# Conjugation

## Transfer of Plasmids Between Bacteria

The transfer of genetic information between two bacterial cells may occur via cell-to-cell contact. This process is known as bacterial conjugation and typically depends on the presence of plasmids. **Transferability** is the ability of certain plasmids to move from one bacterial cell to another. Many medium-sized plasmids, such as the F-type and P-type plasmids, are able to move and are referred to as **Tra$^+$** (transfer-positive) or self-transferable. For transfer to occur, the bacterial cell containing the plasmid must make physical contact with a suitable recipient cell. During bacterial conjugation DNA moves in one direction only, from the plasmid-carrying donor to

Transferable plasmids move from one cell to another via the conjugation bridge.

the recipient . The donor cell manufactures a **sex pilus** that binds to the recipient and draws the two cells together. Next, a **conjugation bridge** forms between the two cells and provides a channel for DNA to move from donor to recipient. In real life, mating bacteria tend to cluster together in groups of 5–10 .

The genes for formation of the sex pilus and conjugation bridge and for overseeing the DNA transfer process are known as *tra* genes and are all found on the plasmid itself. Since plasmid transfer requires over 30 genes, only medium or large plasmids possess this ability. Very small plasmids, such as the ColE plasmids, do not have enough DNA to accommodate the genes needed.

Transferable plasmids move from one cell to another via the conjugation bridge.

A single strand of newly made DNA is transferred from the donor to the recipient cell during conjugation.

The most famous self-transferable plasmid is the **F-plasmid** of *E. coli*, which is approximately 100 kbp long. Donor cells are sometimes known as $F^+$ or "male" and recipient cells as $F^-$ or "female" and conjugation is sometimes referred to as bacterial mating. Note, however, that the "sex" of a bacterial cell is determined by the presence or absence of a plasmid and that DNA transfer is unidirectional, from donor to recipient. When a recipient cell has received the F-plasmid, it becomes $F^+$. From a human perspective it has been transmuted from "female" into a "male"! Thus, bacterial mating is not at all equivalent to sexual reproduction among higher organisms.

Although we talk about "plasmid transfer," in reality both the donor cell and the recipient cell end up with a copy of the plasmid. Thus the transfer mechanism includes the synthesis of a second copy of the plasmid. Plasmid DNA transfer involves replication by the rolling circle mechanism (Fig. 28.14). First, one of the two strands of the double-stranded DNA of the plasmid opens up at the origin of transfer. This linearized single strand of DNA moves through the conjugation bridge from the donor into the recipient cell. An unbroken single-stranded circle of plasmid DNA remains inside the donor cell. This is used as a template for the synthesis of a new second strand to replace the one that just left. As the linear single strand of plasmid DNA enters the female cell, a new complementary strand of DNA is made

using the incoming strand as template. Thus, only one strand of plasmid DNA is transferred from the donor to the recipient.

The detailed physical mechanism of DNA transfer via the conjugation bridge was only solved relatively recently. The earliest proposals were that DNA traveled through the central channel of the sex pilus itself. Although this is incorrect, the DNA does in fact travel through the central channel of the basal structure on which the pilus is built. When the sex pilus is assembled, its protein subunits travel through the channel in the basal structure (also known as the transfer apparatus). After donor and recipient have made contact, the pilus is retracted and the pilus subunits return through the same channel. This brings the two cells into close contact and leaves the basal structure bridging the inner and outer membranes of the donor and in contact with the recipient. DNA then moves through the channel of the basal structure into the recipient. The basal structure belongs to the family of type IV secretion systems. These are used by a variety of bacteria for protein secretion as well as DNA uptake and DNA transfer (Fig. 28.15).

**conjugation bridge** Junction that forms between two cells and provides a channel for DNA to move from donor to recipient during conjugation.
**F-plasmid** Fertility plasmid that allows *E. coli* to donate DNA by conjugation.
**sex pilus** Protein filament made by donor bacteria that binds to a suitable recipient and draws the two cells together.

- Transfer of DNA by ***direct contact*** between two ***living*** bacteria

- Contact is made, and DNA is transferred, by way of a conjugation ***pilus*** (conjugation bridge/sex pilus/F pilus)

- Much larger amounts of DNA can be transferred by conjugation than by transformation or transduction

## Types of cells involved in conjugation

| | |
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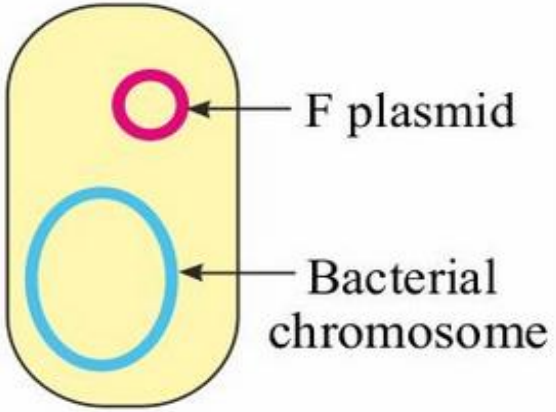
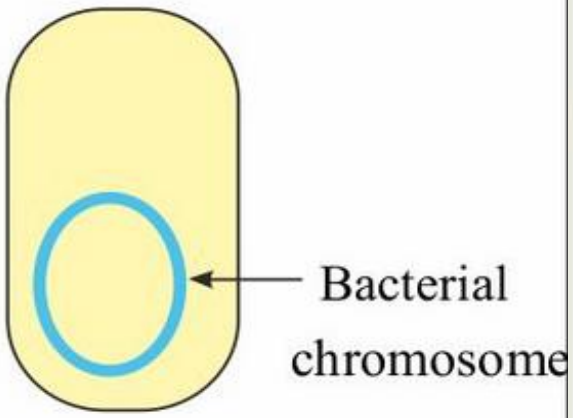
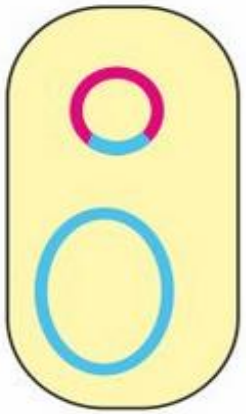
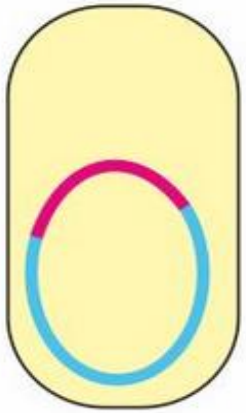
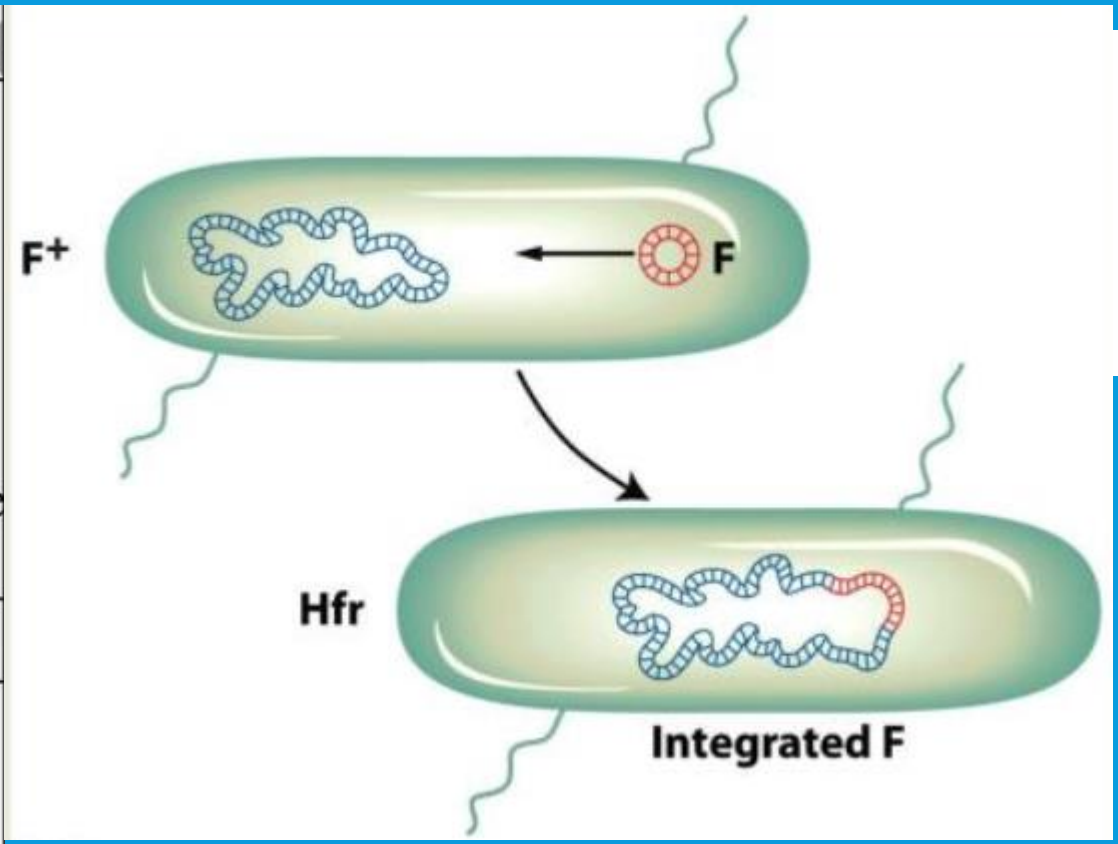
|  |  |
| $F^+$ cell – with F plasmid | $F^-$ cell – without F plasmid |
|  |  |
| F′ cell – with F plasmid containing few chromosomal genes | Hfr cell – F plasmid integrated in the bacterial chromosome |



