MICROBIOLOGY, CELL, AND MOLECULAR BIOLOGY

UNIT I: Scope and Techniques of Microbiology

History and Contribution of Key Figures

- Antonie van Leeuwenhoek: Known as the "Father of Microbiology," he was the first to observe and describe microorganisms using a microscope he developed.
- Louis Pasteur: Pioneered the field of microbiology with his discoveries of the principles of vaccination, microbial fermentation, and pasteurization.
- Robert Koch: Established Koch's postulates, which are critical in linking specific pathogens to specific diseases, and discovered the bacteria responsible for tuberculosis and cholera.
- Joseph Lister: Introduced antiseptic surgical techniques, significantly reducing postoperative infections.
- Alexander Fleming: Discovered penicillin, the first true antibiotic, which revolutionized the treatment of bacterial infections.

Ultrastructure of Bacteria and Growth Curve

- Ultrastructure: Bacterial cells consist of a cell wall, plasma membrane, cytoplasm, ribosomes, nucleoid, plasmids, and sometimes additional structures like capsules, pili, and flagella.
- Growth Curve: Bacterial growth in a closed system typically follows four phases: lag phase, log (exponential) phase, stationary phase, and death phase.

Pure Culture Techniques

- Methods to isolate a single species of microorganism include streak plating, pour plating, and spread plating.

Sterilization Techniques

- Physical Methods:
    - Autoclave: Uses steam under pressure to sterilize equipment and media.
    - Hot Air Oven: Utilizes dry heat for sterilization.
    - Incineration: Burns materials to ashes, useful for decontaminating waste.
- Chemical Methods: Involve disinfectants and antiseptics like ethanol, formaldehyde, and chlorine compounds.
- Radiation Methods: Include UV radiation for surface sterilization and gamma radiation for deep penetration sterilization.

Staining Techniques

- Simple Staining: Uses a single dye to color cells, allowing basic morphology to be observed.

- Gram Staining: Differentiates bacteria into Gram-positive (purple) and Gram-negative (pink) based on cell wall structure.
- Acid-Fast Staining: Used to detect Mycobacterium species, which retain the primary stain despite acid-alcohol decolorization.

Introduction

Microbiology is the science that deals with the study of microorganisms. The term microbiology derives its name from three Greek words mikros [small] bios [life] and logos [study]. Microorganisms are tiny and invisible to naked eye. So, they can be looked into and studied only with the help of microscope.

Small subcellular or cellular living beings with milli-micron or micron in size and are not visible to our naked eyes are called micro-organisms.

Micro-organisms are basically classified under the following 2 groups:

1. Prokaryotic microbes: These include subcellular living entities like prions, viroid,

viruses and cellular organisms like bacteria, cyanobacteria etc.

2. Eukaryotic microbes: These include cellular microbe belonging to following groups. Algae. Ex: Chlamydomonas, Diatoms.

b. Fungi. Ex: Yeast, Rhizopus.

c. Protozoans. Ex: Plasmodium, Amoeba.

Micro-organisms are commonly called microbes and they were the first to occupy planet

earth even before man and other creatures. Microbes are present in every part of biosphere.

HISTORY OF MICROBIOLOGY

Although microbes were the first life forms to occupy the planet earth, the knowledge about

microbiology is well developed with new dimension only after the invention of microscopes

and contribution of knowledge to the field of microbiology from various scientists.

Contributions of Antony Van Leeuwenhoek

- He was Dutch Philosopher, born on 24 October 1632.
- He is regarded as Father of „Bacteriology" and „Protozoology", because of his contribution to the field of bacteria and protozoa.
- He invented simple microscope having magnification power up to 300X.
- He observed bacteria from his teeth scrap under the microscope invented by him and he named them as „animalcules".
- He also discovered bacteria in rain water ditch and protozoans like paramecium and amoeba.
- He presented all his observations with illustration before scientist organization „Royal Society of London" in 1683.

Contributions of Louis Pasteur

- He was a French Biochemist, born on 27 December 1822.
- He is regarded as „Father of Microbiology and Immunology".
- He proposed the „Theory of Germ Disease", where diseases of plants, viruses, animals
- and human beings are caused by pathogenic microbes.
- He disproved the theory of abiogenesis by conducting „Swan neck flask experiment".
- He discovered the presence of bacteria in the air and classified the bacteria into aerobic and anaerobic forms.
- He coined the term „microbiology", aerobic, anaerobic.
- He discovered the role of anaerobic microbes in the fermentation of sugar.
- He developed technique to prevent souring of milk and spoilage of wine. His technique is now called Pasteurization technique.
- He first isolated bacteria causing cholera (Vibrio cholerae).
- He developed technique to strengthen immunity against anthrax bacteria by injecting weakened anthrax bacteria to healthy animal.
- Pasteur demonstrated a disease of silkworm was due to a protozoan parasite.
- Contributions of Robert Koch
- He was a German microbiologist born on 11 December 1843.
- His contribution to the field of microbiology and medical science is the most valuable one.
- He developed for the first-time culture technique to culture the bacteria in the laboratory.
- He discovered bacteria caused tuberculosis of man.
- He developed for the first-time staining technique to stain the bacteria with acidic or basic stain.
- He isolated and identified different kinds of bacteria from various sample.
- He proved theory of germ diseases of Louis Pasteur by conducting investigative experiment.
- He was awarded Nobel Prize of medicine in 1905, formulating principles regarding diseases. These are now called „Koch Postulates".
- Some of them are: -

  a. Specific pathogenic microbe causes one specific disease not more than one type of diseases in plants, animals, and human beings.

  b. Specific pathogenic microbes can be isolate from diseased organism and cultured outside the diseased organism.

Contributions of Alexander Fleming

- He was a Scotland doctor and biochemist born in 1881.
- He contributed knowledge about antibiotic Penicillin for this kind of work.
- He was awarded Nobel Prize in 1945.
- His contributions to the field of microbiology can be summarized as below.
- He studied bacterial action in blood and their response to the antibiotic.

- He worked on antimicrobial substances. That is not toxic to human body but toxic to microbial body.
- He discovered bacteriolytic substance lysosome in the animal tissue.
- He developed technic to study sensitivity of the microbes to the antibiotic drugs.

What is Streak Plate Method?

The streak plate method is a microbiological laboratory technique of isolating pure cultures, and/or getting well-isolated colonies of bacteria from a mixed population. It is mostly used to get pure cultures of bacteria; however, yeasts can also be isolated by this method. It is one of the most used aseptic techniques in microbiology to isolate and propagate bacteria. It is a mechanical isolation technique used in microbiology, commonly known as the "**streaking method**".

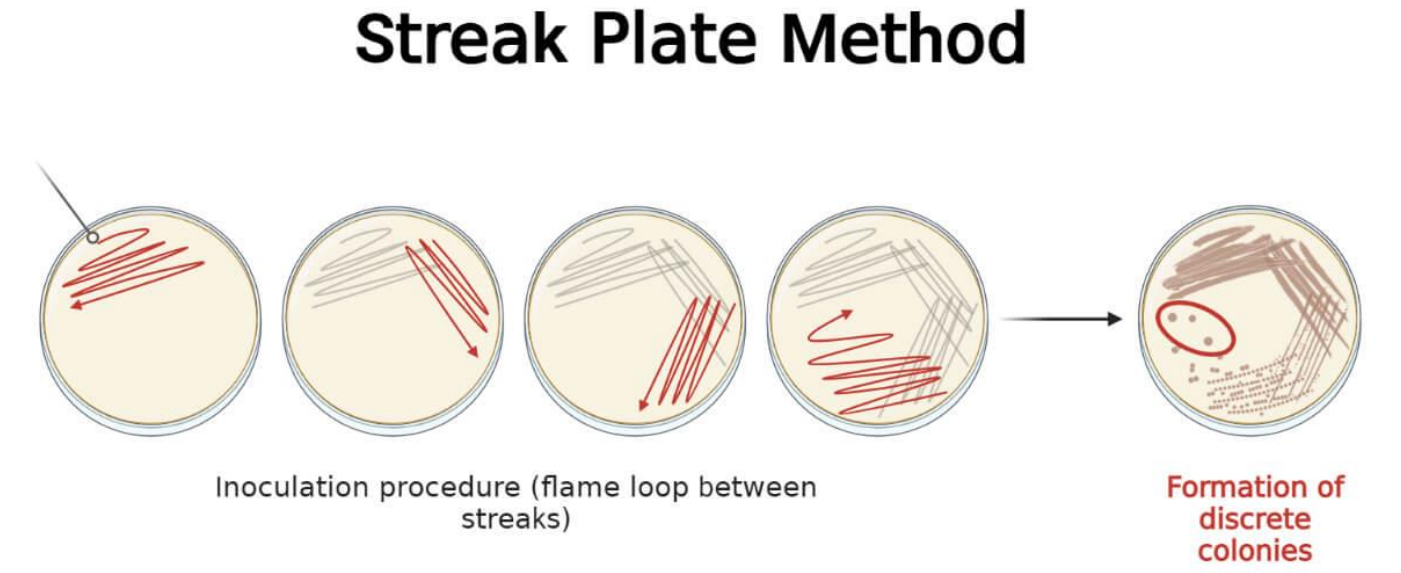


Figure: Streak Plate Method

This method dilutes the bacterial load, over the surface of agar medium, successively as streaking proceeds, and ultimately only a few bacterial cells will be inoculated at the end giving well-isolated colonies in the final streaks. Thus, this method mechanically isolated the bacteria from a mixed population of either the same or different species. After inoculation, the same types of colonies are seen in the terminal streaks if the specimen contained single species, whereas, different types of colonies may be seen if the specimen contained different species.

This is a very old method used in microbiology since the time of Robert Koch. This method was first devised and used by Loeffler and Gaffky in Koch's laboratory to serially dilute bacteria over agar surface and obtain well-isolated colonies. Since that time, it is used as a very important tool in bacteriology.

It is a very simple and reliable aseptic technique that uses tools like cotton swabs, wooden or plastic, metal sticks and toothpicks, or inoculating loop to dilute and spread the specimen over the surface of pre-sterilized specific solid culture media. The specimen used can be either suspension or colonies from the agar surface.

Well isolated colonies can be obtained from successfully performed streaking which allows describing the colony character of the organism on that specific culture media and condition.

Objectives of Streak Plate Method

1. To obtain a pure culture of bacteria from a mixed culture
2. To obtain well-isolated colonies
3. To propagate bacteria

Principle of Streak Plate Method

The streak plate method is based on dilution during the process of mechanical spreading of inoculum over the surface of solidified culture media in order to obtain well-isolated colonies of the sample at the terminal streaks. Sample can be either colony on solid media or suspension in broth. The sample is picked by using different tools, mostly using a sterile inoculating loop or swab. The sample is placed over a surface of sterile solid media at one edge of the petri dish and a smear is prepared. Using the tool, the smear is successively streaked over the agar medium on different patterns. As the streaking proceeds, the inoculum is gradually diluted to the point where bacterial cells are separated as individual cells or as a colony-forming unit (CFU) at a gap of a few millimeters. When these inoculated plates are incubated, the isolated bacterium or a CFU will give rise to a well-isolated colony. This will allow us to get a pure culture as well as describe the colony morphology of the organism.

Types of Streak Plate Method

Based on the pattern of streaking, the streak plate method can be classified into 4 types, viz.: Quadrant Streaking, T-Streaking, Continuous Streaking, and Radiant Streaking.

1. Quadrant Streaking

It is the most commonly used and the most preferred method where four equal-sized sections of the agar plate are streaked. It is also referred to as the “four-quadrant streak” or “four sectors” or “four-way streak” method.

In this method, each plate is divided into four equal sectors and each adjacent sector is streaked sequentially. The sector which is streaked first is called the first sector or the first quadrant, and it has the highest concentration of inoculum. Gradually the second, third, and fourth quadrants will have diluted inoculum. By the time the fourth quadrant is streaked, the inoculum is highly diluted giving rise to isolated colonies following the incubation.

Mostly, a discontinuous fashion of streaking is followed where the loop is sterilized at the end of each quadrant prior to streaking over the next quadrant. However, if the bacterial load is too small (or highly diluted), continuous fashion can also be used. In the latter, the loop needs not be sterilized at the end of every quadrant.

Although being the most popular method, it limits us to use only one specimen per plate. If we try two or more specimens in a single 10 cm plate, this method is not suitable.

2. T-Streaking

It is another method of streaking where the agar Petri plate is divided into three sections and each section is streaked. Hence, this method is also known as the "three-sector streak" method.

The media is divided into three sections by drawing a letter "T" and each adjacent section is streaked sequentially. By the time the final section is being streaked, the inoculum is diluted to the point to give rise to isolated colonies following the incubation. Mostly discontinuous fashion of streaking is followed; however, a continuous fashion can also be used in the very dilute specimen.

As in quadrant streaking, it is difficult to culture two or more samples in a single 10 cm plate using this method.

3. Continuous Streaking

It is another commonly followed method where an inoculum is evenly distributed in a single continuous movement from starting point to the centre of the plate. There is no need to divide the plate and sterilize the loop during the process. It is easy and quick; however, the problem is that we can use it only if the inoculum is either very diluted or we just must propagate pure culture rather than isolate one.

We can divide the 10 cm Petri plate into different sections (mostly 2 to 6), and in each section, we can streak different specimens following this method. Hence, it is used in the clinical laboratory to culture urine, sputum, pus, etc. if multiple samples have arrived at a single time. This will allow us to save media and get maximum output using a minimum resource.

4. Radiant Streaking

It is another method of streaking where the inoculum is first streaked at one edge and spread in vertical lines above the edge. Finally, the vertical lines are cross streaked diagonally. This method is suitable to propagate pure culture, and in the case of a dilute specimen.

There are other modified forms of streaking like:

5. Semi-quantitative Streaking

It is routinely followed in urine culture. It is a modified form of continuous streaking. In this method, a calibrated loop (usually a loop of 1 or 2µl) is used to streak a certain volume of the liquid specimen. A loopful of the specimen is streaked in a horizontal line in the middle of the Petri plate, and the specimen is spread all over the plate in a single continuous back and forth movement. This method allows us to approximately quantify the viable load (in a range, not an exact number) as well as get the pure culture in a single go.

6. Zigzag Streaking

It is another form of continuous streaking where a loopful of the specimen is streaked all over the plate in a zigzag pattern in a single continuous movement. It is commonly done to propagate the pure culture and culture them in large quantities.

Requirements of Streak Plate Method

1. **Streaking tool**

This is a sterile tool used to streak the specimen over the surface of culture media. The tools used for streaking are cotton swabs, inoculating loop (both metal and plastic), toothpicks, and wooden or metal or plastic sticks/wires. The most used one is inoculating loop (nichrome wire loop).

(In this whole article, we will talk about inoculating loop.)

2. **Sample culture**

Sample bacteria may be in the form of suspension, liquid broth, or colonies over solid media. The sample is picked by using an inoculating loop and transferred over the surface of fresh culture media to perform streaking.

3. **Solid culture media**

Specific culture media is used for the isolation and differentiation of suspected (or specific) bacteria. The culture medium is a solid agar medium that is pre-solidified before use.

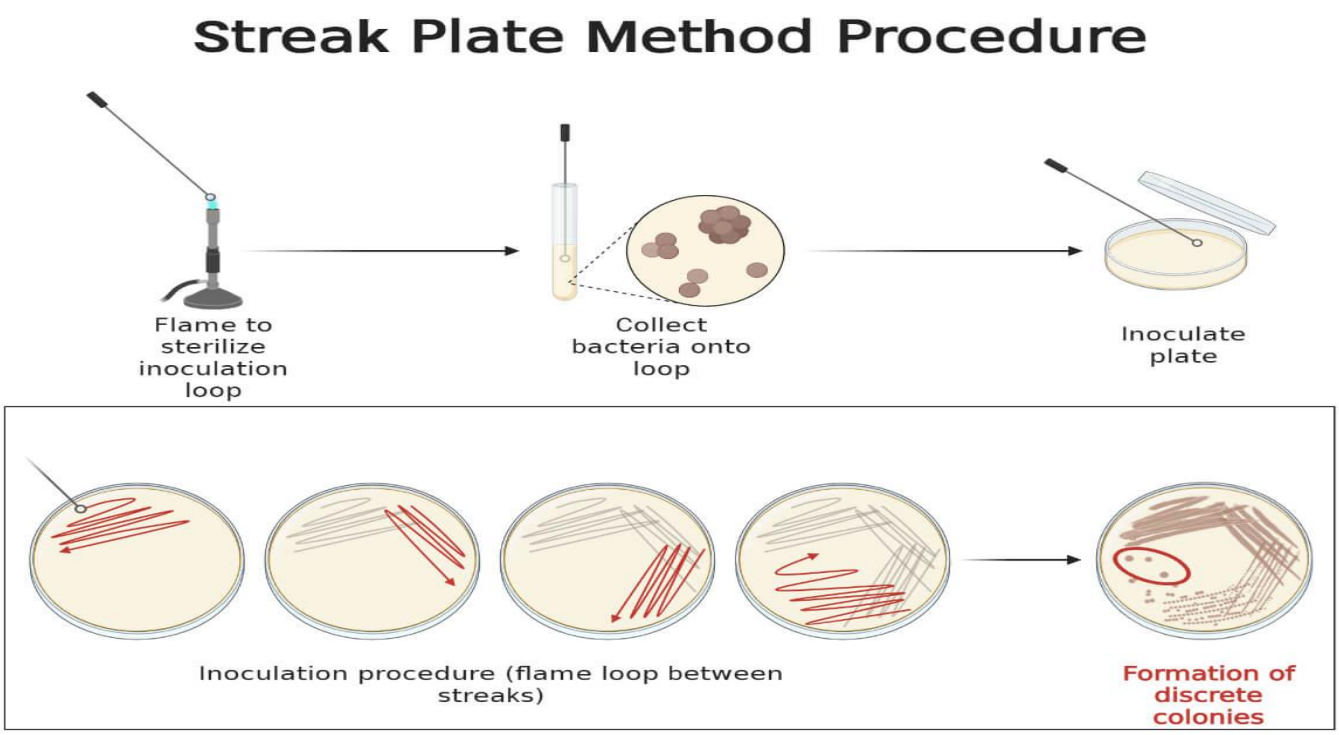
4. **Bunsen burner and other laboratory facilities**

A Bunsen burner is used to sterilize the loop and to create a sterile zone around the flame. Besides, other chemicals, sterilizing materials, and laboratory apparatus are also required.

Procedure or Protocol of Streak Plate Method

The general procedure of the streak plate method can be summarized as:

1. Arrange all the requirements, put on the PPE, sterilize the work surface, and allow all the samples and media to come to room temperature if were refrigerated.
2. If the sample is very concentrated then dilution can be helpful to get the isolated colonies. (But it is not compulsory as the sample will be diluted during the streaking process.)
3. Sterilize the inoculating loop by flaming and allow it to cool. Pick a small portion of the isolated colony. (if the sample is in the suspension, then take a loopful of the sample)



The inoculating procedure is different according to the method of streaking, let us deal with each type:

Quadrant Streaking Procedure

1. Lift the Petri plate in your left hand and hold it at an angle of 60°.
2. The sample is spread over about 1/4th of the media in the Petri plate from the rim to the centre of the plate using a rapid, gentle, back and forth motion.
3. Re-flame the loop and allow it to cool. Turn the Petri plate by 90° anticlockwise, and place the loop to the last streaks of the first quadrant. Move the loop back and forth to spread the inoculum over the last half of the streaks in the first quadrant into the empty second quadrant.
4. Repeat the process *(iii)* for streaking the third quadrant and the fourth quadrant.
5. For the fourth quadrant similar step can be followed. However, many people prefer to draw a few (6 to 7 streaks) well-separated streaks by touching the second half of streaks in the third quadrant. Also, some prefer to make the final streak in a zigzag fashion making a tail.

T-Streaking Procedure

1. Lift the Petri plate in your left hand and hold it at an angle of 60^0.
2. The sample is spread over about 1/3rd of the media in the Petri plate from the rim to the centre of the plate using a rapid, gentle, back and forth motion.
3. Re-flame the loop and allow it to cool. Turn the Petri plate by 90^0 anticlockwise, and place the loop to the last streaks of the first quadrant. Move the loop back and forth to spread the inoculum over the last half of the streaks in the first quadrant into the empty second quadrant.

(Be sure not to move the loop to the streaks in the first half of the first quadrant.)

4. Repeat the process *(iii)* for streaking the third quadrant. As in quadrant streaking, you can follow any one of the streaking patterns at the 3rd quadrant.

Continuous Streaking Procedure

1. Lift the Petri plate in your left hand and hold it at an angle of 60^0.
2. Place the loop at one end of the plate and start streaking the inoculum from that point in a continuous movement to the centre of the plate.
3. Rotate the plate by 180^0 and without sterilizing the loop, follow the step *(ii)* to streak the remaining half of the plate.

Radiant Streaking Procedure

1. Lift the Petri plate in your left hand and hold it at an angle of 60^0.
2. Spread the inoculum over the near edge of the agar plate using a gentle zigzag motion.
3. Sterilize the loop and allow it to cool. Then, the streak from the point of primary spread in a radial direction up to the far edge of the Petri plate.
4. Re-flame the loop and allow it to cool. Then draw horizontal lines crossing the radial streaks.

Semi-quantitative Streaking Procedure

1. Lift the Petri plate in your left hand and hold it at an angle of 60^0.
2. Using a calibrated loop take a loopful of the sample (urine).
3. Draw the sample into a vertical or horizontal streak (primary streak) at the centre of the plate.
4. Using the same loop spread the inoculum by continuously moving the loop in back and forth (zigzag) motion crossing the primary streak.
5. Label at the edge of the bottom of the plate with the date, name, sample ID, and other required information.
6. Incubate the plate in an inverted position under suitable incubation conditions (mostly for 24 hours at 37^0C).

Sterilization Techniques:

Microorganisms play an important role in causing infection and contamination. Therefore, Sterilization is an important technique in microbiology which helps to remove or destroy microorganisms from materials or surfaces.

Sterilization

It is the process by which an article, surface or medium is made free of all microorganisms either in vegetative or spore form.

Disinfection

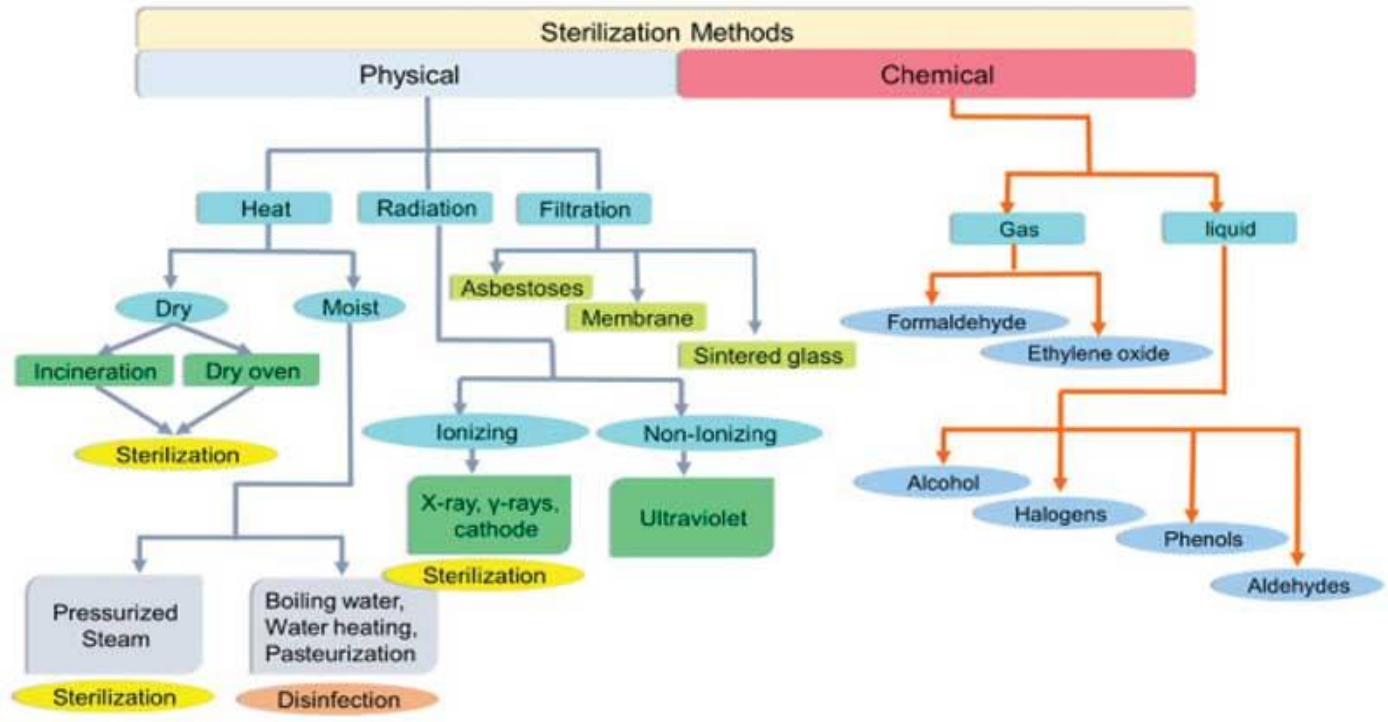
It refers to the destruction of all pathogens or organisms which can cause infection but not necessarily spores. All organisms may not be killed but the number is reduced to a level that no longer harmful to health.

