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Biosynthesis and Localization of the *Autographa californica* Nuclear Polyhedrosis Virus 25K Gene Product

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Mutations of the Ac*M*NPV 25K gene are associated with the "few polyhedra" phenotype (M. J. Fraser *et al.*, 1983, *J. Virol.* 47, 287–300; B. Beames and M. D. Summers, 1989, *Virology* 168, 344–353). Polyclonal antisera was produced and used to investigate the time course of expression and localization of the 25K protein in infected cells. Western blot analysis detected 25K protein in both cytosolic and nuclear extracts from 18–24 hr p.i. through 96 hr p.i. and also in purified viral occlusions, but not in purified virions. Immunogold electron microscopy revealed that 25K protein was predominantly associated with amorphous cytoplasmic structures and to a lesser extent with a more electron-dense structure in the nucleus. Viral occlusions in cell sections were not specifically labeled by 25K antibody. Observations of purified viral occlusions and nuclei prepared for immunogold EM revealed the presence of contaminating amorphous material that was labeled with 25K antibody.

INTRODUCTION

Serial passage of certain members of the nuclear polyhedrosis virus genus of family Baculoviridae (Adams and McClintock, 1991) through cultured cell lines results in the frequent production of "few polyhedra" (FP) mutants (reviewed by Fraser, 1986, 1987). FP mutants exhibit altered plaque morphology, reduced production of viral occlusions, production of occlusions which contain no virions or virions with an abnormal appearance, defective intranuclear envelopment of nucleocapsids, and the enhanced release of infectious virus (Hink and Vail, 1973; Ramoska and Hink, 1974; MacKinnon *et al.*, 1974; Knudson and Harrap, 1976; Potter *et al.*, 1976; Fraser and Hink, 1982; Slavicek *et al.*, 1992). FP mutants frequently contain deletions or insertions in different regions of the genome (Miller and Miller, 1982; Fraser *et al.*, 1983; Kumar and Miller, 1987). The 25K gene locus located at m.u. 37.7–38.8 (*Hin*dIII-I) within the *Autographa californica* nuclear polyhedrosis virus (Ac*M*NPV) genome is a hot spot for such mutations. Deletions of viral DNA and insertions of cellular transposable elements into the 25K gene can produce all of the characteristics commonly associated with the FP phenotype (Fraser *et al.*, 1983, 1985; Beames and Summers, 1988, 1989; Harrison and Summers, unpublished observations). Additionally, Fraser *et al.* (1983) report the absence of a 25 kDa protein from the profiles of cells infected with several different FP mutants containing insertions in the *Hin*dIII-I restriction fragment, further supporting a correlation between the loss or alteration of this protein and the FP phenotype.

The 25K gene is transcribed during the late phase of infection into two 5′ coterminal mRNAs of 0.8 and 1.6 kb length (Beames and Summers, 1989). Both transcripts contain an open reading frame which encodes a 214 amino acid protein of approximately 25 kDa molecular weight. No significant sequence similarity has been detected between the 25K amino acid sequence and sequences in the GenBank database.

In an effort to begin to identify the function of the 25K protein and understand how the loss of function results in the FP phenotype, polyclonal antiserum was produced against bacterially expressed 25K protein and used to study 25K biosynthesis and distribution in cells and virions.

METHODS

Cells and virus

Sf9 cells (cloned from the *Spodoptera frugiperda* IPLB-Sf21-AE cell line, Vaughn *et al.*, 1977) were grown in TNM-FH medium containing 10% heat-inactivated fetal calf serum (JRH Biosciences, Lenexa, KS), amphotericin B, gentamycin, and 0.1% (w/v) pluronic F68 (BASF Wynandotte Corp., Parsippany, NJ; Murhammer and Goochee, 1988). The FP mutants AcFP480-1, AcFP480-2, AcFP875-1, AcFP875-2, and AcFPβgal (Fig. 2A) have been de-

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scribed previously (Beames and Summers, 1988, 1989). The AcMNPV strain E2 (Summers and Smith, 1978) was used as wild-type virus.

Antibody production

The 25K open reading frame was PCR-amplified from a plasmid containing the *Eco*RI-F fragment of Ac*M*NPV, using a 27- and a 28-mer (25Kamp5′, ATGGATCAATTT-GAACAGTTGATTAAC, and 25Kamp3′, CGCTTTTTTACG-AAGTTTAAATTAATTTA). The amplification product was purified by electrophoresis through a low-melting point agarose gel. The termini of the amplification product were repaired with the Klenow fragment of DNA polymerase I and the 5′ ends were phosphorylated. The amplification product was then cloned into the *Stu*I site of the pMAL-c expression vector (New England Biolabs), fusing the first codon of the 25K ORF with the last codon of the factor Xa cleavage site. The 25K insert was sequenced in order to confirm that no errors were introduced during PCR amplification. Purification and cleavage of the *maltose binding protein (MBP)–25K fusion and purification* of 25K away from MBP were carried out according to the manufacturer's directions.

New Zealand White female rabbits were given one primary intramuscular injection of 200 μg of purified 25K protein in Freund's complete adjuvant (Pierce, Rockford, IL) and four secondary subcutaneous injections of 50 μg of protein in Freund's incomplete adjuvant every 4 weeks prior to the final collection of antisera.

Biochemical fractionation of cells and virus

For the generation of cytosolic and nuclear extracts, the detergent-based and detergent-free procedures of Jarvis *et al.* (1992) were followed. For each time point examined, 3×10^6 cells (for the detergent-based procedure) or 9×10^6 cells (for the detergent-free procedure) were infected at an m.o.i. of 10 with Ac*M*NPV-E2, AcFPβ-gal, AcFP480-1, AcFP480-2, AcFP875-1, and AcFP875-2. The cells were harvested and fractions were prepared at 2, 6, 12, 18, 24, 36, 48, 72, and 96 hr postinfection (hpi) for wild-type virus infections and at 48 hpi for the FP mutant virus infections.

For the preparation of protein from occlusion-derived virus (ODV) and budded virus (BV), 3.6×10^8 Sf9 cells were infected with AcMNPV-E2 at an m.o.i. of 10. BV, viral occlusions, and ODV were harvested at 5 days p.i. and ODV and BV envelope and capsid fractions were prepared as described by Braunagel and Summers (1994).

Matrix and envelope ("calyx") fractions were prepared from viral occlusions by the method of Whitt and Manning (1987) with modifications. Briefly, viral occlusions were purified and solubilized by incubation in dilute alkaline solution (DAS; 1 *M* Na_2CO_3, 0.5 *M* NaCl, pH 10.9) in a *ratio of 1 vol DAS: 9 vol polyhedra (40 mg/ml) for 2 hr at* 37°. The dissociated occlusions were centrifuged 10 min at 12,000*g*. The supernatant was used as the matrix fraction, and the pellet was resuspended in DAS and used as the envelope fraction.

