

Applied and Molecular Aspects of Insect Granulosis Viruses†

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INTRODUCTION

Like most other animals, insects are susceptible to diseases caused by viruses. A variety of viruses differing markedly in morphological, biophysical, and biochemical characteristics infect insects. Of the approximately 650 viruses that have been isolated from insects, 83% are from lepidopterans (moths and butterflies), about 14% are from hymenopterans (sawflies), and 3% are from orthopterans (grasshoppers and cockroaches), coleopterans (beetles), and dipterans (flies and mosquitoes) (104). Currently, eight families of insect viruses are recognized (Fig. 1), ranging from the small icosahedral parvoviruses to the larger, more structurally complex poxviruses. They include a recently discovered picornavirus related to *Nudaurelia* β virus (167, 180) and three families in which the viral particles are occluded in a proteinaceous matrix. This feature, which is characteristic of cytoplasmic polyhedrosis viruses, entomopoxviruses, and baculoviruses, is unique to invertebrate viruses. The protein matrix is synthesized and deposited around virions during virus replication and is thought to contribute to the stability of the viruses in the physical environment outside the

host. In general, granulosis virus (GV) enveloped nucleocapsids are singly occluded, whereas in the other occluded viruses several viral particles are embedded randomly within each protein matrix (77, 148, 211) (Fig. 1).

Many insect viruses infect several insect species. The cytoplasmic polyhedrosis viruses and iridoviruses exhibit the widest range of hosts; these viruses have been isolated from a number of insect orders (46, 201). Entomopoxviruses and nuclear polyhedrosis viruses (NPVs) also infect insects belonging to a variety of orders. In contrast, infection by the GVs is limited to lepidopterans (46), and the insect rhabdoviruses presumably infect only *Drosophila* species (177). Generally, insect viruses attack larval forms and cause symptoms ranging from acute infection and insect death to inapparent infection. For recent reviews of the ultrastructures, host ranges, and pathologies of the various insect virus families, see references 46, 77, 148, 149, and 201.

Several of the insect viruses closely resemble vertebrate viruses both in structure and in biochemical properties. In particular, the entomopoxviruses, cytoplasmic polyhedrosis viruses, and sigma viruses show striking similarities to viruses from vertebrate hosts (6, 7, 19, 69, 85, 177, 183). However, significant differences have

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VIRUS FAMILY	NUCLEIC ACID (type)	VERTEBRATE EXAMPLE	INVERTEBRATE (INSECT) EXAMPLE	MORPHOLOGY	
				VIRION	OCCLUDED FORM
REOVIRIDAE	dsRNA	HUMAN REOVIRUS	CYTOPLASMIC POLYHEDROSIS VIRUS		
RHABDOVIRIDAE	ssRNA	VESICULAR STOMATITIS VIRUS	SIGMAVIRUS		—
TOGAVIRIDAE	ssRNA	ARBOVIRUSES	ARBOVIRUSES		—
PARVOVIRIDAE	ssDNA	ADENO-ASSOCIATED VIRUS	DENSOVIRUS		—
PICORNAVIRIDAE	ssRNA	FELINE PICORNAVIRUS	NUDAURELIA CAPENSIS VIRUS		—
POKXVIRIDAE	dsDNA	VACCINIA	ENTOMOPOXVIRUS		
IRIDOVIRIDAE	dsDNA	AFRICAN SWINE FEVER VIRUS	IRIDESCENT VIRUS		—
BACULOVIRIDAE	dsDNA	-----	A) GRANULOSIS VIRUS		
			B) NUCLEAR POLYHEDROSIS VIRUS		
			C) ORYCTES VIRUS		—

FIG. 1. Classification of viruses that infect vertebrates and insects. In the occluded forms, several virions are occluded within a crystalline protein matrix during replication. ds, Double stranded; ss, single stranded; RNA, ribonucleic acid.

been observed in the biological activities and host specificities of these viruses. Cross-transmission of insect viruses to vertebrates has been observed in only a few cases. Densonucleosis virus, which is highly virulent in several tissues of the greater wax moth (*Galleria mellonella*), replicates in mammalian L cells and rat embryo cells (140, 141). A more classic example is the susceptibility of dipteran insects to infection by the arboviruses that they transmit to vertebrate animals (198, 251).

In contrast to other insect virus families, members of the Baculoviridae (the GVs, the NPVs, and the nonoccluded Oryctes and braconid viruses) apparently are restricted to invertebrate hosts (207, 252). No morphologically similar counterpart to the baculoviruses has been detected in vertebrates. This unique characteristic has generated much scientific interest in this group of viruses. In the search for alternatives to chemicals for suppression of insect populations, the use of bacteria, rickettsiae, viruses, parasitic insects, fungi, and nematodes as biological control agents is being investigated (103). Of the insect viruses, only the baculoviruses have

been recommended for field use. A limited host range is an important consideration in the safe implementation of viruses as insecticides (64).

Several studies have shown the effectiveness of the GVs and NPVs against their hosts. In this review we summarize studies that have examined the feasibility of using one group of baculoviruses, the GVs, as insecticides. We also present information on the biochemical and molecular properties of the GVs, much of which has been gathered because of the rigorous characterizations required by governmental agencies for registration of GVs as pesticides. These basic studies not only have provided information useful to the application of insect viruses as insecticides, but also have revealed that the GVs are complex and intriguing animal viruses. For reviews on the NPVs, see references 77, 149, and 201.

APPLICATION AS INSECTICIDES

Insecticidal Potential

A GV infection was first detected in 1926 by Paillot (171), who recognized previously unde-

scribed disease symptoms in larvae of the large white butterfly, *Pieris brassicae*. The disease, which Paillot also found in the cutworm *Agrotis segetum* (172), was not confirmed until 1947, when Steinhaus observed it in the variegated cutworm, *Peridroma margaritosa* (205). Steinhaus called the disease granulosis because light microscopy of the affected tissue revealed cells packed with minute "granules." The viral nature of these granules was demonstrated in 1948 by Bergold (21), who examined diseased tissue of the pine shoot roller (*Choristoneura murinana*) by electron microscopy and observed rod-shaped virus particles.

Susceptibility of Economically Important Pests

Since the initial description of the GVs, more than 80 species of lepidopterans have been reported to be susceptible to GVs (151), including some of the most serious insect pests of agricultural crops and forests. Many destructive insects, such as the potato tuberworm (*Phthorimaea operculella*), the codling moth (*Laspeyresia pomonella*), the almond moth (*Ephestia cautella*), the cabbage looper (*Trichoplusia ni*), and the spruce budworm (*Choristoneura fumiferana*), become extensively diseased when they are infected by GVs (64).

The high pathogenicity of GVs for these and other economically important insects (Table 1) has been largely responsible for the widespread interest in this group of viruses and has led to their consideration for use as biological insecticides. The concept of virological control of in-

sects owes much to the rapid development of strains of insect pests that are resistant to chemical insecticides. For example, pests of stored grain are becoming resistant to malathion, the only chemical insecticide which has been accepted widely for protection of stored commodities (64, 250, 261). More than 300 other species have developed resistance, and this resistance has been countered by higher and more frequent rates of chemical application. In turn, such practices have increased pesticide resistance in insects and have resulted in high levels of pesticide residues. Because of concern about the harmful effects of these residues on the environment and on nontarget organisms, much effort has been devoted to the development of alternative methods of insect control, including viruses.

Large-Scale Application and Field Testing

Some GVs have already been used as insecticides to control pests, and, for many reasons, others appear to be attractive candidates. In initial laboratory studies during the 1950s, the GV of *P. brassicae* was used successfully against early instar larvae (24, 130, 131). Similar control was observed when GV was applied to field populations of another *Pieris* species, *Pieris rapae*, and damage to cabbage plants was reduced (253). More recently, the *P. rapae* GV has played a significant role in reducing populations of this destructive insect in Japan (2, 112) and Canada (114, 115). Another GV which has been used effectively in insect control in Japan is the GV that infects the fruit tree pest *Adoxophyes orana* (113, 193).

TABLE 1. Some insect pests susceptible to GVs

Insect pest	Crop(s) affected	Area of field tests or large-scale application	Reference(s)
Potato tuberworm	Potatoes	Pacific	179
Cotton cutworm	Cotton	Soviet Union	120
Cereal noctuid	Wheat	Soviet Union	64
Cabbage looper	Broccoli, cabbage, cauliflower, celery, lettuce, potatoes, melons	Soviet Union	64
Codling moth	Fruit trees	Canada, United States, Europe, Australia, New Zealand	58, 88, 118, 166, 249
Large white butterfly	Kale crops	Europe	24
Imported cabbage worm	Cabbage and other cruciferous crops	Europe, North America	115
Red-banded leaf roller	Fruit trees, forest trees, herbaceous plants	North America	64
Asiatic rice borer	Rice	Japan	189
Green cloverworm	Soybeans	United States	16
Spruce budworm	Spruce trees	North America	64
Indian meal moth	Stored grains, nuts, raisins	United States	156
Almond moth	Dried fruits, nuts, cereals	United States	98, 99
Fall webworm	Deciduous trees	Japan	223
Summer fruit tortrix	Fruit trees	Japan	113

Large-scale applications of GVs in pest management programs also have been used in the Soviet Union, Czechoslovakia, Yugoslavia, the Peoples' Republic of China, Australia, and Great Britain (121). For example, in the Soviet Union, GVs have been used against the cotton cutworm, on wheat for control of the cereal noctuid (*Hadena sordida*), and on cabbage against the cabbage looper (*T. ni*) (64). Insecticidal preparations of the GV of the fir budworm (*C. murinana*) are being used in forests in Canada to limit defoliation by this insect (190). In Australia, the potato moth GV has been used extensively in the field (153).

The use of viral pathogens as insecticides in the United States and Western Europe has been more restricted due to registration requirements (54, 65, 102, 138, 182). Only the NPVs which suppress the cotton earworm (*Heliothis zea*), the gypsy moth (*Lymantria dispar*), and the douglas fir tussock moth (*Orgria pseudotsugata*) have been approved for use in the United States (192). Of the GVs, the virus infecting the codling moth (*L. pomonella*) has been used most extensively in field studies and is closest to commercial production in the United States. A number of investigations in Canada, Germany, and the United States have demonstrated that this virus reduces codling moth infestations in apple orchards to low levels (31, 60, 86-88, 118, 191). Another GV which has been studied in the United States for its insecticidal potential is the GV of the Indian meal moth, *Plodia interpunctella*. This pest is of international economic concern as it causes extensive damage to grain and other stored commodities. Studies have shown that treatment of corn and wheat under typical storage conditions with this GV (134, 156) or application of the virus to processed products, such as almonds (94, 96) and raisins (97), results in a substantial reduction of feeding damage to these stored products.

