U.S. National Library of Medicine
National Center for Biotechnology Information

NLM Citation: Rohrmann GF. Baculovirus Molecular Biology [Internet]. 4th edition. Bethesda (MD): National Center for Biotechnology Information (US); 2019. Chapter 3, The baculovirus replication cycle: Effects on cells and insects.
Bookshelf URL: https://www.ncbi.nlm.nih.gov/books/

3. The baculovirus replication cycle: Effects on cells and insects

Two types of virions

Baculoviruses, such as AcMNPV, have evolved to initiate infection in the insect midgut and then spread the infection to other tissues throughout the insect. This led to the evolution of two types of virions with major differences in their envelope structure. The environmentally stable, but alkali-soluble occlusion body, contains occluded virions that have a complex envelope that allows survival in the harsh alkaline environment of the midgut that contains a variety of digestive enzymes. These occlusion derived virions (ODV) infect midgut epithelia cells. In contrast, after transiting the midgut epithelium, the environment encountered within the insect has a near-neutral pH, and budded viruses (BV) are produced that have a different envelope structure that facilitates systemic infection. These two types of virions differ in their efficiencies of infection for different tissues; ODV infect midgut epithelial cells up to 10,000-fold more efficiently than BV, whereas BV are up to 1,000-fold more efficient at infecting cultured cells than ODV (1, 2). The major events in systemic infections are illustrated in Figure 1.

The insect midgut

To understand the baculovirus infection cycle, a brief overview of the insect gastrointestinal tract is necessary, since this is the site of the initial infection and several major features of baculovirus biology have evolved to accommodate and exploit this unique environment (3). The insect gastrointestinal tract is composed of three sections: the fore-, mid- and hindgut. In Lepidoptera, the foregut is involved in facilitating the uptake, storage, and physical processing of food. It is lined with a chitin-containing cuticle that is part of the insect exoskeleton. A valve separates the foregut and midgut. The midgut is the major site of digestion of food and lacks a cuticle, but is lined with the peritrophic matrix (PM) (Figure 2). The PM is composed of chitin, mucopolysaccharides, and proteins, and it separates ingested vegetation from the midgut epithelium (4). It is thought that it protects the gut surface from damage caused by abrasive food material and limits the access of microorganisms. It also allows the transfer of liquid and digested substances to the midgut epithelial cells, but prevents the passage of larger food particles. It is worn away by the passage of food and is regenerated from the epithelial cells. The most common midgut epithelial cells are columnar cells with a brush border that is adjacent to the gut lumen. Regenerative cells are present at the base of the epithelium and replenish the columnar epithelial cells that become damaged and are sloughed into the lumen. Goblet cells are also present and may be involved in ion transport that regulates pH (Figure 2). The midgut is involved in enzyme secretion and absorption of digested food and has a gradient of pH values. At the entry and exit of the midgut, the pH is near 7.0, but in the central region it can vary from 10.0 to as high as 12.0, depending on the lepidopteran species (Figure 3) (5). These are

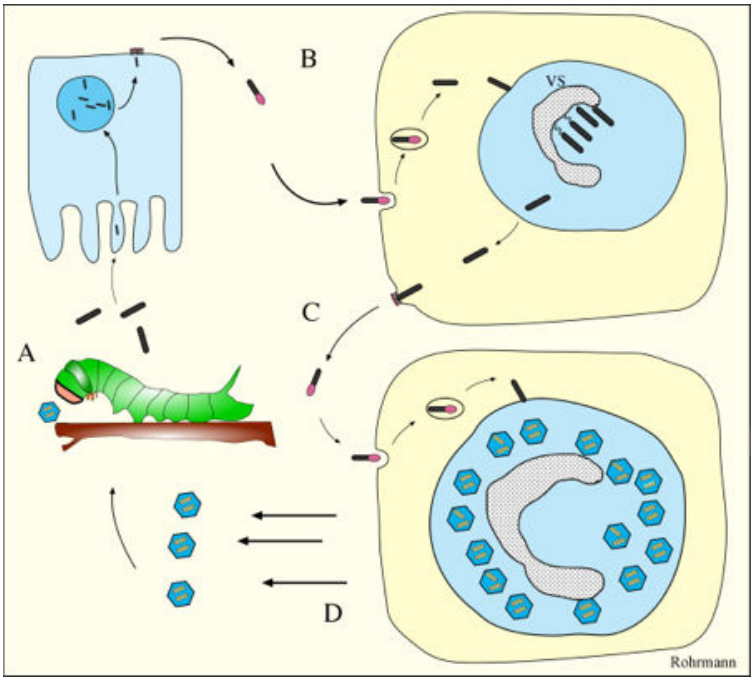

Figure 1. A life cycle of a baculovirus causing systemic infection. Occlusion bodies ingested by an insect dissolve in the midgut, and ODV are released which then infect midgut epithelial cells (A). The virion buds out of the cell in a basal direction and initiate a systemic infection (B). Early in the systemic infection more BV are produced which spread the infection throughout the insect (C). Late in infection occluded virions are produced, and the cell then dies releasing the occlusion bodies (D). The virogenic stroma (VS) is indicated.

among the highest pH values found in biological systems. Another valve separates the midgut and the hindgut. The hindgut is lined with a cuticle similar to the foregut and is involved in uptake of digested material, although to a lesser extent than the midgut.

From occlusion bodies to susceptible midgut cells: transiting the peritrophic matrix (PM)

A common feature of the life cycle of all baculoviruses is the presence of virions embedded in occlusion bodies that are produced in the final stage of the replication cycle and are released upon the death and disintegration of the insect. Occlusion bodies serve to stabilize the virus in the environment and are normally only dissolved under alkaline conditions. High pH environments are rarely, if ever, encountered in most ecosystems and normally are only found in the midguts of some susceptible insects. Upon ingestion, the alkaline conditions of the midgut of larvae cause the dissolution of the occlusion bodies and the release of the ODV (Figure 2, and see Chapter 2, Figure 3). The polyhedron envelope/calyx structure that surrounds the polyhedra is likely degraded by proteinases present in the gut or associated with the polyhedra (see Chapter 2). After their release from occlusion bodies, the first barrier that the viruses encounter in the insect midgut is the peritrophic matrix (PM) (Figure 2). As described in the previous chapter, polyhedra are nonspecifically contaminated by bacterial proteinases that function under alkaline conditions. Also, some baculoviruses encode endopeptidases (metalloproteinases) that contain divalent cations as integral components of their structure. One category of metalloproteinases, called enhancins, is concentrated in occlusion bodies. It is thought that enhancins specifically digest mucin, a PM component, thereby allowing virus access to the epithelial cell surface (6) (7). Enhancins are only encoded by a limited number of baculoviruses and it is unclear how viruses that lack this enzyme pass through the PM. However, some are highly infectious (e.g., AcMNPV) and do not appear to be

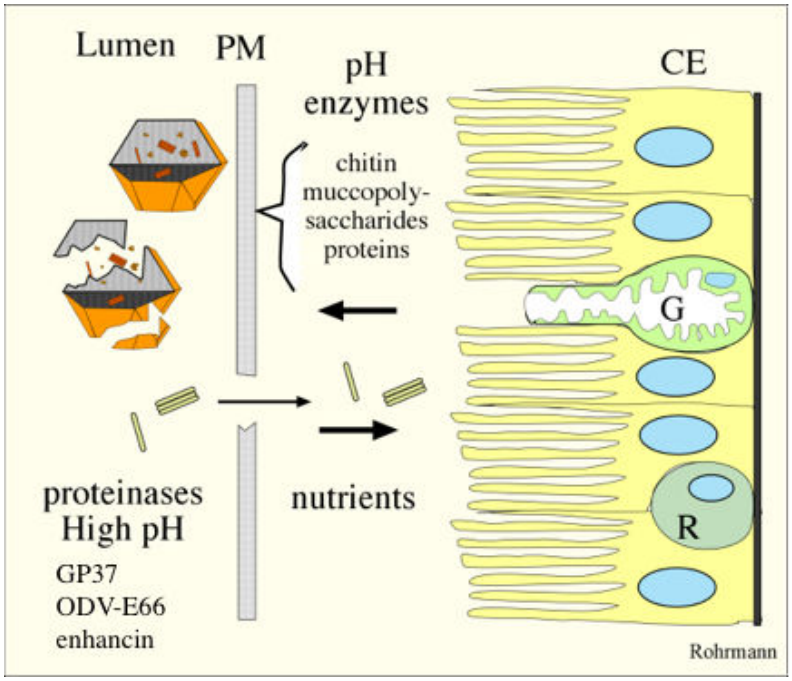

Figure 2. The insect midgut and virus infection. The midgut cells generate the peritrophic matrix (PM) by the synthesis and secretion of chitin, muccopolysaccharides and proteins. They also secrete digestive enzymes and ions that regulate the pH. Occlusion bodies are dissolved by the high pH in the midgut lumen, and are further degraded by proteinases associated with occlusion bodies that may also digest the PM. Chitinases associated with polyhedra or virions including gp37 (Ac64) and ODV-E66 (Ac46) have chitinase activity and gp37 may degrade the PM and be involved with fusion of virions with midgut cells (14). The three major types of midgut cells are indicated: columnar epithelium (CE), goblet cells (G) and regenerative cells (R).

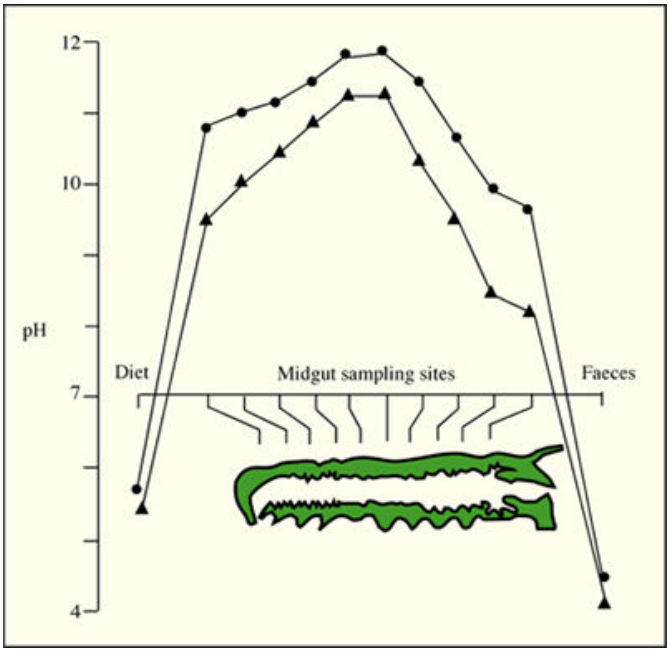

Figure 3. PH profiles along the gut lumens of two lepidopteran species. The pH of the hemolymph was 6.7. The species shown are *Lichnoptera felina* (circles) and *Manduca sexta* (triangles). This figure is modified from (5).

inhibited by the PM, suggesting that there must be alternate or parallel mechanisms, such as the contaminating bacterial proteinases described above that are involved in breaching the PM (8).

AcMNPV encodes at least two enzymes that may facilitate infection by digesting the PM. ODV-E66 (Ac46) (Figure 4) is capable of digesting chondroitin and to a lesser extent hyaluronan polysaccharides that are major cellular components (9) (10). Chondroitin sulfate is present in the PM of *B. mori* and it can be digested by the homolog of Ac46 (Bm37). This has led to the suggestion that Ac46 enhances the primary infection by digesting the chondroitin sulfate in the PM (11). Most Alpha- and Betabaculoviruses encode GP37 (Ac64) and orthologs are also found in entompox viruses. It is polyhedron associated in AcMNPV and N-glycosylated. In entomopox viruses the gp37 ortholog forms crystallized spindle-like structures. These structures have been suggested to digest the peritrophic matrix (12). Structural analysis of these spindles indicated that they contained a globular domain that is related to lytic polysaccharide monooxygenases of chitinivorous bacteria. It is thought that upon ingestion by the host, the spindles are dissolved and the monooxygenase domain is exposed and can then digest the chitin-rich peritrophic matrix (13). It has been reported that GP37 can degrade the peritrophic matrix and also facilitates the binding of ODV to midgut cells (14). The following is an overview of the pathogenesis of AcMNPV and related viruses that cause systemic infections. These viruses, after initial replication in the midgut, spread throughout the organism. In contrast, a variety of less well-characterized viruses limit their replication to midgut cells. At the end of this section, the pathogenesis of viruses that are confined to the midgut and do not cause systemic infections will be reviewed.

Infection of midgut epithelial cells; per os infectivity factors (PIFs)

AcMNPV ODV normally initiate their replication cycle by infecting columnar epithelial cells that are a major cell type lining the midgut, and regenerative cells in *T. ni* (15) or *S. exigua* larvae (16) (Figure 4). There is a combination of factors that appear to be involved in the initiation of midgut infections. These include factors that facilitate binding to the cells, cell receptors to which the virions bind, and virion envelope proteins that may have enzymatic activities that allow viral access to midgut cells or that fuse with the host cell membrane thereby permitting viral entry. A set of gene products called per os infectivity factors (PIFs) (17) are required for infection of midgut cells (Figure 4). Many are specific to ODV and are not found associated with BV. They can be deleted from a viral genome without affecting the ability of the virus to infect cells in culture. However, such deletions reduce the ability of the virus to infect midgut epithelial cells when fed (per os) to insect larvae. In AcMNPV, 10 or more of such genes have been identified and they are described in Chapter 2 (see also Chapter 2, Figure 11). It has been suggested that ODV binds to proteinase sensitive receptors (18) and once bound, the ODV envelope fuses with the epithelial cell membrane releasing the nucleocapsid into the cell cytoplasm.

