RAMA UNIVERSITY

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FACULTY OF ENGINEERING &TECHNOLOGY

DEPARTMENT OF BIOTECHNOLOGY

Conjugation

Conjugation is a process during which genetic information is transferred unidirectionally from a donor (“male) bacterium to a recepient (“Female”) through a cytoplasmic channel between two cells.

The required cell-to-cell contact between the donor and the recepient can be achieved through sex pili (e.g. F pili) as in the case of enteric gut bacteria (e.g. *E.coli* and *Salmonella*) or through agglutinin (Substances that promote cell clumping) or pheromones (chemicals produced by an individual that alters the behaviour of other members of the same species) as in the case of some gram +ive cocci (e.g. *Enterococcus faecalis*).

During conjugation, whole plasmids, some 50 or more genes, and large portion of the bacterial chromosomes can be transferred.

In theory, the entire bacterial chromosome can be transferred during conjugation, but this rarely happens because the cytoplasmic channel usually breaks before transfer is complete.

Types of donor bacteria

There are at least three types of donor bacteria. These are F^+, F', and Hfr bacteria.

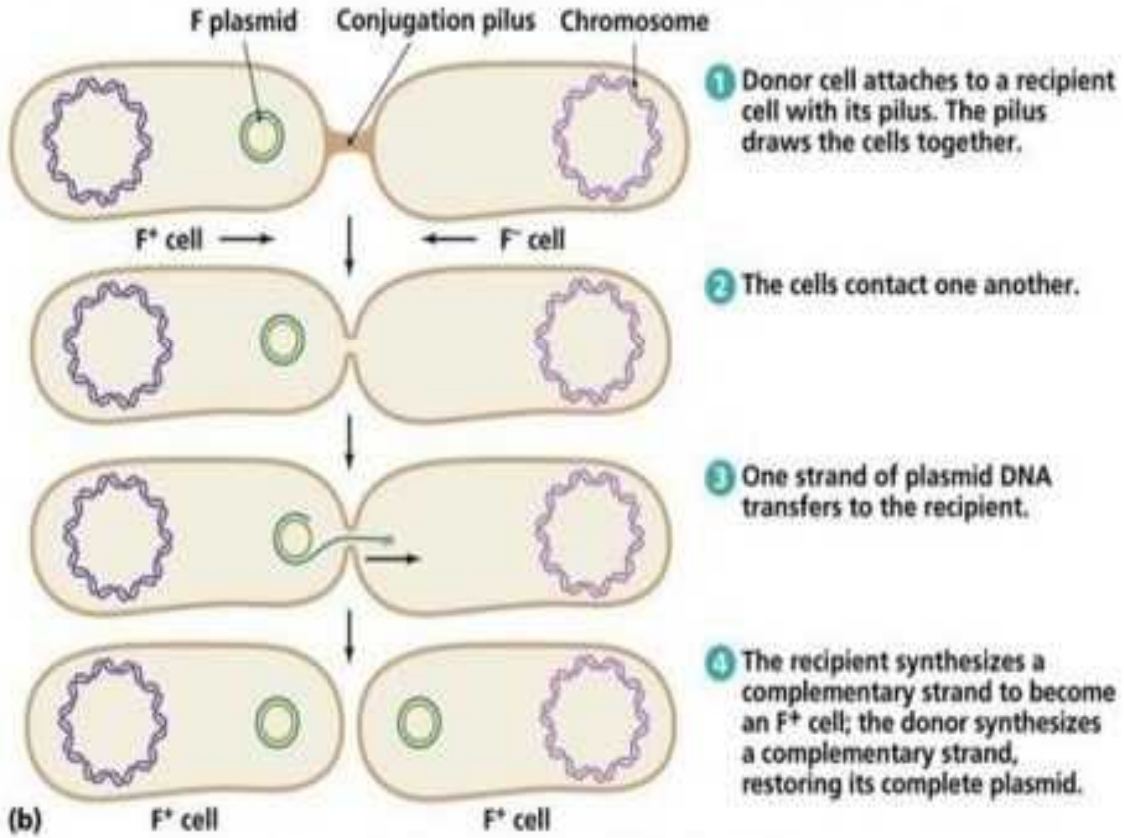
All these three types of bacteria posses sex pili, which form a cytoplasmic bridge with recipient bacteria during conjugation. Recipient bacteria, which lacks the sex pilus, are symbolized F^-.

