Section A-Research paper

BIOLOGY OF BACULOVIRUSES

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Abstract: The Baculoviridae family is a group of large, double-stranded DNA viruses that infect insects. Baculoviruses have been extensively studied as biological control agents against insect pests in agriculture, forestry, and urban settings. They have several advantages over chemical insecticides, including specificity, safety, and environmental friendliness. Baculoviruses are also amenable to genetic manipulation, which has led to the development of recombinant baculoviruses for use as vaccines, gene delivery vectors, and bioinsecticides. The paper reviews the biology of insect-pathogenic viruses, specifically members of the Baculoviridae family. It describes the life cycle of the virus and the temporary pattern of gene expression. Additionally, the paper provides information on the deletion of the egt gene, which is traditionally employed to create bioinsecticides based on baculoviruses.

***Keywords*:** Nucleopolyhedrovirus, Granulovirus, Bombyx mori, Spodoptera frugiperda, Autographa californica, UDP-glucosyltransferase.

Background

The Baculoviridae family includes the only genus Baculovirus, which is divided into two subgroups: subgroup **A** - Nuclear Polyhedrosis Viruses - *Nucleopolyhedrovirus* (NPV) and subgroup **B** - Granulosis Viruses - *Granulovirus* (GV). Subgroup A is further subdivided into two groups: group I and group II. There are over 600 described species of baculoviruses [1], most of which infect the larval stage of arthropods, primarily in the orders *Lepidoptera, Hymenoptera,* and *Diptera* [2].

While most viruses are being investigated for their potential to cause disease in humans or damage the food production system, the study of baculoviruses has been stimulated by their potential to control insect pests and produce large amounts of recombinant proteins in insect cell culture or in the insect itself. The best-studied baculoviruses are NPVs, particularly *Autographa californica* MNPV (AcMNPV, family *Baculoviridae*) and *Bombyx mori* NPV (BmNPV). Despite their close relationship, BmNPV and AcMNPV have significant differences in their infectivity for various insects. For example, although AcMNPV replicates in *Spodoptera frugiperda* 21 (Sf21) cells, it does not infect *B. mori* N (BmN) cells [3;4]. In contrast, BmNPV is highly infectious in BmN cells, but does not replicate in other insect cell lines [3], or it replicates at very low levels [5].

Main Text

The use of baculoviruses as vectors for the expression of foreign genes opens up more and more prospects. Baculoviruses such as AcMNPV (Autographa californica multiple nucleopolyhedrovirus) and BmNPV (Bombyx mori nucleopolyhedrovirus or silkworm nuclear polyhedrosis virus) are widely used as foreign gene expression vectors. Today, this technology has been used to express a large number of genes encoding proteins for medicine and agriculture [1-5].

This expression system is one of the most advanced among other expression systems (bacterial, yeast, mammalian) due to several advantages such as:

• The presence of the correct post-translational modifications of the protein, contributes to the production of a protein that is extremely close in its structure to the analog synthesized in human cells.

• High level of expression of recombinant proteins.

• Baculoviruses are not pathogenic for humans.

• Recombinant proteins can be obtained using cell culture (which is extremely expensive) and insect larvae.

Baculoviruses are insect viruses with a rather complex genome organization for viruses. The genome of baculoviruses is quite large, about 130 kb (thousand base pairs), and encodes many proteins required for the virus. Some of them are involved in virus replication, others are structural proteins, others are responsible for infectivity, and another group of proteins is auxiliary proteins that indirectly contribute to the development of infection.

The presence of such a large genome does not allow the use of conventional methods of genetic engineering. When constructing recombinant baculoviruses encoding foreign proteins, so-called transfer vectors are used, with the help of which a gene that is not essential for virus replication is replaced by the target gene in insect cells. As a result, a recombinant baculovirus is formed, in which the viral gene is replaced by a foreign one. The expression level of a foreign gene obtained using a recombinant virus varies greatly depending on which foreign gene is expressed. The maximum level of foreign gene expression reached was 200 mg/l of cells, which is an excellent result for the expression system of higher eukaryotes.

Even though baculoviruses have been studied as a promising producer of biologically active proteins for the needs of medicine since the late 80s of the last century, the method of creating a recombinant baculovirus by replacing the baculovirus polyhedrin gene with the target gene is still used. Historically, this has developed since the polyhedrin gene was the first studied baculovirus gene. This protein is not essential for baculovirus replication, and its deletion does not affect virus development.