# Historical Background

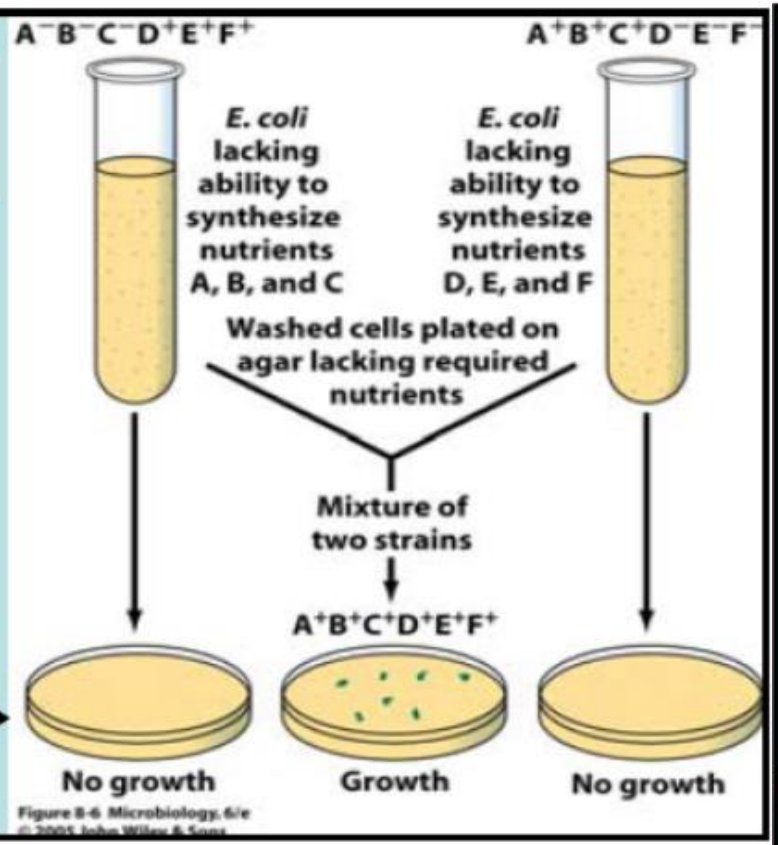
- Discovery: Joshua Lederberg & Edward Tatum (1946).
- They experimented with two auxotrophic strains of E.coli K12 denoted by Strain A and Strain B.
- **STRAIN A** ($met^{-}bio^{-}\ thr^{+}\ leu^{+}\ thi^{+}$) and **STRAIN B** ($met^{+}\ bio^{+}\ thr^{-}leu^{-}\ thi^{-}$) were plated on minimal medium and incubated overnight (CONTROL), no growth observed. Also STRAIN A and B were mixed together and when plated on minimal medium resulted in prototrophs.

Discovery of conjugation: Lederberg experiment

**Auxotrophic** strains of *E. coli*

Only grow on nutrient-rich medium which provides nutrients they cannot make themselves

**Minimal medium** (does NOT provide nutrients A/B/C/D/E/F)




# What happened?

Bacteria of the first strain donated the genes required to synthesize nutrients D/E/F to the second strain

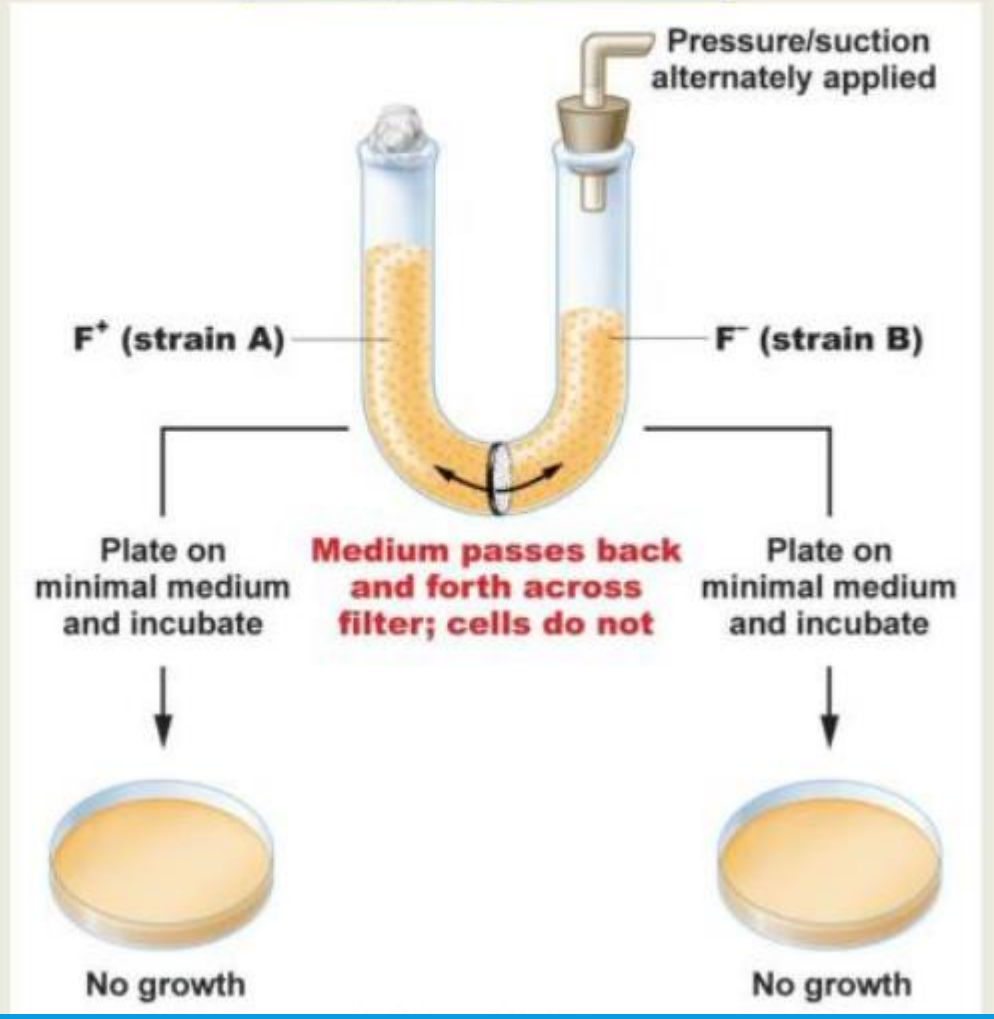
and/or

Bacteria of the second strain donated the genes required to synthesize nutrients A/B/C to the first strain

★ Either way, an *auxotroph* is converted to a *prototroph*

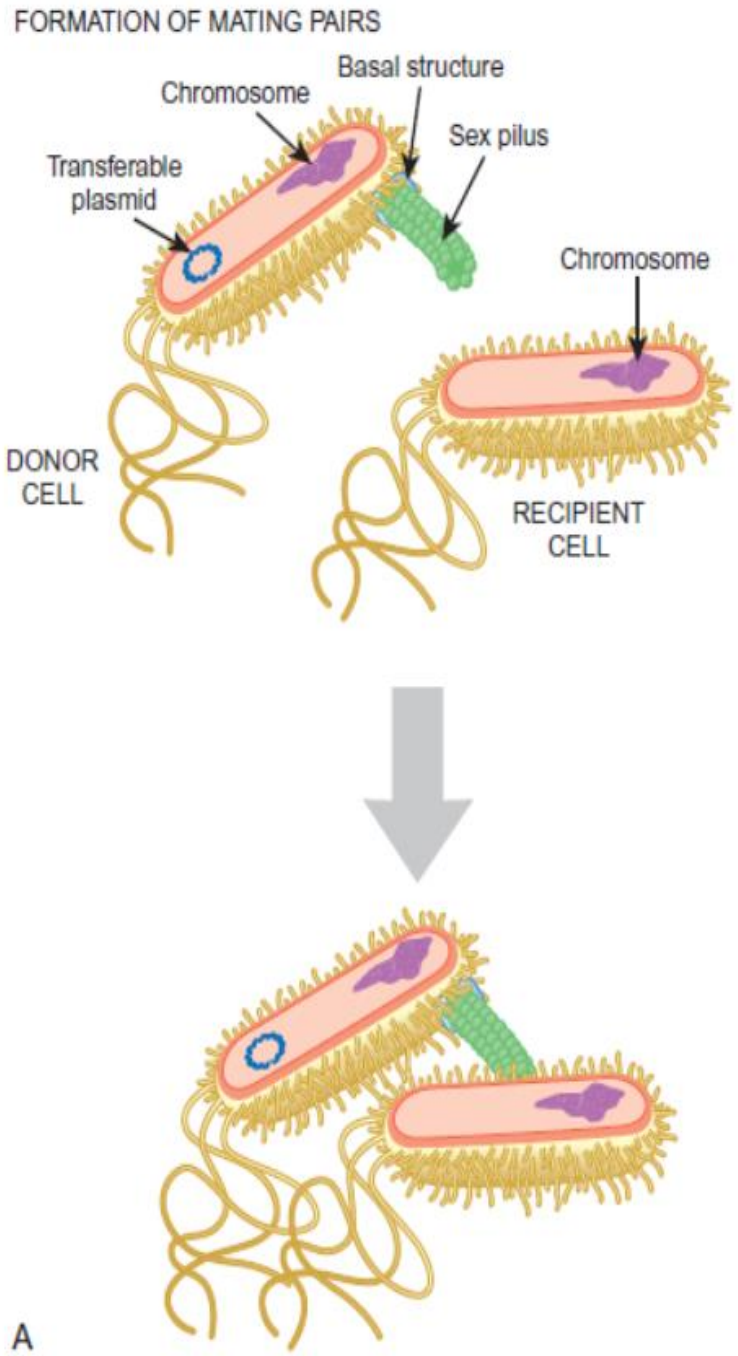
(mutant) (wild type)