Western blot analysis of cell and virus fractions

Cytosolic and nuclear fractions from 5×10^4 cells and 15 μg of protein from the different virion and occlusion fractions were analyzed by SDS–PAGE (Laemmli, 1970) on a 14% gel and either stained with Coomassie blue or transferred to PVDF membranes (Immobilon-P; Millipore, Bedford, MA) using the PolyBlot Electrotransfer semidry apparatus (American Bionetics, Inc, Hayward, CA). After transfer, the membranes were incubated in blocking buffer (25 m*M* Tris (pH 7.4), 140 m*M* NaCl, 0.1% Nonidet P-40, 1% nonfat dry milk, and 0.02% NaN_3) for at least 1 hr. The membranes were then probed with 25K antiserum diluted 1:500 in blocking buffer for 1 hr at room temperature. After three washes in blocking buffer, the membranes were treated with alkaline phosphatase-conjugated *goat anti-rabbit IgG (Sigma Chemical Company,* St. Louis, MO) diluted 1:10,000 in blocking buffer for 1 hr at room temperature. The membranes were washed three more times with blocking buffer and two times with *TBS (25 mM Tris (pH 7.4), 140 mM NaCl)* and then developed with a standard alkaline phosphatase color reaction (Blake *et al.,* 1984).

Immunogold electron microscopy

Sf9 cells (9×10^6/time point) were infected with Ac*M*NPV-E2, AcFPβgal, AcFP480-1, AcFP480-2, AcFP875-1, *and AcFP875-2 at an m.o.i. of 10. The cells* were harvested at 20, 34, and 48 hr postinfection, pelleted, and fixed by incubating 10 min at 4°C in 1% paraformaldehyde–0.5% glutaraldehyde–0.05 *M* sodium cacodylate, pH 7.1, and then 30 min in 2% paraformaldehyde–2.5% glutaraldehyde–0.05 *M* sodium cacodylate, pH 7.1. After washing cells three times with 0.05 *M* sodium cacodylate, pH 7.1, the cells were dehydrated and infiltrated with LR White following a modification of the protocol described by Vandenbosch (1991). Briefly, cells were dehydrated stepwise at 4° in 30, 50, 70, 90, and 100% ethanol for 30 min/step. Following dehydration, the cells were infiltrated with LR White at −20° by incubating first for 1 hr in 1:1 LR White: ethanol and then 1 hr in 3:1 LR White: ethanol, followed by 1 hr in LR White alone, overnight in fresh LR White, and a further 8 hr in fresh LR White. The cells were resuspended in fresh LR White and transferred to gelatin capsules for embedment. The resin was polymerized by incubating capsules at 50° for 48 hr.

Ultrathin sections from blocks of embedded cells were cut on a Reichert–Jung Ultracut E microtome and collected on nickel grids. Gold labeling of sections was *carried out according to a protocol described by Vanden-*

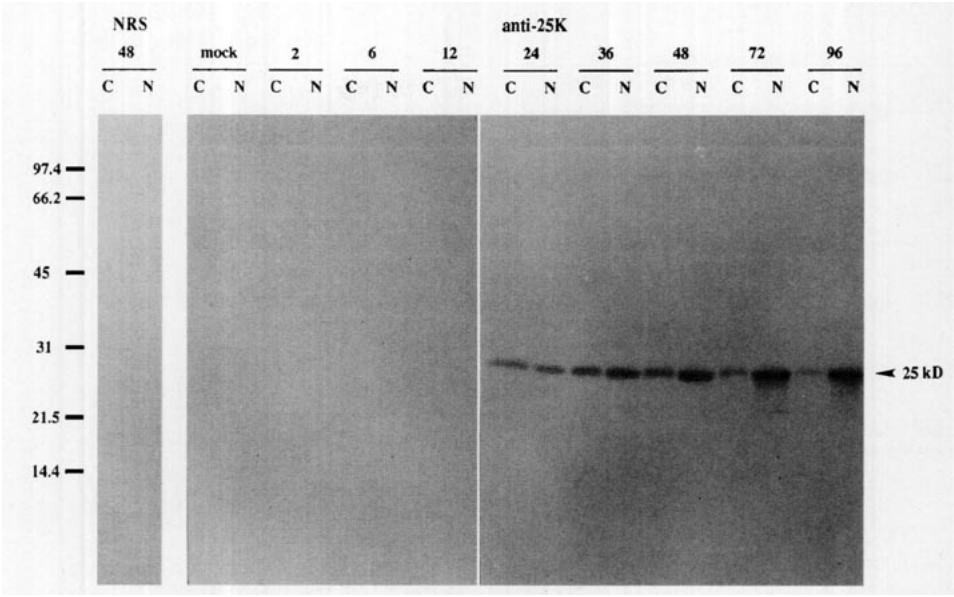

FIG. 1. Western blot analysis of 25K expression. For each time point (indicated in hr p.i. above each pair of lanes), cytosolic (C), and nuclear (N) extracts from 5×10^4 Ac*M*NPV-infected Sf9 cells were analyzed by SDS–PAGE and Western blot with a 1:500 dilution of 25K rabbit polyclonal antiserum. The sizes of protein standards are indicated on the left. NRS, normal rabbit serum.

bosch (1991). Sections were first incubated in blocking buffer (10 m*M* Tris (pH 7.4), 150 m*M* NaCl, 1% [w/v] bovine serum albumin, 0.02% sodium azide, and 0.05% Tween 20) for 15 min. The grids were then immersed in 20-μl drops of primary antibody or preimmune serum at a dilution of 1:100–1:500 in blocking buffer for 1 hr at room temperature. The grids were passed through a series of drops of TBS (10 m*M* Tris (pH 7.4). After removing excess TBS from the grids with filter paper, the grids were immersed in a 20-μl drop of a 1:25 dilution of secondary antibody coupled to 15- to 30-nm gold particles (Amersham Corp., Arlington Heights, IL) for 1 hr at room temperature. After passage through more TBS drops, the sections were either labeled with a second set of primary and secondary antibodies (for double-labeling experiments) or stained 1 min in 2% uranyl acetate and 20 sec in 0.2% lead citrate prepared by the method of Venable and Coggeshall (1965) and examined using a Zeiss 10C transmission electron microscope set at 60 kV.

