Unit 4 – Bacterial Recombinations

Variation is introduced to the genome by mutations of genes between organisms. In Eukaryotes, which are diploid organisms, gene recombination is brought about during fertilization & meiosis. During fertilization haploid male and female gametes fuse to form diploid zygote & two sets of genes are brought together. During meiosis there is random assortment of gene pairs to form haploid gametes.

Bacteria are haploid organism & were formerly thought to multiply only by the binary fission. However, different mechanisms were later discovered for transferring gene from one bacterial cell to another. These mechanisms are----

1) Transformation (uptake of free or naked DNA)
2) Conjugation (sexual transfer of DNA by making cells in contact with one another)
3) Transduction (viral mediated transfer of DNA).

"Changes in the sequence or grouping of genetic nucleotides are called as recombination".

The term recombination is usually applied to permanent changes & excludes folding & unfolding of DNA. The site of recombination is located between the two adjacent nucleotides.

1. BACTERIAL TRANSFORMATION

Transformation is the process of genetic modification in which fragments of DNA from one cell enter and are integrated in another cell DNA.

Bacterial transformation was discovered by **GRIFFITH (**1928), who used two strains of *Diplococcus pneumoniae*. This bacterium causes pneumonia in man. It is pathogenic for mice and may prove fatal. The wild type strains (S) have cells surrounded by a polysaccharide capsule and form a colony with a smooth surface. The strain is virulent (pathogenic) & if injected into the mice, causes severe septicemia & death. A mutant strain (R) does have the capsule,

and forms a colony with a rough surface. It is avirulent (non-pathogenic). Heat-killed smooth cells are rendered avirulent. Injection of avirulent mutant R bacteria or heat-killed virulent S bacteria into mice did not any harm. Injection of a mixture of the bacterial types, however, killed some mice. From these mice the live virulent S bacteria could be recovered. A small bacteria of the R type acquired ability to produce capsules & become virulent. This change induced in the R bacteria was called transformation. GRIFFITH thought that the transforming agent was protein.

In 1944 Avery, Macleod & MacCatry showed that the substance responsible for transformation was DNA. They found that addition of DNA from capsule-producing S cells to a culture of noncapsulated R cells transformed the latter & enabled them to produce capsules.

DNAse removes this transforming ability; an enzyme, which breaks down DNA. DNA was the most important factor in the transformation of genetic information.

Competence: -

The recipient bacteria must be competent if they have to accept DNA and undergo transformation. The competence of bacteria is not a permanent feature but occurs only at certain times in the life cycle. The duration and the time of occurrence of the competence stages vary with bacterial species & condition of growth. Competence is commonly observed at the end of logarithmic phase of growth.

A) Double stranded DNA fragments added to competent cell.
B) Donor (transforming) DNA binds to recipient cell.
C) Localized denaturation of recipient DNA. Penetration of a single strand of transforming DNA into recipient cell.
D) Synapsis of transforming DNA with recipient cell DNA.

E) Integration of transforming DNA with recipient cell DNA. Formation of transformation heteroduplex.

F) Gaps are closed of transforming DNA after covalently integrated into the recipient cell genome.

G) Homoduplex means: Normal DNA.

H) Heteroduplex means: Transformed DNA.

The competence of cell is governed by chromosomal genes & is signaled by certain environmental condition. Many other bacteria do not become competent under ordinary condition but they can be made competent by artificial treatment such as exposure of cells to high concentration of divalent cations [$CaCl_2$] & cold & hot condition.

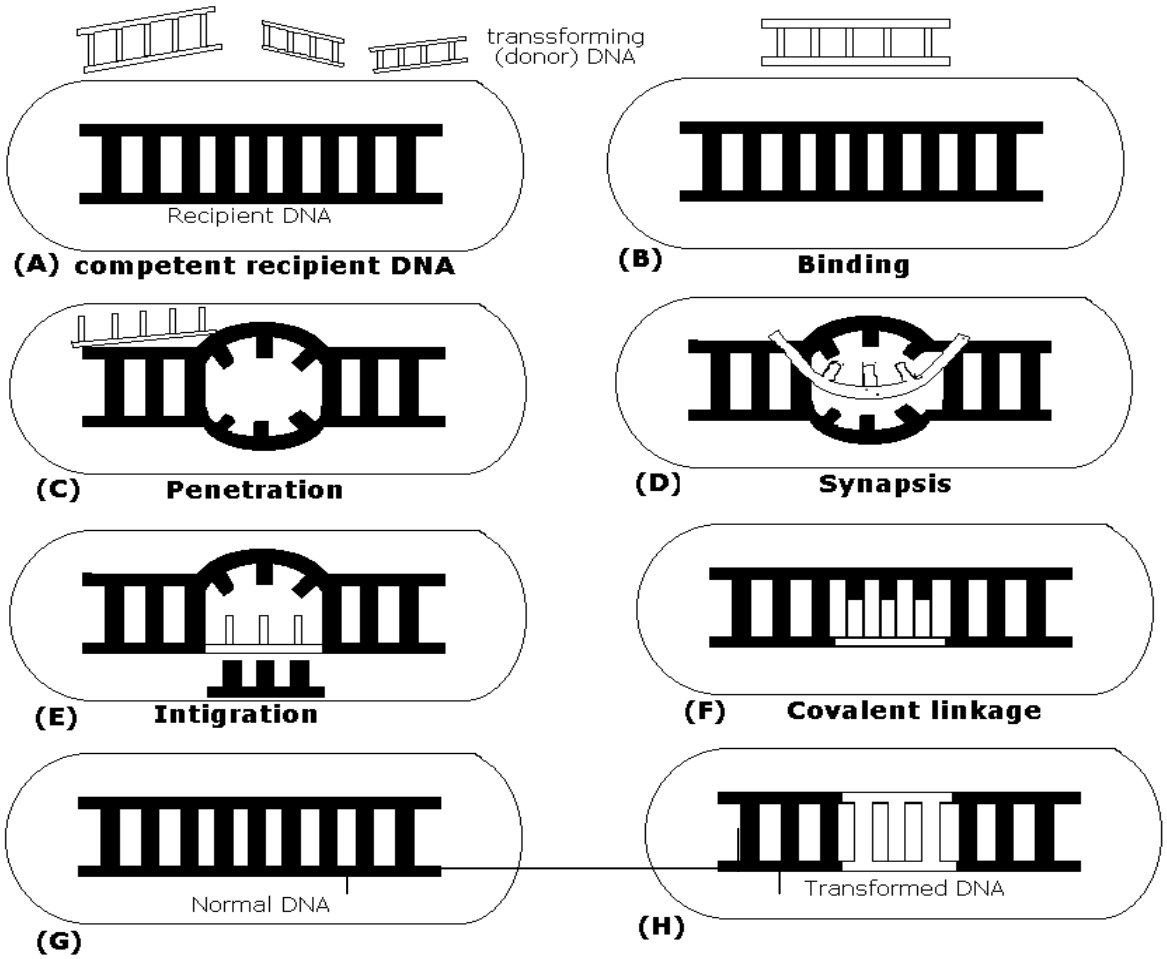


Fig:- A MODEL FOR GENETIC TRANSFORMATION

Transformation in *Diplococcus pneumoniae*

The gram-positive *Diplococcus pneumoniae* rapidly become **competent** during exponential phase by the small protein called as **competence factor**. The cells use these proteins so that cells **adsorb** double stranded DNA to their outer surface of different sites. This DNA is divided into smaller fragments by the surface bound enzyme.