Chitin binding proteins

Ac145 and Ac150, have properties similar to PIF proteins and are described in Chapter 2. Both Ac145 and Ac150 are predicted to encode chitin binding domains (19) and localize to ODV envelopes (20). Although, Ac83 (VP91) (PIF 8), a virion structural protein, is also predicted to encode a chitin binding domain, no chitin binding activity was detected when examined experimentally (21) and the region was predicted to encompass a zinc finger domain (22). There are two possible interactions involving chitin binding that might occur during midgut infection (Figure 2 and Figure 4). One would involve binding to the peritrophic matrix (PM) possibly to facilitate movement through the membrane. However, if the PM is extensively degraded during infection, this binding might be counterproductive. The chitin component of the PM is produced by chitin synthase, an enzyme that is located at the apical tips of brush border microvilli. The enzyme is also found associated with tracheal cells (23, 24). Therefore, an affinity for chitin could facilitate the interaction of the virion with these cells, first to initiate infection of midgut cells (e.g., Ac145 and Ac150) and subsequently to interact with tracheal cells (VP91). Another protein, GP37 (Ac64) has been shown to bind to chitin (25). Although GP37 has not been shown to be a structural protein, it may play a role in the infection of cells that synthesize chitin. It has also been suggested to cause disintegration of the peritrophic matrix and facilitate fusion of virions with the midgut epithelium (14) (see above). Also, ODV-E66 (Ac46), which associates with the PIF complex, is a condroitinase and chondroitin glycosaminoglycans have been found to localize to the apical midgut microvilli of *Anopheles*

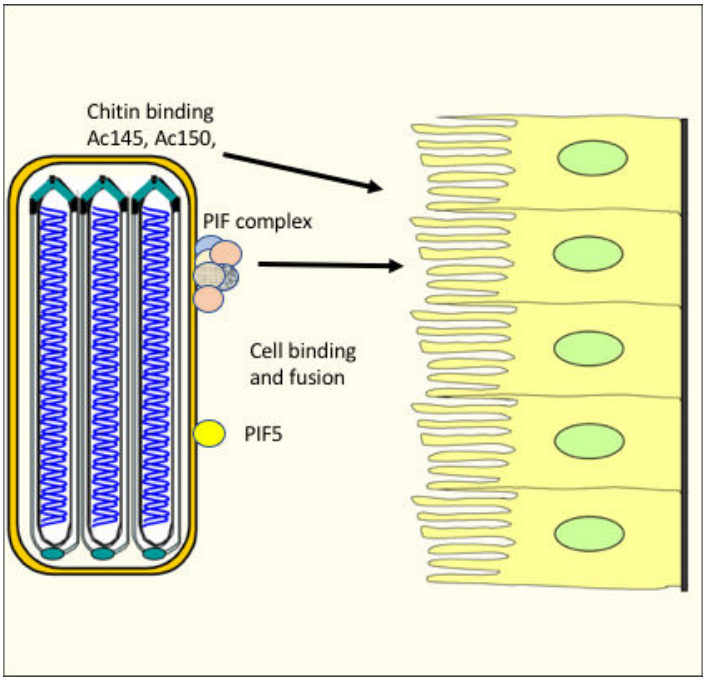

Figure 4. Possible interactions of virion proteins with midgut cells. Ac145 and Ac150 have chitin binding domains, suggesting that they may interact with chitin synthesizing cells. Members of the PIF complex (PIF0-P74, PIF1 and 2) bind to midgut cells.

gambiae (26) suggesting that if Lepidoptera are similar, this enzyme could facilitate recognition or entry into midgut cells.

Viral and host gene expression upon midgut infection

The expression of AcMNPV and T. ni genes in the midguts of T. ni larvae was examined to determine which viral genes might be important in establishing the initial infection and also to identify viral genes expressed to neutralize the host defenses. It was found that the midgut expression profiles for most gene were similar to those of cultured cells infected by the virus. However, fp-25k (Ac61), v-fgf (Ac32), and arif1 (Ac20) were expressed at higher levels (27). These were thought to influence high levels of BV production by the acceleration of systemic infection, and the enhancement of viral movement all of which might result in the efficient establishment and the transition to a systemic infection. It was also found that several host genes were upregulated including orthologs of HMG176, a gene that encodes a protein associated with the basement membrane of midguts of molting and feeding larvae, a hypothetical cuticle protein CPH43 gene, and atlastin encoding a possible anti-viral peptide. Several cytochrome P450 genes that are involed in detoxification of a variety of compounds were found to be downregulated. A number of immune associated genes were also affected. Several interfering RNAs including Dcr-2 and Ago-2 were also upregulated (28).

For virus replication within cells, see Secondary Infections (below).

The initiation of systemic infections: Transiting a midgut barrier - the basal lamina

For some baculoviruses, replication only occurs in midgut cells and there is no systemic infection (see below). However, Alphabaculoviruses cause a systemic infection and therefore must transit the basal lamina to access the interior of the insect. The basal lamina is a structure composed of a fibrous matrix of glycoproteins that surrounds the midgut epithelium and is thought to be a barrier to systemic infection. Furthermore, the initiation of systemic infections may differ based on the insect host. In AcMNPV infected T. ni larvae, newly synthesized GP64 is directed toward the basal and lateral regions of the cell (15). This targets the virions to bud away from

the gut lumen and toward other susceptible tissues, including neighboring cells (29). This polar distribution of GP64 apparently does not occur in other tissues where targeting the infection in a specific direction would not be critical for spreading the infection (15). Subsequent movement of AcMNPV has been examined using viruses expressing a *lacZ* reporter gene so the infection of single cells can be traced. In *T. ni* larvae, secondary infections appeared initially in tracheoblasts and tracheal epidermal cells. It has been reported that in some insects, tracheal cells have projections that penetrate through the basal lamina (30) (31). Such projections could provide access to the tracheal system and allow the virions to move past the basal lamina leading to a systemic infection (29). Further evidence for the ability of trachea to spread infections systemically was the observation that infections could be initiated by exposure of insects to BV through their spiracles. Such infections spread throughout the insect following tracheal tracts (32). However, investigations of AcMNPV using a different insect, *S. exigua*, indicated that rather than the secondary infection specifically localizing to tracheoblasts, other tissues, such as midgut muscle and hemocytes showed simultaneous infection, suggesting that transit through the basal lamina might not have been dependent solely upon tracheoblast infection (16). Surprisingly, it was noted in one of these studies that in most *T. ni* larvae the midgut infection appeared to be transient and although the infection had become systemic, it had been cleared from the midgut (29). This was attributed to sloughing and regeneration of the midgut epithelium, possibly as a response to the infection.

Fibroblast growth factor (FGF), (Ac32) a possible factor in basal lamina transit

It has been suggested that a viral encoded ortholog of fibroblast growth factor (FGF) may be involved in movement of the virus across the basal lamina. Ac32 has homology to FGF and is termed vFGF. Orthologs are found in the genomes of most lepidopteran baculoviruses (NPVs and GVs) and may reflect several independent lineages. AcMNPV *fgf* is closely related to a gene in *D. melanogaster* called *branchless*, whereas a Group II *fgf* ortholog from LdMNPV is less closely related to the insect orthologs, and the GV orthologs show only limited similarity to NPV *fgf* orthologs. In BmNPV, FGF is glycosylated which is essential for its secretion (33, 34) and binds to an insect receptor called *breathless* (35). AcMNPV FGF has been demonstrated to stimulate insect cell motility (36). Although AcMNPV with a deletion of the *vfgf* gene showed no differences from wt in cultured cells (37), the time of death was delayed when it was fed to larvae (38). Similar results were observed for a BmNPV *vfgf* deletion (39). Evidence suggests that vFGF initiates a cascade of events that may accelerate the establishment of systemic infections. This involves two processes; vFGF from virus-infected midgut cells diffuse through the basal lamina and attract tracheal cells so that they are adjacent to infected midgut cells, but separated by the basal lamina. vFGF then activates FGF receptors located on the tips of tracheal cells. This leads to the activation of matrix metalloproteases located in the same subcellular region via a MAP kinase or NFkB pathway. Matrix metalloproteases subsequently activate effector caspases that move extracellularly so that they are positioned for the degradation of the basal lamina by digestion of the laminin component. The delaminated tracheal cells are then susceptible to virus infection. This allows the transit of the virus through tracheal cells to other tissues and results in the systemic infection (40). This theory is supported by evidence for the activation of matrix metalloproteinases, the activation of effector caspases, and the degradation of laminin after the per os infection of midgut cells.

Transiting midgut cells without replication

It has been suggested that under some circumstances nucleocapsids can transit through gut cells, bypassing replication, and bud directly into the hemolymph (41), or tracheal cells (42). This may be a mechanism for accelerating systemic infection and avoiding replication in gut cells that may be sloughed and eliminated from the insect. Genomes of all sequenced Group I viruses have both early and late promoter consensus sequences upstream of their *gp64* envelope fusion protein genes. Expression of *gp64* from the early promoter may prepare cell membranes for budding such that some nucleocapsids might directly transit the cell and bud without undergoing replication. When the early promoter was eliminated in the regulatory region of the AcMNPV *gp64*

gene, a delay in the time course of infections initiated by oral, but not intrahemocelic inoculation of insects was observed (43, 44). In addition, when *T. ni* cells are infected at high moi (200), many virions appear to become associated with the plasma membrane from within the cell (45). This theory is applicable only to the MNPV type of virus, because it is dependent upon a cluster of connected nucleocapsids simultaneously infecting single cells, after which they would have to become separated in the cytoplasm with some entering the nucleus to undergo conventional replication and others proceeding directly to the cell membrane. The virions undergoing replication would direct the early synthesis of GP64 to prepare the cell membrane for budding of the nonreplicated nucleocapsids that transit directly through the cell possibly facilitated by the vFGF pathway described above. The theory is complicated by a lack of understanding of the MNPV type of virion morphology because it appears to lack a genetic determinant (see Chapter 1). A similar combination of early and late promoters is also present upstream of almost all of the F genes of Group II viruses, suggesting that these two types of envelope fusion proteins (F and GP64) may be regulated in a similar manner. Whereas most of the Group I viruses are of the MNPV type (BmNPV may be an exception since single nucleocapsids predominate (46)), Group II viruses can be either MNPV or SNPV. For SNPVs, it is difficult to link the regulation of the fusion protein to the transit of the virus, because it would require extremely high levels of virus to ensure infection of single cells with more than one virion so that both transit and fusion protein synthesis could occur in the same cell. In contrast to the regulatory region of the F gene in Group II viruses, the regulatory region of the F gene orthologs in at least nine sequenced granulovirus genomes lack conventional early promoter consensus sequences (TATA + CAGT) in the proper context, and three were identified (AdorGV, AgseGV, CrleGV) that have late promoter elements within 200 nt upstream of the ATG.

Baculovirus replication: evidence from cultured cells

Secondary infection, cell entry (Figure 1 and Figure 5).

In contrast to occluded virions that have evolved to infect midgut cells, the budded virions that spread the infection within the insect have evolved a completely different mechanism to initiate infection. As described in Chapter 2, either GP64 or an active form of the F protein is required for secondary infection, depending on the virus lineage. GP64 is an envelope fusion protein that, in addition to being required for exit from cells, is involved in initiating infection of other cells. It may also be an attachment factor, although the receptors it interacts with have not been defined. However, investigations have suggested that macropinocytosis, dynamin- and clathrin-dependent endocytosis, and cholesterol in the plasma membrane, all may be involved in the entry of AcMNPV into mammalian cells (47). It has also been suggested that transient forms of GP64 embed hydrophobic side chains into cell membranes triggering endocytosis independent of specific receptor molecules (48). This lack of specific receptors and the affinity of GP64 for cell membranes may explain its ability to facilitate the entry of AcMNPV into a wide variety of different cell types encountered during a systemic infection.

Since AcMNPV is capable of entering many types of cells, including those of vertebrates, receptors may be common molecules such as phospholipids (49). In both insect and vertebrate cells, entry is mediated by GP64 via clathrin-mediated endocytosis (50) (Figure 5). In this process, clathrin becomes concentrated in indentations or pits on the surface of plasma membranes. These structures are involved in the selective uptake of proteins into eukaryotic cells. Viruses commonly attach to receptors located on the surface of these clathrin-coated structures. The receptors contain internalization signals and upon binding to a viral attachment protein are stimulated to cause the pit to invaginate into the cytoplasm of the cell. This internalized vesicle is called an endosome or endocytic vesicle. It subsequently becomes acidified which causes the viral fusion protein to change conformation resulting in the merging of the viral envelope with the endosome membrane. This provides an opening or pore through which the nucleocapsid can enter the cell cytoplasm.

It has also been demonstrated that AcMNPV can enter cells at low pH (pH 4.8) by apparently fusing directly with the cell membrane independent of endocytosis (51). However, it is not clear if the cells and virus would encounter a pH this low under normal conditions.