Life cycle of baculoviruses

Members of the Baculoviridae family possess rod-shaped virions that contain circular, covalently linked DNA [6]. Group A baculoviruses (NPV) have a unique form of the virus known as polyhedra. Polyhedra are a protein matrix in which virions are embedded. Baculoviruses use polyhedra to spread infections from one insect to another.

The infectious cycle of baculoviruses occurs exclusively in the larval stage of the host insect and begins with the larva ingesting the polyhedron along with its food. The alkaline environment of the larva's intestine dissolves the polyhedrin matrix, releasing the virions located inside the polyhedra - ODV (occlusion-derived virions) - into the intestinal lumen. These virions penetrate the peritrophic membrane and use the p74 receptor [7], which is located on the surface of ODV, to specifically attach to the microvilli membrane of intestinal epithelial cells. The nucleocapsids (NC) are then transported to the cell nucleus, initiating the first replication cycle. Inside the infected cell, four generations of viral mRNA are synthesized, encoding proteins that act as activators for the next cycle of synthesis [8]. In the late stage of infection, newly formed nucleocapsids are transported to the cell membrane, where they leave the cell by budding to form extracellular virions - BV. BV then spreads the infection to neighboring cells, as well as to other tissues and organs of the insect through the hemocoel. At a very late stage, the NCs are surrounded by a de novo formed sheath, forming the ODV. The ODVs are embedded in the polyhedrin matrix of the polyhedron, which is mainly composed of the polyhedrin protein produced in large quantities at a very late stage of infection. By the end of the infection, the polyhedra are released when the cell is destroyed.

The life cycle of baculoviruses is quite complex and biphasic, as evident from the information presented above. A characteristic feature of their replication cycle is the formation of two phylogenetically distinct forms of the virus (Fig. 1), which differ in the origin and composition of their membranes [9; 10]. The BV membrane is a modified host cell membrane acquired by the virion through budding. On the other hand, ODVs are formed in the intranuclear annular zone of the nucleus

[11; 12], where they acquire their own membrane. The formed ODVs are surrounded by a paracrystalline matrix consisting of hydrocarbons and specific proteins [13] called polyhedra.

BVs enter cells by endocytosis using a virally encoded glycoprotein, gp64 (Fig. 1), which is unique to BVs [14]. The interaction of BV with the host cell occurs when gp64 is activated by an acidic environment [15; 16]. In contrast, ODV loses the gp64 receptor on its surface and attaches to the cell via the p74 protein [17; 7].

Polyhedra are responsible for spreading the virus from insect to insect and initiating infection in the intestine of the insect (i.e., horizontal transfer). Inside the insect, the virus spreads from cell to cell via BVs.

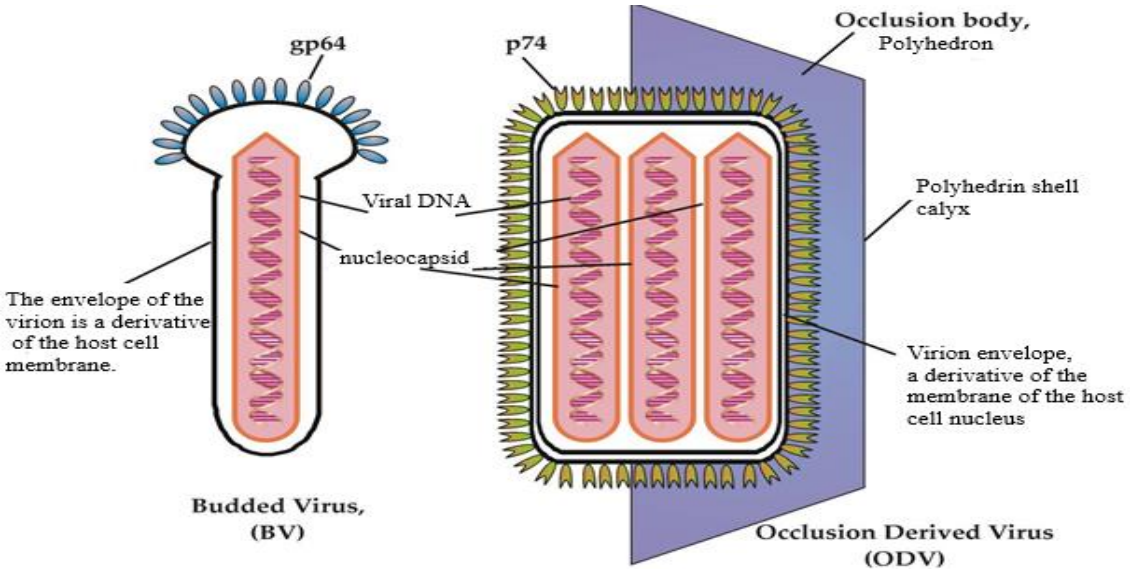

Fig. 1. The structure of the extracellular virion (BV) and the virion located in the polyhedron (ODV)