Then one strand is digested & other strand enters into the cell.

Diplococcus pneumoniae takes up DNA of any source. However, only homologous DNA is integrated into DNA of cell. External DNA is called as **exogenote** & DNA of cell inside is **endogenote**.

Non-homologous DNA is not integrated; it is either **degraded or replicated** independently inside the cell.

Integration of homologous DNA occurs by a process of **strand replacement**. One strand of which is newly entered & other strand is homologous region of the endogenote.

Transformation in Haemophillus influenzae: -

In these gram-positive bacteria no competence factor is produced but cells become competent during their growth in **rich media**. Only homologous DNA source from closely related species is taken. Double stranded DNA is entered inside the cell **without destroying** other.

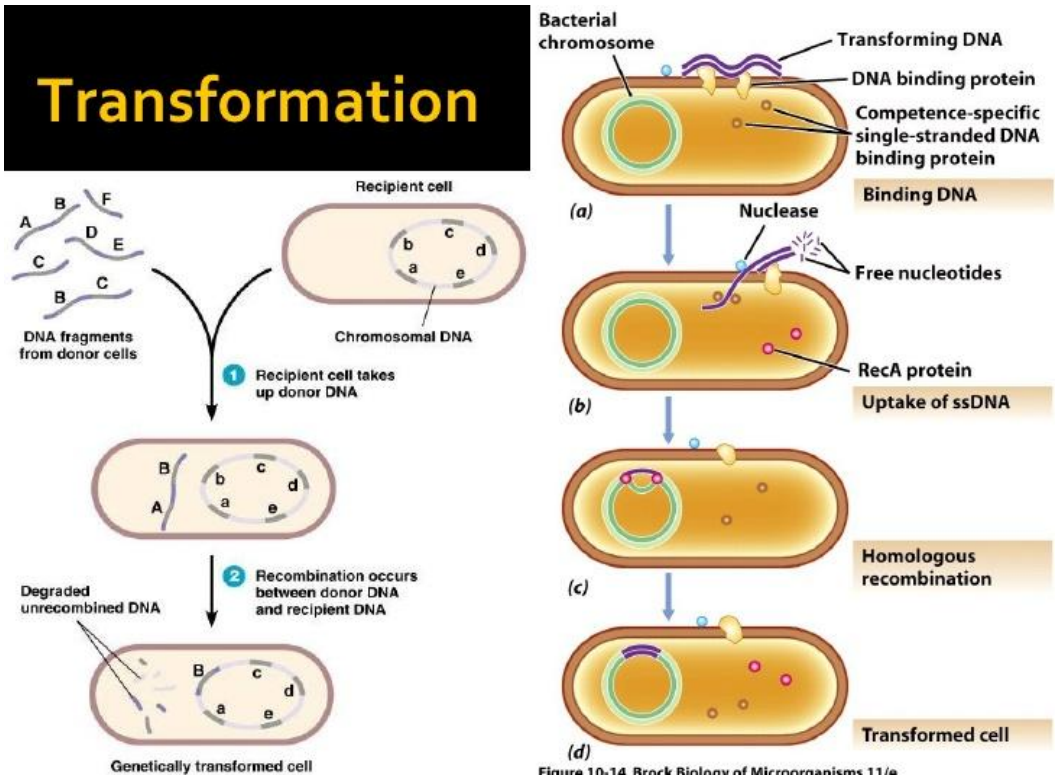
Transformation process: -

1) **Binding: -** DNA comes into contact with the bacterial cell surface as a result of random collision. Donor DNA first binds reversibly & can be removed by exposure to DNAse or extensive washing. DNA binds to the cell surface at several receptor sites, which appear to be associated with the mesosome.
2) **Penetration**: - Permanently bound DNA penetrates the bacterium. Double stranded DNA is converted into single stranded DNA upon entering pneumonecoccus cells. One end of double stranded DNA enters

the cell where an endonuclease digests one of the strands, while the other is pulled into the cell. In *Bacillus subtilis* both strands enter into the cell.

3) **Synapsis:** - The recipient chromosome DNA **unwinds** and initiates pairing with the single-stranded donor DNA. This pairing or Synapsis is the true result of base pairing between donor & recipient DNAs. The donor single stranded DNA is **homologous** but **not identical** with the recipient single stranded DNA with which it pairs.

4) **Integration:** - The unsynapsed recipient DNA strand is cut into two places by an endonuclease. Exogenote then degrades the single stranded DNA strand. Integration of the donor DNA strand involves small amount of repair synthesis to close the gap present followed by final sealing with a ligase. The transformation heteroduplex formed undergoes replication to form transformation Homoduplex one of these is a normal duplex while the other is transformed duplex.



