5.2 Bacterial conjugation

The earliest studies in bacterial genetics revealed the unexpected process of cell conjugation.

Discovery of conjugation

Do bacteria possess any processes similar to sexual reproduction and recombination? The question was answered by the elegantly simple experimental work of Joshua Lederberg and Edward Tatum, who in 1946 discovered a sexlike process in bacteria. They were studying two strains of *Escherichia coli* with different sets of auxotrophic mutations. Strain A would grow only if the medium were supplemented with methionine and biotin; strain B would grow only if it were supplemented with threonine, leucine, and thiamine. Thus, we can designate the strains as

strain A: met^- bio^- thr^+ leu^+ thi^+
strain B: met^+ bio^+ thr^- leu^- thi^-

Figure 5-4a displays in simplified form the design of their experiment. Strains A and B were mixed together, incubated for a while, and then plated on minimal medium, on which neither auxotroph could grow. A small minority of the cells (1 in 10^7) was found to grow as prototrophs and hence must have been wild type,

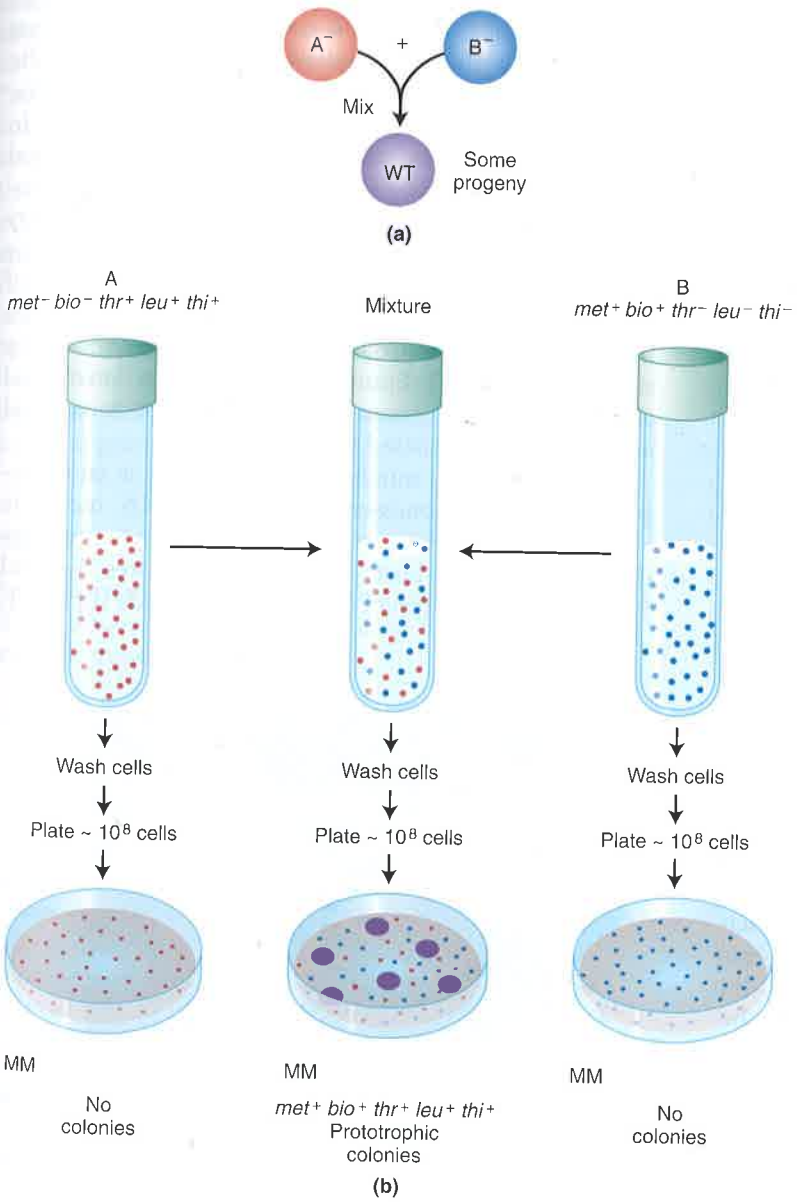


Figure 5-4 Lederberg and Tatum's demonstration of genetic recombination between bacterial cells. (a) The basic concept: two auxotrophic cultures (A^- and B^-) are mixed, yielding prototrophic wild types (WT). (b) Cells of type A or type B cannot grow on an unsupplemented (minimal) medium (MM), because A and B each carry mutations that cause the inability to synthesize constituents needed for cell growth. When A and B are mixed for a few hours and then plated, however, a few colonies appear on the agar plate. These colonies derive from single cells in which an exchange of genetic material has occurred; they are therefore capable of synthesizing all the required constituents of metabolism.

