Bacteriology
2016-2017
2nd year

Lecture 1

Dr. Halah Al-Haideri

-Microbiology: is the study of microscopic organisms, those being unicellular (single cell), multicellular (cell colony), or a cellular (lacking cells).
-A **microorganism** or **microbe** is a microscopic living organism, which may be single-celled or multicellular.

-Microorganisms are very diverse and include all bacteria, archaea and most protozoa. This group also contains some species of fungi, algae, and certain microscopic animals, such as rotifers (commonly called **wheel animals, and presence in fresh water)**.
- Many microscopic animals and plants have microscopic juvenile stages.

- **Bacteriology**: is the science that deals with the study of microorganisms known as bacteria
- The term includes a large group of typically unicellular microscopic organisms which are widely distributed in air, water, soil, still ponds, the bodies of living plants and in the intestinal tract of human and animals.
- The kinds and numbers vary from one place to another, depending upon environmental conditions.
- The great majority of bacteria do not cause disease in animals, humans, or crops, instead, they are beneficial in many ways, ex; organic compounds recycling by plants and animals degradation

History of Bacteriology

❖ The existence of most type of microorganisms like bacteria was firstly reported by **Leeuwenhoek** (1676). He examined a great variety of natural objects by his simple magnifying microscopic lenses.

❖ **Louis Pasteur** (1861). (Father of Microbiology);

-He showed that the changes in the fermenting matter with the resulting formation of alcohol were brought about by living organism like yeast and not by simple chemical process.

-**Facultative anaerobic**: organisms that can live with or with out oxygen

-**Pasteurization**; a process of heating just enough to kill or prevent contaminating organism.

-**Vaccination**; Anthrax, Cholera and rabies

❖**Robert Koch**; express the germ theory of disease and discover the cause of anthrax; a rod-shaped bacterium that formed chains, spores that formed within the bacterial cells produce anthrax when they were injected into mouse.

- **Koch's postulated** (1884):

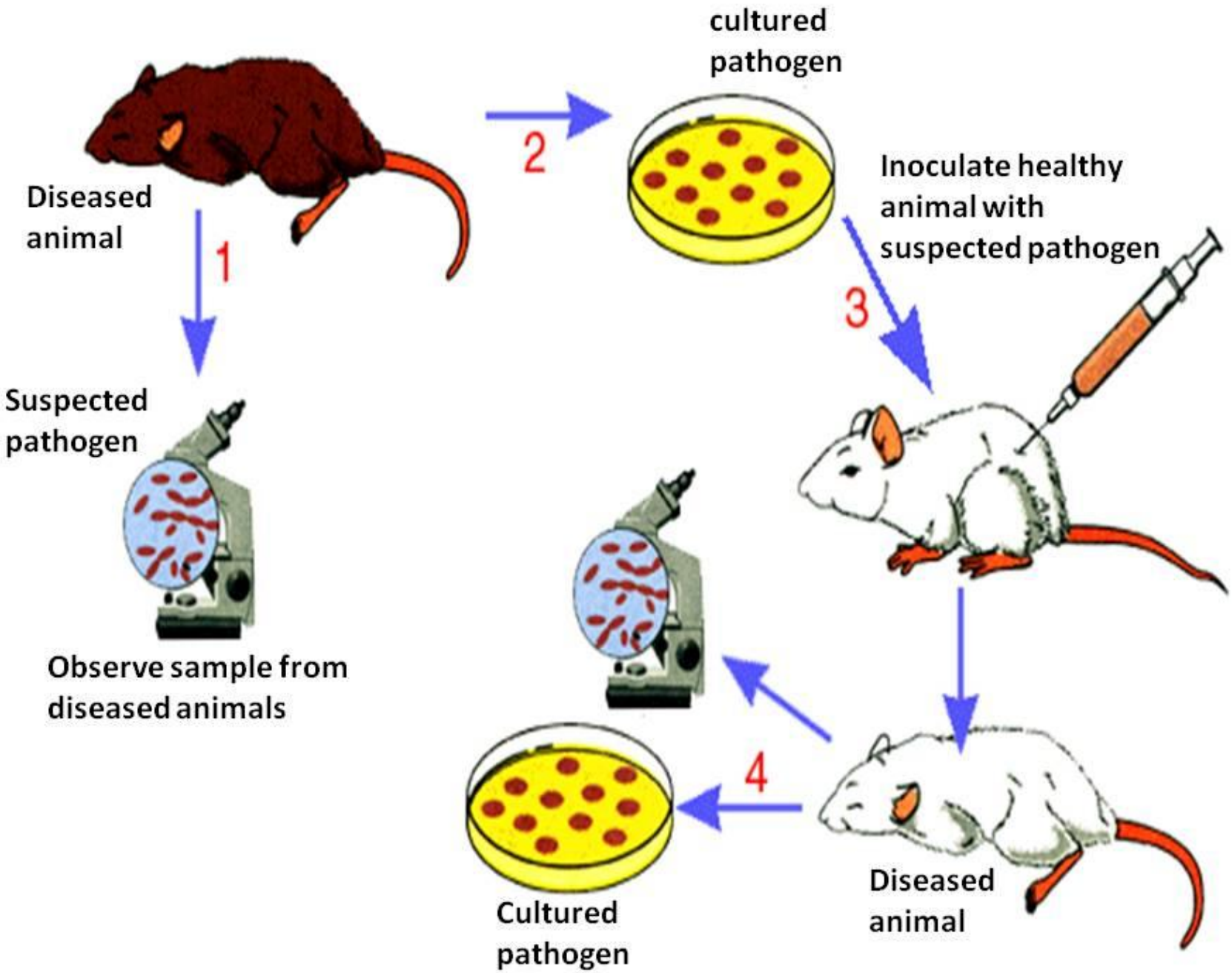
1-The suspected causative agent must be found in every case of the disease and be absent from healthy hosts.

2-The agent must be isolated and grown outside the host.

3-When the agent is introduced to a healthy, susceptible host, the host must get the disease.

4- The same agent must be re-isolated from the diseased experimental host.

- In 1882, announced that *Mycobacterium tuberculosis* is the cause of tuberculosis.


cultured
pathogen
Diseased
animal
2
1
Inoculate healthy
animal with
suspected pathogen
3
Suspected
pathogen
Observe sample from
diseased animals
4
Diseased
animal
Cultured
pathogen

Others

- 1882- Paul Ehrlich : developed acid-fast stain.
- 1884- Christian Gram : developed Gram stain.
- 1887- R.J.Petri : invented Petri Dish.
- 1908 – Paul Ehrlich : discovered cure for syphilis .
- 1929- Alexander Fleming : discovered Penicillin .
- 1980- Carl Richard : defining the archaebacteria as anew Domain of Life .
- 1995- First microbial genomic sequence (*H. influenzae*) published .

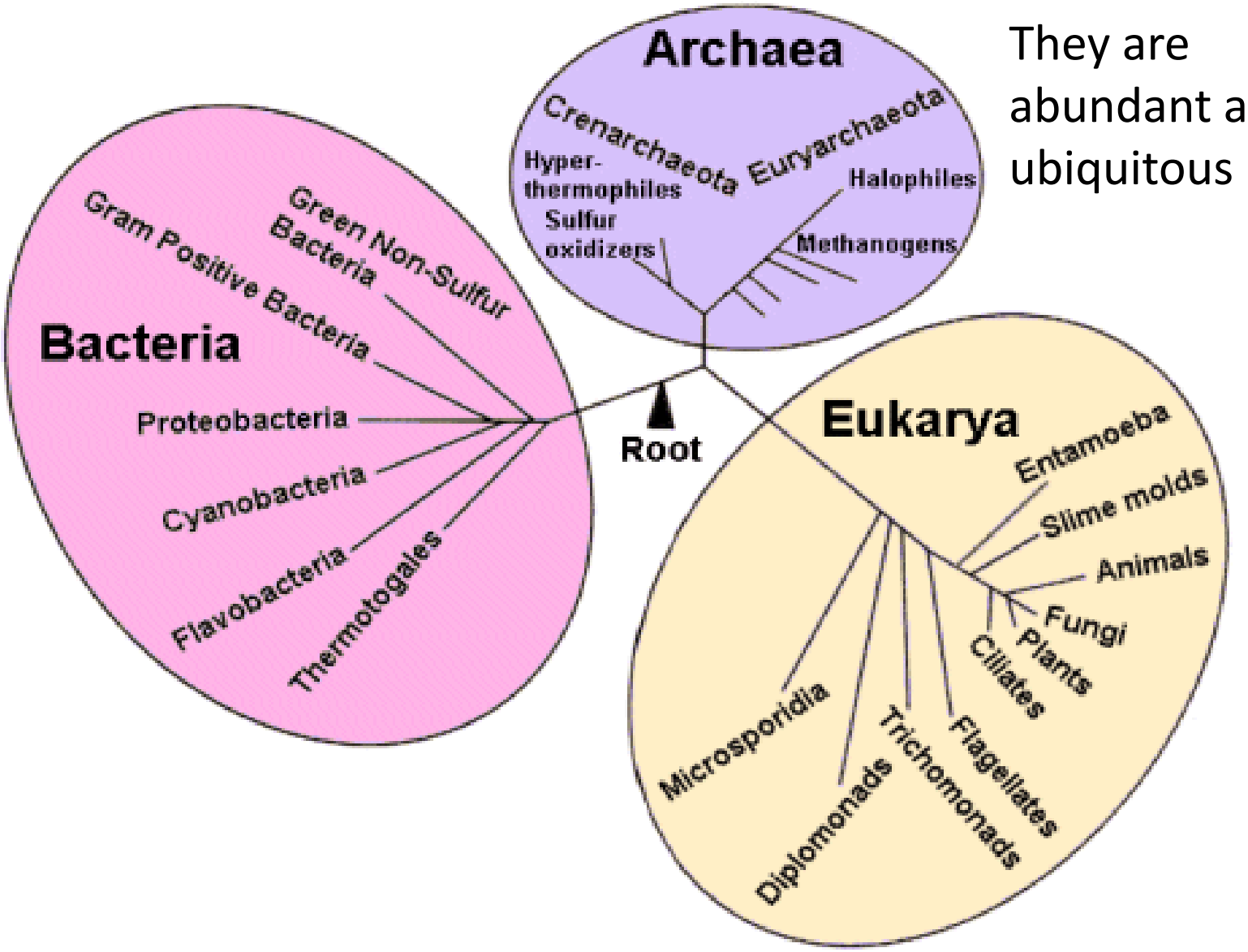
Evolution of Prokaryotes

❖ In the recent past, the living things have been grouped into five kingdoms (Animals, plants, fungi, protists and prokaryotes.

❖Prokaryotes : an organism whose cell or cells are characterized by the absence of a nucleus or any other membrane-bound organelles.

❖In the late 20^{th} century, the difference in the structure of cell membranes and the sequence of small-subunit ribosomal RNA (SSU-rRNA) offers a fundamental way to group organisms on earth into three domains: Bacteria (all organism in the kingdom bacteria), Archaea (the rest of prokaryotes)and Eukaryotes (animal, plant, fungi and protists)

They are abundant and ubiquitous



Properties of Bacteria and Archaea compared with Eucarya.

Property	Eucarya	Bacteria	Archaea
Cell type	eucaryotic	procaryotic	procaryotic
Nuclear membrane	present	absent	absent
Number of chromosomes	>1	1	1
Chromosome shape	linear	circular	circular
Murein in cell wall	-	+	pseudomurein
Cell membrane sterols	present	absent	absent
Organelles (mitochondria and chloroplasts)	present	absent	absent
Ribosome size	80S	70S	70S
Meiosis and mitosis	present	absent	absent
Amino acid initiating protein synthesis	methionine	N-formyl methionine	methionine
Protein synthesis inhibited by streptomycin and chloramphenicol	-	+	-

Characteristics of bacteria:

❖They are microscopic unicellular prokaryotic organisms (1-10 µm) length and (0.2 µm) width, characterized by the lack of a membrane-bound nucleus and membrane-bound organelles like **mitochondria**, **Golgi bodies** and **ER** etc.

❖ are remarkably adaptable to diverse environmental conditions: they are found in the bodies of all living organisms and on all parts of the earth—in land terrains and ocean depths, in arctic ice and glaciers, in hot springs, and even in the stratosphere.

❖Bacteria fall into one of two groups, **Archaebacteria** (ancient forms thought to have evolved separately from other bacteria) and **Eubacteria** (bacteria).

❖They may occur singly or aggregations to form colonies.

❖ They possess rigid cell wall. **Cell wall** is made up of **peptidoglycan (Mureins) and Lipo polysaccharides**.

❖Absence of well defined nucleus.i.e., **DNA** is not enclosed in a nuclear membrane.

❖**Ribosomes** are scattered in the cytoplasmic matrix and are of **70S** type.

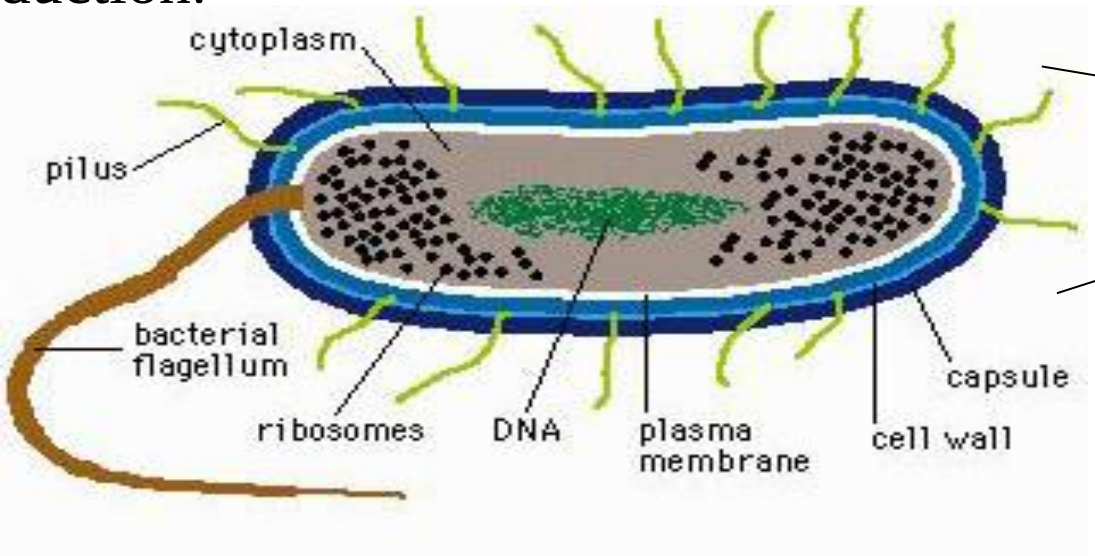
❖The plasma membrane is invaginated to form mesosomes**.**

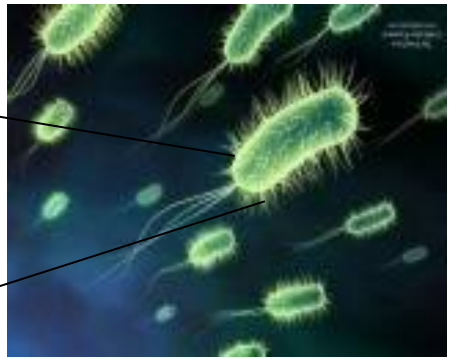
❖Most of the bacteria are heterotrophic. Some bacteria are autotrophic, possess **bacteriochlorophyll**, which is not in plastids., nstead, it is found scattered.

❖Motile bacteria possess one or more **flagella.**

❖The common method of multiplication is **binary fission.**

❖Lacking of true sexual reproduction, whereas, **genetic recombination** occurs by conjugation ,transformation and transduction.

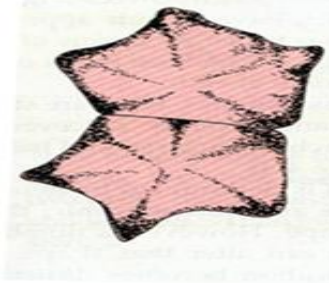


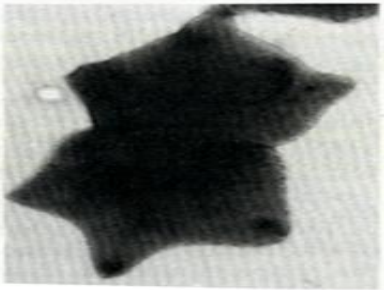


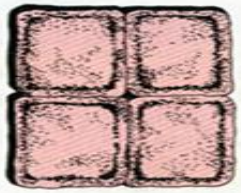
The shape of bacterial cells:

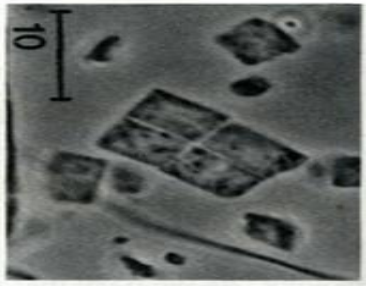
- **Coccus**: cells that are spherical in shape like **Streptococcus** with spherical cells arranged in chains, like beads on a string.
- **Staphylococcus**: a bacterium with spherical cells arranged in clusters, like clusters of grapes.
- **Diplococcus**: spherical cells arranged in pairs likes *Nieisseria*
- **Tetrad**: when cells arranged in a group of four, looks almost like a square under the microscope.
- **Bacillus** (plural, bacilli): a bacterium with rod shaped cells.
- **Spiral:** curved bacteria which can range from a gently curved shape to a corkscrew- like spiral like *Campylobacter jejuni.*
- **Comma-shaped:** with less than complete turn or twisted in the cell. Like *Vibrio cholera* .
- **Filamentous:** very long thin filamentous-shaped bacteria. Some of them form branching filaments resulting in a network of filaments called ‘mycelium’, like *Candidatus* Savagella


Coccus
Rod, or Bacillus
Curved forms: Spirillum/Spirochete
Diplococci (cocci in pairs)
Neisseriae (coffee-bean shape in pairs)
Coccobacilli
Vibrios (curved rods)
Tetrads (cocci in packets of 4)
Sarcinae (cocci in packets of 8,16,32 cells)
Mycobacteria
Corynebacteria (palisades arrangement)
Spirilla
Streptococci (cocci in chains)
Micrococci and staphylococci (large cocci in irregular clusters)
Spore-forming rods
Streptomycetes (moldlike, filamentous bacteria)
Spirochetes





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Lecture 2
Bacterial cell structure

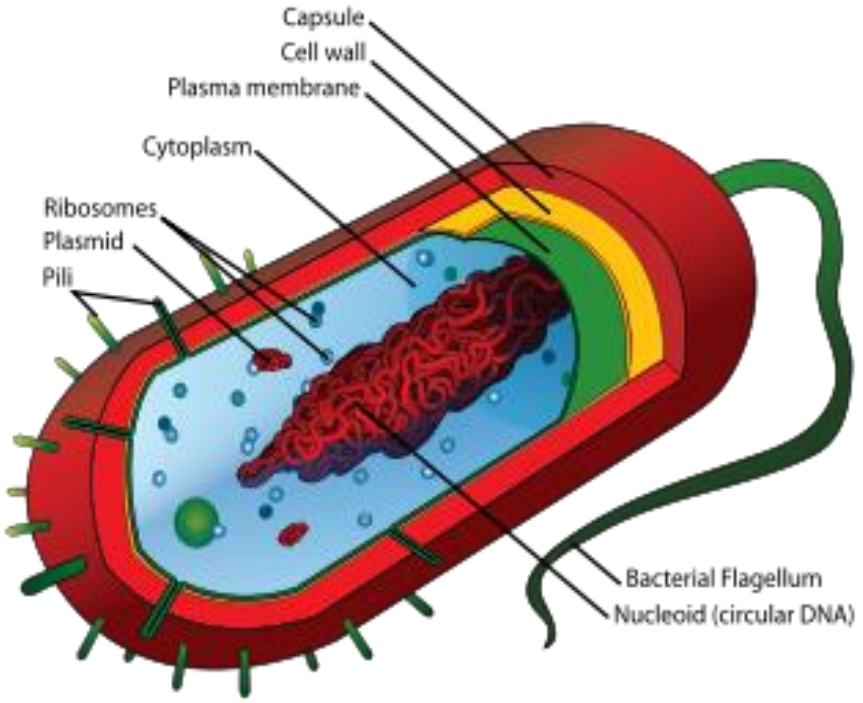
Dr. Halah Al-Haideri

Bacterial cell structure

-Many cells have special external features that enable them to respond to other cells and environment. In prokaryotes, these features include glycocalyces, flagella, fimbriae and pilli.

-Other features belongs to the surface layer, like capsule, cell wall, plasma membrane.