Expression structure of baculovirus genes

The replication of baculoviruses and expression of their genes occur in a cascade-like manner, where the activation of each gene depends on previously synthesized proteins (Fig. 2) [18]. Based on this timing regulation, baculovirus genes can be grouped into three classes: "early", "late", and "very late". Some of the baculovirus genes are transcribed in more than one temporal phase. "Early" genes are transcribed before the start of viral DNA replication and are divided into "immediate early" (**IE**) and "delayed early" (**DE**). "Late" and "very late" genes are activated during or after DNA replication. Some "immediate early" genes do not require the presence of viral gene products for their transcription, but use the host cell's RNA polymerase II complex. Most "early" genes require transactivating factors for full expression.

Late genes are most active between 12 and 24 hours after infection. "Very late" genes are overexpressed following the activation of "late" genes and remain active after the expression of "late" genes decreases. "Early" genes, in most cases, encode proteins that regulate functions such as transcription, replication, and modification of proteins that affect host cell processes. "Late" proteins include proteins involved in the regulation of the expression of "late" and "very late" genes, as well as viral structural proteins. "Very late" proteins are involved in the processes associated with the formation of polyhedra and cell lysis.

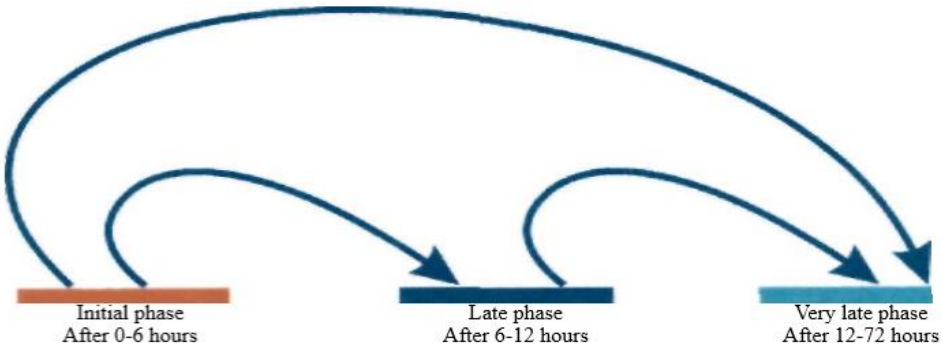

Fig. 2. Temporal regulation of baculovirus gene expression

Time limits are based on AcMNPV infection. BmNPV infections are slower. Baculovirus replication and gene expression can be subdivided into early, late and very late time phases.

Early genes are transcribed by the host RNA polymerase II. Most promoters of early genes contain a functional TATA box [19], which is typical for most promoters of higher eukaryotes using RNA polymerase II. For example, the IE1 gene is transcribed by nuclear extracts prepared from uninfected host cells [20], indicating that the host RNA polymerase II is indeed used for transcription of this gene. In addition to the TATA box, most baculovirus "early" promoters contain a conserved CAGT sequence at or near the transcription start site [21; 22; 23]. However, there are exceptions. For example, transcription of DNA pol by both BmNPV and AcMNPV begins with a G/C-rich sequence (**5'-GCGTGCT-3'** and **5'-AGAGCGT-3'**), with no obvious presence of the **TATA** box [24]. Similarly, the AcMNPV lef4 transcript is initiated from a G/C-rich sequence, and the *Ac-iap* transcript is initiated from a **GAGTTGT** sequence. In addition to the main promoter elements, many enhancer elements have been identified in early promoters.

Most **IE** proteins are involved in regulating viral transcription. One important early gene is the immediate early gene 1 (*ie-1*). Its product is involved in transactivating multiple genes during the late phases of baculovirus infection [25; 26]. Deletion of ie-2 from BmNPV reduces viral DNA replication in *BmN* cells by a factor of 2 [27].

