Microbiology

Bacterial Growth & Genetic

Asst. prof. Dr. Dhay Ali Azeez

2024-2025

(1st) semester

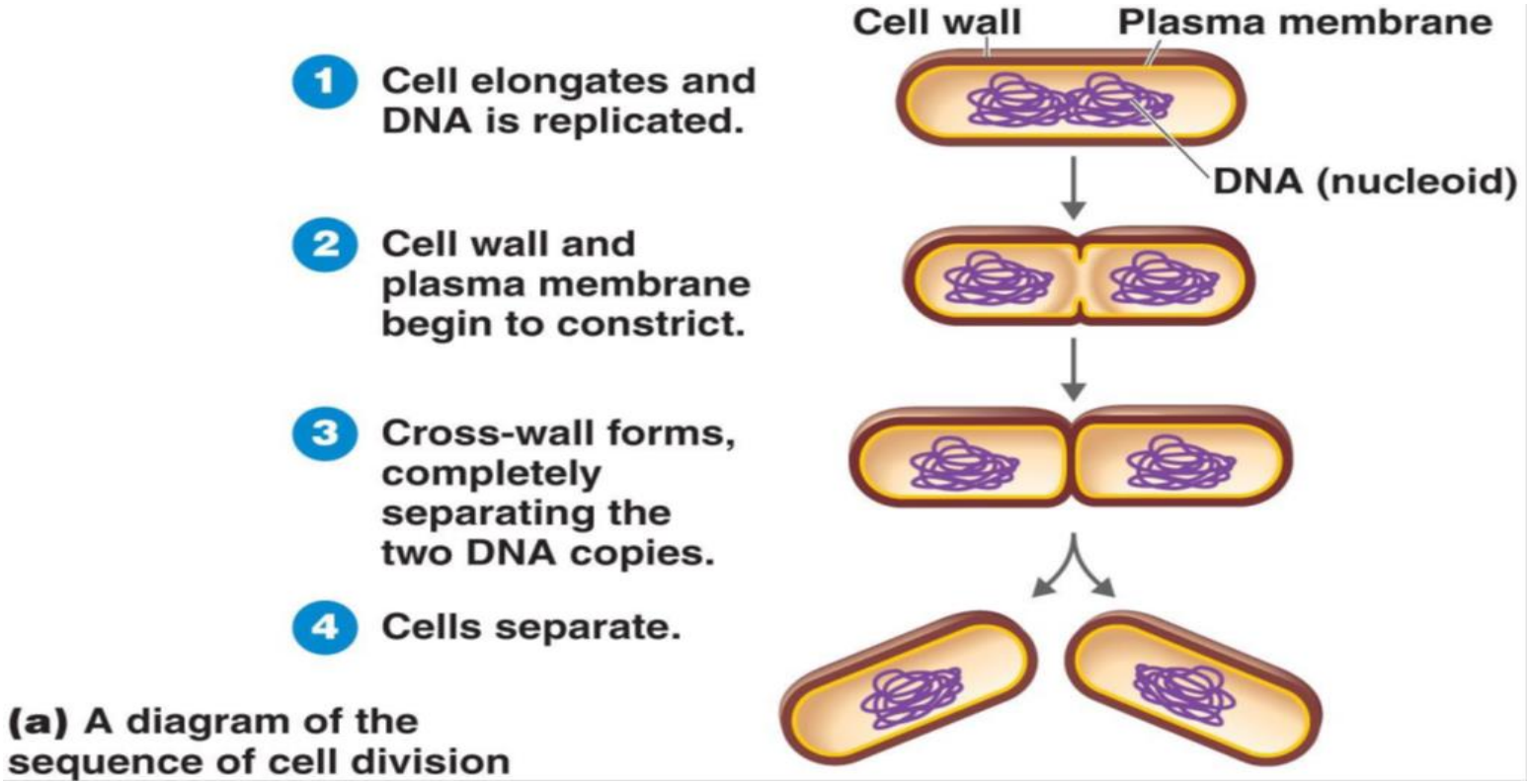
3rd lecture

BACTERIAL GROWTH

BACTERIAL GROWTH

- It is an increase in all the cellular components, which end in multiplication of the cell leading to an increase in population.
- It involves an **increase in the number of individual cells**. Not in cell size.
- Bacteria divide by **binary fission**.

BINARY FISSION



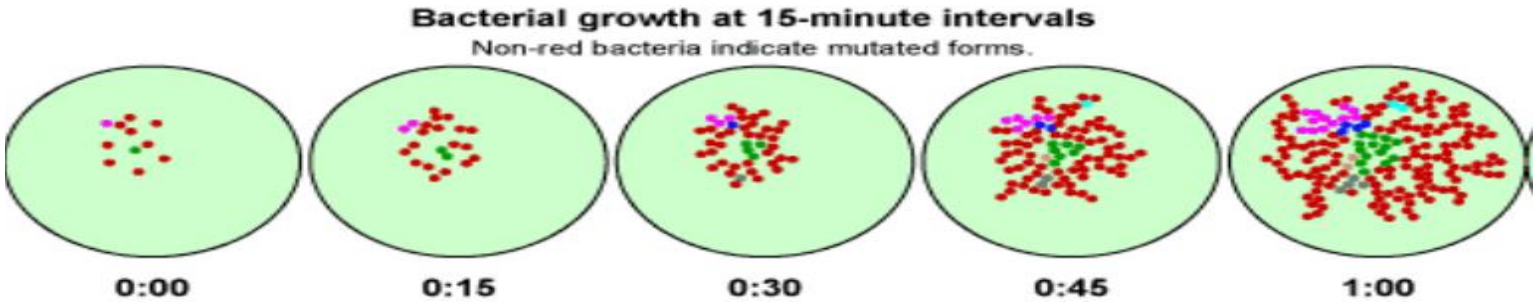


Fig 6.13

Numbers of Cells	Numbers Expressed as a Power of 2	Visual Representation of Numbers
1	2^0	●
2	2^1	●●
4	2^2	●●●●
8	2^3	●●●●●●●●
16	2^4	●●●●●●●●●●●●●●●●
32	2^5	●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●

Generation Number	Number of Cells	Log_{10} of Number of Cells
0	$2^0 = 1$	0
5	$2^5 = 32$	1.51
10	$2^{10} = 1{,}024$	3.01
15	$2^{15} = 32{,}768$	4.52
16	$2^{16} = 65{,}536$	4.82
17	$2^{17} = 131{,}072$	5.12
18	$2^{18} = 262{,}144$	5.42
19	$2^{19} = 524{,}288$	5.72
20	$2^{20} = 1{,}048{,}576$	6.02

GENERATION TIME

- Interval of time between two cell divisions

OR The time required for a bacterium to give rise to two daughter cells under optimum conditions

- Generation time of ***E.coli*** & other medically important bacteria is 20 mins.
- For **tubercle** bacilli is 20 hrs.
- For **lepra** bacilli is 20 days

Microbial Growth

Physical Requirements for Growth: **1.TEMPERATURE**

Bacteria vary in their temperature requirements.

•**Temperature range** – growth does not occur above the maximum or below the minimum.

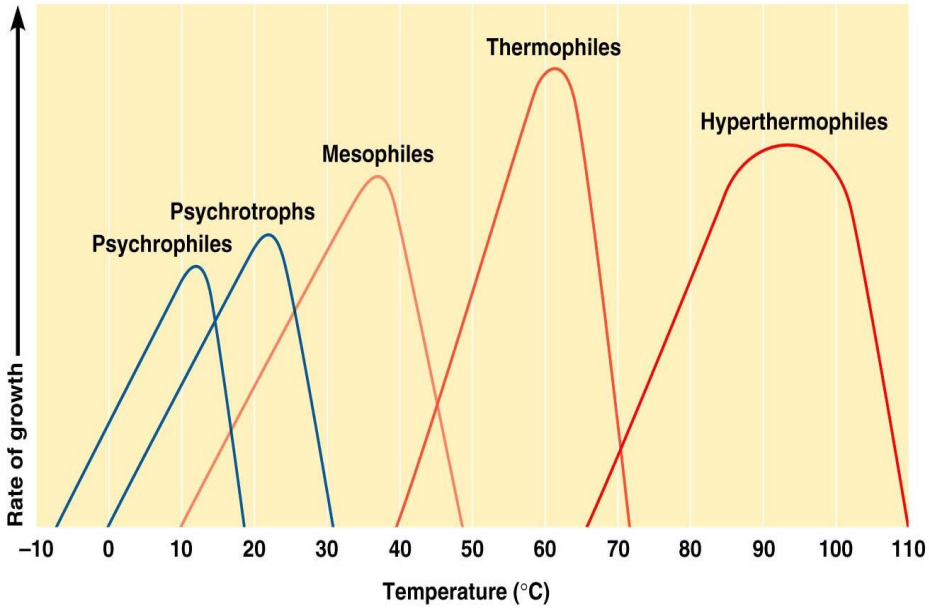
•**Optimum Temperature** – It is the temperature at which growth occurs best, it is 37ºC for most pathogenic

- **Minimum growth temperature**
- **Optimum growth temperature**
- **Maximum growth temperature**

Five groups based on

optimum growth temperature

1. **Psychrophiles**
2. **Psychrotrophs**
3. **Mesophiles**
4. **Thermophiles**
5. **Hyperthermophiles**



1.Mesophilic – grows best between 25ºC and 40ºC. e.g. most pathogenic bacteria

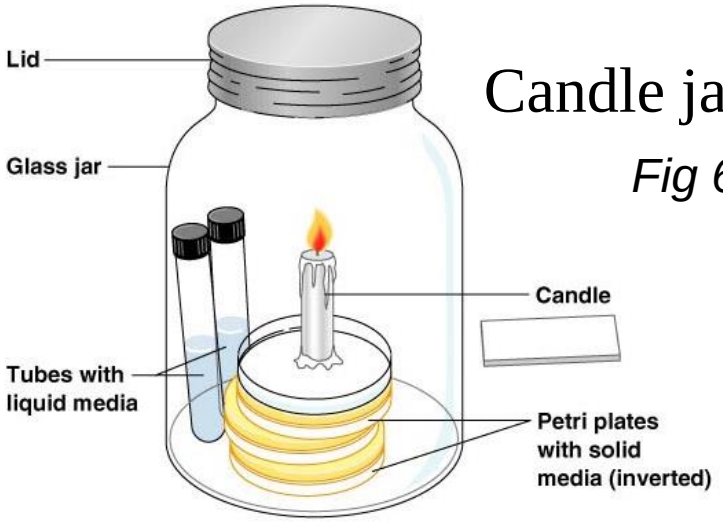
2.Thermophilic – grows best at high temp, 55- 80ºC e.g. ***Bacillus stenothermophilus***

3.Psychrophilic (cold loving) – grows best below 20ºC e.g. ***Flavobacterium*** spps

- **Psychrotrophs cause food spoilage.**
- Listeriosis is a food-borne infection that causes about 2,500 people in the United States to become ill each year, and results in about 500 deaths. It is caused by food contaminated with the bacteria *Listeria monocytogenes*, a rod, coccobacilli shaped bacteria that can be arranged in chains or as single cells. The bacterium is motile at 20°-25°C, and is catalase positive. It is a psychrophile; therefore, it is neither killed nor does it grow slowly at cold temperatures. *Listeria monocytogenes* has been isolated from several sources including: ground beef, chicken and turkey, lunch meats, hot dogs, cheese, and poorly pasteurized milk.
- The disease usually affects pregnant women, newborns, and the immunocompromised.