F^+ = Bacterial strains that contains Fertility factor plasmid (F plasmid). F plasmid is a conjugative plasmid which contains genetic information that codes for pilin, used to make sex pilus necessary for conjugation.

F' = F′ plasmids are derivatives of the F sex plasmid.

Hfr = Bacterial strains exhibiting high frequency of recombination for certain chromosomal gene.

Bacterial Conjugation



Mechanism of plasmid mobilization by conjugation

Conjugative plasmids initiate gene transfer by altering the cell surface to allow contact between the plasmid-containing donor cell (F+ or male) and a plasmid lacking recipients (F- or female)

Sex pilus originates from the donor and establishes conjugative bridge (temporary cytoplasmic bridge) that serves as the conduit for DNA transfer from donor to recipient bacterial cell

Intercellular contact established
A copy of DNA from donor cell (F+ Cell) is transferred to recipient cell (F- cell).
Complementary DNA strand is synthesized in both donor cell and recipient cell.

As the recipient cell now contains F plasmid it behaves as a donor cell.
If F^- cells and F+ cells are mixed in a culture, the entire population quickly becomes F +

Molecular machinery and components involved in bacterial conjugation

Conjugation is brought about by 2 genes in self transmissible plasmid, namely transacting gene ***(Tra* gene)** and Origin of transfer (Ori T) site.

Tra genes further contain **Dtr** (DNA transfer and replication components) and Mpf (Mating pair formation.)

Dtr component prepare plasmid for transfer and it includes components such as relaxses, relaxosome complex and primase.

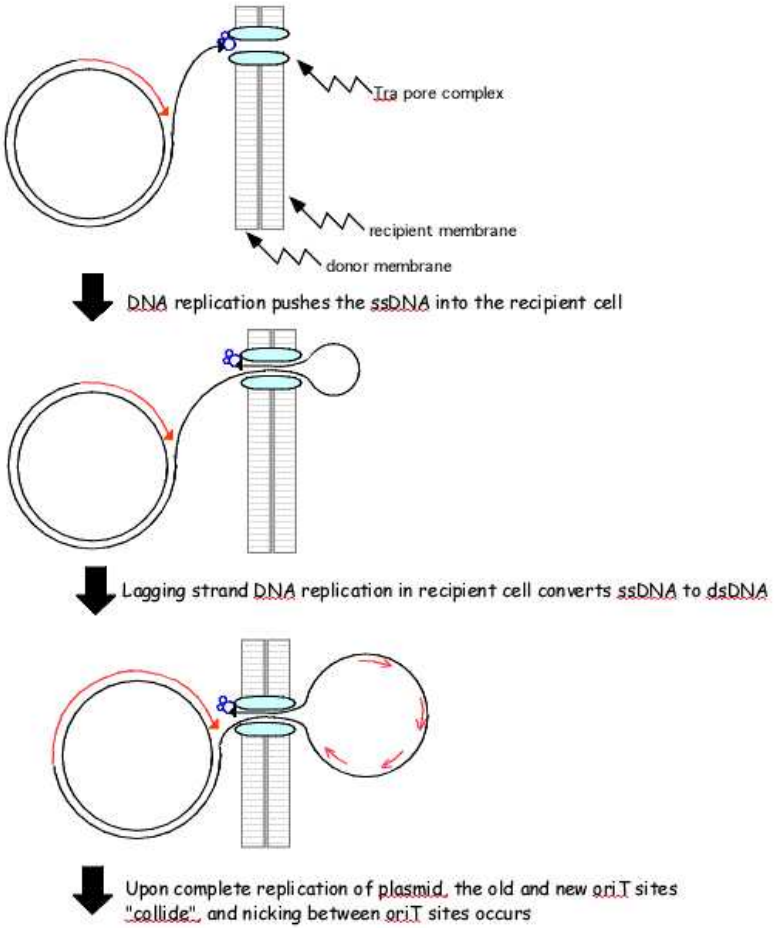
Mpf component holds the donor and recipient cell together, forms a channel through which DNA is transferred and signal Dtr component to initiate transfer. It has 3 components- pilus, channel and coupling protein.

