CONJUGATION

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Introduction:

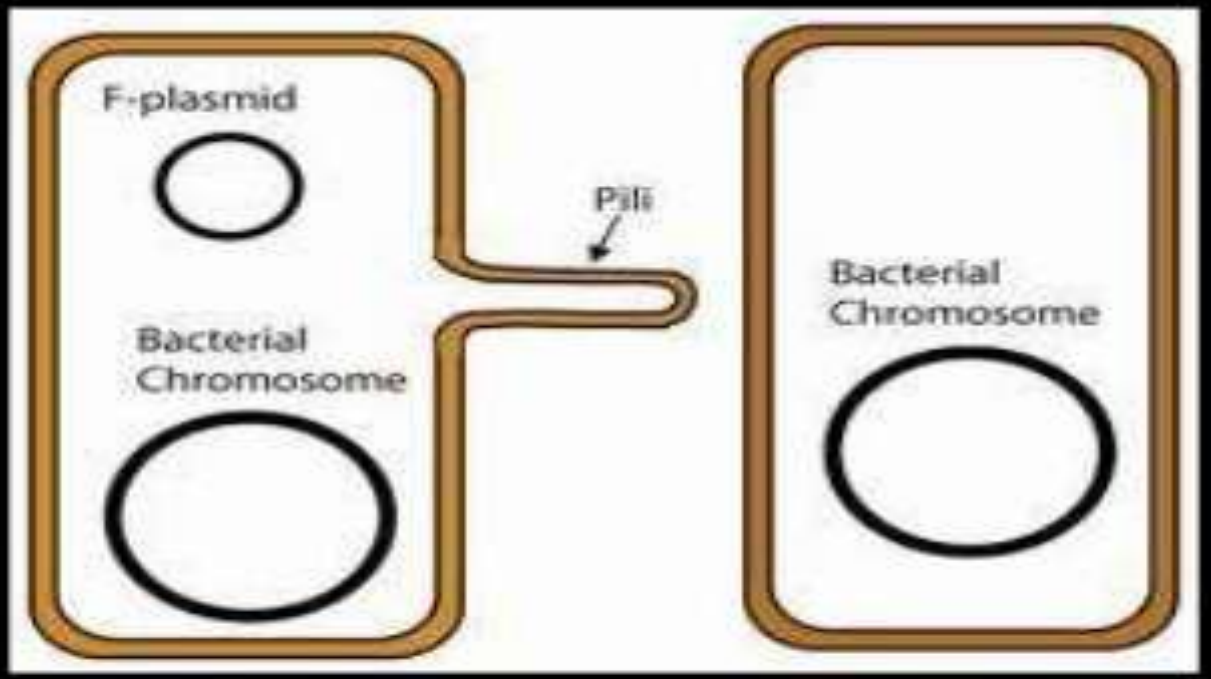
- A remarkable feature of many plasmids is the ability to transfer themselves and other DNA elements from one cell to another in a process called **conjugation.**
- Lederberg and Tatum first observed this process in 1946(1947)
- They mixing some strain of *Escherichia coli* with others resulted in strains that were genetically unlike either of the originals.

- Conjugation is a mechanism of horizontal gene transfer as are transformation and transduction, although these two other mechanisms do not involve cell-to-cell contact.

- Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell to cell contact or by a bridge-like connection between two cells.

