active, and released into the medium. The *E. coli* product was primarily in the form of inclusion bodies, and thus biologically inactive, misfolded, and insoluble. The process to resolubilize and refold the *E. coli* product into active material requires extra steps. The recovery process for the CHO cell material requires five steps, while 16 steps are required for the *E. coli* process. The larger the number of steps, the greater the possibility of yield loss. Total recovery of 47% with the CHO-produced material was possible compared to only 2.8% for the *E. coli*-produced material.

The extra steps in the *E. coli* process are for cell recovery, cell breakage, recovery of inclusion bodies, resolubilization of inclusion bodies, concentration, sulfination, refolding, and concentration of the renatured protein. The difficulty of these processes depends on the nature of the protein; tPA is particularly difficult. With tPA the concentration of tPA had to be maintained at 2.5 mg/l or less, and refolding is slow, requiring 48 hours. A 20% efficiency for renaturation was achieved. Many proteins can be refolded at higher concentrations (up to 1 g/l) and much more quickly. For tPA the result is unacceptably large tanks and very high chemical usage. In this case, five tons of urea and 26 tons of guanidine would be necessary to produce only 11 kg of active tPA.

For tPA the required bioreactor volumes were 14,000 l for the CHO process and 17,300 l for the *E. coli* process. The capital costs were $11.1 million for the CHO process and $70.9 million for the *E. coli* process, with 75% of that capital cost being required for the refolding tanks. Under these conditions, the unit production costs are $10,660/g for the CHO process versus $22,000/g for the *E. coli* process. The rate of return on investment (ROI) for the CHO process was 130% versus only 8% for the *E. coli* process. However, if the refolding step yield were 90% instead of 20%, the overall yield would improve to 15.4%, and the unit production cost would fall to $7,530 with an ROI of 85% for the improved *E. coli* process at production of 11 kg/yr. If the *E. coli* plant remained the original size (17,300 l fermentor) so as to produce 61.3 kg/yr, the unit production cost would drop to $4,400/g. The cost of tPA from the CHO process is very sensitive to cost of serum in the medium. If the price of media dropped from $10.5/l to $2/l (e.g., 10% to 2% serum in the medium), the cost of the CHO cell product would drop to $6,500/g.

A primary lesson from this exercise is the difficulty of making choices of host–vector systems without a fairly complete analysis. The price will depend on the protein, its characteristics, and intended use. Changes in process technology (e.g., low serum medium for CHO cells or protein secretion systems in *E. coli*) can have dramatic effects on manufacturing costs and choice of the host–vector system.

14.4. PROCESS CONSTRAINTS: GENETIC INSTABILITY

There is a tension between the goal of maximal target-protein production and the maintenance of a vigorous culture. The formation of large amounts of foreign protein is always detrimental to the host cell, often lethal. Cells that lose the capacity to make the target protein often grow much more quickly and can displace the original, more productive strain. This leads to *genetic instability* (see Fig. 14.3).

Genetic instability can occur due to *segregational loss, structural instability, host cell regulatory mutations,* and the *growth-rate ratio* of plasmid-free or altered cells to