

the form of lipopolysaccharide [LPS]) at a very high density, this membrane becomes frozen and brittle, with cracks through which macromolecules, including DNA, can pass. After DNA is added to the suspension, the cells are heated to 42°C and then chilled. Under these conditions, cells have been found to take up pieces of DNA through the cytoplasmic membrane, but the molecular mechanisms of the process still remain obscure.

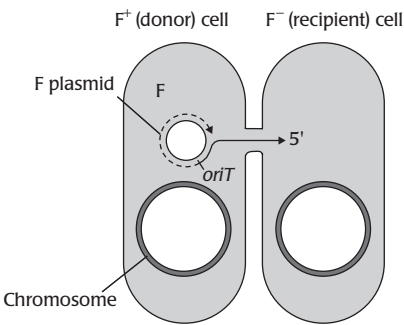
Transformation can be achieved by similar means in certain other bacteria, but there are many species for which this method does not work. One method that works with many organisms (also including *E. coli*) is *electroporation*. In this process, we apply short electrical pulses of very high voltage, which is believed to reorient asymmetric membrane components that carry charged groups, thus creating transient holes in the membrane. DNA fragments can then enter through these openings, either by spontaneous diffusion or driven by the electric charge.

Introduction by Conjugation

We have said that it is difficult to introduce DNA directly into certain species of bacteria. In such cases, taking an indirect route sometimes achieves the desired result. First, a piece of DNA is introduced into an organism (such as *E. coli*) that *can* receive DNA by transformation. This piece of DNA is then transferred from the *E. coli* into the species of interest by another form of genetic exchange in bacteria, conjugation.

The *conjugational transfer* of genes in bacteria was discovered by Joshua Lederberg and Edward L. Tatum in 1946. Subsequent work has shown it to be a unidirectional transfer from a cell containing a sex plasmid, or F-plasmid (for “fertility”), into a cell lacking that plasmid. The transfer of chromosomal genes by conjugation occurs only in rare donor cells, in which the sex plasmid has become integrated into the chromosome. A more frequent process, which occurs with nearly 100% efficiency, is the transfer of just the F-plasmid from a donor to a recipient (Figure 3.1). Conjugation requires that the donor and recipient cells join to form a stable pair connected, at least in the beginning, by a filamentous apparatus (sex pilus).

As we shall see, the first step in the cloning of a fragment of DNA is to insert it into a suitable *vector DNA*, and plasmids are the most frequently used vectors. However, the unmodified sex plasmids are *not* used as vectors. If they were, the job of transferring the recombinant plasmids to other strains and species would be easy, because all the proteins needed for such a transfer are encoded on the plasmid itself. But the procedure could also be potentially dangerous, because if a plasmid-containing strain were to escape into the environment, the recombinant plasmid with the foreign DNA could conceivably start to spread into other, naturally occurring bacteria. The current practice, therefore, is to use as vectors only *nonconjugative* or *non-self-transferring* plasmids (plasmids that lack the information for the cell-to-cell transfer). For these plasmids to be transferred by conjugation, the missing information must be supplied from another plasmid. This procedure is called *plasmid mobilization*. It is useful when DNA must be



**FIGURE 3.1**  
Conjugational transfer of the F-plasmid. One of the strands of the F-plasmid is cut at a specific position (oriT, for “origin of transfer”). This strand becomes elongated by rolling-circle replication (*broken line*), gradually displacing the old part of this strand, which enters into the F<sup>−</sup> cell 5′-end first. A complementary strand is synthesized in the cytoplasm of the recipient cell, and the plasmid is then circularized, converting the recipient cell from F<sup>−</sup> to F<sup>+</sup>.  
Redrawn based on artwork from the first edition (1995), published by W.H. Freeman.