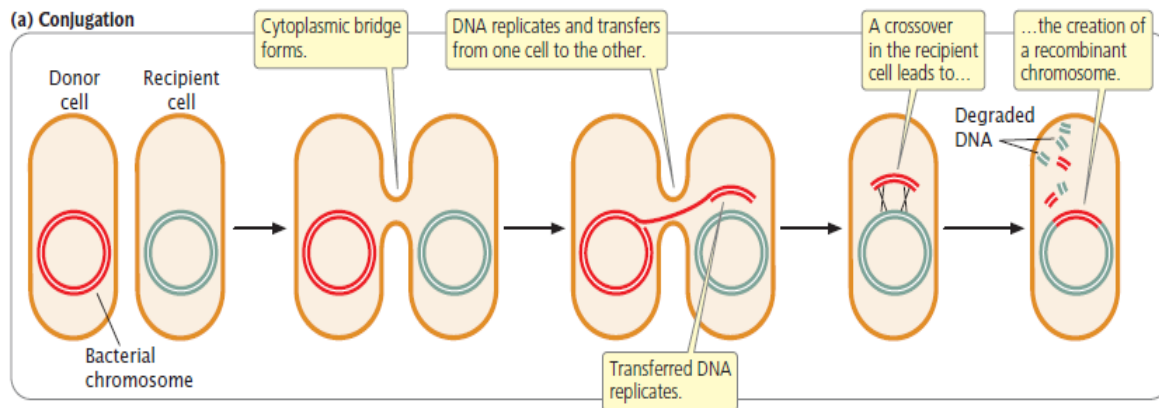


CONJUGATION

Compiled by Srimanta Kanji

Conjugation takes place when genetic material passes directly from one bacterium to another. In conjugation, two bacteria lie close together and a connection forms between them. A plasmid or a part of the bacterial chromosome passes from one cell (the donor) to the other (the recipient). Subsequent to conjugation, crossing over may take place between homologous sequences in the transferred DNA and the chromosome of the recipient cell. In conjugation, DNA is transferred only from donor to recipient, with no reciprocal exchange of genetic material.

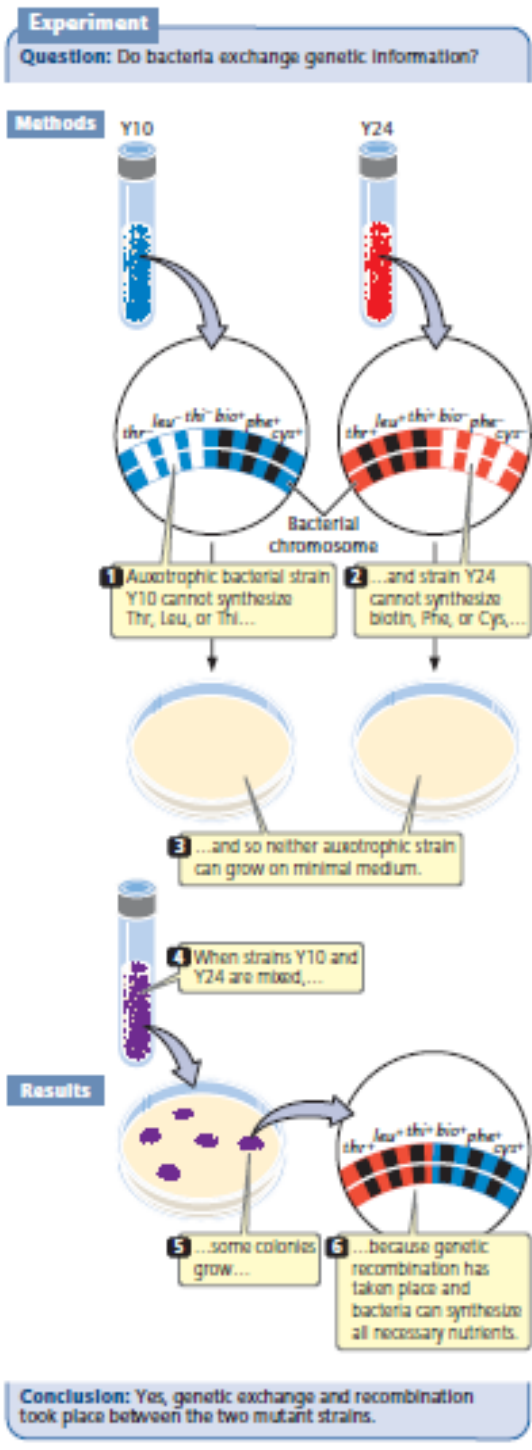


Experiment:

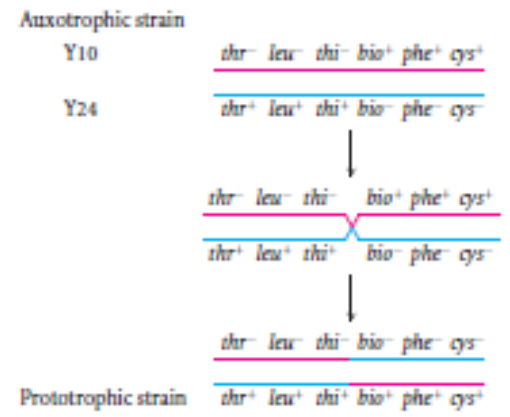
In 1946, Joshua Lederberg and Edward Tatum demonstrated that bacteria can transfer and recombine genetic information, paving the way for the use of bacteria in genetic studies. In the course of their research, Lederberg and Tatum studied auxotrophic strains of *E. coli*. The Y10 strain required the amino acids threonine (and was genotypically *thr*⁻) and leucine (*leu*⁻) and the vitamin thiamine (*thi*⁻) for growth but did not require the vitamin biotin (*bio*⁺) or the amino acids phenylalanine (*phe*⁺) and cysteine (*cys*⁺); the genotype of this strain can be written as *thr*⁻ *leu*⁻ *thi*⁻ *bio*⁺ *phe*⁺ *cys*⁺. The Y24 strain had the opposite set of alleles: it required biotin, phenylalanine, and cysteine in its medium, but it did not require threonine, leucine, or thiamine; its genotype was *thr*⁺ *leu*⁺ *thi*⁺ *bio*⁻ *phe*⁻ *cys*⁻. In one experiment, Lederberg and Tatum mixed Y10 and Y24 bacteria together and plated them on minimal medium (**Figure 8.8**). Each strain was also plated separately on minimal medium.

Alone, neither Y10 nor Y24 grew on minimal medium. Strain Y10 was unable to grow, because it required threonine, leucine, and thiamine, which were absent in the minimal medium; strain Y24 was unable to grow, because it required biotin, phenylalanine, and cysteine, which also were absent from the minimal medium. When Lederberg and Tatum mixed the two strains, however, a few colonies did grow on the minimal medium. These prototrophic bacteria must have had genotype *thr*⁺ *leu*⁺ *thi*⁺ *bio*⁺ *phe*⁺ *cys*⁺. **Where had they come from?** Lederberg and Tatum concluded that some type of genetic transfer and recombination had taken place: What they did not know was *how* it had taken place.

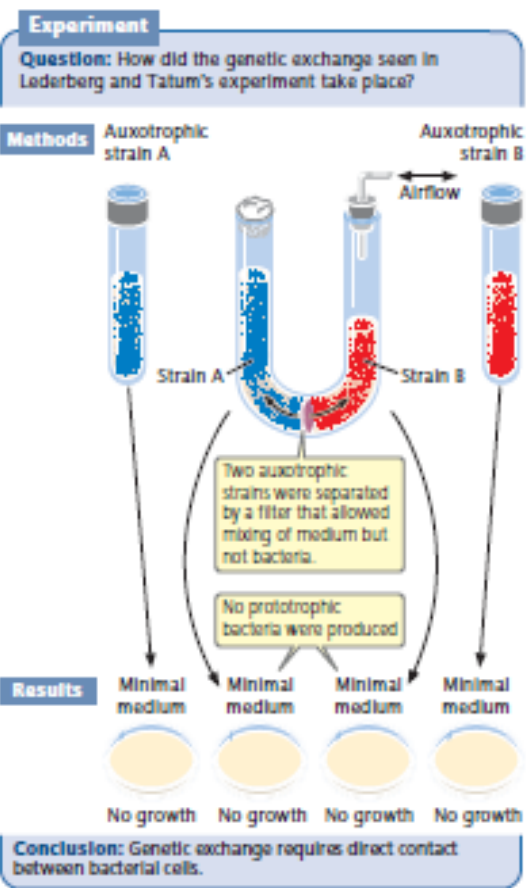
To study this problem, Bernard Davis constructed a U-shaped tube (**Figure 8.9**) that was divided into two compartments by a filter having fine pores. This filter allowed liquid medium to pass from one side of the tube to the other, but the pores of the filter were too small to allow the passage of bacteria. Two auxotrophic strains of bacteria were placed on opposite sides of the filter, and suction was applied alternately to the ends of the U-tube, causing the medium to flow back and forth between the two compartments. Despite hours of incubation in the U-tube, bacteria plated out on minimal medium did not grow; there had been no genetic exchange between the strains. The exchange of bacterial genes clearly required direct contact, or conjugation, between the bacterial cells.



