Baculovirus Entry and Egress from Insect Cells

Gary W. Blissard[1*] and David A. Theilmann[2*]

[1]Boyce Thompson Institute at Cornell University, Ithaca, NY 14853
[2]Summerland Research and Development Center, Agriculture and Agri-Food Canada, Summerland, BC, Canada

*Correspondence:
Gary W. Blissard
Boyce Thompson Institute at Cornell University
533 Tower Road, Ithaca, NY, 14853, USA

David A. Theilmann
Summerland Research and Development Center
Agriculture and Agri-Food Canada
Summerland, BC, Canada

Keywords:

Baculovirus, Budded Virus, Occlusion Derived Virus, Virion Entry, Virion Egress, Virion Phenotype, *Autographa californica multiple nucleopolyhedrovirus,* AcMNPV, BmNPV, OpMNPV, LdMNPV, HearNPV, SeMNPV

Abstract:

Baculoviruses are large DNA viruses of invertebrates that are highly pathogenic in many hosts. In the infection cycle, baculoviruses produce two types of virions. These virion phenotypes are physically and functionally distinct and each serves a critical role in the biology of the virus. One phenotype, the occlusion derived virus (ODV) is occluded within a crystallized protein that facilitates oral infection of the host. A large complex of at least 9 ODV envelope proteins called *Per os* Infectivity Factors (PIF) are critically important for ODV infection of insect midgut epithelial cells. Viral egress from midgut cells is by budding to produce a second virus phenotype, the budded virus (BV). BV binds, enters, and replicates in most other tissues of the host insect. Cell recognition and entry by BV is mediated by a single major envelope glycoprotein: GP64 in some baculoviruses, and F in others. Entry and egress by the two virion phenotypes occur by dramatically different mechanisms and reflect a life cycle in which ODV are specifically adapted for oral infection while BV mediate dissemination of the infection within the animal.

INTRODUCTION

Baculoviruses are pathogenic viruses that infect invertebrates and are widely distributed in the environment. The name baculovirus is derived from the latin “baculum” which refers to the rod-shaped nucleocapsids (app. 50 x 300 nm) characteristic of these viruses. As a group, baculoviruses have been described from host insect species that are mostly within the insect Order Lepidoptera (moths and butterflies), but some baculovirus species also infect insects in the Orders Diptera (mosquitoes) and Hymenoptera (sawflies). Baculoviruses are also well known in biotechnology, as the baculovirus expression vector system has been used extensively for applications that range from routine protein expression in research laboratories, to vaccine production and gene therapy (1-5).

The Family *Baculoviridae*: Baculoviruses have large circular dsDNA genomes ranging from approximately 80-180 kbp. Genome sequences of approximately 70 baculoviruses are available and have guided our current understanding of baculovirus phylogeny (6-9). The family Baculoviridae is subdivided into four genera: *Alphabaculoviruses*, *Betabaculoviruses*, *Deltabaculoviruses*, and *Gammabaculoviruses*. These subdivisions reflect differences in a) phylogeny as determined by relatedness of 38 core genes, b) permissive host species, and c) the manner in which virions are occluded or embedded within their characteristic occlusion bodies (6, 10, 11). It should also be noted that certain other large dsDNA viruses of invertebrates (*Nudiviridae, Hytrosaviruses, Nimaviridae,* and the bracovirus genus of *Polydnaviridae*) share a subset of the 38 baculovirus core genes, suggesting either a common ancestor with the baculoviruses, or an exchange of certain functional groups of genes (6, 7, 12, 13). Among the baculoviruses, several viruses in the *Alphabaculovirus* genus have been studied most intensively, and these include: *Autographa californica multiple nucleopolyhedrovirus* (*AcMNPV*), *Bombyx mori nucleopolyhedrovirus* (*BmNPV*), *Orgyia pseudotsugata multiple nucleopolyhedrovirus* (*OpMNPV*), *Lymantria dispar multiple nucleopolyhedrovirus* (*LdMNPV*), *Spodoptera exigua multiple nucleopolyhedrovirus* (*SeMNPV*), and *Helicoverpa armigera nucleopolyhedrovirus* (*HearNPV*). However, because a large majority of the detailed studies on baculovirus biology have focused on

the model baculovirus *AcMNPV*, this review will focus primarily on our understanding of *AcMNPV*, but with exceptions and examples of diversity noted where appropriate.

Two Virion Phenotypes: A striking feature of the typical baculovirus infection cycle is the production of two physically different types of virions, often referred to as virion phenotypes (Fig. 1). The production of two structurally and functionally distinct virion phenotypes appears to be unique among eukaryotic viruses. The two virion phenotypes carry an identical genome and it was thought earlier that nucleocapsids were identical (see below). Virions of the two phenotypes differ dramatically in several aspects: they are produced at different sites in the infected cell, their envelopes are comprised of different membranes and membrane proteins, and each serves a distinctly different and essential role in the infection cycle in nature. One virion phenotype is referred to as the Occlusion Derived Virus (ODV) and the other is called the Budded Virus (BV), with the names referring to how each virus particle is produced (Fig. 1-2). In the case of ODV virions, nucleocapsids acquire an envelope within the nucleus and virions are subsequently occluded or encased within a crystallized protein matrix to form an occlusion body (OB), a structure that physically protects virions in the environment. ODV spread infection orally from insect to insect and are specifically adapted to infect the epithelial cells of the insect midgut. After infection of midgut cells by ODV, BV bud from the basal surfaces of the polarized midgut epithelial cells into the hemocoel (the open circulatory system of the insect) and they transmit infection systemically from cell-to-cell and tissue-to-tissue within the infected animal. For viruses such as AcMNPV, most tissues within the hemocoel (tracheal epithelium, hemocytes, epidermis, muscle, fatbody, etc.) become infected and produce additional BV, further spreading the infection through the animal. BV are produced in substantial quantities as early as 12-18 h p.i. in cell culture (14). Later in the infection, many nucleocapsids are retained within the nucleus, and are subsequently enveloped by a membrane derived from the nuclear envelope (15). These newly enveloped ODV in the nucleus are then encased in the occlusion body protein, which crystallizes to form the OB. While several distinct types of OBs are produced by baculoviruses, each OB of AcMNPV is large (app. 1.7-3 μm in diameter) and contains many virions (app. 10-30)(Fig. 2B). In addition, each AcMNPV ODV virion typically contains multiple (app. 5-25) nucleocapsids (16). Thus, a single

AcMNPV OB (the oral infectious unit) may carry hundreds of nucleocapsids. OBs are released from infected cells upon cell lysis late in the infection of the animal and typically, infected insects appear to dissolve, a process sometimes called liquefaction. This is a process mediated by at least two viral-encoded enzymes (a chitinase and a cathepsin protease) that catalyze the breakdown of the insect exoskeleton and the release of OBs into the environment (17), completing the infection cycle in nature. Research utilizing cultured cells is believed to be mostly representative of the secondary phase of infection in the animal (non-midgut infections)(Fig. 1B). In cell culture systems, infection is initiated by BV because ODV are poorly infectious in cultured cells (18). However, both BV and ODV are produced in cultured cells. Studies of infection by ODV are typically performed in larval insects as no convenient polarized midgut cell culture system is available. However, much progress has been made toward understanding viral determinants of ODV infection in the midgut, a critically important phase of the infection cycle in nature. Here, we will review some of the important aspects of entry and egress at the cellular level, by baculovirus ODV and BV, and summarize our current understanding of these processes.

I. ODV ENTRY

I.1. ODV Structure

ODV are highly specialized for infection of insect midgut cells and must overcome significant physical and biological barriers. ODV stability in the environment is critically dependent on the occlusion body protein, which surrounds and protects ODV from desiccation and possibly UV inactivation (Fig. 2B). The OB protein (called Polyhedrin in most baculoviruses) forms a natural crystal in the OB, and in that structure Polyhedrin trimers are linked via disulfide bonds to form a dodecamer (19). An N-terminal region of the polyhedrin protein (amino acids 32-48) is highly disordered and it has been proposed that this region may interact with the embedded ODV virions at the interface of the virion and the crystallized OB protein. A critically important characteristic of the OB structure is its rapid disassembly at high pH, a process that occurs in the alkaline environment of the insect gut. The disruption of disulfide bonds between trimer subunits is thought to be an early step in OB disassembly, a process that is accelerated

at high pH (19). Polyhedrin is enriched in tyrosine, with residues clustered at interfaces between trimer subunits. pH effects on these interfaces are thought to also promote OB disassembly at high pH. AcMNPV OBs each contain many embedded ODV, but it should be noted that the number of ODV/OB can vary significantly depending on the baculovirus genus and species. The surface of the OB is covered by an outer layer comprised of carbohydrate and protein which appears to stabilize the OB. ODV of AcMNPV are released into the relatively harsh environment of the midgut lumen which is rich in proteases and is highly alkaline. The peritrophic matrix, a protein-chitin structure that lines the midgut, is another potential obstacle to ODV infection. Chitin-binding fluorochromes known as optical brighteners (e.g. calcofluor) are known to disrupt or degrade the peritrophic matrix and can lower the LD_{50} for baculovirus infections (20). This suggests that the peritrophic matrix is a significant physical barrier to ODV infection. OBs of some alpha- and betabaculoviruses contain a metalloprotease (called Enhancin, Viral Enhancing Factor, VEF; or Synergistic Factor) that can cleave proteins in the peritrophic matrix and increase the efficiency of infection (21-23). While not all baculoviruses sequenced to date contain an identified *enhancin* gene in their genomes, most are thought likely to encode a protein with this function.

I.2. ODV Binding and Entry into Midgut Cells.

ODV enter midgut epithelial cells via direct membrane fusion with microvilli (24, 25). The nature of the receptor on midgut epithelial cells is not known although a specific receptor is suspected since binding of ODV to midgut brush border membrane vesicles is saturable (26, 27). Although definitive experimental evidence is lacking, available current evidence implicates a large protein complex of viral PIF proteins, as mediating ODV binding and fusion with midgut microvillar membranes.

PIF Proteins. The ODV envelope contains at least 13 integral membrane proteins. The first ODV envelope protein found to be important for oral infection and possibly receptor interactions was P74 (*ac138*). Deletion of P74 does not substantially impact BV production but importantly, ODV oral infectivity is lost (27-30). Following the identification of P74, 8 additional ODV envelope proteins required for oral infectivity were identified. The genes encoding these proteins are referred to as *per os infectivity factor* or *pif* genes, and the 9 *pif* genes are named: *pif0* (*p74*), *pif1* (*ac119*), *pif2* (*ac22*),

pif3 (*ac115*), *pif4* (*ac96*), *pif5* (*odv-e56*, *odvp-6e*, or *ac148*), *pif6* (*ac68*), *pif7* (*ac110*), and *pif8* (*ac83*) (31-38). All PIF proteins identified to date have the following properties in common: 1) deletion results in the loss of *per os* infectivity, 2) deletion does not impact assembly of ODV or occlusion of ODV into OBs, 3) deletion has no observable effect on BV production or infectivity, and 4) homologs are present in all baculovirus genomes (*pif* genes represent 9 of the 38 baculovirus core genes). All PIF proteins are associated with the ODV envelope, and PIF8 was also found associated with the nucleocapsid (36). The 9 AcMNPV PIF proteins form a large high molecular weight complex in the ODV envelope (37, 39) (Fig. 3A). Association of PIF0,-5,-6,-7, and -8 with the high molecular weight PIF complex appears to depend on the presence of PIF1-4. Current data suggests that PIF1-4 can form a smaller relatively stable core complex, independent of other PIF proteins (36, 40). An important aspect of the PIF complex is that it exists and functions in an environment rich in proteases and high pH (the midgut) and formation of a stable PIF complex is necessary for resistance to proteolytic degradation (41). Because PIF complex stability is almost certainly critical for oral infectivity, conclusions regarding PIF protein functions (based on gene knockouts and oral infection assays) must take into account the stability and protease resistance of the PIF complex. Thus, conclusions from prior and future studies of *pif* gene deletions should be examined in light of possible effects on PIF complex stability. Still to be determined is the stoichiometry of PIF proteins in the entry complex. Transcriptomic analysis indicates that AcMNPV *pif* gene transcripts are present at significantly different relative levels with *pif5>pif1>pif4>pif8, pif6, pif7, pif2>pif0>pif3* (42). Yeast two-hybrid studies suggest a complex interaction network among PIF proteins. The core complex PIF-1, -2, -3 and -4 all interact with each other, with PIF3 potentially forming a multimer and also binding to PIF5 (also possibly a multimer). In addition, PIF5 interacts with PIF0, and PIF8 binds to PIF1 (36, 43). Other interactions are likely to occur but it is currently unknown how PIF-6 and -7 interact with the complex (Fig. 3A). As all *pif* genes are core genes and thus conserved across all baculoviruses, the ODV entry mechanism appears to be highly conserved in the Baculoviridae. Indeed, the identification of orthologs in other invertebrate viruses also suggests that the same entry mechanism may be used by other virus families. Central questions going forward are: Does the PIF

complex mediate binding and possibly also confer host specificity? Does the PIF complex mediate membrane fusion, and if so, how is fusion with the plasma membrane triggered? To date, no PIF proteins have been identified with similarities to fusion proteins from other viruses although such similarities are often difficult to detect without structural data. Elucidating the predicted binding, fusion and entry mechanisms of PIF complexes and the roles of their components will be one of the most exciting areas of baculovirus research due to their evolutionary conservation in diverse invertebrate DNA viruses. Because ODV membrane fusion events occur in the high pH of the larval midgut, the receptor-binding and membrane fusion mechanisms likely mediated by PIF complexes represent a type of uniquely interesting biological machine that has not been previously studied.

I.3. Nucleocapsid Transit to the Nucleus or Basal Membrane

Upon ODV fusion with a midgut microvillus, a single ODV virion may release one or many nucleocapsids (24, 44). Microvilli typically contain a thick bundle of crosslinked actin filaments and it is not known whether entering nucleocapsid(s) specifically interact with these pre-existing actin filaments. However, based on studies of nucleocapsids released from BV (see below) actin polymerization is likely initiated from one end of the ODV-derived nucleocapsid and the formation of new actin filaments is thought to provide propulsion for transporting nucleocapsids to the nucleus (45-47). The interaction of the nucleocapsid with the nuclear pore complex and transit through the nuclear pore are also thought to be similar to that described during BV entry (47-50) (see Fig. 1B and discussion below). In addition to nucleocapsids that enter the nucleus, nucleocapsids from alphabaculovirus ODV (that contain multiple nucleocapsids) may also circumvent the nucleus and transit directly through the midgut epithelial cell to the basal membranes of those cells, where they bud into the hemocoel (24). The observation by TEM of BV with characteristic GP64-like peplomers budding from basal surfaces of midgut cells within 1-2 hours post infection (h pi) (24), combined with studies of pathogenesis (51) suggest that this midgut cell pass-through process requires early gene expression from nucleocapsids that are simultaneously trafficked to the nucleus (Fig. 1A). Thus for baculoviruses such as AcMNPV, multiple nucleocapsids released

into a midgut cell may permit the infection to rapidly pass through midgut cells and avoid cellular and organismal defenses associated with the midgut.

