A Cytopathological Investigation of *Autographa californica* Nuclear Polyhedrosis Virus p10 Gene Function Using Insertion/Deletion Mutants

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**SUMMARY**

The role of the *Autographa californica* nuclear polyhedrosis virus p10 gene in viral cytopathology and morphogenesis was examined using classes of p10 deletion mutants with and without *lacZ* (β-galactosidase) gene fusion. Mutant-infected cells did not form the fibrillar cytoplasmic and nuclear structures normally observed late in infection with wild-type (wt) virus, and the cells failed to lyse even at 2 weeks post-infection. Based on wt and mutant cytopathology, we suggest lysis may be facilitated by stepwise exhaustion of the host nuclear membrane, and may require a function resident in the carboxy region of p10; this portion of the molecule is also essential for formation of the p10-rich fibrillar bodies. Additional changes in cytopathology were correlated with the level of p10/LacZ fusion protein expression. The insertional mutant designated Ac229, which encodes 51 N-terminal amino acids of p10 fused to LacZ, caused intranuclear accumulation of granular structures at sites corresponding to the fibrillar bodies of wt viral infections. Occlusion body membranes, which associate with the fibrillar bodies in wt infections, were also formed in mutant virus-infected cells. However, membranes did not associate with occlusion bodies in Ac229 infections, and were aberrantly attached to occlusion bodies in cells infected with mutants having simple p10 deletions (represented by Ac231). Loss of the outer membrane increased sensitivity of the occlusion bodies to disruption by physical stress; a partially attached membrane afforded some protection from disruption.

**INTRODUCTION**

The baculovirus *Autographa californica* nuclear polyhedrosis virus (AcNPV) produces two well-characterized RNA transcripts in abundance from late infection until cell lysis (Rohel et al., 1983; Rohel & Faulkner, 1984). These encode the occlusion body matrix protein, polyhedrin (for a review, see Rohrmann, 1986), and a 10K protein of unknown function, designated p10 (Kuzio et al., 1984). The p10 polypeptide is non-structural, and is not considered essential for *in vitro* replication (Crozier et al., 1987; Vlak et al., 1988).

Recent investigations have indicated that p10 shares antigenic similarities with some cytoskeletal elements (Quant-Russell et al., 1987); in addition, the structures associated with p10 in *in vitro* have been identified as large fibrillar masses which accumulate in the host cytoplasm and nucleus (van der Wilk et al., 1987). The fibrillar masses failed to develop in cells infected with a neomycin phosphotransferase insertional p10 mutant (Crozier et al., 1987), and a p10/β-galactosidase (β-gal) fusion (deletion) mutant (Vlak et al., 1988). Also absent from the β-gal but not phosphotransferase mutant virus-infected cells were the fibrous sheet structures previously referred to as 'spacers' (MacKinnon et al., 1974).

To investigate further the role of p10 in NPV replication, we have constructed three classes of p10 deletion mutants, two of which carried the β-gal gene (*lacZ*), and a third class without *lacZ*. Based on morphological and cytopathological effects we make several conclusions regarding the...
role of p10 in baculovirus replication. In addition, the cytological effects of fusion protein over-
production are investigated.

METHODS

Cell lines and virus growth. Spodoptera frugiperda (Vaughn et al., 1977), or Trichoplusia ni TN-368 cells (Hink, 1970) were cultured at 28 °C in BML-TC/10 insect tissue culture medium (Gardiner & Stockdale, 1975) supplemented with 10% foetal calf serum and 50 μg/ml gentamicin sulphate. AcNPV strain HR3 (Brown et al., 1979) is designated wt (wild-type) in the text. It was used at an m.o.i. of 0.1 to 0.2 for preparation of virus stocks, and of 10 to 15 for experimental procedures.

Purification of virus. To prepare occlusion bodies (OBs) for electron microscopy (EM) studies, infected cells were harvested 4 to 5 days post-infection (p.i.), pelleted by centrifugation (1500 g for 10 min), then resuspended in distilled water. Cells were lysed by the addition of 1 vol. of 2% Triton X-100 (1 h, 37 °C), followed by 0.5 vol. of 2% deoxycholate (1 h, 37 °C) (Faulkner & Henderson, 1972). OBs were recovered from the cell extract by pelleting (5000 g, 15 min), then transferred to microfuge tubes and washed by successive rounds of pelleting (in a microfuge for 30 s) in distilled water. Non-occluded virus was harvested from cell cultures as spent media, and concentrated by pelleting through a 20% sucrose cushion.