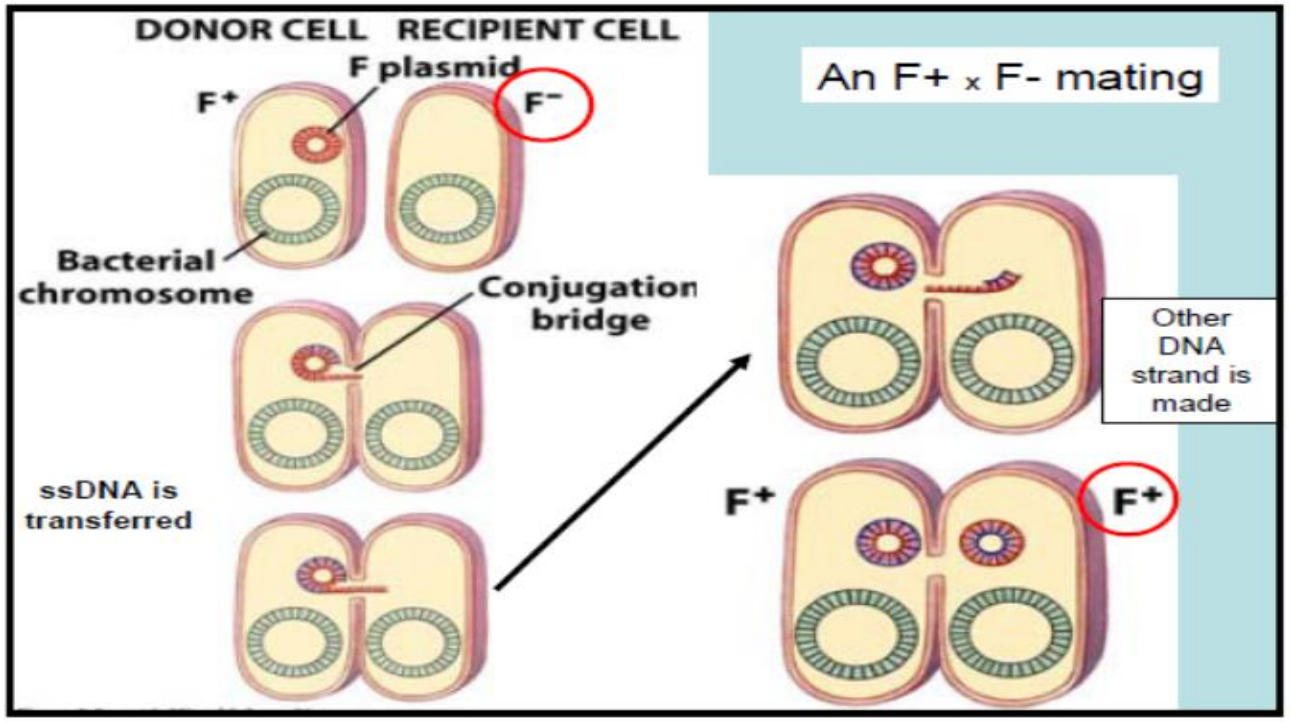
# Cell-cell contact is required for conjugation

