SECTION IV

EXPRESSION OF PROTEINS IN MAMMALIAN CELLS USING VACCINIA VIRAL VECTORS

UNIT 16.15

Overview of the Vaccinia Virus Expression System

16.15

Vaccinia virus was introduced in 1982 as a vector for transient expression of genes in mammalian cells. This expression system differs from others in that transcription occurs in the cytoplasm of the cell rather than in the nucleus. As a vector, vaccinia virus has a number of useful characteristics, including a capacity that permits cloning large fragments of foreign DNA (20 kbp), retention of infectivity after insertion of foreign DNA, a wide host range, a relatively high level of protein synthesis, and "appropriate" transport, secretion, processing, and posttranslational modifications as dictated by the primary structure of the expressed protein and the cell type used. Laboratory applications of vaccinia virus vectors include production of biologically active proteins in tissue culture, analysis of mutant forms of proteins, determination of transport and processing signals, and immunological studies.

Several variations of the vaccinia vector system have been developed. Most commonly, after obtaining the virus stock (*UNIT 16.16*), the gene of interest is placed under control of a vaccinia virus promoter and integrated into the genome of vaccinia so as to retain infectivity (*UNIT 16.17*). Alternatively, expression can be achieved by transfecting a plasmid containing the vaccinia promoter–controlled gene into a cell that has been infected with wild-type vaccinia. These recombinant viruses are then characterized using various methods (*UNIT 16.18*).

VACCINIA REPLICATION CYCLE

Vaccinia is the prototypal member of the Orthopoxvirus genus of the *Poxviridae* family (Fig 16.15.1). Poxviruses differ from other eukaryotic DNA viruses in that they replicate in the cytoplasm rather than in the nucleus. Vaccinia virus has a linear, double-stranded DNA genome of nearly 200,000 bp that encodes most or all proteins needed for replication and transcription in the cytoplasm.

EFFECTS OF VACCINIA INFECTION

Vaccinia virus can productively infect most mammalian and avian cell lines, with a few exceptions such as Chinese hamster ovary (CHO) cells. Infection generally results in rapid inhibition of host nucleic acid and protein synthesis. At the time of maximal late gene expression, host protein synthesis has been largely suppressed, facilitating the identification of viral or recombinant proteins by pulse-labeling with radioactive amino acids.

In fibroblasts, the initial cytopathic effect—which is obvious by several hours postinfection—is cell rounding. Nevertheless, the majority of cells remain intact for ≥48 hr. Approximately 100 to 200 plaque-forming units (pfu), equivalent to ~2500 to 5000 particles, are made per cell within a 20- to 40-hr period. With the commonly used vaccinia virus WR strain, 95% of the infectious virus remains cell-associated. With some other vaccinia virus strains, notably IHD-J, larger amounts of extracellular virus are produced.

VACCINIA VECTOR EXPRESSION SYSTEM

Genes or cDNAs containing open reading frames derived from prokaryotic, eukaryotic, or viral sources have been expressed using vaccinia virus vectors. The gene of interest is usually placed next to a vaccinia promoter and this expression cassette is then inserted into the virus genome by homologous recombination (*UNIT 16.17*). Use of poxvirus promoters is essential because cellular and other viral promoters are not recognized by the vaccinia transcriptional apparatus. Strong late promoters are preferable when high levels of expression are desired.

A number of plasmids have been designed with restriction endonuclease sites for insertion of foreign genes downstream of vaccinia promoters (*UNITS 16.17 & 16.19*). The expression cassette is flanked by vaccinia DNA to permit

