**Orgyia pseudotsugata** baculovirus p10 and polyhedron envelope protein genes: analysis of their relative expression levels and role in polyhedron structure

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To investigate the regulation of p10 and polyhedron envelope protein (PEP) gene expression and their role in polyhedron development, *Orgyia pseudotsugata* multinucleocapsid nuclear polyhedrosis viruses lacking these genes were constructed. Recombinant viruses were produced, in which the p10 gene, the PEP gene or both genes were disrupted with the β-glucuronidase (GUS) or β-galactosidase (lacZ) genes. GUS activity under the control of the PEP protein promoter was observed later in infection and its maximal expression was less than 10% the level for p10 promoter–GUS constructs. Tissues from *O. pseudotsugata* larvae infected with these recombinants were examined by electron microscopy. Cells from insects infected with the p10−viruses lacked p10-associated fibrillar structures, but fragments of polyhedron envelope-like structures were observed on the surface of some polyhedra. Immunogold labelling of cells infected with the p10−GUS+ virus with an antibody directed against PEP showed that the PEP was concentrated at the surface of polyhedra. Although polyhedra produced by p10 and PEP gene deletion mutants demonstrated what appeared to be a polyhedron envelope by transmission electron microscopy, scanning electron microscopy showed that they had irregular, pitted surfaces that were different from wild-type polyhedra. These data suggested that both p10 and PEP are important for the proper formation of the periphery of polyhedra.

**Introduction**

Nuclear polyhedrosis viruses (NPVs) are members of the Baculoviridae family, which include viruses pathogenic for arthropods, primarily insects of the orders Lepidoptera, Hymenoptera and Diptera (Blissard & Rohrmann, 1990). They have large (88 to 160 kbp) supercoiled dsDNA genomes that replicate in the host cell nucleus. NPVs are characterized by a complex infection cycle that culminates in the incorporation of virions into large crystalline occlusion bodies called polyhedra. Polyhedra are surrounded by an electron-dense structure called the polyhedron envelope (PE) or polyhedron calyx. A protein called the PE protein (PEP) appears to be an integral component of the polyhedron envelope. In addition, we showed that the polyhedron envelope from *Orgyia pseudotsugata* multinucleocapsid NPV (OpMNPV) was sensitive to protease treatment indicating that the protein is a major component of this structure (Russell & Rohrmann, 1990). We also examined the expression of the PEP protein using immunological techniques. Western blot analysis showed that PEP was expressed later than the p39 capsid protein (Gombart et al., 1989) and immunoelectron microscopy of baculovirus-infected cells showed that PEP accumulated around developing polyhedra during a late stage in their development [e.g. 72 h post-infection (p.i.)] (Russell & Rohrmann, 1990).

In addition to PEP, a protein called p10 may also be involved in the formation of the PE. P10 is a poorly conserved protein that is highly expressed late in baculovirus infection (Zuidema et al., 1993). Williams et al. (1989) showed that deletion of the p10 gene resulted in the absence of p10-associated fibrillar material and in polyhedra that lacked or had fragmented PEs. They also showed that polyhedra from a mutant virus lacking p10 are fragile and are not released from nuclei late in infection, suggesting that p10 may be involved in cell...
lysall. However, van Oers et al. (1993) recently showed by electron microscopy that a variety of mutants in which the p10 gene was either partially or completely deleted showed evidence of a PE. In addition, they found that a virus with C-terminal deletions past amino acid 79 showed an inability to release polyhedra from cells late in infection, indicating that the disintegration of nuclei did not occur. These data suggest that the p10 gene may have a role in the stability and efficient dissemination of polyhedra.

