BACTERIAL CONJUGATION

Do bacteria possess any processes similar to sexual reproduction and recombination?

The question was answered by the elegantly simple experimental work of **Joshua Lederberg and Edward Tatum**, who in 1946 discovered a sexlike process in bacteria. They were studying two strains of Escherichia coli with different sets of auxotrophic mutations.

Strain A would grow only if the medium were supplemented with methionine and biotin; strain B would grow only if it were supplemented with threonine, leucine, and thiamine.

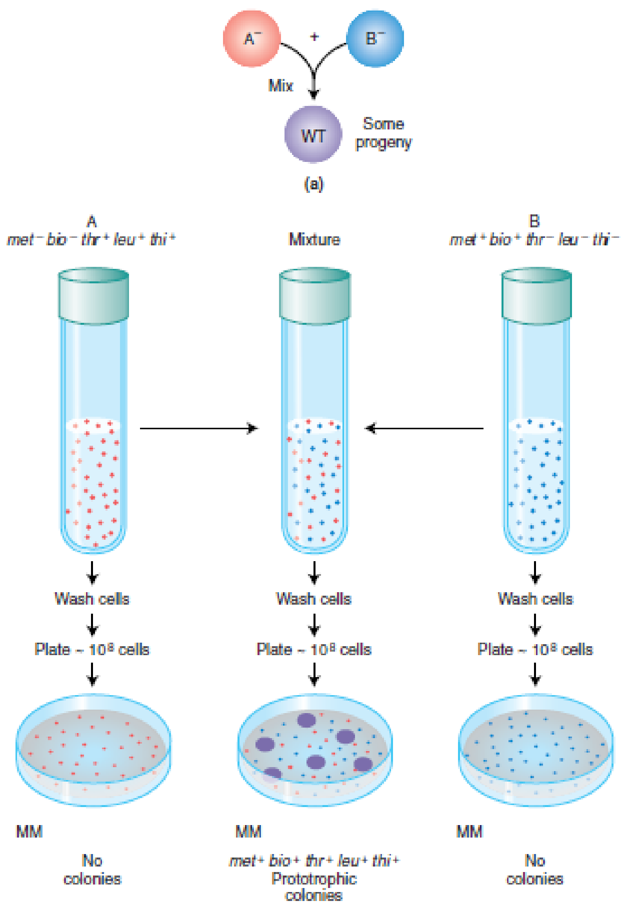
Thus, we can designate the strains as
strain A: met- bio- thr leu thi
strain B: met bio thr- leu- thi-

Strains A and B were mixed together, incubated for a while, and then plated on minimal medium, on which neither auxotroph could grow.

A small minority of the cells (1 in 10^7) was found to grow as prototrophs and hence must have been wild type.

These colonies derive from single cells in which an exchange of genetic material has occurred; they are therefore capable of synthesizing all the required constituents of metabolism.

Some of the dishes were plated only with strain A bacteria and some only with strain B bacteria to act as controls, but from these no prototrophs arose.