Antiseptics

These are chemical disinfectants that are safe to apply on living tissues and used to prevent infection by arresting the growth of the microorganism.

Asepsis

This refers to the technique which helps to prevent the occurrence of infection into an uninfected tissue.



Methods of Sterilization

Physical Methods

Sunlight

Sunlight has an active germicidal effect due to the presence of ultraviolet rays. It is a natural procedure of sterilization which reduces the number of microorganisms in water tanks, lakes, etc.

Heat

Heat is a mostly used method of sterilization. Moreover, it is a highly effective and most reliable process. There are two major methods of using heat in sterilization which are dry heat and moist heat. The principle behind both of these methods is similar. Dry heat induces the denaturation of protein, oxidative damage and toxic effect due to the high level of electrolytes. Moreover, the dry heat can also damage the DNA of the microorganism. As a result, the microorganism got killed. Moist Heat kills the microorganisms by denaturation and coagulation of proteins. There are several factors that can influence the heat killing procedure. Such as

- Temperature and Duration: The duration and temperature are inversely connected to each other. Therefore, in the case of the long duration of heat provided for sterilization, the temperature will be reduced while in case of high temperature the duration will be reduced.
- Characteristic of the Microorganism: Microorganisms can be present in both vegetative and spore form. Spore forms are generally heat resistant. Therefore, the sterilization process will vary on the basis of the characteristic of the microorganism.
- Type of Material: Organic substances often provide protection to the vegetative and spore form of microorganisms which reduces the lethal property of heat. Apart from that, the materials containing the substances are also needed to be heat stable for proper sterilization.

Dry Heat: Procedures

Red Heat

Inoculation loops, wires, forceps tips, needles are needed to be sterilized to inhibit microbial contamination. These instruments are held in the flame of a Bunsen burner until they become red hot.

Flaming

Glass slides, scalpels, and mouths of culture tubes or conical flasks are passed through Bunsen flame without allowing them to become red hot.

Incineration

This procedure is used to reduce the infective material into ashes by burning. The incinerator is used for the process. Soiled dressings, animal carcasses, bedding, and pathological materials are dealt with this method.

Hot Air Oven

It is a widely used method of sterilization by dry heat. The heat inside the oven is maintained by electricity and a fan fitted inside it provides the adequate distribution of hot air inside the chamber. A thermostat is also connected which maintains the temperature inside the chamber. 160^0C for two hours is required for sterilization. There are also some alternative temperatures and holding time which include 170^0C for 1 hour and 180^0C for 30 minutes.

Uses: Sterilization of

- Glassware's like glass syringes, Petri dishes, flasks, pipettes, and test tubes.
- Surgical instruments like scalpels, scissors, forceps, etc.
- Chemicals such as liquid, paraffin, fats, sulphonamides powders etc.

Sterilization control

- The spores of Bacillus subtilis subsp. Niger (NCTC 10075 or ATCC 9372) are kept inside the oven. These spores should be destroyed if the sterilization is proper.
- Thermocouples may also be used.
- Browne's tube with a green spot is available. After proper sterilization, a green color is produced (after two years at 160^0C).

Moist Heat Sterilization: Procedure

Temperature below 100^0C

Pasteurization

There are two different types of pasteurization methods that are used for sterilization of milk, Holder method (63^oC for 30 minutes) and flash method (72^0C for 20 seconds followed by cooling quickly to 13^0C). This method is effective against all non-sporing pathogens such as mycobacteria, *Salmonella*, etc. except *Coxiella burnetii* which survives the holder method due to heat resistant characteristics.

Inspissation

Media like Lowenstein- Jensen's and Loeffler's serum are required to sterile at 80-85^0C for 30 minutes daily on three consecutive days. This process is known as inspissation and the instrument used is called inspissator.

Vaccine bath

It is used for sterilization of bacterial vaccines at 60^0C for one hour. Serum or other body fluids can be sterilized by heating in a water bath at 56^0C for several successive days.

Low-temperature steam formaldehyde sterilization (LTSF)

This method is applicable for materials that cannot withstand 100^0C temperature. In this method, steam at sub atmospheric pressure at 75^0C with formaldehyde vapor is used. *Bacillus stearothermophilus* plays an important role as a biological control to test the efficacy of the test.

At a temperature of 100^0C

Boiling

It is an effective method that can kill vegetative cells. Boiling for 10-30 minutes can kill most of the vegetative cells; however, many spores can withstand this temperature. Boiling can be employed when adequate methods are not available to sterilize glass syringes, rubber stopper, etc.

Tyndallisation

In this case, steam at 100^0C for successive 3 days is used. It is also known as intermittent sterilization. In this case, the first exposure kills the vegetative forms, and in the intervals between the heating and remaining spores germinates into vegetative forms which are killed on subsequent heating. This process is applied for sterilization of egg, serum or sugar-containing media which can be damaged due to exposure in high temperature for a longer period.

Steam sterilizer

Koch's and Arnold's steam sterilization is usually used for media which can easily decompose due to the high temperature in the autoclave. Those media are kept on a perforated tray and steam at 100^0C and at atmospheric pressure passes through the media for 90 minutes. It is an effective method to kill vegetative cells.

Temperature above 100^0C (under pressure)

Autoclave

Steam above 100^0C or saturated steam has a better killing capacity than dry heat. Bacterial proteins coagulate rapidly at moist heat. Saturate steam has the ability to penetrate any porous material. When steam comes into contact with the cooler surface it condenses into water and releases its latent heat to the surface. The large reduction in volume sucks in more steam to the same site and the process continues until the temperature of the substance raised to that of steam. The condensed water produced moist conditions for killing the microbes present.

The autoclave is a modified pressure cooker which contains a vertical or horizontal cylinder. The cylinder is made up of stainless still. A lid on the cylinder is placed and fastened by screw clams to make it airtight. The lid contains a steam discharge unit, a pressure gauge, and a safety valve. Moreover, a thermostat is present to monitor the temperature. Heat is produced by electricity. At the time of sterilization, the cylinder is filled with an adequate amount of water and it is kept for some time for preheating. After that, the materials which are needed to be sterilized are inserted into the cylinder and the lid is then closed tightly. The temperature will increase eventually along with the pressure. When the temperature reaches 121.1^0C and the pressure at 15 psi the sterilization is performed for 15 minutes.

Uses: Sterilization of

- Culture media, rubber material, dressing gloves.
- Materials that are unable to withstand dry heat in a hot air oven.

Sterilization control

- Thermocouple
- Bacterial spores of Bacillus stearothermophilus used at test organisms.
- Browne's tubes contain red solution which turns into green when exposed to the specific temperature for 15 minutes in an autoclave.
- Autoclave tips.

Ozone

Ozone sterilizer uses oxygen, water, and electricity to produce ozone within the sterilizer and provide sterilization without producing toxic chemicals. It runs at 25-35^{0}C temperature. Inside this device, the oxygen is converted into atomic oxygen due to the intense electrical field. The atomic oxygen is then combined with the oxygen molecule to produce ozone. The ozone provides a sterility assurance of 10^{-6} in approximately 4 hours.

Filtration

This process is useful for sterilizing those materials which are unable to withstand heat. There are several types of filters such as

- *Candle filter*: Used for purification of water. These filters consist of hollow candles and water passes through the candles for purification.
- *Asbestos disc filters*: These are made up of magnesium silicate.
- *Sintered glass filters*: These are prepared by fusing finely powdered glass powders.
- *Membrane filters*: These are made up of cellulose esters and are used for water analysis, sterility testing and for the preparation of the solutions. Membrane filters are available in pore size 0.015 to 12 micron. The .22 micron filter is most commonly used as it is smaller than bacteria.
- *Air filters*: These filters are used in laminar airflow chambers to give bacteria-free air supply. These are also known as High-efficiency particulate air (HEPA) filters. These filters can separate particles of 0. Micron or larger.
- *Syringe filters*: Syringes fitted with the membrane of different diameters are available.

A limitation of using the filtration process is that the pores are not small enough for viruses.

Radiation

Ionizing radiation

Ionizing radiations such as gamma rays, X rays, and cosmic rays are used for sterilization process. Due to the high penetrating power, these radiations are lethal for cells. The bacterial cells are killed by damage in the DNA. Gamma radiations from a cobalt 60 source are commercially used for sterilization of disposable items. This procedure is also known as cold sterilization.

Non-ionizing radiation

Infrared radiation and UV radiation comes under this of radiation. Infrared radiation is used for mass sterilization of syringes and catheters. UV radiation with a wavelength of 240nm to 280nm has bactericidal capacity. The UV radiation causes protein denaturation and interferes with DNA replication of bacteria. UV radiations are used for sterilization of close areas, surfaces, operation theaters, laminar airflow, etc.

Chemical Method

Several chemical agents are used as antiseptic and disinfectants. The properties of a chemical antiseptic or disinfectant are following

- The chemical disinfectants need to have a broad spectrum of activity against all microorganisms such as bacteria, viruses, protozoa and fungi.
- The chemical agents should act in the presence of organic matter.
- High penetration power is an important property of the chemical agents
- The chemical agent needs to be chemically stable under both acidic and basic environments.
- The chemical substances should not have any corrosion activity in metals.
- The disinfectants are needed to be non-toxic if absorbed into circulation.
- Finally, the chemical agents are needed to be easily available and less expensive.

Alcohols

Ethyl alcohol and isopropyl alcohol are frequently used as chemical agents for disinfection. Both of the chemicals facilitate the protein denaturation of bacterial proteins. 70% ethyl alcohol is the standard concentration which is used for disinfection. These are used as skin antiseptics. Apart from this methyl alcohol has activity against fungal spores and used to disinfection of inoculation cabinets.

Aldehydes

Formaldehyde

It is known for its bactericidal, sporicidal and virucidal activities. It can be used in both aqueous and gaseous form. A 10% formalin solution is a standard chemical disinfectant. It is used for

- Prevention of tissues for histological examinations.
- Sterilization of bacterial vaccines
- Preparation of toxoids from toxins.

Glutaraldehyde

It has its activity against bacteria (*Mycobacterium tuberculosis),* fungi and viruses (including HIV, hepatitis B, etc). It can also kill spores and is known for its less toxic nature. It is used as a 2% buffered solution. Glutaraldehyde is used for

- Sterilization of cystoscopes, endoscopes, and bronchoscopes
- Sterilization of plastic endotracheal tubes, face masks, metal instruments, etc.

Orthophathalaldehyde

Orthophathalaldehyde (OPA) is a high-level disinfectant and is known for its stability during storage. It has bactericidal effects against mycobacteria. 0.5% OPA is slowly sporicidal and OPA vapors irritate the respiratory tract and eyes, therefore, it must be handled with appropriate safety.

Phenols

Lister (father of antiseptic surgery) used phenol for the first time in the sterilization of surgical instruments. Phenols work as a disinfectant and kill microorganisms by cell membrane damage. It is toxic for the skin. Different derivatives of phenol are used as antiseptics which are following

Cresols

An example of cresol is Lysol which is mostly used for sterilization of infected glassware, floors, etc.

Chlorhexidine

Savlon is an example of a chlorhexidine solution which is widely used in wounds, preoperative disinfection of the skin. It is bactericidal at high dilution. Moreover, it also has fungicidal activity.

Chloroxylenol

Dettol is commercially available as a chloroxylenol solution. It is less toxic and less irritant.

Hexachlorophene

It is bacteriostatic at very high dilution.

Halogens

Chlorine and iodine are commonly used disinfectants. Chlorine is used in water supplies, swimming pools, food, and dairy industries. Chlorine compounds in the form of bleaching powder, sodium hypochlorite, and chloramines. The disinfection action of all the chlorine compounds is due to the release of free chlorine which becomes a strong oxidative agent.

Iodine in alcoholic and aqueous solution is used as a skin disinfectant. It is active against *M tuberculosis* and slightly active against spores. Compounds with iodine with surface-active agents known as iodophors are claimed to be more active than aqueous or alcohol solution.

Oxidizing agents

Hydrogen peroxide

It is effective against most organisms in the concentration of 3-6 %. However, it kills spores at higher concentrations (10-25%). The mode of action is by the liberation of free hydroxyl radical on the decomposition of hydrogen peroxide. These free radicals are active ingredients in the disinfection process.

Peracetic acid

It is an oxidizing agent and is a more potent germicidal agent than hydrogen peroxide.

Salts

Slats of heavy metals have a toxic effect on bacteria. The salts of copper, silver, and mercury are used as a disinfectant. They are protein coagulant ant act by combining with sulfhydryl groups of bacterial proteins and other essential intracellular compounds. Merthiolate (sodium ethyl mercurithiosalicylate) is used in a dilution of 1:10000 for the preservation of sera.

Dyes

Two groups of dyes, aniline and acridine dyes have been used as a skin and wound antiseptics. Both the dyes have bacteriostatic activity. Aniline dyes include crystal violet, brilliant green, and malachite green. Acridine dyes include acriflavine, cuflavin, proflavine, and aminacrine.

Vapor phase Disinfectants

Ethylene Oxide (ETO)

It is a colourless liquid with a boiling point of 10.7^0C. It is effective against all types of microorganisms including viruses and spores. It acts by alkylating the amnio carboxyl, hydroxyl and sulfhydryl groups in protein molecules. In addition, it reacts with DNA and RNA. It is specially used for sterilizing plastic and rubber articles, respirators, heart-lung machines, dental equipment, etc.

Betapropilolactone (BPO)

This is a condensation product of ketane and formaldehyde. It has rapid action and used in0.2%. It is more efficient in fumigation than formaldehyde. BPO is used for the inactivation of vaccines.

UNIT II: Microbial Taxonomy and Metabolism

Microbial Species and Strains

- Microbial species are categorized based on genetic and phenotypic similarities. Strains are variations within a species.

Classification of Bacteria

- Morphology: Shape (cocci, bacilli, spirilla), arrangement (chains, clusters).
- Nutrition: Autotrophs (self-feeding), heterotrophs (feeding on others).
- Environment: Aerobes (require oxygen), anaerobes (do not require oxygen), facultative anaerobes, extremophiles.

Viruses

- General Characteristics: Non-cellular, require host cells to replicate.
- Transmission and Cultivation: Spread through direct contact, air, water, or vectors; cultivated in host cells.
- Examples:
    - Plant Viruses: Tobacco mosaic virus (TMV).
    - Animal Viruses: Newcastle disease virus (NDV).
    - Human Viruses: Human immunodeficiency virus (HIV).
    - Bacterial Viruses: T4 phage.
    - Emerging and Reemerging Viruses: Dengue virus.
    - Zoonotic Viruses: Rabies, SARS-CoV-2.

Microbial Production of Penicillin

- Penicillin is produced by the fungus *Penicillium chrysogenum* through fermentation processes.

Bacterial Toxins and Diseases

- Toxins: Substances produced by bacteria that can cause damage to the host (e.g., exotoxins, endotoxins).
- Diseases:
    - Tuberculosis: Caused by *Mycobacterium tuberculosis*.
    - Typhoid: Caused by *Salmonella typhi*.

Introduction to Fungi, Algae, and Cytoplasm

- Fungi: Eukaryotic organisms, can be unicellular (yeasts) or multicellular (Molds).
- Algae: Photosynthetic organisms found in aquatic environments.

- Cytoplasm: The gel-like substance within cells where cellular activities occurs.

Bacteria are classified and identified to distinguish one organism from another and to group similar organisms by criteria of interest to microbiologists or other scientists.

The classification of bacteria serves a variety of different functions. Because of this variety, bacteria may be grouped using many different typing schemes.

The grounds for the classification commonly used may be:

Interesting Science Videos

What If Ticks Were the Size of Humans?

Morphologic Characteristics

- Both wet-mounted and properly stained bacterial cell suspensions can yield a great deal of information.
- These simple tests can indicate the Gram reaction of the organism; whether it is acid-fast; its motility; the arrangement of its flagella; the presence of spores, capsules, and inclusion bodies; and, of course, its shape.
- This information often can allow identification of an organism to the genus level, or can minimize the possibility that it belongs to one or another group.

Growth Characteristics

- A primary distinguishing characteristic is whether an organism grows aerobically, anaerobically, facultatively (i.e., in either the presence or absence of oxygen), or micro aerobically (i.e., in the presence of a less than atmospheric partial pressure of oxygen). The proper atmospheric conditions are essential for isolating and identifying bacteria.
- Other important growth assessments include the incubation temperature, pH, nutrients required, and resistance to antibiotics. For example, one diarrheal disease agent, *Campylobacter jejuni*, grows well at 42° C in the presence of several antibiotics; another, *Y. enterocolitica*, grows better than most other bacteria at 4° C. *Legionella, Haemophilus,* and some other pathogens require specific growth factors, whereas *E. coli* and most other Enterobacteriaceae can grow on minimal media.

Antigens and Phage Susceptibility

- Cell wall (O), flagellar (H), and capsular (K) antigens are used to aid in classifying certain organisms at the species level, to serotype strains of medically important species for epidemiologic purposes, or to identify serotypes of public health importance.
- Serotyping is also sometimes used to distinguish strains of exceptional virulence or public health importance, for example with *V. cholerae* (O1 is the pandemic strain) and *E. coli* (enterotoxigenic, enter invasive, enterohemorrhagic, and enteropathogenic serotypes).
- Phage typing (determining the susceptibility pattern of an isolate to a set of specific bacteriophages) has been used primarily as an aid in epidemiologic surveillance of diseases caused by *Staphylococcus aureus*, mycobacteria, *P. aeruginosa, V. cholerae*, and *S. Typhi*. Susceptibility to

bacteriocins has also been used as an epidemiologic strain marker. In most cases recently, phage and bacteriocin typing have been supplanted by molecular methods.

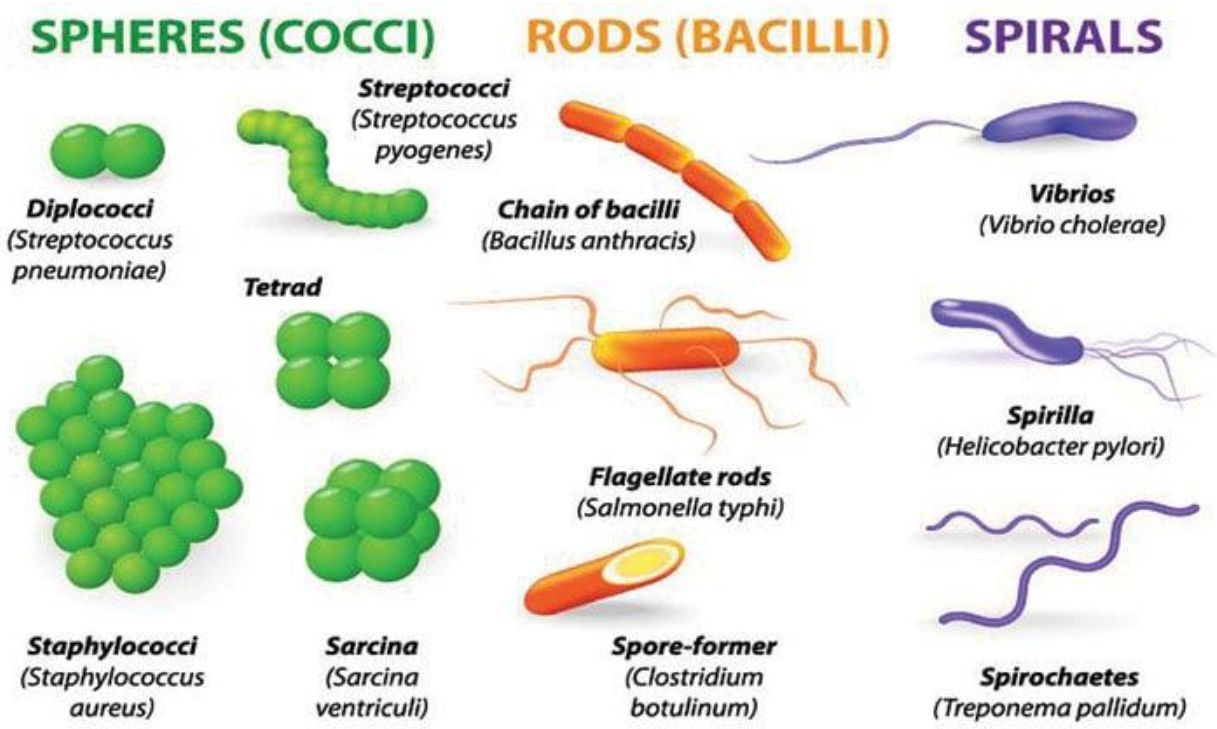
Biochemical Characteristics

- Most bacteria are identified and classified largely on the basis of their reactions in a series of biochemical tests.
- Some tests are used routinely for many groups of bacteria (oxidase, nitrate reduction, amino acid degrading enzymes, fermentation, or utilization of carbohydrates); others are restricted to a single family, genus, or species (coagulase test for staphylococci, pyrrolidonyl arylamidase test for Gram-positive cocci).

1. Classification on the basis of Gram Stain and Bacterial Cell Wall

- Of all the different classification systems, the Gram stain has withstood the test of time. Discovered by H.C. Gram in 1884 it remains an important and useful technique to this day.
- It allows a large proportion of clinically important bacteria to be classified as either **Gram positive or negative** based on their morphology and differential staining properties.
- Slides are sequentially stained with crystal violet, iodine, then destained with alcohol and counter-stained with safranin. Gram positive bacteria stain blue-purple and Gram-negative bacteria stain red.
- The difference between the two groups is believed to be due to a much larger peptidoglycan (cell wall) in Gram positives. As a result, the iodine and crystal violet precipitate in the thickened cell wall and are not eluted by alcohol in contrast with the Gram negatives where the crystal violet is readily eluted from the bacteria.
- As a result, bacteria can be distinguished based on their morphology and staining properties.
- Some bacteria such as mycobacteria are not reliably stained due to the large lipid content of the peptidoglycan. Alternative staining techniques (Kinyoun or acid-fast stain) are therefore used that take advantage of the resistance to destaining after lengthier initial staining.

2. Classification of Bacteria on the Basis of Shape



In the year 1872 scientist Cohn classified bacteria to 4 major types depending on their shapes are as follows:

A) Cocci: These types of bacteria are unicellular, spherical or elliptical shape. Either they may remain as a single cell or may aggregate together for various configurations. They are as follows:

- **Monococcus:** – they are also called micrococcus and represented by single, discrete round Example: *Micrococcus flavus.*
- **Diplococcus:** – the cell of the Diplococcus divides ones in a particular plane and after division, the cells remain attached to each other. Example: *Diplococcus pneumonia*.
- **Streptococcus:** – here the cells divide repeatedly in one plane to form chain of cells. Example: – *Streptococcus pyogenes*.
- **Tetra coccus:** – this consists of four round cells, which defied in two planes at a right angle to one another. Example: – *Gaffkya tetragena. Staphylococcus*: – here the cells divided into three planes forming a structured like bunches of grapes giving and irregular configuration. Example: – *Staphylococcus aureus*.
- **Sarcina:** -in this case the cells divide in three planes but they form a cube like configuration consisting of eight or sixteen cells but they have a regular shape. Example: –*Sarcina lutea*.