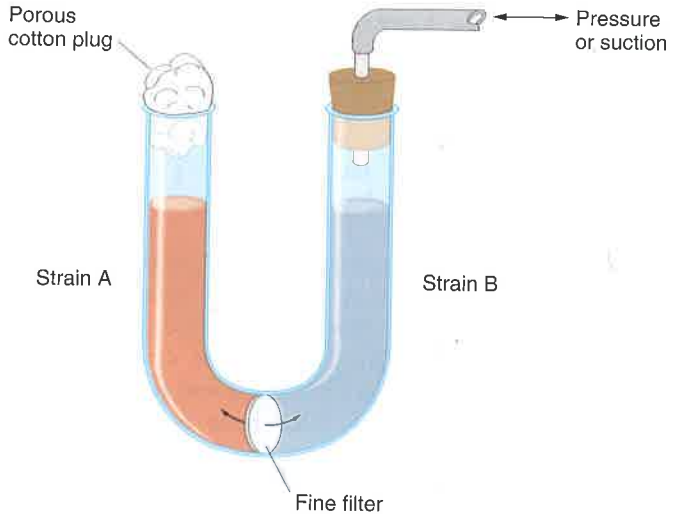


Figure 5-5 Physical contact between bacterial cells is required for genetic recombination. Auxotrophic bacterial strains A and B are grown on either side of a U-shaped tube. Liquid may be passed between the arms by applying pressure or suction, but the bacterial cells cannot pass through the filter. After incubation and plating, no recombinant colonies grow on minimal medium.

having regained the ability to grow without added nutrients. Some of the dishes were plated only with strain A bacteria and some only with strain B bacteria to act as controls, but from these no prototrophs arose. Figure 5-4b illustrates the experiment in more detail. These results suggested that some form of recombination of genes had taken place between the genomes of the two strains to produce the prototrophs.

It could be argued that the cells of the two strains do not really exchange genes but instead leak substances that the other cells can absorb and use for growing. This possibility of "cross feeding" was ruled out by Bernard Davis in the following way. He constructed a U-tube in which the two arms were separated by a fine filter. The pores of the filter were too small to allow bacteria to pass through but large enough to allow easy passage of any dissolved substances (Figure 5-5). Strain A was put in one arm, strain B in the other. After the strains had been incubated for a while, Davis tested the contents of each arm to see if there were any prototrophic cells, but none were found. In other words, *physical contact* between the two strains was needed for wild-type cells to form. It looked as though some kind of genome union had taken place, and genuine recombinants produced. The physical union of bacterial cells can be confirmed under an electron microscope, and is now called **conjugation.**

Discovery of the fertility factor (F)

In 1953, William Hayes discovered that in the above types of "crosses" the conjugating parents acted *unequally* (later we shall see ways to demonstrate this). It seemed that one parent (and *only* that parent) transferred some of or all its genome into another cell. Hence one cell acts as **donor,** and the other cell as a **recipient.** This is quite different from eukaryotic crosses in which parents contribute nuclear genomes equally.

MESSAGE The transfer of genetic material in *E. coli* conjugation is not reciprocal. One cell, the donor, transfers part of its genome to the other cell, which acts as the recipient.

By accident, Hayes discovered a variant of his original donor strain that would not produce recombinants on crossing with the recipient strain. Apparently, the donor-type strain had lost the ability to transfer genetic material and had changed into a recipient-type strain. In working with this "sterile" donor variant, Hayes found that it could regain the ability to act as a donor by association with other donor strains. Indeed the donor ability was transmitted rapidly and effectively between strains during conjugation. A kind of "infectious transfer" of some factor seemed to be taking place. He suggested that donor ability is itself a hereditary state, imposed by a **fertility factor (F).** Strains that carry F can donate, and are designated F^+. Strains that lack F cannot donate and are recipients, designated F^-.

We now know much more about F. It is an example of a small, nonessential circular DNA molecule called a **plasmid** that can replicate in the cytoplasm independent of the host chromosome. Figures 5-6 and 5-7 show how bacteria can transfer plasmids such as F.

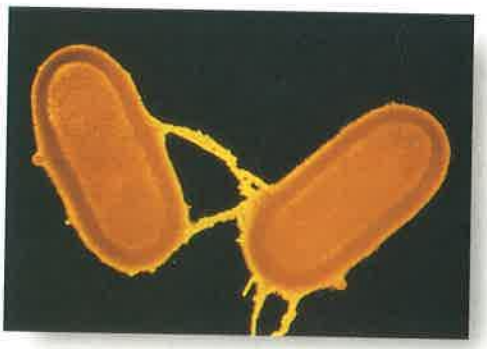


Figure 5-6 Bacteria can transfer plasmids (circles of DNA) through conjugation. A donor cell extends one or more projections—pili—that attach to a recipient cell and pull the two bacteria together. [Oliver Meckes/MPI-Tübingen, Photo Researchers.]

