George F Rohrmann

Baculovirus Molecular Biology

4th edition

Last Updated: 2019

National Center for Biotechnology Information (US)
Bethesda (MD)

National Center for Biotechnology Information (US), Bethesda (MD)

Oregon State University is sponsoring Baculovirus Molecular Biology 4th Edition in association with the author.

NLM Citation: Rohrmann GF. Baculovirus Molecular Biology [Internet]. 4th edition. Bethesda (MD): National Center for Biotechnology Information (US); 2019.

This is the 4th edition of a book that was initiated with the annotation of the function of all the genes in the most commonly studied baculovirus, AcMNPV. As with the previous editions, this information was up-dated and then integrated into chapters covering the major processes central to the replication and pathology of baculoviruses. Topics including taxonomy, genome replication, early and late gene transcription, the application of baculoviruses as insecticides, the molecular basis for the remarkable ability of these viruses to express genes at high levels, and the interrelationships of baculovirus and transposable elements. The 4th edition includes 48 figures and 13 tables, all available for download.

Author

George F Rohrmann
Department of Microbiology, Oregon State University
Corresponding authorrohrmanng@oregonstate.edu

Table of Contents

	Preface	vii
1	Introduction to the baculoviruses, their taxonomy, and evolution	1
2	Structural proteins of baculovirus occlusion bodies and virions	21
3	The baculovirus replication cycle: Effects on cells and insects	57
4	Early events in infection: Virus transcription	83
5	DNA replication and genome processing	97
6	Baculovirus late transcription	125
7	Baculovirus infection: The cell cycle and apoptosis	141
8	Host resistance, susceptibility, and the effect of viral infection on host molecular biology	151
9	Baculoviruses as insecticides: Four examples	163
10	Baculovirus expression technology: Theory and application	169
11	Baculoviruses, retroviruses, DNA transposons (*piggyBac*), and insect cells	185
12	The AcMNPV genome: Gene content, conservation, and function	201
13	Selected baculovirus genes without orthologs in the AcMNPV genome: Conservation and function	277
	Glossary	285

Preface

This is the 4th edition of a book that was initiated with the annotation of the function of all the genes in the most commonly studied baculovirus, AcMNPV. It has been almost six years since I reviewed this literature. As a measure of the research that has occurred over this time, Chapter 12 which reviews all the presumptive genes in the AcMNPV genome went from 481 references to 582, a 21% increase. As with the previous editions, this information is then integrated into chapters covering the major processes central to the replication and pathology of baculoviruses. Topics including taxonomy, the application of baculoviruses as insecticides, the molecular basis for the remarkable ability of these viruses to express genes at high levels, and the interrelationships of baculovirus and transposable elements are also covered. The 4th edition includes 48 figures and 13 tables, all available for download. Some of the most extensive recent reports cover the reaction of cells during viral infection. Whereas, the viral data is fairly straightforward because of the limited number of genes involved, the data from the host involves thousands of genes or their products. They can be put in categories based on function, but the groups are so large that generalization, is difficult, especially in a general review such as this book, because so many of the genes/proteins react differently to the infection. Therefore, although much data has become available, it is up to the researcher to find and interpret it in terms of their own specific interests. For much of the coming metagenomic and proteomic data, this appears to be our future. I am grateful to Drs. T. Ohkawa and Verne Luckow for their comments and suggestions for this edition and Diana Jordan of Bookshelf, NCBI, NLM, NIH for editing this manuscript.

1. Introduction to the baculoviruses, their taxonomy, and evolution

The discovery of baculoviruses

The history of the discovery of baculoviruses is related to the development of the silk industry in China over 5000 years ago. The culture of silkworms spread throughout Asia and reached Japan via Korea by about 300 C.E. arriving in Europe by about 550 C.E. By the 12th century it was established in Italy and Spain spreading to France and England and to Mexico by the 1500s. Silk production has been of major cultural significance in both China and Japan. It was a major item of commerce and in Japan is extensively documented in historic prints (1). Although no longer a major industry, silk production is still practiced symbolically by the Japanese royal family. (For a review of the history of silkworm culture, see (2)). As with any agricultural enterprise, problems were encountered caused by a variety of diseases, and these had to be confronted for the industry to flourish. Even before the germ theory of disease was introduced, a variety of different types of illnesses afflicting silkworms had been described, and methods to mitigate the effects of these diseases had been developed. With the advent of light microscopy, a prominent feature of one of the types of diseases was characterized by the presence of highly refractile occlusion bodies that were symptomatic of the affected insects. These were commonly polyhedron shaped (Figure 1) and led to the naming of the diseases associated with these structures as 'polyhedroses' by the mid-1800s. Although the presence of infectious particles within occlusion bodies had been suggested earlier, it was not until the late 1940s that the presence of rod-shaped virions was convincingly demonstrated by electron microscopy (3). These and other studies demonstrated the crystalline nature of the occlusion body structure. Subsequently, two different types of polyhedrosis diseases were distinguished: those in which the polyhedra developed in nuclei called nuclear polyhedroses (NPVs), and those with occlusion bodies present in the cytoplasm (Figure 2) called cytoplasmic polyhedroses (CPVs) (4). In contrast to the rod-shaped, DNA-containing NPVs, the CPVs have icosahedral capsids and were placed in the Reoviridae (genus Cypovirus), a family of viruses with segmented, double-stranded RNA genomes. A second category of baculovirus characterized by the presence of small, granular, ellipsoidal-shaped occlusion bodies was originally reported in the 1920s (5) was named granulosis viruses (GVs). The division of the baculoviruses into two major groups, the nuclear polyhedrosis viruses (now called nucleopolyhedroviruses (NPV)) (Figure 3) and the granulosis viruses (now called granuloviruses (GVs)) (Figure 4), based on occlusion body morphology defined the major taxonomical divisions of these viruses until the advent of molecular biology.

The terminology for these viruses went through a series of names, and it was not until 1973 that a nomenclature that included Borrelinavirus, Bergoldiavirus, Smithiavirus, Moratorvirus and Vagoiavirus in honor of various historic individuals who had done early research on NPVs and GVs was changed and unified into the Baculoviridae (6). The name baculovirus was proposed by Mauro Martignoni, who because of his Italian-Swiss heritage was a Latin scholar. He suggested that they be named baculoviruses (family Baculoviridae) because of the rod-shape of their virions, which is derived from Latin *baculum* — cane, walking stick, staff. For an excellent biography of Prof. Martignoni, see (7).

The significance of baculoviruses in Nature

Although much of the early interest in baculoviruses was due to the threat they posed to the silk industry, baculoviruses play a major role in the control of natural insect populations. For example, they are a major regulator of gypsy moth populations in North America and in some instances have been shown to be responsible for over 50% of the mortality observed (8). They also are major contributors to the collapse of Douglas-fir tussock moth outbreaks (9). In addition to forest insect populations, they also appear to be important in the natural control of agricultural pests of human food crops, and as a result they may be a substantial contaminant of the human diet. For example, in one study it was found that cabbage purchased from 5 different supermarkets in the Washington D.C. area were all contaminated with baculoviruses to such an extent that each serving (about

100 cm^2 of leaf material) would contain up to 10^8 polyhedra of an NPV pathogenic for the cabbage looper, *Trichoplusia ni* (10)!

The definition is not in the name: Naming baculoviruses

Baculoviruses are normally named for the initial host from which they were isolated. Consequently, the type NPV species, AcMNPV, was named for its host, the alfalfa looper, *Autographa californica* (Ac). This naming would be straightforward except that AcMNPV infects a wide variety of lepidopteran insects and its name originated because of its initial association. Consequently, a variety of other virus isolates e.g., *Galleria mellonella* (GmNPV), *Rachiplusia ou* (RoNPV), and *Plutella xylostella* (PlxyNPV), although having unique names, are closely related (~96-98.5% at the amino acid sequence level) variants of AcMNPV. In addition, viruses were originally named by the first letter of the genus and species of their host. However, as more viruses were discovered, some infected different insects that had names with the same first letters. This resulted in different viruses with the same descriptor. Consequently, the first two letters of the genus and species have become the convention, i.e., AcMNPV should really be AucaMNPV. However, since AcMNPV, GmMNPV and RoMNPV have been used so extensively, the original abbreviations have been retained.

What defines a baculovirus?

Genomes and nucleocapsids

Baculoviruses are a very diverse group of viruses with double-stranded, circular, supercoiled genomes, with sizes varying from about 80 to over 180 kb, that encode between 90 and 180 genes. Of these genes, a common set of about 38 homologous, or core genes, has been identified in all baculovirus genomes, and there are probably others that cannot be recognized because of the extent of changes incorporated over time. The genome is packaged in rod-shaped nucleocapsids that are 230–385 nm in length and 40–60 nm in diameter (11, 12). In the most well characterized baculoviruses, the virions are present as two types, occluded virions (ODV) and budded virions (BV). Although these two types of virions are similar in their nucleocapsid structure, they differ in the origin and composition of their envelopes and their roles in the virus life cycle (see Chapter 2).

Occlusion bodies

Members of the Baculoviridae are characterized by their presence in occlusion bodies called polyhedra for NPVs and granules or capsules for GVs (Figure 3, Figure 4). Polyhedra are about 0.6–2 μM in diameter, whereas granules are oval-shaped with diameters of about 0.2–0.4 μM (11). Occlusion bodies are highly stable and can resist most normal environmental conditions thereby allowing virions to remain infectious indefinitely. Evidence suggests that they can survive passage through the gastrointestinal tract of birds, which can facilitate their dispersal (13, 14). The occlusion body consists of a crystalline matrix composed of a protein called polyhedrin in NPVs and granulin in GVs. Although they have different names, these two proteins are closely related.

Recently, nudiviruses, which may be occluded or non-occluded, have been shown to be related to baculoviruses (15). Therefore, in the future occlusion might not be considered an exclusive feature of the Baculoviridae.

Baculovirus hosts

Over the years, baculoviruses have been reported from a variety of different species of invertebrates. However, the only well-documented hosts are Diptera, Hymenoptera, and Lepidoptera. Convincing documentation has been reported for occluded virions resembling NPVs in a caddis fly (Trichoptera) (16) and a shrimp species (17, 18) (see below). An occluded baculovirus-like virus was also reported for a thysanuran, but it did not appear to affect its host and transmission studies failed (19). Baculoviruses have also been reported from Orthoptera (20), but later these were classified as entomopox viruses, and from Coleoptera, but these are normally not occluded

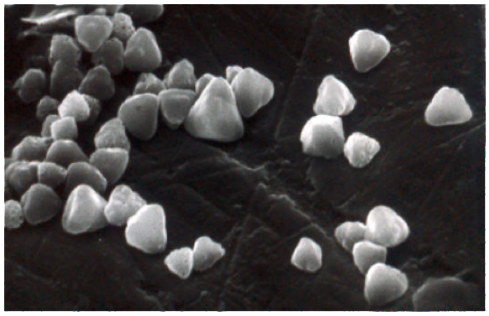

Figure 1. Baculovirus occlusion bodies. Scanning EM by K. Hughes and R. B. Addison.

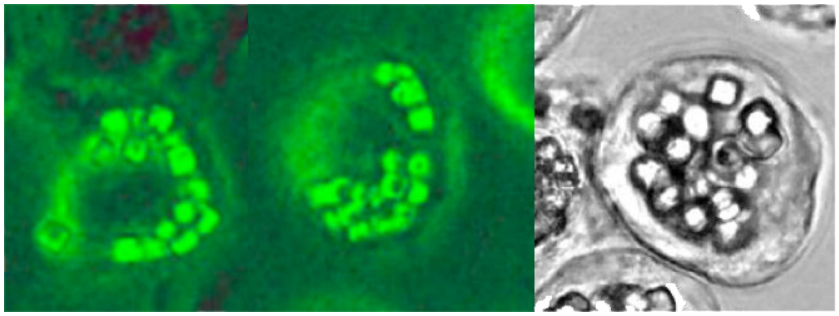

Figure 2. Comparison of cells infected with a cypovirus (CPV – Reoviridae) and a baculovirus. Left panel: CPV of *Orgyia pseudotsugata*. Photo by G. S. Beaudreau. Right Panel: AcMNPV infected *S. frugiperda* cells. From Manji and Friesen (83).

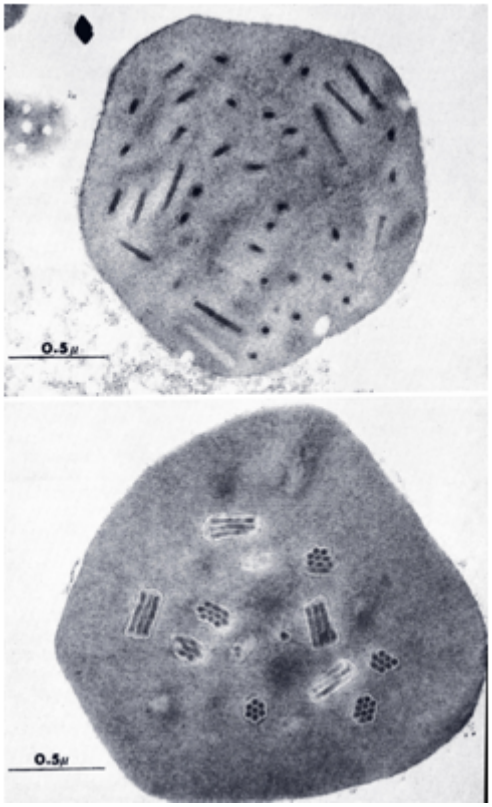

Figure 3. Two nucleopolyhedroviruses pathogenic for *Orgyia pseudotsugata* showing single (top panel) and multiple (bottom panel) nucleocapsids/envelope. Top panel: OpSNPV. Bottom panel: OpMNPV. From Hughes and Addison (38). Copyright 1970 Elsevier. Reproduced with permission via Copyright Clearance Center.

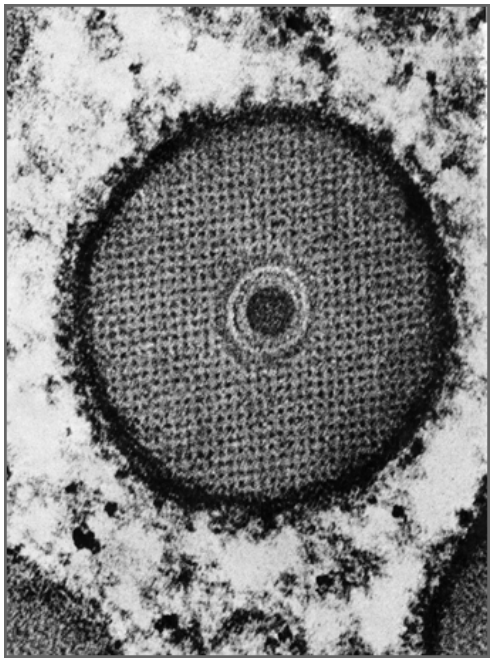

Figure 4. Cross section of a granulovirus of *Plodia interpunctella*. From Arnott and Smith (84). Copyright Elsevier 1967. Reproduced with permission via Copyright Clearance Center.

and were included with the nudiviruses (21). Reports of infection of other insects, e.g., a coleopteran (22) could not be confirmed (Rohrmann, unpublished). In addition, there is a report of a baculovirus infecting a neuropteran (23). However, the infection occurred under laboratory conditions, where neuropterans were fed on lepidoptera that had died of an NPV infection. Consequently, the neuropterans were likely heavily contaminated from their food source, and although they appeared to die of an NPV infection, they were probably exposed to an unusually high virus dose. Naturally infected Neuroptera have not been documented. An example of the distribution of baculoviruses in insects that has been reported in the literature (24) is shown in Table 1. These numbers should be viewed with caution because many of the reports could be of the same virus infecting different species. However, they do give a good general overview of the likely distribution of baculoviruses. Of particular note is that GVs are confined to the Lepidoptera. In addition, all the hymenopteran hosts belong to a suborder called Symphyta that are comprised of sawflies (named because their ovipositor resembles a saw and in some species is used to cut into plants for egg deposition). Sawflies resemble Lepidoptera; they have herbivorous, caterpillar-like larvae and are distinct from the other hymenopteran suborder, Apocrita, which includes bees, ants, and wasps.

Table 1. Distribution1 of baculoviruses in insect orders (24)

Insect orders	NPVs	GVs
Diptera	27	
Hymenoptera (Symphyta)	30	
Lepidoptera	456	148

1 Indicates the number of species that have been reported to be infected

Other defining features of the Baculoviridae: a virus encoded RNA polymerase

In addition to invertebrate hosts, circular, supercoiled double-stranded DNA genomes, rod-shaped enveloped nucleocapsids, and the production of occluded virions, an additional defining feature of baculoviruses is that they encode their own RNA polymerase. The core enzyme is composed of four subunits and functions in the transcription of late promoters that are initiated within a novel sequence element (see Chapter 6). Whereas other DNA viruses of eukaryotes encode their own polymerase, e.g., poxviruses, they replicate in the cytoplasm and therefore do not have access to the host cell transcriptional apparatus located in nuclei. Baculoviruses, in contrast, exploit the host cell transcriptional system for expressing their early genes, but after initiation of DNA replication they are dependent upon their own RNA polymerase for transcription of their late and very late genes. The closely related Nudiviridae also likely encoded their own RNA polymerase related to the baculovirus enzyme.

Baculovirus Diversity

Phylogenetic analysis suggests that large DNA viruses of insects evolved several hundred million years ago along with the first insects (25) (26) and consequently as different insects evolved and proliferated, so did their associated viruses. The recent proliferation of baculovirus genome sequences has greatly expanded our understanding of their diversity and evolution. This has yielded distinct patterns of virus relatedness (Figure 5) in which virus lineages are associated by the host that they infect. The Baculoviridae are divided into four genera (27). The viruses of Lepidoptera are divided into Alpha- and Betabaculoviruses encompassing the NPVs and GVs, respectively, and those infecting Hymenoptera and Diptera are named Gamma- and Deltabaculoviruses, respectively (Table 2). Such patterns of host-associated virus diversity were first observed for small DNA viruses (papilloma and polyoma viruses) of mammals (28) and originally suggested for baculoviruses based on N-terminal polyhedrin and granulin sequences (29). In this process, viruses associate with a host, and as their host becomes genetically isolated and speciates, so does the virus in a process called host-dependent evolution. As more sequence data has become available, this process has been more convincingly demonstrated for baculoviruses (30) and is clearly reflected in Figure 5 in which the major lineages are clustered into clades based on the host insect that they infect. In GVs, host-dependent evolution has been suggested at the level of insect families (31). A major division has been observed in the lepidopteran NPVs that has resulted in the separation of this lineage into two major Groups, I and II (32). These two groups differ significantly in gene content, most notably Group I NPVs use GP64 as their budded virus (BV) fusion protein, whereas Group II NPVs lack *gp64* and utilize a protein called F (33). There are also 11 other genes in addition to *gp64* that appear to be found only in Group I NPVs (Table 3) (34). It has been suggested that the Group I lineage originated when a NPV variant uniquely containing these genes incorporated *gp64* which stimulated their evolution as a distinct lineage (35) (36) (see Chapter 2). Although the list of host insects provides information on baculovirus distribution, sequence analysis of common sets of genes from a wide variety of baculoviruses has provided a picture of the actual extent of their diversity (37).

Table 2. Genera of the Baculoviridae

Genus	Members
Alphabaculovirus	Lepidopteran NPVs
Betabaculovirus	Lepidopteran GVs
Gammabaculovirus	Hymenopteran NPVs
Deltabaculovirus	Dipteran NPVs

Table 3. Genes1 found in and unique to most sequenced Group I NPV genomes

Ac1 (ptp), Ac5, Ac16 (BV-ODV26), Ac27 (iap-1), Ac30, Ac72, Ac73, Ac114, Ac124, Ac128 (gp64), Ac 132, Ac151 (ie2)

[1] Genes are designated by their AcMNPV orf number

Multiple versus single nucleocapsids

A prominent feature of the nucleocapsids within polyhedra is their organization into either single or multiple aggregates of nucleocapsids within an envelope (Figure 3). For example, in some NPVs (called multiple or MNPVs) there can be from 1 to 15 nucleocapsids per envelope, with bundles of 5 to 15 predominating. In contrast, strains defined as having a single nucleocapsid per envelope (called SNPVs) rarely show more than one nucleocapsid per envelope (11, 38). It has been suggested that the MNPV phenotype may accelerate the ability of the virus to establish the infection because if multiple nucleocapsids infect a cell simultaneously, some could enter the nuclei and begin replication whereas others could transit the cell and spread the infection elsewhere (this is discussed in Chapter 3). Another benefit of groups of nucleocapsids infecting a cell would be that they could repair damaged DNA via recombination. Fatally damaged genomes could recombine to produce a viable molecule. Because this feature is so distinctive and characteristic of specific isolates, it was incorporated into the early nomenclature such that NPVs were categorized as either MNPV or SNPVs (also previously called multiply or singly embedded virions (MEV and SEV)). In addition, whereas MNPVs and SNPVs were both found in lepidopteran viruses, only SNPVs were observed in other insect orders. GVs were also categorized as singly enveloped; however, multiple GVs, although rare, have been described (39). With the accumulation of DNA sequence data that allowed for the determination of definitive phylogenetic relationships, it was found that the MNPV and SNPV division did not conform to the phylogeny of the viruses. For example, at one point BmNPV was considered the type virus for SNPVs because of its production of predominantly single nucleocapsids (40). However, sequence data indicates that BmNPV is closely related to AcMNPV (both belong to Group I), whereas other MNPVs such as LdMNPV and SeMNPV are more distantly related Group II viruses, which also includes SNPV-type viruses. Drawing conclusions regarding the properties of MNPV and SNPV viruses is fraught with difficulty because their genetic content is often so different that it is not possible to attribute characteristics of a virus to its M- or SNPV phenotype.

Several genes alter the morphotype. For example, AcMNPV deleted for Ac23, a homolog of the F fusion proteins of Group II viruses, shows an elevated percentage of singly enveloped virions (45%) vs. 11–22% for different virus constructs encoding Ac23 (41). It has also been shown that deletion of Ac92, a gene encoding a sulfhydryl oxidase, results in virions that superficially resemble the SNPV phenotype (42). However, they are not infectious, indicating that they are very different from true SNPVs. It was also observed that point mutations in domains II or III of the AcMNPV DNA polymerase gene caused a dramatic change in the ratios of S- and M- NPV types. For example wt AcMNPV showed about 3% S nucleocapsids in infected cell nuclei, whereas a point mutant in domain III or a double mutant in domains II and III resulted in 61 and 89% single nucleocapsids, respectively (43). Therefore, the expression of some baculovirus genes can influence MNPV production and this may be linked to the efficiency of virion replication or virion integrity.

It also appears that the type of cell infected can influence the morphotype. In a report describing the isolation of a virus pathogenic for *Bombyx mandarina* (BomaNPV S2) (a close relative of BmNPV), it was observed that the virus grew in both Bm5 and *T. ni* cells. In Bm5 cells, the virus was present as an SNPV. However, in T. ni cells it had an MNPV phenotype (44). Therefore, the viral morphotype may be influenced by the physiology of the cell, how the cell responds to viral infection, or cell specific factors.

In summary, the significance of the MNPV morphotype is complex; a specific causal genetic component has not been identified and although it occurs in most Group I *Alphabaculoviruses*, MNPVs are also common in Group II viruses and sometimes are found in the *Betabaculovirus* (GVs). Furthermore, MNPV formation can be influenced by the type of cell infected, suggesting an environmental or physiological influence. Despite

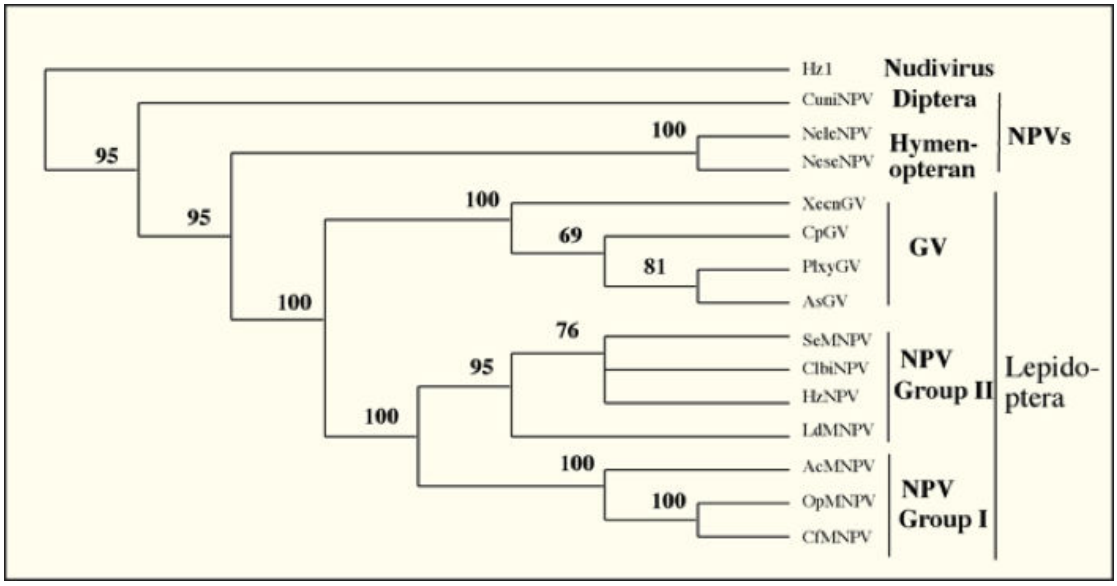

Figure 5. Phylogenetic relatedness of LEF8 from selected baculoviruses. Neighbor joining; bootstrap analysis (1000 reps).

agreement that the MNPV and SNPV designation is not a useful taxonomical trait, it continues to be employed, in part for historical continuity, and also because it can be a convenient method for distinguishing different viruses that are pathogenic for the same host, e.g., OpMNPV and OpSNPV which both infect *Orgyia pseudotsugata* (Figure 3), but are members of Group I and II, respectively. For a detailed review see (45).

Gene content and organization

Although there are major differences in the sizes of baculovirus genomes, a few patterns are evident (Table 4). The viruses of members of the Hymenoptera contain the smallest genomes at a little over 80 kb. In contrast, the GV genomes vary from 101 kb (PlxyGV) up to 178 kb (XecnGV). Group I NPVs cluster around 130 kb, whereas Group II show a much higher degree of diversity, varying from about 130 to almost 170 kb. The small size of the hymenopteran NPV genomes might be attributed to a restricted life cycle confined to replication in insect gut cells (46). Consequently, unlike other baculoviruses that cause systemic infections, genes required for spread throughout the insect, where the virus encounters and replicates in a variety of tissues, might not be necessary. In contrast, the large size of a Group II NPV (LdMNPV), which at 161 kb is about 30 kb larger than many other NPV genomes, can be attributed to a combination of repetitive genes (16 *bro* [baculovirus repeated orf] genes) that add about half of the additional 30 kb, while genes not found in smaller genomes add most of the remainder. These additional genes encode ribonucleotide reductase subunits and two enhancin genes (47). In other viruses with large genomes (e.g., XecnGV), repeated genes including 10 bro and 4 enhancin genes, comprise up to 17% (30 kb) of the genome (48). Despite the large difference in gene content in GV genomes as reflected in their size range, their genomes are surprisingly collinear (49). In contrast, NPVs even from the same order (Lepidoptera) show a high degree of variation (50).

Table 4. Genome size and predicted ORF content* of selected baculoviruses

Virus type	Name of Virus	Size (kb)	Orfs (>50 aa)
Group I (13 members)**	EppoMNPV (85)	119	136
	AnpeNPV (86)	126	145
	AcMNPV (87)	134	~150

Table 4. continued from previous page.

Virus type	Name of Virus	Size (kb)	Orfs (>50 aa)
Group II (20 members)	AdhoNPV (88)	113	125
	SeMNPV(89)	136	139
	AgseNPV (90)	148	153
	LdMNPV(47)	161	163
	LeseNPV (91)	168	169
GV (12 members)	AdorGV (92)	100	119
	CrleGV (49)	111	124
	CpGV (51)	124	143
	XecnGV (48)	179	181
Hymenopt. NPV (3 members)	NeleNPV (93)	82	89
	NeabNPV (94)	84	93
	NeseNPV (95)	86	90
Dipteran NPV (1 member)	CuniNPV (96)	108	109

* Selected from over 50 genome sequences (2010)

**The numbers in brackets indicate the total number of genomes in the category.

Regulatory content: homologous regions (*hrs*)

In addition to containing a set of genes encoding proteins required for productive infection, most baculovirus genomes also contain homologous repeated regions. In AcMNPV, *hrs* are comprised of repeated units of about 70-bp with an imperfect 30-bp palindrome near their center. *Hrs* are repeated at eight locations in the genome with 2 to 8 repeats at each site (see Chapter 4). They are highly variable, and although they are closely related within a genome, they may show very limited homology between different viruses. For example, in the CpGV genome, tandem repeated sequences are not evident, although a 75-bp imperfect palindrome is present at 13 different locations on the genome (51). In addition, in the TnSNPV (group II) sequence, *hrs* were not found (52). *Hrs* have been implicated as both transcriptional enhancers (see Chapter 4) and origins of DNA replication for some baculoviruses (53-58) (see Chapter 5).

Conservation of baculovirus genes: core genes

Despite the diversity in gene content present in different baculovirus genomes, a set of 38 genes that are present in all sequenced baculovirus genomes has been identified (Table 5). Such genes can be very diverse and careful analysis may identify additional core genes. This is particularly true of the dipteran virus that is most distant from the baculoviruses of Lepidoptera and Hymenoptera. Of the conserved genes, about half are virion-associated proteins that are involved in capsid structure, the occlusion-derived virus envelope, and larval infectivity. Most of the others are related to DNA replication or processing, and late or very late transcription. Despite the limited set of genes that baculoviruses share, they do provide insight into some of the major functions required of all baculoviruses such as a common virion structure, the necessity to infect gut cells, and the use of a novel polymerase for the expression of late genes. Furthermore, there are sets of genes specific to, and conserved in, each clade of viruses such as gp64 in Group I NPVs and a number of GV-specific genes (see below). These genes likely reflect major recombination events that altered the properties of the viruses to such an extent that they subsequently developed along distinctive phylogenetic pathways. The observation that different

types of NPVs and GVs and other types of DNA viruses (e.g., entomopox, ascovirus, nudivirus) have the potential to simultaneously infect the same insect indicates how via recombination, genes can be transferred between different viruses and between viruses and the host insect.

Table 5. Conserved genes[1] in baculoviruses, nudiviruses, hytrosaviruses, and polydnaviruses

Conserved in baculoviruses	Conserved in Nudiviruses	Conserved in Hytrosaviruses	Present in polydnavirus (bracovirus)
DNA Replication/processing			
Ac6 Lef2 primase assoc.			
Ac14 Lef1 primase			
Ac65 DNA polymerase	+	+	
Ac95 DNA helicase	+	+	+
Ac133 Alkaline nuclease			
Transcription/RNA polymerase			
Ac50 LEF-8	+	+	+
Ac62 LEF9	+	+	+
Ac40 p47	+		+
Ac90 LEF-4	+		+
Ac77 VLF-1	+		+
Ac99 LEF-5	+	+	+
Structure			
Ac54 vp1054			
Ac66			
Ac80 gp41 tegument			
Ac83 VP91	+		+
Ac89 VP39 capsid	+		+
Ac100 p6.9			
Ac94 ODV-E25			
Ac109			+
Ac142			
Ac143 odv-E18			+
Ac144			
Per os infectivity factors/ODV structure			
Ac22 PIF2	+	+	+
Ac68 PIF6	+		
Ac96 PIF4	+		+
Ac110 Pif7			
Ac115 PIF3	+	+	+
Ac119 PIF1	+	+	+
Ac138 p74 (PIF0)	+	+	+

Table 5. continued from previous page.

Conserved in baculoviruses	Conserved in Nudiviruses	Conserved in Hytrosaviruses	Present in polydnavirus (bracovirus)
Ac148 odv-e56 (pIF5)	+		+
Predicted Enzymes			
Ac53 ubiquitin ligase		+	
Ac92 sulfhydryl oxidase	+	+	+
Ac98 38K phosphatase	+		+
Other			
Ac78			
Ac81	+		
Ac93			
Ac101			
Ac103			

[1] For more details see Chapter 12

Lepidopteran baculovirus (NPV and GV) core genes

In addition to the core genes found in all baculoviruses, lepidopteran baculoviruses encode an additional set of genes that appear to be present in most of their genomes, and about half are also found in Gammabaculovirus (hymenopteran virus) genomes (59) (Table 6). Homologs or functional analogs of many of these genes are likely present in all baculoviruses, but the relationships cannot be detected due to the extent of divergence of the genes. Since they appear to be so central to the replication of the baculoviruses, the single-stranded DNA binding protein, LEF-3, and the transcriptional activator IE-1 may be examples of gene divergence which prevents their detection in the more distantly related viruses. In contrast, homologs of Ac38, an ADP-ribose phosphatase, belong to a family found in many organisms and would likely be detected outside the lepidopteran baculoviruses, if it were present. It acts as a decapping enzyme in vaccinia virus (60) and its presence suggests that the lepidopteran viruses employ molecular strategies significantly different from the other baculovirus genera lacking orthologs of Ac38. Polyhedrin appears to be an example of possible convergent evolution since the occlusion body protein gene of the dipteran virus appears to encode a novel protein (61).

A few genes are present in most Alphabaculoviruses but are found in some, but not all, Betabaculoviruses (34). The most well-characterized of these is Ac9 (orf1629), which is a homolog of WASP proteins that are involved in movement of the virus based on actin polymerization (see Chapter 3). This would suggest that other baculoviruses have major differences in their pathogenesis.

Table 6. Additional Core genes present in all Lepidopteran (Alpha- and Beta-) and some Gammabaculoviruses

AcMNPV orf	Name/function	Presence in Gamma-
Ac8	polyhedrin	+
Ac10	pk-1	
Ac13		
Ac23	related to fusion F protein	

Table 6. continued from previous page.

AcMNPV orf	Name/function	Presence in Gamma-
Ac25	DNA binding protein, dbp	+
Ac28	lef-6	
Ac29		
Ac32	fgf	
Ac36	Pp31/39K	
Ac37	LEF-11	+
Ac46	Odv-E66	
Ac38	ADP-ribose phosphatase, nudix	
Ac53a	LEF-10	
Ac61	Few polyhedra, fp	
Ac67	LEF-3	
Ac75		+
Ac76		+
Ac82	Telokin-like	
Ac102		
Ac106/107		+
Ac110		
Ac139	ME53	
Ac145		+
Ac146	Ie-1	
Ac147	Ie-0	

Granulovirus-specific genes

In addition to their occlusion body morphology, the pathology of GV infection differs from NPVs. For example, their replication is not confined to nuclei as the nucleus and cytoplasm appear to merge during GV infections. The presence of GV specific genes may reflect these differences. Granuloviruses encode a number of genes that are found in most GV genomes. Of these GV genes, 19 appear to be specific to GVs (34), whereas others, although found in all GV genomes, are also found in a few other baculoviruses. Examples of the latter are DNA ligase and helicase-2 homologs that, in addition to GV genomes, are also present in at least one NPV genome (LdMNPV). The helicase-2 homolog is also found in an additional NPV (Maco-B-NPV). There are other GV-specific genes that are found in most, but not all, GV genomes. For additional information or the relatedness of baculovirus genes see (34, 62).

Related viruses: the Nudiviridae

In addition to baculoviruses, there are several groups of viruses pathogenic for invertebrates that have large double-stranded DNA genomes (63). These include nudiviruses, ascoviruses, irridoviruses, and entomopox viruses. However, with the exception of nudiviruses, all these viruses replicate in the cytoplasm or exhibit a

combination of both nuclear and cytoplasmic replication (ascoviruses) (reviewed in (64)). Nudiviruses closely resemble baculoviruses including nuclear replication and rod-shaped, enveloped nucleocapsids. Because of the devastation caused to coconut and oil palm trees by the rhinoceros beetle (*Oryctes rhinoceros*) in the South Pacific in the 1950's, it was of great interest when a virus that could successfully control this insect was discovered in the 1960's (65) (66, 67). Subsequently, similar viruses were identified and were categorized as non-occluded baculoviruses (68). They have double-strand, circular DNA genomes varying in size from 97–230 kb (15). As shown in Table 5, nudivirus genomes contain homologs of up to 20 of the 38 core genes conserved in all baculovirus. These include homologs of genes encoding the novel baculovirus RNA polymerase subunits, virion structural proteins, and a set of genes required for infection of gut tissue called per os infectivity factors or PIFs. Therefore, based on morphology and molecular phylogeny, these two groups of viruses clearly share membership in a distinct viral lineage. They were removed from the Baculoviridae in the 1980s because they were all thought to be nonoccluded and were named nudiviruses (latin *nudi* = naked, bare, uncovered). They also have a somewhat different host range than baculoviruses, having been characterized not only from Lepidoptera and Diptera (69), but also from Coleoptera (the rhinoceros beetle) and Orthoptera (a cricket) (70) and crustacea (Figure 6). Phylogenetic analysis of multiple shared genes shows the separation of the baculovirus and nudivirus into distinct but related lineages (Figure 7) (71).

Examples of two occluded nudiviruses are a dipteran (crane fly), *Tipula oleracea* and the pink shrimp *Penaeus monodon* (Figure 6) both of which were originally thought to be members of the Baculoviridae. However, when their genomes were sequenced, they were both found to be nudiviruses (71). In addition, their occlusion body protein sequences do not appear to be related to baculovirus polyhedrin (29) (71) (72).

Hytrosaviruses

Hytrosaviruses are named for the salivary gland hypertrophy viruses (*hy*per*tro*phy of *sa*livary gland) and have been characterized from several Diptera including the tsetse fly, the vector for sleeping sickness. They are non-occluded and contain large double-stranded DNA genomes and have a virion morphology similar in both size and appearance to baculoviruses. They appear to infect the salivary gland and although not particularly virulent, they can result in a significant reduction in reproductive fitness (73). Genomes of several hytrosaviruses have been sequenced and found to encode homologs of four baculovirus/nudivirus genes involved in per os infectivity including pifs 1-3 and p74 (15). In addition, they also appear to encode orthologs of baculovirus RNA polymerase subunits including LEF-4, -8, -9, but not p47, along with LEF-5 and VLF-1 (74). Phylogenetic analysis indicates that they are a distinct lineage related to baculovirus and nudiviruses (Figure 7).

A polydnavirus connection

Polydnaviruses differ from most viruses because their virions do not contain genetic material for their own replication (75). Their genomes are integrated into their host DNA and they are produced in the ovaries of parasitic wasps. The virus-like particles are injected into host lepidopteran larvae along with the wasp eggs. They were originally named because they contain multiple circular double-stranded DNA molecules (polydispersed DNA) encompassing up to 800 kb that encode gene products that compromise the target host immune system and other processes and are essential for the successful development of the wasp egg. However, another group has been identified that packages virulence proteins rather than DNA. There are two lineages of parasitic wasps that employ these elements; the braconid and ichneumonids, producing bracovirus and ichnovirus polydnavirus virus-like particles, respectively. When cDNAs produced from RNA associated with polydnavirus production in braconid ovaries were analyzed, a number were found to exhibit sequence homology to genes encoded by nudiviruses and baculoviruses (76, 77). Additional such genes were identified by deep sequencing (78) (Table 5). The genes identified included per os infectivity factors that are involved in infection of mid-gut cells by baculoviruses and genes encoding subunits similar to the baculovirus RNA polymerase and a very late transcription factor, VLF-1. When the structural proteins of the polydnavirus virions were analyzed, proteins

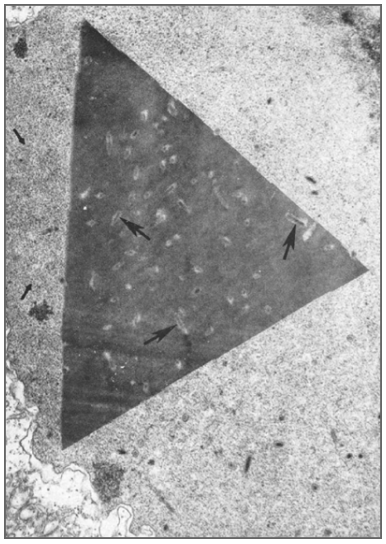

Figure 6. Polyhedral inclusion body of a nudivirus of the pink shrimp, *Penaeus monodon*. Arrows indicated nucleocapsids. From Couch (17). Reproduced with permission of Elsevier Limited via Copyright Clearance Center.

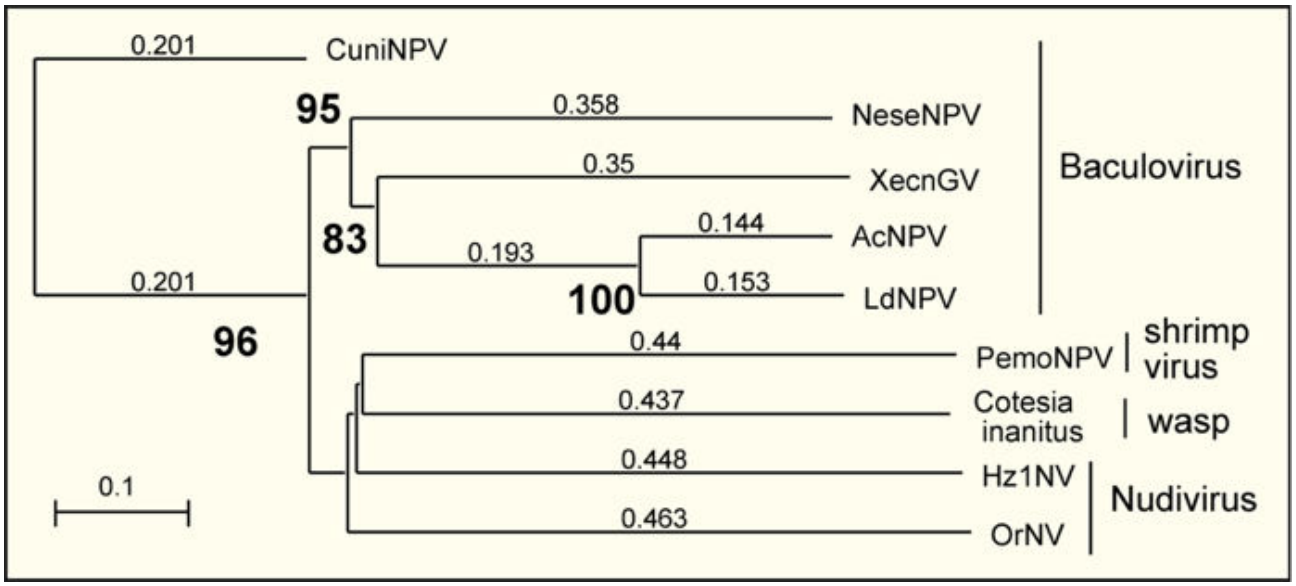

Figure 7. Phylogenetic relatedness of baculoviruses to other viruses. The tree was derived from Maximum likelihood analysis concatenated amino acid multiple alignments of 37 nudivirus-related genes. Bootstraps support (100 replicates) is shown at nodes. *: Diptera-infecting nudiviruses. Modified from (71).

related to per os infectivity factors were also identified. The presence of homologs of these factors suggests that they may be involved in a similar role to that played in baculoviruses by delivering the bracovirus DNA or virulence proteins into cells of the parasitized larvae. Knockdown using RNAi of the expression of the *lef-9* and *lef-4* subunits of the RNA polymerase in female wasps, reduced both the levels of vp39 and p74 transcripts and also the amount packaged DNA suggesting that the baculovirus-like RNA polymerase may be expressed by the wasp and was involved in expressing structural proteins involved in packaging polydnavirus genomes. Likewise, the knockdown of orthologs of per os infectivity factors (p74 and PIF-1) resulted in a reduction in host cells that incorporated a polydnavirus gene, suggesting these factors were involved in cell entry similar to their role in baculovirus infectivity (79). These data suggest that a parasitic wasp was infected by a nudivirus and all or part of the nudivirus genome was incorporated into the wasp genome. Some of these genes are used for the expression

of polydnavirus proteins involved in packaging of the polydnavirus DNA or virulence proteins, thereby facilitating their transmission into the host larvae. A phylogenetic tree shows that the polydnaviruses genes are related to a nudivirus lineage (Figure 7).

A similar analysis of an ichnovirus found that polydnavirion-associated proteins did not show relatedness to known proteins. However, because the genes encoding these products were found clustered in specific locations and are amplified along with the DNA that is packaged in the ichnovirus virions, it was suggested that they are derived from a hitherto unknown or extinct virus that integrated into the host genome similar to the nudivirus-like element that is present in the genomes of braconid wasps (80).

A Whispovirus

The white spot syndrome virus (WSSV) belongs to the genus Whispovirus (*whi*te *spo*t *virus*) and causes severe disease outbreaks in cultured penaeid shrimp, particularly in Asia. It is the only member of the Nimaviridae although there may be several more (81). Nimaviridae is derived from Greek nima=thread referring to its flagellar-like structure at the end of the virion. It is a non-occluded, enveloped, rod-shaped virus with a double-stranded DNA genome of up to 300 kb. It is a highly virulent virus and causes major tissue damage; the infection results in white spots of calcium deposited in the shell (82). Although the genome of WSSV encodes homologs of several baculovirus/nudivirus genes involved in per os infectivity including pifs 1-3 and p74 (15) (81), it is unclear if this represents a direct phylogenetic relatedness to the baculovirus/nudivirus lineage, or whether this is an example of a set of genes important in oral infectivity that was transferred between viruses. The lack of RNA polymerase subunits suggests that their molecular biology may be significantly different from the baculovirus/nudivirus lineage.

Related References

1. Aoki D, Owashi E, Yazaki S. 2001. Ukiyoe VR-Museum. Available at: http://www.biblio.tuat.ac.jp/vr-museum/ukiyoeEng.htm.
2. Steinhaus EA. 1975. Disease in a minor chord. Ohio State University Press, Columbus, Ohio.
3. Bergold GH. Die isolierung des polyeder-virus and die natur der polyeder. Z Naturforsch. 1947;1947:122–143.
4. Xeros N. Cytoplasmic polyhedral virus diseases. Nature. 1952;170:1073. PubMed PMID: 13013322.
5. Paillot A. Sur une nouvelle maladie du noyau au grasserie des chenilles de P. brassicae et un nouveau groupe de microoganismes parasites. Compt Rend. 1926;182:180–2.
6. Vago C, Aizawa K, Ignoffo C, Martignoni ME, Tarasevitch L, Tinsley TW. Editorial: Present status of the nomenclature and classification of invertebrate viruses. J Invertebr Pathol. 1974;23:133–4. PubMed PMID: 4825251.
7. Arif BM. Society of Invertebrate Pathology Founders' Lecture, 2010. Mauro Emilio Martignoni: a renaissance scientist in the 20th century. J Invertebr Pathol. 2010;107:173–8. PubMed PMID: 21762697.
8. Podgwaite JD. 1981. Natural disease within dense gypsy moth populations. *In* Doane CC, McManus ML (ed), The Gypsy Moth: Research Towards Integrated Pest Management. U.S. Dept. of Agriculture, Washington, D.C.
9. Thompson CG. 1978. Nuclear polyhedrosis epizootiology. *In* Brookes MH, Stark RW, Campbell RW (ed), The Douglas-fir tussock moth: a synthesis. U.S. Department of Agriculture, Washington, D.C.
10. Heimpel AM, Thomas ED, Adams JR, Smith LJ. The presence of nuclear polyhedrosis virus of Trichoplusia ni on cabbage from the market shelf. Environ Entomol. 1973;2:72–75.
11. Akermann H-W, Smirnoff WA. A morphological investigation of 23 baculoviruses. J Invertebr Pathol. 1983;41:269–280.
12. Federici BA. 1986. Ultrastructure of baculoviruses, p 61-88. *In* Granados RR, Federici BA (ed), The biology of baculoviruses, vol I. CRC Press, Boca Raton.

13. Entwistle PF, Adams PH, Evans HF. Epizootiology of a nuclear polyhedrosis virus in European spruce sawfly (Gilpinia hercyniae): the rate of passage of infective virus through the gut of birds during cage tests. J Invertebr Pathol. 1978;31:307–12. PubMed PMID: 355558.
14. Hostetter DL, Bell MR. 1985. Natural dispersal of baculoviruses in the envirionment, p 249-284. *In* Maramorosch K, Sherman KE (ed), Virol Insecticides for biological control. Academic Press.
15. Wang Y, Jehle JA. Nudiviruses and other large, double-stranded circular DNA viruses of invertebrates: New insights on an old topic. J Invertebr Pathol. 2009;101:187–93. PubMed PMID: 19460388.
16. Hall DW, Hazard EI. A nuclear polyhedrosis virus of a caddisfly, Neophylax sp. J Invertebr Pathol. 1973;21:323–4.
17. Couch JA. An enzootic nuclear polyhedrosis virus of pink shrimp: ultrastructure, prevalence, and enhancement. J Invertebrate Pathol. 1974;24:311–331. PubMed PMID: 4613755.
18. Couch JA. Free and occluded virus, similar to Baculovirus, in hepatopancreas of pink shrimp. Nature. 1974;247:229–231.
19. Larsson R. Insect pathological investigations on Swedish Thysaura: A nuclear polyhedrosis virus of the bristletail Dilta hibernica. J Invertebr Pathol. 1984;44:172.
20. Henry JE, Jutila JW. The isolation of a polyhedrosis virus from a a grasshopper. J Invertebr Pathol. 1966;8:417–418.
21. Wang Y, Kleespies RG, Ramle MB, Jehle JA. Sequencing of the large dsDNA genome of Oryctes rhinoceros nudivirus using multiple displacement amplification of nanogram amounts of virus DNA. J Virol Methods. 2008;152:106–8. PubMed PMID: 18598718.
22. Ryel RB, Cline GB. Isolation and characterization of a nuclear polyhedral inclusion body from the boll weevil, Athonomas grandis. J Alabama Acad Sci. 1970;41:193.
23. Smith KM, Hills GJ, Rivers CF. Polyhedroses in neuropterous insects. J Insect Pathol. 1959;1:431–437.
24. Martignoni ME, Iwai PJ. 1986. A catalog of viral diseases of insects, mites, and ticks, Fourth ed. USDA Forest Service PNW-195.
25. Theze J, Bezier A, Periquet G, Drezen JM, Herniou EA. Paleozoic origin of insect large dsDNA viruses. Proc Natl Acad Sci U S A. 2011;108:15931–5. PubMed PMID: 21911395.
26. Wu GA, Jun SR, Sims GE, Kim SH. Whole-proteome phylogeny of large dsDNA virus families by an alignment-free method. Proc Natl Acad Sci U S A. 2009;106:12826–31. PubMed PMID: 19553209.
27. Jehle JA, Blissard GW, Bonning BC, Cory JS, Herniou EA, Rohrmann GF, Theilmann DA, Thiem SM, Vlak JM. On the classification and nomenclature of baculoviruses: a proposal for revision. Arch Virol. 2006;151:1257–66. PubMed PMID: 16648963.
28. Soeda E, Maruyama T, Arrand J, Griffin B. Host-dependent evolution of three papova viruses. Nature. 1980;285:165–7. PubMed PMID: 6246442.
29. Rohrmann G, Pearson M, Bailey T, Becker R, Beaudreau G. N-terminal polyhedrin sequences and occluded Baculovirus evolution. J Mol Evol. 1981;17:329–333. PubMed PMID: 7026796.
30. Herniou EA, Olszewski JA, O'Reilly DR, Cory JS. Ancient coevolution of baculoviruses and their insect hosts. J Virol. 2004;78:3244–51. PubMed PMID: 15016845.
31. Bideshi DK, Bigot Y, Federici BA. Molecular characterization and phylogenetic analysis of the Harrisina brillians granulovirus granulin gene. Arch Virol. 2000;145:1933–45. PubMed PMID: 11043952.
32. Zanotto P, Kessing B, Maruniak J. Phylogenetic interrelationships among baculoviruses: evolutionary rates and host associations. J Invertebr Pathol. 1993;62:147–64. PubMed PMID: 8228320.
33. Pearson MN, Rohrmann GF. Transfer, incorporation, and substitution of envelope fusion proteins among members of the Baculoviridae, Orthomyxoviridae, and Metaviridae (insect retrovirus) families. J Virol. 2002;76:5301–5304. PubMed PMID: 11991958.
34. Miele SA, Garavaglia MJ, Belaich MN, Ghiringhelli PD. Baculovirus: molecular insights on their diversity and conservation. Int J Evol Biol. 2011;2011:379424. PubMed PMID: 21716740.
35. Herniou EA, Luque T, Chen X, Vlak JM, Winstanley D, Cory JS, O'Reilly DR. Use of whole genome sequence data to infer baculovirus phylogeny. J Virol. 2001;75:8117–26. PubMed PMID: 11483757.

36. Jiang Y, Deng F, Rayner S, Wang H, Hu Z. Evidence of a major role of GP64 in group I alphabaculovirus evolution. Virus Res. 2009;142:85–91. PubMed PMID: 19428740.
37. Jehle JA, Lange M, Wang H, Hu Z, Wang Y, Hauschild R. Molecular identification and phylogenetic analysis of baculoviruses from Lepidoptera. Virology. 2006;346:180–193. PubMed PMID: 16313938.
38. Hughes KM, Addison RB. Two nuclear polyhedrosis viruses of the Douglas-fir tussock moth. J Invertebr Pathol. 1970;16:196–204.
39. Falcon LA, Hess RT. Electron microscope observations of multiple occluded virions in the granulosis virus of the codling moth, Cydia pomonella. J Invertebr Pathol. 1985;45:356–359.
40. Hukuhara T. Genetic variation of polyhedrosis viruses of insects, p 7-11. *In* (ed),
41. Yu IL, Bray D, Lin YC, Lung O. Autographa californica multiple nucleopolyhedrovirus ORF 23 null mutant produces occlusion-derived virions with fewer nucleocapsids. J Gen Virol. 2009;90:1499–504. PubMed PMID: 19264654.
42. Wu W, Passarelli AL. Autographa californica M nucleopolyhedrovirus Ac92 (ORF92, P33) is required for budded virus production and multiply-enveloped occlusion-derived virus formation. J Virol. in press.
43. Feng G, Thumbi DK, de Jong J, Hodgson JJ, Arif BM, Doucet D, Krell PJ. Selection and Characterization of Autographa californica Multiple Nucleopolyhedrovirus (AcMNPV) DNA Polymerase Mutations. J Virol. 2012;86:13576–13588. PubMed PMID: 23035236.
44. Xu YP, Gu LZ, Lou YH, Cheng RL, Xu HJ, Wang WB, Zhang CX. 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. 2012;93:2480–9. PubMed PMID: 22855783.
45. Rohrmann GF. Baculovirus nucleocapsid aggregation (MNPV vs SNPV): an evolutionary strategy, or a product of replication conditions? Virus Genes. 2014;49:351–7. PubMed PMID: 25224849.
46. Lauzon HA, Garcia-Maruniak A, Zanotto PM, Clemente JC, Herniou EA, Lucarotti CJ, Arif BM, Maruniak JE. Genomic comparison of Neodiprion sertifer and Neodiprion lecontei nucleopolyhedroviruses and identification of potential hymenopteran baculovirus-specific open reading frames. J Gen Virol. 2006;87:1477–89. PubMed PMID: 16690912.
47. Kuzio J, Pearson MN, Harwood SH, Funk CJ, Evans JT, Slavicek J, Rohrmann GF. Sequence and analysis of the genome of a baculovirus pathogenic for *Lymantria dispar*. Virology. 1999;253:17–34. PubMed PMID: 9887315.
48. Hayakawa T, Ko R, Okano K, Seong S, Goto C, Maeda S. Sequence analysis of the *Xestia c-nigrum* granulovirus genome. Virology. 1999;262:277–297. PubMed PMID: 10502508.
49. Lange M, Jehle JA. The genome of the Cryptophlebia leucotreta granulovirus. Virology. 2003;317:220–36. PubMed PMID: 14698662.
50. Hayakawa T, Rohrmann GF, Hashimoto Y. Patterns of genome organization and content in lepidopteran baculoviruses. Virology. 2000;278:1–12. PubMed PMID: 11112474.
51. Luque T, Finch R, Crook N, O'Reilly DR, Winstanley D. The complete sequence of the Cydia pomonella granulovirus genome. J Gen Virol. 2001;82:2531–47. PubMed PMID: 11562546.
52. Willis LG, Siepp R, Stewart TM, Erlandson MA, Theilmann DA. Sequence analysis of the complete genome of Trichoplusia ni single nucleopolyhedrovirus and the identification of a baculoviral photolyase gene. Virology. 2005;338:209–26. PubMed PMID: 15951000.
53. Guarino LA, Gonzalez MA, Summers MD. Complete sequence and enhancer function of the homologous DNA regions of *Autographa californica* nuclear polyhedrosis virus. J Virol. 1986;60:224–229. PubMed PMID: 16789259.
54. Guarino LA, Summers MD. Interspersed homologous DNA of *Autographa californica* nuclear polyhedrosis virus enhances delayed-early gene expression. J Virol. 1986;60:215–223. PubMed PMID: 16789258.
55. Leisy DJ, Rohrmann GF. Characterization of the replication of plasmids containing *hr* sequences in baculovirus-infected *Spodoptera frugiperda* cells. Virology. 1993;196:722–730. PubMed PMID: 8372444.
56. Pearson MN, Bjornson RM, Pearson GD, Rohrmann GF. The *Autographa californica* baculovirus genome: Evidence for multiple replication origins. Science. 1992;257:1382–1384. PubMed PMID: 1529337.

57. Pearson MN, Bjornson RM, Ahrens C, Rohrmann GF. Identification and characterization of a putative origin of DNA replication in the genome of a baculovirus pathogenic for *Orgyia pseudotsugata*. Virology. 1993;197:715–725. PubMed PMID: 8249294.
58. Pearson MN, Rohrmann GF. *Lymantria dispar* nuclear polyhedrosis virus homologous regions: characterization of their ability to function as replication origins. J Virol. 1995;69:213–221. PubMed PMID: 7983712.
59. Arif B, Escasa S, Pavlik L. Biology and genomics of viruses within the genus Gammabaculovirus. Viruses. 2011;3:2214–22. PubMed PMID: 22163341.
60. Parrish S, Resch W, Moss B. Vaccinia virus D10 protein has mRNA decapping activity, providing a mechanism for control of host and viral gene expression. Proc Natl Acad Sci U S A. 2007;104:2139–44. PubMed PMID: 17283339.
61. Perera O, Green TB, Stevens SM Jr, White S, Becnel JJ. Proteins associated with Culex nigripalpus nucleopolyhedrovirus occluded virions. J Virol. 2007;81:4585–90. PubMed PMID: 17301145.
62. Garavaglia MJ, Miele SA, Iserte JA, Belaich MN, Ghiringhelli PD. Ac53, ac78, ac101 and ac103 are newly discovered core genes in the family Baculoviridae. J Virol. 2012;86:12069–79. PubMed PMID: 22933288.
63. Williams T, Bergoin M, van Oers MM. Diversity of large DNA viruses of invertebrates. J Invertebr Pathol. 2017;147:4–22. PubMed PMID: 27592378.
64. Cheng X, Wan X, Xue J, Moore RC. Ascovirus and its evolution. Virolog Sinica. 2007;22:137–147.
65. Huger AM. A virus disease of the Indian rhinoceros beetle, Oryctes rhinoceros(Linnaeus), caused by a new type of insect virus, Rhabdionvirus oryctes gen. n., sp. n. J Invertebr Pathol. 1966;8:38–51. PubMed PMID: 5905536.
66. Ramle M, Wahid MB, Norman K, Glare TR, Jackson TA. The incidence and use of Oryctes virus for control of rhinoceros beetle in oil palm plantations in Malaysia. J Invertebr Pathol. 2005;89:85–90. PubMed PMID: 16039309.
67. Huger AM. The Oryctes virus: its detection, identification, and implementation in biological control of the coconut palm rhinoceros beetle, Oryctes rhinoceros (Coleoptera: Scarabaeidae). J Invertebr Pathol. 2005;89:78–84. PubMed PMID: 16039308.
68. Granados RR, Nguyen T, Cato B. An insect cell line persistently infected with a baculovirus-like particle. Intervirology. 1978;10:309–17. PubMed PMID: 681147.
69. Unckless RL. A DNA virus of Drosophila. PLoS One. 2011;6:e26564. PubMed PMID: 22053195.
70. Wang Y, Kleespies RG, Huger AM, Jehle JA. The genome of Gryllus bimaculatus nudivirus indicates an ancient diversification of baculovirus-related nonoccluded nudiviruses of insects. J Virol. 2007;81:5395–406. PubMed PMID: 17360757.
71. Bezier A, Theze J, Gavory F, Gaillard J, Poulain J, Drezen JM, Herniou EA. The genome of the nucleopolyhedrosis-causing virus from Tipula oleracea sheds new light on the Nudiviridae family. J Virol. 2015;89:3008–25. PubMed PMID: 25540386.
72. Yang YT, Lee DY, Wang Y, Hu JM, Li WH, Leu JH, Chang GD, Ke HM, Kang ST, Lin SS, Kou GH, Lo CF. The genome and occlusion bodies of marine Penaeus monodon nudivirus (PmNV, also known as MBV and PemoNPV) suggest that it should be assigned to a new nudivirus genus that is distinct from the terrestrial nudiviruses. BMC Genomics. 2014;15:628. PubMed PMID: 25063321.
73. Abd-Alla AM, Vlak JM, Bergoin M, Maruniak JE, Parker A, Burand JP, Jehle JA, Boucias DG. Hytrosaviridae: a proposal for classification and nomenclature of a new insect virus family. Arch Virol. 2009;154:909–18. PubMed PMID: 19458899.
74. Jehle JA, Abd-Alla AM, Wang Y. Phylogeny and evolution of Hytrosaviridae. J Invertebr Pathol. 2013;112:S62–7. PubMed PMID: 22841640.
75. Drezen JM, Leobold M, Bezier A, Huguet E, Volkoff AN, Herniou EA. Endogenous viruses of parasitic wasps: variations on a common theme. Curr Opin Virol. 2017;25:41–48. PubMed PMID: 28728099.
76. Bezier A, Annaheim M, Herbiniere J, Wetterwald C, Gyapay G, Bernard-Samain S, Wincker P, Roditi I, Heller M, Belghazi M, Pfister-Wilhem R, Periquet G, Dupuy C, Huguet E, Volkoff AN, Lanzrein B, Drezen

JM. Polydnaviruses of braconid wasps derive from an ancestral nudivirus. Science. 2009;323:926–30. PubMed PMID: 19213916.
77. Bezier A, Herbiniere J, Lanzrein B, Drezen JM. Polydnavirus hidden face: the genes producing virus particles of parasitic wasps. J Invertebr Pathol. 2009;101:194–203. PubMed PMID: 19460382.
78. Burke GR, Strand MR. Deep sequencing identifies viral and wasp genes with potential roles in replication of Microplitis demolitor Bracovirus. J Virol. 2012;86:3293–306. PubMed PMID: 22238295.
79. Burke GR, Thomas SA, Eum JH, Strand MR. Mutualistic polydnaviruses share essential replication gene functions with pathogenic ancestors. PLoS Pathog. 2013;9:e1003348. PubMed PMID: 23671417.
80. Volkoff AN, Jouan V, Urbach S, Samain S, Bergoin M, Wincker P, Demettre E, Cousserans F, Provost B, Coulibaly F, Legeai F, Beliveau C, Cusson M, Gyapay G, Drezen JM. Analysis of virion structural components reveals vestiges of the ancestral ichnovirus genome. PLoS Pathog. 2010;6:e1000923. PubMed PMID: 20523890.
81. Kawato S, Shitara A, Wang Y, Nozaki R, Kondo H, Hirono I. 2018. Crustacean Genome Exploration Reveals the Evolutionary Origin of White Spot Syndrome Virus. J Virol doi: 10.1128/JVI.01144-18:2018. Nov 7. pii: JVI.01144-18.
82. Leu JH, Yang F, Zhang X, Xu X, Kou GH, Lo CF. Whispovirus. Curr Top Microbiol Immunol. 2009;328:197–227. PubMed PMID: 19216439.
83. Volkman LE, Summers MD. Nuclear polyhedrosis virus detection: relative capabilities of clones developed from Trichoplusia ni ovarian cell line TN-368 to serve as indicator cells in a plaque assay. J Virol. 1975;16:1630–7. PubMed PMID: 1104895.
84. Arnott HJ, Smith KM. An ultrastructural study of the development of a granulosis virus in the cells of the moth Plodia interpunctella (Hbn.). J Ultrastruct Res. 1967;21:251–68. PubMed PMID: 5587787.
85. Hyink O, Dellow RA, Olsen MJ, Caradoc-Davies KM, Drake K, Herniou EA, Cory JS, O'Reilly DR, Ward VK. Whole genome analysis of the Epiphyas postvittana nucleopolyhedrovirus. J Gen Virol. 2002;83:957–71. PubMed PMID: 11907346.
86. Fan Q, Li S, Wang L, Zhang B, Ye B, Zhao Z, Cui L. The genome sequence of the multinucleocapsid nucleopolyhedrovirus of the Chinese oak silkworm Antheraea pernyi. Virology. 2007;366:304–15. PubMed PMID: 17540430.
87. Ayres MD, Howard SC, Kuzio J, Lopez-Ferber M, Possee RD. The complete DNA sequence of *Autographa californica* nuclear polyhedrosis virus. Virology. 1994;202:586–605. PubMed PMID: 8030224.
88. Nakai M, Goto C, Kang W, Shikata M, Luque T, Kunimi Y. Genome sequence and organization of a nucleopolyhedrovirus isolated from the smaller tea tortrix, Adoxophyes honmai. Virology. 2003;316:171–83. PubMed PMID: 14599801.
89. Ijkel WFJ, van Strien EA, Jeldens JGM, Broer R, Zuidema D, Goldbach RW, Vlak JM. Sequence and organization of the *Spodoptera exigua* multicapsid nucleopolyhedrovirus genome. J Gen Virol. 1999;80:3289–3304. PubMed PMID: 10567663.
90. Jakubowska AK, Peters SA, Ziemnicka J, Vlak JM, van Oers MM. Genome sequence of an enhancin gene-rich nucleopolyhedrovirus (NPV) from Agrotis segetum: collinearity with Spodoptera exigua multiple NPV. J Gen Virol. 2006;87:537–51. PubMed PMID: 16476975.
91. Xiao H, Qi Y. Genome sequence of Leucania seperata nucleopolyhedrovirus. Virus Genes. 2007;35:845–56. PubMed PMID: 17763934.
92. Wormleaton S, Kuzio J, Winstanley D. The complete sequence of the Adoxophyes orana granulovirus genome. Virology. 2003;311:350–65. PubMed PMID: 12842624.
93. Lauzon HA, Jamieson PB, Krell PJ, Arif BM. Gene organization and sequencing of the Choristoneura fumiferana defective nucleopolyhedrovirus genome. J Gen Virol. 2005;86:945–61. PubMed PMID: 15784888.
94. Duffy SP, Young AM, Morin B, Lucarotti CJ, Koop BF, Levin DB. Sequence analysis and organization of the Neodiprion abietis nucleopolyhedrovirus genome. J Virol. 2006;80:6952–63. PubMed PMID: 16809301.

95. Garcia-Maruniak A, Maruniak JE, Zanotto PM, Doumbouya AE, Liu JC, Merritt TM, Lanoie JS. Sequence analysis of the genome of the Neodiprion sertifer nucleopolyhedrovirus. J Virol. 2004;78:7036–51. PubMed PMID: 15194780.
96. Afonso CL, Tulman ER, Lu Z, Balinsky CA, Moser BA, Becnel JJ, Rock DL, Kutish GF. Genome sequence of a baculovirus pathogenic for Culex nigripalpus. J Virol. 2001;75:11157–65. PubMed PMID: 11602755.

2. Structural proteins of baculovirus occlusion bodies and virions

Infectious baculoviruses can be generated from the transfection of naked viral DNA into susceptible insect cells indicating that the transcription of early genes required for initiating the infection is accomplished via the host cell RNA polymerase. Therefore, viral structural proteins, although necessary for protecting the viral genome and initiating infection, very likely are not required for functions such as transcription or DNA replication. Below is an overview of the proteins that have been associated with major baculovirus virion structures. These include occlusion bodies, budded virus (BV) and occlusion derived virus (ODV) envelopes, and proteins that are components of nucleocapsids. Many of the latter category are shared between BV and ODV (1).

Occlusion body evolution

In temperate climates and in many tropical areas where there are wet and dry seasons, insect populations are transitory and expand dramatically during warm, moist periods, and then collapse with the onset of colder temperatures or drought when food sources are reduced or eliminated. In addition, even during seasons optimal for insect growth and reproduction, their populations are normally limited by predators, pathogens, normal temperature cycles, and food sources. However, under certain circumstances, insect populations can expand dramatically when a combination of conditions greatly increases their food supply, facilitates high levels of reproduction, or eliminates predators and pathogens. These cycles of population expansion are well documented for forest insects and may be separated by long periods of time. For example, the Douglas fir tussock moth, a pest of forests in western North America, has cycles of 7 to 10 years, and other insect epizootics are separated by 5 to 40 years (reviewed in (2)). Viruses often play a major role in causing the collapse of these large insect populations.

A major consequence of the cyclic nature of insect populations is that their pathogens are left without hosts either seasonally, or for much longer periods of time. Viruses, in general, have developed several methods to insure their survival until their hosts reappear. If their hosts are seasonal, such as those of insect pathogens, viruses could be present in the eggs or pupae of over wintering insects, or they might persist in alternate hosts, or some might remain stable outside their hosts. Although there is some evidence for baculoviral persistence within host insects (see Chapter 3), stability outside their hosts mediated by their presence within occlusion bodies appears to be a common feature of all baculoviruses and some nudiviruses. By immobilizing the virus within the crystalline protein lattice of the occlusion body, an environment is provided that allows virions to remain viable indefinitely as long as they are protected from extremes of heat and from UV light. In addition, the stability provided by occlusion may be of such a selective advantage that it has apparently been incorporated into the life cycle of three different types of insect viruses; in Reoviridae (cypoviruses, double-stranded RNA viruses with segmented genomes), the Poxviridae (entomopox viruses-dsDNA viruses with cytoplasmic replication), and in the Baculoviridae/Nudiviridae. No primary amino acid sequence relatedness is evident between these different categories of occlusion body proteins and the primary sequence of the occlusion body protein of a dipteran baculovirus appears to be unrelated to other baculovirus polyhedrins (3), and the polyhedrins from some cypoviruses also appear to be only distantly related to each other (4). Similarly, the occlusion body protein of a nudivirus of the pink shrimp, *Penaeus monodon,* did not appear to be related to baculovirus polyhedrin (5), even though sequence data from other predicted proteins indicated the virus was related to the baculovirus lineage (6). The structure of both cypovirus and baculovirus polyhedrins have been determined, and they appear to be unrelated and, therefore, may be examples of convergent evolution (7, 8), although one report suggests that they may be distantly related (4).

Polyhedra are thought to stabilize baculovirus virions, and in one example it was estimated that only about 0.16% of OpMNPV occlusion bodies remained infectious one year after an epizootic. However, considering that

over 3 x 10^{15} occlusion bodies were estimated to have been produced per hectare during the outbreak, this would still be a substantial number of infectious viruses (reviewed in (2)).

Occlusion body proteins

In addition to virions, occlusion bodies are composed of the matrix that occludes the virions and an outer membrane-like structure on the surface. Several other proteins are also associated with polyhedra (Table 1). The major protein forming occlusion bodies is polyhedrin/granulin.

Table 1 Proteins Associated with Baculovirus Occlusion Bodies

AcMNPV orf # and name	Distribution in the Baculoviridae	Effect of Deletion[2]
Ac8 Polyhedrin	All[1]	Viable
Ac131 Polyhedron envelope/Calyx	All except CuniNPV	Viable
Enhancin	A few NPVs and GVs	Viable
Ac137 p10	Group I/II; some GVs	Viable
Alkaline proteases	Non baculovirus contaminants	

[1] CuniNPV polyhedrin is unrelated to that of other baculoviruses.

[2] For details see Chapter 12

Polyhedrin/Granulin. Polyhedrin and granulin are closely related and are the major structural components of occlusion bodies (polyhedrin in NPVs and granulin in GVs). They initially were challenging to characterize because the occlusion bodies are naturally contaminated with a protease (see below) that caused degradation of the protein upon dissolution of polyhedra. However, once it was found that the proteases could be heat inactivated, polyhedrins from several viruses were characterized and were found to contain about 250 aa (30 kDa) and are one of the most conserved baculovirus proteins. They form a crystalline cubic lattice that is interrupted by and surrounds embedded virions (Figure 1). Orthologs of polyhedrin/granulin are found in all baculovirus genomes, except for that of the dipteran virus (CuniNPV). Surprisingly, CuniNPV has an occlusion body protein that appears to be unrelated in primary amino acid sequence to the polyhedrin of other baculoviruses and is about three times as large (3, 9). The polyhedrin of an NPV pathogenic for pink shrimp also appears to be unrelated to that from other baculoviruses in its primary sequence (5). However, it was reported that it did react with antiserum to AcMNPV and TnGV polyhedrin even though at 50 kDa it appears to be twice as large as the insect baculovirus polyhedrins (10).

Despite forming a natural crystal in nature, determining the structure of polyhedrin by crystallography was unsuccessful because it was not possible to recrystallize it from solution. However, advances in crystallography, plus the observation that a single amino acid change (G25D in AcMNPV polyhedrin) resulted in larger than normal crystals (11) allowed the determination of the structure of polyhedrins from two different Alphabaculoviruses (AcMNPV and *Wiseana* sp NPV) using occlusion bodies produced by viral infection (8) (12). A structure for a granulin from CpGV has also been determined (13). The 30 kDa polyhedrin subunits form trimers that are then arranged into dodecamers (four trimers) via disulfide bonds. This structure interlocks with another dodecamer to form the cubic-shaped unit cell of the crystal (Figure 2). Hydrophobic and salt bridge interactions between the cubes likely form the linkages at the crystal interfaces that are disrupted by the alkaline pH of the insect midgut (see below). Occlusion of virion bundles might displace up to 20,000 polyhedrin subunits and could destabilize the crystals. This possibly led to the evolution of the envelope structure that is thought to stabilize these structures (12). The presence of disulfide bonds may have prevented the attempts to crystallize purified polyhedrin.

The calyx/polyhedron envelope (PE). The calyx/polyhedron envelope is an electron-dense structure that forms a smooth, seamless surface that surrounds polyhedra. The function of the calyx/PE appears to be to seal the surface of polyhedra and to enhance their stability. In the laboratory when polyhedra are subjected to alkaline treatment, the crystalline lattice is dissolved, but the polyhedron envelope remains as a bag-like structure in which many virions are trapped (Figure 3). Although the calyx/polyhedron envelope was originally found to be composed of carbohydrate (14), a phosphorylated protein component was subsequently identified (15). This protein appears to be an integral component of the calyx/PE (16). Homologs of the PE protein (Ac131) are found in the genomes of all lepidopteran NPVs. It is likely that when polyhedra are ingested by susceptible insects, they are dismantled by a combination of the alkaline pH of the insect midgut and proteinases that are present in the midgut and associated with polyhedra. This combination would likely contribute to the disruption of the polyhedron and polyhedron envelope to facilitate virion release. The PE protein is associated with p10 fibrillar structures (Figure 4), and p10 appears to be required for the proper assembly of the polyhedron envelope (17-20). Polyhedra from viruses with either the p10 or PE gene deleted have a similar appearance; they have a rough pitted surface and the PE appears to be fragmented or absent (Figure 5). In addition, in some viral genomes, genes are present that appear to be fusions of both PE and p10 protein domains, further suggesting a fundamental relationship between these two proteins (see below). A partial structure for the PEP of CpGV has been determined (21).

Polyhedron-associated proteins

Ac68 may be involved in polyhedron morphogenesis. Homologs of Ac68 appear to be present in all baculoviruses. When Ac68 was deleted, no major effects were detected other than a longer lethal time in larvae (22). Similar results were obtained when the ortholog in BmNPV (Bm56) was deleted from a bacmid. However, the polyhedra produced by the mutant BmNPV bacmid were abnormal and lacked virions, suggesting that Bm56 may be involved in polyhedron morphogenesis (23).

P10 (Ac137). Although p10 does not appear to be a major occlusion body protein, it colocalizes with the PE protein and appears to be required for the proper formation of the polyhedron envelope. When p10 is phosphorylated, it becomes associated with microtubules (24) (25). This could be related to the structures it forms, including microtubule-associated filaments and tube-like structures that surround the nuclei of infected cells (26, 27). Deletion of P10 results in polyhedra that resemble those produced by mutants lacking the calyx/polyhedron envelope protein; they are fragile, have a rough surface showing cavities where virions have apparently become dislodged, and often show an incomplete calyx/polyhedron envelope (16, 28, 29) (see Figure 5). Serial block-face scanning electron microscopy (SBFSEM) has been used for the 3-D characterization of p10 structures in AcMNPV infected Tn-368 cells (Figure 6) (30). It forms large vermiform structures that surround nuclei and also structures within nuclei that are associated with 'electron dense spacers' which appear to contain Ac131, the polyhedron envelope/calyx associated protein (19). In cultured cells p10 facilitates occlusion body release by nuclear lysis (31) (30) and an increase in infectious virus (28). Homologs of p10 are found in the genomes of all Group I/II NPVs and most GVs, in some instances in multiple copies, e.g., PlxyGV has 3 copies (32). Their phylogeny is complicated somewhat by the fact that some of the GV genes appear to consist of a combination of p10 and polyhedron envelope protein domains (33, 34). A p10 homolog has also been characterized in an entomopox virus (35).

Viral enhancing factors: enhancin. Enhancins are a class of metalloproteinases (36) that are encoded by a few lepidopteran NPVs (e.g., Ld-, Cf-, and MacoNPV) and GVs (e.g., Ag-, As-, Tn-, XcGVs), but not AcMNPV. In one study of TnGV, enhancin was estimated to comprise up to 5% of the mass of occlusion bodies (37). In another virus, LdMNPV, it was found to be associated with ODV envelopes (38). Enhancin genes are often present in multiple copies, e.g., the XecnGV genome has four copies (39). In LdMNPV which encodes two enhancins, deletion of either results in a 2- to 3-fold reduction in potency, whereas deletion of both caused a 12-fold reduction (40). Enhancin is thought to facilitate baculovirus infection by digesting the peritrophic matrix

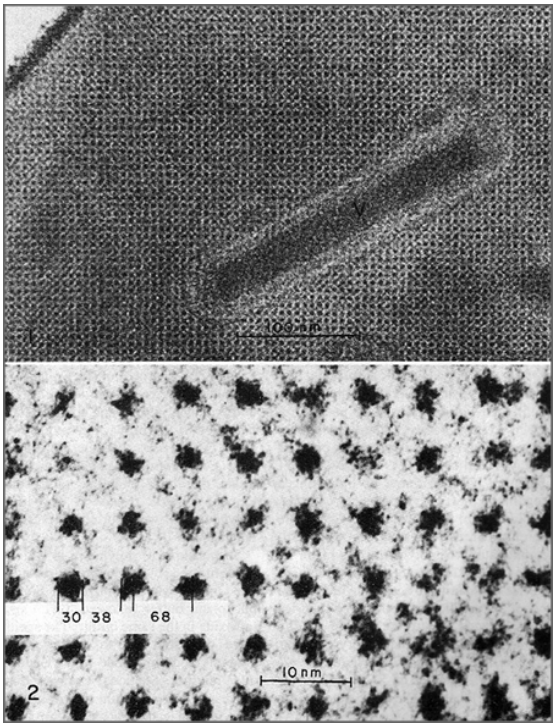

Figure 1. Sections of portions of polyhedra showing crystalline structure. Top panel is from an NPV of *Pseudohazis eglanterina* (western sheep moth). Bottom panel is a higher magnification from an NPV of *Nepytia freemani* (false hemlock looper). Measurements are shown in Angstroms. From K. Hughes (238). Reproduced with permission of Elsevier Limited via Copyright Clearance Center.

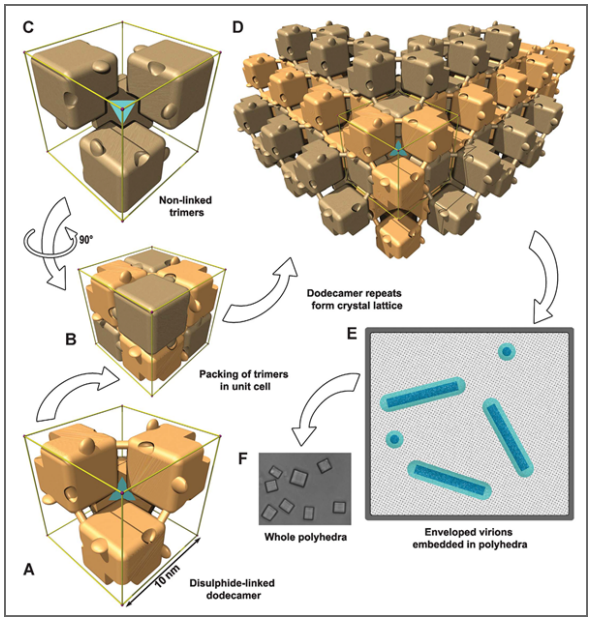

Figure 2. The assembly of polyhedrin into polyhedra. Polyhedrin trimers are depicted as simplified cubic blocks. To clarify interpretation, the edges of the unit cell are shown in gold and a cyan tetrahedron symbolizes the cell center. Within a unit cell, disulphide-linked trimers with one polarity are colored light beech (A) and those with the opposite polarity are colored light brown (C). (B) All eight trimers in the unit cell. The disulphide bond connecting adjoining trimers is shown as a dowel. (D) The crystal lattice is built up from repeats of the dodecameric unit. (E) Sketch of a cross-section through a polyhedron. The lattice spacing of the unit cells is illustrated as a dot pattern into which are embedded nucleocapsids (dark blue) surrounded by an envelope (cyan). (F) Light microscopy image of G25D mutant AcMNPV polyhedra. From Ji et al (12). Reproduced with permission via Copyright Clearance Center.

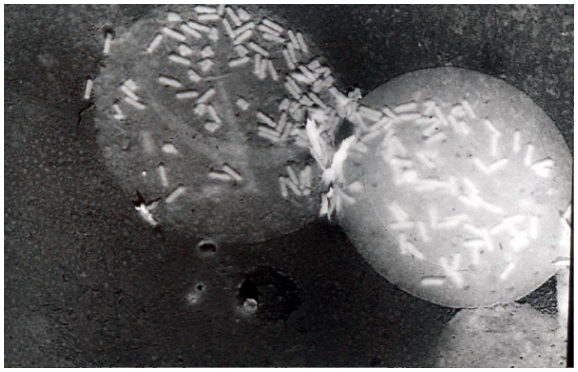

Figure 3. Two adjacent dissolved polyhedra showing rod-shaped virions trapped by the collapsed polyhedron envelope. Photo by K. Hughes.

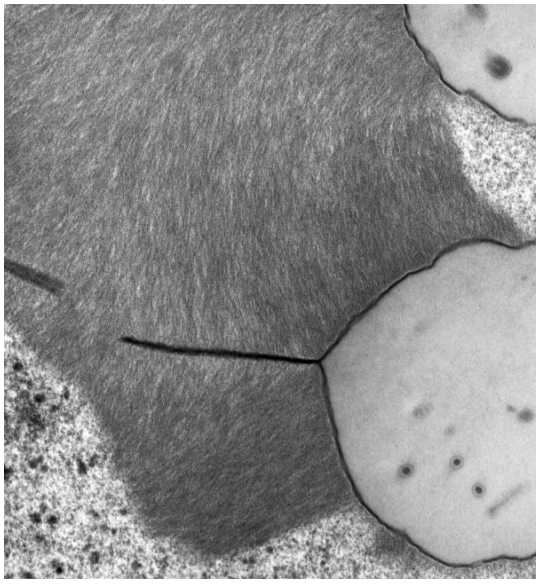

Figure 4. Fibrous p10-containing material aligned with the calyx/polyhedron envelope. Photo courtesy of G. Williams. From (239), with permission.

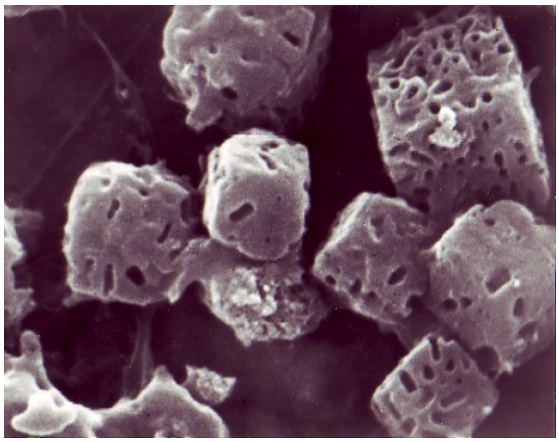

Figure5. Polyhedra from OpMNPV with the polyhedron envelope protein and p10 genes deleted. From (16), with permission.

(PM) (see Figure 2, Chapter 3). The PM forms a barrier in insect guts that prevents the ready access of pathogens to the epithelial cells. The PM is rich in chitin and intestinal mucin protein, and enhancins appear to target the degradation of the mucin, thereby facilitating access of virions to the underlying cells (41). Enhancins show sequence homology with high levels of significance (e.g., 3e-29) to predicted proteins of a number of pathogenic bacteria, e.g., *Clostridium botulinum*, and a variety of *Bacillus* (e.g., *B. anthracis*) and *Yersinia* (e.g., *Y. pestis*) species. To investigate their function, enhancin homologs from *B. cereus*, *Y. pseudotuberculosis*, or TnGV were cloned into a construct of AcMNPV that yielded occluded viruses. Although the LD50 of these constructs was found to be about half of wt, only the construct expressing the TnGV enhancin caused a reduction in survival time. In addition, the bacterial enhancins failed to degrade insect intestinal mucin. It was suggested that the bacterial enhancins may have evolved an activity distinct from their viral homologs (42).

Proteinases. The initial research on occlusion body structure was hindered by the presence of proteinases that degraded the proteins under investigation. These preparations were derived from insect carcasses in various states of disintegration and decay. In hindsight, it is not surprising that these preparations showed proteinase activity. The finding that the proteinases could be heat inactivated (43), led to the ability to purify and eventually sequence a number of polyhedrin proteins before the advent of DNA sequencing technology (44, 45). Subsequently, it was found that the proteinases associated with occlusion bodies had properties similar to enzymes associated with the insect gut (46) and that polyhedra produced in cell culture lacked associated proteinases (47-49). Although many baculoviruses encode a cathepsin-like proteinase, in AcMNPV (Ac127) it was most active under acidic (pH 5) conditions (50). Therefore, the proteinases associated with occlusion bodies are likely a combination of enzymes derived from bacteria, the insect gut, and the virus.

Baculovirus virions: The envelope proteins

For AcMNPV and other relatively well-characterized lepidopteran NPVs, there are two types of virions produced during the virus infection; in insects the infection is initiated by occlusion-derived virus (ODV) that are released into the insect midgut upon dissolution of the occlusion bodies. ODV initiate infection in the midgut epithelium, and the virus propagated in these cells are budded virus (BV) that exit the cells in the direction of the basement membrane and spread the infection throughout the insect. Late in the infection, virions become occluded within the nuclei of infected cells and are released into the environment upon the death and disintegration of the insect. The major difference between BV and ODV is the origin of their envelopes. BVs derive their envelopes as they bud through the host cell plasma membrane that has been modified by viral proteins. In contrast, ODV obtain their envelope in the nucleus and it may be derived from nuclear membranes that are modified with a number of viral proteins. Whereas viral contributions to the BV envelope may be limited to one or two proteins, ODV envelopes are very complex. They appear to contain a number of virally encoded proteins, and in some instances, it is difficult to separate them from capsid proteins.

Cell entry: Baculovirus envelope fusion proteins

Many viruses are surrounded by a lipid envelope and enter cells either by fusion with the cell surface membrane or an endosomal membrane after phagocytosis by a cell. The merging of the membranes is activated by fusion proteins. There are three classes of viral envelope fusion proteins, I, II, and III (reviewed in (51) and baculoviruses employ categories I and III. Class I fusion proteins are activated by cleavage of a trimeric single-chain precursor protein. Cleavage is usually activated by exposure to low pH. Examples are the influenza hemagglutinin (HA), the HIV gp41, and the baculovirus F fusion proteins. Class II fusion proteins are found in small icosahedral viruses such as flaviviruses and alphaviruses and are present in a 1:1 complex with a chaperone protein that inhibits fusion. The inhibition is removed by cleavage of the chaperone. Class III fusion proteins are activated by low pH, but are not cleaved, and are found in rhabdoviruses (e.g. vesicular stomatitis virus - VSV-G), herpes viruses (gB), and some baculoviruses and orthomyxovirues (gp64).

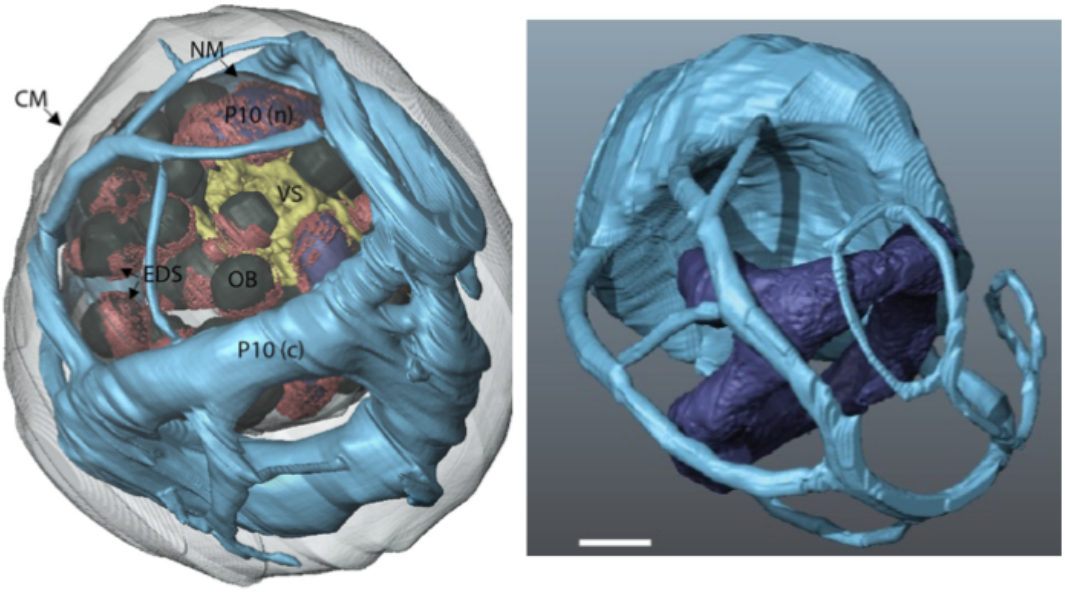

Figure 6. Whole cell 3D reconstruction of P10 structures in AcMNPV infected Tn-368 cells (30) using serial block-face scanning electron microscopy Left image: 72 hpi with the following structures labeled: virogenic stroma (VS); P10 (nuclear, n; cytoplasmic, c); electron dense spacers (EDS); and occlusion bodies (OB) as well as the cytoplasmic (CM) and nuclear membranes (NM). Right image: Nuclear (dark blue) and cytoplasmic P10 (light blue) structures at 96 hpi. Images are from (30) via Creative Commons.

Envelope proteins of budded virions

AcMNPV the most well studied baculovirus may enter cells by two different processes; direct fusion, and by receptor mediated endocytosis (52). Probably the most well characterized baculovirus structural protein is the envelope fusion protein of Group I baculoviruses, GP64 (Class III), because a relatively simple assay for its fusion activity was developed early in its investigation (53). Because of early studies elucidating the importance of this protein for AcMNPV BV infectivity (54, 55), it was unexpected when genome sequence analyses of additional baculoviruses revealed that many of them lacked homologs of the *gp64* gene (56, 57). Furthermore, it was determined that these viruses use a different fusion protein called F (Class I) and that homologs of F are retained in *gp64*-containing viruses (Figure 7). These F homologs in viruses encoding GP64 are inactive as fusion proteins, suggesting that *gp64* was obtained by a baculovirus and displaced the fusion function of the F protein, but the F gene was retained. These proteins are described below.

GP64 (Ac128) is present in all Group I NPVs, a single GV, and certain lineages of orthomyxoviruses. GP64 is a fatty acid acylated glycoprotein (58) and a low pH activated envelope fusion protein (59-61) that is one of the three most abundant proteins, along with vp39 and p6.9, found associated with AcMNPV budded virions (62). The presence of the *gp64* gene is one of the major distinguishing features of the Group I alphabaculoviruses. Deletion of AcMNPV *gp64* results in viruses that replicate in a single cell, but cannot bud out and infect surrounding cells (55, 63). It was originally thought that all Group I viruses use GP64 for the entry of BV into cells, whereas all other baculoviruses lack a *gp64* homolog and appear to use the F (Ac23 homolog) protein as their envelope fusion protein except for hymenopteran NPVs, which lack both genes (Figure 7). However, it was subsequently found that *Diatraea saccharalis* (the sugarcane borer) granulovirus, DisaGV, encodes both F and gp64 fusion protein orthologs (64). This was the first example outside of the Group I nucleopolyhedroviruses where a baculovirus possesses gp64 in addition to the F protein. It is thought that gp64 was incorporated into the Group I viruses in a recombination event (Figure 8) that may have involved up to 11 other additional genes specific to Group I NPVs (Chapter 1, Table 3). After incorporation, it is thought that gp64 displaced the fusion function of the F protein which has been retained but is not an essential gene (65). An AcMNPV bacmid deleted

for gp64 and pseudotyped with DisaGV gp64 was infectious although at a lower level than wt AcMNPV. In cultured sf9 cells, it infected about 60% of the cells, vs wt which infected close to 100%. Also, although DisaGV gp64 was fusogenic for Sf9 cells at low pH, it was less efficient than AcMNPV gp64 (66). DisaGV gp64 shows 67-74% identity to many NPV gp64 sequences in genbank. Phylogenetic analysis of DisaGV gp64 suggests that its incorporation into the GV genome is not a recent event (Figure 9) although it does clearly belong to the baculovirus gp64 lineage. It has not been determined if gp64 is the main fusion protein of DisaGV or if its F protein is active and is also involved in fusion.

GP64 and the Orthomyxoviridae. In addition to the Group I NPVs, orthologs of *gp64* are also found in several genera of the Orthomyxoviridae. Currently, there are 7 genera of Orthomyxoviridae, four that appear to be specific to vertebrates (birds and mammals)(Influenza virus A, B, C, and D), and encode a hemagglutinin (Class I) that facilitates viral attachment to sialic acid residues and fusion. Genera A and B also encode a neuraminidase that digests the receptor and prevents the binding of newly replicated virions to cells, whereas genera C and D encode a combination hemagglutinin-esterase (HE) that has both functions. The Isavirus genus of Orthomyxoviridae (infectious salmon anemia virus -ISAV), a pathogen of Atlantic salmon, appears to encode the attachment and fusion domains on two different molecules. Although evolutionarily distant, structural evidence suggests that the ISAV F protein is a Class I fusion protein related to those from other orthomyxoviruses (67, 68). In contrast, the Quaranjaviruses and Thogotovirus genera lack genes similar to HA and neuraminidase and instead encode an ortholog of baculovirus gp64 (Class III) that is involved in both attachment and fusion. These viruses are associated with ticks and birds, but some have been reported to infect humans. In addition, an unclassified Orthomyxovirus, Sinu virus, associated with mosquitoes also encodes gp64. Primary sequence and structural information indicate that the baculovirus and orthmyxovirus gp64 molecules have a common origin (69). Phylogenetic analysis of gp64 indicates that the Sinu and baculovirus gp64s have a common lineage distinct from the other orthomyxovirus gp64 suggesting that the baculovirus gp64 may have originated from a virus in the Sinu virus lineage (Figure 9). The post-fusion structure of AcMNPV GP64 indicates that six of seven disulfide bonds are conserved between thogotovirus and baculovirus GP64 and one forms an intermolecular bond involved in trimer formation (70). The data indicate that the fusion peptide and receptor binding sites co-localize to a hydrophobic patch located in two loops (L1 and L2) at the tip of the trimer (Figure 10). Further evidence for the involvement of L1 and L2 was subsequently provided in studies that employed alanine scanning mutagenesis (71) (72). A third loop (L3) attached to L2 was not involved in binding or fusion. It was suggested that transient forms of GP64 embed hydrophobic side chains into cell membranes triggering endocytosis independent of specific receptor molecules. This lack of specific receptors (see below) and the affinity of GP64 for cell membranes may explain its ability to facilitate the entry of AcMNPV into a wide variety of different cell types. In addition, it was suggested that the lack of a high affinity receptor is consistent with AcMNPV systemic infections. Since the infection has already been initiated by midgut infection using a mechanism independent of GP64, GP64 has evolved to spread the infection to as many cell types as possible via systemic infection. A comparison of the major structural features of GP64 and F proteins is shown in Figure 10.

Evolution of Group I (GP64 containing) baculoviruses. As indicated in Chapter 1, Group I baculoviruses appear to have been a lineage that had, in addition to gp64, 11 genes not present in other lepidopteran NPVs. Since many groups of NPVs have sets of genes not present in other lineages, this would not have been extraordinary as such genes might be lost and gained over time. However, the incorporation of *gp64* appears to have changed the biology of the lineage enough so that they evolved into a distinct group of viruses (73). In addition, this evolutionary event was recently recapitulated with the demonstration that *gp64* could pseudotype and partially rescue a Group I virus deleted for its F protein (74) (75). However, in those experiments, the recombinant virus lacked both the F protein and the other genes unique to the Group I lineage. The latter includes genes encoding the transactivator IE-2 (Ac151) and the global transactivator ortholog among others that could have facilitated the evolution of this lineage after it incorporated *gp64* (73).

Fusion protein-F, (Ac23). Although F (Ac23) is not an active envelope fusion protein in AcMNPV, in Group II NPVs, GVs and the dipteran virus (CuniNPV), orthologs of F are likely used as the fusion protein, because all these viruses lack homologs of *gp64*. An exception appears to be the hymenopteran NPVs that lack homologs of both the F and GP64 proteins (Figure 7) (see discussion in Chapter 3). Several reports have described investigations in cultured cells on AcMNPV in which *gp64* was substituted with the *F* gene from other baculoviruses. In one study, a construct with Ac23 was not completely rescued by Se8 (77), whereas in another, the presence of Ac23 appeared to be required for the elevation of infectivity to near wt levels (78). It was suggested that these contrasting results might be due to the different strength of the promoters used to express Se8 in the two constructs (78). F proteins of Group II NPVs function as low-pH envelope fusion proteins (79) (80) and can also rescue AcMNPV lacking *gp64* (77) (81). The F protein of granuloviruses has been implicated as a fusion protein due to their lack of a *gp64* ortholog. Although the gene encoding one such protein (PlxyGV orf26) failed to mediate fusion when incorporated into the genome of an AcMNPV mutant bacmid lacking *gp64* (77), another from *Agrotis segetum* GV (Agse orf108) was able to rescue AcMNPV lacking *gp64* (82). Other GV F proteins can also substitute for gp64 (83). F proteins appear to be members of a large and diverse family of viral envelope fusion proteins called Class I (see above), reviewed in (70). They are present as homotrimers and are synthesized as a precursor that is cleaved by a furin-like proteinase into two subunits, and near the amino terminus of C-terminal peptide is a hydrophobic fusion peptide (Figure 10). Class I fusion proteins include many other viral fusion proteins such as influenza HA (e.g., see (84, 85)). In addition, orthologs of the baculovirus F gene are also found as the env gene of insect retroviruses (Figure 8) (reviewed in (65) and are also present in some insect genomes (86, 87) (see Chapter 11). With the recent determination of the structure of a variety of viral envelope fusion proteins, when baculovirus F and insect retrovirus env proteins are analyzed using the Hhpred structure prediction program (88), they all have predicted structures similar to the fusion proteins of paramyxoviruses. Some probabilities are quite high; the PxGV or26, 95%; Kanga env 97%; LD130, 92%. Even the nonfunctional Ac23 from the virus that uses gp64 as its fusion protein, scored about 90%. Paramyxoviruses are negative strand RNA viruses and they only seem to be found in vertebrates, although there is one report from a parasitic insect of birds. Although that data might have been due to the insect feeding on an infected host (89). In addition, recent evidence suggests that the F and GP64 categories of fusion proteins may be distantly related (70).

Although inactive as a fusion protein in Group I viruses, the F protein homolog in OpMNPV (Op21) is glycosylated. Similarly, in Helicoverpa armigera NPV (HearNPV) where F is the active fusion protein, it is N-glycosylated at 5 of 6 sites (76). In OpMNPV it was associated with the envelope of BV and with the membranes of OpMNPV infected cells (90). In AcMNPV, the F homolog (Ac23) is also associated with BV membranes and its deletion from the genome results in infectious virus with titers similar to wt in cultured cells, but the time to kill larvae is somewhat extended (91). In addition, antibodies against some selected regions of GP64 appeared to inhibit binding of BV to Sf9 cells of Ac23 deleted virus to a greater extent than wt virus. This was interpreted to suggest that Ac23 may increase the binding of BV to Sf9 receptor molecules (92). It could also indicate that Ac23 is closely associated with GP64 in the virion envelope and when it is absent, the antibodies have greater access to GP64. Proteomic studies found Ac23 to be associated with AcMNPV ODV as was the homolog in CuniNPV (93, 94). This suggests that it is transported to the nuclei of infected cells. The significance of this is not clear. Whereas AcMNPV is able to enter a variety of vertebrate cells, when the gene encoding its envelope fusion protein, gp64, was replaced by F genes from 5 different NPVs, although some replicated well in insect cells, none of these recombinants was capable of entering vertebrate cells (81) suggesting that they have a much greater cell specificity than GP64.

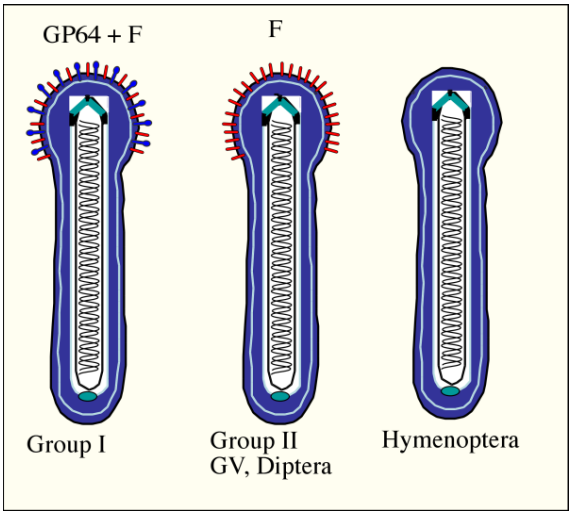

Figure 7. Distribution of envelope fusion proteins. Group I have homologs of both GP64 and F, but F is not a fusion protein. Group II, GVs and dipteran viruses have homologs of F, whereas the hymenopteran viruses have homologs of neither GP64 nor F. As noted in the text, there is one instance of a GV encoding *gp64*.

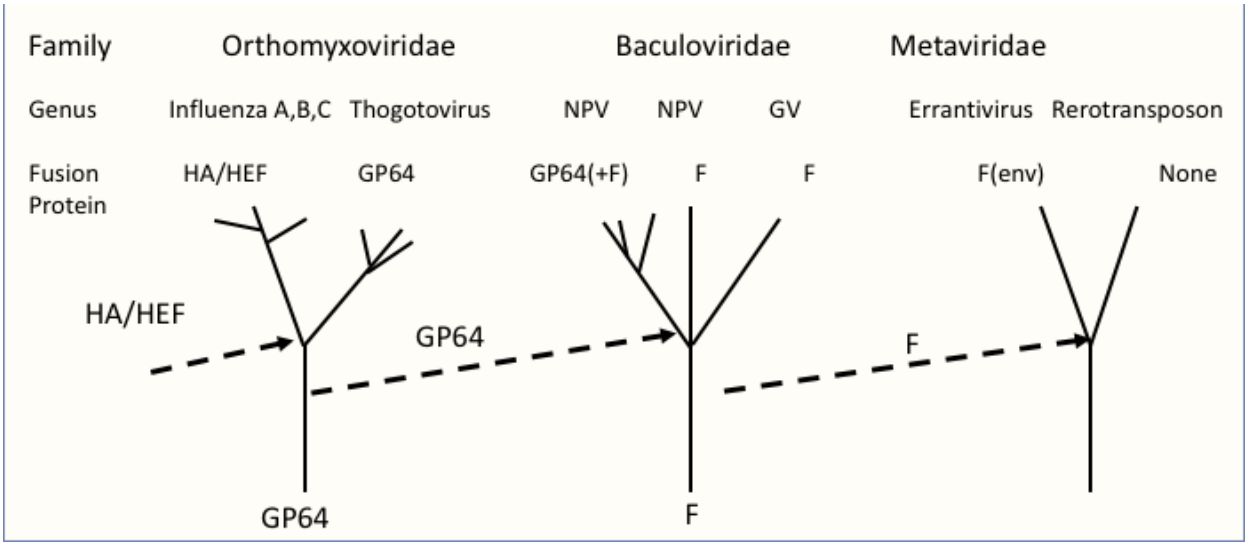

Figure 8. A model for the transfer and incorporation of envelope fusion proteins (EFP) between viral families. Orthomyxoviridae: The original orthomyxoviruses likely had gp64 as their EFP because they originated as pathogens of invertebrates at a time predating the evolution of vertebrates. At some point after vertebrates evolved, one lineage incorporated the HA/HEF EFP and lost GP64. Baculoviridae: A main lineage of baculoviruses had F as its EFP and one lineage incorporated GP64 which displaced the fusion function of F but F was retained. The Metaviridae: An insect retrotransposon lacking an EFP incorporated F from a baculovirus leading to the evolution of the errantivirus lineage.

Identification of virion proteins using mass spectrometry and bacmid knockouts: Is a protein really an essential component of a virion's structure?

A major advance in the enumeration of proteins associated with virion components has involved the use of mass spectrometry. Fractionating BV and ODV into envelope and nucleocapsid fractions and analyzing their content using mass spectrometry in conjunction with data from DNA sequencing, has provided a wealth of information of many proteins associated with these structures. This information can include both host proteins and also modifications of proteins. These studies also identify proteins that would not be predicted to be part of the virion

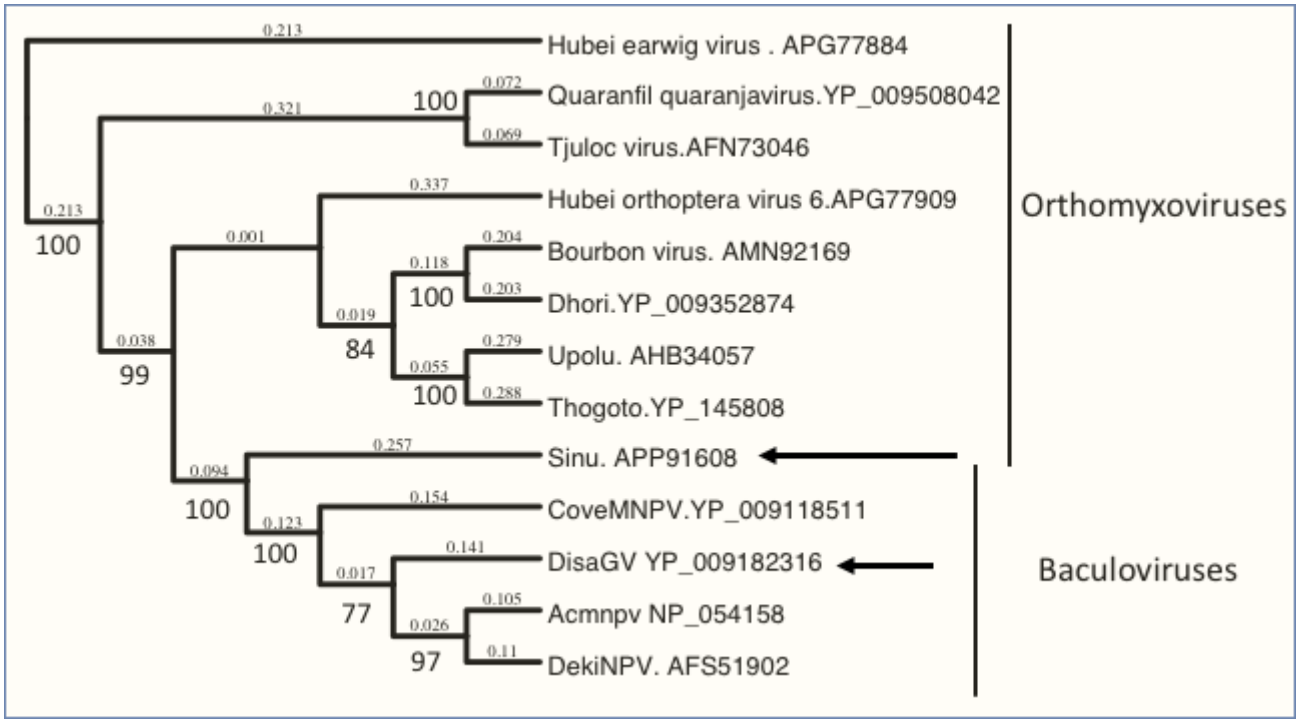

Figure 9. Phylogeny of the GP64 viral envelope fusion protein in the Baculoviridae and Orthomyxoviridae. This is a combination tree: Neighbor Joining Best Tree with Neighbor Joining Bootstrap (1000 replicates). The numbers in bold indicate Bootstrap values ~~(1000 repeats)~~ of over 70%. The GenBank numbers are shown to the right of each viral lineage. The upper arrow indicates the gp64 of the Sinu virus, an orthomyxovirus-like virus. Based on this analysis the Sinu virus gp64 shares a common lineage with baculovirus gp64 with high levels of confidence. The lower arrow indicates the GP64 present in a GV. Baculovirus abbreviations: Cove=Condylorrhiza vestigialis; Disa= Diatraea saccharalis; Deki= Dendrolimus kikuchii.

structure (93-95) including a variety of proteins involved in DNA replication and transcription. It is unknown whether the presence of these proteins is adventitious or if they are bona fide structural proteins and play a role in accelerating the initiation of the infection cycle. The presence of proteins, such as DNA polymerase, likely reflects an intimate relationship between DNA replication and packaging, and nucleocapsid assembly and envelopment. In addition, lipids can be 'sticky,' and proteins in close proximity during virion assembly could adhere to the envelope and co-purify with ODV. Evidence from proteomic analysis of BV support this theory as they appear to lack most proteins associated with DNA replication and transcription that have been reported from ODV, suggesting that they were stripped off the nucleocapsids as they moved through the cell to the cytoplasmic membrane (62) (96). Furthermore, the facultative association of proteins with BV was demonstrated when it was found that BV can trap baculovirus expressed chloramphenicol acetyl transferase (97). Likewise, some proteins that are present in polyhedra and associated with occluded virions may have a lesser affinity for the envelope and may be lost during the ODV purification process. Proteomic analysis combined with the use of bacmid knockout constructs provides additional information regarding whether the protein is required for virion structure. The bacmid data can provide its own set of complications such as when an observed structural defect may be due to a secondary effect on some other structure. An example is the DNA binding protein DBP (Ac25). It is not associated with virions, but when deleted, virions appear to be structurally defective. Since DBP associates with the virogenic stroma, and when deleted, this structure is absent, it has been suggested that the role of DBP in virion structure is caused by the contributions of DBP to the structure of the virogenic stroma. Without a properly formed virogenic stroma, the assembly of virions is aberrant (98). Consequently, the best data is derived from a combination of proteomic, structural investigations using knock out bacmids, and immunological data that can provide definitive information on the location of a protein. Fortunately, the latter information can be often conveniently derived from the use of epitope tagged repair viruses produced using the

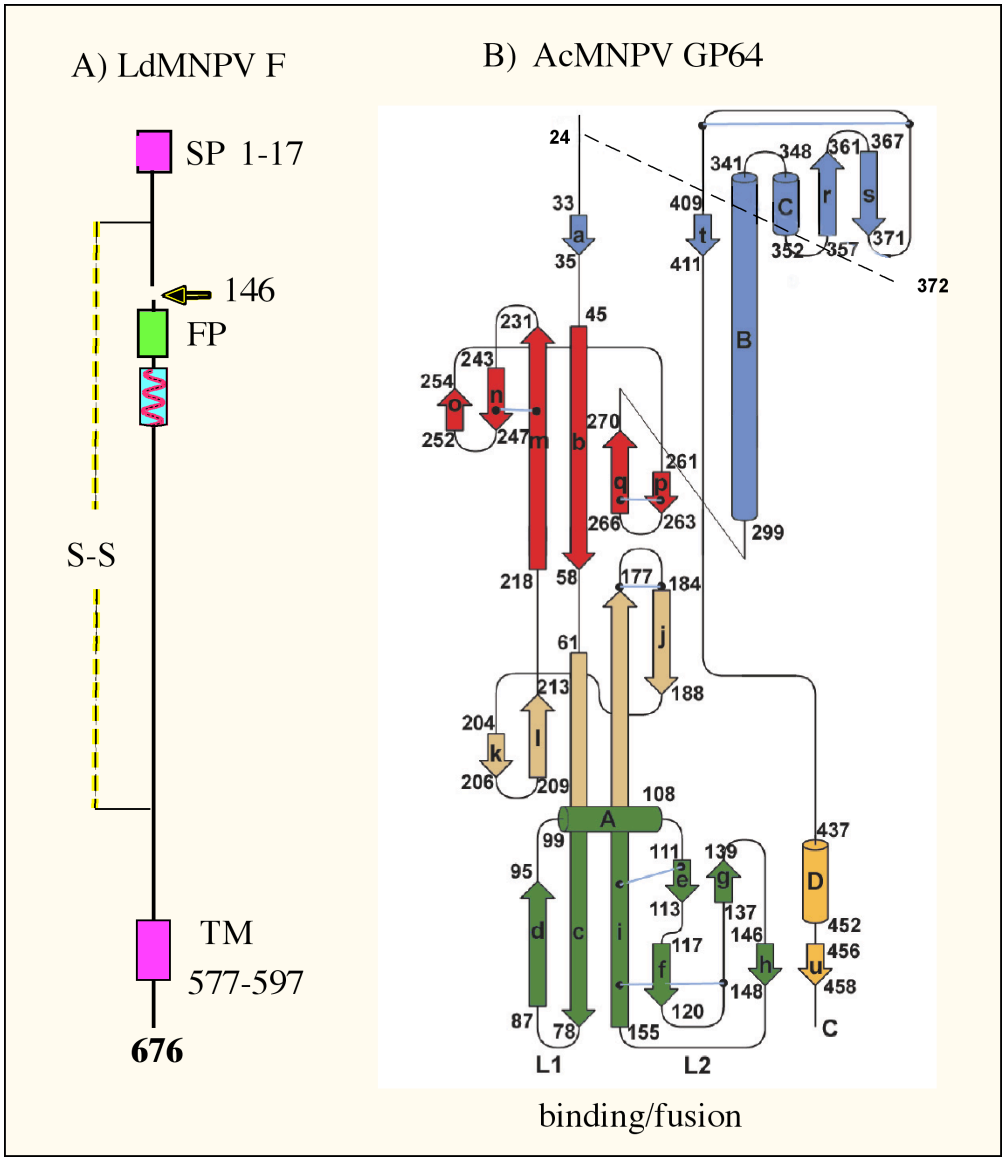

Figure 10. Structure of the baculovirus F (fusion) protein (Ld130) from LdMNPV and AcMNPV GP64. A) Ld130 F protein. Shown is a predicted signal peptide (SP), fusion peptide (FP) and transmembrane domain (TM) including the amino acid coordinates. The cleavage site is indicated by the arrow. A predicted coiled coil domain is also indicated. The disulfide bond is predicted from (240). B) GP64: Shown are 7 disulfide bonds, all of which are intramolecular except for the one from aa 24 which is connected to aa 372 in an adjacent molecule. This and the coiled-coil (299-341) region are involved in trimer formation. All the disulfide bonds are conserved except 178-184 which is not present in thogotovirus GP64. The receptor binding/fusion peptide region is shown at the base of the diagram. GP64 is from Kadlec et al (70). Copyright 2008 by Nature Publishing Group. Reproduced with permission of Nature Publishing Group via Copyright Clearance Center.

bacmid technology. In the following overview, I have concentrated on the proteins for which there is information in addition to that provided by mass spectrometry. Information on AcMNPV proteins, that are not covered below can be found in (1) and Chapter 12.

Host proteins associated with BV envelopes. Evidence for host proteins associated with virions is described at the end of this chapter.

Cell receptors and virus entry for budded virions

Evidence indicates that GP64 is the receptor binding protein of AcMNPV (99). It is also well documented that GP64 can mediate the entry of AcMNPV into a wide variety of vertebrate cell lines (e.g., (100)). However, the identification of the cell receptor for budded virions has remained elusive. In one study, it was suggested that cell surface phospholipids might be involved in the AcMNPV BV (GP64 mediated) entry into vertebrate cells since treating cells with phospholipase C reduced reporter gene expression in cells (101). In addition, acidic phospholipids in giant unilamellar vesicles are required for fusion with AcMNPV BV envelopes (102). A role for membrane spanning heparin sulfate proteoglycans (syndecans) in virus binding has also been proposed for entry into mammalian cells (103). Investigations also have suggested that macropinocytosis, dynamin- and clathrin-dependent endocytosis, and cholesterol in the plasma membrane, all may be involved in the entry of AcMNPV into mammalian cells (103). In mammalian cells, AcMNPV entry is facilitated by low pH. In addition, when the basicity of the basic loop in GP64 was increased, the ability of the virus to enter mammalian cells was elevated. However, this mutant failed to spread between Sf9 cells. Viruses grown in insect cells from different species showed differing efficiency of mammalian cell entry suggesting that some host factors incorporated into the virus might facilitate cell entry (104). As described above, the lack of specific receptors and the affinity of GP64 for cell membranes may explain its ability to facilitate the entry of AcMNPV into a wide variety of different cell types and this might facilitate systemic infections where many different cell types are encountered (104). Other reports indicate that BV of a virus that used an F fusion protein did not have the ability to enter the array of vertebrate cells as AcMNPV. Consequently, it was suggested that the F fusion proteins might use a different receptor from GP64 (105) (106). In contrast, another report described the ability of UV-inactivated HearSNPV expressing either F and GP64 was able to compete for receptors with an F expressing wt virus suggesting that they have similar modes of entry (74). For further information see (1).

Envelope proteins of occlusion-derived virus that are also BV associated

The source and content of the envelope is the major distinguishing feature between BV and ODV. In contrast to BV, where a few virus-encoded proteins have been identified as envelope associated, the ODV envelope is much more complex (Figure 11). There may be five or more such proteins categorized as envelope proteins along with a set of about 9-12 proteins called per os infectivity factors (PIF) (107) (108) (109) that are likely envelope components. Some of these proteins contain an N-terminal hydrophobic sequence in combination with several adjacent positively charged amino acids. These have been predicted to be motifs that target these proteins to intranuclear microvesicles that are the likely precursors from which the envelopes of occluded virus are derived (110). The following proteins (see also Table 2) have been characterized and are likely to be components of ODV envelopes (see also Figure 11).

BV/ODV-E26, (Ac16). Homologs of Ac16 are found in lepidopteran Group I NPVs. Evidence for the association of Ac16 with BV and ODV envelopes has been reported for AcMNPV (111) (93) (62). However, in BmNPV, the homolog of AcMNPV Ac16 (Bm8) was not identified as a virion structural protein (112). It was suggested that these conflicting results were due to the different sources of the antibodies used to detect the protein (110). Ac16 interacts with FP25 (Ac61), forms a complex with cellular actin (111), and is palmitoylated (113). A mutant in which AcMNPV orf16 (called DA26) was insertionally inactivated was viable and showed no difference from wt in infections of *T. ni* or *S. frugiperda* cells or larvae (114). In another report, a virus deleted for Ac16 infected cells showed a delay in BV production (115). It has also been shown to interact with both IE-1 and IE-0 and may be involved in the regulation of these gene products (116).

ODV-E25, (Ac94). Ac94 is a core gene present in all baculovirus genomes (117). The protein encoded by this gene was originally identified in OpMNPV, and immunogold staining with a specific antibody against Ac94 was localized to ODV envelopes (118). It has also been shown to be associated with BV and ODV of AcMNPV and HearNPV (93, 95) (62) (119) and is highly expressed in midgut cells (120). The hydrophobic N-terminal 24 aa of

AcMNPV ODV-E25 appears to be a nuclear targeting signal (121). Ac94 associates with NSF and may be involved in the nuclear entry and egress of BV (122). Deletion of Ac94 resulted in a 100 fold reduction in infectious BV and ODV were not evident and virions were not occluded in polyhedra (123). When ODV-E25 is expressed as an early gene under the IE-1 promoter, it accumulates on the cytoplasmic side of the nuclear membrane rather than within nuclei, and budded virus production is severely reduced. This suggests that it might play a role in the shift from BV to ODV virions. An open reading frame of ODV-E25 encodes a microRNA that down regulates ODV-E25 expression. It was suggested that this might result in a reduction in infectious virus production and be involved in the shift to occluded virus production (124) (125). In addition, expression of Ac94 from the very late polyhedrin or p10 promoter reduced and delayed occlusion body formation suggesting that it may play a role in virion occlusion (126) (127).

ODV-EC43, (Ac109). Ac109 is a core gene present in all baculovirus genomes. Evidence suggests that it is ODV-associated in AcMNPV (93) and *Helicoverpa armigera* NPV (Ha94=ODV-EC43) (95, 128) and also is BV-associated in AcMNPV (62) (129). Four studies have examined deletions of Ac109 and demonstrate that it is an essential gene and when deleted, DNA replication is not affected. One study reported that deletion of Ac109 resulted in a block in nucleocapsid and polyhedron formation (130). However, the other reports described different results. One indicated that polyhedra and virions were produced by Ac109 deletions, but the virus was not infectious (129). Another study found similar results, but also showed that the nucleocapsids had defects in envelopment and the polyhedra lacked virions (131). A fourth report also described similar findings, but indicated that the BV produced by a Ac109 knockout could enter the cytoplasm, but not nuclei, and also noted that the occlusion bodies lacked virions (132).

ODV-E18, (Ac143). Ac143 is a core gene present in the genomes of all baculoviruses. An antibody generated against an Ac143-GST fusion reacted with a protein of 18 kDa in the ODV envelope fraction and Ac143 was named ODV-E18 (133). Ac143 and its HearNPV homolog were found in surveys of ODV-associated proteins by proteomic analysis (93, 95). Deletion of Ac143 results in single cell infections that produce polyhedra and therefore appears to be essential for BV production (134). In addition to its presence in ODV envelopes, it is also BV associated (62). Hhpred (135) predicts the presence of a transmembrane helix and similarity to a protein translocation complex of bacteria.

Ac144 (ODV-EC27) See below.

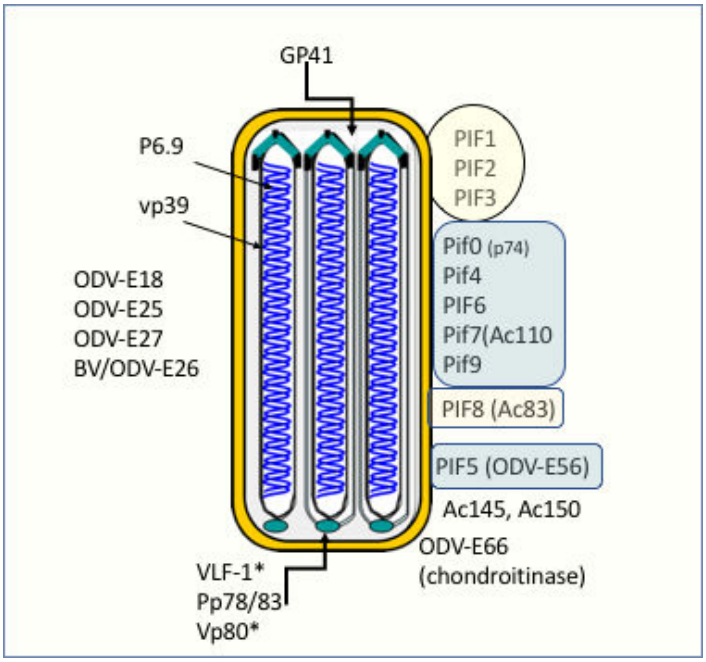

Figure 11. Selected structural proteins of ODV. Shown are envelope associated proteins (ODV-E), the PIF proteins (also envelope associated), the tegument protein, gp41, the DNA binding protein, p6.9, and two basal end-associated proteins, pp78/83 and VLF-1. For convenience, VLF-1 and Vp80 is shown here located at the basal end, however, they could be located at the apical end (227) (167). PIFs 1-3 are the core PIF complex. Without them, the other PIFs will not assemble. PIFs 0, 4, 6, 7, and 9 comprise a larger complex dependent on the pif1-3 complex, while pif8(ac83) binds to that larger complex (108, 109, 136). PIF5 is not part of the complex. There are a variety of other capsid proteins, but they appear to have a more generalized distribution. For details see the text.

Table 2. Occlusion Derived Virus Envelope Proteins and Per os infectivity factors

AcMNPV orf # and name	Distribution in the Baculoviridae	Effect of Deletion
ODV envelope proteins		
Ac16, BV/ODV-E26	Lep. I	Viable (114)
Ac94, ODV-E25	Lep. I, II, GV	Not viable (144)
Ac109, ODV-EC43	All	Not viable (130) (129)
Ac143 ODV-E18	Lep. I, II	Not viable (134)
Per os infectivity factors		
Ac22, pif-2	All	Viable (not by per os) (138)
Ac46, ODV-E66	Lep. I, II, GV	Viable (144) (150)
Ac68, pif-6	All	Viable (not by per os) (242)
Ac83	All	See (145)
Ac96, pif-4	All	Viable (not by per os) (243)
Ac110, pif-7	All	Viable (not by per os) (244)
Ac115, pif-3	All	Viable (not by per os) (138)
Ac119, pif-1	All	Viable (not by per os) (138)
Ac138, pif-0 p74	All	Viable (not by per os) (245)
Ac145	All but CuniNPV	Viable (reduced per os) (155)
Ac148, ODV-E56 pif-5	All	Viable (not by per os) (246)

Table 2. continued from previous page.

AcMNPV orf # and name	Distribution in the Baculoviridae	Effect of Deletion
Ac150	Lep. I (a few)	Viable (155)

Per os infectivity factors (PIF); ODV envelope associated proteins required for midgut infection

Ac22 (*pif*2), Ac68 (*pif*6), Ac83(*pif*8), Ac96 (*pif*4), Ac108(*pif*9), Ac110 (pif7), Ac115 (*pif*3), Ac119 (*pif*1), Ac138 (p74-*pif*0), Ac148 (*pif*5). Per os infectivity factors were originally identified because they were required for infection of insects, but dispensable for infection of cultured cells (107) (108, 109, 136). There are at least 10 *pif* genes encoded by AcMNPV (Figure 11). Orthologs of PIF genes (except PIF9) are present in all baculovirus genomes and most are also found in genomes of nudiviruses (137) and some are found in other virus pathogenic for invertebrates (see Chapter 1). Three additional possible PIF genes have also been noted (Ac46 (ODV-E66), Ac145, and Ac150) (see below). AcMNPV mutants lacking *pif 1*, *2*, or *3* are not orally infectious for *T. ni* or *S. exigua* larvae based on feeding of 10,000 PIB of the deleted virus. They are also not orally infectious for *H. virescens*, except for the PIF2 mutant that shows limited infectivity. In contrast, injection of 1 pfu of the three deletion mutants into third instar larvae of these three species caused over 80% mortality.

PIF function; binding and entry to midgut cells. Most of the PIF proteins contain a hydrophobic region that is thought to be involved integration into the ODV envelope. Nine of the PIF proteins form a complex that is involved in binding and entry of ODV into midgut cells. It includes three that form a stable core complex (PIFs 1-3) and five that associate with this complex (PIFs -0(p74), -4, -6, -7, and -9) to form a larger complex. In addition, Pif8(Ac83) can bind to the larger 8-subunit complex (reviewed in (108) (109, 136) (Figure 11). PIF1, PIF2, and p74 mediate specific binding of ODV to midgut cells, suggesting that they are directly involved in virus cell interaction as an initial step in infection (138) (138, 139). Although PIF-3 appears to be a component of the PIF complex, evidence suggests that it is not involved in specific binding and its function is not clear (140). PIF-5 is apparently not part of the PIF complex (141). A 35-kDa binding partner for AcMNPV P74 was detected in extracts of brush border membrane vesicles from host larvae (*S. exigua*), but not from a non-host (*H. armigera* larvae) (142). The identity of this host protein has not been determined.

Ac83 (VP95)(PIF8); essential for nucleocapsid assembly and is a PIF protein. Ac83 (PIF8) encodes a virion associated protein called VP91 that was originally characterized in OpMNPV (143). It is a core baculovirus gene and is also found in nudiviruses (137) and possibly in several insect genomes, e.g., *Anopheles gambiae* (E = 5e-04). It is an essential gene because a deletion mutant in BmNPV (Bm69) did not produce BV (144). It was found to associate with the PIF complex (141) and although the complete Pif8 gene is required for viability, when a predicted region containing three zinc fingers was deleted, the virus was not infectious when fed to larvae, but could replicate when injected into the haemocoel confirming that it is a PIF protein (145). Originally the zinc finger region was predicted to contain a chitin binding domain, but evidence indicated that AC83 does not bind to chitin (145). The zinc finger region appears to be involved in the assembly and localization of the PIF complex to ODV envelopes and the binding or entry of ODV into midgut cells (146). It was also determined that the ac83 gene contains a cis-acting nucleotide sequence essential for nucleocapsid assembly and is called the nucleocapsid assembly-essential element (NAE) (147). Therefore, the Ac83 gene appears to encode at least two functions; the zinc fingers are involved in the PIF function, whereas the NAE is a nucleotide sequence involved in nucleocapsid assembly. For more information, see Chapter 5.

Ac46 (ODV-E66), a chondroitinase and a PIF protein. Ac46 is a component of ODV envelopes (148) is the only known viral chondroitinase (149). It interacts with several members of the PIF complex (reviewed in (108)). Homologs of Ac46 are found in the genomes of all Group I NPVs, GVs, and most Group II NPVs, but not in hymenopteran or dipteran viruses. Two copies of the gene are present in some genomes (e.g., SeMNPV). In a

study of AcMNPV, an ODV-E66 deletion was observed to kill *Plutella xylostella* larvae as efficiently as wt when injected into larvae, however when infected per os, the LD50 was1000 fold greater for the mutant than wt virus. Therefore it was suggested that ODV-E66 is a per os infectivity factor (150) and could be designated PIF10. A truncated form of Ac46 lacking the N-terminal 66 amino acids was found to be secreted into the medium by infected cells and had chondroitinase activity (149). Chondroitinases have been shown to regulate cytokine and growth factors and can influence a variety of processes including development, inflammation, and organ morphogenesis. Chondroitin sulfate is present in the peritrophic matrix (PM) of *B. mori* and it can be digested by Ac46. This has led to the suggestion that Ac46 enhances the primary infection by digesting the chondroitin sulfate in the PM (151). This activity could be the reason Ac46 is a per os infectivity factor. Chondroitin glycosaminoglycans have been found to localize to the apical midgut microvilli of *Anopheles gambiae* (152) suggesting that if Lepidoptera are similar, this enzyme might also be involved in recognition or entry into midgut cells. Ac46 was previously shown to have hyaluronan lyase activity suggesting that it might be involved in penetrating the extracellular matrix which is composed of hyaluronan (153). However, its activity as a hyaluronan lyase was minimal in the chondroitinase study (149). When the N-terminal 23 amino acids of ODV-E66 are fused to a reporter gene, it is targeted to the nucleus (121). This is consistent it being a protein involved in ODV envelope which develops in association with the nucleus. The crystal structure has been determined (154).

Ac 145 and 150; more possible PIF proteins. These two genes encode small proteins (~9 and 11 kDa, respectively) that are related to one another (23% amino acid sequence identity) and localize to ODV envelopes (155). Close relatives of Ac 145 are found in all baculoviruses except the dipteran NPV. In contrast to Ac145, close relatives of Ac150 are only found in a few NPVs closely related to AcMNPV. Ac145 and 150 are predicted to encode a domain thought to bind to chitin (156). In one study (155), deletion of AcMPNV Ac145 led to a six-fold drop in infectivity in *T. ni,* but not *H. virescens* larvae. An effect of deletion of AcMNPV Ac150 was not detected. Deletion of both genes causes a major (39 fold) reduction of infectivity for *H. virescens*. Injection of BV of the double mutant intrahemocoelically produced the same level of infection as injected wt BV, suggesting that these genes play a role in oral infection. Ac145 and 150 were found to be associated with both BV and ODV (155). In another study (157), occluded virions deleted for Ac150 were found to be significantly less virulent when administered per os than the wt virus in *Heliothis virescens*, *Spodoptera exigua* and *Trichoplusia ni* larvae. Evidence suggested that the mutant had a reduction in its ability to establish primary infections in midgut cells.

Nucleocapsid Structure

Baculovirus nucleocapsids have a defined rod-shaped capsid this is capped by distinct apical and basal structures (Figure 12). Although many proteins appear to be capsid associated, a few appear to be located to end structures. Three of these VP80, VLF-1 and pp78/83 are shown in Figure 11. Although shown located to the basal region, this has not been definitively shown for VP80 or VLF-1.

Essential BV and ODV Nucleocapsid associated proteins encoded by all baculoviruses

As described above many proteins have been associated with BV and ODV nucleocapsids (reviewed in (1) however their role, if any, is unknown. Rather than an exhaustive list of these proteins, in this section I will focus on proteins that evidence suggests are essential components of both BV and ODV nucleocapsids (Table 3). The following are core baculovirus proteins that appear to be nucleocapsid associated.