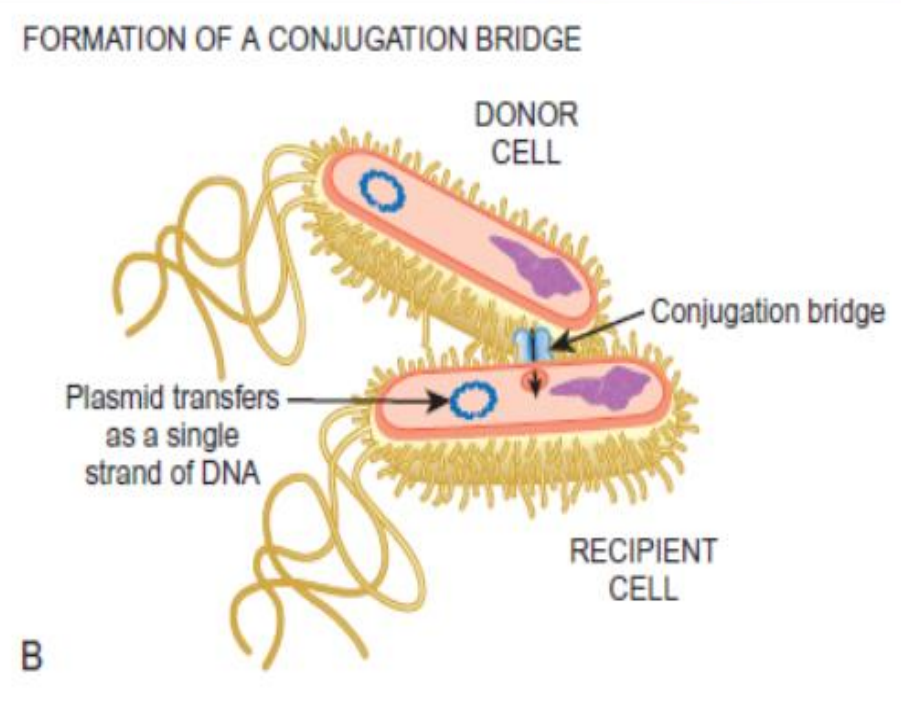


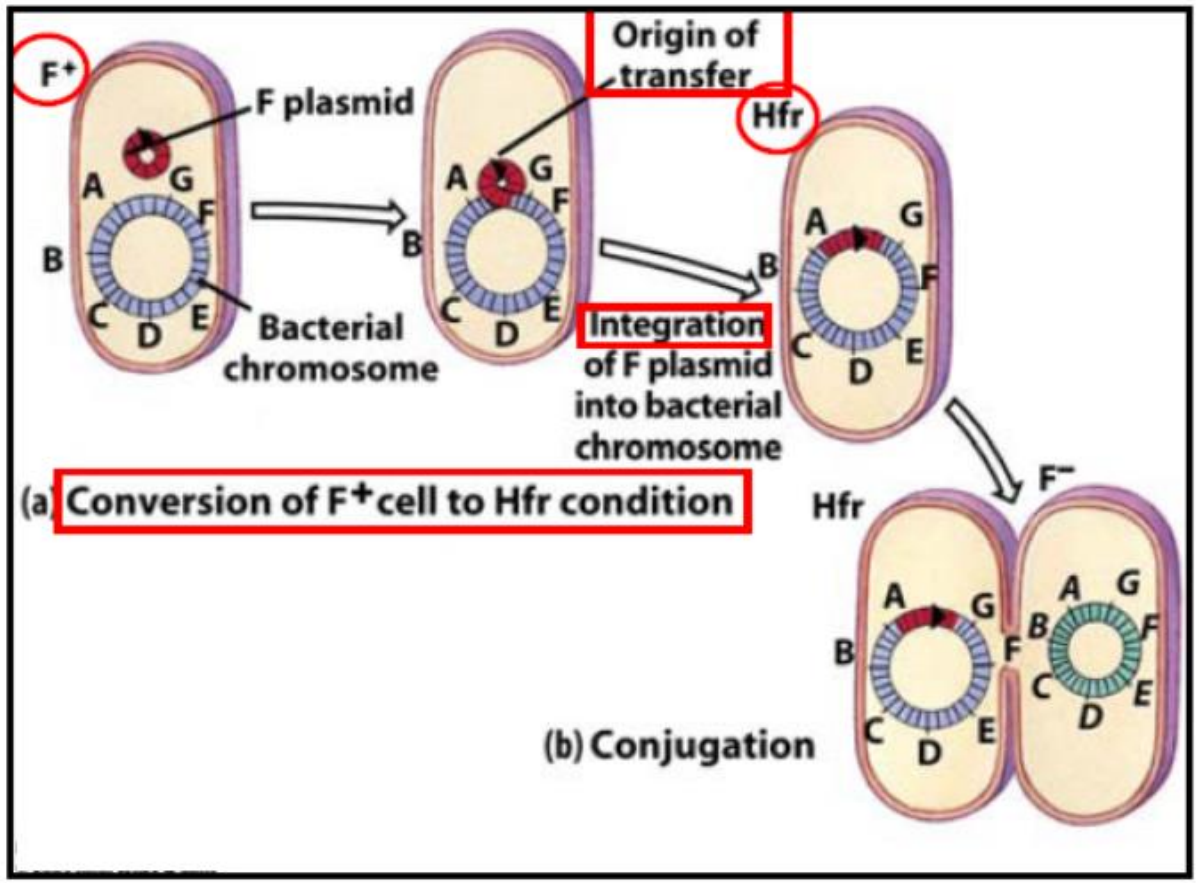
### *Bacterial Conjugation*

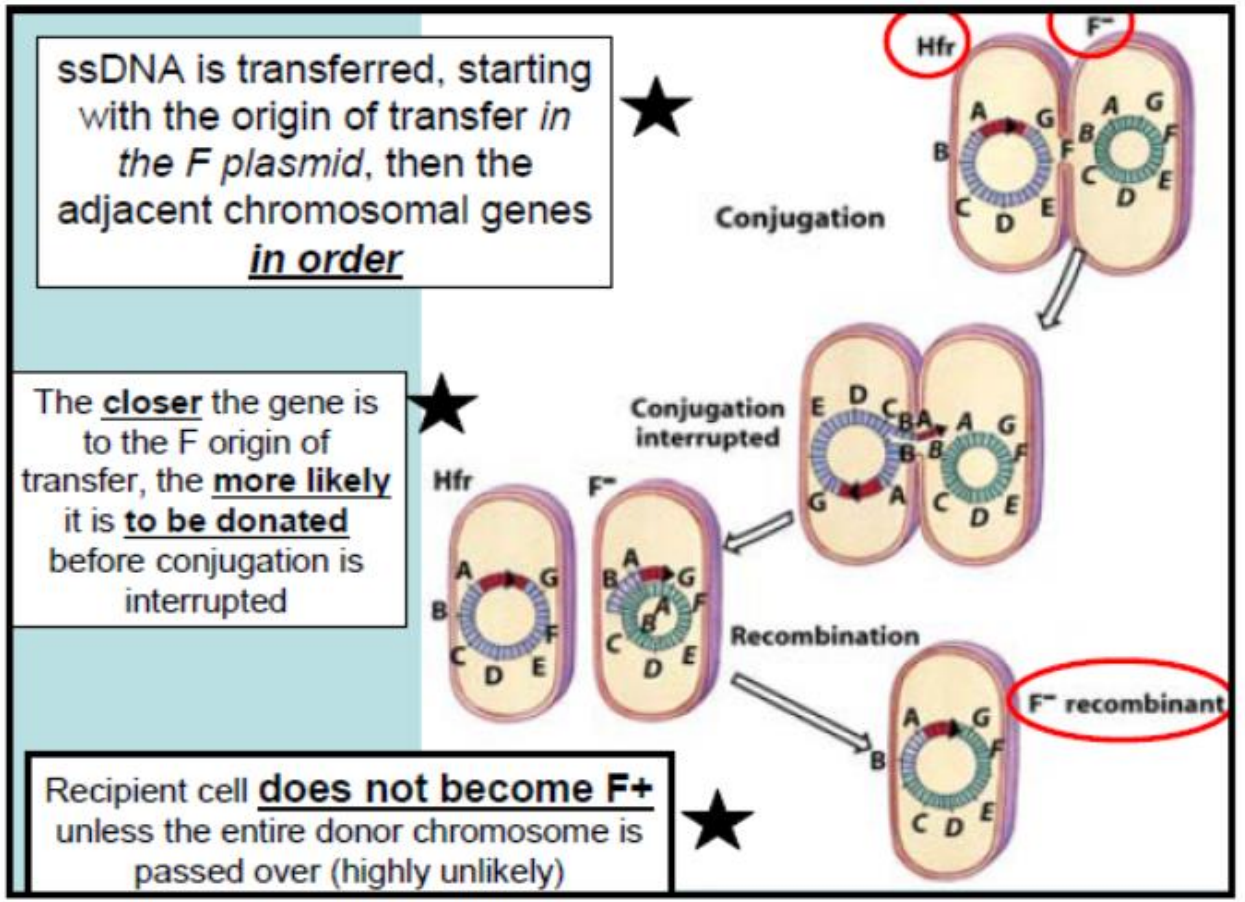
Certain plasmids, called $Tra^+$ or transfer-positive, are able to move a copy of their DNA into a different cell through a mechanism called bacterial conjugation. First, the cell containing a $Tra^+$ plasmid manufactures a rod-like extension on the surface of the outer membrane called a sex pilus. The sex pilus binds to a nearby cell and pulls the two cells together by retracting. Once the cells are in contact, the basal structure of the pilus makes a connection between the two cells known as the conjugation bridge. This connects the cytoplasm of the two cells, so the plasmid can transfer a copy of itself to the recipient cell.



