Review article

Baculovirus structural proteins

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Introduction

Baculovirus biology

The Baculoviridae are a diverse family of virus pathogens which are infectious for arthropods, particularly insects of the order Lepidoptera. They have also been isolated from the insect orders Hymenoptera, Diptera and Trichoptera, as well as from the crustacean order Decapoda (shrimp) (Couch, 1974). Although baculoviruses infect over 600 species of insects (Martignoni & Iwai, 1986), individual isolates normally show a limited host range and infect only closely related species.

Baculoviruses are characterized by large rod-shaped virions containing supercoiled dsDNA genomes ranging in size from 88 to over 160 kbp. A prominent feature is the occlusion of virions in a crystalline protein matrix, and two genera have been distinguished based on the gross structure of the occlusion body. One genus, the nuclear polyhedrosis viruses (NPVs), has polyhedron-shaped occlusion bodies 1 to 15 μm in diameter composed of a protein called polyhedrin which crystallizes around many enveloped nucleocapsids (see Fig. 1 and 2). Different NPVs are characterized by their occluded virions being present either as single (SNPV) or multiple (MNPV) nucleocapsids within the envelope. The other genus contains the granulosis viruses (GVs), which have small occlusion bodies (0.25 to 0.5 μm in cross-section) ellipsoidal in shape. They normally contain a single nucleocapsid which are enveloped and are composed of a protein related to polyhedrin called granulin. Whereas NPVs are found in a number of arthropod orders, GVs are only found within the Lepidoptera.

Occlusion bodies stabilize virions, allowing them to remain viable for long periods in the environment. Infection occurs when an insect ingests the alkali-soluble occlusion body and the virions are released by the high pH of the insect midgut. Occlusion provides such a selective advantage for insect viruses that it has apparently evolved independently at least three times. In addition to baculoviruses, insect viruses from both the Reoviridae (cytoplasmic polyhedrosis viruses) (Payne & Mertens, 1983) and the Poxviridae (entomopox viruses (EPVs)) (Arif, 1984) occlude their virions. Occlusion in both these virus groups is similar to that of baculoviruses in that the occlusion body protein gene is highly expressed, occlusion stabilizes the virus outside the host insect, and occluded virions are released by the high pH encountered in the midgut of a susceptible insect.

For baculoviruses to occlude large numbers of virions efficiently, massive amounts of polyhedrin must be produced during the infectious cycle. This high level transcription of the polyhedrin gene is accomplished by an α-amanitin-resistant RNA polymerase which appears to have a different subunit composition to host RNA polymerases (Yang et al., 1991). Although a hyperexpressed gene product, polyhedrin is not necessary for growth of the virus in cell culture. This has been exploited in the development of the baculovirus expression system, in which the polyhedrin gene is replaced by foreign genes under the control of the polyhedrin promoter (Smith et al., 1983; Pennock et al., 1984). The advantage of this system is that the recombinant baculoviruses express high levels of eukaryotic gene products that are usually folded and processed in a manner similar to the native proteins.

In addition to the current interest in baculoviruses owing to their widespread use as expression vectors, baculoviruses traditionally have been of interest because of their ability to control insect populations selectively. Many insect populations are characterized by cycles of expansion and subsequent collapse. As the number of insects increases, occlusion bodies which contaminate soil or vegetation from past baculovirus epidemics are ingested and initiate an infectious cycle. The dead insects disintegrate and contaminate surrounding vegetation, and the virus is disseminated by wind, birds and insects. This can lead to the spread of the virus over vast areas.
and can significantly contribute to the eventual collapse of an insect population. In forests, insect infestations can encompass several million hectares and baculoviruses often play a prominent role in the eventual collapse of these large populations (Doane & McManus, 1981). With the advent of genetic engineering, genetically modified baculoviruses have been shown to be capable of infecting insects and expressing insect-specific toxin genes, which significantly accelerate the speed with which the virus kills a target insect (Tomalski & Miller, 1991; Stewart et al., 1991; Maeda et al., 1991b).

