MICROBIAL GENETICS

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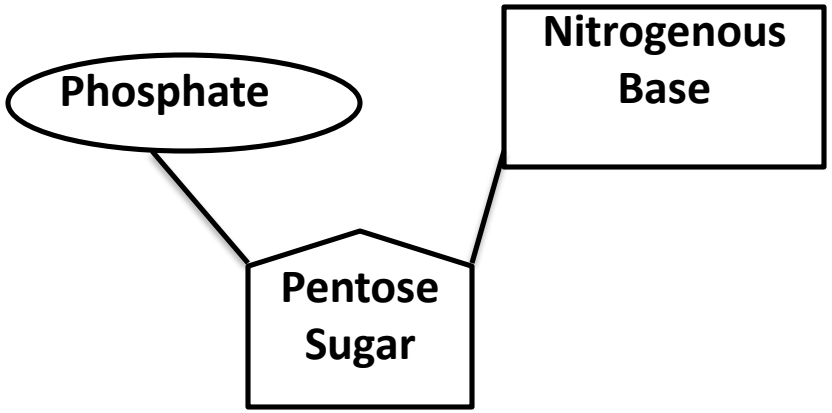
Genotype and Phenotype

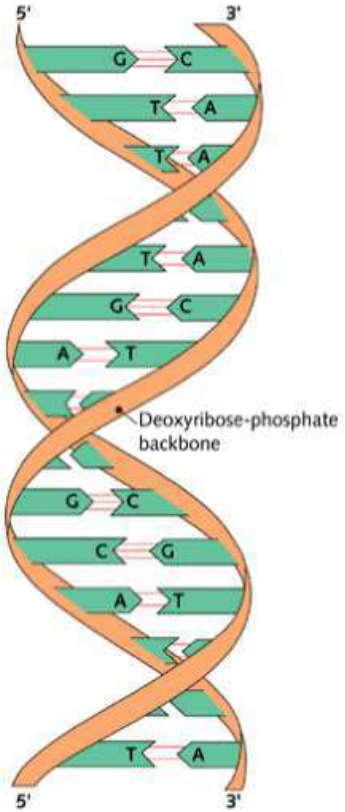
The **genotype** of an organism is its genetic makeup, the information that codes for all the particular characteristics of the organism. The genotype represents *potential properties, but not* the properties themselves. **Phenotype** refers 10 *actual, expressed* properties, such as the organism's ability 10 perform a particular chemical reaction. Phenotype, then, is the manifestation of genotype.

Molecules of Genetics

• The main molecules of genetics are called **nucleic acids.**
• All the genetic information are stored as a sequence of bases through nucleic acids mainly in **DNA and in RNA in some RNA** viruses.

The basic structure of DNA molecules is theNucleotide

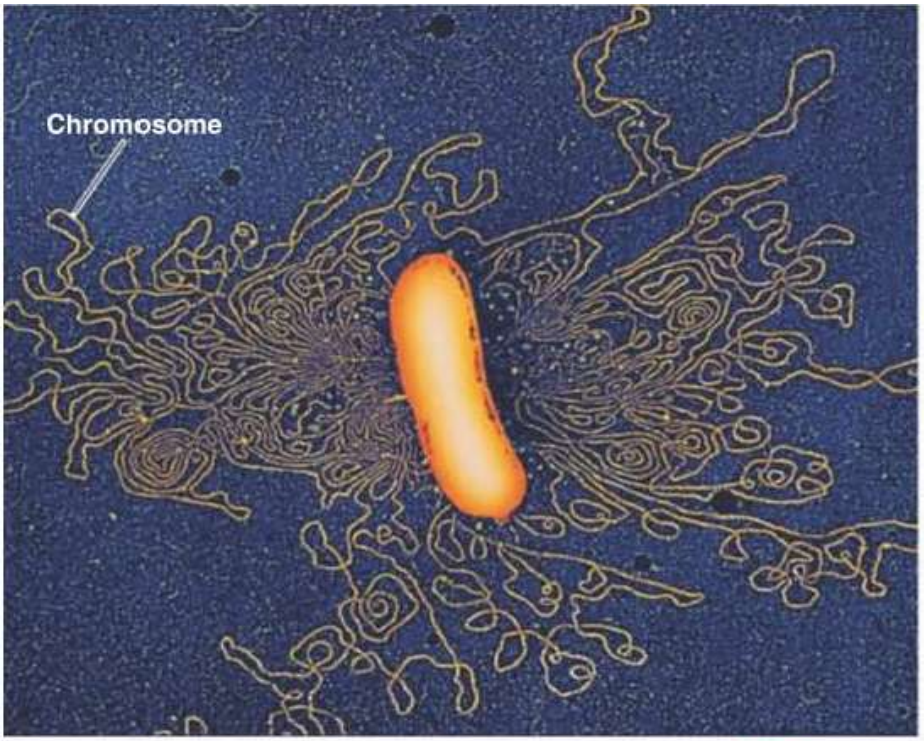




DNA (Deoxyribonucleic acid serves as organism's genetic material.
It is divided into functional units **(genes).**
- Most of DNA is double stranded.
- The two strands held together by hydrogen bonds between **A and T or G and C**
- It consists of non- identical, complementary base sequences

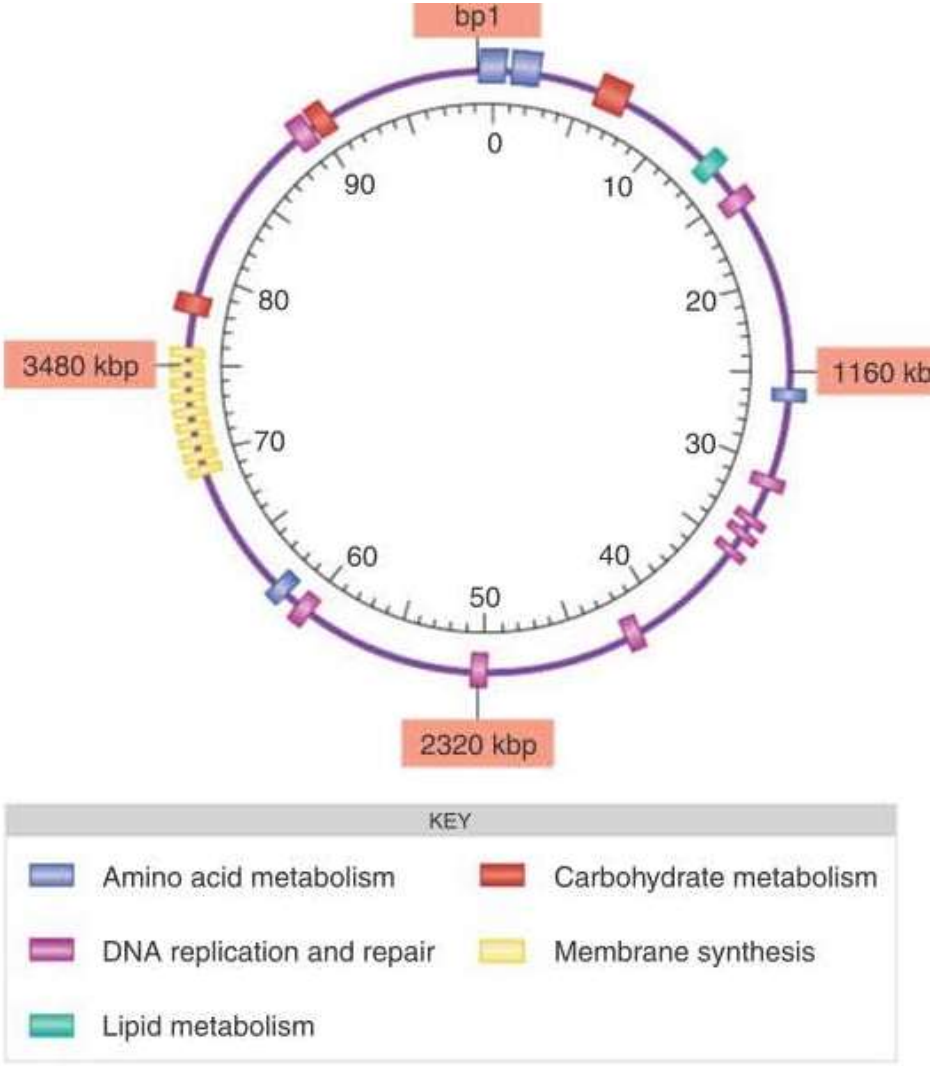
RNA (Ribonucleic acid): Structurally similar to DNA except.
- Most of RNAs are single stranded.
- Sugar is ribose instead of deoxyribose
- Uracil base instead of thymine base

A Prokaryotic Chromosome



(a) The tangled mass and looping strands of DNA emerging from this disrupted *E. coli* cell are part of its single chromosome.

TEM 1 μm



(b) A genetic map of the chromosome of *E. coli*. The numbers inside the circle indicate the number of minutes it takes to transfer the genes during mating between two cells; the numbers in colored boxes indicate the number of base pairs.

Bacterial Chromosome

•Bacteria typically have a single circular chromosome consisting of a single circular molecule of DNA with associated proteins.
•The chromosome is looped and folded and attached at one or several points to the plasma membrane.
•The DNA of £. *coli, the* most-studied bacterial species, has about 4.6 million base pairs and is about 1 mm 10ng- 1000 times longer than the entire cell.
•However, the chromosome takes up only about 10% of the cell's volume because the DNA is twisted, or *supercoiled- much like a telephone cord when you put the* handset back on the receiver.
•The location of genes on a bacterial chromosome can be determined by experiments on the transfer of genes from one cell to another.

In recent years, the complete base sequences of several bacterial chromosomes have been determined . Computers are used to search for *open-reading frames, that is, regions of DNA that are* likely to encode a protein. These are actually base sequences between start and stop codons. The sequencing and molecular characterization of genomes is called **genomics**.

Difference between prokaryotic and eukaryotic chromosome

https://www.easybiologyclass.com/difference-between-prokaryotic-and-eukaryotic-chromosome-a-comparison-table/

https://microbenotes.com/prokaryotic-and-eukaryotic-chromosomes/

https://www.biologyexams4u.com/2012/11/difference-between-prokaryotic-and.html#.XoLCwIgzbIU

Levels of Gene Regulation

The expression of a gene into functional proteins can be regulated at multiple levels:

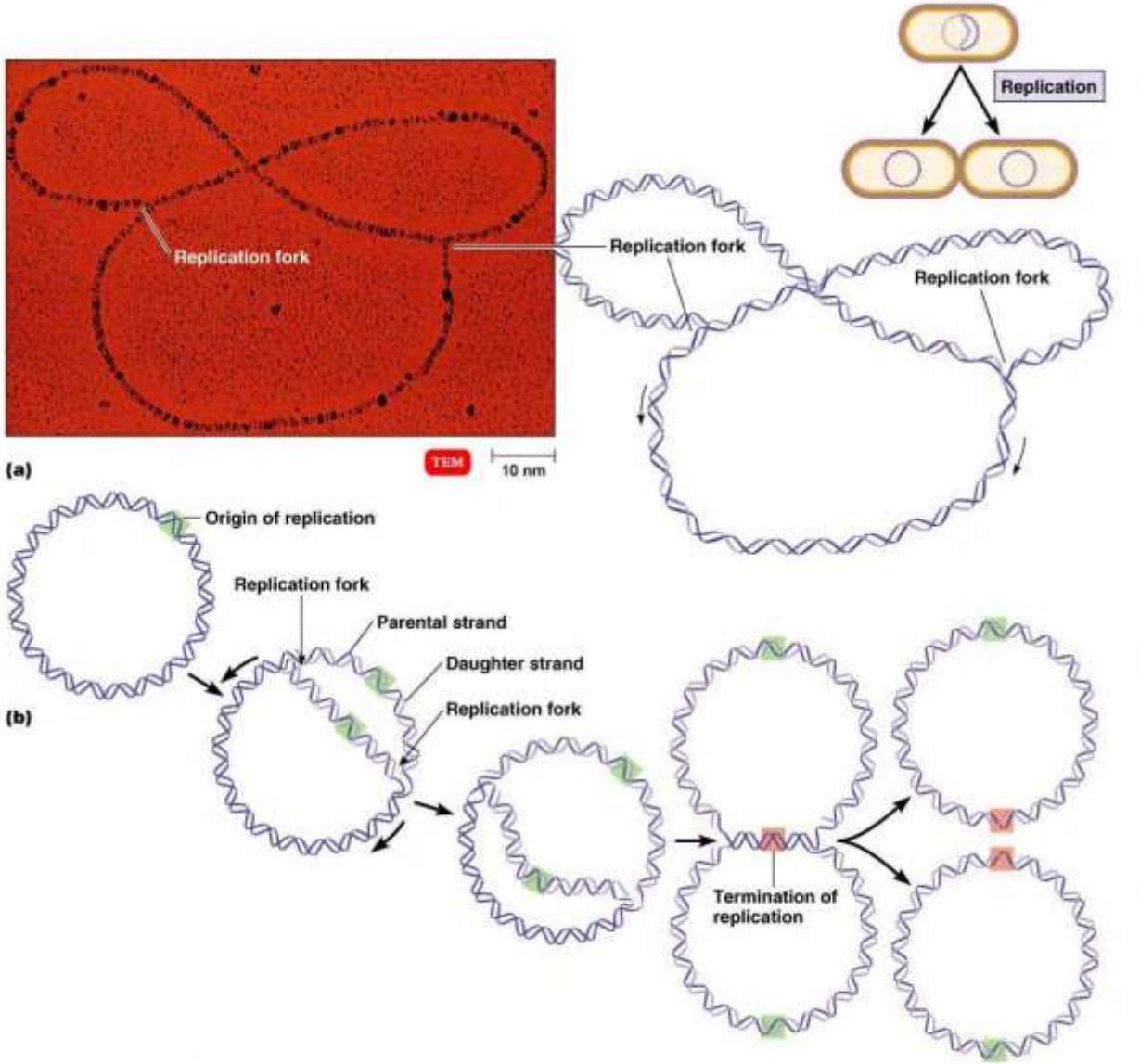
TRANSCRIPTION*
(regulation of rate at which gene is transcribed)

mRNA transcript stability
("half-life" of transcripts)

