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The Baculoviral Genome

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1. Introduction

The molecular biology of Baculoviruses has drawn a great deal of interest due to the variety of applications of these viruses as: 1) agents for biological control of insect pests (Szewczyk et al., 2006); 2) vectors for expression of recombinant proteins in insect cells (Kost et al., 2005); 3) vehicles for gene transduction of mammal cells (Hu, 2006, 2008); and 4) display systems of recombinant epitopes (Makela et al., 2010).

Baculoviridae is a family of insect-specific viruses, with more than 600 reported species, mainly isolated from Lepidoptera (butterflies and moths) and in some cases from Hymenoptera (sawflies) and Diptera (mosquitoes). Baculoviruses have circular, double-stranded DNA genomes ranging in size from approximately 80 to 180 kbp, depending on the species, that are predicted to encode for up to 180 genes. The viral genome associates with proteins forming a nucleocapsid. This structure is surrounded by a membrane envelope to form a rod-shaped virion (hence, the name of the Family: *baculum* is Latin for rod or stick).

During their biological cycle, most baculoviruses produce two different virion phenotypes: the *budded virus* (BV) appears early in infected cells and is responsible for the dissemination of the disease inside the insect body, whereas the *occluded virus* (OV) is produced in the very late stage of the infection and becomes embedded in a protein matrix forming a distinct structure known as *occlusion body* (OB) which is responsible for the horizontal transmission of the virus. OBs are highly stable and protect the virions from damage in the environment.

1.1 Taxonomy

The polyhedral and ovoidal morphology of the different OBs has been used as an initial taxonomic criterion to group baculoviruses in two genera: Nucleopolyhedrovirus (NPVs) and Granulovirus (GVs). The major protein found in polyhedra is polyhedrin, which is

expressed very late in infection. Multiple OVs are embedded within a polyhedron. Also, each OV may contain one or more nucleocapsids. This lead to a grouping of the NPVs as SNPVs (*Single* NPV, one enveloped nucleocapsid per virion) and MNPV (*Multiple* NPV, multiple nucleocapsids per virion). The other genus (Granulovirus) has characteristic OBs that appear as ovoidal granules, with granulin as the major protein component. Usually GVs contain a single virion per OB with only one nucleocapsid (Funk et al., 1997).

More recently, a new classification on the *Baculoviridae* based on DNA sequence data has been proposed and accepted by the ICTV (Carstens and Ball, 2009; Jehle et al., 2006a). It preserves correlation with OB morphology but also reflects host taxonomic classification. Four genera are recognized: *Alphabaculovirus* (NPVs isolated from Lepidoptera); *Betabaculovirus* (GVs isolated from Lepidoptera); *Gammabaculovirus* (NPVs isolated from Hymenoptera) and *Deltabaculovirus* (NPVs isolated from Diptera).

The type baculovirus is *Autographa californica* nucleopolyhedrovirus, AcMNPV, a member of the *Alphabaculovirus* genus (Table 1). The present knowledge about the baculovirus molecular biology is based largely on studies performed with this virus. Consequently, the most of the information presented here is based on AcMNPV.

1.2 Two types of enveloped virions

OBs ingested by susceptible insect larvae are dissolved in the midgut releasing the *occlussion derived virus* (ODV) that initiate the infection of midgut epithelial cells.

Structural differences between BVs and ODVs are due mainly to the origin and composition of the lipoproteic membrane envelope. BVs acquire their envelope from the infected cell membrane (modified by the insertion of viral proteins) during the budding process. On the other hand, ODVs envelope is built at the nuclear stage using the nuclear membrane components and it is much more complex than the envelope of the BVs regarding their protein content (Rohrmann, 2011e).

A distinctive characteristic of the BV phenotype is the presence of a membrane protein that mediates viral entry via an endocytic, pH-dependent mechanism (Blissard and Wenz, 1992; Pearson et al., 2000). There are two types of envelope fusogenic proteins in baculoviruses, GP64 and F. All baculoviruses contain F (the exception being NPVs isolated from hymenoptera) but it does not play a functional role in the early stages of infection in all cases; those expressing GP64 use it as the major player in the early stage of virus entry and infection. Coincident with this difference, NPVs isolated from Lepidoptera have been found to cluster in two phylogenetic subgroups (I and II) based on their polyhedrin sequences as well as the presence or absence of a *gp64* gene, respectively. Those containing GP64 belong to group I (Hefferon et al., 1999; IJkel et al., 2000; Monsma et al., 1996), while those that lack GP64 but have a functional F protein belong to group II (IJkel et al., 2000; Pearson et al., 2000).

1.3 Infectious cycle

The natural infection cycle begins when the insect ingests the OBs contaminating its food (figure 1). Once in the midgut of the insect larvae, the highly alkaline environment contributes to the dissolution of the OBs releasing ODVs. The ODVs must traverse the

peritrofic membrane lining the midgut lumen and fuse with epithelial cell membrane, allowing the entry of the nucleocapsids. These make their way to the nucleus, where the transcription starts in a very finely regulated manner initiating a gene transcription cascade (Friesen, 1997; O' Reilly et al., 1992; Romanowski and Ghiringhelli, 2001).
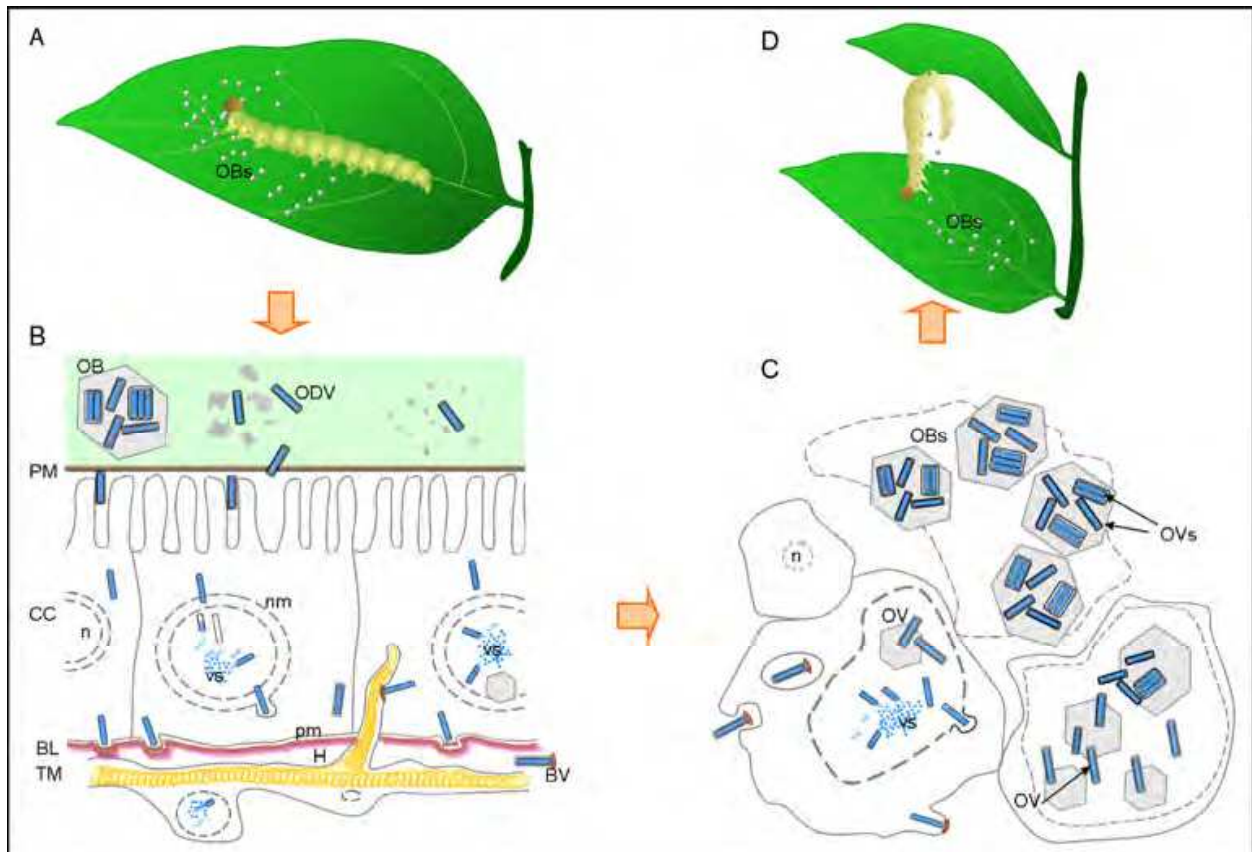