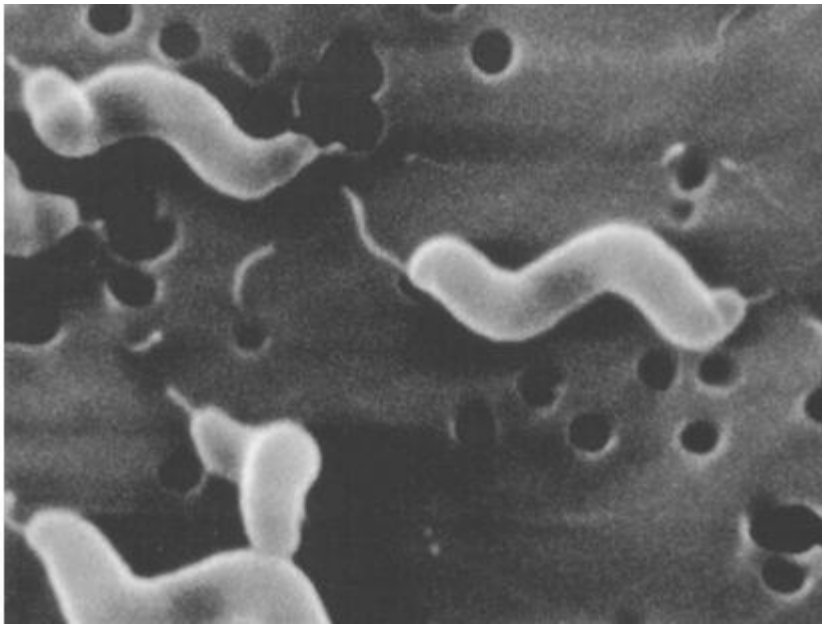
Capnophiles: Aerobic Bacteria Requiring High CO_2

- Low oxygen, high CO_2 conditions resemble those found in
  - intestinal tract
  - respiratory tract and
  - other body tissues where pathogens grow
- *E.g: Campylobacter jejuni*
- Use candle jar, CO_2-generator packets, or CO_2 incubators



Candle jar

Fig 6.7



2-OXYGEN

Depending on the O_2 requirement, bacteria are divided into :

1.(Obligate) Aerobes – require O_2 for growth. e.g. ***Pseudomonas aeruginosa***

2.Strict (Obligate) Anaerobes – grow in the absence of O_2 & may even die on exposure to O_2 . e.g. ***Bacteroides fragilis***

3.Microaerophilic – grow best in the presence of low oxygen levels

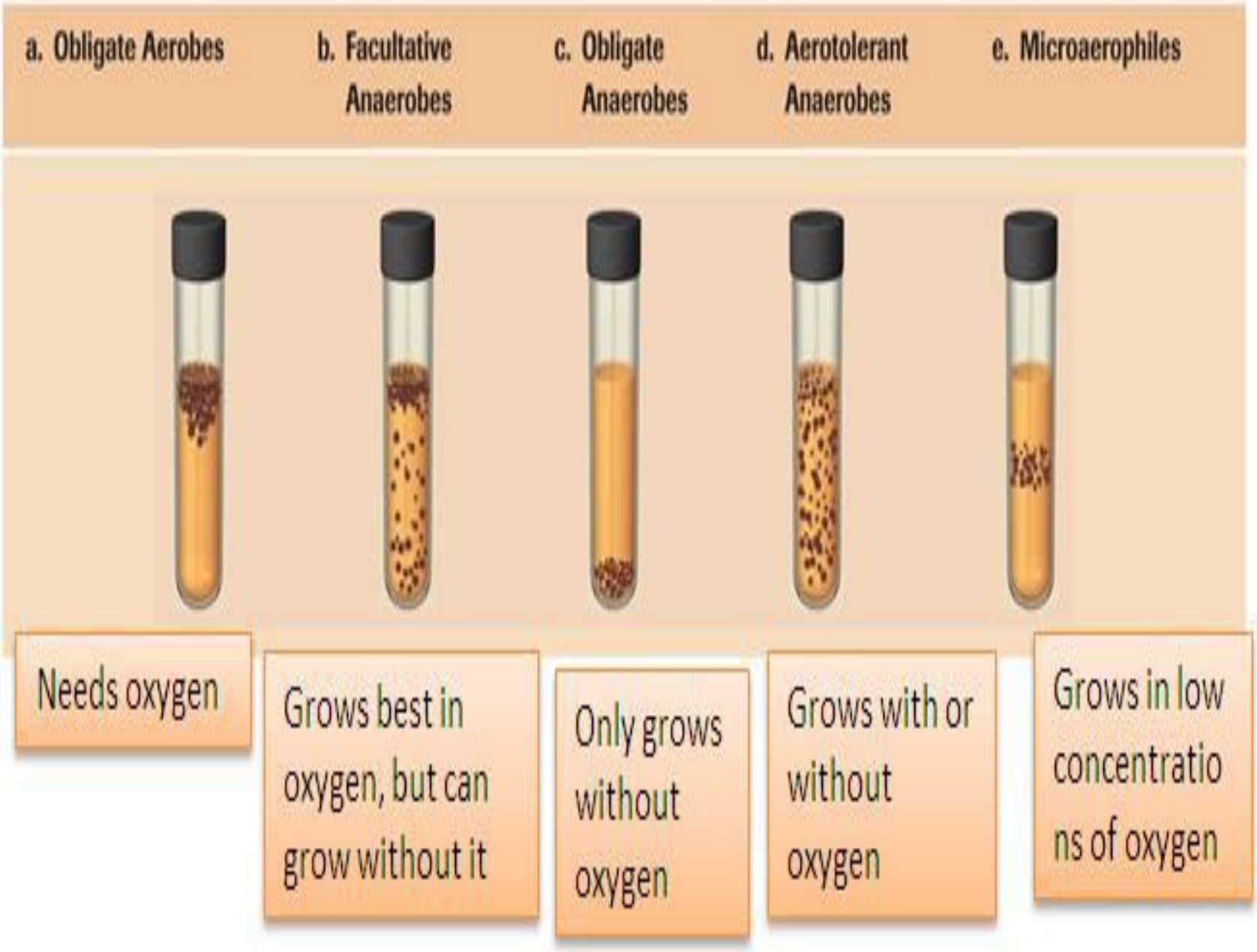
e.g. ***Helicobacter* spp.**

4.Facultative anaerobe – aerobic but can also grow in the absence of O_2 . e.g. ***Staphylococcus* spps**

5.Aerotolerant anaerobe – anaerobic, but tolerates exposure to O_2

e.g. ***Clostridium perfringens***

6.Capnophilic organism – requires high C O_2 levels. eg. ***Neisseria***


a. Obligate Aerobes
b. Facultative Anaerobes
c. Obligate Anaerobes
d. Aerotolerant Anaerobes
e. Microaerophiles
Needs oxygen
Grows best in oxygen, but can grow without it
Only grows without oxygen
Grows with or without oxygen
Grows in low concentrations of oxygen

Physical Requirements for Growth:

3. pH and Osmotic Pressure

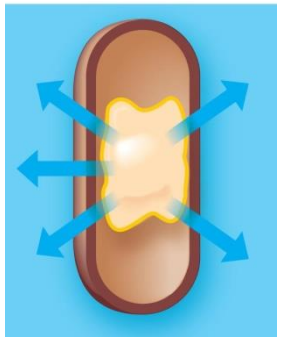
Most bacteria grow best between pH 6.5 and 7.5:
Neutrophils

Some bacteria are very tolerant of acidity or thrive in it:
Acidophiles (preferred pH range 1 to 5)

- ***Lactobacilli*** require **acidic pH**
- ***Vibrio cholerae*** require **alkaline pH**
- Molds and yeasts grow best between pH 5 and 6

Hypertonic environments (increased salt or sugar) cause **plasmolysis**

- Obligate **halophiles**
- facultative **halophiles**



4- MOISTURE AND DRYING

- Water is an essential ingredient of bacteria. Hence drying is lethal to cells.
- Effect of drying varies :

❖ ***Trepanoma pallidum*** are highly **sensitive** to drying

❖ ***Staphylococcus*** **spp.** withstand drying for **months**

❖ **Spores** are resistant to drying and may survive for **several decades**

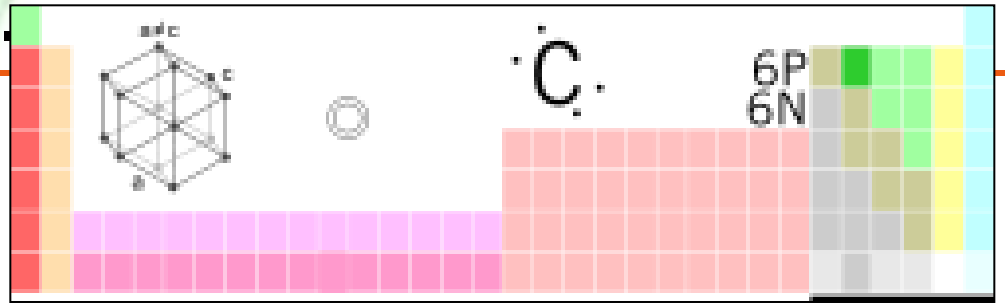
5- RADIATION

X rays & gamma rays exposure – **lethal**

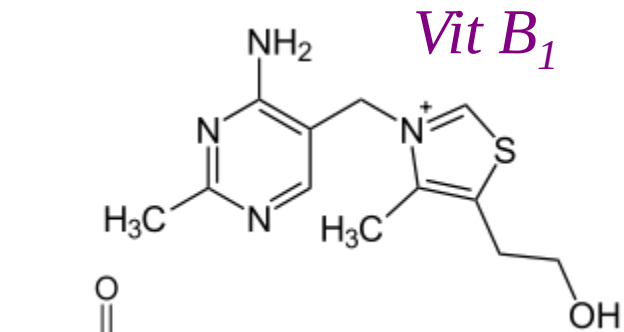
7- MECHANICAL & SONIC STRESS

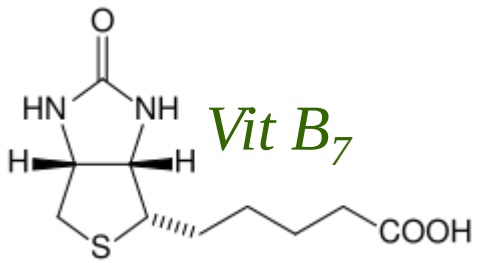
May be ruptured by mechanical stress.

8. Chemical Requirements for Growth: **Carbon, N, S, P**, etc.



- Carbon
  - ~ Half of dry weight
  - Chemoheterotrophs use organic carbon sources
- Nitrogen, Sulfur, Phosphorus
  - Needed for ?
  - Found in amino acids and proteins (most bacteria decompose proteins)
  - S in thiamine and biotin
  - Phosphate ions (PO_4^{3-})





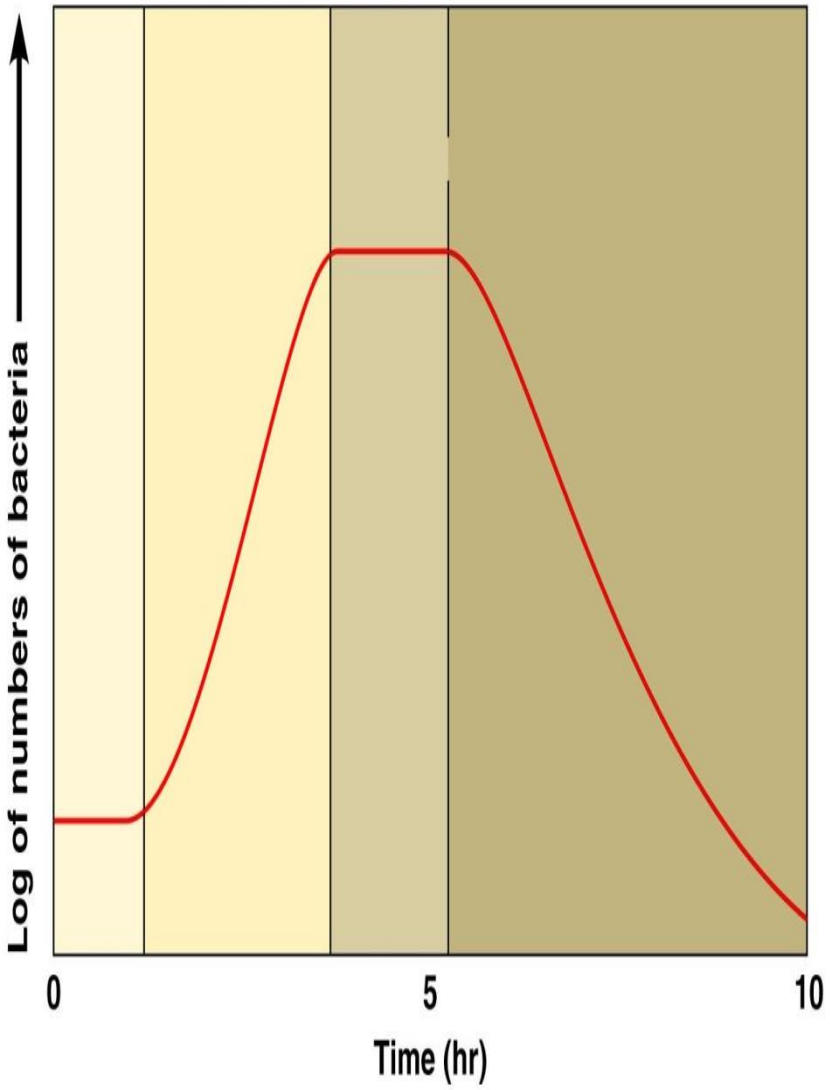
- Also needed K, Mg, Ca, trace elements (as cofactors), and organic growth factors

Bacterial Growth Curve

Phases of growth

- Lag phase
- Exponential or logarithmic (log) phase
- Stationary phase
- Death phase

(decline phase)



PHASES OF GROWTH:

1.Lag phase:

- **no increase** in **number** but there may be an increase in **the size** of the cell, but they are **not** undergoing binary fission.
- however, cells grow larger and are metabolically active, synthesizing proteins needed to grow within the medium
- Bacteria have the maximum cell size towards the end of the lag phase.
- If any cells were damaged or shocked during the transfer to the new medium, repair **takes place** during the lag phase.

- The bacteria are adapting to the new environment and are synthesizing cellular components such as ribosomes, enzymes, and other proteins.
- May last from one hour to several days.
- The duration of the lag phase is determined by many factors, including the species and genetic make-up of the cells, the composition of the medium, and the size of the original inoculum.

