Review Article

Baculovirus: Molecular Insights on Their Diversity and Conservation

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The Baculoviridae is a large group of insect viruses containing circular double-stranded DNA genomes of 80 to 180 kbp. In this study, genome sequences from 57 baculoviruses were analyzed to reevaluate the number and identity of core genes and to understand the distribution of the remaining coding sequences. Thirty one core genes with orthologs in all genomes were identified along with other 895 genes differing in their degrees of representation among reported genomes. Many of these latter genes are common to well-defined lineages, whereas others are unique to one or a few of the viruses. Phylogenetic analyses based on core gene sequences and the gene composition of the genomes supported the current division of the Baculoviridae into 4 genera: Alphabaculovirus, Betabaculovirus, Gammabaculovirus, and Deltabaculovirus.

1. Background

Baculoviruses are arthropod-specific viruses containing large double-stranded circular DNA genomes of 80,000–180,000 bp. The progeny generation is biphasic, with two different phenotypes during virus infection: budded viruses (BVs), during the initial stage of the multiplication cycle, and occlusion-derived viruses (ODVs), at the final stages of replication [1, 2]. In general, primary infection takes place in the insect midgut cells after ingestion of occlusion bodies (OBs). Following this stage, systemic infection is caused by the initial BV progeny [3, 4]. And finally, OBs are produced during the last stage of the infection. These OBs comprise virions embedded in a protein matrix which protects them from the environment [5, 6].

Baculoviruses have been used extensively in many biological applications such as protein expression systems, models of genetic regulatory networks and genome evolution, putative nonhuman viral vectors for gene delivery, and biological control agents against insect pests [7–17].

The Baculoviridae family is divided into four genera according to common biological and structural characteristics: Alphabaculovirus, which includes lepidopteran-specific baculoviruses and is subdivided into Group I or Group II based on the type of fusogenic protein, Betabaculovirus, comprising lepidopteran-specific granuloviruses, Gammabaculovirus, which includes hymenopteran-specific baculoviruses, and finally Deltabaculovirus which, to date, comprises only CuniNPV and possibly the still undescribed dipteran-specific baculoviruses [1, 18–20].

The comparison between known genome sequences of all baculoviruses has been the source for identifying a common set of genes, the baculovirus core genes. However, there are probably more orthologous sequences that may not be identified due to the accumulation of many mutations throughout evolution. Thus, core genes seem to be a key factor for some of the main biological functions, such as those necessary to transcribe viral late genes, produce virion structure, infect gut cells abrogate host metabolism and establish infections [21–24].
Figure 1: GC content in baculovirus genomes. The different histograms contain the distribution of baculovirus genomes according to their GC content and their genus classification. Black bars highlight genomes with a GC content higher than 50%.
For this report, previous data as well as bioinformatic studies conducted on currently available sets of completely sequenced baculovirus genomes were taken into account and have resulted in a summary of gene content and phylogenetic analyses which validates the classification of this important viral family.

2. Baculovirus Ancestral Genes

There are currently 57 complete baculovirus genomes deposited in GenBank (Table 1). These include 41 *Alphabaculoviruses*, 12 *Betabaculoviruses*, 3 *Gammabaculoviruses*, and 1 *Deltabaculovirus*.

As a first approach to perform a comparative analysis, the GC content of the genomes were calculated (Figure 1). The histogram revealed that many baculoviruses have about 41% of GC content although several of them have significantly higher values (CMNPV at 50.1%, *CuniNPV* at 50.9%, *AnpeNPV-L2* at 53.5%, *AnpeNPV-Z* at 53.5%, *LyxyNPV* at 53.5%, *OpMNPV* at 55.1%, and *LdMNVP* at 57.5%). A detailed analysis of DNA content did not show a clear pattern of GC content that could be associated with each genus.

Further characterization of the patterns of gene content and organization may prove useful for establishing evolutionary relationships among members of Baculoviridae. The high variability observed in the number of coding sequences becomes a key feature of viruses with large DNA genomes that infect eukaryotic cells [18].

Insertions, deletions, duplication events, and/or sequence reorganizations by recombination or transposition processes seem to be the main forces of the macroevolution in this particular kind of biological entities. For example, the loss or gain of genetic material could provide new important abilities for colonization of new hosts, or they could improve performance within established hosts. However, there seems to be a set of core genes whose absence would imply the loss of basic biological functions, and that could be typical of the viral family. In view of this, and considering previous reports [1, 19, 22, 23], the amount and identity of baculovirus common genes were reevaluated (Table 2). As a result, P6.9 and Desmoplakin were recognized in this work, as core proteins by using sequence analysis complementary to the standard ones (see Supplementary files available at doi:10.4061/2011/379424).

