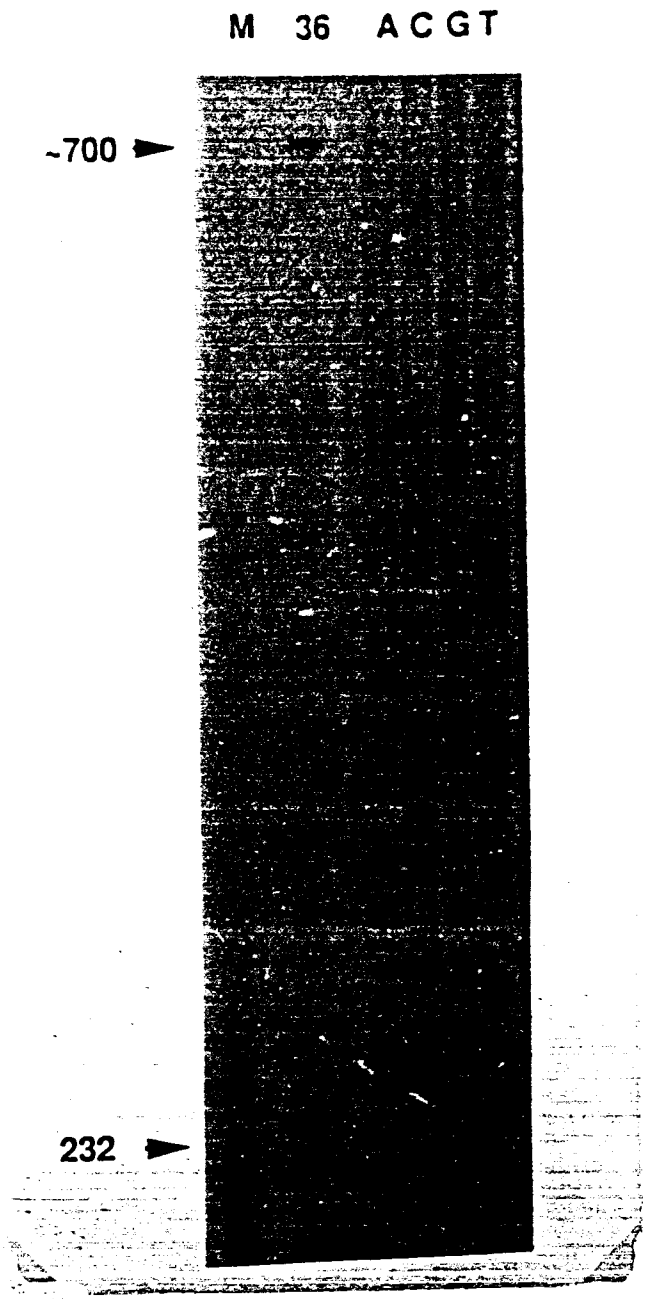

Figure 11.

Figure 12. Transcriptional organization in the HindIII-G and HindIII-O region.

The OpMNPV HindIII map with the cosmid clones (1, 54, 58, 39, 47) and the location of some OpMNPV genes are displayed on the top part. HindIII-J, -G and -O are expanded and open reading frames (ORF's) found are shown below the map. The names of the ORF's are written above and below the arrows indicating the orientation and approximate size of the ORF's. Transcripts present in this area are shown on the bottom part. The late promotor sequence A/GTAAG is indicated upstream of each ORF. λ11 (11) and λ18 (18) inserts and their locations on the genomic map are shown by plain lines and their size is written behind their names in nucleotides (nt).