2.Log or Exponential phase:

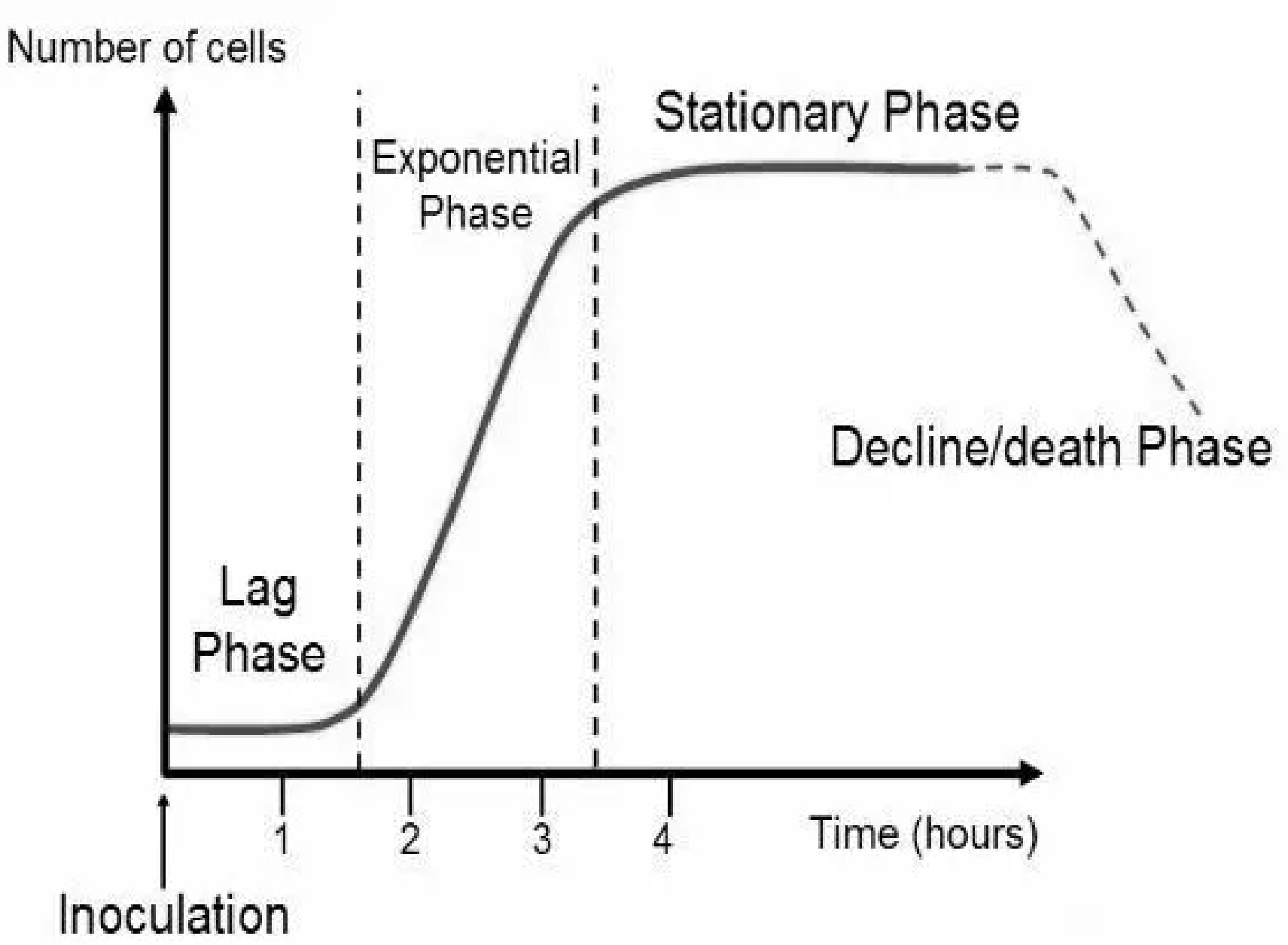
- cells start **dividing** and their **number increases** exponentially. This exponential growth is expressed as the bacteria's generation time.
- During this phase, the conditions are optimal for growth and binary fission occurs.
- Cells are at highest metabolic activity.
- Cells in the this phase show **constant** growth rate and **unifor** metabolic activity. For this reason, cells in the log phase are preferentially used for industrial applications and research work.
- In the log phase, cells are smaller and stain uniformly.

3.Stationary phase:

- Cell division **stops** due to depletion of nutrients & accumulation of toxic products.
- Equilibrium exists between dying cells and the newly formed cells, so viable count remains stationary
- During the stationary phase, cells switch to a survival mode of metabolism.
- As growth slows, so too does the synthesis of peptidoglycans, proteins, and nucleic-acids; thus, stationary cultures are less susceptible to antibiotics that disrupt these processes.
- In bacteria capable of producing endospores, many cell undergo sporulation during the stationary phase.

4.Phase of decline:

- This is the stage named death phase, sometimes called the decline phase.
- The number of dying cells exceeds the number of dividing cells, leading to an exponential decrease in the number of cells, due to the death of cells – autolytic enzymes.
- As a culture medium accumulates toxic waste and nutrients are exhausted, cells die in greater and greater numbers.
- Many cells lyse and release nutrients into the medium, allowing surviving cells to maintain viability and form endospores.


Number of cells
Stationary Phase
Exponential Phase
Decline/death Phase
Lag Phase
1
2
3
4
Time (hours)
Inoculation

Preserving Bacterial Cultures

- **Deep-freezing**: Rapid cooling of pure culture in suspension liquid to –50°to –95°C. Good for several years.
- **Lyophilization** (freeze-drying): Frozen (–54° to –72°C) and dehydrated in a vacuum. Good for many years.

Measuring Microbial Growth - Overview

Direct Methods

- **Plate counts**
- **Filtration**
- *MPN*
- **Direct microscopic count**

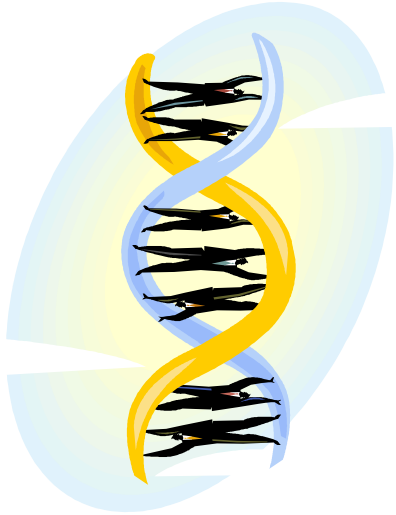
Indirect Methods

- **Turbidity**
- *Metabolic activity*
- *Dry weight*

BACTERIAL GENETICS

DNA Basics

- **DNA is the basis of life because it contains the genetic code for all living organisms**
- **The DNA contain all the organism's genes which make up the blueprint for that organism, and determine its appearance and all of its functions**



Genetics: is the study of genes including the structure of genetic materials what information is stored in the genes.

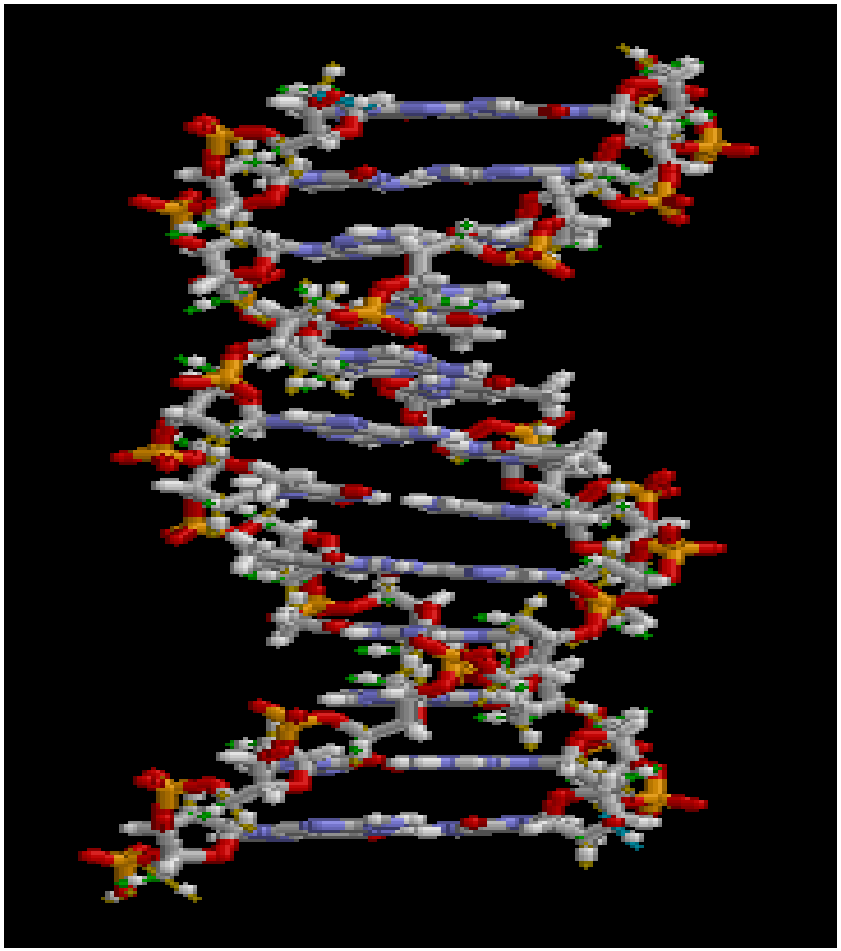
Genotype: the arrangement of genes within organisms.

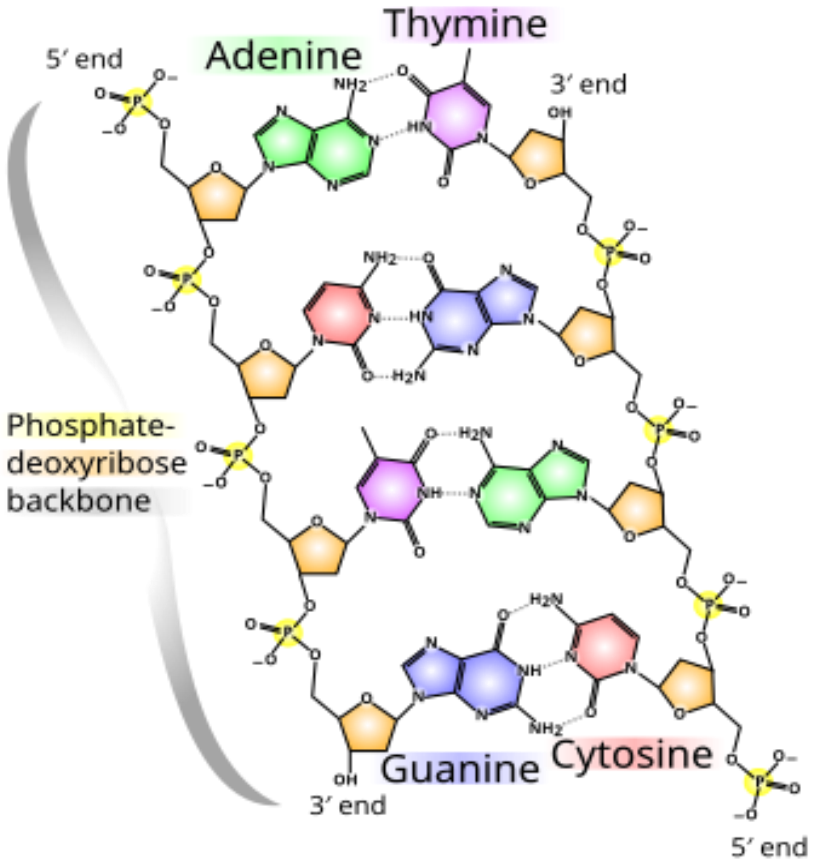
Phenotype: physical characteristics an organism based on its genotype and the interaction with its environment.

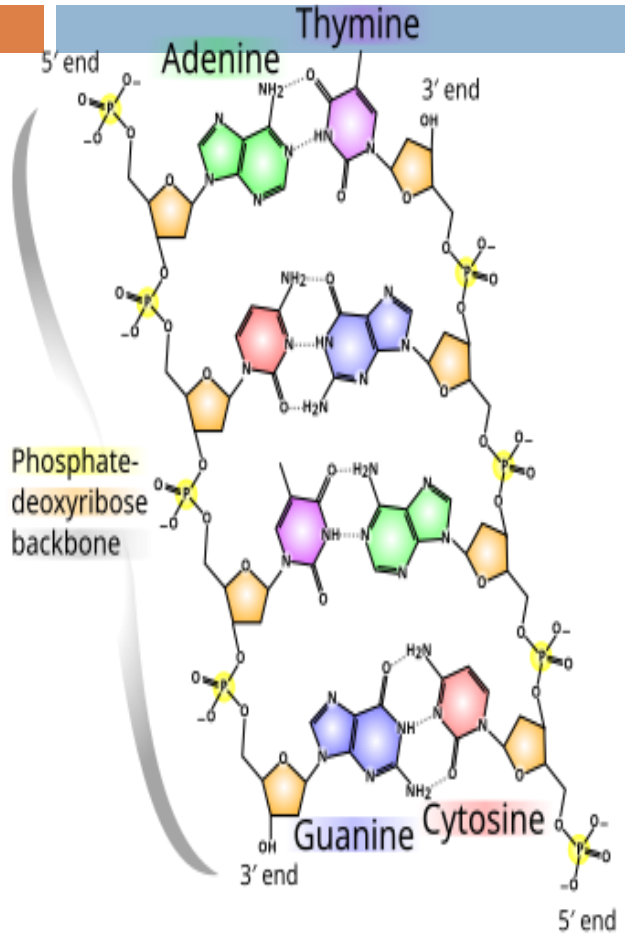
Genes :are sequences of nucleotides within DNA that code for functional proteins.

- The two essential functions of genetic material are replication and expression.