TRANSLATION
(regulation of translation of mRNA)

post-translational modifications
(e.g., cleavage of polypeptides, addition of chemical groups)

DNA Replication in Prokaryotes



begins at the origin of replication (OriC)

can only be completed ifDNA is circularDNA

Mutations

A mutation is *any change in DNA sequence:*

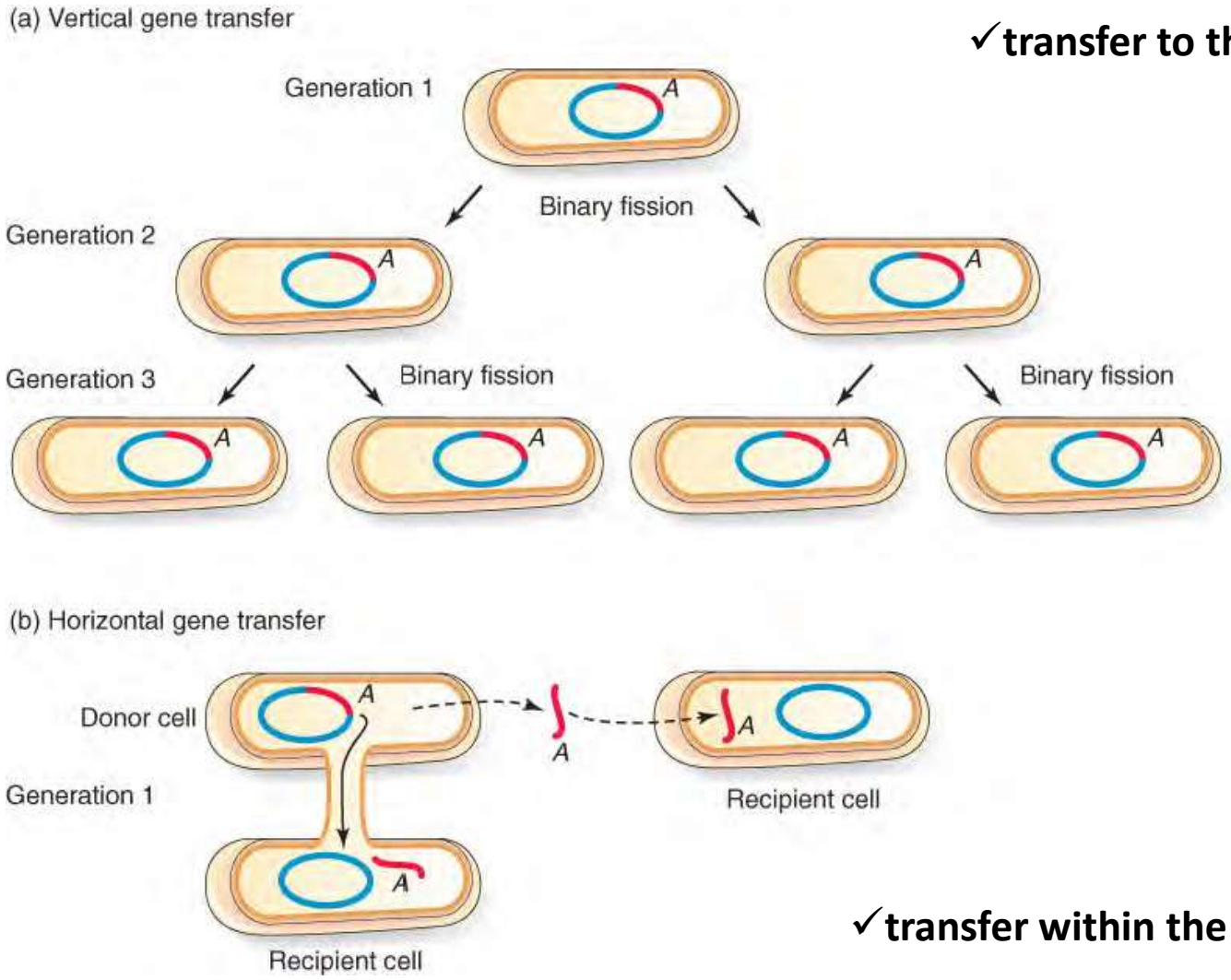
- **change of one nucleotide to another**
- **insertion or deletion of nucleotides or DNA fragments**
- **inversion or recombination of DNA fragments**

What causes mutations?

- **errors in DNA replication, DNA repair**
- **chemical mutagenesis**
- **high energy electromagnetic radiation**
- **UV light, X-rays, gamma rays**

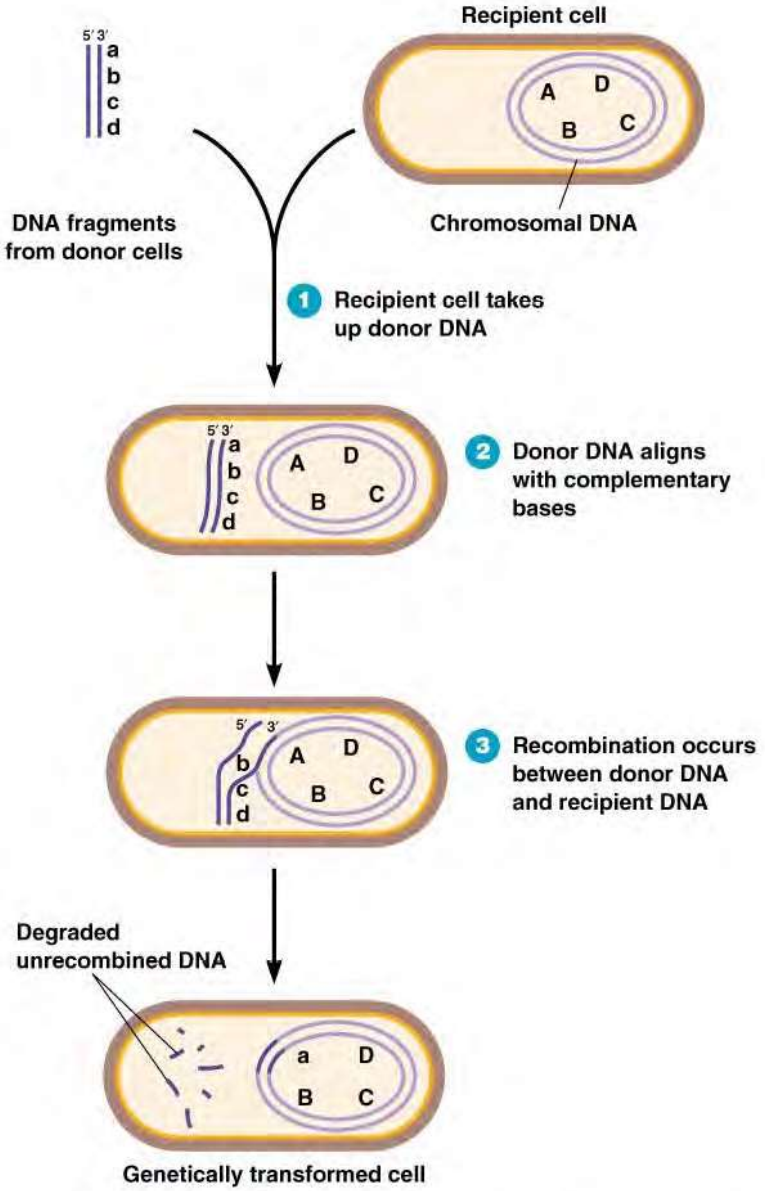
Mechanisms of Gene Transfer

Horizontal vs Vertical Gene Transfer



✓transfer to the next generation

✓transfer within the same generation



Homologous Recombination

Unless transferred DNA is circular w/Ori (plasmid), it must recombine with host DNA to be retained

Recombination can occur between *homologous* (similar) DNA sequences:

- **DNA with "same" genes**
- **facilitated by specialproteins**
- **original DNA is lost**

Mechanism of Genetic Exchange

Bacteria can acquire DNA (i.e., new genes) in 3 basic ways:

1) Transformation

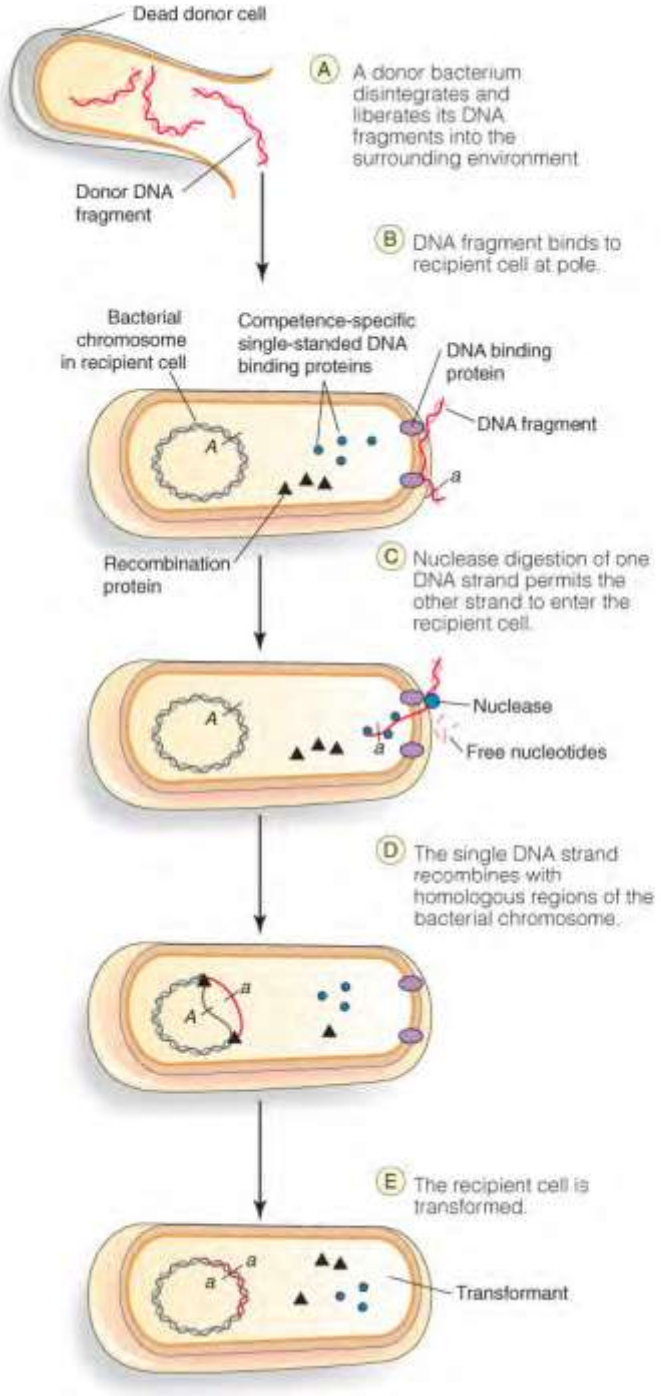
- **uptake and retention of external DNA molecules**