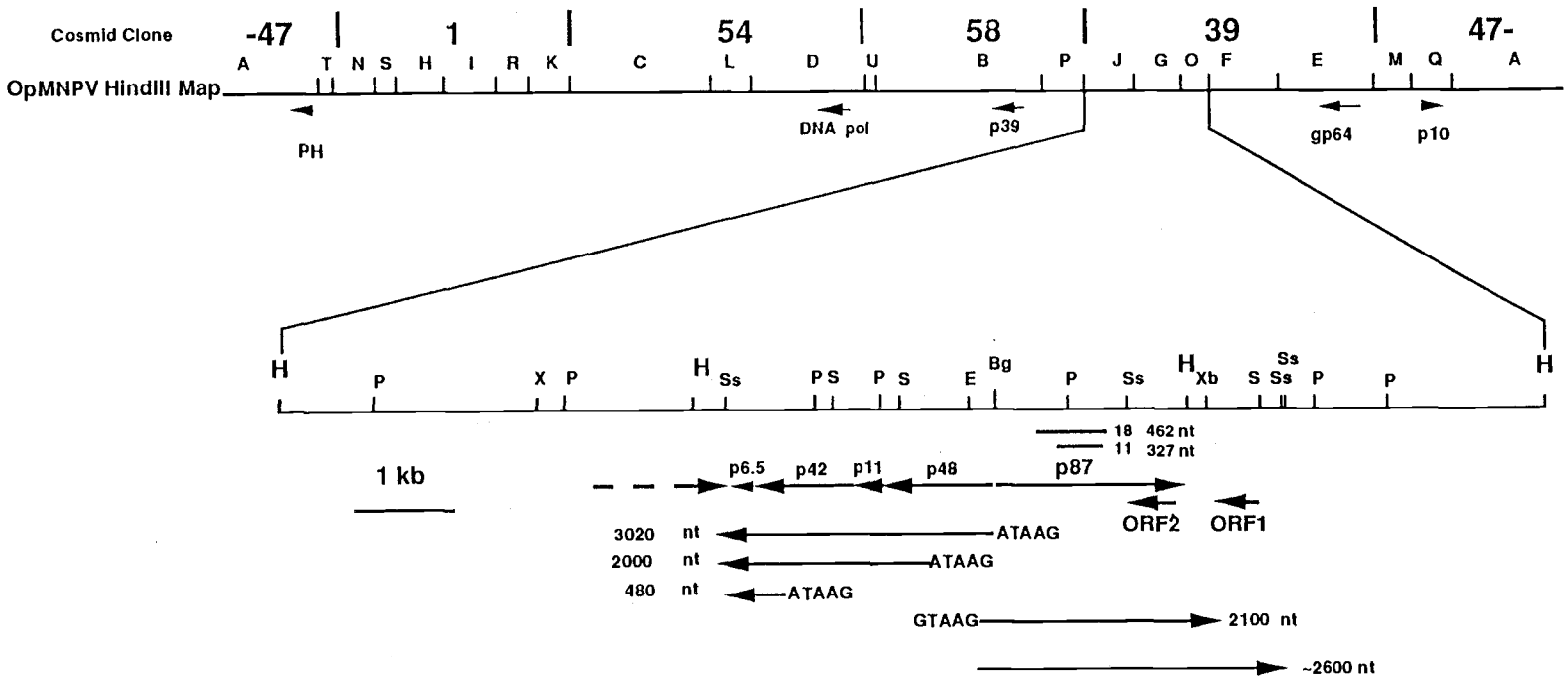
Cosmid Clone
-47
1
54
58
39
47-
A T N S H I R K C L D U B P J G O F E M Q A
OpMNPV HindIII Map
PH
DNA pol
p39
gp64
p10
H P X P H Ss P S P S E Bg P Ss H Xb S Ss Ss P P H
18 462 nt
11 327 nt
1 kb
p6.5 p42 p11 p48 p87
ORF2
ORF1
3020 nt ATAAG
2000 nt ATAAG
480 nt ATAAG
GTAAG 2100 nt
~2600 nt

Figure 13. Construction of a pATH3-p87 gene fusion.

a) Restriction map of Hind-G and Hind-O region the ORF of p87 is indicated by the arrow below the map. The 1.2 kbp PstI/HindIII fragment containing parts of the p87 gene coding for the amino acids 257-625 was excised from subcloned genomic DNA and cloned into a pUC8 plasmid to obtain an in-frame EcoRI site. b) The insert DNA and an additional part of the multiple cloning site of pUC8 were excised with EcoRI and HindIII and cloned into the pATH3 expression vector. This resulted in a gene fusion encoding a polypeptide of 79 kDa. c) The nucleotide sequence of the trpE-p87 junction is shows.