Comparison with Chemical Insecticides

The feasibility of using GVs as insecticides also has been confirmed by comparing the effectiveness of these viruses with that of chemical insecticides. Field studies with chemicals and the GV of the green clover worm (*Plathypena scabra*), on soybeans showed no significant differences between the two methods of control (16). A similar observation was made by Jaques (115) when he compared control by *P. rapae* GV with control by the chemical methomyl. Other field tests have shown that GVs are more effective than chemical insecticides. A single application of potato tuberworm GV gave control equivalent to 8 to 10 applications of chemical

pesticides (203). Populations of the codling moth have been reduced more effectively by virus sprays than by equivalent numbers of organophosphate pesticide treatments (88).

Other Beneficial Features as Insecticides

In addition to being highly virulent to their hosts, GVs have many other characteristics which make them ideally suitable for pest control, as described below.

Infection by GVs is limited to the order Lepidoptera and in most cases is species-specific (39, 101). Because of this selectivity, GVs can be used more precisely in pest management programs, without the detrimental effects on nontarget organisms that are characteristic of many chemical insecticides. Also, outbreaks of secondary pests caused by nonspecific killing of parasite and predatory insects can be avoided.

The influence of environmental factors on the viability of GVs has been reviewed recently by David (47). GVs are stable and can be stored as aqueous suspensions or dried powders for long periods without any loss of activity (50). They are resistant to many chemicals and persist in the soil for many years (51), and their activity is not altered significantly by relative humidity (48), precipitation (49), or prolonged exposure to normal field temperatures (260). On the other hand, GVs deposited on foliage are inactivated rapidly by exposure to the ultraviolet radiation in direct sunlight (45, 52, 114). More than 50% of the activity of these viruses was lost in 2 days after field application (116), and little activity remained after 10 days. As a result, foliar residues, which are a major concern with persistent chemicals, are minimal after application of GVs to crops.

GVs are highly compatible with other methods of pest control and are well suited for use in integrated pest management programs. They can be used concurrently with most chemical insecticides, reducing effective doses of the latter to environmentally acceptable levels (58, 117). For example, the GV of *P. interpunctella* is compatible with certain fumigants (95) and chemical insecticides, such as malathion (157). Combined with malathion, this virus enhanced control of *P. interpunctella*; at the same time, two other serious stored-product insects, the merchant grain beetle (*Oryzaephilus mercator*) and the red flour beetle (*Tribolium castaneum*), were controlled by malathion.

GVs differ significantly from chemical insecticides in that they are components of nature. Large quantities of virus are released into the environment during natural epizootics, which are common, widespread, and often important

in regulating insect population levels (62, 84, 109, 110, 224). There is evidence that the amount of virus which is artificially placed into the environment for insecticidal purposes is minimal compared with the amount produced during such epizootics (232).

Whereas the toxicity and teratogenicity of many chemical insecticides to humans, animals, and plants has been demonstrated clearly (233), the biological effects of the baculoviruses evaluated to date appear to be restricted to the target insects. In vivo and in vitro tests (12, 36, 89, 102, 108, 138, 142, 185) with several vertebrate, invertebrate, and plant species have not demonstrated any pathogenic, toxic, carcinogenic, or teratogenic effects after exposure to these viruses.

Limitations to Commercial Development

The obstacles that have been encountered in the commercial development of GVs as viral insecticides have been described previously (182). Some of the major difficulties include the following.

Efficient processes for large-scale propagation of viruses in living hosts have been developed (31, 105), and encouraging results have been obtained from the growth of NPVs in tissue culture (82, 98, 106, 244, 245). However, the costs of virus production, formulation, and standardization are high (80, 103). Also, replication of GVs in vitro has not been accomplished, so that at present tissue culture systems cannot be used for the commercial production of GVs.

The marketability of individual GV products is limited due to the highly selective host ranges of the viruses.

Applications of viral insecticides must be precisely scheduled and monitored. Because larval susceptibility to virus infection appears to decrease with age, virus preparations must be applied early in insect development if serious economic damage is to be avoided (28).

Inactivation of GVs by ultraviolet radiation necessitates repeated applications of viral insecticides for effective control (32, 108, 111). Various additives, such as ultraviolet-adsorbing materials, are being investigated for their stabilizing effects on virus viability.

Further Investigations

The above-described factors show the great potential and some of the disadvantages that GVs have as biological control agents of insects that attack food, fiber, and forest crops throughout the world. The insecticidal potential of GVs is being further investigated with two main questions in mind.

One question is whether GV insecticides can be used safely. Because the effects of GVs on nontarget species have not been investigated extensively at cellular and molecular levels, the potential risks associated with their use, although most likely minimal, are not fully known (138, 234, 235). There is some evidence for incomplete NPV replication in a vertebrate viper cell line (159) and for persistence of viable *H. zea* NPV in cell cultures from human lung, leukocyte, and amnion tissues (158). These observations warrant further tests for the safe implementation of these viruses as insecticides. Other safety considerations concerning the use of baculoviruses for insect control have been reviewed previously (54, 215a).

The second question is how GVs can be used most effectively and efficiently alone and in integrated pest control programs. What features of GVs are involved in their insecticidal activities, and how can these features be used to best advantage so that the full potential of these viruses for the control of economically important insects is realized?

Answers to these questions are being provided, in part, by basic studies, such as those described below.

CELLULAR BIOLOGY

Ultrastructure

The structure of GVs was first investigated with electron microscopy (21). Characteristically, GVs are ovocylindrical in shape and average 300 to 500 nm in length by 120 to 350 nm in width (23, 90). Thin sectioning of isolated viruses and of infected insect tissues showed that GVs are structurally complex (4, 5, 9, 22, 164, 204, 216). These viruses consist of rod-shaped nucleocapsids surrounded by envelopes. Each enveloped nucleocapsid is embedded within a matrix of protein, which has a regular crystalline lattice (Fig. 2). Interestingly, the continuity of the lattice pattern is not disrupted by the enveloped nucleocapsids (23). As described above, the protein matrix is a feature unique to certain invertebrate viruses and is thought to contribute to their stability. At the periphery of the protein matrix is a structurally distinct electron dense layer (4, 124). The controversy over whether this surface layer in baculoviruses consists of highly condensed matrix protein (73) or a lipoprotein membrane (81) has not been resolved yet. GV particles in their embedded form generally are referred to as occluded enveloped nucleocapsids. The terms "inclusion body" and "capsule" have also been used to indicate the entire GV structure, consisting of nucleocapsid, envelope, and protein matrix (Fig. 2).

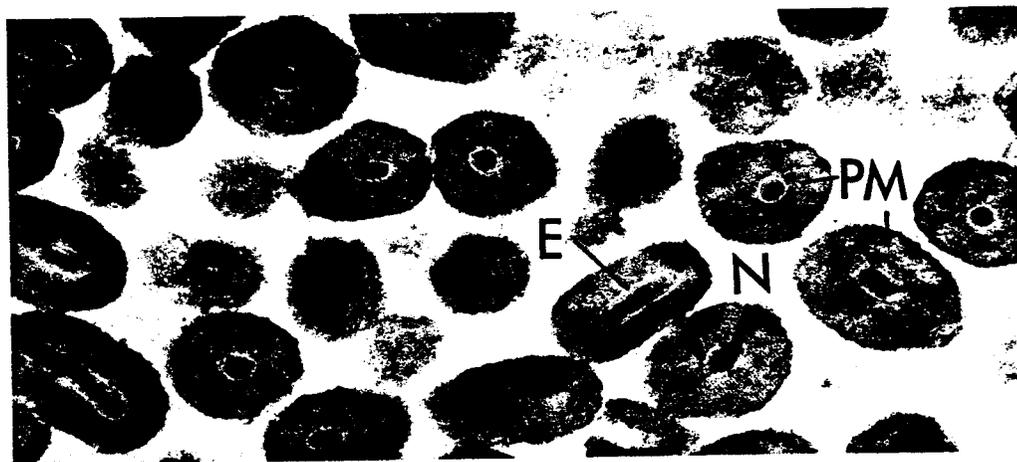


FIG. 2. Electron micrograph of thin-sectioned *P. interpunctella* GV, showing the periodicity of the protein matrix (PM), the viral envelope (E), and the nucleocapsid (N).

Additional structural details of GV components have been revealed by examinations of nonoccluded enveloped nucleocapsids and nucleocapsids. Studies such as those of Beaton and Filshie (13) have shown that the envelope (previously designated the outer or developmental membrane) is a triple-layered membrane which is 5 to 6 nm thick. These investigators studied the fine structures of the GVs in infected cells of *P. rapae* and *P. operculella* and found that the envelope is ultrastructurally similar to a biological lipoprotein unit membrane. Unlike the surfaces of other enveloped animal viruses, the surface of the GV envelope occluded does not appear to be modified by glycoprotein spikes (Fig. 3A).

The rod-shaped nucleocapsids consist of proteinaceous capsids and DNA cores and have average dimensions of 30 to 60 by 260 to 360 nm (Fig. 3B). A number of investigators, including Kozlov and Alexeenko (136) and Summers and Paschke (216), have reported that the opposite ends of the nucleocapsids are morphologically distinct. Whereas one end is blunt and has been referred to as the tail plate, the other end consists of a tail plate into which a caplike structure is inserted (231) (Fig. 3D). Each viral capsid (referred to as an intimate or internal membrane in early work) is made up of subunits assembled in a regular lattice (202, 216). Diffraction studies (13) and electron microscopic measurements (75) have shown that the lattice consists of rings of subunits stacked on top of one another (Fig. 3C). This distinguishes GV capsids from rod-shaped plant virus capsids, in which the subunits are in a helical arrangement (194). Recent studies have suggested that a nucleoprotein complex is contained within each baculovirus capsid (239)

(Fig. 3E and F). The extent to which the ultrastructural features and biochemical nature of this core have been determined is described below.

