14.5. CONSIDERATIONS IN PLASMID DESIGN TO AVOID PROCESS PROBLEMS

When we design vectors for genetic engineering, we are concerned with elements that control plasmid copy number, the level of target-gene expression, and the nature of the gene product, and we must also allow for the application of selective pressure (e.g., antibiotic resistance). The vector must also be designed to be compatible with the host cell.

Different *origins of replication* exist for various plasmids. The origin often contains transcripts that regulate copy number. Different mutations in these regulatory transcripts will yield greatly different copy numbers. In some cases, these transcripts have temperature-sensitive mutations, and temperature shifts can lead to *runaway replication* in which plasmid copy number increases until cell death occurs.

Total protein production depends on both the number of gene copies (e.g., the number of plasmids) and the strength of the promoter used to control transcription from these promoters. Increasing copy number while maintaining a fixed promoter strength increases protein production in a saturable manner. Typically, doubling copy numbers from 25 to 50 will increase protein production twofold, but an increase from 50 to 100 will increase protein production less than twofold. If the number of replicating units is above 50, pure segregational plasmid loss is fairly minimal. Most useful cloning vectors in *E. coli* have stable copy numbers from 25 to 250.

Many promoters exist. Some of the important ones for use in *E. coli* are listed in Table 14.3. An ideal promoter would be both very strong and tightly regulated. A zero basal level of protein production is desirable, particularly if the target protein is toxic to the host cell. A rapid response to induction is desirable, and the inducer should be cheap and safe. Although temperature induction is often used on a small scale, thermal lags in a large fermentation vessel can be problematic. Increased temperatures may also activate a heat-shock response and increased levels of proteolytic enzymes. Many chemical inducers are expensive or might cause health concerns if not removed from the product. Some promoters respond to starvation for a nutrient (e.g., phosphate, oxygen, and energy), but the control of induction with such promoters can be difficult to do precisely. The recent isolation of a promoter induced by oxygen depletion may prove useful, because oxygen levels can be controlled relatively easily in fermenters.

Anytime a strong promoter is used, a strong transcriptional terminator should be used in the construction. Recall from Chapter 4 that a terminator facilitates the release of RNA polymerase after a gene or operon is read. Without a strong terminator, the RNA polymerase may not disengage. If the RNA polymerase reads through, it may transcribe undesirable genes or may disturb the elements controlling plasmid copy numbers. In extreme cases, this might cause runaway replication and cell death.

The nature of the protein and its localization are important considerations in achieving a good process. To prevent proteolytic destruction of the target protein, a hybrid gene for the production of a fusion protein can be made. Typically, a small part of a protein native to the host cell is fused to the sequence for the target protein with a linkage that can be easily cleaved during downstream processing. Also, fusion proteins may be constructed to facilitate downstream recovery by providing a "handle" or "tail" that adheres easily to a particular chromatographic medium.