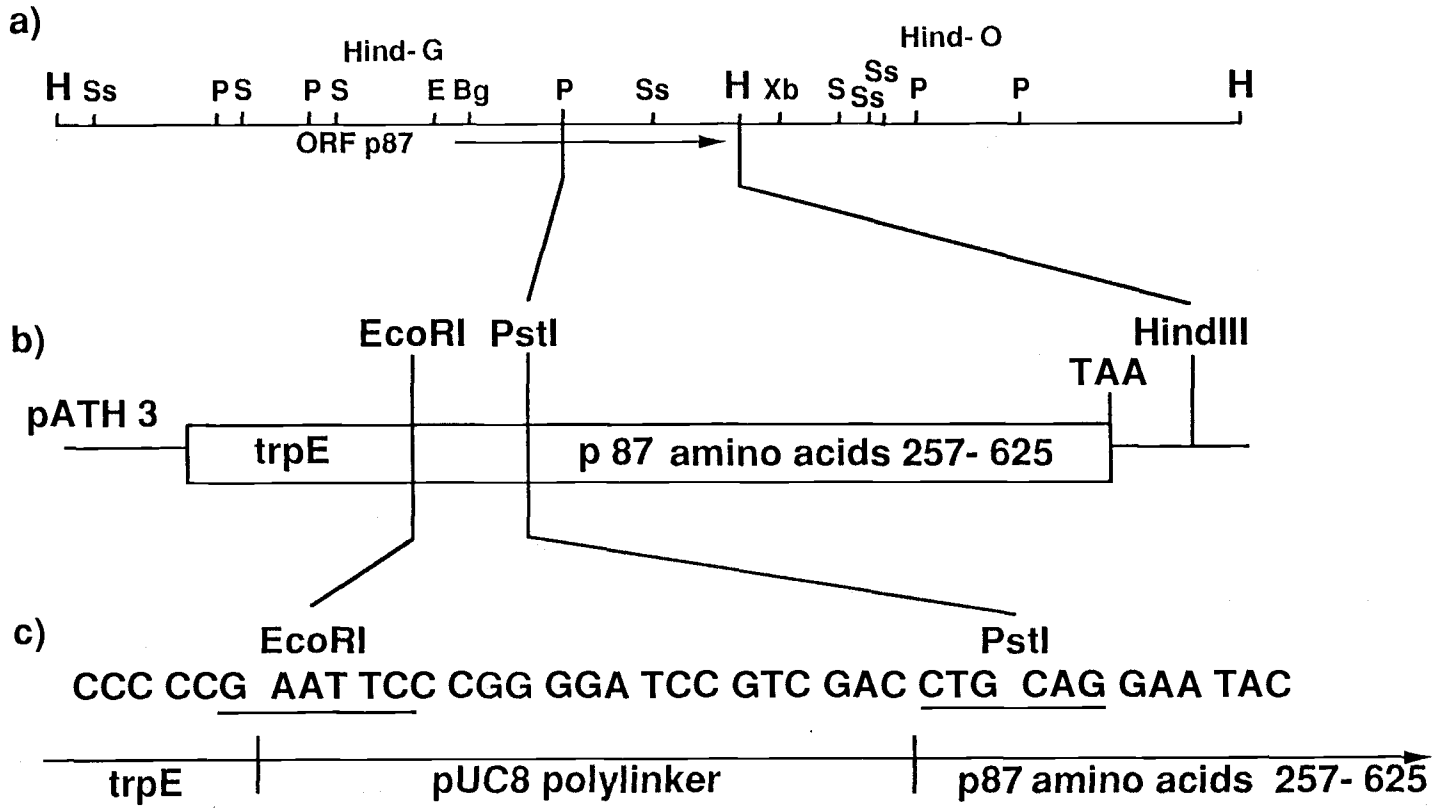
a)
Hind- G
Hind- O
H Ss P S P S E Bg P Ss H Xb S Ss Ss P P H
ORF p87
b)
EcoRI PstI
HindIII
TAA
pATH 3
trpE
p 87 amino acids 257- 625
c)
EcoRI
PstI
CCC CCG AAT TCC CGG GGA TCC GTC GAC CTG CAG GAA TAC
trpE
pUC8 polylinker
p87 amino acids 257- 625

Figure 14. Expression of pATH3-p87 fusion protein.

HB101 Escherichia coli cells were transformed with either the pATH3-p87 plasmid or the wild-type pATH3 plasmid and the proteins were expressed. The HB101 cells containing the expressed proteins were lysed, the lysate was electrophoresed on a SDS-polyacrylamide gel and the proteins were stained with Coomassie brilliant blue. The lanes were loaded (left to right) with BRL prestained protein markers (2.5 μg/lane), nontransformed HB101 cells (2μg/lane), HB101 cells containing the pATH3 wild-type plasmid (2μg/lane) and HB101 cells containing the trpE-p87 fusion plasmid (2μg/lane). The numbers in the left margin represent the molecular weights (kDa) of the protein standards. The molecular weights of the expressed proteins are indicated in the right margins.

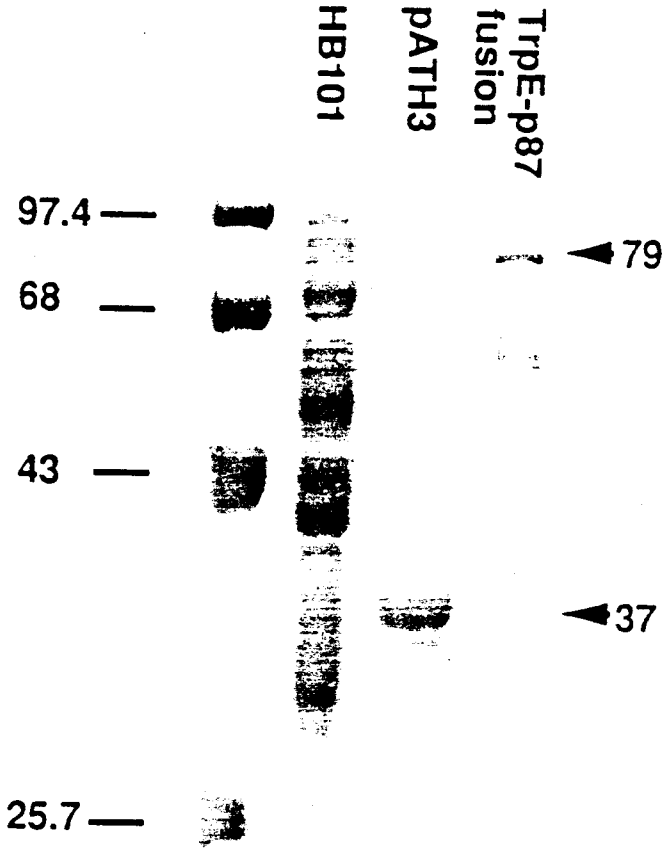

Figure 14.