DNA sequencing. For molecular analysis of deletion or insertion mutants, viral DNA (vDNA) was extracted (Cochran et al., 1982) and digested with appropriate restriction enzymes. The fragments were resolved by agarose gel electrophoresis and visualized by staining with ethidium bromide. Relevant bands were cut from the gels, electro-eluted, then cloned into pUC plasmid vectors (Yanisch-Perron et al., 1985). DNA restriction and modifying enzymes were purchased from Gibco/Bethesda Research Laboratories, Boehringer Mannheim or Pharmacia and used according to manufacturer’s recommendations. All plasmid manipulations were done essentially as described by Maniatis et al. (1982). Supercoiled plasmid DNA was sequenced using the dideoxy method (Bankier & Barrell, 1983), following procedures recommended in the Sequenase kit manual (U.S. Biologicals, Cleveland, Ohio, U.S.A.).

The primer used to initiate sequencing reactions was a 35 nucleotide synthetic fragment homologous to the promoter region of p10 (positions −1 to −35). Sequencing gels were prepared and run as previously described (Kuzio et al., 1984). The programs of Staden (1982) were used for computer analysis of DNA sequences.

Construction of AcNPV p10 mutants. The strategy of deletion and insertion is summarized in Fig. 1. Briefly, the AcNPV restriction fragment EcoRI P (Cochran et al., 1982) was cloned into plasmid pUC7 and amplified in Escherichia coli strain JM83, using procedures detailed by Maniatis et al. (1982). To make deletions, this plasmid (pAcEcoP) was restricted at a unique BgII site within the p10 coding region, then partially digested with the exonuclease Bal 31. The blunt termini were ligated to BamHI linkers, and the plasmid was recircularized and amplified. Some plasmids carrying p10 deletions were further modified within the BamHI site by insertion of the lacZ gene from the plasmid pMC1871, which was a gift of Dr M. Casadaban (Casadaban et al., 1981).

Transfection of insect cells and selection of recombinant virus. S. frugiperda cells were transfected with plasmids containing the modified p10 gene, using a procedure based on the method of Potter & Miller (1980). Briefly, 4 μl sheared herring sperm DNA (stock 10 μg/ml) and 10 μl wt vDNA (stock 10 μg/ml) were added to 5 μg of plasmid DNA in 0.9 ml HEPES buffer (21 mM-HEPES pH 7.1, 137 mM-NaCl, 5 mM-KCl, 0.7 mM-Na2HPO4, 5.5 mM-glucose). Ice-cold 2.5 M-CaCl2 (50 μl) was then added dropwise with gentle mixing. After 20 min at room temperature, 0.5 ml of this transfection mixture was added to 1 x 106 S. frugiperda cells growing in a 35 mm dish. BML-TC/10 medium (1 ml) was added, and cells were rocked for 6 h at 28 °C while a precipitate formed. The monolayer was then washed twice with BML-TC/10 medium, overlaid with an additional 2 ml of the medium, and incubated at 28 °C.

Cotransfection of wt vDNA and pAcEcoP containing p10 deletion/lacZ insertion (a p10/lacZ gene fusion) generated recombinants, which could be selected as blue plaques in the presence of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal; 100 μg/ml), using a 0.8% agarose overlay (Pennock et al., 1984). Some of these recombinants (AcNPV p10−/lacZ+) were cotransfected with pAcEcoP containing simple p10 deletions (lacZ−) (Fig. 1). This second recombination was used to generate AcNPV genome p10 deletions without lacZ; mutants were selected as white plaques (revertants) in the presence of X-gal.

Protein gels. Polyacrylamide analysis was done as described by Rohel & Faulkner (1984). Infected S. frugiperda cells were harvested 48 h p.i. for analysis. Cell pellets were resuspended in electrophoresis sample buffer, and heated at 100 °C. Proteins were separated using 12% polyacrylamide gels, and stained with Coomassie Brilliant Blue.

