UNIT – I FUNDAMENTALS OF CELL STRUCTURE:

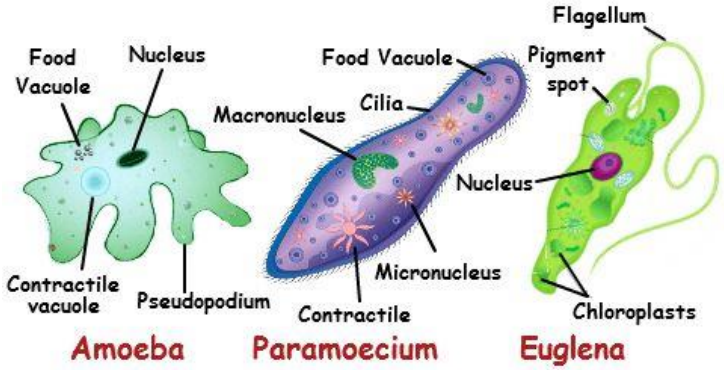
Diversity of cell size and shape. Cell theory, Protoplasm theory, Isolation and growth of cells; Basic properties of cells; Different classes of cells – Prokaryotic and eukaryotic cells. Cell division and cell cycle - Mitosis and meiosis, their regulation, steps in cell cycle, regulation and control of cell cycle.

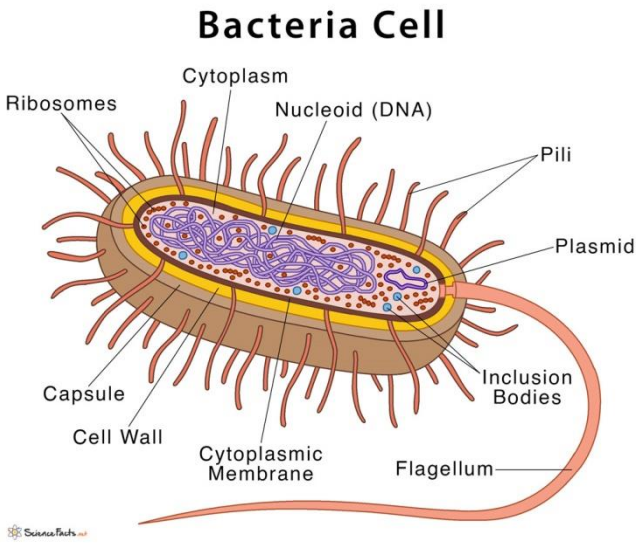
Diversity of cell size and shape

Introduction

A cell is the smallest unit of all living organisms. Cells are the basic building blocks of all organisms and organisms may be made of one cell (like bacteria) or multicellular made of many cells (like a human).

One Cell Organisms





Sizes and Shapes

Living organisms are made up of different types of cells, of different shapes and sizes.

A unicellular organism differs in shape from another unicellular organism. Within a multicellular organism, there are a variety of cells. Some are long while others are short; some are circular while some are oval. Shape and size vary from cell to cell according to their functions and composition. For example, a nerve cell is long and branched, meant for the transmission of signals throughout our body while a muscle cell is small and spindle-shaped which helps in movement.

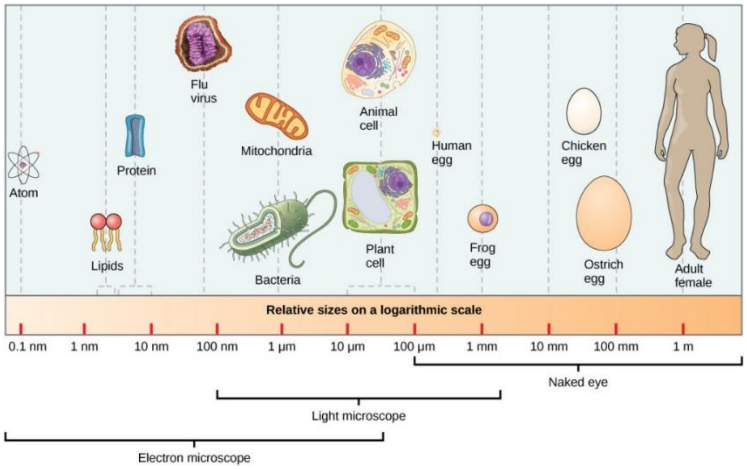
Considering an animal cell, we can generalize the shape of a cell as round (spherical) or irregular. Plant cells are much more rigid and

rectangular in shape. The size of a cell can be as small as 0.0001 mm (mycoplasma) and as large as six to twelve inches (Caulerpa taxifolia). Generally, the unicellular organisms are microscopic, like bacteria. But a single cell like an egg is large enough to touch. Whether regular or irregular in shape, they all consist of the same organelles and help us to perform the daily activities efficiently.

Cell Size

Range of Cell Sizes:

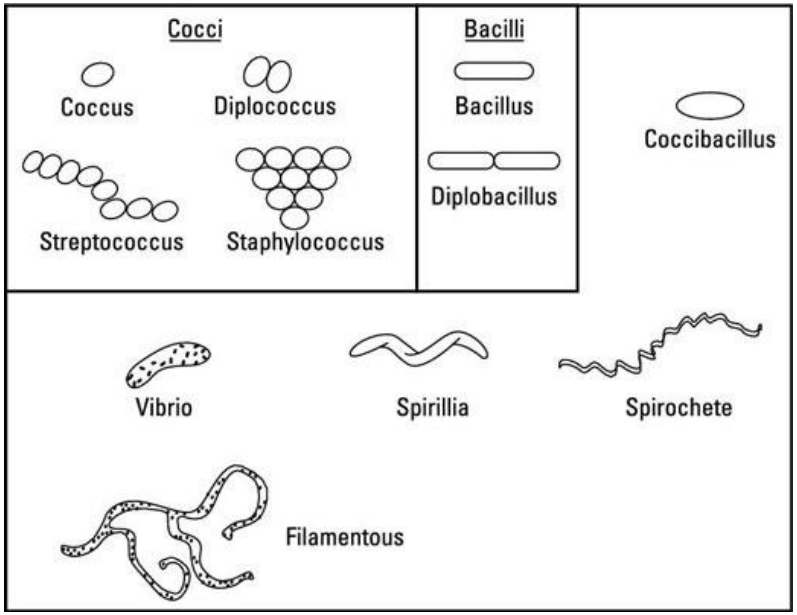
- Smallest cells: Mycoplasma bacteria (~0.1-0.2 micrometers).
- Largest cells: Ostrich egg yolk (up to 5 cm).
- Typical human cells: Red blood cells (~7-8 micrometers in diameter).



Cell Shape

Variety of Shapes: Cells come in various shapes, each suited to its function.

- Spherical (e.g., oocytes).
- Cuboidal (e.g., epithelial cells).
- Columnar (e.g., intestinal lining cells).
- Stellate (e.g., neurons).
- Fusiform (e.g., smooth muscle cells).
- Discoid (e.g., red blood cells).
- Irregular (e.g., white blood cells).



Cell theory

Definition

According to the cell theory, all biological organisms are made up of cells, the basic building blocks of life, and all life evolved from preexisting life. It is the cell theory that emphasizes the unity

underlying the diversity of forms, i.e., the cellular organization of all life forms.

Schleiden & Schwann were the first to introduce the idea of the cell theory in 1839, and it has remained the cornerstone of modern biology ever since. The cell theory continues to be the dominant theory of biology despite the numerous concepts that ultrastructural research and modern molecular biology have introduced.

Progression of Cell Theory

The discovery of cells would not have been possible without the advancement of the microscope. Objects that are too small to be seen with the naked eye are magnified with the help of a microscope.

Robert Hooke was the first person to coin the term cell (L., Cella = hollow space) in 1665. He used a custom-built compound microscope to look at a tiny slice of dry cork that had been cut from a larger piece. He then published a collection of essays under the title Micrographia which described cork as a honeycomb of chambers or "cells". It is now recognized that the chambers or cells are void spaces left behind after the living components of the cell have broken down.

The development of a more sophisticated microscope by Anton van Leeuwenhoek in 1673 led to his observation of numerous minute "animalcules" in water. Additionally, he conducted more research on sperm and red blood cells. Nevertheless, Leeuwenhoek's findings on bacteria and spermatozoa were largely disregarded for a long time.

Marcello Malpighi and Nehemiah Grew performed in-depth analyses of plant cells and confirmed that cellular structures are present throughout the entire plant body.

Malpighi referred to cells as "utriculi" and "saccule" in his 1671 publication "Anatome plantarum." Grew used the terms "bladders", "cells" and "pore" in his book "The Anatomy of Plants" and offered several illustrations of plant material that show he was aware of the cellular structure.

In 1838, a German botanist, Matthias Schleiden noticed that all plants are made up of many types of cells that constitute the plant's tissues after studying many plants.

Theodore Schwann (1839), a British zoologist, researched many animal cell types around the same time and found that cells had a thin outer layer known as the "plasma membrane." Similarly, he also concluded that the presence of a cell wall is a distinctive feature of plant cells based on his research on plant tissues.

Based on this, Schwann proposed the hypothesis that the bodies of animals and plants are composed of cells and products of cells. He summarized his observations into **three conclusions about cells:**

- **The cell is the unit of structure, physiology, and organization in living things.**
- **The cell retains a dual existence as a distinct entity and a building block in the construction of organisms.**
- **Cells form by free-cell formation, similar to the formation of crystals.**

However, Schwann's third conclusion stating that cells formed similarly to crystals, was discounted as this observation refers to the spontaneous generation of life. Schwann's theory also did not explain how new cells were formed.

Rudolf Virchow, a German pathologist, first explained that cells divide and new cells are formed from pre-existing cells and famously wrote "omnis cellula-e cellula." He modified the hypothesis of Schleiden and Schwann to give the cell theory a final shape.

Therefore, the **cell theory states**,

- **All organisms are composed of one or more cells.**
- **The cell is the basic unit of life in all living things.**
- **All cells are produced by the division of pre-existing cells.**

With his "swan-neck" experiment in 1865, Louis Pasteur further provided experimental proof in support of Virchow's extension of the cell hypothesis.

Modern Cell Theory

The original cell theory proposed by Schleiden and Schwann is supplemented by a few additional principles in the modern version; the three basic components of cell theory, plus four additional statements:

- The cell pass information from cell to cell during cell division using DNA.
- All cells have basically the same chemical composition and metabolic activities.
- All cells have basically the same chemical & physiological functions (movement, digestion etc.)
- Cell activity depends on the activities of structures within the cell (organelles, nucleus, plasma membrane).

Understanding the functioning of cells in both healthy and ill conditions paves the way for the creation of novel vaccinations, more potent medications, superior plants, and a greater understanding of how all living things function.

Exception of Cell Theory

Cell theory does not have a universal application, i.e., certain living organisms do not have true cells.

Viruses do not easily fit into the parameters of a true cell. They lack a plasma membrane and metabolic machinery for energy production and the synthesis of proteins.

The protozoan Paramecium, the fungus Rhizopus, and the algae Vaucheria are a few examples of additional organisms that do not fall inside the cell theory's purview. All of these organisms have bodies made up of a single, undivided mass of protoplasm that lacks any cellular organization and has several nuclei.

Protoplasm Theory

What is Protoplasm?

Protoplasm is the living part of the cell, which comprises of different cellular organelles. It is a jelly-like, colourless,

transparent and viscous living substances present within the cell wall.

Definition: The protoplasm theory is the idea that protoplasm is the fundamental living substance in all cells, serving as the physical basis of life. This theory posits that protoplasm is the essential material responsible for the vital activities of cells, making it the core component of both plant and animal life.

Key Points of the Protoplasm Theory

Origin and Development:

Felix Dujardin (1835): Observed a jelly-like substance in protozoa, calling it "sarcode."

Jan Evangelista Purkyně (1839): Used the term "protoplasm" to describe the living material within cells.

Hugo von Mohl (1846): Identified protoplasm as a crucial component in plant cells.

Max Schultze (1861): Unified the concept, stating that protoplasm is the physical basis of life, present in both plant and animal cells.

Composition of Protoplasm:

Water: 70-90%, providing a medium for biochemical reactions.

Proteins: Enzymes and structural proteins critical for cellular functions.

Lipids: Form cell membranes and serve as energy storage.

Carbohydrates: Energy sources and structural elements.

Nucleic Acids: DNA and RNA, responsible for genetic information storage and transfer.

Inorganic Salts: Ions essential for cellular processes like osmoregulation and signaling.

Structure of Protoplasm:

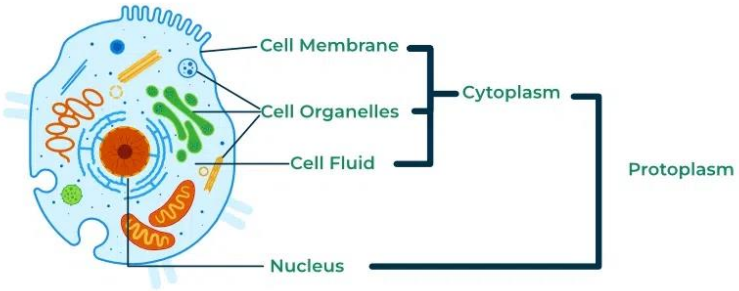
Cytoplasm: The substance outside the nucleus, including:

Cytosol: Fluid part where metabolic reactions occur.

Organelles: Specialized structures such as mitochondria, ER, Golgi apparatus, lysosomes, ribosomes, and the cytoskeleton.

Inclusions: Non-living substances like lipid droplets and pigment granules.

Nucleoplasm: The substance within the nucleus, containing chromatin and the nucleolus.



Functions of Protoplasm:

Metabolism: Encompasses all biochemical reactions necessary for life, including catabolic and anabolic processes.

Growth and Reproduction: Involves cell growth, division, and reproduction through mitosis and meiosis.

Response to Stimuli: Ability to react to environmental changes via signaling pathways.

Movement: Achieved through cytoskeletal elements and motor proteins.

Homeostasis: Maintenance of a stable internal environment.

Impact on Modern Cell Theory: Laid the groundwork for understanding that all living organisms are composed of cells, the basic units of life, and that all cells arise from pre-existing cells.

Shifted the focus from protoplasm as a homogeneous substance to a complex system with specialized organelles and functions.

Importance in Biology and Medicine

Foundation for Cell Biology: The protoplasm theory was a crucial step in recognizing the cell as the fundamental unit of life.

Biotechnological Applications: Understanding protoplasm has implications for genetic engineering, cell culture, and medical research.

Stem Cell and Cancer Research: Provides insights into cell differentiation, abnormal cell behaviors, and potential therapeutic interventions.

Drug Development and Synthetic Biology: Targeting cellular components to treat diseases and designing cells for industrial applications.

Summary

The protoplasm theory underscores the significance of the living material within cells, emphasizing its role in the fundamental processes of life. This theory has evolved to accommodate the complexity of cellular structures and functions, forming the basis of modern cell biology and contributing to advances in biotechnology and medicine.

Isolation and Growth of Cells

Introduction

Isolation and growth of cells are fundamental techniques in cell biology, biotechnology, and medical research. These processes allow scientists to study cell behavior, physiology, and genetic makeup under controlled conditions.

Isolation and Growth of Cells

A. Sources of Cells

Primary Cells: Directly isolated from living tissues (e.g., skin biopsies, blood samples, organs).

Advantages: Closely mimic the in vivo state.

Disadvantages: Limited lifespan, batch-to-batch variability.

Cell Lines: Cells that have been immortalized and can be cultured indefinitely (e.g., HeLa, CHO).

Advantages: Consistent, easy to culture.

Disadvantages: May not accurately represent primary cell behavior.

B. Methods for Isolation and Growth of Cells

Mechanical Methods

Tissue Dissection: Physically cutting tissues into smaller pieces to facilitate further processing and separation of cells.

Homogenization: Using mechanical force, such as blenders or homogenizers, to break down tissues into a cell suspension.

Sieving: Passing homogenized tissue through mesh screens to filter out larger debris and isolate cells.

Enzymatic Digestion

Collagenase: Enzyme that digests collagen, facilitating the release of cells from connective tissues. Commonly used for isolating cells from organs like liver and heart.

Trypsin: Protease that breaks down proteins, used primarily to detach adherent cells from culture surfaces.

Papain: Gentle enzyme often used for dissociating neuronal tissues due to its ability to maintain cell viability.

Density Gradient Centrifugation

Cells are separated based on their density by layering a cell suspension over a gradient medium (e.g., Ficoll or Percoll) and centrifuging it. Different cell types settle at different points in the gradient, allowing for their isolation.

Magnetic-Activated Cell Sorting (MACS)

Cells are labeled with magnetic beads conjugated to antibodies specific to cell surface markers. When passed through a magnetic field, labeled cells are retained, while unlabeled cells pass through. This method is highly specific and efficient.

Fluorescence-Activated Cell Sorting (FACS)

Cells are stained with fluorescently labeled antibodies and passed through a flow cytometer. The cytometer sorts cells based on the presence and intensity of fluorescence, providing high-purity cell populations.

Aseptic Techniques

Ensuring a sterile environment to prevent contamination, including the use of autoclaves for sterilizing equipment, working within laminar flow hoods, and wearing personal protective equipment like gloves, lab coats, and masks.