The events following the onset of viral DNA replication can be subdivided into "late" (6-18 hpi) and "very late" (18-72 hpi) time phases. "Late" and "very late" transcription depend on the expression of early viral genes and viral DNA replication [28; 29]. Transcription of genes during these phases is carried out by the virus's own RNA polymerase. The "late" and "very late" phases coincide with the production of BV and ODV, respectively [30]. During the late phase, genes encoding structural proteins of the viral nucleocapsid, such as p39 and p6.9, the major capsid protein, and the major core protein, are abundantly expressed. Genes encoding proteins associated with polyhedrin bodies, such as polyhedrin, are transcribed at a very late stage. Another highly expressed very late gene is p10. The product of this gene is involved in the formation of the polyhedrin envelope and in the rupture of the cell membrane [31].

The promoters of late and very late genes differ from most promoters that use RNA polymerase II for transcription. They do not contain DNA elements such as the TATA box. The first determining element for the activity of late and very late promoters is the presence of the A/G/TTAAG pentanucleotide, which is localized at the transcription start site of all known late and very late genes [32; 33]. The promoters of the polyhedrin (*polh*) and p10 genes belong to the class of late promoters. The *polh* promoter is highly conserved compared to promoters of other genes among distantly related baculoviruses [33] and has been found to contain a common sequence spanning the transcriptional initiation site (A/T)(A/G)TAAGNA(T/A/C) (T/A)T.

Nineteen late gene expression factors (*lef*) were identified using a mixture of fragments of the baculovirus genome and identifying those fragments that were able to activate late and very late promoters [34; 35; 36]. These genes are necessary for gene expression under the control of "late" and "very late" promoters and can directly activate "late/very late" transcription or have an indirect effect by enhancing the transcription of early genes or mediating DNA replication (through enhancer activity). Information about the lef functions is summarized in Table 1.

Table 1. Expression factors of late BmNPV genes.

Gene	ORF (bp)	promoter	Functions
lef-2	630	E/L	Replication/Transcription
lef-1	810	E/L	Replication
lef-6	519	–	LEF
39k	831	E/L	LEF, phosphoprotein (ne)
lef-11	336	e	LEF
p47	1197	e/E	DNA binding, LEF
lef-8	2631	–	Transcription
lef-10	417	L	LEF
lef-9	1470	–	Transcription
DNApol	2958	e/E	Replication
lef-3	1155	E	Replication
vlf-1	1137	L	Very late expression
lef-4	1395	E	Transcription
DNAhel	3666	E	Replication, host specificity
lef-5	795	–	LEF
lef-7	636	E	Replication (ne)
p35	897	E/L	Apoptosis blocking (ne)
ie-1	1752	E	Transactivator
ie-2	1266	E/L	Transactivator (ne)

E - early; L - late; e - enhancer near the upstream region; ne - not important for replication. LEF is a late expression factor protein encoded by the lef genes.

Nine of the *lef* s (*ie*-1, *ie*-2, *lef*-1, *lef*-2, *lef*-3, *lef*-7, *p143*, *dnapol*, and *p35*) are involved in *hr2*-based DNA replication, while the products of the other nine genes (*lef*-4, *lef*-5, *lef*-6, *lef*-8, *lef*-9, *lef*-10, *lef*-11, *39k*, and *p47*) influence the steady state levels of reporter gene transcripts. Therefore, they are likely involved in some aspects of transcription, transcript processing, or stability [29]. It has recently been shown that a complex of proteins encoded by the *lef*-4, *lef*-8, *lef*-9, and *p47* genes initiates gene transcription under the polh promoter *in vitro* [37].

Experiments on the deletion of these genes from the viral genome have shown that it is possible to obtain viruses that have a deletion of only four genes from this group (*39k*, *ie-2*, *lef-7*, and *p35*) [27]. Mutants with a deletion of the other 15 genes could not be obtained, likely because they are important for viral replication.

A viral gene, *vlf-1* (*very late gene expression factor-1*), has been identified as required to initiate the transcription of very late genes. Mutations in this gene lead to a significant decrease in the level of *polh* and *p10* mRNA [38]. It is noteworthy that the protein encoded by *vlf*-1 has significant homology with the β phage integrase family, and mutations in any of the conserved amino acid sequences of *vlf*-1 have been found to adversely affect virus reproduction [39].

Baculovirus cysteine proteinase (viral cathepsin, V-CATH) and biological aspects of its action.

One of the proteins produced by baculoviruses is cysteine proteinase or viral cathepsin (V-CATH). V-CATH promotes autolysis or 'liquefaction' of insect tissues after death in baculovirus

infection. Cysteine proteinases have been identified in a variety of baculoviruses and likely play a role in the tissue destruction of infected hosts, thereby facilitating horizontal transmission of the virus [10.8].

