Transfer of Genetic Information in Bacteria

- Bacteria reproduce by splitting in two via binary fission. Binary fission makes **clones**, or **genetically identical copies**, of the parent bacterium.
- Binary fission doesn't provide an opportunity for genetic recombination or genetic diversity.
- Genetic variation is key to the survival of a species, allowing groups to adapt to changes in their environment by natural selection. That's true for bacteria as well as plants and animals.
- Prokaryotes can ALSO share genes by three other mechanisms:

  - Conjugation
  - Transformation
  - Transduction

Conjugation

1. Discovered by Joshua Lederberg and Edward Tatum in 1946.
2. Unidirectional transfer of genetic material between donor and recipient bacteria cells by direct contact.
3. In **conjugation**, DNA is transferred from one bacterium to another. After the donor cell pulls itself close to the recipient using a structure called a pilus, DNA is transferred between cells. In most cases, this DNA is in the form of a plasmid.
   - Donor is a cell having fertility factor or F plasmid are also termed as F+ or male cell
   - Recipient is a cell have no any fertility factor or F plasmid are also termed as F- or female cell

Recipients containing donor DNA are called **Transconjugants**.

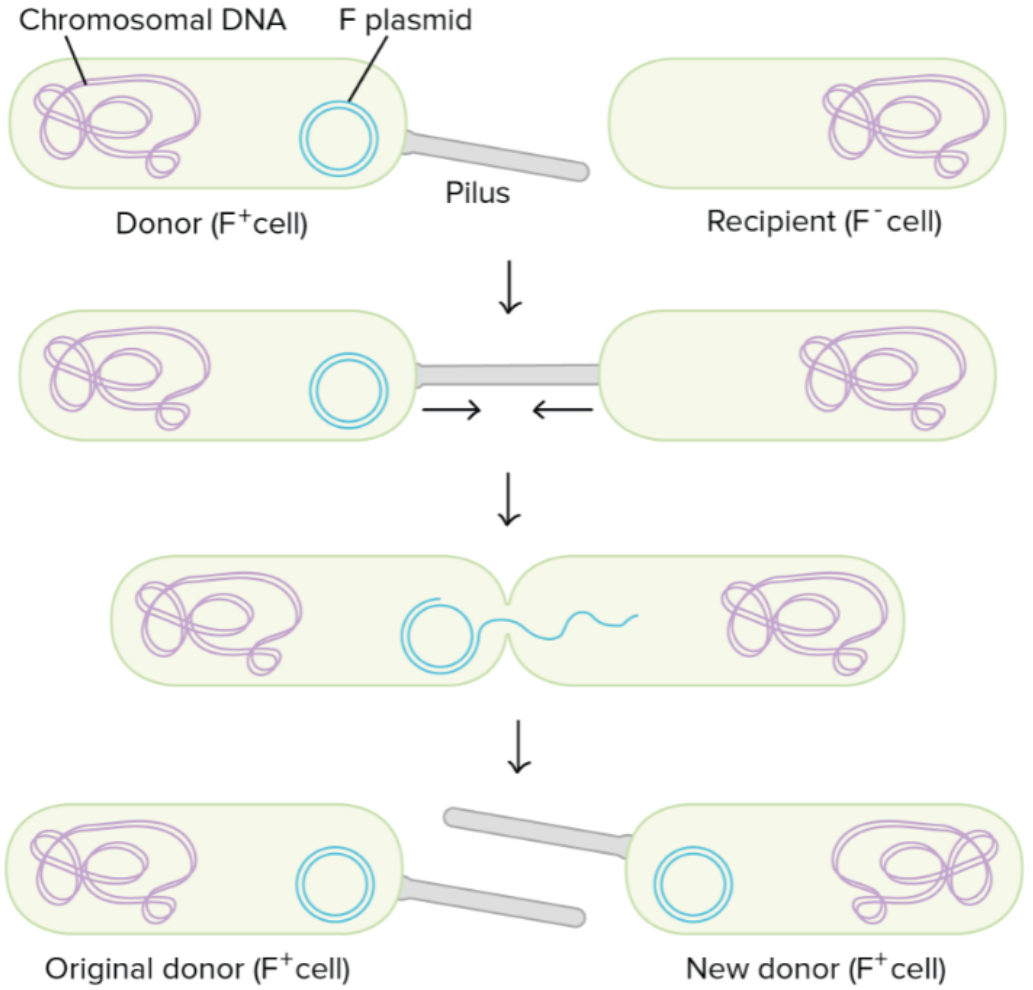


Fig. 15.2, Lederberg & Tatum (1946) Experiment demonstrating recombination in *E. coli*. Recombination of 2 complimentary auxotrophs gives rise to a strain that can synthesize all nutrients.

- **Prototrophic** - (wild type) need only inorganic salts, an organic energy source (sugar, fat, protein) and water to survive and grow. ("minimal medium")

- **Auxotrophic** - (mutant) unable to grow without one or more *essential nutrient(s)*. Auxotrophs are mutant for particular nutrient synthesis pathway enzymes. Such an error is known as an **inborn error of metabolism**, whether it occurs in a bacterium or a eukaryote.

- An auxotroph can be grown only on an **enriched medium** that provides the particular nutrient that the mutant cannot metabolize on its own.