II. ODV Assembly and Egress

Following viral gene expression and DNA replication in the nuclei of infected cells, nucleocapsids are assembled in the virogenic stroma, and then transported to the so-called ring zone, an electron lucent region at the periphery of the nucleus (Fig. 2B). Nuclear transport to the ring zone is dependent on nuclear actin polymerization and nucleocapsid proteins VP80 (Ac104), P78/83 (Ac9), VP1054 (Ac54), and BV/ODV-C42 (Ac101) (45, 52, 53). During assembly or in the ring zone, nucleocapsids may be somehow designated to form either ODV or BV. While the mechanism(s) that direct some nucleocapsids to exit the nucleus (to generate BV) and others to remain within the nucleus (to form ODV) are not understood, several hypotheses have been suggested (discussed below). The genetic content of ODV and BV nucleocapsids appears to be identical and based on viral DNA levels, it has been estimated that 97% of the synthesized viral genomic DNA is found in ODV or remains in the nucleus (54). Thus, only a small percentage of the total viral DNA synthesized is found in BV.

II.1. Nucleocapsid and Envelope Composition.

Although nucleocapsids of the ODV and BV were previously considered to have the same structure, proteomic studies of purified virions indicate that differences exist (55-57). ODV and BV nucleocapsids and envelopes have been analyzed in greatest detail for AcMNPV and HearNPV and the results are summarized in Figure 2A. Of the approximately 55 AcMNPV nucleocapsid proteins, 24 appear to be common to both ODV and BV nucleocapsids. Another 31 proteins are specific to either the ODV or BV nucleocapsids of AcMNPV (Fig. 2A). For HearNPV nucleocapsids, 21 proteins specific to either ODV or BV nucleocapsids were also identified (56). Similarities and differences between virion proteins from the two viruses are indicated in Figure 2A. Depending on the virus analyzed, 3-6 envelope proteins are common to both ODV and BV phenotypes. For AcMNPV these include BV/ODV-E26 (Ac16), F-like protein (Ac23), Ac76, ODV-E25 (Ac94), ODV-E18 (Ac143), and ODV-E56 (PIF5 or Ac148). In contrast, GP64 (Ac128), Viral Ubiquitin (v-Ubi, Ac35), GP37 (Ac64), Ac75, and P18 (Ac93) are

specific to BV envelopes. Envelope proteins specific to AcMNPV ODV include PIF 0-4 and 6-8, ODV-E66 (Ac44), and GP41 (Ac80). Comparisons of AcMNPV and HearNPV show similarities and differences with respect to their protein contents detected for ODV and BV nucleocapsids and envelopes (Fig. 2A). Proteomic studies have also been performed on virions from other baculovirus species and similarly, results have been variable. Future studies with both sensitive and quantitative analytical techniques should permit us to more confidently define the viral and host protein content of ODV and BV.

The assembly and occlusion of ODV in the nucleus requires a complex integration of events that include: trafficking of ODV membrane proteins to the nucleus and formation of intranuclear membranes, the assembly and association of nucleocapsids with intranuclear membranes, and the wrapping or enclosing of nucleocapsids in membranes. Finally, the envelopes of assembled ODV must associate with concentrated occlusion body protein (Polyhedrin) which crystallizes around one or many ODV to form the OB (Fig. 1B and 2B). From TEM studies and electron tomography, a model was recently developed for the sequence of events in ODV envelopment (58).

The ODV envelope. The ODV envelope appears to originate from either the inner nuclear membrane (INM) or both the INM and outer nuclear membrane (ONM) and many of the ODV envelope proteins contain INM sorting motifs (15, 58-67). Microvesicles that are likely derived from the INM appear to begin pinching off or blebbing from the nuclear membrane (Fig. 1B, 2B) and this process was previously shown to require at least three viral proteins: Ac76, Ac75, and Ac93 (68-70). Ac76 is a dimeric integral membrane protein that localizes to the INM and interacts with Ac75 at this location. It is interesting to note that these three proteins are also required for egress of nucleocapsids from the nucleus to ultimately form BV. Thus, these three proteins appear to play roles in both the production of enveloped ODV in the nucleus, and production of BV. Based on TEM studies, it appears that nucleocapsids aggregate concomitant with their interaction with intranuclear virion membranes and subsequently appear to be associated at their ends with somewhat spherical vesicles that likely formed from the INM/ONM-derived microvesicles. Vesicles with associated nucleocapsids then become elongated and nucleocapsids are lined up in a parallel and

polarized fashion (58, 71). Based on gene deletions, three viral proteins have been identified as required for the membrane-nucleocapsid interactions in the nucleus: P45 (Ac103), P49 (Ac142) and ODV-E18 (Ac143) (72-74). Also, P49 (Ac142) and ODV-E18 (Ac143) are found associated with the virus-induced intra-nuclear membranes (62). Later, it appears that the virus-induced membranes wrap around their associated nucleocapsids and close to form large enveloped groups of nucleocapsids, which may be further subdivided by a fission process that generates the ODV. The viral proteins required for this process are not known but deletions of PIF genes do not prevent ODV formation or the incorporation of ODV into OBs (34, 75, 76), indicating that PIF proteins are not required. The intranuclear envelopment of nucleocapsids to form ODV appears to be a process that is unique for eukaryotic viruses and studies of the biochemistry of this complex process should uncover new mechanisms of membrane manipulation and membrane-protein interactions.

II.2. Roles for nuclear F-actin in ODV assembly.

During the later stages of infection (app. 10-20 h pi), nuclear G-actin begins to accumulate in the nucleus and subsequently polymerizes to form F-actin (77). Prior studies indicate that nuclear G- and F-actin are in a dynamic state, requiring capsid protein p78/83 for activation of Arp2/3 followed by polymerization and accumulation of F-actin in the nucleus (45). Further studies indirectly suggest that F-actin may play a role in nucleocapsid morphogenesis and in nucleocapsid transport within the nucleus. In viruses containing a deletion in a gene encoding an F-actin-interacting protein (VP80), nucleocapsids are assembled but not transported from the virogenic stroma to the nuclear periphery, and ODV do not form (53). In addition, it was hypothesized that VP80 may function with another viral protein (Ac66) that is also required for nucleocapsid transport and ODV production (78).

Virions with Single and Multiple Nucleocapsids. It is important to note here that the ODV of all beta-, delta-, and gammabaculoviruses typically have only a single nucleocapsid per virion, and only some alphabaculovirus species form ODV with multiple nucleocapsids per envelope. It is not clear how this trait is determined as the number of nucleocapsids enveloped in the each ODV virion has been reported to be impacted by several viral genes and also by viral replication in different host cell lines

(16, 79-81). It has also been speculated that the multiple nucleocapsid phenotype could be determined by the concentrations and proximity of nucleocapsids in the infected nucleus (9). While a mechanistic explanation is lacking, the envelopment and delivery of multiple nucleocapsids to the midgut cell provides the virus with advantages in rapid movement of infection through the midgut (24, 82), as well as a mechanism for genetic complementation to mitigate the effects of mutations acquired in the environment (83).

After mature ODV are formed, they associate with dense concentrations of the polyhedrin protein, which subsequently crystallize around ODV. The mechanisms that control crystallization at the ODV-polyhedrin interface are unknown. The occlusion process appears to be highly regulated, as OBs of a baculovirus species are relatively uniform in size, occlude a number of ODV (within a predictable range for a virus species), and OBs typically have a peripheral outer exclusion zone in which ODV are not incorporated. An outer layer (known as the calyx or polyhedral envelope) comprised of carbohydrate and protein (Polyhedral Envelope, Ac131) is added to the surface of the mature OB (84-87). The formation of this OB surface structure appears to require a functional P10 protein and is associated with nuclear fibrillar structures (30, 88). In the absence of *ac131* or *p10*, OBs contain no outer calyx layer and are irregular and fragile (30, 88). The occlusion of ODV virions varies substantially between baculovirus genera. For example, betabaculovirus OBs each typically incorporate only a single ODV virion, whereas OBs of the other three genera contain many ODV per OB. The occlusion process is complex and critically important in this group of viruses. Important questions that remain to be addressed include: a) How is occlusion body protein crystallization triggered? b) How do the membrane and membrane proteins of ODV cooperatively interact with the OB protein before and in the crystal state? c) How is the number of ODV incorporated into the OB regulated? and d) How is the size of the growing occlusion body limited? A number of broad questions should also be addressed in the future. Are infections and OB release coordinated at the organismal level, such that coordinated cell lysis and OB release result in maximally efficient OB production and delivery into the environment? Baculoviruses are known to alter the developmental program and behavior of the host insect in order to maximize virus production and distribution (89-91), and it would be surprising if the release of OBs in the dramatic

process of larval melting (or liquefaction) was not also coordinated to optimize OB production and delivery to the environment.

III. BV ENTRY

III.1. BV in nature

In nature, BV transmits infection from cell-to-cell and tissue-to-tissue within the animal. After initially budding from infected midgut cells (and later from other infected tissues), BV circulate in the hemolymph and are capable of binding and entering most cell types within the host. This promiscuous entry of AcMNPV BV into many heterologous cell types has been exploited in a number of biotechnological applications (92-94).

III.2. BV structure

BV Envelopes and Envelope Proteins.

Unlike the ODV envelope, the BV envelope is acquired from the cell plasma membrane during budding, and contains a more limited number of virus-encoded proteins (Fig. 2A). The AcMNPV BV envelope contains one highly abundant viral protein, GP64, and at least six additional virus-encoded membrane proteins that are present at lower levels. These include: F-like protein (Ac23), v-Ubi (Ac35), GP37 (Ac64), ODV-E25 (Ac94), ODV-E18 (Ac143), and BV/ODV-E26 (Ac16) (57, 95). GP64 is essential for virion entry, and two of the other BV envelope proteins are important for the production of infectious BV (ODV-E25 and ODV-E18) (9, 73, 96). However, the other BV envelope proteins (F-like protein, v-Ubi, GP37, and BV/ODV-E26) may impact BV production levels but they are not required for BV production or BV infectivity. BV contain both host and viral-encoded ubiquitin proteins and both associate with the inner surface of the BV envelope through a covalently attached phospholipid anchor (97, 98). Studies of *v-ubi* knockouts in AcMNPV reported substantially reduced BV yields at 24 h pi (99, 100) whereas a *v-ubi* knockout in a closely related virus, BmNPV, was reported to have no apparent effect on cumulative BV production at 72 h pi but earlier times pi were not reported (101). Because ubiquitination of nucleocapsid proteins has also been

reported (see discussion below), and both BV envelopes and nucleocapsids contain viral (v-Ubi) and cellular ubiquitin, the specific role of ubiquitin in the BV envelope is not yet clear.

GP64 and F proteins.

GP64 is a Class III viral fusion protein (102-104) that appears to have been acquired relatively recently in the evolutionary history of the baculoviruses (103, 105, 106). GP64 orthologs within the Baculoviridae show an unusually high level of amino acid sequence conservation and they are found only in a subgroup (group I) of the alphabaculoviruses. Class III fusion proteins are a structurally related group of viral fusion proteins that include members in other seemingly unrelated virus families, such as the gB proteins of herpesviruses, the GP75 proteins of certain orthomyxoviruses, and the G proteins of rhabdoviruses (102, 103, 107). Class III fusion proteins are found as trimers in the membrane and (based on the low pH post-fusion structures of these proteins) have structural characteristics that are distinct from other classes of viral fusion proteins: 1) each monomer contains a long central alpha helix that is one member of a trimer of coiled-coils in the center of the trimer, 2) the monomer contains five characteristic domains which are comprised of alpha helical and beta sheet structures distinct from class I and II fusion proteins, and 3) critical fusion domains (fusion peptides) are found within two internal loops referred to as fusion loops (102, 108). Fusion loops are located at the extremity of the post-fusion structure (Fig. 3B), and contain hydrophobic residues critical for fusion (109-112). Experimental data suggest that they may interact with the host cell membrane bilayer during the process of membrane fusion (104, 109-111). Unlike other viral class III fusion proteins, each monomer of the baculovirus GP64 trimer is covalently linked to the other monomers in the trimer, by inter-molecular disulfide bonds (104, 113).

F proteins. While GP64 is the major envelope glycoprotein in the BV envelope of AcMNPV and other group I alphabaculoviruses (9, 114), the major envelope glycoprotein in group II alphabaculoviruses and other genera of baculoviruses, is called F (for Fusion) protein. GP64 and most F proteins are functional analogs, mediating host cell binding and membrane fusion, but they differ dramatically in structure (104, 115).

GP64 and F proteins also appear to have an interesting evolutionary relationship within this virus family. In group I alphabaculoviruses like AcMNPV, an F (Ac23) protein is also present but it is a minor non-essential component of the BV envelope (116). However, in baculoviruses that do not encode a GP64 protein, F is an abundant BV envelope glycoprotein and is essential, mediating virion binding and membrane fusion (105, 117, 118). F proteins are structurally similar to paramyxovirus F proteins (Class I fusion proteins) although there is little or no recognizable amino acid sequence conservation (115). Although poorly conserved at the sequence level, F proteins related to baculovirus F proteins have also been identified in endogenous retroviruses (119-121) and in the genomes of several insect species (115, 120, 122, 123). Several lines of evidence support the concept that F represents an ancestral BV fusion protein, and that GP64 was more recently acquired by an ancestor of the group I alphabaculoviruses: First, F protein genes are widely distributed in the Baculoviridae and encode proteins with lower levels of amino acid sequence conservation. In contrast, GP64 proteins are restricted to a single baculovirus subgroup (group I of the Alphabaculovirus genus) and have a high level of amino acid sequence conservation. In addition, all group I alphabaculoviruses that encode a *gp64* gene also contain an F protein gene, although it is often called F-like because it no longer functions as a fusion protein in these viruses. Thus, it appears that upon acquisition of GP64 in the progenitor of the group I alphabaculoviruses, the central role of F in entry was displaced (105, 106, 116, 117). However, because the F gene has been retained in viruses carrying GP64, this also suggests positive selection for other function(s) of F. Knockout of the AcMNPV F-like protein gene (Ac23) in AcMNPV has little or no effect on BV production or BV infectivity, but effects on ODV occlusion and pathogenicity have been observed (16, 116). In the group II alphabaculoviruses (which carry no GP64 gene), knockouts of F are lethal (124, 125).

