MEDICAL MICROBIOLOGY

Lec. 3
The Growth, Survival & Death of Microorganisms and bacterial genetics

By:

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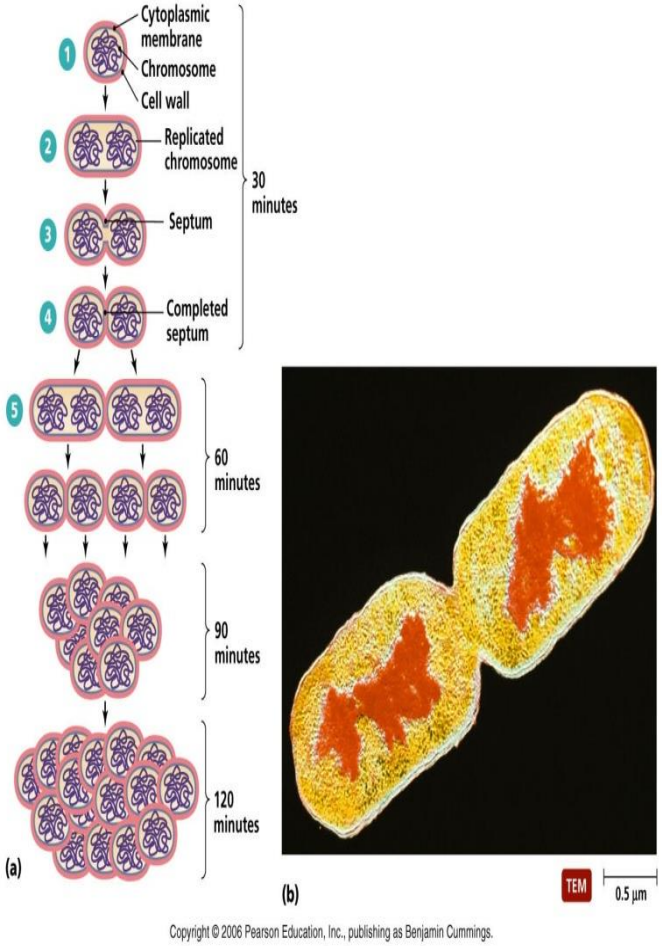
The meaning of growth:

- Growth is the orderly increase in size and the sum of all the components of an organism. This is normally followed by multiplication.
- In unicellular organisms, growth leads to an **increase in the number of individuals** making up a population or culture.
- Bacteria reproduce by ***Binary fission,*** a process by which one parent cell divide to form two progeny cells.
- The time required for a bacterium to give rise to two daughter cells under optimum conditions is known as ***generation time*** or population doubling time.

The growth



The growth curve:

The growth curve of bacteria has four major phases (lag, log or exponential growth, stationary, and decline or death phases).

❖ The lag phase:

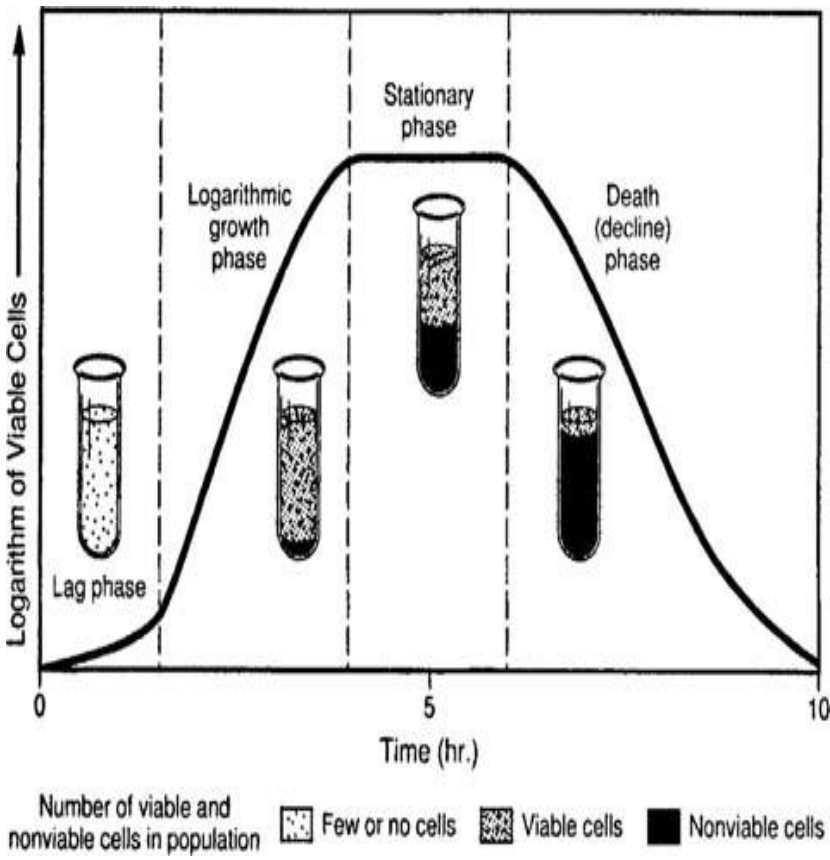
The lag phase represent a period during which vigorous metabolic activity occurs but cells do not divide. This can last for few minutes up to many hours.