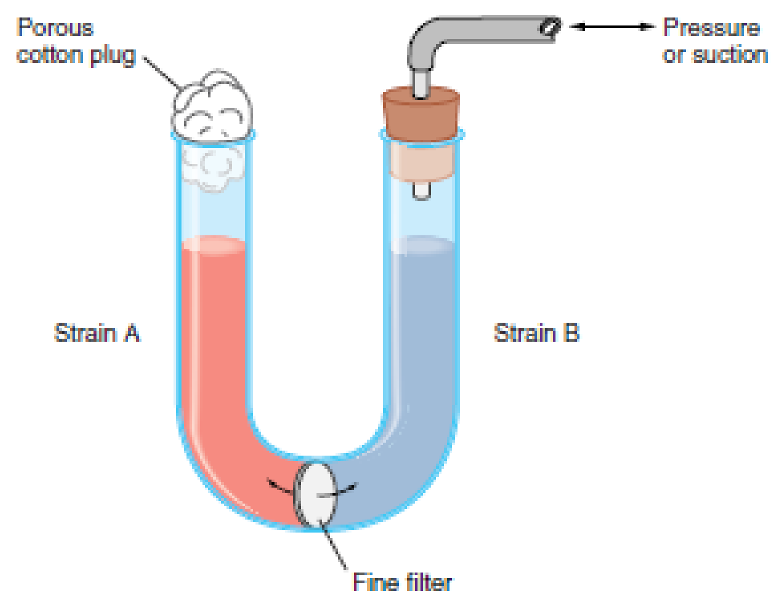


Figure 5-5 Physical contact between bacterial cells is required for genetic recombination. Auxotrophic bacterial strains A and B are grown on either side of a U-shaped tube. Liquid may be passed between the arms by applying pressure or suction, but the bacterial cells cannot pass through the filter. After incubation and plating, no recombinant colonies grow on minimal medium.

It could be argued that the cells of the two strains do not really exchange genes but instead leak substances that the other cells can absorb and use for growing.

This possibility **of "cross feeding" was ruled out by Bernard Davis in the U-shaped tube experiment**

The pores of the filter were too small to allow bacteria to pass through but large enough to allow easy passage of any dissolved substances.

Strain A was put in one arm, strain B in the other.

After the strains had been incubated for a while, Davis tested the contents of each arm to see if there were any prototrophic cells, but none were found.

In other words, physical contact between the two strains was needed for wild-type cells to form.

It looked as though some kind of genome union had taken place, and genuine recombinants produced.

The physical union of bacterial cells can be confirmed under an electron microscope, and is now called conjugation

- In **1953, William Hayes** discovered that in the above types of “crosses” the conjugating parents acted unequally.

- It seemed that one parent (and only that parent) transferred some of or all its genome into another cell.

- Hence one cell acts as donor, and the other cell as a recipient. This is quite different from eukaryotic crosses in which parents contribute nuclear genomes equally.

Donor ability is itself a hereditary state, imposed by a fertility factor (F).

Strains that carry F can donate, and are designated F+. Strains that lack F cannot donate and are recipients, designated F-.

F is an example of a small, nonessential circular DNA molecule called a *plasmid that can replicate in the cytoplasm independent* of the host chromosome.

a) During conjugation, the pilus pulls two bacteria together.

(b) Next, a bridge (essentially a pore) forms between the two cells.

A single-stranded copy of plasmid DNA is produced in the donor cell and then passes into the recipient bacterium, where the single strand, serving as a template, is converted to the double-stranded helix.