2) Conjugation

- **direct transfer of DNA from one bacterium to another**

3) Transduction

- **the transfer of DNA between bacteria by a virus**

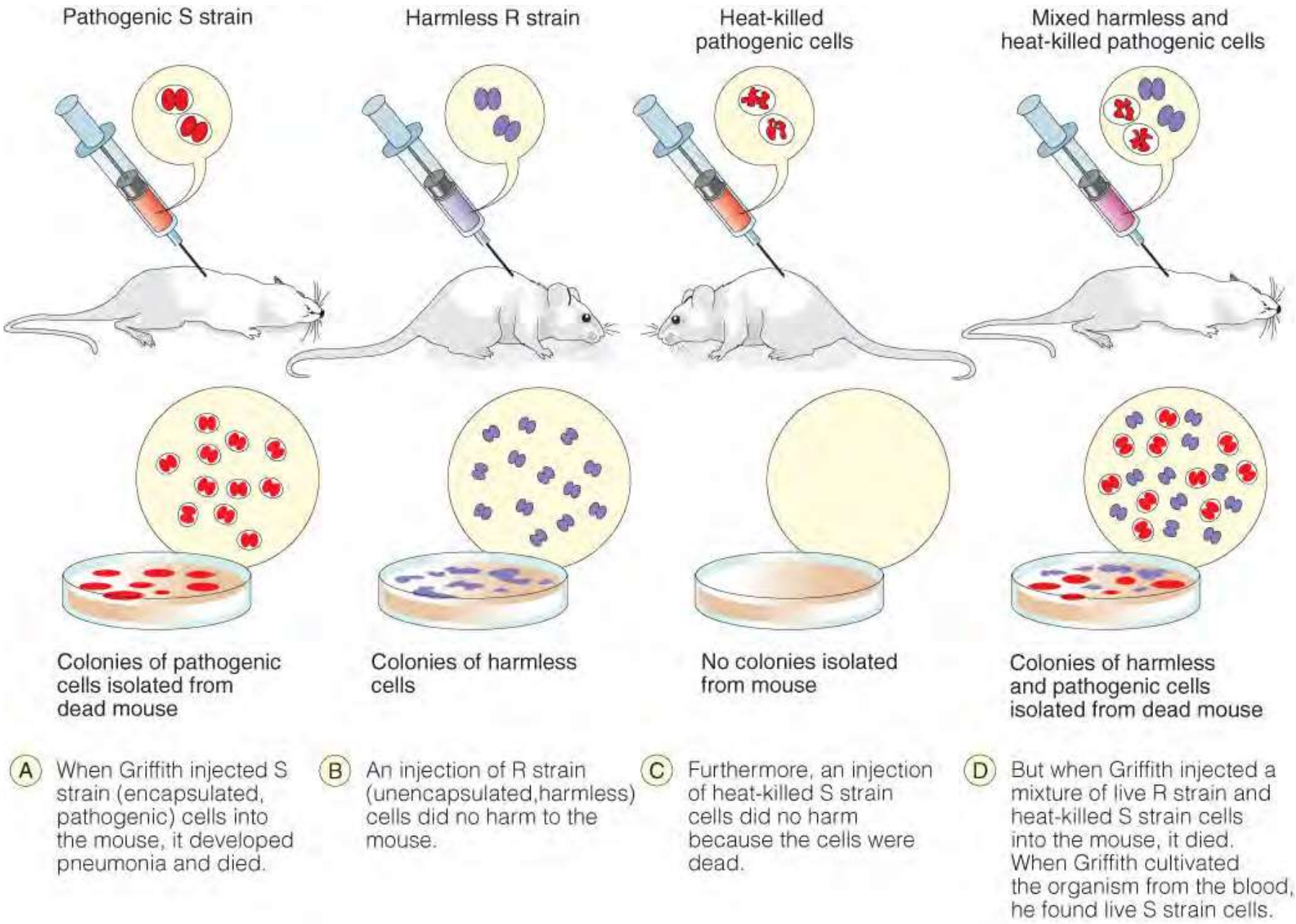


Transformation

Under the right conditions, bacteria can "take in" external DNA fragments (or plasmids) by transformation.

- **DNA binding proteins transfer external DNA across cell envelope**
- **bacterial cells capable of transformation are referred to as competent**
- **homologous recombination can then occur**

Griffith's Transformation Experiment



Plasmids

- Extrachromosomal genetic elements
- Autonomously replicating
- circular DNA - exept. B.burgdorferi
- do not encode essential functions - additional genetic information (phenotypic properties, atb resistance, bacteriocin and toxin production, metabolizing properties)
- Large plasmids – (fertility factor F, resistance transfer factor RTF) - mediate their own transfer - conjugation
- Smaller plasmids - not conjugative - do not encode transfer protein - sedentary - do not transfer
- Conjugation, transduction, incorporation

Classification of Plasmids

1. **Transfer properties**

a. **Conjugative plasmids** - Conjugative plasmids are those that mediated conjugation. These plasmids are usually large and have all the genes necessary for autonomous replication and for transfer of DNA to a recipient (e.g. genes for sex pilus).

b. **Nonconjugative plasmids** - Nonconjugative plasmids are those that cannot mediate conjugation. They are usually smaller than conjugative plasmids and they lack one or more of the genes needed for transfer of DNA. A nonconjugative plasmid can be transferred by conjugation if the cell also harbors a conjugative plasmid.

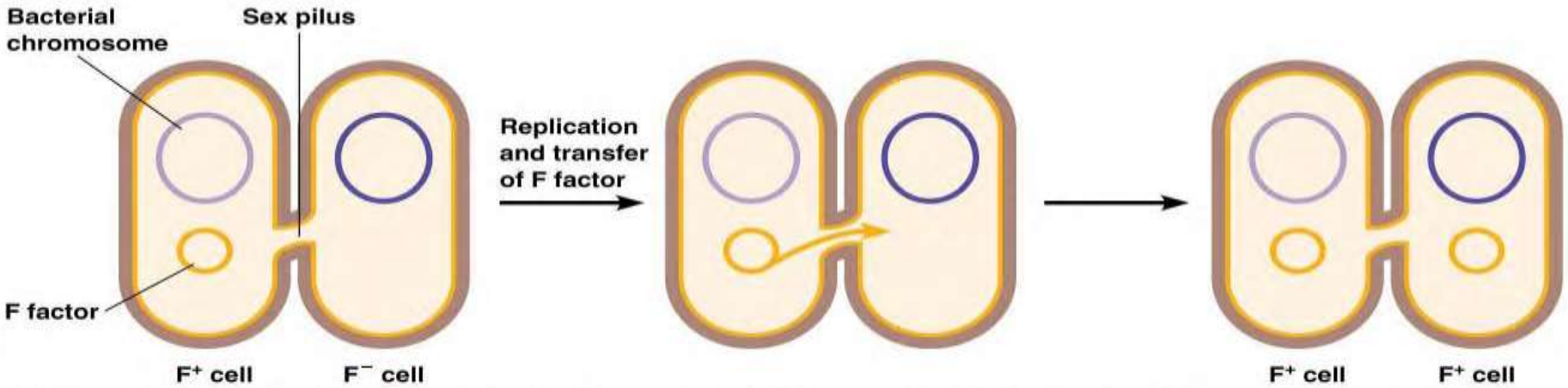
2. **Phenotypic effects**

a. **Fertility plasmid (F factor)**

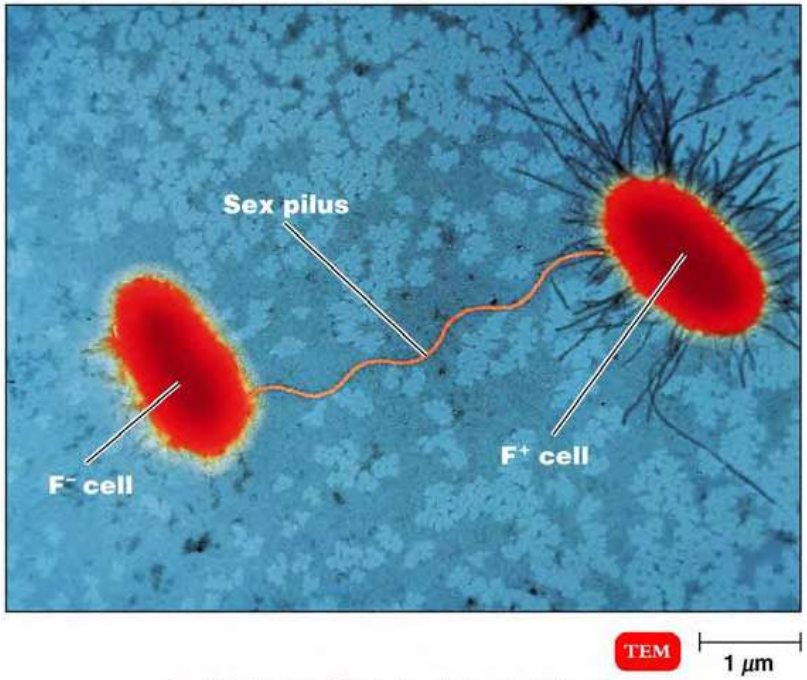
b. **Bacteriocinogenic plasmids** - These plasmids have genes which code for substances that kill other bacteria. These substances are called bacteriocins or colicins.

c. **Resistance plasmids** - These plasmids carry antibiotic resistance genes.

Bacterial Conjugation



(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.

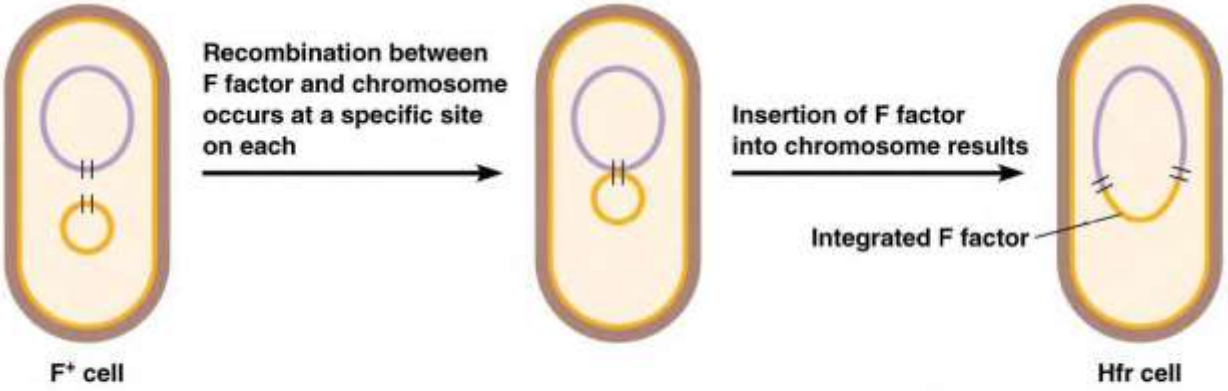


Requires an F factor plasmid

- **has all "conjugation genes"**
- **directs formation of a sex pilus**
- **single DNA strand produced by DNA replication is transferred to F-cell through the sex pilus, recipient produces 2ndstrand**

Hfr Conjugation

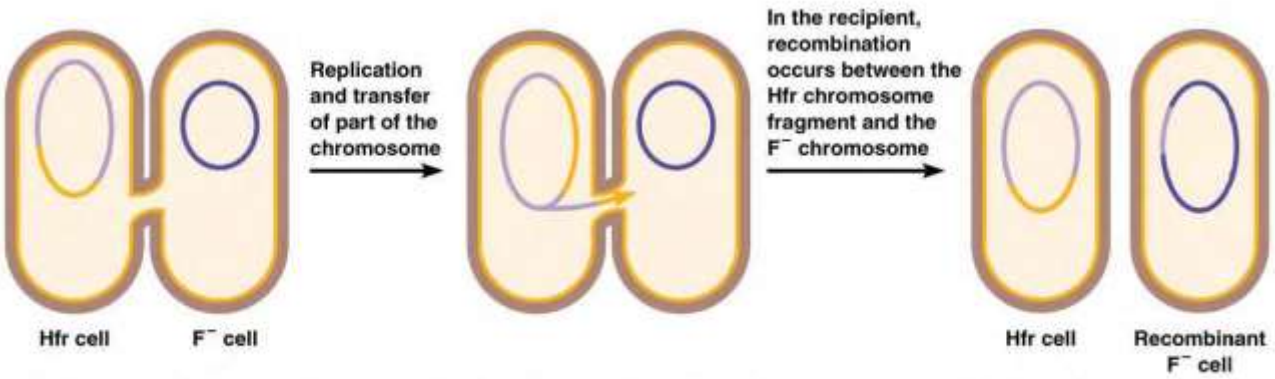
If F factor plasmid is inserted into host chromosome (Hfr cell), this will result in the transfer of the entire DNA complex.



(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.

recipient can incorporate donor cell genes by recombination

****Hfr** = "<u>H</u>igh **<u>f</u>**requency of **<u>r</u>**ecombination**

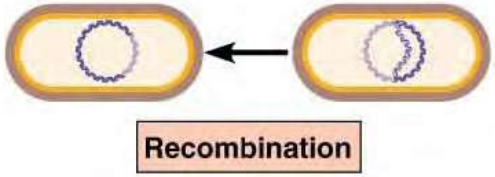


(c) When an Hfr donor passes a portion of its chromosome into an F⁻ recipient, a recombinant F⁻ cell results.