❖ The Exponential phase:

During this phase, the cells are in a steady state. New cells materials are being synthesized at a constant rate & catalytic & the masses increase in exponential manner. This continues until one of two things happens:

1. One or more nutrients in the medium become exhausted.
2. 2. Accumulation of toxic metabolic products & inhibit growth.

Growth curve



❖ The stationary phase:

The exhaustion of nutrients or the accumulation of toxic products finally causes growth to slow until the number of new cells produced balances the cells that die. When this occurs, the total cell count slowly increases although the viable count stays constant.

❖ The decline or death phase:

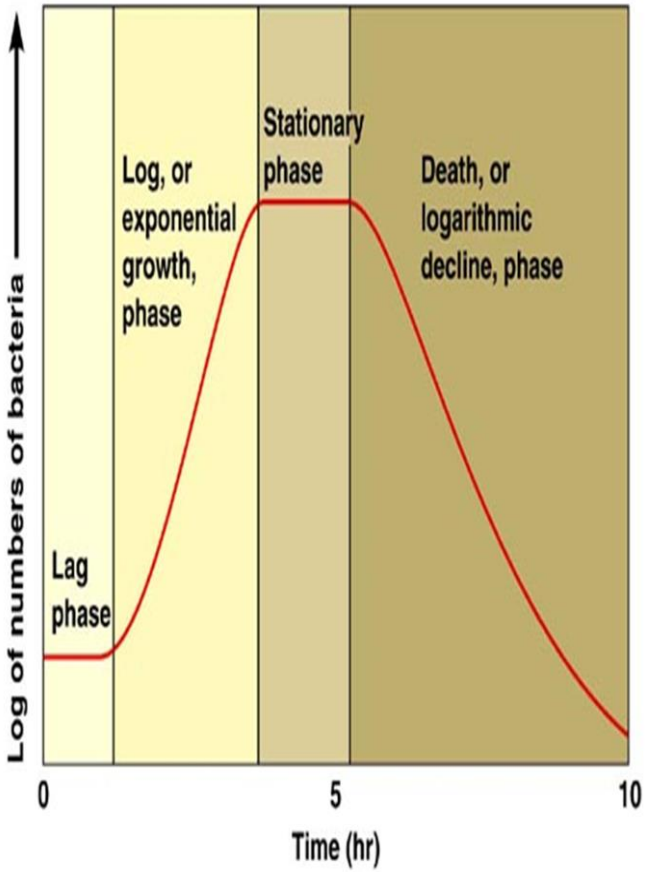
It is marked by a decline in the number of viable bacteria due to death of cells.

Death occurs due to:

- Nutritional exhaustion.
- Accumulation of toxic metabolites.
- Autolytic

➢ A small number of survivors may persist for months. This persistence may reflect cell turnover, a few cells growing at the expense of nutrients released from cells that die & lyses.

Growth curve



The meaning of death:

For microbial cells, death means the irreversible loss of the ability to reproduce (growth & division).

Maintenance of cells in the exponential phase:

Cells can be maintained in exponential phase by transferring them repeatedly into fresh medium of identical composition while they are still growing exponentially.

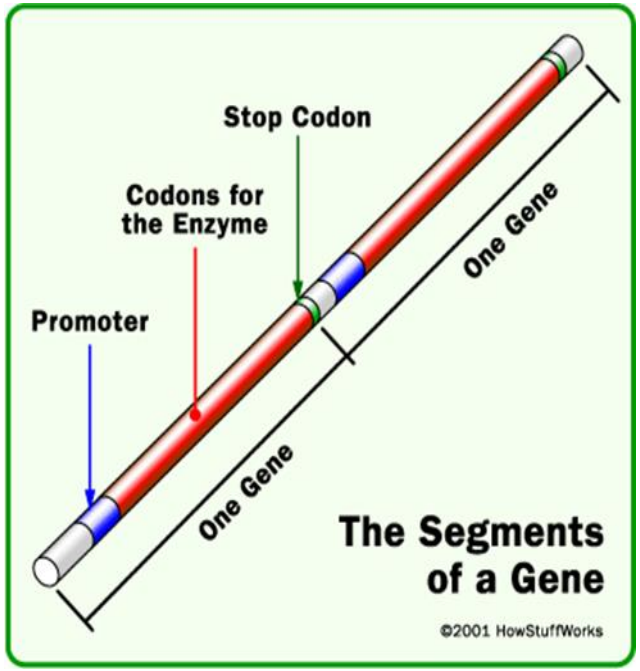
Continuous culture (chemostat):

It is a system in which a continuous supply of fresh nutrients into the culture vessels and continuous removal of the grown bacteria

