JOURNAL OF VIROLOGY, Feb. 1980, p. 866–876
0022-538X/80/02-0866/11$02.00/0

Vol. 33, No. 2

Characterization of an Extremely Basic Protein Derived from Granulosis Virus Nucleocapsids†

KATHLEEN A. TWEETEN,[1] LEE A. BULLA, JR.,[2] AND RICHARD A. CONSIGLI[1]*

Division of Biology, Section of Virology and Oncology, Kansas State University, Manhattan, Kansas 66506,[1] and U.S. Grain Marketing Research Laboratory, Science and Education Administration, Manhattan, Kansas 66502[2]

Nucleocapsids were isolated from purified enveloped nucleocapsids of *Plodia interpunctella* granulosis virus by treatment with Nonidet P-40. When analyzed on sodium dodecyl sulfate-polyacrylamide gels, the nucleocapsids consisted of eight polypeptides. One of these, a major component with a molecular weight of 12,500 (VP12), was selectively extracted from the nucleocapsids with 0.25 M sulfuric acid. Its electrophoretic mobility on acetic acid-urea gels was intermediate to that of cellular histones and protamine. Amino acid analysis showed that 39% of the amino acid residues of VP12 were basic: 27% were arginine and 12% were histidine. The remaining residues consisted primarily of serine, valine, and isoleucine. Proteins of similar arginine content also were extracted from the granulosis virus of *Pieris rapae* and from the nuclear polyhedrosis viruses of *Spodoptera frugiperda* and *Autographa californica*. The basic polypeptide appeared to be virus specific because it was found in nucleocapsids and virus-infected cells but not in uninfected cells. VP12 was not present in polypeptide profiles of granulosis virus capsids, indicating that it was an internal or core protein of the nucleocapsids. Electron microscopic observations suggested that the basic protein was associated with the viral DNA in the form of a DNA-protein complex.

Granulosis virus (GV) and nuclear polyhedrosis virus (NPV) are insect viruses belonging to the family Baculoviridae. They are structurally complex viruses consisting of enveloped nucleocapsids embedded within a thick matrix of protein. The nucleocapsids of GV and NPV are morphologically similar and consist of rod-shaped capsids that contain high-molecular-weight, covalently closed supercoiled DNA molecules (1, 28, 31).

The mechanisms involved in the maturation and assembly of baculovirus nucleocapsids are not well characterized. This is due, in part, to the lack of information on the polypeptide composition of the nucleocapsids. The structural polypeptides of nucleocapsids from only a few NPV (12) and GV (6, 32a) isolates have been identified.

In our studies on the molecular biology of the GV which infects the Indian meal moth, *Plodia interpunctella*, we have become interested in the mode of packaging of the 80 × 10^6-dalton genome of the virus (31) into the viral capsid. Studies with the papovaviruses have demonstrated that cellular histones are tightly associated with the viral DNA, which is also circular and supercoiled, in the form of DNA-protein complexes. These histones have been found to have important functions in the viral replication process, including condensation of the viral DNA (5, 7, 19, 20). Since a major structural polypeptide of the GV nucleocapsids, VP12, was observed to have a molecular weight similar to that of histones, it was isolated and its biochemical properties were investigated to determine whether it was histone-like. The results described in this report demonstrate that VP12 is an extremely basic, arginine-rich polypeptide that can be selectively extracted from GV nucleocapsids with dilute sulfuric acid. Evidence that this protein is located inside the viral capsid as a core component also is presented. Similar basic polypeptides were extracted from a number of other NPVs and GVs, indicating that these proteins are common to baculoviruses.

MATERIALS AND METHODS

Production and purification of virus. The GV of *P. interpunctella* was produced in a laboratory colony of *P. interpunctella* larvae reared as previously described (30). Early third-instar larvae were infected per os with GV, and the virus was purified by differential centrifugation, treatment with 1% deoxycholate, and velocity sedimentation in sucrose gradients (30, 32).

Pieris rapae GV, which was produced in *P. rapae* larvae, was obtained from R. P. Jacques (Canadian

† Contribution no. 80-97-j, Kansas Agricultural Experiment Station, Kansas State University, Manhattan, KS 66506.

Department of Agriculture, Harrow, Ontario) in the form of an insecticide preparation. Sodium dodecyl sulfate (SDS) was added to a final concentration of 0.5%, and the inert ingredients were allowed to settle out. The preparation was filtered through Whatman no. 1 paper, and the virus was then purified by the method utilized for *P. interpunctella* GV.

Autographa californica NPV was produced in vitro in a *Spodoptera frugiperda* cell line (no. IPLB-SF-21AE) obtained from D. L. Knudson (Yale University School of Medicine, New Haven, Conn.). The cells were maintained in Grace's insect tissue culture medium supplemented with 10% fetal calf serum and 0.2% tryptose (Difco Laboratories). The original inoculum of *A. californica* NPV consisted of infected tissue culture supernatant kindly provided by W. F. Hink (Ohio State University, Columbus). Falcon flasks (75 cm^2) were seeded with 10^7 cells and incubated for 24 h at 27°C. The medium was removed, and the cells were infected with NPV inoculum at a multiplicity of infection of 0.01 PFU/cell. The virus was allowed to adsorb for 1 h at room temperature with occasional tilting of the flasks. After adsorption, 12 ml of complete medium was added to each flask. The cultures were harvested 7 days after infection by scraping the infected flasks. The cells and tissue culture fluid were collected and centrifuged at 10,000 rpm (HB-4 rotor) for 30 min. The cell-free supernatant, which contained nonoccluded enveloped nucleocapsids, was layered over 5 ml of 30% (vol/vol) glycerol (in 0.01 M Tris-hydrochloride, pH 7.5) and centrifuged at 25,000 rpm (SW27 rotor) for 1 h. Pelleted virus was resuspended in 0.01 M Tris-hydrochloride, pH 8.5, and utilized for nucleocapsid isolation. The cell pellet, which contained polyhedra, was suspended in distilled water and disrupted with a Sorvall Omnimixer. The preparation was layered on 40 to 65% (wt/wt) sucrose (in distilled water) gradients which were then centrifuged at 25,000 rpm (SW27 rotor) for 1 h at 10°C. The band of virus was recovered from the gradients, diluted with distilled water, and pelleted by centrifugation at 10,000 rpm (HB-4 rotor) for 30 min. The polyhedra were suspended in water and stored at −20°C.

In vivo-grown *S. frugiperda* NPV was obtained from E. Dougherty (U.S. Department of Agriculture, Science, and Education Administration, Beltsville, Md.). The occluded virus was washed with 0.5% SDS for 20 min, filtered through Whatman no. 1 paper, and pelleted by centrifugation at 10,000 rpm (HB-4 rotor) for 20 min. The pellet was suspended in 1 M NaCl in distilled water, incubated for 30 min at room temperature, and pelleted through a 5-ml 40% (wt/wt) sucrose (in distilled water) shelf by centrifugation at 25,000 rpm (SW27 rotor) for 30 min. The pellet was resuspended in distilled water and layered on 40 to 65% (wt/wt) sucrose (in distilled water) gradients which were centrifuged at 25,000 rpm (SW27 rotor) for 1 h at 10°C. The band of virus was recovered from the gradient, diluted with distilled water, and centrifuged at 10,000 rpm (HB-4 rotor) to remove the sucrose. Purified NPV was suspended in water and stored at −20°C.