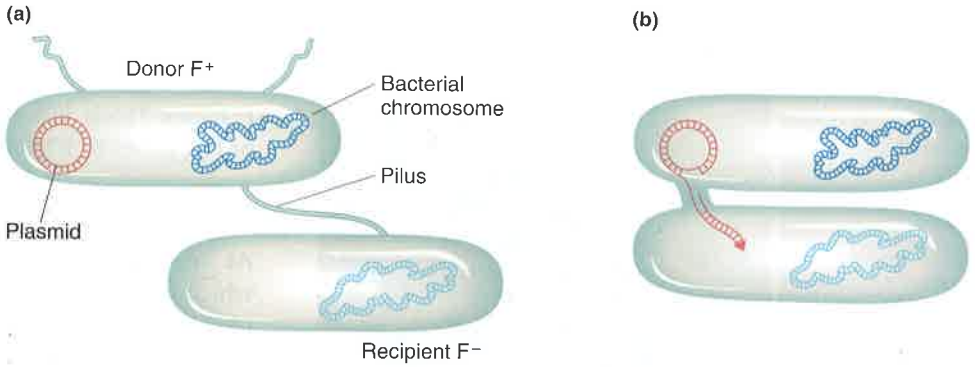


Figure 5-7 Conjugation. (a) During conjugation, the pilus pulls two bacteria together. (b) Next, a bridge (essentially a pore) forms between the two cells. A single-stranded copy of plasmid DNA is produced in the donor cell and then passes into the recipient bacterium, where the single strand, serving as a template, is converted to the double-stranded helix.

The F plasmid directs the synthesis of pili, projections that initiate contact with a recipient (Figure 5-6) and draw it closer. The F DNA in the donor cell makes a single-stranded copy of itself in a peculiar mechanism called **rolling circle replication.** The circular plasmid "rolls," and as it turns, it reels out the single-stranded copy like fishing line. This copy passes through a pore into the recipient cell, where the other strand is synthesized, forming a double helix. Hence a copy of F remains in the donor and another appears in the recipient, as shown in Figure 5-7. Note in the figure that the *E. coli* genome is depicted as a single circular chromosome. (We will examine the evidence for this later.) Most bacterial genomes are circular, a feature quite different from eukaryotic nuclear chromosomes. We shall see that this feature leads to many idiosyncrasies of bacterial genetics.

Hfr strains

An important breakthrough came when Luca Cavalli-Sforza discovered a derivative of an F^+ strain with two unusual properties:

1. On crossing with F^- strains this new strain produced 1000 times as many recombinants as a normal F^+ strain. Cavalli-Sforza designated this derivative an **Hfr** strain to symbolize its ability to promote a *h*igh *f*requency of *r*ecombination.
2. In Hfr $\times$ F^- crosses, virtually none of the F^- parents were converted into F^+ or into Hfr. This result is in contrast with F^+ $\times$ F^- crosses, in which, as we have seen, infectious transfer of F results in a large proportion of the F^- parents being converted into F^+.

It became apparent that an Hfr strain results from the integration of the F factor into the chromosome, as pictured in Figure 5-8. We can now explain the first unusual property of Hfr strains. During conjugation the F factor inserted in the chromosome efficiently drives part or all of that chromosome into the F^- cell. The chromosomal fragment can then engage in recombination with the recipient chromosome. The rare recombinants observed by Lederberg and Tatum in F^+ $\times$ F^- crosses were due to the spontaneous, but rare, formation of Hfr cells in the F^+ culture. Cavalli-Sforza isolated examples of these rare cells from F^+ cultures, and found that indeed they now acted as true Hfr's.

Does an Hfr cell die after donating its chromosomal material to an F^- cell? The answer is no. Just like the F plasmid, during conjugation the Hfr chromosome replicates and transfers a single strand to the F^- cell. The single-stranded nature of the transferred DNA can be demonstrated visually using special strains and antibodies, as shown in Figure 5-9. The replication of the chromosome ensures a complete chromosome for the donor cell after mating. The transferred strand is converted into a double helix in the recipient cell, and donor genes may become incorporated in the recipient's chromosome through crossovers, creating a recombinant cell (Figure 5-10). If there is no recombination, the transferred fragments of DNA are simply lost in the course of cell division.