Fig. 1. Baculovirus (Alphabaculovirus) infection cycle. **A**. Larva ingests food contaminated with OBs. **B**. OBs are dissolved in the alkaline midgut releasing ODVs which upon overcoming the PM infect midgut epithelial cells. Newly formed nucleocapsids bud from the plasma membrane and disseminate inside the larval body, via the tracheal cells or directly through the hemolymph. **C**. In the late stage of infection nucleocapsids acquire their envelope from the nuclear membrane forming OVs (arrows), which may contain one or several nucleocapsids, and are occluded within a polyhedrin matrix forming the OBs. **D.** Dead larva full of OBs typically appears hanging in a the upper part of the plant. OB (Occlusion Body); ODV (Occlusion Derived Virion); PM (Peritrophic Membrane); CC (Columnar Cell); n (nucleus); nm (nuclear membrane); vs (virogenic stroma); BL (Basal Lamina); TM (Tracheal Matrix); H (Hemolymph); OV (Occluded Virion). Figure modified from Federici (1997).

Transcription of viral genes occurs in four stages: immediate early, delayed early, late and very late. Genes of the early stages are transcribed by the cell RNA polymerase II. Immediate early genes are transactivated by host cell transcription factors with no participation of virus-encoded proteins, reflecting the empirical observation that naked baculovirus genomic DNA is infective (Burand et al., 1980; Carstens et al., 1980; Hajos et al., 1998). Transcription of delayed early genes requires the activation by viral gene

products expressed at the previous stage. Among delayed early gene products, those called LEFs (late expression factors) are required for DNA replication and late transcription (Hefferon and Miller, 2002). After the delayed-early stage viral DNA synthesis occurs within the nucleus of the infected cell, in what is called the virogenic stroma. Baculoviral DNA replication is not totally understood but evidence exists that it may occur by a rolling circle mechanism, recombination-dependent mechanism, or by a combination of both. Some sequences called *hrs* (*homologous regions*) behave as functional replication origins (Rohrmann, 2011c).

Genes expressed in the late and very late phases are transcribed by a virus-encoded RNA polymerase. Late genes in AcMNPV are transcribed between 6 and 24 h post infection (p. i.), while very late genes are expressed in an explosive way, beginning at 18 h p. i. approximately and continuing up to 72 h p.i. (Lu et al., 1997). In the late phase, structural nucleocapsid proteins are synthesized, and also GP64 which is essential in the BV structure for the virus systemic infection. GP64 is targeted to the cell membrane, where virions bud between 10 and 24 h p.i. During the very late phase, BV production decreases; nucleocapsids are no longer used in BV formation and they are used in turn to build the occluded virions (OV). In the specific case of NPVs, nucleocapsids are thought to interact with the nuclear membrane in the process to obtain their envelope (Slack and Arif, 2007). Then the OVs become occluded with the very late protein polyhedrin, forming the characteristic refringent polyhedra that can be observed in the infected cell nucleus. Occlusion continues until the nucleus eventually fills with polyhedra. Typically more than 30 polyhedra can be observed in an AcMNPV infected cell. More than 10^{10} polyhedra can be produced in a single infected larva in its last larval stage, before death. These polyhedra can account for up to 30 % of the larva dry weight (Miller et al., 1983). As occlusion progresses fibrillar structures are accumulated in the nucleus, mainly built from a single polypeptide (P10) expressed very late in infection (Van Der Wilk et al., 1987). The function of these fibrillar structures is not absolutely clear but seems to play a role in the controlled disintegration of larvae (Dong et al., 2007; Van Oers et al., 1994; Williams et al., 1989). In the final stage of the infection, virus encoded enzymes, cathepsin and chitinase, aid in the cuticle rupture and liquefaction of the dead larva, leading to the release polyhedra in the environment and making them avaliable for ingestion by a new insect (Hawtin et al., 1997).

2. Baculovirus genomes

Since the first complete sequence of a baculoviral genome was reported (AcMNPV; Ayres et al., 1994), many baculovirus genomes were sequenced to further improve the understanding of the molecular biology of these viruses. To date, there are 58 fully sequenced baculoviral genomes available in GenBank. Forty one belong to the *Alphabaculovirus* genus, thirteen to the *Betabaculovirus*, three to the *Gammabaculovirus* and one to the *Deltabaculovirus* (Table 1). Baculovirus encode 89 (NeleNPV) to 183 (PsunGV) predicted ORFs, in both strands, apparently with no preferred orientation. Typically, the ORF designated as number 1 is that encoding the major occlusion protein (polyhedrin/granulin) and the following ORFs are numbered sequentially in a clockwise direction. In general, baculovirus genomes have low GC content (<50%). The virus with the lowest GC% is NeleNPV (33.3%).

The generally adopted criterion to predict ORFs is to only consider those that code for a polypeptide at least 50 amino acid long (aa) and minimal overlap with other ORFs. Baculovirus genes are not clustered in the genome by function or the time of transcription. Noteworthy, only one expression unit has been detected to contain an intron (*ie0*) (Chisholm and Henner, 1988), which makes it easier to predict ORFs at the DNA sequence level.

The sequencing of complete genomes allowed estimating the whole baculovirus gene content in about 900 genes. All baculovirus genomes sequenced so far encode for a group of 31 genes, known as the core genes. These genes represent a hallmark of the virus family and may play a role in essential biological functions (Miele et al., 2011). According to their function, the core genes (Table 2) can be classified as belonging to the following categories: replication, transcription, packaging and assembly, cell cycle arrest/interaction with host proteins and oral infectivity.

As most of available genomes belong to baculoviruses specific for lepidopteran insects (*Alpha-* and *Betabaculovirus*), there is a good deal of information to characterize a set of genes associated with specificity for Lepidoptera. Likewise, there are some *Betabaculovirus*-specific genes, not found in NPVs, which may be implicated in the differential pathogenesis displayed by these viruses. It is worth noting that GVs (*Beta-*) are not as well studied as *Alpha-* NPVs at the molecular level because of the lack of proper susceptible insect cell lines. So far, the only GV-specific gene characterized at the functional level is a metalloproteinase of XcGV which has orthologs in all GVs (Ko et al., 2000).

Regarding the gene promoters, there are many baculovirus early genes that are preceded by either a TATA-box or a CAGT initiator motif, or both. These motifs are found also in promoters of the host genome and are characteristic of genes transcribed by the RNA polymerase II of the insect cell. Late and very late genes are expressed by the viral RNA polymerase from promoters containing the DTAAG motif. The occurrence of this motif is less frequent than predicted by stochastic distribution, according to its functional role as initiator of late and very late transcription. Some genes contain both early and late promoter motifs and are expected to be expressed throughout the infection. However, not every predicted ORF is preceded by a known motif, which does not imply that it is not expressed. Other elements have been characterized to play a role in baculovirus transcription such as GATA motifs and distal CGT motif (van Oers and Vlak, 2007).

Traditionally, baculovirus gene functions were studied by constructing deletion mutants upon cotransfection of wild type viral DNA and a transfer vector containing an insertion cassette flanked by fragments of homology to the target region in the genome [*e. g.* (Lee et al., 1998)]. As baculoviruses have been widely used as expression vectors, much effort was made to improve the production of recombinant virus. This led to the construction of the first *bacmid* which is a recombinant AcMNPV genome containing the mini-F origin of replication that allows the maintenance and recombination of the virus in *Escherichia coli* (Luckow et al., 1993). This fact led to a new way of studying baculoviral genes: a specific deletion can be made by recombination in *E. coli* and, upon recovering viral DNA from a bacterial culture, it can be transfected in insect cells to study the effect of the modified genome in the viral cycle (Zhao et al., 2003).