Transport within the cytoplasm; the role of PP78/83 (Ac9) a WASP-like protein

Cellular actin is involved in the transport of virions within cells (52) (45) (53, 54) resulting in the production of 'actin comet tails' trailing behind the nucleocapsid (Figure 6). After AcMNPV infection of TN-368 cells, actin is polymerized from G- into F-actin. G-actin is a globular monomeric form of actin, and polymerizes into filamentous, or F-actin. This is a reversible reaction requiring ATP hydrolysis for polymerization. A cellular complex of up to 7 proteins called the Arp2/3 complex is comprised of two actin related proteins (Arp) that resemble the structure of monomeric actin and 5 additional factors. This complex is involved in nucleating the formation of F-actin filaments. Activators are required for this process and they bind both monomeric G actin and the Arp2/3 complex. One category of such activators is called Wiskott-Aldrich syndrome protein (WASP), and an ortholog of WASP (PP78/83) (AcMNPV orf9) is encoded in lepidopteran NPV genomes. A purified truncated version of AcMNPV PP78/83 containing the critical activation domains was found to be capable of stimulating actin polymerization in combination with the Arp2/3 complex in vitro. In addition, a point mutation in the arp2/3 binding region of PP78/83 results in partially defective actin polymerization with reduced actin tails and erratic paths of movement that frequently changed direction (45). The use of actin polymerization for transport of intracellular pathogen has been observed for poxviruses (55) and certain bacteria, such as *Listeria monocytogenes* (56). However, in contrast to these pathogens that employ actin polymerization after replication, AcMNPV uses it immediately upon infection before viral replication (45). It then moves to the nucleus facilitated by actin polymerization (see below) and appears to enter through nuclear pores (41) (45). Transit to nuclei may also be facilitated by the movement of virion-containing endosomes along microtubules (57) (Figure 5).

Entry into nuclei

Several lines of evidence suggest that the nucleocapsids enter nuclei through nuclear pores (Figure 5) (Figure 7). Empty nucleocapsids were originally observed in nuclei of cells early in infection (41). In human cells exposed to AcMNPV at high moi, at 4 hr p.i. about 8% of the nucleocapsids that entered cells were localized to the cytoplasmic side of nuclear pores. When mitosis was blocked, and the nuclear membrane was intact, nucleocapsids were observed in nuclei, suggesting that they do not need dividing cells and the corresponding nuclear membrane breakdown to enter nuclei. Because of their localization to the cytoplasmic side of nuclear pores and their presence in nuclei of cells treated with mitotic inhibitors that prevent the breakdown of the nuclear membrane that occurs during mitosis, these authors concluded that nucleocapsids enter directly through nuclear pores (58). However, electron microscopic investigations have suggested that some baculoviruses inject their DNA through nuclear pores, reviewed in (59). Subsequently, using a virus encoding both a fluorescent tag fused to the vp39 capsid protein in addition to the wt vp39 protein, it was observed that the nucleocapsids localized to nuclear pore complexes and fluorescence was observed within nuclei (45) (Figure 6). This suggests that the nucleocapsids dock with and are transported through the nuclear pore complex. Nuclear pore complexes have been recently characterized and have been calculated to have a channel of 38 - 78 nm (60). Baculovirus virions have been calculated to have dimensions of 30-60 nm in diameter (61) indicating that they should be capable of moving through the pores. Also, AcMNPV nucleocapsids injected into Xenopus oocytes cause nuclear pores in that system to undergo significant reorganization in order to accommodate the transit of the nucleocapsids (62). Evidence suggests that a capsid associated protein, BV/ODV-C42 (Ac101), binds to PP78/83 and transports it into nuclei. Mutant bacmids lacking Ac101 fail to demonstrate polymerization of actin in nuclei (63). In addition, a predicted pocket binding protein site was identified on

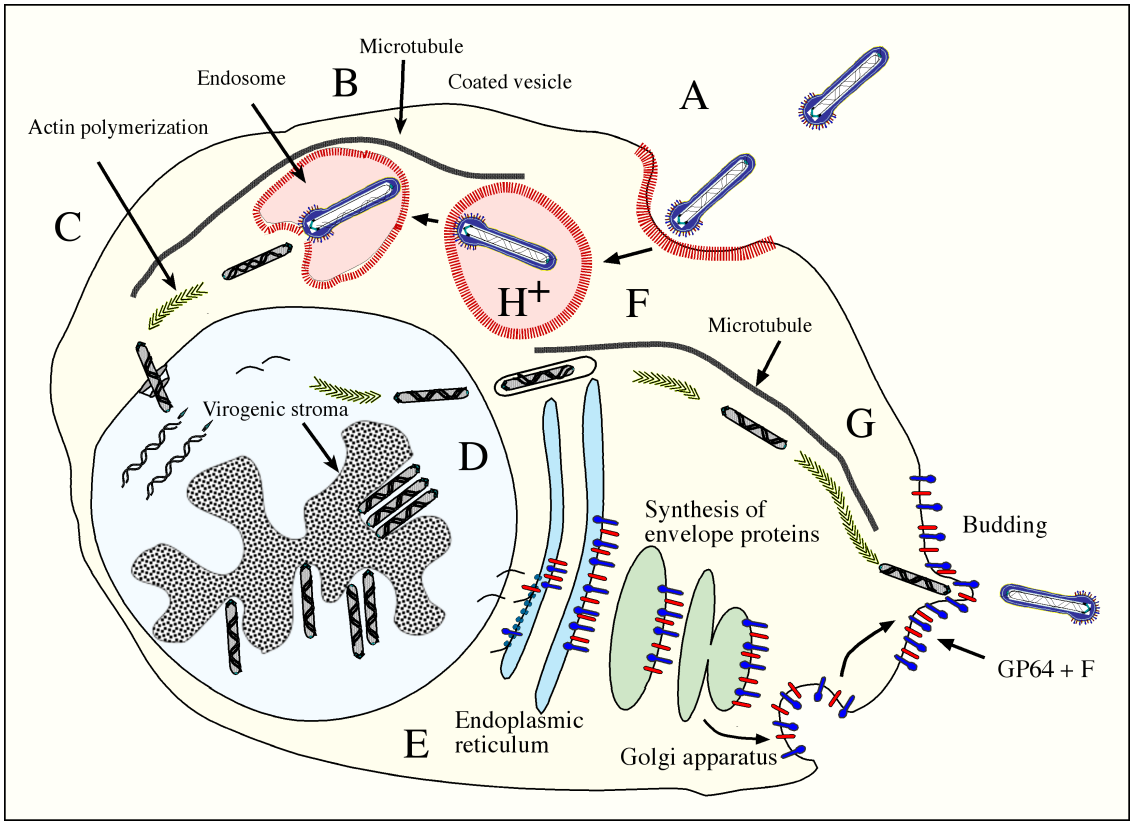

Figure 5. Budded virus infection of a Group I virus. BV attach to receptors located in clathrin coated pits via GP64 and are endocytosed (A). The endocytic vesicle is acidified and this changes the conformation of GP64 and causes the virion envelope to fuse with the endosomal membrane releasing the nucleocapsid into the cytoplasm (B). The nucleocapsid enters the nucleus through a nuclear pore complex (C), genes are transcribed, DNA is replicated and nucleocapsids are assembled in the virogenic stroma (D). In Group I virus, at least two envelope proteins are synthesized, GP64 and F. They are likely translated in association with the endoplasmic reticulum, glycosylated and transported to and incorporated into the cytoplasmic membrane via the Golgi apparatus (E). Nucleocapsids destined to become BV exit the nucleus and are thought to transiently obtain an envelope that is lost (F). Evidence suggests that the transit of the nucleocapsids to nuclei, within nuclei, and towards the cell membrane prior to budding is propelled by actin polymerization (45, 74). Microtubules may also be involved in transport of endosomes and nucleocapsids (57, 77, 148). Upon reaching the F- and GP64-modified cytoplasmic membrane, they bud through, and obtain envelopes (G).

Ac101 that is essential for actin polymerization (64). Once inside the nucleus, the DNA is uncoated and the transcriptional cascade is initiated that eventually results in the production of nucleocapsids.

A major feature of infection by lepidopteran NPVs is the massive reorganization of nuclei in which they expand to such an extent that they fill most of a cell's volume. It has been calculated that the diameter of Sf-9 cells may increase up to 1.45-fold during infection (65). The movement and concentration of actin may contribute significantly to this key feature of these infections (52). P10 also may contribute to cytoskeletal reorganization as it interacts with tubulin and may be involved in modifying microtubules (66, 67).

The virogenic stroma

A novel structure characteristic of NPV-infected cells is the virogenic stroma (Figure 1 and Figure 5). It is an electron-dense, chromatin-like structure surrounding multiple less dense spaces that is found near the center of nuclei of infected cells. It is thought to be a molecular scaffold that is produced for the orderly and coordinated transcription and replication of viral DNA and the subsequent packaging of DNA and assembly of nucleocapsids. The structure of the virogenic stroma is not well understood, but it appears to be composed of RNA and protein with discrete concentrations of DNA that border intrastromal spaces, the sites of virion

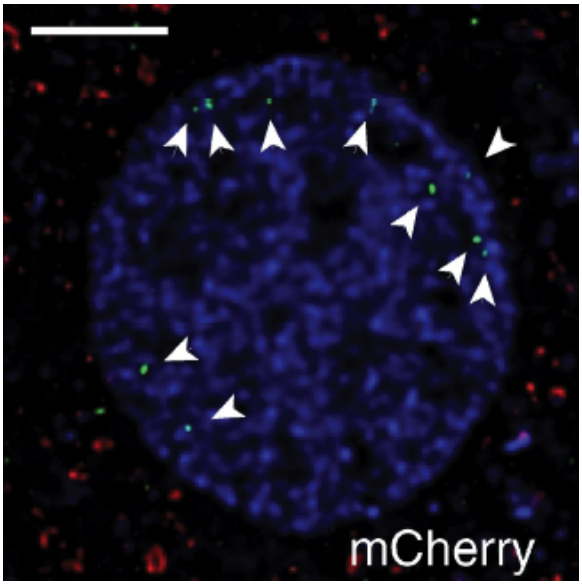

Figure 6. Entry of AcMNPV nucleocapsids through nuclear pores. This shows virus with the capsid protein VP39 fused with the fluorescent label, mCherry. Arrows indicate capsids; bar is 5 µm. Image reproduced from (45), with permission.

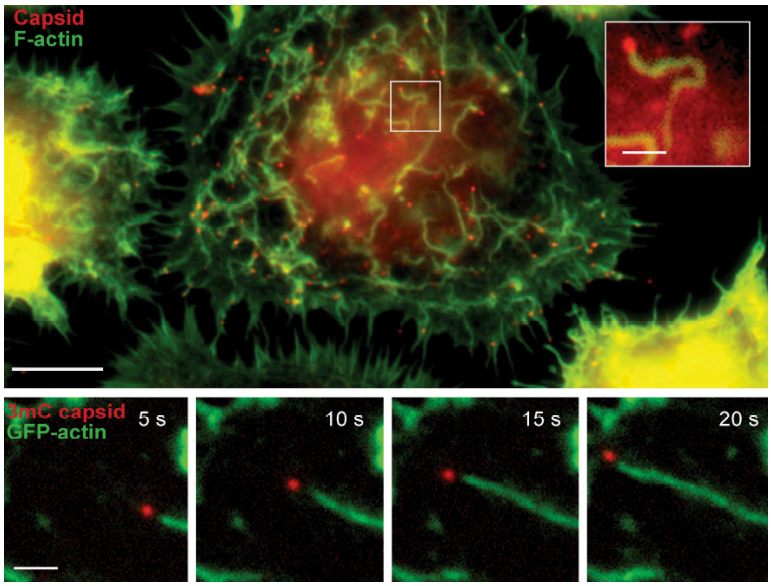

Figure 7. Actin-based motility of AcMNPV within the cytoplasm of the cell. The virus was detected by capsid protein VP39 fused with the fluorescent label, mCherry (red); actin (green; FITC-phalloidin). Inset bar is 10 mm. Bottom: time series at 5 sec intervals. Image reproduced from (45), with permission.

assembly (68, 69). In one study, it was observed that AcMNPV bacmids deleted for the single-stranded DNA binding protein, DBP (Ac25), failed to produce a virogenic stroma and also failed to produce normal-appearing nucleocapsids (70). PP31 (Ac36) also appears to be associated with the virogenic stroma (71), and deletion of Ac36 results in a decrease in the level of transcription of some, if not all, late genes (72). Late in the infection, virions may move from the virogenic stroma to a peripheral area where they become occluded (73), however, very late in infection, occlusion bodies can completely fill the nuclei. For a discussion of the virogenic stroma in relation to DNA replication, see Chapter 5.

