Equation 14.1 can then be applied to yield

$$P = 2^{(1-24)} = 1.2 \times 10^{-7} \tag{14.5}$$

c. When plasmids are not evenly distributed, the probability of forming a plasmid-free cell changes. In this case,

$$\begin{aligned} \frac{1}{2}P_{10} + \frac{1}{2}P_{70} &= \frac{1}{2}[2^{(1-10)}] + \frac{1}{2}[2^{(1-70)}] \\ &= 9.8 \times 10^{-4} + 8.5 \times 10^{-22} = 9.8 \times 10^{-4} \end{aligned} \tag{14.6}$$

Note that although this population has the same average copy number as case a, the probability of forming a plasmid-free cell is 5.4×10^8 greater.

14.4.2. Plasmid Structural Instability

In addition to the problems of segregational instability, some cells retain plasmids but alter the plasmid so as to reduce its harmful effects on the cell (structural instability). For example, the plasmid may encode both for antibiotic resistance and for a foreign protein. The foreign protein drains cellular resources away from growth toward an end product of no benefit to the cell. However, if the investigator has added antibiotics to the medium, the cell will benefit from retaining the gene encoding the antibiotic resistance. Normal mutations will result in some altered plasmids that retain the capacity to encode for desirable functions (for example, antibiotic resistance) while no longer making the foreign protein. In other cases, cellular recombination systems will integrate the gene for antibiotic resistance into the chromosome. Cells containing structurally altered plasmids can normally grow much more quickly than cells with the original plasmids. A culture having undergone a change in which the population is dominated by cells with an altered plasmid has undergone structural instability.

14.4.3. Host Cell Mutations

Mutations in host cells can also occur that make them far less useful as production systems for a given product. These mutations often alter cellular regulation and result in reduced target-protein synthesis. For example, if the promoter controlling expression of the foreign protein utilizes a host cell factor (e.g., a repressor), then modification of the host cell factor may greatly modulate the level of production of the desired plasmid-encoded protein. The lac promoter (see our discussion in Chapter 4 of the lac operon) can be induced by adding chemicals; for a lac promoter lactose or a chemical analog of lactose (e.g., IPTG or isopropyl-β-D-thiogalactoside) can be used. Such promoters are often used in plasmid construction to control the synthesis of a plasmid-encoded protein. If induction of plasmid-encoded protein synthesis from this promoter reduces cellular growth rates (as in Fig. 14.2), then a mutation that inactivates lac permease would prevent protein induction in that mutant cell. The lac permease protein is necessary for the rapid uptake of the inducer. Thus, the mutant cell would grow faster than the desired strain. Alternatively, a host cell mutation in the repressor, so that it would not recognize the inducer, would make induction impossible.