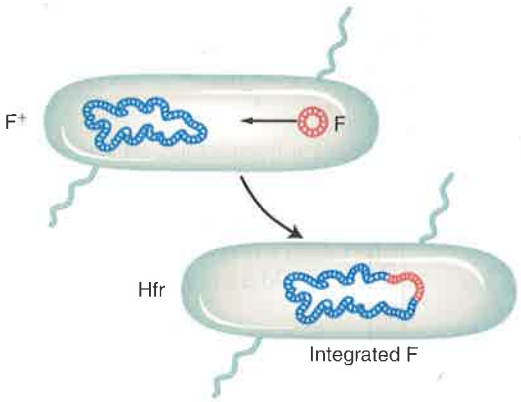


Figure 5-8 Formation of an Hfr. Occasionally, the independent F factor combines with the *E. coli* chromosome, creating an Hfr strain.

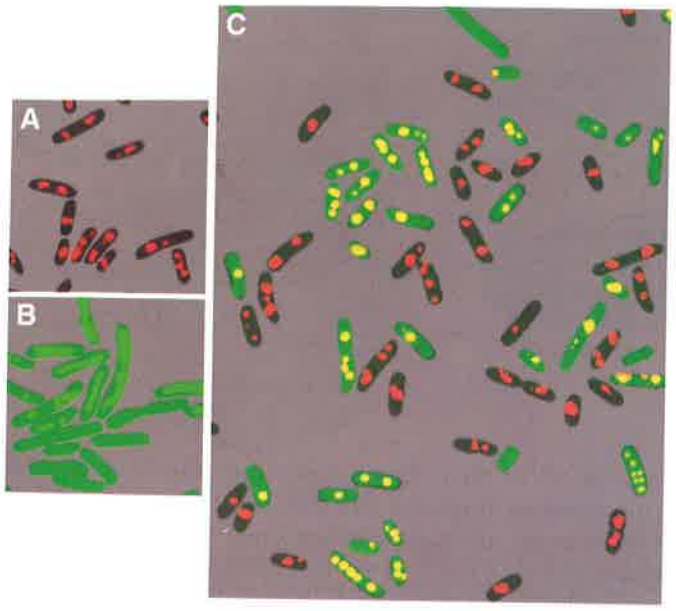


Figure 5-9 Visualization of single-stranded DNA transfer in conjugating *E. coli* cells, using special fluorescent antibodies. Parental Hfr strains (A) are black with red DNA. The red is from binding of an antibody to a protein normally attached to DNA. The recipient F⁻ cells (B) are green due to the presence of the GFP protein; and because they are mutant for a certain gene they do not bind the special protein that binds to antibody. When single-stranded DNA enters the recipient, it promotes atypical binding of the special protein, which fluoresces yellow in this background. Part C shows Hfrs (unchanged) and exconjugants with yellow transferred DNA. A few unmated F⁻ cells are visible. [From Masamichi Kohiyama, Sota Hiraga, Ivan Matic, and Miroslav Radman, "Bacterial Sex: Playing Voyeurs 50 Years Later," *Science* 8 August 2003, p. 803, Figure 1.]

LINEAR TRANSMISSION OF THE HFR GENES FROM A FIXED POINT A clearer view of the behavior of Hfr strains was obtained in 1957, when Elie Wollman and François Jacob investigated the pattern of transmission of Hfr genes to F⁻ cells during a cross. They crossed

Hfr azi^r ton^r lac^+ gal^+ str^s × F⁻ azi^s ton^s lac^- gal^- str^r

At specific times after mixing, they removed samples, which were each put in a kitchen blender for a few seconds to separate the mating cell pairs. This procedure is called **interrupted mating.** The sample was then plated onto a medium containing streptomycin to kill the Hfr donor cells, which bore the sensitivity allele str^s. The surviving str^r cells then were tested for the presence of alleles from the donor genome. Any str^r cell bearing a donor allele must have taken part in conjugation; such cells are called **exconjugants.** Figure 5-11a shows a plot of the results, showing a time course of entry of each donor allele azi^r, ton^r, lac^+, and gal^+. Figure 5-11b portrays the transfer of Hfr alleles.

The key elements in these results are

1. Each donor allele first appears in the F⁻ recipients at a specific time after mating began.
2. The donor alleles appear in a specific sequence.
3. Later donor alleles are present in fewer recipient cells.