CONJUGATION

BACTERIA A

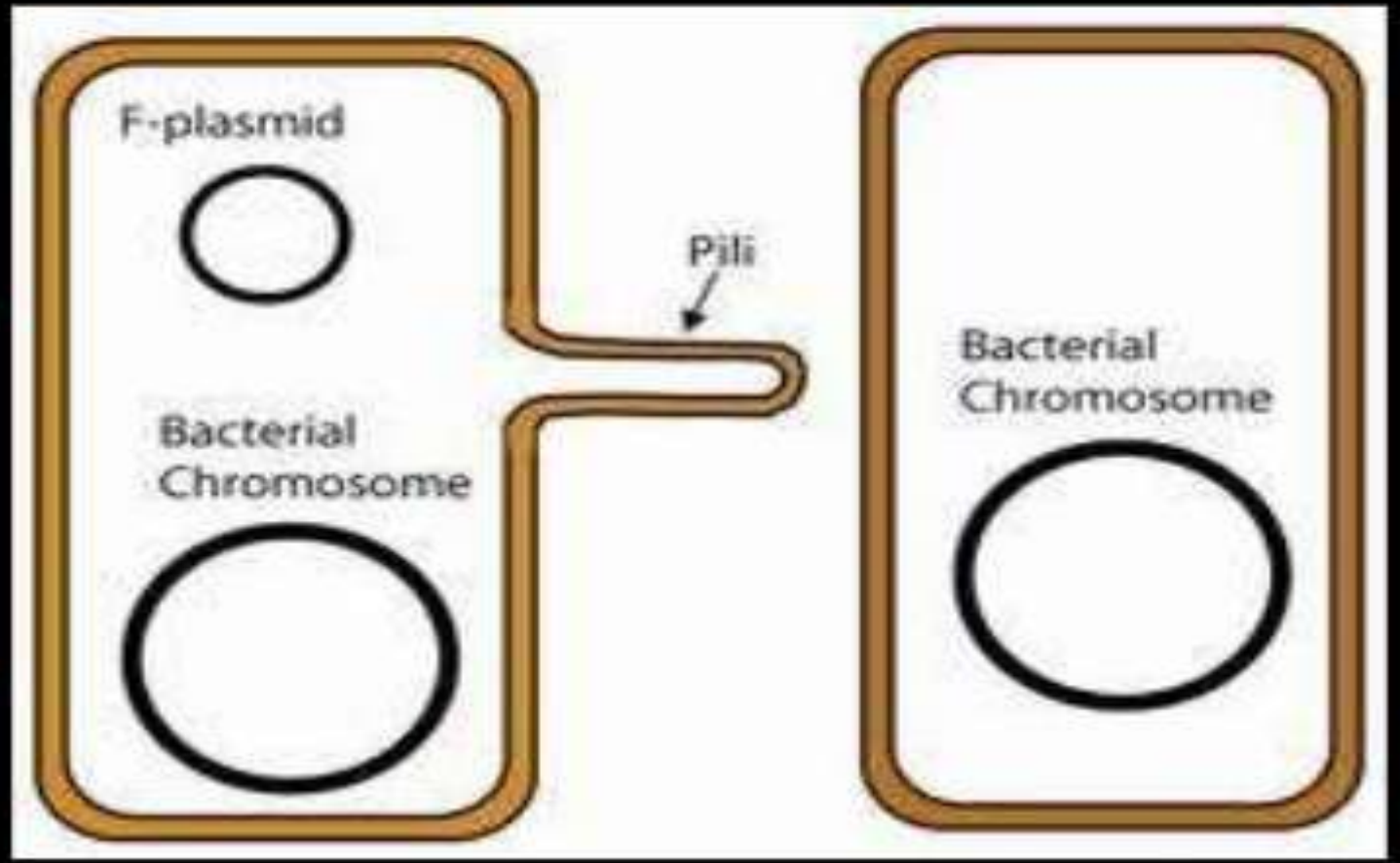


BACTERIA B

TRANSFER OF GENETIC MATERIAL FROM BACTERIA A TO BACTERIA B BY MATING OR CONTACT IS CALLED CONJUGATION.

