JOURNAL OF VIROLOGY, Oct. 1993, p. 5859–5866
0022-538X/93/105859-08$02.00/0

Vol. 67, No. 10

The 1,629-Nucleotide Open Reading Frame Located Downstream of the *Autographa californica* Nuclear Polyhedrosis Virus Polyhedrin Gene Encodes a Nucleocapsid-Associated Phosphoprotein

JORGE E. VIALARD[1] AND CHRISTOPHER D. RICHARDSON[1,2]*

*Department of Microbiology and Immunology, McGill University, 3775 University Street, Montréal, Québec, Canada H3A 2B4,[1] and Virology Group, Biotechnology Research Institute, National Research Council of Canada, 6100 Royalmount Avenue, Montréal, Québec, Canada H4P 2R2[2]**

Received 21 April 1993/Accepted 13 July 1993

A 78-kDa protein was produced in bacteria from a clone of the 1,629-nucleotide open reading frame located immediately downstream from the polyhedrin gene of *Autographa californica* nuclear polyhedrosis virus. The identity of this protein was confirmed by its reactivity with peptide antiserum and amino terminal peptide sequencing after purification from transformed bacteria. The polypeptide was used to produce polyclonal antisera in rabbits. Immunoblot analysis of insect cells infected with the baculovirus indicated that two related proteins with molecular masses of 78 and 83 kDa were synthesized late in infection. Biochemical fractionation studies indicated that both of these proteins were present in purified nucleocapsids from budded and occluded virus preparations. Immunoprecipitation of ^{32}P-labeled proteins and treatment of purified nucleocapsids with alkaline phosphatase demonstrated that the 83-kDa protein was a phosphorylated derivative of the 78-kDa protein. Furthermore, immunoelectron microscopy revealed that the proteins were localized to regions of nucleocapsid assembly within the infected cell and appeared to be associated with the end structures of mature nucleocapsids.

Autographa californica nuclear polyhedrosis virus (Ac*M* NPV) is a member of the *Baculoviridae*, a family of double-stranded DNA viruses with rod-shaped capsids which infect a number of arthropods. After infection, these viruses produce two different types of virions (6). The extracellular, or budded virus (BV), is required for cell-to-cell transmission and acquires an envelope by budding from the plasma membrane of the infected cell. The occluded, or polyhedron-derived virus (PDV), is required for transmission in the external environment. This viral type acquires its envelope in the nuclei of infected cells and may contain single or multiple nucleocapsids. The PDVs are embedded within nuclear occlusion bodies (OBs), also known as polyhedra, which protect them from the environment until they are ingested by a susceptible insect. Once exposed to the alkaline conditions of the insect midgut, the OBs are dissolved, the virions are released, and the infectious cycle proceeds.

Several baculovirus genes encoding virion-associated proteins have been identified previously and are described in a recent review by Rohrmann (37). Some of these proteins appear to be unique to each type of virion. For example, gp67 (or gp64) is an envelope protein required for membrane fusion and is present only in the BV (5, 7, 47), while gp41 (or p40), a protein whose function is not presently known, has been reported to be localized in the space between the PDV nucleocapsid and its envelope (22, 26, 27, 45, 46). In addition, the gene encoding a protein (p74) which is required for PDV infectivity has been identified (15, 19), but it is not presently clear whether this protein is actually associated with the PDV or is a component of the OBs. Recently, Gross et al. (12) described a protein (p16) which appears to be associated with a temporary viral envelope in the cytoplasm of infected cells. This envelope is obtained from the nuclear membrane as the nucleocapsids bud into the cytoplasm and is not present in BV or PDV. In contrast to the proteins described above, the nucleocapsid proteins identified thus far are common to both PDV and BV. The major capsid protein, as determined by its abundance and distribution throughout the nucleocapsid, is p39 (3, 4, 32, 38, 43). A second capsid protein, p87 of *Orgyia pseudotsugata* NPV, and p80, its homolog in Ac*M*NPV, have also been described (21, 25). However, this protein is not as abundant as p39, and its function is presently unknown. Recently, a 24-kDa protein associated with both BV and PDV nucleocapsids was identified and it seems to be evenly distributed throughout the nucleocapsids (2, 11, 28, 47, 51). Finally, a small basically charged DNA-binding protein, p6.5-7.9, is associated with both virion types. This protein may be involved in packaging of the viral genome (23, 39, 48–50).

Recently, a 1,629-nucleotide open reading frame (ORF8) from the *Eco*RI-I fragment of Ac*M*NPV was sequenced and predicted to encode a proline-rich protein with a molecular mass of 60.6 kDa (35). Transcriptional analysis determined that ORF8 is expressed late in infection (29, 33, 35) and may be regulated by transcription from the polyhedrin gene, which is located adjacent to ORF8 (29). In addition, ORF8 transcription differs between cells in culture and in different insect tissues; a cellular homolog(s) may also be present in uninfected insect cells (33). Finally, ORF8 appears to be essential to the viral life cycle in culture because a mutant virus at this locus could not be propagated in the absence of wild-type helper virus (35). In this report, we describe the synthesis, biochemical characterization, and localization of p78/83, the translated product of ORF8. Polyclonal antibodies were raised against p78 produced in bacteria. Subsequent

* Corresponding author.

immunoblot analysis demonstrated that p78/83 is synthesized late in Ac*M*NPV infection and is a component of the nucleocapsid. It is present in phosphorylated and unphosphorylated forms. Finally, immunoelectron microscopy studies suggest that p78/83 is associated with an end structure of baculovirus nucleocapsids.

MATERIALS AND METHODS

Cells and virus. *Spodoptera frugiperda* (Sf9) insect cells and Ac*M*NPV were obtained from Max Summers, Texas A&M University, College Station. Virus and cells were propagated as previously described (41). Infections were performed by incubating cells with virus at a multiplicity of infection of 5 PFU per cell for 1 h followed by removal of the viral inoculum. The zero point of infection corresponded to the addition of virus to cells.