-Intracellular structures include mesosomes, nucleoid, Ribosomes, endospores and inclusion granules



Flagella

-The most notable structures responsible for bacterial movement, long structures that extend beyond the surface of the cell and glycocalyx.

-Consist of three parts: a long thin *filament* (helical propeller), the *hook* (universal joint), and the *basal body* (rotary motor).

-**Filament:** is the largest part, 20 nm in diameter, extend out to the cell's environment.

- Encoded by *FlaA* and *FlaB*

-Composed of many identical globular molecules of protein **(flagellin),** self-assembled to from the hollow core of the flagellum, encoded by *FliC* in *E.coli* and *S. enterica*

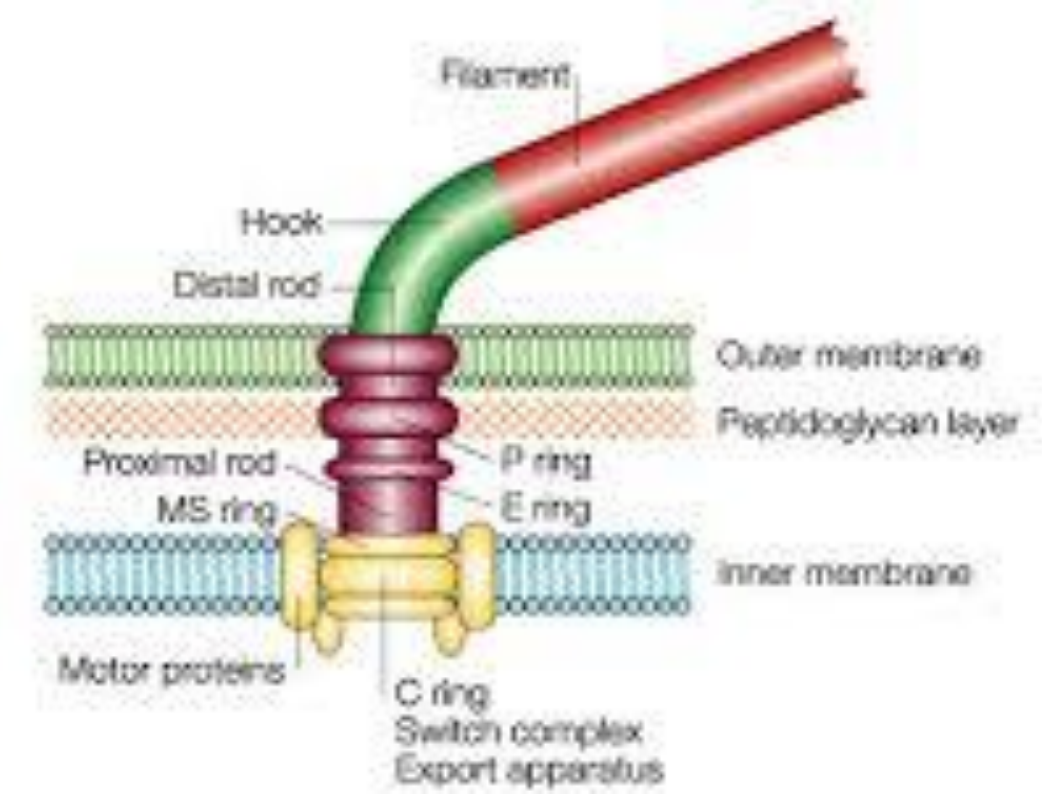
The hook: short tubular structure, connect the filament to the basal body .Universal joint, composed of 120 copies of single protein FlgE. The junction between the hook and filament consist of two proteins FlgK and FlgL.

-The proximal end of the hook is connected to the **basal body**, consists of the rod and coaxially mounted rings: MS, P and L.

-**MS** is embedded in the cytoplasm, **P** and **L** are associated with the peptidoglycan and OM (outer membrane).

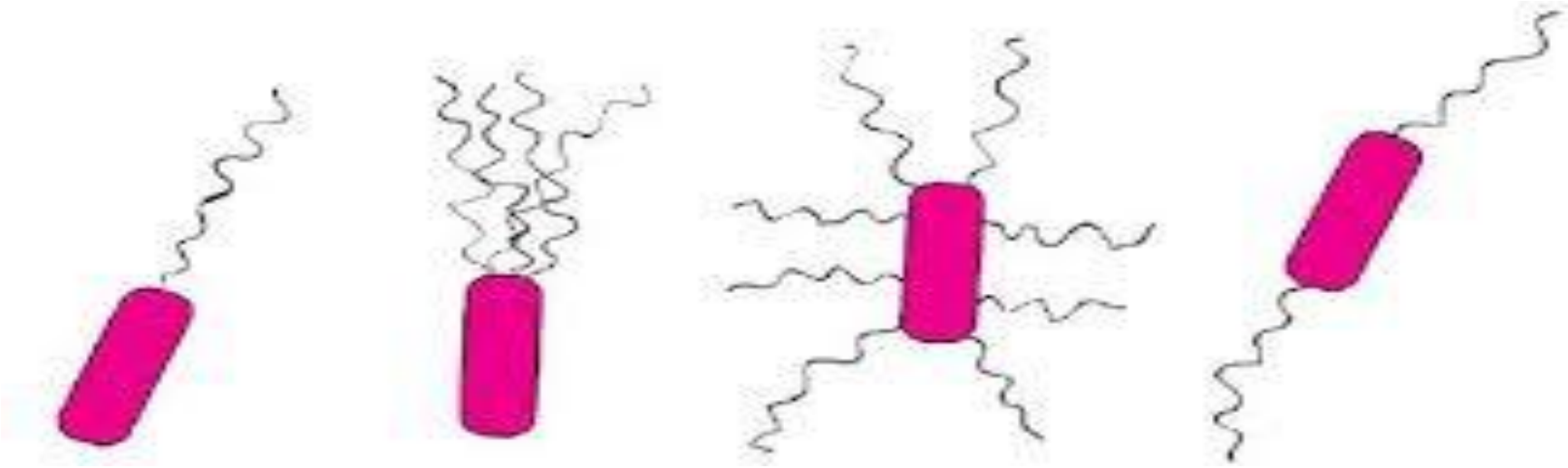
-In Gram-positive , the **LP** rings are present,

C ring is not found.



Flagellar arrangement:

A) Monotrichous: single polar flagellum at one end.
B) Lophotrichous: more than one flagelum at one end.
C) Peritrichous: flagella cover the surface of the cell
D) Amphitrichous: one or more and both ends.



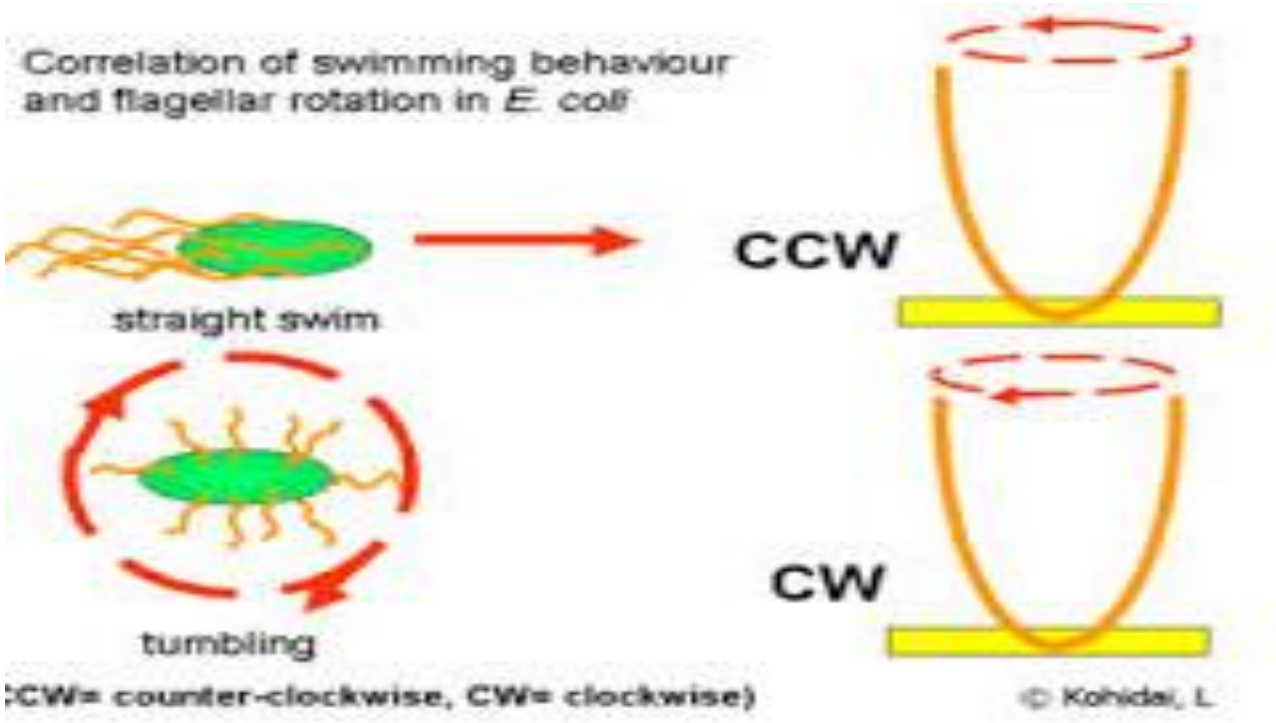
A **B** **C** **D**

The function:

-The exact mechanism by which bacterial flagellum moves is not completely understood.

-Flagella rotate 360°, direct the bacterium through the environment.-

-Direction can be changed from clockwise (CW) to counterclockwise (CCW).



-Bacterial movement occurs in response to stimuli (taxis)
-In the presence of favourable stimuli, the receptors that found on the surface of the cells, send signals to the flagella, which then adjust their speed and direction of rotation.
-The stimulus may be

- light: phototaxis
- Chemical like glucose : chemotaxis
- Presence of Oxygen: aerotaxis
- Response to magnetic field: magnetotaxis

- Movement toward a favourable movement is *positive movement*, whereas, movement away from an unfavourable movement is *negative movement*.

Fimbriae and Pili

-Found in G -ve and some G +ve.

-Sticky, bristle like projection, shorter than flagella

-Pathogens may use fimbriae **to adhere to another host, or to the substances** in the environment , like in *Neisseria gonorrhoeae*

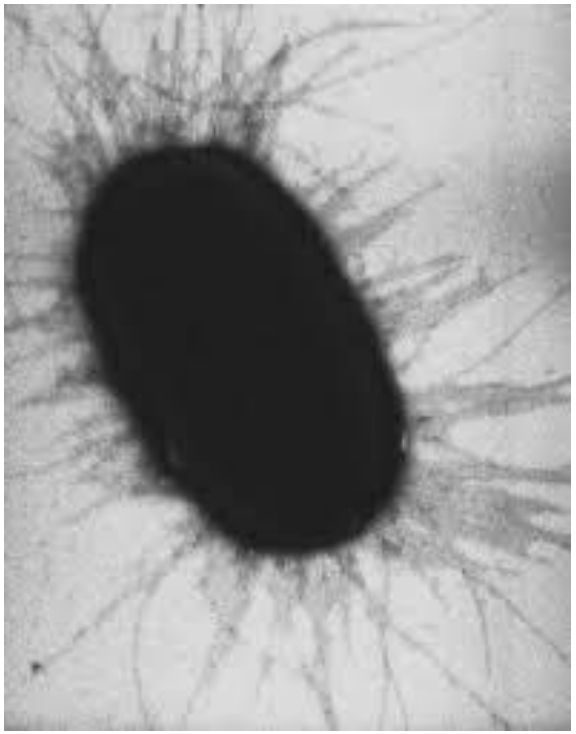
-Serve an important role in **biofilm formation.**

Pili: tubular structure, longer and thinner than flagella.

-Made up of protein pilin

- found in G-ve only.

- Role in bacterial conjugation by cell to cell attachment.



Glycocalyx: gelatinous, sticky substance that surrounds the outside of the cell, also called the sugar cup.

-It may composed of polysaccharide, polypeptide, or both.

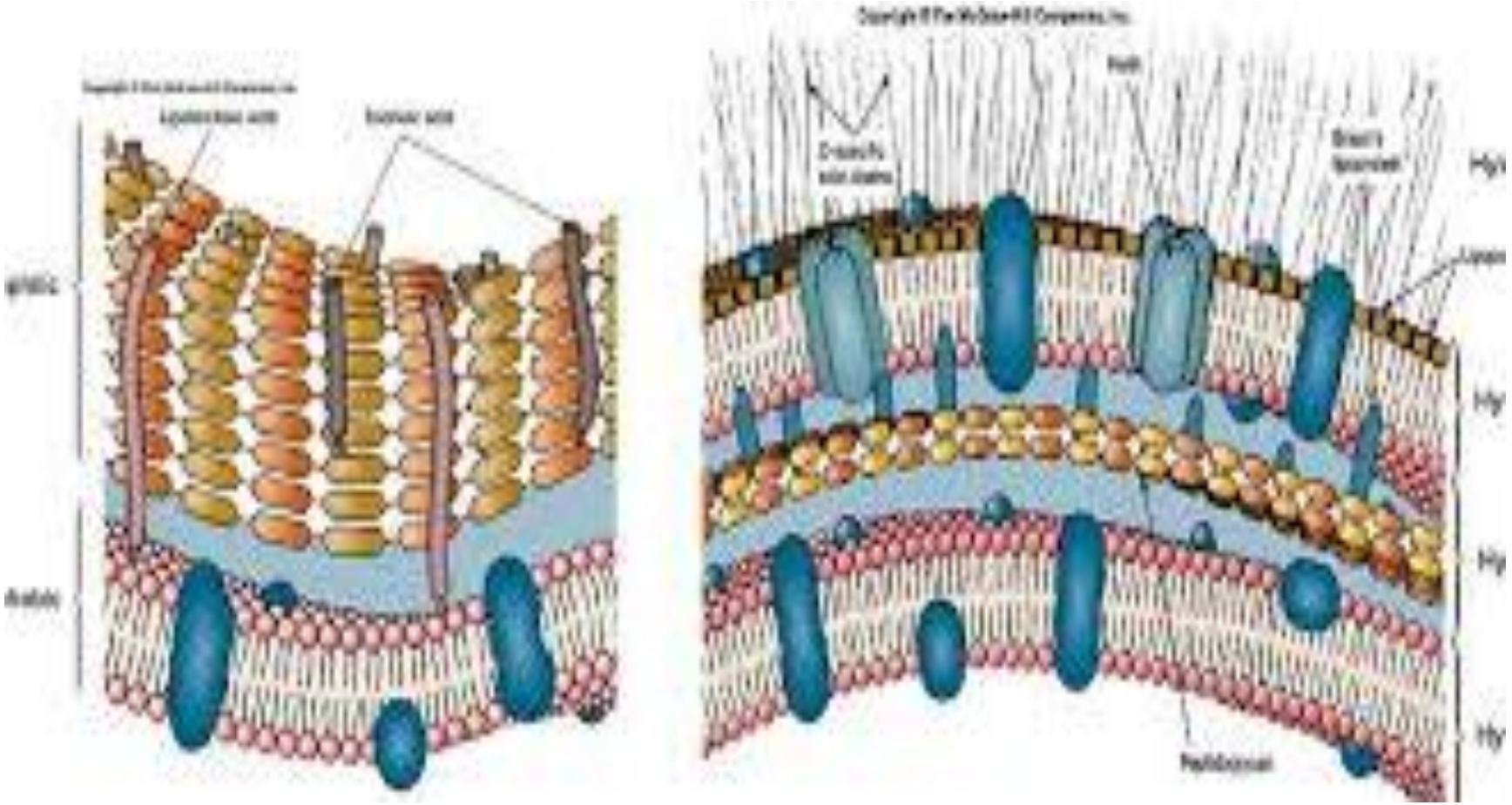
-It also may consist from organized repeating units of organic chemicals firmly attached to the cell surface, is called as **a capsule**, whereas, a slime layer refers to a loose and water soluble glycocalyx.

- Plays a role in the ability of bacterial cell to survive and to cause disease.

-The slim layers enable the oral bacteria to colonize the teeth.

-Capsules may prevent bacteria from being recognized or devoured by defensive cells of the host, like *Streptococcus pneumonia* and *klebsiella pneumonia*.

The cell wall: is the principle stress-bearing and shape-maintaining element in bacteria, and its integrity act as a critical importance to cell viability.



The cell wall

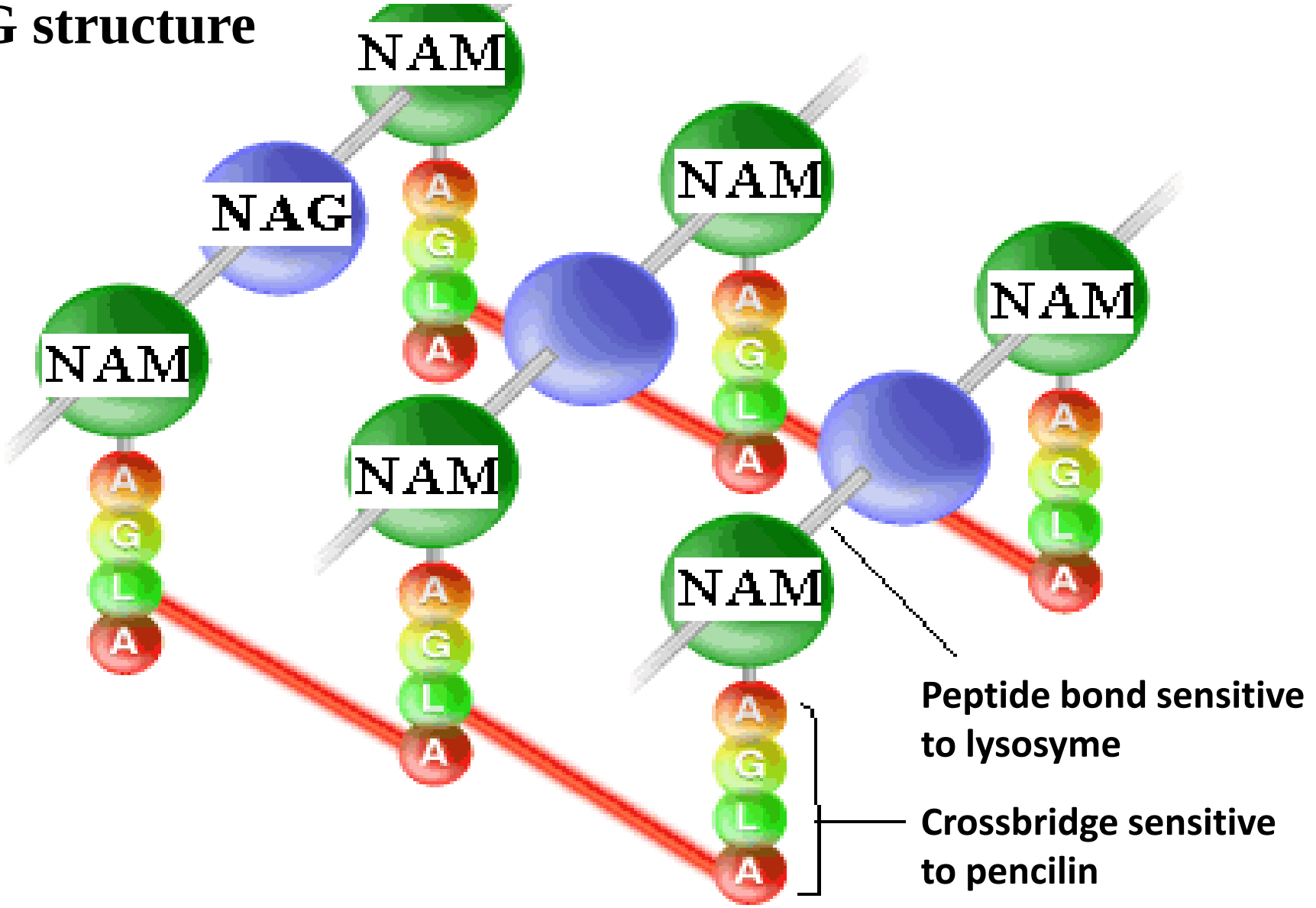
In both G-ve and G+ve, it is composed of cross –linked polymer **peptidoglycan** (PG) or **murein**.

-The basic PG architecture is built up of two types of regularly alternating sugar molecules; ***N-acetylglucosamin* (*NAG*)** and ***N-acetylmuramic acid* (*NAM*)**, which are structurally similar to glucose.

-Millions molecules of NAG and NAM are covalently linked in chains in which NAG alternates with NAM, connected by peptide bond. These chains are the **glycan** portions of PG.