To characterize the expression of PEP further and to examine the role of both p10 and PEP in the structure of the PE, we constructed four recombinants of OpMNPV. In two recombinants, the β-glucuronidase (GUS) gene was expressed under the control of the p10 or PEP promoters and replaced these genes. These constructs permitted us to compare the levels of β-glucuronidase expressed from the p10 and PEP promoters. A third recombinant expressed the β-galactosidase (lacZ) gene under the control of the p10 promoter and the fourth, a double recombinant, expressed β-galactosidase in place of the p10 gene and β-glucuronidase in place of the PEP gene. Examination of polyhedra produced by these mutants allowed us to investigate the influence of these proteins in the formation of the PE.

Methods

Virus, insect cell lines and insects. The cloned isolate of OpMNPV was described previously (Quant-Russell et al., 1987). Virus stocks were titrated by endpoint dilution assays as described by Bradford et al. (1990). For determining the titre of recombinant viruses, reporter gene activity was used to detect the presence of virus as described below. Lymantria dispar (Ld-652y) cells were propagated in T-flasks using TNM-FH medium (Summers & Smith, 1987) supplemented with 10% fetal bovine serum (FBS). For production of budded virus, L. dispar cells adapted to shaker culture (a gift from Stephen Weiss, Gibco) were grown in TNM-FH 10% FBS supplemented with 0.1% Pluronic F-68 (Gibco). The O. pseudotsugata larvae were provided by the U.S. Department of Agriculture Forestry Sciences Laboratory (Corvallis, Oreg., U.S.A.).

Construction of p10 and PEP gene transfer vectors. To construct the p10 transfer vector, a 2.4-kbp HindIII–SalI fragment from the OpMNPV HindIII Q fragment containing the entire p10 gene was cloned into pBlueScribe (pBS) (Stratagene) using standard methods (Sambrook et al., 1989). This plasmid was then digested with XhoI and EcoRI to remove restriction sites from the polylinker, blunt-ended with S1 nucleases, religated, and transformed into Escherichia coli JM 83 cells. Site-directed mutagenesis (Kunkel et al., 1987) was used to produce a unique BamHI site 6 nucleotides (nt) downstream of the translational start site of the p10 gene by changing the sequence ATGTCACAGGCC to ATGTCGAATTC. The primer used to create the mutation was the sequence 5′ CTGCAGATCTCATGTTGTCGATCCAGTGTCGATGGCC 3′. The modified p10 plasmid was digested with BamHI and SstI to delete all but nine 5′-terminal and 48 3′-terminal nucleotides and a 2.1-kbp BamHI–SstI fragment containing the GUS gene (Jefferson et al., 1986) was inserted (Fig. 1a). To construct a p10 promoter–lacZ gene fusion, a 3899 bp BamHI–SstI fragment containing the lacZ gene (Russell et al., 1991) was cloned into the modified p10 plasmid (Fig. 1b).

To construct a PEP gene transfer vector, we used a plasmid that contains the entire PEP gene region from another study (Gross & Rohrmann, 1993). This plasmid was modified by site-directed mutagenesis to contain a BamHI and SstI site at 3 nt and 33 nt after the PEP translational start site, respectively (Gross & Rohrmann, 1993). The PEP construct was digested with BamHI and SstI and the 2.1-kbp BamHI–SstI fragment containing the GUS gene was inserted, resulting in a functional PEP promoter–GUS gene fusion (Fig. 1c).

Construction and purification of recombinant viruses, using β-glucuronidase and β-galactosidase assays. To construct recombinant viruses, L. dispar cells were seeded at 1.5 × 10⁶ cells per well in six-well tissue culture plates. Transfections of these cells using 1 μg of wild-type OpMNPV DNA and 2 μg of a transfer plasmid were performed (Summers & Smith, 1987). After 5 days, the supernatants containing mixtures of wild-type and recombinant virus were collected and recombinant viruses were cloned by a limiting dilution protocol (Summers & Smith, 1987) using either GUS or lacZ gene expression to identify recombinant viruses. GUS activity was assayed by the method of Jefferson (1987), modified for small volumes. Supernatants from the recombination experiment were serially diluted and plated in 96-well microtitre plates containing L. dispar cells, with a final volume of 200 μl/well. After 5 days, 80 μl samples from each well were frozen and thawed once and 150 μl of GUS assay buffer was added. Samples were incubated for several hours at 37 °C and then scored for fluorescence on a long wavelength u.v. (365 nm) transilluminator.