Recombinant bacterial plasmid construction and expression. A DNA fragment containing ORF8 was synthesized by polymerase chain reaction (PCR) with oligonucleotide primers corresponding to the 5′ (ACGAATCGTAGATATGAA) and 3′ (TTAAGCGCTAGATTCTGT) ends of the ORF8 coding sequence (35). The PCR product was cloned into the bacterial expression plasmid pT7-7 (42) at the unique *Eco*RI site which had been made blunt by treatment with DNA polymerase Klenow fragment. This cloning procedure resulted in fusion of a Met-Ala-Arg-Ile peptide derived from the vector to the amino terminus of the ORF8-encoded protein. The resulting plasmid was introduced into *Escherichia coli* BL21(DE3), and the recombinant gene was expressed by induction with 0.4 mM isopropyl-β-D-thiogalactopyranoside (IPTG) as previously described (40).

Antisera and immunoblots. Antipeptide antibodies against amino acids 513 through 527 of the ORF8 translation product (35) were prepared by standard methods (36). Polyclonal antibodies against the ORF8 protein (PAbORF8) were prepared by immunization of rabbits with gel-purified bacterial p78 as follows. Proteins synthesized by the recombinant bacteria described above were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the band corresponding to p78 was excised and electroeluted from the gel. The partially purified protein was subsequently injected into rabbits for the production of polyclonal antibodies (14). For subsequent immunoblot analysis, bacterial and Ac*M*NPV-infected insect cells were suspended in SDS sample buffer (20), and the proteins were resolved by SDS-PAGE, transferred onto nitrocellulose membranes, and probed with antibodies by standard procedures (14). Antibody-antigen complexes were detected by addition of alkaline phosphatase-conjugated donkey anti-rabbit immunoglobulin G antibodies (Jackson Immunoresearch Laboratories) in the presence of the substrates nitroblue tetrazolium, 5-bromo-4-chloro-3-indolyl phosphate, and phenazine methosulfate (Sigma).

Metabolic protein labeling. Proteins were labeled with Tran[^{35}S] label (ICN) as follows. At various times postinfection, 4×10^6 Sf9 cells were incubated in methionine-free medium (41) for 20 min, followed by the addition of 150 μCi of Tran[^{35}S] label per ml in methionine-free medium. After a 45-min incubation period, the labeling medium was removed and the cells were washed with phosphate-buffered saline (PBS).

^{32}P labeling of proteins was performed as follows. At 24 h postinfection, the culture medium was removed and replaced with TNM-FH medium lacking phosphates (41). After a 3-h incubation, 1 mCi of [^{32}P]orthophosphate (ICN) per ml in phosphate-free medium was added and the cells were incubated for a further 3 h. The labeling medium was removed, and the cells were washed with PBS. The ^{32}P-labeled cells were then incubated in 500 μl of lysis buffer (150 mM NaCl, 0.5% Nonidet P-40, 50 mM Tris hydrochloride [pH 8.8]) in the presence of 1 mM phenylmethylsulfonyl fluoride and 4 μg of aprotinin per ml as protease inhibitors. Insoluble matter was removed by centrifugation, and the lysate containing ^{32}P-labeled soluble proteins was stored at −80°C.

Cell fractionation and immunoprecipitations. Unlabeled and ^{35}S-labeled proteins were separated into cytoplasmic and nuclear fractions by detergent treatment of cells followed by centrifugation as previously described (16). Immunoprecipitations were carried out on 100 μl of fractionated lysate or ^{32}P-labeled soluble proteins (described above) in 1 ml of RIPA buffer (150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris hydrochloride [pH 7.5]) containing 0.1% bovine serum albumin (BSA) and protease inhibitors. Immunoprecipitations were cleared with preimmune serum and Immuno-precipitin (Bethesda Research Laboratories) for 1 h followed by centrifugation. The resulting supernatant was then incubated with 2 μl of PAbORF8 for a minimum of 2 h. Antibody-antigen complexes were precipitated by the addition of Immuno-precipitin and incubation for 1 h followed by centrifugation. The pellet was washed twice with RIPA buffer and twice with 10 mM Tris hydrochloride (pH 8.0) prior to SDS-PAGE. The gels were dried, and radiolabeled proteins were detected by autoradiography.

Virion and nucleocapsid purification. BV from infected cell medium was purified through a sucrose gradient as previously described (41), while PDV was obtained from OBs as follows. OBs were purified from infected cells by detergent treatment and sucrose gradient centrifugation essentially as previously described (44) with the following modifications. After cell lysis, OBs were centrifuged through a 30% sucrose cushion at 10,000 × *g* for 15 min. The pellet was resuspended in lysis buffer (44) containing 0.2% SDS, and OBs were purified from a discontinuous sucrose gradient as previously described (24). PDV was obtained from solubilized OBs by alkali treatment with 0.17 M Na_2CO_3–0.01 M dithiothreitol followed by centrifugation through a sucrose gradient as described above for BV. Nucleocapsids were purified from BV and PDV by detergent treatment as previously described for PDV (43).

Phosphatase treatment. PDV was incubated for 4 h at 37°C in the presence of 3.4 U of calf intestinal alkaline phosphatase (Pharmacia) as previously described (30). NaH_2PO_4 (10 mM) was included in some reactions as a phosphatase inhibitor.

Immunoelectron microscopy. Ac*M*NPV-infected Sf9 cells were collected at 60 h postinfection and fixed in 4% paraformaldehyde–0.5% glutaraldehyde in a phosphate buffer. The cells were infiltrated with LR White, polymerized, cut into thin sections, and placed onto nickel grids. Antibody staining was performed as follows. The grids were placed on drops of PBS containing 1% BSA for 30 min followed by a 2-h incubation with protein G-Sepharose-purified PAbORF8 at a 1:500 dilution in PBS–0.05% Tween 20. They were then washed in PBS–0.05% Tween 20 and incubated for 30 min in a 1:20 dilution of donkey anti-rabbit immunoglobulin G conjugated to 12-nm gold particles (the use of protein A rather than secondary antibody resulted in nonspecific immunogold labeling of various infected cell structures). Finally, the grids were washed with PBS–0.05%