Although baculoviruses are of great interest and utility to a large cross-section of the agricultural and biomedical research community, definitive descriptions of proteins contributing to baculovirus structure have appeared only recently. Past reports have described up to 100 proteins associated with virus structure, but most of these descriptions distinguish the proteins only by size. As it was not possible to account for protein breakdown, processing and comigration, these reports may not reflect the actual number of baculovirus structural proteins. Therefore, in this review, only those proteins...
The baculovirus life cycle involves two virion phenotypes

The complexity of baculoviruses is in part due to the presence of two structurally distinct virus phenotypes (Volkman et al., 1976) (Fig. 2), with each phenotype having a specific role during the baculovirus life cycle (for a review see Blissard & Rohrmann, 1990). The budded virus (BV) phenotype (also known as extracellular virus) is produced during the early stages of infection, spreads the infection from cell to cell within the insect and is highly infectious for cultured cells (Volkman & Summers, 1977). In contrast, the polyhedra-derived virion (PDV) phenotype [also known as occluded virus (OV)] is produced during the terminal stages of viral infection, becomes concentrated in the nucleus and is occluded within polyhedra. Occluded PDVs are capable of persisting for extended periods outside the host insect. However, after ingestion by a susceptible insect, polyhedra are dissolved releasing PDV which initiates infection within the midgut cells. PDVs are relatively non-infectious for cultured cells (Volkman & Summers, 1977).

Although the nucleocapsids of the two phenotypes appear to be identical, their envelopes are derived from different sources. During both the early and late phases of viral gene expression a glycoprotein, gp64, is synthesized and transported to the margins of the cell where it is incorporated into the host cell plasma membrane (Volkman et al., 1984; Blissard & Rohrmann, 1989). Once assembled within the nucleus, nucleocapsids destined to become BVs are transported through the cytoplasm and bud out through the gp64-modified plasma membrane. During the budding process, the nucleocapsid becomes enveloped by the modified membrane. In contrast, PDVs become surrounded by an envelope that assembles within the nucleus. Subsequently, they become occluded, and are released upon the death of the cell.

The regulation of baculovirus gene transcription

There are currently three generally accepted categories of baculovirus genes and their relationship to the major events of the viral replicative cycle is shown in Fig. 3.
These include early, late and very late hyperexpressed genes. Early baculovirus genes have promoters which are recognized by host cell RNA polymerase II (Fuchs et al., 1983; Huh & Weaver, 1990; Hoopes & Rohrmann, 1991) and can be transcribed by uninfected cells. A category of 'delayed early genes' has also been described (Guarino & Summers, 1986a), but recent investigations indicate that these genes are transcribed at a low level by uninfected cells (Theilmann & Stewart, 1991) and can also be transcribed by nuclear extracts from uninfected cells (B. Glocker et al., unpublished data). Baculovirus genes encoding trans-activating factors have been identified (Guarino & Summers, 1986a; Carson et al., 1988) and enhancer sequence elements are located in several regions of both the Autographa californica MNPV (AcMNPV) (Guarino & Summers, 1986b; Guarino et al., 1986) and the Orgyia pseudotsugata MNPV (OpMNPV) (Theilmann & Stewart, 1992) genome. Trans-activating factors and enhancers increase the level of transcription of early and late (Guarino & Summers, 1987a) genes, and probably contribute to the speed and facility with which the virus is able to direct the host cell metabolic machinery to transcribe particular genes necessary for viral replication. Genes which are transcribed only after DNA replication begins are classified as late genes. If viral DNA replication is inhibited, no late transcription occurs (Rice & Miller, 1986/87; Friesen & Miller, 1986). Concomitant with the initiation of late baculovirus transcription is the shutdown of some, if not all, host cell nuclear transcription (Ooi & Miller, 1988). Transcription of a number of viral early genes is also shut down during the period of late transcription (Rohel & Faulkner, 1984). Although this suggests that host RNA polymerase II transcription may be inhibited by a late gene product (Ooi & Miller, 1988), some baculovirus early gene mRNAs appear to persist late in infection (Rohel & Faulkner, 1984; Huh & Weaver, 1990). This could reflect mRNA stability or selective shutdown of only certain genes. Late genes are transcribed by an a-amanitin-resistant RNA polymerase (Grula et al., 1981; Fuchs et al., 1983; Huh & Weaver, 1990) that recognizes the baculovirus late promoter, which normally contains the sequence motif ATAAG (Rohrmann, 1986; Rankin et al., 1988; Ooi et al., 1989); the first nucleotide in the motif is sometimes a G, or rarely a T, but the TAAG sequence is invariant. The late mRNAs normally initiate at the T or A residue (positions 1 or 2) of the TAAG sequence. In contrast to late genes, which are transcribed over a defined temporal window (e.g. see Blissard et al., 1989), the very late genes (polyhedrin and p10) begin to be transcribed well after the initiation of late gene transcription, and transcription continues very late into the infection after late gene transcription ceases (Hill-Perkins & Possee, 1990; Thiem & Miller, 1990). Although the nature of late gene transcription and the differential regulation of late and very late genes is not understood, recent evidence indicates that AcMNPV induces the production of an RNA polymerase unrelated to host RNA polymerases (Yang et al., 1991).

