Insect viruses as vectors for highly efficient expression of foreign genes

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Subject: Genetic engineering

(Lecture 7)

Lecture Goal:
To explore the use of insect viruses as vectors for the efficient expression of foreign genes, focusing on the Baculovirus expression system and the Bac-to-Bac hybrid system.

Tasks:

1. Explain the advantages of insect viruses, particularly Baculovirus, as vectors for the expression of foreign genes in biotechnology.
2. Describe the Baculovirus Expression System, including its mechanism and applications for high-level protein expression.
3. Outline the Bac-to-Bac hybrid Baculovirus creation system, detailing its methodology and benefits for producing recombinant viruses.

Keywords: *Insect viruses, Baculovirus, Baculovirus Expression System, Bac-to-Bac system, recombinant protein expression, foreign gene expression, viral vectors, insect cell expression system*

Summary

1. Insect viruses

- Viruses that naturally infect insects and can be engineered to deliver foreign genes.
- Used as vectors due to their high efficiency in infecting insect cells.

2. Baculovirus

- A well-studied insect virus used as a vector for protein expression.
- Has a large double-stranded DNA genome, allowing insertion of large foreign genes.

3. Baculovirus Expression System (BES)

- A platform for producing recombinant proteins in insect cells.
- Combines viral vectors with insect cell culture for high-level protein expression.

4. Bac-to-Bac system

- A modified baculovirus expression system that uses **site-specific transposition** to generate recombinant bacmids.
- Simplifies the construction of recombinant viruses and improves expression efficiency.

5. Recombinant protein expression

- Foreign genes introduced into the baculovirus genome are transcribed and translated in infected insect cells.
- Proteins are often correctly folded and post-translationally modified, similar to eukaryotic systems.

6. Foreign gene expression

- Insect viral vectors enable rapid and high-level expression of genes from various sources (human, animal, plant).
- Useful for research, vaccines, and therapeutic protein production.

7. Viral vectors

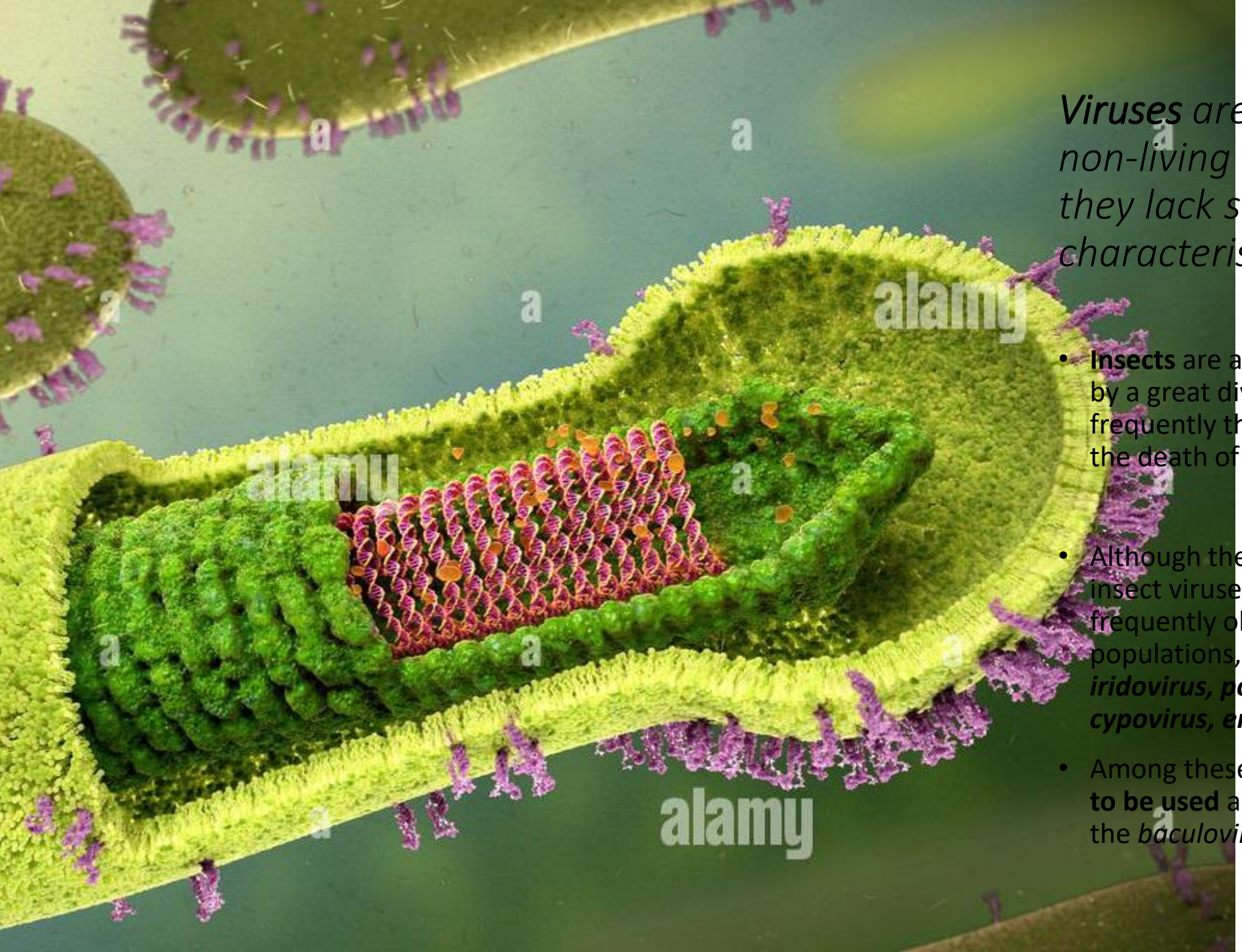
- Engineered viruses used to deliver and express foreign genes in host cells.
- Baculoviruses are safe, non-replicative in mammalian cells, and widely used in biotechnology.

8. Insect cell expression system

- Insect cell lines (e.g., Sf9, Sf21, High Five) are used as hosts for baculovirus-mediated protein production.
- Advantages: eukaryotic protein processing, scalability, and safety.

Key questions

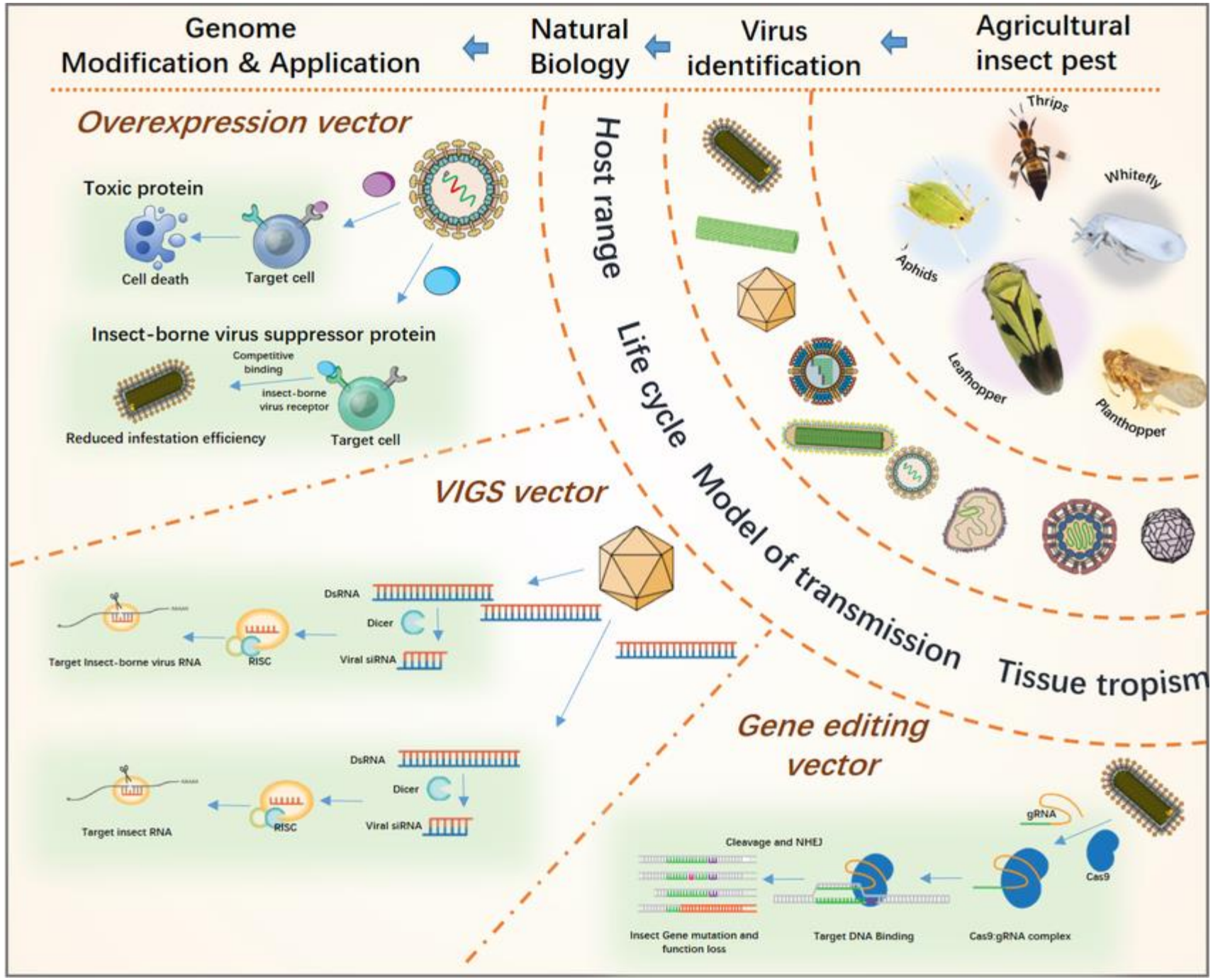
1) What are the advantages of using **insect viruses** as vectors for gene expression?
2) How does the **Baculovirus Expression System** work?
3) What is the **Bac-to-Bac system**, and how does it simplify recombinant protein production?
4) Why are insect cells preferred for baculovirus-mediated recombinant protein expression?
5) What types of proteins can be efficiently expressed using baculovirus vectors?
6) How do viral vectors differ from non-viral methods in terms of gene delivery efficiency?
7) What safety considerations are associated with using baculoviruses for gene expression?



__Viruses__ are often considered non-living things because they lack some of the basic characteristics of life.

- **Insects** are also like human attacked by a great diversity of viruses, and frequently their infection may cause the death of the infected individuals.

- Although there is a great diversity of insect viruses, only a few are frequently observed in insect populations, such as the ***ascovirus, iridovirus, polydnavirus, baculovirus, cypovirus, entomopoxvirus***;
- Among these, only few show **potential to be used** as control agents, mainly the *baculoviruses*.



Overview of insect viruses' identification through next-generation sequencing (NGS) and the prospective applications of recombinant insect viruses for pest control.

- The process involves the collection of field insects, followed by high-throughput sequencing and bioinformatics analysis to identify a diverse array of insect viruses.
- The figure further details the application of gene engineering methods, including the construction of overexpression vectors, gene silencing vectors, and gene editing vectors for these viruses.

Key Features

- ✓ **High expression levels** – Baculovirus systems can produce large amounts of properly folded and post-translationally modified proteins.
- ✓ **Eukaryotic processing** – Insect cells perform glycosylation, disulfide bond formation, phosphorylation, and other modifications similar to mammalian cells.
- ✓ **Safety** – Baculoviruses do not replicate in mammalian cells, which makes them biologically safe for laboratory use.
- ✓ **Versatility** – Suitable for expression of single proteins, multi-subunit complexes, virus-like particles (VLPs), and membrane proteins.

Applications of Insect Virus Expression Systems

1. **Functional studies** – Insect virus-based expression allows researchers to produce active, correctly folded eukaryotic proteins, which can be used to investigate their biological functions, interactions, and regulatory mechanisms in vitro or in cell-based assays.

