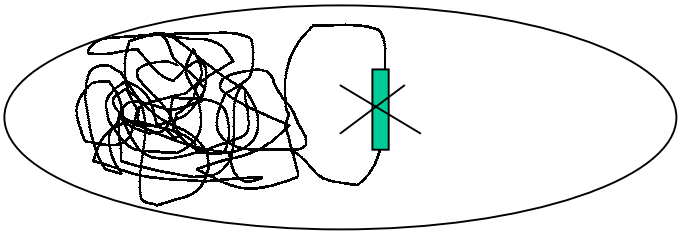
MMG 301, Lec. 26 Gene Transfer

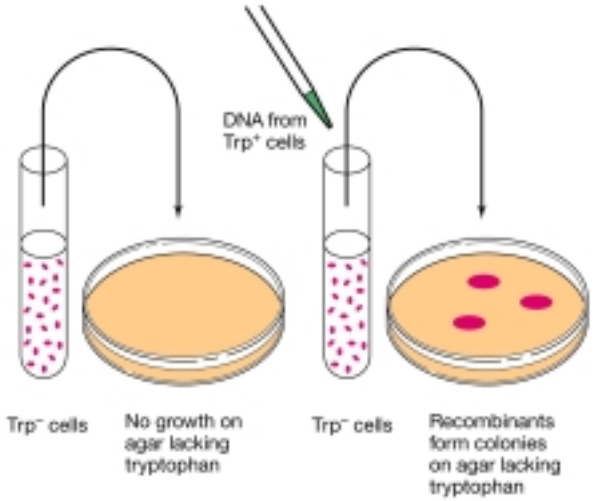
Questions for today:

1. How can we use mutants to study gene transfer?
2. What is transformation and how is it used?
3. What is transduction and how is it used?
4. What is conjugation and how is it used?

Using mutants to study gene transfer

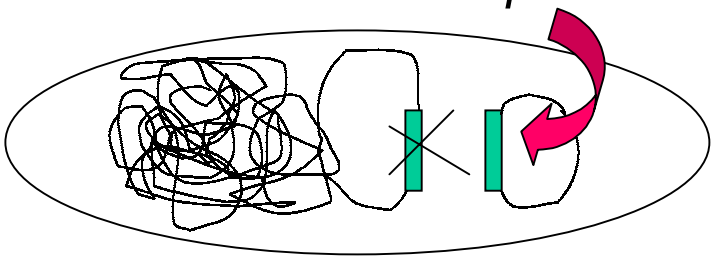


trpA mutant



By transfer of a functional *trpA* gene into the mutant (described below), the defect can be "*complemented*".

Functional *trpA*

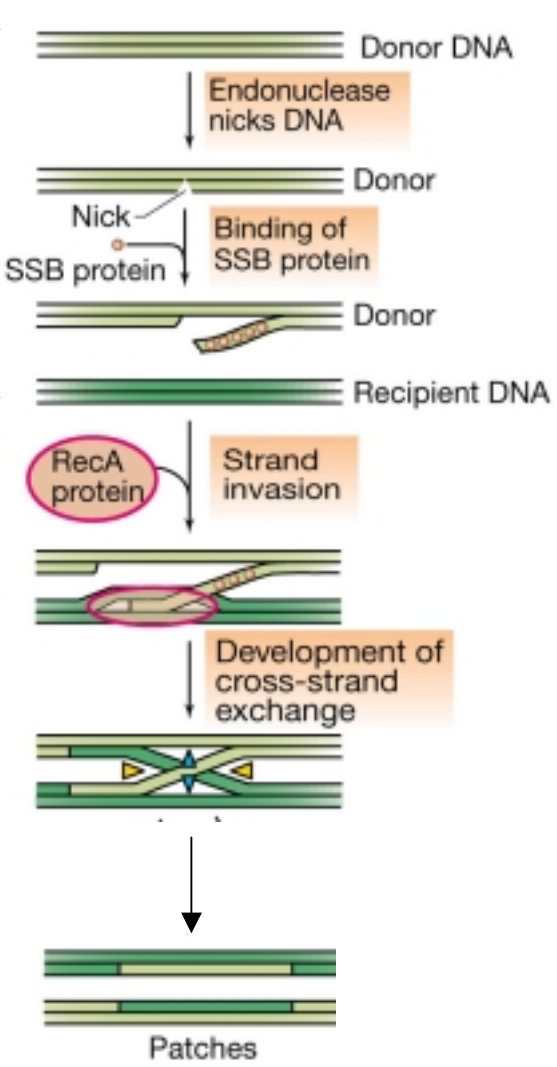


Using "*homologous recombination*" and the RecA protein, the functional gene can replace the defective gene in the chromosome.