-Chains of NAG and NAM are attached to other chains by cross bridges of four amino acids (tetrapeptides); L-alanine, D-glutamic acid, L-Lysyl and D-alanine, which are connected by short crossbridge. This crossbridge is the **peptide** portion of PG.

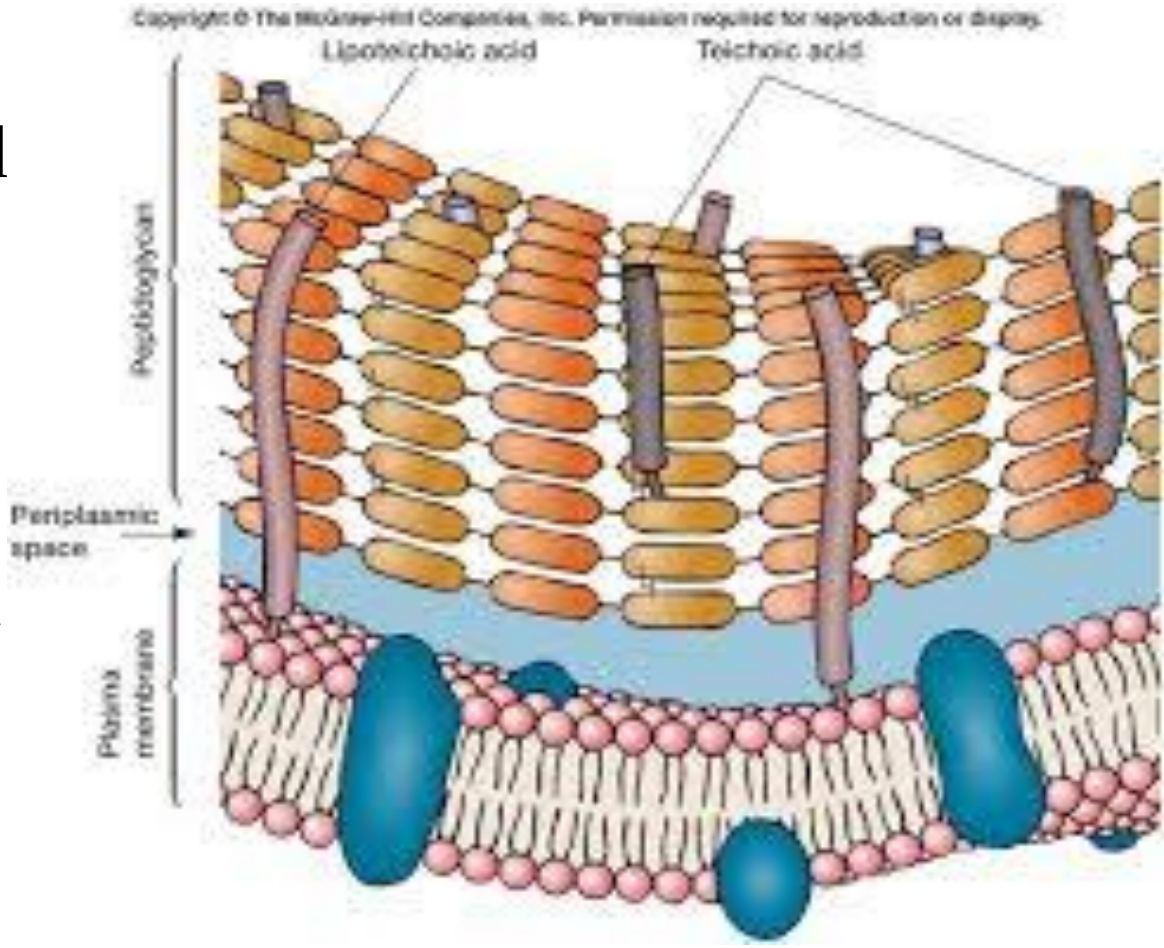
PG structure



Gram –positive cell wall:

-Thick layer of PG, contains unique polyalcoholic acid called teichoic acid. Some of them are covalently linked to lipid (lipoteichoic acid) that anchor the PG to the cell membrane.

- Teichoic acid is a –ve charge, play a role in passage of ions through the wall

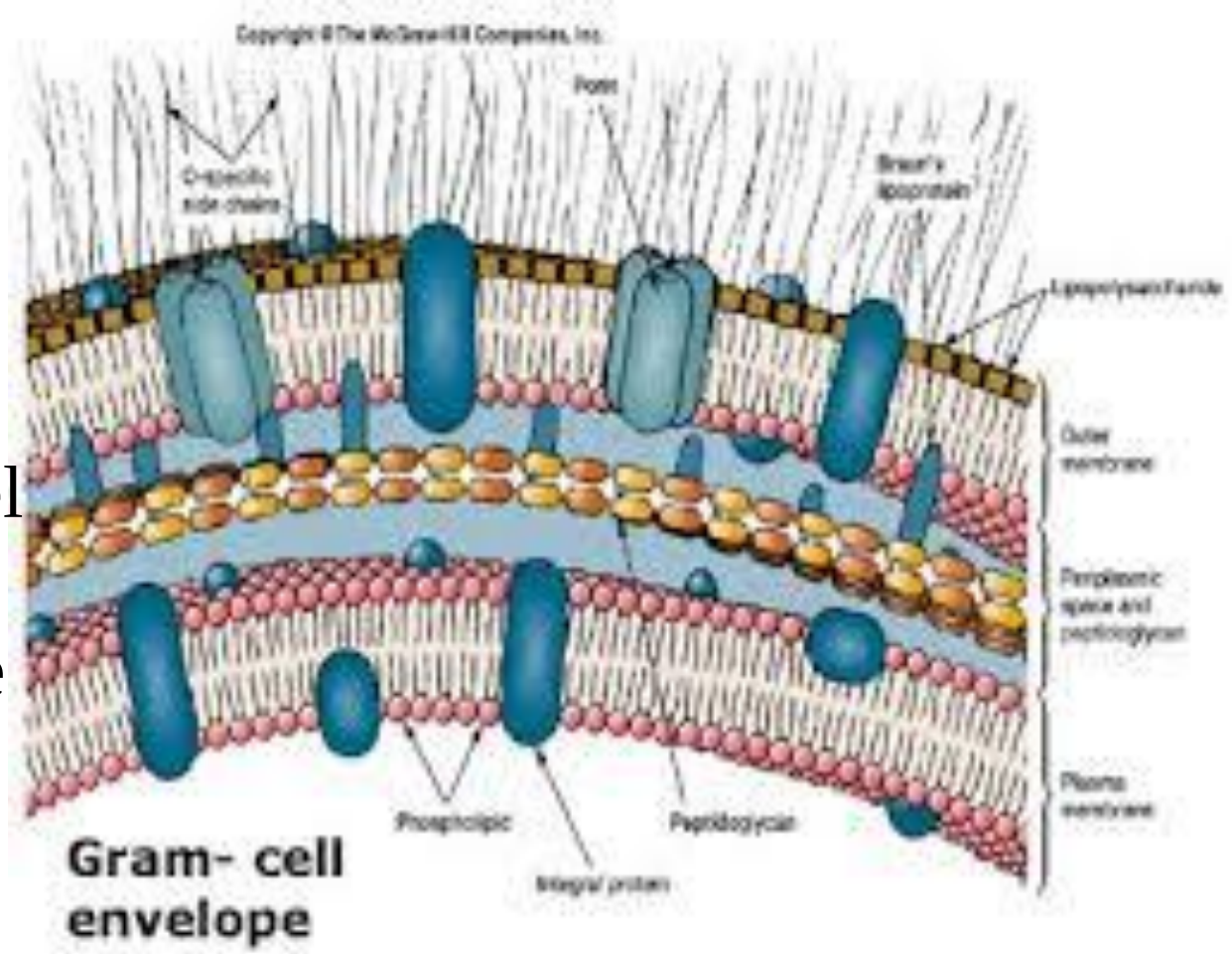


Gram-negative cell wall:

- Thin layer of PG, surrounded with an a symmetrical bilayer membrane on the top, called outer membrane (OM).

-The inner leaflet of the OM is composed of phospholipids and proteins, whereas, the outer leaflet is made of lipopolysaccharide (LPS/LOS).

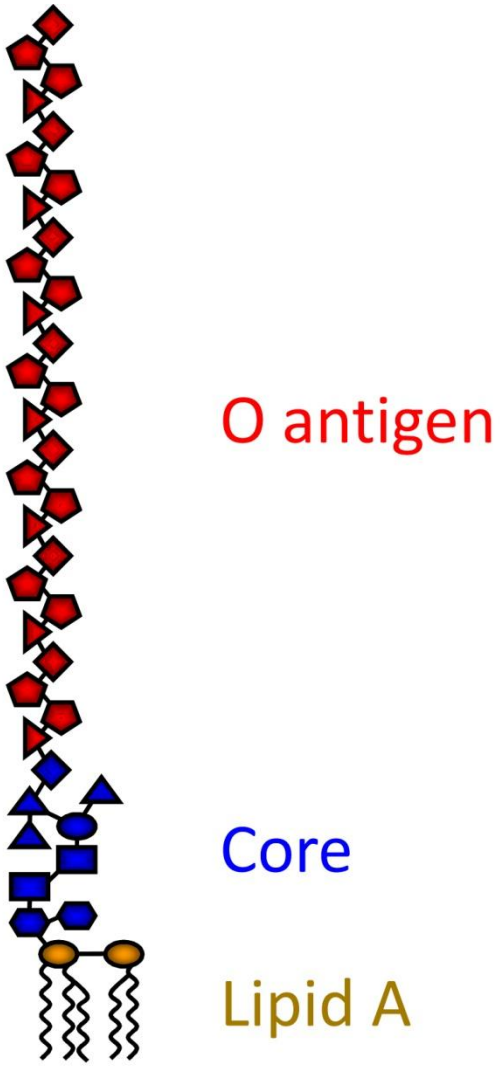
-Porins; is an integral proteins forms channel through both sides of OM, allowing glucose and other mono-saccharides



LPS or endotoxins: is a lipid and sugar, strong stimulators of innate immunity, toxic to animals.

-Consist of typically hydrophobic domain known as **Lipid A (lipid moiety),** non repeating core Oligosaccharide **(glycosidic Part)** of 10 monosaccharide and distal polysaccharide **(O-antigen)** repetitive units of one to eight monosaccharide.

-The first defence mechanism of resistance to antimicrobial peptides in both G+ve and G-ve



-Periplasm space: is the space between the cell membrane and the OM**.**

-Contains the PG and Periplasm

-Periplams contains water, nutrients and substances secreted by the cell such as digestive enzymes, and proteins involved in specific transport.

- enzymes that catabolize large nutrient molecules into smaller molecules, that can be absorbed or transported into the cell.

-<u>Bacteria with out cell wall:</u> like *Mycoplasma pneumonia*, lack the cell wall, small size , similar to prokaryotic cells, have ribosomes, DNA and RNA

Cell wall functions

-Gives the cell a definite shape and structure.

-Provides structural support.

-Protection against infection and mechanical stress.

-Separates interior of the cell from the outer environment.

-It enables transport of substances and information from the cell insides to the exterior and vice versa.

-helps in osmotic-regulation.

-Prevents water loss.

-The physiological and biochemical activity of the cell wall helps in cell-cell communication.

-It prevents the cell from rupturing due to tugor pressure.

-Aids in diffusion of gases in and out of the cell.

- provides mechanical protection from insects and pathogens.

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Lecture 3
Bacterial cell structure

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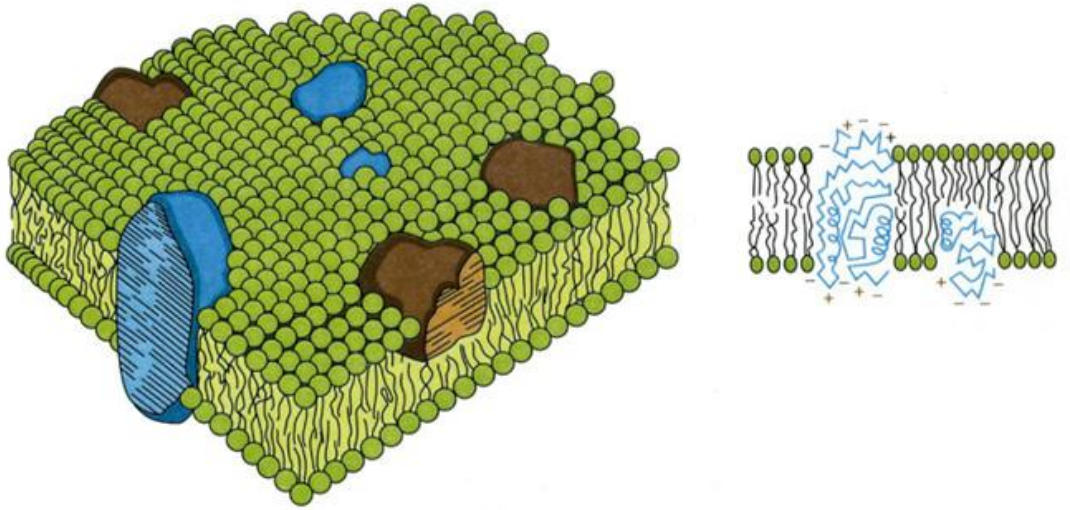
Cytoplasmic membrane : located beneath the glycocalyx, called cell membrane or plasma membrane.

- about 8nm thick and composed of **lipids and integrated proteins**, the fundamental structure of membrane is the **phospholipid bilayer**(a stable barrier between two aqueous compartments; inside and outside of the cell).

-Proteins embedded within the phospholipid bilayer carry out the specific functions of the plasma membrane, **including selective transport of molecules and cell-cell recognition.**

-The phospholipids are **amphoteric** molecules with a polar **hydrophilic glycerol phosphate** "head" attached via an ester bond to two **nonpolar hydrophobic fatty acid tails**, which naturally form a bilayer in aqueous environments.



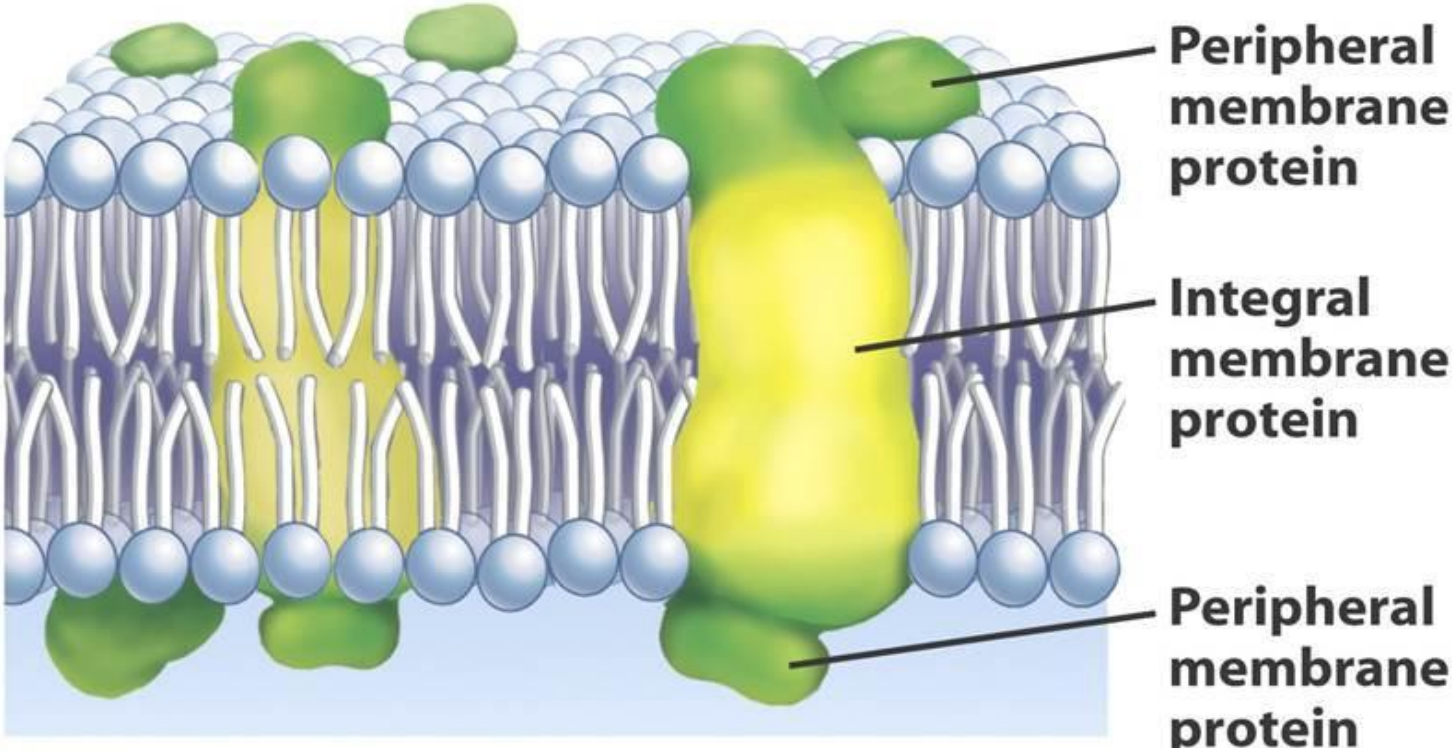
-The hydrocarbon tails of each phospholipid molecule are hydrophobic and huddle together with other tails in the interior of the membrane away from water, whereas, the hydrophilic phosphate head group are attracted to water at the two surfaces of the membrane.

-Integral protein represents about 60% of membrane composition, dispersed with the phospholipid. Some of them are structural proteins, others are enzymes which the carry out most membrane functions., receptors, recognition proteins, carrier or channels.

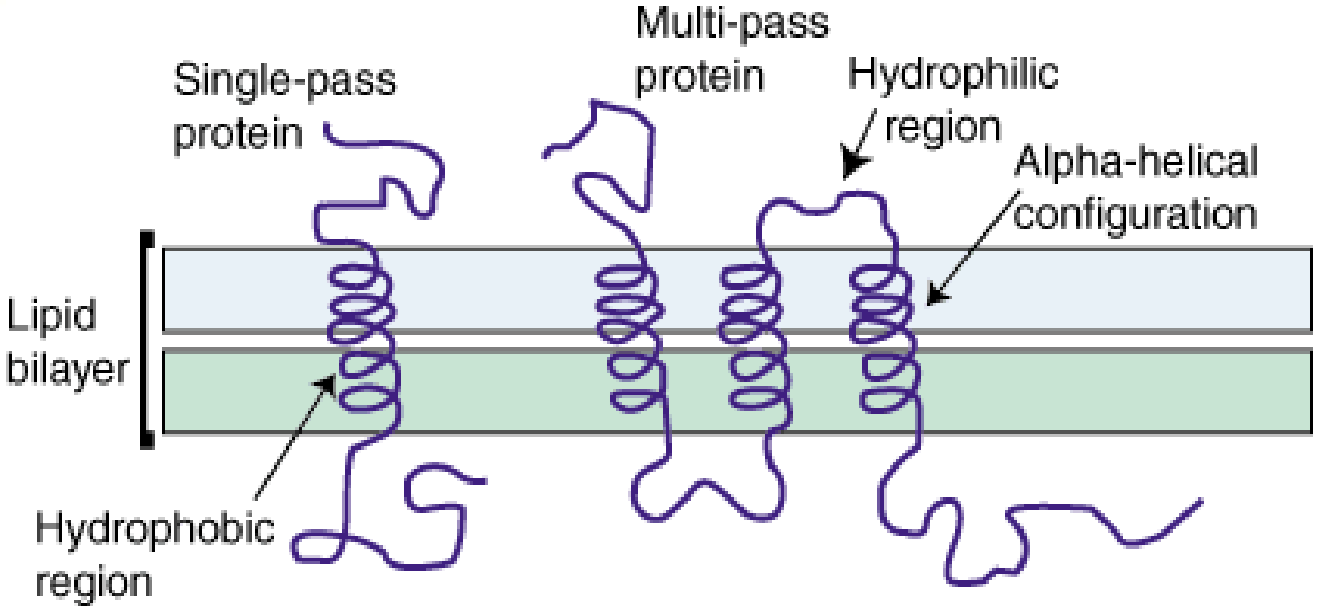
- Some **integral proteins** penetrate the entire bilayer, others are partly inserted, or some are traverse the membrane as channels from outside to the inside. In contrast, **peripheral proteins**; are loosely attached to the membrane on one side or the other. Some membrane proteins are chemically bound to polysaccharide groups called **Glycoproteins.**

-Proteins can move laterally along a surface of the membrane, but it is thermodynamically, unlikely that proteins can be rotated within a membrane, which discounts early theories of how transport systems might work. The arrangement of proteins and lipids to form a membrane is called the **fluid mosaic model**

Types of protein in the plasma membrane



Assembly of proteins in the plasma membrane



Cytoplamsic membrane functions:

-In addition to separate the content of the cell from the outside environment, the cytoplamsic membrane controls the passage of substances into and out of the cell.