The group of conserved sequences found in all baculovirus genomes is consistently estimated at about 30 shared genes, regardless of the increasing number of genomes analyzed [22, 148]. Meanwhile, the role or function assigned to several sequences has been renewed, according to new studies. In particular, it has been identified that 38k (Ac98) gene encodes a protein which is part of the capsid structure [121, 122]; P33 (Ac92) is a sulfhydryl oxidase which could be related to the proper production of virions in the infected cell nucleus [123–125]; ODV-EC43 (Ac109) is a structural component which would be involved in BV and ODV generation [126]; P49 (Ac142) is a capsid
| Genus                          | Name                      | Abbreviation | Code   | Accession number | Genome (bp) | Annotated ORFs | GC% | Ref. |
|-------------------------------|---------------------------|--------------|--------|------------------|-------------|----------------|-----|------|
| **Alphabaculovirus-Group I**  | Antheraea pernyi NPV-Z    | AnpeNPV-Z    | APN    | NC_008035        | 126629      | 145            | 53.5| [27] |
|                              | Antheraea pernyi NPV-L2   | AnpeNPV-L2   | AP2    | EF207986         | 126246      | 144            | 53.5| [28] |
|                              | Anticarsia gemmatalis MNPV-2D | AgMNPV-2D  | AGN    | NC_008520        | 132239      | 152            | 44.5| [29] |
|                              | Autographa californica MNPV-C6 | AcMNPV-C6 | ACN    | NC_001623        | 133894      | 154            | 40.7| [30] |
|                              | Bombyx mori NPV           | BmNPV        | BMN    | NC_001962        | 128413      | 137            | 40.4| [31] |
|                              | Bombyx mandarina NPV      | BomaNPV      | BON    | NC_012672        | 126770      | 141            | 40.2| [32] |
|                              | Choristoneura fumiferana DEF MNPV | CFDEFMNPV | CDN    | NC_005137        | 131160      | 149            | 45.8| [33] |
|                              | Choristoneura fumiferana MNPV | CFMNPV     | CFN    | NC_004778        | 129593      | 145            | 50.1| [34] |
|                              | Epiphyas postvittana NPV  | EppoNPV      | EPN    | NC_003083        | 118584      | 136            | 40.7| [35] |
|                              | Hyphantria cunea NPV      | HycuNPV      | HCN    | NC_007767        | 132959      | 148            | 45.5| [36] |
|                              | Maruca vitrata MNPV       | MaviMNPV     | MVN    | NC_008725        | 111953      | 126            | 38.6| [37] |
|                              | Orgyia pseudotsugata MNPV | OpMNPV       | OPN    | NC_001875        | 131995      | 152            | 55.1| [38] |
|                              | Plutella xylostella MNPV  | PlxyMNPV     | PXN    | NC_008349        | 134417      | 149            | 40.7| U    |
|                              | Rachiplusia ou MNPV       | RoMNPV       | RON    | NC_004323        | 131526      | 146            | 39.1| [39] |
| **Alphabaculovirus-Group II**| Adoxophyes honmai NPV     | AdhoNPV      | AHN    | NC_004690        | 113220      | 125            | 35.6| [40] |
|                              | Adoxophyes orana NPV      | AdorNPV      | AON    | NC_011423        | 111724      | 121            | 35.0| [41] |
|                              | Agrotis ipsilon NPV       | AgipNPV      | AIN    | NC_011345        | 155122      | 163            | 48.6| U    |
|                              | Agrotis segetum NPV       | AgseNPV      | ASN    | NC_007921        | 147544      | 153            | 45.7| [42] |
|                              | Apocheima cinerarium NPV  | ApciNPV      | APO    | FJ914221         | 123876      | 118            | 33.4| U    |
|                              | Chrysodeixis chalcites NPV | ChChNPV     | CCN    | NC_007151        | 149622      | 151            | 39.0| [43] |
|                              | Clanis bilineata NPV      | ClbiNPV      | CBN    | NC_008293        | 135454      | 129            | 37.7| [44] |
|                              | Ectropis obliqua NPV      | EcobNPV      | EON    | NC_008586        | 131204      | 126            | 37.6| [45] |
|                              | Euproctis pseudoconspersa NPV | EupsNPV | EUN    | NC_012639        | 141291      | 139            | 40.4| [46] |
|                              | Helicoverpa armigera NPV-C1 | HearNPV-C1 | HA1    | NC_003094        | 130759      | 135            | 38.9| [47] |
|                              | Helicoverpa armigera NPV-G4 | HearNPV-G4 | HA4    | NC_002654        | 131405      | 135            | 39.0| [47] |
|                              | Helicoverpa armigera MNPV  | HearMNPV     | HAN    | NC_011615        | 154196      | 162            | 40.1| [48] |
|                              | Helicoverpa armigera SNPV-NNg1 | HearSNPV-NNg1 | HAS | NC_011354       | 132425      | 143            | 39.2| [49] |
|                              | Helicoverpa zea SNPV      | HZSNPV       | HZN    | NC_003349        | 130869      | 139            | 39.1| U    |
|                              | Leucania separata NPV-AH1 | LeseNPV-AH1  | LSN    | NC_008348        | 168041      | 169            | 48.6| [50] |
|                              | Lymantria dispar MNPV     | LdMNPV       | LDN    | NC_001973        | 161046      | 163            | 57.5| [51] |
|                              | Lymantria xylina MNPV     | LyxyMNPV     | LNX    | NC_013953        | 156344      | 157            | 53.5| [52] |
| Genus                        | Name                        | Abbreviation | Code  | Accession number | Genome (bp) | Annotated ORFs | GC% | Ref. |
|-----------------------------|-----------------------------|--------------|-------|------------------|-------------|----------------|-----|------|
| *Mamestra configurata*      | NPV-90-2                    | MacoNPV-90-2 | MCN   | NC_003529        | 155060      | 169            | 41.7| [53] |
| *Mamestra configurata*      | NPV-90-4                    | MacoNPV-90-4 | MC4   | AF539999         | 153656      | 168            | 41.7| [54] |
| *Mamestra configurata*      | NPV-B                       | MacoNPV-B    | MCB   | NC_004117        | 158482      | 169            | 40.0| [55] |
| *Orgyia leucostigma*        | NPV                         | OrleNPV      | OLN   | NC_010276        | 156179      | 135            | 39.9| U    |
| *Spodoptera exigua*         | MNPV                        | SeMNPV       | SEN   | NC_002169        | 135611      | 142            | 43.8| U    |
| *Spodoptera frugiperda*      | MNPV-3AP2                   | SfMNPV-3AP2  | SF2   | NC_009011        | 131330      | 143            | 40.2| [56] |
| *Spodoptera frugiperda*      | MNPV-19                     | SfMNPV-19    | SF9   | EU258200         | 132565      | 141            | 40.3| [57] |
| *Spodoptera litura*         | NPV-II                      | SpliNPV-II   | SLN   | NC_011616        | 148634      | 147            | 45.0| U    |
| *Spodoptera litura*         | NPV-G2                      | SpliNPV-G2   | SL2   | NC_003102        | 139342      | 141            | 42.8| [58] |
| *Trichoplusia ni*           | SNPV                        | TnSNPV       | TNN   | NC_007383        | 134394      | 144            | 39.0| [59] |
| *Adoxophyes orana*          | GV                          | AdorGV       | AOG   | NC_005038        | 99657       | 119            | 34.5| [60] |
| *Agrotis segetum*           | GV                          | AgeGV        | ASG   | NC_005859        | 131680      | 132            | 37.3| U    |
| *Choristoneura occidentalis*| GV                          | ChocGV       | COG   | NC_008168        | 104710      | 116            | 32.7| [61] |
| *Cryptophlebia leucotreta*  | GV                          | CrlGV        | CLG   | NC_005068        | 110907      | 129            | 32.4| [62] |
| *Cydia pomonella*           | GV                          | CpGV         | CPG   | NC_002816        | 123500      | 143            | 45.3| [63] |
| *Helicoverpa armigera*       | GV                          | HearGV       | HAG   | NC_010240        | 169794      | 179            | 40.8| [64] |
| *Phthorimea operculella*    | GV                          | PhopGV       | POG   | NC_004062        | 119217      | 130            | 35.7| [65] |
| *Plutella xylostella*       | GV                          | PlxGV        | PXG   | NC_002593        | 100999      | 120            | 40.7| [66] |
| *Pieris rapae*              | GV                          | PiraGV       | PRG   | GQ884143         | 108592      | 120            | 33.2| U    |
| *Pseudoletia unipuncta*     | GV-Hawaiin                  | PsunGV       | PUG   | EU678671         | 176677      | 183            | 39.8| U    |
| *Spodoptera litura*         | GV-KI                       | SpliGV       | SLG   | NC_009503        | 124121      | 136            | 38.8| [67] |
| *Xestia c-nigrum*           | GV                          | XnGV         | XCG   | NC_002331        | 178733      | 181            | 40.7| [68] |
| *Neoapinem abietis*         | NPV                         | NeabNPV      | NAN   | NC_008252        | 84264       | 93             | 33.4| [69] |
| *Neoapinem lecontei*        | NPV                         | NeleNPV      | NLN   | NC_005906        | 81755       | 93             | 33.3| [70, 71] |
| *Neoapinem sertifer*        | NPV                         | NeseNPV      | NSN   | NC_005905        | 86462       | 90             | 33.8| [71, 72] |
| *Culex nigripalpus*         | NPV                         | CuniNPV      | CNN   | NC_003084        | 108252      | 109            | 50.9| [73] |

This table contains all of baculoviruses used in bioinformatic studies, sorted by genus (and within them by alphabetical order). MNPV is the abbreviation of multicapsid nucleopolyhedrovirus; NPV is the abbreviation of nucleopolyhedrovirus; SNPV is the abbreviation of single nucleopolyhedrovirus; GV is the abbreviation of granulovirus. The accession numbers are from National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) and correspond to the sequences of complete genomes. Code is an acronym used for practicality. U: unpublished.