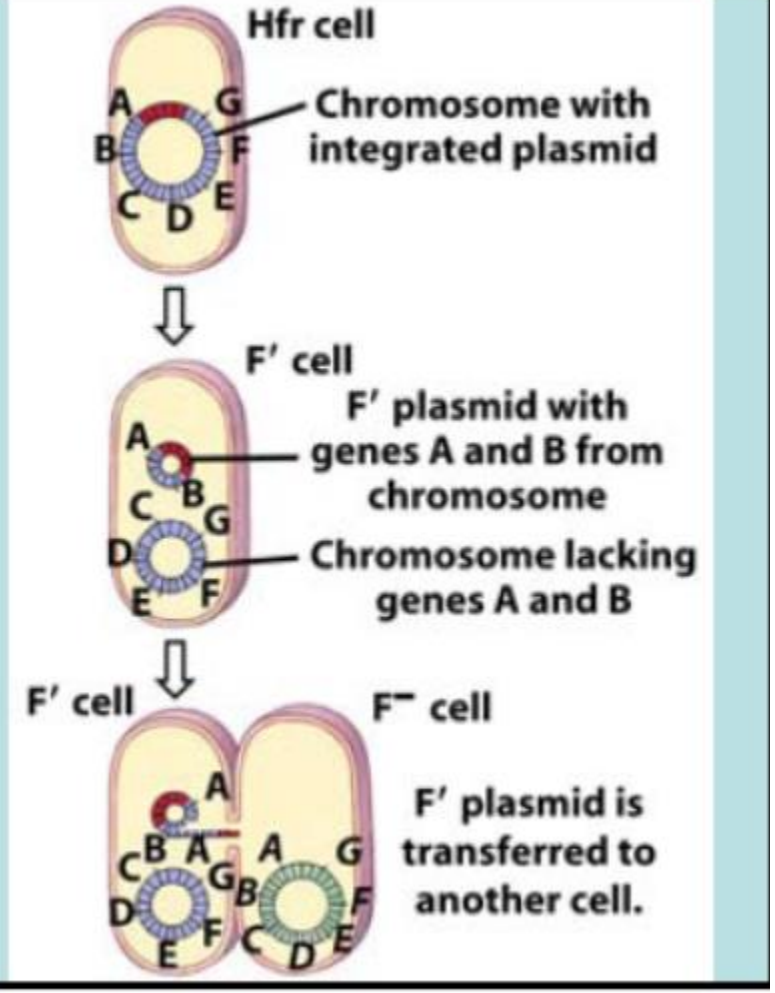



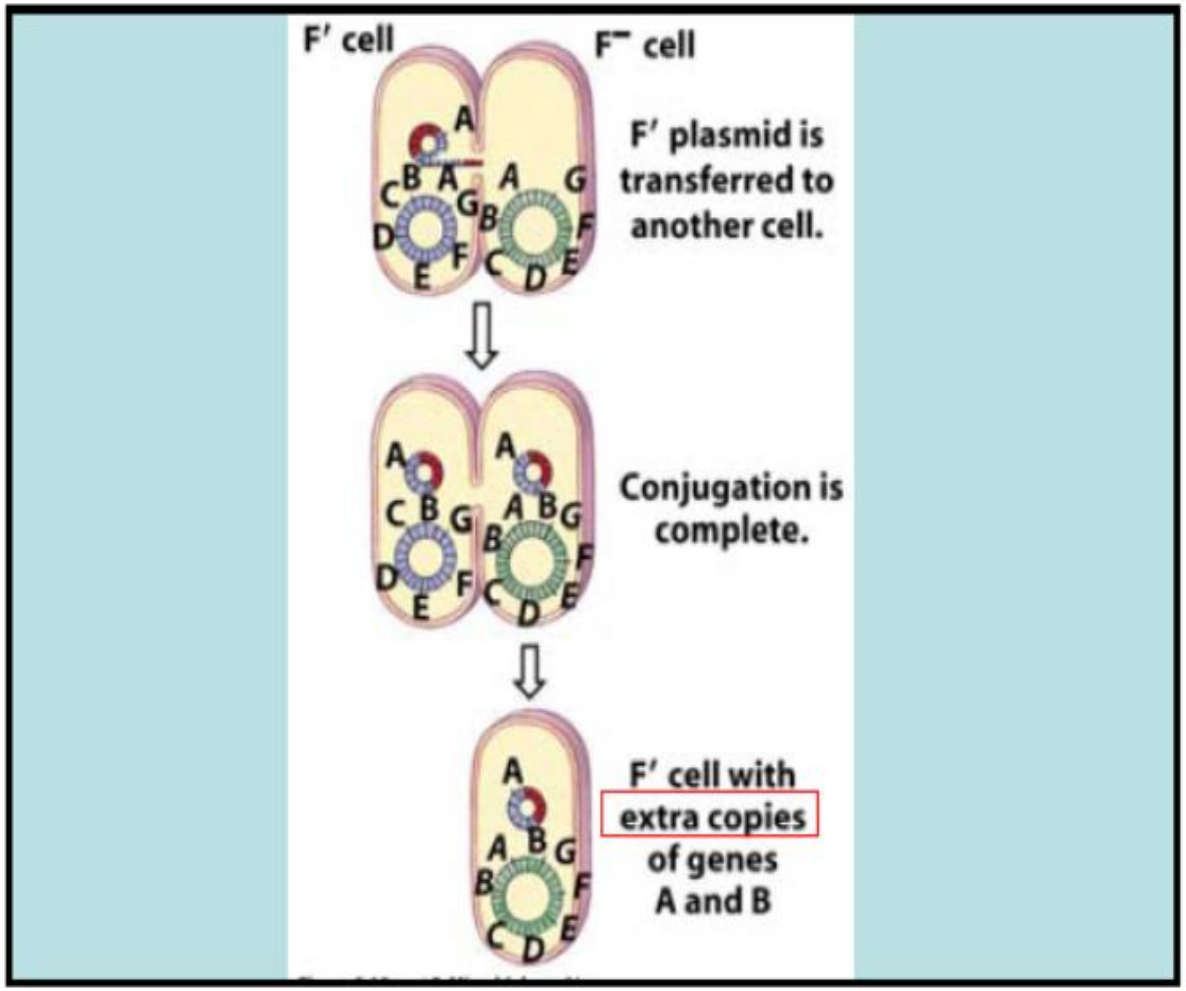




F+
F plasmid
Origin of transfer
Hfr
Bacterial chromosome
Integration of F plasmid into bacterial chromosome
(a) Conversion of F+ cell to Hfr condition
F−
Hfr
(b) Conjugation


ssDNA is transferred, starting with the origin of transfer *in the F plasmid*, then the adjacent chromosomal genes ***in order***
Hfr
F−
Conjugation
The **closer** the gene is to the F origin of transfer, the **more likely** it is **to be donated** before conjugation is interrupted
Conjugation interrupted
Hfr
F−
Recombination
F− recombinant
Recipient cell **does not become F+** unless the entire donor chromosome is passed over (highly unlikely)

Note: F' cell has not "lost" any genes, but they *have moved* from the chromosome to the plasmid



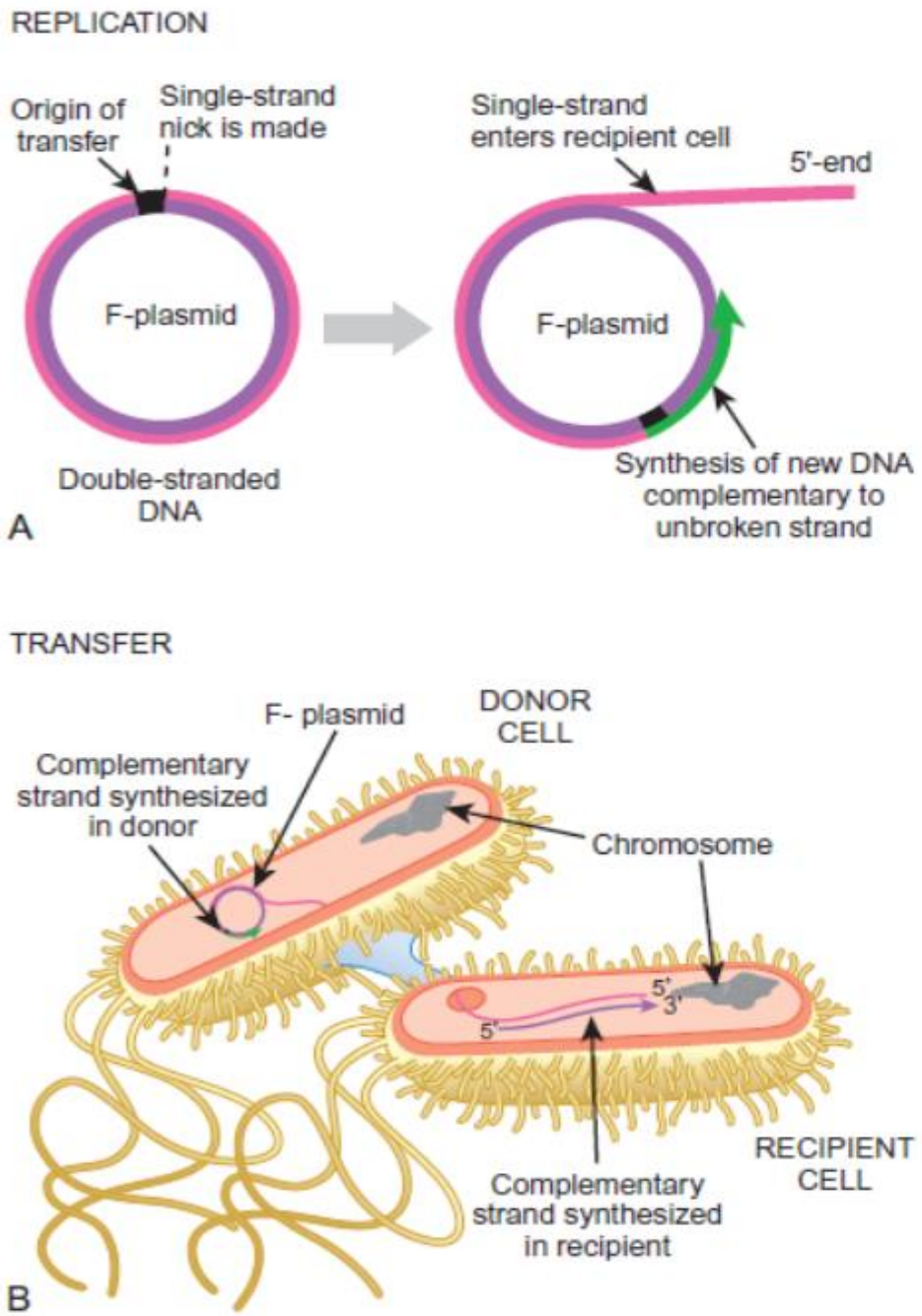
**Results of Selected Conjugations**

| Donor | Recipient | Molecule(s) Transferred | Product |
|---|---|---|---|
| $F^+$ | $F^-$ | F plasmid | $F^+$ cells |
| Hfr | $F^-$ | Initiating segment of F plasmid and variable quantity of chromosomal DNA | $F^-$ with variable quantity of chromosomal DNA |
| $F'$ | $F^-$ | $F'$ plasmid and some chromosomal genes it carries with it | $F'$ cell with some duplicate gene pairs: one on chromosome one on plasmid |



**Conjugating Cells of E. coli**

False-color transmission electron micrograph (TEM) of a male *E. coli* bacterium (bottom-right) conjugating with two females. This male has attached two F-pili to each of the females. The tiny bodies covering the F-pili are bacteriophage MS2, a virus that attacks only male bacteria and binds specifically to F-pili. Magnification: ×11,250. *Credit: Dr. L. Caro, Photo Researchers, Inc.*

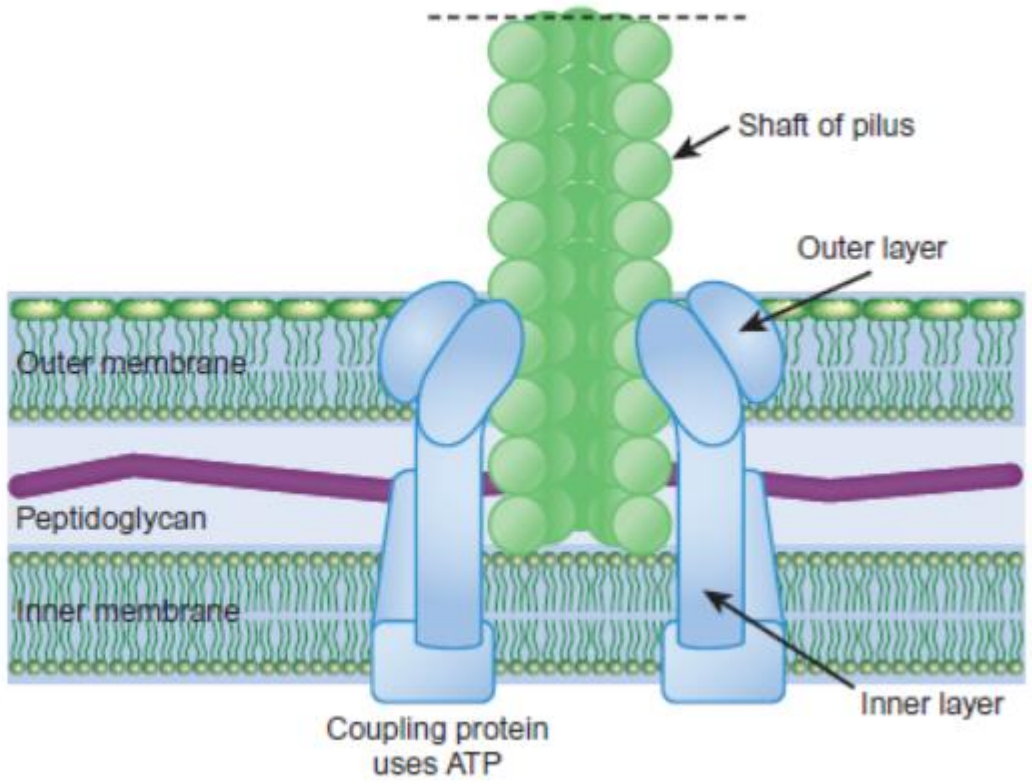



***Plasmid Transfer Involving Rolling Circle Replication***

(A) During bacterial conjugation, the F-plasmid of *E. coli* is transferred to a new cell by rolling circle replication. First, one strand of the F-plasmid is nicked at the origin of transfer. The two strands start to separate and synthesis of a new strand starts at the origin (green strand). (B) The single-strand of F-plasmid DNA that is displaced (pink strand) crosses the conjugation bridge and enters the recipient cell. The second strand of the F-plasmid is synthesized inside the recipient cell. Once the complete plasmid has been transferred, it is re-ligated to form a circle once again.