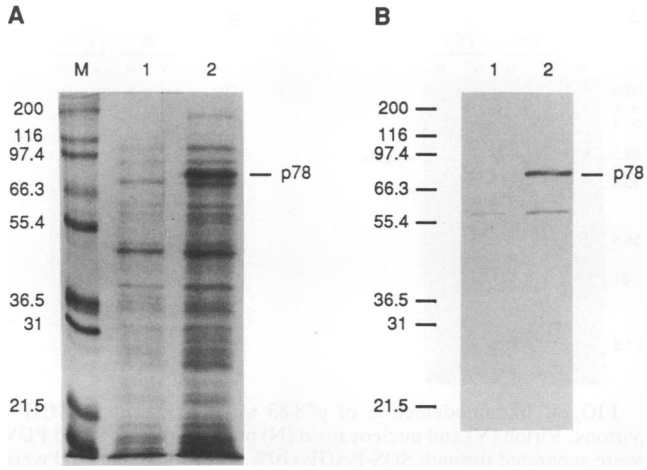

FIG. 1. Expression of the 1,629-nucleotide ORF8 of Ac*M*NPV in bacteria. A Coomassie blue-stained gel (A) and immunoblot (B) of the ORF8 fusion protein (see Materials and Methods) are shown with proteins from bacteria containing ORF8 prior to induction (lane 1) and 4 h after induction (lane 2) with 0.4 mM IPTG. The immunoblot was probed with antiserum raised against a synthetic peptide predicted by the 1,629-nucleotide ORF8 sequences. Lane M contains protein molecular mass markers; numbers correspond to sizes in kilodaltons. The position of p78 is indicated.

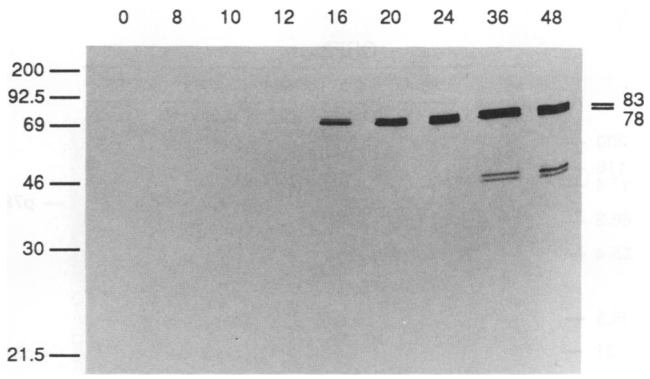

FIG. 2. Time course analysis of p78/83 accumulation in Ac*M* NPV-infected Sf9 cells. Proteins from 2×10^5 cells were collected at various times postinfection, separated through SDS-PAGE (10% polyacrylamide), and reacted with PAbORF8. The numbers along the top indicate hours postinfection. The position of the 78- and 83-kDa doublet is indicated on the right; the positions of molecular mass markers (in kilodaltons) are indicated on the left.

Tween 20 followed by water and stained for 5 min with uranyl acetate and 2 min with lead citrate prior to visualization through a Philips EM-400 microscope.

RESULTS

Bacterial expression of ORF8 and production of antibodies against the p78 protein. The 1,629-nucleotide ORF8 located immediately downstream of the Ac*M*NPV polyhedrin gene is predicted to encode a 543-amino-acid protein with a molecular mass of 60.6 kDa (35). This ORF was synthesized by PCR and cloned into the pT7-7 bacterial expression vector under the control of the T7 RNA polymerase promoter. The resulting plasmid was introduced into a bacterial strain which contains an inducible T7 RNA polymerase (see Materials and Methods). Growth of the bacteria in the presence of the inducer (IPTG) resulted in synthesis of a protein with an apparent molecular mass of 78 kDa as determined by its mobility through SDS-PAGE (Fig. 1A). This protein, p78, reacted with peptide antiserum directed against a portion of the ORF8 translation product (Fig. 1B). This antiserum also cross-reacted with smaller proteins, which were not related to p78 because they were present in both uninduced and induced cells (Fig. 1B) as well as in bacteria which did not contain the expression plasmid (data not shown). The antibodies did not react specifically with any proteins from baculovirus-infected cells (data not shown). This may have been because of low levels of synthesis of the 1,629-nucleotide ORF8 translation product in infected cells.

The 78-kDa protein produced in bacteria was eluted from the SDS-polyacrylamide gel, and its identity was confirmed by microsequencing the amino terminus through Edman degradation. The gel-purified protein was subsequently used to raise polyclonal antibodies (PAbORF8) against the full-length p78 protein (see Materials and Methods).

Time course analysis of p78/83 synthesis and localization in baculovirus-infected cells. Total proteins from Ac*M*NPV-infected cells collected at various times postinfection were resolved by SDS-PAGE, transferred onto nitrocellulose, and subjected to immunoblot analysis with PAbORF8 as described above. The results of this time course analysis are shown in Fig. 2. Two major polypeptides with molecular masses of 78 and 83 kDa were detected late in infection, while a pair of smaller proteins appeared very late in infection. The 78-kDa protein was first observed at 10 h postinfection and was present at very low levels until 16 h postinfection. At this point, a dramatic increase in the expression of the 78-kDa protein was observed, which coincided with the appearance of an 83-kDa polypeptide. Although the 83-kDa protein was not initially as abundant as the 78-kDa form, by 36 h postinfection they were present in nearly equal amounts. At this time in infection, a pair of smaller proteins (49 and 51 kDa) were detected by PAbORF8. These bands were present in much smaller amounts than the 78- and 83-kDa proteins and may represent products of alternate transcription and/or translation start sites of ORF8. Ooi and Miller (29) have previously identified several RNAs which contain 5′ ends within the 1,629-nucleotide ORF8. Translation from these RNAs may result in truncated forms of p78/83. It is also possible that these smaller polypeptides are proteolytic derivatives of full-length p78/83.

The profile of p78/83 synthesis and its intracellular distribution were determined by a combination of immunoprecipitation and Western blot (immunoblot) analyses. Immunoprecipitations of ^{35}S-labeled proteins from cytoplasmic and nuclear fractions at various times postinfection revealed a pattern of p78/83 synthesis typical of baculovirus late proteins. It was actively produced by 18 h postinfection, reaching a maximum between 24 and 48 h, followed by a substantial reduction in synthesis by 65 h postinfection (Fig. 3A). Under the labeling conditions described in Materials and Methods, only the 78-kDa form was detected and it was predominantly cytoplasmic at all times postinfection. Nonetheless, both the 78- and 83-kDa proteins were detected in equal quantities in the cytoplasmic and nuclear fractions when these were analyzed by Western blotting at later times of infection. At 18 h postinfection, the majority of p78/83 was present as the 78-kDa form in both fractions (Fig. 3B). These results suggested that the 83-kDa protein resulted from a