Introduced DNA containing functional gene

Chromosomal DNA containing the mutation

Chromosomal DNA containing the “good” gene



In nature, DNA is introduced by transformation, transduction, or conjugation

Transformation

Uptake of “free DNA” by cells

Characteristics of transformation

Some species of Gram-positive bacteria, Gram-negative bacteria, and Archaea are naturally "*competent*" for DNA uptake.

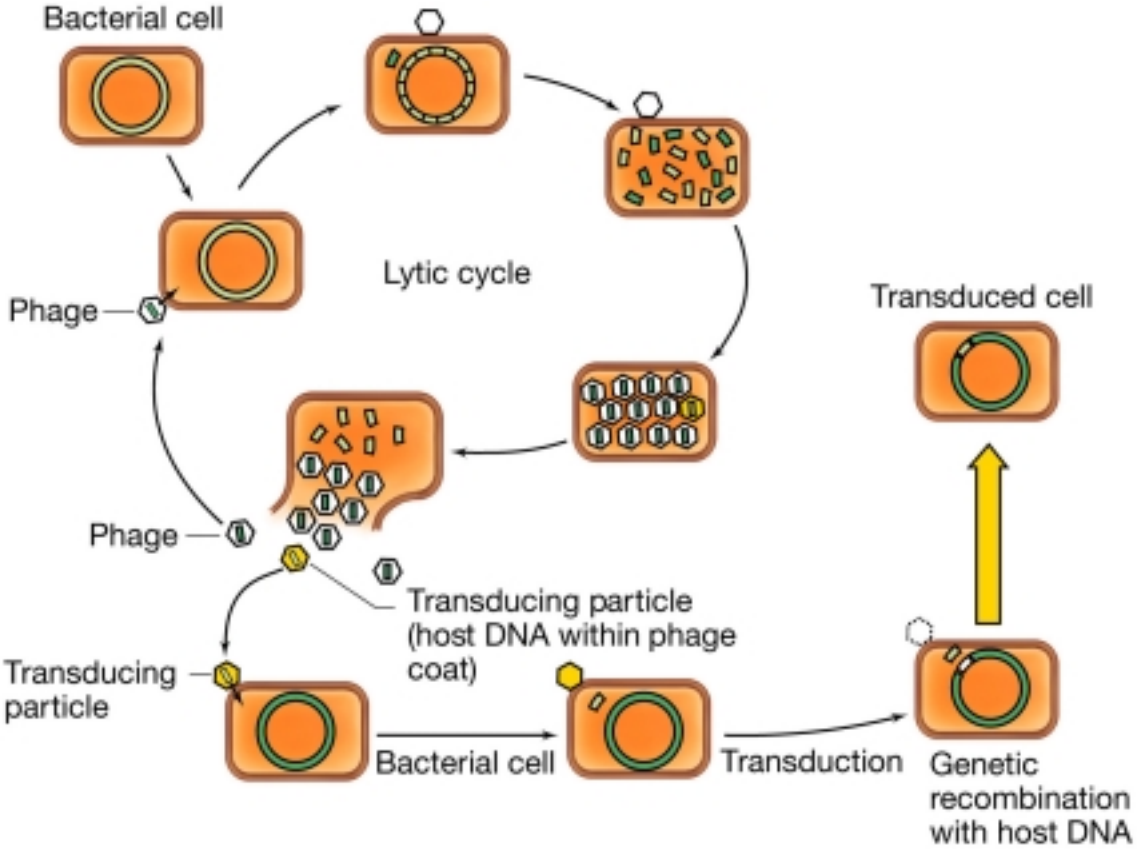
- In these species, competence may be specific to certain stages of growth.
- Other species (e.g., *E. coli*) must be specially treated to induce competence: treatment with high concentrations of Ca plus cold temp. This treatment leads to creation of a Ca-polyphosphate channel in the membrane.
- Alternatively, cells can be zapped with pulses of current to induce transient pores and allow DNA uptake. Such "*electroporation*" does not kill the cells!
- When a competent cell takes up DNA, only ssDNA of a few 1000 bp enters the cell.
- *Haemophilus influenzae* uniquely requires that the DNA taken up must have a particular 11-bp sequence.
- The DNA taken up must become integrated into the chromosome or it is degraded.