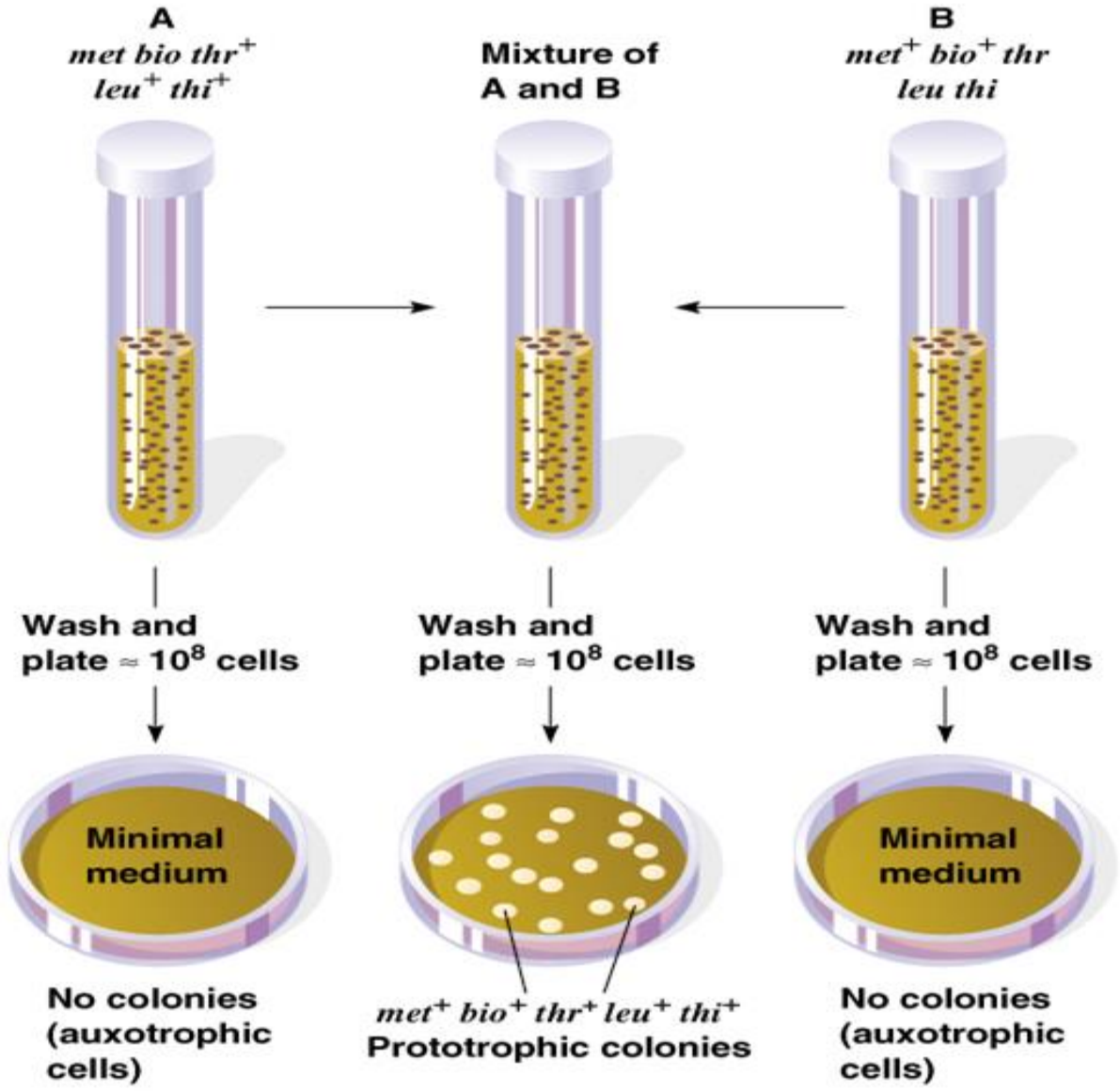
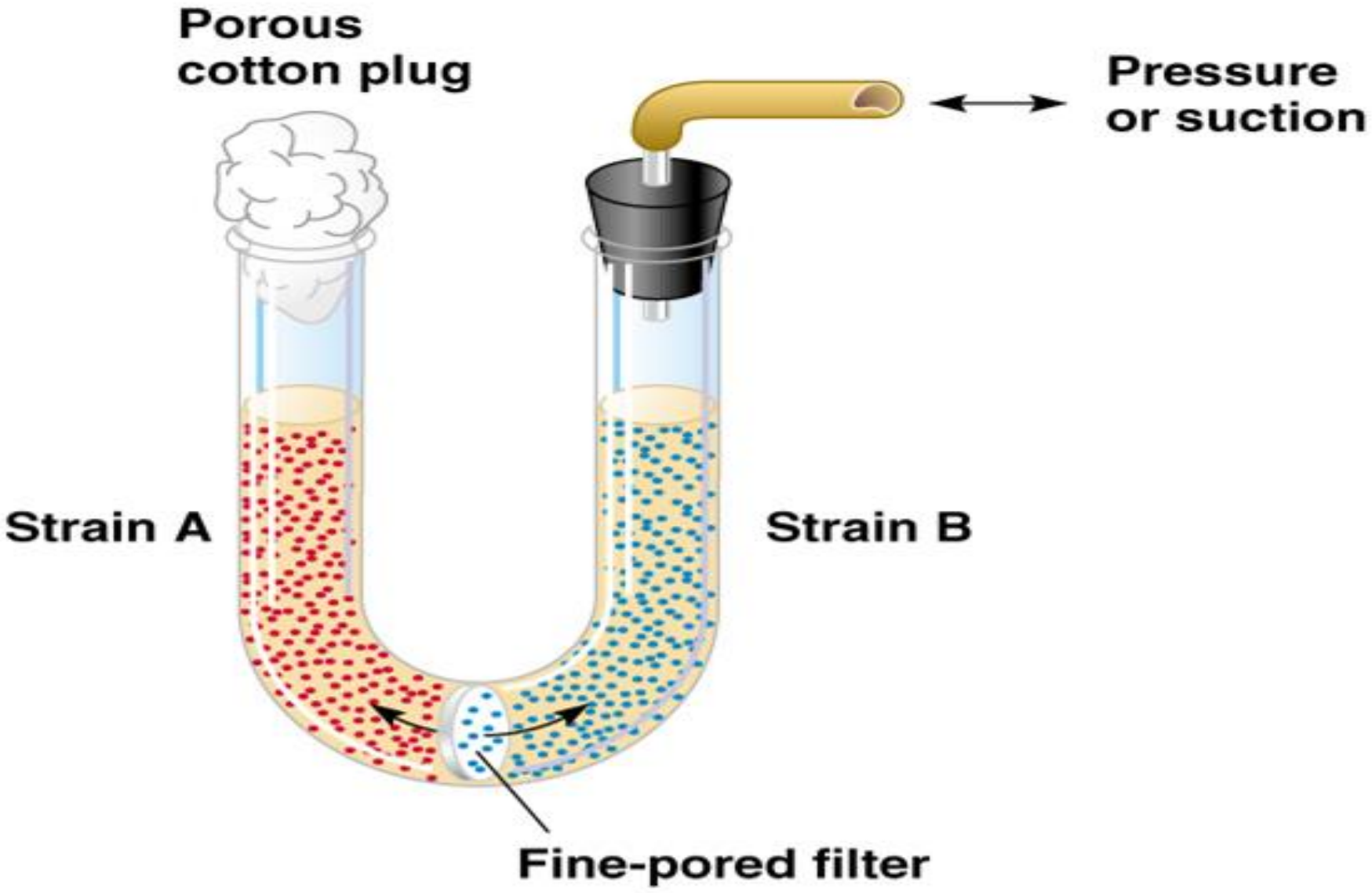
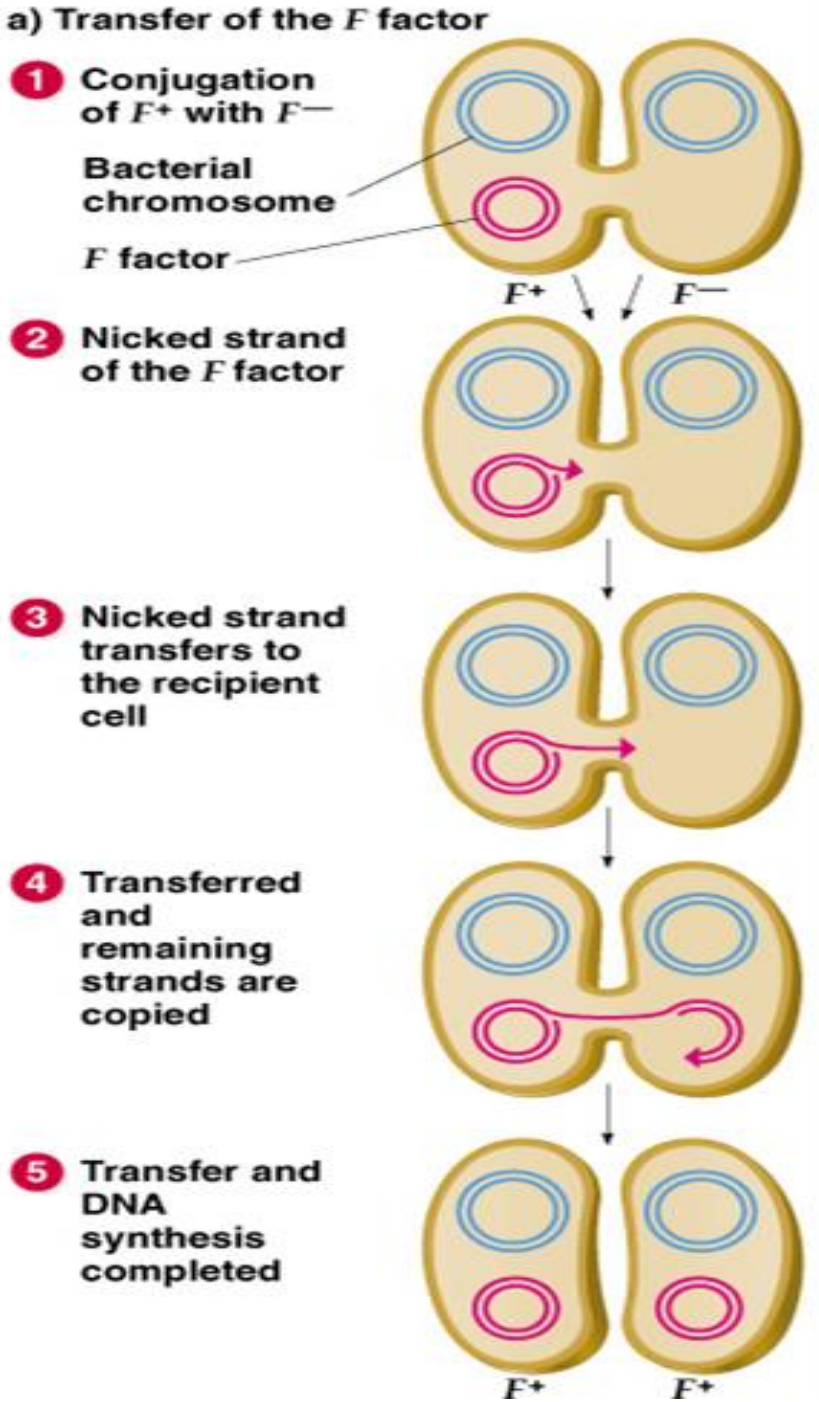


Fig. 15.3, Bernard Davis experiment demonstrated that physical contact is required for bacterial recombination.



Conjugation-transfer of the sex factor *F*:

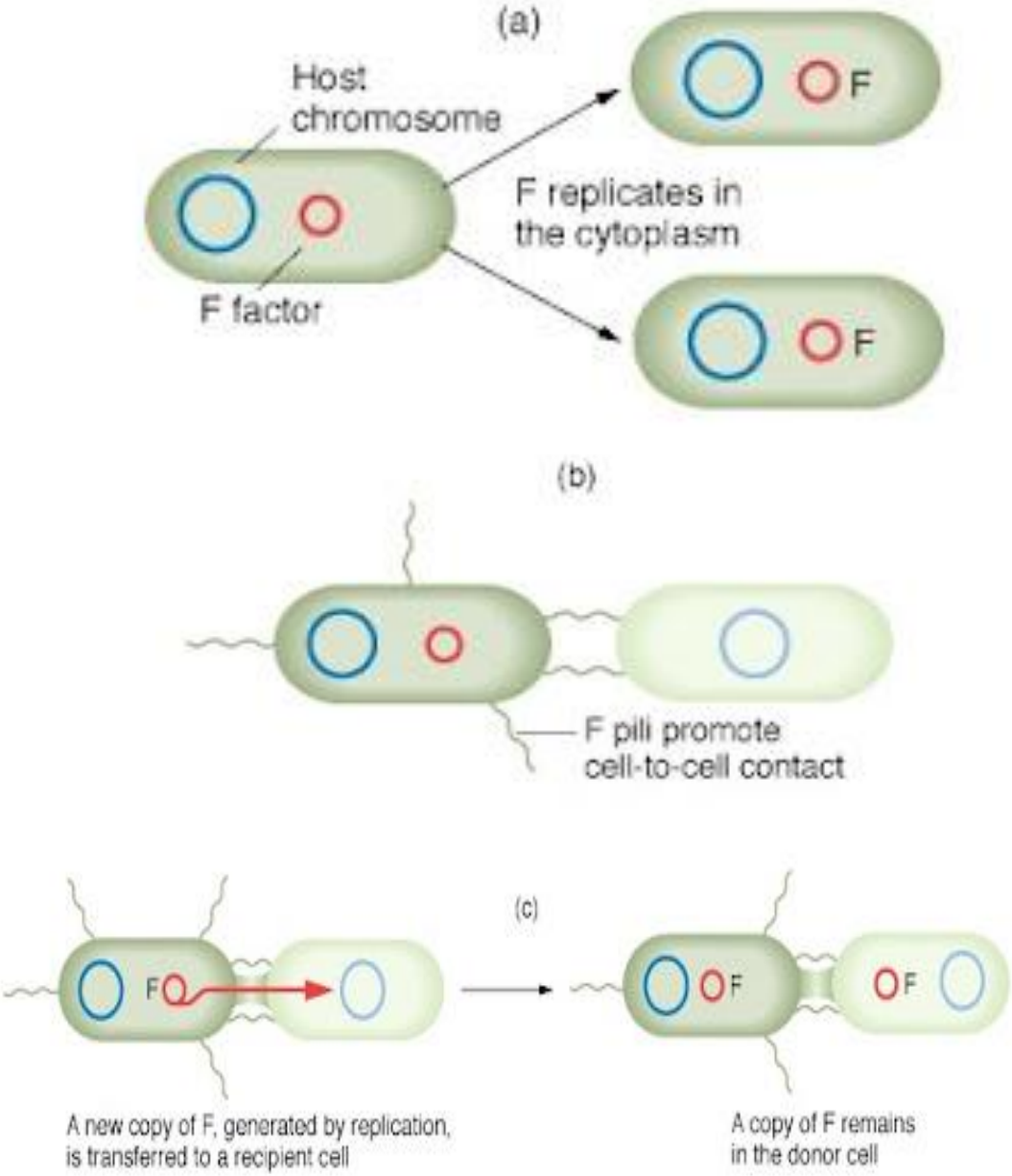
- **William Hayes (1953) demonstrated that genetic exchange in *E. coli* occurs in only one direction, mediated by sex factor *F*.**
- ***F* is a self-replicating, circular DNA plasmid (1/40 the size of the main chromosome).**
- ***F* plasmid contains an origin sequence (*O*), which initiates DNA transfer. It also contains genes for hair-like cell surface (*F*-pili or sex-pili), which aid in contact between cells.**
- **No conjugation can occur between cells of the same mating type.**
- **`**
- **Conjugation begins when the *F* plasmid is nicked at the origin, and a single strand is transferred using the rolling circle mechanism.**
- **When transfer is complete, both cells are F^+ double-stranded.**