The meaning of death

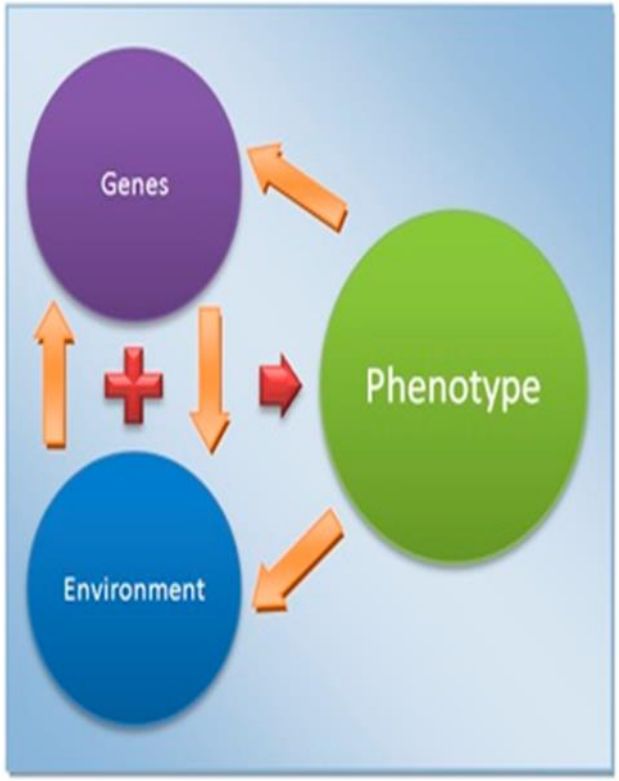
Bacterial genetics

- **Genetics** is the science that defines & analyzes heredity & variation. It aims to understanding the structure & functions of microbial genome, its gene products & their role in infection & disease.
- The unit of heredity is the **gene**.
- The **gene** is a segment or portion of DNA that carries in its nucleotide sequence information for a specific biochemical or physiological property, through coding for a single polypeptide sequence.
- Bacteria are (Haploid)= **single circular chromosome** consisting of double-stranded DNA, therefore having a single copy of each gene.



Bacterial genetics

- **Phenotype** refers to the observable properties (or characters) of an organism which are produced by interaction of genotype with the environment, i.e. the effect of both genes & environment. These include the structural & physiological properties of a cell or an organism (e.g. the eye color in human, resistance to an antibiotic in bacterium).
- **Genotype** refers to the genetic constitution of an organism.
- **Genome** is the sum of the genes of an organism, or the totality of genetic information in an organism.
- **Gene expression** refers to a gene product that can be observed under appropriate condition at the level of phenotype.