B) Bacilli: – These are rod shaped or cylindrical bacteria which either remain singly or in pairs. Example: –*Bacillus cereus*.

C) Vibro: – The vibro are the curved, comma shaped bacteria and represented by a single genus. Example: – *Vibro cholerae*.

D) Spirilla: – These types of bacteria are spiral or spring like with multiple curvature and terminal flagella. Example: –*Spirillum volutans*.

Others

Actinomycetes are branching filamentous bacteria, so called because of a fancied resemblance to the radiating rays of the sun when seen in tissue lesions (from actis meaning ray and mykes meaning fungus).

Mycoplasmas are bacteria that are cell wall deficient and hence do not possess a stable morphology. They occur as round or oval bodies and as interlacing filaments.

3. Classification of Bacteria on the Basis of Mode of Nutrition

1. **Phototrophs:**

- Those bacteria which gain energy from light.
- Phototrops are further divided into two groups on the basis of source of electron.
- **Photolithotrophs:** these bacteria gain energy from light and uses reduced inorganic compounds such as H2S as electron source. Eg. *Chromatium okenii.*
- **Photo organotrophs:** these bacteria gain energy from light and uses organic compounds such as succinate as electron source.

2. **Chemotrophs:**

- Those bacteria gain energy from chemical compounds.
- They cannot carry out photosynthesis.
- Chemotrops are further divided into two groups on the basis of source of electron.
- **Chemolithotrophs:** they gain energy from oxidation of chemical compound and reduces inorganic compounds such as NH3 as electron source. Eg. *Nitrosomonas.*
- **Chemoorganotrophs:** they gain energy from chemical compounds and uses organic compound such as glucose and amino acids as source of electron. eg. *Pseudomonas pseudoflava.*

3. **Autotrophs:**

- Those bacteria which uses carbondioxide as sole source of carbon to prepare its own food.
- Autotrophs are divided into two types on the basis of energy utilized to assimilate carbondioxide. ie. Photoautotrophs and chemoautotrophs.
- **Photoautotrophs:** they utilized light to assimilate CO2. They are further divided into two group on the basis of electron sources. Ie. **Photolithotropic autotrophs** and **Photoorganotropic autotrophs**
- **Chemoautotrophs:** They utilize chemical energy for assimilation of CO2.

4. **Heterotrophs:**

- Those bacteria which uses organic compound as carbon source.

- They lack the ability to fix CO2.
- Most of the human pathogenic bacteria are heterotropic in nature.
- Some heterotrops are simple, because they have simple nutritional requirement. However there are some bacteria that require special nutrients for their growth; known as fastidious heterotrophs.

4. Classification of Bacteria on the Basis of Temperature Requirement

Bacteria can be classified into the following major types on the basis of their temperatures response as indicated below:

1.Psychrophiles:

- Bacteria that can grow at 0°C or below but the optimum temperature of growth is 15 °C or below and maximum temperature is 20°C are called psychrophiles
- Psychrophiles have polyunsaturated fatty acids in their cell membrane which gives fluid nature to the cell membrane even at lower temperature.
- Examples: *Vibrio psychroerythrus, vibrio marinus, Polaromonas vacuolated, Psychroflexus.*

2. **Psychrotrops (facultative psychrophiles):**

- Those bacteria that can grow even at 0°C but optimum temperature for growth is (20-30)°C

3. **Mesophiles:**

- Those bacteria that can grow best between (25-40)^{o} C but optimum temperature for growth is 37C
- Most of the human pathogens are mesophilic in nature.
- Examples: *E. coli, Salmonella, Klebsiella, Staphylococci.*

4. **Thermophiles:**

- Those bacteria that can best grow above 45C.
- Thermophiles capable of growing in mesophilic range are called facultative thermophiles.
- True thermophiles are called as Stenothermophiles, they are obligate thermophiles,
- Thermophils contains saturated fattyacids in their cell membrane so their cell membrane does not become too fluid even at higher temperature.
- Examples: *Streptococcus thermophiles, Bacillus stearothermophilus, Thermus aquaticus.*

5. **Hypethermophiles:**

- Those bacteria that have optimum temperature of growth above 80C.
- Mostly Archeobacteria are hyperthermophiles.
- Monolayer cell membrane of Archeobacteria is more resistant to heat and they adopt to grow in higher remperature.

- Examples: *Thermodesulfobacterium, Aquifex, Pyrolobus fumari, Thermotoga.*

5. Classification of Bacteria on the Basis of Oxygen Requirement

Obligate Aerobes:

- Require oxygen to live.
- Example: *Pseudomonas,* common nosocomial pathogen.

Facultative Anaerobes:

- Can use oxygen, but can grow in its absence.
- They have complex set of enzymes.
- Examples: *E. coli, Staphylococcus*, yeasts, and many intestinal bacteria.

Obligate Anaerobes:

- Cannot use oxygen and are harmed by the presence of toxic forms of oxygen.
- Examples: *Clostridium* bacteria that cause tetanus and botulism.

Aerotolerant Anaerobes:

- Cannot use oxygen, but tolerate its presence.
- Can break down toxic forms of oxygen.
- Example: *Lactobacillus* carries out fermentation regardless of oxygen presence.

Microaerophiles:

- Require oxygen, but at low concentrations.
- Sensitive to toxic forms of oxygen.
- Example: *Campylobacter*.

6. Classification of Bacteria on the Basis of pH of Growth

1. **Acidophiles:**

- These bacteria grow best at an acidic pH.
- The cytoplasm of these bacteria are acidic in nature.
- Some acidopiles are thermophilic in nature, such bacteria are called Thermoacidophiles.
- Examples: *Thiobacillus thioxidans, Thiobacillus, ferroxidans, Thermoplasma, Sulfolobus*

2. **Alkaliphiles:**

- These bacteria grow best at an alkaline pH.
- Example: *Vibrio cholerae* optimum ph of growth is 8.2.

3. **Neutrophiles:**

- These bacteria grow best at neutral pH (6.5-7.5).
- Most of the bacteria grow at neutral pH.
- Example: *E. coli*

7. Classification of Bacteria on the Basis of Osmotic Pressure Requirement

Halophiles:

- Require moderate to large salt concentrations.
- Cell membrane of halophilic bacteria is made up of glycoprotein with high content of negatively charged glutamic acid and aspartic acids. So high concentration of Na+ ion concentration is required to shield the –ve charge.
- Ocean water contains 3.5% salt. Most such bacteria are present in the oceans.
- *Archeobacteria, Halobacterium, Halococcus.*

Extreme or Obligate Halophiles:

- Require a very high salt concentrations (20 to 30%).
- Bacteria in Dead Sea, brine vats.

Facultative Halophiles:

- Do not require high salt concentrations for growth, but tolerate upto 2% salt or more.

8. Classification of Bacteria on the Basis of Number of Flagella

On the basis of flagella the bacteria can be classified as:

1. **Atrichos: –** These bacteria has no flagella. Example: *Corynebacterium diptherae*.
2. **Monotrichous:** – One flagellum is attached to one end of the bacteria cell. Example: – *Vibro cholerae*.
3. **Lophotrichous: –** Bunch of flagella is attached to one end of the bacteria cell.
   Example: *Pseudomonas.*
4. **Amphitrichous:** – Bunch of flagella arising from both end of the bacteria cell.
   Example: *Rhodospirillum rubrum.*
5. **Peritrichous :** – The flagella are evenly distributed surrounding the entire bacterial cell.
   Example: *Bacillus.*

9. Classification of Bacteria on the basis of Spore Formation

1. **Spore forming bacteria:**

- Those bacteria that produce spore during unfavorable condition.

- These are further divided into two groups:

i) Endospore forming bacteria: Spore is produced within the bacterial cell.

Examples. *Bacillus, Clostridium, Sporosarcina* etc

ii) Exospore forming bacteria: Spore is produced outside the cell.

Example. *Methylosinus*

2. **Non sporing bacteria:**

- Those bacteria which do not produce spores.

Eg. *E. coli, Salmonella.*

UNIT III: Cell Structure and Functions

Cellular Organelles

- Endoplasmic Reticulum (ER): Network of membranes involved in protein and lipid synthesis.
- Golgi Bodies: Modify, sort, and package proteins and lipids for transport.
- Mitochondria: Powerhouse of the cell, site of ATP production.
- Ribosomes: Sites of protein synthesis.
- Vacuoles: Storage organelles for nutrients, waste products, and other materials.

Cell Cycle and Cell Division

- Mitosis: Division of a eukaryotic cell's nucleus into two genetically identical nuclei.
- Meiosis: Division that results in four non-identical daughter cells, each with half the number of chromosomes of the parent cell.

Cell Membrane

- Composed of a phospholipid bilayer with embedded proteins; dynamic and selectively permeable.

Cell Signalling and Communication

- Cells communicate through signalling molecules and receptors, coordinating various physiological processes.

Endocytic Pathways

- Processes by which cells internalize substances from their environment (e.g., phagocytosis, pinocytosis).

Cell organelles are specialized entities present inside a particular type of cell that performs a specific function.

There are various cell organelles, out of which, some are common in most types of cells like cell membranes, nucleus, and cytoplasm. However, some organelles are specific to one particular type of cell- like plastids and cell walls in plant cells.

Cell Organelles

Structure and Functions with diagram

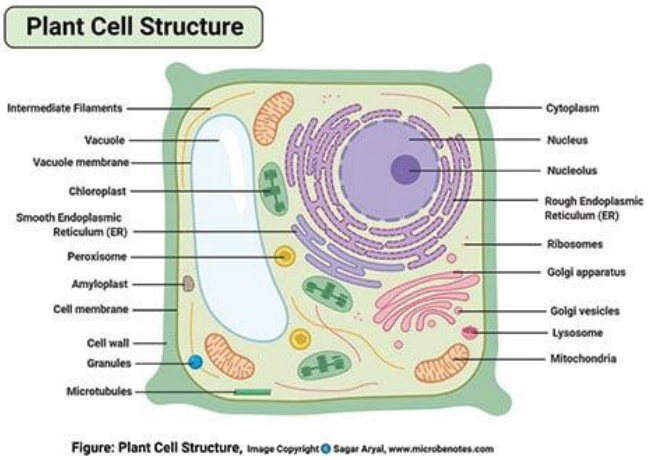


Figure: Plant Cell Structure, Image Copyright © Sagar Aryal, www.microbenotes.com

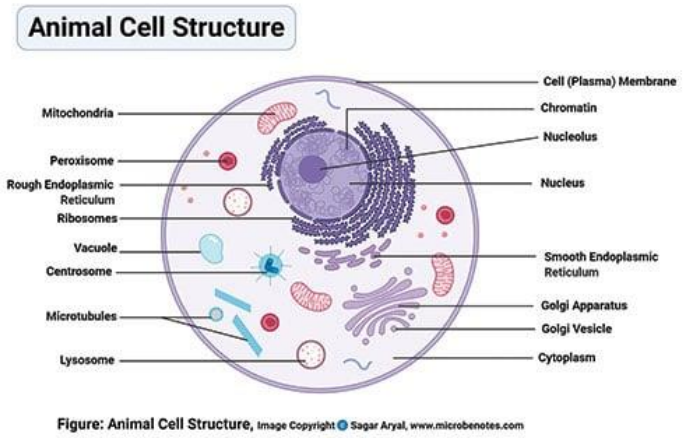


Figure: Animal Cell Structure, Image Copyright © Sagar Aryal, www.microbenotes.com

List of Cell Organelles

1. Cell membrane (Plasma membrane/ Plasmalemma)
2. Cell Wall
3. Centriole
4. Cilia and Flagella
5. Chloroplast
6. Cytoplasm
7. Cytoskeleton
8. Endoplasmic Reticulum (ER)
9. Endosomes
10. Golgi Apparatus/ Golgi Complex/ Golgi Body
11. Intermediate filaments
12. Lysozyme
13. Microfilaments
14. Microtubules

15. Microvilli
16. Mitochondria
17. Nucleus
18. Peroxisomes
19. Plasmodesmata
20. Plastids
21. Ribosomes
22. Storage granules
23. Vacuole
24. Vesicles

Cell membrane (Plasma membrane/ Plasmalemma)

A plasma membrane is composed of lipids and proteins where the composition might fluctuate based on fluidity, external environment, and the different stages of development of the cell.

Structure of Cell Membrane

- Structurally, it consists of a phospholipid bilayer along with two types of proteins viz. embedded proteins and peripheral proteins that function in providing shape and allowing the movement of particles in and out of the cell.
- The most abundant lipid which is present in the cell membrane is a phospholipid that contains a polar head group attached to two hydrophobic fatty acid tails.
- The embedded proteins act as channels for the transfer of particles across the cell with some proteins acting as receptors for the binding of various components.
- The peripheral proteins function as to provide fluidity as well as mechanical support to the structure of the cell.

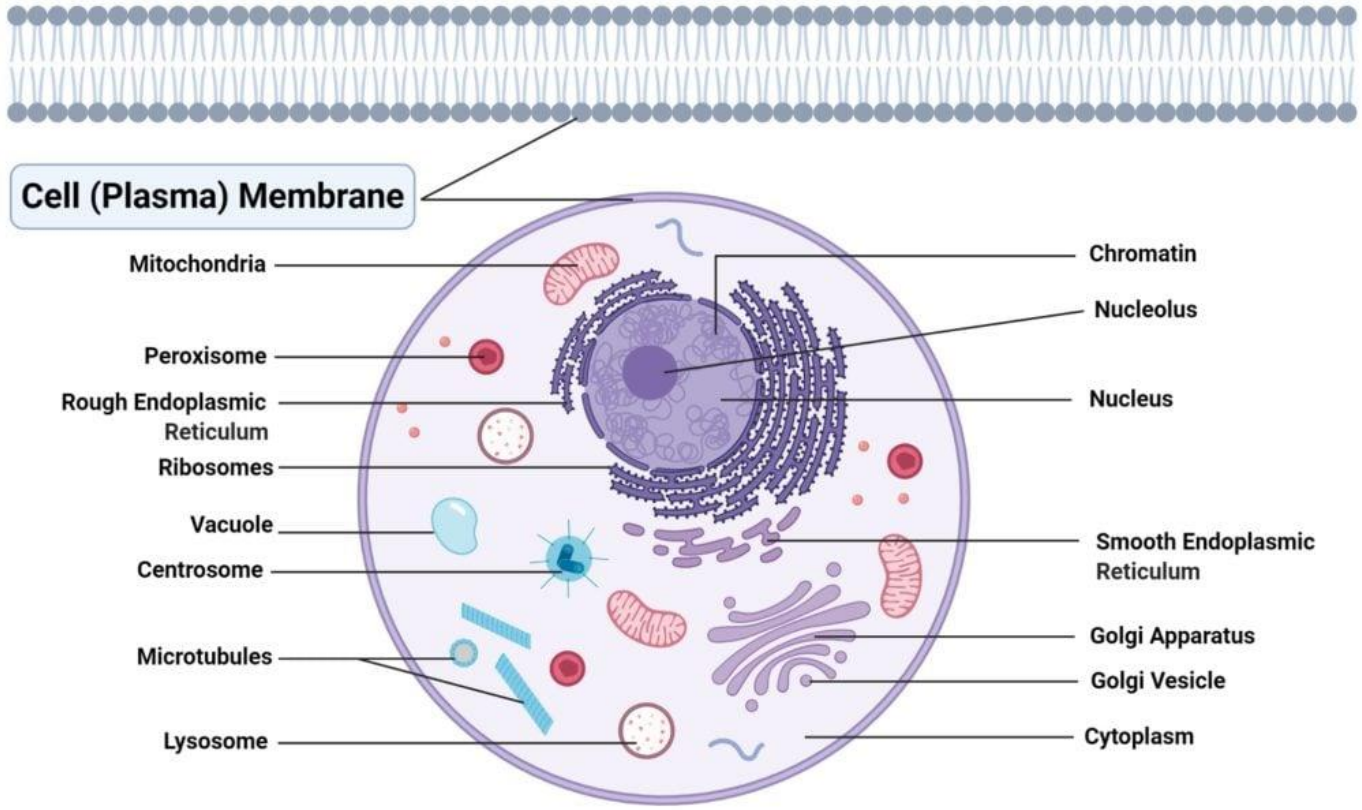


Figure: Animal Cell Structure with Cell (Plasma) Membrane, Image Copyright © Sagar Aryal, www.microbenotes.com

Functions of Cell Membrane

- The cell membrane provides mechanical support that facilities the shape of the cell while enclosing the cell and its components from the external environment.
- It regulates what can be allowed to enter and exit the cell through channels, acting as a semi-permeable membrane, which facilities the exchange of essential compounds required for the survival of the cell.
- It generates and distributes signals in and outside of the cell for the proper functioning of the cell and all the organelles.
- It allows the interaction between cells required during tissue formation and cell fusion.

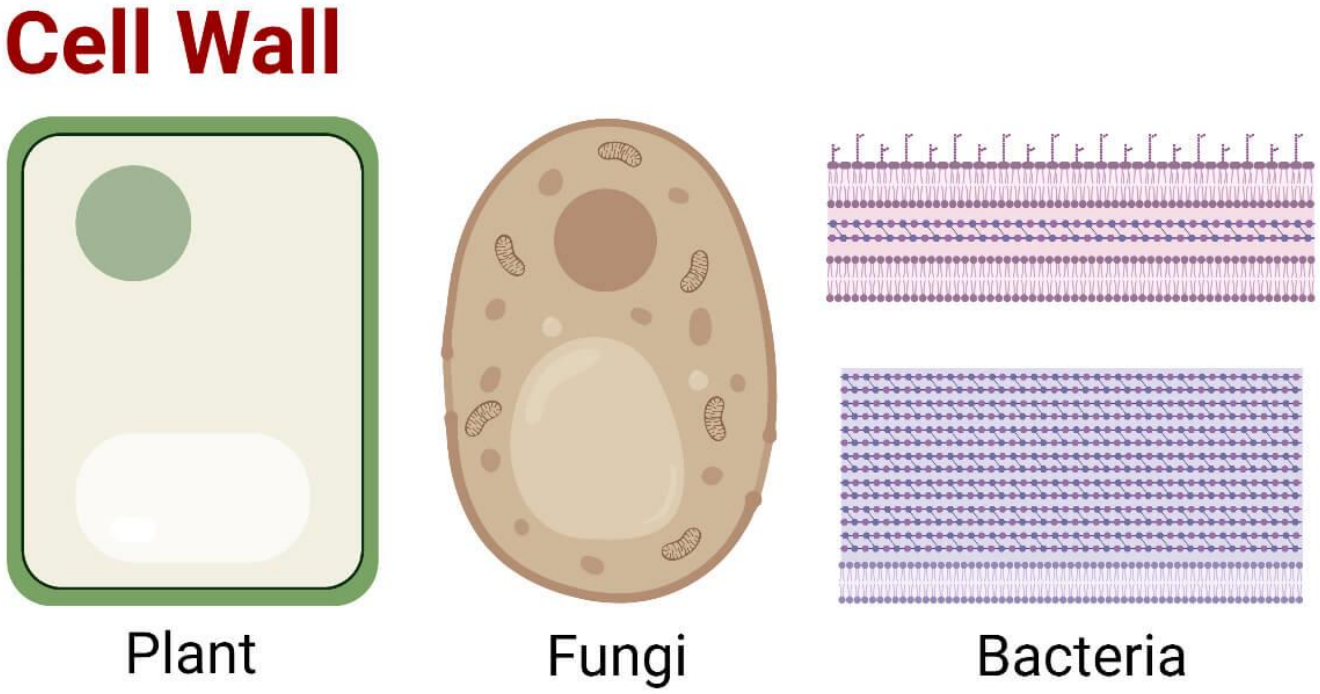
Cell Wall

An additional non-living layer present outside the cell membrane in some cells that provides structure, protection, and filtering mechanism to the cell is the cell wall.

Structure of Cell Wall

- In a plant cell, the cell wall is made up of cellulose, hemicellulose, and proteins while in a fungal cell, it is composed of chitin.
- A cell wall is multilayered with a middle lamina, a primary cell wall, and a secondary cell wall.

- The middle lamina contains polysaccharides that provide adhesion and allow binding of the cells to one another.
- After the middle lamina is the primary cell wall which is composed of cellulose. The last layer, which is not always present, is the secondary cell wall made of cellulose and hemicellulose.



Cell Wall (Plant, Fungal, Bacterial)

Functions of Cell Wall

- The critical function of the cell wall is protecting and maintaining the shape of the cell. It also helps the cell withstand the turgor pressure of the cell.
- It initiates cell division by providing signals to the cell and allows the passage of some molecules into the cell while blocking others.

Centriole

Centrioles are tubular structures mostly found in eukaryotic cells which are composed mainly of the protein tubulin.

Structure of Centriole

- A centriole consists of a cylindrical structure made with nine triplets microtubules that surround the periphery of the centriole while the center has a Y-shaped linker and a barrel-like structure that stabilizes the centriole.
- Another structure called cartwheel is present in a centriole which is made up of a central hub with nine spokes/filaments radiating from it. Each of these filaments/spokes is connected to the microtubules through a pinhead.

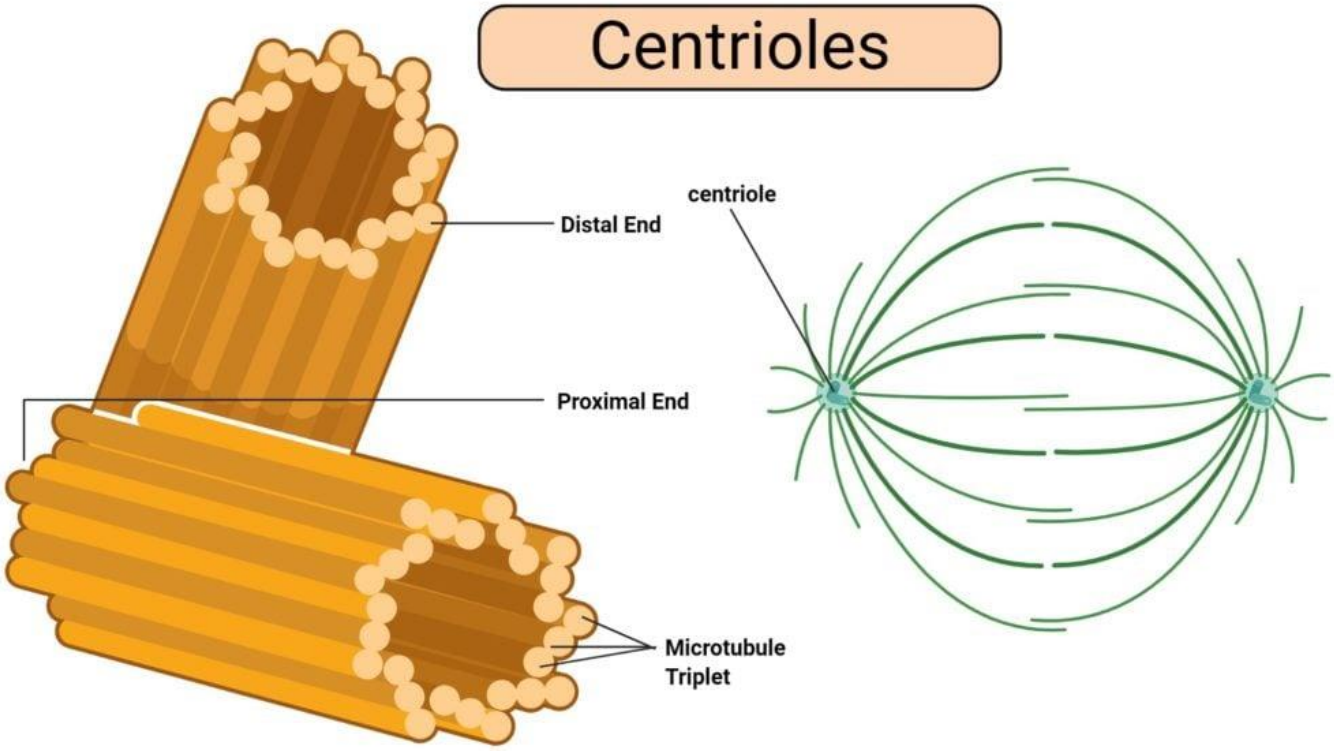


Figure: Centrioles, Image Copyright © Sagar Aryal, www.microbenotes.com

Functions of Centriole

- During cell division, centrioles have a crucial role in forming spindle fibers which assist the movement of chromatids towards their respective sides.
- They are involved in the formation of cilia and flagella.