2. Growth of Cells

A. Cell Culture Environments

Adherent Cultures

- Cells grow attached to a surface like tissue culture plastic or glass.
- Surfaces may be coated with extracellular matrix proteins (e.g., collagen, fibronectin) to promote attachment.

Suspension Cultures

- Cells grow free-floating in the culture medium.
- Suitable for non-adherent cells such as hematopoietic cells and certain tumor cells.

B. Culture Media

Basal Media: Provides essential nutrients, vitamins, minerals, glucose, and buffering agents.

Common types: DMEM, RPMI-1640, MEM.

Serum: Often added to basal media to supply growth factors, hormones, and additional nutrients.

Commonly used: Fetal Bovine Serum (FBS).

Serum-Free Media: Designed to reduce variability and for specific applications like stem cell culture or therapeutic production.

C. Growth Conditions

- Temperature: Typically maintained at 37°C for mammalian cells.
- pH: Maintained around 7.2-7.4 using a CO2 incubator (5-10% CO2).
- Humidity: High humidity levels to prevent media evaporation.
- Oxygen Levels: Can be adjusted to normoxic or hypoxic conditions depending on cell type.

D. Passaging and Subculturing

- Passaging: Regularly transferring cells to new culture vessels to prevent over-confluence and maintain healthy growth.
- Trypsinization: Using trypsin-EDTA to detach adherent cells from the culture surface.
- Counting Cells: Using a hemocytometer or automated cell counter to determine cell density before seeding new cultures.

3. Applications of Cell Isolation and Growth

- Basic Research: Studying cell biology, genetics, and physiology.
- Drug Development: Screening and testing potential therapeutic compounds.
- Regenerative Medicine: Developing cell-based therapies for tissue repair and replacement.
- Cancer Research: Investigating cancer cell behavior, genetics, and treatment responses.

- Toxicology Testing: Assessing the safety and efficacy of chemicals and environmental agents.
- Vaccine Production: Growing virus-infected cells to produce vaccines.

4. **Challenges and Considerations**

- Contamination: Risk of bacterial, fungal, or mycoplasma contamination that can compromise cell cultures.
- Genetic Drift: Cell lines can acquire genetic changes over time, affecting experimental results.
- Ethical Issues: Using primary cells from human tissues requires ethical approval and donor consent.
- Reproducibility: Maintaining consistent culture conditions is crucial for reproducible results.

Summary

Isolation and growth of cells are essential techniques in modern biological research, enabling detailed study of cellular mechanisms and the development of new medical therapies. By understanding and controlling the conditions for cell culture, scientists can achieve significant advancements in various fields of biology and medicine.

Basic properties of cells

Cells, the fundamental units of life, exhibit several key properties that are essential for their function and survival. These properties provide the foundation for understanding cellular behavior, physiology, and their role in the larger context of biological systems.

1. Structural Organization

Plasma Membrane: The cell's boundary, composed of a phospholipid bilayer with embedded proteins, regulates the movement of substances in and out of the cell.

Cytoplasm: The internal environment of the cell, containing the cytosol, organelles, and cytoskeletal elements.

Nucleus: The control center of eukaryotic cells, housing the genetic material (DNA) and coordinating activities such as growth, metabolism, and reproduction.

2. Genetic Material

DNA (Deoxyribonucleic Acid): Stores genetic information necessary for the cell's functions, growth, and reproduction.

RNA (Ribonucleic Acid): Plays a key role in translating genetic information into proteins, with various types including mRNA, tRNA, and rRNA.

3. Metabolism

Anabolism: The process of building complex molecules from simpler ones, requiring energy (e.g., protein synthesis, DNA replication).

Catabolism: The breakdown of complex molecules into simpler ones, releasing energy (e.g., cellular respiration, glycolysis).

4. Energy Utilization

ATP (Adenosine Triphosphate): The primary energy currency of the cell, produced through cellular respiration in mitochondria (eukaryotic cells) or the cytoplasm (prokaryotic cells).

Mitochondria: Organelles in eukaryotic cells responsible for ATP production through oxidative phosphorylation.

5. Growth and Development

Cell Division: Process by which cells reproduce, involving mitosis (for somatic cells) and meiosis (for gametes).

Differentiation: The process by which cells develop specialized functions and structures, crucial for the development of multicellular organisms.

6. Response to Stimuli

Signal Transduction Pathways: Mechanisms by which cells detect and respond to external signals, involving receptors, second messengers, and effector molecules.

Receptors: Proteins on the cell surface or within cells that bind to signaling molecules, triggering a response.

7. Homeostasis

Regulation of Internal Environment: Maintaining stable conditions (e.g., pH, ion concentration, temperature) necessary for optimal cell function.

Transport Mechanisms: Include passive (diffusion, osmosis) and active (pumps, endocytosis, exocytosis) transport to maintain homeostasis.

8. Reproduction and Heredity

Replication of Genetic Material: Ensures genetic information is accurately passed on during cell division.

Inheritance: Transmission of genetic traits from one generation to the next through sexual or asexual reproduction.

9. Cellular Communication

Intercellular Communication: Cells communicate with each other through chemical signals (e.g., hormones, neurotransmitters) and physical contacts (e.g., gap junctions, plasmodesmata).

Intra-cellular Communication: Involves signaling pathways within the cell to coordinate cellular activities.

10. Adaptation and Evolution

Mutation: Changes in DNA sequence that can lead to variations in traits, providing a basis for evolution.

Natural Selection: Process by which advantageous traits become more common in a population over generations.

Summary

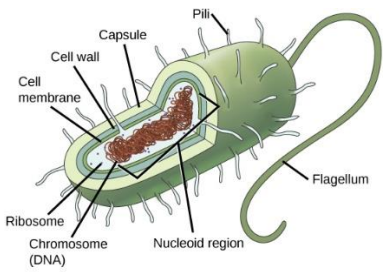
The basic properties of cells, including structural organization, genetic material, metabolism, energy utilization, growth, response to stimuli, homeostasis, reproduction, communication, and

adaptation, are fundamental to their function and survival. Understanding these properties provides insights into the complex behaviors of cells and their role in the larger context of life.

Prokaryotic Cells

All cells share four common components: (1) a plasma membrane, an outer covering that separates the cell's interior from its surrounding environment; (2) cytoplasm, consisting of a jelly-like region within the cell in which other cellular components are found; (3) DNA, the genetic material of the cell; and (4) ribosomes, particles that synthesize proteins. However, prokaryotes differ from eukaryotic cells in several ways.

In this illustration, the prokaryotic cell has an oval shape. The circular chromosome is concentrated in a region called the nucleoid. The fluid inside the cell is called the cytoplasm. Ribosomes, depicted as small circles, float in the cytoplasm. The cytoplasm is encased by a plasma membrane, which in turn is encased by a cell wall. A capsule surrounds the cell wall. The bacterium depicted has a flagellum protruding from one narrow end. Pili are small protrusions that project from the capsule in all directions.



A prokaryotic cell is a simple, single-celled (unicellular) organism that lacks a nucleus, or any other membrane-bound organelle. We will shortly come to see that this is significantly different in eukaryotes. Prokaryotic DNA is found in the central part of the cell: a darkened region called the nucleoid.

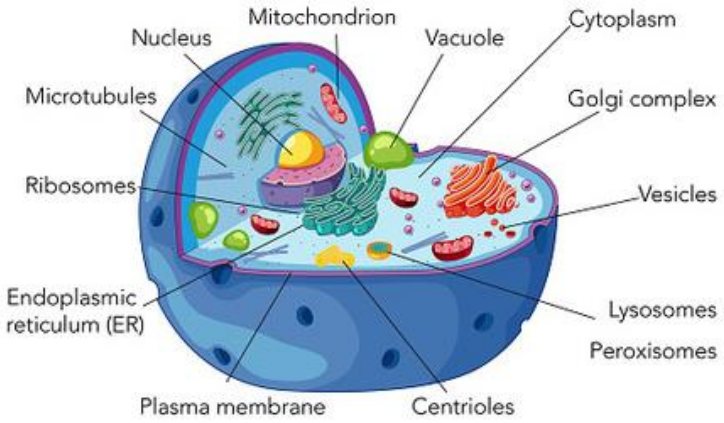
Unlike Archaea and eukaryotes, bacteria have a cell wall made of peptidoglycan, comprised of sugars and amino acids, and many have a polysaccharide capsule (Figure 1). The cell wall acts as an extra layer of protection, helps the cell maintain its shape, and prevents dehydration. The capsule enables the cell to attach to surfaces in its environment. Some prokaryotes have flagella, pili, or fimbriae. Flagella are used for locomotion, while most pili are used to exchange genetic material during a type of reproduction called conjugation.

Eukaryotic Cells

In nature, the relationship between form and function is apparent at all levels, including the level of the cell, and this will become clear as we explore eukaryotic cells. The principle "form follows function" is found in many contexts. It means that, in general, one can deduce the function of a structure by looking at its form, because the two are matched. For example, birds and fish have streamlined bodies that allow them to move quickly through the medium in which they live, be it air or water.

A eukaryotic cell is a cell that has a membrane-bound nucleus and other membrane-bound compartments or sacs, called organelles, which have specialized functions. The word eukaryotic means "true kernel" or "true nucleus," alluding to the presence of the

membrane-bound nucleus in these cells. The word "organelle" means "little organ," and, as we learned earlier, organelles have specialized cellular functions, just as the organs of your body have specialized functions.

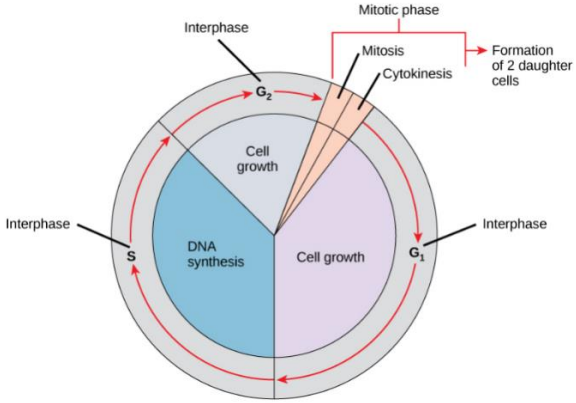


Cell division and cell cycle

Cell cycle

The cell cycle is an ordered series of events involving cell growth and cell division that produces two new daughter cells. Cells on the path to cell division proceed through a series of precisely timed and carefully regulated stages of growth, DNA replication, and nuclear and cytoplasmic division that ultimately produces two identical (clone) cells. The cell cycle has two major phases: interphase and the mitotic phase ((Figure)). During interphase, the cell grows and DNA is replicated. During the mitotic phase, the replicated DNA and cytoplasmic contents are separated, and the cell cytoplasm is typically partitioned by a third process of the cell cycle called cytokinesis. We should note, however, that interphase and mitosis (kayrokinesis) may take place without cytokinesis, in which case cells with multiple nuclei (multinucleate cells) are produced.

The cell cycle in multicellular organisms consists of interphase and the mitotic phase. During interphase, the cell grows and the nuclear DNA is duplicated. Interphase is followed by the mitotic phase. During the mitotic phase, the duplicated chromosomes are segregated and distributed into daughter nuclei. Following mitosis, the cytoplasm is usually divided as well by cytokinesis, resulting in two genetically identical daughter cells.



Interphase

During interphase, the cell undergoes normal growth processes while also preparing for cell division. In order for a cell to move from interphase into the mitotic phase, many internal and external conditions must be met. The three stages of interphase are called G1, S, and G2.

G1 Phase (First Gap)

The first stage of interphase is called the G1 phase (first gap) because, from a microscopic point of view, little change is visible. However, during the G1 stage, the cell is quite active at the biochemical level. The cell is accumulating the building blocks of chromosomal DNA and the associated proteins as well as accumulating sufficient energy reserves to complete the task of replicating each chromosome in the nucleus.

S Phase (Synthesis of DNA)

Throughout interphase, nuclear DNA remains in a semi-condensed chromatin configuration. In the S phase, DNA replication can proceed through the mechanisms that result in the formation of identical pairs of DNA molecules—sister chromatids—that are firmly attached to the centromeric region. The centrosome is also duplicated during the S phase. The two centrosomes of homologous chromosomes will give rise to the mitotic spindle, the apparatus that orchestrates the movement of chromosomes during mitosis. For example, roughly at the center of each animal cell, the centrosomes are associated with a pair of rod-like objects, the centrioles, which are positioned at right angles to each other. Centrioles help organize cell division. We should note, however, that centrioles are not present in the centrosomes of other eukaryotic organisms, such as plants and most fungi.

G2 Phase (Second Gap)

In the G2 phase, the cell replenishes its energy stores and synthesizes proteins necessary for chromosome manipulation and movement. Some cell organelles are duplicated, and the cytoskeleton is dismantled to provide resources for the mitotic phase. There may be additional cell growth during G2. The final preparations for the mitotic phase must be completed before the cell is able to enter the first stage of mitosis.

Cell division

Cell division is the complex phenomenon by which the mature cell divides and forms new daughter cells. It is the final and microscopically visible phase of an underlying change that has occurred at molecular and biochemical level. All cells are produced by the division of the pre-existing cells. Continuity of life depends on cell division. It becomes a means of reproduction in unicellular organisms. Thus, it is central to life of the cell and is essential for the perpetuation of the individual. There are two types of cell division in eukaryotic cells. They are:

1. Mitosis
2. Meiosis

Mitosis

Mitosis is the type of cell division in which a parent cell divides into two daughter cells with same number and type of chromosomes as are present in the parent cell. It occurs in somatic cells and it is meant for the multiplication of cell during embryogenesis and blastogenesis of plants and animals. It is important for replacement of cells lost to wear and tear and for wound healing. The process of mitosis involves a series of events both in nucleus and in cytoplasm. Thus it can be divided into two stages:

a) Karyokinesis or Nuclear Division

b) Cytokinesis or Cytoplasmic Division

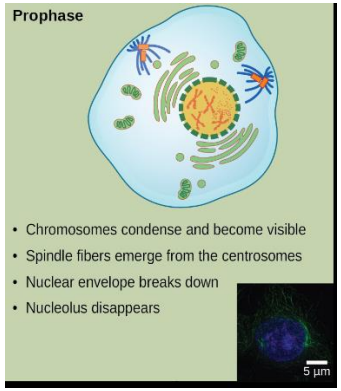
a)Karyokinesis (Division of Nucleus)

The process of Karyokinesis includes the division of nucleus into two daughter nuclei. The nucleus undergoes a number of complex but well organised and regular steps. The process of Karyokinesis can be separated into five phases. They are:

(i) Prophase (ii) Prometaphase (iii) Metaphase (iv) Anaphase

(v) Telophase

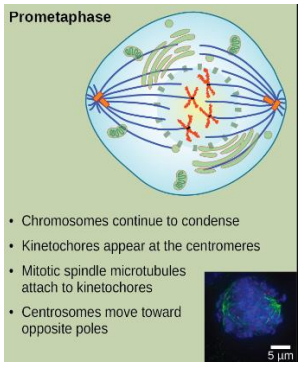
(i) Prophase



The appearance of thin-thread like condensing chromosomes marks the beginning of Prophase. The sister chromatids of each chromosome are held together by centromere. As prophase progresses, the chromatids become shorter and thicker, the chromosomes approach the nuclear envelope, causing the central space of the nucleus to become empty and nucleolus gradually disintegrates. In the cytoplasm of the early prophase, the centrioles become duplicated and each centriole starts migration towards the opposite poles. Between the separating centrioles spindle fibres are formed. In cells of higher plants, however spindle fibre forms without the aid of centriolesand lacks asters. Disappearance of the nuclear envelope marks the end of prophase.

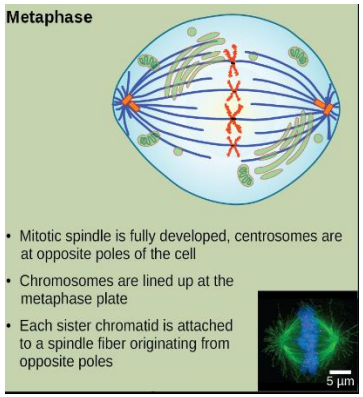
(ii) Prometaphase

The breakdown of nuclear envelope signals the commencement of Prometaphase. The spindle fibres captured the kinetochores of the chromosomes and tried to align them on the metaphase plate. As the metaphase approach, balanced bipolar forces hold chromosomes on the metaphase plate.



(iii) Metaphase

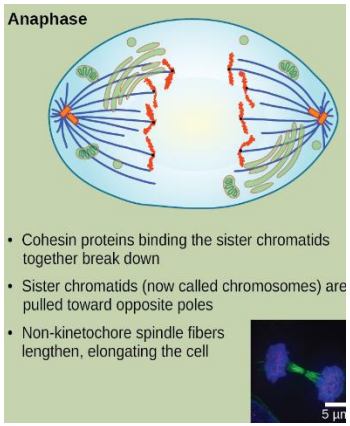
During metaphase the sister chromatids are still held together by centromere, and occupy the equatorial plate. The kinetochores of the two sister chromatids face opposite poles. The metaphase stage continues till all the chromosomes are arranged at the metaphase plate.