RESULTS

Analysis of 25K protein in cytosolic and nuclear extracts

The AcMNPV 25K gene was cloned into the pMal-c bacterial expression vector. The maltose binding protein–25K fusion protein produced in bacteria was cleaved, and the 25K protein was purified and used to produce polyclonal antisera. The antisera was used to probe extracts from cytosolic and nuclear fractions of infected Sf9 cells prepared by a detergent-free procedure (Jarvis *et al.*, 1992). A protein of approximately 25 kDa molecular weight was detected in Ac*M*NPV-infected cells but not in mock-infected cells (Fig. 1). The 25K protein was first detected at 24 hr p.i., although in some experiments a minor amount of 25K protein could be detected at 18 hr p.i. (data not shown). These results are consistent with the assignment of the 25K gene as a member of the late transcriptional class of baculovirus genes (Beames and Summers, 1988, 1989). At 24 hpi the 25K protein was equally distributed between cytosolic and nuclear extracts while by 72 and 96 hr p.i. it was concentrated in the nuclear fractions.

The expression of the 25K gene in five 25K mutants (AcFPβgal, AcFP480-1, AcFP480-2, AcFP875-1, and AcFP875-2; Beames and Summers, 1988, 1989) was also examined. The nature of the alterations in the 25K genes of these viruses are represented in Fig. 2A. Because AcFPβgal, AcFP480-1, and AcFP480-2 encode truncated or fused forms of 25K protein, the relative distribution of 25K protein in cytosolic and nuclear fractions of cells infected with these viruses may indicate the location of potential nuclear targeting or retention signals in the 25K amino acid sequence. The AcFP875-2 virus produces more occlusions and occludes more virions than other FP viruses (Beames and Summers, 1988; Harrison and Summers, submitted for publication). To ascertain the basis of the phenotype of this virus, AcFP875-2 protein was examined to see if wild-type 25K protein was present. The AcFP875-1 virus contains a deletion which encompasses most of the 25K open reading frame, and no transcripts are detected in AcFP875-1 RNA samples with probes derived from the 25K region (Beames and Summers, 1988). Protein samples from cells infected with this virus were examined to see if any protein reacting with the 25K antiserum was present. In Western blots, the 25K-β-galactosidase fusion protein encoded by AcFPβ-gal and the truncated forms of 25K encoded by AcFP480-

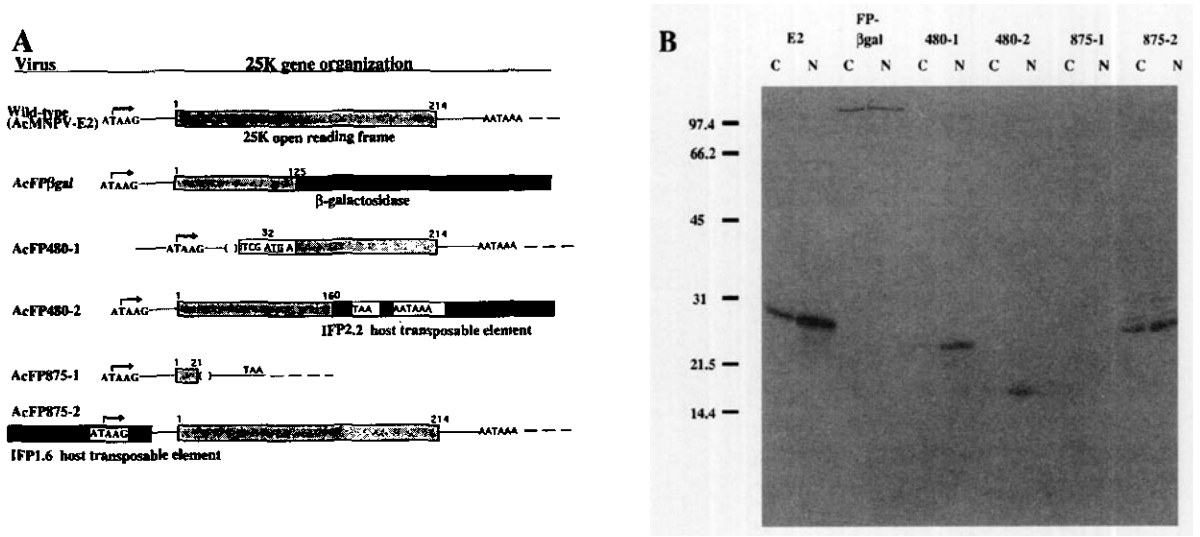

FIG. 2. Expression of mutant forms of 25K. (A) 25K gene organization in wild-type (E2) and FP mutant viruses. Amino acid positions (numbers), termination codons (TAA), polyadenylation signals (AATAAA), and the in-frame initiation codon for the AcFP480-1 25K gene (TCG*ATG*A) are indicated. (B) Western blot analysis of protein from infected Sf9 cells harvested at 48 hr p.i. Cytosolic (C) and nuclear (N) extracts were analyzed as described in the legend of Fig. 1. The molecular weights of size standards are indicated to the left of (B).

1 and AcFP480-2 were detected in both the cytosolic and nuclear fractions of infected cells. The signal from the Western blot of 25K produced by these viruses is less intense than the 25K signal from the wild-type virus. A reduced quantity of 25K protein was detected in cytosolic and nuclear extracts from AcFP875-2-infected cells. The 25K antisera did not react with any protein present in extracts from cells infected with AcFP875-1 (Fig. 2B).

All of the results described above are consistent with results obtained in studies with cellular fractions prepared by a detergent-based procedure (data not shown).

Analysis of 25K protein in virion and occlusion fractions

The 25K antisera was used to detect 25K protein in envelope and capsid fractions of BV and ODV virions and in the envelope and matrix fractions of solubilized viral occlusions. Western blot analysis of virion fractions revealed that, although 25K was detectable in total protein from only 5×10^4 cells, it was not detected in 15 μg of envelope fractions and barely detected in 15 μg of capsid fractions and total virion protein (Fig. 3A). A Coomassie blue-stained gel of the same quantities of protein revealed an enrichment of proteins of molecular weights similar to those of known structural proteins (vp39, gp67) in the virion fractions compared to total cellular protein (Fig. 3B).

Western blot analysis of occlusion protein revealed that 25K was present in 15 μg of protein from both matrix and envelope fractions of viral occlusions (Fig. 4A). The doublet seen in the occlusion protein lanes may be due to degradation of 25K occurring during solubilization of the occlusions in dilute alkaline solution.

Localization of 25K protein by immunogold electron microscopy

Immunogold electron microscopy of infected Sf9 cells was done to confirm the localization of 25K determined by biochemical fractionation. Surprisingly, the 25K antisera most strongly labeled amorphous structures present in the cytoplasm of cells infected with AcMNPV-E2 (Figs. 5a–c). These structures were variable in size and present in one or more copies per cell section (Fig. 5b). A more electron-dense structure present at the periphery of nuclei was also labeled to a lesser extent (Figs. 5a and 5d). We could not positively identify either of these structures, although the nuclear structures resemble chromatin. No specific labeling of these or any other cellular or viral structures was observed in sections from cells infected with AcFPβgal, AcFP480-1, AcFP480-2, or AcFP875-1 (data not shown). In sections of cells infected with AcFP875-2, both structures were labeled at a reduced intensity (Figs. 5e and 5f). No labeling was observed with preimmune serum or with the gold-conjugated secondary antibody alone (data not shown), nor were sections of mock-infected cells labeled with the 25K antiserum (data not shown).

