ter 10). Also, methanol is flammable, and handling large volumes of methanol is a safety concern. Nonetheless, these methylotrophic yeasts are of increasing importance.

Fungi, such as *Aspergillus nidulans* and *Trichoderma reesei,* are also potentially important hosts. They generally have greater intrinsic capacity for protein secretion than *S. cerevisiae.* Their filamentous growth makes large-scale cultivation somewhat difficult. However, commercial enzyme production from these fungi is well established, and the scale-up problems have been addressed. The major limitation has been the construction of expression and secretion systems that can produce as large amounts of extracellular heterologous proteins as some of the native proteins. A better understanding of the secretion pathway and its interaction with protein structure will be critical for this system to reach its potential.

All these lower eucaryotic systems are inappropriate when complex glycosylation and posttranslational modifications are necessary. In such cases, animal cell tissue culture has been employed.

14.3.5. Mammalian Cells

Mammalian cell culture is chosen when the virtual authenticity of the product protein must be complete. Authenticity implies not only the correct arrangement of all amino acids, but also that all posttranslational processing is identical to that in the whole animal. In some cases, the cells in culture may not do the posttranslation modifications identically to those done by the same cell while in the body. But for bioreactor processes, mammalian cell tissue culture will provide the product closest to its natural counterpart. Another advantage is that most proteins of commercial interest are readily excreted.

Slow growth, expensive media, and low protein expression levels all make mammalian cell tissue culture very expensive. As discussed in Chapter 12, a wide variety of reactor systems are being used with animal cell cultures. Although many of these can improve efficiency significantly, processes based on mammalian cells remain very expensive.

Several cell lines have been used as hosts for the production of proteins using recombinant DNA. The most popular hosts are probably lines of CHO (Chinese hamster ovary) cells.

In addition to the cost of production, mammalian cells face other severe constraints. Normal cells from animals are capable of dividing only a few times; these cell lines are *mortal.* Some cells are *immortal* or *continuous* and can divide continuously, just as a bacterium can. Continuous cell lines are *transformed* cells. Cancer cells are transformed also (i.e., have lost the inhibition of cell replication). The theoretical possibility that a cancer-promoting substance could be injected along with the desired product necessitates extreme care in the purification process. It is particularly important to exclude nucleic acids from the product. The use of transformed cells also requires cautions to ensure worker safety.

Additionally, the vectors commonly used with mammalian cell cultures have been derived from primate viruses. Again, there is concern about the reversion of such vectors back to a form that could be pathogenic in humans.

Most of these vectors cannot give high expression levels of the target protein in common host cells (usually < 5% of total protein). However, higher levels of expression can be obtained (e.g., > 100 mg/l of secreted, active protein) through amplification of number of gene copies. It may take six months with a CHO cell line to achieve stable,