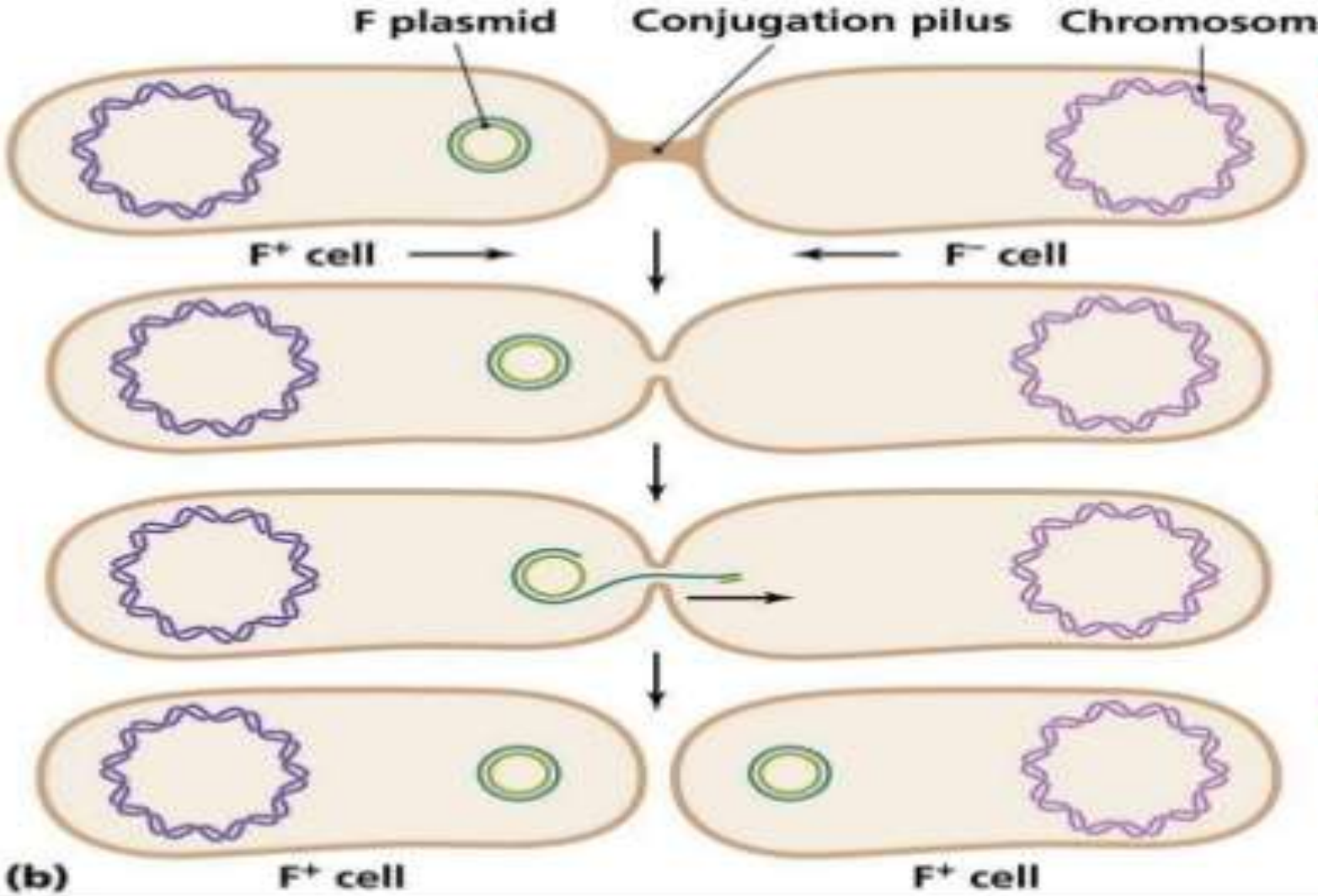
WHO IS A DONOR?