The v-cath gene was discovered in 1991 by Kuzio & Faulkner [62, 63] (GenBank Accession M67451) in the baculovirus AcMNPV. For the first time, a proteinase with properties characteristic of papain, a cysteine proteinase, was described in 1992 by Rawlings et al. A pine-like sequence [64] upstream from the gp64 gene of AcMNPV [65] was identified in the AcMNPV genome. Later, this proteinase was described as a baculovirus endopeptidase [7]. The v-cath gene homolog in the BmNPV (Bombyx mori nucleopolyhedrovirus) genome was named BmNPV-CP (cysteine proteinase), which has 93% identity compared to v-cath AcMNPV [8]. Fifteen other v-cath homologs have been described for other baculovirus species [66]. Their amino acid sequences are identical to V-CATH produced by AcMNPV ranging from 96.3% to 38.1%. The names 'v-cath' or 'cathepsin' have been used for most v-cath homologs.

V-CATH is required for the proteolysis of virus-infected hosts by cell lysis [69], which facilitates the easier spread of polyhedra from a dead insect to others. For baculoviruses of several species, deletion of the v-cath gene has been shown to alter viral pathology so that hosts do not liquefy after death [8-10]. It was also found that the release of polyhedra into the hemolymph does not occur upon deletion of v-cath (v-cath') in baculoviruses [12]. In insect cell culture, cells infected with the v-cath deletion virus are not lysed to release the polyhedra.

Baculoviruses such as AcMNPV and BmNPV are widely used as foreign gene expression vectors. One of the main problems of using baculoviruses as vectors for the expression of foreign genes is the significant degradation of the target protein at the late stages of infection, since it is at this time that the active expression of the baculovirus v-cath gene begins, starting from 84 h.p. [8, 12]. The cysteine proteinase V-CATH is capable of degrading not only host cell proteins but also foreign proteins expressed by baculoviruses [11, 12]. In this regard, a recombinant baculovirus not producing V-CATH is of great advantage for the production of recombinant proteins.

Expression of recombinant proteins using recombinant baculoviruses.

Since baculoviruses have a rather large genome, recombinant baculoviruses are usually constructed in two steps. First, the gene of interest is cloned into a transfer vector under the viral promoter, which is flanked by certain baculovirus DNA sequences surrounding a gene that is not essential for viral replication. The vector is then introduced into the cells simultaneously with wild-type viral DNA. The choice of an appropriate transfer vector can often play an important role in successful gene expression [79, 80, 82]. Transfer vectors currently in use contain the polh promoter and long baculovirus flanking sequences surrounding the polyhedrin gene with defined restriction sites for cloning the foreign gene. The classical polyhedrin promoter provides a high level of expression of most genes during the very late stage of infection [76-78]. This is a very strong baculovirus promoter, but it only ensures expression at the end of the baculovirus life cycle.

Many studies describe the use of promoters earlier than the polh promoter for the expression of functionally active foreign proteins [79-83]. For example, under the control of the 'immediate early' iel promoter, two different genes were expressed that encode proteins of the secretory pathways with enzymatic activity exceeding that of these proteins obtained using conventional vectors (with the polh promoter) even after 48 h.p.i. [82]. Also, using the late promoter of the main protein (pb.9), a biologically active extracellular domain of the thyrotropin receptor was obtained [83]. Some proteins require more time to undergo correct post-translational modifications than they have when the gene is expressed under the control of the 'very late' promoter of the polyhedrin gene. The use of other promoters that initiate gene expression at earlier stages of baculovirus infection, in the case of proteins with 'complex' post-translational modifications, can promote the synthesis of a biologically active protein.

There are a series of transfer vectors that replace the polyhedrin gene with cDNA encoding the target protein. In this case, depending on which vector is used, it is possible to insert a foreign cDNA under the polh, IE1, or gp64 promoters. Recombinant baculoviruses constructed using transfer vectors

containing a modified gp64 promoter make it possible to obtain the expression of the target gene before the onset of virus-induced cytopathic effects incompatible with protein processing, in particular glycosylation and secretion. Such transfer vectors contain an enhanced baculovirus gp64 gene promoter for gene expression that begins earlier than 24 hours after infection. The gp64 promoter in these plasmids is a modified wild-type promoter to enhance expression levels during the late stage of infection [84].

The so-called 'tandem' gp64 promoter in these vectors contains both immediate-early and late promoter sequences and can therefore function in both the late and early stages of baculovirus infection. Thus, with the help of such vectors, a recombinant protein can be obtained that is closer in structure to its natural analog than with vectors based on the polh promoter. The modified gp64 promoter, as well as the immediate early iel promoter, functions as a baculovirus-independent transcription signal for the host RNA polymerase [84]. Therefore, plasmids in which the foreign gene is under the control of these promoters can be directly transfected into an insect cell line for use in transient expression assays as well as to generate stably transformed insect cell lines expressing the target protein.