Structure of DNA

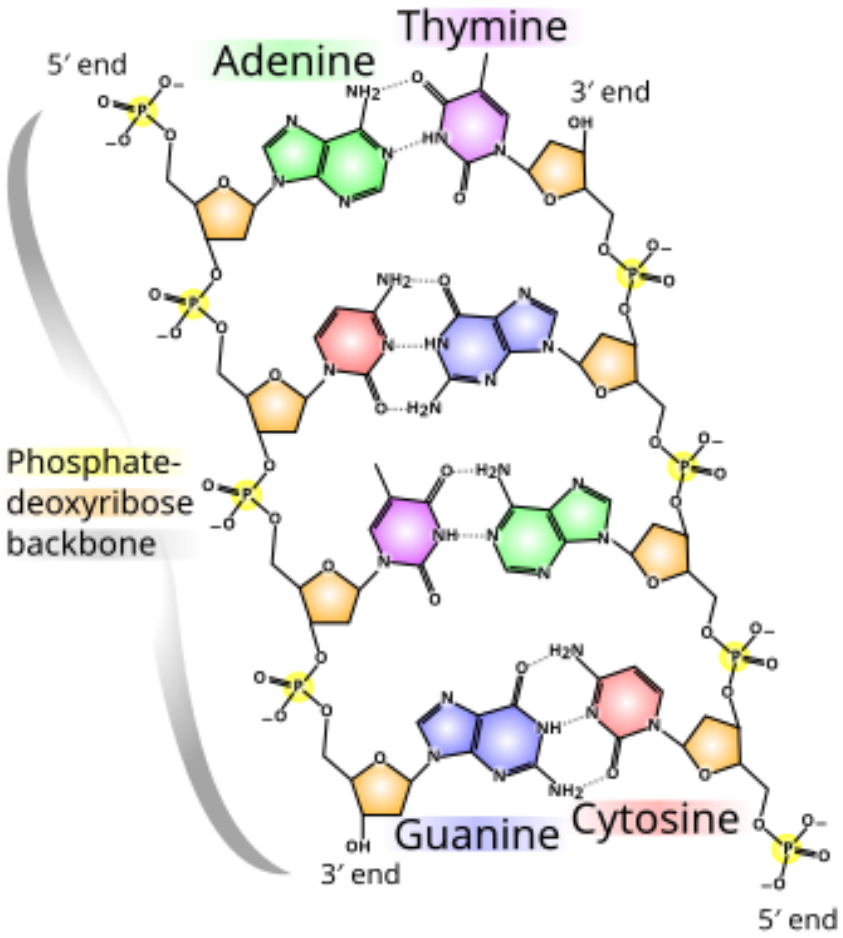




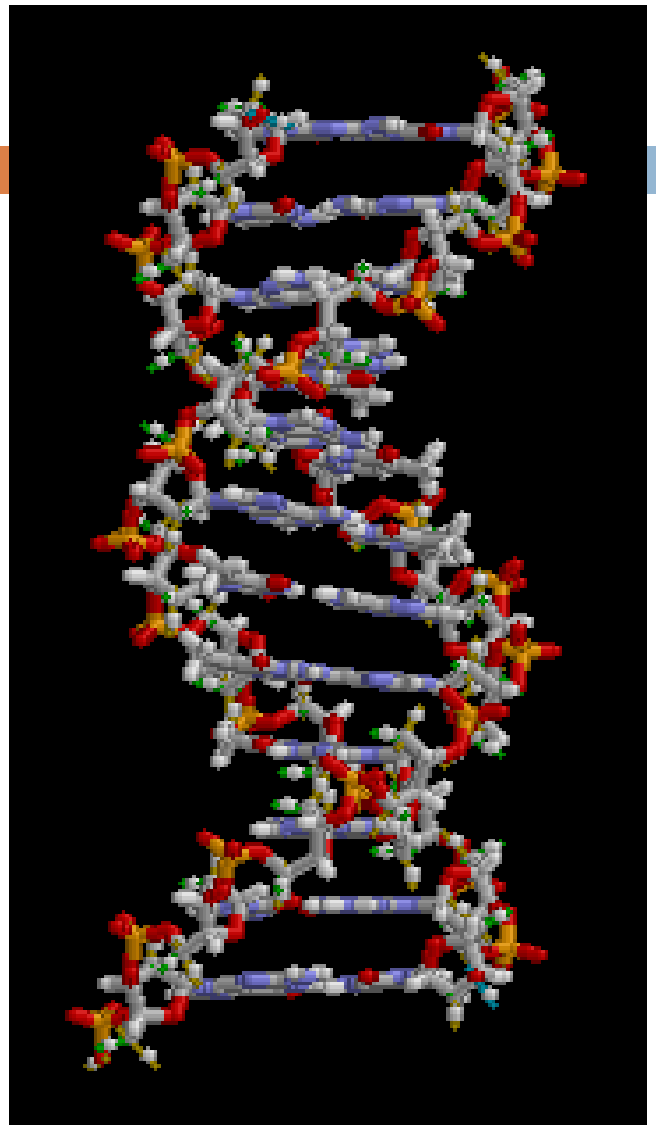


- Each nucleotide unit is composed of
  - a sugar (deoxyribose)
  - a phosphate group
  - a nitrogenous base

Pairing of nucleotide bases in DNA



- **Adenine (A) always pairs with thymine (T)**
- **Cytosine (C) always pairs with guanine (G)**



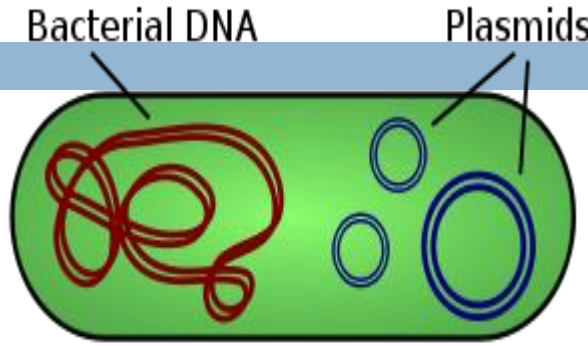
- **Deoxyribonucleic acid = DNA**
- **DNA is a very long, double-stranded, helical molecule made of many nucleotide units**
- **Replication of DNA occurs through the pairing of nucleotide bases to form new strands**

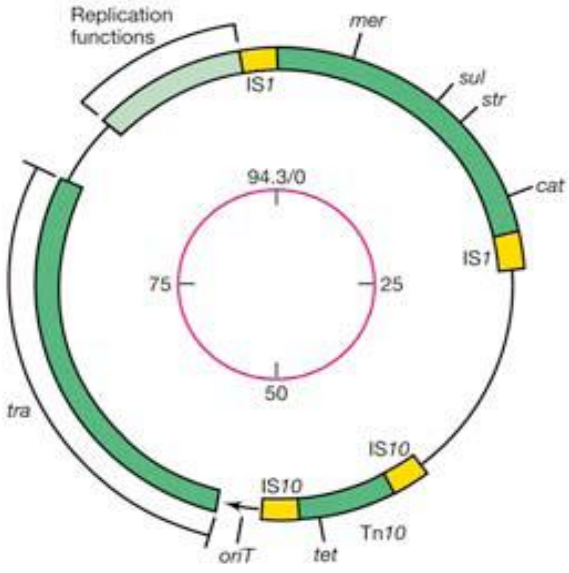
Genome Organization in the bacteria

- **The bacterial chromosome is a circular molecule of DNA that functions as a self-replicating genetic element (replicon).**
- **Extrachromosomal genetic elements such as plasmids and bacteriophages are nonessential replicons which often determine resistance to antimicrobial agents, production of virulence factors, or other functions.**
- **The chromosome replicates semiconservatively; each DNA strand serves as template for synthesis of its complementary strand.**

Plasmids

- **Extrachromosomal genetic elements in bacteria. They are usually much smaller than the bacterial chromosome**
- **Plasmids usually encode traits that are not essential for bacterial viability,and replicate independently of the chromosome.**
- **Most plasmids are supercoiled, circular, double-stranded DNA molecules.**
- **Many plasmids control medically important properties of pathogenic bacteria, including resistance to one or several antibiotics, production of toxins, and synthesis of cell surface structures required for adherence or colonization.**
- **Plasmids that determine resistance to antibiotics are often called R plasmids.**



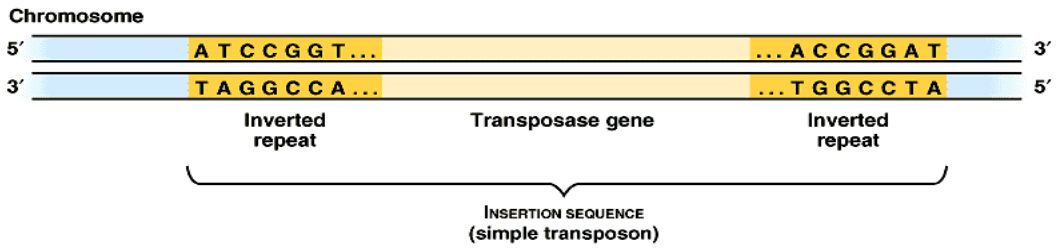


Episomes:

Previously, it was considered synonymous with plasmids. F factors are those plasmids that can code for self transfer to other bacteria. Occasionally such plasmids get spontaneously integrated into chromosome. Plasmids with this capability are called episomes and such bacterial cells are called Hfr cells i.e. high frequency of recombination.

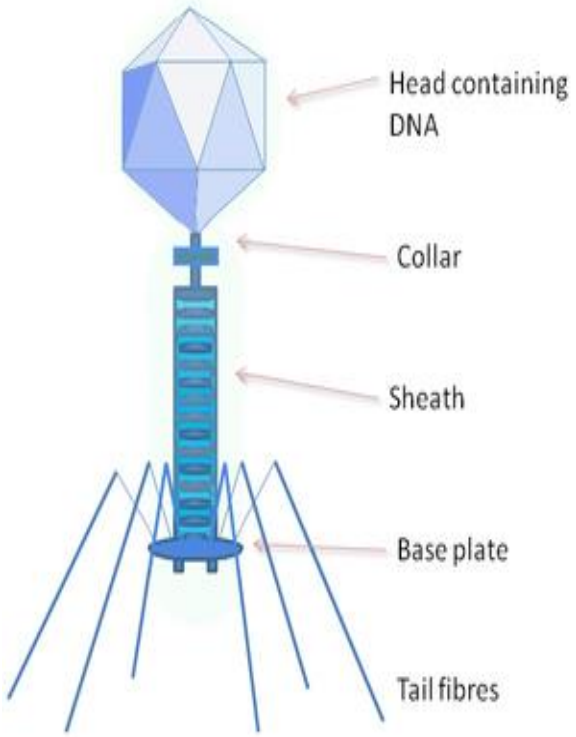
Transposons

- **Transposons** are segments of DNA that can move from one site in a DNA molecule to other target sites in the same or a different DNA molecule. The process is called **transposition**.
- Transposons are important genetic elements because they cause mutations, mediate genomic rearrangements, function as portable regions of genetic homology, and acquire new genes and contribute to their dissemination within bacterial populations.
- Transposon have four domains .on each end is a short DNA sequence of inverted repeats, which involved in the integration of the transposon into recipient DNA. the second is the gene for transposase enzyme which mediate excision and integration process.the third region is the gene for repressor that regulates the synthesis both of the transposase and the gene prodect of the fourth domain, which is an enzyme mediating antibiotic resistance.
- In medically important bacteria, genes that determine production of adherence antigens, toxins, or other virulence factors, or specify resistance to one or more antibiotics, are often located in complex transposons. Well-known examples of complex transposons are Tn5 and Tn10, which determine resistance to kanamycin and tetracycline, respectively.



Bacteriophages

Bacteriophages (bacterial viruses, phages) are infectious agents that replicate as obligate intracellular parasites in bacteria. Extracellular phage particles are metabolically inert and consist principally of proteins plus nucleic acid (DNA or RNA, but not both). The proteins of the phage particle form a protective shell (capsid) surrounding the tightly packaged nucleic acid genome. Phage genomes consist of double-stranded DNA, single-stranded DNA, or RNA. Phage genomes, like plasmids, encode functions not required for replication in bacteria, but unlike plasmids they also encode capsid proteins and nonstructural proteins required for phag assemblye.



Codon

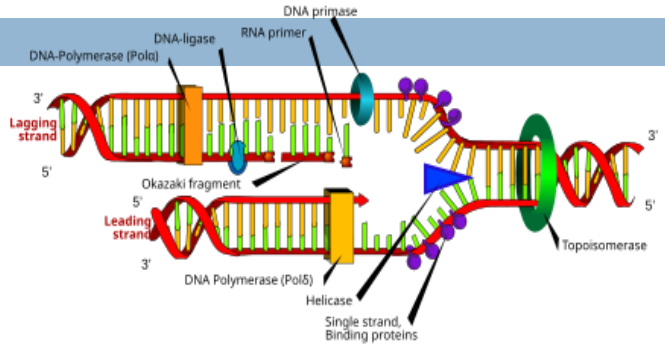
A set of three base pairs constitutes a codon, which codes for a single amino acid. The “triplet code” .