Genus	Virus Name	Acronym	Genome Size (bp)	number of ORFs or CDS	Reference	GenBank Accesion Number
Alphabaculovirus NPVs Group I	*Antheraea pernyi* NPV-Z	AnpeNPV	126.629	147	Nie et al., 2007	DQ486030
	Antheraea pernyi NPV-L2	AnpeNPV	126.246	145	Fan et al., 2007	EF207986
	Anticarsia gemmatalis NPV D2	AgMNPV	132.239	152	Oliveira et al., 2006	DQ813662
	Autographa californica NPV C6	AcMNPV	133.894	156	Ayres et al., 1994	L22858
	Bombyx mandarina NPV	BomanNPV	126.770	141	Xu et al., 2009, unpublished	NC012672
	Bombyx mori NPV T3	BmNPV	128.413	143	Gomi et al., 1999	L33180
	Choristoneura fumiferana DEF NPV	CfDefNPV	131.160	149	Lauzon et al., 2005	AY327402
	Choristoneura fumiferana MNPV	CfMNPV	129.593	146	de Jong et al., 2005	AF512031
	Epiphyas postvittana NPV	EppoNPV	118.584	136	Hyink et al., 2002	AY043265
	Hyphantria cunea NPV	HycuNPV	132.959	148	Ikeda et al., 2006	AP009046
	Maruca vitrata MNPV	MaviMNPV	111.953	126	Chen et al., 2008	EF125867
	Orgyia pseudotsugata MNPV	OpMNPV	131.995	152	Ahrens et al., 1997	U75930
	Plutella xylostella MNPV CL3	PlxyMNPV	134.417	152	Harrison and Lynn, 2007	DQ457003
	Rachiplusia ou MNPV	RoMNPV	131.526	149	Harrison and Bonning, 2003	AY145471
Alphabaculovirus NPVs Group II	*Adoxophyes honmai* NPV ADN001	AdhoNPV	113.220	125	Nakai et al., 2003	AP006270
	Adoxophyes orana NPV	AdorNPV	111.724	121	Hilton and Winstanley, 2008a	EU591746
	Agrotis ipsilon MNPV	AgipMNPV	155.122	163	Harrison, 2009	EU839994
	Agrotis segetum NPV	AgseNPV	147.544	153	Jakubowska et al., 2006	DQ123841
	Apocheima cinerarium NPV	ApciNPV	123876	118	Zhang et. al, unpublished	FJ914221
	Chrysodeixis chalcites NPV	ChchNPV	149.622	151	van Oers et al., 2005	AY864330
	Clanis bilineata NPV DZ1	ClbiNPV	135.454	139	Zhu et al., 2009	DQ504428
	Ecotropis obliqua NPV A1	EcobNPV	131.204	126	Ma et al., 2007	DQ837165
	Euproctis pseudoconspersa NPV	EupsNPV	141.291	139	Tang et al., 2009	NC_012639
	Helicoverpa armigera MNPV	HearMNPV	154.196	162	Tang et al., 2008, unpublished	EU730893
	Helicoverpa armigera NPV C1	HearSNPV	130.759	137	Zhang et al., 2005	AF303045
	Helicoverpa armigera NPV G4	HearSNPV	131.405	135	Chen et al., 2001	AF271059
	Helicoverpa armigera SNPV NNg1	HearSNPV	132.425	143	Ogembo et al., 2009	AP010907
	Helicoverpa zea SNPV	HzSNPV	130.869	139	Chen et al., 2002	AF334030
	Leucania separata NPV AH1	LeseNPV	168.041	169	Xiao and Qi, 2007	AY394490
	Lymantria dispar NPV	LdMNPV	161.046	164	Kuzio et al., 1999	AF081810
	Lymantria xylina MNPV	LyxyMNPV	156.344	157	Nai et al., 2010	GQ202541
	Mamestra configurata NPV A	MacoNPV A-90-2	155.060	169	Li et al., 2002b	U59461

Genus	Virus Name	Acronym	Genome Size (bp)	number of ORFs or CDS	Reference	GenBank Accesion Number
Alphabaculovirus NPVs Group II	*Mamestra configurata* NPV A	MacoNPV A-90-4	153656	168	Li et al., 2005	AF539999
	Mamestra configurata NPV B	MacoNPV B	158.482	168	Li et al., 2002a	AY126275
	Orgyia leucostigma NPV CSF-77	OrleNPV	156.179	135	Eveleigh et al., 2008, unpublished	EU309041
	Spodoptera exigua NPV	SeMNPV	135.611	139	Ijkel et al., 1999	AF169823
	Spodoptera frugiperda MNPV	SfMNPV 19	132.565	141	Wolff et al., 2008	EU258200
	Spodoptera frugiperda MNPV 3AP2	SfMNPV 3AP2	131.330	142	Harrison et al., 2008	EF035042
	Spodoptera litura NPV G2	SpltMNPV	139.342	141	Pang et al., 2001	AF325155
	Spodoptera litura NPV II	SpltNPV II	148.634	147	Li et al., 2008, unpublished	EU780426
	Trichoplusia ni SNPV	TnSNPV	134.394	145	Willis et al., 2005	DQ017380
Betabaculovirus	*Adoxophyes orana* GV	AdorGV	99.657	119	Wormleaton et al., 2003	AF547984
	Agrotis segetum GV	AgseGV	131.680	132	Xiulian et al., 2004, unpublished	AY522332
	Choristoneura occidentalis GV	ChocGV	104.710	116	Escasa et al., 2006	DQ333351
	Clostera anachoreta GV	ClanGV	101487	123	Liang et al., 2011	HQ116624
	Cryptophlebia leucotreta GV CV3	CrleGV	110.907	128	Lange and Jehle, 2003	AY229987
	Cydia pomonella GV	CpGV	123.500	143	Luque et al., 2001	U53466
	Helicoverpa armigera GV	HearGV	169.794	179	Harrison and Popham, 2008	EU255577
	Phthorimaea operculella GV	PhopGV	119.217	130	Croizier et al., 2002, unpublished	AF499596
	Pieris rapae GV	PrGV	108.592	120	Zhang et al., 2010, unpublished	NC_013797
	Plutella xylostella GV K1	PlxyGV K1	100.999	120	Hashimoto et al., 2000	AF270937
	Pseudelatia unipuncta GV	PsunGV	176.677	183	Li et al., 2008, unpublished	EU678671
	Spodoptera litura GV K1	SpltGV K1	124.121	136	Wang et al., 2007, unpublished	DQ288858
	Xestia c-nigrum GV	XecnGV	178.733	181	Hayakawa et al., 1999	AF162221
γ	*Neodiprion abietis* NPV	NeabNPV	84.264	93	Duffy et al., 2006	DQ317692
	Neodiprion sertifer NPV	NeseNPV	86.462	90	Garcia-Maruniak et al., 2004	AY430810
	Neodiprion lecontei NPV	NeleNPV	81.755	89	Lauzon et al., 2004	AY349019
δ	*Culex nigripalpus* NPV	CuniNPV	108.252	109	Afonso et al., 2001	AF403738

Table 1. **Fully sequenced baculovirus genomes**. ORF (**O**pen **R**eading **F**rame) or CDS (protein **Co**ding **S**equence): defined by a start codon ATG followed by at least 50 codons before a stop codon in frame. Gamma and Deltabaculovirus genera are indicated by greek characters.

3. Replication genes

Replication of baculovirus genome is poorly understood. As mentioned above, a rolling circle mechanism has been proposed but there are evidences of recombination being involved as well. Baculovirus genomes contain multiple origins of replication. Sequences that act as origins are called *hrs* (for homologous regions) and are dispersed throughout the genome (explained in section 8). In addition, *non-hr* origins were also found, present only once per genome.

Several viral factors have been demonstrated to be essential for viral replication and others to be stimulatory. IE-1, a known activator of early transcription (see below), was found to be necessary for plasmid replication in transient assays. IE-1 binds to *hr* sequences but it is not clear if this is a requirement for initiation of DNA replication. The other proteins essential for DNA synthesis in AcMNPV are DNA polymerase, DNA helicase, LEF-1, LEF-2 and LEF-3. In addition to polymerization activity by DNA polymerase and DNA unwinding by DNA helicase, a primase activity is associated with LEF-1, and LEF-2 as a primase accessory factor. LEF-3 is a single-stranded DNA binding protein (Mikhailov, 2003; Rohrmann, 2011c).

