The key feature of this category of genetic instability is that a host cell mutation imparts a growth advantage to the mutant, so that it will eventually dominate the culture. In this case the mutant cell will contain unaltered plasmids but will make very little of the target, plasmid-encoded protein.

14.4.4. Growth-rate-dominated Instability

The importance of all three of these factors (segregational loss of plasmid, structural alterations of the plasmid, and host cell mutations) depends on the growth-rate differential of the changed cell-plasmid system to the original host–vector system. If the altered host–vector system has a distinct growth advantage over the original host–vector system, the altered system will eventually dominate (i.e., genetic instability will occur).

The terms used to describe the cause of genetic instability are based on fairly subtle distinctions. For example, if genetic instability is due to segregational instability, we would infer that the rate of formation of plasmid-free cells is high. In this case, the number of plasmid-free cells would be high irrespective of whether the plasmid-free cells had a growth-rate advantage. If, on the other hand, we claimed that the genetic instability is growth-rate dependent, we would imply that the rate of formation of plasmid-free cells is low, but the plasmid-free cells have such a large growth-rate advantage that they outgrow the original host–vector system. In most cases, growth-rate dependent instability and one of the other factors (segregational loss, structural changes in the plasmid, or host cell mutations) are important.

The growth-rate ratio can be manipulated to some extent by the choice of medium (e.g., the use of *selective pressure* such as antibiotic supplementation to kill plasmid-free cells) and the use of production systems that do not allow significant target-protein production during most of the culture period. For example, an inducible promoter can be turned on only at the end of a batch growth cycle when only one or two more cell doublings may normally occur. Before induction, the *metabolic burden* imposed by the formation of the target protein is nil, and the growth ratio of the altered to the original host–vector system is close to 1 (or less if selective pressure is also applied). This *two-phase fermentation* can be done as a modified batch system, or a multistage chemostat could be used. In a two-stage system, the first stage is optimized to produce viable plasmid-containing cells, and production formation is induced in the second stage. The continual resupply of fresh cells to the second stage ensures that many unaltered cells will be present.

The problem of genetic instability is a more significant in commercial operations than in laboratory-scale experiments. The primary reason is that the culture must go through many more generations to reach a density of 10^{10} cells/ml in a 10,000 l tank than in a shake flask with 25 ml. Also, the use of antibiotics as selective agents may not be desirable in the large-scale system, owing either to cost or to regulatory constraints on product quality.

In the next section we will discuss some implications of these process constraints on plasmid design. In the section following, we will discuss how simple mathematical models of genetic instability can be constructed.