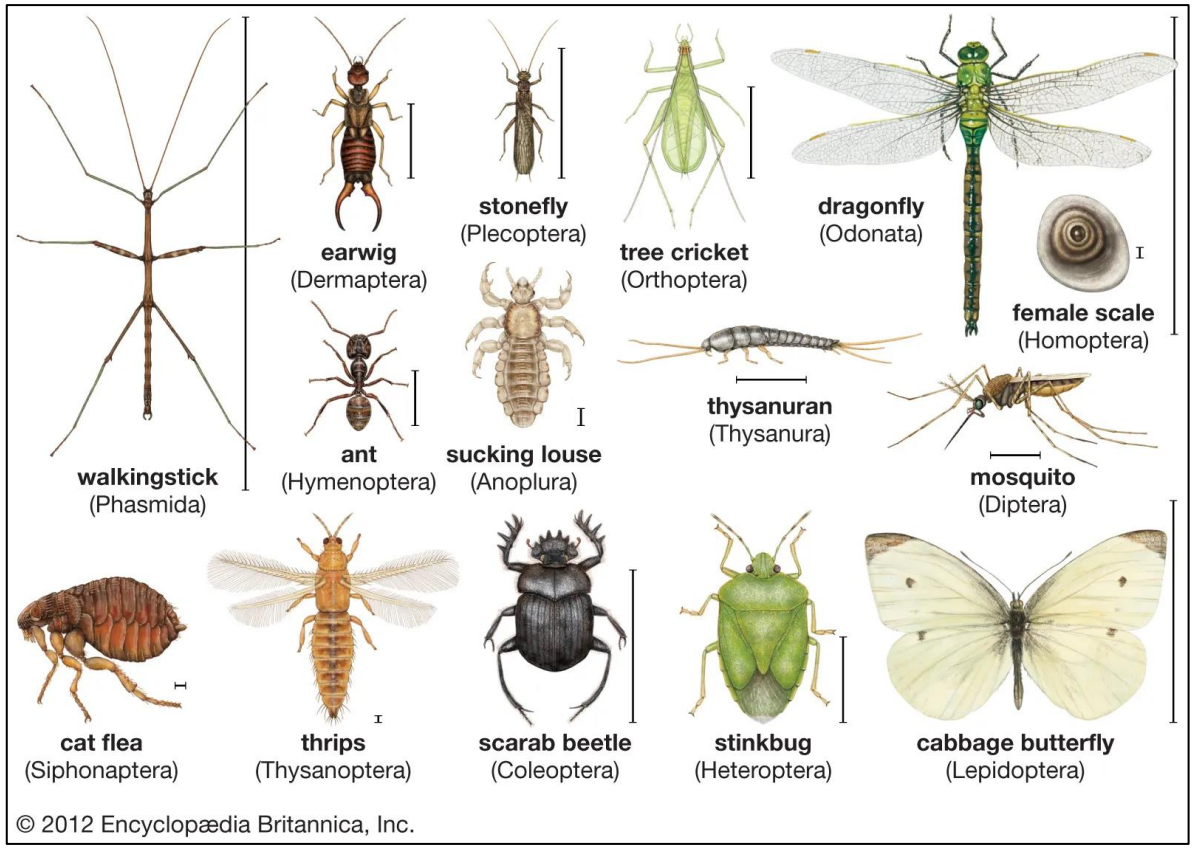
2. **Structural studies** – The system enables large-scale production of purified proteins and multi-subunit complexes suitable for X-ray crystallography, cryo-electron microscopy, and NMR, helping to determine high-resolution 3D structures of complex biomolecules.

3. **Vaccine / antigen / antibodies** – Baculovirus-infected insect cells are used to express viral antigens, virus-like particles (VLPs), and monoclonal antibodies, providing a platform for vaccine development (e.g., HPV, influenza) and for generating recombinant immunogens for diagnostic or therapeutic purposes.

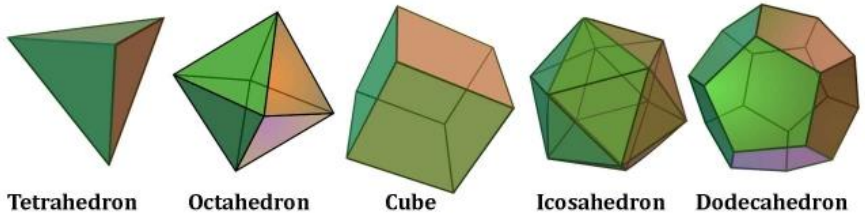
4. **Therapeutic drugs** – The system supports production of biologically active therapeutic proteins such as cytokines, growth factors, and recombinant enzymes, which can be used as biopharmaceuticals in the treatment of various diseases, including metabolic and immune disorders.

5. **Industrial enzymes for the reaction** – Insect cell expression enables the large-scale synthesis of stable and catalytically efficient enzymes used in industrial biotechnology, for example in food processing, biofuel production, biocatalysis, or environmental applications where high activity and proper folding are essential.

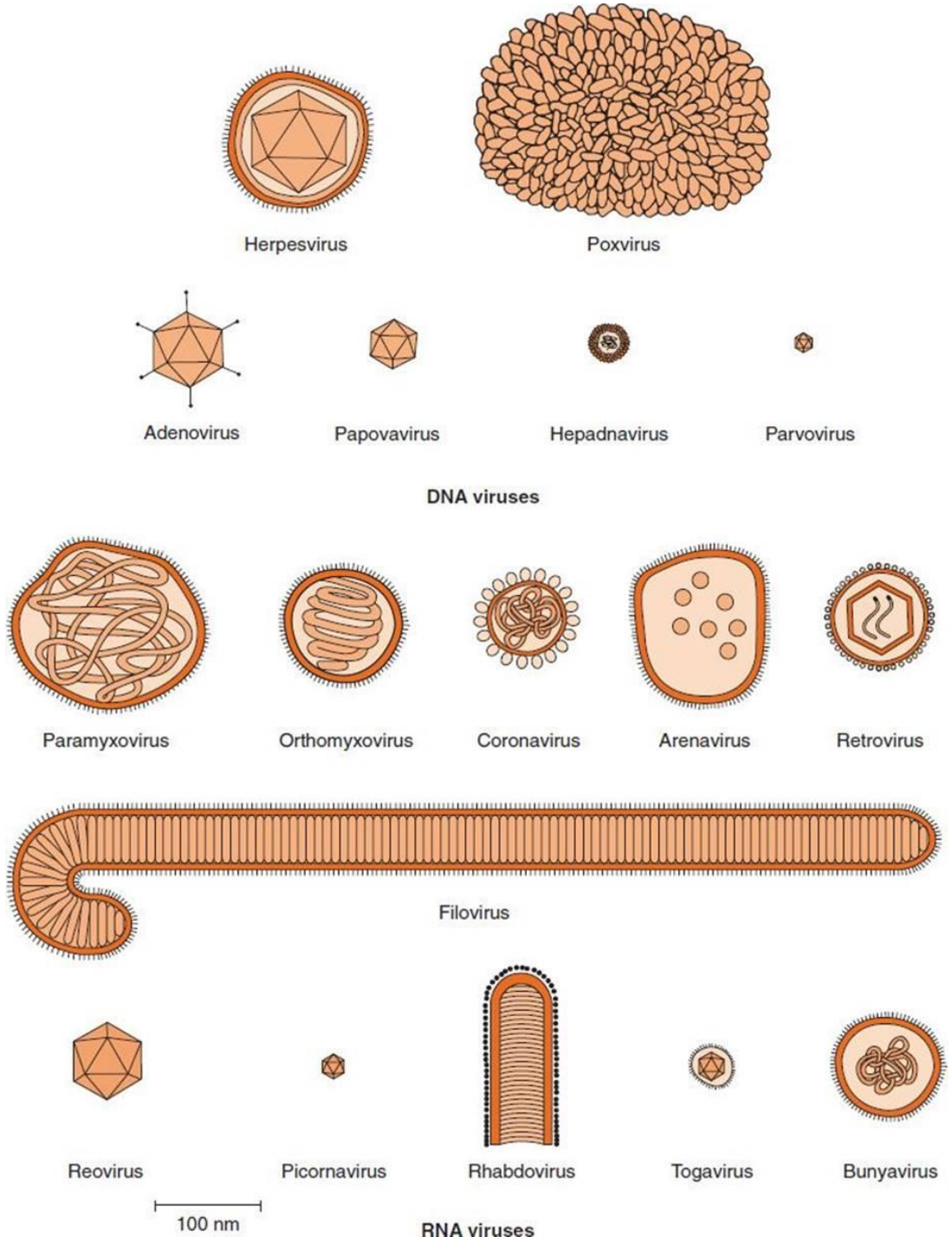
Insects constitute the most divers group of living things on the world.

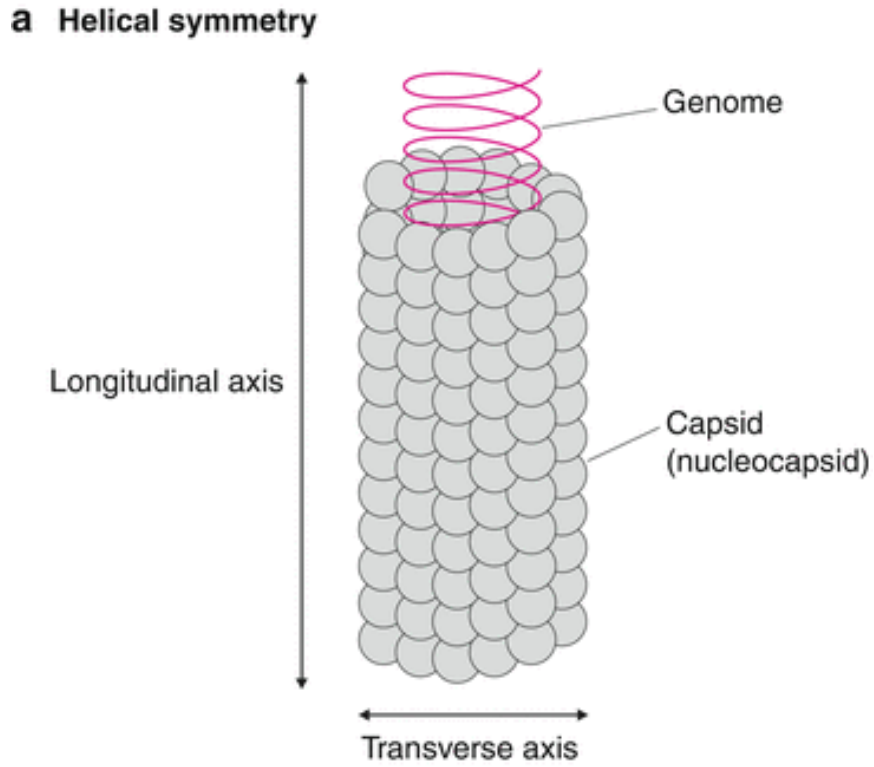


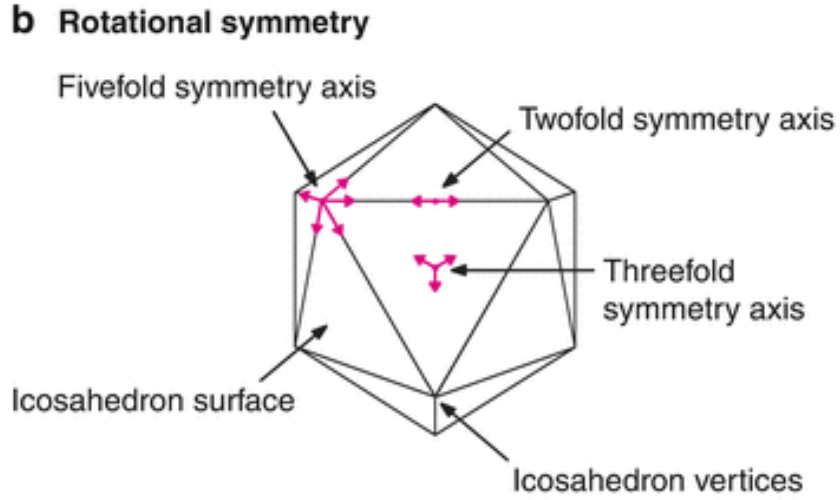
FAMILY	NUCLEIC ACID	NUCLEOCAPSID SIMETRY	OCCLUSION BODY
Baculoviridae	dsDNA	Baciliform	+
Reoviridae	dsRNA	Isometric	+
Poxviridae	dsDNA	Ovoid	+
Iridoviridae	dsDNA	Icosahedral	-
Parvoviridae	ssDNA	Isometric	-
Picornaviridae	ssRNA	Spherical	-
Ascoviridae	dsDNA	Allantoid	-
Polydnaviridae	dsDNA	Ovoid	-
Rhabdoviridae	ssRNA	Baciliform	-
Nodaviridae	ssRNA	Icosahedral	-
Rhabdoviridae	ssRNA	Baciliform	-
NON-CLASSIFIED RNA VIRUSESs			
Divided genome	ssRNA	Isometric	-
β *Nodaurelia*	ssRNA	Isometric	-
Kelply group	ssRNA	Isometric	-
5-virus group	ssRNA	Isometric	-
Minivirus	ssRNA	Isometric	-
Ovoid virases	ssRNA	Ovoid	-



To date studies on the genetics of entomopathogenic viruses are focused on the study of complete genomes as a consequence of the development of genomics. So far, more than 29 complete sequenced genomes have been obtained.