Exiting nuclei and the cell to form BV

After nucleocapsids are replicated in the nucleus, actin polymerization is involved in the propulsion of AcMNPV nucleocapsids within nuclei and is also involved in the disruption of the nuclear membrane to allow virion release into the cytoplasm (74) (Figure 5). It has been reported that AcMNPV infection reduces the lamin concentration and alters the structure of the nuclear envelope (75). Also, it has been suggested that as virions bud out of the nucleus they obtain an envelope from the nuclear membrane (41) (Figure 5). The envelope may contain at least one viral protein, GP16 (76). As described below the host cell ESCRT-III may be involved in nuclear exit. This envelope is lost during transit through the cytoplasm. Two models have been proposed for the movement of nucleocapsids as they exit cells to form BV. In one, it was suggested that VP39 and Ac141 interact with kinesin-1 and this may indicate that nucleocapsids are transported along microtubules (77). It has also been reported that nucleocapsids undergo actin-based motility following escape from the nucleus, and in those studies the microtubule-depolymerizing drug colchicine did not affect viral titer, and the rates of movement were consistent with actin-based motility as opposed to motor-driven transport, suggesting that actin polymerization is critical for nucleocapsid transport through the cytoplasm during egress (74) (Figure 5).

As the infection proceeds, concentrations or patches of envelope proteins accumulate at the plasma membrane (Figure 5). For group I NPVs, these include both GP64 (Ac128) and the F protein (Ac23) (78, 79) (See Figure 5). In other viruses, e.g., members of Group II NPVs that lack *gp64*, this membrane is likely modified by homologs of the F protein (80). The modification of the host cell membrane by GP64 is required for virus budding and consequently for secondary infections (81, 82).

Cellular components important for AcMNPV cell entry, transport, nuclear entry and exit, and budding: SNARE, ESCRT, and NSF complexes

The fusion of viral envelopes with cell membranes is a method by which enveloped viruses enter cells. SNAREs and NSF (*soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor*) (SNARE) proteins are the minimal machinery involved in the fusion of transport vesicles with membranes and are critical for the initiation of infection by enveloped virions (83). Upon AcMNPV infection of Sf9 cells, SNARE gene transcription was observed to be upregulated before its subsequent decline. Interference with SNARE or NSF gene expression inhibited AcMNPV production. Inhibition of NSF caused entering virions to be stalled in the cytoplasm with minimal transport to nuclei. The transport of GP64 to the cell membrane was also inhibited and BV production was greatly reduced and resulted in nucleocapsids being trapped in the perinuclear space between the inner and outer nuclear membranes. NSF was found to be associated with several conserved baculovirus proteins that are involved in BV production including Ac76, Ac78, GP41, Ac93, and Ac103. NSF was also found within BV. These data showed that the SNARE complex is important for AcMNPV infection and that NSF is involved in both entry and nuclear egress of BV (84) (85).

The endosomal sorting complexes required for transport (ESCRT) are involved in a diverse set of processes used in budded virus production including endosomal sorting, vesicular trafficking, and virus budding (86). Dominant negative mutations of Vps4 (Vacuolar Protein Sorting-associated 4), a component of ESCRTIII involved in recycling of the complex, inhibited both AcMNPV entry and egress (87). In addition, RNAi interference assays of components of ESCRT I and III complexes resulted in virions being trapped in the cytoplasm. Components of ESCRT-III, but not ESCRT-I were found to be involved in nuclear egress. A number of conserved AcMNPV proteins were found to interact with Vps4 and proteins of ESCRT-III suggesting that these proteins form an egress complex associated with the nuclear membrane (88) (85).

Viral manipulation of larval metamorphosis: EGT (Ac15)

Another viral protein that can affect the course of an infection is an enzyme, ecdysteroid UDP-glucosyltransferase (89). Egt homologs are found in all lepidopteran NPVs and most GV genomes, but not in

other lineages. Because of its role in insect steroid metabolism, the likely source of a gene encoding this enzyme would be from a host insect, and closely related orthologs of *egt* are found in a variety of insects such as *B. mori*. The function of the viral EGT is to block molting and pupation in infected larvae by catalyzing the transfer of glucose from UDP-glucose to ecdysteroids, thereby inactivating these insect molting hormones (90, 91). Molting can cause severe physiological stress on infected insects and many do not survive this transition. Therefore, the full productivity of the virus infection may not be realized. Evidence suggests that viral EGT prevents this stress by blocking molting. It also prolongs the feeding stage of infected larvae, thereby allowing the virus to replicate over a longer period of time in larger larvae, resulting in a higher yield of virus. The yield of occlusion bodies is increased about 30% in larva infected with wt virus compared to infection by virus lacking the *egt* gene (92, 93). A remarkable feature of NPV infection is that in some instances the insects can grow and continue feeding right up until they die. They appear healthy, yet when examined are heavily infected with high concentrations of occlusion bodies in their cells and hemolymph. EGT likely contributes to this effect. A common method to reduce the time that a virus takes to kill its host is to delete the *egt* gene. Larvae infected with these mutants are smaller and die sooner than wt, thereby reducing the damage caused to crops after the infection.

Budded virus versus cell-associated virus production

A major transition during baculovirus infections is the shift from BV production to the retention of nucleocapsids in the nucleus and their incorporation into occlusion bodies. In a study examining the kinetics of AcMNPV replication in cultured cells, the proportions of BV and cell-associated virus were analyzed using quantitative PCR to measure the number of viral genomes (94) (Table 1). The virus used in this study expressed *lacZ* in place of the polyhedrin gene and did not produce occlusion bodies. It was found that viral DNA doubled every 1.7 hr starting at 6 hr post infection until DNA replication ceased at about 20 hpi, which was correlated with the onset of budding. At this time point, under optimal conditions, virion-associated DNA reached a plateau at about 84,000 genomes per cell. In contrast, only about 2,000 genome equivalents per cell were released into the medium. This suggests that about 2.3% of the viral DNA is budded out of an infected cell. At the cell concentration employed ($>3 \times 10^6$/ml), this would be equivalent to 6×10^9 BV/ml. Since baculovirus titers of about 5×10^8 pfu/ml are commonly achieved, this would suggest that about 10% of the BV were able to form plaques — not an unreasonable number. The numbers measured in the study by Rosinski et al. (94) also provide information on the viral DNA generated per cell during an infection. If, for example, 84,000 genomes are produced per cell, this would be equivalent to 10^{10} bp/cell using 133,000 bp as the AcMNPV genome size. If the DNA content of a diploid *Bombyx mori* cell is ~10^9 bp (95), this would suggest that 10 times more viral DNA was generated during the infection than was present in the cell genome. Although this may seem high, it could be reflected in the expansion that nuclei undergo during the infection. Also, if much of this DNA is packaged into virions, it might be considerably more compact than cellular DNA. In addition, much of this DNA may remain unpackaged, and this could be a factor underlying the ability of baculoviruses to hyperexpress late genes. Under these conditions, high levels of gene expression could be dependent on high gene copy number (see Chapter 5 for more detailed discussion). In an investigation of *Helicoverpa zea* cells infected with the *H. armigera* NPV (HearNPV), it was calculated that about 131,000 viral genomes were produced per cell and the mass of viral DNA was 20 times that of an uninfected diploid cell. They also estimated that extracellular viral genomes from both BV and ODV of about 5,600 and 29,000 at 24 and 48 hpi, respectively (96).

Table 1. DNA produced in AcMNPV infected cells (from (94))

Cells infected (moi = ~20)	AcMNPV genomes/cell	BV genomes/cell (% total genomes)	Host DNA (bp)/cell (95)	Viral DNA (bp)/cell
3×10^6/ml	84,000	2000 (2.3%)	~10^9	~10^{10}
5×10^6/ml	39,500	1750 (4.5%)	~10^9	~5×10^9

The transition from BV to ODV production

The initial production of BV allows the virus to spread throughout the insect, whereas late in infection, the virions are confined to nuclei where they become occluded (Figure 1). The transition from BV to ODV production late in infection could be the result of physical changes in the organization of the nucleus that limit nucleocapsid transit to the cytoplasm, or to the depletion of a component required for transit through the cytoplasm. It also is possible that as the infection progresses, ODV envelope proteins accumulate in the nucleus, and once the nucleocapsids become enveloped they can no longer exit the nucleus. It has been reported that ODV-E25 (Ac94) localizes to the periphery of replication foci, whereas vp39 was found within these structures (97). It has also been demonstrated that if ODV-E25 is expressed as an early gene under the IE-1 promoter, that budded virus production is inhibited and ODV-E25 appears to mostly concentrate in the cytoplasm around the nucleus, rather than localizing to the nucleus (98) (99). Subsequently it was found that the open reading frame of ODV-E25 encodes a microRNA that down regulates ODV-E25 expression. It was suggested that this might result in a reduction in BV production and be involved in the shift to occluded virus production (100) (101). ODV-E25 (Ac94) is highly expressed in midgut cells (27) and associates with NSF and may be involved in the nuclear entry and egress of BV (102). It has also been reported that Ac66 in BV but not ODV is ubiquitinated and this may provide a signal for the selective transit of BV through the cytoplasm (103).

Occlusion, the final stage in virus infection

As described above, most of the assembled nucleocapsids appear to be destined to remain in the nucleus and become occluded. Cell type seems to govern the production of occlusion bodies. For some viruses, midgut cells do not appear to support occlusion body production. However, for other viruses, this is the only locale where occlusion occurs (see below). The reason for this specificity is not clear.

In lepidopteran NPV infections, evidence from electron tomography (ET) along with electron microscopy suggests that both the outer and inner nuclear membrane components can invaginate and contribute to the formation of microvesicles that are thought to be the source of the ODV envelope (104). These are modified with virally encoded ODV-specific envelope proteins (105). A hypothetical model for this process is shown in Figure 8. A feature of the final stage of baculovirus replication that takes place after most DNA replication has occurred is the hyperexpression of very late genes resulting in the production of high levels of polyhedrin and p10. Polyhedrin accumulates in nuclei and at some point crystallizes into a lattice that surrounds virions. It is not clear whether virion occlusion is a concentration-dependent, random event, or if virions serve to enucleate the formation of occlusion bodies. At least one protein (Ac68) may be involved in this process because when the ortholog was deleted from BmNPV (Bm56), the virions produced were not incorporated into the occlusion bodies (106). Also, very late in infection, high levels of P10 are expressed. It forms tube-like structures that penetrate both the nucleus and cytoplasm (67, 107). As occlusion bodies mature, P10 fibrils align with the surface of the polyhedra and appear to be intimately involved with the assembly of the polyhedron envelope on the occlusion body surface (see Chapter 2, Figure 2). The final polyhedron product has a smooth, even surface (see Chapter 1, Figure 1). As discussed previously, if the polyhedron envelope gene is deleted, the polyhedra produced have an uneven surface and virions appear to be prone to becoming dislodged (see Chapter 2, Figure 3).

Virus dispersal: 'tree-top disease' and liquefaction 'melting'

Many newly hatched lepidopteran larvae remain as concentrated populations near the egg mass from which they emerged. Dispersal is largely dependent upon access to food supplies that are rapidly consumed near the egg mass. However, during the final larval stages many Lepidoptera disperse (wander) probably as an evolutionary mechanism to spread the population, reduce predation, and also to find a suitable place to pupate. In *Manduca sexta,* wandering is induced by a limited rise in the ecdysteroid titer in the hemolymph, and is associated with

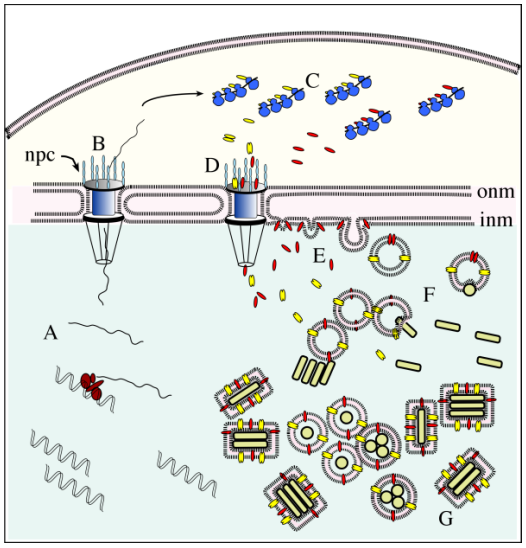

Figure 8. A hypothetical diagram of ODV membrane morphogenesis. In this diagram mRNA encoding ODV envelope proteins are transcribed (A) and exported (B) to the cytoplasm for translation (C) and the proteins are then targeted to the nucleus (D). Some of these proteins may be targeted to the inner nuclear membrane and induce it to invaginate, thereby forming microvesicles (E). The microvesicles may be further modified by the incorporation of additional virally encoded ODV envelope proteins, and virions then become enveloped (F, G). Shown are the outer nuclear membrane, the inner nuclear membrane and nuclear pore complexes (npc).

the cessation of feeding and the commitment to begin pupation ((108) and references therein). Baculovirus infection appears to be capable of enhancing this behavior and can cause a terminally infected insect to migrate to a higher elevation on the branch of a tree or plant. This is thought to facilitate dispersal of the occlusion bodies. This condition was noted in Germany and named 'wipfelkrankeit' (tree-top disease) (109). In one investigation, this phenomena was examined in cabbage moth larvae (*Mamestra brassicae*) on cabbage plants infected with MbNPV (110). It was observed that most of the uninfected larvae were located in the heart of cabbage plants, whereas infected third instar larvae were located at the top or edge of the plant. This behavioral difference appeared to be confined to third instar and was not observed in first or fifth instar larvae (111).