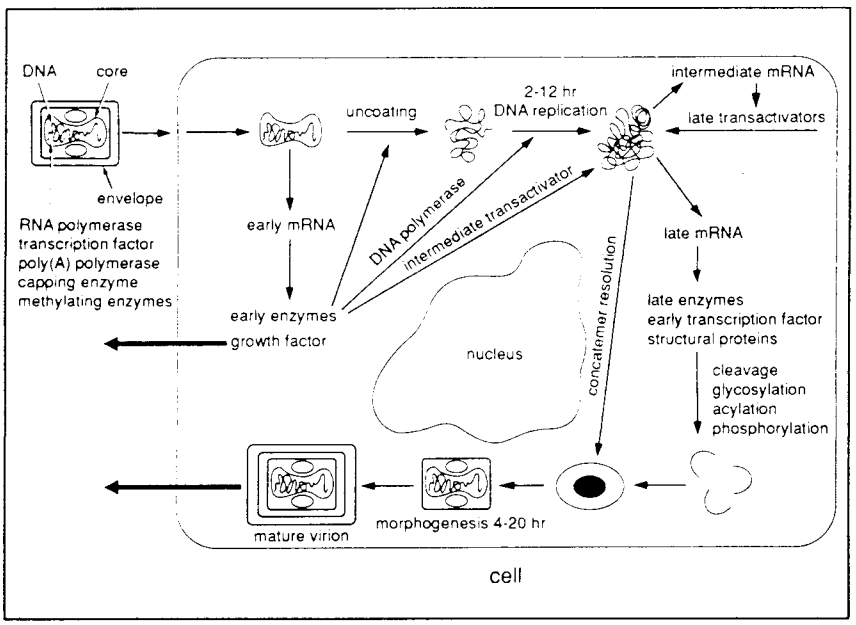

Figure 16.15.1 Replication cycle of vaccinia virus. After entry of vaccinia virus into cells, early genes are expressed, leading to secretion of several proteins (including a growth factor), uncoating of the virus core, synthesis of DNA polymerase (and other replication proteins), RNA polymerase subunits, and transcriptional transactivators of the intermediate class of genes. After DNA replication, intermediate mRNAs are made, some of which encode late transcriptional transactivators, leading to expression of late genes. The latter encode structural proteins, enzymes, and early transcription factors which are packaged in the assembling virus particles. Some mature virions are wrapped in Golgi-derived membranes and are released from the cell. The bold arrows indicate products that exit the cell. Reprinted with permission from Raven Press.

homologous recombination when the plasmid is transfected into cells that have previously been infected with wild-type vaccinia virus. The flanking vaccinia virus DNA is chosen so that recombination will not interrupt an essential viral gene.

Without selection, the ratio of recombinant to parental vaccinia virus is usually ~1:1000. Although this frequency is high enough to permit the use of plaque hybridization (*UNITS 6.3 & 6.4*) or immunoscreening (*UNIT 6.7*) to pick recombinant viruses, a variety of methods to facilitate recombinant-virus identification has been employed. Three widely used selection or screening techniques are described in *UNIT 16.17*. Most commonly, the expression cassette is flanked by segments of the vaccinia thymidine kinase (TK) gene so that recombination results in inactivation of TK. Alternatively, recombinant viruses can be selected by the co-expression of a bacterial antibiotic resistance gene such as guanine phosphoribosyltransferase (*gpt*). Finally, co-expression of the *E. coli lacZ* gene allows color screening of recombinant virus plaques with Xgal (*UNIT 16.17*).

The expression of genes using the vaccinia expression system is presented in detail in *UNITS 16.16-16.18* and outlined in Figure 16.15.2.

SAFETY PRECAUTIONS FOR USING VACCINIA

Vaccinia virus is not to be confused with either variola virus, another member of the Orthopoxvirus genus that caused smallpox prior to its eradication, or with varicella virus, a herpes virus that causes chicken pox. Vaccinia virus was used as a live vaccine to prevent smallpox and therefore many of us already have been immunized with vaccinia. A residual scar, commonly on the upper arm, may be evidence of that vaccination.

To prevent laboratory infections, the Centers for Disease Control (CDC) and the National Institutes of Health (NIH) still recommend that all individuals who come in contact with vaccinia virus receive vaccinations at 10-year intervals. The CDC has supplied vaccine for such purposes when requested by qualified workers. Eczema or an immunodeficiency disorder in the laboratory worker or a personal friend, however, may be a contraindication to vaccination, *which should only be given under medical supervision.* In contrast, the Advisory Committee on Dangerous Pathogens and the Advisory Committee on Genetic Mediators in the United Kingdom do not recommend vaccination except under special circumstances.

Vaccinia virus is very stable and parenteral inoculation, ingestion, and droplet or aerosol exposure of mucous membranes are the primary hazards to laboratory or animal care personnel. Standard safety level 2 (BL-2) practices and class I or II biological safety cabinets should be employed. Institutional biosafety offices should be contacted to determine current policy regarding vaccination and physical containment. Additional precautions may be necessary for expression of certain genes such as toxins or large segments of other viral genomes, and guidelines for recombinant DNA work should be consulted. Approval of local biosafety committees may be necessary.