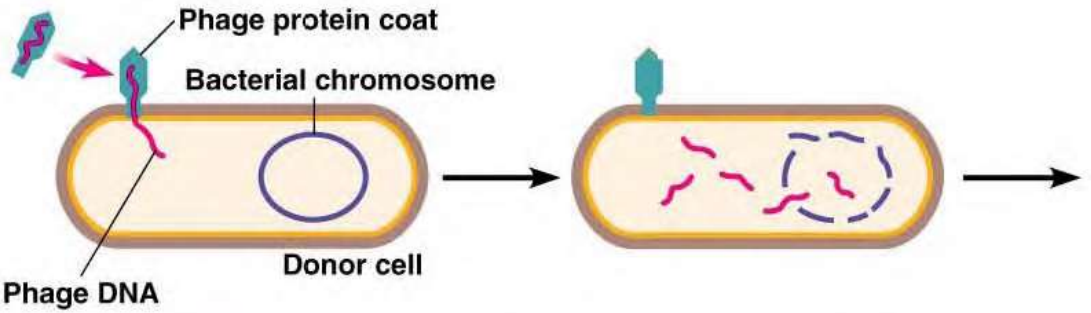
also useful for mapping bacterial genes based on the rate of transfer

Transduction

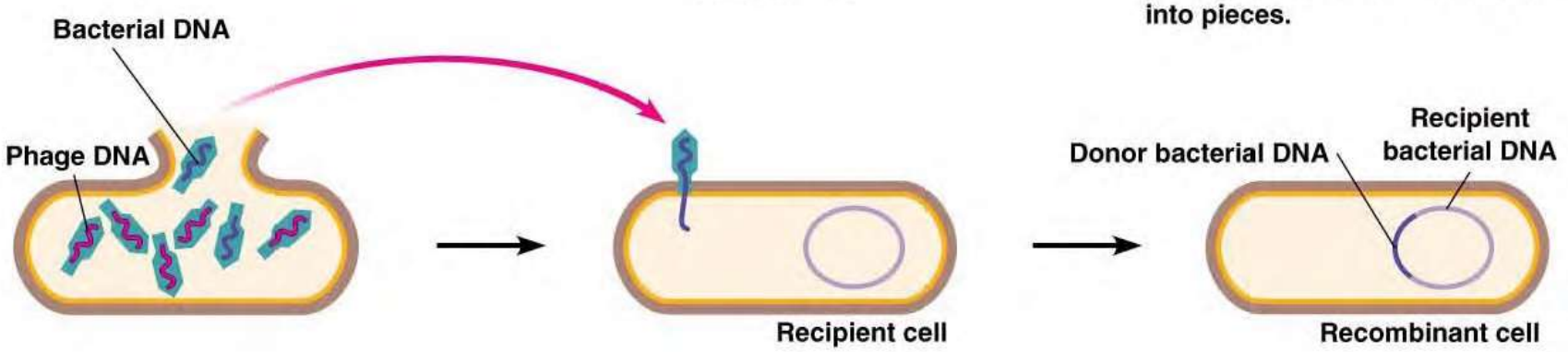
A virus (phage) particle can transfer DNA fragments from one host cell to another followed by recombination



requires a virus to be packaged with bacterial DNA "by mistake"



1. A phage infects the donor bacterial cell.
2. Phage DNA and proteins are made, and the bacterial chromosome is broken into pieces.



3. Occasionally during phage assembly, pieces of bacterial DNA are packaged in a phage capsid. Then the donor cell lyses and releases phage particles containing bacterial DNA.
4. A phage carrying bacterial DNA infects a new host cell, the recipient cell.
5. Recombination can occur, producing a recombinant cell with a genotype different from both the donor and recipient cells.

Episome

•An **episome** is a portion of genetic material that can exist independent of the main body of genetic material (called the chromosome) at some times, while at other times is able to integrate into the chromosome. Examples of **episomes** include insertion sequences and transposons. Viruses are another example of an **episome**.

•An **episome is a plasmid that can exist either** with or without being integrated into the host's chromosome.

•The F factor also has several segments called insertion sequences that assist plasmid integration into the host cell chromosome. Thus the F factor is an episome that can exist outside the bacterial chromosome or be integrated into it.

•The F factor is an episome and can integrate into the bacterial chromosome at several different locations by recombination between homologous insertion sequences present on both the plasmid and host chromosomes. When integrated, the F plasmid's *tra operon is still functional; the plasmid* can direct the synthesis of pili, carry out rolling-circle replication, and transfer genetic material to an F recipient cell. Such a donor is called an **Hfr strain (for high frequency of recombination)** because it exhibits a very high efficiency of chromosomal gene transfer in comparison with F cells. DNA transfer begins when the integrated F factor is nicked at its site of transfer origin.

•Because the F plasmid is an episome, it can leave the bacterial chromosome.

•**Episome**, in bacteria, one of a group of extrachromosomal genetic elements called plasmids, consisting of DNA and capable of conferring a selective advantage upon the bacteria in which they occur. Episomes may be attached to the bacterial cell membrane (such a cell is designated F^+) or become integrated into the chromosome (such a cell is designated Hfr). F^+ and Hfr cells act as donors during conjugation, a mating process in certain bacteria (*e.g., Escherichia, Salmonella, Serratia, Pseudomonas*). During conjugation, cells lacking the episome (called F^- cells) may receive either the episome (from an F^+ cell) or the episome plus the chromosomal genes to which it is attached (from an Hfr cell).

•Some bacterial viruses, called temperate phages, carry DNA that can act as an episome. A bacterial cell into whose chromosome the viral DNA has become integrated is called a prophage.

Episome

•An episome is distinguished from other pieces of DNA that are independent of the chromosome (i.e., plasmids) by their large size.

•Plasmids are different from episomes, as plasmid DNA cannot link up with chromosomal DNA. The plasmid carries all the information necessary for its independent replication. While not necessary for bacterial survival, plasmids can be advantageous to a bacterium. For example, plasmids can carry genes that confer resistance to antibiotics or toxic metals, genes that allow the bacterium to degrade compounds that it otherwise could not use as food, and even genes that allow the bacterium to infect an animal or plants cell. Such traits can be passed on to another bacterium.

•Transposons and insertion sequences are episomes. These are also known as mobile genetic elements. They are capable of existing outside of the chromosome. They are also designed to integrate into the chromosome following their movement from one cell to another. Like plasmids, transposons can carry other genetic material with them, and so pass on resistance to the cells they enter. Class 1 transposons, for example, contain drug resistance genes. Insertion sequences do not carry extra genetic material. They code for only the functions involved in their insertion into chromosomal DNA.

•Transposons and insertion sequences are useful tools to generate changes in the DNA sequence of host cells. These genetic changes that result from the integration and the exit of the mobile elements from DNA, are generically referred to as mutations. Analysis of the mobile element can determine what host DNA is present, and the analysis of the mutated host cell can determine whether the extra or missing DNA is important for the functioning of the cell.

Gene Mapping in Bacteria

Three methods of Recombination in Bacteria:

- Conjugation
- Transformation
- Transduction: Generalized transduction & Specialized transduction

Conjugation

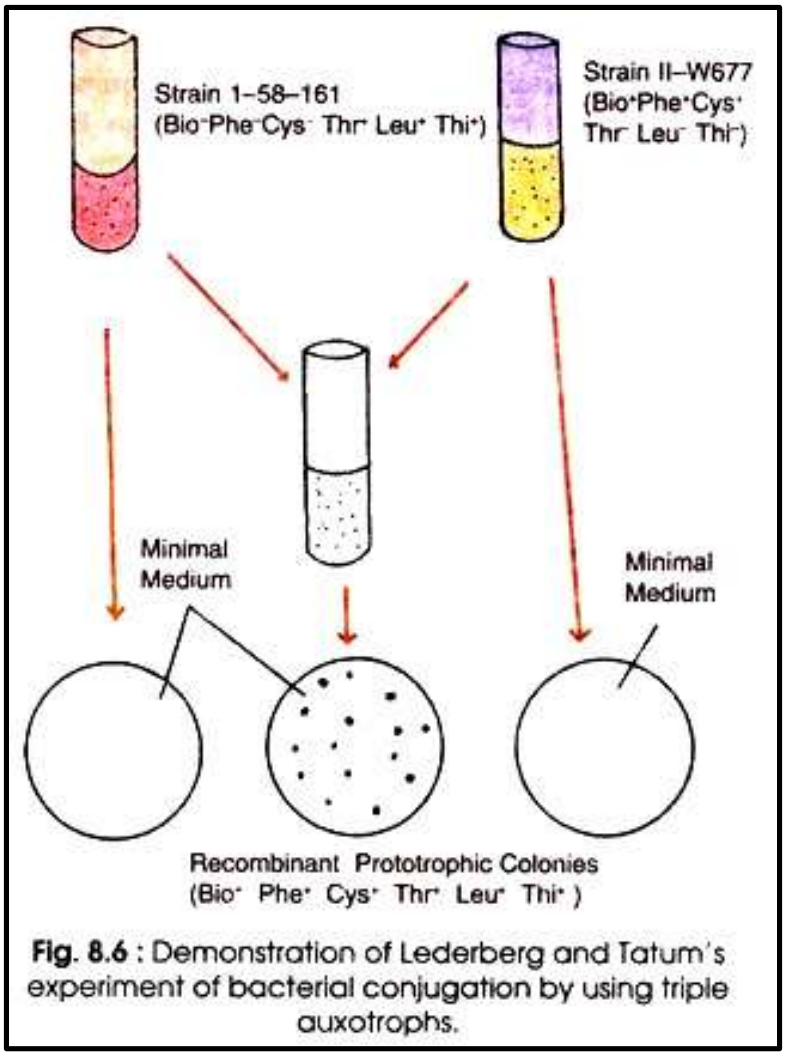


Fig. 8.6 : Demonstration of Lederberg and Tatum's experiment of bacterial conjugation by using triple auxotrophs.

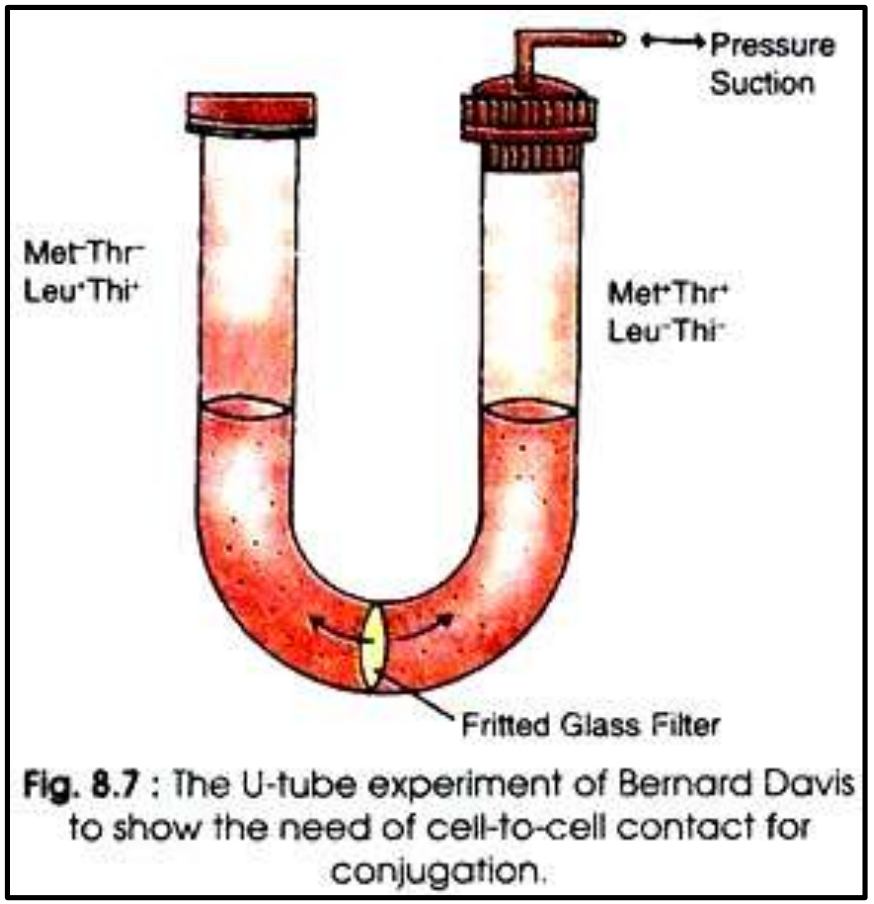


Fig. 8.7 : The U-tube experiment of Bernard Davis to show the need of cell-to-cell contact for conjugation.

Lederberg and Tatum (1946) could not give the proof for physical contact of cells required for gene transfer. The evidence for cell-to-cell contact was provided by Bernard Davis (1950) .

Conjugation Transfer of the Sex-Factor F

•Unidirectional transfer of genetic material between donar and recipient bacterial cells by direct contact.

•Segments (rarely all) of the donor's chromosome recombines with the homologous chromosome.

•Recipients containing donor DNA are called transconjugants.

•Genetic transfer is mediated by sex factor F.

•Donor is F^+ and recipient is 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 haie-like cell surface (F-pili or sex-pili), which aid in contact between cells.

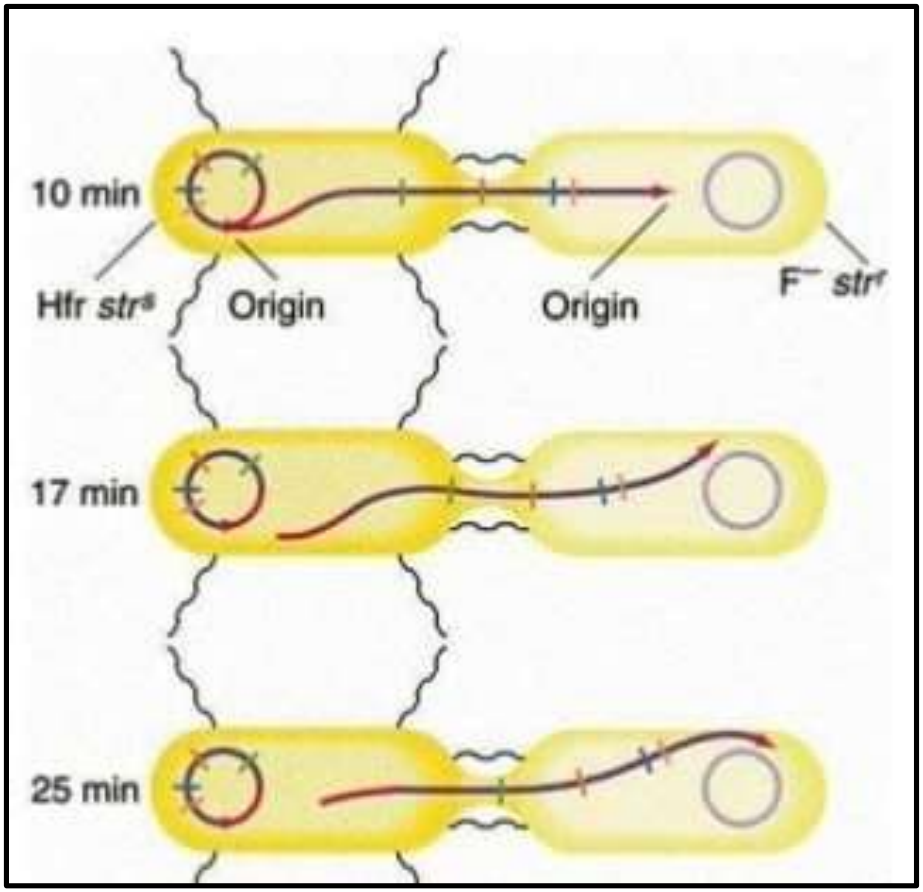
•No conjugation can occur between cells of the mating type.