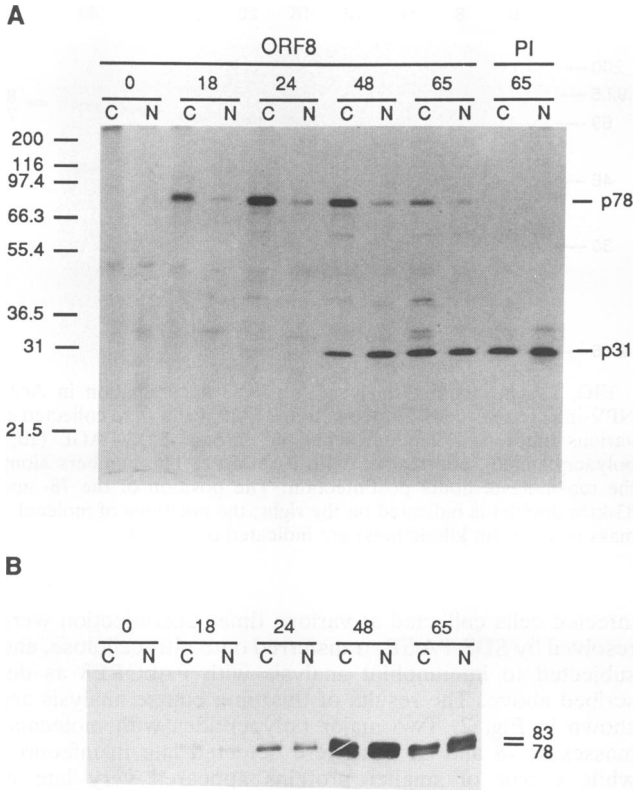

FIG. 3. Synthesis and intracellular distribution of p78/83 at various times postinfection. (A) Autoradiogram of ^{35}S-labeled proteins immunoprecipitated by PAbORF8 (ORF8) or preimmune antiserum (PI) resolved through SDS-PAGE (10% polyacrylamide). The positions of p78 and p31 are indicated on the right. The numbers on the left indicate the positions of molecular mass markers (in kilodaltons). (B) Immunoblot analysis of fractionated proteins resolved through SDS-PAGE (7% polyacrylamide). The positions of the 78- and 83-kDa forms of p78/83 are indicated on the right. The numbers along the top indicate hours postinfection. C, cytoplasmic; N, nuclear.

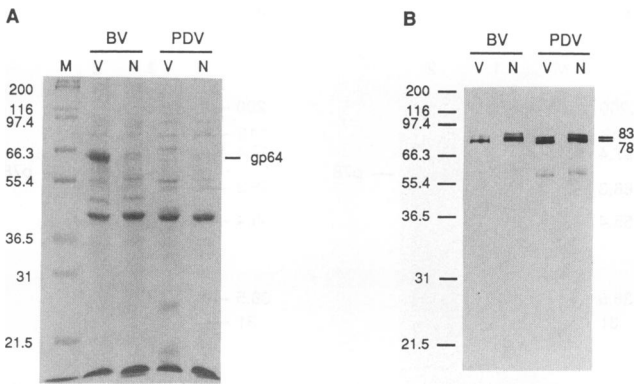

FIG. 4. Immunodetection of p78/83 associated with Ac*M*NPV virions. Virion (V) and nucleocapsid (N) proteins from BV and PDV were separated through SDS-PAGE (10% polyacrylamide) and were detected by Coomassie blue stain (A) or immunoblot analysis with PAbORF8 (B). Lane M contains molecular mass markers (numbers correspond to molecular mass in kilodaltons). The positions of gp64 and the 78- and 83-kDa forms of p78/83 are indicated.

posttranslational modification of the 78-kDa polypeptide and that this process was relatively slow, because the 83-kDa protein was not detected in the immunoprecipitations of ^{35}S-labeled proteins. Also, when proteins were pulse-chase labeled at 24 h postinfection and immunoprecipitated at various times thereafter, the ^{35}S-labeled 83-kDa protein was not detected until 2 h after labeling (data not shown).

In addition to p78, a protein approximately 31 kDa in size was also observed in the immunoprecipitations (Fig. 3A). However, this protein was precipitated nonspecifically by PAbORF8 because it was also present when preimmune serum was used in place of PAbORF8. A similar protein in baculovirus-infected cell immunoprecipitations has been previously reported and may represent the abundant polyhedrin protein (13).

p78/83 is a nucleocapsid protein associated with both the BVs and OBs. The identification of ORF8 as a late gene by transcription (35) and protein synthesis analyses (described above) suggested that it might encode a structural protein. In order to determine the localization of p78/83, BV and PDV were purified from infected-cell medium and solubilized OBs, respectively. The purified virions were treated with detergent to remove their envelopes, and the virion and nucleocapsid proteins were subjected to SDS-PAGE and subsequent immunoblot analysis with PAbORF8. Removal of envelopes from the nucleocapsids was demonstrated by the absence of several polypeptides (including gp64, the BV envelope glycoprotein) in the detergent-treated virions as determined by analysis of Coomassie blue-stained gels (Fig. 4A). The polyclonal antibodies detected both the 78- and 83-kDa polypeptides in BV as well as PDV nucleocapsids (Fig. 4B). However, p78/83 appears to be a minor constituent of Ac*M*NPV nucleocapsids, because a corresponding pair of bands was not readily detected in the protein profiles of purified nucleocapsids stained with Coomassie blue (Fig. 4A). These only became apparent when very large amounts of purified virion or nucleocapsid proteins were resolved by SDS-PAGE (unpublished observations). The smaller bands observed in the Western blot may represent degradation products or truncated forms of p78/83 arising from alternate transcription and/or translation start sites.

Posttranslational modifications of p78/83. The presence of two major polypeptides in the immunoblot analysis of infected cells and purified nucleocapsids suggested that the ORF8 translation product may have undergone posttranslational modifications. The predicted amino acid sequence of ORF8 contains several potential phosphorylation and N-linked glycosylation sites. However, p78/83 does not appear to be a glycoprotein because treatment of infected cells with tunicamycin, an inhibitor of N-linked glycosylation, had no effect on the mobility of p78/83 in SDS-PAGE (data not shown).