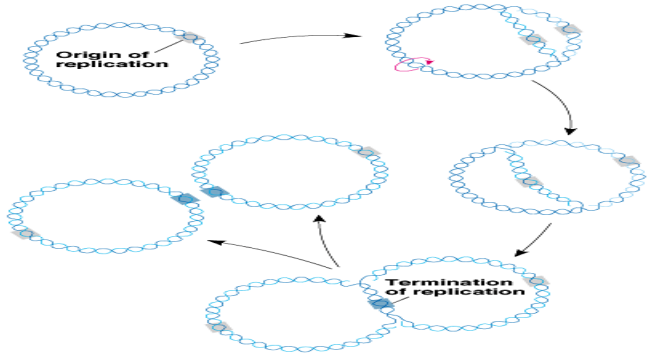
Flow of genetic information

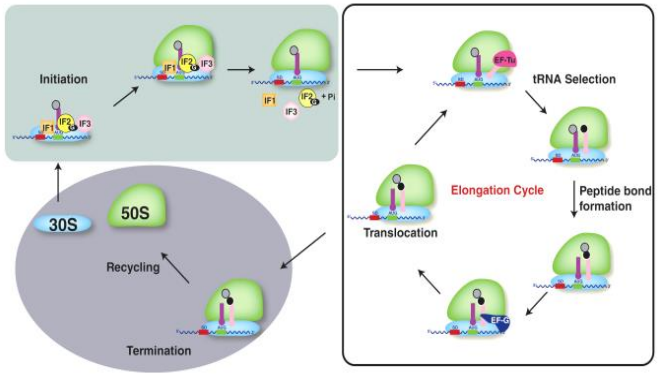
The flow of genetic information includes the replication of DNA to make more DNA, the transcription of the DNA into mRNA and the translation of mRNA into proteins.

Replication of DNA first involves the separation of the two strands of DNA followed by synthesis of new identical DNA strand by enzymes called DNA polymerases.

The RNA strand is synthesized by enzymes called RNA polymerases. The RNA sequence will be complementary to the DNA sequence. The mRNA strands are then guided to the ribosomes for protein translation. Amino acid residues are brought to the mRNA strand on the ribosomes by transfer RNA (tRNA) .





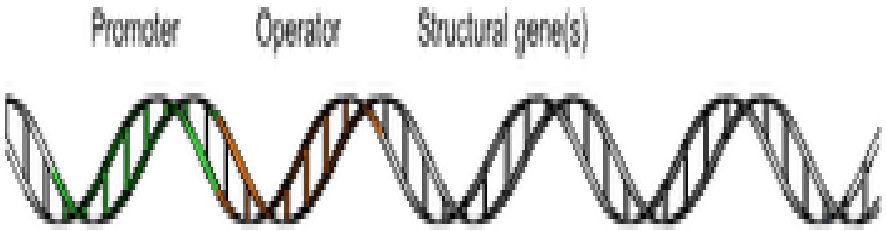


Gene Expression

Genetic information encoded in DNA is expressed by synthesis of specific RNAs and proteins, and information flows from DNA to RNA to protein. The DNA-directed synthesis of RNA is called transcription. The process by which the nucleotide sequence of an mRNA molecule determines the primary amino acid sequence of a protein is called translation.

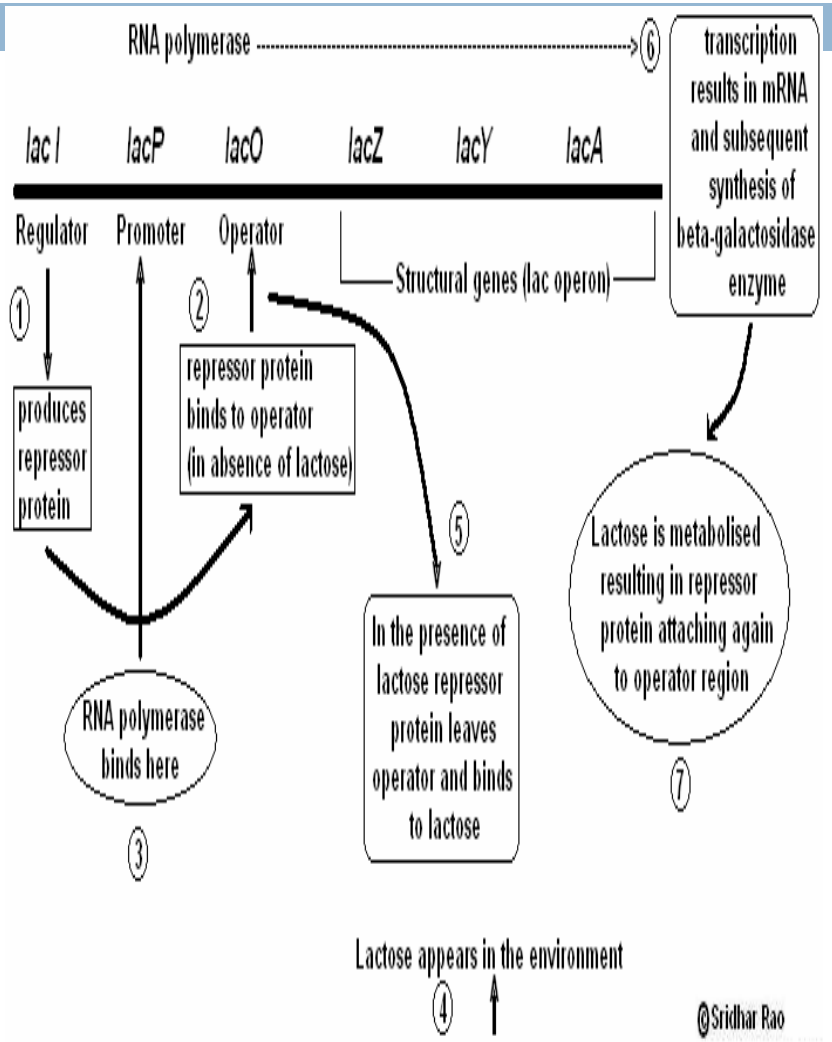
Operon concept

Group of genes expressed together, it is a functioning unit of key nucleotide sequences of DNA including an operator, a common promoter, and one or more structural genes, which is controlled as a unit to produce messenger RNA (mRNA), in the process of transcription by an RNA polymerase.



lac-operon

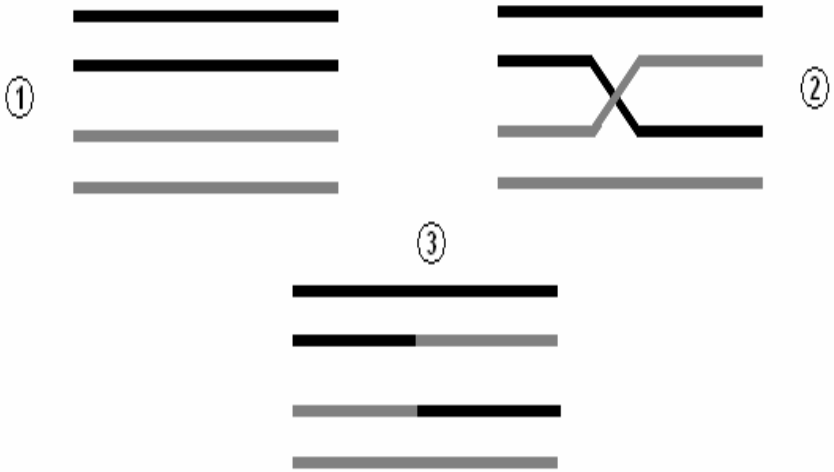
The first operon to be described was the lac-operon in ***Escherichia coli.*** lac operon in E. coli. In order to break down lactose, *E. coli* must use a series of enzymes (beta-galactosidase, galactoside permease and transacetylase). The genes for these three enzymes are located in a row on the DNA and share a single promoter. Genes determining structure of a particular protein are called structural genes and the activity of structural genes are controlled by regulator genes, which lie adjacent to them.



Exchange of Genetic Information

Sometimes when two pieces of DNA come into contact with each other, sections of each DNA strand will be exchanged. This is usually done through a process called crossing over in which the DNA breaks and is attached on the other DNA strand leading to the transfer of genes and possibly the formation of new genes. Genetic recombination is the transfer of DNA from one organism to another.

When two DNA strands having homologous regions come close, one strand from each crosses over to the other side and attaches to the new strand. Thus new genes may be transferred to the other strand. This is homologous recombination.



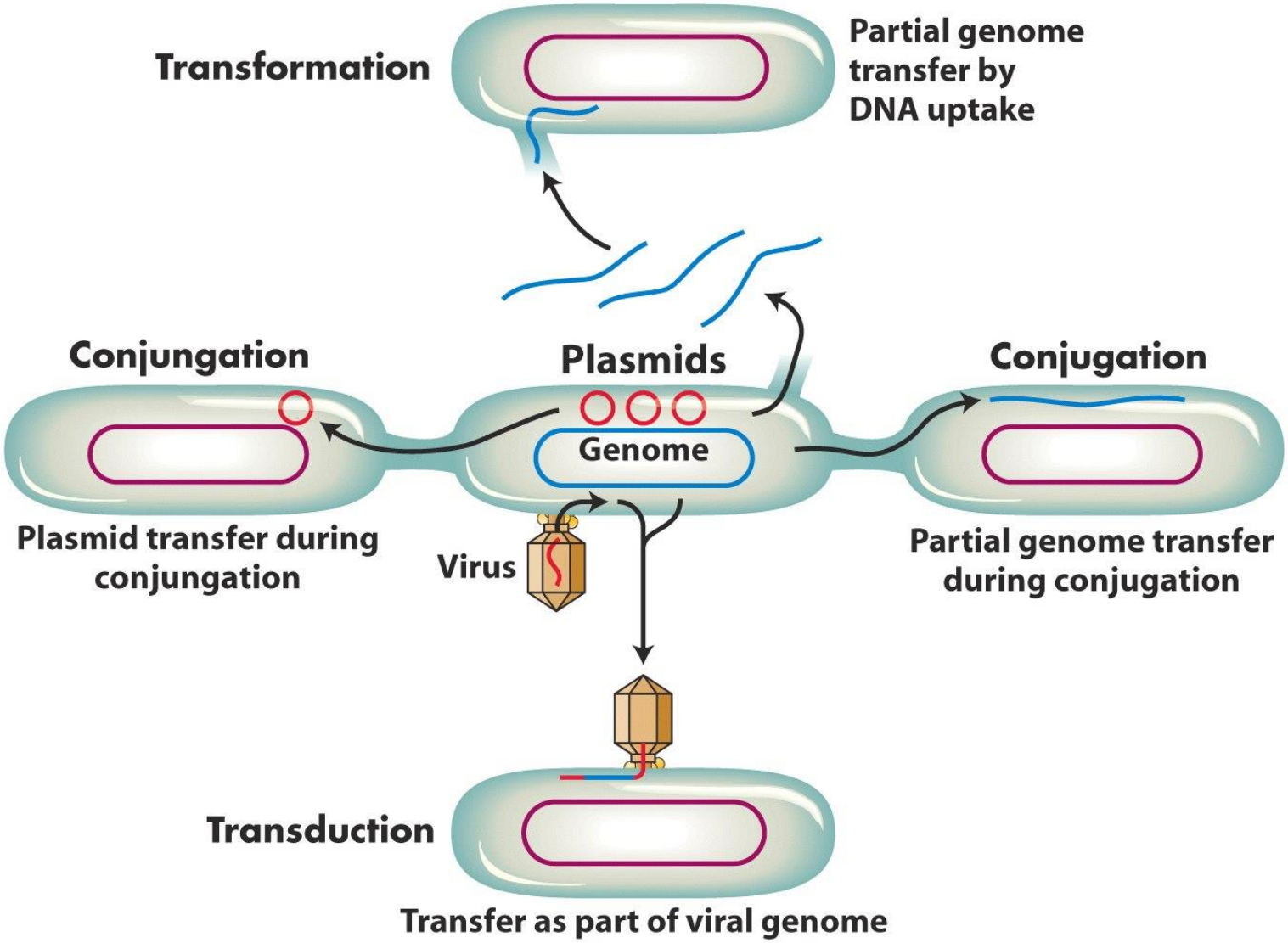
Bacterial Genetics

- "Acquiring genes through gene transfer provides new genetic information to microorganisms, which may allow them to survive changing environments."
- "The major source of variation within a bacterial species is mutation."
- "In mutations, usually only a single gene changes at any one time."
- "In contrast, gene transfer results in many genes being transferred simultaneously, giving the recipient cell much more additional genetic information."

