Journal of **Coastal Development**
Vol. 1, No. 1, 1997: 19-30

INSECT CELLS AS HOSTS FOR RECOMBINANT PROTEINS

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ABSTRACT

Since the development of recombinant baculovirus expression systems, insect cell culture has rapidly gain popularity as the method of choice for production of a variety of biologically active proteins. Up to date tens of recombinant protein have been produced by this method commercially or non-commercially and have been widely used for research. This review describes the basic concept of baculovirus expression vector and the use of insect cells as host for recombinant proteins. Examples of the recombinant proteins produced by this system are given.

Keywords: insect cells, baculovirus, recombinant protein

I. Introduction

Insects are well known as one of the major pests to large number of important crops from an early growth of plant until they are harvested and stored. The shift of interest in controlling insect pests from chemical to biological insecticides comes from the growing concern of environmental pollution, and generation of insecticides-resistant insect caused by the application of the chemical insecticides. The strategy of biological control based on the use of naturally occuring enemies of insect, one of which can be a virus (Christian and Oakeshott, 1989). The primarily pathogenic virus which infects the majority of insects from the Order Lepidoptera (over 600 species insect) is baculovirus from the Family Baculoviridae (Blissard and Rohrman, 1990). The attempt to produce viral insecticides for controlling insect pest has led researches to the development of propagation systems for baculovirus using insect cell culture. From this initial work a new expression system for clone genes has been developed which uses the baculoviridae as vector for expressing the cloned genes in insect cells.

II. Baculovirus Morphology

The family baculovirus is characterized by an enveloped, rod-shape virions containing a circular double-

stranded DNA genomes. The baculovirus is divided into three subgrups based on their morphological properties:
(i) nuclear polyhedrosis viruses (NPVs) which produce polyhedral, pseudo-crystalline proteinnaceous inclusion body (IB). There two types of NPVs, single-nucleocapsid NPV(SNPV) and multi-nucleocapsid NPV (MNPV); (ii) granulosis viruses (GVs), unlike polyhedral shape IB in the first type, produce an oval shape IB; (iii) non-occluded viruses (NOVs) produce virions which are not packed into IB (Bilimoria, 1986).

III. Baculovirus Life Cycle

When larvae of Lepidopteran host ingest any part of plant contaminated with viral occlusions (IB), the crystal is solubilized by the action of highly alkali digestive juices of the midgut lumen, thereby releasing virus particles (Figure 1). The virus particles enter the midgut cells, migrating through the cytoplasm to reach nuclei where the viral DNA is replicated. Secondary infection of other cells and tissues occurs through the budding of virus from plasma membrane and this budded viruses (BV) are referred as extracellular virus (ECV; Granados and Williams, 1986). Late in infection some viruses, in the cell nuclei, are embeded in proteinaceous viral occlusion bodies (Obs) which consists of enveloped bundles of nucleocapsids lying within a paracrystalline protein matrix (Cochran *et al.*, 1982). When the larvae die, they leave millions of IB. The IB protect the embedded virus particle from detrimental environmental factors, thus providing a means for horizontal transmission (Granados and Williams, 1986).

IV. *Spodoptera frugiperda* (Sf-9) Cells as Host for Eucaryotic System

Spodoptera frugiperda cell lines are susceptible to infection by *Autographa californica* multicapsid nuclear polyhedrosis virus (AcMNPV; McIntosh and Ignoffo, 1989) which has been found to be pathogenic to dozens of insect cell species such as alfalfa looper, cabbage looper, and beet armyworm which are widely known as crops pests (Hink, 1982). AcMNPV, therefore, has been used widely as the prototype virus of the family Baculoviridae when studying the propagation of virus as bioinsecticides and for the production of biologically active proteins via the baculovirus cloning system.

V. Regulation of Viral Replication and Gene Expression of AcMNPV

During viral infection, viral genes are expressed in a cascade pattern and host gene expression is terminated in the late phase of infection (Blissard and Rohrman, 1990). The temporally regulated cascade gene expression of AcMNPV is divided in two phase *ie*. an early phase which precedes viral DNA replication and late phase which occurs after viral DNA replication begins (Friesen and Muller, 1986).