Herpesvirus
Poxvirus
Adenovirus
Papovavirus
Hepadnavirus
Parvovirus
DNA viruses
Paramyxovirus
Orthomyxovirus
Coronavirus
Arenavirus
Retrovirus
Filovirus
Reovirus
Picornavirus
Rhabdovirus
Togavirus
Bunyavirus
100 nm
RNA viruses


a Helical symmetry
Genome
Longitudinal axis
Capsid
(nucleocapsid)
Transverse axis

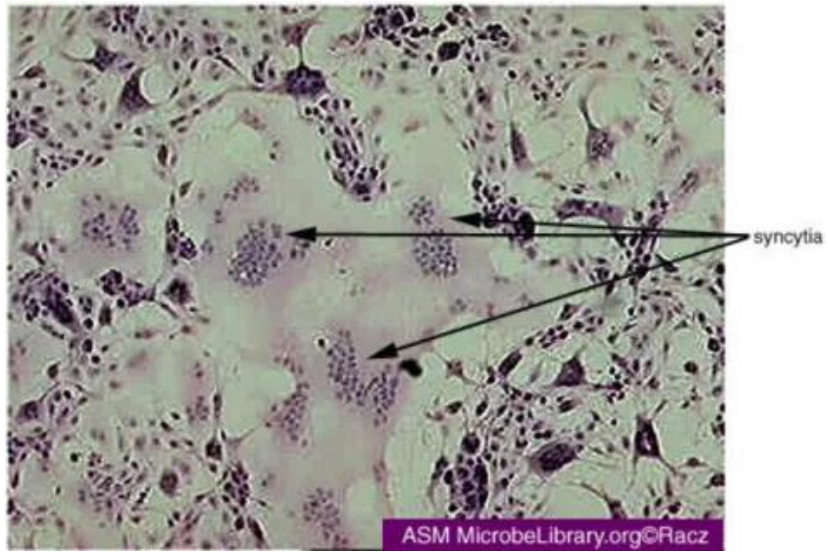

b Rotational symmetry
Fivefold symmetry axis
Twofold symmetry axis
Threefold
symmetry axis
Icosahedron surface
Icosahedron vertices

THESE **INSECT-SPECIFIC VIRUSES** HAVE A STRICT TROPISM AND ARE UNABLE TO REPLICATE IN VERTEBRATE CELLS, THESE PROPERTIES ARE INTERESTING FOR MANY REASONS!

- The first insect-specific virus (ISV) was discovered over 40 years ago by Stollar and Thomas [1]. It was isolated from an *Aedes aegypti* cell culture where a large number of syncytia were observed and the virus was named cell fusing agent virus (CFAV). Further, when inoculated on different vertebrate cell lines no cytopathic effect (CPE) could be observed and the virus could not be re-isolated, suggesting that the virus must be insect-specific [1].

Aedes aegypti

- Insect-specific viruses are believed to be restricted by the innate immune system in the vertebrate cell, but there is evidence pointing to that this is not the only mechanism causing the restriction of host-range [34]. In contrast to arboviruses, who can replicate at temperatures up to 42 °C insect-specific viruses replicate only at **ambient temperatures**.



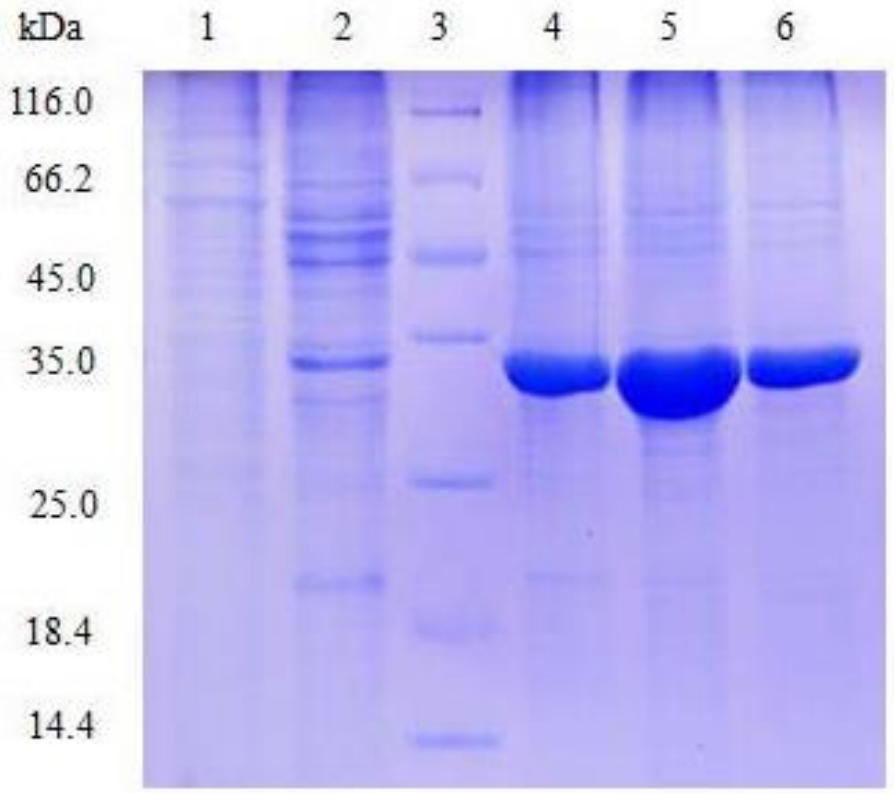
Insect Baculovirus Expression System

- ✓ **Insect baculovirus expression vector system (BEVS)** belongs to the *eukaryotic expression system*, and it's an expression system with high safety.
- ✓ It has a **large genome**, which enables the insertion of **large exogenous genes**, therefore has the great advantage of expressing proteins with large molecular weight.
- ✓ It also has the ability to achieve **complete post-translational modification** and efficiently express exogenous genes.
- ✓ The system consists of **transfer vector, baculovirus vector and the host cell**. The system uses one or more baculovirus **super-strong promoters**, and gets the recombinant virus after the exogenous target gene is inserted into the promoter.
- ✓ The **highly efficient expression** of the exogenous gene is achieved while the recombinant viruses replicate themselves in the insect cells.
- ✓ BEVS is **widely used** in virus vaccine development (such as the development of influenza virus vaccine and HPV vaccine), preparation of cell signaling proteins and cytokines, as well as kinase development, etc.

BACULOVIRUS AS A HIGHLY EFFICIENT EXPRESSION VECTOR IN INSECT AND MAMMALIAN CELLS

Case 1

The protein was highly expressed in our company's Insect baculovirus expression vector system. A clear band was observed from cell lysate by SDS-PAGE. The yield was up to 20 mg/L after purification.



Protein purification image
Lane 1: Flow through.
Lane 2: Cell lysate
Lane 3: Marker
Lane 4: 30 mM imidazole elution
Lane 5: 60 mM imidazole elution
Lane 6: 250 mM imidazole elution

- **Baculovirus** has been widely used for the production of recombinant proteins in insect cells.
- Since the finding that baculovirus can efficiently transduce mammalian cells, the applications of baculovirus have been greatly expanded.

Baculoviruses make up a family of viruses and are grouped into **nuclear polyhedrosis viruses** (NPV) and **granulosis viruses**. More than **500 different types** of baculoviruses have been discovered and the host range is restricted to invertebrates, mostly to insects (eg, moths and butterflies);

- Among the numerous baculoviruses, ***Autographa californica*** multiple NPV (**AcMNPV**) is the most well studied and most extensively used.
- **AcMNPV** has a circular double-stranded DNA genome of approximately 130 kb, which is condensed with a protamine-like protein into the core and packed into the nucleocapsids.
- Nucleocapsids are synthesized in the nucleus of infected cells (typically 40 nm–50 nm in diameter and 200 nm–400 nm in length). Membrane-enveloped nucleocapsids are referred to as virus particles or virions.

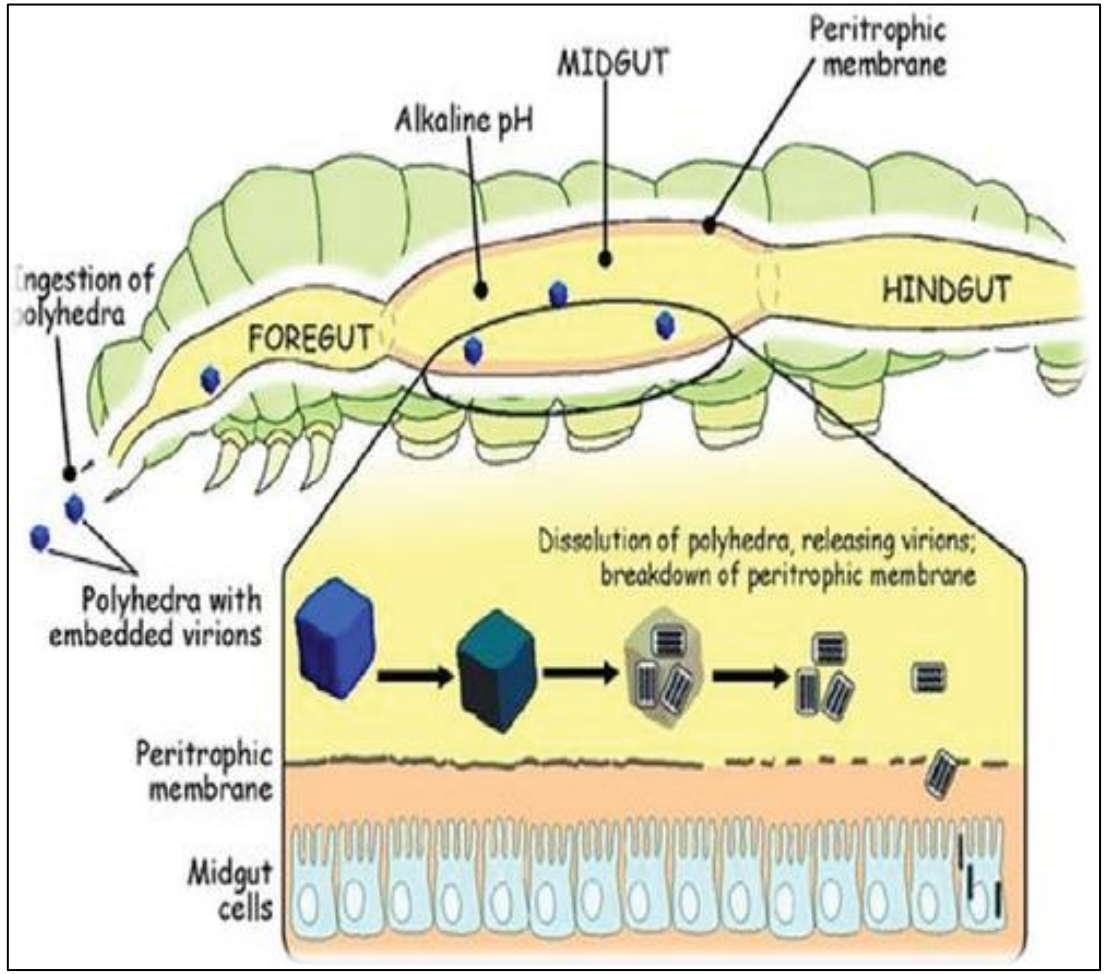
Alfalfa looper

- *Autographa californica* multiple nuclear polyhedrosis virus (Baculovirus)
- The virus usually infects the cells of the *alfalfa* (small beetle) or cutworm insects (and their larvae).
- Uses an ultra-strong promoter from the polyhedron coat protein to enhance protein expression while the virus is inside the insect