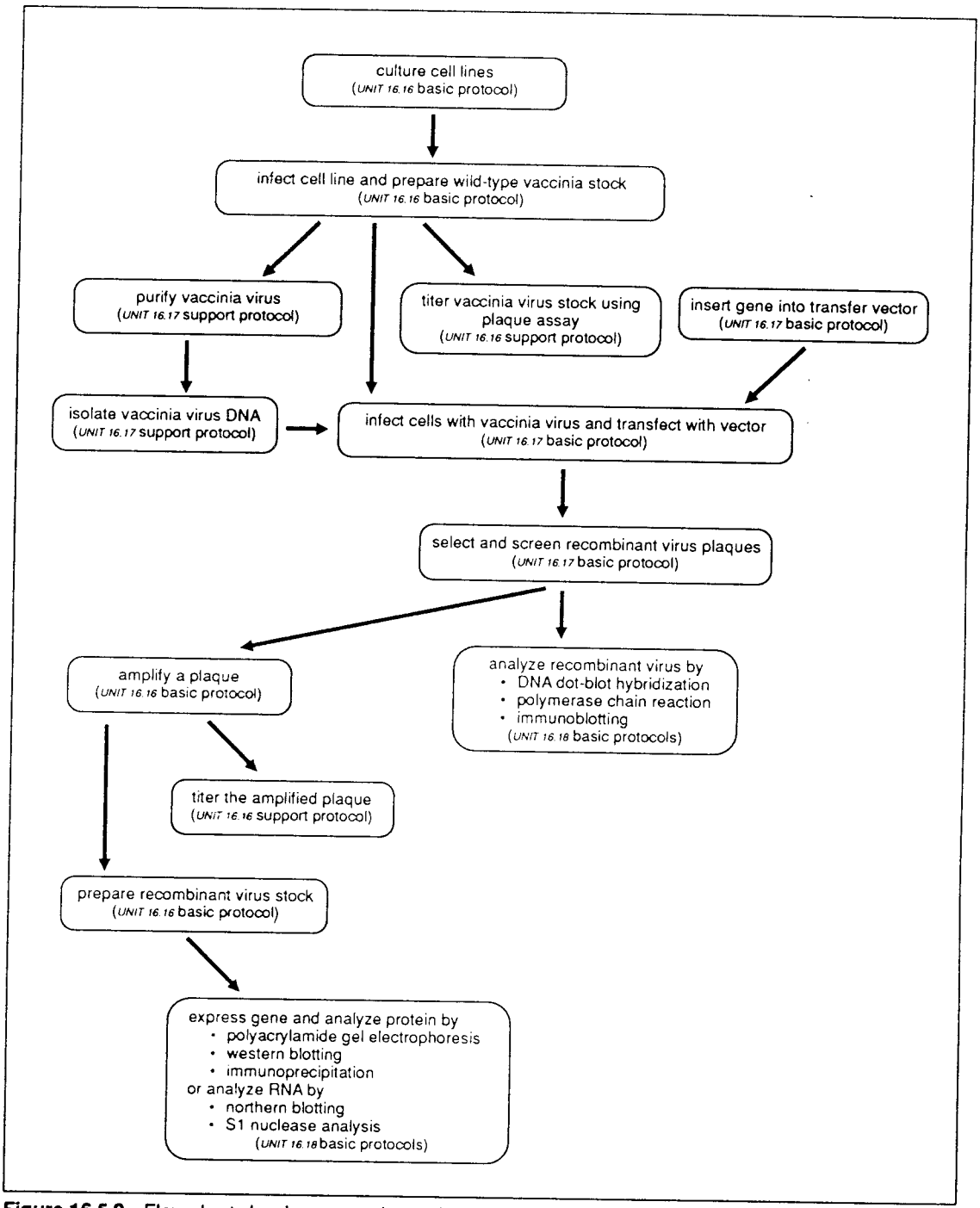

Figure 16.5.2 Flow chart showing protocols used to generate recombinant vaccinia virus.

Reference: Moss, 1990.

Contributors: Bernard Moss and Patricia L. Earl