VP1054, (Ac54). VP1054 was named for the size of its orf and encodes a protein required for nucleocapsid assembly. A ts mutant failed to produce nucleocapsids at the non-permissive temperature, indicating that it is an essential gene. It is found in both BV and ODV (158) and it interacts with 38K (Ac98) (22). Ac98 is likely to be a phosphatase. VP1054 also associates with BV/ODV-C42 and VP80, but not VP39 suggesting that it is critical for

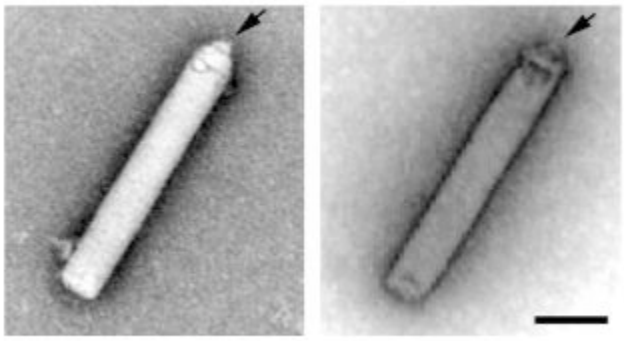

Figure 12. Morphology of AcMNPV capsids stained with uranyl acetate. The figure shows the morphology of the two ends, one with a conical shape (arrow). Scale bar, 50 nm .From Au and Pante (241). Reproduced with permission of Elsevier Limited via Copyright Clearance Center.

nucleocapsid assembly (159). When the vp1054 gene was deleted from a bacmid, the bacmid was not infectious and nucleocapsids appeared to be replaced with tube-like structures. It was suggested that vp1054 is related to a cellular protein called PURα that binds to purine-rich sequences and may be involved in DNA packaging and it was shown to bind to single stranded DNA or RNA sequences that contained runs of GGN. Therefore, it was suggested that it might interact with the orf1629/p/78/83 (ac9) sequence which encodes a series of prolines and therefore is rich in GGN codons (160).

Ac66. Orthologs of Ac66 are present in all baculovirus genomes. An AcMNPV bacmid deleted for Ac66 was severely compromised and BV titers derived from transfected cells were reduced by over 99% compared to wt. In addition, at low titers the mutant BV appeared to infect single cells and was unable to spread to other cells. Although the nucleocapsids appeared to be normal and had an electron-dense core suggesting that they contained DNA, they appeared to be trapped in the virogenic stroma, indicating that Ac66 was required for the efficient egress of virions from nuclei. The deletion did not affect the levels of DNA replication or polyhedrin transcription, but the production of occlusion bodies was eliminated (161). This suggests that Ac66 is required both for egress of virions from nuclei and also may be involved in the nucleation of polyhedra. Ac66 in BV but not ODV is ubiquitinated by the viral ubiquitin and appears to interact with Ac141 (predicted to be an E3 ubiquitin ligase). It was suggested that this might be a signal for BV egress in contrast to ODV that remain in nuclei (162, 163). For more information see Chapter 12.

VLF-1, (Ac77). The very late factor, VLF-1, is a member of the lambda integrase (Int) family of proteins and was originally identified because it influences the hyperexpression of very late genes (164) possibly by binding to their regulatory regions (165). VLF-1 appears to be a structural protein present in both BV and ODV (166) and is clearly required for the production of nucleocapsids. Bacmid deletion mutants produce tube-like structures that stained with vp39 antiserum suggesting that the lack of VLF1 prevents normal capsid assembly. Bacmids with point mutations in the conserved tyrosine form normal appearing capsids, but are also not infectious. VLF-1 localized to the end regions of nucleocapsids further suggesting that it is a structural protein (167). VLF-1 was also associated with ODV virions as determined by proteomic analysis in AcMNPV and CuniNPV, but not HearNPV (93-95). For additional information, see Chapter 5 and Chapter 12.

GP41, tegument protein, (Ac80). GP41 is modified with O-linked N-acetylglucosamine, and is located between the virion envelope and capsid in a structure called the tegument (168, 169). Homologs are present in all baculovirus genomes. Based on the characterization of a ts mutant, it is an essential gene required for the egress of nucleocapsids from the nucleus (170). Under the restrictive temperature, this mutant produces no BV and

infection is limited to single cells. Proteomic studies identified GP41 in both BV and ODV of HearSNPV (119). In addition, it was found that oligomerization of gp41 was required for BV production (171).

VP39, (Ac89). VP39 is thought to be the major capsid protein. It is one of the three most abundant proteins found by proteomic analysis of BV (62). Homologs of vp39 are present in all baculovirus and nudivirus genomes. It has been observed that VP39 interacts with a conserved domain of kinesin 1 and it has been suggested that this interaction is involved in the transport of nucleocapsids destined to become BV to the cell membrane after their assembly in nuclei (172). Based on mutagenic analysis it appears to be required for proper DNA packaging and nucleocapsid assembly (173).

Ac92, (P33), a sulfhydryl oxidase. Ac92 is an essential core gene that encodes a sulfhydryl oxidase, and is likely involved in the production of disulfide bonds in viral proteins (174) (175). It forms a stable complex with the tumor suppressor gene p53 and appears to enhance its apoptotic function (176) (177). P33 was found to be associated with ODV virions by proteomic analysis in several baculoviruses (93-95) (119) and in both AcMNPV BV and ODV in western blot analyses (175) (178). It is unclear whether Ac92 is required for virion structure or is associated with virions because it facilitates disulfide bond formation during virion assembly. Further information on Ac92 is available in Chapters 5, 7, and 12.

Ac98 (38K). Ac98 is encoded by all baculoviruses and is associated with both BV and ODV nucleocapsids. By yeast two-hybrid assays it interacted with VP1054, VP39, VP80, and itself (22). When deleted, tube-like structures devoid of DNA but that stain with vp39 antibody are produced (179). It is related to a set of enzymes including CTD phosphatases and evidence indicates that it dephosphorylates the p6.9 DNA binding protein which allows it to be packaged with the viral DNA (180).

P6.9, (Ac100). P6.9 is a DNA binding protein and one of the three most abundant proteins found in proteomic analysis of BV (62). It is a small (55 aa) arginine/serine/threonine rich protein (181). Homologs appear to be encoded by all baculovirus genomes, but may be difficult to identify in computer analyses because of their small size and repetitive amino acid content. P6.9 was originally identified as a DNA binding protein in a GV (182) and the homolog was subsequently identified from AcMNPV (181). The high concentration of arg and ser/thr residues is similar to protamines that are also small molecules of 44-65 amino acids (183, 184) present in sperm nuclei of many higher eukaryotes and are involved in the production of highly condensed DNA. Arginine has a high affinity for the phosphate backbone of DNA, and the polyarginine tracts in protamines neutralize the phosphodiester backbone, whereas the ser and thr residues interact with other protamine molecules, thereby yielding a neutral, highly compact DNA-protein complex that is biochemically inert. P6.9 localizes to the nuclear matrix during infection (185). Once viral DNA has been delivered to the nucleus, p6.9 is phosphorylated (see below) resulting in both DNA and p6.9 being negatively charged. It is thought that this causes the removal of p6.9 from the viral DNA thereby allowing access to transcription factors (186). AcMNPV has two candidate kinases that could be involved in this process. Protein kinase 1, PK1 (Ac10), is encoded by lepidopteran baculoviruses (GVs and NPVs), whereas PK2 (Ac123) is encoded by only a few baculoviruses closely related to AcMNPV. Neither protein was found to be associated with ODV by mass spectrometry (93-95). However, a kinase was found to be associated with BV and ODV, although its source was not determined (187). P6.9 was found to co-localize with viral DNA and to fractionate with RNA polymerase II at 24 hpi (141). After synthesis, p6.9 is hyperphosphorylated, at least in part by pk-1 and this is associated with high levels of expression of very late genes (188) (189). This occurs immediately upon synthesis and p6.9 is dephosphorylated by the 38k protein (180) before being complexed with DNA (189). Using an AcMNPV bacmid deleted for p6.9, nucleocapsids were not produced although tube-like structures similar to those associated with the deletion of VLF-1 and Ac98 (see above) were observed. The mutant appeared to synthesize normal amounts of DNA, but did not produce infectious virus (190). BmNPV micro RNA-3 (mir-3) appears to regulate, at least in part, the expression of BmNPV p6.9 (191).

BV/ODV-C42, (Ac101). Ac101 is a core gene and encodes a capsid-associated protein found in both BV and ODV (93). In addition, it was reported to interact in a yeast two-hybrid assay and by native gel electrophoresis (192) with the actin nucleation factor pp78/83 (Ac9) (described below) and is required for its transport into nuclei (193). It stabilizes P78/83 by inhibiting its degradation. C42 also interacts with Ac102 and this suppresses ubiquitination of C42 further regulating the stability of P78/83 (194-196). Deletion of Ac101 affected nucleocapsid formation but, although lethal, did not appear to affect DNA synthesis (197).

Ac141 (exon0). Ac141 is conserved in all lepidopteran baculoviruses and is associated with both BV and ODV nucleocapsids (198) (62) and interacts with BV/ODV-C42 (Ac101) and FP25 (Ac61) (199). It appears to be required for the efficient transport of nucleocapsids from nuclei through the cytoplasm (198, 200). It appears to both co-localize with and co-purify with β-tubulin. In addition, inhibitors of microtubules reduced BV production by over 85%. Therefore it has been suggested that the interaction of Ac141 with microtubules might be important in the egress of BV (201). It has also been shown to interact with a conserved domain of kinesin 1, a motor protein involved in transporting cargo along microtubules to the periphery of the cell further supporting a role for microtubules in transport of virions to the cell surface (172, 202). Hhpred (135) predicts with over 90% probability that the C-terminal ~90 amino acids has structural similarity to E3 protein ubiquitin ligase along with several other proteins. Deletion of ac141 and vubi results in single cell infection and BV were not produced. The ubiquitination of Ac141 was essential for optimal production of BV. BV but not ODV nucleocapsids were ubiquitinated by vUbi. The target was Ac66 and it was shown to co-localize with vUbi and Ac141 at the nuclear periphery. It was suggested that the ubiquitination of capsid proteins may be a signal for BV egress from nuclei (162).

Ac142, (p49). Ac142 is encoded by a core gene and is associated with both BV and ODV virions. Deletion of Ac142 appeared to affect nucleocapsid formation but, although lethal, did not appear to affect DNA synthesis (197). Similar results were reported for the homolog in BmNPV (Bm118) (203). Another study describing a different bacmid construct that deleted less of the Ac142 gene showed similar results except that nucleocapsids, although fully formed, were not enveloped in nuclei and were not occluded (204). It is unclear whether the difference in the two studies was due to the removal of a 3' processing signal for the upstream Ac141 gene in the former investigation, or to the presence of a significant portion of the Ac142 orf in the latter study. Proteomic analysis also suggests that Ac142 is ODV-associated in three different viruses (93-95). In addition, it was reported that Ac142 interacts with components of the ESCRT-III complex and may be involved in the release of nucleocapsids at the nuclear membrane (205).

Ac144. Ac144 is a core gene and was originally named ODV-EC27 and it was suggested that it is a cyclin (206). However, another investigation using an HA-tagged Ac144 recombinant virus found that Ac144 was expressed as an ~ 33.5 kDa protein which conforms to the predicted MW (197). In addition, it was found to be BV associated (62, 197). Proteomic analysis suggests that Ac144 is ODV-associated in three different viruses (93-95). A variety of investigations have been conducted on Ac144. Initially, it was confirmed that its transcript initiates at a late promoter element (133). It was reported to interact in a yeast two-hybrid assay with Ac101 described above (also named C42) and with both Ac101 and p78/83 (Ac9) in native gel electrophoresis assays (192). It was also found that deletion of Ac144 resulted in amorphous electron dense structures that stained with vp39-capsid antibodies, but no nucleocapsids were evident. Although lethal, deletion of Ac144 did not appear to affect DNA synthesis (197).

Table 3 Selected Proteins Associated with Baculovirus Nucleocapsids

Name and AcMNPV orf #	Distribution in the Baculoviridae	Effect of Deletion or mutation
Ac100, P6.9 DNA binding	All	Not viable (190)
Ac89, VP39 capsid	All	Not viable (144)
Ac80 GP41 tegument	All	Not viable (170)

Table 3 continued from previous page.

Name and AcMNPV orf #	Distribution in the Baculoviridae	Effect of Deletion or mutation
Ac98 38K	All	Not viable (179)
Ac142	All	Not viable (197, 204)
Ac144	All	Not viable (197)
Ac66	All	Severely compromised (161)
Ac92 (P33)	All	Not viable (175, 178)
Ac54 (VP1054)	All	Not viable (158)
Ac77 VLF-1	All	Not viable (247)
Ac104, VP80	Lep. I and II NPV	Not viable (225)
Ac9, PP78/83	Lep. I and II NPV	Not viable(211)
Ac129, P24	Lep. I, II, GV	Viable (235)

Other structural proteins

The following structural proteins are found in some, but not all baculovirus genomes. This could indicate either that they are not present or have evolved to such an extent that their relatedness can no longer be identified in the genomes in which they are not found.

PP78/83, (Ac9). PP78/83 is a phosphorylated protein that is located at one end of nucleocapsids (207, 208). It is a Wiskott-Aldrich syndrome protein (WASP)-like protein. Such proteins are involved in nuclear actin assembly, and it has been demonstrated that pp78/83 serves this function during AcMNPV infection (209) (210) (see Chapter 3). Homologs of pp78/83 are found in all lepidopteran NPV genomes. It is an essential gene, and because it is located adjacent to the polyhedrin gene, it was originally manipulated via complementation to elevate the frequency of obtaining recombinant baculoviruses at the polyhedrin locus (211).

GP37 (Ac64). Orthologs of gp37 have been found in the genomes of all alpha- and betabaculoviruses (212). In AcMNPV it is expressed as a late gene (213) and is nonessential for replication in cell culture or *T. ni* larvae (214). The BmNPV homolog (Bm52) was also found to be non essential in BmN cells (144). The gp37 homolog in SpltNPV has been reported to contain chitin binding domains and is capable of binding to chitin (215). The gp37 of CpGV also binds chitin and was able to enhance per os infections (216). Insect proteins, such as the coagulation protein hemolectin, also have chitin-binding domains (217). Whether gp37 somehow inhibits or redirects such pathways remains to be determined. GP37 was reported to be polyhedron associated in AcMNPV and to be N-glycosylated (218). It was also found to be BV associated (62). In OpMNPV infected *L. dispar* cells, GP37 was found to be an N-glycosylated protein located in cytoplasmic occlusions late in infection (219). In entomopox viruses the gp37 ortholog forms crystallized spindle-like structures. These structures have been suggested to digest the peritrophic matrix (220). Structural analysis of these spindles indicated that they contained a globular domain that is related to lytic polysaccharide monooxygenases of chitinovorous bacteria. It is thought that upon ingestion by the host, the spindles are dissolved and the monooxygenase domain is exposed and can then digest the chitin-rich peritrophic matrix (221) thereby facilitating the passage of virions through the peritrophic matrix and allowing them access to midgut cells. It has been reported that GP37 can degrade the peritrophic matrix and also facilitates the binding of ODV to midgut cells (222). The *Spodoptera litura* NPV genome was found to contain a gene that is a fusion of ubiquitin and gp37 (for discussion in Chapter 12, see *Ac35, ubiquitin*) and the protein was associated with the envelopes of BV and ODV (223). For more information see Chapter 12.

Vp80 (Ac104). Orthologs of Ac104 are found in all Group I and II lepidopteran NPV genomes, but not in those of GVs or hymenopteran or dipteran NPVs. It is capsid associated in both OpMNPV (224) and AcMNPV (93, 225, 226) and interacts with 38K (Ac98) (22). Deletion of Vp80 showed that it is an essential gene and resulted in nucleocapsids that were unable to move from the virogenic stroma (227). It appears to localize in nuclei near actin scaffolds that may connect the virogenic stroma to the nuclear envelope. In addition, it co-immunoprecipitates with actin. It also appears to localize to one end of nucleocapsids and contains sequences similar to paramyosin motifs that may be involved in the transport of virions to the periphery of nuclei (227). It forms dimers, contains a C-terminal region that was predicted to contain a basic helix-loop-helix domain, and binds to DNA (228).

Ac109 (ME53). Homologs of *ac139* are present in the genomes of all the lepidopteran NPVs and GVs, but have not been reported in hymenopteran or dipteran baculovirus genomes. It is BV and ODV associated (229). One study indicated that AcMNPV deleted for this gene is not viable and fails to replicate its DNA and does not produce nucleocapsids. However, cells transfected with DNA from the mutant showed early stages of cpe, including nuclear enlargement and the formation of granular material in the nucleus (230). This suggests that the mutant is blocked in an early gene function. This is consistent with its original characterization as a major early gene (231). However, another study showed that deletion of Ac139 did not alter DNA replication, but results in a 1000-fold reduction in BV titer. In addition, it was found that it appears to be required both early and late in infection (229). ME53 fused to GFP localized mostly to the cytoplasm early and to nuclei late in infection. However, foci of ME53 were also noted at the cell periphery late in infection and co-localized with gp64 and VP39-capsid and was capsid associated in BV. It was suggested that it may provide a connection between the nucleocapsid and the viral envelope (232).

P24-capsid, (Ac129). Ac129 (P24) was found to be associated with both BV and ODV of AcMNPV and OpMNPV by Western blot and electron microscopic analyses (233). It is likely to be nonessential as interruption of this gene with a transposable element in a strain of AcMNPV has been reported (234, 235) and it can also be deleted from BmNPV with no detectable effects (144). Homologs of Ac129 are present in all Group I /II and GV genomes. The Ac129 homolog was not reported to be associated with ODV of HearNPV (95). It was also reported to be BV associated (62).

Additional virion associated proteins. Many more proteins have been found to be associated with AcMNPV virions. Information on these and summarized in (1) and Chapter 12.

Host proteins and protein modification

Host proteins. Analysis of BV from AcMNPV using mass spectrometry identified 48 virally encoded proteins and 11 host proteins (62). In addition, a variety of host proteins were also associated with preparations of HearSNPV ODV and BV, with 21 and 101 host proteins identified, respectively. Many of these proteins could be simply trapped as the virions are assembled or are present as normal components of the membranes from which their viral envelopes were derived. However, some components could be essential for virion function. One such protein, cyclophilin A was associated with both HearSNPV BV and ODV. Cyclophilin A catalyzes the isomerization of peptide bonds from *trans* to *cis* at proline residues, a process that can be important in protein folding. In HIV-1, but not other primate immunodeficiency viruses, cyclophilin A associates with a proline rich region of the GAG polyprotein and is required for the production of infectious virions (236). The 11 host proteins that were identified in AcMNPV BV (62) included cytoplasmic actin and actin depolymerizing factor, perhaps reflecting the role actin plays in BV transport (210). In addition, it was observed that baculoviruses grown in different insect cells show differing efficiencies in mammalian cell entry suggesting that some host factor may facilitate cell entry (104).

Post translational modification of structural proteins. Proteomic analysis using several techniques was applied to both BV and ODV of the HearSNPV (119). They reported N-glycosylated proteins in only BV, but not in

ODV. These included F, FGF, V-CATH, ChiA, P26 (=Ac136), and ODV-E18 (=Ac143). One of these, ODV-E18, is present in both BV and ODV. They also observed differing phosphorylation profiles between the two phenotypes with 38 sites in ODV associated proteins, and 4 in BV. Serine was the predominant phosphorylation site (72.1%) followed by Thr (22.3%) and Tyr (4.6%). The phosphorylated proteins (phosphoproteome) of B. mori cells infected with BmNPV has been examined. Many phosphorylated host and viral proteins were identified, some of which were hyperphosphorylated including p6.9 (237).

References

1. Blissard GW, Theilmann DA. Baculovirus Entry and Egress from Insect Cells. Annu Rev Virol. 2018;5:113–139. PubMed PMID: 30004832.
2. Evans HF. 1986. Ecology and epizootiology of baculoviruses, p 89-132. *In* Granados RR, Federici BA (ed), The biology of baculoviruses, vol II. CRC Press, Inc., Boca Raton.
3. Perera OP, Valles SM, Green TB, White S, Strong CA, Becnel JJ. Molecular analysis of an occlusion body protein from Culex nigripalpus nucleopolyhedrovirus (CuniNPV). J Invertebr Pathol. 2006;91:35–42. PubMed PMID: 16376931.
4. Ji X, Axford D, Owen R, Evans G, Ginn HM, Sutton G, Stuart DI. Polyhedra structures and the evolution of the insect viruses. J Struct Biol. 2015;192:88–99. PubMed PMID: 26291392.
5. Chaivisuthangkura P, Tawilert C, Tejangkura T, Rukpratanporn S, Longyant S, Sithigorngul W, Sithigorngul P. Molecular isolation and characterization of a novel occlusion body protein gene from Penaeus monodon nucleopolyhedrovirus. Virology. 2008;381:261–7. PubMed PMID: 18829059.
6. Wang Y, Jehle JA. Nudiviruses and other large, double-stranded circular DNA viruses of invertebrates: New insights on an old topic. J Invertebr Pathol. 2009;101:187–93. PubMed PMID: 19460388.
7. Coulibaly F, Chiu E, Ikeda K, Gutmann S, Haebel PW, Schulze-Briese C, Mori H, Metcalf P. The molecular organization of cypovirus polyhedra. Nature. 2007;446:97–101. PubMed PMID: 17330045.
8. Coulibaly F, Chiu E, Gutmann S, Rajendran C, Haebel PW, Ikeda K, Mori H, Ward VK, Schulze-Briese C, Metcalf P. The atomic structure of baculovirus polyhedra reveals the independent emergence of infectious crystals in DNA and RNA viruses. Proc Natl Acad Sci U S A. 2009;106:22205–10. PubMed PMID: 20007786.
9. Afonso CL, Tulman ER, Lu Z, Balinsky CA, Moser BA, Becnel JJ, Rock DL, Kutish GF. Genome sequence of a baculovirus pathogenic for Culex nigripalpus. J Virol. 2001;75:11157–65. PubMed PMID: 11602755.
10. Summers MD. 1977. Characterization of shrimp baculovirus. EPA Ecological Research Series:EPA-600/3-77-130.
11. Carstens EB, Krebs A, Gallerneault CE. l986. Identification of an amino acid essential to the normal assembly of *Autographa californica* nuclear polyhedrosis virus polyhedra. J Virol.58:684–688. PubMed PMID: 3528527.
12. Ji X, Sutton G, Evans G, Axford D, Owen R, Stuart DI. How baculovirus polyhedra fit square pegs into round holes to robustly package viruses. EMBO J. 2010;29:505–514. PubMed PMID: 19959989.
13. Gati C, Oberthuer D, Yefanov O, Bunker RD, Stellato F, Chiu E, Yeh SM, Aquila A, Basu S, Bean R, Beyerlein KR, Botha S, Boutet S, DePonte DP, Doak RB, Fromme R, Galli L, Grotjohann I, James DR, Kupitz C, Lomb L, Messerschmidt M, Nass K, Rendek K, Shoeman RL, Wang D, Weierstall U, White TA, Williams GJ, Zatsepin NA, Fromme P, Spence JC, Goldie KN, Jehle JA, Metcalf P, Barty A, Chapman HN. Atomic structure of granulin determined from native nanocrystalline granulovirus using an X-ray free-electron laser. Proc Natl Acad Sci U S A. 2017;114:2247–2252. PubMed PMID: 28202732.
14. Minion FC, Coons LB, Broome JR. Characterization of the polyhedral envelope of the nuclear polyhedrosis virus of *Heliothis virescens*. J Invertebr Pathol. 1979;34:303–307.
15. Whitt MA, Manning JS. A phosphorylated 34-kDa protein and a subpopulation of polyhedrin are thiol-linked to the carbohydrate layer surrounding a baculovirus occlusion body. Virology. 1988;163:33–42. PubMed PMID: 3279702.

16. Gross CH, Russell RLQ, Rohrmann GF. The Orgyia pseudotsugata baculovirus p10 and polyhedron envelope protein genes: analysis of their relative expression levels and role in polyhedron structure. J Gen Virol. 1994;75:1115–1123. PubMed PMID: 8176372.
17. Gombart AF, Pearson MN, Rohrmann GF, Beaudreau GS. A baculovirus polyhedral envelope-associated protein: genetic location, nucleotide sequence, and immunocytochemical characterization. Virology. 1989;169:182–193. PubMed PMID: 2646825.
18. Russell RLQ, Rohrmann GF. A baculovirus polyhedron envelope protein: Immunogold localization in infected cells and mature polyhedra. Virology. 1990;174:177–184. PubMed PMID: 2403704.
19. Russell RLQ, Pearson MN, Rohrmann GF. Immunoelectron microscopic examination of *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus-infected *Lymantria dispar* cells: time course and localization of major polyhedron-associated proteins. J Gen Virol. 1991;72:275–283. PubMed PMID: 1993872.
20. Lee SY, Poloumienko A, Belfry S, Qu X, Chen W, MacAfee N, Morin B, Lucarotti C, Krause M. A common pathway for p10 and calyx proteins in progressive stages of polyhedron envelope assembly in AcMNPV-infected Spodoptera frugiperda larvae. Arch Virol. 1996;141:1247–58. PubMed PMID: 8774685.
21. Busby JN, K.N.Goldie, Metcalf P. 2009. Structural studies of granulovirus envelope fibres. doi: 10.2210/pdb4YE7/pdb, p]. Protein Data Bank.
22. Wu W, Liang H, Kan J, Liu C, Yuan M, Liang C, Yang K, Pang Y. Autographa californica Multiple Nucleopolyhedrovirus 38K Is a Novel Nucleocapsid Protein That Interacts with VP1054, VP39, VP80 and Itself. J Virol. 2008;82:12356–64. PubMed PMID: 18922869.
23. Xu H, Yang Z, Zhao J, Tian C, Ge J, Tang X, Bao Y, Zhang C. Bombyx mori nucleopolyhedrovirus ORF56 encodes an occlusion-derived virus protein and is not essential for budded virus production. J Gen Virol. 2008;89:1212–1219. PubMed PMID: 18420799.
24. Cheley S, Kosik KS, Paskevich P, Bakalis S, Bayley H. Phosphorylated baculovirus p10 is a heat-stable microtubule-associated protein associated with process formation in Sf9 cells. J Cell Sci. 1992;102:739–752. PubMed PMID: 1331130.
25. Raza F, McGouran JF, Kessler BM, Possee RD, King LA. Phosphorylation Induces Structural Changes in the Autographa californica Nucleopolyhedrovirus P10 Protein. J Virol. 2017.:91. PubMed PMID: 28424279.
26. Carpentier DCJ, Griffiths CM, King LA. The baculovirus P10 protein of Autographa californica nucleopolyhedrovirus forms two distinct cytoskeletal-like structures and associates with polyhedral occlusion bodies during infection. Virology. 2008;371:278–91. PubMed PMID: 17991504.
27. Patmanidi AL, Possee RD, King LA. Formation of P10 tubular structures during AcMNPV infection depends on the integrity of host-cell microtubules. Virology. 2003;317:308–20. PubMed PMID: 14698669.
28. Vlak JM, Klinkenberg FA, Zaal KJM, Usmany M, Klinge-roode EC, Geervliet JBF, Roosien J, Lent JWV. Functional studies on the p10 gene of *Autographa californica* nuclear polyhedrosis virus using a recombinant expressing a p10-b-galactosidase fusion gene. J Gen Virol. 1988;69:765–776. PubMed PMID: 3128641.
29. Williams GV, Rohel DZ, Kuzio J, Faulkner P. A cytopathological investigation of *Autographa californica* nuclear polyhedrosis virus p10 gene function using insertion/deletion mutants. J Gen Virol. 1989;70:187–202. PubMed PMID: 2659726.
30. Graves LP, Hughes LC, Irons SL, Possee RD, King LA. In cultured cells the baculovirus P10 protein forms two independent intracellular structures that play separate roles in occlusion body maturation and their release by nuclear disintegration. PLoS Pathog. 2019.:15. PubMed PMID: 31181119.
31. van Oers MM, Flipsen JTM, Reusken CBEM, Sliwinsky EL, Goldbach RW, Vlak JM. Functional domains of the p10 protein of Autographa californica nuclear polyhedrosis virus. J Gen Virol. 1993;74:563–574. PubMed PMID: 8468550.
32. Hashimoto Y, Hayakawa T, Ueno Y, Fugita T, Sano Y, Matsumoto T. Sequence analysis of the *Plutella xylostella* granulovirus genome. Virology. 2000;275:358–372. PubMed PMID: 10998336.
33. Escasa SR, Lauzon HA, Mathur AC, Krell PJ, Arif BM. Sequence analysis of the Choristoneura occidentalis granulovirus genome. J Gen Virol. 2006;87:1917–33. PubMed PMID: 16760394.

34. Lange M, Jehle JA. The genome of the Cryptophlebia leucotreta granulovirus. Virology. 2003;317:220–36. PubMed PMID: 14698662.
35. Alaoui-Ismaili MH, Richardson CD. Insect virus proteins (FALPE and p10) self-associate to form filaments in infected cells. J Virol. 1998;72:2213–23. PubMed PMID: 9499079.
36. Lepore LS, Roelvink PR, Granados RR. Enhancin, the granulosis virus protein that facilitates nucleopolyhedrovirus (NPV) infections, is a metalloprotease. J Invertebr Pathol. 1996;68:131–40. PubMed PMID: 8858909.
37. Hashimoto Y, Cosaro BG, Granados RR. Location and nucleotide sequence of the gene encoding the viral enhancing factor of the *Trichoplusia ni* granulosis virus. J Gen Virol. 1991;72:2645–2651. PubMed PMID: 1940861.
38. Slavicek JM, Popham HJ. The Lymantria dispar nucleopolyhedrovirus enhancins are components of occlusion-derived virus. J Virol. 2005;79:10578–88. PubMed PMID: 16051850.
39. Hayakawa T, Ko R, Okano K, Seong S, Goto C, Maeda S. Sequence analysis of the *Xestia c-nigrum* granulovirus genome. Virology. 1999;262:277–297. PubMed PMID: 10502508.
40. Popham HJ, Bischoff DS, Slavicek JM. Both Lymantria dispar nucleopolyhedrovirus enhancin genes contribute to viral potency. J Virol. 2001;75:8639–48. PubMed PMID: 11507209.
41. Wang P, Granados RR. An intestinal mucin is the target substrate for a baculovirus enhancin. Proc Natl Acad Sci U S A. 1997;94:6977–82. PubMed PMID: 9192677.
42. Galloway CS, Wang P, Winstanley D, Jones IM. Comparison of the bacterial Enhancin-like proteins from Yersinia and Bacillus spp. with a baculovirus Enhancin. J Invertebr Pathol. 2005;90:134–7. PubMed PMID: 16081094.
43. Kozlov EA, Sidorova NM, Serebryani SB. Proteolytic cleavage of polyhedral protein during dissolution of inclusion bodies of the nuclear polyhedrosis viruses of Bombyx mori and Galleria mellonella under alkaline conditions. J Invertebr Pathol. 1975;25:97–101. PubMed PMID: 1089735.
44. Kozlov EA, Levitina TL, Gusak NM, Serebryani SB. Comparison of the amino acid sequences of inclusions body proteins of *Bombyx mori, Porthetria dispar*, and *Galleria mellonella*. Biorganic Khimia. 1981;7:1008–1015.
45. Serebryani SB, Levitina TL, Kautsman ML, Radavski YL, Gusak NM, Ovander MN, Sucharenko NV, Kozlov EA. The primary structure of the polyhedral protein of nuclear polyhedrosis virus (NPV) of *Bombyx mori*. J Invertebr Pathol. 1977;30:442–443.
46. Rubinstein R, Polson A. Midgut and viral associated proteases of *Heliothis armigera*. Intervirology. 1983;19:16–25. PubMed PMID: 6337972.
47. McCarthy WJ, DiCapua RA. Characterization of solubilized proteins from tissue culture and host-derived nuclear polyhedra of *Lymantria dispar* and *Autographa californica*. Intervirology. 1979;11:174–181. PubMed PMID: 372133.
48. Wood HA. *Autographa californica* nuclear polyhedrosis virus-induced proteins in tissue culture. Virology. 1980;102:21–27. PubMed PMID: 18631644.
49. Zummer M, Faulkner P. Absence of protease in baculovirus polyhedral bodies propagated in vitro. J Invertebr Pathol. 1979;33:383–384.
50. Slack JM, Kuzio J, Faulkner P. Characterization of *v-cath*, a cathepsin L-like proteinase expressed by the baculovirus *Autographa californica* multiple nuclear polyhedrosis virus. J Gen Virol. 1995;76:1091–1098. PubMed PMID: 7730794.
51. Harrison SC. Viral membrane fusion. Virology. 2015;479-480:498–507. PubMed PMID: 25866377.
52. Dong S, Wang M, Qiu Z, Deng F, Vlak JM, Hu Z, Wang H. Autographa californica multicapsid nucleopolyhedrovirus efficiently infects Sf9 cells and transduces mammalian cells via direct fusion with the plasma membrane at low pH. J Virol. 2010;84:5351–9. PubMed PMID: 20219938.
53. Blissard GW, Wenz JR. Baculovirus gp64 envelope glycoprotein is sufficient to mediate pH-dependent membrane fusion. J Virol. 1992;66:6829–6835. PubMed PMID: 1404622.
54. Oomens AGP, Monsma SA, Blissard GW. The baculovirus gp64 envelope fusion protein: synthesis, oligomerization, and processing. Virology. 1995.:592–603. PubMed PMID: 7778291.

55. Monsma SA, Oomens AGP, Blissard GW. The gp64 envelope fusion protein is an essential baculovirus protein required for cell-to-cell transmission of infection. J Virol. 1996;70:4607–4616. PubMed PMID: 8676487.
56. Kuzio J, Pearson MN, Harwood SH, Funk CJ, Evans JT, Slavicek J, Rohrmann GF. Sequence and analysis of the genome of a baculovirus pathogenic for *Lymantria dispar*. Virology. 1999;253:17–34. PubMed PMID: 9887315.
57. Ijkel WFJ, van Strien EA, Jeldens JGM, Broer R, Zuidema D, Goldbach RW, Vlak JM. Sequence and organization of the *Spodoptera exigua* multicapsid nucleopolyhedrovirus genome. J Gen Virol. 1999;80:3289–3304. PubMed PMID: 10567663.
58. Roberts TE, Faulkner P. Fatty acid acylation of the 67K envelope glycoprotein of a baculovirus: *Autographa californica* nuclear polyhedrosis virus. Virology. 1989;172:377–381. PubMed PMID: 2672565.
59. Hohmann AW, Faulkner P. Monoclonal antibodies to baculovirus structural proteins: determination of specificities by western blot analysis. Virology. 1983;125:432–444. PubMed PMID: 6340331.
60. Whitford M, Stewart S, Kuzio J, Faulkner P. Identification and sequence analysis of a gene encoding gp67 an abundant envelope glycoprotein of the baculovirus, *Autographa californica* nuclear polyhedrosis virus. J Virol. 1989;63:1393–1399. PubMed PMID: 2644449.
61. Volkman LE. l986. The 64K envelope protein of budded *Autographa californica* nuclear polyhedrosis virus. Curr Top Microbiol Immunol.131:103–118. PubMed PMID: 3545692.
62. Wang R, Deng F, Hou D, Zhao Y, Guo L, Wang H, Hu Z. Proteomics of the Autographa californica Nucleopolyhedrovirus Budded Virions. J Virol. 2010;84:7233–7242. PubMed PMID: 20444894.
63. Oomens AG, Blissard GW. Requirement for GP64 to drive efficient budding of *Autographa californica* multicapsid nucleopolyhedrovirus. Virology. 1999;254:297–314. PubMed PMID: 9986796.
64. Ardisson-Araujo DM, Pereira BT, Melo FL, Ribeiro BM, Bao SN. A betabaculovirus encoding a gp64 homolog. BMC Genomics. 2016;17:94. de AZPM, Moscardi F, Kitajima EW, Sosa-Gomez DR, Wolff JL. PubMed PMID: 26847652.
65. Pearson MN, Rohrmann GF. Transfer, incorporation, and substitution of envelope fusion proteins among members of the Baculoviridae, Orthomyxoviridae, and Metaviridae (insect retrovirus) families. J Virol. 2002;76:5301–5304. PubMed PMID: 11991958.
66. Ardisson-Araujo DM, Melo FL, Clem RJ, Wolff JL, Ribeiro BM. A Betabaculovirus-Encoded gp64 Homolog Codes for a Functional Envelope Fusion Protein. J Virol. 2016;90:1668–72. PubMed PMID: 26537678.
67. Cook JD, Soto-Montoya H, Korpela MK, Lee JE. Electrostatic Architecture of the Infectious Salmon Anemia Virus (ISAV) Core Fusion Protein Illustrates a Carboxyl-Carboxylate pH Sensor. J Biol Chem. 2015;290:18495–504. PubMed PMID: 26082488.
68. Cook JD, Sultana A, Lee JE. Structure of the infectious salmon anemia virus receptor complex illustrates a unique binding strategy for attachment. Proc Natl Acad Sci U S A. 2017;114:E2929–E2936. PubMed PMID: 28320973.
69. Peng R, Zhang S, Cui Y, Shi Y, Gao GF, Qi J. Structures of human-infecting Thogotovirus fusogens support a common ancestor with insect baculovirus. Proc Natl Acad Sci U S A. 2017;114:E8905–E8912. PubMed PMID: 29073031.
70. Kadlec J, Loureiro S, Abrescia NG, Stuart DI, Jones IM. The postfusion structure of baculovirus gp64 supports a unified view of viral fusion machines. Nat Struct Mol Biol. 2008;10:1024–30. PubMed PMID: 18776902.
71. Dong S, Blissard GW. Functional analysis of the Autographa californica multiple nucleopolyhedrovirus GP64 terminal fusion loops and interactions with membranes. J Virol. 2012;86:9617–28. PubMed PMID: 22740400.
72. Yu Q, Blissard GW, Liu TX, Li Z. Autographa californica multiple nucleopolyhedrovirus GP64 protein: Analysis of domain I and V amino acid interactions and membrane fusion activity. Virology. 2016;488:259–70. PubMed PMID: 26655244.

73. Jiang Y, Deng F, Rayner S, Wang H, Hu Z. Evidence of a major role of GP64 in group I alphabaculovirus evolution. Virus Res. 2009;142:85–91. PubMed PMID: 19428740.
74. Wang M, Yin F, Shen S, Tan Y, Deng F, Vlak JM, Hu Z, Wang H. Partial Functional Rescue of HearNPV Infectivity by Substitution of F Protein with GP64 from AcMNPV. J Virol. 2010;84:11505–14. PubMed PMID: 20739531.
75. Wang M, Wang J, Yin F, Tan Y, Deng F, Chen X, Jehle JA, Vlak JM, Hu Z, Wang H. Unraveling the entry mechanism of baculoviruses and its evolutionary implications. J Virol. 2014;88:2301–11. PubMed PMID: 24335309.
76. Shen S, Wang M, Li X, Li S, van Oers MM, Vlak JM, Braakman I, Hu Z, Deng F, Wang H. Mutational and functional analysis of N-linked glycosylation of envelope fusion protein F of Helicoverpa armigera nucleopolyhedrovirus. J Gen Virol. 2016;97:988–99. PubMed PMID: 26769631.
77. Lung O, Westenberg M, Vlak JM, Zuidema D, Blissard GW. Pseudotyping *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV): F proteins from group II NPVs are functionally analogous to AcMNPV GP64. J Virol. 2002;76:5729–36. PubMed PMID: 11992001.
78. Wang M, Tan Y, Yin F, Deng F, Vlak JM, Hu Z, Wang H. The F-like protein Ac23 enhances the infectivity of the budded virus of gp64-null AcMNPV pseudotyped with baculovirus envelope fusion protein F. J Virol. 2008;82:9800–4. PubMed PMID: 18653446.
79. Pearson MN, Groten C, Rohrmann GF. Identification of the *Lymantria dispar* nucleopolyhedrovirus envelope fusion protein provides evidence for a phylogenetic division of the Baculoviridae. J Virol. 2000;74:6126–6131. PubMed PMID: 10846096.
80. Ijkel WFJ, Westernberg M, Goldbach RW, Blissard GW, Vlak JM, Zuidema D. A novel baclovirus envelope fusion protein with a proprotein convertase cleavage site. Virology. 2000;274:30–41. PubMed PMID: 11017785.
81. Westenberg M, Soedling HM, Hirani N, Nicholson LJ, Mann DA, Dolphin CT. Seamless replacement of Autographa californica multiple nucleopolyhedrovirus gp64 with each of five novel type II alphabaculovirus fusion sequences generates pseudotyped virus that fails to transduce mammalian cells. J Gen Virol. 2012;93:1583–90. PubMed PMID: 22492915.
82. Yin F, Wang M, Tan Y, Deng F, Vlak JM, Hu Z, Wang H. A functional F analogue of AcMNPV GP64 from the Agrotis segetum Granulovirus. J Virol. 2008;82:8922–6. PubMed PMID: 18562524.
83. Yin F, Wang M, Tan Y, Deng F, Vlak JM, Hu Z, Wang H. 2013. Betabaculovirus F proteins showed different efficiencies when rescuing the infectivity of gp64-null Autographa californica nucleopolyhedrovirus. Virology 436:2013 Feb 5;436(1):59-66.
84. Eckert DM, Kim PS. Mechanisms of viral membrane fusion and its inhibition. Annu Rev Biochem. 2001;70:777–810. PubMed PMID: 11395423.
85. Skehel J, Wiley D. Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin. Annu Rev Biochem. 2000;69:531–69. PubMed PMID: 10966468.
86. Rohrmann GF, Karplus PA. Relatedness of baculovirus and gypsy retrotransposon envelope proteins. BMC Evol Biol. 2001;1:1. PubMed PMID: 11244578.
87. Malik HS, Henikoff S. Positive Selection of Iris, a Retroviral Envelope-Derived Host Gene in Drosophila melanogaster. PLoS Genet. 2005;1:429–43. PubMed PMID: 16244705.
88. Soding J. Protein homology detection by HMM-HMM comparison. Bioinformatics. 2005;21:951–60. PubMed PMID: 15531603.
89. Gresikova M, Nosek J, Ciampor F, Sekeyova M, Turek R. Isolation of paramyxovirus type 4 from Oeciacus hirundinis bugs. Acta Virol. 1980;24:222–3. PubMed PMID: 6107039.
90. Pearson MN, Russell R, Rohrmann GF. Characterization of a baculovirus encoded protein that is associated with infected-cell membranes and budded virions. Virology. 2001;291:22–31. PubMed PMID: 11878873.
91. Lung OY, Cruz-Alvarez M, Blissard GW. Ac23, an envelope fusion protein homolog in the baculovirus Autographa californica multicapsid nucleopolyhedrovirus, is a viral pathogenicity factor. J Virol. 2003;77:328–39. PubMed PMID: 12477838.

92. Zhou J, Blissard GW. Identification of a GP64 subdomain involved in receptor binding by budded virions of the baculovirus AcMNPV. J Virol. 2008;82:4449–60. PubMed PMID: 18287233.
93. Braunagel SC, Russell WK, Rosas-Acosta G, Russell DH, Summers MD. Determination of the protein composition of the occlusion-derived virus of Autographa californica nucleopolyhedrovirus. Proc Natl Acad Sci U S A. 2003;100:9797–802. PubMed PMID: 12904572.
94. Perera O, Green TB, Stevens SM Jr, White S, Becnel JJ. Proteins associated with Culex nigripalpus nucleopolyhedrovirus occluded virions. J Virol. 2007;81:4585–90. PubMed PMID: 17301145.
95. Deng F, Wang R, Fang M, Jiang Y, Xu X, Wang H, Chen X, Arif BM, Guo L, Wang H, Hu Z. Proteomics analysis of Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus identified two new occlusion-derived virus-associated proteins, HA44 and HA100. J Virol. 2007;81:9377–85. PubMed PMID: 17581982.
96. Hou D, Zhang L, Deng F, Fang W, Wang R, Liu X, Guo L, Rayner S, Chen X, Wang H, Hu Z. Comparative Proteomics Reveal Fundamental Structural and Functional Differences between the Two Progeny Phenotypes of a Baculovirus. J Virol. 2013;87:829–39. PubMed PMID: 23115289.
97. Carbonell LF, Miller LK. Baculovirus interaction with nontarget organisms: a virus-borne reporter gene is not expressed in two mammalian cell lines. Appl Environ Microbiol. 1987;53:1412–1417. PubMed PMID: 3116925.
98. Vanarsdall AL, Mikhailov VS, Rohrmann GF. Characterization of a baculovirus lacking the DBP (DNA-binding protein) gene. Virology. 2007;364:475–85. PubMed PMID: 17449080.
99. Hefferon KL, Oomens AG, Monsma SA, Finnerty CM, Blissard GW. Host cell receptor binding by baculovirus GP64 and kinetics of virion entry. Virology. 1999;258:455–68. PubMed PMID: 10366584.
100. Hofmann C, Sandig V, Jennings G, Rudolph M, Schlag P, Strauss M. Efficient gene transfer into human hepatocytes by baculovirus vectors. Proc Natl Acad Sci U S A. 1995;92:10099–103. PubMed PMID: 7479733.
101. Tani H, Nishijima M, Ushijima H, Miyamura T, Matsuura Y. Characterization of cell-surface determinants important for baculovirus infection. Virology. 2001;279:343–53. PubMed PMID: 11145915.
102. Kamiya K, Kobayashi J, Yoshimura T, Tsumoto K. Confocal microscopic observation of fusion between baculovirus budded virus envelopes and single giant unilamellar vesicles. Biochim Biophys Acta. 2010;1798:1625–1631. PubMed PMID: 20493165.
103. Kataoka C, Kaname Y, Taguwa S, Abe T, Fukuhara T, Tani H, Moriishi K, Matsuura Y. Baculovirus GP64-mediated entry into mammalian cells. J Virol. 2012;86:2610–20. PubMed PMID: 22190715.
104. O'Flynn NM, Patel A, Kadlec J, Jones IM. Improving promiscuous mammalian cell entry by the baculovirus Autographa californica multiple nuclear polyhedrosis virus. Biosci Rep. 2012;33:23–36. PubMed PMID: 23035899.
105. Westenberg M, Uijtdewilligen P, Vlak JM. Baculovirus envelope fusion proteins F and GP64 exploit distinct receptors to gain entry into cultured insect cells. J Gen Virol. 2007;88:3302–6. PubMed PMID: 18024899.
106. Liang C, Song J, Chen X. The GP64 protein of Autographa californica multiple nucleopolyhedrovirus rescues Helicoverpa armigera nucleopolyhedrovirus transduction in mammalian cells. J Gen Virol. 2005;86:1629–35. PubMed PMID: 15914840.
107. Kikhno I, Guitiérrez S, Croizier L, Croizier G, Lopéz-Ferber ML. Characterization of pif, a gene required for the per os infectivity of Spodoptera littoralis nucleopolyhedrovirus. J Gen Virol. 2002;83:3013–3022. PubMed PMID: 12466478.
108. Boogaard B, van Oers MM, van Lent JWM. An Advanced View on Baculovirus per Os Infectivity Factors. Insects. 2018.:9. PubMed PMID: 30018247.
109. Zheng Q, Shen Y, Kon X, Zhang J, Feng M, Wu X. Protein-protein interactions of the baculovirus per os infectivity factors (PIFs) in the PIF complex. J Gen Virol. 2017;98:853–861. PubMed PMID: 28141488.
110. Braunagel SC, Summers MD. Molecular Biology of the Baculovirus Occlusion-Derived Virus Envelope. Curr Drug Targets. 2007;8:1084–1095. PubMed PMID: 17979668.
111. Beniya H, Braunagel SC, Summers MD. Autographa californica nuclear polyhedrosis virus: subcellular localization and protein trafficking of BV/ODV-E26 to intranuclear membranes and viral envelopes. Virology. 1998;240:64–75. PubMed PMID: 9448690.

112. Imai N, Kurihara M, Matsumoto S, Kang WK. Bombyx mori nucleopolyhedrovirus orf8 encodes a nucleic acid binding protein that colocalizes with IE1 during infection. Arch Virol. 2004;149:1581–94. PubMed PMID: 15290382.
113. Burks JK, Summers MD, Braunagel SC. BV/ODV-E26: a palmitoylated, multifunctional structural protein of Autographa californica nucleopolyhedrovirus. Virology. 2007;361:194–203. PubMed PMID: 17169392.
114. O'Reilly DR, Passarelli AL, Goldman IF, Miller LK. Characterization of the DA26 gene in a hypervariable region of the Autographa californica nuclear polyhedrosis virus genome. J Gen Virol. 1990;71(Pt 5):1029–37. PubMed PMID: 2189022.
115. Nie Y, Theilmann DA. Deletion of AcMNPV AC16 and AC17 results in delayed viral gene expression in budded virus infected cells but not transfected cells. Virology. 2010;404:168–79. PubMed PMID: 20627351.
116. Nie Y, Fang M, Theilmann DA. AcMNPV AC16 (DA26, BV/ODV-E26) regulates the levels of IE0 and IE1 and binds to both proteins via a domain located within the acidic transcriptional activation domain. Virology. 2009;385:484–95. PubMed PMID: 19150105.
117. Yuan M, Huang Z, Wei D, Hu Z, Yang K, Pang Y. Identification of Autographa californica nucleopolyhedrovirus ac93 as a core gene and its requirement for intranuclear microvesicle formation and nuclear egress of nucleocapsids. J Virol. 2011;85:11664–74. PubMed PMID: 21880748.
118. Russell RLQ, Rohrmann GF. A 25 kilodalton protein is associated with the envelopes of occluded baculovirus virions. Virology. 1993;195:532–540. PubMed PMID: 8337828.
119. Hou D, Zhang L, Deng F, Fang W, Wang R, Liu X, Guo L, Rayner S, Chen X, Wang H, Hu Z. 2012. Comparative Proteomics Reveal Fundamental Structural and Functional Differences between the Two Progeny Phenotypes of a Baculovirus. J Virol doi:JVI.02329-12 [pii] doi: 10.1128/JVI.02329-12.
120. Shrestha A, Bao K, Chen YR, Chen W, Wang P, Fei Z, Blissard GW. Global Analysis of Baculovirus Autographa californica Multiple Nucleopolyhedrovirus Gene Expression in the Midgut of the Lepidopteran Host Trichoplusia ni. J Virol. 2018.:92. PubMed PMID: 30209166.
121. Hong T, Summers M, Braunagel S. N-terminal sequences from Autographa californica nuclear polyhedrosis virus envelope proteins ODV-E66 and ODV-E25 are sufficient to direct reporter proteins to the nuclear envelope, intranuclear microvesicles and the envelope of occlusion derived virus. Proc Natl Acad Sci U S A. 1997;8:4050–4055. PubMed PMID: 9108103.
122. Guo YJ, Fu SH, Li LL. Autographa californica multiple nucleopolyhedrovirus ac75 is required for egress of nucleocapsids from the nucleus and formation of de novo intranuclear membrane microvesicles. PLoS One. 2017;12:e0185630. PubMed PMID: 28968422.
123. Chen L, Hu X, Xiang X, Yu S, Yang R, Wu X. Autographa californica multiple nucleopolyhedrovirus odv-e25 (Ac94) is required for budded virus infectivity and occlusion-derived virus formation. Arch Virol. 2012;157:617–25. PubMed PMID: 22218963.
124. Zhu M, Wang J, Deng R, Xiong P, Liang H, Wang X. A MicroRNA Encoded by Autographa californica Nucleopolyhedrovirus Regulates Expression of Viral Gene ODV-E25. J Virol. 2013;87:13029–34. PubMed PMID: 24027316.
125. Zhu M, Wang J, Deng R, Wang X. Functional Regulation of an Autographa californica Nucleopolyhedrovirus-Encoded MicroRNA, AcMNPV-miR-1, in Baculovirus Replication. J Virol. 2016;90:6526–6537. PubMed PMID: 27147751.
126. Luo XC, Wang SS, Zhang J, Qian DD, Wang SM, Li LL. Effects of Early or Overexpression of the Multiple Nucleopolyhedrovirus (ODV-E25) on Virus Replication. PLoS One. 2013;8:e65635. PubMed PMID: 23825525.
127. Chen L, Yang R, Hu X, Xiang X, Yu S, Wu X. The formation of occlusion-derived virus is affected by the expression level of ODV-E25. Virus Res. 2013;173:404–14. PubMed PMID: 23298549.
128. Fang M, Wang H, Wang H, Yuan L, Chen X, Vlak JM, Hu Z. Open reading frame 94 of Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus encodes a novel conserved occlusion-derived virion protein, ODV-EC43. J Gen Virol. 2003;84:3021–7. PubMed PMID: 14573807.
129. Fang M, Nie Y, Theilmann DA. Deletion of the AcMNPV core gene ac109 results in budded virions that are non-infectious. Virology. 2009;389:66–74. PubMed PMID: 19411088.

130. Lin L, Wang J, Deng R, Ke J, Wu H, Wang X. ac109 is required for the nucleocapsid assembly of Autographa californica multiple nucleopolyhedrovirus. Virus Res. 2009;144:130–5. PubMed PMID: 19393701.
131. Lehiy CJ, Wu W, Berretta MF, Passarelli AL. Autographa californica M nucleopolyhedrovirus open reading frame 109 affects infectious budded virus production and nucleocapsid envelopment in the nucleus of cells. Virology. 2013;435:442–52. PubMed PMID: 23149091.
132. Alfonso V, Maroniche GA, Reca SR, Lopez MG, Del Vas M, Taboga O. AcMNPV Core Gene ac109 Is Required for Budded Virion Transport to the Nucleus and for Occlusion of Viral Progeny. PLoS One. 2012;7:e46146. PubMed PMID: 23049963.
133. Braunagel SC, He H, Ramamurthy P, Summers MD. Transcription, translation, and cellular localization of three Autographa californica nuclear polyhedrosis virus structural proteins: ODV-E18, ODV-E35 and ODV-EC27. Virology. 1996;222:100–114. PubMed PMID: 8806491.
134. McCarthy CB, Theilmann DA. AcMNPV ac143(odv-e18) is essential for mediating budded virus production and is the 30th baculovirus core gene. Virology. 2008;375:277–91. PubMed PMID: 18328526.
135. Zimmermann L, Stephens A, Nam SZ, Rau D, Kubler J, Lozajic M, Gabler F, Soding J, Lupas AN, Alva V. A Completely Reimplemented MPI Bioinformatics Toolkit with a New HHpred Server at its Core. J Mol Biol. 2018;430:2237–2243. PubMed PMID: 29258817.
136. Wang X, Shang Y, Chen C, Liu S, Chang M, Zhang N, Hu H, Zhang F, Zhang T, Wang Z, Liu X, Lin Z, Deng F, Wang H, Zou Z, Vlak JM, Wang M, Hu Z. Baculovirus per os Infectivity Factor Complex: Components and Assembly. J Virol. 2019. doi: 10.1128/JVI.02053-18. PubMed PMID: 30602603.
137. Wang Y, Kleespies RG, Huger AM, Jehle JA. The genome of Gryllus bimaculatus nudivirus indicates an ancient diversification of baculovirus-related nonoccluded nudiviruses of insects. J Virol. 2007;81:5395–406. PubMed PMID: 17360757.
138. Ohkawa T, Washburn JO, Sitapara R, Sid E, Volkman LE. 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. 2005;79:15258–64. PubMed PMID: 16306597.
139. Peng K, van Oers MM, Hu Z, van Lent JW, Vlak JM. Baculovirus per os infectivity factors form a complex on the surface of occlusion-derived virus. J Virol. 2010;84:9497–504. PubMed PMID: 20610731.
140. Li X, Song J, Jiang T, Liang C, Chen X. The N-terminal hydrophobic sequence of Autographa californica nucleopolyhedrovirus PIF-3 is essential for oral infection. Arch Virol. 2007;152:1851–8. PubMed PMID: 17585368.
141. Peng K, van Lent JW, Boeren S, Fang M, Theilmann DA, Erlandson MA, Vlak JM, van Oers MM. Characterization of novel components of the baculovirus per os infectivity factor complex. J Virol. 2012;86:4981–8. PubMed PMID: 22379094.
142. Zhou W, Yao L, Xu H, Yan F, Qi Y. The function of envelope protein p74 from Autographa californica M nucleopolyhedrovirus in primary infection to host. Virus Genes. 2005;30:139–150. PubMed PMID: 15744572.
143. Russell RL, Rohrmann GF. Characterization of P91, a protein associated with virions of an Orgyia pseudotsugata baculovirus. Virology. 1997;233:210–23. PubMed PMID: 9201231.
144. Ono C, Kamagata T, Taka H, Sahara K, Asano S, Bando H. Phenotypic grouping of 141 BmNPVs lacking viral gene sequences. Virus Res. 2012;165:197–206. PubMed PMID: 22421381.
145. Zhu S, Wang W, Wang Y, Yuan M, Yang K. The baculovirus core gene ac83 is required for nucleocapsid assembly and per os infectivity of Autographa californica nucleopolyhedrovirus. J Virol. 2013;87:10573–86. PubMed PMID: 23864639.
146. Javed MA, Biswas S, Willis LG, Harris S, Pritchard C, van Oers MM, Donly BC, Erlandson MA, Hegedus DD, Theilmann DA. Autographa californica Multiple Nucleopolyhedrovirus AC83 is a Per Os Infectivity Factor (PIF) Protein Required for Occlusion-Derived Virus (ODV) and Budded Virus Nucleocapsid Assembly as well as Assembly of the PIF Complex in ODV Envelopes. J Virol. 2017;91:02115–16. PubMed PMID: 28031365.

147. Huang Z, Pan M, Zhu S, Zhang H, Wu W, Yuan M, Yang K. The Autographa californica Multiple Nucleopolyhedrovirus ac83 Gene Contains a cis-Acting Element That Is Essential for Nucleocapsid Assembly. J Virol. 2017.:91. PubMed PMID: 28031366.
148. Hong T, Braunagel SC, Summers MD. Transcription, translation, and cellular localization of PDV-E66: A structural protein of the PDV envelope of Autographa californica nuclear polyhedrosis virus. Virology. 1994;204:210–224. PubMed PMID: 8091653.
149. Sugiura N, Setoyama Y, Chiba M, Kimata K, Watanabe H. Baculovirus envelope protein ODV-E66 is a novel chondroitinase with distinct substrate specificity. J Biol Chem. 2011;286:29026–34. PubMed PMID: 21715327.
150. Xiang X, Chen L, Hu X, Yu S, Yang R, Wu X. Autographa californica multiple nucleopolyhedrovirus odv-e66 is an essential gene required for oral infectivity. Virus Res. 2011;158:72–8. PubMed PMID: 21440017.
151. Sugiura N, Ikeda M, Shioiri T, Yoshimura M, Kobayashi M, Watanabe H. Chondroitinase from baculovirus Bombyx mori nucleopolyhedrovirus and chondroitin sulfate from silkworm Bombyx mori. Glycobiology. 2013;23:1520–30. PubMed PMID: 24052236.
152. Dinglasan RR, Alaganan A, Ghosh AK, Saito A, van Kuppevelt TH, Jacobs-Lorena M. Plasmodium falciparum ookinetes require mosquito midgut chondroitin sulfate proteoglycans for cell invasion. Proc Natl Acad Sci U S A. 2007;104:15882–7. PubMed PMID: 17873063.
153. Vigdorovich V, Miller AD, Strong RK. Ability of hyaluronidase 2 to degrade extracellular hyaluronan is not required for its function as a receptor for jaagsiekte sheep retrovirus. J Virol. 2007;81:3124–9. PubMed PMID: 17229709.
154. Kawaguchi Y, Sugiura N, Onishi M, Kimata K, Kimura M, Kakuta Y. Crystallization and X-ray diffraction analysis of chondroitin lyase from baculovirus: envelope protein ODV-E66. Acta Crystallogr Sect F Struct Biol Cryst Commun. 2012;68:190–2. PubMed PMID: 22297996.
155. Lapointe R, Popham HJ, Straschil U, Goulding D, O'Reilly DR, Olszewski JA. Characterization of two Autographa californica nucleopolyhedrovirus proteins, Ac145 and Ac150, which affect oral infectivity in a host-dependent manner. J Virol. 2004;78:6439–48. PubMed PMID: 15163737.
156. Dall D, Luque T, O'Reilly DR. Insect-virus relationships: sifting by informatics. Bioessays. 2001;23:184–193. PubMed PMID: 11169592.
157. Zhang JH, Ohkawa T, Washburn JO, Volkman LE. Effects of Ac150 on virulence and pathogenesis of Autographa californica multiple nucleopolyhedrovirus in noctuid hosts. J Gen Virol. 2005;86:1619–27. PubMed PMID: 15914839.
158. Olszewski J, Miller LK. Identification and characterization of a baculovirus structural protein, VP1054, required for nucleocapsid formation. J Virol. 1997;71:5040–5050. PubMed PMID: 9188569.
159. Guan Z, Zhong L, Li C, Wu W, Yuan M, Yang K. The Autographa californica Multiple Nucleopolyhedrovirus ac54 Gene Is Crucial for Localization of the Major Capsid Protein VP39 at the Site of Nucleocapsid Assembly. J Virol. 2016;90:4115–4126. PubMed PMID: 26865720.
160. Marek M, Romier C, Galibert L, Merten O, van Oers MM. Baculovirus VP1054 is an acquired cellular PURα, a nucleic-acid6n binding protein specific for GGN repeats. J Virol. 2013;87:8465–80. PubMed PMID: 23720732.
161. Ke J, Wang J, Deng R, Wang X. 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. 2008;374:421–31. PubMed PMID: 18241908.
162. Biswas S, Willis LG, Fang M, Nie Y, Theilmann DA. Autographa californica Nucleopolyhedrovirus AC141 (Exon0), a Potential E3 Ubiquitin Ligase, Interacts with Viral Ubiquitin and AC66 To Facilitate Nucleocapsid Egress. J Virol. 2018.:92. PubMed PMID: 29142135.
163. Feng G, Krell PJ. Autographa californica multiple nucleopolyhedrovirus DNA polymerase C terminus is required for nuclear localization and viral DNA replication. J Virol. 2014;88:10918–33. PubMed PMID: 25008932.
164. McLachlin JR, Miller LK. Identification and characterization of vlf-1, a baculovirus gene involved in very late gene expression. J Virol. 1994;68:7746–7756. PubMed PMID: 7966564.

165. Yang S, Miller LK. Activation of baculovirus very late promoters by interaction with very late factor 1. J Virol. 1999;73:3404–9. PubMed PMID: 10074194.
166. Yang S, Miller LK. Expression and mutational analysis of the baculovirus very late factor 1 (vlf-1) gene. Virology. 1998;245:99–109. PubMed PMID: 9614871.
167. Vanarsdall AL, Okano K, Rohrmann GF. Characterization of the role of VLF-1 in baculovirus capsid structure and DNA processing. J Virol. 2006;80:1724–1733. PubMed PMID: 16439529.
168. Whitford M, Faulkner P. A structural polypeptide of the baculovirus Autographa californica nuclear polyhedrosis virus contains 0-linked N-acetylglucosamine. J Virol. 1992;66:3324–3329. PubMed PMID: 1583718.
169. Whitford M, Faulkner P. Nucleotide sequence and transcriptional analysis of a gene encoding gp41, a structural glycoprotein of the baculovirus Autographa californica nuclear polyhedrosis virus. J Virol. 1992;66:4763–4768. PubMed PMID: 1629955.
170. Olszewski J, Miller LK. A role for baculovirus GP41 in budded virus production. Virology. 1997;233:292–301. PubMed PMID: 9217053.
171. Li Y, Shen S, Hu L, Deng F, Vlak JM, Hu Z, Wang H, Wang M. The Functional Oligomeric State of Tegument Protein GP41 Is Essential for Baculovirus Budded Virion and Occlusion-Derived Virion Assembly. J Virol. 2018.:92. PubMed PMID: 29643237.
172. Danquah JO, Botchway S, Jeshtadi A, King LA. Direct interaction of baculovirus capsid proteins VP39 and EXON0 with kinesin-1 in insect cells determined by fluorescence resonance energy transfer-fluorescence lifetime imaging microscopy. J Virol. 2012;86:844–53. PubMed PMID: 22072745.
173. Katsuma S, Kokusho R. A Conserved Glycine Residue Is Required for Proper Functioning of a Baculovirus VP39 Protein. J Virol. 2017.:91. PubMed PMID: 28077638.
174. Long CM, Rohrmann GF, Merrill GF. The conserved baculovirus protein p33 (Ac92) is a flavin adenine dinucleotide-linked sulfhydryl oxidase. Virology. 2009;388:231–5. PubMed PMID: 19409596.
175. Wu W, Passarelli AL. Autographa californica M nucleopolyhedrovirus Ac92 (ORF92, P33) is required for budded virus production and multiply-enveloped occlusion-derived virus formation. J Virol. 2010;84:12351–61. PubMed PMID: 20861245.
176. Prikhod'ko GG, Wang Y, Freulich E, Prives C, Miller LK. Baculovirus p33 binds human p53 and enhances p53-mediated apoptosis. J Virol. 1999;73:1227–34. PubMed PMID: 9882325.
177. Wu W, Clem RJ, Rohrmann GF, Passarelli AL. The baculovirus sulfhydryl oxidase Ac92 (P33) interacts with the Spodoptera frugiperda P53 protein and oxidizes it in vitro. Virology. 2013;447:197–207. PubMed PMID: 24210115.
178. Nie Y, Fang M, Theilmann DA. Autographa californica multiple nucleopolyhedrovirus core gene ac92 (p33) is required for the efficient budded virus production. Virology. 2011;409:38–45. PubMed PMID: 20965540.
179. Wu W, Lin T, Pan L, Yu M, Li Z, Pang Y, Yang K. Autographa californica multiple nucleopolyhedrovirus nucleocapsid assembly is interrupted upon deletion of the 38K gene. J Virol. 2006;80:11475–85. PubMed PMID: 16987976.
180. Lai Q, Wu W, Li A, Wang W, Yuan M, Yang K. The 38K-Mediated Specific Dephosphorylation of the Viral Core Protein P6.9 Plays an Important Role in the Nucleocapsid Assembly of Autographa californica Multiple Nucleopolyhedrovirus. J Virol. 2018.:92. PubMed PMID: 29444944.
181. Wilson ME, Mainprize TH, Friesen PD, Miller LK. l987. Location, transcription, and sequence of a baculovirus gene encoding a small arginine-rich polypeptide. J Virol.61:66l–666.
182. Tweeten KA, Bulla LA, Consigli RA. Characterization of an extremely basic protein derived from granulosis virus nucleocapsid. J Virol. 1980;33:866–876. PubMed PMID: 16789190.
183. Balhorn R. A model for the structure of chromatin in mammalian sperm. J Cell Biol. 1982;93:298–305. PubMed PMID: 7096440.
184. Nakano M, Kasai K, Yoshida K, Tanimoto T, Tamaki Y, Tobita T. Conformation of the fowl protamine, galline, and its binding properties to DNA. J Biochem. 1989;105:133–137. PubMed PMID: 2738040.
185. Wilson ME, Price KH. Association of Autographa californica nuclear polyhedrosis virus (AcMNPV) with the nuclear matrix. Virology. 1988;167:233–241. PubMed PMID: 3055664.

186. Wilson ME, Consigli RA. Functions of a protein kinase activity associated with purified capsids of the granulosis virus infecting *Plodia interpunctella*. Virology. 1985;143:526–535. PubMed PMID: 18639856.
187. Miller LK, Adang MJ, Browne D. Protein kinase activity associated with the extracellular and occluded forms of the baculovirus *Autographa californica* NPV. J Virol. 1983;46:275–278. PubMed PMID: 16789240.
188. Li A, Zhao H, Lai Q, Huang Z, Yuan M, Yang K. Posttranslational Modifications of Baculovirus Protamine-Like Protein P6.9 and the Significance of Its Hyperphosphorylation for Viral Very Late Gene Hyperexpression. J Virol. 2015;89:7646–59. PubMed PMID: 25972542.
189. Oppenheimer DI, Volkman LE. Proteolysis of p6.9 induced by cytochalasin D in Autographa californica M nuclear polyhedrosis virus-infected cells. Virology. 1995;207:1–11. PubMed PMID: 7871717.
190. Wang M, Tuladhar E, Shen S, Wang H, van Oers MM, Vlak JM, Westenberg M. Specificity of baculovirus P6.9 basic DNA-binding proteins and critical role of the C terminus in virion formation. J Virol. 2010;84:8821–8. PubMed PMID: 20519380.
191. Singh CP, Singh J, Nagaraju J. bmnpv-miR-3 facilitates BmNPV infection by modulating the expression of viral P6.9 and other late genes in Bombyx mori. Insect Biochem Mol Biol. 2014;49:59–69. PubMed PMID: 24698834.
192. Braunagel SC, Guidry PA, Rosas-Acosta G, Engelking L, Summers MD. 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. 2001;75:12331–8. PubMed PMID: 11711623.
193. Wang Y, Wang Q, Liang C, Song J, Li N, Shi H, Chen X. Autographa californica multiple nucleopolyhedrovirus nucleocapsid protein BV/ODV-C42 mediates the nuclear entry of P78/83. J Virol. 2008;82:4554–61. PubMed PMID: 18287235.
194. Wang Y, Zhang Y, Han S, Hu X, Zhou Y, Mu J, Pei R, Wu C, Chen X. Identification of a novel regulatory sequence of actin nucleation promoting factor encoded by Autographa californica multiple nucleopolyhedrovirus. J Biol Chem. 2015;290:9533–41. PubMed PMID: 25691574.
195. Zhang Y, Hu X, Mu J, Hu Y, Zhou Y, Zhao H, Wu C, Pei R, Chen J, Chen X, Wang Y. Ac102 Participates in Nuclear Actin Polymerization by Modulating BV/ODV-C42 Ubiquitination during Autographa californica Multiple Nucleopolyhedrovirus Infection. J Virol. 2018.:92. PubMed PMID: 29618641.
196. Hepp SE, Borgo GM, Ticau S, Ohkawa T, Welch MD. Baculovirus AC102 Is a Nucleocapsid Protein That Is Crucial for Nuclear Actin Polymerization and Nucleocapsid Morphogenesis. J Virol. 2018.:92. PubMed PMID: 29540600.
197. Vanarsdall AL, Pearson MN, Rohrmann GF. Characterization of baculovirus constructs lacking either the Ac 101, Ac 142, or the Ac 144 open reading frame. Virology. 2007;367:187–95. PubMed PMID: 17585983.
198. Fang M, Dai X, Theilmann DA. Autographa californica multiple nucleopolyhedrovirus EXON0 (ORF141) is required for efficient egress of nucleocapsids from the nucleus. J Virol. 2007;81:9859–69. PubMed PMID: 17626083.
199. Fang M, Nie Y, Dai X, Theilmann DA. Identification of AcMNPV EXON0 (ac141) domains required for efficient production of budded virus, dimerization and association with BV/ODV-C42 and FP25. Virology. 2008;375:265–76. PubMed PMID: 18313716.
200. Dai X, Stewart TM, Pathakamuri JA, Li Q, Theilmann DA. Autographa californica multiple nucleopolyhedrovirus exon0 (orf141), which encodes a RING finger protein, is required for efficient production of budded virus. J Virol. 2004;78:9633–44. PubMed PMID: 15331696.
201. Fang M, Nie Y, Theilmann DA. AcMNPV EXON0 (AC141) which is required for the efficient egress of budded virus nucleocapsids interacts with beta-tubulin. Virology. 2009;385:496–504. PubMed PMID: 19155039.
202. Biswas S, Blissard GW, Theilmann DA. Trichoplusia ni Kinesin-1 Associates with Autographa californica Multiple Nucleopolyhedrovirus Nucleocapsid Proteins and Is Required for Production of Budded Virus. J Virol. 2016;90:3480–95. PubMed PMID: 26763996.
203. Yang ZN, Xu HJ, Park EY, Zhang CX. 2008. Characterization of Bombyx mori nucleopolyhedrovirus with a deletion of bm118. Virus Res.

204. McCarthy CB, Dai X, Donly C, Theilmann DA. Autographa californica multiple nucleopolyhedrovirus ac142, a core gene that is essential for BV production and ODV envelopment. Virology. 2008;372:325–39. PubMed PMID: 18045640.
205. Yue Q, Yu Q, Yang Q, Xu Y, Guo Y, Blissard GW, Li Z. Distinct Roles of Cellular ESCRT-I and ESCRT-III Proteins in Efficient Entry and Egress of Budded Virions of Autographa californica Multiple Nucleopolyhedrovirus. J Virol. 2018.:92. PubMed PMID: 29046462.
206. Belyavskyi M, Braunagel SC, Summers MD. The structural protein ODV-EC27 of Autographa californica nucleopolyhedrovirus is a multifunctional viral cyclin. Proc Natl Acad Sci U S A. 1998;95:11205–10. PubMed PMID: 9736714.
207. Vialard JE, Richardson CD. The 1,629-nucleotide open reading frame located downstream of the Autographa californica nuclear polyhedrosis virus polyhedrin gene encodes a nucleocapsid-associated phosphoprotein. J Virol. 1993;67:5859–5866. PubMed PMID: 8371345.
208. Russell RLQ, Funk CJ, Rohrmann GF. Association of a baculovirus encoded protein with the capsid basal region. Virology. 1997;227:142–152. PubMed PMID: 9007067.
209. Goley ED, Ohkawa T, Mancuso J, Woodruff JB, D'Alessio JA, Cande WZ, Volkman LE, Welch MD. Dynamic nuclear actin assembly by Arp2/3 complex and a baculovirus WASP-like protein. Science. 2006;314:464–7. PubMed PMID: 17053146.
210. Ohkawa T, Volkman LE, Welch MD. Actin-based motility drives baculovirus transit to the nucleus and cell surface. J Cell Biol. 2010;190:187–95. PubMed PMID: 20660627.
211. Kitts PA, Possee RD. A method for producing recombinant baculovirus expression vectors at high frequency. BioTechniques. 1993;14:810–816. PubMed PMID: 8512707.
212. Garavaglia MJ, Miele SA, Iserte JA, Belaich MN, Ghiringhelli PD. Ac53, ac78, ac101 and ac103 are newly discovered core genes in the family Baculoviridae. J Virol. 2012;86:12069–79. PubMed PMID: 22933288.
213. Wu J, Miller LK. Sequence, transcription, and translation of a late gene of the Autographa californica nuclear polyhedrosis virus encoding a 34.8 K polypeptide. Journal of General Virology. 1989;70:2449–2459. PubMed PMID: 2674327.
214. Cheng X, Krell P, Arif B. P34.8 (GP37) is not essential for baculovirus replication. J Gen Virol. 2001;82:299–305. PubMed PMID: 11161266.
215. Li Z, Li C, Yang K, Wang L, Yin C, Gong Y, Pang Y. Characterization of a chitin-binding protein GP37 of Spodoptera litura multicapsid nucleopolyhedrovirus. Virus Res. 2003;96:113–22. PubMed PMID: 12951271.
216. Liu X, Ma X, Lei C, Xiao Y, Zhang Z, Sun X. Synergistic effects of Cydia pomonella granulovirus GP37 on the infectivity of nucleopolyhedroviruses and the lethality of Bacillus thuringiensis. Arch Virol. 2011;156:1707–15. PubMed PMID: 21643992.
217. Lemaitre B, Hoffmann J. The host defense of Drosophila melanogaster. Annu Rev Immunol. 2007;25:697–743. PubMed PMID: 17201680.
218. Vialard JE, Yuen L, Richardson CD. Identification and characterization of a baculovirus occlusion body glycoprotein which resembles spheroidin, an entomopoxvirus protein. Journal of Virology. 1990;64:5804–5811. PubMed PMID: 2243377.
219. Gross CH, Wolgamot GM, Russell RLQ, Pearson MN, Rohrmann GF. A 37 kda glycoprotein from a baculovirus of *Orgyia pseudotsugata* is localized to cytoplasmic inclusion bodies. Journal of Virology. 1993;67:469–475. PubMed PMID: 8380088.
220. Mitsuhashi W, Miyamoto K. Disintegration of the peritrophic membrane of silkworm larvae due to spindles of an entomopoxvirus. J Invertebr Pathol. 2003;82:34–40. PubMed PMID: 12581717.
221. Chiu E, Hijnen M, Bunker RD, Boudes M, Rajendran C, Aizel K, Olieric V, Schulze-Briese C, Mitsuhashi W, Young V, Ward VK, Bergoin M, Metcalf P, Coulibaly F. Structural basis for the enhancement of virulence by viral spindles and their in vivo crystallization. Proc Natl Acad Sci U S A. 2015;112:3973–8. PubMed PMID: 25787255.

222. Liu X, Fang W, Fan R, Zhang L, Lei C, Zhang J, Nian W, Dou T, An S, Zhou L, Sun X. Granulovirus GP37 Facilitated ODVs Cross Insect Peritrophic Membranes and Fuse with Epithelia. Toxins. 2019.:11. PubMed PMID: 30836616.
223. Li Z, Gong Y, Yin C, Wang L, Li C, Pang Y. Characterization of a novel ubiquitin-fusion gene Uba256 from Spodoptera litura nucleopolyhedrovirus. Gene. 2003;303:111–9. PubMed PMID: 12559572.
224. Müller R, Pearson MN, Russell RLQ, Rohrmann GF. A capsid-associated protein of the multicapsid nuclear polyhedrosis virus of *Orgyia pseudotsugata*: Genetic location, sequence, transcriptional mapping, and immunocytochemical characterization. Virology. 1990;176:133–144. PubMed PMID: 2184573.
225. Marek M, van Oers MM, Devaraj FF, Vlak JM, Merten OW. Engineering of baculovirus vectors for the manufacture of virion-free biopharmaceuticals. Biotechnol Bioeng. 2011;108:1056–67. PubMed PMID: 21449023.
226. Lu A, Carstens EB. Nucleotide sequence and transcriptional analysis of the p80 gene of Autographa californica nuclear polyhedrosis virus: a homologue of the Orgyia pseudotsugata nuclear polyhedrosis virus capsid-associated gene. Virology. 1992;190:201–209. PubMed PMID: 1529529.
227. Marek M, Merten OW, Galibert L, Vlak JM, van Oers MM. Baculovirus VP80 protein and the F-actin cytoskeleton interact and connect the viral replication factory with the nuclear periphery. J Virol. 2011;85:5350–62. PubMed PMID: 21450830.
228. Marek M, Merten OW, Francis-Devaraj F, Oers MM. Essential C-terminal region of the baculovirus minor capsid protein VP80 binds DNA. J Virol. 2012;86:1728–38. PubMed PMID: 22090126.
229. de Jong J, Arif BM, Theilmann DA, Krell PJ. Autographa californica multiple nucleopolyhedrovirus me53 (ac140) is a nonessential gene required for efficient budded-virus production. J Virol. 2009;83:7440–8. PubMed PMID: 19457997.
230. Xi Q, Wang J, Deng R, Wang X. Characterization of AcMNPV with a deletion of me53 gene. Virus Genes. 2007;34:223–32. PubMed PMID: 17096186.
231. Knebel-Mörsdorf D, Kremer A, Jahnel F. Baculovirus gene ME53, which contains a putative zinc finger motif, is one of the major early-transcribed genes. J Virol. 1993;67:753–8. PubMed PMID: 8093490.
232. de Jong J, Theilmann DA, Arif BM, Krell PJ. Immediate-early protein ME53 forms foci and colocalizes with GP64 and the major capsid protein VP39 at the cell membranes of Autographa californica multiple nucleopolyhedrovirus-infected cells. J Virol. 2011;85:9696–707. PubMed PMID: 21775466.
233. Wolgamot GM, Gross CH, Russell RLQ, Rohrmann GF. Immunocytochemical characterization of p24, a baculovirus capsid-associated protein. J Gen Virol. 1993;74:103–107. PubMed PMID: 8423444.
234. Gombart AF, Blissard GW, Rohrmann GF. Characterization of the genetic organization of the HindIII-M region of the multicapsid nuclear polyhedrosis virus of Orgyia pseudotsugata reveals major differences among baculoviruses. J Gen Virol. 1989;70:1815–1828. PubMed PMID: 2661722.
235. Schetter C, Oellig C, Doerfler W. An insertion of insect cell DNA in the 81-map-unit segment of *Autographa californica* nuclear polyhedrosis virus DNA. J Virol. 1990;64:1844–1850. PubMed PMID: 2157067.
236. Franke EK, Yuan HE, Luban J. Specific incorporation of cyclophilin A into HIV-1 virions. Nature. 1994;372:359–62. PubMed PMID: 7969494.
237. Shobahah J, Xue S, Hu D, Zhao C, Wei M, Quan Y, Yu W. Quantitative phosphoproteome on the silkworm (Bombyx mori) cells infected with baculovirus. Virol J. 2017;14:117. PubMed PMID: 28629377.
238. Hughes KM. The macromolecular lattices of polyhedra. J Invertebr Pathol. 1978;31:217–224.
239. Rohrmann GF. Baculovirus structural proteins. J Gen Virol. 1992;73:749–761. PubMed PMID: 1634870.
240. Westenberg M, Wang H, IJkel WF, Goldbach RW, Vlak JM, Zuidema D. Furin is involved in baculovirus envelope fusion protein activation. J Virol. 2002;76:178–84. PubMed PMID: 11739683.
241. Au S, Pante N. Nuclear transport of baculovirus: revealing the nuclear pore complex passage. J Struct Biol. 2012;177:90–8. PubMed PMID: 22100338.
242. Nie Y, Fang M, Erlandson MA, Theilmann DA. Analysis of the autographa californica multiple nucleopolyhedrovirus overlapping gene pair lef3 and ac68 reveals that AC68 is a per os infectivity factor

and that LEF3 is critical, but not essential, for virus replication. J Virol. 2012;86:3985–94. PubMed PMID: 22278232.
243. Fang M, Nie Y, Harris S, Erlandson MA, Theilmann DA. Autographa californica multiple nucleopolyhedrovirus core gene ac96 encodes a per Os infectivity factor (PIF-4). J Virol. 2009;83:12569–78. PubMed PMID: 19759145.
244. Jiantao L, Zhu L, Zhang S, Deng Z, Huang Z, Yuan M, Wu W, Yang K. The Autographa californica multiple nucleopolyhedrovirus ac110 gene encodes a new per os infectivity factor. Virus Res. 2016;221:30–7. PubMed PMID: 27212681.
245. Kuzio J, Jaques R, Faulkner P. Identification of p74, a gene essential for virulence of baculovirus occlusion bodies. Virology. 1989;173:759–763. PubMed PMID: 2688302.
246. Harrison RL, Sparks WO, Bonning BC. 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. 2010;91:1173–82. PubMed PMID: 20032203.
247. Vanarsdall AL, Okano K, Rohrmann GF. Characterization of a baculovirus with a deletion of vlf-1. Virology. 2004;326:191–201. PubMed PMID: 15262507.

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

Two types of virions

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

The insect midgut

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

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

A common feature of the life cycle of all baculoviruses is the presence of virions embedded in occlusion bodies that are produced in the final stage of the replication cycle and are released upon the death and disintegration of the insect. Occlusion bodies serve to stabilize the virus in the environment and are normally only dissolved under alkaline conditions. High pH environments are rarely, if ever, encountered in most ecosystems and normally are only found in the midguts of some susceptible insects. Upon ingestion, the alkaline conditions of the midgut of larvae cause the dissolution of the occlusion bodies and the release of the ODV (Figure 2, and see Chapter 2, Figure 3). The polyhedron envelope/calyx structure that surrounds the polyhedra is likely degraded

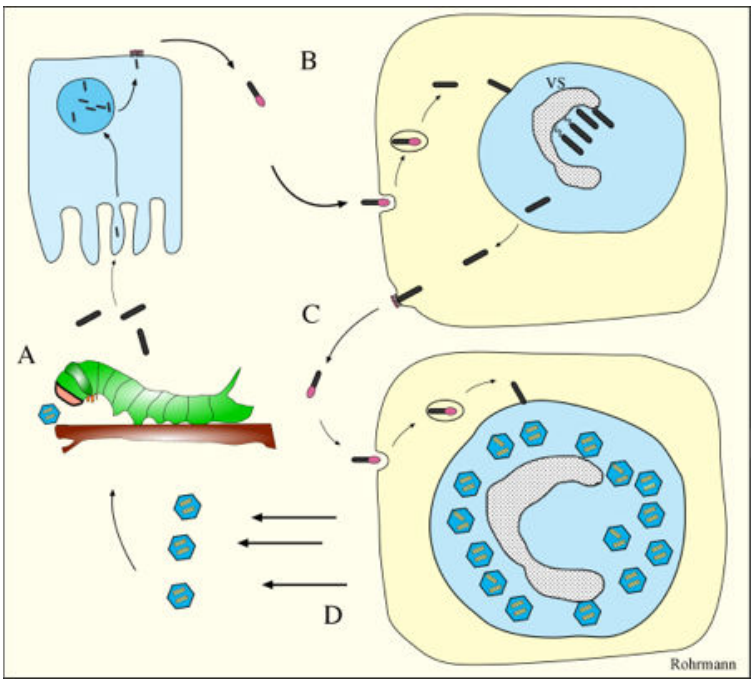

Figure 1. A life cycle of a baculovirus causing systemic infection. Occlusion bodies ingested by an insect dissolve in the midgut, and ODV are released which then infect midgut epithelial cells (A). The virion buds out of the cell in a basal direction and initiate a systemic infection (B). Early in the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

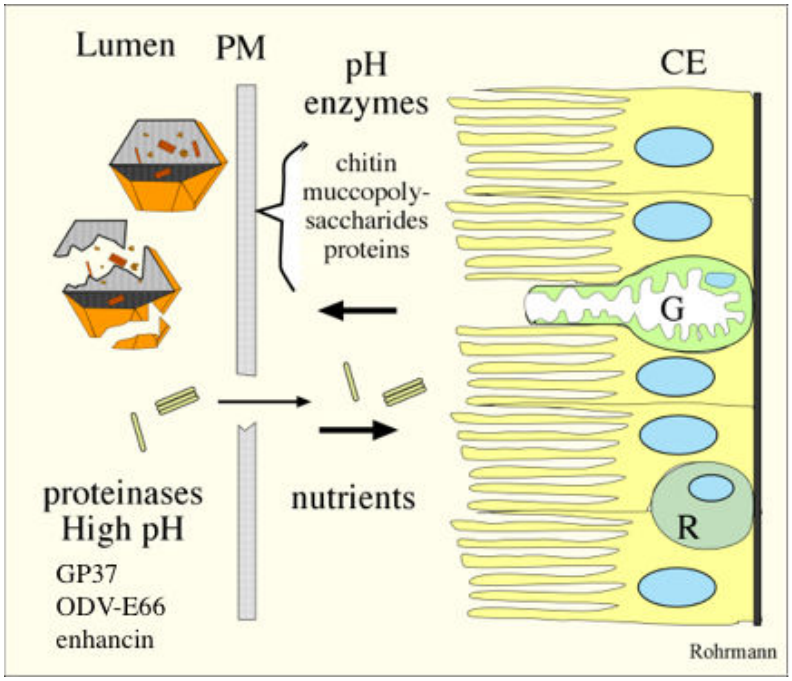

Figure 2. The insect midgut and virus infection. The midgut cells generate the peritrophic matrix (PM) by the synthesis and secretion of chitin, muccopolysaccharides and proteins. They also secrete digestive enzymes and ions that regulate the pH. Occlusion bodies are dissolved by the high pH in the midgut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

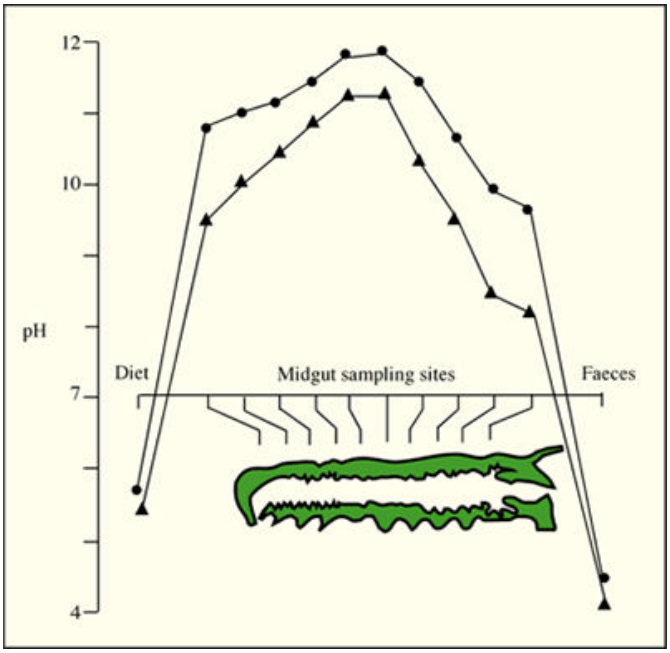

Figure 3. PH profiles along the gut lumens of two lepidopteran species. The pH of the hemolymph was 6.7. The species shown are *Lichnoptera felina* (circles) and *Manduca sexta* (triangles). This figure is modified from (5).

by proteinases present in the gut or associated with the polyhedra (see Chapter 2). After their release from occlusion bodies, the first barrier that the viruses encounter in the insect midgut is the peritrophic matrix (PM) (Figure 2). As described in the previous chapter, polyhedra are nonspecifically contaminated by bacterial proteinases that function under alkaline conditions. Also, some baculoviruses encode endopeptidases (metalloproteinases) that contain divalent cations as integral components of their structure. One category of metalloproteinases, called enhancins, is concentrated in occlusion bodies. It is thought that enhancins specifically digest mucin, a PM component, thereby allowing virus access to the epithelial cell surface (6) (7). Enhancins are only encoded by a limited number of baculoviruses and it is unclear how viruses that lack this enzyme pass through the PM. However, some are highly infectious (e.g., AcMNPV) and do not appear to be inhibited by the PM, suggesting that there must be alternate or parallel mechanisms, such as the contaminating bacterial proteinases described above that are involved in breaching the PM (8).

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

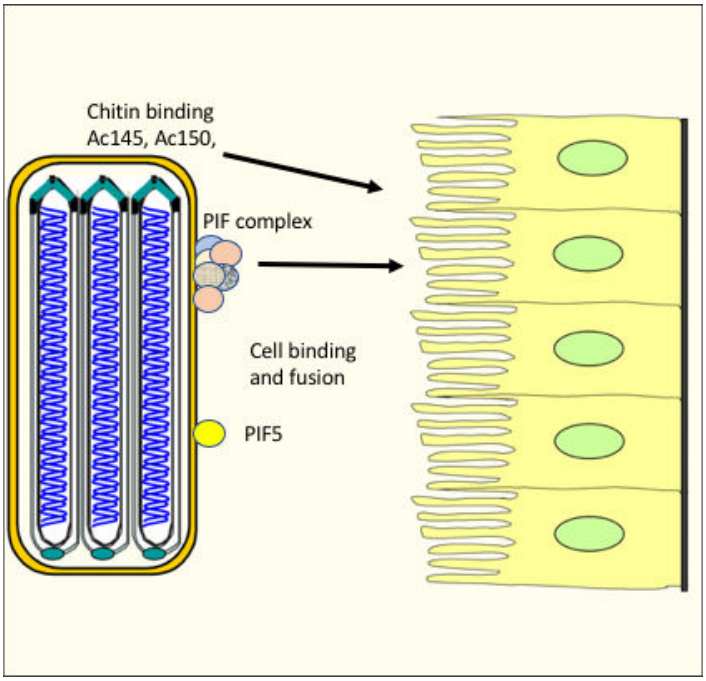

Figure 4. Possible interactions of virion proteins with midgut cells. Ac145 and Ac150 have chitin binding domains, suggesting that they may interact with chitin synthesizing cells. Members of the PIF complex (PIF0-P74, PIF1 and 2) bind to midgut cells.

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

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

Chitin binding proteins

Ac145 and Ac150, have properties similar to PIF proteins and are described in Chapter 2. Both Ac145 and Ac150 are predicted to encode chitin binding domains (19) and localize to ODV envelopes (20). Although, Ac83 (VP91) (PIF 8), a virion structural protein, is also predicted to encode a chitin binding domain, no chitin binding activity was detected when examined experimentally (21) and the region was predicted to encompass a zinc finger domain (22). There are two possible interactions involving chitin binding that might occur during midgut infection (Figure 2 and Figure 4). One would involve binding to the peritrophic matrix (PM) possibly to facilitate movement through the membrane. However, if the PM is extensively degraded during infection, this binding might be counterproductive. The chitin component of the PM is produced by chitin synthase, an enzyme that is located at the apical tips of brush border microvilli. The enzyme is also found associated with tracheal cells (23, 24). Therefore, an affinity for chitin could facilitate the interaction of the virion with these cells, first to initiate infection of midgut cells (e.g., Ac145 and Ac150) and subsequently to interact with tracheal

cells (VP91). Another protein, GP37 (Ac64) has been shown to bind to chitin (25). Although GP37 has not been shown to be a structural protein, it may play a role in the infection of cells that synthesize chitin. It has also been suggested to cause disintegration of the peritrophic matrix and facilitate fusion of virions with the midgut epithelium (14) (see above). Also, ODV-E66 (Ac46), which associates with the PIF complex, is a condroitinase and chondroitin glycosaminoglycans have been found to localize to the apical midgut microvilli of *Anopheles gambiae* (26) suggesting that if Lepidoptera are similar, this enzyme could facilitate recognition or entry into midgut cells.

Viral and host gene expression upon midgut infection

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

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

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

For some baculoviruses, replication only occurs in midgut cells and there is no systemic infection (see below). However, Alphabaculoviruses cause a systemic infection and therefore must transit the basal lamina to access the interior of the insect. The basal lamina is a structure composed of a fibrous matrix of glycoproteins that surrounds the midgut epithelium and is thought to be a barrier to systemic infection. Furthermore, the initiation of systemic infections may differ based on the insect host. In AcMNPV infected T. ni larvae, newly synthesized GP64 is directed toward the basal and lateral regions of the cell (15). This targets the virions to bud away from the gut lumen and toward other susceptible tissues, including neighboring cells (29). This polar distribution of GP64 apparently does not occur in other tissues where targeting the infection in a specific direction would not be critical for spreading the infection (15). Subsequent movement of AcMNPV has been examined using viruses expressing a *lacZ* reporter gene so the infection of single cells can be traced. In *T. ni* larvae, secondary infections appeared initially in tracheoblasts and tracheal epidermal cells. It has been reported that in some insects, tracheal cells have projections that penetrate through the basal lamina (30) (31). Such projections could provide access to the tracheal system and allow the virions to move past the basal lamina leading to a systemic infection (29). Further evidence for the ability of trachea to spread infections systemically was the observation that infections could be initiated by exposure of insects to BV through their spiracles. Such infections spread throughout the insect following tracheal tracts (32). However, investigations of AcMNPV using a different insect, *S. exigua*, indicated that rather than the secondary infection specifically localizing to tracheoblasts, other tissues, such as midgut muscle and hemocytes showed simultaneous infection, suggesting that transit through the basal lamina might not have been dependent solely upon tracheoblast infection (16). Surprisingly, it was noted in one of these studies that in most *T. ni* larvae the midgut infection appeared to be transient and although the infection had become systemic, it had been cleared from the midgut (29). This was attributed to sloughing and regeneration of the midgut epithelium, possibly as a response to the infection.

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

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

Transiting midgut cells without replication

It has been suggested that under some circumstances nucleocapsids can transit through gut cells, bypassing replication, and bud directly into the hemolymph (41), or tracheal cells (42). This may be a mechanism for accelerating systemic infection and avoiding replication in gut cells that may be sloughed and eliminated from the insect. Genomes of all sequenced Group I viruses have both early and late promoter consensus sequences upstream of their *gp64* envelope fusion protein genes. Expression of *gp64* from the early promoter may prepare cell membranes for budding such that some nucleocapsids might directly transit the cell and bud without undergoing replication. When the early promoter was eliminated in the regulatory region of the AcMNPV *gp64* gene, a delay in the time course of infections initiated by oral, but not intrahemocelic inoculation of insects was observed (43, 44). In addition, when *T. ni* cells are infected at high moi (200), many virions appear to become associated with the plasma membrane from within the cell (45). This theory is applicable only to the MNPV type of virus, because it is dependent upon a cluster of connected nucleocapsids simultaneously infecting single cells, after which they would have to become separated in the cytoplasm with some entering the nucleus to undergo conventional replication and others proceeding directly to the cell membrane. The virions undergoing replication would direct the early synthesis of GP64 to prepare the cell membrane for budding of the nonreplicated nucleocapsids that transit directly through the cell possibly facilitated by the vFGF pathway described above. The theory is complicated by a lack of understanding of the MNPV type of virion morphology because it appears to lack a genetic determinant (see Chapter 1). A similar combination of early and late promoters is also present upstream of almost all of the F genes of Group II viruses, suggesting that these two types of envelope fusion proteins (F and GP64) may be regulated in a similar manner. Whereas most of the Group I viruses are of the MNPV type (BmNPV may be an exception since single nucleocapsids predominate (46)), Group II viruses can be either MNPV or SNPV. For SNPVs, it is difficult to link the regulation of the fusion protein to the transit of the virus, because it would require extremely high levels of virus to ensure infection of single cells with more than one virion so that both transit and fusion protein synthesis could occur

in the same cell. In contrast to the regulatory region of the F gene in Group II viruses, the regulatory region of the F gene orthologs in at least nine sequenced granulovirus genomes lack conventional early promoter consensus sequences (TATA + CAGT) in the proper context, and three were identified (AdorGV, AgseGV, CrleGV) that have late promoter elements within 200 nt upstream of the ATG.

Baculovirus replication: evidence from cultured cells

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

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

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

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

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

Cellular actin is involved in the transport of virions within cells (52) (45) (53, 54) resulting in the production of 'actin comet tails' trailing behind the nucleocapsid (Figure 6). After AcMNPV infection of TN-368 cells, actin is polymerized from G- into F-actin. G-actin is a globular monomeric form of actin, and polymerizes into filamentous, or F-actin. This is a reversible reaction requiring ATP hydrolysis for polymerization. A cellular complex of up to 7 proteins called the Arp2/3 complex is comprised of two actin related proteins (Arp) that resemble the structure of monomeric actin and 5 additional factors. This complex is involved in nucleating the formation of F-actin filaments. Activators are required for this process and they bind both monomeric G actin and the Arp2/3 complex. One category of such activators is called Wiskott-Aldrich syndrome protein (WASP), and an ortholog of WASP (PP78/83) (AcMNPV orf9) is encoded in lepidopteran NPV genomes. A purified truncated version of AcMNPV PP78/83 containing the critical activation domains was found to be capable of

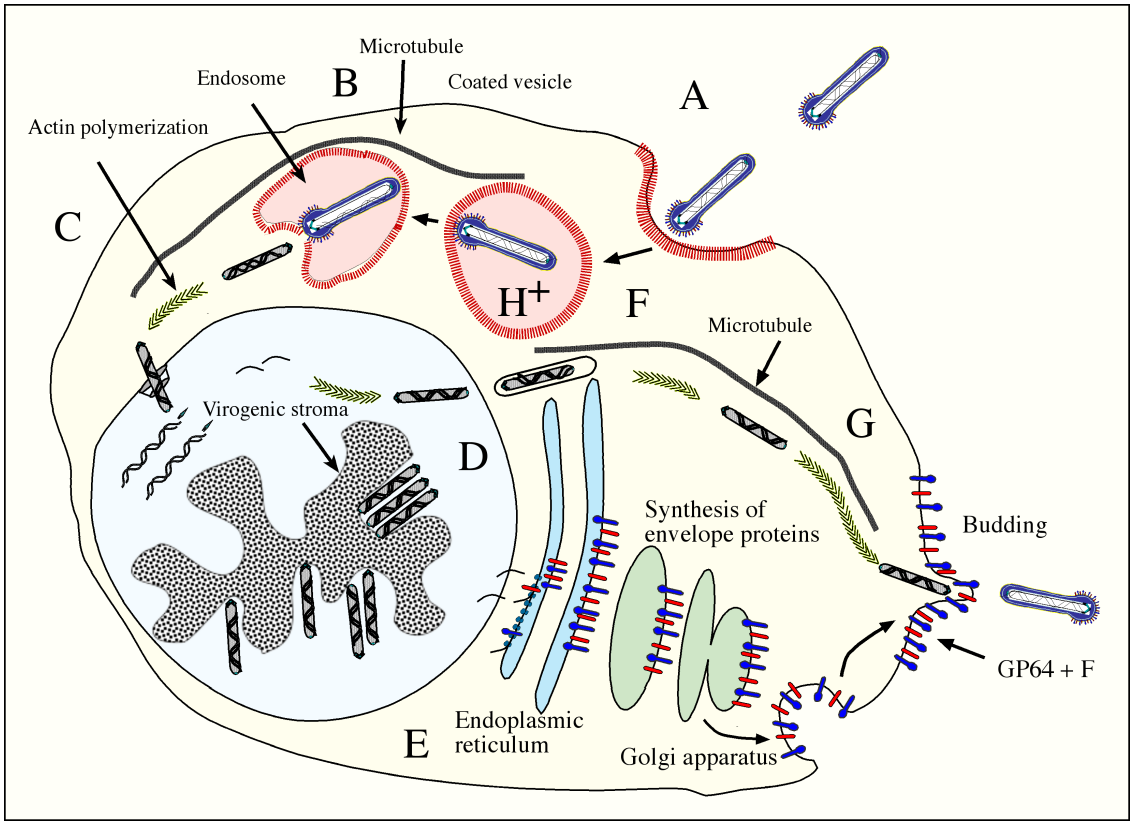

Figure 5. Budded virus infection of a Group I virus. BV attach to receptors located in clathrin coated pits via GP64 and are endocytosed (A). The endocytic vesicle is acidified and this changes the conformation of GP64 and causes the virion envelope to fuse with the endosomal membrane releasing the nucleocapsid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

stimulating actin polymerization in combination with the Arp2/3 complex in vitro. In addition, a point mutation in the arp2/3 binding region of PP78/83 results in partially defective actin polymerization with reduced actin tails and erratic paths of movement that frequently changed direction (45). The use of actin polymerization for transport of intracellular pathogen has been observed for poxviruses (55) and certain bacteria, such as *Listeria monocytogenes* (56). However, in contrast to these pathogens that employ actin polymerization after replication, AcMNPV uses it immediately upon infection before viral replication (45). It then moves to the nucleus facilitated by actin polymerization (see below) and appears to enter through nuclear pores (41) (45). Transit to nuclei may also be facilitated by the movement of virion-containing endosomes along microtubules (57) (Figure 5).

Entry into nuclei

Several lines of evidence suggest that the nucleocapsids enter nuclei through nuclear pores (Figure 5) (Figure 7). Empty nucleocapsids were originally observed in nuclei of cells early in infection (41). In human cells exposed to AcMNPV at high moi, at 4 hr p.i. about 8% of the nucleocapsids that entered cells were localized to the cytoplasmic side of nuclear pores. When mitosis was blocked, and the nuclear membrane was intact, nucleocapsids were observed in nuclei, suggesting that they do not need dividing cells and the corresponding

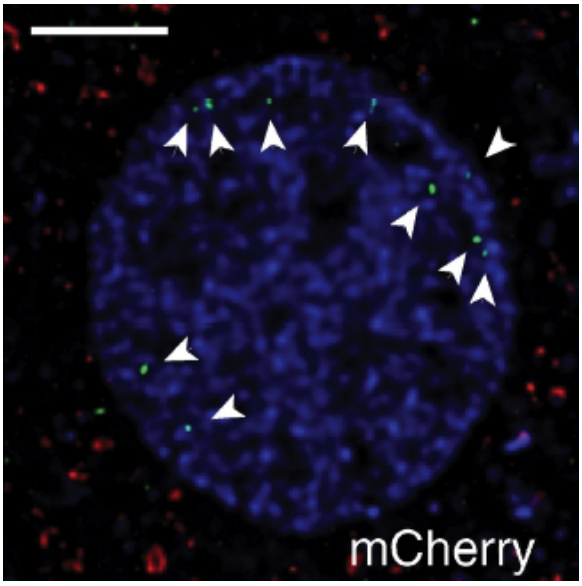

Figure 6. Entry of AcMNPV nucleocapsids through nuclear pores. This shows virus with the capsid protein VP39 fused with the fluorescent label, mCherry. Arrows indicate capsids; bar is 5 µm. Image reproduced from (45), with permission.

nuclear membrane breakdown to enter nuclei. Because of their localization to the cytoplasmic side of nuclear pores and their presence in nuclei of cells treated with mitotic inhibitors that prevent the breakdown of the nuclear membrane that occurs during mitosis, these authors concluded that nucleocapsids enter directly through nuclear pores (58). However, electron microscopic investigations have suggested that some baculoviruses inject their DNA through nuclear pores, reviewed in (59). Subsequently, using a virus encoding both a fluorescent tag fused to the vp39 capsid protein in addition to the wt vp39 protein, it was observed that the nucleocapsids localized to nuclear pore complexes and fluorescence was observed within nuclei (45) (Figure 6). This suggests that the nucleocapsids dock with and are transported through the nuclear pore complex. Nuclear pore complexes have been recently characterized and have been calculated to have a channel of 38 - 78 nm (60). Baculovirus virions have been calculated to have dimensions of 30-60 nm in diameter (61) indicating that they should be capable of moving through the pores. Also, AcMNPV nucleocapsids injected into Xenopus oocytes cause nuclear pores in that system to undergo significant reorganization in order to accommodate the transit of the nucleocapsids (62). Evidence suggests that a capsid associated protein, BV/ODV-C42 (Ac101), binds to PP78/83 and transports it into nuclei. Mutant bacmids lacking Ac101 fail to demonstrate polymerization of actin in nuclei (63). In addition, a predicted pocket binding protein site was identified on Ac101 that is essential for actin polymerization (64). Once inside the nucleus, the DNA is uncoated and the transcriptional cascade is initiated that eventually results in the production of nucleocapsids.

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

The virogenic stroma

A novel structure characteristic of NPV-infected cells is the virogenic stroma (Figure 1 and Figure 5). It is an electron-dense, chromatin-like structure surrounding multiple less dense spaces that is found near the center of nuclei of infected cells. It is thought to be a molecular scaffold that is produced for the orderly and coordinated transcription and replication of viral DNA and the subsequent packaging of DNA and assembly of

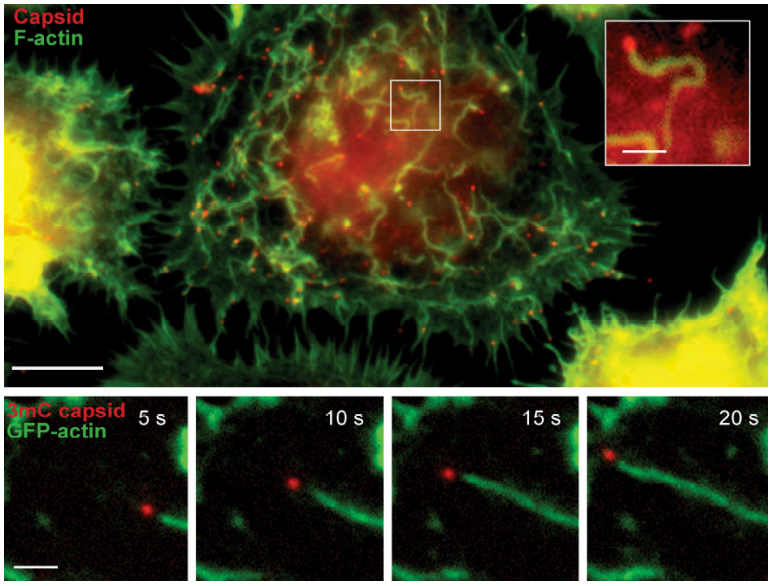

Figure 7. Actin-based motility of AcMNPV within the cytoplasm of the cell. The virus was detected by capsid protein VP39 fused with the fluorescent label, mCherry (red); actin (green; FITC-phalloidin). Inset bar is 10 mm. Bottom: time series at 5 sec intervals. Image reproduced from (45), with permission.

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

Exiting nuclei and the cell to form BV

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

As the infection proceeds, concentrations or patches of envelope proteins accumulate at the plasma membrane (Figure 5). For group I NPVs, these include both GP64 (Ac128) and the F protein (Ac23) (78, 79) (See Figure 5). In other viruses, e.g., members of Group II NPVs that lack *gp64*, this membrane is likely modified by homologs

of the F protein (80). The modification of the host cell membrane by GP64 is required for virus budding and consequently for secondary infections (81, 82).

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

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

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

Viral manipulation of larval metamorphosis: EGT (Ac15)

Another viral protein that can affect the course of an infection is an enzyme, ecdysteroid UDP-glucosyltransferase (89). Egt homologs are found in all lepidopteran NPVs and most GV genomes, but not in other lineages. Because of its role in insect steroid metabolism, the likely source of a gene encoding this enzyme would be from a host insect, and closely related orthologs of *egt* are found in a variety of insects such as *B. mori*. The function of the viral EGT is to block molting and pupation in infected larvae by catalyzing the transfer of glucose from UDP-glucose to ecdysteroids, thereby inactivating these insect molting hormones (90, 91). Molting can cause severe physiological stress on infected insects and many do not survive this transition. Therefore, the full productivity of the virus infection may not be realized. Evidence suggests that viral EGT prevents this stress by blocking molting. It also prolongs the feeding stage of infected larvae, thereby allowing the virus to replicate over a longer period of time in larger larvae, resulting in a higher yield of virus. The yield of occlusion bodies is increased about 30% in larva infected with wt virus compared to infection by virus lacking the *egt* gene (92, 93). A remarkable feature of NPV infection is that in some instances the insects can grow and continue feeding right up until they die. They appear healthy, yet when examined are heavily infected with high concentrations of occlusion bodies in their cells and hemolymph. EGT likely contributes to this effect. A common method to reduce the time that a virus takes to kill its host is to delete the *egt* gene. Larvae infected with these mutants are smaller and die sooner than wt, thereby reducing the damage caused to crops after the infection.

Budded virus versus cell-associated virus production

A major transition during baculovirus infections is the shift from BV production to the retention of nucleocapsids in the nucleus and their incorporation into occlusion bodies. In a study examining the kinetics of

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

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

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

The transition from BV to ODV production

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

Occlusion, the final stage in virus infection

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

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

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

Many newly hatched lepidopteran larvae remain as concentrated populations near the egg mass from which they emerged. Dispersal is largely dependent upon access to food supplies that are rapidly consumed near the egg mass. However, during the final larval stages many Lepidoptera disperse (wander) probably as an evolutionary mechanism to spread the population, reduce predation, and also to find a suitable place to pupate. In *Manduca sexta,* wandering is induced by a limited rise in the ecdysteroid titer in the hemolymph, and is associated with the cessation of feeding and the commitment to begin pupation ((108) and references therein). Baculovirus infection appears to be capable of enhancing this behavior and can cause a terminally infected insect to migrate to a higher elevation on the branch of a tree or plant. This is thought to facilitate dispersal of the occlusion bodies. This condition was noted in Germany and named 'wipfelkrankeit' (tree-top disease) (109). In one investigation, this phenomena was examined in cabbage moth larvae (*Mamestra brassicae*) on cabbage plants infected with MbNPV (110). It was observed that most of the uninfected larvae were located in the heart of cabbage plants, whereas infected third instar larvae were located at the top or edge of the plant. This behavioral difference appeared to be confined to third instar and was not observed in first or fifth instar larvae (111).

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

Late in infection, after the wandering stage, the insects become torpid and undergo what is termed 'melting'. Melting refers to the disintegration or liquefaction of the insect and is caused by some, but not all viruses. When

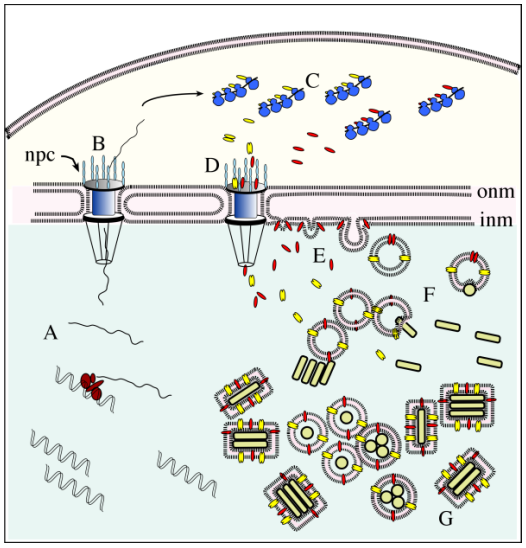

Figure 8. A hypothetical diagram of ODV membrane morphogenesis. In this diagram mRNA encoding ODV envelope proteins are transcribed (A) and exported (B) to the cytoplasm for translation (C) and the proteins are then targeted to the nucleus (D). Some of these proteins may be targeted to the inner nuclear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

on an even surface, the infected insects appear to flatten out and all the tissues appear to melt together and liquefy. Clearly, the disintegration of insects at a higher elevation on a plant and the subsequent contamination of lower vegetation could result in the infection of additional insect hosts, in contrast to simply dying, falling on the ground, and decaying into the soil.

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

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

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

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

The cytopathology of GVs

The above descriptions have focused on the replicative cycle of the most well-characterized baculoviruses, namely NPVs of Lepidoptera that can be easily cultivated and genetically manipulated. However, there are a variety of other viruses that cause a more limited infection. Although some GVs have been investigated, understanding their cytopathology has been hindered by the lack of an efficient cell culture system. In one cell culture system that has been described, both infectious hemolymph and BV titers were low (the highest BV titer was 10^6 $TCID_{50}$/ml) and BV titers decreased with passage (123). These low titers could reflect the insensitivity of the GV cell culture system and has made the generation of recombinant GVs challenging. Although GVs cause systemic infection, most GVs that have been characterized show a different pattern of cytopathology (Figure 9) from the lepidopteran NPVs described above. In addition, it is difficult to generalize regarding pathology because of the variation of gene content in different types of GVs. For example, half the GV genomes characterized lack cathepsin/chitinase genes and some also lack EGT genes. After their initial replication in midgut cells, subsequent infections vary with different GVs (reviewed in (124, 125)). Some GV infections are limited to the midgut, whereas others cause systemic infections and can replicate in a wide variety of tissues similar to NPVs. In addition, others appear to spread to and are limited to replicating only in fat body tissues. The cytopathology that occurs in the different cell types after GV infection, however, appears to be similar for all GVs and differs from NPVs (Figure 9). In infected cultured cells, the nucleus enlarges and the interior becomes clear as electron dense material becomes concentrated at the periphery near the nuclear membrane. At this stage, nucleocapsids that are likely destined to develop into BV are evident in the nuclei, but occlusion bodies are not present. Later in the infection the nuclear membrane appears to disintegrate and the nucleoplasm and cytoplasm merge. After this point, occlusion bodies become evident (123). Similar patterns of pathology have been found in infected insects with some cells showing a well-developed virogenic stroma in nuclei before the disintegration of the nuclear membrane (124).

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

In some types of baculoviruses, the infection appears to be confined to the midgut. These viruses include NPVs of Diptera, e.g., mosquitos (CuniNPV) (126), Hymenoptera, and certain types of GVs mentioned above (127). In the mosquito virus, CuniNPV, viral replication appears to proceed from an early production of virions that bud into the cytoplasm and the later development of occluded virions. The role of BV in spreading the infection to other cells is unclear (126). The development of infections by NPVs of Hymenoptera is also not well characterized. Most surprising is the lack of a homolog of either gp64 or the F protein in the three hymenopteran NPV genomes that have been sequenced. Two candidates for genes encoding possible fusion proteins of 217 and

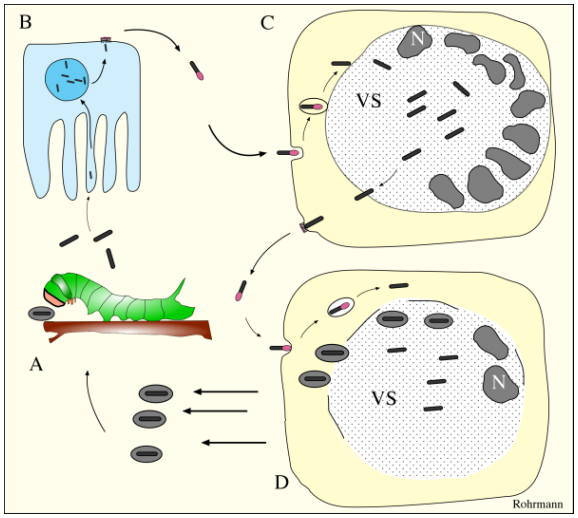

Figure 9. A granulovirus life cycle with systemic infection. Many features of a systemic GV infection are likely to be similar to that of NPVs, including the infection of insect midgut and the systemic spread to other tissues (A, B). However, the GV infection leads to the clearing of the nucleus with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

74 aa have been identified (128). However, fusion proteins of such a small size would be unprecedented for baculoviruses. Because of the requirement of fusion proteins for both BV cell egress and entry, it is unclear how infections by these viruses might spread. It is possible that infected cells are sloughed or disintegrate into the midgut lumen and release a mixture of occluded and nonoccluded virions containing an ODV envelope and other proteins that would allow infection of other midgut cells. In this case, ODV envelope and PIF proteins could facilitate this process. Genes predicted to encode homologs of these factors (including all the PIF proteins) have been identified in the genomes of the NPVs of Hymenoptera. A feature of at least some of these infections has been described as infectious diarrhea (125). This could reflect extensive midgut cell death. Conserved proteinases that could be involved in this process are described below.

Possible dissemination strategies for GVs, and hymenopteran and dipteran NPVs

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

Therefore, the presence of a trypsin-like protein may facilitate the dispersal of virus from the gut cells, both by releasing the virus into the environment, and also by providing inocula for the infection of other gut cells.

Viruses with other tissue specificities

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

Covert baculovirus infections

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

The possible presence of persistent baculoviruses has been suggested based on spontaneous outbreaks in controlled insect colonies, or by induction from exposing insects to physiological stressors such as cold temperature (136). Such occurrences have been reported for several different baculoviruses (reviewed in (137)). Other stressors include, overcrowding, high temperatures, uv light, humidity, ingestion of toxic chemicals, parasitism, and dietary conditions (reviewed in (133)). Although evidence of covert infections has been noted for many years, it was not until the advent of molecular biological technology including the polymerase chain reaction that methods became available to investigate these observations. In a review of over 35 studies using PCR in several Alpha- and Betabaculovirus, it was noted that over half of the investigations detected the presence of baculovirus genes at a prevalence of over 50% of the insects sampled (133). For example, a virus, MbNPV, has been found to persist in most populations of the cabbage moth (*Mamestra brassicae*) in the United Kingdom (138). The latent virus can be activated when the persistently infected insects are fed either a closely related virus, *Panolis flammea* (pine beauty moth) NPV (PfNPV), or the more distantly related AcMNPV. PCR amplification identified MbNPV polyhedrin gene sequences in all insect stages including eggs, larvae, pupae, and adults. In the fourth instar larvae, the latent virus was found only in fat body tissue. A cell line was derived from this PCR-positive tissue that contained the latent virus (139) and it was estimated that the cell line contained 13-20 copies of the viral genome per cell (140). In addition, it was found that virus-free *M. brassicae* larvae died of an MbNPV-like infection when they were fed fat body cells from the latently infected laboratory strain of the insect. In another study, plasmids containing baculovirus late and very late promoters fused to the CAT gene were activated when transfected into latently infected cells. But when these plasmids were transfected into virus-free cells, such activation did not occur. This was interpreted to indicate that low levels of baculovirus genes were expressed in the latently infected cells, and the proteins produced were able to activate the expression of the late promoter constructs (141). Covert infections of laboratory cultures of *Spodoptera exigua* by *S. exigua* multiple nucleopolyhedrovirus (SeMNPV) and *Mamestra brassicae* NPV (MbNPV) have also been reported

(142). Covert infections have also been reported for field collected and laboratory colonies of *Choristoneura fumiferana* (143) and were found to be induced in false codling moth, *Thaumatotibia leucotreta*, by overcrowding (144). In an investigation involving a GV of the Indian meal moth, *Plodia interpunctella*, it was found that transcripts of the PlinGV granulin were present in 60-80% of the offspring of insects that had survived exposure to a ~10% lethal dose of PlinGV. Inheritance of the virus was also observed in offspring of either exposed males or females mated with naïve insects (145).

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

References

1. Volkman LE, Summers MD. *Autographa californica* nuclear polyhedrosis virus: Comparitive infectivity of the occluded, alkali-liberated, and nonoccluded forms. J Invertebr Pathol. 1977;30:102–103. PubMed PMID: 336795.
2. Volkman LE, Summers MD, Hsieh CH. Occluded and nonoccluded nuclear polyhedrosis virus grown in *Trichoplusia ni*: comparative neutralization, comparative infectivity, and in vitro growth studies. J Virol. 1976;19:820–832. PubMed PMID: 787558.
3. Lehane MJ, Billingsley PF. 1996. Biology of the insect midgut. *In* Lehane MJ, Billingsley PF (ed), Biology of the insect midgut. Chapman and Hall, London.
4. Hegedus D, Erlandson M, Gillott C, Toprak U. New insights into peritrophic matrix synthesis, architecture, and function. Annu Rev Entomol. 2009;54:285–302. PubMed PMID: 19067633.
5. Dow JA. pH gradients in lepidopteran midgut. J Exp Biol. 1992;172:355–375. PubMed PMID: 9874748.
6. Wang P, Granados RR. An intestinal mucin is the target substrate for a baculovirus enhancin. Proc Natl Acad Sci U S A. 1997;94:6977–82. PubMed PMID: 9192677.
7. Toprak U, Harris S, Baldwin D, Theilmann D, Gillott C, Hegedus DD, Erlandson MA. Role of enhancin in Mamestra configurata nucleopolyhedrovirus virulence: selective degradation of host peritrophic matrix proteins. J Gen Virol. 2012;93:744–53. PubMed PMID: 22238230.
8. Washburn JO, Kirkpatrick BA, Volkman LE. Comparative pathogenesis of Autographa californica M nuclear polyhedrosis virus in larvae of Trichoplusia ni and Heliothis virescens. Virology. 1995;209:561–8. PubMed PMID: 7778288.
9. Vigdorovich V, Miller AD, Strong RK. Ability of hyaluronidase 2 to degrade extracellular hyaluronan is not required for its function as a receptor for jaagsiekte sheep retrovirus. J Virol. 2007;81:3124–9. PubMed PMID: 17229709.
10. Sugiura N, Setoyama Y, Chiba M, Kimata K, Watanabe H. Baculovirus envelope protein ODV-E66 is a novel chondroitinase with distinct substrate specificity. J Biol Chem. 2011;286:29026–34. PubMed PMID: 21715327.
11. Sugiura N, Ikeda M, Shioiri T, Yoshimura M, Kobayashi M, Watanabe H. Chondroitinase from baculovirus Bombyx mori nucleopolyhedrovirus and chondroitin sulfate from silkworm Bombyx mori. Glycobiology. 2013;23:1520–30. PubMed PMID: 24052236.
12. Mitsuhashi W, Miyamoto K. Disintegration of the peritrophic membrane of silkworm larvae due to spindles of an entomopoxvirus. J Invertebr Pathol. 2003;82:34–40. PubMed PMID: 12581717.

13. Chiu E, Hijnen M, Bunker RD, Boudes M, Rajendran C, Aizel K, Olieric V, Schulze-Briese C, Mitsuhashi W, Young V, Ward VK, Bergoin M, Metcalf P, Coulibaly F. Structural basis for the enhancement of virulence by viral spindles and their in vivo crystallization. Proc Natl Acad Sci U S A. 2015;112:3973–8. PubMed PMID: 25787255.
14. Liu X, Fang W, Fan R, Zhang L, Lei C, Zhang J, Nian W, Dou T, An S, Zhou L, Sun X. Granulovirus GP37 Facilitated ODVs Cross Insect Peritrophic Membranes and Fuse with Epithelia. Toxins. 2019.:11. PubMed PMID: 30836616.
15. Keddie BA, Aponte GW, Volkman LE. The pathway of infection of Autographa californica nuclear polyhedrosis virus in an insect host. Science. 1989;243:1728–1730. PubMed PMID: 2648574.
16. Flipsen JT, Martens JW, van Oers MM, Vlak JM, van Lent JW. Passage of Autographa californica nuclear polyhedrosis virus through the midgut epithelium of Spodoptera exigua larvae. Virology. 1995;208:328–35. PubMed PMID: 11831715.
17. Kikhno I, Guitiérrez S, Croizier L, Croizier G, Lopéz-Ferber ML. Characterization of pif, a gene required for the per os infectivity of Spodoptera littoralis nucleopolyhedrovirus. J Gen Virol. 2002;83:3013–3022. PubMed PMID: 12466478.
18. Horton HM, Burand JP. Saturable attachment sites for polyhedron-derived baculovirus on insect cells and evidence for entry via direct membrane fusion. J Virol. 1993;67:1860–1868. PubMed PMID: 8445715.
19. Dall D, Luque T, O'Reilly DR. Insect-virus relationships: sifting by informatics. Bioessays. 2001;23:184–193. PubMed PMID: 11169592.
20. Lapointe R, Popham HJ, Straschil U, Goulding D, O'Reilly DR, Olszewski JA. Characterization of two Autographa californica nucleopolyhedrovirus proteins, Ac145 and Ac150, which affect oral infectivity in a host-dependent manner. J Virol. 2004;78:6439–48. PubMed PMID: 15163737.
21. Zhu S, Wang W, Wang Y, Yuan M, Yang K. The baculovirus core gene ac83 is required for nucleocapsid assembly and per os infectivity of Autographa californica nucleopolyhedrovirus. J Virol. 2013;87:10573–86. PubMed PMID: 23864639.
22. Javed MA, Biswas S, Willis LG, Harris S, Pritchard C, van Oers MM, Donly BC, Erlandson MA, Hegedus DD, Theilmann DA. Autographa californica Multiple Nucleopolyhedrovirus AC83 is a Per Os Infectivity Factor (PIF) Protein Required for Occlusion-Derived Virus (ODV) and Budded Virus Nucleocapsid Assembly as well as Assembly of the PIF Complex in ODV Envelopes. J Virol. 2017;91:02115–16. PubMed PMID: 28031365.
23. Zimoch L, Hogenkamp DG, Kramer KJ, Muthukrishnan S, Merzendorfer H. Regulation of chitin synthesis in the larval midgut of Manduca sexta. Insect Biochem Mol Biol. 2005;35:515–27. PubMed PMID: 15857758.
24. Broehan G, Zimoch L, Wessels A, Ertas B, Merzendorfer H. A chymotrypsin-like serine protease interacts with the chitin synthase from the midgut of the tobacco hornworm. J Exp Biol. 2007;210:3636–43. PubMed PMID: 17921165.
25. Li Z, Li C, Yang K, Wang L, Yin C, Gong Y, Pang Y. Characterization of a chitin-binding protein GP37 of Spodoptera litura multicapsid nucleopolyhedrovirus. Virus Res. 2003;96:113–22. PubMed PMID: 12951271.
26. Dinglasan RR, Alaganan A, Ghosh AK, Saito A, van Kuppevelt TH, Jacobs-Lorena M. Plasmodium falciparum ookinetes require mosquito midgut chondroitin sulfate proteoglycans for cell invasion. Proc Natl Acad Sci U S A. 2007;104:15882–7. PubMed PMID: 17873063.
27. Shrestha A, Bao K, Chen YR, Chen W, Wang P, Fei Z, Blissard GW. Global Analysis of Baculovirus Autographa californica Multiple Nucleopolyhedrovirus Gene Expression in the Midgut of the Lepidopteran Host Trichoplusia ni. J Virol. 2018.:92. PubMed PMID: 30209166.
28. Shrestha A, Bao K, Chen W, Wang P, Fei Z, Blissard GW. Transcriptional responses of the Trichoplusia ni midgut to oral infection by the baculovirus Autographa californica Multiple Nucleopolyhedrovirus. J Virol. 2019. doi: 10.1128/JVI.00353-19. PubMed PMID: 31043536.

29. Engelhard EK, Kam-Morgan LN, Washburn JO, Volkman LE. The insect tracheal system: a conduit for the systemic spread of Autographa californica M nuclear polyhedrosis virus. Proc Natl Acad Sci U S A. 1994;91:3224–3227. PubMed PMID: 8159729.
30. Maina JN. Scanning and transmission electron microscopic study of the tracheal air sac system in a grasshopper Chrotogonus senegalensis (Kraus)--Orthoptera: Acrididae: Pyrgomorphinae. Anat Rec. 1989;223:393–405. PubMed PMID: 2712353.
31. Volkman LE. Baculovirus infectivity and the actin cytoskeleton. Curr Drug Targets. 2007;8:1075–83. PubMed PMID: 17979667.
32. Kirkpatrick BA, Washburn JO, Engelhard EK, Volkman LE. Primary infection of insect tracheae by Autographa californica M nuclear polyhedrosis virus. Virology. 1994;203:184–6. PubMed PMID: 8030276.
33. Katsuma S, Shimada T, Kobayashi M. Characterization of the baculovirus Bombyx mori nucleopolyhedrovirus gene homologous to the mammalian FGF gene family. Virus Genes. 2004;29:211–7. PubMed PMID: 15284481.
34. Katsuma S, Daimon T, Horie S, Kobayashi M, Shimada T. N-linked glycans of Bombyx mori nucleopolyhedrovirus fibroblast growth factor are crucial for its secretion. Biochem Biophys Res Commun. 2006;350:1069–75. PubMed PMID: 17046716.
35. Katsuma S, Daimon T, Mita K, Shimada T. Lepidopteran ortholog of Drosophila breathless is a receptor for the baculovirus fibroblast growth factor. J Virol. 2006;80:5474–81. PubMed PMID: 16699027.
36. Detvisitsakun C, Berretta MF, Lehiy C, Passarelli AL. Stimulation of cell motility by a viral fibroblast growth factor homolog: proposal for a role in viral pathogenesis. Virology. 2005;336:308–17. PubMed PMID: 15892971.
37. Detvisitsakun C, Hutfless EL, Berretta MF, Passarelli AL. Analysis of a baculovirus lacking a functional viral fibroblast growth factor homolog. Virology. 2006;346:258–65. PubMed PMID: 16476460.
38. Detvisitsakun C, Cain EL, Passarelli AL. The Autographa californica M nucleopolyhedrovirus fibroblast growth factor accelerates host mortality. Virology. 2007;365:70–8. PubMed PMID: 17459443.
39. Katsuma S, Horie S, Daimon T, Iwanaga M, Shimada T. In vivo and in vitro analyses of a Bombyx mori nucleopolyhedrovirus mutant lacking functional vfgf. Virology. 2006;355:62–70. PubMed PMID: 16904150.
40. Means JC, Passarelli AL. Viral fibroblast growth factor, matrix metalloproteases, and caspases are associated with enhancing systemic infection by baculoviruses. Proc Natl Acad Sci U S A. 2010;107:9825–30. PubMed PMID: 20457917.
41. Granados RR, Lawler KA. l98l. In vivo pathway of *Autographa californica* baculovirus invasion and infection. Virology l08:297-308.
42. Washburn JO, Lyons EH, Haas-Stapleton EJ, Volkman LE. Multiple nucleocapsid packaging of Autographa californica nucleopolyhedrovirus accelerates the onset of systemic infection in Trichoplusia ni. J Virol. 1999;73:411–6. PubMed PMID: 9847346.
43. Washburn JO, Chan EY, Volkman LE, Aumiller JJ, Jarvis DL. Early synthesis of budded virus envelope fusion protein GP64 enhances Autographa californica multicapsid nucleopolyhedrovirus virulence in orally infected Heliothis virescens. J Virol. 2003;77:280–90. PubMed PMID: 12477833.
44. Zhang JH, Washburn JO, Jarvis DL, Volkman LE. Autographa californica M nucleopolyhedrovirus early GP64 synthesis mitigates developmental resistance in orally infected noctuid hosts. J Gen Virol. 2004;85:833–42. PubMed PMID: 15039526.
45. Ohkawa T, Volkman LE, Welch MD. Actin-based motility drives baculovirus transit to the nucleus and cell surface. J Cell Biol. 2010;190:187–95. PubMed PMID: 20660627.
46. Hukuhara T. Genetic variation of polyhedrosis viruses of insects, p 7-11. *In* (ed),
47. Kataoka C, Kaname Y, Taguwa S, Abe T, Fukuhara T, Tani H, Moriishi K, Matsuura Y. Baculovirus GP64-mediated entry into mammalian cells. J Virol. 2012;86:2610–20. PubMed PMID: 22190715.
48. Kadlec J, Loureiro S, Abrescia NG, Stuart DI, Jones IM. The postfusion structure of baculovirus gp64 supports a unified view of viral fusion machines. Nat Struct Mol Biol. 2008;15:1024–30. PubMed PMID: 18776902.

49. Tani H, Nishijima M, Ushijima H, Miyamura T, Matsuura Y. Characterization of cell-surface determinants important for baculovirus infection. Virology. 2001;279:343–53. PubMed PMID: 11145915.
50. Long G, Pan X, Kormelink R, Vlak JM. Functional entry of baculovirus into insect and mammalian cells is dependent on clathrin-mediated endocytosis. J Virol. 2006;80:8830–3. PubMed PMID: 16912330.
51. Dong S, Wang M, Qiu Z, Deng F, Vlak JM, Hu Z, Wang H. Autographa californica multicapsid nucleopolyhedrovirus efficiently infects Sf9 cells and transduces mammalian cells via direct fusion with the plasma membrane at low pH. J Virol. 2010;84:5351–9. PubMed PMID: 20219938.
52. Goley ED, Ohkawa T, Mancuso J, Woodruff JB, D'Alessio JA, Cande WZ, Volkman LE, Welch MD. Dynamic nuclear actin assembly by Arp2/3 complex and a baculovirus WASP-like protein. Science. 2006;314:464–7. PubMed PMID: 17053146.
53. Au S, Wu W, Zhou L, Theilmann DA, Pante N. A new mechanism for nuclear import by actin-based propulsion used by a baculovirus nucleocapsid. J Cell Sci. 2016;129:2905–11. PubMed PMID: 27284005.
54. Mueller J, Pfanzelter J, Winkler C, Narita A, Le Clainche C, Nemethova M, Carlier MF, Maeda Y, Welch MD, Ohkawa T, Schmeiser C, Resch GP, Small JV. Electron tomography and simulation of baculovirus actin comet tails support a tethered filament model of pathogen propulsion. PLoS Biol. 2014;12:e1001765. PubMed PMID: 24453943.
55. Cudmore S, Cossart P, Griffiths G, Way M. Actin-based motility of vaccinia virus. Nature. 1995;378:636–8. PubMed PMID: 8524400.
56. Theriot JA, Mitchison TJ, Tilney LG, Portnoy DA. The rate of actin-based motility of intracellular Listeria monocytogenes equals the rate of actin polymerization. Nature. 1992;357:257–60. PubMed PMID: 1589024.
57. Qin F, Xu C, Hu J, Lei C, Zheng Z, Peng K, Wang H, Sun X. Dissecting the cell entry pathway of baculovirus by single particle tracking and quantitative electron microscopic analysis. J Virol. 2019. doi: 10.1128/JVI.00033-19. PubMed PMID: 30760565.
58. van Loo ND, Fortunati E, Ehlert E, Rabelink M, Grosveld F, Scholte BJ. Baculovirus infection of nondividing mammalian cells: mechanisms of entry and nuclear transport of capsids. J Virol. 2001;75:961–70. PubMed PMID: 11134309.
59. Au S, Wu W, Pante N. Baculovirus Nuclear Import: Open, Nuclear Pore Complex (NPC) Sesame. Viruses. 2013;5:1885–900. PubMed PMID: 23881277.
60. Alber F, Dokudovskaya S, Veenhoff LM, Zhang W, Kipper J, Devos D, Suprapto A, Karni-Schmidt O, Williams R, Chait BT, Rout MP, Sali A. Determining the architectures of macromolecular assemblies. Nature. 2007;450:683–94. PubMed PMID: 18046405.
61. Jehle JA, Blissard GW, Bonning BC, Cory JS, Herniou EA, Rohrmann GF, Theilmann DA, Thiem SM, Vlak JM. On the classification and nomenclature of baculoviruses: a proposal for revision. Arch Virol. 2006;151:1257–66. PubMed PMID: 16648963.
62. Au S, Pante N. Nuclear transport of baculovirus: revealing the nuclear pore complex passage. J Struct Biol. 2012;177:90–8. PubMed PMID: 22100338.
63. Wang Y, Wang Q, Liang C, Song J, Li N, Shi H, Chen X. Autographa californica multiple nucleopolyhedrovirus nucleocapsid protein BV/ODV-C42 mediates the nuclear entry of P78/83. J Virol. 2008;82:4554–61. PubMed PMID: 18287235.
64. Li K, Wang Y, Bai H, Wang Q, Song J, Zhou Y, Wu C, Chen X. The putative pocket protein binding site of Autographa californica nucleopolyhedrovirus BV/ODV-C42 is required for virus-induced nuclear actin polymerization. J Virol. 2010;84:7857–68. PubMed PMID: 20484515.
65. Gotoh T, Fukuhara M, Kikuchi K. Mathematical model for change in diameter distribution of baculovirus-infected Sf-9 insect cells. Biochem Engin J. 2008;40:379–386.
66. Cheley S, Kosik KS, Paskevich P, Bakalis S, Bayley H. Phosphorylated baculovirus p10 is a heat-stable microtubule-associated protein associated with process formation in Sf9 cells. J Cell Sci. 1992;102:739–752. PubMed PMID: 1331130.
67. Patmanidi AL, Possee RD, King LA. Formation of P10 tubular structures during AcMNPV infection depends on the integrity of host-cell microtubules. Virology. 2003;317:308–20. PubMed PMID: 14698669.