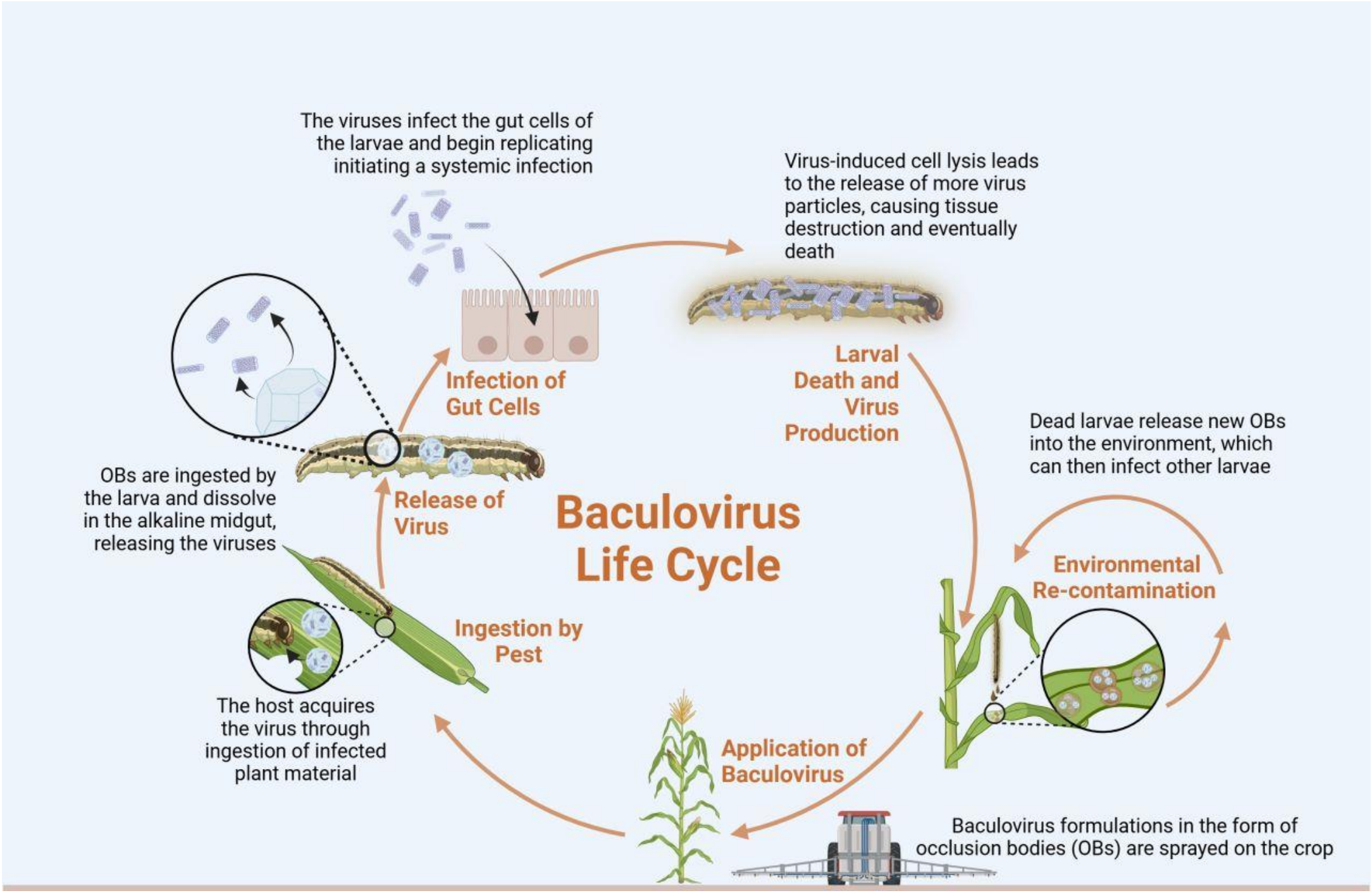
Autographica californica

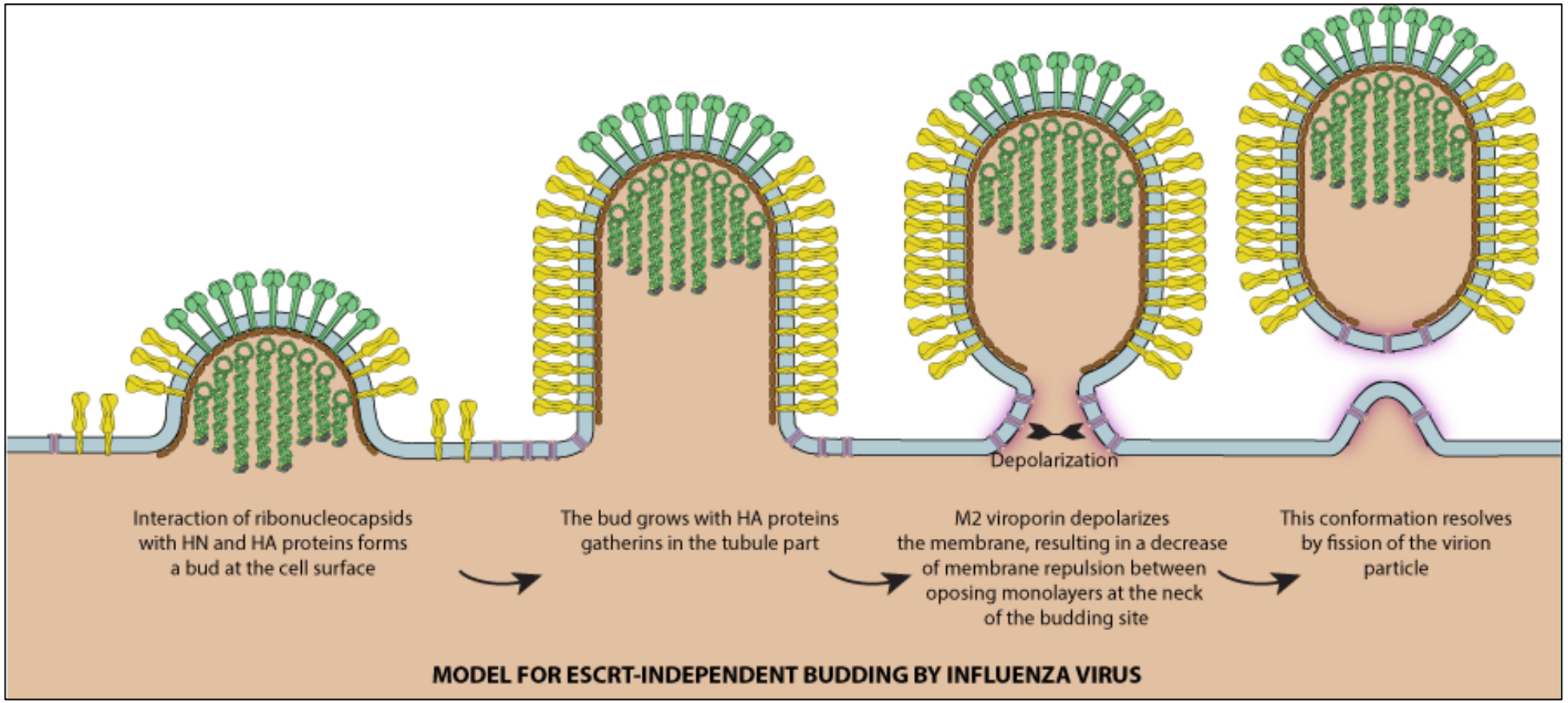
- In nature, **AcMNPV** are occluded in a polyhedron (2 µm–15 µm in size) mainly consisting of polyhedrin protein.
- After ingestion by insects, the polyhedrin matrix is dissolved in the **alkaline midgut**, thus releasing the embedded virions, which subsequently infect the epithelial cells of the intestine.
- Early in the infection cycle, the DNA genome is replicated and transcribed in the nucleus and the nucleocapsids are assembled.
- The budding of nucleocapsids through plasma membrane results in the release of budded virus, which is responsible for systemic transmission within an infected insect.
- Late in the infection cycle, progeny nucleocapsids become membrane-bound within the nucleus and are embedded into the polyhedra.
- After cell death and lysis, the polyhedra are released in the wild to spread the infection.

➢ **Budded virus is highly infectious to cultured insect cells and is the primary form used in the laboratory as an expression vector.**



The mode action of nucleopolyhedrovirus (NPV) on insect host.


The viruses infect the gut cells of the larvae and begin replicating initiating a systemic infection
Virus-induced cell lysis leads to the release of more virus particles, causing tissue destruction and eventually death
Infection of Gut Cells
Larval Death and Virus Production
Dead larvae release new OBs into the environment, which can then infect other larvae
OBs are ingested by the larva and dissolve in the alkaline midgut, releasing the viruses
Release of Virus
Baculovirus Life Cycle
Environmental Re-contamination
Ingestion by Pest
The host acquires the virus through ingestion of infected plant material
Application of Baculovirus
Baculovirus formulations in the form of occlusion bodies (OBs) are sprayed on the crop



Advantages of Budding Viruses:

- ✓ Non-lytic release – cells stay alive, continuous production
- ✓ Native membrane protein display – correct folding + orientation
- ✓ Eukaryotic post-translational modifications

- ✓ Safe design – can be made non-replicating / pseudotyped
- ✓ Flexible engineering – surface display, gene delivery, antigen presentation
- ✓ Targeted delivery – customizable viral envelope proteins
- ✓ Scalable production – suitable for suspension cultures & bioreactors

General Properties of Baculoviruses

1. **Large circular dsDNA genomes** (80–180+ kb)
2. **Obligate insect pathogens** – do not replicate in mammals
3. **Two virion phenotypes**:
   - **ODV (occlusion-derived virus)** – for transmission between insects
   - **BV (budded virus)** – for systemic spread inside host cells
4. **Used in biotechnology**: protein expression, VLP vaccines, gene delivery

Class / Genus	Host Range	Occlusion Body Type	Genome Type	Key Features
Alphabaculovirus (NPVs)	Lepidoptera (moths, butterflies)	**Polyhedra** (polyhedral occlusion bodies)	dsDNA, circular	Most widely used in BEVS; *AcMNPV*, *BmNPV*
Betabaculovirus (GVs)	Lepidoptera	**Granules** (granular occlusion bodies)	dsDNA, circular	Slower infection; high host specificity
Gammabaculovirus	Hymenoptera (sawflies)	Polyhedra	dsDNA, circular	Infect larvae of sawflies; not used in expression systems
Deltabaculovirus	Diptera (mosquitoes)	Polyhedra	dsDNA, circular	Only known baculoviruses infecting mosquitoes

1. Alphabaculovirus (Nucleopolyhedroviruses, NPVs)

Infect **Lepidoptera** (moths and butterflies).

Characterized by **polyhedral occlusion bodies (OBs)** containing multiple virions.

Genome size typically ranges from 120 to 160 kb.

Includes the most studied and biotechnologically relevant species such as **Autographa californica multiple nucleopolyhedrovirus (AcMNPV)** and **Bombyx mori NPV (BmNPV)**.

Exhibit two virion phenotypes: **ODV (occlusion-derived virus)** for horizontal transmission between insects and **BV (budded virus)** for systemic spread within the host.

Main group used in the Baculovirus Expression Vector System (BEVS) due to broad cell line compatibility (Sf9, Sf21, High Five cells).

2. Betabaculovirus (Granuloviruses, GVs)

Also infect **Lepidoptera**, but differ by producing **granular occlusion bodies** that typically contain a single virion per granule.

Genome size is smaller (around 100–120 kb).

Show **high host specificity** and slower infection cycles compared to NPVs.

Not widely used in biotechnology because of narrow host range and limited cell culture systems, but have potential applications in **biological pest control**.

3. Gammabaculovirus

Infect **Hymenoptera**, particularly sawfly larvae (*Symphyta*).

Produce polyhedral occlusion bodies similar to NPVs but differ in gene content and replication strategy.

Replication occurs exclusively in the **midgut epithelium**, making them less systemic.

Not currently used for recombinant protein expression, but relevant for ecological and evolutionary studies.

4. Deltabaculovirus

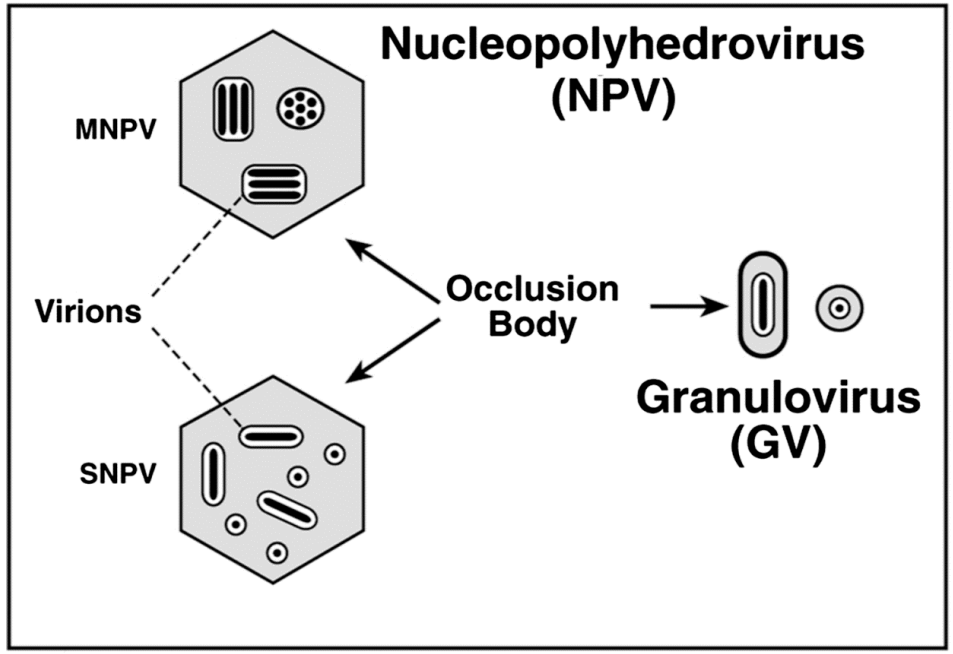
Infect **Diptera**, mainly mosquito larvae.

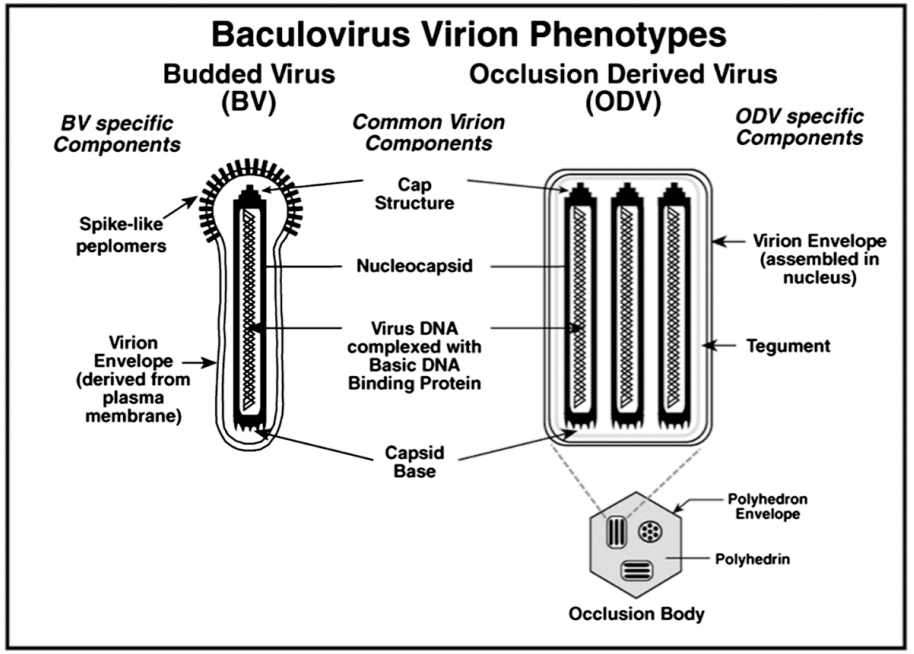
Represent the only baculoviruses outside Lepidoptera/Hymenoptera host range.