In order to determine the phosphorylation states of p78/83, infected-cell proteins were metabolically labeled with [^{32}P] orthophosphate and immunoprecipitated with PAbORF8. The results of these immunoprecipitations are shown in Fig. 5. Although several phosphorylated proteins were observed in the immunoprecipitations, only one protein band was specific for PAbORF8; this phosphoprotein was not precipitated by preimmune serum. The PAbORF8-specific phosphoprotein migrated through SDS-PAGE somewhat more slowly than ^{35}S-labeled p78. Western blot analysis of immunoprecipitated ^{32}P-labeled proteins detected both forms of the protein in the immunoprecipitations, but when this membrane was subjected to autoradiography, only the 83-

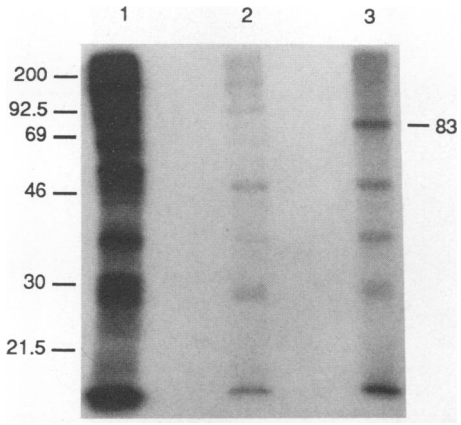

FIG. 5. Analysis of p78/83 phosphorylation in Ac*M*NPV-infected Sf9 cells at 24 h postinfection. An autoradiogram of ^{32}P-labeled proteins separated through SDS-PAGE (10% polyacrylamide) is shown. Lanes: 1, soluble Ac*M*NPV-infected Sf9 cell proteins; 2, proteins immunoprecipitated with preimmune antiserum; 3, proteins immunoprecipitated with PAbORF8. The position of p83 is indicated on the right. Numbers correspond to sizes (in kilodaltons) of molecular mass markers.

kDa protein was detected (data not shown). This result suggested that the 83- and 78-kDa polypeptides represented the phosphorylated and unphosphorylated translation products of ORF8, respectively. In order to confirm this hypothesis, purified PDV was treated with calf intestinal alkaline phosphatase, the proteins were separated through SDS-PAGE, and then the proteins were subjected to immunoblot analysis. Phosphatase treatment resulted in the disappearance of the 83-kDa protein but had no effect on the 78-kDa form (Fig. 6). This effect was prevented in the presence of the phosphatase inhibitor, NaH_2PO_4. These data, together with the immunoblot analysis of ^{32}P-labeled proteins,

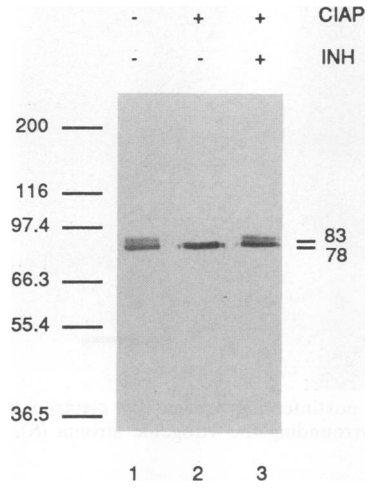

FIG. 6. Alkaline phosphatase treatment of p78/83. Immunoblot of purified PDV subjected to phosphatase treatment and separated through SDS-PAGE (7% polyacrylamide). The presence (+) and absence (−) of calf intestinal alkaline phosphatase (CIAP) and phosphatase inhibitor (INH) are indicated above each lane. The positions of the 78- and 83-kDa forms of p78/83 are indicated on the right. The positions and sizes of molecular mass markers (in kilodaltons) are indicated on the left.

strongly indicate that the 83-kDa protein is a phosphorylated form of p78/83.

Immunoelectron microscopy. The association of p78/83 with virions and nucleocapsids was verified by immunogold staining of baculovirus-infected cells at 60 h postinfection. PAbORF8-specific staining was observed in areas of nucleocapsid assembly surrounding the virogenic stroma and in mature virions within OBs (Fig. 7). However, whereas p78/83 appeared to be randomly distributed throughout the areas of nucleocapsid assembly (Fig. 7A), staining of mature virions (within the OBs) appeared to be specific to the ends of the nucleocapsids (Fig. 7B). Immunogold staining was not observed along the lengths of the nucleocapsids or in cross sections through the middle of the nucleocapsids. It was not conclusive from these studies whether both or only one end of the nucleocapsids contained p78/83. Even though p78/83 was shown to be present in both BV and PDV nucleocapsids by Western blot analysis (described above), immunogold staining of BV nucleocapsids in the cytoplasm or at the plasma membrane was not detected. This may have been due to a combination of the relative paucity of BV as opposed to PDV in infected cells and the requirement for cross sections to bisect the nucleocapsids at the ends in order for the p78/83 protein to be labeled. Preimmune antiserum and nonspecific antibodies did not result in staining of either nucleocapsids or virions (data not shown).

DISCUSSION

The results of this study reveal that the 1,629-nucleotide ORF8 immediately downstream of the polyhedrin gene of Ac*M*NPV encodes a protein, p78/83, which is produced late in infection and is a component of both the BV and PDV nucleocapsids. The protein is present in phosphorylated and nonphosphorylated forms and appears to be associated with structures at the end(s) of the baculovirus nucleocapsid.

The translated product of ORF8 located in the *Eco*RI-I fragment of Ac*M*NPV has a predicted molecular mass of 60.6 kDa (35). However, immunoblot analysis of the gene product demonstrated that it is expressed in Ac*M*NPV-infected Sf9 cells as two polypeptides with apparent molecular masses of 78 and 83 kDa according to SDS-PAGE (Fig. 2). The large discrepancy between the calculated and observed molecular size does not appear to be due to post-translational modification events. Although the amino acid sequence of ORF8 contains several potential N-linked glycosylation and phosphorylation sites, p78/83 is not glycosylated (unpublished observations) and the nonphosphorylated form migrates as a 78-kDa protein (Fig. 6). Further evidence for the size of unmodified p78/83 was derived from immunoblot analysis of p78 produced in bacteria (Fig. 1) and an in vitro translation system (34). Thus, the retarded migration of p78/83 in SDS-PAGE appears to be due to the high content and uneven distribution of proline residues in the protein (35). The presence of these proline-rich regions may result in an extended, rigid conformation which affects the mobility of p78/83 in SDS-PAGE.