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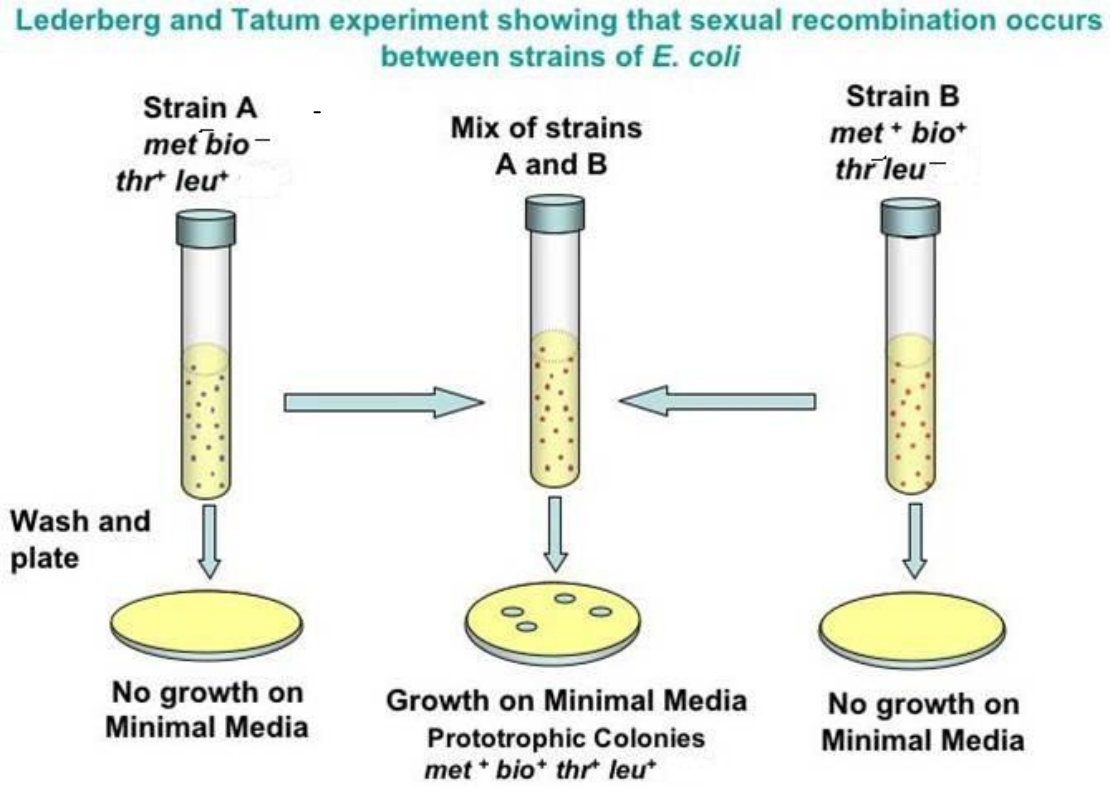
2. Bacterial Conjugation

In 1946 **Leaderberg and Tautum** discovered the process of conjugation in bacteria. In conjugation there is **physical contact** between two bacterial cells of same or closely related species. **Conjugation tube** is established between two conjugates. Genetic material is transferred from one cell (**male as donor**) to the other (**female as recipient**). The conjugation tube is formed by **sex pilli**, specified by a **Plasmid, the F factor** in the donor. The recipient cell possesses specific receptor site for conjugation. There is no exchange of genetic material thus is unilateral transfer.

Leaderberg and Tautum crossed two **Auxotrophic** strains of *E. coli* and found that **prototroph** could be recovered. They produced two auxotrophic strains (58-161 & W677) of *E. coli* K_{12} by mutation. The strain 58- 161 cannot synthesize biotin & methionine and hence denoted as **bio$^-$ met $^-$**. The strain W677 cannot synthesize threonine and leucine and it is denoted as **thre $^-$ leu$^-$** . The genotype of the two parental types are shown bellow—

Parental type I (58-161) → bio$^-$ met$^-$ thre$^+$ leu$^+$

Parental type II (W677) → bio$^+$ met$^+$ thre$^-$ leu$^-$

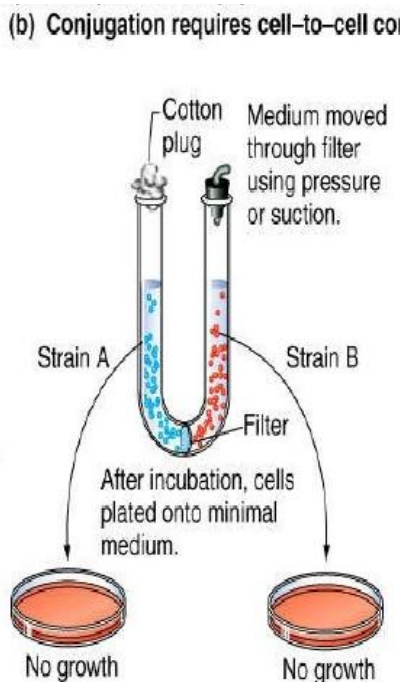


Mixing bacteria of the two parental types and plating on a medium containing none of the growth factors produced the prototrophy.

bio^+ met^+ $thre^+$ leu^+

These cells have the capacity to synthesize all four growth factor.

Devis (1950) repeated the experiments by using a **U shaped tube**, each arm of which contained one auxotrophic strain. The two arms of the U shaped tube were separated by sintered glass filter, which do not permit the *E. coli* cells. It permits the small molecules & nutrients. Here no prototroph appeared in either arm. It proves that contact between two parent cells was essential for formation recombinant bacterial genomes.



THE F FACTORS: -

The cells in which the F factor is present are designated as F^+ (male or donor), Cells in which the F factor is absent are called as F^- (female or recipient cells).

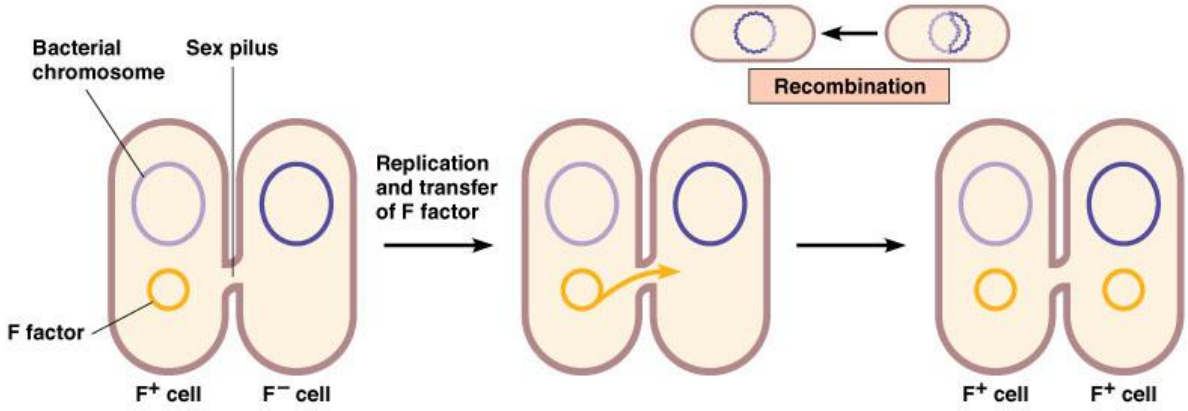
Some F factors are **plasmids** and others are **episomes**. The plasmids occur as independent ring of DNA in the cytoplasm, when a plasmid is **integrated** with chromosomal DNA it is called an episome. *E coli* F^+ male have only one F plasmid. There is simultaneous replication of plasmid and chromosomes.