Preparation of radiolabeled GV and NPV. Radioactively labeled *P. interpunctella* GV was produced in vivo by injection of 1 μl (0.5 μCi) of [^{3}H]thymidine (Schwarz/Mann) into larvae at 96 and 120 h after infection. The GV was purified from injected larvae 8 days after infection.

Radiolabeled *A. californica* NPV was prepared by growing infected cells in Grace's insect tissue culture medium (GIBCO Laboratories) containing 10% fetal calf serum, 0.2% tryptose, and 10 μCi of [^{3}H]thymidine per ml. Polyhedra and extracellular enveloped nucleocapsids were harvested and purified as described above.

Isolation and purification of nucleocapsids. Nucleocapsids were isolated by treatment of enveloped nucleocapsids with Nonidet P-40 (Shell Chemical Co.). To obtain GV enveloped nucleocapsids, purified *P. interpunctella* or *P. rapae* GV was incubated in 0.05 M sodium carbonate–0.05 M NaCl, pH 10.6, for 30 min at room temperature. The dissociated virus was layered on 30 to 70% (vol/vol) glycerol (in 0.01 M Tris-hydrochloride, pH 7.5) gradients which were centrifuged at 25,000 rpm (SW41 rotor) for 1 h at 10°C. The band of enveloped nucleocapsids was recovered from the gradients and centrifuged at 25,000 rpm (SW41 rotor) for 1 h to remove the glycerol. A similar procedure was used to obtain NPV enveloped nucleocapsids, except the alkaline solubilization consisted of incubating the NPVs in 0.1 M sodium carbonate for 2 h at 37°C. The dissociated virus was layered on 10 to 50% (wt/wt) sucrose in 0.01 M Tris-hydrochloride, pH 7.5) gradients which were centrifuged at 17,500 rpm (SW41 rotor) for 30 min at 10°C. The bands of enveloped nucleocapsids were recovered from the gradients and pelleted by centrifugation at 25,000 rpm (SW41 rotor) for 1 h. For the isolation of nucleocapsids, the NPV and GV enveloped nucleocapsids were incubated in 1% (vol/vol) Nonidet P-40 (in 0.01 M Tris-hydrochloride, pH 8.5) for 30 min with stirring at room temperature. The nucleocapsids were separated from the solubilized envelope proteins by sedimentation on 30 to 70% (vol/vol) glycerol (in 0.01 M Tris-hydrochloride, pH 8.5) gradients by centrifugation at 30,000 rpm (SW41 rotor) for 1 h. The band of nucleocapsids was recovered, diluted with 0.01 M Tris-hydrochloride (pH 8.5), and centrifuged at 25,000 rpm (SW41 rotor) for 1 h to remove glycerol. Freshly prepared nucleocapsids were utilized for acid extraction or gel electrophoresis.

Capsid isolation. Nucleocapsids were incubated in 2% Nonidet P-40–0.01 M EDTA–1 M NaCl in 0.01 M Tris-hydrochloride, pH 8.5, for 12 h at 37°C (29). The preparation was then sedimented on a preformed cesium chloride gradient made in 0.01 M Tris-hydrochloride, pH 8.5, and ranging in density from 1.20 to 1.50 g/cm^3. Centrifugation was at 34,000 rpm (SW41 rotor) for 2 h at 10°C. The visible band of capsids was recovered, diluted with 0.01 M Tris-hydrochloride (pH 7.5), and pelleted by centrifugation at 30,000 rpm (SW50.1 rotor) for 30 min at 10°C.

Electron microscopy. For examination of intact nucleocapsids or capsids, samples were placed on Formvar-coated grids and were negatively stained with 2% uranyl acetate. For degradation studies, purified nucleocapsids were air dried onto Formvar-coated grids. The grids were incubated for 30 min in droplets of 0.01 M EDTA in 0.01 M Tris-hydrochloride (pH 7.0), followed by a 30-min incubation in droplets of 5 mM dithiothreitol in 0.01 M Tris-hydrochloride (pH 7.0). Specimens were then stained with 2% uranyl

acetate. Grids were examined with a Philips EM 201 electron microscope at 60 kV.

Acid extraction. For acid extraction, nucleocapsids were suspended in distilled water, and an equal volume of cold 0.5 N sulfuric acid was added. After incubation for 16 h at 4°C, the acid-insoluble protein was pelleted by centrifugation at 15,000 rpm (SS34 rotor) for 1 h at 4°C. The supernatant was centrifuged to insure the removal of acid-insoluble proteins. The supernatant was recovered and mixed with 4 volumes of cold 100% ethanol. After incubation overnight at −20°C, the precipitated protein was collected by centrifugation at 10,000 rpm (SS34 rotor) for 30 min. The resulting pellet, along with the acid-insoluble pellets, was washed two times with cold 95% ethanol and dried under a stream of nitrogen.

Acid-soluble proteins were also extracted from whole cells, and nuclei were obtained from *P. interpunctella* larvae. Whole cells were prepared by homogenizing 25 fourth-instar larvae in 10 ml of cold 0.15 M NaCl–0.01 M sodium citrate–0.05 M sodium bisulfite, pH 7.8. The homogenate was filtered through gauze and centrifuged at 5,500 rpm (HB-4 rotor) for 15 min. The pellet of cells was suspended in 1 ml of distilled water, and cold sulfuric acid was added to a final concentration of 0.4 N for extraction of the tissue. The acid-soluble protein was then recovered as described above. The larval cell homogenates were also utilized for nuclei isolation. After centrifugation at 5,500 rpm (HB-4 rotor) for 15 min, the cellular pellet was suspended in 2 ml of 0.15 M NaCl–0.01 M sodium citrate–0.05 M sodium bisulfite–1% Triton X-100, pH 8.0. The mixture was stirred for 10 min at 4°C and was then centrifuged at 5,500 rpm (HB-4 rotor) for 15 min. The nuclear pellet was washed two times in 0.15 M NaCl–0.01 M sodium citrate–0.05 M sodium bisulfite, pH 7.8, and suspended in 1 ml of distilled water. With a 26-gauge needle and syringe, the nuclei were disrupted and were then extracted with 0.4 M sulfuric acid as described above.

SDS-polyacrylamide gel electrophoresis. Nucleocapsids, capsids, acid-soluble proteins, and acid-insoluble proteins were subjected to electrophoresis on 15% SDS-polyacrylamide slab gels (1.5 by 14 by 18 cm; model SE 500; Hoefer Scientific Instruments), using the discontinuous buffer system of Laemmli (18). Samples were prepared for electrophoresis by boiling for 3 min in 2% SDS–5% 2-mercaptoethanol–0.0625 M Tris-hydrochloride (pH 6.8)–10% glycerol. Electrophoresis was carried out at 20 mA/slab. Gels were stained overnight at 0.1% Coomassie brilliant blue R (Sigma Chemical Co.)–50% methanol–7.5% acetic acid. Destaining was in 50% methanol–7.5% acetic acid for 1 h and 25% methanol–1.5% acetic acid for 48 h. Molecular weights were determined by the method of Weber and Osborne (33), using cytochrome *c* (molecular weight, 11,700), chymotrypsinogen (molecular weight, 27,500), ovalbumin (molecular weight, 43,000), and bovine serum albumin (molecular weight, 68,500) (Schwarz/Mann) as standards. Gels were dried with an SE-540 Hoefer Scientific Instruments slab gel dryer.

