MOLECULAR BIOLOGY

CONJUGATION

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CONJUGATION

In 1946 Joshua Lederberg and Tatum discovered that some bacteria can transfer genetic information to other bacteria through a process known as conjugation. Bacterial conjugation is the transfer of DNA from a living donor bacterium to a recipient bacterium.

Plasmids are small autonomously replicating circular pieces of double-stranded circular DNA. Conjugation involves the transfer of plasmids from donor bacterium to recipient bacterium.

Plasmid transfer in Gram-negative bacteria occurs only between strains of the same species or closely related species. Some plasmids are designated as F factor (F plasmid, fertility factor or sex factor) because they carry genes that mediate their own transfer. The F factor can replicate autonomously in the cell. These genes code for the production of the sex pilus and enzymes necessary for conjugation.

Cells possessing F plasmids are F+ (male) and act as donors. Those cells lacking this plasmid are F- (female) and act as recipient. All those plasmids, which confer on their host cells to act as donors in conjugation are called transfer factor.

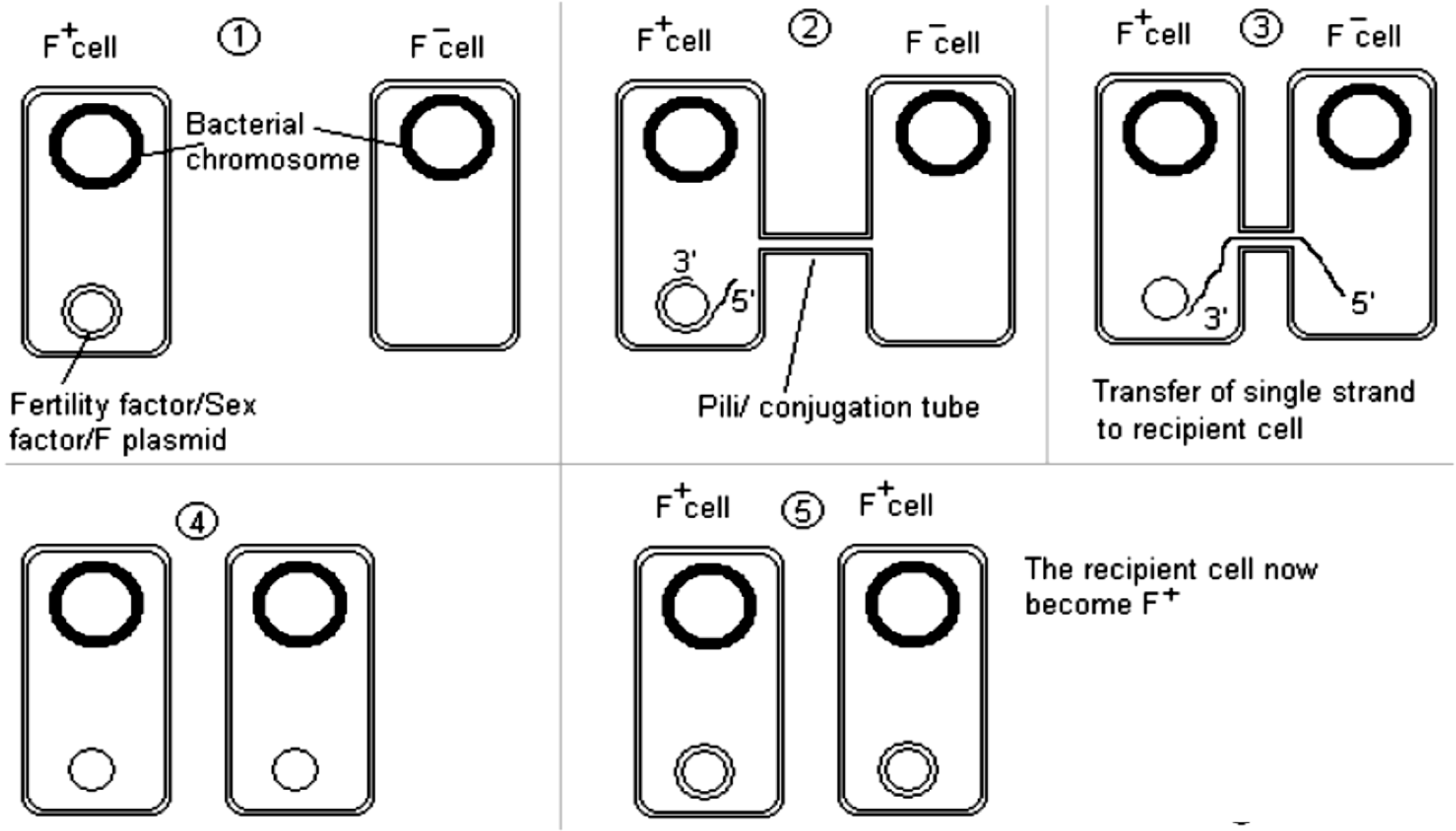
Each Gram negative F+ bacterium has 1 to 3 sex pili that bind to a specific outer membrane protein on recipient bacteria to initiate mating. The sex pilus then retracts, bringing the two bacteria in contact and the two cells become bound together at a point of direct envelope-to-envelope contact. In Gram-positive bacteria sticky surface molecules are produced which bring the two bacteria into contact. Gram-positive donor bacteria produce adhesins that cause them to aggregate with recipient cells, but sex pili are not involved. DNA is then transferred from the donor to the recipient. Plasmid-mediated conjugation occurs in Bacillus subtilis, Streptococcus lactis, and Enterococcus faecalis but is not found as commonly in the Gram-positive bacteria as compared to the Gram-negative bacteria.