Ori T site: It is the site where plasmid DNA transfer initiates in donor cell and the site for recyclization in the recipient cell.

Molecular view of step 5 (complementary strand synthesis)

In the last step, the donor cell and the recipient cell, both containing single-stranded DNA of F-plasmid

A complementary strand is then synthesized in both donor and recipient cell, Now the recipient cell also contains a copy of F-plasmid and become a donor cell.


Tra pore complex
recipient membrane
donor membrane
DNA replication pushes the ssDNA into the recipient cell
Lagging strand DNA replication in recipient cell converts ssDNA to dsDNA
Upon complete replication of plasmid, the old and new oriT sites "collide", and nicking between oriT sites occurs

F –plasmid

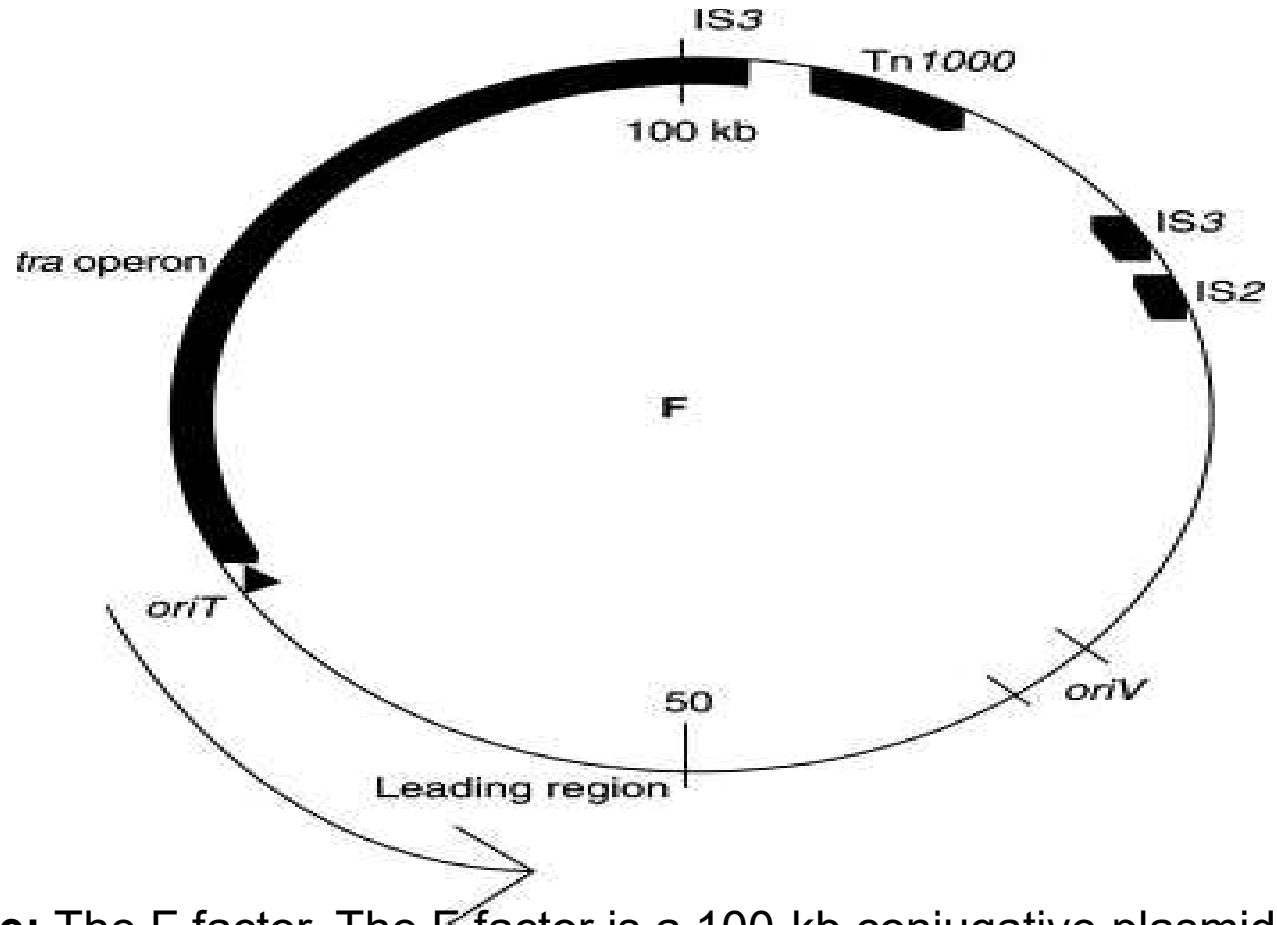
The F plasmid or Fertility is a large plasmid, which contains genes that allow the plasmids DNA to be transferred between cells.