Acetic acid-urea gels. Viral proteins were also electrophoresed on 15% polyacrylamide slab gels containing 6.25 M urea. Gels were prepared and preelectrophoresed by the method of Panyim and Chalkley (23) to give a final pH of 3.2. Samples were prepared for electrophoresis by dissolving them in 0.9 N acetic acid–10 M urea–2% 2-mercaptoethanol–10% glycerol. The buffer was 0.9 N acetic acid, and electrophoresis was carried out at 20 mA/slab until the methyl green tracking dye eluted. Gels were stained, destained, and dried as described above.

Amino acid analysis. Samples were hydrolyzed in 1.0 ml of 6 N hydrochloric acid in evacuated, sealed tubes at 110°C for 24 h. After removal of the hydrochloric acid by a nitrogen stream, amino acid analysis was performed on a Beckman 120C analyzer. Cystine and cysteine were determined as cysteic acid and methionine was determined as the sulfone after performic acid oxidation (13).

Isoelectric focusing of VP12. VP12 was acid extracted from nucleocapsids and precipitated as described above. The isolated protein was suspended in 1% carrier ampholytes (pH 9 to 11; Brinkmann Instruments Inc.)–12.5% sucrose in deionized water. Isoelectric focusing was conducted in 7.5% polyacrylamide slab gels containing 2% carrier ampholytes (pH 9 to 11), 5% urea, 0.1% lysine, and 0.1% arginine. Gels were prefocused for 60 min at 10 mA. After sample application, the gels were electrophoresed at 15 mA until the voltage reached 500 V and the pH gradient formed. The voltage was then set at 100 V and increased by 100 V every 15 min until 1,000 V was attained. Electrophoresis was continued until the current dropped to 5 mA. The gel was fixed in 20% (vol/vol) trichloroacetic acid for 90 min and washed in 25% methanol–10% acetic acid for 10 min. Staining was overnight in 0.2% Coomassie brilliant blue R in 45% methanol–10% acetic acid. The pH gradient was determined on a section of gel removed before fixing and staining with a Desaga/Brinkmann flat membrane glass electrode.

RESULTS

Polypeptide composition of nucleocapsids. When nucleocapsids isolated from the GV of *P. interpunctella* were electrophoresed on SDS-polyacrylamide gels, eight structural polypeptides were observed (Fig. 1). The nucleocapsids were composed primarily of two of these proteins, having molecular weights of 12,500 (VP12) and 31,000 (VP31). The remaining proteins were present in minor amounts and ranged in molecular weight from 30,100 to 64,200. The nucleocapsids were found to be free of envelope protein contamination by SDS discontinuous and gradient gel electrophoresis and by surface radioiodination studies (32a). Thus, these eight structural polypeptides are unique constituents of GV nucleocapsids.

Acid extraction of nucleocapsids. To determine whether any of the structural polypeptides were basic in nature, we treated the GV nucleocapsids with cold 0.25 M sulfuric acid. The preparation was then separated into acid-soluble and acid-insoluble fractions by centrifugation. The acid-soluble supernatant was re-

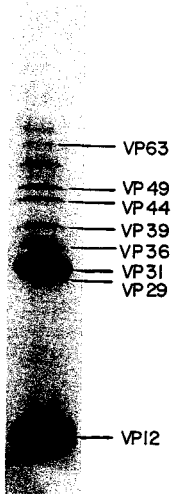

FIG. 1. *SDS-polyacrylamide gels electrophoresis of P. interpunctella GV nucleocapsids. Nucleocapsids were isolated as described in the text and prepared for electrophoresis by boiling in 2% SDS–5% 2-mercaptoethanol–10% glycerol. Numerical designations refer to the molecular weight ($\times 10^{-3}$) of each polypeptide determined by a comparison with molecular weight standards.*

FIG. 2. *Electrophoresis of the acid extract of nucleocapsids from P. interpunctella GV on an acetic acid-urea gel. Nucleocapsids were incubated for 16 h at 4°C in 0.25 M sulfuric acid. Acid-insoluble proteins were removed by centrifugation at 15,000 rpm (SS34 rotor), and the acid-soluble proteins in the supernatant were precipitated with ethanol. The pellet was dissolved in 0.9 N acetic acid–10 M urea–2% 2-mercaptoethanol–10% glycerol and electrophoresed on 15% polyacrylamide slab gels containing 6.25 M urea at pH 3.2. (A) Calf thymus histones; (B) acid extract of GV nucleocapsids. Arrow indicates the migration of the dye marker.*

covered for characterization by precipitating it with ethanol. When the acid-soluble extract from the nucleocapsids was electrophoresed on polyacrylamide gels containing 6.25 M urea at pH 3, a single polypeptide species was observed (Fig. 2, lane B). To identify which nucleocapsid polypeptide this protein corresponded to, we analyzed it by SDS-polyacrylamide gel electrophoresis. A polypeptide with a molecular weight of 12,500 was present in the SDS-polyacrylamide gels, indicating that the acid-soluble nucleocapsid protein was VP12 (data described below).

The extreme basicity of this nucleocapsid protein was revealed with the acetic acid-urea gel system. In these gels, calf thymus histones, which range in molecular weight from 11,000 to 21,000, had relatively similar electrophoretic mobilities (Fig. 2, lane A). The much greater cathodic mobility of the 12,500-dalton VP12 in comparison with the histones suggested that it had a higher arginine content than did these proteins. The electrophoretic mobility of VP12 was not, however, as extreme as that of the very arginine-rich polypeptide protamine sulfate, which eluted from the gel with the tracking dye (Fig. 2, arrow).

Amino acid analysis and isoelectric focusing of VP12. For a further evaluation of the basicity and arginine content of VP12, it was isolated from GV nucleocapsids by acid extraction, hydrolyzed with 6 N hydrochloric acid, and analyzed for its amino acid composition. The analysis (Table 1) showed that more than one-third, approximately 39%, of the amino acid residues of VP12 were basic: 27% were arginine and 12% were histidine. Only trace amounts of lysine were present in the polypeptide. The overall amino acid composition of the protein was relatively simple, with the seven amino acids histidine, arginine, serine, valine, isoleucine, tyrosine, and proline contributing over 90% of the

TABLE 1. *Amino acid analysis of VP12*

Amino acid residue	mol/100 mol
Lysine	1.31
Histidine	12.45
Arginine	26.61
Aspartic acid	0.97
Threonine	0.00
Serine	16.31
Glutamic acid	0.39
Proline	4.14
Half cystine	0.28
Glycine	1.65
Alanine	1.32
Valine	16.89
Methionine	0.00
Isoleucine	13.28
Leucine	0.10
Tyrosine	5.11
Phenylalanine	0.41

total amino acid residues. Other than the basic amino acids, the most prominent residues were serine (16.3%), valine (16.9%), and isoleucine (13.3%). Only minor amounts of aspartic acid, glutamic acid, glycine, and alanine were detected in VP12.