The following result have been obtained by crossing growing F^+ and F^- strains together

1. $F^- X F^-$ crosses are always sterile.
2. $F^+ X F^-$ crosses are fertile, but it is one in a million rarities.
3. $F^+ X F^+$ crosses are also fertile but at very low level, the fact is that F^+ cells are converted into F^- cells.
4. When F^+ and F^- strains are grown together up to 70 % F^- cells are converted into F^+ cells.
5. The F^+ cells transfer F factor to F^- cells without much chromosomal transfer. The F^+ cells however, remains F^+.
6. Prolonged incubation with acridine dye converts F^+ strains into F^- .
7. Certain F^+ subsrtains show a X 1000 increase in the rate of recombination with F^- strains & are called as HFr **(High frequency of recombination) strains.** The HFr strains are produced by the integration of F factor within the bacterial chromosome.

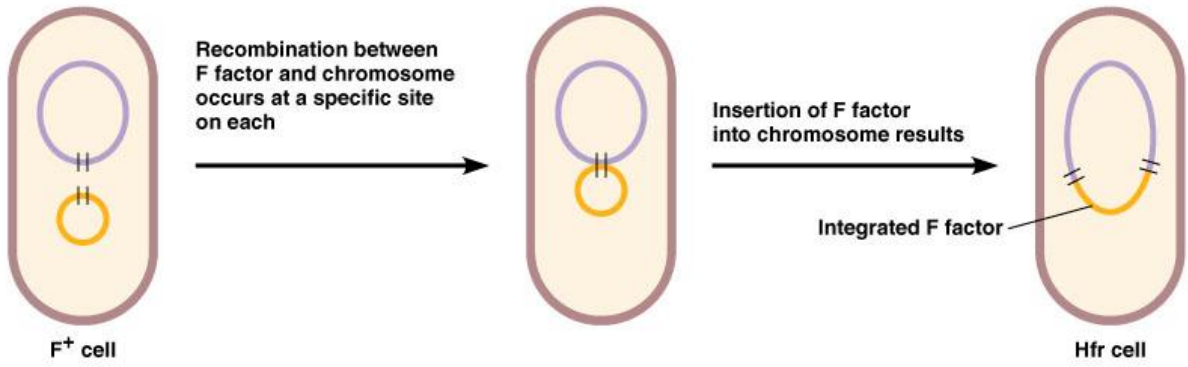
Donor cell are of three types F^+, HFr & F prime (F').

i) **F^+ Cells:** The F factor (plasmid) is present as independent double stranded ring of DNA. During conjugation there is a break in one of the two strands of the plasmid. This strand passes into the recipient cell. The donor cell retains one strand. The strand in both donor and recipient cell now synthesize a complementary strand. Thus two intact duplexes are formed, one in each cell. Thus F^+ remains as F^+.



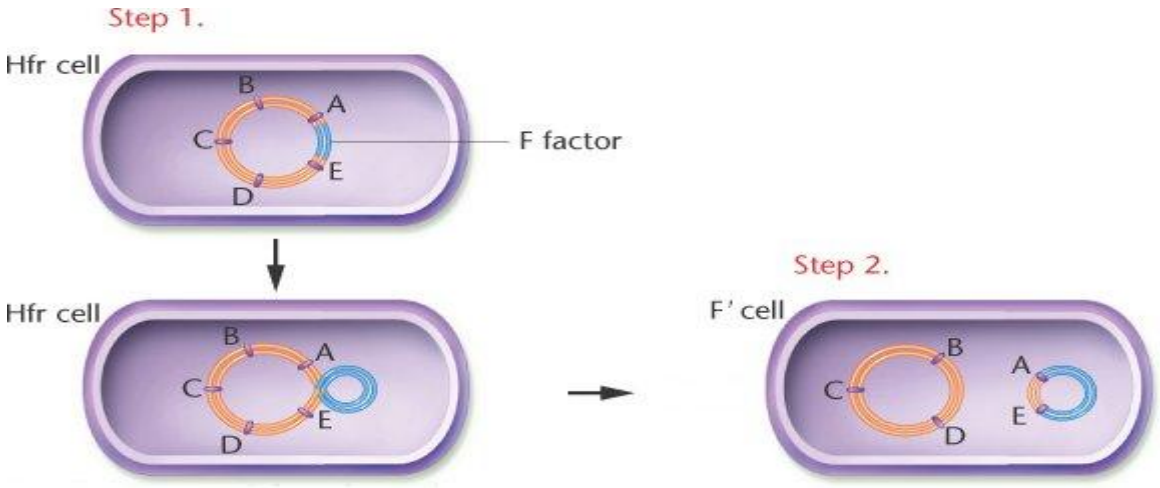
(a) When an F factor (a plasmid) is transferred from a donor (F^+) to a recipient (F^-), the F^- cell is converted into an F^+ cell.

ii) **The HFr cells (episomes)**: It is formed by the integration of F factor into the chromosome. The probability of HFr formation is about 10^5 per cell, per generation. The break occurs in both strands of plasmid as well as chromosome having similarity of nucleotides sequence. The plasmid DNA is inserted into chromosome to form HFr donor cell. During conjugation of HFr cell with F^- cell, one strand of the F plasmid breaks, and part of the plasmid strand is transferred to the recipient cell along with chromosome.



(b) When an F factor becomes integrated into the chromosome of an F^+ cell, it makes the cell a high frequency of recombination (Hfr) cell.

iii) **The F prime (F') cells:** These are formed by the excision the F factor from the chromosome along with the segment of chromosome. This factor was isolated by **Adelberg** & **Burns (1960)**. The F prime factor consists of the plasmid along with segment picked up from the chromosome. It carries certain chromosomal genes but takes a maximum part of the F factor.



Process of F' cell formation

- F^+ donor cell with chromosome F factor
- conjugation break in one strand of plasmid ring of donor cell.
- plasmid strand passes into recipient cell.
- Each plasmid strand synthesizes a complementary DNA ring. Both cell F^+ cells.
- breaks occur in the both strands of plasmid as well as chromosomal DNA rings.
- Plasmid is inserted into homologous regions of chromosome.
- integrated ring of plasmid and chromosomal DNA.
- breakage of plasmid ring from the chromosomes. Breakage & reunion occur at a different point from plasmid incorporation.
- chromosome & plasmid rings with interchanged segments. F prime donor formed.