Cilia and Flagella

Cilia and Flagella are tiny hair-like projections from the cell made of microtubules and covered by the plasma membrane.

Structure of Cilia and Flagella

- Cilia are hair-like projections that have a 9+2 arrangement of microtubules with a radial pattern of 9 outer microtubule doublet that surrounds two singlet microtubules. This arrangement is attached to the bottom with a basal body.
- Flagella is a filamentous organelle, the structure of which, is different in prokaryotes and eukaryotes.
- In prokaryotes, it is made up of the protein called flagellin wrapped around in a helical manner creating a hollow structure at the center throughout the length.
- In eukaryotes, however, the protein is absent and the structure is replaced with microtubules.

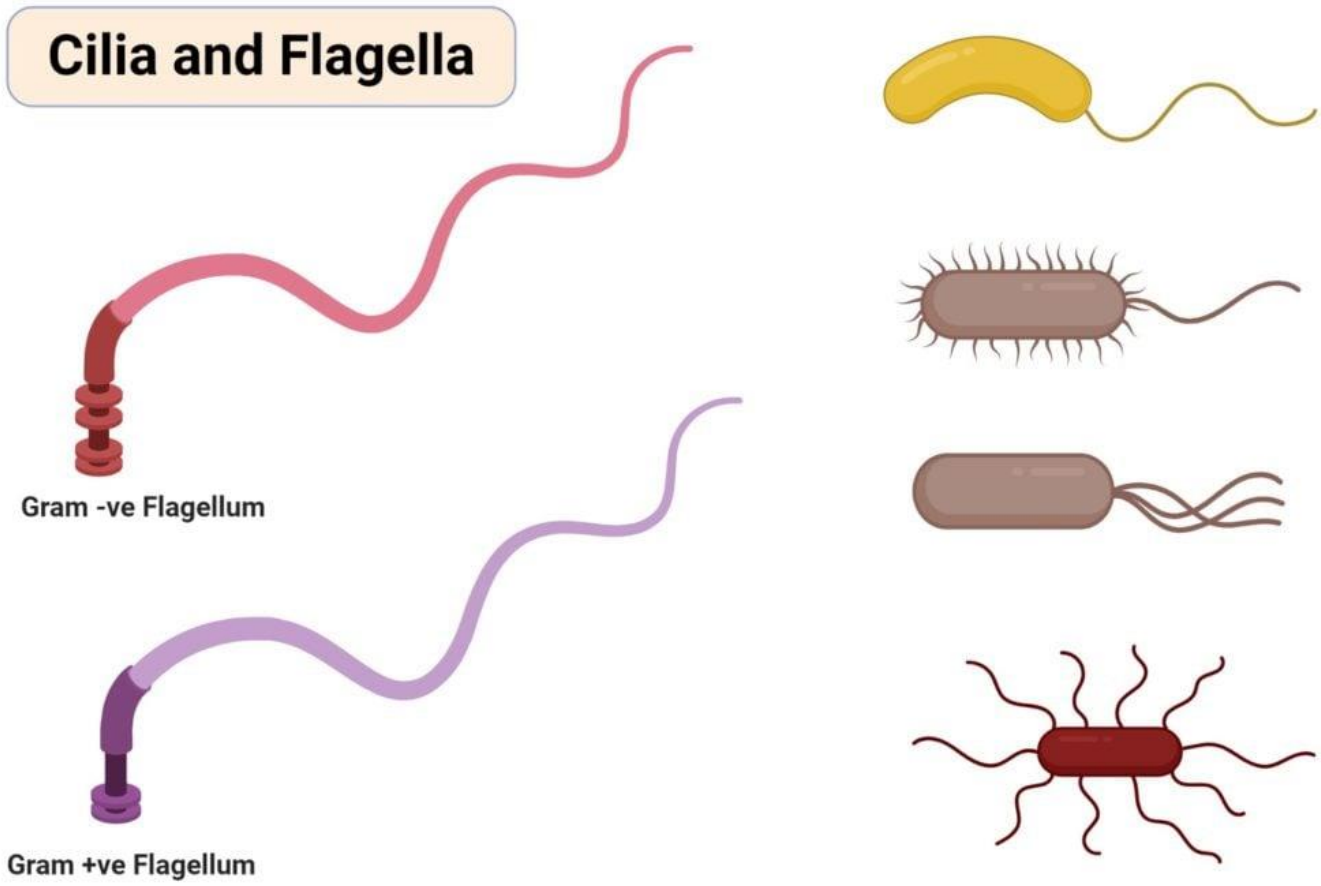


Figure: Cilia and Flagella, Image Copyright © Sagar Aryal, www.microbenotes.com

Functions of Cilia and Flagella

- The most critical role of cilia and flagella is movement. These are responsible for the movement of the organisms as well as for the movement of various particles present around the organisms.
- Some cilia present in some organs may have the function of sense. The cilium in the blood vessels, which helps in controlling the flow of blood is an example.

Chloroplast

A chloroplast is a type of plastic that is involved in photosynthesis in plants and algae. Chloroplast contains an essential pigment called chlorophyll necessary to trap sunlight to produce glucose.

Structure of Chloroplast

- It is a double-membraned structure with its own DNA which is inherited from the previous chloroplast.
- These are usually lens-shaped with shape and number varying according to cells. They have an outer membrane, an inner membrane, and a thylakoid membrane that enclosed the gel-like matric called the stroma.

- The outer and inner membrane is porous and allows transport of materials while the stroma contains DNA, chloroplast ribosomes, proteins, and starch granules.

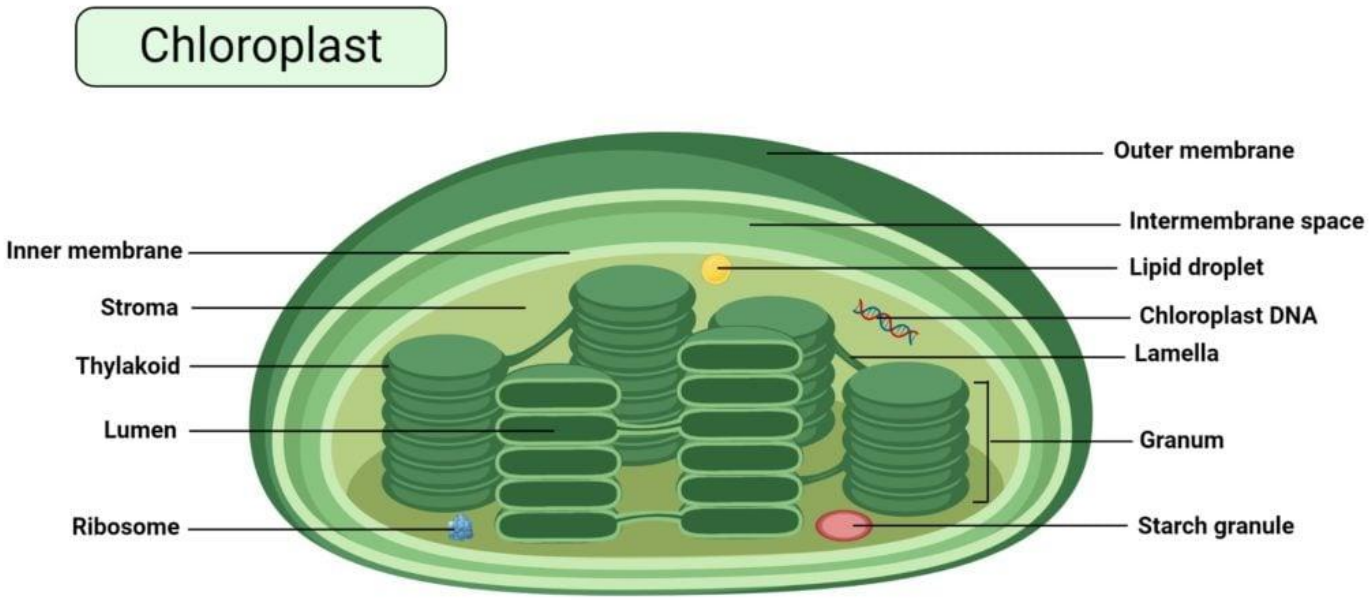


Figure: Chloroplast, Image Copyright © Sagar Aryal, www.microbenotes.com

Functions of Chloroplast

- The chloroplast is the primary center for light-dependent and light-independent reactions during photosynthesis.
- Different proteins present in chlorophyll are involved in the regulation of photorespiration.

Cytoplasm

Cytoplasm refers to everything present inside the cell except the nucleus.

Structure of Cytoplasm

- The cytoplasm consists of a cytosol; a gel-like substance that contains other matter; cell organelles; smaller cell-like bodies bound by separate membranes; and cytoplasmic inclusions; insoluble molecules that store energy and are not surrounded by any layer.
- The cytoplasm is colorless and has about 80% water along with various nutrients required for the cell.
- It is known to have the properties of both viscous matter as well as elastic matter. Under its elasticity, cytoplasm helps in the movement of materials inside the cell by a process termed cytoplasmic streaming.

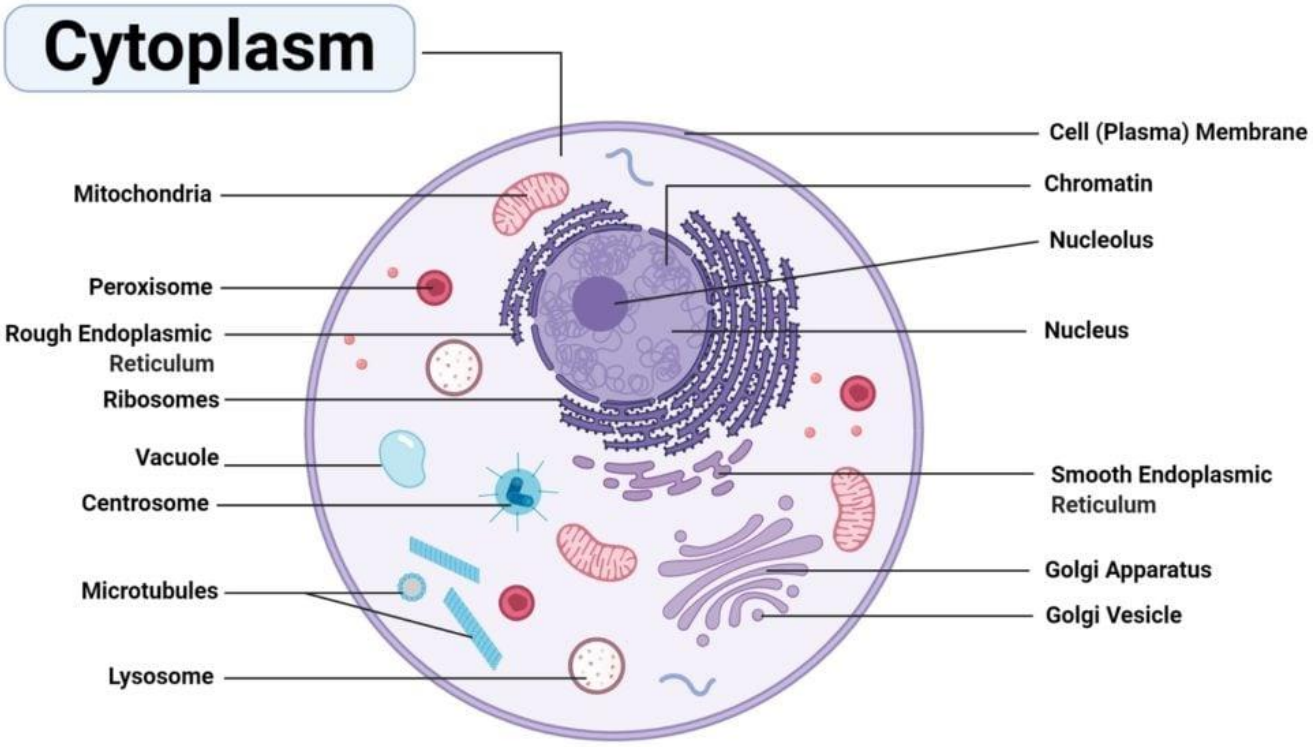


Figure: Animal Cell Structure with Cytoplasm, Image Copyright © Sagar Aryal, www.microbenotes.com

Functions of Cytoplasm

- Most of the vital cellular and enzymatic reactions like cellular respiration and translation of mRNA into proteins occur in the cytoplasm.
- It acts as a buffer and protects genetic materials as well as other organelles from damage due to collision or change in the pH of the cytosol.
- The process called cytoplasmic streaming helps in the distribution of various nutrients and facilitates the movement of cell organelles within the cell.

Cytoskeleton

A number of fibrous structures are present in the cytosol that helps give shape to the cell while supporting cellular transport.

Structure of Cytoskeleton

- Around three different classes of fibers make up the cytoskeleton which is: microtubules, microfilaments, and intermediate filaments.
- These are separated based on a protein present in them.

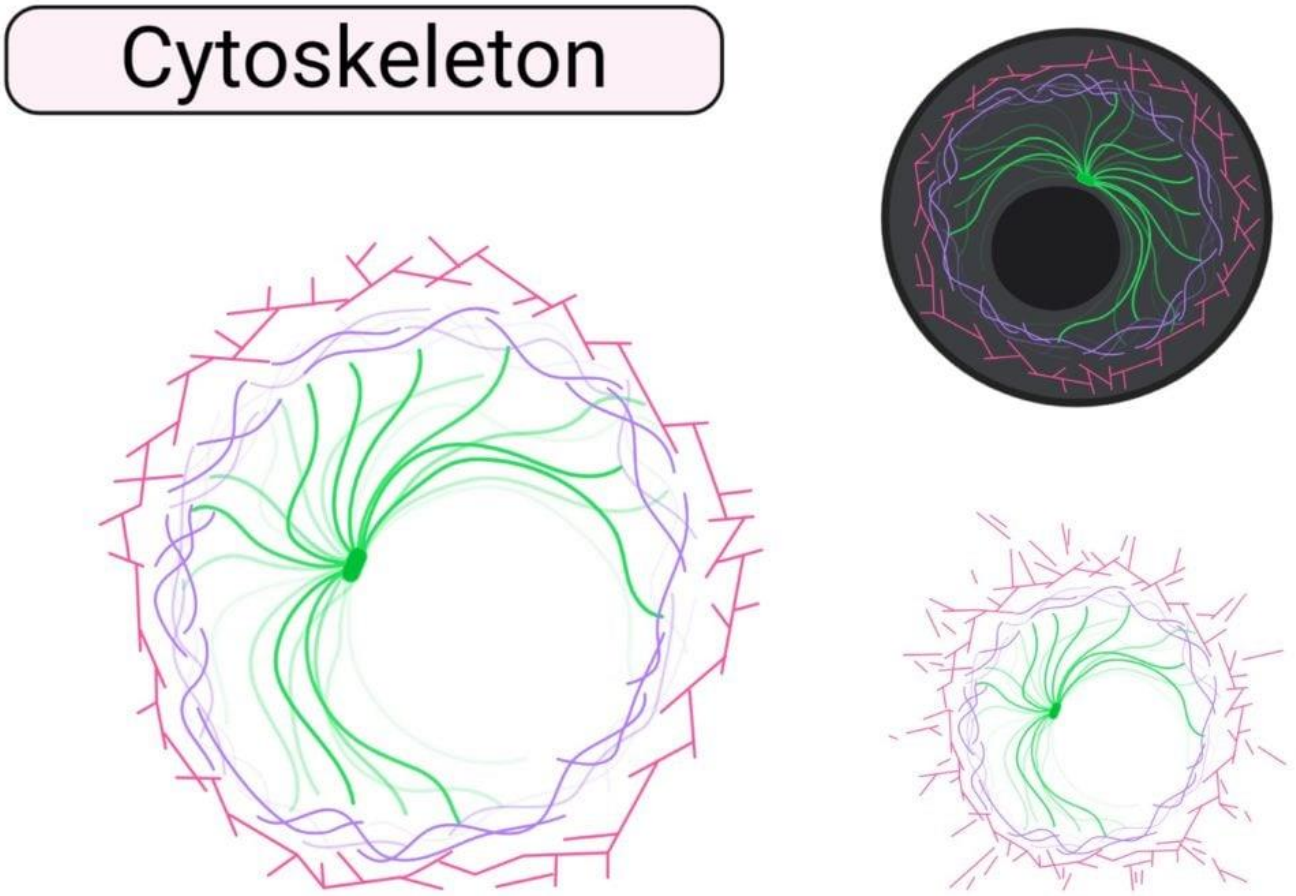


Figure: Cytoskeleton, Image Copyright © Sagar Aryal, www.microbenotes.com

Functions of Cytoskeleton

- The critical function of the cytoskeleton is to provide shape and mechanical support to the cell against deformation.
- It allows the expansion and contraction of the cell which assists in the movement of the cell.
- It is also involved in the intracellular and extracellular transport of materials.

Endoplasmic Reticulum (ER)

Endoplasmic Reticulum (ER) is present as an interconnection of tubules that are connected to the nuclear membrane in eukaryotic cells.

There are two types of ER based on the presence or absence of ribosomes on them:

- Rough ER (RER) with ribosomes attached on the cytosolic face of Endoplasmic Reticulum and thus is involved in protein synthesis
- Smooth ER (SER) lacks ribosomes and has a function during lipid synthesis.

Structure of Endoplasmic Reticulum (ER)

- Endoplasmic Reticulum exists in three forms viz. cisternae, vesicles, and tubules.
- Cisternae are sac-like flattened; unbranched structures that remain stacked one on top of another.
- Vesicles are spherical structures that carry proteins throughout the cell.
- Tubules are tubular branched structures forming a connection between cisternae and vesicles.

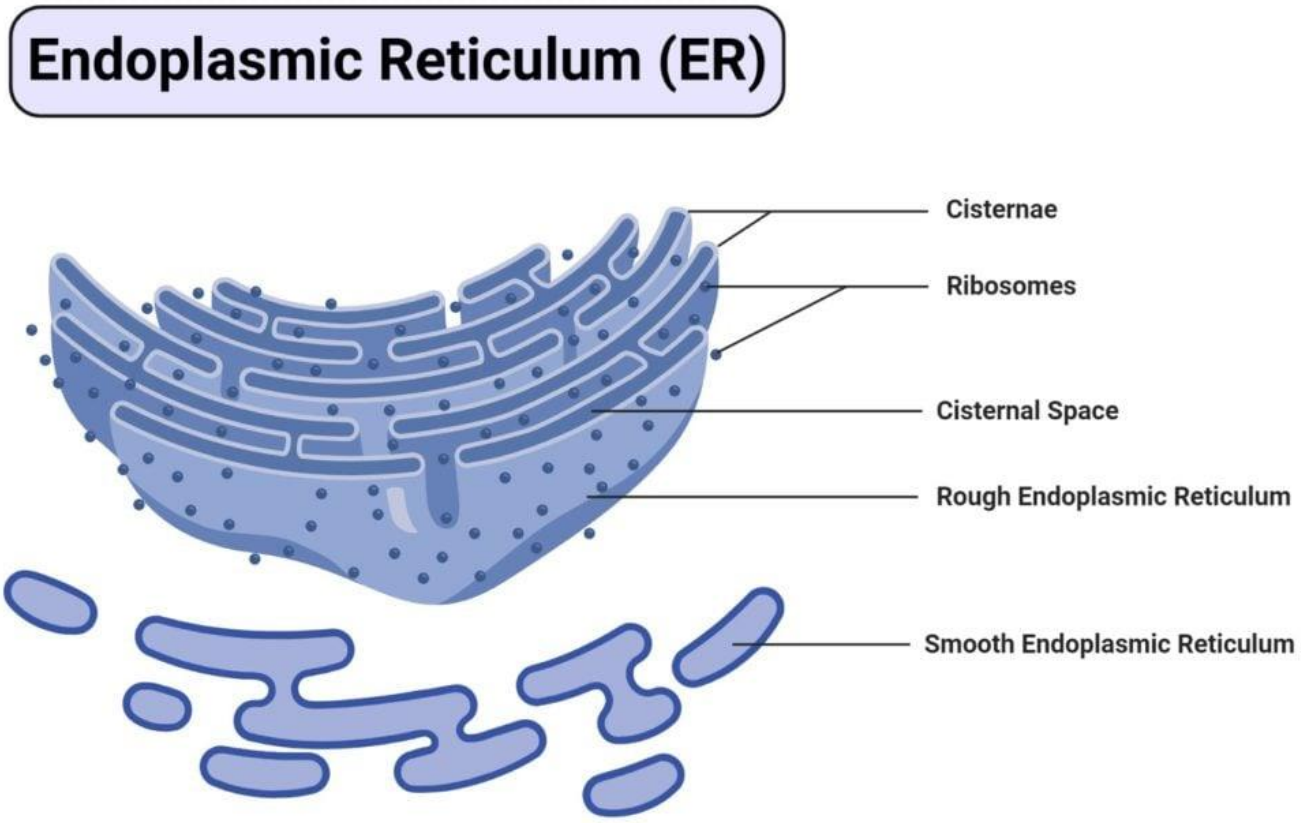


Figure: Endoplasmic Reticulum (ER), Image Copyright © Sagar Aryal, www.microbenotes.com

Functions of Endoplasmic Reticulum (ER)

- ER contains many of the enzymes required for several metabolic processes, and the surface of the ER is essential for other operations like diffusion, osmosis, and active transport.
- One of the crucial functions of ER is the synthesis of lipids like cholesterol and steroids.
- Rough ER allows for the modification of polypeptides emerging out of the ribosomes to prepare secondary and tertiary structures of the protein.
- ER also synthesizes various membrane proteins and has a crucial role in preparing the nuclear envelope after cell division.

Endosomes

Endosomes are membrane-bound compartments within a cell originating from the Golgi network

Structure of Endosomes

- There are different types of endosomes based on morphology and the time it takes for the endocytosed materials to reach them.
- The early endosomes are made with the tubular-vesicular network while the late endosomes lack tubules but contain many close-packed intraluminal vesicles. The recycling endosomes are found with microtubules and are mainly composed of tubular structures.