Although several baculovirus genes such as ptp (Ac1) and egt (Ac15) have been implicated in influencing the upward movement of larvae late in infection, such behavior likely involves a complex interplay of viral and host genes and to date no gene has been definitively proven responsible for this behavior. In fact, recent investigations have shown that light is a key factor in inducing this phenomenon (112, 113). These observations and the variability of earlier data emphasizes how challenging it may be to delineate the mechanisms involved in this behavior.

Late in infection, after the wandering stage, the insects become torpid and undergo what is termed 'melting'. Melting refers to the disintegration or liquefaction of the insect and is caused by some, but not all viruses. When on an even surface, the infected insects appear to flatten out and all the tissues appear to melt together and liquefy. Clearly, the disintegration of insects at a higher elevation on a plant and the subsequent contamination of lower vegetation could result in the infection of additional insect hosts, in contrast to simply dying, falling on the ground, and decaying into the soil.

Enzymes facilitating liquefaction: Chitinase (Ac126) and cathepsin (Ac127)

Some baculoviruses express enzymes that facilitate the disintegration of infected larvae late in infection. That an insect virus would obtain a chitinase gene to facilitate its dispersal might have been expected because chitin and chitinases are integral components of their host insects. The insect exoskeleton is composed of chitin, and

because it is rigid, it must be periodically removed and reconstructed for insect larvae to grow. Therefore, insect larvae pass through various growth stages, or instars. The transition between these stages involves the digestion and absorption of part of the exoskeleton and shedding of the rest. Consequently, viruses infecting insects very likely had ready access to chitinase genes from their host insects. Chitinase genes are present in most lepidopteran Group I and II NPV and several GV genomes. The chitinase of these viruses is phylogenetically clustered with several lepidopteran chitinases, e.g., the AcMNPV (Ac126) and BmNPV (Bm103) proteins show over 60% aa sequence identity to *B. mori* chitinase. They are also similar (60% identical) to the chitinase of the bacteria, *Serratia marcescens* (114). Although closely related, the insect and viral enzymes have different properties; the AcMNPV chitinase is retained in the endoplasmic reticulum and functions under alkaline conditions, whereas the host enzyme is secreted and has reduced activity at higher pH (115-117). In conjunction with another enzyme, a viral proteinase (cathepsin, Ac127, see below), chitinase participates in the liquefaction of insects late in infection. Insects infected with viruses in which either the chitinase or the cathepsin gene had been deleted remained intact for several days after death (118). The retention of the viral chitinase in the ER may prevent the premature liquefaction of infected insects, allowing the virus to continue to replicate. The facility with which a virus (AgNPV) can be processed as a biocontrol agent for use against the soybean pest *Anticarsia gemmatalis* has been attributed to its lack of these two genes, thereby allowing collection of the virus from intact rather than disintegrated insects (119).

Orthologs of the viral cathepsins (Ac127) have a similar distribution to Ac126 (chitinase) and are present in most alphabaculovirus and several GV genomes and they appear to work in concert. As with the viral and insect chitinase genes, the viral cathepsins are closely related to insect cathepsins, e.g., the Ac127 cathepsin is 39% identical to an *Apis mellifera* cathepsin. To further optimize the role of the viral cathepsin in insect liquefaction, it is synthesized in an inactive form that is activated upon death of the insect (120). Therefore, both chitinase and cathepsin appear to have developed mechanisms to prevent their premature activation, thereby prolonging the infection. For more information on baculovirus chitinase, cathepsins, and enzymes of other baculoviruses that are involved in disintegration of the host larvae see (121).

Because of the dramatic and gruesome nature of the final events in some baculovirus infections, it was incorporated into popular fiction by a science writer. In this novel, a baculovirus was engineered to replicate in human brain cells and the resulting mayhem it causes when released into the human population is the story line (122).

The cytopathology of GVs

The above descriptions have focused on the replicative cycle of the most well-characterized baculoviruses, namely NPVs of Lepidoptera that can be easily cultivated and genetically manipulated. However, there are a variety of other viruses that cause a more limited infection. Although some GVs have been investigated, understanding their cytopathology has been hindered by the lack of an efficient cell culture system. In one cell culture system that has been described, both infectious hemolymph and BV titers were low (the highest BV titer was 10^6 $TCID_{50}$/ml) and BV titers decreased with passage (123). These low titers could reflect the insensitivity of the GV cell culture system and has made the generation of recombinant GVs challenging. Although GVs cause systemic infection, most GVs that have been characterized show a different pattern of cytopathology (Figure 9) from the lepidopteran NPVs described above. In addition, it is difficult to generalize regarding pathology because of the variation of gene content in different types of GVs. For example, half the GV genomes characterized lack cathepsin/chitinase genes and some also lack EGT genes. After their initial replication in midgut cells, subsequent infections vary with different GVs (reviewed in (124, 125)). Some GV infections are limited to the midgut, whereas others cause systemic infections and can replicate in a wide variety of tissues similar to NPVs. In addition, others appear to spread to and are limited to replicating only in fat body tissues. The cytopathology that occurs in the different cell types after GV infection, however, appears to be similar for all GVs and differs from NPVs (Figure 9). In infected cultured cells, the nucleus enlarges and the interior becomes

clear as electron dense material becomes concentrated at the periphery near the nuclear membrane. At this stage, nucleocapsids that are likely destined to develop into BV are evident in the nuclei, but occlusion bodies are not present. Later in the infection the nuclear membrane appears to disintegrate and the nucleoplasm and cytoplasm merge. After this point, occlusion bodies become evident (123). Similar patterns of pathology have been found in infected insects with some cells showing a well-developed virogenic stroma in nuclei before the disintegration of the nuclear membrane (124).

Viruses that are confined to the midgut: hymenopteran and dipteran NPVs

In some types of baculoviruses, the infection appears to be confined to the midgut. These viruses include NPVs of Diptera, e.g., mosquitos (CuniNPV) (126), Hymenoptera, and certain types of GVs mentioned above (127). In the mosquito virus, CuniNPV, viral replication appears to proceed from an early production of virions that bud into the cytoplasm and the later development of occluded virions. The role of BV in spreading the infection to other cells is unclear (126). The development of infections by NPVs of Hymenoptera is also not well characterized. Most surprising is the lack of a homolog of either gp64 or the F protein in the three hymenopteran NPV genomes that have been sequenced. Two candidates for genes encoding possible fusion proteins of 217 and 74 aa have been identified (128). However, fusion proteins of such a small size would be unprecedented for baculoviruses. Because of the requirement of fusion proteins for both BV cell egress and entry, it is unclear how infections by these viruses might spread. It is possible that infected cells are sloughed or disintegrate into the midgut lumen and release a mixture of occluded and nonoccluded virions containing an ODV envelope and other proteins that would allow infection of other midgut cells. In this case, ODV envelope and PIF proteins could facilitate this process. Genes predicted to encode homologs of these factors (including all the PIF proteins) have been identified in the genomes of the NPVs of Hymenoptera. A feature of at least some of these infections has been described as infectious diarrhea (125). This could reflect extensive midgut cell death. Conserved proteinases that could be involved in this process are described below.

Possible dissemination strategies for GVs, and hymenopteran and dipteran NPVs

Although chitinase and cathepsin are found in almost all lepidopteran group I and II viruses, they are present in only a few GV genomes, and neither one is found in the genomes of the hymenopteran and dipteran viruses. However, there are other enzymes encoded in these viruses that might compensate for the lack of chitinase and cathepsin, especially when the infection is localized to the midgut. One such enzyme is a metalloproteinase (distinct from enhancin and cathepsin) with homologs present in all sequenced GV genomes. They have about 30% amino acid sequence identity to a catalytic domain in a stromelysin1 metalloproteinase of humans and sea urchins. The GV enzyme may be non-secreted and continuously active because it lacks both a signal peptide and a cysteine switch that maintains the homologous enzymes in an inactive form. The metalloproteinase from XcGV is capable of digesting proteins and is inhibited by metalloproteinase inhibitors (129). The presence of metalloproteinase homologs in GV genomes may be involved in assisting in viral transmission by facilitating the disintegration of cells after the GV replicative cycle is complete. This might be reflected in infection-associated diarrhea reported for some GVs (125). Likewise, although hymenopteran viruses lack homologs of chitinase and cathepsin, they all encode a trypsin-like protein (130) that shows high levels of sequence identity (~50%) to insect trypsin-like orthologs. For baculovirus infections limited to gut tissues such as hymenopteran NPVs (see above), chitinase may not be necessary because chitin is not a major structural component of midgut cells. Therefore, the presence of a trypsin-like protein may facilitate the dispersal of virus from the gut cells, both by releasing the virus into the environment, and also by providing inocula for the infection of other gut cells.

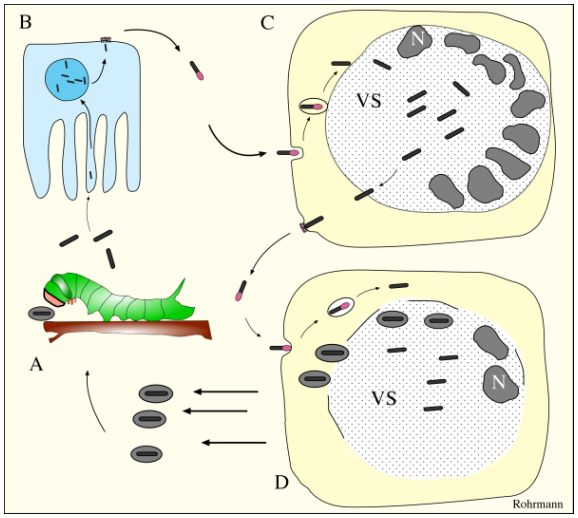

Figure 9. A granulovirus life cycle with systemic infection. Many features of a systemic GV infection are likely to be similar to that of NPVs, including the infection of insect midgut and the systemic spread to other tissues (A, B). However, the GV infection leads to the clearing of the nucleus with nuclear material (N) locating to the margins (C) and the virogenic stroma (VS) distributed throughout the nucleus. Later in the infection the nuclear membrane becomes fragmented and the nuclear and cytoplasmic regions merge (D). This figure is interpreted from (123, 124).

Viruses with other tissue specificities

In contrast to viruses that are confined to replication in midgut cells, there has been one report of an NPV of a crane fly (*Tipula paludosa*) that specifically replicates in hemocytes (131) and the virus of the pink shrimp replicates in the hepatopancreas, an organ analogous to the vertebrate liver and insect fat body (132). However, these viruses were subsequently found to members of the Nudiviridae, indicating their distance from the Baculoviridae (see Chapter 1).

Covert baculovirus infections

Two types of covert baculovirus infections have been described (reviewed in (133). These include persistent infections that may result from semi permissive infection in which the virus can replicate, but causes differing degrees of pathology, but is normally not lethal. In contrast during latent infections, the virions do not replicate and only a few viral genes are expressed. A classic example of a latent infection is with human herpesvirus in which the latency associated transcript (LAT) is the major transcript expressed and is processed into several micro RNAs that block expression of anti-apoptotic and some early genes thereby preventing viral replication, but concurrently preserving the latently infected cell. A similar type of latency has been characterized in the nudivurus Hz-1 in which the persistent-associated gene 1 (*pag1)* is the only viral transcript detected (134) (reviewed in (133)). In addition, the exploitation of a covert infection by a host insect has been demonstrated in certain parasitoid wasps, in which genes derived from a nudivirus have been integrated into the host genome and evolved to be used for the production of virus-like particles that package host genes. These particles are injected along with wasp eggs into lepidopteran host larvae and the packaged genes when expressed can immunocompromise the host larvae leading to successful parasitization (135).

The possible presence of persistent baculoviruses has been suggested based on spontaneous outbreaks in controlled insect colonies, or by induction from exposing insects to physiological stressors such as cold temperature (136). Such occurrences have been reported for several different baculoviruses (reviewed in (137)). Other stressors include, overcrowding, high temperatures, uv light, humidity, ingestion of toxic chemicals, parasitism, and dietary conditions (reviewed in (133)). Although evidence of covert infections has been noted

for many years, it was not until the advent of molecular biological technology including the polymerase chain reaction that methods became available to investigate these observations. In a review of over 35 studies using PCR in several Alpha- and Betabaculovirus, it was noted that over half of the investigations detected the presence of baculovirus genes at a prevalence of over 50% of the insects sampled (133). For example, a virus, MbNPV, has been found to persist in most populations of the cabbage moth (*Mamestra brassicae*) in the United Kingdom (138). The latent virus can be activated when the persistently infected insects are fed either a closely related virus, *Panolis flammea* (pine beauty moth) NPV (PfNPV), or the more distantly related AcMNPV. PCR amplification identified MbNPV polyhedrin gene sequences in all insect stages including eggs, larvae, pupae, and adults. In the fourth instar larvae, the latent virus was found only in fat body tissue. A cell line was derived from this PCR-positive tissue that contained the latent virus (139) and it was estimated that the cell line contained 13-20 copies of the viral genome per cell (140). In addition, it was found that virus-free *M. brassicae* larvae died of an MbNPV-like infection when they were fed fat body cells from the latently infected laboratory strain of the insect. In another study, plasmids containing baculovirus late and very late promoters fused to the CAT gene were activated when transfected into latently infected cells. But when these plasmids were transfected into virus-free cells, such activation did not occur. This was interpreted to indicate that low levels of baculovirus genes were expressed in the latently infected cells, and the proteins produced were able to activate the expression of the late promoter constructs (141). Covert infections of laboratory cultures of *Spodoptera exigua* by *S. exigua* multiple nucleopolyhedrovirus (SeMNPV) and *Mamestra brassicae* NPV (MbNPV) have also been reported (142). Covert infections have also been reported for field collected and laboratory colonies of *Choristoneura fumiferana* (143) and were found to be induced in false codling moth, *Thaumatotibia leucotreta*, by overcrowding (144). In an investigation involving a GV of the Indian meal moth, *Plodia interpunctella*, it was found that transcripts of the PlinGV granulin were present in 60-80% of the offspring of insects that had survived exposure to a ~10% lethal dose of PlinGV. Inheritance of the virus was also observed in offspring of either exposed males or females mated with naïve insects (145).