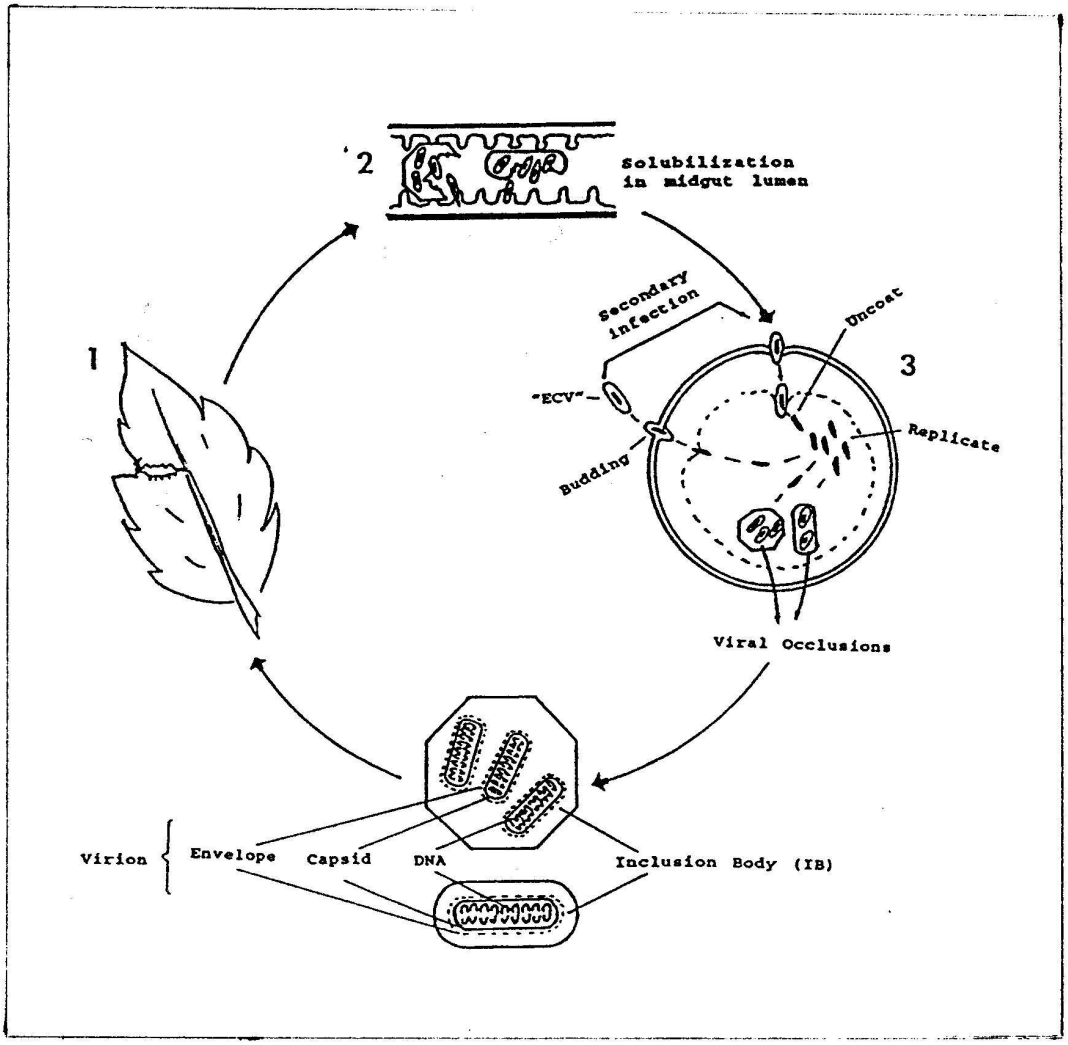

Figure 1. Life Cycle of Occluded Baculovirus

1. Insect larvae ingest viral occlusions (Ibs) from foligae.
2. Alkali juices of midgut lumen solubilize the viral occlusion, releasing virions
3. Nucleocapsids enter the midgut cell cytoplasm, migrate to nuclei, uncoate, and the viral DNA replicates. Some virus particles bud through the plasma membrane (ECV) to infect other cells and tissues. Late in infection some virus particles are embedded into IB.
4. When the larvae die, they release Ibs into the surroundings.