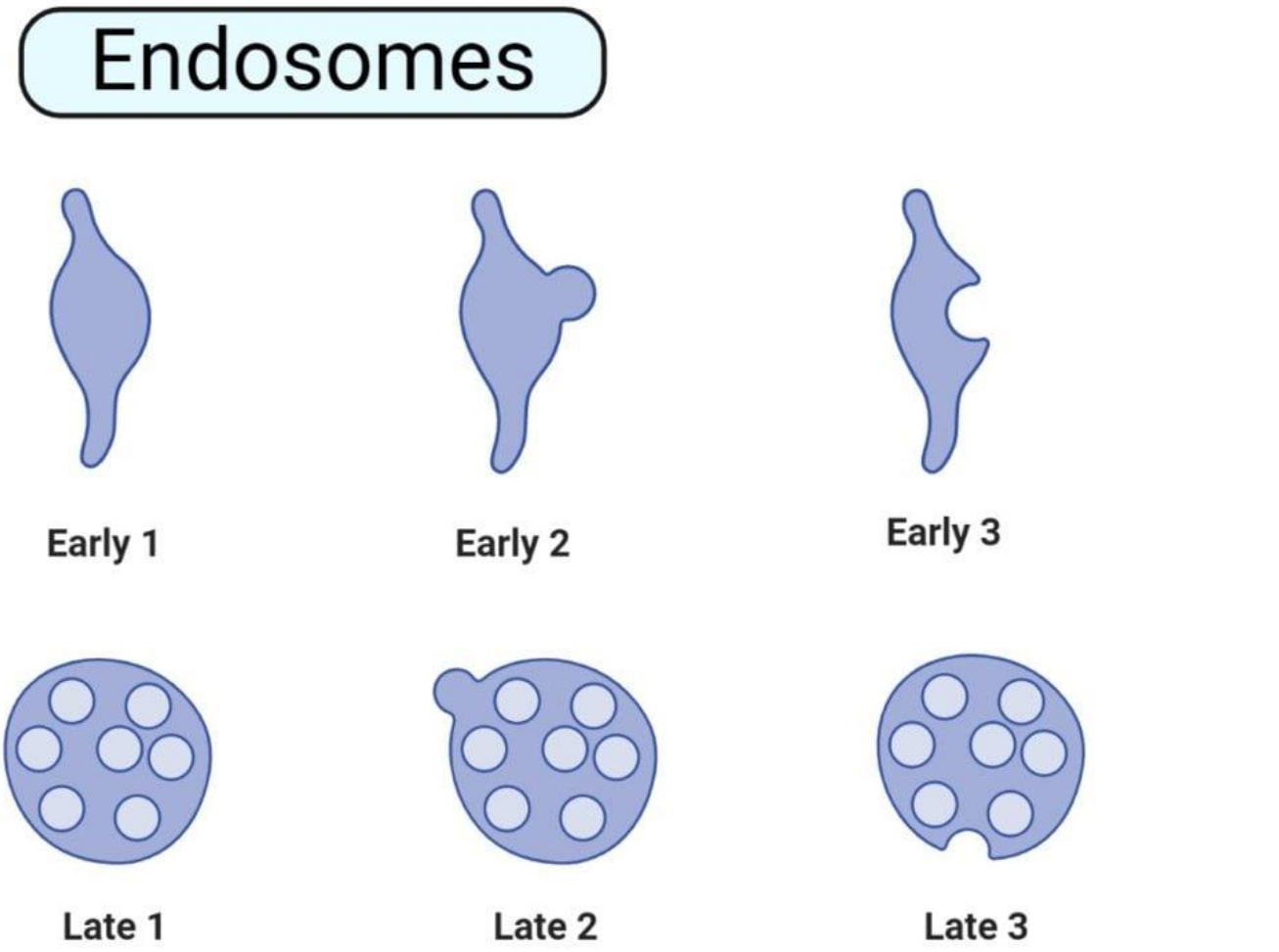


Figure: Endosomes, Image Copyright © Sagar Aryal, www.microbenotes.com

Functions of Endosomes

- Endosomes allow the sorting and delivery of internalized materials from the cell surface and transport of materials to the Golgi or the lysosomes.

Golgi Apparatus/ Golgi Complex/ Golgi Body

The Golgi Apparatus is the cell organelle mostly present in eukaryotic cells which is responsible for the packaging of macromolecules into vesicles so that they can be sent out to their site of action.

Structure of Golgi Apparatus

- The structure of the Golgi Complex is pleomorphic; however, it typically exists in three forms, i.e. cisternae, vesicles, and tubules.
- The cisternae, which is the smallest unit of the Golgi Complex, has a flattened sac-like structure that is arranged in bundles in a parallel fashion.
- Tubules are present as tubular and branched structures that radiate from the cisternae and are fenestrated at the periphery.
- Vesicles are spherical bodies that are divided into three groups as transitional vesicles, secretory vesicles, and clathrin-coated vesicles.

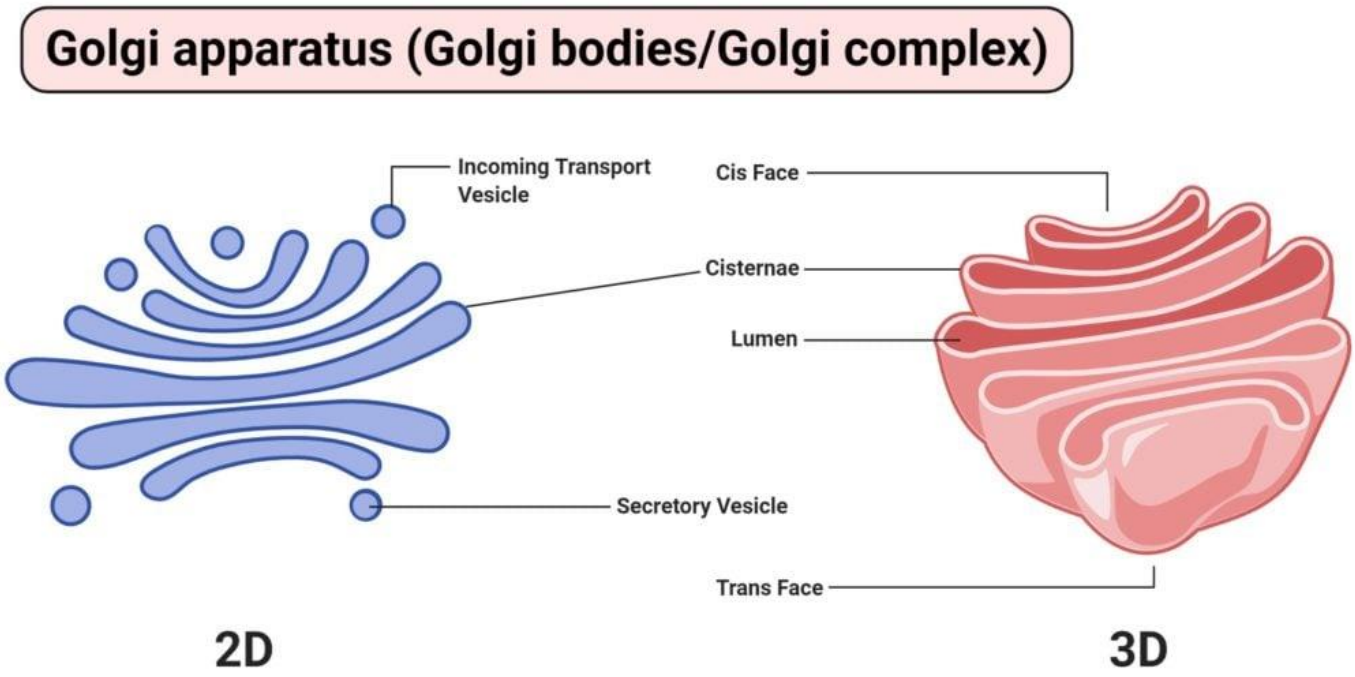


Figure: Golgi apparatus (Golgi bodies/Golgi complex), Image Copyright © Sagar Aryal, www.microbenotes.com

Functions of Golgi Apparatus

- Golgi Complex has an essential purpose of directing proteins and lipids to their destination and thus, act as the "traffic police" of the cell.
- They are involved in the exocytosis of various products and proteins like zymogen, mucus, lactoprotein, and parts of the thyroid hormone.
- Golgi Complex is involved in the synthesis of other cell organelles like a cell membrane, lysozymes, among others.
- They are also involved in the sulfation of various molecules.

Intermediate filaments

The third class of filament that makes up the cytoskeleton is the intermediate filaments. They are designated as intermediate filaments because of the intermediate diameter of the filaments as compared to microfilaments and myosin proteins.

Structure of Intermediate filaments

- Intermediate filaments contain a family of related proteins.
- The individual filaments are coiled around each other in a helical structure called coiled-coil structure.

Functions of Intermediate filaments

- Intermediate filaments contribute to the structural integrity of a cell while playing a crucial role in holding tissues of various organs like the skin.

Lysozyme

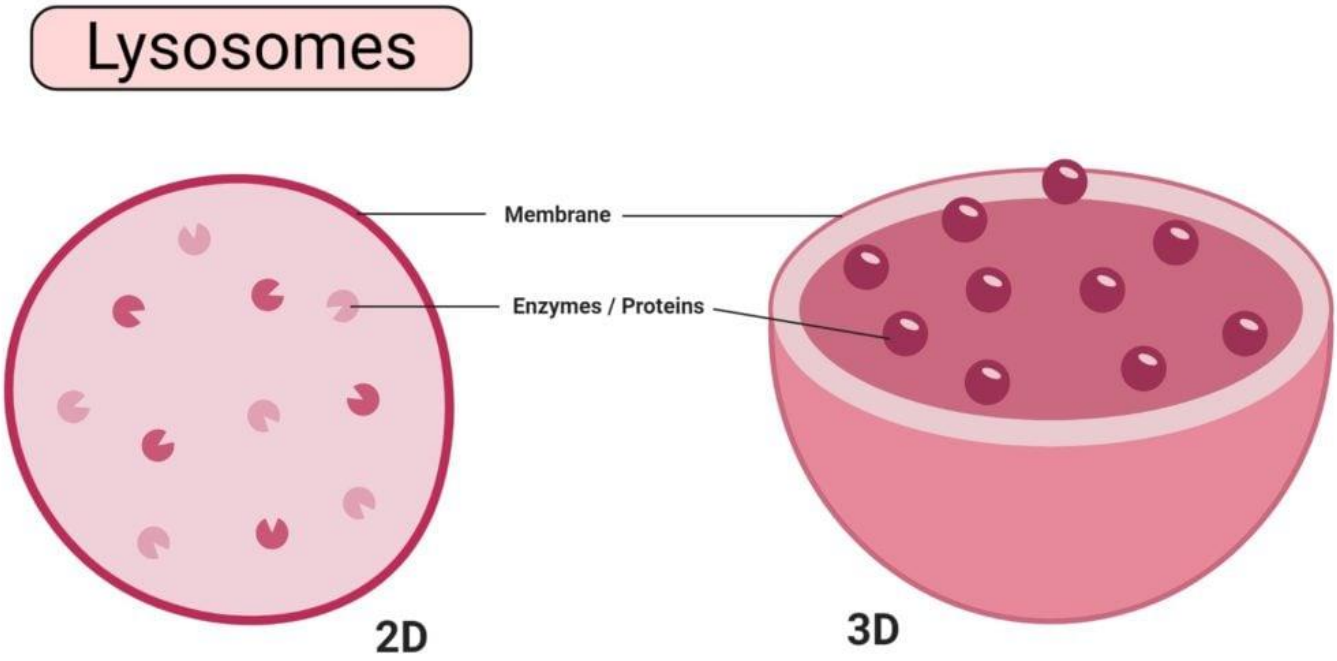
Lysozymes are membrane-bound organelles that occur in the cytoplasm of animal cells. These organelles contain an array of hydrolytic enzymes required for the degradation of various macromolecules.

There are two types of lysozymes:

- **Primary lysozyme** containing hydrolytic enzymes like lipases, amylases, proteases, and nucleases.
- **Secondary lysozyme** formed by the fusion of primary lysozymes containing engulfed molecules or organelles.

Structure of Lysozyme

- The shape of lysozymes is irregular or pleomorphic; however, mostly, they are found in spherical or granular structures.
- Lysozymes are surrounded by a lysosomal membrane that contains the enzymes within the lysosome and protects the cytosol with the rest of the cell from the harmful action of the enzymes.



Functions of Lysozyme

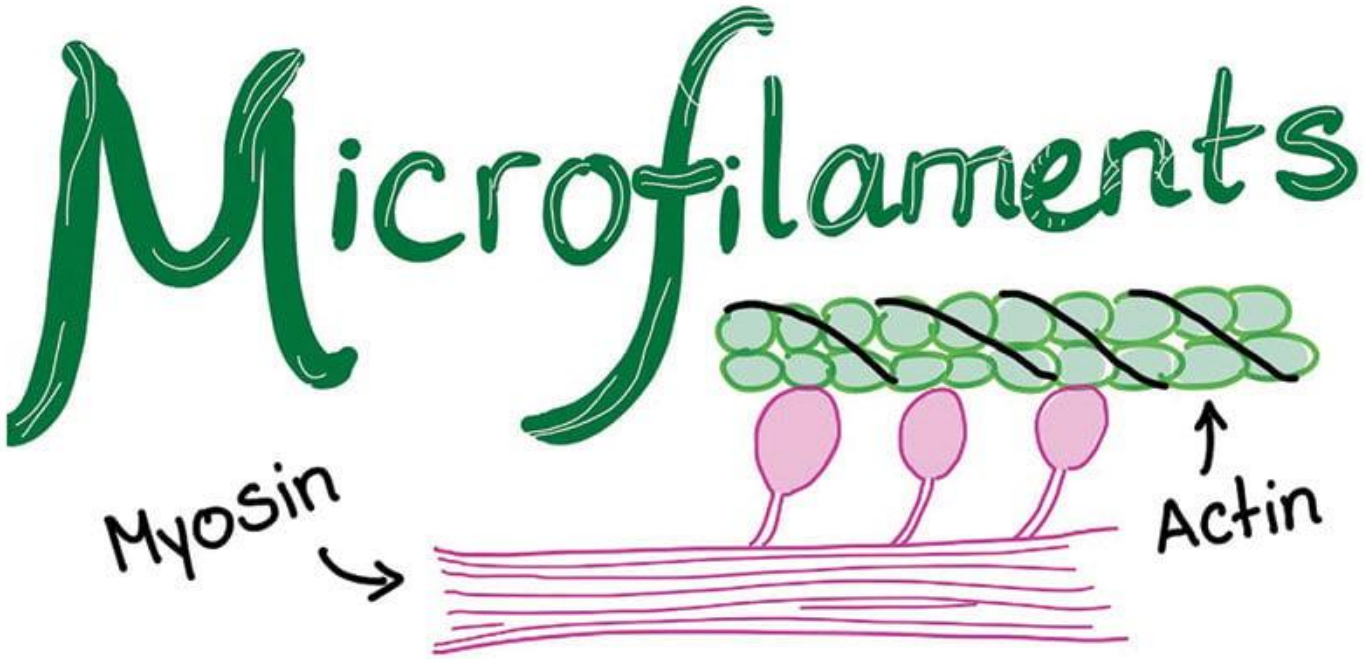
- These organelles are responsible for intracellular digestion where the larger macromolecules are degraded into smaller molecules with the help of enzymes present in them.
- Lysozymes also perform the critical function of the autolysis of unwanted organelles within the cytoplasm.
- Besides these, the lysosome is involved in various cellular processes, including secretion, plasma membrane repair, cell signaling, and energy metabolism.

Microfilaments

Microfilaments are a part of the cytoskeleton of a cell made up of actin protein in the form of parallel polymers. These are the smallest filaments of the cytoskeleton with high rigidity and flexibility, providing strength and movement to the cell.

Structure of Microfilaments

- The filaments are present either in cross-linked forming networks or as bundles. The chains of protein remain twisted around each other in a helical arrangement.
- One of the polar ends of the filament is positively charged and barbed, whereas the other end is negatively charged and pointed.



Functions of Microfilaments

- It generates the strength for the structure and movement of the cell in association with myosin protein.
- They help in cell division and are involved in the products of various cell surface projections.

Microtubules

Microtubules are also a part of the cytoskeleton differing from microfilaments in the presence of tubulin protein

Structure of Microtubules

- They are long hollow, beaded tubular structures of a diameter of about 24nm.
- The wall of the microtubules consists of globular subunits present at a helical array of a and b tubulin.
- Similar to microfilaments, the ends of microtubules also have a defined polarity with one end being positively charged while the other being negatively charged.

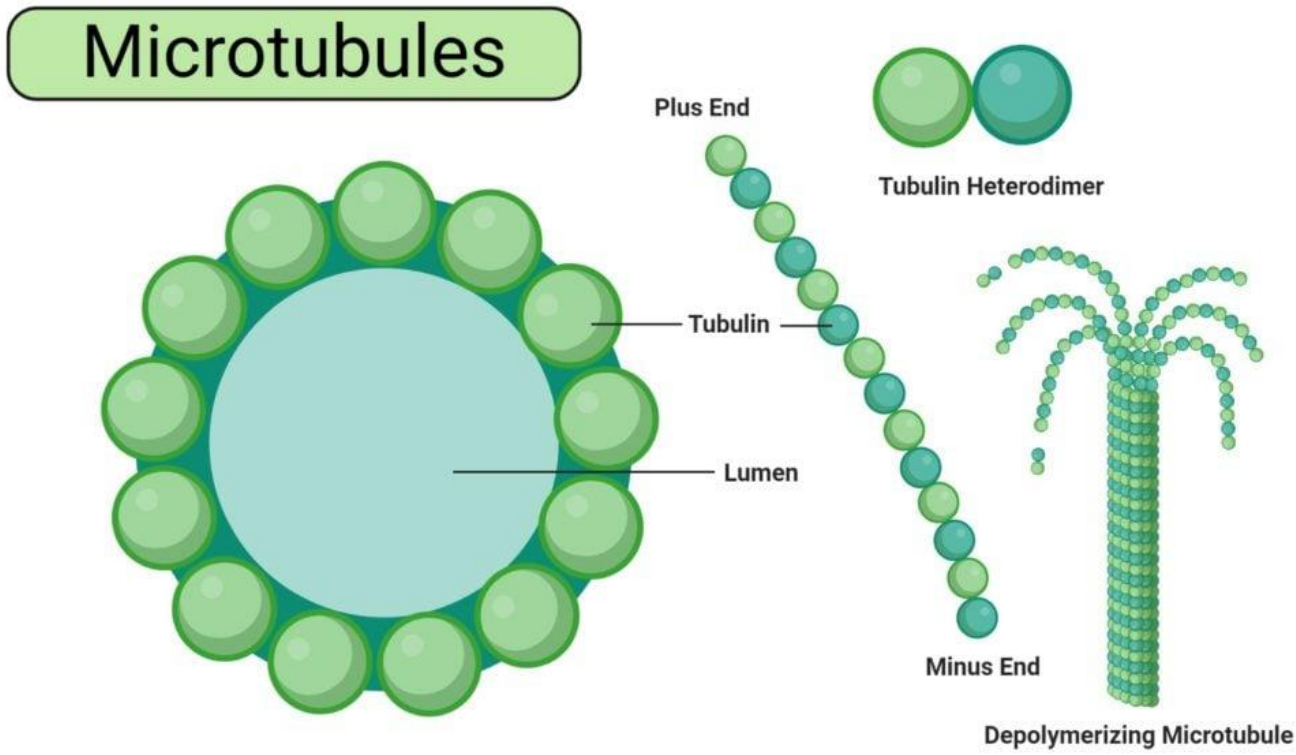


Figure: Microtubules, Image Copyright © Sagar Aryal, www.microbenotes.com

Functions of Microtubules

- As a part of the cytoskeleton, they provide shape and movement to the cell.
- Microtubules facilitate the movement of other cell organelles within the cell through binding proteins.

Microvilli

Microvilli are tiny finger-like structures that project on or out of the cells. These exist either on their own or in conjunction with villi.

Structure of Microvilli

- Microvilli are bundles of protuberances loosely arranged on the surface of the cell with little or no cellular organelles.
- These are surrounded by a plasma membrane enclosing cytoplasm and microfilaments.
- These are bundles of actin filaments bound by fimbrin, villin, and epsin.

Microvilli

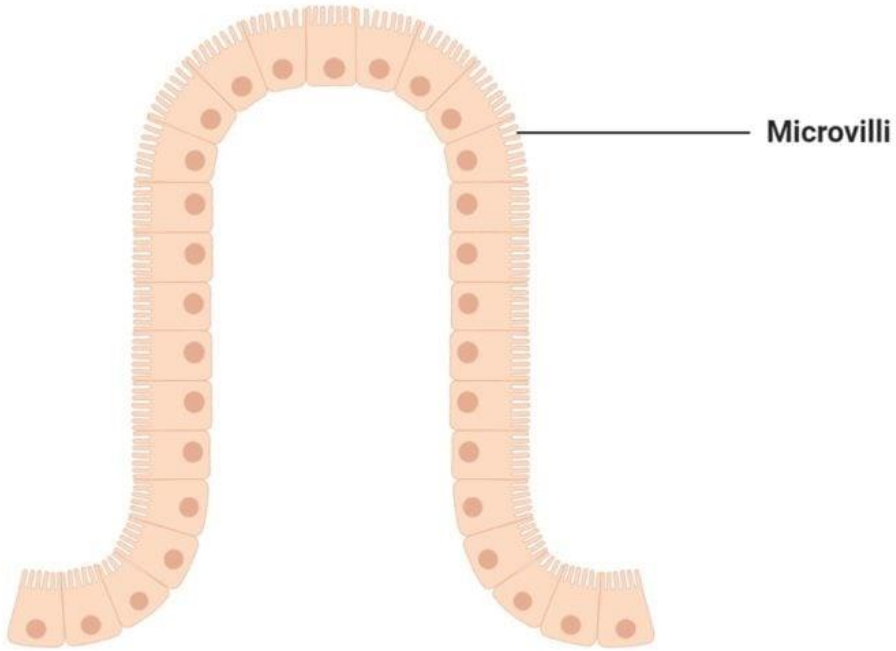


Figure: Microvilli, Image Copyright © Sagar Aryal, www.microbenotes.com

Functions of Microvilli

- Microvilli increase the surface area of the cell, thus, enhancing the absorption and secretion functions.
- The membrane of microvilli is packed with enzymes that allow the break down of larger molecules into smaller allowing more effective absorption.
- Microvilli act as an anchoring agent in white blood cells and in sperms during fertilization.

Mitochondria

Mitochondria are double membrane-bound cell organelles responsible for the supply and storage of energy for the cell. The oxidation of various substrates in the cell to release energy in the form of ATP (Adenosine Triphosphate) is the primary purpose of mitochondria.

Structure of Mitochondria

- A mitochondrion contains two membranes with the outer layer being smooth while the inner layer is marked with folding and finger-like structures called cristae.
- The inner mitochondrial membrane contains various enzymes, coenzymes, and components of multiple cycles along with pores for the transport of substrates, ATP, and phosphate molecules.

- Within the membranes is a matrix that contains various enzymes of metabolic processes like Kreb's cycle.
- In addition to these enzymes, mitochondria are also home to single or double-stranded DNA called mtDNA that is capable of producing 10% of the proteins present in the mitochondria.

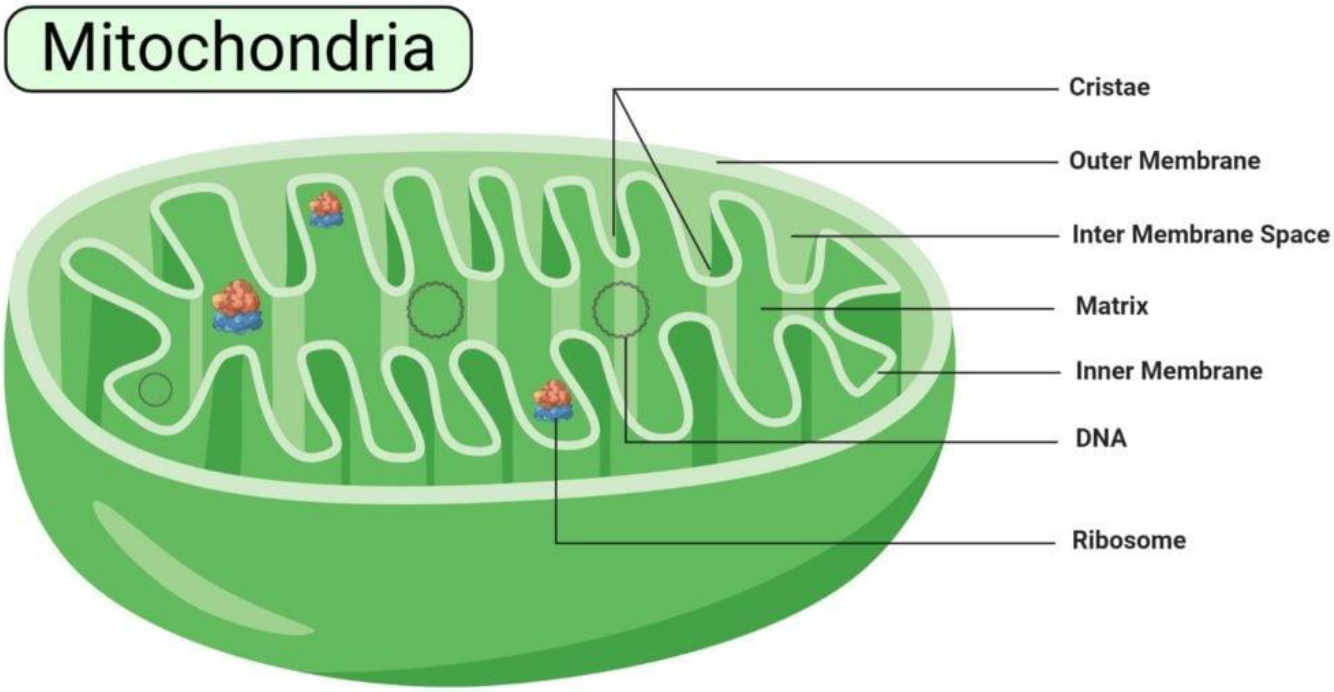


Figure: Mitochondria, Image Copyright © Sagar Aryal, www.microbenotes.com

Functions of Mitochondria

- The primary function of mitochondria is the synthesis of energy in the form of ATP required for the proper functioning of all the cell organelles.
- Mitochondria also help in balancing the amount of Ca+ ions within the cell and assists the process of apoptosis.
- Different segments of hormones and components of blood are built within mitochondria.
- Mitochondria in the liver have the ability to detoxify ammonia.