The isoelectric point of VP12 was obtained by a direct measurement of the gel pH after isoelectric focusing. An alkaline pH range gel was needed to resolve VP12 which was observed to have an isoelectric point of approximately 9.8 to 10.0.

Acid extraction of uninfected and GV-infected whole cells and nuclei. Acid extracts were also prepared from uninfected and GV-infected *P. interpunctella* larvae to determine whether the arginine-rich VP12 was specific to virus infection. To analyze for the presence of VP12, we subjected the acid extracts to electrophoresis in acetic acid-urea gels (Fig. 3). Lanes C through F contained increasing amounts of the acid-soluble proteins derived from uninfected larval cell nuclei. In no case was a protein as basic as VP12 (Fig. 3, lane B) observed. Similar results were obtained when whole cells from uninfected larvae were acid extracted, indicating that the arginine-rich protein was not a normal constituent of the host cell protein composition. On the other hand, a polypeptide with an electrophoretic mobility in acetic acid-urea gels characteristic of VP12 was present in acid extracts of virus-infected cells and nuclei from infected cells (data not shown). These results suggest that VP12 is coded for by the viral genome. It is also possible that VP12 is a host-contributed polypeptide that is induced and synthesized during virus infection.

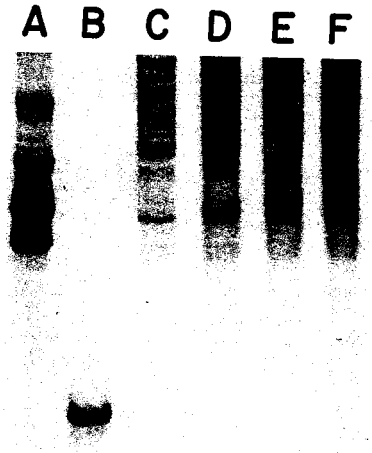

FIG. 3. *Acetic acid-urea gel electrophoresis of the acid extract of GV nucleocapsids and of nuclei from uninfected P. interpunctella larvae. For the isolation of nuclei, larvae were homogenized and the cells were pelleted by centrifugation at 5,500 rpm (HB-4 rotor). The cells were incubated in 0.15 M NaCl–0.01 M sodium citrate, pH 8.0, containing 1% Triton X-100 and 0.05 M sodium bisulfite for 10 min at 4°C and then centrifuged at 5,500 rpm (HB-4 rotor). The nuclear pellet was suspended in distilled water and extracted with 0.4 M sulfuric acid as described in the legend to Fig. 2. (A) Calf thymus histones; (B) acid extract of GV nucleocapsids; (C, D, E, and F) 50, 100, 150, and 200 μl, respectively, of the acid extract from uninfected larval nuclei.*

Isolation of capsids and comparison of their polypeptide composition with that of nucleocapsids. Because of its basicity, it was speculated that VP12 was associated with the viral DNA and, thus, would be an internal component of the nucleocapsids. To determine whether this was the case, capsids, devoid of DNA and any core proteins, were prepared. This was accomplished by treating purified nucleocapsids with 1 M NaCl–0.01 M EDTA in 0.01 M Tris-hydrochloride, pH 8.5, followed by velocity sedimentation in cesium chloride gradients. The band of capsids was recovered from the gradients and examined by electron microscopy (Fig. 4B). The tubular structures no longer took up the uranyl acetate stain as did intact nucleocapsids (Fig. 4A), indicating that they had lost their DNA core. In addition, when capsids were isolated from [^{3}H]thymidine-labeled nucleocapsids, no radioactivity was associated with the resulting capsid preparations, demonstrating that the viral DNA had been removed from them (data not shown).

The polypeptide composition of the capsids was then compared with that of nucleocapsids by SDS-polyacrylamide gel electrophoresis (Fig. 5). Present in the nucleocapsids, (Fig. 5, lane A) were the eight polypeptides described earlier, with VP31 and VP12 being the predominant components. Electrophoresed in lane B was the protein extracted from the nucleocapsids by the sulfuric acid treatment. It consisted of one of the major nucleocapsid structural polypeptides, VP12. Lane C contained the nucleocapsid proteins that were insoluble in the acid. All of the nucleocapsid proteins, except VP12, were observed. The SDS-polyacrylamide gel profile of the capsids of GV (Fig. 5, lane D) revealed that it closely resembled the profile of the acid-insoluble nucleocapsid proteins (lane C). Present in the capsid preparations were all of the nucleocapsid proteins except the basic protein, VP12, and VP44. The absence of VP12 from the capsids suggests that it is an internal or core protein that was removed from the capsids along with the viral DNA. The fact that VP44, an acid-insoluble polypeptide, was not present in the capsids may indicate that it also is a core protein