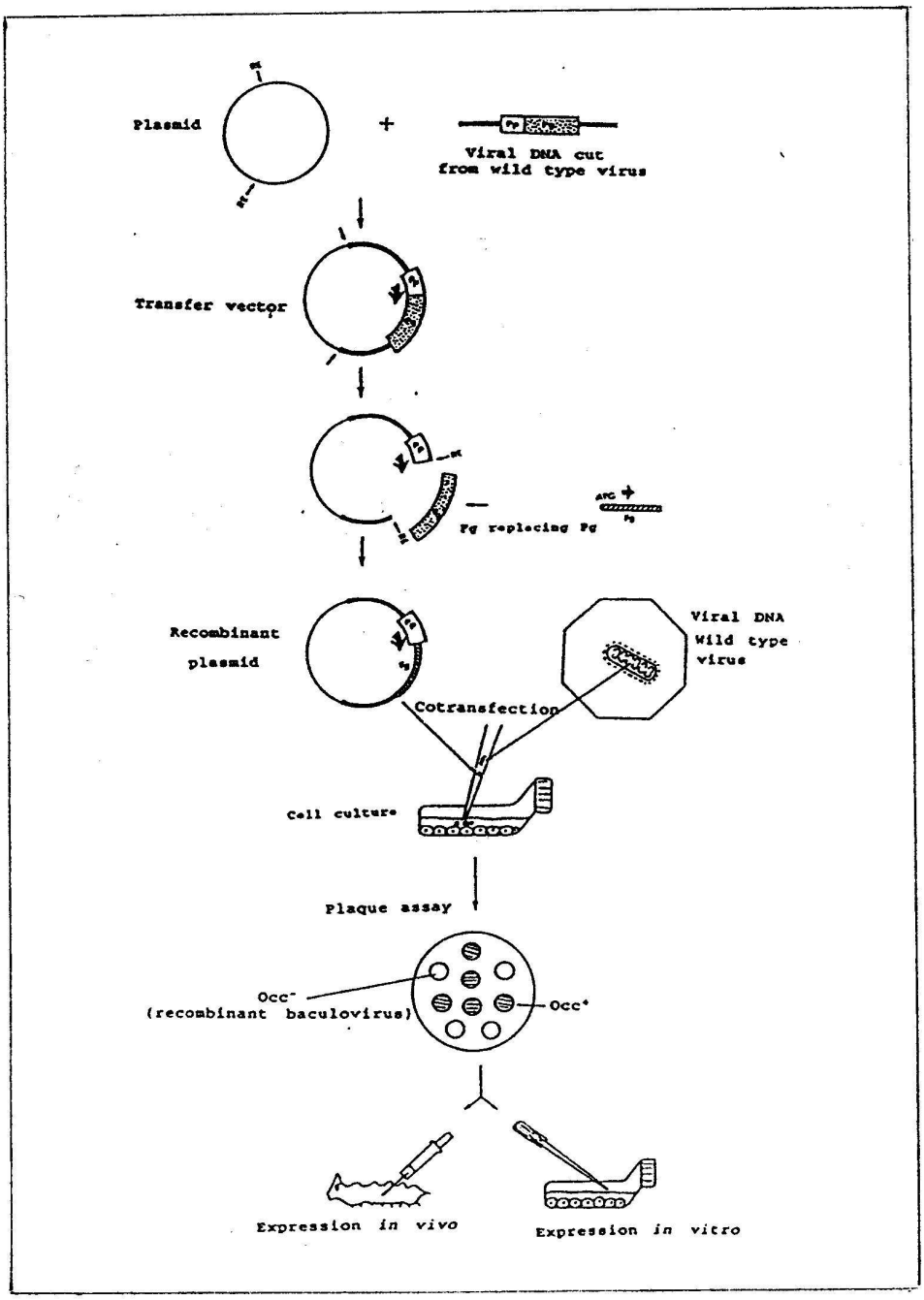

Figure 2.

Schemes of construction of recombinant baculovirus expression vector (rBEV) system.

(——) bacterial palsmid, (▬) viral DNA sequences, (Pp) polyhedrin promoter, (Pg) polyhedrin gene, (Fg) foreign gene with ATG start codon, (→/RE) restriction sites, (➡) direction of transcription. Schemes are not scale.

The early phase consists of immediate early (IE) genes which require no viral gene product for expression and can be transcribed by uninfected insect cells, and delayed early (DE) genes which require viral gene product for their transcription. The α-aminitin sensitive host RNA polymerase II are believed to be responsible for the transcription of the early genes (IE and DE; Blissard and Rohrman, 1990). The gene product of the ie-1 gene ,IE-1 has been shown to transactive the DE promoter (Guariono and Summer, 1988).

The late phase consists of late (L) and very late (VL) genes which are transcribed after or concurrently with the onset of viral DNA synthesis and transcribed from the late virus promoter. The α-aminitin resistant RNA polymerase is thought to be responsible for the transcription of the late genes (Yang *et al.*, 1991). Three early genes required for the expression of L and VL genes have been identified as ie-1, ie-n, and late expresion factor-2 (Passarelli and Miller, 1993).

The swicth of expression from L to VL stage is characterized by a gradual shut down of L genes and intensified expression of VL resulting an over expression of the VL genes. The swicth is important as it underlies the transition of ECV to IB or occluded virus (OV) production (Wei and Volkman, 1992).