Although splicing does not appear to play a major role in the regulation of most baculovirus genes, the mRNA of the AcMNPV trans-activating factor gene, IE-1, appears to be spliced and this may lead to differentially expressed forms of IE-1 (Chisholm & Henner, 1988; Kovacs et al., 1991).

### Baculovirus structural proteins

Baculoviruses have very large genomes with the potential to encode about 100 genes. Because transfection of susceptible cultured insect cells with purified virus DNA results in the production of viable virus (Burand et al., 1980; Carstens et al., 1980; Kelly & Wang, 1981), it is apparent that under these conditions no virion-associated proteins are essential for viral replication. This is in contrast to the complexity of some viruses which are unable to infect cells productively via DNA transfection (e.g. poxvirus virions carry an extensive array of enzymes which are essential for early gene transcription and other functions). Although the production of virus from transfected DNA suggests that the virion structure functions as a delivery system for introduction of the virus genome into cells, it is likely that a variety of proteins with enzymatic activities are associated with the virion envelope, or are present as nucleocapsid structural components or reside within polyhedra. There is at least one protein (p74) required for infection of insects (but not cultured cells) which may be associated with PDVs of AcMNPV (Kuzio et al., 1989). Some virion-associated proteins, although not absolutely required, may enhance the efficiency of viral replication. The adventitious association of some proteins within occlusion bodies and with the membrane of the BV is also likely to occur.

The genomic location of AcMNPV structural protein genes discussed in this review are indicated in Fig. 4. Genes encoding structural proteins are distributed throughout the genome with no obvious pattern to their location. In addition, the initial characterization of the Lymatricia dispar MNPV (LdMNPV) genome, which included the locating and sequencing several structural protein genes, has demonstrated that the surrounding genetic milieu is often not conserved (R. Bjorson & G. F. Rohrmann, unpublished results). This suggests that many of these genes can be expressed irrespective of their exact location in the genome or proximity to other specific genes.
Structural proteins of polyhedra

Besides the structural components of occluded virions (PDV), polyhedra are constructed of two well characterized components, polyhedrin and the polyhedron envelope (PE) protein. Another protein, p10, appears to be associated with the formation of polyhedra.