Lipid composition of the BV Envelope.

In the limited studies that have examined and compared the lipids of BV and ODV envelopes (and host cells) it was determined that the BV and ODV envelope lipid compositions differ substantially (64, 126, 127). The envelope phospholipids of

AcMNPV BV generated from infection of Sf9 cells were reported to be comprised of approximately 50% phosphatidylserine (PS) and lower amounts of sphingomyelin (SPH, 13%), phosphatidylinositol (PI, 12%), phosphatidylcholine (PC, 11%), phosphatidylethanolamine (PE, 8%) and lysophosphatidylcholine (LPC, 6%)(64). In contrast, ODV envelopes contain much higher levels of PC (39%) and PE (30%), while PS is present at somewhat reduced levels (20%) and SPH and LPC were reported at extremely low levels (<2 and 1%, respectively). While lipid compositions of the BV and ODV envelopes, as well as that of the host or target cells have received very little attention, they are likely to play important roles in virus binding and entry, as well as ODV and BV assembly and egress.

III.3. BV Binding

Cellular Receptor

Receptor binding by baculovirus BV is only poorly understood. AcMNPV BV binding to host cells is mediated by the major envelope glycoprotein GP64 and its cognate receptor has not been clearly identified. While the AcMNPV F-like protein, Ac23, is non-essential for infection (116), experiments in a heterologous system suggest that Ac23 could enhance entry in some cases (105). Early studies indicated that AcMNPV BV binding to Sf9 cells (and 5 other cell lines) was spatially saturated, essentially covering the cell surface (128). Current data suggest that GP64 binds to either acidic phospholipids in the cell's plasma membrane (110), or to a highly abundant and widely distributed cellular protein or class of proteins (128-130). Studies of BV transduction of mammalian cells (that are non-permissive for viral replication) have also implicated charged cell surface proteins as important for BV binding in those cells (131, 132), although it is not clear whether such binding is relevant to entry into permissive insect cells. The lack of a requirement for a protein receptor is also suggested by a number of studies demonstrating AcMNPV BV binding to liposomes that contain no protein (110, 126, 133). BV binding to liposomes is enhanced when liposome membranes contain acidic phospholipids, suggesting that GP64 interactions with the charged heads of phospholipids may be the critical factor for BV binding to the host cell. It is of special note that in studies of receptor binding by another promiscuously-binding class III fusion

protein (VSV G), a definitive protein receptor has not been identified and phospholipids have also been implicated as a likely cellular receptor (134-137). In support of a phospholipid receptor for AcMNPV BV, prior treatment of mammalian cells with phospholipase, and studies with cell lines deficient in phospholipid synthesis showed reduced BV transduction efficiency (138). Combined, these and other studies suggest that lipid composition plays an important if not critical role in BV binding and entry.

GP64 Binding.

In studies of GP64-binding to host cells, it was reported that binding was neutralized by anti-peptide antisera directed against regions of GP64 that contain the fusion loops (139). In addition, substitution mutations of several critical residues within the GP64 fusion loops resulted in decreased binding of a soluble GP64 protein to liposomes (110). These data suggest that the GP64 fusion loops may serve roles in both BV binding and membrane fusion (see below). Recently, a similar model for binding was proposed for the structurally related VSV G protein. In this model, reversible structural changes expose fusion loop domains that subsequently interact with target membranes as a step in host cell binding (136). It was proposed that binding by VSV G was established via reversible extension of the fusion loops at neutral pH, with more extensive interactions occurring at lower pH. Experimental data from GP64 and BV interactions with liposomes (110, 126, 133, 140, 141) suggest the possibility that a similar mechanism may mediate GP64 binding to permissive host cells.

III.4. Endocytosis

After binding at the cell surface, BV enter cells by clathrin-mediated endocytosis (142-144) and this appears to be the case for both permissive insect cells and non-permissive mammalian cells (144, 145). Little is known regarding the endosomal trafficking that occurs immediately following entry from the cell surface but several studies have identified cellular components of vesicle formation and trafficking that are necessary or important for entry (146-149). These include components of the endosomal sorting complex required for transport (ESCRT) pathway and N-ethylmaleimide sensitive fusion protein (NSF), a key regulator of soluble NSF attachment protein receptor (SNARE) function. The cellular ESCRT pathway is best

known to mediate the formation and fission of multivesicular bodies in healthy cells, but this pathway plays a variety of additional roles in normal cellular physiology (150, 151). Many viruses hijack components of the cellular ESCRT machinery for virion budding during viral egress (152, 153). ESCRT proteins are also involved in entry by a variety of viruses, including rhabdoviruses, arenaviruses, flaviviruses, herpesviruses, bunyaviruses, and rotaviruses (153-158). NSF is an AAA ATPase that is critical for the disassembly and recycling of SNARE complexes, and disruption of NSF function results in disrupted SNARE function. SNARE proteins mediate the carefully regulated fusion between intracellular vesicles and their target membranes. While these studies point to the importance of specific components of the cellular vesicle trafficking system in the trafficking of virion-containing endosomes during entry, understanding the precise mechanistic roles of ESCRT and SNARE proteins in endosomal trafficking during baculovirus entry will require additional study. Following internalization of BV, endosomes are acidified by proton pumps in the endosome membrane and the acidification triggers GP64-mediated membrane fusion, releasing the nucleocapsid into the cytoplasm. Using inhibitors of endosome acidification, it was determined that nucleocapsids are released from the endosome with a half-time of approximately 25 min (142). Because the pH threshold for GP64-mediated membrane fusion was measured at approximately pH 5.5 (159), this suggests that fusion and nucleocapsid release occurs in late endosomes.

III.5. Membrane fusion by GP64:

Baculovirus GP64 proteins mediate low pH triggered membrane fusion in a pH dependent manner, through a series of discrete steps that include: receptor binding, low pH induced GP64 conformational change, outer membrane leaflet merger and membrane mixing (hemifusion), membrane pore formation, and finally pore enlargement which releases the nucleocapsid into the cytoplasm. How various fusion proteins accomplish membrane fusion is a subject of intense study, yet the mechanistic details of membrane fusion are only poorly understood in the best cases (160). While only the postfusion (low pH) structure of the GP64 protein is available (104)(Fig. 3B), both pre- and post-fusion structures are available for the structurally related class III fusion protein, VSV G, which also shares functional characteristics (promiscuous binding and

reversible structural changes) with GP64. A model for the neutral and low pH triggered conformational changes, and the extension of fusion loops has been proposed for VSV G (136). Like VSV G, GP64 requires no proteolytic activation and after exposure to low pH, conformational changes in the protein appear to be reversible (161). Because the pKa of histidine residues is around pH 6.0, the GP64 conformational change induced by low pH is believed to result from protonation of histidine residues. A cluster of 3 histidine residues was identified as necessary for stabilizing the prefusion structure of GP64, and thus these histidine residues are likely participants in the low pH triggering GP64 (162). Following triggering, Class III fusion proteins such as GP64 are thought to form an extended structure such that the two fusion loops at the extremity interact with the adjacent cell membrane. Subsequent refolding of the protein (apposition) then pulls the cell membrane into close proximity with the viral envelope membrane. Residues within and in close proximity to the viral envelope membrane (the transmembrane, TM, and the pre-transmembrane, PTM domains, respectively) are thought to play a role in disrupting the bilayer structure to facilitate membrane mixing and formation of a hemifusion state in which the outer layers of the two adjacent bilayers are merged (163). Experimental studies have identified amino acid positions in the GP64 fusion loops (102, 104, 110, 126, 139) as well as in the TM and PTM domains (164-166), that are important for several of the sequential steps in GP64-membrane interactions and fusion. In addition, domains important for stabilizing the pre-fusion and post-fusion structures of GP64 have been examined (162, 167). Understanding the mechanisms of GP64 triggering and membrane fusion will ultimately require a prefusion structure of GP64, as well as intermediate structures in the conformational change. Based on patch-clamping studies, the GP64-induced fusion pore was described as a large pore that opens rapidly, when compared with the influenza HA-induced fusion pore, which forms small rapidly opening and closing pores that gradually enlarge (168-170). During membrane fusion, a higher order fusion complex of approximately 10 GP64 trimers appears to form (170, 171). Although a number of studies have examined GP64 domains and their roles in membrane fusion, much work will be required to fully understand the mechanisms of GP64 receptor recognition, pH triggering and

conformational changes, and the complex interactions of GP64 domains with the viral envelope and host cell membrane during the process of membrane fusion.

III.6. Actin Propulsion of Nucleocapsids

Within a few hours following infection, dynamic changes in the actin cytoskeleton can be observed. Actin accumulates at the periphery of the cell near the plasma membrane and actin cables form in the cytoplasm (172, 173). A non-essential viral gene called *actin rearrangement -inducing factor 1* (*arif-1*) was identified as required for the peripheral actin localization (173, 174). Following release of BV nucleocapsids from endosomes, a nucleocapsid structural protein, P78/83 (Ac9), recruits cellular Arp2/3 which is a nucleator of actin polymerization (45). P78/83 is a viral WASP-like protein that contains a domain that binds G-actin, and another domain that binds the Arp2/3 complex. The polymerizing F-actin generates “comet tails” which may represent or be related to the actin cables observed within a few hours after infection. Using AcMNPV BV (containing mCherry-labeled nucleocapsids) to infect cells expressing EGFP-actin, nucleocapsids were observed being propelled by actin comet tails at a velocity of approximately 7-22 μm/min, similar to the actin-based motility described from Listeria, Shigella, and vaccinia virus (47). The actin polymerization activity of P78/83 appears to be at least partially regulated by viral protein BV/ODV-C42 (Ac101) (175-177). A recent study of AcMNPV nucleocapsid propulsion by actin, using electron tomography in model vertebrate cells, suggested that nucleocapsids may be continuously tethered to branching actin filaments in what was described as a “fishbone-like array” and it was suggested that nucleocapsid propulsion likely results from an average of 4 filaments pushing a nucleocapsid (178). The authors also proposed that directionality may be imposed by P78/83 through the restriction of actin filament branching in a biased manner. It will be exciting to follow future studies that help us to better understand the mechanistic details of these engines of nucleocapsid propulsion in the cell.

III.7. Nuclear entry

It is now clear that nucleocapsids of the alphabaculoviruses enter the nucleus by transport through the nuclear pore complex (NPC). Initial evidence of nucleocapsid-NPC interaction came from TEM observations of baculovirus infected cells (24). Nucleocapsids were observed to interact with the NPC via the nucleocapsid cap end,

and electron dense nucleocapsids were observed in the nucleoplasm, leading to the conclusion that nucleocapsids transited the NPC. Nucleocapsids labeled with mCherry can also be observed co-localize with nuclear pores, followed by their detection in the nucleoplasm (47). Nuclear entry can also be blocked by a truncated form of importin-beta which inhibits NPC transport of large cargoes by binding to NPC proteins called nucleoporins. Using electron microscopy and electron tomography, nucleocapsids have been shown to interact with cytoplasmic filaments and transit the NPC (48). Deletion of *ac132* results in nucleocapsids that appear to dock at the nucleus but do not enter, indicating that Ac132 may be required for NPC trafficking at a step following docking (179). Normally, transit of cargo across the NPC using the importin-beta superfamily requires the RAN-GTPase cycle. However, recent studies found that nucleocapsid transit into the nucleus required only actin polymerization mediated by Arp2/3 and not the RAN-GTPase cycle (50). These results are the basis of a model for a novel mechanism of nuclear transport in which actin polymerization is the propulsive force driving cargo through the NPC and overcoming the normal size limit of the NPC central channel (47, 48). This type of mechanism for NPC transit has not been described previously and may be unique for alphabaculovirus nuclear entry. It should be noted here that other baculovirus genera may utilize another mechanism for nuclear entry. In TEM studies of betabaculovirus infected cells, nucleocapsids were observed docking at the NPC (but not entering through the NPC). Empty nucleocapsids were also observed docked on the cytoplasmic side of the NPC suggesting that betabaculovirus nucleocapsids may dock at the NPC and release the viral genome into the nucleus through a nuclear pore (180, 181).

IV. BV EGRESS

BV egress has been studied largely in the alphabaculoviruses. In those viruses, a subset of the nucleocapsids assembled within the nucleus will exit the nucleus to produce the BV, while another subset remains in the nucleus and are enveloped to form the ODV (Fig. 1B). Although the mechanism by which nucleocapsids are selectively tagged for nuclear egress or retention is unknown, differences in the protein

compositions of nucleocapsids isolated from BV and ODV (55-57)(Fig. 2A) could suggest that one or more of the BV- or ODV-specific nucleocapsid proteins may regulate nuclear egress or retention (see Fig. 2A). Substantial differences in the levels of ubiquitination of nucleocapsids from BV and ODV were also reported, with BV-derived nucleocapsids ubiquitinated at much higher levels than those from ODV (100), and it was speculated that nucleocapsid ubiquitination (potentially catalyzed by the viral E3 ubiquitin ligase Ac141 or Exon0), may serve as a tag for nucleocapsid egress. A potential target for ubiquitination was identified as Ac66, and the ubiquitinated Ac66 was detected only in BV nucleocapsids. Supporting a possible role for these proteins in nucleocapsid egress, deletion of either Ac66 or Ac141 results in nucleocapsids that are unable to exit the nucleus (78, 182). It is also possible that other post-translational modifications that have not yet been examined could be involved in regulating this process and studies that further refine and extend the structural analysis of nucleocapsids could yield important information in this area. Based on protein-protein interactions, gene knockouts, RNAi, and use of dominant-negative proteins, it was recently proposed that nucleocapsids may exit the nucleus via an egress complex at the nuclear membrane (146). The proposed complex of interacting proteins includes viral proteins Ac142, Ac146, Ac103, Ac93, Ac78, Ac76, and host cell proteins VPS20, Snf7, VPS24, VPS2, VPS46, VPS60, and VPS4. From studies based largely on TEM analysis, nucleocapsid egress from the nucleus is thought to occur through a process of budding from the nuclear membrane (Fig. 1B, 2B). Cage-like outpockets of the nuclear membrane appear during the peak of nucleocapsid egress and this nucleocapsid budding from the nucleus appears to produce a so-called “transport vesicle” in the cytoplasm, with the nucleocapsid(s) surrounded by two membranes (24, 183). It appears that the envelopes of transport vesicles are lost in the cytoplasm since free nucleocapsids are observed there and at the plasma membrane. Trafficking of enveloped nucleocapsids (transport vesicles) may occur via microtubules as inhibitors of microtubule formation disrupt BV production (184, 185). Production of transport vesicles or nucleocapsid release from them could also involve membrane fusion by cellular fusion proteins such as SNARE proteins since dominant negative forms of NSF (which regulates SNARE fusion of cellular transport vesicles) have been shown to

inhibit nuclear egress or release of nucleocapsids (147). In contrast to the nuclear egress of nucleocapsids (described for alphabaculoviruses here), betabaculovirus appear to differ substantially in this regard as TEM studies show that the nuclear membrane is lost late in infection, by a mechanism that is not understood. After release of nucleocapsids from alphabaculovirus transport vesicles, nucleocapsids may be trafficked by actin polymerization as has been described during entry and early stages of infection (47). Alternatively however, because Kinesin 1 may interact directly with VP39 and Ac141 (Exon0), it was proposed that free nucleocapsids could be transported to the plasma membrane along microtubules (185). Because the details and mechanisms of these events remain somewhat enigmatic, further work will be necessary to disentangle the complex nature of nucleocapsid tagging, nuclear egress, and transport to the plasma membrane.