Putting all these observations together, Wollman and Jacob deduced that in the conjugating Hfr, single-stranded DNA transfer begins from a fixed point on the donor chromosome, termed the **origin (O),** and continues in a linear fashion. The point O is now known to be the site at which the F plasmid is inserted. The farther an gene is from O, the later it is transferred to the F⁻. The transfer process will

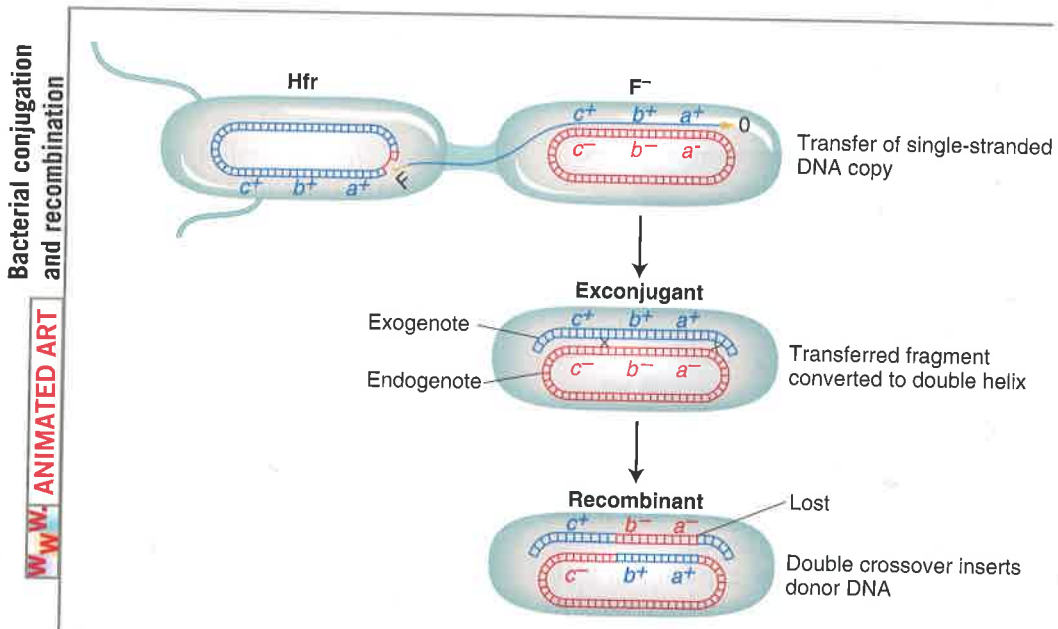


Figure 5-10 Bacterial conjugation and recombination. Transfer of single-stranded fragment of donor chromosome and recombination with recipient chromosome.

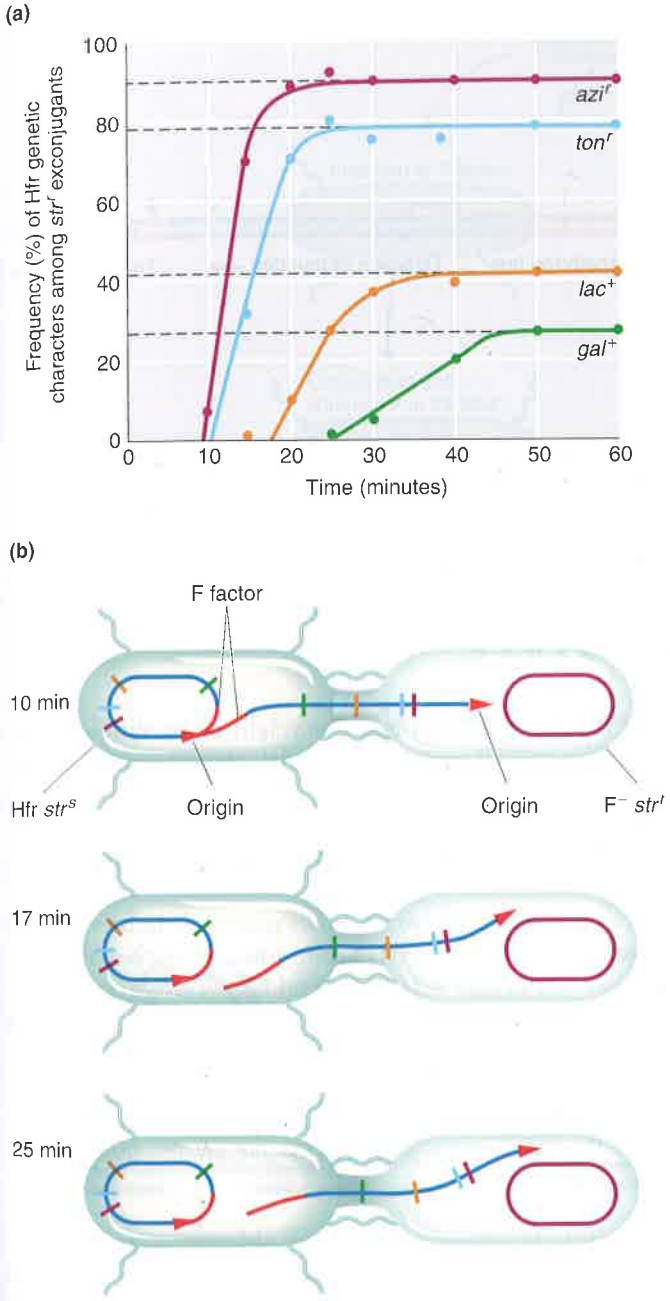
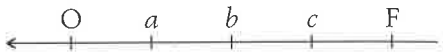