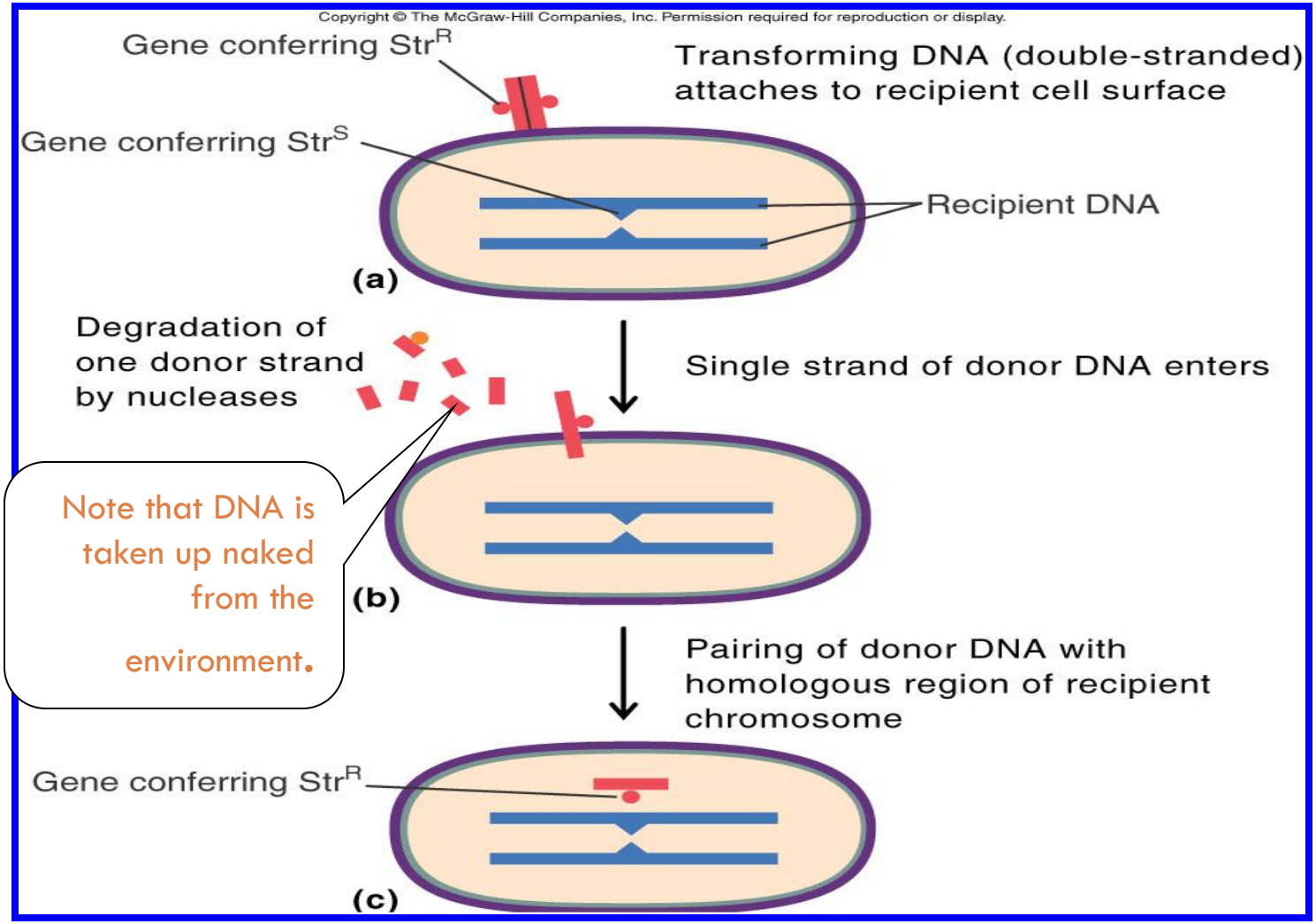
There are **THREE MAJOR TYPES** of genetic transfer found in bacteria

- 1.Transformation – fragments of DNA released from donor bacteria are taken up by competent recipient bacteria.(**absorb from environment)**
- 2.Conjugation - DNA is transferred from one bacteria cell to another, via "sex pilli". **(plasmid-mediated transfer)**
- 3.Transduction - DNA is introduced into bacteria by injection from a bacteriophage **(bacteriophage-mediated transfer)**

Methods of recombination



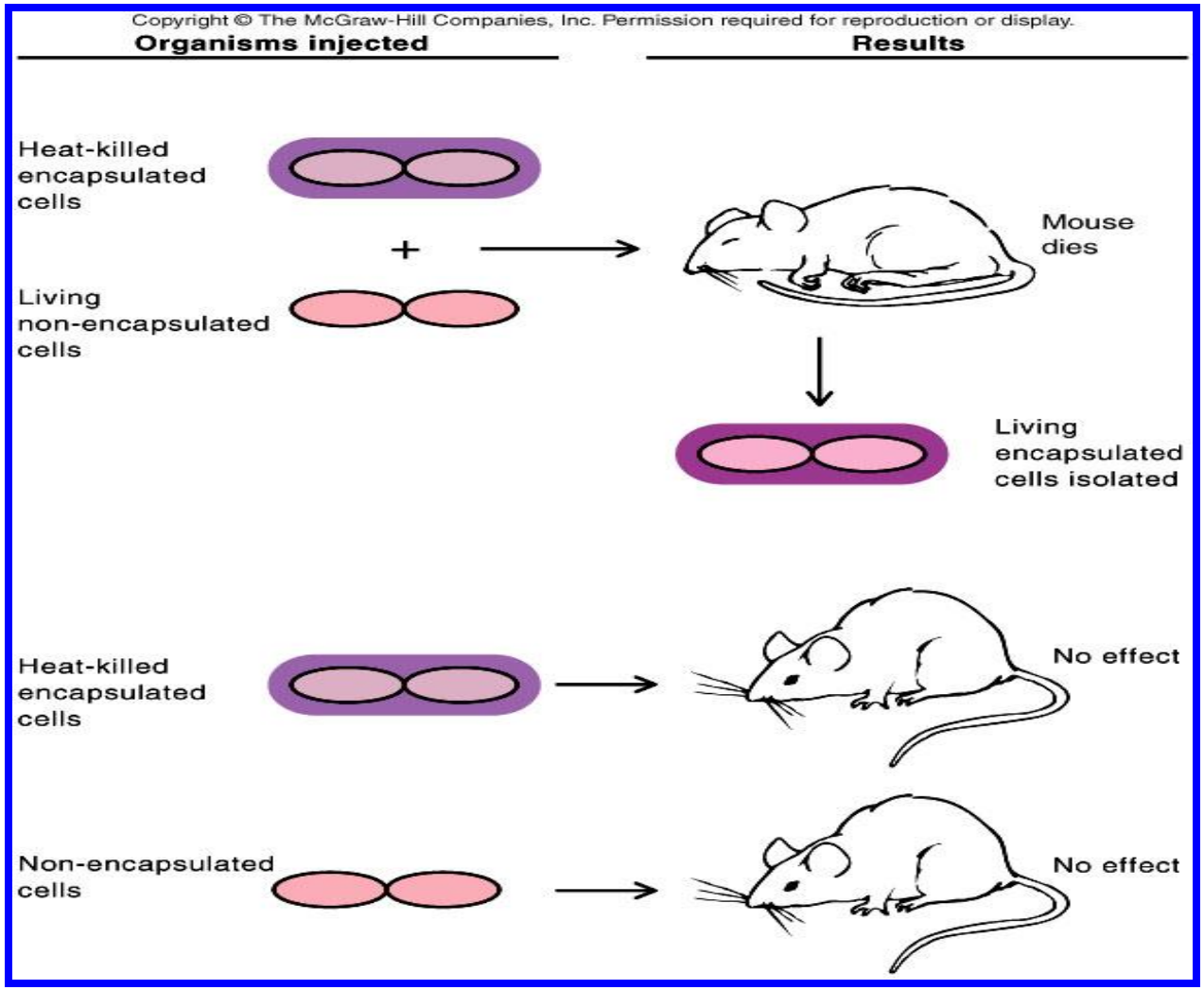
DNA-Mediated Transformation



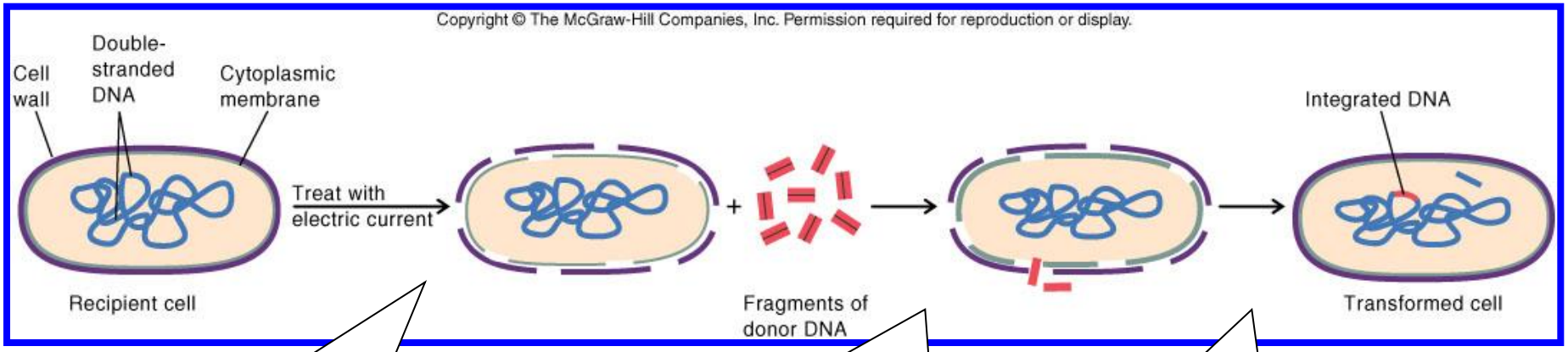
The First demonstration of **bacterial transformation**.

Experiments done by Frederick Griffith (in London) in 1928 found there were two different types of the bacterium *Streptococcus pneumoniae:*

Original Transformation Exp.
F. Griffith (1928) using pneumococci



Artificial Competence by Electroporation

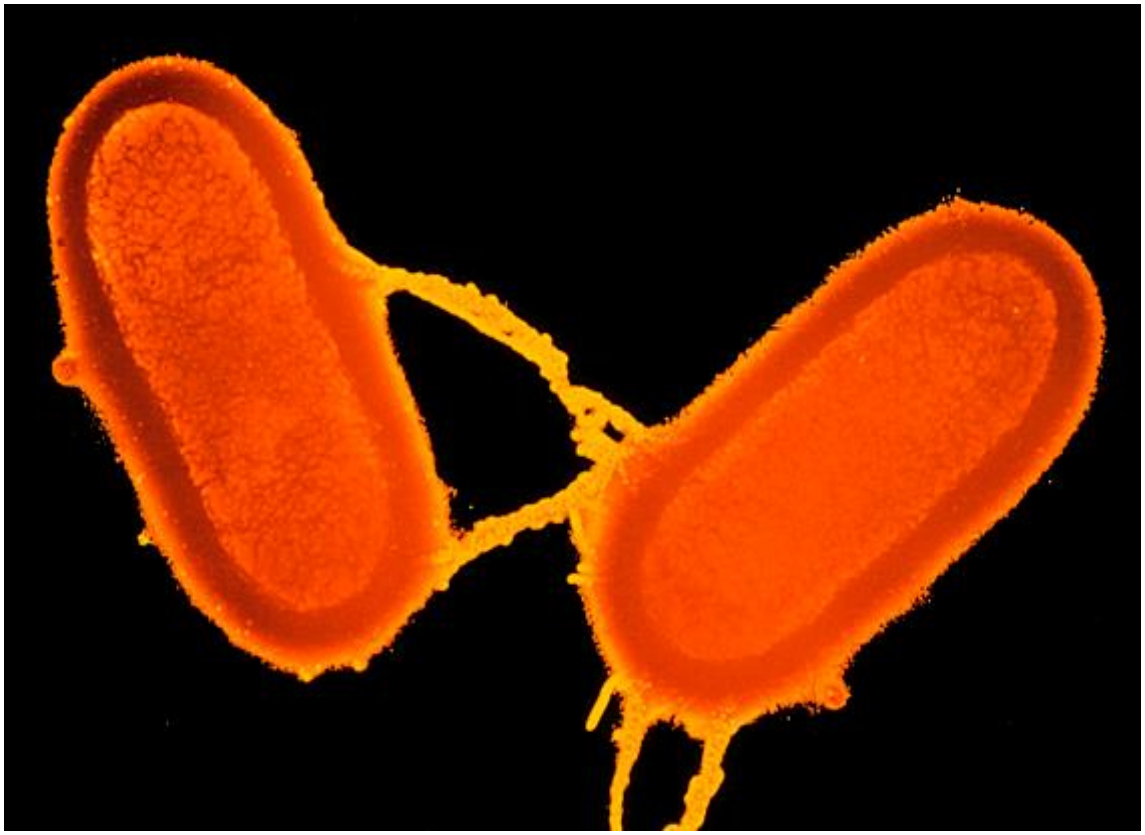


Competence denotes the ability to take up DNA naked from the environment.

Most bacteria are not naturally competent but many can be made artificially so.

Artificially induced competence is very important to gene cloning.

BACTERIAL CONJUGATION



Conjugation and Chromosome Mobilization

- Conjugation is a plasmid-encoded mechanism, but can mobilize host chromosome as well.
- The F plasmid of *E. coli* first confirmed the occurrence of conjugation.
- Conjugation involves a donor cell containing a conjugative plasmid and a recipient cell, which does not.
- Sex pilus: may be specified by the plasmid, allowing for specific pairing between donor and recipient. The pillus formed by the F plasmid is called the F pillus.