Specimen preparation for EM. For transmission EM (TEM), cells were pelleted in microfuge tubes (20 s), rinsed with fixing buffer (0.1 M-PIPES pH 7.2, 5 mM-EGTA, 2 mM-MgCl2, 10 mM-KCl), and fixed for 40 min to 1 h with 4% paraformaldehyde and 1.75% glutaraldehyde (J.B. EM Services, Montreal, Canada) in the same buffer. Pellets were washed, then post-fixed with 1% OsO4 (30 min) in 0.1 M-cacodylate buffer pH 7.2. Fixed pellets were washed in cacodylate for 1 h, cold-dehydrated up to 70% ethanol (4 °C) and left overnight. Samples were rinsed briefly in...
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80% ethanol, then infiltrated with the acrylate embedding resin L.R. White (London Resin, U.K.). Procedures used were modified from Taugner et al. (1985) and Graber & Kreutzberg (1985) in order to decrease polymerization damage. Briefly, dehydrated pellets were treated with mixtures of L.R. White : absolute ethanol at 4 °C (1:1 then 2:1: 1 h each) and finally with pure resin (twice for 1 h each, then overnight). Before use, the L.R. White resin was pre-treated by the addition of 20% (v/v) activated molecular sieves (BDH, type 4A). Sieving was done in the cold (4 °C) for at least 48 h, with occasional mixing. The embedding procedure, which incorporated use of a stabilizing buffer during fixation and overnight cold dehydration, greatly improved ultrastructural preservation in this resin.

Infiltrated specimens were transferred to fresh resin and brought to room temperature, then transferred to pre-dried gelatin capsules filled with sieved resin. Capsules were sealed tightly and polymerized at 54 °C (24 to 36 h). Ultrathin sections were mounted on 300-mesh high transmission nickel grids (J.B. EM Services), stained with 7% uranyl acetate (in 70% methanol, 15 min) and 0.1% alkaline lead citrate (10 s). Stained specimens were photographed on a Hitachi H500 TEM operating at 75 kV.

For scanning EM (SEM), purified OBs were seeded onto metal stubs pre-coated with cytochrome c (0.1 mg/ml solution), allowed to adhere, and fixed as for TEM. Samples were dehydrated to absolute ethanol, cleared with Freon-113, and critical point-dried and sputter-coated with gold by standard methods. Samples were observed with a Hitachi S-450 SEM and photographed on Polaroid type 52 film.

RESULTS

Construction of AcNPV p10 deletion mutants having β-galactosidase (LacZ) fusion protein

Recombinant plasmids were prepared with an insertion of the β-gal gene (lacZ) within deletions in the AcNPV p10 coding region. Selected plasmids were cotransfected with AcNPV wt DNA into S. frugiperda cells (Fig. 1), and recombinant virus was purified from infectious supernatants by plaque assay in the presence of X-gal. The mutants could be classified as either high galactosidase producers, having intense blue plaques, or low galactosidase producers, having pale blue plaques. A mutant from each class was selected and designated Ac229 (high producer) and Ac228 (low producer). Initially, it was thought that mutants such as Ac228 produced low amounts of galactosidase as a result of illegitimate recombination within the AcNPV genome. Thus, low level expression could result from the p10 promoter driving expression of a lacZ gene integrated outside the p10 coding region. However, Southern blots of the Ac228 genome using both lacZ- and EcoRI P-specific probes showed that recombination had occurred within the EcoRI P (p10 coding) region of the genome as an insertional mutant (data not shown). Furthermore, SDS–PAGE analysis confirmed that Ac228 did not produce p10 or detectable amounts of the Mr 130K p10/lacZ fusion protein, although the latter was easily detectable in extracts of Ac229-infected cells (Fig. 2).

After restriction analysis, relevant fragments from the genomes of Ac228 and Ac229 were cloned into plasmid vectors and the fusion sites were sequenced. The lacZ gene was out of frame in Ac228, and in frame in Ac229 (Fig. 3).

To assess the effect of large quantities of lacZ gene product on viral cytopathology, a third type of p10 deletion mutant was selected which did not encode lacZ. For easy selection of mutant plaques, Ac228 vDNA stocks were cotransfected with plasmids having deletions within the p10 coding region (see Fig. 1), and revertant (white) plaques were selected in the presence of X-gal. The virus from one such plaque, designated Ac231, was examined by restriction analysis and Southern blotting (not shown). The affected fragment was cloned and sequenced to confirm the loss of the lacZ coding region. Sequence data indicated that translation of p10 would terminate immediately after a unique double BamHI restriction site (Fig. 3).