In contrast with the results from the cellular fractionation studies, the most intense labeling of cells occurred with the amorphous structures in the cytoplasm of infected cells, suggesting that most of the detectable 25K protein in infected cells is present in the cytoplasm. Also, viral occlusions were not specifically labeled by the 25K antisera. Occlusions produced by both wild-type (E2) virus and AcFP875-1 (which is missing most of the 25K open reading frame and does not produce any protein recognized by the 25K antisera) were weakly labeled

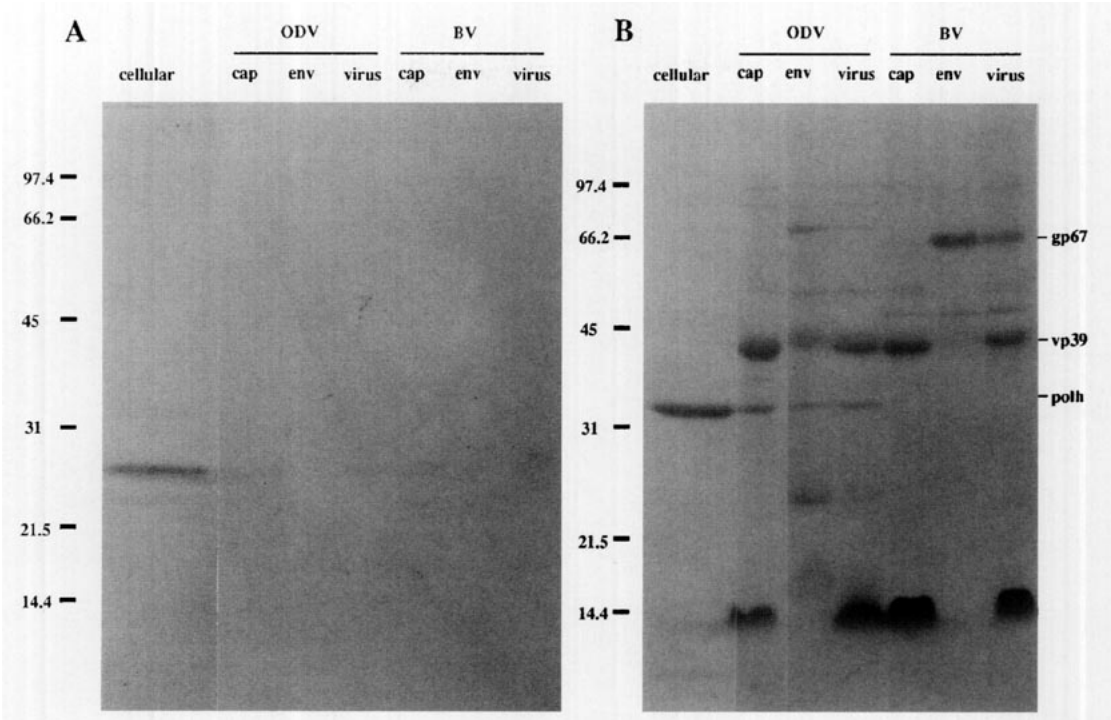

FIG. 3. Analysis of virion protein for the presence of 25K. (A) Western blot analysis of protein from 5×10^4 cells harvested at 56 hr p.i., 15 μg of capsid (cap) and envelope (env) fractions and unfractionated virion protein (virus) of occlusion-derived virus (ODV) or budded virus (BV). (B) Coomassie blue-stained SDS–PAGE gel of the same amounts of protein analyzed in (A). Proteins of the molecular weight expected for gp67 (Whitford *et al.*, 1989), vp39 (Thiem and Miller, 1989), and polyhedrin (polh; Smith *et al.*, 1983) are indicated to the right. The molecular weights of size standards are indicated to the left of (A) and (B).

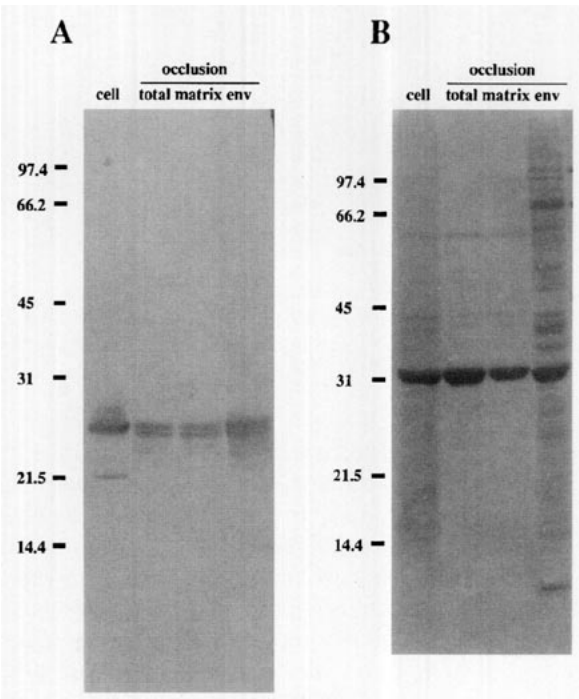

FIG. 4. Analysis of viral occlusion protein for the presence of 25K. (A) Western blot analysis of protein from 5×10^4 cells harvested at 56 hr p.i., 15 μg of unfractionated protein (total) from purified, solubilized occlusions, envelope (env), and matrix fractions of viral occlusions. (B) Coomassie blue-stained SDS–PAGE gel of the same amounts of protein analyzed in (A). The molecular weights of size standards are indicated to the left of (A) and (B).

(compare Figs. 5g and 5h), indicating that the labeling of occlusions by the 25K antibodies was nonspecific. The apparent discrepancy between our cellular fractionation and immunogold EM analyses could be explained if the amorphous cytoplasmic structures copurified with nuclei and viral occlusions in the gradients used to isolate each. To test this, nuclei and viral occlusions were prepared by the methods employed for generating nuclear extracts and purified occlusion protein. The nuclei and viral occlusions were fixed, embedded, and examined by immunogold EM. Both the nuclei (Figs. 6a and 6b) and the viral occlusions (Fig. 6c) were contaminated with structures resembling the amorphous cytoplasmic structures present in whole cells labeled with the 25K antibody. These structures were present in nuclei prepared by both the detergent-based and the detergent-free methods (data not shown). These data suggest that the relatively high proportion of 25K protein in nuclear extracts and presence of 25K in preparations of viral occlusion protein are due to the copurification of 25K-associated structures with nuclei and occlusions.