F plasmid OR fertility factor

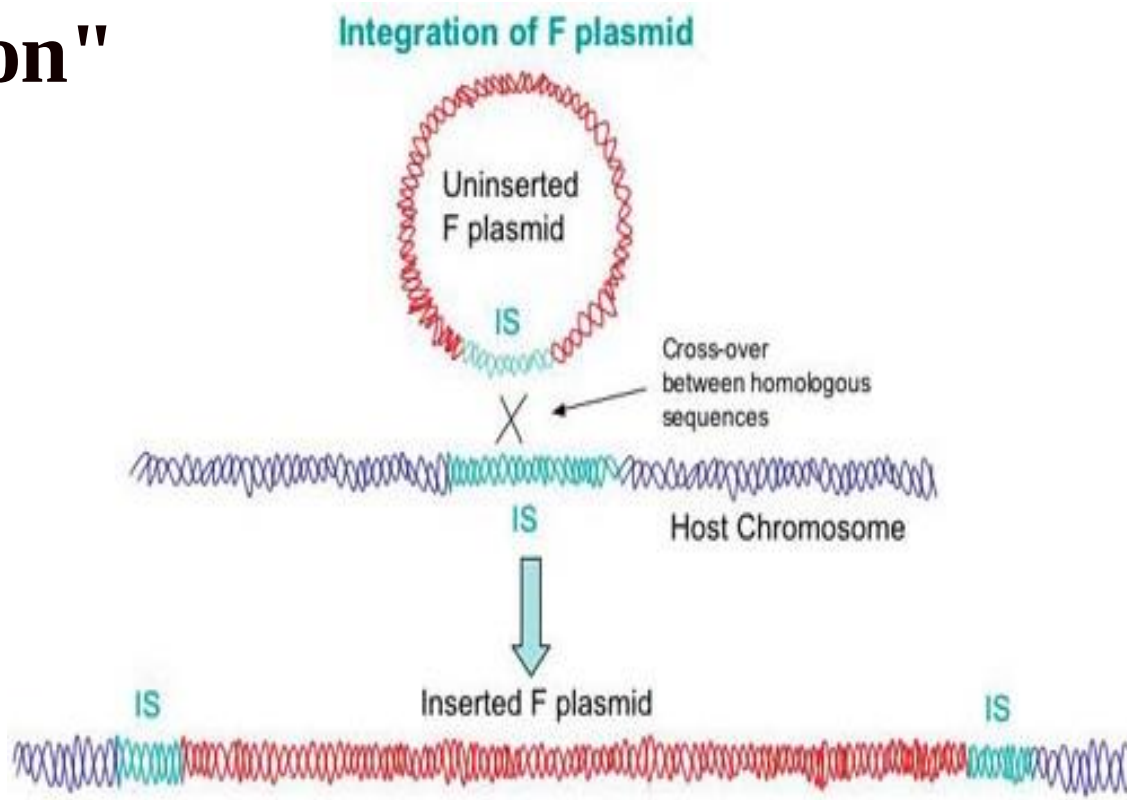
F is a small ,circular DNA element, that act like a minichromosome.
F contain approx 100 genes

1. It can replicates by its own therefore allows to be maintained in a cellular population.
2. Cells carrying F produce pili (singular, pilus), a minute proteinaceous tubule that allow the F+ cells to attach to other cells.
3. F+ cells can transfer the the newly synthesized copy of the circular F plasmid to recipient (F-)cell that lack such plasmid. After successful transfer, recipient cell now become F+ cell and donor also have its own copy.
4. Occasionally, F leaves the cytoplasm and integrates itself into the host bacterial chromosome. F+ bacteria with an *incorporated* F-factor are called **Hfr--"high frequency of recombination"--** strains.

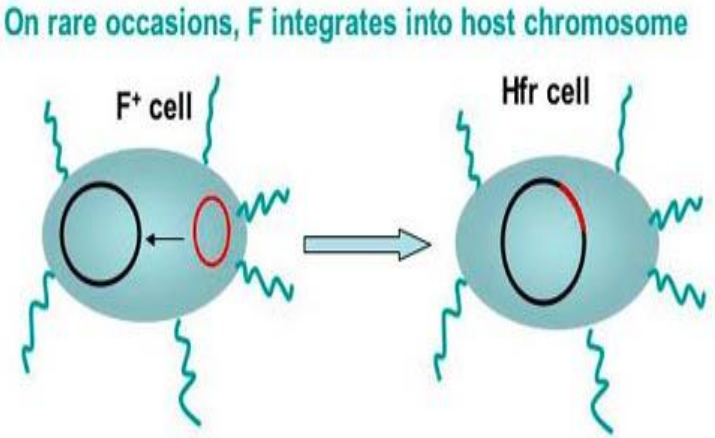


Hfr--"high frequency of recombination"

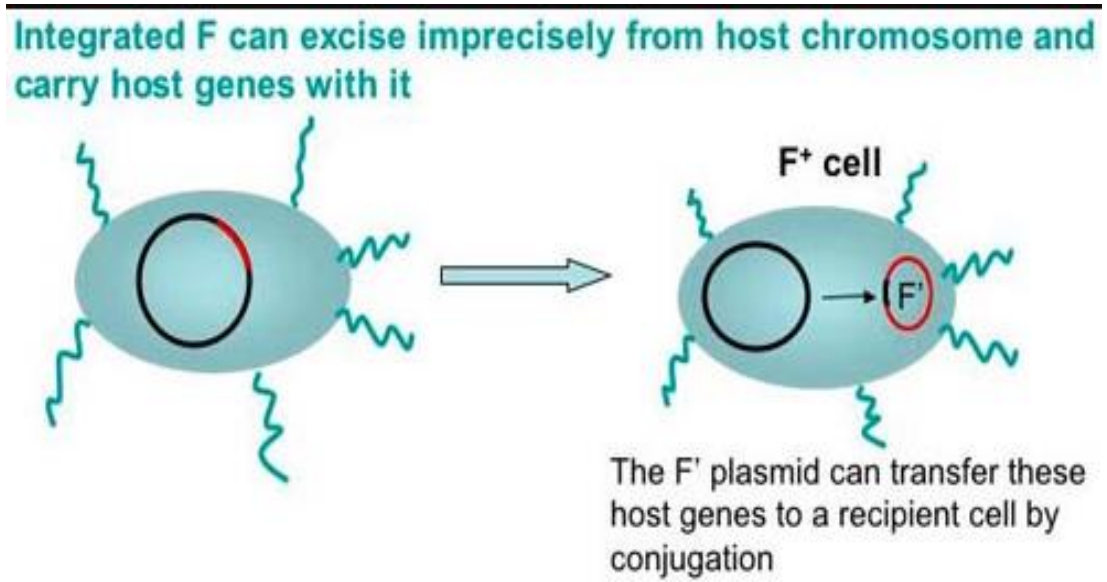
- If the F plasmid carried by a bacterium happens to contain an IS element (**Bacterial insertion sequence**, is required for integration), it can promote a crossover into the bacterium's main chromosome, inserting some or all of the F plasmid into the recipient's genome.
- These promote *incorporation* of DNA fragments into the host's genome.
- This incorporation is rare, and happens in only a few members of a given conjugating population of bacteria.

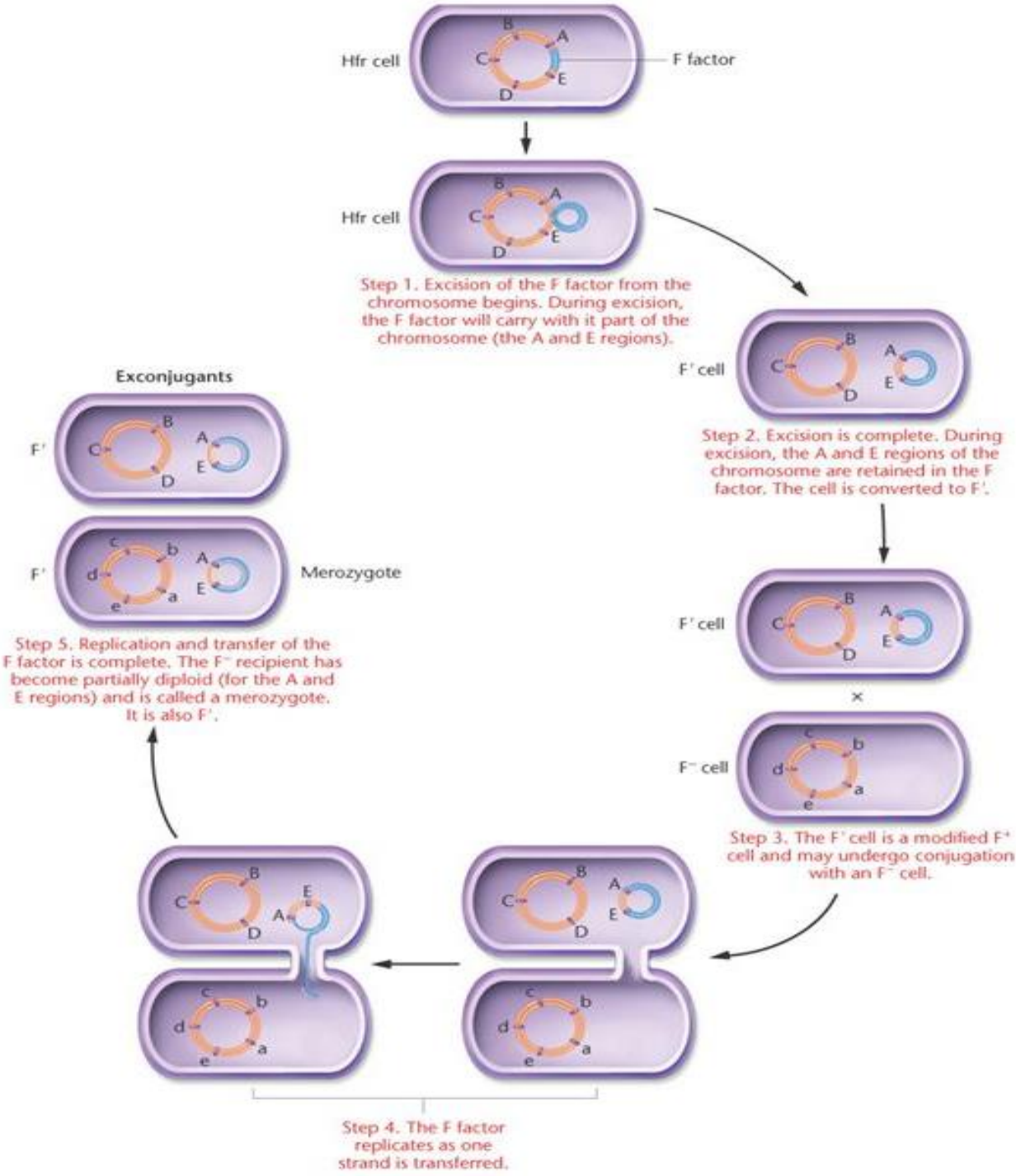


- F+ bacteria with an *incorporated* F-factor are called **Hfr--"high frequency of recombination"**—strains (can produce 1000 times more recombinant than a normal F+).
- Hfr individuals can be isolated from an F+ strain and allowed to multiply in culture until the investigator has a pure strain of Hfr bacteria. Because Hfr promote crossing over when they conjugate with F- strains, they are most useful for bacterial gene mapping.