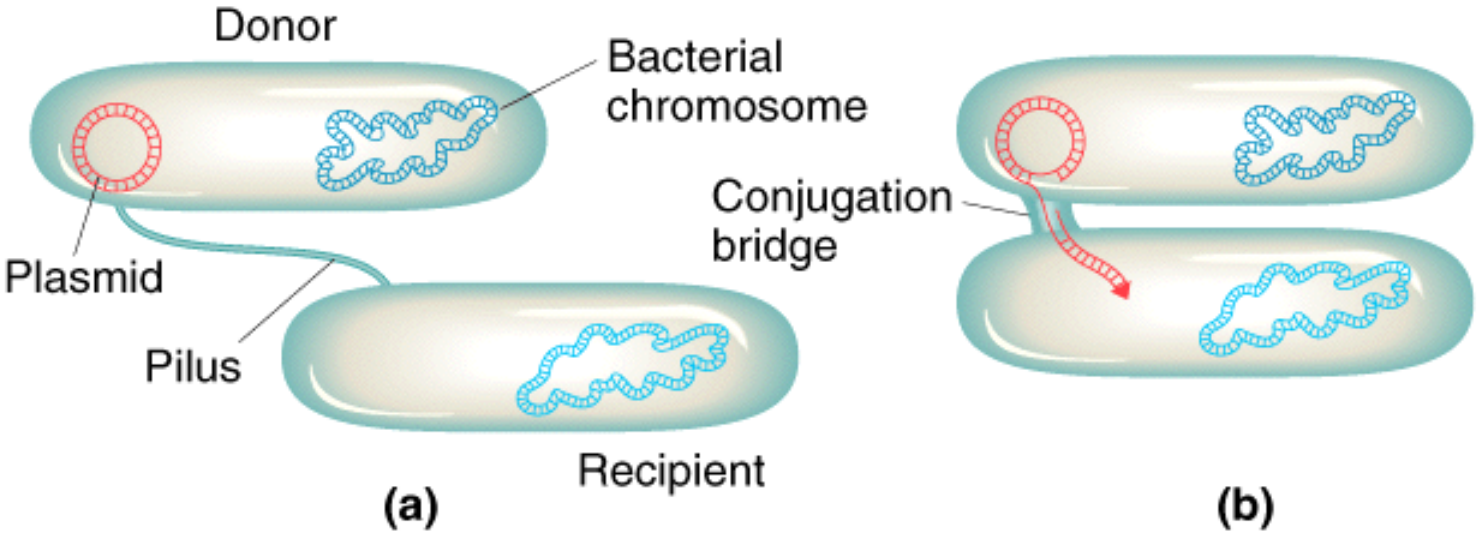
DNA Transfer During Conjugation

- DNA synthesis is necessary for DNA transfer to occur.
- Rolling circle replication: DNA transfer during conjugation. This process is triggered by cell-to-cell contact.
- At the end of the process, both donor and recipient possess plasmids and the recipient can become a donor, spreading the plasmids between populations like infectious agents.

F Plasmid

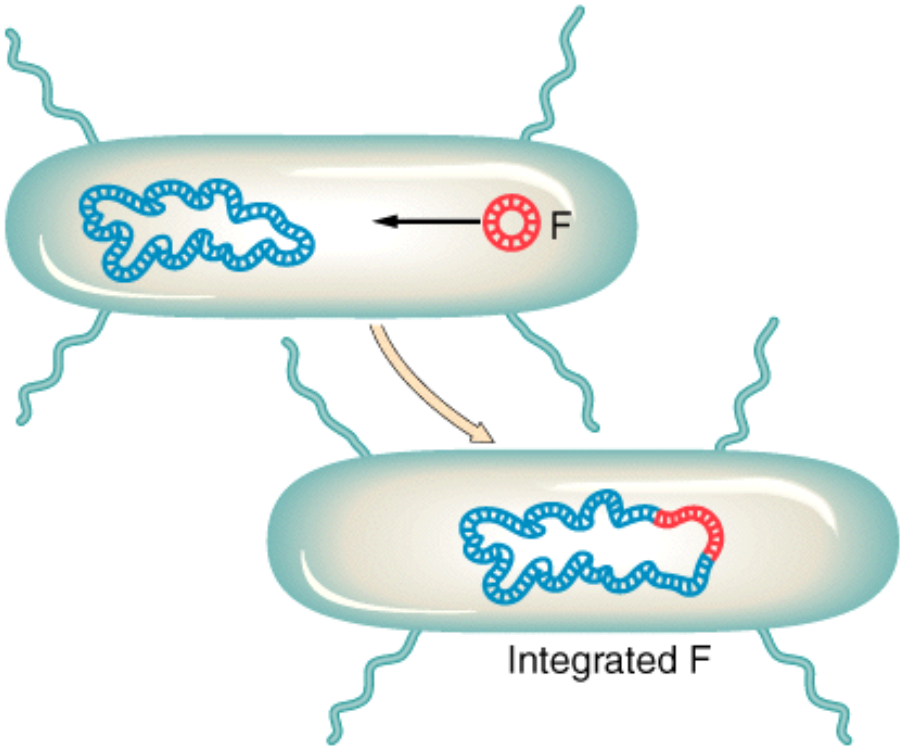
- Cells having an F plasmid are able to synthesize and F pillus, mobilize DNA for transfer to another cell, and alter surface receptors so that the cell can no longer serve as a recipient.
- The F plasmid can integrate into the host chromosome at sites called insertion sequences (IS) . Once integrated, the F plasmid no longer controls its own replication.
- Usually, because of breakage of the DNA strand during transfer, only part of the donor chromosome is transferred. Although Hfr strains transmit chromosomal genes at high frequency, they usually do not convert F- strains to F+ or Hfr because the entire F plasmid is rarely transferred. However, F+ strains can convert F- strains to F+ because the entire F plasmid is transferred.

BACTERIAL CONJUGATION



- **fertility factor – F plasmid**
- **F^+ donor & F^- recipient strains**
- **F^+ x F^- ➔ both F^+**
- **unidirectional rolling circle plasmid replication**
- **F DNA transferred through a pore in the pilus**

BACTERIAL CONJUGATION



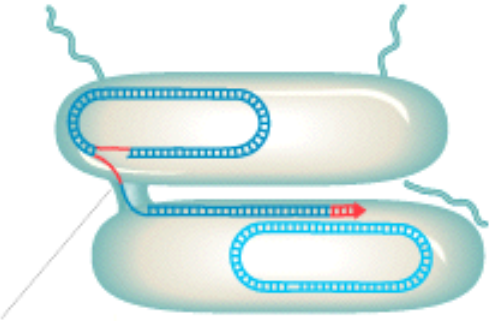
F is integrated into the host chromosome

(a)

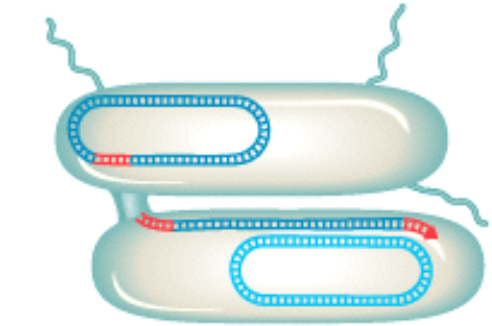
- the F plasmid *can* integrate into the host chromosome
- generates a high frequency recombinant strain ... Hfr

BACTERIAL CONJUGATION

- Hfr transfers part of the host genome during conjugation
- Hfr x F^- ➔ F^- rarely converted to Hfr or F^+



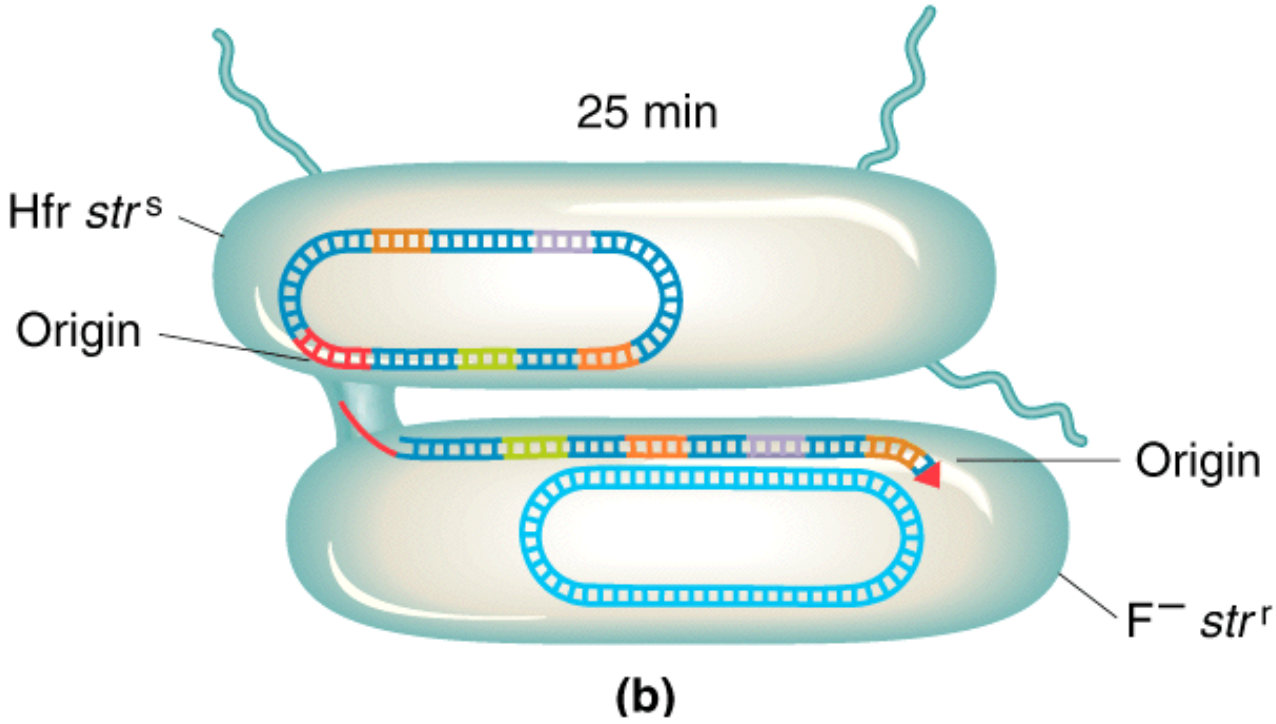
A single strand of F is transferred, along with a copy of part of the host chromosome, to a recipient cell, where a second strand is synthesized.



A copy of the host chromosome with F integrated (both generated by replication) remains in the donor cell after replication of the remaining single strand.

(b)

BACTERIAL CONJUGATION

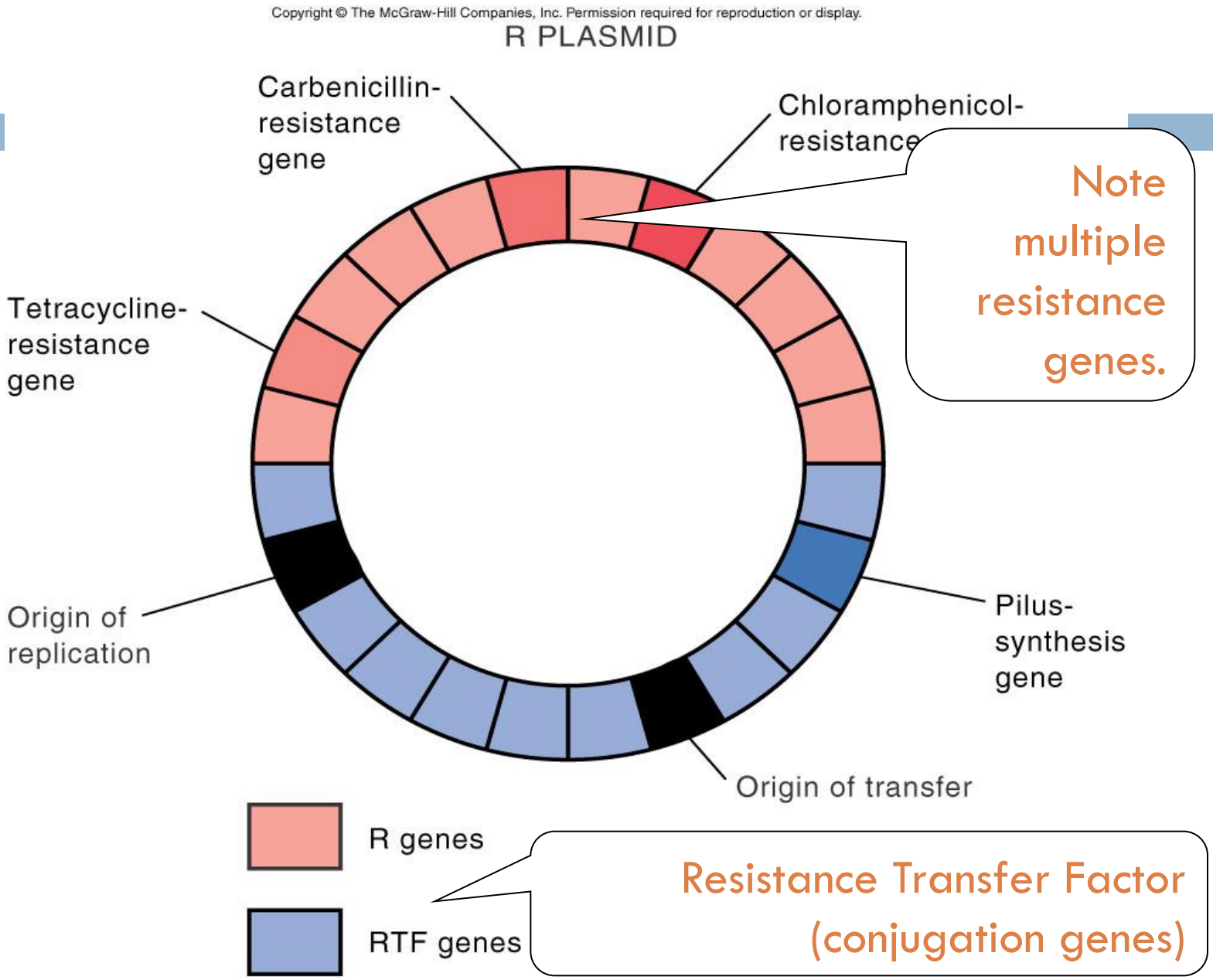


Hfr x F^{-} → recombination of donor genes in host F

Hfr (High Frequency of Recombination) Strains

- F plasmid: conjugative, can integrate into host chromosome (= **episome**), and can also mobilize chromosome transfer.
- Cells with an unintegrated F plasmid = F^+, while those having a chromosome-integrated F plasmid = Hfr, and cells without and F plasmid = F^-.
- Conjugation with Hfr donor → transfer of host chromosome.
- After transfer, an Hfr strain remains Hfr since it retains a copy of the F plasmid in the chromosome.

