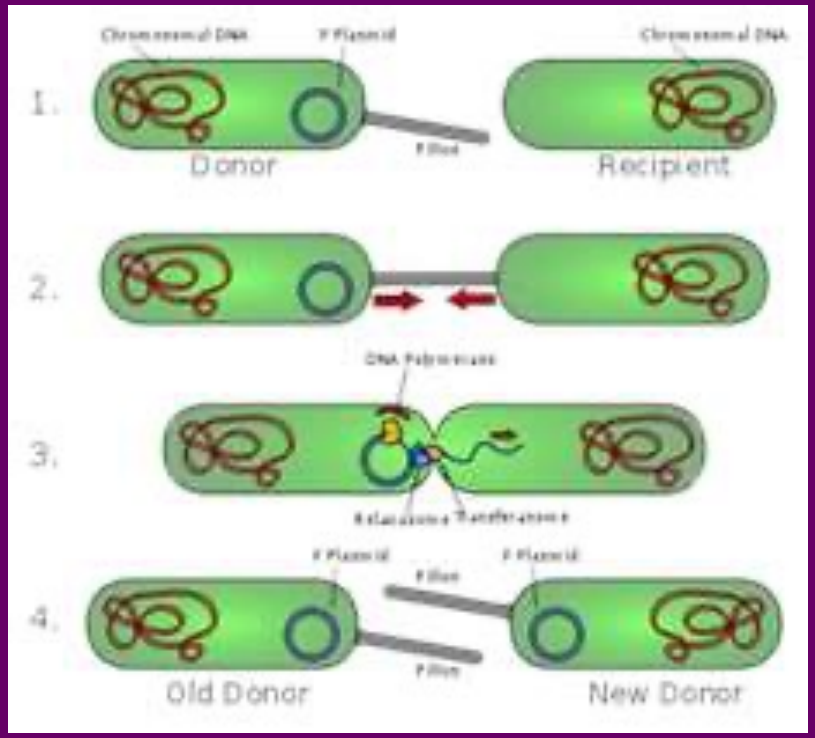
RECOMBINATION IN BACTERIA



1.Gene transfer in bacteria can be accomplished through transformation, conjugation and transduction by phages.

2.Conjugation, transformation, and generalized transduction have in common one important property. Each process introduces a DNA fragment into the recipient cell; then a double-crossover event must take place if the fragment is to be incorporated into the recipient genome and subsequently inherited. Unincorporated fragments cannot replicate and are diluted out and lost from the population of daughter cells.

3. Specialized transduction by phages is a processes in which, a specific and limited set of bacterial genes is introduced into the recipient cell. After transfer, the F’ factor replicates in the bacterial cytoplasm as a separate entity, whereas specialized transducing-phage DNA is recombined into the bacterial chromosome by a phage-encoded recombination system.

Genetic Recombination in Bacteria

Transformation

Transformation involves acquisition of DNA from the environment

Conjugation

conjugation involves acquisition of DNA directly from another bacterium

Transduction

transduction involves acquisition of bacterial DNA via a bacteriophage intermediate

- ❖Generalised
- ❖Specialised

- Genetic recombination - transfer of DNA from one organism (donor) to another recipient. The transferred donor DNA may then be integrated into the recipient's nucleoid by various mechanisms (homologous, non-homologous).

- Homologous recombination- homologous DNA sequences having nearly the same nucleotide sequences are exchanged by means of Rec A proteins. This involves breakage and reunion of paired DNA segments
- Natural mechanisms of genetic recombination in bacteria include:

  a. transformation
  b. transduction
  c. conjungation

Transformation

- Genetic recombination in which a DNA fragment from a dead, degraded bacterium enters a competent recipient bacterium and it is exchanged for a piece of the recipient's DNA.

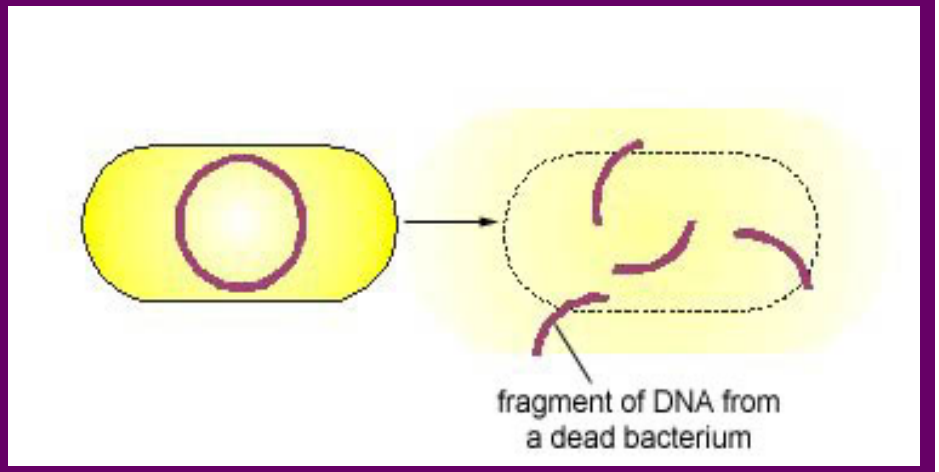
- The first demonstration of bacterial transformation was done with *Streptococcus pneumoniae* and led to the discovery that DNA is the substance of the genes. The path leading to this discovery began in 1928 with the work of an English bacteriologist, Fred Griffith.

- In order to become successfully transformed, bacteria must be **competent**. This means that the bacteria are expressing the appropriate enzymes (the 'transformation machinery') required to transport the exogenous DNA into the cell. Therefore, the correct genes must be expressed in order to carry out transformation. Expression of these genes depends on the growth conditions: bacteria most likely to be competent are dividing rapidly, but nutrients in the environment are becoming limited