-Nutrients are brought into the cell, and wastes are removed.

-Energy storage , and harvest the light energy and converted to chemical energy in photosynthetic prokaryotes.

-Electron transport system, that couples **aerobic respiration and ATP synthesis.**

- procaryotic membranes may contain **sensing proteins** that measure concentrations of molecules in the environment or **binding proteins** that translocate signals to genetic and metabolic machinery in the cytoplasm.

-Membranes also contain **enzymes** involved in many metabolic processes such as **cell wall synthesis, septum formation, membrane synthesis, DNA replication, CO_2 fixation and ammonia oxidation**.

-The cytoplasmic membrane is considered to be **selectively permeable**; that is, it allow some substances to cross it, while preventing the crossing of others, **but**, how does a membrane exert control over the contents of the cell, and the substances that move across it?

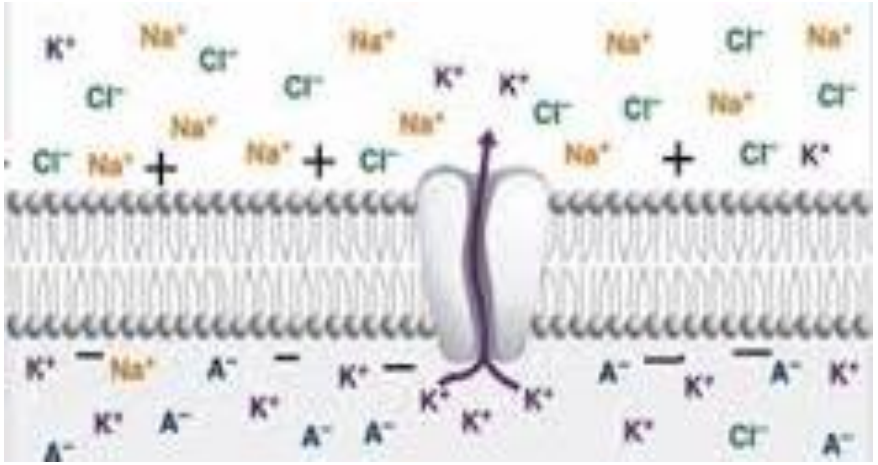
-Naturally, the phospholipid bilayer is impermeable to most substances, large molecules cant cross through it; ions and molecule with electrical charge are repelled by it; and hydrophilic substances cannot easily cross its hydrophobic interior, **however**, cytoplasmic membranes contain proteins that allow substances to cross the membrane by channels, pores or carries.

-So movement across the cyoplasmic membrane occurs by two process: **Passive:** do not required ATP storage; and **active** process, which ATP dependent.

-Another feature of cytoplasmic membrane is: its ability to maintain a concentration gradients OR Electrical potential of cytoplasmic membarne

- Membranes enable a cell to concentrate chemicals on one side of the membrane or the other.
- The difference in concentration of a chemical on the two sides of the membrane is its concentration gradient OR chemical gradient.
- Many of the substances that have concentration gradient across the membrane are electrically charged chemicals or voltage.
- For example: membrane are permeable to K ions than Na ions, thus lead to segregate the negative charged inside more than that of outside, therefore, the tendency to repel the negatively charged chemicals and attract of positively charged chemicals.

Electrical potential of cytoplasmic membrane: the electric potential exists across the membrane , because there are more negative charge s inside the cell than outside

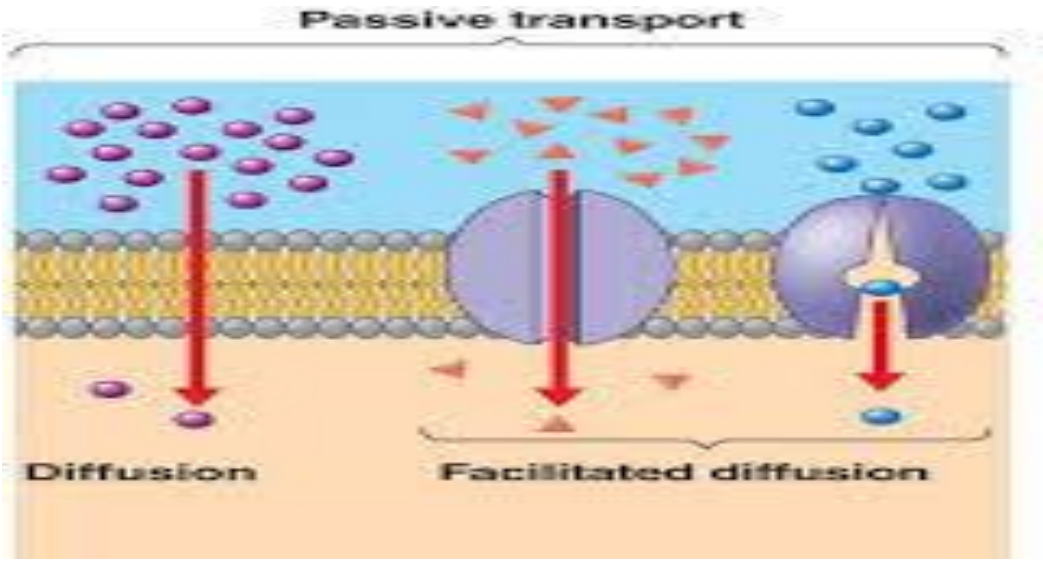


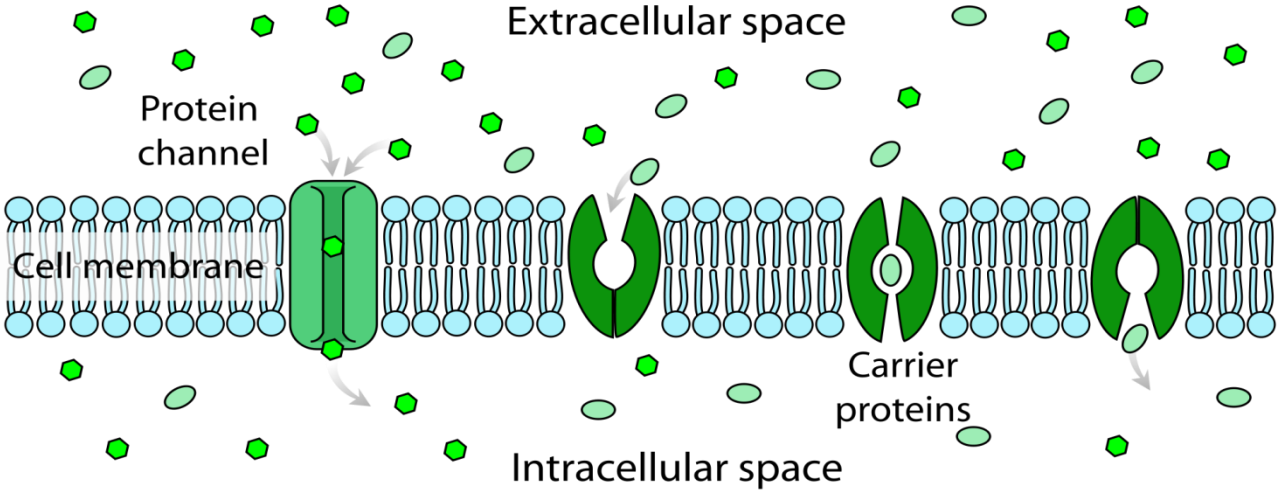
Passive transport or process: a source of energy is provided by electrochemical gradient, so the cell does not expand its ATP energy. This type includes:

1- Diffusion: is the movement of a chemical from **an area of higher concentration to an area of lower concentration**. It requires no energy output by the cell. Only small chemicals and lipid soluble can diffuse through the lipid portion of the membrane. Other examples like: oxygen, carbon dioxide, alcohol and fatty acid, can freely diffuse through the cytoplasmic membrane, whereas, glucose and proteins cannot.

2- **Facilitated diffusion:** the phospholipid bilayer blocks the movement of large or electrically charged molecules, so they cannot cross the membrane, unless there is a pathway for diffusion. Because of cell membranes contain integral proteins, some of these proteins act as channels or carriers to allow certain molecules to diffuse into or out of the cell. The electrochemical gradient provides all of the required energy.

- Some channel proteins allow the passage of a range of chemicals that have the right size or electrical charge, **whereas**, others are more specific, carrying only certain substrates, these are called **permeases**

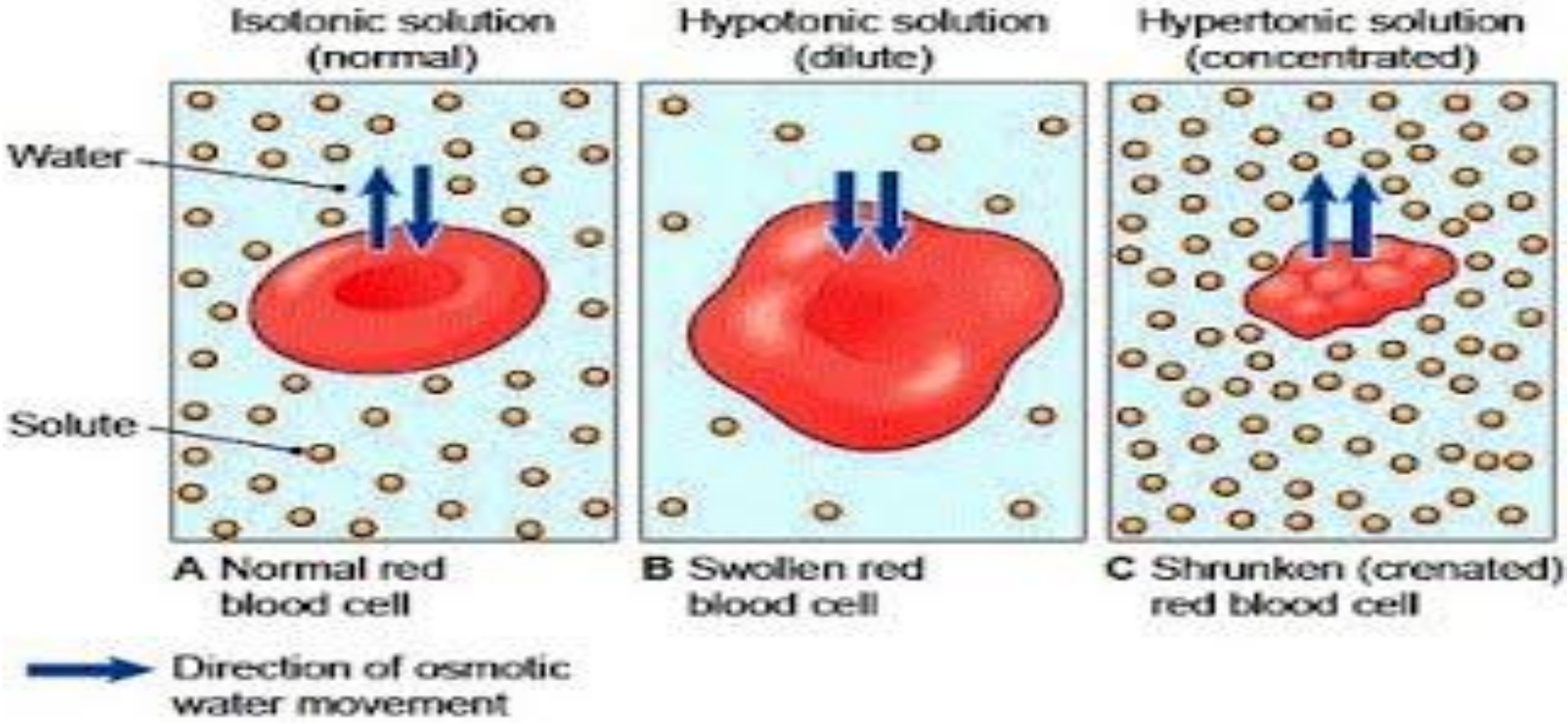




3- Osmoses: diffusion of water across a selectively permeable membrane, which is permeable to water but not all solutes that are present like **proteins, amino acids, salt or glucose**. Because of these solutes cannot freely penetrate the membrane, therefore, cannot freely diffuse, instead, water can be diffused from the side of the membrane that contains **higher concentration of water but lower concentration of solute** to the side that contains **lower concentration of water but higher concentration of solutes.**

-Osmosis continued until equilibrium is reached.

-Commonly, solution can be classified in to three classes according to their concentration of solutes: isotonic, hypertonic and hypotonic.


Isotonic solution
(normal)
Hypotonic solution
(dilute)
Hypertonic solution
(concentrated)
Water
Solute
A Normal red
blood cell
B Swollen red
blood cell
C Shrunken (crenated)
red blood cell
Direction of osmotic
water movement

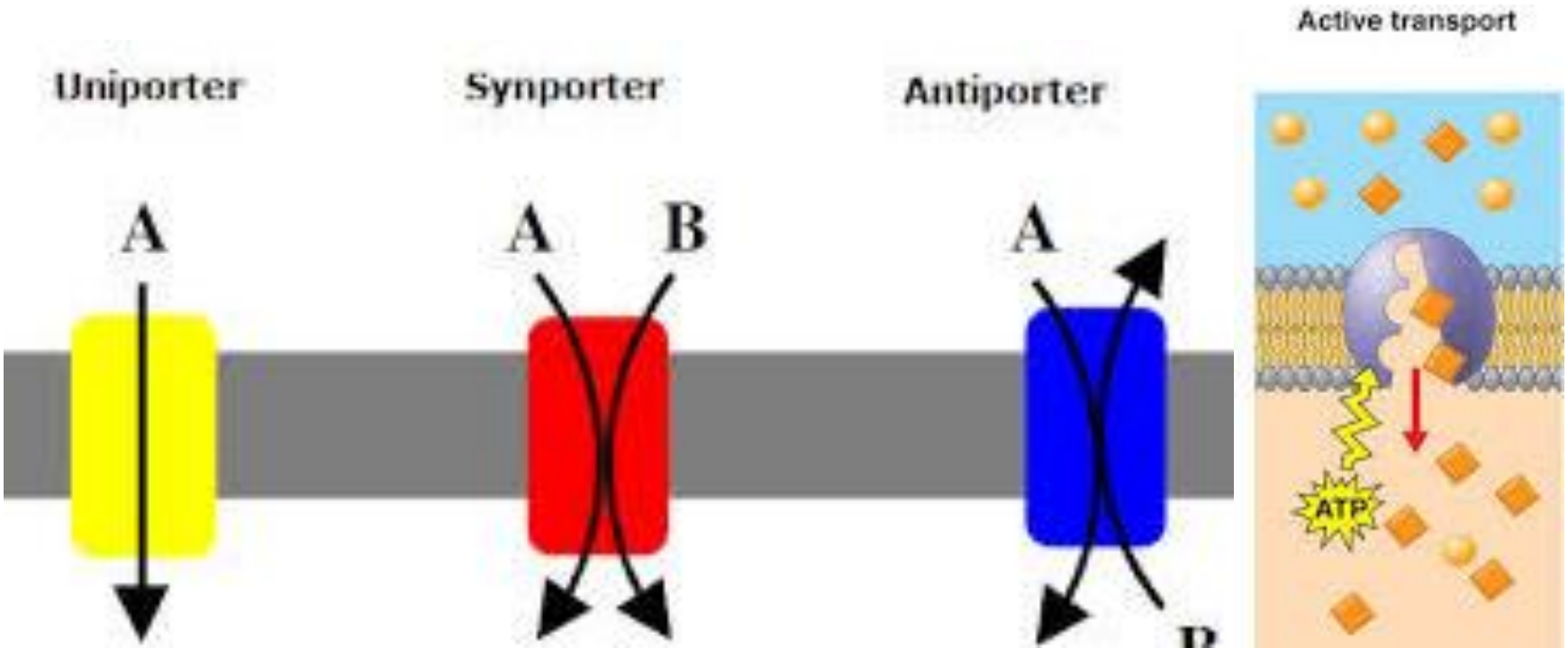
-Active process: utilises transmembarne permease proteins and these protein requires the cell to expand ATP to transport molecules across the membrane.

-Some proteins are controlled and called **gated proteins**, when the cell is in need of a substance , the protein become functional (**the gate opens**), at other times, the gate is **close.**

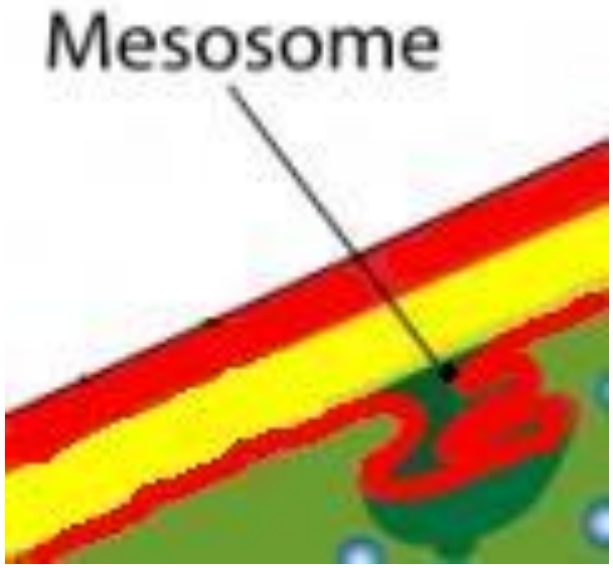
A- Uniport: one substance is transported at a time

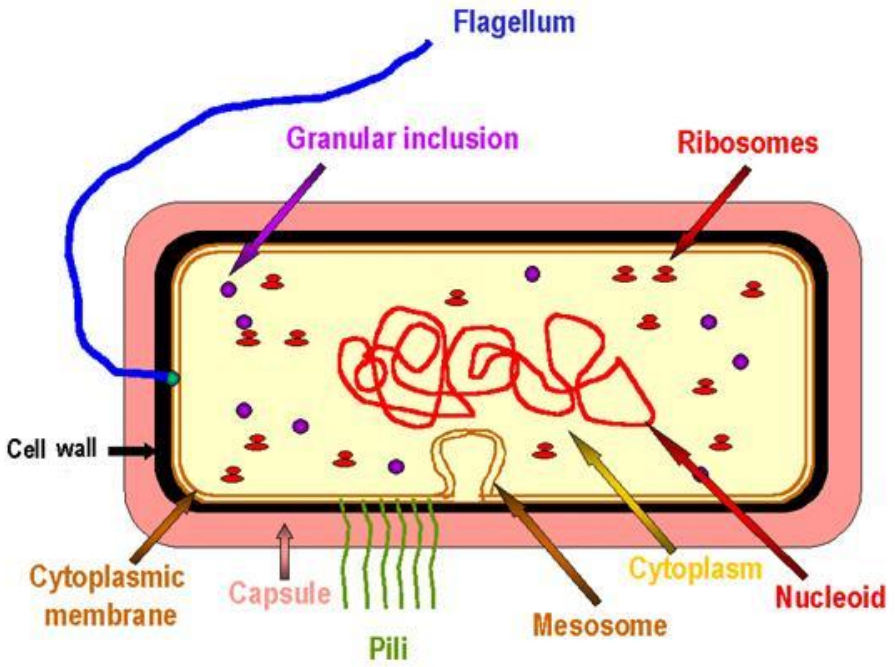
B- Symport: two substances are transported in one direction

C-Antiport: two chemicals are transported in opposite direction



-**Mesosomes** are structures of prokaryotic cells formed by folded invaginations of the plasma membrane, contains all the enzymes associated with **respiration** and **oxidative phosphorylation process** of the prokaryotic cell (bacteria). Not all prokaryotic cells have mesosomes.
-function in cell injury and physiological cellular processes, such as replication and separation of nucleoids and oxidative phosphorylation.



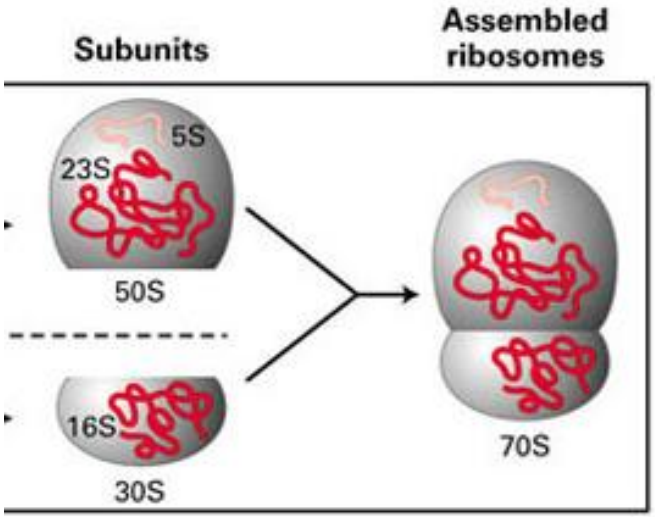


-**Ribosomes:** are the site of protein synthesis in cell. Thousands of ribosomes are found in prokaryotic cells in their cytoplasim.