In Vitro Replication

Unlike the NPVs (66), the GVs have not been propagated successfully in vitro. The only reported infection was in primary cultures of *L. dispar* ovaries inoculated with GV-infected fat bodies from *P. brassicae* (242). Virus production was irregular and incomplete, with nuclear hypertrophy and formation of occluded virus detected in only some of the cultures. More recently, investigators were not able to replicate GVs in cell lines derived from *Spodoptera frugiperda*, *T. ni*, and *H. zea* (67). In *T. ni* cells, an aberrant infection occurred, in which numerous membrane structures and long cylindrical forms resembling capsids, but no normal nucleocapsids, were synthesized. A similar incomplete development of virus was reported after infection of alternate insect hosts with GVs and NPVs (99) or after prolonged undiluted serial passage of NPVs in vitro (147).

Although other attempts to infect cultured cells with GVs have apparently been made (67), the cellular responses have not been reported. If such information was made available, perhaps some insight could be obtained concerning the influence of homologous and heterologous cell lines, cell types, cell nutrition, and sources of viral inoculum (hemolymph, infected tissues, viral deoxyribonucleic acid [DNA]) on the ability to produce GVs in vitro.

In Vivo Replication

Although many species of lepidopteran larvae

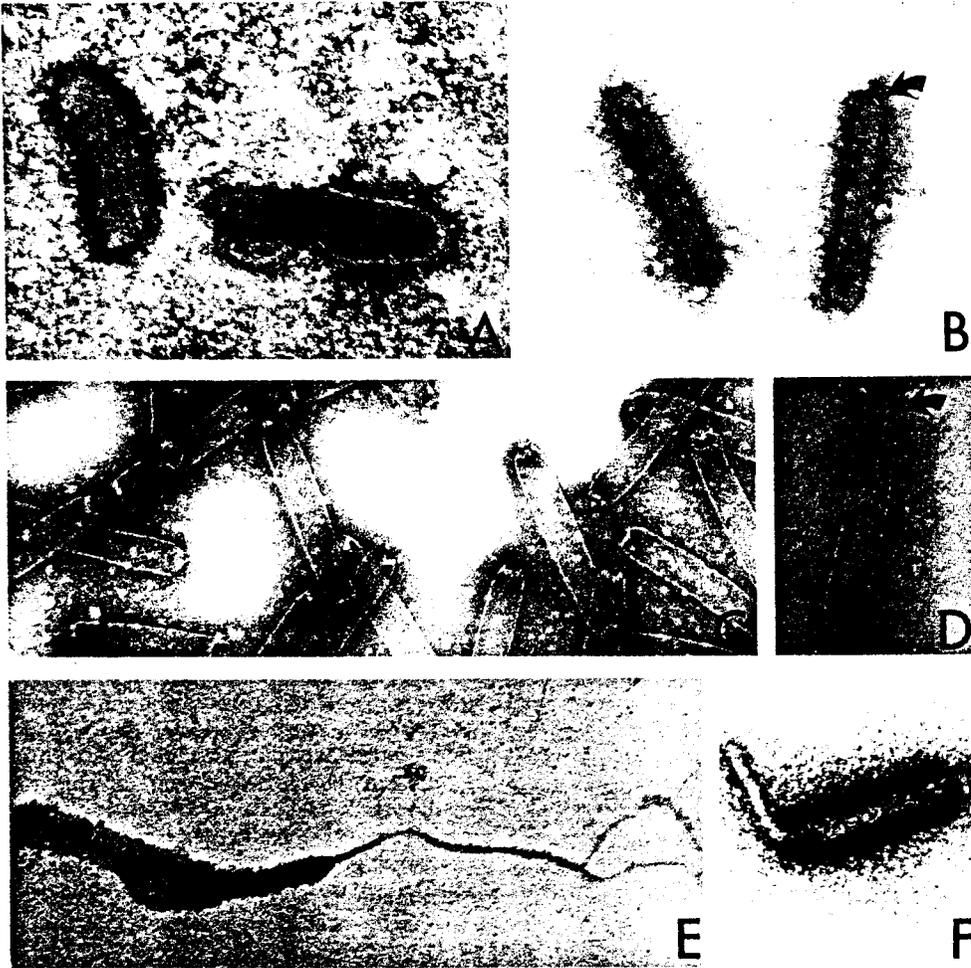


FIG. 3. Electron micrographs of *P. interpunctella* GV structural components. (A) Enveloped nucleocapsids. (B) Nucleocapsids. (C) and (D) Capsids. The arrows in (B) and (D) indicate the caplike structure present at one end. (E) and (F) Fibrillar DNA core released from within capsids by treatment with a chelating agent.

are infected by GVs, the replication cycles of these viruses have been examined extensively in only a few of these insects. The results which have been obtained so far, primarily from electron microscopic observations of infected tissues, have shown that GV infection is a complex and intriguing process. The predominant organs infected by GVs are the fat bodies (4, 11, 38, 71, 90). This insect tissue serves as a major site of intermediary metabolism and storage of fat, protein, and glycogen. In some species, other tissues, such as the epidermis (15, 47, 124), tracheal matrix (15, 100), hemocytes (204), and Malpighian tubules (100, 188, 204), may be infected.

GV infection is initiated when an insect ingests an occluded virus and the virus is transported to the lumen of the midgut (208, 209, 228). The

complexity of GV infection arises, in part, from the process through which the virus interacts with its host, leading from the gut to eventual replication in the fat bodies and other hemocoelic tissues. The sequence of events apparently consists of primary infection of midgut cells in which progeny virus that are responsible for secondary infections of hemocoelic tissues are generated.

Because the infectious form of GVs is the enveloped nucleocapsid, disruption of the protein matrix releasing the enveloped nucleocapsids is the first essential step in the invasion and replication of GVs. The conditions suitable for solubilization of the matrix (an alkaline environment and other undefined factors) appear to be present only in the gut (61, 150). In an insect

hemocoel, where the pH is approximately 6.4 to 6.5, occluded virus remains intact (51, 90). The midgut dissolution process has been examined by Summers (209) in GV-infected larvae of *T. ni*. Observations of gut tissues shortly after viral ingestion revealed the protein matrix in various stages of disruption. Matrix dissociation occurred at each end of the occluded virus and from areas adjacent to the enveloped nucleocapsids outward (Fig. 4). As the protein matrix split open, the enveloped nucleocapsids were released and became associated with the microvilli of

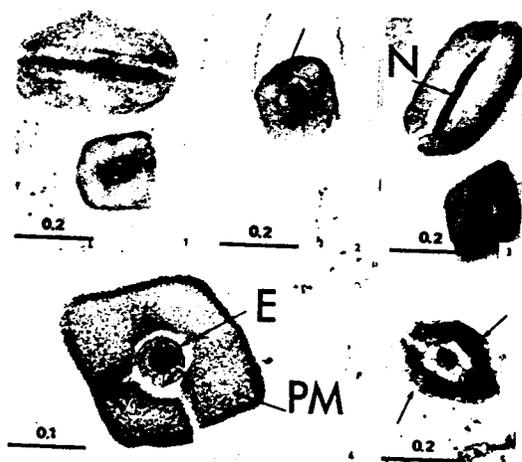


FIG. 4. Disruption of the protein matrix (PM) of *T. ni* GV in the insect midgut. E, Envelope; N, nucleocapsid. From reference 209.

midgut columnar cells. Only nucleocapsids free of envelope were observed inside microvilli, indicating that viral penetration occurred by fusion of the viral envelope with host cell membranes. A similar mode of entry into midgut cells was detected in NPV-infected *Rachiplusia ou* and *Aglais urticae* (72, 126).

The studies of Summers on *T. ni* larvae revealed a unique mechanism for the uncoating of GV nucleocapsids at the nuclear membrane (209). At 2 to 6 h postinfection, intact nucleocapsids frequently were associated end-on with nuclear pores (Fig. 5). Empty or partially empty capsids were then observed outside the nucleus, suggesting that the viral genome was inserted into the nucleus without the capsid. A similar association of nucleocapsids with nuclear pores was observed in three different NPV infections (126, 178, 226).

After an eclipse period of approximately 12 to 18 h in GV-infected *T. ni*, progeny nucleocapsids are detected in the midgut cell nuclei in regions of dense, aggregated material distinct from host chromatin (100, 209). Whereas the nuclear membranes appear disorganized and disrupted in some cells, most midgut cells are not affected adversely by GV replication. Several processes of envelope acquisition have been observed. Some nucleocapsids which are released into the cytoplasm when the integrity of the nuclear membrane is lost obtain loose-fitting envelopes from intracytoplasmic membranes which have proliferated after virus infection (228). Other nucleocapsids are inserted into infoldings of the



FIG. 5. Electron micrographs showing the association of GV nucleocapsids with nuclear pores (NP). Capsids (C) are present outside the nucleus (N), suggesting that the viral genome has been inserted into the nucleus. From reference 209.

nuclear membrane. The enveloped nucleocapsids appear to be incorporated into vacuole-like structures in the cytoplasm, in which they are transported to the basement membrane of the midgut cells (100, 184). The enveloped nucleocapsids are then released from the vacuoles into the hemocoel by an undetermined process. Still another form of envelope acquisition consists of budding of free nucleocapsids through the plasma membrane (Fig. 6). The budding process occurs in regions of the membrane which have been modified by the insertion of peplomeric structures (Fig. 6). These structures are retained as the enveloped nucleocapsids move into the hemocoel (184, 209).

Regardless of the mechanism of envelope acquisition, the enveloped nucleocapsids rarely are occluded in matrix protein. The newly replicated virus particles are thought to initiate secondary infections in cells of the fat body and other susceptible tissues in the body cavity (78). Tracheolar cells, which are situated near the basal lamina of midgut cells, are frequently infected 48 h after the primary infection.

Although infection of midgut cells by GVs is fairly well documented, the possibility that enveloped nucleocapsids originally released from occluded viruses in the gut lumen may infect

hemocoelic tissues has not been ruled out. Tanada and Leutenegger (228) have proposed that some of the enveloped nucleocapsids pass directly from the gut into the hemocoel through intercellular spaces.