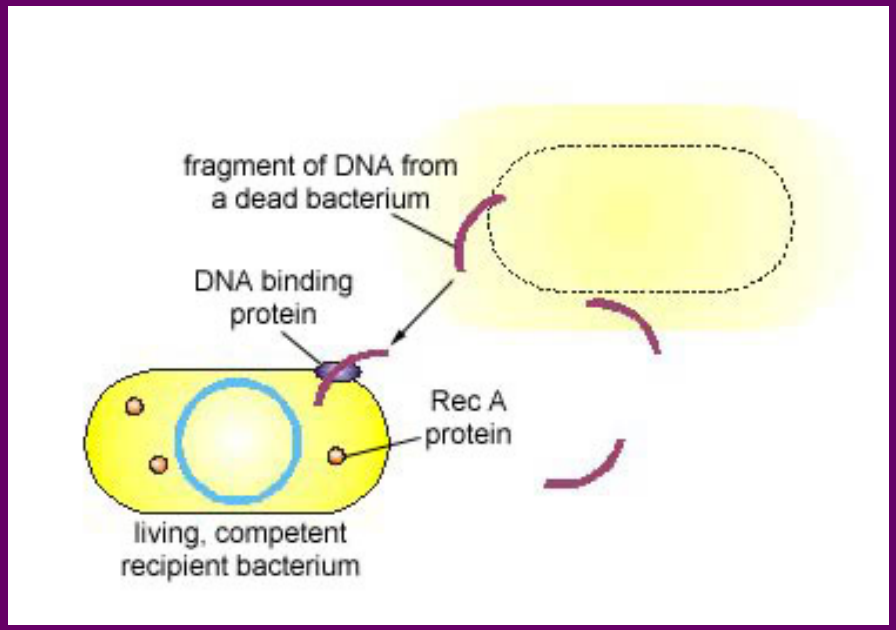
Transformation

- A cell surface receptor binds to DNA in the environment.
- The DNA is transported across the membrane by the transformation machinery.
- As this occurs, one strand of the DNA is digested away by an exonuclease, so that the DNA that enters the cell is single stranded.
- This promotes recombination, as long as the DNA taken up is sufficiently homologous to the host DNA to allow recombination to occur.
- The recombination that occurs is one-way (non-reciprocal); in this case the new DNA will simply replace a strand of the host DNA.
- The replaced segment of host DNA will be degraded. If the new DNA is of a different allelic nature than the host DNA, a gene conversion event can occur.
- This is what happened in the example when avirulent *Streptococcus pneumoniae* became virulent by being exposed to heat-killed virulent cells: the avirulent strain of *S. pneumoniae* had a mutation in a gene required for production of the bacterial capsule. Heat killing the virulent cells (which contained the wild-type capsule gene) caused the release of fragments of the dead cells' genomes.
- Some of the avirulent cells picked up a piece of DNA containing the wild-type capsule gene, and underwent gene conversion so that they were wild type for that gene, causing them to become virulent

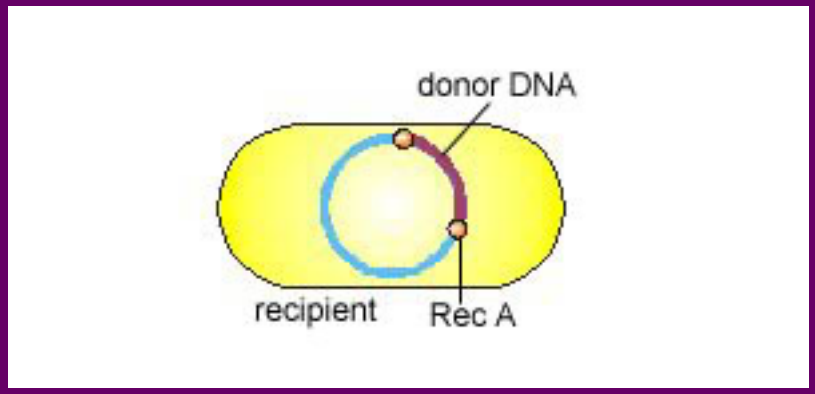
The 4 steps in Transformation



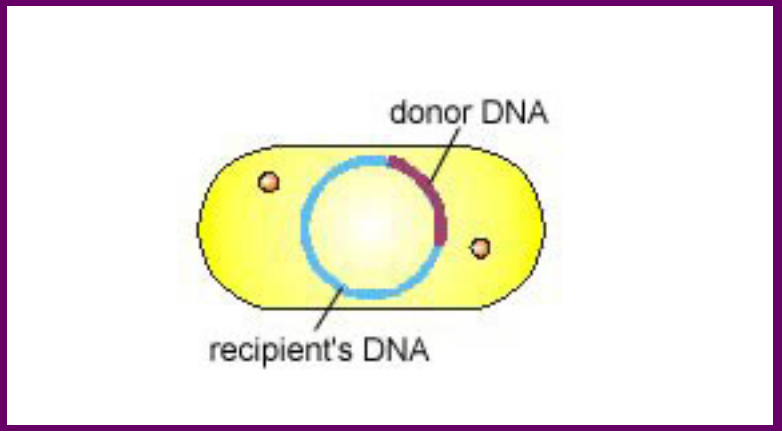
1. A donor bacterium dies and is degraded



2. A fragment of DNA from the dead donor bacterium binds to DNA binding proteins on the cell wall of a competent, living recipient bacterium



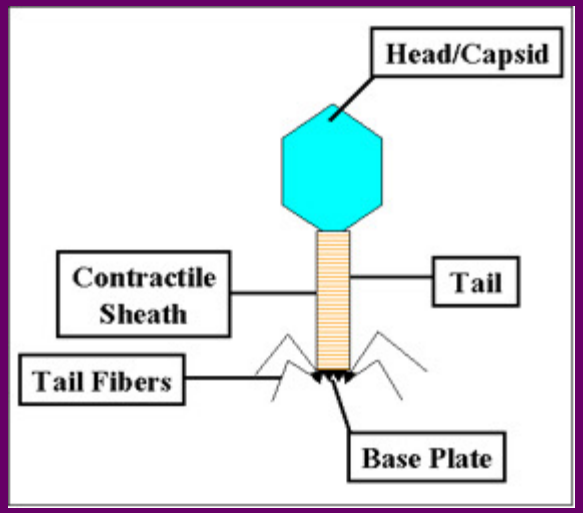
3. The Rec A protein promotes genetic exchange between a fragment of the donor's DNA and the recipient's DNA



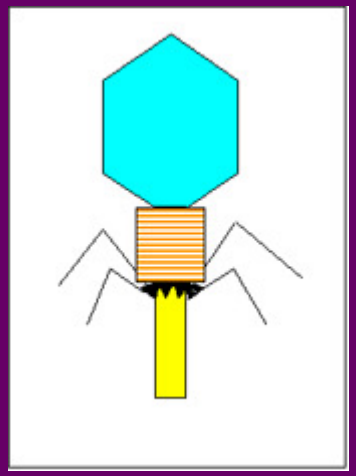
4. Exchange is complete

Transduction

- Genetic recombination in which a DNA fragment is transferred from one bacterium to another by a bacteriophage
- Transduction is discovered by Zinder and Lederberg in 1952