Don't confuse prokarotic transformation with eukaryotic transformation: a process in which tumor viruses cause eukaryotic cells to exhibit uncontrolled grow—a cancerous state.

Transduction

The use of phage to transfer DNA by either of two methods.

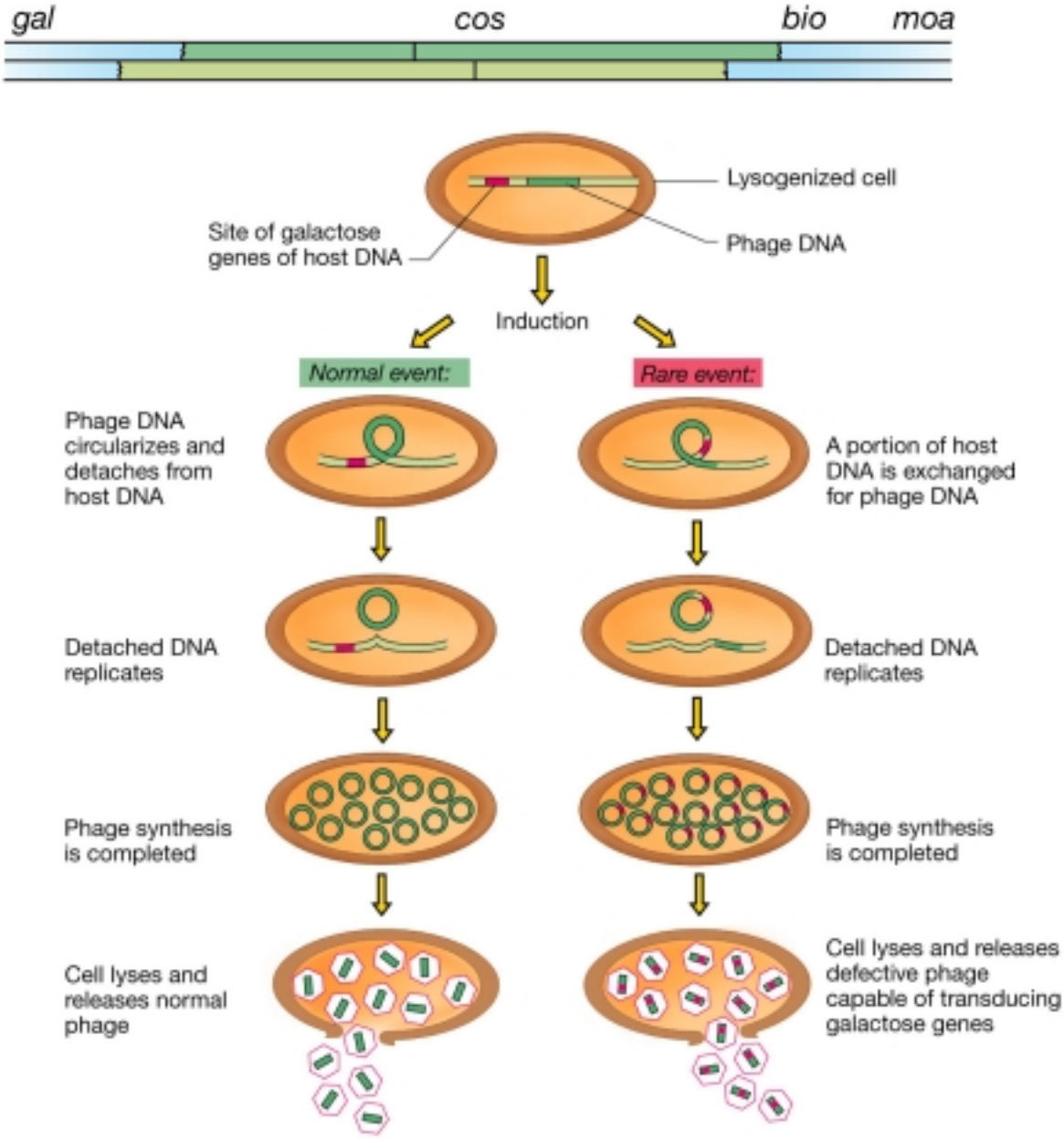
Generalized transduction: During a lytic infection, some of the host DNA is mis-packaged into the virion. This defective “transducing particle” cannot replicate, but it can deliver the DNA to a host cell.