68. Young JC, MacKinnon EA, Faulkner P. The architecture of the virogenic stroma in isolated nuclei of *Spodoptera frugiperda* cells in vitro infected by *Autographa californica* nuclear polyhedrosis virus. J Struct Biol. 1993;110:141–153.
69. Fraser MJ. Ultrastructural observations of virion maturation in *Autographa californica* nuclear polyhedrosis virus infected *Spodoptera frugiperda* cell cultures. J Ultrastruct Mol Struct Res. 1986;95:189–195.
70. Vanarsdall AL, Mikhailov VS, Rohrmann GF. Characterization of a baculovirus lacking the DBP (DNA-binding protein) gene. Virology. 2007;364:475–85. PubMed PMID: 17449080.
71. Guarino LA, Dong W, Xu B, Broussard DR, Davis RW, Jarvis DL. The baculovirus phosphoprotein pp31 is associated with the virogenic stroma. J Virol. 1992;66:7113–7120. PubMed PMID: 1433508.
72. Yamagishi J, Burnett ED, Harwood SH, Blissard GW. The AcMNPV pp31 gene is not essential for productive AcMNPV replication or late gene transcription but appears to increase levels of most viral transcripts. Virology. 2007;365:34–47. PubMed PMID: 17467768.
73. Williams GV, Faulkner P. 1997. Cytological changes and viral morphogenesis during baculovirus infection. *In* Miller LK (ed), The Baculoviruses. Plenum, New York.
74. Ohkawa T, Welch MD. 2018. Baculovirus Actin-Based Motility Drives Nuclear Envelope Disruption and Nuclear Egress. Curr Biol 28:2153-2159 e4.
75. Wei W, Wang H, Li X, Fang N, Yang S, Liu H, Kang X, Sun X, Ji S. Cloning and Characterization of Sf9 Cell Lamin and the Lamin Conformational Changes during Autographa californica multiple nucleopolyhedrovirus Infection. Viruses. 2016.:8. PubMed PMID: 27164127.
76. Gross CH, Wolgamot GM, Russell RLQ, Pearson MN, Rohrmann GF. A baculovirus encoded 16 kDa glycoprotein localizes near the nuclear membrane of infected cells. Virology. 1993;192:386–390. PubMed PMID: 8517029.
77. Biswas S, Blissard GW, Theilmann DA. Trichoplusia ni Kinesin-1 Associates with Autographa californica Multiple Nucleopolyhedrovirus Nucleocapsid Proteins and Is Required for Production of Budded Virus. J Virol. 2016;90:3480–95. PubMed PMID: 26763996.
78. Blissard GW, Rohrmann GF. Location, sequence, transcriptional mapping, and temporal expression of the gp64 envelope glycoprotein gene of the *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus. Virology. 1989;170:537–555. PubMed PMID: 2658304.
79. Pearson MN, Russell R, Rohrmann GF. Characterization of a baculovirus encoded protein that is associated with infected-cell membranes and budded virions. Virology. 2001;291:22–31. PubMed PMID: 11878873.
80. Pearson MN, Groten C, Rohrmann GF. Identification of the *Lymantria dispar* nucleopolyhedrovirus envelope fusion protein provides evidence for a phylogenetic division of the Baculoviridae. J Virol. 2000;74:6126–6131. PubMed PMID: 10846096.
81. Oomens AG, Blissard GW. Requirement for GP64 to drive efficient budding of *Autographa californica* multicapsid nucleopolyhedrovirus. Virology. 1999;254:297–314. PubMed PMID: 9986796.
82. Monsma SA, Oomens AGP, Blissard GW. The gp64 envelope fusion protein is an essential baculovirus protein required for cell-to-cell transmission of infection. J Virol. 1996;70:4607–4616. PubMed PMID: 8676487.
83. Hernandez JM, Podbilewicz B. The hallmarks of cell-cell fusion. Development. 2017;144:4481–4495. PubMed PMID: 29254991.
84. Guo Y, Yue Q, Gao J, Wang Z, Chen YR, Blissard GW, Liu TX, Li Z. Roles of Cellular NSF Protein in Entry and Nuclear Egress of Budded Virions of Autographa californica Multiple Nucleopolyhedrovirus. J Virol. 2017.:91. PubMed PMID: 28747507.
85. Blissard GW, Theilmann DA. Baculovirus Entry and Egress from Insect Cells. Annu Rev Virol. 2018;5:113–139. PubMed PMID: 30004832.
86. Ahmed I, Akram Z, Iqbal HMN, Munn AL. The regulation of Endosomal Sorting Complex Required for Transport and accessory proteins in multivesicular body sorting and enveloped viral budding - An overview. Int J Biol Macromol. 2019;127:1–11. PubMed PMID: 30615963.

87. Li Z, Blissard GW. Cellular VPS4 is required for efficient entry and egress of budded virions of Autographa californica multiple nucleopolyhedrovirus. J Virol. 2012;86:459–72. PubMed PMID: 22072775.
88. Yue Q, Yu Q, Yang Q, Xu Y, Guo Y, Blissard GW, Li Z. Distinct Roles of Cellular ESCRT-I and ESCRT-III Proteins in Efficient Entry and Egress of Budded Virions of Autographa californica Multiple Nucleopolyhedrovirus. J Virol. 2018.:92. PubMed PMID: 29046462.
89. Wang Y, Schwedes JF, Parks D, Mann K, Tegtmeyer P. Interaction of p53 with its consensus DNA-binding site. Mol Cell Biol. 1995;15:2157–65. PubMed PMID: 7891710.
90. O'Reilly DR, Miller LK. A baculovirus blocks insect molting by producing ecdysteroid UDP-glucosyl transferase. Science. 1989.:1110–1112. PubMed PMID: 2505387.
91. O'Reilly DR, Miller LK. Regulation of expression of a baculovirus ecdysteroid UDPglucosyltransferase gene. J Virol. 1990;64:1321–1328. PubMed PMID: 2106039.
92. O'Reilly DR, Miller LK. Improvement of a baculovirus pesticide by deletion of the egt gene. Bio/Technology. 1991;9:1086–1089.
93. O'Reilly DR, Hails RS, Kelly TJ. The impact of host developmental status on baculovirus replication. J Invertebr Pathol. 1998;72:269–75. PubMed PMID: 9784350.
94. Rosinski M, Reid S, Nielsen LK. Kinetics of baculovirus replication and release using real-time quantitative polymerase chain reaction. Biotechnol Bioeng. 2002;77:476–80. PubMed PMID: 11787021.
95. Mita K, Kasahara M, Sasaki S, Nagayasu Y, Yamada T, Kanamori H, Namiki N, Kitagawa M, Yamashita H, Yasukochi Y, Kadono-Okuda K, Yamamoto K, Ajimura M, Ravikumar G, Shimomura M, Nagamura Y, Shin-I T, Abe H, Shimada T, Morishita S, Sasaki T. The genome sequence of silkworm, Bombyx mori. DNA Res. 2004;11:27–35. PubMed PMID: 15141943.
96. Nguyen Q, Chan LC, Nielsen LK, Reid S. Genome scale analysis of differential mRNA expression of Helicoverpa zea insect cells infected with a H. armigera baculovirus. Virology. 2013;444:158–70. PubMed PMID: 23827436.
97. Kawasaki Y, Matsumoto S, Nagamine T. Analysis of baculovirus IE1 in living cells: dynamics and spatial relationships to viral structural proteins. J Gen Virol. 2004;85:3575–3583. PubMed PMID: 15557230.
98. Luo XC, Wang SS, Zhang J, Qian DD, Wang SM, Li LL. Effects of Early or Overexpression of the Multiple Nucleopolyhedrovirus (ODV-E25) on Virus Replication. PLoS One. 2013;8:e65635. PubMed PMID: 23825525.
99. Chen L, Yang R, Hu X, Xiang X, Yu S, Wu X. The formation of occlusion-derived virus is affected by the expression level of ODV-E25. Virus Res. 2013;173:404–14. PubMed PMID: 23298549.
100. Zhu M, Wang J, Deng R, Xiong P, Liang H, Wang X. A MicroRNA Encoded by Autographa californica Nucleopolyhedrovirus Regulates Expression of Viral Gene ODV-E25. J Virol. 2013;87:13029–34. PubMed PMID: 24027316.
101. Zhu M, Wang J, Deng R, Wang X. Functional Regulation of an Autographa californica Nucleopolyhedrovirus-Encoded MicroRNA, AcMNPV-miR-1, in Baculovirus Replication. J Virol. 2016;90:6526–6537. PubMed PMID: 27147751.
102. Guo YJ, Fu SH, Li LL. Autographa californica multiple nucleopolyhedrovirus ac75 is required for egress of nucleocapsids from the nucleus and formation of de novo intranuclear membrane microvesicles. PLoS One. 2017;12:e0185630. PubMed PMID: 28968422.
103. Biswas S, Willis LG, Fang M, Nie Y, Theilmann DA. Autographa californica Nucleopolyhedrovirus AC141 (Exon0), a Potential E3 Ubiquitin Ligase, Interacts with Viral Ubiquitin and AC66 To Facilitate Nucleocapsid Egress. J Virol. 2018.:92. PubMed PMID: 29142135.
104. Shi Y, Li K, Tang P, Li Y, Zhou Q, Yang K, Zhang Q. Three-dimensional visualization of the Autographa californica multiple nucleopolyhedrovirus occlusion-derived virion envelopment process gives new clues as to its mechanism. Virology. 2015;476:298–303. PubMed PMID: 25569457.
105. Braunagel SC, Summers MD. Molecular Biology of the Baculovirus Occlusion-Derived Virus Envelope. Curr Drug Targets. 2007;8:1084–1095. PubMed PMID: 17979668.

106. Xu H, Yang Z, Zhao J, Tian C, Ge J, Tang X, Bao Y, Zhang C. Bombyx mori nucleopolyhedrovirus ORF56 encodes an occlusion-derived virus protein and is not essential for budded virus production. J Gen Virol. 2008;89:1212–1219. PubMed PMID: 18420799.
107. Carpentier DC, Griffiths CM, King LA. The baculovirus P10 protein of Autographa californica nucleopolyhedrovirus forms two distinct cytoskeletal-like structures and associates with polyhedral occlusion bodies during infection. Virology. 2008;371:278–91. PubMed PMID: 17991504.
108. Chamberlin ME, King ME. Changes in Midgut Active Ion Transport and Metabolism During the Fifth Instar of the Tobacco Hornworm (Manduca sexta). J Exp Zool. 1998;280:135–141.
109. Evans HF. 1986. Ecology and epizootiology of baculoviruses, p 89-132. *In* Granados RR, Federici BA (ed), The biology of baculoviruses, vol II. CRC Press, Inc., Boca Raton.
110. Jehle JA, Lange M, Wang H, Hu Z, Wang Y, Hauschild R. Molecular identification and phylogenetic analysis of baculoviruses from Lepidoptera. Virology. 2006;346:180–193. PubMed PMID: 16313938.
111. Goulson D. Wipfelkrankheit : modification of host behaviour during baculoviral infection. Oecologia. 1997;109:219–228. PubMed PMID: 28307172.
112. Han Y, van Houte S, van Oers MM, Ros VID. Timely trigger of caterpillar zombie behaviour: temporal requirements for light in baculovirus-induced tree-top disease. Parasitology. 2018;145:822–827. PubMed PMID: 29144213.
113. Bhattarai UR, Li F, Katuwal Bhattarai M, Masoudi A, Wang D. Phototransduction and circadian entrainment are the key pathways in the signaling mechanism for the baculovirus induced tree-top disease in the lepidopteran larvae. Sci Rep. 2018;8:17528. PubMed PMID: 30510155.
114. Fukamizo T, Sato H, Mizuhara M, Ohnuma T, Gotoh T, Hiwatashi K, Takahashi S. Chitinase from Autographa californica Multiple Nucleopolyhedrovirus: Rapid Purification from Sf-9 Medium and Mode of Action. Biosci Biotechnol Biochem. 2011;75:1763–9. PubMed PMID: 21897030.
115. Daimon T, Katsuma S, Kang W, Shimada T. Comparative studies of Bombyx mori nucleopolyhedrovirus chitinase and its host ortholog, BmChi-h. Biochem Biophys Res Commun. 2006;345:825–33. PubMed PMID: 16701557.
116. Thomas CJ, Brown HL, Hawes CR, Lee BY, Min MK, King LA, Possee RD. Localization of a baculovirus-induced chitinase in the insect cell endoplasmic reticulum. J Virol. 1998;72:10207–12. PubMed PMID: 9811762.
117. Saville GP, Patmanidi AL, Possee RD, King LA. Deletion of the Autographa californica nucleopolyhedrovirus chitinase KDEL motif and in vitro and in vivo analysis of the modified virus. J Gen Virol. 2004;85:821–31. PubMed PMID: 15039525.
118. Hawtin RE, Zarkowska T, Arnold K, Thomas CJ, Gooday GW, King LA, Kuzio JA, Possee RD. Liquefaction of Autographa californica nucleopolyhedrovirus-infected insects is dependent on the integrity of virus-encoded chitinase and cathepsin genes. Virology. 1997;238:243–253. PubMed PMID: 9400597.
119. Oliveira JV, Wolff JL, Garcia-Maruniak A, Ribeiro BM, de Castro ME, de Souza ML, Moscardi F, Maruniak JE, Zanotto PM. Genome of the most widely used viral biopesticide: Anticarsia gemmatalis multiple nucleopolyhedrovirus. J Gen Virol. 2006;87:3233–50. PubMed PMID: 17030857.
120. Hom LG, Ohkawa T, Trudeau D, Volkman LE. Autographa californica M nucleopolyhedrovirus ProV-CATH is activated during infected cell death. Virology. 2002;296:212–8. PubMed PMID: 12069520.
121. Ishimwe E, Hodgson JJ, Clem RJ, Passarelli AL. Reaching the melting point: Degradative enzymes and protease inhibitors involved in baculovirus infection and dissemination. Virology. 2015;479-480:637–49. PubMed PMID: 25724418.
122. Preston R. 1997. The Cobra Event. Ballantine Books, New York.
123. Winstanley D, Crook NE. Replication of Cydia pomonella granulosis virus in cell cultures. J Gen Virol. 1993;74(Pt 8):1599–609. PubMed PMID: 8345351.
124. Tanada Y, Hess RT. 1991. Baculoviridae. Granulosis Viruses. *In* Adams JR, Bonami JR (ed), Atlas of invertebrate viruses. CRC Press, Inc., Boca Raton.
125. Federici BA. 1997. Baculovirus pathogenesis. *In* Miller LK (ed), The Baculoviruses. Plenum, New York.

126. Moser B, Becnel J, White S, Afonso C, Kutish G, Shanker S, Almira E. Morphological and molecular evidence that Culex nigripalpus baculovirus is an unusual member of the family Baculoviridae. J Gen Virol. 2001;82:283–297. PubMed PMID: 11161265.
127. Federici BA, Stern VM. Replication and occlusion of a granulosis virus in larval and adult midgut epithelium of the western grapeleaf skeletonizer, Harrisina brillians. J Invertebr Pathol. 2000;56:401–414.
128. Duffy SP, Young AM, Morin B, Lucarotti CJ, Koop BF, Levin DB. Sequence analysis and organization of the Neodiprion abietis nucleopolyhedrovirus genome. J Virol. 2006;80:6952–63. PubMed PMID: 16809301.
129. Ko R, Okano K, Maeda S. Structural and functional analysis of the Xestia c-nigrum granulovirus matrix metalloproteinase. J Virol. 2000;74:11240–6. PubMed PMID: 11070022.
130. Garcia-Maruniak A, Maruniak JE, Zanotto PM, Doumbouya AE, Liu JC, Merritt TM, Lanoie JS. Sequence analysis of the genome of the Neodiprion sertifer nucleopolyhedrovirus. J Virol. 2004;78:7036–51. PubMed PMID: 15194780.
131. Smith KA. Intranuclear changes in the polyhedrosis of Tipula paludosa. Nature. 1955;176:255. [Diptera]. PubMed PMID: 13244670.
132. Couch JA. Free and occluded virus, similar to Baculovirus, in hepatopancreas of pink shrimp. Nature. 1974;247:229–231.
133. Williams T, Virto C, Murillo R, Caballero P. Covert Infection of Insects by Baculoviruses. Front Microbiol. 2017;8:1337. PubMed PMID: 28769903.
134. Wu YL, Wu CP, Liu CY, Hsu PW, Wu EC, Chao YC. A non-coding RNA of insect HzNV-1 virus establishes latent viral infection through microRNA. Sci Rep. 2011;1:60. PubMed PMID: 22355579.
135. Drezen JM, Leobold M, Bezier A, Huguet E, Volkoff AN, Herniou EA. Endogenous viruses of parasitic wasps: variations on a common theme. Curr Opin Virol. 2017;25:41–48. PubMed PMID: 28728099.
136. Biever KD, Wilkinson JD. A stress-induced gruanosis virus of pieris rapae. Environ Entomol. 1978;7:572–573.
137. Kukan B. Vertical transmission of nucleopolyhedrovirus in insects. J Invertebr Pathol. 1999;74:103–11. PubMed PMID: 10486221.
138. Burden JP, Possee RD, Sait SM, King LA, Hails RS. Phenotypic and genotypic characterisation of persistent baculovirus infections in populations of the cabbage moth (Mamestra brassicae) within the British Isles. Arch Virol. 2006;151:635–49. PubMed PMID: 16328143.
139. Hughes DS, Possee RD, King LA. Activation and detection of a latent baculovirus resembling Mamestra brassicae nuclear polyhedrosis virus in M. brassicae insects. Virology. 1993;194:608–15. PubMed PMID: 8503177.
140. Hughes DS, Possee RD, King LA. Quantification of latent Mamestra brassicae nuclear polyhedrosis virus in M. brassicae insects using a PCR-scintillation proximity assay. J Virol Methods. 1994;50:21–7. PubMed PMID: 7714043.
141. Hughes DS, Possee RD, King LA. Evidence for the presence of a low-level, persistent baculovirus infection of Mamestra brassicae insects. J Gen Virol. 1997;78(Pt 7):1801–5. PubMed PMID: 9225059.
142. Murillo R, Hussey MS, Possee RD. Evidence for covert baculovirus infections in a Spodoptera exigua laboratory culture. J Gen Virol. 2011;92:1061–70. PubMed PMID: 21228125.
143. Kemp EM, Woodward DT, Cory JS. Detection of single and mixed covert baculovirus infections in eastern spruce budworm, Choristoneura fumiferana populations. J Invertebr Pathol. 2011;107:202–5. PubMed PMID: 21616077.
144. Opoku-Debrah JK, Hill MP, Knox C, Moore SD. Overcrowding of false codling moth, Thaumatotibia leucotreta (Meyrick) leads to the isolation of five new Cryptophlebia leucotreta granulovirus (CrleGV-SA) isolates. J Invertebr Pathol. 2013;112:219–28. PubMed PMID: 23277142.
145. Burden JP, Griffiths CM, Cory JS, Smith P, Sait SM. Vertical transmission of sublethal granulovirus infection in the Indian meal moth, Plodia interpunctella. Mol Ecol. 2002;11:547–55. PubMed PMID: 11918789.
146. Lee JC, Chen HH, Chao YC. Persistent baculovirus infection results from deletion of the apoptotic suppressor gene p35. J Virol. 1998;72:9157–65. PubMed PMID: 9765462.

147. Weng Q, Yang K, Xiao W, Yuan M, Zhang W, Pang Y. Establishment of an insect cell clone that harbours a partial baculoviral genome and is resistant to homologous virus infection. J Gen Virol. 2009;90:2871–6. PubMed PMID: 19675188.
148. Danquah JO, Botchway S, Jeshtadi A, King LA. Direct interaction of baculovirus capsid proteins VP39 and EXON0 with kinesin-1 in insect cells determined by fluorescence resonance energy transfer-fluorescence lifetime imaging microscopy. J Virol. 2012;86:844–53. PubMed PMID: 22072745.

4. Early events in infection: Virus transcription

Transcriptional activators, enhancers and the host RNA polymerase

An underlying feature of gene expression in eukaryotic cells is that relatively few genes are concurrently transcribed and the population of genes that are expressed may change during development. Critical for regulating gene expression is a somewhat scarce set of proteins that comprise the transcriptional apparatus. The heart of this complex is an enzyme, RNA polymerase, that synthesizes RNA. However, for RNA polymerase to selectively transcribe specific genes, a variety of transcription factors are required. These factors have different functions and can include: selecting the genes that will be transcribed; attracting and stabilizing the RNA polymerase complex; and facilitating RNA synthesis. Another category of critical factors are transcriptional activators that elevate the levels of specific RNA synthesis. These often function in conjunction with enhancers that are DNA sequences that bind transcription factors and bring them in close proximity to the RNA polymerase complex, thereby elevating the levels of RNA synthesis. Collectively these DNA sequences, enzymes, and factors regulate transcription by influencing the rate at which RNA polymerase is recruited to, or stabilized, at a specific promoter and its subsequent ability to efficiently transcribe RNA. In higher eukaryotes, different transcriptional activators can cause the expression of ordered sets of genes at each stage of development, or can lead to the transcription of tissue-specific genes in differentiated tissues.

Viruses destined for productive infections are confronted with a dilemma upon entry into a cell: they must compete with the cell for control of its well-organized and programmed transcriptional machinery that is strictly regulated for carrying out cellular processes. Without special mechanisms to take control of the cell and focus its replicative machinery on the synthesis of viral products, the viral genome would languish in the cell and could be overwhelmed by competition from cellular genes. To outcompete the cell and focus transcription on the viral genome, viruses rely on several mechanisms. They can selectively inhibit host cell transcription or eliminate host mRNA. They also can encode their own set of transcriptional activators and enhancer sequences, which facilitate the assembly of the host cell transcription complex at the site of RNA initiation of viral genes on the viral genome, rather than elsewhere, e.g., the host genome. Transcriptional activators can coordinate both the assembly of the transcription complex, and by interaction with enhancer sequences, they can bring enhancer-bound cellular transcription factors into close proximity to a gene promoter region. In some cases, this can elevate expression levels of viral genes several thousand-fold, thereby allowing the virus to redirect cellular processes for its own replication. Many DNA viruses use this process to cause a transcriptional cascade upon infection. In this process, one of the first viral genes synthesized is a transcription factor that activates a set of genes including a second transcription factor. This second factor activates transcription of a second set of genes and so on until the transcription cascade is complete. This produces a regulated progression through a transcription program of categories of early and late genes that carry out the functions necessary for each stage of viral replication. In the case of herpes simplex virus, the transcriptional program is initiated by a transcriptional activator (vp16) that is a structural component of the virion and is transported into the nucleus during the initiation of infection. Other nuclear replicating viruses, e.g., adenoviruses, express a transcriptional activator early after infection, thereby initiating the transcriptional cascade. Throughout the transcriptional programs employed by these two viruses, the virus is dependent upon regulating the host RNA polymerase to transcribe its genes and thereby carry out its replication.

Baculovirus infection: selective effects on host cell gene expression

Early investigations using pulse labeling with ^{35}S methionine to examine protein synthesis in baculovirus infected cells indicated that as the infection proceeded, the host cell proteins faded and the viral proteins became

more prominent (1). This was subsequently correlated with the down regulation of host cell mRNA levels (2, 3). It could also be a result of a phenomenon called translational arrest (4). Although the precise mechanism is not known, since the virus is dependent on a variety of cell functions including RNA polymerase II, a generalized inhibition of all host protein expression is unlikely in a permissive infection. More recent experiments have indicated that a number of host genes are up regulated during infection. For example, certain mitogen activated protein kinases (MAPKs) appear to be required for BmNPV gene expression. Inhibition of the expression of some MAPK genes results in reduced virus production (5), suggesting that any inhibition of host gene expression that occurs is selective or that the virus is able to up regulate certain categories of host genes. During AcMNPV infection of *T. ni* cells, the majority of host transcripts decrease between 6 to 48 hr post infection (pi) such that at the latter time they comprise about 10% of the total. However, about 6% of host genes are upregulated from 0-6 hr pi and then decrease for the remainder of the infection. In addition, a small group of genes related to metabolism and stress response were elevated at 18-24 hr pi and then declined. Concomitant to the decline in host RNA, the viral RNA increased such that it comprised over 50% of the total by about 13 hr pi infection and continued increasing thereafter (6). Using 2-D gel comparisons in combination with proteomic analysis, a comparison of protein expression in cells permissive, semi- and non-permissive for AcMNPV infection was reported (7). It has also been observed that in *S. exigua* larvae infected with AcMNPV, some host genes are up-regulated while others are down regulated (8) suggesting that in whole insects the general shut down of host genes does not occur. It has also been shown that baculovirus DNA replication can trigger both apoptosis and translational arrest, thereby influencing the expression of host genes (9). The conflicting evidence regarding levels of host protein synthesis could be due to the level of expression of the up-regulated genes. Many of the most abundant host proteins could be shut down, while other genes, particularly those with an essential enzymatic function, might be up regulated, but are not evident because their expression is relatively low. For additional discussion of the regulation of RNA polymerase II, see the section Shutoff of Early Transcription below.

The baculovirus transcription cascade: the evolution of a novel strategy

Baculoviruses begin their infection cycle similar to other large DNA viruses and employ enhancers and transcriptional activators to exploit the host transcriptional apparatus. This early program is focused on establishing the infection and producing the components necessary for initiating viral DNA replication and other early functions. However, whereas these early events are dependent on genes transcribed by the host RNA polymerase, the later genes are transcribed by a baculovirus-encoded RNA polymerase (Figure 1). Consequently, early in infection transcription is carried out by the host RNA polymerase, whereas the viral RNA polymerase is involved late in infection. Although some bacterial viruses, e.g., T7, also exploit their host's RNA polymerase early and employ their own RNA polymerase later in infection, baculoviruses are the only nuclear replicating DNA viruses of eukaryotes that employ this combination of cellular and viral polymerases.

Other eukaryotic DNA viruses that encode their own RNA polymerases, such as pox viruses, replicate in the cytoplasm and do not have ready access to the cellular transcriptional apparatus because of their separation from the nucleus. The evolutionary logic for baculoviruses to encode their own RNA polymerase is unclear. However, it may have originally been a major advantage for the virus to be able to regulate its own polymerase in the context of cells that were undergoing the shock of virus infection. This advantage could include the mitigation of possible effects caused by low levels of host RNA polymerase that might be normally present or caused by the infection (see Chapter 6).

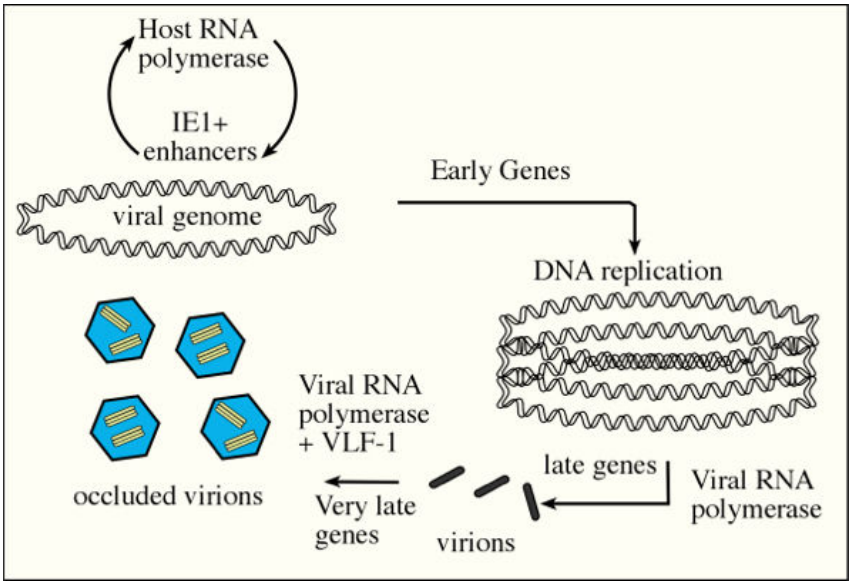

Figure 1. The baculovirus transcriptional cascade showing the interrelationship of host and viral RNA polymerases and DNA replication and VLF-1.

Transcriptional enhancers

Similar to other viruses, enhancers and transcriptional activators play a major role in the early events in baculovirus infections. Enhancers are specific DNA sequences (Figure 2) that are able to bind and concentrate transcription factors and can deliver them to the regulatory region of a gene, thereby facilitating transcriptional initiation (Figure 3). They can be located near or at some distance from the site of transcriptional initiation. It is thought that, when distant, the enhancer DNA with its associated transcription factors can bend around and be positioned in close proximity to the site of transcriptional initiation (Figure 3). Bending of the DNA can be facilitated by transcriptional activators that bind to both the enhancer sequence, and to the RNA polymerase complex. In this process, the RNA polymerase complex would bind to the gene promoter and the activator could then bind to the enhancer region and bring it and its associated transcription factors into close proximity to the RNA polymerase-promoter complex (Figure 3). Whereas one might consider an enhancer sequence distant from a promoter, activators binding to the enhancer located on the same DNA molecule as a promoter would likely be in much closer proximity to the promoter than if they were floating free in the cell.

Baculovirus enhancers:*hrs* (homologous regions)

A characteristic feature of most baculovirus genomes is the presence of homologous regions, or *hrs*. In AcMNPV, *hrs* are comprised of repeated units of about 70-bp with an imperfect 30-bp palindrome near their center (Figure 2). They are present at eight locations in the genome with 2 to 8 repeats at each site. They are highly variable, and although they are closely related within a genome, they may show very limited homology between different viruses. For example, in the CpGV genome, tandem repeated sequences are not evident, although a 75-bp imperfect palindrome is present at 13 different locations on the genome (10). In addition, in the TnNPV (group II) and several other genomes, *hr*-like sequences were not found (11), reviewed in (12).

Hrs have been implicated both as transcriptional enhancers and origins of DNA replication for a number of baculoviruses (13-18). In AcMNPV, they bind the transcriptional activator IE1 (Ac147) (19-21) and this binding can elevate the levels of IE1 transactivation up to 1000-fold (13). In addition, *hr* binding may cause IE1 to localize to sites that may be a prelude to replication foci (22). *Hrs* contain cAMP and 12-O-tetradecanoylphorbol 13-acetate (TPA) response elements (CRE and TRE)-like sequences located between the palindromes Figure 2).

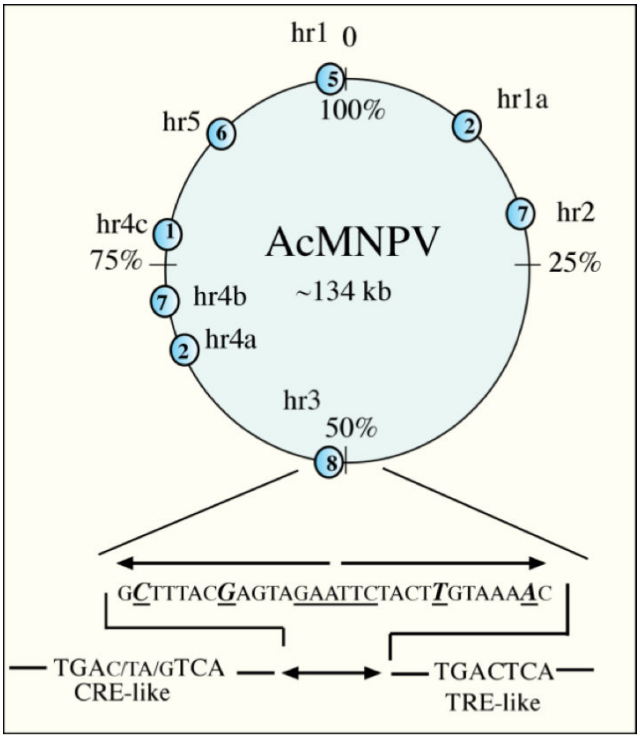

Figure 2. Diagram of the AcMNPV genome showing *hrs*. The circled numbers indicate the number of repeats in each *hr*. Below is shown a representative palindrome with mismatches shown as underlined, italicized larger type. The EcoRI site at the center of the palindrome is also underlined. At the bottom is a schematic showing the CRE- and TRE-like sequences that are located between the major palindromes.

A survey of baculovirus genomes found that these elements were preferentially concentrated within the *hr* sequences. In AcMNPV, although *hrs* comprise less than 3% of the total genome sequence, they contain 94% (48 of 51) of the CRE and 62% (13 of 21) of the TRE motifs. One to two of these elements are found between each pair of *hr* palindromes (Figure 2). These are evolutionarily conserved throughout the lepidopteran NPVs, but are not found in GVs, or dipteran and hymenopteran NPVs. In other systems, these elements bind cellular transcription factors and stimulate RNA polymerase II dependent transcription. In AcMNPV, the CRE and TRE were found to bind to host cell proteins and activate transcription in transient assays (23). Despite the concentration of these motifs in *hrs*, their ability to activate transcription appears to be adapted to each virus, probably due to a reduction in the affinity of IE1 from one virus to the *hrs* of another. For example, AcMNPV IE1 is much more efficient in activating a reporter gene linked to an AcMNPV *hr* than is OpMNPV IE1 (24). The consensus sequences of the *hr* palindromes between the two viruses are about 57% (17/30) identical (25).

It has been shown that deletion of individual or combinations of two *hrs* did not appear to affect AcMNPV replication in cultured cells (26). This is probably due to the ability of transcriptional activators to interact with *hrs* in other locations of the genome, allowing them to be brought into close proximity to the promoters being activated by bending of the DNA (Figure 3). However, the removal of up to five *hrs* reduced BV titers about 10-fold and the removal of all eight AcMNPV *hrs* caused a reduction in BV production of over 10,000-fold, and the number of cells producing polyhedra was reduced to a few percent compared to wt infection. In addition, the expression levels of LEF3, GP64 and VP39(capsid) was greatly reduced when eight *hrs* were removed (27). Since *hr* sequences serve are origins of DNA replication in transient assays (see Chapter 5), the severe effects of removing all the AcMNPV *hrs* could be due to a reduction in DNA replication or transcriptional activation or a combination of both.

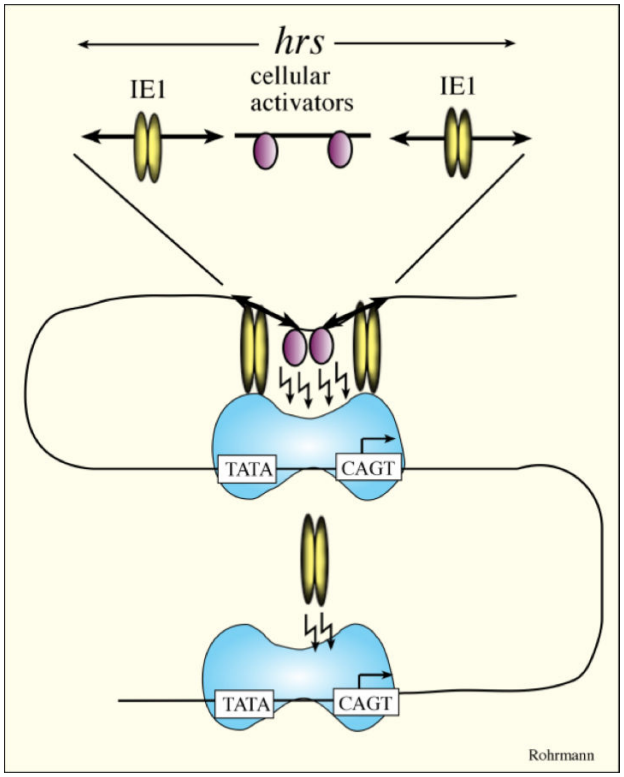

Figure 3. Activation of baculovirus early gene transcription by *hrs* and IE1. This diagram shows two possible mechanism by which IE1 activates transcription. Transcription might be activated directly by IE1 as shown in the bottom of the diagram, or it can interact with both RNA polymerase II (in blue) and *hr* sequences thereby bringing *hr*-bound transcriptional activators in close proximity to RNA polymerase II as shown in the top of the figure.

Transactivation of early genes.

There are five genes that are expressed as immediate early genes and they all have been reported to have transactivator function. They include *ie0*, *ie1*, *ie2*, *me53*, and *pe38*. An analysis of all five genes indicated that elevated levels of IE gene expression is related to the activation of this set of genes. However, IE0 has a repressor function caused by its interaction with IE1 and the role of Me53 is unclear (28).

A major transcriptional activator of early genes, immediate early gene 1 (IE1, Ac147). To start the transcriptional cascade that initiates the baculovirus replication cycle, many baculoviruses employ the transcriptional activator, IE1. IE1 was originally identified because of its ability to transactivate early promoters of AcMNPV (29). It is transcribed early in infection (hence the name: immediate early gene 1, *ie1*) and continues to be transcribed through the late phase (30). Orthologs of IE1 have been identified in all Group I and II NPV genomes sequenced. They also appear to be present in GV genomes, but the similarity is very low, e.g., XcGVorf9 and Ac147 are about 10% identical. However, the orientation and position of XcGV orf9 relative to more conserved orfs is similar to Ac147, which suggests that Ac147 and XcGV orf9 are related. Part of the IE1 population is called IE0 and is translated from a larger spliced mRNA which is the only major spliced transcript described for baculoviruses (see below Ac147-0). In AcMNPV, either IE1 or IE0 can support viral replication (see below). AcMNPV IE1 contains two separate domains: an acidic activation domain located in the N-terminal region, and a DNA binding domain present in the C-terminal region (31, 32). It also contains a dimerization domain that is associated with a predicted helix-loop-helix sequence near the C terminus (21, 33). A positively charged domain adjacent to the dimerization domain is involved in nuclear import and is dependent on IE1 dimerization (34). A conserved 10-aa basic region (aa 152-161-KIKLRPKYKK) separating two acidic activation domains was found to be required for DNA binding. It was suggested that this basic region

neutralizes the adjacent acidic activation domains. In this model, when the basic region interacts with DNA, the acidic domains are exposed, allowing them to participate in activation (35). IE1 from a related virus, OpMNPV, has activation domains similar to AcMNPV IE1 (36) and a region in the 65 N-terminal amino acids that is involved in both transcriptional activation and DNA replication. The replication domain was specific to the virus and other sequences that supported transactivation did not support replication in an OpMNPV transient assay system (37). The ability of IE1 to transactivate transient transcription is greatly enhanced when the activated gene is linked to *hrs* (14). IE1 may also participate in the negative regulation of some genes (38) (see below). IE1 is required for transient DNA replication (39, 40) and is an essential gene as a bacmid deleted for this gene was not viable (41). Similar results have been reported for BmNPV (42).

Binding of IE1 to *hr* sequences

Although IE1 has been extensively investigated, purification of biologically active IE1 has not been reported. This suggests that it might be unstable and inactivated during purification procedures or that it is only active in combination with another protein(s). However, IE1 has been shown to bind to *hr* sequences independent of insect cell proteins. This was accomplished by transcribing the *ie1* gene in vitro, and then translating the resulting RNA in a rabbit reticulocyte system. The product was able to specifically bind to *hr* sequences indicating that additional insect cell proteins are not required for binding to occur (19, 43).

Many proteins, including type II restriction enzymes and numerous transcription factors, recognize and bind to palindrome sequences. Such proteins are normally found as homodimers and interact in such a way that the DNA binding site of each monomer recognizes the identical sequence, but on opposite sides of the palindrome. IE1 appears to follow this pattern in its interaction with the palindromes of *hr* sequences, and both halves of a palindrome are necessary for activation (21). Footprinting analyses have not been straightforward, which may be due to the presence of two types of binding to DNA. If mixtures of these two types of binding occur, especially when mutants are being examined, the actual pattern of binding may be obscured. However, foot printing of wt sequences suggests that IE1 interacts with both halves of the palindrome, but on opposite DNA strands as described above. Although altering a palindrome half-site by mutation prevents activation, IE1 still binds to the remaining site. However, the binding properties are changed, such that IE1 binds to both strands of the remaining half site (44). It was suggested that this could explain the mechanism by which IE1 negatively regulated the expression of certain genes that have an *hr* half-site sequence located between their TATA promoter and mRNA start site motif (38). By binding in this region, IE1 could prevent the binding and function of RNA polymerase II, thereby preventing transcription of the gene.

Despite their apparently critical role as transcriptional enhancers and probable role as origins of DNA replication, it is not clear how *hrs* are recognized by the proteins involved in these functions. It was suggested that they may form cruciform structures in the DNA that would provide landmarks for the binding of transcriptional activators. This has been observed in other palindrome-like structures, e.g., the high affinity binding of the cyclic AMP response element binding protein (CREB) to enkephalin enhancers (45). However, studies in vitro were unable to detect the formation of cruciform-like structures by AcMNPV *hr*-like imperfect palindromes, although such structures were formed by perfect palindromes in which mismatches in the predicted *hr* structure were eliminated. In addition, IE1 was able to bind to both these structures (46).

Splicing in AcMNPV; Ac147-0 (ie0) is a major spliced gene

Part of the IE1 population is called IE0 and is translated from a larger spliced mRNA which was the only major spliced transcript described for baculoviruses, and in AcMNPV results in an additional 54 amino acids at the N-terminus of IE1 for a total of 636 amino acids (47). In a survey of the AcMNPV transcriptome in infected *T. ni* cells, 11 spliced mRNAs in addition to IE1 were identified (48). However, only 3 of these sites were located within coding regions, the rest being in the 5' or 3' UTRs. For *ie1*, the spliced form is abundant early and

comprised over 80% of the ie1 mRNA at 6 hpi, but declined to 2% by 48 hpi. Other splice sites in reading frames included p47 that resulted in a reading frame change and in exon 0, however these were minor components. With the exception of IE1, most of the spliced mRNAs are minor components of the transcript population, however, the majority of the SOD and orf114 transcripts have spliced 3' UTRs that are present throughout the infection.

Another example of a spliced mRNA was reported from OpMNPV, in which a 2.8 kb intron was removed. This resulted in the fusion of Op18 mRNA downstream of its stop codon with Op21 downstream of the Op21 ATG. This mRNA would include all the Op18 orf and its stop codon and would be unlikely to produce a novel protein because of the location of a stop codon upstream of the splice site (49).

AcMNPV IE1 is present as a homodimer but also can form a heterodimer with IE0 that has been reported to suppress IE1 activation (28). Either IE1 or IE0 can support the production of infectious virus; however, there are subtle differences in the timing of events and of BV and polyhedra production depending on which gene is being expressed, suggesting that both IE0 and IE1 are required for wt levels of infection (41). In contrast, in LdMNPV, only the spliced form is able to transactivate transient transcription and DNA replication (50).

Two additional transcriptional activators

AcMNPV appears to encode two additional factors that may be involved in activating transcription. It is likely that both these proteins form homodimers, but unlike IE1, specific DNA binding sites have not been identified, suggesting that their activation may be based on their interaction with other proteins.

Ac151 (ie2/ie-n). Homologs of *ie2* are limited to Group I lepidopteran NPVs. IE2 contains a predicted RING finger domain indicating that it likely interacts with other proteins, and in *B. mori* has been found to interact with itself (51). IE2 was found to augment activation by IE1 (52-54) and was required for optimal origin-specific plasmid DNA replication when transfected into Sf-21 cells, but had little effect in Tn-368 cells (55). AcMNPV mutants deleted for *ie2* show a delay in infection in Sf-21. However, this delay was not observed in *T. ni* cells. In larvae of *T. ni* and *S. frugiperda*, the mutant viruses were significantly less infectious than wt which appeared to be due to the lack of virions in the occlusion bodies produced by the mutants. However, the intrahemocelic injection of mutant BV caused infections similar to wt (56). IE-2 may also be involved in cell cycle regulation (57).

Ac153 (pe38). The other potential transcriptional activator in AcMNPV is *Ac153*. Homologs of Ac153 have an unusual distribution being found in all Group I NPV and at least four GV sequenced genomes. Ac153 was originally identified because of its early transcription profile and the presence of predicted zinc finger and leucine zipper motifs. It was named after a cDNA clone that hybridized to a PstI/EcoRI fragment (58). Although it was not found to activate transient late transcription in one assay (59), evidence from OpMNPV indicated that it is a transactivator (60). When AcMNPV *pe38* was deleted, although the expression of several genes was unaffected, a number of others were delayed (61). This appears to delay the infection and results in a 99% reduction in BV production, reduced levels of DNA synthesis, and a reduction in oral infectivity in larvae (62). In addition, it appears to activate DNA replication in transient assays (39).

RNA polymerase II signals regulating early virus gene transcription

Upstream of baculovirus early genes are insect cell regulatory sequences including promoters that are recognized by the host cell RNA polymerase II and the associated transcriptional apparatus. The insect RNA polymerase II is able to initiate transcription from baculovirus promoters and even promoters that normally are transcribed by vertebrate cells such as the adenovirus major late promoter (63). Two major sequences have been identified in baculovirus early genes including a TATA promoter motif and a transcriptional initiation consensus sequence,

CAGT. Transient transcription of a minimal gp64 promoter was eliminated when either of these sequences was mutated, suggesting that they both are necessary for transcription (64). The CAGT sequence is apparently required for positioning the location where the mRNA sequence is started and can also act independently as an initiator. Initiators facilitate the assembly of the RNA polymerase II preinitiation complex and can promote transcription in the absence of a TATA sequence. This has also been demonstrated in baculoviruses for the *ie1* gene (65). Many baculovirus early genes have TATA promoter sequences along with consensus CAGT (or CATT — see below) transcriptional initiation sequences located about 30 bp downstream. These consensus sequences are two of the most prevalent motifs found in regulatory regions of genes from *Drosophila* (66). Several sequences have been identified both upstream and downstream of the promoter region that elevate early gene transcription; however, the mechanism underlying their function has not been characterized (67).

Genome-wide analysis of baculovirus promoters

A survey of predicted baculovirus promoter sequences was conducted on 26 baculovirus genomes (68). In this investigation, sequence patterns were characterized for the 300 bp of DNA upstream of the ATG initiation codons of baculovirus orfs. The significance of the prevalence of these predicted promoter sequences was determined by comparing their frequency in the 300-bp downstream of the ATG. There was a strong correlation for TATA sequences upstream of an ORF. Particularly significant were sequences such as TATAAGG that contain both TATA and late promoter sequences (ATAAG) in combination. These were about 7 times as frequent in the upstream location. Furthermore, about 70% of the TATA sequences present in the genome are clustered in the 100-bp upstream of the ATG. TATA sequences combined with mRNA start site consensus sequences CAGT or CATT separated by ~30-bp were 3 times as frequent in the upstream region. Similarly, putative initiator sequences (69) in combination with a TCGT motif ~30-bp downstream were almost 5 times more prevalent upstream of the ATG. Some other sequences such as GTAG, AGTC and TAGG were about 20 times more frequent in the promoter (upstream) region. They were often present in combination with early or late promoter elements, suggesting that they are involved in optimizing transcription from these sequences.

In a study of the transcriptome from AcMNPV infected *T. ni* cells (48), the sequences near the early transcription start site (TSS) had the following frequency: CAGT, 15%; CATT, 9%; and CAAT, 8.7%. In addition, the TSS was located ~32 nt downstream of the TATAA sequence.

For more information on transcriptional initiation sites, see Chapter 6.

Temporal expression of early and late genes (see Chapter 6).

How are baculovirus early genes activated?

IE1 can activate transcription, and the level of activation is increased many fold in the presence of *hr* enhancer sequences. In addition, *hr* sequences appear to be capable of binding to cellular factors that activate transcription. Since IE1 can activate transcription in the absence of *hrs*, it likely has the ability to interact with the RNA polymerase II-containing transcription complex and facilitate either the recruitment of other factors, or to elevate the levels of transcription itself. Since *hrs* appear to bind both cellular transcription factors and IE1, IE1 could recruit the *hr* bound factors to the RNA polymerase II complex (Figure 3). In this manner it would appear to be able to influence the rate at which RNA polymerase is recruited to or stabilized at a specific promoter and its subsequent ability to efficiently transcribe RNA.

Shutoff of early transcription

A feature of most baculovirus early genes is that they appear to be transiently expressed and are shut off at late times post-infection. The mechanism for this shutoff is not understood. Some early genes avoid being shut off early by having late in addition to early promoter elements. These genes can be continuously expressed through

both early and late times post-infection. In addition, some genes such as *ie1* appear to be transcribed throughout infection by an undetermined mechanism (30).

A mechanism for the regulation of RNA polymerase II entails the addition or removal of phosphates to its carboxyl terminal domain (CTD), and CTD phosphatases are involved in this process. Ac98 is a core gene and has homology to CTD phosphatases and is required for viral viability (70), may stimulate both early and late gene expression in transient assays (71) and dephosphorylates the p6.9 DNA binding protein which allows it to be packaged with the viral DNA (72). Dephosphorylation of the CTD can negatively regulate RNA polymerase II by inhibiting RNA elongation (73, 74). Although Ac98 may stimulate early transcription in a transient assay, this needs to be investigated in the context of viral infections as Ac98 itself could be regulated to selectively stimulate baculovirus early genes.

Caveats and qualifications

Despite the complexity of the structure of baculovirus virions and the presence of nonstructural proteins that are likely packaged with budded and occluded virions, it is possible that none of these proteins are required for the initiation of infection once the DNA is uncoated in the nucleus. Consequently, although many proteins have been reported to be associated with baculovirus virions, none have been shown to be required for the initiation of gene expression or DNA replication. Additional evidence for independence from virion proteins is the observation that purified viral DNA devoid of proteins is capable of initiating baculovirus infection when transfected into susceptible cells (75-78). Therefore, no viral proteins are required to initiate a baculovirus infection; however, this observation does not rule out the possibility that they facilitate the efficiency of the infection. In fact, IE1 was found to be associated with BV but not ODV of OpMNPV (79). In addition, a low level of a variety of late transcripts was observed at early times in the transcriptome of AcMNPV infected *T. ni* cells suggesting that they may have been transferred with the virion during infection (48). It is not clear whether they contribute to the initiation of the infection.

Another caveat regarding the molecular events involved in viral transcription and replication is that almost all this information is derived from AcMNPV, and to a lesser extent, the closely related BmNPV. Whereas much of this information is likely applicable to most baculoviruses, there are major differences, not only in gene content, but also in gene sequence homology between AcMNPV and many other baculoviruses. In addition, there are major differences in the number and distribution of *hr* enhancer sequences. All these factors likely have major, but currently unknown, influences on these events in other baculoviruses.

References

1. Kelly DC, Lescott T. Baculovirus replication: protein synthesis in *Spodoptera frugiperda* cells infected with *Trichoplusia ni* nuclear polyhedrosis virus. Microbiologica. 1981;4:35–47.
2. Nobiron I, O'Reilly DR, Olszewski JA. Autographa californica nucleopolyhedrovirus infection of Spodoptera frugiperda cells: a global analysis of host gene regulation during infection, using a differential display approach. J Gen Virol. 2003;84:3029–39. PubMed PMID: 14573808.
3. Ooi BG, Miller LK. Regulation of host RNA levels during baculovirus infection. Virology. 1988;166:515–523. PubMed PMID: 2459844.
4. Thiem SM. Baculovirus genes affecting host function. In Vitro Cell Dev Biol Anim. 2009;45:111–26. PubMed PMID: 19247726.
5. Katsuma S, Mita K, Shimada T. ERK- and JNK-dependent signaling pathways contribute to Bombyx mori nucleopolyhedrovirus infection. J Virol. 2007;81:13700–9. PubMed PMID: 17913811.
6. Chen YR, Zhong S, Fei Z, Gao S, Zhang S, Li Z, Wang P, Blissard GW. Transcriptome responses of the host Trichoplusia ni to infection by the baculovirus Autographa californica multiple nucleopolyhedrovirus. J Virol. 2014;88:13781–97. PubMed PMID: 25231311.

7. Popham HJ, Grasela JJ, Goodman CL, McIntosh AH. Baculovirus infection influences host protein expression in two established insect cell lines. J Insect Physiol. 2010;56:1237–45. PubMed PMID: 20362582.
8. Choi JY, Roh JY, Wang Y, Zhen Z, Tao XY, Lee JH, Liu Q, Kim JS, Shin SW, Je YH. Analysis of genes expression of Spodoptera exigua larvae upon AcMNPV infection. PLoS One. 2012;7:e42462. PubMed PMID: 22860129.
9. Schultz K, Friesen P. Baculovirus DNA replication-specific expression factors trigger apoptosis and shutoff of host protein synthesis during infection. J Virol. 2010;83:11123–32. PubMed PMID: 19706708.
10. Luque T, Finch R, Crook N, O'Reilly DR, Winstanley D. The complete sequence of the Cydia pomonella granulovirus genome. J Gen Virol. 2001;82:2531–47. PubMed PMID: 11562546.
11. Willis LG, Siepp R, Stewart TM, Erlandson MA, Theilmann DA. Sequence analysis of the complete genome of Trichoplusia ni single nucleopolyhedrovirus and the identification of a baculoviral photolyase gene. Virology. 2005;338:209–26. PubMed PMID: 15951000.
12. Thumbi DK, Eveleigh RJ, Lucarotti CJ, Lapointe R, Graham RI, Pavlik L, Lauzon HA, Arif BM. Complete sequence, analysis and organization of the Orgyia leucostigma nucleopolyhedrovirus genome. Viruses. 2011;3:2301–27. PubMed PMID: 22163346.
13. Guarino LA, Gonzalez MA, Summers MD. Complete sequence and enhancer function of the homologous DNA regions of *Autographa californica* nuclear polyhedrosis virus. J Virol. 1986;60:224–229. PubMed PMID: 16789259.
14. Guarino LA, Summers MD. Interspersed homologous DNA of *Autographa californica* nuclear polyhedrosis virus enhances delayed-early gene expression. J Virol. 1986;60:215–223. PubMed PMID: 16789258.
15. Leisy DJ, Rohrmann GF. Characterization of the replication of plasmids containing *hr* sequences in baculovirus-infected *Spodoptera frugiperda* cells. Virology. 1993;196:722–730. PubMed PMID: 8372444.
16. Pearson MN, Bjornson RM, Pearson GD, Rohrmann GF. The *Autographa californica* baculovirus genome: Evidence for multiple replication origins. Science. 1992;257:1382–1384. PubMed PMID: 1529337.
17. Pearson MN, Bjornson RM, Ahrens C, Rohrmann GF. Identification and characterization of a putative origin of DNA replication in the genome of a baculovirus pathogenic for *Orgyia pseudotsugata*. Virology. 1993;197:715–725. PubMed PMID: 8249294.
18. Pearson MN, Rohrmann GF. *Lymantria dispar* nuclear polyhedrosis virus homologous regions: characterization of their ability to function as replication origins. J Virol. 1995;69:213–221. PubMed PMID: 7983712.
19. Choi J, Guarino LA. The baculovirus transactivator IE1 binds to viral enhancer elements in the absence of insect cell factors. J Virol. 1995;69:4548–4551. PubMed PMID: 7769721.
20. Leisy DJ, Rasmussen C, Kim H-T, Rohrmann GF. The *Autographa californica* nuclear polyhedrosis virus homologous region 1a: Identical sequences are essential for DNA replication activity and transcriptional enhancer function. Virology. 1995;208:742–752. PubMed PMID: 7747446.
21. Rodems SM, Friesen PD. Transcriptional enhancer activity of *hr*5 requires dual-palindrome half sites that mediate binding of a dimeric form of the baculovirus transregulator IE1. J Virol. 1995;69:5368–5375. PubMed PMID: 7636981.
22. Nagamine T, Kawasaki Y, Iizuka T, Matsumoto S. Focal Distribution of Baculovirus IE1 Triggered by Its Binding to the hr DNA Elements. JVirol. 2005;79:39–46. PubMed PMID: 15596799.
23. Landais I, Vincent R, Bouton M, Devauchelle G, Duonor-Cerutti M, Ogliastro M. Functional analysis of evolutionary conserved clustering of bZIP binding sites in the baculovirus homologous regions (hrs) suggests a cooperativity between host and viral transcription factors. Virology. 2006;344:421–31. PubMed PMID: 16198391.
24. Theilmann DA, Stewart S. Identification and characterization of the IE-1 gene of *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus. Virology. 1991;180:492–508. PubMed PMID: 1989381.
25. Ahrens CH, Russell R, Funk CJ, Evans JT, Harwood SH, Rohrmann GF. The sequence of the *Orgyia pseudotsugata* multinucleocapsid nuclear polyhedrosis virus genome. Virology. 1997;229:381–399. PubMed PMID: 9126251.

26. Carstens EB, Wu Y. No single homologous repeat region is essential for DNA replication of the baculovirus Autographa californica multiple nucleopolyhedrovirus. J Gen Virol. 2007;88:114–22. PubMed PMID: 17170443.
27. Bossert M, Carstens EB. Sequential deletion of AcMNPV homologous regions leads to reductions in budded virus production and late protein expression. Virus Res. 2018;256:125–133. PubMed PMID: 30121325.
28. Ono C, Sato M, Taka H, Asano S, Matsuura Y, Bando H. Tightly regulated expression of Autographa californica multicapsid nucleopolyhedrovirus immediate early genes emerges from their interactions and possible collective behaviors. PLoS One. 2015;10:e0119580. PubMed PMID: 25816136.
29. Guarino LA, Summers MD. Functional mapping of a trans-activating gene required for expression of a baculovirus delayed-early gene. J Virol. 1986;57:563–57l. PubMed PMID: 3944847.
30. Kovacs GR, Guarino LA, Graham BL, Summers MD. Identification of spliced baculovirus RNAs expressed late in infection. Virology. 1991;185:633–643. PubMed PMID: 1962442.
31. Kovacs GR, Choi J, Guarino LA, Summers MD. Functional dissection of the *Autographa californica* nuclear polyhedrosis virus immediate-early 1 transcriptional regulatory protein. J Virol. 1992;66:7429–7437. PubMed PMID: 1433524.
32. Slack JM, Blissard GW. Identification of two independent transcriptional activation domains in the Autographa californica multicapsid nuclear polyhedrosis virus IE1 protein. J Virol. 1997;71:9579–87. PubMed PMID: 9371622.
33. Olson VA, Wetter JA, Friesen PD. Oligomerization mediated by a helix-loop-helix-like domain of baculovirus IE1 is required for early promoter transactivation. J Virol. 2001;75:6042–51. PubMed PMID: 11390606.
34. Olson VA, Wetter JA, Friesen PD. Baculovirus transregulator IE1 requires a dimeric nuclear localization element for nuclear import and promoter activation. J Virol. 2002;76:9505–15. PubMed PMID: 12186932.
35. Olson VA, Wetter JA, Friesen PD. The highly conserved basic domain I of baculovirus IE1 is required for hr enhancer DNA binding and hr-dependent transactivation. J Virol. 2003;77:5668–77. PubMed PMID: 12719559.
36. Forsythe IJ, Shippam CE, Willis LG, Stewart S, Grigliatti T, Theilmann DA. Characterization of the acidic domain of the IE1 regulatory protein from Orgyia pseudotsugata multicapsid nucleopolyhedrovirus. Virology. 1998;252:65–81. PubMed PMID: 9875318.
37. Pathakamuri JA, Theilmann DA. The acidic activation domain of the baculovirus transactivator IE1 contains a virus-specific domain essential for DNA replication. J Virol. 2002;76:5598–604. PubMed PMID: 11991988.
38. Leisy DJ, Rasmussen C, Owusu O, Rohrmann GF. A mechanism for negative gene regulation in *Autographa californica* multicapsid nuclear polyhedrosis virus. J Virol. 1997;71:5088–5094. PubMed PMID: 9188574.
39. Kool M, Ahrens C, Goldbach RW, Rohrmann GF, Vlak JM. Identification of genes involved in DNA replication of the *Autographa californica* baculovirus. Proc Natl Acad Sci USA. 1994;91:11212–11216. PubMed PMID: 7972036.
40. Lu A, Miller LK. The roles of eighteen baculovirus late expression factor genes in transcription and DNA replication. J Virol. 1995;69:975–982. PubMed PMID: 7815565.
41. Stewart TM, Huijskens I, Willis LG, Theilmann DA. The Autographa californica multiple nucleopolyhedrovirus ie0-ie1 gene complex is essential for wild-type virus replication, but either IE0 or IE1 can support virus growth. J Virol. 2005;79:4619–29. PubMed PMID: 15795248.
42. Gomi S, Zhou CE, Yih W, Majima K, Maeda S. Deletion analysis of four of eighteen late gene expression factor gene homologues of the baculovirus, BmNPV. Virology. 1997;230:35–47. PubMed PMID: 9126260.
43. Rodems SM, Pullen SS, Friesen PD. DNA-dependent transregulation by IE1 of Autographa californica nuclear polyhedrosis virus: IE1 domains required for transactivation and DNA binding. J Virol. 1997;71:9270–9277. PubMed PMID: 9371585.
44. Leisy DJ, Rohrmann GF. The Autographa californica nucleopolyhedrovirus IE-1 protein complex has two modes of specific DNA binding. Virology. 2000;274:196–202. PubMed PMID: 10936100.

45. McMurray CT, Wilson WD, Douglass JO. Hairpin formation within the enhancer region of the human enkephalin gene. Proc Natl Acad Sci USA. 1991;88:666–670. PubMed PMID: 1988963.
46. Rasmussen C, Leisy DJ, Ho PS, Rohrmann GF. Structure-function analysis of the *Autographa californica* multinucleocapsid nuclear polyhedrosis virus homologous region palindromes. Virology. 1996;224:235–245. PubMed PMID: 8862418.
47. Chisholm GE, Henner DJ. Multiple early transcripts and splicing of the *Autographa californica* nuclear polyhedrosis virus IE-1 gene. J Virol. 1988;62:3193–3200. PubMed PMID: 3043024.
48. Chen YR, Zhong S, Fei Z, Hashimoto Y, Xiang JZ, Zhang S, Blissard GW. The transcriptome of the baculovirus Autographa californica Multiple Nucleopolyhedrovirus (AcMNPV) in Trichoplusia ni cells. J Virol. 2013;87:6391–405. PubMed PMID: 23536684.
49. Pearson MN, Russell RLQ, Rohrmann GF. Transcriptional mapping of two genes encoding baculovirus envelope-associated proteins. J Gen Virol. 2002;83:937–943. PubMed PMID: 11907344.
50. Pearson MN, Rohrmann GF. Splicing is required for transactivation by the immediate early gene 1 of the Lymantria dispar multicapsid nuclear polyhedrosis virus. Virology. 1997;235:153–165. PubMed PMID: 9300047.
51. Imai N, Kang W, Iwabuchi K, Sato K, Maeda S. Analysis of interaction between molecules of Bombyx mori nucleopolyhedrovirus IE-2 using a yeast two-hybrid system. Acta Virol. 2000;44:199–202. PubMed PMID: 11155366.
52. Carson DD, Guarino LA, Summers MD. Functional mapping of an AcNPV immediate early gene which augments expression of the IE-1 trans-activated 39K gene. Virology. 1988;162:444–451. PubMed PMID: 3277328.
53. Carson DD, Summers MD, Guarino LA. Molecular analysis of a baculovirus regulatory gene. Virology. 1991;182:279–286. PubMed PMID: 2024466.
54. Yoo S, Guarino LA. The *Autographa californica* nuclear polyhedrosis virus *ie2* gene encodes a transcriptional regulator. Virology. 1994;202:746–753. PubMed PMID: 8030237.
55. Lu A, Miller LK. Differential requirements for baculovirus late expression factor genes in two cell lines. J Virol. 1995;69:6265–6272. PubMed PMID: 7666527.
56. Prikhod'ko EA, Lu A, Wilson JA, Miller LK. In vivo and in vitro analysis of baculovirus ie-2 mutants. J Virol. 1999;73:2460–8. PubMed PMID: 9971831.
57. Prikhod'ko EA, Miller LK. Role of baculovirus IE2 and its RING finger in cell cycle arrest. J Virol. 1998;72:684–92. PubMed PMID: 9420274.
58. Krappa R, Knebel-Mörsdorf D. Identification of the very early transcribed baculovirus gene PE-38. J Virol. 1991;65:805–812. PubMed PMID: 1987375.
59. Passarelli AL, Miller LK. Three baculovirus genes involved in late and very late gene expression: ie-1, ie-n, and lef-2. J Virol. 1993;67:2149–2158. PubMed PMID: 8445724.
60. Wu X, Stewart S, Theilmann DA. Alternative transcriptional initiation as a novel mechanism for regulating expression of a baculovirus *trans* activator. J Virol. 1993;67:5833–5842. PubMed PMID: 8371344.
61. Jiang SS, et al. Temporal transcription program of recombinant Autographa californica multiple nucleopolyhedrosis virus. J Virol. 2006;80:8989–99. PubMed PMID: 16940511.
62. Milks ML, Washburn JO, Willis LG, Volkman LE, Theilmann DA. Deletion of pe38 attenuates AcMNPV genome replication, budded virus production, and virulence in Heliothis virescens. Virology. 2003;310:224–34. PubMed PMID: 12781710.
63. Hoopes RR Jr, Rohrmann GF. *In vitro* transcription of baculovirus immediate early genes: Accurate mRNA initiation by nuclear extracts from both insect and human cells. Proc Nat Acad Sci USA. 1991;88:4513–4517. PubMed PMID: 2034691.
64. Blissard GW, Kogan PH, Wei R, Rohrmann GF. A synthetic early promoter from a baculovirus: Roles of the TATA box and conserved mRNA start site CAGT sequence in basal levels of transcription. Virology. 1992;190:783–793. PubMed PMID: 1519359.
65. Pullen SS, Friesen PD. The CAGT motif functions as an initiator element during early transcription of the baculovirus transregulator ie-1. J Virol. 1995;69:3575–83. PubMed PMID: 7745705.

66. Ohler U, Liao GC, Niemann H, Rubin GM. 2002. Computational analysis of core promoters in the Drosophila genome. Genome Biol 3:0087.1.
67. Friesen PD. 1997. Regulation of baculovirus early gene expression. *In* Miller LK (ed), The Baculoviruses. Plenum, NY.
68. Xing K, Deng R, Wang J, Feng J, Huang M, Wang X. Analysis and prediction of baculovirus promoter sequences. Virus Res. 2005;113:64–71. PubMed PMID: 15908030.
69. Hariharan MJ, Driver DA, Townsend K, Brumm D, Polo JM, Belli BA, Catton DJ, Hsu D, Mittelstaedt D, McCormack JE, Karavodin L, Dubensky TW Jr, Chang SM, Banks TA. DNA immunization against herpes simplex virus: enhanced efficacy using a Sindbis virus-based vector. J Virol. 1998;72:950–8. PubMed PMID: 9444987.
70. Wu W, Lin T, Pan L, Yu M, Li Z, Pang Y, Yang K. Autographa californica multiple nucleopolyhedrovirus nucleocapsid assembly is interrupted upon deletion of the 38K gene. J Virol. 2006;80:11475–85. PubMed PMID: 16987976.
71. Hefferon KL. ORF98 of Autographa californica nucleopolyhedrosisvirus is an auxiliary factor in late gene expression. Can J Microbiol. 2003;49:157–63. PubMed PMID: 12795401.
72. Lai Q, Wu W, Li A, Wang W, Yuan M, Yang K. The 38K-Mediated Specific Dephosphorylation of the Viral Core Protein P6.9 Plays an Important Role in the Nucleocapsid Assembly of Autographa californica Multiple Nucleopolyhedrovirus. J Virol. 2018.:92. PubMed PMID: 29444944.
73. Yeo M, Lin PS. Functional characterization of small CTD phosphatases. Methods Mol Biol. 2007;365:335–46. PubMed PMID: 17200573.
74. Yeo M, Lin PS, Dahmus ME, Gill GN. A novel RNA polymerase II C-terminal domain phosphatase that preferentially dephosphorylates serine 5. J Biol Chem. 2003;278:26078–85. PubMed PMID: 12721286.
75. Burand JP, Summers MD, Smith GE. Transfection with baculovirus DNA. Virology. 1980;101:286–290. PubMed PMID: 18631639.
76. Carstens EB, Tjia ST, Doerfler W. Infectious DNA from *Autographa californica* nuclear polyhedrosis virus. Virology. 1980;101:311–314. PubMed PMID: 16945844.
77. Potter KN, Miller LK. Transfection of two invertebrate cell lines with DNA of *Autographa californica* nuclear polyhedrosis virus. J Invertebr Pathol. 1980;36:431–432.
78. Kelly DC, Wang X. 1981. The infectivity of nuclear polyhedrosis virus DNA. Annals of Virology (Institute Pasteur) 132 E:247-259.
79. Theilmann DA, Stewart S. Analysis of the *Orgyia pseudotsugata* multcapsid nuclear polyhedrosis virus trans-activators IE-1 and IE-2 using monoclonal antibodies. J Gen Virol. 1993;74:1819–1826. PubMed PMID: 8376961.

5. DNA replication and genome processing

Cells encode all of the genes necessary for the replication of their own DNA. Viruses that infect these cells can either exploit this apparatus for their DNA replication, or they can independently encode some or all the genes involved in this process. However, it is likely that most viruses that replicate in nuclei use a combination of host and virally encoded proteins. Determining the viral factors involved in DNA replication provides fundamental information on how viral DNA synthesis is accomplished. In addition, because so much is known about the proteins involved in DNA replication in other systems, the identification of proteins that are encoded by the virus can lead by inference to proteins that are likely contributed by the host cell.

Some questions are fundamental to understanding viral DNA replication. These include: i) the identification of sequences, called origins of replication, that specify where DNA replication begins; ii) the identification of gene products involved in DNA replication; iii) understanding the role of these proteins in DNA replication; iv) understanding how the DNA is replicated; and v) understanding how the DNA is incorporated into nucleocapsids. Rapid advances in the first three of these areas were made for baculoviruses by the application of approaches developed for similar studies in other viruses. However, these methods often employ transient assays that involve the transfection of combinations of cloned viral DNA into insect cells, and although they have yielded significant information, they do not result in the production of mature, functional genomes. Below is a summary of the current information in this area.

Identification of origins of viral DNA replication

When a DNA virus that replicates in nuclei infects a cell, it must distinguish its DNA from that of the host in order to facilitate specific replication of viral DNA, rather than that of the host. One method of distinguishing viral DNA from host DNA is the presence of unique viral DNA sequences that specify sites for the initiation of DNA synthesis. These sequences are called origins of replication. By directing the synthesis of a protein that recognizes a viral replication origin, a virus can specify DNA sequences that will be replicated.

Two approaches have been employed to identify viral origins of DNA replication. In one, plasmids that contain viral DNA are transfected into virus infected cells. To distinguish newly replicated DNA from input DNA, a restriction enzyme (DpnI) is used because it cuts at a site that is methylated in the input plasmid DNA (derived from bacteria), but not at that site in DNA that has replicated in insect cells because it is not methylated. Therefore, if a plasmid is replicated in insect cells, it is not methylated at its DpnI sites and therefore is resistant to DpnI and is not digested by the enzyme. The other approach is to pass the virus at high multiplicity of infection (moi). This results in the enrichment of defective genomes with major deletions that replicate rapidly due to their small size. They rely on the presence of complete genomes with a full complement of replication genes to support their replication. The defective genomes are often very small and are enriched for replication origins because these sequences are preferentially amplified and can, in some instances, evolve to become the most prevalent sequences produced after extended high moi viral passage. For AcMNPV both these approaches identified homologous regions (*hrs*, see Chapter 4) as causing the infection-dependent replication of plasmids (1, 2). However, using the same two techniques, non-*hr* sequences that acted as origins of replication were also identified (3, 4). Subsequently, early promoters were also identified as having origin activity (5). Using another type of assay based on PCR of DNA extracted from infected cells, both a non-*hr* origin and an early promoter were shown to have origin activity (6). Consequently, although *hr* and non-*hr* origin sequences have been examined in some detail, it is still not clear which, if any, of these sequences is the preferred site of replication initiation in vivo.

It is possible that DNA replication may initiate at any sequences that become unwound, thereby providing an entry point for the replication complex. The baculovirus transcriptional transactivator IE-1 binds to *hr* sequences and may be involved in facilitating entry at these sites. Likewise, RNA polymerase II would cause unwinding at early promoter sequences. Non-*hr* origins may provide sequences of less specificity that are

involved in DNA unwinding. However, understanding the role of these sequences is complicated by a lack of understanding of the structure of baculovirus replication intermediates. All available information suggests that baculovirus DNA is replicated either by a rolling circle mechanism (7, 8) or one that includes extensive recombination or a combination of both these mechanisms (9). If recombination-dependent replication and rolling circle mechanisms are major contributors to baculovirus genome amplification, then origins of replication ultimately may play a minor role in this process. Once genome replication is initiated, subsequent amplification might be independent of specific sequences.

Defective interfering particles and DNA replication

Defective interfering (DI) viruses (10) are commonly produced by many viruses as a consequence of passage in cell culture, especially at high multiplicity. They consist of populations that have varying amounts of their genomes deleted including at least one essential gene thereby rendering them defective. However, they retain the signals that are required for genome replication and packaging. Since they are non-infectious they depend on the presence of helper viruses to encode the missing genes required for their replication. The DI populations often predominate and can result in severe declines in viral titers. It is thought that they out compete (interfere with) the wt virus replication because their genomes are smaller and thus are more efficiently replicated and consequently can greatly outnumber the helper virus. The presence of DIs in baculovirus populations has been well documented and in two instances, the characterization of DIs led to the identification of putative origins of DNA replication. Restriction enzyme analysis of viral DNA isolated after 40 serial passages resulted in seven supermolar *Eco*RI fragments that hybridized to sequences flanking *hrs* (2). This indicated that *hrs* were selectively retained in defective genomes. In similar studies (3, 11), defective genomes analyzed after 81 passages were found to be heterogeneous in size with the majority migrating at about 50 kb as determined by pulsed-field gel electrophoresis. However, in contrast to the results of (2), these defective genomes appeared to retain multiple repeats of a non-*hr*–containing sequence of less than 2.8 kb derived from the HindIII-K region of the AcMNPV genome. The HindIII-K region spans portions of p94 and p35 genes (12). Subsequently it was observed in SeMNPV, that if a non-*hr* origin was deleted, it enhanced the stability of a recombinant bacmid after serial passaging (13). In another study, the transposon insertion sites in the fp25k gene region were altered. This resulted in not only a reduction in the FP phenotype, but also led to a delay in DI particle formation. It was suggested that DIs are formed by a process that involves the insertion of transposons followed by deletion of sequences between the inserted transposons and this may be an important step in DI formation (14).

Genes required for DNA synthesis

The genes involved in baculovirus DNA synthesis were originally identified by a combination of the characterization of temperature sensitive mutants (15) and by transient replication assays (16, 17). Transient replication assays involve the transfection of regions of the viral genome along with a plasmid containing a replication origin and the use of the DpnI digestion assay described above. Starting with enough of the genome to give positive replication, subsequent experiments involve the elimination of sequences until a minimal set of genes is identified. Since the original experiments were conducted on AcMNPV before the complete genome sequence was published, the final set of plasmids was sequenced which allowed identification of the essential genes. Six genes were identified that are essential for transient DNA replication and several others were found to be stimulatory. The essential genes include *dnapol*, *helicase (p143)*, *lef-1 (late expression factor-1)*, *lef-2*, *lef-3* and *ie1* (Table 1). In addition to *dnapol* and *helicase*, these genes have the following functions: LEF-1, primase; LEF-2, primase accessory factor; LEF-3, single stranded DNA binding protein (SSB); and IE-1, unknown but it may bind to origins of replication. Homologs of the first four have been identified in all sequenced baculovirus genomes (18). The lack of homologs of *lef-3* and *ie1* does not necessarily imply their absence in evolutionary distant genomes. It could reflect the incorporation of so many changes that the homology of the primary sequences can no longer be identified. This set of genes is similar to those identified as being required for herpes simplex 1 transient DNA replication (19) (Table 1). In both these viruses it is likely that host enzymes also

participate in this process. The similarity of the complement of herpes virus and baculovirus replication and other genes has led to the suggestion that these virus are members of a lineage distinct from other large DNA viruses of eukaryotes (20).

Initiation of DNA replication in well-characterized systems such as *E. coli* is an orderly process that begins with an origin binding protein interacting with an origin of replication and unwinding this region, thereby allowing access by a helicase that induces more extensive unwinding allowing access by the DNA polymerase. Many of the factors involved in baculovirus DNA replication are similar to those found in other organisms and are described below starting with IE1 that has properties somewhat similar to an origin binding protein (also see Figure 1).

Table 1. Viral genes essential for DNA replication

Gene	Baculovirus	Herpesvirus
DNA pol	+ (Ac65)	+ (UL30)
Ssb	+ (LEF-3) (Ac67)	+ (ICP8)
primase	+ (LEF-1) (Ac14)	+ (UL52)
Primase accessory factor	+ (LEF-2) (Ac6)	+ (UL8)
helicase	+ (p143) (Ac95)	+ (UL5)
Origin binding protein	+ IE1 (?) (Ac147)	+ (UL9)
Processivity factor	-	UL42

IE1 (Ac147). IE1 has been identified in all NPV genomes sequenced from the Lepidoptera and also appears to be present in GV genomes, but the homology is very low. Homologs have not been identified in the hymenopteran and dipteran virus genomes that have been sequenced (18). As described in Chapter 4, IE1 is a transcriptional activator that binds to *hr* sequences. The identification of *hrs* as putative replication origins and of IE1 as an *hr* binding protein would suggest that IE1 is an origin binding protein and might serve as a marker for enucleating the assembly of a replication complex. This would allow viral DNA to be distinguished from host DNA and would lead to the specific replication of viral sequences. However, the identification of non- *hr* sequences that also act as replication origins and to which IE1 does not appear to specifically bind, suggests that IE1 might not be an origin binding protein in all cases. It was observed that the N-terminal 23 residues of AcMNPV IE1 are involved in specifically binding DNA replication origins, but was not required for transcriptional activation. Furthermore, a conserved cyclin-dependent kinase phosphorylation motif was observed within this 23-amino sequence that was required for IE1 phosphorylation. Mutagenesis of this motif inactivated the ability of IE1 to mediate DNA replication (21). Because IE1 acts as a transcriptional transactivator of baculovirus early genes, and since all the viral genes involved in DNA replication are early genes, IE1 could act as a transactivator of all these genes. Without transactivation by IE1, the replication genes might not be synthesized to a high enough concentration for transient DNA replication to be detected.

As described above, although IE1 may be an origin binding protein, it should be noted that a host protein may be involved as it was observed that the *B. mori* origin recognition complex subunit 2 had the highest phosphorylation ratio (5.69) of all the cellular proteins during BmNPV infection (22) suggesting that it may be involved in the infection.

LEF-3 (Ac67), a single-stranded DNA binding protein (SSB). A feature of DNA replication systems is a dependence on the presence of SSBs. SSBs bind tightly to ssDNA and prevent the formation of secondary structure. They also prevent the renaturation of ssDNA and may inhibit non-specific interactions of ssDNA with other proteins. They bind in a cooperative manner and can interact with replication proteins.

AcMNPV LEF-3 was identified as being required for DNA replication and was subsequently characterized as an SSB (23). It is a 385 aa protein with a molecular weight of about 44.5 kDa. LEF-3 homologs are present in all lepidopteran baculovirus genomes, but have not been reported in baculoviruses from Hymenoptera or Diptera (18). A LEF-3 homolog may very likely be present in these viruses, but its primary amino acid sequence may have evolved to such an extent that it is not readily identified. For example, the putative XecnGV LEF-3 and AcMNPV LEF-3 show only 14% aa sequence identity.

In addition to being an SSB, LEF-3 has a number of other roles. LEF-3 interacts with alkaline nuclease and may regulate the function of this enzyme (24, 25); it is capable of both unwinding and annealing DNA depending on its concentration or redox state (26, 27); and it can facilitate the production of recombination intermediates via strand exchange between donor and recipient molecules in vitro (28). LEF-3 binds to other LEF-3 molecules which may facilitate its function. LEF-3 also interacts with helicase and is required for its transport into nuclei (29). The nuclear localization signal was localized to about 5 amino acids and was also found to facilitate nuclear transport in mammalian cells (30). LEF-3 is also required for DNA replication independent of this role (31). This feature was localized to the N-terminal 125 amino acids (32).

Helicase (p143) (Ac95). DNA helicases are critical enzymes involved in DNA replication. They are motor proteins that move directionally and unwind DNA by disrupting the hydrogen bonds between bases in the double helix. This exposes the bases so that complementary DNA strands can be synthesized. Energy provided by the cleavage of ATP by an ATPase domain in helicase is required for strand separation.

A helicase homologue was identified in an AcMNPV ts mutant that was defective in DNA replication (15). Helicase was subsequently found to be required for DNA replication in transient assays and was shown to have the biochemical properties of a DNA helicase with both ATPase and helicase activities, and the capacity to bind ss and dsDNA (33). As described above, helicase interacts with LEF-3 and this could facilitate the interaction of helicase with ssDNA.

A DNA primase, LEF-1 (Ac14) and a primase-associated factor, LEF-2 (Ac6). Once a segment of DNA is unwound and stabilized by its interaction with SSBs, DNA replication can be initiated. However, DNA polymerases are unable to independently initiate the synthesis of a new strand of DNA; they can only elongate from an -OH group on an existing strand. Therefore, a short sequence of oligonucleotides that serves as a primer must be synthesized first. Primers are generated by a category of RNA polymerases called primases. This process results in hybrid molecules containing small regions of RNA linked to DNA. Eventually, the RNA primer is removed and replaced by DNA.

In AcMNPV, LEF-1 and LEF-2 were originally identified as factors required for DNA replication in the transient assay system. Subsequently it was found that LEF-1 interacts with LEF-2 (34). It was originally suspected that LEF-1 was a DNA primase because it contained a conserved primase domain (WVVDAD). When this domain was altered to WVVQAD, transient DNA replication activity was eliminated (34). Purified LEF-1 was found to have primase activity, and products of several hundred nucleotides or more were synthesized when M13 ssDNA was employed as a template. Elution profiles of LEF-1 and LEF-2 from ssDNA cellulose and DEAE resin suggested that LEF-2 may bind to both DNA and LEF-1. Although it is essential for transient DNA replication, the exact role of LEF-2 has not been clearly defined (35).

Based on limited amounts of DNA synthesis by a *lef-2* bacmid knockout in transfected cells, it was suggested that Lef-2 is not required for the initiation of DNA replication. This was in contrast to a bacmid with the helicase gene deleted that showed no synthesis (36). However, the data in this report could be interpreted in a different manner. The transfected DNA is likely nicked, and therefore, the ends of the nicks could act as primers, resulting in limited amounts of leading strand DNA synthesis in the absence of an active primase complex. A control with the helicase deleted showed no DNA synthesis, indicating that it is required for all DNA synthesis.

Phylogenetic analysis suggests that LEF-1 and LEF-2 are members of a primase lineage common to archaea and eukarya that is distinct from the primase lineage of a number of other large eukaryotic DNA viruses (37).

Baculovirus primers. In contrast to leading strand synthesis, lagging strand synthesis of double-stranded DNA is not continuous and results in the synthesis of non-contiguous oligomers called Okazaki fragments that fill in the single-stranded region generated as the leading strand is synthesized (Figure 1). These relatively short oligomers are transient because they become ligated to the lagging strand. When short-lived DNA intermediates were characterized in *S. frugiperda* cells, fragments of 80–200 bp were observed. These are similar in size to the Okazaki fragments observed in other eukaryotic cells. However, when these intermediates were characterized in AcMNPV infected cells, they were much larger (0.2–5.0 kb) with an average of 1–2 kb. This is similar in size to what has been observed in prokaryotes, bacteriophage, and herpes virus and may reflect a lower frequency of priming than the host cell. This would result in longer fragments being synthesized to span the distance between the primed regions (38).

DNA polymerase (Ac65). As described above, DNA polymerases extend DNA from the 3' -OH of an RNA primer in a 5' to 3' direction. This results in the synthesis of a leading strand that can continue indefinitely. However, synthesis of the second or lagging DNA strand must occur in the opposite direction of the leading strand in order to progress in a 5' to 3' direction. As the leading strand is synthesized, additional DNA is exposed on the complementary strand. Consequently, whereas leading strand synthesis can be continuous, lagging strand synthesis occurs in segments called Okazaki fragments.

DNA polymerases often have associated exonuclease activities. A 3' to 5' exonuclease activity allows the removal of newly synthesized DNA in the reverse direction of synthesis. This activity is associated with proofreading and allows the polymerase to correct mistakes as it is synthesizing DNA. This may cause DNA polymerase to repeatedly shuttle between polymerizing and editing modes (39). The other activity involves a 5' to 3' exonuclease and is used for the removal of RNA primers. This would be particularly important in lagging strand synthesis because the extended strands would invariably collide with the RNA primer of the upstream Okazaki fragment as they synthesize DNA in the direction opposite of the replication fork. Some DNA polymerases such as DNA pol I of *E. coli* have both 3’ to 5’ and 5’ to 3’ exonuclease activities so they can proofread and repair newly synthesized DNA and also remove RNA primers. However, many DNA polymerases lack the 3' to 5' activity.

All DNA viruses that infect animal cells appear to encode their own DNA polymerases, except for some with small genomes (polyoma, papilloma and parvoviruses). It has been suggested that this benefits the virus because there may be insufficient levels of the cellular DNA polymerase in infected cells since they are not dividing, and therefore, are not in S phase (40). In fact it has been shown that the mRNA and protein levels of several DNA polymerases increase by three-fold or more during S phase (41). However, the benefits of viruses encoding their own polymerases could also be due to the isolation of viral DNA replication to specific foci within nuclei, or simply that it is more efficient to have the DNA polymerase under regulatory control of the virus so that its expression can be coordinated with the infection. A baculovirus specific DNA polymerase was originally characterized from *Bombyx mori* infected with BmNPV (42). Subsequently, a gene with homology to DNA polymerase was identified in the AcMNPV genome using hybridization of primers to conserved DNA polymerase sequences (43). This gene is most closely related to members of the DNA polymerase B family. Enzymes in this family synthesize both leading and lagging strands of DNA and have a high degree of fidelity that is associated with a strong 3' to 5' exonuclease activity. The enzymes from BmNPV and AcMNPV were shown to have a 3’ to 5’ exonuclease activity (42, 44, 45), and therefore likely have the capacity to proofread newly synthesized DNA and remove DNA sequences if defects such as mismatching are detected. In contrast, a 5' to 3' exonuclease activity was not observed (45). Since this activity is associated with removal of the primer used for the initiation of DNA synthesis, it is not clear how primers are removed during baculovirus DNA replication (see below).

An unexpected observation described in one report employing transient assays for the identification of baculovirus replication genes suggested that DNA polymerase was not essential, since significant levels of DNA replication (12%) were observed in the absence of *dnapol* (17). This led to the suggestion that it might be stimulatory rather than essential and that the host DNA polymerase may be involved in initiating baculoviral DNA replication. Furthermore, it was observed that in transient replication assays the AcMNPV DNA polymerase could be substituted with the ortholog from OpMNPV (46) or even from an ascovirus (47). This further suggested that the DNA polymerase may be interchangeable in the context of the other AcMNPV replication proteins. However, when the DNA pol gene was deleted from a bacmid containing the AcMNPV genome, no viral DNA replication was observed (48). This suggested that the DpnI resistant DNA generated in the absence of DNA polymerase in the transient assays could be due to repair of the origin containing plasmid caused by the other baculovirus genes and a host cell DNA polymerase.

Additional genes that influence DNA replication

A variety of other genes have been identified that influence DNA replication, but their role in this process has not been characterized. These include the following:

DBP (Ac25), A second SSB. AcMNPV encodes two proteins that possess properties typical of SSBs, LEF-3, and a protein referred to as DNA-binding protein (DBP). Homologs of dbp are found in all sequenced baculovirus genomes except that of the dipteran (CuniNPV), and in some instances, multiple copies of the dbp gene are present. It has properties similar to LEF-3 in that it interacts with itself and is capable of both unwinding and annealing DNA (49). DBP was also able to compete with LEF-3 for binding sites on ssDNA templates and protected ssDNA against hydrolysis by a baculovirus alkaline nuclease (AN)/LEF-3 complex. It is an essential gene, as bacmids lacking Ac25 were non-infectious and appeared to produce defective nucleocapsids; however, unlike LEF-3 it is not required for transient DNA replication. Although not a virion structural protein, fractionation studies indicated that DBP is tightly associated with subnuclear structures, suggesting that it is a component of the virogenic stroma (49). It was found to localize to the virogenic stroma by immuno-electron microscopy and when *dbp* was deleted from an AcMNPV bacmid, cells transfected with this construct appeared to lack a virogenic stroma and failed to produce normal-appearing nucleocapsids. This suggested that *dbp* is required for the production of nucleocapsids and the virogenic stroma (50). In addition, although viral DNA synthesis occurred in cells transfected with the *dbp* knockout, the levels were less than that of the control virus, indicating that DBP may be required for normal levels of DNA synthesis, or for stability of nascent viral DNA. Analysis of the viral DNA replicated by the *dbp* knockout by pulsed-field gel electrophoresis resulted in DNA of apparent high molecular weight that is retained in the wells of the gel and fragments that are shorter than the full-size viral genome (50). The DNA fragments might represent degradation products of viral genomes or replicative intermediates. Two different activities of DBP might be responsible for these observations. First, DBP may prevent the enzymatic degradation of viral genomes. It has been shown that DBP inhibits hydrolysis of DNA by the proofreading activity of phage T4 DNA polymerase (51) or by the nuclease activity of the AN/L3 complex (49). Therefore, DBP may protect mature viral genomes against nucleases and stabilize them at stages that precede packaging into virions. On the other hand, the unwinding and renaturation activity of DBP may be required for processing of replicative intermediates by annealing and strand invasion reactions involved in DNA recombination that may be essential for the complete replication and processing of baculovirus genomes (9, 52, 53).

LEF-11 (Ac37). Homologs of LEF-11 are present in all baculovirus genomes except the dipteran CuniNPV. No homology to known proteins associated with DNA replication was identified in the LEF-11 sequence. It was found to stimulate late gene transcription, but was not required for transient DNA replication (17). However, an AcMNPV bacmid deleted for lef-11 was unable to synthesize DNA or carry out late gene transcription when transfected into Sf-9 cells (54). In BmNPV, oligomerization of LEF-11 is required for DNA replication (55). Although LEF-11 localizes to nuclei of infected cells, its role in DNA replication is not known (54). BmNPV

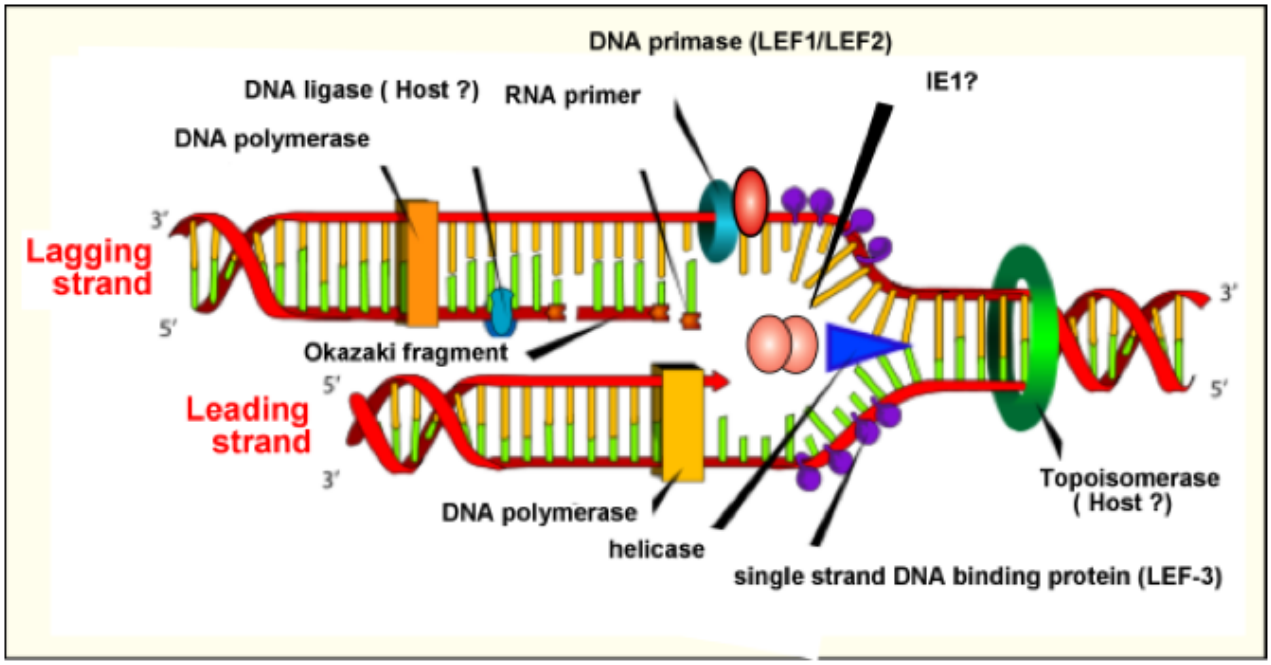

Figure 1. AcMNPV DNA replication. This diagram shows a hypothetical relationship of the baculovirus replication factors along with several factors, DNA ligase and topoisomerase, that are likely contributed by the host. IE1 is also shown, although its role is unknown. Diagram courtesy of and modified from a figure by Mariana Ruiz.

LEF-11 interacts with and upregulates both a host ATPase and HSPD1 (HSP60) proteins and this appears to facilitate viral DNA replication (56). BmNPV LEF-11 has a novel nuclear localization signal (57, 58)and appears to interact with host importin α-3 and within nuclei it co-localizes with IE-1 and interacts with LEF-3(57).

Ac139 (ME53). Homologs of Ac139 are present in the genomes of all lepidopteran baculoviruses. AcMNPV bacmids deleted for this gene are not viable, fail to replicate their DNA, and do not produce nucleocapsids. However, cells transfected with DNA from the deletion mutant showed early stages of cytopathogenic effects including nuclear enlargement and the formation of granular material in the nucleus (59). No homology to known proteins associated with DNA replication was identified in the Ac139 sequence.

Lef-7 (Ac125). Lef-7 is stimulatory for transient DNA replication (17, 60). When deleted, infection was unaffected in Tn368 cells, but in Sf21 and *S. exigua* cells DNA replication was 10% of wt (61). When deleted from BmNPV, a reduction in DNA synthesis was also observed (62). Homologs are present in most Group I, a few group II NPVs, and a few GV genomes. LEF-7 was found to be involved in the regulation of the DNA damage response (DDR). It is an F-box protein that interacts with host S-phase kinase-associated protein 1 (SKP1). SKP1 is a component of a complex that interacts with and targets proteins for polyubiquitination. Deletion of *lef-7* from the AcMNPV genome resulted in the accumulation of phosphorylated H2AX and activation of the DDR that led to a major reduction in late gene expression and reduced infectious virus production by 100-fold. It was suggested that LEF-7 may interfere with the phosphorylation of H2AX thereby diverting host DDR proteins from cellular chromatin, so that they can be exploited for viral DNA replication (63).

Proliferating cell nuclear antigen (PCNA) (Ac49). Ac 49 has homology to proliferating cell nuclear antigen (PCNA). PCNA homologs have been found in the genomes of a few Group I and Group II lepidopteran NPVs and appear to have been derived from two different lineages (see Chapter 12). Although eukaryotic PCNA lacks an enzymatic function, it plays a role in DNA synthesis, DNA repair, and cell cycle progression. It functions as a sliding circular clamp that mediates protein interactions with DNA and is required for the coordinated synthesis

of both leading and lagging strands at the replication fork during DNA replication (64). In AcMNPV it is not an essential gene (65, 66) and did not appear to elevate DNA replication in transient replication assays (16). However, it did appear to be involved in the timing of late gene expression (67). Over expression under the control of the p10 promotor of EGFP fusions of AcMNPV-PCNA and host cell Sf-PCNA has been examined in Sf9 cells. Both stimulated BV production and DNA replication of both host and virus and Ac-PCNA elevated transcription of selected late genes and increased the larval mortality rate (69).

Baculovirus DNA replication genes: What's missing?

Topoisomerase and DNA ligase. Based on the general model of DNA replication, AcMNPV encodes most of the genes involved in this process (Figure 1). However, there are two major factors that have not been identified. These include a topoisomerase and a DNA ligase. As helicases advance and unwind DNA they cause an increase in the number of twists or topoisomers that accumulate in the DNA in advance of the enzyme. This can cause a barrier to the advance of the helicase. This problem is alleviated by topoisomerases that break and rejoin DNA strands thereby allowing the DNA helix to unwind and relieving the barrier to helicase progression. Integrase homologs from other viruses show topoisomerase activity (70). Therefore, it was thought that VLF-1, a homolog of integrase encoded by all baculovirus genomes, may be involved in this process; however, purified VLF-1 showed no topoisomerase activity (71).

Evidence implicating host cell topoisomerase and DNA ligase. Analysis of data of AcMNPV infected *T. ni* cells from (72), shows that DNA ligase 1 and topoisomerase 1b and -II transcripts increased by a factor of over 1.3 by 6 hr pi, stayed relatively high through 12 hr pi and then declined by about 75% at 18 hr and were down to 3-6% of the 6 hr level at 48 hr pi. This is in contrast to the bulk of the host transcripts that had declined by about 50% by 13 hr pi (72). This suggests that AcMNPV is capable of stimulating host cell topoisomerase and ligase transcription early in infection such that they can be exploited for viral genome replication. It has also been shown in BmNPV infected *B. mori* cells that the host cell DNA topoisomerase II is phosphorylated with a ratio of 1.43 compared to uninfected cells (22).

Dealing with Okazaki fragments: two DNA ligases and a second helicase. DNA ligase is necessary for the ligation of Okazaki fragments to one another. For viruses lacking a DNA ligase homolog, it is assumed that a host enzyme supplies this function (see above). However, ligase homologs are present in most granulovirus genomes and at least two NPV genomes (LdMNPV and MacoNPV-B). These ligases are most similar to ligase III that is involved in DNA repair. The DNA ligase of LdMNPV has been shown to have enzymatic activity and is able to form a covalent link with (α32P)ATP and to ligate double-stranded synthetic DNA substrates containing a single nick, suggesting that it would be capable of ligating Okazaki fragments. It was not required or stimulatory for DNA replication in transient replication assays, although these assays may not be dependent upon ligase activity (73). A striking feature of the baculovirus ligase homologs is that they are almost always (except MacoNPV-B) accompanied with a helicase homolog that is not found in any of the genomes lacking ligase. This helicase is related to the PIF1 family (73) (note: this is not a per os infectivity factor). Members of this family have a preference for RNA-DNA hybrids and could be involved in the maturation of Okazaki fragments (74). This may involve displacement of the RNA primer producing an RNA flap that would then be cleaved by a flap endonuclease (FEN) (75) or digested by a 5' to 3' exonuclease. Interestingly, the genome of a GV encodes a fusion of helicase 2 and alkaline nuclease (76) suggesting that the helicase may unwind overlapping RNA and DNA hybrids and the alkaline nuclease might remove the RNA although its ability to digest single strand RNA has not been tested. DNA polymerase would then fill in the gap by extending the Okazaki fragment and the ligase could join the fragments. Another enzyme that may be a candidate for a DNA ligase in some viruses is Ac105 (also called HE65). Computer predictions indicate that it contains a domain homologous to eukaryotic DNA ligases. Homologs of Ac105 are present in some Group I and II NPVs and at least two GVs. Therefore, although there are at least two candidate ligases, it is not clear what role they play in baculovirus DNA replication. Ac105 is probably non-essential as insertion/deletion of this gene in BmNPV (Bm89) had no

apparent effect on infectivity (77). Vaccinia virus also encodes a DNA ligase most closely related to ligase III. When it is deleted, the virus was still capable of replication and the host cell ligase I was implicated in this process (78).

Rationale for the composition of baculovirus replication genes

As described above, baculoviruses and herpes viruses encode many, but not all, genes that are likely to be involved in DNA replication. To understand the evolution of these systems, it is important to determine the theoretical rationale for the genes that these viruses encode. A main reason for encoding replication genes rather than using host genes, would be to separate the viral genome replication from dependence on the host cell. Since most host genes are likely shut down upon infection (79) or are present in low concentration because the cells are not dividing and therefore are not replicating their DNA, encoding a replication system independent of host cell control may be of compelling importance to the virus. However, this still does not explain the pattern of replication genes that are virally encoded. As shown in Table 1, these two DNA viruses encode a DNA polymerase, an SSB, and a primase. The presence of the SSB may be due to a requirement for its abundant expression, because of all the replication proteins, it may have to be present in the highest concentration (Figure 1). In addition, the presence of virally encoded DNA polymerase/primase may also be due to the need for optimal concentrations higher than the host cell can provide — especially if the host is stalled at the G2/M stage by the viral infection (see Chapter 7). In particular, each nucleotide polymerized would have to be manipulated by the polymerase. Likewise, each nucleotide of the DS DNA would have to be separated by the DNA helicase. In contrast, the lack of the necessity for a viral encoded ligase could be due to it being required only rarely, especially if long Okazaki fragments are produced as may be the case (see above) (38). Under these circumstances, the low levels of ligase present in the host cell may be sufficient. In addition, if the viral DNA is nicked and not covalently closed during initial replication, a topoisomerase activity may not be required to relieve torsional stress during replication of the virus genome. However, it would be necessary for the production of the final covalently closed supercoiled DNA that is packaged into virions. Whether this requires only low levels of a host enzyme remains to be determined.

Location of baculovirus DNA replication; development of the virogenic stroma

As described in Chapter 3, the virogenic stroma is the site of viral genome replication and nucleocapsid assembly. Several investigations have examined the viral gene products involved in the formation of this structure. IE-1 appeared to localize to specific foci before DBP and LEF-3. After DNA replication begins, the foci enlarge and occupy over half the nucleus and DBP, IE-1, and LEF-3 along with newly replicated DNA co-localize to this region. When DNA replication was inhibited with aphidicolin, foci containing all three proteins were present at early times post infection, but were not as uniform as in the absence of the drug. The number of IE-1 foci appears to be restricted to about 15, suggesting that there are a limited number of preexisting sites where DNA replication could be initiated. It was suggested that these might be equivalent to nuclear domain 10 (ND10) sites found in mammalian cells (80). ND10 are sites of concentrations of proteins involved in a variety of cellular processes, and these sites are often associated with virus assembly (81, 82). Similar data on the localization of replication proteins has been described by others, and it was suggested that IE2 may also be associated with these sites (83). Subsequently, it was found that *hr* sequences were sufficient to cause IE1 to form foci (84) and that the presence of the viral DNA helicase was necessary for LEF-3 to localize to these structures (85). This is consistent with the requirement of LEF-3 for the transport of helicase into nuclei described above (29). In addition, it was suggested that the replication factors DNA helicase, LEF-3, IE1 along with *hr* sequences are all that is required to produce foci capable of recruiting other replication factors (85). Studies on the relationship of the IE1 foci with virion structural proteins indicated that ODV-E25 (Ac94) and vp91 (Ac83) localize to the periphery of these structures, whereas vp39 was found within the IE1-associated foci (86). It has

also been suggested that IE1 interacts with another structural protein BV-ODV-E26 (Ac16) and serves to recruit it to replication sites (87). Using fluorescent-tagged histone H4, the effect of baculovirus infection on this histone was examined. It was found to relocate to the margins of infected nuclei and appeared to be excluded from the viral replication compartment. This marginalization of histone H4 could also be induced by ie1, lef3, p143-helicase and an hr (88).

Additional baculovirus genes: hints of DNA repair

Although, as described above, the 3' to 5' exonuclease activity of the baculovirus DNA polymerase suggests that it has the ability to proofread DNA as it is synthesized, there do not appear to be other DNA repair systems common to all baculoviruses. However, there are enzymes produced by a limited set of baculoviruses that suggest that some viruses may encode additional pathways for repairing their DNA. This suggests that viruses lacking these pathways may be able to co-opt the homologous proteins from the host cell.

Photolyase. A common threat to occluded baculoviruses in the environment is their inactivation by UV light (89). UV light causes crosslinking of adjacent pyrimidine residues. These crosslinked dimers can cause the DNA to bend and this may inhibit the ability of the DNA replication complex to copy beyond the damaged site or cause the incorporation of incorrect nucleotides. This may result in lethal mutations or the inhibition of the interaction of proteins involved in gene regulation. Photolyases are enzymes that bind to the site of such mutations and, after being activated by light, can catalyze the separation of the mutant dimer, thereby correcting the mutation. Homologs of photolyase genes have been found in the genomes of Group II baculovirus that are members of a lineage that infects insects of the subfamily Plusiinae of the family Noctuidae (90) (91) (92). *Chrysodeixis chalcites* encodes two photolyase genes that are predicted to encode proteins with 45% amino acid sequence identity. When tested, one copy showed photoreactivating activity, whereas the other copy did not (93). Transfection of egfp fusions of photolyase genes into *T. ni* cells resulted in fluorescence localized to chromosomes and spindles and other structures associated with mitosis. Baculovirus infection of the transfected cells caused fluorescence to localize to the virogenic stroma (94). Evidence suggests that they are associated with mitotic structures (94) and may be involved in circadian clock regulation (95). The incorporation of an algal virus photolyase gene as a means to cause resistance to UV inactivation of AcMNPV has been described. However, although BV survival was increased after exposure to UV light, occluded virion survival was not (96). An active photolyase is also encoded by some poxviruses (97).

dUTPase. Deoxyuridine triphosphate (dUTP) can be mutagenic if incorporated into DNA. The enzyme dUTPase dephosphorylates dUTP to dUMP, which is a substrate for thymidine biosynthesis. Homologs of dUTPase are present in many NPVs and at least one GV genome (98). Baculoviruses may have incorporated this gene to either supplement or substitute for the host gene. The viruses that encode a *dUTPase* homolog also normally encode both subunits of ribonucleotide reductase (RR). The presence of RR may have selected for the incorporation of *dutpase* to mitigate the production of the dUTP mutagen by ribonucleotide reductase.

Viral three-prime repair exonuclease (v-trex). A gene with homology to 3' to 5' exonucleases from other systems has been identified in at least two baculovirus genomes. The enzyme from both AgMNPV and CfMNPV demonstrated 3' to 5' exonucleolytic activity (99, 100). These enzymes would appear to carry out proofreading functions similar to those employed by the DNA polymerase. However, it has been suggested that trex proteins may associate with DNA polymerase and increase the fidelity of DNA replication under conditions that cause error-prone nucleotide polymerization (39).

Ac79, a member of the UvrC endonuclease superfamily? It has been suggested that Ac79 is a member of the UvrC superfamily of endonucleases that are involved in DNA repair (101). Homologs of Ac79 are present in all group I and about half group II lepidopteran NPV and also in a few GV genomes. It has homology to orfs found in other insect viruses and a variety of bacteria. It is predicted to be homologous to an endonuclease in a number of these organisms. When Ac79 was deleted from AcMNPV, titers were reduced and plaque sizes were smaller,

however differences in DNA replication and protein synthesis and occlusion body production were not observed, but the quantity of infectious virions appeared to be reduced and aberrant capsid-like structures were observed (102). In another study it was found to be associated with AcMNPV ODV (103).

PARP. A homolog of poly (ADP-ribose) polymerase (PARP) is found in one baculovirus genome (AgMNPV – Ag31) (104) (105). PARP is an enzyme found in nuclei that is activated by DNA strand breaks and uses NAD+ as a substrate to synthesize polymers of ADP-ribose on acceptor proteins that are involved in the repair of single-strand breaks in DNA by activating and recruiting DNA repair enzymes. It is also involved in telomere elongation, chromatin structure, and the transcription of a variety of genes involved in immunity, stress resistance, hormone responses, and the possible silencing of retroelements (106). It may also be involved in the regulation of a mitochondrial protein that induces apoptosis (107). PARP is a caspase-3 substrate and its cleavage is used as a measure of apoptosis.

PARG (Ac114). Poly (ADP-ribose) glycohydrolase (PARG) is an enzyme that reverses the products produced by PARP (108). Homologs of Ac114 are found in most Group I NPV genomes. Hhpred analysis (109) indicates that it is a Poly(ADP-ribose) glycohydrolase (PARG) with almost 100% probability. This appears to be a Group I PARG that was not previously identified. The original baculovirus PARG is specific to Group II baculoviruses. The Group I and II PARGs show low levels of relatedness (e.g. AcMNPV and LdMNPV are less than 15% identical) and appear to represent two different lineages of this enzyme. PARG catalyzes the hydrolysis of glycosidic (1'–2') linkages in poly(ADP-ribose) to produce ADP-ribose (110). Therefore, whereas PARP stimulates a variety of processes (see above), PARG reverses the products of PARP. In AcMNPV it appears to be an ODV (103, 111) and BV associated protein (112) and in HaSNPV, PARG (Ha100) it was ODV associated (113). When deleted in a BmNPV bacmid, the resulting virus appeared similar to wt (77), whereas a deletion mutant of HaSNPV was similar to wt except the lethal time was longer and the LC50 was higher than wt (114).

Nicotinamide riboside kinase 1 (NRK1). Orthologs of NRK1 are found in most group II NPVs and in at least 5 GVs. It plays a role in nicotinamide adenine dinucleotide (NAD+) synthesis. It phosphorylates nicotinamide riboside yielding nicotinate mononucleotide (115). Since PARG reverses the ADP-ribosylation of proteins by PARP and NRK1 is part of the nicotinamide adenine dinucleotide pathway, it is possible that the presence of PARG and NRK1 in many group II baculoviruses is indicative of their ability to manipulate these processes – possibly with NRK1 participating in the salvage of ADP-ribose generated by PARG.

Genes involved in nucleotide biosynthesis

Most baculoviruses do not encode genes involved in nucleotide biosynthesis. However, many Group II and several GVs encode both subunits of ribonucleotide reductase. In addition, these same viruses normally also encode dUTPase (see above).

Ribonucleotide reductase. Ribonucleotide reductase is a heterodimer composed of large and small subunits (RR1 and RR2, respectively). It is involved in the catalysis of ribonucleotides to deoxyribonucleotides as a pathway for providing nucleotides for DNA synthesis. Well-documented RR1 and RR2 genes have been reported in the genomes of at least three GVs, 10 Group II, and a single Group I (OpMNPV) NPVs (98). Two different RR2 genes have been reported for LdMNPV (116). Based on the phylogeny of baculovirus RR1 genes, it is postulated that two different capture events resulted in baculoviruses obtaining this gene (117). For the OpMNPV and LdMNPV RR1 gene lineage, the source was a bacterium, whereas the other lineage (e.g., Spodoptera exigua MNPV (SeMNPV)) appears to have been derived from a eukaryote, most likely an insect. The two RR2 genes from LdMNPV appear to be derived independently, one from each different source rather than via gene duplication. No enzymology has been described for baculovirus RR and it is not known whether they have enzymatic activity, or how they integrate with or substitute for the homologous host enzymes.

Baculovirus infection and the DNA damage response (DDR)

The DNA of individual human cells encounters tens of thousands of lesions each day. If they are not repaired correctly, they can result in mutations incorporated into the cell genome with subsequent threats to the viability of the organism. In additions to errors during replication such as mismatch incorporation or abortive strand breaks introduced by topoisomerase, other factors such as reactive oxygen or nitrogen species, or environmental toxins are major causes of DNA damage. It has been estimated that DNA damage events can total 100,000 per day per cell and that a day in the sun can cause an additional 100,000 lesions per exposed cell per hour. Furthermore, uv induced inflammation can also cause high levels of oxidative damage [reviewed in (118) (119) (120)]. Consequently, a complex system called the DNA damage response (DDR) has evolved to identify and address this damage.

The DNA damage response is a surveillance network maintained in eukaryotic cells that monitors the integrity of their DNA. When DNA damage is detected, the DDR pathway is activated to prevent mutations from being permanently incorporated into DNA due to double or single-strand breaks, or stalled replication forks. A major feature of the DDR is the phosphorylation of a type of histone that acts as signal for the DDR. Histones form protein spools around which DNA is wound, and there are 5 classes, H1, H2A, H2B, H3 and H4. H2AX is a variant of histone H2A and comprises 2-25% of the H2A population. In humans it is 13 amino acids longer than histone H2A.1 and this extension contains a conserved serine that is located 4 amino acid residues from the carboxyl terminus (121). In response to double stranded DNA breaks, this serine is rapidly and massively phosphorylated by members of the phosphatidylinositol-3 kinase-like kinase family (PIKK). The site of phosphorylation may comprise thousands of nucleosomes spanning up to 2 megabases of chromatin surrounding the DNA break and marks them for repair via the DDR. This can lead to the recruitment of an extensive array of repair-related proteins that can immobilize the ends of the break and facilitate their repair [reviewed in (122)].

The outcome of the DDR can be a pause in the cell cycle, repair of the DNA, and the resumption of the cell cycle. However, if the damage it too extensive, it can lead to apoptosis and the death of the cell. The DDR provides a variety of challenges and opportunities for viruses and several have been shown to be dependent upon it for full levels of replication (123). There may be compelling reasons for viral exploitation of this response. Since virus genomes are limited in size and, although larger viral genomes may encode genes involved in DNA replication, they are still dependent on the host cell for a variety of enzymes involved in these processes. As described above, most baculovirus do not encode enzymes required for nucleotide biosynthesis and lack DNA ligases and topoisomerases. Consequently, the activation of the DNA damage response may provide a variety of enzymes that viruses require for their successful replication.

Several studies have investigated the relationships with baculoviruses and the DDR. It was found that the DDR appeared to be activated by factors associated with viral DNA replication, and inhibition of the DDR resulted in up to a 10^5 fold reduction in BV titers (124) (125). The tumor suppressor gene, p53 is a regulator of apoptosis in many systems. It was found that AcMNPV infection caused the accumulation of P53 and also the phosphorylation of H2AX, the signal for DDR described above. It was observed that despite the accumulation of p53 under conditions of both DNA damage and AcMNPV infection, silencing p53 did not affect the induction of apoptosis suggesting that it might not be absolutely required for activating apoptosis in Sf9 cells (124).

In another study (125), the induction of the DDR in the non-permissive *Drosophila* cell system was examined. They observed that the DDR was induced by baculovirus DNA replication and H2AX was phosphorylated. They found that activation of the DDR also promoted apoptosis. In contrast, in permissive *S. frugiperda* cells, they found that the phosphorylation of H2AX was delayed and did not occur until 24 h pi. They suggest that this indicates that the virus might manipulate the activation of the DDR in novel ways to facilitate its replication. Subsequently they found that LEF-7 was involved in the regulation of the DDR. LEF-7 appears to be an F-box protein that interacts with host S-phase kinase-associated protein 1 (SKP1). SKP1 is a component of a complex that interacts with and targets proteins for polyubiquitination. Deletion of *lef-7* from the AcMNPV genome

resulted in the accumulation of phosphorylated H2AX and activation of the DDR that led to a major reduction in late gene expression and also reduced infectious virus production by 100-fold. They suggested that LEF-7 may interfere with the phosphorylation of H2AX thereby diverting host DDR proteins from cellular chromatin, so that they can be exploited for viral DNA replication (63).

How are baculovirus genomes replicated?

Whereas some genes have been identified that are required for transient DNA synthesis and major advances have been made in understanding the function of most of these genes, it is still not clear how baculovirus genomes are replicated. Evidence suggests that baculovirus replication results in DNA that is larger than unit length genomes. This DNA could be produced by rolling circle replication, DNA recombination, or by a combination of both these processes. The evidence for these two processes is described below.

Rolling circle replication. It was originally suggested that baculovirus genomes replicate by a rolling circle type mechanism because plasmids that were replicated in the transient system showed a ladder of multiples of unit length-sized DNA fragments when they were partially digested by a restriction enzyme with a single site in the plasmid (7). It is not clear how closely the plasmid reflects the viral genome replication because of its much smaller size and minimal complexity. However, evidence has been presented suggesting that AcMNPV might replicate its genome in this manner (8). Furthermore, it was found that plasmid DNA, when co-transfected with AcMNPV DNA, replicated to become high-molecular weight concatemers, some of which were integrated at a variety of locations in the viral genome. This was interpreted to suggest that both rolling circle replication and recombination may be involved in baculovirus DNA replication (5).

Recombination-dependent replication. Baculovirus replication induces a highly recombinogenic state (52, 53, 126, 127), and this contributed to their development as expression vectors because foreign genes could be so readily incorporated into the genomes by homologous recombination. Recombination-dependent replication is a complex yet common mode by which many viruses replicate their DNA. It is unclear why systems have evolved this mode of replication, but evidence for it playing major roles in genome replication is found throughout DNA viruses with large genomes. T4 phage begins DNA replication in an origin-dependent manner and then switches to a recombination-dependent mode (128). Lambda phage also provides an important model for recombination-dependent replication. Lambda DNA replication initially is of the theta type in which DNA is synthesized bi-directionally from a replication origin initially producing a bubble-like structure that progresses to resemble the Greek letter theta. However, later in infection, concatemers are generated by either rolling circle replication or recombination. Lambda encodes a recombination system called the Red system after mutations that were found to be *re*combination *d*efective. This system includes *red* α, β and γ that encode an exonuclease, an SSB, and an inhibitor of a host recombination system, respectively. RED α, the exonuclease, digests DNA in a 5' to 3' direction thereby generating 3' overhangs that anneal with complementary strands or invade homologous double strands; RED β, the SSB, facilitates annealing of DNA strands (129). In addition, RED α and β interact forming heterodimers. There is a complex interaction between the host and phage recombination systems. A lambda-type phage, P22, is dependent on recombination and if both the host and phage recombination systems are inactivated, the phage will not replicate. Viability can be restored by the incorporation of the Red system into P22 (130).

It is thought that the replication of herpes virus genomes is recombination-dependent. DNA isolated from herpes simplex virus 1 (HSV-1) infected cells has a nonlinear, apparently branched structure, and much of it will not enter a pulsed-field gel even after digestion with a restriction enzyme that cuts at a single site in the genome. In addition, SV40 DNA normally employs theta replication, but yields complex high MW DNA resembling HSV-1 DNA when replicated in an HSV-1 dependent system (131). HSV-1 encodes a gene (UL12) homologous to the lambda *red* α exonuclease. The HSV-1 exonuclease also interacts with an SSB (UL29) (132). The two

HSV-1 proteins facilitate strand exchange in a manner similar to the lambda Red system (133). When the HSV-1 exonuclease gene is inactivated, the production of infectious virions is severely compromised (134-136).

Similar to herpes virus, baculoviruses produce high molecular weight DNA that fails to enter a pulsed-field gel. When digested with an enzyme that cuts at a single site in the viral genome, much of the DNA is retained in the well. Also similar to herpes viruses, when SV40 is replicated in a baculovirus system, complex, high-molecular weight DNA is produced rather than products of theta replication (137). In addition, all baculoviruses encode a homolog of the lambda *red* α exonuclease called alkaline nuclease (AN). It forms a stable complex with an SSB, LEF-3, and possesses both a 5'->3' exonuclease and endonuclease activity (24, 25). LEF-3 has been shown to facilitate strand exchange in vitro (28). These activities are consistent with the AN-LEF-3 complex being involved in DNA recombination. An AcMNPV bacmid with the AN gene deleted did not produce infectious virions after transfection into Sf9 cells. Also, although DNA replication levels appeared to be normal, much of the DNA generated appeared to be significantly smaller than observed in the control. These data demonstrated that *an* is an essential baculovirus gene (138) and suggested that it may be involved in the generation of larger than genome length fragments consistent with its role in a recombination system (9). In one baculovirus, the AN gene was found fused to a helicase gene suggesting that the two genes may function together during baculovirus replication (76).

In summary, replication of baculovirus DNA has properties similar to other systems. These include the generation of what appear to be structurally complex DNA molecules that are larger than genome length, and the expression of two proteins, an exonuclease and an SSB that interact with one another and have the properties of a system that is involved in DNA recombination in phage and other eukaryotic viruses. Furthermore, in viruses from phage to herpes viruses, homologs of the exonuclease are present and in all cases they interact with an SSB.

Implications of recombination-dependent replication: Multiple replication origins, a covalently closed circular genome, and multiple nucleocapsids per envelope

If recombination plays a major role in baculovirus DNA replication, it could explain several features unique to baculoviruses. These could include the possible use of multiple replication origins. Such origins could greatly amplify the amount of viral DNA that can be produced in a given time. The constraints of identifying and initiating replication at a specific site would be avoided, and replication could originate simultaneously at a number of sites. Upon recombination, molecules destined to become genomes would be produced that could begin or end anywhere as long as they were greater than genome length. In addition, the production of covalently closed circular DNA could be mediated by a final recombination event in which two homologous areas near the ends of a greater than unit length linear replication intermediate are joined (Figure 2). Whereas insect cells likely have extensive DNA recombination and repair systems, this final event in baculovirus DNA processing may be crucial for the production of an infectious virus. It is also possible that the propensity for some lepidopteran viruses to produce multiple nucleocapsids in a single envelope may facilitate recombination and repair of those that might be damaged (139). Such damage could occur during their production, after they are released, or during infection in the midgut or within cells.

Processing and packaging of genome-size DNA

Although recombination-based replication appears to solve several problems that are confronted by the replication of baculovirus genomes, recombination in combination with secondary initiation of replication might result in complex branched structures that would likely need to be resolved into covalently closed circular genomes of unit length before they could be packaged. The mechanism leading to the resolution of these

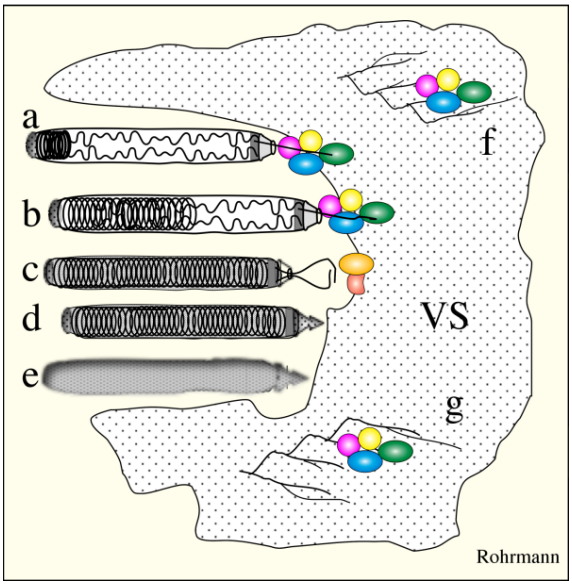

Figure 2. Theoretical diagram of different stages of DNA replication and packaging. Shown is replication coordinated with packaging (a and b) and DNA replication that is independent of packaging showing extensive recombination (f,g). The large stippled area is the virogenic stroma (VS). The circularization of the genome by recombination is also indicated (c). Mature virions are represented by (d) and (e).

structures is not clear. In addition to the extensive effects that alkaline nuclease and LEF-3 may have on genome processing, another protein, VLF-1 may also be involved.

Very late factor 1 (VLF-1) (Ac77). VLF-1 has been implicated in genome processing although its exact role is not clear. It is a member of the lambda integrase (Int) family of proteins. Integrases are a large group of site-specific DNA recombinases that catalyze DNA rearrangements and are found in a variety of organisms including viruses where they are involved in the integration and excision of viral genomes and decatenation of newly replicated chromosomes. A feature of these enzymes is that a conserved tyrosine forms a covalent link with DNA during the cleavage process. VLF-1 homologs are found in all sequenced baculovirus genomes suggesting that it plays a critical role in baculovirus biology. VLF-1 likely has two different functions in baculovirus biology. It is involved in the hyperexpression of very late genes (140) (see Chapter 6) and it also may have another function, independent of transcription, involved in genome processing.

An AcMNPV bacmid with *vlf-1* deleted appears to have normal levels of DNA replication but fails to produce infectious virus. Aberrant tube-like capsids are produced by this mutant and appear to lack DNA. Furthermore, viral DNA in this mutant appeared to be completely accessible to DNAse (141). In contrast, other mutants with defective capsid production (e.g., a deletion of alkaline nuclease) showed significant levels of DNase protection (~35%) (Vanarsdall and Rohrmann, unpublished), suggesting that VLF-1 is required for DNA protection. When the bacmid with *vlf-1* deleted was rescued with a copy of VLF-1 that carries a mutation of the highly conserved tyrosine (Y355F), it restored the production of nucleocapsids with a normal appearance, but they were not infectious. Furthermore, the mutant appeared to be defective in the movement of nucleocapsids out of the virogenic stroma, suggesting that without active VLF-1, a final virion maturation step was blocked, possibly leaving the capsids tethered to the virogenic stroma by incompletely processed, larger than unit length DNA. Finally, VLF-1 appears to localize to an end region of the nucleocapsid. Collectively, these results indicate that VLF-1 is required for normal capsid assembly and serves an essential function during the final stages of the DNA packaging process (141-143).

In addition to evidence suggesting that VLF-1 is required for both proper capsid and genome structure, VLF-1 is capable of binding to several types of DNA structures that may further suggest that it has a possible role in DNA

processing. It shows high affinity binding to cruciform DNA that mimics a structure common to recombination intermediates. No binding was evident to single and double stranded structures, and very low binding was observed to Y-shaped forks (71). These results are consistent with the involvement of VLF-1 in the processing of branched DNA molecules at the late stages of viral genome replication. The ability of VLF-1 to bind homologous region (hr) sequences was also examined and it was found to bind to hrs that form hairpin and H-shaped structures. Although VLF-1 is capable of binding to branched DNA structures, enzymatic activity (endonuclease and topoisomerase) was not detected, suggesting that enzymatic activity of VLF-1, if present, may depend on accessory cellular or viral factors.

DNA packaging and nucleocapsid assembly. Early in the infection, DNA replication may result in the rapid amplification of DNA sequences that are used as templates for the expression of late genes that encode a variety of proteins, including those involved in capsid structure. However, once capsids begin to be assembled and the concentration of viral DNA increases, genome production may be coordinated with the packaging of DNA into preformed capsids. The preassembly of capsids has been suggested by electron microscopy (144) and is a common feature of some large ds DNA viruses. However, genes that affect DNA replication and processing also appear to affect nucleocapsid structure, suggesting that DNA is an integral component of nucleocapsid structure and without it, the capsid itself cannot assemble properly. A good example is the VLF-1 knockout described above that produced empty tube-like capsids. Similar defects are observed with a deletion of the DNA binding protein, DBP (50).

Packaging DNA into a preformed capsid is an energetically unfavorable process and requires an ATP-driven packaging motor. Packaging motor complexes include a channel for the insertion of the DNA into the capsid and a set of enzymes that can compress the genome through the channel into the capsid. In tailed bacteriophage, the channel is composed of a ring-shaped portal structure embedded in the capsid. This forms a point for the nucleation of DNA packaging enzymes. Genome packaging motors of dsDNA viruses are comprised of a pair of components that are not part of the virion structure; the larger component binds to the procapsid, while the smaller component binds to DNA (145, 146) (147). The motor protein associated with genome packaging of lambda phage has an endonuclease activity indicating that it may be involved in both packaging and DNA processing (148).

DNA recognition can be involved in packaging in two ways: a specific sequence may be recognized to initiate DNA packaging, and it may contact the motor complex during packaging. The specific sequence would ensure that only viral DNA would be packaged. Upon packaging within the capsid, DNA is concentrated up to 100-fold, often as a spool of concentric rings, so that it becomes highly condensed to near liquid crystalline density (149). Viruses with concatemeric precursor DNA use either a 'headful' mechanism to measure DNA incorporation or utilize specific recognition sequences that bracket a complete genome. The cleavage at these sequences results in the excision of a complete genome sequence. It has been suggested that the 'headful' mechanism may cause a conformational change in the portal region as the head becomes full, thereby triggering a mechanism to terminate packaging.

Energy for packaging can be derived either from the hydrolysis of ATP or by an electrochemical potential (proton gradient) generated across a membrane. Motor proteins contain active sites that bind ATP and catalyze its cleavage to ADP and Pi, releasing energy that causes a conformation change in the protein, thereby driving the motor. Each cleaved ATP molecule can result in a processive, ratchet type movement driving the DNA into the capsid. In T4, the motor has a packaging rate of 700–2000 bp/sec with a force of >60 pN, one of the most powerful motors documented (150). If similar, it would take less than 5 min to package the AcMNPV genome.

A candidate for a motor protein that might be involved in DNA packaging is Ac66. Homologs of *ac66* appear to be present in all baculoviruses. It is related to a variety of motor proteins including myosin heavy chain, a centromere protein, and Smc, a chromosome segregation ATPase that is involved in cell division. Hhpred, the structure prediction program, (109) suggested it was related to formins that are involved in actin polymerization

and are associated with the fast-growing end of actin filaments. Ac66 was found to be associated with AcMNPV and HearNPV ODV (103, 113). However, an *ac66* knockout bacmid, although not viable, appeared to be normal and produced nucleocapsids with an electron dense core, suggesting that they contained DNA (151). It has been noted that during infection, actin that is normally in the cytoplasm is transported to the nucleus (152) and interacts with structural components of the virion (153). In addition, reagents that block actin polymerization may result in the production of aberrant capsids that appear to lack DNA (154). Collectively, these observations indicate that components of the cytoskeleton could be involved in the insertion of DNA into nucleocapsids.

It has been suggested that a protein called vp1054 (ac54) may be related to a cellular protein called PURα that binds to purine-rich sequences and may be involved in DNA packaging. It was found to be capable of binding single strand DNA or RNA sequences that contained runs of GGN. Therefore it was suggested that it might interact with the orf1629/p/78/83 (ac9) sequence which encodes a series of prolines and therefore is rich in GGN sequences (155).

DNA signals for packaging or processing

Two investigations have identified DNA sequences that may be involved in the regulation of genome packaging or processing. In one investigation, it was observed that an AT-rich conserved non-protein-coding element (CNE) located in the ac152 region of AcMNPV appears to be an essential *cis*-acting sequence for virion production (156). In the other report, a nucleocapsid assembly element (NAE) was located within Ac83, a virion structural protein (157). These sequences were found only in Alphabaculoviruses and appeared to have a conserved position relative to one another.

Coordinating DNA replication and packaging: A process for avoiding DNA recombination? A common feature of investigations that identify proteins associated with ODV is the presence of some proteins involved in DNA replication (103, 113). This association may reflect remnants of macromolecular complexes that are 'frozen' in association with nucleocapsids by the occlusion process. Therefore, the presence of these replication-associated proteins with the nucleocapsid could reflect a highly coordinated set of reactions, including DNA synthesis, processing, and packaging in close proximity to the nucleocapsids. The insertion of DNA into nucleocapsids as it is synthesized could protect the partially packaged DNA from strand invasion or nuclease attack (Figure 3). In addition, it is possible that DNA replication is partitioned such that DNA destined for genome production is highly coordinated with packaging to prevent extensive recombination.

Unpackaged DNA is required for very late transcription. Is it the basis for very late gene hyperexpression? The coordination of DNA synthesis with packaging would protect the nascent genomic DNA from random recombination. However, evidence suggests that there is a large component of DNA that is not packaged as genomes. This DNA would be free to recombine and be destined to become templates for gene expression, particularly for very late hyperexpressed genes involved in occlusion body formation. These genes are transcribed after genomic DNA is packaged and inaccessible to transcription (Figure 3) and would be lost after occlusion is completed. Therefore, a major contribution to the ability of baculoviruses to hyperexpress very late genes may be dependent upon the gene copy number required for the massive production of polyhedrin and p10 after genomic DNA is packaged. This is a theory, but in one set of experiments it was observed that late in infection over half the viral DNA in a cell is DNAse sensitive, suggesting that it was not packaged into nucleocapsids (141). Therefore, if this DNA is accessible to the baculovirus RNA polymerase, it could provide a high copy number of very late genes to serve as templates for mRNA synthesis.

A problem with the model of coordination of genome replication with packaging to prevent recombination? A problem with this model of two types of DNA - circular genomic DNA destined for packaging, and the highly branched DNA required for hyperexpression of very late genes model is presented by the ability of the DNA to readily undergo recombination in the laboratory for the production of recombinant virions for gene expression studies. This suggests that DNA destined for viral genome production is not protected from recombination.

However, recombinant genomes may be relatively rare under normal replication conditions. In contrast, recombinant production in the laboratory normally involves co-transfection of high concentrations of both viral and target DNA. Under these conditions, recombination might be favored because the DNA is not packaged or in a natural form when it enters nuclei.

More unanswered questions

The lack of genome isomerization. Similar to baculoviruses, herpes simplex virus I (HSV-1) and other herpes viruses are highly recombinogenic (reviewed in (133, 158)). During HSV-1 replication, intra-genomic inversions between two repeated elements within the genome result in a population of four different genome isomers. Since most baculovirus genomes appear to be punctuated with homologous repeated sequences that are distributed throughout their genomes (e.g., AcMNPV has 8 *hrs*, see above and see Chapter 4, Figure 2) it is surprising that their genomes are replicated with such fidelity. It is clear that inversions do occur as there are examples of inversions bracketed by *hrs* between different viruses. For example, AcMNPV orfs 1–10 are bordered by *hr*1 and *hr*1a and are inverted relative to the homologous sequences in the *Orgyia pseudotsugata* MNPV genome (159). However, there is no evidence that major populations of isomers are packaged into virions during normal virus replication similar to what occurs in herpes viruses. Restriction enzyme digestion of baculovirus genomic DNA results in a single characteristic pattern for each enzyme, and that pattern conforms to the sequence of the genome. Since one might expect that *hr* inversions and other forms of recombination between these elements would be common during baculovirus replication, a mechanism must exist to either minimize these events or to eliminate such recombinants from the genome population. As described above, this could involve the partitioning of DNA replication such that DNA destined to become virion genomes is packaged as it is synthesized, whereas other DNA destined for use as templates for transcription is synthesized in a less coordinated manner and is subject to high levels of recombination.