Figure 15. Western blot of p87 expression in OpMNPV-infected L. dispar cells.

The numbers on the top of each well indicate hours post infection. 6 μl of cell lysate diluted 1:80 (equivalent of 300 cells) was run per lane. The polyclonal antiserum was used at a 1:500 dilution. The protein sizes of the standard are indicated on the left.

Black and white photocopy. Best scan available.

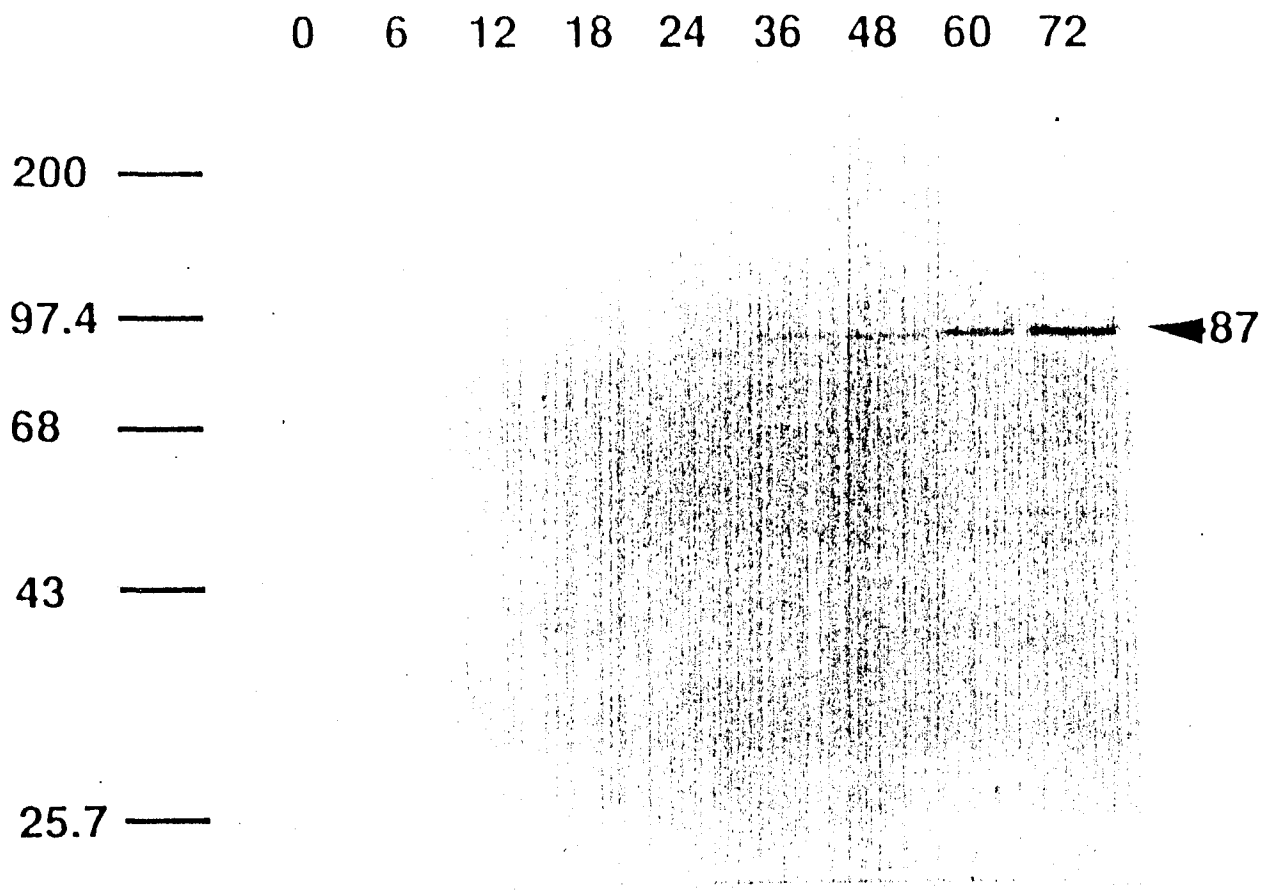

Figure 15.

Figure 16. Western blot of cell lysate from OpMNPV infected L. dispar cells.

The numbers on the top of each well indicate hours post infection. 6 μl of the cell lysate 1:10 (equivalent of 2400 cells) diluted were loaded per lane and p87 antiserum was used at 1:500.