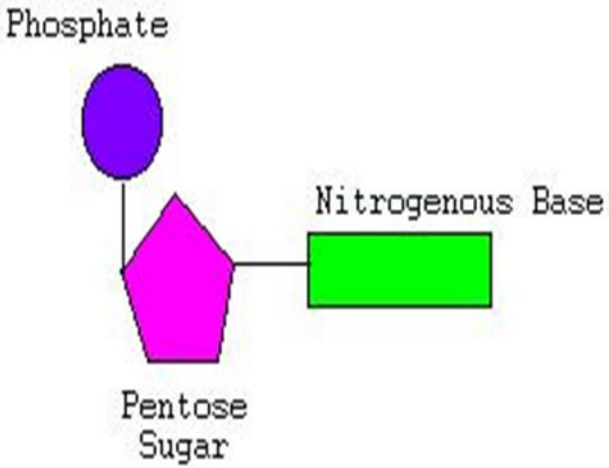


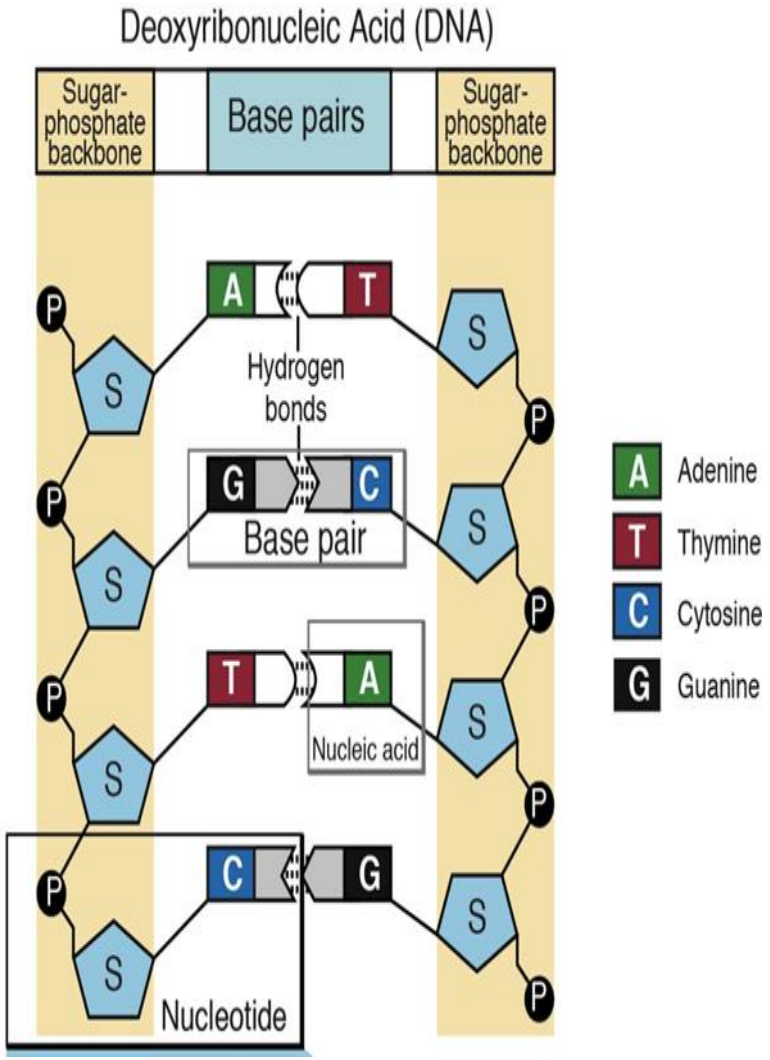
Bacterial genetics

The genomic structure consists of 3 types of genetic information: **chromosome, plasmids** and **bacteriophages**, the later two structures provide additional genetic information & are transient in some instances.

1- Chromosomal structure:

- Double helix
- DNA strand structure
- Nucleotide structure
- Four nitrogen base (A, T, G, C)





Bacterial genetics

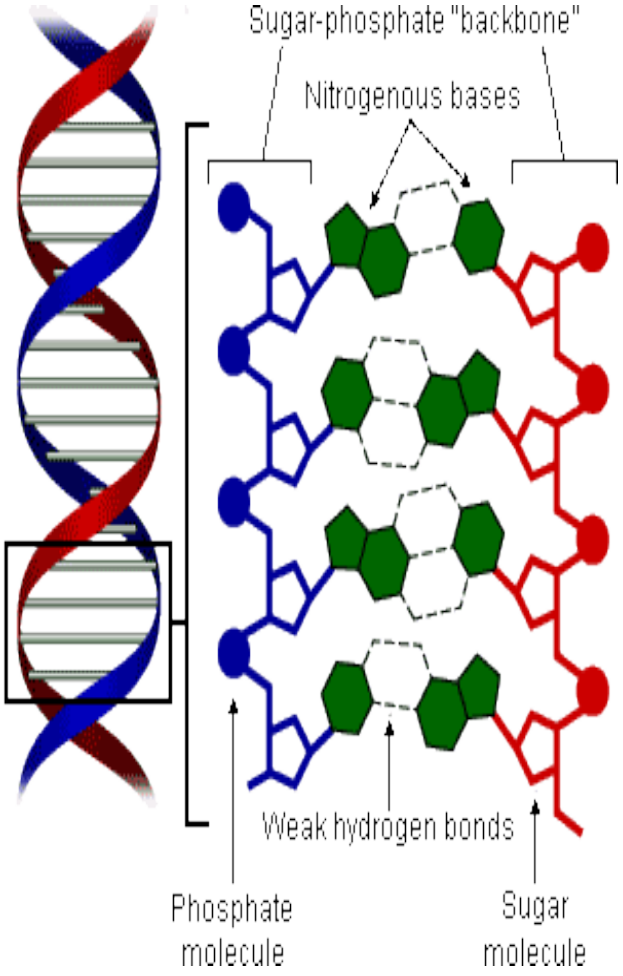
Replication of bacterial DNA:

As bacteria replicate by binary fission, the daughter cells produced are usually indistinguishable genetically. Replication of chromosome in bacteria begins at a specific location called origin of replication.

During replication, the purine [**adenine (A) & guanine (G)]** & pyrimidine [**thymine (T) & cytocine (C)**] Each of the 4 bases is bound to phospho-deoxyribose to form a nucleotide.

Adenine always pairs with thymine and the guanine pairs with cytocine by hydrogen bonds in the center of the molecule.

nucleotides in each DNA strand are accurately copied into two new ds daughter molecules. Each of these molecules is composed of a strand from the parent molecule & a newly synthesized complementary strand, a process termed **semi-conservative replication.**

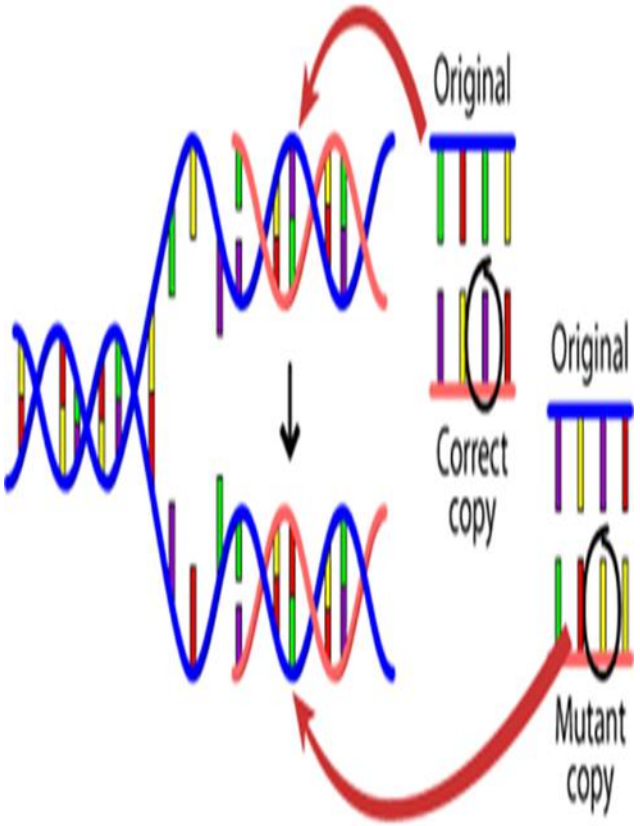


Bacterial genetics

Semi-conservative replication:

As the two parent strands of the helical DNA unwind under the influence of the enzyme **DNA helicase**, each acts as a template for the synthesis of a complementary strand. In this manner, two identical helical DNA molecules are formed through the action of replicating enzyme **DNA polymerase**. The ends of the new fully formed strands are joined by **DNA ligase.**

RNA most frequently occurs in single stranded form. The base **uracil (U)** serves in RNA the hybridization function that thymine (T) serves in DNA. So the complementary bases that determine the structure of RNA are A-U and C-G.



Mechanisms of genetic variation:

Mutations: are changes in the DNA sequence.

Mutations can occur due to **base substitution, frame shift, and transposon or insertion mutation**.

- **Base substitution mutation:** one base is inserted in place of another during DNA replication, due to an error in DNA polymerase function or mutagen alters the hydrogen bonds of the base being used as a template. It can be divided to:

- **Missense:** when the mutation results in a codon that simply causes a different amino acid
- **Nonsense:** when the base substitution results in a termination codon that stops protein synthesis.

- **Frame shift mutation :** due to insertion or deletion of one or more base pair.

- **Transposon or insertion sequence mutation:**

It results when transposon or or insertion sequence are integrated into DNA and cause a profound changes in the genes into which they inserted or alter the expression of nearby genes.

Spontaneous mutations for a given gene mutation generally occur with a frequency of 10^{-8} - 10^{-6} in a population derived from a single bacterium.

Mutagens: the frequency of mutation is greatly enhanced by exposure of cells to mutagens.

- **Physical mutagen**:

- Ultraviolet light (UV) that damage DNA by linking neighboring thymine bases to form dimer.
- X-ray that damage the DNA by either forming free radicals, braking bonds that holds DNA backbones, or altering the electron in the bases and changing their hydrogen bonds.

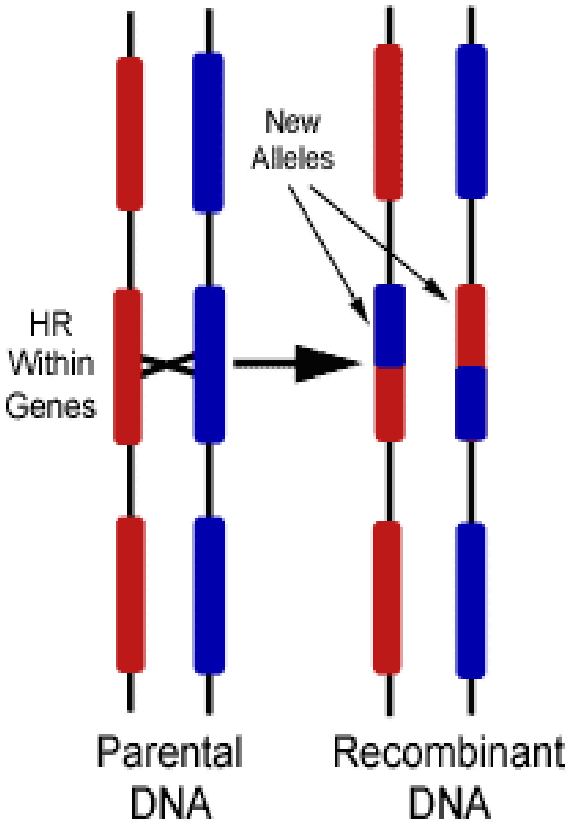
- **Chemical mutagens** altering either the chemical or the physical structure of DNA, or acting as base analogues.
- **Biological mutagens** refers to those pathogens that are able to induce alterations in the host DNA.

Bacterial genetics

- **Conditional-lethal mutations:** it indicates that the mutation is expressed only under certain conditions.
- **Temperature-sensitive mutation:** the temperature- sensitive organism can grow at permissive temperature (32° C) but can not grow at restrictive temperature (37 ° C).