Genomes are among the smallest in the family (~80–90 kb).

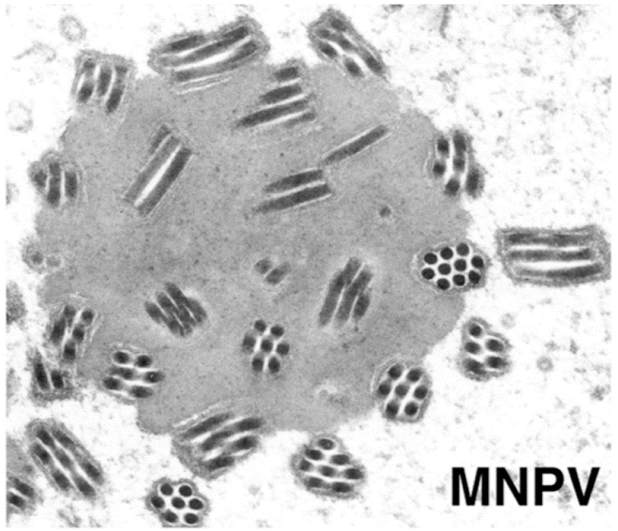
Potentially interesting for mosquito population control and as vectors for delivering antipathogen genes in disease control strategies.

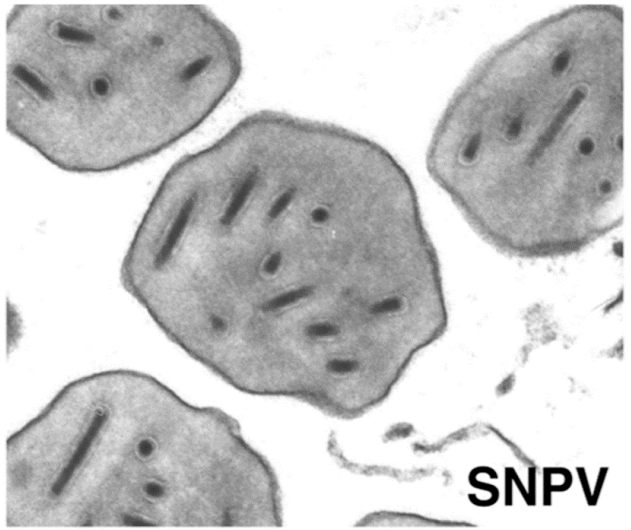
Baculovirus

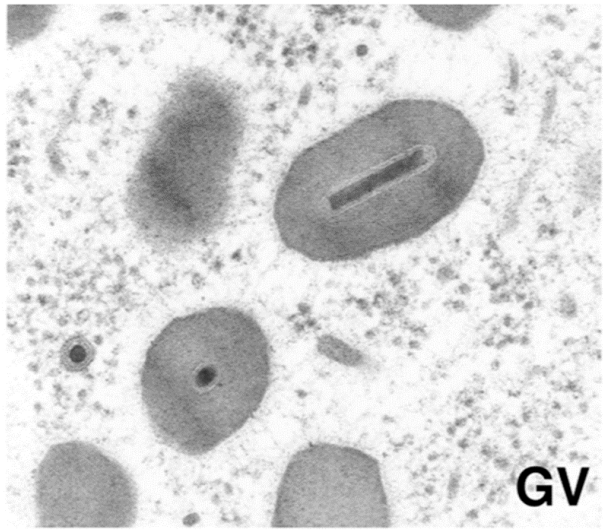




Occlusion Bodies







NPV – Nucleopolyhedrovirus

- NPV stands for Nucleopolyhedrovirus, a type of baculovirus that produces large polyhedral occlusion bodies (POBs) containing many virions.

Key features:

- Infect mainly Lepidoptera (moths and butterflies).
- Occlusion bodies contain multiple virions, each packaged inside a protein matrix made of polyhedrin.
- Genome size: ~120–160 kb, circular dsDNA.
- Two phases of infection: ODV (Occlusion-Derived Virus) – for oral infection in insect larvae. BV (Budded Virus) – for systemic spread inside host tissues.
- Widely used in biotechnology (AcMNPV is the main vector in the baculovirus expression system).

GV – Granulovirus

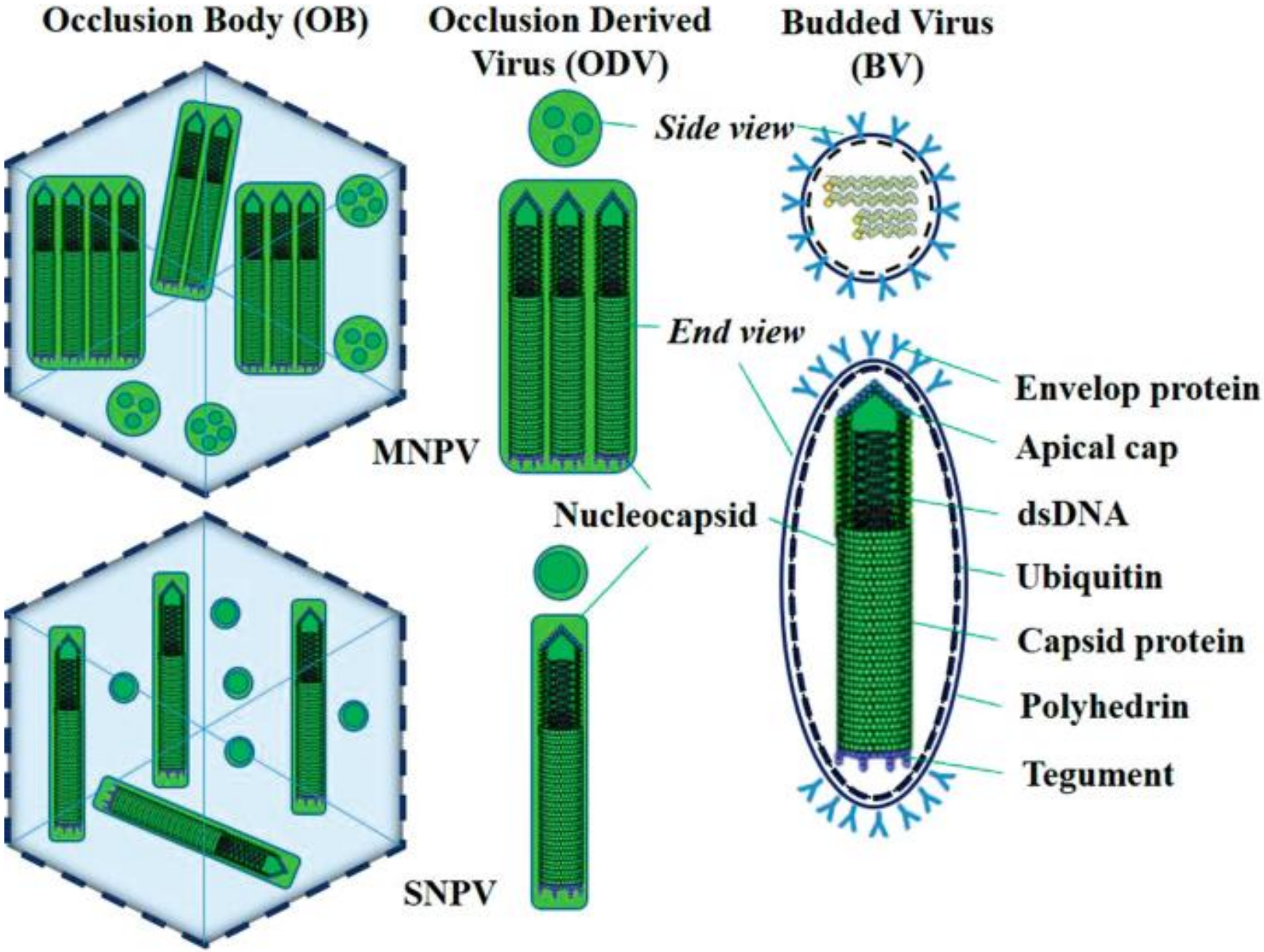
- **GV** stands for **Granulovirus**, another type of baculovirus, but with different occlusion morphology and infection characteristics.

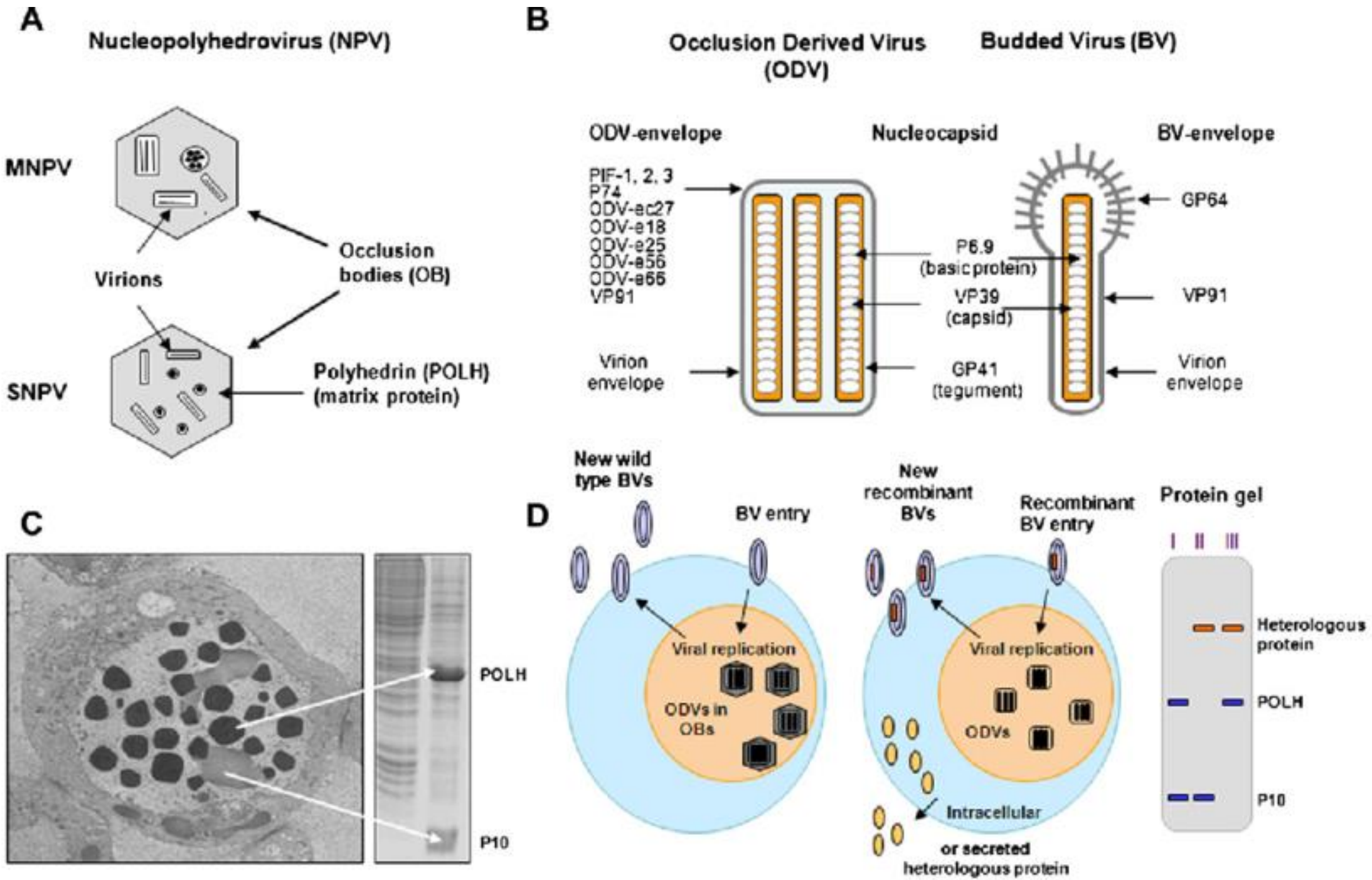
Key features:

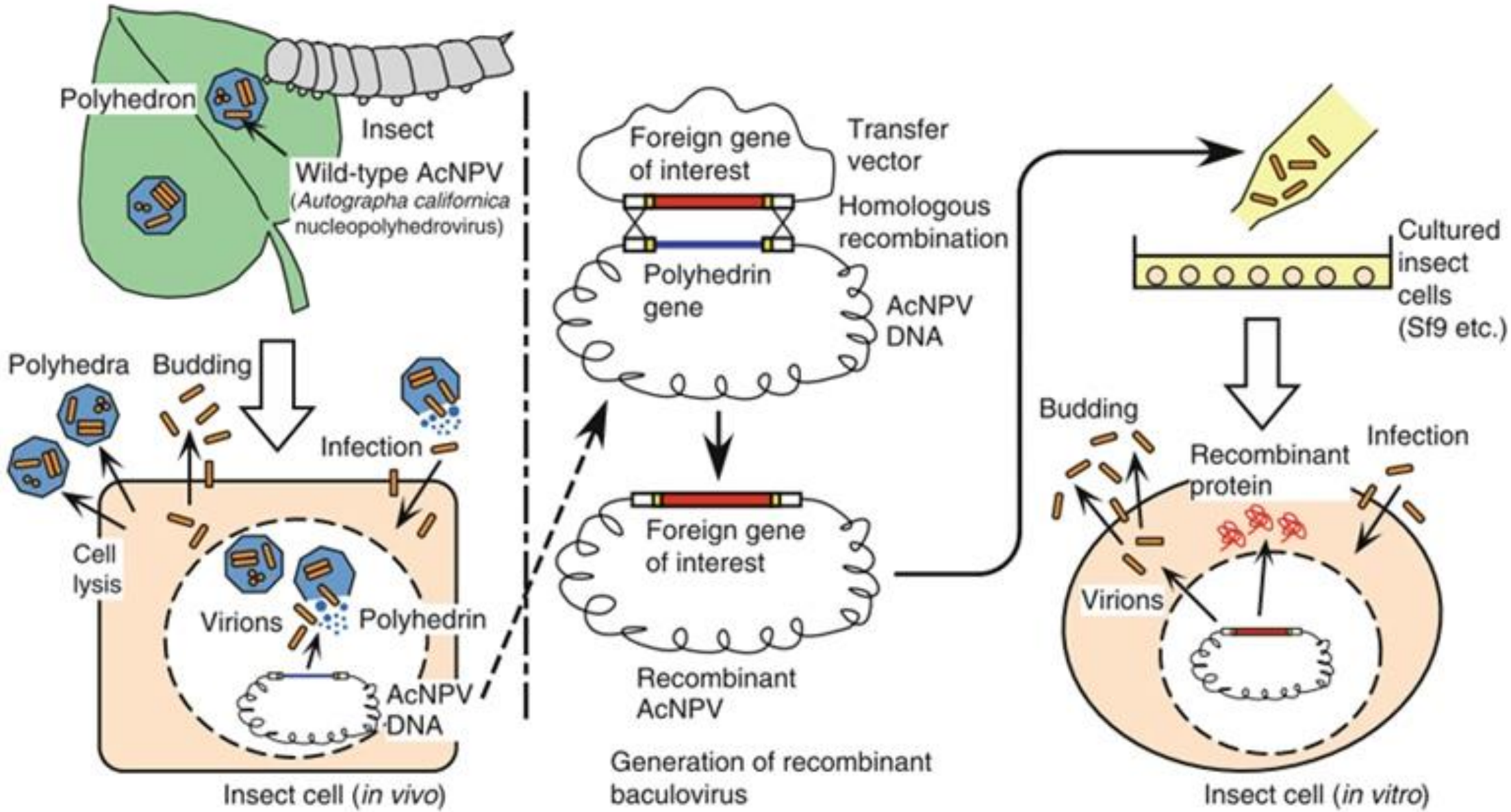
• Also infect **Lepidoptera**, but with **narrower host specificity** than NPVs.
• Form **granular occlusion bodies (GOBs)**, also called **granules**, usually containing **a single virion per granule**.
• Occlusion body protein is **granulin**, not polyhedrin.
• Genome size: ~100–120 kb.
• Mostly used as **biological insecticides** in pest control, not for protein expression.

SNPV – Single Nucleopolyhedrovirus

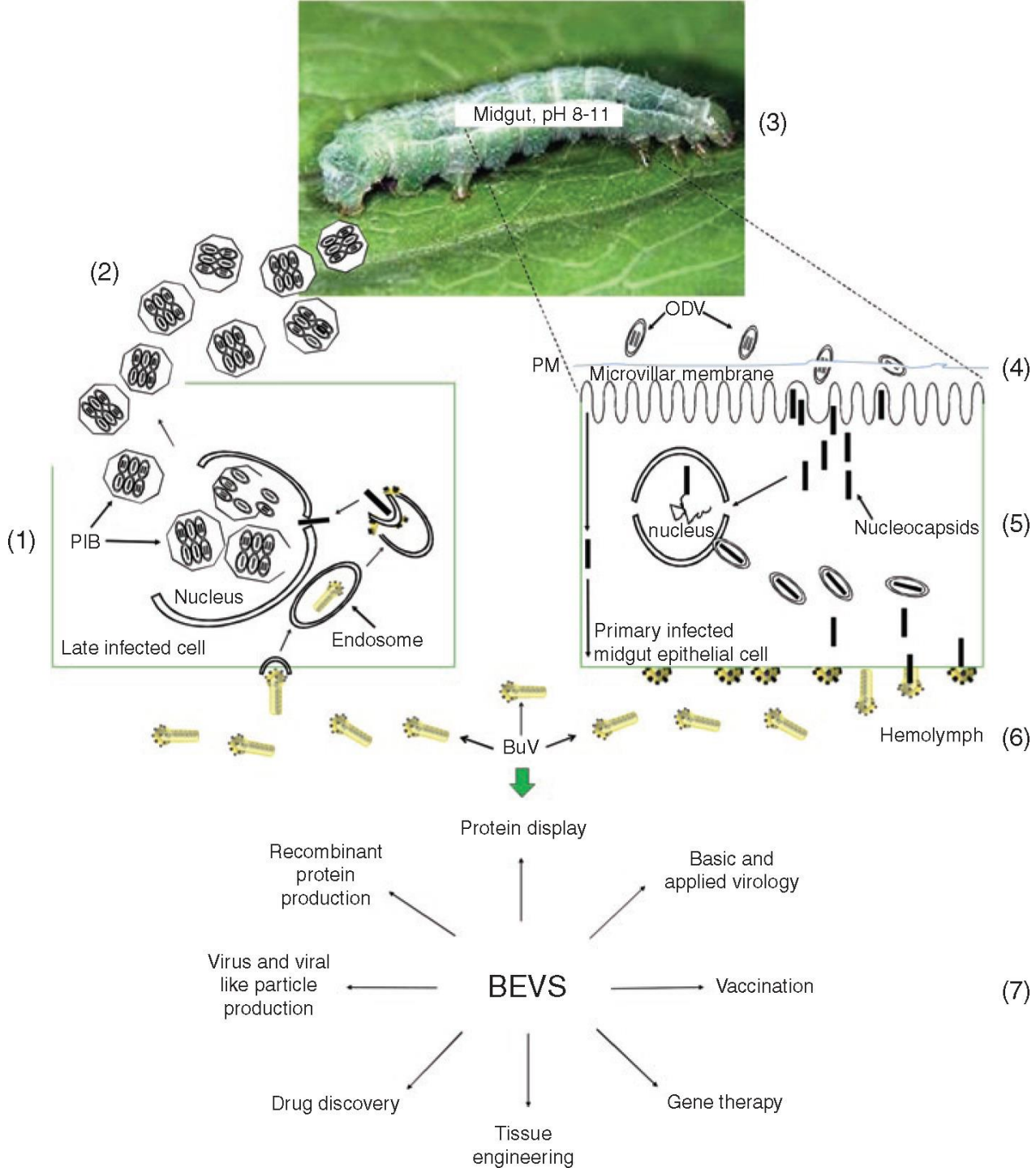
- **SNPV** means **Single Nucleopolyhedrovirus**, a subtype of NPV.
  It refers to NPVs where **each virion inside the occlusion body contains a single nucleocapsid**.
- **SNPV = occlusion body with many virions, but each virion has 1 nucleocapsid.**
- Example: *Autographa californica* MNPV (AcMNPV) is actually an **MNPV**, not SNPV (meaning *multiple* nucleocapsids).
- Opposite term: **MNPV (Multiple Nucleopolyhedrovirus)** – virions contain **multiple nucleocapsids inside a single envelope**.


Occlusion Body (OB)
Occlusion Derived Virus (ODV)
Budded Virus (BV)
Side view
End view
MNPV
Nucleocapsid
SNPV
Envelop protein
Apical cap
dsDNA
Ubiquitin
Capsid protein
Polyhedrin
Tegument


A
Nucleopolyhedrovirus (NPV)
MNPV
Virions
Occlusion bodies (OB)
SNPV
Polyhedrin (POLH) (matrix protein)
B
Occlusion Derived Virus (ODV)
Budded Virus (BV)
ODV-envelope
Nucleocapsid
BV-envelope
PIF-1, 2, 3
P74
ODV-ec27
ODV-e18
ODV-e25
ODV-e56
ODV-e66
VP91
Virion envelope
P6.9 (basic protein)
VP39 (capsid)
GP41 (tegument)
GP64
VP91
Virion envelope
C
POLH
P10
D
New wild type BVs
BV entry
Viral replication
ODVs in OBs
New recombinant BVs
Recombinant BV entry
Viral replication
ODVs
Intracellular
or secreted heterologous protein
Protein gel
I II III
Heterologous protein
POLH
P10

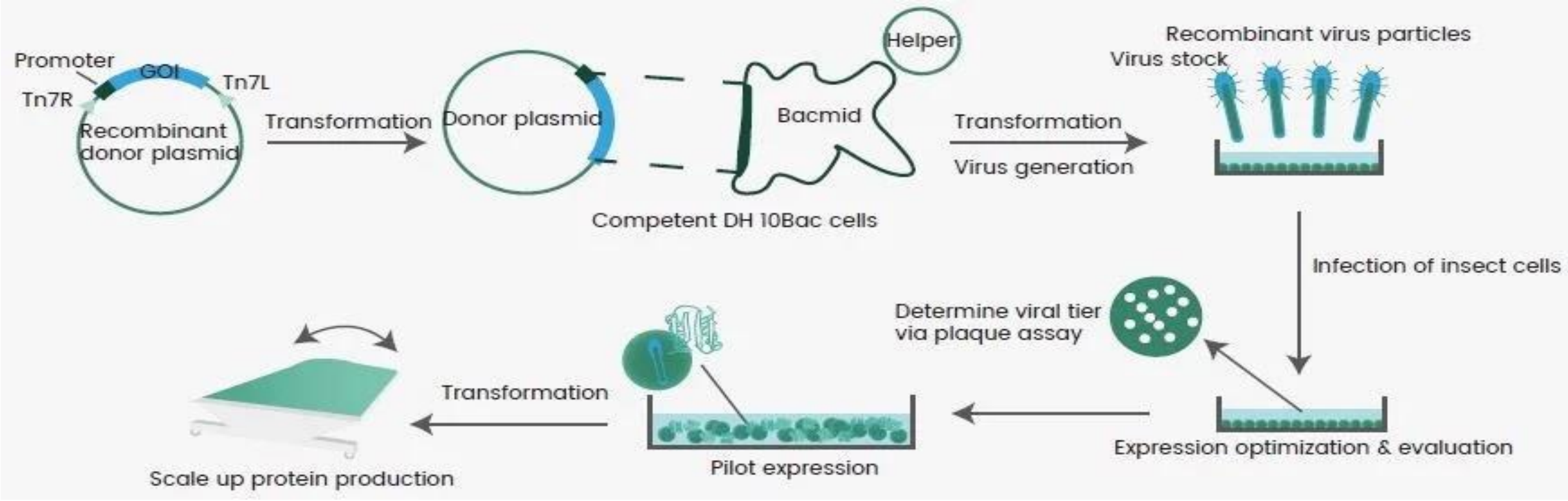


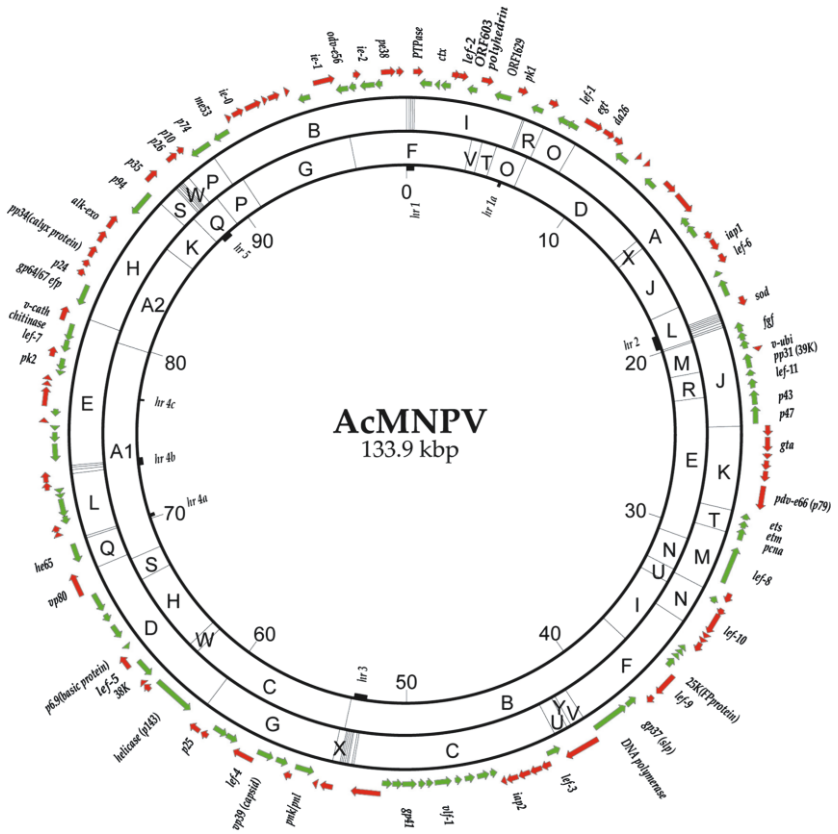
Recombinant protein production in the baculovirus-insect system
(Antibody Expression and Production, 2010)