These proteins were found in all baculoviruses sequenced to date but some other proteins have been identified to have an influence on DNA replication. These are P35, IE-2; PE38; LEF-7; VLF-1, Alcaline Exonuclease (AN); DBP, LEF-11, ME53 and PCNA (Mikhailov, 2003).

Some baculovirus genomes code also for other proteins that may be involved in DNA replication like DNA ligase, and a second helicase. In addition, genes encoding enzymes related to DNA repair: photolyase (present in some group II nucleopolyhedrovirus; (Xu et al., 2008), Ac79 (homolog to UvrC endonuclease superfamily), V-trex exonuclease (present in AgMNPV and CfMNPV), polyADP-ribose polymerase (PARP, found in AgMNPV) and polyADP-ribose glycohydrolase (PARG, present in all sequenced group II NPVs) (Rohrmann, 2011c). Nucleotide biosynthesis seems to be another aspect of DNA replication that some baculoviruses may influence since they have genes for ribonucleotide reductase subunits and dUTPase, both related to dTTP biosynthesis (Herniou et al., 2003).

4. Transcription genes

Transcription of baculovirus genes occurs in several temporal stages. As mentioned above, early genes are transcribed by the host RNA pol II and after DNA replication, late gene transcription proceeds through the action of a viral RNA polymerase. One of the first proteins to be transcribed is IE-1, which functions as a transcriptional activator of itself and delayed early genes. It is known that IE-1 binds to *hr* sequences as a dimer and it is thought that this complex interacts with the host transcription machinery to enhance expression of early genes. Although there are no other recognizable IE-1 binding sites, an *hr*-independent mechanism of transactivation is likely to occur also (Friesen, 1997). Part of the IE-1 population is called IE-0 which is the translation product of the only spliced mRNA described in AcMNPV. IE-1 orthologs appear in all *Alpha* and *Betabaculovirus* genomes. Other transcription factors encoded by AcMNPV and other baculoviruses that were found to transactivate early genes are IE-2 and PE-38 (Cohen et al., 2009; Rohrmann, 2011d).

In the late stage of the infection additional genes are implicated in transcription. Viral RNA polymerase is made of four subunits coded by four core genes: *lef-4*, *lef-8*, *lef-9* and *p47* (Guarino et al., 1998). LEF-8 and LEF-9 have motifs common to the largest subunits of bacterial and eukaryotic RNA polymerases. LEF-8 contains the essential C-terminal region conserved in RNA polymerases, while the rest of the polypeptide shows no sequence homology to other known RNA polymerases. LEF-9 contains the Mg^{2+} binding site of the catalytic centre found in other RNA polymerases. LEF-4 is an RNA capping enzyme and P47 does not show homology with other RNA polymerase subunits (van Oers and Vlak, 2007). Two other core genes are implicated in late transcription: *lef-5* and *vlf-1*. LEF-5 appears to be an initiation factor in AcMNPV (Guarino et al., 2002). VLF-1 (Very Late Expression Factor-1) is involved in the expression of the very late genes *polyhedrin* (*polh*) and *p10*. VLF-1 was found to interact with the so-called "burst sequence" present downstream of the very late genes triggering their hyperexpression (Yang and Miller, 1999). Other proteins required for late transcription as revealed by transient expression assays are LEF-6 (a putative mRNA export factor), LEF-10, LEF-12 and PP31 (Rohrmann, 2011b). Additional proteins may be involved: a methyltrasferase (Ac69), probably implicated in mRNA capping (Wu and Guarino, 2003), an ADP-ribose pyrophosphatase of the nudix superfamily (Ge et al., 2007) which is a putative decapping enzyme, LEF-2 which apart from being an essential replication factor (see above) is also implicated in the very late transcription (Merrington et al., 1996); and PK1 (Mishra et al., 2008)

5. Structural genes

While nucleocapsids are essentially the same in both baculovirus phenotypes, BVs and ODVs differ in the origin and protein composition of their envelope. Moreover, ODVs are occluded in a proteinaceus matrix forming the OBs, which are essential structures for maintenance of orally infective virus. No structural protein is needed for the initiation of transcription once viral DNA enters the nucleus of the cell; therefore the structural proteins found in virions are supposed to focus on overcoming the barriers for cell entry. In a simmilar fashion some OB proteins are involved in facilitating horizontal transmission and invasion of the midgut altogether. As expected, baculovirus genome encodes many genes for proteins that are included in the the virion and OB structures, as well as genes whose products may not be present in the final structure but are important for its assembly (Funk et al., 1997).

5.1 Occlusion body

Baculovirus OB is formed by the major occlusion body protein polyhedrin, for NPVs, or granulin, for GVs. Polyhedrin and granulin are closely related. The occlusion body protein for the only dipteran baculovirus completely sequenced, CuniNPV, does not show sequence homology to its lepidopteran counterparts and is a much larger protein. Hymenopteran baculoviruses occlusion protein is homologous to Alphabaculoviruses polyhedrin (Garcia-Maruniak et al., 2004). *Polyhedrin* is a very late gene that is expressed at very high levels. This characteristic has been exploited for the expression of recombinant proteins in insect cells. In the natural cycle of a NPV, polyhedrin forms a crystalline cubic lattice that surrounds the ODVs. The structure of the polyhedron was recently determined (Ji et al., 2010).

Other proteins apart from the major occlusion protein are present in the structure of the OB or play a role in its morphogenesis. The polyhedron is surrounded by a protein layer which

provides the OB with a smooth, sealed surface that enhances its stability (Gross et al., 1994). The viral protein responsible of this envelope is the calyx/PE. During OBs formation calyx/PE is found associated with fibrillar structures formed by P10, the other protein that is highly expressed at the very late phase. Although it is not part of the OB, P10 plays a role in its correct morphogenesis (Williams et al., 1989).
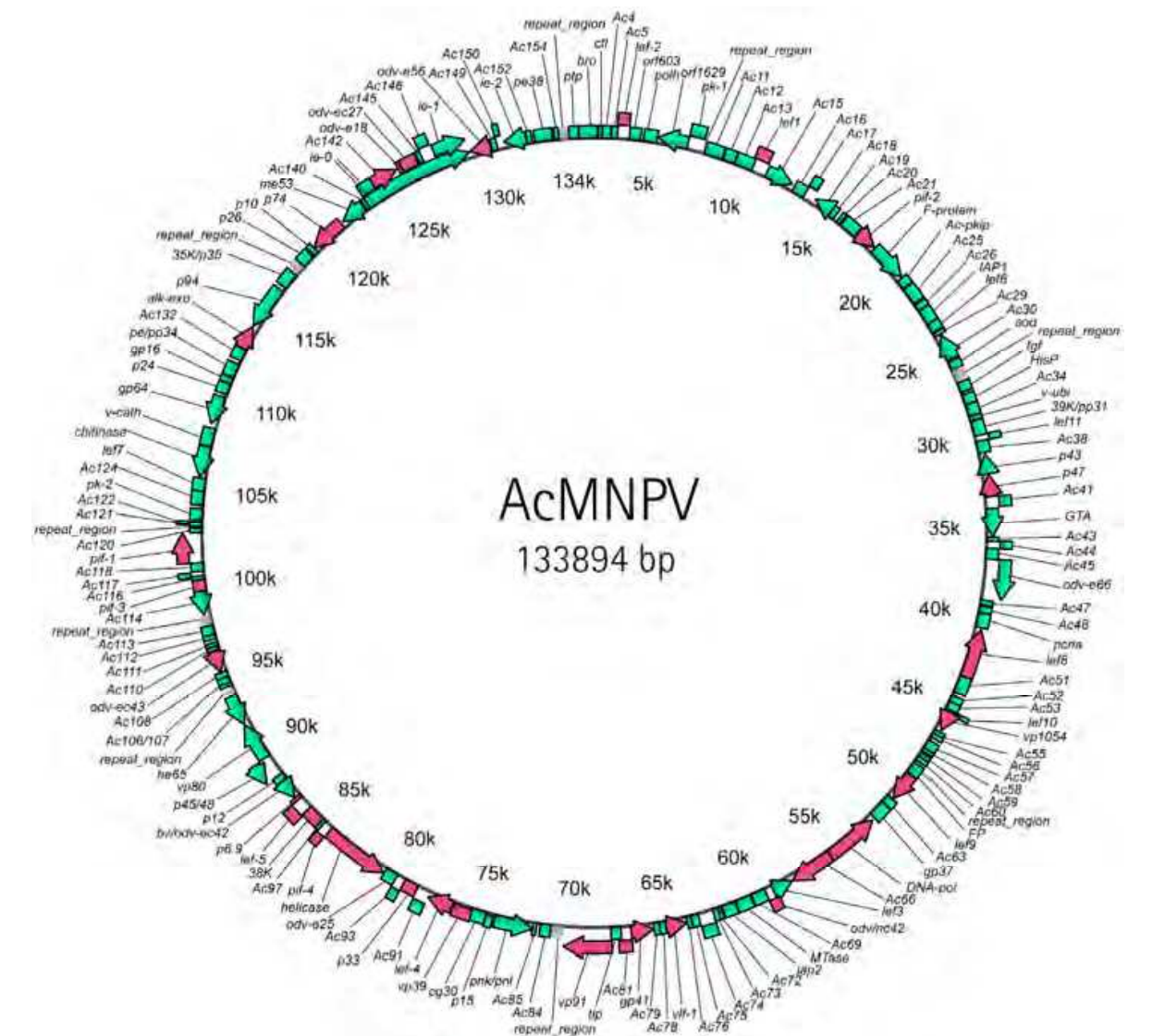