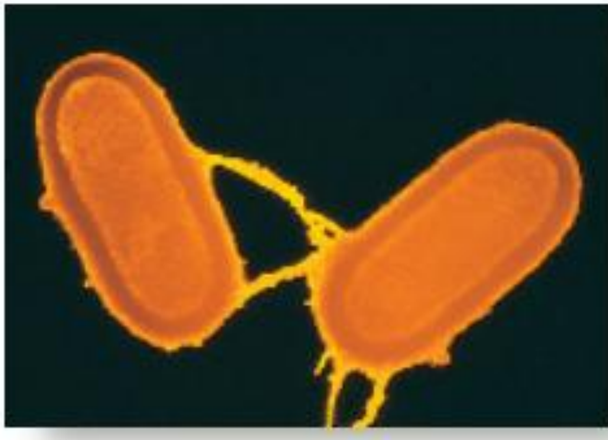
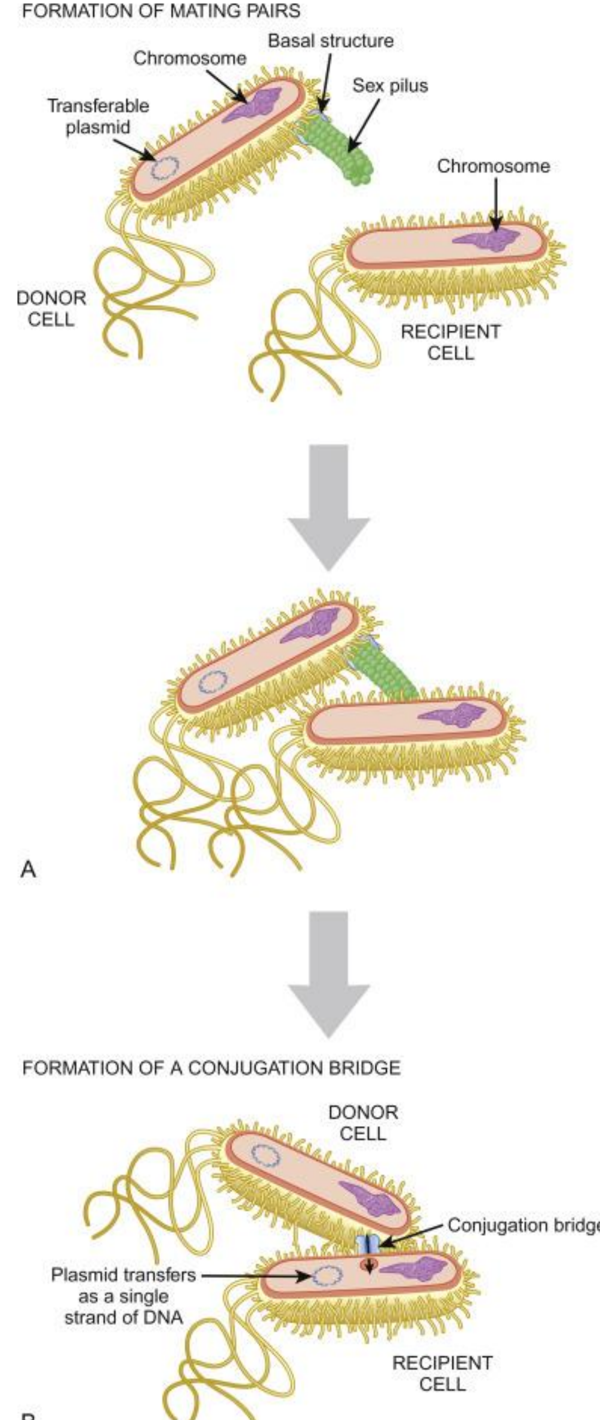