Our virion fractionation results indicate that p78/83 is associated with Ac*M*NPV nucleocapsids (Fig. 4). Homology searches through protein data banks resulted in the identification of various proline-rich proteins which generally belong to either transcriptional or structural classes of proteins; p78/83 appears to belong to the latter. Virus-encoded transcription proteins associated with the virion are usually required for transcription of immediate-early viral genes (e.g., VP16 of herpes simplex virus). However, the baculo-

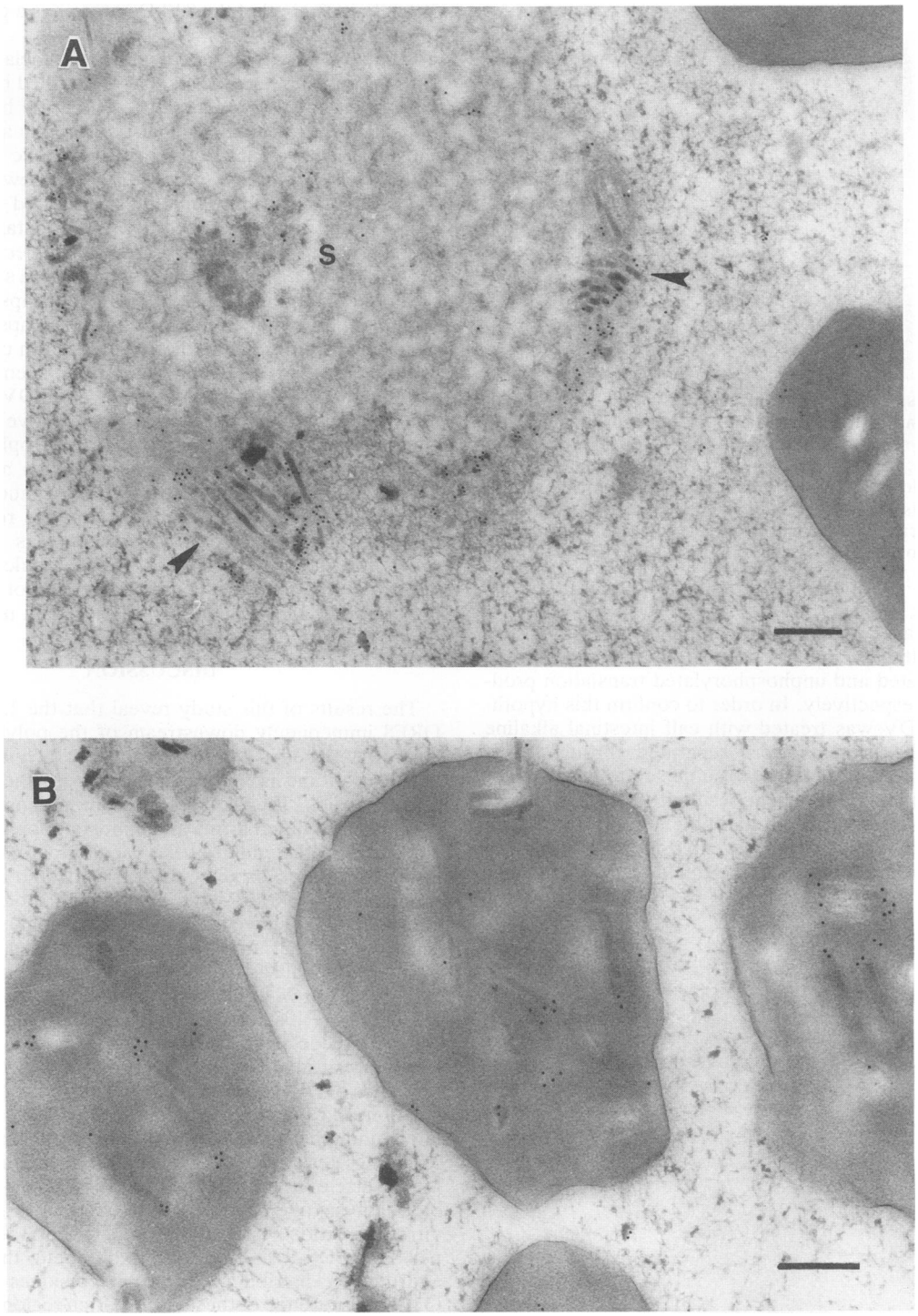

FIG. 7. Immunogold staining of nuclear structures from infected cells at 60 h postinfection viewed by electron microscopy. (A) Association of p78/83 with areas of nucleocapsid assembly (indicated by arrows) surrounding the virogenic stroma (S). (B) Staining of nucleocapsids within OBs. Each bar represents 0.25 μm.

virus immediate-early genes are transcribed by the host cell machinery in the absence of any viral proteins, as demonstrated by the ability of transfected baculovirus DNA to initiate a productive infectious cycle. In addition, it has been recently reported that the presence of the 1,629-nucleotide gene has no effect on the levels of transcription from late and very late baculovirus promoters in a transient expression assay (31). This does not exclude p78/83 from having an effect on other aspects of baculovirus transcription, such as tissue specificity or timing of gene expression. However, it seems more likely that p78/83 is a structural component of the baculovirus nucleocapsids.

Previous immunogold microscopy studies determined that two other baculovirus proteins, p39 (the major capsid protein) and p24, are distributed throughout the nucleocapsids (38, 51). The precise localization of a third capsid protein, p87, has not yet been determined (25). Immunogold staining of Ac*M*NPV-infected Sf9 cells revealed that p78/83 is associated with the ends of nucleocapsids in mature virions found embedded within OBs (Fig. 7). Baculovirus nucleocapsids contain distinctive end structures (1, 9) which are apparently composed of proteins different from the rest of the nucleocapsid (8). The appearances of the cap structures are different at each end of the nucleocapsid and have been previously termed the apical cap and basal structure (10). These end structures (the apical cap in particular) have been implicated in various processes, including packaging of the nucleoprotein core (10), initiation of nucleocapsid envelopment within the infected-cell nucleus (10, 17), and budding through the nuclear and cytoplasmic membranes (10, 18). The presence of p78/83 in the areas in which nucleocapsid assembly and PDV envelope acquisition occur is consistent with any of the functions described above and may explain the essential requirement for the p78/83 gene in the baculovirus life cycle (35).