For many viruses, the budding and fission (scission) event that releases the budded virion from the plasma membrane is mediated by viral recruitment of components of the cellular ESCRT pathway to budding sites (151-153). The ESCRT pathway is a series of protein complexes that mediate cargo recruitment, bud formation, and scission of vesicles in the formation of multivesicular bodies in healthy cells. The baculovirus AcMNPV may also use this mechanism for budding at the plasma membrane as a functioning ESCRT pathway is required for BV production (146, 149). However, because ESCRT pathway components may be involved both in nuclear egress and budding at the plasma membrane, additional studies will be required to separately understand each process. The roles of viral proteins are unclear as regards BV budding at the plasma membrane. The major envelope glycoprotein GP64 dramatically influences the efficiency of BV budding, but is not absolutely essential for budding since low levels of non-infectious BV are produced in the absence of GP64 (186). The F-like protein (Ac23) does not appear to influence budding in AcMNPV infected cells (116). While no known matrix protein has been identified from baculovirus BV, viral protein ME53 (Ac140) has been shown to co-localize with GP64 at the plasma membrane (possibly at budding sites), and deletion of the *me53* gene results in an approximately 1000 fold reduction in infectious BV, suggesting that ME53 may play a role in BV budding at the plasma membrane (187, 188). Viral envelope and

nucleocapsid proteins that are necessary for recruitment of an ESCRT complex, or are otherwise required for the budding or scission process, have not been identified. Discovery of the specific requirements for and mechanism of BV budding will be important for understanding the biology of this virus, and should have important applications in biotechnology.

V. Challenges and Future Studies

The production of two virion phenotypes is an effective biological adaptation for virus survival in the environment, efficient oral transmission, and rapid viral amplification in individuals and populations of insects. While much progress has been made toward understanding specific details of the structures and functions of each virion phenotype, many fundamental questions remain to be addressed. Although critical components of the ODV have been identified and characterized, details of binding and entry mechanisms are not well understood and remain challenging. Dissecting the details of virus-host receptor interactions is experimentally challenging as studies of ODV entry have been limited largely to *in vivo* studies in animals. While more sensitive and quantitative analytical tools for understanding the physical composition of the ODV and protein-protein interactions should be forthcoming, perhaps most challenging will be the development of biological tools such as cell lines that permit synchronous infections of cultured cells. The development of engineered insect cell lines that are permissive for ODV infection, or the generation of polarized insect midgut epithelial cell cultures could greatly accelerate our understanding of ODV interactions during binding and entry. New tools such as CRISPR-Cas9 combined with host genome sequences should also lead to the development of new cell lines and transgenic host insects for detailed studies of midgut cell proteins and their interactions with ODV and PIF complexes. While the understanding of BV structure and entry have advanced substantially over the last decade, a number of key questions remain. While GP64 and F proteins utilize different receptors, unequivocal identification of a receptor for either remains elusive. The mechanism of binding and fusion-related conformational changes in GP64 or F also remain unknown. While crystal structures representing post-fusion conformations of

GP64 and F have been reported and analyzed, the next major step in understanding the mechanisms for triggering conformational change or mediating membrane fusion, will require pre-fusion structures for each protein. In addition, much remains to be discovered about the roles of cellular trafficking proteins and pathways that are critical for nucleocapsid transport and BV egress at the nuclear and plasma membranes. This is particularly significant in the midgut which represents the first cellular barrier to infection and the most critical step in successful infection of the organism.

REFERENCES

1. Smith GE, Summers MD, Fraser MJ. 1983. Production of human beta interferon in insect cells infected with a baculovirus expression vector. *Mol. Cell. Biol.* 3: 2156-65
2. Pennock GD, Shoemaker C, Miller LK. 1984. Strong and regulated expression of Escherichia coli beta -galactosidase in insect cells with a baculovirus vector. *Mol. Cell Biol* 4: 399-406
3. O'Reilly DR, Miller LK, Luckow VA. 1992. *Baculovirus expression vectors, a laboratory manual.* New York: W. H. Freeman and Co.
4. Cox MM. 2012. Recombinant protein vaccines produced in insect cells. *Vaccine*
5. Felberbaum RS. 2015. The baculovirus expression vector system: A commercial manufacturing platform for viral vaccines and gene therapy vectors. *Biotechnol J* 10: 702-14
6. Herniou EA, Jehle JA. 2007. Baculovirus phylogeny and evolution. *Curr Drug Targets* 8: 1043-50
7. Williams T, Bergoin M, van Oers MM. 2016. Diversity of large DNA viruses of invertebrates. *J Invertebr Pathol*
8. Viruses ICoTo. 2016. Virus Taxonomy: 2016 Release. International Committee on Taxonomy of Viruses (ICTV)
9. Rohrmann GF. 2013. Baculovirus Molecular Biology: Third Edition. In *Baculovirus Molecular Biology: Third Edition*. Bethesda (MD): National Center for Biotechnology Information (US)
10. Jehle JA, Blissard GW, Bonning BC, Cory JS, Herniou EA, et al. 2006. On the classification and nomenclature of baculoviruses: a proposal for revision. *Arch Virol* 151: 1257-66
11. Hu ZH, Arif BM, Jin F, Martens JW, Chen XW, et al. 1998. Distinct gene arrangement in the Buzura suppressaria single- nucleocapsid nucleopolyhedrovirus genome. *J Gen Virol* 79: 2841-51
12. Drezen JM, Gauthier J, Josse T, Bezier A, Herniou E, Huguet E. 2016. Foreign DNA acquisition by invertebrate genomes. *J Invertebr Pathol*
13. Theze J, Takatsuka J, Nakai M, Arif B, Herniou EA. 2015. Gene Acquisition Convergence between Entomopoxviruses and Baculoviruses. *Viruses* 7: 1960-74
14. Milks ML, Washburn JO, Willis LG, Volkman LE, Theilmann DA. 2003. Deletion of pe38 attenuates AcMNPV genome replication, budded virus production, and virulence in heliothis virescens. *Virology* 310: 224-34
15. Braunagel SC, Summers MD. 2007. Molecular biology of the baculovirus occlusion-derived virus envelope. *Curr Drug Targets* 8: 1084-95

16. Yu IL, Bray D, Lin YC, Lung O. 2009. Autographa californica multiple nucleopolyhedrovirus ORF 23 null mutant produces occlusion-derived virions with fewer nucleocapsids. *J Gen Virol* 90: 1499-504

17. Hawtin RE, Zarkowska T, Arnold K, Thomas CJ, Gooday GW, et al. 1997. Liquefaction of Autographa californica nucleopolyhedrovirus-infected insects is dependent on the integrity of virus-encoded chitinase and cathepsin genes. *Virology.* 238: 243-53

18. Lynn DE. 2003. Comparative susceptibilities of twelve insect cell lines to infection by three baculoviruses. *J Invertebr Pathol* 82: 129-31

19. Ji X, Sutton G, Evans G, Axford D, Owen R, Stuart DI. 2010. How baculovirus polyhedra fit square pegs into round holes to robustly package viruses. *Embo J* 29: 505-14

20. Wang P, Granados RR. 2000. Calcofluor disrupts the midgut defense system in insects. *Insect Biochem Mol Biol* 30: 135-43

21. Toprak U, Harris S, Baldwin D, Theilmann DA, Gillott C, et al. 2012. The role of enhancin in Mamestra configurata nucleopolyhedrovirus virulence: Selective degradation of host peritrophic matrix proteins. *J Gen Virol*

22. Ohba M, Tanada Y. 1983. A synergistic factor enhances the in vitro infection of an insect baculovirus. *Naturwissenschaften* 70: 613-14

23. Wang P, Granados RR. 1997. An intestinal mucin is the target substrate for a baculovirus enhancin. *Proc. Natl. Acad. Sci. U S A* 94: 6977-82

24. Granados RR, Lawler KA. 1981. *In vivo* pathway of *Autographa californica* baculovirus invasion and infection. *Virology* 108: 297-308

25. Kawanishi CY, Summers MD, Stoltz DB, Arnott HJ. 1972. Entry of an insect virus *in vivo* by fusion of viral envelope and microvillus membrane. *J. Invertebr. Pathol.* 20: 104-08

26. Horton HM, Burand JP. 1993. Saturable attachment sites for polyhedron-derived baculovirus on insect cells and evidence for entry via direct membrane fusion. *J Virol* 67: 1860-8

27. Haas-Stapleton EJ, Washburn JO, Volkman LE. 2004. P74 mediates specific binding of Autographa californica M nucleopolyhedrovirus occlusion-derived virus to primary cellular targets in the midgut epithelia of Heliothis virescens Larvae. *Journal of virology* 78: 6786-91

28. Faulkner P, Kuzio J, Williams GV, Wilson JA. 1997. Analysis of p74, a PDV envelope protein of Autographa californica nucleopolyhedrovirus required for occlusion body infectivity in vivo. *J Gen Virol* 78 (Pt 12): 3091-100

29. Kuzio J, Jaques R, Faulkner P. 1989. Identification of p74 a gene essential for virulence of baculovirus occlusion bodies. *Virology* 173: 759-63

30. Williams GV, Rohel DZ, Kuzio J, Faulkner P. 1989. A cytopathological investigation of Autographa californica nuclear polyhedrosis virus p10 gene function using insertion-deletion mutants. *J Gen Virol* 70: 187-202

31. Ohkawa T, Washburn JO, Sitapara R, Sid E, Volkman LE. 2005. Specific binding of Autographa californica M nucleopolyhedrovirus occlusion-derived virus to midgut cells of Heliothis virescens larvae is mediated by products of pif genes Ac119 and Ac022 but not by Ac115. *J. Virol.* 79: 15258-64

32. Fang M, Nie Y, Harris S, Erlandson MA, Theilmann DA. 2009. Autographa californica multiple nucleopolyhedrovirus core gene ac96 encodes a per Os infectivity factor (PIF-4). *J Virol* 83: 12569-78

33. Sparks WO, Rohlfing A, Bonning BC. 2011. A peptide with similarity to baculovirus ODV-E66 binds the gut epithelium of Heliothis virescens and impedes infection with Autographa californica multiple nucleopolyhedrovirus. *J Gen Virol*

34. Nie Y, Fang M, Erlandson MA, Theilmann DA. 2012. Analysis of the AcMNPV overlapping gene pair lef3 and ac68 reveals that AC68 is a per os infectivity factor (PIF6) and LEF3 is critical, but not essential for virus replication. *J Virol*

35. Jiantao L, Zhu L, Zhang S, Deng Z, Huang Z, et al. 2016. The Autographa californica multiple nucleopolyhedrovirus ac110 Gene Encodes a New Per Os Infectivity Factor. *Virus Res*

36. Javed MA, Biswas S, Willis LG, Harris S, Pritchard C, et al. 2017. 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* 91

37. Wang X, Liu X, Makalliwa GA, Li J, Wang H, et al. 2017. Per os infectivity factors: a complicated and evolutionarily conserved entry machinery of baculovirus. *Sci China Life Sci*

38. Huang Z, Pan M, Zhu S, Zhang H, Wu W, et al. 2016. The Autographa californica nucleopolyhedrovirus ac83 gene contains a cis-acting element that is essential for nucleocapsid assembly. *J Virol*

39. Peng K, van Oers MM, Hu Z, van Lent JWM, Vlak JM. 2010. Baculovirus Per Os Infectivity Factors Form a Complex on the Surface of Occlusion-Derived Virus. *J. Virol.* 84: 9497-504

40. Peng K, van Lent JW, Boeren S, Fang M, Theilmann DA, et al. 2012. Characterization of novel components of the baculovirus per os infectivity factor (PIF) complex. *J Virol*

41. Boogaard B, van Lent JWM, Theilmann DA, Erlandson MA, van Oers MM. 2017. Baculoviruses require an intact ODV entry-complex to resist proteolytic degradation of per os infectivity factors by co-occluded proteases from the larval host. *J Gen Virol*

42. Chen YR, Zhong S, Fei Z, Hashimoto Y, Xiang JZ, et al. 2013. The transcriptome of the baculovirus *Autographa californica* Multiple Nucleopolyhedrovirus (AcMNPV) in *Trichoplusia ni* cells. *J Virol* 87: 6391-405

43. Zheng Q, Shen Y, Kon X, Zhang J, Feng M, Wu X. 2017. Protein-Protein Interactions of the Baculovirus Per Os Infectivity Factors in the PIF Complex. *J Gen Virol*

44. Granados RR. 1978. Early events in the infection of *Heliothis zea* midgut cells by a baculovirus. *Virology* 90: 170-74

45. Goley ED, Ohkawa T, Mancuso J, Woodruff JB, D'Alessio JA, et al. 2006. Dynamic nuclear actin assembly by Arp2/3 complex and a baculovirus WASP-like protein. *Science* 314: 464-7

46. Goley ED, Rammohan A, Znameroski EA, Firat-Karalar EN, Sept D, Welch MD. 2010. An actin-filament-binding interface on the Arp2/3 complex is critical for nucleation and branch stability. *Proc Natl Acad Sci U S A* 107: 8159-64