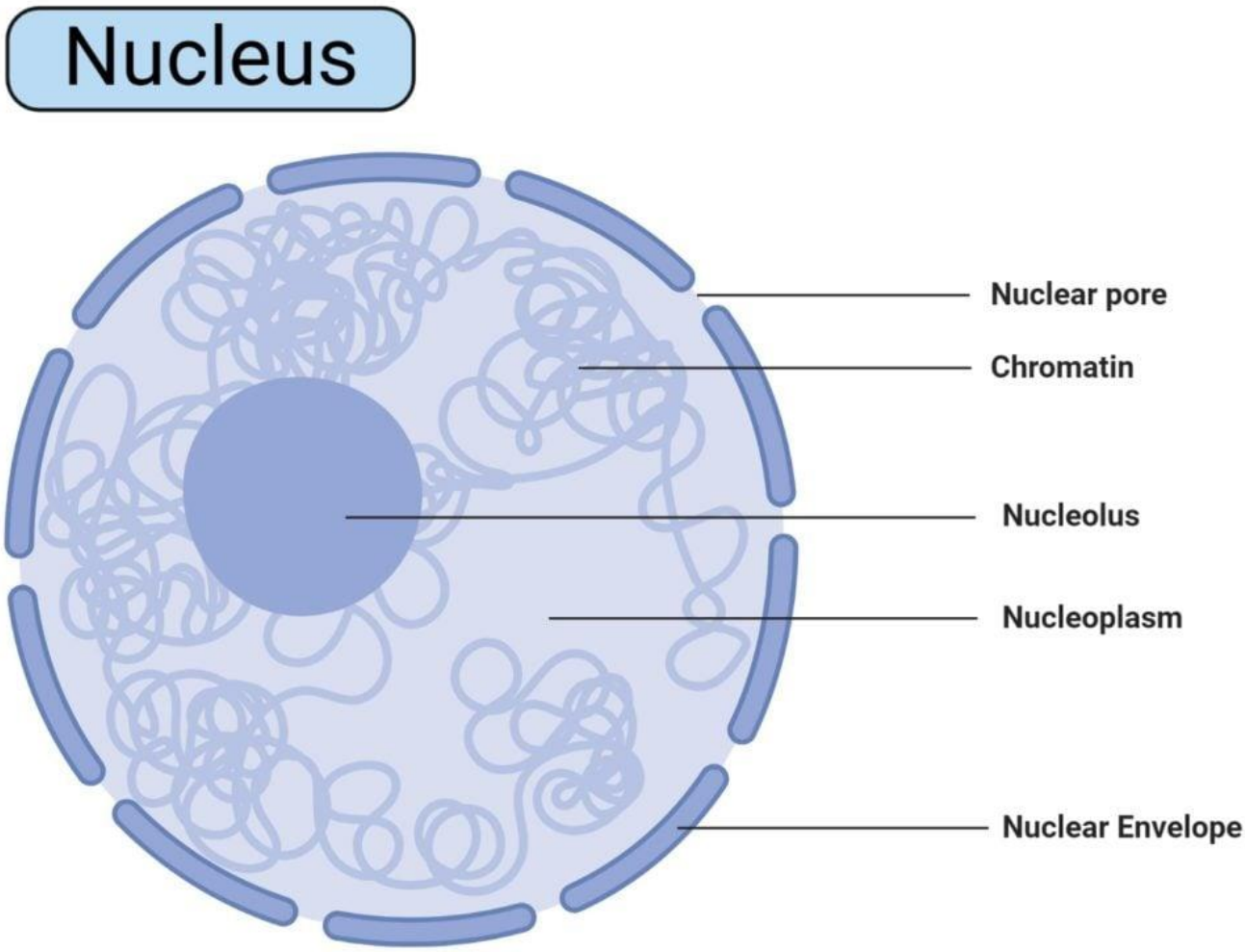
Nucleus

The nucleus is a double membrane-bound structure responsible for controlling all cellular activities as well as a center for genetic materials, and it's transferring. It is one of the large cell organelles occupying 10% of the total space in the cell. It is often termed the "brain of the cell" as it provides commands for the proper functioning of other cell organelles. A nucleus is clearly defined in the case of a eukaryotic cell; however, it is absent in prokaryotic organisms with the genetic material distributed in the cytoplasm.

Structure of Nucleus

- Structurally, the nucleus consists of a nuclear envelope, chromatin, and nucleolus.

- The nuclear envelope is similar to the cell membrane in structure and composition. It has pores that allow the movement of proteins and RNA in and outside the nucleus. It enables the interaction with other cell organelles while keeping nucleoplasm and chromatin within the envelope.
- The chromatin in the nucleus contains RNA or DNA along with nuclear proteins, as genetic material that is responsible for carrying the genetic information from one generation to another. It is present in a sense and compact structure which might be visible as a chromosome under powerful magnification.
- The nucleolus is like a nucleus within the nucleus. It is a membrane-less organelle that is responsible for the synthesis of rRNA and the assembly of ribosomes required for protein synthesis.



Functions of Nucleus

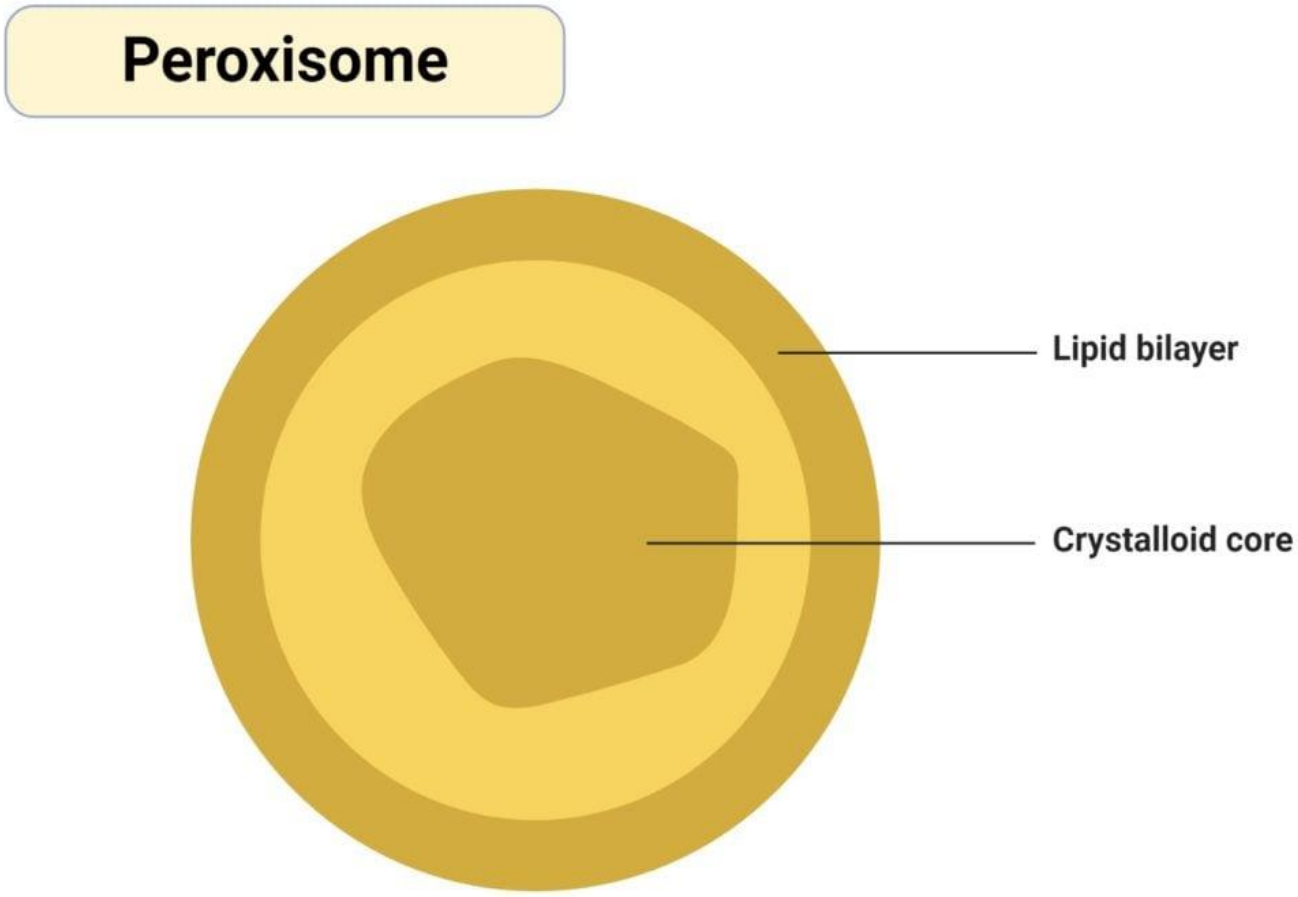
- The nucleus is responsible for storage as well as the transfer of genetic materials in the form of DNA or RNA.
- It aids in the process of transcription by the synthesis of mRNA molecules.
- The nucleus controls the activity of all other organelles while facilitating processes like cell growth, cell division, and the synthesis of proteins.

Peroxisomes

Peroxisomes are oxidative membrane-bound organelles found in the cytoplasm of all eukaryotes. The name is accredited due to their hydrogen peroxide generating and removing activities.

Structure of Peroxisomes

- Peroxisome consists of a single membrane and granular matrix scattered in the cytoplasm.
- They exist either in the form of interconnected tubules or as individual peroxisomes.
- The compartments within every peroxisome allow the creation of optimized conditions for different metabolic activities.
- They consist of several types of enzymes with major groups being urate oxidase, D-amino acid oxidase, and catalase.



Functions of Peroxisomes

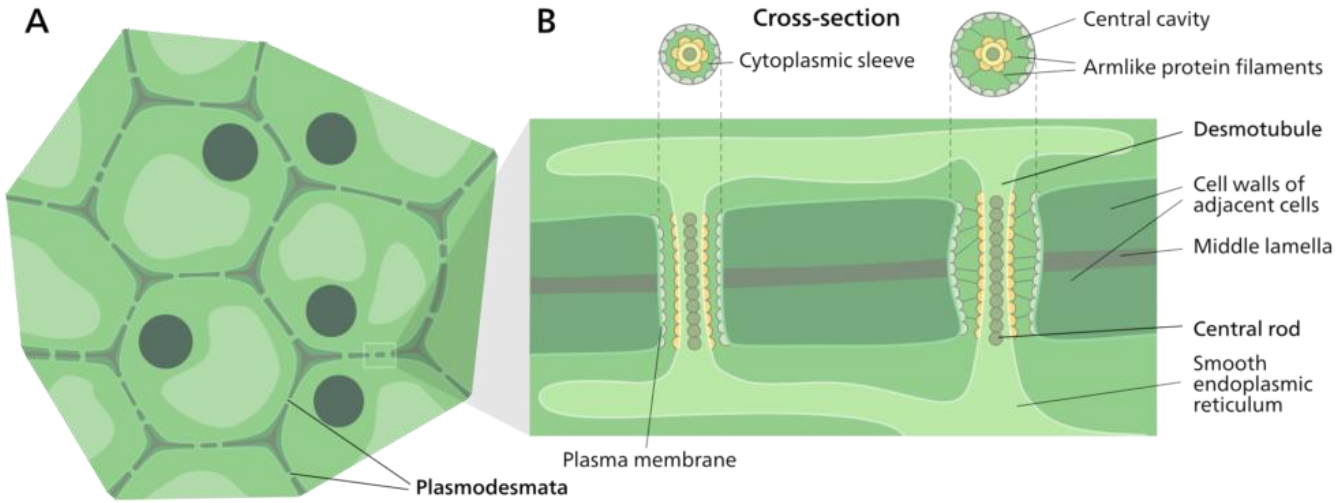
- Peroxisomes are involved in the production and elimination of hydrogen peroxide during biochemical processes.
- Oxidation of fatty acids takes place within peroxisomes.
- Additionally, peroxisomes are also involved in the synthesis of lipid-like cholesterol and plasmalogens.

Plasmodesmata

Plasmodesmata are tiny passages or channels that allow the transfer of material and communication between different cells.

Structure of Plasmodesmata

- There are 103 – 105 plasmodesmata connecting two adjacent cells with 50-60 nm in diameter.
- A plasmodesma has three layers:
    - The plasma membrane is continuous with the plasma membrane of the cell and has the same phospholipid bilayer.
    - The cytoplasmic sleeve is continuous with the cytosol that allows the exchange of materials between two cells.
    - Desmotubule which is a part of the endoplasmic reticulum that provides a network between two cells and allows the transport of some molecules.



Functions of Plasmodesmata

- Plasmodesmata are the primary site for the communication of two cells. It allows the transfer of molecules like proteins, RNA, and viral genomes.

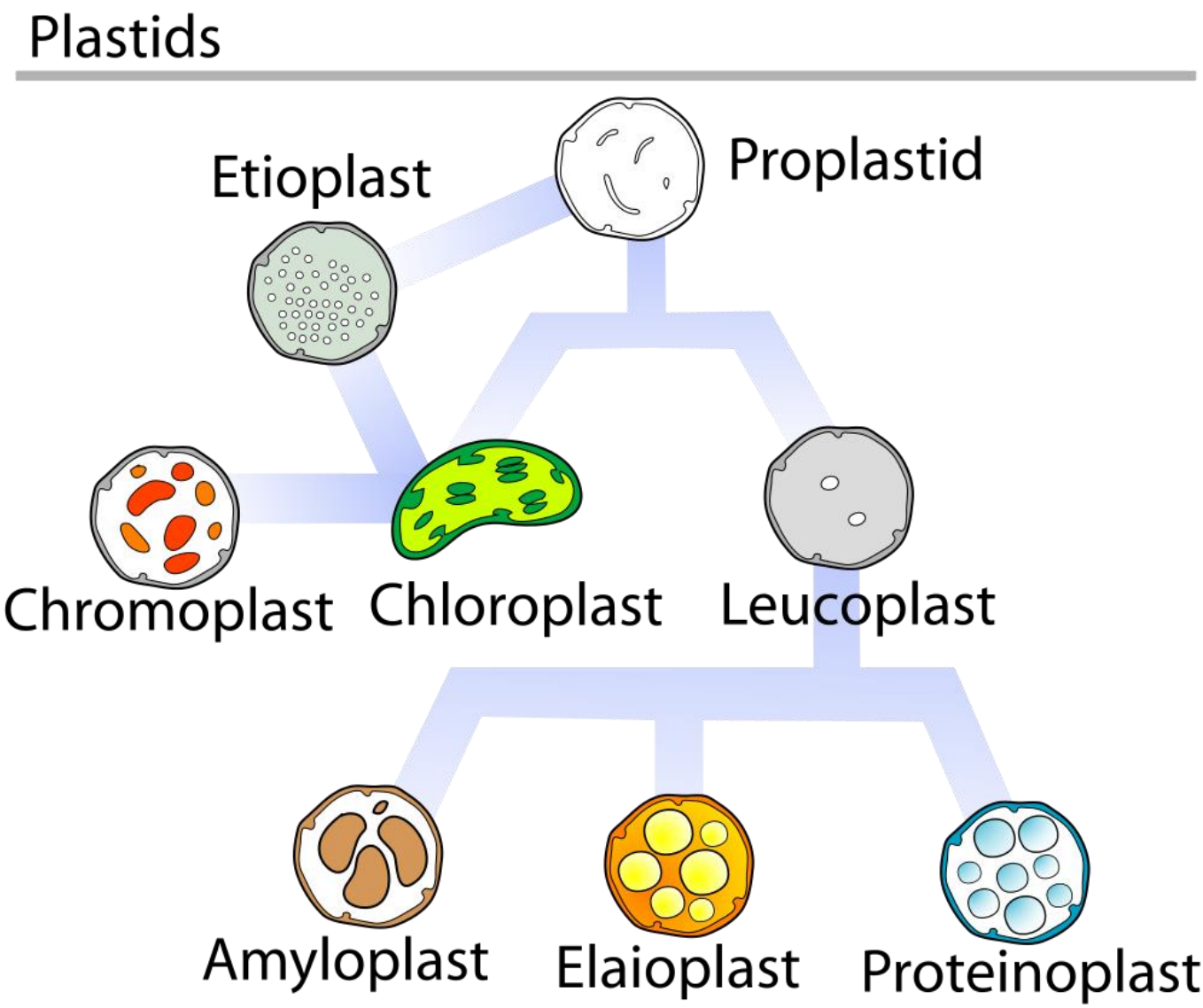
Plastids

Plastids are double membrane-bound structures present in plants and other eukaryotes involved in the synthesis and storage of food.

Structure of Plastids

- Plastids are usually oval or spherical with an outer and an inner membrane between which lies the intermembrane space.
- The inner membrane enclosed a matrix called stroma that contains small structures called grana.
- Each granum consists of several sac-like thylakoids piled one on the other and connected by stroma lamellae.

- Plastids contain DNA and RNA that allows it to synthesize necessary proteins for different processes.



Functions of Plastids

- Chloroplasts are the center for many metabolic activities, including photosynthesis as it contains enzymes and other components required for it.
- They are also involved in the storage of food, primarily starch.

Ribosomes

Ribosomes are ribonucleoproteins containing equal parts RNA and proteins along with an array of other essential components required for protein synthesis. In prokaryotes, they exist freely while in eukaryotes, they are found either free or attached to the endoplasmic reticulum.

Structure of Ribosomes

- The ribonucleoprotein consists of two subunits.

- In the case of prokaryotic cells, the ribosomes are of the 70S with the larger subunit of 50S and the smaller one of 30S.
- Eukaryotic cells have 80S ribosomes with 60S larger subunit and 40S smaller subunit.
- Ribosomes are short-lived as after the protein synthesis, the subunits split up and can be either reused or remain broken up.

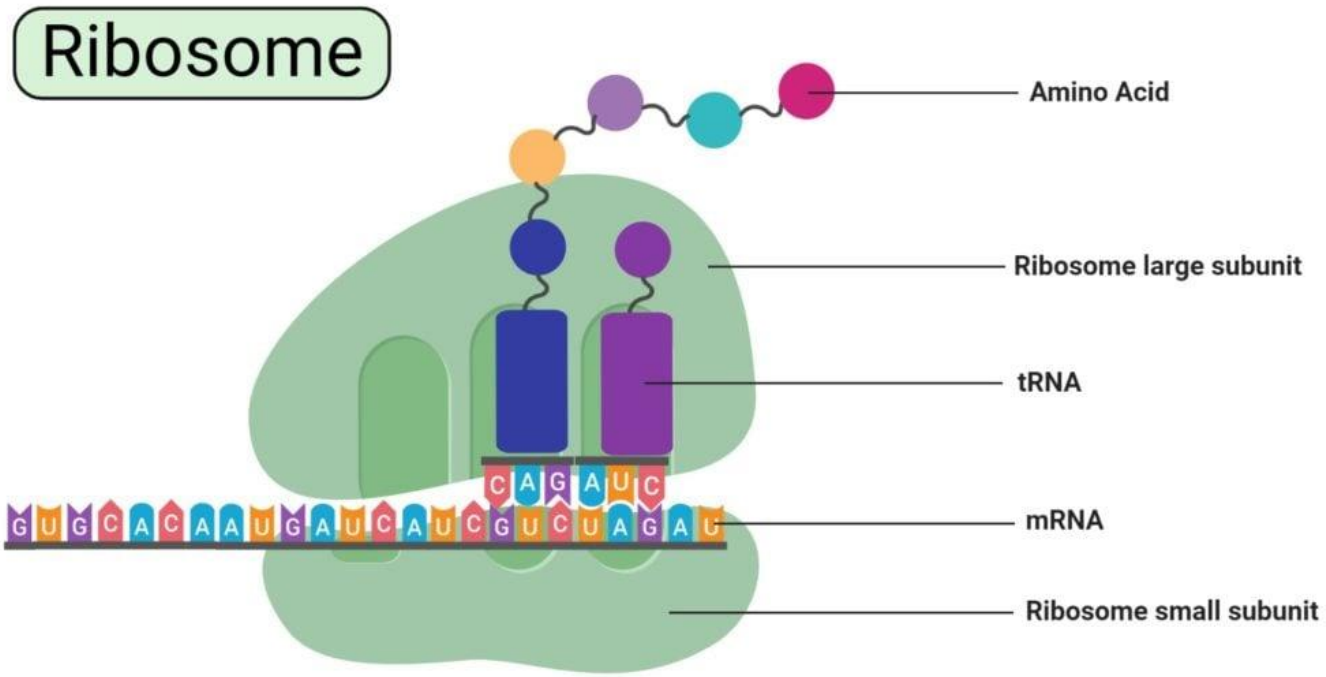


Figure: Ribosome, Image Copyright © Sagar Aryal, www.microbenotes.com

Functions of Ribosomes

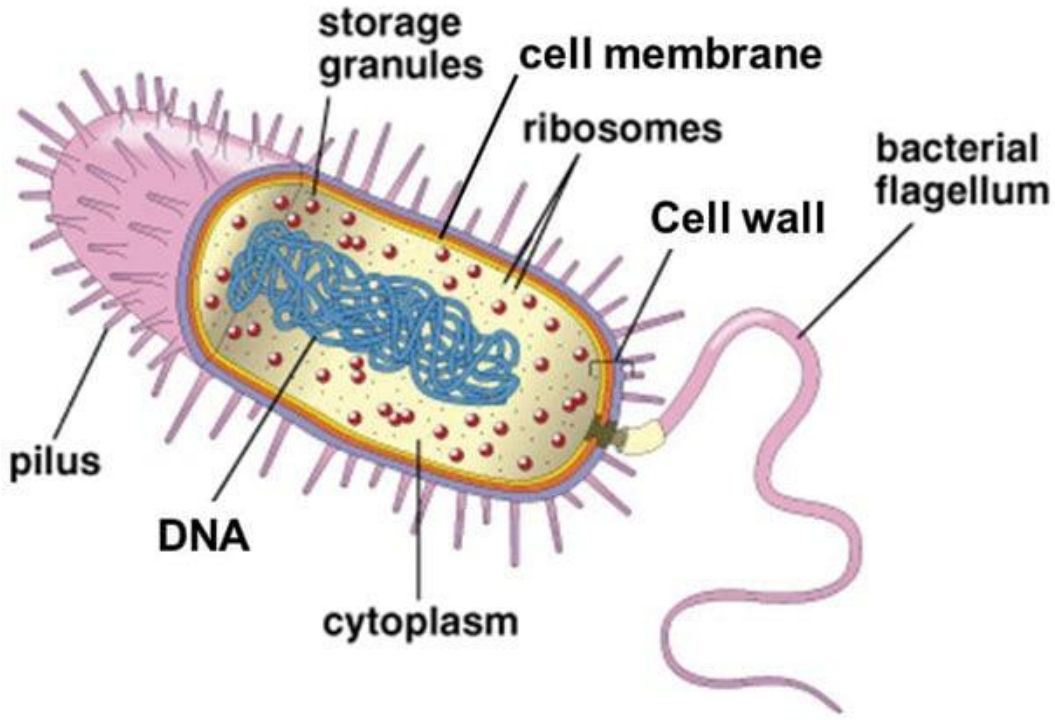
- Ribosomes are the site of biological protein synthesis in all living organisms.
- They arrange the amino acids in the order indicated by tRNA and assist in protein synthesis.

Storage granules

Storage granules are membrane-bound organelles, also called zymogen granules storing cells' energy reserve and other metabolites.

Structure of Storage granules

- These granules are surrounded by a lipid bilayer and are composed mostly of phosphorus and oxygen.
- The components inside these storage granules depend on their location in the body with some even containing degradative enzymes yet to participate in digestive activities.