(iv) Anaphase

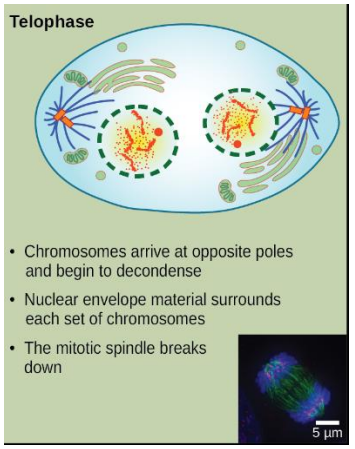
The anaphase begins abruptly with the synchronous splitting of each chromosome into its sister chromatids, and become chromosomes.

The daughter chromosomes move apart and migrate towards the opposite poles. During their migration, the centromeres remain foremost and the arms of the daughter chromosomes directed towards the equator so that the chromosomes characteristically appear U, V or J- shaped.



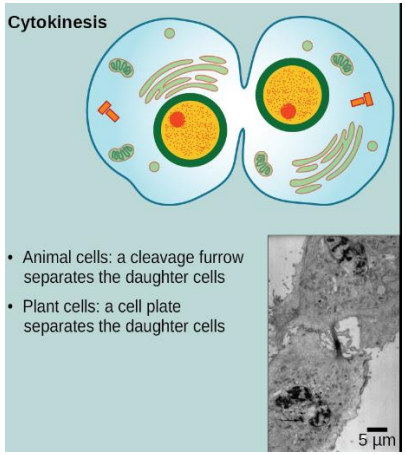
(v) Telophase

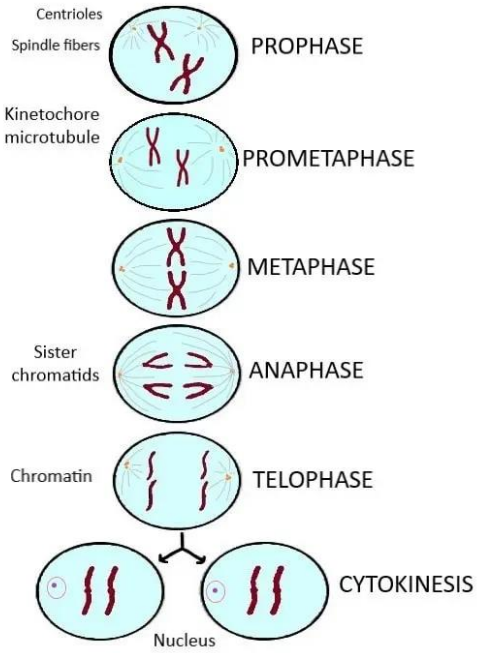
The telophase begins with the migrating daughter chromosomes reached the opposite poles.During it, the nuclear envelope reassembles around each group of chromosomes to form two daughter nuclei. The mitotic apparatus except the centrioles disappear, high viscosity of the cytoplasm decreases, the chromosomes resume their long, slender, extended form and RNA synthesis restarts causing the nucleolus to reappear.



b) Cytokinesis

The cytokinesis begins with the unfolding of the chromosomes and formation of the nuclear envelope. The cytoplasm constricts in the equatorial region and this constriction is accentuated and deepened until the cell divides. During it the cytoplasmic components including the mitochondria and Golgi complex are distributed to the two daughter cells.





1. Meiosis

It is a type of cell division in which the diploid parent cell duplicates its chromosomes only once, but divides twice in succession resulting in the production of four cells containing half the number of chromosomes present in the parent cell. It occurs in germ cells of sexually reproducing organisms. The two successive divisions take place without an intervening interphase. In the first division, a diploid parent cell produces two haploid cells and is known as reduction division. The exchange of genetic materials between the two non-sister chromatids occurs in this division. In the second meiotic division, the two haploid cells divide mitoticallyand result into four haploid cells. No pairing of the homologous chromosomes and exchange of genetic material takes place in the second meiotic division. Both the meiotic division I and division II are further divided into different stages.

Meiotic Division I

Meiotic division I is divided into five stages:

1. Prophase I
2. Prometaphase I
3. Metaphase I
4. Anaphase I
5. Telophase I

1.Prophase I

Prophase I is the longest stage of the meiotic division and includes the following substages:

(i) Proleptotene

In proleptotene the chromosomes are extremely thin, long and uncoiled and closely resembles with the early mitotic prophase.

(ii) Leptotne

In leptotene, the nucleus has increased in size and the chromosomes have become more apparent. Frequently the chromosomes have a definite polarisation and form loops whose ends are attached to the nuclear envelope at points near the centrioles.

(iii) Zygotene

The first essential phenomenon of meiosis that is synapsis occurs in the Zygotene. The homologous chromosomes become aligned and undergo pairing. The pairing may start from within the chromosomes and continue towards the end of the chromosome; in other cases, fusion occurs simultaneously at various places along the length of the chromosomes. The pairing is associated with the formation of highly organised but complex protein called synaptonemal complex.

(iv) Pachytene

In pachytene, the chromosomes contract longitudinally and they become thicker and shorter. In the middle of the Pachytene, each homologous chromosome splits lengthwise to form two chromatids. In late Pachytene, a line of separation perpendicular to the plane of pairing appears and four chromatids become visible. The exchange of genetic materials between the chromatids of the homologous chromosomes known as crossing over takes place during this substage.

(v) Diplotene

The separation of the paired homologous chromosome known as desynapsis starts at Diplotene. The synaptonemal complex appears dissolved, leaving participating chromatids of the paired homologous chromosomes physically joint at one or more discrete points called chiasmata.

(vi) Diakinesis

In the diakinesis, the bivalent chromosomes become more condensed and evenly distributed in the nucleus. The nucleolus detaches from the nucleolar organizer and ultimately disappears. The nuclear envelope breaks down. The chiasmata move from the centromere towards the end of the chromosomes and the intermediate chiasmata diminish. The chromatids remain connected by the terminal chiasma and these exist up to the metaphase.

2. Prometaphase

In the Prometaphase, the nuclear envelope disintegrates and spindle fibre arises in between the two centrioles which occupy the opposite poles. The chromosomes become greatly coiled, attached by the spindle fibres at their centromere and hold them at the equatorial plate.

3. Metaphase I

During metaphase I, the spindle fibres are attached to the centromeres of the homologous chromosomes. The centromere of each chromosome is directed towards the opposite poles. The repulsive forces between the homologous chromosomes increase greatly and the chromosomes become ready to separate.

4. Anaphase I

At anaphase I homologues chromosomes are freed from each other and due to the shortening of chromosomal fibres each homologous chromosome with its two chromatids and undivided centromere move towards the opposite poles of the cell. The actual reduction in the number of chromosomes occurs at this stage.

5. Telophase I

The arrival of a haploid set of chromosomes at each pole defines the onset of telophase I, during which nuclei are reassembled, the endoplasmic reticulum forms the nuclear envelope around the chromosomes and the chromosomes become uncoil. The nucleolus reappears and, thus, two daughter nuclei are formed. After the karyokinesis, cytokinesis occurs and two haploid cells are formed. Both the cells pass through a short resting interphase. During interphase, no DNA replication occurs.

Meiotic Division II

The second meiotic division is actually the mitotic division which divides each haploid meiotic cell into two haploid cells. The second meiotic division includes following four stages.

1. Prophase II

In the second prophase, each centriole divides into two and they migrate to the opposite poles. The spindle fibres are arranged at the right angle of the spindle of first meiosis. The nuclear membrane and

the nucleolus disappear. The chromosomes with two chromatids become short and thick.

2. Metaphase II

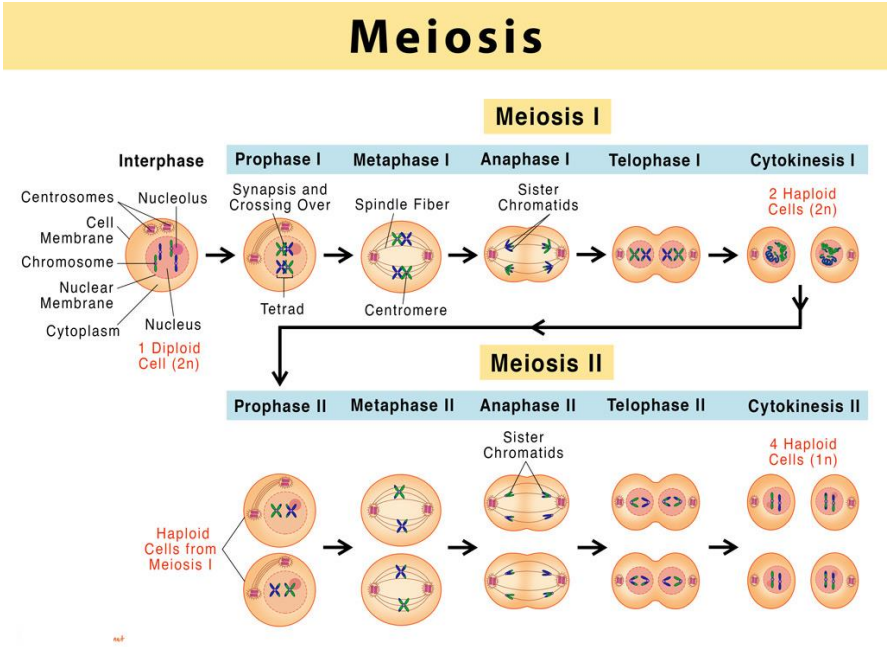
During metaphase II, the chromosomes get arranged on the equator of the spindle. The centromere divides into two and, thus, each chromosome produces two daughter chromosomes. The spindle fibres are attached with the centromere of the chromosomes.

3. Anaphase II

The daughter chromosomes move towards the opposite poles due to the shortening of chromosomal microtubules and stretching of interzonal microtubules of the spindle.

4. Telophase II

The chromosomes reach the opposite poles. The endoplasmic reticulum forms the nuclear envelope around the chromosomes and the nucleolus reappears. After the karyokinesis, in each haploid meiotic cell, the cytokinesis occurs and, thus, four haploid cells are resulted.



Comparison between Mitosis and Meiosis

1. Mitosis occurs continuously in the somatic cells while Meiosis occurs in the germ cells.
2. In mitosis each nuclear division is preceded by an interphase stage while in meiosis oneinterphase is followed by two successive nuclear divisions.
3. The prophase of mitosis is of short duration and includes no substage while theprophase of meiosis is of longer duration and it completes in six successive sub-stages.
4. The process of synapsis, crossing over and chiasmata formation do not take place betweenthe homologous chromosomes in mitosis while synapsis, crossing over and chiasmataformation is done between the homologous chromosomes in meiosis.

5. In the mitotic metaphase, the chromatids occur in the form of dyads while in meioticmetaphase I, the chromatids of the two homologous chromosomes occur as tetrads.
6. In the mitotic anaphase, the chromosomes have single chromatid while in meioticanaphase I the chromosomes have two chromatids and single centromere.
7. In mitosis the telophase always occurs while in meiosis the first telophase is sometimesomitted.
8. A diploid cell produces two diploid cells by a mitotic division while a diploid cell producesfour haploid cells by a meiotic division.

Conclusion

For the continuity of a cell and perpetuation of an individual, cell division is required. The division of the cell may be for the purpose of the growth of the individual, for the replacement of cells lost to wear and tear or for reproduction. Thus the cell division may be mitosis or meiosis. Mitosis results in the production of daughter cells which have chromosome as that of the parent cell, both quantitatively as well as qualitatively. The process of meiosis is much elongated in comparison to mitosis. It consists of two nuclear divisions after the interphase that results in the production of daughter cells having half the number of chromosomes present in the parent cell. During meiosis, the exchange of genetic materials between the non-sister chromatids occurred that led to the recombination of genes. It is genetically significant that it increases the chances of variation which is necessary for natural selection and evolution.

Regulation and control of cell cycle.

The length of the cell cycle is highly variable, even within the cells of a single organism. In humans, the frequency of cell turnover ranges from a few hours in early embryonic development, to an average of two to five days for epithelial cells, and to an entire human lifetime spent in G_0 by specialized cells, such as cortical neurons or cardiac muscle cells.

There is also variation in the time that a cell spends in each phase of the cell cycle. When rapidly dividing mammalian cells are grown in a culture (outside the body under optimal growing conditions), the length of the cell cycle is about 24 hours. In rapidly dividing human cells with a 24-hour cell cycle, the G_1 phase lasts approximately nine hours, the S phase lasts 10 hours, the G_2 phase lasts about four and one-half hours, and the M phase lasts approximately one-half hour. By comparison, in fertilized eggs (and early embryos) of fruit flies, the cell cycle is completed in about eight minutes. This is because the nucleus of the fertilized egg divides many times by mitosis but does not go through cytokinesis until a multinucleate “zygote” has been produced, with many nuclei located along the periphery of the cell membrane, thereby shortening the time of the cell division cycle. The timing of events in the cell cycle of both “invertebrates” and “vertebrates” is controlled by mechanisms that are both internal and external to the cell.

Regulation of the Cell Cycle by External Events

Both the initiation and inhibition of cell division are triggered by events external to the cell when it is about to begin the replication process. An event may be as simple as the death of nearby cells or as sweeping as the release of growth-promoting hormones, such as human growth hormone (HGH or hGH). A lack of HGH can *inhibit* cell division, resulting in dwarfism, whereas too much HGH can result in gigantism. Crowding of cells can also inhibit cell division. In contrast, a factor that can initiate cell division is the size of the cell: As a cell grows, it becomes physiologically inefficient due to its

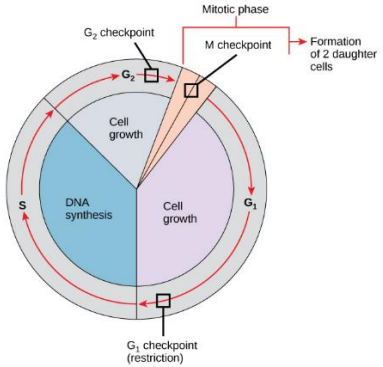
decreasing surface-to-volume ratio. The solution to this problem is to divide.

Whatever the source of the message, the cell receives the signal, and a series of events within the cell allows it to proceed into interphase. Moving forward from this initiation point, every parameter required during each cell cycle phase must be met or the cycle cannot progress.

Regulation at Internal Checkpoints

It is essential that the daughter cells produced be exact duplicates of the parent cell. Mistakes in the duplication or distribution of the chromosomes lead to mutations that may be passed forward to every new cell produced from an abnormal cell. To prevent a compromised cell from continuing to divide, there are internal control mechanisms that operate at three main cell-cycle checkpoints: A checkpoint is one of several points in the eukaryotic cell cycle at which the progression of a cell to the next stage in the cycle can be halted until conditions are favorable. These checkpoints occur near the end of G_1, at the G_2/M transition, and during metaphase ((Figure)).

The cell cycle is controlled at three checkpoints. The integrity of the DNA is assessed at the G_1 checkpoint. Proper chromosome duplication is assessed at the G_2 checkpoint. Attachment of each kinetochore to a spindle fiber is assessed at the M checkpoint.



The G_1 Checkpoint

The G_1 checkpoint determines whether all conditions are favorable for cell division to proceed. The G_1 checkpoint, also called the restriction point (in yeast), is a point at which the cell irreversibly commits to the cell division process. External influences, such as growth factors, play a large role in carrying the cell past the G_1 checkpoint. In addition to adequate reserves and cell size, there is a check for genomic DNA damage at the G_1 checkpoint. A cell that does not meet all the requirements will not be allowed to progress into the S phase. The cell can halt the cycle and attempt to remedy the problematic condition, or the cell can advance into G_0 and await further signals when conditions improve.

The G_2 Checkpoint

The G_2 checkpoint bars entry into the mitotic phase if certain conditions are not met. As at the G_1 checkpoint, cell size and protein reserves are assessed. However, the most important role of the G_2 checkpoint is to ensure that all of the chromosomes have been replicated and that the replicated DNA is not damaged. If the checkpoint mechanisms detect problems with the DNA, the cell cycle is halted, and the cell attempts to either complete DNA replication or repair the damaged DNA.

The M Checkpoint

The M checkpoint occurs near the end of the metaphase stage of karyokinesis. The M checkpoint is also known as the spindle checkpoint, because it determines whether all the sister chromatids are correctly attached to the spindle microtubules. Because the separation of the sister chromatids during anaphase is an irreversible step, the cycle will not proceed until the kinetochores of each pair of sister chromatids are firmly anchored to at least two spindle fibers arising from opposite poles of the cell.

UNIT – II CELLULAR MEMBRANES AND MATRICES:

Chemical composition and fluidity of membranes; lipid bilayer and membrane protein.Dynamic nature of membranes; membrane potentials; transportation across the cellmembrane; Diffusion, osmosis, ion channels, active transport, membrane pumps,mechanism of sorting and regulation of intracellular transport, Extracellular matrices –structure and function; cytoskeleton – structure and function.

Chemical composition and fluidity of membranes

The outer thin membrane or the layer of the living cell is known as the cell membrane.

Cell Membrane Overview:

The cell membrane, also known as the plasma membrane in animal cells and plasmalemma in plant cells, is the outermost layer of a living cell, providing protection and regulating substance movement.

The term "cell membrane" was introduced by Nageli and Cramer in 1885.

Types of Cell Membranes:

Cytoplasmic Membrane: Encloses the entire protoplasm, separating the cell's interior from its external environment.

Internal Membranes: Surround organelles and vacuoles, creating distinct compartments within the cell.

Composition:

Proteins (40-50%): Integral and peripheral proteins are embedded or attached to the lipid bilayer, involved in various functions including transport, signaling, and structural support.