CHROMOSOME TRANSFER

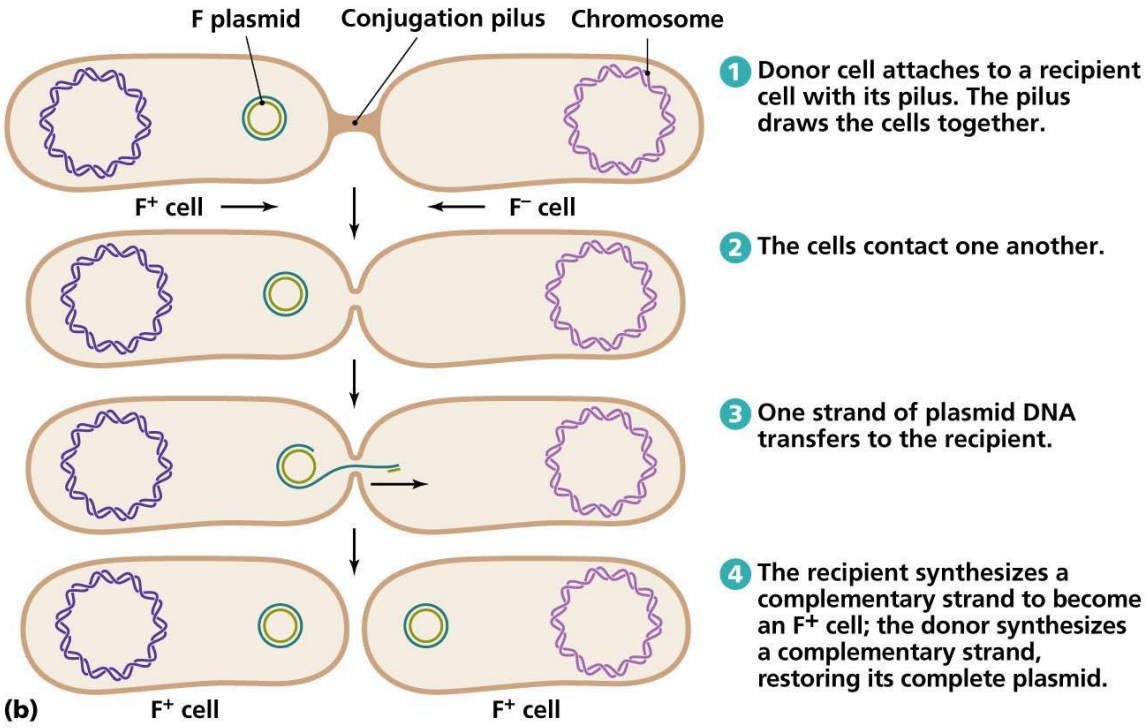
The origin of transfer is located within the sex factor (plasmid). The sex factor breaks at the origin, & the DNA is transferred on a linear structure.

There are three general classes of models for chromosome transfer.

(A) According to one model, the chromosome to be transferred is previously synthesized in the donor. One chromosome remains in the donor while the second is transferred to the recipient which gains two pre-existing strands.

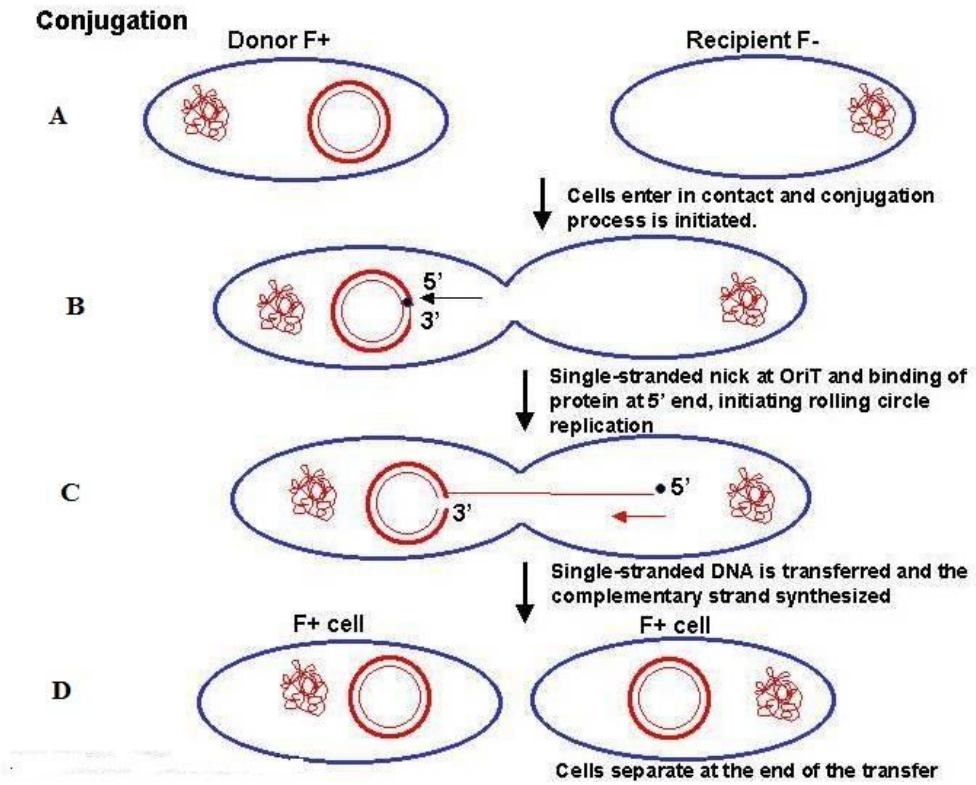
(B) IInd model- transfer & replication takes place simultaneously in the donor cell. The recipient cell requires pre-existing strand & one newly synthesizes strand.

(C) IIIrd model- only one strand of DNA chromosome passes into the recipient cell. There is transfer of only one pre-existing strands. This model is the rolling circle model of **Gilbert** & **Pressler** (1968).



A) Model I- Transfer of pre-existing duplex chromosome.
B) Model II- Transfer of newly synthesized duplex chromosome consisting of one pre-existing & one newly synthesized strand.
C) Model III- Transfer of single pre-existing strand.

Experiment with radioactive isotopes demonstrated that only one of the two previously existing parental donor strands is transferred to the recipient cell. Genetic & biochemical analysis show that only one strand of the donor chromosome is transferred to the recipient cell therefore it supports the rolling circle model. The plasmid transfer takes place as shown in figure.



A. Mating pair connected by conjugation tube, one strand of the chromosome is nicked at the origin (arrow).
B. Nicked strand transferred by 5' end into recipient cell.
C. Transfer of nicked strand continues. Newly DNA synthesis begins in both cells.
D. Recircularization of both plasmids.

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3. Transduction

Transfer of fragments of DNA from one bacteria cell to another bacterial cell through the agency of virus (Bacteriophages) is called Transduction.

Transduction is of two types

1) Generalized
2) Specialized

1) GENERLIZED TRANSDUCTION: -

Phage mediated transduction of a small segment from any region of the bacterial chromosome is called generalized transduction.