Midgut, pH 8-11
(3)
(2)
ODV
PM
Microvillar membrane
(4)
(1)
PIB
Nucleus
Late infected cell
Endosome
nucleus
Nucleocapsids
(5)
Primary infected
midgut epithelial cell
Hemolymph
(6)
BuV
Protein display
Recombinant
protein
production
Basic and
applied virology
Virus and viral
like particle
production
BEVS
Vaccination
(7)
Drug discovery
Tissue
engineering
Gene therapy

The baculovirus has been widely used for the production of numerous recombinant proteins in insect cells because it has the following advantages:

(i) proper post-translational modification, because insect cells are higher eukaryotes;
(ii) a high capacity for multiple genes or a large insert, because of the huge and flexible viral genome (130 kb);
(iii) biosafety, because baculovirus naturally does not infect humans;
(iv) a very high yield driven by the strong promoters polyhedrin or p10.





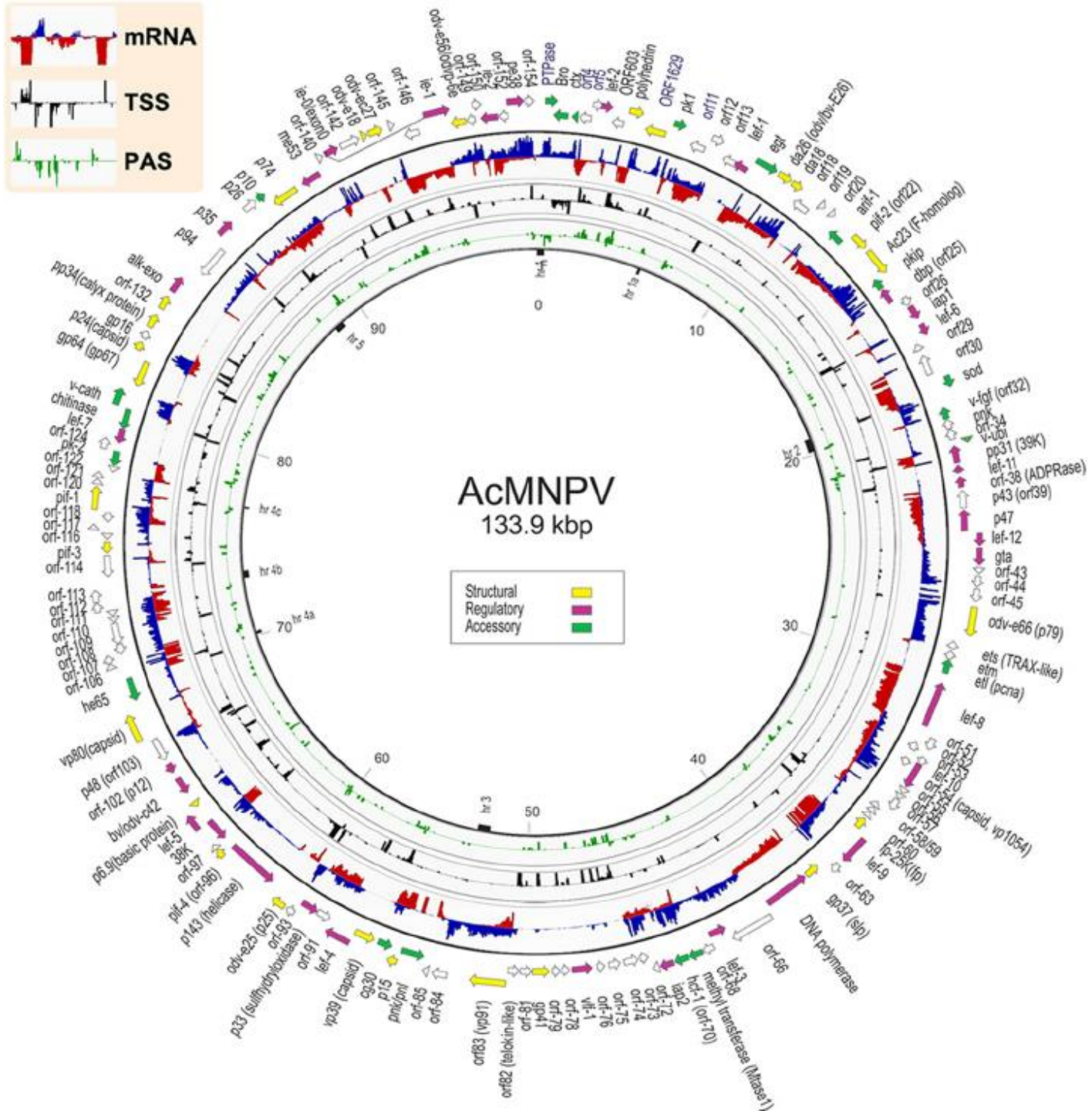
Temporal Gene Expression Program
Baculovirus genes are expressed in a **highly ordered temporal cascade** after infection. They are divided into **four major classes** based on timing and promoter type:

Gene Class	Timing	Promoter Type	Function
Immediate-early (IE)	0–2 h post infection	Host RNA polymerase II	Activate early genes, regulate transcription (e.g., **ie-1**)
Early (E)	2–6 h	Host RNA pol II	DNA replication, immune modulation, host takeover (e.g., **dnapol**, **p35**)
Late (L)	6–18 h	Viral RNA polymerase	Structural proteins, nucleocapsid assembly (e.g., **vp39**, **p6.9**)
Very late (VL)	18–72 h	Strong viral promoters **polh** and **p10**	Massive expression of occlusion body proteins (polyhedrin, p10)

Major Functional Gene Groups
1. **Replication genes** – DNA polymerase, helicase, primase, LEF proteins (Late Expression Factors).
2. **Transcription regulation genes** – IE-1, IE-2, LEF-3, viral RNA polymerase subunits.
3. **Structural genes** – capsid proteins, envelope proteins (GP64 or F protein).
4. **Host manipulation genes** – apoptosis inhibitors (p35, iap), immune suppressors (egt), actin modifiers.
5. **Occlusion body genes** – **polh (polyhedrin)** and **p10**, expressed at extremely high levels in the very late phase.

✓ The polyhedrin (polh) promoter is one of the strongest known eukaryotic promoters, **producing up to 50%** of total cell protein in the late stages.

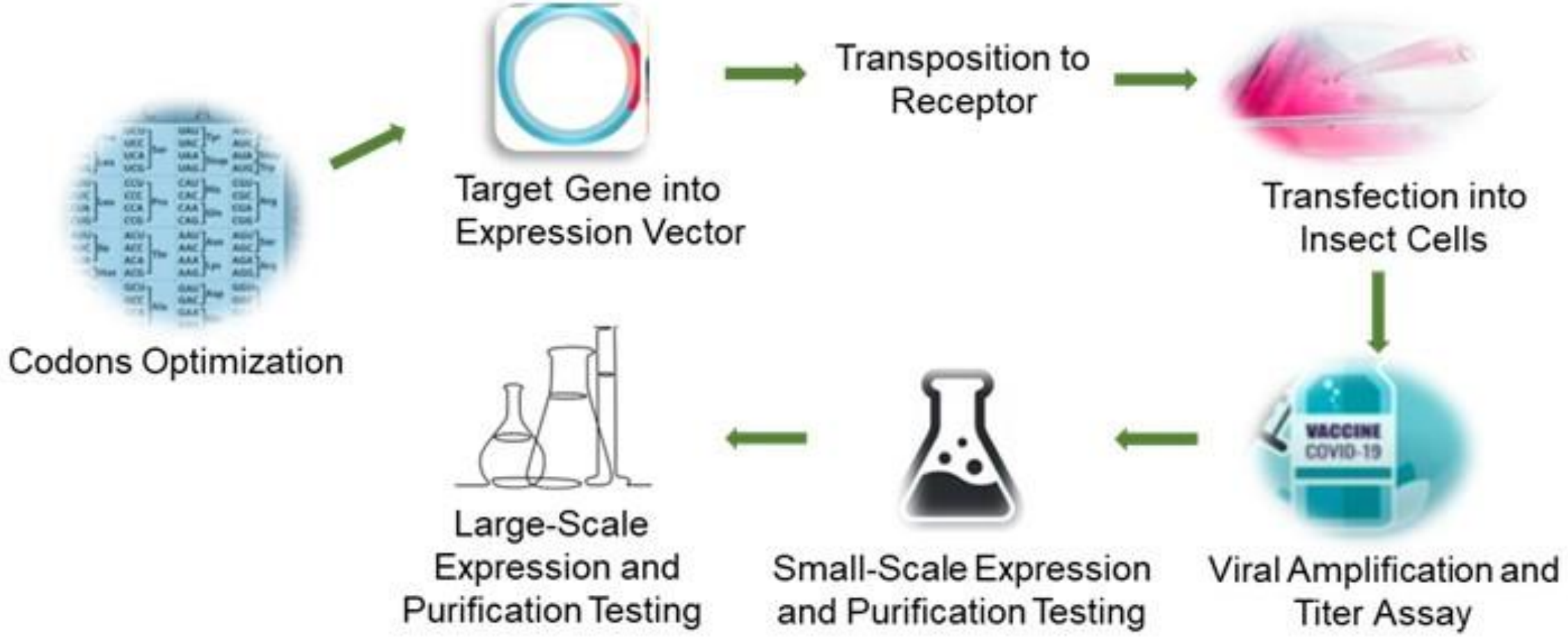
→ This is why it is used in the baculovirus expression vector system (BEVS) to express foreign recombinant proteins at very high yield.



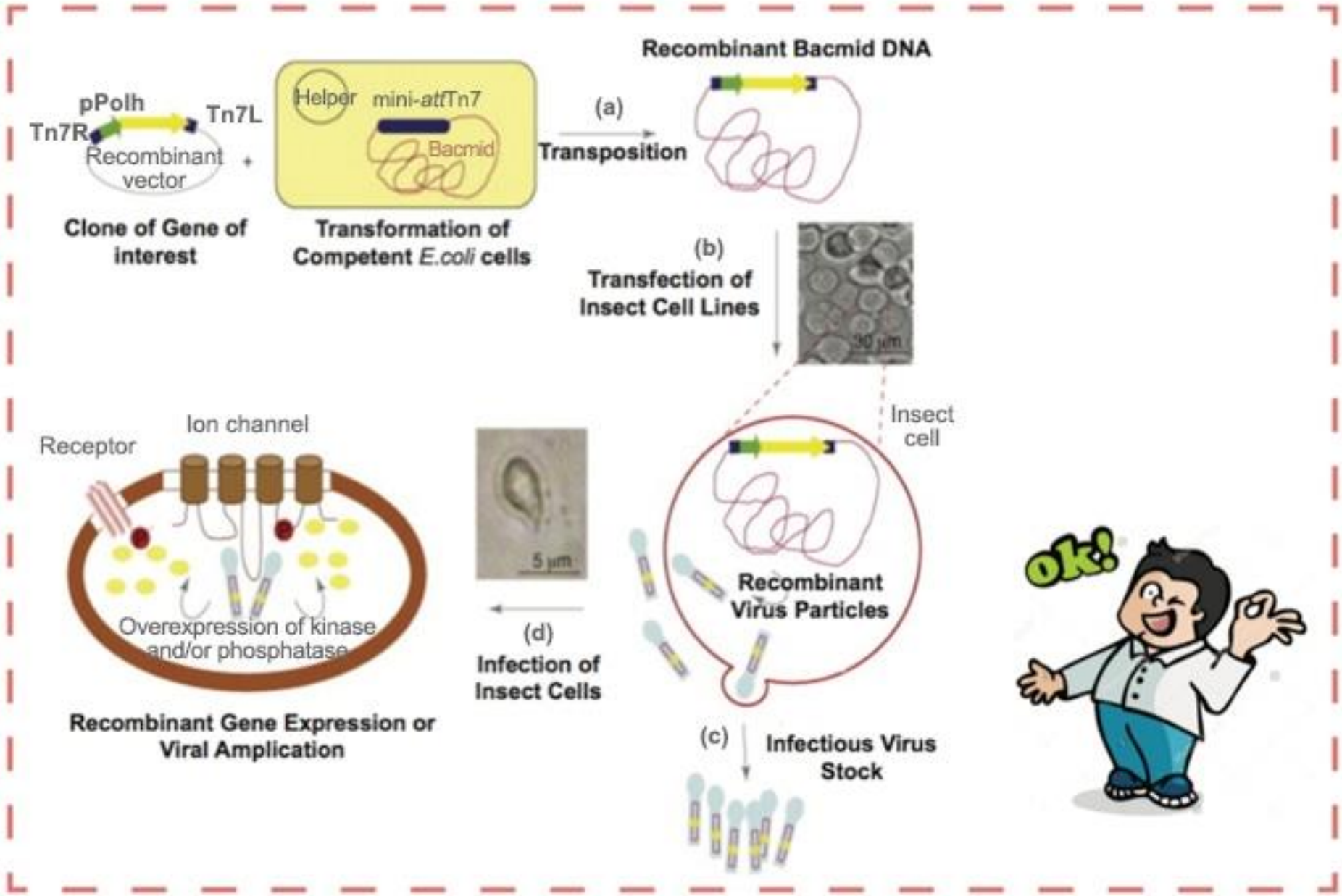
Circular diagram of the AcMNPV genome and summary of AcMNPV transcription initiation sites, polyadenylation sites, and abundance.