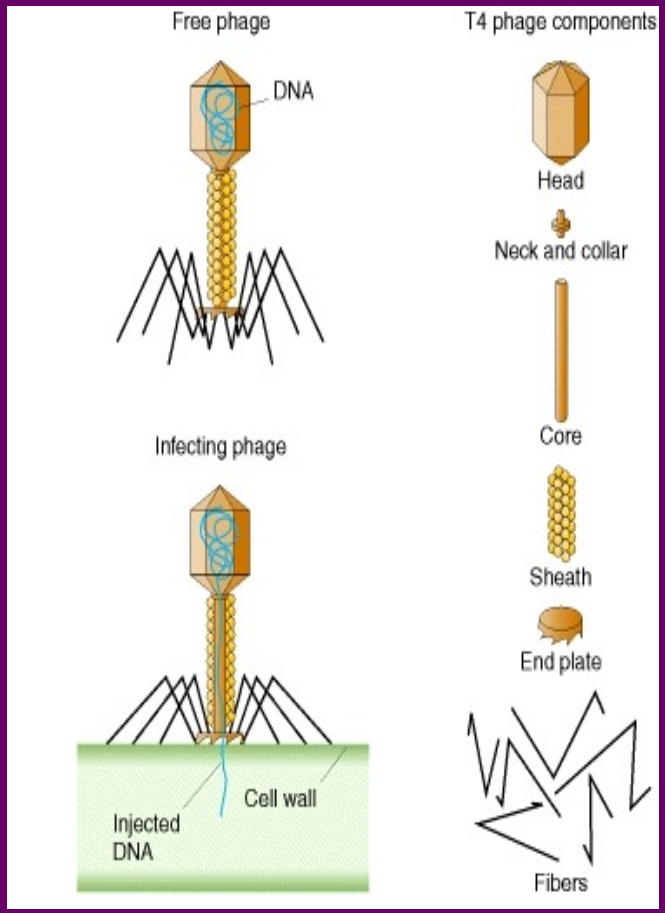
Structure of T4 bacteriophage



Contraction of the tail sheath of T4

What are Bacteriophages?

Bacteriophage (phage) are obligate intracellular parasites that multiply inside bacteria by making use of some or all of the host biosynthetic machinery (i.e., viruses that infect bacteria)



Phage T4, depicted in its free state and in the process of infecting an *E. coli* cell. The infecting phage injects DNA through its core structure into the cell. On the right, a phage has been diagrammatically exploded to show its highly ordered three-dimensional structure

When a phage infects a bacterial cell, it injects its DNA into the cell.

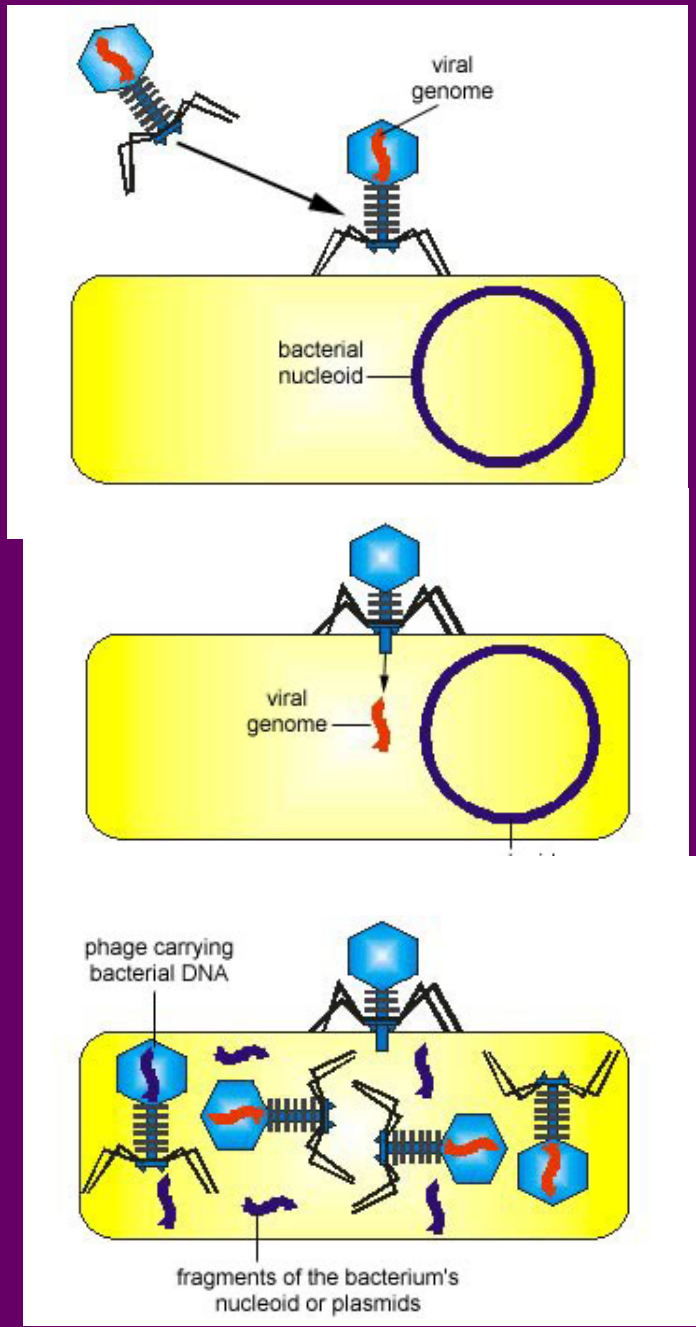
The viral DNA is replicated numerous times, and viral genes are expressed, producing the proteins that make up the viral capsid (or protein coat) and nucleases that digest the host genome into fragments.

The newly replicated viral DNA molecules are packaged into viral capsids, and the bacterial cell is lysed (burst, and therefore killed), releasing hundreds of viral progeny, which then go on to infect other cells.

1. Generalized Transduction

- Sometimes, during bacteriophage replication, a mistake is made, and a fragment of the host DNA gets packaged into a viral capsid.
- The resulting phage would be able to infect another cell, but it would not have any viral genes, so it would not be able to replicate.
- The cell infected by this phage would survive, and would have an extra piece of bacterial DNA present, which could undergo recombination with the host chromosome, and perhaps cause a gene conversion event.
- Because it is a random fragment that gets packaged into the viral capsid, any segment of the bacterial DNA can be transferred this way (hence the name 'generalized').