1. F+ CONJUGATION

This results in the transfer of an F+ plasmid (coding only for a sex pilus) but not chromosomal DNA from a male donor bacterium to a female recipient bacterium. The two strands of the plasmid separate. One strand enters the recipient bacterium progressing in the 5' to 3' direction while one strand remains in the donor. The complementary strands are synthesized in both donor and recipient cells. The recipient then becomes an F+ male and can make a sex pilus.

During conjugation, no cytoplasm or cell material except DNA passes from donor to recipient. The mating pairs can be separated by shear forces and conjugation can be interrupted. Consequently, the mating pairs remain associated for only a short time.

After conjugation, the cells break apart. Following successful conjugation the recipient becomes F+ and the donor remains F+.


F+cell
①
F−cell
Bacterial chromosome
Fertility factor/Sex factor/F plasmid
F+cell
②
F−cell
3'
5'
Pili/ conjugation tube
F+cell
③
F−cell
3'
5'
Transfer of single strand to recipient cell
④
F+cell
⑤
F+cell
The recipient cell now become F+

2. RESISTANCE PLASMID CONJUGATION

- Some Gram-negative bacteria harbor plasmids that contain antibiotic resistance genes, such plasmids are called **R factors**.
- The R factor has two components, one that codes for self transfer (like F factor) called **RTF** (resistance transfer factor) and the other R determinant that contains genes coding for antibiotic resistance.
- R plasmids may confer resistance to as many as five different antibiotics at once upon the cell and by conjugation; they can be rapidly disseminated through the bacterial population.
- The difference between F factor and R factor is that the latter has **additional genes coding for drug resistance**.