47. Ohkawa T, Volkman LE, Welch MD. 2010. Actin-based motility drives baculovirus transit to the nucleus and cell surface. *J Cell Biol* 190: 187-95

48. Au S, Panté N. 2012. Nuclear transport of baculovirus: Revealing the nuclear pore complex passage. *J Struct Biol* 177: 90–98

49. Au S, Wu W, Pante N. 2013. Baculovirus nuclear import: open, nuclear pore complex (NPC) sesame. *Viruses* 5: 1885-900

50. Au S, Wu W, Zhou L, Theilmann DA, Pante N. 2016. A new mechanism for nuclear import by actin-based propulsion used by a baculovirus nucleocapsid. *J Cell Sci* 129: 2905-11

51. Washburn JO, Chan EY, Volkman LE, Aumiller JJ, Jarvis DL. 2003. Early synthesis of budded virus envelope fusion protein GP64 enhances Autographa californica multicapsid nucleopolyhedrovirus virulence in orally infected Heliothis virescens. *J. Virol.* 77: 280-90

52. Guan Z, Zhong L, Li C, Wu W, Yuan M, Yang K. 2016. The Autographa californica multiple nucleopolyhedrovirus ac54 gene is crucial for the localization of the major capsid protein VP39 at the site of nucleocapsid assembly. *J Virol*

53. Marek M, Merten OW, Galibert L, Vlak JM, van Oers MM. 2011. Baculovirus VP80 protein and the F-actin cytoskeleton interact connecting the viral replication factory with the nuclear periphery. *J Virol* 85: 5350-62

54. Rosinski M, Reid S, Nielsen LK. 2002. Kinetics of baculovirus replication and release using real-time quantitative polymerase chain reaction. *Biotechnol Bioeng* 77: 476-80

55. Braunagel SC, Russell WK, Rosas-Acosta G, Russell DH, Summers MD. 2003. Determination of the protein composition of the occlusion-derived virus of *Autographa californica* nucleopolyhedrovirus. *Proc. Natl. Acad. Sci. U S A* 100: 9797-802

56. Hou D, Zhang L, Deng F, Fang W, Wang R, et al. 2013. Comparative Proteomics Reveal Fundamental Structural and Functional Differences between the Two Progeny Phenotypes of a Baculovirus. *J Virol* 87: 829-39

57. Wang R, Deng F, Hou D, Zhao Y, Guo L, et al. 2010. Proteomics of the *Autographa californica* Nucleopolyhedrovirus budded virions. *J Virol* 84: 7233-42

58. Shi Y, Li K, Tang P, Li Y, Zhou Q, et al. 2015. Three-dimensional visualization of the Autographa californica multiple nucleopolyhedrovirus occlusion-derived virion envelopment process gives new clues as to its mechanism. *Virology* 476c: 298-303

59. Tanada Y, Hess RT. 1976. *Development of a nuclear polyhedrosis virus in midgut cells and penetration of the virus into the hemocoel of the armyworm, Pseudaletia unipuncta*: Journal of invertebrate pathology. July 1976, 28 (1). p. 67-76.

60. Braunagel SC, Burks JK, Rosas-Acosta G, Harrison RL, Ma H, Summers MD. 1999. Mutations within the Autographa californica nucleopolyhedrovirus FP25K gene decrease the accumulation of ODV-E66 and alter its intranuclear transport. *J. Virol.* 73: 8559-70

61. Braunagel SC, Cox V, Summers MD. 2009. Baculovirus data suggest a common but multifaceted pathway for sorting proteins to the inner nuclear membrane. *J Virol* 83: 1280-8

62. Braunagel SC, He H, Ramamurthy P, Summers MD. 1996. Transcription, translation, and cellular localization of three *Autographa californica* nuclear polyhedrosis virus structural proteins: ODV-E18, ODV-E35, and ODV-EC27. *Virology* 222: 100-14

63. Braunagel SC, Elton DM, Ma H, Summers MD. 1996. Identification and analysis of an *Autographa californica* Nuclear Polyhedrosis Virus structural protein of the occlusion-derived virus envelope: ODV-E56. *Virology.* 217: 97-110

64. Braunagel SC, Summers MD. 1994. Autographa californica nuclear polyhedrosis virus, PDV, and ECV viral envelopes and nucleocapsids: Structural proteins, antigens, lipid and fatty acid profiles. *Virology* 202: 315-28

65. Braunagel SC, Williamson ST, Ding Q, Wu X, Summers MD. 2007. Early sorting of inner nuclear membrane proteins is conserved. *Proc. Natl. Acad. Sci. U S A*

66. Burks JK, Summers MD, Braunagel SC. 2007. BV/ODV-E26: a palmitoylated, multifunctional structural protein of Autographa californica nucleopolyhedrovirus. *Virology* 361: 194-203

67. Braunagel SC, Williamson ST, Saksena S, Zhong Z, Russell WK, et al. 2004. Trafficking of ODV-E66 is mediated via a sorting motif and other viral proteins: facilitated trafficking to the inner nuclear membrane. *Proc. Natl. Acad. Sci. U S A* 101: 8372-7

68. Hu Z, Yuan M, Wu W, Liu C, Yang K, Pang Y. 2010. Autographa californica multiple nucleopolyhedrovirus ac76 is involved in intranuclear microvesicle formation. *J Virol* 84: 7437-47

69. Wei D, Wang Y, Zhang X, Hu Z, Yuan M, Yang K. 2014. Autographa californica Nucleopolyhedrovirus Ac76: a dimeric type II integral membrane protein that contains an inner nuclear membrane-sorting motif. *J Virol* 88: 1090-103

70. Yuan M, Huang Z, Wei D, Hu Z, Yang K, Pang Y. 2011. Identification of Autographa californica nucleopolyhedrovirus ac93 as a core gene and its requirement for intranuclear microvesicle formation and nuclear egress of nucleocapsids. *J Virol*

71. Fraser MJ. 1986. Ultrastructural observations of virion maturation in *Autographa californica* nuclear polyhedrosis virus infected *Spodoptera frugiperda* cell cultures. *J Ultrastruct Mol Struct Res* 95: 189-95

72. Yuan M, Wu W, Liu C, Wang Y, Hu Z, et al. 2008. A highly conserved baculovirus gene p48 (ac103) is essential for BV production and ODV envelopment. *Virology* 379: 87-96

73. McCarthy CB, Theilmann DA. 2008. AcMNPV ac143 (odv-e18) is essential for mediating budded virus production and is the 30th baculovirus core gene. *Virology* 375: 277-91

74. McCarthy CB, Dai X, Donly C, Theilmann DA. 2008. Autographa californica multiple nucleopolyhedrovirus ac142, a core gene that is essential for BV production and ODV envelopment. *Virology* 372: 325-39

75. Song J, Wang R, Deng F, Wang H, Hu Z. 2008. Functional studies of per os infectivity factors of Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus. *J Gen Virol* 89: 2331-8

76. Harrison RL, Sparks WO, Bonning BC. 2010. Autographa californica multiple nucleopolyhedrovirus ODV-E56 envelope protein is required for oral infectivity and can be substituted functionally by Rachiplusia ou multiple nucleopolyhedrovirus ODV-E56. *J Gen Virol* 91: 1173-82

77. Volkman LE, Talhouk SN, Oppenheimer DI, Charlton CA. 1992. Nuclear F Actin a Functional Component Of Baculovirus-Infected Lepidopteran Cells? *J Cell Sci* 103: 15-22

78. Ke J, Wang J, Deng R, Wang X. 2008. Autographa californica multiple nucleopolyhedrovirus ac66 is required for the efficient egress of nucleocapsids from the nucleus, general synthesis of preoccluded virions and occlusion body formation. *Virology* 374: 421-31

79. Wu W, Passarelli AL. 2010. Autographa californica M nucleopolyhedrovirus Ac92 (ORF92, P33) is required for budded virus production and multiply-enveloped occlusion-derived virus formation. *J Virol* 84: 12351-61

80. Feng G, Thumbi DK, de Jong J, Hodgson JJ, Arif BM, et al. 2012. Selection and characterization of Autographa californica multiple nucleopolyhedrovirus DNA polymerase mutations. *J Virol* 86: 13576-88

81. Xu YP, Gu LZ, Lou YH, Cheng RL, Xu HJ, et al. 2012. A baculovirus isolated from the wild silkworm encompasses the host ranges of Bombyx mori NPV and Autographa californica MNPV in cultured cells. *J Gen Virol*

82. Washburn JO, Trudeau D, Wong JF, Volkman LE. 2003. Early pathogenesis of *Autographa californica* multiple nucleopolyhedrovirus and *Helicoverpa zea* single nucleopolyhedrovirus in *Heliothis virescens*: a comparison of the 'M' and 'S' strategies for establishing fatal infection. *J Gen Virol* 84: 343-51

83. Lopez-Ferber M, Simon O, Williams T, Caballero P. 2003. Defective or effective? Mutualistic interactions between virus genotypes. *Proc Biol Sci* 270: 2249-55

84. Minion FC, Coons LB, Broome JR. 1979. Characterization of the polyhedral envelope of the nuclear polyhedrosis virus of *Heliothis virescens*. *J. Invertebr. Pathol.* 34: 303-07

85. Lee SY, Poloumienko A, Belfry S, Qu X, Chen W, et al. 1996. A common pathway for p10 and calyx proteins in progressive stages of polyhedron envelope assembly in AcMNPV-infected Spodoptera frugiperda larvae. *Arch Virol* 141: 1247-58

86. Gombart AF, Pearson MN, Rohrmann GF, Beaudreau GS. 1989. A baculovirus polyhedral envelope-associated protein: genetic location, nucleotide sequence, and immunocytochemical characterization. *Virology* 169: 182-93

87. Whitt MA, Manning JS. 1988. A phosphorylated 34-kDa protein and a subpopulation of polyhedrin are thiol linked to the carbohydrate layer surrounding a baculovirus occlusion body. *Virology* 163: 33-42

88. Vlak JM, Klinkenberg FA, Zaal K, Usmany M, KlingeRoode EC, Geervliet J. 1988. Functional studies on the p10 gene of Autographa californica nuclear polyhedrosis virus using a recombinant expressing a p10- beta -galactosidase fusion gene. *J. Gen. Virol.* 69: 765-76

89. Ros VI, van Houte S, Hemerik L, van Oers MM. 2015. Baculovirus-induced tree-top disease: how extended is the role of egt as a gene for the extended phenotype? *Mol Ecol* 24: 249-58

90. Hoover K, Grove M, Gardner M, Hughes DP, McNeil J, Slavicek J. 2011. A gene for an extended phenotype. *Science* 333: 1401

91. Kamita SG, Nagasaka K, Chua JW, Shimada T, Mita K, et al. 2005. A baculovirus-encoded protein tyrosine phosphatase gene induces enhanced locomotory activity in a lepidopteran host. *Proc Natl Acad Sci U S A* 102: 2584-9

92. Hu Y. 2006. Baculovirus vectors for gene therapy. In *Advances in Virus Research*, ed. BC Bonning, K Maramorosch, JS Aaron, pp. 287-320: Academic Press

93. Hu YC. 2008. Baculoviral vectors for gene delivery: a review. *Curr Gene Ther* 8: 56-65

94. Boyce FM, Bucher NLR. 1996. Baculovirus-mediated gene transfer into mammalian cells. *Proc. Natl. Acad. Sci. USA* 93: 2348-52

95. Deng F, Wang R, Fang M, Jiang Y, Xu X, et al. 2007. Proteomics analysis of Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus identified two new occlusion-derived virus-associated proteins, HA44 and HA100. *J Virol* 81: 9377-85

96. Volkman LE, Goldsmith PA, Hess RT, Faulkner P. 1984. Neutralization of budded *Autographa californica* NPV by a monoclonal antibody: Identification of the target antigen. *Virology* 133: 354-62

97. Guarino LA, Smith G, Dong W. 1995. Ubiquitin is attached to membranes of baculovirus particles by a novel type of phospholipid anchor. *Cell* 80: 301-09

98. Haas AL, Katzung DL, Reback PM, Guarino LA. 1996. Functional characterization of the ubiquitin variant encoded by the baculovirus Autographa californica. *Biochemistry.* 35: 5385-94

99. Reilly LM, Guarino LA. 1996. The viral ubiquitin gene of Autographa californica nuclear polyhedrosis virus is not essential for viral replication. *Virology* 218: 243-47

100. Biswas S, Willis LG, Fang M, Nie Y, Theilmann DA. 2017. Autographa californica nucleopolyhedrovirus AC141 (Exon0), a potential E3 ubiquitin ligase, interacts with viral ubiquitin and AC66 to facilitate nucleocapsid egress. *J Virol*

101. Katsuma S, Tsuchida A, Matsuda-Imai N, Kang W, Shimada T. 2011. Role of the ubiquitin-proteasome system in Bombyx mori nucleopolyhedrovirus infection. *Journal of General Virology* 92: 699-705

102. Backovic M, Jardetzky TS. 2009. Class III viral membrane fusion proteins. *Curr Opin Struct Biol* 19: 189-96

103. Garry CE, Garry RF. 2008. Proteomics computational analyses suggest that baculovirus GP64 superfamily proteins are class III penetrenes. *Virol J* 5: 28

104. Kadlec J, Loureiro S, Abrescia NG, Stuart DI, Jones IM. 2008. The postfusion structure of baculovirus gp64 supports a unified view of viral fusion machines. *Nat Struct Mol Biol* 15: 1024-30

105. Wang M, Wang J, Yin F, Tan Y, Deng F, et al. 2013. Unraveling the entry mechanism of baculoviruses and evolutionary implications. *J Virol*

106. Pearson MN, Rohrmann GF. 2002. Transfer, incorporation, and substitution of envelope fusion proteins among members of the Baculoviridae, Orthomyxoviridae, and Metaviridae (insect retrovirus) families. *J. Virol.* 76: 5301-4

107. Garry CE, Garry RF. 2009. Proteomics computational analyses suggest that the bornavirus glycoprotein is a class III viral fusion protein (gamma penetrene). *Virol J* 6: 145

108. Backovic M, Jardetzky TS. 2011. Class III viral membrane fusion proteins. *Adv Exp Med Biol* 714: 91-101

109. Sun X, Belouzard S, Whittaker GR. 2008. Molecular architecture of the bipartite fusion loops of vesicular stomatitis virus glycoprotein G, a class III viral fusion protein. *J Biol Chem* 283: 6418-27