It is found in the bacterium *E. coli*. Within the *E. coli* cells, the F plasmid has one or two copies making it a low-copy-number plasmid. During the cell cycle, it replicates once and segregates to both daughter cells.

Structure of F-plasmid

The F factor is 100 kb of duplex DNA with two replication-origin regions (Figure below).
The *oriV* or vegetative replication region contains two replication origins, one of which is used for bidirectional maintenance replication of the plasmid when it is not being transferred to another cell.

oriT, the transfer origin, promotes a special mode of unidirectional, single-(leading) strand replication used during conjugative transfer of the F factor to another cell. The copy number control of the F factor is similar to that of the chromosome such that there are one or two copies per bacterial chromosome.



Structure: The F factor. The F factor is a 100-kb conjugative plasmid. The *tra* operon encodes functions required for conjugative transfer of the F factor. Transposable elements are indicated: IS*3*, IS*2*, and Tn*1000*, and the direction of transfer is indicated by the thin arrow. Insertion sequence (IS) enables integration of chromosomes of F-plasmid in given cells.

Some properties of F-plasmid

Cells carrying the F plasmid are designated **F^+**, and those lacking it are **F^-**. The F plasmid contains approximately 100 genes, which give the plasmid several important properties:

1. The F plasmid can replicate its own DNA, allowing the plasmid to be maintained in a dividing cell population.

2. Cells carrying the F plasmid promote the synthesis of pili (singular, pilus) on the bacterial cell surface. **Pili** are minute proteinaceous tubules that allow the F^+ cells to attach to other cells and maintain contact with them; that is, to conjugate .

3. F^+ and F^- cells can conjugate. When conjugation occurs, the F^+ cells can act as F donors. The F plasmid DNA replicates and the newly synthesized copy of the circular F molecule is transferred to the F^- recipient. However, a copy of F always remains behind in the donor cell. The recipient cell becomes converted into F^+, because it now contains a circular F genome. The transfer of the F plasmid from F^+ to F^- is rapid, so the F plasmid can spread like wildfire throughout a population from strain to strain.

4. F^+ cells are usually inhibited from making contact with other F^+ cells; therefore the F plasmid is not transferred from F^+ to F^+.