Cells infected with the recombinant virus expressing the lacZ gene were assayed for β-galactosidase (Miller, 1972) by adding 80 μl of cell extract prepared as described above to 120 μl of buffer Z (60 mM-Na₂HPo₄, 40 mM-NaH₂PO₄, 10 mM-KCl, 1 mM-MgSO₄ and 0.27% 2-mercaptoethanol). After incubation at 37 °C for 5 min, 40 μl of 4 mg/ml o-nitrophenyl-β-D-galactoside in 0.1M-Na₂PO₄ (pH 7.0) were added, the samples were incubated at 37 °C for several hours and scored for the presence of β-galactosidase by colour.

To construct the double recombinant lacking both p10 and PEP genes, the p10–LacZ+ transfer vector (Fig. 1b) was recombined with the PEP GUS+ recombinant (Fig. 1c). Screening for both LacZ and GUS allowed isolation of a mutant virus lacking both the PEP and p10 genes.

After the recombinant viruses had been cloned by three rounds of endpoint dilution, 150 ml of L. dispar cells grown in shaker flasks was infected, budded virus was purified, and viral DNA was isolated (Summers & Smith, 1987). Restriction site analysis was performed to confirm that the proper recombination events had occurred and that the virus stocks were pure.

Western blot analysis. To verify that the recombinant viruses did not express the inactivated genes, Western blot analyses (Quant-Russell et al., 1987) of p10 and PEP expression were performed. L. dispar cells infected with recombinant virus were lysed in 2× SDS-PAGE sample buffer at 60 h p.i. and an amount equivalent to approximately 4.8 × 10⁴ cells per lane was electrophoresed through a 10% SDS-polyacrylamide gel (Laemmli, 1970). Antibodies against p10 (MAb 236), PEP, and the antibodies used for positive controls [p39 (MAb 236) and p24] are described in Gombart et al. (1989), Pearson et al. (1988), Quant-Russell et al. (1987) and Wolgastoi et al. (1993), respectively. The antisera were diluted as follows: p39 (MAb 236) 1:7000; p10 (MAb 210) 1:5000; PEP 1:500 and p24 1:1000 in TBS-T (20 mM-Tris–HCl pH 7.5, 500 mM-NaCl, 0.05% Tween 20).

Assay of reporter gene expression at various times p.i. To examine the expression of the reporter genes during a time course of virus infection, L. dispar cells (1.5 × 10⁶) were seeded into six-well tissue culture plates and allowed to attach for 3 h. Cells were infected at a TCID₅₀ of 10
Characterization of OpMNPV \( p10 \) and PEP

(a) \( p10 \)-GUS\(^+\) transfer plasmid

\[
\text{pBS} \quad 620 \text{ nt} \quad 5' \quad \text{p10} \\
\text{HindIII} \quad \text{AAAGTCGTTG} \\
\text{p10 start codon} \\
\text{p10 stop codon} \\
\]

\[
\text{ATGTCGATCCC} \\
70th codon p10 \\
\]

\[
\text{GUS} = 2100 \text{ nt} \\
\]

\[
\text{Sacl} \\
\]

(b) \( p10 \)-LacZ\(^+\) transfer plasmid

\[
\text{pBS} \quad 620 \text{ nt} \quad 5' \quad \text{p10} \\
\text{HindIII} \quad \text{AAAGTCGTTG} \\
\text{p10 start codon} \\
\text{p10 stop codon} \\
\]

\[
\text{ATGTCGATCCC} \\
\]

\[
\text{BamHI} \\
9th codon lacZ \\
\]

\[
\text{I2 SauI} \\
12th codon p10 \\
\]

\[
\text{KpnI} \quad \text{GGGTACC} \\
\]

\[
\text{Sacl} \\
\text{GAGCT} \\
70th codon p10 \\
\]

\[
\text{l} \quad \text{l} \\
\]