One late gene, p74, has been iden-tified and known to be essential for virulence of baculovirus occlusion body (Kuzio et al., 1989). Three other late genes identified as p24 (Wolgamot et al., 1993), p23 (Pearson et al., 1988) and p87 (Lu and Carsten, 1991) have been shown to be associated with AcmNPV capsid-protein.

Two very late genes, identified as polyhedrin and p10, are hyper-expressed very late in infection from approximately 20 - 72 h post infection (Miller, 1988). Both genes are dispensable for replication of ECV (Vlak et al., 1987). Polyhedrin is important for polyhedron envelope (polyhedral IB) formation. In infected Sf-9 cell culture polyhedrin is produced abundantly, comprising 50 - 77% of the total stainable protein of the cell detected on SDS-polycrylamide gel (Summer and Smith, 1987). The p10 gene product is associated with fibrous structure in the nucleus and sytoplasm of infected-cells and thought to be the procecursor of polyhedron membrane (Vlak et al., 1988). Miller (1988) speculated that p10 gene product is associated with maturation of the polyhedron envelope.

Infection of insect cells with AcMNPV devoid of polyhedrin gene produced NOV, and infection with AcMNPV devoid of p10 produced virus that fail to lyse the cell late in infection (Blissard and Rohrman, 1990).

VI. Recombinat Baculovirus Expression Vector (rBEV) System

AcMNPV as vector for expressing foreign genes was developed based on the replacement of the abundantly expressed polyhedrin gene with a foreign gene of interest under the control of strong polyhedrin promotor (Miller, 1988).

The transfer vector was constructed from a plasmid shuttle vector

containing viral DNA flanking the polyhedrin promoter. The foreign gene was inserted either upstream from polyhedrin ATG start codon to express non-fused protein or down-stream from the intact or mutated start codon to express fused-protein. For non-fused protein production, the foreign gene insert requires an open reading frame fused in phase with th initiation codon ATG. Another construct in which the foreign gene was inserted out of phase down stream from the ATG expresses non-fused protein (Luckow and Summer, 1988).

An rBEV was produced by co-transfection of the recombinant transfer vector above with wild-type virus DNA into insect cells. The foreign gene would replace the wild type polyhedrin gene during viral DNA replication through homologous recombination (Miller, 1988).

When the cultured insect cells were infected with rBEV, they produced Occ^- which are distinctively different from the wild type virus producing Occ^+ in plaque assay. This different morphology provides a selection method for recombinant virus. Other baculovirus vectors have been constructed using the p10 promoter in place of or in addition to the polyhedrin promoter. In this vector, a ß-galactosidase gene under the control of the p10 promoter was inserted in addition to a foreign gene replacing the polyhedrin. When ß-galactosidase indicator (e.g. 5-bromo-4chloro-3indolyl-ß-D-glactosidase) was present in the agarose overlay used in plaque assay, the Occ^- virus forms blue plaque (Vialard et al., 1990) providing more rapid visual selection for recombinant virus. The recombinant virus was cloned by successive plaque purification and the clone was used to infect cells for large propagation of foreign proteins. Figure 2 summarised the system for recombinant baculovirus expression vector.

VII. Recombinant Protein Production

Insect cell-baculovirus expression systems have demonstrated a remarkable capacity to produce a variety of different fucntional proteins ranging from bacteriae, invertebrate, plant, to mammalian proteins (Table 1). Baculovirus-infected insect cells have been shown to carry out the post-translational modification process which is characteristic property of eukaryotic/mammalian gene expression systems. A major post-translational modification is the addition of carbohydrate moities to produce glycoproteins. Various baculovirus-produced mammalian gene products have been reported to be glycosylated; eg. Interveron-γ receptor (Fountoulakis et al., 1991) was N- and O-linked glycosylated, neutral endopeptidase (Fossie et al., 1991), human laminin B1 (Pikkarainen et al., 1992), and human plasminogen (Davidson and Castellino, 1991) were N-glycosylated. Other types of post translational modification eg. Phosphorylation (HIV core protein, Mills and Jones, 1990; Rat choline acetyl-tranferase, Habert et al., 1992) and cleavage (β-interferon, Smith et al., 1983; α-interferon, Maeda et al., 1985; mouse interleukin-3, Miyajima et al., 1987; soluble human and mouse inter-feron-γ receptor, Gentz et al., 1992) were also reported to occur in insect cells. Many baculovirus-infected cells secrete soluble forms of

protein which are biologically active as shown by induced immune response. Although the exact nature of the post-translational modification process by insect cells is not yet fully understood, the amount of proteins secreted, the range of protein types, and the simplicity and ease of production has led the baculovirus expression system to become a popular choice for expression of eucaryotic proteins.