5. Sometimes F carries within its genome one or more IS (insertion-sequence) elements . An **IS element** is a mobile segment of DNA that moves from place to place within the host chromosome or between chromosome and plasmid. The existence of a specific IS element both in the plasmid *and* in the chromosome affords a site at which homologous crossing-over occasionally occurs. A crossover between the two circular DNAs leads to the integration of the plasmid into the bacterial chromosome. When this integration occurs, F can drive the transfer of the entire host chromosome into the recipient cell, along with its own integrated F DNA

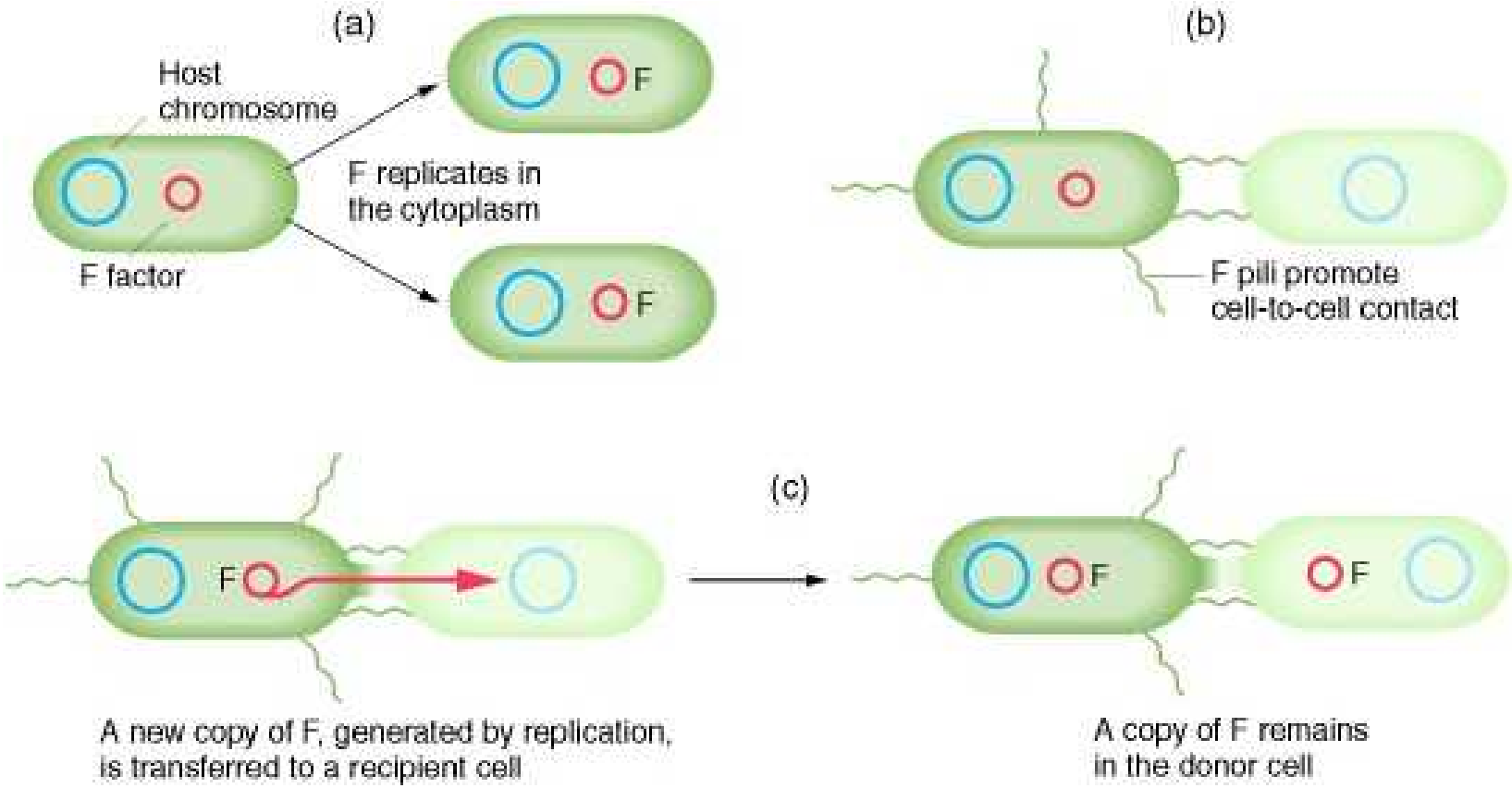


Figure: Diagrammatic representation of F-plasmid characteristics

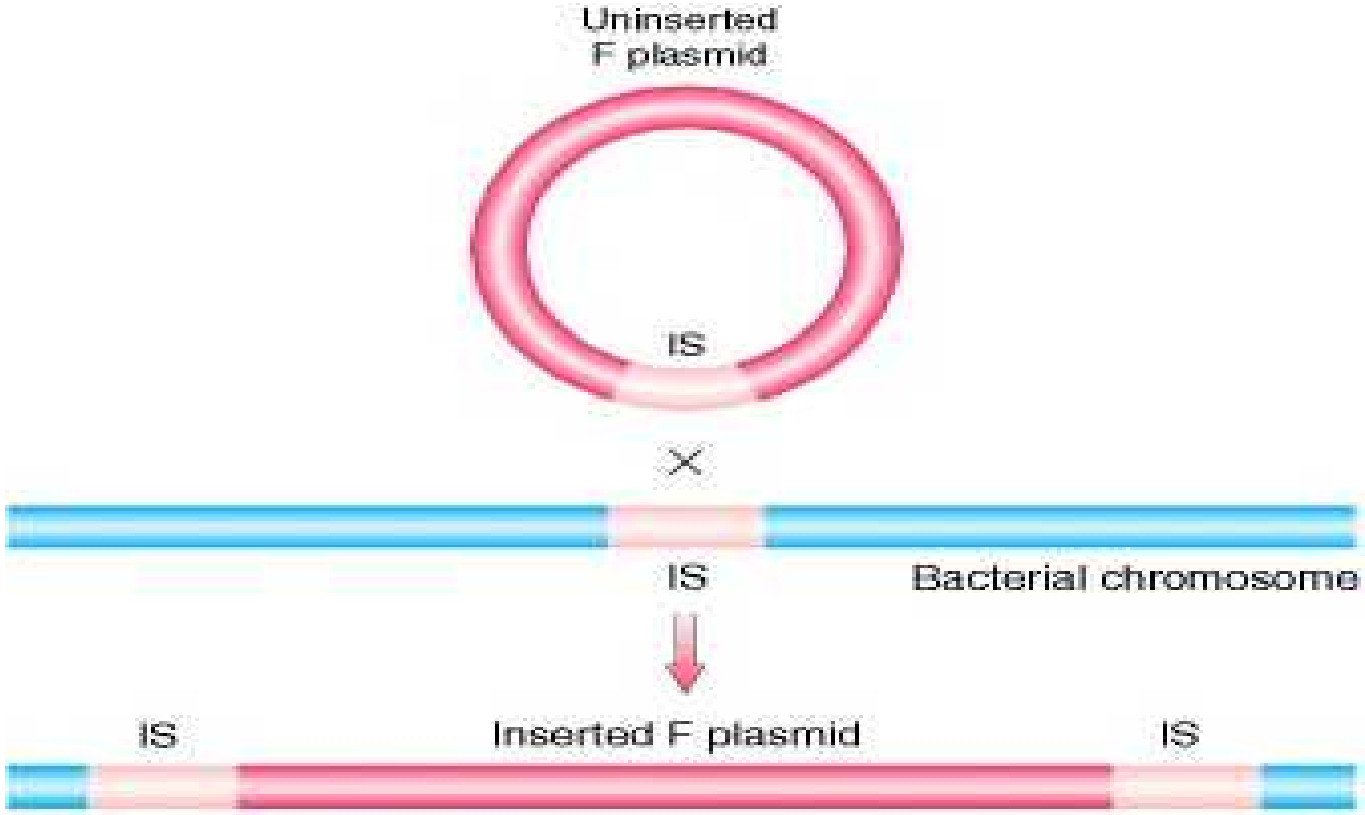


Figure: Insertion of the F-plasmid into the bacterial chromosome. IS indicates homologous insertion sequence.