(c) PEP-GUS\(^+\) transfer plasmid

\[
\text{pBS} \quad 995 \text{ nt} \quad 5' \quad \text{PEP} \\
\text{HpaI} \\
\]

\[
\text{ATG GAT CCC} \\
\]

\[
\text{BamHI} \\
7th codon PEP \\
\]

\[
\text{CGAGCTCGC} \\
11th codon PEP \\
\]

\[
\text{Sacl} \\
\]

\[
\text{GUS} = 2100 \text{ nt} \\
\]

\[
\text{HindIII} \\
\]

Fig. 1. Maps of the transfer plasmids used for constructing the recombinant viruses. The construction of this plasmid is described in detail in Methods. (a) \( p10 \) promoter–GUS gene fusion transfer vector (\( p10 \)-GUS\(^+\)). (b) \( p10 \) promoter–LacZ gene fusion transfer vector (\( p10 \)-LacZ\(^+\)). (c) PEP promoter–GUS gene fusion transfer vector (PEP-GUS\(^+\)). The solid bars represent the pBS sequences; the stippled and cross-hatched bars represent \( p10 \) and PEP flanking sequences, respectively; the thin lines with the arrows at the end represent the lacZ or GUS reporter genes. The nucleotide sequences at the cloning junctions are indicated.

with either the \( p10 \)-GUS\(^+\) or PEP-GUS\(^+\) virus in 800 \( \mu \)l of medium. After 1 h, the medium was replaced with 1 ml of fresh medium. For each time point, three independent wells were analysed. Cells were harvested at various times p.i. with a rubber policeman, transferred to microfuge tubes and centrifuged at 4000 g for 3 min. Supernatants were removed, cell pellets were resuspended in 500 \( \mu \)l of PBS (120 mM-NaCl, 2.7 mM-KCl, 10 mM-Na\(_2\)HPO\(_4\), 1.8 mM-KH\(_2\)PO\(_4\), pH 7.4) and frozen at \(-80^\circ\)C. To assay for reporter gene activity, cells were lysed by one freezing and thawing cycle, debris was pelleted and 5 \( \mu \)l of supernatant was transferred to a 24-well plate. GUS assay buffer (595 \( \mu \)l at \( 37^\circ\)C) (Jefferson, 1987) was added to each well and incubated at \( 37^\circ\)C for 15 min. Samples (100 \( \mu \)l) were transferred to a microfuge tube containing 900 \( \mu \)l of 0.2 M-Na\(_2\)CO\(_3\). The quantity of the fluorogenic product from each time point was measured in a fluorimeter.

Electron microscopy. Fourth and fifth instar \( O. \) pseudotsugata larvae were injected with 50 000 TCID\(_{50}\) of budded virus. Fat body tissue was removed at 4 days p.i. and fixed in 2.5% glutaraldehyde, dehydrated with ethanol, embedded in LR White resin, and labelled with immunogold as previously described (Russell & Rohrmann, 1990).