protein important in DNA processing, packaging, and capsid morphogenesis [129]; Ac81 interacts with Actin 3 in the cytoplasm but does not appear in BVs or in ODVs [135]; ODV-E18 (Ac143) would mediate BV production [131]; desmoplakin (Ac66) seems to be essential in releasing processes from virogenic stroma to cytoplasm [132]; PIF-4 (Ac96) and PIF-5 (ODV-56, Ac148) are ODV envelope proteins with an essential role in per os infection route [145, 147]; Ac68 may be involved in polyhedron morphogenesis [130].
Table 2: Core genes.

| Replication                  | ACN | LDN | CPG | NSN | CNN |
|------------------------------|-----|-----|-----|-----|-----|
| lef-1 [74]                   | 14  | 123 | 74  | 68  | 45  |
| lef-2 [74]                   | 6   | 137 | 41  | 57  | 25  |
| DNA pol [75–78]              | 65  | 83  | 111 | 28  | 91  |
| Helicase [79–90]             | 95  | 97  | 90  | 61  | 89  |
| *Transcription*              |     |     |     |     |     |
| lef-4 [91–95]                | 90  | 93  | 95  | 62  | 96  |
| lef-8 [91, 96]               | 50  | 51  | 131 | 81  | 26  |
| lef-9 [95, 97]               | 62  | 64  | 117 | 40  | 59  |
| p47 [91, 98]                 | 40  | 48  | 68  | 49  | 73  |
| lef-5 [98–101]               | 99  | 100 | 87  | 58  | 88  |
| *Packaging, assembly, and release* |     |     |     |     |     |
| p6.9 [102–104]               | 100 | 101 | 86  | 36  | 23  |
| vp39 [105–108]               | 89  | 92  | 96  | 89  | 24  |
| vlf-1 [100, 109–113]         | 77  | 86  | 106 | 45  | 18  |
| alk-exo [114–116]            | 133 | 157 | 125 | 31  | 53  |
| vp1054 [117]                 | 54  | 57  | 138 | 85  | 8   |
| vp91/p95 [118]               | 83  | 91  | 101 | 84  | 35  |
| gp41 [119, 120]              | 80  | 88  | 104 | 47  | 33  |
| 38 k [121, 122]              | 98  | 99  | 88  | 59  | 87  |
| p33 [123–125]                | 92  | 94  | 93  | 95  | 14  |
| odv-ec43 [126–128]           | 109 | 107 | 55  | 90  | 69  |
| p49 [129]                    | 142 | 20  | 15  | 63  | 30  |
| odv-nc42 [130]               | 68  | 80  | 114 | 41  | 58  |
| odv-e18 [131]                | 143 | 19  | 14  | 65  | 31  |
| desmoplakin [132]            | 66  | 82  | 112 | 29  | 92  |
| *Cell cycle arrest and/or interaction with host proteins* |     |     |     |     |     |
| odv-c27 [133, 134]           | 144 | 18  | 97  | 66  | 32  |
| ac81 [135]                   | 81  | 89  | 103 | 48  | 106 |
| *Oral infectivity*           |     |     |     |     |     |
| pif-0/p74 [136–141]          | 138 | 27  | 60  | 50  | 74  |
| pif-1 [142–144]              | 119 | 155 | 75  | 79  | 29  |
| pif-2 [136, 142]             | 22  | 119 | 48  | 55  | 38  |
| pif-3 [1142]                 | 115 | 143 | 35  | 69  | 46  |
| pif-4/19k/odv-e28 [145]      | 96  | 98  | 89  | 60  | 90  |
| pif-5/odv-e56 [146, 147]     | 148 | 14  | 18  | 38  | 102 |

The virus names are indicated in three letter code according to established in Table 1. Numbers in columns indicates the corresponding ORFs of each genome.

The number and identity of shared orthologous genes in every accepted member of each genus were investigated, and the unique sequences typical of each clade as well as those shared between different phylogenetic groups were identified (Figure 2).

This analysis shows that the four accepted baculovirus genera have accumulated a large number of genes during evolution. Probably, many of these sequences have been incorporated into viral genomes prior to diversification processes since they are found in members of different genera. In contrast, other genes are unique to each genus, suggesting that they have been incorporated more recently and after diversification (Table 3). The possibility that non-shared genes found only in one genus which represent baculovirus ancestral sequences deleted in the other lineages should also be considered. In any case, a set of particular genes which could help in an appropriate genus taxonomy of new baculoviruses with partial sequence information were obtained from this analysis.

3. Whole Baculovirus Gene Content

The study of all genes reported in the 57 completely sequenced viral genomes revealed the existence of about
895 different ORFs, a set of sequences that might be called the whole baculovirus gene content. This high number of potential coding sequences contrasts with the range of gene content among the family members, which is between 90–181 genes (Alphabaculovirus: 118–169; Betabaculovirus: 116–181; Gammabaculovirus: 90–93; Deltabaculovirus: 109) as well as with the proportion of core genes which represents only 3%. This curious biological feature supports the hypothesis that highlights the great importance of structural mutations in the macroevolution of viruses with large DNA genomes. From this view, the set of genes shared by all members belonging to each baculovirus genus was compared to those corresponding to the whole genus gene content (Figure 3).

The analysis shows that Group I alphabaculoviruses and gammabaculoviruses have a lower diversity of gene content with respect to the rest of lineages. This information, coupled with the significant number of genome sequences obtained from Group I alphabaculoviruses, suggests that this lineage of viruses would constitute the newest clade in baculovirus evolution history [149]. This is based on the assumption that Group I alphabaculoviruses have had less time to incorporate new sequences from different sources (host genomes, other viral genomes, bacterial genomes, etc.) since the appearance of their common ancestor.

4. Baculovirus Core Gene Phylogeny

Traditional attempts to infer relationships between baculoviruses were performed by amino acid or nucleotide sequence analyses of single genes encoding proteins such as polyhedrin/granulin (the major component of OBs), the envelope fusion polypeptides known as F protein and GP64, or DNA polymerase protein, among many other examples [149–152].
Figure 4: Baculovirus genome phylogeny. Cladogram based on amino acid sequence of core genes. The 31 identified core genes from Baculoviridae family were independently aligned using MEGA 4 [25] program with gap open penalty = 10, gap extension penalty = 1, and dayhoff matrix [26]. Then, a concatemer was generated and phylogeny inferred using the same software (UPGMA; bootstrap with 1000 replicates; gap/missing data = complete deletion; model = amino (dayhoff matrix); patterns among sites = same (homogeneous); rates among sites = different (gamma distributed); gamma parameter = 2.25). Baculoviruses are identified by the acronyms given in Table 1, and the accepted distribution in lineages and genera are also indicated. Gammabaculovirus and Deltabaculovirus are referenced by Greek letters. The proposed clades of Betabaculoviruses are shown in bold letters.
Mostly, the evolutionary inferences were in agreement with much stronger subsequent studies based on sequence analyses derived from sets of genes with homologous sequences in all baculoviruses. Thus, these new approaches were based on the construction of common-protein-concatemers which were used to propose evolution patterns for baculoviruses [149].

Then, the fact that a viral family consists of members who share a common pattern of genes and functions and whose proliferation cycle continuously challenges the viral viability turns it essential to take into account their higher or lesser tolerance to the molecular changes. Molecular constraints regarding tolerance to changes in core genes are different from those of other genes. Therefore, core genes should be considered the most ancestral genes which may have diverged in higher or lesser degrees. According to this, a phylogenetic study was performed based on concatemers obtained from multiple alignments of the 31 proteins recognized in this work as core genes for the 57 available baculoviruses with sequenced genomes (Figure 4).