Recombination:

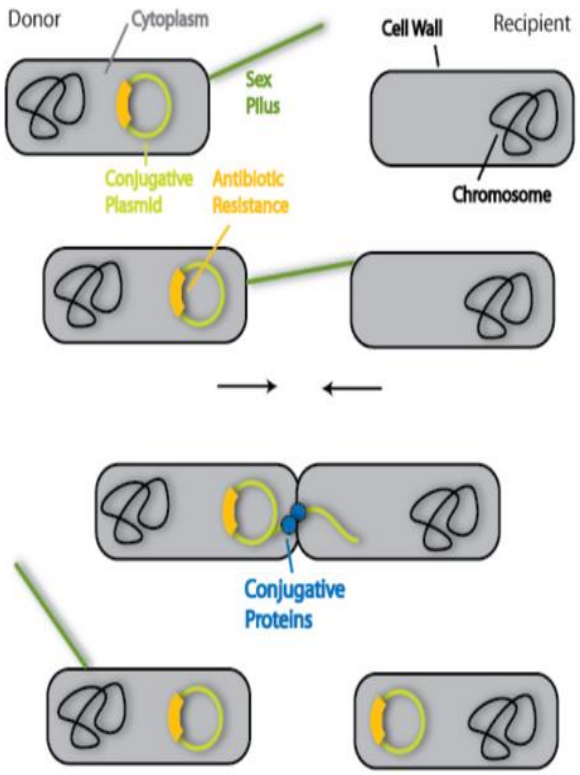
Recombination occurs when sequences of DNA from 2 separate sources are integrated in bacteria, recombination includes an unexpected inheritable change due to introduction of new genetic material from a different cell. This genetic material can be introduce by: **Conjugation, Transduction or Transformation.**



Bacterial genetics

1. Conjugation:

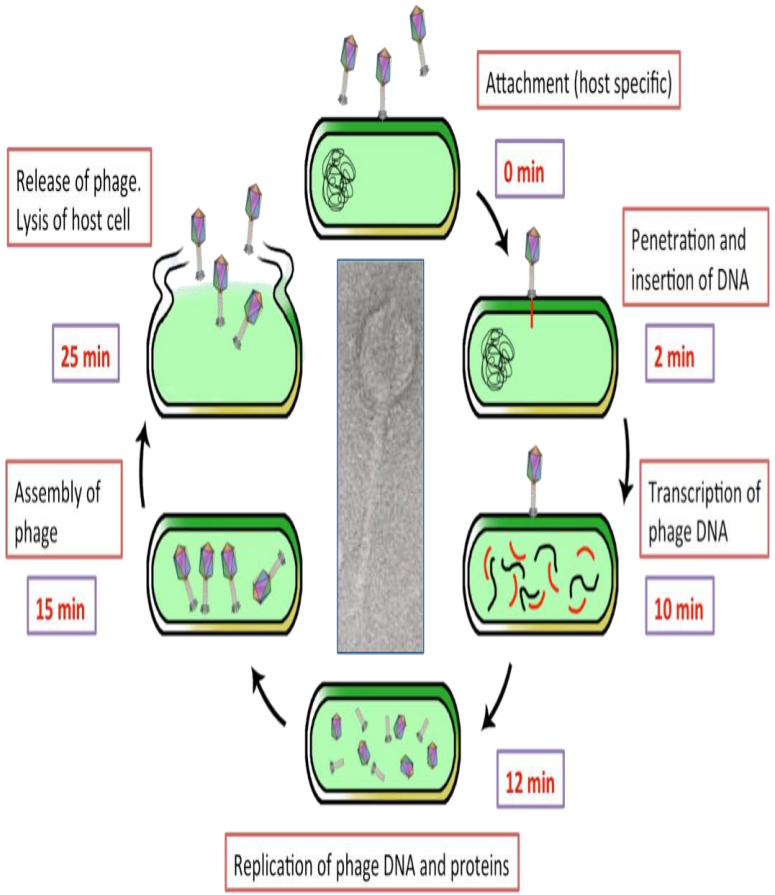
- Is the mating of 2 bacterial cells during which the DNA is transferred from the donor to recipient cell.
- This process is controlled by F (fertility) factor.
- The process begin when the sex pilus from f+ cell attached to receptor on f- cell.
- Only one strand of DNA is transferred. The recipient cell completes the structure of ds-DNA by synthesizing the complementary strand
- Some F+ cells have their F plasmid integrated into the bacterial DNA and acquire the capability of transferring the chromosome into another cell. These cells are called **high-frequency recombination HFr**.