For scanning electron microscopy (SEM), larvae at 4 days p.i. were frozen and polyhedra were subsequently purified either using detergent and sodium deoxycholate or without these reagents. For the former method two or three infected larvae (about 0.65 g) were placed in a
1.5 ml microfuge tube with 0.5 ml of 2% Triton X-100, macerated with a pipette tip and incubated for 1 h at 37 °C. 0.25 ml of 2% sodium deoxycholate was then added and the sample was incubated for 1 h at 37 °C. The suspended material was transferred to a new tube using a pipette, leaving the exoskeleton of the larvae. The suspension was microfuged at 9000 r.p.m. for 15 min, the supernatant removed, and the pellet suspended in 1.0 ml of TNS (10 mM-Tris-HCl, 0.15 M-NaCl, 0.0025% SDS, pH 8.0). The sample was microfuged for 30 s and the pellet was resuspended in 200 μl TNS, layered on 1.0 ml of 50% sucrose in TNS and microfuged for 30 min. The sucrose solution was removed and the pellet was resuspended in 50 μl TNS. To prepare polyhedra without Triton X-100 or sodium deoxycholate treatment, two larvae (about 0.4 g) were added to 0.5 ml of TES (10 mM-NaCl, 20 mM-Tris-HCl pH 7.5, 0.1 mM-EDTA) and the larvae were macerated with a pipette tip. TES (0.5 ml) was added and the suspension was microfuged for 30 s. The pellet was resuspended in 1.0 ml TES and microfuged for 30 s. The pellet was resuspended in 300 μl TES layered on the surface of 1.0 ml of 50% sucrose and microfuged for 15 min. The pellet was resuspended in 400 μl TES, microfuged for 5 min and then resuspended in 100 μl TES and frozen at -20 °C.

For SEM, samples were prepared in a manner similar to that described by Williams et al. (1989). Polyhedra were spread on metal stubs coated with 0.1 mg/ml cytochrome c, allowed to dry, and fixed for 45 min in 2% paraformaldehyde-2% glutaraldehyde (pH 7.2). Samples were washed for 20 min in phosphate buffer (0.028 M-Na2HPO4, H2O, 0.0387 M-KH2PO4 pH 7.2), post-fixed in 1% OsO4 for 30 min and washed for 1 h. Samples were then dehydrated with 100% ethanol, followed by dehydration with trichlorotrifluoroethane. Samples were critical point-dried, sputter-coated with gold and observed in an AMR model 1000A scanning electron microscope.

For polyhedron stability studies, polyhedra were sonicated for 5 min with a Heat System Ultrasonics Sonicator Model I-10 at setting 5 at 4 °C.

**Results**

**Construction of recombinant viruses**

We initially planned to produce p10-GUS+ and PEP-LacZ+ recombinants and then use the PEP-LacZ+ virus to construct a double recombinant containing p10-GUS+/PEP-LacZ+. Although construction of the p10-GUS+ recombinant was straightforward (Fig. 1a), we were unable to isolate a PEP-LacZ+ recombinant. This was apparently due to a combination of the weakness of the PEP promoter and the poor sensitivity of the LacZ assay. However, construction of a PEP-GUS+ recombinant was successful (Fig. 1c). Therefore, a p10-LacZ+ transfer vector was constructed (Fig. 1b) and used for the construction of both a p10-LacZ+ recombinant and a p10-LacZ+/PEP-GUS+ double recombinant.

**Western blot analysis of wild-type OpMNPV and recombinant viruses**

To verify that the p10 and PEP were not expressed in the appropriate recombinant viruses, extracts from infected L. dispar cells, 60 h p.i., were subjected to Western blot analysis. The p10-LacZ- and p10-GUS- recombinant viruses showed no expression of the p10 protein (Fig. 2a, lanes 1 and 2, respectively). The double recombinant virus (p10-LacZ+/PEP-GUS+) showed no expression of either p10 protein (Fig. 2a, lane 3) or PEP (Fig. 2b, lane 1). The PEP-GUS+ recombinant virus showed no PEP expression (Fig. 2b, lane 2). The wild-type OpMNPV expressed both p10 (Fig. 2a, lane 4) and PEP (Fig. 2b, lane 3). The p39-capsid MAb (MAb 236) (Pearson et al., 1988) was used as a positive control for the analysis of p10 expression (Fig. 2a, lanes 1 to 4). The p24 polyclonal antiserum (Wolgamot et al., 1993) was used as a positive control in the analysis of PEP expression (Fig. 2b, lanes 1 to 3).