Figure 5-11 Interrupted-mating conjugation experiments. F^- streptomycin-resistant cells with mutations in *azi*, *ton*, *lac*, and *gal* are incubated for varying times with Hfr cells that are sensitive to streptomycin and carry wild-type alleles for these genes. (a) A plot of the frequency of donor alleles in exconjugants as a function of time after mating. (b) A schematic view of the transfer of markers (shown in different colors) over time. [Part a after E. L. Wollman, F. Jacob, and W. Hayes, *Cold Spring Harbor Symp. Quant. Biol.* 21, 1956, 141.]

generally stop before the farthermost genes are transferred, resulting in their being included in fewer exconjugants.

How can we explain the second unusual property of Hfr crosses, that F^- exconjugants are rarely converted into Hfr or F^+? When Wollman and Jacob allowed Hfr × F^- crosses to continue for as long as 2 hours before disruption, they found that in fact a few of the exconjugants were converted into Hfr. In other words, the part of F that confers donor ability was eventually transmitted but at a very low frequency. The rareness of Hfr exconjugants suggested that the inserted F was transmitted as the *last* element of the linear chromosome. We can summarize this with the following map, in which the arrow indicates the process of transfer, beginning with O:



Thus almost none of the F^- recipients are converted because the fertility factor is the last element transmitted, and usually the transmission process will have stopped before getting that far.

MESSAGE The Hfr chromosome, originally circular, unwinds and is transferred to the F^- cell in a linear fashion, with the F factor entering last.

INFERRING INTEGRATION SITES OF F AND CHROMOSOME CIRCULARITY Wollman and Jacob went on to shed more light on how and where the F plasmid integrates to form an Hfr, and in doing so deduced the circularity of the chromosome. They performed interrupted-mating experiments using different, separately derived Hfr strains. Significantly, the order of transmission of the alleles differed from strain to strain, as in the following examples:

Hfr strain	
H	O *thr pro lac pur gal his gly thi* F
1	O *thr thi gly his gal pur lac pro* F
2	O *pro thr thi gly his gal pur lac* F
3	O *pur lac pro thr thi gly his gal* F
AB 312	O *thi thr pro lac pur gal his gly* F

Each line can be considered a map showing the order of alleles on the chromosome. At first glance, there seems to be a random shuffling of genes. However, when the identical alleles of the different Hfr maps are lined up, the similarity in sequence becomes clear.

H	F *thi gly his gal pur lac pro thr* O
(written backwards)	
1	O *thr thi gly his gal pur lac pro* F
2	O *pro thr thi gly his gal pur lac* F
3	O *pur lac pro thr thi gly his gal* F
AB 312	F *gly his gal pur lac pro thr thi* O
(written backwards)	