110. Dong S, Blissard GW. 2012. Functional analysis of the *Autographa californica* multiple nucleopolyhedrovirus GP64 terminal fusion loops and interactions with membranes. *J Virol* 86: 9617-28

111. Falanga A, Tarallo R, Vitiello G, Vitiello M, Perillo E, et al. 2012. Biophysical characterization and membrane interaction of the two fusion loops of glycoprotein B from herpes simplex type I virus. *PLoS ONE* 7: e32186

112. Heldwein EE, Lou H, Bender FC, Cohen GH, Eisenberg RJ, Harrison SC. 2006. Crystal structure of glycoprotein B from herpes simplex virus 1. *Science* 313: 217-20

113. Li Z, Blissard GW. 2010. Baculovirus GP64 disulfide bonds: The intermolecular disulfide bond of AcMNPV GP64 is not essential for membrane fusion and virion budding. *J. Virol.* 84: 8584-89

114. Zanotto PM, Kessing BD, Maruniak JE. 1993. Phylogenetic interrelationships among baculoviruses: evolutionary rates and host associations. *J Invertebr Pathol* 62: 147-64

115. Wang Q, Vlak(advisor) J. 2015. *Structural and functional analysis of the baculovirus envelope fusion protein F*. Ph.D thesis. Wageningen University, Wageningen University, NL

116. Lung OY, Cruz-Alvarez M, Blissard GW. 2003. Ac23, an envelope fusion protein homolog in the baculovirus *Autographa californica* multicapsid nucleopolyhedrovirus, is a viral pathogenicity factor. *J. Virol.* 77: 328-39

117. Lung O, Westenberg M, Vlak JM, Zuidema D, Blissard GW. 2002. Pseudotyping *Autographa californica* multicapsid Nucleopolyhedrovirus (AcMNPV): F proteins from Group II NPVs are functionally analogous to AcMNPV GP64. *J. Virol.* 76: 5729-36

118. Pearson MN, Groten C, Rohrmann GF. 2000. Identification of the *Lymantria dispar* nucleopolyhedrovirus envelope fusion protein provides evidence for a phylogenetic division of the Baculoviridae. *J. Virol.* 74: 6126-31

119. Rohrmann GF, Karplus PA. 2001. Relatedness of baculovirus and gypsy retrotransposon envelope proteins. *BMC Evol Biol* 1:1

120. Malik HS, Henikoff S, Eickbush TH. 2000. Poised for contagion: evolutionary origins of the infectious abilities of invertebrate retroviruses. *Genome Res* 10: 1307-18.

121. Pearson MN, Rohrmann GF. 2006. Envelope gene capture and insect retrovirus evolution: the relationship between errantivirus and baculovirus envelope proteins. *Virus Res* 118: 7-15

122. Lung O, Blissard GW. 2005. A cellular *Drosophila melanogaster* protein with similarity to baculovirus F envelope fusion proteins. *J. Virol.* 79: 7979-89

123. Malik HS, Henikoff S. 2005. Positive Selection of Iris, a Retroviral Envelope-Derived Host Gene in Drosophila melanogaster. *PLoS Genet* 1: e44

124. Westenberg M, Vlak JM. 2008. GP64 of group I nucleopolyhedroviruses cannot readily rescue infectivity of group II f-null nucleopolyhedroviruses. *J Gen Virol* 89: 424-31

125. Wang M, Tan Y, Yin F, Deng F, Vlak JM, et al. 2008. The F protein of Helicoverpa armigera single nucleopolyhedrovirus can be substituted functionally with its homologue from Spodoptera exigua multiple nucleopolyhedrovirus. *J Gen Virol* 89: 791-8

126. O'Flynn NM, Patel A, Kadlec J, Jones IM. 2012. Improving promiscuous mammalian cell entry by the baculovirus AcMNPV. *Biosci Rep*

127. Marheineke K, Grunewald S, Christie W, Reilander H. 1998. Lipid composition of Spodoptera frugiperda (Sf9) and Trichoplusia ni (Tn) insect cells used for baculovirus infection. *FEBS Lett* 441: 49-52

128. Wickham T, Granados RR, Wood HA, Hammer DA, Shuler ML. 1990. General analysis of receptor-mediated viral attachment to cell surfaces. *Biophys. J.* 58: 1501-16

129. Wang P, Hammer DA, Granados RR. 1997. Binding and fusion of *Autographa californica* nucleopolyhedrovirus to cultured insect cells. *J. Gen. Virol.* 78: 3081-9

130. Wickham TJ, Shuler ML, Hammer DA, Granados RR, Wood HA. 1992. Equilibrium and kinetic analysis of *Autographa californica* Nuclear Polyhedrosis Virus attachment to different insect cell lines. *J. Gen. Virol.* 73: 3185-94

131. Makkonen KE, Turkki P, Laakkonen JP, Yla-Herttuala S, Marjomaki V, Airenne KJ. 2013. 6-O sulfated and N-sulfated Syndecan-1 promotes baculovirus binding and entry into mammalian cells. *J Virol*

132. Duisit G, Saleun S, Douthe S, Barsoum J, Chadeuf G, Moullier P. 1999. Baculovirus vector requires electrostatic interactions including heparan sulfate for efficient gene transfer in mammalian cells. *J Gene Med* 1: 93-102

133. Kamiya K, Kobayashi J, Yoshimura T, Tsumoto K. 2010. Confocal microscopic observation of fusion between baculovirus budded virus envelopes and single giant unilamellar vesicles. *Biochim Biophys Acta* 1798: 1625-31

134. Schlegel R, Tralka TS, Willingham MC, Pastan I. 1983. Inhibition of VSV binding and infectivity by phosphatidylserine: is phosphatidylserine a VSV-binding site? *Cell* 32: 639-46

135. Coil DA, Miller AD. 2004. Phosphatidylserine is not the cell surface receptor for vesicular stomatitis virus. *J Virol* 78: 10920-6

136. Kim IS, Jenni S, Stanifer ML, Roth E, Whelan SP, et al. 2017. Mechanism of membrane fusion induced by vesicular stomatitis virus G protein. *Proc Natl Acad Sci U S A* 114: E28-e36

137. Albertini AA, Baquero E, Ferlin A, Gaudin Y. 2012. Molecular and cellular aspects of rhabdovirus entry. *Viruses* 4: 117-39

138. Tani H, Nishijima M, Ushijima H, Miyamura T, Matsuura Y. 2001. Characterization of cell-surface determinants important for baculovirus infection. *Virology* 279: 343-53.

139. Zhou J, Blissard GW. 2008. Identification of a GP64 subdomain involved in receptor binding by budded virions of the baculovirus AcMNPV. *J. Virol.* 82: 4449–60

140. Nishigami M, Mori T, Tomita M, Takiguchi K, Tsumoto K. 2017. Membrane fusion between baculovirus budded virus-enveloped particles and giant liposomes generated using a droplet-transfer method for the incorporation of recombinant membrane proteins. *Colloids Surf B Biointerfaces* 155: 248-56

141. Kamiya K, Tsumoto K, Arakawa S, Shimizu S, Morita I, et al. 2010. Preparation of connexin43-integrated giant liposomes by a baculovirus expression-liposome fusion. *Biotechnol Bioeng*

142. Hefferon K, Oomens A, Monsma S, Finnerty C, Blissard GW. 1999. Host cell receptor binding by baculovirus GP64 and kinetics of virion entry. *Virology* 258: 455-68

143. Volkman LE, Goldsmith PA. 1985. Mechanism of neutralization of budded *Autographa californica* nuclear polyhedrosis virus by a monoclonal antibody: Inhibition of entry by adsorptive endocytosis. *Virology* 143: 185-95

144. Long G, Pan X, Kormelink R, Vlak JM. 2006. Functional entry of baculovirus into insect and mammalian cells is dependent on clathrin-mediated endocytosis. *J. Virol.* 80: 8830-3

145. Kataoka C, Kaname Y, Taguwa S, Abe T, Fukuhara T, et al. 2011. Baculovirus GP64-mediated entry into mammalian cells. *J Virol*

146. Yue Q, Yu Q, Yang Q, Xu Y, Guo Y, et al. 2018. 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*

147. Guo Y, Yue Q, Gao J, Wang Z, Chen YR, et al. 2017. Roles of cellular NSF protein in entry and nuclear egress of budded virions of Autographa californica multiple nucleopolyhedrovirus. *J Virol* 91: e01111-17

148. Li Z, Blissard G. 2015. The vacuolar protein sorting genes in insects: A comparative genome view. *Insect Biochem Mol Biol* 62: 211-25

149. Li Z, Blissard GW. 2012. Cellular VPS4 is required for efficient entry and egress of budded virions of *Autographa californica* multiple Nucleopolyhedrovirus. *J. Virol.* 86: 459-72

150. Henne WM, Buchkovich NJ, Emr SD. 2011. The ESCRT pathway. *Dev Cell* 21: 77-91

151. Hurley JH. 2015. ESCRTs are everywhere. *Embo j*

152. Chen BJ, Lamb RA. 2008. Mechanisms for enveloped virus budding: can some viruses do without an ESCRT? *Virology* 372: 221-32

153. Votteler J, Sundquist WI. 2013. Virus budding and the ESCRT pathway. *Cell Host Microbe* 14: 232-41

154. Shtanko O, Nikitina RA, Altuntas CZ, Chepurnov AA, Davey RA. 2014. Crimean-Congo hemorrhagic fever virus entry into host cells occurs through the multivesicular body and requires ESCRT regulators. *PLoS Pathog* 10: e1004390

155. Veettil MV, Kumar B, Ansari MA, Dutta D, Iqbal J, et al. 2016. ESCRT-0 Component Hrs Promotes Macropinocytosis of Kaposi's Sarcoma-Associated Herpesvirus in Human Dermal Microvascular Endothelial Cells. *J Virol* 90: 3860-72

156. Pasqual G, Rojek JM, Masin M, Chatton JY, Kunz S. 2011. Old world arenaviruses enter the host cell via the multivesicular body and depend on the endosomal sorting complex required for transport. *PLoS Pathog* 7: e1002232

157. Luyet PP, Falguieres T, Pons V, Pattnaik AK, Gruenberg J. 2008. The ESCRT-I subunit TSG101 controls endosome-to-cytosol release of viral RNA. *Traffic* 9: 2279-90

158. Silva-Ayala D, Lopez T, Gutierrez M, Perrimon N, Lopez S, Arias CF. 2013. Genome-wide RNAi screen reveals a role for the ESCRT complex in rotavirus cell entry. *Proc Natl Acad Sci U S A* 110: 10270-5

159. Blissard GW, Wenz JR. 1992. Baculovirus GP64 envelope glycoprotein is sufficient to mediate pH dependent membrane fusion. *J. Virol.* 66: 6829-35

160. Harrison SC. 2015. Viral membrane fusion. *Virology* 479-480: 498-507

161. Zhou J, Blissard GW. 2006. Mapping the conformational epitope of a neutralizing antibody (AcV1) directed against the AcMNPV GP64 protein. *Virology* 352: 427-37

162. Li Z, Blissard GW. 2011. The AcMNPV GP64 protein: Roles of Histidine residues in triggering membrane fusion and fusion pore expansion. *J. Virol.* 85: 12492-504

163. White JM, Delos SE, Brecher M, Schornberg K. 2008. Structures and Mechanisms of Viral Membrane Fusion Proteins: Multiple Variations on a

Common Theme. *Critical Reviews in Biochemistry and Molecular Biology* 43: 189 - 219

164. Li Z, Blissard GW. 2009. The pre-transmembrane domain of the *Autographa californica* Multicapsid Nucleopolyhedrovirus GP64 protein is critical for membrane fusion and virus infectivity. *J. Virol.* 83: 10993-1004

165. Li Z, Blissard GW. 2009. The *Autographa californica* Multicapsid Nucleopolyhedrovirus (AcMNPV) GP64 protein: Analysis of transmembrane (TM) domain length and sequence requirements *J. Virol.* 83: 4447–61

166. Li Z, Blissard GW. 2008. Functional analysis of the transmembrane (TM) domain of the *Autographa californica* multicapsid nucleopolyhedrovirus GP64 protein: substitution of heterologous TM domains. *J. Virol.* 82: 3329-41

167. Yu Q, Blissard GW, Liu TX, Li Z. 2015. *Autographa californica multiple nucleopolyhedrovirus* GP64 protein: Analysis of domain I and V amino acid interactions and membrane fusion activity. *Virology* 488: 259-70

168. Plonsky I, Cho MS, Oomens AGP, Blissard GW, Zimmerberg J. 1999. An analysis of the role of the target membrane on the gp64-induced fusion pore. *Virology* 253: 65-76

169. Plonsky I, Kingsley DH, Rashtian A, Blank PS, Zimmerberg J. 2008. Initial size and dynamics of viral fusion pores are a function of the fusion protein mediating membrane fusion. *Biol Cell* 100: 377-86

170. Plonsky I, Zimmerberg J. 1996. The initial fusion pore induced by baculovirus GP64 is large and forms quickly. *J. Cell Biol.* 135: 1831-39

171. Markovic I, Pulyaeva H, Sokoloff A, Chernomordik LV. 1998. Membrane fusion mediated by baculovirus gp64 involves assembly of stable gp64 trimers into multiprotein aggregates. *J. Cell Biol.* 143: 1155-66

172. Charlton CA, Volkman LE. 1991. Sequential rearrangement and nuclear polymerization of actin in baculovirus-infected *Spodoptera frugiperda* cells. *J. Virol.* 65: 1219-27

173. Roncarati R, Knebel-Moersdorf D. 1997. Identification of the early actin-rearrangement-inducing factor gene, arif-1, from Autographa californica multicapsid nuclear polyhedrosis virus [published erratum appears in J. Virol. 1998 Jan;72(1):888-9]. *J. Virol.* 71: 7933-41

174. Dreschers S, Roncarati R, Knebel-Moersdorf D. 2001. Actin Rearrangement-Inducing Factor of Baculoviruses Is Tyrosine Phosphorylated and Colocalizes to F-Actin at the Plasma Membrane. *J. Virol.* 75: 3771-8.