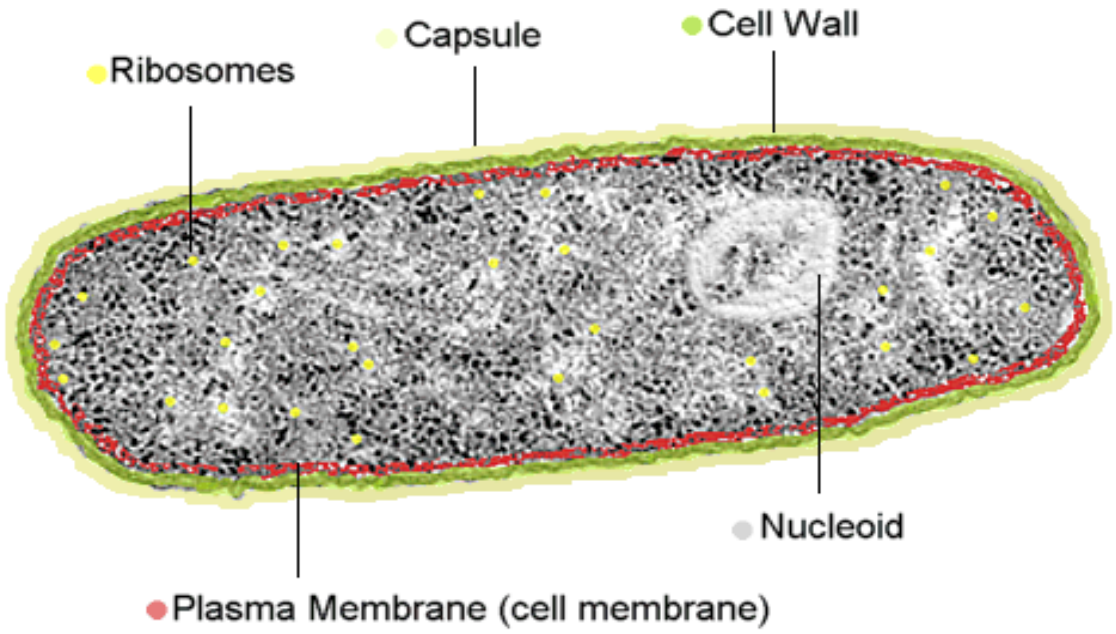
-The size of ribosomes and other cellular structure is expressed in Svedberg's (S), and is determent by their sedimentation rate, the rate at which they move to the bottom of a test tube during centrifugation. So, large, compact and heavy particles sediment faster than small, loosely packed or light ones, and so are assigned a higher number.

-Examples; prokaryotic ribosomes are 70S, in contrast, eukaryotes have larger ribosomes 80S.

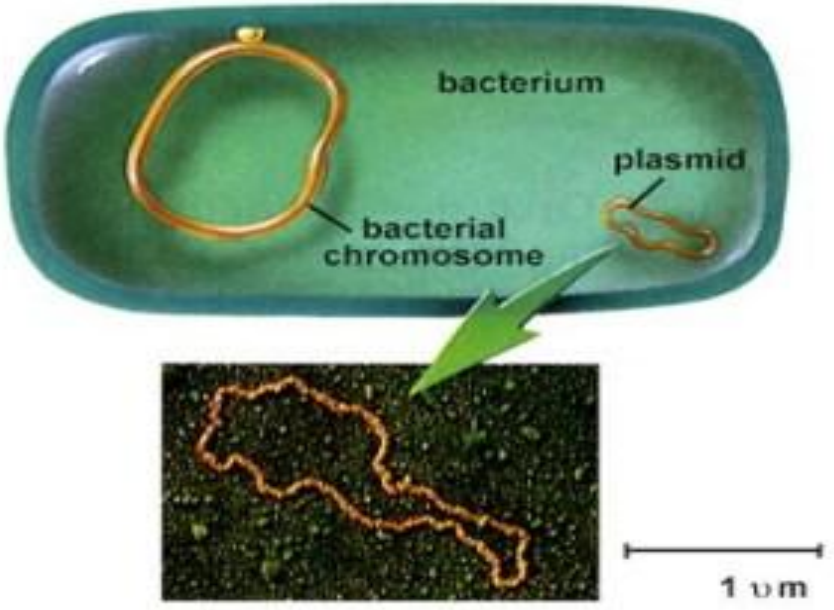
-All ribosomes are composed of two subunits, each of which is composed of polypeptides and molecule of RNA called rRNA or ribosomal RNA.

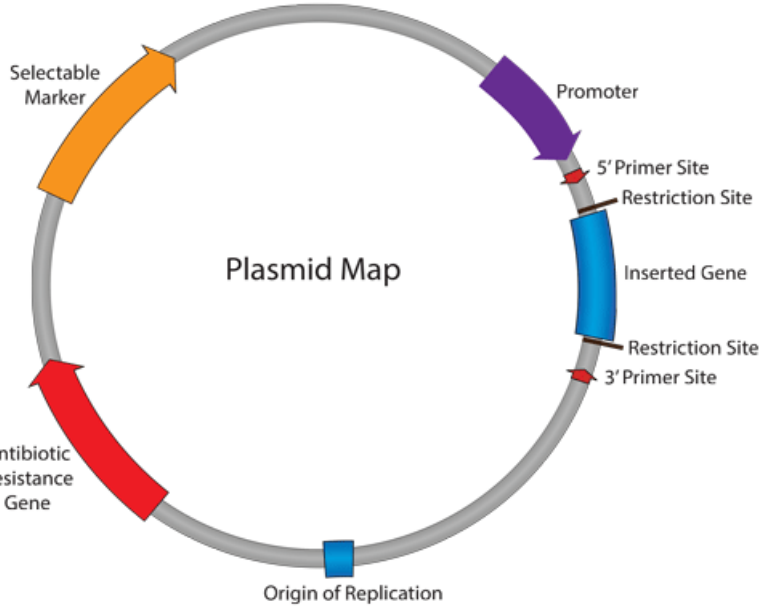


The nucleoid: The *nucleoid* (meaning nucleus-like) is an irregularly-shaped region within the cell of a prokaryote that contains all or most of the genetic material. Unlike the nucleus of eukaryotic cell, it is not surrounded by a nuclear membrane. The genome of prokaryotic organisms generally is a circular, double-stranded piece of DNA, it present as super coiled or **C**ovalently **C**losed **C**ircular molecules (CCC) of which multiple copies may exist at any time. The length of a genome varies widely, but is generally at least a few million base pairs.



-The chromosomal DNA carries most of the genetic information.
-Bacteria often contain plasmids – small circular DNA molecules.
-Bacteria can pick up new plasmids from other bacterial cells (during conjugation) or from the environment. They can also readily lose them – for instance, when a bacterium divides in two, one of the daughter cells might miss out on getting a plasmid.
-Every plasmid has its own 'origin of replication' – a stretch of DNA that ensures it gets replicated (copied) by the host bacterium.
- Plasmids can confer resistance to antibiotics like R plasmid, OR, it can transfer the genetic information from one cell to another like F plasmid.





Inclusions: are found within the cytosol of prokaryotes, may contain reserve deposits of lipids, starch or compounds containing nitrogen, phosphate or sulfur,. Such chemicals may be taken in and stored in the cytosol when nutrients are in abundance, and then utilized when nutrients are scarce. Several types:

- Glycogen: where many bacteria and archaea store carbon and energy in molecules of glycogen, polymer of glucose molecules, or as lipid polymer.
- Gas vacuoles: found in many aquatic cyanobacteria (blue-green photosynthetic prokaryotes) that store gases in protein sacs.
- Magnetosomes: small crystals of magnetite, stored by *Magnetobacteria*.
- **Volutin granules:** are an intracytoplasmic (inside the cytoplasm of a cell) storage form of complexed inorganic polyphosphate, the production of which is used as one of the identifying criteria when attempting to isolate *Corynebacterium diphtheriae*

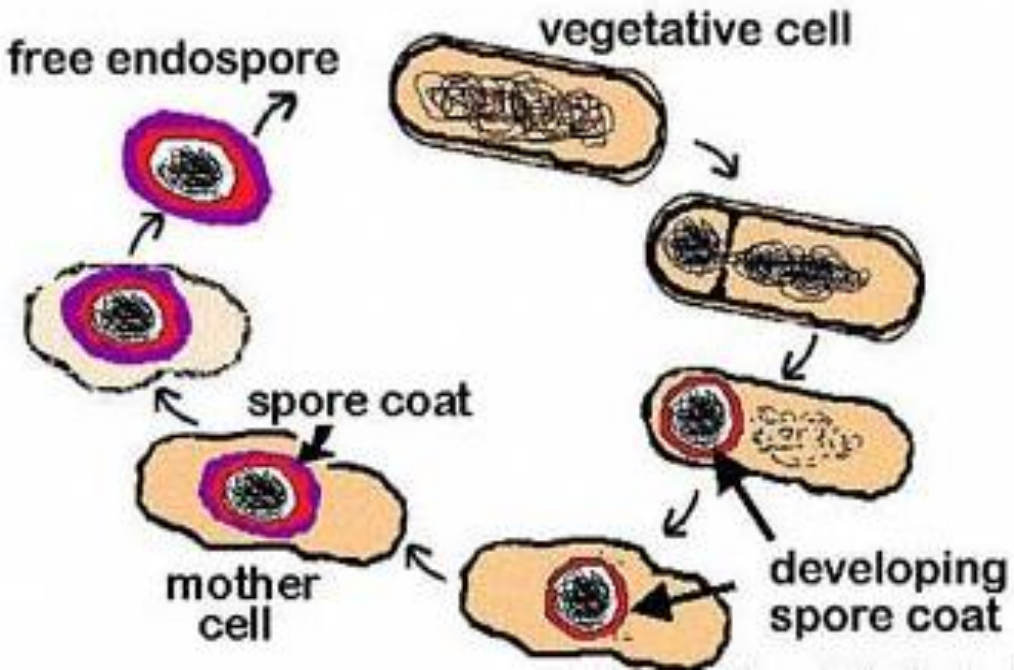
Endospores: unique structure produced by some bacteria like *Bacillus* and *Clostridium*, important for their durability and potential pathogenicity, and constitute a defensive strategy against hostile or unfavourable conditions .

-Endospores are not reproductive structure , because it produce only one vegetative cell after germination.

-Endospores are formed by a process called **sporulation,** at which two membranes , a thick layer of peptidoglycan and spore coat form around a copy of cell's DNA and a small portion of cytoplasm.

-The spore then is surrounded by Spore coat, and then is released to the environment after vegitable cell lyses.

-The endospore will be either centrally, subtermenally (near one End), or terminally (near one end)



-Endospores, are extremely resistant to drying, heat, radiation and lethal chemicals, ex, they remain alive in boiling water for several hours. Unharmed by alcohol, peroxide, bleach and other toxic chemicals.

Microbial growth

Bacterial growth refers to an increase in cell numbers rather than an increase in cell size. The process by which bacterial cells divide to reproduce themselves is known as **binary transverse fission**. The time taken from cell formation to cell division is called **the generation time**. The generation time can therefore be defined as the time taken for the cell count to double.

- When bacteria are inoculated into a liquid medium, there are four distinct phases to a population's growth curve; the lag, log, stationary and death

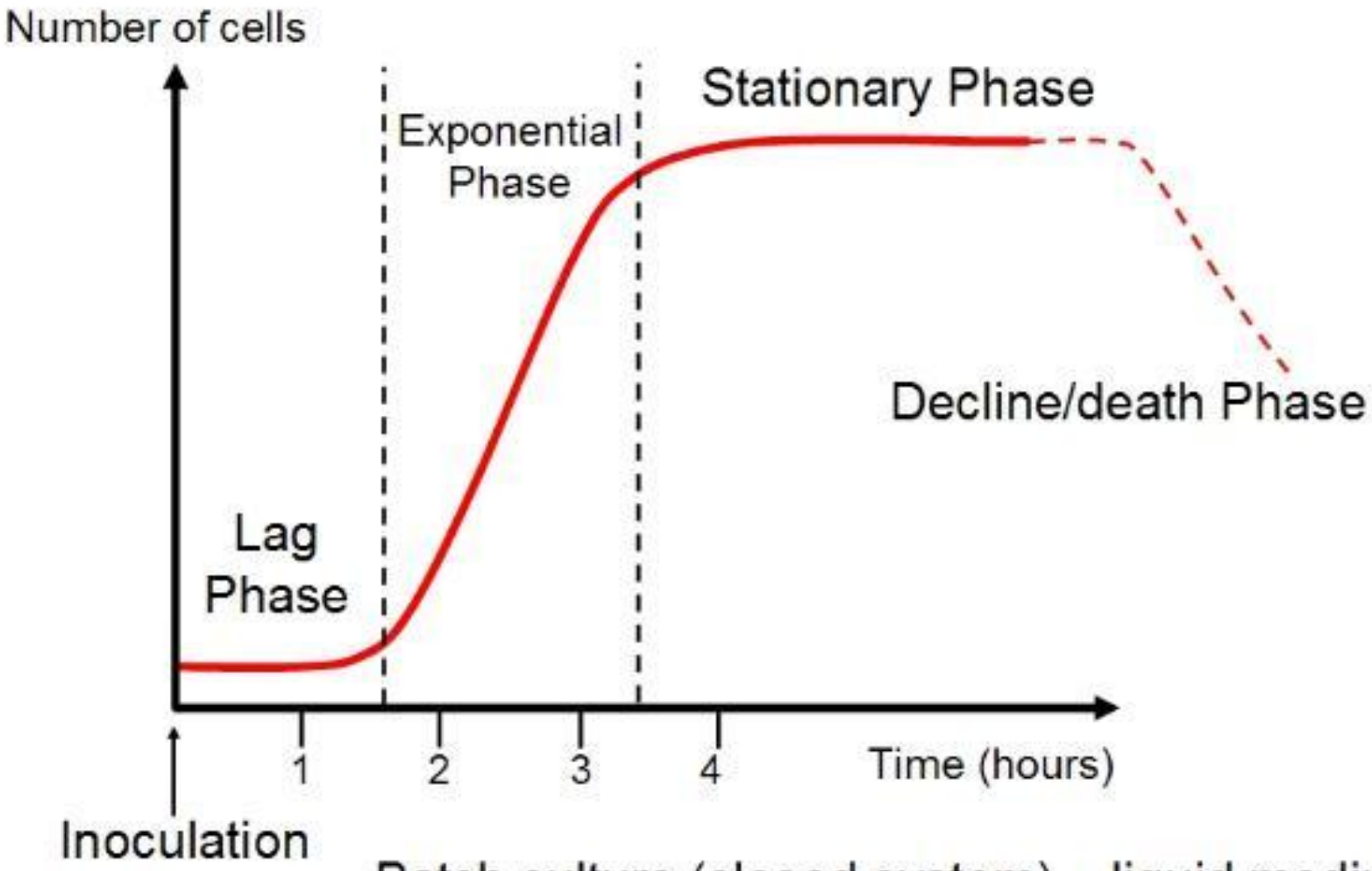
1-Lag phase: the cells are adjusting to their new environment, most cell do not reproduce immediately, instead, actively synthesise enzymes to utilize novel nutrients in the medium. Depending on the species, chemical and physical conditions of the medium, lag phase can last less than an hour or for days.

2- Log phase: the bacteria synthesised the necessary chemicals for conducting metabolism in their new environment, rapid chromosome replication, growth and reproduction. The log phase is so called because the population increases logarithmically, and the reproductive rate reaches a constant as DNA and protein syntheses are maximised.

3-Stationary phase: the rate of reproductive decreased, as the nutrients are depleted, and waste is accumulated. The number of dying cells equal the number of cell being produced. The size of population become stationary , and the metabolic rate declines.

4-Death phase: some cells remain alive and continue metabolizing and reproducing , but the number of dying cells exceeds the number of new cells produced.

Bacteria - Population Growth Curve



Batch culture (closed system) – liquid medium

Bacteriology
2016-2017
2nd year

Lecture 4
Microbial metabolism

Dr. Halah Al-Haideri

Microbial metabolism: is all of the chemical reactions in an organism, which can be divided into two major classes of reactions; **catabolism** and **anabolism.** A series of such these reactions called **pathway.**

- Catabolic pathway: is breaking down the larger molecules into smaller products, like breakdown lipids into glycerol and fatty acids. Thus release energy , that is , catabolic pathways are ***exergonic***. Cells store some of this released energy in the bonds of ATP, though much of the energy is lost as heat. Catabolic pathways also resulted in production of numerous smaller molecules, some of which are precursor metabolites of anabolism.

- Example; *E. coli* can synthesize every thing in their cells from just precursor metabolites, other organisms must acquire some anabolic building blocks from out side their cells as nutrients So that catabolic pathway produce ATP, or metabolites or both.

• Anabolic pathway: is synthesize large molecules from the smaller products of catabolism, and thus require more energy than they release, that is, anabolic pathway is *endergonic*, because building of any thing requires energy. This energy is come from ATP molecules produced during catabolism. Like synthesis of lipid for cell membrane form glycerol and fatty acid.

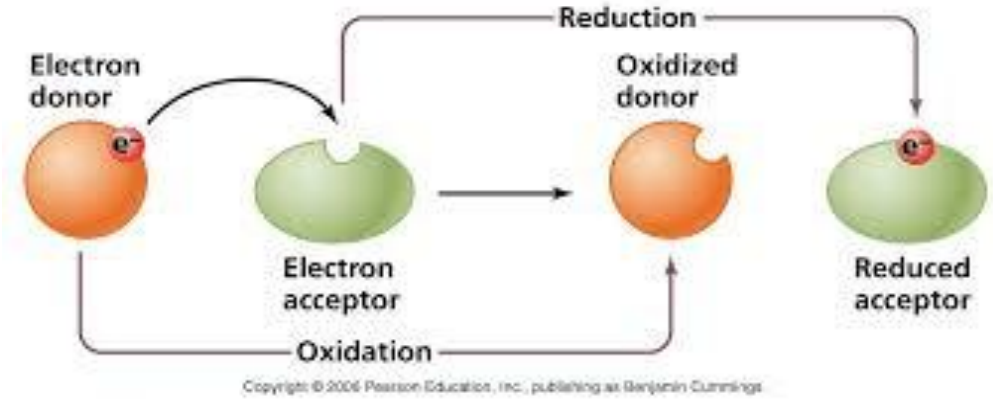
Oxidation and reduction reactions

-Is so called electron transfers

-It is the transfers of electrons from a molecule that donates an electron (called an *electron donor*), to a molecule that accepts an electron (called an *electron acceptor*).

-An electron receptor= **reduced** (reduced the overall electrical Charge)

-An electron donor= **oxidized** (electron donated to oxygen atom)



• Electrons rarely exist freely in the cytoplasm, instead, they orbit atomic nuclei, therefore, cells use electron carrier molecules to carry electrons (hydrogen atoms)from one location in a cell to another.

•Three important electron carriers, derived from vitamins:

•Nicotinamide adenine dinucleotide (NAD^+)

•Nicotinamide dinucleotude phosphate ($NADP^+$)

•Flavin adenine dinucleotide (FAD^+)

-Cells use each of these molecules in specific metabolic pathways to carry pairs of electrons. One of the electrons carried by either \ NAD^+ or $NADP^+$....as a part of hydrogen atoms, forming NADH and NADPH.

OR

-Two electrons are carried by FAD^+..as a part of hydrogen atom $FADH_2$.

-Many metabolic pathways require such electron carrier molecules.