While the polh promoter construct begins to produce detectable amounts of protein only after 24 hours post-infection (HPI), which increases rapidly after 48 HPI [85], it should be noted that the expression levels obtained using these vectors probably also depend on the individual properties of the proteins [76, 86]. However, currently available transfer vectors have one important drawback. All of these (or at least the vast majority of them) result in the deletion of the polyhedrin gene in the resulting recombinant baculovirus. Indeed, polyhedrin is the most convenient selective marker, as recombinant viruses do not form polyhedra. Unfortunately, this entails the impossibility of infecting the larvae per os. Such a method of infection could greatly facilitate the infection of insects to obtain large-scale expression in larvae and consequently reduce the cost of the recombinant protein, since the production of recombinant proteins in culture cells is a rather expensive process. To solve this problem, a transfer vector can be constructed that promotes the deletion of some other gene that is not important for the replication, assembly, and infectivity of the virus.

Construction of recombinant baculoviruses using conventional homologous recombination.

Homologous recombination in cell culture is most commonly used to construct recombinant viruses expressing the target protein. The target gene is transferred into wild-type viral DNA using a specific transfer vector. The recombinant vector is constructed and propagated in *E. coli*. The transfer vector DNA, together with wild-type virus DNA, is then used to co-transfect the insect cell culture. Since the vector contains an extended viral DNA fragment homologous to a region flanking a gene (e.g., polh) in the viral genome, the vector and wild-type viral DNA are recombined such that the viral gene (e.g., polyhedrin gene) is replaced by the target cDNA. At the same time, the promoter that controls the expression of the foreign gene is located in the correct position and is regulated in the same way as in the wild-type virus.

According to published data, homologous recombination occurs at a frequency of 0.1-1% [6]. This is unsatisfactory for the industrial synthesis of recombinant proteins. To facilitate more convenient selection, many biotechnology companies have begun to use reporter genes encoding a convenient marker protein that is present in the transfer vector. For example, Novagen Inc (Madison, Wis, USA) offers transfer vectors of the pBAC series containing the β-glucuronidase gene as a GUS reporter gene. As a result of the transfection of a mixture of such a vector with wild-type viral DNA in the presence of the chromogenic substrate X-gluc, blue plaques are formed, which are quite easy to identify. However, the first significant achievement to increase the yield of recombinants was the use of wild-type linear baculovirus DNA together with a vector for co-transfection of insect cell culture. A unique Bsu36I site was introduced into wild-type viral DNA (within ORF603, located before the polyhedrin gene) and used for its linearization. Due to this, the frequency of recombination increased up to 30% due to the restoration of viral DNA. This is because the replication of the linear form of baculovirus DNA is much less efficient than the circular formof viral DNA. This leads to the prevalence of the recombinant virus over its wild form. Recombination with a transfer vector that

contains viral DNA sequences flanking the cleavage site repairs the viral DNA and returns it to its circular form, which increases the proportion of recombinant viral molecules in the total DNA pool in the cell [87].

Baculovirus expression system Bac-to-Bac.

Another option for generating recombinant baculoviruses is the Bac-to-Bac™ system. This system does not require homologous recombination in insect cells. The entire baculovirus genome replicates in the bacterial artificial chromosome (BAC) [90, 91]. This approach makes it possible to obtain recombinant baculoviruses in a shorter time.

The Bac-to-Bac system has several advantages over methods for producing recombinant baculoviruses in insect tissues by homologous recombination. The recombinant viral DNA is isolated from individual bacterial colonies, not mixed with wild-type viral DNA, eliminating the need for multiple rounds of virus purification with plaques. As a result, the time required to obtain the recombinant virus is reduced from the 4-6 weeks required for the traditional method to 7-10 days.