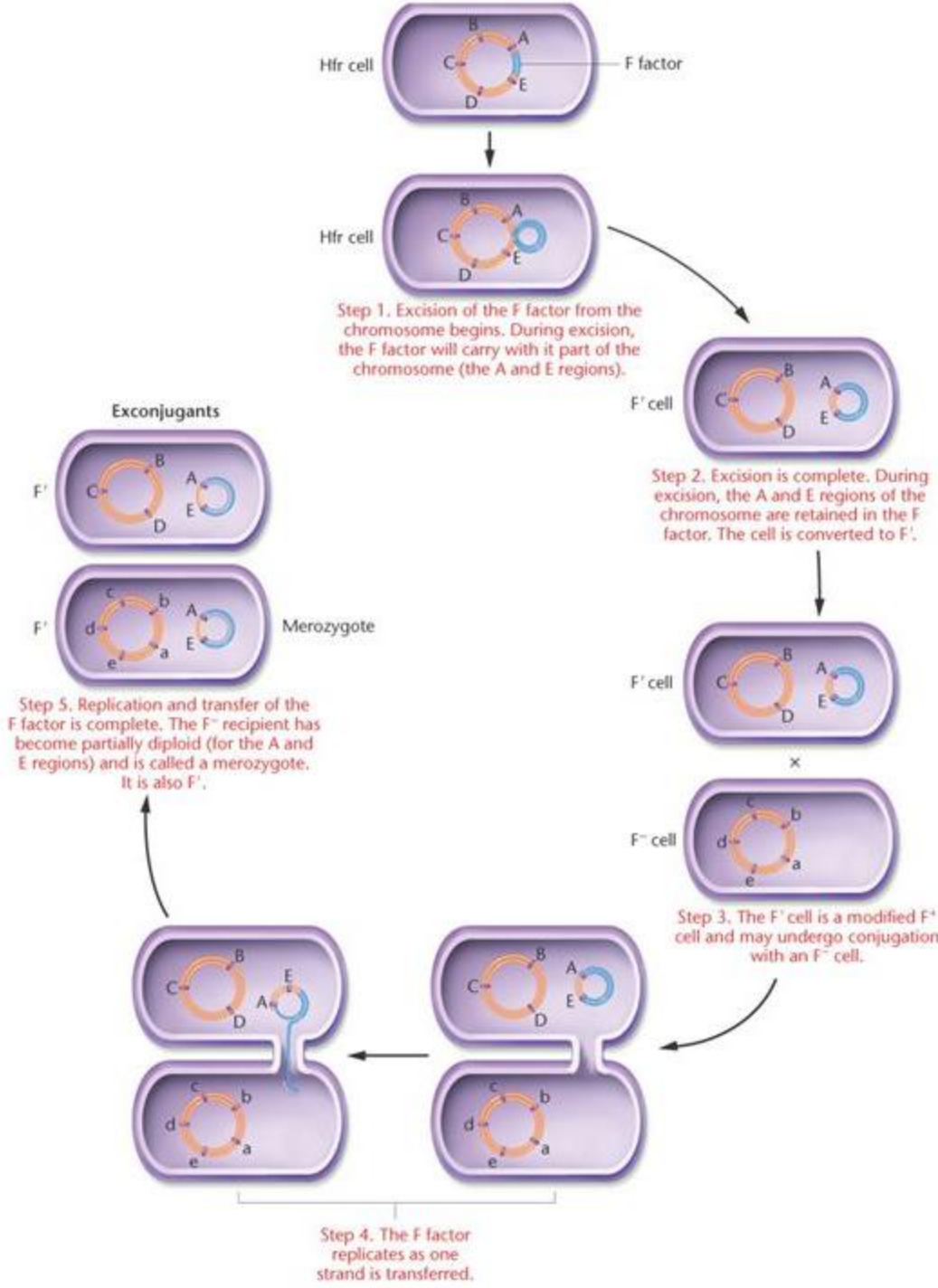
- Sometimes an integrated F factor (in an Hfr bacterium) pops back out of the host DNA, and--rarely--may also carry a few of the host's genes with it.
- The modified F factor (carrying host genes) is called an F' plasmid. (If the F' is known to be carrying a specific gene, it may be named for that gene, as in: F'-lac, F'-str, etc.)
- The F' is still an F factor: it still promotes the bacterium carrying it to seek out F- for sex. But now it can infect the F- recipient cells with genes from the original donor bacterial cell.
- The process of infecting F- cells with a plasmid via Hfr bacterial conjugation is known as **sexduction.**



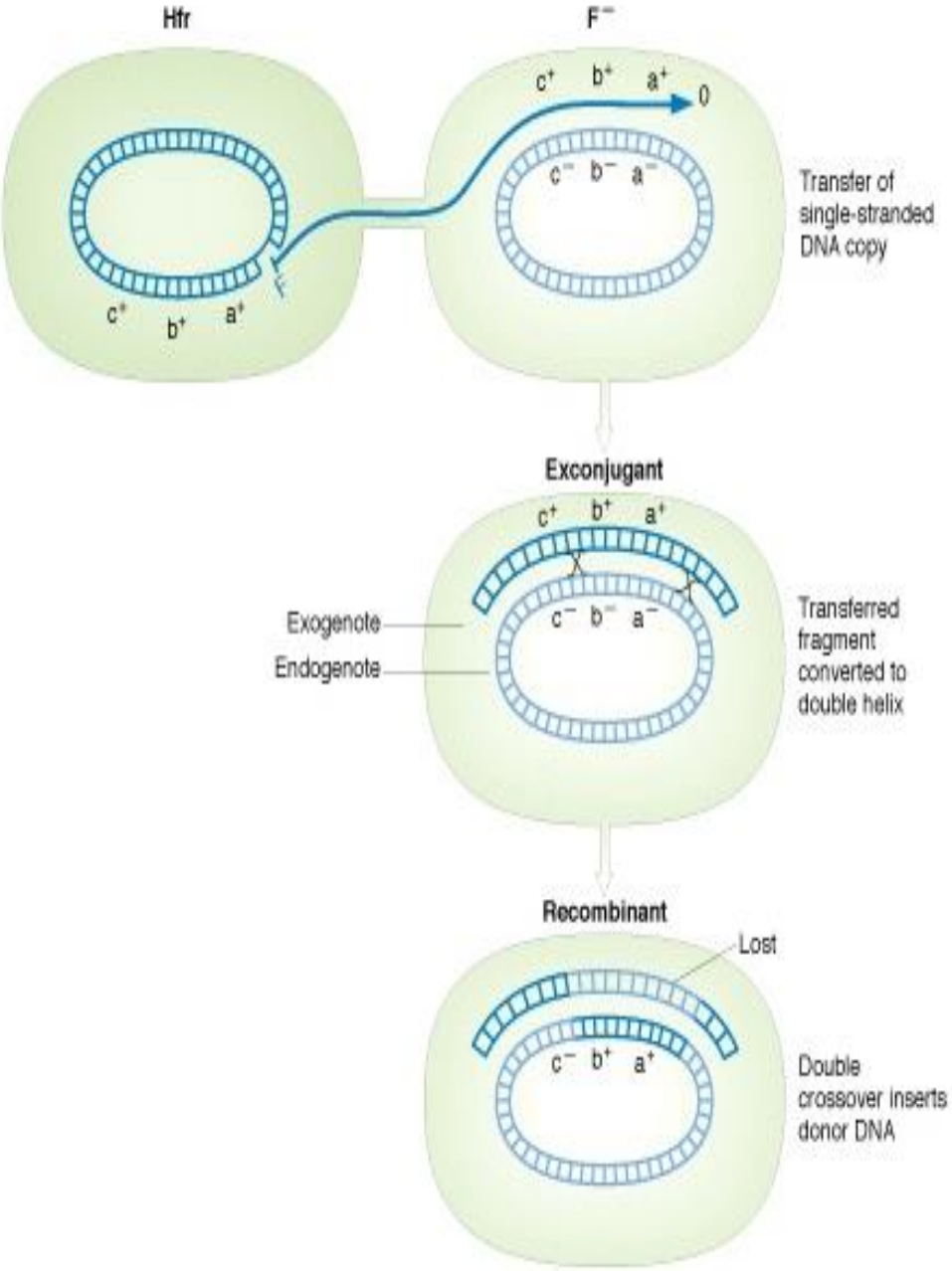


Recombinant Frequency Mapping

- Usually wild type bacteria are **haploid**, they will express whatever allele they carry, whether it is dominant or recessive.
- The F factor can both insert *and* detach from a host bacterial chromosome, and in some cases, a detached F factors will tear off and carry a bit of bacterial genome along with it (F' plasmid).
- If this F' factor later re-inserts into a new bacterium during sexduction, the new host can be **artificially diploid** for whatever donor loci are carried along with the F factor. Such a bacterium is known as a **Merozygote.**
- Bacteria that are diploid for a few loci can be produced, and these are useful for studying the properties of those particular genes.
- Recombination frequency provides an estimate or approximation of physical distance between genes.
- Pair of genes with a larger recombination frequency are likely farther apart, while a pair with a smaller recombination frequency are likely closer together.