Seven steps in Generalised Transduction

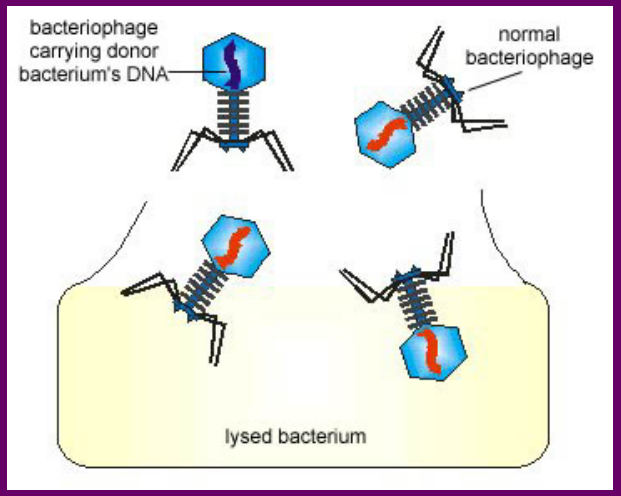


1. A lytic bacteriophage adsorbs to a susceptible bacterium.

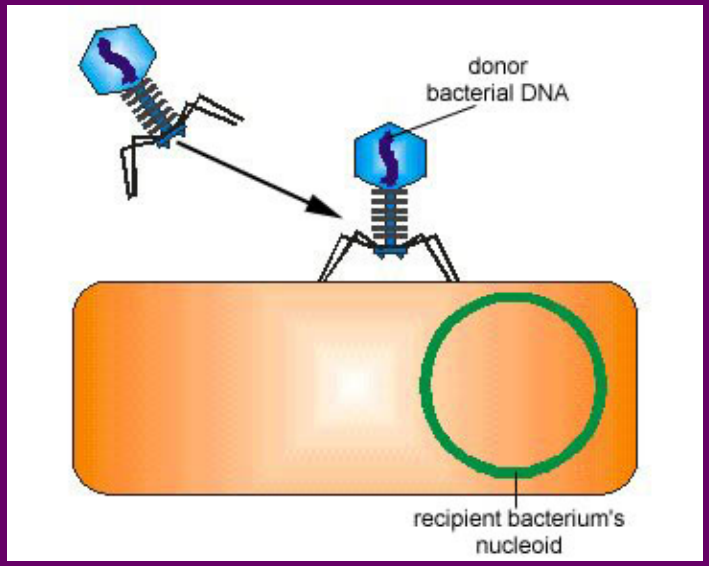
2. The bacteriophage genome enters the bacterium. The genome directs the bacterium's metabolic machinery to manufacture bacteriophage components and enzymes

3. Occasionally, a bacteriophage head or capsid assembles around a fragment of donor bacterium's nucleoid or around a plasmid instead of a phage genome by mistake.

Seven steps in Generalised Transduction (cont'd)

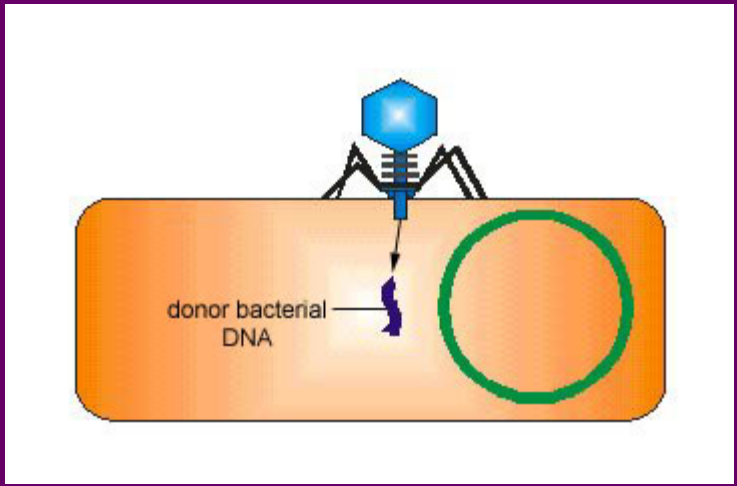


4. The bacteriophages are released.

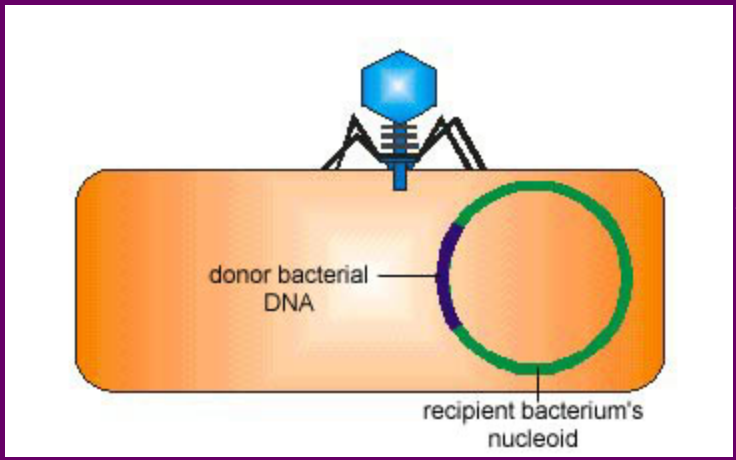


5. The bacteriophage carrying the donor bacterium's DNA adsorbs to a recipient bacterium

Seven steps in Generalised Transduction (contd)



6. The bacteriophage inserts the donor bacterium's DNA it is carrying into the recipient bacterium .

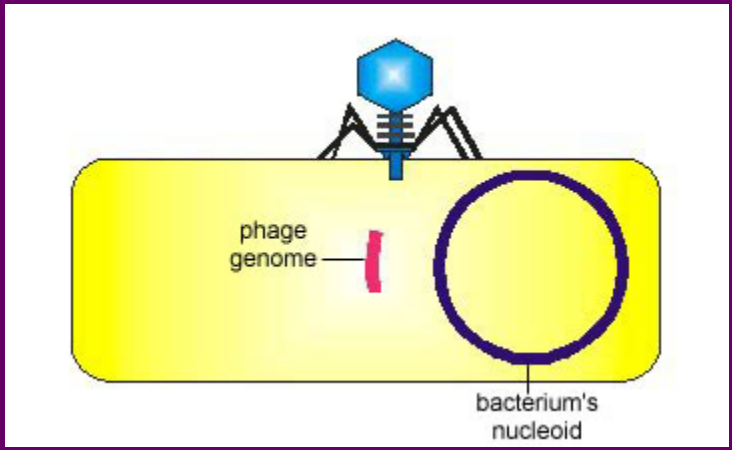


7. The donor bacterium's DNA is exchanged for some of the recipient's DNA.

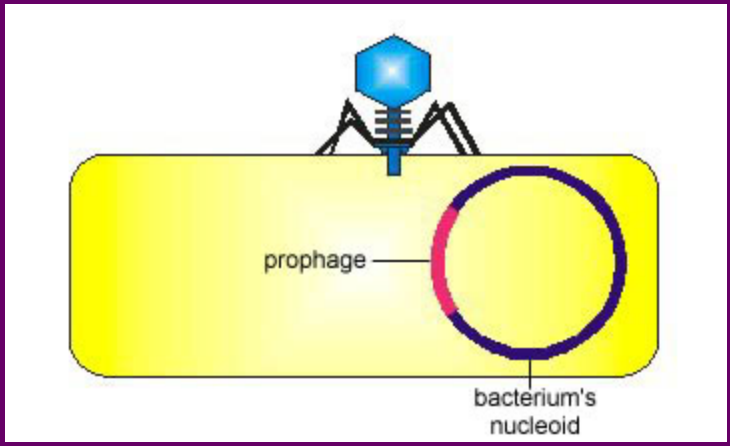
2. Specialized Transduction

- In specialized transduction, bacteriophage transfer only a few restricted gene (DNA fragments) from donor bacteria to recipient bacteria. Specialized transduction is carried only by temperate bacteriophage which undergoes lysogenic cycle in donor cell.
- At first temperate bacteriophage enter into donor bacteria and then its genome gets integrated with host cell's DNA at certain location and remains dormant and pass generation to generation into daughter cell during cell division. The bacteriophage which follows lysogenic cycle is known as temperate phage.
- When such lysogenic cell is exposed to certain stimulus such as some chemicals or UV lights, it causes induction of virus genome from host cell genome and begins lytic cycle.
- On induction from donor DNA, this phage genome sometimes carries a part of bacterial DNA with it. The bacterial DNA lies on sides of integrated phage DNA are only carried during induction.
- When such bacteriophage carries a part of donor bacterial DNA infects a new bacteria, it can transfer that donor DNA fragments into new recipient cell. So, in this specialized transduction only those restricted gene are situated on the side of integrated viral genome have a chance to enter into recipient cell.