Fig. 2. Baculovirus genome (AcMNPV). Core genes are shown in pink.

Depending on the species there may be other proteins associated with the polyhedron: enhancin/viral enhancing factor (Vef) and proteinases. Enhancins are metalloproteinases that help disrupt the peritrophic membrane (PM) of the insect midgut. PM is the first barrier baculoviruses must overcome when ingested in order to get midgut epithelial cells. PM is made of mucin proteins and chitin. Enhancin degrades mucin helping this way ODVs pass through the PM (Wang and Granados, 1997). Not all the baculoviruses encode for enhancins but, for example, XcGV has four copies (Hayakawa et al., 1999). Alkaline proteinases were found associated to the OBs that may aid in the dissolution of OBs and subsequent ODVs release. However since there is not such a gene identified in baculovirus genomes, those could be bacterial or insect contaminants present in the OB preparation (Rohrmann, 2011e).

5.2 BV and ODV

As mentioned above, although both BVs and ODVs carry the same genetic information there are several differences between them in function and structure. BVs are the first virion phenotype produced in an infected cell and consist of a nucleocapsid which acquire their envelope as they bud from the cell membrane previously modified with the GP64 (group I *Alphabaculovirus*) or F protein (rest of the *Baculoviridae*). On the other hand OVs obtain their envelope from the nuclear membrane, may include several nucleocapsids per virion -in the case of MNPVs- and their protein content seems to be more complex than that of BVs.

Genomic DNA associates with proteins to form nucleocapsids. A small basic protein, P6.9, directly interacts with DNA and is involved in the assembly of highly condensed DNA (Kelly et al., 1983). VP39 is the major nucleocapsid protein and, along with P6.9, is a core gene. Both proteins are two of the three most abundant proteins in AcMNPV BV, being GP64 the third one (Wang et al., 2010b). VLF-1 is also a core gene and it was first described as the factor necessary for the expression of very late genes. But later it was shown that VLF-1 is present in both, BV and ODV, localizing at one end of the nucleocapsid. This protein belongs to the lambda integrase family and is involved in the production of nucleocapsids (Vanarsdall et al., 2006).

Other core gene products are GP41 (tegument protein), 38K, P49 and ODV-EC27 (Table 2). They seem to be associated with the nucleocapsid, and consequently found in both BVs and ODVs.

	AcMNPV ORF number	References		AcMNPV ORF number	References
Replication			**Cell cycle arrest and/or interaction with host**		
lef-1	14	(Evans et al., 1997)	*odv-e27*	144	(Belyavskyi et al., 1998)
lef-2	6	(Evans et al., 1997)	*ac81*	81	(Chen et al., 2007)
DNApol	65	(McDougal and Guarino, 1999)			
Helicase	95	(McDougal and Guarino, 2000)			
Transcription			**Packaging, assembly, and release**		
lef-4	90	(Jin et al., 1998)	*p6.9*	100	(Wang et al., 2010a)
lef-8	50	(Titterington et al., 2003)	*vp39*	89	(Thiem and Miller, 1989)
lef-9	62	(Iorio et al., 1998)	*vlf-1*	77	(Vanarsdall et al., 2006)
p47	40	(Guarino et al., 1998)	*alk-exo*	133	(Mikhailov et al., 2003)
lef-5	99	(Guarino et al., 2002)	*vp1054*	54	(Olszewski and Miller, 1997a)
Oral infectivity			*vp91*	83	(Russell and Rohrmann, 1997)
			gp41	80	(Olszewski and Miller, 1997b)
p74	138	(Faulkner et al., 1997)	*38k*	98	(Wu et al., 2006)
pif-1	119	(Kikhno et al., 2002)	*p33*	92	(Wu and Passarelli, 2010)
pif-2	22	(Pijlman et al., 2003)	*odv-ec43*	109	(Fang et al., 2003)
pif-3	115	(Ohkawa et al., 2005)	*p49*	142	(McCarthy et al., 2008)
pif-4	96	(Fang et al., 2009)	*odv-nc42*	68	(Li et al., 2008)
pif-5/odv-e56	148	(Sparks et al., 2011)	*odv-e18*	143	(McCarthy and Theilmann, 2008)
			desmoplakin	66	(Ke et al., 2008)

Table 2. Baculovirus core genes

Proteins included in ODV and BV structures of some baculoviruses have been identified by high throughput techniques based on mass spectrometry. Those are the cases of the ODVs of AcMNPV (*Alphabaculovirus*) (Braunagel et al., 2003), CuniNPV (*Deltabaculovirus*) (Perera et al., 2007), HearNPV (*Alphabaculovirus*) (Deng et al., 2007) and PrGV (*Betabaculovirus*) (Wang et al., 2011), and the BVs of AcMNPV (Wang et al., 2010b). These studies demonstrated that baculovirus virions are complex: in addition to *ca.*, 40 virally encoded proteins host proteins may be present as well.

5.3 *Per os* infectivity factors

Per os infectivity factors (PIFs) are baculovirus proteins essential for oral infection of insect hosts but not relevant in cell culture propagation. Six proteins have been described to play this role and are encoded by 6 core genes *p74*, *pif-1*, *pif-2*, *pif-3*, *pif-4* and *pif-5* (*odv-e56*). PIF-1, PIF-2 and PIF-3 form a stable complex on the surface of AcMNPV ODV in association with P74. It was proposed that these four proteins form an evolutionarily conserved complex on ODV surface that may play an essential role in the initial stage of infection (Peng et al., 2010). PIF-4 was found to be essential for oral infection of AcMNPV in *Trichoplusia ni* larvae (Fang et al., 2009). In recent studies ODV-E56 was demonstrated to be a *PIF* (PIF-5) in AcMNPV (Sparks et al., 2011) and BmNPV (Xiang et al., 2011).

6. Auxiliary genes

Baculovirus whole gene content is wide and diverse. As already noted, there is a group of 31 core genes that are present in all the baculoviruses sequenced to date. However, each particular baculovirus species codes for many more than 31 genes. A recent study determined the whole gene content based on the information of 57 baculovirus genomes and came up to a sum of 895 different ORFs (Miele et al., 2011). This means that there may be genes that are not essential but capable of modulating the infection of viruses with a particular gene subset. Moreover, some genes might have evolved a particular function and play a role only in the context of species-specific virus-host interactions. Those genes are commonly categorized as auxiliary genes. Other genes that could be included in this group, may participate in processes other than replication and transcription or may code for structural genes essential for a particular virus to succeed in the infection of a specific host.

This section focuses on some of the auxiliary genes that are widely distributed in the family and/or their function has been described.