It has been reported that persistent baculovirus infections can be caused by the infection of cells with AcMNPV that lacks the *p35* anti-apoptosis gene. These cell lines were resistant to subsequent challenge by AcMNPV infection, and some of the cells contained high levels of viral DNA and exhibited early gene expression (146). Under these conditions it would appear that there might be a balance between gene expression and the apoptotic pathway, and replication of viruses that lack *p35* selects for virus that do not induce apoptosis, thereby allowing them to persist. It has also been reported that serial undiluted passage of SeMNPV in Se301 cells can yield persistently infected cells that contain a partial SeMNPV genome. Although they grow slower than wt cells, they are somewhat resistant to infection by the homologous virus resulting in reduced yields of polyhedra and an about a 10-fold reduction in BV titers. The replication of the a heterologous virus, AcMNPV appeared to be unaffected (147).

References

1. Volkman LE, Summers MD. *Autographa californica* nuclear polyhedrosis virus: Comparitive infectivity of the occluded, alkali-liberated, and nonoccluded forms. J Invertebr Pathol. 1977;30:102–103. PubMed PMID: 336795.
2. Volkman LE, Summers MD, Hsieh CH. Occluded and nonoccluded nuclear polyhedrosis virus grown in *Trichoplusia ni*: comparative neutralization, comparative infectivity, and in vitro growth studies. J Virol. 1976;19:820–832. PubMed PMID: 787558.
3. Lehane MJ, Billingsley PF. 1996. Biology of the insect midgut. *In* Lehane MJ, Billingsley PF (ed), Biology of the insect midgut. Chapman and Hall, London.
4. Hegedus D, Erlandson M, Gillott C, Toprak U. New insights into peritrophic matrix synthesis, architecture, and function. Annu Rev Entomol. 2009;54:285–302. PubMed PMID: 19067633.
5. Dow JA. pH gradients in lepidopteran midgut. J Exp Biol. 1992;172:355–375. PubMed PMID: 9874748.

6. Wang P, Granados RR. An intestinal mucin is the target substrate for a baculovirus enhancin. Proc Natl Acad Sci U S A. 1997;94:6977–82. PubMed PMID: 9192677.
7. Toprak U, Harris S, Baldwin D, Theilmann D, Gillott C, Hegedus DD, Erlandson MA. Role of enhancin in Mamestra configurata nucleopolyhedrovirus virulence: selective degradation of host peritrophic matrix proteins. J Gen Virol. 2012;93:744–53. PubMed PMID: 22238230.
8. Washburn JO, Kirkpatrick BA, Volkman LE. Comparative pathogenesis of Autographa californica M nuclear polyhedrosis virus in larvae of Trichoplusia ni and Heliothis virescens. Virology. 1995;209:561–8. PubMed PMID: 7778288.
9. Vigdorovich V, Miller AD, Strong RK. Ability of hyaluronidase 2 to degrade extracellular hyaluronan is not required for its function as a receptor for jaagsiekte sheep retrovirus. J Virol. 2007;81:3124–9. PubMed PMID: 17229709.
10. Sugiura N, Setoyama Y, Chiba M, Kimata K, Watanabe H. Baculovirus envelope protein ODV-E66 is a novel chondroitinase with distinct substrate specificity. J Biol Chem. 2011;286:29026–34. PubMed PMID: 21715327.
11. Sugiura N, Ikeda M, Shioiri T, Yoshimura M, Kobayashi M, Watanabe H. Chondroitinase from baculovirus Bombyx mori nucleopolyhedrovirus and chondroitin sulfate from silkworm Bombyx mori. Glycobiology. 2013;23:1520–30. PubMed PMID: 24052236.
12. Mitsuhashi W, Miyamoto K. Disintegration of the peritrophic membrane of silkworm larvae due to spindles of an entomopoxvirus. J Invertebr Pathol. 2003;82:34–40. PubMed PMID: 12581717.
13. Chiu E, Hijnen M, Bunker RD, Boudes M, Rajendran C, Aizel K, Olieric V, Schulze-Briese C, Mitsuhashi W, Young V, Ward VK, Bergoin M, Metcalf P, Coulibaly F. Structural basis for the enhancement of virulence by viral spindles and their in vivo crystallization. Proc Natl Acad Sci U S A. 2015;112:3973–8. PubMed PMID: 25787255.
14. Liu X, Fang W, Fan R, Zhang L, Lei C, Zhang J, Nian W, Dou T, An S, Zhou L, Sun X. Granulovirus GP37 Facilitated ODVs Cross Insect Peritrophic Membranes and Fuse with Epithelia. Toxins. 2019.:11. PubMed PMID: 30836616.
15. Keddie BA, Aponte GW, Volkman LE. The pathway of infection of Autographa californica nuclear polyhedrosis virus in an insect host. Science. 1989;243:1728–1730. PubMed PMID: 2648574.
16. Flipsen JT, Martens JW, van Oers MM, Vlak JM, van Lent JW. Passage of Autographa californica nuclear polyhedrosis virus through the midgut epithelium of Spodoptera exigua larvae. Virology. 1995;208:328–35. PubMed PMID: 11831715.
17. Kikhno I, Guitiérrez S, Croizier L, Croizier G, Lopéz-Ferber ML. Characterization of pif, a gene required for the per os infectivity of Spodoptera littoralis nucleopolyhedrovirus. J Gen Virol. 2002;83:3013–3022. PubMed PMID: 12466478.
18. Horton HM, Burand JP. Saturable attachment sites for polyhedron-derived baculovirus on insect cells and evidence for entry via direct membrane fusion. J Virol. 1993;67:1860–1868. PubMed PMID: 8445715.
19. Dall D, Luque T, O'Reilly DR. Insect-virus relationships: sifting by informatics. Bioessays. 2001;23:184–193. PubMed PMID: 11169592.
20. Lapointe R, Popham HJ, Straschil U, Goulding D, O'Reilly DR, Olszewski JA. Characterization of two Autographa californica nucleopolyhedrovirus proteins, Ac145 and Ac150, which affect oral infectivity in a host-dependent manner. J Virol. 2004;78:6439–48. PubMed PMID: 15163737.
21. Zhu S, Wang W, Wang Y, Yuan M, Yang K. The baculovirus core gene ac83 is required for nucleocapsid assembly and per os infectivity of Autographa californica nucleopolyhedrovirus. J Virol. 2013;87:10573–86. PubMed PMID: 23864639.
22. Javed MA, Biswas S, Willis LG, Harris S, Pritchard C, van Oers MM, Donly BC, Erlandson MA, Hegedus DD, Theilmann DA. Autographa californica Multiple Nucleopolyhedrovirus AC83 is a Per Os Infectivity Factor (PIF) Protein Required for Occlusion-Derived Virus (ODV) and Budded Virus Nucleocapsid Assembly as well as Assembly of the PIF Complex in ODV Envelopes. J Virol. 2017;91:02115–16. PubMed PMID: 28031365.

23. Zimoch L, Hogenkamp DG, Kramer KJ, Muthukrishnan S, Merzendorfer H. Regulation of chitin synthesis in the larval midgut of Manduca sexta. Insect Biochem Mol Biol. 2005;35:515–27. PubMed PMID: 15857758.
24. Broehan G, Zimoch L, Wessels A, Ertas B, Merzendorfer H. A chymotrypsin-like serine protease interacts with the chitin synthase from the midgut of the tobacco hornworm. J Exp Biol. 2007;210:3636–43. PubMed PMID: 17921165.
25. Li Z, Li C, Yang K, Wang L, Yin C, Gong Y, Pang Y. Characterization of a chitin-binding protein GP37 of Spodoptera litura multicapsid nucleopolyhedrovirus. Virus Res. 2003;96:113–22. PubMed PMID: 12951271.
26. Dinglasan RR, Alaganan A, Ghosh AK, Saito A, van Kuppevelt TH, Jacobs-Lorena M. Plasmodium falciparum ookinetes require mosquito midgut chondroitin sulfate proteoglycans for cell invasion. Proc Natl Acad Sci U S A. 2007;104:15882–7. PubMed PMID: 17873063.
27. Shrestha A, Bao K, Chen YR, Chen W, Wang P, Fei Z, Blissard GW. Global Analysis of Baculovirus Autographa californica Multiple Nucleopolyhedrovirus Gene Expression in the Midgut of the Lepidopteran Host Trichoplusia ni. J Virol. 2018.:92. PubMed PMID: 30209166.
28. Shrestha A, Bao K, Chen W, Wang P, Fei Z, Blissard GW. Transcriptional responses of the Trichoplusia ni midgut to oral infection by the baculovirus Autographa californica Multiple Nucleopolyhedrovirus. J Virol. 2019. doi: 10.1128/JVI.00353-19. PubMed PMID: 31043536.
29. Engelhard EK, Kam-Morgan LN, Washburn JO, Volkman LE. The insect tracheal system: a conduit for the systemic spread of Autographa californica M nuclear polyhedrosis virus. Proc Natl Acad Sci U S A. 1994;91:3224–3227. PubMed PMID: 8159729.
30. Maina JN. Scanning and transmission electron microscopic study of the tracheal air sac system in a grasshopper Chrotogonus senegalensis (Kraus)--Orthoptera: Acrididae: Pyrgomorphinae. Anat Rec. 1989;223:393–405. PubMed PMID: 2712353.
31. Volkman LE. Baculovirus infectivity and the actin cytoskeleton. Curr Drug Targets. 2007;8:1075–83. PubMed PMID: 17979667.
32. Kirkpatrick BA, Washburn JO, Engelhard EK, Volkman LE. Primary infection of insect tracheae by Autographa californica M nuclear polyhedrosis virus. Virology. 1994;203:184–6. PubMed PMID: 8030276.
33. Katsuma S, Shimada T, Kobayashi M. Characterization of the baculovirus Bombyx mori nucleopolyhedrovirus gene homologous to the mammalian FGF gene family. Virus Genes. 2004;29:211–7. PubMed PMID: 15284481.
34. Katsuma S, Daimon T, Horie S, Kobayashi M, Shimada T. N-linked glycans of Bombyx mori nucleopolyhedrovirus fibroblast growth factor are crucial for its secretion. Biochem Biophys Res Commun. 2006;350:1069–75. PubMed PMID: 17046716.
35. Katsuma S, Daimon T, Mita K, Shimada T. Lepidopteran ortholog of Drosophila breathless is a receptor for the baculovirus fibroblast growth factor. J Virol. 2006;80:5474–81. PubMed PMID: 16699027.
36. Detvisitsakun C, Berretta MF, Lehiy C, Passarelli AL. Stimulation of cell motility by a viral fibroblast growth factor homolog: proposal for a role in viral pathogenesis. Virology. 2005;336:308–17. PubMed PMID: 15892971.
37. Detvisitsakun C, Hutfless EL, Berretta MF, Passarelli AL. Analysis of a baculovirus lacking a functional viral fibroblast growth factor homolog. Virology. 2006;346:258–65. PubMed PMID: 16476460.
38. Detvisitsakun C, Cain EL, Passarelli AL. The Autographa californica M nucleopolyhedrovirus fibroblast growth factor accelerates host mortality. Virology. 2007;365:70–8. PubMed PMID: 17459443.
39. Katsuma S, Horie S, Daimon T, Iwanaga M, Shimada T. In vivo and in vitro analyses of a Bombyx mori nucleopolyhedrovirus mutant lacking functional vfgf. Virology. 2006;355:62–70. PubMed PMID: 16904150.
40. Means JC, Passarelli AL. Viral fibroblast growth factor, matrix metalloproteases, and caspases are associated with enhancing systemic infection by baculoviruses. Proc Natl Acad Sci U S A. 2010;107:9825–30. PubMed PMID: 20457917.

