SECTION II

EXPRESSION OF PROTEINS IN INSECT CELLS USING BACULOVIRAL VECTORS

UNIT 16.8

Overview of the Baculoviral Expression System

16.8

Baculoviruses have emerged as a popular system for overproducing recombinant proteins in eukaryotic cells for several reasons. First, unlike bacterial expression systems, the baculovirus-based system is eukaryotic and thus uses many of the protein modification, processing, and transport systems present in higher eukaryotic cells. In addition, the baculoviral expression system uses a helper-independent virus that can be propagated to high titers in insect cells adapted for growth in suspension cultures, making it possible to obtain large amounts of recombinant protein with relative ease. The majority of this overproduced protein remains soluble in insect cells by contrast with the insoluble proteins often obtained from bacteria. Furthermore, the viral genome is large (130 kbp) and thus can accommodate large segments of foreign DNA. Finally, baculoviruses are noninfectious to vertebrates, and the polyhedrin promoter has been shown to be inactive in mammalian cells—an advantage over other systems when expressing oncogenes or potentially toxic proteins.

BACULOVIRUS LIFE CYCLE

Currently, the most widely used baculovirus expression system utilizes a lytic virus known as *Autographa californica* multiply enveloped nuclear polyhedrosis virus (AcMNPV; hereafter called baculovirus). This virus is the prototype of the family *Baculoviridae*. It is a large, enveloped, double-stranded DNA virus that infects arthropods. The baculoviral expression system takes advantage of some unique features of the viral life cycle (Fig. 16.8.1). See Doerfler and Bohm (1986) for a comprehensive review.

Two types of viral progeny are produced during the life cycle of the virus: extracellular virus particles (nonoccluded viruses) and polyhedra-derived virus particles (occluded viruses). Occluded viral particles are embedded in proteinaceous viral occlusions called polyhedra within the nucleus of infected cells. The polyhedrin protein (29 kDa) is the major protein component of the occlusion bodies. Although the polyhedrin protein is essential for survival of the virus in nature, it is dispensable for virus survival and propagation in tissue culture cells.

BACULOVIRAL EXPRESSION SYSTEM

To produce a recombinant virus that expresses the gene of interest, the gene is first cloned into a special vector (see below). The gene, once cloned into the expression vector, is flanked both 5′ and 3′ by polyhedrin-specific sequences and is positioned downstream of the polyhedrin promoter. Next, the recombinant vector is transfected along with purified wild-type viral

DNA into insect cells. In a homologous recombination event, the foreign gene is inserted into the viral genome and the polyhedrin gene is excised. Recombinant viruses lack the polyhedrin gene and in its place contain the inserted gene, whose expression is controlled by the polyhedrin promoter.

Homologous recombination occurs at low frequency (1-5%); thus, the majority of the virus produced after cotransfection is still wild-type virus. Therefore, a method is necessary to identify recombinant viruses. One of the beauties of this expression system is a visual screen allowing recombinant viruses to be distinguished. The polyhedrin protein is produced at very high levels in the nuclei of infected cells at late times after viral infection and accumulates to form occlusion bodies that also contain embedded virus particles. These occlusion bodies, ≤15 μm in size, are highly refractile, giving them a bright shiny appearance under the light microscope. Cells infected with recombinant viruses lack occlusion bodies. To distinguish recombinant virus from wild-type virus, the transfection supernatant is plaqued onto a monolayer of insect cells (*UNIT 16.10*). The plaques are then screened under the light microscope for the presence (wild-type virus) or absence (recombinant virus) of occlusion bodies.