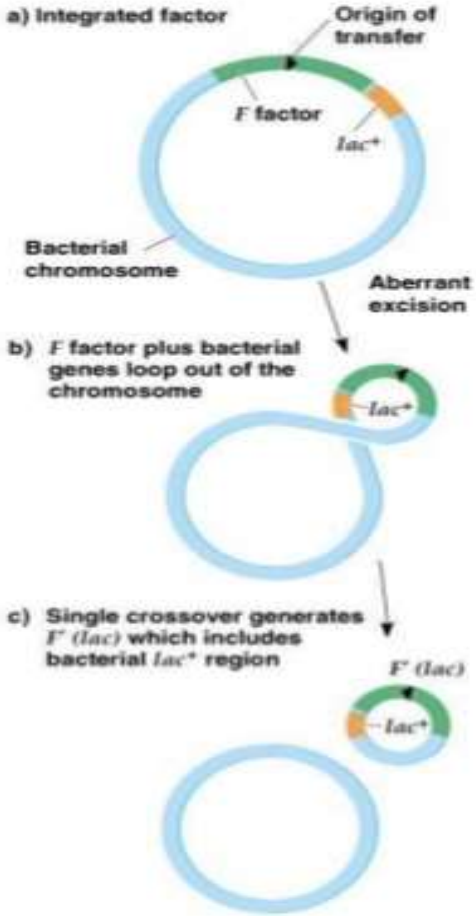
•Conjugation begins when the F plasmid is nicked at the origin, and a single strand is transferred using the rolling circle mechanism.

•When transfer is complete, both cells are F^+.

Transfer of the chromosome by Hfr during conjugation

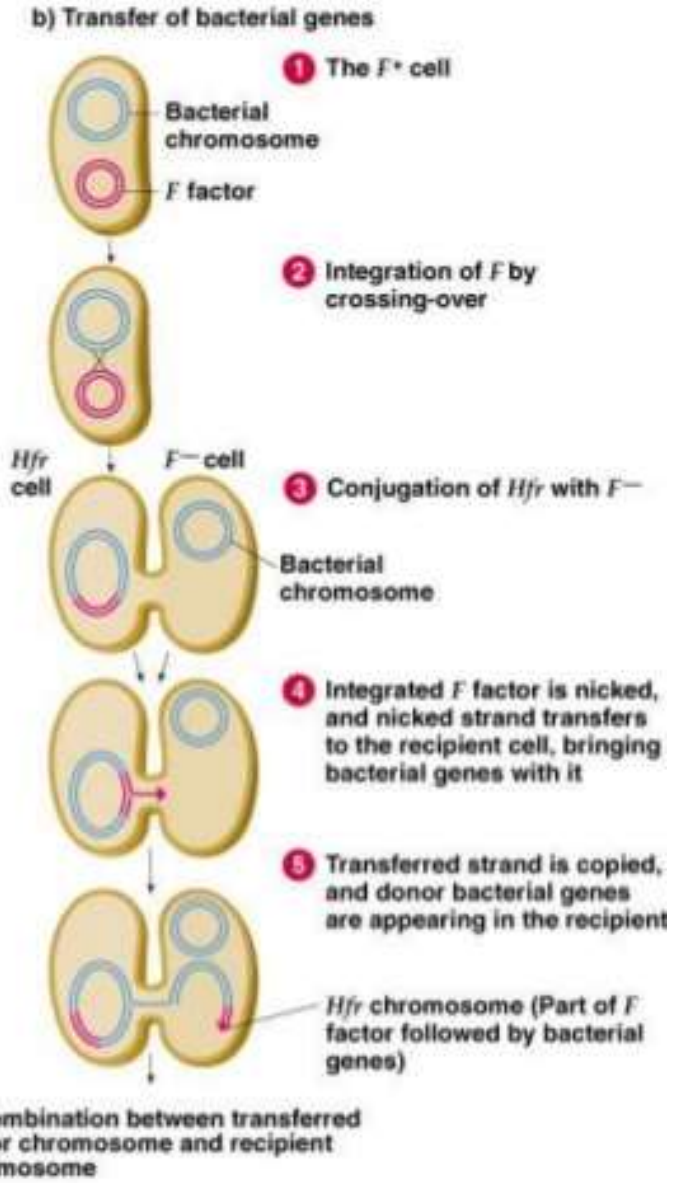
- Frequency 10^{-4} per each Hfr cell.
- Hfr strains replicate F factor as part of their main chromosome.
- Complete F^+ sequence (or complete chromosomal DNA) is rarely transferred (1/10000) because bacteria separate randomly before DNA synthesis completes.
- Recombinants are produced by cross-over of the recipient chromosome and the donar DNA containing F^+.





Transfer of the Hfr F+ factor

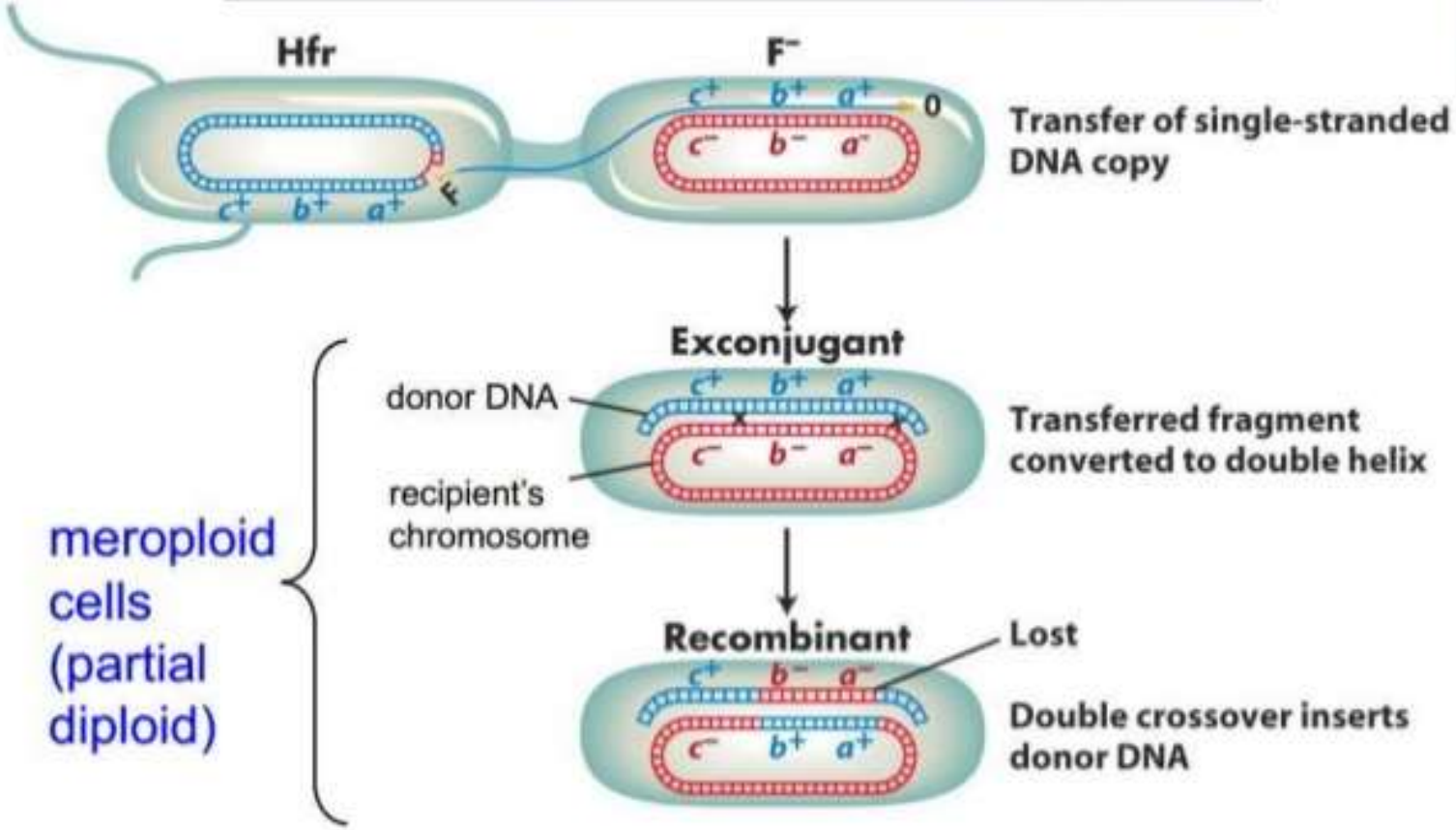
- Longer period- not all genes transferred
- F- cell remains F-



Excision of the F+ factor also occurs spontaneously at low frequency

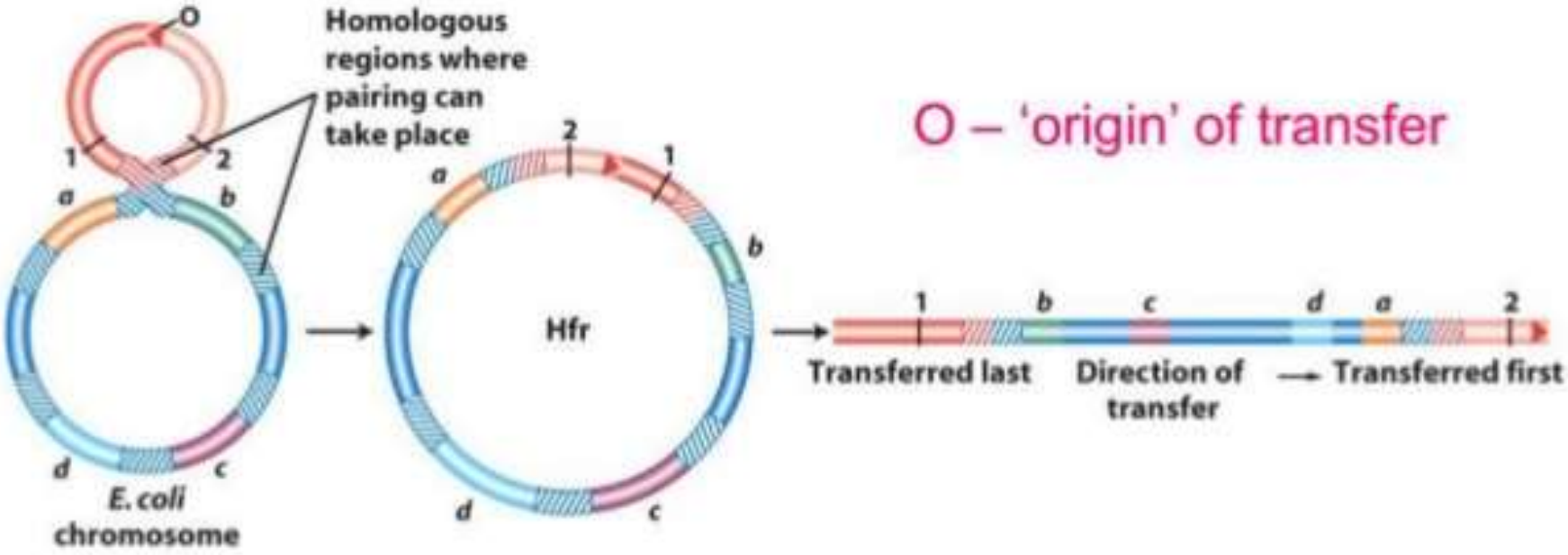
- Begin with Hfr cell containing F+
- Small section of host chromosome also may be excised, creating an F' plasmid
- F' plasmid is named for the gene it carries. e.g., F' (lac)

Recombination during bacterial conjugation



Single crossover would make the chromosome linear and the cell would die

Insertion of F factor into bacterial chromosome



Merozygotes (partial diploid cells)