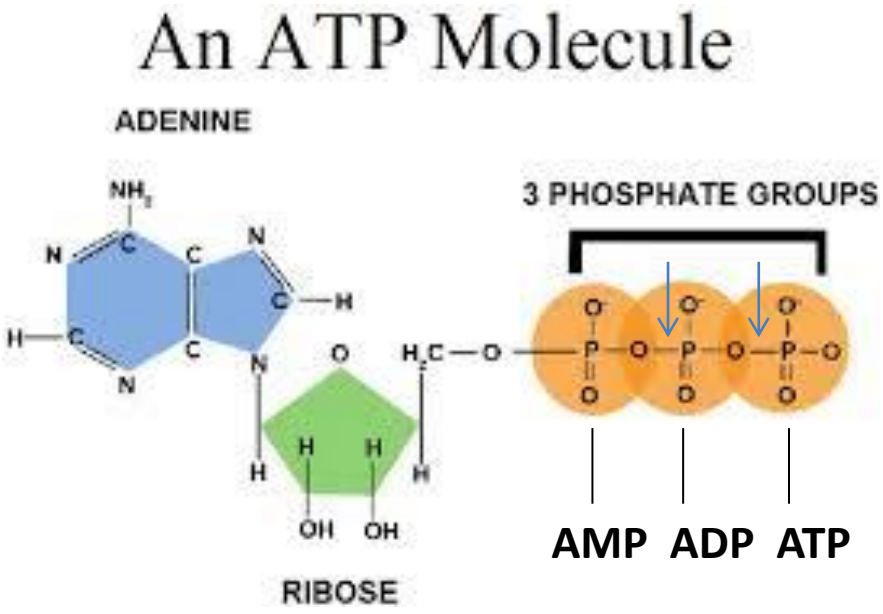
ATP production and energy storage

-Nutrients contain energy, but that energy is spread throughout their chemical bond, and it is not concentrated enough to use in anabolic reactions.

-During catabolism, organisms release energy from nutrients that can be then concentrated and stored in high-energy phosphate bond of ATP molecules. This happen by a general process called **phosphorylation,** In which inorganic phosphate [PO^{2-}_4] is added to a substrate.

Ex:

Cells phosphorylate ADP to form ATP



Phosphorylation of ADP to ATP is mediated by three specific ways:

1- *Substrate-level phosphorylation*: which involves the transfer of phosphate to ADP from another phosphorylated organic compound.

2- *Oxidation phosphorylation :* energy from redox reactions of respiration is used to attach inorganic phosphate to ADP.

3- Photophosphorylation: in which light energy is used to phosphorylate ADP with inorganic phosphate.

-After ADP is phosphorylated to produce ATP , anabolic pathways use some energy of ATP by breaking a phosphate bond to re-back to ADP. Thus the cyclic inter-conversion of ADP and ATP functions as re-chargeable batteries. That is ADP molecules can be recharged to ATP again and again.

Enzymes: are chemicals that increased the likelihood of a reaction, but are not permanently changed in process.

1- *Hydrolases*: catabolise molecules by adding water in a de-composition process called as hydrolysis. (depolymerisation of macromolecules)

2- *Isomerases*: rearrange the atoms within a molecule, but do not add or remove anything (so they are neither catabolic nor anabolic).

3- *Ligases or polymerases*: join two molecules together, often use energy supplied by ATP.

4- Lyases: split large molecules without using water in the process.

5- Oxidoreductases: remove electrons from (oxidize), or add electrons to (reduce) various substrates. They are used in both catabolic ad anabolic pathways.

6- Transeferases: transfer functional groups, such as amino group (NH_4), a phosphate group, or a two carbon group (acetyl), between molecules.

Carbohydrate catabolism:

-Many organisms oxidize carbohydrates as their primary energy source for anabolic reaction. They use glucose most commonly, other sugars, amino acids and fat, which converting them to glucose.

-Glucose can be catabolised via one of two process

-cellular respiration: process resulted in complete breakdown of glucose to carbon dioxide and water.

-or fermentation: which result in organic waste product.

Both cellular respiration and fermentation start with *glycolysis* , a process that catabolize a single molecules of glucose to two molecules of pyruvic acid or (pyruvate), and result in small amount of ATP production.

-Respiration is continued via the Kreps cycle and the electron transport chain, which results in a significant amount of ATP.

-Fermentation: involved the conversion of pyruvic acid into other organic compounds and much less of ATP production.

Growth requirements

-In general, organisms use a variety of chemicals (nutrients) to get their energy needs, and to build organic molecules and cellular structure.

-The most common nutrients are compounds containing necessary elements as carbon, oxygen, nitrogen and hydrogen.

-Microbes obtain nutrients from a variety of sources in their environment, so that , organisms can be categorized into two broad groups based on their source of carbon:

-*Autotrophs*: organisms that utilize an organic source of carbon as a sole carbon source; make organic compounds from CO2 from the same organism.

-*Heterotrophs:* organisms that catabolise reduced organic molecules such as proteins carbohydrates, amino acids, and fatty acids , which they acquire from another organism.

-Organisms can be also grouped according to whether they use chemicals or light as a source of energy for cellular process anabolism, intracellular transport and motility:

-*Chemotrophs:* organisms that acquire energy from redox reactions involving inorganic and organic chemicals via either aerobic respiration, anaerobic respiration or fermentation.

-*Phototrophs*: organisms that use light as their energy source.

-So, organisms can be classified into four groups according to their carbon and energy source:

-Photoautotrophs (such as plant, some protozoa and algae).

-Chemoautotrophs

-Photoheterotrophs

-Chemoheterotrophs (animals, fungi and protozoa).

-In addition, the cells of all organism s require electrons or hydrogen atoms for redox reactions, at which hydrogen is the most common chemicals in cells and it is so common in organic molecules and water.
- Hydrogen is essential for hydrogen bonding and in electron transfer.

-*Organotrophs*: organisms that acquire electrons from teh same organic molecules that provide them carbon and energy.
-*Lithotrophs:* organisms that acquire electrons or hydrogen atoms from inorganic sources, such as H_2, NO^{2-}, H_2S and Fe^{2+}

-Oxygen requirement
Organisms varies according to their oxygen requirement:
-Aerobic or obligate aerobic: oxygen is serve as the final electron acceptor of electron transport chains, which produce most of the ATP.
- Anaerobic: oxygen is a deadly poison.
-Facultative anaerobic: can live in varies oxygen concentrations such as *E. coli.*

-Aerotolerant anaerobic: do not use aerobic metabolism, but they tolerate oxygen by having some of the enzymes that detoxify oxygen's poisonous forms (superoxide radicals and peroxide anion). Ex; lactobacilli that transform cucumber into pickles).
-Microaerophilic: require oxygen less than present in the atmosphere, 2-10 % , the have limited ability to detoxify hydrogen peroxide an superoxide radicals, such as *Helicobacter pylori.*

Nitrogen: is found in many organic compounds, including the amino group of amino acids and as apart of nucleotide bases.
-Is a growth-limiting nutrient for many organisms, that is, their anabolism ceases because they don't have sufficient nitrogen to build up proteins and nucleotides
-Nitrogen is acquired from organic and inorganic nutrients, most photosynthetic organisms can reduce nitrate (NO_3^-) to ammonium (NH_4^+), which can be used for biosynthesis.-
-All cells recycle their nitrogen from their amino acid and nucleotide.

Other chemical requirements: includes phosphorus (is a component of DNA, RNA and ATP and proteins), sulfur (is a component of sulfur-containing amino acids which binds via disulfied bond and vitamins), calcium, manganese, copper and iron.

-Other elements is called trace elements, because they are required in very small amounts.

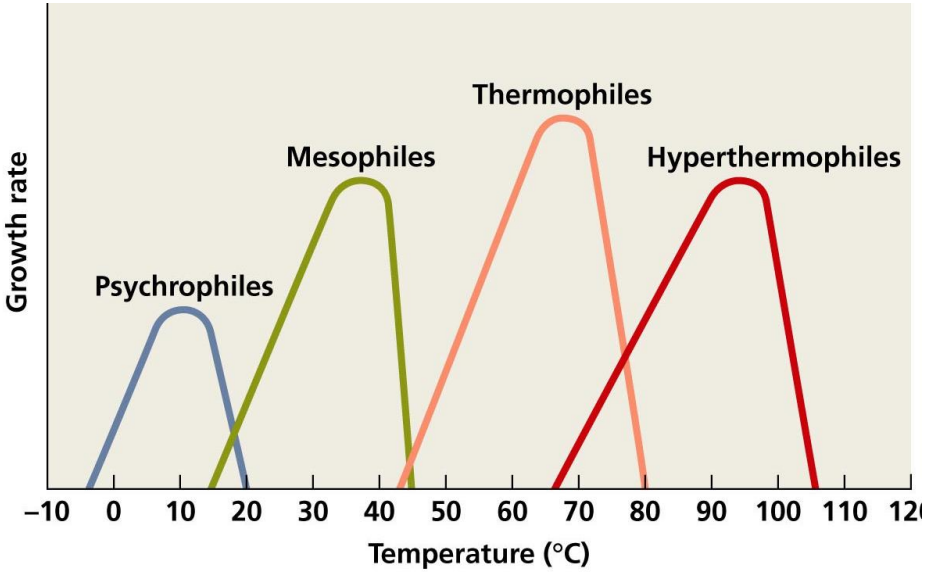
Physical requirements

-Temperature: plays an important role in microbial life through its effect on the three-dimentional configurations of biological molecules (protein denatures).

-In addition, lipid is temperature sensitive, as it is the main component of the membrane. If the temperature is too low, membranes become rigid and fragile; if the temperature is to high, the membranes become too fluid, and it cannot contain the cells and organelle.

-Organisms can be categorised into four overlapping groups based on preferred temperature:

-Psychrophiles: best growth at temperature below 15˚C or even below 0 ˚C. like algae, fungi and bacteria and Archaea, live in snowfield, ice and cold water. Non pathogenic.
-Mesophiles: grow best in temperature ranged from 20-40˚C. pathogenic.
-Thermophiles: grow at temperature above 45˚C in hot springs.
-Hyperthermophiles: grow in water above 80˚C, such as Archaea.



-**pH:** organisms are sensitive to change in acidity because hydrogen ions and hydroxyl ions interfere with hydrogen bonding within proteins and nucleic acid.
- Neutrophiles: most bacteria and protozoa that grow in narrow range around the a neutral pH (6.5-7.5).
-Acidophiles: other bacteria and many fungi grow best in acidic hapitats.
Alkalinophiles: live in alkaline soil and water up to pH 11.5, such as *Vibrio cholerae.*

Water: is needed to dissolve enzymes and nutrients, and an important reactant in many metabolic reactions.
- The physiological effect of water is the osmotic pressure and hydrostatic pressure

Bacteriology
2016-2017
2nd year

Lecture 5 continued to lecture 4

Microbial metabolism

Dr. Halah Al-Haideri

-Osmotic pressure of a solution; is the pressure exerted on a semipermeable membrane by a solution containing solutes (dissolved materials) that cannot freely cross the membrane.

-Osmotic pressure is related to the concentration of dissolved molecules and ions in a solution. So, solution with greater concentrations of such solutes are *hypertonic* relative to those with a lower solute concentration, which are *hypotonic*.

-For example: a cell placed in freshwater (a hypertonic solution relative to the cell's cytoplasm) gains water from its environment and swells to the limit of its cell wall, whereas, a cell placed in seawater, which is a solution containing about 3.5 %solutes and thus hypotonic to most cells, loses water into the surrounding saltwater . Such cells can die from crenation, or shriveling of its cytoplasm.

- Obligate halophiles, are adapted to growth under high osmotic pressure such as exists in the Great Salt Lake and small salt ponds. They will grow in up to 30% slat and will burst if placed in fresh water.

-Facultative halophiles: they do not require high salt concentrations, such as *S. aureus*, can tolerate up to 20 % salt, which allow them to colonize the surface of the skin.

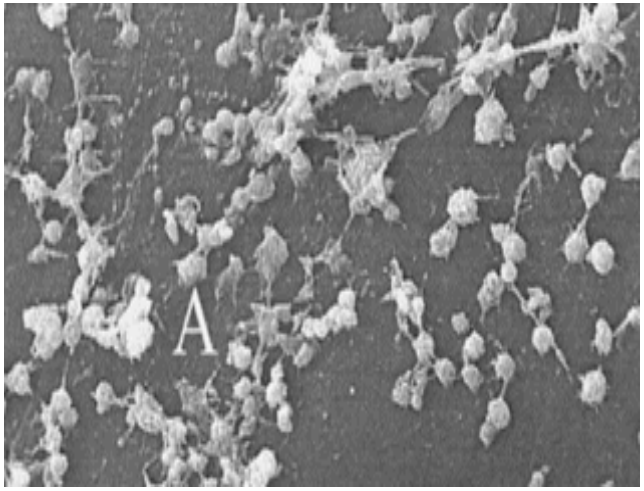
-Hydrostatic pressure: water exerts pressure in proportion to its depth. For every additional 10 m of depth, water pressure increases 1 atmosphere (atm), therefore, the pressure at 100 m below the surface is 10 atm (ten times greater than at the surface).

-In deep ocean basins (thousand of meter below the surface, the pressure is tremendous, the organisms that live under such extreme pressure are called **barophiles.**

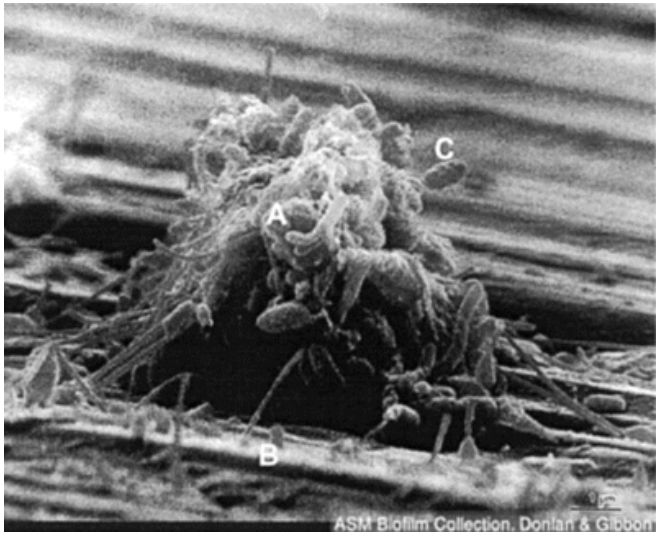
- Their membrane and enzymes do not merely tolerate pressure, but depend on pressure to maintain their three dimensional functional shapes. Thus barophiles brought to the surface , die quickly due to their protein denature.

Biofilm formation: is a process whereby microorganisms irreversibly attach to and grow on a surface and produce extracellular polymers that facilitate attachment and matrix formation.

- Matrix composed of DNA, proteins and fiber of polysaccharides of the cell's glycocalyces.
- The matrix adheres cells to one another, sticks the biofilm to the substrate, forms microenvironments within the biofilm, sequesters nutrients, and may protect individuals in the biofilm from environmental stresses, including UV, antimicrobial drugs and changes in pH, TM and humidity

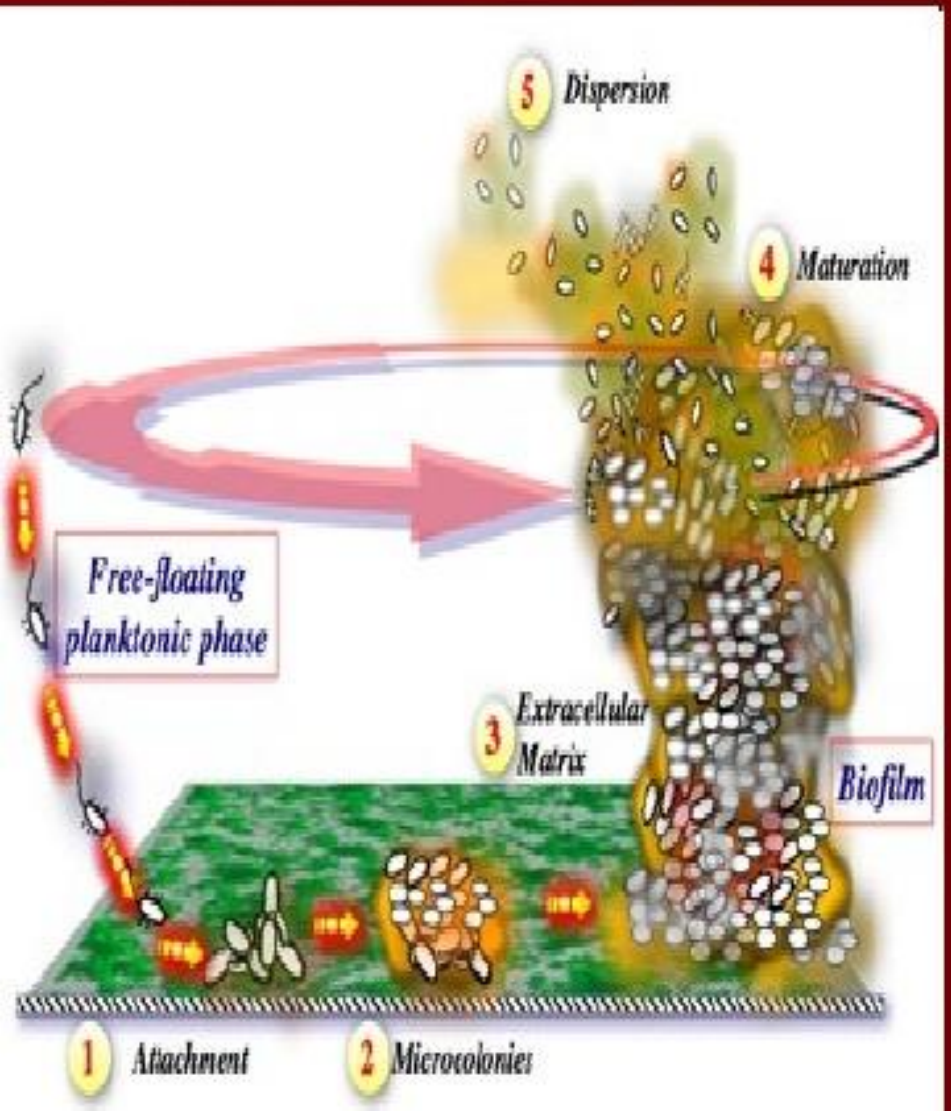


Staphylococcal biofilm



Scanning electron micrograph depicting a developed biofilm

Steps in Biofilm Development



- Biofilm development can be divided into several key steps including attachment, micro colony formation, biofilm maturation and dispersion; and in each step bacteria may recruit different components and molecules including flagella, type IV pili, DNA and exo polysaccharides.

Bacteriology
2016-2017
2nd year

Virulence Factors
Lecture 6
Dr. Halah Al-Haideri

Virulence factors

Virulence factors are molecules produced by pathogens (bacteria, viruses, fungi and protozoa) that contribute to the pathogenicity of the organism and enable them to achieve colonization and attachment, immunoevasion, evasion and inhibition of the host's immune response, entry into and exit out of cells, and aquire nutrition from the cell.

-Specific pathogens possess a wide array of virulence factors. Some are chromosomally encoded and intrinsic to the bacteria (e.g. capsules and endotoxin), whereas others are obtained from mobile genetic elements like plasmids and bacteriophage (e.g. some exotoxins).

Gram positive bacteria secrete a variety of virulence factors at host pathogenic interface, via membrane vesicle trafficking like bacterial outer membrane trafficking for invasion, nutrition and other cell-cell communications.

-bacterial virulence factors have two different routes used to help them survive and grow:
1- The factors are used to assist and promote colonization of the host. These factors include adhesins, invasins, and antifagocytic factors.
The factors, including toxins, hemolysine, and proteases, bring damage to the host.

Types of virulence factors

1- Adherence Factors: Many pathogenic bacteria colonize mucosal sites by using *pili* (fimbriae) to adhere to cells.

2-Invasion Factors: Surface components that allow the bacterium to invade host cells can be encoded on plasmids, but more often are on the chromosome.

3- Capsules: Many bacteria are surrounded by capsules that protect them from opsonization and phagocytosis.

4- Endotoxins: The lipopolysaccharide endotoxins on Gram-negative bacteria cause fever, changes in blood pressure, inflammation, lethal shock, and many other toxic events.

The innate (cellular-mediated) immune system is able to recognise a broad range of pathogens, including the LPS endotoxin. This ability is mediated by Toll-like receptors (TLR) – a series of receptors able to detect a variety of pathogen epitopes. The TLR responsible for recognising LPS is TLR-4.

5- Exotoxins: include several types of protein toxins and enzymes produced and/or secreted from pathogenic bacteria. Major categories include cytotoxins, neurotoxins, and enterotoxins. The toxin is the major factor in determining virulence, e.g. strains of *E. coli* without the exotoxins are low/non-virulent.

The toxins can remain toxic even at very low concentrations.
Exotoxins are typically named descriptively to show where the toxin acts, for example; neurotoxin, leukotoxin, enterotoxin and haemolysin

6-Siderophores: Siderophores are iron-binding factors that allow some bacteria to compete with the host for iron, which is bound to hemoglobin, transferrin, and lactoferrin.

Mechanism of exotoxins

Damage to cell membranes – For example, *Clostridium perfringens* α-toxin has phospholipase C activity which causes degradation of the cell membrane. *Staphylococcus aureus* α-toxin causes the formation of a pore in the membrane of target cells. This pore alters ion influx/efflux and can lead to swelling/lysis of the cell.