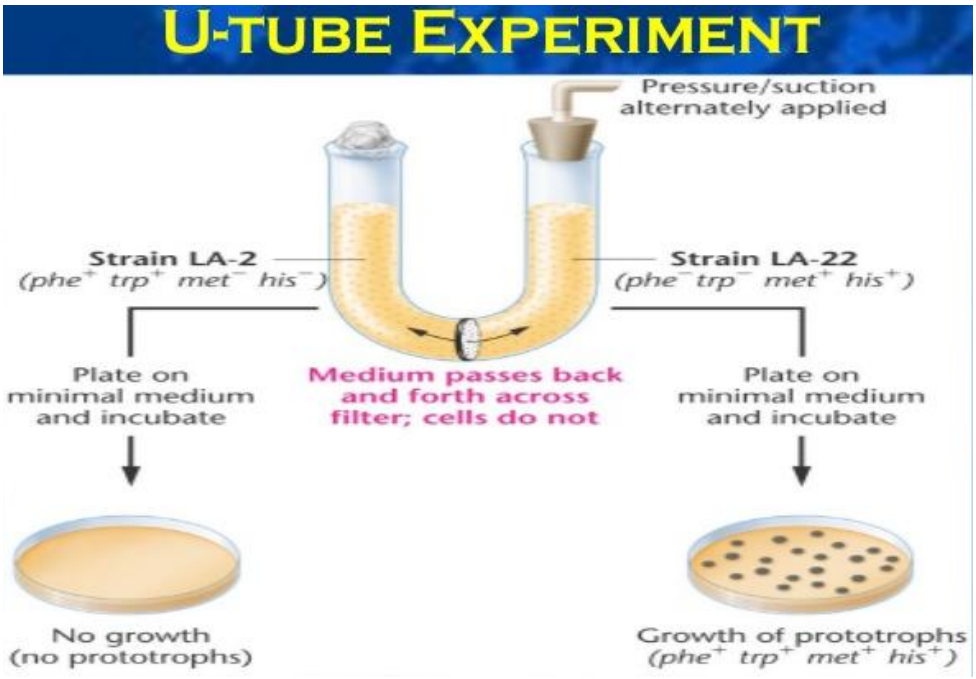
In 1952 **ZINDER & LIDERBERG** discovered that bacteriophage particles could transfer bacterial gene from one bacterium to another.

Zinder & Lederberg initially began their experiments with the objective of discovering whether the *E. coli* type of genetic exchange also existed in *Salmonella typhimurium.* Various strains of auxotroph were crossed on an amino acid minimal medium in an attempt to find new prototrophic combinations. The LA22 strain was unable to synthesize the amino acid phenyl-alanine & tryptophan (phe^- trp^- strain) but could synthesize methionine & histidine. The LA2 strain was unable to synthesize methionine & histidine (met^- his^- strain) but could synthesize phenyl-alanine and tryptophan. Crossing of the LA22 & LA2 strain resulted in a wild type, which could synthesize all four amino acids (phe^+ trp^+ $meth^+$ his^+).

Each strain is placed in one arm of a Davis U tube. The two arm of the tube were separated by a sintered glass filter which was impervious to bacterial cells (and thus prevented conjugation) but allowed the free passage of nutrient medium and particles smaller than 0.1µ.The culture medium was made to pass through the filter from one arm to the other by alternating suction & pressure. Thus the two auxotrophic strains, although physically separate were grown in the same medium.

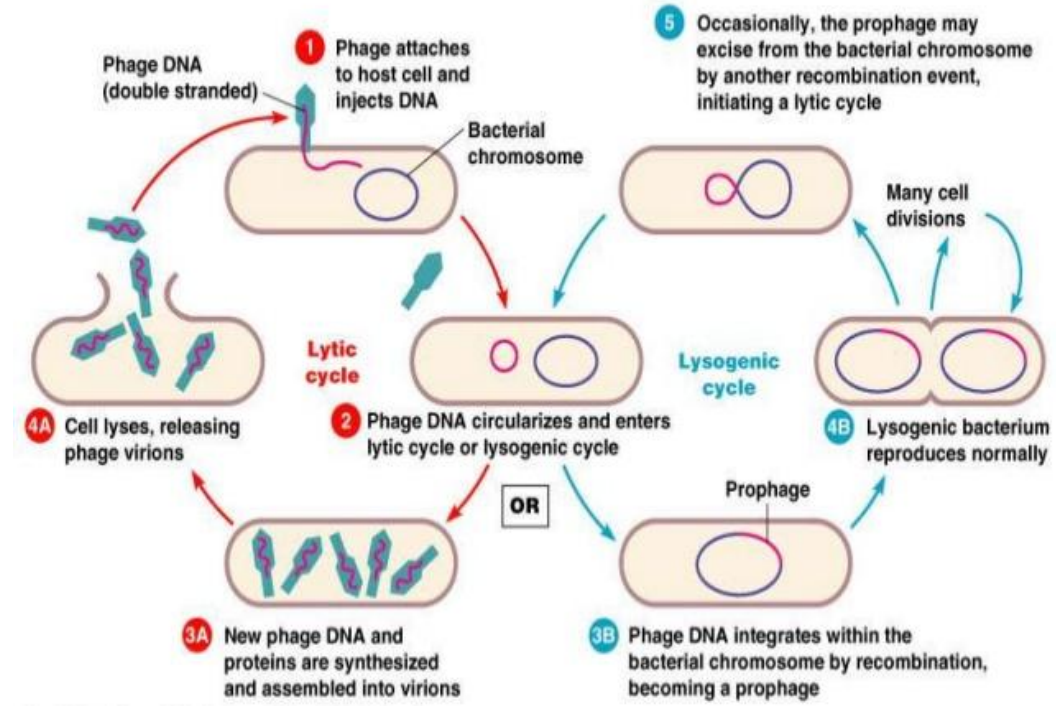
Prototrophs appeared in the LA22 arm of the U tube but not in the LA2 arm. A genetically active **Filterable Agent (FA)** was produced by LA2. When LA2 was grown separately from LA22 it did not produce an active FA that the FA was not an ordinary transforming agent (DNA molecule) was shown by the facts that it was resistant to DNAse treatment & was larger than DNA.

