high-level expression. It is often easier to obtain high titers (or product concentration) when producing monoclonal antibodies from hybridoma cultures.

While the quality of the protein product may change upon scale-up with any system, this issue is particularly important with animal cell cultures. This contention is due, in part, to the fact that animal cell cultures are used primarily because authenticity of the protein product is a major concern. Since culture conditions (shear, glucose, amino sugars, DO, etc.) can change upon scale-up, the efficiency of cellular protein processing can change, altering the level of posttranslational processing. Further, it has been shown that protein quality may change with harvest time in batch cultures. This change may be due to alterations in intracellular machinery, but often it is due to release of proteases and siladase (an enzyme that removes the silalic acid cap from glycosylated proteins) from dead cells. Also, excessive levels of protein production may saturate the intracellular protein-processing organelles (i.e., ER and Golgi), leading to incompletely processed proteins. These problems can significantly impact process strategy. One well-known company has been forced to harvest 24 h early to maintain the silalic acid/protein ratio specified for the product. Early harvest resulted in a 30% loss in protein concentration (as compared with delayed harvest).

Strategies to reduce such problems include selection of cell lines or genetic manipulation of cell lines with reduced levels of siladase production or enhanced protein processing capacity. Redesign of medium can be beneficial; chemicals that inhibit undesirable extracellular enzyme activity can be added or precursors added (e.g., amino sugars) to improve processing. Cell lysis can be reduced by adding genes (e.g., *bcl* 2) to the host cell that reduce apotosis. An engineering solution is to remove the product from the medium as it is formed. For example, perfusion systems with an integrated product capture step can be used.

14.3.6. Insect Cell–Baculovirus System

A popular alternative for protein production at small (< 100 l) or laboratory scale is the insect cell–baculovirus system. This system is particularly attractive for rapidly obtaining biologically active protein for characterization studies. Typical host cell lines come from the fall armyworm (*Spodoptera frugiperda*) and the cabbage looper (*Trichoplusia ni*). The baculovirus, *Autographa californica* nuclear polyhedrosis virus (Ac NPV), is used as a vector for insertion of recombinant DNA into the host cell.

This virus has an unusual biphasic replication cycle in nature. An insect ingests the occluded form, in which multiple virus particles are embedded in a protein matrix. The protein matrix protects the virus when it is on a leaf from environmental stresses (e.g., UV radiation). This protein matrix is from the polyhederin protein. In the mid-gut of the insect, the matrix dissolves, allowing the virus to attack the cells lining the insect's gut; this is the primary infection. These infected cells release a second type of virus; it is nonoccluded (no polyhederin matrix) and buds through the cell envelope. The nonoccluded virus (NOV) infects other cells throughout the insect (secondary infection).

In insect cell culture, only NOVs are infectious, and the polyhederin gene is unnecessary. The polyhederin promoter is the strongest known animal promoter and is expressed late in the infection cycle. Replacing the polyhederin structural gene with the gene for a target protein allows high-level target protein production (up to 50% of cellular protein). Proteins that are secreted and glycosylated are often made at much lower levels than nonsecreted proteins.