The obtained cladogram reproduces the current baculovirus classification based on 4 genera. Additionally, this approach consistently separates the alphabaculoviruses into two lineages: Group I and Group II. And the same can be observed when analyzing Group I, where the presence of two different clades can be clearly inferred (clade a and clade b). These groupings result in accordance with previous reports [20, 150]. In Group II alphabaculoviruses, a clear clustering may not be identified and would not allow to suggest a subdivision.

In contrast, in the Betabaculovirus genus, it is possible to propose their separation into two different clades: clade a (XnGV, HearGV, PsunGV, SpliGV, AgseGV, and PlxyGV), and clade b (AdorGV, PhopGV, CpGV, CrleGV, PiragGV, ChocGV).

Despite the evolutionary inference based on core genes, there was a remaining question: “is the tolerance to changes in all core genes the same?” The answer could be reached by an individual core gene variability analysis for which studies of sequence distance for each baculovirus core gene were performed (Figure 5).

The resulting order of core genes shows that pif-2 was the most conserved baculovirus ancestral sequence, whereas desmoplakin was the gene with evidence of greatest variability. This analysis reveals that genomes can be evolutionarily constrained in different ways depending on the proteins they encode.

The gain of access to new hosts might be an important force for gene evolution. During an infection process, the genome variants that appear with mutations introduced by errors in the replication/reparation machinery could be quickly incorporated into the virus population if the nucleotide changes offered a better biological performance when proteins were translated. The DNA helicase gene was considered as an important host range factor being, for this study, the second core sequence showing more variability [87]. However, other sequences like pif-2 gene would not accumulate mutations because the protein encoded might lose vital functions not necessarily associated with the nature of the host.

5. Conclusions

Baculoviridae is a large family of viruses which infect and kill insect species from different orders. The valuable applications of these viruses in several fields of life sciences encourage their constant study with the goal of
understanding the molecular mechanisms involved in the generation of progeny in the appropriate cells as well as the processes by which they evolve. The establishment of solid bases to recognize their phylogenetic relationships is necessary to facilitate the generation of new knowledge and the development of better methodologies.

In view of this, many researchers have proposed and used different bioinformatic methodologies to identify genes as well as related baculoviruses. Some of them were based on gene sequences [150], gene content [17], or genome rearrangements [152]. In this work, a combination of core gene sequence and gene content analyses were applied to reevaluate Baculoviridae classification. To our knowledge, the most important fact is that this report is the first work which identifies the whole baculovirus gene content and the shared genes that are unique in different genera and subgenera. All this information should be taken into account to group and classify new virus isolates and to propose molecular methodologies to diagnose baculoviruses based on proper gene targets according to gene variability and gene content.

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References

[1] J. A. Jehle, G. W. Blissard, B. C. Bonning et al., "On the classification and nomenclature of baculoviruses: a proposal for revision," Archives of Virology, vol. 151, no. 7, pp. 1257–1266, 2006.
[2] G. W. Blissard and G. F. Rohrmann, "Baculovirus diversity and molecular biology," Annual Review of Entomology, vol. 35, no. 1, pp. 127–155, 1990.
[3] V. A. Kozlov, T. L. Levitin, and N. M. Gusak, "The primary structure of baculovirus inclusion body proteins. Evolution and structure-function aspects," Current Topics in Microbiology and Immunology, vol. 131, pp. 135–164, 1986.
[4] G. F. Rohrmann, "Baculovirus structural proteins," Journal of General Virology, vol. 73, no. 4, pp. 749–761, 1992.
[5] T. Ohkawa, J. O. Washburn, R. Sitpara, E. Sid, and L. E. Volkman, "Specific binding of Autographa california M nucleopolyhedrovirus occlusion-derived virus to midgut cells of heliothis virescens larvae is mediated by products of pig genes Ac119 and Ac022 but not by Ac115," Journal of Virology, vol. 79, no. 24, pp. 15258–15264, 2005.
[6] R. Jackes, E. Marom, and K. Sherman, Stability of Insect Viruses in the Environment. Viral Insecticides for Biological Control, Academic Press, New York, NY, USA, 1985.
[7] G. Zhang, "Research, development and application of Heliothis viral pesticide in China," Resources and Environment in the Yangtze Valley, vol. 3, pp. 1–6, 1994.
[8] R. D. Possee, "Baculoviruses as expression vectors," Current Opinion in Biotechnology, vol. 8, no. 5, pp. 569–572, 1997.
[9] F. Moscardi, "Assessment of the application of baculoviruses for control of lepidoptera," Annual Review of Entomology, vol. 44, pp. 257–289, 1999.
[10] T. A. Kost and J. P. Condreay, "Recombinant baculoviruses as expression vectors for insect and mammalian cells," Current Opinion in Biotechnology, vol. 10, no. 5, pp. 428–433, 1999.
[11] A. B. Inceoglu, S. G. Kamita, A. C. Hinton et al., "Recombinant baculoviruses for insect control," Pest Management Science, vol. 57, no. 10, pp. 981–987, 2001.
[12] T. A. Kost, J. P. Condreay, and D. L. Jarvis, "Baculovirus as versatile vectors for protein expression in insect and mammalian cells," Nature Biotechnology, vol. 23, no. 5, pp. 567–575, 2005.
[13] M. D. Summers, "Milestones leading to the genetic engineering of baculoviruses as expression vectors and viral pesticides," Advances in Virus Research, vol. 68, pp. 3–73, 2006.
[14] A. B. Inceoglu, S. G. Kamita, and B. D. Hackett, "Genetically modified baculoviruses: a historical overview and future outlook," Advances in Virus Research, vol. 68, pp. 323–360, 2006.
[15] X. Shi and D. L. Jarvis, "Protein N-glycosylation in the baculovirus-insect cell system," Current Drug Targets, vol. 8, no. 10, pp. 1116–1125, 2007.
[16] J. P. Condreay and T. A. Kost, "Baculovirus expression vectors for insect and mammalian cells," Current Drug Targets, vol. 8, no. 10, pp. 1126–1131, 2007.
[17] X. L. Sun and H. Y. Peng, "Recent advances in biological control of pest insect by using viruses in China," Virologica Sinica, vol. 22, no. 2, pp. 158–162, 2007.
[18] A. E. Hernioui, T. Luque, X. Chen et al., "Use of whole genome sequence data to infer baculovirus phylogeny," Journal of Virology, vol. 75, no. 17, pp. 8117–8126, 2001.
[19] A. E. Hernioui, J. A. Olszewski, J. S. Cory, and D. R. O’Reilly, "The genome sequence and evolution of baculoviruses," Annual Review of Entomology, vol. 48, pp. 211–234, 2003.
[20] J. A. Jehle, M. Lange, H. Wang, Z. Hu, Y. Wang, and R. Hauschild, "Molecular identification and phylogenetic analysis of baculoviruses from Lepidoptera," Virology, vol. 346, no. 1, pp. 180–193, 2006.
[21] M. M. van Oers and J. M. Vlak, "Baculovirus genomics," Current Drug Targets, vol. 8, no. 10, pp. 1051–1068, 2007.
[22] G. F. Rohrmann, Baculovirus Molecular Biology, National Library of Medicine (US), NCBI, Bethesda, MD, USA, 2008.
[23] T. Hayakawa, G. F. Rohrmann, and Y. Hashimoto, "Patterns of genome organization and content in lepidopteran baculoviruses," Virology, vol. 278, no. 1, pp. 1–12, 2000.
[24] C. B. McCarthy and D. A. Theilmann, "AcMNPV ac143 (odv-e18) is essential for mediating budded virus production and is the 30th baculovirus core gene," Virology, vol. 375, no. 1, pp. 277–291, 2008.
[25] K. Tamura, J. Dudley, M. Nei, and S. Kumar, "MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0," Molecular Biology and Evolution, vol. 24, no. 8, pp. 1596–1599, 2007.
[26] R. M. Schwartz and M. O. Dayhoff, “Matrices for detecting distant relationships,” in Atlas of Protein Sequences, M. O. Dayhoff, Ed., pp. 353–358, National Biomedical Research Foundation, 1979.