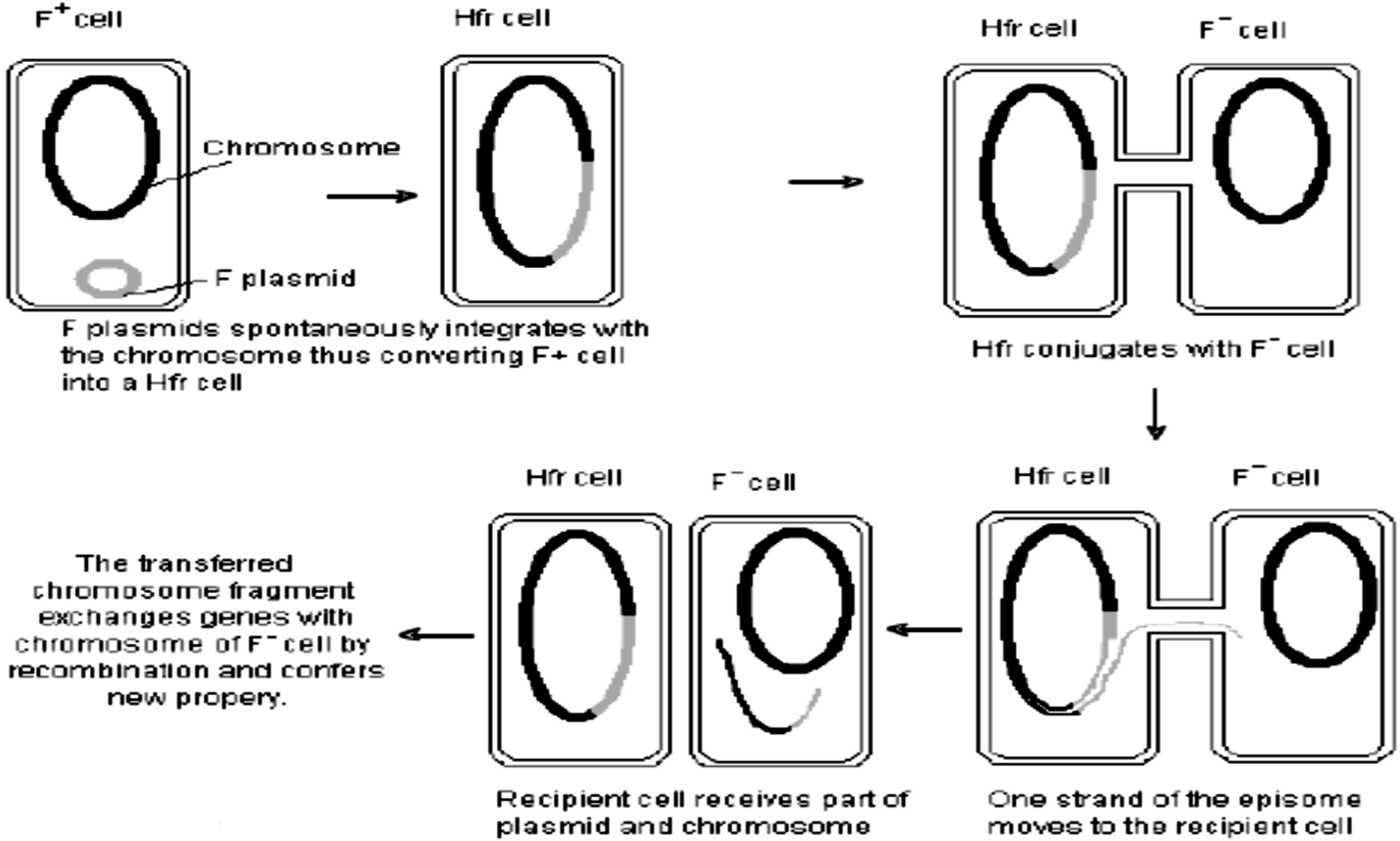
During conjugation there is transfer of resistance plasmid (R plasmid) from a donor bacterium to a recipient. One plasmid strand enters the recipient bacterium while one strand remains in the donor. Each strand then makes a complementary copy. R-plasmid has genes coding for multiple antibiotic resistance as well as sex pilus formation. The recipient becomes multiple antibiotic resistant and male, and is now able to transfer R-plasmids to other bacteria.

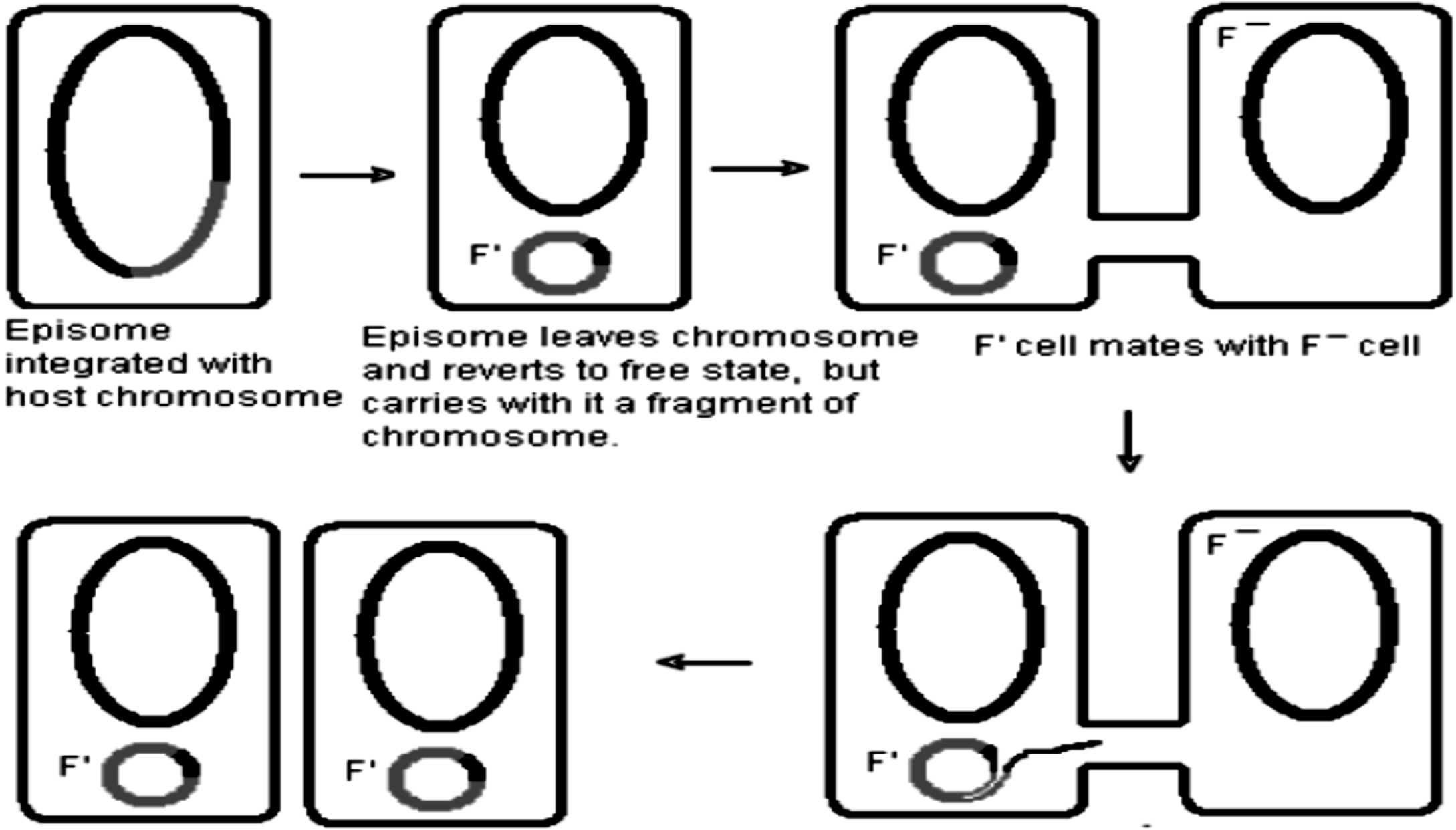
When the recipient cells acquire entire R factor, it too expresses antibiotic resistance.