![Fig. 2. Western blot analysis of extracts of cells infected with wild-type and recombinant OpMNPV viruses. (a) Western blot analysis using the p39 (MAb 236) and p10 (MAb 210) MAb Abs. Lane 1, p10-LacZ+ virus; lane 2, p10-GUS+ virus; lane 3, p10-LacZ+/PEP-GUS+ virus; lane 4, wild-type OpMNPV-infected cell extracts. (b) Western blot analysis using the PEP and p24 polyclonal antisera. Lane 1, p10-LacZ+/PEP-GUS+ double recombinant virus; lane 2, PEP-GUS+ recombinant virus; lane 3, wild-type OpMNPV-infected cell extracts.](image-url)
Expression of the p10 and PEP genes

To examine the time course and relative levels of expression of the p10 and PEP genes, L. dispar cells were infected with the appropriate recombinant virus at a TCID\textsubscript{50} of 10 and assayed for GUS activity at various times p.i. Trace amounts of GUS expression from infection with p10-GUS \textsuperscript{*} (p10 promoter–GUS), were first observed at 20 h p.i. and the level increased throughout the time course (Fig. 3). In contrast, PEP promoter–GUS expression (from infection by PEP-GUS \textsuperscript{+}) appeared later (after 28 h p.i.) and increased throughout the time course. The ratio of p10 promoter–GUS to PEP promoter–GUS expression remained constant (at about 12-fold) after 42 h p.i. Therefore p10 promoter–GUS expression can be detected about 8 h earlier and was expressed at a substantially higher level than PEP promoter–GUS expression.

**SEM of mutant polyhedra**

SEM was used to examine mutant and wild-type polyhedra further. Whereas wild-type polyhedra had a smooth surface (Fig. 5a, b), most polyhedra produced by mutant viruses are smaller, have pitted surfaces, and the PEP\textsuperscript{*} constructs produced polyhedra with a more cubic profile (Fig. 5d to f). It was not determined what caused the production of polyhedra with this shape. The rod-shaped pits on the mutant polyhedra surfaces appear to be due to the loss of virion bundles. Many of the polyhedra from the mutant lacking both the p10 and PEP genes had indistinct margins and appeared aggregated into larger groups (Fig. 5e, f). To determine whether the lack of p10 or PEP affected the physical stability of mutant polyhedra, preparations of polyhedra were sonicated and then examined by SEM. Both sonicated and untreated wild-type polyhedra show a few fractures or tears on the surface (Fig. 5a, b). Sonication of polyhedra from the PEP-GUS\textsuperscript{*}/p10\textsuperscript{*}-LacZ\textsuperscript{*} recombinant appeared to cause more extensive pitting on the surface which may be due to the dislodging of virion bundles (compare Fig. 5e, f). In addition, the surface of polyhedra appear irregular suggesting that some surface fragmentation may have occurred.

Although examination with TEM showed many mutant polyhedra that had what appeared to be a PE-like structure, SEM showed that they had a surface that appeared different suggesting that the PE may be modified. It was thought that the Triton X-100–sodium deoxycholate treatment may have removed the PE.
However, when polyhedra were purified using a protocol that lacked Triton X-100–sodium deoxycholate treatment (see Methods), and examined by SEM, the polyhedron surfaces were similar, suggesting that this treatment was not responsible for altering the surface (data not shown).

**Discussion**

Although PEP is a prominent structural component of polyhedra and may play an important role in polyhedron stability, our investigations suggest that PEP gene expression is governed by a weak promoter. GUS activity in extracts of cells infected with the PEP-GUS+ virus appeared later and was expressed at less than 10% of the level of GUS expressed by the p10'GUS' construct. In a previous study, developing polyhedra were observed at 48 h p.i. by electron microscopy but detectable concentrations of PEP were not observed until 72 h p.i. (Russell & Rohrmann, 1990). The low levels of PEP promoter–GUS expression relative to p10 promoter–GUS expression and the late appearance of PEP in infected cells suggests that the PEP promoter is one of the weakest baculovirus late promoters yet characterized.