Functions of Storage granules

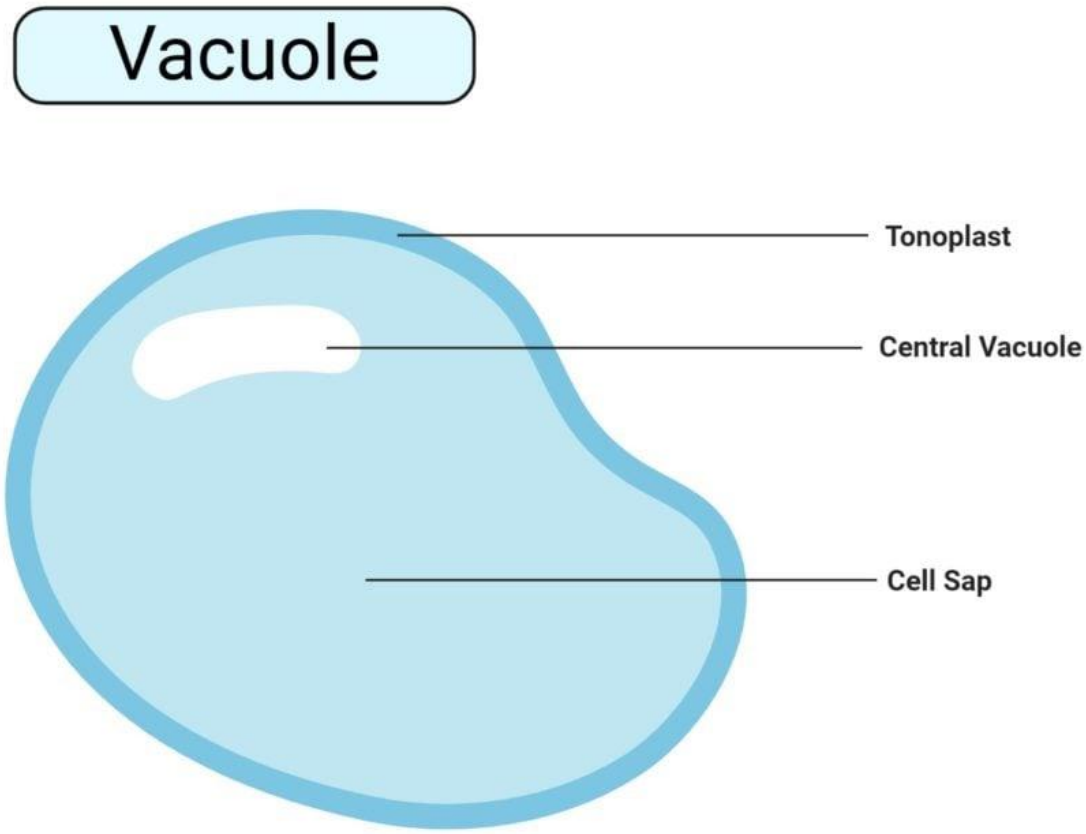
- Many prokaryotes and eukaryotes store nutrients and reserves in the form of storage granules in the cytoplasm.
- Sulfur granules are characteristic of prokaryotes that utilize hydrogen sulfide as a source of energy.

Vacuole

Vacuoles are membrane-bound structures varying in size in cells of different organisms.

Structure of Vacuoles

- The vacuole is surrounded by a membrane called tonoplast, which encloses fluid containing inorganic materials like water and organic materials like nutrients and even enzymes.
- These are formed by the fusion of various vesicles, so vacuoles are very similar to vesicles in structure.



Functions Vacuoles

- Vacuoles act as a storage for nutrients as well as waste materials to protect the cell from toxicity.
- They have an essential function of homeostasis as it allows the balance of pH of the cell by influx and outflow of H+ ions to the cytoplasm.
- Vacuoles contain enzymes that play an important role in different metabolic processes.

Vesicles

Vesicles are structures present inside the cell that are either formed naturally during processes like exocytosis, endocytosis, or transport of materials throughout the cell, or they might form artificially, which are called liposomes. There are different types of vesicles like vacuoles, secretory, and transport vesicles based on their function

Structure of Vesicles

- A vesicle is a structure containing liquid or cytosol which is enclosed by a lipid bilayer.
- The outer layer enclosing the liquid is called a lamellar phase which is similar to the plasma membrane. One end of the lipid bilayer it hydrophobic whereas the other end is hydrophilic.

Vesicles

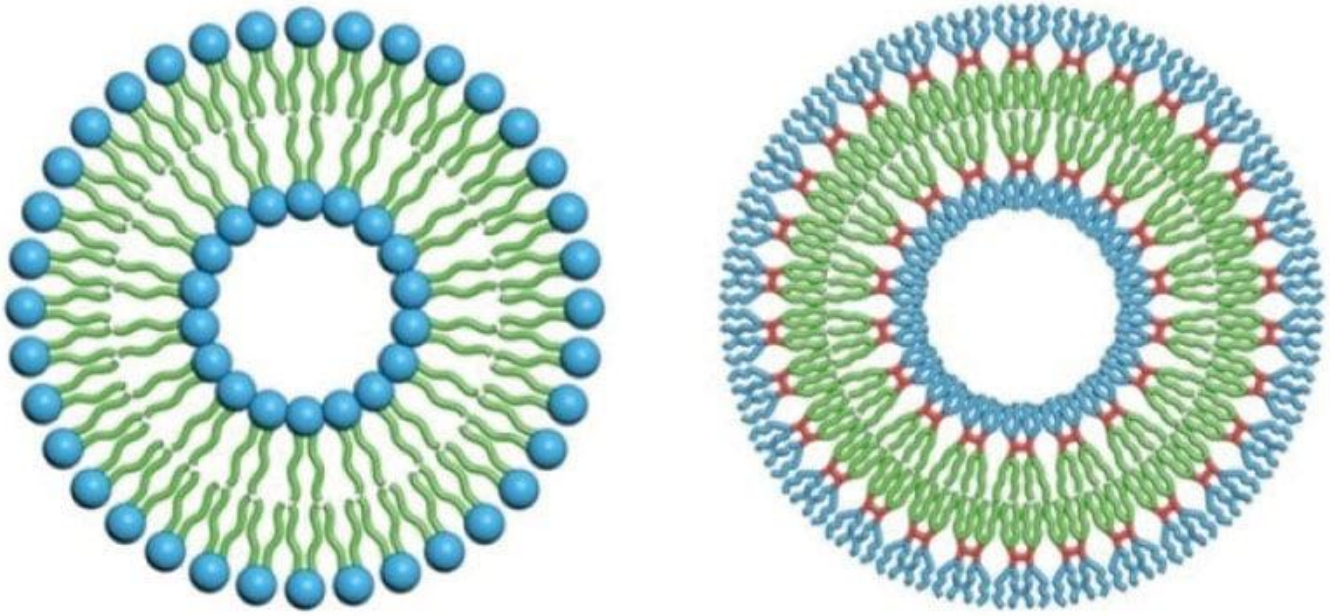


Figure: A liposome (left) and dendrimer some. The blue parts of their molecules are hydrophilic, the green parts are hydrophobic. Credit: Image courtesy of University of Pennsylvania

Functions of Vesicles

- Vesicles facilitate the storage and transport of materials in and outside the cell. It even allows the exchange of molecules between two cells.
- Because vesicles are enclosed inside a lipid bilayer, vesicles also function in metabolism and enzyme storage.
- They allow temporary storage of food and also control the buoyancy of the cell.

UNIT IV: DNA Replication, Repair, and Regulation of Gene Expression

DNA Replication

- Prokaryotic vs. Eukaryotic: Prokaryotic replication is simpler and faster; eukaryotic replication involves more complex machinery and multiple origins of replication.
- Mechanism: Involves initiation, elongation, and termination; key enzymes include DNA polymerase, helicase, ligase.
- Models: Semiconservative is the accepted model, where each new DNA molecule consists of one old strand and one new strand.

DNA Damage and Repair

- Types of Damage: UV radiation, chemicals, replication errors.
- Repair Mechanisms: Direct repair, excision repair, mismatch repair.

Regulation of Gene Expression

- Lac Operon: Model of gene regulation in prokaryotes; regulates lactose metabolism.
- Trp Operon: Regulates tryptophan synthesis in prokaryotes.

DNA Replication: Enzymes, Mechanism, Steps, Applications

November 2, 2023 by Rajat Thapa

Edited By: Sagar Aryal

DNA replication is the process of producing two identical copies of DNA from one original DNA molecule.

- DNA is made up of millions of nucleotides, which are composed of deoxyribose sugar, with phosphate and a base.
- The complementary pairing of these bases keeps the double strands intact. So, to make two copies of one DNA, these hydrogen bonds in between the bases should be broken to begin replication.
- DNA replication is semi-conservative, meaning that each strand in the DNA acts as a template for the synthesis of a new complementary strand. Semi conservative because once DNA molecule is synthesized it has one strand from the parent and the other strand is a newly formed strand.
- DNA replication starts by taking one DNA molecule and giving two daughter molecules, with each newly synthesized molecule containing one new and one old strand.
- DNA replication simply is the process by which a DNA makes a copy of itself. Though easy as it may sound it’s a complex process happening inside of our cells, and many enzymes, proteins, and metal ions should work coherently to make this process happen.

Table of Contents

Interesting Science Videos

What If Your Body Could Be Recycled?

Mechanism of DNA Replication

Summary: DNA replication takes place in three major steps.

1. Opening of the double-stranded helical structure of DNA and separation of the strands
2. Priming of the template strands

3. Assembly of the newly formed DNA segments.

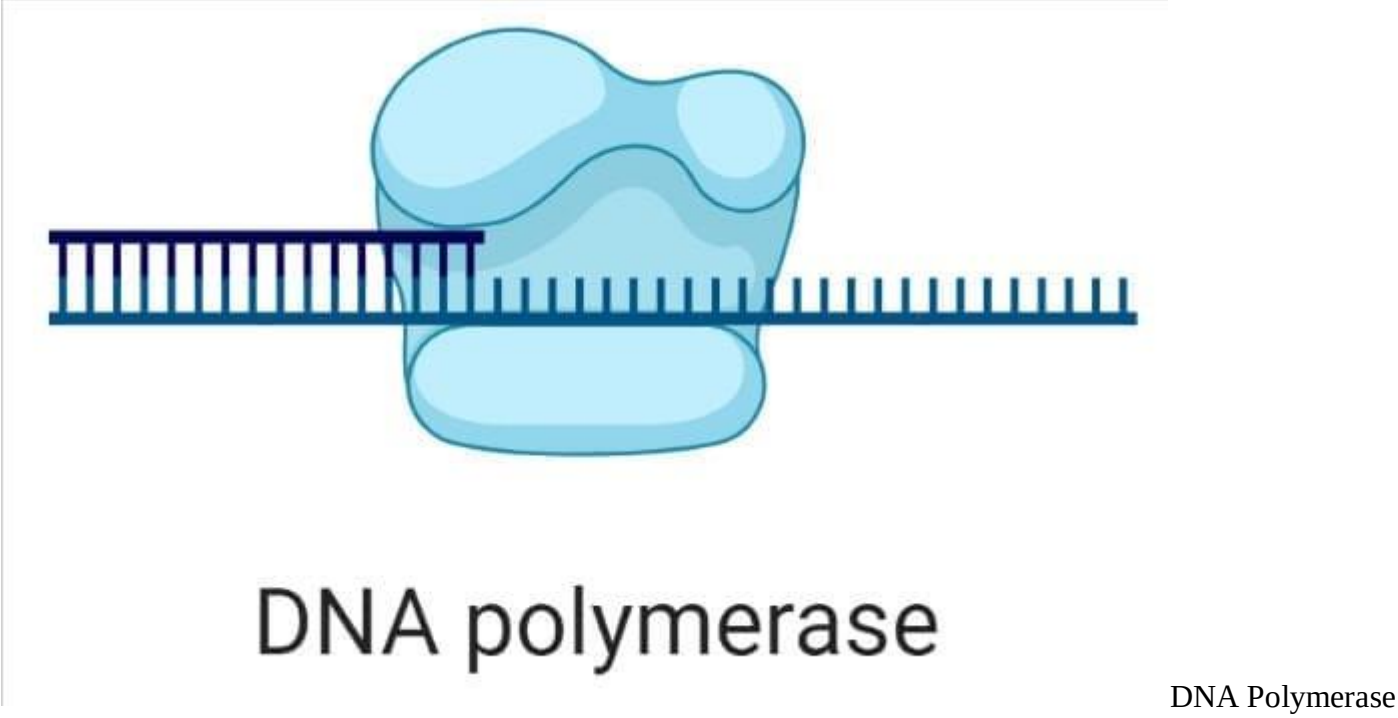
- During the separation of DNA, the two strands uncoil at a specific site known as the **origin**. With the involvement of several enzymes and proteins, they prepare (prime) the strands for duplication.
- At the end of the process, DNA polymerase enzyme starts to organize the assembly of the new DNA strands.
- These are the general steps of DNA replication for all cells but they may vary specifically, depending on the organism and cell type.
- Enzymes play a major role in DNA replication because they catalyze several important stages of the entire process.
- DNA replication is one of the most essential mechanisms of a cell's function and therefore intensive research has been done to understand its processes.
- The mechanism of DNA replication is well understood in *Escherichia coli*, which is also similar to that in eukaryotic cells.
- In E.coli, DNA replication is initiated at the oriClocus (oriC), to which DnaA protein binds while hydrolyzing of ATP takes place.

Enzymes and Proteins Used in DNA Replication

Nucleases

- A nuclease is an enzyme that can cleave the phosphodiester bonds present in between the nucleotides.
- On the basis where they cleave, they are characterized as Exo and endonucleases.
- Exonucleases cleave nucleotides from their respective ends. Corresponding to this fact, these exonucleases show activity from both directions 5′ to 3′ and 3′ to 5′.
- Endonucleases act on the region in the middle of the targeted nucleotide. They are also endonucleases that are selective to which molecule they cleave and are sub-divided as DNase for DNA for cleaving and RNase for RNA cleaving. Additionally, recently discovered nucleases are also being used for gene editing such as Cas9 in the CRISPR genome editing technique.
- Restrictive endonuclease or restriction enzymes are the ones that cleave DNA into fragments at or near the specific recognition sites within the molecule known as restriction sites.
- To cleave the DNA, restriction endonuclease makes two incisions, once through each sugar-phosphate backbone of the DNA double helix. These endonucleases recognize a specific sequence of nucleotides and produce a double-stranded cut in the DNA.
- This specific sequence is usually 4 – 8 bases and is present in the recognition site.

DNA Polymerase



DNA Polymerase

- DNA polymerases are the enzyme that is responsible for adding new nucleotides and synthesizing a new strand of DNA by taking the old fragmented strand as a template.
- DNA Polymerases also possess exonuclease activity, that cuts incorrectly added nucleotides, and allows the DNA replication to happen without errors.
- DNA Polymerase is of many types and functions based on the cell they are found in.
- In prokaryotic cells, there are three DNA polymerases: DNA Polymerase I, DNA Polymerase II and DNA Polymerase III.
- DNA polymerase I is a repair polymerase with 5′ to 3′ and 3′ to 5′ exonuclease activity. It is involved in the processing of Okazaki fragments during lagging strand synthesis.
- DNA polymerase II has 3′ to 5′ exonuclease activity and participated in DNA repair with 5′ to 3′ polymerase activity.
- DNA polymerase III is the primary enzyme involved in the DNA replication of *E.coli*. It has 3′ to 5′ exonuclease activity and 5′ to 3′ polymerase activity.
- In eukaryotic cells, there are five DNA polymerases: DNA Polymerase α, β, γ, δ and ε
- DNA polymerase α is a repair polymerase, with 3′ to 5′ exonucleases activities and 5′ to 3′ polymerase activities.
- DNA Polymerase β is a repair polymerase.
- DNA Polymerase γ shows polymerase activity 5′ to 3′ and exonucleases activity 3′ to 5′, it is involved in Mitochondrial DNA replication
- DNA Polymerase δ shows 3′ to 5′ exonuclease activity and 5′ to 3′ polymerase activity. This enzyme is involved in lagging strand synthesis.

- DNA Polymerase ε shows 3′ to 5′ and 5′ to 3′ exonucleases activities. This enzyme not only repairs but also synthesizes the leading strand efficiently in a 5′ to 3′ direction. It is the prime enzyme involved in DNA replication.

DNA ligase

DNA ligase

- DNA ligase is a specific type of enzyme that facilitates the joining of DNA strands together by catalyzing the formation of a phosphodiester bond.
- This enzyme joins the 3′ hydroxyl group of one nucleotide with the 5′ phosphate end of another nucleotide at an expense of ATP.

DNA helicase

- DNA helicase is a motor protein that moves directionally along a nucleic acid phosphodiester backbone, separating two nucleotides of DNA molecule.
- They separate double-stranded DNA molecules into single strands allowing each strand to be copied.
- During DNA replication, this DNA helicase unwinds DNA at the origin, a site where the replication is to be initiated.
- DNA helicase continues to unwind the double helix of DNA and thus forms a structure called replication fork, named after the forked appearance of two strands of DNA when unzipped apart.
- It is an energy-driven process as it involves the breaking of Hydrogen bonds between annealed nucleotide bases.

DNA primase

- Primase is an enzyme that is capable to synthesize short stretches of RNA sequences known as a primer.
- Primers are an integral part of DNA replication. These primers serve as an initiating site for the addition of nucleotides by DNA polymerase.
- DNA polymerase can only add nucleotide at pre-existing 3′ Hydroxyl group which is thus provided by the primers.

- As we can see that primers are short stretches of RNA, but replication is of DNA, so therefore after elongation of the chains of nucleotides, these primers are replaced by DNA.

DNA topoisomerase

- DNA topoisomerase is a class of enzymes that release helical tension during transcription and replication by creating transient nicks within the phosphate backbone on one or both strands of the DNA.
- This tension is aroused when the DNA molecule unwinds due to helicase activity and forms a replication fork. The progress of the replication fork generates supercoils, making it hard for other machinery involved to access the DNA molecule.
- Class I DNA topoisomerase makes a single-stranded break to relax the helix and progress the process.
- Class II DNA topoisomerase break both the strands of DNA helix, this class of topoisomerases is also very important during the cell cycle for the condensation of chromosomes.

Single strand binding proteins

- The single-strand binding (SSB) protein are DNA binding proteins, that binds to single-stranded DNA to facilitate DNA replication.
- SSB proteins prevent the hardening of strands during DNA replication. It also protects strands from nuclease degradation and prevents the rewinding of DNA.
- These proteins destabilize helical duplexes so that DNA polymerase can hold onto the DNA during DNA replication, recombination, and repair.
- It also removes unwanted secondary structures on strands for easy access of the strands to the machinery involved in DNA replication.
- Thus, SSB proteins stabilize the single-stranded DNA structure that is important for genomic progression.

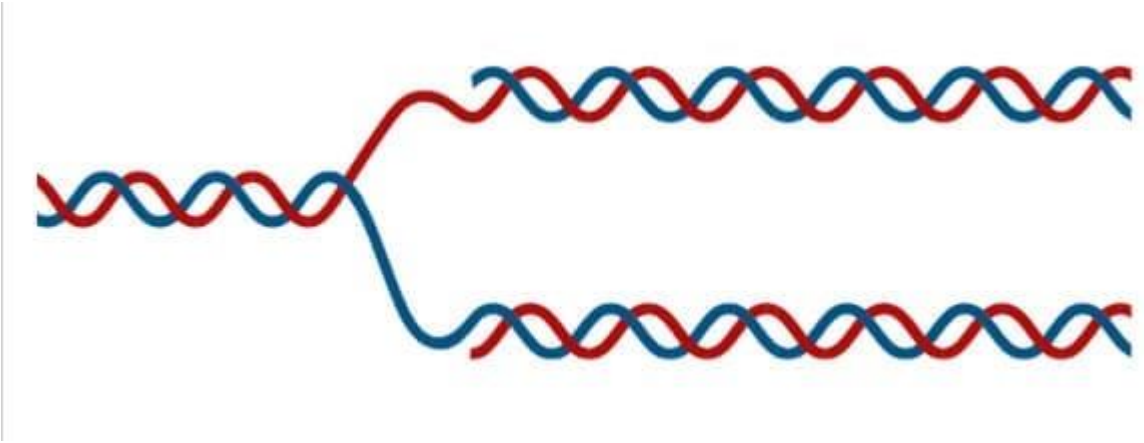
So, in summary here are the list of 7 enzymes and proteins used in DNA Replication:

1. Nucleases
2. DNA Polymerase
3. DNA ligase
4. DNA helicase
5. DNA primase
6. DNA topoisomerase
7. Single strand binding proteins

Steps in DNA Replication

Step 1: Formation of Replication Fork

- Before DNA can replicate, this double-stranded molecule must unwind into two single strands to initiate the replication process.
- DNA unwinds when the complementary base pairing between the double-stranded is broken, and the site to initiate this unwinding is denoted by specific regions (Adenine and Thymine rich).
- These specific coding regions are referred to as Origin of Replication (Ori) and thus the replication process begins.
- These origins are targeted by initiator proteins, which go on to recruit more proteins that can help the replication process by forming a replication fork around the Ori.
- Within this replication protein complex is an enzyme DNA helicase, which starts to unwind the DNA from its Ori and exposes two strands resembling a Y-like structure referred to as replication fork.
- The activity of helicase causes topological stress to the un-winded strand forming supercoiled DNA, this stress is relieved by Topoisomerase by negative supercoiling.
- The replication fork is bidirectional; one strand is oriented to 3′ to 5′ direction (leading strand) and the other strand is oriented to 5′ to 3′ direction (lagging strand) but the addition of nucleotide progress only in 5′ to 3′ direction.
- The formation of a replication fork exposing two single-stranded strands marks the beginning of Initiation.

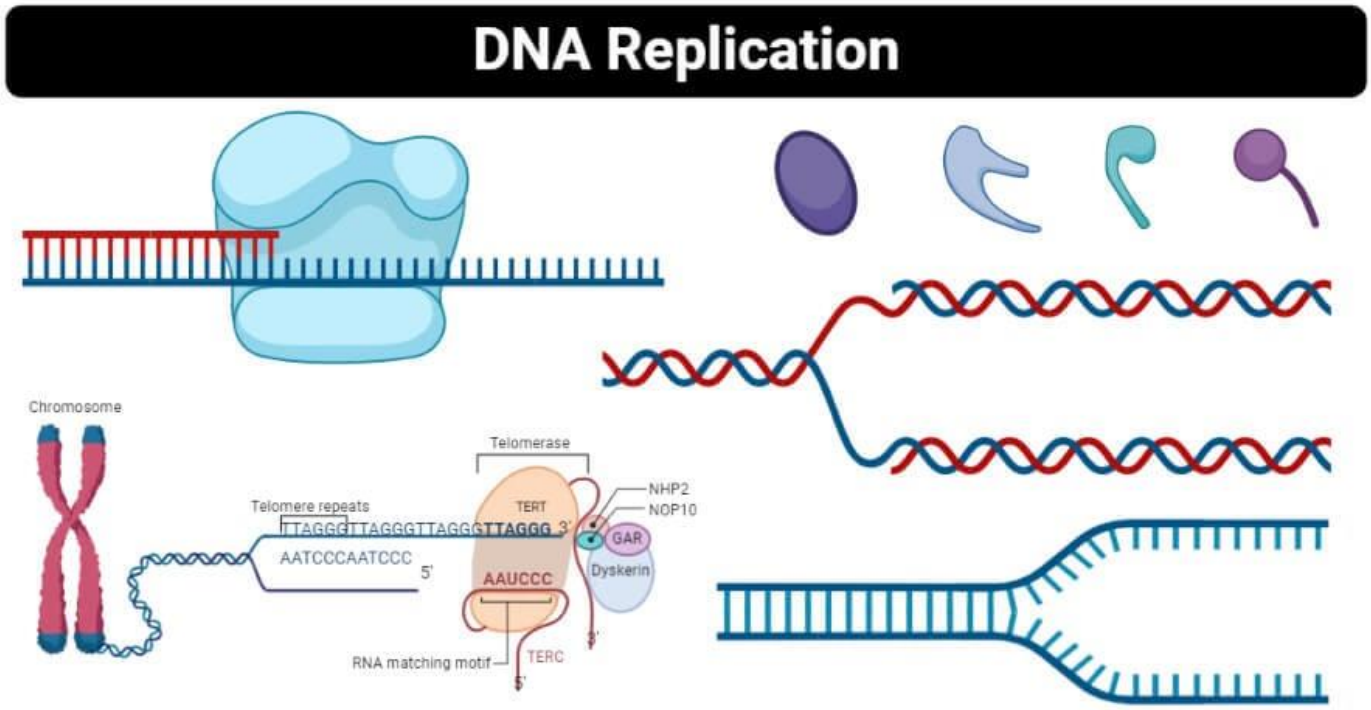


Formation of Replication Fork

Step 2: Initiation

- One strand runs from 3′ to 5′ direction towards the replication fork and is referred to as leading strand and the other strand runs from 5′ to 3′ away from the replication fork and is referred to as lagging strands.
- To this exposed single-stranded DNA, SSB proteins are adhered to prevent recoiling of DNA and to stabilize it.