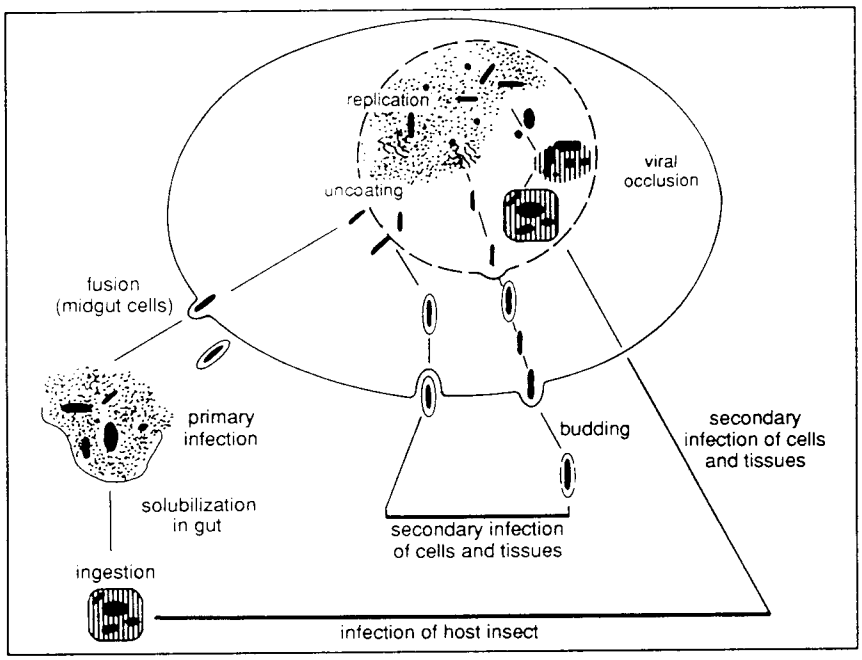

Figure 16.8.1 Baculovirus life cycle. Reproduced from Summers and Smith (1987) with permission from the Texas Agricultural Experiment Station. Viruses enter cells by adsorptive endocytosis and move to the nucleus where their DNA is released. Both DNA replication and and viral assembly take place in the nuclei of infected cells to generate two types of viral progeny. These include extracellular (nonoccluded) virus particles and polyhedra-derived (occluded) virus particles. Extracellular virus is released from the cell by budding, starting at ~12 hr postinfection or until cellular lysis. Polyhedra-derived virus is embedded in proteinaceous viral occlusions, the major protein component of which is the viral polyhedrin protein. Secondary infection of cells and tissues occurs by two pathways. In the first, the extracellular virus, once budded from the site of primary infection, is free to infect neighboring cells by the pathway just described. Alternatively, polyhedra-derived virus is released from occlusion bodies after an infected food source is ingested by a new host.

PROTEIN PROCESSING IN INSECT CELLS

Because baculoviruses infect invertebrate cells, it is possible that processing of proteins produced by them are different from proteins produced by vertebrate cells. While this seems to be the case for some posttranslational modifications, it is not the case for others. For further information on protein processing in insect cells, see Jarvis and Summers (1990) and Luckow and Summers (1988).

STEPS FOR OVERPRODUCING PROTEINS USING THE BACULOVIRAL EXPRESSION SYSTEM

Overproduction of recombinant proteins using the baculoviral system is presented in *UNITS 16.9-16.11*. A brief overview is presented in Fig. 16.8.2.

REAGENTS AND SOLUTIONS FOR THE BACULOVIRAL EXPRESSION SYSTEM

Commonly used reagents and solutions are summarized below.

1. *Spodoptera frugiperda* (*Sf*9) cells (derived from fall armyworm ovaries) from American Type Culture Collection, accession #CRL 1711.

2. Insect cell culture medium (Grace's Antheraea medium), both 1× and 2× strength in powdered or liquid form, from GIBCO/BRL or JR Scientific. For instructions on media preparation from individual components, see Summers and Smith (1987).

3. Difco lactalbumen hydrolysate and yeastolate from VWR Scientific.

4. Incubator set to 27±1°C; CO_2 is not required.

5. Magnetic spinner flasks from Bellco.

6. Stir plate for multiple spinners (Bellco #7760-06005).

7. Fetal bovine serum (FBS). Obtain and test different lots from a number of suppliers. The lot that promotes the best growth rate and cell viability should be purchased in bulk.

8. SeaPlaque agarose from FMC Bioproducts (#50102).

9. Lux 60-mm tissue culture plates from Nunc Intermed (#5220).

10. Antibiotics (optional)—gentamicin and amphotericin B (Fungizone from Flow Laboratories).

11. Microscope—either an inverted light microscope or a dissecting scope.

12. A kit containing appropriate cloning vectors, manual of methods, and wild-type baculovirus is available from Dr. Max D. Summers, Department of Entomology, Texas Agricultural Experiment Station, Texas A & M University, College Station, TX 77843 (409-845-9730). It is necessary to sign a licensing agreement before kit will be sent. A commercial kit, MaxBac, is also available from Invitrogen (*APPENDIX 4*).