8.8 Lederberg and Tatum's experiment demonstrated that bacteria undergo genetic exchange.



What they did not know was how it had taken place. To study this problem, Bernard Davis constructed a U-shaped tube (Figure 8.9) that was divided into two compart-



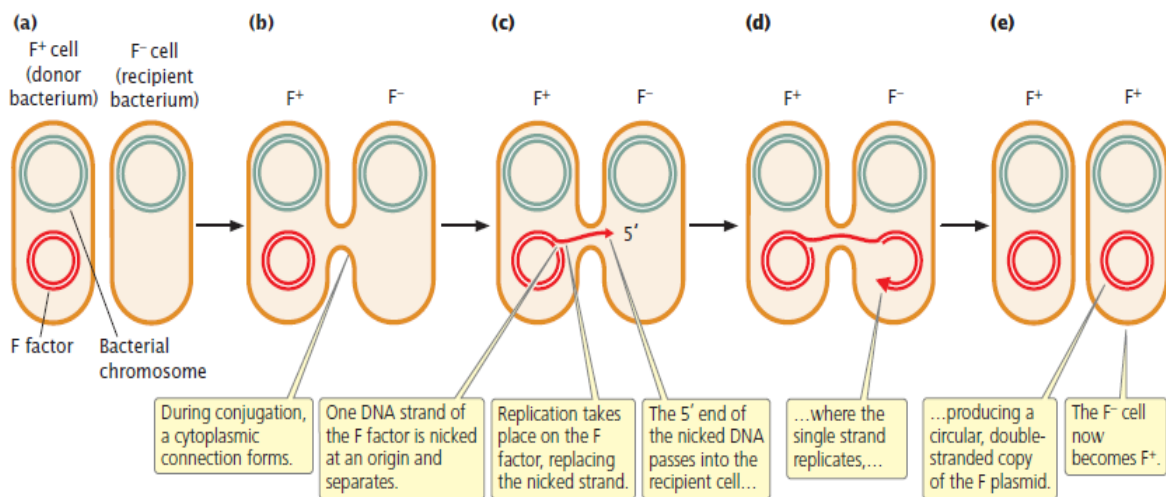
8.9 Davis's U-tube experiment

F⁺ and F⁻ cells:

In most bacteria, conjugation depends on a fertility (F) factor that is present in the donor cell and absent in the recipient cell. Cells that contain F are referred to as F⁺, and cells lacking F are F⁻. The F factor contains an origin of replication and a number of genes required for conjugation (see Figure 8.6). For example, some of these genes encode sex **pili** (singular, pilus), slender extensions of the cell membrane. A cell containing F produces the sex pili, one of which makes contact with a receptor on an F⁻ cell (**Figure 8.10**) and pulls the two cells together. DNA is then transferred from the F⁺ cell to the F⁻ cell. Conjugation can take place only between a cell that possesses F and a cell that lacks F. In most cases, the only genes transferred during conjugation between an F⁺ and F⁻ cell are those on the F factor (**Figure 8.11a and b**). Transfer is initiated when one of the DNA strands on the F factor is nicked at an origin (*oriT*). One end of the nicked DNA separates from the circle and passes into the recipient cell (**Figure 8.11c**). Replication takes place on the nicked strand, proceeding around the circular plasmid in the F⁺ cell and replacing the transferred strand (**Figure 8.11d**). Because the plasmid in the F⁺ cell is always nicked at the *oriT* site, this site always enters the recipient cell first, followed by the rest of the plasmid. Thus, the transfer of genetic material has a defined direction. Inside the recipient cell, the single strand replicates, producing a circular, double-stranded copy of the F plasmid (**Figure 8.11e**). If the entire F factor is transferred to the recipient F⁻ cell, that cell becomes an F⁺ cell.