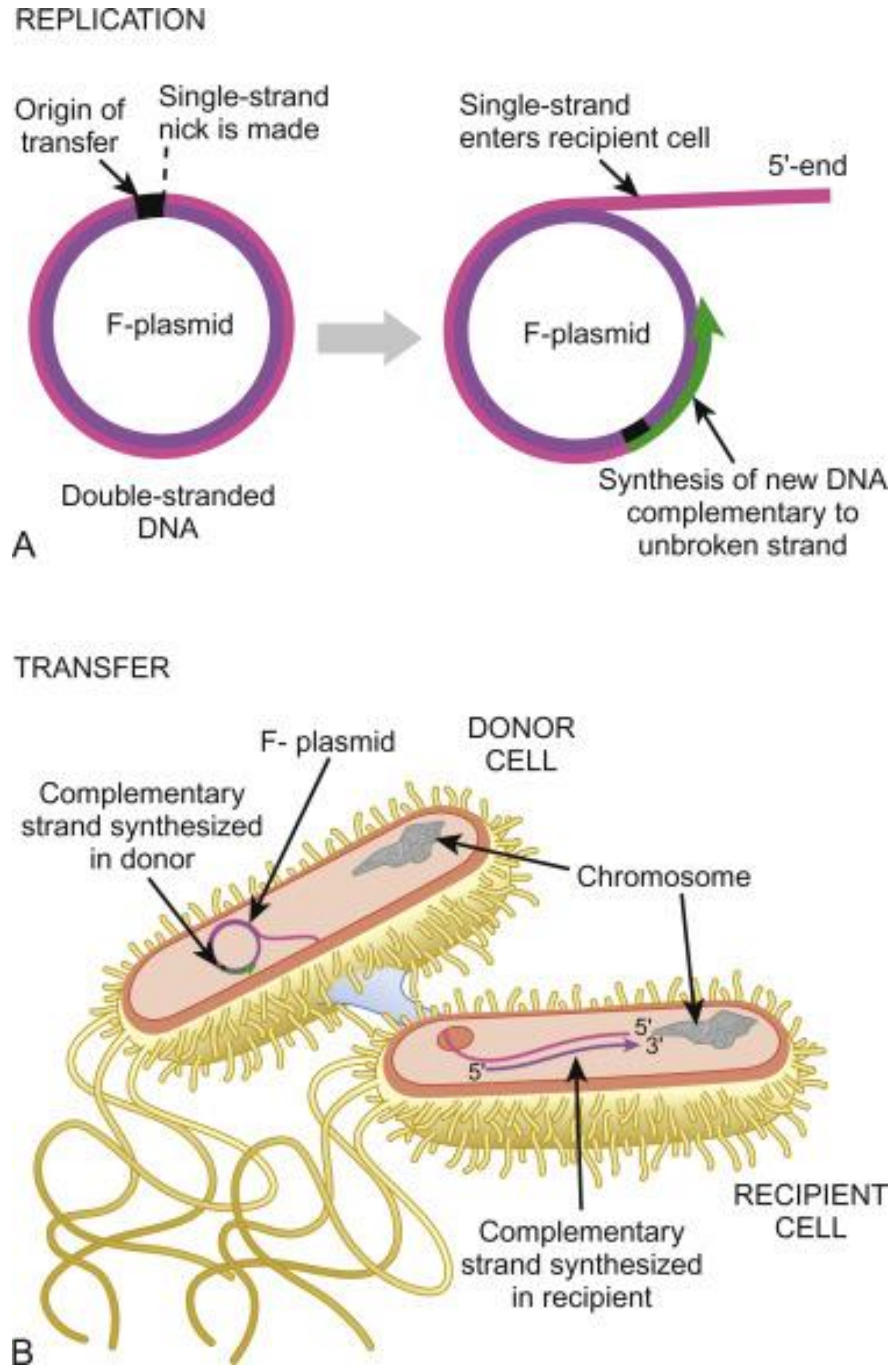
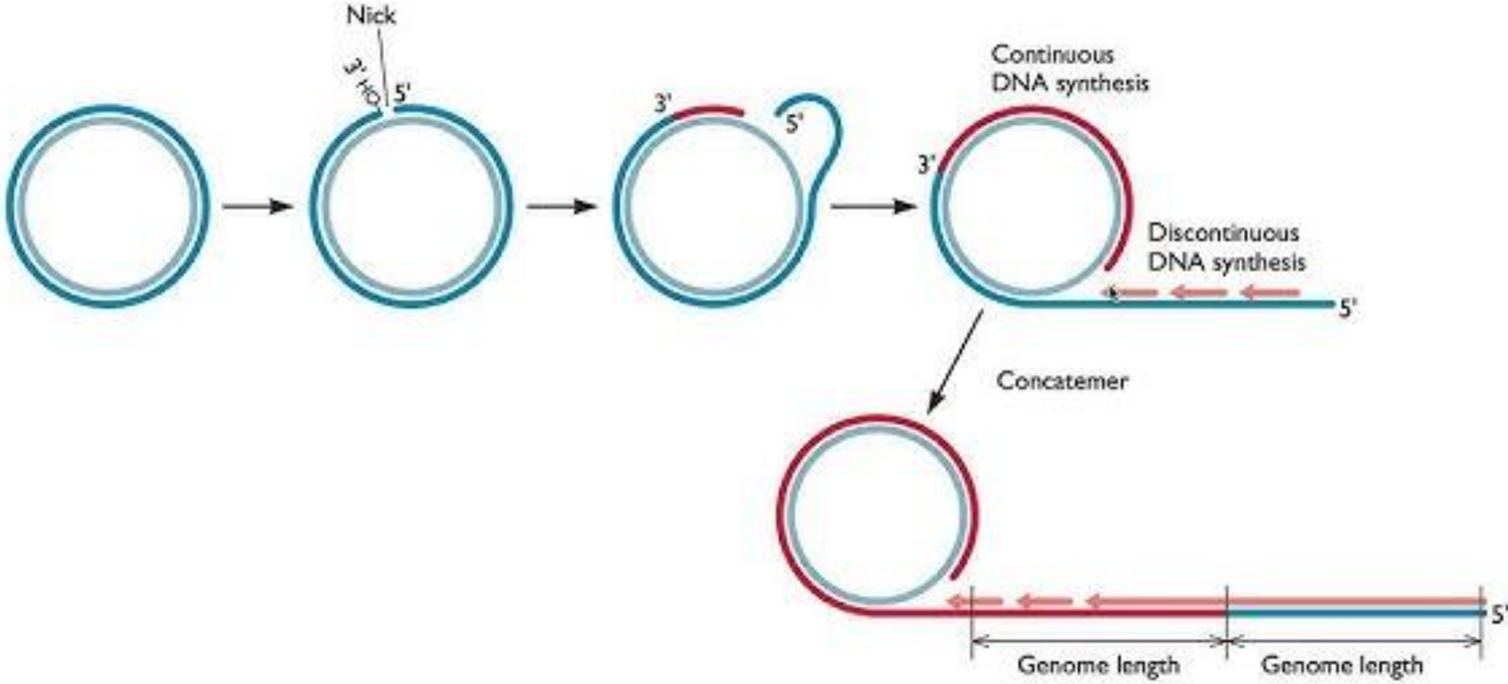