Comparison of SDS–PAGE profiles of Ac229 and Ac231 showed that neither virus produced the p10 polypeptide and that Ac229 synthesized large amounts of β-gal, with concomitant reduction in polyhedrin synthesis (Fig. 2). These observations were consistent within each mutant class. In addition, the predicted small p10 fragment produced in Ac231-infected cells could not be located in high percentage polyacrylamide gels even after [35S]methionine labelling (not shown), indicating either secretion or degradation of the truncated peptide. None of the mutants used in this study expressed antigenically recognizable p10 in S. frugiperda or T. ni cells at the light microscope level, as determined by fluorescence and peroxidase labelling techniques, and using either polyclonal or monoclonal antibodies.
Fig. 1. Construction of AcNPV p10 deletion mutants. A cloned AcNPV EcoRI P fragment (a) was linearized at the unique BgII site, then partially digested with Bal31. BamHI linkers were added, and plasmids were either recircularized (T4 ligase) with the insertion of the β-gal gene (lacZ) (b), or remained without further modification (c). The p10/lacZ fusions (b) were cotransfected with wt viral DNA to produce lacZ+ recombinants (d). These could be cotransfected again with the straight p10 deletions produced in (c), to yield white plaque revertants defective in the p10 gene (e). B, BamHI; Bg, BgII; E, EcoRI.

Morphology and cytopathology of cells infected with AcNPV−p10 deletion mutants

Phase microscopic examination of cells showed that the different classes of p10 mutants had distinctive cytopathic effects compared to cells infected with wt virus (Fig. 4). At 4 days p.i. cells infected with Ac231 and Ac228 had large swollen nuclei that were filled with occlusion bodies (Fig. 4c). By contrast, Ac229-infected cells had swollen nuclei containing few OBs (Fig. 4b). A striking cytological observation was that cells infected with viruses having p10 mutations did not progress to cell lysis, whereas cells infected with wt virus began to lyse by 2 days p.i. (Fig. 4a). The delay in lysis could be observed until at least 2 weeks p.i.

To determine the morphological changes incurred by deletion of p10, cells infected with wt virus and the mutants Ac229 and Ac231 were examined by EM. Late in infection (36 h p.i.), wt virus-infected cells contained numerous highly ordered structures, especially within the nucleus (Fig. 5). A lattice-like virogenic stroma was generally found in the centre (core) of the nucleus, with all other elements being displaced peripherally and comprising a ring zone (Fig. 5a). The structures included host (marginated) chromatin and nucleoli, mature enveloped and non-enveloped nucleocapsids, forming OBs, and large fibrillar masses associated with sheets of OB membrane precursor material (fibrous sheets; FS) (Fig. 5b).

Cells infected with both mutants lacked the large cytoplasmic and nuclear fibrillar masses seen in wt infections at all the time points examined (18 to 48 h p.i.), suggesting that the p10 polypeptide is a major constituent of these bodies. OB membrane sheets were present in the nucleus of cells infected with both mutants (Fig. 6, 7); their morphogenesis followed the same time course as in cells infected with wt virus, indicating that p10 gene expression was not required for their formation.
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Fig. 2. SDS-PAGE (12%) of wt- and deletion mutant-infected S. frugiperda cells at 3 days p.i. The peptide p10 is present only in wt virus-infected cells (lane 1). The Ac229-infected cells (lane 3) contain large amounts of β-gal/p10 fusion protein, but decreased polyhedrin (PH). Fusion protein cannot be detected in the Ac228-infected cell extract (lane 2). Ac231-infected cell extract is shown in lane 4. Approximate $M_r$ values ($\times 10^3$) are indicated.

Other features of morphogenesis and cytopathology were altered in cells infected with the mutants, and were distinctive for each class. The virogenic stroma (VS) of Ac229-infected cells seemed to intermix with the peripheral nuclear matrix (Fig. 6a), and was frequently displaced to one side of the nucleus by clusters of forming OBs (Fig. 4b). Large aggregates of spherical particles were observed within the VS and around the forming OBs (Fig. 6b). These structures had an electron-dense core (37 to 55 nm) and an outer halo of lower electron density (100 to 120 nm).