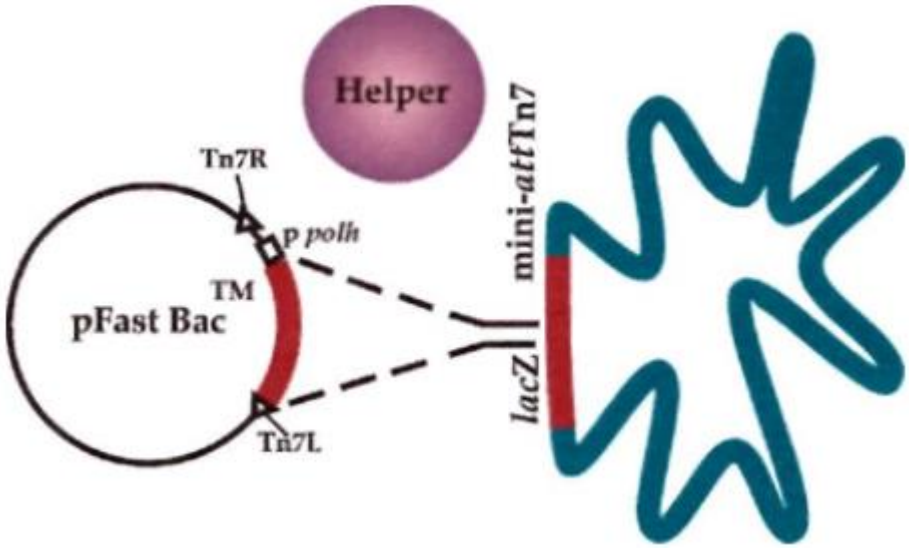

Fig. 3. Diagram of the Bacto-Bac® System.

Figure 3 depicts recombinant baculovirus generation and expression of your gene of interest using the Bac-to-Bac® Baculovirus Expression System.

The essence of the method is as follows: Alien genes are transferred into the BAC-cloned baculoviral genome (bacmids) by site-specific transposition between the transposable elements Tn7L and Tn7R flanking the foreign gene in the donor plasmid transfer vector (pFastBac) and the Tn7 mini-att site of the bacmids, which was previously introduced into the viral genome. The bacmids contain a baculovirus genome, a mini-F replicon that allows for stable replication in E. coli, and a Tn7 mini-att site inserted in lacZa. Transposition requires a helper plasmid containing the tnsA-E transposase genes. To obtain recombinant baculovirus, E. coli DH10B cells carrying bacmid and helper plasmid were transformed with a donor plasmid.

The yield of target recombinants decreased upon competitive transposition into functional chromosomal Tn7 att, which is present in E. coli DH10B. Therefore, it was proposed to use a modified DH10B strain with a blocked chromosomal Tn7 att. With this strain, the yield of recombinants was (1.0-1.5) x 10 per μg of the donor plasmid. When selecting recombinants, various antibiotic resistance genes present in each plasmid, lacZa bacmids, and temperature sensitivity of the donor plasmid (stable at 30°C) were used. The only thing to do after transfection is to select a recombinant bacmid colony,

purify the DNA, and transfect insect cells, which will then produce the recombinant protein. Due to this ease of implementation, the Bac-to-Bac system today dominates the field of obtaining recombinant proteins.

However, this system has already shown itself to be unsuitable for long-term expression of target genes. Recently, one of the most prominent research groups studying baculoviruses published data demonstrating the instability of the Bac-to-Bac system as a baculovirus expression system. Pijlman and others havedemonstrated that a BAC vector containing an expression cassette is spontaneously deleted from the viral genome upon passage of the recombinant virus into insect cells. This cassette included a bacterial mini-F replicon, two antibiotic resistance genes, and two foreign genes (CSFV-E2 and GFP) under the control of p10 and polh baculovirus promoters. Spontaneous baculovirus mutants carrying this deletion quickly became dominant during passage, resulting in a sharp drop in the level of production of recombinant proteins.

4. Gene ecdysteroid UDP-glucosyltransferase

The enzyme UDP-glucosyltransferase, encoded by the egt gene, transfers glucose from UDP-glucose to ecdysteroids, thus changing the conformation of the insect hormone responsible for molting and thereby inhibiting the molting process.

Ecdysteroids are a family of steroid hormones that control molting and pupation. Adding sugar to these hormones inactivates them. It follows that the addition of UDP-sugar to ecdysteroids due to the action of the egt gene leads to the suppression of molting of the host insect. This was first demonstrated by obtaining an AcMNPV mutant deleted at the functional egt gene. Comparison of the penultimate or last instar development of Spodoptera frugiperda larvae infected with wild-type AcMNPV or egt mutant confirmed that egt expression by wild-type AcMNPV inhibits the development of infected insects. Similar results were obtained in various other systems, including Trihoplusia ni and Heliothis virescens infected with AcMNPV and Lymantria dispar infected with LdMNPV [44].