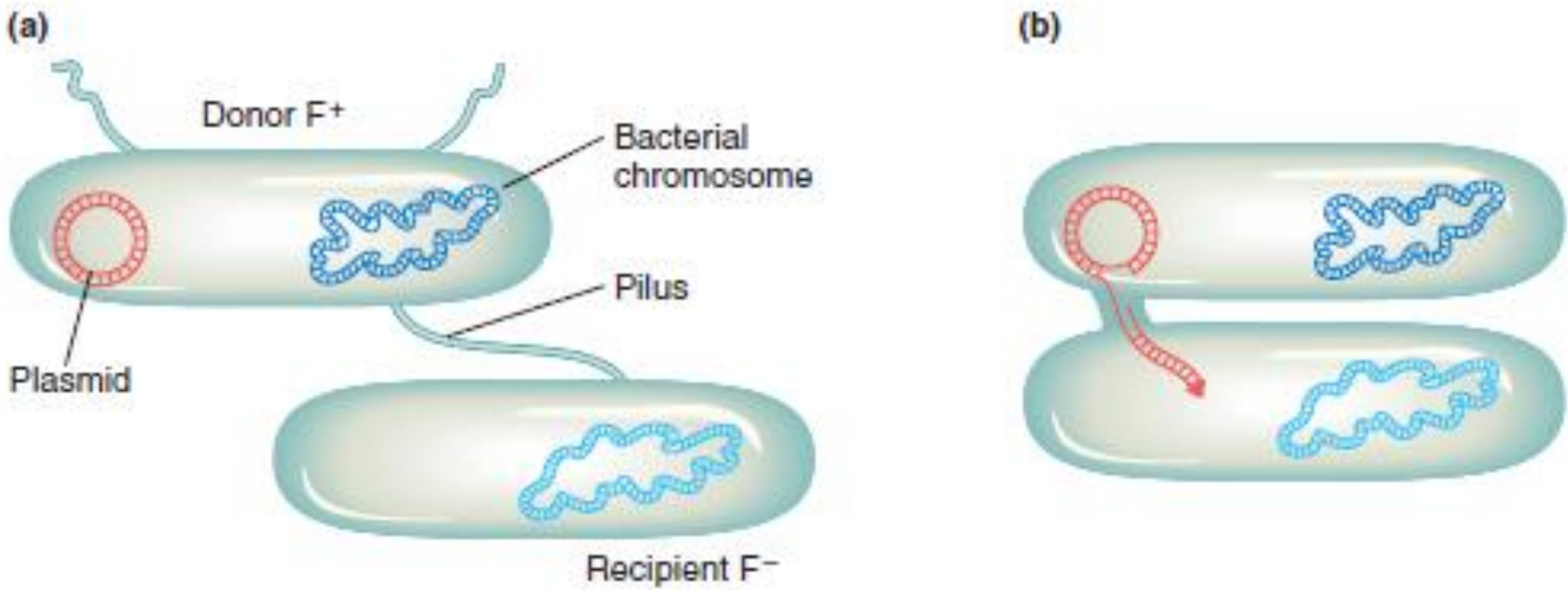
Figure 5-6 Bacteria can transfer plasmids (circles of DNA) through conjugation. A donor cell extends one or more projections—pili—that attach to a recipient cell and pull the two bacteria together. [Oliver Meckes/MPI-Tübingen, Photo Researchers.]