The PEP and p10 genes are commonly considered to be non-essential loci, allowing for the insertion of foreign
Fig. 5. SEM of wild-type and mutant polyhedra. (a) Wild-type polyhedra; (b) Sonicated wild-type polyhedra; (c) p10\textsuperscript{0} LacZ\textsuperscript{+}; (d) PEP\textsuperscript{−} GUS\textsuperscript{+}; (e) p10\textsuperscript{0} LacZ\textsuperscript{+}/PEP\textsuperscript{−} GUS\textsuperscript{+}; (f) Sonicated p10\textsuperscript{0} LacZ\textsuperscript{+}/PEP\textsuperscript{−} GUS\textsuperscript{+}. Bar marker in (e) represents 1 μm for (a) to (f).
genes without sacrificing the occluded phenotype. To investigate the possible roles of these genes in the structure of polyhedra, we examined polyhedra produced by mutants with deletions of these genes. Previous investigations of AcMNPV polyhedra produced from virions with mutant pl0 genes showed that when the codons for the N-terminal amino acids of pl0 were fused to the β-galactosidase (lacZ) gene in a recombinant virus and grown in cell culture, the resulting polyhedra did not display a PE structure (Williams et al., 1989). Another mutant virus was examined in which 65 amino acids were deleted from the C terminus of pl0. This virus produced polyhedra with a PE that was patchy and not properly attached to the surface of the polyhedra (Williams et al., 1989). van Oers et al. (1993) recently showed that all members of a series of AcMNPV mutants in which the pl0 gene was either partially or completely deleted showed evidence of a PE. In addition, reports indicate that AcMNPV recombinants that expressed the lacZ gene under the control of the PEP promoter do not produce polyhedra with a PE (van Lent et al., 1990; Zuidema et al., 1989).

As summarized above, a number of studies based on TEM have not agreed on the relationship of the deletion of the pl0 gene from AcMNPV to the presence or absence of a PE. Our results are based on a different virus (OpMNPV) from these studies and we investigated polyhedra produced in insect larvae rather than in cultured cells. We found that some polyhedra from both pl0- and PE- mutants had what appeared to be at least partial PE-like structures. However, SEM showed that polyhedra resulting from mutant virus infection had rough and highly pitted surfaces that did not resemble the surface of wild-type polyhedra which indicates that they may lack a PE. This suggests that the PE-like structure observed by TEM may be an artefact of the fixation protocol, or is an incomplete or improperly assembled PE and may be lost during the purification of polyhedra from infected cells. In addition, the presence of aggregates of polyhedra produced by the PEP/GUS+/pl0-LacZ+ recombinant may indicate that the surface of mutant polyhedra is altered such that polyhedra aggregate during the purification protocol. One possible function of a properly assembled PE could be to prevent the fusion or aggregation of polyhedra. Such a function would be important in ensuring their maximal dissemination.

The mutant OpMNPV polyhedra produced from virus lacking the p10 and PEP genes resemble AcMNPV pl0- mutants described by Williams et al. (1989), but appeared to be more stable. In contrast to the AcMNPV pl0- mutants, vigorous and extended sonication of the OpMNPV PEP-GUS+/p10-LacZ+ polyhedra did not cause extensive fragmentation of these particles. These results could be due to differences between the viruses in the roles of p10 and PEP. However, the AcMNPV was grown in cell culture, whereas the OpMNPV mutants were grown in insects which may contribute a factor(s) that stabilizes polyhedra.

These investigations indicate that PEP, in conjunction with p10, probably plays a major role in the morphology of polyhedra. p10 and PEP appear to be involved in sealing the periphery of polyhedra, thereby ensuring retention of nucleocapsids that may otherwise become dislodged and lost. The PE may also stabilize polyhedra and protect them from mechanical damage and prevent aggregation. All these features could contribute to the ability of the virus to transmit a maximal number of intact virions in individual stable polyhedra.

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References


Characterization of OpMNPV p10 and PEP


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