In Ac231 infections the cytopathology more closely resembled that caused by wt virus (Fig. 7a). VS, although poorly staining compared to wt infections, was usually located centrally in the nucleus and did not mix with matrix or other de novo structures (Fig. 7b). Occasionally, large
Fig. 3. (a) Construct maps of the mutants used in the study. The last p10 amino acid (aa) encoded is numbered, along with the nucleotide number (nt) corresponding to the start of the BamHI linker. Numbers in parentheses represent the non-p10, non-β-gal amino acids created by the linker sequence. Note that the Ac228 β-gal coding region extends past the wt p10 coding region. (b) Sequence analysis of mutants, indicating the p10 amino acids (capital letters). The last p10 amino acid (number) is given, and BamHI restriction sites are underlined. The stop codon is indicated by three asterisks.

aggregates of tubular structures were seen in association both with FS and forming OBs (Fig. 7c). Periodic electron-dense regions observed along these tubules were similar in appearance to complete nucleocapsids, suggesting there may be aberrant (excessive) nucleocapsid formation.

**Transmission EM examination of OBs**

The ultrastructure of OBs from cells infected with the p10 mutants was investigated following the observations that deletions in the gene affected cellular events associated with their morphogenesis. Vigorous washing procedures during purification were found to dissolve the
Fig. 4. Phase microscopic examination of wt and mutant late-infected S. frugiperda cells (m.o.i. > 10) at 4 days p.i. Cells infected with wt (a) or Ac-231 (c) produce large numbers of OBs relative to Ac229 infection (b). Note the release of OBs in wt-infected preparations (a), and the large, swollen but intact cells of the mutants (b, c).
Fig. 5. AcNPV wt-infected *T. ni* cells (a) 30 h p.i. embedded in L.R. White resin. The VS is in the centre of the nucleus, and is distinct from the surrounding (peripheral) matrix. This difference was exaggerated by omission of buffer additives during fixation, and demonstrates the sensitivity of cells to damage by the L.R. White resin. Seen in the matrix region are forming OBs, nucleolus (Nu), fibrillar bodies (FB), and nucleocapsid bundles (N). (b) Fixation at 38 h p.i. with buffer additives demonstrates the intranuclear FB associated with FS. A nearby OB has an occlusion membrane (arrowheads). Note the nuclear membrane is very difficult to see at this stage of infection. Bar markers represent 2 μm (a) and 0·5 μm (b).
mutant OBs partially, leaving a white powdery residue along the sides of the centrifuge tube, and notably decreasing the yield of pure OBs. Very gentle procedures gave yields more comparable to wt preparations; these samples were fixed and embedded in epoxy resin for examination by TEM.
Fig. 7. Ac231-infected T. ni cells (38 h p.i.). (a) Cells have a poorly differentiated VS located centrally in the nucleus. FS structures are present in the peripheral matrix, as are forming OBs. A region is shown at higher magnification (b) demonstrating FS attached to forming OBs (arrowheads). Nearby are profiles of unattached FS. Occasionally, large bundles of aberrant nucleocapsids (N) are seen associated with OBs and FS (c). Bar markers represent 2 μm (a) and 1 μm (b, c).
Fig. 8. Viral OBs from infected *T. ni* cells, prepared using gentle washing procedures. (a, b) wt OB; (c, d) Ac229 OB; (e, f) Ac231 OB. The occlusion membrane (arrowheads) was present on wt OBs (b), but absent from Ac229 OBs (d), and loosely attached to Ac231 OBs (f). Mutant OBs shed matrix protein during preparation, as indicated by the dark staining of the embedding matrix (c, e) compared to wt (a). Bar markers represent 1 μm (a, c, e) and 100 nm (b, d, f).
Fig. 9. Scanning EM of wt and mutant OBs. (a to d) OBs prepared using gentle wash procedures. (a) wt occlusions were cuboidal with smooth sides. Ac231 OBs (b, d) were similar to wt, although less regular in shape. Ac229 occlusions (c) were consistently fragmented with pitted surfaces. Vigorous washing procedures caused severe damage to Ac229 and Ac231 occlusions (e), relative to only slightly damaged wt occlusions (f). Bar markers represent 10 μm (a, b, e, f), and 2 μm (c, d).
OBs from wt virus infections (Fig. 8a, b) were discrete structures comprising a paracrystalline matrix (polyhedrin) containing enveloped virions, and covered with a mature FS (the OB membrane). Little or no staining was visible in the embedding matrix surrounding the OBs. In contrast, mutant OBs were always seen with high background staining of the embedding matrix (Fig. 8c, e), suggesting a constant shedding of occlusion matrix. The OB membrane was entirely missing from the surface of Ac229 OBs (Fig. 8d), and large profiles of FS were loosely associated with Ac231 OBs (Fig. 8e). Since FS was present in cells infected with both mutants, but was not co-purified with Ac229 occlusions, the FS profiles associated with Ac231 preparations had probably been attached to OBs, and had become separated during processing. OB membranes were attached in an aberrant manner at the surface of Ac231 OBs, and shedding of the OB matrix (polyhedrin) was obvious (Fig. 8f).