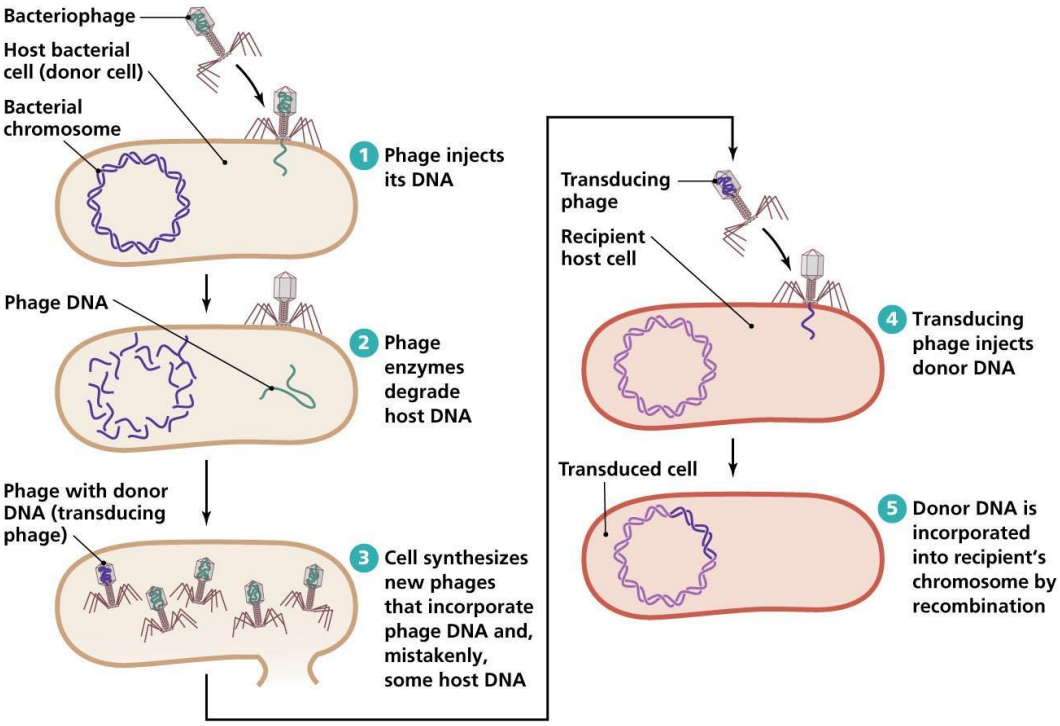
The FA was shown to be the **temperate Salmonella phage P_{22},** which was carried in one of the parent strains as a prophase. This was confirmed when it was shown that the FA is destroyed by exposure to P_{22} anti-serum.



P_{22} ordinarily exists in the lysogenic state in the LA22 strain of salmonella. Occasionally it passes into the proliferative vegetative state and lyses the bacterial host cell. The phage particles now pass through filter and infect the non-lysogenic LA2 strain. They become associated with genetic material from LA2. Some of this material was wild type for the mutant recessive genes carried by LA22. Recombination between LA2 material and LA22 chromosome produces Prototrophs. The phase particles have brought transduction of the LA22 strain. The non-lysogenic LA2 does not carry a temperate phage and hence can transfer material in the presence of the lysogenic LA22. The transducing frequency is low and only one in 10^5 -10^7 cells undergo transduction.

Only a small segment of the bacterial chromosome material undergoes transduction by the phage particle.





Generalized transduction

1. Phage injects its DNA into bacterium.
2. Phage enzymes bacterial DNA.

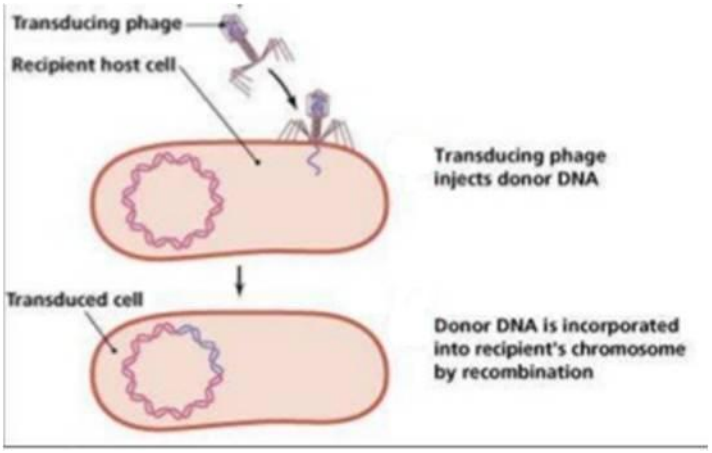
3. Cell synthesizes new phages that incorporate Phage DNA and mistakenly some host DNA. Replication of phage DNA to form multiple copies and bacterial cell undergoes lysis.
4. The released phage infects another bacterial cell (donor cell) & injects its DNA into it.
5. Recipient bacterial cell is infected by a phage caring DNA (x') from donor bacterium. Donor DNA incorporated into recipient cell chromosome.

Role of defective phage: -

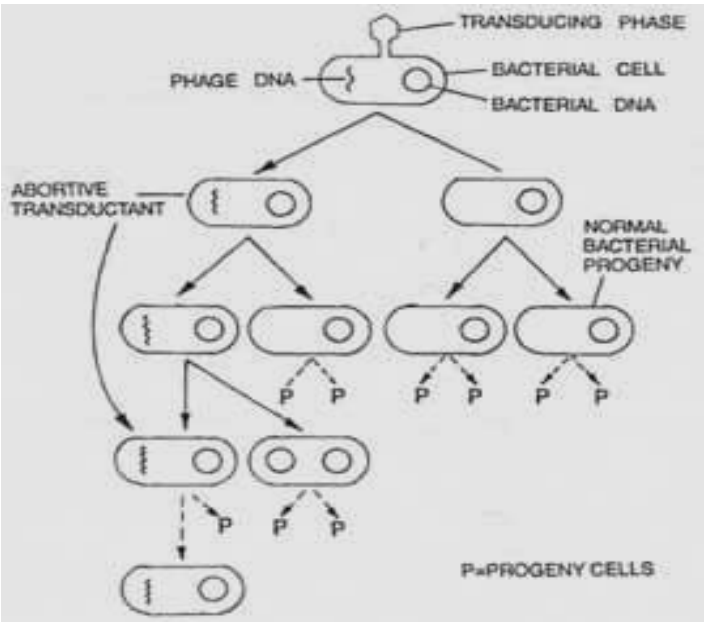
Transducing phages are ordinarily defective, and do not have a structurally or functionally normal genome. The phage does not have the space to carry its own DNA and a large piece of foreign DNA enclosed in a coat. The bulk of transductants do not contain any detectable phage genes.

<u>Complete</u> & <u>abortive transduction</u>: -

When a transducing phage introduces DNA (exogenate) into a bacterial cell, the DNA may either become inserted into the bacterial chromosome (endogenate) or may remain in the free state in the cytoplasm. The **insertion of the exogenate into the bacterial chromosome** takes place after pairing and crossover and produces a recombinant chromosome. Such type of transduction is called as **complete transduction.** When the transduced cell divides the recombinant chromosome replicates & the integrated gene is transmitted into the daughter cell.



When the **exogenate is not inserted into the bacterial chromosome,** it results in the abortive transduction. The DNA fragment remains in the cytoplasm as a free particle, which cannot undergo replication. When the bacterial cell divides, only one daughter cell in each generation contains the exogenate, which is transmitted unilinearly.