F PLASMID
DONOR
F +
MALE CELL

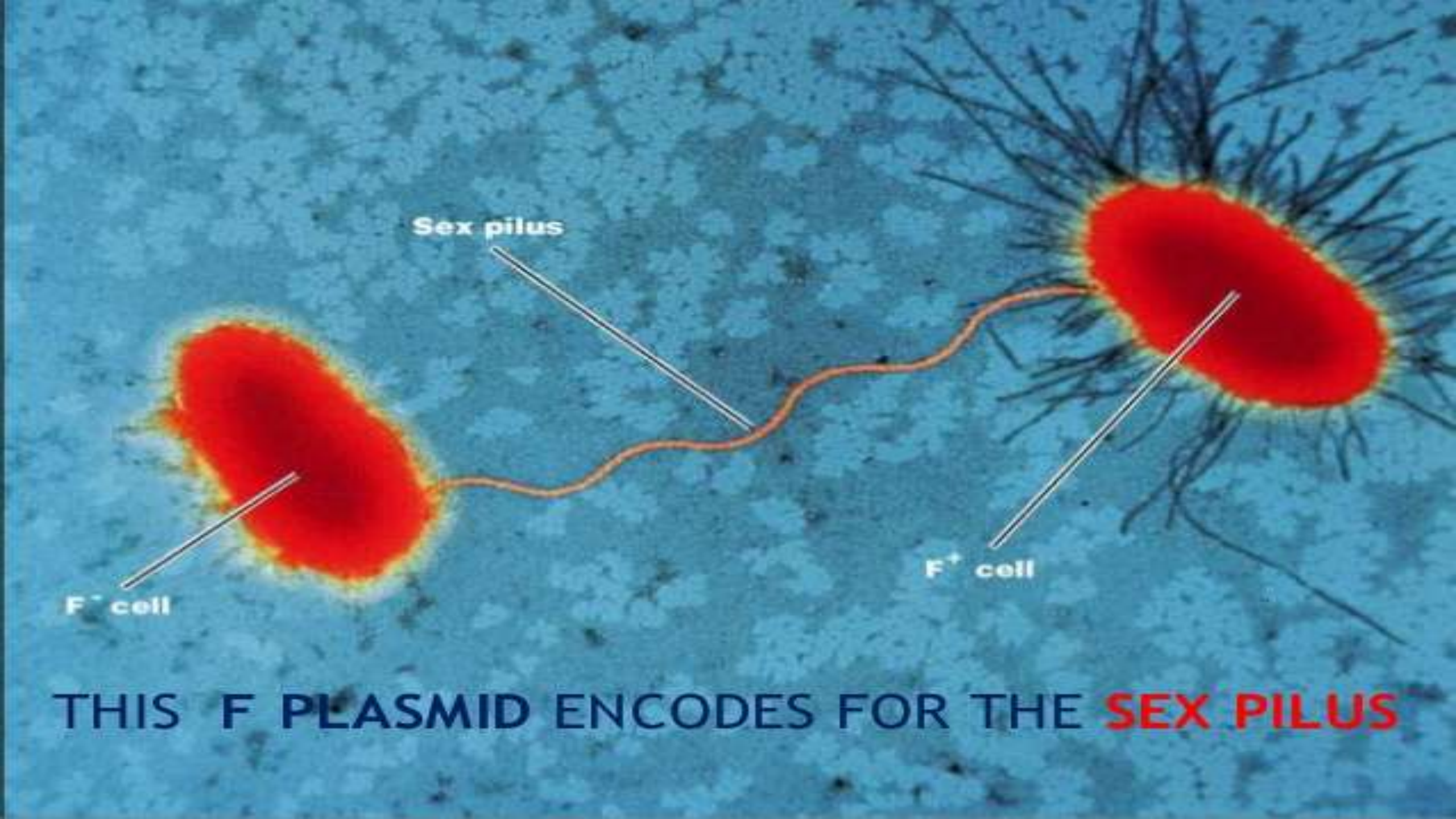


ABSENT
RECIPIENT
F –
FEMALE

WHAT HAPPENS DURING CONJUGATION?



1. Donor cell attaches to a recipient cell with its pilus. The pilus draws the cells together.
2. The cells contact one another.
3. One strand of plasmid DNA transfers to the recipient.
4. The recipient synthesizes a complementary strand to become an F^+ cell; the donor synthesizes a complementary strand, restoring its complete plasmid.



THIS **F PLASMID** ENCODES FOR THE **SEX PILUS**

self transmissible plasmids:

- These plasmids can transfer themselves.
- They encode all the functions they need to move among cells, and sometimes they also aid in the transfer of mobilizable plasmids(non-conjugative) because they encode not all of the proteins required for transfer.
- These non-conjugative plasmids transfer by a process termed mobilization.