**Scanning EM examination of OBs**

Mutant and wt OBs were prepared by both gentle and vigorous wash procedures. They were seeded onto SEM stubs coated with cytochrome c, then fixed and processed. The wt and Ac231 OBs (gently purified) were nearly identical in appearance (Fig. 9a, b), except that the surface of Ac231 OBs was slightly more irregular than that of wt (Fig. 9b, d). However, Ac229 OBs were very irregular in shape regardless of the method of purification (Fig. 9c), and had sharp angles and pitted surfaces where virus had been shed during preparation.

Vigorous washing procedures caused major disruption of both types of mutant OBs (Fig. 9e), compared with minimal damage to wt OBs (Fig. 9f).

**DISCUSSION**

Fibrillar bodies (Fig. 5b) have been seen to accumulate in the cytoplasm and nucleus of cells infected with nuclear polyhedrosis viruses (Harrap, 1970; MacKinnon et al., 1974; Chung et al., 1980). The speculation that p 10 is a major component of these structures is supported by recent immunocytochemical observations (van der Wilk et al., 1987) and the finding that the structures are absent in cells infected with the p10 deletion mutants (Fig. 6, 7). Further, an analysis of morphogenesis and cytopathology of the mutants allows some general conclusions on the role of p10 in baculovirus replication. The peptide is first detected in infected cells at about 10 h p.i. (Rohel & Faulkner, 1984), and the fibrillar bodies appear in the nucleus starting at approximately 21 to 24 h p.i. (MacKinnon et al., 1974). Electron-dense bilaminar sheets (FS) are associated with these intranuclear fibrillar structures and have previously been referred to as spacers (MacKinnon et al., 1974), and cisternae (Hess & Falcon, 1978). Based on comparative examination of cells infected with mutant and wt virus, we conclude that the FS (spacers) and OB membranes are comprised of the same structure at different developmental stages. Previous investigators thought that the fibrillar bodies and occlusion membranes may arise from condensation of polyhedrin (reviewed by Vaughn & Dougherty, 1985). These structures were thought to represent early morphogenic stages in the formation (ontogeny) of OBs (Chung et al., 1980). However, deletion of the p10 gene caused loss of the fibrillar structures while production of OBs and OB membranes were not affected (Fig. 6, 7). Instead, the transport and attachment of FS to the OB surface was rendered ineffective (Fig. 8). The presence of the occlusion membrane was shown to stabilize OBs from disruption by physical stress, as simulated by vigorous vortexing and centrifugation (Fig. 9). Hence we suggest that the function of this structure is to enhance the physical stability of OBs in the environment. A similar function for the FS was recently postulated by Whitt & Manning (1988) in their study on the role of the phosphoprotein pp34, but is demonstrated here for the first time.

Our observations of virus morphogenesis using p10/lacZ deletion/insertion mutants are different from those of Vlak et al. (1988), who noted a total absence of FS in cells infected with β-gal-producing p10 deletion mutants. Absence of the membrane sheets from Ac229 OBs cannot be due to lack of the sheet structures in the infected cells, since we always found them in abundance (Fig. 6a). We conclude that the de novo granular bodies associated with forming OBs (Fig. 6b) probably interfere with the attachment of the sheets to the OBs, and attribute their presence to accumulation of the p10/lacZ fusion product.
The fusion protein is produced in large quantities (Fig. 2) and includes 51 N-terminal amino acids of p10 (Fig. 3). Signal sequences in this segment of p10 could account for site-specific intranuclear accumulations of the granules, while signals for formation of the fibrillar bodies may reside in the carboxy 43 amino acids of the wt p10 molecule.

Similar granular structures were also observed in mutant-infected cells by Vlak et al. (1988), and found to react with antisera raised against β-gal and p10, as determined by immunogold labelling. With Ac229, the presence of fusion protein interfered with the passive transfer of FS onto the OB surface, a process which occurred, albeit inefficiently, in Ac231-infected cells (Fig. 7b, 8). The de novo fusion product structures may act as a simple physical barrier or, more speculatively, may sequester enzymes or molecules required in the attachment process.