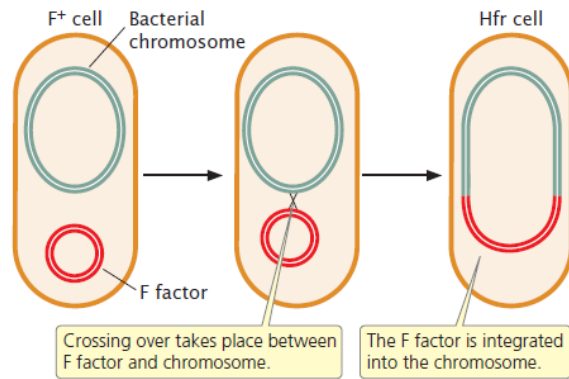
8.10 A sex pilus connects F⁺ and F⁻ cells during bacterial conjugation. [Dr. Dennis Kunkel/Phototake.]



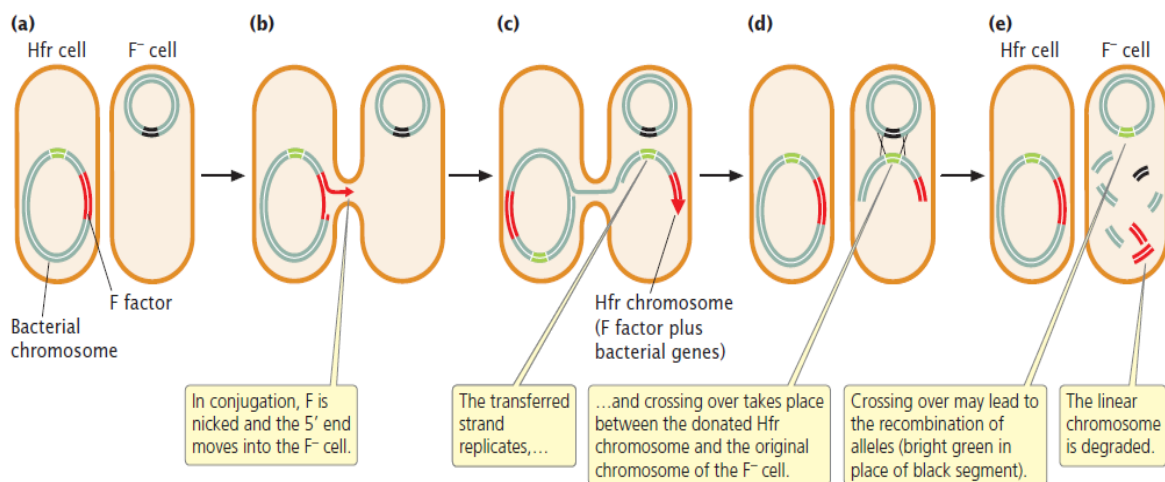
8.11 The F factor is transferred during conjugation between an F⁺ and F⁻ cell.

Hfr cells:

Conjugation transfers genetic material in the F plasmid from F⁺ to F⁻ cells but does not account for the transfer of chromosomal genes observed by Lederberg and Tatum. In Hfr (high-frequency) strains, the F factor is integrated into the bacterial chromosome (**Figure 8.12**). Hfr cells behave as F⁺ cells, forming sex pili and undergoing conjugation with F⁻ cells. In conjugation between Hfr and F⁻ cells (**Figure 8.13a**), the integrated F factor is nicked, and the end of the nicked strand moves into the F⁻ cell (**Figure 8.13b**), just as it does in conjugation between F⁺ and F⁻ cells. Because, in an Hfr cell, the F factor has been integrated into the bacterial chromosome, the chromosome follows it into the recipient cell. How much of the bacterial chromosome is transferred depends on the length of time that the two cells remain in conjugation. Inside the recipient cell, the donor DNA strand replicates (**Figure 8.13c**), and crossing over between it and the original chromosome of the F⁻ cell (**Figure 8.13d**) may take place. This gene transfer between Hfr and F⁻ cells is how the recombinant prototrophic cells observed by Lederberg and Tatum were produced. After crossing over has taken place in the recipient cell, the donated chromosome is degraded and the recombinant recipient chromosome remains (**Figure 8.13e**), to be replicated and passed on to later generations by binary fission. The F plasmid in F⁺ cells integrate into the bacterial chromosome, causing an F⁺ cell to become Hfr, at a frequency of only about 1/10,000. This low frequency accounts for the low rate of recombination observed by Lederberg and Tatum in their F⁺ cells. The F factor is excised from the bacterial chromosome at a similarly low rate, causing a few Hfr cells to become F⁺.