Polyhedrin

Polyhedrin is a protein of about 245 amino acids (29K) and is the major component of polyhedra (Fig. 1). Polyhedrin was the subject of a previous review (Rohrmann, 1986), so it will not be discussed extensively here. Polyhedrin has received considerable attention because it is hyperexpressed, constituting up to 18% (Quant et al., 1984) or more of total cellular alkali-soluble protein late in infection. The polyhedrin gene is not essential for viral replication in cell culture, and is the major component of polyhedra (Fig. 1). Polyhedrin is a protein of about 245 amino acids (29K) and is the major component of polyhedra (Fig. 1).

Fig. 5. Amino acid sequences of selected polyhedrins and granulins. The LdMNPV sequence is from Smith et al. (1988) and the NsSNPV sequence is from M. Harris & R. Possee (unpublished results). For other sequences, see Rohrmann (1986). The dots indicate amino acid identity; the dashes indicate gaps; the numbers indicate amino acid positions.

NsSNPV may have undergone host-dependent evolution rather than originating from cross-infection from the Lepidoptera into the Hymenoptera as was previously suggested (Rohrmann, 1986). In host-dependent evolution a pathogen becomes genetically isolated and speciates with its host.

The PE protein

Polyhedra are surrounded by an electron-dense envelope (Fig. 1) which has been termed the PE, polyhedron membrane or polyhedron calyx; PE will be used in this review. The PE of one NPV was originally reported to be composed of carbohydrate (Minion et al., 1989). How-
Fig. 6. Microscopic localization of baculovirus structural proteins. (a) Immunogold staining of the PE protein in a cross-section of a polyhedron of OpMNPV. A polyclonal antibody produced against a TrpE fusion protein was used (for details see Russell & Rohrmann, 1990a). (b) Immunogold staining of the periphery of p10 fibrillar structures in OpMNPV-infected L. dispar cells with antibodies against the PE protein. For details see Russell et al. (1991). (c) Immunogold staining of the p39 capsid protein in a cross-section of an OpMNPV-infected L. dispar cell. An MAb directed against the p39 protein was used (for details see Russell et al., 1991). (d) Immunofluorescence localization of the gp64 protein in OpMNV-infected L. dispar cells at 36 h p.i. An MAb produced against the AcMNPV gp64 protein was used (for details see Blissard & Rohrmann, 1989). The bar markers represent (a to c) 0.25 μm, (d) 25 μm.

However, a protein component of the PE of AcMNPV was identified by Whitt & Manning (1988) using immunofluorescence microscopy and monoclonal antibodies (MAbs) prepared against polyhedra. They suggested that the PE protein is phosphorylated and may be thiolly linked to the carbohydrate component of the envelope. Subsequently, Gombart et al. (1989a) located the gene encoding the PE protein in both the OpMNPV and AcMNPV genomes. Immunoelectron microscopic examination of cross-sections of OpMNPV polyhedra showed that the PE protein appears to be a major component of the PE (Fig. 6a) (Russell & Rohrmann, 1990a). Similar results have been observed in AcMNPV-infected Spodoptera frugiperda cells (van Lent et al.,...
dispar polyhedra was not evident until 72 h p.i. The PE has the margins of pl0 fibrillar structures (see next section) ported. The PE protein has also been shown to localize to predominantly of carbohydrate as was originally re-

Comparison of the predicted proteins shows them to vary from LdMNPV has recently been sequenced (R. Russell &

Fig. 7. Amino acid sequences of PE proteins. AcMNPV and OpMNPV sequences are from Gambert et al. (1989a) and Oellig et al. (1987), respectively. The LdMNPV sequence was provided by R. Bjornson & G. F. Rohrmann (unpublished results). The underlined amino acids are identical in OpMNPV and LdMNPV. See Fig. 5 for details.