The sequence of events during GV replication in the fat body has been characterized in several larval species and differs in certain aspects from the sequence observed in midgut epithelial cells (4, 5, 10, 18, 100, 204, 209). Many more cells are infected, the yields of virus per cell are much greater, and a complete cycle of replication (including occlusion) takes place. The mechanism of attachment and penetration of enveloped nucleocapsids into cells of the fat body and other hemocoelic tissues is not known. If this mechanism is similar to the process which has been observed in NPV infections, it may involve specific attachment of the modified end of the viral envelope to host cell membranes, followed by viropexis (1, 83, 123).

Because of difficulties in obtaining synchronous infection of fat body cells in insects the process of nucleocapsid uncoating in these cells has not been studied. The first event which has been observed is an increase in the size of the nucleus and redistribution of host chromatin to the periphery of the nuclear membrane. This is



FIG. 6. Electron micrograph showing *P. brassicae* GV nucleocapsids acquiring envelopes by budding through the cell membrane. From reference 184.

followed by development of an electron-dense network of chromatin-like material in the nucleus (4). This network, which is much more extensive than the network observed in midgut cells, has been termed the virogenic stroma because it consists of aggregated strands that are readily distinguished from host chromatin (256). As the stroma develops, it becomes increasingly Feulgen positive, and the host chromatin degenerates and finally disappears. The stroma appears to be the site of viral DNA synthesis and nucleocapsid assembly because capsids in various stages of electron density are observed at its highly condensed periphery (Fig. 7). Depending on the insect species infected by GV, fat body cell nuclear membranes may disintegrate, mixing the viral chromatin with cytoplasmic contents. In infected cells of *P. interpunctella* and *C. murinana*, hypertrophied nuclei rupture very early in infection, so that newly replicated nucleocapsids are first detected in the cytoplasm (4, 25, 91). In *C. pomonella*, *T. ni*, and *P. operculella* cells, the nuclei remain intact long enough to allow observable nucleocapsid development in the nuclei (18, 204, 209). After breakdown of the nuclear membrane, in some species nucleocapsid assembly continues in the former nuclear area of the cell (18, 204). In other species the virogenic network spreads throughout the cell (25).

As in the midgut, the acquisition of envelopes by nucleocapsids in fat body cells appears to occur through several processes. Whereas some envelopes are obtained by de novo synthesis (26, 175, 206, 209), others are acquired by budding through nuclear or cytoplasmic membranes. In *P. interpunctella* cells, envelopes appear to be acquired from large masses of smooth endoplasmic reticulum that accumulate in infected cells (Fig. 8). Nucleocapsids orient in regular arrays along the endoplasmic reticulum and then are inserted individually into envelopes (4). Similar arrays of nucleocapsids closely associated with endoplasmic reticulum have been observed in GV-infected larvae of *Plutella xylostella* (8) and *Cadra cautella* (98). There is evidence from studies on NPVs that the initial association of an envelope with a nucleocapsid begins at the capped end of the nucleocapsid (122).

As the stroma is depleted, matrix formation begins with deposition of the matrix protein on the outer surface of the viral envelope (Fig. 8). This process is selective, as no cellular components other than enveloped nucleocapsids are occluded in matrix protein (23). The crystallization appears to begin either at one side or at one end of the envelope (Fig. 8) and proceeds around the particle (8, 98, 175, 248). Unlike GVs replicated in midgut cells, the majority of enveloped nucleocapsids present in fat body cells

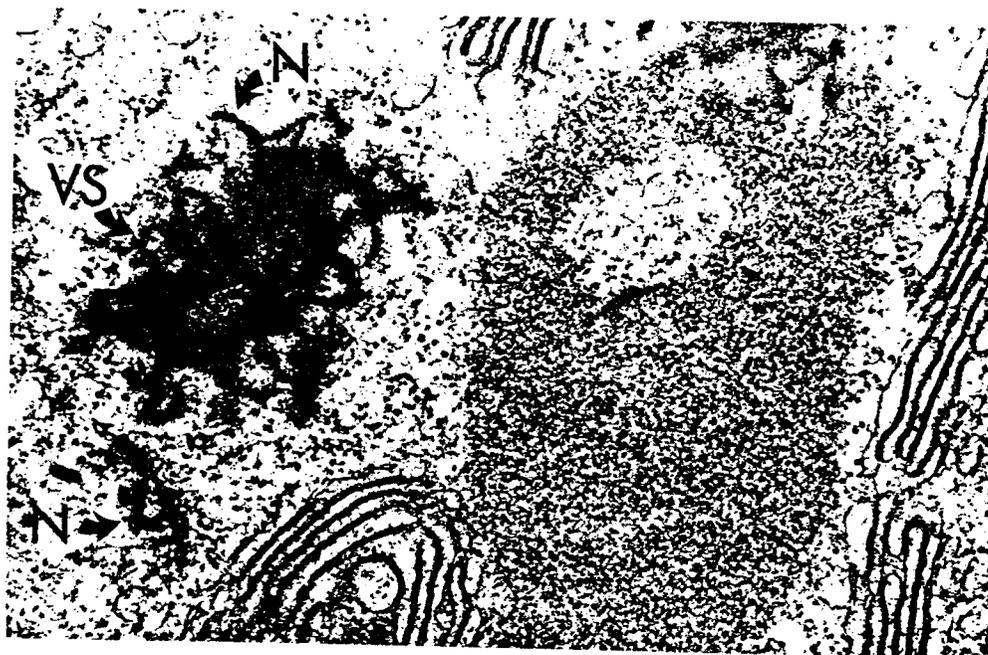


FIG. 7. Electron micrograph of a GV-infected fat body cell of *P. interpunctella*, showing the electron-dense virogenic stroma (VS) which appears to be the site of viral DNA synthesis and nucleocapsid (N) assembly. From reference 4.

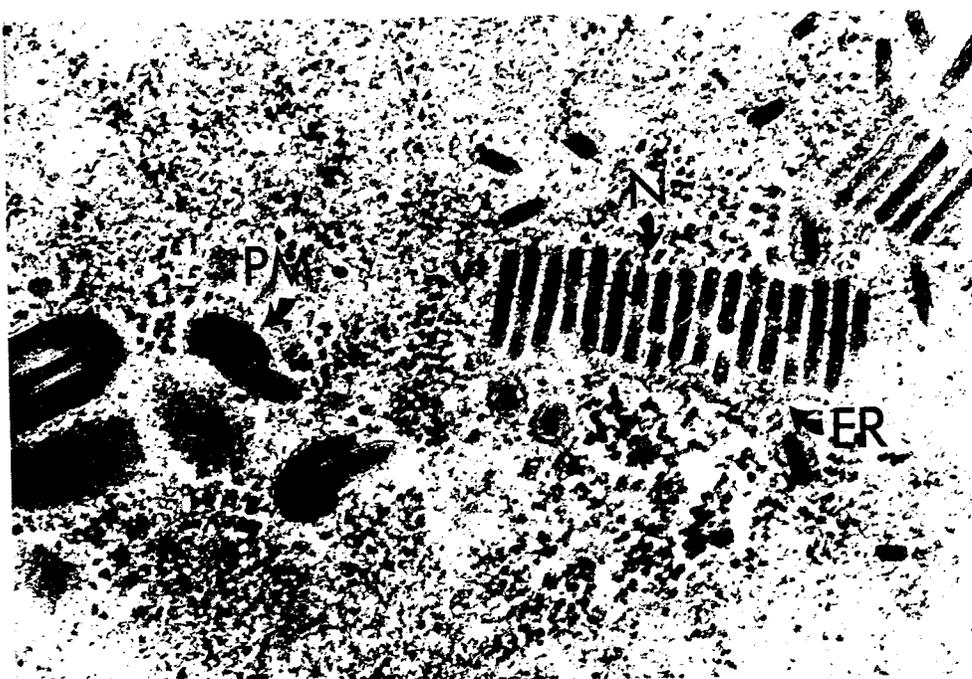


FIG. 8. Electron micrograph of a GV-infected *C. cautella* fat body cell, showing the association of nucleocapsids (N) with endoplasmic reticulum (ER) and the occlusion of enveloped nucleocapsids within the protein matrix (PM). From reference 98.

become occluded in the matrix. A few of the enveloped nucleocapsids which remain nonoccluded may be the source for further infection, along with nucleocapsids budding through the plasma membrane. Indeed, Summers and Volkman (221) have shown that in an NPV system the infectious material in the insect hemolymph consists primarily of enveloped nucleocapsids. A similar sequence of events has been observed in fat body cells of larvae and in cultured cells infected with NPVs except that the entire replication process occurs within the nuclei (1, 66, 67, 72, 79, 122, 135).

There have been few studies concerning the relationship of DNA, ribonucleic acid, and protein metabolism to the sequence of steps in viral replication and assembly. Injection of [³H]uridine or [³H]thymidine into larvae of *C. pomonella* at different times after infection revealed that, before any cytopathological changes in the fat body cells occurred, there was a sharp increase in ribonucleic acid synthesis in the cytoplasm, chromatin, and nucleoli (18). This synthesis was accompanied by a swelling of the nucleoli and, after reaching a maximum at approximately 25 h postinfection, was localized primarily in nucleoli (247). From about 24 to 30 h postinfection, when host chromatin was being redistributed to the peripheral regions of the

nuclei, the nucleoli degenerated, and the levels of ribonucleic acid synthesis returned to normal. Throughout this initial period of infection, DNA synthesis was generally comparable to DNA synthesis in uninfected cells. In some insects, an increase in DNA synthesis was observed early in the infection process, suggesting that host DNA synthesis may be induced by the virus. The most substantial incorporation of label into DNA was detected between 30 and 40 h postinfection, during which hypertrophy of the nucleus and formation of the virogenic stroma occurred. As the nuclear membrane became disorganized, a tremendous increase in [³H]thymidine incorporation was observed in the peripheral regions of the viral chromatin network (248). DNA synthesis increased to a rate approximately 30 times the rate in uninfected cells at 60 to 70 h postinfection, after which it decreased. The burst of DNA synthesis was accompanied by an increase in ribonucleic acid synthesis, primarily in the strands of the stroma. Incorporation into ribonucleic acid continued at an increased rate until most of the enveloped nucleocapsids were occluded. Completion of matrix formation was followed by a rapid decrease in ribonucleic acid synthesis.

Time course studies of protein synthesis during GV replication are incomplete (229). Incom-

poration of [^3H]tyrosine by infected fat body cells of *Hyphantria cunea* provided evidence for the association of newly synthesized protein with the virogenic stroma (248).

