

the test plate at positions identical to those on the master plate. After incubation (approximately 24 hours for *E. coli*), the test plate is compared to the master plate. Colonies that appear at the same positions on both plates arise from wild-type cells, while colonies that exist only on the master plate must arise from the auxotrophic mutants.

Another class of mutants is called *conditional* mutants. Mutations that would normally be lethal to the cell could not be detected by methods we have described so far. However, mutated proteins are often more temperature sensitive than normal proteins. Thus, temperature sensitivity can often be used to select for conditionally lethal mutations. For example, the mutant may be unable to grow at the normal growth temperature (e.g., 37°C for *E. coli*) but will grow satisfactorily at a lower temperature (e.g., 25°C). Table 8.1 summarizes a variety of mutants and how they may be detected.

Mutation and selection have been used to tremendous advantage to probe the basic features of cell physiology and regulation. They also have been the mainstay of industrial programs for the improvement of production strains. Mutation and selection programs have been primarily responsible for increasing the yield of penicillin from 0.001 g/l in 1939 to current values of about 50 g/l of fermentation broth.

8.3. NATURAL MECHANISMS FOR GENE TRANSFER AND REARRANGEMENT

Bacteria can gain and express wholly different biochemical capabilities (e.g., the ability to degrade an antibiotic or detoxify a hazardous chemical in their environment) literally overnight. These alterations cannot be explained through inheritance and small evolutionary changes in the chromosome. Rather, they arise from gene transfer from one organism to another and/or large rearrangements in chromosomal DNA. In this section we will discuss genetic recombination, gene transfer, and genetic rearrangements—all mechanisms that can be exploited to genetically engineer cells. (See Table 8.1.)

8.3.1. Genetic Recombination

Genetic recombination is a process that brings genetic elements from two different genomes into one unit, resulting in new genotypes in the absence of mutations. Genetic recombination in procaryotes is a rare event, but sufficiently frequent to be important industrially and ecologically. The three main mechanisms for gene transfer are *transformation*, *transduction*, and *conjugation*. Transformation is a process in which free DNA is taken up by a cell. Transduction is a process in which DNA is transferred by a bacteriophage, and conjugation is DNA transfer between intact cells that are in direct contact with one another.

Once *donor DNA* is inside a cell, the mechanism for recombination is essentially independent of how the donor DNA was inserted. Figure 8.4 summarizes the molecular-level events in general recombination. The donor DNA must be homologous, or nearly so, to a segment of DNA on the recipient DNA.[†] Under the right conditions, cellular enzymes

[†] *Illegitimate recombination* between nonhomologous regions of DNA is possible, but rare. See later discussion on transposons.