Lipids (50-60%): Key components of the membrane, including:

Phospholipids (55% in plasma membrane): Form the primary structure of the lipid bilayer.

Glycolipids (5% in plasma membrane): Involved in cell recognition and communication.

Steroids (20% in plasma membrane): Cholesterol stabilizes the membrane's fluidity.

Other Lipids (20% in plasma membrane): Include various minor lipids with specialized functions.

Bacterial Membranes: Differ from eukaryotic membranes with higher cholesterol content (70%) and lower phospholipid content (30%).

Membrane Fluidity

Definition: The ability of lipid and protein molecules to move laterally within the lipid bilayer, contributing to the dynamic nature of the membrane.

Factors Affecting Fluidity:

Lipid Composition: Saturated fatty acids (more rigid) vs. unsaturated fatty acids (increase fluidity).

Cholesterol Content: Acts as a buffer, preventing the membrane from becoming too fluid at high temperatures and too rigid at low temperatures.

Temperature: Higher temperatures increase fluidity, while lower temperatures decrease it.

Importance of Fluidity:

Membrane Functionality: Essential for processes like diffusion, membrane protein function, vesicle formation, and cell signaling.

Adaptation: Cells adjust membrane fluidity to maintain function under varying environmental conditions.

Lipid Bilayer and Membrane Proteins

1. Lipid Bilayer

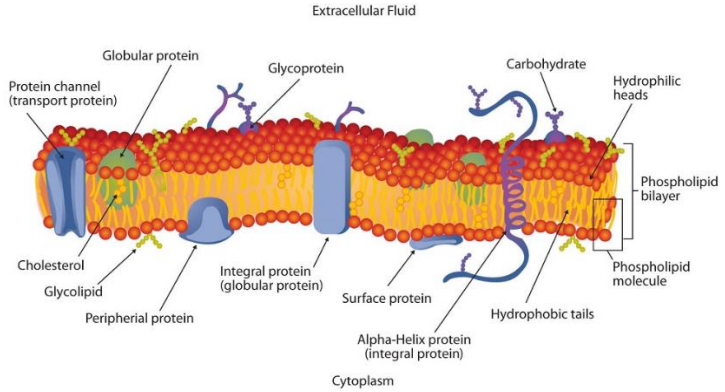
Structure:

Basic Composition: The lipid bilayer is composed primarily of phospholipids, which spontaneously arrange themselves into two opposing layers.

Outer Layer (Extracellular): Hydrophilic heads face outward, interacting with the aqueous external environment.

Inner Layer (Cytoplasmic): Hydrophilic heads face inward, towards the aqueous cytosol.

Hydrophobic Tails: The fatty acid tails of the phospholipids face each other in the interior of the bilayer, forming a hydrophobic core that excludes water-soluble substances.



Amphipathic Nature:

Hydrophilic Heads: Composed of a phosphate group and a polar molecule (e.g., choline, serine, inositol), these heads are attracted to water.

Hydrophobic Tails: Consist of long hydrocarbon chains (usually 16-18 carbon atoms) that are repelled by water.

Fluid Mosaic Model:

Fluidity: The lipid bilayer is fluid, meaning that lipids and proteins can diffuse laterally within their respective layers. This fluidity is crucial for membrane function and cellular processes such as endocytosis, exocytosis, and signal transduction.

Mosaic Pattern: The membrane's surface is a mosaic of different proteins and lipids, which are distributed unevenly.

Functions:

Barrier Formation: The lipid bilayer forms a selective barrier, preventing the free passage of ions and most water-soluble molecules while allowing the diffusion of small nonpolar molecules (e.g., oxygen, carbon dioxide).

Compartmentalization: Separates different cellular compartments and the cell from its external environment, creating distinct biochemical environments necessary for various cellular functions.

Flexibility and Self-Healing: The bilayer can self-heal if damaged, due to the spontaneous rearrangement of lipids. This flexibility is vital for maintaining cell integrity and function.

Fluidity Regulation:

Lipid Composition: The presence of unsaturated fatty acids in phospholipids introduces kinks in the hydrocarbon chains, preventing tight packing and increasing fluidity. Saturated fatty acids, in contrast, lead to a more rigid and less fluid membrane.

Cholesterol: Interspersed between phospholipids, cholesterol stabilizes membrane fluidity. It decreases fluidity at high temperatures by restraining phospholipid movement and prevents the membrane from becoming too rigid at low temperatures by disrupting the close packing of phospholipids.

Temperature: Membrane fluidity is temperature-dependent. Higher temperatures increase fluidity by providing more kinetic energy to lipid molecules, while lower temperatures decrease fluidity by reducing molecular movement.

Phospholipids

1. Overview

Phospholipids are a major class of lipids that form the structural foundation of cellular membranes. They are amphipathic molecules, meaning they have both hydrophilic (water-attracting) and hydrophobic (water-repelling) regions. This unique property allows them to form bilayers that are fundamental to the structure and function of biological membranes.

2. Structure of Phospholipids

Glycerol Backbone:

A three-carbon alcohol that serves as the foundation for the phospholipid molecule.

Fatty Acid Chains:Two long hydrocarbon chains attached to the glycerol backbone via ester bonds. These chains can vary in length and degree of saturation (saturated or unsaturated).

Saturated Fatty Acids: Have no double bonds between carbon atoms, resulting in straight chains that pack tightly.

Unsaturated Fatty Acids: Have one or more double bonds, introducing kinks in the chain that prevent tight packing and increase membrane fluidity.

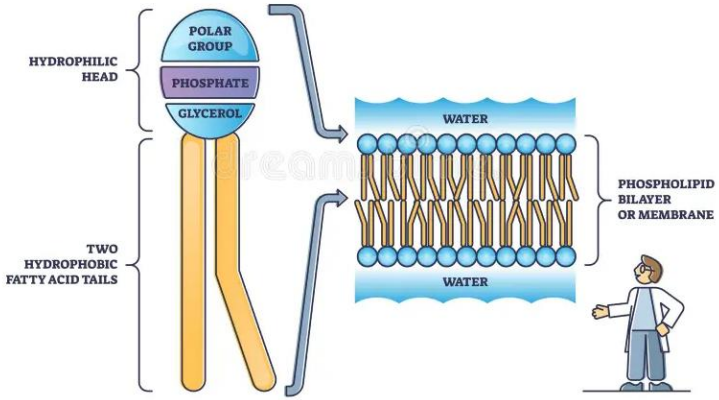
Phosphate Group:

Attached to the third carbon of the glycerol backbone. The phosphate group is hydrophilic and interacts with the aqueous environment.

Head Group:

Attached to the phosphate group. The nature of the head group determines the specific type of phospholipid (e.g., choline, serine, inositol).

General Phospholipid Structure:



Hydrophilic Head: Composed of the phosphate group and the head group, facing outward toward the aqueous environments (both intracellular and extracellular).

Hydrophobic Tails: Two fatty acid chains facing inward, away from water, forming the internal hydrophobic core of the bilayer.

3. Types of Phospholipids

Phosphatidylcholine (PC):

Structure: Glycerol backbone, two fatty acids, phosphate group, and choline head group.

Functions: Major component of cell membranes, involved in lipid metabolism, supports liver function, and contributes to cognitive health by aiding acetylcholine production.

Sources: Egg yolks, soybeans, sunflower seeds.

Phosphatidylserine (PS):

Structure: Glycerol backbone, two fatty acids, phosphate group, and serine head group.

Functions: Maintains membrane integrity, involved in cell apoptosis (programmed cell death), and supports cognitive functions such as memory and learning.

Sources: Soy lecithin, white beans, supplements.

Phosphatidylinositol (PI):

Structure: Glycerol backbone, two fatty acids, phosphate group, and inositol head group.

Functions: Essential for signal transduction, membrane dynamics, and organization of the cytoskeleton. Acts as a precursor for important signaling molecules (e.g., inositol trisphosphate).

Sources: Soybeans, sunflower seeds, and synthesized within the body.

4. Functions of Phospholipids

Membrane Formation:

Form the basic structure of biological membranes, creating a bilayer that acts as a barrier to separate the cell's interior from the external environment.

Fluidity and Flexibility:

The presence of unsaturated fatty acids contributes to membrane fluidity and flexibility, essential for membrane dynamics and function.

Signaling:

Some phospholipids are involved in signaling pathways, such as phosphatidylinositol, which plays a role in cell signaling and regulation.

Cell Recognition and Adhesion:

Phospholipids with carbohydrate modifications (glycolipids) are involved in cell recognition and adhesion processes.

5. Phospholipid Bilayer Formation

Self-Assembly:

Phospholipids spontaneously form bilayers when placed in an aqueous environment. This self-assembly is driven by the hydrophobic effect, where hydrophobic tails avoid water and aggregate in the interior, while hydrophilic heads interact with water.

Dynamic Structure:

The bilayer is not static; it exhibits fluidity, with lipids and proteins moving laterally within their respective layers. This fluid mosaic model supports various cellular processes, including membrane fusion, transport, and cell signaling.

Membrane Proteins

Types:

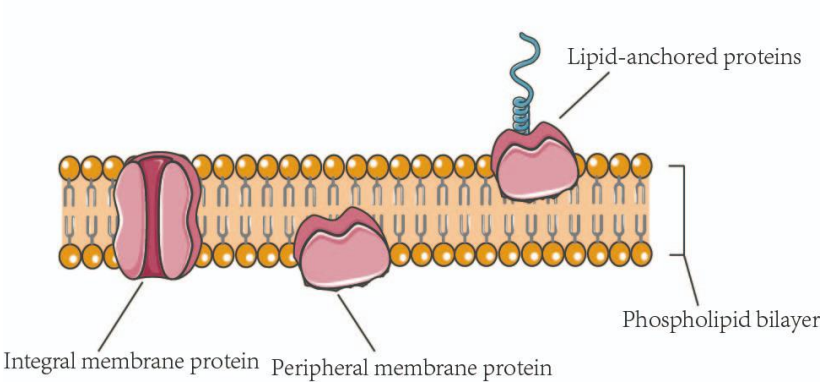
Integral Proteins:

Transmembrane Proteins: Span the entire lipid bilayer, with regions exposed on both the extracellular and cytoplasmic sides of the membrane. They include:

Channel Proteins: Form pores that allow specific ions or molecules to pass through the membrane.

Carrier Proteins: Bind to specific molecules and undergo conformational changes to shuttle them across the membrane.

Monotropic Proteins: Attached to only one side of the membrane, interacting with one leaflet of the bilayer without crossing it.



Peripheral Proteins:

Attachment: Loosely attached to the lipid bilayer or integral proteins through electrostatic interactions or hydrogen bonds.

Functions: Often involved in signaling pathways, maintaining cell shape, and facilitating communication between cells. They can be easily dissociated from the membrane by changes in ionic strength or pH.

Lipid-anchored membraneproteins

Attachment: Lipid-anchored proteins are covalently linked to membrane lipids via GPI anchors, prenylation, palmitoylation, or myristoylation, positioning them on the extracellular or cytoplasmic side of the membrane.

Functions: They play key roles in cell signaling, adhesion, membrane organization, and dynamic regulation by stabilizing proteins, organizing lipid rafts, and facilitating protein interactions.

Functions:

Transport:

Passive Transport: Utilizes channel and carrier proteins to facilitate the movement of molecules down their concentration gradient without energy expenditure (e.g., facilitated diffusion).

Active Transport: Involves carrier proteins that move molecules against their concentration gradient, requiring energy (usually from ATP) to drive the process (e.g., sodium-potassium pump).

Enzymatic Activity:

Catalysis: Membrane proteins can act as enzymes that catalyze chemical reactions at the membrane surface or within the membrane.

Examples: ATPases, which hydrolyze ATP to provide energy for various cellular processes.

Signal Transduction:

Receptors: Membrane proteins act as receptors that bind to extracellular signaling molecules (e.g., hormones) and transmit signals into the cell, initiating a cellular response.

Examples: G-protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs).

Cell-Cell Recognition:

Glycoproteins and Glycolipids: Carbohydrate chains attached to membrane proteins and lipids that are involved in cell recognition, adhesion, and communication.

Examples: Major Histocompatibility Complex (MHC) molecules and blood group antigens.

Structural Support:

Cytoskeleton Linkage: Membrane proteins anchor to the cytoskeleton, helping maintain cell shape and stability.

Examples: Integrins and spectrin.

Dynamic nature of membranes; membrane potentials

Dynamic Nature of Membranes

The biological membrane is not static but exhibits remarkable **dynamic behavior** that is essential for its function. It is a semi-permeable structure primarily composed of lipids, proteins, and carbohydrates.

1. Key Components of Membrane Dynamics

- **Lipid Bilayer Fluidity**:
  - Composed of phospholipids and cholesterol, the bilayer allows lateral diffusion of its components.
  - Factors influencing fluidity:
    - **Fatty Acid Composition**: Unsaturated fatty acids increase fluidity.
    - **Cholesterol**: Acts as a fluidity buffer, stabilizing membranes across temperature changes.
    - **Temperature**: Higher temperatures increase fluidity; lower temperatures decrease it.
- **Membrane Proteins**:
  - Can be integral (embedded) or peripheral (loosely associated).
  - Exhibit lateral mobility within the membrane, crucial for signal transduction and cell communication.
- **Asymmetry of Lipids**:
  - Different lipid compositions exist in the inner and outer leaflets of the bilayer.
  - Lipid flipping and flopping occur via **flippases**, **floppases**, and **scramblases**, aiding in cellular signaling and homeostasis.

2. Membrane Dynamics in Function

- **Endocytosis and Exocytosis**:
  - Membranes dynamically invaginate (endocytosis) or fuse (exocytosis) for material transport.
- **Membrane Trafficking**:
  - Vesicles bud from organelles like the ER and Golgi to transport molecules.
- **Signal Transduction**:
  - Receptors in the membrane detect and transmit external signals through conformational changes.

Transportation Across the Cell Membrane

The cell membrane facilitates the movement of substances in and out of the cell, maintaining homeostasis and enabling cellular functions.

1. Diffusion

- **Definition**: Movement of molecules from a region of higher concentration to a region of lower concentration.
- **Types**:

- **Simple Diffusion**: Passive movement of small, nonpolar molecules directly through the lipid bilayer.
- **Facilitated Diffusion**: Uses **carrier proteins** or **channel proteins** for the transport of polar or larger molecules (e.g., glucose, ions).

2. Osmosis

- **Definition**: The movement of water molecules through a selectively permeable membrane from a region of lower solute concentration to higher solute concentration.
- **Key Components**:
  - **Aquaporins**: Specialized channels facilitating water movement.
  - **Tonicity**:
    - **Hypertonic**: Water moves out, causing cell shrinkage.
    - **Hypotonic**: Water moves in, causing cell swelling.
    - **Isotonic**: No net water movement.

3. Ion Channels

- **Definition**: Proteins that form pores in the membrane to allow selective ion passage.
- **Types**:
  - **Voltage-Gated Channels**: Open/close in response to changes in membrane potential.
  - **Ligand-Gated Channels**: Respond to specific chemical signals (e.g., neurotransmitters).
  - **Mechanically Gated Channels**: Open due to physical stress (e.g., stretch).

4. Active Transport

- **Definition**: Energy-dependent movement of molecules against their concentration gradient.
- **Key Features**:
  - Requires ATP.
  - Involves **transport proteins** like pumps.

5. Membrane Pumps

- Utilize ATP to move ions/molecules against their gradients.

6. Mechanism of Sorting and Regulation of Intracellular Transport

- **Endoplasmic Reticulum (ER)**: Synthesizes and sorts proteins for secretion or organelle targeting.
- **Golgi Apparatus**: Modifies and packages proteins and lipids for transport.
- **Vesicles**: Aid in intracellular transport and exocytosis.
- **Regulation**:
  - Signal sequences on proteins direct them to their specific destinations.
  - Rab proteins and SNARE complexes regulate vesicle docking and fusion.

Extracellular Matrices (ECM)

Structure

- Composed of **proteins** (e.g., collagen, elastin), **glycoproteins** (e.g., fibronectin, laminin), and **proteoglycans** (e.g., hyaluronic acid).

- Organized into a complex network surrounding cells, particularly in connective tissues.

Function

- Provides **structural support** to tissues.
- Facilitates **cell adhesion** and **migration**.
- Regulates cellular behavior through **mechanotransduction**.
- Acts as a reservoir for growth factors.

Cytoskeleton

Structure

The cytoskeleton is a dynamic network of protein filaments, including:

1. **Microfilaments**: Composed of **actin**, these filaments support cell shape and enable movement.
2. **Intermediate Filaments**: Provide tensile strength and structural integrity (e.g., keratin).
3. **Microtubules**: Composed of **tubulin**, they form tracks for intracellular transport and contribute to cell division.

Function

- Maintains cell shape.
- Facilitates intracellular transport via motor proteins:
  - **Kinesin**: Moves cargo toward the cell periphery
  - **Dynein**: Moves cargo toward the cell center
- Plays a role in cell motility (e.g., pseudopodia in amoeboid movement).
- Essential for mitosis and cytokinesis by forming the mitotic spindle.