In the experiments described, insects were infected with large doses of the virus at various stages of development. During the research, it was found that the size of the insect, the development of which was suspended, directly depended on the dose of the virus they received. Additionally, the size of a given insect was found to be inversely proportional to the time of infection within its age. In other words, the older the infected insect was, the less likely it was to be inhibited in its development. These data suggest that the developmental process of an infected insect depends on a balance between egt activity and ecdysteroid levels. A low amount of virus inoculum or a late phase of infection age makes it almost impossible to express the megt gene to inactivate host insect ecdysteroids before molting [45].

The analysis of the development of insects infected with egt-deleted AcMNPV revealed that the function of this gene is not to prolong the feeding time of the insect after infection. Normally, uninfected insects stop feeding during molting and before pupation. However, insects infected with AcMNPV encoding an active egt gene (wild-type virus) feed for longer periods of time than they otherwise might. At the same time, insects infected with the recombinant virus, as well as healthy individuals, experience a suspension in feeding, as their development is not impaired. However, these insects die from the viral infection faster than those infected with the native virus. For both *Spodoptera frugiperda* and *Trihoplusia ni,* the 50% survival time (ST^{50}) of insects infected with egt-negative AcMNPV is reduced by 20 to 30% compared to insects infected with the wild-type virus. On the contrary, the dependence parameters of insect mortality on the virus dose change insignificantly. By visual observation, it can be seen that most insects infected with the egt-negative virus die during or immediately after molting. Based on this, it can be assumed that the stress associated with molting is poorly tolerated by infected insects [46]. This property of the egt gene has been utilized in the construction of recombinant baculoviruses intended for the biocontrol of crop pests [47, 48, 49].

Understanding the role of egt in baculovirus replication would be incomplete without understanding the advantage gained by the virus through the production of this gene. The expression of the egt gene provides an undoubted advantage by increasing the yield of viral progeny. Insects that have entered the late phase of pupation can produce up to five times fewer viral offspring than insects

that have lingered in the early stage of the last instar. These data indicate that the function of egt is to increase the progeny yield of the virus by delaying the development of the insect host, thereby maximizing the spread of the virus in the environment.

However, as noted earlier, the developmental delay observed during infection depends on both the virus dose and the exact time of infection within the age of the insect. Thus, the magnitude of the increase in yield will vary greatly from insect to insect, depending on the dose received and the stage of development at infection. On the other hand, the expression of the egt gene delays the onset of insect death, and therefore, the release of viral progeny is also delayed. This is a disadvantageous property for the virus in terms of propagation time in the host population. Thus, due to the egt gene, there is a trade-off between increasing the yield of virus progeny and delaying the release of virus progeny. The main takeaway in understanding the biological significance of egt is to find the balance at which this trade-off occurs.

The expression of egt may also have a function other than directly increasing the yield of virus progeny. For example, the purpose of egt may be to change the behavior of an infected insect. Many Lepidoptera leave the host plant and burrow before pupation, which is undesirable for the spread of the virus in the insect population. Thus, the function of egt may be to keep fifth-instar insects (which produce most of the OB offspring) on the plant. Another possibility is that egt does not specifically enhance the ability to transmit the virus. Insects that die at the pupation stage have a strong cuticle, and it is much easier to destroy the cuticle if the insect is caught at an early stage of the fifth instar. The offspring of the virus can thus spread over a smaller area from such a pupa, and egt, by blocking the development of pupation, can indirectly influence the spread of the virus. None of these possibilities is mutually exclusive, and a combination of all these effects seems to be the most beneficial [50].

Conclusions. Baculoviruses are known for their high specificity for certain insect species, which makes them ideal for pest control without harming beneficial insects or other non-target organisms. Baculoviruses have been used successfully in a variety of agricultural settings, including controlling pests in crops like cotton, corn, and vegetables.Furthermore, the genetic manipulation of baculoviruses has allowed for the creation of recombinant baculoviruses, which have shown potential as vaccines for human and animal diseases, as well as gene delivery vectors for gene therapy. Taking into account the information provided above, we have attempted to provide a more comprehensive overview of the biology of baculoviruses. This includes information on specific genes of baculoviruses and their functions, as well as their life cycle.

List of Abbreviations

GV – Granulovirus
NPV – Nucleopolyhedrovirus
AcMNPV - Autographa californica Multi Nucleopolyhedrovirus
BmNPV - Bombyx mori Nucleopolyhedrovirus
Sf21 - Spodoptera frugiperda 21
ODV - occlusion-derived virions
NC – nucleocapsids
BV – Baculovirus
h.p.i - hours post-infection

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