REPLICATION
Origin of transfer
Single-strand nick is made
F-plasmid
Double-stranded DNA
A
Single-strand enters recipient cell
5'-end
F-plasmid
Synthesis of new DNA complementary to unbroken strand
TRANSFER
F- plasmid
DONOR CELL
Complementary strand synthesized in donor
Chromosome
5'
3'
5'
RECIPIENT CELL
Complementary strand synthesized in recipient
B

Rolling circle replication

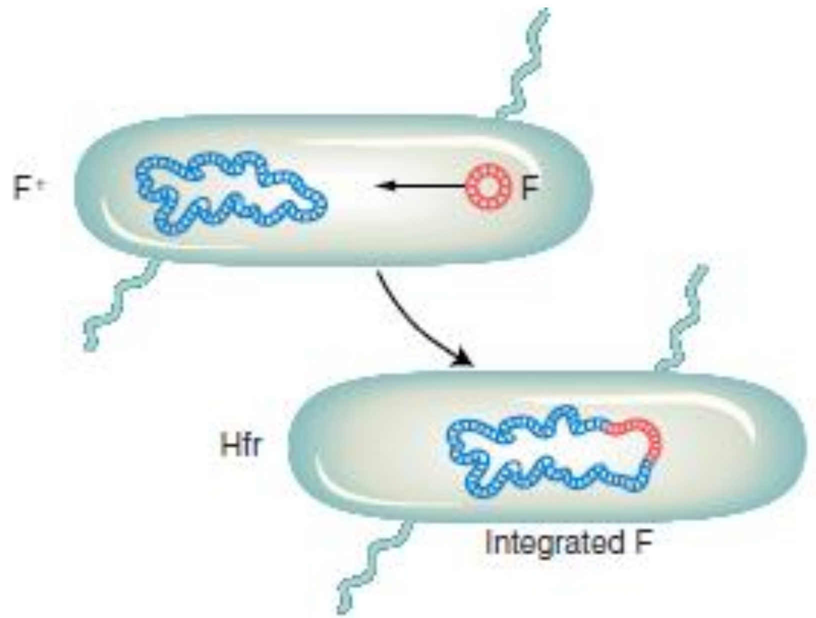




https://www.youtube.com/watch?v=hm8SZaFmlWg

The F plasmid directs the synthesis of pili, projections that initiate contact with a recipient (Figure 5-6) and draw it closer. The F DNA in the donor cell makes a single-stranded copy of itself in a peculiar mechanism called **rolling circle replication. The circular plasmid** "rolls," and as it turns, it reels out the single-stranded copy like fishing line. This copy passes through a pore into the recipient cell, where the other strand is synthesized, forming a double helix.
Hence a copy of F remains in the donor and another appears in the recipient,

An Hfr strain results from the integration of the F factor into the chromosome,



Does an Hfr cell die after donating its chromosomal material to an F cell?
The answer is no.

Just like the F plasmid, during conjugation the Hfr chromosome replicates and transfers a single strand to the F cell.

The single-stranded nature of the transferred DNA can be demonstrated visually using special strains and antibodies.

The replication of the chromosome ensures a complete chromosome for the donor cell after mating.

The transferred strand is converted into a double helix in the recipient cell, and donor genes may become incorporated in the recipient's chromosome through crossovers, creating a recombinant cell.

If there is no recombination, the transferred fragments of DNA are simply lost in the course of cell division.

Putting all these observations together, Wollman and Jacob deduced that in the conjugating Hfr, single-stranded DNA transfer begins from a fixed point on the donor chromosome, termed the **origin (O), and continues in a linear** fashion.

The point O is now known to be the site at which the F plasmid is inserted. The farther an gene is from O, the later it is transferred to the F.