Nucleocapsid length/genome size. Another major unanswered question involves the parameters that determine the length of the capsid and the size of the DNA molecule that is packaged. An examination of capsids associated with defective viral genomes suggested that capsid length may be flexible in response to genome size (160). If capsids are both preassembled and can vary in length, it would suggest that they can be expanded or reduced in response to the size of the genome as part of the packaging process. The facility with which baculoviruses can be engineered to contain additional genetic material could also indicate that a unit size capsid may have some flexibility in the length of DNA that can be accommodated. How the virus senses that a genome is complete and terminates the encapsidation process remains to be determined.

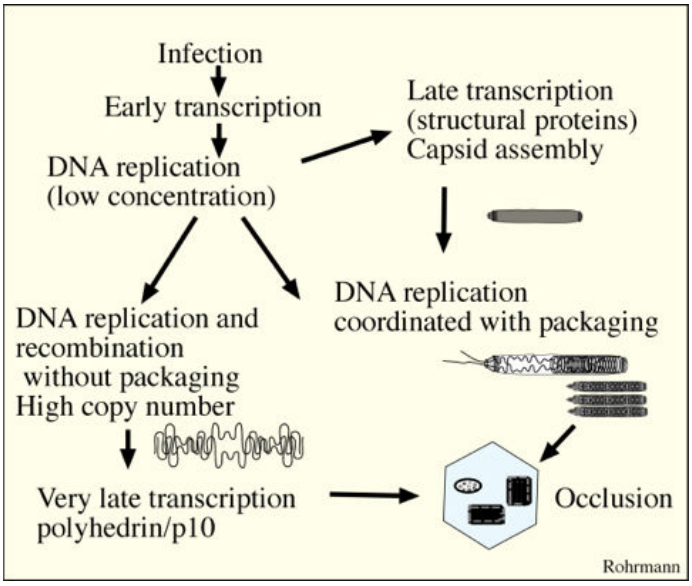

Figure 3. Hypothetical diagram for roles of two types of baculovirus DNA: genomic DNA that is packaged and DNA that is not packaged and is essential for very late transcription.

References

1. Pearson MN, Bjornson RM, Pearson GD, Rohrmann GF. The *Autographa californica* baculovirus genome: Evidence for multiple replication origins. Science. 1992;257:1382–1384. PubMed PMID: 1529337.
2. Kool M, Van Den Berg PM, Tramper J, Goldbach RW, Vlak JM. Location of two putative origins of DNA replication of Autographa californica nuclear polyhedrosis virus. Virology. 1993;192:94–101. PubMed PMID: 8517035.
3. Lee HY, Krell PJ. Generation and analysis of defective genomes of Autographa californica nuclear polyhedrosis virus. J Virol. 1992;66:4339–4347. PubMed PMID: 1602548.
4. Pearson MN, Bjornson RM, Ahrens C, Rohrmann GF. Identification and characterization of a putative origin of DNA replication in the genome of a baculovirus pathogenic for *Orgyia pseudotsugata*. Virology. 1993;197:715–725. PubMed PMID: 8249294.
5. Wu Y, Liu G, Carstens EB. Replication, integration, and packaging of plasmid DNA following cotransfection with baculovirus viral DNA. J Virol. 1999;73:5473–80. PubMed PMID: 10364295.
6. Habib S, Hasnain SE. Differential activity of two non-hr origins during replication of the baculovirus autographa californica nuclear polyhedrosis virus genome. J Virol. 2000;74:5182–9. PubMed PMID: 10799593.
7. Leisy DJ, Rohrmann GF. Characterization of the replication of plasmids containing *hr* sequences in baculovirus-infected *Spodoptera frugiperda* cells. Virology. 1993;196:722–730. PubMed PMID: 8372444.
8. Oppenheimer DI, Volkman LE. Evidence for rolling circle replication of *Autographa californica* M nucleopolyhedrovirus genomic DNA. Arch Virol. 1997;142:2107–2113. PubMed PMID: 9413520.
9. Okano K, Vanarsdall AL, Rohrmann GF. A Baculovirus alkaline nuclease knock out construct produces fragmented DNA and aberrant capsids. Virology. 2007;359:46–54. PubMed PMID: 17046043.
10. Marriott AC, Dimmock NJ. Defective interfering viruses and their potential as antiviral agents. Rev Med Virol.20:51–62. PubMed PMID: 20041441.
11. Lee HY, Krell PJ. Reiterated DNA fragments in defective genomes of Autographa californica nuclear polyhedrosis virus are competent for AcMNPV-dependent DNA replication. Virology. 1994;202:418–429. PubMed PMID: 8009853.

12. Kool M, Goldbach RW, Vlak JM. A putative non-hr origin of DNA replication in the HindIII-K fragment of Autographa californica multiple nucleocapsid nuclear polyhedrosis virus. J Gen Virol. 1994;75(Pt 12): 3345–52. PubMed PMID: 7996129.
13. Pijlman GP, Dortmans JC, Vermeesch AM, Yang K, Martens DE, Goldbach RW, Vlak JM. Pivotal role of the non-hr origin of DNA replication in the genesis of defective interfering baculoviruses. J Virol. 2002;76:5605–11. PubMed PMID: 11991989.
14. Giri L, Feiss MG, Bonning BC, Murhammer DW. Production of baculovirus defective interfering particles during serial passage is delayed by removing transposon target sites in fp25k. J Gen Virol. 2012;93:389–99. PubMed PMID: 21994323.
15. Lu A, Carstens EB. Nucleotide sequence of a gene essential for viral DNA replication in the baculovirus *Autographa californica* nuclear polyhedrosis virus. Virology. 1991;181:336–347. PubMed PMID: 1994581.
16. Kool M, Ahrens C, Goldbach RW, Rohrmann GF, Vlak JM. Identification of genes involved in DNA replication of the *Autographa californica* baculovirus. Proc Natl Acad Sci USA. 1994;91:11212–11216. PubMed PMID: 7972036.
17. Lu A, Miller LK. The roles of eighteen baculovirus late expression factor genes in transcription and DNA replication. J Virol. 1995;69:975–982. PubMed PMID: 7815565.
18. Okano K, Vanarsdall AL, Mikhailov VS, Rohrmann GF. Conserved molecular systems of the Baculoviridae. Virology. 2006;344:77–87. PubMed PMID: 16364739.
19. Wu CA, Nelson NJ, McGeoch DJ, Challberg MD. Identification of Herpes Simplex Virus Type I genes required for origin-dependent DNA synthesis. J Virol. 1988;62:435–443. PubMed PMID: 2826806.
20. Iyer LM, Balaji S, Koonin EV, Aravind L. Evolutionary genomics of nucleo-cytoplasmic large DNA viruses. Virus Res. 2006;117:156–84. PubMed PMID: 16494962.
21. Taggart DJ, Mitchell JK, Friesen PD. A conserved N-terminal domain mediates required DNA replication activities and phosphorylation of the transcriptional activator IE1 of Autographa californica multicapsid nucleopolyhedrovirus. J Virol. 2012;86:6575–85. PubMed PMID: 22496221.
22. Shobahah J, Xue S, Hu D, Zhao C, Wei M, Quan Y, Yu W. Quantitative phosphoproteome on the silkworm (Bombyx mori) cells infected with baculovirus. Virol J. 2017;14:117. PubMed PMID: 28629377.
23. Hang X, Dong W, Guarino LA. The *lef-3* gene of *Autographa californica* nuclear polyhedrosis virus encodes a single-stranded DNA-binding protein. J Virol. 1995;69:3924–3928. PubMed PMID: 7745748.
24. Mikhailov V, Okano K, Rohrmann G. Baculovirus Alkaline Nuclease Possesses a 5'->3' Exonuclease Activity and Associates with the DNA-Binding Protein LEF-3. J Virol. 2003;77:2436–2444. PubMed PMID: 12551981.
25. Mikhailov V, Okano K, Rohrmann G. Specificity of the Endonuclease Activity of the Baculovirus Alkaline Nuclease for Single-stranded DNA. J Biol Chem. 2004;279:14734–14745. PubMed PMID: 14736888.
26. Mikhailov VS, Okano K, Rohrmann GF. The Redox State of the Baculovirus Single-stranded DNA-binding Protein LEF-3 Regulates Its DNA Binding, Unwinding, and Annealing Activities. J Biol Chem. 2005;280:29444–53. PubMed PMID: 15944160.
27. Mikhailov V. Helix-destabilizing properties of the baculovirus single-stranded DNA-binding protein (LEF-3). Virology. 2000;270:180–9. PubMed PMID: 10772990.
28. Mikhailov VS, Okano K, Rohrmann GF. Structural and functional analysis of the baculovirus single-stranded DNA-binding protein LEF-3. Virology. 2006;346:469–478. PubMed PMID: 16375940.
29. Wu Y, Carstens EB. A baculovirus single-stranded DNA binding protein, LEF-3, mediates the nuclear localization of the putative helicase P143. Virology. 1998;247:32–40. PubMed PMID: 9683569.
30. Au V, Yu M, Carstens EB. Characterization of a baculovirus nuclear localization signal domain in the late expression factor 3 protein. Virology. 2009;385:209–17. PubMed PMID: 19073335.
31. Chen Z, Carstens EB. Identification of domains in Autographa californica multiple nucleopolyhedrovirus late expression factor 3 required for nuclear transport of P143. J Virol. 2005;79:10915–22. PubMed PMID: 16103143.

32. Yu M, Carstens EB. Identification of a domain of the baculovirus Autographa californica multiple nucleopolyhedrovirus single-strand DNA-binding protein LEF-3 essential for viral DNA replication. J Virol. 2010;84:6153–62. PubMed PMID: 20357098.
33. McDougal VV, Guarino LA. The autographa californica nuclear polyhedrosis virus p143 gene encodes a DNA helicase. J Virol. 2000;74:5273–9. PubMed PMID: 10799604.
34. Evans JT, Leisy DJ, Rohrmann GF. Characterization of the interaction between the baculovirus replication factors, LEF-1 and LEF-2. J Virol. 1997;71:3114–3119. PubMed PMID: 9060674.
35. Mikhailov VS, Rohrmann GF. The Baculovirus Replication Factor LEF-1 is a DNA Primase. J Virol. 2002;76:2287–2297. PubMed PMID: 11836407.
36. Wu CP, Huang YJ, Wang JY, Wu YL, Lo HR, Wang JC, Chao YC. Autographa californica multiple nucleopolyhedrovirus LEF-2 is a capsid protein required for amplification but not initiation of viral DNA replication. J Virol. 2010;84:5015–24. PubMed PMID: 20219928.
37. Iyer LM, Koonin EV, Leipe DD, Aravind L. Origin and evolution of the archaeo-eukaryotic primase superfamily and related palm-domain proteins: structural insights and new members. Nucleic Acids Res. 2005;33:3875–96. PubMed PMID: 16027112.
38. Mikhailov VS, Rohrmann GF. Characterization of short-lived intermediates produced during replication of baculovirus DNA. Virus Res. 2009;145:106–11. PubMed PMID: 19560496.
39. Shevelev IV, Hubscher U. The 3' 5' exonucleases. Nat Rev Mol Cell Biol. 2002;3:364–76. PubMed PMID: 11988770.
40. Coen DW. 1996. Viral DNA polymerases. *In* DePamphilis ML (ed), DNA replication in eukaryotic cells. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
41. Zeng XR, Hao H, Jiang Y, Lee MY. Regulation of human DNA polymerase delta during the cell cycle. J Biol Chem. 1994;269:24027–33. PubMed PMID: 7929054.
42. Mikhailov V, Marlyev K, Ataeva J, Kullyev P, Atrzhev A. Characterization of 3' -> 5' exonuclease associated with DNA polymerase of silkworm nuclear polyhedrosis ivrus. Nucleic Acids Research. 1986;14:3841–3857. PubMed PMID: 3012482.
43. Tomalski MD, Wu J, Miller LK. The location, sequence, transcription, and regulation of a baculovirus DNA polymerase gene. Virology. 1988;167:591–600. PubMed PMID: 3059678.
44. McDougal VV, Guarino LA. Autographa californica nuclear polyhedrosis virus DNA polymerase: measurements of processivity and strand displacement. J Virol. 1999;73:4908–18. PubMed PMID: 10233952.
45. Hang X, Guarino LA. Purification of Autographa californica nucleopolyhedrovirus DNA polymerase from infected insect cells. J Gen Virol. 1999;80:2519–26. PubMed PMID: 10501509.
46. Ahrens CH, Rohrmann GF. The DNA polymerase and helicase genes of a baculovirus of *Orgyia pseudotsugata*. J Gen Virol. 1996;77:825–837. PubMed PMID: 8609478.
47. Pellock BJ, Lu A, Meagher RB, Weise MJ, Miller LK. Sequence, function, and phylogenetic analysis of an ascovirus DNA polymerase gene. Virology. 1996;216:146–157. PubMed PMID: 8614981.
48. Vanarsdall AL, Okano K, Rohrmann GF. Characterization of the replication of a baculovirus mutant lacking the DNA polymerase gene. Virology. 2005;331:175–180. PubMed PMID: 15582664.
49. Mikhailov VS, Vanarsdall AL, Rohrmann GF. Isolation and characterization of the DNA-binding protein (DBP) of the Autographa californica multiple nucleopolyhedrovirus. Virology. 2007;370:415–29. PubMed PMID: 17935748.
50. Vanarsdall AL, Mikhailov VS, Rohrmann GF. Characterization of a baculovirus lacking the DBP (DNA-binding protein) gene. Virology. 2007;364:475–85. PubMed PMID: 17449080.
51. Mikhailov VS, Mikhailova AL, Iwanaga M, Gomi S, Maeda S. *Bombyx mori* nucleopolyhedrovirus encodes a DNA -binding protein capable of destabilizing duplex DNA. J Virol. 1998;72:3107–3116. PubMed PMID: 9525636.
52. Hajós JP, Pijnenburg J, Usmany M, Zuidema D, Závodszky P, Vlak JM. High frequency recombination between homologous baculoviruses in cell culture. Arch Virol. 2000;145:159–164. PubMed PMID: 10664413.

53. Kamita SG, Maeda S, Hammock BD. High-frequency homologous recombination between baculoviruses involves DNA replication. J Virol. 2003;77:13053–61. PubMed PMID: 14645562.
54. Lin G, Blissard GW. Analysis of an Autographa californica nucleopolyhedrovirus lef-11 knockout: LEF-11 is essential for viral DNA replication. J Virol. 2002;76:2770–9. PubMed PMID: 11861844.
55. Dong ZQ, Hu N, Zhang J, Chen TT, Cao MY, Li HQ, Lei XJ, Chen P, Lu C, Pan MH. Oligomerization of Baculovirus LEF-11 Is Involved in Viral DNA Replication. PLoS One. 2015;10:e0144930. PubMed PMID: 26660313.
56. Dong ZQ, Hu N, Dong FF, Chen TT, Jiang YM, Chen P, Lu C, Pan MH. Baculovirus LEF-11 Hijack Host ATPase ATAD3A to Promote Virus Multiplication in Bombyx mori cells. Sci Rep. 2017;7:46187. PubMed PMID: 28393927.
57. Zhang J, Dong ZQ, Zhang CD, He Q, Chen XM, Cao MY, Li HQ, Xiao WF, Lu C, Pan MH. Identification of a novel nuclear localization signal of baculovirus late expression factor 11. Virus Res. 2014;184:111–9. PubMed PMID: 24607592.
58. Chen T, Dong Z, Hu N, Hu Z, Dong F, Jiang Y, Li J, Chen P, Lu C, Pan M. Baculovirus LEF-11 nuclear localization signal is important for viral DNA replication. Virus Res. 2017;238:133–140. PubMed PMID: 28648848.
59. Xi Q, Wang J, R. D, Wang X. Characterization of AcMNPV with a deletion of me53 gene. Virus Genes. 2007.:223–32. PubMed PMID: 17096186.
60. Morris TD, Todd JW, Fisher B, Miller LK. Identification of *lef-7*: A baculovirus gene affecting late gene expression. Virology. 1994;200:360–369. PubMed PMID: 8178427.
61. Chen CJ, Thiem SM. Differential infectivity of two Autographa californica nucleopolyhedrovirus mutants on three permissive cell lines is the result of lef-7 deletion. Virology. 1997;227:88–95. PubMed PMID: 9007061.
62. Gomi S, Zhou CE, Yih W, Majima K, Maeda S. Deletion analysis of four of eighteen late gene expression factor gene homologues of the baculovirus, BmNPV. Virology. 1997;230:35–47. PubMed PMID: 9126260.
63. Mitchell JK, Byers NM, Friesen PD. Baculovirus F-Box Protein LEF-7 Modifies the Host DNA Damage Response To Enhance Virus Multiplication. J Virol. 2013;87:12592–9. PubMed PMID: 24027328.
64. Johnson A, O'Donnell M. CELLULAR DNA REPLICASES: Components and Dynamics at the Replication Fork. Ann Rev Biochem. 2005;74:283–315. PubMed PMID: 15952889.
65. Iwahori S, Ikeda M, Kobayashi M. Generation and characterization of *Autographa californica* nucleopolyhedrovirus mutants defective in *pcna* gene homologoue. J Insect Biotechnol Sericology. 2002;71:129–139.
66. Iwahori S, Ikeda M, Kobayashi M. Association of Sf9 cell proliferating cell nuclear antigen with the DNA replication site of Autographa californica multicapsid nucleopolyhedrovirus. J Gen Virol. 2004;85:2857–62. PubMed PMID: 15448347.
67. Crawford AM, Miller LK. Characterization of an early gene accelerating expression of late genes of the baculovirus Autographa californica nuclear polyhedrosis virus. J Virol. 1988;62:2773–2781. PubMed PMID: 3292791.
68. Gomi S, Majima K, Maeda S. Sequence analysis of the genome of Bombyx mori nucleopolyhedrovirus. J Gen Virol. 1999;80:1323–37. PubMed PMID: 10355780.
69. Fu Y, Wang R, Liang A. Function analysis of Ac-PCNA and Sf-PCNA during the Autographa californica multiple nucleopolyhedrovirus infection process. Mol Cell Biochem. 2018;443:57–68. PubMed PMID: 29075988.
70. Craig NL, Nash HA. The mechanism of phage lambda site-specific recombination: site-specific breakage of DNA by Int topoisomerase. Cell. 1983;35:795–803. PubMed PMID: 6317202.
71. Mikhailov V, Rohrmann G. Binding of the baculovirus very late expression factor 1 (VLF-1) to different DNA structures. BMC Mol Biol. 2002;3:14. PubMed PMID: 12350233.
72. Chen YR, Zhong S, Fei Z, Gao S, Zhang S, Li Z, Wang P, Blissard GW. Transcriptome responses of the host Trichoplusia ni to infection by the baculovirus Autographa californica multiple nucleopolyhedrovirus. J Virol. 2014;88:13781–97. PubMed PMID: 25231311.

73. Pearson MN, Rohrmann GF. Characterization of a baculovirus-encoded ATP-dependent DNA ligase. J Virol. 1998;72:9142–9149. PubMed PMID: 9765460.
74. Boule JB, Zakian VA. The yeast Pif1p DNA helicase preferentially unwinds RNA DNA substrates. Nucleic Acids Res. 2007;35:5809–18. PubMed PMID: 17720711.
75. Lieber MR. The FEN-1 family of structure-specific nucleases in eukaryotic DNA replication, recombination and repair. Bioessays. 1997.:233–40. PubMed PMID: 9080773.
76. Ferrelli ML, Salvador R, Biedma ME, Berretta MF, Haase S, Sciocco-Cap A, Ghiringhelli PD, Romanowski V. Genome of Epinotia aporema granulovirus (EpapGV), a polyorganotropic fast killing betabaculovirus with a novel thymidylate kinase gene. BMC Genomics. 2012;13:548. PubMed PMID: 23051685.
77. Ono C, Kamagata T, Taka H, Sahara K, Asano S, Bando H. Phenotypic grouping of 141 BmNPVs lacking viral gene sequences. Virus Res. 2012;165:197–206. PubMed PMID: 22421381.
78. Paran N, De Silva FS, Senkevich TG, Moss B. Cellular DNA ligase I is recruited to cytoplasmic vaccinia virus factories and masks the role of the vaccinia ligase in viral DNA replication. Cell Host Microbe. 2009;6:563–9. PubMed PMID: 20006844.
79. Nobiron I, O'Reilly DR, Olszewski JA. Autographa californica nucleopolyhedrovirus infection of Spodoptera frugiperda cells: a global analysis of host gene regulation during infection, using a differential display approach. J Gen Virol. 2003;84:3029–39. PubMed PMID: 14573808.
80. Okano K, Mikhailov VS, Maeda S. Colocalization of baculovirus IE-1 and two DNA-binding proteins, DBP and LEF-3, to viral replication factories. J Virol. 1999;73:110–119. PubMed PMID: 9847313.
81. Negorev D, Maul GG. Cellular proteins localized at and interacting within ND10/PML nuclear bodies/ PODs suggest functions of a nuclear depot. Oncogene. 2001;20:7234–42. PubMed PMID: 11704851.
82. Wileman T. Aggresomes and pericentriolar sites of virus assembly: cellular defense or viral design? Annu Rev Microbiol. 2007;61:149–67. PubMed PMID: 17896875.
83. Mainz D, Quadt I, Knebel-Morsdorf D. Nuclear IE2 structures are related to viral DNA replication sites during baculovirus infection. J Virol. 2002;76:5198–207. PubMed PMID: 11967334.
84. Nagamine T, Kawasaki Y, Iizuka T, Matsumoto S. Focal Distribution of Baculovirus IE1 Triggered by Its Binding to the hr DNA Elements. JVirol. 2005;79:39–46. PubMed PMID: 15596799.
85. Nagamine T, Kawasaki Y, Matsumoto S. Induction of a subnuclear structure by the simultaneous expression of baculovirus proteins, IE1, LEF3, and P143 in the presence of hr. Virology. 2006;352:400–7. PubMed PMID: 16780915.
86. Kawasaki Y, Matsumoto S, Nagamine T. Analysis of baculovirus IE1 in living cells: dynamics and spatial relationships to viral structural proteins. J Gen Virol. 2004;85:3575–3583. PubMed PMID: 15557230.
87. Kang W, Imai N, Kawasaki Y, Nagamine T, Matsumoto S. IE1 and hr facilitate the localization of Bombyx mori nucleopolyhedrovirus ORF8 to specific nuclear sites. J Gen Virol. 2005;86:3031–8. PubMed PMID: 16227225.
88. Nagamine T, Kawasaki Y, Abe A, Matsumoto S. Nuclear marginalization of host cell chromatin associated with expansion of two discrete virus-induced subnuclear compartments during baculovirus infection. J Virol. 2008;82:6409–18. PubMed PMID: 18434402.
89. Griego VM, Martignoni ME, Claycomb AE. Inactivation of nuclear polyhedrosis virus (Baculovirus subgroup A) by monochromatic UV radiation. Appl Environ Microbiol. 1985;49:709–10. PubMed PMID: 3888111.
90. van Oers MM, Herniou EA, Usmany M, Messelink GJ, Vlak JM. Identification and characterization of a DNA photolyase-containing baculovirus from *Chrysodeixis chalcites*. Virology. 2004;330:460–470. PubMed PMID: 15567439.
91. Willis LG, Siepp R, Stewart TM, Erlandson MA, Theilmann DA. Sequence analysis of the complete genome of Trichoplusia ni single nucleopolyhedrovirus and the identification of a baculoviral photolyase gene. Virology. 2005;338:209–26. PubMed PMID: 15951000.
92. Xu F, Vlak JM, van Oers MM. Conservation of DNA photolyase genes in group II nucleopolyhedroviruses infecting plusiine insects. Virus Res. 2008;136:58–64. PubMed PMID: 18513819.

93. van Oers MM, Lampen MH, Bajek MI, Vlak JM, Eker AP. Active DNA photolyase encoded by a baculovirus from the insect Chrysodeixis chalcites. DNA Repair (Amst). 2008;7:1309–18. PubMed PMID: 18547877.
94. Xu F, Vlak JM, Eker AP, van Oers MM. DNA photolyases of Chrysodeixis chalcites nucleopolyhedrovirus are targeted to the nucleus and interact with chromosomes and mitotic spindle structures. J Gen Virol. 2010;91:907–14. PubMed PMID: 19955559.
95. Biernat MA, Eker AP, van Oers MM, Vlak JM, van der Horst GT, Chaves I. A baculovirus photolyase with DNA repair activity and circadian clock regulatory function. J Biol Rhythms. 2012;27:3–11. PubMed PMID: 22306969.
96. Petrik DT, Iseli A, Montelone BA, Van Etten JL, Clem RJ. Improving baculovirus resistance to UV inactivation: increased virulence resulting from expression of a DNA repair enzyme. J Invertebr Pathol. 2003;82:50–56. PubMed PMID: 12581719.
97. Bennett CJ, Webb M, Willer DO, Evans DH. Genetic and phylogenetic characterization of the type II cyclobutane pyrimidine dimer photolyases encoded by Leporipoxviruses. Virology. 2003;315:10–9. PubMed PMID: 14592755.
98. Esteban DJ, Da Silva M, Upton C. New bioinformatics tools for viral genome analyses at Viral Bioinformatics - Canada. Pharmacogenomics. 2005;6:271–80. PubMed PMID: 16013958.
99. Slack JM, Shapiro M. Anticarsia gemmatalis multicapsid nucleopolyhedrovirus v-trex gene encodes a functional 3' to 5' exonuclease. J Gen Virol. 2004;85:2863–71. PubMed PMID: 15448348.
100. Yang DH, de Jong JG, Makhmoudova A, Arif BM, Krell PJ. Choristoneura fumiferana nucleopolyhedrovirus encodes a functional 3'-5' exonuclease. J Gen Virol. 2004;85:3569–73. PubMed PMID: 15557229.
101. Aravind L, Walker DR, Koonin EV. Conserved domains in DNA repair proteins and evolution of repair systems. Nucleic Acids Res. 1999;27:1223–42. PubMed PMID: 9973609.
102. Wu W, Passarelli AL. The Autographa californica M nucleopolyhedrovirus ac79 gene encodes an early gene product with structural similarities to UvrC and intron-encoded endonucleases that is required for efficient budded virus production. J Virol. 2012;86:5614–25. PubMed PMID: 22419804.
103. Braunagel SC, Russell WK, Rosas-Acosta G, Russell DH, Summers MD. Determination of the protein composition of the occlusion-derived virus of Autographa californica nucleopolyhedrovirus. Proc Natl Acad Sci U S A. 2003;100:9797–802. PubMed PMID: 12904572.
104. Oliveira JV, Wolff JL, Garcia-Maruniak A, Ribeiro BM, de Castro ME, de Souza ML, Moscardi F, Maruniak JE, Zanotto PM. Genome of the most widely used viral biopesticide: Anticarsia gemmatalis multiple nucleopolyhedrovirus. J Gen Virol. 2006;87:3233–50. PubMed PMID: 17030857.
105. de Castro Oliveira JV, de Melo FL, Romano CM, Iamarino A, Rizzi TS, Yeda FP, Hársi CM, Wolff JL, Zanotto PM. Structural and phylogenetic relationship of ORF 31 from the Anticarsia gemmatalis MNPV to poly (ADP-ribose) polymerases (PARP). Virus Genes. 2008;37:177–84. PubMed PMID: 18648922.
106. Tulin A, Naumova NM, Menon AK, Spradling AC. Drosophila poly(ADP-ribose) glycohydrolase mediates chromatin structure and SIR2-dependent silencing. Genetics. 2006;172:363–71. PubMed PMID: 16219773.
107. Yu SW, Wang H, Poitras MF, Coombs C, Bowers WJ, Federoff HJ, Poirier GG, Dawson TM, Dawson VL. Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. Science. 2002;297:259–63. PubMed PMID: 12114629.
108. Chen X, IJkel W, Tarchini R, Sun X, Sandbrink H, Wang H, Peters S, Zuidema D, Lankhorst R, Vlak J, Hu Z. The sequence of the *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus genome. J Gen Virol. 2001;82:241–257. PubMed PMID: 11125177.
109. Zimmermann L, Stephens A, Nam SZ, Rau D, Kubler J, Lozajic M, Gabler F, Soding J, Lupas AN, Alva V. A Completely Reimplemented MPI Bioinformatics Toolkit with a New HHpred Server at its Core. J Mol Biol. 2018;430:2237–2243. PubMed PMID: 29258817.
110. Miwa M, Tanaka M, Matsushima T, Sugimura T. Purification and properties of glycohydrolase from calf thymus splitting ribose-ribose linkages of poly(adenosine diphosphate ribose). J Biol Chem. 1974;249:3475–82. PubMed PMID: 4831224.

111. Liang G, Li G, Chen K, Yao Q, Chen H, Zhou Y. Bombyx mori nucleopolyhedrovirus ORF94, a novel late protein is identified to be a component of ODV structural protein. Curr Microbiol. 2010;61:190–6. PubMed PMID: 20135318.
112. Wang R, Deng F, Hou D, Zhao Y, Guo L, Wang H, Hu Z. Proteomics of the Autographa californica Nucleopolyhedrovirus Budded Virions. J Virol. 2010;84:7233–7242. PubMed PMID: 20444894.
113. Deng F, Wang R, Fang M, Jiang Y, Xu X, Wang H, Chen X, Arif BM, Guo L, Wang H, Hu Z. Proteomics analysis of Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus identified two new occlusion-derived virus-associated proteins, HA44 and HA100. J Virol. 2007;81:9377–85. PubMed PMID: 17581982.
114. Luo S, Zhang Y, Xu X, Westenberg M, Vlak JM, Wang H, Hu Z, Deng F. Helicoverpa armigera nucleopolyhedrovirus occlusion-derived virus-associated protein, HA100, affects oral infectivity in vivo but not virus replication in vitro. J Gen Virol. 2011;92:1324–31. PubMed PMID: 21325478.
115. Magni G, Amici A, Emanuelli M, Orsomando G, Raffaelli N, Ruggieri S. Enzymology of NAD+ homeostasis in man. Cell Mol Life Sci. 2004;61:19–34. PubMed PMID: 14704851.
116. Kuzio J, Pearson MN, Harwood SH, Funk CJ, Evans JT, Slavicek J, Rohrmann GF. Sequence and analysis of the genome of a baculovirus pathogenic for *Lymantria dispar*. Virology. 1999;253:17–34. PubMed PMID: 9887315.
117. Hughes AL, Friedman R. Genome-Wide Survey for Genes Horizontally Transferred from Cellular Organisms to Baculoviruses. Mol Biol Evol. 2003;20:979–8. PubMed PMID: 12716988.
118. Hoeijmakers JH. DNA damage, aging, and cancer. N Engl J Med. 2009;361:1475–85. PubMed PMID: 19812404.
119. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2009;461:1071–8. PubMed PMID: 19847258.
120. Ciccia A, Elledge SJ. The DNA damage response: making it safe to play with knives. Mol Cell. 2010;40:179–204. PubMed PMID: 20965415.
121. Mannironi C, Bonner WM, Hatch CL. H2A.X. a histone isoprotein with a conserved C-terminal sequence, is encoded by a novel mRNA with both DNA replication type and polyA 3' processing signals. Nucleic Acids Res. 1989;17:9113–26. PubMed PMID: 2587254.
122. Fernandez-Capetillo O, Lee A, Nussenzweig M, Nussenzweig A. H2AX: the histone guardian of the genome. DNA Repair (Amst). 2004;3:959–67. PubMed PMID: 15279782.
123. Weitzman MD, Lilley CE, Chaurushiya MS. Genomes in conflict: maintaining genome integrity during virus infection. Annu Rev Microbiol. 2010;64:61–81. PubMed PMID: 20690823.
124. Huang N, Wu W, Yang K, Passarelli AL, Rohrmann GF, Clem RJ. Baculovirus infection induces a DNA damage response that is required for efficient viral replication. J Virol. 2011;85:12547–56. PubMed PMID: 21917957.
125. Mitchell JK, Friesen PD. Baculoviruses modulate a proapoptotic DNA damage response to promote virus multiplication. J Virol. 2012;86:13542–53. PubMed PMID: 23035220.
126. Martin DW, Weber PC. DNA replication promotes high-frequency homologous recombination during Autographa californica multiple nuclear polyhedrosis virus infection. Virology. 1997;232:300–309. PubMed PMID: 9191843.
127. Crouch EA, Passarelli AL. Genetic requirements for homologous recombination in Autographa californica nucleopolyhedrovirus. J Virol. 2002;76:9323–34. PubMed PMID: 12186915.
128. Mosig G. Recombination and recombination-dependent DNA replication in bacteriophage T4. Annu Rev Genet. 1998;32:379–413. PubMed PMID: 9928485.
129. Li Z, Karakousis G, Chiu SK, Reddy G, Radding CM. The beta protein of phage lambda promotes strand exchange. J Mol Biol. 1998;276:733–44. PubMed PMID: 9500923.
130. Poteete A. What makes the bacteriophage lambda Red system useful for genetic engineering: molecular mechanism and biological function. FEMS Microbiol Lett. 2001;201:9–14. PubMed PMID: 11445160.
131. Blumel J, Graper S, Matz B. Structure of simian virus 40 DNA replicated by herpes simplex virus type 1. Virology. 2000;276:445–54. PubMed PMID: 11040135.

132. Thomas MS, Gao M, Knipe DM, Powell KL. Association between the herpes simplex virus major DNA-binding protein and alkaline nuclease. J Virol. 1992;66:1152–61. PubMed PMID: 1309895.
133. Wilkinson DE, Weller SK. The role of DNA recombination in herpes simplex virus DNA replication. IUBMB Life. 2003;55:451–8. PubMed PMID: 14609200.
134. Porter I, Stow N. Virus particles produced by the herpes simplex virus type 1 alkaline nuclease null mutant ambUL12 contain abnormal genomes. J Gen Virol. 2004.:85. PubMed PMID: 14993641.
135. Porter I, Stow N. Replication, recombination and packaging of amplicon DNA in cells infected with the herpes simplex virus type 1 alkaline nuclease null mutant ambUL12. J Gen Virol. 2004;85:3501–10. PubMed PMID: 15557223.
136. Martinez R, Sarisky RT, Weber PC, Weller SK. Herpes simplex virus type 1 alkaline nuclease is required for efficient processing of viral DNA replication intermediates. J Virol. 1996;70:2075–85. PubMed PMID: 8642627.
137. Martin DW, Weber PC. Replication of simian virus 40 origin-containing DNA during infection with a recombinant Autographa californica multiple nuclear polyhedrosis virus expressing large T antigen. J Virol. 1997;71:501–506. PubMed PMID: 8985377.
138. Okano K, Vanarsdall AL, Rohrmann GF. Characterization of a baculovirus lacking the Alkaline Nuclease Gene. J Virol. 2004;78:10650–10656. PubMed PMID: 15367632.
139. Blissard GW, Rohrmann GF. Location, sequence, transcriptional mapping, and temporal expression of the gp64 envelope glycoprotein gene of the *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus. Virology. 1989;170:537–555. PubMed PMID: 2658304.
140. McLachlin JR, Miller LK. Identification and characterization of vlf-1, a baculovirus gene involved in very late gene expression. J Virol. 1994;68:7746–7756. PubMed PMID: 7966564.
141. Vanarsdall AL, Okano K, Rohrmann GF. Characterization of the role of VLF-1 in baculovirus capsid structure and DNA processing. J Virol. 2006;80:1724–1733. PubMed PMID: 16439529.
142. Vanarsdall AL, Okano K, Rohrmann GF. Characterization of a baculovirus with a deletion of vlf-1. Virology. 2004;326:191–201. PubMed PMID: 15262507.
143. Li Y, Wang J, Deng R, Zhang Q, Yang K, Wang X. vlf-1 deletion brought AcMNPV to defect in nucleocapsid formation. Virus Genes. 2005;31:275–84. PubMed PMID: 16175333.
144. Fraser MJ. Ultrastructural observations of virion maturation in *Autographa californica* nuclear polyhedrosis virus infected *Spodoptera frugiperda* cell cultures. J Ultrastruct Mol Struct Res. 1986;95:189–195.
145. Yang K, Baines JD. The putative terminase subunit of herpes simplex virus 1 encoded by UL28 is necessary and sufficient to mediate interaction between pUL15 and pUL33. J Virol. 2006;80:5733–9. PubMed PMID: 16731912.
146. Thoma C, Borst E, Messerle M, Rieger M, Hwang JS, Bogner E. Identification of the interaction domain of the small terminase subunit pUL89 with the large subunit pUL56 of human cytomegalovirus. Biochemistry. 2006;45:8855–63. PubMed PMID: 16846228.
147. Aathavan K, Politzer AT, Kaplan A, Moffitt JR, Chemla YR, Grimes S, Jardine PJ, Anderson DL, Bustamante C. Substrate interactions and promiscuity in a viral DNA packaging motor. Nature. 2009;461:669–73. PubMed PMID: 19794496.
148. Ortega ME, Gaussier H, Catalano CE. The DNA Maturation Domain of gpA, the DNA Packaging Motor Protein of Bacteriophage Lambda, Contains an ATPase Site Associated with Endonuclease Activity. J Mol Biol. 2007;373:851–65. PubMed PMID: 17870092.
149. Smith DE, Tans SJ, Smith SB, Grimes S, Anderson DL, Bustamante C. The bacteriophage phi29 portal motor can package DNA against a large internal force. Nature. 2001;413:748–52. PubMed PMID: 11607035.
150. Fuller DN, Raymer DM, Kottadiel VI, Rao VB, Smith DE. Single phage T4 DNA packaging motors exhibit large force generation, high velocity, and dynamic variability. Proc Natl Acad Sci U S A. 2007;104:16868–73. PubMed PMID: 17942694.

151. Ke J, Wang J, Deng R, Wang X. 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. 2008;374:421–31. PubMed PMID: 18241908.
152. Volkman LE, Talhouk SN, Oppenheimer DI, Charlton CA. Nuclear F-actin: a functional component of baculovirus-infected lepidopteran cells? J Cell Sci. 1992;103:15–22.
153. Lanier LM, Volkman LE. Actin binding and nucleation by Autographa california M nucleopolyhedrovirus. Virology. 1998;243:167–77. PubMed PMID: 9527926.
154. Volkman LE. Autographa californica MNPV nucleocapsid assembly: inhibition by cytochalasin D. Virology. 1988;163:547–53. PubMed PMID: 3281373.
155. Marek M, Romier C, Galibert L, Merten O, van Oers MM. Baculovirus VP1054 is an acquired cellular PURα, a nucleic-acid6n binding protein specific for GGN repeats. J Virol. 2013;87:8465–80. PubMed PMID: 23720732.
156. Kikhno I. Identification of a Conserved Non-Protein-Coding Genomic Element that Plays an Essential Role in Alphabaculovirus Pathogenesis. PLoS One. 2014;9:e95322. PubMed PMID: 24740153.
157. Huang Z, Pan M, Zhu S, Zhang H, Wu W, Yuan M, Yang K. The Autographa californica Multiple Nucleopolyhedrovirus ac83 Gene Contains a cis-Acting Element That Is Essential for Nucleocapsid Assembly. J Virol. 2017.:91. PubMed PMID: 28031366.
158. Thiry E, Meurens F, Muylkens B, McVoy M, Gogev S, Thiry J, Vanderplasschen A, Epstein A, Keil G, Schynts F. Recombination in alphaherpesviruses. Rev Med Virol. 2005;15:89–103. PubMed PMID: 15546129.
159. Ahrens CH, Russell R, Funk CJ, Evans JT, Harwood SH, Rohrmann GF. The sequence of the *Orgyia pseudotsugata* multinucleocapsid nuclear polyhedrosis virus genome. Virology. 1997;229:381–399. PubMed PMID: 9126251.
160. Kool M, Voncken JW, Van Lier FLJ, Tramper J, Vlak JM. Detection and analysis of Autographa californica nuclear polyhedrosis virus mutants with defective interfering properties. Virology. 1991;183:739–746. PubMed PMID: 1853572.

6. Baculovirus late transcription

Activation of baculovirus late genes

A common feature of many viruses is the division of transcription into early and late categories. These divisions may be further separated into immediate early, early, late and very late. The separation between early and late transcription is always the onset of DNA replication. Despite this being a common feature of viruses, the mechanism underlying the linkage of DNA replication to the activation of late gene transcription is unclear. Two major features of newly replicated DNA may be involved in this process. Immediately after DNA replication there may be a transient period when proteins are not bound to the DNA and this might expose late promoters and facilitate their activation. In poxviruses, a set of transcriptional activators that facilitate the expression of late genes are expressed from newly replicated (naked) DNA (1). In baculoviruses, the generation of DNA free of proteins might facilitate the activation of late promoters until p6.9 or other DNA binding proteins accumulate to such a level that all viral DNA is coated and transcription is inhibited. Another major feature of DNA in the process of being replicated is the numerous nicks and RNA-DNA junctions generated by the synthesis of Okazaki fragments during lagging strand synthesis. In viruses, e.g., T-4, it has been suggested that the unligated junctions of Okazaki fragments may serve as transient enhancers of late transcription by acting as loading sites for late gene activators that move along the DNA until late promoters are recognized (2). Once DNA replication is completed, these loading sites for late gene activators might be eliminated by the removal of the RNA primers and ligation of the lagging strand, thereby terminating late transcription. In baculoviruses, essential activators of late gene transcription have not been identified in in vitro analyses, however, several additional factors have been characterized in transient assays that may be essential for late transcription (see below).

Baculovirus late promoter elements

Initially, because the sequence of the AcMNPV polyhedrin gene region revealed a conventional-appearing TATA sequence upstream of the reading frame, it was suggested that baculovirus late genes were transcribed using a conventional RNA polymerase II promoter (3). However, the OpMNPV polyhedrin gene lacked this sequence, and the mapping of its mRNA start site indicated that it initiated at an ATAAG sequence. When several other baculovirus late genes were sequenced (4-7), they were all found to have this sequence. This led to the proposal that ATAAG was the baculovirus late promoter sequence and that mRNA initiated within this sequence (8). Subsequently, this was demonstrated experimentally (9) and eventually it was determined that the core sequence is normally ATAAG, GTAAG, or TTAAG, but that CTAAG is apparently not used. A survey of predicted baculovirus promoter sequences was conducted on 26 baculovirus genomes (10) (see Chapter 5). The purpose of this investigation was to determine whether baculovirus promoter elements could be correlated with their position relative to the ATG translation initiation codons. They found a number of clear correlations. There was a strong correlation of TAAG sequences upstream of the ATG, particularly when combined with a TATA early promoter, e.g., TATAAGG. These were about 7 times as frequent in the upstream location and reflect genes that are transcribed by both the host RNA polymerase II and the baculovirus RNA polymerase and are expressed both early and late in infection. TAAG sequences were almost 6 times more prevalent in the promoter (upstream) region. Of the TAAG sequences, ATAAG and GTAAG sequences were most prevalent, followed by TTAAG. In contrast, CTAAG, which does not appear to function as a late promoter element, was only slightly more prevalent in the upstream promoter regions.

In comprehensive investigations using genome wide analyses, strand-specific RNAseq technology, and the sequencing of 5' RACE PCR products (11), 218 transcriptional start sites (TSS) were mapped for 156 AcMNPV orfs from infected *Trichoplusia ni* cells. The majority of the TSS (126) were located at consensus later promoter motifs (with the sequence TAAG) upstream of 101 orfs, or about 65% of the AcMNPV orfs. In addition, 92 non late promoter TSS were identified of which 89 were located immediately upstream of 77 predicted orfs, or about

half of the orfs. There were 21 orfs identified that had multiple late TSS and another 21 that had both early and late TSS.

Late TSS always initiated at the second nt of the TAAG late promoter element. About 13% of the orfs have more than one late TSS. Although no additional consensus nt were identified near the TAAG sequence, they were located in the context of an AT-rich sequence, with the 6-7 nt upstream and downstream of the TAAG being about 75% AT, vs 59% for the genome as a whole. In addition, no other consensus nucleotides were identified that were associated with the relative strength of the late promoters (11).

An implication of the novel promoter and mRNA start site of late and very late baculovirus genes is that their transcripts would all begin with the sequence AAG (Figure 1). Whether this serves as a signal for selective sorting or transport of these mRNAs remains to be determined.

Temporal gene expression

In the characterization of the transcriptome of AcMNPV infected *T. ni* cells, it was observed that at 6 hpi, the majority of transcripts were from early genes and comprised about 3% of the total mRNA in the cell. The genes expressed included some involved in DNA replication (lef-2 – the primase accessory factor, lef-3 – the SSB), anti-apoptosis (p35), and gp64, the envelope fusion protein. Other abundantly expressed genes included dbp, pp31(39K), and lef6. For genes that have both early and late promoters, such as gp64 and pp31, the late promoters were expressed more highly than the early promoters at 6 hpi. This suggests that early promoter activity might facilitate access to the late promoter by the viral RNA polymerase or enhance its activity in some other manner. At 12 hpi, which corresponds to the beginning of DNA replication, the viral mRNA population greatly expanded to comprise 38% of the total mRNA in the cells and likely reflects the amplification of the templates and also the accessibility of the late promoter elements possibly due to their nascent synthesis. Several transcripts encoding virion associated structures including p6.9, odv-ec27, odv-e18 were 2 – 3-fold higher in concentration than other transcripts. By 24 hpi, polyhedrin and p10 were the most abundant mRNAs and by 48 hpi, they were up to 4-fold higher than the mRNA of the next highest group that included p6.9, odv-e18 and odv-ec27. At this time polyhedrin and p10 mRNA comprised 24% and 7.5% of the total RNA of the cell, respectively. Overall, genes that had conventional early promoters (TATAA and CAGT-like sequences) were expressed highest at 6-12 hpi, whereas those with a later promoter element were expressed the highest at 12-18 hpi or later (11).

Insect virus RNA polymerases and occlusion body protein hyperexpression

Baculoviruses encode a novel RNA polymerase composed of four subunits that transcribes late and very late genes and that recognizes the unique promoter consensus sequence described above. It is not clear why a virus that replicates in the nucleus would encode its own RNA polymerase, since many such viruses depend on exploiting the host enzyme for transcribing all their genes. The remarkable ability of baculoviruses to hyper express very late genes might be considered the impetus for the evolution of a system that is independent of host transcription. Although several families of cytoplasmic RNA and DNA viruses, such as the Poxviridae and Reoviridae, encode their own RNA polymerases, most lineages of these viruses do not hyper express genes. However, both these families have occluded genera, the cypoviruses and entomopox viruses respectively, that are pathogenic for insects and express occlusion body proteins at very high levels reminiscent of baculovirus very late genes, e.g., entomopox spheroidin, can comprise 30-40% of total protein (12). These viruses are able to accomplish this utilizing RNA polymerases related to those of other members of their viral family that do not hyper express genes, indicating that the evolution of the polymerase might not be specifically linked to its ability to hyper express genes. However, the independence of transcription from the host cell RNA polymerase could have facilitated the evolution of the extraordinary levels of gene expression of the polymerases of the occluded

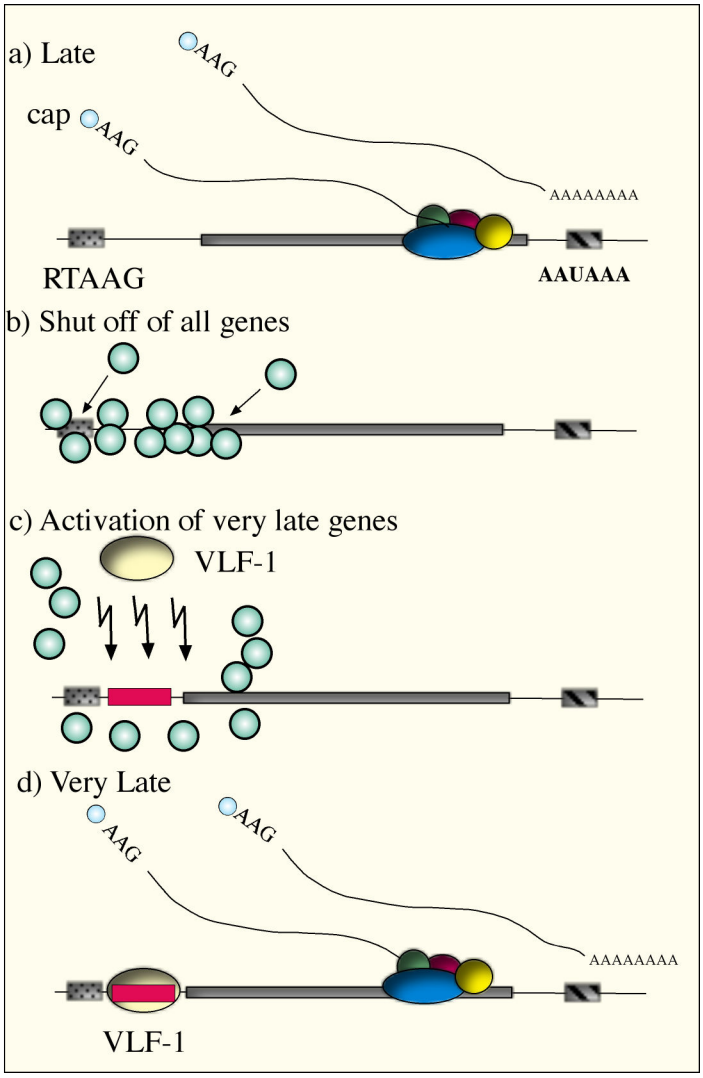

Figure 1. Comparison of late and very late gene transcription. Shown is: (a) late gene transcription; (b) a hypothetical mechanism for the shutoff of late transcription; and (c and d) activation of very late gene transcription involving VLF-1 interacting with the burst sequence (shown as a red rectangle). Late transcription initiates within the late promoter element. Therefore, all late messages likely begin with the sequence AAG.

viruses. In the case of baculoviruses, the independence from the host enzyme may have initially provided regulatory advantages, such as independence from low levels of RNA transcription factors in nondividing cells. This could have provided the enzymatic platform for the evolution of their ability to hyper express very late genes involved in occlusion body formation. The combination of factors that likely influence high levels of baculovirus gene expression is discussed in detail in Chapter 10.

The baculovirus RNA polymerase

Hints of the presence of a novel baculovirus RNA polymerase had been suggested by the observation that the expression of baculovirus late genes was α-amanitin resistant (13, 14). Alpha-amanitin is a fungal toxin that inhibits RNA polymerase II and hence mRNA synthesis at very low concentrations. The resistance to α-amanitin, along with the observation that baculovirus late genes employed a novel late promoter element, suggested that there was a distinct RNA polymerase involved in the expression of baculovirus late genes. Using a transient assay system that was dependent upon transcription from a late promoter element to express a reporter gene led to the identification of baculovirus genes that are involved in late gene transcription (15, 16). This assay

eventually implicated 19 genes in late transcription; however, since the assay was dependent upon DNA replication, it included genes that were involved in that process. The DNA replication genes were identified using a separate assay (see Chapter 5), and the genes involved in late transcription were inferred by subtracting the replication genes. Subsequently, a protein complex of about 560 kDa was isolated that was able to support in vitro transcription from late promoter-containing DNA templates. This complex includes late expression factors (LEF) -4, -8, -9, and p47 with predicted molecular masses of 54, 102, 55 and 47 kDa, respectively. The combined predicted molecular mass of these four proteins is about 260 kDa. Because they appear to be present in equimolar amounts, it was suggested that the baculovirus RNA polymerase complex contains two molecules of each peptide (17). The significance of this is unclear, as such a subunit composition has not been reported for other RNA polymerases. Homologs of these gene products are present in all baculovirus genomes that have been sequenced. Two of these subunits (LEF-8 and -9) have significant levels of similarity to the two largest subunits found in bacterial and eukaryotic polymerases, respectively (Figure 2). These are called β and β' subunits, but they can vary greatly in size and consequently their size may be independent of their lineage.

LEF-9 is related to the large RNA polymerase subunits. LEF 9 contains a 7-amino acid motif (NTDCDGD) similar to the Mg++ binding sequence (NADFDGD) found in the catalytic center in the large RNA polymerase subunits of DNA-dependent RNA polymerases (18) (Figure 2). The D residues are critical components of the sequences because they coordinate the binding of Mg++ that is necessary for the activity of the polymerase. These three residues are conserved in all the orthologous large subunit sequences. AcMNPV LEF-9, at 516 amino acids, is much smaller than the other large RNA polymerase subunits, e.g., that of *Drosophila* is almost 1900 amino acids. Alignments indicate that LEF-9 is most closely related to the N-terminal 60% of the larger orthologous subunits. However, the homology is very low and other domains found conserved in this subunit from bacteria through eukaryotes and vaccinia virus (19) have not been reported in the baculovirus protein.

LEF-8 is related to the second largest RNA polymerase subunit. LEF-8 shows limited homology with the second largest subunit of a number of other organisms at the conserved 13-amino acid sequence GDKXX(s/g)RHG(q/n)KG(v/i/t). This homology is shown in Figure 2 and compared to the orthologous *D. melanogaster* subunit and has been proposed to be part of the catalytic site (20, 21) (Figure 3).

P47, a unique subunit. The third subunit, P47, was originally identified as the site of a ts mutation that caused a defect in late gene expression (22, 23). It has not been convincingly shown to be related to RNA polymerase subunits from other organisms.

LEF-4 is an RNA capping enzyme. The 5' RNA cap is a novel feature of mRNAs from eukaryotic cells and viruses that is not present in bacteria and archaea, because they lack the necessary enzymes for its synthesis. The cap is added to the 5' end of mRNA and consists of a guanine nucleotide linked to the mRNA with a 5' to 5' triphosphate linkage. The guanosine is methylated at the N-7 position by an enzyme called methyl transferase. It can be further modified by an additional methylation of the 2' hydroxyl groups of the ribose sugars at the 5' end of the mRNA (**Figure 4**). This results in the RNA resembling the 3' end of an RNA molecule -- the 5' carbon of the cap ribose is blocked whereas the 3' position is free. Capping involves an RNA 5' triphosphatase that removes the terminal phosphate of the RNA, a guanylyl transferase to add the guanine, and two different methylases. The first two activities are present in LEF-4 and are also present on a single protein located at the N- and C-termini, respectively, in metazoans and plants. Although having similar activity, LEF-4 is unrelated to this category of capping enzyme, but is a member of the metal dependent group of capping enzymes found in fungi and protozoa (24). The enzymes that are involved in the two methylase reactions are not known, although AcMNPV does encode a methyltransferase homolog (Ac69) that would be capable of carrying out the second methylase reaction (see below).

The 5' cap structure is thought to have several major roles associated with mRNA function. These include: i) nuclear export: it binds a protein that is recognized by the nuclear pore complex and is exported; ii) protection from exoribonucleases: this can be important because the export process can take significant amounts of time

during which the RNA is subjected to exonuclease exposure; iii) promotion of translation: the cap serves to recruit initiation factors, which in turn recruit ribosomes (25). Capping can also be involved in intron excision; however, this may not be a major role in baculoviruses because of the limited amount of splicing that occurs.

Capping in eukaryotes involves an enzyme that associates with the highly repetitive carboxyl terminal domain (CTD) of the β' subunit of RNA polymerase II. Because the baculovirus polymerase lacks a similar domain, it is likely that it evolved to include the enzyme as part of the RNA polymerase complex. Similarly, vaccinia virus also lacks a CTD on its β' subunit, and its capping enzyme is also associated with the RNA polymerase complex, and capping occurs during transcription when the nascent RNA oligomer is about 30 nt long (26); however, it does not appear to be an integral component of the polymerase enzyme.

Assuming that in vitro transcription reactions are free from exoribonuclease, it is not clear why LEF-4 is required for transcription in vitro assays. These assays monitor RNA transcripts that would not need to be capped in order to be detected. This suggests that LEF-4 may play a structural role in the organization of the polymerase subunits or it may have some other function. Furthermore, inactivation of both the LEF-4 RNA triphosphatase domain and the RNA triphosphatase encoded by Ac orf1 did not affect the viability of a double mutant virus. Therefore, if capping is required in cultured cells, these data suggest that a host enzyme can carry out the triphosphatase reaction. In addition, the chemical composition of the baculovirus cap structure does not appear to be identical to the conventional motif (27). However, variant cap structures have been described in at least one other virus (28). Complicating an interpretation of the role of LEF-4 in capping is evidence indicating that the 5' untranslated region of p10 mRNA facilitates translation in a cap independent manner (29). Since commonly used internal ribosome entry sites are apparently not active in certain insect cell systems, the p10 5' untranslated region has been used as a translational enhancer of uncapped mRNA in investigations employing insect cells (30).

Baculovirus protein involved in RNA capping

Ac69, a methyltransferase (MTase). Ac69 encodes a methyltransferase and it is found in most Group I NPV, about one-half the Group II NPV and in the NeseNPV genomes. Ac69 was found to stimulate late gene transcription in a transient assay (31). The gene encodes a protein with RNA Cap (nucleoside-2-O)-methyltransferase activity. AcMNPV, with a null mutation of the gene, replicated normally in cell culture (32). Similar results were observed for a knockout of the homolog (Bm57) in BmNPV (33). Therefore, it is not clear whether a host gene can carry out this function or the viral gene may not be necessary for viral replication in cell culture.

Ac38—a baculovirus decapping enzyme? Ac38 is an ADP-ribose pyrophosphatase (ADPRase), a member of the subfamily of nudix pyrophosphatases. Purified Bm29 also showed ADPRase activity (34). Orthologs are found in all lepidopteran NPV and GV genomes. Proteins of the nudix superfamily are common in all organisms and have been reported in other viruses, including T4 bacteriophage, African swine fever virus (family : Asfarviridae), and poxvirus. An AcMNPV Ac38 deletion mutant was severely compromised and produced BV at 1% the level of wt (35). Vaccinia virus also encodes a nudix protein and it may be important in negatively regulating gene expression by acting as a decapping enzyme (36) by removing the 7-methylguanosine diphosphate. Deletion of the gene in vaccinia resulted in smaller plaques and lower virus yield (37), similar to the *Ac38*-deleted AcMNPV.

The categories of RNA polymerases

There are four major lineages of RNA polymerases that appear to be unrelated. These include: i) RNA-dependent RNA polymerases of RNA viruses, ii) the DNA-dependent RNA polymerases of some phage such as T7, iii) primases that produce short RNA transcripts for priming DNA replication, and iv) the DNA-dependent RNA polymerases of eubacteria, archaea, and eukaryotic cells. The DNA-dependent RNA polymerases of bacteria and

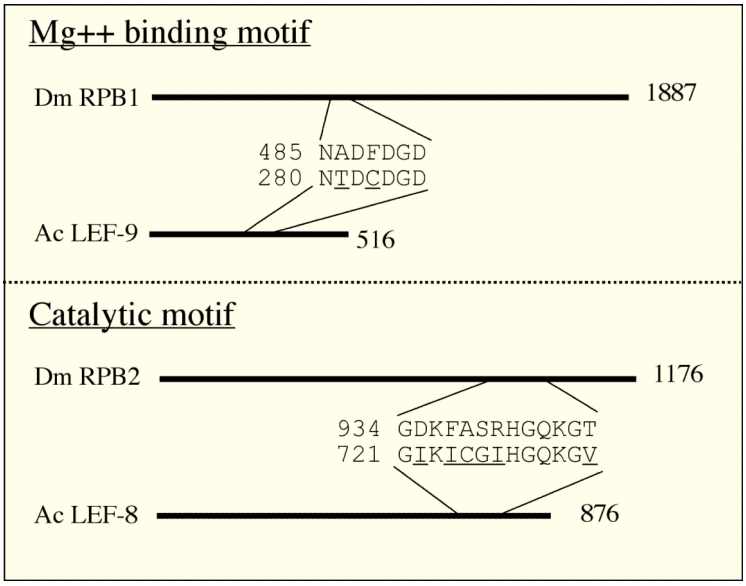

Figure 2. Homology of baculovirus RNA polymerase subunits. The similarity of the LEF-8 and -9 subunits to the two largest RNA polymerase subunits of *Drosophila melanogaster* RNA polymerase II is shown. The numbers at the end of the lines indicate the size of each protein in amino acids. The numbers before the sequences indicate the location of the domain within the sequence. The underlined amino acids are not conserved.

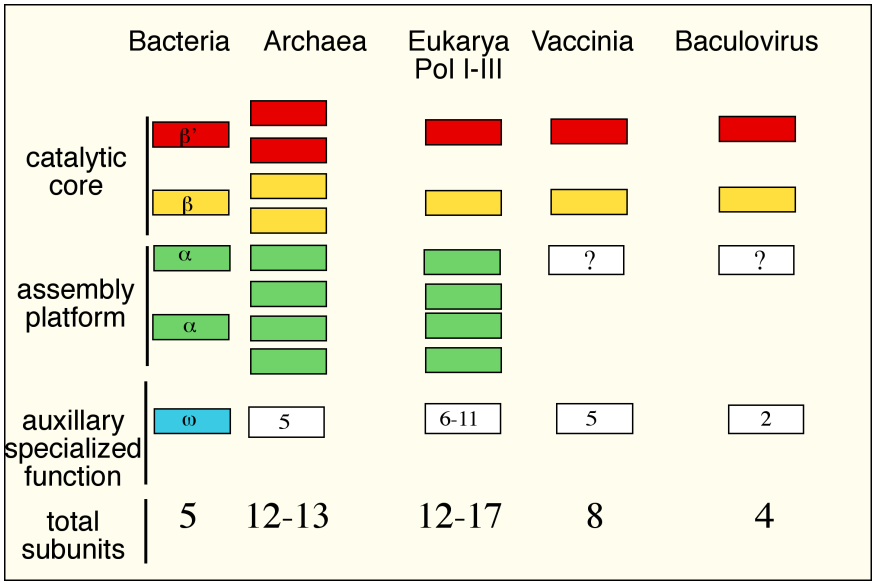

Figure 3. Relationships of RNA polymerase subunits. The large subunits, β' and β, in some archaea have undergone fission and are present as either 3 or 4 components. The subunits are color-coded to indicate similar function. This figure is based on the data of (38, 42, 44, 83, 84). For description of baculovirus homology, see text.

eukaryotes are highly conserved enzymes and are composed in most organisms of 5 to 15 subunits. Four of these correspond to α, β, β' and ω subunits of bacteria and form the conserved core, and orthologs are present in all the cellular enzymes (Figure 3). The β and β' subunits interact with each other, and the active site is formed by their interface [(38) and references therein]. The β' subunit contains a catalytic site and has three invariant aspartate residues that interact with a Mg2+ ion and the α-phosphate of the NTP during polymerization (39). Subunits related to the β and β' polypeptides are also encoded by the genomes of a number of families of large DNA viruses that are pathogenic for eukaryotes (40). The α subunit is involved in the initiation of RNA polymerase assembly and sequence specific protein-DNA interactions that result in promoter recognition, and is

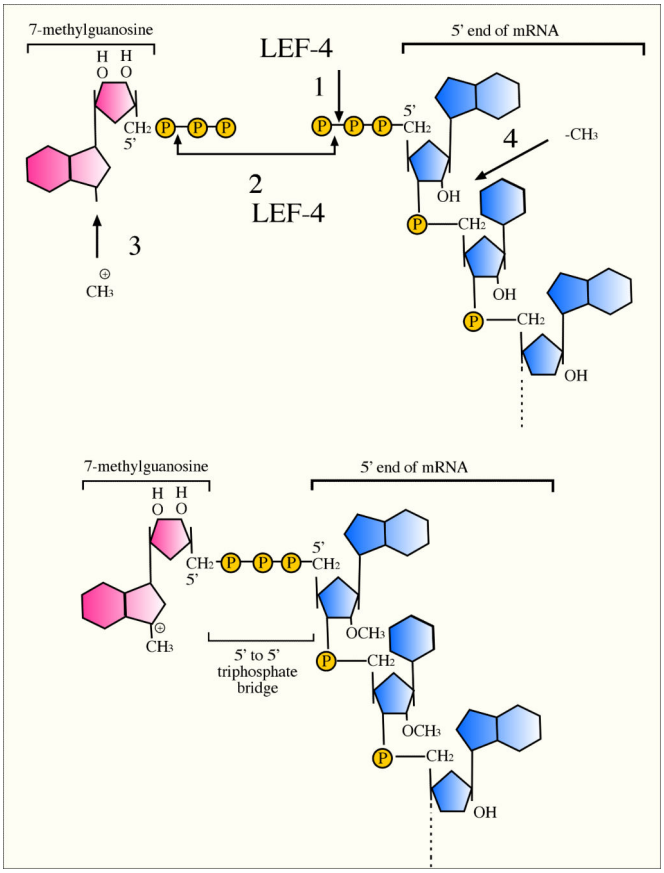

Figure 4. Capping baculovirus mRNA. The four modifications that occur during capping are summarized in the following steps: 1) LEF-4 has the potential to dephosphorylate the 5' end of mRNA; 2) and to transfer guanosine to the 5' end of the mRNA; 3) the guanosine is methylated--enzyme is unknown; 4) The 2' OH group of the terminal A is methylated by an unknown methyltransferase. The final structure is shown at the bottom.

also a target for transcriptional activation (41). Two identical α subunits are present in bacterial RNA polymerase and four homologs of the α subunit are found in the polymerases of archaea and eukarya (Figure 3). Surprisingly, a homolog of the α subunit has not been described for the vaccinia RNA polymerase (42).

In addition to the subunits that comprise the RNA polymerase, transcription of genes often requires other additional factors. Bacterial RNA polymerases recognize specific promoter elements by interacting with sigma factors that specify transcription of specific categories of genes. However, transcription from the eukaryotic RNA polymerases involves a variety of transcription factors, some of which are composed of multiple subunits. Perhaps as a consequence of the size and complexity of eukaryotic polymerases, the promoters to which the polymerases bind and the location of the transcription start sites are separated by 30 bases or more. Although not as complex, the promoter and start site region of bacterial polymerases encompasses up to 35 bp. Finally, the RNA polymerases of phage such as T7 are composed of a single subunit unrelated to those described above, and have a compact polymerase binding RNA start site of about 20-bp (43). In contrast, as described above, both the baculovirus RNA polymerase promoter and mRNA start site are very compact and located at TAAG sequences.

The relationship of baculovirus RNA polymerase to other RNA polymerases

Although there is homology to the β and β' subunits of RNA polymerase, as summarized above, the degree of relatedness is very limited making it difficult to determine the lineage of the baculovirus polymerase. In addition, no other RNA polymerase appears to have a promoter similar in sequence to that of late baculovirus

genes (see above). The initiator sequences of RNA polymerase II (Chapter 4) are similar in that transcription initiates at the promoter sequence. However, the extent of the interaction of the baculovirus RNA polymerase with promoter sequences has not been determined, possibly because the complex is so unstable that it cannot be footprinted using conventional techniques. Analysis of the RNA polymerase subunits of bacteria, archaea and eukaryotes found that they almost all encode four subunits related to the bacterial β and β', α and ω (38). The RNA polymerase of the poxvirus, vaccinia, is similar to those of eukarya as it has a complex subunit composition with at least eight subunits, seven of which appear to be orthologs of RNA polymerase II subunits β and β' plus RPB 5, 6, 7, and 10 and the transcription factor SII (42, 44) (Figure 3). The baculovirus RNA polymerase is distinct from these other major DNA dependent RNA polymerases in that it is composed of four subunits. Since the catalytic cleft is located on the two largest peptides, and baculoviruses possess orthologs of these two subunits, the basic enzyme core is retained. However, based on current analyses, the phylogenetic origin of the baculovirus RNA polymerase is unclear.

In vitro transcription assays

It should be noted that much of the information regarding baculovirus RNA polymerase has been derived from in vitro transcription assays (17, 45) and these systems involve the addition of DNA templates containing late promoter elements. Since this DNA is purified and presumably protein free, it is not clear how well these systems reflect late transcription in infected cells. Future analysis of transcription from templates complexed with proteins may yield information on additional proteins involved in the activation of late transcription. In addition, whereas the baculovirus RNA polymerase complex is limited to four proteins, a number of other proteins are required for late transcription in transient assays (see below).

Termination and polyadenylation of early and late mRNAs

In the study of AcMNPV transcripts in cultured *T. ni* cells (11), it was observed that most early and late orfs have a single polyadenylation site (PAS) located 18-22 nt downstream of a consensus AAUAAA consensus sequence. About 10% of the ORFs had multiple PAS. Also, a number of sets of tandem orfs oriented in the same direction shared the same PAS such that their transcripts, although initiating at different locations, overlapped one or more downstream transcripts, all of which terminated at the same location. For example, there are 7 sets of 5 or more orfs that share a common PAS. The PAS are located in an AT rich region and the 40 nt surrounding the PAS was 80% AT compared to the AcMNPV genome average of (59%). Whereas 3' processing of early transcripts is carried out by the host cell system resulting in polyadenylated transcripts, the situation with late genes has been unclear. It was suggested that late genes transcribed using purified baculovirus RNA polymerase in a cell free system have 3'ends formed by the presence of T-rich sequences that destabilize the late transcription complex (46). However, in cultured cells, most (87%) of the transcripts reflected conventional 3' processing by the host cell downstream of an AAUAAA sequence (Figure 1) (11).

Very late gene expression

Very late factor 1 (VLF-1, Ac77). A novel feature of baculoviruses is their ability to express genes at high levels very late in infection. This phenomenon has been exploited in the development of baculoviruses as expression vectors. Two highly expressed very late genes have been characterized, polyhedrin and p10. Polyhedrin is the occlusion body protein, whereas the role of p10 is not clear, although it appears to form fibrillar structures that may be involved in the assembly of the polyhedron envelope during the maturation of polyhedra (47) (see Chapter 2, Figure 2) and may influence the lysis of terminally infected nuclei (48). Because these genes appear to be involved in polyhedron morphogenesis, which is a very late step in the baculovirus life cycle that occurs after virions destined for occlusion have been assembled, it is likely that they are transcribed from DNA that does not become packaged as virion genomes. Consequently, it remains accessible to the very late RNA polymerase complex. Both polyhedrin and p10 genes contain an A/T-rich sequence downstream of a late promoter sequence

that is involved in their high level expression (49). This sequence was called the 'burst sequence' because it caused a burst of transcription very late in infection. VLF-1 was originally identified because it influences the hyperexpression of very late genes (50). Subsequently, it was found that VLF-1 interacts with the burst sequence in gel shift assays (51) and the presence of this sequence stimulates the level of VLF-1. In vitro transcription assays suggest that VLF-1 can stimulate very late transcription about 10-fold. In addition, when the burst sequence is removed, the level of stimulation is reduced about fourfold (52) (Figure 1). Homologs of VLF-1 are found in all sequenced baculovirus genomes, and they belong to a family of proteins that includes lambda integrase. Integrases are a large group of site-specific DNA recombinases that catalyze DNA rearrangements and are found in a variety of organisms including viruses where they are involved in the integration and excision of viral genomes and decatenation of newly replicated chromosomes. Evidence suggests that VLF-1 may also be involved in the processing or packaging of baculovirus genomes (see Chapter 5).

LEF-2 (Ac6) and PK-1 (Ac10). In addition to VLF-1, LEF-2 (Ac6) appears to be involved in very late transcription. Although it is an essential replication gene where it apparently functions as a primase accessory factor (see Chapter 5), LEF-2 mutants of AcMNPV have been characterized that appear to affect very late transcription, indicating that it may have roles in both replication and transcription (53). In BmNPV, LEF-2 has also been shown to activate late transcription (54) independent of its influence on DNA replication. It has been suggested that Ac10, which encodes a serine/threonine kinase (called PK-1), also influences very late gene expression. Orthologs of Ac10 are found in all lepidopteran baculovirus genomes. The inhibition of PK-1 expression by either a ts mutation (55) or by DNAzyme technology causes a reduction in the expression from the polyhedrin promoter (56). PK-1 also may be associated with a very late transcription complex and be involved in the phosphorylation of LEF-8 (57). PK1 of SpltNPV-I and AcMNPV may interact with the polyhedrin promoter, suggesting that it might act as a very late gene transcription factor (58) (59).

Other genes involved in late transcription

With the removal of the six essential replication genes and the four genes encoding the late RNA polymerase complex from the total of 19 genes that have been implicated in influencing late gene transcription (16), the roles of nine genes are left unexplained. These genes are required for or stimulatory for late transcription in the transient assay system.

P35, LEF-7, LEF-11, and IE2. P35 blocks apoptosis and therefore, by preserving the viability of cells, promotes both viral DNA replication and late transcription. LEF-7 is stimulatory for DNA replication and deletion of *lef-7* from the AcMNPV genome led to a major reduction in late gene expression and a 100-fold reduction in infectious virus production. It was suggested that LEF-7 is involved in the regulation of the host cell DNA damage response (DDR) and diverts host DDR proteins from cellular chromatin, so that they can be exploited for viral DNA replication (60) (see Chapter 5). LEF-11 was not identified as being involved in DNA replication in the transient assay system, however a *lef-11* knockout construct is defective in DNA synthesis (see Chapter 5). IE-2 may influence late transcription because of its role in the activation of early transcription (see Chapter 4). Therefore, LEF-7 and IE-2 could affect late gene transcription by influencing DNA replication. This leaves five genes that may specifically influence late gene transcription. These are described below.

Lef-5 (Ac99), a possible ortholog of TFIIS. Orthologs of *lef-5* are found in all baculoviruses and are also present in nudiviruses (61). LEF-5 was originally identified as being required for transient late gene transcription (62). It was demonstrated to interact with itself and to contain a domain similar to that of the RNA polymerase II elongation factor TFIIS (63). Subsequent investigations indicated that LEF-5, although highly stimulatory for in vitro transcription, did not enable the baculovirus polymerase to transit pause sites, and it was concluded that it functions as an initiation factor, rather than an elongation factor (64). Deletion is probably lethal as interrupted mutants of this gene in BmNPV (Bm83) could not be isolated (65).

Lef-6 (Ac28), a possible mRNA export factor. Homologs of *lef-6* are found in the genomes of all lepidopteran NPVs and GVs. It was originally identified because it was required for transient transcription of late genes (66). A bacmid deleted for lef-6 was infectious, but the infection was delayed and the titer was reduced to about 10% of wt. The major effect appeared to be reflected in a delay in the onset of late transcription (67). LEF-6 is not particularly well conserved. Although AcMNPV and LdMNPV showed only 27% amino acid sequence identity, using the HHpred program described above, both showed over 80% probability of encoding a region that is related to the RNA binding domain of a factor called TAP that is involved in the export of mRNA out of nuclei through their interaction with nuclear pore proteins (68, 69). If LEF-6 performs a similar function, it might compensate for the nuclear transport pathway if it is compromised by the infection. It might also be essential for the transport of the high levels of mRNA produced by very late genes. BmNPV LEF-6 is highly phosphorylated during infection (70).

Lef-10 (Ac53a); a prion? This orf was named Ac53a because it was not identified in the original AcMNPV genome sequence because it is a small orf encoding 78 aa and about half the coding region at the 3' end overlaps the 5' region of Ac 54. Homologs of lef-10 are found in all Group I and most Group II NPV and GV genomes. Lef-10 was originally identified because it was required for late gene expression (18). It is likely an essential gene as an insertion mutant in the BmNPV homolog (Bm42a) could not be isolated (65). When linked to EGFP, Lef-10 formed punctate spots (71). It has been reported that LEF10 behaves as a prion (72). The full-length protein or its predicted prion-forming domain can functionally replace the prion domain of the prion, Sup35, of yeast. A high multiplicity of infection can lead to conversion of LEF10 to an aggregated state that inhibits late gene expression (71, 72).

Ac41 (Lef-12). Lef-12 is found in about half the Group I and Group II NPV genomes sequenced. Although 18 genes were originally identified as being involved in transient expression from a late promoter (15), when a set of these genes were individually cloned, they failed to support late transcription. Because of its close proximity to Ac 40 (p47), Ac41 (*lef-12*) had not been identified in the initial screen. It was subsequently demonstrated to be required for transient late gene transcription *in S. frugiperda* cells (16, 31), but not required for late transcription in *T. ni* cells (16). Mutants with *lef-12* interrupted by insertional mutagenesis or by mutation of the ATG translation initiation codon were viable in both *S. frugiperda* and *T. ni* cells, although reduced yields of BV were observed (20-40% of wt) in both cell lines, and the infection cycle appear to be slowed (73). It was suggested that *lef-12* may be functionally redundant in the AcMNPV genome, and therefore it is not essential for late transcription when the rest of the virus genome is present (73). Analysis by Hhpred (74, 75) predicts that Lef-12 may be structurally related to TFIIA with a probability of about 70%. TFIIA is involved in transcriptional initiation of RNA polymerase II (76).

pp31 (39K -Ac36). Pp31 was originally identified because it contains an early promoter that is stimulated by IE-1 (77). Homologs are present in all lepidopteran NPV and GV genomes. It is phosphorylated and localizes to the virogenic stroma of infected cells, and is capable of binding to DNA, but is not a virion structural protein (78). Purified PP31 binds to single-stranded and double-stranded DNA with equal affinities and inhibited transcription in vitro (79). Phosphorylation of PP31 is a dynamic process (80). Several basic regions appeared to be involved in nuclear localization, and one of these regions is involved in DNA binding (81). It was found to stimulate late gene transcription in a transient transcription assay (15). Deletion of the *pp31* homolog in BmNPV (Bm27) resulted in virus that, although viable, showed a reduction in late gene transcription, a 100-fold reduction in BV production, and improper formation of the virogenic stroma (65). Similar results were obtained for an AcMNPV bacmid deleted for *pp31,* and it was observed that the deletion resulted in a significant decrease of the transcription of several late genes (82). It is not clear whether this gene acts directly on late transcription or may be a structural protein of the virogenic stroma and is required for the optimal organization of this structure, thereby indirectly influencing late transcription.

How can baculoviruses express very late genes at such high levels?

There is no evidence that the baculovirus RNA polymerase has an intrinsic ability for high level gene expression. In fact, it may not bind to the very late promoter region with high levels of tenacity as reflected in the fact that the footprint of the polymerase on genomic DNA has never been reported despite an extensive effort by at least one laboratory (Rohrmann, unpublished).

It is likely that high levels of gene expression are influenced by several features of baculovirus biology. These include: i) the amplification of genes by DNA replication (see Chapter 5); ii) the shutoff of most late transcription, possibly by DNA binding proteins that coat the DNA and thereby make RNA polymerase available for very late transcription; iii) the efficiency of the late polymerase and VLF-1 in recognizing and initiating from very late promoter elements; iv) the efficiency of LEF-4 in capping the mRNA (see Figure 4), and v) a possible role for LEF-2 and PK-1. As mentioned above, the 5' untranslated region of p10 mRNA appears to be capable of facilitating cap-independent translation, which may reduce the reliance of these transcripts on LEF-4 activity (29). Other factors that might enhance translation of very late expressed mRNAs have not been identified. For a detailed discussion of the factors influencing very late transcription, see Chapter 10.

References

1. Keck JG, Baldick CJ, Moss B. Role of DNA replication in vaccinia virus gene expression: A naked template is required for transcription of three late trans-activator genes. Cell. 1990;61:801–809. PubMed PMID: 2344616.
2. Brody EN, Kassavetis GA, Ouhammouch M, Sanders GM, Tinker RL, Geiduschek EP. Old phage, new insights: two recently recognized mechanisms of transcriptional regulation in bacteriophage T4 development. FEMS Microbiol Lett. 1995;128:1–8. PubMed PMID: 7744235.
3. Hooft van Iddekinge BJL, Smith GE, Summers MD. Nucleotide sequence of the polyhedrin gene of *Autographa californica* nuclear polyhedrosis virus. Virology. 1983;131:561–565. PubMed PMID: 18639177.
4. Kuzio JD, Rohel Z, Curry CJ, Krebs A, Carstens EB, Faulkner P. Nucleotide sequence of the p10 polypeptide gene of *Autographa californica* nuclear polyhedrosis virus. Virology. 1984;139:414–418. PubMed PMID: 18639833.
5. Iatrou K, Ito K, Witkiewicz H. Polyhedrin gene of Bombyx mori nuclear polyhedrosis virus. J Virol. 1985;54:436–445. PubMed PMID: 3886932.
6. Chakerian R, Rohrmann GF, Nesson MH, Leisy DJ, Beaudreau GS. The nucleotide sequence of the Pieris brassicae granulosis virus granulin gene. J Gen Virol. 1985. PubMed PMID: 3891914.
7. Akiyoshi D, Chakerian R, Rohrmann GF, Nesson MH, Beaudreau GS. Cloning and sequencing of the granulin gene from the *Trichoplusia ni* granulosis virus. Virology. 1985;141:328–332. PubMed PMID: 4082501.
8. Rohrmann GF. Polyhedrin structure. J Gen Virol. 1986;67:1499–1513. PubMed PMID: 3525744.
9. Rankin C, Ooi BG, Miller LK. Eight base pairs encompassing the transcriptional start point are the major determinant for baculovirus polyhedrin gene expression. Gene. 1988;70:39–49. PubMed PMID: 3071492.
10. Xing K, Deng R, Wang J, Feng J, Huang M, Wang X. Analysis and prediction of baculovirus promoter sequences. Virus Res. 2005;113:64–71. PubMed PMID: 15908030.
11. Chen YR, Zhong S, Fei Z, Hashimoto Y, Xiang JZ, Zhang S, Blissard GW. The transcriptome of the baculovirus Autographa californica Multiple Nucleopolyhedrovirus (AcMNPV) in Trichoplusia ni cells. J Virol. 2013;87:6391–405. PubMed PMID: 23536684.
12. Li Y, Hall RL, Yuan SL, Moyer RW. High-level expression of Amsacta moorei entomopoxvirus spheroidin depends on sequences within the gene. J Gen Virol. 1998;79(Pt 3):613–22. PubMed PMID: 9519842.
13. Grula MA, Buller PL, Weaver RF. Alpha amanitin-resistant viral RNA synthesis in nuclei isolated from nuclear polyhedrosis virus-infected *Heliothis zea* larvae and *Spodoptera frugiperda* cells. J Virol. 1981;38:916–921. PubMed PMID: 16789208.

14. Fuchs YL, Woods MS, Weaver RF. Viral transcription during Autographa californica nuclear polyhedrosis virus infection: A novel RNA polymerase induced in infected Spodoptera frugiperda cells. J Virol. 1983;48:641–646. PubMed PMID: 16789246.
15. Todd JW, Passarelli AL, Miller LK. Eighteen baculovirus genes, including *lef-11, p35, 39K*, and *p47*, support late gene expression. J Virol. 1995;69:968–974. PubMed PMID: 7815564.
16. Rapp JC, Wilson JA, Miller LK. Nineteen baculovirus open reading frames, including LEF-12, support late gene expression. J Virol. 1998;72:10197–206. PubMed PMID: 9811761.
17. Guarino LA, Xu B, Jin J, Dong W. A virus-encoded RNA polymerase purified from baculovirus-infected cells. J Virol. 1998;72:7985–7991. PubMed PMID: 9733837.
18. Lu A, Miller LK. Identification of three late expression factor genes within the 33.8- to 43.4-map-unit region of *Autographa californica* nuclear polyhedrosis virus. J Virol. 1994;68:6710–6718. PubMed PMID: 8084003.
19. Broyles SS, Moss B. Homology between RNA polymerases of poxviruses, prokaryotes, and eukaryotes: nucleotide sequence and transcriptional analysis of vaccinia virus genes encoding 147-kDa and 22-kDa subunits. Proc Natl Acad Sci U S A. 1986;83:3141–5. PubMed PMID: 3517852.
20. Passarelli AL, Todd JW, Miller LK. A baculovirus gene involved in late gene expression predicts a large polypeptide with a conserved motif of RNA polymerases. J Virol. 1994;68:4673–4678. PubMed PMID: 8207843.
21. Titterington JS, Nun TK, Passarelli AL. Functional dissection of the baculovirus late expression factor-8 gene: sequence requirements for late gene promoter activation. J Gen Virol. 2003;84:1817–26. PubMed PMID: 12810876.
22. Partington S, Yu H, Lu A, Carstens EB. Isolation of temperature sensitive mutants of Autographa californica nuclear polyhedrosis virus: phenotype characterization of baculovirus mutants defective in very late gene expression. Virology. 1990;175:91–102. PubMed PMID: 2408231.
23. Carstens EB, Lu AL, Chan HLB. Sequence, transcriptional mapping, and overexpression of p47, a baculovirus gene regulating late gene expression. J Virol. 1993;67:2513–2520. PubMed PMID: 8474157.
24. Gross CH, Shuman S. RNA 5'-triphosphatase, nucleoside triphosphatase, and guanylyltransferase activities of baculovirus LEF-4 protein. J Virol. 1998;72:10020–8. PubMed PMID: 9811740.
25. Shuman S. What messenger RNA capping tells us about eukaryotic evolution. Nat Rev Mol Cell Biol. 2002;3:619–25. PubMed PMID: 12154373.
26. Hagler J, Shuman S. A freeze-frame view of eukaryotic transcription during elongation and capping of nascent mRNA. Science. 1992;255:983–6. PubMed PMID: 1546295.
27. Li Y, Guarino LA. Roles of LEF-4 and PTP/BVP RNA triphosphatases in processing of baculovirus late mRNAs. J Virol. 2008;82:5573–83. PubMed PMID: 18385232.
28. Ogino T, Banerjee AK. Formation of guanosine(5')tetraphospho(5')adenosine (GppppA) cap structure by an unconventional mRNA capping enzyme of vesicular stomatitis virus. J Virol. 2008;82:7729–34. PubMed PMID: 18495767.
29. Scheper GC, Vries RG, Broere M, Usmany M, Voorma HO, Vlak JM, Thomas AA. Translational properties of the untranslated regions of the p10 messenger RNA of Autographa californica multicapsid nucleopolyhedrovirus. J Gen Virol. 1997;78:687–96. PubMed PMID: 9049423.
30. Kohl A, Hart TJ, Noonan C, Royall E, Roberts LO, Elliott RM. A bunyamwera virus minireplicon system in mosquito cells. J Virol. 2004;78:5679–85. PubMed PMID: 15140965.
31. Li L, Harwood SH, Rohrmann GF. Identification of additional genes that influence baculovirus late gene expression. Virology. 1999;255:9–19. PubMed PMID: 10049816.
32. Wu X, Guarino LA. Autographa californica nucleopolyhedrovirus orf69 encodes an RNA cap (nucleoside-2'-O)-methyltransferase. J Virol. 2003;77:3430–40. PubMed PMID: 12610118.
33. Ono C, Kamagata T, Taka H, Sahara K, Asano S, Bando H. Phenotypic grouping of 141 BmNPVs lacking viral gene sequences. Virus Res. 2012;165:197–206. PubMed PMID: 22421381.
34. Chen HQ, Zhou YJ, Chen KP, Yu Q. Expression and functional validation of Bombyx mori nucleopolyhedrovirus ORF29, a conserved Nudix motif protein. Acta Virol. 2013;57:442–6. PubMed PMID: 24294958.

35. Ge J, Wei Z, Huang Y, Yin J, Zhou Z, Zhong J. AcMNPV ORF38 protein has the activity of ADP-ribose pyrophosphatase and is important for virus replication. Virology. 2007;361:204–11. PubMed PMID: 17174373.
36. Parrish S, Resch W, Moss B. Vaccinia virus D10 protein has mRNA decapping activity, providing a mechanism for control of host and viral gene expression. Proc Natl Acad Sci U S A. 2007;104:2139–44. PubMed PMID: 17283339.
37. Parrish S, Moss B. Characterization of a vaccinia virus mutant with a deletion of the D10R gene encoding a putative negative regulator of gene expression. J Virol. 2006;80:553–61. PubMed PMID: 16378957.
38. Iyer LM, Koonin EV, Aravind L. Evolutionary connection between the catalytic subunits of DNA-dependent RNA polymerases and eukaryotic RNA-dependent RNA polymerases and the origin of RNA polymerases. BMC Struct Biol. 2003;3:1. PubMed PMID: 12553882.
39. Wang D, Bushnell DA, Westover KD, Kaplan CD, Kornberg RD. Structural basis of transcription: role of the trigger loop in substrate specificity and catalysis. Cell. 2006;127:941–54. PubMed PMID: 17129781.
40. Iyer LM, Balaji S, Koonin EV, Aravind L. Evolutionary genomics of nucleo-cytoplasmic large DNA viruses. Virus Res. 2006;117:156–84. PubMed PMID: 16494962.
41. Ebright RH, Busby S. The Escherichia coli RNA polymerase alpha subunit: structure and function. Curr Opin Genet Dev. 1995;5:197–203. PubMed PMID: 7613089.
42. Knutson BA, Broyles SS. Expansion of poxvirus RNA polymerase subunits sharing homology with corresponding subunits of RNA polymerase II. Virus Genes. 2008;36:307–11. PubMed PMID: 18264749.
43. Turingan RS, Liu C, Hawkins ME, Martin CT. Structural confirmation of a bent and open model for the initiation complex of T7 RNA polymerase. Biochemistry. 2007;46:1714–23. PubMed PMID: 17253774.
44. Broyles SS. Vaccinia virus transcription. J Gen Virol. 2003;84:2293–303. PubMed PMID: 12917449.
45. Glocker B, Hoopes RR Jr, Hodges L, Rohrmann GF. In vitro transcription from baculovirus late gene promoters: accurate mRNA initiation by nuclear extracts prepared from infected *Spodoptera frugiperda* cells. J Virol. 1993;67:3771–3776. PubMed PMID: 8510206.
46. Jin J, Guarino LA. 3'-end formation of baculovirus late RNAs. J Virol. 2000;74:8930–8937. PubMed PMID: 10982336.
47. Russell RLQ, Pearson MN, Rohrmann GF. Immunoelectron microscopic examination of *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus-infected *Lymantria dispar* cells: time course and localization of major polyhedron-associated proteins. J Gen Virol. 1991;72:275–283. PubMed PMID: 1993872.
48. van Oers MM, Vlak JM. The baculovirus 10-kDa protein. J Invertebr Pathol. 1997;70:1–17. PubMed PMID: 9217463.
49. Ooi BG, Rankin C, Miller LK. Downstream sequences augment transcription from the essential initiation site of a baculovirus polyhedrin gene. J Mol Biol. 1989;210:721–736. PubMed PMID: 2693741.
50. McLachlin JR, Miller LK. Identification and characterization of vlf-1, a baculovirus gene involved in very late gene expression. J Virol. 1994;68:7746–7756. PubMed PMID: 7966564.
51. Yang S, Miller LK. Activation of baculovirus very late promoters by interaction with very late factor 1. J Virol. 1999;73:3404–9. PubMed PMID: 10074194.
52. Mistretta TA, Guarino LA. Transcriptional activity of baculovirus very late factor 1. J Virol. 2005;79:1958–60. PubMed PMID: 15650223.
53. Merrington CL, Kitts PA, King LA, Possee RD. An Autographa californica nucleopolyhedrovirus lef-2 mutant: consequences for DNA replication and very late gene expression. Virology. 1996;217:338–348. PubMed PMID: 8599220.
54. Sriram S, Gopinathan KP. The potential role of a late gene expression factor, lef2, from Bombyx mori nuclear polyhedrosis virus in very late gene transcription and DNA replication. Virology. 1998;251:108–22. PubMed PMID: 9813207.
55. Fan X, Thirunavukkarasu K, Weaver RF. Temperature-sensitive mutations in the protein kinase-1 (*pk-1*) gene of the *Autographa californica* nuclear polyhedrosis virus that block very late gene expression. Virology. 1996;224:1–9. PubMed PMID: 8862393.

56. Mishra G, Chadha P, Chaudhury I, Das RH. Inhibition of Autographa californica nucleopolyhedrovirus (AcNPV) polyhedrin gene expression by DNAzyme knockout of its serine/threonine kinase (pk1) gene. Virus Res. 2008;135:197–201. PubMed PMID: 18353480.
57. Mishra G, Chadha P, Das RH. Serine/threonine kinase (pk-1) is a component of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) very late gene transcription complex and it phosphorylates a 102kDa polypeptide of the complex. Virus Res. 2008;137:147–9. PubMed PMID: 18577405.
58. Mishra G, Gautam HK, Das RH. Serine/Threonine kinase dependent transcription from the polyhedrin promoter of SpltNPV-I. Biochem Biophys Res Commun. 2007;358:942–7. PubMed PMID: 17512903.
59. Liang C, Su X, Xu G, Dai X, Zhao S. Autographa californica multiple nucleopolyhedrovirus PK1 is a factor that regulates high-level expression of very late genes in viral infection. Virology. 2017;512:56–65. PubMed PMID: 28915406.
60. Mitchell JK, Byers NM, Friesen PD. Baculovirus F-Box Protein LEF-7 Modifies the Host DNA Damage Response To Enhance Virus Multiplication. J Virol. 2013;87:12592–9. PubMed PMID: 24027328.
61. Wang Y, Kleespies RG, Huger AM, Jehle JA. The genome of Gryllus bimaculatus nudivirus indicates an ancient diversification of baculovirus-related nonoccluded nudiviruses of insects. J Virol. 2007;81:5395–406. PubMed PMID: 17360757.
62. Passarelli AL, Miller LK. Identification of genes encoding late expression factors located between 56.0 and 65.4 map units of the Autographa californica nuclear polyhedrosis virus genome. Virology. 1993;197:704–714. PubMed PMID: 8249293.
63. Harwood SH, Li L, Ho PS, Preston AK, Rohrmann GF. AcMNPV late expression factor-5 interacts with itself and contains a zinc ribbon domain that is required for maximal late transcription activity and is homologous to elongation factor TFIIS. Virology. 1998;250:118–134. PubMed PMID: 9770426.
64. Guarino LA, Dong W, Jin J. In vitro activity of the baculovirus late expression factor LEF-5. J Virol. 2002;76:12663–75. PubMed PMID: 12438592.
65. Gomi S, Zhou CE, Yih W, Majima K, Maeda S. Deletion analysis of four of eighteen late gene expression factor gene homologues of the baculovirus, BmNPV. Virology. 1997;230:35–47. PubMed PMID: 9126260.
66. Passarelli AL, Miller LK. Identification and transcriptional regulation of the baculovirus lef-6 gene. J Virol. 1994;68:4458–4467. PubMed PMID: 8207819.
67. Lin G, Blissard GW. Analysis of an Autographa californica multicapsid nucleopolyhedrovirus lef-6-null virus: LEF-6 is not essential for viral replication but appears to accelerate late gene transcription. J Virol. 2002;76:5503–14. PubMed PMID: 11991978.
68. Liker E, Fernandez E, Izaurralde E, Conti E. The structure of the mRNA export factor TAP reveals a cis arrangement of a non-canonical RNP domain and an LRR domain. Embo J. 2000;19:5587–98. PubMed PMID: 11060011.
69. Matzat LH, Berberoglu S, Levesque L. Formation of a Tap/NXF1 Homotypic Complex Is Mediated through the Amino-Terminal Domain of Tap and Enhances Interaction with Nucleoporins. Mol Biol Cell. 2008;19:327–38. PubMed PMID: 17978099.
70. Shobahah J, Xue S, Hu D, Zhao C, Wei M, Quan Y, Yu W. Quantitative phosphoproteome on the silkworm (Bombyx mori) cells infected with baculovirus. Virol J. 2017;14:117. PubMed PMID: 28629377.
71. Xu X, Zhou X, Nan H, Zhao Y, Bai Y, Ou Y, Chen H. Aggregation of AcMNPV LEF-10 and Its Impact on Viral Late Gene Expression. PLoS One. 2016;11:e0154835. PubMed PMID: 27152613.
72. Nan H, Chen H, Tuite MF, Xu X. A viral expression factor behaves as a prion. Nat Commun. 2019;10:359. PubMed PMID: 30664652.
73. Guarino LA, Mistretta TA, Dong W. Baculovirus lef-12 is not required for viral replication. J Virol. 2002;76:12032–43. PubMed PMID: 12414945.
74. Soding J. Protein homology detection by HMM-HMM comparison. Bioinformatics. 2005;21:951–60. PubMed PMID: 15531603.
75. Zimmermann L, Stephens A, Nam SZ, Rau D, Kubler J, Lozajic M, Gabler F, Soding J, Lupas AN, Alva V. A Completely Reimplemented MPI Bioinformatics Toolkit with a New HHpred Server at its Core. J Mol Biol. 2018;430:2237–2243. PubMed PMID: 29258817.

76. Louder RK, He Y, Lopez-Blanco JR, Fang J, Chacon P, Nogales E. Structure of promoter-bound TFIID and model of human pre-initiation complex assembly. Nature. 2016;531:604–9. PubMed PMID: 27007846.
77. Guarino LA, Summers MD. Functional mapping of a trans-activating gene required for expression of a baculovirus delayed-early gene. J Virol. 1986;57:563–57l. PubMed PMID: 3944847.
78. Guarino LA, Dong W, Xu B, Broussard DR, Davis RW, Jarvis DL. The baculovirus phosphoprotein pp31 is associated with the virogenic stroma. J Virol. 1992;66:7113–7120. PubMed PMID: 1433508.
79. Guarino LA, Mistretta TA, Dong W. DNA binding activity of the baculovirus late expression factor PP31. Virus Res. 2002;90:187–95. PubMed PMID: 12457973.
80. Broussard DR, Guarino LA, Jarvis DL. Dynamic phosphorylation of Autographa californica nuclear polyhedrosis virus pp31. J Virol. 1996;70:6767–74. PubMed PMID: 8794314.
81. Broussard DR, Guarino LA, Jarvis DL. Mapping functional domains in AcMNPV pp31. Virology. 1996;222:318–31. PubMed PMID: 8806516.
82. Yamagishi J, Burnett ED, Harwood SH, Blissard GW. The AcMNPV pp31 gene is not essential for productive AcMNPV replication or late gene transcription but appears to increase levels of most viral transcripts. Virology. 2007;365:34–47. PubMed PMID: 17467768.
83. Werner F. Structure and function of archaeal RNA polymerases. Mol Microbiol. 2007;65:1395–404. PubMed PMID: 17697097.
84. Minakhin L, Bhagat S, Brunning A, Campbell EA, Darst SA, Ebright RH, Severinov K. Bacterial RNA polymerase subunit omega and eukaryotic RNA polymerase subunit RPB6 are sequence, structural, and functional homologs and promote RNA polymerase assembly. Proc Natl Acad Sci U S A. 2001;98:892–7. PubMed PMID: 11158566.

7. Baculovirus infection: The cell cycle and apoptosis

The cell cycle and apoptosis are interrelated. If a cell detects perturbations in different stages of its replicative cycle such as those caused by viral infection, the cell can be induced to undergo apoptosis. Because baculovirus replication causes major alterations in the cell cycle, it has evolved several mechanisms to interfere with apoptosis.

Baculovirus infection and the cell cycle

Cell division involves a cycle of defined stages during which DNA precursors and other cellular components are synthesized, chromosomes are duplicated and segregated to opposite poles of a cell during mitosis, followed by division of the cell. These stages in cell growth have been named gap or G phases (G1, G2, and G0), S for the synthetic phase, and M for the mitotic phase (Figure 1). G1 follows mitosis, during which the cell is prepared for DNA replication by the production of synthetic enzymes; S follows G1 and involves the DNA synthesis phase during which chromosomes are duplicated; G2 follows S and involves preparation for cell division, which occurs in M or the mitosis phase. If a cell becomes dormant and is not in a replication cycle it is in stationary or G0 phase. The cell cycle is highly regulated, and the orderly progression through each phase is critical for chromosome replication, separation, and production of daughter cells. The transitions between the different stages of the cycle are governed by the phosphorylation state of a number of proteins called cyclins. They are phosphorylated by cyclin dependent kinases (cdks) (Figure 1). Progress through the cell cycle is also governed by molecular checkpoints that can prevent the continuation of the cell cycle at specific phases. Deregulation of the cell cycle can result in uncontrolled cell proliferation, a hallmark of cancer development. Consequently, regulation of the cell cycle has been intensively investigated.

The cell cycle can be monitored experimentally by measuring the DNA content in each cell in a population by flow cytometry. In this process cells are fixed in ethanol, RNA is removed by treatment with RNase, and the DNA is stained with propidium iodide and quantified for each cell by flow cytometry. Two major populations of cells are normally present, those in G1 phase that contain a set of diploid chromosomes, and those in G2 and M phase (G2/M), in which the chromosomes are doubled and have twice the DNA content of diploid cells. Normally, a diffuse population of cells in S phase is present, and unless the cells have been synchronized, it is not particularly distinct.

Many viruses block the cell cycle (1). This may seem logical, because it would block competition by a dividing cell for the cellular replicative apparatus and components and allow these to be directed towards viral replication. However, blockage of the cell cycle can result in very low levels of enzymes required for transcription and DNA synthesis because they are no longer needed for cell division. For example, in some cells replicative DNA polymerases and other factors associated with DNA replication are amplified threefold or more during S-phase (2). A number of DNA viruses do not encode the substrates required for DNA synthesis. When these viruses infect cells that are in interphase and are not undergoing cell division, there is a reduced availability of compounds necessary for DNA synthesis. Consequently, viruses have devised several methods to induce nonreplicating cells to either enter S phase or to induce a pseudo S phase in which the cellular synthetic machinery is activated, allowing for virus replication independent of cell replication. Some viruses can induce cells to enter S phase by expressing proteins that inactivate a protein called Rb. It is named for the retinoblastoma susceptibility gene that was first implicated in cancer of the retina, a disease that usually occurs before the age of five in about 1 in every 20,000 children. In this tumor, the Rb protein is inactivated and that led to its discovery. Rb governs the G1/S check point by binding to a transcription factor (E2F). If Rb binds to certain viral proteins, it releases the transcription factor (E2F) that can then activate S phase. Normally Rb is regulated by its phosphorylation state—it binds to the S-phase activator when it is not phosphorylated. The inactivation of Rb can lead not only to the entry of the cell into S phase, but to unregulated cell growth and division, which is a characteristic feature of virus-induced tumors. Therefore, the byproduct of the necessity of the virus to induce S phase can be a tumor. This is especially true when the portion of the virus genome encoding the inhibitor of Rb

integrates into the cell genome, thereby permanently deregulating the cell cycle. Examples of viruses capable of causing this mutation are tumor viruses, such as human papilloma, SV40, and adenovirus.

Another checkpoint governs the transition from G2 to M. It is thought that regulation of this transition is involved in monitoring for DNA damage. If DNA damage is detected, then progression into mitosis is inhibited and the cell may undergo apoptosis. This prevents the establishment of cells with defective DNA genomes. Many viruses appear to block the cell cycle at G2/M, thereby rendering the cells unable to divide (1). This may be caused by the cell checkpoint monitoring systems that detect viral DNA replication and regards it as damaged cellular DNA and thereby stops the transition to metaphase. Concomitant with blocking the transition to M phase, a number of viruses appear to induce what is called a 'pseudo' S phase. Baculoviruses likely fall into this category as several reports have indicated that infection causes cells to be blocked at G2/M. One report found that most non-synchronized cells were blocked at G2/M after AcMNPV infection (3). In another report, synchronized Sf9 cells infected in G2/M were arrested in that stage, whereas those infected at G1 were arrested in S phase (4). In yet another study it was reported that cells infected at G2/M, G1, or S phases, were arrested in the S or G2/M phase (5). Investigations on infection with another virus, HaSNPV, in a different cell line, also resulted in a block at G2/M. Normally cyclin B1 is degraded at the onset of metaphase allowing progression through this phase, however in these cells it accumulates and remained at high levels (6). An AcMNPV protein (Ac144) was identified that may contribute to the blockage at G2/M (7) (for more information about Ac144, see Chapter 12).

The various observations regarding baculovirus infection and the cell cycle could be a reflection of the blockage of cellular DNA replication upon infection and the subsequent replication of viral DNA, which would obscure the phase of the host cell cycle. Whereas blocking the cell cycle may be necessary for diverting the cellular replicative machinery for virus replication, it is not understood how the cellular replicative apparatus is exploited by the virus. Clearly, however, for viruses that are dependent on host enzymatic pathways and synthetic machinery, the activity of these systems would either be preserved or elevated during the infection.

Viral synthetic pathways: Induction of a 'pseudo' or 'viral' S phase environment

Several factors likely contribute to the ability of baculoviruses to replicate independently of the cell cycle and produce a 'pseudo' S phase-like environment, which might be more appropriately termed 'viral' S phase because of the components that the virus contributes to macromolecular synthesis. These include the ability of cells to selectively transcribe viral genes through the transactivator IE1 in combination with *hr* enhancer sequences, the synthesis of a set of DNA replication proteins so that virus replication is independent of host proteins for DNA replication, and the production of an RNA polymerase so that they are independent of the host RNA polymerase II. These factors are summarized in Figure 1. Another contributing factor would be the shut down of most host RNA polymerase II transcription (8). In addition, there are likely a few other cellular pathways that are exploited by the virus for DNA synthesis. Hints of some of these are likely reflected in enzymes involved in biosynthetic pathways encoded by some, but not all baculoviruses (see Chapter 5). It is likely that there are advantages for the genes encoding these enzymes to be incorporated into the viral genome because then they would be under the regulatory control of the virus. However, in viruses lacking these genes, they likely remain dependent on the host pathways. An example of one such enzyme is ribonucleotide reductase (RR) that is encoded by a minority of baculoviruses and consists of two genes that encode a heterodimer composed of large and small subunits (RR1 and RR2, respectively). RR is involved in the catalysis of ribonucleotides to deoxyribonucleotides as a pathway for providing nucleotides for DNA synthesis. It is found in most Group II NPV, at lease one Group 1 NPV (OpMNPV) and a few GV genomes that have been sequenced. Although it is not clear why some viruses encode this enzyme and others do not, it does suggest that it may be a required component for optimizing the cellular environment for viral replication and indicates that viruses that lack the enzyme are dependent on the host for this pathway. In all of the NPVs and one of the GVs encoding the RR genes, they are accompanied with a

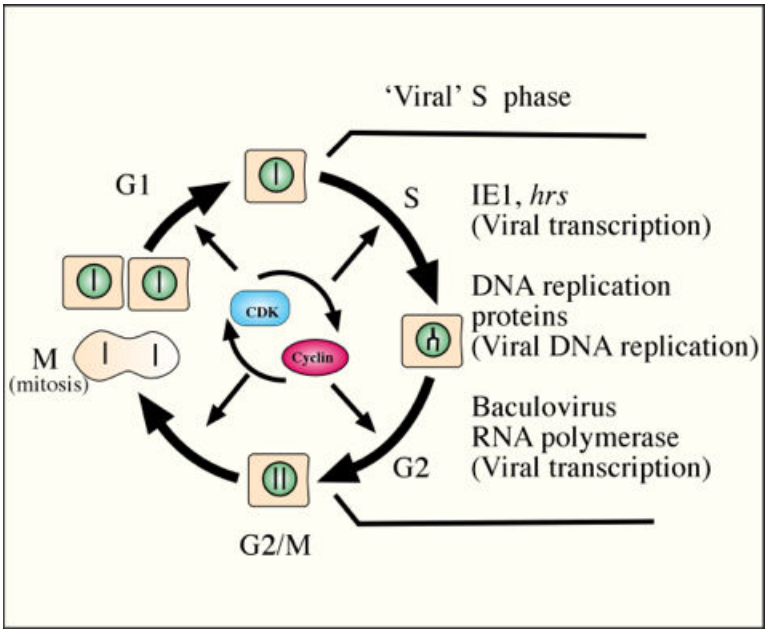

Figure 1. The cell cycle is regulated by the phosphorylation state of different cyclin molecules. Baculovirus infection appears to block the cell cycle and prevents cells from undergoing mitosis. They likely induce a 'pseudo' S phase-like environment in which the virus transcriptional activator IE1, along with *hr* enhancer sequences preferentially activate early viral genes leading to DNA replication and the production of the baculovirus RNA polymerase.

companion enzyme, dUTPase. One of the products of RR can be dUTP, which if incorporated into DNA, can be mutagenic. Consequently, the presence of both RR and dUTPase suggests that the latter enzyme mitigates the mutagenic potential of dUTP, which is inactivated by dUTPase.

It has also been noted that many of the most prevalent promoter sequences present in baculovirus genomes are combinations of both early and late promoters (see Chapter 4) (9) such that the genes can be initially expressed by the host RNA polymerase II, but can also be expressed by the viral polymerase. This promoter arrangement would ensure that sufficient levels of the protein are present independent of host cell at both early and late times post-infection.

Apoptosis and baculovirus infections

Apoptosis or programmed cell death is a pathway that is thought to have evolved to allow multicellular organisms to eliminate cells that are no longer required either to facilitate the development of the organism or because they are damaged or malfunctioning. A classic example of this process is the transformation that occurs during insect pupation that causes the metamorphosis of a worm-like insect larva into an adult insect often capable of flight. During this process, larval structures are dismantled, which frees up their components for reuse in the production of new structures. In addition, in some instances apoptosis is induced by malfunctions in the cell cycle. This can include DNA damage or a variety of features of cells that are undergoing virus infection, such as unscheduled DNA replication or RNA synthesis that is independent of the cell cycle. Therefore, apoptosis is also a major method by which organisms can limit and control viral infections.

Apoptosis is characterized by a specific series of events that are associated with cell death. These include loss of attachment to adjacent cells, cell shrinkage, nuclear fragmentation, chromatin condensation and DNA fragmentation and subsequent disintegration or blebbing of the cell into apoptotic bodies that are eliminated by phagocytosis. For a video of this process see (10). Apoptosis is induced by processes associated with baculovirus DNA replication in *Spodoptera frugiperda* cells (11) and involves the activation of a series of proteases called caspases. There are two types of caspases involved in apoptosis, initiator and effector caspases. Initiator caspases

activate inactive forms of effector caspases (procaspases) by cleaving them. Effector caspases then cleave other cellular proteins resulting in apoptosis. Some effector caspases specifically target cytoskeletal proteins thereby causing the initial morphological changes including detachment and cell shrinkage. The activation of nucleases and the targeting of cell structural elements cause the subsequent fragmentation of the cell. Although it is thought that apoptosis evolved for the removal of cells that are no longer needed by an organism, it was adapted as a cellular defense mechanism for the elimination of cells infected by viruses. This greatly reduces the ability of viruses to establish infections. In AcMNPV infections, yield can be reduced up to 15,000-fold for viruses lacking the anti-apoptotic gene, p35 (12) (13). In some aspects, the monitoring for virus infection is thought to be integrally involved with the monitoring of the cell cycle. When a cell is determined by checkpoint monitoring to have aberrant features such as DNA that is damaged beyond repair, the cell can be directed to undergo apoptosis, and thereby be eliminated. Apoptosis can be induced by DNA damage caused by ionizing radiation and by a variety of toxic chemicals. It is thought that the apparatus that monitors the cell cycle interprets viral DNA replication as aberrant or damaged DNA and induces the cell to undergo apoptosis. In AcMNPV, it was found that by silencing genes required for DNA replication using RNAi, apoptosis could be significantly reduced. In contrast, silencing genes specific to late gene expression did not reduce apoptosis (11) (14). From this it was concluded that either the replication genes themselves, or DNA replication were responsible for the induction of apoptosis.

The baculovirus anti apoptosis gene, p35.

The study of apoptosis is a relatively new field, and baculoviruses have played a major role in understanding this process. Much of the early work on baculoviruses and apoptosis was done in the laboratory of Dr. Lois Miller. For a biography of Dr. Miller, see (15) and for a history of the early discoveries, see (16).

Baculoviruses became widely used as vectors for protein expression in the mid-1980s. Initially, the protocol involved the production of recombinants by homologous recombination at the polyhedrin locus. The polyhedrin gene was the selectable marker, and recombination resulted in occlusion negative virions that could be identified by examination of the plaques that they formed in cultured cells. The first baculovirus apoptotic inhibitor was discovered when a laboratory identified an occlusion negative plaque that was not expressing the intended recombinant protein, but that had aberrant features, including a small plaque size. These investigators brought this to the attention of Lois Miller's laboratory that specialized in baculovirus research. The mutant virus was found to induce apoptosis and consequently had a small plaque size and reduced budded virus production. Investigation of the genome of the mutant virus led to the discovery of a baculovirus gene called p35, much of which was deleted in this aberrant virus. It was found that under normal conditions p35 was capable of blocking the apoptotic pathway in cells infected with the wt virus, but in this mutant it was inactivated (17).

A second fortuitous observation occurred when a laboratory was expressing one of the proteinases involved in apoptosis, caspase 1 (also called ICE) in the baculovirus system and found that it consistently co-purified with a contaminating protein. This contaminant was subsequently shown to be p35 and it was found to be a substrate for an effector caspase, caspase 1, but in the process of its cleavage it irreversibly binds to and inactivates caspase 1. Therefore, when caspase 1 was expressed in the baculovirus system, it bound to p35 that was also being expressed by the baculovirus. This led to understanding how p35 was able to block the apoptotic pathway (18) (Figure 2). Subsequently, p35 was found to block other categories of caspases in a similar manner (19). Although baculoviruses encoding p35 would appear to benefit by its presence, its evolutionary lineage is not clear. Closely related orthologs are only found in a few Group I baculoviruses closely related to AcMNPV. Orthologs of p35 have also been reported in a GV of *Choristoneura occidentalis* (ChocGV) (20), and in an entomopox virus. The entompox virus p35 ortholog functions similarly to P35 in blocking effector caspases (21). Despite its limited distribution to a few insect viruses, p35 is capable of blocking the apoptotic pathway in diverse organisms from invertebrates to mammals and has been a critical reagent in understanding the molecular interactions involved

in the apoptotic pathway. In infected insect cells evidence suggests that the induction of apoptosis is initiated by p53 at the onset of viral DNA replication (22).

P49, a variant of P35. A variant of P35 called P49 was found in a Group II NPV (SpliNPV) (23). In contrast to the inactivation of effector caspases by p35, P49 inhibits both initiator and effector caspases (24) (25), reviewed in (26) (Figure 2). Furthermore, whereas P35 functions as a monomer, P49 acts as a dimer and is capable of binding two caspase molecules and targets a different cleavage motif (27).

The discovery of the IAP family of antiapoptotic proteins

After the initial discovery of P35, to understand its distribution and diversity, its presence in other baculoviruses was investigated. This involved a granulovirus (CpGV) and an NPV, OpMNPV, that had been shown to have a deletion at the position where *p35* was predicted to be located (28). Complementation was used to rescue an AcMNPV mutant deleted for *p35*. Unexpectedly, in both these viruses, a gene other than p35 was found to compensate for the lack of p35 (29, 30). This gene was called inhibitor of apoptosis (*iap*). Subsequently, in contrast to the limited distribution of *p35*, *iap* orthologs were found in genomes of almost all baculoviruses and the related Nudiviridae and Hytrosaviridae. Orthologs are also found in the Asfarviridae, Iridoviridae, Ascoviridae, Entompoxvirinae, and Malacoherpesviridae (reviewed in (16)). They are also widely distributed in eukaryotes, from yeast to mammals.

In addition to their widespread distribution throughout eukaryotes, where it is often present in multiple copies, six lineages of iap genes (iap 1-6) have been identified in baculoviruses (16). Often an individual baculovirus will have representatives of several iap groups in their genomes, e.g., OpMNPV has representatives of iap -1, 2, -3, and -4 (31). In addition, some viruses have two members of one of the lineages (32). The iap-5 lineage appears to be confined to Betabaculovirus (GV) genomes as is iap-6 which is present in only a few GV genomes. Although, iap-1, -2, -4 are found only in Alphabaculoviruses, iap-3 has a wider distribution and is found in some Alpha-, Beta-, and Gammabaculoviruses (16). Although the genomes of CpGV and OpMNPV were not completely sequenced when their iap genes were initially identified, both of these were later found to be members of the iap-3 lineage. Iap-3 genes are closely related to *iap* genes of insects. For example, OpMNPV IAP-3 is 57% identical to IAP from *B. mori*. In addition, iap from *S. frugiperda* has similar properties to IAP-3 in terms of its structure and function (33). The role of other *iap* genes in baculovirus biology is not clear (reviewed in (16)). For example, in EppoMNPV, deletion of the *iap-1* gene delayed the onset, but did not prevent apoptosis induced by actinomycin. However the *iap-2* ortholog from this virus was found to have anti apoptotic activity when expressed from a CMV promoter in *S. frugiperda* cells (34) (35). It is possible that the different iap genes may function in different cell types (19).

IAP Domains

IAP sequences have a number of distinguishing domains. These include baculovirus IAP repeat (BIR) domains of about 70 amino acids that coordinates a Zn ion. BIR domains are often present in multiple copies, with two copies present in many baculovirus IAPs and up to three copies in some cellular IAPs and is involved in protein-protein interactions. Important interactions include the interaction and inactivation of caspases and the interaction with factors that dissociate IAP-caspase complexes thereby freeing up caspases so that they can participate in apoptosis, reviewed in (36). A RING ('really interesting new gene') finger domain of about 40 amino acids is also often present near the C-terminus of the protein and is required for dimerization and for E3 ubiquitin ligase activity. Such enzymes transfer ubiquitin from E2 ubiquitin conjugating enzymes to target proteins.

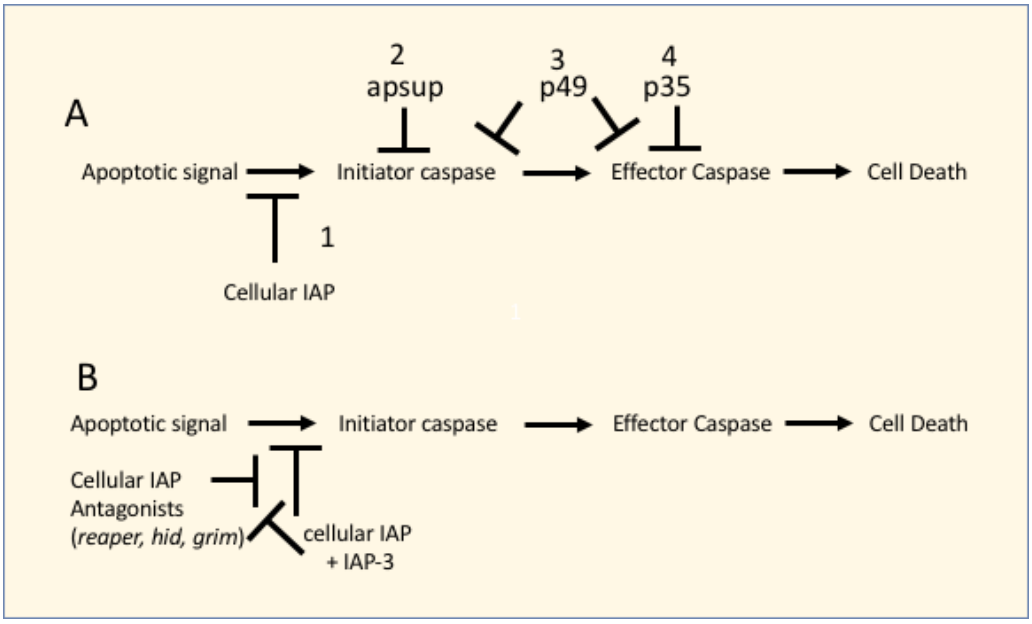

Figure 2. Cell Death regulation by baculovirus proteins that block apoptosis. A. Roles of P35, P49, and Apsup. 1) Cellular iap blocks apoptosis but has a short half-life. 2) Apsup interacts with and prevents the cleavage of Dronc, an initiator caspase. 3) P49 inhibits both initiator and effector caspases. 4) p35 is a cleaved by and then irreversibly binds to and inactivates caspase 1. B. The role of IAP-3 in stabilizing cIAP. Cellular iaps blocks apoptosis but have a short half-life and can be inactivated by antagonists such as reaper, hid and grim. IAP-3 binds to and stabilizes cIAP thereby preserving its ability to block apoptosis.

Cellular IAP genes

Although initially discovered in baculoviruses, homologs of IAP genes were subsequently found to be present in many organisms including yeast, insects, and mammals. It has been found that the BIR domains of cellular IAPs block selected caspases. For example, in human X-linked iap (XIAP), the BIR2 domain binds to and inhibits caspase 3 and 7, whereas BIR3 inhibits caspase-9 (37). Other IAPs have ubiquitin ligase activity associated with their RING domain and thereby target caspases for ubiquitination and subsequent degradation (38). In *Drosophila* there are several proteins, e.g., HID, Grim and Reaper, that block the activity of *Drosophila* IAPs and are called IAP antagonists (Figure 2). These antagonists are necessary to allow for normal insect development that is based on the orderly removal of unneeded tissues via apoptosis. Therefore, they facilitate apoptosis by blocking proteins that would inhibit this process.

Baculovirus Iap-3 function. Whereas, XIAP inhibits caspases, the most well-characterized baculovirus IAP, Op-IAP-3 increases the stability of cellular IAPs. Cellular IAP proteins serve as a primary mechanism for blocking apoptosis. Virus infection triggers the rapid depletion of cellular IAP thereby initiating caspase-mediated apoptosis leading to cell death. *Spodoptera frugiperda* IAP (SfIAP) and related IAPs from other insects contain an instability motif (degron) in their N-terminal leader region that regulates cellular IAP turnover. The baculovirus IAP-3 proteins lack an equivalent degron motif which contributes to their stability. Op-IAP3 can form a heterodimer with SfIAP and prevent its degradation thereby blocking apoptosis. Op-IAP3 also increases the levels and increased the half-life of SfIAP thereby enhancing its anti-apoptotic function. (39, 40)

In summary, evidence suggests that homologs of the IAP proteins are capable of blocking apoptosis by several mechanisms including direct interference with caspases, targeting caspases for degradation via ubiquitin ligase activity, interference with antagonists of other proteins that block apoptosis, and increasing the stability of the host cell IAP. Unlike p35 that has a limited distribution and indeterminate origin, the baculovirus IAP genes have multiple homologs in their insect hosts that would have provided a source for the incorporation of an IAP gene into viral genomes on multiple occasions.

Apsup, a third baculovirus antiapoptotic gene family

The apoptotic suppressor (*apsup*) was discovered in the genome (orf109) of the *Lymantria dispar* MNPV. Ld109 encodes a protein with a predicted mass 39.3 kDa and does not appear to be related to other proteins in the database. It blocks initiator caspases (Figure 2). Homologs were identified in *Lymantria xylina* MNPV and also in AcMNPV (Ac112/113). Ac112/113 shows about 30% amino acid sequence identity to APSUP, but is truncated and lacks 79 amino acids at its C-terminus and also lacks anti apoptotic activity (26, 35, 41). Apsup blocked apoptosis by inhibiting the proteolytic processing of an initiator caspase (Dronc) of *L. dispar*. This inhibition may be caused by it physically interacting with Dronc (42).

Baculovirus CIDE domain proteins

The cell death-inducing DFF45-like effector (CIDE) domain is usually present near the N-terminus of a DNAse that is activated by caspase cleavage and is associated with the degradation of DNA during apoptosis and lipid homeostasis (43). CIDE_N domains have been identified (ORF38) in the Mythimna unipuncta GV (MyunGV)) (44) and in a Group I NPV, Choristoneura fumiferana def (CfdefMNPV) (orf142). MyunGV orf38 is related to orfs from 5 other GVs. In contrast, CfdefMNPV orf142 is most closely related to an orf from another Group I NPV (*Neophasia* sp – the pine butterfly – NespNPV. Both lineages showed structural relatedness to CIDE domains from *Mus musculus and Drosophila melanogaster* with a probability of almost 100% by Hhpred (45). The predicted CIDE domain proteins from the NPVs and GVs are only distantly related suggesting that this protein may have been incorporated into baculovirus genomes on at least two independent occasions.

AcMNPV orf92 (p33) – a link between apoptosis and the cell cycle?

Orthologs of Ac92 are present in all sequenced baculovirus genomes and it is an essential gene as viable recombinants deleted for this gene have not been isolated (46)(47). Ac92 has been demonstrated to have sulfhydryl oxidase activity, suggesting that it is involved in the formation of disulfide bonds (48). It is also found associated with BV and ODV (46) (47), suggesting that it may be involved in the formation of disulfide bonds in the nuclei of infected cells. The crystal structure of Ac92 was described as a novel dimer composed of two pseudodimers (49). The structure of Ac92 ortholog in BmNPV (Bm75) has also been reported (50). Ac92 also interacts with p53. P53 has been called 'the guardian of the genome' because of a number of roles it plays in protecting cells from damage. Although normally inactive or expressed at low levels, it is induced by factors causing DNA damage, ribonucleotide depletion, and deregulated oncogene expression and can facilitate blocking the division of such cells. This interruption in the cell cycle allows time for the cell to repair the damaged DNA or it can lead to the induction of apoptosis and the destruction of the cell. Similar to the interaction with Rb, some viruses express proteins that inactivate p53 by interfering with its regulation of the cell cycle and preventing it from inducing apoptosis. Therefore, it was of considerable interest when it was observed that Ac92 (p33) forms a stable complex with human p53 (51). When expressed by itself, Ac92 shows diffuse cytoplasmic staining and punctate staining of nuclei. However, when co-expressed with p53, it exclusively localizes to nuclei. Expression of human p53 in Sf cells causes apoptosis which can be blocked by co-expression of baculovirus anti-apoptotic suppressors p35 or OpIAP. However, co-expression of p53 with Ac92 elevated the induction of apoptosis about two-fold. Proteins with sulfhydryl oxidase activity have been implicated in the protection of cells from oxidative stress caused by apoptosis (52) (53). When the role of p53 in baculovirus infection was examined it was found that AcMNPV infection caused the accumulation of P53. It was observed that despite the accumulation of p53 under conditions of both DNA damage and AcMNPV infection, silencing p53 did not affect the induction of apoptosis suggesting that it might not be absolutely required for activating apoptosis in Sf9 cells (54). An ortholog of P53 has been described for *S. frugiperda* (55) and similar to human p53, Sfp53 was found to interact with Ac92 (56). It interacts with the Sfp53 DNA binding domain and a point

mutation in Sfp53 that inactivated DNA binding also inactivated binding of Ac92 to Sfp53. Ac92 was also shown to oxidize Sfp53 in vitro. However, despite the ability of p33 to interact with and oxidize Sfp53 in cultured cells, no effects on Sfp53-mediated apoptosis or virus replication were observed (56). Effects on other cell types or in whole insects was not ruled out by these studies.

References

1. Davy C, Doorbar J. G2/M cell cycle arrest in the life cycle of viruses. Virology. 2007;368:219–26. PubMed PMID: 17675127.
2. Zeng XR, Hao H, Jiang Y, Lee MY. Regulation of human DNA polymerase delta during the cell cycle. J Biol Chem. 1994;269:24027–33. PubMed PMID: 7929054.
3. Braunagel SC, Parr R, Belyavskyi M, Summers MD. Autographa californica nucleopolyhedrovirus infection results in Sf9 cell cycle arrest at G2/M phase. Virology. 1998;244:195–211. PubMed PMID: 9581791.
4. Ikeda M, Kobayashi M. Cell-cycle perturbation in Sf9 cells infected with Autographa californica nucleopolyhedrovirus. Virology. 1999;258:176–88. PubMed PMID: 10329579.
5. Saito T, Dojima T, Toriyama M, Park EY. The effect of cell cycle on GFPuv gene expression in the baculovirus expression system. J Biotechnol. 2002;93:121–9. PubMed PMID: 11738719.
6. Zhou R, Yu ZH, Li XQ, Jia F, Wu JH, Chen X. Heliocoverpa armigera single nucleocapsid nucleopolyhedrovirus induces Hz-AM1 cell cycle arrest at the G2 phase with accumulation of cyclin B1. Virus Res. 2004;105:113–20. PubMed PMID: 15351484.
7. Belyavskyi M, Braunagel SC, Summers MD. The structural protein ODV-EC27 of Autographa californica nucleopolyhedrovirus is a multifunctional viral cyclin. Proc Natl Acad Sci U S A. 1998;95:11205–10. PubMed PMID: 9736714.
8. Nobiron I, O'Reilly DR, Olszewski JA. Autographa californica nucleopolyhedrovirus infection of Spodoptera frugiperda cells: a global analysis of host gene regulation during infection, using a differential display approach. J Gen Virol. 2003;84:3029–39. PubMed PMID: 14573808.
9. Xing K, Deng R, Wang J, Feng J, Huang M, Wang X. Analysis and prediction of baculovirus promoter sequences. Virus Res. 2005;113:64–71. PubMed PMID: 15908030.
10. Manji GA, Friesen PD. Apoptosis in motion. An apical, P35-insensitive caspase mediates programmed cell death in insect cells. J Biol Chem. 2001;276:16704–10. PubMed PMID: 11278634.
11. Schultz KL, Friesen PD. Baculovirus DNA replication-specific expression factors trigger apoptosis and shutoff of host protein synthesis during infection. J Virol. 2009;83:11123–32. PubMed PMID: 19706708.
12. Clem RJ, Miller LK. Apoptosis reduces both the in vitro replication and the in vivo infectivity of a baculovirus. J Virol. 1993;67:3730–8. PubMed PMID: 8510202.
13. Herschberger PA, Dickson JA, Friesen PD. Site-specific mutagenesis of the 35-kilodalton protein gene encoded by Autographa californica nuclear polyhedrosis virus: cell line-specific effects on virus replication. J Virol. 1992;66:5525–5533. PubMed PMID: 1501287.
14. Schultz KL, Wetter JA, Fiore DC, Friesen PD. Transactivator IE1 is required for baculovirus early replication events that trigger apoptosis in permissive and nonpermissive cells. J Virol. 2009;83:262–72. PubMed PMID: 18945761.
15. Passarelli AL, Westpheling J, Espelie EM, Espelie K. 2014. Lois K. Miller, A Biograhical Memoir. Available at: http://wwwnasonlineorg/publications/biographical-memoirs/memoir-pdfs/miller-loispdf.
16. Clem RJ. Viral IAPs, then and now. Semin Cell Dev Biol. 2015;39:72–9. PubMed PMID: 25652775.
17. Clem RJ, Fechheimer M, Miller LK. Prevention of apoptosis by a baculovirus gene during infection of insect cells. Science. 1991;254:1388–1390. PubMed PMID: 1962198.
18. Bump NJ, Hackett M, Hugunin M, Seshagiri S, Brady K, Chen P, Ferenz C, Franklin S, Ghayur T, Li P, et al. Inhibition of ICE family proteases by baculovirus antiapoptotic protein p35. Science. 1995;269:1885–8. PubMed PMID: 7569933.
19. Clem RJ. Baculoviruses and apoptosis: a diversity of genes and responses. Curr Drug Targets. 2007;8:1069–74. PubMed PMID: 17979666.

20. Escasa SR, Lauzon HA, Mathur AC, Krell PJ, Arif BM. Sequence analysis of the Choristoneura occidentalis granulovirus genome. J Gen Virol. 2006;87:1917–33. PubMed PMID: 16760394.
21. Means JC, Penabaz T, Clem RJ. Identification and functional characterization of AMVp33, a novel homolog of the baculovirus caspase inhibitor p35 found in Amsacta moorei entomopoxvirus. Virology. 2007;358:436–47. PubMed PMID: 17010407.
22. Makino S, Hamajima R, Saito A, Tomizaki M, Iwamoto A, Kobayashi M, Yamada H, Ikeda M. Bombyx mori homolog of tumor suppressor p53 is involved in apoptosis-mediated antiviral immunity of B. mori cells infected with nucleopolyhedrovirus. Dev Comp Immunol. 2018;84:133–141. PubMed PMID: 29448034.
23. Du Q, Lehavi D, Faktor O, Qi Y, Chejanovsky N. Isolation of an apoptosis suppressor gene of the Spodoptera littoralis nucleopolyhedrovirus. J Virol. 1999;73:1278–85. PubMed PMID: 9882332.
24. Zoog SJ, Schiller JJ, Wetter JA, Chejanovsky N, Friesen PD. Baculovirus apoptotic suppressor P49 is a substrate inhibitor of initiator caspases resistant to P35 in vivo. Embo J. 2002;21:5130–40. PubMed PMID: 12356729.
25. Jabbour AM, Ekert PG, Coulson EJ, Knight MJ, Ashley DM, Hawkins CJ. The p35 relative, p49, inhibits mammalian and Drosophila caspases including DRONC and protects against apoptosis. Cell Death Differ. 2002;9:1311–20. PubMed PMID: 12478468.
26. Ikeda M, Yamada H, Hamajima R, Kobayashi M. Baculovirus genes modulating intracellular innate antiviral immunity of lepidopteran insect cells. Virology. 2013;435:1–13. PubMed PMID: 23217611.
27. Guy MP, Friesen PD. Reactive-site cleavage residues confer target specificity to baculovirus P49, a dimeric member of the P35 family of caspase inhibitors. J Virol. 2008;82:7504–14. PubMed PMID: 18508888.
28. Gombart AF, Blissard GW, Rohrmann GF. Characterization of the genetic organization of the HindIII-M region of the multicapsid nuclear polyhedrosis virus of Orgyia pseudotsugata reveals major differences among baculoviruses. J Gen Virol. 1989;70:1815–1828. PubMed PMID: 2661722.
29. Crook NE, Clem RJ, Miller LK. An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif. Journal of Virology. 1993;67:2168–2174. PubMed PMID: 8445726.
30. Birnbaum MJ, Clem RJ, Miller LK. An apoptosis-inhibiting gene from a nuclear polyhedrosis virus encoding a polypeptide with Cys/His sequence motifs. J Virol. 1994;68:2521–2528. PubMed PMID: 8139034.
31. Ahrens CH, Russell R, Funk CJ, Evans JT, Harwood SH, Rohrmann GF. The sequence of the *Orgyia pseudotsugata* multinucleocapsid nuclear polyhedrosis virus genome. Virology. 1997;229:381–399. PubMed PMID: 9126251.
32. Esteban DJ, Da Silva M, Upton C. New bioinformatics tools for viral genome analyses at Viral Bioinformatics - Canada. Pharmacogenomics. 2005;6:271–80. PubMed PMID: 16013958.
33. Cerio RJ, Vandergaast R, Friesen PD. Host Insect Inhibitor-of-Apoptosis SfIAP Functionally Substitutes for Baculovirus IAP but is Differentially Regulated by its N-terminal Leader. J Virol. 2010;84:11448–60. PubMed PMID: 20739517.
34. Maguire T, Harrison P, Hyink O, Kalmakoff J, Ward VK. The inhibitors of apoptosis of Epiphyas postvittana nucleopolyhedrovirus. J Gen Virol. 2000;81:2803–11. PubMed PMID: 11038395.
35. Yamada H, Shibuya M, Kobayashi M, Ikeda M. Baculovirus Lymantria dispar multiple nucleopolyhedrovirus IAP2 and IAP3 do not suppress apoptosis, but trigger apoptosis of insect cells in a transient expression assay. Virus Genes. 2012;45:370–9. PubMed PMID: 22798056.
36. Mace PD, Shirley S, Day CL. Assembling the building blocks: structure and function of inhibitor of apoptosis proteins. Cell Death Differ. 2010;17:46–53. PubMed PMID: 19373243.
37. Eckelman BP, Salvesen GS, Scott FL. Human inhibitor of apoptosis proteins: why XIAP is the black sheep of the family. EMBO Rep. 2006;7:988–94. PubMed PMID: 17016456.
38. Vaux DL, Silke J. IAPs, RINGs and ubiquitylation. Nat Rev Mol Cell Biol. 2005;6:287–97. PubMed PMID: 15803136.
39. Byers NM, Vandergaast RL, Friesen PD. Baculovirus Inhibitor-of-Apoptosis Op-IAP3 Blocks Apoptosis by Interaction with and Stabilization of a Host Insect Cellular IAP. J Virol. 2016;90:533–44. PubMed PMID: 26491164.

