In addition to high expression levels, the insect–baculovirus system offers safety advantages over mammal–retrovirus systems. The insect cell lines derived from ovaries or embryos are continuous but not transformed. The baculovirus is not pathogenic toward either plants or mammals. Thus, the insect–baculovirus system offers potential safety advantages. Another important advantage is that the molecular biology and high-level expression of correctly folded proteins can be achieved in less than a month.

This system also has the cellular machinery to do almost all the complex posttranslational modifications that mammalian cells do. However, even when the machinery is present, at least some of the proteins produced in the insect cell–baculovirus system are not processed identically to the native protein. In some cases, their slight variations may be beneficial (e.g., increased antigenic response in the development of an AIDS vaccine), while in others they may be undesirable. While complex glycoforms (including silaic acid) have been made, it is more common to observe only simple glycoforms. Production of complex forms requires special host cell lines and is sensitive to culture conditions.

The insect cell–baculovirus system is a good system to illustrate a holistic perspective on heterologous protein production. Any bioprocess for protein production is complex, consisting of the nonlinear interaction of many subcomponents. Thus the optimal process is not simply the sum of individually optimized steps. Figure 14.2 presents a holistic view for the insect cell–baculovirus system. Because of the viral component, this system is even more complex than most other bioprocesses, as the infection process and resulting protein expression kinetics must be considered. One factor is the ratio of infectious particles to cells (e.g., multiplicity of infection or MOI), which alters the synchrony of infection and the resulting protein expression kinetics. Another is the genetic design of the virus (which shares many of the general features of vector design). Also, the quality of the virus stock is important; if the virus stock is maintained incorrectly, mutant virus can form. One example is the formation of *defective interfering particles* (DIPs) that reduce protein expression in the culture by 90% when high MOIs are used.

You should work from the bottom of Fig. 14.2 toward the top. What is the desired product quality? What is the product worth? These questions then guide selection of bioprocess strategies to achieve the cost and quality desired. To develop that strategy requires an understanding of the basic kinetics and capabilities of the biological system. Understanding these requirements guides selection of the specific host cell line, the medium, and the molecular design of the virus. For example, the addition of serum to the medium of some *Ti ni* cell lines results in production of proteins with complex N-linked glycosylation, including a sialic acid cap, which may be a requirement for product quality. However, the use of serum alters growth kinetics, expression levels (often less), and the difficulty of purification, which may alter cost. Such trade-offs need to be considered with respect to alternative approaches (e.g., development of a genetically engineered host that could perform the same reactions, but in serum-free medium).

14.3.7. Transgenic Animals

In some cases proteins with necessary biological activity cannot be made in animal cell culture. While posttranslational protein processes, such as N-linked glycosylation, can be done in cell culture, other more subtle forms of posttranslational processing may not be done satisfactorily. An alternative to cell culture is the use of transgenic animals. Animals