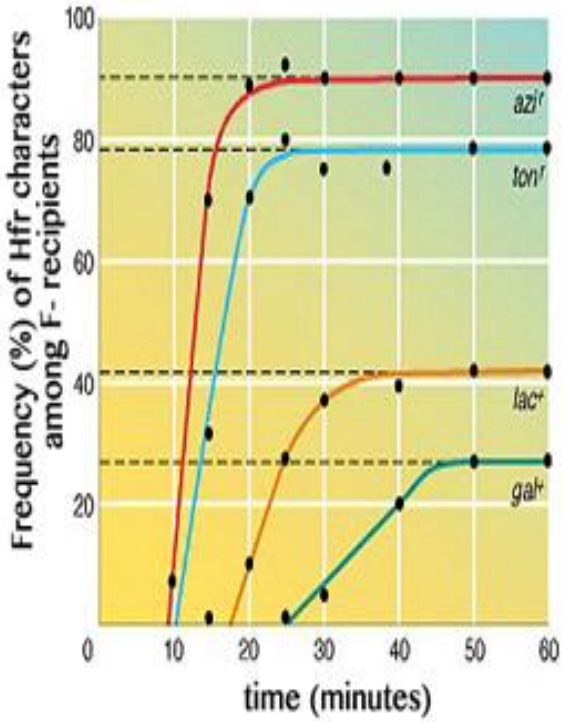


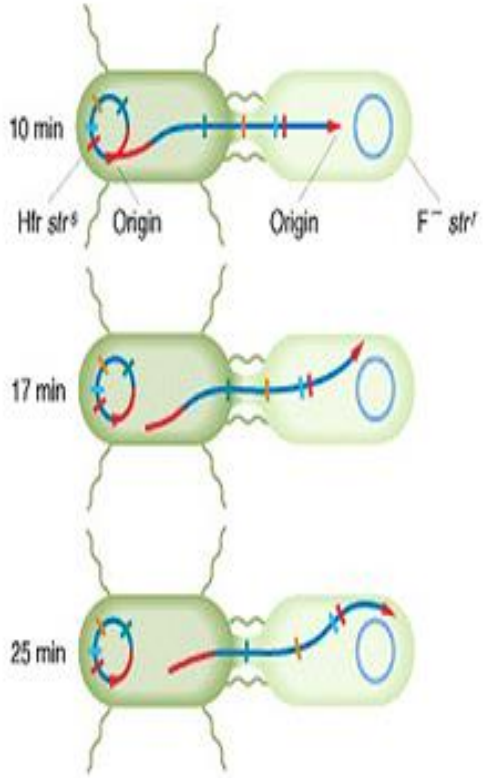
- Once an F' factor (along with its IS elements) is incorporated into a new host cell, the possibility for mapping exists, since there will be crossing over between the F' factor (carrying genes) and the recipient's chromosome.
- Only Hfr x F- crosses will yield crossing over. That's the whole point of isolating the Hfr strains: to map the bacterial genome.
- Once contact is made via pilus, the Hfr bacterium spews out a single strand of DNA which travels through the pilus to the recipient F- cell.
- The specific point of transfer of the Hfr gene occurs at a site on the integrated F called the **origin (O)**.
- Because the DNA is transferred from cell to cell from O forward, the genes closest to the O are transferred first. The farther away from the O site, the longer it takes for the gene to get into the new cell.
- A DOUBLE CROSSOVER BETWEEN THE LINEAR F FACTOR AND THE CIRCULAR RECIPIENT CHROMOSOME WILL ALLOW SOME OF THE F FACTOR GENES TO REMAIN IN THE RECIPIENT CELL AND BE EXPRESSED AS PART OF THE GENOME.

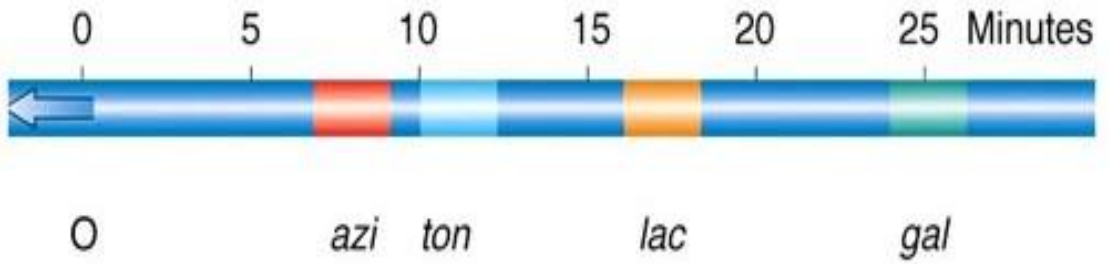


Mapping by Interrupting Conjugation

- If the investigator allows the Hfr to start conjugating with a strain of F- bacteria, but interrupts them at specific times during their mating, s/he is essentially allowing differing lengths of the F plasmid to be transferred into the host cells.
- (Remember, it starts at O and proceeds in a linear fashion into the recipient).
- For example: Allow conjugation for 5 minutes, then take a sample of the breeding culture and kill all the Hfr's.
- Plate out the remaining F- bacteria and determine which phenotypes show up.
- Doing this for longer and longer time intervals reveals the order of the genes relative to the O site.
- For example, if the investigator knows the bacteria have various mutant alleles that code for things like...
- inability to digest lactose (*lac*)
- resistance to sodium azide (*azi*)
- sensitivity to T1 bacteriophage (*ton*)
- resistance to streptomycin (*str*)
- inability to digest galactose (*gal*)





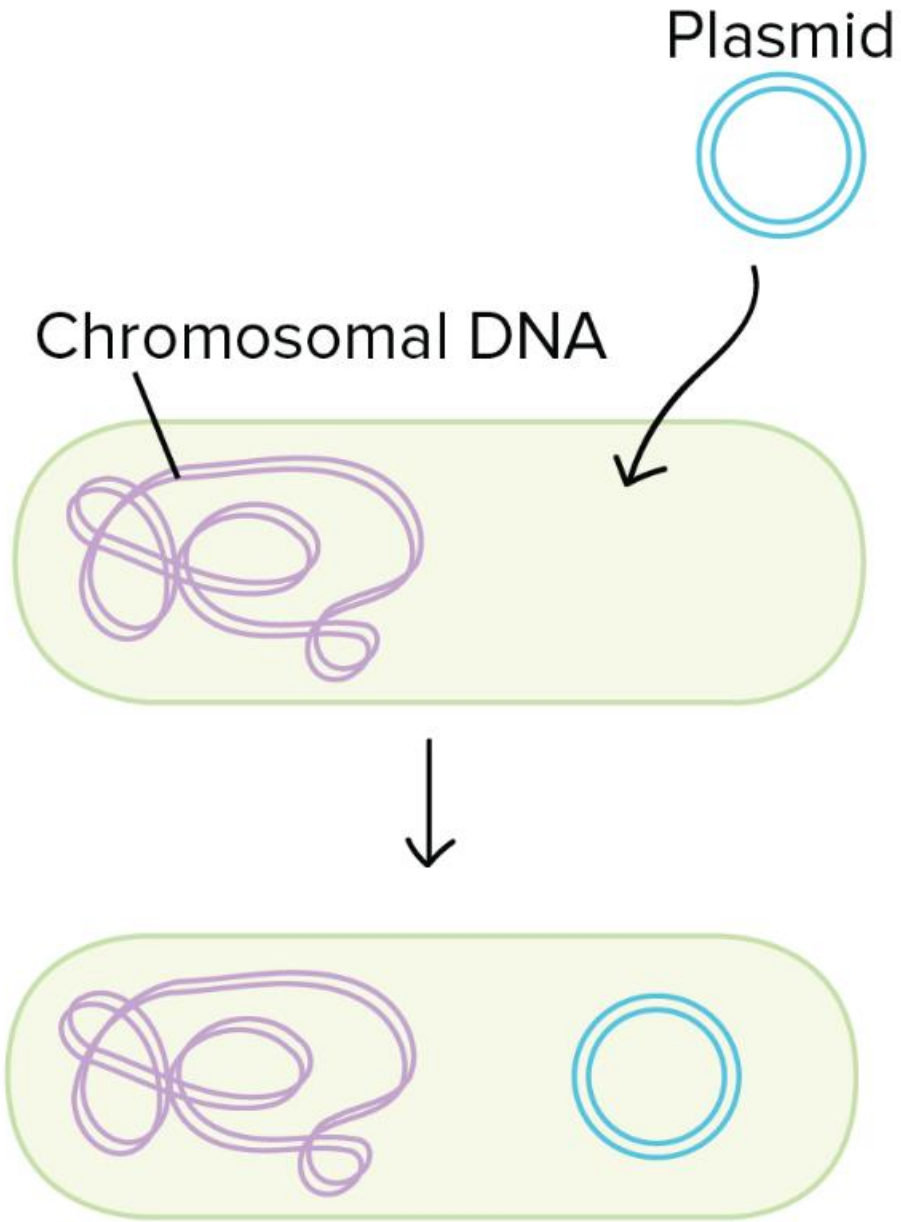


Genetic map-results of interrupted *E. coli* mating experiment.

Transformation

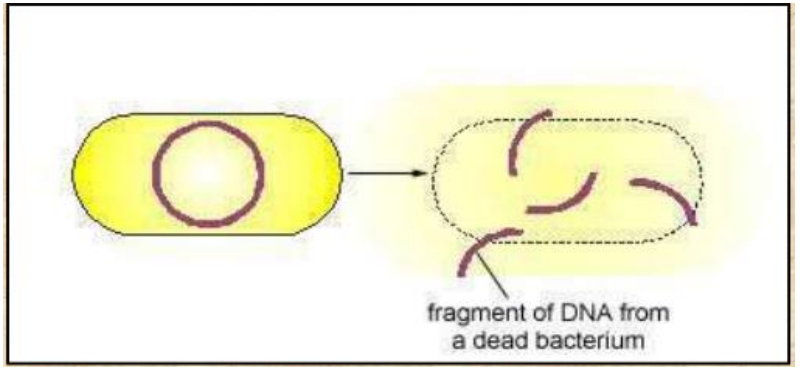
- In **transformation**, a bacterium uptake DNA of relatively small fragments from its environment

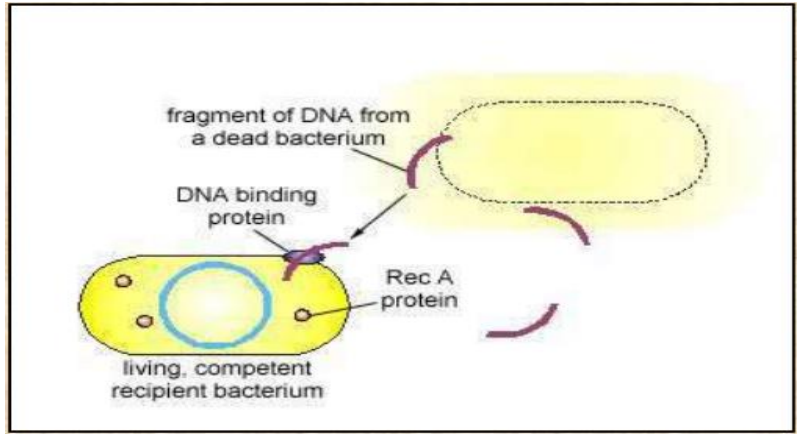
- A harmless bacterium takes up DNA for a toxin gene from a pathogenic (disease-causing) species of bacterium. If the receiving cell incorporates the new DNA into its own chromosome (which can happen by a process called homologous recombination), it too may become pathogenic.