[27] Q. Fan, S. Li, L. Wang et al., “The genome sequence of the multinucleocapsid nucleopolyhedrovirus of the Chinese oak silkworm Antheraea pernyi,” Virology, vol. 366, no. 2, pp. 304–315, 2007.

[28] Z. M. Nie, Z. F. Zhang, D. Wang et al., “Complete sequence and organization of Antheraea pernyi nucleopolyhedrovirus, a ds-rich baculovirus,” BMC Genomics, vol. 8, Article ID 248, 2007.

[29] J. V. de Castro Oliveira, J. L. C. Wolff, A. Garcia-Maruniak et al., “Genome of the most widely used viral biopesticide: anticarsia gemmatalis multiple nucleopolyhedrovirus,” Journal of General Virology, vol. 87, no. 11, pp. 3233–3250, 2006.

[30] M. D. Ayres, S. C. Howard, J. Kuzio, M. Lopez-Ferber, and R. D. Possee, “The complete DNA sequence of Autographa californica nuclear polyhedrosis virus,” Virology, vol. 202, no. 2, pp. 586–605, 1994.

[31] S. Gomi, K. Majima, and S. Maeda, “Sequence analysis of the genome of Bombyx mori nucleopolyhedrovirus,” Journal of General Virology, vol. 80, no. 5, pp. 1323–1337, 1999.

[32] Y. P. Xu, Z. P. Ye, C. Y. Niu et al., “Comparative analysis of the genomes of Bombyx mori mandarina and Bombyx mori nucleopolyhedroviruses,” Journal of Microbiology, vol. 48, no. 1, pp. 102–110, 2010.

[33] J. G. de Jong, H. A. M. Lauzon, C. Dominy et al., “Analysis of the Choristoneura funeferana nucleopolyhedrovirus genome,” Journal of General Virology, vol. 86, no. 4, pp. 929–943, 2005.

[34] H. A. M. Lauzon, P. B. Jamieson, P. J. Krell, and B. M. Arif, “Gene organization and sequencing of the Choristoneura funeferana defective nucleopolyhedrovirus genome,” Journal of General Virology, vol. 86, no. 4, pp. 945–961, 2006.

[35] O. Hyink, R. A. Dellow, M. J. Olsen et al., “Whole genome analysis of the Epiphaga postvittana nucleopolyhedrovirus,” Journal of General Virology, vol. 83, no. 4, pp. 957–971, 2002.

[36] M. Ikeda, M. Shikata, N. Shirata, S. Chaeychomrsi, and M. Kobayashi, “Gene organization and complete sequence of the Hyphantria cunea nucleopolyhedrovirus genome,” Journal of General Virology, vol. 87, no. 9, pp. 2549–2562, 2006.

[37] Y. R. Chen, C. Y. Wu, S. T. Lee et al., “Genomic and host range studies of Maruca vitrata nucleopolyhedrovirus,” Journal of General Virology, vol. 89, no. 9, pp. 2315–2330, 2008.

[38] C. H. Ahrens, R. L. Q. Russell, C. J. Funk, J. T. Evans, S. H. Harwood, and G. E. Rohrmann, “The sequence of the Orgyia pseudotsugata multinucleocapsid virus polyhedrosis virus genome,” Virology, vol. 229, no. 2, pp. 381–399, 1997.

[39] R. L. Harrison and B. C. Bonning, “The nucleopolyhedroviruses of Rachiphusia ou and Anagropa falcifera are isolates of the same virus,” Journal of General Virology, vol. 80, no. 10, pp. 2793–2798, 1999.

[40] M. Nakai, C. Goto, W. Kang, M. Shikata, T. Luque, and Y. Kunimi, “Genome sequence and organization of a nucleopolyhedrovirus isolated from the smaller tea tortrix, Adoxophyes honmai,” Virology, vol. 316, no. 1, pp. 171–183, 2003.

[41] S. Hilton and D. Winstanley, “Genomic sequence and biochemical characterization of a nucleopolyhedrovirus isolated from the summer fruit tortrix, Adoxophyes orana,” Journal of General Virology, vol. 89, no. 11, pp. 2898–2908, 2008.

[42] A. K. Jakubowska, S. A. Peters, J. Ziemnicka, J. M. Vlak, and M. M. van Oers, “Genome sequence of an enhacin gene-rich nucleopolyhedrovirus (NPV) from Agrotis segetum: collinearity with Spodoptera exigua multiple NPV,” Journal of General Virology, vol. 87, no. 3, pp. 357–351, 2006.

[43] M. M. van Oers, M. H. C. Abma-Henkens, E. A. Herniou, J. C. W. de Groot, S. Peters, and J. M. Vlak, “Genome sequence of Chrysodeixis chalcites nucleopolyhedrovirus, a baculovirus with two DNA photolyase genes,” Journal of General Virology, vol. 86, no. 7, pp. 2069–2080, 2005.

[44] S. Y. Zhu, J. P. Yi, W. D. Shen et al., “Genomic sequence, organization and characteristics of a new nucleopolyhedrovirus isolated from Clania bilineata larva,” BMC Genomics, vol. 10, Article ID 91, 9 pages, 2009.

[45] X. C. Ma, J. Y. Shang, Z. N. Yang, Y. Y. Bao, Q. Xiao, and C. X. Zhang, “Genome sequence and organization of a nucleopolyhedrovirus that infects the tea looper caterpillar, Ectropis obliqua,” Virology, vol. 360, no. 1, pp. 235–246, 2007.

[46] X. D. Tang, Q. Xiao, X. C. Ma, Z. R. Zhu, and C. X. Zhang, “Morphology and genome of Euprotis pseudococonspera nucleopolyhedrovirus,” Virus Genes, vol. 38, no. 3, pp. 495–506, 2009.

[47] C. X. Zhang, X. C. Ma, and Z. J. Guo, “Comparison of the complete genome sequence between C1 and G4 isolates of the Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus,” Virology, vol. 333, no. 1, pp. 190–199, 2005.

[48] J. G. Ogembo, S. Chaeychomrsi, K. Kamiya et al., “Cloning and comparative characterization of nucleopolyhedroviruses isolated from African bollworm, Helicoverpa armigera, (Lepidoptera: Noctuidiae) in different geographic regions,” Journal of Insect Biotechnology and Sericology, vol. 76, no. 1, pp. 39–49, 2007.

[49] X. Chen, W. F. J. Ijkel, C. Dominy et al., “Identification, sequence analysis and phylogeny of the lef-2 gene of Helicoverpa armigera single-nucleocapsid baculovirus,” Virus Research, vol. 65, no. 1, pp. 21–32, 1999.

[50] H. Xiao and Y. Qi, “Genome sequence of Leucania separata nucleopolyhedrovirus,” Virus Genes, vol. 35, no. 3, pp. 845–856, 2007.