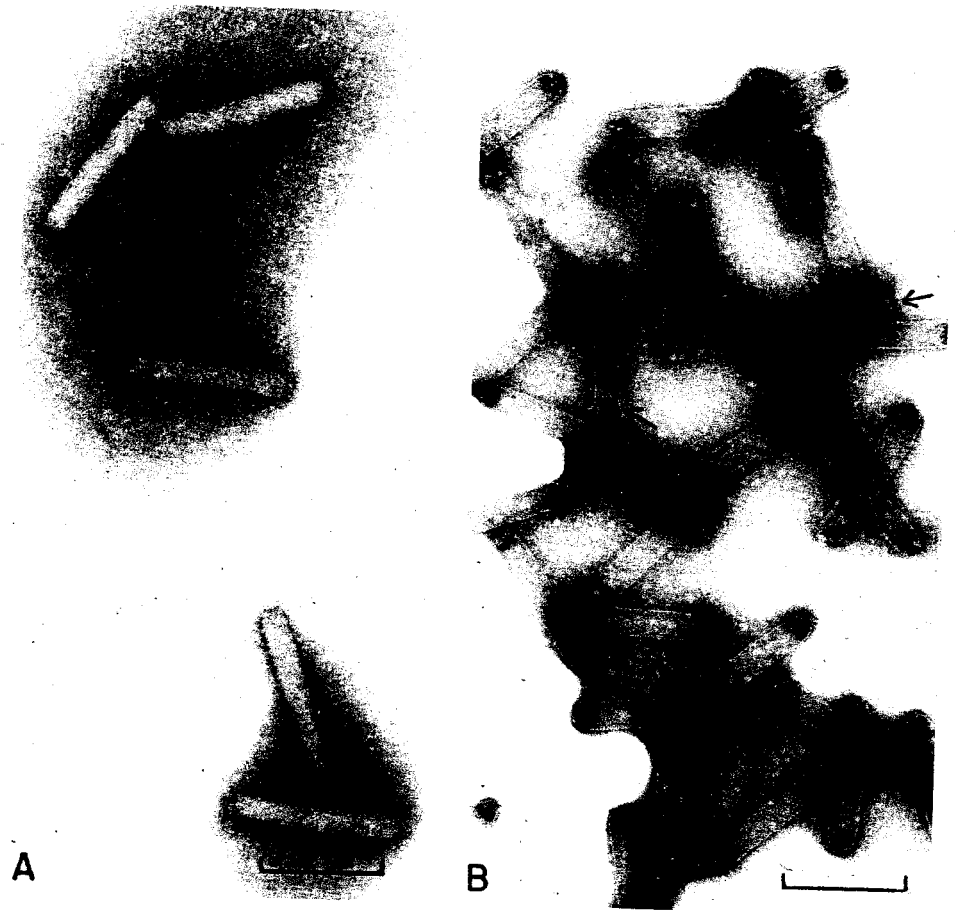

FIG. 4. *Electron micrographs of P. interpunctella GV nucleocapsids and capsids. Capsids were isolated by incubating nucleocapsids in 2% Nonidet P-40–0.01 M EDTA–1 M NaCl in 0.01 M Tris-hydrochloride, pH 8.5, for 12 h at 37°C. The preparation was then centrifuged on a preformed cesium chloride gradient (1.20 to 1.50 g/cm³ in 0.01 M Tris-hydrochloride, pH 8.5) at 34,000 rpm (SW41 rotor) for 2 h. The band of capsids recovered from the gradient and nucleocapsids were mounted on Formvar-coated grids and negatively stained with 2% uranyl acetate. Bar = 200 nm. (A) Nucleocapsids; (B) capsids.*

or that it comprises the structures located at the ends of the capsids. These structural components appear to have been "blown out" or removed during the isolation of the capsids (Fig. 4B, arrows) such that the capsids look like hollow cylinders.

Acid extraction of other baculovirus nucleocapsids. It was of interest to determine whether a basic protein similar to that obtained from the nucleocapsids of *P. interpunctella* GV was also a structural component of other baculoviruses. To investigate this possibility, we prepared nucleocapsids from the GVs of *P. rapae* and *S. frugiperda* and from the NPVs of *A. californica* and *S. frugiperda*. Each nucleocapsid preparation was extracted with 0.25 M sulfuric acid, and the resulting acid-soluble fractions were analyzed on acetic acid-urea gels. The results of two such electrophoretic analyses (lanes A to C and lanes D to G) are shown in Fig. 6. Electrophoresed in both gels as reference proteins were calf thymus histones (lanes A and G) and the basic protein, VP12, isolated from *P. interpunctella* GV (lanes B and F). An acid-extractable protein having a fast electrophoretic mobility in acetic acid-urea gels was obtained from all of the baculoviruses examined. The acid extract of *A. californica* NPV nucleocapsids prepared from nonoccluded enveloped nucleocapsids consisted of a single polypeptide (lane C) migrating slightly behind VP12. A similar protein was acid extracted from nucleocapsids isolated from *A. californica* polyhedra (data not shown). The proteins acid extracted from nucleocapsids of the NPV of *S. frugiperda* and the GV of *P. rapae* are shown in lanes D and E,

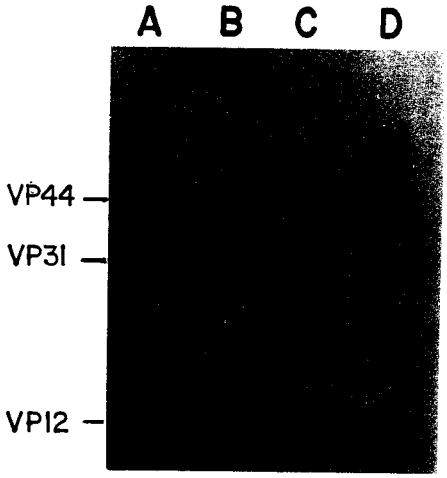

FIG. 5. *SDS-polyacrylamide gel electrophoresis of nucleocapsids, capsids, and the acid-soluble and -insoluble fractions from nucleocapsids of P. interpunctella GV. Acid-soluble and -insoluble nucleocapsid proteins and capsids were prepared as described in the legends to Fig. 2 and 4, respectively. Samples were boiled in 2% SDS–5% 2-mercaptoethanol–10% glycerol and electrophoresed on 15% polyacrylamide slab gels. (A) Nucleocapsids; (B) acid-soluble nucleocapsid proteins; (C) acid-insoluble nucleocapsid proteins; (D) capsids.*

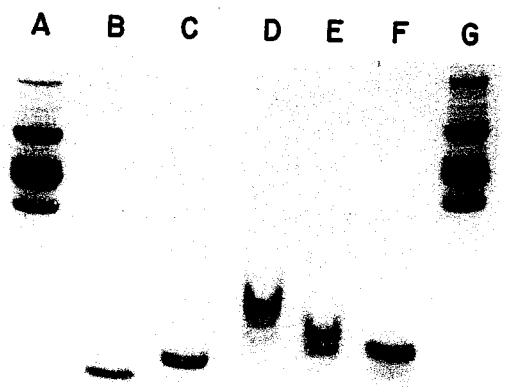

FIG. 6. *Acetic acid-urea gel electrophoresis of acid extracts of nucleocapsids from various baculoviruses. Acid extraction of nucleocapsids and electrophoresis were conducted as described in the legend to Fig. 2. This figure is a composite of two separate gels: A to C and D to G. (A and G) Calf thymus histones; (B and F) acid extract from nucleocapsids of P. interpunctella GV; (C, D, and E) acid extracts from nucleocapsids of A. californica NPV, S. frugiperda NPV, and P. rapae GV, respectively.*

respectively. Once again, the migration of these proteins in the acid-urea gels was characteristic of arginine-rich proteins. The multiple bands of protein observed in the acid extracts of these two baculoviruses may be due to a modification of the basic proteins by phosphorylation or acetylation since SDS-polyacrylamide gel electrophoresis of each of these preparations resolved only a single polypeptide species (data described below). When the acid-soluble fraction obtained from nucleocapsids of the GV of *S. frugiperda* was analyzed on acetic acid-urea gels, it also was found to have an electrophoretic mobility similar to that of VP12 (data not shown).

SDS-polyacrylamide gel analysis of baculovirus nucleocapsids and acid-soluble extracts. Nucleocapsids isolated from the GV of *P. rapae* and the NPV of *A. californica* and their acid extracts were subjected to electrophoresis on SDS-polyacrylamide gels. This was done to determine the molecular weights of the basic proteins derived from these viruses so that comparisons could be made with the *P. interpunctella* GV basic protein, VP12. As shown in Fig. 7, the basic proteins extracted from *P. rapae* GV (lane B) and from *A. californica* NPV (lane E) were both low-molecular-weight polypeptides, having molecular weights of 12,400 and 13,000, respectively. These polypeptides, as in the case