1990). Examination of OpMNPV-infected Lymantria dispar cells indicated that, although polyhedra containing mature PDVs were present at 48 h post-infection (p.i.), the association of PE protein with the periphery of polyhedra was not evident until 72 h p.i. The PE has been shown to be sensitive to a protease (Russell & Rohrmann, 1990a) which suggests that protein is an integral component of this structure and is not composed predominantly of carbohydrate as was originally re-

Despite the apparent importance of pl0 in the formation of the PE and the significant role it plays in the virus life cycle, the p10 protein shows relatively limited sequence conservation. Only 42% and 26% amino acid sequence identity exists between the p10 protein of AcMNPV (Kuzio et al., 1984) and those from OpMNPV (Leisy et al., 1986) and S. exigua baculovirus (SeMNPV) (D. Zuidema, personal communication), respectively (Fig. 8); the SeMNPV p10 protein is more similar to that of OpMNPV (38% amino acid identity). Of approximately 25 complete and partial open reading frames (ORFs) sequenced in both AcMNPV and OpMNPV, only one shows less amino acid sequence conservation than p10 (27%, ORF 4; Gambert et al., 1989b; G. F. Rohrmann, unpublished data).

Other proteins associated with occlusion bodies

Viral enhancing factor (VEF)

The VEF is a 104K protein which forms about 5% of the occlusion bodies of the Trichoplusia ni granulosis virus
(TnGV). The insect gut is lined with a chitin-rich structure called the peritrophic membrane which may function as a barrier to invasion of gut cells by insect pathogens. The VEF protein appears to facilitate GV infection by disrupting the peritrophic membrane, thereby allowing virions access to the surface of gut cells (Derksen & Granados, 1988). The TnGV gene encoding this protein has been sequenced and cross-hybridizing regions in the genomes of closely related GVs have been found; however, related sequences have not been identified in NPVs (Hashimoto et al., 1991), although a VEF activity appears to be associated with AcMNPV polyhedra (Derksen & Granados, 1988). It is not clear whether the VEF is an integral component of a virus structure or simply trapped in granules during the occlusion process.

Spheroidin-like protein (SLP)
The EPVs occlude their virions in ellipsoid-shaped occlusion bodies termed spherules, which are composed largely of a protein called as spheroidin. A gene reported to encode the spheroidin of Choristoneura biennis EPV (CbEPV) has been sequenced (Yuen et al., 1990), and encodes a 38.5K protein. Although it demonstrates no evident sequence homology to baculovirus polyhedrin, Vialard et al. (1990) have found that it is closely related (39% similarity) to another baculovirus gene which encodes an Nglycosylated 37K polypeptide, described previously by Wu & Miller (1989). This protein has been termed the SLP. Although antiserum directed against the CbEPV spheroidin did not bind to the PDV or BV of AcMNPV in Western blots, it did bind to a protein of the size predicted for SLP associated with AcMNPV occlusion bodies. Immunofluorescence microscopy with anti-spheroidin antibodies suggested that the SLP is a component of the PE. The OpMNPV genome contains a gene which encodes a protein with over 60% amino acid sequence identity to the SLP of AcMNPV. Western blot and immunoelectron microscopic analyses of a variety of virion and polyhedron preparations, using an antiserum specific to the OpMNPV SLP, indicates that it is not associated with the PE in this virus (C. Gross, personal communication).

Alkaline proteases
Until the presence of alkaline proteases associated with insect-produced polyhedra was documented (Yamafuji et al., 1958; Kozlov et al., 1975), polyhedrin could not be characterized because it was extensively degraded during the alkaline dissolution of polyhedra. Subsequently, it was demonstrated that the proteases associated with baculoviruses have properties similar to those isolated from the insect gut (Rubinstein & Polson, 1983). These data, along with the finding that polyhedra produced in cell culture lack protease activity (Zummer & Faulkner, 1979; McCarthy & Dicapua, 1979; Wood, 1980), indicate that the protease is probably a contaminant derived from the insect gut or bacteria, and becomes associated with polyhedra when the insect dies and disintegrates.

Structural proteins of the nucleocapsid
The nucleocapsids of BV and PDV appear to have a similar structure (Fig. 2). In addition to containing the genome, the nucleocapsid is composed of at least one small putative DNA-binding protein, a capsid protein termed p39 and another putative capsid protein termed p87.