6.1 Genes affecting cellular metabolism

To succeed in infection a virus needs to circumvent host cell apoptosis. It is well known that apoptosis is one of the mechanisms an organism uses to clear an infection: a cell detected as being infected is set to die. All baculoviruses encode anti-apoptotic genes to counteract this cell response in order to complete their replicative cycle. There are two types of antiapoptotic genes in baculoviruses: P35/P49 homologs and IAPs. P35/P49 function directly inhibiting the effector action of caspases and they have been found in some NPVs and one GV (Escasa et al., 2006). IAPs are metalloproteinases that act upstream P35 in the apoptotic pathway (van Oers and Vlak, 2007).

A gene coding for a superoxide dismutase (*sod*) is widely distributed among baculoviruses. Its function seems to be the removal of free radicals in infected hemocytes, which are superoxide producers (Rohrmann, 2011a).

Most lepidopteran baculoviruses encode a viral ubiquitin. It was suggested that baculoviruses carry this gene in order to inhibit steps in the host degradative pathways in a strategy to stabilize viral proteins that otherwise would be short-lived (Haas et al., 1996).

Most of the baculovirus genes are present in a single copy in the genome. But there is the special case of *bro* (*Baculovirus repeated orf*) genes that are a multigene family present in several baculoviruses. They appear in different number of copies: from 0 to 16, in the LdMNPV genome. Most *bro* genes share a core sequence but show different degrees of similarity in other regions (Kuzio et al., 1999). Although *bro* genes are similar among them, they have no homology with other known proteins, making it difficult to predict their function. A study of BmNPV *bro* genes showed that these proteins have DNA binding activity, preferentially to single stranded DNA, and two of them were speculated to function as DNA binding proteins that influence host DNA replication and/or transcription (Zemskov et al., 2000).

6.2 Genes affecting the insect host as an organism

Baculoviruses that infect lepidoptera are characterized by the systemic infection of the host rather than being restricted to the midgut epithelial cells. It was proposed that, in order to spread from this primary site of infection, they use the insect tracheal system. Fibroblast growth factor (FGF) involvement in the attraction and motility of tracheal cells has been well studied in *Drosophila melanoganster* (Sutherland et al., 1996). Alpha and Betabaculoviruses carry viral *fgf* homologs (*v-fgf*) in their genomes. Conversely, this gene is absent in Gamma and Deltabaculoviruses which cause midgut-restricted infections. Interestingly, it was found that the presence of *v-fgf* accelerates larval death as knockouts of these genes in AcMNPV and BmNPV caused a retardation in host death compared to infection with wild type viruses (Passarelli, 2011).

Several lepidopteran baculoviruses code for a protein designated GP37, which is homologous to fusolin, encoded by entomopoxviruses. Fusolin, as well as GP37, is a glycoprotein that contains chitin binding domains. Fusolin was demonstrated to form spindle-like bodies that enhance the entomopoxvirus oral infection in host larvae. The mechanism of action for these spindles appears to be associated with the disruption of the peritrophic membrane (PM) allowing the virions to reach the midgut epithelial cells (Mitsuhashi et al., 2007). Except for the case of CfDEFMNPV (Li et al., 2000), in baculoviruses no spindle bodies have been observed, although GP37 was found to be associated with OBs in AcMNPV (Vialard et al., 1990). On the other hand, in OpMNPV and MbMNPV this protein was found in cytoplasmic inclusion bodies that accumulate late in infection (Gross et al., 1993; Phanis et al., 1999). In SpliMNPV infected cells GP37 was found to localize in the cytoplasm and the nucleus as well as in the envelopes of BVs and ODVs. Its chitin binding capacity was demonstrated suggesting that it may bind to the chitin component of the PM (Li et al., 2003).

Another strategy for the baculoviruses success is the delay of larval molting. Ecdysteroid-UDP-glycosytransferase (EGT) mediates the inactivation of molting hormones (ecdysone) in insects. The *egt* gene is present in most baculovirus genomes. The virus benefits from the

presence of this gene product that prevents the infected larva from molting as it keeps feeding, thus allowing higher virus progeny yields (O'Reilly and Miller, 1989).

In the final stage of infection, after insect death, the larva liquefies releasing baculovirus OBs to the environment. This liquefaction is mediated by the two viral-encoded enzymes: cathepsin and chitinase. Cathepsin is a protease that acts together with chitinase disrupting the insect exoskeleton and promoting the release and spread of progeny virus (Hawtin et al., 1997).

7. Host range

One of the characteristic features of baculoviruses is their narrow host range. Due to their exquisite specificity, most baculoviruses can be regarded as "magic bullets" targeting a single host organism and, therefore, are excellent candidates for biological pest control. From the environmental point of view baculoviruses are safe alternatives for pest control as their host range is generally restricted to one insect species, not affecting other organisms. But from the economical point of view the narrow host range represents a disadvantage when more than one pest is to be controlled simultaneously in the same field.

On the other hand, as a consequence of their narrow host range, baculoviruses are innocuous to vertebrates. Moreover, as they are able to enter mammalian cells, they have been widely studied as viral vectors for gene therapy (Hu, 2006). For these reasons, baculoviral genes affecting host range and the interaction with the host are an important object of study.

One of the first studies in this field was conducted on two closely related viruses, *i.e.* AcMNPV and BmNPV. Despite the high similarity of these viruses their host specificities do not overlap. AcMNPV infects Sf-9 cells (derived from *Spodoptera frugiperda*) but not BmN cells (derived from *Bombyx mori*). Conversely, BmNPV does infect BmN cells but not Sf-9 cells. In coinfection assays a recombinant BmNPV was obtained that could replicate in both cell lines. The characterization of this virus revealed that its altered host range was due to a recombinant sequence in the helicase gene (Maeda et al., 1993), being a single amino acid change enough for this phenotypic change (Kamita and Maeda, 1997).

Another example of host range expansion due to a single gene was the case of AcMNPV modified by the insertion of a LdMNPV gene, the *host range factor 1* (*hrf-1*). This modification allowed AcMNPV to replicate in *Lymantria dispar* cells and larvae (Thiem et al., 1996). HRF-1 is present in the genome of LdMNPV and OpMNPV, both of which are able to replicate in Ld652Y cells, derived from *L. dispar*. Moreover, other NPVs modified by the incorporation of this factor were found to replicate in these cells, that are non-permissive for the corresponding wild type viruses. It was suggested that this factor is important in the progression of the infection after DNA replication and that the global protein synthesis shutoff is the major factor that restricts NPV replication in Ld652Y cells, being HRF-1 a crucial viral factor that counteracts this antiviral mechanism active in NPV-infected Ld652Y cells (Ishikawa et al., 2004).

Other genes that play a role in baculovirus host range have been detected and studied: *host cell factor 1* (*hcf-1*), *p35*, *iap* and *lef-7* (Miller and Lu, 1997; Thiem, 1997).

8. Homologous regions and replication origins

Homologous regions (*hrs*) are repeated sequences present in baculovirus genomes that vary widely in terms of length, sequence and copy number between species (Berretta and Romanowski, 2008). They occur also in other viruses of invertebrates that appear to be phylogenetically related to baculoviruses (van Oers and Vlak, 2007). In general, each repeat consists of an imperfect palindrome and a number of repeats with similar sequences are distributed in the genome as singletons or arranged in tandem with variable number of copies. *Hrs* have been found in genomes of the four genera of the current baculovirus classification, including all non-lepidopteran species with fully sequenced genomes. However, *hrs* could not be found in the genomes of some species such as TnSNPV (Willis et al., 2005), ChchNPV (van Oers et al., 2005), and AgseGV (Hilton and Winstanley, 2008b). *Hrs* are A-T rich compared to the overall genome nucleotide composition. They represent part of the non-coding regions that account for less than 10% of baculovirus genomes, although in some GVs, they overlap predicted genes likely to be transcribed (Hilton and Winstanley, 2008). AcMNPV has nine *hrs* that contain a total of 38 repeats with a copy number ranging from one to eight (Ayres et al., 1994). Each repeat consists of a 28 bp-long imperfect palindrome that diverges slightly from a consensus sequence. Similar to *hrs* found in other NPVs, AcMNPV *hrs* displaying several palindromic repeats, have a modular organization in which each palindrome is embedded within a direct or inverted repeat in tandem. Frequently, *hr* palindromes are bisected by a restriction enzyme site (eg EcoRI in AcMNPV hrs). In GVs the majority of *hrs* are less structured, although imperfect palindromes may be as long as *ca.* 300 bp. Repeats are poorly conserved except for 13 bp at their ends (Hilton and Winstanley, 2008b). Regarding to their function, *hrs* act as enhancers of transcription of early genes in those NPVs in which they were studied and there are indirect evidences that they serve as origins of replication in NPVs and GVs. Non-homologous sequences within many NPV *hrs* have motifs known to bind cellular transcription factors of the bZIP family (Landais et al., 2006) but the enhancing activity of *hrs* depends primarily on viral factor IE-1 binding to palindromic repeats. In AcMNPV, IE-1 binds to the 28-mer element as a dimer and this interaction stimulates transcription of *cis*-linked promoters that are responsive to the RNA pol II activity in transient assays (Rodems and Friesen, 1995).