175. Braunagel SC, Guidry PA, Rosas-Acosta G, Engelking L, Summers MD. 2001. Identification of BV/ODV-C42, an Autographa californica nucleopolyhedrovirus orf101-encoded structural protein detected in infected-cell complexes with ODV-EC27 and p78/83. *J. Virol.* 75: 12331-8

176. Li K, Wang Y, Bai H, Wang Q, Song J, et al. 2010. The Putative Pocket Protein Binding Site of Autographa californica Nucleopolyhedrovirus BV/ODV-C42 Is Required for Virus-induced Nuclear Actin Polymerization. *J Virol*

177. Wang Y, Zhang Y, Han S, Hu X, Zhou Y, et al. 2015. Identification of a Novel Regulatory Sequence of Actin Nucleation Promoting Factor Encoded by Autographa californica Multiple Nucleopolyhedrovirus. *J Biol Chem*

178. Mueller J, Pfanzelter J, Winkler C, Narita A, Le Clainche C, et al. 2014. Electron tomography and simulation of baculovirus actin comet tails support a tethered filament model of pathogen propulsion. *PLoS Biol* 12: e1001765

179. Fang Z, Li C, Wu W, Yuan M, Yang K. 2016. The Autographa californica multiple nucleopolyhedrovirus Ac132 plays a role in nuclear entry. *J Gen Virol* 97: 3030-38

180. Summers MD, Arnott HJ. 1969. Ultrastructural studies on inclusion formation and virus occlusion in nuclear polyhedrosis and granulosis virus-infected cells of Trichoplusia ni (Hubner). *J Ultrastruct Res* 28: 462-80

181. Summers MD. 1971. Electron microscopic observations on granulosis virus entry, uncoating and replication processes during infection of the midgut cells of Trichoplusia ni. *J Ultrastruct Res* 35: 606-25

182. Fang M, Dai X, Theilmann DA. 2007. Autographa californica Multiple Nucleopolyhedrovirus EXON0 (ORF141) Is Required for Efficient Egress of Nucleocapsids from the Nucleus. *J. Virol.* 81: 9859-69

183. Williams GV, Faulkner P. 1997. Cytological Changes and Viral Morphogenesis during Baculovirus Infection. *In The Baculoviruses. Ed. L. K. Miller. Plenum Press. New York*: pp 61-108

184. Biswas S, Blissard GW, Theilmann DA. 2016. *Trichoplusia ni* Kinesin-1 associates with *Autographa californica* Multiple Nucleopolyhedrovirus nucleocapsid proteins and is required for production of budded virus. *J Virol* 90: 3480-95

185. Danquah JO, Botchway S, Jeshtadi A, King LA. 2011. 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* 86: 844-53

186. Oomens AGP, Blissard GW. 1999. Requirement for GP64 to drive efficient budding of *Autographa californica* Multicapsid Nucleopolyhedrovirus. *Virology* 254: 297-314

187. de Jong J, Theilmann DA, Arif BM, Krell PJ. 2011. Immediate early protein ME53 forms foci and co-localizes with GP64 and the major capsid protein, VP39, at the cell membrane of AcMNPV infected cells. *J Virol*

188. de Jong J, Arif BM, Theilmann DA, Krell PJ. 2009. Autographa californica multiple nucleopolyhedrovirus me53 (ac140) is a non- essential gene required for efficient budded virus production. *J Virol* 83: 7440-8

189. Adams JR, Bonami JR, eds. 1991. *Atlas of Invertebrate Viruses*. Boca Raton: CRC Press

190. Wang Q, Bosch BJ, Vlak JM, van Oers MM, Rottier PJ, van Lent JW. 2016. Budded baculovirus particle structure revisited. *J Invertebr Pathol* 134: 15-22

191. Peng K, van Lent JW, Vlak JM, Hu Z, van Oers MM. 2011. In situ cleavage of the baculovirus occlusion derived virus receptor binding protein P74 in the peroral infectivity complex. *J Virol*

FIGURE LEGENDS

Figure 1.

1A. Baculovirus virion phenotypes and their roles in the infection of host tissues. The diagram illustrates the Primary and Secondary phases of infection. The primary phase of infection occurs when occlusion bodies (OB) disassemble in response to high pH and release occlusion derived virions (ODV) into the lumen of the midgut. After traversing the peritrophic membrane (PM), ODV bind and fuse with microvilli of polarized epithelial cells of the midgut epithelium, releasing nucleocapsids into the cytoplasm. Following viral replication and nucleocapsid assembly in the nucleus (or direct pass-through), nucleocapsids are transported to the basal plasma membrane where they bud to generate the budded virus (BV) phenotype. The BV may infect some cells directly (Tracheal cells and Hemocytes) or may circulate in the hemolymph, infecting other tissues such as Fatbody and Muscle, among others. Infection of other tissues in this Secondary phase of infection, results in the generation of additional BV which further disseminates infection in the animal. ODV and OBs are produced in all cell types and are subsequently released when cells lyse and the animal dissolves or liquefies.

1B. Baculovirus entry, replication, and egress in non-midgut host cells. The diagram represents infection by the alphabaculovirus budded virus (BV) and subsequent viral replication and production of BV and ODV. Following BV binding and entry by clathrin-mediated endocytosis (CME), endosomes are likely transported along microtubules (MT) and upon acidification of the endosome, the nucleocapsid (blue) is released. P78/83 recruitment of the Arp2/3 complex results in initiation of actin polymerization which provides a propulsive force for transporting the nucleocapsid in the cytoplasm and traversing the nuclear pore. Nucleocapsid uncoating in the nucleus results in viral gene expression,

DNA replication, and assembly of progeny nucleocapsids (blue and green) in the virogenic stroma (VS). Of the progeny nucleocapsids, some are tagged for egress from the nucleus (blue) and others for ODV production (green). Nucleocapsids that exit the nucleus are observed in cytoplasmic vesicles (transport vesicles) which appear to later release nucleocapsids into the cytoplasm. Transport of nucleocapsids to the plasma membrane may involve microtubules, actin polymerization, or both. ESCRT pathway proteins are involved in egress at either or both nuclear egress and budding at the plasma membrane. Nucleocapsids destined to become ODV interact with membranes derived from the inner nuclear membrane (INM) to eventually form the ODV which are subsequently occluded by the occlusion body protein (Polyhedrin).

Figure 2.

2A. Comparison of protein components of the two virion phenotypes: BV and ODV from the baculoviruses AcMNPV and HearNPV. Proteins are predicted primarily from proteomic analyses (55-57) as well as a variety of additional studies. Proteins are listed beside representations of BV and ODV. Proteins reported from the envelope or associated with nucleocapsids are subdivided into groups that are either specific to one phenotype (ODV or BV) or common to both. Proteins in common, within the same grouping of AcMNPV and HearNPV, are shown in bold italic and blue. The detailed analysis of HearNPV proteins (56) found some proteins associated with both the envelope and nucleocapsid fractions (*) suggesting that some may be tegument proteins. For simplicity, those proteins are listed in the nucleocapsid fraction. The nomenclature used for all proteins is for the AcMNPV protein unless the protein is specific to HearNPV, in which case the HearNPV protein name is used.

2B. Electron micrographs of AcMNPV replication and virion phenotypes. a) Transmission Electron Micrograph (TEM) showing the various stages of BV and ODV development in an AcMNPV-infected Sf9 cell at approximately 24 h p.i. Membrane microvesicles and nucleocapsids associated with membranes and in the nucleus (N) are indicated by green arrows. Nucleocapsids that are exiting the nucleus in transport vesicles are indicated by red arrows in the cytoplasm (C). The nuclear envelope (NE) and virogenic stroma (S) are also indicated. Blue arrows show nucleocapsids budding at the plasma membrane. A black arrow shows a nucleocapsid being assembled in the stroma. Yellow arrows show non-membrane associated nucleocapsids in the cytoplasm. b) TEM of a section showing end on association of nucleocapsids with membranes in the nucleus and envelopment of nucleocapsids to form ODV, prior to occlusion. c) TEM of a cross section of a mature AcMNPV OB showing embedded ODV with multiple NCs per envelope. Surrounding the OB is the polyhedral calyx (arrow). d) SEM of AcMNPV OBs. e) TEM cross section showing the detailed structure of the ODV. f) Cryo EM image showing detailed structure of AcMNPV BV including the prominent spike proteins in the envelope.
Images b, d, and e are reprinted from reference (189) and f is reprinted from reference (190).

Figure 3.

3A. Schematic diagram depicting the ODV PIF complex comprised of the nine baculovirus core proteins, PIF0-8. While little is known about the topology of the PIF complex, the hypothetical model presented is based on biochemical studies, protein interaction analysis between PIF proteins, and consensus membrane topology predictions resulting from analysis of multiple PIF homologs using the TOPCONS server (http:

http://topcons.cbr.su.se) (36, 41, 43, 191). The PIF complex containing all nine PIF proteins appears to depend on the formation of the core complex consisting of PIF1-4. PIF8 is known to associate with the nucleocapsid as well as the ODV membrane, and PIF8 binds to PIF1 via a central Zinc finger domain.

3B. The crystal structure of the postfusion form of the AcMNPV GP64 protein (104) is shown as a ribbon diagram of the trimer (left) and monomer (center). Locations of the fusion loops (FL1 and FL2) are indicated. An end-on surface view of the fusion loop end of the trimer is shown in the upper right, with the fusion loops 1 (FL1) and 2 (FL2) indicated. A lateral view of the two fusion loops in the monomer is shown at the bottom right.

Fig. 1A

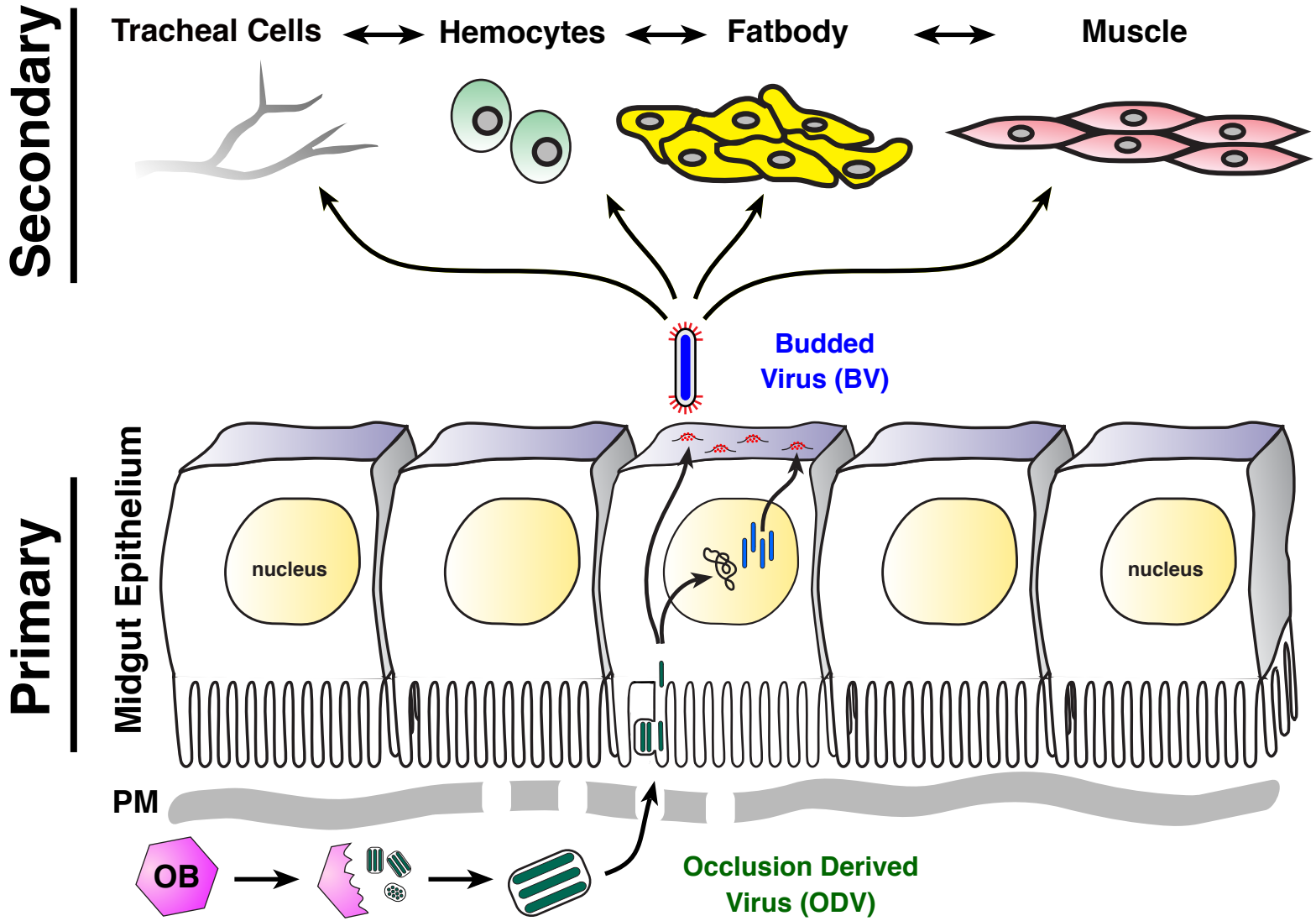
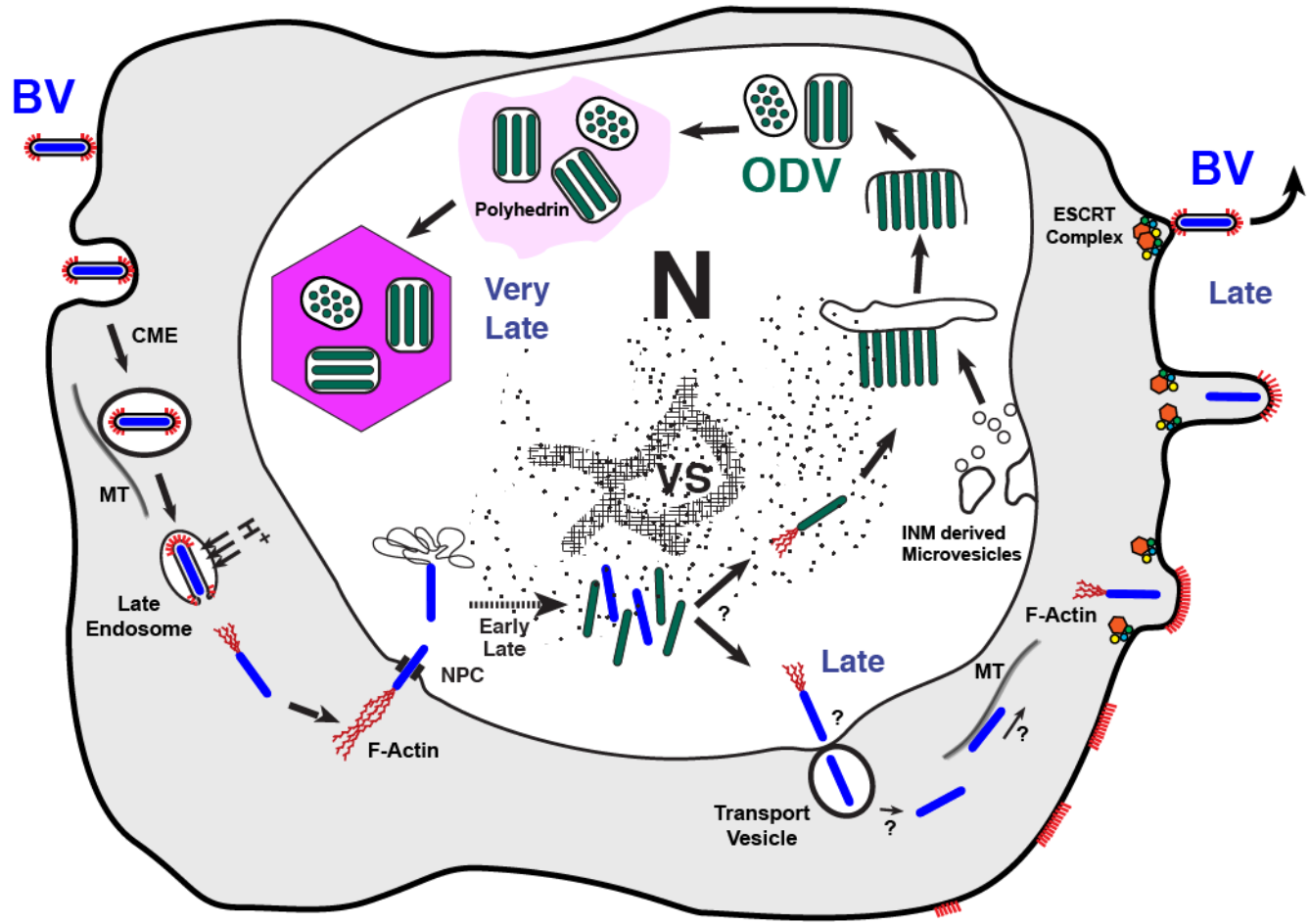