Six steps in Specialised Transduction

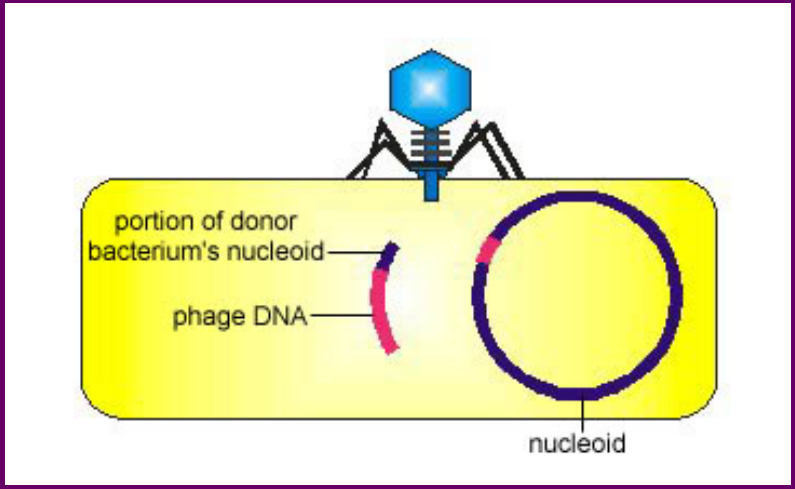


1. A temperate bacteriophage adsorbs to a susceptible bacterium and injects its genome .

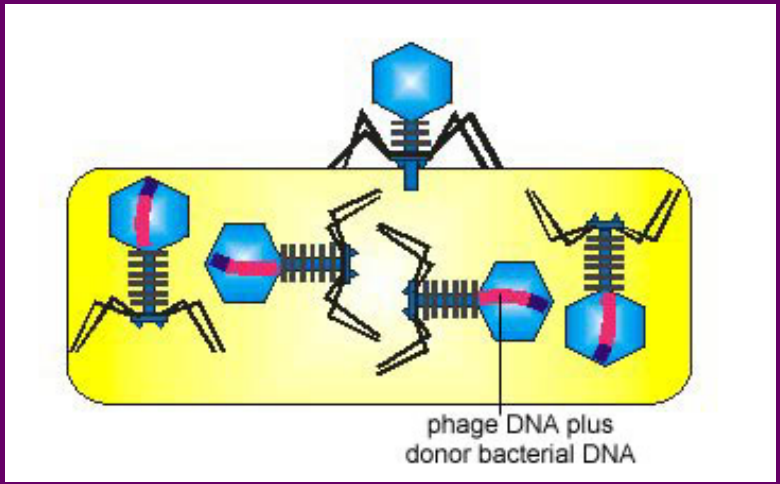


2. The bacteriophage inserts its genome into the bacterium's nucleoid to become a prophage.

Six steps in Specialised Transduction (cont'd)

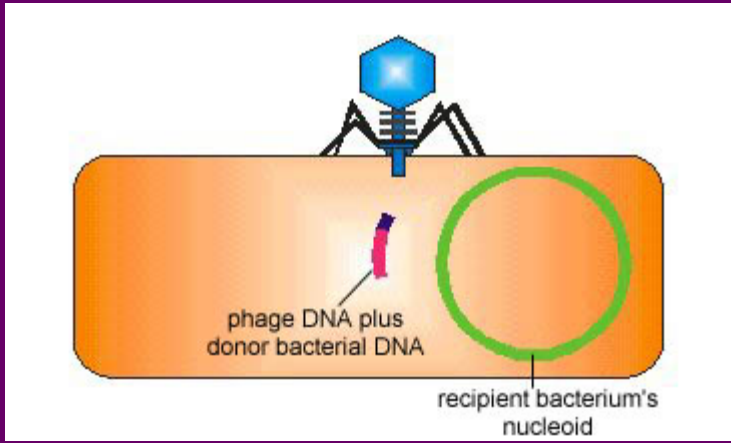


3. Occasionally during spontaneous induction, a small piece of the donor bacterium's DNA is picked up as part of the phage's genome in place of some of the phage DNA which remains in the bacterium's nucleoid.

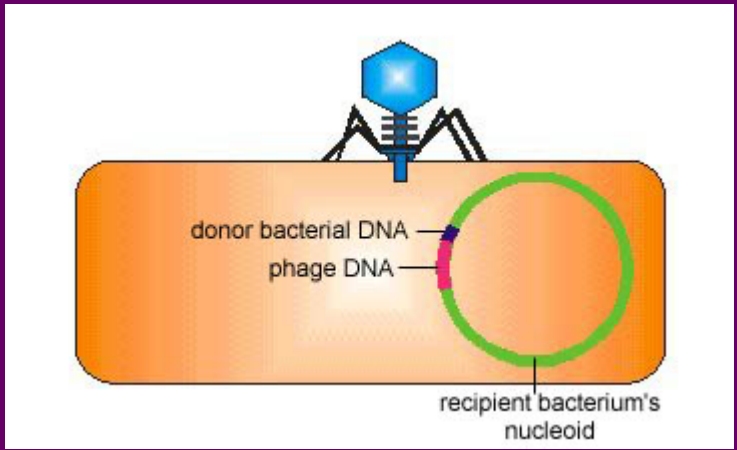


4. As the bacteriophage replicates, the segment of bacterial DNA replicates as part of the phage's genome. Every phage now carries that segment of bacterial DNA.