Summary Table

Feature	Key Role
Diffusion	Passive movement of small molecules across the membrane.
Osmosis	Water movement through a membrane driven by solute concentration differences.
Ion Channels	Facilitate ion flow to maintain cellular potential and signaling.
Active Transport	Moves molecules against gradients using ATP (e.g., Na+/K+Na^+/K^+Na+/K+ pump).
ECM	Provides structural support, regulates signaling, and anchors cells.
Cytoskeleton	Maintains cell shape, aids transport, and enables division and motility.

UNIT –III CELLULAR ORGANELLES IN METABOLISM:

Endoplasmic reticulum (ER)– smooth & rough; functions of ER; Golgi complex – structure and function; Ribosomes – Types, structure and function; Morphology and functions of peroxisomes and glyoxisomes; Plant cell vacuoles; endocytic pathways – endocytosis, phagocytosis; membrane trafficking.

Endoplasmic reticulum (ER)

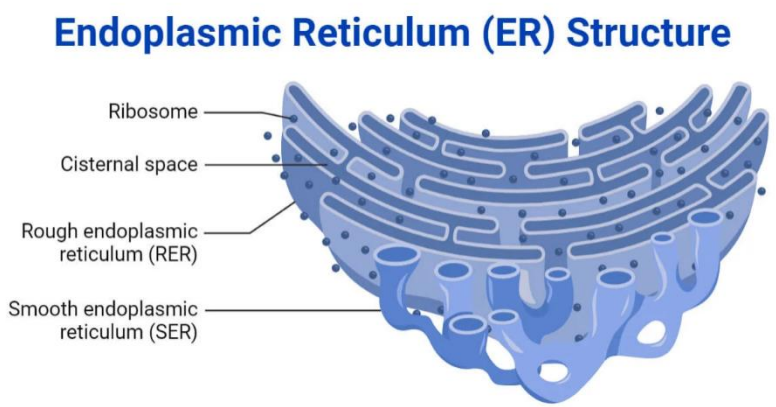
Endoplasmic Reticulum is a complex network of tubular membranes exclusively present in the cytoplasm of the eukaryotic cell.

Structure of Endoplasmic Reticulum (ER)

- The membrane of the endoplasmic reticulum is 50 to 60 Aº thickness and fluid-mosaic like the unit membrane of the plasma membrane.
- The membranes of the endoplasmic reticulum are found to contain many kinds of enzymes that are needed for various important synthetic activities.
- The membrane of endoplasmic reticulum remains continuous with the membranes of the plasma membrane, nuclear membrane, and Golgi apparatus.
- The cavity of the endoplasmic reticulum is well developed and acts as a passage for the secretory products.

The endoplasmic reticulum may occur in the following three forms:

- Lamellar form or cisternae
- Vesicular form or vesicle and
- Tubular form or tubules.



The Cisternae

- RER usually exists as cisternae that occur in those cells which have synthetic roles as the cells of the pancreas, notochord, and brain.
- The cisternae are long, flattened, sac-like, unbranched tubules having a diameter of 40 to 50 µm.
- They remain arranged parallelly in bundles or stakes.

The Vesicles

- The vesicles are oval; membrane-bound vacuolar structures having a diameter of 25 to 500 µm.
- They often remain isolated in the cytoplasm and occur in most cells but especially abundant in the SER.

The Tubules

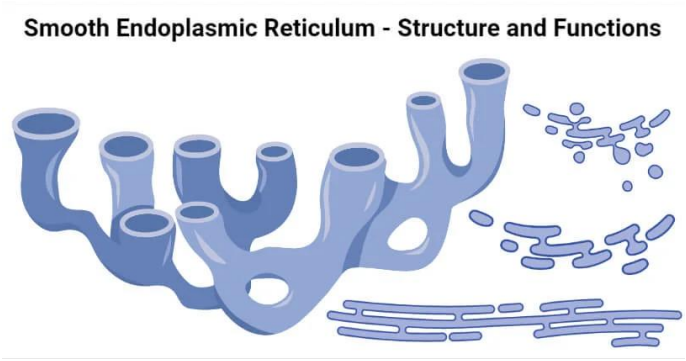
- The tubules are branched structures forming the reticular system along with the cisternae and vesicles.

- They usually have a diameter from 50 to 190 µm and occur almost in all the cells.
- Tubular form of ER is often found in SER and is dynamic in nature, i.e., it is associated with membrane movements, fission and fusion between membranes of cytocavity network.

Types of Endoplasmic Reticulum (ER)

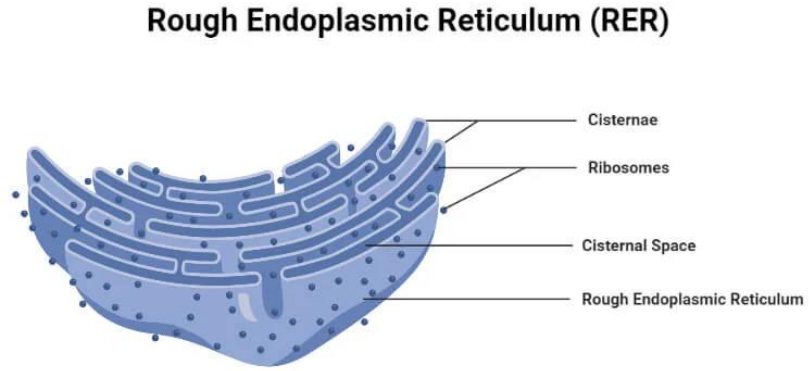
1. Smooth Endoplasmic Reticulum

- They are also called as the agranular endoplasmic reticulum.
- This type of endoplasmic reticulum possesses smooth walls because the ribosomes are not attached to its membranes.
- The smooth type of endoplasmic reticulum occurs mostly in those cells, which are involved in the metabolism of lipids (including steroids) and glycogen. Eg. adipose cells, interstitial cells, glycogen storing cells of the liver, conduction fibers of heart, spermatocytes, and leucocytes.



2. Rough Endoplasmic Reticulum

- It possesses rough walls because the ribosomes remain attached to its membranes.
- On their membranes, rough ER (RER) contains certain ribosome specific, transmembrane glycoproteins, called ribophorins I and II, to which are attached the ribosomes while engaged in polypeptide synthesis.
- The rough type of endoplasmic reticulum is found abundantly in those cells which are active in protein syntheses such as pancreatic cells, plasma cells, goblet cells, and liver cells.



Functions of Endoplasmic Reticulum (ER)

- ✓ Functions of smooth ER include lipid metabolism (both catabolism and anabolism; they synthesize a variety of phospholipids, cholesterol, and steroids).
- ✓ Glycogenolysis (degradation of glycogen; glycogen being polymerized in the cytosol).
- ✓ Drug detoxification (by the help of the cytochrome P-450).

- ✓ The endoplasmic reticulum provides an ultrastructural skeletal framework to the cell and gives mechanical support to the colloidal cytoplasmic matrix.
- ✓ The exchange of molecules by the process of osmosis, diffusion and active transport occurs through the membranes of the endoplasmic reticulum.
- ✓ The endoplasmic reticulum is the main component of the endomembrane system, also called the cytoplasmic vacuolar system or cytocavity network.
- ✓ The endoplasmic membranes contain many enzymes that perform various synthetic and metabolic activities. Further, the endoplasmic reticulum provides an increased surface for various enzymatic reactions.
- ✓ The endoplasmic reticulum acts as an intracellular circulatory or transporting system.
- ✓ As a growing secretory polypeptide emerges from the ribosome, it passes through the RER membrane and gets accumulated in the lumen of RER. Here, the polypeptide chains undergo tailoring, maturation, and molecular folding to form functional secondary or tertiary protein molecules.
- ✓ RER pinches off certain tiny protein-filled vesicles which ultimately get fused to cis Golgi.
- ✓ The ER membranes are found to conduct intra-cellular impulses. For example, the sarcoplasmic reticulum transmits impulses from the surface membrane into the deep region of the muscle fibers.
- ✓ The ER membranes form the new nuclear envelope after each nuclear division.
- ✓ The SER contains several key enzymes that catalyze the synthesis of cholesterol which is also a precursor substance for the biosynthesis of two types of compounds— the steroid hormones and bile acids.
- ✓ RER also synthesize membrane proteins and glycoproteins which are cotranslationally inserted into the rough ER membranes. Thus, the endoplasmic reticulum is the site of the biogenesis of cellular membranes.

Golgi complex – structure and function

The Golgi apparatus or the Golgi body or Golgi complex or simply Golgi is a cellular organelle present in most of the cells of the eukaryotic organisms.

It is referred to as the manufacturing and the shipping center of the cell.

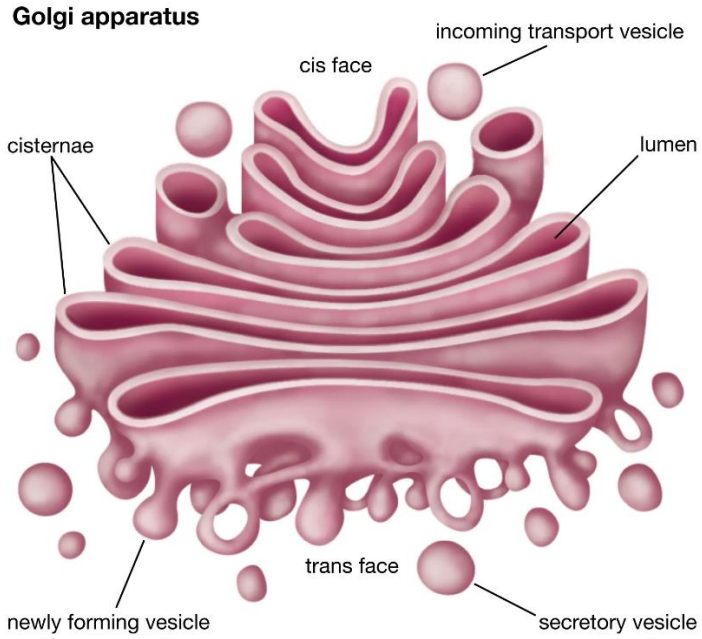
Golgi is involved in the packaging of the protein molecules before they are sent to their destination. These organelles help in processing and packaging the macromolecules like proteins and lipids that are synthesized by the cell and hence act as the 'post office' of the cell.

Golgi apparatus was discovered in the year 1898 by an Italian biologist Camillo Golgi.

Structure of Golgi Apparatus

- Under the electron microscope, the Golgi apparatus is seen to be composed of stacks of flattened structures that contain numerous vesicles containing secretory granules.
- The Golgi apparatus is morphologically very similar in both plant and animal cells. However, it is extremely pleomorphic: in some cell types it appears compact and limited, in others spread out and reticular (net-like).

- Typically, however, Golgi apparatus appears as a complex array of **interconnecting tubules, vesicles, and cisternae.**



A. Cisternae

- It is the simplest unit of the Golgi apparatus is the cisterna.
- Cisternae (about 1 µm in diameter) are central, flattened, plate-like or saucer-like closed compartments that are held in parallel bundles or stacks one above the other.
- In each stack, cisternae are separated by a space of 20 to 30 nm which may contain rod-like elements or fibers.
- Each stack of cisternae forms a dictyosome which may contain 5 to 6 Golgi cisternae in animal cells or 20 or more cisternae in plant cells.
- Each cisterna is bounded by a smooth unit membrane (7.5 nm thick), having a lumen varying in width from about 500 to 1000 nm.
- The margins of each cisterna are gently curved so that the entire dictyosome of the Golgi apparatus takes on a bow-like appearance.
- The cisternae at the convex end of the dictyosome comprise proximal, forming or cis-face and cisternae at the concave end of the dictyosome comprise the distal, maturing or trans-face.

B. Tubules

- A complex array of associated vesicles and anastomosing tubules (30 to 50 nm diameter) surround the dictyosome and radiate from it. In fact, the peripheral area of the dictyosome is fenestrated (lace-like) in structure.

C. Vesicles

The vesicles (60 nm in diameter) are of three types:

(i) Transitional vesicles are small membrane limited vesicles which are thought to form as blebs from the transitional ER to migrate and converge to cis face of Golgi, where they coalesce to form new cisternae.

(ii) Secretory vesicles are varied-sized membrane-limited vesicles that discharge from margins of cisternae of Golgi. They, often, occur between the maturing face of Golgi and the plasma membrane.

(iii) Clathrin-coated vesicles are spherical protuberances, about 50 µm in diameter and with a rough surface. They are found at the periphery of the organelle, usually at the ends of single tubules, and are morphologically quite distinct from the secretory vesicles. The clathrin-coated vesicles are known to play a role in intracellular traffic of membranes and of secretory products, i.e., between ER and Golgi, as

well as, between the GELR region and the endosomal and lysosomal compartments.

Functions of Golgi Apparatus

1. Golgi vesicles are often, referred to **as the "traffic police" of the cell**. They play a key role in sorting many of the cell's proteins and membrane constituents, and in directing them to their proper destinations.

To perform this function, the Golgi vesicles contain different sets of enzymes in different types of vesicles— cis, middle and trans cisternae—that react with and modify secretory proteins passing through the Golgi lumen or membrane proteins and glycoproteins that are transiently in the Golgi membranes as they are en route to their final destinations.

The Golgi apparatus hence acts as the assembly factory of the cell where the raw materials are directed to the Golgi apparatus before being passed out from the cell.

2. In animals, the Golgi apparatus is involved in the packaging and exocytosis.

3. It is also involved in the formation of certain cellular organelles such as plasma membrane, lysosomes, acrosome of spermatozoa and cortical granules of a variety of oocytes.

4. They are also involved in the transport of lipid molecules around the cell.

5. The Golgi complex also plays an important role in the production of proteoglycans. The proteoglycans are molecules that are present in the extracellular matrix of the animal cells.

6. It is also a major site of synthesis of carbohydrates. These carbohydratres include the synthesis of glycosaminoglycans, Golgi attaches to these polysaccharides which then attaches to a protein produced in the endoplasmic reticulum to form proteoglycans.

7. The Golgi involves in the sulfation process of certain molecules.

8. The process of phosphorylation of molecules by the Golgi requires the import of ATP into the lumen of the Golgi.

9. In plants, Golgi apparatus is mainly involved in the secretion of materials of primary and secondary cell walls (e.g., formation and export of glycoproteins, lipids, pectins and monomers for hemicellulose, cellulose, lignin, etc.)

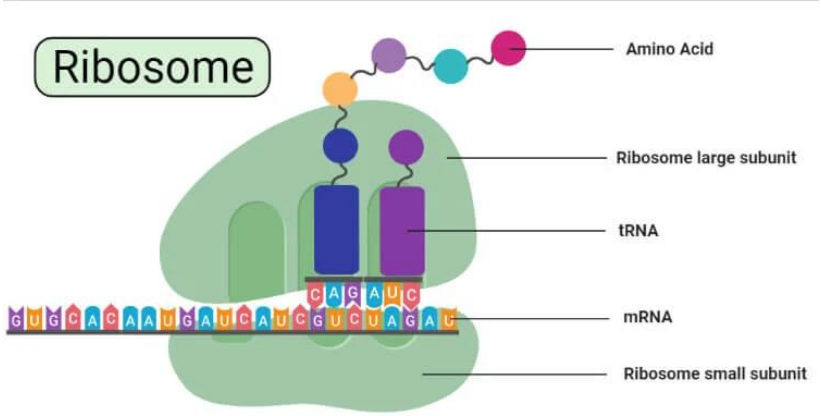
Ribosomes – Types, structure and function

The ribosome word is derived – 'ribo' from ribonucleic acid and 'somes' from the Greek word 'soma' which means 'body'.

Ribosomes are tiny spheroidal dense particles (of 150 to 200 A^0 diameters) that are primarily found in most prokaryotic and eukaryotic.

- They are sites of **protein synthesis**.
- They are structures containing approximately equal amounts of RNA and proteins and serve as a scaffold for the ordered interaction of the numerous molecules involved in protein synthesis.
- The ribosomes occur in cells, both prokaryotic and eukaryotic cells.
- In prokaryotic cells, the ribosomes often occur freely in the cytoplasm.
- In eukaryotic cells, the ribosomes either occur freely in the cytoplasm or remain attached to the outer surface of the membrane of the endoplasmic reticulum.
- The location of the ribosomes in a cell determines what kind of protein it makes.
- If the ribosomes are floating freely throughout the cell, it will make proteins that will be utilized within the cell itself.

- When ribosomes are attached to the endoplasmic reticulum, it is referred to as rough endoplasmic reticulum or rough ER.
- Proteins made on the rough ER are used for usage inside the cell or outside the cell.
- The number of ribosomes in a cell depends on the activity of the cell.
- On average in a mammalian cell, there can be about 10 million ribosomes.



Structure of Ribosomes

- A ribosome is made from complexes of RNAs and proteins and is, therefore, a ribonucleoprotein.
- Around 37 to 62% of RNA is comprised of RNA and the rest is proteins.
- Each ribosome is divided into two subunits:

1. **A smaller subunit** which binds to a larger subunit and the mRNA pattern, and
2. **A larger subunit** which binds to the tRNA, the amino acids, and the smaller subunit.