Self-Transmissible R Plasmid

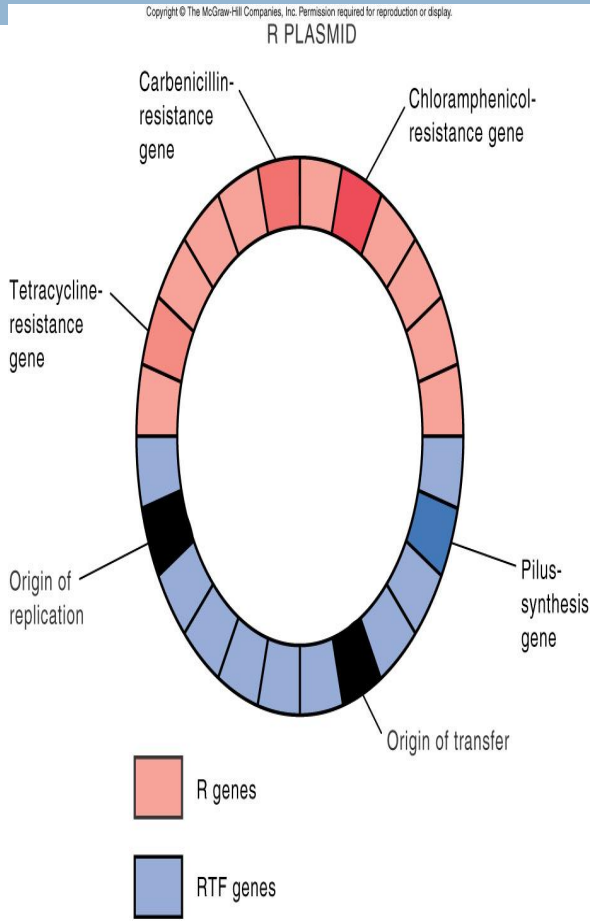


Note multiple resistance genes.

Resistance Transfer Factor (conjugation genes)

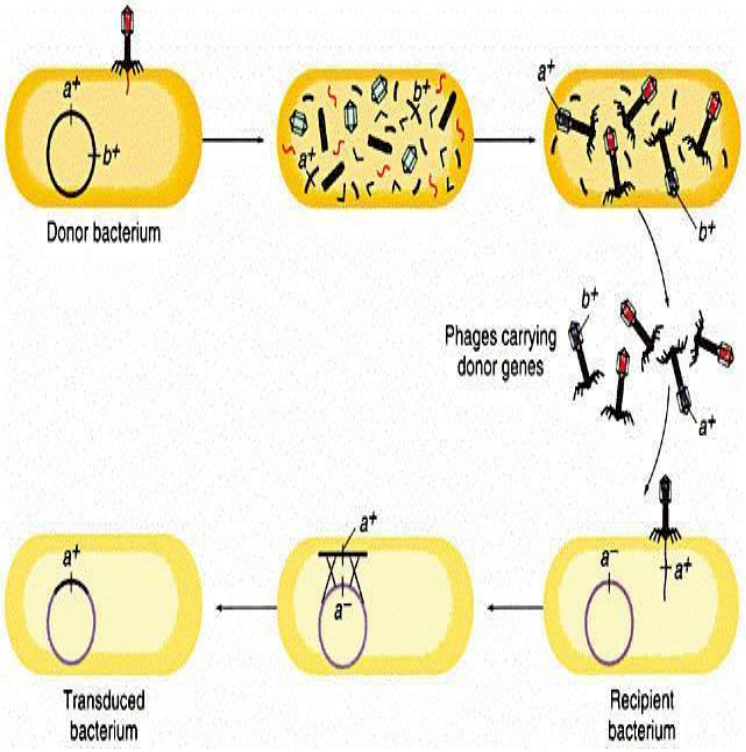
Self-Transmissible R Plasmid

Some Gram-negative bacteria harbor plasmids that contain antibiotic resistance genes, such plasmids are called R factors. The R factor has two components, one that codes for self transfer (like F factor) called RTF (resistance transfer factor) and the other R determinant that contains genes coding for antibiotic resistance. R plasmids may confer resistance to as many as five different antibiotics at once upon the cell and by conjugation; they can be rapidly disseminated through the bacterial population. The difference between F factor and R factor is that the latter has additional genes coding for drug resistance. During conjugation there is transfer of resistance plasmid (Rplasmid) from a donor bacterium to a recipient. One plasmid strand enters the recipient bacterium while one strand remains in the donor. Each strand then makes a complementary copy. R-plasmid has genes coding for multiple antibiotic resistance as well as sex pilus formation. The recipient becomes multiple antibiotic resistant and is now able to transfer R-plasmids to other bacteria. When the recipient cells acquire entire R factor, it too expresses antibiotic resistance.



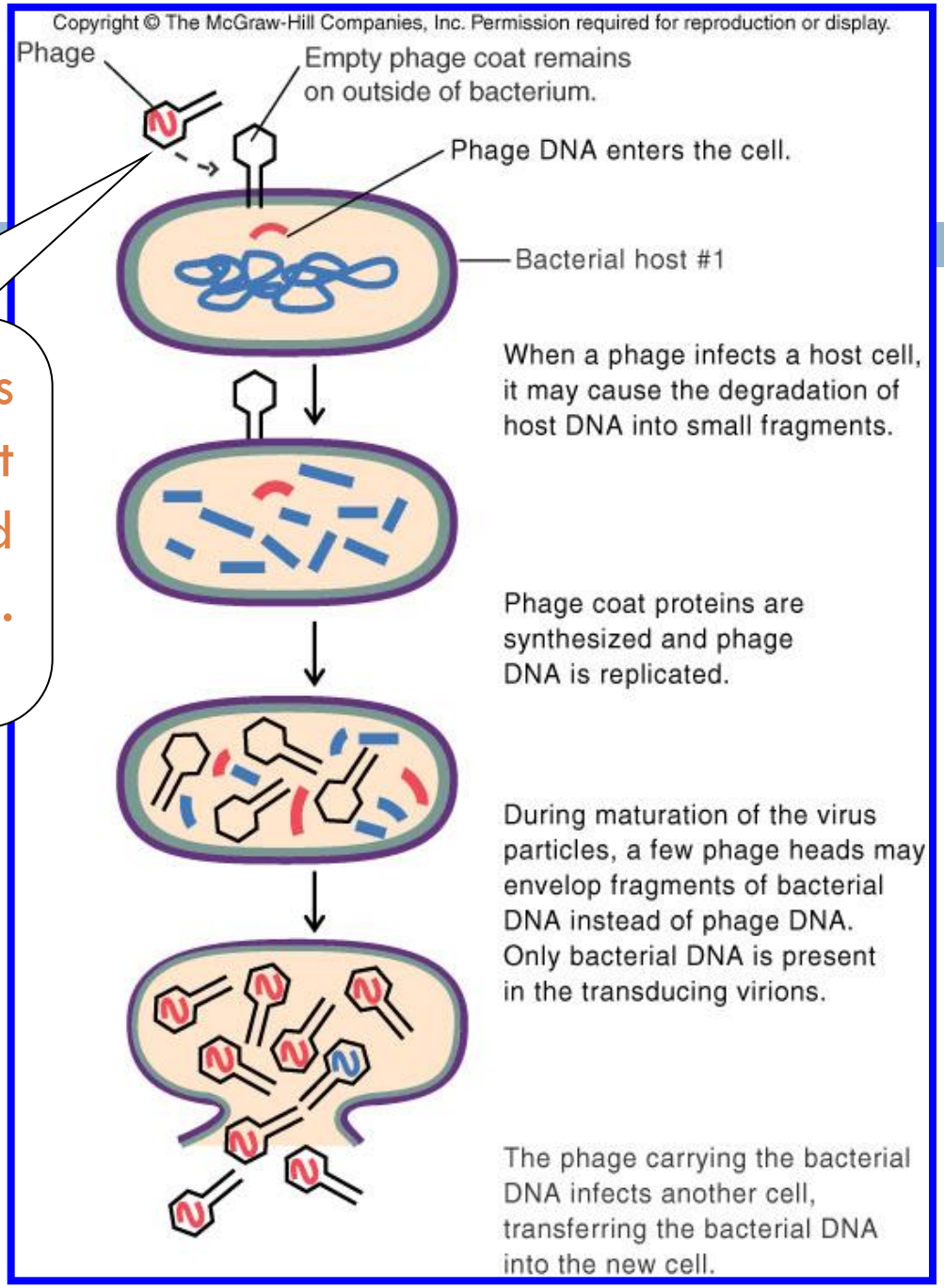
TRANSDUCTION:

Bacteriophage are viruses that parasitize bacteria and use their machinery for their own replication. During the process of replication inside the host bacteria the bacterial chromosome or plasmid is erroneously packaged into the bacteriophage capsid. Thus newer progeny of phages may contain fragments of host chromosome along with their own DNA or entirely host chromosome. When such phage infects another bacterium, the bacterial chromosome in the phage also gets transferred to the new bacterium. This fragment may undergo recombination with the host chromosome and confer new property to the bacterium. Life cycle of bacteriophage may either by lytic or lysogenic. In the former, the parasitized bacterial cell is killed with the release of mature phages while in the latter the phage DNA gets incorporated into the bacterial chromosome as prophage.

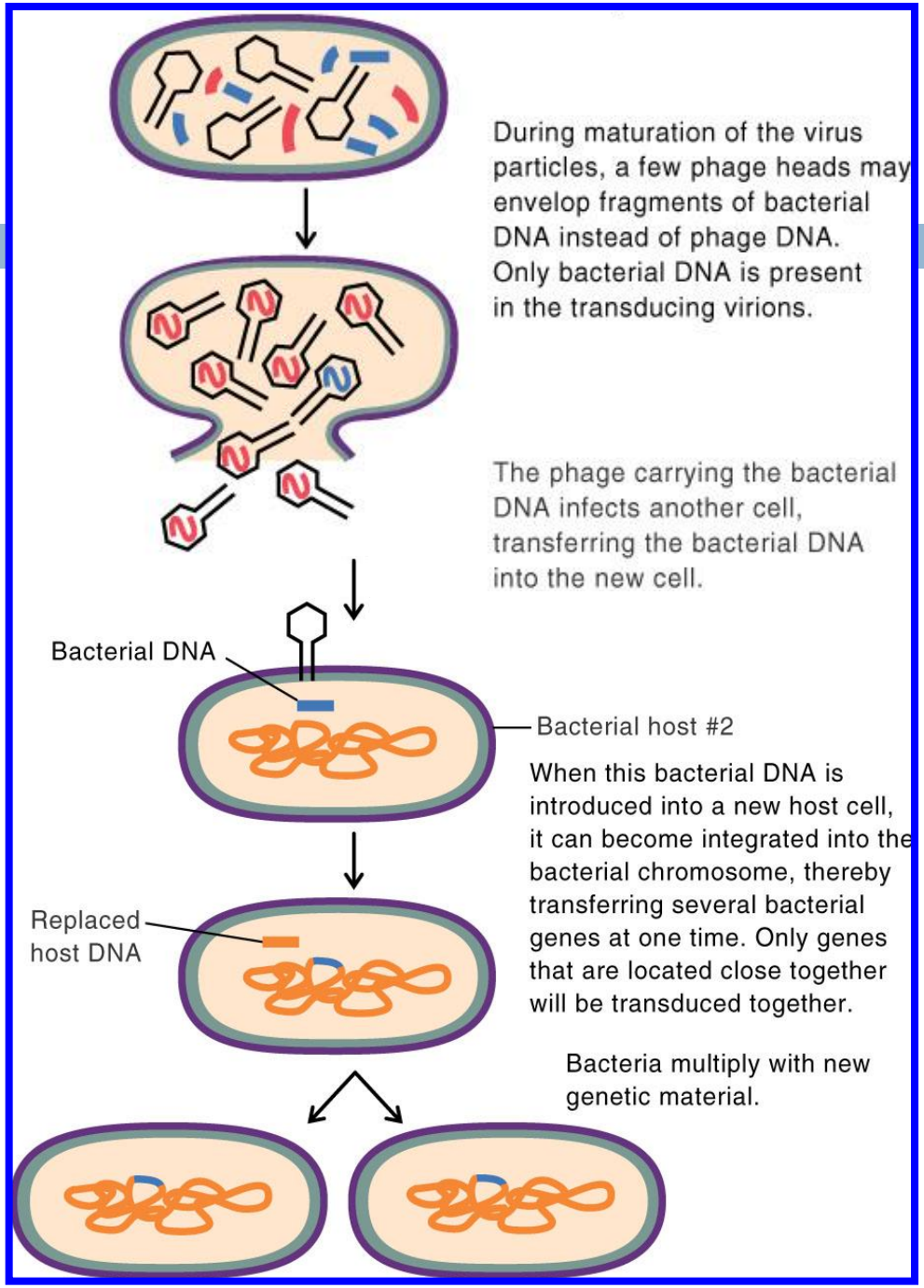


Generalized Transduction

Bacteriophages are viruses that only infect (and can kill) bacteria.



Generalized Transduction




GOOD
BAY