Secondary
Tracheal Cells
Hemocytes
Fatbody
Muscle
Budded
Virus (BV)
Primary
Midgut Epithelium
nucleus
nucleus
PM
OB
Occlusion Derived
Virus (ODV)

Fig. 1B


BV
CME
MT
Late
Endosome
H+
F-Actin
NPC
Early
Late
Polyhedrin
Very
Late
N
ODV
VS
INM derived
Microvesicles
Late
?
Transport
Vesicle
?
MT
?
F-Actin
ESCRT
Complex
BV
Late

Baculovirus Virion Proteins

Nucleocapsid Associated

BV Specific

AcMNPV	HearNPV
Ac1 PTP	Ac17 EC
Ac2 BRO	Ac24 PKIP*
Ac36 PP31	Ac28 LEF6*
Ac51	Ac32 FGF*
Ac53	Ac64 GP37*
Ac71 IAP-2	Ac75
Ac73	Ac103*
Ac82	Ac136 p26
Ac98 38K	Ac139 ME53
Ac124	HA57
Ac126 chitinase	
Ac127 v-cath	
Ac131 PEP	
Ac145	

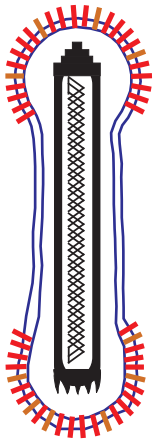
Common to BV and ODV

AcMNPV	HearNPV
Ac9 P78/83	***Ac9-1629 P78/83****
Ac49 PCNA	***Ac58 (HA44)***
Ac54 VP1054	***Ac59 (HA44)***
Ac58	Ac61 FP*
Ac59	***Ac66****
Ac65 DNAPOL	Ac80 GP41*
Ac66	***Ac89 VP39***
Ac74	Ac98 38K
Ac75	***Ac100 P6.9***
Ac77 VLF1	***Ac101 BV/ODV-C42***
Ac83 PIF8	Ac102 P3.1
Ac89 VP39	***Ac104 VP80****
Ac100 P6.9	***Ac129 P24***
Ac101 BV/ODV-C42	Ac131 PEP
Ac104 VP80	Ac144 ODV-EC27
Ac109	***Ac142 49K****
Ac114	Ha44
Ac129	HA100*
Ac132	
Ac139 ME53	
Ac141 EXON0	
Ac142 49K	
Ac144 ODV-EC27	
Ac150	

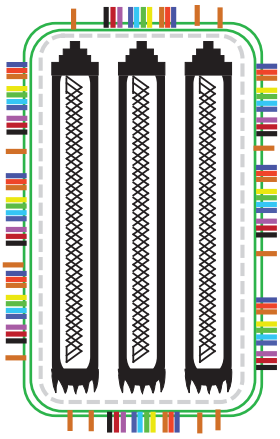
ODV Specific

AcMNPV	HearNPV
Ac5	Ac26*
Ac14-LEF1	Ac51
Ac22	Ac54 VP1054
Ac30	Ac60
Ac39	Ac75
Ac61 FP	Ac77 VLF1*
Ac67 LEF3	Ac81*
Ac70 HCF1	***Ac92 P33***
Ac79	Ac109*
Ac86 PNK/PNL	HOAR*
Ac88 CG30	HA45
Ac92 P33	
Ac95 HELICASE	
Ac102	
Ac103	
Ac133 ALK-EXO	
Ac147 IE1	

Budded Virus (BV)



Occlusion Derived Virus (ODV)



Envelope

BV Specific

AcMNPV	HearNPV
Ac35 vUBI	Ac23 F
Ac64 GP37	Ac74
Ac75	Ac126 ChiA
Ac93 P18	
Ac128 GP64	

Common to BV and ODV

AcMNPV	HearNPV
Ac16BV/OD V-E26	Ac35 v-UBI
Ac23 F	***Ac94 ODV-E25***
Ac76	***Ac143 ODV-E18***
Ac94 ODV-E25	
Ac143 ODV-E18	
Ac148 ODV-E56 PIF5	

ODV Specific

AcMNPV	HearNPV
Ac22 PIF2	***Ac22 PIF2***
Ac46 ODV-E66	Ac31 SOD
Ac68 PIF6	***Ac46 ODV-e66***
Ac80 GP41	***Ac68 PIF6***
Ac83 PIF8	Ac75
Ac96 PIF4	Ac78
Ac110 PIF7	***Ac83 PIF8***
Ac115 PIF3	***Ac96 PIF4***
Ac119 PIF1	***Ac110 PIF7***
Ac138 p74 PIF0	***Ac115 PIF3***
	Ac119 PIF1
	Ac138 p74 PIF0

Fig. 2B

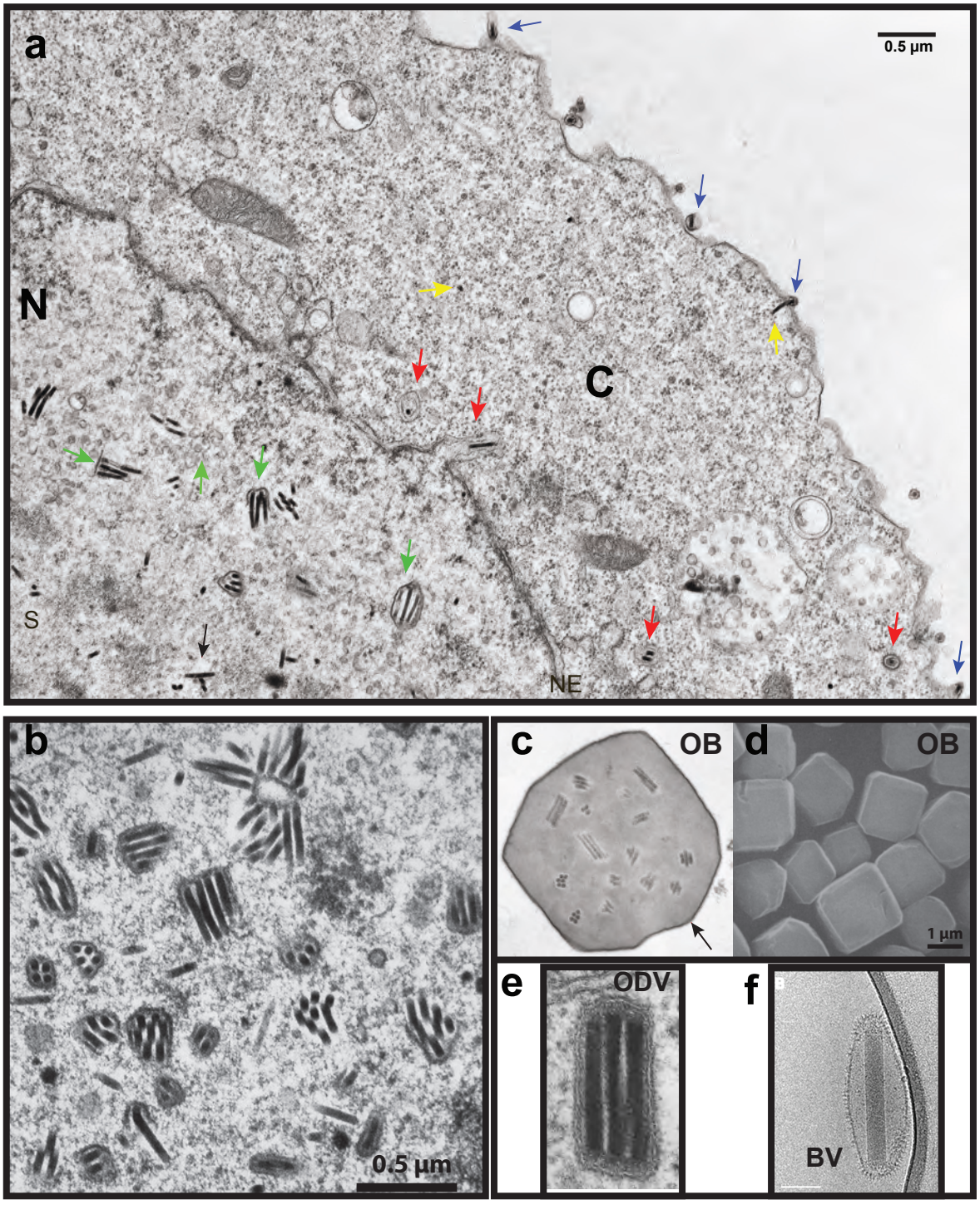
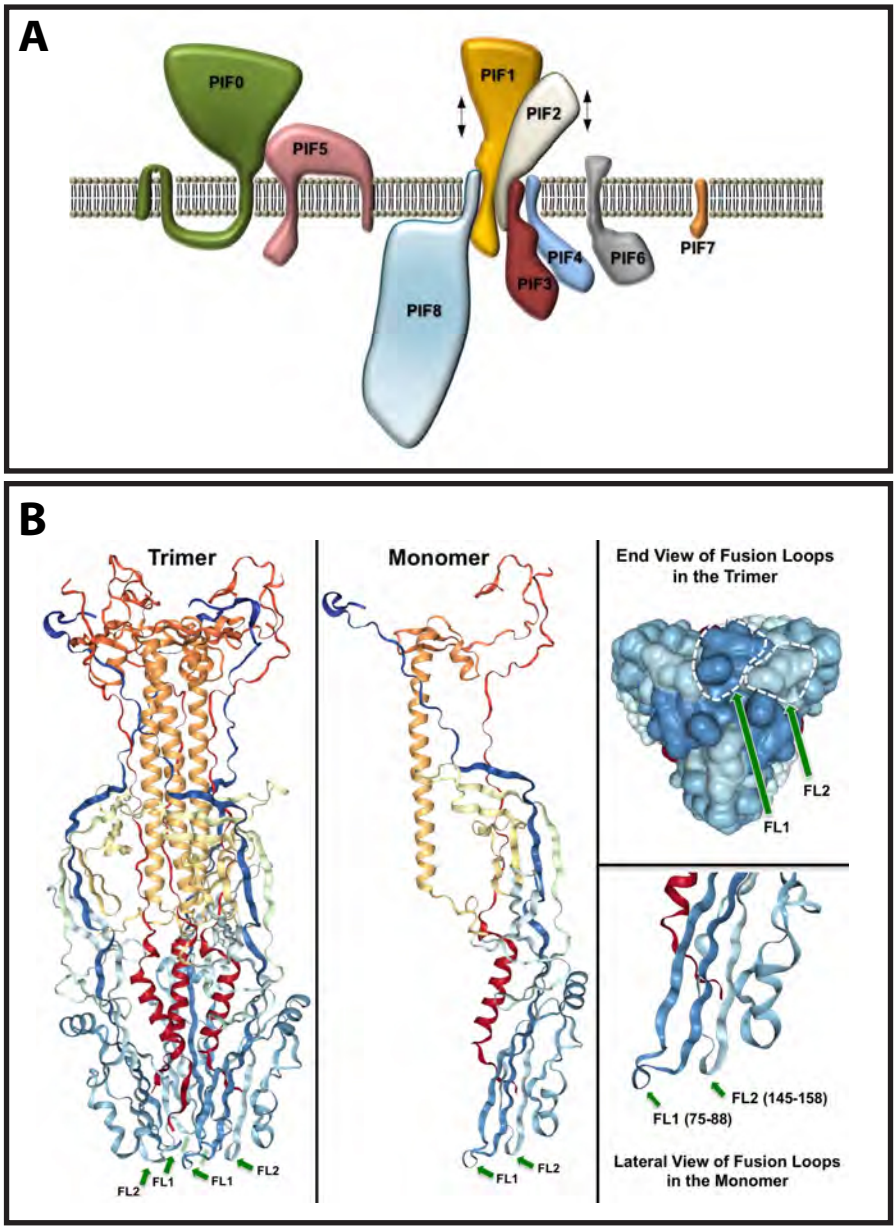


Figure 3


A
PIF0
PIF5
PIF1
PIF2
PIF8
PIF4
PIF3
PIF6
PIF7
B
Trimer
Monomer
End View of Fusion Loops in the Trimer
FL2
FL1
FL2
FL1
FL1
FL2
FL1
FL2
FL2 (145-158)
FL1 (75-88)
Lateral View of Fusion Loops in the Monomer