- Prokaryotes have 70S ribosomes respectively subunits comprising the little subunit of 30S and the bigger subunit of 50S.
- Their small subunit has a 16S RNA subunit (consisting of 1540 nucleotides) bound to 21 proteins.
- The large subunit is composed of a 5S RNA subunit (120 nucleotides), a 23S RNA subunit (2900 nucleotides) and 31 proteins.
- Eukaryotes have 80S ribosomes respectively comprising of little (40S) and substantial (60S) subunits.
- The smaller 40S ribosomal subunit is prolate ellipsoid in shape and consists of one molecule of 18S ribosomal RNA (or rRNA) and 30 proteins (named as S1, S2, S3, and so on).
- The larger 60S ribosomal subunit is round in shape and contains a channel through which growing polypeptide chain makes its exit.
- It consists of three types of rRNA molecules, i.e., 28S rRNA, 5.8 rRNA and 5S rRNA, and 40 proteins (named as L1, L2, L3 and so on).
- The differences between the ribosomes of bacterial and eukaryotic are used to create antibiotics that can destroy bacterial infection without harming human cells.
- The ribosomes seen in the chloroplasts of mitochondria of eukaryotes are comprised of big and little subunits composed of proteins inside a 70S particle.
- The ribosomes share a core structure that is similar to all ribosomes despite differences in its size.
- The two subunits fit together and work as one to translate the mRNA into a polypeptide chain during protein synthesis.
- Because they are formed from two subunits of non-equal size, they are slightly longer in the axis than in diameter.
- During protein synthesis, when multiple ribosomes are attached to the same mRNA strand, this structure is known as polysome.

- The existence of ribosomes is temporary, after the synthesis of polypeptide the two sub-units separate and are reused or broken up.

Types of Ribosomes

Based on the size and the sedimentation coefficient (S), ribosomes are of two types:

- 70S ribosome
- 80S ribosome

70S ribosome

- They are smaller in size.
- Sedimentation coefficient: 70S
- Molecular weight: 2.7× 106 daltons.
- They are found in:
- prokaryotic cells of the blue-green algae and bacteria.
- mitochondria and chloroplasts of eukaryotic cells.

80S ribosome

- Sedimentation coefficient: 80S
- Molecular weight: 40 × 106 daltons.
- They are found in the eukaryotic cells i.e. in plants and animals.
- The ribosomes present in mitochondria and chloroplasts are smaller than 80S cytoplasmic ribosomes.
- In the 80S ribosome of yeast, 79r-protein are present where only 12 r-protein are found to be specific.

Functions of Ribosomes

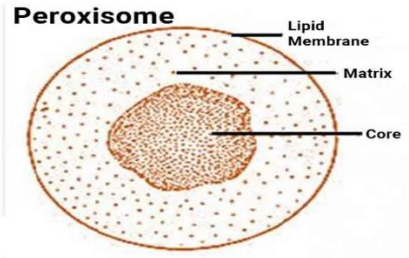
- The ribosome is a complex molecular machine, found within all living cells, that serves as the site of biological protein synthesis (translation).
- Ribosomes link amino acids together in the order specified by messenger RNA (mRNA) molecules.
- Ribosomes act as catalysts in two extremely important biological processes called peptidyl transfer and peptidyl hydrolysis.
- The nascent polypeptide chain is protected from the activity of protein digestive enzymes.

Peroxisomes

Peroxisomes are small, membrane-enclosed cellular organelles containing oxidative enzymes that are involved in a variety of metabolic reactions, including several aspects of energy metabolism.

- They are considered as an important type of microbody found in both plants and animal cells.
- They were identified as organelles by Belgian cytologist Christian de Duve in 1967 after already been described.
- First peroxisomes to be discovered were isolated from leaf homogenate of spinach.
- They are most abundantly found in detoxifying organs such as the liver and kidney cells. However, they can be induced to proliferate in response to metabolic needs.

Structure of Peroxisomes



- They are membrane-bound spherical bodies of 0.2 to 1.5 µm in diameter found in all eukaryotic organisms including both plants and animal cells.
- They are found floating freely in the cytoplasm in close association of ER, mitochondria or chloroplast within the cell.
- They are among the simplest of eukaryotic organelles.
- They exist either in the form of a network of interconnected tubules called peroxisome reticulum or as individual microperoxisomes.
- They are variable in size and shape according to the cell and usually circular in cross-section.
- They range from 0.2 -1.5 µm in diameter.
- It consists of a single limiting membrane of lipid and protein molecules enclosing the granular matrix.
- The matrix consists of fibrils or a crystalloid structure containing enzymes.

Peroxisomal Enzymes

- Approximately 60 known enzymes are present in the matrix of peroxisomes.
- They are responsible to carry out oxidation reactions leading to the production of hydrogen peroxide.
- The main groups of enzymes include:

1. Urate oxidase
2. D-amino acid oxidase
3. Catalase

Functions of Peroxisomes

1. **Hydrogen Peroxide Metabolism:**

- Enzymes present in the peroxisomes both lead to the production and elimination of H_20_2 which is a reactive oxygen species.

2. **Fatty acid oxidation:**

- Oxidation of fatty acids, in animal cells, occurs in both peroxisomes and mitochondria, but in yeasts and plants, only limited to peroxisomes.
- Oxidation is accompanied by the production of H_20_2 which is decomposed by **catalase** enzyme. This provides a major source of metabolic energy.

3. **Lipid biosynthesis**

- Synthesis of cholesterol and dolichol occurs in both ER and peroxisomes. Bile acid synthesis takes place from cholesterol in the liver.
- Peroxisomes contain enzymes to synthesize plasmalogens, a family of phospholipids which are important membrane components of tissues of the heart and brain.

4. **Germination of seeds**

- Peroxisomes in seeds responsible for the conversion of stored fatty acids to carbohydrates, critical to providing energy and raw materials for the growth of germinating plants.

5. **Photorespiration**

- Peroxisomes in leaves particularly in the green ones carry out the photorespiration process along with chloroplasts.

6. **Degradation of purines**

- Carry out the catabolism of purines, polyamines and amino acids especially by uric acid oxidase

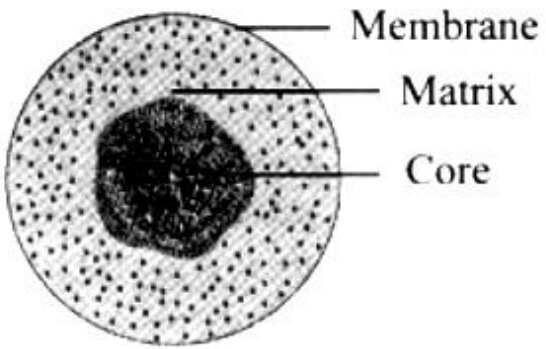
7. **Bioluminescence**

- Luciferase enzyme found in the peroxisomes of fireflies help in bioluminescence and thus aid the flies in finding a mate or its meal.

Glyoxysomes

Glyoxysomes were discovered by Breidenbach in 1967. These organelles are regarded as a distinct variety of peroxisomes.

- These are the microbodies that contain the fatty acid oxidation and glyoxylate pathway enzymes.
- These are the biggest microbodies and are only present in plants.
- Glyoxysomes are the structures that are present in the cells of some fungi and the fatty seeds that are germination (such as castor, groundnut, etc.) up until and unless the stored fat is eaten.
- Enzymes are found inside glyoxysomes, which have a single outer membrane.
- Acetyl CoA is created when fatty acids are oxidized. The glyoxylate cycle breaks down the latter to create carbs.
- After serving their purpose, the glyoxysomes are changed into peroxisomes.
- For the purpose of lipid mobilisation and breakdown, these structures emerge in senescent plant tissues.



Glyoxysomes

Plant cell vacuoles

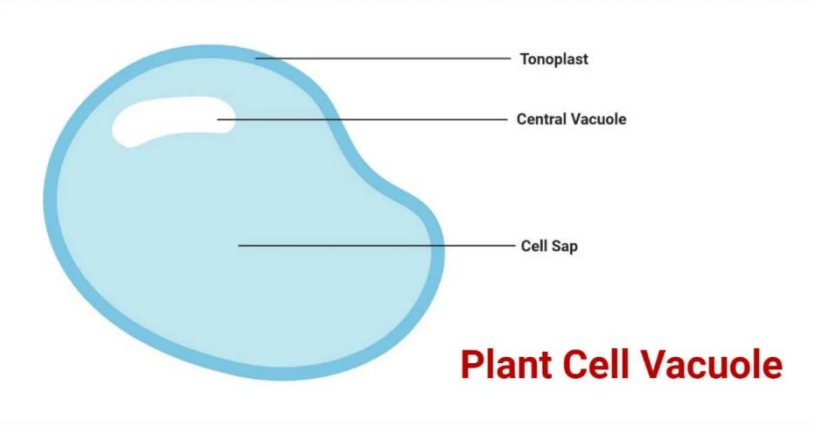
The vacuole is a very large, fluid-filled vesicle which is present in the cytoplasm of a plant cell. The biosynthetic and endocytic pathways form it. The term 'vacuole' was first introduced by the French biologist FlexisDujardin, and it represents the space of a protozoan contractile vesicle.

In a single cell, there can be many vacuoles. Tonoplast separates it from the cytoplasm, which is also a single unit **membrane.** Vacuole tends to be very large and occupy more than 30% of the cell volume, but they vary from 5 to 90% according to the cell types in mature plant cells.

Vacuoles provide structural support. They also provide functions such as storage, maintaining water balance, and disposing of waste materials. But in immature and actively dividing plant cells, the vacuoles are quite small.

Different types of cellular components are present in vacuoles such as protein, sugar, salts, acid, nitrogenous compound (such as alkaloid and anthocyanin pigment), ions, and secondary metabolites. They play a crucial role in the plant signaling system.

Structure of Plant Cell Vacuole



- A vacuole is a membrane-bound structure found in the cytoplasmic matrix cell.
- Generally, they have no basic shape or size. Its structure varies according to the requirement of the cell.
- The membrane surrounding the vacuole is termed the Tonoplast, separating the vacuolar content from the cell's cytoplasm.
- It is an important and highly integrated component of the plant's internal membrane network (endomembrane).
- The movement of ions is regulated by the vacuole.
- It also isolates the harmful materials from the cells.
- Vacuoles are functionally and structurally related to lysosomes in animal cells and may contain many hydrolytic enzymes.
- In addition, they usually contain sugars, salts, acid, ions, and nitrogenous compounds such as alkaloids and anthocyanin pigment in their cell sap.
- The membranes are embedded with proteins that help in transporting molecules across the membrane.
- Different combination of these protein helps the vacuoles to hold different materials.
- The large vacuole slowly develops as it matures by the fusion of smaller vacuoles derived from the ER and Golgi apparatus.
- The pH of plant vacuole ranges from 3 to 10.
- Due to an abundant quantity of alkaline substances, its pH may increase to 9-10.
- Similarly, due to the presence of acids like citric acid, oxalic acid, and tartaric acids, pH can lower to 3.
- Two types of vacuole appear sequentially during embryogenesis.

Types of Plant Cell Vacuole

1. Lytic Vacuoles

- It is a plant specialized vacuole equivalent to animal lysosomes or yeast vacuoles, functioning as a compartment for degradation and waste storage.

2. Protein storage vacuole

- Protein storage vacuole assembles a large amount of protein in the storage tissue of the plant.

- In most seeds, protein storage vacuole contains three morphologically distinct regions: the matrix, crystalloid, and globoid.

3. Cell sap

- It is a fluid that is found in the plant cell vacuole.
- It contains a variable amount of food, waste material, inorganic salts, nitrogenous compound, water, amino acid, and glucose.
- That provides mechanical support and serves as a storage material, especially in non-woody plants.
- It plays a vital role in plant cell osmosis.

Functions of Plant Cell Vacuole

- Turgor pressure created by the vacuole helps to maintain the shape of the cell.
- It also helps to cope with extreme conditions.
- Turgor pressure is the pressure that is exerted on the cell wall by the water present in the vacuoles.

Central vacuole is used by the developing seed cells for protein storage.

The plant vacuole stores salts, minerals, nutrients, proteins, and pigments, which help in plant growth.

For both the nutrients and the waste products, it acts as the storage organelle.

Vacuole plays the role in maintaining the homeostatic condition in plant cells with respect to the different alterations in the environment.

To maintain the acidic condition in the content of the vacuole, H+ ions are pumped inside. The in and out movement is controlled by the Tonoplast or vacuolar membrane selectively.

Inside the vacuole, the smaller vacuoles are retained, but the water can move freely.

The trypsin inhibitors commonly found in seeds and the wound-induced protease inhibitors of leaf cells (to inhibit both insect and microbial proteases), both accumulate in the vacuole and are presumably designed to interfere with the digestive processes of herbivores.

Endocytic pathways – endocytosis, phagocytosis; membrane trafficking.

1. Endocytosis

Structure:

Clathrin-Mediated Endocytosis:

Clathrin-Coated Pits: Specialized areas of the plasma membrane coated with clathrin protein, forming a basket-like structure.

Vesicle Formation: Clathrin and adaptor proteins (e.g., AP-2) help invaginate the membrane to form a vesicle.

Dynamin: A GTPase that pinches off the vesicle from the membrane.

Caveolae-Mediated Endocytosis:

Caveolae: Small, flask-shaped invaginations enriched in cholesterol and caveolin proteins.

Vesicle Formation: Caveolae internalize substances and form vesicles without the need for clathrin.

Macropinocytosis:

Ruffle Formation: The cell membrane extends outward to form ruffles that engulf extracellular fluid.

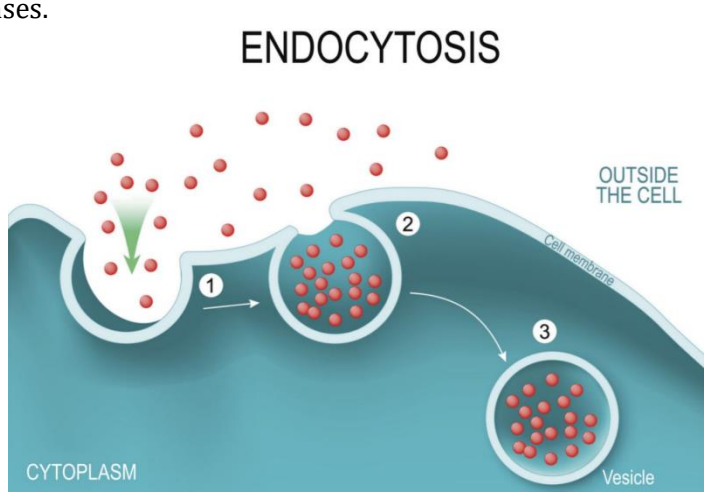
Vesicle Formation: The ruffles fold back to form large macropinosomes.

Function:

Clathrin-Mediated Endocytosis: Internalizes specific molecules like growth factors, nutrients, and receptors.

Caveolae-Mediated Endocytosis: Involved in the uptake of certain lipids and receptors, and plays a role in signaling.

Macropinocytosis: Allows cells to take in large volumes of extracellular fluid and solutes, aiding in nutrient uptake and cellular responses.



2. Phagocytosis

Structure:

Phagocyte Membrane:

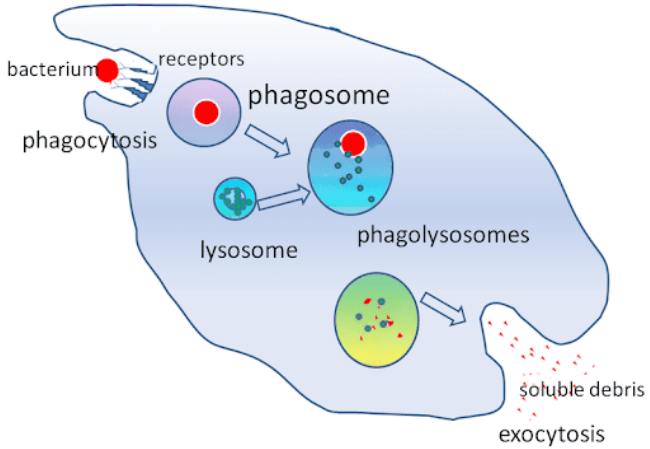
Pseudopodia: Extensions of the cell membrane that surround and engulf large particles. They are driven by actin polymerization and form the phagocytic cup.

Phagosome: The vesicle formed around the engulfed particle, which then fuses with lysosomes to form a phagolysosome.

Phagolysosome:

Fusion: The phagosome fuses with lysosomes, creating a phagolysosome where the engulfed material is exposed to digestive enzymes.

Digestion: Enzymes in the phagolysosome break down the engulfed particles, leading to their degradation and removal.



Function:

Particle Clearance: Phagocytosis is essential for removing large particles such as pathogens, dead cells, and cellular debris from the body. This process is crucial for maintaining tissue homeostasis and immune defense.

Immune Response: Phagocytes, including macrophages and neutrophils, play a central role in the immune system by engulfing and destroying pathogens. They also present antigens to other immune cells to initiate an adaptive immune response.

3. Membrane Trafficking

Structure:

Vesicles:

Formation: Vesicles are formed by budding off from donor membranes such as the endoplasmic reticulum (ER) or Golgi apparatus. They are coated with proteins (e.g., COPI, COPII) that help in the budding and transport processes.

Cargo: Vesicles carry proteins, lipids, and other molecules between cellular compartments.

Cytoskeleton:

Microtubules and Actin Filaments: Serve as tracks for vesicle movement. Motor proteins travel along these tracks, carrying vesicles to their destination.

Motor Proteins: Kinesins (for anterograde transport) and dyneins (for retrograde transport) move vesicles along microtubules, while myosins move vesicles along actin filaments.

Fusion and Sorting:

Docking: Vesicles dock at specific target membranes using docking proteins and SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors).