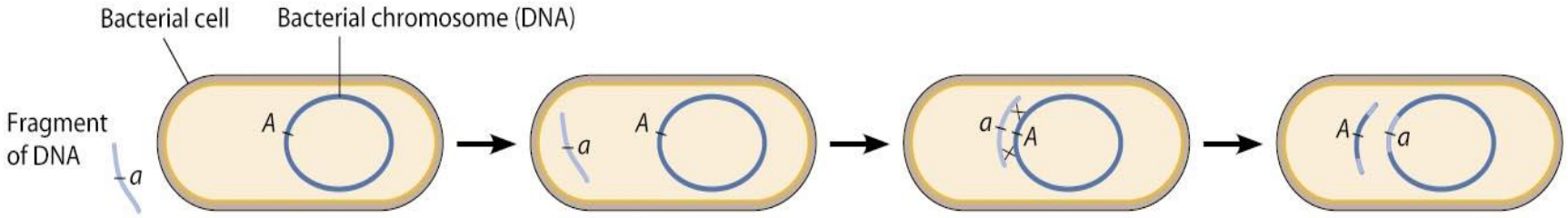
Bacterial genetics

2. Trasduction:

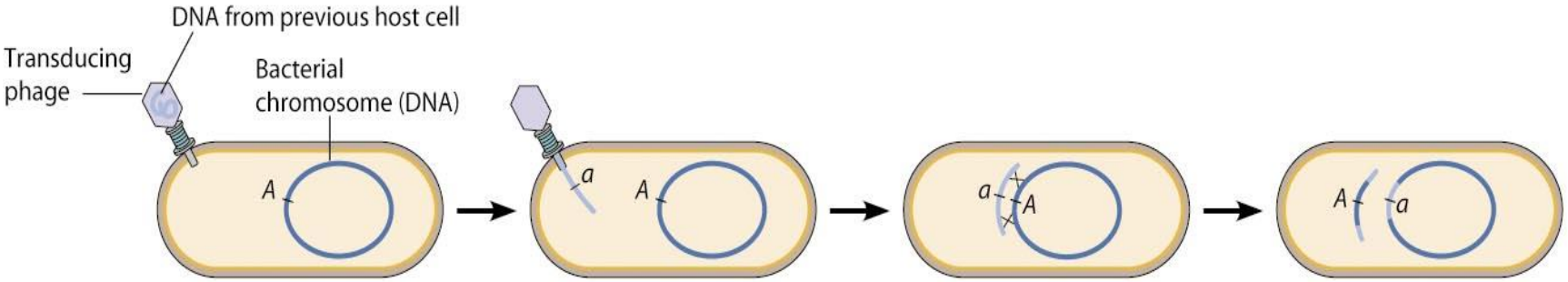
- Transfer of bacterial DNA by the mean of bacterial virus (bacteriophage).
- During the growth of the virus within the cell, a piece of bacterial DNA is incorporated into the virus & transferred to recipient during infection.
- Within the recipient cell, the phage DNA can integrate in the cell DNA and the cell acquire new feature.
- There are two types of transduction: generalized, and specialized.



Bacterial transduction



(a) Transformation. Transformation involves uptake by the bacterial cell of exogenous DNA, which occasionally becomes integrated into the bacterial genome by two crossover events (indicated by X's). The exogenous DNA will be detectable in progeny cells only if integrated into the bacterial chromosome, because the fragment of DNA initially taken up does not normally have the capacity to replicate itself autonomously in the cell. (The main exception is an intact plasmid.)



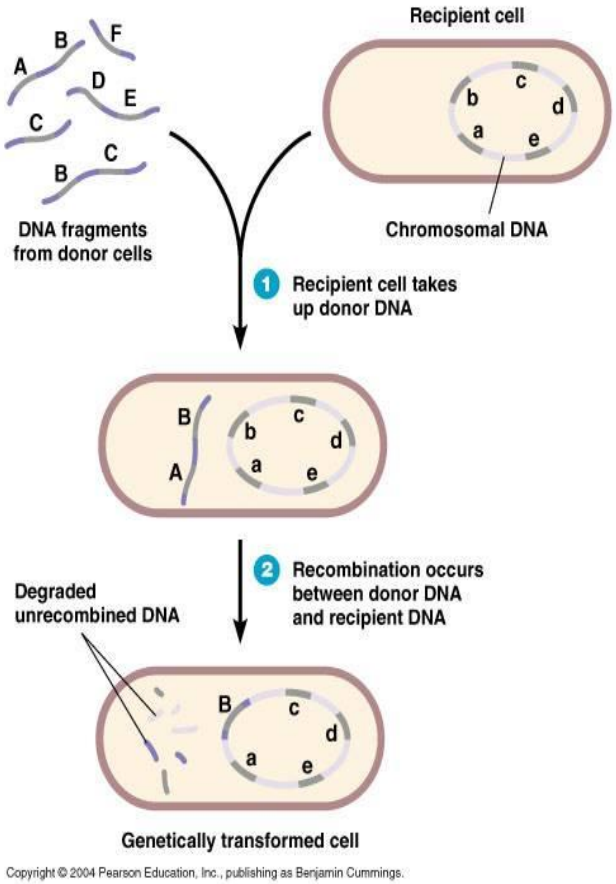
(b) Transduction. Transduction involves the introduction of exogenous DNA into a bacterial cell by a phage. Once injected into the host cell, the DNA can become integrated into the bacterial genome in the same manner as in transformation. In both cases, linear fragments of DNA that end up outside the bacterial chromosome are eventually degraded by nucleases.

Bacterial genetics

Transformation:

is the transfer of free (nacked) DNA itself:

- In nature, dying bacteria may release their DNA, which may be taken up by a recipient.
- In laboratory, extraction of DNA from one cell then introduction of it to another cell
- Many bacteria unable to undergo natural transformation, can be forced to incorporate plasmids by treatment with calcium chloride & temperature shock. This process is the cornerstone of modern molecular biology, because it enable DNA from diverse biological sources to be incorporated as part of well-characterized bacterial replicon.



Bacterial genetics

Genetic engineering:

Specified DNA fragments can be isolated & amplified, & their genes can be expressed at high levels. The nucleotide specificity required for cleavage by restriction enzymes allows fragments containing genes or parts of genes to be covalently bound to plasmids " **vector**" that can be inserted into bacterial host. Bacterial colonies or **clones** carrying specified genes can be identified by **hybridization** of DNA or RNA with chemical or radiochemical **probes**.