A DNA-binding protein
Baculoviruses have large genomes which must become highly condensed in order to be efficiently packaged within a nucleocapsid. Although histones neutralize the electrostatic repulsion of neighbouring DNA sequences in cellular DNA, they do not appear to be associated with DNA packaged within nucleocapsids (Wilson & Miller, 1986). However, an arginine-serine-threonine-rich protein has been shown to be present in virions of the GV of Plodia interpunctella (PiGV) (Tweeten et al., 1980). Subsequently a small gene encoding 54 amino acids (termed p6.9) was identified in AcMNPV; this has an amino acid composition similar to that of the putative DNA-binding protein of PiGV (Wilson et al., 1987). Homologues to the AcMNPV p6.9 gene have been isolated from both OpMNPV (Russell & Rohrmann, 1990b) and the NPV of Bombyx mori (BmNPV) (Maeda et al., 1991a). The predicted core amino acid sequences of these proteins are closely related (over 76% similarity), but the BmNPV gene contains an additional 12 amino acid, arginine-serine-rich sequence (Fig. 9a). All three of the proteins contain more than 40% arginine and approximately 30% serine or threonine residues.

The high concentration of arginine, serine and threonine in these proteins is similar to that of protamines, which are a class of proteins present in many fish, avian and mammalian sperm nuclei and are involved in the production of highly condensed DNA (Fig. 9b). Protamines are small molecules (44 to 65 amino acids) composed of 55 to 70% arginine (Balhorn, 1982; Nakano et al., 1989). Arginine has the highest affinity of all amino acids for interaction with the phosphate backbone of DNA. It is thought that the central polyarginine segment in protamines binds in the minor groove of DNA, cross-linking and neutralizing the
phosphodiester backbone of DNA, whereas the N- and C-terminal regions of protamine form bonds with other protamine molecules (Balhorn, 1982). Such an arrangement generates a neutral insoluble chromatin complex of compact volume, which is to a large degree biochemically inert.

In a sense, viral nucleocapsids and sperm have a similar function: the transport of genetic information in a highly compact form to a recipient cell. In baculoviruses, it has been suggested that the basic arginine residues of the DNA-binding protein neutralize the acidic residues of viral DNA. Upon entry into an insect cell, the DNA-binding protein may become phosphorylated by a protein kinase that results in the unpackaging of the viral DNA. This theory is supported by the presence of a protein kinase associated with purified capsids of GVs (Wilson & Consigli, 1985a, b), and with both the BV and PDV of AcMNPV (Miller et al., 1989), respectively. This region in AcMNPV and OpMNPV is concentrated in the nucleus late in OpMNPV infection (Lu & Carstens, 1991).

**p39 capsid**

MAbs produced against the p39 protein of OpMNPV bind to a 39K protein on Western blots of both PV and BV proteins (Pearson et al., 1988). Immunoelectron microscopy confirms that the p39 protein is a component of the capsid (Fig. 6c) (Russell et al., 1991) and shows that it is randomly distributed over the surface of the nucleocapsid. This indicates that p39 is a component of the capsid rather than part of a more specialized structure (such as an end structure of the nucleocapsid). The p39 capsid gene has been located and sequenced in AcMNPV (Thiem & Miller, 1989), OpMNPV (Pearson et al., 1988; Blissard et al., 1989) and LdMNPV (R. Bjornson & G. F. Rohrmann, unpublished data). The AcMNPV and OpMNPV p39 amino acid sequences are 59% identical, and they are 39% and 47% identical, respectively, to the LdMNPV p39 sequence (Fig. 10). However, the C-terminal 65 amino acids of the LdMNPV p39 sequence show only 11% and 22% sequence identity with AcMNPV and OpMNPV p39, respectively. This region in AcMNPV and OpMNPV is also variable (38% identity). Another feature of the p39 sequence is that it is a highly conserved (81 to 92%) relative to the nucleotide sequence of the p39 ORF.