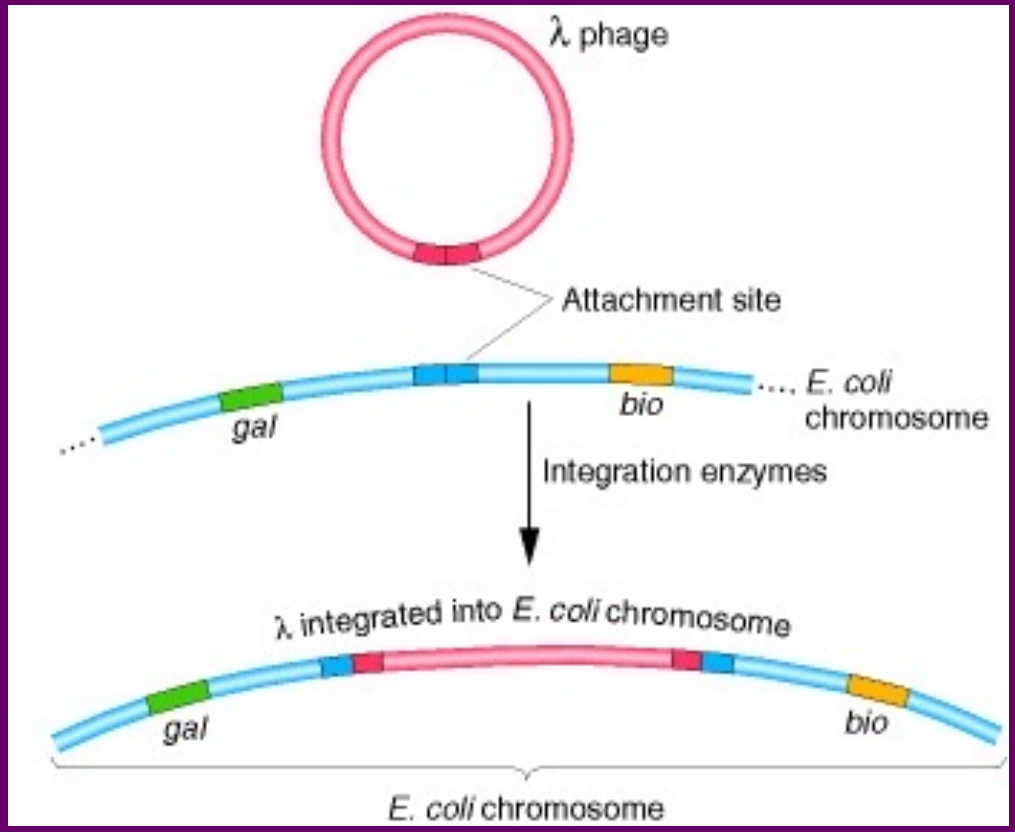
Six steps in Specialised Transduction (cont'd)



5. The bacteriophage adsorbs to a recipient bacterium and injects its genome.



6. The bacteriophage genome carrying the donor bacterial DNA inserts into the recipient bacterium's nucleoid.



Model for the integration of phage λ into the *E. coli* chromosome. Reciprocal recombination takes place between a specific attachment site on the circular λ DNA and a specific region on the bacterial chromosome between the *gal* and *bio* genes

Bacterial Conjugation

Bacterial Conjugation is genetic recombination in which there is a transfer of DNA from a living donor bacterium to a recipient bacterium. Often involves a sex pilus.

Conjugation was discovered by Lederberg and Tatum in 1946

Some bacteria, *E. coli* is an example, can transfer a portion of their chromosome to a recipient with which they are in direct contact. As the donor replicates its chromosome, the copy is injected into the recipient. At any time that the donor and recipient become separated, the transfer of genes stops. Those genes that successfully made the trip replace their equivalents in the recipient's chromosome

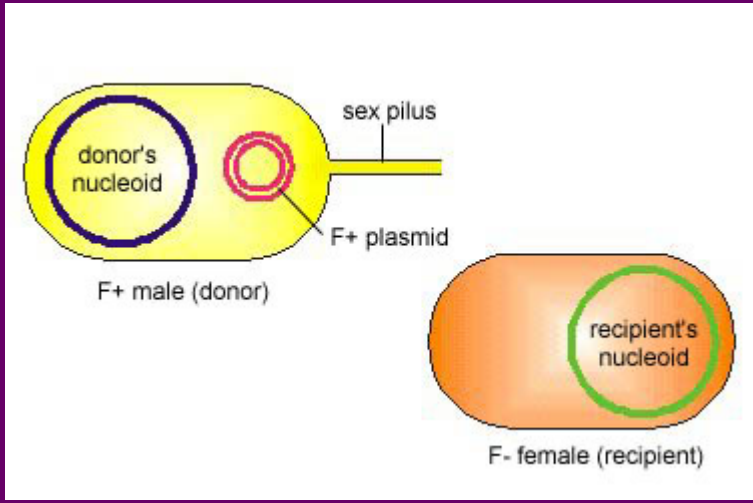
- **The 3 conjugative processes**
  1. **F^+ conjugation**
  2. **Hfr conjugation**
  3. **Resistance plasmid conjugation**

I. F+ Conjugation Process

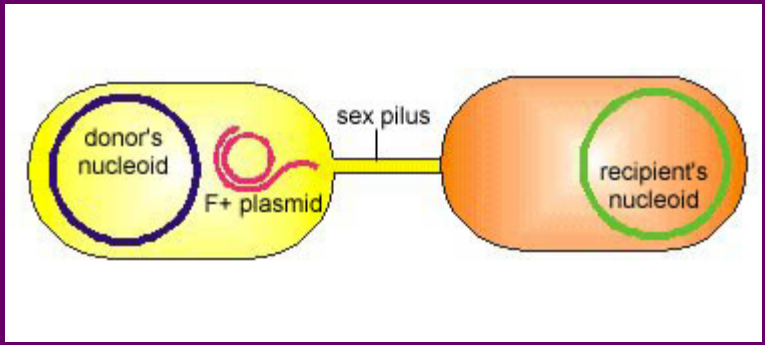
F+ Conjugation- Genetic recombination in which there is a transfer of an F+ plasmid (coding only for a sex pilus) but not chromosomal DNA from a male donor bacterium to a female recipient bacterium. Involves a sex (conjugation) pilus. Other plasmids present in the cytoplasm of the bacterium, such as those coding for antibiotic resistance, may also be transferred during this process.

- It involves transfer of genetic information from one bacterial cell to another, and requires physical contact between the two bacteria involved. The contact between the cells is via a protein tube called an F or sex pilus, which is also the conduit for the transfer of the genetic material.
- Basic conjugation involves two strains of bacteria: F+ and F-. The difference between these two strains is the presence of a Fertility factor (or F factor) in the F+ cells. The F factor is an episome that contains 19 genes and confers the ability to conjugate upon its host cell. Genetic transfer in conjugation is from an F+ cell to an F- cell, and the genetic material transferred is the F factor itself

The 4 stepped F+ Conjugation

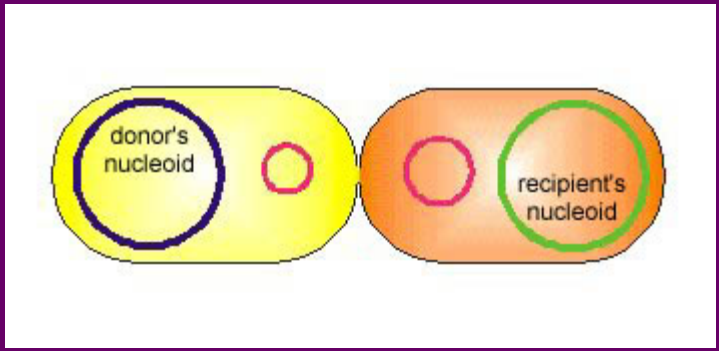


1. The F+ male has an F+ plasmid coding for a sex pilus and can serve as a genetic donor



2. The sex pilus adheres to an F- female (recipient). One strand of the F+ plasmid breaks

The 4 stepped F+ Conjugation (cont'd)