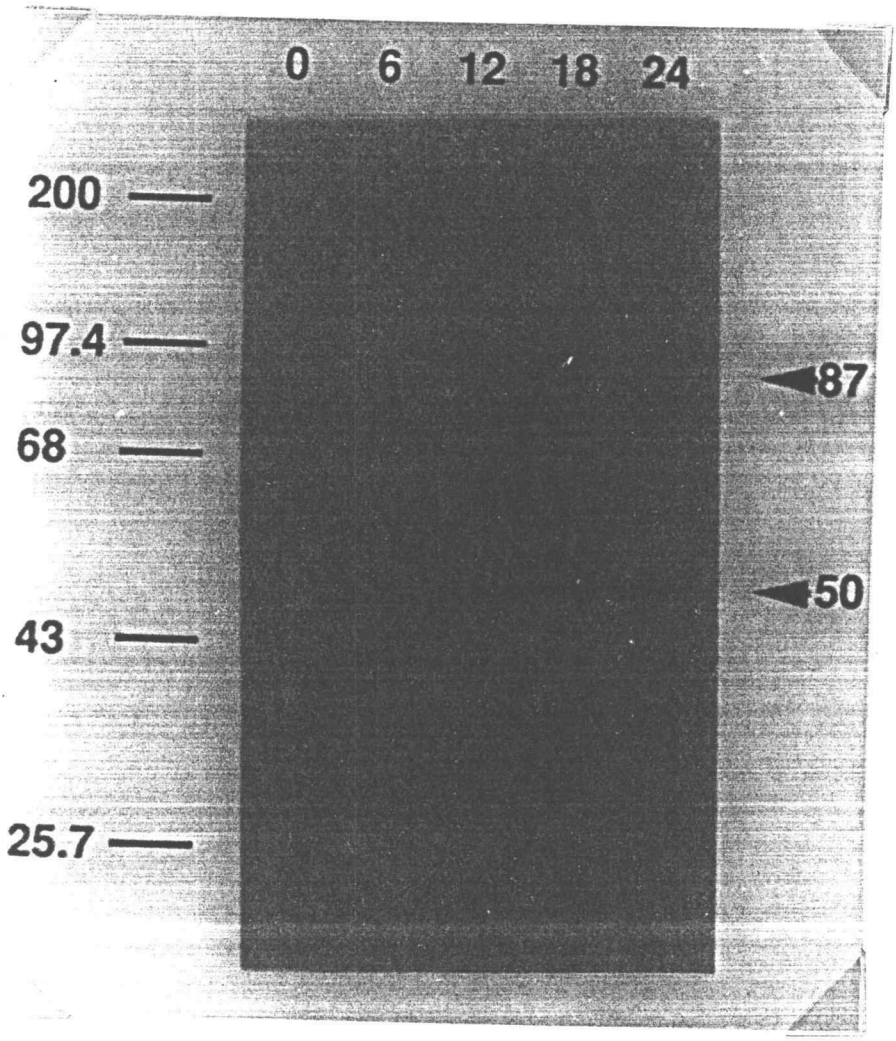

Figure 16.

Figure 17. Western blot of purified PDV, BV and capsid.

The wells were loaded with purified PDV (PDV) (approximately 2 μg), purified BV (BV) (approximately 2 μg) and purified capsid (Cp) (approximately 2 μg) and electrophoresed on a 9% SDS protein gel. The numbers indicate the molecular weight of the markers in kDa, the numbers with arrows indicate the molecular weight of the reactive proteins. The western blot was reacted with αp87 (1:3000 diluted), αp39 (tissue culture fluid undiluted) and αgp64 (tissue culture fluid undiluted).

Black and white photocopy. Best scan available.