The *Orgyia pseudotsugata M*NPV gp37 protein (Gross *et al.*, 1993) was reported to localize to inclusions in the cytoplasm of Op*M*NPV-infected Ld-652Y cells. To determine whether 25K and gp37 are associated with the same cytoplasmic structure, gp37 antibodies were used to probe sections of Ac*M*NPV-infected Sf9 cells. The gp37 antiserum did not label the amorphous 25K-associ-

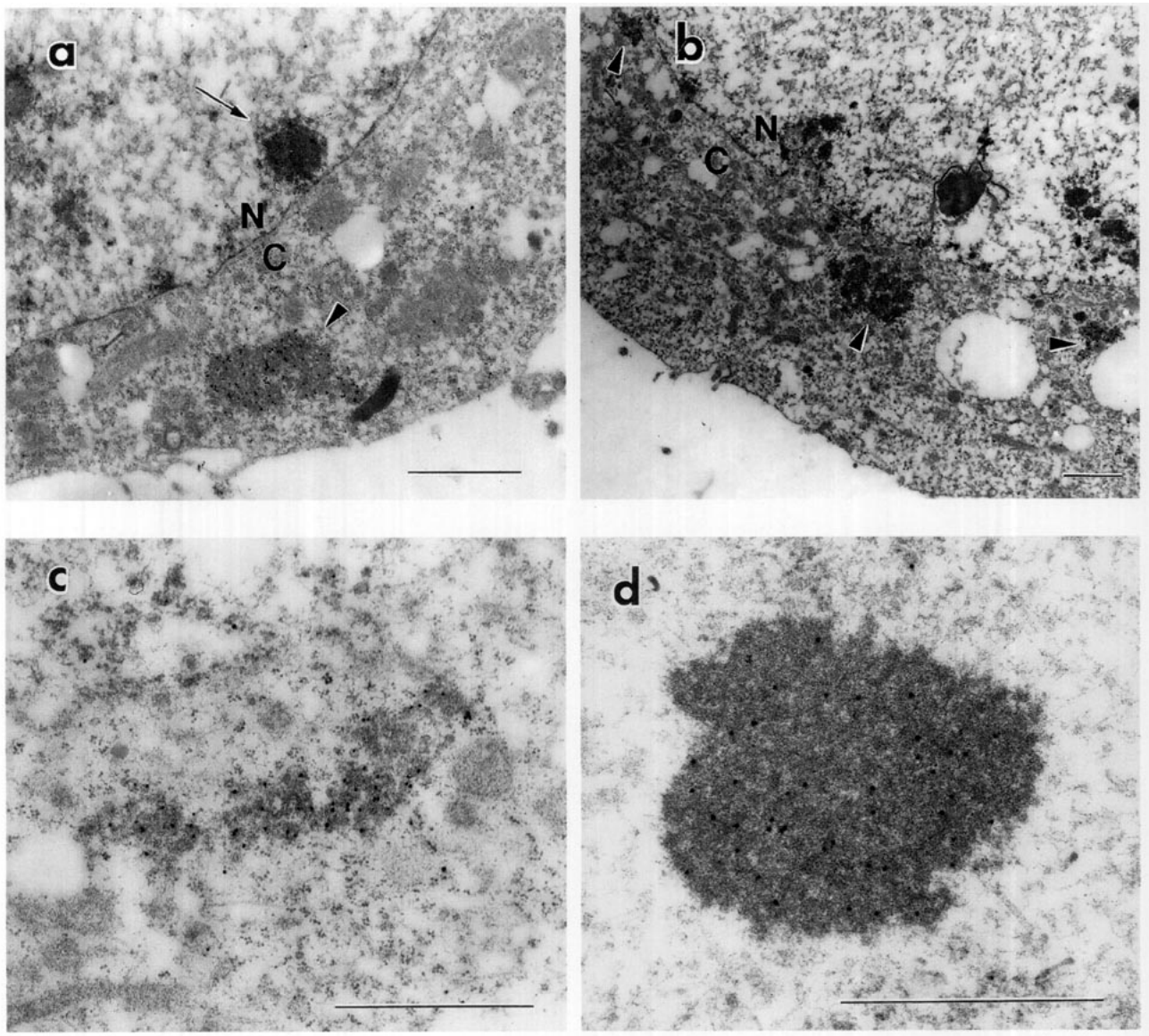

FIG. 5. Immunogold staining of infected Sf9 cells. (a–d, g) Cells infected with wild-type (E2) Ac*M*NPV and harvested at 34 hr p.i. (a,b,g) or 48 hr p.i. (c,d). (e,f) Cells infected with AcFP875-2 and harvested at 48 hr p.i. (h) Cells infected with AcFP875-1 and harvested at 48 hr p.i. The 25K antiserum was diluted 1:400 (a–f, h) or 1:500 (g). Arrowheads, cytoplasmic labeled structures; arrows, nuclear labeled structures; C, cytoplasm; N, nucleus. Bars, 1.0 μm.

ated structures. Instead, rhombus-shaped structures which were much smaller than the 25K-associated structures and resembled the structures labeled in Op*M*NPV-infected cells (Gross *et al.*, 1993; Fig. 6) were labeled by anti-gp37 (*data not shown*). Also, neither the cytoplasmic nor the nuclear 25K-associated structures were labeled by polyhedrin monoclonal antibodies in a double-labeling *experiment (data not shown)*.

DISCUSSION

A mutation in the Ac*M*NPV 25K gene is sufficient to cause all the characteristics commonly associated with the FP phenotype (Fraser *et al.*, 1983; Beames and Summers, 1989; Harrison and Summers, submitted for publication). In this study, 25K antibodies were produced and used to characterize its biosynthesis and cellular localization. Western blot analysis confirmed that 25K protein is a late gene expression product. The reduced proportion of 25K protein in cytosolic extracts of cells harvested at 72 and 96 hpi might be accounted for by the lysis of cells at this late stage of infection and by the premature breakage of cells upon washing with hypotonic buffer prior to dounce homogenization. It was observed that cells harvested at this time of infection required fewer strokes with the pestle to achieve 95% cell breakage.