40. Vandergaast R, Mitchell JK, Byers NM, Friesen PD. Insect inhibitor-of-apoptosis (IAP) proteins are negatively regulated by signal-induced N-terminal degrons absent within viral IAP proteins. J Virol. 2015;89:4481–93. PubMed PMID: 25653450.
41. Yamada H, Shibuya M, Kobayashi M, Ikeda M. Identification of a novel apoptosis suppressor gene from the baculovirus Lymantria dispar multicapsid nucleopolyhedrovirus. J Virol. 2011;85:5237–42. PubMed PMID: 21411519.
42. Yamada H, Kitaguchi K, Hamajima R, Kobayashi M, Ikeda M. Novel apoptosis suppressor Apsup from the baculovirus Lymantria dispar multiple nucleopolyhedrovirus precludes apoptosis by preventing proteolytic processing of initiator caspase Dronc. J Virol. 2013;87:12925–34. PubMed PMID: 24067961.
43. Choi JY, Qiao Q, Hong SH, Kim CM, Jeong JH, Kim YG, Jung YK, Wu H, Park HH. CIDE domains form functionally important higher-order assemblies for DNA fragmentation. Proc Natl Acad Sci U S A. 2017;114:7361–7366. PubMed PMID: 28652364.
44. Harrison RL, Rowley DL, Mowery J, Bauchan GR, Theilmann DA, Rohrmann GF, Erlandson MA. The Complete Genome Sequence of a Second Distinct Betabaculovirus from the True Armyworm, Mythimna unipuncta. PLoS One. 2017;12:e0170510. PubMed PMID: 28103323.
45. Zimmermann L, Stephens A, Nam SZ, Rau D, Kubler J, Lozajic M, Gabler F, Soding J, Lupas AN, Alva V. A Completely Reimplemented MPI Bioinformatics Toolkit with a New HHpred Server at its Core. J Mol Biol. 2018;430:2237–2243. PubMed PMID: 29258817.
46. Wu W, Passarelli AL. Autographa californica M nucleopolyhedrovirus Ac92 (ORF92, P33) is required for budded virus production and multiply-enveloped occlusion-derived virus formation. J Virol. 2010;84:12351–61. PubMed PMID: 20861245.
47. Nie Y, Fang M, Theilmann DA. Autographa californica multiple nucleopolyhedrovirus core gene ac92 (p33) is required for the efficient budded virus production. Virology. 2011;409:38–45. PubMed PMID: 20965540.
48. Long CM, Rohrmann GF, Merrill GF. The conserved baculovirus protein p33 (Ac92) is a flavin adenine dinucleotide-linked sulfhydryl oxidase. Virology. 2009;388:231–5. PubMed PMID: 19409596.
49. Hakim M, Mandelbaum A, Fass D. Structure of a baculovirus sulfhydryl oxidase, a highly divergent member of the erv flavoenzyme family. J Virol. 2011;85:9406–13. PubMed PMID: 21752922.
50. Hou Y, Xia Q, Yuan YA. Crystal structure of Bombyx mori nucleopolyhedrovirus ORF75 reveals a pseudo-dimer of thiol oxidase domains with a putative substrate-binding pocket. J Gen Virol. 2012;93:2142–51. PubMed PMID: 22764321.
51. Prikhod'ko GG, Wang Y, Freulich E, Prives C, Miller LK. Baculovirus p33 binds human p53 and enhances p53-mediated apoptosis. J Virol. 1999;73:1227–34. PubMed PMID: 9882325.
52. Morel C, Adami P, Musard JF, Duval D, Radom J, Jouvenot M. Involvement of sulfhydryl oxidase QSOX1 in the protection of cells against oxidative stress-induced apoptosis. Exp Cell Res. 2007;313:3971–82. PubMed PMID: 17927979.
53. Thirunavukkarasu C, Wang LF, Harvey SA, Watkins SC, Chaillet JR, Prelich J, Starzl TE, Gandhi CR. Augmenter of liver regeneration: an important intracellular survival factor for hepatocytes. J Hepatol. 2008;48:578–88. PubMed PMID: 18272248.
54. Huang N, Wu W, Yang K, Passarelli AL, Rohrmann GF, Clem RJ. Baculovirus infection induces a DNA damage response that is required for efficient viral replication. J Virol. 2011;85:12547–56. PubMed PMID: 21917957.
55. Huang N, Clem RJ, Rohrmann GF. Characterization of cDNAs encoding p53 of Bombyx mori and Spodoptera frugiperda. Insect Biochem Mol Biol. 2011;41:613–9. PubMed PMID: 21497653.
56. Wu W, Clem RJ, Rohrmann GF, Passarelli AL. 2013. The baculovirus sulfhydryl oxidase Ac92 (P33) interacts with the Spodoptera frugiperda P53 protein and oxidizes it in vitro. 447:197-207.

8. Host resistance, susceptibility, and the effect of viral infection on host molecular biology

Viruses are dependent on a variety of cellular functions to successfully infect an organism. For a productive infection, cellular structures and molecular pathways must be compatible with the virus for all the major events in virus replication including attachment, entry, uncoating, replication, assembly and exit. Insects, like many other organisms, have evolved methods to inhibit or block virus infections. Several methods by which organisms inhibit virus infection are summarized below. For a more detailed review of some of the topics in this chapter, see (1).

The insect immune system: hemocytes, melanization and encapsulation

Although lacking an adaptive immune system similar to vertebrates, insects have a variety of methods to resist infection by pathogens. Many of these defense mechanisms are similar to those of higher organisms and include physical barriers along with local and systemic responses. The latter includes the production of antimicrobial peptides and highly specialized cells called hemocytes. Hemocytes are circulating cells found in the hemolymph of insects and other arthropods that are similar to neutrophils and carry out roles in immunity such as phagocytosis, encapsulation and melanization as a first line of defense after injury or invasion by microorganisms. Although not employed by mammals, melanization causes a blackening of a wound site, which results from the synthesis and deposition of melanin. It is an important defense against infections because it can encapsulate and isolate pathogens similar to the blood clotting system in vertebrates. In addition, the intermediates of the melanization reaction can involve the production of reactive oxygen species that can be directly toxic to pathogenic microbes. In arthropods, an inactive form of phenoloxidase (prophenoloxidase) is synthesized and secreted into the hemolymph. Melanization is regulated by a cascade of serine proteases that cleave and activate prophenoloxidase (to phenoloxidase) that is then able to catalyze the oxidation of phenols (e.g., tyrosine) to non-aromatic ring compounds called quinones, which then polymerize and form melanin (2). It has been suggested that phenol oxidase may have an antiviral effect in the plasma of insects in addition to its role in encapsulation (3). The Toll pathway is another component of the innate immune response in many organisms. It acts by the recognition of structurally conserved molecules associated with pathogens such as viruses that are not present in the host organism. This recognition activates a cellular immune response. In *Drosophila*, melanization requires activation of the Toll pathway and is dependent on the removal of a serine protease inhibitor (Serpin27A), thereby allowing the cleavage of prophenoloxidase. The Toll pathway has been shown to be required for the efficient inhibition of Drosophila X virus. Inactivation of this system led to rapid death of infected insects and elevated virus titers (4).

Although most of the characterization of components of insect immune systems has been described from *Drosophila*, investigations have been conducted on baculovirus infection of Lepidoptera that suggest that they have similar defense systems. *Heliothis virescens* (tobacco bud worm) and *Helicoverpa zea* (corn earworm) are both members of the Noctuidae, but *H. zea* is 1,000-fold less susceptible to AcMNPV infection than *H. virescens*. Larvae of the two species are similar in their susceptibility to primary infection of the midgut and secondary infections of the tracheal epidermis. However, in *H. zea,* the foci of infections in the tracheal epidermis become melanized and encapsulated, and hemocytes appear to be resistant to infection and are capable of removing the virus from the hemolymph. Therefore, AcMNPV infection of *H. zea* appears to be able to activate the host melanization and encapsulation responses that reduce viral titers in the hemolymph and inhibit the progression of the infection. In contrast, in *H. virescens* this pathway appears to be much less active (5). *Spodoptera littoralis* is also highly resistance to AcMNPV infection and has been shown to be capable of efficiently encapsulating the virus in tracheoblast cells that serve the midgut columnar cells. These viral foci were removed during molting thereby eliminating the infection (6).

A sequence related to lepidopteran serpins was found in the genome of a baculovirus of *Hemileuca* sp. (HespNPV) (7). Serpins, (serine protease inhibitors), were named because of their ability to inhibit chymotrypsin-like serine proteases. Serpins bind serine proteases and then undergo cleavage. This results in a major change in the conformation of the serpin and the formation of a covalent linkage with the protease leading to its inactivation. In cowpox virus, a serpin, crmA, has been implicated in mitigating both the inflammatory and apoptotic responses. Although crmA is a member of the serpin group, in contrast to other serpins, it is capable of inhibiting cysteine caspases (8). Similar to serpins, crmA binds the active caspases as a pseudosubstrate and has been shown to irreversibly bind to caspase-1 and is also capable of inhibiting caspases 4, 5, 9 and 10, reviewed in (9). The baculovirus serpin was able to inhibit a subset of serine proteases and also inhibited phenol oxidase activation in vitro indicating that it is a functional serpin. When expressed in AcMNPV it accelerated BV production and reduced the dose to produce an LD50 in T. ni larvae 4 fold (10).

RNA interference

Viral RNAi. RNA interference (RNAi) has been shown to inhibit the infection of insects by some RNA viruses. In *Drosophila*, evidence suggests that virus-specific dsRNA replication intermediates of RNA viruses are released upon cell lysis and taken up and processed by other cells, thereby generating systemic virus-specific RNAi based interference (11). However, some viruses can also interfere with this response. In one well-studied example, Flock house virus encodes a protein called B2 that suppresses RNAi by inhibiting the ability of Dicer to cleave long dsRNAs, thereby preventing the generation of siRNAs. In addition, by binding to siRNAs, B2 prevents their incorporation into the RISC complex that is dependent on their incorporation to identify and cleave homologous RNA sequences, thereby blocking the cleavage of target RNAs (12). Although it was originally thought that flies were not able to generate a systemic RNAi response, it was shown that an uptake pathway for dsRNA exists in *Drosophila*. This pathway is essential for antiviral immunity in adult flies. Mutants defective for this system had up to a 100-fold increase in viral titers (11).

Baculovirus and cellular RNAi. Similar to other viruses, baculoviruses encode microRNAs (13) that are involved in the regulation of both host and viral genes. In one study, four miRNAs were predicted from analyses of the BmNPV genome (14). The data suggested that they were targeted against 8 viral and 64 cellular genes. The viral targets included genes involved in replication, transcription (e.g., dbp, DNA polymerase, and lef-8). Two different bro genes, chitinase, and several other genes were also targeted. Some of the cellular targets involved host defense pathways that are likely involved in resisting the virus infection. These include prophenoloxidase, which is integrally involved in the melanization response described above. Hemolin, another antiviral compound, also was predicted to be targeted. It is involved in pattern recognition, hemocyte aggregation, and phagocytosis. Several proteins involved in the pre-miRNA response and mRNA initiation were also identified. In another investigation of host and viral miRNAs of BmNPV and *B. mori*, it was found that BmNPV encodes a miRNA (miR-1) that down regulates the host GTP-binding protein RAN. RAN is an essential component of the Exportin-5 mediated transport machinery that is involved in the transport of small RNA molecules from the nucleus to the cytoplasm. Blocking BmNPV miR-1 results in elevated expression of RAN and a decrease in BmNPV replication. In contrast blocking a host miRNA (bmo-miR-8) that is targeted at BmNPV IE-1 transcripts resulted in a 3 fold increase in *ie-1* transcripts and an about 8 fold increase of BmNPV DNA accumulation in fat body tissue (15). It has also been reported that BmNPV-mir-3 down regulates the DNA binding protein, p6.9 (16). It also has imperfect matches with several other baculovirus late genes. It was suggested that this may delay viral maturation in order to allow the virus to escape host defenses which may become less effective later in the infection (13). AcMNPV and BmNPV mir-3 do not apper to be related. In contrast to BmNPV mir-3 that regulates p6.9, AcMNPV mir-3 is encoded on the opposite strand of Ac101 and appears to be involved in its down regulation and may be involved in regulating BV and ODV production (17).

In another report it would found that miRNAs appeared to be conserved between *S.frugiperda* and *B. mori* and were down regulated during baculovirus infection (18). In contrast to the implication of small RNAs in host cell

defense, in *Helicoverpa armigera* larvae infected with HaSNPV, a large population of small RNAs were found that mapped to a variety of viral structural and replication genes. The authors suggest that the down regulation of these genes may make the infection more efficient by preventing the over replication and premature death of the cell (19). It has been found in AcMNPV that the open reading frame of ODV-E25 (Ac94) encodes a miRNA that down regulates ODV-E25 expression. Evidence suggested that this miRNA causes a reduction in infectious virus production and may be involved in the shift from budded virus to occluded virus production (20). The BmNPV ie-1 gene also appears to be a target of B. mori bmo-miR-2819 (21).

In another investigation characterizing baculovirus miRNA, SpliNPV infection of Sf21 cells was examined. At 12 hpi >95% of the host proteins were stable, but by 72 hpi, over half the host proteins were down regulated by 2-fold or more. This was reflected in the down regulation of translation, transcription, protein export and oxidative phosphorylation pathways. 117 host genes were identified that were possible targets of 10 viral miRNAs. Two miRNAs were examined experimentally and 9 host genes were identified that were down regulated by these molecules (22).

It was also observed that components of the RNAi system such as Dicer-2, R2D2, and Ago2 were upregulated in the T. ni midgut in apparent response to AcMNPV infection (23). It is not clear whether these are used by a viral or host RNAi response.

Additional mechanisms of virus and host resistance

Antimicrobial peptides. Antimicrobial peptides have also been implicated in protection against viral infection. One of these, gloverin, a cationic protein with antibacterial properties was found to be induced by AcMNPV infection. The addition of gloverin to infected cells was found to reduce AcMNPV BV production to 8 to 27% of untreated samples (24). However, when cDNA sequences were examined in infected cells, it was found that AcMNPV infection of *Spodoptera exigua* larvae down regulated three forms of gloverin along with attacin, another antimicrobial peptide (25). In addition, a member of the atlastin family, BmAtlastin-n, was shown to have an antiviral effect against BmNPV in Bombyx mori larva (26). It was observed to be upregulated in T. ni larvae infected with AcMNPV (23).

Developmental resistance. It was also observed that *H. virescens* larvae demonstrate increasing resistance to fatal AcMNPV infections as they age. In one investigation it was found that the progression of infection is much slower in fifth- compared to fourth-instar larvae that had been orally infected. It was suggested that resistance was caused by different physiology of the midgut or tracheal cells. In particular, it was noted that midgut cells might undergo major changes since their composition is altered late in the fifth instar as the gut develops as part of metamorphosis to adult cell types. It was also suggested that midgut cells may be sloughed at a higher rate in later larval instars (27). Similar patterns of resistance to baculovirus infection has been observed in *Lymantria dispar*. Newly molted fourth-instar larvae showed lower LD_{50} titers compared to larvae infected at 2–3 days post molting. Also, the 2–3-day post-molt insects showed a higher number of foci of infection that had been melanized and a reduced number of infected haemocytes (28, 29).

Inactivation of superoxides, Ac31, superoxide dismutase (SOD). Insect hemocytes are phagocytic cells (see above) and can destroy invading pathogens by the production of reactive oxygen such as superoxide (30). SOD can inactivate superoxide by converting it to hydrogen peroxide, which is also toxic, but can itself be inactivated with catalase to yield water and O_2. Many baculoviruses may infect hemocytes and in this manner can spread an infection throughout an insect. Orthologs of SOD are found in the genomes of almost all lepidopteran baculovirus. Ac31 (SOD) is closely related to SOD from a variety of insects including *B. mori* (E = 8E-49). The expression of viral SOD might mitigate the effects of superoxide production by hemocytes. However, AcMNPV deleted for *sod* replicated normally in cultured cells and insect larvae. The *sod*-deleted viruses showed no reduction in replication when grown in the presence of paraquat, a superoxide anion inducer (31). Whereas these data suggest that SOD of baculoviruses may be involved in some other role in virus biology, it could also

indicate the conditions used to investigate its activity were not sensitive enough to determine its role. In contrast to AcMNPV, in BmNPV it was found that *sod* (Bm23) was essential for replication in BmN cells (32).

Other inhibitors of viral replication. In field populations of *Cydia pomonella* exposed to the granulovirus (CpGV), some populations have been identified with up 10,000-fold resistance. Using several approaches, changes in the innate immune systems, and altered peritrophic membranes and midgut receptors were ruled out as causing resistance. Using Q-PCR to examine viral replication in different tissues, no viral DNA was detected in the fat body, hemocytes or midgut cells of the tissues of resistant insects suggesting that the virus was unable to replicate in these cells. Furthermore, resistance could not be conferred by the transfusion of hemolymph from a resistant to a susceptible insect. This led the authors to conclude that resistance occurs within cells and must inhibit a very early step in viral replication (33).

Host resistance to baculovirus infection in the midgut

The peritrophic matrix (PM). In addition to protection from external invasion by pathogens that is provided by the chitin-containing exoskeleton, an example of another physical barrier unique to insects that might influence resistance to viral infection is the PM. The ability of a virus to access the midgut epithelium would be one of the first problems confronting a virus when it is initiating infection. In some insects that are resistant to infection it was found that differing features of the PM can influence the susceptibility of an insect to viral infection. In *Anticarsia gemmatalis*, it was observed that insects that were more resistant to infection had a relatively thicker PM, a higher chitin content, and bound wheat germ agglutinin more intensely than the more susceptible insects (34) (35).

Midgut interactions. It has also been demonstrated that the oral infectivity of a virus could be influenced by the ability of the ODV to bind to the midgut of insects. It was observed that compared to SfMNPV, AcMNPV ODV had a reduced affinity for midgut cells of *S. frugiperda* larvae. Evidence was also presented suggesting that SfMNPV might bind to a different receptor(s) on columnar epithelial cells and this could contribute to the efficiency of its ability to initiate infection (36).

Other factors influencing Baculovirus host range

A variety of phenomena that govern the selective infectivity of baculoviruses has been covered previously and there is little to add to their review (37). However, there have been recent contributions to understanding specific genes that have been observed to affect host range in insects. Most investigations on the molecular basis or host range specificity of baculoviruses have been done using BmNPV, AcMNPV, and LdMNPV. All of these viruses can be grown in cell culture and BmNPV and AcMNPV are closely related with homologous orfs showing ~90% nt and ~93% aa sequence identity (38). In contrast, LdMNPV is a member of the Group II baculoviruses and its orfs show about 41% aa identity with their AcMNPV homologs (39). Despite the similarity of AcMNPV and BmNPV, AcMNPV infects a much more diverse set of insects and insect cell lines than BmNPV (40). Whereas some of the limits on host range that were initially observed in cultured cells extend through to infection of the host insects, other host range effects are limited to a cell line and are not as restrictive in other cell lines from the same insects or in insect larvae of the species from which the cell lines were derived. This section will focus on investigations of host range in AcMNPV and BmNPV.

Investigations on BmNPV and AcMNPV host range in *B. mori* and *S. frugiperda* cells

Although the baculoviruses of BmNPV and AcMNPV are closely related, they differ significantly in their infectivity spectrum. For example, BmNPV replicates in *B. mori* (BmN) cells, but not *S. frugiperda* (Sf) cells. Conversely, AcMNPV replicates in Sf, but not BmN cells. Although the two viruses do not appear to

productively infect the heterologous cell line, their patterns of gene expression differ in the nonpermissive cell lines. For example, almost all of the AcMNPV genes were found to be expressed in both BmN and Sf9 cells, although peak levels were delayed by about 12 hr in the nonpermissive BmN cells and polyhedrin and p10 expression were substantially reduced. In contrast, although almost all of the BmNPV genes were expressed in BmN cells, their expression in Sf9 cells was greatly reduced (41). Several different laboratories have investigated the factors responsible for the inability of these viruses to replicate in the heterologous cells. Some of these studies are summarized below.

Implication of DNA helicase in specifying host range in BmNPV and AcMNPV. By characterizing mixed infections of BmNPV and AcMNPV, a variant of BmNPV was isolated that was able to replicate in both BmN and Sf-21 cells. A 572 bp fragment of the BmNPV DNA helicase gene was found to be responsible for this altered host range (42). Further characterization of this region identified a single amino acid in the helicase orf (Asn564Ser) responsible for this change (43). Using a similar approach, other investigators found that altering three closely spaced amino acids in the AcMNPV helicase gene with the amino acids from BmNPV located at these positions, i.e., Val556Leu, Ser564Asn, Phe577Leu, allowed AcMNPV to replicate in *B. mori* cells (44). When AcMNPV mutants selected for their ability to replicate in BmN cells were passed through *B. mori* larvae, two amino acid changes (Ser564Asn, Phe577Leu) were found to be required to cause death of the larvae (45). It is unclear what role helicase plays in governing the inability of the virus to replicate in the heterologous cell lines. However, co-infection of BmN cells with wt AcMNPV and wt BmNPV causes premature cessation of both viral and host protein synthesis, although viral transcription appears to be normal. This effect is not observed when BmN cells are infected with both the recombinant AcMNPV and wt BmNPV. It has been suggested that since these few changes in the helicase gene have such a major effect on infection in BmN cells, the wt AcMNPV helicase gene may be toxic to the cell by eliciting an antiviral defense mechanism, by interacting with a host cell protein or nucleic acid, or by interfering with the translational apparatus (46). A wild silkworm, *B. mandarina* was found that had a host range allowing it to replicate in Bm5, Sf9, and T. ni cells. The BoMANPV helicase exhibited two mutations Asp291Asn and Asp300Asn. However, due a variety of other differences in many other genes, the significance of these changes remains to be determined (47).

BmNPV may be infectious for Sf-9 and other cells, but at a low level. In contrast to the reports described above, other laboratories using viral constructs expressing reporter genes have detected replication of BmNPV and AcMNPV in heterologous cell lines. In one study, a BmNPV construct expressing the LacZ gene under the polyhedrin promoter in Sf-9, Sf-21 and Hi-5 cells was examined. Compared with BmNPV infection in Bm5 cells, the BmNPV infections in the other cell lines were delayed with DNA replication detectable 3 to 5 days after being observed in BmN cells. In addition, the viral titers were much lower, varying from 0.7 to 7% (10^6 to 10^7 pfu/ml) the level in BmN cells (48). Since this report utilized LacZ expression to trace the virus, it may be more sensitive than previous studies that utilized the visualization of polyhedron production to monitor viral replication. Indeed, these authors detected either very few or no polyhedra in the heterologous infections using a wt BmNPV with an intact polyhedrin gene. In another investigation, using an AcMNPV construct that expressed the firefly luciferase gene under the *Drosophila* heat shock promoter, luciferase activity was detected in both virus infected larvae and in larvae of the next generation (49). Therefore, both these studies indicate that viral replication is occurring in the heterologous cell lines. Another report described AcMNPV DNA replication in BmN cells, but budded virions were not produced. In contrast, BmNPV DNA replication and BV production only occurred in Sf cells when they were superinfected with AcMNPV (50).

Implication of a host factor in specifying host range in BmNPV and AcMNPV (51). In studies conducted by the intrahemocoelic injection of AcMNPV into 31 different strains of *B. mori* larvae, 14 permissive insect strains were identified. A series of genetic crosses implicated a dominant host gene or set of linked genes that prevented AcMNPV infection in the resistant insects, but that are not present or do not interact with the virus in a negative manner in the susceptible insects.

GP64: an AcMNPV and BmNPV host range determinant. The one study that is difficult to reconcile with the investigations described above examined the replication of BmNPV in Sf-9 and Tn-5 (Hi-5) cells. This investigation suggested that the barrier to infection was caused by the inability of BmNPV to be translocated to the nuclei of the Sf-9 or Tn-5 cells. It was found that BmNPV constructs that lacked the BmNPV *gp64* envelope fusion protein gene, but contained the AcMNPV *gp64* gene, were able to be translocated to nuclei. Although this process resulted in a productive infection in Hi-5 cells, replication in Sf-9 cells was still compromised. There are 22 codons that are different between these two *gp64* genes. There is also the potential that the BmNPV *gp64* orf may encode 19 additional amino acids at the N-terminus in transcripts initiated from late promoter elements (40). This study indicated that abortive replication was caused by the inability of BmNPV GP64 to facilitate fusion with the endosomal membrane. This would suggest that BmNPV GP64 has a major difference in biological properties compared to AcMNPV GP64, which has been demonstrated to allow entry into a wide array of vertebrate cells (52). Recent evidence suggests that a single amino is the host range determinant in AcMNPV GP64. It is HIS155 and the equivalent amino acid in BmNPV is TYR153. Changing AcMNPV gp64 to HIS155TYR and that of BmNPV to TYR153HIS decreased and increased the replication and cell spread of the respective viruses (53). One possible difference between GP64 expression in the two viruses is the observation that, in contrast to the AcMNPV *gp64* gene, which is shut off late in infection of Sf9 cells, in BmNPV infected BmN cells, *gp64* is not shut off late in infection (54).

Global protein synthesis shutdown.

Global protein synthesis shutdown has been observed in a variety of combinations of baculoviruses and cell types, reviewed in (55) and can involve either transcriptional or translational shut down. This phenomenon appears to be a host response to viral infection in some cell lines and consequently viruses have developed mechanisms to counteract it. Helicase has been implicated in this process (see above), but there are also several other genes that act as host range factors because they allow the virus to replicate in certain cells that otherwise would be able to shut down the virus infection. Several such genes are described below.

Host cell-specific factor-1 (*hcf-1*, Ac70): AcMNPV specificity for *T. ni* cells. The AcMNPV gene host cell-specific factor-1 (hcf-1, Ac70) has been implicated in global protein synthesis shutdown. Homologs of hcf-1 are present in a few other baculoviruses. Two are close relatives of AcMNPV and their HCF-1 orfs are 99% (PlxyNPV) and 84% (RoMNPV) identical to that of AcMNPV, whereas the homolog in ClbiNPV is more distantly related (21% identical). HCF-1 was found to be required for transient expression of a late promoter-reporter gene by a late expression factor library in Tn368 cells, but not SF-21 cells (56). AcMNPV with null mutations of *hcf-1* appeared to replicate normally in both Sf-21 cells and *S. frugiperda* larvae. However, in Tn-368 cells replication was impaired, including defects in DNA replication, late gene transcription, and virus production. This was reflected in the global shutdown of both host and viral protein synthesis. Such severe effects were not observed in another *T. ni* cell line. In *T. ni* larvae the oral infectivity of the null mutant was relatively normal, although the insects died more slowly than when infected with wt (57). This would suggest that HCF-1 is required for the productive infection of some, but not all cell types in *T. ni* larvae. Therefore, whereas AcMNPV deleted for *hcf*-1 was unable to replicate in cells similar to Tn-368, it can replicate in other types of *T. ni* cells, thereby allowing infection of larvae that contain a variety of different cell types.

Host range factor-1 (*hrf-1*): An LdMNPV gene that allows AcMNPV to replicate in *L. dispar* cells and insects. The Ld652Y cell line is semi-permissive for AcMNPV replication and all categories of viral genes are transcribed, but both viral and host mRNA translation is blocked late in infection (58). Cotransfection of LdMNPV and AcMNPV DNA into Ld652Y cells results in the production of budded virus that can replicate in Sf cells, suggesting that AcMNPV had been altered such that it could replicate in Ld652Y cells. To determine the LdMNPV gene responsible for this change, AcMNPV DNA was co-transfected with cloned segments of the LdMNPV genome, and an LdMNPV gene was identified, *hrf-1*, that permitted AcMNPV to replicate in Ld652Y cells (59). This recombinant AcMNPV was also able to infect *L. dispar* larvae in concentrations similar to wt

AcMNPV in permissive insects (60). It was found that wt AcMNPV infection of *L. dispar* cells resulted in a shutdown of protein synthesis late in the infection (61). This protein synthesis inhibition was at the level of mRNA translation and could be rescued with tRNA from uninfected cells. This suggested that infection of *L. dispar* cells with wt AcMNPV results in the depletion or blockage of tRNA synthesis (62). It was also found that AcMNPV, lacking the apoptotic inhibitor p35 did not cause translational arrest, suggesting that the inhibition of apoptosis, which normally prevents cell death, in this case induces translational arrest (63). It was found that HRF-1 facilitated the replication of two other viruses in *L. dispar* cells (64).

Apoptosis and the specificity of baculovirus infections. Insect cells have different abilities to detect the presence of a virus infection and initiate an apoptotic program. In some cells, virus infection will induce apoptosis, whereas in others, virus replication is unaffected. In addition, baculoviruses are able to interfere with apoptosis by the expression of apoptotic inhibitors. This has led to advances in understanding of apoptotic pathways and to the identification of baculovirus proteins that can block this process. It has also led to an understanding of the role apoptosis can play in determining the host range of a virus. An overview of apoptosis with relation to baculoviruses is included in Chapter 7.

The reaction of host cells to baculovirus infection: The challenge of interpreting data from proteomics, microarrays, and expression analyses

During AcMNPV infection of *T. ni* cells, the majority of host transcripts decrease between 6 h pi – 48 hpi such that at the latter time they comprise about 10% of the total. However, about 6% of host genes are upregulated from 0-6 hr pi and then decrease for the remainder of the infection. In addition, a small group of genes related to metabolism and stress response were elevated at 18-24 hpi and then declined. Concomitant to the decline in host RNA, the viral RNA increased such that it comprised over 50% of the total by about 13 hr pi infection and continued increasing thereafter (65). The challenge of providing an overview of the response of insect cells to baculovirus infection is that when the expressed transcripts are compartmentalized into broad categories, the categories are often so large that as many transcripts are up-regulated as down-regulated. For example, in AcMNPV infected T. ni cells at 6 hr pi, transcripts of genes that have catalytic activity comprised 31% of those upregulated, but also 24% of those down regulated (65). Therefore, these investigations have major value when an individual has a specific question in mind and then accesses the data and can hopefully obtain the desired information. An example of this was described in Chapter 5, where it was found that host genes that are required for DNA replication (DNA ligase and topoisomerase) but that are not encoded the AcMNPV, were up-regulated and stabilized during the initial stages of infection. Also, in some instances when authors have a particular interest they will parse and interpret their data for publication. Examples of this are cell transcripts involved in cell entry, midgut expression and the ESCRT and NSF pathways (66-69). Similar generalization regarding the changes in the proteome of baculovirus infected cells are also challenging, for example at 72 hpi, similar sized categories of the same type of proteins are both constant or down regulated (22). Therefore, although the data is there, it is up to the individual to find and interpret the relevant information.

References

1. Thiem SM. Baculovirus genes affecting host function. In Vitro Cell Dev Biol Anim. 2009;45:111–26. PubMed PMID: 19247726.
2. Lemaitre B, Hoffmann J. The host defense of Drosophila melanogaster. Annu Rev Immunol. 2007;25:697–743. PubMed PMID: 17201680.
3. Popham HJ, Shelby KS, Brandt SL, Coudron TA. Potent virucidal activity in larval Heliothis virescens plasma against Helicoverpa zea single capsid nucleopolyhedrovirus. J Gen Virol. 2004;85:2255–61. PubMed PMID: 15269366.

4. Zambon RA, Nandakumar M, Vakharia VN, Wu LP. The Toll pathway is important for an antiviral response in Drosophila. Proc Natl Acad Sci U S A. 2005;102:7257–62. PubMed PMID: 15878994.
5. Trudeau D, Washburn JO, Volkman LE. Central role of hemocytes in Autographa californica M nucleopolyhedrovirus pathogenesis in Heliothis virescens and Helicoverpa zea. J Virol. 2001;75:996–1003. PubMed PMID: 11134313.
6. Rivkin H, Kroemer JA, Bronshtein A, Belausov E, Webb BA, Chejanovsky N. Response of immunocompetent and immunosuppressed Spodoptera littoralis larvae to baculovirus infection. J Gen Virol. 2006;87:2217–25. PubMed PMID: 16847117.
7. Rohrmann GF, Erlandson MA, Theilmann DA. The genome of a baculovirus isolated from Hemileuca sp. encodes a serpin ortholog. Virus Genes. 2013;47:357–364. PubMed PMID: 23852342.
8. Ekert PG, Silke J, Vaux DL. Caspase inhibitors. Cell Death Differ. 1999;6:1081–6. PubMed PMID: 10578177.
9. Turner S, Kenshole B, Ruby J. Viral modulation of the host response via crmA/SPI-2 expression. Immunol Cell Biol. 1999;77:236–41. PubMed PMID: 10361255.
10. Ardisson-Araujo DM, Rohrmann GF, Ribeiro BM, Clem RJ. Functional characterization of hesp018, a baculovirus-encoded serpin gene. J Gen Virol. 2015;96:1150–60. PubMed PMID: 25573886.
11. Saleh MC, Tassetto M, van Rij RP, Goic B, Gausson V, Berry B, Jacquier C, Antoniewski C, Andino R. Antiviral immunity in Drosophila requires systemic RNA interference spread. Nature. 2009;458:346–50. PubMed PMID: 19204732.
12. Venter PA, Schneemann A. Recent insights into the biology and biomedical applications of Flock House virus. Cell Mol Life Sci. 2008. PubMed PMID: 18516498.
13. Tang Q, Qiu L, Li G. Baculovirus-Encoded MicroRNAs: A Brief Overview and Future Prospects. Curr Microbiol. 2018. doi: 10.1007/s00284-018-1443-y. PubMed PMID: 29487989.
14. Singh J, Singh CP, Bhavani A, Nagaraju J. Discovering microRNAs from Bombyx mori nucleopolyhedrosis virus. Virology. 2010;407:120–128. PubMed PMID: 20800868.
15. Singh CP, Singh J, Nagaraju J. A baculovirus-encoded MicroRNA (miRNA) suppresses its host miRNA biogenesis by regulating the exportin-5 cofactor Ran. J Virol. 2012;86:7867–79. PubMed PMID: 22593162.
16. Singh CP, Singh J, Nagaraju J. bmnpv-miR-3 facilitates BmNPV infection by modulating the expression of viral P6.9 and other late genes in Bombyx mori. Insect Biochem Mol Biol. 2014;49:59–69. PubMed PMID: 24698834.
17. Jiao Y, Wang J, Deng R, Yu X, Wang X. AcMNPV-miR-3 is a miRNA encoded by Autographa californica nucleopolyhedrovirus and regulates the viral infection by targeting ac101. Virus Res. 2019. doi: 10.1016/j.virusres.2019.05.004. PubMed PMID: 31077766.
18. Mehrabadi M, Hussain M, Asgari S. MicroRNAome of Spodoptera frugiperda cells (Sf9) and its alteration following baculovirus infection. J Gen Virol. 2013;94:1385–97. PubMed PMID: 23407421.
19. Jayachandran B, Hussain M, Asgari S. RNA interference as a cellular defense mechanism against the DNA virus baculovirus. J Virol. 2012;86:13729–34. PubMed PMID: 23055564.
20. Zhu M, Wang J, Deng R, Xiong P, Liang H, Wang X. A MicroRNA Encoded by Autographa californica Nucleopolyhedrovirus Regulates Expression of Viral Gene ODV-E25. J Virol. 2013;87:13029–34. PubMed PMID: 24027316.
21. Wu P, Shang Q, Dweteh OA, Huang H, Zhang S, Zhong J, Hou Q, Guo X. Over expression of bmo-miR-2819 suppresses BmNPV replication by regulating the BmNPV ie-1 gene in Bombyx mori. Mol Immunol. 2019;109:134–139. PubMed PMID: 30947109.
22. Nayyar N, Kaur I, Malhotra P, Bhatnagar RK. Quantitative proteomics of Sf21 cells during Baculovirus infection reveals progressive host proteome changes and its regulation by viral miRNA. Sci Rep. 2017;7:10902. PubMed PMID: 28883418.
23. Shrestha A, Bao K, Chen W, Wang P, Fei Z, Blissard GW. Transcriptional responses of the Trichoplusia ni midgut to oral infection by the baculovirus Autographa californica Multiple Nucleopolyhedrovirus. J Virol. 2019. doi: 10.1128/JVI.00353-19. PubMed PMID: 31043536.

24. Moreno-Habel DA, Biglang-awa IM, Dulce A, Luu DD, Garcia P, Weers PM, Haas-Stapleton EJ. Inactivation of the budded virus of Autographa californica M nucleopolyhedrovirus by gloverin. J Invertebr Pathol. 2012;110:92–101. PubMed PMID: 22401766.
25. Choi JY, Roh JY, Wang Y, Zhen Z, Tao XY, Lee JH, Liu Q, Kim JS, Shin SW, Je YH. Analysis of genes expression of Spodoptera exigua larvae upon AcMNPV infection. PLoS One. 2012;7:e42462. PubMed PMID: 22860129.
26. Liu TH, Dong XL, Pan CX, Du GY, Wu YF, Yang JG, Chen P, Lu C, Pan MH. A newly discovered member of the Atlastin family, BmAtlastin-n, has an antiviral effect against BmNPV in Bombyx mori. Sci Rep. 2016;6:28946. PubMed PMID: 27353084.
27. Kirkpatrick BA, Washburn JO, Volkman LE. AcMNPV pathogenesis and developmental resistance in fifth instar Heliothis virescens. J Invertebr Pathol. 1998;72:63–72. PubMed PMID: 9647703.
28. McNeil J, Cox-Foster D, Slavicek J, Hoover K. Contributions of immune responses to developmental resistance in Lymantria dispar challenged with baculovirus. J Insect Physiol. 2010;56:1167–77. PubMed PMID: 20350551.
29. McNeil J, Cox-Foster D, Gardner M, Slavicek J, Thiem S, Hoover K. Pathogenesis of Lymantria dispar multiple nucleopolyhedrovirus in L. dispar and mechanisms of developmental resistance. J Gen Virol. 2010;91:1590–600. PubMed PMID: 20164260.
30. Bergin D, Reeves EP, Renwick J, Wientjes FB, Kavanagh K. Superoxide production in Galleria mellonella hemocytes: identification of proteins homologous to the NADPH oxidase complex of human neutrophils. Infect Immun. 2005;73:4161–70. PubMed PMID: 15972506.
31. Tomalski MD, Eldridge R, Miller LK. A baculovirus homolog of a Cu/Zn superoxide dismutase gene. Virology. 1991.:149–161. PubMed PMID: 1871962.
32. Katsuma S, Kawaoka S, Mita K, Shimada T. Genome-wide survey for baculoviral host homologs using the Bombyx genome sequence. Insect Biochem Mol Biol. 2008;38:1080–6. PubMed PMID: 19280699.
33. Asser-Kaiser S, Radtke P, El-Salamouny S, Winstanley D, Jehle JA. Baculovirus resistance in codling moth (Cydia pomonella L.) caused by early block of virus replication. Virology. 2011;410:360–7. PubMed PMID: 21190707.
34. Levy SM, Falleiros AM, Moscardi F, Gregorio EA. Susceptibility/resistance of Anticarsia gemmatalis larvae to its nucleopolyhedrovirus (AgMNPV): Structural study of the peritrophic membrane. J Invertebr Pathol. 2007;96:183–6. PubMed PMID: 17568609.
35. Levy SM, Falleiros AM, Moscardi F, Gregorio EA. The role of peritrophic membrane in the resistance of Anticarsia gemmatalis larvae (Lepidoptera: Noctuidae) during the infection by its nucleopolyhedrovirus (AgMNPV). Arthropod Struct Dev. 2012;40:429–34. PubMed PMID: 21775204.
36. Haas-Stapleton EJ, Washburn JO, Volkman LE. Spodoptera frugiperda resistance to oral infection by Autographa californica multiple nucleopolyhedrovirus linked to aberrant occlusion-derived virus binding in the midgut. J Gen Virol. 2005;86:1349–55. PubMed PMID: 15831946.
37. Miller LK, Lu A. 1997. The molecular basis of baculovirus host range. *In* Miller LK (ed), The Baculoviruses. Plenum, New York.
38. Gomi S, Majima K, Maeda S. Sequence analysis of the genome of Bombyx mori nucleopolyhedrovirus. J Gen Virol. 1999;80:1323–37. PubMed PMID: 10355780.
39. Kuzio J, Pearson MN, Harwood SH, Funk CJ, Evans JT, Slavicek J, Rohrmann GF. Sequence and analysis of the genome of a baculovirus pathogenic for *Lymantria dispar*. Virology. 1999;253:17–34. PubMed PMID: 9887315.
40. Katou Y, Ikeda M, Kobayashi M. Abortive replication of Bombyx mori nucleopolyhedrovirus in Sf9 and High Five cells: defective nuclear transport of the virions. Virology. 2006;347:455–65. PubMed PMID: 16412489.
41. Iwanaga M, Takaya K, Katsuma S, Ote M, Tanaka S, Kamita SG, Kang W, Shimada T, Kobayashi M. Expression profiling of baculovirus genes in permissive and nonpermissive cell lines. Biochem Biophys Res Commun. 2004;323:599–614. PubMed PMID: 15369793.

42. Maeda S, Kamita SG, Kondo A. Host range expansion of *Autographa californica* nuclear polyhedrosis virus (NPV) following recombination of a 0.6-kilobase-pair DNA fragment originating from *Bombyx mori* NPV. J Virol. 1993;67:6234–6238. PubMed PMID: 8396678.
43. Kamita SG, Maeda S. Sequencing of the putative DNA helicase-encoding gene of the Bombyx mori nuclear polyhedrosis virus and fine-mapping of a region involved in host range expansion. Gene. 1997;190:173–9. PubMed PMID: 9185864.
44. Croizier G, Croizier L, Argaud O, Poudevigne D. Extension of *Autographa californica* nuclear polyhedrosis virus host range by interspecific replacement of a short DNA sequence in the p143 helicase gene. Proc Natl Acad Sc USA. 1994;91:48–52. PubMed PMID: 8278405.
45. Argaud O, Croizier L, Lopez-Ferber M, Croizier G. Two key mutations in the host-range specificity domain of the p143 gene of Autographa californica nucleopolyhedrovirus are required to kill Bombyx mori larvae. J Gen Virol. 1998;79(Pt 4):931–5. PubMed PMID: 9568990.
46. Kamita SG, Maeda S. Inhibition of *Bombyx mori* nuclear polyhedrosis virus (NPV) replication by the putative DNA helicase gene of *Autographa californica* NPV. J Virol. 1993;67:6239–6245. PubMed PMID: 7690422.
47. Xu YP, Gu LZ, Lou YH, Cheng RL, Xu HJ, Wang WB, Zhang CX. 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. 2012;93:2480–9. PubMed PMID: 22855783.
48. Woo SD, Roh JY, Choi JY, Jin BR. Propagation of Bombyx mori Nucleopolyhedrovirus in nonpermissive insect cell lines. J Microbiol. 2007;45:133–8. PubMed PMID: 17483798.
49. Mori H, Yamao M, Nakazawa H, Sugahara Y, Shirai N, Matsubara F, Sumida M, Imamura T. Transovarian transmission of a foreign gene in the silkworm, Bombyx mori, by Autographa californica nuclear polyhedrosis virus. Biotechnology (N Y). 1995;13:1005–7. PubMed PMID: 9636278.
50. Rahman MM, Gopinathan KP. Analysis of host specificity of two closely related baculoviruses in permissive and nonpermissive cell lines. Virus Res. 2003;93:13–23. PubMed PMID: 12727338.
51. Guo T, Wang S, Guo X, Lu C. Productive infection of Autographa californica nucleopolyhedrovirus in silkworm Bombyx mori strain Haoyue due to the absence of a host antiviral factor. Virology. 2005;341:231–7. PubMed PMID: 16087208.
52. Hofmann C, Sandig V, Jennings G, Rudolph M, Schlag P, Strauss M. Efficient gene transfer into human hepatocytes by baculovirus vectors. Proc Natl Acad Sci U S A. 1995;92:10099–103. PubMed PMID: 7479733.
53. Katou Y, Yamada H, Ikeda M, Kobayashi M. A single amino acid substitution modulates low-pH-triggered membrane fusion of GP64 protein in Autographa californica and Bombyx mori nucleopolyhedroviruses. Virology. 2010;404:204–14. PubMed PMID: 20627345.
54. Rahman MM, Gopinathan KP. Characterization of the gene encoding the envelope fusion glycoprotein GP64 from Bombyx mori nucleopolyhedrovirus. Virus Res. 2003;94:45–57. PubMed PMID: 12837556.
55. Ikeda M, Yamada H, Hamajima R, Kobayashi M. Baculovirus genes modulating intracellular innate antiviral immunity of lepidopteran insect cells. Virology. 2013;435:1–13. PubMed PMID: 23217611.
56. Lu A, Miller LK. Differential requirements for baculovirus late expression factor genes in two cell lines. J Virol. 1995;69:6265–6272. PubMed PMID: 7666527.
57. Lu A, Miller LK. Species-specific effects of the hcf-1 gene on baculovirus virulence. J Virol. 1996;70:5123–30. PubMed PMID: 8764020.
58. Guzo D, Rathburn H, Guthrie K, Dougherty E. Viral and host cellular transcription in Autographa californica nuclear polyhedrosis virus-infected gypsy moth cell lines. J Virol. 1992;66:2966–72. PubMed PMID: 1560533.
59. Thiem SM, Du X, Quentin ME, Berner ML. Identification of a baculovirus gene that promotes *Autographa californica* nuclear polyhedrosis virus replication in a nonpermissive insect cell line. J Virol. 1996;70:2221–2229. PubMed PMID: 8642646.

60. Chen CJ, Quentin ME, Brennan LA, Kukel C, Thiem SM. *Lymantria dispar* nucleopolyhedrovirus *hrf*-1 expands the larval host range of *Autographa californica* nucleopolyhedrovirus. J Virol. 1998;72:2526–2531. PubMed PMID: 9499118.
61. Du X, Thiem SM. Responses of insect cells to baculovirus infection: protein synthesis shutdown and apoptosis. J Virol. 1997;71:7866–7872. PubMed PMID: 9311875.
62. Mazzacano CA, Du X, Thiem SM. Global protein synthesis shutdown in Autographa californica nucleopolyhedrovirus-infected Ld652Y cells is rescued by tRNA from uninfected cells. Virology. 1999;260:222–31. PubMed PMID: 10417257.
63. Thiem SM, Chejanovsky N. The role of baculovirus apoptotic suppressors in AcMNPV-mediated translation arrest in Ld652Y cells. Virology. 2004;319:292–305. PubMed PMID: 14980489.
64. Ishikawa H, Ikeda M, Alves CA, Thiem SM, Kobayashi M. Host range factor 1 from Lymantria dispar Nucleopolyhedrovirus (NPV) is an essential viral factor required for productive infection of NPVs in IPLB-Ld652Y cells derived from L. dispar. J Virol. 2004;78:12703–8. PubMed PMID: 15507661.
65. Chen YR, Zhong S, Fei Z, Gao S, Zhang S, Li Z, Wang P, Blissard GW. Transcriptome responses of the host Trichoplusia ni to infection by the baculovirus Autographa californica multiple nucleopolyhedrovirus. J Virol. 2014;88:13781–97. PubMed PMID: 25231311.
66. Hodgson JJ, Buchon N, Blissard GW. Identification of insect genes involved in baculovirus AcMNPV entry into insect cells. Virology. 2018;527:1–11. PubMed PMID: 30445201.
67. Shrestha A, Bao K, Chen YR, Chen W, Wang P, Fei Z, Blissard GW. Global Analysis of Baculovirus Autographa californica Multiple Nucleopolyhedrovirus Gene Expression in the Midgut of the Lepidopteran Host Trichoplusia ni. J Virol. 2018.:92. PubMed PMID: 30209166.
68. Yue Q, Yu Q, Yang Q, Xu Y, Guo Y, Blissard GW, Li Z. Distinct Roles of Cellular ESCRT-I and ESCRT-III Proteins in Efficient Entry and Egress of Budded Virions of Autographa californica Multiple Nucleopolyhedrovirus. J Virol. 2018.:92. PubMed PMID: 29046462.
69. Guo Y, Yue Q, Gao J, Wang Z, Chen YR, Blissard GW, Liu TX, Li Z. Roles of Cellular NSF Protein in Entry and Nuclear Egress of Budded Virions of Autographa californica Multiple Nucleopolyhedrovirus. J Virol. 2017.:91. PubMed PMID: 28747507.

9. Baculoviruses as insecticides: Four examples

One of the earliest references for using natural pathogens for insect control was reported by V. Audouin in 1839, who tells of a sericulturist who emptied fungus-contaminated silkworm rearing trays out a window onto trees infested with defoliating insect larvae. Within a few days all of the defoliating insects had died of the fungus. More explicit suggestions for the microbial control of insects were made by J.L. LeConti in 1874 in an address to the AAAS, in which he recommended the study of epidemic diseases of insects and advocated their use to control insects. At the same time, Louis Pasteur, who had spent considerable effort investigating a microsporidian parasite of silkworms, recommended the use of this pathogen against an insect pest of grapes, described in (1).

In ecosystems, baculoviruses often play a major role in the suppression of a variety of different types of insects. For example, the virus of the gypsy moth, *Lymantria dispar,* is considered to be the major natural regulator of dense populations of this moth (2). Likewise, baculoviruses of the Douglas fir tussock moth, *Orgyia pseudotsugata*, are also major factors in the control of this insect (3). They also are found to naturally control pest insects of cultivated crops. For example, they were found to be major contaminants of cabbage purchased from supermarkets in the Washington, D.C. area, and it was estimated that an average serving of cole slaw would contain over 100 million occlusion bodies (4). Once the role these viruses played in controlling natural insect populations was understood, they were considered for a variety of insect control programs, particularly of forest pests (5).

There have been numerous reviews on the development of baculoviruses to control insects, e.g., (6-10), and despite this widespread interest and intrinsic attractiveness of their application, the acceptance and use of viruses for insect control has been limited. This can be attributed to their slow speed of kill, their limited host range (such that one preparation can only be used on a few insects), and to a certain degree, the complexity of producing standardized viral preparations. The slow speed of kill may be of particular advantage to the virus, because it results in greatly increased viral yields. However, delays in the death of the host result in more vegetation being consumed by the infected insect. A variety of recombinant viruses have been investigated that have been designed to enhance the efficacy of the virus by reducing the time it takes to kill target insects or by causing the cessation of feeding. These recombinants express insect specific toxins, insect hormones or enzymes, or are deleted for the EGT gene. Significant public resistance was encountered when a plan to test spray an AcMNPV expressing a scorpion toxin gene was announced in 1994 (11) (12).

Another contributing factor to the limited use of baculoviruses for biocontrol is that production of viral insecticides is labor intensive; consequently, their use has been limited to high value crops, particularly those that have become resistant to chemical insecticides or to crops in countries with access to relatively inexpensive labor. An exception appears to be their use against forest insects in North America. However, relative to the size of forested areas, these applications are also limited. In addition, the use of *Bacillus thuringiensis* preparations is highly competitive compared to baculoviruses because of the simplicity of their growth and formulation. In this chapter, I will review four instances where viruses have been relatively extensively applied in the field. Recent reviews include (13, 14).

An NPV of the velvet bean caterpillar, *Anticarsia gemmatalis*: Application in Brazil – A major setback

The most extensive program employing baculoviruses for insect control was developed in Brazil and to a lesser extent in Paraguay and involves a virus that infects the velvet bean caterpillar (*Anticarsia gemmatalis*), a pest of soybeans (15-17). Virus preparations are applied at 1.5×10^{11} occlusion bodies per hectare (about 20 g or 50 larval equivalents). This program was initiated in the early 1980s, and by 2005, the area treated had expanded to over two million ha, ((10) and references therein). Initially, laboratory production was not found to be economically viable, and virus production was carried out in farmers' fields. Plots of soybeans that were

naturally infested with *A. gemmatalis* were sprayed with virus and then the dead larvae were collected 8 -10 days after virus application. Individuals were able to collect about 1.8 kg of larvae/day at a cost in the mid 1990s of about $15. Production varied in the 1990s from enough virus to treat 650,000 to 1.7 million ha/year. By 1999, the production of virus was not sufficient to meet the demand.

Problems occurred with field production. i) The quantity of infected larvae was dependent upon the natural prevalence of the host insect that could vary from year to year; ii) There was a change in the collection procedure - the plants were shaken over drop cloths which led to a poor quality product because the material included other insects, debris, and *A. gemmatalis* that were not in the final stages of the virus infection cycle. The final product was of lower infectivity and also caused problems with application – the debris caused clogging of equipment used for application.

To address these problems a laboratory was established for the growth of larvae on an artificial diet and their infection under controlled conditions. Although slightly more expensive than virus produced under field conditions, it was of much higher quality. The company involved in this project scaled up to employ 45 people and infect 800,000 to 1,000,000 larvae per day resulting in the ability to produce enough virus to treat up to 2 million ha/yr. The infected larvae were processed into a wettable powder that involved milling the infected larvae and formulating them into a mixture that contains kaolin. Kaolin is an aluminosilicate compound first discovered in Kao-lin, China that is commonly used as an inert carrier or filler.

Some of the reasons for the feasibility of this viral control program were caused by specific features of AgMNPV. First, the virus is highly virulent for *A. gemmatalis* and usually only needs to be applied one time. In contrast, chemical insecticides often need to be applied twice. Furthermore, AgMNPV lacks the chitinase and cathepsin genes, so the insects die without 'melting,' and the dead insects can be more readily collected and processed than if they had disintegrated (18) (see Chapter 3). Another factor contributing to the initial success of the program is that soybean plants can endure significant defoliation without a reduction in yield. The virus can also be spread by insect predators and can survive passage through the digestive tract of beetles and Hemiptera (17). Overall, the use of the viruses was 20-30% less expensive than chemical insecticides, and it was estimated that the use of up to 17 million liters of chemical insecticides was been eliminated since the beginning of the program. Limitations included the reluctance of farmers to monitor their fields to determine the optimal timing for virus application and its use in regions that have low mean temperatures, which lengthens the time required to kill the insects. Extended periods of drought also adversely affect the efficacy of the virus preparation, ((10) and references therein).

Recently, a major shift in the method of soybean cultivation resulted in a dramatic decline in the use of the virus (19). This involved a change to a no-till system that involves the application of herbicides prior to planting. The farmers mixed broad-spectrum insecticides with the herbicides such that all plants and insects were indiscriminately eliminated. At about 2 to 3 weeks after soybean emergence, the fields were again treated with an herbicide-insecticide mixture. This resulted in the reduction of beneficial insects and the emergence of several other insects including mites, *Pseudoplusia includens* (the soybean looper), white flies, and species of *Spodoptera* that previously had been considered secondary pests. Consequently, since they could not be controlled by the AgMNPV preparation, alternative broad spectrum insecticides had to be used. The situation was further complicated by the introduction of soybean rust that required the application of fungicides that reduced the presence of naturally occurring entomopathogenic fungi that had served to naturally control some of the pest species. Collectively these changes led to a reduction in the area treated with AgMNPV to about 300,000 ha/year and resulted in the termination of the laboratory production of virus.

A granulovirus of the codling moth, *Cydia pomonella*: Application in North America and Europe

Whereas the use of AgMNPV has been limited predominantly to one major area in Brazil, a granulovirus of the codling moth *Cydia pomonella* (CpGV) has been used in a number of countries in North America and Europe for the control of the insect on pear and apple crops. CpGV was originally isolated from *C. pomonella* in Mexico in 1963 (20). Because of the development of resistance of codling moth to several chemical insecticides and for a variety of other safety and environmental reasons, the use of CpGV has increased in Europe and North America since 2000. The virus is used on a hundred thousand or more hectares on these continents. Currently, commercial preparations of the virus are available from several different companies and include preparations called Cyd-X and VirosoftCP4 in North America and in Europe include Carpovirusine™ (France), Madex™ and Granupom™ (Switzerland), Granusal™ (Germany), and Virin-CyAP (21) (10). The virus is highly virulent for codling moth with LD50's as low as 1.2-5 occlusion bodies per insect. The codling moth lays eggs on fruit trees, and after hatching, the larvae browse on leaves before entering fruit. They need to feed inside fruit for normal development, which can result in severe damage. Depending on the climate, there can be from one to three generations per season, and to ensure exposure during the brief window from hatching to entry into fruit requires the application of CpGV at least at weekly intervals up to six times in a season.

Recently, resistance to the virus has been described in Europe, with these insects able to tolerate CpGV over 1,000 times higher than previously observed. In laboratory experiments, it was determined that a gene conferring resistance is located on the male (Z) chromosome, and it was found that females with a single Z chromosome could be selected that were almost 100,000 times less susceptible to the CpGV infection (22). Because of the complexity of baculovirus replication it was often assumed that it would be challenging for an insect to develop resistance. However, these results clearly indicate that the alteration of a single or limited number of linked genes can severely compromise the infectivity of these viruses. Although the mechanism of CpGV resistance is not clear, its evolution emphasizes how dependent baculoviruses are on their hosts for carrying out their replication cycle and how a change in a single receptor or other protein, such as would be required for DNA replication, can interfere with virus infectivity.

An NPV of the cotton bollworm, *Helicoverpa armigera*: Application in China

The cotton bollworm, *Helicoverpa armigera,* is a major pest of cotton and other crops, and with the intensive use of chemical insecticides it has developed resistance in many parts of the world. One approach to counteract this resistance has been the use of baculoviruses for control of this insect. In China, HearNPV has been produced for use against the cotton bollworm. In 2014 there were 17 different products from 10 companies that incorporated HearNPV as the insecticidal component of their products and about 968 tons of this product were produced (13). The insects were grown on an artificial diet composed of mainly corn and wheat, and the infected larvae were processed after removal of lipids into wettable powders or emulsions. Treatment involves spraying fields 3 to 5 times per growing season to control two generations of the cotton bollworm. It was estimated that the virus preparation was used on 200,000 to 300,000 hectares of cotton in 2005. In India, it has been reported that insects are collected by shaking larvae off pea plants onto blankets. HearNPV is then produced by feeding the larvae virus-contaminated chickpea seeds (10).

A recombinant HearNPV is being evaluated in China that expresses a gene encoding an insect-specific toxin (AaIT) from a scorpion found in the Middle East and Africa called *Androctonus australis*. The use of this recombinant baculovirus is limited to experimental plots of about 2 hectares. In this construct, the AaIT gene is inserted at the EGT locus. This causes problems with the production of the recombinant virus in infected insect larvae. Since the toxin is active against larvae, and deletion of the EGT gene reduces the time it takes the virus to

kill the insect, the levels of production are significantly affected; under optimal conditions virus yield is less than 50% of wt. However, the yield from cotton plants treated with this virus is about 25% higher than from plants treated with wt virus. Consequently, this recombinant has significant advantages over the wt virus (23).

A variety of other viruses that are being produced in China range from 120 tons of AcMNPV to less than 50 tons of several other viruses in 2005. These were used to control a variety of insects mostly on vegetables and tea. The data described above is from (23) and Xiu-lian Sun (pers. Comm.)(13).

A nudivirus of the coconut palm rhinoceros beetle, *Oryctes rhinoceros*

Although the *Oryctes* virus is not a member of the Baculoviridae, it is of a related lineage and is an interesting example of the advantages of employing viruses for insect control. This section was based on a review by Alois Huger (24). The coconut palm rhinoceros beetle, *Oryctes rhinoceros* was accidentally introduced into a number of Pacific Islands in first half of the 20th century from tropical Asia. They feed on the developing fronds of several types of palm trees including coconut and oil palms. The damage reportedly caused the death of about 50% of the coconut palms in some locations. Attempts to control the insects using chemical insecticides were unsuccessful due to the inaccessibility of the pesticide to the insects.

In 1963 Alois Huger of the Institute for Biological Control in Darmstadt, Germany was hired for 4 months to attempt to isolate pathogens of the beetle. He focused his search on Malaysia where a native indigenous population of the insects were present. He visited oil palm plantations that provided an abundance of all stages of the insect that inhabited rotting palm logs and stumps. This material resulted from older trees that had been cut down as the plantations were replanted on a 30-35 year cycle. Larvae with an apparent disease were identified; they showed a negative geotropism and congregated on the surface of their feeding substrate (buckets of rotting sawdust) and were lethargic, showed a variety of pathological symptoms, and died within 1 to 4 weeks. Extracts from the diseased larvae were fed to healthy larvae resulting in similar symptoms which allowed for the investigation of the disease. It was found to be caused by a rod-shaped virus that replicated in virogenic stroma and was predominantly non-occluded. The virus was initially classified as a member of the Baculoviridae, but decades later viral genome sequence data indicated that it was more similar to members of the Nudiviridae, a viral family in the same lineage as baculoviruses (25).

In 1967 an experimental release of the virus was conducted on two islands in Samoa in which rotting sawdust containing the virus was used to contaminate rotting coconut logs, a major breeding substrate of the beetle. This treatment led to the collapse of the beetle populations on the treated islands and within 18 months diseased larvae were identified on a third island that had not been treated. It was subsequently determined that the adult beetles were also susceptible to the virus and became heavily infected in their midguts. The infected adults could survive for many weeks during which they defecated large amounts of virus into their breeding and feeding sites. The virus not only resulted in the death of infected larvae, but also caused a major reduction in the fecundity of infected female adult beetles. Subsequently, control of the insects was initiated by the contamination of adult insects. This led to a reduction in tree damage of up to 95%. Recent data suggests that the insect may have developed resistance to the virus (26).

Possible unintended consequences. The Japanese rhinoceros beetle, *Allomyrina dichotoma*, is found in Southeast Asia and is raised commercially for medicinal use, as children's pets, and for entertainment and gambling via staged beetle combat. In 2012 it was reported that *A. dichotoma* larvae on a farm in Korea died en masse. By 2014 similar incidents were reported throughout Korea. Analysis by pcr using primers specific to the *Oryctes rhinoceros* nudivirus (OrNV) gave positive products that were 98% identical to the OrNV genome sequence (27). It was suggested that the virus, which was not previously reported in Korea, had somehow been

transported there. In contrast to the *Oryctes rhinoceros* beetle, *A. dichotoma* is not an agricultural pest, but a part of a niche farming economy. The source of this virus has not been determined.

References

1. Steinhaus EA. 1975. Disease in a minor chord. Ohio State University Press, Columbus, Ohio.
2. Podgwaite JD. 1981. Natural disease within dense gypsy moth populations. *In* Doane CC, McManus ML (ed), The Gypsy Moth: Research Towards Integrated Pest Management. U.S. Dept. of Agriculture, Washington, D.C.
3. Thompson CG. 1978. Nuclear polyhedrosis epizootiology. *In* Brookes MH, Stark RW, Campbell RW (ed), The Douglas-fir tussock moth: a synthesis. U.S. Department of Agriculture, Washington, D.C.
4. Heimpel AM, Thomas ED, Adams JR, Smith LJ. The presence of nuclear polyhedrosis virus of Trichoplusia ni on cabbage from the market shelf. Environ Entomol. 1973;2:72–75.
5. Huber J. 1986. Use of baculoviruses in pest management programs, p 181-202. *In* Granados RR, Federici BA (ed), The biology of baculoviruses, vol II. CRC, Boca Raton.
6. Black BC, Brennan LA, Dierks PM, Gard IE. 1997. Commercialization of baculoviral insecticides, p 341-387. *In* Miller LK (ed), The Baculoviruses. Plenum Press, New York.
7. Inceoglu AB, Kamita SG, Hammock BD. Genetically modified baculoviruses: a historical overview and future outlook. Adv Virus Res. 2006;68:323–60. PubMed PMID: 16997016.
8. (ed). 2012. Recent Advances in Our Knowledge of Baculovirus Molecular Biology and Its Relevance for the Registration of Baculovirus-Based Products for Insect Pest Population Control. Intech, Accessed
9. (ed). 2012. Baculoviruses: Members of Integrated Pest Management Strategies. Intech, Accessed
10. Szewczyk B, Hoyos-Carvajal L, Paluszek M, Skrzecz I, Lobo de Souza M. Baculoviruses — re-emerging biopesticides. Biotechnol Adv. 2006;24:143–160. PubMed PMID: 16257169.
11. Coghlan A. Focus: Will the scorpion gene run wild? - Fears over genetic engineering have been heightened by an Oxford scientist's plan to release a virus that carries a gene for scorpion venom. New Sci. 1994.
12. Coghlan A. Scorpion gene virus on trial in Oxford. New Sci. 1994.
13. Sun X. History and current status of development and use of viral insecticides in China. Viruses. 2015;7:306–19. PubMed PMID: 25609304.
14. Haase S, Sciocco-Cap A, Romanowski V. Baculovirus insecticides in Latin America: historical overview, current status and future perspectives. Viruses. 2015;7:2230–67. PubMed PMID: 25941826.
15. Moscardi F. 1989. Use of viruses for pest control in brazil: The case of the nuclear polyhedrosis virus of the soybean caterpillar, *Anticarsia gemmatalis*. Memorias do Instituto Oswaldo Cruz, Rio de Janeiro 84:51-56.
16. Moscardi F. Assessment of the application of baculovirus for control of lepidoptera. Ann Rev Entomol. 1999;44:257–289. PubMed PMID: 15012374.
17. Moscardi F, Sosa-Gomez DR. 1993. A case study in Biological control: soybean defoliating caterpillars in Brazil, abstr 1st International Crop Science Congress, Madison, WI, 1993. Crop Science Society of America, Inc.,
18. Oliveira JV, Wolff JL, Garcia-Maruniak A, Ribeiro BM, de Castro ME, de Souza ML, Moscardi F, Maruniak JE, Zanotto PM. Genome of the most widely used viral biopesticide: Anticarsia gemmatalis multiple nucleopolyhedrovirus. J Gen Virol. 2006;87:3233–50. PubMed PMID: 17030857.
19. Moscardi F, de Souza ML, de Castro MLM, Moscardi L, Szewczyk B. 2011. Baculovirus Pesticides: Present State and Future Perspectives, p 415-445. *In* Ahmad I, Ahmad F, Pichtel J (ed), Baculovirus Pesticides: Present State and Future Perspectives. Springer, New York.
20. Tanada Y. A granulosis virus of the codling moth, Carpocapsae pomonella (Linnaeus) (Olethreutidae, Lepidoptera). J Insect Pathol. 1964;6:78–80.
21. Neumann JR, Morency CA, Russian KO. A novel rapid assay for chloramphenicol acetyl transferase gene expression. Biotechniques. 1987;5:444–447.

22. Asser-Kaiser S, Fritsch E, Undorf-Spahn K, Kienzle J, Eberle KE, Gund NA, Reineke A, Zebitz CP, Heckel DG, Huber J, Jehle JA. Rapid emergence of baculovirus resistance in codling moth due to dominant, sex-linked inheritance. Science. 2007;317:1916–8. PubMed PMID: 17901332.
23. Sun X, Peng H. Recent advances in biological control of pest insects by using viruses in China. Virolog Sinica. 2007;22:158–162.
24. Huger AM. The Oryctes virus: its detection, identification, and implementation in biological control of the coconut palm rhinoceros beetle, Oryctes rhinoceros (Coleoptera: Scarabaeidae). J Invertebr Pathol. 2005;89:78–84. PubMed PMID: 16039308.
25. Wang Y, van Oers MM, Crawford AM, Vlak JM, Jehle JA. Genomic analysis of Oryctes rhinoceros virus reveals genetic relatedness to Heliothis zea virus 1. Arch Virol. 2007;152:519–31. PubMed PMID: 17106621.
26. Reil JB, Doorenweerd C, San Jose M, Sim SB, Geib SM, Rubinoff D. Transpacific coalescent pathways of coconut rhinoceros beetle biotypes: Resistance to biological control catalyses resurgence of an old pest. Mol Ecol. 2018;27:4459–4474. PubMed PMID: 30252975.
27. Lee S, Park KH, Nam SH, Kwak KW, Choi JY. First report of Oryctes rhinoceros nudivirus (Coleoptera: Scarabaeidae) causing severe disease in Allomyrina dichotoma in Korea. J Insect Sci. 2015.:15. PubMed PMID: 25765317.

10. Baculovirus expression technology: Theory and application

Some History

I participated in a symposium on microbial pesticides organized by Lois Miller as part of a regional American Society for Microbiology meeting in Moscow, Idaho in mid-June, 1983. At that meeting she showed me some pictures of a baculovirus expression system — blue β-galactosidase plaques on *S. frugiperda* cells — developed in her laboratory by an undergraduate student, Greg Pennock. She indicated that she was using them as cover pictures for an issue of *Cell* to which she had submitted a manuscript describing this work. Later that summer, at the most riveting American Society for Virology workshop that I ever attended, she described this system as did Gale Smith, who showed baculovirus expression of an interferon. Gale Smith and Max Summers published their report later in the year (1). Lois Miller's report was rejected by *Cell,* thereby delaying publication until 1984 (2) and giving Max Summers and Gale Smith clear precedence with regard to this technology. In addition, their production of a practical manual detailing the use of the system (3) and distribution of its major components greatly facilitated its widespread application before it was commercially available. The development of this technology gave birth to a new era in baculovirology based on targeted gene recombination and has resulted directly or indirectly in much of the progress in understanding the biology of these viruses along with advances in many other biological systems dependent on protein expression technology. The application of this technology culminated with the licensing by the FDA of vaccines produced by recombinant baculovirus that are directed against human papilloma virus and influenza (4, 5). Veterinary vaccines against porcine circovirus and swine fever virus are also in use, reviewed in (6). The variations and modifications of the baculovirus expression system are not within the scope of this book and have been ably covered in several recent reviews (7). However, I will give a brief overview of the combination of biological features of insects and baculoviruses that have contributed to the evolution of a system of such utility for biotechnology. These are summarized in Table 1. I will then cover the major advances in the development of the bacmid based systems that has revolutionized baculovirus expression technology.

Table 1. Major factors influencing high levels of baculovirus very late gene transcription

	Factor	Result
1	Ability of virus to cause systemic infection	Allows virus to exploit insect synthetic systems, e.g., the fat body
2	Shut off of early baculovirus and host gene transcription	Makes host cell biosynthetic systems available for baculovirus gene expression
3	Shut off of baculovirus late gene expression	Makes baculovirus RNA polymerase available for very late gene expression
4	DNA replication and a high concentration of unpackaged viral DNA	High copy numbers of very late genes are accessible for transcription
5	Efficiency of the baculovirus RNA polymerase	Facilitates high level mRNA production and RNA capping
6	The biosynthetic capacity of insect cells	Allows high levels of very late gene expression

Initiating infection: environmental stability and the insect midgut

Insect species are the predominant terrestrial eukaryotic animals both in terms of species numbers and also in their contribution to the terrestrial biomass. After Coleoptera, members of the family Lepidoptera are some of the most numerous in terms of species numbers. Members of this family are phytophagous, and in temperate climates and in many tropical areas where there are distinct wet and dry seasons, insect populations are

transitory and expand dramatically during warm, moist periods, and then collapse with the onset of colder temperatures or drought when food sources are reduced or eliminated. In addition, even during seasons optimal for insect growth and reproduction, their populations are normally limited by predators, pathogens, normal temperature cycles, and food sources. However, under certain circumstances, insect populations can expand dramatically when a combination of conditions greatly increases their food supply, facilitates high levels of reproduction, or eliminates predators and pathogens. These cycles of population expansion are well-documented for forest insects and may be separated by long periods of time. The brief life cycle of most Lepidoptera, often limited to a few weeks, allows them to rapidly respond to such environmental conditions. For example, typical lepidopteran larvae, e.g., *M. sexta*, are capable of exponential growth that can be particularly dramatic in the last larval instar, during which a 12-fold increase in mass (from 1g to 12 g) in as few as 4 days has been documented (8). However, considering the small size of their eggs, an overall increase in mass of several thousand-fold occurs over the 3 weeks of their development. The capacity of Lepidoptera to both ingest and assimilate large amounts of food combined with their prolific reproductive abilities are correlated with the devastating losses that they can inflict on both native and cultivated vegetation. One of the purposes of these levels of synthesis is for the production of lipids and proteins that are involved in egg production in females and also in the major transformations that occur during metamorphosis (9). In addition, adult Lepidoptera lack mandibles and either shift to nectar feeding via an extendable proboscis, or do not feed at all, thereby necessitating the storage of energy during the larval stage. Consequently, because of their ability to proliferate to high population densities and their metabolic potential, Lepidoptera provided an attractive target for viral parasitization.

Several major problems had to be solved to allow for baculoviruses to successfully infect Lepidoptera. First, they had to evolve a method of infection. All baculoviruses appear to normally infect insects via the digestive tract as contaminants of the food supply. The insect midgut provided viral access to cells that lacked a resistant chitinous surface. Second, they had to develop a method of persistence so that they could survive the interludes between the population cycles of their hosts. Third, they had to confront the environment of the insect midgut that attains some of the highest pH levels recorded in Nature. At the entry and exit of the midgut, the pH is commonly near 7.0; however, in the central region, levels of 10.0 to as high as 12.0 have been recorded (10) (see Chapter 3, Figure 3). The problems of environmental stability and the high pH of the insect midgut have been solved by several apparently unrelated viruses in a similar manner. This solution involves incorporation of virions into an alkali-soluble occlusion body that provides stability outside the host insect, allowing them to persist in the environment between insect populations. It also provides for the release of the virions when an environment conducive to infection is encountered. Few if any areas of such high pH are present in nature, except for the lepidopteran midgut. The combination of occlusion with alkali solubility has been so successful that it has apparently evolved independently on at least three occasions: with the baculoviruses, the cypoviruses, and the entomopox viruses. All are insect viruses that infect their hosts via the midgut, but are from completely different viral families. In all cases, the consequence of occlusion is the fact that all these different viruses have developed the ability to express one or two proteins at very high levels. These form the occlusion body matrices.

The infection of insects requires that the occluded virions be liberated from the protein matrix, and this is accomplished by the alkaline conditions encountered in the midgut. Surviving and initiating infection under these conditions is apparently such a highly specialized task that many of the proteins that are involved in this process are conserved throughout not only the Baculoviridae, but also in related groups of nonoccluded viruses, the nudiviruses, and hytrosaviruses. Many of what are considered the more primitive baculoviruses appear to limit their infection to the gut cells. However, when limited to this tissue, their reproductive potential is greatly diminished because they do not have access to systemic infection — in particular, to fat body cells that comprise the major metabolic organ of lepidopteran larvae.

Evolution of a biphasic replication cycle that allowed exploitation of the biosynthetic capacity of insect systems

The ability of baculoviruses to exploit the power of the lepidopteran metabolic system was likely facilitated by their ability to move from the midgut to cause systemic infections. This required the production of a viral type that could initiate infection in a completely different environment: one in which the pH was near neutral and that lacked the high concentrations of enzymes that the occluded virions had evolved to survive. Consequently, baculoviruses appear to have incorporated a more conventional envelope fusion protein, called the F protein, which is similar to the pH-dependent fusion proteins of many viruses. This protein is expressed by the virus, modifies the cytoplasmic membrane and is obtained by the virus when it buds out of the infected cells. It allows the virus to infect a wide variety of cells throughout the insect and thereby provides access to the prodigious metabolic potential of the insect larvae. Subsequently, another fusion protein, GP64, displaced the fusion function of the F protein in one baculovirus lineage (including AcMNPV) and is the focus of much of the research in biotechnology (see Chapter 2).

Optimizing the cellular environment: The viral RNA polymerase and the shut-down of most viral and host genes late in infection

Upon infecting cells, baculoviruses appear to be able to focus transcription on their own genes probably by the expression of the powerful transactivator IE1 along with the presence of enhancer sequences on the viral genome (see Chapter 5). In addition, a major innovation was their incorporation of an RNA polymerase. The viral RNA polymerase allows exploitation of the insect synthetic machinery independent of the constraints of host cell transcription.

In addition to exploiting the synthetic capacity of insects that became accessible by the evolution of systemic infections, several other factors contributed to the evolution of high-level baculovirus gene expression. Identification of one of these factors was based on the observation that most baculovirus genes are shutdown after they have been expressed. An example is the gp64 gene of OpMNPV that has both early and late promoter elements (11). The early promoter is activated shortly after infection (6 hr pi in *L. dispar* cells) and continues until 36 hr after which it declines. Activation of the late promoters occurs between 24 and 36 hr p.i. and then also declines. The vp39 gene, which only has a late promoter element, showed similar kinetics (12). In contrast, the polyhedrin and p10 gene transcripts are present very late in infection (60 hr p.i.) (13, 14). In addition, it has been demonstrated that AcMNPV infections of *S. frugiperda* cells leads to a reduction in levels of almost all host cell mRNAs by 24 hr p.i. (15). The shutoff of most host genes would likely make both the cellular transcriptional and translational apparatus available so that it can be focused on the expression of viral genes. Similarly, the shutoff of viral late genes likely frees up the viral RNA polymerase so that it can focus on the transcription of polyhedrin and p10. It is not clear what governs the shut off of viral and host genes. The viral transactivator IE1, along with *hr* enhancer sequences, may divert early transcription from host cell genes to viral genes. This could lead to the shut-down of most host genes. The expression of baculovirus late genes occurs after DNA replication is initiated. This could be facilitated by the presence of newly replicated (naked) DNA, or by the presence of the un-ligated junctions of Okazaki fragments that may serve as transient enhancers of late transcription by acting as loading sites for late gene activators that track along the DNA until late promoters are recognized (16) (see Chapter 6). Once DNA replication is completed, these loading sites for late gene activators are no longer available and this may be reflected in the turn off of late genes.

Very late gene (p10 and polyhedrin) activation and transcription

If viral late genes are shut down by the accumulation of DNA binding proteins, very late gene transcription may be induced by the expression of a transcriptional activator (see Chapter 6, Figure 1). As described in Chapter 6, VLF-1 is one of the few genes that has been implicated in the selective up regulation of very late genes and has been shown to interact with the burst sequence of p10 and polyhedrin regulatory regions in gel shift assays (17). LEF-2 (18, 19) and protein kinase 1 (Ac10) (20, 21) (18) (22) may also be involved in this process. In addition to focusing very late transcription on polyhedrin and p10, the high levels of transcription could be influenced by the efficiency of the baculovirus RNA polymerase in transcribing these two genes and also the ability of LEF4 to cap the very late transcripts so that they can be transported and translated efficiently.

A role for gene copy number and non-encapsidated viral DNA

Another major contributing factor to high levels of very late gene expression is likely to be a high copy number of polyhedrin and p10 genes. Baculovirus very late gene expression is fundamentally concerned with the occlusion of nucleocapsids. However, in order for very late genes to be transcribed, it is likely that a significant portion of the DNA synthesized during infection is never packaged into nucleocapsids and is lost at the end of the infection cycle. It is critical that this DNA is not incorporated into nucleocapsids, because once incorporated, it would not be accessible to the RNA polymerase. Indeed, it has been observed that, whereas 100% of BV DNA is resistant to DNAse, up to 70% of viral DNA present in extracts of cells late in infection was sensitive to DNAse, suggesting that it was not packaged and is therefore accessible to digestion (23). The magnitude of gene copy number is reflected in the number of genomes synthesized per cell. AcMNPV and *H. armigera* NPV (HearNPV) were reported to yield up to 81,000 and 131,000 genomes per infected cell, respectively (24) (25). Furthermore, it has been observed that, whereas a portion of the DNA in infected cells can be converted to unit length DNA, most of the DNA appears to be present in complex, possibly branched structures (26). This has led to the suggestion that there are two categories of baculovirus DNA (see Chapter 5): DNA destined to be incorporated into nucleocapsids, and DNA that is never packaged because it is required for very late transcription. The synthesis of DNA incorporated into nucleocapsids may be coordinated with its packaging into virions, whereas the extra-viral DNA is synthesized independent of packaging and undergoes extensive recombination because it is not protected from this process by incorporation into virions. The likely complex structure of the unpackaged DNA would probably not interfere with high levels of very late transcription.

Baculovirus gene expression and biotechnology

As described above, the biosynthetic capacity of baculoviruses is derived from the intersection of their infections with the growth of their hosts. This results in the diversion of the cellular macromolecular material and energetic capacity to viral rather than insect growth. The ability to recreate this environment in the laboratory was dependent upon the isolation of insect cell lines permissive for baculovirus infection, and the identification of growth conditions that allow for optimal viral replication in these cells. The isolation and development of a variety of insect cell lines to be both permissive for baculovirus replication and to have the capacity to undergo exponential growth has allowed for the exploitation of the combination of both the insect synthetic capacity and the virus in the form of the baculovirus expression system.

Summary and conclusions

It is likely that several factors have combined to make baculoviruses highly efficient in gene expression. This includes gene amplification, the shutoff of most other genes very late in infection, the specific activation of very late genes, efficient gene transcription, and access to the protein synthetic machinery of the host insect, which

has evolved to synthesize proteins at high levels to allow the insect larvae to undergo growth and development in a very compact time frame. This is summarized in Table 1.

Whereas equally high levels of gene expression are present in other eukaryotic systems, e.g., the production of ovalbumin in the avian oviduct and the production of various milk proteins in mammals, the expression of these proteins is limited to specific tissues and does not occur systemically. In addition, whereas transgenic animals have been engineered to exploit these systems, they can in no way compare with the ease of manipulating baculoviruses and their cell culture systems for protein production.

Baculovirus Expression Technology: Application

Homologous recombination

The original method for the construction of recombinant baculoviruses was based on homologous recombination and involved the co-transfection of viral DNA along with a plasmid that contained the foreign gene under the control of the polyhedrin promoter and flanked by sequences from the polyhedrin region (1) (2). The selection of recombinants was based on the elimination of the polyhedrin gene and the detection of occlusion negative plaques in cell monolayers. These plaques often occurred at low frequency and their identification required skill and familiarity with the system and often involved extensive screening to confirm that the recombinant expressed the correct insert. This system was improved by a variety of innovations that favored the selection of recombinant viruses, reviewed in (27, 28). One of the protocols involved the linearization of the virus at the polyhedrin locus. Recombination would re-circularize the viral genome and this favored replication (amplification) of the genome (29). Various combinations of this approach were also employed including the incorporation of a lacZ gene at the polyhedrin locus and subsequent linearization, or the further engineering of unique restriction enzyme sites into an essential gene (orf1629) that flanks the polyhedrin locus (30). Linearization using these sites results in a defective genome. The portion of the orf1629 gene deleted in the baculovirus genome was incorporated into the transfer plasmid such that orf1629 function would be restored in recombinants. This along with the elimination of lacZ expression as proof of recombination, greatly facilitated the ability to screen recombinants. However, in recent years the most common approach employs variations of the bacmid system described below.

The bacmid system(31)

The bacmid system exploits two remarkable technologies: the ability of the bacterial transposon Tn7 to specifically integrate adjacent to a defined sequence in bacterial genomes, and the identification of the mini-F plasmid replicon that, when incorporated into the baculovirus genome, allows its coordinated replication in bacteria as an artificial chromosome. The bacmid technology is based on the incorporation of the components of the Tn7 system into a transfer plasmid containing the Tn7 integration signal, and then using a helper plasmid with transposase functions, and a bacmid that contained the specific Tn7 target sequence within a baculovirus genome, as a targeted method of recombination in bacterial cells. Subsequent isolation of recombinant DNA from bacteria and transfection into insect cells normally results in a viable virus. Because all the added genes except the foreign gene are expressed from bacterial promoters, the genes added to the baculovirus genome are likely to be inactive in insect cells.

The bacmid system: The theoretical basis.

This system exploits a novel transposon called Tn7 that, in contrast to many transposons that insert randomly or into a small target sequence present in many locations, specifically inserts near a highly conserved 36 nt sequence at high frequency.

This system is composed of the transposon Tn7 that was originally identified inserted into a plasmid called R483 because it carried resistance genes to two antibiotics; trimethoprim and streptomycin/spectinomycin) (32). This plasmid was originally isolated from calves that were being fed high doses of trimethoprim (33). In addition to the two resistance genes, Tn7 contains 5 genes that are involved in transposition and flanking sequences of 150-bp (Tn7L) and 90-bp (Tn7R) that are involved in this process (see below). Tn7L contains 3 and Tn7R contains 4 22-bp transposase binding sites. The complete sequence comprises about 14 kb (Figure 1A). In addition to the transposon, the Tn7 system includes a target or attachment site (attTn7) that is the 12 C-terminal codons (36-bp) of the 609-amino acid (in *E. coli*) bacterial protein glmS (34) (35) (Figure 1C). This sequence encodes part of the active site of GlmS, an enzyme involved in the production of N-acetyl-glucosamine, that is a component of an essential cell wall peptidoglycan. Although Tn7 recognizes this sequence, it actually inserts into a sequence located 23 nt downstream of the glmS orf (Figure 1C). Consequently, insertion of Tn7 does not affect the viability of bacteria. In addition, the insertion is normally orientation specific such that Tn7 transcription is in the same orientation as glmS. Although Tn7L and Tn7R show sequence relatedness to each other, they are not related to the AttTn7 sequence (36).

The transposase helper plasmid (Figure 1B)

This construct contains all the genes involved in Tn7 transposition (TnsA-E), a selectable marker TetR, and an origin of replication from the plasmid pBR322. Although it supplies the transposase functions, since it lacks a sequence involved in integration (Tn7L), it will not cause its own integration into other sequences.

Transposition

Transposition is thought to be accomplished as follows: TnsA and TnsB recognize the ends of the transposon (Tn7R and Tn7L), excise it, and then join it to the target DNA. TnsA is related to a category of type II restriction enzymes that cleave DNA downstream of their recognition site in a non-specific sequence– it has been compared structurally to FokI (37), whereas TnsB is related to retroviral integrase. TnsD and E are involved in target selection and activate the transposase machinery that is composed of the TnsA and TnsB. TnsC is an ATPase and is involved in the control of transposition. It interacts with TnsA, activates the TnsAB transposase and is also able to bind to distorted target DNA thereby linking the transposase to the site of insertion.

The baculovirus bacmid

The mini-F replicon

The F (Fertility) plasmid in E. coli K-12 was the first plasmid/bacterial host system to be identified because of its ability to transfer its DNA via a tube-like pilus to bacteria lacking the F plasmid. The F plasmid genome is about 100 kb and encodes about 100 genes that are involved in three main functions i) pilus structure which is essential for cell recognition, contact, and physical transfer of plasmid DNA, ii) conjugative transfer of plasmid DNA, and iii) replication and maintenance of the plasmid in its bacterial host. This latter region was discovered when it was found that a 9 kb EcoRI fragment supported replication of a kanamycin resistant plasmid in *E. coli* and was termed the mini-F replicon (38) (39) (40).

Subsequently the mini-F replicon was exploited in the construction of bacterial artificial chromosomes such as bacmids. The incorporation of the mini-F replicon into, for example, a baculovirus genome allows it to replicate and be stably maintained in bacterial cells such that upon division each daughter cell inherits 1 or 2 copies (41) (42). For the construction of the original baculovirus bacmid, this mini-F replicon sequence was further trimmed to about 7 kb by the elimination of a BamHI-EcoR1 sequence at one end which included an origin of replication, oriV, and two other genes. The remaining sequence contains *oriS,* a unidirectional origin of replication, *repE,* a protein essential for replication that also influences copy number, partitioning (par) proteins *sobA*(*parA)* and *sobB(parB)* that are involved in the segregation of DNA during cell division thereby insuring

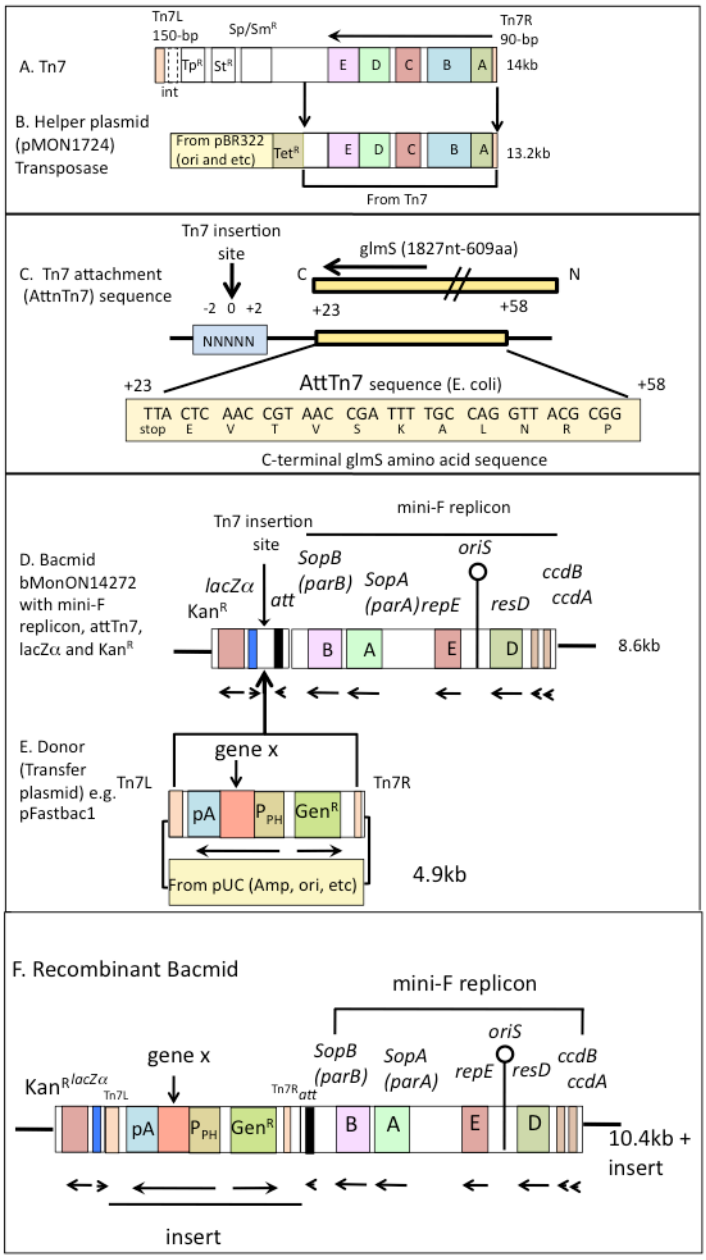

Figure 1. Maps of major constructs used for the production of the bacmid system. A) Map of Tn7. Shown are the genes A-E that are involved in transposition (see text) and at the left end int associated with an inactive integron system and three antibiotic resistance genes including Tp^r (trimethoprim), streptothricin St^r, and streptomycin and spectinomycin Sp/Sm^r (37) (36). B.) Map of the helper plasmid showing the components of Tn7 used in its construction. C) Tn7 attachment sequence. Map of the C-terminal end of the glmS gene showing the Tn7 insertion site 21-25 nt downstream. D). This map shows the organization of the integration site along with the region from the fertility (F) plasmid (mini F-replicon). The genes shown are involved in the coordination of the replication of the bacmid with the bacterial genome (see text). Also shown is the Tn7 integration site (*att*) and the kanamycin resistance gene (Kan^r) and *lacZα*. E.). Map of the donor (transfer) plasmids such as pFastbac1. The location of the polyadenylation signal (pA) polyhedrin promoter (P_{ph}), the selectable marker gentamicin resistance (Gen^R) along with the location of the gene being expressed (gene x). The sequences involved in integration into the att site, Tn7L and Tn7R, are also shown. The rest of the map are the components of the pUC plasmid used for making the transfer plasmid). F.). This is a map of an example of a recombinant bacmid. For abbreviations see above.

that daughter cells contain an appropriate DNA complement, *resD* that is required for the resolution of cointegrates, and *ccdA* and *ccdB* (coupled cell division) that are involved in the inhibition of cell division (Figure 1D). The bacmid is further modified by the addition of a Kanamycin resistance gene, the lacZa sequence for color selection, and the Tn7 transposase attachment (att) site (Figure 1D).

The transposon target site

This sequence is from a 550 bp HincII fragment from E.coli glmS C-terminal region and contains the attTn7 sequence (43) (Figure 1C). This is the target sequence where the Tn7 sequences (Tn7L Tn7R) bordering the insert in the transfer plasmid integrate.

The donor (transfer) plasmid (Figure 1E)

The donor plasmid is the cloning vector used for positioning the foreign gene of interest such that it can be transposed into the Tn7 integration site in the bacmid. As an example, pFastBac1 from Invitrogen is shown. The transposable region is bracketed by Tn7R and Tn7L and includes a selectable marker for gentamicin resistance and the 94-bp polyhedrin promoter region which extends from a remnant EcoRV half-site upstream of the ATAAG promoter down to the AT of the polyhedrin ATG. Downstream of the promoter there is a polylinker region that can also include other sequences such as 6xHis tag to facilitate protein purification, a protease sequence allowing subsequent removal of the tag, and by a SV40 polyadenylation signal.

Recombinant bacmid production

The transposase helper plasmid (Figure 1B) is maintained in bacteria by Tet selection along with the bacmid sequence that is maintained by Kan selection (Figure 1D). In the presence of the transfer plasmid (Figure 1E), the transposase causes the insertion of the portion of the transfer plasmid bordered by Tn7L and Tn7R downstream of the att site (Figure 1D) resulting in the recombinant bacmid shown in Figure 1F. Selection is by gentamicin. In addition, the insertion inactivates the production of lacZa, thereby allowing selection of recombinant white colonies using Xgal. Transposition could also occur into the bacterial genome because it encodes the essential glmS gene that contains the attTn7 target sequence, however these colonies should be blue.

Further modification: the Multibac system (44)

The bacmid system has been further modified for the production of multisubunit proteins on a single bacmid. For example, the 1.1 mDa 13 subunit anaphase promoting complex (APC/C) was expressed from two recombinant baculoviruses (8 subunits in one and 5 in the other) (45). In another project the 223 kDa 7 subunit mediator head complex was expressed in a single baculovirus and used for structural determination by X-ray crystallography (46). The multibac vectors use dual very late promoters (polyhedrin and p10) that are inserted into the polyhedrin locus using a system similar to that described above (Figure 1A). It also contains a loxP site in the *v-cath* and *chiA* (Ac126 and Ac127) locus (Ac126 and Ac127 are non-essential in cell culture and are disrupted in this construct). *Vcath* encodes a protease (cathepsin) and elimination of this activity stabilizes expression products, whereas *vchi* encodes a chitinase and its elimination facilitates the application of chitin-affinity chromatography for protein purification. The loxP site allows recombination with a plasmid also containing a loxP site in the presence of cre recombinase that is expressed on a separate plasmid. A set of additional technologies have also been applied. These include the use of internal ribosome entry sites (IRES) to allow translation of tandem protein coding sequences from a single mRNA, the insertion of self-cleaving polyprotein constructs or by the co-expression of a tobacco etch virus protease (TEVP) and the incorporation of TEVP recognition sequence between the domains of a polyprotein sequence.

Acceptor and donor plasmids

The number of genes incorporated into a transfer vector can be increased using the concept of 'acceptor' and 'donor' plasmids. Acceptor plasmids resemble standard plasmids and contain an origin of replication that allows propagation in *E. coli*. In contrast, the donor plasmids contain a conditional origin of replication that requires an additional protein that is expressed from a modified host *E. coli* strain. Both plasmids contain baculovirus promoters, different antibiotic resistance genes, and loxP sites. Consequently. when the donor and acceptor plasmid DNA along with a plasmid expressing cre recombinase are transfected into the normal E.coli host (that

will not support replication of the donor plasmid) and subjected to selection with both antibiotics, the surviving plasmid will be a recombinant expressing both antibiotic genes. The plasmids also contain unique endonuclease sites, e.g. BstXI or homing endonuclease sites that can facilitate the insertion of additional genes. Homing endonucleases are encoded by introns or inteins and have long recognition sequences. The insert region is flanked by the Tn7L/Tn7R sequences and once the desired set of genes is assembled, it can be inserted into the bacmid sequence at the *att* site using the Tn7 transposase system (Figure 1). Further genes can be incorporated into donor plasmids and recombined into the *v-cath/chiA* locus. For construction of the APC/C 8-subunit complex, a plasmid containing 6 genes was inserted using the Tn7 system, whereas the other two genes were located on another plasmid and inserted into the *v-cath/chiA* locus using *cre* recombinase. The other bacmid was made by incorporating 5 genes into the Tn7 *att* locus (45).

The flashbac system. A hybrid bacmid-homologous recombination system (28)

This novel technology for the production of recombinant baculoviruses combines the advantages of bacmid technology with homologous recombination in insect cells. A defective baculovirus/bacmid construct is used that replicates in bacteria, but when co-transfected into insect cells with the transfer vector, the only viable recombinants are ones that are derived from a plasmid that repairs the defect in the baculovirus genome. To accomplish this, a bacmid was engineered that lacks a portion of the C-terminal region of orf1629 (Ac9) that is adjacent to the polyhedrin locus. Orf1629 is an essential gene and encodes a structural protein located at the base of the virion that is involved in actin assembly (see Chapter 3). Orf1629 is repaired during the recombination event and results in a viable virus with the gene of interest inserted into the polyhedrin locus. This approach has a number of advantages over other baculovirus expression systems including the elimination of the need for i) antibiotic or lacZ screening, ii) the transposase encoding helper plasmid, and iii) plaque purification. In the process of recombination, the mini-F replicon region is eliminated since it is flanked by the lef2 (ac6) and orf1629 genes that border the polyhedrin gene locus and are the sites of recombination (Figure 2).

In the original *flash*BAC construct, the chitinase (*chiA*) (Ac126) gene was deleted because it was observed to interfere with the secretory pathway in the endoplasmic reticulum. Deleting *chiA* improved the expression of secreted and membrane proteins. In a subsequent vector, *flash*BACGOLD, the baculovirus cathepsin (*vcath*) (Ac127) gene that encodes a proteinase was deleted in addition to *chiA*. This reduces the proteolysis of expressed proteins. A subsequent vector, *flash*BACULTRA has an additional three adjacent genes deleted, p26 (Ac136), p10 (Ac137), and p74 (Ac 138). Since p10 is a highly expressed very late gene, its elimination reduces competition with the polyhedrin very late promoter, p74 is required for infection of insect midgut cells and its removal prevents insect transmission by the recombinant virus. The mutation does not affect replication in cultured cells. P26 is not well characterized, but its deletion appears to have no effects in cell culture.

These constructs are also available as a BacMagic series from EMD Millipore, Inc., a division of Merck, KGa.

OmniBac

OmniBac is a system allowing either transposon mediated recombination or homologous recombination based on the orf1629/lef2/orf603 sequences for insertion of multiple genes into baculovirus vectors. It allows the use of all baculoviruses using either of these two approaches (47).

Post translational processing of baculovirus expressed proteins

Glycosylation

The baculovirus expression system has been widely used because of its ability to process proteins similar to other higher eukaryotes. This leads to proteins that are often biologically active as a result of being folded properly, trafficked to their native locations in the cell, and posttranslationally modified similar to higher eukaryotic cells. One of the areas of difference between the insect system and that of higher eukaryotes is in glycosylation.

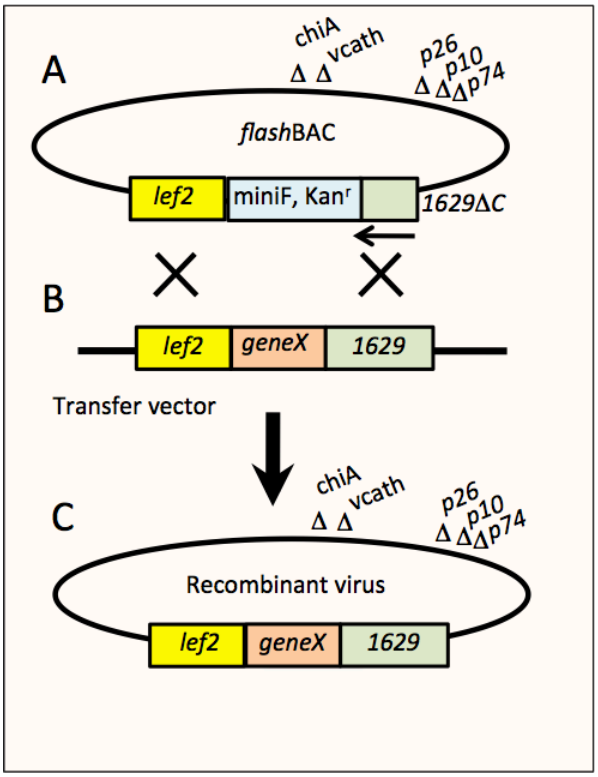

Figure 2. The *flash*BAC baculovirus expression system. A.). A map of the *flash*BAC bacmid. The deleted genes are indicated. The mini-F insert contains genes from the F plasmid involved in coordination of replication of the bacmid with the bacterial genome (see Fig. 1D). A kanamycin resistance gene is also indicated. The essential Orf 1629ΔC is truncated at its C- terminus. This prevents the bacmid from replicating as a virus in insect cells. B). The transfer vector which contains a flanking gene, lef2, the insert, and a complete orf1629 gene. C.). A recombinant virus with a repaired orf1629 gene. The BAC sequences are eliminated by recombination.

Whereas higher eukaryotes such as mammals have complex N-glycans with sialic acid residues at their termini, those produced in the baculovirus system have a simpler side chain terminating in mannose residues with the addition of 1-3 fucose residues. Although it has been reported that some insect cell lines are capable of the synthesis of more complex sialated glycans, the proteins examined in these reports may have been contaminated with a sialoglycoprotein in the culture medium (48). The fucose residues found in insect derived glycoproteins are of concern because they can elicit a strong allergic response in some humans due to the presence of cross-reactive IgE derived from environmental exposure to the fucose epitope; reviewed in (49).

In both insects and mammals, the initial N-glycan complex is added to asparagine residues (in the context of Asn-X-Ser/Thr) in the endoplasmic reticulum or Golgi apparatus. This normally involves the addition of a pre-assembled precursor glycan comprised of two N-acetyl-glucoseamine residues attached to a branched complex of mannose molecules with one branch further extended by the addition of glucose molecules (Figure 3A). This complex is then processed by the addition or removal of different carbohydrate molecules. Two main reactions are involved; glycosidases and mannosidases remove (50) glucose and mannose molecules, respectively (Figure 3B), whereas a glycosylaminotransferase is involved in the addition of a GlcNac residue (Figure 3C). Although the initial N-glycosylation structure added to the protein and the preliminary trimming reactions are similar between insects and mammalian cells, the succeeding processing reactions differ significantly.

In the insect pathway, the action of fucosyltransferases and an N-acetyglucosamidase, result in one non-fucosylated and two different fucosylated variants (Figure 3E). In contrast the mammalian pathway can yield several sialated and non-sialated variants (Figure 3F).

Because of the possibility of the fucosylated proteins eliciting an allergic reaction, efforts has been made to identify the enzymes involved in the production of glycan modifications and incorporate them into insect cells so that N-glycans that resemble those found in mammalian cells are produced; reviewed in (51). This has resulted a commercially available transgenic *Spodoptera frugiperda* cell line (SfWT-series) (Mimic cells, Invitrogen, Inc.) capable of producing terminally sialylated N-glycans similar to those found in mammalian systems (52) (53).

A recent system called Sweetbac (54) incorporates genes coding for bovine β1,4-galactosyltransferase I and Caenorhabditis elegans N-acetylglucosaminyltransferase II into a multibac vector (see above) such that proteins expressed by this virus had a mammalian-like N-glycan structures.

Another innovation involves the problem of sialylation which involves the added expense of media supplementation with a precursor of sialic acid, N-acetylmannosamine. To eliminate the need for this supplement, the gene encoding *E. coli* N-acetylglucosamine-6-phosphate 2'-epimerase (GNPE) was engineered into insect cells. It was found that, although GNPE normally degrades sialic acid, in insect cells which have the degradation product, but not sialic acid, the reverse reaction (sialic acid synthesis) occurs (55).

Further baculovirus manipulation: Synthetic baculovirus technology

Using PCR and transformation-associated recombination in yeast, a viable synthetic AcMNPV genome was constructed. This technology may facilitate the investigation of gene function which could be important in advances in gene expression technology and basic biology (56).

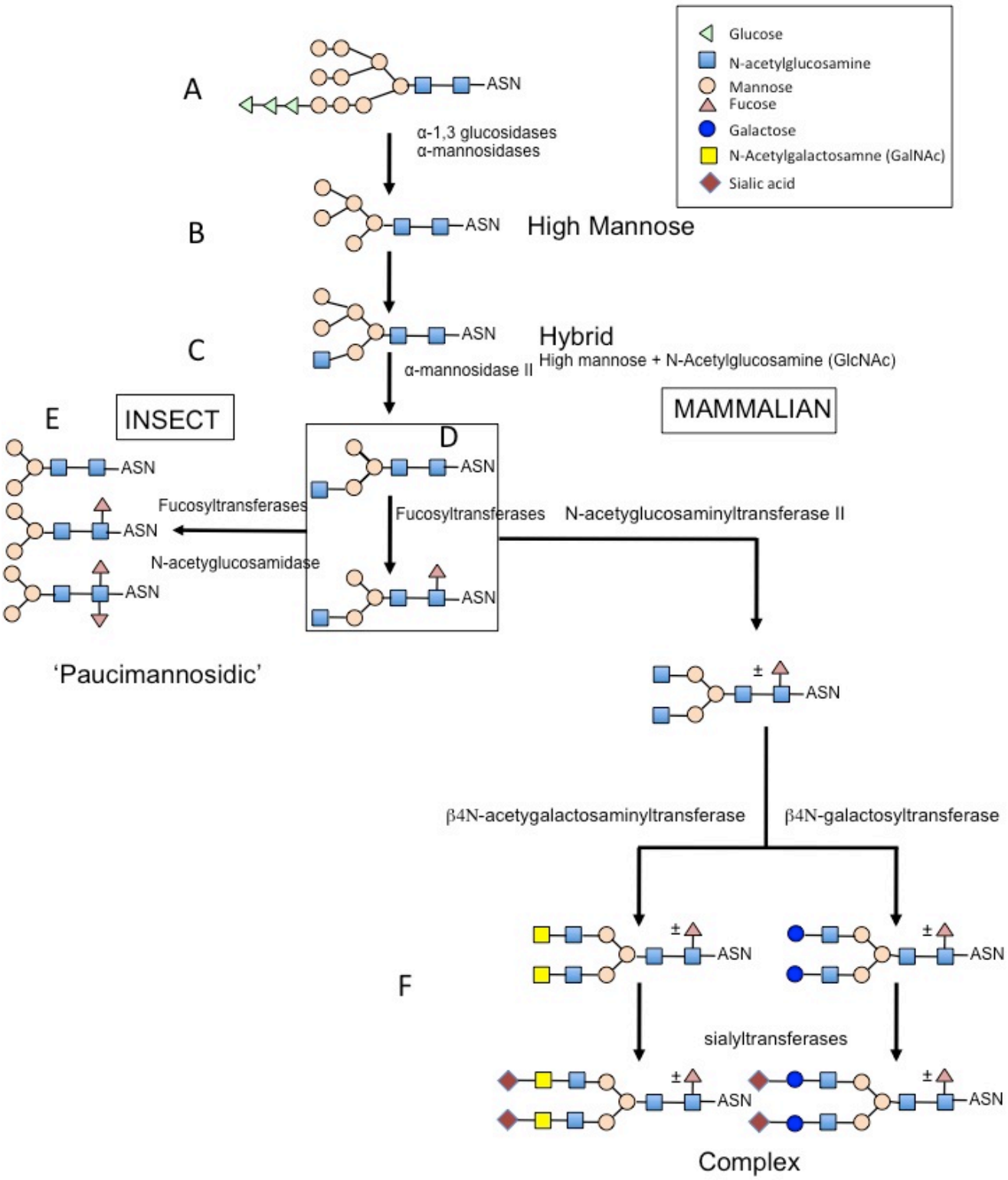

Figure 3. A comparison of insect and mammalian N-glycosylation pathways. The symbols are defined in the key. The major difference is that insect derivatives may contain fucose (D), whereas the mammalian derivatives may or may not incorporate fucose (E) and contain N-acetylgalactosamine (GalNac) or with or without sialic acid (F). Modified from (51).

References

1. Smith GE, Summers MD, Fraser MJ. Production of human beta interferon in insect cells infected with a baculovirus expression vector. Mol Cell Biol. 1983;3:2156–2165. PubMed PMID: 6318086.
2. Pennock GD, Shoemaker C, Miller LK. Strong and regulated expression of *Escherichia coli* B-galactosidase in insect cells with a baculovirus vector. Mol Cell Biol. 1984;4:399–406. PubMed PMID: 6325875.
3. Summers MD, Smith GE. 1987. A manual of methods for baculovirus vectors and insect cell culture procedures. Texas Agricultural Experiment Station, Bulletin No. 1555.
4. Treanor JJ, El Sahly H, King J, Graham I, Izikson R, Kohberger R, Patriarca P, Cox M. Protective efficacy of a trivalent recombinant hemagglutinin protein vaccine (FluBlok(R)) against influenza in healthy adults: a randomized, placebo-controlled trial. Vaccine. 2011;29:7733–9. PubMed PMID: 21835220.
5. Inglis S, Shaw A, Koenig S. 2006. Chapter 11: HPV vaccines: commercial research & development. Vaccine 24 Suppl 3:S3/99-105.
6. Legastelois I, Buffin S, Peubez I, Mignon C, Sodoyer R, Werle B. Non-conventional expression systems for the production of vaccine proteins and immunotherapeutic molecules. Hum Vaccin Immunother. 2017;13:947–961. PubMed PMID: 27905833.
7. Bonning BC (ed). 2006. Insect Viruses: Biotechnological Applications. Academic Press, Amsterdam.
8. Nijhout HF, Davidowitz G, Roff DA. A quantitative analysis of the mechanism that controls body size in Manduca sexta. J Biol. 2006;5:16. PubMed PMID: 16879739.
9. Telfer WH, Kunkel JG. The function and evolution of insect storage hexamers. Annu Rev Entomol. 1991;36:205–28. PubMed PMID: 2006868.
10. Dow JA. pH gradients in lepidopteran midgut. J Exp Biol. 1992;172:355–375. PubMed PMID: 9874748.
11. Blissard GW, Rohrmann GF. Location, sequence, transcriptional mapping, and temporal expression of the gp64 envelope glycoprotein gene of the *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus. Virology. 1989;170:537–555. PubMed PMID: 2658304.
12. Blissard GW, Quant-Russell RL, Rohrmann GF, Beaudreau GS. Nucleotide sequence, transcriptional mapping, and temporal expression of the gene encoding p39, a major structural protein of the multicapsid nuclear polyhedrosis virus of *Orgyia pseudotsugata*. Virology. 1989;168:354–362. PubMed PMID: 2644736.
13. Leisy D, Rohrmann G, Beaudreau G. The nucleotide sequence of the polyhedrin gene region from the multicapsid baculovirus of *Orgyia pseudotsugata*. Virology. 1986;153:280–288. PubMed PMID: 3526710.
14. Leisy DJ, Rohrmann GF, Nesson M, Beaudreau GS. Nucleotide sequence and transcriptional mapping of the p10 gene region of the *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus. Virology. 1986;153:157–167. PubMed PMID: 3526709.
15. Nobiron I, O'Reilly DR, Olszewski JA. Autographa californica nucleopolyhedrovirus infection of Spodoptera frugiperda cells: a global analysis of host gene regulation during infection, using a differential display approach. J Gen Virol. 2003;84:3029–39. PubMed PMID: 14573808.
16. Brody EN, Kassavetis GA, Ouhammouch M, Sanders GM, Tinker RL, Geiduschek EP. Old phage, new insights: two recently recognized mechanisms of transcriptional regulation in bacteriophage T4 development. FEMS Microbiol Lett. 1995;128:1–8. PubMed PMID: 7744235.
17. Yang S, Miller LK. Activation of baculovirus very late promoters by interaction with very late factor 1. J Virol. 1999;73:3404–9. PubMed PMID: 10074194.
18. Sriram S, Gopinathan KP. The potential role of a late gene expression factor, lef2, from Bombyx mori nuclear polyhedrosis virus in very late gene transcription and DNA replication. Virology. 1998;251:108–22. PubMed PMID: 9813207.
19. Merrington CL, Kitts PA, King LA, Possee RD. An Autographa californica nucleopolyhedrovirus lef-2 mutant: consequences for DNA replication and very late gene expression. Virology. 1996;217:338–348. PubMed PMID: 8599220.

20. Mishra G, Chadha P, Chaudhury I, Das RH. Inhibition of Autographa californica nucleopolyhedrovirus (AcNPV) polyhedrin gene expression by DNAzyme knockout of its serine/threonine kinase (pk1) gene. Virus Res. 2008;135:197–201. PubMed PMID: 18353480.
21. Mishra G, Chadha P, Das RH. Serine/threonine kinase (pk-1) is a component of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) very late gene transcription complex and it phosphorylates a 102kDa polypeptide of the complex. Virus Res. 2008;137:147–9. PubMed PMID: 18577405.
22. Fan X, McLachlin JR, Weaver RF. Identification and characterization of a protein kinase-interacting protein encoded by the Autographa californica nuclear polyhedrosis virus. Virology. 1998;240:175–182. PubMed PMID: 9454690.
23. Vanarsdall AL, Okano K, Rohrmann GF. Characterization of the role of VLF-1 in baculovirus capsid structure and DNA processing. J Virol. 2006;80:1724–1733. PubMed PMID: 16439529.
24. Rosinski M, Reid S, Nielsen LK. Kinetics of baculovirus replication and release using real-time quantitative polymerase chain reaction. Biotechnol Bioeng. 2002;77:476–80. PubMed PMID: 11787021.
25. Nguyen Q, Chan LC, Nielsen LK, Reid S. Genome scale analysis of differential mRNA expression of Helicoverpa zea insect cells infected with a H. armigera baculovirus. Virology. 2013;444:158–70. PubMed PMID: 23827436.
26. Okano K, Vanarsdall AL, Rohrmann GF. A Baculovirus alkaline nuclease knock out construct produces fragmented DNA and aberrant capsids. Virology. 2007;359:46–54. PubMed PMID: 17046043.
27. Hitchman RB, Locanto E, Possee RD, King LA. Optimizing the baculovirus expression vector system. Methods. 2011;55:52–7. PubMed PMID: 21945427.
28. Hitchman RB, Possee RD, King LA. High-throughput baculovirus expression in insect cells. Methods Mol Biol. 2012;824:609–27. PubMed PMID: 22160923.
29. Kitts PA, Ayres MD, Possee RD. Linearization of baculovirus DNA enhances the recovery of recombinant virus expression vectors. Nucleic Acids Res. 1990;18:5667–72. PubMed PMID: 2216760.
30. Kitts PA, Possee RD. A method for producing recombinant baculovirus expression vectors at high frequency. BioTechniques. 1993;14:810–816. PubMed PMID: 8512707.
31. Luckow VA, Lee SC, Barry GF, Olins PO. Efficient generation of infectious recombinant baculoviruses by site-specific transposon-mediated insertion of foreign genes into a baculovirus genome propagated in Escherichia coli. J Virol. 1993;67:4566–79. PubMed PMID: 8392598.
32. Barth PT, Datta N, Hedges RW, Grinter NJ. Transposition of a deoxyribonucleic acid sequence encoding trimethoprim and streptomycin resistances from R483 to other replicons. J Bacteriol. 1976;125:800–10. PubMed PMID: 767328.
33. Hedges RW, Datta N, Fleming MP. R factors conferring resistance to trimethoprim but not sulphonamides. J Gen Microbiol. 1972;73:573–5. PubMed PMID: 4571517.
34. Gay NJ, Tybulewicz VL, Walker JE. Insertion of transposon Tn7 into the Escherichia coli glmS transcriptional terminator. Biochem J. 1986;234:111–7. PubMed PMID: 3010949.
35. Mitra R, McKenzie GJ, Yi L, Lee CA, Craig NL. Characterization of the TnsD-attTn7 complex that promotes site-specific insertion of Tn7. Mob DNA. 2010;1:18. PubMed PMID: 20653944.
36. Craig NL. 2002. Tn7, p 423-456. *In* Craig NL, Craigie R, Gellert M, Lambowitz AM (ed), Mobile DNA II. ASM Press, Washington, D.C.
37. Peters JE, Craig NL. Tn7: smarter than we thought. Nat Rev Mol Cell Biol. 2001;2:806–14. PubMed PMID: 11715047.
38. Timmis K, Cabello F, Cohen SN. Cloning, isolation, and characterization of replication regions of complex plasmid genomes. Proc Natl Acad Sci U S A. 1975;72:2242–6. PubMed PMID: 1094460.
39. Lovett MA, Helinski DR. Method for the isolation of the replication region of a bacterial replicon: construction of a mini-F'kn plasmid. J Bacteriol. 1976;127:982–7. PubMed PMID: 783124.
40. Willetts N, Skurray R. 1987. Structure and function of the F factor and mechanism of conjugation, p 1110-1133. *In* Neidhardt FC (ed), Escherichia coli and Salmonella typhimurium Cellular and Molecular Biology, vol 1. American Society for Microbiology, Washington, D.C.

41. Tsutsui H, Matsubara K. Replication control and switch-off function as observed with a mini-F factor plasmid. J Bacteriol. 1981;147:509–16. PubMed PMID: 7021532.
42. Shizuya H, Birren B, Kim UJ, Mancino V, Slepak T, Tachiiri Y, Simon M. Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in Escherichia coli using an F-factor-based vector. Proc Natl Acad Sci U S A. 1992;89:8794–7. PubMed PMID: 1528894.
43. Lichtenstein C, Brenner S. Site-specific properties of Tn7 transposition into the E. coli chromosome. Mol Gen Genet. 1981;183:380–7. PubMed PMID: 6276688.
44. Bieniossek C, Imasaki T, Takagi Y, Berger I. MultiBac: expanding the research toolbox for multiprotein complexes. Trends Biochem Sci. 2012;37:49–57. PubMed PMID: 22154230.
45. Schreiber A, Stengel F, Zhang Z, Enchev RI, Kong EH, Morris EP, Robinson CV, da Fonseca PC, Barford D. Structural basis for the subunit assembly of the anaphase-promoting complex. Nature. 2012;470:227–32. PubMed PMID: 21307936.
46. Imasaki T, Calero G, Cai G, Tsai KL, Yamada K, Cardelli F, Erdjument-Bromage H, Tempst P, Berger I, Kornberg GL, Asturias FJ, Kornberg RD, Takagi Y. Architecture of the Mediator head module. Nature. 2012;475:240–3. PubMed PMID: 21725323.
47. Vijayachandran LS, Thimiri Govinda Raj DB, Edelweiss E, Gupta K, Maier J, Gordeliy V, Fitzgerald DJ, Berger I. Gene gymnastics: Synthetic biology for baculovirus expression vector system engineering. Bioengineered. 2013;4:279–87. PubMed PMID: 23328086.
48. Hillar A, Jarvis DL. Re-visiting the endogenous capacity for recombinant glycoprotein sialylation by baculovirus-infected Tn-4h and DpN1 cells. Glycobiology. 2010;20:1323–30. PubMed PMID: 20574041.
49. Altmann F. The role of protein glycosylation in allergy. Int Arch Allergy Immunol. 2007;142:99–115. PubMed PMID: 17033195.
50. Miller LK, Trimarchi RE, Browne D, Pennock GD. A temperature-sensitive mutant of the baculovirus *Autographa californica* nuclear polyhedrosis virus defective in an early function required for further gene expression. Virology. 1983;126:376–380. PubMed PMID: 18638939.
51. Shi X, Jarvis DL. Protein N-glycosylation in the baculovirus-insect cell system. Curr Drug Targets. 2007;8:1116–25. PubMed PMID: 17979671.
52. Jarvis DL, Kawar ZS, Hollister JR. Engineering N-glycosylation pathways in the baculovirus-insect cell system. Curr Opin Biotechnol. 1998;9:528–33. PubMed PMID: 9821284.
53. Aumiller JJ, Hollister JR, Jarvis DL. A transgenic insect cell line engineered to produce CMP-sialic acid and sialylated glycoproteins. Glycobiology. 2003;13:497–507. PubMed PMID: 12626399.
54. Palmberger D, Wilson IB, Berger I, Grabherr R, Rendic D. SweetBac: a new approach for the production of mammalianised glycoproteins in insect cells. PLoS One. 2012;7:e34226. PubMed PMID: 22485160.
55. Geisler C, Jarvis DL. Innovative use of a bacterial enzyme involved in sialic acid degradation to initiate sialic acid biosynthesis in glycoengineered insect cells. Metab Eng. 2012;14:642–52. PubMed PMID: 23022569.
56. Shang Y, Wang M, Xiao G, Wang X, Hou D, Pan K, Liu S, Li J, Wang J, Arif BM, Vlak JM, Chen X, Wang H, Deng F, Hu Z. Construction and Rescue of a Functional Synthetic Baculovirus. ACS Synth Biol. 2017;6:1393–1402. PubMed PMID: 28383905.

11. Baculoviruses, retroviruses, DNA transposons (*piggyBac*), and insect cells

There are a variety of classes of transposable elements (TEs) integrated into the genomes of all cells and they are often major components of cellular genomes. For example, from 0.3% of bacterial genomes up to almost 80% of some vertebrate genomes are composed of these elements (1). This chapter will focus on two types of TEs that were discovered because they were found integrated into the AcMNPV genome. These include a DNA transposon called *piggyBac* that is of major interest as a vector for engineering cell lines and organisms. In addition to DNA transposons, many TEs are retroelements, which have an RNA intermediate. They often encode a reverse transcriptase, which can convert the RNA of the retroelement into cDNA that is then integrated into the host cell genome. A major category of retroelements includes the retroviruses that are infectious and can spread between organisms. The genome of the retrovirus becomes integrated into a host genome and is called a provirus and contains long terminal repeats (LTR) at either end that encode regulatory elements, a gag gene that encodes a structural (capsid) protein, the polymerase gene that encodes several enzymatic functions, and the envelope gene, env, that provides the virus with the ability to infect other cells (Figure 1A). There are several different categories of retroelements; some lack LTRs, while others lack an env gene, and are normally confined to a cell and are not infectious. In addition, some do not encode a reverse transcriptase, but rely on other retroelements to supply this enzyme. The focus of this chapter is on a group of retroelements found in insects that resemble retroviruses.

A baculovirus-associated errantivirus (retrovirus)

Few polyhedra (FP) mutants are a readily observable baculovirus phenotype that result in reduced numbers of polyhedra and an elevated titer of budded virus. Such mutants often contain an insert in the *fp-25k* gene (ac61), although the phenotype can result from mutations elsewhere in the genome. In the process of characterizing AcMNPV FP mutants produced after 25 passages in *Trichoplusia ni* cells, an isolate, (FP-D), was found to contain an integrated retrotransposon that originated from the host genome. It was called transposable element D or TED (2). This element had features of a retrovirus, including long terminal repeats and was demonstrated to express gag, pol, and env-like genes that are capable of being incorporated into virus-like particles (3) (4) (5). Normally retroviruses that integrate into a genome remain integrated and are spread and amplified by the RNA intermediate that is transcribed from the integrated provirus by the host cell RNA polymerase II. In contrast, the TED provirus was found to be unstable and upon excision, a copy of one LTR of about 270 bp remained in the baculovirus genome (6). This instability was probably due to the large size of the TED genome relative to the baculovirus genome. Viruses related to TED have been found in other insects with the retrotransposon called *gypsy* from *Drosophila* being the most well studied example.

The insect retroelements that encode an env gene are called the errantiviruses (from Latin *errans*, to wander). Although similar to retroviruses, they have not been included within the Retroviridae because they are a distinct lineage, and evidence that they are infectious is indirect (see below). Kanga and roo-like retroviruses also encode a related env gene (7). These retrovirus-like elements are often found in multiple copies and are present as apparent complete and truncated or defective genomes. For example, in the *Drosophila melanogaster* genome there are five different categories of errantiviruses encompassing 78 complete or partial sequences that range from a single full-length copy of *gypsy* to 18 full length/39 partial copies of the element *297*. Other categories include *17.6* (7 full length/5 partial) and *idefix* (2 full length/5 partial). A fifth category, *zam*, was not found in this sequence, indicating the variability of errantivirus distribution between *D. melanogaster* strains (8).

The fp25-locus (Ac61); a remnant of a LINE-1 integration? Fp-25k (Ac61) is present in the genomes of most if not all Alpha-, Beta-, and Gammabaculoviruses. Analysis using the structure prediction program Hhpred (9) indicates that fp25k is related to orf1p of the Line-1 group of retrotransposons with a probability of 99.8%. Orf1p acts as a nucleic acid chaperone and similar to orf1p, fp25k has a coiled-coil domain and a predicted RNA

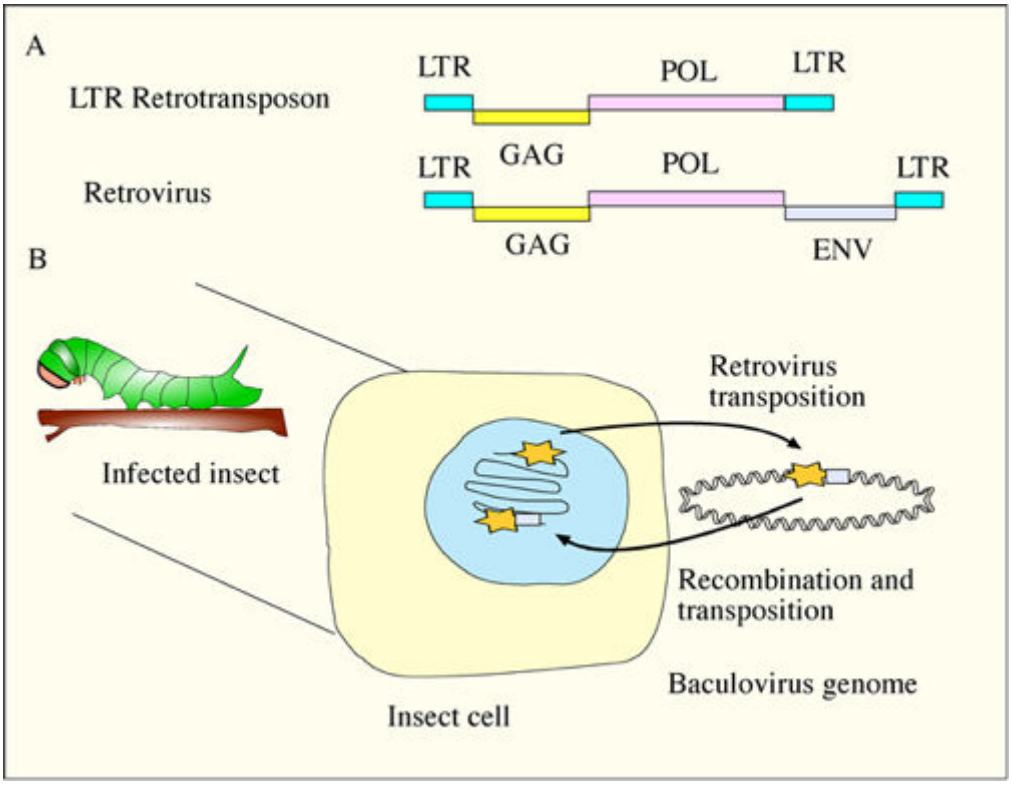

Figure 1. The conversion of an LTR retrotransposon to a retrovirus by obtaining the env gene. A) The difference between LTR retrotransposons and retroviruses is the env gene. B) A theoretical diagram of the incorporation of an LTR retrotransposon into a baculovirus genome adjacent to the envelope fusion protein. By DNA recombination, the DNA would have incorporated the envelope fusion protein and then transposed into the genome of an insect cell.

binding motif (10). Deletion or mutations in the fp-25 locus are not lethal, but results in a 'few polyhedra phenotype' (fp) (11, 12). FP mutants are defective in virion occlusion and nucleocapsid envelopment in nuclei and release two- to fivefold more infectious BV than wt in infected Sf9 cells (12, 13). Although the significance the relatedness is compelling, how this gene adapted to baculovirus biology is unclear.

Errantiviruses in Lepidoptera

Two of the main cell lines used for baculovirus research were derived from primary explants of pupal ovarian tissue from moths of the family Noctuidae. One is from the fall army worm (*Spodoptera frugiperda* (Sf)) (14), while the other is derived from the cabbage looper, *Trichoplusia ni* (Tn) (15). In a survey of the genomes of these cell lines, using degenerate oligomers targeted to a conserved region of the errantivirus reverse transcriptase gene, over 20 different PCR products from each cell line were amplified, cloned, and sequenced. Analysis of these sequences resulted in the identification of over 20 lineages that could be grouped into several major clades (Figure 2). Three of the sequences were identical to the TED errantivirus described above (16). Phylogenetic analyses indicated that most of the Sf and Tn sequences were closely related to each other and to sequences from other Lepidoptera. The next most closely related sequences were from the *Drosophila* (Diptera). However, there are several sequences from both Sf and Tn that form lineages distinct from the majority of the lepidopteran or dipteran sequences. This research was confirmed for *S. frugiperda* cells when genome and transcriptome assemblies were characterized (17). Thirteen different errantivirus sequences were identified, nine were similar to the lineages previously identified from partial sequences (16). Most of these elements were closely related to each other and to the TED element from T. ni although two lineages were significantly different. Five of the elements appeared to be transcribed and had relative abundances of 763, 292, 67, 19, and 8. The elements with

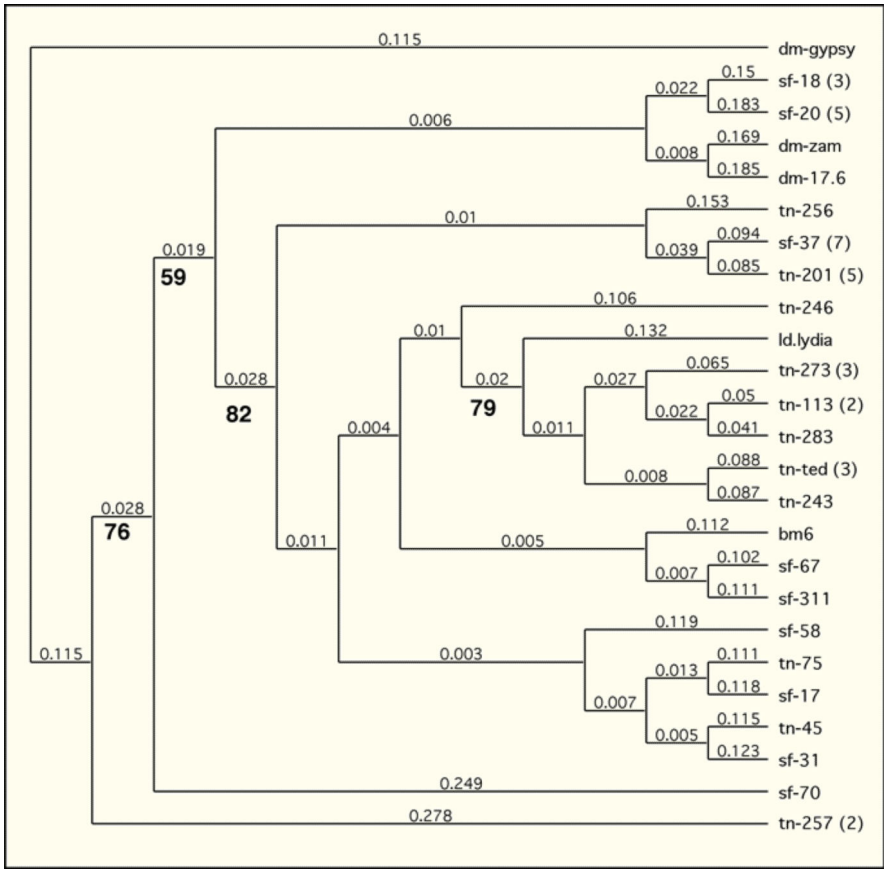

Figure 2. Phylogenetic tree of *S. frugiperda* and *T. ni errantivirus* sequences. The sequences were PCR amplified from the reverse transcriptase region. Nodes with bootstrap values greater than 50% are indicated as bold numbers. The numbers in brackets indicate the number of times the sequence was found in the data set. Bm6 is from *B. mori*, Ld-lydia is from *L. dispar*, and dm-gypsy, dm-17.6, and dm-zam are from *Drosophila*. For details see (16).

the highest level of transcripts present (763 and 292) corresponded to sf37 and sf20 that were also the two most abundant sf lineages in the previous study (16).

Relationships between insect retroviruses and baculoviruses: the env gene

Phylogenetic analyses of the errantivirus env gene and baculovirus genome sequence data resulted in the unexpected observation that the errantivirus env gene is related to the baculovirus envelope fusion protein, or F protein lineage (18, 19) (20). The sequence similarity was most striking in the region that includes the furin cleavage signal (RxxR) and a predicted fusion peptide immediately downstream.

This fusion peptide of about 20 amino acids is highly hydrophobic except for 2 D and 1 K residue (Figure 3). The errantiviruses most likely obtained the F protein from a recombination event that occurred when a retrotransposon integrated into a baculovirus genome (Figure 1). Evidence for such an event is compelling because the errantivirus TED was found integrated into a baculovirus genome as described above (2). Baculoviruses have two different envelope fusion proteins: gp64 and F. GP64 appears to have been recently incorporated into one baculovirus lineage called Group I. Whereas the Group I baculoviruses retain a copy of the F gene, it no longer functions independently as a fusion protein. In contrast, most baculoviruses (Group II) lack gp64 and contain only a copy of the F gene, suggesting that it is the fusion protein in these viruses (21) (see Figure 2, Chapter 2).

In addition to the strong evidence for the capture of a baculovirus envelope fusion protein leading to the evolution of errantiviruses, this phenomenon appears to be a relatively common event in virus evolution and may have occurred a number of times (18, 22). It has not only occurred with elements such as retrotransposons which commonly integrate into DNA, but also has been observed for a variety of other categories of viruses, including members of the Orthomyxoviridae and with the baculovirus gp64 gene described above (21) (see Figure 7, Chapter 2).

Cellular homologs of baculovirus F/errantivirus env proteins

In addition to the relatedness of the baculovirus F and errantivirus env genes, a cellular homolog in the *Drosophila* genome sequence was also identified (19). However, this protein is not cleaved (see below), does not have membrane fusion activity, and appears to localize to intracellular organelles rather than cell membranes (23). This gene was determined to have entered the Drosophila lineage once, and another time into a mosquito lineage. In *Drosophila* it is expressed in most tissues analyzed in both adult males and females. It was suggested that it was incorporated into and retained by the insect genome because its expression could protect the host cell from infection by retroviruses or baculoviruses that shared a related env protein. This could be accomplished if the cellular homolog binds to and interferes with the viral receptors on the cell surface or if they act as dominant negative inhibitors in which the endogenous env would complex with and inactivate the viral env protein (7).