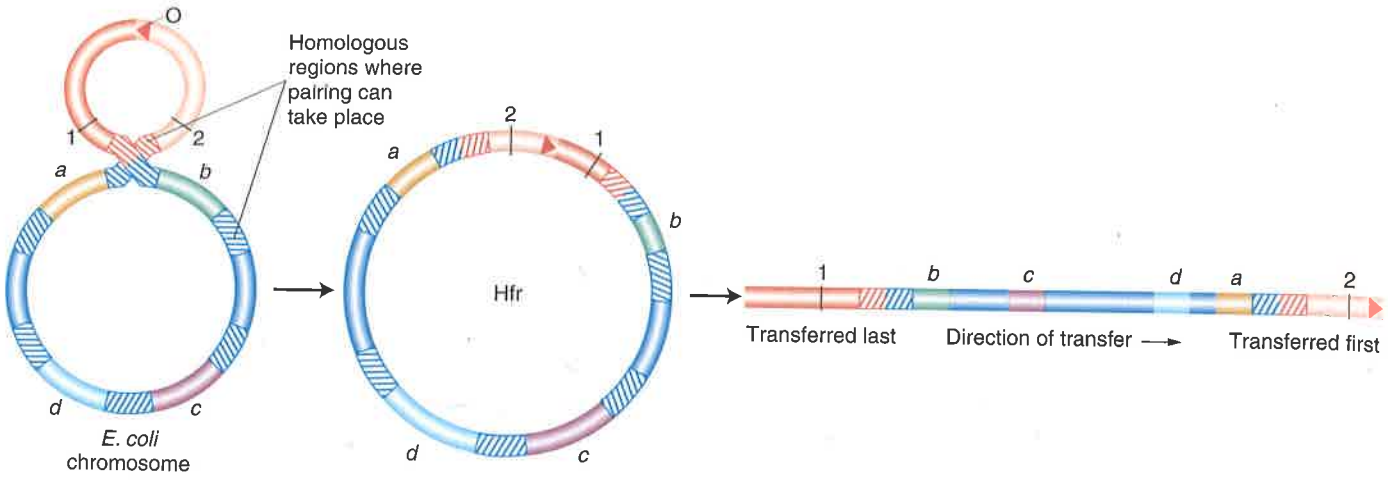


Figure 5-12 Insertion of the F factor into the *E. coli* chromosome by crossing-over. Hypothetical markers 1 and 2 are shown on F to depict the direction of insertion. The origin (O) is the mobilization point where insertion into the *E. coli* chromosome occurs; the pairing region is homologous with a region on the *E. coli* chromosome; *a*–*d* are representative genes in the *E. coli* chromosome. Fertility genes on F are responsible for the F^+ phenotype. Pairing regions (hatched) are identical in plasmid and chromosome. They are derived from mobile elements called *insertion sequences* (see Chapter 13). In this example, the Hfr cell created by the insertion of F would transfer its genes in the order *a*, *d*, *c*, *b*.

The relationship of the sequences to one another is explained if each map is the segment of a circle. This was the first indication that bacterial chromosomes are circular. Furthermore, Allan Campbell proposed a startling hypothesis that accounted for the different Hfr maps. He proposed that if F is a ring, then insertion might be by simple crossover between F and the bacterial chromosome (Figure 5-12). That being the case, any of the linear Hfr chromosomes could be generated simply by insertion of F into the ring in the appropriate place and orientation (Figure 5-13).

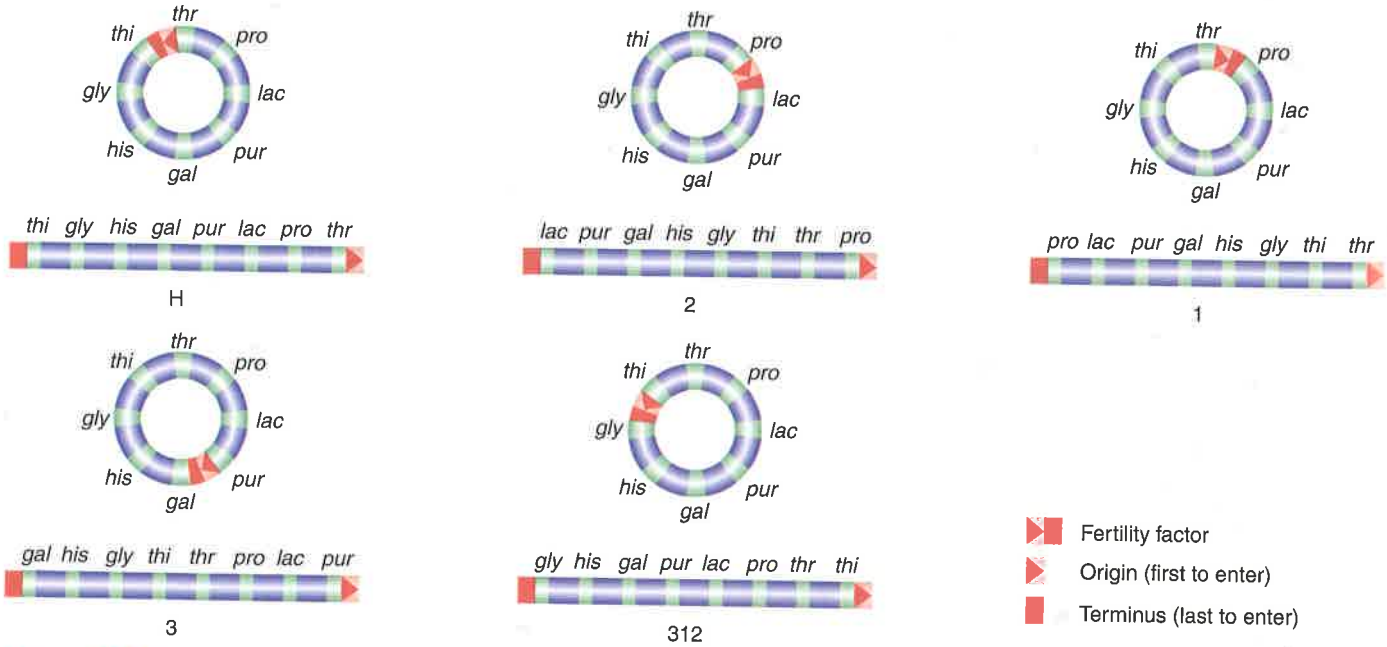


Figure 5-13 Order of gene transfer. The five *E. coli* Hfr strains shown each have different F factor insertion points and orientations. All strains share the same order of genes on the *E. coli* chromosome. The orientation of the F factor determines which gene enters the recipient cell first. The gene closest to the terminus enters last.