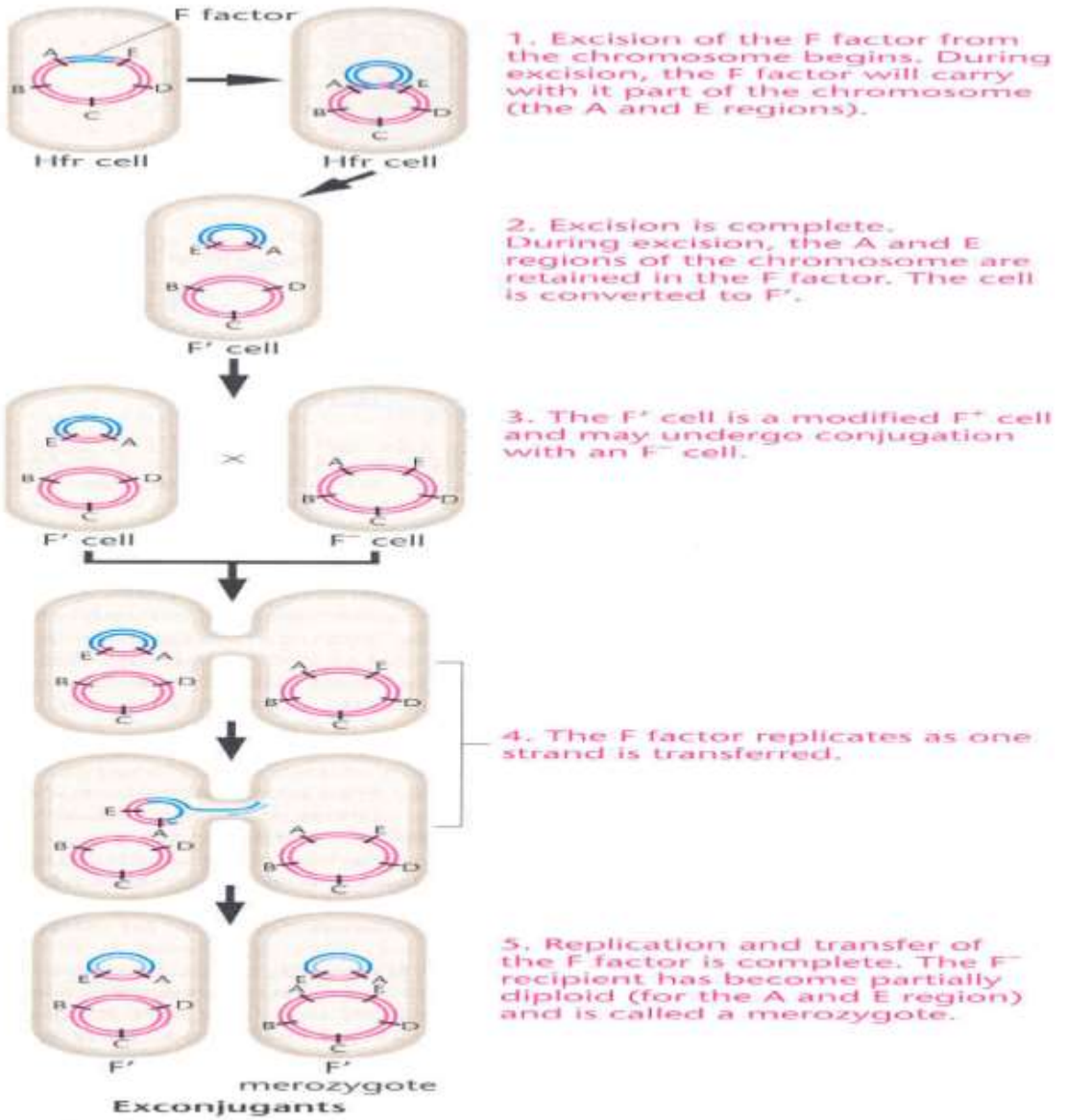
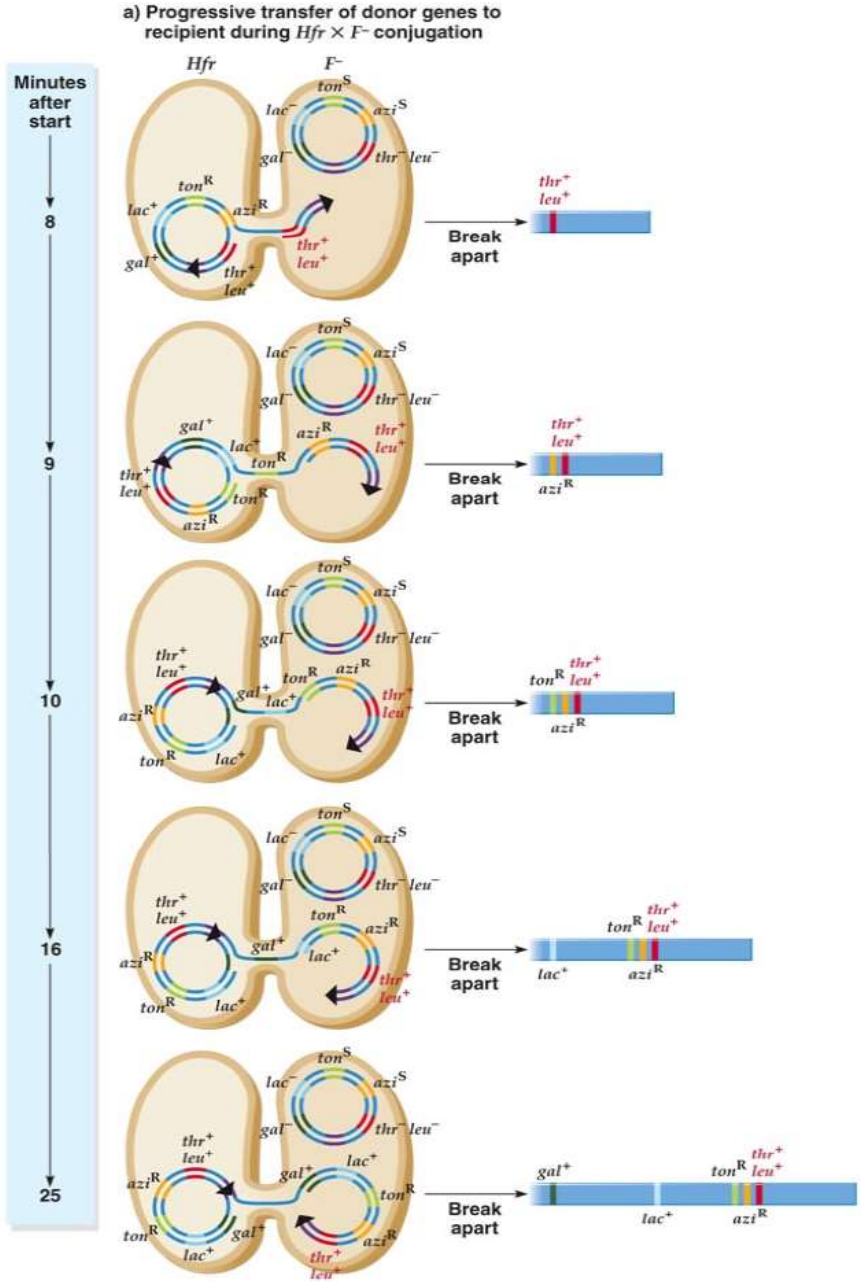
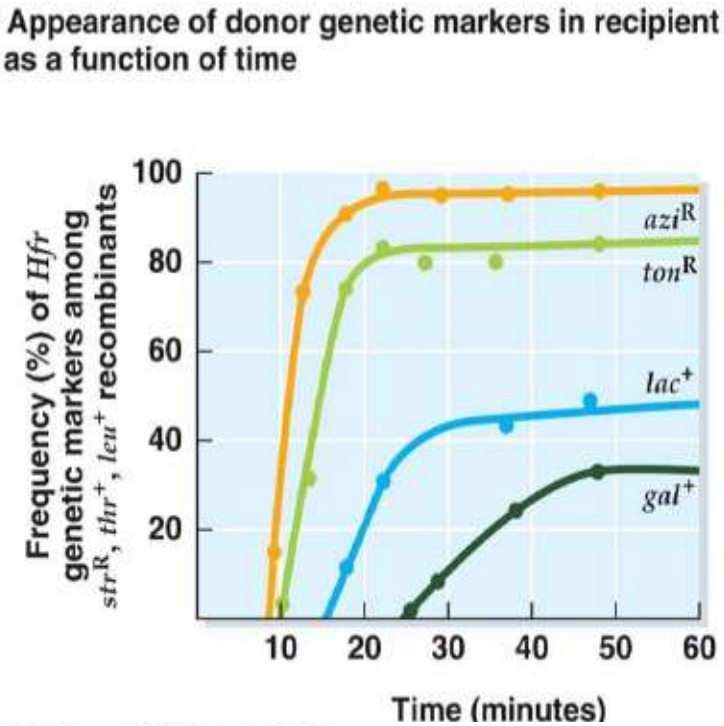
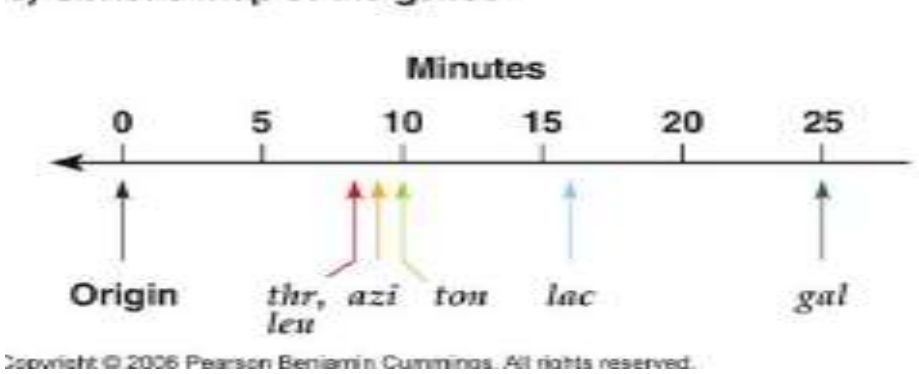


FIGURE 16–11 Conversion of an Hfr bacterium to F′ and its subsequent mating with an F⁻ cell. The conversion occurs when the F factor loses its integrated status. During excision from the chromosome, the F factor may carry with it one or more chromosomal genes (*A* and *E*). Following conjugation with an F⁻ cell, the recipient cell becomes partially diploid and is called a merozygote. It also behaves as an F⁺ donor cell.

Gene Mapping





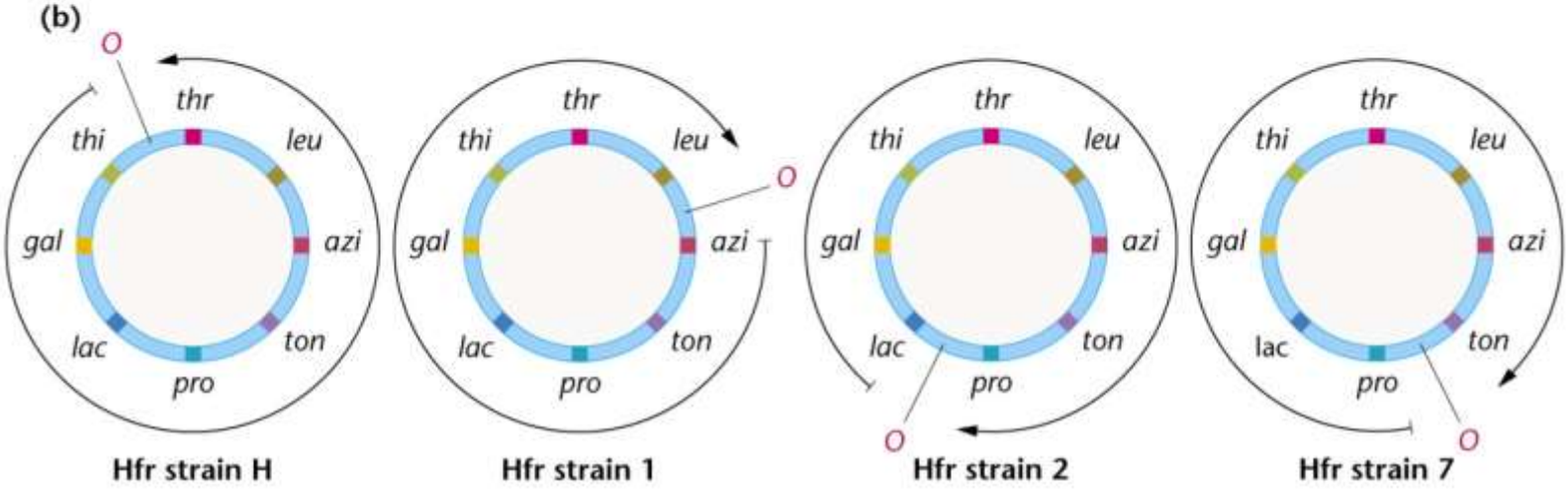


Gene Mapping

(a)

Hfr strain	Order of transfer (Earliest → Latest)													
H	*thr*	–	*leu*	–	*azi*	–	*ton*	–	*pro*	–	*lac*	–	*gal*	–	*thi*
1	*leu*	–	*thr*	–	*thi*	–	*gal*	–	*lac*	–	*pro*	–	*ton*	–	*azi*
2	*pro*	–	*ton*	–	*azi*	–	*leu*	–	*thr*	–	*thi*	–	*gal*	–	*lac*
7	*ton*	–	*azi*	–	*leu*	–	*thr*	–	*thi*	–	*gal*	–	*lac*	–	*pro*