The significance of p78/83 phosphorylation could not be resolved from this study, but it does not seem to be involved in determining either the localization of the protein within the infected cell or the localization of the protein to BV or PDV. Both forms of p78/83, phosphorylated and nonphosphorylated, are present in the cytoplasm and in the nucleus (Fig. 3) as well as in both virion types (Fig. 4). Nonetheless, phosphorylation of p78/83 may play a role in nucleocapsid assembly through interactions with the nucleoprotein core or other structural proteins; the amino acid sequence of p78/83 encodes a potential leucine zipper domain which may direct protein-protein interactions. Alternatively, phosphorylation may be involved in regulating interactions with the envelopes during the maturation of virions.

ACKNOWLEDGMENTS

We thank Cathy Iorio for help in raising polyclonal antibodies, Kathy Hewitt for assistance with electron microscopy, and France Dumas for protein microsequencing. We also thank Brian Taylor for providing the pT7-7 plasmid.

This work was supported by Medical Research Council of Canada grant MA10638 and Natural Sciences and Engineering Research Council of Canada Postgraduate scholarship 157882.

REFERENCES

1. **Adams, J. R., and J. T. McClintock.** 1991. Nuclear polyhedrosis viruses of insects, p. 89–204. *In* J. R. Adams and J. R. Bonami (ed.), Atlas of invertebrate viruses. CRC Press, Boca Raton, Fla.
2. **Bjornson, R. L., and G. F. Rohrmann.** 1992. Nucleotide sequence of the polyhedron envelope protein gene region of the *Lymantria dispar* nuclear polyhedrosis virus. J. Gen. Virol. **73**:1499–1504.
3. **Bjornson, R. L., and G. F. Rohrmann.** 1992. Nucleotide sequence of the p39-capsid gene region of the *Lymantria dispar* nuclear polyhedrosis virus. J. Gen. Virol. **73**:1505–1508.
4. **Blissard, G. W., R. L. Quant-Russell, G. F. Rohrmann, and G. S. Beaudreau.** 1989. Nucleotide sequence, transcriptional mapping, and temporal expression of the gene encoding p39, a major structural protein of the multicapsid nuclear polyhedrosis virus of *Orgyia pseudotsugata*. Virology **168**:354–362.
5. **Blissard, G. W., and G. F. Rohrmann.** 1989. Location, sequence, transcriptional mapping, and temporal expression of the gp64 envelope glycoprotein gene of the *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus. Virology **170**:537–555.
6. **Blissard, G. W., and G. F. Rohrmann.** 1990. Baculovirus diversity and molecular biology. Annu. Rev. Entomol. **35**:127–155.
7. **Blissard, G. W., and J. R. Wenz.** 1992. Baculovirus gp64 envelope glycoprotein is sufficient to mediate pH-dependent membrane fusion. J. Virol. **66**:6829–6835.
8. **Burley, S. K., A. Miller, K. A. Harrap, and D. C. Kelly.** 1982. Structure of the *Baculovirus* nucleocapsid. Virology **120**:433–440.
9. **Federici, B. A.** 1986. Ultrastructure of baculoviruses, p. 61–88. *In* R. R. Granados and B. A. Federici (ed.), The biology of baculoviruses, vol. 1. CRC Press, Boca Raton, Fla.
10. **Fraser, M. J.** 1986. Ultrastructural observations of virion maturation in *Autographa californica* nuclear polyhedrosis virus infected *Spodoptera frugiperda* cell cultures. J. Ultrastruct. Mol. Struct. Res. **95**:189–195.
11. **Gombart, A. F., G. W. Blissard, and G. F. Rohrmann.** 1989. Characterization of the genetic organization of the *Hin*dIII M region of the multicapsid nuclear polyhedrosis virus of *Orgyia pseudotsugata* reveals major differences among baculoviruses. J. Gen. Virol. **70**:1815–1828.
12. **Gross, C. H., G. M. Wolgamot, R. L. Q. Russell, M. N. Pearson, and G. F. Rohrmann.** 1993. A baculovirus encoded 16-kDa glycoprotein localizes near the nuclear membrane of infected cells. Virology **192**:386–390.
13. **Guarino, L. A., W. Dong, B. Xu, D. R. Broussard, R. W. Davis, and D. L. Jarvis.** 1993. Baculovirus phosphoprotein pp31 is associated with virogenic stroma. J. Virol. **66**:7113–7120.
14. **Harlow, E., and D. Lane.** 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Hill, J. E., J. Kuzio, J. A. Wilson, E. A. MacKinnon, and P. Faulkner.** 1993. Nucleotide sequence of the p74 gene of a baculovirus pathogenic to the spruce budworm, *Choristoneura fumiferana* multicapsid nuclear polyhedrosis virus. Biochim. Biophys. Acta **1172**:187–189.
16. **Jarvis, D. L., D. A. Bohlmeyer, and A. Garcia, Jr.** 1991. Requirements for nuclear localization and supramolecular assembly of a baculovirus polyhedrin protein. Virology **185**:795–810.
17. **Kawamoto, F., N. Kumada, and M. Kobayashi.** 1977. Envelopment of the nuclear polyhedrosis virus of the oriental tussock moth, *Euproctis subflava*. Virology **77**:867–871.
18. **Kawamoto, F., C. Suto, N. Kumada, and M. Kobayashi.** 1977. Cytoplasmic budding of a nuclear polyhedrosis virus and comparative ultrastructural studies of envelopes. Microbiol. Immunol. **21**:255–265.
19. **Kuzio, J., R. Jacques, and P. Faulkner.** 1989. Identification of p74, a gene essential for virulence of baculovirus occlusion bodies. Virology **173**:759–763.
20. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
21. **Lu, A., and E. B. Carstens.** 1992. Nucleotide sequence and transcriptional analysis of the p80 gene of *Autographa californica* nuclear polyhedrosis virus: a homologue of the *Orgyia pseudotsugata* nuclear polyhedrosis virus capsid-associated gene. Virology **190**:201–209.
22. **Ma, S.-W., B. G. Corsaro, P. E. Klebba, and M. J. Fraser.** 1993. Cloning and sequence analysis of a p40 structural protein gene of *Helicoverpa zea* nuclear polyhedrosis virus. Virology **192**: 224–233.
23. **Maeda, S., S. G. Kamita, and H. Kataoka.** 1991. The basic DNA-binding protein of *Bombyx mori* nuclear polyhedrosis virus: the existence of an additional arginine repeat. Virology **180**:807–810.
24. **McCarthy, W. J., R. R. Granados, and D. W. Roberts.** 1974. Isolation and characterization of entomopox virions from virus-containing inclusions of *Amsacta moorei* (Lepidoptera: Arctiidae). Virology **59**:59–69.
25. **Müller, R., M. N. Pearson, R. L. Q. Russell, and G. F.**

Rohrmann. 1990. A capsid-associated protein of the multicapsid nuclear polyhedrosis virus of *Orgyia pseudotsugata*: genetic location, sequence, transcriptional mapping, and immunocytochemical characterization. Virology **176:**133–144.