Inhibition of protein synthesis – Toxins which inhibit protein synthesis target the elongation factors and ribosomal RNA which are associated with protein synthesis. By targeting these factors, the cell is prevented from synthesising protein and the cell dies. An example of such a toxin is the diptheria toxin.

Interfere with cell signalling – These toxins target the proteins associated with signal transduction, either blocking or altering the signalling pathways. Such alteration of these pathways disrupts cellular function. For example *E. coli* cytotoxic necrotising factors modify RHO GTP-binding proteins, their modification disrupts the cell cyctoskeleton and thus the cell membrane.

Inhibition of neurotransmitters – These toxins target proteins of the synaptic cleft. They prevent the release of neurotransmitters from the presynaptic membrane. For example *Clostridium botulinum* neurotoxin or the *Clostridium tetani* tetanus toxin.

Affecting immune response – One example of altering the immune response is the super antigen TSS-1 released by *Staphylococcus aureus* which causes Toxic shock syndrome. The toxin interacts with T-cells of the host immune system in an abnormal manner and provokes the release of enormous amounts of inflammatory cytokines, which are harmful to the host.

Bacteriology
2016-2017
2nd year

Lecture 7

Dr. Halah Al-Haideri

Bacterial Genetics

A bacterial genome = the total amount of DNA in an organism, the genome of each species contains unique arrangement of genes. The genome of prokaryotes such as bacteria consist of a few thousand genes and it is typically a single circular chromosome.

*The first bacterial genome to be completely sequenced was that of *Haemophilus influenzae* (1995).

*The first archaeal genome to be completely sequenced was that of Methanococcus sp (1997).

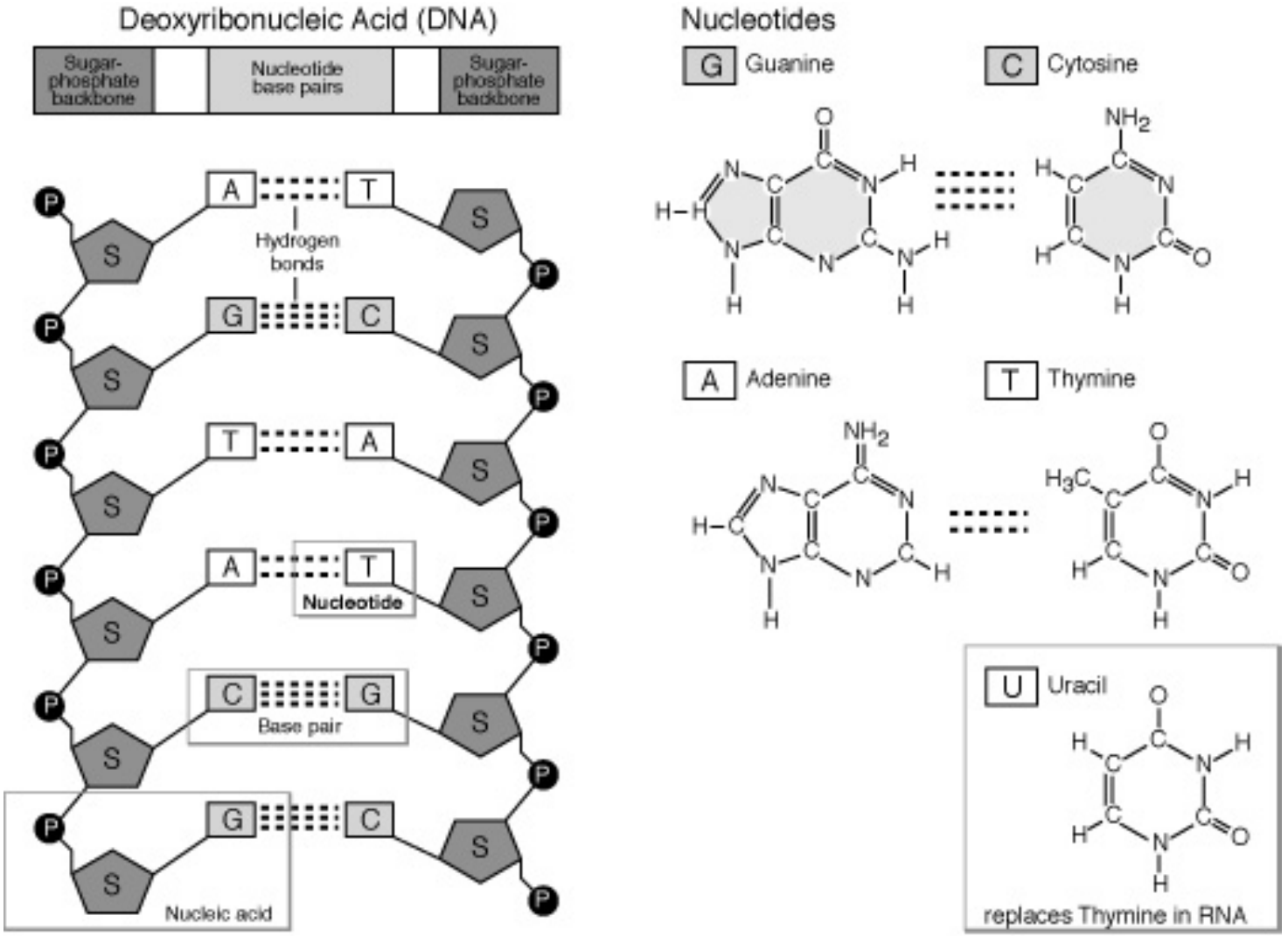
Nucleic acids:

-Nucleic acids are biopolymers, or large biomolecules, essential for all known forms of life. Nucleic acids, which include DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), are made from monomers known as nucleotides.

-Each nucleotide has three components**: a 5-carbon sugar, a phosphate group, and a nitrogenous base**. If the sugar is deoxyribose, the polymer is DNA. If the sugar is ribose, the polymer is RNA. When all three components are combined, they form a nucleotide.

-Nucleotides are also known as phosphate nucleotides.

-The nucleobases found in the two nucleic acid types are different: **adenine, cytosine, and guanine** are found in both RNA and DNA, while **thymine** occurs in DNA and **uracil** occurs in RNA.

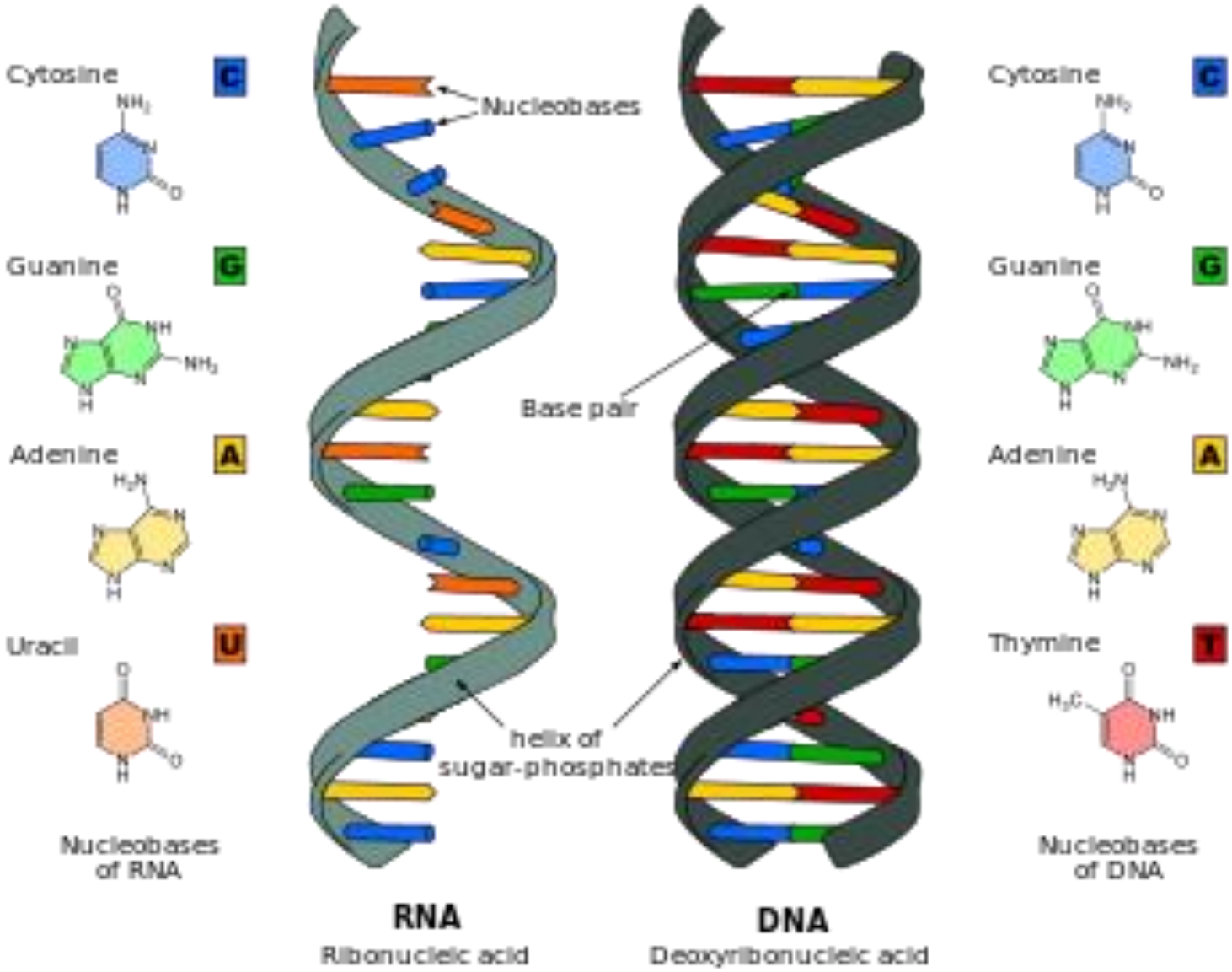


The structure of DNA and nucleotides from the National Human Genome Research Institute (NHGRI)

-Nucleic acids are among the most important biological macromolecules (others being amino acids/proteins, sugars/carbohydrates, and lipids/fats). They are found in abundance in all living things, where their function in encoding, transmitting and expressing genetic information, in other words, information is conveyed through the nucleic acid sequence, or the order of nucleotides within a DNA or RNA molecule. Strings of nucleotides strung together in a specific sequence are the mechanism for storing and transmitting hereditary, or genetic information via protein synthesis.

-Nucleic acids were discovered by Friedrich Miescher in 1869. Experimental studies of nucleic acids constitute a major part of modern biological and medical research, and form a foundation for genome and forensic science, as well as the biotechnology and pharmaceutical industries.

DNA	RNA
DNA is a double stranded molecule	RNA is a single stranded molecules
sugar is deoxyribose	sugar is ribose
DNA is responsible to storing and transferring genetic information	RNA directly codes **for amino acids** and as acts as a messenger between DNA and ribosomes to make proteins like **mRNA** (messenger ribonucleic acid)
DNA is stable under alkaline conditions	Is not stable
Purine basses : Adenine, Guanine Pyrimidine bases: Thymine, Cytosine	Purine basses : Adenine, Guanine Pyrimidine bases: Uracil, Cytosine


Cytosine
C
Guanine
G
Adenine
A
Uracil
U
Nucleobases of RNA
Nucleobases
Base pair
helix of sugar-phosphates
RNA
Ribonucleic acid
DNA
Deoxyribonucleic acid
Cytosine
C
Guanine
G
Adenine
A
Thymine
T
Nucleobases of DNA

DNA:

• DNA is a molecule that carries most of the genetic instructions used in the development, functioning and reproduction of all known living organisms and many viruses.

• DNA stores biological information. The DNA backbone is resistant to cleavage, and both strands of the double-stranded structure store the same biological information. Biological information is replicated as the two strands are separated. A significant portion of DNA (more than 98% for humans) is non-coding, meaning that these sections do not serve as patterns for protein sequences.

•The two strands of DNA run in opposite directions to each other and are therefore anti-parallel. Attached to each sugar is one of four types of nucleobases (informally, bases). It is the sequence of these four nucleobases along the backbone that encodes biological information. Under the genetic code, RNA strands are translated to specify the sequence of amino acids within proteins. These RNA strands are initially created using DNA strands as a template in a process called **transcription**.

• Prokaryotes (bacteria and archaea) store their DNA only in the cytoplasm. Within the chromosomes, chromatin proteins such as histones compact and organize DNA. These compact structures guide the interactions between DNA and other proteins, helping control which parts of the DNA are transcribed.

Structure of chromosome

In contrast to the linear chromosomes found in eukaryotic cells, most bacteria have single, covalently closed, circular chromosomes. Not all bacteria have a single circular chromosome: some bacteria have multiple circular chromosomes, and many bacteria have linear chromosomes and linear plasmids. Multiple chromosomes have also been found in many other bacteria, including Brucella, Leptospira interrogans, Burkholderia and Vibrio cholerae.

-Borrelia and Streptomyces have linear chromosomes and most strains contain both linear and circular plasmids. The chromosome of *E coli* has a length of approximately 1.35 mm, several hundred times longer than the bacterial cell, but the circular DNA is then looped and supercoiled to allow the chromosome to fit into the small space inside the cell.

Codon

A set of three base pairs constitutes a codon, which codes for a single amino acid. The “triplet code” is said to be degenerate or redundant because more than codon may exist for the same amino acid. For example, the codons AGA, AGG, CGU, CGC, CGA and CGG all code for arginine. There are 64 codons, of which 3 (UAA, UAG and UGA) are nonsense codons. They don’t code for any amino acid, but act as stop codons. There are specific codons which code for start and stop sequences.

-The start codon (AUG) indicates the beginning of the sequence to be translated, and the stop codons (UAA, UGA, UAG) terminate the protein synthesis. With the exception of methionine, all amino acids are coded for by more than one codon. The DNA in a gene that are expressed into the protein product are called exons and the non-coding DNA segments are called introns. There are no introns in bacterial chromosome. A segment of DNA carrying codons specifying a particular polypeptide is called a cistron or a gene.

Flow of genetic information

The central dogma of molecular biology is that DNA carries all 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).

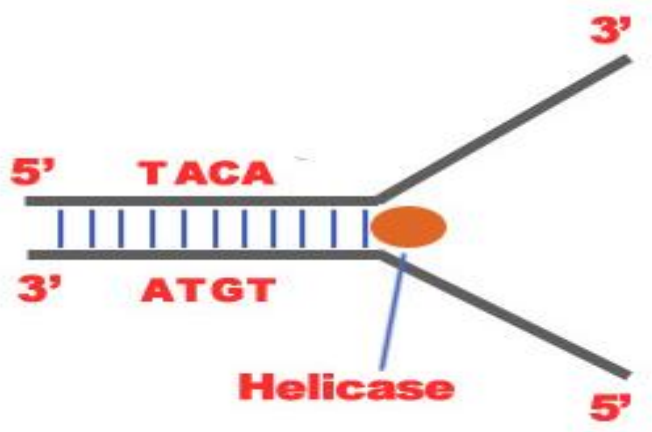
DNA replication

DNA replication is the process of producing two identical replicans from one original DNA molecule. This biological process occurs in all living organisms and is the basis for biological inheritance. DNA is made up of two strands and each strand of the original DNA molecule serves as a template for the production of the complementary strand, a process referred to as semiconservative replication. Cellular proofreading and error-checking mechanisms ensure near perfect fidelity for DNA replication.

Steps of DNA Replication

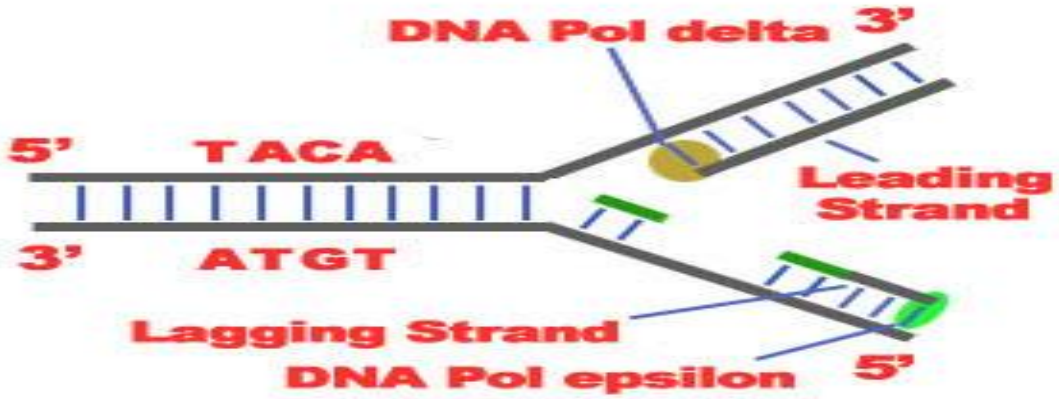
Initiation:

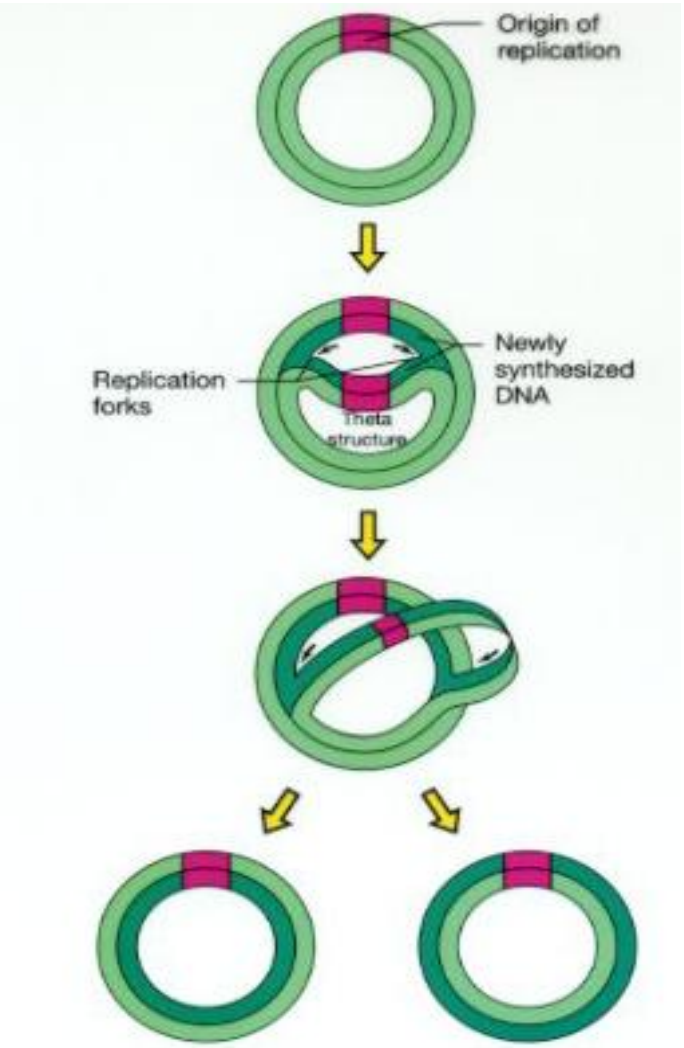
The first major step for the **DNA Replication** to take place is the breaking of hydrogen bonds between bases of the two antiparallel strands. The unwounding of the two strands is the starting point. The splitting happens in places of the chains which are rich in A-T. That is because there are only two bonds between Adenine and Thymine (there are three hydrogen bonds between Cytosine and Guanine). **Helicase** is the enzyme that splits the two strands. The initiation point where the splitting starts is called "origin of replication". The structure that is created is known as "**Replication Fork**".