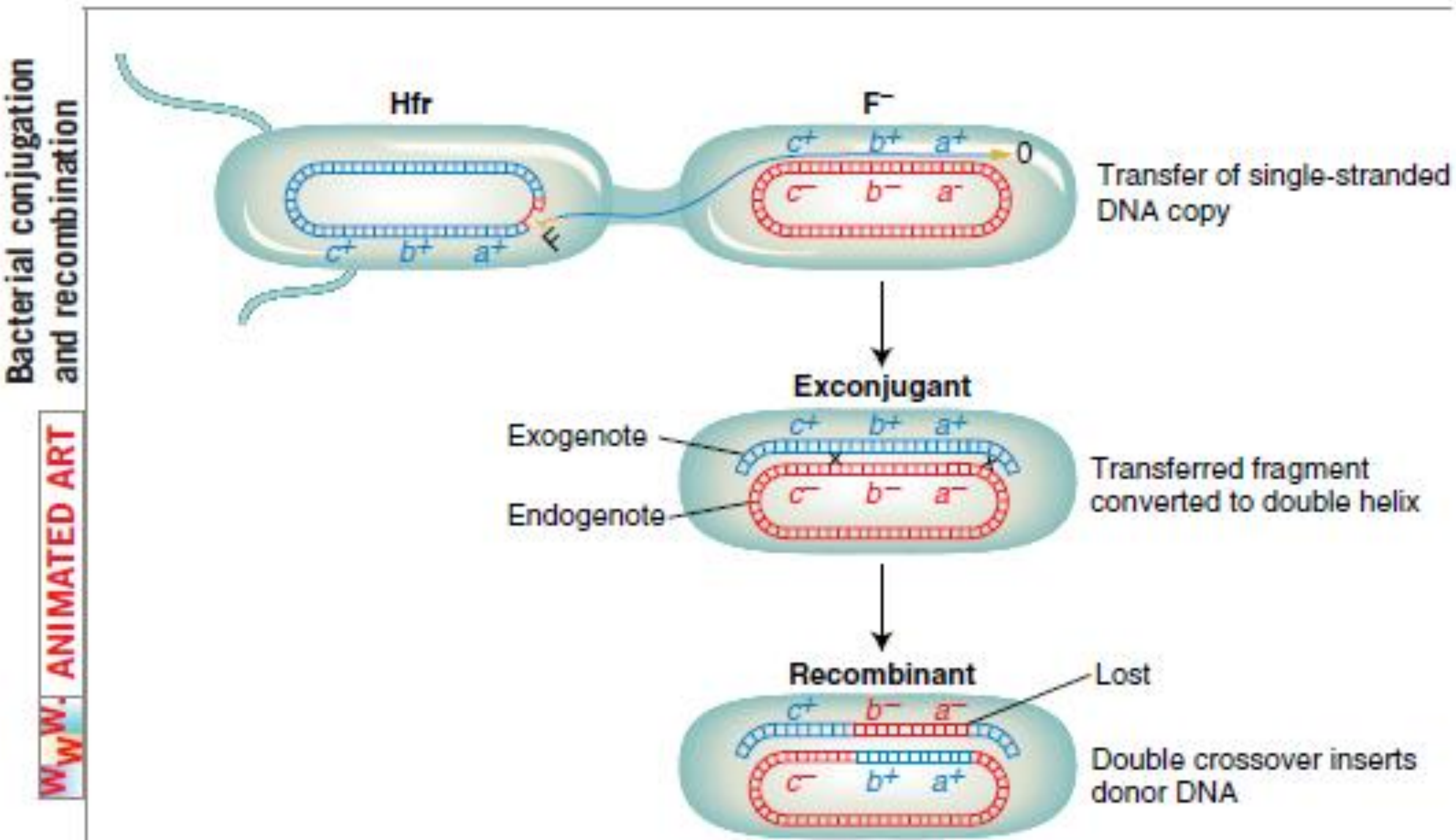


Figure 5-10 Bacterial conjugation and recombination. Transfer of single-stranded fragment of donor chromosome and recombination with recipient chromosome.

Some special features of the recombination event in bacteria.

Note that recombination does not take place between two whole genomes, as it does in eukaryotes.

In contrast, it takes place between one *complete genome, from the F, called the* **endogenote, and an *incomplete one, derived from the Hfr*** donor and called the **exogenote.**

The cell at this stage has two copies of one segment of DNA—one copy is the exogenote and one copy is part of the endogenote.

Thus at this stage the cell is a *partial diploid, called a* **merozygote.**