3. The sex pilus retracts and a bridge is created between the two bacteria. One strand of the F+ plasmid enters the recipient bacterium

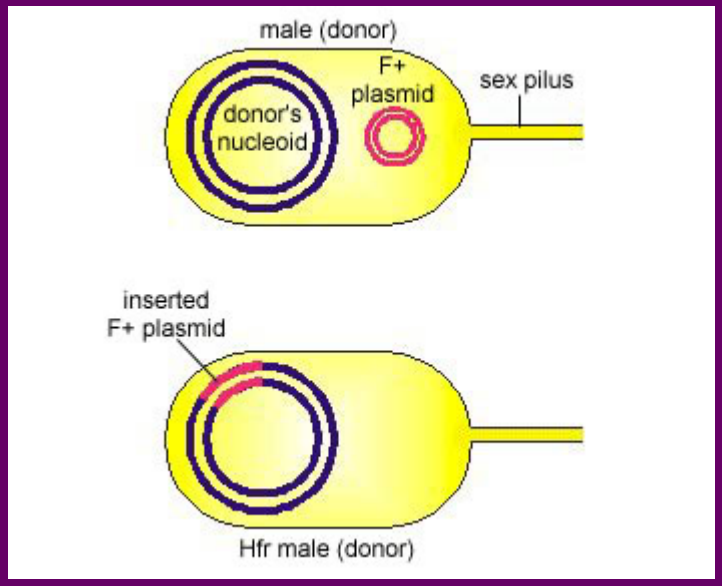
4. Both bacteria make a complementary strand of the F+ plasmid and both are now F+ males capable of producing a sex pilus. There was no transfer of donor chromosomal DNA although other plasmids the donor bacterium carries may also be transferred during F+ conjugation.

II. Hfr Conjugation

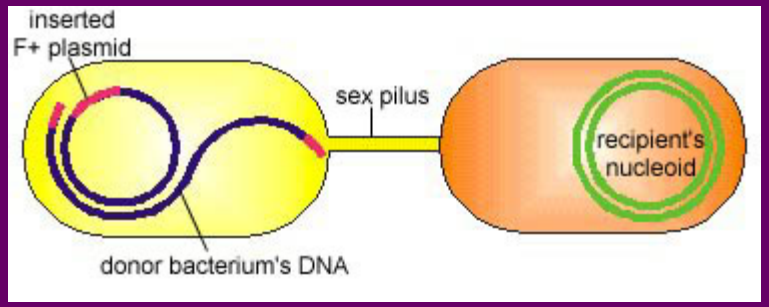
Genetic recombination in which fragments of chromosomal DNA from a male donor bacterium are transferred to a female recipient bacterium following insertion of an F+ plasmid into the nucleoid of the donor bacterium. Involves a sex (conjugation) pilus.

Occasionally, the F factor integrates into a random position in the bacterial chromosome. When this happens, the bacterial cell is called Hfr instead of F+. Hfr bacteria are still able to initiate conjugation with F- cells, but the outcome is completely different from conjugation involving F+ bacteria

5 stepped Hfr Conjugation

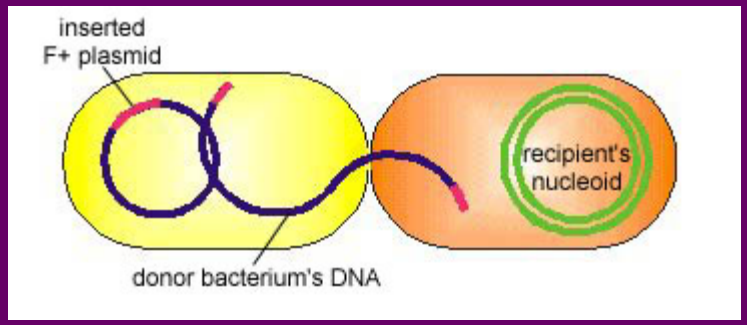


1. An F+ plasmid inserts into the donor bacterium's nucleoid to form an Hfr male.

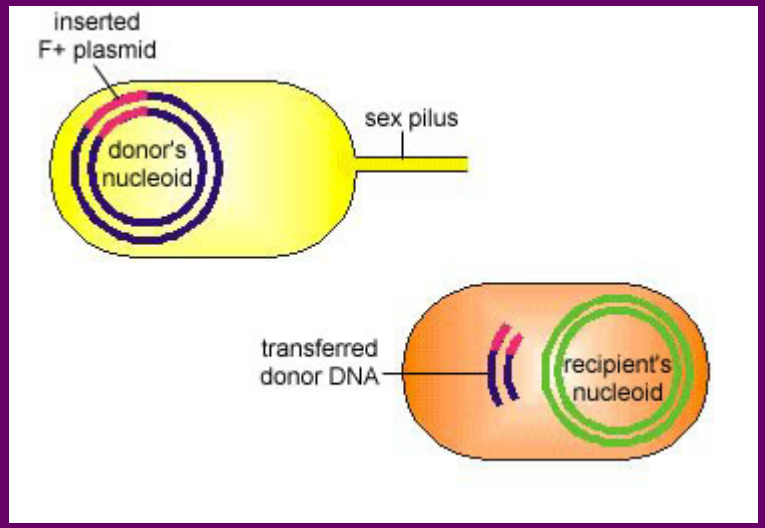


2. The sex pilus adheres to an F- female (recipient). One donor DNA strand breaks in the middle of the inserted F+ plasmid.

5 stepped Hfr Conjugation (cont'd)

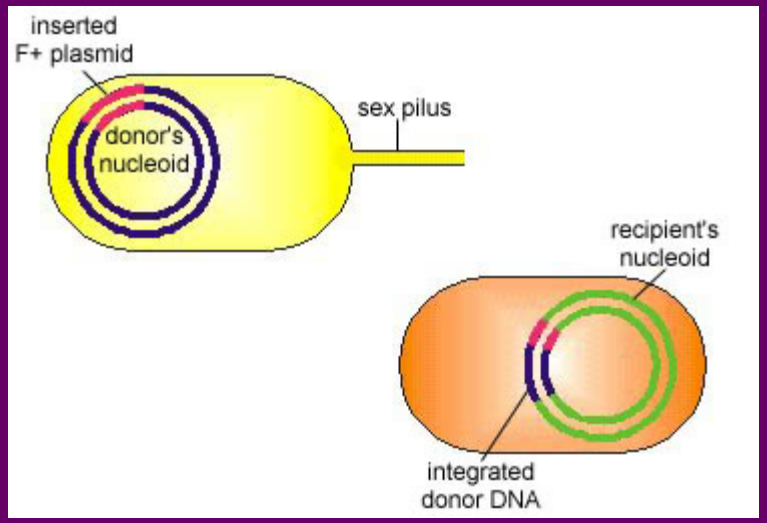


3. The sex pilus retracts and a bridge forms between the two bacteria. One donor DNA strand begins to enter the recipient bacterium. The two cells break apart easily so the only a portion of the donor's DNA strand is usually transferred to the recipient bacterium.