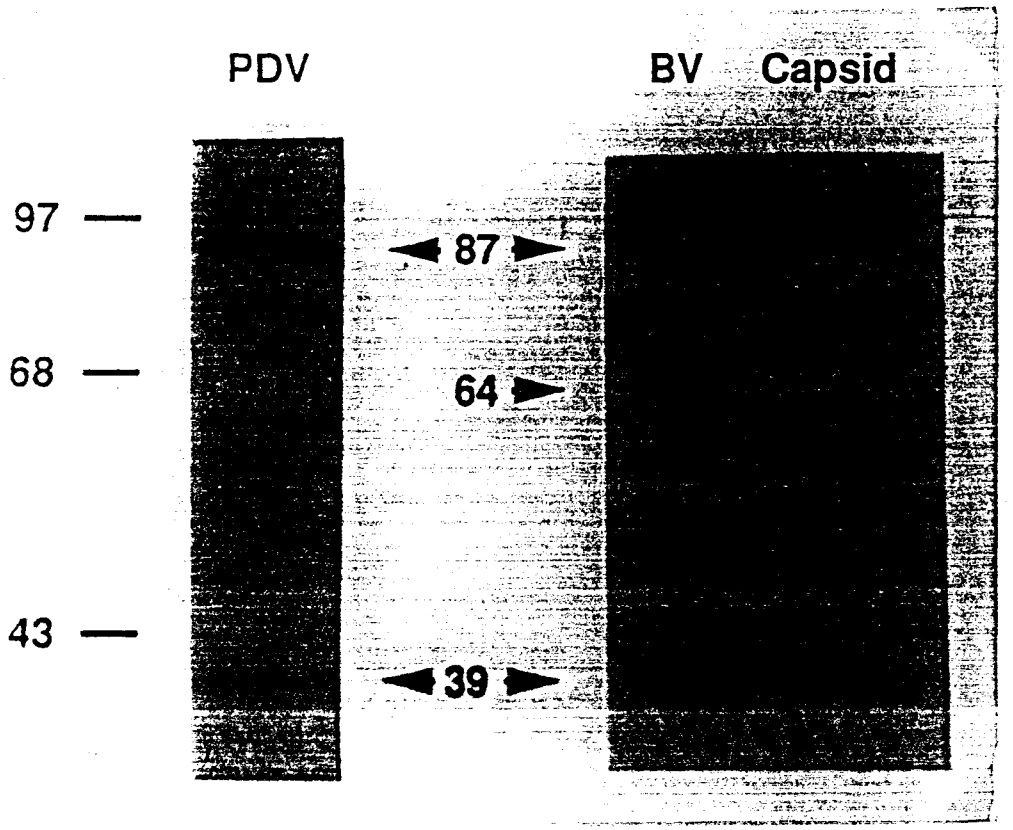

Figure 17.

BIBLIOGRAPHY

Blissard, G.W. and Rohrmann, G.F. (1989). Location, sequence, transcriptional mapping, and temporal expression of the gp64 gene of the Orgyia pseudotsugata multicapsid nuclear polyhedrosis virus. Virology 170, 537-555.

Blissard, G., Russell, R., Rohrmann, G.F.and Beaudreau, G.S. (1989). Nucleotide sequence and expression of the gene encoding p39, a major structural protein of the multicapsid nuclear polyhedrosis virus of Orgyia pseudotsugata. Virology 168,354-362.

Burgess, S. (1977). Molecular weights of lepidopteran baculovirus DNAs: derivation by electron microscopy. J. gen. Virol. 37, 501-510.

Carson, D.D., Guarino, L.A. and Summers, M.D. (1988). Functional mapping of an AcNPV immediate early gene which augments expression of the IE-1 trans-activated 39 k gene. Virology 162, 444-451.

Couch, J.A. (1974). An enzootic nuclear polyhedrosis virus of pink shrimp: ultrastructure, prevalence, and enhancement. J.Invertebr. Pathol. 24, 311-331.

Creighton, T.E., (1984). Proteins, Structure and Molecular Properties. W.H. Freeman and Company, New York.

Chapman, A.J. and Ignoffo, C.M. (1972). Influence of rate and spray volume of a nucleopoyhedrosis virus on control of Heliothis in in cotton. J. Invertebr. Pathol. 20, 183.

Dieckmann, C.L. and Tzagoloff, A. (1985). Assembly of the mitochondrial membrane system. J. Biol. Chem. 260, 1513-1520.

Falcon, L.A. (1976). Problems associated with the use of arthropod viruses in pest control. Annu. Rev. Entomol. 21, 305.

Favaloro, J., Treisman, R. and Kamen, R. (1980). Transcriptional maps of polyoma virus-specific RNA: analysis by two dimensional nuclease S1 gel mapping. Methods in Enzymology 65, 718-749.

Feinberg, A.P. and Vogelstein, B. (1984). A technique for radiolabelling DNA restriction fragments to high specific activity. Analytical Biochemistry 132, 6-13.

Fraser, M.J., Smith G.E. and Summers M.D. (1983). Aquisition of host cell DNA sequences by Baculovirus; Relationship between host DNA insertions and FP mutants of Autographa californica and Galleria mellonella nuclear polyhedrosis virus, J. Virol. 47, 287-300.

Franz, J.M. and Huber, J. (1979). Feldversuche mit insectenpathogenen Viren in Europa. Entomophaga, 24, 333.

Friesen, P.D. and Miller, L.K. (1985). Temporal regualation of baculovirus RNA: overlapping early and late transcripts. J. Virol. 54, 392-400.

Friesen, P.D. and Miller, L.K. (1987). Divergent transcription of early 35- and 94-kilodalton protein genes encoded by the HindIII K genome fragment of the baculovirus Autographa californica nuclear polyhedrosis virus. J. Virol. 61, 2264-2272.

Fuchs, Y. L., Woods M.S., and Weaver R.F. (1983). Viral transcription during Autographa californica nuclear polyhedrosis virus infection: A novel RNA polymerase induced in infected Spodoptera frugiperda cells. J. Virol. 48, 641-646.

Gombart, A.F., Pearson, M.N., Rohrmann, G.F. and Beaudreau, G.S. (1989a). A baculovirus polyhedral envelope-associated protein: Genetic location, nucleotide sequence and immunocytochemical characterization. Virology 169, 182-193.

Gombart, A.F., Rohrmann, G.F., Blissard G.F. and Beaudreau, G.S. (1989b). Characterization of the genetic organization of the HindIII-M region of the MNPV of Orgyia pseudotsugata reveals major differences among baculovirus. J. gen. Virol. 70, 1815-1828.