The 25K mutant viruses AcFPβgal, AcFP480-1, and AcFP480-2 produce forms of 25K protein which are recognized by 25K antibodies. However, each mutant virus exhibits a level of impaired occlusion production, virion

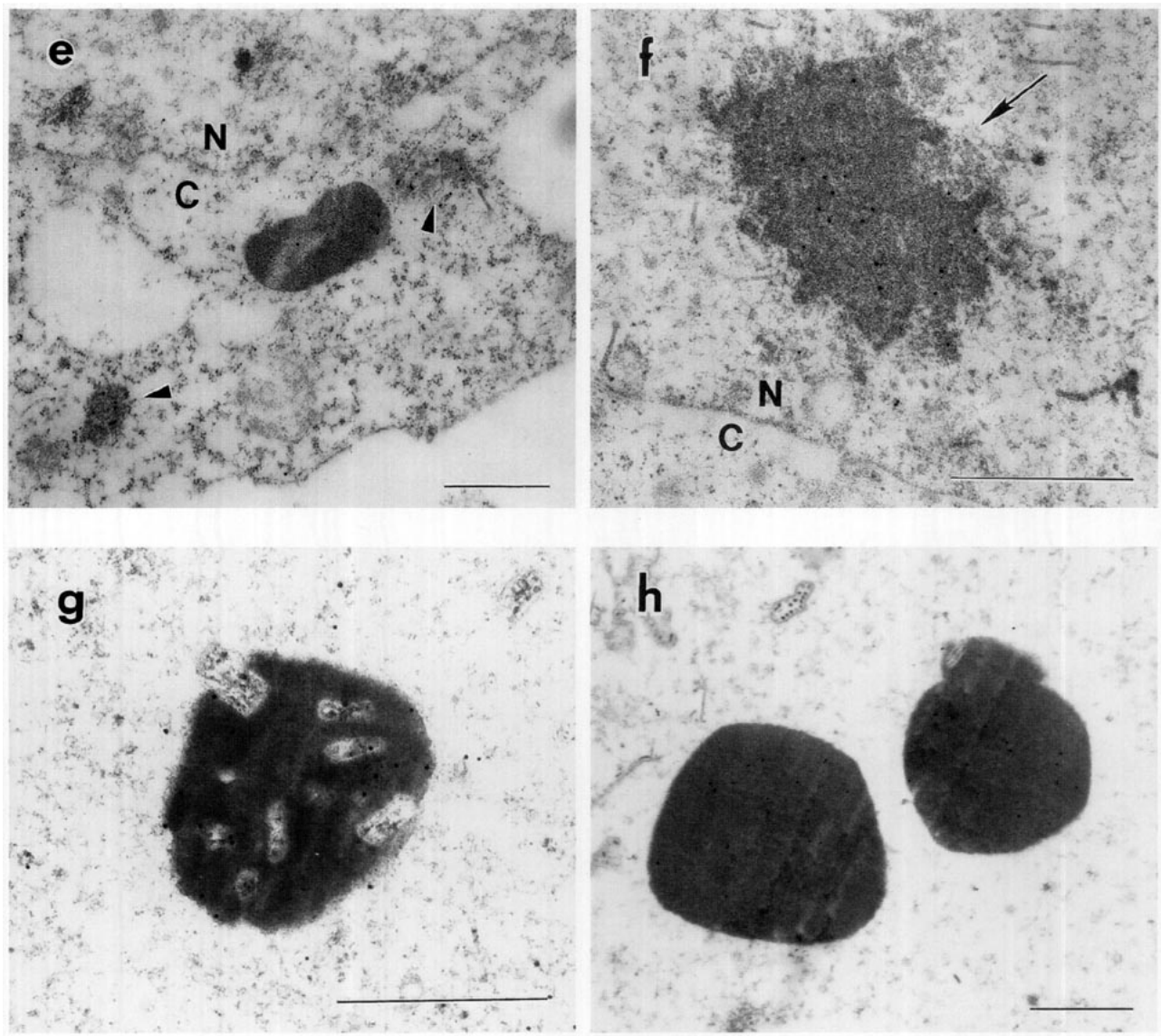

FIG. 5—*Continued*

occlusion, and intranuclear nucleocapsid envelopment which is indistinguishable from that seen with AcFP875-1 (Harrison and Summers, submitted). AcFP875-1 produces no antigen recognizable by 25K antiserum (Fig. 2B). With truncated forms of 25K produced by AcFP480-1 and AcFP480-2, it is uncertain whether the reduction in 25K protein is due to the reduced levels of transcripts which could encode these proteins (Beames and Summers, 1988) or to destabilization of the proteins caused by deletions of the amino acid sequence. Alternatively, the weaker signal obtained with the truncated forms of 25K may be due to a reduced affinity of the antibody for the truncated proteins. It is difficult to conclude on the basis of these data whether the FP phenotypes of AcFP480-1 and AcFP480-2 are caused by the reduced quantity of 25K protein or by the N- and C-terminal truncations of 25K proteins encoded by these viruses.

The 25K proteins encoded by AcFPβgal, AcFP480-1, and AcFP480-2 are detectable in nuclear fractions (Fig. 2B). However, the presence of nuclear localization signals in these proteins could not be clearly identified because of contamination of the nuclear fractions with cytoplasmic 25K protein (Figs. 5a and 5b). It is possible that the occurrence of 25K proteins encoded by AcFPβgal, AcFP480-1, and AcFP480-2 in the nuclear fractions might be due to contamination of nuclei with the cytoplasmic structures with which 25K associates. In this case, the amino acid sequences required for the association of 25K with these structures would need to be present in the mutant forms of the protein. Finally, it is interesting to note the correlation between the reduced level of wild-type 25K produced by AcFP875-2 and (1) a level of occlusion production which is intermediate between wild type and FP (Beames and Summers, 1988), along with (2)