In the advanced stages of an infection, large masses of cells are completely packed with GV (4, 90). Eventually, the cells rupture, liberating occluded viruses into the hemocoel of the host insect. Such large numbers of GVs are produced that the fat body becomes opaque white and the hemolymph becomes turbid and milky. Macroscopically, this can usually be detected by a change in the color of the infected larvae. For example, *P. interpunctella* larvae turn from tan to an opaque white (201), and *L. pomonella* larvae become more intensely pink (225). Infected larvae finally die from GV infections, and the occluded viruses released into the environment infect other insects.

MOLECULAR BIOLOGY

It has become obvious that the molecular properties of baculoviruses must be investigated if we are to take full advantage of using them as insect control agents. One of the major problems in using baculoviruses as insecticides has been the inability to identify and quantitate these viruses easily. Increasing numbers of baculoviruses are being isolated from a variety of insects, and, until recently, little methodology has been available to distinguish one viral isolate from another. The classical means of differentiating baculoviruses consists of naming a virus for the host insect from which it was isolated originally. However, certain baculoviruses are able to replicate in more than one insect species, and unrelated types of baculoviruses have been shown to replicate simultaneously in a single insect host. To overcome the inadequacies of this identification system, investigators have turned to a variety of biochemical techniques for characterizing and determining relationships among viral isolates. These techniques have been reviewed by Harrap and Payne (77) and include polyacrylamide gel electrophoretic analysis of viral structural proteins, restriction endonuclease digestion of viral DNA, and serology. With these procedures, information on the physical, chemical, and biological properties of the baculoviruses is being gathered, and insight is being gained into which techniques are most useful for identifying and monitoring these viruses. This information is also being used in the development of sensitive probes for (i) studying the effects of baculoviruses on nontarget species at the cellular level, (ii) monitoring the quality of commercially produced viruses and the biological activity of viral residues, and (iii) detecting of viral strain mu-

tations and the possible broadening of the host range which may develop during the use of baculoviruses as insecticides.

Genome

GVs were first identified as DNA viruses by Wyatt (254, 255), who analyzed the base compositions of the nucleic acids of the GVs of *C. murinana* and *C. fumiferana* and found that they contain adenine, guanine, cytosine, and thymine but no uracil. Shvedchikova and Tarasevich (195) demonstrated the double-stranded nature of GV DNA and determined from electron microscopic studies that molecules obtained from the GVs of the Siberian silkworm (*Dendrolimus sibiricus*), and the cutworm (*A. segetum*) had high molecular weights. These investigators also observed some circular molecules in the viral DNA preparations and were the first to propose a cyclic structure for the GV genome (195, 196). Further evidence for a circular conformation of GV DNA was obtained by sedimentation analyses of the DNAs of *S. frugiperda* and *T. ni* GVs in alkaline sucrose and cesium chloride-ethidium bromide gradients (210, 212, 213). Two species of DNA were isolated on these gradients. Whereas 30% of the DNA sedimented in a manner characteristic of covalently closed circular DNA, about 60% sedimented as relaxed circles. Calculations based on sedimentation analyses of the DNAs showed that the molecular weights of the genomes of these viruses are more than 90×10^6 . Electron microscopic observations by Tweeten et al. (237) confirmed the circular and superhelical nature of GV DNA and provided an estimate of 76×10^6 for the molecular weight of the *P. interpunctella* GV genome (Fig. 9A and B). DNA molecules of similar size and conformation (Fig. 9C) have since been obtained from a variety of GV isolates (33, 37, 240). Estimates of genome molecular weights have been obtained by using sedimentation analysis, reassociation kinetics, electron microscopy, and restriction enzyme digestion. Depending on the viral isolate, the molecular weight of GV DNA ranges from 69×10^6 to 111×10^6 (Table 2). Biophysical properties, such as thermal melting points, densities, and guanine-plus-cytosine contents also have been determined for several GV DNAs and have been reviewed recently by Harrap and Payne (77).

The techniques of reassociation kinetics and restriction endonuclease digestion have provided additional information on the structure of GV DNA. In studies with *P. brassicae* (33) and *P. interpunctella* (D. Scharnhorst and R. Weaver, personal communication) GV DNAs,

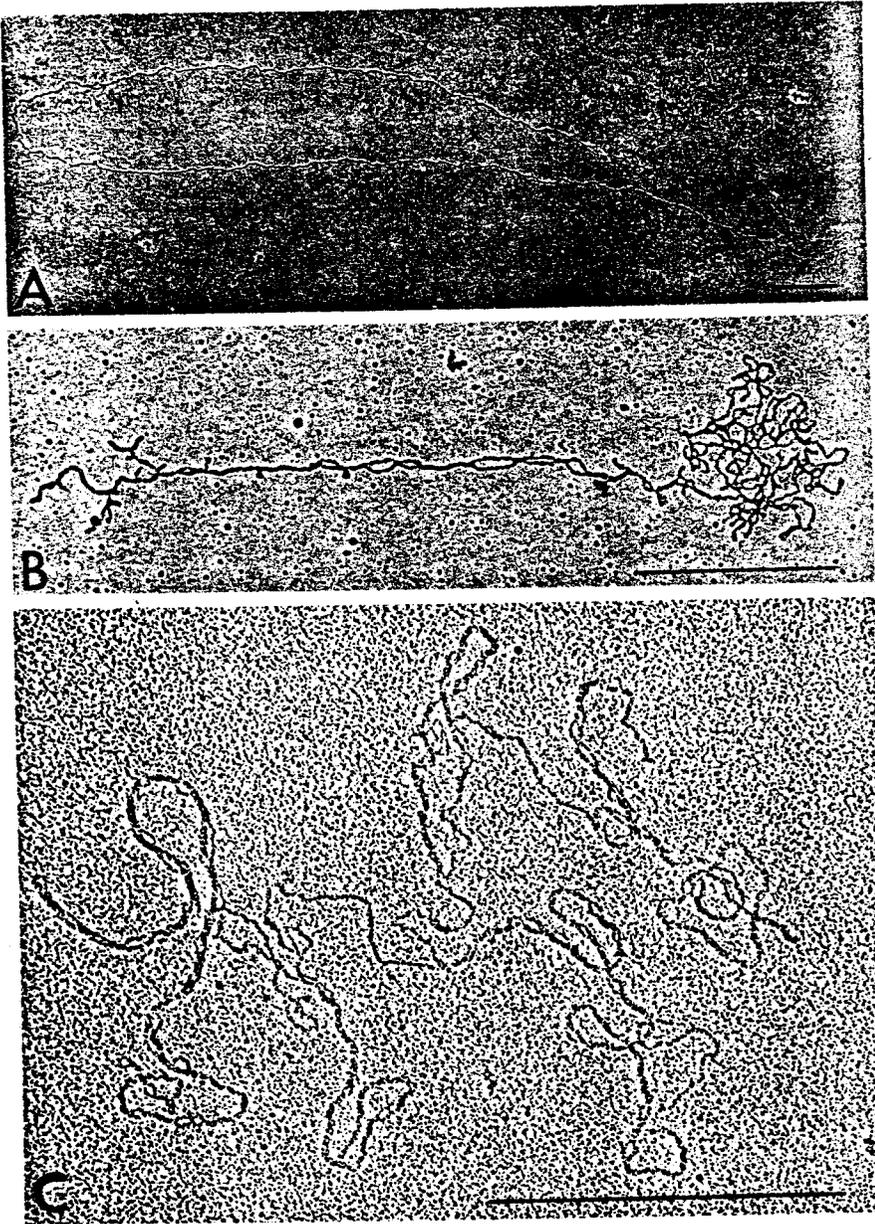


FIG. 9. Electron micrographs of GV genomes. (A) Relaxed circular molecule of *P. interpunctella* GV DNA. (B) and (C) Supercoiled molecules of *P. interpunctella* and *P. brassicae* GV DNAs, respectively. Bars = 1 μ m. (C) is from reference 33.

workers have found genomic molecular weights of 74.6×10^6 and 80.2×10^6 , respectively. Because these values closely correspond to molecular weights estimated by other means, GV DNA appears to consist primarily of single copy sequences. Restriction fragment patterns of GV DNAs have been used to define differences in GVs isolated from various hosts at the genetic

level. The DNAs that have been compared to date (from GVs of *S. frugiperda*, *T. ni*, *P. rapae*, and *P. interpunctella*) do not appear to be closely related. The fragments which were generated from these viral DNAs by digestion with several restriction enzymes differed markedly in number and size. Restriction enzyme analyses of NPV DNAs have been more extensive. Restriction

TABLE 2. Molecular weights of DNAs from GVs

Insect	DNA mol wt ($\times 10^6$)	Reference(s)
<i>A. segetum</i>	30 ^a	196
<i>D. sibiricus</i>	80 ^a	195
<i>Heliothis armigera</i>	95 ^a	37
<i>L. pomenella</i>	71.2 ^a	37
<i>P. operculella</i>	70.6 ^a	37
<i>P. brassicae</i>	69.8 ^a	33
	74.6 ^b	33
<i>P. interpunctella</i>	76.4 ^a	237
	81.0 ^c	237
	78.0 ^d	237
	72.0 ^e	240
<i>S. frugiperda</i>	91-114 ^f	213
	80, 96 ^d	213, 214
	77.3 ^e	199
<i>T. ni</i>	99-119 ^c	213
	89, 104 ^d	213, 214
	111 ^e	199

^a Determined by electron microscopy.

^b Determined by reassociation kinetics.

^c Determined by sedimentation in neutral sucrose gradients.

^d Determined by sedimentation in alkaline sucrose gradients.

^e Determined by restriction endonuclease digestion.

tion fragment patterns have been used to identify viral isolates from various hosts (161, 187, 199) and to distinguish among closely related genomic variants (143, 162, 199).

Physical maps of a number of NPV DNAs have been developed, and the genomic regions coding for specific viral polypeptides have been described (200, 220). When similar studies are conducted with GV DNAs, it will be possible to make detailed comparisons of the structures and organizations of the genomes of the baculovirus subgroups.

The infectivity of purified GV DNA has been investigated only to a limited extent. Transfection of a continuous line of *T. ni* cells with DNAs from the GVs of *S. frugiperda* and *T. ni* did not produce plaque formation (35). Interpretation of these results is difficult at present because viral replication was not detected in the cells when enveloped nucleocapsids were used as the infecting agent.