- Gene names are shown on the outside of the circle beside arrows representing ORFs.
- Transcript features are indicated below the ORF map.
- ORF colors represent classes of genes.
- Transcript abundance at 12 h postinfection is represented by a graphic representation of strand-specific RNA sequencing data shown directly below the ORFs, with the orientation of transcripts indicated in blue (plus strand; clockwise orientation) and red (minus strand; counterclockwise orientation).
- The transcription start sites (TSS) and polyadenylation sites (PAS) identified by Illumina 5 = and 3 = RACE sequencing are shown as black and green marks, respectively, on the inner circles.
- The inner scale indicates map units (MU; 1 MU б 134 bp).

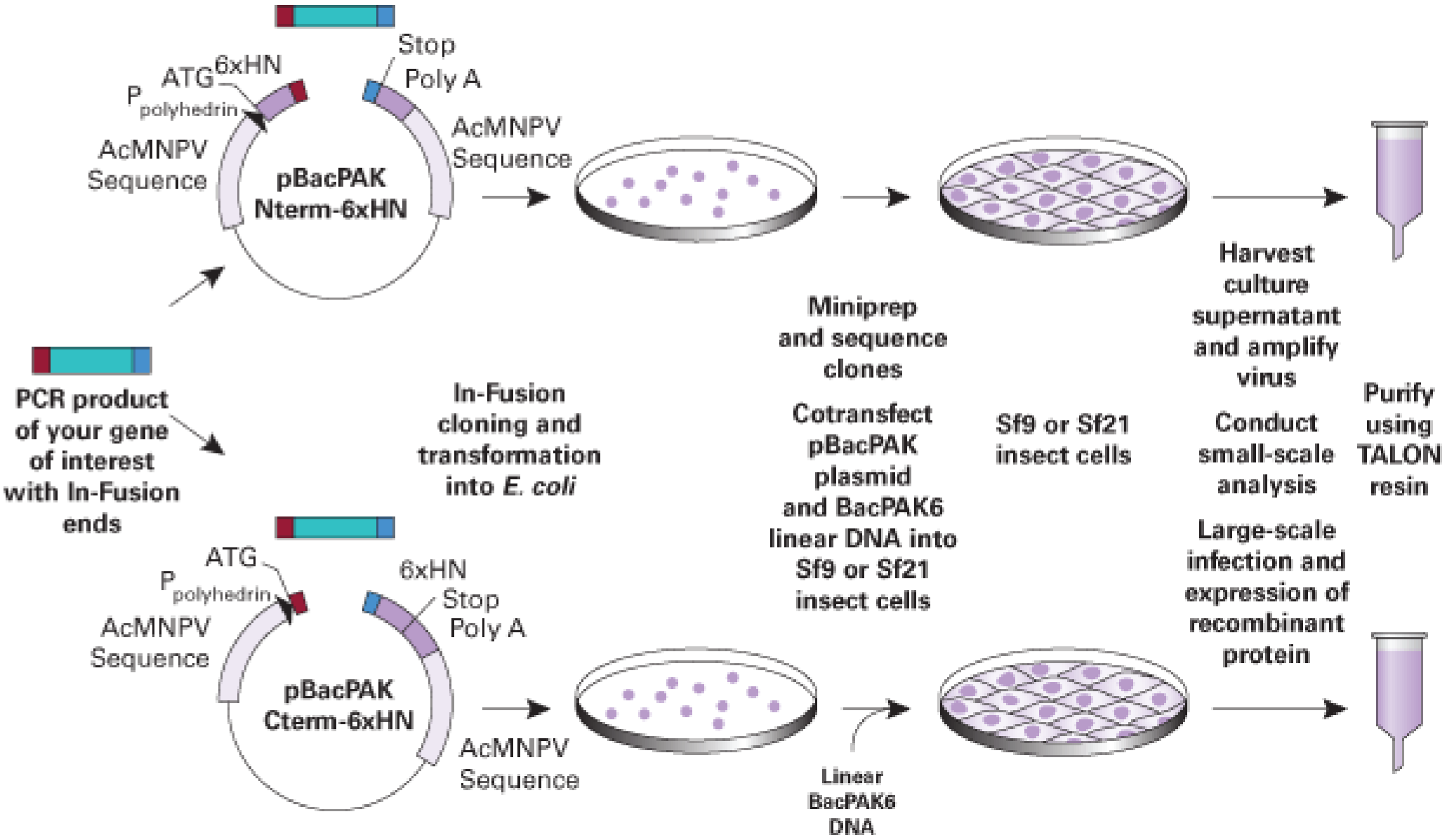
Bac-to-Bac Baculovirus Expression System - Lifeasible



Generation of Recombinant Baculovirus and Gene Expression



Baculovirus expression system: a complete system



BAC-TO-BAC HYBRID BACULOVIRUS CREATION SYSTEM

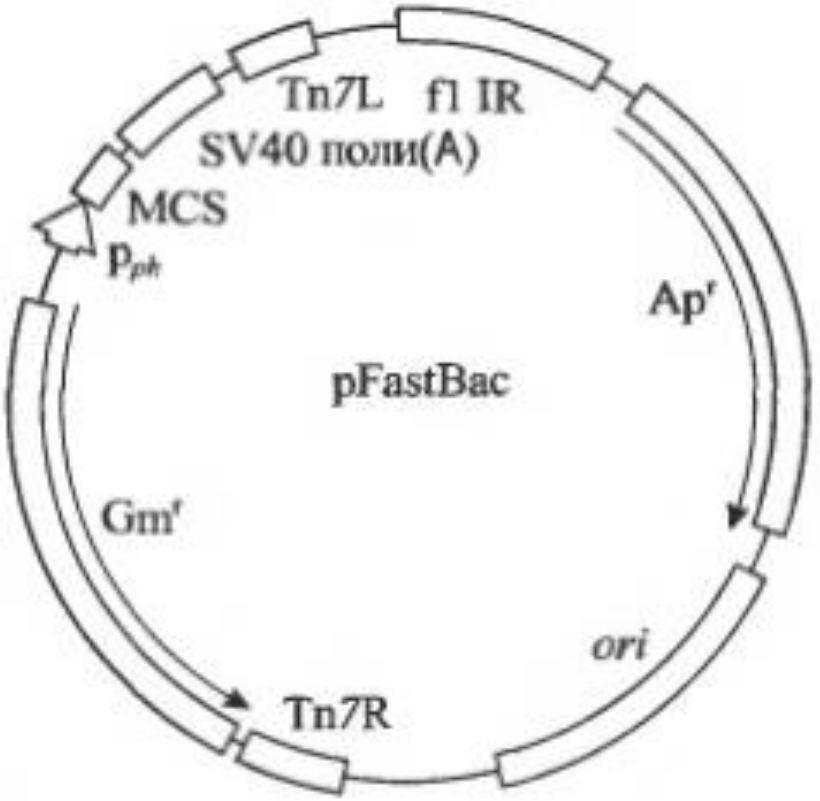
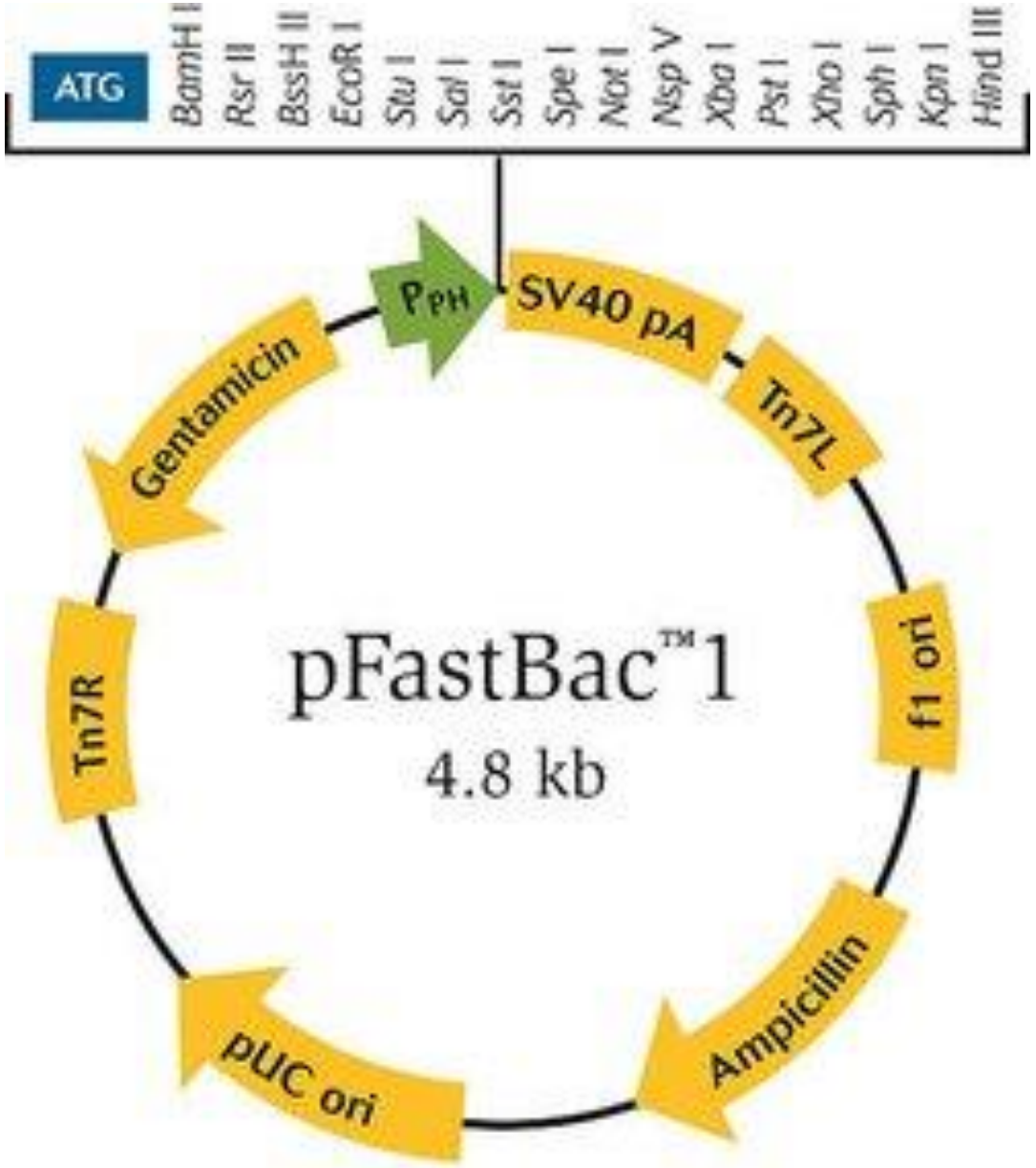


Рис. 15.6. Векторная экспрессионная плазмида pFastBac, предназначенная для направленной встройки целевых генов в бакмиду, содержащую геном вируса ядерного полиэдроза.

MCS — полилинкер для встройки целевого гена



Baculovirus systems

Disadvantages:

- Grows very slowly (10-12 days to prepare);
- Cell culture is only viable for 4-5 days;
- Preparing takes a lot of time, not as easy as yeast.

Advantages:

- Can express large proteins (>50 kDa).
- Proper glycosylation and removal of signal peptide.
- It has chaperonins that help fold “strong” proteins.
- Very high yields, cheap

References

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3) Hong M. et al. «Genetic engineering of baculovirus-insect cell system to improve protein production.» Frontiers in Bioengineering and Biotechnology. 2022. Frontiers
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