26. **Nagamine, T., and M. Kobayashi.** 1991. Preparation and characterization of monoclonal antibodies against occluded virions of *Bombyx mori* nuclear polyhedrosis virus. J. Invertebr. Pathol. **57:**311–324.
27. **Nagamine, T., H. Sugimori, K. Nakamura, S. Saga, and M. Kobayashi.** 1991. Nucleotide sequence of the gene coding for p40, an occluded virion-specific polypeptide of *Bombyx mori* nuclear polyhedrosis virus. J. Invertebr. Pathol. **58:**290–293.
28. **Oellig, C., B. Happ, T. Müller, and W. Doerfler.** 1987. Overlapping sets of viral RNAs reflect the array of polypeptides in the *Eco*RI J and N fragments (map positions 81.2 to 85.0) of the *Autographa californica* nuclear polyhedrosis virus genome. J. Virol. **61:**3048–3057. (Erratum, **63:**1494, 1989.)
29. **Ooi, B. G., and L. K. Miller.** 1990. Transcription of the baculovirus polyhedrin gene reduces the levels of an antisense transcript initiated downstream. J. Virol. **64:**3126–3129.
30. **Parthun, M. R., and J. A. Jaehning.** 1992. A transcriptionally active form of GAL4 is phosphorylated and associated with GAL80. Mol. Cell. Biol. **12:**4981–4987.
31. **Passarelli, A. L., and L. K. Miller.** 1993. Three baculovirus genes involved in late and very late gene expression: *ie-1*, *ie-n*, and *lef-2*. J. Virol. **67:**2149–2158.
32. **Pearson, M. N., R. L. Quant-Russell, G. F. Rohrmann, and G. S. Beaudreau.** 1988. P39, a major baculovirus structural protein: immunocytochemical characterization and genetic location. Virology **167:**407–413.
33. **Pham, D. Q.-D., and N. Sivasubramanian.** 1992. *In vivo* transcriptional analysis of three baculovirus genes: evidence of homology between viral and host transcripts. Virology **190:**288–297.
34. **Pham, D. Q.-D., and N. Sivasubramanian.** 1992. Sequence and in vitro translational analysis of a 1629-nucleotide ORF in *Autographa californica* nuclear polyhedrosis virus strain E2. Gene **122:**345–348.
35. **Possee, R. D., T.-P. Sun, S. C. Howard, M. D. Ayres, M. Hill-Perkins, and K. L. Gearing.** 1991. Nucleotide sequence of the *Autographa californica* nuclear polyhedrosis 9.4 kbp *Eco*RI-I and -R (polyhedrin gene) region. Virology **185:**229–241.
36. **Richardson, C. D., A. Berkovich, S. Rozenblatt, and W. J. Bellini.** 1985. Use of antibodies directed against synthetic peptides for identifying cDNA clones, establishing reading frames, and deducing the gene order of measles virus. J. Virol. **54:**186–193.
37. **Rohrmann, G. F.** 1992. Baculovirus structural proteins. J. Gen. Virol. **73:**749–761.
38. **Russell, R. L. Q., M. N. Pearson, and G. F. Rohrmann.** 1991. Immunoelectron microscopic examination of *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus-infected *Lymantria dispar* cells time course and localization of major polyhedron-associated proteins. J. Gen. Virol. **72:**275–283.
39. **Russell, R. L. Q., and G. F. Rohrmann.** 1990. The p6.5 region of a nuclear polyhedrosis virus of *Orgyia pseudotsugata*: DNA sequence and transcriptional analysis of four late genes. J. Gen. Virol. **71:**551–560.
40. **Studier, F. W., and B. A. Moffatt.** 1986. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. J. Mol. Biol. **189:**113–130.
41. **Summers, M. D., and G. E. Smith.** 1987. A manual of methods for baculovirus vectors and insect cell culture procedures. Bulletin 1555. Texas Agricultural Experiment Station and Texas A&M University, College Station.
42. **Tabor, S., and C. C. Richardson.** 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA **82:**1074–1078.
43. **Thiem, S. M., and L. K. Miller.** 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.
44. **Vialard, J. E., L. Yuen, and C. D. Richardson.** 1990. Identification and characterization of a baculovirus occlusion body glycoprotein which resembles spheroidin, an entomopoxvirus protein. J. Virol. **64:**5804–5811.
45. **Whitford, M., and P. Faulkner.** 1992. A structural polypeptide of the baculovirus *Autographa californica* nuclear polyhedrosis virus contains O-linked *N*-acetylglucosamine. J. Virol. **66:**3324–3329.
46. **Whitford, M., and P. Faulkner.** 1992. Nucleotide sequence and transcriptional analysis of a gene encoding gp41, a structural glycoprotein of the baculovirus *Autographa californica* nuclear polyhedrosis virus. J. Virol. **66:**4763–4768. (Erratum, **67:**2427, 1993.)
47. **Whitford, M., S. Stewart, J. Kuzio, and P. Faulkner.** 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.
48. **Wilson, M. E., T. H. Mainprize, P. D. Friesen, and L. K. Miller.** 1987. Location, transcription, and sequence of a baculovirus gene encoding a small, arginine-rich polypeptide. J. Virol. **61:**661–666.
49. **Wilson, M. E., and K. H. Price.** 1988. Association of *Autographa californica* nuclear polyhedrosis virus (AcMNPV) with the nuclear matrix. Virology **167:**233–241.
50. **Wilson, M. E., and K. H. Price.** 1991. Expression and location of a baculovirus DNA-binding protein within the host cell. J. Invertebr. Pathol. **57:**264–268.
51. **Wolgamot, G. M., C. H. Gross, R. L. Q. Russell, and G. F. Rohrmann.** 1993. Immunocytochemical characterization of p24, a baculovirus capsid-associated protein. J. Gen. Virol. **74:**103–107.