The probability that a particular gene will be packaged and delivered to another cell is very small, about 1 per 10^6 to 10^8 virions.

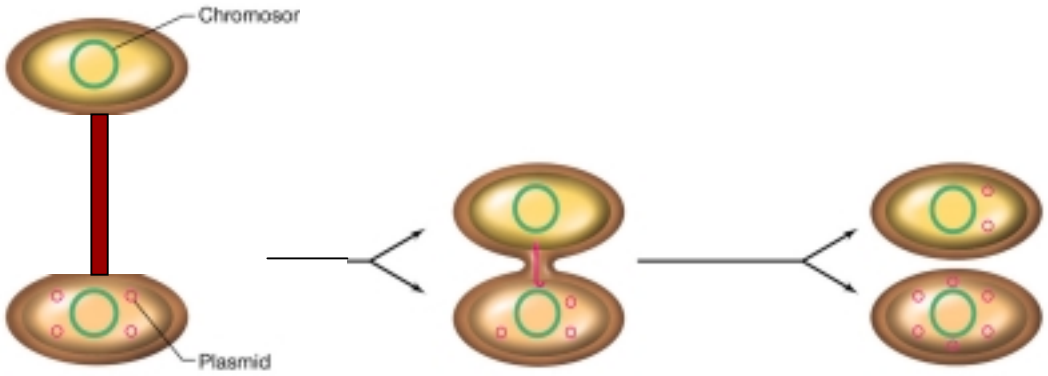
Specialized Transduction: This only occurs with lysogenic phage and results from a mistake during prophage excision so that some host DNA is taken along with the phage DNA.

Recall that Lambda inserts between *gal* and *bio* genes of *E. coli*. One of these genes may be incorporated when lambda excises.