[51] J. Kuzio, M. N. Pearson, S. H. Harwood et al., “Sequence and analysis of the genome of a baculovirus pathogenic for Lymastra dispars,” Virology, vol. 253, no. 1, pp. 17–34, 1999.

[52] Y. S. Nai, C. Y. Wu, T. C. Wang et al., “Genomic sequencing and analyses of Lymastra xylina multiply nucleopolyhedrovirus,” BMC Genomics, vol. 11, no. 1, Article ID 116, 2010.

[53] S. Li, M. Erlandson, D. Moody, and C. Gillott, “A physical map of the Mamestra configurata nucleopolyhedrovirus genome and sequence analysis of the polyhedrin gene,” Journal of General Virology, vol. 78, no. 1, pp. 265–271, 1997.

[54] L. Li, Q. Li, L. G. Willis, M. Erlandson, D. A. Theilmann, and C. Donly, “Complete comparative genomic analysis of two field isolates of Mamestra configurata nucleopolyhedrovirus,” Journal of General Virology, vol. 86, no. 1, pp. 91–105, 2005.

[55] L. Li, C. Donly, Q. Li et al., “Identification and genomic analysis of a second species of nucleopolyhedrovirus isolated from Mamestra configurata,” Virology, vol. 297, no. 2, pp. 226–244, 2002.

[56] R. L. Harrison, B. Puttler, and H. J. R. Popham, “Genomic sequence analysis of a fast-killing isolate of Spodoptera frugiperda multiple nucleopolyhedrovirus,” Journal of General Virology, vol. 89, no. 3, pp. 775–790, 2008.
H. A. Lauzon, A. Garcia-Maruniak, P. M. A. Zanotto et al., “Sequence analysis of the genome of Neodiprion sertifer nucleopolyhedrovirus,” Journal of Virology, vol. 80, no. 13, pp. 7036–7051, 2004.

C. L. Afonso, E. R. Tulman, Z. Lu et al., “Genome sequence of a baculovirus pathogenic for Culex nigripalpus,” Journal of Virology, vol. 75, no. 22, pp. 11157–11165, 2001.

J. T. Evans, D. J. Leisy, and G. F. Rohrmann, “Characterization of the interaction between the baculovirus replication factors LEF-1 and LEF-2,” Journal of Virology, vol. 71, no. 4, pp. 3114–3119, 1997.

A. L. Vanarsdall, K. Okano, and G. F. Rohrmann, “Characterization of the replication of a baculovirus mutant lacking the DNA polymerase gene,” Virology, vol. 331, no. 1, pp. 175–180, 2005.

J. Huang and D. B. Levin, “Expression, purification and characterization of the Spodoptera littoralis nucleopolyhedrovirus (SplNPV) DNA polymerase and interaction with the SplNPV non-hr origin of DNA replication,” Journal of General Virology, vol. 82, no. 7, pp. 1767–1776, 2001.

V. V. McDougall and L. A. Guarino, “Autographa californica nuclear polyhedrosis virus DNA polymerase: measurements of processivity and strand displacement,” Journal of Virology, vol. 73, no. 6, pp. 4908–4918, 1999.

X. Hang and L. A. Guarino, “Purification of Autographa californica nucleopolyhedrovirus DNA polymerase from infected insect cells,” Journal of General Virology, vol. 80, no. 9, pp. 2519–2526, 1999.

J. G. M. Heldens, Y. Liu, D. Zuidema, R. W. Goldbach, and J. M. Vlak, “Characterization of a putative Spodoptera exigua multicapsid nucleopolyhedrovirus helicase gene,” Journal of General Virology, vol. 78, no. 12, pp. 3101–3114, 1997.

S. Maeda, S. G. Kamita, and A. Kondo, “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,” Journal of Virology, vol. 67, no. 10, pp. 6234–6238, 1993.

E. Ito, D. Sahri, R. Knippers, and E. B. Carstens, “Baculovirus proteins IE-1, LEF-3, and P143 interact with DNA in vivo: a formaldehyde cross-linking study,” Virology, vol. 329, no. 2, pp. 337–347, 2004.

V. V. McDougall and L. A. Guarino, “The Autographa californica nuclear polyhedrosis virus p143 gene encodes a DNA helicase,” Journal of Virology, vol. 74, no. 11, pp. 5273–5279, 2000.

D. K. Bideshi and B. A. Federici, “DNA-independent ATPase activity of the Trichoplusia ni granulovirus DNA helicase,” Journal of General Virology, vol. 81, no. 6, pp. 1601–1604, 2000.

G. E. Liu and E. B. Carstens, “Site-directed mutagenesis of the AcMNPV p143 gene: effects on baculovirus DNA replication,” Virology, vol. 253, no. 1, pp. 125–136, 1999.

D. K. Bideshi and B. A. Federici, “The Trichoplusia ni granulovirus helicase in unable to support replication of Autographa californica multicapsid nucleopolyhedrovirus in cells and larvae of T. ni,” Journal of General Virology, vol. 81, no. 6, pp. 1593–1599, 2000.

J. T. Evans, G. S. Rosenblatt, D. J. Leisy, and G. F. Rohrmann, “Characterization of the interaction between the baculovirus ssDNA-binding protein (LEF 3) and putative helicase (P143),” Journal of General Virology, vol. 80, no. 2, pp. 493–500, 1999.
[87] O. Argaud, L. Crozier, M. López-Ferber, and G. Crozier, “Two key mutations in the host-range specificity domain of the p143 gene of Autographa californica nucleopolyhedrovirus are required to kill Bombyx mori larvae,” Journal of General Virology, vol. 79, no. 4, pp. 931–935, 1998.

[88] S. G. Kamita and S. Maeda, “Abortive infection of the baculovirus Autographa californica nuclear polyhedrosis virus in Sf-9 cells after mutation of the putative DNA helicase gene,” Journal of Virology, vol. 70, no. 9, pp. 6244–6250, 1996.

[89] G. Crozier, L. Crozier, O. Argaud, and D. Poudouvigne, “Extension of Autographa californica nuclear polyhedrosis virus host range by interspecific replacement of a short DNA sequence in the p143 helicase gene,” Proceedings of the National Academy of Sciences of the United States of America, vol. 91, no. 1, pp. 48–52, 1994.

[90] G. Liu and E. B. Carstens, “Site-directed mutagenesis of the AcMNPV p 143 gene: effects on baculovirus DNA replication,” Virology, vol. 253, no. 1, pp. 125–136, 1999.

[91] L. A. Guarino, B. Xu, J. Jin, and W. Dong, “A virus-encoded RNA polymerase purified from baculovirus-infected cells,” Journal of Virology, vol. 72, no. 10, pp. 7985–7991, 1998.

[92] L. A. Guarino, J. Jin, and W. Dong, “Guanylyltransferase activity of the LEF-4 subunit of baculovirus RNA polymerase,” Journal of Virology, vol. 72, no. 12, pp. 10003–10010, 1998.

[93] C. H. Gross and S. Shuman, “RNA 5′-triphosphatase, nuclease triphosphatase, and guanylyltransferase activities of baculovirus LEF-4 protein,” Journal of Virology, vol. 72, no. 12, pp. 10020–10028, 1998.

[94] J. Jin, W. Dong, and L. A. Guarino, “The LEF-4 subunit of baculovirus RNA polymerase has RNA 5′-triphosphatase and ATPase activities,” Journal of Virology, vol. 72, no. 12, pp. 10011–10019, 1998.