Fusion: Vesicle and target membrane fusion is mediated by SNARE proteins, leading to the release of vesicle contents into the target compartment.

Sorting: Ensures that vesicle contents are directed to appropriate destinations such as lysosomes, endosomes, or the plasma membrane.

Function:

Transport: Membrane trafficking is essential for moving proteins, lipids, and other molecules between different cellular compartments, such as from the ER to the Golgi apparatus and then to the plasma membrane or lysosomes.

Sorting and Processing: Involves sorting molecules for proper localization, modification, and function. This includes sorting of newly synthesized proteins for secretion or delivery to organelles.

Homeostasis: Maintains cellular organization and function by ensuring that essential molecules reach their correct cellular destinations.

UNIT – IV ENERGY METABOLISM:

Mitochondria –Morphology – structural variations; Chemical compositions and Functions; Enzyme system of Mitochondria; Mode of energy production – oxidation of carbohydrates: Glycolysis, oxidative decarboxylation, Krebs cycle; respiratory chain andoxidative phosphorylation; Chloroplast – structure and function. Import and Sorting of Chloroplast Proteins. Photosynthesis; Electron Flow through Photosystems I and II, Cyclic Electron Flow, ATP Synthesis.

Mitochondria

Mitochondria are membrane-bound organelles found in nearly all eukaryotic cells. They are known as the “powerhouses” of the cell because they are the main site of ATP (adenosine triphosphate) production, which is the energy currency of the cell.

1. Morphology (Structure)

Size and Shape:

- Mitochondria are typically rod-shaped or oval.
- They vary in size from 0.5 to 10 micrometers (μm) in length.
- The shape of mitochondria is dynamic, meaning they can change based on the cell’s energy needs and environmental conditions. They may be round, elongated, or even branched.

Number of Mitochondria:

The number of mitochondria in a cell depends on the cell's energy requirements. Cells with high energy demands, such as muscle cells, may contain hundreds or thousands of mitochondria.

Membranes:

Outer Membrane:

- The outer membrane is smooth and surrounds the entire organelle.
- It contains proteins known as porins, which allow molecules smaller than 5,000 daltons to pass freely in and out of the mitochondrion.

Inner Membrane:

- The inner membrane is folded into structures called cristae. These folds increase the surface area available for energy production (ATP synthesis).
- The inner membrane contains a large amount of proteins involved in the electron transport chain and ATP synthesis.

Mitochondrial Matrix:

- The space inside the inner membrane is called the matrix.
- The matrix contains enzymes, mitochondrial DNA (mtDNA), ribosomes, and other molecules involved in cellular metabolism, particularly the citric acid cycle (Krebs cycle).

2. Structural Variations

- Mitochondria can vary structurally based on the type of cell and its specific function:
- In muscle cells, mitochondria are long and tubular, helping to efficiently meet the energy demands of muscle contraction.
- In liver cells, mitochondria tend to be smaller but more numerous, allowing the liver to perform its numerous metabolic functions.
- Mitochondria can also undergo fusion (joining together) and fission (splitting apart), depending on the cell's energy needs and the health of the mitochondria.

3. Chemical Composition

Lipids and Proteins:

- The outer and inner mitochondrial membranes are made up of a lipid bilayer, primarily composed of phospholipids.
- The inner membrane has a unique lipid called cardiolipin, which is critical for the function of several enzymes involved in energy production.
- The inner membrane contains a very high concentration of proteins (about 75% by weight) compared to lipids, reflecting its role in biochemical reactions.

Mitochondrial DNA (mtDNA):

- Mitochondria have their own circular DNA, known as mitochondrial DNA (mtDNA), which encodes some of the proteins and RNAs needed for mitochondrial function.
- mtDNA is inherited maternally (only from the mother).

Ribosomes:

Mitochondria contain their own ribosomes, which are similar to bacterial ribosomes (70S type). These ribosomes synthesize proteins encoded by the mitochondrial DNA.

4. Functions of Mitochondria

ATP Production (Oxidative Phosphorylation):

Mitochondria are the primary site of ATP production through a process called oxidative phosphorylation, which takes place in the inner membrane.

During oxidative phosphorylation, energy is extracted from food molecules (such as glucose) and converted into ATP, which powers cellular processes.

Citric Acid Cycle (Krebs Cycle):

The matrix of mitochondria contains enzymes for the citric acid cycle (also called the Krebs cycle or tricarboxylic acid cycle).

This cycle generates high-energy molecules (NADH and FADH2), which are used in the electron transport chain to produce ATP.

Apoptosis (Programmed Cell Death):

Mitochondria play a key role in triggering apoptosis, a process by which cells undergo programmed death in response to cellular damage or stress.

Mitochondria release cytochrome c, which activates a series of enzymes called caspases, leading to cell death.

Calcium Storage:

Mitochondria help regulate calcium levels in the cell, which is important for cellular signaling, muscle contraction, and metabolism.

Heat Production (Thermogenesis):

In specialized cells (such as brown fat cells), mitochondria generate heat by a process called non-shivering thermogenesis. This is important in maintaining body temperature, especially in newborns and hibernating animals.

Synthesis of Biomolecules:

Mitochondria are involved in the synthesis of steroids, heme (a component of hemoglobin), and amino acids.

5. Enzyme System of Mitochondria

The mitochondria are equipped with a series of enzymes that perform various functions:

Electron Transport Chain (ETC): The ETC is located on the inner membrane and is composed of five complexes (I-V):

- Complex I (NADH dehydrogenase): Transfers electrons from NADH to ubiquinone.
- Complex II (Succinate dehydrogenase): Transfers electrons from FADH2 to ubiquinone.
- Complex III (Cytochrome bc1 complex): Transfers electrons from ubiquinol to cytochrome c.
- Complex IV (Cytochrome c oxidase): Transfers electrons to oxygen, forming water.
- Complex V (ATP synthase): Uses the proton gradient generated by the electron transport chain to convert ADP into ATP.

The passage of electrons through the ETC generates a proton gradient across the inner membrane, which drives ATP synthesis by ATP synthase (Complex V).

Krebs Cycle Enzymes:

These enzymes are located in the matrix and catalyze reactions in the citric acid cycle. The cycle produces high-energy molecules (NADH, FADH2) that feed into the electron transport chain.

Beta-Oxidation Enzymes:

Mitochondria contain enzymes involved in beta-oxidation, the process by which fatty acids are broken down to generate acetyl-CoA, which then enters the citric acid cycle.

Conclusion

Mitochondria are essential organelles responsible for generating the energy needed to power cellular functions. In addition to energy production, they play key roles in regulating apoptosis, calcium storage, and the synthesis of certain biomolecules. Their structure and enzyme systems are highly specialized to perform these diverse functions efficiently.

Mode of Energy Production: Oxidation of Carbohydrates

Cellular respiration is a series of metabolic processes that cells use to produce energy (ATP) by oxidizing carbohydrates. The breakdown of glucose occurs in multiple steps: **Glycolysis**, **Oxidative Decarboxylation**, **Krebs Cycle**, and **Oxidative Phosphorylation**. Below is a detailed explanation of each step involved in energy production through glucose oxidation.

1. Glycolysis

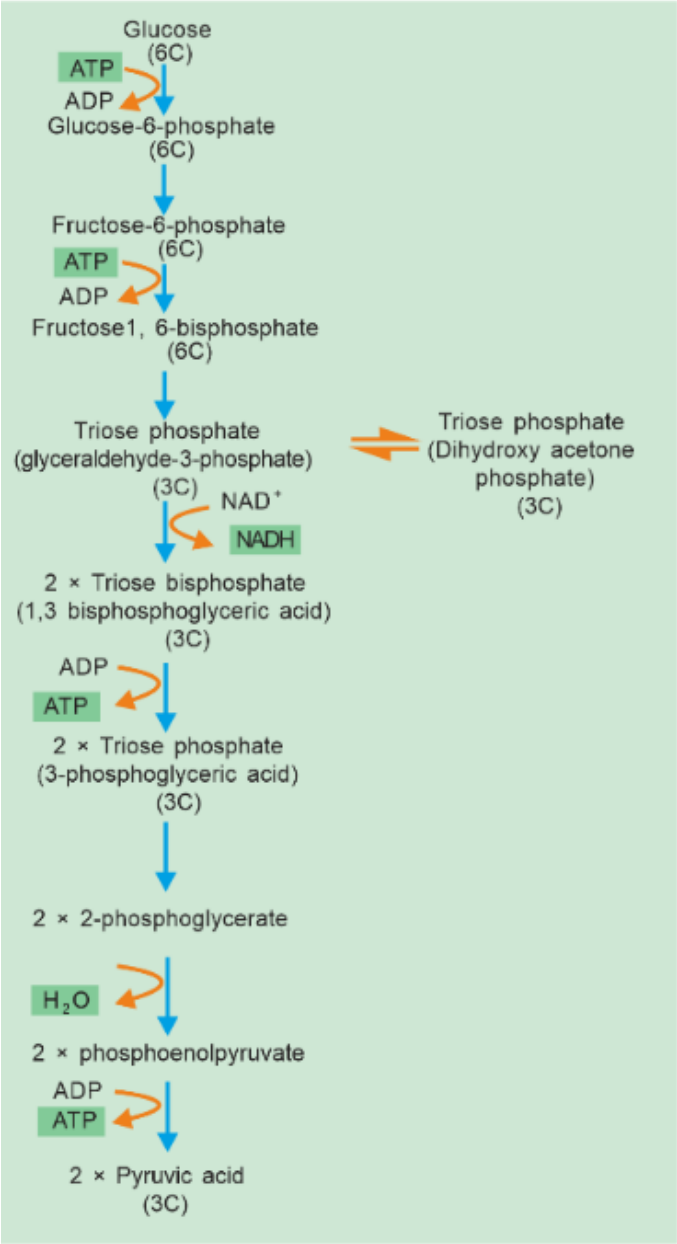
- **Location:** Cytoplasm of the cell.
- **Definition:** Glycolysis is the anaerobic breakdown of one molecule of glucose (6-carbon) into two molecules of **pyruvate** (3-carbon).

Key Reactions:

1. **Glucose is phosphorylated** by ATP to form **glucose-6-phosphate**.
2. Through a series of enzyme-mediated steps, glucose-6-phosphate is converted into **fructose-**

1,6-bisphosphate, which is then cleaved into two 3-carbon molecules.

3. Each 3-carbon molecule is further oxidized, producing **2 NADH** and **4 ATP** (net gain of 2 ATP as 2 ATP are used in earlier steps).
4. The end product is **2 pyruvate** molecules.



Net Energy Yield:

- **2 ATP** (net).
- **2 NADH** (will be used later in the electron transport chain for more ATP production).

2. Oxidative Decarboxylation (Pyruvate to Acetyl-CoA)

- **Location:** Mitochondrial matrix.
- **Definition:** Each pyruvate molecule produced in glycolysis is transported into the mitochondrion and converted to **acetyl-CoA**. This step links glycolysis to the Krebs cycle.

Key Reactions:

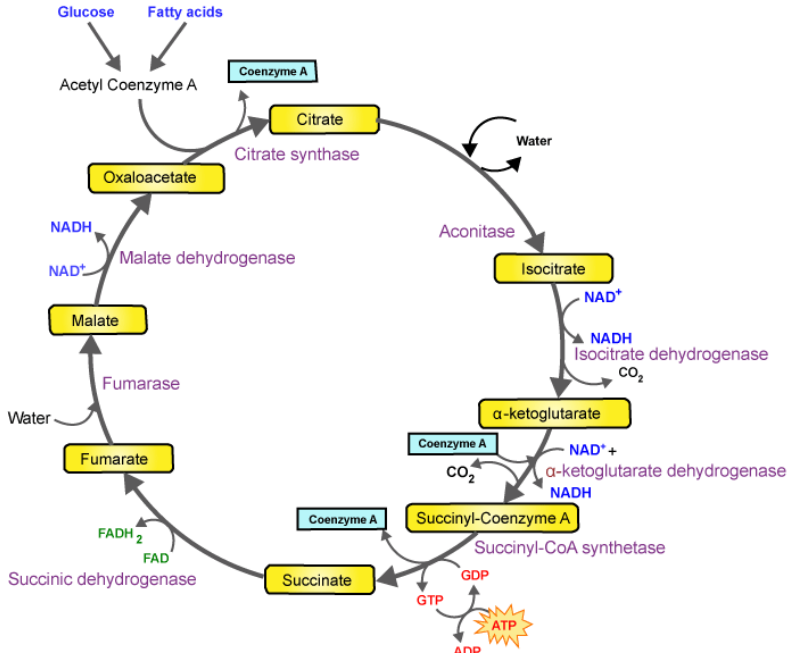
1. **Pyruvate is decarboxylated** (removal of CO_2), producing a two-carbon molecule called **acetyl group**.
2. The acetyl group combines with **Coenzyme A** to form **Acetyl-CoA**.
3. NAD^+ is reduced to form **NADH** in the process.

End Products:

- **Per pyruvate**: 1 NADH, 1 CO_2, and 1 Acetyl-CoA.
- **Per glucose**: 2 NADH, 2 CO_2, and 2 Acetyl-CoA (since one glucose produces two pyruvates).

3. Krebs Cycle (Citric Acid Cycle)

- **Location:** Mitochondrial matrix.
- **Definition:** The Krebs cycle completes the oxidation of glucose by fully oxidizing Acetyl-CoA to carbon dioxide.



Key Reactions:

1. Acetyl-CoA (2-carbon) combines with **oxaloacetate** (4-carbon) to form **citrate** (6-carbon).
2. Citrate undergoes a series of reactions that lead to the release of **2 molecules of CO_2**, and the regeneration of **oxaloacetate**.
3. During the cycle, **NAD^+** is reduced to **NADH**, **FAD** is reduced to **$FADH_2$**, and **ATP (or GTP)** is synthesized.

End Products (per Acetyl-CoA):

- 3 NADH.
- 1 $FADH_2$.
- 1 ATP (or GTP).
- 2 CO_2.

End Products (per glucose):

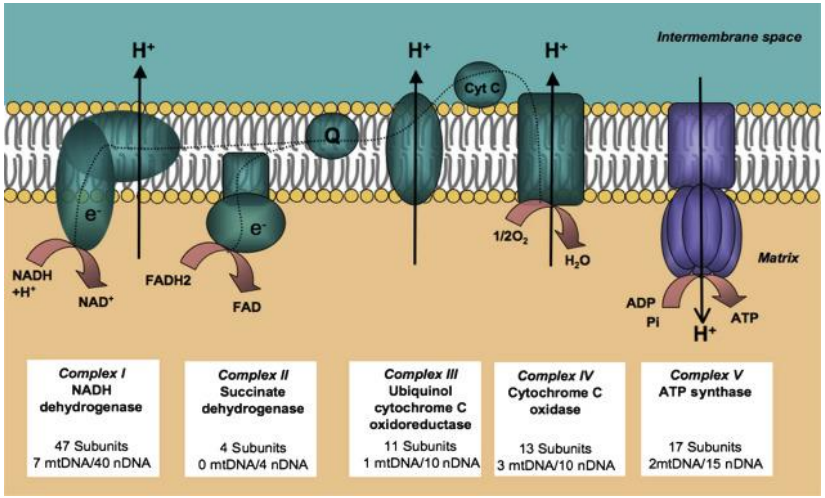
- 6 NADH.
- 2 $FADH_2$.
- 2 ATP.
- 4 CO_2.

4. Electron Transport Chain (Respiratory Chain) and Oxidative Phosphorylation

- **Location:** Inner mitochondrial membrane.
- **Definition:** The electron transport chain (ETC) is a series of protein complexes that use electrons from NADH and $FADH_2$ to generate a **proton gradient** across the inner mitochondrial membrane, which is then used to drive **ATP synthesis**.

Key Reactions:

1. **NADH and $FADH_2$** donate electrons to the complexes in the ETC (Complex I-IV).
2. Electrons are passed from one complex to the next, releasing energy to pump protons (H^+) into the **intermembrane space**, creating a proton gradient (proton motive force).
3. **Oxygen** is the final electron acceptor, combining with electrons and protons to form **water** (H_2O).
4. Protons flow back into the mitochondrial matrix through the enzyme **ATP synthase**, which uses the energy of the proton gradient to convert **ADP** into **ATP** (oxidative phosphorylation).



ATP Production:

- Each NADH can generate **2.5 ATP**.
- Each $FADH_2$ can generate **1.5 ATP**.

Summary of ATP Production per Glucose Molecule

1. **Glycolysis**:
   - 2 ATP (net).
   - 2 NADH (5 ATP in ETC).
2. **Oxidative Decarboxylation**:
   - 2 NADH (5 ATP in ETC).
3. **Krebs Cycle**:
   - 2 ATP (direct).
   - 6 NADH (15 ATP in ETC).
   - 2 $FADH_2$ (3 ATP in ETC).
4. **Total ATP Yield**:
   - **30-32 ATP** per glucose molecule.

Conclusion

The oxidation of carbohydrates, primarily glucose, occurs through a well-organized series of reactions that efficiently extract energy in the form of ATP. **Glycolysis** and the **Krebs cycle** generate a small amount of ATP directly, but most of the energy is harvested in the form of **NADH** and **$FADH_2$**, which donate electrons to the **Electron Transport Chain**. **Oxidative phosphorylation** is the stage where the majority of ATP is produced, making it the most significant energy-yielding step in the entire process.