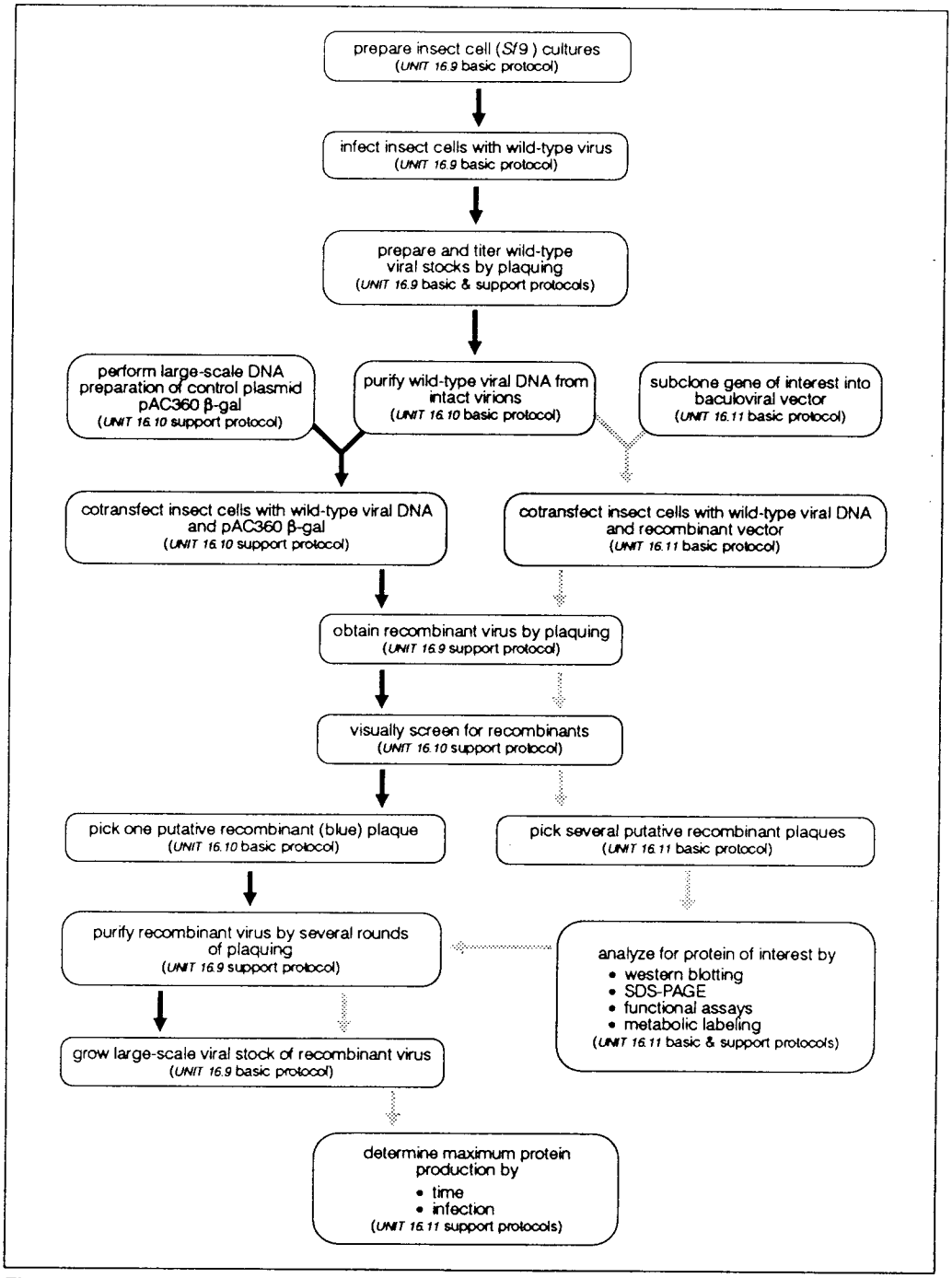

Figure 16.8.2 Flow chart for expression of proteins in insect cells using baculoviral vectors. The black arrows indicate the protocols used to optimize conditions and the light arrows indicate the protocols used to generate recombinant baculoviruses expressing the protein of interest..

References: Doerfler and Bohm, 1986; Jarvis and Summers, 1990; Luckow and Summers, 1988; Summers and Smith, 1987.

Contributor: Helen Piwnica-Worms