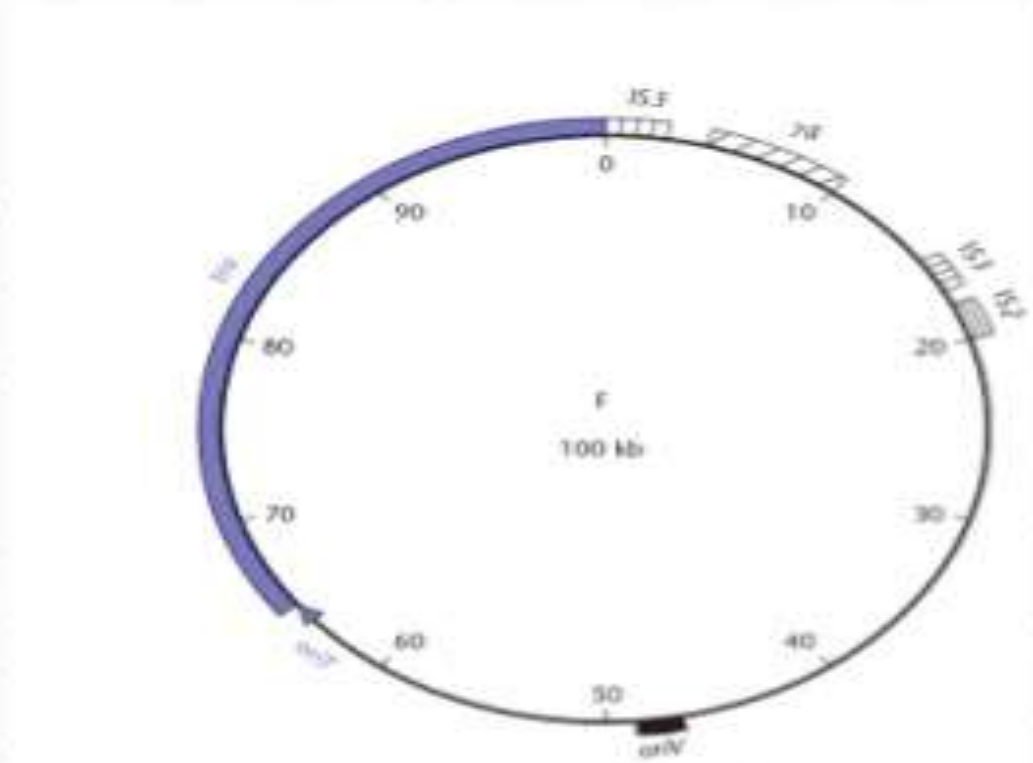
- Any bacterium harboring a self-transmissible plasmid is a potential donor(male strains) , because it can transfer DNA to other bacteria.
- Bacteria that lack the self-transmissible plasmid are potential recipient(transconjugant).
- Self-transmissible plasmids probably exist in all types of bacteria but those that have been studied most extensively are from the gram-negative genera *Escherichia* and *Pseudomonas* and the gram-positive genera *Enterococus,Streptococcus,Bacillus,Staphylococcus, Streptomyces*

Classification of self-transmissible plasmids:

- Plasmids are usually classified by their incompatibility(Inc)group.
- The F-type plasmids use a transfer system known as the Tra system of IncF plasmids or RP4 plasmid uses the Tra system of IncP plasmids

Transfer (tra) Genes

- Conjugation is a complicated process that requires the products of many genes,the genes required for transfer are called tra genes.



Partial genetic and physical map of Fplasmid

- The tra genes of self-transmissible plasmid required for plasmid transfer can be divided in two components:
- 1- Dtr component (DNA Transfer and Replication)
- 2- Mpf component (Mating-Pair Formation)

Mpf system

Functions:

1- hold a donor cell and a recipient cell together during the mating process.

2- form a channel through which protein and DNA are transferred during the mating.

3- includes the protein that communicates news of Mpf to the Dtr system,beginning the transfer of plasmid.

- The Mpf system includes :

1. The pilus
2. The channel
3. Coupling proteins

Pilus:

A tube like structure that sticks out of the cell surface,these pili are 10 nm or more in diameter with a central channel.

The structure of the pilus differs markedly among plasmid transfer system. for example:

- F plasmid encodes a long,thin,flexible pilus
- pkm101 makes a long,rigid pilus
- RP4 make a short,thick,rigid pilus
- Col1b-P9 make 2 pili: a long thin and a short rigid

The channel:

- In addition to a pilus , the Mpf system encodes a channel or pore which DNA passes during conjugation .
- Some of the tra gene products making up this pore.