- After which another enzyme DNA primase comes into action to synthesize a short stretch of RNA primer, which provides a free 3′ hydroxyl group for DNA polymerase can now add nucleotides and extend the new chain of nucleotides.



DNA Replication. Created with BioRender.com

Step 3: Elongation

- Now that primer is added to unzipped two single-stranded DNA, these strands now act as a template for synthesizing new DNAs.
- The enzyme DNA polymerase synthesizes new nucleotide to match the template and add on to the free 3′ hydroxyl group provided by the primer in each single-stranded DNA.
- The leading strand runs from 3′ to 5′ so the addition of nucleotides by DNA polymerase happens from 5′ to 3′ direction. As the replication fork progresses the addition of nucleotide is continuous thus only requiring the primer once.
- However, lagging strands is antiparallel and run from the 5′ to 3′ direction, the continuous addition of nucleotides is not possible as the replication fork progresses, DNA polymerase cannot add complementary nucleotides to the 5′ end. Therefore, multiple primers are required.
- Due to this phenomenon, the DNA nucleotides synthesis from lagging strands occurs in fragments. These fragments are termed Okazaki fragments.
- Hence, the leading strand using only one primer synthesizes nucleotides continuously, while the lagging strand uses multiple primers and thus synthesizes nucleotides discontinuously.

Step 4: Termination

- RNA primers of both leading and lagging strands are cleaved out or degraded by exonucleases activity of DNA polymerase, and the nicks or gaps so formed are filled with DNA and sealed by the enzyme DNA ligase.
- DNA polymerase also shows proofreading activity and check, remove and replace any errors.
- Interestingly, in eukaryotic organisms having linear DNA, when RNA primer at 5′ end of daughter strand is removed, there is not a preceding 3′ OH such that DNA polymerase can use it to replace with DNA.
- So, at the 5′ end of daughter strands, there is a gap (missing DNA). This missing DNA can cause a loss of information contained in that region. This gap must be filled before the next round of replication.
- For solving this end replication problem, researchers have found that linear ends of DNA called telomere are used which contain specific G: C rich repeats. These sequences are known as telomere sequences.
- These telomere sequences do not code anything but are essential to fill in the gap in the daughter strand and maintain the integrity of DNA.
- Eventually, the replication forks terminate at terminating recognizing sequences (ter).
- The ter sequences are of around 23 base pairs which facilitate as the binding sites for TUS protein.
- This ter- TUS complex arrest replication fork and terminate.

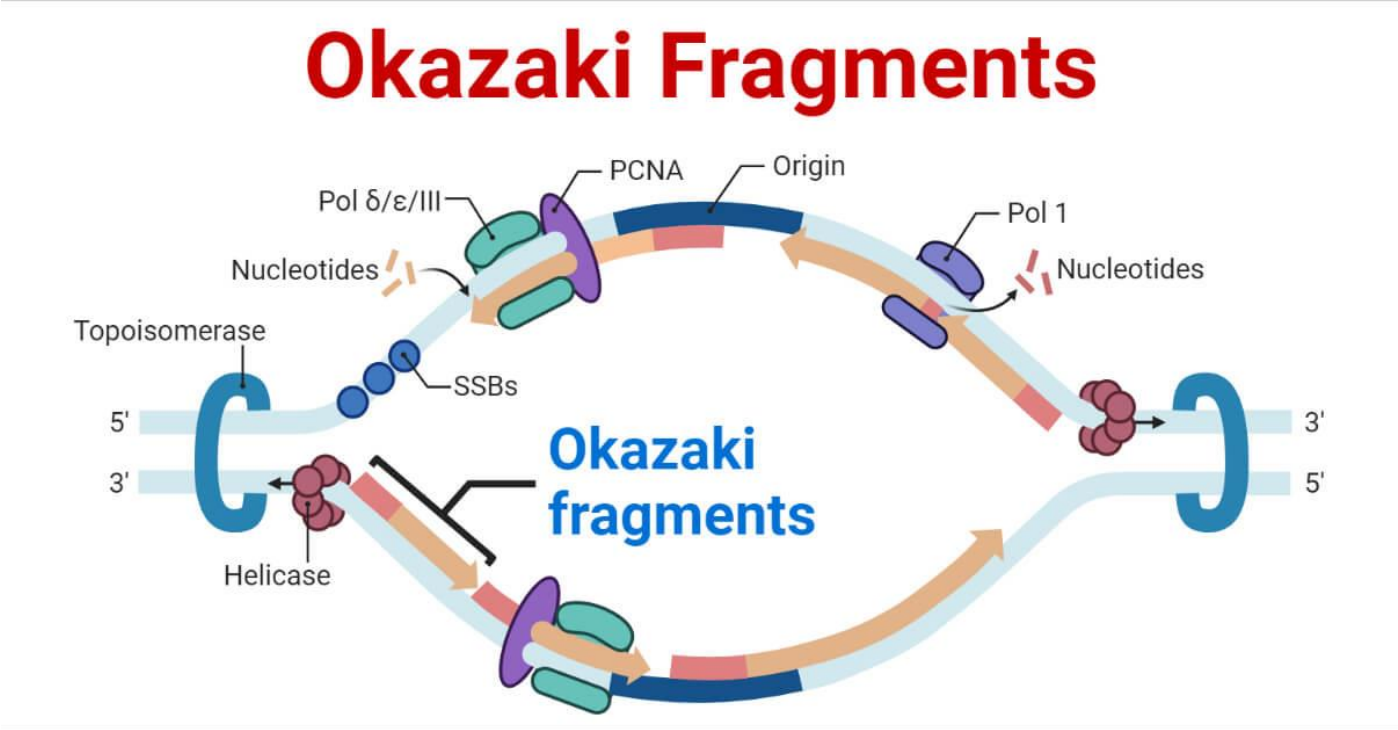
So, in summary, these are the 4 steps of DNA Replication:

1. Formation of Replication Fork
2. Initiation
3. Elongation
4. Termination

Okazaki Fragments

- The two DNA strands run in opposite or antiparallel directions, and therefore to continuously synthesize the two new strands at the replication fork requires that one strand is synthesized in the 5’to3′ direction while the other is synthesized in the opposite direction, 3’to 5′.
- However, DNA polymerase can only catalyze the polymerization of the dNTPs only in the 5’to 3’direction.
- This means that the other opposite new strand is synthesized differently. But how?
- By the joining of discontinuous small pieces of DNA that are synthesized backward from the direction of movements of the replication fork. These small pieces or fragments of the new DNA strand are known as the **Okasaki Fragments.**

- The Okasaki fragments are then joined by the action of DNA ligase, which forms an intact new DNA strand known as the lagging strand.
- The lagging phase is not synthesized by the primer that initiates the synthesis of the leading strand.
- Instead, a short fragment of RNA serves as a primer (RNA primer) for the initiation of replication of the lagging strand.
- RNA primers are formed during the synthesis of RNA which is initiated de novo, and an enzyme known as primase synthesizes these short fragments of RNA, which are 3-10 nucleotides long and complementary to the lagging strand template at the replication fork.
- The Okazaki fragments are then synthesized by the extension of the RNA primers by DNA polymerase.
- However, the newly synthesized lagging strand is that it contains an RNA-DNA joint, defining the critical role of RNA in DNA replication.



Okazaki Fragments

Replication Fork Formation and its function

- The replication fork is the site of active DNA synthesis, where the DNA helix unwinds and single strands of the DNA replicates.
- Several sites of origin represent the replication forks.
- The replication fork is formed during DNA strand unwinding by the helicase enzyme which exposes the origin of replication. A short RNA primer is synthesized by primase and elongation done by DNA polymerase.

- The replication fork moves in the direction of the new strand synthesis. The new DNA strands are synthesized in two orientations, i.e. 3′ to 5′ direction which is the leading strand, and the 5′ to 3′ orientation which is the lagging strand.
- The two sides of the new DNA strand (leading and lagging strand) are replicated in two opposite directions from the replication fork.
- Therefore, the replication fork is bi-directional.

Leading Strand

- The leading strand is the new DNA strand that is continuously synthesized by the DNA polymerase enzyme.
- It is the simplest strand that is synthesized during replication.
- The synthesis starts after the DNA strand has unzipped and separated. This generates a short piece of RNA known as a **primer**, by the DNA primase enzyme.
- The primer binds to the 3′ end (start) of the strand, thus initiating the synthesize of the new strand (leading strand).
- The synthesis of the leading strand is a continuous process.

The Lagging Strand

- This is the template strand (5′ to 3′) that is synthesized in a discontinuous manner by RNA primers.
- During the synthesis of the leading strand, it exposes small, short strands, or templates that are then used for the synthesis of the Okazaki fragments.
- The Okazaki fragments synthesize the lagging strand by the activity of DNA polymerase which adds the pieces of DNA (the Okazaki fragments) to the strand between the primers.
- The formation of the lagging strand is a discontinuous process because the newly formed strand (lagging strand) is the fragmentation of short DNA strands.

Applications of DNA Replication

- DNA replication makes the transfer of genetic information from one generation to another possible.
- It is an important phenomenon happening inside our cells, that allows the body to maintain homeostasis and integrity of the body.
- With the available information about DNA replication, scientific communities today have a proper idea of genome sequencing which has now been applied in different expertise ranging from clinical diagnosis to possible treatment of genetic diseases.
- DNA replication has made sequencing of whole human genome sequencing possible.
- Cloning of genes has also been possible by DNA replication.
- Enzymes involved in DNA replication have now been greatly studied due to their wider applications. The recent breakthrough Cas9/ CRISPR technology where nucleases are used to cleave the desired

portion of DNA and replace it with required nucleotides is the prime example of how we can use these enzymes and make potential advancements in them thus broadening and exploring their uses.

- Polymerase Chain Reaction uses DNA polymerases to repeatedly replicate DNA in-vitro and has numerous applications in diagnosis, sequencing, and recombinant DNA technology.
- The formation of complementary DNA (cDNA) can also be considered as an example of a wider application of the enzymes involved in DNA replication.
- There are various applications of DNA replication, we can even consider that if there is any technique involving genes, some way or the other DNA replication is applied.

DNA Replication Stress

During DNA replication, the process and the DNA genome undergoes various stress arising from the mechanism. these stresses an result in stalled replication and stalled replication fork formation. Several events contribute to these stresses, including;

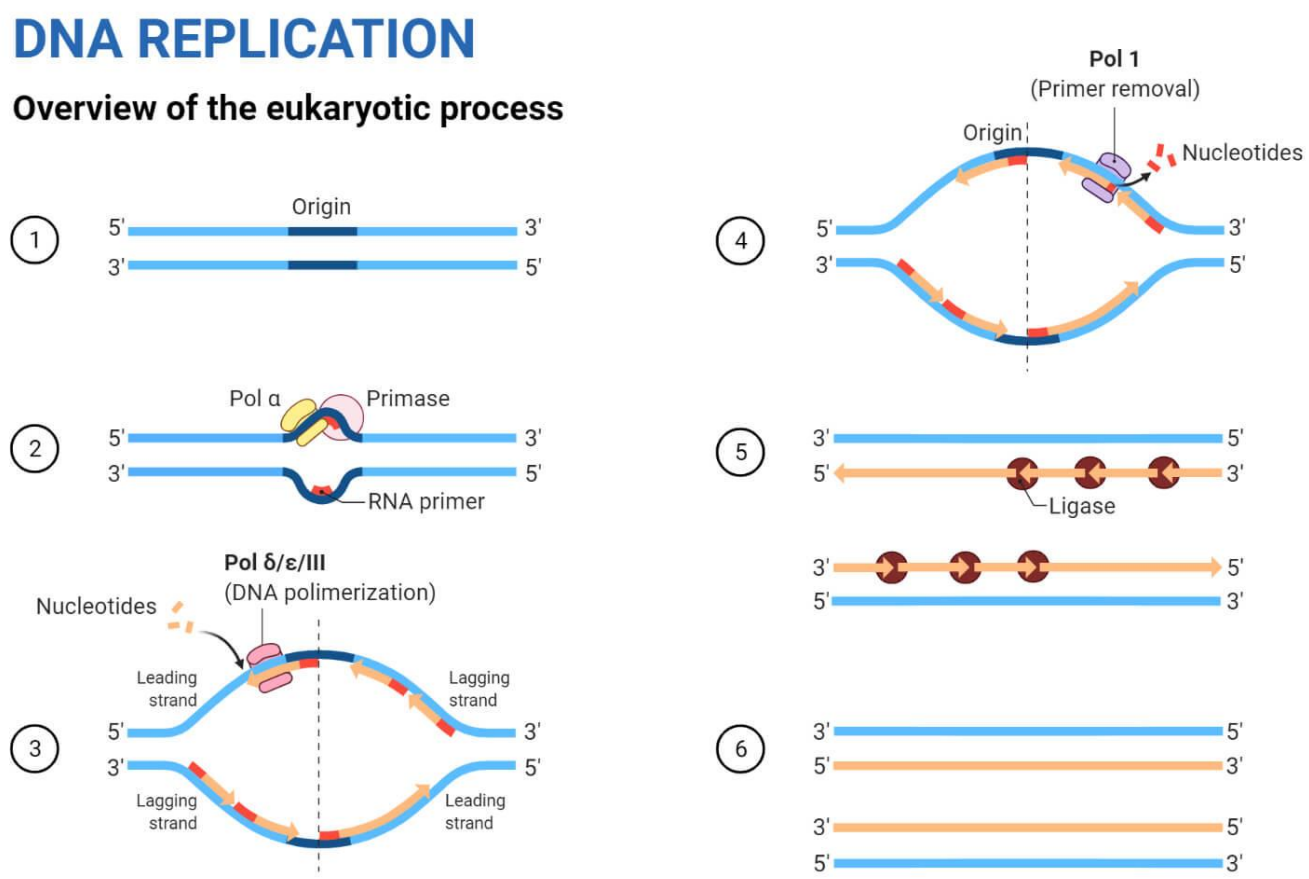
- Unusual DNA structure
- Mismatched ribonucleotides
- Tensions arising from concurrent mechanisms of replication and transcription
- Inadequate availability of important replication factors
- Fragile sites on the replicating DNA strand
- Overexpression or constitutive activation of oncogenes
- Inaccessible chromatins

Kinase regulatory proteins such as ATM (**ATM serine/threonine kinase**) and ATP are proteins that assist in alleviating replication stress. These proteins get recruited and activated by DNA damages.

Stalled replication forks may collapse if the regulatory proteins do not stabilize, and if and when this happens, initiation of repairing mechanisms to reassembling of the replication fork takes place. this helps to amend damages the damaged ends of DNA.

Similarities between Prokaryotic and Eukaryotic DNA Replication

- The unwinding mechanism of DNA before replication is initiated is the same for both Prokaryotes and eukaryotes.
- In both organisms, the DNA polymerase enzyme coordinated the synthesis of new DNA strands.
- Additionally, both organisms use the semi-conservative replication pattern, making the leading and lagging strands in different directions. Okasaki fragments make the lagging strand.
- Lastly, both organisms initiate DNA replication using a short RNA primer.



Eukaryotic DNA Replication

Eukaryotic vs. Prokaryotic DNA Replication (11 Major Differences)

S.N.	Eukaryotic DNA Replication	Prokaryotic DNA replication
1.	Occurs in eukaryotic cells.	Occurs in a prokaryotic cell.
2.	This process takes place in the cell's nucleus.	This process takes place in the cell's cytoplasm.
3.	There are multiple sites for the origin of replication per DNA molecule.	There is a single site for the origin of replication per DNA molecule.
4.	Initiation of DNA replication is carried out by multi-subunit proteins, origin recognition complex.	Initiation of DNA replication is carried out by protein DnaA and DnaB.
5.	Multiple replication forks are formed in a DNA molecule.	Only two replication forks are formed in a DNA molecule.

6.	Okazaki fragments are short of around 100-200 nucleotides in length	Okazaki fragments are large, around 1000-2000 nucleotides in length.
7.	It is a slow process with around 100 nucleotides added per second.	It is a fast process with around 2000 nucleotides added per second.
8.	DNA is linear and double-stranded.	DNA is circular and double-stranded.
9.	DNA polymerase involved in eukaryotic DNA replication is DNA polymerases ε, α, and δ.	DNA polymerase involved in prokaryotic DNA replication is DNA polymerase I, and III.
10.	Eukaryotic cells have telomeres at the end of DNA thus they are replicated.	Prokaryotic cells have circular DNA thus they are not replicated.
11.	DNA gyrase (Telomerase) is needed.	DNA gyrase (Telomerase) is not needed.

UNIT V: Central Dogma of Molecular Biology

Genome Organization

- Prokaryotic: Typically, circular DNA with no introns.
- Eukaryotic: Linear chromosomes with introns and exons.

Genetic Code

- Triplet code where three nucleotides code for one amino acid; universal and degenerate.

Transcription

- Prokaryotic: Occurs in the cytoplasm, involves simpler machinery.
- Eukaryotic: Occurs in the nucleus, involves complex processing (capping, polyadenylation, splicing).

Translation

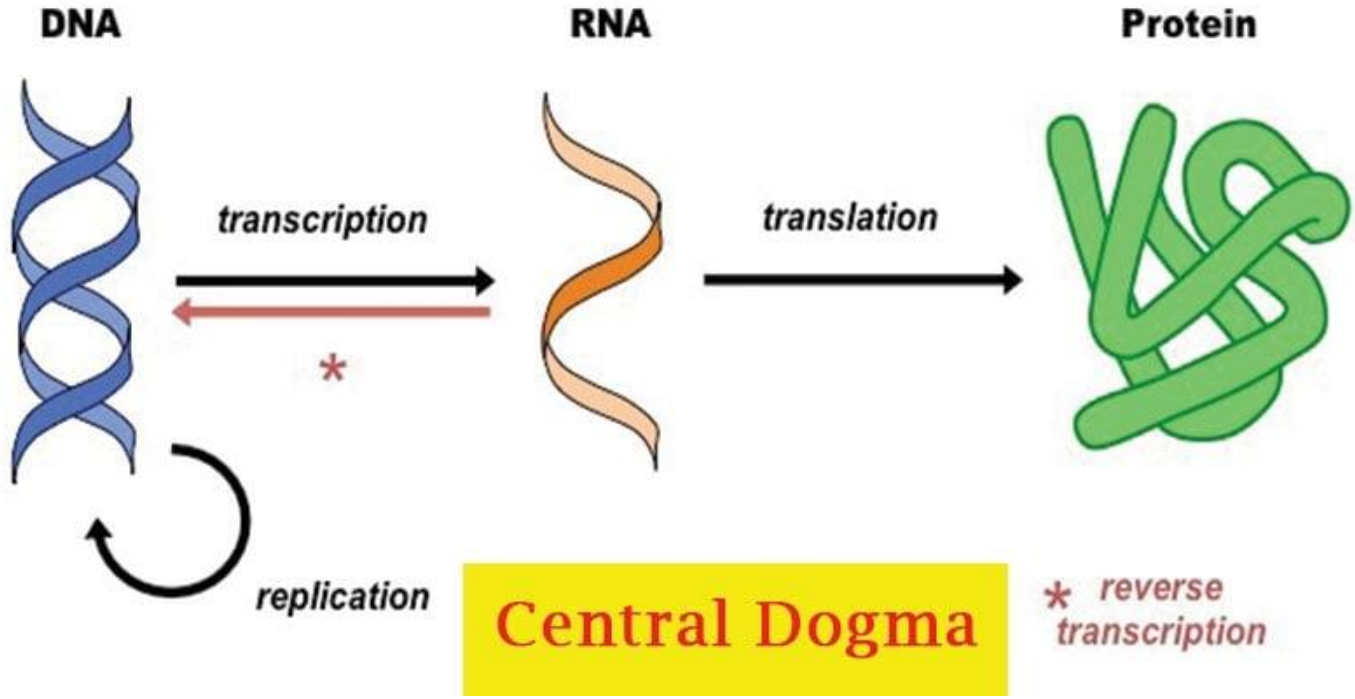
- Mechanism: Initiation, elongation, termination; involves ribosomes, mRNA, tRNA.
- Post-Translational Modification: Includes glycosylation and phosphorylation, modifying protein function and activity.

Central Dogma: Replication, Transcription, Translation

- **DNA** contains the complete genetic information that defines the structure and function of an organism.
- Proteins are formed using the **genetic code** of the DNA.
- Conversion of DNA encoded information to **RNA** is essential to form **proteins**.
- Thus, within most cells, the genetic information flows from – DNA to RNA to protein.
- The flow of information is followed through three different processes which are responsible for the inheritance of genetic information and for its conversion from one form to another:

1. **Replication:** a double stranded nucleic acid is duplicated to give identical copies. This process perpetuates the genetic information.
2. **Transcription:** a DNA segment that constitutes a gene is read and transcribed into a single stranded sequence of RNA. The RNA moves from the nucleus into the cytoplasm.
3. **Translation:** the RNA sequence is translated into a sequence of amino acids as the protein is formed. During translation, the ribosome reads three bases (a codon) at a time from the RNA and translates them into one **amino acid**.

- This flow of information is unidirectional and irreversible.



This explanation is the simplest way in which the **Central Dogma of Molecular Biology** is interpreted.

- In the bigger picture, the central dogma of molecular biology is an explanation of the flow of genetic information within a biological system.

- It was first stated by Francis Crick in 1958, as"Once 'information' has passed into protein it cannot get out again. In more detail, the transfer of information from nucleic acid to nucleic acid or from nucleic acid to protein may be possible, but transfer from protein to protein, or from protein to nucleic acid is impossible."

The Dogmas

- The dogma is a framework for understanding the transfer of sequence information between information-carrying biopolymers, DNA and RNA (both nucleic acids), and protein.
- There are 3×3=9 conceivable direct transfers of information that can occur between these.
- The dogma classes these into 3 groups of 3:

A. Three general transfers

- It describes the normal flow of biological information: DNA can be copied to DNA (DNA replication), DNA information can be copied into mRNA (transcription), and proteins can be synthesized using the information in mRNA as a template (translation).
- It is believed to occur normally in most cells.

B. Three special transfers

- The special transfers describe: RNA being copied from RNA (RNA replication), DNA being synthesised using an RNA template (reverse transcription), and proteins being synthesised directly from a DNA template without the use of mRNA.
- Temin (1970) reported the existence of an enzyme "RNA dependent DNA polymerase" (inverse transcriptase) which could synthesize DNA from a single stranded RNA template.
- Baltimore (1970) also reported the activity of this enzyme in certain RNA tumour viruses.
- This exciting finding in molecular biology gave rise to the concept of **central dogma reverse**" or teminism, suggesting that the sequence of information flow is not necessarily from DNA to RNA to protein but can also take place from RNA to DNA.
- It is known to occur, but only under specific conditions in case of some viruses or in a laboratory.

C. Three unknown transfers

- The unknown transfers describe: a protein being copied from a protein, synthesis of RNA using the primary structure of a protein as a template, and DNA synthesis using the primary structure of a protein as a template
- These are not thought to naturally occur.

Significance of the Central Dogma of Molecular Biology

Thus, the central dogma provides the basic framework for how genetic information flows from a DNA sequence to a protein product inside cells and thus give an insight to the important processes going on inside the cells.