Purification of Occluded Virus and Its Structural Components

Definitive characterization of GV proteins has been dependent upon the development of procedures for separating and isolating the structural components (protein matrixes, enveloped nucleocapsids, nucleocapsids, capsids) of these

viruses. Through analyses of individual components, the localizations and organizations of the polypeptides in the structurally complex GVs have begun to be elucidated. Because all studies to date have necessarily utilized GVs produced in insects, the first important step in determining viral protein composition has been the isolation of occluded viruses from infected larvae. Several methods for the purification of baculoviruses have been described (74, 76, 132). Typically, these procedures include homogenization of larvae, followed by differential centrifugation and velocity sedimentation in sucrose gradients. A critical examination of the adequacy of such procedures in removing host contamination was provided by the mixing experiments of Tweeten et al. (236). Uninfected, radioactively labeled larvae were mixed with infected, unlabeled larvae, and the level of radioactive material was monitored at each step of virus purification. The resulting virus was free of detectable host contaminants and retained its physical and biological integrity. These investigators determined that treatment of the virus preparation with a detergent was an essential step in purification. Significant quantities of larval contamination remained associated with the virus if the detergent (1% [vol/vol] deoxycholate) was omitted. Another detergent which is useful in providing structurally intact, purified GVs is 1% sodium dodecyl sulfate (215).

The technique generally used to isolate the protein matrix is treatment of occluded GVs with sodium carbonate (pH 9 to 11). This well-established procedure results in dissociation of the protein matrix from the enveloped nucleocapsids (20, 53, 74, 132, 155, 216). Several other chemicals, such as 5 M guanidine, 7 M urea, and *n*-propanol, can be used to induce fragmentation of the protein matrix. However, these chemicals also cause damage to the other viral components (53, 124). Of the various compounds that have been tested, dissolution by carbonate most closely resembles the solubilization of matrix and the release of enveloped nucleocapsids observed in vivo. The rate of matrix solubilization is dependent on temperature, pH, and carbonate concentration, and the optimum conditions for matrix dissolution must be defined for each GV isolate (53). Recently, Yamamoto and Tanada (258) have shown that the protein matrix of *Pseudaletia unipuncta* GV also can be solubilized readily by 0.02 M NaOH (pH 12) with no apparent effect on the integrity of the viral envelope. With either method, the solubilized matrix is separated from the other viral components by differential centrifugation or by sedimentation on sucrose or glycerol gradients. After cen-

trifugation, the matrix proteins are recovered for further analysis from the top of the gradients or in the supernatant.

A number of techniques for recovering alkali-liberated enveloped nucleocapsids have been investigated. Depending on the manner in which the enveloped nucleocapsids are handled after matrix dissolution, particles of varying degrees of structural integrity, purity, and infection capability have been obtained (74, 125, 128). Optimum yields of enveloped nucleocapsids generally result when the nucleocapsids are removed directly from the carbonate dissolution mixture by differential centrifugation or by sedimentation on gradients (17, 155, 216, 241). Dilution of the mixture before centrifugation with buffers that reduce the pH below approximately 8.5 must be avoided. At lower pH values, precipitation of matrix protein occurs, resulting in contamination of enveloped nucleocapsids by matrix constituents and the loss of enveloped nucleocapsids through aggregation (3, 76, 216). The stability of the isolated enveloped nucleocapsids varies with the viral species but usually is limited. Most preparations are contaminated to some extent with envelopes and nucleocapsids. The use of brief times of exposure to alkaline conditions and the removal of glycerol or sucrose by pelleting rather than by dialysis minimize viral degradation and loss of infectivity (241, 258).

More recently, methods to release GV and NPV nucleocapsids from viral envelopes have been described. Of the materials that have been investigated, the nonionic detergent Nonidet P-40 (NP-40) appears to be the most effective in envelope removal. Originally, Harrap and Longworth (76) used a 30-min incubation in 0.2% (vol/vol) NP-40 to isolate *Melanchnra persicaria* GV nucleocapsids. However, Arif and Brown (3) found that this treatment resulted in incomplete removal of the envelope. These investigators used centrifugation of enveloped nucleocapsids through a layer of 2% NP-40 on top of sucrose gradients and recovered relatively intact, envelope-free nucleocapsids. Higher NP-40 concentrations resulted in degradation of nucleocapsids. Modifications of this latter procedure have been used to isolate nucleocapsids from the GVs of *P. brassicae* and *P. interpunctella* (33, 241). Enveloped nucleocapsids from these viruses were incubated in 1% (vol/vol) NP-40 for 30 min at 26 to 30°C. The nucleocapsids then were separated from the solubilized envelope proteins by sedimentation on sucrose or glycerol gradients. The resulting nucleocapsids were structurally intact and were free of contaminating envelope fragments, as determined by

electron microscopy. Triton X-100 has also been used to dissolve GV envelopes. Yamamoto and Tanada (259) used 0.1% Triton X-100 to solubilize the viral envelopes from two strains of *P. unipuncta* GV, and this treatment provided nucleocapsids that appeared to be intact. Tweeten et al. (241) found that nucleocapsid aggregation, which frequently has been observed by others, can be minimized if preparations are maintained in 0.01 M tris(hydroxymethyl)aminomethane hydrochloride (pH 8.5) during both incubation in detergent and gradient centrifugation. Below pH 8.5, clumping of nucleocapsids occurs, and most nucleocapsids are lost by pelleting during centrifugation.

As with the isolation of other GV components, the conditions used for removing GV viral envelopes vary with the viral isolate. The envelopes surrounding *S. frugiperda* GV nucleocapsids are particularly resistant to solubilization (219). Enveloped nucleocapsids from this virus remain stable even after prolonged exposure to 2% NP-40-6 M urea, which causes extensive degradation of envelopes and nucleocapsids from other GVs (see below).

A procedure for isolating baculovirus capsids was introduced by Summers and Smith (219). This procedure consists of incubating enveloped nucleocapsids in 0.1 M tris(hydroxymethyl)aminomethane hydrochloride-2% NP-40-1 M NaCl for 18 h at 37°C, followed by velocity sedimentation on preformed cesium chloride gradients. The tubular capsids band at a density of approximately 1.33 g/ml in the gradients. Tweeten et al. (239) adapted this technique for generating capsids from nucleocapsids by omitting the detergent. Treatment of nucleocapsids with 0.6 to 1.0 M NaCl in 0.01 M tris(hydroxymethyl)aminomethane hydrochloride (pH 8.5) was sufficient for complete extraction of the DNA and the core proteins from the capsids. Capsids prepared in this manner appeared to have lost the caplike structures located at one end of the capsids. This suggests that whereas the rest of the capsid is stable at high salt concentrations, these caplike structures are not. Also, it is possible that the cap structure is associated with the viral genome and, consequently, is removed from the capsid when the DNA is ejected.

Structural Proteins

Protein matrix. The most extensively characterized protein of GVs is the protein of the crystalline matrix. The first studies revealed that alkaline carbonate-solubilized matrix consists of a heterogeneous mixture of peptides. Several

proteins were found by gel electrophoresis and sedimentation analysis (146, 215, 228). The presence of multiple protein species also was indicated by carboxy- and amino-terminal analyses and serological tests, which showed the presence of at least two antigenic components. These results suggested a complex composition and a possible subunit structure for the matrix.

Further clarification of the biochemical and biophysical properties of the solubilized matrix was provided by the discovery that an alkaline protease was associated with the protein matrix of NPVs (55, 218). This protease was activated by the conditions used to solubilize the matrix and degraded matrix components to a mixture of lower-molecular-weight polypeptides. Several additional baculoviruses have been investigated, and similar protease activities have been detected (42, 57, 137, 155, 238). Inhibition of this activity by $HgCl_2$ or by heat treatment at 70°C for 30 min has allowed the matrix to be solubilized and recovered in a nondegraded form (57, 218, 238). After such treatment, the matrix exhibits one predominant 12S component when it is analyzed by velocity sedimentation. This molecule can be further dissociated into a single low-molecular-weight polypeptide by treatment with sodium dodecyl sulfate and 2-mercaptoethanol (217, 218). Comparative studies of species of this protein, called granulins and polyhedrin in GVs and NPVs, respectively, revealed close similarities in molecular weight. All of the baculovirus matrix proteins examined to date have molecular weights in the range from 25,000 to 30,000 (Table 3). These studies have indicated that the 12S molecule consists of eight subunits of granulins or polyhedrin. Recent immunochemical studies with the polyhedrins from two *O. pseudotsugata* NPVs and *T. ni* NPV suggest that the 12S aggregate contains one of the major antigenic determinants observed in matrix prep-

arations (56, 186). The other antigenic site appears to be present in the monomeric polypeptide.

In addition to similarities in structure and size, granulins from several GVs are similar in amino acid composition. These proteins are characterized by high contents of aspartic and glutamic acid residues. Also prominent are the hydrophobic amino acids valine, isoleucine, and leucine. Comparative peptide mapping studies of granulins and polyhedrins have shown that many common peptides are present in the matrix proteins (152, 217). As suggested by Rohrmann (186) and Maruniak and Summers (152), these peptides may represent regions of the protein that contribute to its aggregative properties and, thus, have been conserved through evolution. Other peptides are unique to individual matrix proteins and can be used to distinguish viral species. The granulins from *T. ni* and *P. interpunctella* GV appear to be phosphorylated (218, 241). Whether this is typical of granulins and polyhedrins is not known.

Yamamoto and Tanada (257a) have reported that a 126,000-molecular-weight component containing phospholipid and protein is associated with matrix preparations from the GV of *P. unipuncta*. This component enhanced infection of *P. unipuncta* by an NPV, and it has been suggested that this component is involved in the attachment of enveloped nucleocapsids to mid-gut cell membranes (230).

Enveloped nucleocapsids and envelope proteins. Enveloped nucleocapsids from only a few GVs have been purified and analyzed for their polypeptide compositions. Polyacrylamide gel electrophoretic analyses of enveloped nucleocapsids from *S. frugiperda*, *T. ni*, *P. brassicae*, *P. unipuncta*, and *P. interpunctella* GVs have revealed several structural proteins (33, 219, 241, 259). From 12 to 18 polypeptides have been observed, with each virus having a unique protein composition. The molecular weights of the enveloped nucleocapsid structural polypeptides are shown in Table 4. Several additional minor viral protein species have been detected in enveloped nucleocapsids of *P. interpunctella* GV by using sodium dodecyl sulfate-polyacrylamide gradient gels (241). Several polypeptides having similar electrophoretic mobilities were resolved in these gels. These results indicate that gel systems with resolving powers over various molecular weight ranges must be used to reveal the number of GV structural polypeptides adequately.