[95] C. H. Gross and S. Shuman, “Characterization of a baculovirus-encoded RNA 5′-triphosphatase,” Journal of Virology, vol. 72, no. 9, pp. 7057–7063, 1998.

[96] J. S. Titterington, T. K. Nunn, and A. L. Passarelli, “Functional dissection of the baculovirus late expression factor-8 gene: sequence requirements for late gene promoter activation,” Journal of General Virology, vol. 84, no. 7, pp. 1817–1826, 2003.

[97] C. Iorio, J. E. Viallard, S. McCracken, M. Lagacé, and C. D. Richardson, “The late expression factors 8 and 9 and possibly the phosphoprotein p78/83 of Autographa californica multicapsid nucleopolyhedrovirus are components of the virus-induced RNA polymerase,” Interdisciplinary Virology, vol. 41, no. 1, pp. 35–46, 1998.

[98] J. R. McLachlin and L. K. Miller, “Identification and characterization of vi1-1, a baculovirus gene involved in very late gene expression,” Journal of Virology, vol. 68, no. 12, pp. 7746–7756, 1994.

[99] A. Lu and L. K. Miller, “The roles of eighteen baculovirus late expression factor genes in transcription and DNA replication,” Journal of Virology, vol. 69, no. 2, pp. 975–982, 1995.

[100] J. W. Todd, A. L. Passarelli, A. Lu, and L. K. Miller, “Factors regulating baculovirus late and very late gene expression in transient-expression assays,” Journal of Virology, vol. 70, no. 4, pp. 2307–2317, 1996.

[101] A. L. Passarelli and L. K. Miller, “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, vol. 197, no. 2, pp. 704–714, 1993.

[102] M. E. Wilson and L. K. Miller, “Changes in the nucleoprotein complexes of a baculovirus DNA during infection,” Virology, vol. 151, no. 2, pp. 315–328, 1986.

[103] M. E. Wilson, T. H. Mainprize, P. D. Friesen, and L. K. Miller, “Location, transcription, and sequence of a baculovirus gene encoding a small arginine-rich polypeptide,” Journal of Virology, vol. 61, no. 3, pp. 661–666, 1987.

[104] M. Wang, E. Tuladhar, S. Shen et al., “Specificity of baculovirus P6.9 basic DNA-binding proteins and critical role of the C terminus in virion formation,” Journal of Virology, vol. 84, no. 17, pp. 8821–8828, 2010.

[105] S. M. Thiem and L. K. Miller, “Identification, sequence, and transcriptional mapping of the major capsid protein gene of the baculovirus Autographa californica nuclear polyhedrosis virus,” Journal of Virology, vol. 63, no. 5, pp. 2008–2018, 1989.

[106] M. N. Pearson, R. L. Q. Russell, G. F. Rohrmann, and G. S. Beaudreau, “p39, a major baculovirus structural protein: immunocytochemical characterization and genetic location,” Virology, vol. 167, no. 2, pp. 407–413, 1988.

[107] G. W. Blissard, R. L. Quant-Russell, G. F. Rohrmann, and G. S. Beaudreau, “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, vol. 168, no. 2, pp. 354–362, 1989.

[108] S. Lu, G. Ge, and Y. Qi, “Ha-VP39 binding to actin and the influence of F-actin on assembly of progeny virions,” Archives of Virology, vol. 149, no. 11, pp. 2187–2198, 2004.

[109] J. R. McLachlin and L. K. Miller, “Identification and characterization of vlf-1, a baculovirus gene involved in very late gene expression,” Journal of Virology, vol. 68, no. 12, pp. 7746–7756, 1994.

[110] S. Yang and L. K. Miller, “Control of baculovirus polyhedrin gene expression by very late factor 1,” Virology, vol. 248, no. 1, pp. 131–138, 1998.

[111] S. Yang and L. K. Miller, “Expression and mutational analysis of the baculovirus very late factor 1 (vlf-1) gene,” Virology, vol. 245, no. 1, pp. 99–109, 1998.

[112] V. S. Mikhailov and G. F. Rohrmann, “Binding of the baculovirus very late expression factor 1 (VLF-1) to different DNA structures,” BMC Molecular Biology, vol. 3, Article ID 14, 2002.

[113] A. L. Vanarsdall, K. Okano, and G. F. Rohrmann, “Characterization of the role of very late expression factor 1 in baculovirus capsid structure and DNA processing,” Journal of Virology, vol. 80, no. 4, pp. 1724–1733, 2006.

[114] V. S. Mikhailov, K. Okano, and G. F. Rohrmann, “Baculovirus alkaline nuclease possesses a 5′→3′ exonuclease activity and associates with the DNA-binding protein LEF-3,” Journal of Virology, vol. 77, no. 4, pp. 2436–2444, 2003.

[115] V. S. Mikhailov, K. Okano, and G. F. Rohrmann, “Specificity of the endonuclease activity of the baculovirus alkaline nuclease for single-stranded DNA,” Journal of Biological Chemistry, vol. 279, no. 15, pp. 14734–14745, 2004.

[116] K. Okano, A. L. Vanarsdall, and G. F. Rohrmann, “Characterization of a baculovirus lacking the alkaline nuclease gene,” Journal of Virology, vol. 78, no. 19, pp. 10650–10656, 2004.

[117] J. Olszewski and L. K. Miller, “Identification and characterization of a baculovirus structural protein, VP1054, required for nucleocapsid formation,” Journal of Virology, vol. 71, no. 7, pp. 5040–5050, 1997.

[118] R. L. Q. Russell and G. F. Rohrmann, “Characterization of P91, a protein associated with virions of an Orgyia pseudotsugata baculovirus,” Virology, vol. 233, no. 1, pp. 210–223, 1997.
[119] M. Whitford and P. Faulkner, “A structural polypeptide of the baculovirus Autographa californica nuclear polyhedrosis virus contains O-linked N-acetylgalcosamine,” Journal of Virology, vol. 66, no. 6, pp. 3324–3329, 1992.

[120] J. Olszewski and L. K. Miller, “A role for baculovirus GP41 in budded virus production,” Virology, vol. 233, no. 2, pp. 292–301, 1997.

[121] W. Wu, T. Lin, L. Pan et al., “Autographa californica multiple nucleopolyhedrovirus nucleocapsid assembly is interrupted upon deletion of the 38K gene,” Journal of Virology, vol. 80, no. 23, pp. 11475–11485, 2006.

[122] W. Wu, H. Liang, J. Kan et al., “Autographa californica multiple nucleopolyhedrovirus 38K is a novel nucleocapsid protein that interacts with VP1054, VP39, VP80, and itself,” Journal of Virology, vol. 82, no. 24, pp. 12356–12364, 2008.

[123] C. M. Long, G. F. Rohrmann, and G. F. Merrill, “The structural polypeptide of Autographa californica nucleopolyhedrovirus nucleocapsid assembly is interrupted upon deletion of the 38K gene,” Journal of Virology, vol. 80, no. 23, pp. 11475–11485, 2006.

[124] W. Wu and A. L. Passarelli, “Autographa californica multiple nucleopolyhedrovirus Ac92 (ORF92, P33) is required for budded virus production and multiply enveloped occlusion-derived virus formation,” Journal of Virology, vol. 84, no. 23, pp. 12351–12361, 2010.