4. The donor bacterium makes a complementary copy of the remaining DNA strand and remains an Hfr male. The recipient bacterium makes a complementary strand of the transferred donor DNA.

5 stepped Hfr Conjugation (cont'd)

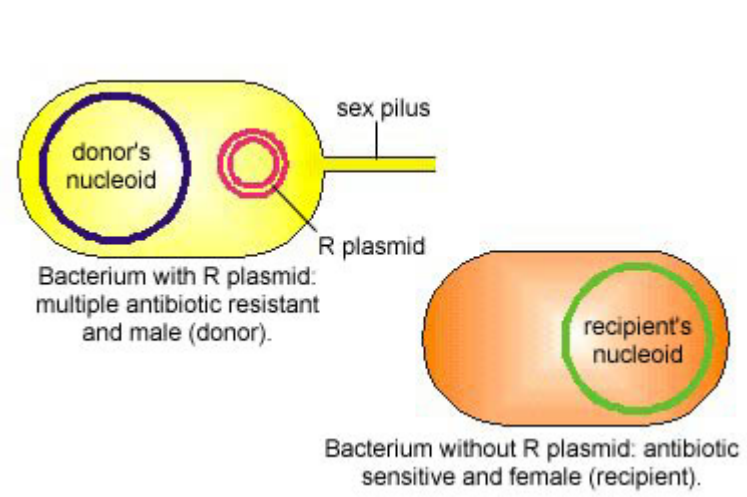


5. The donor DNA fragment undergoes genetic exchange with the recipient bacterium's DNA. Since there was transfer of some donor chromosomal DNA but usually not a complete F+ plasmid, the recipient bacterium usually remains F-

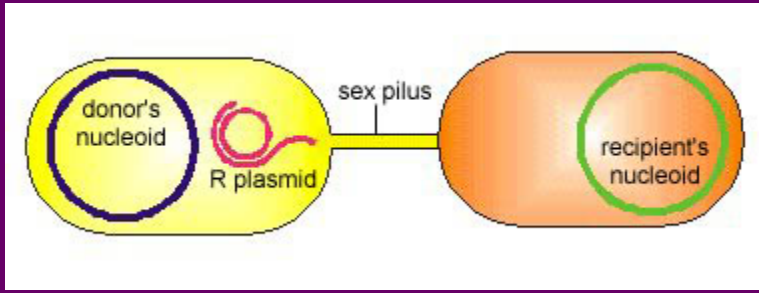
III. Resistant Plasmid Conjugation

Genetic recombination in which there is a transfer of an R plasmid (a plasmid coding for multiple antibiotic resistance and often a sex pilus) from a male donor bacterium to a female recipient bacterium. Involves a sex (conjugation) pilus

4 steped Resistant Plasmid Conjugation

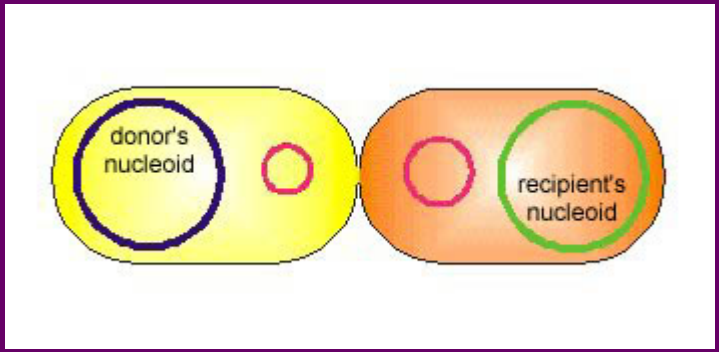


1. The bacterium with an R-plasmid is multiple antibiotic resistant and can produce a sex pilus (serve as a genetic donor).

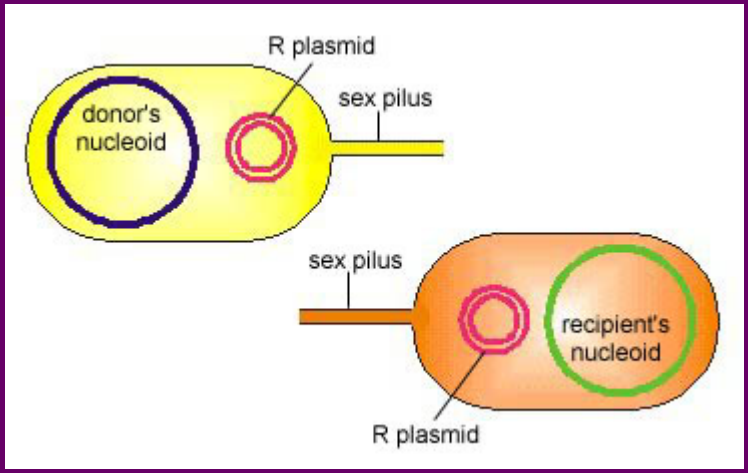


2. The sex pilus adheres to an F- female (recipient). One strand of the R-plasmid breaks.

4 stepped Resistant Plasmid Conjugation (cont'd)



3. The sex pilus retracts and a bridge is created between the two bacteria. One strand of the R-plasmid enters the recipient bacterium.



4. Both bacteria make a complementary strand of the R-plasmid and both are now multiple antibiotic resistant and capable of producing a sex pilus.

SUMMARY

- Bacteria can pick up loose DNA in their environment through the process of transformation. The newly acquired DNA is rendered single stranded, and can recombine with the host chromosome.
- Bacteria can exchange DNA through the process of conjugation. The F factor confers the ability to initiate conjugation. If the F factor alone is transferred, no recombination will occur. Under certain circumstances, chromosomal DNA can be transferred to the recipient cell. In these cases, recombination will occur.
- Bacteria can receive bacterial DNA from viruses through the process of transduction. Bacterial viruses can accidentally pick up pieces of bacterial DNA. When they subsequently infect a cell, they transfer the peice of bacterial DNA, which can undergo recombination with the host bacterial chromosome.
- The result of recombination in the above cases may be gene conversion, in which a mutant allele becomes wild-type or vice versa.
- Conjugation involving Hfr bacteria can be used to map genes along the bacterial chromosome. This is done by determining in what order genes are transferred during conjugation, what the time difference is between the transfer of genes.

Significance of genetic recombination in Bacteria

- The completion of the sequence of the entire genome of a variety of different bacteria (and archaea) suggest that genes have in the past moved from one species to another. This phenomenon is called **lateral gene transfer** (LGT).
- The remarkable spread of resistance to multiple antibiotics may have been aided by the transfer of resistance genes within populations and even between species.
- Many bacteria have enzymes that enable them to destroy foreign DNA that gets into their cells. In any case, these **restriction enzymes** have provided the tools upon which the advances of molecular biology and the biotechnology industry depend.