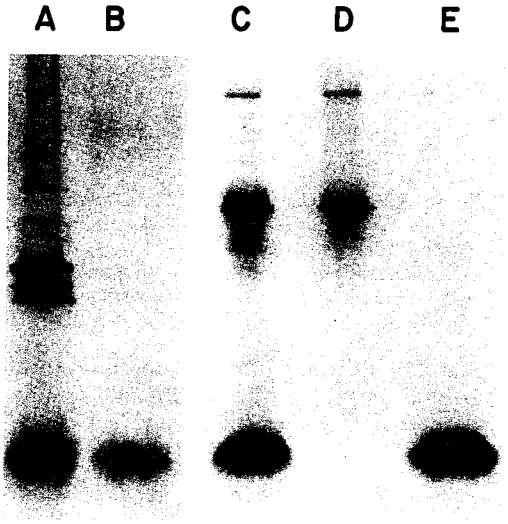

FIG. 7. *SDS-polyacrylamide gel electrophoresis of nucleocapsids, capsids, and nucleocapsid acid extracts from P. rapae GV and A. californica NPV. Acid-soluble nucleocapsid proteins and capsids were isolated as described in the legends to Fig. 2 and 4, respectively. This figure is a composite of two separate gels (A to B and C to F), prepared as described in the legend to Fig. 5. (A) P. rapae GV nucleocapsids; (B) P. rapae GV nucleocapsid acid extract; (C, D, and E) nucleocapsids, capsids, and nucleocapsid acid extract, respectively, from the NPV of A. californica.*

of VP12, were major components of the nucleocapsids (*P. rapae* GV, lane A; *A. californica* NPV, lane C) of these viruses. Capsids also were prepared from nucleocapsids of these baculoviruses, and their protein composition was determined. Like *P. interpunctella* GV capsids, they lacked the arginine-rich protein (*A. californica* NPV, lane E; *P. rapae*, data not shown). These results suggest that a low-molecular-weight, extremely basic core protein is characteristic of the baculoviruses.

Visualization of GV nucleoprotein complex by electron microscopy. Preliminary evidence that the arginine-rich protein isolated from GV nucleocapsids was associated with the viral DNA in the form of a DNA-protein complex was obtained by electron microscopy of dissociated nucleocapsids. Nucleocapsids from *P. interpunctella* GV were exposed briefly to a chelator (0.01 M EDTA) and a reducing agent (0.005 M dithiothreitol). These agents have been found to efficiently dissociate polyoma virions to capsomeres and a DNA-protein complex (4, 5). After treatment, the disrupted nucleocapsids were stained with uranyl acetate and examined by electron microscopy (Fig. 8B to G). Observations revealed that subterminal openings had been generated in the nucleocapsids through which a compact, rod-shaped structure was seen to emerge. The compactness and staining properties of these structures were characteristic of a DNA-protein complex (16, 17). Exposure of the nucleocapsids, on the other hand, to 1.0 M NaCl resulted in the release of naked viral DNA which was visualized as long, thin strands (Fig. 8H to J). It appeared that the salt treatment not only disrupted the capsids but also removed the proteins associated with the viral DNA.

DISCUSSION

The results presented in this paper demonstrate that one of the major structural polypeptides of nucleocapsids from *P. interpunctella* GV, VP12, is an extremely basic protein. The basic nature of VP12 was initially revealed by its acid solubility, a property characteristic of basic proteins such as histones or protamines (14). When GV nucleocapsids were treated with diluted, strong acid, VP12 was readily and selectively extracted from the viral preparation.

Also indicative of the basicity of the nucleocapsid polypeptide were its fast electrophoretic mobility in acetic acid-urea gels, high isoelectric point, and amino acid composition. When analyzed on urea gels, VP12 migrated to a position intermediate to that of calf thymus histones and protamine sulfate, a very arginine-rich protein. Because the molecular weight of VP12 was similar to that of the histones, its greater cathodic mobility suggested that it was considerably more basic than the histones. This was confirmed by amino acid analysis of isolated VP12, which indicated that 27% of the residues were arginine as compared with 14% for the arginine-rich histones (14). Also contributing to the basicity of VP12 was histidine, which accounted for 12% of the amino acid residues.

The chemical composition of VP12 was found to be unique. The high arginine content and apparent lack of lysine are characteristic of protamines, suggesting that VP12 is more protamine-like than histone-like. Its lack of aspartic acid and glutamic acid, along with its relatively simple amino acid composition, also suggests a more protamine-like character. In addition, serine, valine, and isoleucine were present in higher proportions than are typically found in vertebrate and invertebrate histones (35). VP12, however, was distinguishable from both mammalian and insect histones and most protamines by its unusually high histidine content and low glycine and alanine contents.

The basic polypeptide appears to be a structural component characteristic of the baculoviruses. Nucleocapsids of all of the NPVs and GVs examined in the present study contained an acid-extractable polypeptide that exhibited fast electrophoretic mobility in acetic acid-urea gels. Electrophoretic analysis on SDS-polyacrylamide gels revealed that, like VP12, the basic proteins extracted from the other baculoviruses were of low molecular weight, ranging from 12,400 for *P. rapae* GV to 13,000 for *A. californica* NPV. In addition, the basic polypeptides accounted for a substantial amount of the protein associated with the nucleocapsids from the various baculoviruses examined, being the major constituent of nucleocapsids from the GVs of *P. interpunctella* and *P. rapae* and the NPV of *A. californica*. The basic proteins present in the GV and NPV of *S. frugiperda* appear to be exceptions. Proteins demonstrating electrophoretic mobilities similar to that of VP12 were observed in acetic acid-urea gels of the acid extracts from nucleocapsids of these viruses. However, when these preparations were electrophoresed on SDS-polyacrylamide gels, no Coomassie brilliant blue-stained bands of protein were observed. One explanation may be that the arginine-rich proteins which are components of the baculoviruses that infect *S. frugiperda* are of such low molecular weight that they eluted from the gels along with the tracking dye.

It is interesting to note that most of the NPV and GV enveloped nucleocapsids whose structural polypeptide compositions have been determined contain a low-molecular-weight poly-