[125] Y. Nie, M. Fang, and D. A. Theilmann, “Autographa californica multiple nucleopolyhedrovirus core gene ac92 (p33) is required for efficient budded virus production,” Virology, vol. 409, no. 1, pp. 38–45, 2011.

[126] M. Fang, H. Wang, H. Wang et al., “Open reading frame 94 of Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus encodes a novel conserved occlusion-derived virion protein, ODV-EC43,” Journal of General Virology, vol. 84, no. 11, pp. 3021–3027, 2003.

[127] F. Deng, R. Wang, M. Fang et al., “Proteomics analysis of Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus identified two new occlusion-derived virus-associated proteins, HA44 and HAI00,” Journal of Virology, vol. 81, no. 17, pp. 9377–9385, 2007.

[128] KE. Peng, M. Wu, F. Deng et al., “Identification of protein-protein interactions of the occlusion-derived virus-associated proteins of Helicoverpa armigera nucleopolyhedrovirus,” Journal of General Virology, vol. 91, no. 3, pp. 659–670, 2010.

[129] A. L. Vanarsdall, M. N. Pearson, and G. F. Rohrmann, “Characterization of baculovirus constructs lacking either the Ac 101, Ac 142, or the Ac 144 open reading frame,” Virology, vol. 367, no. 1, pp. 187–195, 2007.

[130] G. Li, J. Wang, R. Deng, and X. Wang, “Characterization of AcMNPV with a deletion of ac68 gene,” Virus Genes, vol. 37, no. 1, pp. 119–127, 2008.

[131] C. B. McCarthy and D. A. Theilmann, “AcMNPV ac143 (ovd-e18) is essential for mediating budded virus production and is the 30th baculovirus core gene,” Virology, vol. 375, no. 1, pp. 277–291, 2008.

[132] J. Ke, J. Wang, R. Deng, and X. Wang, “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, vol. 374, no. 2, pp. 421–431, 2008.

[133] M. Belyavskiy, S. C. Braunagel, and M. D. Summers, “The structural protein ODV-EC27 of Autographa californica nucleopolyhedrovirus is a multifunctional viral cyclin,” Proceedings of the National Academy of Sciences of the United States of America, vol. 95, no. 19, pp. 11205–11210, 1998.

[134] S. C. Braunagel, H. He, P. Ramamurthy, and M. D. Summers, “Transcription, translation, and cellular localization of three Autographa californica nuclear polyhedrosis virus structural proteins: ODV-E18, ODV-E35, and ODV-EC27,” Virology, vol. 222, no. 1, pp. 100–114, 1996.

[135] H. Q. Chen, KE. P. Chen, Q. Yao, Z. J. Guo, and L. L. Wang, “Characterization of a late gene, ORF67 from Bombyx mori nucleopolyhedrovirus,” FEBS Letters, vol. 581, no. 30, pp. 5836–5842, 2007.

[136] O. Simón, S. Gutiérrez, T. Williams, P. Caballero, and M. López-Ferber, “Nucleotide sequence and transcriptional analysis of the pif gene of Spodoptera frugiperda nucleopolyhedrovirus (SfMNPV),” Virus Research, vol. 108, no. 1–2, pp. 213–220, 2005.

[137] G. P. Pijilman, A. J. P. Pruijssers, and J. M. Vlak, “Identification of pif-2, a third conserved baculovirus gene required for per os infection of insects,” Journal of General Virology, vol. 84, no. 8, pp. 2041–2049, 2003.

[138] P. Faulkner, J. Kuzio, G. V. Williams, and J. A. Wilson, “Analysis of p74, a PDV envelope protein of Autographa californica nucleopolyhedrovirus required for occlusion body infectivity in vivo,” Journal of General Virology, vol. 78, no. 12, pp. 3091–3100, 1997.

[139] W. Zhou, L. Yao, H. Xu, F. Yan, and Y. Qi, “The function of envelope protein p74 from Autographa californica multiple nucleopolyhedrovirus in primary infection to host,” Virus Genes, vol. 30, no. 2, pp. 139–150, 2005.

[140] E. J. Haas-Stapleton, J. O. Washburn, and L. E. Volkman, “P74 mediates specific binding of Autographa californica M nucleopolyhedrovirus occlusion-derived virus to primary cellular targets in the midgut epithelium of Heliothis virescens larvae,” Journal of Virology, vol. 78, no. 13, pp. 6786–6791, 2004.

[141] L. Yao, W. Zhou, H. Xu, Y. Zheng, and Y. Qi, “The Heliothis armigera single nucleocapsid nucleopolyhedrovirus envelope protein P74 is required for infection of the host midgut,” Virus Research, vol. 104, no. 2, pp. 111–121, 2004.

[142] S. C. Braunagel, W. K. Russell, G. Rosas-Acosta, D. H. Russell, and M. D. Summers, “Determination of the protein composition of the occlusion-derived virus of Autographa californica nucleopolyhedrovirus,” Proceedings of the National Academy of Sciences of the United States of America, vol. 100, no. 17, pp. 9797–9802, 2003.

[143] I. Kikhno, S. Gutiérrez, L. Croizier, G. Crozier, and M. López Ferber, “Characterization of pif, a gene required for the per os infectivity of Spodoptera littoralis nucleopolyhedrovirus,” Journal of General Virology, vol. 83, no. 12, pp. 3013–3022, 2002.

[144] T. Ohkawa, J. O. Washburn, R. Sitapara, E. Sid, and L. E. Volkman, “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,” Journal of Virology, vol. 79, no. 24, pp. 15258–15264, 2005.

[145] M. Fang, Y. Nie, S. Harris, M. A. Erlandson, and D. A. Theilmann, “Autographa californica multiple nucleopolyhedrovirus core gene ac96 encodes a per os infectivity factor (pif-4),” Journal of Virology, vol. 83, no. 23, pp. 12569–12578, 2009.

[146] S. C. Braunagel, D. M. Elton, H. Ma, and M. D. Summers, “Identification and analysis of an Autographa californica nuclear polyhedrosis virus structural protein of the occlusion-derived virus envelope: ODV-ES6,” Virology, vol. 217, no. 1, pp. 97–110, 1996.
[147] W. O. Sparks, R. L. Harrison, and B. C. Bonning, “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*, vol. 409, no. 1, pp. 69–76, 2011.

[148] D. P. A. Cohen, M. Marek, B. G. Davies, J. M. Vlak, and M. M. van Oers, “Encyclopedia of *Autographa californica* nucleopolyhedrovirus genes,” *Virologica Sinica*, vol. 24, no. 5, pp. 359–414, 2009.

[149] Y. Jiang, F. Deng, S. Rayner, H. Wang, and Z. Hu, “Evidence of a major role of GP64 in group I alphabaculovirus evolution,” *Virus Research*, vol. 142, no. 1-2, pp. 85–91, 2009.

[150] E. A. Herniou and J. A. Jehle, “Baculovirus phylogeny and evolution,” *Current Drug Targets*, vol. 8, no. 10, pp. 1043–1050, 2007.

[151] P. M. de Andrade Zanotto and D. C. Krakauer, “Complete genome viral phylogenies suggests the concerted evolution of regulatory cores and accessory satellites,” *PLoS ONE*, vol. 3, no. 10, Article ID e3500, 2008.

[152] D. Goodman, N. Ollikainen, and C. Sholley, “Baculovirus phylogeny based on genome rearrangements,” in *Proceedings of the International Conference on Comparative Genomics*, vol. 4751 of *Lecture Notes in Computer Science*, pp. 69–82, 2007.