Recently, studies have been initiated to localize the polypeptides within the enveloped nucleocapsid structure. Dissolution of the viral en-

TABLE 3. Molecular weights of granulins from GV isolates

Insect	Granulin mol wt	Reference(s)
<i>Cirphis unipuncta</i>	26,300	43
<i>L. pomonella</i>	28,000	43
<i>Mamestra oleracea</i>	26,400	43
<i>P. brassicae</i>	28,200, 27,500	43, 33
<i>P. interpunctella</i>	28,000	238
<i>Pseudaletia unipuncta</i>		
Hawaiian strain	28,700	258
Oregon strain	29,100	258
<i>Pygera anastomosis</i>	26,900	43
<i>S. frugiperda</i>	28,000, 26,000	217, 219
<i>T. ni</i>	28,000	218, 219
<i>Zeiraphera diniana</i>	27,200	43

TABLE 4. Structural polypeptides of GV enveloped nucleocapsids^a

<i>S. frugiperda</i> GV (219) ^b	<i>T. ni</i> GV (219)	<i>P. brassicae</i> GV (33)	<i>P. unipuncta</i> GV (259)	<i>P. interpunctella</i> GV (241) ^c
VP160				
VP95	VP90	VP91	VP97	VP97
VP89	VP85		VP89	VP88
	VP77			
VP71		VP73		
		VP68		
	VP64	VP64		
	VP63			VP63
VP58	VP57	VP58		
	VP56			
	VP52		VP52	
VP48	VP47	VP47	VP48	VP48
				VP46
				VP44 ^c
VP42		VP42	VP42	VP42
VP39	VP39			VP39 ^c
	VP38		VP38	VP38
VP37			VP37	
		VP34	VP34	
VP31	VP31	VP30		VP31
VP29			VP29	VP29 ^c
VP26				VP26
	VP25			
VP24	VP24	VP24	VP24	
VP21.5	VP21.5			
VP20.5	VP20.5			
VP18	VP18	VP18		
VP17	VP17			VP17 ^c
VP16	VP16			VP16
		VP12	VP14	VP12

^a The numbers in the designations refer to the molecular weights ($\times 10^3$) of the viral proteins, as determined by comparisons with molecular weight standards.

^b Numbers in parentheses are reference numbers.

^c Phosphorylated polypeptides.

velopes with detergents has allowed polyacrylamide gel analyses of nucleocapsids and solubilized envelope proteins. Nucleocapsids from *P. brassicae* and *P. interpunctella* GVs contain at least eight polypeptides, ranging in molecular weight from 12,000 to 73,000 (33, 239, 241). Approximately four or five proteins are removed by NP-40 and thus appear to be associated with the viral envelope. However, treatment of *P. unipuncta* GV enveloped nucleocapsids with Triton X-100 produced nucleocapsids with only three polypeptides and an envelope fraction containing as many as nine proteins (259). Although these results may reflect intrinsic differences in the organizations and complexities of GV nucleocapsids, they also may have been due to differences in the extractabilities of the various structural polypeptides by the two detergents. A more direct method for identifying GV envelope

proteins has been used by Tweeten et al. (241). These investigators used solid-phase bound lactoperoxidase and ¹²⁵I to radiolabel the proteins external to the viral envelope. Autoradiograms obtained from electropherograms of iodinated enveloped nucleocapsids indicated that five proteins were located on the outer surface of the viral envelope. These polypeptides (VP17, VP39, VP42, VP48, and VP97) also were the major polypeptides released from the virus structure by detergent treatment.

In addition to the proteins identified as envelope components, there are a few other viral polypeptides that are present in *P. interpunctella* GV enveloped nucleocapsids but not in nucleocapsids (241). Although this observation suggests that these proteins are envelope proteins, these proteins did not appear to be labeled by the radioiodination procedure. It is possible that these proteins are located internally in the viral membrane and are not available to the lactoperoxidase. They also may be components of an intermediate layer; Kawamoto et al. (122) have proposed that such a layer exists between baculovirus envelopes and nucleocapsids. This intermediate layer may be analogous to the membrane proteins associated with the envelopes of rhabdoviruses, orthomyxoviruses, and paramyxoviruses, which may serve as recognition sites for assembly of nucleocapsids into envelopes (168).

An analysis of the lipid content of GV envelopes has been conducted with the GV of *P. unipuncta* (257). Chloroform-methanol extraction and thin-layer chromatography revealed the presence of phosphatidyl choline, phosphatidyl ethanolamine, and an unidentified phospholipid specific to virus infection. The neutral lipid and glycolipid contents of GV envelopes have not been reported, nor have the viral lipids and the midgut and fat body cell membrane lipids been compared.

Nucleocapsid and capsid proteins. Comparisons between the polypeptide compositions of GV nucleocapsids and the polypeptide compositions of capsids have provided further information on the locations and functions of some of the structural proteins. As described above, nucleocapsids from the GVs of *P. interpunctella*, *P. brassicae*, and *P. unipuncta* have been isolated and analyzed (33, 241, 259). Whereas the gel patterns differ for several of the minor protein components of these nucleocapsids, there is a substantial resemblance in electrophoretic mobilities for the two major components (Table 5). Evidence indicates that the higher-molecular-weight protein (molecular weight range, 31,000 to 34,000) is the major capsid protein. This protein is the predominant polypeptide in empty

TABLE 5. Structural polypeptides of GV nucleocapsids and capsids

Nucleocapsids ^a		Capsids ^a		
<i>P. brassicae</i> GV (33) ^b	<i>P. interpunctella</i> GV (241)	<i>P. unipuncta</i> GV (259)	<i>P. interpunctella</i> GV (239)	<i>T. ni</i> GV (219)
VP73				
	VP64		VP64	
VP58				
VP47	VP49 ^c		VP49	
VP42	VP44 ^c			
	VP39	VP38	VP39	
	VP36		VP36	
VP34 ^d	VP31 ^d	VP34 ^d	VP31 ^d	VP31 ^d
VP30	VP29 ^c		VP29	VP29
VP24				VP26
				VP17
VP12 ^d	VP12 ^d	VP14 ^d		

^a The numbers in the designations refer to the molecular weights ($\times 10^3$) of the viral proteins, as determined by comparisons with molecular weight standards.

^b Numbers in parentheses are reference numbers.

^c Phosphorylated polypeptides.

^d Predominant structural polypeptides in nucleocapsids or capsids.

capsids of *T. ni* and *P. interpunctella* GVs (219, 239). A protein of similar molecular weight is present in most NPV and GV enveloped nucleocapsids that have been characterized, suggesting that this protein functions as the major structural component of baculovirus capsids (33, 40, 70, 78, 160, 174).

The other protein present in large amounts in nucleocapsids has a low molecular weight ranging from 12,000 to 14,000. After isolation of this protein from nucleocapsids of *P. interpunctella* GV, an investigation of its biochemical properties revealed that it is an extremely basic, arginine-rich polypeptide (239). With approximately 39% of its amino acid residues consisting of arginine and histidine, this viral protein was acid soluble, exhibited an electrophoretic mobility in acid-urea gels intermediate between the mobilities of histones and protamine, and had an isoelectric point of 9.8 to 10.0. Polyacrylamide gel analysis showed that this basic protein is not present in capsids, suggesting that it is an internal or core protein associated with viral DNA. Electron microscopic observations by Tweeten et al. (239) provided further evidence that this is the case. Treatment of *P. interpunctella* GV nucleocapsids with chelating agents resulted in rupture of the ends of the capsids and release of a thick fiber from within the capsids (Fig. 3E and F). This putative nucleoprotein complex was sensitive to salt, which caused decondensation of the compact structure into long thin

strands of naked viral DNA. These observations are consistent with the speculation that a protein, most likely the arginine-rich polypeptide, is bound to the DNA.

The basic polypeptide appears to be a structural component which is characteristic of baculoviruses. Proteins of similar basicity and molecular weight have been extracted from within nucleocapsids of *P. rapae* GV and *A. californica* and *S. frugiperda* NPVs (239). Most other NPV and GV enveloped nucleocapsids whose structural protein compositions have been determined contain predominant polypeptides with molecular weights ranging from 12,000 to 16,000 (33, 40, 70, 78, 160, 174). It is likely that these low-molecular-weight proteins also are arginine-rich, core-associated proteins. The apparent absence of such a protein in the enveloped nucleocapsids of *T. ni* and *S. frugiperda* GVs (Table 4) perhaps can be accounted for by the gel system used for the analysis of these enveloped nucleocapsids (219). We have been unable to detect polypeptides having molecular weights less than 14,000 with gels containing 11% or less polyacrylamide.

SEROLOGY

There is evidence which suggests that intrinsic differences in baculovirus genomes and structural polypeptides are reflected in serological properties (78). Thus, another promising system for classification of baculoviruses is a system based on antigenicity. Several immunological techniques in which antibodies formed against matrix proteins, enveloped nucleocapsids, and nucleocapsids are used are being investigated for their sensitivity and specificity in detection of baculovirus antigens. By comparative analyses of the antigenicities of polypeptides from these viral components, structural proteins that may determine virus strain-specific antigens can be identified (78). Of particular interest is the use of this information for developing procedures for reliable identification and monitoring of baculovirus levels in field insects and soil samples (154).

Serological tests, such as complement fixation and hemagglutination inhibition, appear to detect only the group-specific determinants contained in polyhedrins and granulins (44, 93, 169). As a result, these procedures have shown much serological cross-reactivity among matrix proteins from several baculoviruses. On the other hand, sensitive immunodiffusion and radioimmunoassay systems have revealed the presence of virus-specific antigens and have been used to discriminate among distantly and closely related NPVs and GVs (41, 119, 170, 186). The latter

technique and enzyme-linked immunosorbent assays appear to be sensitive enough to detect polyhedrin and enveloped nucleocapsid antigens in soil samples and infected larvae (41, 119, 129, 146).