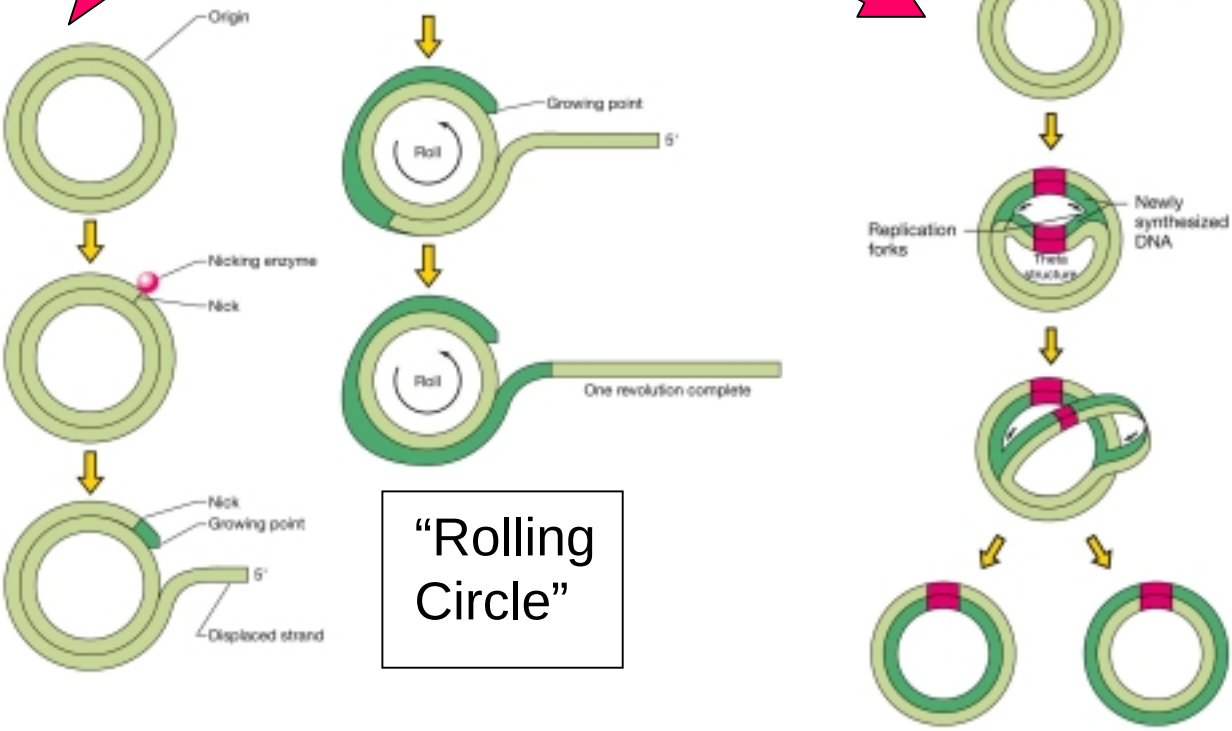
Conjugation

This requires cell-to-cell contact initiated by a pilus.



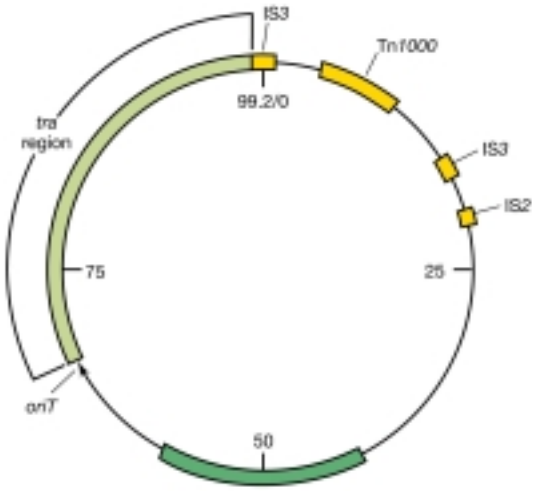
DNA transfer can occur for “conjugative plasmids” (as above) or with the entire chromosome (later).

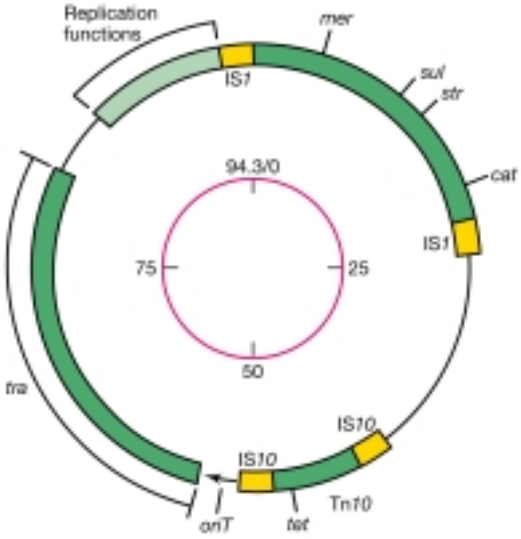
Conjugative plasmids: replicate by “*unidirectional replication*”, a process that differs from the “*bidirectional replication*” of the chromosome and other plasmids.



“Rolling Circle”

Conjugative plasmids require a *tra* (transfer) region in addition to an origin of transfer *(oriT):*