41. Granados RR, Lawler KA. l98l. In vivo pathway of *Autographa californica* baculovirus invasion and infection. Virology l08:297-308.
42. Washburn JO, Lyons EH, Haas-Stapleton EJ, Volkman LE. Multiple nucleocapsid packaging of Autographa californica nucleopolyhedrovirus accelerates the onset of systemic infection in Trichoplusia ni. J Virol. 1999;73:411–6. PubMed PMID: 9847346.
43. Washburn JO, Chan EY, Volkman LE, Aumiller JJ, Jarvis DL. Early synthesis of budded virus envelope fusion protein GP64 enhances Autographa californica multicapsid nucleopolyhedrovirus virulence in orally infected Heliothis virescens. J Virol. 2003;77:280–90. PubMed PMID: 12477833.
44. Zhang JH, Washburn JO, Jarvis DL, Volkman LE. Autographa californica M nucleopolyhedrovirus early GP64 synthesis mitigates developmental resistance in orally infected noctuid hosts. J Gen Virol. 2004;85:833–42. PubMed PMID: 15039526.
45. Ohkawa T, Volkman LE, Welch MD. Actin-based motility drives baculovirus transit to the nucleus and cell surface. J Cell Biol. 2010;190:187–95. PubMed PMID: 20660627.
46. Hukuhara T. Genetic variation of polyhedrosis viruses of insects, p 7-11. *In* (ed),
47. Kataoka C, Kaname Y, Taguwa S, Abe T, Fukuhara T, Tani H, Moriishi K, Matsuura Y. Baculovirus GP64-mediated entry into mammalian cells. J Virol. 2012;86:2610–20. PubMed PMID: 22190715.
48. Kadlec J, Loureiro S, Abrescia NG, Stuart DI, Jones IM. The postfusion structure of baculovirus gp64 supports a unified view of viral fusion machines. Nat Struct Mol Biol. 2008;15:1024–30. PubMed PMID: 18776902.
49. Tani H, Nishijima M, Ushijima H, Miyamura T, Matsuura Y. Characterization of cell-surface determinants important for baculovirus infection. Virology. 2001;279:343–53. PubMed PMID: 11145915.
50. Long G, Pan X, Kormelink R, Vlak JM. Functional entry of baculovirus into insect and mammalian cells is dependent on clathrin-mediated endocytosis. J Virol. 2006;80:8830–3. PubMed PMID: 16912330.
51. Dong S, Wang M, Qiu Z, Deng F, Vlak JM, Hu Z, Wang H. Autographa californica multicapsid nucleopolyhedrovirus efficiently infects Sf9 cells and transduces mammalian cells via direct fusion with the plasma membrane at low pH. J Virol. 2010;84:5351–9. PubMed PMID: 20219938.
52. Goley ED, Ohkawa T, Mancuso J, Woodruff JB, D'Alessio JA, Cande WZ, Volkman LE, Welch MD. Dynamic nuclear actin assembly by Arp2/3 complex and a baculovirus WASP-like protein. Science. 2006;314:464–7. PubMed PMID: 17053146.
53. Au S, Wu W, Zhou L, Theilmann DA, Pante N. A new mechanism for nuclear import by actin-based propulsion used by a baculovirus nucleocapsid. J Cell Sci. 2016;129:2905–11. PubMed PMID: 27284005.
54. Mueller J, Pfanzelter J, Winkler C, Narita A, Le Clainche C, Nemethova M, Carlier MF, Maeda Y, Welch MD, Ohkawa T, Schmeiser C, Resch GP, Small JV. Electron tomography and simulation of baculovirus actin comet tails support a tethered filament model of pathogen propulsion. PLoS Biol. 2014;12:e1001765. PubMed PMID: 24453943.
55. Cudmore S, Cossart P, Griffiths G, Way M. Actin-based motility of vaccinia virus. Nature. 1995;378:636–8. PubMed PMID: 8524400.
56. Theriot JA, Mitchison TJ, Tilney LG, Portnoy DA. The rate of actin-based motility of intracellular Listeria monocytogenes equals the rate of actin polymerization. Nature. 1992;357:257–60. PubMed PMID: 1589024.
57. Qin F, Xu C, Hu J, Lei C, Zheng Z, Peng K, Wang H, Sun X. Dissecting the cell entry pathway of baculovirus by single particle tracking and quantitative electron microscopic analysis. J Virol. 2019. doi: 10.1128/JVI.00033-19. PubMed PMID: 30760565.
58. van Loo ND, Fortunati E, Ehlert E, Rabelink M, Grosveld F, Scholte BJ. Baculovirus infection of nondividing mammalian cells: mechanisms of entry and nuclear transport of capsids. J Virol. 2001;75:961–70. PubMed PMID: 11134309.
59. Au S, Wu W, Pante N. Baculovirus Nuclear Import: Open, Nuclear Pore Complex (NPC) Sesame. Viruses. 2013;5:1885–900. PubMed PMID: 23881277.

60. Alber F, Dokudovskaya S, Veenhoff LM, Zhang W, Kipper J, Devos D, Suprapto A, Karni-Schmidt O, Williams R, Chait BT, Rout MP, Sali A. Determining the architectures of macromolecular assemblies. Nature. 2007;450:683–94. PubMed PMID: 18046405.
61. Jehle JA, Blissard GW, Bonning BC, Cory JS, Herniou EA, Rohrmann GF, Theilmann DA, Thiem SM, Vlak JM. On the classification and nomenclature of baculoviruses: a proposal for revision. Arch Virol. 2006;151:1257–66. PubMed PMID: 16648963.
62. Au S, Pante N. Nuclear transport of baculovirus: revealing the nuclear pore complex passage. J Struct Biol. 2012;177:90–8. PubMed PMID: 22100338.
63. Wang Y, Wang Q, Liang C, Song J, Li N, Shi H, Chen X. Autographa californica multiple nucleopolyhedrovirus nucleocapsid protein BV/ODV-C42 mediates the nuclear entry of P78/83. J Virol. 2008;82:4554–61. PubMed PMID: 18287235.
64. Li K, Wang Y, Bai H, Wang Q, Song J, Zhou Y, Wu C, Chen X. The putative pocket protein binding site of Autographa californica nucleopolyhedrovirus BV/ODV-C42 is required for virus-induced nuclear actin polymerization. J Virol. 2010;84:7857–68. PubMed PMID: 20484515.
65. Gotoh T, Fukuhara M, Kikuchi K. Mathematical model for change in diameter distribution of baculovirus-infected Sf-9 insect cells. Biochem Engin J. 2008;40:379–386.
66. Cheley S, Kosik KS, Paskevich P, Bakalis S, Bayley H. Phosphorylated baculovirus p10 is a heat-stable microtubule-associated protein associated with process formation in Sf9 cells. J Cell Sci. 1992;102:739–752. PubMed PMID: 1331130.
67. Patmanidi AL, Possee RD, King LA. Formation of P10 tubular structures during AcMNPV infection depends on the integrity of host-cell microtubules. Virology. 2003;317:308–20. PubMed PMID: 14698669.
68. Young JC, MacKinnon EA, Faulkner P. The architecture of the virogenic stroma in isolated nuclei of *Spodoptera frugiperda* cells in vitro infected by *Autographa californica* nuclear polyhedrosis virus. J Struct Biol. 1993;110:141–153.
69. Fraser MJ. Ultrastructural observations of virion maturation in *Autographa californica* nuclear polyhedrosis virus infected *Spodoptera frugiperda* cell cultures. J Ultrastruct Mol Struct Res. 1986;95:189–195.
70. Vanarsdall AL, Mikhailov VS, Rohrmann GF. Characterization of a baculovirus lacking the DBP (DNA-binding protein) gene. Virology. 2007;364:475–85. PubMed PMID: 17449080.
71. Guarino LA, Dong W, Xu B, Broussard DR, Davis RW, Jarvis DL. The baculovirus phosphoprotein pp31 is associated with the virogenic stroma. J Virol. 1992;66:7113–7120. PubMed PMID: 1433508.
72. Yamagishi J, Burnett ED, Harwood SH, Blissard GW. The AcMNPV pp31 gene is not essential for productive AcMNPV replication or late gene transcription but appears to increase levels of most viral transcripts. Virology. 2007;365:34–47. PubMed PMID: 17467768.
73. Williams GV, Faulkner P. 1997. Cytological changes and viral morphogenesis during baculovirus infection. *In* Miller LK (ed), The Baculoviruses. Plenum, New York.
74. Ohkawa T, Welch MD. 2018. Baculovirus Actin-Based Motility Drives Nuclear Envelope Disruption and Nuclear Egress. Curr Biol 28:2153-2159 e4.
75. Wei W, Wang H, Li X, Fang N, Yang S, Liu H, Kang X, Sun X, Ji S. Cloning and Characterization of Sf9 Cell Lamin and the Lamin Conformational Changes during Autographa californica multiple nucleopolyhedrovirus Infection. Viruses. 2016.:8. PubMed PMID: 27164127.
76. Gross CH, Wolgamot GM, Russell RLQ, Pearson MN, Rohrmann GF. A baculovirus encoded 16 kDa glycoprotein localizes near the nuclear membrane of infected cells. Virology. 1993;192:386–390. PubMed PMID: 8517029.
77. Biswas S, Blissard GW, Theilmann DA. Trichoplusia ni Kinesin-1 Associates with Autographa californica Multiple Nucleopolyhedrovirus Nucleocapsid Proteins and Is Required for Production of Budded Virus. J Virol. 2016;90:3480–95. PubMed PMID: 26763996.
78. Blissard GW, Rohrmann GF. Location, sequence, transcriptional mapping, and temporal expression of the gp64 envelope glycoprotein gene of the *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus. Virology. 1989;170:537–555. PubMed PMID: 2658304.

79. Pearson MN, Russell R, Rohrmann GF. Characterization of a baculovirus encoded protein that is associated with infected-cell membranes and budded virions. Virology. 2001;291:22–31. PubMed PMID: 11878873.
80. Pearson MN, Groten C, Rohrmann GF. Identification of the *Lymantria dispar* nucleopolyhedrovirus envelope fusion protein provides evidence for a phylogenetic division of the Baculoviridae. J Virol. 2000;74:6126–6131. PubMed PMID: 10846096.
81. Oomens AG, Blissard GW. Requirement for GP64 to drive efficient budding of *Autographa californica* multicapsid nucleopolyhedrovirus. Virology. 1999;254:297–314. PubMed PMID: 9986796.
82. Monsma SA, Oomens AGP, Blissard GW. The gp64 envelope fusion protein is an essential baculovirus protein required for cell-to-cell transmission of infection. J Virol. 1996;70:4607–4616. PubMed PMID: 8676487.
83. Hernandez JM, Podbilewicz B. The hallmarks of cell-cell fusion. Development. 2017;144:4481–4495. PubMed PMID: 29254991.
84. Guo Y, Yue Q, Gao J, Wang Z, Chen YR, Blissard GW, Liu TX, Li Z. Roles of Cellular NSF Protein in Entry and Nuclear Egress of Budded Virions of Autographa californica Multiple Nucleopolyhedrovirus. J Virol. 2017.:91. PubMed PMID: 28747507.
85. Blissard GW, Theilmann DA. Baculovirus Entry and Egress from Insect Cells. Annu Rev Virol. 2018;5:113–139. PubMed PMID: 30004832.
86. Ahmed I, Akram Z, Iqbal HMN, Munn AL. The regulation of Endosomal Sorting Complex Required for Transport and accessory proteins in multivesicular body sorting and enveloped viral budding - An overview. Int J Biol Macromol. 2019;127:1–11. PubMed PMID: 30615963.
87. Li Z, Blissard GW. Cellular VPS4 is required for efficient entry and egress of budded virions of Autographa californica multiple nucleopolyhedrovirus. J Virol. 2012;86:459–72. PubMed PMID: 22072775.
88. Yue Q, Yu Q, Yang Q, Xu Y, Guo Y, Blissard GW, Li Z. Distinct Roles of Cellular ESCRT-I and ESCRT-III Proteins in Efficient Entry and Egress of Budded Virions of Autographa californica Multiple Nucleopolyhedrovirus. J Virol. 2018.:92. PubMed PMID: 29046462.
89. Wang Y, Schwedes JF, Parks D, Mann K, Tegtmeyer P. Interaction of p53 with its consensus DNA-binding site. Mol Cell Biol. 1995;15:2157–65. PubMed PMID: 7891710.
90. O'Reilly DR, Miller LK. A baculovirus blocks insect molting by producing ecdysteroid UDP-glucosyl transferase. Science. 1989.:1110–1112. PubMed PMID: 2505387.
91. O'Reilly DR, Miller LK. Regulation of expression of a baculovirus ecdysteroid UDPglucosyltransferase gene. J Virol. 1990;64:1321–1328. PubMed PMID: 2106039.
92. O'Reilly DR, Miller LK. Improvement of a baculovirus pesticide by deletion of the egt gene. Bio/Technology. 1991;9:1086–1089.
93. O'Reilly DR, Hails RS, Kelly TJ. The impact of host developmental status on baculovirus replication. J Invertebr Pathol. 1998;72:269–75. PubMed PMID: 9784350.
94. Rosinski M, Reid S, Nielsen LK. Kinetics of baculovirus replication and release using real-time quantitative polymerase chain reaction. Biotechnol Bioeng. 2002;77:476–80. PubMed PMID: 11787021.
95. Mita K, Kasahara M, Sasaki S, Nagayasu Y, Yamada T, Kanamori H, Namiki N, Kitagawa M, Yamashita H, Yasukochi Y, Kadono-Okuda K, Yamamoto K, Ajimura M, Ravikumar G, Shimomura M, Nagamura Y, Shin-I T, Abe H, Shimada T, Morishita S, Sasaki T. The genome sequence of silkworm, Bombyx mori. DNA Res. 2004;11:27–35. PubMed PMID: 15141943.
96. Nguyen Q, Chan LC, Nielsen LK, Reid S. Genome scale analysis of differential mRNA expression of Helicoverpa zea insect cells infected with a H. armigera baculovirus. Virology. 2013;444:158–70. PubMed PMID: 23827436.
97. Kawasaki Y, Matsumoto S, Nagamine T. Analysis of baculovirus IE1 in living cells: dynamics and spatial relationships to viral structural proteins. J Gen Virol. 2004;85:3575–3583. PubMed PMID: 15557230.
98. Luo XC, Wang SS, Zhang J, Qian DD, Wang SM, Li LL. Effects of Early or Overexpression of the Multiple Nucleopolyhedrovirus (ODV-E25) on Virus Replication. PLoS One. 2013;8:e65635. PubMed PMID: 23825525.