### *Basal Structure of the Sex Pilus*

The basal structure of the sex pilus resembles a type IV secretion system. It crosses both the inner and outer membranes and its central channel is large enough for the transit of proteins or DNA. The details of individual components vary somewhat between organisms, depending on the specific role of the system. This diagram is a simplified version showing the common core structures.



Plasmids unable to transfer themselves may be able to hitchhike using the transfer systems of other plasmids.

Although small plasmids such as ColE are not self-transferable, they are often mobilizable ($Mob^+$). A transferable plasmid, such as the F-plasmid, can mobilize the ColE plasmid if they both inhabit the same cell. The F-plasmid oversees conjugation and forms the conjugation bridge and the ColE plasmid is transferred through this. The *mob* (mobilization) genes of the ColE plasmid are responsible for making a single-stranded nick at the origin of transfer of ColE and for unwinding the strand to be transferred. Other small plasmids are neither self-transferable nor mobilizable.

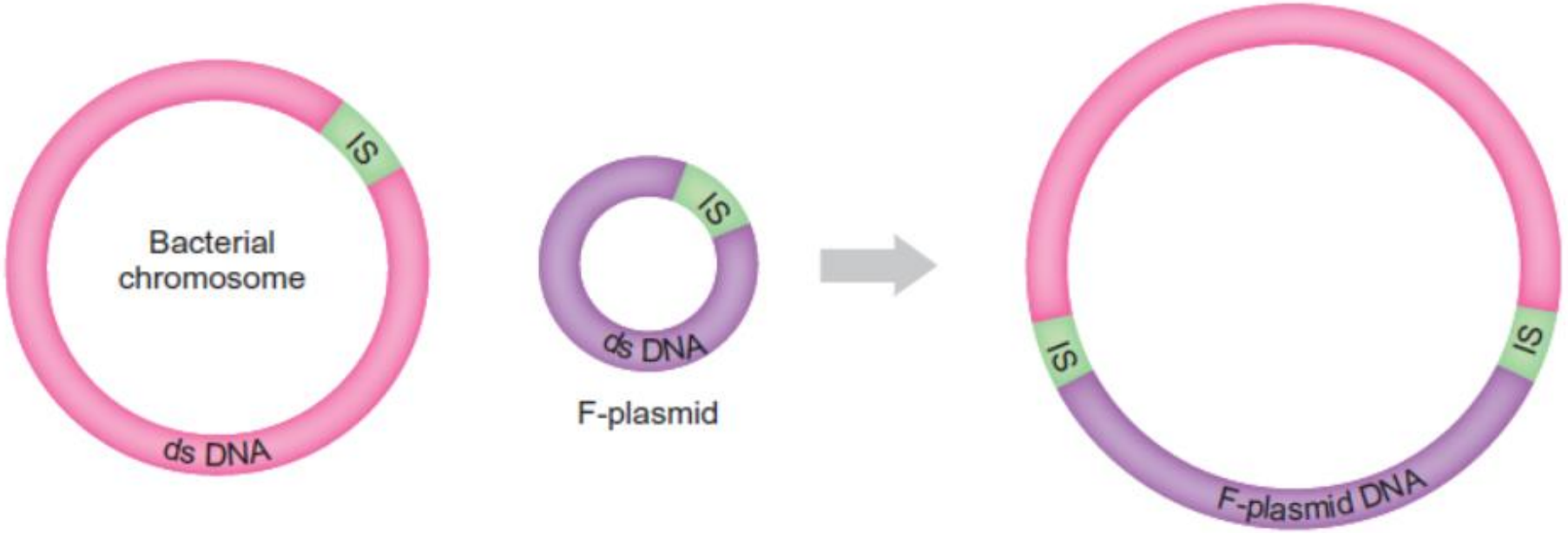
## 5.1. Transfer of Chromosomal Genes Requires Plasmid Integration

Transferable plasmids sometimes move chromosomal DNA from one cell to another.

Although many plasmids allow the cells carrying them to conjugate, usually only the plasmid itself is transferred through the conjugation bridge. But occasionally, plasmids mediate transfer of the host chromosome when they move from one bacterial cell to another. Plasmids, such as the F-plasmid of *E. coli*, that enable a cell to donate host chromosomal DNA are called **fertility plasmids**. In order to transfer chromosomal genes, a plasmid must first physically integrate itself into the chromosome of the bacterium. This event requires pairs of identical (or nearly identical) DNA sequences, one on the plasmid and the other on the chromosome. For example, the F-plasmid uses **insertion sequences** (see Chapter 25: Mobile DNA) for integration into the chromosome of *E. coli* (Fig. 28.16).

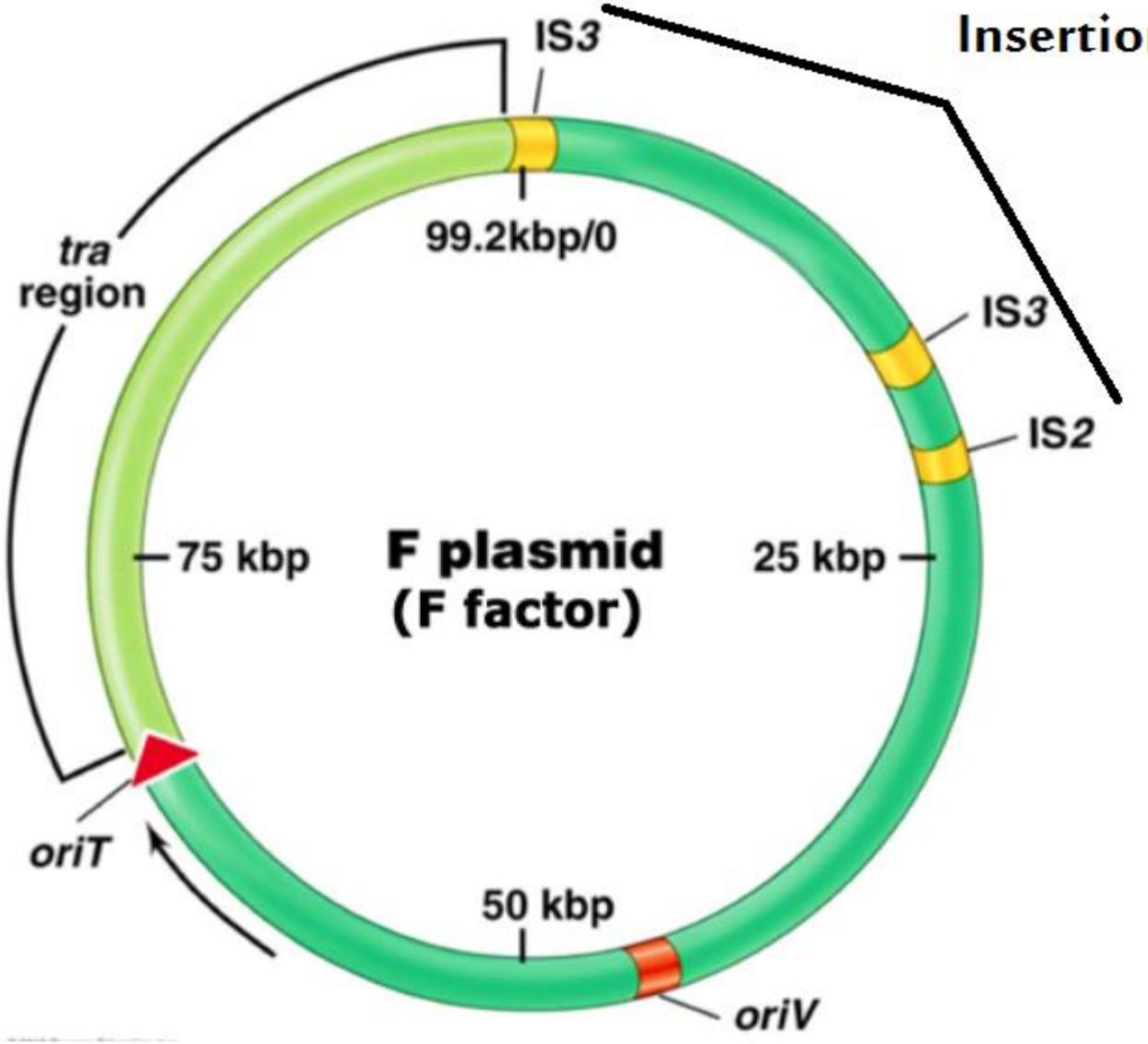
A variety of different insertion sequences are found on the chromosome of *E. coli* and in its plasmids and viruses. The F-plasmid has three insertion sequences (Fig. 28.17): Two copies of IS3 and a single copy of IS2. The chromosome of a typical laboratory strain of *E. coli* has 13 copies of IS2 and 6 copies of IS3 scattered

**fertility plasmid** Plasmid that enables a cell to donate DNA by conjugation.
**insertion sequence** A simple transposon consisting only of inverted repeats surrounding a gene that encodes transposase.