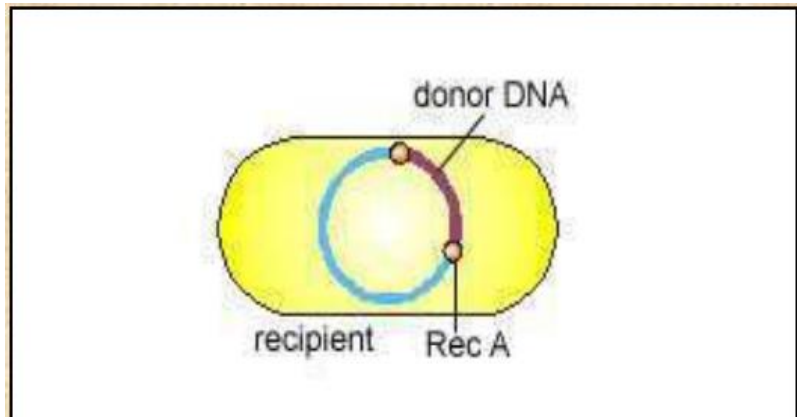


Process of Transformation

1. dsDNA from died or degraded bacterium relesed into Enivirоment
2. Nucleases cut the release DNA into fragments (around 20 genes long)
3. These fragments bind to DNA binding proteins present on the surface of a competent recipient bacterium, and translocated in the cytoplasm of recipient bacterium
4. DNA fragment from the donor is then exchanged from the recepient's DNA by means of Rec A proteins.



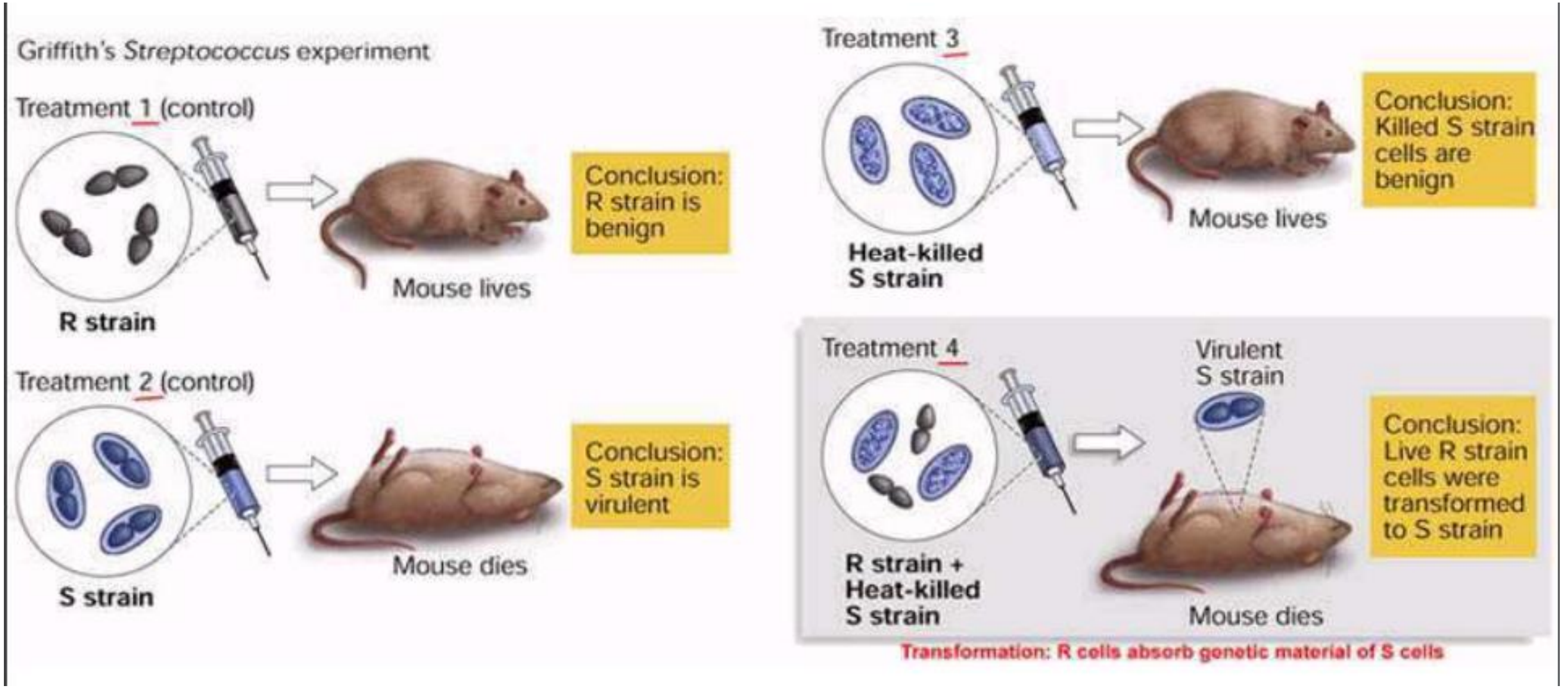




Factors affecting Transformation

- **DNA size and state**
  Sensitive to nucleases (at least 5 X 105 daltons)
- **Competence of the recipient** (Bacillus, Haemophilus, Neisseria, Streptococcus)
- The ability to take up DNA from the environment is known as competence
  only DNA from closely related bacteria (competent cells) would be successfully transformed
- Competence factor (a specific protein produced at a particular time in the growth cycle of competent bacteria and enable it to take up DNA naturally)
- Induced competence, e.g. by CaCl2

Griffth's Experiment for Transformation



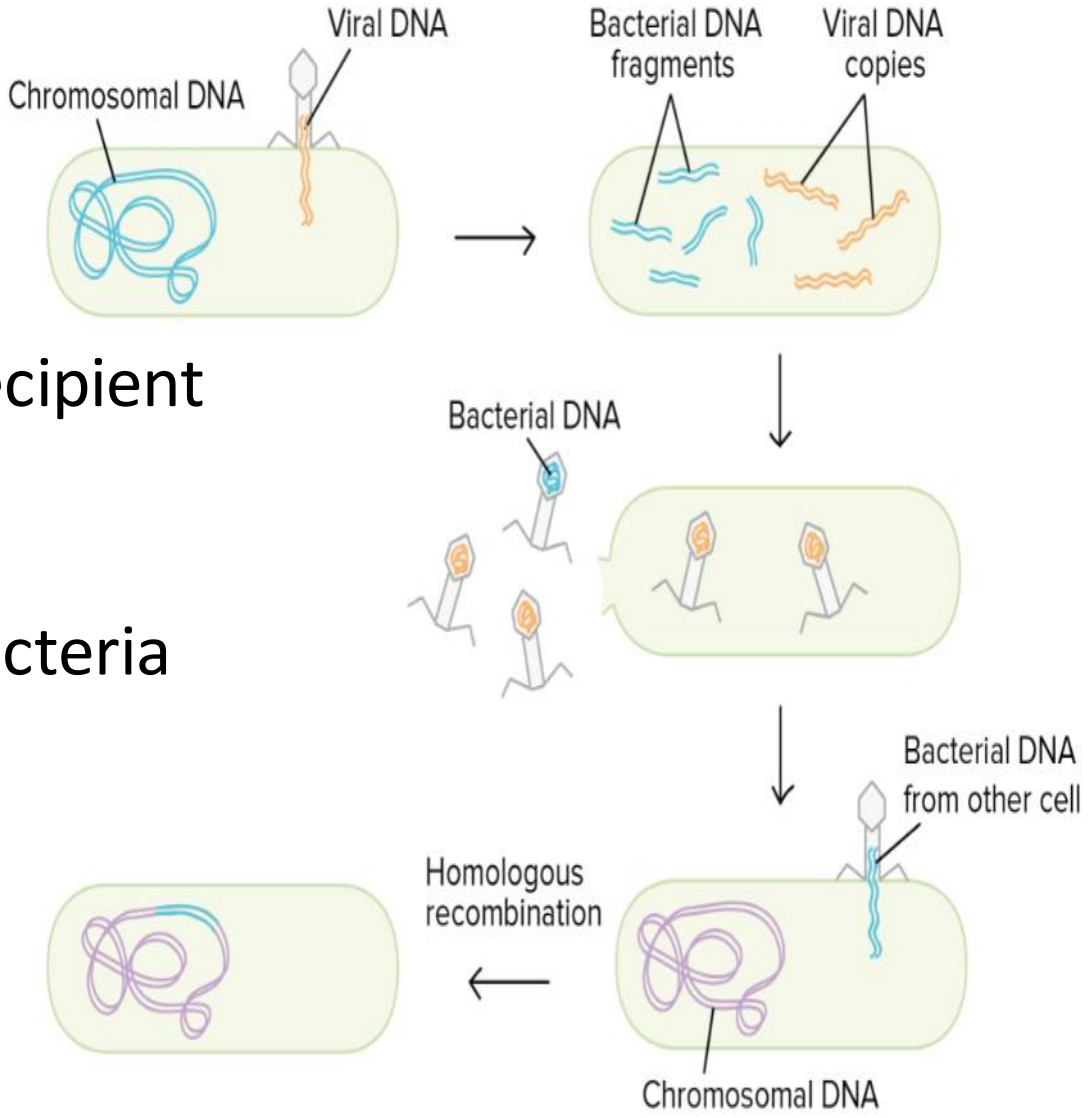
Transduction

Definition: Gene transfer from a donor to a recipient bacteria through a bacteriophage

Bacteriophage (phage): A virus that infects bacteria

Types of transduction

- Generalized
- Specialized.