**p87 capsid**

In addition to p39, another protein, p87, has been found associated with capsids of OpMNPV (Müller et al., 1990). Monospecific antiserum produced against this protein binds to a protein of 87K on Western blots of both PDVs, BVs and purified capsids. The p87 ORF encodes a protein of predicted Mr 71K. No evidence of N-linked glycosylation has been found and the cause of the difference between the predicted size of the ORF and the size calculated from Western blots has not been determined. A homologue of the gene has been found in the AcMNPV genome in a position similar to that of OpMNPV (Lu & Carstens, 1991). Immunofluorescence microscopy has demonstrated that p87 is concentrated in the nucleus late in OpMNPV infection.
infection, similarly to the p39 capsid protein. However, attempts to localize the protein with this antiserum by immunoelectron microscopy have been unsuccessful (R. Russell, personal communication). Therefore, the exact association of this protein within baculovirus nucleocapsids remains to be elucidated.

**ORF 1 (p21)**

A gene referred to as ORF 1 is one of five contiguous ORFs oriented in the same direction (Oellig et al., 1987; Gombart et al., 1989a, b) (the third ORF encodes the PE protein). ORF 1 is a highly variable gene; in one strain of AcMNPV it contains a transposable element (Gombart et al., 1989a, b), and in the LdMNPV genome only the C-terminal half of the gene appears to be present (R. Bjornson & G. F. Rohrmann, unpublished data). Monospecific antiserum produced against a fusion protein containing the ORF 1 protein of OpMNPV reacts with a protein of 21K associated with both the PDV and BV (C. Gross, personal communication).

**Proteins of the BV envelope**

**gp64**

To date only one virus-encoded protein has been found associated with the BV envelope (Fig. 2). This protein is termed gp64 (or gp67) and has been characterized in both AcMNPV (Whitford et al., 1989) and OpMNPV (Blissard & Rohrmann, 1989). Immunoelectron and immunofluorescence (Fig. 6d) microscopy indicate that gp64 concentrates at the plasma membrane (Volkman et al., 1984; Blissard & Rohrmann, 1989, respectively). Nucleocapsids become enveloped within the gp64-modified plasma membrane during their exit from the cells. BV primarily enters cells by endocytosis, a pathway in which the entire virion is endocytosed into an intracellular vesicle called an endosome. The acidification of the endosome is thought to initiate fusion of the virus envelope with the endosomal membrane, releasing the viral nucleocapsid into the cytoplasm. Antibodies to gp64 block infectivity but not adsorption, and inhibitors that prevent acidification of the endosome also block infectivity, suggesting that gp64 may be involved in the pH-dependent fusion of the virus envelope with the endosomal membrane (Volkman & Goldsmith, 1985). More recent studies of isolated gp64 have confirmed that it is a pH-dependent fusion protein (G. Blissard, personal communication).

Transcriptional characterization of this gene in OpMNPV has demonstrated that gp64 has one early promoter and four late promoter motifs, with the two most distal late promoters involved in initiating late gene expression (Blissard & Rohrmann, 1989). Gp64 is the only characterized baculovirus structural protein that has both early and late promoters, although this has been found in other non-structural baculovirus genes [e.g. the nuclear matrix-associated gene known as 39K (Guarino & Smith, 1990)]. The presence of an early promoter may permit the expression of the gene so that the plasma membrane is modified in advance of the production of nucleocapsids (Blissard & Rohrmann, 1990). The late promoter elements initiate the expression of the protein during the entire cycle of virion production and assembly. Synthesis of gp64 from the late promoters allows the replacement of gp64 in the plasma membranes after depletion caused by BV production.