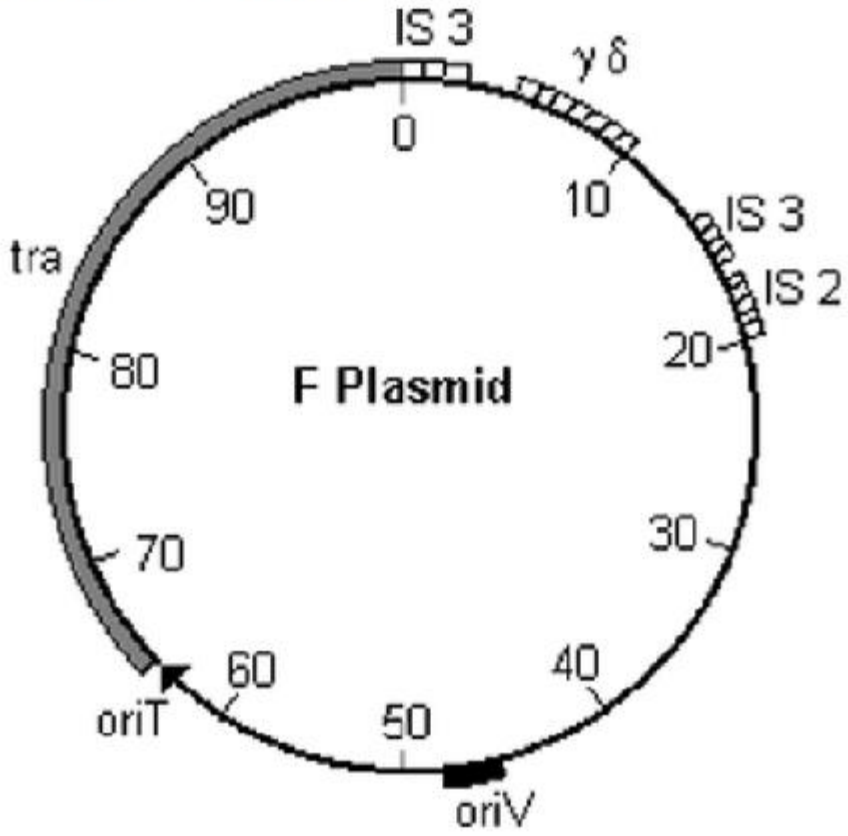



***Integration of F-Plasmid Into Chromosome***

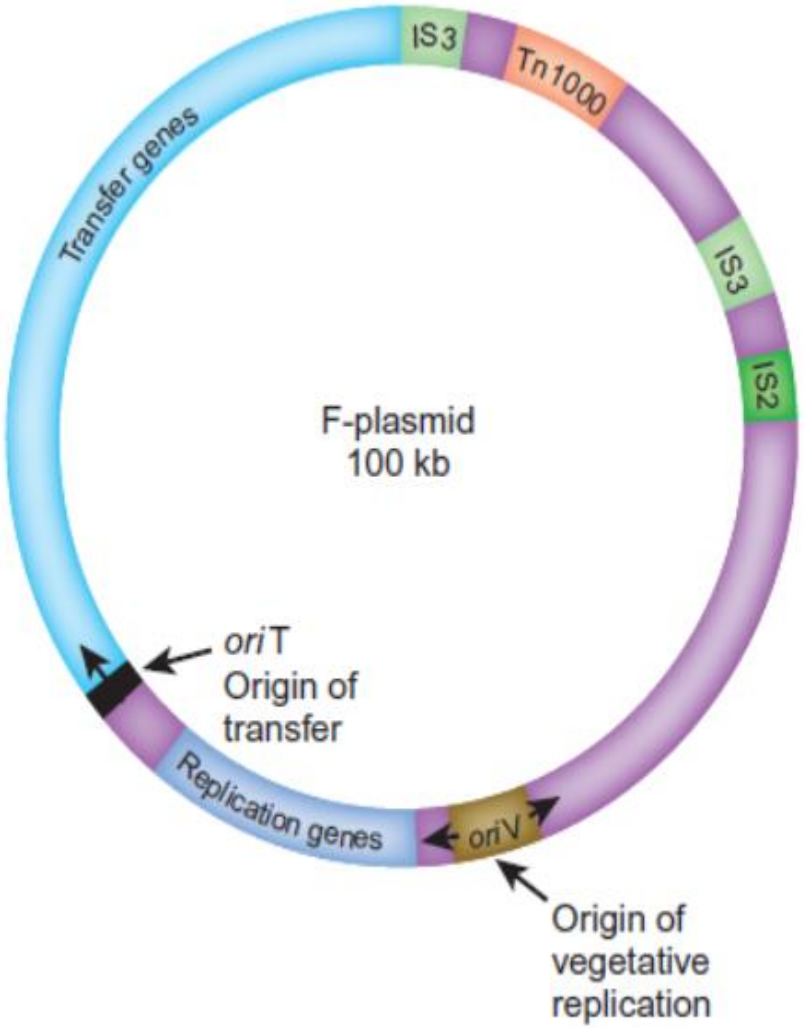
If recombination occurs between two insertion sequences, one on the F-plasmid and one on the host bacterial chromosome, the entire F-plasmid becomes integrated into the chromosome.




## Insertion and excision




IS 3 & IS 2 = insertion sequences
γδ = transposon Tn1000
oriV = origin of replication
oriT = origin of conjugal transfer
tra = tra functions




## *Insertion Sequences on F-Plasmid and Chromosome*

Insertion sequences are scattered throughout the F-plasmid and chromosome of *E. coli*. The F-plasmid has two IS3 elements and one IS2 element. Even more copies of IS2 and IS3 are found on the chromosome (not shown). A recombination event between any of the chromosomal IS2 or IS3 elements and the corresponding element on the F-plasmid will integrate the entire F-plasmid into the chromosome. Tn1000 (also known as $\gamma\delta$) is another insertion sequence, although not generally involved in F-plasmid integration in *E. coli*.

around more or less at random. Integration of the F-plasmid may occur in either orientation at any of these 19 sites.