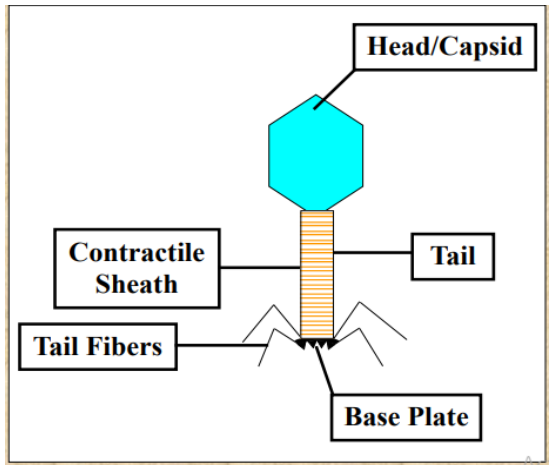
Phage composition & Structure

Composition

- Nucleic acid (DNA/RNA): Modified bases (protect from host nucleases
- Potein= Protection & Infection

Structure (T4)

- Size (80 X 100 nm)
- Head or capsid
- Tail (contractile sheath, base plate, tail fibres)



Types of Bacteriophage

Virulent phage: a phage that multiply within the host cell, lyse the cell and release progeny phage (e.g. T4) – lytic cycle

Temperate phage: a phage that can either multiply via the lytic cycle or enter a quiescent integrated state in the bacterial cell.

lysogenic cycle

– Expression of most phage genes repressed

– Prophage: Phage DNA in the quiescent integrated

state

– Lysogen – Bacteria harboring a prophage

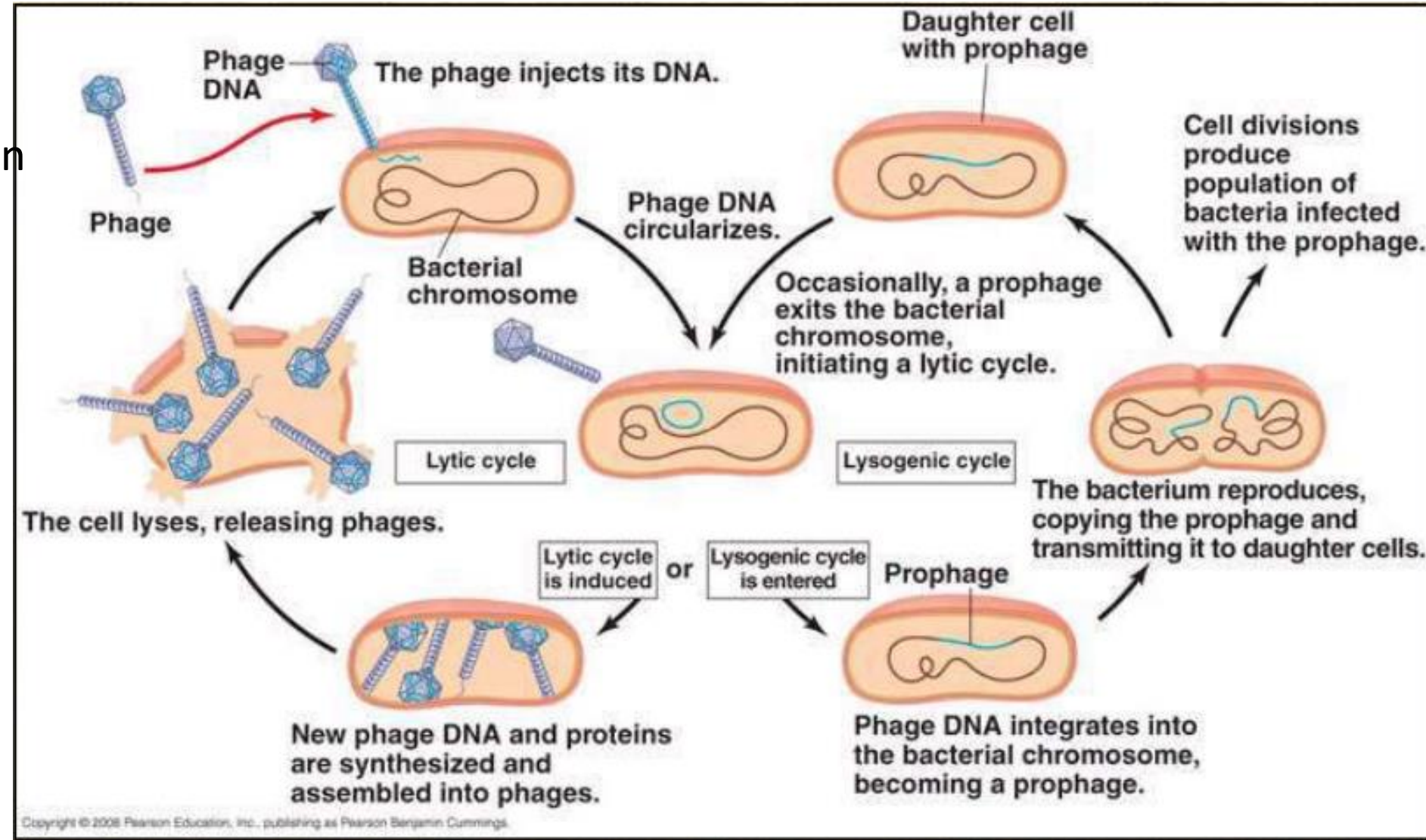
Infection of Host Cells by Phages

Adsorption

- Tail fibers
- Receptor is LPS for T4

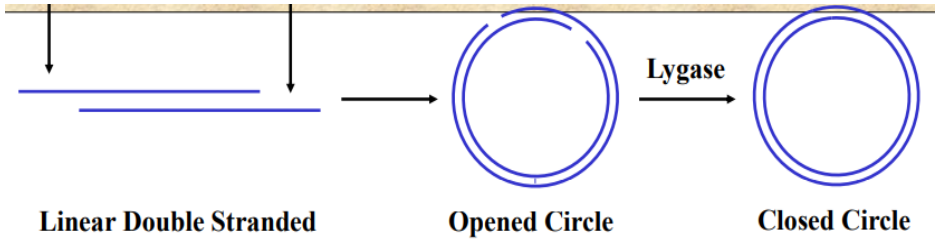
Irreversible attachment

- Base plate
- Nucleic acid injection
- Sheath Contraction
- DNA uptake



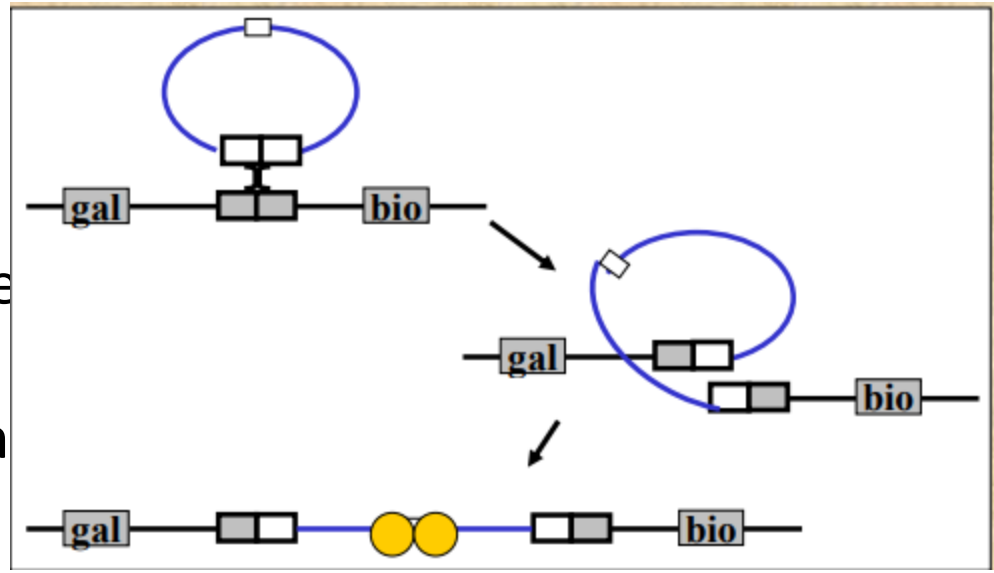
Events leading to Lysogeny

1. **Circularization of the phage DNA**
– Cohesive ends: double stranded linear molecule with small single stranded regions at the 5' ends.
– Cohesive ends promotes circularization of phage DNA
– e.g. lambda phage



2. Site-specific recombination – recombination occurs between a particular site on the circularized phage DNA and a particular site on the host chromosome DNA – phage coded enzyme helps .

3. Repression of the phage genome – phage coded repressor protein – binds to a particular site on the phage DNA, called the operator, and shuts off transcription of most phage genes EXCEPT the repressor gene resulting in a stable repressed phage genome