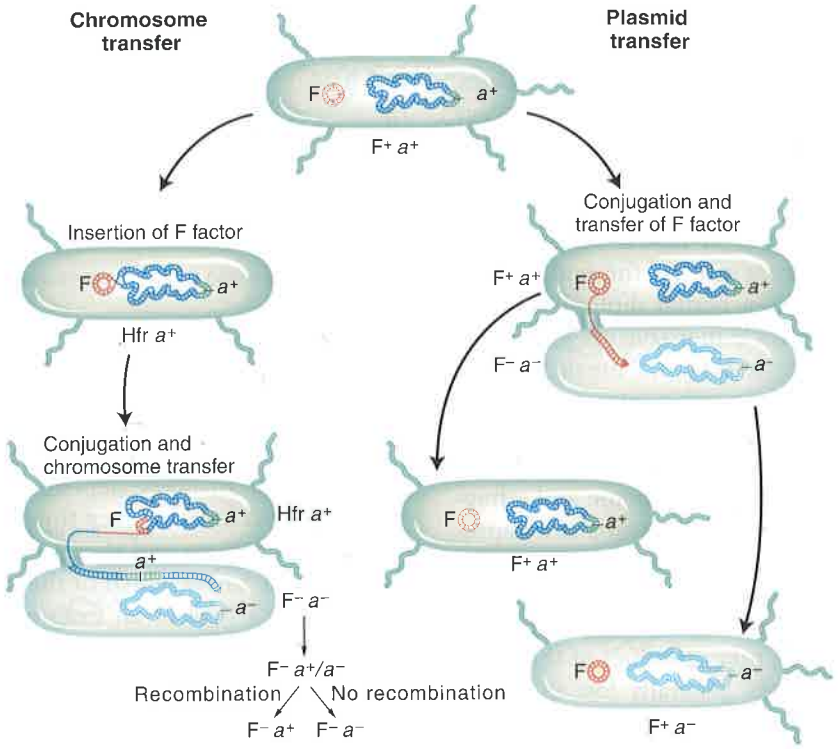


Figure 5-14 Conjugation summary. Summary of the various events that take place in the conjugational cycle of *E. coli*.

Several hypotheses—later supported—followed from Campbell's proposal.

1. One end of the integrated F factor would be the **origin,** where transfer of the Hfr chromosome begins. The **terminus** would be at the other end of F.
2. The orientation in which F is inserted would determine the order of entry of donor alleles. If the circle contains genes *A*, *B*, *C*, and *D*, then insertion between *A* and *D* would give the order *ABCD* or *DCBA*, depending on orientation. Check the different orientations of the insertions in Figure 5-12.

How is it possible for F to integrate at different sites? If F DNA had a region homologous to any of several regions on the bacterial chromosome, any one of these could act as a pairing region at which pairing could be followed by a crossover. These regions of homology are now known to be mainly segments of transposable elements called *insertion sequences*. For a full explanation of these, see Chapter 13.

The fertility factor thus exists in two states:

1. The plasmid state: as a free cytoplasmic element F is easily transferred to F^- recipients.
2. The integrated state: as a contiguous part of a circular chromosome F is transmitted only very late in conjugation.

The *E. coli* conjugation cycle is summarized in Figure 5-14.

Mapping of bacterial chromosomes

BROAD-SCALE CHROMOSOME MAPPING USING TIME OF ENTRY Wollman and Jacob realized that it would be easy to construct linkage maps from the interrupted-mating results, using as a measure of "distance" the times at which the donor alleles first appear after mating. The units of map distance in this case are minutes. Thus, if b^+ begins to enter the F^- cell 10 minutes after a^+ begins to enter, then a^+ and b^+ are 10 units apart. Like eukaryotic maps based on crossovers, these linkage maps were originally purely genetic constructions. At the time they were originally devised, there was no way of testing their physical basis.

FINE-SCALE CHROMOSOME MAPPING BY RECOMBINANT FREQUENCY For an exconjugant to acquire donor genes as a permanent feature of its genome, the donor fragment must recombine with the recipient chromosome. However, note that time-of-entry mapping is not based on recombinant frequency. Indeed the units are minutes, not RF. Nevertheless it is possible to use recombinant frequency for a more fine scale type of mapping in bacteria, and this is the method to which we now turn.