Features of baculovirus F and insect retrovirus env proteins: Class I fusion proteins

The baculovirus F and errantivirus env proteins appear to be members of the Class I (24) group of envelope fusion proteins common to many vertebrate viruses. Although, in general, they lack sequence relatedness, it has long been suggested that a number of envelope fusion proteins from a variety of disparate viruses are related. This is based on their requirement for cleavage to be activated and the fact that one of the resulting peptides is membrane associated via a transmembrane domain. In addition, the membrane-associated peptide contains a hydophobic fusion peptide domain downstream of a cleavage site followed by predicted coiled-coil domains that are involved in forming hairpin-like structures that are important in virus-cell fusion (25, 26). Such structures have been characterized in fusion proteins from viruses as diverse as filoviruses, retroviruses, orthomyxoviruses, and paramyxoviruses (24). Evidence suggests that baculovirus F proteins are members of this group (Figure 3), and it has been demonstrated that they require cleavage, most likely by the host cell furin protease, for activation (27, 28). Errantivirus env proteins also have similar features (19) consistent with their being members of this group. Furthermore, *gypsy* env accumulates at the cell membrane as would be expected for a viral envelope protein and contains a predicted furin cleavage site; it is cleaved when expressed in both *Drosophila* S2 and Sf9 cells (29). Recently with the determination of structures of a variety of viral fusion proteins, the baculovirus F proteins are predicted to have a high degree of structural similarity to the F proteins of paramxyoviruses by the Hhpred structure prediction program (30) (see Chapter 2).

Additional relationships of insect retroviruses and baculoviruses

The relatedness of the errantivirus *env* protein and the baculovirus *F* homologs may reflect more than a fortuitous recombination event between these two viruses. The errantivirus TED is a mid-repetitive element (about 50 copies/genome) in *Trichoplusia ni* (6) and is capable of transposition from the insect into the baculovirus genome. A key feature of the relationship that may have led to the capture of a baculovirus F gene by a pre-errantivirus retrotransposon involves the ability of baculoviruses to express genes at very high levels. This feature appears to be due at least in part to the fact that they encode an RNA polymerase (31) capable of high levels of transcription in the context of the virus replication program. This polymerase recognizes a unique promoter sequence (A/G/TTAAG) (32) that is found in the TED LTR as a palindrome. Late in the baculovirus

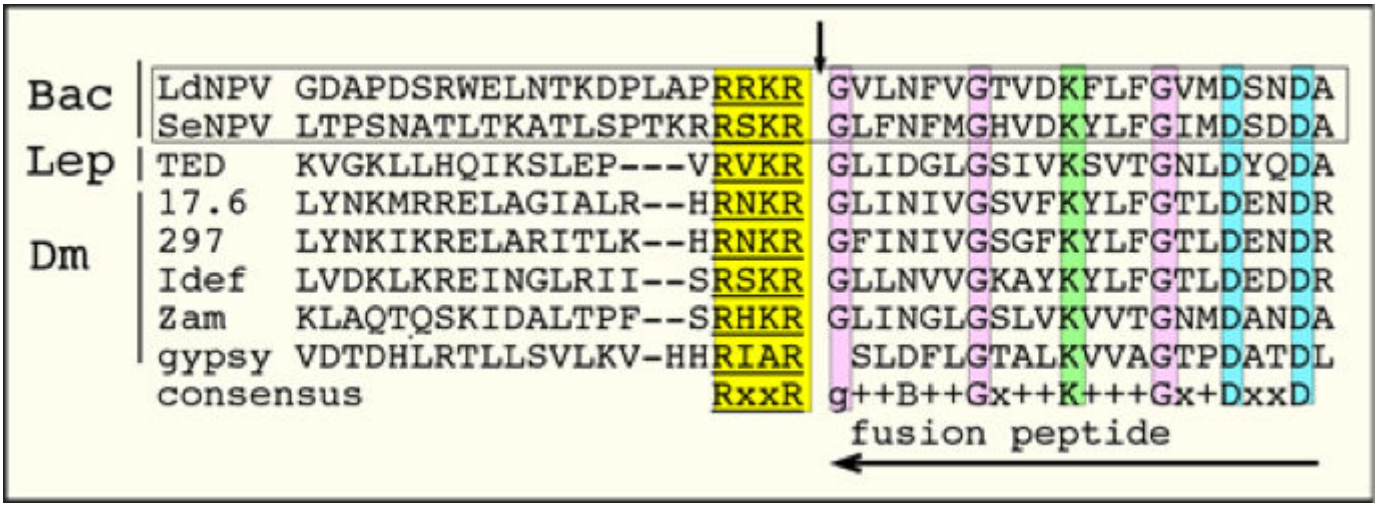

Figure 3. Homology at the cleavage site of selected baculovirus F proteins, a lepidopteran (Lep) errantivirus env (79), and 5 env sequences from *Drosophila melanogaster* (Dm). The two sequences at the top (in box) are from baculoviruses (Bac). The furin cleavage signal (RxxR) is underlined and colored yellow. The vertical arrow shows the furin cleavage site. Other conserved amino acids are also colored. The predicted fusion peptide is indicated by the horizontal arrow. In the consensus sequence hydrophobic amino acids are indicated by (+), N or D by (B), and variable amino acids by (x). For more information see (19).

infection, mRNA is expressed from these LTRs at high levels (6). Therefore, integration into a baculovirus genome may reflect a strategy to exploit baculovirus late gene expression to express the integrated retrotransposon/retrovirus genome at high levels. This could result in the production of a mixture of retrovirus particles and occluded baculoviruses containing integrated retroviruses and would provide two methods of escape from an insect with a fatal baculovirus infection: they could either survive by integration into baculovirus genomes, or possibly as infectious virus particles (Figure 4). The evolution of this relationship between a baculovirus and a primordial LTR-type retrotransposon provides a clear pathway, via DNA recombination, for the transposable element to incorporate the baculovirus F homolog into its genome, thereby converting it into a potentially infectious retrovirus (Figure 1B).

Are errantiviruses infectious?

Early on it was noted that retrovirus-like particles and reverse transcriptase activity were present in *Drosophila* cells (33). Subsequently, *gypsy* became the most intensively studied retrovirus-like element in *D. melanogaster*. Indirect evidence suggests that *gypsy* is infectious for *Drosophila* (34, 35). These data were obtained by feeding a strain of *Drosophila* that lacks active *gypsy* transposition with either purified virus like particles (vlps) from insects with transpositionally active *gypsy*, or extracts derived from such insects, and then documenting increased levels of transposition in the recipient insects. Similarly, it was observed that *gypsy* could be transmitted between cells in culture (36).

Does env play a role in errantivirus infectivity?

The evidence implicating env in errantivirus infectivity is varied. In one study, it was found that a preparation of two monoclonal antibodies against *gypsy* env mixed with the vlp fraction reduced the number of insertion events in insect feeding experiments (37). In addition, evidence suggests that an integrated Moloney leukemia virus-luciferase construct pseudotyped with a *gypsy* envelope is infectious for *Drosophila* cells (38). This suggested that *gypsy* env is capable of mediating infection of *Drosophila* cells. Although *gypsy* may be infectious, its infectivity appears to be very limited. It has been suggested that since they are adapted for integration into the cell genome, they no longer require propagation via infection (39). However, this does not explain why they have retained,

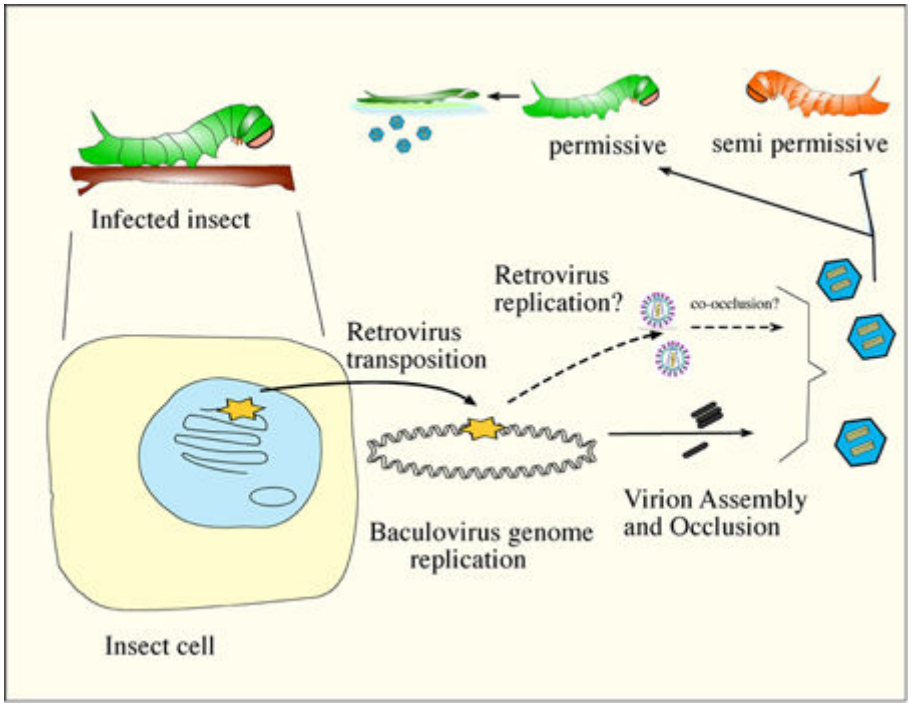

Figure 4. Possible pathways of retrovirus survival and transmission in a baculovirus-infected host. A retrovirus integrated into an insect genome might have two mechanisms for surviving a fatal infection. It could transpose into a baculovirus genome and as a provirus become incorporated into a baculovirus virion and subsequently occluded. As a provirus it could likely remain stable in the occlusion body indefinitely. The other mechanism is more theoretical and is shown by dashed arrows. Retrovirus could be produced from the provirus in the baculovirus genome. Retrovirus genomic RNA would be produced from the late promoter elements in the LTR; these could be translated into proteins and mature virions could be produced. These could also be occluded in occlusion bodies and would likely be stable similar to the baculovirus virion. Upon encountering an insect semi-permissive for virus infection, the retrovirus could invade the new host during abortive replication.

conserved, and continue to express an *env* gene. Since envelope proteins often play major roles in the virulence of other viruses (40), the errantivirus *env* proteins may allow the viruses to be infectious and spread between different organisms, but this infectivity may be restricted by features of their env proteins. It has also been suggested that *gypsy* env has fusogenic properties (41).

The invasion and amplification of retroelements

TED, the baculovirus associated errantivirus described above that was originally found integrated into the AcMNPV genome is a particularly useful example of how a virus could spread a transposable element to other species. One can imagine a situation in insects where a baculovirus abortively infects a species that might be semi permissive for virus replication (Figure 4). The infection could result in low levels of baculovirus replication that would allow mRNA expression, cDNA production and transposition of a TE from the invading baculovirus into host cell DNA, but the host would eventually overcome and survive the baculovirus infection. An example of such virus/insect combinations could be AcMNPV pathogenesis in *Helicoverpa zea* which is over 1000-fold more resistant to fatal infection than *Heliothis virescens* (42). The primary midgut infection and secondary infection of the tracheal epidermis are similar in the two insects, but *H. zea* larvae are able to encapsidate infected cells, thereby limiting the infection. Under these conditions, the surviving insects could have been exposed to and possibly parasitized by a TE carried by the invading baculovirus, whereas in contrast, the carrier baculovirus would have been eliminated.

Once a retroelement integrates into a genome, they can be transcribed into RNA, reverse transcribed into cDNA that can integrate elsewhere in the genome. Via this process they can greatly amplify their copy number and the

size of the genome of their host organism. Depending on the element, some TEs can transpose at a rate of from $10–^{3}$ to $10–^{5}$ per element per generation. Consequently, they can be more significant in the production of genetic change that normal nucleotide base changes which are altered at about $10–^{8}$–$10–^{9}$ per nucleotide per generation. Bursts of transpositional activity are thought to have been a major force in the evolution of new species (1). For example, the activation of a gypsy-like retrotransposon in plants of the genus Gossypium, which includes cotton, is responsible for a three-fold difference in the genome size between some species. Similarly, the amplification of several TE families has led to the doubling of the size of the rice genome. These elements often accumulate in heterochromatic regions of the genome. Such regions normally contain repetitive DNA and are transcriptionally inactive and include centromeres and telomeres (43).

What prevents retroelements from amplifying continuously?

The insertion of transposable elements can interrupt genes, alter regulatory regions such as promoters and enhancers, and disrupt patterns of splicing and 3' transcriptional processing and therefore can be highly mutagenic. Mutations in germ line cells could affect the success of progeny. In somatic cells, mutations could cause localized disruption of cell function, or they could cause more generalized effects if they alter the regulation of functions such as cell division that could lead to the production of oncogenic cells. Consequently, molecular systems have evolved to defend cells against TEs. Therefore, although molecular evidence suggests that amplification of TEs is a major feature of eukaryotic genome evolution, most are eventually silenced by the host. If a lineage survives the invasion and amplification of a TE, at some point its further proliferation is quelled. This is accomplished by several epigenetic mechanisms that involve inherited processes that do not affect the DNA sequence. These include post-transcriptional silencing by RNAi, chromatin modifications by changes in DNA methylation patterns, histone modifications including methylation, and changes in chromatin condensation and packing. It is likely that these processes work cooperatively to both suppress transcription and to eliminate RNA that is expressed.

Suppression of transposable elements by DNA and histone methylation

Although endogenous retroviruses are normally silenced, their active transcription can be detected at different stages during development. For example, a number of retroelements in Drosophila show patterns of spatially regulated expression during embryogenesis (44). DNA methylation involves the addition of a methyl group to the cytocine in CpG sequences. CpG sequences are often found concentrated in 'islands' present in promoter regions. Such regions are often characterized by increased histone occupancy with a corresponding reduction in the binding of transcription factors. Methylated DNA can also attract methyl-binding proteins that also can inhibit transcription. It has been suggested that one of the primary roles for this phenomenon is to prevent transcription of TEs thereby protecting the host from endogenous retroviruses, reviewed in (45, 46). Although some insect genomes are highly methylated, the patterns of these modifications may differ from other organisms. It may be associated with non-CpG dinucleotides and may not be focused on mobile sequences that are often heavily methylated in the genomes of vertebrates and plants (47, 48). Transcription can also regulated both positively and negatively by the pattern of histone methylation (49).

Suppression of transposable elements by RNA interference

Three pathways involved in RNA silencing have been identified in both mammals and insects and a major focus of these pathways is the suppression of endogenous transposable elements. These pathways include: i) RNA interference (RNAi) that employs small interfering RNAs (siRNAs) that are derived from exogenous double stranded RNA (dsRNA) and can act as a defense against viral infection by targeting TE RNA for degradation. In addition, siRNAs can also be derived from endogenous sequences and are involved in suppressing the expression

of endogenous transposable elements in somatic cells; ii) microRNAs (miRNAs) involve endogenous small RNAs that repress partially complementary mRNAs(50); and iii) Piwi-interacting RNAs (piRNA) that repress transposons in germ line cells and can also activate transcription in heterochromatin which is a gene-poor, highly condensed, DNA-protein complex, reviewed in (51). Therefore, both exogenous and endogenous RNAs can be inactivated and different mechanisms can be involved in germ line and somatic cells.

The Argonautes: proteins with RNAse activity that are critical in RNA interference

Key to the function of interfering RNAs is their interaction with Argonaute proteins (52). Although in Greek mythology the Argonautes were sailors on the ship Argo who accompanied Jason in his search for the Golden Fleece, in molecular biology the term was originally used to describe the shape of the leaves of a mutant of *Arabidopsis thaliana*, *AGO1*, because they resembled the squid *Argonauta argo* (53). The Argonaute proteins facilitate both the processing of some micro RNAs by eliminating the non-active siRNA strand and also use small RNAs as guides to identify and repress complementary transcripts by degradation (via endonuclease activity) or by inhibition of translation. They appear to have evolved from the RNAse H family of endonucleases but have substituted ssRNA for ssDNA as the template to target RNA, reviewed in (52).

Suppression of transposable elements in gonadal cells

A major category of piRNAs (54) (55) include rasiRNAs (repeat associated siRNAs) that are involved in the silencing of transposable elements. PiRNAs map to repetitive elements throughout the *Drosophila* genome, however a limited number of loci called piRNA clusters appear to match most piRNAs. The transposons in the clusters involved in piRNA production appear to be truncated or defective relicts and are probably not capable of autonomous expression or transposition. It has been suggested that piRNAs are derived from long single stranded precursor RNAs in which a 5' cleavage occurs at a uridine residue. The sequence then becomes incorporated into a Piwi protein complex where a second cleavage occurs generating the specific size. The piRNA then targets the piwi complex to RNA expressed from transposable elements and can interfere with splicing of the retroelement (56). Further evidence for the role of Piwi type proteins in the suppression of transposable elements in gonadal tissue was the observation that a mutation in piwi reduced the repression of *gypsy* in restricted tissues leading to up to a 150 fold increase in gypsy RNA levels (57, 58) (54). PiRNAs have also been described from Bombyx mori and they appear to include a major subclass of rasiRNAs and are thought to be involved in transposon silencing and development of germ line cells (59, 60) (61). The evolution of piRNA clusters has been examined experimentally by incorporating GFP into silkworm cells via *piggyBac* transposition and then isolating cell lines. It was observed that these lines amplified piRNAs capable of silencing GFP providing a system to examine a pathway for the development of piRNAs against a new insertion (62). Similar investigations have also been carried out with Drosophila and show that a piRNA complex can be activated against an invading mobile element within a single generation, reviewed in (63). Although it has been thought that suppression of TEs might prevent their accumulation, it has been argued that, in fact, by silencing their activity it promotes their incorporation by preventing the subsequent damage that they can cause. It was also suggested that this was important for genome expansion (64).

In *Drosophila; flamenco*, a source for piRNAs

A locus called *flamenco* controls the activity of retroviral elements *gypsy* (65), *idefix*, and *zam* (66). The *flamenco* locus was mapped to a region that corresponds to a piRNA cluster spanning a region of 179 kb and is comprised of nested transposable elements and fragments including those specific to *gypsy*, *idefix* and *zam*, in addition to other transposable element-specific sequences. Such regions are called piRNA clusters and essentially lack protein coding sequences and are comprised of truncated or damaged copies of TEs that appear to lack the

capacity to be mobilized and are concentrated in the pericentromeric or telomeric heterochromatin (58, 67). Other loci that are involved piRNA production have also been described (68, 69) and are reviewed in (54).

Activation of endogenous retroelement sequences during a baculovirus infection

A major question is if the baculovirus infection influences the host cell silencing systems. In an investigation examining the expression of HzSNPV in hemocytes *of H. virescens*, it was observed that over a hundred genes that were differentially regulated after infection were derived from sequences related to endogenous retroelements. Many of these genes were upregulated from 3 to 11 fold compared to controls (70). Similarly, infection of *Antherea pernyi* with ApNPV, 12 full-length LTR-retrotransposons significantly changed their levels of expression with 6 being up- and 6 being down-regulated (71). Also in *Helicoverpa zea* cells reverse transcriptase activity was up-regulated after infection with HearNPV (72). This may be related to the reduction in piRNA population in sf21 cells 12 hpi with SpliNPV (73). This suggests that in these systems virus infection might relax or inhibit the silencing systems present in their host cells thereby facilitating gene expression and possibly amplification of endogenous TEs. This is not unexpected as viruses have been demonstrated to interfere with RNAi systems in a variety of organisms (74). However, it is not clear how the non-specific effects of baculovirus replication might influence this process. For instance, it is well documented that transcription of many RNA polymerase II transcribed genes is turned off as the baculovirus infection progresses (see Chapter 4 and 10) (75-77). It has also been shown that RNA polymerase II transcripts of viral mRNA are degraded late in infection, e.g. see (78). This could interfere with RNAi production if it was carried out by this polymerase. How these characteristics of baculovirus infection might affect the RNA pol II transcripts derived from the integrated provirus such as TED (79) remains to be determined.

Piggybac, a transposon from *Trichoplusia ni*, that was originally isolated from a baculovirus.

Isolation of piggyBac

The adaptation of cell lines from *Trichoplusia ni* and *Spodoptera frugiperda* that were permissive AcMNPV and related viruses established the basis for understanding the molecular events of baculovirus replication. These cell systems supported both budded virus production early in infection and occluded virions later in the replication cycle. Because they were readily visualized, occlusion bodies provided a convenient marker of infection. As investigators became familiar with this system, it was noted that occlusion body mutants occasionally occurred in which there were fewer than the normal number of occlusion bodies produced. These were called 'few polyhedra' or FP mutants and were also characterized by elevated levels of budded virus production such that in cell culture they had a selective advantage and could out compete wt virus (80, 81). Characterization of these mutants indicated that they were derived by the insertion of cellular DNA at a specific location in the genome. This locus was eventually determined to be AcMNPV orf61 that encodes a predicted protein of 214 amino acids (25 kDa) and was called the 'few polyhedra' or *fp* gene (11) (82). Subsequently, it was determined that one of the inserts, IFP2 was found in the genome of AcMNPV grown in T. ni cells. It inserted at TTAA sites that were duplicated upon insertion, contained 13-bp inverted terminal repeats and encoded a 594 amino acid transposase that could facilitate its own transposition and it was renamed *piggyBac* (83) (84). *PiggyBac* shows precise excision upon transposition such that the TTAA target site is not duplicated and therefore leaves no footprint upon removal (82) (83).

Up to 98 copies of sequences similar to piggyBac were found in the *B. mori* genome, although only 5 appeared to be complete (85). A related transposon was also identified in the silkworm genome (86) and named *yabusame* element after a type of Japanese archery performed while riding a horse. (Perhaps the bow represents the

transposase, the arrow the transposon, DNA is the target, and the archer on the horse is the cell?). Related transposons have now been found in many different species from plants, insects and mammals, reviewed in (87).

Although *piggyBac* demonstrates limited sequence similarity with other transposon superfamilies, its transposase contains DDD acidic residues in its catalytic site suggesting that it belongs to the DDE superfamily of recombinases. In these recombinases, the acidic amino acids DDE or DDD coordinate metal ions required for activity (88).

PiggyBac has potential in many different systems

PiggyBac has demonstrated widespread potential as a genetic tool because of its ability to integrate into a variety of heterologous systems including numerous insect species (89), mammals including human (90) (91) and mice (92), planaria (93), the malaria parasite *Plasmodium falciparum* (94) and the schistosome, *Schistosoma mansoni* (95). In addition to its widespread host range, it is regarded as having great potential for applications in the production of transgenic animals and in gene therapy. It is normally delivered in a combination of two plasmids; a helper plasmid encoding the transposase and the transfer plasmid containing the transgene flanked by the specific inverted terminal repeats that bind the transposase that subsequently cleaves out the transgene and directs it to and facilitates its integration into the target site (Figure 5). The advantages of *piggybac* have been reviewed by (92) (96), and elsewhere. They include: i) its ability to be efficiently inserted into germline cells and the levels of insertion can be increased by optimizing the transposase codon preferences to the host species being engineered. In addition, the inserts are stably integrated when the *piggyBac* transposase is absent and there is a lower level of silencing of *piggyBac* relative to other transposons such as *Sleeping Beauty* or *Tol2*. It also has the potential to be engineered for more specificity of its integration site (97); ii) single copies of the transgene are inserted rather than concatemers that are often produced by other systems. Such multiple inserts can be detrimental because they can result in higher than normal levels of the expression of the insert; iii) the sites of integration can be rapidly identified using inverse PCR allowing rapid analysis of their potential effects at the integration site. iv) *PiggyBac* allows the integration of inserts of up to 100 kb (98), significantly larger than other vectors such as those derived from retroviruses and adeno associated viruses. These features have led Meir et al (96) to conclude 'Collectively, *piggyBac* is currently the most promising DNA transposon for gene and stem cell therapy because of its highly effective transposition activity, large cargo capacity, ability of stably expressing transgene, capability to be molecularly engineered to achieve site-specific gene targeting, and the unique feature of generating foreign DNA-free iPSCs (induced pluripotent stem cells).'

Another DNA Transposon found in a baculovirus genome.

In the course of a genome sequencing project another DNA transposon encoding a transposase and a novel adjacent orf were found integrated into the genome of an NPV isolated from an oak looper, *Lambdina fiscellaria* (99). The transposase is most closely related (49% amino acid identity) to an orf in *Amyelois transitella*, the navel orange worm moth, also a lepidopteran. The second orf was also most closely related to an orf in this same insect.

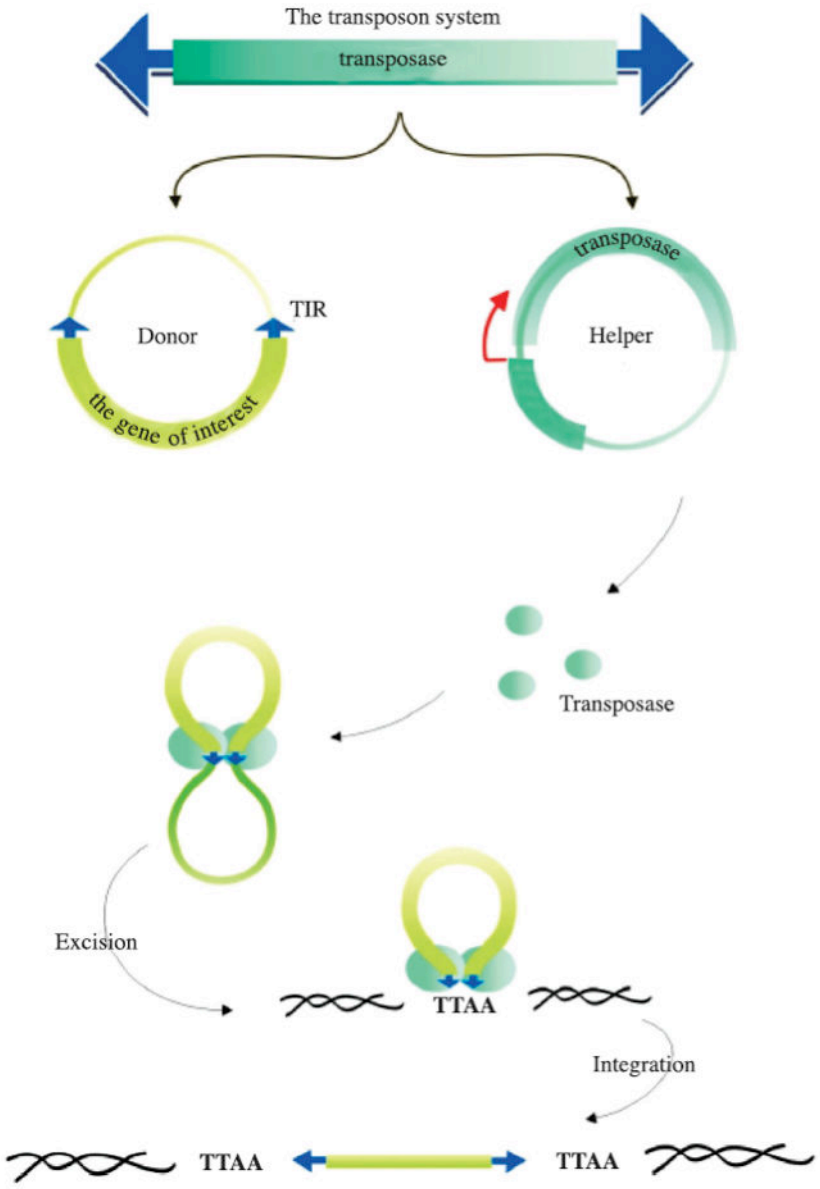

Figure 5. A two-plasmid transposition system. This system is divided into two parts. One plasmid (helper) encodes the transposase. The other plasmid (donor) contains the gene of interest located between terminal inverted repeats (TIRS). The transposase is expressed from the helper plasmid and binds to the TIRs and removes the gene of interest from the donor plasmid. The gene is then transported to the donor site by the transposes and integrated. This figure is from (96).

References

1. Biemont C, Vieira C. Genetics: junk DNA as an evolutionary force. Nature. 2006;443:521–4. PubMed PMID: 17024082.
2. Miller DW, Miller LK. A virus mutant with an insertion of a copia-like transposable element. Nature (London). 1982;299:562–564. PubMed PMID: 6289125.
3. Hajek KL, Friesen PD. Proteolytic processing and assembly of gag and gag-pol proteins of TED, a baculovirus-associated retrotransposon of the gypsy family. J Virol. 1998;72:8718–24. PubMed PMID: 9765414.
4. Lerch RA, Friesen PD. The baculovirus-integrated retrotransposon TED encodes gag and pol proteins that assemble into viruslike particles with reverse transcriptase. J Virol. 1992;66:1590–601. PubMed PMID: 1371168.

5. Ozers MS, Friesen P. The Env-like open reading frame of the baculovirus-integrated retrotransposon TED encodes a retrovirus-like envelope protein. Virology. 1996;226:252–9. PubMed PMID: 8955045.
6. Friesen PD, Rice WC, Miller DW, Miller LK. Bidirectional transcription from a solo long terminal repeat of the retrotransposon TED: Symmetrical RNA start sites. Mol Cell Biol. 1986;6:1599–1607. PubMed PMID: 3023897.
7. Malik HS, Henikoff S. Positive Selection of Iris, a Retroviral Envelope-Derived Host Gene in Drosophila melanogaster. PLoS Genet. 2005;1:429–43. PubMed PMID: 16244705.
8. Kaminker J, Bergman C, Kronmiller B, Carlson J, Svirskas R, Patel S, Frise E, Wheeler D, Lewis S, Rubin G, Ashburner M, Celniker S. The transposable elements of the Drosophila melanogaster euchromatin: a genomics perspective. Genome Biol. 2002.:3. PubMed PMID: 12537573.
9. Zimmermann L, Stephens A, Nam SZ, Rau D, Kubler J, Lozajic M, Gabler F, Soding J, Lupas AN, Alva V. A Completely Reimplemented MPI Bioinformatics Toolkit with a New HHpred Server at its Core. J Mol Biol. 2018;430:2237–2243. PubMed PMID: 29258817.
10. Garretson TA, McCoy JC, Cheng XW. Baculovirus FP25K Localization: Role of the Coiled-Coil Domain. J Virol. 2016;90:9582–9597. PubMed PMID: 27512078.
11. Beames B, Summers MD. Location and nucleotide sequence of the 25k protein missing from baculovirus few polyhedra (FP) mutants. Virology. 1989;168:344–353. PubMed PMID: 2644735.
12. Harrison RL, Jarvis DL, Summers MD. The role of the AcMNPV 25K gene, "FP25," in baculovirus polh and p10 expression. Virology. 1996;226:34–46. PubMed PMID: 8941320.
13. Fraser MJ, Brusca JS, Smith GE, Summers MD. Transposon mediated mutagenesis of a baculovirus. Virology. 1985;145:356–361. PubMed PMID: 2992159.
14. Vaughn JL, Goodwin RH, Tompkins GJ, McCawley P. The establishment of two cell lines from the insect *Spodoptera frugiperda* (Lepidoptera: Noctuidae). In Vitro. 1977;13:213–217. PubMed PMID: 68913.
15. Hink WF. Established insect cell line from the cabbage looper, *Trichoplusia ni*. Nature. 1970;226:466–467. PubMed PMID: 16057320.
16. Menzel T, Rohrmann GF. Diversity of errantivirus (retrovirus) sequences in two cell lines used for baculovirus expression, Spodoptera frugiperda and Trichoplusia ni. Virus Genes. 2008;36:583–586. PubMed PMID: 18363091.
17. Geisler C. A new approach for detecting adventitious viruses shows Sf-rhabdovirus-negative Sf-RVN cells are suitable for safe biologicals production. BMC Biotechnol. 2018;18:8. PubMed PMID: 29415704.
18. Malik HS, Henikoff S, Eickbush TH. Poised for contagion: evolutionary origins of the infectious abilities of invertebrate retroviruses. Genome Res. 2000;10:1307–18. PubMed PMID: 10984449.
19. Rohrmann GF, Karplus PA. Relatedness of baculovirus and gypsy retrotransposon envelope proteins. BMC Evol Biol. 2001;1:1. PubMed PMID: 11244578.
20. Terzian C, Pélisson A, Bucheton A. Evolution and phylogeny of insect endogenous retroviruses. BMC Evol Biol. 2001.:1. PubMed PMID: 11591216.
21. Pearson MN, Rohrmann GF. Transfer, incorporation, and substitution of envelope fusion proteins among members of the Baculoviridae, Orthomyxoviridae, and Metaviridae (insect retrovirus) families. J Virol. 2002;76:5301–5304. PubMed PMID: 11991958.
22. Kim FJ, Battini JL, Manel N, Sitbon M. Emergence of vertebrate retroviruses and envelope capture. Virology. 2004;318:183–91. PubMed PMID: 14972546.
23. Lung O, Blissard GW. A cellular Drosophila melanogaster protein with similarity to baculovirus F envelope fusion proteins. J Virol. 2005;79:7979–89. PubMed PMID: 15956544.
24. Harrison SC. Viral membrane fusion. Virology. 2015;479-480:498–507. PubMed PMID: 25866377.
25. Eckert DM, Kim PS. Mechanisms of viral membrane fusion and its inhibition. Annu Rev Biochem. 2001;70:777–810. PubMed PMID: 11395423.
26. Skehel J, Wiley D. Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin. Annu Rev Biochem. 2000;69:531–69. PubMed PMID: 10966468.
27. Westenberg M, Wang H, IJkel WF, Goldbach RW, Vlak JM, Zuidema D. Furin is involved in baculovirus envelope fusion protein activation. J Virol. 2002;76:178–84. PubMed PMID: 11739683.

28. Pearson MN, Russell RLQ, Rohrmann GF. Functional analysis of a conserved region of the baculovirus envelope fusion protein, LD130. Virology. 2002;304:81–88. PubMed PMID: 12490405.
29. Pearson MN, Rohrmann GF. Conservation of a proteinase cleavage site between an insect retrovirus (gypsy) env protein and a baculovirus envelope fusion protein. Virology. 2004;322:61–68. PubMed PMID: 15063117.
30. Soding J. Protein homology detection by HMM-HMM comparison. Bioinformatics. 2005;21:951–60. PubMed PMID: 15531603.
31. Guarino LA, Xu B, Jin J, Dong W. A virus-encoded RNA polymerase purified from baculovirus-infected cells. J Virol. 1998;72:7985–7991. PubMed PMID: 9733837.
32. Rohrmann GF. Polyhedrin structure. J Gen Virol. 1986;67:1499–1513. PubMed PMID: 3525744.
33. Heine CW, Kelly DC, Avery RJ. The detection of intracellular retrovirus-like entities in Drosophila melanogaster cell cultures. J Gen Virol. 1980;49:385–95. PubMed PMID: 6160197.
34. Song SU, Gerasimova T, Kurkulos M, Boeke JD, Corces VG. An env-like protein encoded by a Drosophila retroelement: evidence that gypsy is an infectious retrovirus. Genes Dev. 1994;8:2046–57. PubMed PMID: 7958877.
35. Kim A, Terzian C, Santamaria P, Pelisson A, Prud'homme N, Bucheton A. Retroviruses in invertebrates: the gypsy retrotransposon is apparently an infectious retrovirus of *Drosophila melanogaster*. Proc Natl Acad Sci U S A. 1994;91:1285–9. PubMed PMID: 8108403.
36. Syomin BV, Fedorova LI, Surkov SA, Ilyin YV. The endogenous Drosophila melanogaster retrovirus gypsy can propagate in Drosophila hydei cells. Mol Gen Genet. 2001;264:588–94. PubMed PMID: 11212913.
37. Song SU, Kurkulos M, Boeke JD, Corces VG. Infection of the germ line by retroviral particles produced in the follicle cells: a possible mechanism for the mobilization of the gypsy retroelement of Drosophila. Development. 1997;124:2789–98. PubMed PMID: 9226450.
38. Teysset L, Burns JC, Shike H, Sullivan BL, Bucheton A, Terzian C. A Moloney murine leukemia virus-based retroviral vector pseudotyped by the insect retroviral gypsy envelope can infect Drosophila cells. J Virol. 1998;72:853–6. PubMed PMID: 9420299.
39. Pelisson A, Mejlumian L, Robert V, Terzian C, Bucheton A. Drosophila germline invasion by the endogenous retrovirus gypsy: involvement of the viral env gene. Insect Biochem Mol Biol. 2002;32:1249–1256. PubMed PMID: 12225916.
40. Steinhauer DA. Role of hemagglutinin cleavage for the pathogenicity of influenza virus. Virology. 1999;258:1–20. PubMed PMID: 10329563.
41. Misseri Y, Cerutti M, Devauchelle G, Bucheton A, Terzian C. Analysis of the Drosophila gypsy endogenous retrovirus envelope glycoprotein. J Gen Virol. 2004;85:3325–31. PubMed PMID: 15483247.
42. Trudeau D, Washburn JO, Volkman LE. Central role of hemocytes in Autographa californica M nucleopolyhedrovirus pathogenesis in Heliothis virescens and Helicoverpa zea. J Virol. 2001;75:996–1003. PubMed PMID: 11134313.
43. Slotkin RK, Martienssen R. Transposable elements and the epigenetic regulation of the genome. Nat Rev Genet. 2007;8:272–85. PubMed PMID: 17363976.
44. Ding D, Lipshitz HD. Spatially regulated expression of retrovirus-like transposons during Drosophila melanogaster embryogenesis. Genet Res. 1994;64:167–81. PubMed PMID: 7698641.
45. Maksakova IA, Mager DL, Reiss D. Keeping active endogenous retroviral-like elements in check: the epigenetic perspective. Cell Mol Life Sci. 2008;65:3329–47. PubMed PMID: 18818875.
46. Miranda TB, Jones PA. DNA methylation: the nuts and bolts of repression. J Cell Physiol. 2007;213:384–90. PubMed PMID: 17708532.
47. Field LM, Lyko F, Mandrioli M, Prantera G. DNA methylation in insects. Insect Mol Biol. 2004;13:109–15. PubMed PMID: 15056357.
48. Schaefer M, Lyko F. DNA methylation with a sting: an active DNA methylation system in the honeybee. Bioessays. 2007;29:208–11. PubMed PMID: 17295216.
49. Cheng X, Blumenthal RM. Coordinated chromatin control: structural and functional linkage of DNA and histone methylation. Biochemistry. 2010;49:2999–3008. PubMed PMID: 20210320.

50. Grassmann R, Jeang KT. The roles of microRNAs in mammalian virus infection. Biochim Biophys Acta. 2008. PubMed PMID: 18549828.
51. Ghildiyal M, Seitz H, Horwich MD, Li C, Du T, Lee S, Xu J, Kittler EL, Zapp ML, Weng Z, Zamore PD. Endogenous siRNAs derived from transposons and mRNAs in Drosophila somatic cells. Science. 2008;320:1077–81. PubMed PMID: 18403677.
52. Hutvagner G, Simard MJ. Argonaute proteins: key players in RNA silencing. Nat Rev Mol Cell Biol. 2008;9:22–32. PubMed PMID: 18073770.
53. Bohmert K, Camus I, Bellini C, Bouchez D, Caboche M, Benning C. AGO1 defines a novel locus of Arabidopsis controlling leaf development. Embo J. 1998;17:170–80. PubMed PMID: 9427751.
54. Senti KA, Brennecke J. The piRNA pathway: a fly's perspective on the guardian of the genome. Trends Genet. 2010;26:499–509. PubMed PMID: 20934772.
55. Leslie M. The immune system's compact genomic counterpart. Science. 2013;339:25–7. PubMed PMID: 23288523.
56. Teixeira FK, Okuniewska M, Malone CD, Coux RX, Rio DC, Lehmann R. piRNA-mediated regulation of transposon alternative splicing in the soma and germ line. Nature. 2017;552:268–272. PubMed PMID: 29211718.
57. Sarot E, Payen-Groschene G, Bucheton A, Pelisson A. Evidence for a piwi-dependent RNA silencing of the gypsy endogenous retrovirus by the Drosophila melanogaster flamenco gene. Genetics. 2004;166:1313–21. PubMed PMID: 15082550.
58. Brennecke J, Aravin AA, Stark A, Dus M, Kellis M, Sachidanandam R, Hannon GJ. Discrete small RNA-generating loci as master regulators of transposon activity in Drosophila. Cell. 2007;128:1089–103. PubMed PMID: 17346786.
59. Kawaoka S, Hayashi N, Katsuma S, Kishino H, Kohara Y, Mita K, Shimada T. Bombyx small RNAs: genomic defense system against transposons in the silkworm, Bombyx mori. Insect Biochem Mol Biol. 2008;38:1058–65. PubMed PMID: 18801438.
60. Kawaoka S, Minami K, Katsuma S, Mita K, Shimada T. Developmentally synchronized expression of two Bombyx mori Piwi subfamily genes, SIWI and BmAGO3 in germ-line cells. Biochem Biophys Res Commun. 2008;367:755–60. PubMed PMID: 18191035.
61. Kawaoka S, Hayashi N, Suzuki Y, Abe H, Sugano S, Tomari Y, Shimada T, Katsuma S. The Bombyx ovary-derived cell line endogenously expresses PIWI/PIWI-interacting RNA complexes. RNA. 2009;15:1258–64. PubMed PMID: 19460866.
62. Kawaoka S, Mitsutake H, Kiuchi T, Kobayashi M, Yoshikawa M, Suzuki Y, Sugano S, Shimada T, Kobayashi J, Tomari Y, Katsuma S. A role for transcription from a piRNA cluster in de novo piRNA production. RNA. 2011;18:265–73. PubMed PMID: 22194309.
63. Levine MT, Malik HS. Learning to protect your genome on the fly. Cell. 2011;147:1440–1. PubMed PMID: 22196722.
64. Fedoroff NV. Presidential address. Transposable elements, epigenetics, and genome evolution. Science. 2012;338:758–67. PubMed PMID: 23145453.
65. Pelisson A, Song SU, Prud'homme N, Smith PA, Bucheton A, Corces VG. Gypsy transposition correlates with the production of a retroviral envelope-like protein under the tissue-specific control of the Drosophila flamenco gene. Embo J. 1994;13:4401–11. PubMed PMID: 7925283.
66. Desset S, Meignin C, Dastugue B, Vaury C. COM, a heterochromatic locus governing the control of independent endogenous retroviruses from Drosophila melanogaster. Genetics. 2003;164:501–9. PubMed PMID: 12807771.
67. Aravin AA, Hannon GJ, Brennecke J. The Piwi-piRNA pathway provides an adaptive defense in the transposon arms race. Science. 2007;318:761–4. PubMed PMID: 17975059.
68. Saito K, Inagaki S, Mituyama T, Kawamura Y, Ono Y, Sakota E, Kotani H, Asai K, Siomi H, Siomi MC. A regulatory circuit for piwi by the large Maf gene traffic jam in Drosophila. Nature. 2009;461:1296–9. PubMed PMID: 19812547.

69. Klattenhoff C, Xi H, Li C, Lee S, Xu J, Khurana JS, Zhang F, Schultz N, Koppetsch BS, Nowosielska A, Seitz H, Zamore PD, Weng Z, Theurkauf WE. The Drosophila HP1 homolog Rhino is required for transposon silencing and piRNA production by dual-strand clusters. Cell. 2009;138:1137–49. PubMed PMID: 19732946.
70. Breitenbach JE, Shelby KS, Popham HJR. Baculovirus Induced Transcripts in Hemocytes from the Larvae of Heliothis virescens. Viruses. 2011;3:2047–2064. PubMed PMID: 22163334.
71. Feng M, Ren F, Zhou Y, Zhang N, Lu Q, Swevers L, Sun J. Correlation in Expression between LTR Retrotransposons and Potential Host Cis-Targets during Infection of Antherea pernyi with ApNPV Baculovirus. Viruses. 2019.:11. PubMed PMID: 31064084.
72. Nguyen Q, Chan LC, Nielsen LK, Reid S. Genome scale analysis of differential mRNA expression of Helicoverpa zea insect cells infected with a H. armigera baculovirus. Virology. 2013;444:158–70. PubMed PMID: 23827436.
73. Kharbanda N, Jalali SK, Ojha R, Bhatnagar RK. Temporal expression profiling of novel Spodoptera litura nucleopolyhedrovirus-encoded microRNAs upon infection of Sf21 cells. J Gen Virol. 2015;96:688–700. PubMed PMID: 25481752.
74. Berkhout B, Haasnoot J. The interplay between virus infection and the cellular RNA interference machinery. FEBS Lett. 2006;580:2896–902. PubMed PMID: 16563388.
75. Ooi BG, Miller LK. Regulation of host RNA levels during baculovirus infection. Virology. 1988;166:515–523. PubMed PMID: 2459844.
76. Nobiron I, O'Reilly DR, Olszewski JA. Autographa californica nucleopolyhedrovirus infection of Spodoptera frugiperda cells: a global analysis of host gene regulation during infection, using a differential display approach. J Gen Virol. 2003;84:3029–39. PubMed PMID: 14573808.
77. Katsuma S, Mita K, Shimada T. ERK- and JNK-dependent signaling pathways contribute to Bombyx mori nucleopolyhedrovirus infection. J Virol. 2007;81:13700–9. PubMed PMID: 17913811.
78. Blissard GW, Rohrmann GF. Location, sequence, transcriptional mapping, and temporal expression of the gp64 envelope glycoprotein gene of the *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus. Virology. 1989;170:537–555. PubMed PMID: 2658304.
79. Friesen PD, Nissen MS. Gene organization and transcription of TED, a Lepidopteran retrotransposon integrated within the baculovirus genome. Mol Cell Biol. 1990;10:3067–3077. PubMed PMID: 1692964.
80. Fraser MJ, Hink WF. The isolation and characterization of the MP and FP variants of Galleria mellonella nuclear polyhedrosis virus. Virology. 1982;117:366–378. PubMed PMID: 18635122.
81. Fraser MJ, Smith GE, Summers MD. Aquisition of host cell DNA sequences by Baculoviruses; Relationships between host DNA insertions and FP mutants of *Autographa californica* and *Galleria mellonella* nuclear polyhedrosis virus. J Virol. 1983;47:287–300. PubMed PMID: 16789244.
82. Cary LC. Transposon mutagenesis of baculoviruses: analysis of Trichoplusia ni transposon IFP2 insertions within the FP locus of nuclear polyhedrosis viruses. Virology. 1989;172:156–69. M. G, Corsaro BG, Wang H, Rosen E, Fraser MJ. PubMed PMID: 2549707.
83. Fraser MJ, Cary L, Boonvisudhi K, Wang HG. Assay for movement of Lepidopteran transposon IFP2 in insect cells using a baculovirus genome as a target DNA. Virology. 1995;211:397–407. PubMed PMID: 7645244.
84. Elick TA, Bauser CA, Fraser MJ. Excision of the piggyBac transposable element in vitro is a precise event that is enhanced by the expression of its encoded transposase. Genetica. 1996;98:33–41. PubMed PMID: 8765680.
85. Xu HF, Xia QY, Liu C, Cheng TC, Zhao P, Duan J, Zha XF, Liu SP. Identification and characterization of piggyBac-like elements in the genome of domesticated silkworm, Bombyx mori. Mol Genet Genomics. 2006;276:31–40. PubMed PMID: 16685528.
86. Daimon T, Mitsuhiro M, Katsuma S, Abe H, Mita K, Shimada T. Recent transposition of yabusame, a novel piggyBac-like transposable element in the genome of the silkworm, Bombyx mori. Genome. 2010;53:585–93. PubMed PMID: 20725145.
87. Yusa K. 2015. piggyBac Transposon. Microbiol Spectr 3:MDNA3-0028-2014.

88. Mitra R, Fain-Thornton J, Craig NL. piggyBac can bypass DNA synthesis during cut and paste transposition. EMBO J. 2008;27:1097–109. PubMed PMID: 18354502.
89. Handler AM. Use of the piggyBac transposon for germ-line transformation of insects. Insect Biochem Mol Biol. 2002;32:1211–20. PubMed PMID: 12225912.
90. Wilson MH, Coates CJ, George AL Jr. PiggyBac transposon-mediated gene transfer in human cells. Mol Ther. 2007;15:139–45. PubMed PMID: 17164785.
91. Tipanee J, VandenDriessche T, Chuah MK. Transposons: Moving Forward from Preclinical Studies to Clinical Trials. Hum Gene Ther. 2017;28:1087–1104. PubMed PMID: 28920716.
92. Ding S, Wu X, Li G, Han M, Zhuang Y, Xu T. Efficient transposition of the piggyBac (PB) transposon in mammalian cells and mice. Cell. 2005;122:473–83. PubMed PMID: 16096065.
93. Gonzalez-Estevez C, Momose T, Gehring WJ, Salo E. Transgenic planarian lines obtained by electroporation using transposon-derived vectors and an eye-specific GFP marker. Proc Natl Acad Sci U S A. 2003;100:14046–51. PubMed PMID: 14615580.
94. Balu B, Shoue DA, Fraser MJ Jr, Adams JH. High-efficiency transformation of Plasmodium falciparum by the lepidopteran transposable element piggyBac. Proc Natl Acad Sci U S A. 2005;102:16391–6. PubMed PMID: 16260745.
95. Morales ME, Mann VH, Kines KJ, Gobert GN, Fraser MJ Jr, Kalinna BH, Correnti JM, Pearce EJ, Brindley PJ. piggyBac transposon mediated transgenesis of the human blood fluke, Schistosoma mansoni. FASEB J. 2007;21:3479–89. PubMed PMID: 17586730.
96. Meir YJ, Wu SC. Transposon-based vector systems for gene therapy clinical trials: challenges and considerations. Chang Gung Med J. 2011;34:565–79. PubMed PMID: 22196059.
97. Kettlun C, Galvan DL, George AL Jr, Kaja A, Wilson MH. Manipulating piggyBac transposon chromosomal integration site selection in human cells. Mol Ther. 2011;19:1636–44. PubMed PMID: 21730970.
98. Li MA, Turner DJ, Ning Z, Yusa K, Liang Q, Eckert S, Rad L, Fitzgerald TW, Craig NL, Bradley A. Mobilization of giant piggyBac transposons in the mouse genome. Nucleic Acids Res. 2012;39:e148. PubMed PMID: 21948799.
99. Rohrmann GF, Erlandson MA, Theilmann DA. Genome Sequence of an Alphabaculovirus Isolated from the Oak Looper, Lambdina fiscellaria, Contains a Putative 2-Kilobase-Pair Transposable Element Encoding a Transposase and a FLYWCH Domain-Containing Protein. Genome Announc. 2015.:3. PubMed PMID: 26021909.

12. The AcMNPV genome: Gene content, conservation, and function

The *Autographa californica* nucleopolyhedrovirus (AcMNPV) was originally described in the early 1970s (1) and research on its genetics began later in that decade. This was stimulated by the facility with which the virus replicated in cells from *Spodoptera frugiperda* (2) and *Trichoplusia ni* (3). Subsequently, this led to the development of technology for the deletion of genes (4, 5), and allowed for targeted studies on the function of specific genes, particularly if the deletion or mutation of the target gene was not lethal to the virus. The publication of the genome sequence of AcMNPV (6) was a landmark in the investigation of this virus because it put all previous and future investigations into context. It also revealed genes that are shared with other organisms, and provided the basis for understanding baculovirus diversity. Subsequently, the development of the bacmid system, which allowed for the production of recombinants via transposition of recombinant plasmids into the AcMNPV genome incorporated into a bacterial artificial chromosome, allowed for manipulation of the whole AcMNPV genome in bacteria (7). The adaptation of this technology to making targeted deletions provided a method for constructing baculovirus gene knockouts in bacteria that could then be investigated via transfection into insect cell lines (8). The modification of this technology using a lambda red recombinant system (9) allowed for the more efficient production of knockouts (10). This has resulted in a proliferation of studies on AcMNPV essential genes that were previously difficult to investigate because knockouts were lethal and they could not be readily generated without the production of complementary cell lines. With bacmid knockouts the function of the target gene can be inferred from the examination of cells transfected with DNA of the mutant. The adaptation of similar bacmid systems for other baculovirus genomes has allowed for parallel studies on these viruses. Concomitant with the development of techniques for investigating gene function has been a proliferation of complete genome sequence data for many baculoviruses. Because of the widespread use of AcMNPV, not only for fundamental investigations on gene function, but also because of its use as an expression vector, I have attempted to annotate the AcMNPV genome in terms of understanding the function of each of the genes.

Adjustments to the AcMNPV genome sequence: There are approximately 150 orfs

This analysis is based on the first AcMNPV genome to be sequence, the C-6 strain (6). Several regions of the AcMNPV genome have been re-sequenced by one or more laboratories. This, in combination with genome sequences of variants of AcMNPV that have become available including the commonly used E2 strain (11), has revealed revisions that should be incorporated into the sequence. The following summarizes the physical corrections to the AcMNPV genome that have been described. The original orfs Ac20/21, 58/59, 106/107 and 112/113 (6) should be combined into single orfs. In addition, resequencing also indicated that Ac17, 52, 131, and 143 are longer than originally reported (12). The total number of orfs reported for AcMNPV was originally calculated to be 154; this adjustment reduces it by 4. However, the discovery of lef-10 (Ac53a), which was missed in the original annotation because of its significant overlap with the 5' region of an adjacent gene (Ac54) (13), adds an additional orf. Therefore, there appear to be 151 orfs present in the genome based on the original criteria of an orf comprising 50 amino acids. However, Ac85 (53 aa) is only found in two other NPVs that are variants of AcMNPV (PlxyNPV) (14) and RoMNPV (12). Two orfs, Ac97 (56 aa) and Ac140 (60 aa), are only present in AcMNPV. Of these three orfs, only Ac97 was predicted to be preceded by transcriptional regulatory sequences (6). Although these three orfs may not be valid, others may be present that have not yet been detected. Therefore, it would appear that there are 'about' 150 orfs in the AcMNPV genome.

In this review, I have attempted to include pertinent information on the function of all the orfs present in the AcMNPV genome. This is not an exhaustive review, but an attempt to infer function from either actual experimentation on the AcMNPV gene or to homology from related viruses. A significant proportion of the data

included comes from the *Bombyx mori* NPV (BmNPV). It is closely related to AcMNPV and is the second most studied NPV and a complete study based on the generation of BmNPV bacmid knockouts of all its open reading frames has been described (15). In addition, comparisons with orfs from Helicoverpa armigera SNPV, a group II alphabaculovirus are also included because it is being intensively investigated in a number of labs. The annotation for this chapter is based on Accession Numbers NC_001623 (AcMNPV C-6 strain - originally L22858), L33180 (BmNPV), and AF271059 (HearSNPV).

Table 1 lists all the genes that have been named in the AcMNPV genome followed by the orf number. It cannot be emphasized enough how important it is to incorporate both orf numbers and the AcMNPV homolog (if present) into any description of a baculovirus gene no matter which baculovirus it is from. Without this reference, it is difficult, if not impossible, to put reports into any sort of perspective. Following the index is a review of what I could find regarding all the genes in the AcMNPV genome. This is not a complete review of these genes but focuses particularly on AcMNPV. When a report of the homolog from another virus is relevant, that material is also included.

Table 1. Index to named AcMNPV genes/gene products from the C-6 strain (6).

ARIF1 (Actin rearranging factor1)	**Ac20/21**
Alkaline nuclease	***Ac133**
Apsup	**Ac112/113**
BRO (Baculovirus repeated orf)	**Ac2**
BV/ODV-E26	**Ac16**
BV/ODV-C42	***Ac101**
Calyx, polyhedron envelope	**Ac131**
Cathepsin	**Ac127**
ChaB homolog	**Ac59**
ChaB homolog	**Ac60**
Cg30	**Ac88**
C42	**Ac102**
Chitinase	**Ac126**
Chondroitinase, odv-e66	**Ac46**
Conotoxin like (Ctl)	**Ac3**
DA26	**Ac16**
DNA primase	**Ac14**
Desmoplakin-like	***Ac66**
DBP (DNA binding protein)	**Ac25**
DnaJ domain protein	**Ac51**
DNA polymerase	***Ac65**
EGT	**Ac15**
ETL (PCNA)	**Ac49**
ETM	**Ac48**
ETS	**Ac47**

Table 1. continued from previous page.

Exon-0	**Ac141**
F (fusion protein homolog)	**Ac23**
FGF (fibroblast growth factor)	**Ac32**
FP (few polyhedra), fp-25k	**Ac61**
Fusolin (gp37)	**Ac64**
GP16	**Ac130**
GP37	**Ac64**
GP41	**Ac80**
GP64	**Ac128**
Gta (global transactivator)	**Ac42**
Hcf-1 (host cell factor 1)	**Ac70**
Helicase, p143	***Ac95**
He65	**Ac105**
Histodinol phosphatase	**Ac33**
Homologous regions	**Hrs** (see end of chapter)
Iap-1	**Ac27**
Iap-2	**Ac71**
Ie1	**Ac147**
Ie0	**Ac147-0**
Ie2	**Ac151**
J domain	**Ac51**
Lef1	***Ac14**
Lef2	***Ac6**
Lef3	**Ac67**
Lef4	***Ac90**
Lef5	***Ac99**
Lef6	**Ac28**
Lef7	**Ac125**
Lef8	***Ac50**
Lef9	***Ac62**
Lef10	**Ac53a**
Lef11	***Ac37**
Lef12	***Ac41**
Me53	**Ac139**
MTase (methyl transferase)	**Ac69**
Nudix	**Ac38**
ODV-E18	***Ac143**
ODV-E25, p25, 25k	***Ac94**

Table 1. continued from previous page.

ODV-EC27	**Ac144**
ODV-E56, Pif-5	***Ac148**
ODV-E66	**Ac46**
P6.9	***Ac100**
P10	**Ac137**
P11	**Ac145**
P12	**Ac102**
P15	**Ac87**
P18	**Ac93**
P24	**Ac129**
P26	**Ac136**
P33 sulfhydryl oxidase	***Ac92**
P35	**Ac135**
P40	***Ac102**
P43	**Ac39**
P45, p48	***Ac103**
P47	***Ac40**
P49	***Ac142**
P74, Pif-0	**Ac138**
P94	**Ac134**
P95, p91	***Ac83**
P143 (helicase)	***Ac95**
PCNA	**Ac49**
Pe38	**Ac153**
PEP polyhedron envelope protein	**Ac131**
Pif-0, p74	***Ac138**
Pif-1	***Ac119**
Pif-2	***Ac22**
Pif-3	***Ac115**
Pif-4 (19K)	***Ac96**
Pif-5, odv-e56	***Ac148**
Pif-6	***Ac68**
Pif-7	***Ac110**
Pif-8, vp91, vp94	***Ac83**
PK1 (Protein kinase 1)	**Ac10**
PK2 (Protein kinase 2)	**Ac123**
PKIP (Protein kinase interacting factor)	**Ac24**

Table 1. continued from previous page.

PNK polynucleotide kinase	**Ac33**
PNK/PNL polynucleotide kinase/ ligase	**Ac86**
Polyhedrin	**Ac8**
Pp31;39K	**Ac36**
Pp34, polyhedron envelope	**Ac131**
Pp78/83;orf1629	**Ac9**
Primase	**Ac14**
Primase accessory factor	**Ac6**
Protein tyrosine phosphatase (ptp)	**Ac1**
Single stranded DNA binding protein (SSB)	**Ac67**
SOD superoxide dismutase	**Ac31**
Sulfhydryl oxidase, sox	***Ac92**
TLP telokin-like	**Ac82**
TRAX-like	**Ac47**
Ubiquitin	**Ac35**
VLF-1 very late factor 1	***Ac77**
Vp39, capsid	***Ac89**
Vp80, vp87	**Ac104**
Vp91, p95	***Ac83**
Vp1054	***Ac54**
19K (pif-4)	***Ac96**
38K	***Ac98**
39K, pp31	**Ac36**
49K	***Ac142**

* Core genes

Annotation of the AcMNPV genome

Below is an annotation of the orfs in the AcMNPV genome based on their orf number from (6). An asterisk (*) indicates a core gene with homologs found in all sequenced baculovirus genomes. The orf size in amino acids, followed by the molecular mass in kDa is indicated after each orf as reported by (6, 12). The same information is also included for the BmNPV (16) and *Helicoverpa armigera* (HaSNPV) (17) orthologs of these genes. Both AcMNPV and BmNPV are Group I alphabaculoviruses whereas HaSNPV is a group II alphabaculovirus and lacks some of the genes found in AcMNPV and BmNPV but also has some additional genes. The survey of information regarding each gene was updated in early 2019.

Ac1 (168aa:19.3kDa), (Bm130:168aa:19.3kDa), (protein tyrosine phosphatase (ptp); baculovirus phosphatase (bvp)).

Ac1 homologs are present in the genomes of all lepidopteran Group I NPVs, but not those of other baculoviruses. Closely related orfs are found in a variety of invertebrates, e.g., *Drosophila* (E = 7e-30) and vertebrates, e.g., human (E = 2e-27). It is expressed from a late promoter (18). It was originally identified because of its relatedness to protein tyrosine phosphatases and its ability to dephosphorylate proteins at ser, thr and tyr residues (19). However, it was later found that Ac1 is an RNA 5'-triphosphatase and hydrolyzes the gamma phosphate of triphosphate-terminated poly(A) and also hydrolyzes ATP to ADP and GTP to GDP (20, 21). The crystal structure has been determined (22). Ac1 is predicted to have one of the same enzymatic activities of LEF-4 (Ac90) that is involved in preparing RNA for cap formation. Although deletion is not lethal, AcMNPV mutants are partially defective in occluded virus production in Sf-21, but not Tn-368 cells (23). I addition, it was found to be required for AcMNPV replication in in *S. exigua* larvae (24), but not for replication of BmNPV in *B. mori* larvae (25). A host *B. mori* ptp was shown to be induced by BmNPV infection and RNAi knockdown reduced viral replication (26). Although not reported to be associated with AcMNPV ODV by proteomic analysis, it was detected in BV preparations (27). It was reported to influence BmNPV infected larval wandering late in infection in one study (28), but had no effect in another (29).

Ac2 (328aa:38.8kDa), (Bm131:349aa:40.1kDa, Bm22:317aa:35.4kDa, Bm80:239:27.5kDa, Bm81:318aa:35.9kDa, Bm132:241aa:27.8kDa), (Ha59:244aa:28.2,Ha60:527aa:59.7kDa,Ha105:501aa:58.3kDa) (Baculovirus repeated orf—BRO).

Derivation: In American inner-city dialect 'bro' means 'brother', but not necessarily a close relationship.

Homologs of Ac2 have a widespread distribution in lepidopteran NPVs and GVs and are also found in the dipteran, but not hymenopteran NPV genomes. Related orfs are also found in double-stranded DNA phage, prokaryotic class II transposons, and a variety of DNA viruses pathogenic for insects, including entomopox viruses, iridoviruses and ascoviruses (30). Twenty-three copies of *bro* genes have been reported in a *Heliothis virescens* ascovirus genome sequence (31). Although there is only one copy of the *bro* gene in AcMNPV, the number can vary in different baculoviruses from none in the closely related *Rachiplusia ou* MNPV — its orfs are 96% identical in sequence to AcMNPV (12), two in the more closely related PlxyNPV (14) (orfs are 98.5% identical to AcMNPV), to up to 16 copies in LdMNPV (32). AcMNPV Ac 2 deletion mutants are viable, but some differences in polyhedron production in infected cells were noted (30). One of the major differences in gene content between BmNPV and AcMNPV is the presence of 3-5 copies of *bro* genes in BmNPV vs. a single copy in AcMNPV (16). The BmNPV *bro* genes are present in three locations with duplicate genes in two of the locations. In contrast, another BmNPV strain only shows 3 copies of the gene, one at each of the three locations (33, 34). This suggests that duplication/loss of these genes might be common. In the strain with five genes, individual mutants of four of the genes were isolated in BmNPV, but a mutant of one gene (*bro-d*) could not be isolated. Also, a double mutant of *bro-a* and *bro-c* could not be produced, suggesting that they complement an essential function and that *bro-d* has a unique essential function (35). BmNPV BRO proteins have DNA binding activity (36), and all the BmNPV *bro* genes appeared to be expressed as early genes and are distributed in both the nucleus and cytoplasm (35). One of the *bro* gene products was found to interact with laminin, a glycoprotein that is a major constituent of the basal lamina and is involved in cell attachment (37). In BmNPV infections, BRO proteins were found to be phosphorylated (36) and BRO-D was found to be highly phosphorylated (38). In AcMNPV, *bro* was highly expressed in T. ni midguts throughout infection (39).

Ac3 (53aa:5.6kDa) (Conotoxin-like (*ctl*) genes).

Conotoxins are small disulfide-rich ion channel antagonists isolated from snails (genus *Conus)* (40). Homologs of *ctl* are found in about half of the sequenced Group I and Group II lepidopteran NPV genomes and two GVs (Xcni- and HaGV). Although in AcMNPV a single *ctl* gene is present, several other viruses, e.g., OpMNPV and LdMNPV, encode two *ctl* genes of different lineages (*ctl-1* and *ctl-2*). Homologs are found in the *Amsacta moori* entomopoxvirus (E = 0.006), a few mosquito species, a funnel web spider, a wasp, and a bacterium. The EPV (*A. moori* gene falls within the baculovirus *ctl-2* lineage. In a study examining the AcMNPV *ctl* (*ctl*-1) gene, no differences in mortality, motility, or weight gain were observed when either neonate or late instar *Spodoptera frugiperda* larvae were infected with an AcMNPV mutant deleted for *ctl-1*, compared with infection with wt virus (41). It was found to inhibit melanization and when conjugated to gfp, it localized to cell membranes (42).

Ac4 (151aa:17.6kDa), (Bm133:151aa:17.7kDa).

Homologs of Ac4 are found in most Group I, and a few Group II and GV genomes, but not in other lineages. Ac4/Bm133 is likely to be nonessential because when it was deleted the virus appeared normal (15, 43, 44). However, in BmNPV, Bm133 and 134 were found to be distributed throughout the cell during infection and deletion of either gene appeared to reduce the number of occluded virions(45).

Ac 5 (109aa:12.4kDa), (Bm134:109aa:12.4kDa).

Homologs of Ac5 are found in most Group I NPV genomes. Ac5/Bm134 is likely to be nonessential because when Bm134 was deleted, the virus appeared normal (15, 28). However, in BmNPV, Bm133 and 134 were found to be distributed throughout the cell during infection and deletion of either gene appeared to reduce the number of occluded virions and Bm134 appeared to localize to polyhedra(45, 46). Although Ac5 was suggested to form a complex with PIF proteins (47), this was not observed by others (46, 48).

*Ac6 (210aa:23.9kDa), (Bm135:210aa:23.8kDa) (Ha117:241aa: 27.8kDa)(Lef-2)(DNA primase accessory factor).

LEF-2 is a DNA primase accessory factor and is encoded by all baculovirus genomes. It interacts with LEF-1 (49), the baculovirus DNA primase. It has homology to the large subunit of DNA primase in several archaea. It is required for transient DNA replication (50, 51). It is an essential gene as AcMNPV or BmNPV bacmids deleted for *lef-2* were unable to produce infectious virus (15, 52).

Based on limited amounts of DNA synthesis by a *lef-2* bacmid knockout in transfected cells, it was suggested that Lef-2 is not required for the initiation of DNA replication. This was in contrast to a bacmid with the helicase gene deleted that showed no synthesis (52). However, the transfected DNA is likely nicked and therefore the ends of the nicks could act as primers, resulting in limited amounts of leading strand DNA synthesis in the absence of an active primase complex. Elution profiles of LEF-1 and LEF-2 from ssDNA cellulose and DEAE resin, suggested that LEF-2 may bind to both DNA and LEF-1 (53). LEF-2 mutants have been characterized that appear to affect very late transcription, indicating that it may have roles in both replication and transcription (54). In addition, BmNPV LEF-2 was shown to activate late transcription (55).

Ac7 (201aa:23.6kDa) (Orf 603).

This gene is only found in three baculovirus genomes in addition to AcMNPV, including ClbiNPV, PlxyNPV and RoMNPV. Deletion from AcMNPV did not affect replication in cell culture or in *T. ni* larvae (56).

Ac8 (245aa:28.6kDa), (Bm1:245aa:28.8kDa) (Ha1:245aa: 26.7kDa) (polyhedrin, occlusion body protein).

Homologs of polyhedrin (called granulin in GVs) are found in all baculovirus genomes, except for that of the dipteran virus (CuniNPV). Surprisingly, CuniNPV has an occlusion body protein unrelated and about three times as large as polyhedrin of other baculoviruses (57, 58). Because of unexpected patterns of phylogeny of AcMNPV polyhedrin, it has been suggested that it is a chimera derived from both Group I and II sequences (59). It is generally thought that polyhedrin serves to stabilize baculovirus virions in the environment allowing them to persist indefinitely. The polyhedrin gene is nonessential in cell culture, and occlusion-positive and negative plaques can be readily be distinguished. This, in combination with the strength of the polyhedrin promoter, led to the use of the polyhedrin locus as the site for the production of recombinant baculoviruses (4, 5). The crystal structure of AcMNPV polyhedra and Cydia pomonella GV granula have been described (60) (61).

Ac9 (543aa:60.7kDa), (Bm2:542aa:60.9kDa) (Ha9:413aa:45.9kDa), (pp78/83, Orf1629).

Homologs of pp78/83 are found in all lepidopteran NPV (I and II) genomes. It is an essential gene. Because it is located adjacent to the polyhedrin gene, it was originally manipulated via complementation to elevate the frequency of obtaining recombinant baculoviruses at the polyhedrin locus (62). It is phosphorylated (63) and is a structural protein located at one end of nucleocapsids (63, 64). Ac9 is a Wiskott-Aldrich syndrome protein (WASP)-like protein involved in nuclear actin assembly during the baculovirus infection that leads to movement of virions through the cytoplasm of the infected cell (65, 66).

Ac10 (272aa:32kDa), (Bm3:275aa:32.4kDa) (Ha10:267aa:31.5kDa), (Protein kinase-1 (PK-1)).

Homologs of pk-1 are found in lepidopteran NPV and GV genomes, but not in other lineages. AcMNPV PK-1 shows significant relatedness to some insect orfs, e.g., *Anopheles gambiae* (E = 3e-18). Purified PK-1 was able to phosphorylate histone substrates (67). A bacmid with a knockout of pk-1 produced no viral progeny, although DNA replication was unaffected. It was also found that the kinase catalytic domain was required for infectivity. Cells transfected with the mutant bacmids showed extensive arrays of tubular structures that appeared to lack DNA suggesting that the phosphorylation of one or more proteins was required for capsid morphogenesis (68). Ts mutants characterized for defects in very late gene expression contained mutations in *pk-1* (69). In addition, inactivation of pk-1 mRNA using DNAzyme technology caused a reduction in the expression from the polyhedrin promoter (70). PK-1 also may be associated with a very late transcription complex and be involved in the phosphorylation of LEF-8 (71). PK1 of SpltNPV-I and AcMNPV may interact with the polyhedrin promoter, suggesting that it might act as a very late gene transcription factor (72) (73).

Ac11 (340aa:40.1kDa), (Bm4:340aa:39.8kDa).

Homologs of Ac11 are found in all lepidopteran Group I NPV and in one Group II (LdMNPV) genomes, but not in other lineages. Although it was suggested that Bm4 was nonessential (15), an examination of Ac11 suggested that it was required for BV production and ODV envelopment (74). In addition, it was reported that Ac11 interacts with components of the ESCRT-III complex and may be involved in the release of nucleocapsids at the nuclear membrane(75).

Ac12 (217aa:25.4kDa).

Homologs of Ac12 are found in many alphabaculoviruses and a few betabaculoviruses. It has an F-box domain and interacts with S phase kinase associated protein1 and may be involved in ubiquitin pathways, but is a non-

essential gene (76). It shows limited similarity to the tryptophan repeat gene family proteins of *Amsacta moorei* entomopoxvirus (E = 0.12) (see also Ac30).

Ac13 (327aa:38.7kDa), (Bm5:331aa:39.3kDa)(Ha123:385aa:44.5kDa).

Homologs of Ac13 are found in all Group I, II, and GV genomes, but not in other lineages. Analysis by HHpred (77) predicts that it contains coiled-coil regions and has some structural similarity to some membrane proteins. Ac13/Bm5 may be nonessential because when it was deleted, the virus appeared normal (15). However, Bm5 appears to encode a late expressed protein not associated with BV or ODV (78) and it was also found that Bm5 localizes to the nuclear membrane and its inactivation caused lower titers of BV, fewer occlusion bodies, and seemed to interfere with the expression of some very late genes, and resulted a delay in larval death (79). In another investigation, Bm5 was termed BION (baculovirus protein associated with both the inner- and outer nuclear membranes) because it was found to be present in both the inner- and outer nuclear membranes (80).

*Ac14 (266aa:30.8kDa), (Bm6:270aa:31.1kDa), (Ha124:245aa:29kDa) (Lef-1, DNA primase).

LEF-1 is a core gene present in all baculovirus genomes. Purified LEF-1 has DNA primase activity (53). It interacts with Ac6 (LEF-2) (49), the baculovirus DNA primase accessory factor and is required for transient DNA replication (50, 51). Lef-1 is likely an essential gene as a deletion/insertion mutant in the BmNPV homolog (Bm6) could not be isolated (15, 81).

Ac15 (506aa:57kDa), (Bm7:506aa:57.0kDa), (Ha126:515:58.9kDa), EGT.

Ac15 encodes ecdysteroid UDP-glucosyltransferase (82) and homologs are found in all Group I, II, and most GV genomes, but not in other lineages. Homologs are found in a variety of insects, e.g., *B. mori* (E = 3e-50). The function of EGT is to block molting and pupation in infected larvae by catalyzing the transfer of glucose from UDP-glucose to ecdysteroids, thereby inactivating these insect molting hormones (83, 84). This is thought to prolong the feeding stage of infected larvae, thereby allowing the virus to replicate over a longer period in larger larvae, resulting in a higher yield of virus. AcMNPV and BmNPV mutants in which the *egt* gene is inactivated are viable (84) but their survival time is reduced (44, 85). Spontaneous deletions of the *egt* gene commonly occur in cell culture (86).

Ac16 (225aa:25.9kDa), (Bm8:229aa:26.2kDa) (BV/ODV-E26).

Homologs of Ac16 are found in all lepidopteran Group I NPV genomes, but not in other lineages. Ac16 interacts with fp25 (Ac61), forms a complex with cellular actin (87) and is palmitoylated (88). It was found to be associated with the envelopes of both BV and ODV and was called BV/ODV-E26, and was also identified as being associated with ODV by mass spectrometry analysis (89). However, in BmNPV, the homolog of AcMNPV Ac16 (Bm8) was not identified as a virion structural protein (90). It was suggested that the difference in the results was due to the source of the antibody (91). In BmNPV, Bm8 directly interacts with IE-1 (92). Similar observations were made in AcMNPV with Ac16 interacting with both IE-1 and IE-0 (93). In addition, Bm8 was found to interact with cellular membrane-bound proteins or secreted proteins (94). A mutant, in which AcMNPV orf16 (called DA26) was insertionally inactivated was viable and showed no difference from wt in *T. ni* or *S. frugiperda* cells or larvae (95). Initial attempts at isolation of null mutants of BmNPV (Bm8) were not successful, but a C-terminal deletion mutant was viable (90), although this mutant was unable to produce BV titers as high as wt. However, a bacmid deleted for Bm8 was viable (15). It was also found that Bm8 appears to inhibit occlusion body production in middle silk glands and its deletion accelerated the death of infected insects (44). In another investigation with BmNPV, inactivation of Bm8 resulted in reduced levels of BV and more occlusion bodies in both cultured cells and larvae and appeared to accelerate viral infection in various tissues suggesting that it might be involved in tissue tropism (96). In AcMNPV, deletion of Ac16 resulted in a delay in

full levels of DNA synthesis and BV production in one study (97), but had lesser effects in another (93). Analysis by HHpred (77) predicts that it contains a coiled-coil region.

Ac17 (209aa:23.9kDa), (Bm9:210aa:24.1kDa), (Ha128:266aa:30.4kDa).

Homologs of Ac17 are found in most Group I and II NPV genomes. It is an early expressed gene with a product of about 19 kDa that localizes to the cytoplasm (98). A homolog of Ac17 in HearNPV (He128) is expressed late and was found in the cytoplasm (99). Deletion of Ac17 did not affect DNA synthesis, although BV production was reduced by up to a factor of 10 to 100 (97). Deletion of both Ac16 and Ac17 was similar to the Ac17 deletion although there were more significant delays for DNA replication and BV titers to reach levels similar to the Ac17 deletion alone (97). Similar results were reported for a BmNPV bacmid knock out. In addition, the mutant BmNPV took significantly longer to kill larvae and required higher titers BV to achieve an LD_{50} (100). The predicted size of Ac17 is longer than previously reported (12).

Ac18 (353aa:40.9kDa), (Bm10:356aa:41.5kDa).

Homologs of Ac18 are found in all Group I and II NPV genomes. An AcMNPV bacmid deleted for Ac18 was infectious with an LD50 in *T. ni* larvae similar to wt, but took somewhat longer to kill larvae (101). It was observed that Ac18 was upregulated about 2 fold by AcMNPV-miR-1 and was suggested that Ac18 interacts with fp25 and Ac18 upregulation may affect the function of fp25(102). Analysis by Hhpred (77) suggested that it was related to nucleotidyl transferase with over 80% probability.

Ac19 (108aa:12.2kDa), (Bm11:110aa:12.5kDa), (Ha115:129aa: 15.3kDa).

Homologs of Ac19 are found in all Group I and most Group II NPVs. Ac19 is likely to be nonessential because when it a BmNPV Bm11 deletion was viable (15, 44). However, more recent investigations on Bm11 indicated that it is a late gene, localized to the ring zone, and its inactivation reduced OB production and the occlusion of ODV (103).

Ac20 (Ac20/21 (417aa:47.7kDa), (Bm12:440aa:50.0kDa), (Ha131: 265aaa:30.4kDa)(arif-1).

The two orfs, Ac20/21, were likely caused by a sequencing error in the original C6 sequence. This region has been re-sequenced in several AcMNPV strains including C6 and in all cases it was found that Ac20 and 21 are a single orf (104), see also (12). Homologs of arif-1 are present in all Group I and most Group II genomes. AcMNPV orf20/21 is called actin-rearrangement-inducing factors (arif-1). It is expressed as an early gene and transfection of a plasmid containing this gene into Tn-368 cells is able to induce actin rearrangement (105). Arif-1 was found to colocalize with F-actin at the plasma membrane and deletion mutants showed a loss of actin concentration at the plasma membrane. Deletions of the C-terminal half of the gene or insertion of the LacZ gene near the center of the orf resulted in constructs that showed no significant loss of infectivity in Tn-368 or Sf cells (104). Arif-1 was more highly expressed in midgut than cultured cells suggesting it may be important in establishing the infection (39). Defective mutants of BmNPV Arif-1 caused a significant delay in the infection in several tissues including trachea, fat body, suboesophageal ganglion and brain (106).

*Ac22 (382aa:43.8kDa), (Bm13:382aa:43.8kDa), (Ha132:383:44.5kDa) (PIF-2).

This gene is a member of the per os infectivity factor (PIF) (107) gene group. These genes can be deleted and the mutants are still infectious for cultured cells, but are not orally infectious for insects (108). It is present in all

baculovirus genomes and is also present in a nudivirus (109) and an *Apis mellifera* filamentous virus genome (110). For more information see Ac138 (p74) and Chapter 3.

Ac23 (690aa:79.9kDa), (Bm14:673aa:78.0kDa), (Ha133:677aa: 78.2kDa), F (Fusion) protein.

Ac23 is a homolog of the predicted fusion protein (F) of Group II lepidopteran NPVs, GVs, and the dipteran virus (CuniNPV). Homologs are found in all baculoviruses except the hymenopteran viruses. In Group II viruses such as LdMNPV and HaSNPV, Ac23 homologs (*ld130, Ha133*) encode low pH-activated fusion proteins. In Group I viruses, they appear to be inactive as fusion proteins and have been replaced by *gp64*, which is not found in the Group II viruses, GVs, or hymenopteran or dipteran viruses. Orthologs of the F gene are also found as the env gene of insect retroviruses (111), and are also present in some insect genomes (112). They also appear to be related to the fusion (F) proteins of the paramyxoviridae (see Chapter 2). Although inactive in Group I viruses, the Acorf23 homolog is glycosylated and associated with the envelope of BV and with the membranes of OpMNPV-infected cells (113). In AcMNPV, Ac23 is also associated with BV membranes and its deletion from the genome results in infectious virus, but the time to kill larvae was somewhat extended (10). Ac23 was found to be associated with ODV as was the homolog in CuniNPV (89, 114). Ac23 increases viral infectivity and may be involved in cell binding (115, 116). In BmNPV, deletion of Bm14 had a variety of effects including reduced production of BV, a delay in the lethal time in infected larvae, reduced production of OB, fewer virions in OBs, and irregular OB with pitted surfaces(117).

Ac24 (169aa:19.2kDa), (Bm15:169aa:19.4kDa), Protein kinase interacting protein (PKIP).

Homologs are found in all Group I and II NPV genomes, however, they are not very closely related suggesting that they may be of different lineages. PKIP was found to interact with AcMNPV protein kinase I (see Ac10) in a yeast two-hybrid assay. It stimulates PK-1 activity in vitro. PKIP appears to be essential as attempts to isolate a deletion mutant were unsuccessful (118). Ts mutants in PKIP are defective in very late transcription, show a delay in shutoff of host protein synthesis, and produce few, if any, BV (119). BmNPV bacmids deleted for Bm15 were viable, but the infection spread slower than wt in cultured cells (15).

Ac25 (316aa:36.6kDa), (Bm16:317aa:36.7kDa),(Ha25:324aa:37.6kDa), DNA binding protein (DBP).

Ac25 encodes a single-stranded DNA binding protein called DBP. Homologs are found in all sequenced baculovirus genomes, except that of the dipteran (CuniNPV) (although an orf is present with ~50% identity over 22 aa) and in some instances, multiple copies of the *dbp* gene are present. It has properties similar to the other baculovirus SSB, LEF-3 (Ac67), in that it interacts with itself and is capable of both unwinding and annealing DNA (120). It is an essential gene, as bacmids lacking Ac25 are noninfectious and appear to produce defective nucleocapsids. Although not a virion structural protein, DBP exhibits a tight association with subnuclear structures, suggesting that it is a component of the virogenic stroma (120), and when DBP was deleted from an AcMNPV bacmid, cells transfected with this construct appeared to lack a virogenic stroma. This suggested that *dbp* is required for the production of nucleocapsids and the virogenic stroma (121). In BmNPV, it was also essential as deletion mutants failed to produce virions(122).

Ac26 (129aa:14.6kDa), (Bm17:129aa:14.5kDa), Ha26:133aa:15kDa).

Homologs of Ac26 are found in the genomes all Group I, and most Group II NPV genomes. Bm17 is expressed as a late gene and is localized to both the nucleus and cytoplasm (123, 124). When Bm17 was deleted from a BmNPV bacmid, the virus was viable, but the infection spread slowly in cell culture (15).

Ac27 (286aa:33.3kDa), (Bm18:292aa:34.0kDa), iap-1.

Ac27 is a member of the inhibitor of apoptosis (*iap*) gene family. Up to 6 *iap* homologs are found in baculovirus genomes (125) (126), however AcMNPV has two copies, *iap-1* and *-2*. Unlike *iap-2*, which is found in both Group I and II NPVs, *iap-1* appears to be confined to Group I lepidopteran NPVs and is a member of a lineage distinct from the other iap homologs. Deletion mutants of *iap-1* were similar to wt in their replication in cells lines and larvae of *T. ni* and *S. frugiperda*. However, when they were co-infected with wt virus in Tn-368 (but not Sf-21) cells, the mutants appeared to out compete wt virus (127). Evidence suggested that transfection of AcMNPV iap-1 into T. ni cells suppressed apoptosis by HearNPV infections, although a recombinant HearNPV expressing iap-1 also suppressed apoptosis, BV production was not rescued (128). In another investigation in EppoMNPV, The iap-1 gene was able to delay apoptosis onset caused by inducing agents such as actinomycin but was not able to prevent apoptosis upon prolonged exposure of the cells to the inducer (129). In another study, transient expression of iap-1 from a variety of viruses induced apoptosis and it was suggested that this might facilitate dissemination of the virus (130). For a review see (126).

Ac28 (173aa:20.4kDa), (Bm19:173aa:20.3kDa), (Ha24:187aa:22.2kDa), Lef-6.

Homologs of lef-6 are found in the genomes of all lepidopteran NPVs and GVs. It was originally identified because it was required for transient transcription of late genes (131). A bacmid deleted for lef-6 was infectious, but the virus was severely compromised. The major effect appeared to be reflected in a delay in the onset of late transcription (132). Using the HHpred program (77), AcMNPV LEF-6 showed over 80% probability of being related to the retroviral mRNA export factor TAP (133). BmNPV LEF-6 is highly phosphorylated during infection (38).

Ac29 (71aa:8.6kDa), (Bm20:71aa:8.6kDa), (Ha23:67aa:8.3kDa).

Homologs of Ac29 are found in all Group I and most Group II and GV genomes. A BmNPV bacmid deleted for Bm20 was viable (15). Using the HHpred program (77), Ac29 showed over 80% probability of being related to the amphiphysin BAR domain from Drosophila. These are sensors of membrane curvature.

Ac30 (463aa:54.7kDa), (Bm21:472aa:55.8kDa).

Homologs of Ac30 are present in the genomes of all Group 1 NPVs. It has homology to a family of genes that encode tryptophan repeat gene family proteins (see also Ac12) that are also found in entomopox viruses. e.g., *Melanoplus sanguinipes* entomopoxvirus (E = 1e-11). These proteins contain 3 to 12 copies of a 23-amino acid sequence containing tryptophan, leucine and isoleucine residues (134). Ac30 is likely to be nonessential because when the ortholog (Bm21) was interrupted by insertional/deletion mutagenesis in BmNPV, the virus appeared to be normal, although it resulted in a longer survival time suggesting that the mutant was less virulent that wt (135).

Ac31 (151aa:16.2kDa), (Bm23:151aa:16.3kDa), SOD.

Ac31 has homology to super oxide dismutase (136). Homologs are found in the genomes of almost all lepidopteran baculovirus genomes (it appears to be absent only in EppoNPV, a member of Group II, and SpliGV). It has a high degree of similarity to SOD from a variety of insects, including *B. mori* (E = 8E-49). Insect hemocytes are phagocytic cells similar to neutrophils and can destroy invading pathogens by the production of superoxide (137). Superoxide can be inactivated by SOD by converting it to hydrogen peroxide, which is also toxic, but can itself be inactivated with catalase yielding water and O_2. Many baculoviruses may infect hemocytes and in this manner can spread an infection throughout an insect. The expression of viral SOD might mitigate the effects of superoxide production by hemocytes. An enzymatic activity could not be confirmed for

AcMNPV SOD and AcMNPV deleted for *sod* replicated normally in cultured cells and insect larvae. The *sod*-deleted viruses showed no reduction in replication when grown in the presence of paraquat, a superoxide anion inducer (136). In one study, deletion of the *sod* gene from BmNPV (Bm23) indicated that it was essential for replication in BmN cells (138), however, another report indicated that a Bm23-deleted bacmid was viable (15).

Ac32 (181aa:20.6kDa), (Bm24:182aa:20.8kDa), (Ha113:301aa: 34.4kDa) fgf.

Ac32 has homology to fibroblast growth factor (FGF). Orthologs are found in the genomes of all lepidopteran baculoviruses and may reflect several independent lineages. AcMNPV *fgf* is most closely related to a non-baculovirus gene in *D. melanogaster* called *branchless* (E = 3e-24). In contrast, a Group II *fgf* homolog from LdMNPV is less closely related to the insect homologs (e.g. *D. melanogaster* branchless E = 2e-10) and the GV homologs show only limited similarity to NPV *fgf* homologs (e.g., AcMNPV vs CpGV FGFs = ~10% identity). It has been suggested all of the NPV *fgf*s are monophyletic, however the possibility of at least two capture events of fgf in the GVs has not been ruled out (139). AcMNPV FGF is a secreted protein that stimulates insect cell motility (140). In BmNPV, the FGF homolog is glycosylated, which is essential for its (141, 142) and binds to an insect receptor of FGF/*branchless* called *breathless* (141). Although the deletion of *fgf* in AcMNPV showed no differences from wt on cultured cells (143), the time of death was delayed when fed to two insect species (144). Similar results were observed for a BmNPV *fgf* (145) and HearNPV (146) *fgf* deletion mutants. vFGF was more highly expressed in midgut than cultured cells suggesting it may be important in establishing the infection (39). It has been suggested that FGF may play a role in dissemination of the virus within the host insect (144). vFGF appears to initiate a cascade of events that may accelerate the establishment of systemic infections. This involves two processes. vFGF from virus infected midgut cells diffuse through the basal lamina and attract tracheal cells so that they are adjacent to infected midgut cells but separated by the basal lamina. vFGF then activates FGF receptors located on the tips of tracheal cells. This leads to the activation of matrix metalloproteases located in the same subcellular region via a MAP kinase or NFkB pathway. Matrix metalloproteases subsequently activate effector caspases that move extracellularly so that they are positioned for the degradation of the basal lamina by digestion of the laminin component. The delaminated tracheal cells are then susceptible to virus infection. This allows the transit of the virus through tracheal cells to other tissues and results in the systemic infection (147). This theory is supported by evidence for the activation of matrix metalloproteinases, the activation of effector caspases, and the degradation of laminin after the per os infection of midgut cells.

Ac33 (182aa:20.8kDa) (polynucleotide kinase (PNK)).

Orthologs of Ac33 are found in most Group II and a few Group I and GV genomes. Homology searches indicate that it has significant similarity to polynucleotide kinase-3'-phosphatase of *Apis mellifera* (E = 9e-36) and somewhat lesser to the human homolog (E = 1e-28). In other organisms this enzyme has functions similar to T4 PNK. It is predicted to have structural similarity to Chain B of a DNA repair enzyme, polynucleotide kinase with an E value of $1.0e\text{-}^{32}$ (148). As previously suggested, Ac33 also has structural similarity to histidinol-phosphatase (an enzyme in the histidine biosynthesis pathway) (149). Orthologs are not present in BmNPV (16) or HaSNPV (17).

Ac34 (215aa:24.9kDa), (Bm25:215aa:24.8kDa), (Ha27:255aa:29.5kDa).

Orthologs of Ac34 are found in Group I and II NPV genomes. Ac34 localizes to both the nuclei and cytoplasm of infected cells but does not appear to be a structural protein. Deletion of Ac34 from an AcMNPV bacmid resulted a delay in late gene expression, a100-fold reduction the viral titer, but did not appear to affect DNA replication. This mutant was unable to establish a fatal infection in the larvae of *T. ni* via *per os* exposure (150). In another study, deletion of Ac34 resulted in no BV production, but reduction of Ac34 expression by RNAi, elevated the expression of a heterologous gene expressed from the polyhedrin promoter (151). Deletion of Bm25 also resulted

in a compromised virus (15). Subsequently, Ac34 was implicated in Arp2/3 (actin nucleator actin-related protein complex) nuclear accumulation and Ac34 inhibits the CRM1 (chromosome maintenance 1) pathway that is involved in the nuclear export of proteins and RNA, resulting in nuclear retention of the Arp2/3 complex. It was also shown that Ac34 binds to P40, P34, and P20 of the Arp2/3 complex in Sf9 cells (152) and that it contains a zinc finger domain that appears to be essential for nuclear localization (153). Ac34 has also been reported to increase the transcription of late genes, inhibit the proliferation of sf9 cells, caused the abundant expression of Sf953, and activated the JNK pathway which triggers apoptosis

(154).

Ac35 (77aa:8.7kDa), (Bm26:77aa:8.7kDa), (Ha28:83aa:9.2kDa), ubiquitin-like protein.

Although it was suggested baculoviruses were the only viral lineage encoding a ubiquitin-like protein (155), they were recently identified in avipoxvirus genomes. Orthologs of ubiquitin have been found in the genomes of most alpha and beta baculoviruses, but are not present in hymenopteran or dipteran baculoviruses (156) (155). Ac35 is expressed from both early and late promoter elements (157, 158). It was observed that late in infection recombinant virus expressing GFP fusions of ubiquitin from BmNPV localized throughout the nuclei with a few concentrated foci in the cytoplasm, whereas that of HaSNPV localized mostly to the peripheral regions of nuclei (159). The phylogenetic tree indicates that, whereas the ubiquitin of most eukaryotes is almost invariant, the baculovirus tree shows a higher degree of phylogenetic diversity, particularly between GVs and NPVs, suggesting that it may have been independently incorporated into a viral genome more than once. In addition, there appear to be two different lineages of alphabaculovirus ubiquitin (155). Viral ubiquitin is BV associated (27) and appears to be present on the inner surface of viral envelopes (160). A viral mutant with a frameshift of Ac35 is viable, but a 5–10-fold reduction in BV was observed (161). It has been suggested that viral ubiquitin may inhibit steps in the host degradative pathway to stabilize what would otherwise be a short-lived viral protein (162). Ac35 interacts with Ac141(exon0) and deletion of ac141 and *vubi* limited viral infection to single cells. Nucleocapsids of BV, but not ODV are ubiquitinated by vUbi. Ac66, a BV specific nucleocapsid protein is ubiquitinated by vUbi and interacts with Ac141 and vUbi at the margins of nuclei suggesting that vUbi may be involved as a signal regulating whether nucleocapsids exit nuclei and form BV, or remain and form ODV (163). The *Spodoptera litura* NPV genome was found to contain a gene that is a fusion of *ubiquitin* and *gp37* (=Ac64). In addition, it was noted that unfused homologs of both these proteins are found in entomopox viruses (164). Although the significance of the linkage of these two proteins is not known, other such proteins have been termed 'rosetta stone' proteins because they reveal proteins that interact with one another and participate in the same molecular pathways. Consequently, when a mutation event occurs that leads to the fusion of two proteins that normally function together, the mutation is preserved because such a linkage is a normal feature of the two proteins. The presence of homologs of both these proteins in two disparate families of viruses along with the presence of a fused orf in the SlNPV genome, suggests that these orfs may participate in the same pathway, possibly as participants in a ubiquitin pathway or in ubiquitin inhibition.

Ac36 (275aa:31.3kDa), (Bm27:277aa:31.5kDa), (Ha31:311aa:35.2kDa), 39K;pp31.

Pp31 was originally identified because it contains an early promoter that is stimulated by IE-1 (165). Homologs are found in all lepidopteran NPV and GV genomes. It is phosphorylated and localized to the virogenic stroma of infected cells, and is capable of binding to DNA but is not a virion structural protein (166), although it was reported to be BV associated in a proteomic study (27). Purified PP31 was found to bind to single-stranded and double-stranded DNA with equal affinities and inhibited transcription in vitro (167). Phosphorylation of pp31 appeared to be a dynamic process (168). Several basic regions were identified that may be involved in nuclear localization or DNA binding (169). Pp31 stimulates late gene expression in a transient transcription assay (170).

Deletion of the *pp31* homolog in BmNPV (Bm27) resulted in virus that, although viable, showed a reduction in late gene transcription, a 100 fold reduction in BV production, and improper formation of the virogenic stroma (81). Similar results were obtained for an AcMNPV bacmid deleted for *pp31* and it was observed that the deletion resulted in a significant decrease of the transcription of six late genes (171).

Ac 37 (112aa:13.1kDa), (Bm28:112aa:13.1kDa), (Ha32:127aa:14.6 kDa), Lef-11.

Lef-11 is present in all baculovirus genomes, except the dipteran CuniNPV. It was identified as being stimulatory for late gene expression in a transient transcription assay (51). An AcMNPV bacmid deleted for *lef-11* failed to replicate and no DNA synthesis or late gene transcription were evident, indicating that it is an essential gene. In BmNPV, oligomerization of LEF-11 is required for DNA replication (172). Although LEF-11 localizes to nuclei of infected cells, its role in DNA replication is not known (132). BmNPV LEF-11 interacts with and upregulates both a host ATPase and HSPD1 (HSP60) proteins and this appears to facilitate viral DNA replication (173). BmNPV LEF-11 has a novel nuclear localization signal (174, 175)and appears to interact with host importin α-3 (involved in the import of proteins into nuclei) and within nuclei it co-localizes with IE-1 and interacts with LEF-3(174).

Ac38 (216aa:25.3kDa), (Bm29:217aa:25.5kDa), (Ha33:238aa:28.4kDa), Nudix, ADP-ribose pyrophosphatase (ADPRase).

Homologs of Ac38 are found in all lepidopteran NPVs and GVs. It contains a conserved Nudix (nucleotide diphosphate X) motif ($GX_5EX_7REUXEEXGU$; X= any aa, U represents l, L, or V) (176) and has a homology to ADPRase, a subfamily of Nudix pyrophosphatases. Ac38 was shown to have ADPRase activity and a deletion mutant was severely compromised and produced BV at 1% the level of wt (177). Purified Bm29 also showed ADPRase activity (178). Proteins of the nudix superfamily are common in all organisms and have been reported in other viruses including T4 bacteriophage, African swine fever virus (ASFV), and poxviruses. A vaccinia virus nudix protein may negatively regulate viral gene expression by acting as a decapping enzyme (179). Deletion of the gene in vaccinia resulted in smaller plaque and low virus yield (180), similar to the *Ac38*-deleted AcMNPV. Ha33 of the *Helicoverpa armigera* NPV is a homolog Ac38 and was found to be associated with the envelope of budded virions (181).

Ac39 (363aa:43.5kDa), (Bm30:362aa:43.4kDa).

Homologs of Ac39 are found in a few Group I NPV closely related to AcMNPV and at least two Group II (AdhoNPV and ClbiNPV) genomes. Deletion showed no effects on growth curves or virus production (182).

*Ac40 (401aa:47.5kDa), (Bm31:399aa:47.3kDa), (Ha35:3590aa:39kDa), P47, a subunit of the baculovirus polymerase.

P47 homologs are found in all baculovirus genomes. P47 was originally identified as the site of a ts mutation that caused a defect in late gene expression (183, 184). P47 was found to be required for transient late gene transcription (170) and to be a component of the baculovirus late polymerase complex (185). It is likely an essential gene as a deletion/insertion mutant in the BmNPV *lef-8* homolog (Bm31) could not be isolated (15, 81).

Ac41 (181aa:21.1kDa), (Bm32:183aa:21.1kDa), (Ha36:223aa:25.8kDa), Lef-12.

Lef-12 is found in about one-half the Group I and Group II NPV genomes sequenced. Although 18 genes were originally identified as being involved in transient expression from a late promoter (170), when individually cloned, the genes failed to support late transcription. Because of its close proximity to Ac 40 (p47), Ac41 (lef-12)

had not been identified in the initial screen. It was subsequently demonstrated to be required for transient late gene transcription in *S. frugiperda* cells (186, 187), but not in *T. ni* cells (186). Mutants with *lef-12* interrupted by insertional mutagenesis or by mutation of the ATG translation initiation codon were viable in both *S. frugiperda* and *T. ni* cells, although reduced yields of BV were observed (20-40% or wt) in both cell lines and the infection cycle appeared to be slowed (188). Although expressed as an (aphidicolin sensitive) late gene, initiation of *lef-12* mRNA did not appear to occur at conventional late (or early) promoter elements. It was suggested that *lef-12* may be functionally redundant in the AcMNPV genome and, therefore, it is not essential for late transcription when the rest of the virus genome is present (188). Analysis by Hhpred (77, 189)predicts that Lef-12 may be structurally related to TFIIA with a probability of about 70%. TFIIA is involved in transcriptional initiation of RNA polymerase II (190).

Ac42 (506aa:59.1kDa), (Bm33:506aa:59.2kDa), gta.

Ac42 has homology to 'global transactivator,' the DEAD-like helicase superfamily that are enzymes involved in ATP-dependent unwinding of DNA or RNA. They contain an SNF2 family N-terminal domain that is present in proteins involved in some processes, such as regulation of transcription, DNA recombination and repair, chromatin unwinding, and other functions. Homologs of this gene are found in all Group I NPV genomes. It has significant similarity to a wide variety of orfs from bacteria to marsupials, e.g., (E = 9e-61). Deletion of the Ac43 homolog from BmNPV (Bm33) did not cause any defects in BV or ODV production in BmN cells. Assays in *B. mori* larvae showed that the mutant, although similar in infectivity to the wt, took about 15 hr longer to kill when administered either by injection or per os (191).

Ac43 (77aa:8.8kDa), (Bm34:78aa:9.0kDa), (Ha37:80aa:9.5).

Homologs of Ac43 are found in all Group I and most Group II NPV genomes. It appears to be involved in late and very late gene expression as deletion of BmNPV Bm34 resulted in a reduction in occlusion body production and a lengthening of the time to death in larvae. These effects were attributed to a down regulation of *vlf-1* which is required for very late gene expression along with the reduction expression of the *fp25k* (Ac61) gene (192). A knockout of Ac43 did not appear to affect BV production, but resulted in reduced expression of polyhedrin, but those present were larger than normal. They appeared contain more singly enveloped nucleocapsids than wt (193). Analysis by Hhpred (189) predicts that Ac43 may be structurally related to several proteins including the N-terminal domain of TFIIB from *Pyrococcus furiosus* with a probability of greater than 70%.

Ac44 (131aa:15kDa), (Bm35:131aa:15.0kDa), iap related?

Homologs of Ac44 are found in most Group I and at least one Group II (SeMNPV) lepidopteran NPVs. Analysis by Hhpred (189) predicts that Ac44 is related to iap family members with a probability greater than 97% and a Blast search indicates homology to E3 ubiquitin-protein ligase family members. Ac44 may be nonessential as a BmNPV bacmid with a Bm35 knock out appeared to be normal (15).

Ac45 (192aa:22.7kDa), (Bm36:193aa:22.5kDa).

Homologs of Ac45 are found in four close relatives of AcMNPV (PlxyNPV, BmNPV, RoMNPV, and MaviMNPV). Sequences located within Ac45 appeared to be required for Ac41 expression in a transient late transcription assay (187). Analysis by Hhpred (189)predicts that Ac45 is structurally similar to a S-phase kinase associated/F-box protein with a probability greater that 70%. F-box domains mediate protein-protein interactions. Ac45 may be nonessential as a BmNPV bacmid with a Bm36 knock out appeared to be normal (15).

Ac46 (704aa:79.1kDa). (Bm37:702aa:79.2kDa), (Ha96:672aa:76kDa), ODV-E66.

Ac46 (ODV-E66) is the only known viral chondroitinase (194) and is a component of ODV envelopes (195). Homologs of this gene are found in the genomes of most alpha- and betabaculoviruses, but not in hymenopteran or dipteran viruses. Two copies of the gene are present in some genomes (e.g., SeMNPV). When the N-terminal 23 amino acids of ODV-E66 are fused to a reporter gene, it is targeted to the nucleus (196). Chondroitinases have been shown to regulate cytokine and growth factors and can influence a variety of processes including development, inflammation, and organ morphogenesis. The crystal structure has been determined and it is most similar to bacterial chondroitin lyases suggesting it was obtained from a bacterium via horizontal gene transfer (197). ODV-E66 may be involved in the digestion of the peritrophic matrix as it contains low levels of chondroitin sulfate (194) (198). When the homolog of ODV-E66 was inactivated in BmNPV (Bm37), the mutant, although viable, took more time to kill insect larvae (199). In a study of AcMNPV, an ODV-E66 deletion was observed to kill *Plutella xylostella* larvae as efficiently as wt, however when infected per os, the LD50 was1000 fold greater for the mutant than wt virus. Therefore it was suggested that ODV-E66 is a per os infectivity factor (PIF) (200). It has also been shown that a truncated form lacking the N-terminal 66 amino acids was secreted into the medium by infected cells and had chondroitinase activity. A peptide of about 12 aa that was similar to the AcMNPV ODV-E66 sequence was observed to bind to the epithelium of guts *Heliothis virescens* and inhibited infection by AcMNPV (201). For more information on PIFs see Chapters 2 and 3.

Ac47 (88aa:10.5kDa), (Bm38:89aa:10.5kDa), TRAX-like.

Ac 47 homologs are found in several Group I lepidopteran NPVs closely related to AcMNPV. It shows 27% identity to homologs of a protein called translin-associated factor X (Trax) that binds to DNA breakpoints. Although TRAX interacts with translin, which may be involved in responses to DNA damage, transport of RNA, and control of translation, its function is not known (202). Ac47 may be nonessential as a BmNPV bacmid with a Bm38 knock out appeared to be normal (15). In AcMNPV his gene was referred to as ETS and transcriptional data for Ac47 in relation to PCNA has been described (203).

Ac48 (113aa:12.9kDa).

Ac48 homologs are found in the genomes of most Group I lepidopteran NPVs. A homolog of Ac48 is not present in the BmNPV genome (16). This gene was referred to as ETM (the mid-sized orf in the EcoRI T fragment) and transcriptional data for Ac48 in relation to PCNA has been described (203).

Ac49 (285aa:32.1kDa) (PCNA).

Ac 49 has homology to proliferating cell nuclear antigen (PCNA). PCNA homologs have been found in a few Group I and Group II alphabaculovirus genomes. The Group I PCNA homologs appear to be insect-derived and show a high degree of similarity to insect PCNAs, e.g., *S. frugiperda* (E = 6e-65). In contrast, the PCNA homologs of two Group II viruses (TnSNPV and ChchNPV) belong to a different lineage and do not show such a close relationship to insect PCNAs, e.g., *S. frugiperda* (E = 7e-20) and are even more distantly related to the Group I baculovirus PCNAs than to those of insects. Although eukaryotic PCNA lacks an enzymatic function, it plays a role in DNA synthesis, DNA repair, and cell cycle progression. It functions as a sliding circular clamp that mediates protein interactions with DNA and is required for the coordinated synthesis of both leading and lagging strands at the replication fork during DNA replication (204). In AcMNPV it is not an essential gene (203, 205, 206) and did not appear to elevate DNA replication in transient replication assays (50). A homolog of PCNA is not present in the genome of BmNPV (16). It is not clear what role the viral PCNA plays in the infection, however over expression under the control of the p10 promotor of EGFP fusions of AcMNPV-PCNA and host cell Sf-PCNA has been examined in Sf9 cells. Both stimulated BV production and DNA replication of

host and virus and Ac-PCNA elevated transcription of selected late genes and increased the larval mortality rate (207).

*Ac50 (876aa:101.8kDa), (Bm39:877aa:101.8kDa), (Ha38:901aa:105 kDa), Lef-8, baculovirus RNA polymerase subunit.

Lef-8 was originally identified as a gene required for transient late gene expression (208). Homologs are found in all baculoviruses and are also in nudiviruses. LEF-8 contains a conserved motif found in other RNA polymerases and it is thought that this is part of the catalytic site (208, 209). It is a component of the baculovirus late RNA polymerase complex (185, 210). It is likely an essential gene; in BmNPV a ts mutant located in lef-8 (A542V) was defective for BV production at the non-permissive temperature (211). In addition, a deletion mutant in the BmNPV lef-8 homolog (Bm39) was not viable (15). Also, a ts mutation L531S in Ac50 abolished very late transcription at 33 ° C (212). Inactivation of BmNPV *lef-8* prevented virus replication (213).

Ac51 (318aa:37.5kDa), (Bm40:319aa:37.8kDa), (Ha39 :194aa : 22.5kDa), DnaJ domain protein.

Homologs of Ac51 are found in all Group I and II lepidopteran NPV genomes. It is a DNA J domain protein and shows homology to a variety of bacterial proteins. In *E. coli*, DnaJ has been demonstrated to have chaperone activity and aids in folding of other proteins (214). The homolog of Ac51 in HearNPV (ha39) has an RNA recognition motif, localizes to the cytoplasm and is associated with BV (27, 215). A BmNPV bacmid with Bm40 deleted was defective for viral spread (15). Another investigation demonstrated that bm40 is a late gene that localized in nuclei and become concentrated near the nuclear membrane; deletion inactivated the virus, although there was no effect on DNA replication. EM analysis suggested the Bm40 is required for nucleocapsid egress from the nucleus, envelopment to form ODV, and embedding into OB (216). Investigations on Ac51 indicated that it is a late gene and was present in the cytoplasm and nuclei of infected cells. Upon deletion of ac51, BV production was reduced 1000 fold. However, DNA replication, virus gene expression, nucleocapsid assembly, and ODV formation were not affected. The defect in BV production appeared to be caused by a decrease in the egress of nucleocapsids from nuclei (217).

Ac52 (194aa:123.2kDa), (Bm41:194aa:23.3kDa), (Ha42:180aa: 21.3kDa).

Homologs of Ac52 are found in about one-half the Group I and II alphabaculovirus genomes. Deletion of Bm41 resulted in reduction in BV production by 1000-fold and appeared to disrupt normal nucleocapsid envelopment and polyhedron formation in infected nuclei and resulted in a 14-fold elevation of LD_{50} in larvae and an increase in time to death (218). The predicted size of Ac52 is longer (194 vs 123 aa) than previously reported (12).

*Ac53 (139aa:17kDa), (Bm42:139aa:16.9kDa), (Ha43:136aa:16.4kDa).

Homologs of Ac53 are found in all baculoviruses. Deletion of Ac53 indicated that it was an essential gene. The mutant bacmid was able to replicate DNA, but the virions were defective and appear to lack the nucleoprotein core (219). Ac53 is predicted to contain domains structurally similar to the U-box/RING-like domains found in the E3 ubiquitin ligase family (156).

Ac53a (78aa:8.6kDa), (Bm42a:78aa:8.6kDa), (Ha46:71aa:7.7kDa), Lef-10.

This orf was named Ac53a because it was not identified in the original AcMNPV genome sequence because it is a small orf encoding 78 aa and about half the 3' coding region overlaps the 5' region of Ac54. Homologs of lef-10

are found in the genomes of all Group I and most Group II NPV and GV genomes. Lef-10 was originally identified because it was required for late gene expression (13). When Bm42a was deleted from a BmNPV bacmid, the bacmid was not viable (15). It was shown that AcMNPV Lef-10 was required for viral replication and interacted with itself. A truncated form (amino acids 1-48) also supported replication and could self-aggregate. When linked to EGFP, Lef-10 formed punctate spots (220). It has been reported that LEF10 behaves as a prion (221). The full-length protein or its predicted prion-forming domain can functionally replace the prion domain of Sup35 of yeast. A high multiplicity of infection can lead to conversion of LEF10 to an aggregated state that inhibits late gene expression (220, 221).

*Ac54 (vp1054) (365aa:42.1kDa), (Bm43:365aa:42.0kDa), (Ha47:351aa:41.7kDa), Capsid protein

Homologs of Ac54 are found in all baculovirus genomes. It encodes a protein required for nucleocapsid assembly. A ts mutant failed to produce nucleocapsids at the non-permissive temperature, indicating that it is an essential gene and it was associated with both BV and ODV (222). It interacts with 38K (Ac98) (223). When the vp1054 gene was deleted from a bacmid, the bacmid did not appear to be viable and nucleocapsids appeared to be replaced with tube-like structures. It was suggested that vp1054 may be related to a cellular protein called PURa that binds to purine-rich sequences and may be involved in DNA packaging. Ac54 was shown to bind to single stranded DNA or RNA sequences that contained runs of GGN (224). Inactivation of Ac54 disrupted nucleocapsid assembly and resulted in aberrant capsid structures that were located with vp39 in the nuclear periphery. Using immunoelectron microscopy it was observed that BV/ODV-C42, PP78/83, and 38K did not associate with capsid structures when VP1054 was absent. It was found the VP1054 associates with BV/ODV-C42 and VP80 but not VP39 suggesting that VP1054 is critical for nucleocapsid assembly (225).

Ac55 (73aa:8.2kDa), (Bm44:77aa:8.6kDa), (Ha48:68aa:8.0kDa).

Homologs of Ac55 are found in the genomes of all Group I and most of the Group II NPVs. It appears to be nonessential because a BmNPV mutant deleted for this gene appeared to be normal (15).

Ac56 (84aa:9.9kDa), (Bm45:84aa:9.9kDa), (Ha49:64aa:7.4kDa).

Homologs of this orf are found in genomes of most Alphabaculoviruses. It appears to be nonessential because a BmNPV mutant deleted for this gene appeared to be normal (15).

Ac57 (161aa:19kDa), (Bm46:161aa:20.2kDa), (Ha50:171aa:20.7kDa).

Homologs of this orf are found in most Group I and II NPVs. It appears to be nonessential because a BmNPV mutant deleted for this gene appeared to be normal (15). It also appeared to be non-essential for HearSNPV (226).

Ac58, Ac58/59 (172aa:20.3kDa), (Bm47:171aa:37.8kDa), (Ha51:160aa:19.0kDa), ChaB-like.

Ac58, Ac58/59 is a single gene, as they were found to be joined when the region was re-sequenced in the C-6 strain (12). This results in an orf predicted to encode 172 amino acids. Homologs of this orf are found in the genomes of most alphabaculoviruses. It has a ChaB domain. In *E. coli*, ChaB is thought to regulate ChaA, a cation transporter protein. It was found to localize to nuclei of infected cells (227) and was associated with AcMNPV ODV (89). It is also BV associated (27) and is hyperphosphorylated is cell extracts at 24 hpi (38). It appears to be nonessential because a BmNPV mutant deleted for this gene appeared to be normal (15).

Ac60 (87aa:10.1kDa), Bm48:83aa:9.7kDa), (Ha52:88aa:10.2kDa), (ChaB-like).

Homologs of Ac60 are found in the genomes of all Group I and II NPVs and most GVs. Similar to Ac58/59, Ac60 also has a ChaB domain. It is surprising that Ac58/59 and Ac60 are both predicted to encode ChaB domains as they do not show much sequence similarity. Alignment of the sequences resulted in an amino acid sequence identity of 15%; however, it required the insertion of several gaps, so the significance of the relatedness is not clear. In SpliNPV, two adjacent ChaB homologs were also identified. Evidence suggests that they may be DNA binding proteins (228). It appears to be nonessential because a BmNPV mutant deleted for this gene appeared to be normal (15).

Ac61 (214aa:25.2kDa), (Bm49:214aa:25.3kDa), (Ha53:217aa:25.4kDa), Few polyhedra (fp, fp-25k).

Although orthologs of Ac61 have been reported in the genomes of all alpha-, beta-, and gammabaculoviruses (156), I was unable to confirm their presence in gammabaculovirus genomes. Whereas both Ac61 and XcGV orf140 were predicted by Hhpred to be similar in structure to Line-1 orf1p (see below), the gammabaculovirus fp25K (Neab54) from (156) was not predicted to be similar. Also, I could find no relationships via PSI Blast. AC61 has been shown to be BV associated (27). Deletion is not lethal, but results in a 'few polyhedra phenotype' (fp) (229, 230). Fp-25k was more highly expressed in midgut than cultured cells suggesting it may be important in establishing the infection (39). FP mutants are defective in virion occlusion and nucleocapsid envelopment in nuclei and release two- to fivefold more infectious BV than wt in infected Sf9 cells (230, 231). The nonlethal, but readily distinguishable phenotype has facilitated investigations on this gene. FP mutations often result from the insertion of host DNA into the fp gene (229, 232). They can also be the result of errors in DNA replication (233). Mutations in the *fp* gene result in a reduction in polyhedrin gene (but not *p10*) transcription (230). Mutations also appear to affect the levels and nuclear transport of Ac46 (ODV-E66), an ODV envelope protein (234, 235). In BmNPV *fp* mutants, the few virions that were occluded appear to lack envelopes (236). A combination of reduction in the level of polyhedrin and an ODV envelope protein could contribute to the FP phenotype. The defect in occlusion and in the ODV envelope could lead to the availability of more virions for budding. A reduced level of liquefaction of larvae was also noted with an fp mutant in BmNPV (236). This was attributed to the involvement of Bm49 in the regulation of v-cathepsin expression (237). In *T. ni* cells, double *p35* (see Ac135) and *fp* mutants underwent apoptosis, whereas p35 mutants alone did not, suggesting that the *fp* gene may have pro-apoptotic properties in this cell line (238). In contrast, in BmNPV infected BmN cells, deletion of fp25k did not affect apoptosis induced by a virus with p35 deleted (239). Fp25k mutants of AcMNPV produce virions that are occluded with different efficiencies depending on the cell line or insect. When replicated in Sf cells or insects the efficiency was lower than in *T. ni* Hi F cells or larvae (240). The fp25k gene was also found to contain two hypermutable sequences of 7 adenine residues and the mutant 25k genes expressed different amounts of polyhedrin mRNA and protein depending on the cell line (241). It was suggested that fp25k may destabilize the genome thereby elevating the level of mutagenesis (242). Evidence indicates that fp25k is related to the orf1p of the Line-1 group of retrotransposons with a probability of 99.8% according to Hhpred (189). Orf1p acts as a nucleic acid chaperone and similar to orf1p, fp25k has a coiled-coil domain and a predicted RNA binding motif (243). Although the significance the relatedness is compelling, how this gene adapted to baculovirus biology is unclear.

*Ac62 (516aa:59.3kDa), (Bm50:490aa:56.4kDa), (Ha55:519aa: 60.0kDa), Lef-9, baculovirus RNA polymerase subunit.

Lef-9 homologs are present in all baculovirus and also nudivirus genomes. It was found to be required for transient late gene expression (13) and subsequently shown to be a subunit of the baculovirus RNA polymerase

(185). It contains a 7-amino acid motif (NTDCDGD or NRDCDGD except NADFDGD in the dipteran virus) similar to the Mg++ binding sequence (NADFDGD) found in the catalytic center in large RNA polymerase subunits of a few DNA-dependent RNA polymerases (13). The D residues bind Mg++ and are conserved in all these sequences. It is likely an essential gene as an insertion/deletion mutant in the BmNPV homolog (Bm50) could not be isolated (81) or did not replicate (15). Another Bm50 knockout was found to have limited effect on viral genome replication, but transcription of several early and late genes was greatly diminished (244).

Ac63 (155aa:18.5kDa), (Bm51:155aa:18.5kDa), (Ha121:154aa: 18.5kDa).

Homologs of Ac63 are found in several Group I and Group II alphabaculoviruses. A homolog in a nudivirus has been reported (245). It appears to be associated with BV envelopes (246). It appears to be nonessential because a BmNPV mutant deleted for this gene appeared to be normal (15).

Ac64 (302aa:34.8kDa), (Bm52:294aa:33.8kDa), (Ha58:279aa:32.1kDa), (GP37/P34.8, spindlin, fusolin, spheroidin-like protein).

The terminology of Ac64 has a confusing history and, in addition to GP37, has been referred to as p34.8, spindlin, fusolin, or spheroidin-like protein because of homology with an entomopox virus gene (247, 248). Evidence suggests that it is not a spheroidin-like homolog (249). Orthologs of *gp37* have been found in the genomes of most alpha and beta baculoviruses (156). In AcMNPV it is expressed as a late gene (250). In addition to entomopox, it is related to orfs in a variety of eubacteria, e.g., *Vibrio alginolyticus* (E = 5e-27). It has been suggested that the granulovirus *gp37* lineage is more closely related to the entompox lineage than to *gp37s* from NPVs (251). A homolog of Ac64 is referred to as chitinase B in the marine bacterium *Pseudoalteromonas* sp. and was found to bind to, but not digest chitin (252). The GP37 homolog in SpltNPV has been reported to contain chitin binding domains and is capable of binding to chitin (253). The GP37 of CpGV also bound chitin and was able to enhance per os infections (254). Insect proteins, such as the coagulation protein hemolectin, also have chitin-binding domains (255). GP37 was reported to be polyhedron associated in AcMNPV and to be N-glycosylated (248). It was also found to be BV associated (27). In OpMNPV infected *L. dispar* cells, GP37 was found to be an N-glycosylated protein located in cytoplasmic occlusions late in infection (256). In entomopox viruses the GP37 ortholog forms crystallized spindle-like structures. These structures have been suggested to digest the peritrophic matrix (257). Structural analysis of these spindles indicated that they contained a globular domain that is related to lytic polysaccharide monooxygenases of chitinovorous bacteria. It is thought that upon ingestion by the host, the spindles are dissolved and the monooxygenase domain is exposed and can then digest the chitin-rich peritrophic matrix (258). It has been reported that GP37 can degrade the peritrophic matrix and also facilitates the binding of ODV to midgut cells (259). The *gp37* gene is nonessential for replication in cell culture or *T. ni* larvae (247). Similar results were observed for the BmNPV homolog (Bm52) (15). The *Spodoptera litura* NPV genome was found to contain a gene that is a fusion of ubiquitin and *gp37* (for discussion, see *Ac35, ubiquitin*) and the protein was associated with the envelopes of BV and ODV (164) .

*Ac65 (984aa:114.3kDa), (Bm53:986aa:114.4kDa), (Ha67:1020aa: 119.3kDa) (DNA polymerase).

Homologs are found in all baculoviruses. The non-baculovirus homologs showing the highest level of similarity are found in herpesviruses, e.g., human herpes virus 7 (E = 1e-25), several protozoans, and archaea. A DNA polymerase homolog was originally identified in the AcMNPV genome by hybridization with degenerate primers designed based on a highly conserved domain in other DNA polymerases (260). A 3'→5' exonuclease activity specific for single-stranded DNA was shown to be associated with the DNA polymerase from *Bombyx mori* NPV (BmNPV) (261) and AcMNPV (262), suggesting that a proofreading activity was associated with this enzyme. A purified DNA polymerase from AcMNPV was characterized as being active on singly primed M13

templates (263). The polymerase is highly processive on poly (dA)-oligo dT (262). Mutations of AcMNPV DNA polymerase resistant to a variety of inhibitors have been described (264). The N-terminal 186 aa was determined to be important in the function of the protein (265), and the C-terminal ~200 aa was predicted to contain two sequences for nuclear localization and both appeared to be required (266). DNA polymerase is an essential gene because deletion is lethal in AcMNPV (267) and BmNPV (15, 81).

*Ac66 (808aa:94kDa), (Bm54:805aa:93.3kDa), (Ha66:785aa:88.9kDa).

Homologs of *Ac66* appear to be present in all baculoviruses. Many baculoviruses have two copies of Ac66, and some may have three copies (125). Ac66 is oriented in the opposite direction of DNA polymerase (Ac65) and its promoter region overlaps with the 5' region of the DNA pol orf. This orientation is conserved in many, if not all baculoviruses. Consequently, it is likely that Ac66 is conserved throughout the baculoviruses. Ac66 is transcribed as a late gene and its expression does not affect the expression of DNA pol (268). Ac66 is closely related to a variety of proteins including an actin binding protein, myosin heavy chain, and centromere protein E. Although the orf adjacent to DNA polymerase in *Neodiprion sertifer* NPV (NeseNPV) shows little homology to Ac66, it and Ac66 show homology to the same proteins, i.e., they both show almost 100% probability of being related to formins that are involved in actin nucleation, myosin, and components of a splicing complex using the HHpred program (77). Ac66 is associated with AcMNPV (89) and HearNPV (Ha66) (269) ODV and AcMNPV BV (27). Ac66 in BV but not ODV is ubiquitinated by the viral ubiquitin and appears to interact with Ac141 (predicted to be a an E3 ubiquitin ligase). It was suggested that this might be a signal for BV egress in contrast to ODV that remain in nuclei (163, 266). An AcMNPV bacmid deleted for Ac66 was severely compromised and BV titers derived from transfected cells were reduced by over 99% compared with wt. In addition, at low titers the mutant BV appeared to infect single cells and was unable to spread. Although the nucleocapsids appeared to be normal and had an electron dense core, suggesting that they contained DNA, they appeared to be trapped in the virogenic stroma, suggesting that Ac66 was required for the efficient egress of virions from nuclei. Deletion of Ac66 did not affect the levels of DNA replication or polyhedrin transcription, but the production of occlusion bodies was eliminated (270). This suggests that Ac66 is required both for egress of virions from nuclei and also may be involved in the enucleation of polyhedra. A bacmid deleted from Bm54 produced non-infectious BV and appeared to be properly assembled. Polyhedron formation also appeared to be affected (271). For additional discussion, see Chapter 5.

Ac67 (385aa:44.6kDa), (Bm55:385aa:44.9kDa), (Ha65:379aa:44.0kDa), LEF-3, (Single stranded DNA binding protein – SSB).

Lef-3 was originally found to be essential for DNA replication in transient assays (50, 51). Homologs of *lef-3* are found in the genomes of lepidopteran NPVs and GVs, but not those of hymenopteran or the dipteran NPVs. LEF-3 is a single-stranded DNA binding protein (SSB) (272) and interacts with itself as a homo-oligomer (273) (273). It also binds to helicase (274), facilitates its transport into the nucleus (275) and can drive nuclear transport into mammalian cells (276). It also may have a function in DNA replication in addition to its requirement as a helicase transport factor (277, 278). LEF-3 also interacts with alkaline nuclease and may regulate the function of this enzyme (279, 280); it is capable of both unwinding and annealing DNA depending on its concentration or redox state (281, 282); and it can facilitate the production of structures resembling recombination intermediates via strand exchange between donor and recipient molecules in vitro (283). The lack of conservation of LEF-3 in baculoviruses might not be unexpected because, although homologs of alkaline nuclease are present in many organisms (see *Ac133*) and many have been reported to interact with an SSB (e.g., herpesvirus, ICP8 and lambda phage, red-beta), clear sequence relationships between the various SSBs are not evident (284). Insertion/deletion mutants of *lef-3* are lethal (81, 278). In another report a bacmid deleted for LEF-3 showed some evidence for limited amounts of DNA replication and late gene expression, and some infectious virus was observed although over100,000 times less than wt (285).

*Ac68 (192aa:22.3kDa), (Bm56:134aa:15.8kDa), (Ha64:133aa:15.6kDa) PIF-6.

Homologs of Ac68 are present in all baculoviruses. A frame shift in this gene did not affect transient late gene expression (187) and a deletion of Ac68 resulted in no major effects on AcMNPV production and TCID50, and no differences in the number, size, and shape of polyhedra were noted, although the lethal time was longer in T. in larvae (286). In contrast, another report suggested that Ac68 was a per os infectivity factor (PIF-6) as a deletion mutant, although producing normal appearing polyhedra, they failed to kill *T. ni* larvae (285). In addition, it is reported to be associated with the AcMNPV PIF complex (47) (287). When the homolog in BmNPV (Bm56) was deleted in a bacmid, no effects on titers in cultured cells or in BV-injected larvae were detected, although the lethal time in larvae was longer. Although enveloped ODV were present, the polyhedra produced by the mutant bacmid were abnormal and lacked virions, suggesting that Bm56 is involved in polyhedron morphogenesis (288).

Ac69 (262aa:30.4kDa), (Bm57:262aa:30.4kDa), (Ha63:274aa:31.6kDa), (MTase).

Ac69 encodes a methyltransferase and orthologs are found in the genomes of most Group I NPVs and about one-half of Group II NPVs and one hymenopteran NPV. The homolog present in the hymenopteran NPV (NeseNPV) falls within an insect, rather than a baculovirus lineage. Homologs are found in a nudivirus (Hz-1) and a variety of insects, e.g., *Anopheles gambiae* (E = 8e-18) and other invertebrates and vertebrates. Ac69 was found to stimulate late gene transcription in a transient assay (187). The gene encodes a protein with RNA Cap (Nucleoside-2′-O)-Methyltransferase activity. AcMNPV, with a null mutation of the gene, replicated normally in cell culture (289). Similar results were observed for a knockout of the homolog (Bm57) in BmNPV (15).

Ac70 (290aa:34.4kDa), host cell-specific factor-1 (hcf-1).

Homologs are present in only three other baculoviruses; two are close relatives of AcMNPV and their HCF-1 orfs are 99% (PlxyNPV) and 84% (RoMNPV) identical to that of AcMNPV, whereas the homolog in ClbiNPV is more distantly related (21% identical). HCF-1 was found to be required for transient expression of a late promoter-reporter gene by a late expression factor library in *T. ni* cells, but not SF-21 cells (290, 291). AcMNPV with null mutations in hcf-1 were found to replicate normally in both Sf-21 cells and *S. frugiperda* larvae. However, in *T. ni* cells, replication was impaired and in *T. ni* larvae the mutant showed a significantly reduced infectivity by intrahemocelic injection. Although oral infectivity was relatively normal in *T. ni* larvae, the insects died more slowly than when infected with wt (292). It was suggested that HCF-1 is a RING finger-containing protein that is dependent upon self-association and gene repression for its activity (293).

Ac71 (249aa:28.6kDa), (Bm58:249aa:28.7kDa), (Ha62:250aa:29.3kDa), iap-2.

Ac71 encodes an inhibitor of apoptosis-2 (*iap-2*) gene. Up to 6 *iap* homologs are found in baculovirus genomes (125) (126). Homologs of *iap-2* are found in the genomes of most alphabaculoviruses, and as with all *iap* genes, more distant relatives are found in many organisms. It is BV associated in AcMNPV (27). Deletion of *iap-2* had no effect on viral replication in cell culture; however, this may have been due to the presence of another apoptotic suppressor, *p35* (294). In contrast, deletion of *iap-2* (bm58) from the BmNPV genome indicated that it was required for replication in BmN cells (138). Evidence suggested that transfection of AcMNPV iap-2 into *T. ni* cells suppressed apoptosis by HearNPV infections, and although a recombinant HearNPV expressing iap-2 also suppressed apoptosis, BV production was not rescued (128). In *Epiphyas postvittana* NPV, the *iap-2* homolog was found to have anti apoptotic activity when expressed from a CMV promoter in *S. frugiperda* cells (129). However, it was observed that *iap-2* of *Lymantria dispar* MNPV induced apoptosis when transfected into

Ld652Y cells. It was suggested apoptosis was suppressed by the virus because of the presence of the apoptotic repressor, *apsup* in LdMNPV (295).

Ac72 (60aa:7.1kDa), (Bm58a,60aa,7.1kDa).

Homologs of Ac72 are found in the genomes of most sequenced Group I NPVs, but not in other viruses. Bm58a localizes to the cell membrane at the late stage of infection. When Bm58a was deleted, the infected cells failed to lyse and larvae did not undergo liquefaction (296). However, in another report, deletion of this in BmNPV caused no observable differences from wt (15)

Ac73 (99aa:11.5kDa), (Bm59:99aa:11.5kDa), a BAG protein.

Homologs of Ac73 are found in the genomes of most sequenced Group I NPVs, but not in other viruses. It is BV associated in AcMNPV (27). Bm59 is an early gene and when deleted, the infection appears to progress normally (297). Analysis by Hhpred (189) indicates that it is a BAG (Bcl-2-associated athanogene) protein with greater than 99% probability. BAG proteins are regulators of molecular chaperones and share a BAG domain (BD) which binds to and regulates the Hsp70/Hsc70 family of proteins.

Ac74 (265aa:30.6kDa), (Bm60:268aa:31.0kDa), (Ha68:152aa:17.6kDa).

Homologs of Ac74 are found in the genomes of most Group I and about half Group II NPVs, but is not present in hymenopteran or dipteran NPVs or GVs. Bm60 was found to be expressed as a late gene and was localized to both the cytoplasm and nucleus of infected cells (298). It is BV associated in AcMNPV (27) and was found to be associated with AcMNPV ODV (89), but not in HearNPV ODV (269). Deletion of Bm60 from BmNPV resulted in a reduction and delay in DNA synthesis, a reduction in BV production by about 10-fold, and a lengthening of the time to kill larvae (299).

Ac75 (133aa:15.5kDa), (Bm61:133aa:15.5kDa), (Ha69:127aa:14.9kDa).

Homologs of Ac75 are present in all lepidopteran NPV, GV and hymenopteran NPV genomes, but not in the dipteran virus genome. It was found to be associated with both BV and ODV of BmNPV and localized to the ring zone of infected cells (300). In BmNPV it appears to be essential because when deleted, no BV were detected and the virions appeared to be retained in the nuclei (301). Other evidence indicates it is involved in nuclear egress of nucleocapsids and the formation of intranuclear microvesicles (302) (303).

Ac76 (84aa:9.4kDa), (Bm62:85aa:9.6kDa) (Ha70:85aa:10.0kDa).

Homologs of Ac76 are present in the genomes of all lepidopteran NPVs, GVs, and hymenopteran NPVs, but have not been reported in the dipteran virus genome. Ac76 localized to the ring zone late in infection. It is an essential gene, as deletion of ac76 resulted in a mutant bacmid able to produce DNA to normal levels, but was deficient in intra nuclear microvesicles and was unable to produce BV (304). Ac76 appears to be present as a stable dimer that is resistant to denaturation and functions as a type II integral membrane protein in which the C-terminus is located in the ER lumen and the N-terminus interacts with the cytosol (305). Ac76 interacted with NSF and may be involved in the nuclear entry and egress of BV (302). It also interacts with components of the ESCRT-III complex and may be involved in the release of nucleocapsids at the nuclear membrane (75).

*Ac77 (379aa:44.4kDa), (Bm63:379aa:44.3.0kDa), (Ha71:412aa: 47.9kDa), Very late factor-1 (Vlf-1).

Homologs of Vlf-1 are found in all baculoviruses. It is a member of the lambda integrase (306) family of proteins. Integrases are a large group of site-specific DNA recombinases that catalyze DNA rearrangements and are involved in the integration and excision of viral genomes and decatenation of newly replicated

chromosomes. A feature of these enzymes is that a conserved tyrosine forms a covalent link with DNA during the cleavage process. VLF-1 was originally identified because it influences the hyperexpression of very late genes (306). It was found to bind near the regulatory region of very late genes (307). Whereas mutations to the region that affected very late gene transcription were not lethal, other mutations, including mutation of the conserved tyrosine, appeared to be lethal to the virus (308). VLF-1 is present in both BV and ODV (308) and localizes to the ends of nucleocapsids, suggesting that it is a structural protein (309) and is required for the production of nucleocapsids. Although *vlf-1* is an essential gene, an AcMNPV bacmid with *vlf-1* knocked out (309-311) was able to synthesize viral DNA at levels similar to control bacmids. However, the mutant produces tube-like capsids that appear to lack DNA. Characterization of a bacmid with a mutation of the conserved tyrosine indicated the nucleocapsids were unable to be released from the virogenic stroma, suggesting that the protein may be involved in a final maturation step of the nucleocapsid (309). VLF-1 showed structure-dependent binding to DNA substrates with the highest binding affinity to cruciform DNA that mimics a structure common to recombination intermediates (312). See also Chapters 5 and 6.

*Ac78 (109aa:12.5kDa), (Bm64:110aa:12.7kDa), (Ha72:110aa: 12.7kDa).

Ac78 is a core gene (156) and in BmNPV it appeared to be essential (15). When deleted from AcMNPV, DNA replication was unaffected, nucleocapsids were confined to nuclei, infectious BV were not produced, and polyhedra lacked occluded virions. It is important in BV production and per os infectivity, but did not appear to be involved in DNA replication or ODV maturation (313). Ac78 was envelope associated in both BV and ODV (314) and analysis using Hhpred (189) shows over 90% probability that part of it is similar in structure to an integrin transmembrane domain. The homolog of Ac78 in HearNPV was associated with the ODV envelopes (315) and essential for production of infectious viruses and interacted with the baculovirus sulfhydryl oxidase, p33 (316). In addition, it was reported that Ac78 associates with NSF and may be involved in the nuclear entry and egress of BV (302). It also interacts with components of the ESCRT-III complex and may be involved in the egress of nucleocapsids at the nuclear membrane(75).