2) <u>Specialized (restricted) transduction</u>:-

In specialized transduction a particular bacteriophage strain can transfer only certain genes. In 1956 **<u>MORSE</u> & <u>LEDERBURG</u>** found that in the Lambda (λ) phage. The transducing activity was restricted to the **galactose locus**. Only the gene loci adjacent to the site of insertion of the prophage are incorporated. Wild type bacterial prototroph used as donors for lambda phage transduction affect recipient auxotroph only by modifying gal^- to gal^+, other loci such as thr, trp, lac etc. are never transduced. Restricted transduction is possible only with lysate produced by the induction of the prophase. In contrast to the general transduction, it is not possible with lysate produced by lytic infection.

When the lambda chromosome enters the bacterial host cells it either enters the lytic cycle or is incorporated into *E coli* chromosome by recombination & becomes a prophage. In the later case the host survives integration & become lysogenic. In the prophage condition the phage DNA replicates each time the *E coli* chromosome replicates. Under certain condition e.g. exposure to ultra-violet light, the prophage enters the lytic cycle & kills the

hosts. The prophage is excited from the bacterial chromosome and carries away with it pieces of the DNA of the hosts. The transduced genes are linked to the viral chromosome.

Normal excision takes place in the majority of cells, giving rise to circular viral chromosome and nonlysogenic bacterial chromosome. The viral chromosome replicates by the rolling circle model. Each unit length DNA is then packaged into infectious particles.

When abnormal excision takes place (1 cell at of 1,00,000) a circular molecule containing some host DNA is formed. When this replicates, it gives rise to infectious particles containing a segment of bacterial DNA, which has replaced a segment of viral DNA. Depending upon the site of abnormal excision, different virus variants are formed.

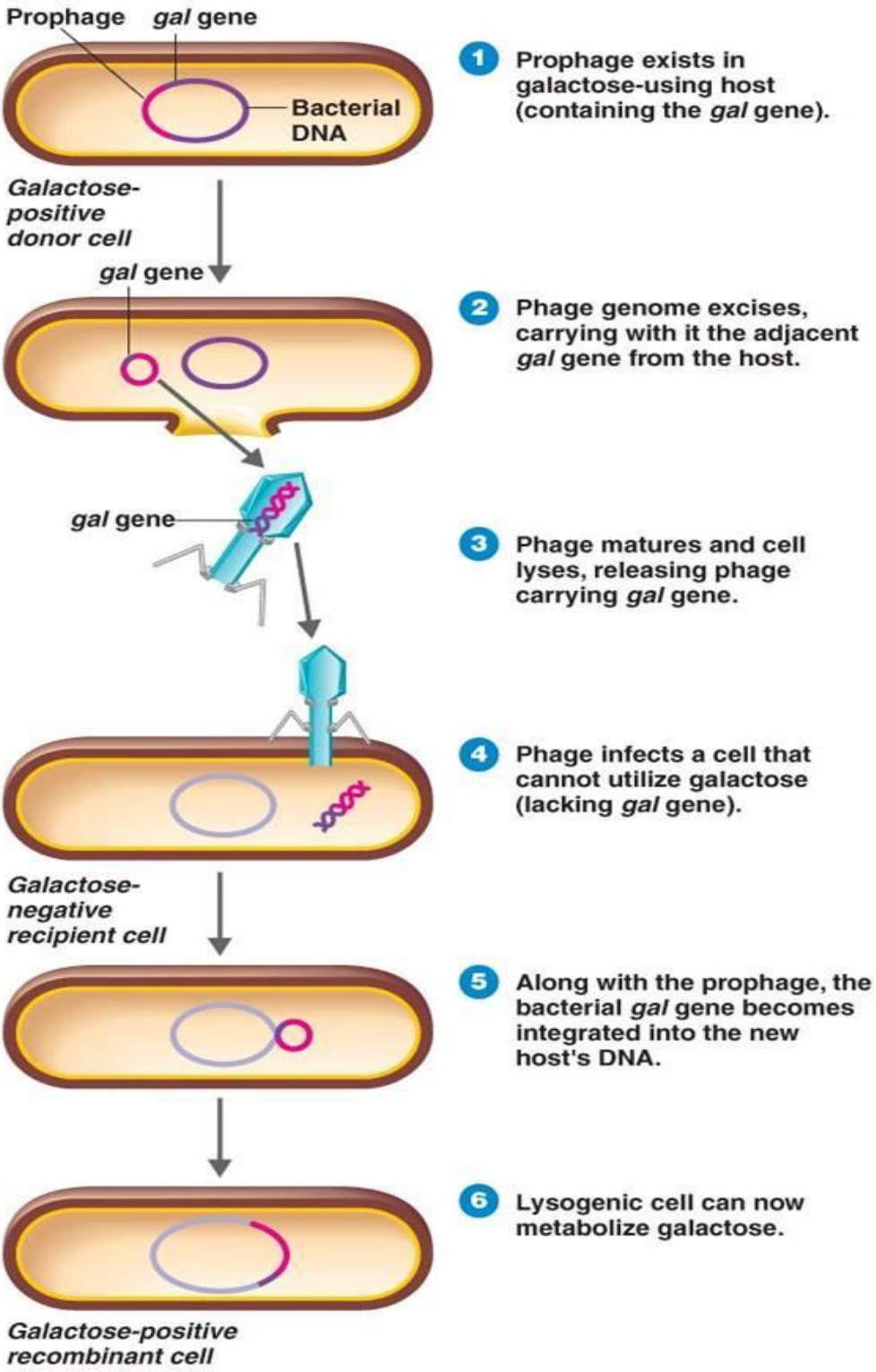
LOW FREQUENCY TRANSDUCTION

The lambda prophage can undergo exchange with nearby gal segment of bacterial DNA. The vegetative lambda now contains a segment of bacterial chromosome inducing gal. (A part of the phage genome the h region) is transferred to the bacterial chromosome in this exchange. The particle is defective & carries gal, & is hence known as **λ dg (or lambda defective gal.)**

Lambda dg particles constitute only a **minute fraction of the lysate** particles. The lysate is, therefore called as low frequency- transduction (LFT) lysate.

HIGH FREQUENCY TRANSDUCTION: -

When induced by **ultraviolet** light the lysogenic gal^+ / gal^- heterozygotes had a very high transducing power nearly half of the phage particles had the capacity to transduce gal^- recipient cell into gal^+. The term, *high frequency transduction* (HFT) was used for such lysate, in contrast to the original low frequency transduction (LFT). HFT lysates can transduce only the gal character, producing mostly unstable gal+ / gal- heterozygotes, which in turn yield HFT lysate upon ultra-violate irradiation.



Specialized transduction