The coupling protein

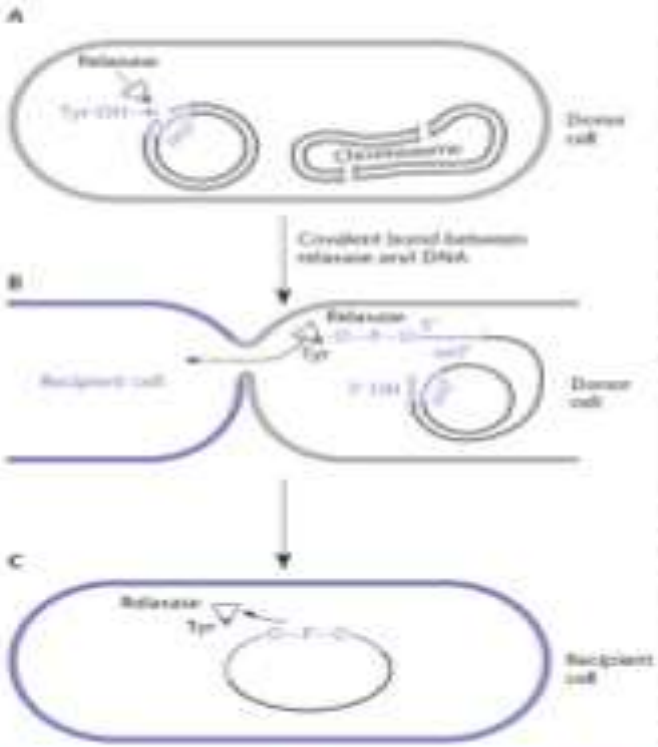
- The communication between the Dtr and Mpf systems is provided by proteins called coupling proteins,which are part of the Mpf system.
- these proteins is bound to the membrane channel.
- These proteins provide the specificity for the transport process so that only some plasmids are transferred
- Also these proteins specifically recognizes the relaxase of the Dtr component of certain plasmids as well as any other proteins to be transferred.

Dtr system

- The Dtr is involved in preparing the plasmid DNA for transfer.
- a number of protein make up this component such as Relaxase and Primase.

Relaxase

- A central part of the Dtr component of plasmids is the relaxase.
- This is a specific DNA endonuclease which makes a single-strand break or "nick" at the specific site called the nic site in the oriT sequence to initiate the transfer process.
- Relaxase works is similar to the action of Rep protein in rolling-circle plasmid replication.
- Relaxase breaks a phosphodiester bond at the nic site by transferring the bond from a deoxynucleotide to one of its own tyrosines(transesterificarion)and requires very little energy because there is no net breakage or formation of new chemical bonds.



A. Relaxase nicks the DNA at a specific site in oriT
B. Relaxase is transferred to the recipient cell,dragging the DNA along with it.
C. In a reversal of the original transesterification reaction,the phosphate is transferred back to the 3OH of the other end of the DNA,recyclizing the DNA and releasing the relaxase.

Reaction performed by the relaxase

Relaxosome :

The relaxase protein in the donor cell is part of a larger structure called **relaxosome** ,which is made up of a number of proteins that are normally bound to the oriT sequence of the plasmid.

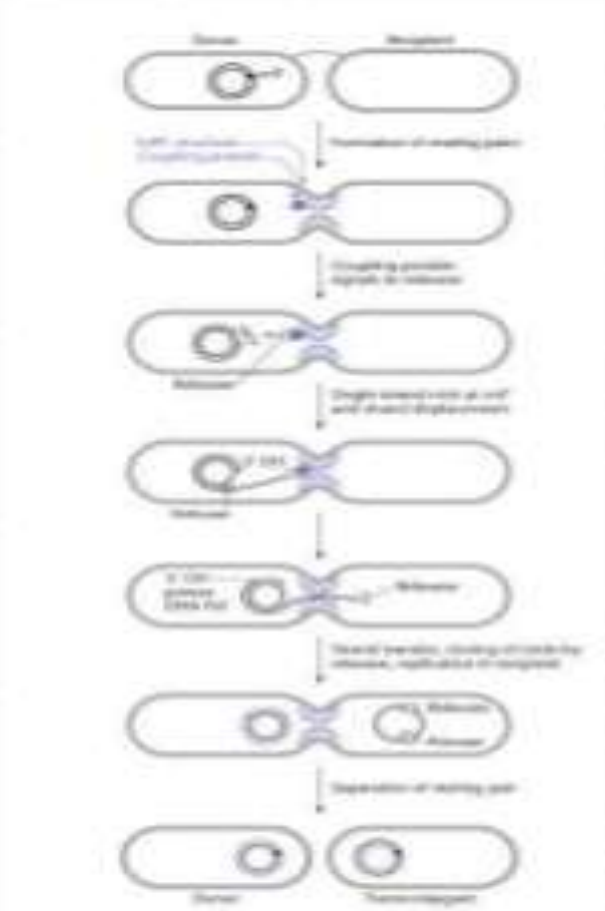
in some plasmids, one of the other proteins of the relaxosome may be the **helicase** ,which helps seprate the strands of DNA beginning at the oriT sequence,while in others the helicase seems to be part of the relaxase protein itself.