The first evidence that *hrs* are putative origins of DNA replication was the accumulation of *hrs* in defective genomes obtained by serial passages of AcMNPV in cultured cells (Kool et al., 1993). These viral particles have genomes smaller than the wild type virus, which means a replicative advantage for those retaining *ori* sequences in the molecule. In infection-dependent replication assays, performed in different virus/permissive cell line systems, *hrs* were found to confer plasmids the ability to replicate (Broer et al., 1998; Hilton and Winstanley, 2008b; Pearson et al., 1992). It was observed that viruses promote replication of *hr*-containing plasmids only when the *hr* comes from the same or a closely related viral species. The stringency of this specificity is higher than that observed associated with the function of *hrs* as enhancers (Berretta and Passarelli, 2006). This may come as a result of more viral factors involved in the replication process and possible interactions thereof. IE-1 binding to *hr* sequences is also thought to play a role in replication possibly by recruiting the components of the replication machinery (Nagamine et al., 2006). Since the replication mechanism of baculoviruses is not well understood, the function of *hrs* as origins during the infective cycle remains to be confirmed. Deletion of up to two *hrs* from AcMNPV did not impair replication of the virus (Carstens and Wu, 2007). Moreover, there are other *non-hr*

sequences that function as *oris* in transient assays, including promoters of early genes (Kool et al., 1993; Wu and Carstens, 1996). *Hrs* may produce cruciform structures in the DNA, although *in vitro* studies were unable to detect such forms in AcMNPV imperfect palindromes. This kind of branched structures are likely to form if baculovirus replication involves recombination events. VLF-1 protein was found to bind cruciform DNA as well as certain *hr* sequences; this capacity is consistent with its requirement during the DNA packaging process (Rohrmann, 2011c). Whether *hrs* participate in the final stages of genome processing is not known. Consistent with this possibility it has been suggested that hrs constitute factors of genome plasticity as mediators of intra- and inter-molecular recombination events during baculovirus evolution (van Oers and Vlak, 2007).

9. Baculoviral microRNAs

MicroRNAs (miRNAs) are small non coding RNAs that play a role in the regulation of the expression of genes in a wide variety of cellular processes. Typically they are molecules of about 22 nucleotides obtained by the processing of a longer primary RNA (pri-miRNA). In most cases this pri-miRNA is transcribed by the RNA polymerase II, and contains a ~80 nt hairpin that is recognized by the RNaseIII-like enzyme Drosha that removes it from the pri-miRNA to give the pre-miRNA. Pre-miRNA is exported to the cytosol. Once there, it is processed by Dicer which cleaves the terminal loop of the hairpin. One strand of the remaining dsRNA is incorporated by the RISC complex in order to target a specific mRNA and inhibit its translation. Recently it was found that viruses also encode miRNAs. These are from DNA virus families and were first discovered in herpesvirus (Grundhoff and Sullivan, 2011).

More recently, microRNAs were discovered in baculovirus; Singh et al., (2010) demonstrated that BmNPV encodes four miRNAs by sequencing small RNAs followed by *in silico* analysis and validation using other techniques. Micro RNAs were searched in two different tissues of infected larvae. As the genome of *Bombyx mori* is available it was possible to discriminate the miRNAs encoded by the virus from those encoded by the host. Other related baculoviruses were searched to see if these miRNAs were conserved. All four miRNAs were found to be present with 100% identity in AcMNPV, BomaNPV and PlxyMNPV. Three miRNAs were conserved in RoMNPV and one in MaviNPV. This conservation is strongly suggesting that the miRNAs play some kind of crucial role in the viral cycle. Regarding their targeting, the *in silico* analysis revealed that these miRNAs have more than one target that could be either viral or host-cell in origin. Primarily, miRNAs bind to 3'UTR of target mRNA, but there are recent reports of miRNAs binding to 5'UTR or the coding sequence triggering the translation repression, as well. Two of the predicted viral targets of BmNPV miRNAs are *dna binding protein* and *chitinase* mRNAs targeted by two different miRNAs (*bmnpv-miR-3* and *bmnpv-miR-2*). Interestingly, they were found to bind to the complementary region from which they were transcribed. Other viral targets are *bro-I*, *bro-III*, *lef-8*, *fusolin*, *DNA polymerase*, *p25* and *ORF 3* of BmNPV. Another interesting finding was that the computationally identified cellular targets such as prophenoloxidase and hemolin are related to different antiviral host defense mechanisms. Other important cellular targets were GTP binding nuclear protein Ran, DEAD box polypeptides and eukaryotic translation initiation factors that play an important role in small RNA-mediated gene regulation. It was proposed that these viral miRNAs are important for regulating cellular activities in order to easily establish infection in the host (Singh et al., 2010).

10. Baculovirus phylogeny

Before the advent of rapid automatic sequencing methods, when only a restricted number of complete genomes sequences was available, baculovirus phylogeny studies were performed using single homologous genes. Initially, the preferred gene product was polyhedrin/granulin, the major occlusion body protein. It is highly expressed; therefore, easily purified and its N-terminal region could be sequenced. Also, as it is a conserved protein it was easy to identify in new baculovirus isolations (Herniou and Jehle, 2007). These first phylogenetic studies revealed that baculoviruses were divided in 4 different groups (Rohrmann, 1986): (i) dipteran-specific baculovirus with OB protein unrelated to Polh/Gran; (ii) hymenopteran-specific baculovirus with OB protein being poorly related to Polh/Gran; (iii) lepidopteran nucleopolyhedroviruses and (iv) granuloviruses. The analyses of Polh/Gran also revealed a subgrouping of lepidopteran NPVs in groups I and II (Zanotto et al., 1993). Interestingly, this separation was correlated with the different utilization of fusogenic protein of the BV, GP64 or F, respectively (Lung et al., 2002).

The use of single genes to infer phylogeny must follow, at least, two criteria: the gene must be present in all members of the virus family and its level of conservation must reflect evolutionary distance (Herniou and Jehle, 2007). The studies using genes such as *lef-8* and *pif-2* supported the grouping mentioned above (Herniou et al., 2004). When several complete genomes became available better phylogenetic analyses could be undertaken based on the sequence of all genes that were present all the genomes (Herniou et al., 2001). One approach consists in concatenating the amino acid sequences of all these gene products to perform the analysis. This approach is convenient because each gene contributes to the overall phylogenetic signal and a synergistic effect is produced by the combination of all the signals (Herniou et al., 2003). The first report comprising whole-genome data was based on nine complete genomes available at that moment, which only represented lepidopteran baculoviruses and 63 common genes were detected and employed in the analysis (Herniou et al., 2001). When more genomes became available, especially those from non-lepidopteran baculoviruses, a group of about 30 genes were found to be present in all the baculoviruses, allowing to perform more significant phylogenetic studies. One important consequence of this increasing amount of sequence data was the proposal of a new classification of the *Baculoviridae*, based on 29 core genes among 29 baculovirus genomes, including the dipteran and hymenopteran ones (Jehle et al., 2006a). A recent report utilized 57 baculovirus genomes of which a group of 31 core genes was determined and used to perform an up-to-date phylogeny (Miele et al., 2011). In this report the cladogram obtained reproduced the current baculovirus classification. Also it was consistently reproduced the separation of Alphabaculoviruses in groups I and II as well as the subdivision of group I in clades Ia and Ib previously reported (Herniou and Jehle, 2007; Jehle et al., 2006b). The Betabaculovirus genus clade also reveals a subdivision in two groups (Miele et al., 2011).