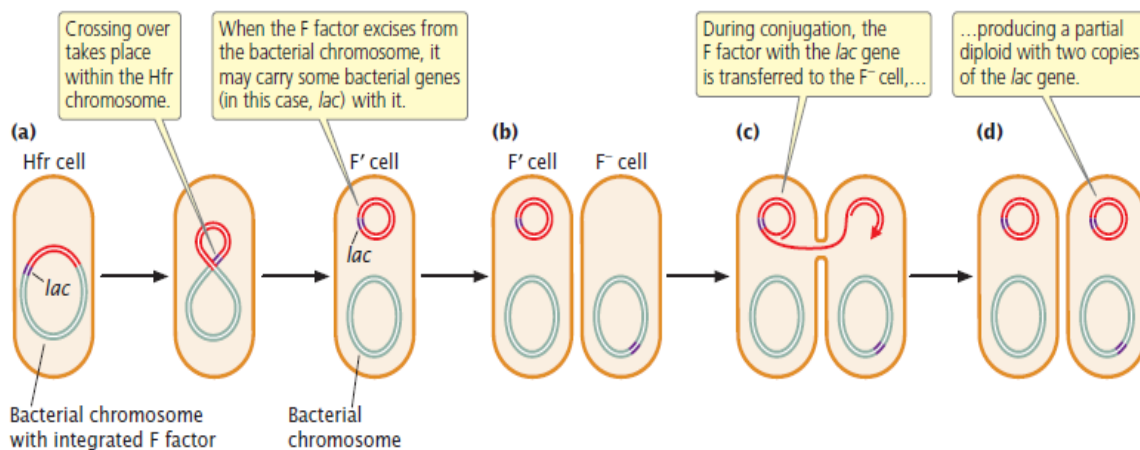
8.12 The F factor is integrated into the bacterial chromosome in an Hfr cell.



8.13 Bacterial genes may be transferred from an Hfr cell to an F⁻ cell in conjugation. In an Hfr cell, the F factor has been integrated into the bacterial chromosome.

F' cells:

When an F factor does excise from the bacterial chromosome, a small amount of the bacterial chromosome may be removed with it, and these chromosomal genes will then be carried with the F plasmid (Figure 8.14). Cells containing an F plasmid with some bacterial genes are called F prime (F'). For example, if an F factor integrates into a chromosome adjacent to the *lac* genes (genes that enable a cell to metabolize the sugar lactose), the F factor may pick up *lac* genes when it excises, becoming F'*lac*. F' cells can conjugate with F⁻ cells because F' cells possess the F plasmid with all the genetic information necessary for conjugation and gene transfer. During conjugation between an F'*lac* cell and an F⁻ cell, the F plasmid is transferred to the F⁻ cell, which means that any genes on the F plasmid, including those from the bacterial chromosome, may be transferred to F⁻ recipient cells. This process is called **sexduction**. It produces **partial diploids**, or **merozygotes**, which are cells with two copies of some genes, one on the bacterial chromosome and one on the newly introduced F plasmid.



8.14 An Hfr cell may be converted into an F' cell when the F factor excises from the bacterial chromosome and carries bacterial genes with it. Conjugation produces a partial diploid.

Sexduction: The recombination of bacteria in which the transmission of the bacterial gene to a recipient cell take place through their incorporation in to an autonomous conjugative plasmid, is called **sexduction**. It is a special type of conjugation, where the F factor (in Hfr cell) may dissociate and go back to cytoplasm again, as a result Hfr males are reverted to F⁺ males. The F factor may carry some bacterial genes during dissociation and termed as F'. when F' factor is transferred, the recipient becomes heterozygous for a part of DNA.

Concepts:

Conjugation in *E. coli* is controlled by an episome called the F factor. Cells containing F (F⁺ cells) are donors during gene transfer; cells lacking F (F⁻ cells) are recipients. Hfr cells possess F integrated into the bacterial chromosome; they donate DNA to F⁻ cells at a high frequency. F' cells contain a copy of F with some bacterial genes.

Table 8.2 Characteristics of *E. coli* cells with different types of F factor

Type	F Factor Characteristics	Role in Conjugation
F ⁺	Present as separate circular DNA	Donor
F ⁻	Absent	Recipient
Hfr	Present, integrated into bacterial chromosome	High-frequency donor
F'	Present as separate circular DNA, carrying some bacterial genes	Donor

Table 8.3 Results of conjugation between cells with different F factors

Conjugating	Cell Types Present after Conjugation
F ⁺ × F ⁻	Two F ⁺ cells (F ⁻ cell becomes F ⁺)
Hfr × F ⁻	One Hfr cell and one F ⁻ (no change)*
F' × F ⁻	Two F' cells (F ⁻ cell becomes F')

*Rarely, the F⁻ cell becomes F⁺ in an Hfr × F⁻ conjugation if the entire chromosome is transferred during conjugation.