Sometimes RTF may disassociate from the R determinant and the two components may exist as separate entities. In such cases though the host cell remains resistant to antibiotics, it can not transfer this resistance to other cells. Sometimes RTF can have other genes (such as those coding for hemolysin, enterotoxin) apart from R determinants attached to it.

3. HFR (HIGH FREQUENCY RECOMBINANT) CONJUGATION

Plasmids may integrate into the bacterial chromosome by a recombination event depending upon the extent of DNA homology between the two. After integration, both plasmid and chromosome will replicate as a single unit. A plasmid that is capable of integrating into the chromosome is called an **episome**. If the F plasmid is integrated into the chromosome it is called an **Hfr cell**. After integration, both chromosome and plasmid can be conjugally transferred to a recipient cell. Hfr cells are called so because **they are able to transfer chromosomal genes to recipient cells with high frequency.**


F+ cell
Hfr cell
Chromosome
F plasmid
F plasmids spontaneously integrates with the chromosome thus converting F+ cell into a Hfr cell
Hfr cell
F⁻ cell
Hfr conjugates with F⁻ cell
Hfr cell
F⁻ cell
One strand of the episome moves to the recipient cell
Hfr cell
F⁻ cell
Recipient cell receives part of plasmid and chromosome
The transferred chromosome fragment exchanges genes with chromosome of F⁻ cell by recombination and confers new propery.



Episome integrated with host chromosome

Episome leaves chromosome and reverts to free state, but carries with it a fragment of chromosome.

F' cell mates with F⁻ cell

One strand of F' is transferred to the recipient cell

Both donor and recipient have F' factors

The DNA is nicked at the origin of transfer and is replicated. One DNA strand begins to passes through a cytoplasmic bridge to the F- cell, where its complementary strand is synthesized. Along with the portion of integrated plasmid, the chromosome is also transmitted to the F- cell. The bacterial connection usually breaks before the transfer of the entire chromosome is completed so the remainder of the F+ plasmid rarely enters the recipient. Usually only a part of the Hfr chromosome as well as the plasmid is transferred during conjugation and the recipient cell does not receive complete F factor. After conjugation the Hfr cell remains Hfr but the F- cell does not become F+ and continues to remain F- . However the transferred chromosome fragment recombines with the chromosome of F- cell thereby transferring some new property to the recipient cell.

The integration of episome into the chromosome is not stable and the episomes are known to revert back to free state. While doing so, the episomes sometimes carry fragments of chromosomal genes along with it. **Such an F factor that incorporates some chromosomal genes is called F prime (F') factor**. When such a F' cell mates with F- recipient cell, it not only transfers the F factor but also the host genes that it carried with it. This process of transfer of chromosomal genes along with F factor is known is **sexduction**.

SIGNIFICANCE

Among the Gram negative bacteria this is the major way that bacterial genes are transferred. Transfer can occur between different species of bacteria. Transfer of multiple antibiotic resistance by conjugation has become a major problem in the treatment of certain bacterial diseases. Since the recipient cell becomes a donor after transfer of a plasmid, an antibiotic resistance gene carried on a plasmid can quickly convert a sensitive population of cells to a resistant one.