11. Transposable elements

Transposons have been found in almost all eukaryotic organisms, being a central component in many genomes (Wicker et al., 2007). These sequences, also known as transposable elements (TEs), are characterized by the ability to move and replicate through various mechanisms according to their genetic nature. In view of this, TEs are not innocuous for genomes, because their activity may affect the genetic endowment of a species. Their

prominent role in biological evolution has been thoroughly reviewed and the conclusion is that TEs provide plasticity to the genomes and are an important source of variability.

In Eukarya, TEs show a great diversity in gene content, size and mechanism of transposition. According to shared characteristics, these sequences are classified into two main groups: Class I (retrotransposons) and Class II (DNA transposons). A crucial difference between them resides in the existence of an RNA intermediate in the Class I TEs. Other properties are also used to subdivide into subclasses, including the size of the target site duplication, the occurrence and gene content (Wicker et al., 2007). Retrotransposons can be grouped into two subclasses: the LTR retrotransposons and the non-LTR retrotransposons or retroposons (Capy, 2005). This is mainly based on the presence/absence of LTRs (Long Terminal Repeats), but other features are also considered. In all cases, reverse transcription processes are involved. On the other hand, Class II transposons or DNA TEs are mobilized in the genomes using a single or double-stranded DNA intermediate. These sequences can be divided into three major subclasses: those that excise as dsDNA and reinsert elsewhere in the genome ("cut-and-paste" transposons); (ii) those that utilize a mechanism related to rolling-circle replication (helitrons), and Mavericks, whose mechanism of transposition is not yet well understood, but that likely replicate using a self-encoded DNA polymerase (Feschotte and Pritham, 2007).

Because of their biological activity, TEs behave like selfish sequences that impact on genomic architecture. However, it has also been reported that some TEs participate in other biological functions such as transcription, translation and DNA replication, localization and movement (Ponicsan et al., 2010; von Sternberg and Shapirob, 2005). In any case, TEs can be mobilized within a genome or between genomes. It is at this latter point where viruses take a leading role, because they can be recipients of TEs and transport them to other individuals in subsequent infections. In particular, the genomes of baculoviruses can be the targets for the isertion of different insect TEs when they replicate in the host cells. Taking into account that one of the main sources of genome variability in viruses with large dsDNA genomes are structural mutations, the possible sequence rearrangements produced by transposition processes (gene interruption, deletions, inversions, translocations, etc.) could actively participate in their evolution (Herniou et al., 2001).

TEs have also been exploided for genetic modification in the laboratory. One of the transposons most widely used in biotechnology is probably piggyBac, an insect DNA TE. This sequence was identified in AcMNPV propagated in TN-368 cell line (Fraser et al., 1985). The trans-mobilization between host chromosome and virus genome was discovered because the transposition occurred into 25k gene, producing a distinct "few polyhedra" phenotype. Later, other reports acknowledged the presence of TEs in baculoviral genomes, including the description of TED -a retrotransposon in AcMNPV- and TC14.7 -a DNA TE in CpGV (Friesen and Nissen, 1990; Jehle et al., 1995). Two additional DNA TEs have been described, one from CpGV designated TCp3.2 (Jehle et al, 1997), and the other, a new piggyBac-related transposon isolated from AgMNPV and designated IDT for *iap* disruptor transposon (Carpes et al., 2009). Considering these evidences, gene transfer processes could be more common than initially realized.

TEs may play an important role in baculovirus biology and evolution. They can provide mechanisms for horizontal transfer of genes between virus species replicating in the same

host cell, between the host genome and the viral genome, or between this and the genome of other entities such as pathogenic bacteria. The high similarity between baculovirus and insect sequences (*egt* and *sod* genes), or between baculovirus and other pathogens (v-*chitinase* gene) could be the consequence of transposition events that were selected during evolution of baculoviruses.
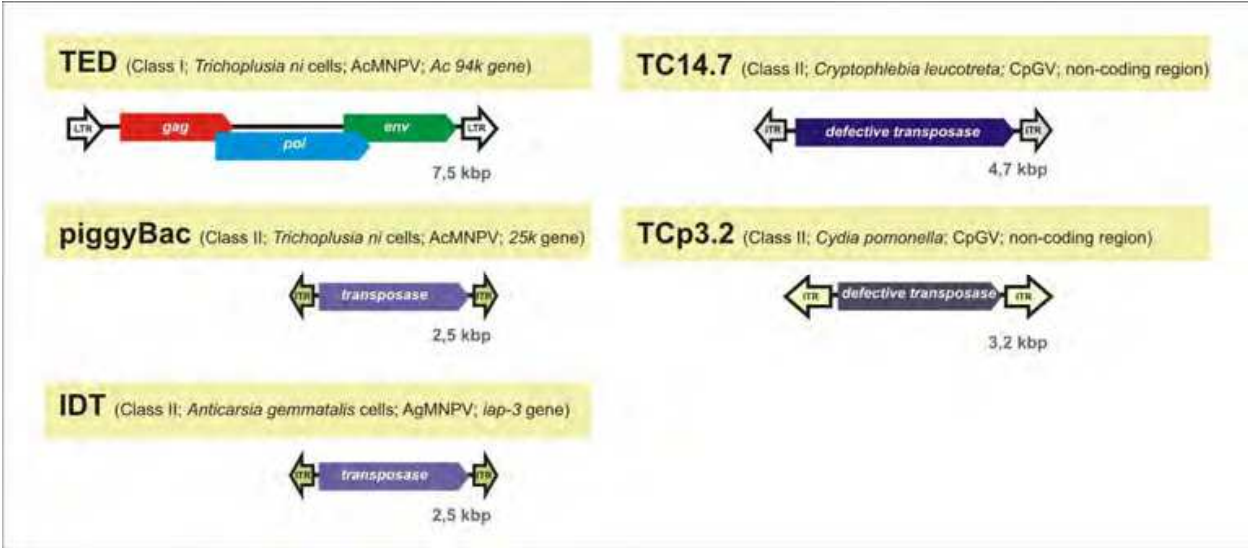

Fig. 3. **Types of transposable elements** (TE) found and described in baculovirus genomes. ORFs contained in each transposon are shown as coloured block arrows indicating their predicted function. The size of the TE is indicated in kilobasepairs; class of transposon, the species of donor insect genome, baculovirus species, viral gene sequence interrupted by the TE are indicated in brackets. Signature sequences for different TEs are indicated by arrows: LTR (Long Terminal Repeats) and ITR (Inverted Terminal Repeats).

12. Concluding remarks

Baculoviruses are a family of insect specific viruses with quite diverse and interesting applications. Therefore the knowledge of their gene content and molecular biology is a matter of growing interest. For instance, discovery and characterization of genes implicated in host range are subject of investigations for improvement of their application as designer biopesticides. Another focus of studies is interaction of baculoviruses with non target cells (*e.g.* mammalian cells) to assess the biosafety of using them for efficient gene transduction in therapeutic applications. As more baculovirus full genome sequences become available (especilly dipteran and hymenopteran-specific viruses), the bioinformatic analysis and experimental validation will help to establish a better defined set of genes characteristic of the family and those that are involved with the host specificity. In addition a more robust and detailed evolutionary tree will be probably assembled.

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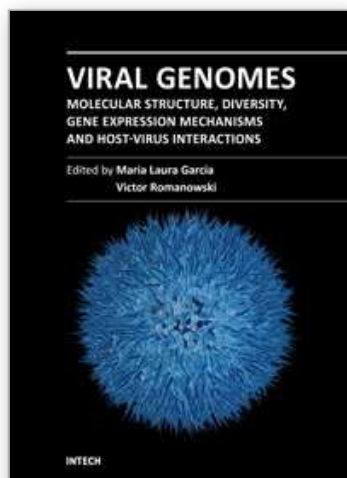
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Viral Genomes - Molecular Structure, Diversity, Gene Expression Mechanisms and Host-Virus Interactions
Edited by Prof. Maria Garcia

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