Gracella P., Wang, C.L.A., Stafford, W.F. (1988). Caldesmon. Molecular weight and subunit composition by analytical ultracentrifugation. J. Biol. Chem. 263, 14196-14202.

Granados, R.R., and Lawler, K.A. (1981). In vivo pathway of Autographa californica baculovirus invasion and infection. Virology 108, 297-308.

Granados, R. R. and Williams, K.K. (1986). In vivo infection and replication of baculoviruses. pp. 89-108. In: Granados, R.R. and Federichi, B.A. eds. The biology of baculoviruses Volume I biological properties and molecular biology. CRC Press, Inc., Boca Raton, Florida USA.

Grula, M.A., Buller, P.L. and Weaver, R.F. (1981). Alpha amanitin-resistant viral RNA synthesis in nuclei from nuclea polyhedrosis virus-infected Heliotis zea larvae and Spodoptera fruguperda cells. J. Virol. 38, 916-921.

Guarino, L.A. and Summers, M.D. (1987). Nucleotides sequence and temporal expression of a baculovirus regulatory gene. J. Virol. 61, 2091-2099.

Guarino, L.A. and Summers, M.D. (1986). Functional mapping of a trans-activating gene required for expression of a baculovirus delayed-early gene. J. Virol. 57, 563-571.

Henikoff, S. (1984). Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene 28, 351-359.

Hopp T.P. and Woods K.R. (1981). Prediction of protein antigenic determinants from amino acid sequences. Proc. Natl. Acad. Sci. USA 78, 3824-3828.

Ignoffo, C.M. (1968). Specificity of insect viruses. Bull. Entomol. Soc. Am. 14, 265.

Jaques, R.P., Laing, J.E., MacLellan, C.R., Proverbs, M.D., Sanford, K.H., and Trottier, R. (1981). Apple orchard tests on the efficacy of the granulosis virus of the codling moth, Laspeyresia pomonella. Entomophaga, 26, 111.

Kawamoto, F. and Asayama, T. (1975). Studies on the arrangement patterns of nucleocapsid within the envelope of nuclear polyhedrosis virus in the fat body cells of the brown tail moth Euroctis similis. J. Invertebr. Pathol. 26, 47-55

Keddie, A. and Volkmann, L.E. (1985). Infectivity difference between the two phenotyoes of Autographa californica nuclear polyhedrosis virus: Importance if the 64k envelope glycoprotein. J. gen. Virol. 52, 313-319.

Kleid, D.G., Yanasura, D., Small, B., Dowbenko, D., Moore, D.M., Grubman, M.J., McKercher, P.D., Morgan, D.O., Robertson, B.H., and Bachrach, H.L. (1981). Cloned viral protein for foot-and-mouth disease: responses in cattle and swine. Science 241, 1125-1129.

Kozak, M. (1986). Point mutations define a sequence flanking the AUG initator codon that modulates translation by eukaryotic ribosomes. Cell 44, 283-292.

Kuzio, J. D., Rohel, Z., Curry, C.J., Krebs, A., Carstens, E.B. & Faulkner, P. (1984). Nucleotide sequence of the p10 polypeptide gene of Autographa californica nuclear polyhedrosis virus. Virology 139, 414-418.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227, 680-685.

Leisy, D.J., Rohrmann, G.F. and Beaudreau, G.S. (1984). Conservation of genome organization in two multicapsid nuclear polyhedrosis viruses. J. Virol. 52, 699-702.

Leisy, D.J., Rohrmann, G.F., Nesson, M., and Beaudreau, G.S. (1986). Location and nucleotide sequencing of the Orgyia pseudotsugata SNPV polyhedrin gene. J. gen. Virol., 67, 1073-1079.

Maeda, S. (1989). Expression of foreign genes in insects using baculovirus vectors. Ann. Rev. Entomol. 34, 351-372

Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982). Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Minion, F.C., Coons, L.B., and Broome, J.R. (1979). Characterization of the polyhedral envelope of the nuclear polyhedrosis virus of *Heliothis virescens*. J. Invertebr. Pathol. 34, 303-307.

Pearson, M.N., Quant-Russell, R.L., Rohrmann, G.F., and Beaudreau, G.S. (1988) P39, a major baculovirus structural protein: Immunocytochemical characterization and genetic location. Virology, 167, 407-413.

Pennock, G.D., Shoemaker, C., Miller, L.K. (1984). Strong and regulated expressiln of Escherichia coli ß-galactosidase in insect cells with a baculovirus vector. Mol. Cell. Biol. 4, 399-406.

Quant-Russell, R.L., Pearson, M.N., Rohrmann, G.F., and Beaudreau, G.S. (1987). Characterization of baculovirus p10 synthesis using monoclonal antibodies. Virology 160, 9-19.

Quant, R.L., Pearson, M.N., Rohrmann, G.F., and Beaudreau, G.S. (1984). Production of polyhedrin monoclonal antibodies for distinguishing two *Orgyia pseudotsugata* baculoviruses. Appl. Environ. Microbiol. 48, 732-736.