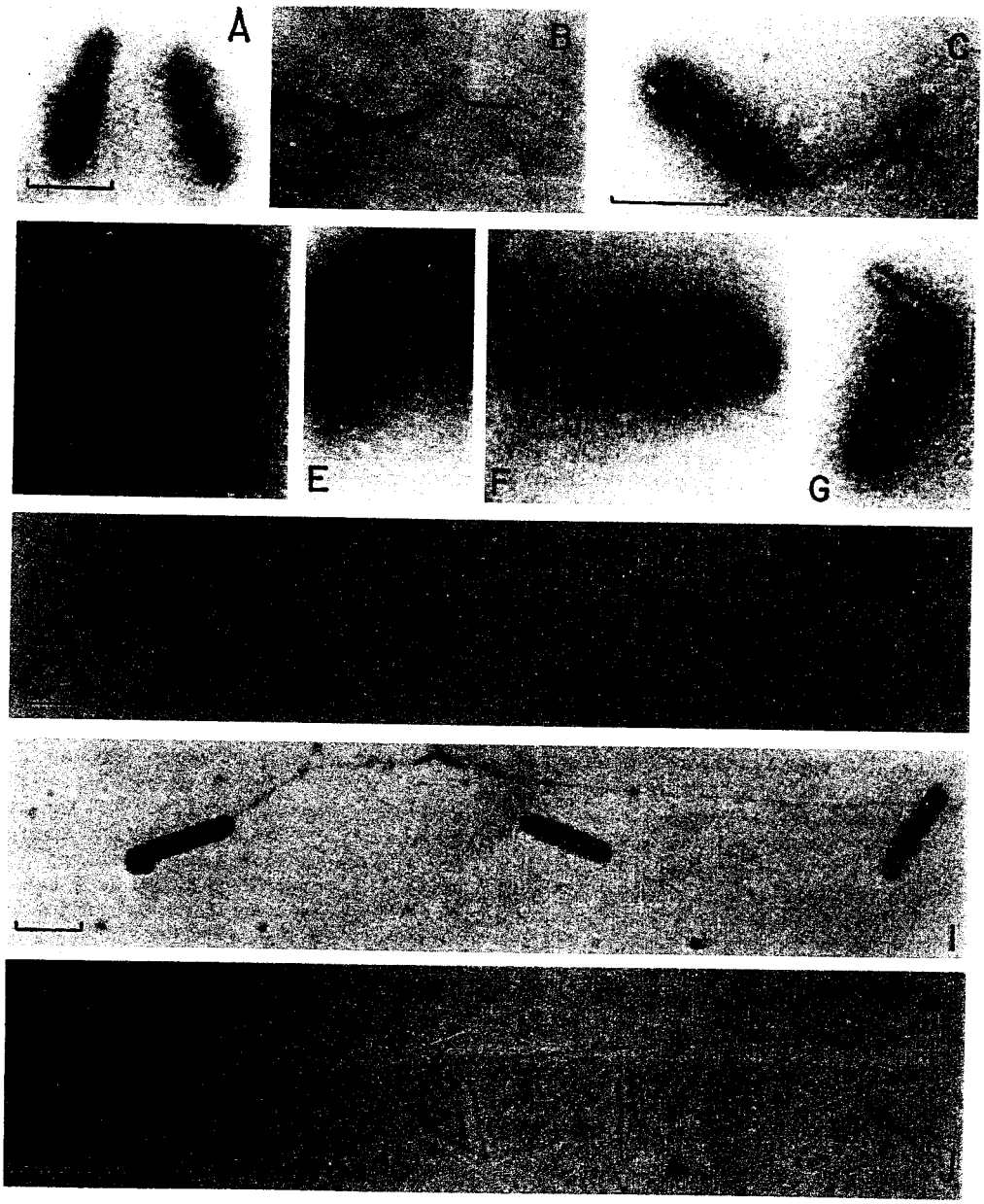

FIG. 8. *Electron micrographs of intact and disrupted P. interpunctella GV nucleocapsids. (A) Intact nucleocapsids; (B to G) nucleocapsids air dried on Formvar-coated grids and then incubated in droplets of 0.01 M EDTA–0.005 M dithiothreitol in 0.01 M Tris-hydrochloride, pH 7.0, for 30 min; (H to J) nucleocapsids incubated in 0.01 M EDTA–1 M NaCl in 0.01 M Tris-hydrochloride, pH 8.5, for 30 min. All specimens were stained with 2% uranyl acetate. Bar = 200 nm.*

peptide as their major component. In the nonoccluded baculovirus of *Oryctes rhinoceros* (25) and the enveloped nucleocapsids of the GV of *Pieris brassicae* (6) and of the NPVs of *Rachiplusia ou*, *Trichoplusia ni*, and *Galleria mellonella* (8, 21), the predominant polypeptide has a molecular weight of 12,000 to 12,600. For the NPVs of *Spodoptera littoralis*, *Tipula paludosa*, and *Lymantria dispar*, this protein is of a slightly higher molecular weight, ranging from 14,000 to 16,000 (11, 12, 21). Based on the observations made in the present study, it is likely

that these low-molecular-weight polypeptides are also arginine-rich, core-associated proteins. Because they comprise such a significant amount of the protein found in the nucleocapsids, these proteins probably are important structural and functional components of the baculoviruses.

Histones and histone-like proteins have been isolated from a number of animal viruses. Associated with the DNA of papovaviruses such as simian virus 40 (7), polyoma virus (5, 20), and human papillomaviruses (9) are cellular histones. Another DNA virus, adenovirus, contains four basic proteins in its core which resemble arginine-rich histones. One of these adenovirus proteins, polypeptide μ, closely resembles protamine in its arginine content and extreme electrophoretic mobility (15). However, unlike the GV-derived basic protein, it is present only as a minor component of the virion.

Histones and protamines are usually found associated with DNA in the form of DNA-protein complexes. From a functional point of view, all of the supercoiling present in the covalently closed DNA of polyoma virus and simain virus 40 is accounted for by the binding of the cellular histones (10). The arginine-rich histones H3 and H4 particularly play fundamental roles in nucleosome formation and condensation of the viral DNA (2, 34). The factors responsible for the supercoiling of the high-molecular-weight DNA of the baculoviruses are not known. Most likely, these same factors play a part in the condensation of the DNA necessary for packaging of the genome within the GV and NPV capsids. Protamines and other arginine-rich proteins have been observed to replace histones on DNA during late stages of spermatogenesis (3) in a number of vertebrate and invertebrate species. These proteins bind strongly to DNA and have been implicated in condensing DNA and in rendering it transcriptionally inactive (14). It is possible that the arginine-rich proteins isolated from the baculovirus nucleocapsids perform similar functions during viral maturation. The following two lines of evidence supporting this theory were obtained. (i) Experiments designed to localize the basic protein in the nucleocapsid structure revealed that, although it was the major constituent of the nucleocapsids, it was absent from capsids. This observation suggests that the arginine-rich polypeptides are internal or core proteins. (ii) Electron microscopic observations provided evidence that the core of GV nucleocapsids consists of a nucleoprotein complex. Rupture of the ends of the capsids with chelating agents resulted in the release of a thick fiber from within the capsid. Fibrillar structures similar in diameter to those present in *P. interpunctella* GV nucleocapsids also have been released from baculoviruses exposed to alkaline carbonate or thioglycolate (22, 26). Compact but less stable structures have been demonstrated after disruption of NPV or GV enveloped nucleocapsids by thermal shock (24, 27). Strands ranging in diameter from 25 to 30 nm to a size characteristic of naked duplex DNA (2.5 nm) were observed, probably representing various stages of DNA decondensation. In these earlier studies, the factors responsible for the aggregation of the viral DNA were not determined. The sensitivity of the compact structure found in *P. interpunctella* GV nucleocapsids to salt is consistent with the speculation that protein is bound to the DNA. If this indeed is the case, the data presented in this report strongly suggest that the protein bound to baculovirus DNA is the extremely basic nucleocapsid polypeptide.

Experiments are currently in progress to isolate the nucleoprotein complex in an intact form so that its biochemical properties and associated proteins can be identified and characterized. As the properties of the complex are investigated, insight should be gained into the function of the arginine-rich nucleocapsid protein. For example, is its appearance in infected cells correlated with the condensing and packing of the viral DNA into capsids? Does it interact with the viral genome only during assembly, or does it remain associated with the DNA after uncoating of the nucleocapsids and act to regulate transcription of the viral DNA? Answers to these questions not only will lead to a better understanding of the GV infection process, but also will provide information on what appears to be a very unique group of proteins.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant ES 02036 from the National Institute of Environmental Health Services. K.A.T. was supported by a research associateship from the U.S. Grain Marketing Research Laboratory, U.S. Department of Agriculture, Science and Education Administration, and from the Kansas Agricultural Experiment Station.

We thank Kimberly Osborne, Dennis K. Anderson, Diane Potts, and Viola Hill for their excellent technical assistance.