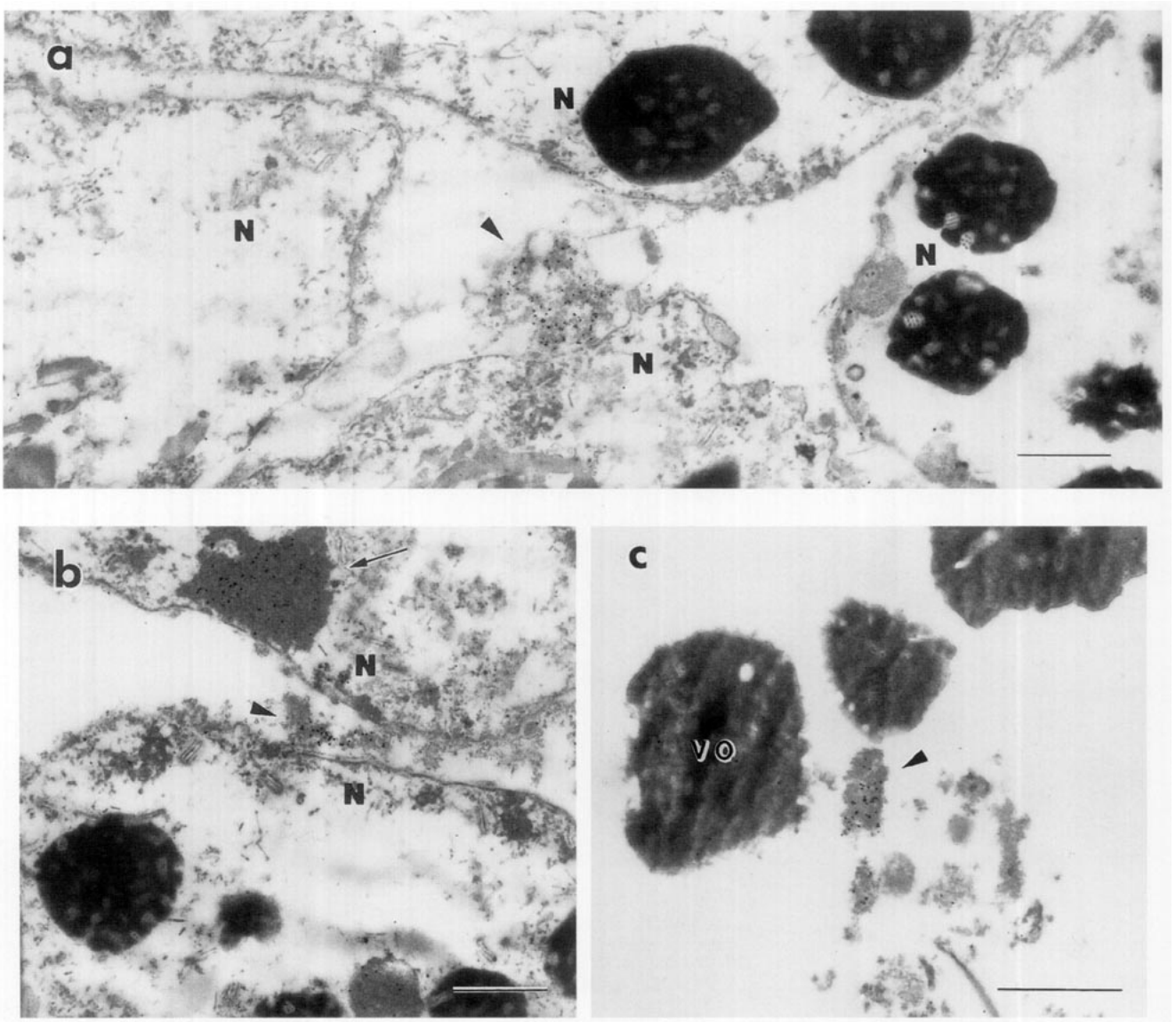

FIG. 6. Immunogold staining of nuclei (a,b) and viral occlusions (c) purified from Ac*M*NPV-infected Sf9 cells. Nuclei were prepared at 48 hr p.i.; viral occlusions were prepared 5 days p.i. The 25K antiserum was diluted 1:400. Arrowheads, cytoplasmic labeled structures; arrows, nuclear labeled structures; C, cytoplasm; N, nucleus; VO, viral occlusion. Bars, 1.0 μm.

impairment of intranuclear envelopment and a reduction in virion occlusion (Harrison and Summers, submitted for publication).

Wood (1980) speculated that a defect in an ODV envelope protein responsible for nucleation of the polyhedrin crystalline lattice around ODV virions could account for the FP phenotype, at least with respect to the lack of virions within viral occlusions and the reduced number of occlusions produced. Our analysis of virion envelope and capsid fractions failed to demonstrate that the 25K protein is a part of the ODV envelope or of any other component of ODV or BV virions. Rather, the 25K protein was shown to be associated primarily with an amorphous cytoplasmic structure of unknown identity, and to a lesser extent with a nuclear structure that resembles chromatin. Structures labeled by the 25K antiserum *which resembled the labeled structures in the cytoplasm* of infected cells were present in preparations of purified nuclei and viral occlusions. This observation can account for the initial biochemical results indicating that 25K was found in viral occlusion protein and at the relatively high levels in nuclear extracts. It is difficult to tell whether the cytoplasmic structure is viral in origin, virally induced, or cellular, because the only clearly distinguishing feature which these structures possess is their capacity to be labeled with 25K antiserum. Identification of these structures in uninfected cells or cells infected with AcFP875-1 is uncertain. High magnification micrographs of the structures reveal that ribosomes are associated with the edges of the structure and can also sometimes be spotted within the structure, suggesting that the cytoplasmic structures might be associated with protein synthesis.

However, ribosomes and polyribosomes that are not associated with the labeled cytoplasmic structures are also observed throughout the cytoplasm. The nuclear labeled structure resembles chromatin, but only one "patch" of this relatively electron-dense material was observed to be labeled per nucleus. This nuclear structure might be the nucleolus of the cell. However, these structures lack the substructural features commonly associated with nucleoli (Goessens, 1984).

The inability to label the cytoplasmic and nuclear structures in sections from cells infected with AcFPβgal, AcFP480-1, and AcFP480-2 may be due to the reduced amount of 25K protein produced by these mutants or to the loss or unavailability of epitopes which are still recognized by the 25K antisera after fixation and embedment in resin. Alternatively, the 25K proteins produced by these viruses may not be sufficiently concentrated in any region in the cell to be detectable above background by immunogold EM.

The relationship between the location of the 25K gene product and phenotype caused by 25K mutation is unclear. The results of this study do not support the possibility that the 25K protein participates directly in intranuclear nucleocapsid envelopment or virion occlusion. The report of Jarvis *et al.* (1992) that polyhedrin synthesis and nuclear localization are reduced in cells infected with AcFPβgal suggests that the 25K protein may influence occlusion assembly indirectly by playing a role in the expression of the genes involved in the occlusion assembly pathway. Wei and Volkman (1992) report an inverse correlation between levels of polyhedrin and p10 mRNA synthesis and levels of actin mRNA synthesis in Ac*M*NPV-infected cells, suggesting a negative regulatory role for actin in the accumulation of polyhedrin mRNA. The possibility that the 25K protein may interact with actin to prevent an actin-mediated reduction in polyhedrin gene expression was tested by an immunogold double-labeling experiment with actin and 25K antibodies. The 25K-associated structures were not labeled by an actin monoclonal antibody (data not shown), indicating that the 25K protein and actin did not colocalize in the cell. Another possibility is that the 25K protein, which is located primarily in the cytoplasm, could play a role in the translation or stability of mRNAs encoding proteins required for the correct assembly of ODV and viral occlusions. Studies are currently underway to explore these possibilities and define the role of the 25K protein in baculovirus gene expression.

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REFERENCES

Adams, J. R., and McClintock, J. T. (1991). Baculoviridae. Nuclear polyhedrosis viruses, part 1: Nuclear polyhedrosis viruses of insects. *In* "Atlas of Invertebrate Viruses" (J. R. Adams and J. R. Bonami, Eds.), pp. 87–204. CRC Press, Boca Raton, FL.