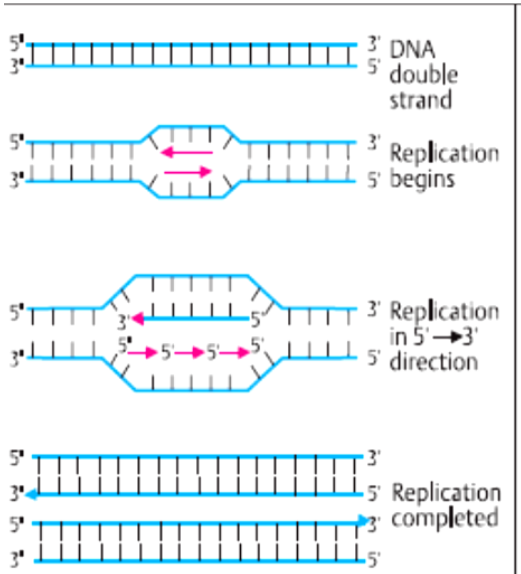


Elongation

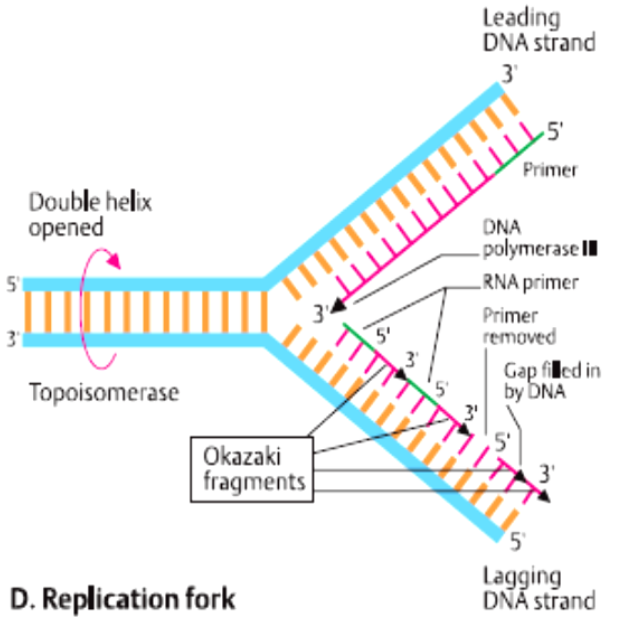
2) One of the most important steps of DNA Replication is the binding of RNA Primase in the the initiation point of the 3'-5' parent chain. RNA Primase can attract RNA nucleotides which bind to the DNA nucleotides of the 3'-5' strand due to the hydrogen bonds between the bases. RNA nucleotides are the primers (starters) for the binding of DNA nucleotides.







C. Scheme of replication



D. Replication fork

Translation: is the process in which cellular ribosomes create proteins. In translation, messenger RNA (mRNA)—produced by transcription from DNA—is decoded by a ribosome to produce a specific amino acid chain, or polypeptide. The polypeptide later folds into an active protein and performs its functions in the cell. The ribosome facilitates decoding by inducing the binding of complementary tRNA anticodon sequences to mRNA codons. The tRNAs carry specific amino acids that are chained together into a polypeptide as the mRNA passes through and is "read" by the ribosome. The entire process is a part of gene expression.

- Breifly, translation proceeds in four phases:

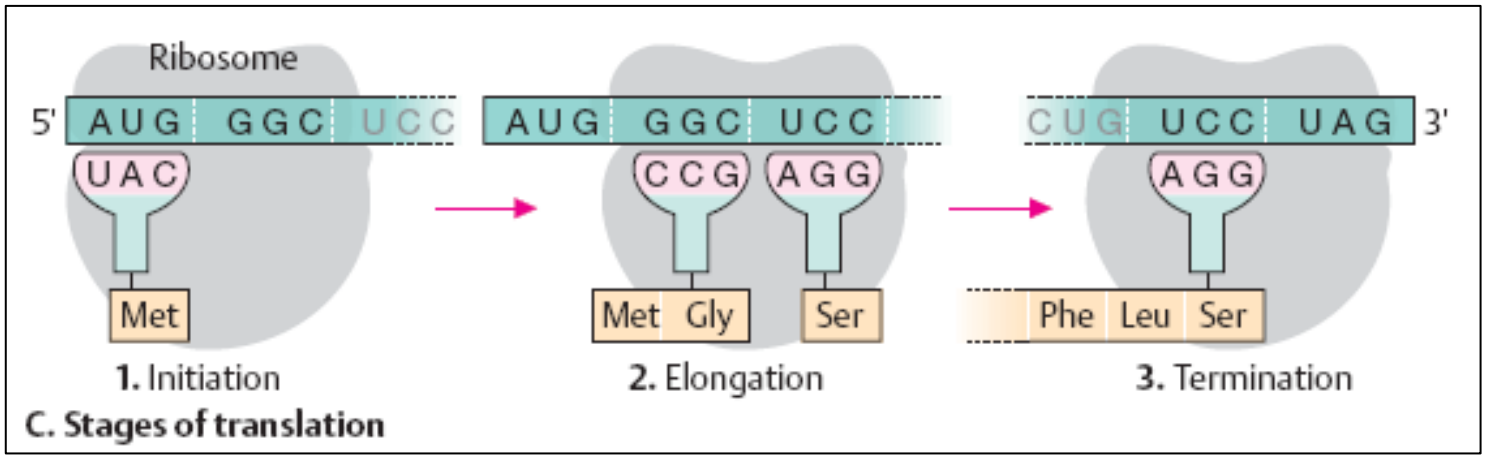
Initiation: The ribosome assembles around the target mRNA. The first tRNA is attached at the start codon.

Elongation: The tRNA transfers an amino acid to the tRNA corresponding to the next codon.

Translocation: The ribosome then moves (translocates) to the next mRNA codon to continue the process, creating an amino acid chain.

Termination: When a stop codon is reached, the ribosome releases the polypeptide.

-In bacteria, translation occurs in the cell's cytoplasm, where the large and small subunits of the ribosome bind to the mRNA. In eukaryotes, translation occurs in the cytosol or across the membrane of the endoplasmic reticulum in a process called vectorial synthesis. In many instances, the entire ribosome/mRNA complex binds to the outer membrane of the rough endoplasmic reticulum (ER); the newly created polypeptide is stored inside the ER for later vesicle transport and secretion outside of the cell.



Mutations:

-The term “mutation”, which is derived from Latin word meaning “to change”. Mutations are heritable changes in genotype that can occur spontaneously or be induced by chemical or physical treatments. (Organisms selected as reference strains are called wild type, and their progeny with mutations are called mutants.) The process of mutation is called mutagenesis and the agent inducing mutations is called mutagen. –

-Changes in the sequence of template DNA (mutations) can drastically affect the type of protein end product produced. For a particular bacterial strain under defined growth conditions, the mutation rate for any specific gene is constant and is expressed as the probability of mutation per cell division.

-Spontaneous mutation occurs naturally about one in every million to one in every billion divisions. Mutation rates of individual genes in bacteria range from 10-2 to 10-10 per bacterium per division.

- Most spontaneous mutations occur during DNA replication.

Mechanisms of mutation

A- Substitution of a nucleotide: Base substitution, also called point mutation, involves the changing of single base in the DNA sequence. This mistake is copied during replication to produce a permanent change. If one purine [A or G] or pyrimidine [C or T] is replaced by the other, the substitution is called a **transition.** If a purine is replaced by a pyrimidine or vice-versa, the substitution is called **transversion**. This is the most common mechanism of mutation.

b. Deletion or addition of a nucleotide: deletion or addition of a nucleotide during DNA replication. When a transposon (jumping gene) inserts itself into a gene, it leads to disruption of gene and is called insertional mutation. Results of mutation

C T A C T A C T C T A

a. Missense mutation: Missing mutations are DNA mutations which lead to changes in the amino acid sequence (one wrong codon and one wrong amino acid) of the protein product. This could be caused by a single point mutation or a series of mutations.

b. Nonsense mutation: A mutation that leads to the formation of a stop codon is called a nonsense mutation. Since these codon cause the termination of protein synthesis, a nonsense mutation leads to incomplete protein products.

c. Silent mutation: Sometimes a single substitution mutation change in the DNA base sequence results in a new codon still coding for the same amino acid. Since there is no change in the product, such mutations are called silent.

d. Frameshift mutation: Frameshift mutations involve the addition or deletion of base pairs causing a shift in the "reading frame" of the gene. This causes a reading frame shift and all of the codons and all of the amino acids after that mutation are usually wrong. Since the addition of amino acids to the protein chain is determined by the three base codons, when the overall sequence of the gene is altered, the amino acid sequence may be altered as well.

e. Lethal mutation: Sometimes some mutations affect vital functions and the bacterial cell become nonviable. Hence those mutations that can kill the cell are called lethal mutation.

f. Suppressor mutation: It is a reversal of a mutant phenotype by another mutation at a position on the DNA distinct from that of original mutation. True reversion or back mutation results in reversion of a mutant to original form, which occurs as a result of mutation occurring at the same spot once again.

g. Conditional lethal mutation: Sometimes a mutation may affect an organism in such a way that the mutant can survive only in certain environmental condition. Example; a temperature sensitive mutant can survive at permissive temperature of 35o C but not at restrictive temperature of 39o C.

h. Inversion mutation: If a segment of DNA is removed and reinserted in a reverse direction, it is called inversion mutation.

PLASMIDS:

Plasmids are extrachromosomal elements found inside a bacterium. These are not essential for the survival of the bacterium but they confer certain extra advantages to the cell. Number and size: A bacterium can have no plasmids at all or have many plasmids (20-30) .

Plasmid. Usually they are closed circular molecules; however they occur as linear molecule in *Borrelia burgdorferi*. Their size can vary from 1 Kb to 400 Kb. Multiplication: Plasmids multiply independently of the chromosome and are inherited regularly by the daughter cells. Types of plasmids: R factor, Col factor, RTF and F factor.

F factor: This is also known as fertility factor or sex factor. Most plasmids are unable to mediate their own transfer to other cells. Vertical (inheritance) or horizontal (transfer) transmissions maintain plasmids. F factor is a plasmid that codes for sex pili and its transfer to other cells. Those bacteria that possess transfer factor are called F+, such bacteria have sex pili on their surface. Those cells lacking this factor are designated F-. The F factor plasmid istransferred to other cells through conjugation. An F- cell will become F+ when it receives the fertility factor from another F+ cell.

R factor: Those plasmids that code for the transmissible drug resistance are called **R factor**. These plasmids contain genes that code for resistance to many antibiotics. R factors may be transferred by conjugation and its transfer to other bacteria is independent of the F factor. Bacteria possessing such plasmids are resistant to many antibiotics and this drug resistance is transferred to closely related species. R factors may simultaneously confer resistance to five antibiotics. They are usually transferred to related species along with RTF.

Heavy–metal resistance plasmid

There are several bacterial strains that contain genetic determinants of resistance to heavy metals, such as Hg^{++}, Ag^{+}, $Cd^{++,}$ CrO_4, Cu^{++}, Ni^{++}, Pb^{+++}, Zn^{++}, and so forth. These determinants for resistance are often found on plasmids and transposons. Bacteria that have been found resistant to heavy metals are *E. coli*, *Pseudomonas aeruginosa*.

Virulence plasmid:

Formation of invasin due to its virulence plasmid makes *Shigella flexneri* (a human intestinal pathogen) able to penetrate intestinal mucosa

Degradative plasmids:

consist of genes that equip the bacteria (e.g., Pseudomonas sps.) with special enzymes or enzyme system to enable them to digest unusual substances (Xenobiotics) like chlorinated aromatic or hydrocarbon compounds. For example, the camphor (CAM) plasmid of P. putida encodes enzymes for degradation of camphor, octane (OCT) plasmid helps it degrade octane, XYL–plasmid helps degrade xylene and toluene, NAH–plasmid helps degrade naphthalene, and SAL–plasmid helps it degrade salicilate. These plasmids are conjugative.

Industrial Bacteriology

The economic importance of bacteria derives from the fact that bacteria are exploited by humans in a number of beneficial ways. Despite the fact that some bacteria play harmful roles, such as causing disease and spoiling food, the economic importance of bacteria includes both their useful and harmful aspects

Biotechnology and bacteria:

Biotechnology is defined as the use of micro organism such as bacteria, fungi and algae for the manufacturing and services industries. These include-:

-Fermentation processes, such as brewing, baking, cheese and butter manufacturing, Bacteria, often Lactobacillus in combination with yeasts and fungi, have been used for thousands of years in the preparation of fermented foods such as cheese, pickles, , vinegar, wine, and yogurt.

-Chemical manufacturing such as ethanol, acetone, organic acid, enzymes, perfumes etc. In the chemical industry, bacteria are most important in the production pharmaceuticals

Genetic engineering and bacteria

Genetic engineering is the manipulation of genes. It is also called recombinant DNA technology. In genetic engineering, pieces of DNA (genes) are introduced into a host by means of a carrier (vector) system. The foreign DNA becomes a permanent feature of the host, being replicated and passed on to daughter cells along with the rest of its DNA. Bacterial cells are transformed and used in production of commercially important products. The examples are production of human insulin (used against diabetes), human growth hormone (somatotrophin used to treat pituitary dwarfism), and infections which can be used to help fight viral diseases.

Using biotechnology techniques,or bio medical technology bacteria can also be bioengineered for the production of therapeutic proteins.

Food Microbiology

Conditions for Spoilage

1– Water availability (aw): amount of water in food (pure water is 1.0) most bacteria require >0.90

2– pH: most pathogens not grow at pH<4.5 (except Lactic acid bacteria)

3– Nutrients

4- Storage temperature <0 no growth (water crystallizes), Refrigerator: 4C to 10C (enzyme runs very slow or non-existent

5- Atmosphere: availability of O_2

- **Food spoilage:** results from growth of microbes in food**,** resulted in altering food visibly and in other ways, rendering it unsuitable for consumption**.** It also involves predictable succession of microbes, so that different foods undergo different types of spoilage processes**,** toxins are sometimes produced**.** Approximately 1/3rd of all food manufactured in world is lost to spoilage.

- **Spoilage: Meat**

  -Cutting board contamination, Conveyor belts, Temperature, Failure to distribute quickly, Fecal bacteria from intestines, Fish, Polluted waters, Transportation boxes, Poultry and Eggs, Human contact, Penetration by bacteria, Milk and Dairy Products, Lactobacillus and Streptococcus species that survive pasturization (sour milk), Breads Spores and fungi that survive baking, Grains, Fungi produce toxins.

Food-Borne Diseases

Two primary types of food –borne disease:

1-food-borne infections

2- food intoxications: ingestion of toxins in foods in which microbes have grown include staphylococcal food poisoning, botulism, *Clostridium perfringens* food poisoning, and *Bacillus cereus* food poisoning.

Toxins:

1-Ergotism: toxic condition caused by growth of a fungus in grains

2- Aflatoxins: carcinogens produced in fungus-infected grains and nut products

3- Fumonisins : carcinogens produced in fungus-infected corn.

Removal of Microorganisms

Usually achieved by filtration, commonly used for water, beer, wine, juices, soft drinks, and other liquids.

-Low Temperature: refrigeration at 5°C retards but does not stop microbial growth, however, psychrophiles and psychrotrophs can still cause spoilage, and growth at temperatures below -10°C has been observed.

-High Temperature: includes

-Pasteurization: kills pathogens and substantially reduces number of spoilage organisms, different pasteurization procedures heat for different lengths of time, shorter heating times result in improved flavor.

-Canning: food heated in special containers (retorts) to 115 °C for 25 to 100 minutes, kills spoilage microbes, but not necessarily all microbes in food.

GRAS

Chemical agents "generally recognized as safe", pH of food impacts effectiveness of chemical preservative.

Radiation:

1-Ultraviolet (UV) radiation

- used for surfaces of food-handling equipment

- does not penetrate foods

2-Gamma radiation

-use of ionizing radiation (gamma radiation) to extend shelf life or sterilize meat, seafoods, fruits, and vegetables.

Bacteria causes food spoilage

Clostridium botulinum*:* cause botulism

Staphylococcus aureus*: cause* (Food poisoning)

Salmonella typhii: Causes typhoid fever (enteric fever)

Shigella dysenteriae**:** causes dysentery

Vibrio cholerae

Escherichia coli**:** causes hemorrhagic colitis& causes diarrhea

Listeria monocytogenes**:** Infects GI tract

Bacillus cereus cause food poisoning

Taxonomy and Classification of bacteria

Bacterial Classification

Taxonomy- It is the science of organism classification

Classification: is the assignment of organisms (species) into an organized scheme of naming.

These schemes are based on evolutionary relationships (i.e., the more similar the name, the closer the evolutionary relationship)
• Classification is concerned with the criteria for identifying organisms and assignment to groups (what belongs where)
Taxon (Singular- taxa)– It is a group or category of related organisms
• Members of lower level taxa (e.g., species) are more similar to each other than are members of higher level taxa (e.g., kingdoms or domain)
• Members of specific taxa are more similar to each other than any are to members of different specific taxa found at the same hierarchical level (e.g., humans are more similar to apes, i.e., comparison between species, than either is similar to, for example, Escherichia coli).

Binomial nomenclature

- The naming of organisms is called as binomial nomenclature (viruses are exceptions)
- Binomial nomenclature employs the names of the two lower level taxa, genus and species, to name a species
- Genus comes before species (e.g., Escherichia coli)
- Genus name is always capitalized (e.g., Escherichia)
- Species name is in small letter (e.g., coli)
- Both names are always either italicized or underlined (e.g., Escherichia coli)
- The genus name may be used alone, but not the species name (i.e., saying or writing "Escherichia," alone is legitimate while saying or writing "coli" is not)

Bacterial species- A bacterial species is defined by the similarities found among its members. Properties such as biochemical reactions, chemical composition, cellular structures, genetic characteristics, and immunological features are used in defining a bacterial species

I.The five-kingdom system – It was firstly proposed in 1969
The five kingdoms include:

1. Plantae (the plants)
2. Fungi (the fungi)
3. Animalia (the animals)
4. Protista (the unicellular eucaryotes**)**
5. Monera (the prokaryotes)

Kingdom Monera –Includes the eubacteria, the cyanobacteria, and the archaeobacteria

- The eubacteria are our common, every-day bacteria, some of which are disease-causing
- The cyanobacteria are photosynthetic eubacteria
- The archaeobacteria are distinctive in their adaptation to extreme environments (e.g., very hot, salty, or acidic) though not all archaeobacteria live in extreme environments

Kingdom Protista

- Protista, like Monera, consists mostly of unicellular organisms
- Some members of protista are multicellular, however

Kingdom Fungi

- This group includes eukaryotic fungi
- They are nutrient absorbers plus have additional distinctive features
- Unicellular fungi are called as yeasts

Kingdom Plantae –Includes all plants
Kingdom animalia- includes all animals

I. Phenotypic classification systems:

1. Gram stain: H.C. Gram in 1884 invented this technique. Bacteria are classified into Gram positive or negative based on their morphology and differential staining properties
Gram positive- Purple; Gram negative – Pink color
2. Growth Requirements: Microorganisms can be grouped on the basis of their need for oxygen to
grow.

- Facultatively anaerobes- bacteria that grows in high oxygen or low oxygen content
- Strict anaerobes - bacteria that grows in the absence of oxygen environment. Ex bacteroides
  found in the large bowel
- Strict aerobes- Bacteria that grows only in the presence of oxygen. Ex. Pseudomonas aeruginosa,
- Microaerophiles- bacteria grows under conditions of reduced oxygen and sometimes also require increased levels of carbon dioxide. Ex; Neisseria

3.Biochemical reactions: Clinical microbiology laboratories typically will identify a pathogen in
a clinical sample, purify the microorganism by plating a single colony of the microorganism on a separate plate, and then perform a series of biochemical studies that will identify the bacterial
species.

4. Serologic systems:

- Selected antisera can be used to classify different bacterial species.
- This may be based on either carbohydrate or protein antigens from the bacterial cell wall or the capsular polysaccharide.
- (Group A streptococcal M proteins or O and H polysaccharide antigens of salmonella).

II. Genotypic classification system:

It is based in the Genetic homology- A homology is a similarity between two organisms that exists.

The similarity of the DNA (or RNA) of organisms may be determined by a number of means including determinations of base composition, nucleotide sequence, or DNA hybridization rates

Base composition-Chargaff's rule says that- adenines (A's) and thymines (T's) are always present in DNA in equal proportions, and that the same is true for cytosines (C's) and guanines (G's)

Distinguishing strains- Very closely related organisms, i.e., members of the same species, are typically sufficiently similar that there exist additional methods that are able to distinguish the small differences seen between them. These methods include:

- Protein profiling
- Ribosomal RNA (rRNA) sequence analysis
- Phage typing
- Molecular subtyping:

Protein profile

- Various techniques exist for isolating (separating) and then visualizing the proteins from cells
- By distinguishing proteins in terms of their sizes and/or charges one can construct reproducible patterns that are typical of a given organism
- More-similar organisms display more-similar protein patterns

Phage typing

Typing of bacteria using bacteriophages are called as phage typing

•Different phages will have specific receptors for different bacteria

Ribosomal RNA (rRNA) sequence analysis:

- This has emerged as a major method for classification.
- It has been used to establish a phylogenetic tree.

Molecular subtyping:

• This may be done by examining the biochemical studies or the antibiotic susceptibility profile but a more reliable method is by molecular analysis.
• Pulsed Field Gel Electrophoresis (PFGE) is the most frequently used molecular technique
Bergey's Manual- Methods for distinguishing and identifying bacteria are assembled into Bergey's Manual of Determinative Bacteriology.