Since we show that large accumulations of the fusion product interfere with normal cytopathology, it is difficult to assess whether the complete lack of FS in mutant virus-infected cells previously reported (Vlak et al., 1988) is a legitimate difference from the mutants described here, or is another lacZ-induced artefact. Recent analysis suggests that loss of the FS could be due to other mutations outside the p10 coding region (J. M. Vlak, personal communication).

For gene function analysis, the use of a recombinant baculovirus generated by the white plaque strategy avoids the complication of altered cytopathology while allowing relatively simple selection of recombinants. High production of lacZ was manifested by an uncontrollably intense colour reaction in the presence of X-gal, and made selection of recombinants difficult. Low expression of lacZ, such as in Ac228-infected cells, did not alter gross cytopathology relative to straight deletion (Ac231) or the level of polyhedrin expression (Fig. 2). Sequence analysis determined that lacZ was out of frame in Ac228, but the presence of light blue in the plaques indicated that some active β-galactosidase was produced. Since plaque characteristics were stable and uniform, mutation was not considered a viable explanation. We attribute this expression to a translational frameshift (Hizi et al., 1987; Moore et al., 1987).

The observed decrease in polyhedrin synthesis in Ac229-infected cells (Fig. 2) probably illustrates a metabolic imbalance caused by overproduction of the p10/β-galactosidase fusion product. Polyhedrin synthesis was affected only when lacZ was overproduced; correction of the reading frame of an Ac228-like mutant was associated with decreased polyhedrin. Further, restriction analysis and Southern blotting failed to demonstrate any genome alterations apart from the EcoRI P segment deletions/insertions.

Although study of the deletion mutants has helped to define the intranuclear function of p10, a function for the cytoplasmic form remains unknown. The peptide does not appear in the nucleus until at least 21 h p.i. Instead, it accumulates and persists in the cytoplasm from about 10 h p.i. as a discrete structure. Van der Wilk et al. (1987) speculated that the cytoplasmic accumulations may act as reservoirs of p10. However, Quant-Russell et al. (1987) demonstrated a cross-reactivity of some p10 monoclonal antibodies to the cytoskeleton. The distinct morphology of the cytoplasmic p10 bodies, their persistence through infection, and the antigenic similarity to cytoskeletal elements are not consistent with the proposed functionless storage form of the molecule.

Cytoplasmic and nuclear p10 may have a role in controlling the release of OBs from infected cells, since p10 deletion mutants failed to cause cell lysis (Fig. 4). This could be one process in which cytoplasmic p10 is important, since the fibrillar bodies adjacent to or abutting the nuclear membrane late in infection are associated with severe membrane vesiculation (G. V. Williams, unpublished observations). Also, by late infection the nuclear membrane became very indistinct (Fig. 5b). We suggest that stepwise exhaustion of the host nuclear membrane by p10 leads to eventual rupture. The accumulation of OBs, large fibrillar structures, and persistence of the VS causes severe nuclear swelling (Fig. 4), which would be an indication of internal pressure. Sudden release of nuclear contents would probably cause a sufficient pressure surge to lyse also the plasma membrane.

Other features of viral morphogenesis seem to be affected by deletion of p10. For example, bundles of aberrant tubular structures were seen in the peripheral nucleoplasm of Ac231-infected cells (Fig. 7c), which have been described previously as excess or aberrant capsid formation (MacKinnon et al., 1974; Summers, 1971; Vaughn & Dougherty, 1985). Association
of these nucleocapsid bundles with FS and forming OBs suggests that p10 may be required to separate various processes, perhaps by physically partitioning space until individual components have matured. The simplified morphology of the VS in mutant virus-infected cells separates various processes, perhaps by physically partitioning space until individual transport of viral components, at least those within the nucleus. A fibrillar structure described in epithelial cells infected in vivo (see Harrap, 1970), is morphologically similar to the in vitro nuclear p10 accumulations (Fig. 5b). Mature virus nucleocapsids are aligned on these structures, perhaps being transported to sites of envelopment or incorporation into OBs. We are currently investigating the distribution of p10 in vivo, and examining the consequences of p10 deletion on in vivo replication.

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REFERENCES


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