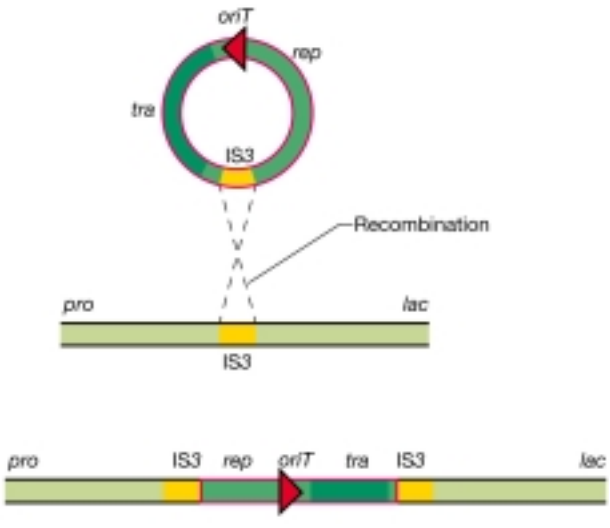


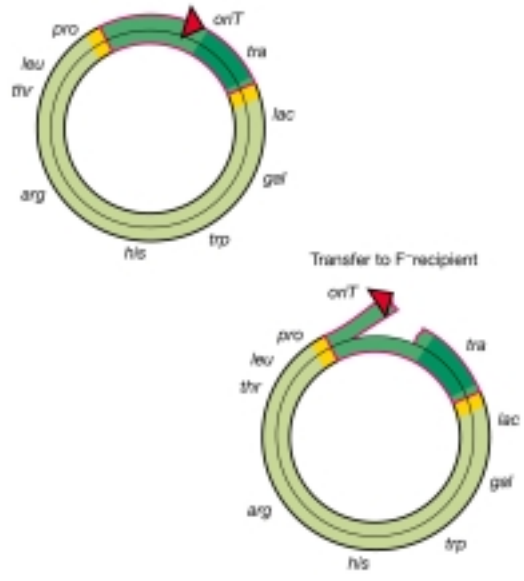


F (fertility) plasmid
(An F+ strain can converts an F- strain to become F+)

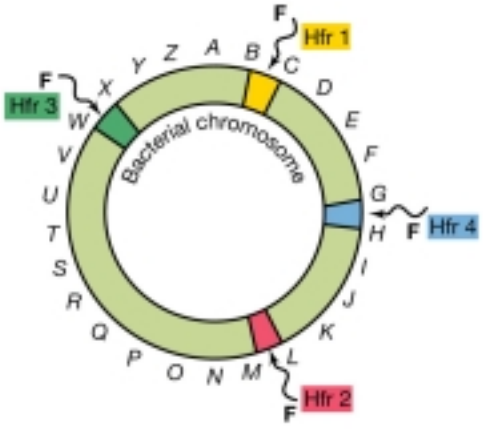
Resistance plasmid (chloramphenicol, sulfonamide, tet, steptomycin)

Chromosomal transfer: IS elements may lead to insertion of a plasmid into the chromosome. Then the entire chromosome can be transferred.





A series of such strains can be used to identify the sequence of genes on the chromosome! 100 min for complete *E. coli* chromosome. Waring blender leads to “interrupted mating”



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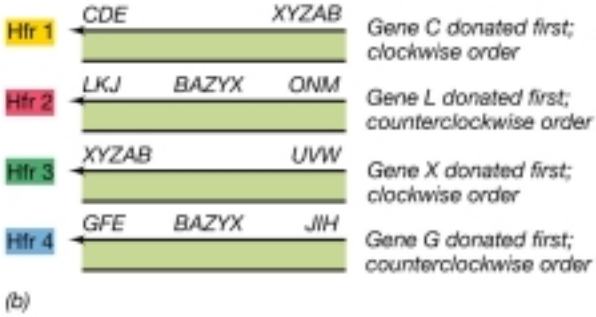

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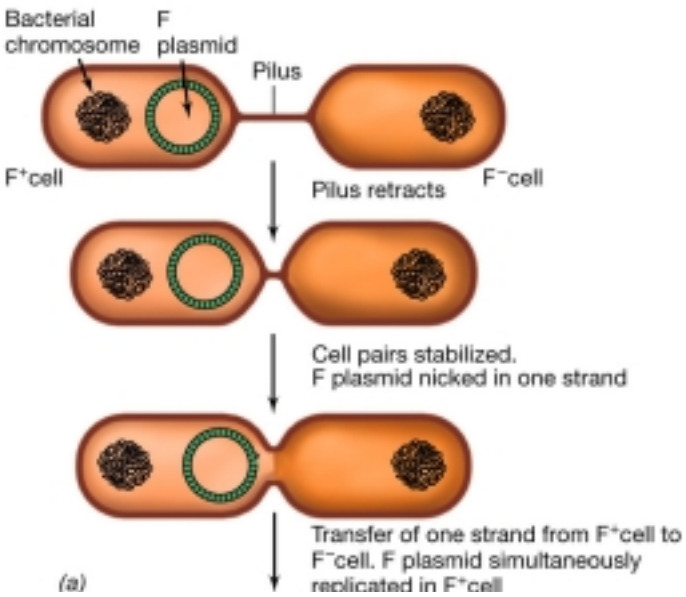

(a)