VIII. Conclusion

Baculovirus is a natural infectious virus for insect. This property of baculovirus is used for the development of baculovirus expression vestor and the use of insect cells as host for recombinant protein production. The baculovirus expression vector was contracted by replacing polyhedrin gene which is abundantly expressed but does not affect viral replication in vitro, with the foreign gene of interest under the control of the strong polyhedrin promoter. As this foreign gene-carriying baculovirus vector infect cells, the virus replicates and during the replication the foreign gene is expressed and translated into protein. The protein produced by this system is biologically active.

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Table 1. Protein Produced Using Recombinant Baculovirus Expression Vector System

Transfer Vector	Protein Products	Cell Culture System	Yield	Notes	Reference
IpDC125	Papain precursor	Sf-9 cells in TNMH complete media	400 nmol protein/l culture	*in-vitro* activated precursor is indistinguishable from natural papain	Vernet *et al.*, 1990
pAcC5	Pheromone binding protein (PBP) of silkmoth *A. pernyi*	Sf-9 cells in TNMH complete media	1-2 mg/l	Secreted in mature form into the culture media. Molecular mass, signal peptide cleavage, and anti-PBP serum are similar to PBP isolated from moth antennae	Krieger *et al.*, 1992.
pAV6	α-subunit of carp gonadotropin	IPLB-SF-21 in TNMH complete	-	biologically active	Huang *et al.*, 1991
pVL941	Bovine immuno-deficiency virus	Sf-9 cells in Grace media + 7.5% FBS	15-50 μg per ml culture	similar to the immature forms produced in infected mammalian cells	Rasmussen *et al.*, 1990
pACJM3	Insecticidal crystal protein: *cry*IA (b) from *Bacillus thuringiensis*	IPLB-SF-21 in TNMH complete	5% of total cell protein	crystal ultrastructure is similar to native crystal with toxicity comparable to that produced in *E. coli*	Martens *et al.*, 1990.
pVL941	Human and mouse interferon-γ receptor	sf-9 cells in a. IPL-41 Media+0.4% yeastolate + 1,5% FCS +cod liver oil b. Excell-400+2% FCS	5-15 μg/ml culture	soluble, N- and O-linked glycosylated, binds interferon-γ	a. Fountoulakis *et al.*, 1991; b. Gentz *et al.*, 1992
pAcRP14	HIVcore protein, p24	sf-9 cells in TC-100 media	>50 mg/l culture	Phosphorilated, can serves as diagnostic reagent for detecting p24 antobodies from sera of AIDS patients	Mills and Jones, 1990
pAcVC3	Bluetongue virus (BTV) rorelike particles (VP3 and VP7)	sf-9 cells in SF900-II in shake flasks	53-60 mg/l culture	Size, appearance, and stoichiometric arrangement of VP3 to VP7 (2:15) are similar to authentic BTV cores	Baker *et al.*, 1993
pVL1393	Human transcobalamin II isoprotein (TCII)	sf-9 cells in TNMH	2-4 μg/ml culture media	The recombinant TCII polypeptides are identical to TCII polypeptides purified from human plasma	Quadross *et al.*, 1993.

Table 1. (contonued)

Transfer Vector	Protein Products	Cell Culture System	Yield	Notes	Reference
pAC373	Human para influenza virus type-3	Sf-9 and IPLB-SF-21 cells in TNMH complete	-	Low dose of vaccination protecting rat against live virus challenge, and the immunogenicity is equivalent to CHO cell-expressed virus	Lehman *et al.*, 1993 Weyer and Possee, 1991
pAV6	Hepatitis B virus X protein	IPLB-SF-21-AE	-	Functionally identical to that produce in mammalian cells	Spandau *et al.*, 1991
pAc373 pVL941	murine β-2-micro globulin	Sf-9 cells in TC-100+0.33% Lactalbumin + 4% FBS	10 μg/10^6 cells	biologically active and indistinguishable from that produced by mouse cells	Godeau *et al.*, 1991
pVL	Human nerve growth factor (NGF)	Sf-9 cells in TNMH complete	2-3 mg/l	Fully folded and properly processed, binds to NGF receptor, and stimulates neurite outgrowth	Buxser *et al.*, 1991
pAc373	Human CD-23	Sf-9 cells in 10% FCS containing TC-100 media	-	Glycosylated and in the presence of interleukin-1 promotes survival of germinal centre B cells. Antigenically similar to natural CD23 produced in RPMI 8866 cells	Graber *et al.*, 1992.