Termination of Lysogeny

Induction

- Adverse conditions

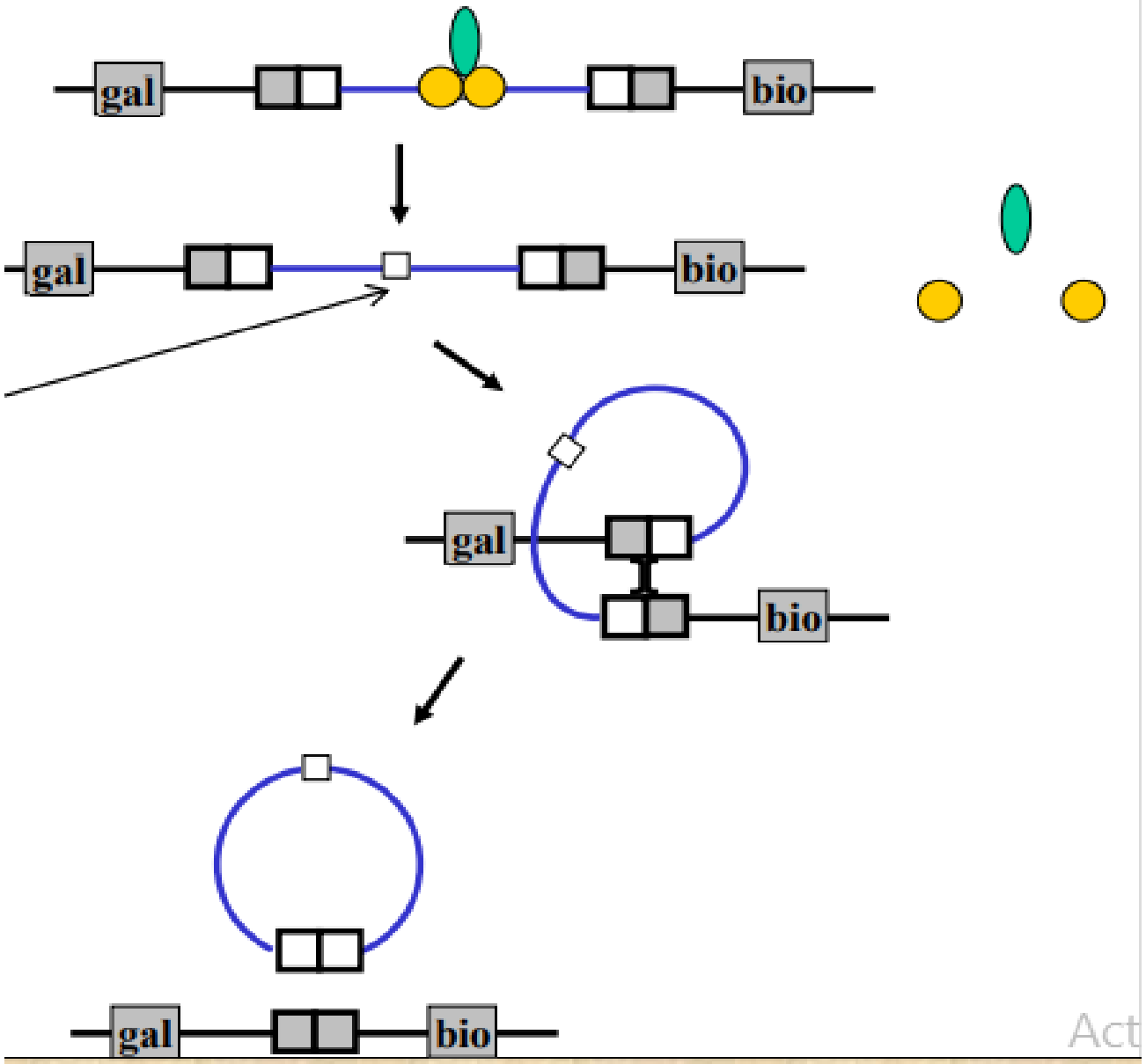
Role of proteases

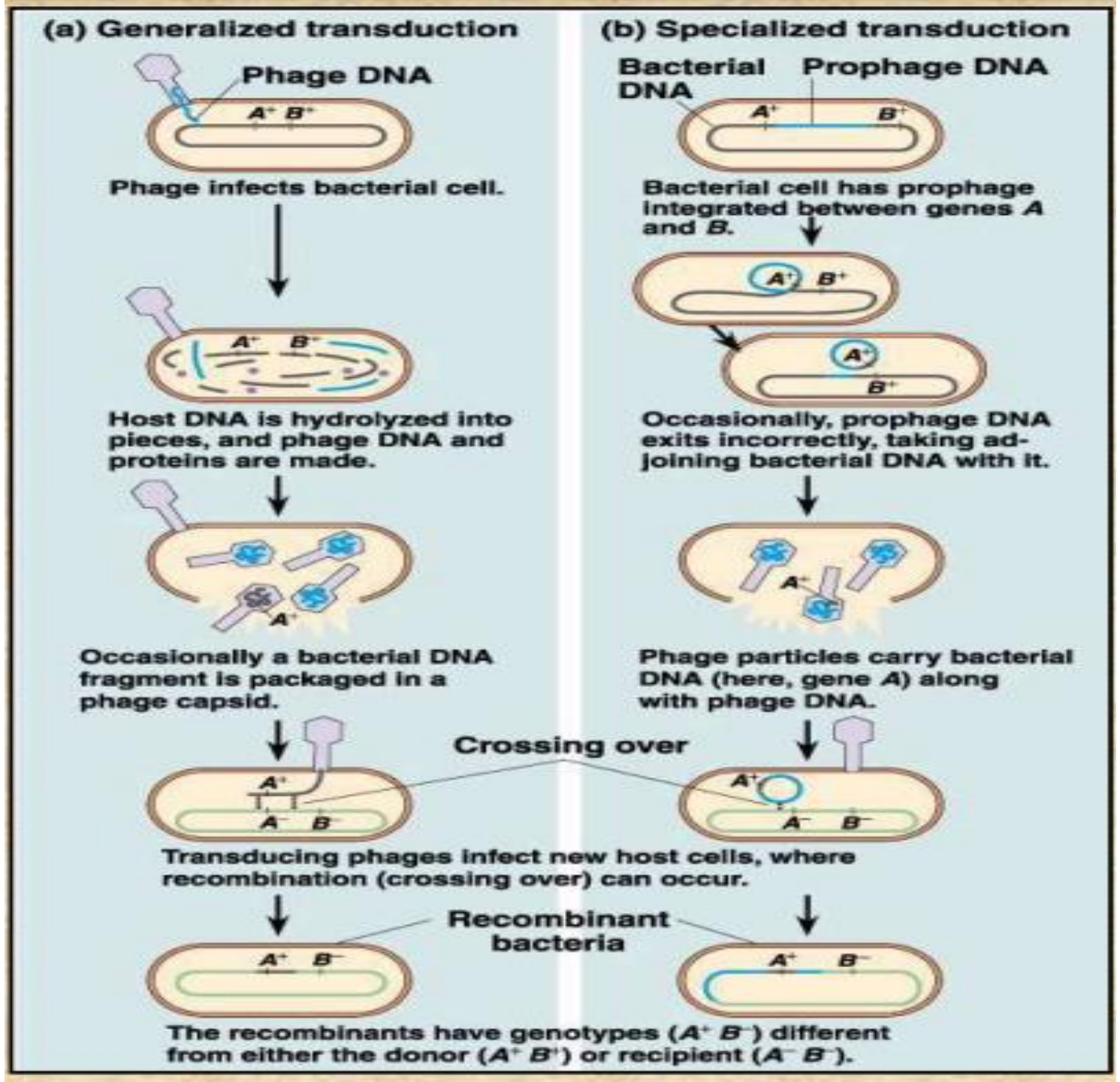
- recA protein
- destruction of phage repressor

Phage Gene Expression

Excision of phage

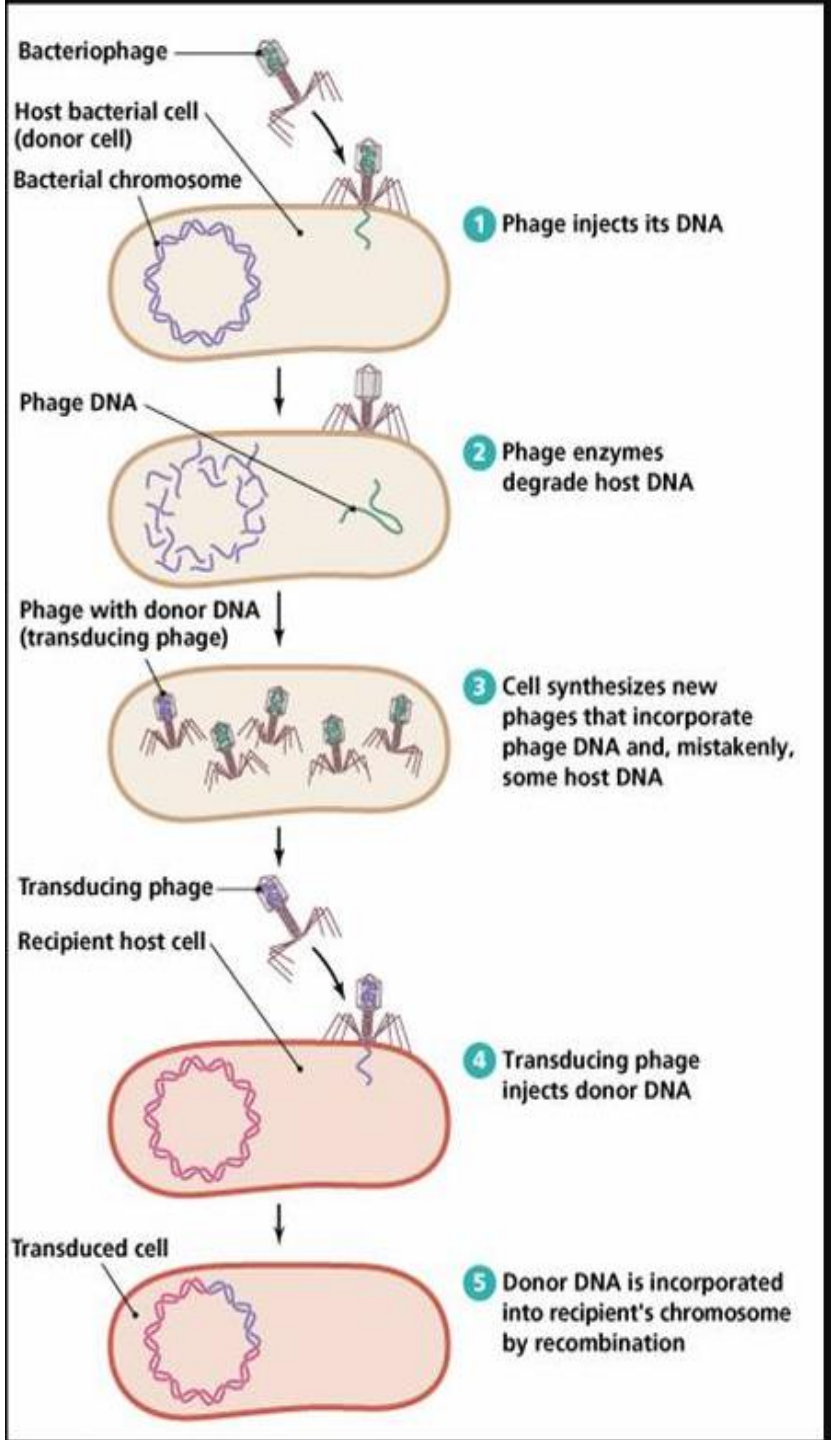
Lytic growth




(a) Generalized transduction
Phage DNA
A+ B+
Phage infects bacterial cell.
A+ B+
Host DNA is hydrolyzed into pieces, and phage DNA and proteins are made.
A+
Occasionally a bacterial DNA fragment is packaged in a phage capsid.
(b) Specialized transduction
Bacterial DNA
Prophage DNA
A+
B+
Bacterial cell has prophage integrated between genes A and B.
A+ B+
A+
B+
Occasionally, prophage DNA exits incorrectly, taking adjoining bacterial DNA with it.
A+
Phage particles carry bacterial DNA (here, gene A) along with phage DNA.
Crossing over
A+
A− B
A+
A− B
Transducing phages infect new host cells, where recombination (crossing over) can occur.
Recombinant bacteria
A+ B−
A+ B−
The recombinants have genotypes (A+ B−) different from either the donor (A+ B+) or recipient (A− B−).

Generalized Transduction

- Generalized transduction can transfer any gene of donor bacteria to recipient bacteria
- During the replication of a lytic phage the capsid sometimes enclose a small fragment of lysed bacterial DNA, instead of phage DNA, by a "head-full" mechanism. **This is a defective phage:** Such a phage cannot lyse another bacterium because the DNA in the phage head does not have the genetic information to produce phage genome and proteins.
- On infection of another bacterium defective phage injects the fragment of donor bacterial DNA into the recipient bacteria, where it can be exchanged for a piece of the recipient's DNA, if their sequences are homologous.



Specialized transduction

- A transduction in which only certain donor genes can be transferred to the recipient
- Occur during the lysogenic life cycle of a temperate phage. During spontaneous induction of lysogeny, a small piece of bacterial, DNA may sometimes be exchanged for a piece of phage genome.
- This piece of bacterial DNA replicates as a part of the phage genome and is incorporated into capsid of each phage progeny
- On infection of a recipient bacteria, the phage DNA containing donor bacerium genes are injected into the recipient bacterium where donor, DNA fragment can be exchanged for a piece of the recipient's DNA, if their sequences are homologous
- Different phages may transfer different genes but an individual phage can only transfer certain genes
- Lysogenic (phage) conversion occurs in nature and is the source of virulent strains of bacteria.