99. Chen L, Yang R, Hu X, Xiang X, Yu S, Wu X. The formation of occlusion-derived virus is affected by the expression level of ODV-E25. Virus Res. 2013;173:404–14. PubMed PMID: 23298549.
100. Zhu M, Wang J, Deng R, Xiong P, Liang H, Wang X. A MicroRNA Encoded by Autographa californica Nucleopolyhedrovirus Regulates Expression of Viral Gene ODV-E25. J Virol. 2013;87:13029–34. PubMed PMID: 24027316.
101. Zhu M, Wang J, Deng R, Wang X. Functional Regulation of an Autographa californica Nucleopolyhedrovirus-Encoded MicroRNA, AcMNPV-miR-1, in Baculovirus Replication. J Virol. 2016;90:6526–6537. PubMed PMID: 27147751.
102. Guo YJ, Fu SH, Li LL. Autographa californica multiple nucleopolyhedrovirus ac75 is required for egress of nucleocapsids from the nucleus and formation of de novo intranuclear membrane microvesicles. PLoS One. 2017;12:e0185630. PubMed PMID: 28968422.
103. Biswas S, Willis LG, Fang M, Nie Y, Theilmann DA. Autographa californica Nucleopolyhedrovirus AC141 (Exon0), a Potential E3 Ubiquitin Ligase, Interacts with Viral Ubiquitin and AC66 To Facilitate Nucleocapsid Egress. J Virol. 2018.:92. PubMed PMID: 29142135.
104. Shi Y, Li K, Tang P, Li Y, Zhou Q, Yang K, Zhang Q. Three-dimensional visualization of the Autographa californica multiple nucleopolyhedrovirus occlusion-derived virion envelopment process gives new clues as to its mechanism. Virology. 2015;476:298–303. PubMed PMID: 25569457.
105. Braunagel SC, Summers MD. Molecular Biology of the Baculovirus Occlusion-Derived Virus Envelope. Curr Drug Targets. 2007;8:1084–1095. PubMed PMID: 17979668.
106. Xu H, Yang Z, Zhao J, Tian C, Ge J, Tang X, Bao Y, Zhang C. Bombyx mori nucleopolyhedrovirus ORF56 encodes an occlusion-derived virus protein and is not essential for budded virus production. J Gen Virol. 2008;89:1212–1219. PubMed PMID: 18420799.
107. Carpentier DC, Griffiths CM, King LA. The baculovirus P10 protein of Autographa californica nucleopolyhedrovirus forms two distinct cytoskeletal-like structures and associates with polyhedral occlusion bodies during infection. Virology. 2008;371:278–91. PubMed PMID: 17991504.
108. Chamberlin ME, King ME. Changes in Midgut Active Ion Transport and Metabolism During the Fifth Instar of the Tobacco Hornworm (Manduca sexta). J Exp Zool. 1998;280:135–141.
109. Evans HF. 1986. Ecology and epizootiology of baculoviruses, p 89-132. *In* Granados RR, Federici BA (ed), The biology of baculoviruses, vol II. CRC Press, Inc., Boca Raton.
110. Jehle JA, Lange M, Wang H, Hu Z, Wang Y, Hauschild R. Molecular identification and phylogenetic analysis of baculoviruses from Lepidoptera. Virology. 2006;346:180–193. PubMed PMID: 16313938.
111. Goulson D. Wipfelkrankheit : modification of host behaviour during baculoviral infection. Oecologia. 1997;109:219–228. PubMed PMID: 28307172.
112. Han Y, van Houte S, van Oers MM, Ros VID. Timely trigger of caterpillar zombie behaviour: temporal requirements for light in baculovirus-induced tree-top disease. Parasitology. 2018;145:822–827. PubMed PMID: 29144213.
113. Bhattarai UR, Li F, Katuwal Bhattarai M, Masoudi A, Wang D. Phototransduction and circadian entrainment are the key pathways in the signaling mechanism for the baculovirus induced tree-top disease in the lepidopteran larvae. Sci Rep. 2018;8:17528. PubMed PMID: 30510155.
114. Fukamizo T, Sato H, Mizuhara M, Ohnuma T, Gotoh T, Hiwatashi K, Takahashi S. Chitinase from Autographa californica Multiple Nucleopolyhedrovirus: Rapid Purification from Sf-9 Medium and Mode of Action. Biosci Biotechnol Biochem. 2011;75:1763–9. PubMed PMID: 21897030.
115. Daimon T, Katsuma S, Kang W, Shimada T. Comparative studies of Bombyx mori nucleopolyhedrovirus chitinase and its host ortholog, BmChi-h. Biochem Biophys Res Commun. 2006;345:825–33. PubMed PMID: 16701557.
116. Thomas CJ, Brown HL, Hawes CR, Lee BY, Min MK, King LA, Possee RD. Localization of a baculovirus-induced chitinase in the insect cell endoplasmic reticulum. J Virol. 1998;72:10207–12. PubMed PMID: 9811762.

117. Saville GP, Patmanidi AL, Possee RD, King LA. Deletion of the Autographa californica nucleopolyhedrovirus chitinase KDEL motif and in vitro and in vivo analysis of the modified virus. J Gen Virol. 2004;85:821–31. PubMed PMID: 15039525.
118. Hawtin RE, Zarkowska T, Arnold K, Thomas CJ, Gooday GW, King LA, Kuzio JA, Possee RD. Liquefaction of Autographa californica nucleopolyhedrovirus-infected insects is dependent on the integrity of virus-encoded chitinase and cathepsin genes. Virology. 1997;238:243–253. PubMed PMID: 9400597.
119. Oliveira JV, Wolff JL, Garcia-Maruniak A, Ribeiro BM, de Castro ME, de Souza ML, Moscardi F, Maruniak JE, Zanotto PM. Genome of the most widely used viral biopesticide: Anticarsia gemmatalis multiple nucleopolyhedrovirus. J Gen Virol. 2006;87:3233–50. PubMed PMID: 17030857.
120. Hom LG, Ohkawa T, Trudeau D, Volkman LE. Autographa californica M nucleopolyhedrovirus ProV-CATH is activated during infected cell death. Virology. 2002;296:212–8. PubMed PMID: 12069520.
121. Ishimwe E, Hodgson JJ, Clem RJ, Passarelli AL. Reaching the melting point: Degradative enzymes and protease inhibitors involved in baculovirus infection and dissemination. Virology. 2015;479-480:637–49. PubMed PMID: 25724418.
122. Preston R. 1997. The Cobra Event. Ballantine Books, New York.
123. Winstanley D, Crook NE. Replication of Cydia pomonella granulosis virus in cell cultures. J Gen Virol. 1993;74(Pt 8):1599–609. PubMed PMID: 8345351.
124. Tanada Y, Hess RT. 1991. Baculoviridae. Granulosis Viruses. *In* Adams JR, Bonami JR (ed), Atlas of invertebrate viruses. CRC Press, Inc., Boca Raton.
125. Federici BA. 1997. Baculovirus pathogenesis. *In* Miller LK (ed), The Baculoviruses. Plenum, New York.
126. Moser B, Becnel J, White S, Afonso C, Kutish G, Shanker S, Almira E. Morphological and molecular evidence that Culex nigripalpus baculovirus is an unusual member of the family Baculoviridae. J Gen Virol. 2001;82:283–297. PubMed PMID: 11161265.
127. Federici BA, Stern VM. Replication and occlusion of a granulosis virus in larval and adult midgut epithelium of the western grapeleaf skeletonizer, Harrisina brillians. J Invertebr Pathol. 2000;56:401–414.
128. Duffy SP, Young AM, Morin B, Lucarotti CJ, Koop BF, Levin DB. Sequence analysis and organization of the Neodiprion abietis nucleopolyhedrovirus genome. J Virol. 2006;80:6952–63. PubMed PMID: 16809301.
129. Ko R, Okano K, Maeda S. Structural and functional analysis of the Xestia c-nigrum granulovirus matrix metalloproteinase. J Virol. 2000;74:11240–6. PubMed PMID: 11070022.
130. Garcia-Maruniak A, Maruniak JE, Zanotto PM, Doumbouya AE, Liu JC, Merritt TM, Lanoie JS. Sequence analysis of the genome of the Neodiprion sertifer nucleopolyhedrovirus. J Virol. 2004;78:7036–51. PubMed PMID: 15194780.
131. Smith KA. Intranuclear changes in the polyhedrosis of Tipula paludosa. Nature. 1955;176:255. [Diptera]. PubMed PMID: 13244670.
132. Couch JA. Free and occluded virus, similar to Baculovirus, in hepatopancreas of pink shrimp. Nature. 1974;247:229–231.
133. Williams T, Virto C, Murillo R, Caballero P. Covert Infection of Insects by Baculoviruses. Front Microbiol. 2017;8:1337. PubMed PMID: 28769903.
134. Wu YL, Wu CP, Liu CY, Hsu PW, Wu EC, Chao YC. A non-coding RNA of insect HzNV-1 virus establishes latent viral infection through microRNA. Sci Rep. 2011;1:60. PubMed PMID: 22355579.
135. Drezen JM, Leobold M, Bezier A, Huguet E, Volkoff AN, Herniou EA. Endogenous viruses of parasitic wasps: variations on a common theme. Curr Opin Virol. 2017;25:41–48. PubMed PMID: 28728099.
136. Biever KD, Wilkinson JD. A stress-induced gruanosis virus of pieris rapae. Environ Entomol. 1978;7:572–573.
137. Kukan B. Vertical transmission of nucleopolyhedrovirus in insects. J Invertebr Pathol. 1999;74:103–11. PubMed PMID: 10486221.
138. Burden JP, Possee RD, Sait SM, King LA, Hails RS. Phenotypic and genotypic characterisation of persistent baculovirus infections in populations of the cabbage moth (Mamestra brassicae) within the British Isles. Arch Virol. 2006;151:635–49. PubMed PMID: 16328143.

139. Hughes DS, Possee RD, King LA. Activation and detection of a latent baculovirus resembling Mamestra brassicae nuclear polyhedrosis virus in M. brassicae insects. Virology. 1993;194:608–15. PubMed PMID: 8503177.
140. Hughes DS, Possee RD, King LA. Quantification of latent Mamestra brassicae nuclear polyhedrosis virus in M. brassicae insects using a PCR-scintillation proximity assay. J Virol Methods. 1994;50:21–7. PubMed PMID: 7714043.
141. Hughes DS, Possee RD, King LA. Evidence for the presence of a low-level, persistent baculovirus infection of Mamestra brassicae insects. J Gen Virol. 1997;78(Pt 7):1801–5. PubMed PMID: 9225059.
142. Murillo R, Hussey MS, Possee RD. Evidence for covert baculovirus infections in a Spodoptera exigua laboratory culture. J Gen Virol. 2011;92:1061–70. PubMed PMID: 21228125.
143. Kemp EM, Woodward DT, Cory JS. Detection of single and mixed covert baculovirus infections in eastern spruce budworm, Choristoneura fumiferana populations. J Invertebr Pathol. 2011;107:202–5. PubMed PMID: 21616077.
144. Opoku-Debrah JK, Hill MP, Knox C, Moore SD. Overcrowding of false codling moth, Thaumatotibia leucotreta (Meyrick) leads to the isolation of five new Cryptophlebia leucotreta granulovirus (CrleGV-SA) isolates. J Invertebr Pathol. 2013;112:219–28. PubMed PMID: 23277142.
145. Burden JP, Griffiths CM, Cory JS, Smith P, Sait SM. Vertical transmission of sublethal granulovirus infection in the Indian meal moth, Plodia interpunctella. Mol Ecol. 2002;11:547–55. PubMed PMID: 11918789.
146. Lee JC, Chen HH, Chao YC. Persistent baculovirus infection results from deletion of the apoptotic suppressor gene p35. J Virol. 1998;72:9157–65. PubMed PMID: 9765462.
147. Weng Q, Yang K, Xiao W, Yuan M, Zhang W, Pang Y. Establishment of an insect cell clone that harbours a partial baculoviral genome and is resistant to homologous virus infection. J Gen Virol. 2009;90:2871–6. PubMed PMID: 19675188.
148. Danquah JO, Botchway S, Jeshtadi A, King LA. Direct interaction of baculovirus capsid proteins VP39 and EXON0 with kinesin-1 in insect cells determined by fluorescence resonance energy transfer-fluorescence lifetime imaging microscopy. J Virol. 2012;86:844–53. PubMed PMID: 22072745.

License