(b)



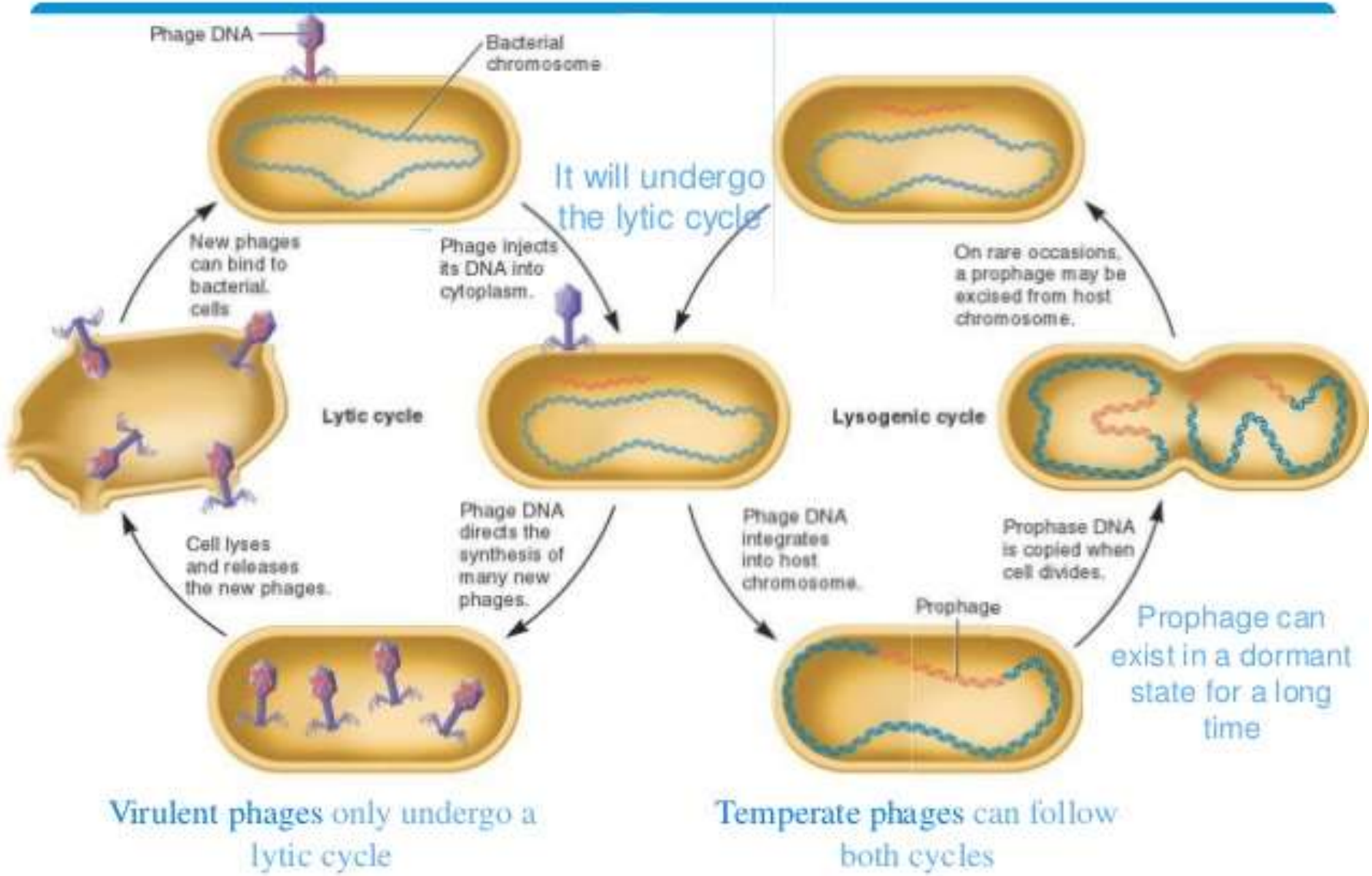
Transduction

•Transduction is the transfer of DNA from one bacterium to another via a bacteriophage

•A bacteriophage is a virus that attacks bacterial cells

•It is composed of genetic material surrounded by a protein coat.

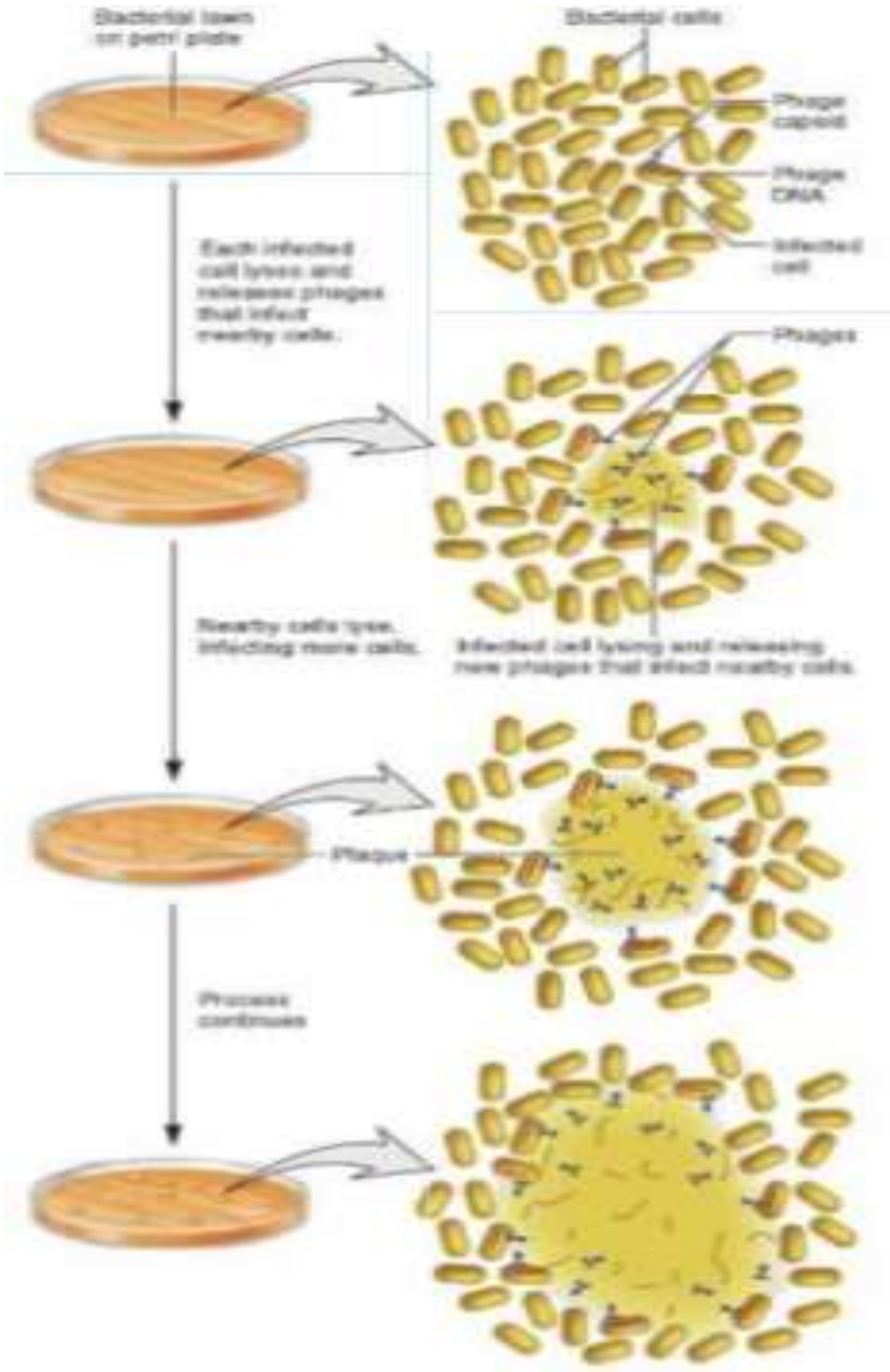
•It can undergo two types of cycles
-Lytic
-Lysogenic



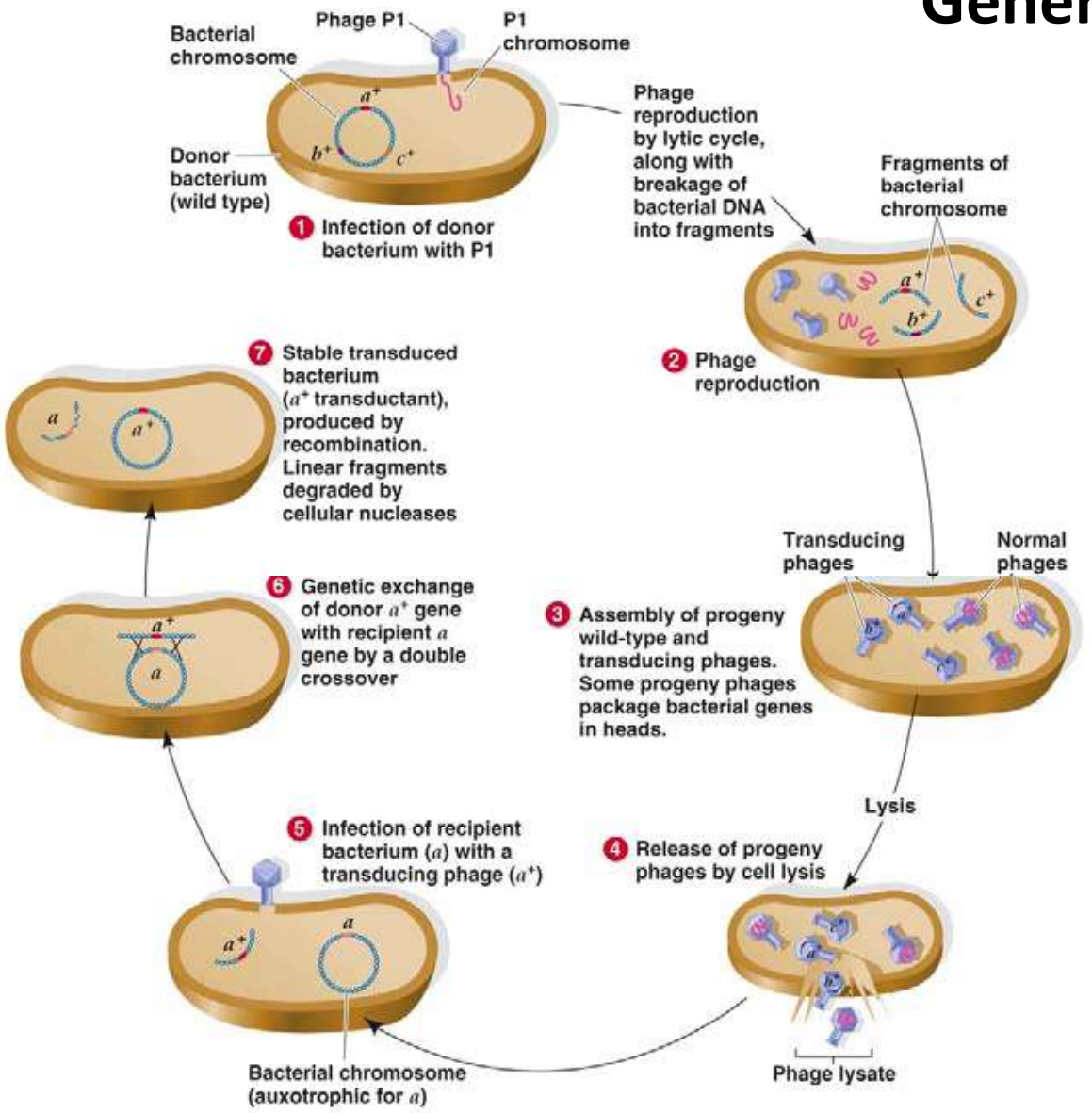
Plaques

•A plaque is a clear area on an otherwise opaque bacterial lawn on the agar surface of a petridish

•It is caused by the lysis of bacterial cells as a result of the growth and reproduction of phages