The gp64 gene contains a signal peptide sequence at the N terminus (Fig. 11) which appears to be removed in the mature protein (N. Sivasubramanian, personal communication). Signal peptides are associated with nascent secretory and membrane proteins, and are involved in directing proteins across the membrane of rough endoplasmic reticulum (ER), during which they are normally cleaved off. From within the ER, secretory proteins move to the cell surface via the Golgi complex, in which glycosylation may occur. A putative C-terminal anchor domain has also been identified on gp64 (Fig. 11). The anchor is a membrane-spanning domain (which is hydrophobic) followed by a hydrophilic domain that remains on the cytosol side of the membrane; such domains serve to anchor a protein into the plasma membrane.

The proteins of AcMNPV and OpMNPV are 78% similar in amino acid sequence (Fig. 11). The gp64 protein has been shown to be highly glycosylated (as much as 10K of the Mr is contributed by glycosidic

![Fig. 11. Amino acid sequence of gp64 proteins. AcMNPV and OpMNPV sequences are from Whitford et al. (1989) and Blissard & Rohrmann (1989), respectively. The putative N-glycosylation signals are indicated by underlining and overlining in the AcMNPV and OpMNPV sequences, respectively. The signal peptide (N. Sivasubramanian, personal communication) and the hydrophobic putative anchor domain (Roberts & Faulkner, 1989) are also indicated. See Fig. 5 for details.](attachment:image-url)
residues) (T. Roberts, personal communication) and examination of the two amino acid sequences indicates that they contain between five and seven sites for N-linked glycosylation (Fig. 11). Using inhibitors of N-glycosylation, T. Roberts (personal communication) has shown that glycosylation of AcMNPV gp64 is required for incorporation of the envelope protein into virions. In addition, palmitic acid is ester-linked to the AcMNPV gp64 peptide making it the major acylated protein associated with the BV (Roberts & Faulkner, 1989). It is believed that acylation of proteins may be involved in anchoring to membranes, membrane fusion or regulation of intracellular transport.

Although other virus-specific components of the BV envelope might be expected to exist, none have yet been identified.

The PDV envelope and other structures

Although PDVs display a classic bilayer envelope structure (Fig. 6c), to date no proteins specific to this structure have been identified. Several candidate proteins for this structure include peptides of 14.2K, 18K and 26-4K (T. Roberts, personal communication). The PDV envelope may play an important role in the initiation of infection in the insect gut and could also be essential for the association of the PDV with polyhedrin in the nucleus.

p74

The p74 gene is located downstream and in the opposite orientation to the p10 gene in both the AcMNPV (Kuzio et al., 1989) and OpMNPV (Leisy et al., 1986) genomes (Fig. 4). p74 is not glycosylated (T. Roberts, personal communication) and although it is expressed at low levels late in infection, a conventional late promoter sequence is not found near the mRNA start sites. If levels late in infection, a conventional late promoter might be expected to exist, none have yet been identified.

Future studies

Although substantial progress has been made in the elucidation of the structural components of baculoviruses, many questions remain unanswered. In particular, our knowledge of the structure of the envelope of both the BV and PDV is limited. It is likely that the BV envelope may contain major components contributed by the host membrane. Identification of the genes encoding these proteins will probably require the use of cDNA expression libraries derived from host mRNA. It is also very likely that minor but essential structural components of virus are present that will be difficult to identify using currently available techniques. The eventual identification of the individual components of the virion structure is essential for understanding the architecture of the virion and the role of its structure in the process of infection.

The author wishes to thank the following: G. Williams for preparation of and permission to use the electron micrograph (Fig. 1); R. Bjornson, T. Roberts, M. Harris, R. Possee and D. Zuidema for providing data prior to its publication; G. Schroth for helpful discussions; and R. Russell, M. Pearson, C. Rasmussen, B. Gläcker, C. Gross, G. Blissard, D. Leisy, D. Thielmann, L. Volkman, P. Faulkner and J. Kuzio for their suggestions and criticisms of this manuscript. This work was supported by grants from the NIH, USDA and Sandoz Crop Protection, Inc. This is Technical Report no. 9725 from the Oregon State University Agricultural Experiment Station.

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