PROSPECTS FOR FUTURE RESEARCH

In recent years, significant progress in GV research has been made. Many details of the viral replication process have been established, and the biochemical properties of viral genomes and several structural polypeptides have been determined. However, the information currently available provides only a beginning to our understanding of these genetically and biologically complex animal viruses. A number of features of GV infection and GV molecular biology remain to be elucidated.

Little is known about the viral and host components responsible for the host specificity exhibited by GVs. Such knowledge is of primary importance in the selection of GVs with restricted host ranges for use as biological insecticides and for detection of changes in host range that may occur after the application of these viruses for insect control. The peritrophic mem-

brane (Fig. 10A), a network of chitin, hyaluronic acid, and protein which lines the midgut, may be a specific barrier to infection (14, 30, 173). Although penetration of this membrane may be through nonselective discontinuities, it is also possible that it is mediated by virus-associated enzymes. Another factor which may define host range is the insect gut environment. Various GVs differ in their susceptibilities to alkali, and, therefore, appropriate conditions must be present in the gut for solubilization of the protein matrix to occur (Fig. 10B). Degradation of the matrix may also be aided by host or virus-associated alkaline proteases (238). However, such protease activity may not be essential for GV infection, as *P. brassicae* GV and tissue culture-derived NPVs, which lack alkaline proteases, are highly infectious in vivo (33, 243, 245).

The mechanism of entry of GV enveloped nucleocapsids into cells appears to depend on the tissue type being infected. In the midgut, penetration occurs through fusion of the viral envelope with columnar cell membranes (Fig. 10C and D). On the other hand, entry into hemocoelic tissues, such as tracheae and fat bodies, may be primarily through viropexis (Fig.

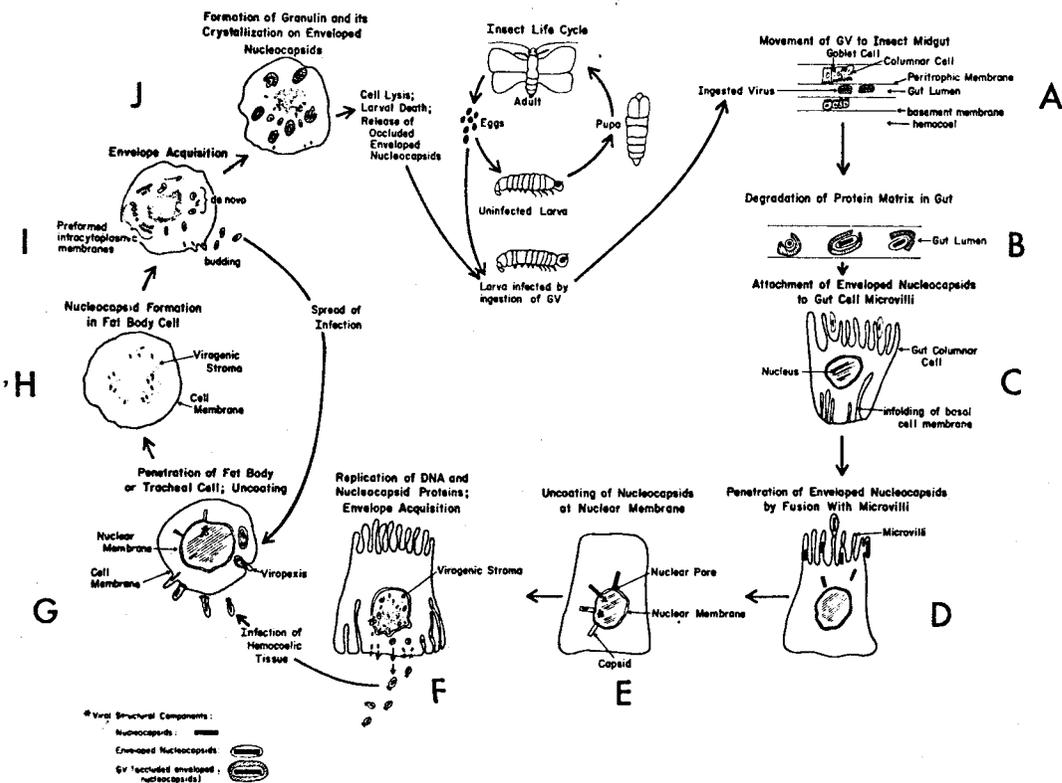


FIG. 10. Diagrammatic summary of the GV infection process.

10G). There is evidence which suggests that the interaction between enveloped nucleocapsids and host cells is dependent on the nature of the viral envelope. Virus particles that acquire envelopes through budding are much more infectious by intrahemocoelic inoculation than per os (127, 246). This suggests that the envelopes may contain receptors necessary for specific attachment to cells in the hemolymph. Indeed, structural modifications which appear to be analogous to the glycoprotein spikes of enveloped vertebrate viruses are present on the budding ends of these envelopes (Fig. 10F). These surface projections are not present on enveloped nucleocapsids which become occluded and exhibit very low infectivity for hemocoelic cells (79, 221). The GV structural polypeptides that are responsible for the different properties and functions of the two forms of enveloped nucleocapsids have not been identified. Preliminary studies with the NPV of *A. californica* have revealed qualitative and quantitative differences in the polypeptide compositions of the occluded and budded enveloped nucleocapsids (199). Whether these differences are due to modifications of proteins by glycosylation or phosphorylation or are restricted to viral envelope proteins is not known. Further investigations in this area will be important because specific envelope proteins are likely to contribute significantly to the host and tissue specificities of GVs.

Electron microscopic studies have indicated that uncoating of GV nucleocapsids occurs at or near the nuclear membrane, probably in association with nuclear pores (Fig. 10E). Similar interactions with nuclear pores have been observed for baculoviruses that infect species of *Apanteles* and *Chelonus* wasps (207). The wasp viruses have appendages attached to one end of nucleocapsids which are analogous to bacteriophage tails. After penetration of an appendage into a nucleus through nuclear pores, the viral DNA appears to be injected into the nucleus. Although GV nucleocapsids lack taillike structures, their ends are morphologically distinct. Structural polypeptides at one of these termini might interact specifically with the nuclear pore complex. The mechanism which triggers the uncoating event(s) is unknown. Preliminary experiments in our laboratory have shown that cations are associated with *P. interpunctella* GV and that chelation of the cations causes nucleocapsids to release their DNA cores (239). During infection, a similar mechanism for uncoating may be operational. The contribution of nuclear membrane components to NPV uncoating may differ from the contribution in GV infections. NPV nucleocapsids appear to enter nuclei and

uncoat within them (68, 83, 135).

Information concerning the biochemical events that occur during the period from uncoating to the appearance of newly replicated nucleocapsids on the virogenic stroma is also limited (Fig. 10H). There is some evidence that the virus initially may stimulate infected cells to divide, thus amplifying the cellular enzymes and nucleic acid machinery needed for viral replication (18, 165). It is possible that this effect is mediated by virus-specified enzymes or early viral polypeptides. The GV genome certainly contains enough genetic information to code for nonstructural proteins having regulatory or enzymatic activities. To synthesize the total number of structural polypeptides identified so far in these viruses, only 25 to 30% of the viral DNA molecule would be needed. The maturation of nucleocapsids appears to consist of formation of tubular capsids into which viral genomes are then inserted. With electron microscopy, GV capsids in the process of being filled with fibrous strands (presumably DNA) have been observed in infected cells of *Archips argyrospila* (175). A similar sequence of assembly has been described for NPV nucleocapsids (92, 139). The NPV capsids also are associated with filamentous material and exhibit increasing electron density as infection proceeds. The mechanism through which the large baculovirus genome is condensed and packaged within a capsid has not been well characterized. Shvedchikova et al. (197) have proposed that the molecule is condensed through a series of sequential spiralizations. However, more consistent with the maturation process observed in infected cells is the suggestion by Tweeten et al. (239) that an extremely basic protein becomes associated with the viral DNA during packaging. What is evident from the observations of several investigators is that DNA is present within capsids in the form of a highly organized structure (163, 176, 181, 197, 239). Further ultrastructural and biochemical analyses of this structure, which appears to be more analogous to DNA-protamine complexes than to DNA-histone complexes (27, 29), will be needed to obtain further insight into the mode of baculovirus DNA packaging.

Equally undefined is the final sequence of events in GV replication, the polymerization of granulin around enveloped nucleocapsids (Fig. 10J). Recent studies have led to speculation on the process by which granulin crystallization occurs. One possible mechanism may involve interaction of the hydrophobic amino acid residues in the matrix protein (133). Also proposed is neutralization of dicarboxylic acids (the predominant amino acid residues in granulin) by

cellular cations concentrated in the region of the matrix assembly (186). It is not clear that granulins are synthesized in midgut cells, although formation of occluded viruses in this tissue is rare. In fat body cells, where the majority of enveloped nucleocapsids become occluded, the envelope appears to be essential for matrix deposition. Only nucleocapsids which have envelopes are occluded, and in some insect species, envelopes alone may be incorporated into the protein matrix. Apparently, the viral envelope contains granulins-specific attachment sites (79, 208). The relationship between the GV structural polypeptides involved in this interaction and the envelope proteins which function in fusion with host cell membranes remains to be elucidated. The mechanism by which matrix polymerization ceases also is not known. Harrap (73) has suggested that formation of the as-yet uncharacterized electron-dense layer at the periphery of the matrix may be the determining factor.

A major breakthrough needed to resolve many of the questions discussed above is the development of tissue culture systems in which GVs can be replicated. The ability to prepare immunological reagents selective for each baculovirus structural component and, in some cases, individual viral polypeptides is currently being realized (222). Individual sera and combinations of sera are being investigated for use in typing systems for baculoviruses. The use of these reagents as sensitive probes for analyses of the biochemistry and kinetics of GV replication can only be critically accomplished in cell lines. Tissue culture systems also will allow the selection of viral mutants for genetic mapping and further investigation of the GV infection process. Such studies are presently being conducted with NPVs in cultured cells (34, 144).

A further evaluation of the insecticidal activities of the GVs must also be made. Particular attention must be given to defining the field or storage situations in which viral insecticide usage would be the most suitable form of insect control. Studies on the integration of viruses with other new approaches to the regulation of economic pests, including pheromones, growth hormones, sterilants, and appropriate crop cultivation techniques, are also needed.

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