LITERATURE CITED

1. **Beaton, C. D., and B. K. Filshie.** 1976. Comparative ultrastructural studies of insect granulosis and nuclear polyhedrosis viruses. J. Gen. Virol. **31**:151–161.
2. **Bina-Stein, M., and R. T. Simpson.** 1977. Specific folding and contraction of DNA by histones H3 and H4. Cell **11**:609–618.
3. **Bloch, D. P.** 1969. A catalog of sperm histones. Genetics **61**(Suppl.):93–111.
4. **Brady, J. N., V. D. Winston, and R. A. Consigli.** 1977. Dissociation of polyoma virus by the chelation of calcium ions found associated with purified virions. J. Virol. **23**:717–724.
5. **Brady, J. N., V. D. Winston, and R. A. Consigli.** 1978. Characterization of a DNA-protein complex and cap-

somere subunits derived from polyoma virus by treatment with ethyleneglycol-bis-*N,N'*-tetraacetic acid and dithiothreitol. J. Virol. **27**:193–204.

6. **Brown, D. H., H. M. Bud, and D. C. Kelly.** 1977. Biophysical properties of the structural components of a granulosis virus isolated from the cabbage white butterfly, (*Pieris brassicae*). Virology **81**:317–327.
7. **Christiansen, G., T. Landers, J. Griffith, and P. Berg.** 1977. Characterization of components released by alkali disruption of simian virus 40. J. Virol. **21**:1079–1084.
8. **Cibulsky, R. J., J. D. Harper, and R. T. Gudauskas.** 1977. Biochemical comparison of virion proteins from five nuclear polyhedrosis viruses infecting plusiine larvae (Lepidoptera: Noctuidae). J. Invertebr. Pathol. **30**: 303–313.
9. **Favre, M., F. Breitburd, O. Croissant, and G. Orth.** 1977. Chromatin-like structures obtained after alkaline disruption of bovine and human papillomaviruses. J. Virol. **21**:1205–1209.
10. **Germond, J. E., B. Hirt, P. Oudet, M. Gross-Bellard, and P. Chambon.** 1975. Folding of the DNA double helix in chromatin-like structures from simian virus 40. Proc. Natl. Acad. Sci. U.S.A. **72**:1843–1847.
11. **Guelpa, B., M. Bergoin, and G. Crozier.** 1977. La proteine d'inclusion et les proteines du virion du Baculovirus du diptere *Tipula paludosa* (Meigen). C.R. Acad. Sci. **284**:779–782.
12. **Harrap, K. A., C. C. Payne, and J. S. Robertson.** 1977. The properties of three baculoviruses from closely related hosts. Virology **79**:14–31.
13. **Hirs, C. H. W.** 1967. Determination of cystine as cysteic acid. Methods Enzymol. **2**:59–62.
14. **Hnilica, L. S.** 1972. Chemical properties of histones, p. 3–45. *In* R. C. Weast (ed.), The structure and biological function of histones. CRC Press, Cleveland, Ohio.
15. **Hosokawa, K., and M. T. Sung.** 1976. Isolation and characterization of an extremely basic protein from adenovirus type 5. J. Virol. **17**:924–934.
16. **Kierszenbaum, A. L., and L. L. Tres.** 1975. Structural and transcriptional features of the mouse spermatid genome. J. Cell Biol. **65**:258–270.
17. **Kleinschmidt, A., and R. K. Zahn.** 1960. Morphologie geloster Deoxyribonucleinsaure-Praparate und einige ihrer Eigenschafter in Oberflachen-Mischfilmen. Proc. 4th Int. Congr. Electron Micros. (Berlin), p. 115–118.
18. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
19. **Mackay, R. L., and R. A. Consigli.** 1976. Early events in polyoma virus infection: attachment, penetration, and nuclear entry. J. Virol. **19**:620–636.
20. **McMillen, J., and R. A. Consigli.** 1974. Characterization of polyoma DNA-protein complexes. I. Electrophoretic identification of the proteins in a nucleoprotein complex isolated from polyoma-infected cells. J. Virol. **14**:1326–1336.
21. **Merdan, A., L. Crozier, J. C. Veyrunes, and G. Crozier.** 1977. Etude comparee des proteines des polyedres et des virions de trois isolats de Baculovirus de *Spodoptera littoralis*. Entomophaga **22**:413–420.
22. **Monsarrat, P., B. Revet, and I. Gourevitch.** 1975. Mise en evidence stabilisation et purification d'une structure nucleoproteique intracapsidaire chez le Baculovirus d'*Oryctes rhinoceros* L. C.R. Acad. Sci. **281**:1439–1442.
23. **Panyim, S., and R. Chalkley.** 1969. High resolution acrylamide gel electrophoresis of histones. Arch. Biochem. Biophys. **130**:337–346.
24. **Pawar, V. M.** 1975. Structure, development and physicochemical properties of the nuclear polyhedrosis virus of *Spodoptera litura* (Fabricius) and associated biochemical changes in the hemolymph of the host. Entomol. Newsl. **5**:14–15.
25. **Payne, C. C., D. Compson, and S. M. DeLooze.** 1977. Properties of the nucleocapsids of a virus isolated from *Oryctes rhinoceros*. Virology **77**:269–280.
26. **Ponsen, M. B.** 1965. Electron microscopy of DNA-cores in nuclear polyhedral viruses. Neth. J. Plant Pathol. **71**: 54–56.
27. **Shvedchikova, N. G., V. P. Ulanov, and L. M. Tarasevich.** 1969. Structure of the granulosis virus of Siberian silkworm *Dendrolimus sibiricus* Tschetw. Mol. Biol. **3**:283–287.
28. **Summers, M. D., and D. L. Anderson.** 1973. Characterization of nuclear polyhedrosis virus DNAs. J. Virol. **12**:1336–1346.
29. **Summers, M. D., and G. E. Smith.** 1978. Baculovirus structural polypeptides. Virology **84**:390–402.
30. **Tweeten, K. A., L. A. Bulla, Jr., and R. A. Consigli.** 1977. Isolation and purification of a granulosis virus from infected larvae of the Indian meal moth, *Plodia interpunctella*. Appl. Environ. Microbiol. **34**:320–327.
31. **Tweeten, K. A., L. A. Bulla, Jr., and R. A. Consigli.** 1977. Supercoiled circular DNA of an insect granulosis virus. Proc. Natl. Acad. Sci. U.S.A. **74**:3574–3578.
32. **Tweeten, K. A., L. A. Bulla, Jr., and R. A. Consigli.** 1978. Characterization of an alkaline protease associated with a granulosis virus of *Plodia interpunctella*. J. Virol. **26**:702–711.

32a. **Tweeten, K. A., L. A. Bulla, Jr., and R. A. Consigli.** 1980. Structural polypeptides of the granulosis virus of *Plodia interpunctella*. J. Virol. **33**:877–886.

33. **Weber, K., and M. Osborne.** 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. **244**: 4406–4412.
34. **Weihe, A., C. VonMickwitz, K. Grade, and R. Lindigkeit.** 1978. Complexes of DNA with arginine-rich and slightly lysine-rich histones. Transcription and electron microscopy. Biochim. Biophys. Acta **518**:172–176.
35. **Yoshida, M., K. Yokotsuka, and K. Shimura.** 1966. Amino acid composition and heterogeneity of histones from posterior silkgland. J. Biochem. (Tokyo) **60**:586–588.