Primase:

- Another component of the Dtr system made in the donor is the **primase** .

primase are needed for prime plasmid replication.

primase encoded either by the host or by the plasmid.



Mechanism of DNA transfer during conjugation

The oriT sequence:

A self-transmissible plasmid mobilizes any DNA that contains its oriT sequence.

The oriT sequence of F-plasmid is known to be shorter than 300 bp,contains inverted repeated sequences,a region rich in AT base pairs.

Plasmid transfer initiates specifically at the oriT site because the specific relaxase encoded by one of the tra genes cuts DNA only at this sequence.

Male-specific phages:

Some phages use the pilus of a self-transmissible plasmid as their adsorption site that are called **male-specific phages** because they infect only donor or male cells. For example:

M13 and R17 infect only cells carrying the F plasmid

Pf3 and PRR1 infect only cells containning an **IncP** plasmid such as RP4.

Mating cells :

Donor or F^+ :

The ability of a bacterium to be a donor is a consequence of the presence in the cell of an extra piece of DNA called the F-factor

(fertility factor or sex factor).

F-factor codes sex pilus(F pilus) on the surface of the bacterium.

Recipient or F^- :

The ability to acts as a recipient is a consequence of the lack of the F-factor.

THE ONCE FEMALE RECIPIENT CELL NOW BECOMES THE MALE DONOR CELL

THIS CHARACTERISTIC OF MALENESS (F+) IN BACTERIA IS TRANSMISSIBLE OR INFECTIOUS

Gene Mapping

Mapping - determining the location of elements within a genome, with respect to identifiable landmarks.

Types of mapping…with tools/resources utilized

- **Genetic mapping – linear description of DNA markers/genes on a given chromosome with closely placed markers being inherited together more often.**
  - linkage mapping
  - pedigree
  - polymorphic markers
- **Physical mapping – physical location on the chromosome, relating more towards exact positioning of gene elements.**
  - cytogenetic mapping
  - somatic cell mapping
  - radiation hybrid mapping
  - restriction mapping - *PFGE, BAC contigs, sequencing*
- **Comparative mapping**
  - **gene sequences**
  - **databases**
  - **DNA chips**

GENOME/GENETIC MAPS - Graphic representation of the relative positions of genes on a DNA sequences.

CLASSICAL EXPERIMENT OF MAPPING

If two alleles are far apart on the chromosome, as it is more likely that a cross-over will occur between them and they will be separated. Genes inherited in this way are said to be linked, and are referred to as "*linkage groups.*" For example, in fruit flies the genes affecting eye color are inherited together because they appear on the same chromosome.

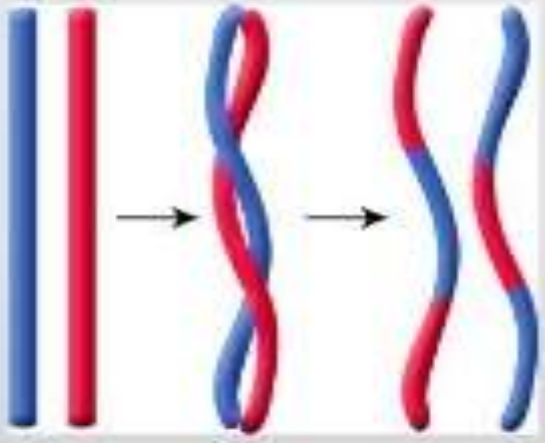
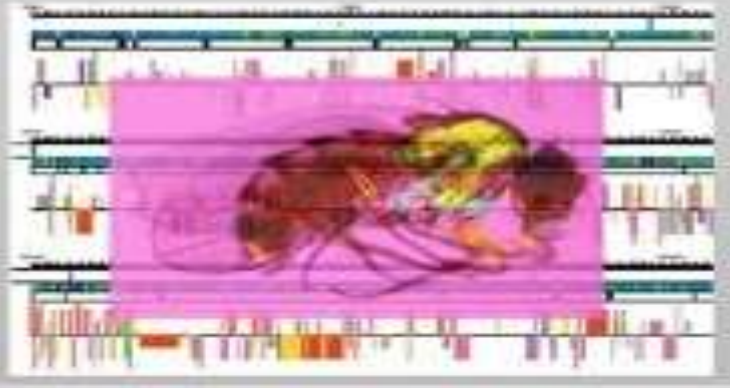