The protein products encoded by these genes can be recognized either by enzyme activity or by immunological techniques.

The protein products of isolated genes offers great promise as **vaccine** because it can be prepared without genes that encode the replication of viral nucleic acid. Moreover, protein ,e.g. insulin can be prepared in large quantities from bacteria that express cloned genes.

Bacterial genetics

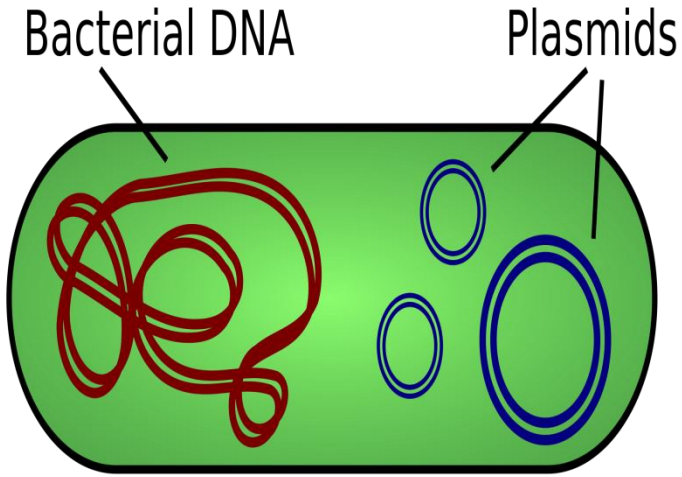
Mobile genetic elements:

Plasmids:

are small extra-chromosomal genetic elements capable of independent replication from chromosome. plasmids carry genes associated with specialized functions (such as virulence factors & antibiotic resistance) & genes that mediate their transfer from one organism to another. Types of plasmids:

1- transmissible plasmid: can be transferred from cell to cell by conjugation. They are large and present in few copies

2- non-transmissible plasmid: they are small and do not contain transfer genes and present in many copies in cell.



Mobile genetic elements

Transposons:

they are pieces of DNA that move readily from one genetic locus to another either within or between bacterial DNAs, plasmid, bacteriophages. Unlike plasmids, transposons do not contain genetic information necessary for their own replication. Therefore, they depend on their physical integration with a bacterial replicon.

They can cause mutation in the gene into which they insert or alter the expression of nearby genes.

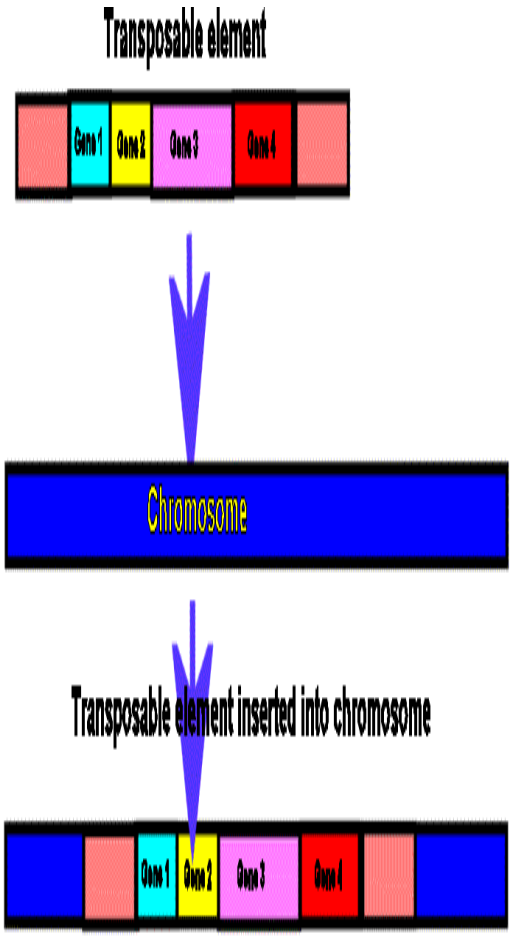
They have four identifiable domains:

1- on each end, DNA sequence of inverted repeats: involved in the integration of transposon in the recipient cell.

2- gene of transposase; mediate integration and excision processes.

3- repressor gene: regulates synthesis both of transposase and gene product domain

4- gene product domain



Mobile genetic elements

Bacteriophages (phages):

Viruses that infect bacteria. Many phages contain syringe-like structure that bind to receptors on the cell surface & inject the phage nucleic acid into the host cell. Replication of phage is similar to that of animal viruses.

Types of phages:

1. **Lytic phage:** produces many copies of themselves & kill their host cells. e.g. T2, T4 phage of *E. coli*
2. **Temperate phage:** are able to enter a non-lytic prophage state in which replication of its NA is linked to the replication of host cell DNA. Bacteria carry prophage are called lysogenic (e.g. *E. coli* phage lambda).
3. **Filamentous phage:** (e.g. *E. coli* phage M13) their filaments contain ss-RNA with protein that extruded from their host cell, which are debilitated but not killed by the phage infection.