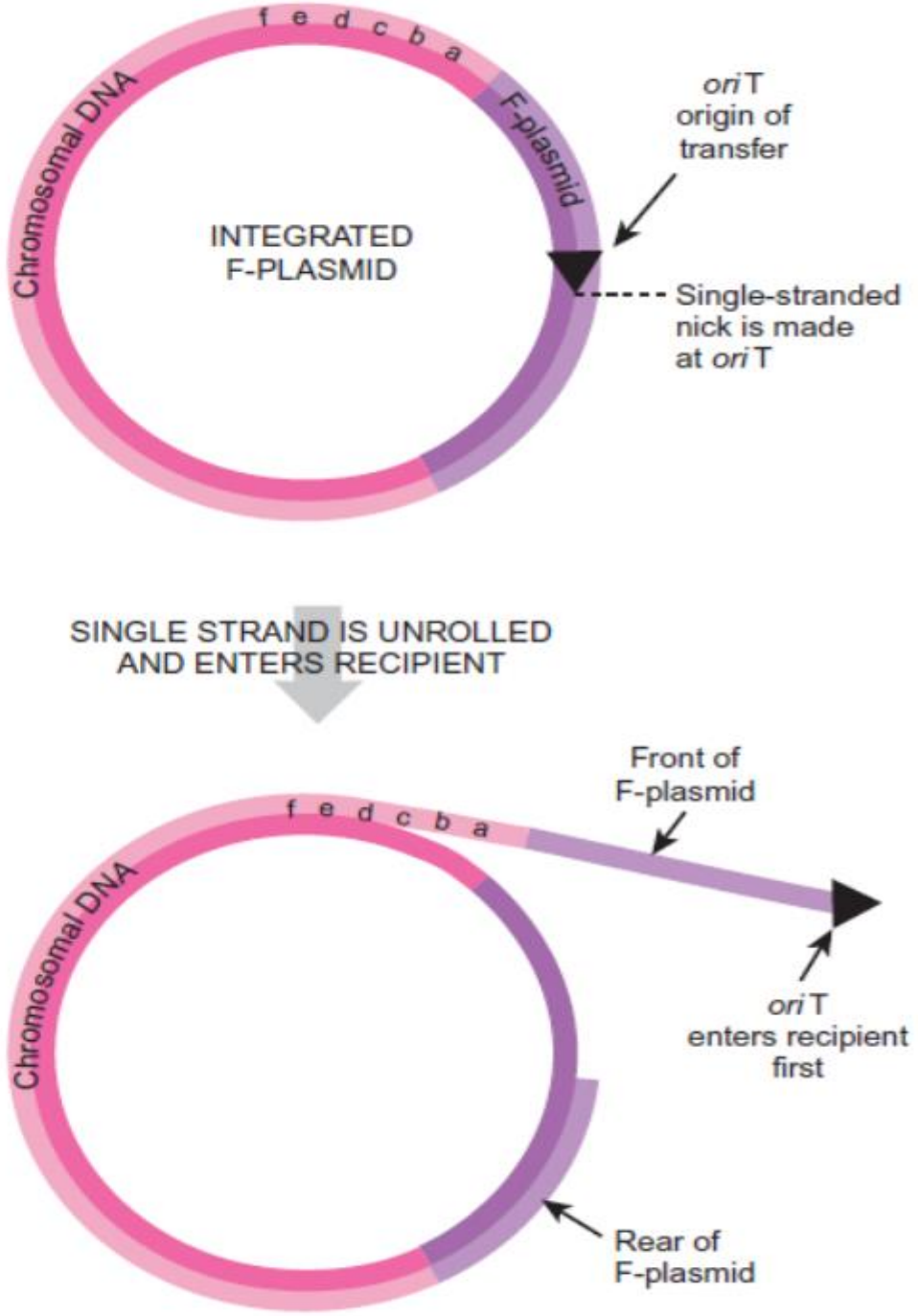
When an F-plasmid that is integrated into the chromosome transfers itself by conjugation, it drags along the chromosomal genes to which it is attached (Fig. 28.18). Just as for the unintegrated F-plasmid, only a single strand of the DNA moves and the recipient cell has to make the complementary strand itself. Bacteria with an F-plasmid integrated into the chromosome are known as **Hfr strains** because they transfer chromosomal genes at high frequency. A prolonged mating of 90 minutes or so is needed to transfer the whole chromosome of *E. coli*. More often, bacteria break off after a shorter period of, say, 15–30 minutes, and only part of the chromosome is transferred. Since different Hfr strains have their F-plasmids inserted at different sites on the bacterial chromosome, transfer of chromosomal genes begins at different points. In addition, the F-plasmid may be inserted in either orientation. Consequently, chromosomal gene transfer may be either clockwise or counterclockwise for any particular Hfr strain.

In order to mobilize chromosomal DNA, the plasmid must first integrate into the chromosome.

**Hfr-strain** Bacterial strain that transfers chromosomal genes at high frequency due to an integrated fertility plasmid (F-plasmid).

## *Transfer of Chromosomal Genes by F-Plasmid*

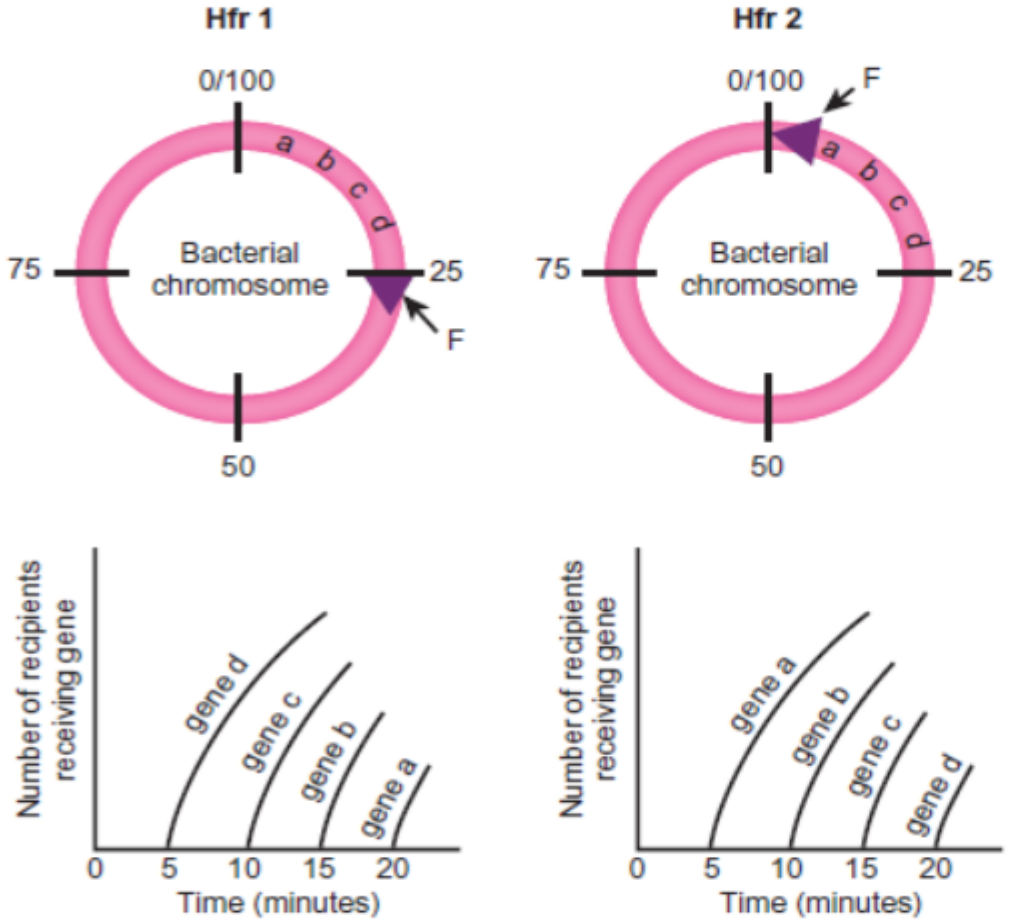
An integrated F-plasmid can still induce bacterial conjugation and rolling circle transfer of DNA into another bacterial cell. Since rolling circle replication does not stop until the entire circle is replicated, the attached chromosome is also transferred into the recipient cell. First, a single-stranded nick is made at the *ori*T, or transfer origin of the integrated plasmid. The free 5′ end (black triangle) enters the recipient cell through the conjugation bridge. Notice that the transfer of the single-stranded DNA does not end with the F-plasmid DNA and continues into the chromosomal DNA. Genes closest to the site of plasmid integration are transferred first (in the order a, b, c, d, e, f, in this example). The amount of chromosomal DNA that is transferred depends on how long the two bacteria remain attached by the conjugation bridge.



Hfr strains were used in earlier times to identify the order of genes on the *E. coli* chromosome. To monitor whether the recipient has received a particular gene, the donor and recipient strains must have different alleles of this gene that can be distinguished phenotypically, usually by their growth properties. For example, the recipient might have a mutation in the *lac* operon that prevents growth on lactose as carbon source. The donor Hfr strain would have an allele that restores the ability to use lactose. Using this method, genetic maps were constructed by two major approaches. First, the **cotransfer frequency** of two genes was measured. If two genes were close to each other, a donor Hfr strain would transfer them together at high frequency. Conversely, if two genes were far apart on the chromosome, an Hfr strain would usually only transfer one of them, and the cotransfer frequency would be low.

Secondly, time-of-entry measurements were made to determine gene order. Hfr strains transfer chromosomal genes starting where the F-plasmid is integrated and proceeding sequentially around the circular chromosome (Fig. 28.19). The length of time it takes for a gene to enter the recipient gives an estimate of its relative distance from the origin of transfer of the Hfr strain. For time of entry mapping the site and orientation of the F-plasmid must be known. In addition, mutations in the genes being studied (a, b, c, and d) must give recognizable phenotypes. Finally, the recipient must be resistant to some antibiotic (e.g., streptomycin) so that it can be selected on medium that prevents growth of the Hfr strain. Different Hfr strains will transfer the same genes in different orders and at different times, depending on their location relative to the integration site of the F-plasmid.

**cotransfer frequency** Frequency with which two genes remain associated during transfer of DNA between bacterial cells.




F-plasmids can excise themselves from the chromosome by reversing the integration process. Sometimes they excise carrying pieces of chromosomal DNA, which creates **F′- or F-prime plasmids**. This typically occurs by recombination between a different pair of IS sequences than used during integration. Such F′-plasmids may be transferred to F-minus recipients, carrying with them the chromosomal segment from their previous host. If the chromosomal segment is homologous, the F′ can reintegrate via homologous recombination. Historically, F-primes were used to carry part of the *lacZ* gene in the **alpha-complementation** method for screening recombinant plasmids

***Time of Entry by Conjugation***

To determine the time of entry by conjugation, the Hfr strain is mixed with a recipient strain carrying a defective copy of a particular gene, "a." After conjugation has proceeded for a specific time, a sample of the mixture is removed. This is plated on agar, which prevents growth of the Hfr and only allows growth of strains carrying the wild-type version of gene "a." Survivors are derivatives of the recipient that have gained the wild-type version of gene "a" from the Hfr. This is repeated for several time points. The whole procedure is then repeated for the other genes. In strain Hfr 1 (left panel), the integrated F-plasmid is closest to gene "d" and only begins transferring gene "a" after about 20 min. In strain Hfr 2 (right panel), the F-plasmid is integrated closer to gene "a," which therefore begins to appear in the recipient as early as 5 min after transfer begins.