Ac79 (104aa:12.2kDa), (Bm65:104aa:12.2kDa).

Homologs are present in most Group I, about half the Group II NPV and GV genomes. It was found to be associated with AcMNPV ODV(89). It has a high degree of relatedness to ascovirus orfs from *T. ni*, and *S. frugiperda*, Chilo iridescent virus, and orfs from a variety of bacteria and archaea. It is predicted with over 99% probability by Hhpred (189) to be similar in structure to the GIY-YIG N-terminal endonuclease domain of UvrC involved in DNA repair (317) (318). Bacmids deleted for Ac79 resulted in reduced BV production and smaller plaque size, and showed some tube-like structures that may be aberrant capsids. Point mutations in conserved motifs shared by Ac79 and the endonuclease superfamily did not result in tube-like structures, but one of the mutations caused a reduction in BV production (318). One study of BmNPV suggested that Bm65 is an essential gene (319), whereas another study indicated that it produced BV and could spread between cells, but with reduced efficiency (15).

*Ac80 (409aa:45.4kDa), (Bm66:403aa:44.9kDa), (Ha73:322aa: 36.6kDa), GP41, tegument protein.

GP41 is a tegument protein modified with O-linked N-acetylglucosamine, located between the virion envelope and capsid (320, 321). It was found to be associated with ODV by mass spectrometry (89, 269). Homologs are present in all baculovirus genomes. Based on the characterization of a ts mutant, Ac80 is an essential gene required for the egress of nucleocapsids from the nucleus (322). A deletion mutant of the homologous gene in BmNPV (Bm66) indicated that it produced BV and could spread between cells but with reduced efficiency (15).

Deletion of AcMNPV *gp41* blocked the formation of BV and ODV. In addition, it was found that oligomerization of gp41 was required for BV production

(323). It was reported that gp4 associates with NSF and may be involved in the nuclear entry and egress of BV (302). Also, it interacts with components of the ESCRT-III complex and may be involved in the release of nucleocapsids at the nuclear membrane (75).

*Ac81 (233aa:26.9kDa), (Bm67:234aa:27.0kDa), (Ha74:241aa: 27.7kDa).

Homologs of this orf appear to be present in all baculovirus genomes and in nudiviruses (245). The BmNPV homolog (Bm67) appears to be a late expressed nonstructural gene that localizes to the cytoplasm (324). It may be essential because a deletion mutant of the homologous gene in BmNPV (Bm67) was severely compromised and did not appear to produce BV (15).

Ac82 (180aa:19.8kDa), (Bm68:181aa:20.1kDa), (Ha75:225aa:24.9kDa), Telokin-like protein (TLP).

Telokin-like protein is not like telokin! Homologs of Ac82 are found in the genomes of all lepidopteran NPVs and GVs. Telokin is the myosin binding fragment of myosin light chain kinase and is involved in muscle contraction. A polyclonal antibody prepared against smooth muscle telokin reacted with a protein from cell extracts of AcMNPV-infected Sf9 cells. This protein was called telokin-like protein. Clones that reacted with the antibody were isolated from a cDNA library of AcMNPV infected sf9 cells (325). The clones showed no sequence homology to telokin but when expressed in a pET vector, the product reacted with the telokin polyclonal antiserum. The AcMNPV sequence that produced the reactive protein contains portions of Ac82. The crystal structure of AcMNPV TLP was determined, but showed no similarity to telokin or any other characterized protein (326). Therefore, although this protein has been called telokin-like protein, its resemblance to telokin appears to be an artifact of the polyclonal antiserum. Ac82 is likely to be nonessential because when it was deleted in BmNPV (Bm68) the virus appeared to be normal, but production of BV and DNA replication was somewhat delayed. Although it showed nuclear localization and did not concentrate at the plasma cell membrane, it was found to be associated with the envelope/tegument of budded virions (327). In AcMNPV a TS mutant appeared to cause a major reduction in BV production (212). Bm68 was hyperphosphorylated during infection (38).

*Ac83, VP91, PIF-8 (847aa:96.2kDa), (Bm69:839aa:95.8kDa), (Ha76:816aa:93.5kDa)

Ac83 (PIF8) encodes a virion capsid protein called VP91 that was originally characterized in OpMNPV (328). It has also been shown to be ODV associated in AcMNPV, CuniNPV, and HaSNPV by mass spectrometry (89, 114, 329) and was found as a component of the per os infectivity factor (PIF) complex (47). Homologs are encoded by all baculovirus and are also found in nudiviruses (245) and possibly in several insect genomes. Ac83 is predicted to contain a chitin binding domain and has a high degree of predicted structural similarity by HHpred (77) to tachycitin, a 73aa antimicrobial peptide (330). However, chitin binding by Ac83 has not been detected (331) and structural predictions suggested that it lacked critical features of a chitin binding domain and may be a second zinc finger domain (332). When *ac83* was deleted from an AcMNPV bacmid, the bacmid was non-infectious. However when just the chitin binding domain region was deleted, the virus was unable to infect larvae via the midgut, but could infect via intrahaemocoelic injection, indicating that Ac83 is a per os infectivity factor and that the predicted chitin binding domain may play a major role in the ability of the virus to initiate midgut infection (331). A deletion mutant of this gene in BmNPV (Bm69) did not produce BV and results in the production of tubular structures (333). AC83 is associated with ODV nucleocapsids and envelopes and contains

a cis-acting nucleotide sequence essential for nucleocapsid assembly and is called the nucleocapsid assembly-essential element (NAE) (334). In addition, three contiguous zinc finger domains were predicted that are critical for per os infectivity and it was suggested that they are involved in the localization of the PIF complex to ODV envelopes and interaction with the midgut cell membrane (332). Therefore, Ac83 has at least two functions; the gene contains a DNA sequence necessary for virion assembly and it is a PIF.

Ac84 (188aa:21.7kDa).

This orf is only found in a few other NPVs: PlxyNPV, ChchNPV, and RoMNPV, and TnSNPV. A homolog is also found in ascoviruses, e.g., *T. ni* ascovirus (E = 3e-10). It is not found in the BmNPV or the HaSNPV genomes.

Ac85 (53aa:6.4kDa).

This small orf encoding 53 aa is only found in two other NPVs that are AcMNPV variants: PlxyNPV and RoMNPV. This gene is not found in the BmNPV or HaSNPV genomes. Hhpred (189) indicates that Ac85 is structurally similar to a carbohydrate esterase with a probability of about 90%.

Ac86 (684aa:80.8kDa) (PNK/PNL).

This gene encodes a protein with RNA ligase, polynucleotide 5'-kinase, and polynucleotide 3'-phosphatase activities and may be part of an RNA repair pathway (335). Homologs are only found in a few baculovirus genomes, three are closely related to AcMNPV including AgMNPV, ApNPV, and RoMNPV, whereas the other is in a GV, SpliGV. A closely related orf is also found in a *T. ni* ascovirus (E = 2e-125). Ac86 appears to be a nonessential gene expressed early in infection (336). This gene is not found in the BmNPV or HaSNPV genomes.

Ac87 (126aa:15kDa), (Bm70:126aa:15.1kDa).

This gene appears to be present in most Group I lepidopteran NPV genomes. It was suggested that the homolog in BmNPV (Bm70) might encode a capsid protein called p15 (337). It is likely nonessential, as a deletion mutant in BmNPV (Bm70) appeared normal (15).

Ac88 (264aa:30.1kDa), (Bm71:267aa:30.7kDa), (Ha77:283aa:32.3kDa), CG30.

Homologs of Ac88 appear to be present in the genomes of most Group I and II NPVs, and also may be present in a single GV (SpliGV). An orf in *Clostridium perfringens* showed significant similarity (E = 2e-06). Ac88 contains predicted zinc finger and leucine finger domains (338). It was found to be associated with AcMNPV (89), but not in HearNPV ODV (269). Deletion of this gene from AcMNPV resulted in only subtle differences from wt (339). However, deletion of the gene from BmNPV (Bm71) resulted in a 10 to 100 fold reduction in titer and showed a longer lethal time (340). In another study of Bm71, a deletion and two RING finger mutants were constructed. The deletion mutant produced fewer BV and fewer occlusion bodies were released into the hemolymph of infected larvae. The RING finger mutants released fewer OBs into larval hemolymph. They also noted that cg30 localized to nuclei of infected cells (341). It is predicted by Hhpred (189) to be structurally similar to several proteins involved in splicing, ubiquitin ligation, transcription, and translation with a probability of over 99%.

*Ac89 (347aa:39kDa), (Bm72:350aa:39.3kDa), (Ha78:293aa:33.4kDa), VP39.

This gene encodes the major capsid protein VP39. It is present in all baculovirus genomes. It was originally characterized in OpMNPV (342) and AcMNPV (343). It interacts with 38K (Ac98) (223). Deletion of Bm72 from BmNPV resulted in no apparent BV production (15). It has been observed that VP39 interacts with a

conserved domain of kinesin 1 and it has been suggestion that this interaction is involved in the transport of nucleocapsids destined to become BV to the cell membrane after their assembly in nuclei (344). A conserved glycine at 276 is essential for infectivity and appeared to influence very late gene expression. Based on mutagenic analysis it appears to be required for proper DNA packaging and nucleocapsid assembly (345).

*Ac90 (464aa:53.9kDa), (Bm73:465aa:54.0kDa), (Ha79:3461aa: 54.0kDa), LEF-4.

LEF-4 is a component of the late baculovirus RNA polymerase (185). It is present in all baculovirus genomes and is also present in nudivirus genomes (245). This gene was originally identified as being essential for late transcription (170). LEF-4 was subsequently found to be an RNA capping enzyme (346, 347). The addition of an mRNA 5' cap structure involves the hydrolysis of the gamma phosphate of the 5'-triphosphate of the first nucleotide of pre-mRNA and the capping reaction that involves the transfer of GMP from GTP. The two reactions involve two different enzymatic activities: an RNA 5' triphosphatase to remove the terminal gamma phosphate and the addition of GMP by guanylytransferase. These two activities are present on a single protein located at the N- and C-termini, respectively, in metazoans and plants. Although having similar activity, LEF-4 is unrelated to this category of capping enzyme, but is a member of metal dependent group of capping enzymes found in fungi and protozoa (346, 348, 349). The 5' cap structure appears to serve two roles. It protects the 5' end of the mRNA from degradation by exonucleases and it interacts with translation initiation factors, thereby facilitating the initiation of translation. Capping in eukaryotes involves an enzyme that associates with the highly repetitive carboxy terminal domain (CTD) of the β' subunit of RNA polymerase II. Because the baculovirus polymerase lacks a similar domain, it is likely that it evolved to include the enzyme as part of the RNA polymerase complex. However, assuming these reactions are free from exonuclease, it is not clear why LEF-4 is required for transcription in in vitro assays. These assays monitor RNA transcripts that would not need to be capped in order to be detected. This suggests that LEF-4 may play a structural role in the organization of the polymerase subunits, or it may have some other function. LEF-4 is an essential gene and could not be deleted (350). For more information see Chapter 6.

Ac91 (224aa:24.1kDa), (Bm74:154aa:17.3kDa).

Homologs of this gene are found in genomes of all Group I lepidopteran NPV and at least two GV (CpGV and PlxyGV) genomes. It has an unusual predicted amino acid sequence: 31% proline and 18% ser/thr residues. In HearNPV the Ac91 homolog (ha80) was expressed first cytoplasmically and then in nuclei, but did not appear to be a structural protein of BV or ODV (351). Deletion of Bm74 causes few differences from wt and repair viruses in DNA synthesis or BV titers. However, the lethal time in larvae was longer by 14.7 hr (352).

*Ac92 (259aa:30.9kDa), (Bm75:259aa:30.9kDa), (Ha80:254aa: 30.8kDa), p33, sulfhydryloxidase (sox).

Ac92 is a flavin adenine dinucleotide (FAD)-linked sulfhydryl oxidase (353) (354). Proteins with sulfhydryl oxidase activity have been implicated in the protection of cells from oxidative stress caused by apoptosis (355) (356). Orthologs of Ac92 are present in all sequenced baculovirus genomes, it is associated with BV and ODV, and it is an essential gene as viable recombinants deleted for this gene have not been isolated (354) (357). Ac92 is able to form a stable complex with the human tumor suppressor gene p53 when it was expressed in a baculovirus system. When expressed by itself, p33 shows diffuse cytoplasmic staining and punctate staining of nuclei. However, when co-expressed with p53, it exclusively localizes to nuclei. Expression of human p53 in Sf cells causes apoptosis that can be blocked by co-expression of baculovirus anti-apoptotic suppressors p35 or OpIAP. However, co-expression of p53 with p33 elevated the induction of apoptosis about two-fold. By proteomic analysis, p33 appears to be an ODV-associated protein in AcMNPV (89) and HearNPV (269, 329). The crystal structure of Ac92 was described as a novel dimer composed of two pseudodimers (358). The structure of Bm75

has also been reported (359). In addition, by mutagenesis and crystallography, three domains including the active site, the dimer interface, and a salt bridge at R127-E183 were shown to be required for sulfhydryl oxidase activity (360). An ortholog of P53 has been described for *S. frugiperda* (361) and similar to human p53, Sfp53 was found to interact with Ac92 (362). It interacts with the Sfp53 DNA binding domain and a point mutation in Sfp53 that inactivated DNA binding also inactivated binding of Ac92 to Sfp53. Ac92 was also shown to oxidize Sfp53 in vitro. However, despite the ability of p33 to interact with and oxidize Sfp53 in cultured cells, no effects on Sfp53-mediated apoptosis or virus replication were observed (362). Effects on other cell types or in whole insects was not ruled out by these studies.

*Ac93 (161aa:18.4kDa), (Bm76:161aa:18.4kDa) (Ha81:162aa:19.1kDa).

This gene appears to be present in all baculovirus genomes (363). An Ac93 knockout did not produce infectious BV and may be involved in the formation of intranuclear microvesicles, (363). Ac93 interacted with NSF and may be involved in the nuclear entry and egress of BV (302). It also interacts with components of the ESCRT-III complex and may be involved in the release of nucleocapsids at the nuclear membrane (75). A BmNPV bacmid deleted for Bm76 did not produce BV (15).

*Ac94 (228aa:25.5kDa), (Bm77:228aa:25.6kDa), (Ha82:230aa: 25.9kDa), ODV-E25 (p25, 25k, e25).

Ac94 appears to be encoded by all baculovirus genomes (363). The protein encoded by this gene was originally identified in OpMNPV, and immunogold staining with a specific antibody against Ac94 was localized to ODV envelopes (364). It has also been shown to be associated with BV and ODV of AcMNPV and HearNPV (27, 89, 269, 329). The hydrophobic N-terminal 24 aa of AcMNPV ODV-E25 appears to be a nuclear targeting signal (196). Deletion of Ac94 resulted in a 100-fold reduction in infectious BV. In addition, ODV were not evident and although polyhedra were produced, they lacked virions (365). It has been reported that when ODV-E25 is expressed as an early gene under the IE-1 promoter, it accumulated on the cytoplasmic side of the nuclear membrane rather than within nuclei, and budded virus production was severely reduced. This suggests that it might play a role in the shift from BV to ODV virions. In addition, expression from the very late polyhedrin or p10 promoter reduced and delayed occlusion body formation suggesting that it may play a role in virion occlusion (366) (367). Subsequently it was found that the open reading frame of ODV-E25 encodes a microRNA that down regulates ODV-E25 expression. It was suggested that this might result in a reduction in infectious virus production and be involved in the shift to occluded virus production (368) (102). Ac94 is highly express in midgut cells (39) and associates with NSF and may be involved in the nuclear entry and egress of BV (302).

*Ac95 (1221aa:143kDa), (Bm78:1222aa:143.6kDa), (Ha84:1253aa: 146.0kDa), DNA helicase (p143).

Homologs of DNA helicase are present in all baculovirus genomes. This gene was initially identified as a ts mutant that was unable to synthesize DNA at 33° (369). The defect was localized to a homolog of DNA helicase with a predicted mass of 143 kDa (370). P143 is required for transient DNA replication (50, 51) and shows ATPase activity and is able to unwind a DNA primer annealed to a larger DNA molecule in an ATPase-dependent manner (371). Helicase is dependent on an interaction with LEF-3 for transport to the nucleus (see Ac67, LEF-3) (275). P143 been implicated in affecting viral host range (372, 373) (374) (375) and specific amino acids of the Ac-p143 protein cause rRNA degradation in BmN cells and this may be a signal for apoptosis(376) (377). Deletion is probably lethal as deletion/insertion mutants of this gene in BmNPV (Bm84) could not be isolated (81).

*Ac96 (173aa:19.8kDa), (Bm79:182aa:21.0kDa), (Ha85:173aa:19.8kDa) PIF-4.

This orf appears to be present in all baculoviruses and homologs also appear to be present in nudiviruses (245). The homolog in BmNPV (Bm79) is an ODV envelope associated protein (378) and was also found associated with the envelopes of BV (379). Deletion of Ac96 from a bacmid construct resulted in a virus that could replicate in cell lines, but not insects. Consequently it was concluded that Ac96 is a per os infectivity factor (PIF4), (379). Similar results were obtained for Bm79 (380). Pif-4 interacts with PIF-1 and PIF-3 (48) and forms part of a core complex (332) and may provide proteolytic resistance to the core structure on dissolution of polyhedral in the insect midgut (287)

Ac97 (56aa:6.5kDa).

This is a small orf (56 aa) and appears to be present only in AcMNPV. There is no homolog in BmNPV and it is positioned at the location of the apparent insertion of two bro (Ac2) homologs (see Ac2). The lack of this orf in closely related viruses may indicate that it is not a functional orf.

*Ac98 (320aa:38kDa), (Bm82:320aa:38.0kDa), (Ha86:321aa:37.9kDa), 38K.

Ac98 encodes a predicted protein of 38k and orthologs are present in all baculovirus genomes. AcMNPV Ac98 interacts with itself, VP1054 (Ac54), VP39 (Ac89), and VP80 (Ac104) and is associated with BV and ODV nucleocapsids (223). In HearNPV it interacted with itself, ODV-E56 (Ac148), GP41 (Ac80), PIF-2 (Ac22) and PIF-3 (Ac115) (381). In BmNPV, 38K interacted with itself, FP (Ac61), C41 (Ac102), EC27 (Ac144), GP64(Ac128), ODV-E25(Ac94), ODV-E18 (Ac143), and vCATH (Ac127), PKIP (Ac24), P48(Ac103), pp31 (Ac36), gp37 (Ac64) (382). An AcMNPV bacmid deletion construct, although unable to produce infectious virions, was capable of DNA synthesis, but nucleocapsid formation was disrupted. Tube-like structures that appeared to lack DNA, but stained with an anti-vp39 antibody were observed (383). It has also been suggested that Ac98 is capable of stimulating transcription in a transient transcription assay (384). It is related to a set of enzymes including CTD phosphatases and evidence indicates that it dephosphorylates the p6.9 DNA binding protein which allows it to be packaged with the viral DNA (385). Although it is required for nucleocapsid formation, it does not appear to be a structural component of ODV as determined by proteomic analysis (89, 269). However, it was detected in CuniNPV ODV (114).

*Ac99 (265aa:31kDa), (Bm83:265aa:31.1kDa), (Ha87:315aa:37.0kDa), (LEF-5).

Homologs of lef-5 are found in all baculoviruses and are also present in nudiviruses (245). LEF-5 was originally identified as being required for transient late gene expression (386). It was demonstrated to interact with itself and to contain a domain similar to that of the RNA polymerase II elongation factor TFIIS (387). Subsequent investigations indicated that LEF-5 did not enable the baculovirus polymerase to transit pause sites, and it was concluded that it functions as an initiation factor, rather than an elongation factor (388). A lef-5 knockout bacmid appeared to express early genes and replicate DNA normally, but was defective in late gene transcription, and did not yield any detectable virus when transfected in to Sf9 cells. (389).

*Ac100 (55aa:6.9kDa), (Bm84:65aa: 8.1kDa), (Ha88:109aa:11.5kDa), p6.9.

P6.9 is a small (55 aa) arginine/serine/threonine-rich DNA binding protein (390). Homologs appear to be found in all baculoviruses, but are apparently difficult to detect because of their small size and repetitive amino acid

content (391). It was originally shown to be a DNA binding protein in a GV (392), and the homolog was isolated from AcMNPV (390). The high concentration of arginine and ser/thr residues is similar to protamines that are present in sperm nuclei of many higher eukaryotes and are involved in the production of highly condensed DNA. Protamines are also small molecules of 44-65 amino acids (393, 394). Arginine is positively charged, and the polyarginine tracts in protamines neutralize the phosphodiester backbone, whereas the ser and thr residues interact with other protamine molecules, thereby yielding a neutral, highly compact complex that is biochemically inert. P6.9 localizes to the nuclear matrix during infection (395). It was found to elevate virus transcription at 12-24 hpi, but did not appear to be involved in basal levels of virus transcription. It was also found to co-localize with viral DNA during this same time frame and to fractionate with RNA polymerase II at 24 hpi (396). Some localizes to the virogenic stroma but the majority was found near the inner nuclear membrane throughout the infection. It shows distinct patterns of phosphorylation with multiple forms present in association with ODV, however, only the dephosphorylated form was associated with BV (397). After synthesis, p6.9 is hyperphosphorylated, at least in part by pk-1 and this appears to be essential for high levels of expression of very late genes (398). Phosphorylation appears to occur immediately upon synthesis and p6.9 is dephosphorylated by the 38k protein (385) before being complexed with DNA (399). Using an AcMNPV bacmid deleted for p6.9, nucleocapsids were not produced although tube-like structures similar to those associated with the deletion of VLF-1 and Ac98 (see above) were observed. The mutant appeared to synthesize normal amounts of DNA, but did not produce infectious virus (400). BmNPV mir-3 appears to regulate, at least in part, the expression of BmNPV p6.9 (401).

*Ac101 (361aa:41.5kDa), (Bm85:362aa:41.6kDa), (Ha89:369aa: 42.6kDa), BV/ODV-C42.

Ac101 encodes a capsid-associated protein of both BV and ODV (89). Homologs have been identified in all sequenced baculovirus genomes (156). It was reported to interact in a yeast two-hybrid assay and by native gel electrophoresis (402) with pp78/83 (Ac8) that has been shown to localize to the basal end region of nucleocapsids (63, 64). Evidence suggests that it binds to PP78/83 and transports it into nuclei (403) and is involved in actin polymerization (404). It also interacts with FP25K (Ac61) and Ac141 (405). Deletion of Ac101 from an AcMNPV bacmid appeared to affect nucleocapsid formation but, although lethal, did not appear to affect DNA synthesis (406). Deletion/mutation of Ac101 was also reported to be lethal by others (403, 407). C42 functions to stabilize the actin nucleation factor, P78/83, by inhibiting its degradation. C42 in turn interacts with Ac102 and suppresses ubiquitination of C42 further regulating the stability of P78/83 (408-410). Micro RNA-3 (Mir-3) is encoded on the opposite strand of Ac101 and appears to be involved in its down regulation and may be involved in regulating BV and ODV production (411).

Ac102 (122aa:13.3kDa), (Bm86:123aa:13.5kDa), (Ha90:122aa: 13.8kDa), p12.

Homologs of Ac102 are found in all lepidopteran NPV, and GV genomes, but not in hymenopteran or dipteran viruses. It appears to encode an ODV-associated protein (89) in AcMNPV and HearNPV (329). It is involved in the nuclear localization of G-actin (43, 412). Deletion is lethal as insertion/deletion mutants of this gene could not be isolated in AcMNPV (43, 407) and viral spread to other cells was not observed with a BmNPV (Bm86) knockout (15). Ac102 is expressed as a late protein and is required for F-actin assembly in infected nuclei. It is a nucleocapsid protein and interacts with EC27 (Ac144), C42, and P78/83 (410). The interaction of Ac102 with C42 regulates the stability of P78/83 (see Ac101 above) (409).

*Ac103 (387aa:45.3kDa), (Bm87:387aa:45.4kDa), (Ha91:377aa: 44.0kDa), p45.

Homologs of Ac103 are present in all baculovirus genomes (156). Deletion of Ac103 was lethal, no viable BV were detected, and the constructs appeared to be deficient in the envelopment of ODV and their incorporation into occlusion bodies (413). Ac103 associates with NSF and may be involved in the nuclear entry and egress of BV (302). It was reported that Ac103 interacts with components of the ESCRT-III complex and may be involved in the release of nucleocapsids at the nuclear membrane(75).

Ac104 (691aa:79.9kDa), (Bm88:692aa:79.9kDa), (Ha92:605aa: 69.7kDa), vp80 capsid, vp87.

Homologs of Ac104 are found in all Group I and II lepidopteran NPV genomes, but not in those of GVs or hymenopteran or dipteran NPVs. It is capsid associated in both OpMNPV (414) and AcMNPV (89, 415, 416) and interacts with 38K (Ac98) (223). Deletion of Vp80 showed that it is an essential gene and resulted in nucleocapsids that were unable to move from the virogenic stroma (417). It appears to localize in nuclei near actin scaffolds that connect the virogenic stroma to the nuclear envelope. In addition, it co-immunoprecipitates with actin. It also appears to localize to one end of nucleocapsids and contains sequences similar to paramysin motifs that may be involved in the transport of virions to the periphery of nuclei (415). It forms dimers, contains a C-terminal region that was predicted to contain a basic helix-loop-helix domain, and binds to DNA (418).

Ac105 (553aa:65.6kDa), (Bm89:289aa:34.3kDa), He65, RNA editing ligase.

Homologs of Ac105 are found in the genomes of most Group I, about one-half the Group II and several GVs including Agse-, Ha-, and XecnGV. It is a member of a distinct family of ligases that includes editing ligases of trypanosomes, putative RNA ligases of many species of archaea, and also baculoviruses and an entomopoxviruses (419). It is an early-transcribed gene (420). It may be involved in the nuclear localization of G-actin (412). It appears to be a non-essential gene in AcMNPV (43) and BmNPV (15).

Ac106/107 (243aa:28.3kDa), (Bm90:249aa:28.9kDa) (Ha101:253aa: 29.0kDa).

These two orfs were found to be joined when the region was re-sequenced in the C-6 strain (12). Homologs are found in all Group I and II lepidopteran NPV, GV and hymenopteran NPV genomes, but not in that of the dipteran NPV. Deletion of Bm90 resulted in a mutant that was unable to spread between cells indicating that it is an essential gene (15).

Ac108 (105aa:11.8kDa), (Bm91:105aa:11.8kDa) (Ha95:94aa:11.0kDa) (PIF9).

Orthologs of Ac108 have been found in the genomes of all alpha, beta, and gamma baculoviruses (156). Although Ac108 was not found to be ODV-associated in AcMNPV (89) or HearNPV (269), the homolog in the *Antheraea pernyi* nucleopolyhedrovirus (p11) was found to be associated with ODV (421). In later studies it was found in the PIF complex of AcMNPV (47) and was identified as a PIF protein(422). Furthermore, the ortholog in *Spodoptera frugiperda* MNPV (sf58) appeared to be a per os infectivity factor (PIF) (423). Ac108 associates with PIF8 and is part of the complete PIF complex and when it was deleted, the virus was not orally infectious and it has been designated as PIF9 (424). In contrast, in BmNPV, Bm91 was ODV associated, but appeared to be non-essential in oral assays, although its deletion did extend the lethal time (15, 425) (425). Hhpred (189) predicted that Ac108 was similar in structure to several proteins with a probability of over 80% including

Vesicle-associated membrane protein 2 of a SNARE complex and the membrane domain of E. coli histidine kinase receptor QseC.

*Ac109 (390aa:44.8kDa), (Bm92:391aa:45.0kDa), (Ha94:361aa: 41.5kDa).

Homologs of Ac109 are present in all baculovirus genomes. Evidence suggests that it is ODV-associated in AcMNPV (89) and *Helicoverpa armigera* NPV (Ha94-ODV-EC43) (269, 426) and also is BV-associated in AcMNPV (27, 427). Four studies have examined deletions of Ac109 and demonstrate that it is an essential gene and when deleted, DNA replication is not affected. One study reported that deletion of Ac109 resulted in a block in nucleocapsid and polyhedron formation (428). However, the other reports described different results. One indicated that polyhedra and virions were produced by Ac109 deletions, but the virus was not infectious (427). Another study found similar results, but also showed that the nucleocapsids had defects in envelopment and the polyhedra lacked virions (429). A fourth report also described similar findings, but indicated that the BV produced by an Ac109 knockout could enter the cytoplasm, but not nuclei, and also noted that the occlusion bodies lacked virions (430).

*Ac110 (56aa:6.8kDa), (Bm92a), (Ha93:58aa:6.9kDa), PIF-7.

Orthologs ofAc110 are found in all baculoviruses and it is a per os infectivity factor, PIF-7 (431) (332).

Ac111 (67aa:8.2kDa), (Bm93:67aa: 8.2kDa), (Ha116:71aa:8.2kDa).

Homologs of Ac111 are present in genomes of most Group I, and several Group II, e.g. Hear-, Heze-, Ld-, and LeseNPV and at least two GVs (SpliGV and XecnGV). Deletion of Bm93 had no effects on virus replication (15). Deletion of Ac111 did not affect BV production and had no effect on per os infectivity in *S. exigua* larvae, but infectivity was reduced by 5-fold in *T. ni* larvae (432) suggesting that it may be a per os infectivity factor, depending on the host insect.

Ac112/113 (258aa:30.9kDa), Apsup.

These two orfs were found to be joined when the region was re-sequenced in the C-6 strain (12). Homologs are present in several Group I and II NPV and GV genomes. It shows highly significant homology (over 50% identity) to an orf in several avian poxvirus genomes. A related orf is not present in BmNPV (16). The ortholog of this orf in LdMNPV (ld109) is an apoptosis suppressor called *apsup*. They show about 30% amino acid sequence identity, but Ac112/113 did not show any apoptotic suppressor activity (433).

Ac114 (424aa:49.3kDa), (Bm94:424aa:49.4kDa) PARG.

Homologs of Ac114 are found in most Group I NPV genomes. Hhpred analysis (189) indicates that it is a Poly(ADP-ribose) glycohydrolase (PARG) with almost 100% probability. This appears to be a baculovirus Group I PARG lineage that was not previously identified. The original baculovirus PARG is specific to Group II baculoviruses. The Group I and II PARGs show low levels of relatedness (e.g. AcMNPV and LdMNPV are less than 15% identical) and appear to represent two different lineages of this enzyme. In AcMNPV it is an ODV (89, 434) and BV associated protein (27). It is likely to be nonessential, as a BmNPV bacmid deleted for this gene (Bm94) appeared similar to wt (15).

*Ac115 (204aa:23kDa), (Bm95:204aa:23.0kDa), (Ha90:199aa:22.4kDa), pif-3.

Homologs of *pif-3* appear to be present in all baculovirus genomes. It is also present in nudivirus genomes (245). Like other *pif* genes, *pif-3* is required for oral infectivity of insect but not for infection of cultured cells (108). It

forms a complex with PIF-1, -2, and -4 (381) (287) (48) and also interacts with ODV-E56 (435). For more information see Chapter 2.

Ac116 (56aa:6.4kDa), (Bm95a).

Homologs of Ac116 are found in a few Group I NPVs (Ac-, Ro-, Bm-, and PlxyNPV). Deletion of Bm95a and Bm96 showed no defects as did deletion of Bm96 alone, therefore Bm95 appears to be non-essential (15).

Ac117 (95aa:11kDa), (Bm96:95aa:10.9kDa), (Ha110:88aa:10.1kDa).

Homologs of Ac117 are found in the genomes of all Group I and some Group II NPVs. It is likely to be nonessential, as insertion/deletion mutants of this gene in BmNPV (Bm96) were similar to wt, although a slight effect on the motility of infected larvae was noted (15, 199).

Ac118 (157aa:18.7kDa).

Homologs are found in a few Group I NPVs (Ac-, Ro- and PlxyNPV) genomes. A related orf is not present in BmNPV.

*Ac119 (530aa:59.8kDa), (Bm97:527aa:59.8kDa), (Ha111:528aa: 60.3kDa), pif-1.

Homologs of *pif-1* are present in all baculovirus genomes and are also present in nudivirus genomes (245). This gene can be deleted and the mutant is still infectious for cultured cells, but is not orally infectious for insects (108). It forms a complex with PIF2 -3, and -4 (381) (287) (48). For more information see Chapter 2.

Ac120 (82aa:9.5kDa), (Bm98:82aa:9.5kDa).

Homologs of Ac120 are found in all Group I and most Group II genomes. It is likely to be nonessential, as an insertion/deletion mutation of this gene in BmNPV (Bm98) had no apparent effect on infectivity (15). Analysis using Hhpred (189) indicates with a probability of over 96% that Ac120 is related to the MIT domain (microtubule interacting and transport) of vacuolar protein sorting associated protein 4 (vps4).

Ac121 (58aa:6.7kDa), (Bm98a).

Homologs of Ac121 are only found in the genomes of Bm- and PlxyNPV. It may be a transcriptional activator of some early genes, including IE1 and pp31 (436). However, it does not appear to activate late gene expression (187). In BmNPV, it appeared to be non-essential (15).

Ac122 (62aa:7.2kDa), (Bm99:61aa:7.1kDa).

Homologs of Ac122 are present in most Group I genomes. It is likely to be nonessential, as an insertion mutation of this gene in BmNPV (Bm99) had no apparent effect on infectivity (15).

Ac123 (215aa:25kDa), (Bm100:225aa:26.0kDa), Protein kinase 2 (PK2).

Homologs of Ac123 are found in only a few NPVs including BmNPV, PlxyNPV and RoMNPV and they are all over 98% identical to AcPK2. PK2 is closely related to translation initiation factor eIF 2α kinase (e.g., B. mori (E = 2e-20)), and the homology appears to be focused on the C-terminal region of the kinase domain (437). An AcMNPV mutant deleted for *pk2* displayed no differences from wt in its infectivity to cultured cells and insect larvae (437). In contrast, PK2 (Bm100) from BmNPV was found to be required for replication in BmN cells (138). In addition, a novel eIF-2α kinase called BeK was identified from B. mori. It has a distinct N-terminal regulatory region not shared by other eIF-2α kinases. BmNPV PK2 was capable of inhibiting the enzymatic

activity of BeK (438). AcMNPV PK2 inhibits yeast and human eIF2α kinases (439). Insect cells infected with wt showed reduced eIF2α phosphorylation and increased translational activity that was not observed in cells infected with the *pk2* deletion mutant. It was suggested that PK2 may be involved in a mechanism for inactivating a host stress response to virus infection (439). Sf9 cell infection by AcMNPV deleted for PK2 or by wt virus both encoding the p35 anti-apoptotic gene was found to induce the expression of BiP. BiP is a stress marker of the endoplasmic reticulum and a chaperone. It was observed, however, that the UV induction of eIF2alpha phosphorylation and the activation of caspase were mitigated more effectively by the wt virus than the mutant virus that lacks pK2, which is an eIF2α kinase inhibitor (440). The translation initiation factor eIF2α is phosphorylated by the eIF2α family of kinases. Phosphorylation of eIF2α inhibits cell mRNA translation and can lead to apoptosis. This often occurs under stress conditions such as viral infection. PK2 interferes with the function of an eIF2α kinase by binding its kinase domain thereby preventing the phosphorylation of eIF2α (441). This prevents the apoptotic signal caused by phosphorylation of eIF2α and allows viral mRNAs to be translated.

Ac124 (247aa:28.5kDa), (Bm101:244aa:28.1kDa).

Homologs of Ac124 are present in the genomes of most Group I lepidopteran NPVs. The deletion of Ac124 had few differences from wt; however, although the LD50 for larvae appeared unaltered, the time to larval death was extended (442). In BmNPV, it is associated with the envelopes of BV and in infected cells is present in the cytosol and cytoplasmic membrane (443). In AcMNPV it has been shown to be BV associated (27). A Bm101 knockout virus appear to replicate DNA normally, but did not produce infectious BV (444). In an earlier investigation it was reported that Bm101 appeared to be a non-essential gene (15). Also, a knockout of AcMNPV Ac124 had no detectable effect on the viral infection cycle and it was suggested that it may influence elevate the expression of the chitinase gene (445).

Ac125 (226aa:26.6kDa), (Bm102:227aa:26.6kDa), lef-7.

Homologs of lef-7 are present in the genomes of all Group I, and several Group II including Se-, Sf- and MacoNPV A and several GVs including Ha-, Xecn- and SfGV and can be present in two copies (446). It is a F-Box protein; the F-box is a motif of about 50 amino acids and is involved in protein-protein interaction. SfGV LEF-7 contains 3 F-Box domains (447). Lef-7 is stimulatory for transient DNA replication (51, 448). When deleted, infection was unaffected in Tn368 cells, but in Sf21 and Se1c cells DNA replication was 10% of wt (449). Deletion of BmNPV lef-7 also caused a reduction in BmNPV DNA synthesis (81). LEF-7 was found to be involved in the regulation of the DNA damage response (DDR) and it interacts with host S-phase kinase-associated protein 1 (SKP1). SKP1 is a component of a complex that interacts with and targets proteins for polyubiquitination. Deletion of *lef-7* from the AcMNPV genome resulted in the accumulation of phosphorylated H2AX and activation of the DDR that led to a major reduction in late gene expression and reduced infectious virus production by 100-fold. It was suggested that LEF-7 may interfere with the phosphorylation of H2AX thereby diverting host DDR proteins from cellular chromatin, so that they can be exploited for viral DNA replication (450).

Ac126 (551aa:61.4kDa), (Bm103:552aa:61.8kDa), (Ha41:570aa: 65.5kDa), chitinase.

Homologs of chitinase were reported in genomes of most Alphabaculoviruses and several Betabaculoviruses(451) and is phylogenetically clustered with a number of lepidopteran chitinases, i.e., it shows 63% aa sequence identity to *B. mori* chitinase. Comparison of BmNPV and *B. mori* chitinases indicated that, although closely related, they have different properties; the viral chitinase is retained in the cell and functions under alkaline conditions, whereas the host enzyme is secreted and has reduced activity at higher pH (452). Phylogenetic studies indicate that it is more closely related to the chitinase of proteobacteria that employ the enzyme to degrade fungal chitins (453). It has a mode of action similar to *Serratia marcescens* chitinase to which

it is 60.5% identical and processively hydrolyzes beta-chitin (454). In conjunction with Ac127 (cathepsin), chitinase participates in the liquefaction of insects late in infection. It is a late expressed gene and its product is localized to the cytoplasm (455) and also is BV associated (27). When it is deleted along with Ac127 (cathepsin), insects remained intact for several days after death (456). Chitinase is localized to the endoplasmic reticulum in infected cells by KEDL, an endoplasmic reticulum retention motif (457, 458). The retention in the ER may prevent the premature death and liquefaction of infected insects, allowing the virus to continue to replicate. It is thought that the presence of chitinase and cathepsin assists in the dissemination of the virus by degrading the insect upon its death. The facility with which a virus (*Anticarsia gemmatalis* NPV) can be processed for use as a biocontrol agent has been attributed to its lack of these two genes, thereby allowing collection of the virus from intact rather than disintegrated insects (459).

Ac127 (323aa:36.9kDa), (Bm104:323aa:36.9kDa), (Ha56:365aa: 42.0kDa), cathepsin, vcath, a metalloprotease.

Homologs of Ac127, cathepsin, have a similar distribution to Ac126 (chitinase) and are present in the genomes of most alphabaculoviruses and several GVs (451). The baculovirus genes are closely related to insect cathepsins, i.e. Ac127 is 39% identical to an *Apis mellifera* cathepsin. The baculovirus cathepsin appears to participate along with chitinase in the liquefaction of infected insects (see Ac126) (460). When it is deleted along with Ac126 (chitinase), insects remained intact for several days after death (456). It has been suggested that Ac127 is synthesized in an inactive form that is activated upon death of the insect by lysosomal proteinases (461). It was subsequently demonstrated that AcMNPV and CfMNPV cathepsins are expressed as pre-proenzymes that are cleaved in infected cells (462). Viral chitinase (see above) is apparently synthesized before cathepsin to facilitate the retention of cathepsin in the ER. Cathepsin is synthesized as an inactive precursor (preproV-CATH) and upon translation the N-terminal 22 amino acids encompassing the signal peptide causes the localization to the ER during which the signal peptide is cleaved. Within the ER the viral chitinase appears to interact with the proV-CATH and assists in the proper folding and causes its retention in the ER (463). Upon death of the host, proV-CATH is cleaved and activated and released from the ER along with chitinase to facilitate the degradation of the insect and release of the virus.

Ac128 (530aa:60.6kDa), (Bm105:530aa:60.6kDa), gp64, gp67.

gp64 encodes a low pH activated envelope fusion protein, and homologs are present in all Group I genomes. It is one of the major distinguishing features of these viruses. It is thought that all Group I viruses use GP64 for the entry of BV into cells, whereas all other baculoviruses lack a *gp64* homolog and use the F protein (ac23 homolog) except for hymenopteran NPVs which lack both genes. However, an ortholog of gp64 was identified in a GV (464). Orthologs of *gp64* are also found in thogotoviruses, which are members of the Orthomyxoviridae (465). GP64 (466-468) is a fatty acid acylated glycoprotein (469). Deletion of gp64 is lethal and results in viruses that replicate in a single cell, but cannot bud out and infect surrounding cells (470, 471). The postfusion structure of GP64 has been described (472). It was found that a ubiquitin-protein ligase SINA-like 10 (SINAL10) interacted with GP64 and overexpression elevated viral production whereas inhibition caused their reduction (473). For more information see Chapter 2.

Ac129 (198aa:22.1kDa), (Bm106:195aa:21.8kDa), (Ha118:248aa: 28.4kDa), p24-capsid.

Homologs of Ac129 are present in the genomes of all Group I /II and GV genomes. Ac129 (p24) is associated with both BV and ODV of AcMNPV and OpMNPV (27, 474). Its presence in AcMNPV ODV was confirmed, however, the HearNPV homolog, He118, was not found associated with ODV (269). It is likely to be nonessential, as interruption of this gene with a transposable element in a strain of AcMNPV has been reported (475, 476). In addition, insertion/deletion mutations of this gene in BmNPV (Bm106), although viable, took

slightly longer to kill insects than wt (199). Also, in LdMNPV, the original strain sequenced, lacked this gene, whereas it is present in other strains (477).

Ac130 (106aa:12.1kDa), (Bm107:106aa:12.1kDa), (Ha119:94aa:10.7kDa), gp16.

Homologs of Ac130 are present in the genomes of most alphabaculoviruses. Ac130 localized to near the nuclear membrane and was present in a membrane fraction. Deletion caused the survival time (ST50) to increase about 6 hr (478). In OpMNPV, the homolog (Op128) is glycosylated and localized near the nuclear membrane in the cytoplasm. Although it appeared to be associated with envelopes of nucleocapsids in the cytoplasm, it was not associated with either ODV or BV (479). It appears to be a non-essential gene in BmNPV (15). Analysis by Hhpred (189), indicates that it may be related to several proteins with a probability above 80% including bacterial toxins, a heat shock binding protein, and a subunit of a filovirus fusion protein.

Ac131 (322aa:36.4kDa), (Bm108:315aa:35.4kDa), (Ha120:340aa:39.1kDa), calyx, polyhedron envelope (PE) protein, pp34.

The predicted size of Ac131 is longer (322 vs 252 aa) than previously reported (12). Orthologs of Ac131 have been found in the genomes of most alpha, beta, and gamma baculoviruses (156)(173). In addition, domains of PE may be present as fusion with segments of p10 in some GVs (see below). The calyx/PE appears to be applied in layers on the polyhedron surface (480). The calyx/PE was originally found to contain carbohydrate (481); subsequently a phosphorylated protein component was identified (482) (483). Similar results were obtained for OpMNPV and it was also found to be associated with p10 fibrillar structures (484-487). In addition, in some viral genomes, genes are present that appear to be fusions of both PE and p10 protein domains (488, 489). The Ac131 encoded protein appears to be an integral component of the calyx/PE, and when the gene is deleted, polyhedra lack an intact calyx/PE, and have a rough surface showing cavities where virions have apparently been dislodged (490). It has also been reported to be BV associated (27) although what role it may play in this phenotype is not clear. The function of the calyx/PE appears to be to encase the occlusion body in order to enhance its stability. Based on Hhpred (189) it is predicted to be similar in structure to Ac13. See Chapter 2 for additional information.

Ac132 (219aa:25.1kDa), (Bm109:220aa:25.2kDa).

Homologs of Ac132 are present in most Group I genomes. Ac132 was identified as being associated with AcMNPV ODV(89) and BV (27). Bm109 was also reported to be ODV associated (491). In BmNPV, it appear to be an essential gene (15). A knockout of Ac132 resulted in a single cell phenotype indicating that it is essential for viral propagation. However, DNA replication and the expression of some late genes appeared unaffected, but the formation of the virogenic stroma was delayed and the numbers of enveloped nucleocapsids was reduced. OB formation was also delayed and mainly occluded singly enveloped nucleocapsids Ac132 interacted with ODV-E18 and p6.9 (492). Another report characterizing a deletion mutant indicated that BV are released from transfected cells and could enter cells but could not enter nuclei thereby preventing replication (493).

*Ac133 (419aa:48.3kDa), (Bm110:420aa:48.5kDa), (Ha114:428aa:49.4kDa), Alkaline nuclease (AN).

Homologs of alkaline nuclease (AN) are found in all baculovirus genomes. They are also found in a variety of other viruses such as lambda phage and herpes viruses. In these viruses, the AN homolog associates with an SSB and has an exonuclease activity which generates 3' single-strand DNA ends that can participate in DNA recombination. In AcMNPV, AN interacts and co-purifies with the SSB LEF-3 and has both a 5'→3' exonuclease and an endonuclease activity (279, 280, 494). Deletion of Ac133 is lethal (495, 496). It is thought that AN is involved in DNA recombination. Homologs are also present in nudiviruses and hytrosaviruses. In *Epinotia*

aporema granulovirus (EpapGV), the ortholog of Ac133 is fused with an ortholog of helicase 2 (497) (see Chapter 13) suggesting that they might act together possibly for the maturation of Okazaki intermediates. Ac133 may act as Fen nuclease in this process as its endonuclease and the single strand specific 5' to 3' exonuclease could be involved in the digestion of the primer overhangs generated by helicases. Hhpred (189) indicated the expected relationship with other exonucleases, but also showed a high degree of structural similarity to BIR-contain iap proteins near the C-terminal region.

Ac134 (Bm111:803aa:94.5kDa) (p94).

Homologs of Ac134 are present in the genomes of most Group I, a few Group II and a few GVs. Homologs are found in several polydnaviruses, e.g., *Cotesia congregata* bracovirus (E = 7e-40). The disruption of the p94 gene showed no effect on the ability of AcMNPV to infect *S. frugiperda* larvae by either the oral or intrahaemocelic route (498). Analysis by Hhpred (189) predicted relatedness of the first 131 amino acids to several proteins with a probability of over 90%. These include integrin and tumor endothelial marker 8.

Ac135 (299aa:34.8kDa), (Bm112:299aa:34.5kDa), p35.

P35 is an inhibitor of apoptosis, and homologs are limited to a few Group I NPVs closely related to AcMNPV. A homolog has also been reported in a GV of *Choristoneura occidentalis* (ChocGV) (488), and a variant (p49) is found in a Group II NPV (SpliNPV) genome (499, 500). Furthermore, a homolog most closely related to SpliNPV p49 has also been identified in an entomopox virus genome (501). P35 is able to block apoptosis in *S. frugiperda* cells caused by AcMNPV infection (502). Although deletion mutants are viable, they are severely compromised in BV production in Sf cells (502, 503). The crystal structure of p35 has been described (504) (505). For additional information, see Chapter 7.

Ac136 (240aa:27.3kDa), (Bm113:240aa:27.3kDa), (Ha22:267aa: 30.5kDa), p26.

Homologs of p26 are present in the genomes of most Group I and Group II Alphabaculoviruses, but are not present in those of GVs. Multiple copies of the gene may be present. Homologs are also found in the genomes of numerous pox viruses, e.g., Vaccinia (E = 0.15, 25% identity over 201 aa). Ac126 forms homodimers and is primarily a cytoplasmic protein (506). The examination of an AcMNPV deleted for p26 revealed no differences from wt in the cells and larvae tested (507). However, a deletion of p26 along with p10 and p74 resulted in polyhedra lacking virions (508).

Ac137 (94aa:10.3kDa), (Bm114:70aa:7.5kDa), (Ha21:87aa:9.3kDa), p10.

Homologs of p10 are found in the genomes of most Group I and II NPVs and many GVs, in some instances in multiple copies (509). They are also present in all hymenopteran NPV genomes. A p10 homolog has been characterized in an entomopox virus (510). P10 was originally identified as a very late hyper-expressed gene (511) and therefore the p10 promoter has been used in expression vectors (512). It was observed that an inhibitor of the microRNA, *bantam*, resulted in the expression level of p10 to increase 40-fold (513). P10 interacts with tubulin (514) and forms two different types of structures; microtubule-associated filaments, and tube-like structures that surround the nucleus (515). As noted above, p10 appears to be associated with the PE protein (Ac131) and in some viral genomes, genes are present that appear to be fusions of both PE and p10 protein domains (488, 489). Deletions of P10 result in polyhedra that resemble those produced by mutants lacking the calyx/polyhedron envelope protein (Ac131); they are fragile, have a rough surface showing cavities where virions have apparently been dislodged, and often show an incomplete calyx/polyhedron envelope (490, 516, 517). AcMNPV p10 is phosphorylated at serine 93 and the serine was critical for the formation of p10 associated tube-like structures (509). Hhpred (189) indicated that Ac137 was structurally related to the reovirus

sigma 1 tail with a probability of about 90%. The 3 orthologs of p10 from the PxGV genome (px002, px021, and px050)] all were predicted to have a similar structure to the reovirus protein. For more information, see Chapter 2.

*Ac138 (645aa:73.9kDa), (Bm115:645aa:74.0kDa), (Ha20:688aa: 78.4kDa), p74-pif.

P74 was the first per os infectivity factor (PIF) to be identified (107, 518-521). PIFs are required for oral infection of insects, but are dispensable for infection of cultured cells. Homologs of *p74* are present in all baculovirus genomes and are also found in genomes of nudiviruses (245). Three other *pif* genes were identified in BmNPV (522) and subsequently their homologs were characterized in AcMNPV. In addition, PIF1, PIF2, and p74 mediate specific binding of occlusion derived virus to midgut cells, suggesting that they are directly involved in virus cell interaction as an initial step in infection (108). Co-infection with a wt- and a *p74*-deleted virus expressing *gfp* resulted in per os infection by the *gfp* expressing virus, suggesting the p74 did not have to be directly associated with a virus to facilitate per os infection. In addition, a 35-kDa binding partner for AcMNPV P74 was detected in extracts of brush border membrane vesicles from host larvae (*Spodoptera exigua*), but not from a non-host (*Helicoverpa armigera* larvae) (521). The identity of this host protein has not been determined. By proteomic analysis, P74 was found associated with AcMNPV, HearNPV and CuniNPV ODV (89, 269) (114); however, the other PIF proteins showed differing associations, e.g., CuniNPV (PIF-1,2,3), AcMNPV (PIF-2) and HearNPV (PIF-1). P74 appears to be cleaved into two fragments by a protease associated with occlusion bodies produced in insects. This cleavage does not occur in polyhedra produced in cell culture. The significance of the cleavage is not clear because cell culture and insect produced polyhedra appear to be equally infectious (523). P74 does not associate with the core PIF complex of (48) (287). It has been shown to be required for ODV binding (524).

Ac 139 (449aa:52.6kDa), (Bm116:451aa:52.6kDa), (Ha16:284aa: 33.6kDa), ME53.

Homologs of *ac139* are present in the genomes of all the lepidopteran NPVs and GVs, but have not been reported in hymenopteran or dipteran baculovirus genomes. It is BV and ODV associated (525). One study indicated that AcMNPV deleted for this gene is not viable and fails to replicate its DNA and does not produce nucleocapsids. However, cells transfected with DNA from the mutant showed early stages of cpe, including nuclear enlargement and the formation of granular material in the nucleus (526). This suggests that the mutant is blocked in an early gene function. This is consistent with its original characterization as a major early (ME) gene (527). However, another study showed that deletion of Ac139 did not alter DNA replication, but results in a 1000-fold reduction in BV titer. In addition, it was found that it appears to be required both early and late in infection (525). ME53 fused to GFP localized mostly to the cytoplasm early and to nuclei late in infection. However, foci of ME53 were also noted at the cell periphery late in infection and co-localized with gp64 and VP39-capsid and was capsid associated in BV. It was suggested that it may provide a connection between the nucleocapsid and the viral envelope (528). Amino acids 109-137 contains a nuclear translocation domain and aa 101-398 are essential for BV production whereas aa 1-100 and the C-terminal 50 aa were dispensable for BV production (529).

Ac140 (60aa:7.1kDa).

This orf encodes 60 aa and is only found in AcMNPV.

Ac141 (261aa:30.1kDa), (Bm117:261aa:30.1kDa), (Ha8:285aa: 33.2kDa), exon0.

In one of few baculovirus splicing events, an internal splice site in Ac141 results in the N-terminal 38 amino acids of Ac141 also being present at the N-terminus of IE1 (Ac147) resulting in IE0, the spliced form of IE1. Ac141 is found in all lepidopteran NPVs, and orfs with low homology are also found in GV genomes. In AcMNPV it is associated with both BV and ODV nucleocapsids (27, 530) and interacts with BV/ODV-C42 (Ac101) and FP25 (Ac61) (405). Ac141 contains a predicted RING finger domain (531) that is a type of zinc finger comprising 40-60 residues that binds two zinc atoms and may be involved in protein-protein interactions. Deletion of Ac141 severely compromises BV production and results in virus that appear to be restricted to cells initially infected (530, 531). It appears to both co-localize with and co-purify with β-tubulin, and inhibitors of microtubules reduced BV production by over 85% (532). It has also been shown to interact with a conserved domain of kinesin 1, a motor protein involved in transporting cargo along microtubules to the periphery of the cell supporting a role for microtubules in the transport of virions to the cell surface (344) (533). Therefore, it has been suggested that the interaction of Ac141 with microtubules might be involved in the egress of BV. Hhpred (189) predicts with over 90% probability that the C-terminal ~90 amino acids has structural similarity to E3 protein ubiquitin ligase. Deletion of ac141 and vubi results in single cell infection and BV were not produced. The ubiquitination of Ac141 was essential for optimal production of BV. BV but not ODV nucleocapsids were ubiquitinated by vUbi. The target was Ac66 and it was shown to co-localize with vUbi and Ac141 at the nuclear periphery. It was suggested that the ubiquitination of capsid proteins may be a signal for BV egress from nuclei (163).

*Ac142 (477aa:55.4kDa), (Bm118:476aa:55.5kDa), (Ha9:468aa: 55.3kDa), p49.

Homologs of Ac142 have been identified in all sequenced baculovirus genomes. Ac142 is associated with both BV and ODV virions, and deletion of Ac142 appeared to affect nucleocapsid formation but, although lethal, did not appear to affect DNA synthesis (406). Another study describing a different bacmid construct that deleted less of the Ac142 gene showed similar results except that some nucleocapsids appeared to be fully formed, but were un-enveloped in the nucleus and were not occluded (534). It is unclear whether the difference in the two studies was due to the removal of a 3' processing signal for the upstream Ac141 gene in the former investigation, or to the presence of a significant portion of the Ac142 orf in the latter study. A BmNPV deleted for Bm118 failed to produce BV and produced polyhedra lacking virions. There appeared to be a defect in nucleocapsid formation as elongated capsid-like particles apparently devoid of DNA were observed (535). Mass spectrometry also suggests that Ac142 is ODV-associated in three different viruses (89, 114, 269). In addition, it was reported that Ac142 interacts with components of the ESCRT-III complex and may be involved in the release of nucleocapsids at the nuclear membrane (75).

*Ac143 (90aa:9.7kDa), (Bm119:101aa:10.4kDa), (Ha10:81aa:8.8kDa), ODV-E18.

Homologs of Ac143 are present in the genomes of all baculoviruses. An antibody generated against an Ac143-GST fusion reacted with a protein of 18 kDa in the ODV envelope fraction, and Ac143 was named ODV-E18 (536). Ac143 and its HearNPV homolog were found in surveys of ODV-associated proteins by mass spectrometry (89, 269). Ac143 was found to be BV associated in a proteomic analysis (27) and the predicted size of Ac143 is longer than previously reported (90 vs 62aa) (12). No BV is produced when Ac143 is deleted (537). Hhpred (189) predicts the presence of a transmembrane helix and similarity to a protein translocation complex.

*Ac144 (290aa:33.5kDa), (Bm120:290aa:33.5kDa), (Ha11:284aa: 33.3kDa).

Homologs of Ac144 are present in all sequenced baculovirus genomes. Ac144 was originally named ODV-EC27 and suggested that it is a cyclin of 27kDa (538). However, another investigation using an HA-tagged Ac144 recombinant virus and anti-HA monoclonal antibodies found that Ac144 was expressed as an ~ 33.5 kDa protein which conforms to the predicted MW (406). In addition, it was found to be BV associated (27, 406). Mass spectrometry also suggests that Ac144 is ODV-associated in three different viruses (89, 114, 269). A variety of investigations have been conducted on Ac144. Initially, it was confirmed that its transcript initiates at a late promoter element (536). It was reported to interact in a yeast two-hybrid assay with Ac101 described above (also named C42) and with both Ac101 and p78/83 (Ac9) in native gel electrophoresis assays (402). Deletion of Ac144 resulted in amorphous electron dense structures that stained with vp39-capsid antibodies, but no nucleocapsids were evident. Although lethal, deletion of Ac144 did not appear to affect DNA synthesis (406).

Ac145 (97.0aa:8.9kDa), (Bm121:95aa:11.0kDa), (Ha12:92aa:10.8kDa), (pif?).

Ac145 and Ac150 encode small proteins (~9 and 11 kDa, respectively) that are related to one another (23% aa sequence identity) and are also related to a gene encoding an 11-kDa protein in an entomopox virus of *Heliothis armigera*. The predicted size of Ac145 is longer (97 vs 77 aa) than previously reported (12). Close relatives of Ac145 are found in all baculovirus genomes including lepidopteran NPVs and GVs, and hymenopteran NPVs, but not the dipteran NPV. In contrast to Ac145, close relatives of Ac150 are only found in a few NPVs closely related to AcMNPV. However, it shows significant homology (E = ~10^{-3}) to predicted proteins from several dipteran insects. Ac145 and 150 are predicted to encode a domain thought to bind to chitin (539). In one study (540), deletion of Ac145 led to a six-fold drop in infectivity in *T. ni,* but not *H. virescens* larvae. An effect of deletion of Ac150 was not detected. Deletion of both genes causes a major (39-fold) reduction of infectivity for *H. virescens*. Injection of BV of the double mutant intrahemocoelically was as infectious as wt suggesting that these genes play a role in oral infection and are pif genes. Products of *ac145* and *ac150* were found to be associated with both BV and ODV and with ODV they localized to the envelope (540). In another study (541), occluded virions deleted for Ac150 were found to be significantly less virulent when administered per os than the wt virus in *Heliothis virescens*, *S. exigua* and *T. ni* larvae. Evidence suggested that the mutant had a reduction in its ability to establish primary infections in midgut cells. The Ac145 homolog in HearSNPV was found to bind to chitin (542). Hhpred (189) predicts Ac145 is structurally related to tachycitin, an antimicrobial chitin binding protein with a probability of almost 100%.

Ac146 (201aa:22.9kDa), (Bm122:201aa:22.9kDa), (Ha13:203aa: 22.9kDa).

Homologs of Ac146 are present in the genomes of all lepidopteran NPV and GV genomes, but are not present in those of hymenopteran or dipteran. It is likely an essential gene, as infectious BV was not produced by an mutant deleted for Ac146 (543). When Bm122 was fused with gfp, nuclear localization was observed (544). Ac146 is expressed at late times pi. Deletion resulted in a defective virus that did not produce BV. A HA-tagged Ac146 bacmid construct indicated that Ac146 was associated with both BV nucleocapsids, but not envelopes of ODV suggesting that it may be a structural protein. In BV it appeared as a polypeptide of 23kDa that conforms to its predicted mass, whereas in ODV, there were two sizes, one of 23kDa and the other of 34kDa. In addition, it was reported that Ac146 interacts with components of the ESCRT-III complex and may be involved in the release of nucleocapsids at the nuclear membrane(75).

Ac147 (582aa:66.9kDa), (Bm123:584aa:66.9kDa), (Ha14:655aa: 76.0kDa), immediate early gene-1 (IE-1).

Homologs of IE-1 have been identified in all Group I and II genomes sequenced. They also appear to be present in all GV genomes, but the homology is very low, e.g., XcGVorf9 vs. Ac147 show about 10% amino acid sequence identity. However, the orientation and position of XcGV orf9 relative to more conserved orfs is similar to Ac147, suggesting that the limited homology might be real. In addition, the limited identity is located in conserved regions that are identified by other more convincing alignments, e.g., Ac-Ie1 vs. Ld-Ie1 (23% identity). Part of the IE-1 population is called IE-0 and is translated from a larger spliced mRNA, which is the only major spliced transcript described for baculoviruses (see below Ac147-0). IE-1 was originally identified because of its ability to transactivate early promoters of AcMNPV (165). The ability of IE-1 to transactivate transcription is greatly enhanced when the activated gene is linked to *hr* sequences (545). It also may participate in the negative regulation of some genes (546). IE-1 is required for transient DNA replication (50, 51). Whereas deletions of either IE-1 or IE-0 can support infectious virus production, inactivation/deletion of both these genes is lethal (547). Similar results were reported for BmNPV (81).

Ac147-0 (636aa:72kDa) (ie-0).

Part of the IE-1 population is called IE-0 and is translated from a larger spliced mRNA, which is the only major spliced transcript described for baculoviruses and in AcMNPV results in an additional 54 amino acids at the N-terminus of IE-1 for a total of 636 amino acids (548). The 5' splice site is within Ac141 and the 3' site is upstream of the Ac147 ATG. This results in 38 amino acids being identical between Ac141 and Ac147-0 with an addition 16 aa derived from the RNA upstream of the Ac147 ATG. AcMNPV IE-1 is present as a homodimer but also can form a heterodimer with IE-0, and either IE-1 or IE-0 can support infectious virus production; however, there were subtle differences in timing of events and production of BV and polyhedra, depending on which gene is being expressed, suggesting that both *ie-0* and *ie-1* are required for wt levels of infection. As described above, *ie-0* can be eliminated, as long as IE-1 is being produced (547). It was found that IE0 preferentially activates a set of viral genes at very early times in infection and it is thought that this accelerates replication and BV production (549). IE0 has a repressor function that is caused by its interaction with ie1(550). In contrast to AcMNPV, in LdMNPV only the spliced form is able to transactivate transient transcription and DNA replication (551).

*Ac148 (376aa:40.9kDa), (Bm124:375aa:41.3kDa), (Ha15:354aa: 38.9kDa), odv-e56, PIF-5.

Homologs of odv-e56 are present in the genomes of all baculovirus and are also present in nudivirus genomes (245). ODV-E56 localizes to the envelopes of occluded virions (552) in AcMNPV and other baculoviruses (553) and has also been reported to be associated with AcMNPV BV (27). An insertion mutant, in which the lacZ gene was placed in frame at about amino acid 139 (out of 376) was viable (552). Ac148 and its homologs in HearNPV and CuniNPV were found to be ODV associated (89, 114, 269). Deletion of Ac148 from a bacmid construct resulted in a virus that could replicate in cell lines, but not insects. Consequently, it was concluded that Ac148 is a per os infectivity factor (554, 555). Similar conclusions were drawn for a BmNPV deletion of Bm124 (333).

Ac149 (107aa:12.4kDa), (Bm125:106aa:12.3kDa).

Homologs of Ac149 are present in 4 other Group I viruses closely related to AcMNPV (Bm-, Mavi-, Plxy-, and RoNPV). It is likely to be nonessential as BmNPV with insertion/deletion mutations of this gene (Bm125) appeared normal (15).

Ac150 (99aa:11.2kDa), (Bm126:115aa:13.4kDa), pif?

Ac150 is related to Ac145. In contrast to Ac145 homologs that are found in lepidopteran NPV and GV and hymenopteran NPV genomes, Ac150 is only found in a few Group I NPVs closely related to AcMNPV. in AcMNPV, deletion results in less infectivity by occluded virions for larvae (541). In BmNPV, deletion resulted in no apparent difference in BV production of mean lethal dose by occlusion bodies although the lethal time was extended somewhat (556). For more information, see Ac145 above. Hhpred (189) predicts Ac150 is structurally related to chitin binding proteins with a probability of almost 100%.

Ac151 (408aa:47kDa), (Bm127:422aa:48.8kDa), ie-2/ie-n.

Homologs of *ie-2* are limited to the genomes of most Group I lepidopteran NPVs. IE-2 contains a predicted RING finger domain and a coiled-coil domain and has ubiquitin ligase activity (557) and shows significant levels of similarity to a protein of *Trichomonas vaginalis* (E = 1e-06), an anaerobic, parasitic flagellated protozoan. IE-2 was found to augment activation by IE-1 (558-560). BmNPV IE-2 interacts with itself (561). IE-2 was required for optimal origin specific plasmid DNA replication in Sf-21 cells, but had little effect in Tn-368 cells (290). *Ie-2* deletion mutants behaved differently in Sf-21 cells in which the infection was delayed vs. Tn-5B1-4 cells, in which the infection was not delayed. In insect larvae, the mutant viruses were significantly less infectious than wt, which appeared to be due to a lack of virions in the occlusion bodies (562). IE-2 may also be involved in cell cycle regulation (563). IE2 has been shown to induce the expression of heat shock proteins and the facilitate transactivation by IE2 (564).

Ac152 (92aa:10.8kDa).

Homologs of Ac152 are present in the genomes of four Group I NPVs closely related to AcMNPV, and three Group II NPVs. It is associated with the nuclear localization of G-actin (412). Deletion resulted in reduced BV titers (43). Orthologs are not found in the BmNPV or HaSNPV genomes.

Ac153 (321aa:37.4kDa), (Bm128:309aa:36.1kDa), pe38.

Ac153 homologs have an unusual distribution being found in all Group I NPV and four GV genomes. Duplicate copies appear to be present in some of the genomes. Ac153 was originally identified because of its early transcription profile and the presence of predicted zinc finger and leucine zipper motifs (565). However, in OpMNPV it was shown to be expressed as full length (34 kda) and truncated (20 kda) forms with the larger variant functioning as a transcriptional transactivator of an early promoter (566). In addition, it appears to activate DNA replication in transient assays (50). Deletion of *pe38* results in a reduction in the expression of several genes, a delay in DNA replication, a 99% reduction in BV production, and reduced levels of DNA synthesis and was less orally infectious in larvae (567, 568). An 8-amino acid repeat in pe38 (DTVDDTVD) was shown cause insects to be resistant to the virus, whereas those lacking this sequence were sensitive to the virus (569). Hhpred (189) predicts that about 170 aa near the center of the orf is structurally related to E3 ubiquitin ligase with a probability of almost 100%. A RING finger domain is also predicted within this region.

Ac154 (81aa:9.4kDa), (Bm129:77aa:8.9kDa).

Homologs of Ac154 are present in 4 other Group I viruses closely related to AcMNPV (Bm-, Mavi-, Plxy-, and RoNPV). It is likely to be nonessential, as an insertion mutation of this gene in BmNPV (Bm129) had no apparent effect on infectivity (15).

Hrs (homologous regions).

In AcMNPV, *hrs* are comprised of repeated units of about 70-bp with an imperfect 30-bp palindrome near their center. They are repeated at eight locations in the genome with 2 to 8 repeats at each site. They are highly

variable, and although they are closely related within a genome, they may show very limited homology between different viruses. For example, in the CpGV genome, tandem repeated sequences are not evident, although a 75-bp imperfect palindrome is present at 13 different locations on the genome (570). In addition, in the TnSNPV (Group II) and several other genomes, *hrs* were not found (571), reviewed in (572). *Hrs* have been implicated both as transcriptional enhancers and origins of DNA replication for several baculoviruses (545, 573-577). They bind the transcriptional activator IE-1 (Ac147) (578-580) and this binding may cause IE-1 to localize into foci which may be a prelude to replication loci (581). *Hrs* contain a high concentration of cAMP and TPA response elements (CRE and TRE) that bind cellular transcription factors and stimulate RNA polymerase II dependent transcription and enhance activation by IE-1 (582). In AcMNPV, deletion of individual *hrs* or combinations of up to five *hrs* does not significantly affect virus replication in cultured cells. The deletion of 7 hrs resulted in a 10-fold reduction in BV titers, but DNA replication appeared normal, and polyhedra were still produced. However, when all eight hrs were removed, BV production was reduced by over 1000-fold, DNA replication was severely reduced, and few polyhedral were produced (583, 584).

References

1. Vail P, Sutter G, Jay D, Gough D. Recipocal infectivity of nuclear polyhedrosis viruses of the cabbage looper and alfalfa looper. J Invertebr Pathol. 1971;17:383–388.
2. Vaughn JL, Goodwin RH, Tompkins GJ, McCawley P. The establishment of two cell lines from the insect *Spodoptera frugiperda* (Lepidoptera: Noctuidae). In Vitro. 1977;13:213–217. PubMed PMID: 68913.
3. Hink WF. Established insect cell line from the cabbage looper, *Trichoplusia ni*. Nature. 1970;226:466–467. PubMed PMID: 16057320.
4. Smith GE, Summers MD, Fraser MJ. Production of human beta interferon in insect cells infected with a baculovirus expression vector. Mol Cell Biol. 1983;3:2156–2165. PubMed PMID: 6318086.
5. Pennock GD, Shoemaker C, Miller LK. Strong and regulated expression of *Escherichia coli* B-galactosidase in insect cells with a baculovirus vector. Mol Cell Biol. 1984;4:399–406. PubMed PMID: 6325875.
6. Ayres MD, Howard SC, Kuzio J, Lopez-Ferber M, Possee RD. The complete DNA sequence of *Autographa californica* nuclear polyhedrosis virus. Virology. 1994;202:586–605. PubMed PMID: 8030224.
7. Luckow VA, Lee SC, Barry GF, Olins PO. Efficient generation of infectious recombinant baculoviruses by site-specific transposon-mediated insertion of foreign genes into a baculovirus genome propagated in Escherichia coli. J Virol. 1993;67:4566–79. PubMed PMID: 8392598.
8. Bideshi DK, Federici BA. The Trichoplusia ni granulovirus helicase is unable to support replication of Autographa californica multicapsid nucleopolyhedrovirus in cells and larvae of T. ni. J Gen Virol. 2000;81:1593–9. PubMed PMID: 10811943.
9. Datsenko KA, Wanner BL. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc Natl Acad Sci U S A. 2000;97:6640–5. PubMed PMID: 10829079.
10. Lung OY, Cruz-Alvarez M, Blissard GW. Ac23, an envelope fusion protein homolog in the baculovirus Autographa californica multicapsid nucleopolyhedrovirus, is a viral pathogenicity factor. J Virol. 2003;77:328–39. PubMed PMID: 12477838.
11. Maghodia AB, Jarvis DL, Geisler C. Complete Genome Sequence of the Autographa californica Multiple Nucleopolyhedrovirus Strain E2. Genome Announc. 2014.:2. PubMed PMID: 25502662.
12. Harrison RL, Bonning BC. Comparative analysis of the genomes of Rachiplusia ou and Autographa californica multiple nucleopolyhedroviruses. J Gen Virol. 2003;84:1827–42. PubMed PMID: 12810877.
13. Lu A, Miller LK. Identification of three late expression factor genes within the 33.8- to 43.4-map-unit region of *Autographa californica* nuclear polyhedrosis virus. J Virol. 1994;68:6710–6718. PubMed PMID: 8084003.
14. Harrison RL, Lynn DE. Genomic sequence analysis of a nucleopolyhedrovirus isolated from the diamondback moth, Plutella xylostella. Virus Genes. 2007;35:857–73. PubMed PMID: 17671835.
15. Ono C, Kamagata T, Taka H, Sahara K, Asano S, Bando H. Phenotypic grouping of 141 BmNPVs lacking viral gene sequences. Virus Res. 2012;165:197–206. PubMed PMID: 22421381.

16. Gomi S, Majima K, Maeda S. Sequence analysis of the genome of Bombyx mori nucleopolyhedrovirus. J Gen Virol. 1999;80:1323–37. PubMed PMID: 10355780.
17. Chen X, IJkel W, Tarchini R, Sun X, Sandbrink H, Wang H, Peters S, Zuidema D, Lankhorst R, Vlak J, Hu Z. The sequence of the *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus genome. J Gen Virol. 2001;82:241–257. PubMed PMID: 11125177.
18. Kim D, Weaver RF. Transcriptional mapping and functional analysis of the protein tyrosine/serine phosphatase (PTPase) gene of the Autographa californica nuclear polyhedrosis virus. Virology. 1993;195:587–595. PubMed PMID: 8337833.
19. Sheng Z, Charbonneau H. The baculovirus Autographa californica encodes a protein tyrosine phosphatase. J Biol Chem. 1993;268:4728–4733. PubMed PMID: 8444848.
20. Gross CH, Shuman S. Characterization of a Baculovirus-Encoded RNA 5'-Triphosphatase. J Virol. 1998;72:7057–7063. PubMed PMID: 9696798.
21. Takagi T, Taylor GS, Kusakabe T, Charbonneau H, Buratowski S. A protein tyrosine phosphatase-like protein from baculovirus has RNA 5'-triphosphatase and diphosphatase activities. Proc Natl Acad Sci U S A. 1998;95:9808–12. PubMed PMID: 9707557.
22. Changela A, Martins A, Shuman S, Mondragón A. Crystal structure of baculovirus RNA triphosphatase complexed with phosphate. J Biol Chem. 2005;280:17848–56. PubMed PMID: 15713658.
23. Li Y, Miller LK. Properties of a baculovirus mutant defective in the protein phosphatase gene. J Virol. 1995;69:4533–4537. PubMed PMID: 7769718.
24. van Houte S, Ros VI, Mastenbroek TG, Vendrig NJ, Hoover K, Spitzen J, van Oers MM. Protein Tyrosine Phosphatase-Induced Hyperactivity Is a Conserved Strategy of a Subset of BaculoViruses to Manipulate Lepidopteran Host Behavior. PLoS One. 2012;7:e46933. PubMed PMID: 23077534.
25. Katsuma S, Koyano Y, Kang W, Kokusho R, Kamita SG, Shimada T. The baculovirus uses a captured host phosphatase to induce enhanced locomotory activity in host caterpillars. PLoS Pathog. 2012;8:e1002644. PubMed PMID: 22496662.
26. Wang F, Xue R, Li X, Hu C, Xia Q. Characterization of a protein tyrosine phosphatase as a host factor promoting baculovirus replication in silkworm, Bombyx mori. Dev Comp Immunol. 2016;57:31–7. PubMed PMID: 26684065.
27. Wang R, Deng F, Hou D, Zhao Y, Guo L, Wang H, Hu Z. Proteomics of the Autographa californica Nucleopolyhedrovirus Budded Virions. J Virol. 2010;84:7233–7242. PubMed PMID: 20444894.
28. Kamita SG, Nagasaka K, Chua JW, Shimada T, Mita K, Kobayashi M, Maeda S, Hammock BD. A baculovirus-encoded protein tyrosine phosphatase gene induces enhanced locomotory activity in a lepidopteran host. Proc Natl Acad Sci U S A. 2005;102:2584–9. PubMed PMID: 15699333.
29. Katsuma S. Phosphatase activity of Bombyx mori nucleopolyhedrovirus PTP is dispensable for enhanced locomotory activity in B. mori larvae. J Invertebr Pathol. 2015;132:228–32. PubMed PMID: 26550695.
30. Bideshi DK, Renault S, Stasiak K, Federici BA, Bigot Y. Phylogenetic analysis and possible function of bro-like genes, a multigene family widespread among large double-stranded DNA viruses of invertebrates and bacteria. J Gen Virol. 2003;84:2531–44. PubMed PMID: 12917475.
31. Asgari S, Davis J, Wood D, Wilson P, McGrath A. Sequence and organization of the Heliothis virescens ascovirus genome. J Gen Virol. 2007;88:1120–32. PubMed PMID: 17374755.
32. Kuzio J, Pearson MN, Harwood SH, Funk CJ, Evans JT, Slavicek J, Rohrmann GF. Sequence and analysis of the genome of a baculovirus pathogenic for *Lymantria dispar*. Virology. 1999;253:17–34. PubMed PMID: 9887315.
33. Lopez Ferber M, Argaud O, Croizier L, Croizier G. Diversity, distribution, and mobility of bro gene sequences in Bombyx mori nucleopolyhedrovirus. Virus Genes. 2001;22:247–54. PubMed PMID: 11450942.
34. Zhou JB, Li XQ, De-Eknamkul W, Suraporn S, Xu JP. Identification of a new Bombyx mori nucleopolyhedrovirus and analysis of its bro gene family. Virus Genes. 2012;44:539–47. PubMed PMID: 22311430.

35. Kang W, Suzuki M, Zemskov E, Okano K, Maeda S. Characterization of baculovirus repeated open reading frames (bro) in Bombyx mori nucleopolyhedrovirus. J Virol. 1999;73:10339–45. PubMed PMID: 10559352.
36. Zemskov EA, Kang W, Maeda S. Evidence for nucleic acid binding ability and nucleosome association of *Bombyx mori* nucleopolyhedrovirus BRO proteins. J Virol. 2000;74:6784–9. PubMed PMID: 10888617.
37. Kang WK, Imai N, Suzuki M, Iwanaga M, Matsumoto S, Zemskov EA. Interaction of Bombyx mori nucleopolyhedrovirus BRO-A and host cell protein laminin. Arch Virol. 2003;148:99–113. PubMed PMID: 12536298.
38. Shobahah J, Xue S, Hu D, Zhao C, Wei M, Quan Y, Yu W. Quantitative phosphoproteome on the silkworm (Bombyx mori) cells infected with baculovirus. Virol J. 2017;14:117. PubMed PMID: 28629377.
39. Shrestha A, Bao K, Chen YR, Chen W, Wang P, Fei Z, Blissard GW. Global Analysis of Baculovirus Autographa californica Multiple Nucleopolyhedrovirus Gene Expression in the Midgut of the Lepidopteran Host Trichoplusia ni. J Virol. 2018.:92. PubMed PMID: 30209166.
40. Olivera BM, Miljanich GP, Ramachandran J, Adams ME. Calcium channel diversity and neurotransmitter release: The w-conotoxins and w-agatoxins. Ann Rev Biochem. 1994;63:823–867. PubMed PMID: 7979255.
41. Eldridge R, Li Y, Miller LK. Characterization of a baculovirus gene encoding a small conotoxin-like polypeptide. J Virol. 1992;66:6563–6571. PubMed PMID: 1404603.
42. Cao Q, Zhu SY, Wu Y, Liu Y, Zhu J, Wang W. The Effect of a Small Conotoxin-Like ctx Gene from Autographa californica Nuclear Polyhedrosis Virus (AcMNPV) on Insect Hemolymph Melanization. Pol J Microbiol. 2012;61:183–189. PubMed PMID: 29334052.
43. Gandhi KM, Ohkawa T, Welch MD, Volkman LE. Nuclear localization of actin requires AC102 in Autographa californica multiple nucleopolyhedrovirus-infected cells. J Gen Virol. 2012;93:1795–803. PubMed PMID: 22592260.
44. Katsuma S, Kobayashi J, Koyano Y, Matsuda-Imai N, Kang W, Shimada T. Baculovirus-encoded protein BV/ODV-E26 determines tissue tropism and virulence in lepidopteran insects. J Virol. 2012;86:2545–55. PubMed PMID: 22190721.
45. Shen Y, Wang H, Xu W, Wu X. Bombyx mori nucleopolyhedrovirus orf133 and orf134 are involved in the embedding of occlusion-derived viruses into polyhedra. J Gen Virol. 2018;99:717–729. PubMed PMID: 29624165.
46. Wang X, Chen C, Zhang N, Li J, Deng F, Wang H, Vlak JM, Hu Z, Wang M. The group I alphabaculovirus-specific protein, AC5, is a novel component of the occlusion body but is not associated with ODVs or the PIF complex. J Gen Virol. 2018;99:585–595. PubMed PMID: 29465345.
47. Peng K, van Lent JW, Boeren S, Fang M, Theilmann DA, Erlandson MA, Vlak JM, van Oers MM. Characterization of novel components of the baculovirus per os infectivity factor complex. J Virol. 2012;86:4981–8. PubMed PMID: 22379094.
48. Zheng Q, Shen Y, Kon X, Zhang J, Feng M, Wu X. Protein-protein interactions of the baculovirus per os infectivity factors (PIFs) in the PIF complex. J Gen Virol. 2017;98:853–861. PubMed PMID: 28141488.
49. Evans JT, Leisy DJ, Rohrmann GF. Characterization of the interaction between the baculovirus replication factors, LEF-1 and LEF-2. J Virol. 1997;71:3114–3119. PubMed PMID: 9060674.
50. Kool M, Ahrens C, Goldbach RW, Rohrmann GF, Vlak JM. Identification of genes involved in DNA replication of the *Autographa californica* baculovirus. Proc Natl Acad Sci USA. 1994;91:11212–11216. PubMed PMID: 7972036.
51. Lu A, Miller LK. The roles of eighteen baculovirus late expression factor genes in transcription and DNA replication. J Virol. 1995;69:975–982. PubMed PMID: 7815565.
52. Wu CP, Huang YJ, Wang JY, Wu YL, Lo HR, Wang JC, Chao YC. Autographa californica multiple nucleopolyhedrovirus LEF-2 is a capsid protein required for amplification but not initiation of viral DNA replication. J Virol. 2010;84:5015–24. PubMed PMID: 20219928.
53. Mikhailov VS, Rohrmann GF. The Baculovirus Replication Factor LEF-1 is a DNA Primase. J Virol. 2002;76:2287–2297. PubMed PMID: 11836407.

54. Merrington CL, Kitts PA, King LA, Possee RD. An Autographa californica nucleopolyhedrovirus lef-2 mutant: consequences for DNA replication and very late gene expression. Virology. 1996;217:338–348. PubMed PMID: 8599220.
55. Sriram S, Gopinathan KP. The potential role of a late gene expression factor, lef2, from Bombyx mori nuclear polyhedrosis virus in very late gene transcription and DNA replication. Virology. 1998;251:108–22. PubMed PMID: 9813207.
56. Gearing KL, Possee RD. Functional analysis of a 603 nucleotide open reading frame upstream of the polyhedrin gene of Autographa californica nuclear polyhedrosis virus. J Gen Virol. 1990;71:251–62. PubMed PMID: 2407804.
57. Afonso CL, Tulman ER, Lu Z, Balinsky CA, Moser BA, Becnel JJ, Rock DL, Kutish GF. Genome sequence of a baculovirus pathogenic for Culex nigripalpus. J Virol. 2001;75:11157–65. PubMed PMID: 11602755.
58. Perera OP, Valles SM, Green TB, White S, Strong CA, Becnel JJ. Molecular analysis of an occlusion body protein from Culex nigripalpus nucleopolyhedrovirus (CuniNPV). J Invertebr Pathol. 2006;91:35–42. PubMed PMID: 16376931.
59. Jehle JA. The mosaic structure of the polyhedrin gene of the Autographa californica nucleopolyhedrovirus (AcMNPV). Virus Genes. 2004;29:5–8. PubMed PMID: 15215679.
60. Ji X, Sutton G, Evans G, Axford D, Owen R, Stuart DI. How baculovirus polyhedra fit square pegs into round holes to robustly package viruses. EMBO J. 2010;29:505–514. PubMed PMID: 19959989.
61. Gati C, Oberthuer D, Yefanov O, Bunker RD, Stellato F, Chiu E, Yeh SM, Aquila A, Basu S, Bean R, Beyerlein KR, Botha S, Boutet S, DePonte DP, Doak RB, Fromme R, Galli L, Grotjohann I, James DR, Kupitz C, Lomb L, Messerschmidt M, Nass K, Rendek K, Shoeman RL, Wang D, Weierstall U, White TA, Williams GJ, Zatsepin NA, Fromme P, Spence JC, Goldie KN, Jehle JA, Metcalf P, Barty A, Chapman HN. Atomic structure of granulin determined from native nanocrystalline granulovirus using an X-ray free-electron laser. Proc Natl Acad Sci U S A. 2017;114:2247–2252. PubMed PMID: 28202732.
62. Kitts PA, Possee RD. A method for producing recombinant baculovirus expression vectors at high frequency. BioTechniques. 1993;14:810–816. PubMed PMID: 8512707.
63. Vialard JE, Richardson CD. The 1,629-nucleotide open reading frame located downstream of the Autographa californica nuclear polyhedrosis virus polyhedrin gene encodes a nucleocapsid-associated phosphoprotein. J Virol. 1993;67:5859–5866. PubMed PMID: 8371345.
64. Russell RLQ, Funk CJ, Rohrmann GF. Association of a baculovirus encoded protein with the capsid basal region. Virology. 1997;227:142–152. PubMed PMID: 9007067.
65. Goley ED, Ohkawa T, Mancuso J, Woodruff JB, D'Alessio JA, Cande WZ, Volkman LE, Welch MD. Dynamic nuclear actin assembly by Arp2/3 complex and a baculovirus WASP-like protein. Science. 2006;314:464–7. PubMed PMID: 17053146.
66. Ohkawa T, Volkman LE, Welch MD. Actin-based motility drives baculovirus transit to the nucleus and cell surface. J Cell Biol. 2010;190:187–95. PubMed PMID: 20660627.
67. Reilly LM, Guarino LA. The pk-1 gene of Autographa californica multinucleocapsid nuclear polyhedrosis virus encodes a protein kinase. J Gen Virol. 1994;75:2999–3006. PubMed PMID: 7964609.
68. Liang C, Li M, Dai X, Zhao S, Hou Y, Zhang Y, Lan D, Wang Y, Chen X. Autographa californica multiple nucleopolyhedrovirus PK-1 is essential for nucleocapsid assembly. Virology. 2013. in press. PubMed PMID: 23768784.
69. Fan X, Thirunavukkarasu K, Weaver RF. Temperature-sensitive mutations in the protein kinase-1 (*pk-1*) gene of the *Autographa californica* nuclear polyhedrosis virus that block very late gene expression. Virology. 1996;224:1–9. PubMed PMID: 8862393.
70. Mishra G, Chadha P, Chaudhury I, Das RH. Inhibition of Autographa californica nucleopolyhedrovirus (AcNPV) polyhedrin gene expression by DNAzyme knockout of its serine/threonine kinase (pk1) gene. Virus Res. 2008;135:197–201. PubMed PMID: 18353480.
71. Mishra G, Chadha P, Das RH. Serine/threonine kinase (pk-1) is a component of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) very late gene transcription complex and it phosphorylates a 102kDa polypeptide of the complex. Virus Res. 2008;137:147–9. PubMed PMID: 18577405.

72. Mishra G, Gautam HK, Das RH. Serine/Threonine kinase dependent transcription from the polyhedrin promoter of SpltNPV-I. Biochem Biophys Res Commun. 2007;358:942–7. PubMed PMID: 17512903.
73. Liang C, Su X, Xu G, Dai X, Zhao S. Autographa californica multiple nucleopolyhedrovirus PK1 is a factor that regulates high-level expression of very late genes in viral infection. Virology. 2017;512:56–65. PubMed PMID: 28915406.
74. Tao XY, Choi JY, Kim WJ, An SB, Liu Q, Kim SE, Lee SH, Kim JH, Woo SD, Jin BR, Je YH. Autographa californica multiple nucleopolyhedrovirus ORF11 is essential for budded-virus production and occlusion-derived-virus envelopment. J Virol. 2015;89:373–83. PubMed PMID: 25320313.
75. Yue Q, Yu Q, Yang Q, Xu Y, Guo Y, Blissard GW, Li Z. Distinct Roles of Cellular ESCRT-I and ESCRT-III Proteins in Efficient Entry and Egress of Budded Virions of Autographa californica Multiple Nucleopolyhedrovirus. J Virol. 2018.:92. PubMed PMID: 29046462.
76. Costa Navarro GS, Amalfi S, Lopez MG, Llauger G, Arneodo JD, Taboga O, Alfonso V. The Autographa californica multiple nucleopolyhedrovirus Ac12: a non-essential F box-like protein that interacts with cellular SKP1 component of the E3 ubiquitin ligase complex. Virus Res. 2019;260:67–77. PubMed PMID: 30472094.
77. Soding J. Protein homology detection by HMM-HMM comparison. Bioinformatics. 2005;21:951–60. PubMed PMID: 15531603.
78. Zhou Y, Chen K, Yao Q, Shen H, Liang G, Li X, Wang N, Li Y. Characterization of a late expression gene of Bombyx mori nucleopolyhedrovirus. Z Naturforsch C. 2010;65:508–18. PubMed PMID: 20737922.
79. Kokusho R, Koh Y, Fujimoto M, Shimada T, Katsuma S. Bombyx mori nucleopolyhedrovirus BM5 protein regulates progeny virus production and viral gene expression. Virology. 2016;498:240–249. PubMed PMID: 27614700.
80. Nagamine T, Inaba T, Sako Y. A nuclear envelop-associated baculovirus protein promotes intranuclear lipid accumulation during infection. Virology. 2019;532:108–117. PubMed PMID: 31055062.
81. Gomi S, Zhou CE, Yih W, Majima K, Maeda S. Deletion analysis of four of eighteen late gene expression factor gene homologues of the baculovirus, BmNPV. Virology. 1997;230:35–47. PubMed PMID: 9126260.
82. Wang Y, Schwedes JF, Parks D, Mann K, Tegtmeyer P. Interaction of p53 with its consensus DNA-binding site. Mol Cell Biol. 1995;15:2157–65. PubMed PMID: 7891710.
83. O'Reilly DR, Miller LK. A baculovirus blocks insect molting by producing ecdysteroid UDP-glucosyl transferase. Science. 1989.:1110–1112. PubMed PMID: 2505387.
84. O'Reilly DR, Miller LK. Regulation of expression of a baculovirus ecdysteroid UDPglucosyltransferase gene. J Virol. 1990;64:1321–1328. PubMed PMID: 2106039.
85. O'Reilly DR, Miller LK. Improvement of a baculovirus pesticide by deletion of the egt gene. Bio/Technology. 1991;9:1086–1089.
86. Kumar S, Miller LK. Effects of serial passage of AcNPV in cell culture. Virus Res. 1987;7:335–349. PubMed PMID: 2887077.
87. Beniya H, Braunagel SC, Summers MD. Autographa californica nuclear polyhedrosis virus: subcellular localization and protein trafficking of BV/ODV-E26 to intranuclear membranes and viral envelopes. Virology. 1998;240:64–75. PubMed PMID: 9448690.
88. Burks JK, Summers MD, Braunagel SC. BV/ODV-E26: a palmitoylated, multifunctional structural protein of Autographa californica nucleopolyhedrovirus. Virology. 2007;361:194–203. PubMed PMID: 17169392.
89. Braunagel SC, Russell WK, Rosas-Acosta G, Russell DH, Summers MD. Determination of the protein composition of the occlusion-derived virus of Autographa californica nucleopolyhedrovirus. Proc Natl Acad Sci U S A. 2003;100:9797–802. PubMed PMID: 12904572.
90. Imai N, Kurihara M, Matsumoto S, Kang WK. Bombyx mori nucleopolyhedrovirus orf8 encodes a nucleic acid binding protein that colocalizes with IE1 during infection. Arch Virol. 2004;149:1581–94. PubMed PMID: 15290382.
91. Braunagel SC, Summers MD. Molecular Biology of the Baculovirus Occlusion-Derived Virus Envelope. Curr Drug Targets. 2007;8:1084–1095. PubMed PMID: 17979668.

92. Kang W, Imai N, Kawasaki Y, Nagamine T, Matsumoto S. IE1 and hr facilitate the localization of Bombyx mori nucleopolyhedrovirus ORF8 to specific nuclear sites. J Gen Virol. 2005;86:3031–8. PubMed PMID: 16227225.
93. Nie Y, Fang M, Theilmann DA. AcMNPV AC16 (DA26, BV/ODV-E26) regulates the levels of IE0 and IE1 and binds to both proteins via a domain located within the acidic transcriptional activation domain. Virology. 2009;385:484–95. PubMed PMID: 19150105.
94. Kang W, Katsuma S, Matsuda-Imai N, Kurihara M, Yoshiga T, Shimada T, Matsumoto S. Identification and characterization of host factors interacting with Bombyx mori nucleopolyhedrovirus ORF8. J Microbiol. 2012;50:469–77. PubMed PMID: 22752911.
95. O'Reilly DR, Passarelli AL, Goldman IF, Miller LK. Characterization of the DA26 gene in a hypervariable region of the Autographa californica nuclear polyhedrosis virus genome. J Gen Virol. 1990;71(Pt 5):1029–37. PubMed PMID: 2189022.
96. Hikida H, Kokusho R, Kobayashi J, Shimada T, Katsuma S. Inhibitory role of the Bm8 protein in the propagation of Bombyx mori nucleopolyhedrovirus. Virus Res. 2018;249:124–131. PubMed PMID: 29574100.
97. Nie Y, Theilmann DA. Deletion of AcMNPV AC16 and AC17 results in delayed viral gene expression in budded virus infected cells but not transfected cells. Virology. 2010;404:168–79. PubMed PMID: 20627351.
98. An SH, Xing LP, Shi WJ, Zhang CX. Characterization of Autographa californica multiple nucleopolyhedrovirus ORF17. Acta Virol. 2006;50:17–23. PubMed PMID: 16599181.
99. An SH, Wang D, Zhang-Nv Y, Guo ZJ, Xu HJ, Sun JX, Zhang CX. Characterization of a late expression gene, Open reading frame 128 of Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus. Arch Virol. 2005;150:2453–66. PubMed PMID: 16052278.
100. Yang ZN, Xu HJ, Thiem SM, Xu YP, Ge JQ, Tang XD, Tian CH, Zhang CX. Bombyx mori nucleopolyhedrovirus ORF9 is a gene involved in the budded virus production and infectivity. J Gen Virol. 2009;90:162–9. PubMed PMID: 19088285.
101. Wang Y, Wu W, Li Z, Yuan M, Feng G, Yu Q, Yang K, Pang Y. ac18 is not essential for the propagation of Autographa californica multiple nucleopolyhedrovirus. Virology. 2007;367:71–81. PubMed PMID: 17573091.
102. Zhu M, Wang J, Deng R, Wang X. Functional Regulation of an Autographa californica Nucleopolyhedrovirus-Encoded MicroRNA, AcMNPV-miR-1, in Baculovirus Replication. J Virol. 2016;90:6526–6537. PubMed PMID: 27147751.
103. Wang H, Xu W, Kong X, Fan Y, Wu X. Bombyx mori nucleopolyhedrovirus protein Bm11 is involved in occlusion body production and occlusion-derived virus embedding. Virology. 2018;527:12–20. PubMed PMID: 30447410.
104. Roncarati R, Knebel-Morsdorf D. Identification of the early actin-rearrangement-inducing factor gene, arif-1, from Autographa californica multicapsid nuclear polyhedrosis virus. J Virol. 1997;71:7933–41. PubMed PMID: 9311884.
105. Dreschers S, Roncarati R, Knebel-Morsdorf D. Actin rearrangement-inducing factor of baculoviruses is tyrosine phosphorylated and colocalizes to F-actin at the plasma membrane. J Virol. 2001;75:3771–8. PubMed PMID: 11264366.
106. Kokusho R, Kawamoto M, Koyano Y, Sugano S, Suzuki Y, Shimada T, Katsuma S. Bombyx mori nucleopolyhedrovirus actin rearrangement-inducing factor 1 enhances systemic infection in B. mori larvae. J Gen Virol. 2015;96:1938–46. PubMed PMID: 25809914.
107. Pijlman GP, Dortmans JC, Vermeesch AM, Yang K, Martens DE, Goldbach RW, Vlak JM. Pivotal role of the non-hr origin of DNA replication in the genesis of defective interfering baculoviruses. J Virol. 2002;76:5605–11. PubMed PMID: 11991989.
108. Ohkawa T, Washburn JO, Sitapara R, Sid E, Volkman LE. 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. 2005;79:15258–64. PubMed PMID: 16306597.

109. Cheng C, Liu S, Chow T, Hsiao Y, Wang D, Huang J, H C. Analysis of the Complete Genome Sequence of the Hz-1 Virus Suggests that It Is Related to Members of the Baculoviridae. J Virol. 2002;76:9024–9034. PubMed PMID: 12186886.
110. Gauthier L, Cornman S, Hartmann U, Cousserans F, Evans JD, de Miranda JR, Neumann P. The Apis mellifera Filamentous Virus Genome. Viruses. 2015;7:3798–815. PubMed PMID: 26184284.
111. Pearson MN, Rohrmann GF. Transfer, incorporation, and substitution of envelope fusion proteins among members of the Baculoviridae, Orthomyxoviridae, and Metaviridae (insect retrovirus) families. J Virol. 2002;76:5301–5304. PubMed PMID: 11991958.
112. Malik HS, Henikoff S. Positive Selection of Iris, a Retroviral Envelope-Derived Host Gene in Drosophila melanogaster. PLoS Genet. 2005;1:429–43. PubMed PMID: 16244705.
113. Pearson MN, Russell R, Rohrmann GF. Characterization of a baculovirus encoded protein that is associated with infected-cell membranes and budded virions. Virology. 2001;291:22–31. PubMed PMID: 11878873.
114. Perera O, Green TB, Stevens SM Jr, White S, Becnel JJ. Proteins associated with Culex nigripalpus nucleopolyhedrovirus occluded virions. J Virol. 2007;81:4585–90. PubMed PMID: 17301145.
115. Wang M, Tan Y, Yin F, Deng F, Vlak JM, Hu Z, Wang H. The F-like protein Ac23 enhances the infectivity of the budded virus of gp64-null AcMNPV pseudotyped with baculovirus envelope fusion protein F. J Virol. 2008;82:9800–4. PubMed PMID: 18653446.
116. Wang M, Wang J, Yin F, Tan Y, Deng F, Chen X, Jehle JA, Vlak JM, Hu Z, Wang H. Unraveling the entry mechanism of baculoviruses and its evolutionary implications. J Virol. 2014;88:2301–11. PubMed PMID: 24335309.
117. Xu W, Fan Y, Wang H, Feng M, Wu X. Bombyx mori nucleopolyhedrovirus F-like protein Bm14 affects the morphogenesis and production of occlusion bodies and the embedding of ODVs. Virology. 2018;526:61–71. PubMed PMID: 30342303.
118. Fan X, McLachlin JR, Weaver RF. Identification and characterization of a protein kinase-interacting protein encoded by the Autographa californica nuclear polyhedrosis virus. Virology. 1998;240:175–182. PubMed PMID: 9454690.
119. McLachlin JR, Yang S, Miller LK. A baculovirus mutant defective in PKIP, a protein which interacts with a virus-encoded protein kinase. Virology. 1998;246:379–91. PubMed PMID: 9657956.
120. Mikhailov VS, Vanarsdall AL, Rohrmann GF. Isolation and characterization of the DNA-binding protein (DBP) of the Autographa californica multiple nucleopolyhedrovirus. Virology. 2007;370:415–29. PubMed PMID: 17935748.
121. Vanarsdall AL, Mikhailov VS, Rohrmann GF. Characterization of a baculovirus lacking the DBP (DNA-binding protein) gene. Virology. 2007;364:475–85. PubMed PMID: 17449080.
122. Zhao C, Zhang C, Chen B, Shi Y, Quan Y, Nie Z, Zhang Y, Yu W. A DNA Binding Protein Is Required for Viral Replication and Transcription in Bombyx mori Nucleopolyhedrovirus. PLoS One. 2016;11:e0159149. PubMed PMID: 27414795.
123. Shen H, Zhou Y, Zhang W, Nin B, Wang H, Wang X, Shao S, Chen H, Guo Z, Liu X, Yao Q, Chen K. Characterization of Bombyx mori nucleopolyhedrovirus with a knockout of Bm17. Cytotechnology. 2012;64:711–8. PubMed PMID: 22476564.
124. Shen H, Wang R, Han Q, Zhang W, Nin B, Zhou Y, Shao S, Yao Q, Chen K, Liu X. Characterization of Bombyx mori nucleopolyhedrovirus Bm17. J Basic Microbiol. 2013;53:808–14. PubMed PMID: 22961820.
125. Esteban DJ, Da Silva M, Upton C. New bioinformatics tools for viral genome analyses at Viral Bioinformatics - Canada. Pharmacogenomics. 2005;6:271–80. PubMed PMID: 16013958.
126. Clem RJ. Viral IAPs, then and now. Semin Cell Dev Biol. 2015;39:72–9. PubMed PMID: 25652775.
127. McLachlin JR, Escobar JC, Harrelson JA, Clem RJ, Miller LK. Deletions in the Ac-iap1 gene of the baculovirus AcMNPV occur spontaneously during serial passage and confer a cell line-specific replication advantage. Virus Res. 2001;81:77–91. PubMed PMID: 11682127.

128. Zeng X, Nan F, Liang C, Song J, Wang Q, Vlak JM, Chen X. Functional analysis of the Autographa californica nucleopolyhedrovirus IAP1 and IAP2. Sci China C Life Sci. 2009;52:761–70. PubMed PMID: 19727595.
129. Maguire T, Harrison P, Hyink O, Kalmakoff J, Ward VK. The inhibitors of apoptosis of Epiphyas postvittana nucleopolyhedrovirus. J Gen Virol. 2000;81:2803–11. PubMed PMID: 11038395.
130. Ikeda M, Yamada H, Ito H, Kobayashi M. Baculovirus IAP1 induces caspase-dependent apoptosis in insect cells. J Gen Virol. 2011;92:2654–63. PubMed PMID: 21795471.
131. Passarelli AL, Miller LK. Identification and transcriptional regulation of the baculovirus lef-6 gene. J Virol. 1994;68:4458–4467. PubMed PMID: 8207819.
132. Lin G, Blissard GW. Analysis of an Autographa californica nucleopolyhedrovirus lef-11 knockout: LEF-11 is essential for viral DNA replication. J Virol. 2002;76:2770–9. PubMed PMID: 11861844.
133. Teplova M, Wohlbold L, Khin NW, Izaurralde E, Patel DJ. Structure-function studies of nucleocytoplasmic transport of retroviral genomic RNA by mRNA export factor TAP. Nat Struct Mol Biol. 2011;18:990–8. PubMed PMID: 21822283.
134. Afonso CL, Tulman ER, Lu Z, Oma E, Kutish GF, Rock DL. The genome of Melanoplus sanguinipes entomopoxvirus. J Virol. 1999;73:533–52. PubMed PMID: 9847359.
135. Huang J, Hao B, Deng F, Sun X, Wang H, Hu Z. Open reading frame Bm21 of Bombyx mori nucleopolyhedrovirus is not essential for virus replication in vitro, but its deletion extends the median survival time of infected larvae. J Gen Virol. 2008;89:922–30. PubMed PMID: 18343833.
136. Tomalski MD, Eldridge R, Miller LK. A baculovirus homolog of a Cu/Zn superoxide dismutase gene. Virology. 1991.:149–161. PubMed PMID: 1871962.
137. Bergin D, Reeves EP, Renwick J, Wientjes FB, Kavanagh K. Superoxide production in Galleria mellonella hemocytes: identification of proteins homologous to the NADPH oxidase complex of human neutrophils. Infect Immun. 2005;73:4161–70. PubMed PMID: 15972506.
138. Katsuma S, Kawaoka S, Mita K, Shimada T. Genome-wide survey for baculoviral host homologs using the Bombyx genome sequence. Insect Biochem Mol Biol. 2008;38:1080–6. PubMed PMID: 19280699.
139. Popovici C, Roubin R, Coulier F, Birnbaum D. An evolutionary history of the FGF superfamily. Bioessays. 2005;27:849–57. PubMed PMID: 16015590.
140. Detvisitsakun C, Berretta MF, Lehiy C, Passarelli AL. Stimulation of cell motility by a viral fibroblast growth factor homolog: proposal for a role in viral pathogenesis. Virology. 2005;336:308–17. PubMed PMID: 15892971.
141. Katsuma S, Daimon T, Mita K, Shimada T. Lepidopteran ortholog of Drosophila breathless is a receptor for the baculovirus fibroblast growth factor. J Virol. 2006;80:5474–81. PubMed PMID: 16699027.
142. Katsuma S, Shimada T, Kobayashi M. Characterization of the baculovirus Bombyx mori nucleopolyhedrovirus gene homologous to the mammalian FGF gene family. Virus Genes. 2004;29:211–7. PubMed PMID: 15284481.
143. Detvisitsakun C, Hutfless EL, Berretta MF, Passarelli AL. Analysis of a baculovirus lacking a functional viral fibroblast growth factor homolog. Virology. 2006;346:258–65. PubMed PMID: 16476460.
144. Detvisitsakun C, Cain EL, Passarelli AL. The Autographa californica M nucleopolyhedrovirus fibroblast growth factor accelerates host mortality. Virology. 2007;365:70–8. PubMed PMID: 17459443.
145. Katsuma S, Horie S, Daimon T, Iwanaga M, Shimada T. In vivo and in vitro analyses of a Bombyx mori nucleopolyhedrovirus mutant lacking functional vfgf. Virology. 2006;355:62–70. PubMed PMID: 16904150.
146. Yin F, Du R, Kuang W, Yang G, Wang H, Deng F, Hu Z, Wang M. Characterization of the viral fibroblast growth factor homolog of Helicoverpa armigera single nucleopolyhedrovirus. Virol Sin. 2016;31:240–8. PubMed PMID: 27142667.
147. Means JC, Passarelli AL. Viral fibroblast growth factor, matrix metalloproteases, and caspases are associated with enhancing systemic infection by baculoviruses. Proc Natl Acad Sci U S A. 2010;107:9825–30. PubMed PMID: 20457917.

148. Wang Y, Addess KJ, Chen J, Geer LY, He J, He S, Lu S, Madej T, Marchler-Bauer A, Thiessen PA, Zhang N, Bryant SH. MMDB: annotating protein sequences with Entrez's 3D-structure database. Nucleic Acids Res. 2007;35:D298–300. PubMed PMID: 17135201.
149. Lauzon HA, Jamieson PB, Krell PJ, Arif BM. Gene organization and sequencing of the Choristoneura fumiferana defective nucleopolyhedrovirus genome. J Gen Virol. 2005;86:945–61. PubMed PMID: 15784888.
150. Cai Y, Long Z, Qiu J, Yuan M, Li G, Yang K. An ac34 Deletion Mutant of Autographa californica Nucleopolyhedrovirus Exhibits Delayed Late Gene Expression and a Lack of Virulence In Vivo. J Virol. 2012;86:10432–43. PubMed PMID: 22787232.
151. Salem TZ, Zhang F, Thiem SM. Reduced expression of Autographa californica nucleopolyhedrovirus ORF34, an essential gene, enhances heterologous gene expression. Virology. 2012;435:225–38. PubMed PMID: 23131351.
152. Mu J, Zhang Y, Hu Y, Hu X, Zhou Y, Chen X, Wang Y. The role of viral protein Ac34 in nuclear relocation of subunits of the actin-related protein 2/3 complex. Virol Sin. 2016;31:480–489. PubMed PMID: 27900558.
153. Qiu J, Tang Z, Yuan M, Wu W, Yang K. The 91-205 amino acid region of AcMNPV ORF34 (Ac34), which comprises a potential C3H zinc finger, is required for its nuclear localization and optimal virus multiplication. Virus Res. 2017;228:79–89. PubMed PMID: 27894868.
154. Zhang B, Liang A, Fu Y. Ac34 protein of AcMNPV promoted progeny virus production and induced the apoptosis in host Sf9 cells. Biotechnol Lett. 2019;41:147–158. PubMed PMID: 30421092.
155. Ma SS, Zhang Z, Xia HC, Chen L, Yang YH, Yao Q, Chen KP. Evolutionary analysis of the ubiquitin gene of baculovirus and insect hosts. Genet Mol Res. 2015;14:9963–73. PubMed PMID: 26345932.
156. Garavaglia MJ, Miele SA, Iserte JA, Belaich MN, Ghiringhelli PD. Ac53, ac78, ac101 and ac103 are newly discovered core genes in the family Baculoviridae. J Virol. 2012;86:12069–79. PubMed PMID: 22933288.
157. Guarino LA. Identification of a viral gene encoding a ubiquitin-like protein. Proc Natl Acad Sci U S A. 1990;87:409–13. PubMed PMID: 2153300.
158. Lin XA, Chen Y, Xu WH, Yi YZ, Zhang ZF. Characterization of the Autographa californica nucleopolyhedrovirus ubiquitin gene promoter. Z Naturforsch [C]. 2008;63:277–83. PubMed PMID: 18533474.
159. Guo ZJ, Zhu YM, Li GH, Chen KP, Zhang CX. Ubiquitins of Bombyx mori nucleopolyhedrovirus and Helicoverpa armigera nucleopolyhedrovirus show distinct subcellular localization in infected cells. Acta Virol. 2011;55:101–6. PubMed PMID: 21692557.
160. Guarino LA, Smith G, Dong W. Ubiquitin is attached to membranes of baculovirus particles by a novel type of phospholipid anchor. Cell. 1995;80:301–309. PubMed PMID: 7834750.
161. Reilly LM, Guarino LA. The viral ubiquitin gene of Autographa californica nuclear polyhedrosis virus is not essential for viral replication. Virology. 1996;218:243–7. PubMed PMID: 8615029.
162. Haas AL, Katzung DJ, Reback PM, Guarino LA. Functional characterization of the ubiquitin variant encoded by the baculovirus Autographa californica. Biochemistry. 1996;35:5385–94. PubMed PMID: 8611528.
163. Biswas S, Willis LG, Fang M, Nie Y, Theilmann DA. Autographa californica Nucleopolyhedrovirus AC141 (Exon0), a Potential E3 Ubiquitin Ligase, Interacts with Viral Ubiquitin and AC66 To Facilitate Nucleocapsid Egress. J Virol. 2018.:92. PubMed PMID: 29142135.
164. Li Z, Gong Y, Yin C, Wang L, Li C, Pang Y. Characterization of a novel ubiquitin-fusion gene Uba256 from Spodoptera litura nucleopolyhedrovirus. Gene. 2003;303:111–9. PubMed PMID: 12559572.
165. Guarino LA, Summers MD. Functional mapping of a trans-activating gene required for expression of a baculovirus delayed-early gene. J Virol. 1986;57:563–57l. PubMed PMID: 3944847.
166. Guarino LA, Dong W, Xu B, Broussard DR, Davis RW, Jarvis DL. The baculovirus phosphoprotein pp31 is associated with the virogenic stroma. J Virol. 1992;66:7113–7120. PubMed PMID: 1433508.
167. Guarino LA, Mistretta TA, Dong W. DNA binding activity of the baculovirus late expression factor PP31. Virus Res. 2002;90:187–95. PubMed PMID: 12457973.

168. Broussard DR, Guarino LA, Jarvis DL. Dynamic phosphorylation of Autographa californica nuclear polyhedrosis virus pp31. J Virol. 1996;70:6767–74. PubMed PMID: 8794314.
169. Broussard DR, Guarino LA, Jarvis DL. Mapping functional domains in AcMNPV pp31. Virology. 1996;222:318–31. PubMed PMID: 8806516.
170. Todd JW, Passarelli AL, Miller LK. Eighteen baculovirus genes, including *lef-11, p35, 39K*, and *p47*, support late gene expression. J Virol. 1995;69:968–974. PubMed PMID: 7815564.
171. Yamagishi J, Burnett ED, Harwood SH, Blissard GW. The AcMNPV pp31 gene is not essential for productive AcMNPV replication or late gene transcription but appears to increase levels of most viral transcripts. Virology. 2007;365:34–47. PubMed PMID: 17467768.
172. Dong ZQ, Hu N, Zhang J, Chen TT, Cao MY, Li HQ, Lei XJ, Chen P, Lu C, Pan MH. Oligomerization of Baculovirus LEF-11 Is Involved in Viral DNA Replication. PLoS One. 2015;10:e0144930. PubMed PMID: 26660313.
173. Dong ZQ, Hu N, Dong FF, Chen TT, Jiang YM, Chen P, Lu C, Pan MH. Baculovirus LEF-11 Hijack Host ATPase ATAD3A to Promote Virus Multiplication in Bombyx mori cells. Sci Rep. 2017;7:46187. PubMed PMID: 28393927.
174. Zhang J, Dong ZQ, Zhang CD, He Q, Chen XM, Cao MY, Li HQ, Xiao WF, Lu C, Pan MH. Identification of a novel nuclear localization signal of baculovirus late expression factor 11. Virus Res. 2014;184:111–9. PubMed PMID: 24607592.
175. Chen T, Dong Z, Hu N, Hu Z, Dong F, Jiang Y, Li J, Chen P, Lu C, Pan M. Baculovirus LEF-11 nuclear localization signal is important for viral DNA replication. Virus Res. 2017;238:133–140. PubMed PMID: 28648848.
176. Mildvan AS, Xia Z, Azurmendi HF, Saraswat V, Legler PM, Massiah MA, Gabelli SB, Bianchet MA, Kang LW, Amzel LM. Structures and mechanisms of Nudix hydrolases. Arch Biochem Biophys. 2005;433:129–43. PubMed PMID: 15581572.
177. Ge J, Wei Z, Huang Y, Yin J, Zhou Z, Zhong J. AcMNPV ORF38 protein has the activity of ADP-ribose pyrophosphatase and is important for virus replication. Virology. 2007;361:204–11. PubMed PMID: 17174373.
178. Chen HQ, Zhou YJ, Chen KP, Yu Q. Expression and functional validation of Bombyx mori nucleopolyhedrovirus ORF29, a conserved Nudix motif protein. Acta Virol. 2013;57:442–6. PubMed PMID: 24294958.
179. Parrish S, Resch W, Moss B. Vaccinia virus D10 protein has mRNA decapping activity, providing a mechanism for control of host and viral gene expression. Proc Natl Acad Sci U S A. 2007;104:2139–44. PubMed PMID: 17283339.
180. Parrish S, Moss B. Characterization of a vaccinia virus mutant with a deletion of the D10R gene encoding a putative negative regulator of gene expression. J Virol. 2006;80:553–61. PubMed PMID: 16378957.
181. Wang D, An SH, Guo ZJ, Xu HJ, Zhang CX. Characterization of Helicoverpa armigera nucleopolyhedrovirus orf33 that encodes a novel budded virion derived protein, BV-e31. Arch Virol. 2005;150:1505–15. PubMed PMID: 15834653.
182. Yu M, Carstens EB. Characterization of an Autographa californica multiple nucleopolyhedrovirus mutant lacking the ac39(p43) gene. Virus Res. 2011;155:300–6. PubMed PMID: 20974197.
183. Partington S, Yu H, Lu A, Carstens EB. Isolation of temperature sensitive mutants of Autographa californica nuclear polyhedrosis virus: phenotype characterization of baculovirus mutants defective in very late gene expression. Virology. 1990;175:91–102. PubMed PMID: 2408231.
184. Carstens EB, Lu AL, Chan HLB. Sequence, transcriptional mapping, and overexpression of p47, a baculovirus gene regulating late gene expression. J Virol. 1993;67:2513–2520. PubMed PMID: 8474157.
185. Guarino LA, Xu B, Jin J, Dong W. A virus-encoded RNA polymerase purified from baculovirus-infected cells. J Virol. 1998;72:7985–7991. PubMed PMID: 9733837.
186. Rapp JC, Wilson JA, Miller LK. Nineteen baculovirus open reading frames, including LEF-12, support late gene expression. J Virol. 1998;72:10197–206. PubMed PMID: 9811761.

187. Li L, Harwood SH, Rohrmann GF. Identification of additional genes that influence baculovirus late gene expression. Virology. 1999;255:9–19. PubMed PMID: 10049816.
188. Guarino LA, Mistretta TA, Dong W. Baculovirus lef-12 is not required for viral replication. J Virol. 2002;76:12032–43. PubMed PMID: 12414945.
189. Zimmermann L, Stephens A, Nam SZ, Rau D, Kubler J, Lozajic M, Gabler F, Soding J, Lupas AN, Alva V. A Completely Reimplemented MPI Bioinformatics Toolkit with a New HHpred Server at its Core. J Mol Biol. 2018;430:2237–2243. PubMed PMID: 29258817.
190. Louder RK, He Y, Lopez-Blanco JR, Fang J, Chacon P, Nogales E. Structure of promoter-bound TFIID and model of human pre-initiation complex assembly. Nature. 2016;531:604–9. PubMed PMID: 27007846.
191. Katsuma S, Fujii T, Kawaoka S, Shimada T. Bombyx mori nucleopolyhedrovirus SNF2 global transactivator homologue (Bm33) enhances viral pathogenicity in B. mori larvae. J Gen Virol. 2008;89:3039–46. PubMed PMID: 19008391.
192. Katsuma S, Shimada T. Bombyx mori nucleopolyhedrovirus ORF34 is required for efficient transcription of late and very late genes. Virology. 2009;392:230–7. PubMed PMID: 19651423.
193. Tao XY, Choi JY, Wang Y, Roh JY, Lee JH, Liu Q, Park JB, Kim JS, Kim W, Je YH. Functional characterization of Autographa californica multiple nucleopolyhedrovirus ORF43 and phenotypic changes of ORF43-knockout mutant. J Microbiol. 2013;51:515–21. PubMed PMID: 23990304.
194. Sugiura N, Setoyama Y, Chiba M, Kimata K, Watanabe H. Baculovirus envelope protein ODV-E66 is a novel chondroitinase with distinct substrate specificity. J Biol Chem. 2011;286:29026–34. PubMed PMID: 21715327.
195. Hong T, Braunagel SC, Summers MD. Transcription, translation, and cellular localization of PDV-E66: A structural protein of the PDV envelope of Autographa californica nuclear polyhedrosis virus. Virology. 1994;204:210–224. PubMed PMID: 8091653.
196. Hong T, Summers M, Braunagel S. N-terminal sequences from Autographa californica nuclear polyhedrosis virus envelope proteins ODV-E66 and ODV-E25 are sufficient to direct reporter proteins to the nuclear envelope, intranuclear microvesicles and the envelope of occlusion derived virus. Proc Natl Acad Sci U S A. 1997;8:4050–4055. PubMed PMID: 9108103.
197. Kawaguchi Y, Sugiura N, Onishi M, Kimata K, Kimura M, Kakuta Y. Crystallization and X-ray diffraction analysis of chondroitin lyase from baculovirus: envelope protein ODV-E66. Acta Crystallogr Sect F Struct Biol Cryst Commun. 2012;68:190–2. PubMed PMID: 22297996.
198. Sugiura N, Ikeda M, Shioiri T, Yoshimura M, Kobayashi M, Watanabe H. Chondroitinase from baculovirus Bombyx mori nucleopolyhedrovirus and chondroitin sulfate from silkworm Bombyx mori. Glycobiology. 2013;23:1520–30. PubMed PMID: 24052236.
199. Gomi S, Kamita SG, Maeda S. Deletion analysis of all genes of Bombyx mori nucleopolyhedrovirus (BmNPV). RIKEN Review. 1999;22:39–41.
200. Xiang X, Chen L, Hu X, Yu S, Yang R, Wu X. Autographa californica multiple nucleopolyhedrovirus odv-e66 is an essential gene required for oral infectivity. Virus Res. 2011;158:72–8. PubMed PMID: 21440017.
201. Sparks WO, Rohlfing A, Bonning BC. 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. 2011;92:1051–60. PubMed PMID: 21228132.
202. Claussen M, Koch R, Jin ZY, Suter B. Functional characterization of Drosophila Translin and Trax. Genetics. 2006;174:1337–47. PubMed PMID: 17028328.
203. Crawford AM, Miller LK. Characterization of an early gene accelerating expression of late genes of the baculovirus Autographa californica nuclear polyhedrosis virus. J Virol. 1988;62:2773–2781. PubMed PMID: 3292791.
204. Johnson A, O'Donnell M. CELLULAR DNA REPLICASES: Components and Dynamics at the Replication Fork. Ann Rev Biochem. 2005;74:283–315. PubMed PMID: 15952889.
205. Iwahori S, Ikeda M, Kobayashi M. Generation and characterization of *Autographa californica* nucleopolyhedrovirus mutants defective in *pcna* gene homologoue. J Insect Biotechnol Sericology. 2002;71:129–139.

206. Iwahori S, Ikeda M, Kobayashi M. Association of Sf9 cell proliferating cell nuclear antigen with the DNA replication site of Autographa californica multicapsid nucleopolyhedrovirus. J Gen Virol. 2004;85:2857–62. PubMed PMID: 15448347.
207. Fu Y, Wang R, Liang A. Function analysis of Ac-PCNA and Sf-PCNA during the Autographa californica multiple nucleopolyhedrovirus infection process. Mol Cell Biochem. 2018;443:57–68. PubMed PMID: 29075988.
208. Passarelli AL, Todd JW, Miller LK. A baculovirus gene involved in late gene expression predicts a large polypeptide with a conserved motif of RNA polymerases. J Virol. 1994;68:4673–4678. PubMed PMID: 8207843.
209. Titterington JS, Nun TK, Passarelli AL. Functional dissection of the baculovirus late expression factor-8 gene: sequence requirements for late gene promoter activation. J Gen Virol. 2003;84:1817–26. PubMed PMID: 12810876.
210. Crouch EA, Cox LT, Morales KG, Passarelli AL. Inter-subunit interactions of the Autographa californica M nucleopolyhedrovirus RNA polymerase. Virology. 2007;367:265–74. PubMed PMID: 17604071.
211. Shikata M, Sano Y, Hashimoto Y, Matsumoto T. Isolation and characterization of a temperature-sensitive mutant of Bombyx mori nucleopolyhedrovirus for a putative RNA polymerase gene. J Gen Virol. 1998;79(Pt 8):2071–8. PubMed PMID: 9714259.
212. Gauthier D, Thirunavukkarasu K, Faris BL, Russell DL, Weaver RF. Characterization of an Autographa californica multiple nucleopolyhedrovirus dual mutant: ORF82 is required for budded virus production, and a point mutation in LEF-8 alters late and abolishes very late transcription. J Gen Virol. 2012;93:364–73. PubMed PMID: 22031528.
213. Ioannidis K, Swevers L, Iatrou K. Bombyx mori nucleopolyhedrovirus lef8 gene: effects of deletion and implications for gene transduction applications. J Gen Virol. 2016;97:786–96. PubMed PMID: 26701681.
214. Langer T, Lu C, Echols H, Flanagan J, Hayer MK, Hartl FU. Successive action of DnaK, DnaJ and GroEL along the pathway of chaperone-mediated protein folding. Nature. 1992;356:683–9. PubMed PMID: 1349157.
215. Xu HJ, Liu YH, Yang ZN, Zhang CX. Characterization of ORF39 from Helicoverpa armigera single-nucleocapsid nucleopolyhedrovirus, the gene containing RNA recognition motif. J Biochem Mol Biol. 2006;39:263–9. PubMed PMID: 16756754.
216. Shen Y, Feng M, Wu X. Bombyx mori nucleopolyhedrovirus ORF40 is essential for budded virus production and occlusion-derived virus envelopment. J Gen Virol. 2018;99:837–850. PubMed PMID: 29676725.
217. Qiu J, Tang Z, Cai Y, Wu W, Yuan M, Yang K. The Autographa californica multiple nucleopolyhedrovirus ac51 gene is required for efficient nuclear egress of nucleocapsids and is essential for in vivo virulence. J Virol. 2019.:93. PubMed PMID: 30429334.
218. Tian CH, Zhao JF, Xu YP, Xue J, Zhang BQ, Cui YJ, Zhang MJ, Bao YY, Zhang CX. Involvement of Bombyx mori nucleopolyhedrovirus ORF41 (Bm41) in BV production and ODV envelopment. Virology. 2009;387:184–92. PubMed PMID: 19249804.
219. Liu C, Li Z, Wu W, Li L, Yuan M, Yang K, Pang Y. Autographa californica multiple nucleopolyhedrovirus ac53 plays a role in nucleocapsid assembly. Virology. 2008;382:59–68. PubMed PMID: 18851866.
220. Xu X, Zhou X, Nan H, Zhao Y, Bai Y, Ou Y, Chen H. Aggregation of AcMNPV LEF-10 and Its Impact on Viral Late Gene Expression. PLoS One. 2016;11:e0154835. PubMed PMID: 27152613.
221. Nan H, Chen H, Tuite MF, Xu X. A viral expression factor behaves as a prion. Nat Commun. 2019;10:359. PubMed PMID: 30664652.
222. Olszewski J, Miller LK. Identification and characterization of a baculovirus structural protein, VP1054, required for nucleocapsid formation. J Virol. 1997;71:5040–5050. PubMed PMID: 9188569.
223. Wu W, Liang H, Kan J, Liu C, Yuan M, Liang C, Yang K, Pang Y. Autographa californica Multiple Nucleopolyhedrovirus 38K Is a Novel Nucleocapsid Protein That Interacts with VP1054, VP39, VP80 and Itself. J Virol. 2008;82:12356–64. PubMed PMID: 18922869.

224. Marek M, Romier C, Galibert L, Merten O, van Oers MM. Baculovirus VP1054 is an acquired cellular PURα, a nucleic-acid6n binding protein specific for GGN repeats. J Virol. 2013;87:8465–80. PubMed PMID: 23720732.
225. Guan Z, Zhong L, Li C, Wu W, Yuan M, Yang K. The Autographa californica Multiple Nucleopolyhedrovirus ac54 Gene Is Crucial for Localization of the Major Capsid Protein VP39 at the Site of Nucleocapsid Assembly. J Virol. 2016;90:4115–4126. PubMed PMID: 26865720.
226. Chen Y, Zheng F, Tao L, Zheng Z, Liu Y, Wang H. Helicoverpa armaigera nucleopolyhedrovirus ORF50 is an early gene not essential for virus propagation in vitro and in vivo. Virus Genes. 2012;45:149–60. PubMed PMID: 22581445.
227. Guo ZJ, Wang DX, Yao Q, Chen KP, Zhang CX. Identification of a novel functional nuclear localization signal in the protein encoded by open reading frame 47 of Bombyx mori nucleopolyhedrovirus. Arch Virol. 2010;155:1943–50. PubMed PMID: 20706853.
228. Li Z, Li L, Yu H, Li S, Pang Y. Characterization of two homologues of ChaB in Spodoptera litura multicapsid nucleopolyhedrovirus. Gene. 2006;372:33–43. PubMed PMID: 16488560.
229. Beames B, Summers MD. Location and nucleotide sequence of the 25k protein missing from baculovirus few polyhedra (FP) mutants. Virology. 1989;168:344–353. PubMed PMID: 2644735.
230. Harrison RL, Jarvis DL, Summers MD. The role of the AcMNPV 25K gene, "FP25," in baculovirus polh and p10 expression. Virology. 1996;226:34–46. PubMed PMID: 8941320.
231. Fraser MJ, Brusca JS, Smith GE, Summers MD. Transposon mediated mutagenesis of a baculovirus. Virology. 1985;145:356–361. PubMed PMID: 2992159.
232. Beames B, Summers MD. Sequence comparison of cellular and viral copies of host cell DNA insertions found in Autographa californica nuclear polyhedrosis virus. Virology. 1990;174:354–63. PubMed PMID: 2154879.
233. Bischoff DS, Slavicek JM. Phenotypic and genetic analysis of Lymantria dispar nucleopolyhedrovirus few polyhedra mutants: mutations in the 25K FP gene may be caused by DNA replication errors. J Virol. 1997;71:1097–1106. PubMed PMID: 8995630.
234. Braunagel SC, Burks JK, Rosas-Acosta G, Harrison RL, Ma H, Summers MD. Mutations within the Autographa californica nucleopolyhedrovirus FP25K gene decrease the accumulation of ODV-E66 and alter its intranuclear transport. J Virol. 1999;73:8559–70. PubMed PMID: 10482609.
235. Rosas-Acosta G, Braunagel SC, Summers MD. Effects of deletion and overexpression of the Autographa californica nuclear polyhedrosis virus FP25K gene on synthesis of two occlusion-derived virus envelope proteins and their transport into virus-induced intranuclear membranes. J Virol. 2001;75:10829–42. PubMed PMID: 11602724.
236. Katsuma S, Noguchi Y, Zhou CL, Kobayashi M, Maeda S. Characterization of the 25K FP gene of the baculovirus Bombyx mori nucleopolyhedrovirus: implications for post-mortem host degradation. J Gen Virol. 1999;60:783–91. PubMed PMID: 10092020.
237. Katsuma S, Nakanishi T, Shimada T. Bombyx mori nucleopolyhedrovirus FP25K is essential for maintaining a steady-state level of v-cath expression throughout the infection. Virus Res. 2009;140:155–60. PubMed PMID: 19100790.
238. Kelly BJ, King LA, Possee RD, Chapple SD. Dual mutations in the Autographa californica nucleopolyhedrovirus FP-25 and p35 genes result in plasma-membrane blebbing in Trichoplusia ni cells. J Gen Virol. 2006;87:531–6. PubMed PMID: 16476974.
239. Nakanishi T, Shimada T, Katsuma S. Characterization of a Bombyx mori nucleopolyhedrovirus mutant lacking both fp25K and p35. Virus Genes.41:144–8. PubMed PMID: 20508980.
240. Cheng XH, Kumar S, Arif BM, Krell PJ, Zhang CX, Cheng XW. Cell-dependent polyhedra production and virion occlusion of AcMNPV fp25k mutants in vitro and in vivo. J Gen Virol. 2012;94:177–86. PubMed PMID: 22993192.
241. Cheng XH, Hillman CC, Zhang CX, Cheng XW. Reduction of polyhedrin mRNA and protein expression levels in Sf9 and High Five cell lines but not in Sf21 infected with AcMNPV fp25k mutants. J Gen Virol. 2012;94:166–76. PubMed PMID: 23015742.

242. Li S, Wang M, Shen S, Hu Z, Wang H, Deng F. The FP25K Acts as a Negative Factor for the Infectivity of AcMNPV Budded Virus. PLoS One. 2015;10:e0128471. PubMed PMID: 26020780.
243. Garretson TA, McCoy JC, Cheng XW. Baculovirus FP25K Localization: Role of the Coiled-Coil Domain. J Virol. 2016;90:9582–9597. PubMed PMID: 27512078.
244. Zhang Y, Shi Y, Yu H, Li J, Quan Y, Shu T, Nie Z, Zhang Y, Yu W. Functional characterization of Bombyx mori nucleopolyhedrovirus mutant lacking late expression factor 9. Acta Virol. 2016;60:281–9. PubMed PMID: 27640438.
245. Wang Y, Kleespies RG, Huger AM, Jehle JA. The genome of Gryllus bimaculatus nudivirus indicates an ancient diversification of baculovirus-related nonoccluded nudiviruses of insects. J Virol. 2007;81:5395–406. PubMed PMID: 17360757.
246. Tian CH, Tang XD, Xu HJ, Ge JQ, Miao YG, Zhang CX. Bombyx mori nucleopolyhedrovirus ORF51 encodes a budded virus envelope associated protein. Virus Genes. 2008. PubMed PMID: 19085095.
247. Cheng X, Krell P, Arif B. P34.8 (GP37) is not essential for baculovirus replication. J Gen Virol. 2001;82:299–305. PubMed PMID: 11161266.
248. Vialard JE, Yuen L, Richardson CD. Identification and characterization of a baculovirus occlusion body glycoprotein which resembles spheroidin, an entomopoxvirus protein. Journal of Virology. 1990;64:5804–5811. PubMed PMID: 2243377.
249. Hall RL, Moyer RW. Identification of an *Amsacta* spheroidin-like protein within occlusion bodies of *Choristoneura* entomopoxviruses. Virology. 1993;192:179–187. PubMed PMID: 8517016.
250. Wu J, Miller LK. Sequence, transcription, and translation of a late gene of the Autographa californica nuclear polyhedrosis virus encoding a 34.8 K polypeptide. Journal of General Virology. 1989;70:2449–2459. PubMed PMID: 2674327.
251. Salvador R, Ferrelli ML, Berretta MF, Mitsuhashi W, Biedma ME, Romanowski V, Sciocco-Cap A. Analysis of EpapGV gp37 gene reveals a close relationship between granulovirus and entomopoxvirus. Virus Genes. 2012;45:610–3. PubMed PMID: 22899338.
252. Techkarnjanaruk S, Goodman AE. Multiple genes involved in chitin degradation from the marine bacterium Pseudoalteromonas sp. strain S91. Microbiology. 1999;145:925–934. PubMed PMID: 10220172.
253. Li Z, Li C, Yang K, Wang L, Yin C, Gong Y, Pang Y. Characterization of a chitin-binding protein GP37 of Spodoptera litura multicapsid nucleopolyhedrovirus. Virus Res. 2003;96:113–22. PubMed PMID: 12951271.
254. Liu X, Ma X, Lei C, Xiao Y, Zhang Z, Sun X. Synergistic effects of Cydia pomonella granulovirus GP37 on the infectivity of nucleopolyhedroviruses and the lethality of Bacillus thuringiensis. Arch Virol. 2011;156:1707–15. PubMed PMID: 21643992.
255. Lemaitre B, Hoffmann J. The host defense of Drosophila melanogaster. Annu Rev Immunol. 2007;25:697–743. PubMed PMID: 17201680.
256. Gross CH, Wolgamot GM, Russell RLQ, Pearson MN, Rohrmann GF. A 37 kda glycoprotein from a baculovirus of *Orgyia pseudotsugata* is localized to cytoplasmic inclusion bodies. Journal of Virology. 1993;67:469–475. PubMed PMID: 8380088.
257. Mitsuhashi W, Miyamoto K. Disintegration of the peritrophic membrane of silkworm larvae due to spindles of an entomopoxvirus. J Invertebr Pathol. 2003;82:34–40. PubMed PMID: 12581717.
258. Chiu E, Hijnen M, Bunker RD, Boudes M, Rajendran C, Aizel K, Olieric V, Schulze-Briese C, Mitsuhashi W, Young V, Ward VK, Bergoin M, Metcalf P, Coulibaly F. Structural basis for the enhancement of virulence by viral spindles and their in vivo crystallization. Proc Natl Acad Sci U S A. 2015;112:3973–8. PubMed PMID: 25787255.
259. Liu X, Fang W, Fan R, Zhang L, Lei C, Zhang J, Nian W, Dou T, An S, Zhou L, Sun X. Granulovirus GP37 Facilitated ODVs Cross Insect Peritrophic Membranes and Fuse with Epithelia. Toxins. 2019.:11. PubMed PMID: 30836616.
260. Tomalski MD, Wu J, Miller LK. The location, sequence, transcription, and regulation of a baculovirus DNA polymerase gene. Virology. 1988;167:591–600. PubMed PMID: 3059678.