Image of crossing over occuring in chromosomes

Sturtevant from the analyze of the fruit fly genome found, for example, that leg length was inherited with eye color more often than with wing length, and that wing length was inherited with eye color more often than with leg length. .

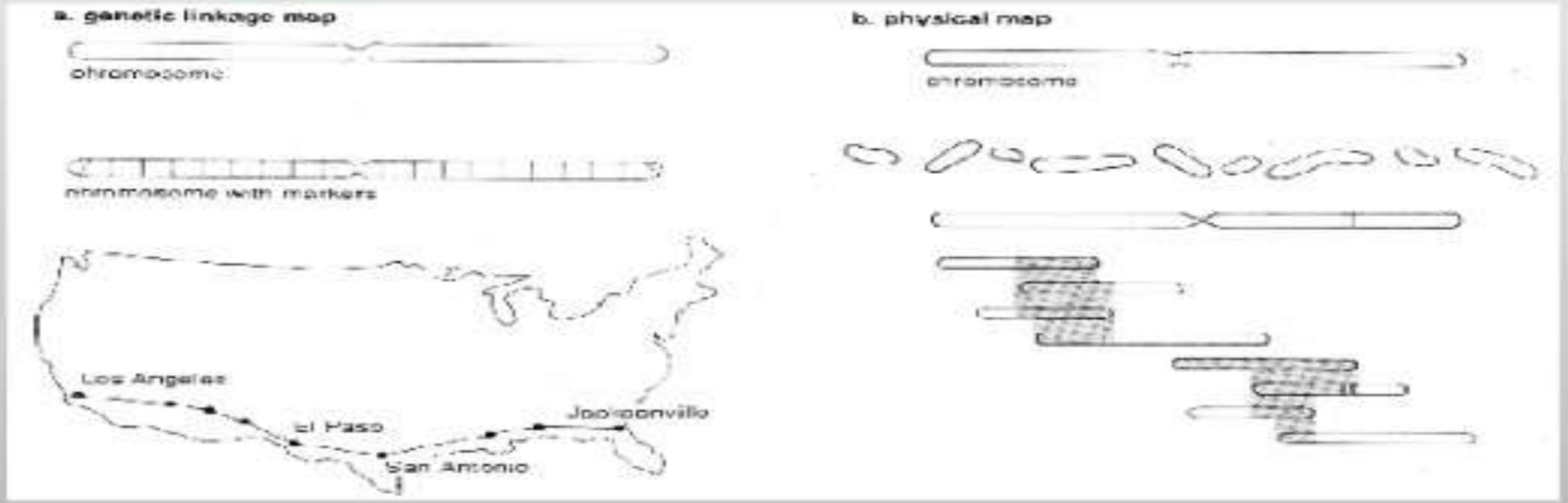
Thus, he concluded, the gene for eye color must be between the genes for wing length and leg length.



Linkage and Physical Mapping

Linkage map versus physical map

It follows that a linkage map is a chromosome shows the position of its known genes and/or markers relative to each other in terms of recombination frequency, rather than as specific physical distance along each chromosome as in physical maps



Approaches to Genetic mapping

1. Experimental crosses
   - Backcrosses
   - F2 intercrosses
   - Introgression lines
   - Recombinant inbred (RI) lines
2. Pedigree analysis
3. Association studies (Linkage disequilibrium, LD mapping)
   - With candidate genes (direct approach)
   - Localized association studies (chromosomal region)
   - Whole-genome association studies


Thank You