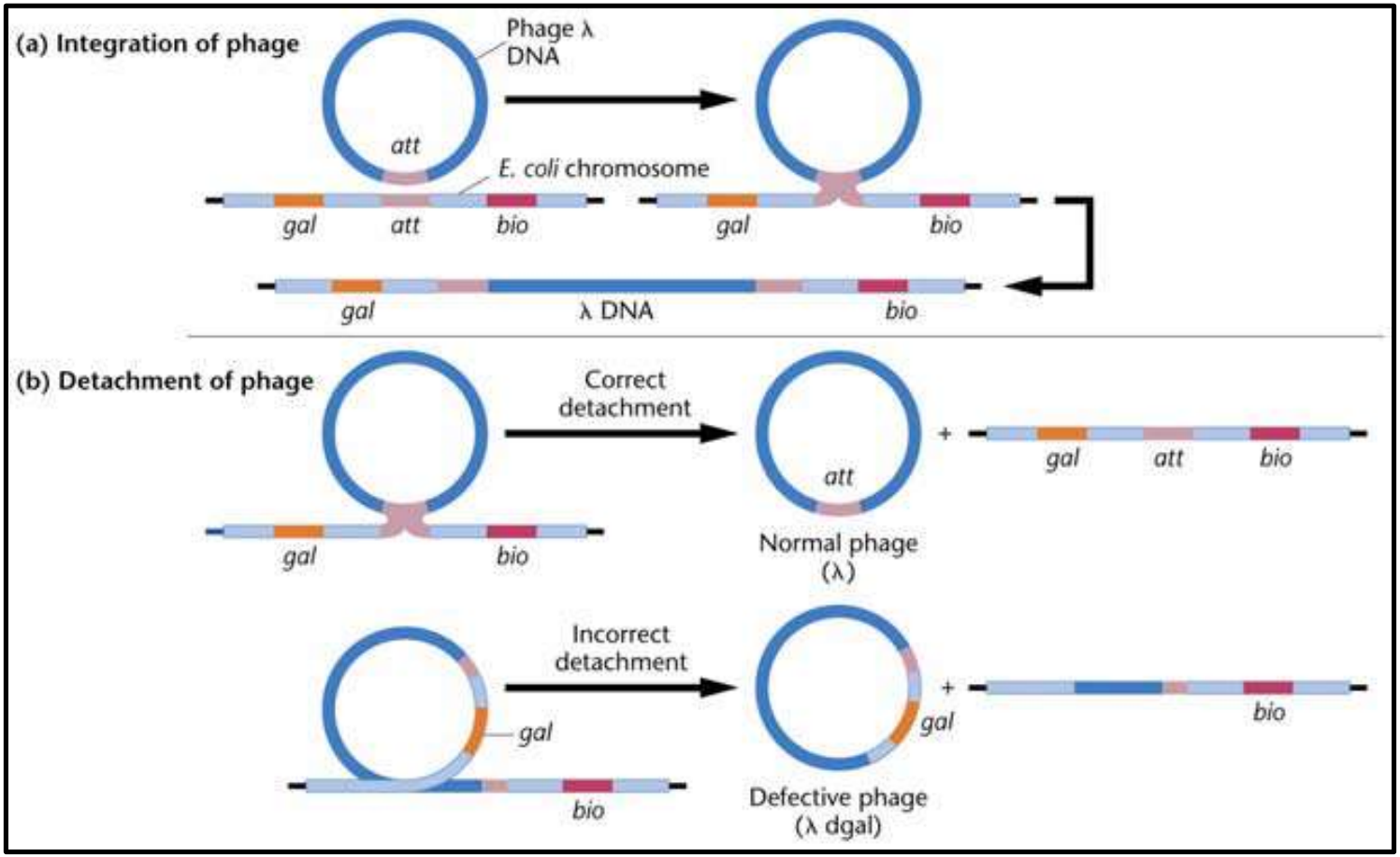
Generalized transduction

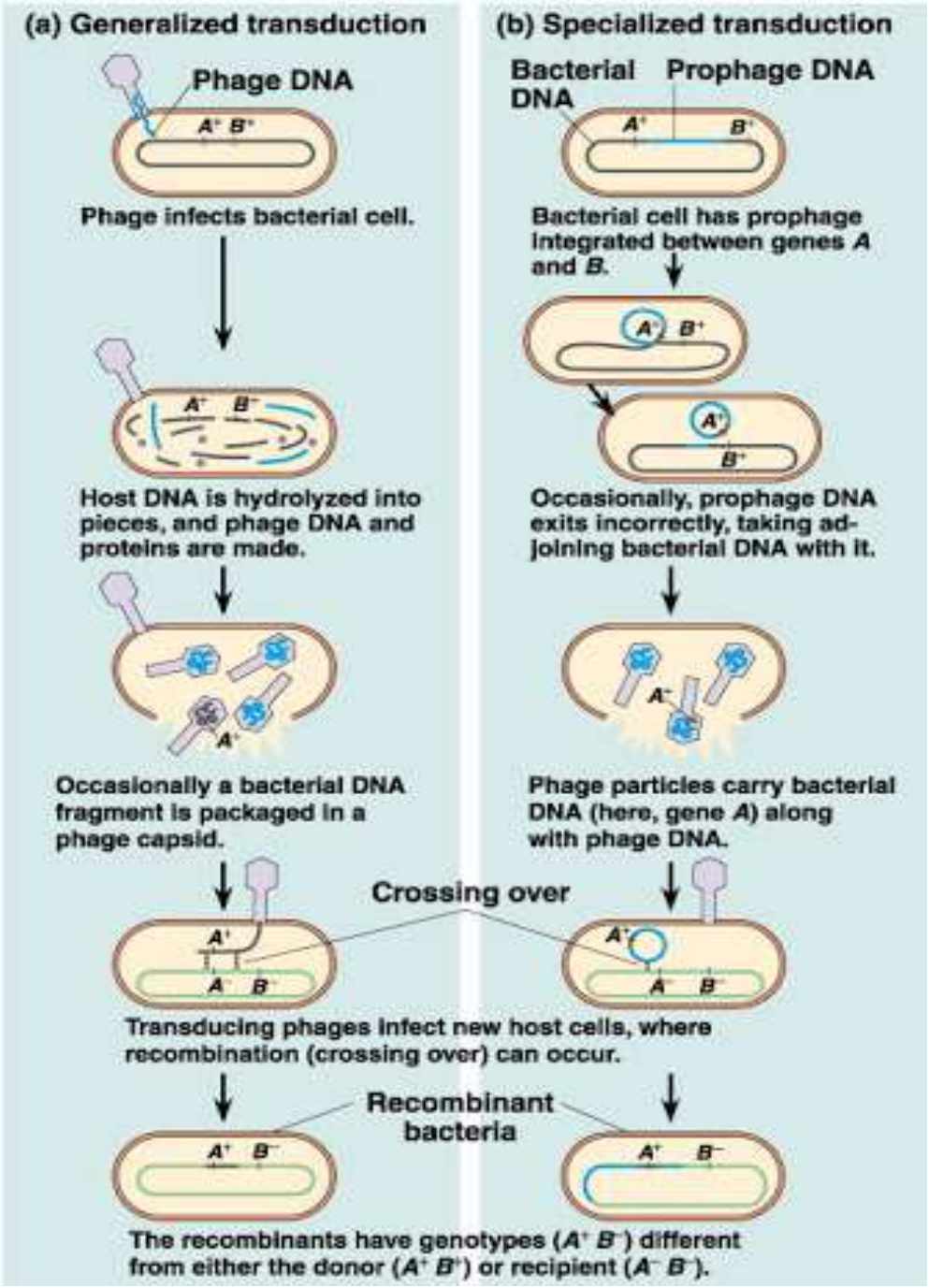


- Any piece of bacterial DNA can be incorporated into the phage (random incorporation).
- This type of transduction is termed generalized transduction.
- Lytic cycle of virus

Specialized Transduction

- Lysogenic cycle of virus
- Incorporates into bacterial genome
- Excises out; can carry bacterial genes
- More specific genes





Mapping Genes Using Bacteriophage

•Infect bacteria with phages of different genotypes using two, three or four gene crossesii.e., crossover.

•Count recombinant phage phenotypes by determining differences in cleared areas (no bacterial growth) on a bacterial lawn.

•Different phage genes induce different types of clearing (small/large clearing with fuzzy/distinct borders).

Transformation

•Bacteria take up extracellular DNA

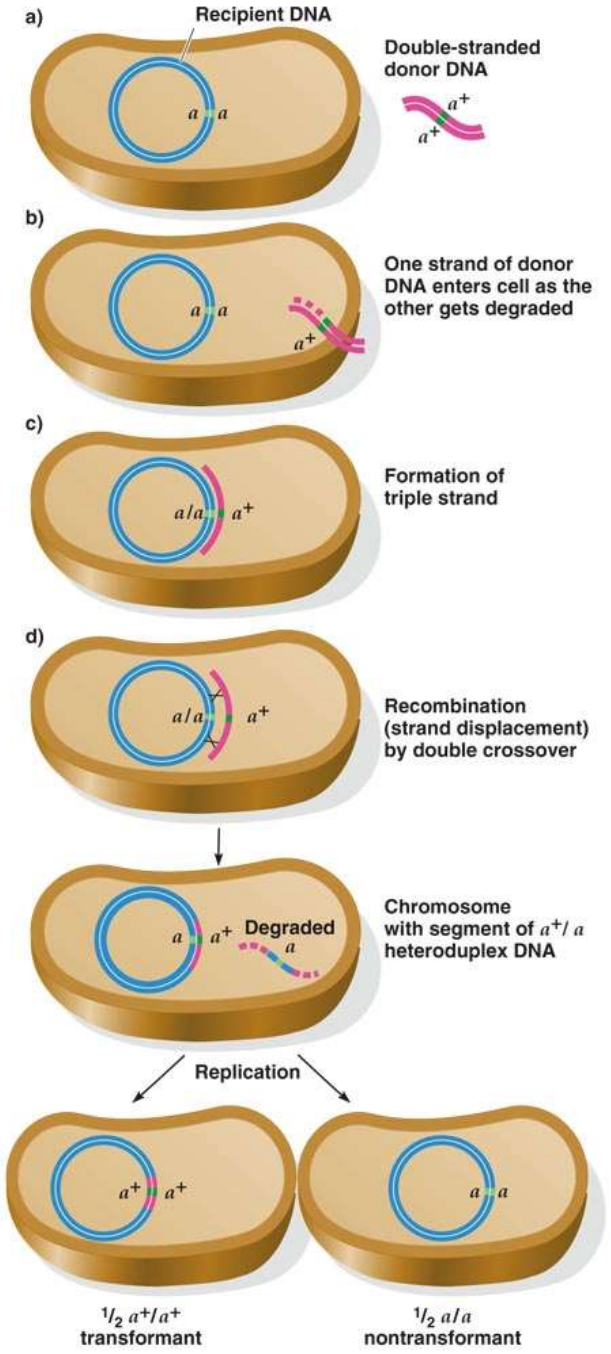
•Discovered by Frederick Griffith, 1928

•There are two types
-Natural transformation
DNA uptake occurs without outside help
-Artificial transformation
DNA uptake occurs with the help of special techniques

•Natural transformation occurs in a wide variety of bacteria

•It depends on competence of cells

•The prerequisite for **bacteria** to undergo **transformation** is its ability to take up free, extracellular genetic material. Such **bacteria** are termed as **competent** cells.



•If the DNA that enters the cell is homologous to a portion of gene on the chromosome then that DNA will be incorporated into the genome by homologous recombination

•Sometimes, the DNA that enters the cell is not homologous to any genes on the chromosome

•It may be incorporated at a random site on the chromosome- the process termed as non-homologous recombination

•Like cotransduction, transformation mapping is used for genes that are relatively close together

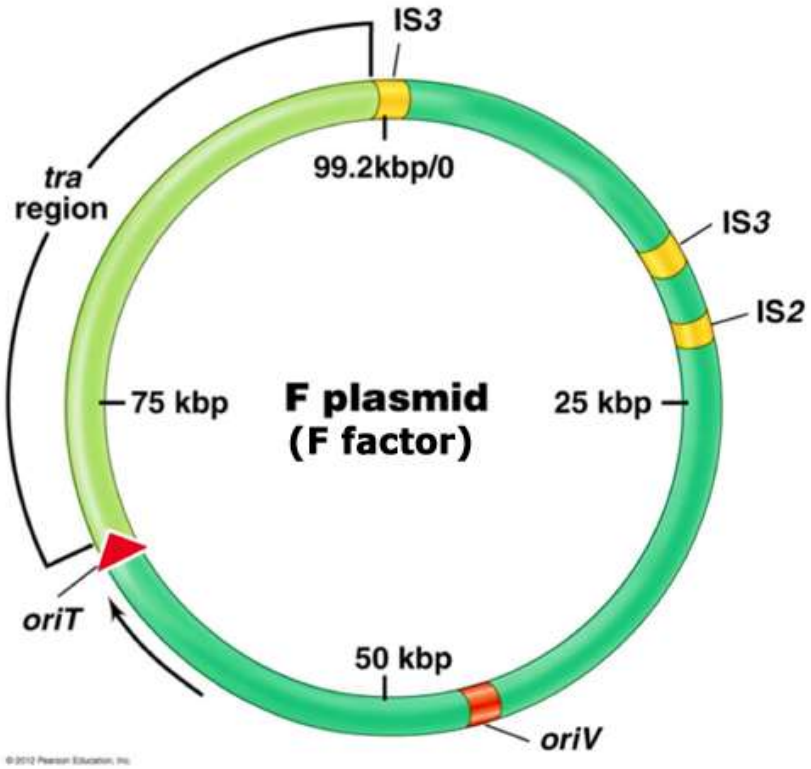
Mapping Using Transformation

1. Recombination frequencies are used to infer gene order.

   p+ q+ o+ x p q o

2. If p+ and q+ frequently cotransform, order is p-q-o.

3. If p+ and o+ frequently cotransform, order is p-o-q.



The F plasmid belongs to a class of plasmids that control sexual functions in bacteria. The F plasmid consists of

***OriT* (Origin of Transfer):** starting point of conjugative transfer

***OriV* (Origin of Vegetative Replication):** start of sequence replicated in a recipient cell

***tra*-region (transfer genes):** genes encoding the F-Pilus and DNA transfer elements.

IS (Insertion Sequences): DNA fragments ("selfish genes") that integrate copies of themselves into a recipient chromosome