261. Mikhailov V, Marlyev K, Ataeva J, Kullyev P, Atrzhev A. Characterization of 3' -> 5' exonuclease associated with DNA polymerase of silkworm nuclear polyhedrosis ivrus. Nucleic Acids Research. 1986;14:3841–3857. PubMed PMID: 3012482.
262. McDougal VV, Guarino LA. Autographa californica nuclear polyhedrosis virus DNA polymerase: measurements of processivity and strand displacement. J Virol. 1999;73:4908–18. PubMed PMID: 10233952.
263. Hang X, Guarino LA. Purification of Autographa californica nucleopolyhedrovirus DNA polymerase from infected insect cells. J Gen Virol. 1999;80:2519–26. PubMed PMID: 10501509.
264. Feng G, Thumbi DK, de Jong J, Hodgson JJ, Arif BM, Doucet D, Krell PJ. Selection and Characterization of Autographa californica Multiple Nucleopolyhedrovirus DNA Polymerase Mutations. J Virol. 2012;86:13576–88. PubMed PMID: 23035236.
265. Chen G, Yan Q, Fang Y, Wu L, Krell PJ, Feng G. The N Terminus of Autographa californica Multiple Nucleopolyhedrovirus DNA Polymerase Is Required for Efficient Viral DNA Replication and Virus and Occlusion Body Production. J Virol. 2018.:92. PubMed PMID: 29563284.
266. Feng G, Krell PJ. Autographa californica multiple nucleopolyhedrovirus DNA polymerase C terminus is required for nuclear localization and viral DNA replication. J Virol. 2014;88:10918–33. PubMed PMID: 25008932.
267. Vanarsdall AL, Okano K, Rohrmann GF. Characterization of the replication of a baculovirus mutant lacking the DNA polymerase gene. Virology. 2005;331:175–180. PubMed PMID: 15582664.
268. Ohresser M, Morin N, Cerutti M, Delsert C. Temporal regulation of a complex and unconventional promoter by viral products. J Virol. 1994;68:2589–2597. PubMed PMID: 8139038.
269. Deng F, Wang R, Fang M, Jiang Y, Xu X, Wang H, Chen X, Arif BM, Guo L, Wang H, Hu Z. Proteomics analysis of Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus identified two new occlusion-derived virus-associated proteins, HA44 and HA100. J Virol. 2007;81:9377–85. PubMed PMID: 17581982.
270. Ke J, Wang J, Deng R, Wang X. 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. 2008;374:421–31. PubMed PMID: 18241908.
271. Zhang MJ, Tian CH, Fan XY, Lou YH, Cheng RL, Zhang CX. Bombyx mori nucleopolyhedrovirus ORF54, a viral desmoplakin gene, is associated with the infectivity of budded virions. Arch Virol. 2012;157:1241–51. PubMed PMID: 22446883.
272. Hang X, Dong W, Guarino LA. The *lef-3* gene of *Autographa californica* nuclear polyhedrosis virus encodes a single-stranded DNA-binding protein. J Virol. 1995;69:3924–3928. PubMed PMID: 7745748.
273. Downie K, Adetola G, Carstens EB. Characterization of protein-protein interaction domains within the baculovirus Autographa californica multiple nucleopolyhedrovirus late expression factor LEF-3. J Gen Virol. 2013;94:2530–5. PubMed PMID: 23997180.
274. Evans JT, Rosenblatt GS, Leisy DJ, Rohrmann GF. Characterization of the interaction between the baculovirus ssDNA- binding protein (LEF-3) and putative helicase (P143). J Gen Virol. 1999;80:493–500. PubMed PMID: 10073712.
275. Wu Y, Carstens EB. A baculovirus single-stranded DNA binding protein, LEF-3, mediates the nuclear localization of the putative helicase P143. Virology. 1998;247:32–40. PubMed PMID: 9683569.
276. Au V, Yu M, Carstens EB. Characterization of a baculovirus nuclear localization signal domain in the late expression factor 3 protein. Virology. 2009;385:209–17. PubMed PMID: 19073335.
277. Chen Z, Carstens EB. Identification of domains in Autographa californica multiple nucleopolyhedrovirus late expression factor 3 required for nuclear transport of P143. J Virol. 2005;79:10915–22. PubMed PMID: 16103143.
278. Yu M, Carstens EB. Identification of a domain of the baculovirus Autographa californica multiple nucleopolyhedrovirus single-strand DNA-binding protein LEF-3 essential for viral DNA replication. J Virol. 2010;84:6153–62. PubMed PMID: 20357098.

279. Mikhailov V, Okano K, Rohrmann G. Baculovirus Alkaline Nuclease Possesses a 5'->3' Exonuclease Activity and Associates with the DNA-Binding Protein LEF-3. J Virol. 2003;77:2436–2444. PubMed PMID: 12551981.
280. Mikhailov V, Okano K, Rohrmann G. Specificity of the Endonuclease Activity of the Baculovirus Alkaline Nuclease for Single-stranded DNA. J Biol Chem. 2004;279:14734–14745. PubMed PMID: 14736888.
281. Mikhailov VS, Okano K, Rohrmann GF. The Redox State of the Baculovirus Single-stranded DNA-binding Protein LEF-3 Regulates Its DNA Binding, Unwinding, and Annealing Activities. J Biol Chem. 2005;280:29444–53. PubMed PMID: 15944160.
282. Mikhailov V. Helix-destabilizing properties of the baculovirus single-stranded DNA-binding protein (LEF-3). Virology. 2000;270:180–9. PubMed PMID: 10772990.
283. Mikhailov VS, Okano K, Rohrmann GF. Structural and functional analysis of the baculovirus single-stranded DNA-binding protein LEF-3. Virology. 2006;346:469–478. PubMed PMID: 16375940.
284. Iyer LM, Koonin EV, Aravind L. Classification and evolutionary history of the single-strand annealing proteins, RecT, Redbeta, ERF and RAD52. BMC Genomics. 2002;3:8. PubMed PMID: 11914131.
285. Nie Y, Fang M, Erlandson MA, Theilmann DA. Analysis of the autographa californica multiple nucleopolyhedrovirus overlapping gene pair lef3 and ac68 reveals that AC68 is a per os infectivity factor and that LEF3 is critical, but not essential, for virus replication. J Virol. 2012;86:3985–94. PubMed PMID: 22278232.
286. Li G, Wang J, Deng R, Wang X. Characterization of AcMNPV with a deletion of ac68 gene. Virus Genes. 2008;37:119–27. PubMed PMID: 18483845.
287. Boogaard B, van Oers MM, van Lent JWM. An Advanced View on Baculovirus per Os Infectivity Factors. Insects. 2018.:9. PubMed PMID: 30018247.
288. Xu H, Yang Z, Zhao J, Tian C, Ge J, Tang X, Bao Y, Zhang C. Bombyx mori nucleopolyhedrovirus ORF56 encodes an occlusion-derived virus protein and is not essential for budded virus production. J Gen Virol. 2008;89:1212–1219. PubMed PMID: 18420799.
289. Wu X, Guarino LA. Autographa californica nucleopolyhedrovirus orf69 encodes an RNA cap (nucleoside-2'-O)-methyltransferase. J Virol. 2003;77:3430–40. PubMed PMID: 12610118.
290. Lu A, Miller LK. Differential requirements for baculovirus late expression factor genes in two cell lines. J Virol. 1995;69:6265–6272. PubMed PMID: 7666527.
291. Tachibana A, Hamajima R, Tomizaki M, Kondo T, Nanba Y, Kobayashi M, Yamada H, Ikeda M. HCF-1 encoded by baculovirus AcMNPV is required for productive nucleopolyhedrovirus infection of non-permissive Tn368 cells. Sci Rep. 2017;7:3807. PubMed PMID: 28630398.
292. Lu A, Miller LK. Species-specific effects of the hcf-1 gene on baculovirus virulence. J Virol. 1996;70:5123–30. PubMed PMID: 8764020.
293. Wilson JA, Forney SD, Ricci AM, Allen EG, Hefferon KL, Miller LK. Expression and mutational analysis of Autographa californica nucleopolyhedrovirus HCF-1: functional requirements for cysteine residues. J Virol. 2005;79:13900–14. PubMed PMID: 16254326.
294. Griffiths CM, Barnett AL, Ayres MD, Windass J, King LA, Possee RD. In vitro host range of Autographa californica nucleopolyhedrovirus recombinants lacking functional p35, iap1 or iap2. J Gen Virol. 1999;80:1055–1066. PubMed PMID: 10211976.
295. Yamada H, Shibuya M, Kobayashi M, Ikeda M. Baculovirus Lymantria dispar multiple nucleopolyhedrovirus IAP2 and IAP3 do not suppress apoptosis, but trigger apoptosis of insect cells in a transient expression assay. Virus Genes. 2012;45:370–9. PubMed PMID: 22798056.
296. Yang R, Zhang J, Feng M, Wu X. Identification of Bombyx mori nucleopolyhedrovirus bm58a as an auxiliary gene and its requirement for cell lysis and larval liquefaction. J Gen Virol. 2016;97:3039–3050. PubMed PMID: 27667819.
297. Hu X, Shen Y, Zheng Q, Wang G, Wu X, Gong C. Bm59 is an early gene, but is unessential for the propagation and assembly of Bombyx mori nucleopolyhedrovirus. Mol Genet Genomics. 2016;291:145–54. PubMed PMID: 26210473.

298. Du MF, Yin XM, Guo ZJ, Zhu LJ. Characterization of a late gene, ORF60 from Bombyx mori nucleopolyhedrovirus. J Biochem Mol Biol. 2006;39:737–42. PubMed PMID: 17129410.
299. Guo ZJ, Qiu LH, An SH, Yao Q, Park EY, Chen KP, Zhang CX. Open reading frame 60 of the Bombyx mori nucleopolyhedrovirus plays a role in budded virus production. Virus Res. 2010;151:185–91. PubMed PMID: 20576538.
300. Shen H, Chen K, Yao Q, Zhou Y. Characterization of the Bm61 of the Bombyx mori nucleopolyhedrovirus. Curr Microbiol. 2009;59:65–70. PubMed PMID: 19319594.
301. Shen H, Chen K. BM61 of Bombyx mori nucleopolyhedrovirus: its involvement in the egress of nucleocapsids from the nucleus. FEBS Lett. 2012;586:990–5. PubMed PMID: 22569252.
302. Guo YJ, Fu SH, Li LL. Autographa californica multiple nucleopolyhedrovirus ac75 is required for egress of nucleocapsids from the nucleus and formation of de novo intranuclear membrane microvesicles. PLoS One. 2017;12:e0185630. PubMed PMID: 28968422.
303. Shi A, Hu Z, Zuo Y, Wang Y, Wu W, Yuan M, Yang K. Autographa californica Multiple Nucleopolyhedrovirus ac75 Is Required for the Nuclear Egress of Nucleocapsids and Intranuclear Microvesicle Formation. J Virol. 2018.:92. PubMed PMID: 29212928.
304. Hu Z, Yuan M, Wu W, Liu C, Yang K, Pang Y. Autographa californica multiple nucleopolyhedrovirus ac76 is involved in intranuclear microvesicle formation. J Virol. 2010;84:7437–47. PubMed PMID: 20484514.
305. Wei D, Wang Y, Zhang X, Hu Z, Yuan M, Yang K. Autographa californica nucleopolyhedrovirus Ac76: a dimeric type II integral membrane protein that contains an inner nuclear membrane-sorting motif. J Virol. 2014;88:1090–103. PubMed PMID: 24198428.
306. McLachlin JR, Miller LK. Identification and characterization of vlf-1, a baculovirus gene involved in very late gene expression. J Virol. 1994;68:7746–7756. PubMed PMID: 7966564.
307. Yang S, Miller LK. Activation of baculovirus very late promoters by interaction with very late factor 1. J Virol. 1999;73:3404–9. PubMed PMID: 10074194.
308. Yang S, Miller LK. Expression and mutational analysis of the baculovirus very late factor 1 (vlf-1) gene. Virology. 1998;245:99–109. PubMed PMID: 9614871.
309. Vanarsdall AL, Okano K, Rohrmann GF. Characterization of the role of VLF-1 in baculovirus capsid structure and DNA processing. J Virol. 2006;80:1724–1733. PubMed PMID: 16439529.
310. Vanarsdall AL, Okano K, Rohrmann GF. Characterization of a baculovirus with a deletion of vlf-1. Virology. 2004;326:191–201. PubMed PMID: 15262507.
311. Li Y, Wang J, Deng R, Zhang Q, Yang K, Wang X. vlf-1 deletion brought AcMNPV to defect in nucleocapsid formation. Virus Genes. 2005;31:275–84. PubMed PMID: 16175333.
312. Mikhailov V, Rohrmann G. Binding of the baculovirus very late expression factor 1 (VLF-1) to different DNA structures. BMC Mol Biol. 2002;3:14. PubMed PMID: 12350233.
313. Chen L, Shen Y, Yang R, Wu X, Hu W, Shen G. Bombyx mori nucleopolyhedrovirus (BmNPV) Bm64 is required for BV production and per os infection. Virol J. 2015;12:173. PubMed PMID: 26497116.
314. Tao XY, Choi JY, Kim WJ, Lee JH, Liu Q, Kim SE, An SB, Lee SD, Jin BR, Je YH. The Autographa californica multiple nucleopolyhedrovirus ORF78 is essential for BV production and general occlusion body formation. J Virol. 2013;87:8441–50. PubMed PMID: 23698311.
315. Hou D, Zhang L, Deng F, Fang W, Wang R, Liu X, Guo L, Rayner S, Chen X, Wang H, Hu Z. Comparative Proteomics Reveal Fundamental Structural and Functional Differences between the Two Progeny Phenotypes of a Baculovirus. J Virol. 2013;87:829–39. PubMed PMID: 23115289.
316. Huang H, Wang M, Deng F, Hou D, Arif BM, Wang H, Hu Z. The ha72 core gene of baculovirus is essential for budded virus production and occlusion-derived virus embedding, and amino acid K22 plays an important role in its function. J Virol. 2014;88:705–709. PubMed PMID: 24089571.
317. Aravind L, Walker DR, Koonin EV. Conserved domains in DNA repair proteins and evolution of repair systems. Nucleic Acids Res. 1999;27:1223–42. PubMed PMID: 9973609.
318. Wu W, Passarelli AL. The Autographa californica M nucleopolyhedrovirus ac79 gene encodes an early gene product with structural similarities to UvrC and intron-encoded endonucleases that is required for efficient budded virus production. J Virol. 2012;86:5614–25. PubMed PMID: 22419804.

319. Tang Q, Li G, Yao Q, Chen L, Feng F, Yuan Y, Chen K. Bm65 is Essential for the Propagation of Bombyx mori Nucleopolyhedrovirus. Curr Microbiol. 2013;66:22–9. PubMed PMID: 23053485.
320. Whitford M, Faulkner P. A structural polypeptide of the baculovirus Autographa californica nuclear polyhedrosis virus contains 0-linked N-acetylglucosamine. J Virol. 1992;66:3324–3329. PubMed PMID: 1583718.
321. Whitford M, Faulkner P. Nucleotide sequence and transcriptional analysis of a gene encoding gp41, a structural glycoprotein of the baculovirus Autographa californica nuclear polyhedrosis virus. J Virol. 1992;66:4763–4768. PubMed PMID: 1629955.
322. Olszewski J, Miller LK. A role for baculovirus GP41 in budded virus production. Virology. 1997;233:292–301. PubMed PMID: 9217053.
323. Li Y, Shen S, Hu L, Deng F, Vlak JM, Hu Z, Wang H, Wang M. The Functional Oligomeric State of Tegument Protein GP41 Is Essential for Baculovirus Budded Virion and Occlusion-Derived Virion Assembly. J Virol. 2018.:92. PubMed PMID: 29643237.
324. Chen HQ, Chen KP, Yao Q, Guo ZJ, Wang LL. Characterization of a late gene, ORF67 from Bombyx mori nucleopolyhedrovirus. FEBS Lett. 2007;581:5836–5842. PubMed PMID: 18053810.
325. Raynes DA, Hartshorne DJ, Guerriero VJ. Sequence and expression of a baculovirus protein with antigenic similarity to telokin. J Gen Virol. 1994.:1807–1809. PubMed PMID: 7517434.
326. Holden HM, Wesenberg G, Raynes DA, Hartshorne DJ, Guerriero VJ, Rayment I. Molecular structure of a proteolytic fragment of TLP20. Acta Crystallogr D Biol Crystallogr. 1996;52:1153–60. PubMed PMID: 15299576.
327. Iwanaga M, Kurihara M, Kobayashi M, Kang W. Characterization of Bombyx mori nucleopolyhedrovirus orf68 gene that encodes a novel structural protein of budded virus. Virology. 2002;297:39–47. PubMed PMID: 12083834.
328. Russell RL, Rohrmann GF. Characterization of P91, a protein associated with virions of an Orgyia pseudotsugata baculovirus. Virology. 1997;233:210–23. PubMed PMID: 9201231.
329. Hou D, Zhang L, Deng F, Fang W, Wang R, Liu X, Guo L, Rayner S, Chen X, Wang H, Hu Z. 2012. Comparative Proteomics Reveal Fundamental Structural and Functional Differences between the Two Progeny Phenotypes of a Baculovirus. J Virol doi:JVI.02329-12 [pii] doi: 10.1128/JVI.02329-12.
330. Suetake T, Tsuda S, Kawabata S, Miura K, Iwanaga S, Hikichi K, Nitta K, Kawano K. Chitin-binding proteins in invertebrates and plants comprise a common chitin-binding structural motif. J Biol Chem. 2000;275:17929–32. PubMed PMID: 10770921.
331. Zhu S, Wang W, Wang Y, Yuan M, Yang K. The baculovirus core gene ac83 is required for nucleocapsid assembly and per os infectivity of Autographa californica nucleopolyhedrovirus. J Virol. 2013;87:10573–86. PubMed PMID: 23864639.
332. Javed MA, Biswas S, Willis LG, Harris S, Pritchard C, van Oers MM, Donly BC, Erlandson MA, Hegedus DD, Theilmann DA. Autographa californica Multiple Nucleopolyhedrovirus AC83 is a Per Os Infectivity Factor (PIF) Protein Required for Occlusion-Derived Virus (ODV) and Budded Virus Nucleocapsid Assembly as well as Assembly of the PIF Complex in ODV Envelopes. J Virol. 2017;91:02115–16. PubMed PMID: 28031365.
333. Xiang X, Chen L, Guo A, Yu S, Yang R, Wu X. The Bombyx mori nucleopolyhedrovirus (BmNPV) ODV-E56 envelope protein is also a per os infectivity factor. Virus Res. 2011;155:69–75. PubMed PMID: 20817055.
334. Huang Z, Pan M, Zhu S, Zhang H, Wu W, Yuan M, Yang K. The Autographa californica Multiple Nucleopolyhedrovirus ac83 Gene Contains a cis-Acting Element That Is Essential for Nucleocapsid Assembly. J Virol. 2017.:91. PubMed PMID: 28031366.
335. Martins A, Shuman S. Characterization of a baculovirus enzyme with RNA ligase, polynucleotide 5'-kinase, and polynucleotide 3'-phosphatase activities. J Biol Chem. 2004;279:18220–31. PubMed PMID: 14747466.
336. Durantel D, Croizier L, Ayres MD, Croizier G, Possee RD, Lopez-Ferber M. The pnk/pnl gene (ORF 86) of Autographa californica nucleopolyhedrovirus is a non-essential, immediate early gene. J Gen Virol. 1998;79(Pt 3):629–37. PubMed PMID: 9519844.

337. Lu M, Iatrou K. Characterization of a domain of the genome of BmNPV containing a functional gene for a small capsid protein and harboring deletions eliminating three open reading frames that are present in AcNPV. Gene. 1997;185:69–75. PubMed PMID: 9034315.
338. Thiem SM, Miller LK. A baculovirus gene with a novel transcription pattern encodes a polypeptide with a zinc finger and a leucine zipper. Journal of Virology. 1989;63:4489–4497. PubMed PMID: 2507791.
339. Passarelli AL, Miller LK. In vivo and in vitro analyses of recombinant baculoviruses lacking a functional cg30 gene. J Virol. 1994;68:1186–90. PubMed PMID: 8289348.
340. Zhang MJ, Cheng RL, Lou YH, Ye WL, Zhang T, Fan XY, Fan HW, Zhang CX. Disruption of Bombyx mori nucleopolyhedrovirus ORF71 (Bm71) results in inefficient budded virus production and decreased virulence in host larvae. Virus Genes. 2012;45:161–8. PubMed PMID: 22585339.
341. Ishihara G, Shimada T, Katsuma S. Functional characterization of Bombyx mori nucleopolyhedrovirus CG30 protein. Virus Res. 2013;174:52–9. PubMed PMID: 23507307.
342. Pearson MN, Russell RLQ, Rohrmann GF, Beaudreau GS. P39, a major baculovirus structural protein: Immunocytochemical characterization and genetic location. Virology. 1988;167:407–413. PubMed PMID: 3059676.
343. Thiem SM, Miller LK. Identification, sequence, and transcriptional mapping of the major capsid protein gene of the baculovirus *Autographa californica* nuclear polyhedrosis virus. J Virol. 1989;63:2008–2018. PubMed PMID: 2649691.
344. Danquah JO, Botchway S, Jeshtadi A, King LA. Direct interaction of baculovirus capsid proteins VP39 and EXON0 with kinesin-1 in insect cells determined by fluorescence resonance energy transfer-fluorescence lifetime imaging microscopy. J Virol. 2012;86:844–53. PubMed PMID: 22072745.
345. Katsuma S, Kokusho R. A Conserved Glycine Residue Is Required for Proper Functioning of a Baculovirus VP39 Protein. J Virol. 2017.:91. PubMed PMID: 28077638.
346. Gross CH, Shuman S. RNA 5'-triphosphatase, nucleoside triphosphatase, and guanylyltransferase activities of baculovirus LEF-4 protein. J Virol. 1998;72:10020–8. PubMed PMID: 9811740.
347. Jin J, Dong W, Guarino LA. The LEF-4 subunit of baculovirus RNA polymerase has RNA 5'- triphosphatase and ATPase activities. J Virol. 1998;72:10011–9. PubMed PMID: 9811739.
348. Martins A, Shuman S. Mutational analysis of baculovirus capping enzyme Lef4 delineates an autonomous triphosphatase domain and structural determinants of divalent cation specificity. J Biol Chem. 2001;276:45522–9. PubMed PMID: 11553638.
349. Martins A, Shuman S. The domain order of mammalian capping enzyme can be inverted and baculovirus phosphatase can function in cap formation in vivo. Virology. 2002;304:167–75. PubMed PMID: 12504559.
350. Knebel-Morsdorf D, Quadt I, Li Y, Montier L, Guarino LA. Expression of baculovirus late and very late genes depends on LEF-4, a component of the viral RNA polymerase whose guanyltransferase function is essential. J Virol. 2006;80:4168–73. PubMed PMID: 16571832.
351. Wang D, Zhang CX. Helicoverpa armigera nucleopolyhedrovirus ORF80 encodes a late, nonstructural protein. J Biochem Mol Biol. 2007;40:65–71. PubMed PMID: 17244484.
352. Shen H, Chen K, Yao Q, Yu W, Pan Y, Huo J, Xia H, Huang G. Characterization of Bombyx mori nucleopolyhedrovirus orf74, a novel gene involved in virulence of virus. Virus Genes. 2009;38:487–94. PubMed PMID: 19340569.
353. Long CM, Rohrmann GF, Merrill GF. The conserved baculovirus protein p33 (Ac92) is a flavin adenine dinucleotide-linked sulfhydryl oxidase. Virology. 2009;388:231–5. PubMed PMID: 19409596.
354. Wu W, Passarelli AL. Autographa californica M nucleopolyhedrovirus Ac92 (ORF92, P33) is required for budded virus production and multiply-enveloped occlusion-derived virus formation. J Virol. 2010;84:12351–61. PubMed PMID: 20861245.
355. Morel C, Adami P, Musard JF, Duval D, Radom J, Jouvenot M. Involvement of sulfhydryl oxidase QSOX1 in the protection of cells against oxidative stress-induced apoptosis. Exp Cell Res. 2007;313:3971–82. PubMed PMID: 17927979.

356. Thirunavukkarasu C, Wang LF, Harvey SA, Watkins SC, Chaillet JR, Prelich J, Starzl TE, Gandhi CR. Augmenter of liver regeneration: an important intracellular survival factor for hepatocytes. J Hepatol. 2008;48:578–88. PubMed PMID: 18272248.
357. Nie Y, Fang M, Theilmann DA. Autographa californica multiple nucleopolyhedrovirus core gene ac92 (p33) is required for the efficient budded virus production. Virology. 2011;409:38–45. PubMed PMID: 20965540.
358. Hakim M, Mandelbaum A, Fass D. Structure of a baculovirus sulfhydryl oxidase, a highly divergent member of the erv flavoenzyme family. J Virol. 2011;85:9406–13. PubMed PMID: 21752922.
359. Hou Y, Xia Q, Yuan YA. Crystal structure of Bombyx mori nucleopolyhedrovirus ORF75 reveals a pseudo-dimer of thiol oxidase domains with a putative substrate-binding pocket. J Gen Virol. 2012;93:2142–51. PubMed PMID: 22764321.
360. Kuang W, Zhang H, Wang M, Zhou NY, Deng F, Wang H, Gong P, Hu Z. Three Conserved Regions in Baculovirus Sulfhydryl Oxidase P33 Are Critical for Enzymatic Activity and Function. J Virol. 2017.:91. PubMed PMID: 28904203.
361. Huang N, Clem RJ, Rohrmann GF. Characterization of cDNAs encoding p53 of Bombyx mori and Spodoptera frugiperda. Insect Biochem Mol Biol. 2011;41:613–9. PubMed PMID: 21497653.
362. Wu W, Clem RJ, Rohrmann GF, Passarelli AL. 2013. The baculovirus sulfhydryl oxidase Ac92 (P33) interacts with the Spodoptera frugiperda P53 protein and oxidizes it in vitro. 447:197-207.
363. Yuan M, Huang Z, Wei D, Hu Z, Yang K, Pang Y. Identification of Autographa californica nucleopolyhedrovirus ac93 as a core gene and its requirement for intranuclear microvesicle formation and nuclear egress of nucleocapsids. J Virol. 2011;85:11664–74. PubMed PMID: 21880748.
364. Russell RLQ, Rohrmann GF. A 25 kilodalton protein is associated with the envelopes of occluded baculovirus virions. Virology. 1993;195:532–540. PubMed PMID: 8337828.
365. Chen L, Hu X, Xiang X, Yu S, Yang R, Wu X. Autographa californica multiple nucleopolyhedrovirus odv-e25 (Ac94) is required for budded virus infectivity and occlusion-derived virus formation. Arch Virol. 2012;157:617–25. PubMed PMID: 22218963.
366. Luo XC, Wang SS, Zhang J, Qian DD, Wang SM, Li LL. Effects of Early or Overexpression of the Multiple Nucleopolyhedrovirus (ODV-E25) on Virus Replication. PLoS One. 2013;8:e65635. PubMed PMID: 23825525.
367. Chen L, Yang R, Hu X, Xiang X, Yu S, Wu X. The formation of occlusion-derived virus is affected by the expression level of ODV-E25. Virus Res. 2013;173:404–14. PubMed PMID: 23298549.
368. Zhu M, Wang J, Deng R, Xiong P, Liang H, Wang X. A MicroRNA Encoded by Autographa californica Nucleopolyhedrovirus Regulates Expression of Viral Gene ODV-E25. J Virol. 2013;87:13029–34. PubMed PMID: 24027316.
369. Gordon JD, Carstens E. Phenotypic characterization and physical mapping of a terperature-sensitive mutant of AcMNPV defective in DNA synthesis. Virology. 1984;138:69–81. PubMed PMID: 6093362.
370. Lu A, Carstens EB. Nucleotide sequence of a gene essential for viral DNA replication in the baculovirus *Autographa californica* nuclear polyhedrosis virus. Virology. 1991;181:336–347. PubMed PMID: 1994581.
371. McDougal VV, Guarino LA. The autographa californica nuclear polyhedrosis virus p143 gene encodes a DNA helicase. J Virol. 2000;74:5273–9. PubMed PMID: 10799604.
372. Maeda S, Kamita SG, Kondo A. Host range expansion of *Autographa californica* nuclear polyhedrosis virus (NPV) following recombination of a 0.6-kilobase-pair DNA fragment originating from *Bombyx mori* NPV. J Virol. 1993;67:6234–6238. PubMed PMID: 8396678.
373. Croizier G, Croizier L, Argaud O, Poudevigne D. Extension of *Autographa californica* nuclear polyhedrosis virus host range by interspecific replacement of a short DNA sequence in the p143 helicase gene. Proc Natl Acad Sc USA. 1994;91:48–52. PubMed PMID: 8278405.
374. Hill T, Unckless RL. Baculovirus Molecular Evolution via Gene Turnover and Recurrent Positive Selection of Key Genes. J Virol. 2017.:91. PubMed PMID: 28814516.
375. Nagamine T, Sako Y. A Role for the Anti-Viral Host Defense Mechanism in the Phylogenetic Divergence in Baculovirus Evolution. PLoS One. 2016;11:e0156394. PubMed PMID: 27244571.

376. Hamajima R, Kobayashi M, Ikeda M. Identification of amino acid residues of AcMNPV P143 protein involved in rRNA degradation and restricted viral replication in BM-N cells from the silkworm Bombyx mori. Virology. 2015;485:244–51. PubMed PMID: 26313611.
377. Hamajima R, Kobayashi M, Ikeda M. P143 proteins from heterologous nucleopolyhedroviruses induce apoptosis in BM-N cells derived from the silkworm Bombyx mori. Virus Res. 2017;233:70–76. PubMed PMID: 28315706.
378. Xu HJ, Yang ZN, Wang F, Zhang CX. Bombyx mori nucleopolyhedrovirus ORF79 encodes a 28-kDa structural protein of the ODV envelope. Arch Virol. 2006;151:681–95. PubMed PMID: 16328137.
379. Fang M, Nie Y, Harris S, Erlandson MA, Theilmann DA. Autographa californica multiple nucleopolyhedrovirus core gene ac96 encodes a per Os infectivity factor (PIF-4). J Virol. 2009;83:12569–78. PubMed PMID: 19759145.
380. Dong ZQ, Zhang J, Chen XM, He Q, Cao MY, Wang L, Li HQ, Xiao WF, Pan CX, Lu C, Pan MH. Bombyx mori nucleopolyhedrovirus ORF79 is a per os infectivity factor associated with the PIF complex. Virus Res. 2014;184:62–70. PubMed PMID: 24583368.
381. Peng K, van Oers MM, Hu Z, van Lent JW, Vlak JM. Baculovirus per os infectivity factors form a complex on the surface of occlusion-derived virus. J Virol. 2010;84:9497–504. PubMed PMID: 20610731.
382. Zhang J, Feng M, Fan Y, Xu W, Zheng Q, Wu X. Networks of protein-protein interactions among structural proteins of budded virus of Bombyx mori nucleopolyhedrovirus. Virology. 2018;518:163–171. PubMed PMID: 29510333.
383. Wu W, Lin T, Pan L, Yu M, Li Z, Pang Y, Yang K. Autographa californica multiple nucleopolyhedrovirus nucleocapsid assembly is interrupted upon deletion of the 38K gene. J Virol. 2006;80:11475–85. PubMed PMID: 16987976.
384. Hefferon KL. ORF98 of Autographa californica nucleopolyhedrosisvirus is an auxiliary factor in late gene expression. Can J Microbiol. 2003;49:157–63. PubMed PMID: 12795401.
385. Lai Q, Wu W, Li A, Wang W, Yuan M, Yang K. The 38K-Mediated Specific Dephosphorylation of the Viral Core Protein P6.9 Plays an Important Role in the Nucleocapsid Assembly of Autographa californica Multiple Nucleopolyhedrovirus. J Virol. 2018.:92. PubMed PMID: 29444944.
386. Passarelli AL, Miller LK. Identification of genes encoding late expression factors located between 56.0 and 65.4 map units of the Autographa californica nuclear polyhedrosis virus genome. Virology. 1993;197:704–714. PubMed PMID: 8249293.
387. Harwood SH, Li L, Ho PS, Preston AK, Rohrmann GF. AcMNPV late expression factor-5 interacts with itself and contains a zinc ribbon domain that is required for maximal late transcription activity and is homologous to elongation factor TFIIS. Virology. 1998;250:118–134. PubMed PMID: 9770426.
388. Guarino LA, Dong W, Jin J. In vitro activity of the baculovirus late expression factor LEF-5. J Virol. 2002;76:12663–75. PubMed PMID: 12438592.
389. Su J, Lung O, Blissard GW. The Autographa californica multiple nucleopolyhedrovirus lef-5 gene is required for productive infection. Virology. 2011;416:54–64. PubMed PMID: 21601232.
390. Wilson ME, Mainprize TH, Friesen PD, Miller LK. l987. Location, transcription, and sequence of a baculovirus gene encoding a small arginine-rich polypeptide. J Virol.61:66l–666.
391. Garcia-Maruniak A, Maruniak JE, Zanotto PM, Doumbouya AE, Liu JC, Merritt TM, Lanoie JS. Sequence analysis of the genome of the Neodiprion sertifer nucleopolyhedrovirus. J Virol. 2004;78:7036–51. PubMed PMID: 15194780.
392. Tweeten KA, Bulla LA, Consigli RA. Characterization of an extremely basic protein derived from granulosis virus nucleocapsid. J Virol. 1980;33:866–876. PubMed PMID: 16789190.
393. Balhorn R. A model for the structure of chromatin in mammalian sperm. J Cell Biol. 1982;93:298–305. PubMed PMID: 7096440.
394. Nakano M, Kasai K, Yoshida K, Tanimoto T, Tamaki Y, Tobita T. Conformation of the fowl protamine, galline, and its binding properties to DNA. J Biochem. 1989;105:133–137. PubMed PMID: 2738040.
395. Wilson ME, Price KH. Association of Autographa californica nuclear polyhedrosis virus (AcMNPV) with the nuclear matrix. Virology. 1988;167:233–241. PubMed PMID: 3055664.

396. Peng Y, Li K, Pei RJ, Wu CC, Liang CY, Wang Y, Chen XW. The protamine-like DNA-binding protein P6.9 epigenetically up-regulates Autographa californica multiple nucleopolyhedrovirus gene transcription in the late infection phase. Virol Sin. 2012;27:57–68. PubMed PMID: 22270807.
397. Liu X, Zhao H, Fang Z, Yuan M, Yang K, Pang Y. Distribution and phosphorylation of the basic protein P6.9 of Autographa californica nucleopolyhedrovirus. J Virol. 2012;86:12217–27. PubMed PMID: 22951830.
398. Li A, Zhao H, Lai Q, Huang Z, Yuan M, Yang K. Posttranslational Modifications of Baculovirus Protamine-Like Protein P6.9 and the Significance of Its Hyperphosphorylation for Viral Very Late Gene Hyperexpression. J Virol. 2015;89:7646–59. PubMed PMID: 25972542.
399. Oppenheimer DI, Volkman LE. Proteolysis of p6.9 induced by cytochalasin D in Autographa californica M nuclear polyhedrosis virus-infected cells. Virology. 1995;207:1–11. PubMed PMID: 7871717.
400. Wang M, Tuladhar E, Shen S, Wang H, van Oers MM, Vlak JM, Westenberg M. Specificity of baculovirus P6.9 basic DNA-binding proteins and critical role of the C terminus in virion formation. J Virol. 2010;84:8821–8. PubMed PMID: 20519380.
401. Singh CP, Singh J, Nagaraju J. bmnpv-miR-3 facilitates BmNPV infection by modulating the expression of viral P6.9 and other late genes in Bombyx mori. Insect Biochem Mol Biol. 2014;49:59–69. PubMed PMID: 24698834.
402. Braunagel SC, Guidry PA, Rosas-Acosta G, Engelking L, Summers MD. 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. 2001;75:12331–8. PubMed PMID: 11711623.
403. Wang Y, Wang Q, Liang C, Song J, Li N, Shi H, Chen X. Autographa californica multiple nucleopolyhedrovirus nucleocapsid protein BV/ODV-C42 mediates the nuclear entry of P78/83. J Virol. 2008;82:4554–61. PubMed PMID: 18287235.
404. Li K, Wang Y, Bai H, Wang Q, Song J, Zhou Y, Wu C, Chen X. The putative pocket protein binding site of Autographa californica nucleopolyhedrovirus BV/ODV-C42 is required for virus-induced nuclear actin polymerization. J Virol. 2010;84:7857–68. PubMed PMID: 20484515.
405. Fang M, Nie Y, Dai X, Theilmann DA. Identification of AcMNPV EXON0 (ac141) domains required for efficient production of budded virus, dimerization and association with BV/ODV-C42 and FP25. Virology. 2008;375:265–76. PubMed PMID: 18313716.
406. Vanarsdall AL, Pearson MN, Rohrmann GF. Characterization of baculovirus constructs lacking either the Ac 101, Ac 142, or the Ac 144 open reading frame. Virology. 2007;367:187–95. PubMed PMID: 17585983.
407. Lu A, Craig A, Casselman R, Carstens EB. Nucleotide sequence, insertional mutagenesis, and transcriptional mapping of a conserved region of the baculovirus Autographa californica nuclear polyhedrosis virus (map unit 64.8-66.9). Can J Microbiol. 1996;42:1267–73. PubMed PMID: 8989865.
408. Wang Y, Zhang Y, Han S, Hu X, Zhou Y, Mu J, Pei R, Wu C, Chen X. Identification of a novel regulatory sequence of actin nucleation promoting factor encoded by Autographa californica multiple nucleopolyhedrovirus. J Biol Chem. 2015;290:9533–41. PubMed PMID: 25691574.
409. Zhang Y, Hu X, Mu J, Hu Y, Zhou Y, Zhao H, Wu C, Pei R, Chen J, Chen X, Wang Y. Ac102 Participates in Nuclear Actin Polymerization by Modulating BV/ODV-C42 Ubiquitination during Autographa californica Multiple Nucleopolyhedrovirus Infection. J Virol. 2018.:92. PubMed PMID: 29618641.
410. Hepp SE, Borgo GM, Ticau S, Ohkawa T, Welch MD. Baculovirus AC102 Is a Nucleocapsid Protein That Is Crucial for Nuclear Actin Polymerization and Nucleocapsid Morphogenesis. J Virol. 2018.:92. PubMed PMID: 29540600.
411. Jiao Y, Wang J, Deng R, Yu X, Wang X. AcMNPV-miR-3 is a miRNA encoded by Autographa californica nucleopolyhedrovirus and regulates the viral infection by targeting ac101. Virus Res. 2019. doi: 10.1016/j.virusres.2019.05.004. PubMed PMID: 31077766.
412. Ohkawa T, Rowe AR, Volkman LE. Identification of six Autographa californica multicapsid nucleopolyhedrovirus early genes that mediate nuclear localization of G-actin. J Virol. 2002;76:12281–9. PubMed PMID: 12414968.

413. Yuan M, Wu W, Liu C, Wang Y, Hu Z, Yang K, Pang Y. A highly conserved baculovirus gene p48 (ac103) is essential for BV production and ODV envelopment. Virology. 2008;379:87–96. PubMed PMID: 18656219.
414. Müller R, Pearson MN, Russell RLQ, Rohrmann GF. A capsid-associated protein of the multicapsid nuclear polyhedrosis virus of *Orgyia pseudotsugata*: Genetic location, sequence, transcriptional mapping, and immunocytochemical characterization. Virology. 1990;176:133–144. PubMed PMID: 2184573.
415. Marek M, Merten OW, Galibert L, Vlak JM, van Oers MM. Baculovirus VP80 protein and the F-actin cytoskeleton interact and connect the viral replication factory with the nuclear periphery. J Virol. 2011;85:5350–62. PubMed PMID: 21450830.
416. Lu A, Carstens EB. Nucleotide sequence and transcriptional analysis of the p80 gene of Autographa californica nuclear polyhedrosis virus: a homologue of the Orgyia pseudotsugata nuclear polyhedrosis virus capsid-associated gene. Virology. 1992;190:201–209. PubMed PMID: 1529529.
417. Marek M, van Oers MM, Devaraj FF, Vlak JM, Merten OW. Engineering of baculovirus vectors for the manufacture of virion-free biopharmaceuticals. Biotechnol Bioeng. 2011;108:1056–67. PubMed PMID: 21449023.
418. Marek M, Merten OW, Francis-Devaraj F, Oers MM. Essential C-terminal region of the baculovirus minor capsid protein VP80 binds DNA. J Virol. 2012;86:1728–38. PubMed PMID: 22090126.
419. Ho CK, Shuman S. Bacteriophage T4 RNA ligase 2 (gp24.1) exemplifies a family of RNA ligases found in all phylogenetic domains. Proc Natl Acad Sci U S A. 2002;99:12709–14. PubMed PMID: 12228725.
420. Becker D, Knebel-Morsdorf D. Sequence and temporal appearance of the early transcribed baculovirus gene HE65. J Virol. 1993;67:5867–72. PubMed PMID: 8371346.
421. Shi SL, Pan MH, Lu C. Characterization of Antheraea pernyi nucleopolyhedrovirus p11 gene, a homologue of Autographa californica nucleopolyhedrovirus orf108. Virus Genes. 2007;35:97–101. PubMed PMID: 17072759.
422. Boogaard B, Evers F, van Lent JWM, van Oers MM. The baculovirus Ac108 protein is a per os infectivity factor and a component of the ODV entry complex. J Gen Virol. 2019. doi: 10.1099/jgv.0.001200. PubMed PMID: 30694169.
423. Simon O, Palma L, Williams T, Lopez-Ferber M, Caballero P. Analysis of a naturally-occurring deletion mutant of Spodoptera frugiperda multiple nucleopolyhedrovirus reveals sf58 as a new per os infectivity factor of lepidopteran-infecting baculoviruses. J Invertebr Pathol. 2012;109:117–26. PubMed PMID: 22041202.
424. Wang X, Shang Y, Chen C, Liu S, Chang M, Zhang N, Hu H, Zhang F, Zhang T, Wang Z, Liu X, Lin Z, Deng F, Wang H, Zou Z, Vlak JM, Wang M, Hu Z. Baculovirus per os Infectivity Factor Complex: Components and Assembly. J Virol. 2019. doi: 10.1128/JVI.02053-18. PubMed PMID: 30602603.
425. Tang Q, Li G, Yao Q, Chen L, Lv P, Lian C, Chen K. Bm91 is an envelope component of ODV but is dispensable for the propagation of Bombyx mori nucleopolyhedrovirus. J Invertebr Pathol. 2013;113:70–7. PubMed PMID: 23391406.
426. Fang M, Wang H, Wang H, Yuan L, Chen X, Vlak JM, Hu Z. Open reading frame 94 of Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus encodes a novel conserved occlusion-derived virion protein, ODV-EC43. J Gen Virol. 2003;84:3021–7. PubMed PMID: 14573807.
427. Fang M, Nie Y, Theilmann DA. Deletion of the AcMNPV core gene ac109 results in budded virions that are non-infectious. Virology. 2009;389:66–74. PubMed PMID: 19411088.
428. Lin L, Wang J, Deng R, Ke J, Wu H, Wang X. ac109 is required for the nucleocapsid assembly of Autographa californica multiple nucleopolyhedrovirus. Virus Res. 2009;144:130–5. PubMed PMID: 19393701.
429. Lehiy CJ, Wu W, Berretta MF, Passarelli AL. Autographa californica M nucleopolyhedrovirus open reading frame 109 affects infectious budded virus production and nucleocapsid envelopment in the nucleus of cells. Virology. 2013;435:442–52. PubMed PMID: 23149091.
430. Alfonso V, Maroniche GA, Reca SR, Lopez MG, Del Vas M, Taboga O. AcMNPV Core Gene ac109 Is Required for Budded Virion Transport to the Nucleus and for Occlusion of Viral Progeny. PLoS One. 2012;7:e46146. PubMed PMID: 23049963.

431. Jiantao L, Zhu L, Zhang S, Deng Z, Huang Z, Yuan M, Wu W, Yang K. The Autographa californica multiple nucleopolyhedrovirus ac110 gene encodes a new per os infectivity factor. Virus Res. 2016;221:30–7. PubMed PMID: 27212681.
432. Li S, Li L, Zhao H, Liu W. Disruption of Autographa Californica Multiple Nucleopolyhedrovirus ac111 Results in Reduced per os Infectivity in a Host-Dependent Manner. Viruses. 2018.:10. PubMed PMID: 30262719.
433. Yamada H, Shibuya M, Kobayashi M, Ikeda M. Identification of a novel apoptosis suppressor gene from the baculovirus Lymantria dispar multicapsid nucleopolyhedrovirus. J Virol. 2011;85:5237–42. PubMed PMID: 21411519.
434. Liang G, Li G, Chen K, Yao Q, Chen H, Zhou Y. Bombyx mori nucleopolyhedrovirus ORF94, a novel late protein is identified to be a component of ODV structural protein. Curr Microbiol. 2010;61:190–6. PubMed PMID: 20135318.
435. Peng K, Wu M, Deng F, Song J, Dong C, Wang H, Hu Z. Identification of protein-protein interactions of the occlusion-derived virus-associated proteins of Helicoverpa armigera nucleopolyhedrovirus. J Gen Virol. 2010;91:659–70. PubMed PMID: 19906939.
436. Gong M, Jin J, Guarino LA. Mapping of ORF121, a factor that activates baculovirus early gene expression. Virology. 1998;244:495–503. PubMed PMID: 9601517.
437. Li Y, Miller LK. Expression and functional analysis of a baculovirus gene encoding a truncated protein kinase homolog. Virology. 1995;206:314–323. PubMed PMID: 7831787.
438. Prasad MD, Han SJ, Nagaraju J, Lee WJ, Brey PT. Cloning and characterization of an eukaryotic initiation factor-2alpha kinase from the silkworm, Bombyx mori. Biochim Biophys Acta. 2003;1628:56–63. PubMed PMID: 12850273.
439. Dever TE, Sripriya R, McLachlin JR, Lu J, Fabian JR, Kimball SR, Miller LK. Disruption of cellular translational control by a viral truncated eukaryotic translation initiation factor 2alpha kinase homolog. Proc Natl Acad Sci U S A. 1998.:4164–9. PubMed PMID: 9539707.
440. Aarti I, Rajesh K, Ramaiah KV. Phosphorylation of eIF2 alpha in Sf9 cells: a stress, survival and suicidal signal. Apoptosis. 2010;15:679–92. PubMed PMID: 20162453.
441. Li JJ, Cao C, Fixsen SM, Young JM, Ono C, Bando H, Elde NC, Katsuma S, Dever TE, Sicheri F. Baculovirus protein PK2 subverts eIF2alpha kinase function by mimicry of its kinase domain C-lobe. Proc Natl Acad Sci U S A. 2015;112:E4364–73. PubMed PMID: 26216977.
442. Liang C, Lan D, Zhao S, Liu L, Xue Y, Zhang Y, Wang Y, Chen X. The Ac124 protein is not essential for the propagation of Autographa californica multiple nucleopolyhedrovirus, but it is a viral pathogenicity factor. Arch Virol. 2015;160:275–84. PubMed PMID: 25380680.
443. Chen H, Li M, Huang G, Mai W, Chen K, Zhou Y. Bombyx mori nucleopolyhedrovirus ORF101 encodes a budded virus envelope associated protein. Curr Microbiol. 2014;69:158–63. PubMed PMID: 24677040.
444. Chen H, Li M, Mai W, Tang Q, Li G, Chen K, Zhou Y. Analysis of BmNPV orf101 disruption: orf101 is essential for mediating budded virus production. Cytotechnology. 2014;66:1021–9. PubMed PMID: 25300342.
445. Fang Z, Que Y, Li J, Zhang Z. The deletion of the AcMNPV ac124 gene resulted in a decrease in chitinase transcription. Virus Res. 2019;263:151–158. PubMed PMID: 30711578.
446. Harrison RL, Mowery JD, Rowley DL, Bauchan GR, Theilmann DA, Rohrmann GF, Erlandson MA. The complete genome sequence of a third distinct baculovirus isolated from the true armyworm, Mythimna unipuncta, contains two copies of the lef-7 gene. Virus Genes. 2018;54:297–310. PubMed PMID: 29204787.
447. Ferrelli ML, Pidre ML, Ghiringhelli PD, Torres S, Fabre ML, Masson T, Cedola MT, Sciocco-Cap A, Romanowski V. Genomic analysis of an Argentinean isolate of Spodoptera frugiperda granulovirus reveals that various baculoviruses code for Lef-7 proteins with three F-box domains. PLoS One. 2018;13:e0202598. PubMed PMID: 30133523.
448. Morris TD, Todd JW, Fisher B, Miller LK. Identification of *lef-7*: A baculovirus gene affecting late gene expression. Virology. 1994;200:360–369. PubMed PMID: 8178427.

449. Chen CJ, Thiem SM. Differential infectivity of two Autographa californica nucleopolyhedrovirus mutants on three permissive cell lines is the result of lef-7 deletion. Virology. 1997;227:88–95. PubMed PMID: 9007061.
450. Mitchell JK, Byers NM, Friesen PD. Baculovirus F-Box Protein LEF-7 Modifies the Host DNA Damage Response To Enhance Virus Multiplication. J Virol. 2013;87:12592–9. PubMed PMID: 24027328.
451. Ishimwe E, Hodgson JJ, Clem RJ, Passarelli AL. Reaching the melting point: Degradative enzymes and protease inhibitors involved in baculovirus infection and dissemination. Virology. 2015;479-480:637–49. PubMed PMID: 25724418.
452. Daimon T, Katsuma S, Kang W, Shimada T. Comparative studies of Bombyx mori nucleopolyhedrovirus chitinase and its host ortholog, BmChi-h. Biochem Biophys Res Commun. 2006;345:825–33. PubMed PMID: 16701557.
453. Hughes AL, Friedman R. Genome-Wide Survey for Genes Horizontally Transferred from Cellular Organisms to Baculoviruses. Mol Biol Evol. 2003;20:979–8. PubMed PMID: 12716988.
454. Fukamizo T, Sato H, Mizuhara M, Ohnuma T, Gotoh T, Hiwatashi K, Takahashi S. Chitinase from Autographa californica Multiple Nucleopolyhedrovirus: Rapid Purification from Sf-9 Medium and Mode of Action. Biosci Biotechnol Biochem. 2011;75:1763–9. PubMed PMID: 21897030.
455. Hawtin R, Arnold LH, Chappell MD, Ayres MD, Kitts PA, Gooday GW, Possee RD. Identification of a chitinase gene in the *Autographa californica* nuclear polyhedrosis virus, p 41. *In* (ed),
456. Hawtin RE, Zarkowska T, Arnold K, Thomas CJ, Gooday GW, King LA, Kuzio JA, Possee RD. Liquefaction of Autographa californica nucleopolyhedrovirus-infected insects is dependent on the integrity of virus-encoded chitinase and cathepsin genes. Virology. 1997;238:243–253. PubMed PMID: 9400597.
457. Thomas CJ, Brown HL, Hawes CR, Lee BY, Min MK, King LA, Possee RD. Localization of a baculovirus-induced chitinase in the insect cell endoplasmic reticulum. J Virol. 1998;72:10207–12. PubMed PMID: 9811762.
458. Saville GP, Patmanidi AL, Possee RD, King LA. Deletion of the Autographa californica nucleopolyhedrovirus chitinase KDEL motif and in vitro and in vivo analysis of the modified virus. J Gen Virol. 2004;85:821–31. PubMed PMID: 15039525.
459. Oliveira JV, Wolff JL, Garcia-Maruniak A, Ribeiro BM, de Castro ME, de Souza ML, Moscardi F, Maruniak JE, Zanotto PM. Genome of the most widely used viral biopesticide: Anticarsia gemmatalis multiple nucleopolyhedrovirus. J Gen Virol. 2006;87:3233–50. PubMed PMID: 17030857.
460. Slack JM, Kuzio J, Faulkner P. Characterization of *v-cath*, a cathepsin L-like proteinase expressed by the baculovirus *Autographa californica* multiple nuclear polyhedrosis virus. J Gen Virol. 1995;76:1091–1098. PubMed PMID: 7730794.
461. Hom LG, Ohkawa T, Trudeau D, Volkman LE. Autographa californica M nucleopolyhedrovirus ProV-CATH is activated during infected cell death. Virology. 2002;296:212–8. PubMed PMID: 12069520.
462. Hodgson JJ, Arif BM, Krell PJ. Autographa californica multiple nucleopolyhedrovirus and Choristoneura fumiferana multiple nucleopolyhedrovirus v-cath genes are expressed as pre-proenzymes. J Gen Virol. 2009;90:995–1000. PubMed PMID: 19264635.
463. Hodgson JJ, Arif BM, Krell PJ. Interaction of Autographa californica multiple nucleopolyhedrovirus cathepsin protease progenitor (proV-CATH) with insect baculovirus chitinase as a mechanism for proV-CATH cellular retention. J Virol. 2011;85:3918–29. PubMed PMID: 21289117.
464. Ardisson-Araujo DM, Melo FL, Clem RJ, Wolff JL, Ribeiro BM. A Betabaculovirus-Encoded gp64 Homolog Codes for a Functional Envelope Fusion Protein. J Virol. 2016;90:1668–72. PubMed PMID: 26537678.
465. Morse MA, Marriott AC, Nuttall PA. The glycoprotein of Thogoto virus (a tick-borne orthomyxo-like virus) is related to the baculovirus glycoprotein gp64. Virology. 1992;186:640–646. PubMed PMID: 1733105.
466. Hohmann AW, Faulkner P. Monoclonal antibodies to baculovirus structural proteins: determination of specificities by western blot analysis. Virology. 1983;125:432–444. PubMed PMID: 6340331.

467. Whitford M, Stewart S, Kuzio J, Faulkner P. Identification and sequence analysis of a gene encoding gp67 an abundant envelope glycoprotein of the baculovirus, *Autographa californica* nuclear polyhedrosis virus. J Virol. 1989;63:1393–1399. PubMed PMID: 2644449.
468. Volkman LE. l986. The 64K envelope protein of budded *Autographa californica* nuclear polyhedrosis virus. Curr Top Microbiol Immunol.131:103–118. PubMed PMID: 3545692.
469. Roberts TE, Faulkner P. Fatty acid acylation of the 67K envelope glycoprotein of a baculovirus: *Autographa californica* nuclear polyhedrosis virus. Virology. 1989;172:377–381. PubMed PMID: 2672565.
470. Monsma SA, Oomens AGP, Blissard GW. The gp64 envelope fusion protein is an essential baculovirus protein required for cell-to-cell transmission of infection. J Virol. 1996;70:4607–4616. PubMed PMID: 8676487.
471. Oomens AG, Blissard GW. Requirement for GP64 to drive efficient budding of *Autographa californica* multicapsid nucleopolyhedrovirus. Virology. 1999;254:297–314. PubMed PMID: 9986796.
472. Kadlec J, Loureiro S, Abrescia NG, Stuart DI, Jones IM. The postfusion structure of baculovirus gp64 supports a unified view of viral fusion machines. Nat Struct Mol Biol. 2008;10:1024–30. PubMed PMID: 18776902.
473. Feng M, Kong X, Zhang J, Xu W, Wu X. Identification of a novel host protein SINAL10 interacting with GP64 and its role in Bombyx mori nucleopolyhedrovirus infection. Virus Res. 2018;247:102–110. PubMed PMID: 29447976.
474. Wolgamot GM, Gross CH, Russell RLQ, Rohrmann GF. Immunocytochemical characterization of p24, a baculovirus capsid-associated protein. J Gen Virol. 1993;74:103–107. PubMed PMID: 8423444.
475. Gombart AF, Blissard GW, Rohrmann GF. Characterization of the genetic organization of the HindIII-M region of the multicapsid nuclear polyhedrosis virus of Orgyia pseudotsugata reveals major differences among baculoviruses. J Gen Virol. 1989;70:1815–1828. PubMed PMID: 2661722.
476. Schetter C, Oellig C, Doerfler W. An insertion of insect cell DNA in the 81-map-unit segment of *Autographa californica* nuclear polyhedrosis virus DNA. J Virol. 1990;64:1844–1850. PubMed PMID: 2157067.
477. Slavicek JM, Hayes-Plazolles N. The Lymantria dispar nucleopolyhedrovirus contains the capsid-associated p24 protein gene. Virus Genes. 2003;26:15–8. PubMed PMID: 12680688.
478. Yang M, Huang C, Qian DD, Li LL. Functional characterization of Autographa californica multiple nucleopolyhedrovirus gp16 (ac130). Virology. 2014;464-465:341–352. PubMed PMID: 25113905.
479. Gross CH, Wolgamot GM, Russell RLQ, Pearson MN, Rohrmann GF. A baculovirus encoded 16 kDa glycoprotein localizes near the nuclear membrane of infected cells. Virology. 1993;192:386–390. PubMed PMID: 8517029.
480. Sajjan DB, Hinchigeri SB. Structural Organization of Baculovirus Occlusion Bodies and Protective Role of Multilayered Polyhedron Envelope Protein. Food Environ Virol. 2016;8:86–100. PubMed PMID: 26787118.
481. Minion FC, Coons LB, Broome JR. Characterization of the polyhedral envelope of the nuclear polyhedrosis virus of *Heliothis virescens*. J Invertebr Pathol. 1979;34:303–307.
482. Whitt MA, Manning JS. A phosphorylated 34-kDa protein and a subpopulation of polyhedrin are thiol-linked to the carbohydrate layer surrounding a baculovirus occlusion body. Virology. 1988;163:33–42. PubMed PMID: 3279702.
483. van Lent JWM, Groenen JTM, Klinge-Roode EC, Rohrmann GF, Zuidema D, Vlak JM. Localization of the 34kDa polyhedron envelope protein in *Spodoptera frugiperda* cells infected with *Autographa californica* nuclear polyhedrosis virus. Archives of Virology. 1990;111:103–114. PubMed PMID: 2109589.
484. Gombart AF, Pearson MN, Rohrmann GF, Beaudreau GS. A baculovirus polyhedral envelope-associated protein: genetic location, nucleotide sequence, and immunocytochemical characterization. Virology. 1989;169:182–193. PubMed PMID: 2646825.
485. Russell RLQ, Rohrmann GF. A baculovirus polyhedron envelope protein: Immunogold localization in infected cells and mature polyhedra. Virology. 1990;174:177–184. PubMed PMID: 2403704.
486. Russell RLQ, Pearson MN, Rohrmann GF. Immunoelectron microscopic examination of *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus-infected *Lymantria dispar* cells: time course and

localization of major polyhedron-associated proteins. J Gen Virol. 1991;72:275–283. PubMed PMID: 1993872.
487. Lee SY, Poloumienko A, Belfry S, Qu X, Chen W, MacAfee N, Morin B, Lucarotti C, Krause M. A common pathway for p10 and calyx proteins in progressive stages of polyhedron envelope assembly in AcMNPV-infected Spodoptera frugiperda larvae. Arch Virol. 1996;141:1247–58. PubMed PMID: 8774685.
488. Escasa SR, Lauzon HA, Mathur AC, Krell PJ, Arif BM. Sequence analysis of the Choristoneura occidentalis granulovirus genome. J Gen Virol. 2006;87:1917–33. PubMed PMID: 16760394.
489. Lange M, Jehle JA. The genome of the Cryptophlebia leucotreta granulovirus. Virology. 2003;317:220–36. PubMed PMID: 14698662.
490. Gross CH, Russell RLQ, Rohrmann GF. The Orgyia pseudotsugata baculovirus p10 and polyhedron envelope protein genes: analysis of their relative expression levels and role in polyhedron structure. J Gen Virol. 1994;75:1115–1123. PubMed PMID: 8176372.
491. Zhou Y, Yao Q, Shen H, Xia H, Lin F, Chen K. Characterization of Bombyx mori Nucleopolyhedrovirus ORF109 that Encodes a 25-kDa Structural Protein of the Occlusion-Derived Virion. Curr Microbiol. 2010;61:451–7. PubMed PMID: 20372907.
492. Yang M, Wang S, Yue XL, Li LL. Autographa californica multiple nucleopolyhedrovirus orf132 encodes a nucleocapsid-associated protein required for budded-virus and multiply enveloped occlusion-derived virus production. J Virol. 2014;88:12586–98. PubMed PMID: 25142609.
493. Fang Z, Li C, Wu W, Yuan M, Yang K. The Autographa californica multiple nucleopolyhedrovirus Ac132 plays a role in nuclear entry. J Gen Virol. 2016;97:3030–3038. PubMed PMID: 27608627.
494. Li L. Characterization of a baculovirus alkaline nuclease. J Virol. 2000;74:6401–6407. Rohrmann. PubMed PMID: 10864651.
495. Okano K, Vanarsdall AL, Rohrmann GF. Characterization of a baculovirus lacking the Alkaline Nuclease Gene. J Virol. 2004;78:10650–10656. PubMed PMID: 15367632.
496. Okano K, Vanarsdall AL, Rohrmann GF. A Baculovirus alkaline nuclease knock out construct produces fragmented DNA and aberrant capsids. Virology. 2007;359:46–54. PubMed PMID: 17046043.
497. Ferrelli ML, Salvador R, Biedma ME, Berretta MF, Haase S, Sciocco-Cap A, Ghiringhelli PD, Romanowski V. Genome of Epinotia aporema granulovirus (EpapGV), a polyorganotropic fast killing betabaculovirus with a novel thymidylate kinase gene. BMC Genomics. 2012;13:548. PubMed PMID: 23051685.
498. Clem RJ, Robson M, Miller LK. Influence of infection route on the infectivity of baculovirus mutants lacking the apoptosis-inhibiting gene *p35* and the adjacent gene *p94*. J Virol. 1994;68:6759–6762. PubMed PMID: 8084009.
499. Du Q, Lehavi D, Faktor O, Qi Y, Chejanovsky N. Isolation of an apoptosis suppressor gene of the Spodoptera littoralis nucleopolyhedrovirus. J Virol. 1999;73:1278–85. PubMed PMID: 9882332.
500. Zoog SJ, Schiller JJ, Wetter JA, Chejanovsky N, Friesen PD. Baculovirus apoptotic suppressor P49 is a substrate inhibitor of initiator caspases resistant to P35 in vivo. Embo J. 2002;21:5130–40. PubMed PMID: 12356729.
501. Means JC, Penabaz T, Clem RJ. Identification and functional characterization of AMVp33, a novel homolog of the baculovirus caspase inhibitor p35 found in Amsacta moorei entomopoxvirus. Virology. 2007;358:436–47. PubMed PMID: 17010407.
502. Clem RJ, Fechheimer M, Miller LK. Prevention of apoptosis by a baculovirus gene during infection of insect cells. Science. 1991;254:1388–1390. PubMed PMID: 1962198.
503. Herschberger PA, Dickson JA, Friesen PD. Site-specific mutagenesis of the 35-kilodalton protein gene encoded by Autographa californica nuclear polyhedrosis virus: cell line-specific effects on virus replication. J Virol. 1992;66:5525–5533. PubMed PMID: 1501287.
504. dela Cruz WP, Friesen PD, Fisher AJ. Crystal structure of baculovirus P35 reveals a novel conformational change in the reactive site loop after caspase cleavage. J Biol Chem. 2001;276:32933–9. PubMed PMID: 11402050.
505. Fisher AJ, Cruz W, Zoog SJ, Schneider CL, Friesen PD. Crystal structure of baculovirus P35: role of a novel reactive site loop in apoptotic caspase inhibition. Embo J. 1999;18:2031–9. PubMed PMID: 10205157.

506. Goenka S, Weaver RF. The p26 gene of the Autographa californica nucleopolyhedrovirus: Timing of transcription, and cellular localization and dimerization of product. Virus Res. 2008;31:136–44. PubMed PMID: 17935816.
507. Simon O, Williams T, Caballero P, Possee RD. Effects of Acp26 on in vitro and in vivo productivity, pathogenesis and virulence of Autographa californica multiple nucleopolyhedrovirus. Virus Res. 2008;136:202–5. PubMed PMID: 18538883.
508. Wang L, Salem TZ, Campbell DJ, Turney CM, Kumar CM, Cheng XW. Characterization of a virion occlusion-defective Autographa californica multiple nucleopolyhedrovirus mutant lacking the p26, p10 and p74 genes. J Gen Virol. 2009;90:1641–8. PubMed PMID: 19264658.
509. Raza F, McGouran JF, Kessler BM, Possee RD, King LA. Phosphorylation Induces Structural Changes in the Autographa californica Nucleopolyhedrovirus P10 Protein. J Virol. 2017.:91. PubMed PMID: 28424279.
510. Alaoui-Ismaili MH, Richardson CD. Insect virus proteins (FALPE and p10) self-associate to form filaments in infected cells. J Virol. 1998;72:2213–23. PubMed PMID: 9499079.
511. Kuzio JD, Rohel Z, Curry CJ, Krebs A, Carstens EB, Faulkner P. Nucleotide sequence of the p10 polypeptide gene of *Autographa californica* nuclear polyhedrosis virus. Virology. 1984;139:414–418. PubMed PMID: 18639833.
512. Weyer U, Possee RD. A baculovirus dual expression vector derived from the Autographa californica nuclear polyhedrosis virus polyhedrin and p10 promoters: co-expression of two influenza virus genes in insect cells. J Gen Virol. 1991;72(Pt 12):2967–74. PubMed PMID: 1765769.
513. Shi X, Ran Z, Li S, Yin J, Zhong J. The Effect of MicroRNA bantam on Baculovirus AcMNPV Infection in Vitro and in Vivo. Viruses. 2016.:8. PubMed PMID: 27196923.
514. Patmanidi AL, Possee RD, King LA. Formation of P10 tubular structures during AcMNPV infection depends on the integrity of host-cell microtubules. Virology. 2003;317:308–20. PubMed PMID: 14698669.
515. Carpentier DC, Griffiths CM, King LA. The baculovirus P10 protein of Autographa californica nucleopolyhedrovirus forms two distinct cytoskeletal-like structures and associates with polyhedral occlusion bodies during infection. Virology. 2008;371:278–91. PubMed PMID: 17991504.
516. Vlak JM, Klinkenberg FA, Zaal KJM, Usmany M, Klinge-roode EC, Geervliet JBF, Roosien J, Lent JWV. Functional studies on the p10 gene of *Autographa californica* nuclear polyhedrosis virus using a recombinant expressing a p10-b-galactosidase fusion gene. J Gen Virol. 1988;69:765–776. PubMed PMID: 3128641.
517. Williams GV, Rohel DZ, Kuzio J, Faulkner P. A cytopathological investigation of *Autographa californica* nuclear polyhedrosis virus p10 gene function using insertion/deletion mutants. J Gen Virol. 1989;70:187–202. PubMed PMID: 2659726.
518. Faulkner P, Kuzio J, Williams GV, Wilson JA. Analysis of p74, a PDV envelope protein of Autographa californica nucleopolyhedrovirus required for occlusion body infectivity in vivo. J Gen Virol. 1997;78:3091–3100. PubMed PMID: 9400957.
519. Haas-Stapleton EJ, Washburn JO, Volkman LE. P74 mediates specific binding of Autographa californica M nucleopolyhedrovirus occlusion-derived virus to primary cellular targets in the midgut epithelia of Heliothis virescens larvae. J Virol. 2004;78:6786–6789. PubMed PMID: 15194753.
520. Kuzio J, Jaques R, Faulkner P. Identification of p74, a gene essential for virulence of baculovirus occlusion bodies. Virology. 1989;173:759–63. PubMed PMID: 2688302.
521. Zhou W, Yao L, Xu H, Yan F, Qi Y. The function of envelope protein p74 from Autographa californica M nucleopolyhedrovirus in primary infection to host. Virus Genes. 2005;30:139–150. PubMed PMID: 15744572.
522. Ohkawa T. 1997. Identification and characterization of genes of the baculovirus Bombyx mori nucleopolyhedrovirus (BmNPV) involved in viral pathogenesis. . PhD thesis University of California, Davis, Calif.
523. Peng K, van Lent JW, Vlak JM, Hu Z, van Oers MM. In situ cleavage of baculovirus occlusion-derived virus receptor binding protein P74 in the peroral infectivity complex. J Virol. 2011;85:10710–8. PubMed PMID: 21849453.

524. Mu J, van Lent JW, Smagghe G, Wang Y, Chen X, Vlak JM, van Oers MM. Live imaging of baculovirus infection of midgut epithelium cells: a functional assay of per os infectivity factors. J Gen Virol. 2014;95:2531–9. PubMed PMID: 25006078.
525. de Jong J, Arif BM, Theilmann DA, Krell PJ. Autographa californica multiple nucleopolyhedrovirus me53 (ac140) is a nonessential gene required for efficient budded-virus production. J Virol. 2009;83:7440–8. PubMed PMID: 19457997.
526. Xi Q, Wang J, Deng R, Wang X. Characterization of AcMNPV with a deletion of me53 gene. Virus Genes. 2007;34:223–32. PubMed PMID: 17096186.
527. Knebel-Mörsdorf D, Kremer A, Jahnel F. Baculovirus gene ME53, which contains a putative zinc finger motif, is one of the major early-transcribed genes. J Virol. 1993;67:753–8. PubMed PMID: 8093490.
528. de Jong J, Theilmann DA, Arif BM, Krell PJ. Immediate-early protein ME53 forms foci and colocalizes with GP64 and the major capsid protein VP39 at the cell membranes of Autographa californica multiple nucleopolyhedrovirus-infected cells. J Virol. 2011;85:9696–707. PubMed PMID: 21775466.
529. Liu Y, de Jong J, Nagy E, Theilmann DA, Krell PJ. Nuclear Translocation Sequence and Region in Autographa californica Multiple Nucleopolyhedrovirus ME53 That Are Important for Optimal Baculovirus Production. J Virol. 2016;90:3953–3965. PubMed PMID: 26842471.
530. Fang M, Dai X, Theilmann DA. Autographa californica multiple nucleopolyhedrovirus EXON0 (ORF141) is required for efficient egress of nucleocapsids from the nucleus. J Virol. 2007;81:9859–69. PubMed PMID: 17626083.
531. Dai X, Stewart TM, Pathakamuri JA, Li Q, Theilmann DA. Autographa californica multiple nucleopolyhedrovirus exon0 (orf141), which encodes a RING finger protein, is required for efficient production of budded virus. J Virol. 2004;78:9633–44. PubMed PMID: 15331696.
532. Fang M, Nie Y, Theilmann DA. AcMNPV EXON0 (AC141) which is required for the efficient egress of budded virus nucleocapsids interacts with beta-tubulin. Virology. 2009;385:496–504. PubMed PMID: 19155039.
533. Biswas S, Blissard GW, Theilmann DA. Trichoplusia ni Kinesin-1 Associates with Autographa californica Multiple Nucleopolyhedrovirus Nucleocapsid Proteins and Is Required for Production of Budded Virus. J Virol. 2016;90:3480–95. PubMed PMID: 26763996.
534. McCarthy CB, Dai X, Donly C, Theilmann DA. Autographa californica multiple nucleopolyhedrovirus ac142, a core gene that is essential for BV production and ODV envelopment. Virology. 2008;372:325–39. PubMed PMID: 18045640.
535. Yang ZN, Xu HJ, Park EY, Zhang CX. 2008. Characterization of Bombyx mori nucleopolyhedrovirus with a deletion of bm118. Virus Res.
536. Braunagel SC, He H, Ramamurthy P, Summers MD. Transcription, translation, and cellular localization of three Autographa californica nuclear polyhedrosis virus structural proteins: ODV-E18, ODV-E35 and ODV-EC27. Virology. 1996;222:100–114. PubMed PMID: 8806491.
537. McCarthy CB, Theilmann DA. AcMNPV ac143(odv-e18) is essential for mediating budded virus production and is the 30th baculovirus core gene. Virology. 2008;375:277–91. PubMed PMID: 18328526.
538. Belyavskyi M, Braunagel SC, Summers MD. The structural protein ODV-EC27 of Autographa californica nucleopolyhedrovirus is a multifunctional viral cyclin. Proc Natl Acad Sci U S A. 1998;95:11205–10. PubMed PMID: 9736714.
539. Dall D, Luque T, O'Reilly DR. Insect-virus relationships: sifting by informatics. Bioessays. 2001;23:184–193. PubMed PMID: 11169592.
540. Lapointe R, Popham HJ, Straschil U, Goulding D, O'Reilly DR, Olszewski JA. Characterization of two Autographa californica nucleopolyhedrovirus proteins, Ac145 and Ac150, which affect oral infectivity in a host-dependent manner. J Virol. 2004;78:6439–48. PubMed PMID: 15163737.
541. Zhang JH, Ohkawa T, Washburn JO, Volkman LE. Effects of Ac150 on virulence and pathogenesis of Autographa californica multiple nucleopolyhedrovirus in noctuid hosts. J Gen Virol. 2005;86:1619–27. PubMed PMID: 15914839.

542. Wang D, Zhang CX. HearSNPV orf83 encodes a late, nonstructural protein with an active chitin-binding domain. Virus Res. 2006;117:237–43. PubMed PMID: 16313991.
543. Dickison VL, Willis LG, Sokal NR, Theilmann DA. Deletion of AcMNPV ac146 eliminates the production of budded virus. Virology. 2012;431:29–39. PubMed PMID: 22682215.
544. Ge JQ, Zhao JF, Shao YM, Tian CH, Zhang CX. Characterization of an early gene orf122 from Bombyx mori nucleopolyhedrovirus. Mol Biol Rep. 2009;36:543–8. PubMed PMID: 18246445.
545. Guarino LA, Summers MD. Interspersed homologous DNA of *Autographa californica* nuclear polyhedrosis virus enhances delayed-early gene expression. J Virol. 1986;60:215–223. PubMed PMID: 16789258.
546. Leisy DJ, Rasmussen C, Owusu O, Rohrmann GF. A mechanism for negative gene regulation in *Autographa californica* multicapsid nuclear polyhedrosis virus. J Virol. 1997;71:5088–5094. PubMed PMID: 9188574.
547. Stewart TM, Huijskens I, Willis LG, Theilmann DA. The Autographa californica multiple nucleopolyhedrovirus ie0-ie1 gene complex is essential for wild-type virus replication, but either IE0 or IE1 can support virus growth. J Virol. 2005;79:4619–29. PubMed PMID: 15795248.
548. Chisholm GE, Henner DJ. Multiple early transcripts and splicing of the *Autographa californica* nuclear polyhedrosis virus IE-1 gene. J Virol. 1988;62:3193–3200. PubMed PMID: 3043024.
549. Sokal N, Nie Y, Willis LG, Yamagishi J, Blissard GW, Rheault MR, Theilmann DA. Defining the roles of the baculovirus regulatory proteins IE0 and IE1 in genome replication and early gene transactivation. Virology. 2014;468-470:160–171. PubMed PMID: 25173193.
550. Ono C, Sato M, Taka H, Asano S, Matsuura Y, Bando H. Tightly regulated expression of Autographa californica multicapsid nucleopolyhedrovirus immediate early genes emerges from their interactions and possible collective behaviors. PLoS One. 2015;10:e0119580. PubMed PMID: 25816136.
551. Pearson MN, Rohrmann GF. Splicing is required for transactivation by the immediate early gene 1 of the Lymantria dispar multicapsid nuclear polyhedrosis virus. Virology. 1997;235:153–165. PubMed PMID: 9300047.
552. Braunagel SC, Elton DM, Ma H, Summers MD. Identification and analysis of an *Autographa californica* nuclear polyhedrosis virus structural protein of the occlusion-derived virus envelope: ODV-E56. Virology. 1996;217:97–110. PubMed PMID: 8599240.
553. Theilmann DA, Chantler JK, Stewart S, Flipsen HTM, Vlak JM, Crook NE. Characterization of a highly conserved baculovirus structural protein that is specific for occlusion-derived virions. Virology. 1996;218:148–158. PubMed PMID: 8615018.
554. Harrison RL, Sparks WO, Bonning BC. 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. 2010;91:1173–82. PubMed PMID: 20032203.
555. Sparks WO, Harrison RL, Bonning BC. Autographa californica multiple nucleopolyhedrovirus ODV-E56 is a per os infectivity factor, but is not essential for binding and fusion of occlusion-derived virus to the host midgut. Virology. 2011. PubMed PMID: 20970820.
556. Hao B, Huang J, Sun X, Deng F, Zhang Y, Wang H, Chen H, Hu Z. Variants of open reading frame Bm126 in wild-type Bombyx mori nucleopolyhedrovirus isolates exhibit functional differences. J Gen Virol. 2009;90:153–61. PubMed PMID: 19088284.
557. Imai N, Matsumoto S, Kang W. Formation of Bombyx mori nucleopolyhedrovirus IE2 nuclear foci is regulated by the functional domains for oligomerization and ubiquitin ligase activity. J Gen Virol. 2005;86:637–44. PubMed PMID: 15722524.
558. Carson DD, Guarino LA, Summers MD. Functional mapping of an AcNPV immediate early gene which augments expression of the IE-1 trans-activated 39K gene. Virology. 1988;162:444–451. PubMed PMID: 3277328.
559. Carson DD, Summers MD, Guarino LA. Molecular analysis of a baculovirus regulatory gene. Virology. 1991;182:279–286. PubMed PMID: 2024466.
560. Yoo S, Guarino LA. The *Autographa californica* nuclear polyhedrosis virus *ie2* gene encodes a transcriptional regulator. Virology. 1994;202:746–753. PubMed PMID: 8030237.

561. Imai N, Kang W, Iwabuchi K, Sato K, Maeda S. Analysis of interaction between molecules of Bombyx mori nucleopolyhedrovirus IE-2 using a yeast two-hybrid system. Acta Virol. 2000;44:199–202. PubMed PMID: 11155366.
562. Prikhod'ko EA, Lu A, Wilson JA, Miller LK. In vivo and in vitro analysis of baculovirus ie-2 mutants. J Virol. 1999;73:2460–8. PubMed PMID: 9971831.
563. Prikhod'ko EA, Miller LK. Role of baculovirus IE2 and its RING finger in cell cycle arrest. J Virol. 1998;72:684–92. PubMed PMID: 9420274.
564. Tung H, Wei SC, Lo HR, Chao YC. Baculovirus IE2 Stimulates the Expression of Heat Shock Proteins in Insect and Mammalian Cells to Facilitate Its Proper Functioning. PLoS One. 2016;11:e0148578. PubMed PMID: 26863132.
565. Krappa R, Knebel-Mörsdorf D. Identification of the very early transcribed baculovirus gene PE-38. J Virol. 1991;65:805–812. PubMed PMID: 1987375.
566. Wu X, Stewart S, Theilmann DA. Alternative transcriptional initiation as a novel mechanism for regulating expression of a baculovirus *trans* activator. J Virol. 1993;67:5833–5842. PubMed PMID: 8371344.
567. Milks ML, Washburn JO, Willis LG, Volkman LE, Theilmann DA. Deletion of pe38 attenuates AcMNPV genome replication, budded virus production, and virulence in Heliothis virescens. Virology. 2003;310:224–34. PubMed PMID: 12781710.
568. Jiang SS, Chang IS, Huang LW, Chen PC, Wen CC, Liu SC, Chien LC, Lin CY, Hsiung CA, Juang JL. Temporal transcription program of recombinant Autographa californica multiple nucleopolyhedrosis virus. J Virol. 2006;80:8989–99. PubMed PMID: 16940511.
569. Gebhardt MM, Eberle KE, Radtke P, Jehle JA. Baculovirus resistance in codling moth is virus isolate-dependent and the consequence of a mutation in viral gene pe38. Proc Natl Acad Sci U S A. 2014;111:15711–6. PubMed PMID: 25331863.
570. Luque T, Finch R, Crook N, O'Reilly DR, Winstanley D. The complete sequence of the Cydia pomonella granulovirus genome. J Gen Virol. 2001;82:2531–47. PubMed PMID: 11562546.
571. Willis LG, Siepp R, Stewart TM, Erlandson MA, Theilmann DA. Sequence analysis of the complete genome of Trichoplusia ni single nucleopolyhedrovirus and the identification of a baculoviral photolyase gene. Virology. 2005;338:209–26. PubMed PMID: 15951000.
572. Thumbi DK, Eveleigh RJ, Lucarotti CJ, Lapointe R, Graham RI, Pavlik L, Lauzon HA, Arif BM. Complete sequence, analysis and organization of the Orgyia leucostigma nucleopolyhedrovirus genome. Viruses. 2011;3:2301–27. PubMed PMID: 22163346.
573. Guarino LA, Gonzalez MA, Summers MD. Complete sequence and enhancer function of the homologous DNA regions of *Autographa californica* nuclear polyhedrosis virus. J Virol. 1986;60:224–229. PubMed PMID: 16789259.
574. Leisy DJ, Rohrmann GF. Characterization of the replication of plasmids containing *hr* sequences in baculovirus-infected *Spodoptera frugiperda* cells. Virology. 1993;196:722–730. PubMed PMID: 8372444.
575. Pearson MN, Bjornson RM, Ahrens C, Rohrmann GF. Identification and characterization of a putative origin of DNA replication in the genome of a baculovirus pathogenic for *Orgyia pseudotsugata*. Virology. 1993;197:715–725. PubMed PMID: 8249294.
576. Pearson MN, Bjornson RM, Pearson GD, Rohrmann GF. The *Autographa californica* baculovirus genome: Evidence for multiple replication origins. Science. 1992;257:1382–1384. PubMed PMID: 1529337.
577. Pearson MN, Rohrmann GF. *Lymantria dispar* nuclear polyhedrosis virus homologous regions: characterization of their ability to function as replication origins. J Virol. 1995;69:213–221. PubMed PMID: 7983712.
578. Choi J, Guarino LA. The baculovirus transactivator IE1 binds to viral enhancer elements in the absence of insect cell factors. J Virol. 1995;69:4548–4551. PubMed PMID: 7769721.
579. Leisy DJ, Rasmussen C, Kim H-T, Rohrmann GF. The *Autographa californica* nuclear polyhedrosis virus homologous region 1a: Identical sequences are essential for DNA replication activity and transcriptional enhancer function. Virology. 1995;208:742–752. PubMed PMID: 7747446.

580. Rodems SM, Friesen PD. The hr5 transcriptional enhancer stimulates early expression from the *Autographa californica* nuclear polyhedrosis virus genome but is not required for virus replication. J Virol. 1993;67:5776–5785. PubMed PMID: 8371342.
581. Nagamine T, Kawasaki Y, Iizuka T, Matsumoto S. Focal Distribution of Baculovirus IE1 Triggered by Its Binding to the hr DNA Elements. JVirol. 2005;79:39–46. PubMed PMID: 15596799.
582. Landais I, Vincent R, Bouton M, Devauchelle G, Duonor-Cerutti M, Ogliastro M. Functional analysis of evolutionary conserved clustering of bZIP binding sites in the baculovirus homologous regions (hrs) suggests a cooperativity between host and viral transcription factors. Virology. 2006;344:421–31. PubMed PMID: 16198391.
583. Carstens EB, Wu Y. No single homologous repeat region is essential for DNA replication of the baculovirus Autographa californica multiple nucleopolyhedrovirus. J Gen Virol. 2007;88:114–22. PubMed PMID: 17170443.
584. Bossert M, Carstens EB. Sequential deletion of AcMNPV homologous regions leads to reductions in budded virus production and late protein expression. Virus Res. 2018;256:125–133. PubMed PMID: 30121325.

13. Selected baculovirus genes without orthologs in the AcMNPV genome: Conservation and function

Below is a non-inclusive list of baculovirus genes that are not present in the AcMNPV genome, but that either have homology with well-characterized genes from other organisms, or that have been investigated in baculoviruses. Following this list is a summary of investigations on each gene.

- Apsup
- CIDE domain protein
- Collagenase
- DNA ligase
- dUTPase
- Enhancin
- Eukaryotic translation initiation factor 5
- G protein-coupled receptor
- Helicase-2
- HOAR
- Iap-3
- Metalloproteinase
- Nicotinamide riboside kinase 1
- PARP
- Phosphotransferase
- PTP-2
- Photolyase
- Ribonucleotide reductase Large subunit
- Ribonucleotide reductase Small Subunit
- Serpin
- SWI/SNF Chromatin remodelers
- Thymidylate kinase
- Trypsin-like
- V-TREX

Apsup: a third baculovirus antiapoptotic gene family

The apoptotic suppressor (*apsup*) was discovered in the genome of the *Lymantria dispar* MNPV. It encodes a protein with a predicted mass 39.3 kDa and does not appear to be related to other proteins in the database. It blocks initiator caspases. Homologs were identified in *Lymantria xylina* MNPV and also in AcMNPV (Ac112/113). Ac112/113 shows about 30% amino acid sequence identity to APSUP, but is truncated and lacks 79 amino acids at its C-terminus and also lacks anti apoptotic activity (1-3).

CIDE domain proteins. The cell death-inducing DFF45-like effector (CIDE) domain is usually present near the N-terminus of a DNAse that is activated by caspase cleavage and is associated with the degradation of DNA during apoptosis and lipid homeostasis (4). CIDE_N domains have been identified (ORF38) of the Mythimna unipuncta GV (MyunGV)) (5) and in a Group I NPV Choristoneura fumiferana def (CfdefMNPV) (orf142). MyunGV orf38 is related to orfs from 5 other GVs. In contrast, CfdefMNPV orf142 is most closely related to an orf from another Group I NPV (*Neophasia* sp – the pine butterfly – NespNPV. Both lineages showed structural relatedness to CIDE domains from Mus musculus and Drosophila melanogaster with a probability of almost 100% by Hhpred (6). The predicted CIDE domain proteins from the NPVs and GVs are only distantly related suggesting that this protein may have been incorporated into baculovirus genomes on two independent occasions.

Collagenase. Most group II alphabaculoviruses encode proteins of over 800 amino acids related to collagenases from Clostridium. In Clostridium these are extracellular enzymes (reviewed in (7)).

DNA Ligase. A DNA ligase would be involved in the ligation of Okazaki fragments during lagging strand synthesis. Homologs of DNA ligase are present in all sequenced granulovirus genomes and at least two NPV genomes (LdMNPV and MacoNPV-B). The GV ligases are similar to ligase I, whereas the LdMNPV is similar to ligase III (8). Vaccinia also encodes an ortholog of ligase III (9). The DNA ligase of LdMNPV was characterized and found to be capable of ligating double-stranded synthetic DNA substrates containing a single nick (10). A striking feature of the baculovirus ligase homologs is that they are always (except MacoNPV-B) accompanied with a helicase homolog that is not found in any of the genomes lacking ligase. This helicase is related to the PIF1 family (10) (note: this is not a per os infectivity factor). Members of this family have a preference for RNA-DNA hybrids and could be involved in the maturation of Okazaki fragments (11). This may involve displacement of the RNA primer, producing an RNA flap that would then be cleaved by a flap endonuclease (FEN) (12) or digested by a 5' to 3' exonuclease. DNA polymerase would then fill in the gap by extending the Okazaki fragment, and the ligase could join the fragments.

dUTPase. Deoxyuridine triphosphate (dUTP) can be mutagenic if incorporated into DNA. The enzyme dUTPase dephosphorylates dUTP to dUMP, which is a substrate for thymidine biosynthesis. Homologs of dUTPase are present in many NPVs (most in Group II) and a few GV genomes (13). Baculoviruses may have incorporated this gene to either supplement or substitute for the host gene. The viruses that encode a *dutpase* homolog also normally encode both subunits of ribonucleotide reductase (RR) (see below). The presence of RR may have selected for the incorporation of *dutpase* in order to mitigate the production of the dUTP mutagen by ribonucleotide reductase. In one study of an NPV, dUTPase first appeared in cell nuclei, but late in the infection it appeared to be excluded from the nucleus, but was diffusely located in the cytoplasm (14). An orf in *Perigonia lusca* single nucleopolyhedrovirus (PeluSNPV), (*pelu112)* was predicted to encode a fusion of *dUTP and thymidylate kinase* (*tmk*) (15).

Enhancin. Metalloproteinases are endopeptidases that contain divalent cations as integral components of their structure (16). Enhancins are members of this proteinase group and are encoded by a few lepidopteran NPVs and GVs. In one study of TnGV, enhancin was estimated to comprise up to 5% of the mass of occlusion bodies (17). In LdMNPV, enhancin was found to be associated with ODV (18). Enhancin genes are often present in multiple copies, e.g., the XecnGV genome has four copies (19). In LdMNPV, which encodes two enhancins, deletion of either results in a 2- to 3-fold reduction in potency, whereas deletion of both caused a 12-fold reduction (20). Enhancin is thought to facilitate baculovirus infection by digesting the peritrophic membrane (PM). The PM forms a barrier in insect guts that prevents the ready access of pathogens to the epithelial cells. The PM is rich in chitin and insect intestinal mucin, and enhancins appear to target the degradation of intestinal mucin, thereby facilitating access of virions to the underlying cells (21) (22). Enhancins show sequence homology with high levels of significance (e.g., E = 3e-29) to predicted proteins of some pathogenic bacteria, e.g., *Clostridium botulinum*, and a variety of *Bacillus* (e.g., *B. anthracis*) and *Yersinia* (e.g., *Y. pestis*) species. To investigate their function, enhancins from *B. cereus*, *Y. pseudotuberculosis*, or TnGV were cloned into a construct of AcMNPV that yielded occluded viruses. Although the LD50 of these constructs was found to be about half of wt, only the construct expressing the TnGV enhancin caused a reduction in survival time. In addition, the bacterial enhancins failed to degrade insect intestinal mucin. It was suggested that the bacterial enhancins may have evolved an activity distinct from their viral homologs (23).

Eukaryotic translation initiation factor 5. Orthologs of eukaryotic translation initiation factor 5 have been found in at least two baculoviruses including *Choristoneura rosaceana* NPV (ChroNPV) and *Choristoneura occidentalis* GV (ChocGV). They are closely related (72% sequence identity) and are members of a lepidopteran lineage indicating that the gene was likely captured from a host insect and subsequently one virus obtained it from the other during a co-infection (24) (25).

G protein-coupled receptor (*GPCR*). A predicted G protein-coupled receptor (GPCR) was found in the Diatraea saccharalis *GV (DisaGV-Disa-038) genome. It has not been reported in other baculoviruses, but orthologs have been identified in a number of entompox viruses. The DisaGV GPCR was most closely related to those from Lepidoptera (26). The DisaGV GPCR was predicted to encode a signal peptide and 7 trans membrane domains suggesting that it belongs to the Secretin family of GPCRs (27).* A human herpesvirus (Epstein-Barr virus) encodes a GPCR that hijacks the signaling pathways of the cell (28).

Helicase-2. A second helicase homolog has been found in many GV and a few NPV genomes (13). The homology to one of the NPVs, from *Spodoptera littoralis* (SpliNPV)-ORF 40, is minimal. The *hel-2* gene from LdMNPV (29) is related to a yeast helicase that is important in recombination and repair of mitochondrial DNA. It had no effect on DNA replication in a transient replication assay and could not substitute for helicase-p143 (10). With one exception, *Mamestra configurata* NPV (MacoNPV-B) (and the limited homology of SpliNPV-ORF40 described above), the *hel-2* and *DNA ligase* genes (see above) are found in the same genomes (predominantly GVs), suggesting that they may participate in the same metabolic pathway in these viruses (see Chapter 5). In one GV genome, the hel-2 gene was fused to the alkaline exonuclease gene (30), suggesting that the fused gene may encode a protein involved in the separating and digestion of the RNA primer component of Okazaki fragments during lagging strand synthesis. A closely related ortholog of *hel-2* is also present in ascoviruses.

HOAR. According to Prof. David Tribe the name is derived as follows: 'H refers to Heliothis, O open reading frame and Hoar sounds like the given second name of the student Hoa (TH Le) who determined the sequence.' HOAR is predicted to contain a RING-finger domain and the gene appears to be somewhat unstable and shows a high degree of variation in a repeated region (31). Based on Hhpred it has a 96% probability of being a ubiquitin ligase. It is found in many group II alphabaculoviruses

Iap-3. Inhibitor of apoptosis-3. Although 6 lineages of *iap* genes have been identified in baculoviruses, the *iap-3* lineage is the most well-characterized and is a powerful inhibitor of apoptosis in certain cell lines. It is not found in the AcMNPV genome, although related *iap* genes are present. This lack of *iap-3* is likely compensated by the presence of *p35*, another apoptotic inhibitor. Members of the *iap*-3 lineage are found in Group I, II, GVs and hymenopteran NPVs. *Iap-3* genes are closely related to *iap* genes of insects. OpMNPV IAP-3 is 57% identical to IAP from *B. mori,* indicating that the *iap* gene was likely captured by viruses on one or more occasions. In addition, *iap* from *S. frugiperda* has similar properties to IAP-3 in terms of its structure and function (32). For additional information, see Chapter 7.

Metalloproteinase. As described above, metalloproteinases are endopeptidases that contain divalent cations. Baculoviruses encode three distinct metalloproteinases, cathepsin, enhancin, and a stromelysin1-like metalloproteinase. Although cathepsin homologs are found most lepidopteran group I and II NPVs, they are only found in four GV genomes and are not present in the hymenopteran and dipteran viruses. However, there are other enzymes encoded in GVs that might compensate for the lack of cathepsin. One such enzyme is a metalloproteinase that has homologs in all sequenced GV genomes, but is not present in NPV genomes. They have about 30% amino acid sequence identity to a catalytic domain in a stromelysin1 metalloproteinase of humans and sea urchins. The GV enzyme lacks a signal peptide and a cysteine switch that maintains the other enzymes in an inactive form. The stromelysin1-like metalloproteinase from XcGV was characterized and found be capable of digesting proteins and was inhibited by metalloproteinase inhibitors (33). It is possible that the universal presence of metalloproteinase homologs in the GV genomes is involved in assisting in their viral transmission by facilitating the disintegration of cells after the GV replicative cycle is complete.

Nicotinamide riboside kinase 1 (NRK1). Orthologs of NRK1 are found in most group II NPVs and in some GVs. It plays a role in nicotinamide adenine dinucleotide (NAD+) synthesis. It phosphorylates nicotinamide riboside yielding nicotinate mononucleotide (34). Since PARG reverses the ADP-ribosylation of proteins by

PARP and NRK1 is part of the nicotinamide adenine dinucleotide pathway, it is possible that the presence of PARG and NRK1 in many group II baculoviruses is indicative of their ability to manipulate these processes.

PARP. A homolog of poly (ADP-ribose) polymerases (PARP) has only been reported in a single baculovirus genome, *Anticarsia gemmatalis* (AgMNPV), Ag31 (35) (36). PARP is an enzyme found in nuclei. It is activated by DNA strand breaks and signals enzymatic pathways involved in DNA repair. Upon detecting a single strand break, it binds to the DNA and uses NAD+ as a substrate to synthesize polymers of ADP-ribose on acceptor proteins that in turn act as signals for recruiting enzymes involved in DNA repair. It is also involved in telomere elongation, chromatin structure, and the transcription of a variety of genes involved in immunity, stress resistance, hormone responses, and the possible silencing of retroelements (37) (38). It may also be involved in the regulation of a mitochondrial protein that induces apoptosis (39). PARP is a caspase-3 substrate and its cleavage is used as a measure of apoptosis.

Phosphotransferase. Homologs to RNA 2′-phosphotransferase are found in all three of the sequenced gammabaculovirus genomes (40). The substrates of this enzyme are [[2'-phospho-[ligated tRNA]]] and NAD+ and yields mature tRNA.

Protein tyrosine phosphatase-2. All group Group I alphabaculoviruses encode an ortholog of protein tyrosine phosphatase. The Group I viruses are divided into two major lineages and most of the viruses in one of these lineages encode another ptp gene called ptp-2. In addition, most Group II baculoviruses and at least two betabaculoviruses (GVs) also encode orthologs to ptp-2 (41). In OpMNPV, ptp-1 (op10) shows 60% aa sequence identity to AcMNPV PTP-1, but only ~20% identity to Op9 (PTP-2). PTP-2 is more closely related to a vaccinia and a human PTP with sequence identity of ~27% (42). The PTP2 of Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) was found to induce mild apoptosis when transiently expressed in *Spodoptera frugiperda* (Sf) 21 cells and the larvae infected. Deletion of ptp2 from SeMNPV resulted in a reduced yield of viral inclusion bodies (43). The crystal structure of ptp-2 from Cydia pomonella virus (CpGV) has been reported and it was found to contain a similar fold and active site structure to other phosphatases. It also contains a C-terminal extension in a region that corresponds to the interface of poxvirus dimeric phosphates that belong to the Tyr-Ser homology group(44).

Photolyase. Homologs of photolyase genes have been found in the genomes of Group II baculovirus that are members of a lineage that infects insects of the subfamily Plusiinae of the family Noctuidae (45-47). Orthologs are also found in some poxviruses (48) including entomopox viruses (49). They have also been observed to be associated with mitotic structures (50). Photolyases are involved in the repair of DNA damage caused by ultraviolet light. *Chrysodeixis chalcites* NPV (ChchNPV) encodes two photolyase genes that are predicted to encode proteins with 45% amino acid sequence identity. When both were tested, only one copy showed photoreactivating activity (51). Transfection of egfp fusions of photolyase genes into *T. ni* cells resulted in fluorescence localized to chromosomes and spindles and other structures associated with mitosis. Baculovirus infection of the transfected cells caused fluorescence to localize to the virogenic stroma (50). It was observed that one of the ChchNPV binds a CLOCK protein and represses CLOCK/BMAL1- transcription affected the oscillation of embryonic mouse fibroblasts indicating that it may be involved in circadian clock regulation (52). The incorporation of an algal virus photolyase gene as a means to cause resistance to UV inactivation of AcMNPV has been described. However, although BV survival was increased after exposure to UV light, occluded virion survival was not affected (53).

Ribonucleotide reductase. Ribonucleotide reductase is a heterodimer composed of large and small subunits (RR1 and RR2, respectively). It is involved in the catalysis of ribonucleotides to deoxyribonucleotides as a pathway for providing nucleotides for DNA synthesis. Well-documented RR1 and RR2 genes have been reported in the genomes of a few GVs, many Group II NPVs, and at least one Group I NPV (OpMNPV) (13). Two different RR2 genes have been reported for LdMNPV (29). Based on the phylogeny of baculovirus RR1 genes, it has been postulated that two different capture events resulted in baculoviruses obtaining this gene (8). One

source was from a bacterium for the OpMNPV and LdMNPV RR1 gene lineage, whereas the other lineage (e.g., *Spodoptera exigua* MNPV (SeMNPV)) appears to have been derived from eukaryotes, most likely insects. The two RR2 genes from LdMNPV appear to be derived independently, one from each different source, rather than via gene duplication.

Serpin. Serpins, (serine protease inhibitors), were named because of their ability to inhibit chymotrypsin-like serine proteases. A sequence related to lepidopteran serpins was found in the genome of a baculovirus of Hemileuca sp., a member of the Saturniidae. It shows about 34% amino acid sequence identity to serpins from *Manduca sexta* and *Bombyx mori* suggesting that it was captured from a host insect (54). No other baculoviruses have been reported to encode this gene. Expression of the HespNPV serpin in AcMNPV increased the virulence of infection by four fold in T. ni larvae (55). It was found that in *Helicoverpa armigera* inhibition of melanization by serpin-9 and -5 elevated the levels of baculovirus infection (56).

SWI/SNF Chromatin remodelers. Blast analysis indicates that *Lonomia obliqua* NPV (LoobMNPV) (57) encodes a protein (loob035) closely related to Transcription termination factor 2 (TTF2)(57). Hhpred (6) analysis of this same orf predicts with about 100% probability that it is related to the SWI/SNF family of chromatin remodelers.

Thymidylate kinase. A gene encoding an ortholog of Thymidylate kinase was observed in the genome of a GV pathogenic for *Epinotia aporema* (30). Thymidylate kinase is involved in adding a phosphate to thymidine 5' monophosphate and converting it to thymidine 5' diphosphate. It is important for the production of dTTP for DNA synthesis. An orf in *Perigonia lusca* single nucleopolyhedrovirus (PeluSNPV), (*pelu112)* was predicted to encode a fusion of *dUTP and thymidylate kinase* (*tmk*) (15).

Transcription termination factor 2. See WI/SNF Chromatin remodelers above.

Trypsin-like. Although hymenopteran lack homologs of chitinase and cathepsin, they all encode a trypsin-like protein (e.g., Nese7) (58) that shows high levels of aa sequence identity (e.g., 50%) to insect trypsin-like homologs. It is possible that the presence of this enzyme compensates for the absence of chitinase and cathepsin and facilitates the release of virus from infected gut cells into the environment and to provide inoculum for the re-infection of other gut cells.

V-TREX (Viral three-prime repair exonuclease). A gene with homology to 3' to 5' exonucleases from other systems has been identified in three Group I NPVs, AgMNPV, CfMNPV and AnpeNPV. The enzyme from both AgMNPV and CfMNPV demonstrated to 3' to 5' exonucleolytic activity. It is thought that they may be involved in DNA repair (59, 60).

References

1. Yamada H, Shibuya M, Kobayashi M, Ikeda M. Identification of a novel apoptosis suppressor gene from the baculovirus Lymantria dispar multicapsid nucleopolyhedrovirus. J Virol. 2011;85:5237–42. PubMed PMID: 21411519.
2. Yamada H, Shibuya M, Kobayashi M, Ikeda M. Baculovirus Lymantria dispar multiple nucleopolyhedrovirus IAP2 and IAP3 do not suppress apoptosis, but trigger apoptosis of insect cells in a transient expression assay. Virus Genes. 2012;45:370–9. PubMed PMID: 22798056.
3. Ikeda M, Yamada H, Hamajima R, Kobayashi M. Baculovirus genes modulating intracellular innate antiviral immunity of lepidopteran insect cells. Virology. 2013;435:1–13. PubMed PMID: 23217611.
4. Choi JY, Qiao Q, Hong SH, Kim CM, Jeong JH, Kim YG, Jung YK, Wu H, Park HH. CIDE domains form functionally important higher-order assemblies for DNA fragmentation. Proc Natl Acad Sci U S A. 2017;114:7361–7366. PubMed PMID: 28652364.

5. Harrison RL, Rowley DL, Mowery J, Bauchan GR, Theilmann DA, Rohrmann GF, Erlandson MA. The Complete Genome Sequence of a Second Distinct Betabaculovirus from the True Armyworm, Mythimna unipuncta. PLoS One. 2017;12:e0170510. PubMed PMID: 28103323.
6. Zimmermann L, Stephens A, Nam SZ, Rau D, Kubler J, Lozajic M, Gabler F, Soding J, Lupas AN, Alva V. A Completely Reimplemented MPI Bioinformatics Toolkit with a New HHpred Server at its Core. J Mol Biol. 2018;430:2237–2243. PubMed PMID: 29258817.
7. Ishimwe E, Hodgson JJ, Clem RJ, Passarelli AL. Reaching the melting point: Degradative enzymes and protease inhibitors involved in baculovirus infection and dissemination. Virology. 2015;479-480:637–49. PubMed PMID: 25724418.
8. Hughes AL, Friedman R. Genome-Wide Survey for Genes Horizontally Transferred from Cellular Organisms to Baculoviruses. Mol Biol Evol. 2003;20:979–8. PubMed PMID: 12716988.
9. Paran N, De Silva FS, Senkevich TG, Moss B. Cellular DNA ligase I is recruited to cytoplasmic vaccinia virus factories and masks the role of the vaccinia ligase in viral DNA replication. Cell Host Microbe. 2009;6:563–9. PubMed PMID: 20006844.
10. Pearson MN, Rohrmann GF. Characterization of a baculovirus-encoded ATP-dependent DNA ligase. J Virol. 1998;72:9142–9149. PubMed PMID: 9765460.
11. Boule JB, Zakian VA. The yeast Pif1p DNA helicase preferentially unwinds RNA DNA substrates. Nucleic Acids Res. 2007;35:5809–18. PubMed PMID: 17720711.
12. Lieber MR. The FEN-1 family of structure-specific nucleases in eukaryotic DNA replication, recombination and repair. Bioessays. 1997.:233–40. PubMed PMID: 9080773.
13. Esteban DJ, Da Silva M, Upton C. New bioinformatics tools for viral genome analyses at Viral Bioinformatics - Canada. Pharmacogenomics. 2005;6:271–80. PubMed PMID: 16013958.
14. Lin T, Chang R, Wei C, Lang G. Characterization of the deoxyuridine triphosphatase gene of Ophiusa disjungens nucleopolyhedrovirus. Acta Virol. 2012;56:241–6. PubMed PMID: 23043604.
15. Ardisson-Araujo DM, Lima RN, Melo FL, Clem RJ, Huang N, Bao SN, Sosa-Gomez DR, Ribeiro BM. Genome sequence of Perigonia lusca single nucleopolyhedrovirus: insights into the evolution of a nucleotide metabolism enzyme in the family Baculoviridae. Sci Rep. 2016;6:24612. PubMed PMID: 27273152.
16. Lepore LS, Roelvink PR, Granados RR. Enhancin, the granulosis virus protein that facilitates nucleopolyhedrovirus (NPV) infections, is a metalloprotease. J Invertebr Pathol. 1996;68:131–40. PubMed PMID: 8858909.
17. Hashimoto Y, Cosaro BG, Granados RR. Location and nucleotide sequence of the gene encoding the viral enhancing factor of the *Trichoplusia ni* granulosis virus. J Gen Virol. 1991;72:2645–2651. PubMed PMID: 1940861.
18. Slavicek JM, Popham HJ. The Lymantria dispar nucleopolyhedrovirus enhancins are components of occlusion-derived virus. J Virol. 2005;79:10578–88. PubMed PMID: 16051850.
19. Hayakawa T, Ko R, Okano K, Seong S, Goto C, Maeda S. Sequence analysis of the *Xestia c-nigrum* granulovirus genome. Virology. 1999;262:277–297. PubMed PMID: 10502508.
20. Popham HJ, Bischoff DS, Slavicek JM. Both Lymantria dispar nucleopolyhedrovirus enhancin genes contribute to viral potency. J Virol. 2001;75:8639–48. PubMed PMID: 11507209.
21. Wang P, Granados RR. An intestinal mucin is the target substrate for a baculovirus enhancin. Proc Natl Acad Sci U S A. 1997;94:6977–82. PubMed PMID: 9192677.
22. Toprak U, Harris S, Baldwin D, Theilmann D, Gillott C, Hegedus DD, Erlandson MA. Role of enhancin in Mamestra configurata nucleopolyhedrovirus virulence: selective degradation of host peritrophic matrix proteins. J Gen Virol. 2012;93:744–53. PubMed PMID: 22238230.
23. Galloway CS, Wang P, Winstanley D, Jones IM. Comparison of the bacterial Enhancin-like proteins from Yersinia and Bacillus spp. with a baculovirus Enhancin. J Invertebr Pathol. 2005;90:134–7. PubMed PMID: 16081094.
24. Escasa SR, Lauzon HA, Mathur AC, Krell PJ, Arif BM. Sequence analysis of the Choristoneura occidentalis granulovirus genome. J Gen Virol. 2006;87:1917–33. PubMed PMID: 16760394.

25. Thumbi DK, Beliveau C, Cusson M, Lapointe R, Lucarotti CJ. Comparative Genome Sequence Analysis of Choristoneura occidentalis Freeman and C. rosaceana Harris (Lepidoptera: Tortricidae) Alphabaculoviruses. PLoS One. 2013;8:e68968. PubMed PMID: 23861954.
26. Ardisson-Araujo DM, Pereira BT, Melo FL, Ribeiro BM, Bao SN. A betabaculovirus encoding a gp64 homolog. BMC Genomics. 2016;17:94. de AZPM, Moscardi F, Kitajima EW, Sosa-Gomez DR, Wolff JL. PubMed PMID: 26847652.
27. Krishnan A, Almen MS, Fredriksson R, Schioth HB. The origin of GPCRs: identification of mammalian like Rhodopsin, Adhesion, Glutamate and Frizzled GPCRs in fungi. PLoS One. 2012;7:e29817. PubMed PMID: 22238661.
28. Nijmeijer S, Leurs R, Smit MJ, Vischer HF. The Epstein-Barr virus-encoded G protein-coupled receptor BILF1 hetero-oligomerizes with human CXCR4, scavenges Galphai proteins, and constitutively impairs CXCR4 functioning. J Biol Chem. 2010;285:29632–41. PubMed PMID: 20622011.
29. Kuzio J, Pearson MN, Harwood SH, Funk CJ, Evans JT, Slavicek J, Rohrmann GF. Sequence and analysis of the genome of a baculovirus pathogenic for *Lymantria dispar*. Virology. 1999;253:17–34. PubMed PMID: 9887315.
30. Ferrelli ML, Salvador R, Biedma ME, Berretta MF, Haase S, Sciocco-Cap A, Ghiringhelli PD, Romanowski V. Genome of Epinotia aporema granulovirus (EpapGV), a polyorganotropic fast killing betabaculovirus with a novel thymidylate kinase gene. BMC Genomics. 2012;13:548. PubMed PMID: 23051685.
31. Le TH, Wu T, Robertson A, Bulach D, Cowan P, Goodge K, Tribe D. Genetically variable triplet repeats in a RING-finger ORF of Helicoverpa species baculoviruses. Virus Res. 1997;49:67–77. PubMed PMID: 9178498.
32. Cerio RJ, Vandergaast R, Friesen PD. Host Insect Inhibitor-of-Apoptosis SfIAP Functionally Substitutes for Baculovirus IAP but is Differentially Regulated by its N-terminal Leader. J Virol. 2010;84:11448–60. PubMed PMID: 20739517.
33. Ko R, Okano K, Maeda S. Structural and functional analysis of the Xestia c-nigrum granulovirus matrix metalloproteinase. J Virol. 2000;74:11240–6. PubMed PMID: 11070022.
34. Magni G, Amici A, Emanuelli M, Orsomando G, Raffaelli N, Ruggieri S. Enzymology of NAD+ homeostasis in man. Cell Mol Life Sci. 2004;61:19–34. PubMed PMID: 14704851.
35. Oliveira JV, Wolff JL, Garcia-Maruniak A, Ribeiro BM, de Castro ME, de Souza ML, Moscardi F, Maruniak JE, Zanotto PM. Genome of the most widely used viral biopesticide: Anticarsia gemmatalis multiple nucleopolyhedrovirus. J Gen Virol. 2006;87:3233–50. PubMed PMID: 17030857.
36. de Castro Oliveira JV, de Melo FL, Romano CM, Iamarino A, Rizzi TS, Yeda FP, Hársi CM, Wolff JL, Zanotto PM. Structural and phylogenetic relationship of ORF 31 from the Anticarsia gemmatalis MNPV to poly (ADP-ribose) polymerases (PARP). Virus Genes. 2008;37:177–84. PubMed PMID: 18648922.
37. Tulin A, Naumova NM, Menon AK, Spradling AC. Drosophila poly(ADP-ribose) glycohydrolase mediates chromatin structure and SIR2-dependent silencing. Genetics. 2006;172:363–71. PubMed PMID: 16219773.
38. Jagtap P, Szabo C. Poly(ADP-ribose) polymerase and the therapeutic effects of its inhibitors. Nat Rev Drug Discov. 2005;4:421–40. PubMed PMID: 15864271.
39. Yu SW, Wang H, Poitras MF, Coombs C, Bowers WJ, Federoff HJ, Poirier GG, Dawson TM, Dawson VL. Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. Science. 2002;297:259–63. PubMed PMID: 12114629.
40. Arif B, Escasa S, Pavlik L. Biology and genomics of viruses within the genus Gammabaculovirus. Viruses. 2011;3:2214–22. PubMed PMID: 22163341.
41. van Houte S, Ros VI, Mastenbroek TG, Vendrig NJ, Hoover K, Spitzen J, van Oers MM. Protein Tyrosine Phosphatase-Induced Hyperactivity Is a Conserved Strategy of a Subset of BaculoViruses to Manipulate Lepidopteran Host Behavior. PLoS One. 2012;7:e46933. PubMed PMID: 23077534.
42. Ahrens CH, Russell R, Funk CJ, Evans JT, Harwood SH, Rohrmann GF. The sequence of the *Orgyia pseudotsugata* multinucleocapsid nuclear polyhedrosis virus genome. Virology. 1997;229:381–399. PubMed PMID: 9126251.

43. Han Y, van Houte S, van Oers MM, Ros VID. Baculovirus PTP2 Functions as a Pro-Apoptotic Protein. Viruses. 2018.:10. PubMed PMID: 29642442.
44. Huang G, Oliver MR, Keown JR, Goldstone DC, Metcalf P. Crystal structure of protein tyrosine phosphatase-2 from Cydia pomonella granulovirus. Acta Crystallogr F Struct Biol Commun. 2019;75:233–238. PubMed PMID: 30950823.
45. van Oers MM, Herniou EA, Usmany M, Messelink GJ, Vlak JM. Identification and characterization of a DNA photolyase-containing baculovirus from *Chrysodeixis chalcites*. Virology. 2004;330:460–470. PubMed PMID: 15567439.
46. Willis LG, Siepp R, Stewart TM, Erlandson MA, Theilmann DA. Sequence analysis of the complete genome of Trichoplusia ni single nucleopolyhedrovirus and the identification of a baculoviral photolyase gene. Virology. 2005;338:209–26. PubMed PMID: 15951000.
47. Xu F, Vlak JM, van Oers MM. Conservation of DNA photolyase genes in group II nucleopolyhedroviruses infecting plusiine insects. Virus Res. 2008;136:58–64. PubMed PMID: 18513819.
48. Bennett CJ, Webb M, Willer DO, Evans DH. Genetic and phylogenetic characterization of the type II cyclobutane pyrimidine dimer photolyases encoded by Leporipoxviruses. Virology. 2003;315:10–9. PubMed PMID: 14592755.
49. Afonso CL, Tulman ER, Lu Z, Oma E, Kutish GF, Rock DL. The genome of Melanoplus sanguinipes entomopoxvirus. J Virol. 1999;73:533–52. PubMed PMID: 9847359.
50. Xu F, Vlak JM, Eker AP, van Oers MM. DNA photolyases of Chrysodeixis chalcites nucleopolyhedrovirus are targeted to the nucleus and interact with chromosomes and mitotic spindle structures. J Gen Virol. 2010;91:907–14. PubMed PMID: 19955559.
51. van Oers MM, Lampen MH, Bajek MI, Vlak JM, Eker AP. Active DNA photolyase encoded by a baculovirus from the insect Chrysodeixis chalcites. DNA Repair (Amst). 2008;7:1309–18. PubMed PMID: 18547877.
52. Biernat MA, Eker AP, van Oers MM, Vlak JM, van der Horst GT, Chaves I. A baculovirus photolyase with DNA repair activity and circadian clock regulatory function. J Biol Rhythms. 2012;27:3–11. PubMed PMID: 22306969.
53. Petrik DT, Iseli A, Montelone BA, Van Etten JL, Clem RJ. Improving baculovirus resistance to UV inactivation: increased virulence resulting from expression of a DNA repair enzyme. J Invertebr Pathol. 2003;82:50–56. PubMed PMID: 12581719.
54. Rohrmann GF, Erlandson MA, Theilmann DA. The genome of a baculovirus isolated from Hemileuca sp. encodes a serpin ortholog. Virus Genes. 2013;47:357–364. PubMed PMID: 23852342.
55. Ardisson-Araujo DM, Rohrmann GF, Ribeiro BM, Clem RJ. Functional characterization of hesp018, a baculovirus-encoded serpin gene. J Gen Virol. 2015;96:1150–60. PubMed PMID: 25573886.
56. Yuan C, Xing L, Wang M, Wang X, Yin M, Wang Q, Hu Z, Zou Z. Inhibition of melanization by serpin-5 and serpin-9 promotes baculovirus infection in cotton bollworm Helicoverpa armigera. PLoS Pathog. 2017;13:e1006645. PubMed PMID: 28953952.
57. Spadacci-Morena DD, Soares MA, Moraes RH, Sano-Martins IS, Sciani JM. The urticating apparatus in the caterpillar of Lonomia obliqua (Lepidoptera: Saturniidae). Toxicon. 2016;119:218–24. PubMed PMID: 27319295.
58. Garcia-Maruniak A, Maruniak JE, Zanotto PM, Doumbouya AE, Liu JC, Merritt TM, Lanoie JS. Sequence analysis of the genome of the Neodiprion sertifer nucleopolyhedrovirus. J Virol. 2004;78:7036–51. PubMed PMID: 15194780.
59. Slack JM, Shapiro M. Anticarsia gemmatalis multicapsid nucleopolyhedrovirus v-trex gene encodes a functional 3' to 5' exonuclease. J Gen Virol. 2004;85:2863–71. PubMed PMID: 15448348.
60. Yang DH, de Jong JG, Makhmoudova A, Arif BM, Krell PJ. Choristoneura fumiferana nucleopolyhedrovirus encodes a functional 3'-5' exonuclease. J Gen Virol. 2004;85:3569–73. PubMed PMID: 15557229.

Glossary

Arp — A cellular complex of up to 7 proteins called the Arp2/3 complex is comprised of two actin related proteins (Arp) that resemble the structure of monomeric actin and 5 additional proteins.

Bacmid — Bacmids are baculovirus genomes that contain a bacterial origin of replication so that they can replicate in bacteria as a plasmid (1). The term is derived from a contraction of baculovirus and plasmid (Luckow, pers. comm.). The original construct contained the AcMNPV genome, a bacterial origin of replication, the target site for the bacterial transposon Tn7, and a selectable kanamycin resistance marker gene, and the lacZ gene all in the polyhedrin locus. This construct allowed for the manipulation and recombination via transposition of the viral genome in bacteria; however, the construct is viable upon transfection into insect cells.

Baculoviridae — A family of occluded viruses pathogenic for insects and possibly other invertebrates. They have large circular, supercoiled, double-stranded DNA genomes and replicate in nuclei.

Basal lamina — This is a fibrous structure that separates the insect midgut epithelium from the hemocele. It is a barrier that baculoviruses might have to cross to cause a systemic infection.

BmN, (BmN 1, 4, 5) — Variants of a cell line derived from *Bombyx mori* that are permissive for infection of BmNPV. The original cell line was isolated by Dr. T. D. Grace. The derivation of the different subclonal lines is ambiguous and is discussed in (2).

Burst Sequence — This is an A/T-rich regulatory sequence between the promoter and translational start site of the very late hyperexpressed genes, polyhedrin and p10. Evidence suggests that it binds VLF-1 (3).

Budded virus (BV) — A type of baculovirus that buds out of infected cells and spreads the infection within an insect and within cell culture. The BVs derive their envelope from modified cell membranes.

Caspase — A category of proteases activated in the apoptotic pathway. There are two types: initiator and effector. Initiator caspases are regulatory and cleave and thereby activate effector caspases. Effector caspases carry out the apoptotic program.

CIDE Domain Protein — Cell death-inducing DFF45-like effector (CIDE) domain is usually present near the N-terminus of a DNAse that is activated by caspase cleavage and is associated with the degradation of DNA during apoptosis and lipid homeostasis (4).

E (expect) value — The E-value is an indication of the statistical significance of a specific pairwise sequence alignment and reflects a combination of the number of sequences in a database and the scoring system employed. The lower the E-value, the more significant the relatedness. An alignment with an E-value of 0.001 indicates that this amount of relatedness has a 1 in 1000 probability of occurring by chance alone. Although E-values often indicate convincing relatedness, they can be distorted by repeated amino acids and a variety of other factors.

Encapsulation — Encapsulation is a defense response in insects that is directed against objects that are too large to be phagocytose. It involves the accumulation of hemocytes that form a capsule around the object and often is accompanied with melanin deposition.

Enhancin — See Metalloproteinases

Errantivirus — Errantiviruses are insect retroelements that encode an env gene and their name is derived from Latin *errans*, to wander. Although similar to retroviruses, they have not been included within the Retroviridae because they are a distinct lineage, and evidence that they are infectious is indirect (see Chapter 11). Their env gene is related to the baculovirus F protein (4) (5).

ESCRT — Endosomal sorting complexes required for transport (ESCRT) are involved in a diverse set of processes used in budded virus production including endosomal sorting, vesicular trafficking, and virus budding

F protein — This protein is thought to be the fusion protein of most baculoviruses with the exception of Group I, which use gp64 and hymenopteran viruses that do not encode homologs of either gp64 or F. F protein homologs are present, as env proteins in insect retroviruses, also called errantiviruses. Homologs are also present in some insect lineages.

Few Polyhedra (FP) mutants — Few polyhedra (FP) mutants are a readily observable baculovirus phenotype that result in reduced numbers of polyhedra and an elevated titer of budded virus. Such mutants often contain an insert in the *fp* gene (ac61), although the phenotype can result from mutations elsewhere in the genome.

GP64 — The envelope fusion protein used by Group I NPVs. It is related to the envelope fusion protein of a group of arthropod-borne orthomyxoviruses, the thogotoviruses.

Granulosis viruses; granuloviruses (GV) — A lineage of baculoviruses pathogenic for Lepidoptera, which normally have a single virion per ovoid-shaped occlusion body.

Group I — One of two major lineages of lepidopteran NPVs; it is distinguished from other baculoviruses by using a different envelope fusion protein, gp64. Several other genes are also unique to this lineage.

Group II — One of two major lineages of lepidopteran NPVs; members are thought to use a fusion protein (F) to initiate infection

HOAR — A predicted open reading frame described in (6). According to Prof. David Tribe the name is derived as follows: 'H refers to *Heliothis*, O for open reading frame and Hoar sounds like the given second name of the student Hoa (T.H. Le) who determined the sequence.' It is found in many group II Alphabaculoviruses and has sequence similarity to ubiquitin ligase.

Homolog (homologous protein; ortholog, paralog) — Homolog is an inclusive term that indicates that two proteins are evolutionarily related. Homologs are divided into orthologs and paralogs. **Orthologs** (ortho = exact) have homology resulting from speciation and are directly related evolutionarily to one another from a common ancestor via vertical descent, whereas **paralogs** (para = parallel) show relatedness that results from gene duplication. Often, this leads to one copy evolving a different function. Due to the presence of more than one lineage and the possible loss of one duplicate in some lineages, the phylogeny of paralogous proteins may not reflect a direct phylogenetic relationship via a common ancestor.

***Hrs,* homologous repeated sequences** — These are often located at several sites in a baculovirus genome, which have been implicated as origins of DNA replication and transcriptional enhancers.

Hytrosavirus. — Hytrosaviruses are also known as the salivary gland hypertrophy viruses and have been characterized from several of Diptera including the tsetse fly, the vector for sleeping sickness. They are non-occluded and contain large, circular, double stranded DNA genomes within enveloped rod-shaped nucleocapsids. They appear to infect the salivary gland and although not particularly virulent, they can result in a significant reduction in reproductive fitness. They encode several per os infectivity factor proteins related to those of baculoviruses and nudiviruses. The name is derived from the symptoms (hypertrophy of the salivary gland)(7).

Inhibitor of apoptosis (IAP) — The IAP gene family was originally discovered in baculoviruses (8). Subsequently homologs have been found in almost all baculovirus and also entomopox and irridoviruses, and they are also widely distributed in eukaryotes, from yeast to mammals. IAP sequences have a number of distinguishing domains. These include baculovirus IAP repeat (BIR) domains of about 70 amino acids. BIR domains are often present in multiple copies with two copies present in many baculovirus IAPs and up to three

copies in some cellular IAPs. A zinc (RING) finger domain is also often present near the C-terminus of the protein. For more information see Chapter 7.

LEF, late expression factor — In AcMNPV, these are factors that are involved in transient DNA replication or late transcription.

Melanization — In insects, melanization involves the synthesis and deposition of melanin at the site of injury. It is regulated by a cascade of serine proteases that cleave and activate prophenoloxidase (to phenoloxidase) that is then able to catalyze the oxidation of phenols (e.g., tyrosine) to quinones (nonaromatic ring compounds), which then polymerize and form melanin (9).

Metalloproteinases — Metalloproteinases are peptidases that contain divalent cations as integral components of their structure (10). Baculoviruses encode a several members of this group of enzymes. They include enhancin, which is thought to enhance infectivity of some viruses by digesting the peritrophic membrane. Enhancin is found in a few lepidopteran NPVs (e.g., Ld-, Cf-, and MacoNPV) and GVs (e.g., Ag-, As-, Tn-, XcGVs). Another group consists of stromelysin1-like metalloproteinases. Orthologs of this family are found in all sequenced GV genomes. The third metalloproteinase is cathepsin, which is found in most lepidopteran group I and II viruses, but is only present in three GVs genomes. It is not present in the genomes of the hymenopteran and dipteran viruses.

Midgut — The site of that baculovirus occlusion bodies are dissolved and infection is initiated. It is where food digestion takes place in lepidopteran larvae.

MNPV/SNPV, Multiple (M) or singly (S) enveloped nucleocapsids — The morphology of nucleocapsids, in which multiple or single nucleocapsids are present within an envelope. MNPVs are found in Group I and II lepidopteran NPVs and are normally not present in GVs, or hymenopteran, or dipteran NPVs. Although characteristic of viral lineages, it does not appear to be a phylogenetic trait.

NSF — See SNARES.

Nimaviridae — See Whispovirus.

Nuclear polyhedrosis virus; nucleopolyhedrovirus (NPV) — The most widely distributed type of baculovirus. NPVs replicate in the nucleus and usually produce polyhedron-shaped occlusion bodies containing more than one virion.

Nudiviruses — A group of viruses pathogenic for invertebrates and related to baculoviruses. They have enveloped, rod-shaped nucleocapsids with large circular DNA genomes and share about 15 core genes with baculoviruses. However, they are not occluded and therefore are not included in the Baculoviridae.

Occlusion-derived virus (ODV) — Viruses that are derived from occlusion bodies. They obtain their envelope within the nucleus. Also called OV.

P35 — P35 is an inhibitor of apoptosis encoded by AcMNPV. Closely related orthologs are only found in a few Group I baculoviruses closely related to AcMNPV. A homolog has also been reported in a GV of *Choristoneura occidentalis* (ChocGV) (11) and a variant (p49) is found in a Group II NPV (SpliNPV) (12). In addition, a p35 ortholog was identified in an entomopox virus. P35 is a substrate for an effector caspase, caspase 1, but in the process of its cleavage it irreversibly binds to and inactivates caspase 1 (13). Subsequently p35 was found to block other categories of caspases in a similar manner - reviewed in (14). For more information see Chapter 7.

Peritrophic matrix (PM) — A tube-like structure that separates food from the midgut epithelium. It is composed of chitin and protein.

per os (per mouth) — This refers to the route of infection of insects by ingestion.

PIB, Polyhedral inclusion bodies — This refers to NPV occlusion bodies.

PIF; per os infectivity factors — Factors that are required for oral infection by ODV. An abbreviation that preceded this refers to yeast 'petite integration frequency' mutants (15).

Polydnaviruses — Polydnaviruses are not true viruses because they do not contain genetic material for their own replication. They are produced in the ovaries of parasitic wasps and the virus-like particles contain genetic material from the wasp and are injected into host lepidopteran larvae along with the wasp eggs. They are named because they contain multiple circular double-stranded DNA molecules (polydispersed DNA) encompassing up to 560 kb that encode gene products that compromise the target host immune system and other processes and are essential for the successful development of the wasp egg. There are two lineages of parasitic wasps that employ these elements; the braconid and ichneumonids and they produce bracovirus and ichnovirus polydnavirus elements, respectively. Evidence indicates that the structural proteins of the virus-like particles of the braconid lineage are related to per os infectivity factors and are derived from an integrated nudivirus-like virus (16).

Ring zone — The ring zone is a less electron dense region near the margins of nuclei and surrounding the virogenic stroma of NPV infected cells. Polyhedra initially form in this zone, but they eventually can fill the whole nucleus.

Sf-9, Sf-21 — Sf-21 is a cell line that was derived from ovarian tissue from *Spodoptera frugiperda* pupae (17). Sf-9 cells are a clonal isolate derived from Sf21 cells. Both cell lines are permissive for AcMNPV infection.

TED — In the process of characterizing AcMNPV FP mutants produced after 25 passages in *Trichoplusia ni* cells, an isolate, (FP-D), was found to contain an integrated retrotransposon that originated from the host genome. It was called transposable element D or TED (18). This element had features of a retrovirus including long terminal repeats and was demonstrated to express gag, pol, and env-like genes that are capable of being incorporated into virus-like particles (19-21).

SNARES and NSF — SNAREs and NSF (soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor) proteins are the minimal machinery involved in the fusion of transport vesicles with membranes and are critical for the initiation of infection by enveloped virions.

Tn-368. — A cell line derived from *Trichoplusia ni* that is permissive for AcMNPV infection. It was derived from ovarian tissue of a virgin adult (22).

Virogenic stroma — This is an electron dense chromatin-like structure in nuclei of baculovirus infected insects. A molecular scaffold that is believed to be produced for the replication of viral genomes and the assembly of nucleocapsids.

WASP — Wiskott-Aldrich syndrome protein (WASP). The Arp complex is involved in nucleating the formation of F-actin filaments. Activators are required for this process and they bind both monomeric G actin and the Arp2/3 complex. One category of such activators is called Wiskott-Aldrich syndrome protein (WASP), and an ortholog of WASP (PP78/83) (AcMNPV orf9) is encoded by all lepidopteran NPV genomes. The Wiskott-Aldrich syndrome is a rare, inherited, X-linked, recessive disease caused by mutations in the WASp gene. It is associated with immune dysregulation.

White spot syndrome virus — See Whispovirus.

Whispovirus — *Whispovirus* is the only genus in the family Nimaviridae and contain a single species, white spot syndrome virus (WSSV) that causes disease in a wide variety of crustaceans. Severe disease outbreaks of the disease are a major problem in cultured penaeid shrimp, particularly in Asia. WSSV is a non-occluded, enveloped, rod-shaped virus with a double stranded DNA genome of about 300 kb. It is highly virulent and causes major tissue damage and the infection results in white spots of calcium deposited in the shell (23). The

name *Whispovirus* is from *wh*ite *spo*t syndrome virus. Nimaviridae is from Latin nima "thread", that refers to a flagellum-like structure protruding from the nucleocapid.

References

1. Luckow VA, Lee SC, Barry GF, Olins PO. Efficient generation of infectious recombinant baculoviruses by site-specific transposon-mediated insertion of foreign genes into a baculovirus genome propagated in Escherichia coli. J Virol. 1993;67:4566–79. PubMed PMID: 8392598.
2. O'Reilly DR, Miller LK, Luckow VA. 1994. Baculovirus expression vectors. A laboratory manual. Oxford University Press, New York.
3. Yang S, Miller LK. Activation of baculovirus very late promoters by interaction with very late factor 1. J Virol. 1999;73:3404–9. PubMed PMID: 10074194.
4. Malik HS, Henikoff S, Eickbush TH. Poised for contagion: evolutionary origins of the infectious abilities of invertebrate retroviruses. Genome Res. 2000;10:1307–18. PubMed PMID: 10984449.
5. Rohrmann GF, Karplus PA. Relatedness of baculovirus and gypsy retrotransposon envelope proteins. BMC Evol Biol. 2001;1:1. PubMed PMID: 11244578.
6. Le TH, Wu T, Robertson A, Bulach D, Cowan P, Goodge K, Tribe D. Genetically variable triplet repeats in a RING-finger ORF of Helicoverpa species baculoviruses. Virus Res. 1997;49:67–77. PubMed PMID: 9178498.
7. Abd-Alla AM, Vlak JM, Bergoin M, Maruniak JE, Parker A, Burand JP, Jehle JA, Boucias DG. Hytrosaviridae: a proposal for classification and nomenclature of a new insect virus family. Arch Virol. 2009;154:909–18. PubMed PMID: 19458899.
8. Birnbaum MJ, Clem RJ, Miller LK. An apoptosis-inhibiting gene from a nuclear polyhedrosis virus encoding a polypeptide with Cys/His sequence motifs. J Virol. 1994;68:2521–2528. PubMed PMID: 8139034.
9. Lemaitre B, Hoffmann J. The host defense of Drosophila melanogaster. Annu Rev Immunol. 2007;25:697–743. PubMed PMID: 17201680.
10. Lepore LS, Roelvink PR, Granados RR. Enhancin, the granulosis virus protein that facilitates nucleopolyhedrovirus (NPV) infections, is a metalloprotease. J Invertebr Pathol. 1996;68:131–40. PubMed PMID: 8858909.
11. Escasa SR, Lauzon HA, Mathur AC, Krell PJ, Arif BM. Sequence analysis of the Choristoneura occidentalis granulovirus genome. J Gen Virol. 2006;87:1917–33. PubMed PMID: 16760394.
12. Du Q, Lehavi D, Faktor O, Qi Y, Chejanovsky N. Isolation of an apoptosis suppressor gene of the Spodoptera littoralis nucleopolyhedrovirus. J Virol. 1999;73:1278–85. PubMed PMID: 9882332.
13. Bump NJ, Hackett M, Hugunin M, Seshagiri S, Brady K, Chen P, Ferenz C, Franklin S, Ghayur T, Li P, et al. Inhibition of ICE family proteases by baculovirus antiapoptotic protein p35. Science. 1995;269:1885–8. PubMed PMID: 7569933.
14. Clem RJ. Baculoviruses and apoptosis: a diversity of genes and responses. Curr Drug Targets. 2007;8:1069–74. PubMed PMID: 17979666.
15. Foury F, Lahaye A. Cloning and sequencing of the PIF gene involved in repair and recombination of yeast mitochondrial DNA. EMBO J. 1987;6:1441–1449. PubMed PMID: 3038524.
16. Bezier A, Herbiniere J, Lanzrein B, Drezen JM. Polydnavirus hidden face: the genes producing virus particles of parasitic wasps. J Invertebr Pathol. 2009;101:194–203. PubMed PMID: 19460382.
17. Vaughn JL, Goodwin RH, Tompkins GJ, McCawley P. The establishment of two cell lines from the insect *Spodoptera frugiperda* (Lepidoptera: Noctuidae). In Vitro. 1977;13:213–217. PubMed PMID: 68913.
18. Miller DW, Miller LK. A virus mutant with an insertion of a copia-like transposable element. Nature (London). 1982;299:562–564. PubMed PMID: 6289125.
19. Hajek KL, Friesen PD. Proteolytic processing and assembly of gag and gag-pol proteins of TED, a baculovirus-associated retrotransposon of the gypsy family. J Virol. 1998;72:8718–24. PubMed PMID: 9765414.

20. Lerch RA, Friesen PD. The baculovirus-integrated retrotransposon TED encodes gag and pol proteins that assemble into viruslike particles with reverse transcriptase. J Virol. 1992;66:1590–601. PubMed PMID: 1371168.
21. Ozers MS, Friesen P. The Env-like open reading frame of the baculovirus-integrated retrotransposon TED encodes a retrovirus-like envelope protein. Virology. 1996;226:252–9. PubMed PMID: 8955045.
22. Hink WF. Established insect cell line from the cabbage looper, *Trichoplusia ni*. Nature. 1970;226:466–467. PubMed PMID: 16057320.
23. Leu JH, Yang F, Zhang X, Xu X, Kou GH, Lo CF. Whispovirus. Curr Top Microbiol Immunol. 2009;328:197–227. PubMed PMID: 19216439.