Beames, B., and Summers, M. D. (1988). Comparisons of host cell DNA insertions and altered transcription at the site of insertions in few polyhedra baculovirus mutants. *Virology* **162,** 206–220.

Beames, B., and Summers, M. D. (1989). Location and nucleotide sequence of the 25 K protein missing from baculovirus few polyhedra (FP) mutants. *Virology* **168,** 344–353.

Blake, M. S., Johnston, K. H., Russell-Jones, G. J., and Gotschlich, E. C. (1984). A rapid, sensitive method for detection of alkaline phosphatase conjugated anti-antibody on Western blots. *Anal. Biochem.* **36,** 175–179.

Braunagel, S. C., and Summers, M. D. (1994). *Autographa californica* nuclear polyhedrosis virus, PDV, and ECV viral envelopes and nucleocapsids: Structural proteins, antigens, lipid and fatty acid profiles. *Virology* **202,** 315–328.

Fraser, M. J., and Hink, W. F. (1982). The isolation and characterization of the MP and FP plaque variants of *Galleria mellonella* polyhedrosis virus. *Virology* **117,** 366–378.

Fraser, M. J., Smith, G. E., and Summers, M. D. (1983). Acquisition of host cell DNA sequences by baculoviruses: relationship between host DNA insertions and FP mutants of *Autographa californica* and *Galleria mellonella* nuclear polyhedrosis viruses. *J. Virol.* **47,** 287–300.

Fraser, M. J., Brusca, J. S., Smith, G. E., and Summers, M. D. (1985). Transposon-mediated mutagenesis of a baculovirus. *Virology* **145,** 356–361.

Fraser, M. J. (1986). Transposon-mediated mutagenesis of baculoviruses: transposon shuttling and implications for speciation. *Ann. Entomol. Soc. Am.* **79,** 773–783.

Fraser, M. J. (1987). FP mutation of nuclear polyhedrosis viruses: A novel system for the study of transposon-mediated mutagenesis. *In* "Biotechnology in Invertebrate Pathology and Cell Culture" (K. Maramorosch, Ed.), pp. 265–293. Academic Press, New York.

Goessens, G. (1984). Nucleolar structure. *Int. Rev. Cytol.* **87,** 107–158.

Gross, C. H., Wolgamot, G. M., Russell, R. L. Q., Pearson, M. N., and Rohrmann, G. F. (1993). A 37-kilodalton glycoprotein from a baculovirus of *Orgyia pseudotsugata* is localized to cytoplasmic inclusion bodies. *J. Virol.* **67,** 469–475.

Hink, W. F., and Vail, P. V. (1973). A plaque assay for titration of alfalfa looper nuclear polyhedrosis virus in a cabbage looper (TN-368) cell line. *J. Invertebr. Pathol.* **22,** 168–174.

Jarvis, D. L., Bohlmeyer, D. A., and Garcia, Jr., A. (1992). Enhancement of polyhedrin nuclear localization during baculovirus infection. *J. Virol.* **66,** 6903–6911.

Knudson, D. L., and Harrap, K. A. (1976). Replication of a nuclear polyhedrosis virus in a continuous cell culture of *Spodoptera frugiperda:* Microscopy study of the sequence of events of the virus infection. *J. Virol.* **17,** 254–268.

Kumar, S., and Miller, L. K. (1987). Effects of serial passage of *Autographa californica* nuclear polyhedrosis virus in cell culture. *Virus Res.* **7,** 335–349.

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

MacKinnon, E. A., Henderson, J. F., Stoltz, D. B., and Faulkner, P. (1974). Morphogenesis of nuclear polyhedrosis virus under conditions of prolonged passage *in vitro*. *J. Ultrastruct. Res.* **49,** 419–435.

Miller, D. W., and Miller, L. K. (1982). A virus mutant with an insertion of a copia-like transposable element. *Nature* (London) **299,** 562–564.

Murhammer, D. W., and Goochee, C. F. (1988). The scaleup of insect cell cultures: protective effects of pluronic F-68. *Bio/Technology* **6**, 1411–1418.

Potter, K. N., Faulkner, P., and MacKinnon, E. A. (1976). Strain selection during serial passage of *Trichoplusia ni* nuclear polyhedrosis virus. *J. Virol.* **18**, 1040–1050.

Ramoska, W. A., and Hink, W. F. (1974). Electron microscope examination of two plaque variants from a nuclear polyhedrosis virus of the alfalfa looper, *Autographa californica*. *J. Invertebr. Pathol.* **23**, 197–201.

Slavicek, J. M., Podgewaite, J., and Lanner-Herrera, C. (1992). Properties of two *Lymantra dispar* nuclear polyhedrosis virus isolates obtained from the microbial pesticide Gypchek. *J. Invertebr. Pathol.* **59**, 142–148.

Smith, G. E., Vlak, J. M., and Summers, M. D. (1983). Physical analysis of *Autographa californica* nuclear polyhedrosis virus transcripts for polyhedrin and 10,000-molecular-weight protein. *J. Virol.* **45**, 215–225.

Summers, M. D., and Smith, G. E. (1978). Baculovirus structural polypeptides. *Virology* **84**, 390–402.

Summers, M. D., and Smith, G. E. (1987). "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures." Tex. Agric. Exp. Stn. Bull. 1555.

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

Vandenbosch, K. A. (1991). Immunogold labeling. *In* "Electron Microscopy of Plant Cells" (J. L. Hall and C. Hawes, Eds.), pp. 181–218. Academic Press, New York.

Vaughn, J. L., Goodwin, R. H., Tompkins, G. J., and McCawley, P. (1977). The establishment of two cell lines from the insect *Spodoptera frugiperda* (Lepidoptera; Noctuidae). *In Vitro* **13**, 213–217.

Venable, J. H., and Coggeshall, R. (1965). A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* **25**, 407–408.

Wei, N., and Volkman, L. E. (1992). Hyperexpression of baculovirus polyhedrin and p10 is inversely correlated with actin synthesis. *Virology* **191**, 42–48.

Whitford, M., Stewart, S., Kuzio, J., and Faulkner, P. (1989). Identification and sequence analysis of a gene encoding gp67, an abundant envelope glycoprotein of the baculovirus *Autographa californica* nuclear polyhedrosis virus. *J. Virol.* **63**, 1393–1399.

Whitt, M. A., and Manning, J. S. (1987). Role of chelating agents, monovalent anion and cation in the dissociation of *Autographa californica* nuclear polyhedrosis virus occlusion body. *J. Invertebr. Pathol.* **49**, 61–69.

Wood, H. A. (1980). Isolation and replication of an occlusion body-deficient mutant of the *Autographa californica* nuclear polyhedrosis virus. *Virology* **105**, 338–344.