Reed, D.K. and Hall, I.M. (1972). Electron microscopy of a rod-shaped noninclusion virus infecting the citus red mite. J. Invertebr. Pathol. 20, 272-278.

Rohrmann, G.F., Martignoni, M.E., and Beaudreau, G.S. (1982). DNA sequence homology between Autographa californica and Orgyia pseudotsugata nuclear polyhedrosis viruses. J. gen. Virol. 62,. 137-143.

Rohrmann, G.F., (1986). Polyhedrin Structure. J. gen. Virol. 67, 1499-1513.

Sanger, F., Coulson, A.R., Barrell, B.G., Smith, A.J.H. and Roe, B.A. (1980). Cloning in single stranded bacteriophage as an aid to rapid DNA sequencing. Journal of Molecular Biology 143, 161-178.

Singh, S.P., Gudauskas, R.T. and Harper, J.D. (1983). High resolution two-dimensional gel electrophoresis of structural proteins of baculoviruses of Autographa californica and Porthetria (Lymantria) dispar.. Virology 125, 370-380.

Smith, G. E., and Summers, M.D. (1981). Application of a novel radioimmunoassay to identify baculovirus structural proteins that share interspecies antigenic determinats. J. Virol.39, 125-137.

Smith, G. E., and Summers, M. D. (1978). Analysis of baculovirus genomes with restriction endonucleases. Virology 89, 517-527.

Spindler, K.R., Rosser, D.S.E. and A.J.Berk. (1984). Analysis of adenovirus transforming proteins from early regions 1A and 1B with antisera to inducible fusion antigens produced in *Escherichia coli*. J. Virol. 49, 132-141.

Stelzer,M.J., Neisess J., and Thompson, C.G. (1975) Aerial application of a nucleopoyhedrosis virus and Bacillus thuringiensis against the Douglas-fir tussock moth. J. Econ. Entomol., 68, 269.

Summers, M.D., and L.E. Volkmann (1976). Comparison of biophysical and morphological properties of occluded and extracellular nonoccluded baculovirus from in vivo and in vitro host systems. J. Virol. 17, 962-972

Thiem S.M. and Miller L.K. (1989). Identification, sequence and transcriptional mapping of the major capsid protein of the baculovirus Autographa californica nuclear polyhedrosis virus. J.gen.Virol. 63, 2008-2018.

Tinsley, T. W. and Harrpa, K.A. (1979). Viruses of invertebrates. "Comprehensive Virology" (.Fraenkel-Conrat and R.R. Wagner, eds.). Plenum Press, New York. 12, 1-110

Tollefson, A.E. and Wold, W.S.M. (1988). Identification and gene mapping of a 14,700-molecular-weight protein encoded by region E3 of group C adenoviruses. J. Virol. 62, 33-39.

Toneguzzo,F., Glynn, S., Leci, E., Mijolsness, S., and Hayday, A. (1988). Use of a chemically modified T7 polymerase for manual and automated sequencing of supercoiled DNA. BioTechniques 6, 460-469.

Vlak, J.M., Klinkenberg, F.A., Zaal, K.J.M., Usmany, M., Klinge-roode, E.C., Geervliet, J.B.F., Roosien, J. and Lent, J.W.V. (1988). Functional studies on the p10 gene of Autographa californica nuclear polyhedrosis virus using a recombinant expressing a p10-ß-galactosidase fusion gene. J. gen. Virol. 69, 765-776

Vlak, J.M., and Rohrmann, G.F. (1985). The Nature of Polyhedrin. Pp. 489-544. In: Maramorosch, K. and Sherman, K.E. eds. Viral Insecticides for Biological Control. Academic Press, Orlando, FL USA.

Volkman, L.E. (1986). The 64K envelope protein of budded *Autographa californica* nuclear polyhedrosis virus. Curr. Top. Microbiol. Immunol. 131, 103-117.

Volkmann, L.E., Goldsmith, P.A., Hess, R.T. and Faulkner, P. (1984). Neutralization of budded Autographa californica NPV by a monoclonal antibody: Identification of the target antigen. Virology 133, 354-362.

Volkmann, L.E. and Goldsmith, P.A. (1985). Mechanism of neutralization of budded Autographa californica polyhedrosis virus by a monclonal antibody: inhibition of entry by adsorptive endocytosis. Virology 143, 185-195.

Williams, G.V., Rohel, D.Z., Kuzio,J. and Faulkner, P. (1989). A cytopathlogical investigation of Autographa californica nuclear polyhedrosis virus p10 gene function using insertion/geletion mutants. J.gen.Virol.70, 187-202.

Wilson, M.E., Mainprize, T.H., Friesen, P.D., and Miller, L.K. (1987). Location, transcription, and sequence of a baculovirus gene encoding a small arginine-rich polypeptide. J. Virol. 61, 661-666.

Wilson, M.E. and Consigli, R.A. (1985). Functions of a protein kinase activity associated with purified capsids of the granulosis virus infecting Plodia interpunctella. Virology 143, 526-535.