Chloroplast: Structure and Function

Structure of Chloroplast

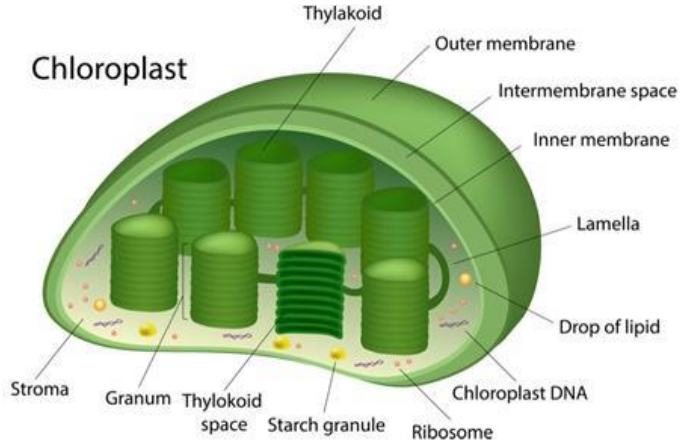
- **Location:** Chloroplasts are found in plant cells and some algae, responsible for photosynthesis. They are lens-shaped, membrane-bound organelles.

Key Structural Components:

1. **Outer Membrane:** A smooth, permeable membrane that allows small molecules to pass easily.
2. **Inner Membrane:** Less permeable and contains transport proteins to regulate molecule movement.
3. **Intermembrane Space:** The space between the outer and inner membranes.
4. **Stroma:** The aqueous matrix inside the inner membrane where the Calvin cycle occurs. It contains enzymes, ribosomes, chloroplast DNA, and starch granules.
5. **Thylakoid Membrane System:**
    - **Thylakoids** are flat, disk-like structures that stack to form **grana**.
    - **Grana (plural of granum):** Stacks of thylakoids that increase the surface area for photosynthesis.
    - **Thylakoid Lumen:** The space inside the thylakoid discs.
    - **Photosynthetic pigments** (chlorophyll) and electron carriers involved in the light reactions of photosynthesis are embedded in the thylakoid membrane.

Other Features:

- **Chlorophyll pigments** (chlorophyll a and b) absorb light energy, essential for capturing sunlight.
- **DNA and Ribosomes:** Chloroplasts contain their own DNA and ribosomes for the synthesis of some proteins.



Functions of Chloroplast

- **Photosynthesis:** Conversion of solar energy into chemical energy (ATP and NADPH) in the **light reactions**, followed by the **Calvin cycle** to produce glucose from CO_2.
- **ATP Production:** Similar to mitochondria, chloroplasts produce ATP during the light-dependent reactions of photosynthesis.
- **Biosynthesis:** Chloroplasts are involved in the biosynthesis of lipids, amino acids, and hormones.

- **Oxygen Production:** As a byproduct of water splitting during light reactions, oxygen is released.

Import and Sorting of Chloroplast Proteins

Chloroplasts contain their own genome, but most of their proteins are encoded by nuclear genes and must be imported from the cytosol. This import system is essential for chloroplast biogenesis and function.

1. **Protein Targeting to Chloroplasts:**
   - Proteins are synthesized in the cytosol and have a **chloroplast transit peptide** (a specific signal sequence) at their N-terminus that directs them to the chloroplast.
2. **Translocation into Chloroplast:**
   - Proteins are transported across the **outer chloroplast membrane** via the **Toc complex** (Translocon at the Outer membrane of Chloroplast).
   - They are then transferred to the **Tic complex** (Translocon at the Inner membrane of Chloroplast) to cross the inner membrane into the **stroma**.
3. **Sorting to Specific Locations:**
   - **Stromal proteins** are released into the stroma after the transit peptide is cleaved.
   - **Thylakoid proteins** are further directed to the thylakoid membrane via other pathways, including the **Sec pathway**, **Tat pathway**, and **SRP pathway**.
4. **Protein Folding and Assembly:** Once inside the chloroplast, proteins fold and assemble into functional complexes, such as those in the **photosystems** or **ATP synthase**.

Photosynthesis

Photosynthesis is the process by which plants, algae, and some bacteria convert light energy into chemical energy stored in glucose.

- **Overall Equation:** 6CO2+6H2O+light energy→C6H12O6+6O26CO_2 + 6H_2O + \text{light energy} → $C_6H_{12}O_6$ + 6$O_2$6CO2+6H2 O+light energy→C6H12O6+6O2

Photosynthesis occurs in two main stages:

1. **Light Reactions (Light-Dependent Reactions):** Occur in the **thylakoid membrane**.
2. **Calvin Cycle (Light-Independent Reactions):** Occur in the **stroma**.

Electron Flow through Photosystems I and II

The **light reactions** of photosynthesis involve two main protein complexes called **Photosystem I (PSI)** and **Photosystem II (PSII)**. These complexes work together to drive the flow of electrons and produce ATP and NADPH.

1. **Photosystem II (PSII):**

- **Location:** Thylakoid membrane.
- **Function:** PSII captures light energy, which excites electrons in the chlorophyll molecule. These high-energy electrons are passed to the **primary electron acceptor**.
- **Water Splitting (Photolysis):** PSII splits water molecules into oxygen, protons (H^+), and electrons to replenish its electron supply.
- **Electron Transport:** The electrons travel through a series of electron carriers (plastoquinone, cytochrome b_6f) and generate a **proton gradient** across the thylakoid membrane.

2. **Photosystem I (PSI):**
   - **Location:** Thylakoid membrane.
   - **Function:** After electrons pass through the electron transport chain, they are passed to PSI. PSI re-energizes the electrons using light energy.
   - **NADPH Formation:** The energized electrons are passed to **$NADP^+$** reductase, reducing it to form **NADPH**, which is used in the Calvin cycle for carbon fixation.

Cyclic Electron Flow

- In **cyclic electron flow**, electrons from PSI are recycled back into the electron transport chain instead of being transferred to $NADP^+$.
- This generates additional **ATP** but no NADPH or oxygen.
- Cyclic flow occurs when the demand for ATP is greater than NADPH in the chloroplast.

ATP Synthesis

- **Location:** Thylakoid membrane.
- **Process:** ATP synthesis in the chloroplast occurs via **chemiosmosis**.
  - **Proton Gradient:** The electron transport chain pumps protons into the **thylakoid lumen**, creating a proton gradient (high proton concentration inside the thylakoid and low in the stroma).
  - **ATP Synthase:** Protons flow back into the stroma through **ATP synthase**, driving the conversion of ADP and inorganic phosphate (Pi) into **ATP**.

Summary

- **Chloroplast structure** includes outer and inner membranes, thylakoids, and the stroma. It contains pigments like chlorophyll, which are essential for photosynthesis.
- **Chloroplast proteins** are imported from the cytosol through specific protein complexes (Toc and Tic) and sorted to their functional locations within the chloroplast.
- **Photosynthesis** is the process by which chloroplasts convert light energy into chemical energy, with **Photosystems I and II** playing crucial roles in electron flow and energy capture.

- **ATP synthesis** in chloroplasts occurs through chemiosmosis, driven by a proton gradient generated by the electron transport chain.

UNIT – V TOOLS AND TECHNIQUES IN CELL BIOLOGY:

Resolving power of Microscope- Light microscope, Principles and applications of Bright field, Dark field, Phase contrast, Fluorescent Microscope; Electron microscopes- Transmission electron Microscope (TEM) and Scanning electron microscope (SEM). Cell fractionation and centrifugation; Autoradiography.

Resolving power of Microscope

The resolving power of a microscope refers to its ability to distinguish two close objects as separate entities. It is one of the most important factors determining the quality of the image produced by the microscope. The resolving power depends on the wavelength of light used and the numerical aperture (NA) of the objective lens.

Formula for Resolving Power:

The resolving power (R) is given by the equation:

$$R = \frac{\lambda}{2 \times NA}$$

Where:

- λ is the wavelength of light used,
- NA is the numerical aperture of the microscope's objective lens.

Key Factors Influencing Resolving Power:

Wavelength of Light (λ): Shorter wavelengths of light (e.g., blue light) provide better resolution. This is why electron microscopes, which use electron beams with much shorter wavelengths than visible light, have much higher resolving power than light microscopes.

Numerical Aperture (NA): This is a measure of the lens's ability to gather light and resolve fine specimen detail at a fixed object distance. Higher numerical aperture results in better resolution.

Types of Microscopes and Resolving Power:

Light Microscope: The typical resolving power is around 0.2 micrometers (200 nanometers), meaning it can distinguish objects that are at least 200 nm apart.

Electron Microscope: The resolving power of transmission electron microscopes can go down to about 0.1 nanometers due to the shorter wavelength of electrons.

Light microscope

What is a light microscope?

A light microscope is a biology laboratory instrument or tool that uses visible light to detect and magnify very small objects and enlarge them.

Types of light microscopes (optical microscope)

With the evolved field of Microbiology, the microscopes used to view specimens are both simple and compound light microscopes, all using lenses. The difference is simple light microscopes use a single lens for magnification while compound lenses use two or more lenses for magnifications. This means, that a series of lenses are placed in an order such that, one lens magnifies the image further than the initial lens.

The modern types of Light Microscopes include:

- Bright field Light Microscope
- Phase Contrast Light Microscope
- Dark-Field Light Microscope
- Fluorescence Light Microscope

Brightfield Light Microscope (Compound light microscope)

This is the most basic optical Microscope used in microbiology laboratories which produces a dark image against a bright background. Made up of two lenses, it is widely used to view plant and animal cell organelles including some parasites such as Paramecium after staining with basic stains. Its functionality is based on being able to provide a high-resolution image, which highly depends on the proper use of the microscope. This means that an adequate amount of light will enable sufficient focusing of the image, to produce a quality image. It is also known as a compound light microscope.

It is composed of:

- Two lenses which include the objective lens and the eyepiece or ocular lens.
- Objective lens is made up of six or more glasses, which make the image clear from the object
- The condenser is mounted below the stage which focuses a beam of light onto the specimen. It can be fixed or movable, to adjust the quality of light, but this entirely depends on the microscope.
- They are held together by a sturdy metallic curved back used as an arm and a stand at the bottom, known as the base, of the microscope. The arm and the base hold all the parts of the microscope.
- The stage where the specimen is placed, allowing movement of the specimen around for better viewing with the flexible knobs and it is where the light is focused on.

- Two focusing knobs i.e the fine adjustment knob and the coarse adjustment knob, found on the microscopes' arm, which can move the stage or the nosepiece to focus on the image. the sharpen the image clarity.
- It has a light illuminator or a mirror found at the base or on the microbes of the nosepiece.
- The nosepiece has about three to five objective lenses with different magnifying power. It can move round to any position depending on the objective lens to focus on the image.
- An aperture diaphragm also is known as the contrast, which controls the diameter of the beam of light that passes through the condenser, in that, when the condenser is almost closed, the light comes through to the center of the condenser creating high contrast. But when the condenser is widely open, the image is very bright with very low contrast.

Applications of the Bright Field Light Microscope (Compound light microscope)

- Vastly used in Microbiology, this microscope is used to view fixed and live specimens, that have been stained with basic stains. This gives contrast for easy visibility under the microscope. Therefore it can be used to identify basic bacteria cells and parasitic protozoans such as *Paramecium*.

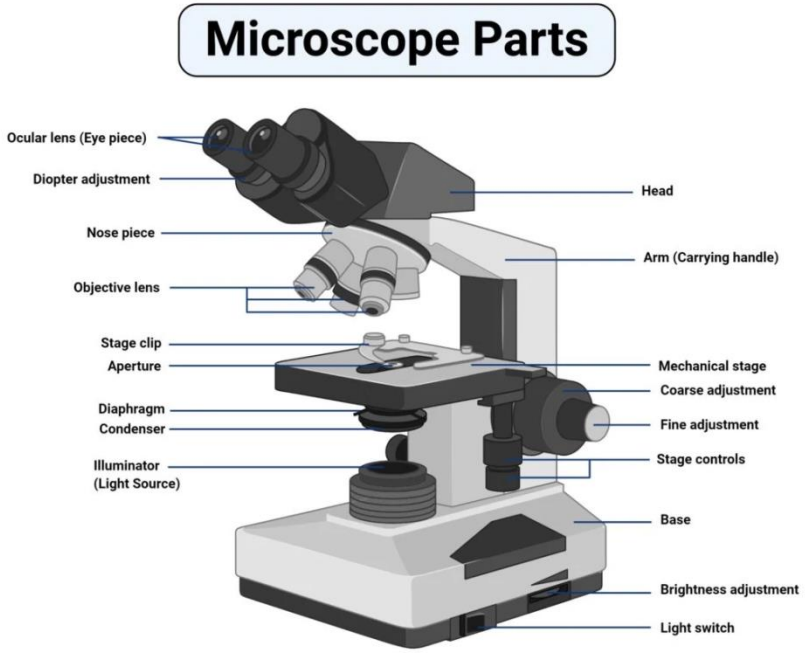


Figure: Parts of a microscope, Image Copyright © Sagar Aryal, www.microbenotes.com

Parts of a bright-field microscope (Compound light microscope)

Phase Contrast Microscope

- This is a type of optical microscope whereby small light deviations known as **phase shifts** occur during light penetration into the unstained specimen. These phase shifts are converted into the image to mean, when light passes through the opaque specimen, the phase shifts

brighten the specimen forming an illuminated (bright) image in the background.

- The phase-contrast microscope produces high contrast images when using a transparent specimen more so those of microbial cultures, thin tissue fragments, cell tissues, and subcellular particles.
- The principle behind the working of the phase-contrast microscope is the use of an optical method to transform a specimen into an amplitude image, that's viewed by the eyepiece of the microscope.
- The PCM can be used to view unstained cells also known as the **phase objects**, which means that the morphology of the cell is maintained and the cells can be observed in their natural state, in high contrast and efficient clarity. This is because if the specimens are stained and fixed, they kill most cells, a characteristic that is uniquely undone by the brightfield light microscope.
- The shifts that occur during light penetration, become converted to changes in amplitude which causes the image contrast.
- Coupled with contrast-enhancing elements such as fluorescence, they produce better visuals of the specimens' image.

Parts of the Phase Contrast Microscope

The instrumentation of the Phase Contrast Microscope is based on its light pathways from receiving the source of light to the visualization of the image.

Therefore its sequentially made up of:

Light source (Mercury arc lamp)
Collective lens
Aperture
Condenser
Condenser annular
Specimen
Objective
Phase plate
Deflected light
Phase ring
Phase Contrast Microscopy

The functioning of the Phase Contrast microscope

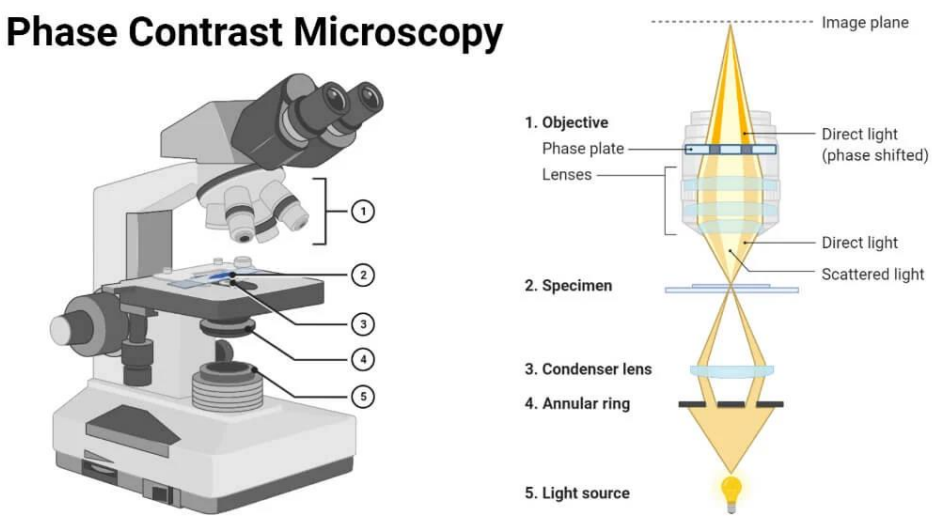
- The change is caused by the deviated scattered (Deflected) light and the undeviated light that reaches the specimen which is absorbed, create at a certain wavelength, producing color. The difference created by the scattered light and that of the absorbed light is known as **amplitude variations**. These amplitude variations are sensitive to allowing visualization by photographic equipment like the Phase Contrast Microscope, hence seen by the human eye.
- The Condenser of the phase-contrast microscope has an opaque disk that is known as an annular ring, with a transparent ring that produces a cone of light, that passes through a specimen. Due to light variations some light bends at the specimen, caused by variations in light density, forming an image at the objective lens. The

undeviated light will strike the phase ring on the phase plate and the deviated light will miss the phase ring passing through the phase plate directly, this forms an image.

- The Phase-Contrast Microscope is designed with objective lenses that have the ability to perform multiple functions when combined with contrast-enhancing techniques, for example, fluorescence. The objective lenses are located in the internal phase plate with variation in the light absorption and phase displacement i.eundiffraction, creating a wide spectrum for contrasting the specimen and forming a strong contrast in the background.

Applications of Phase-Contrast Microscope

- Determine morphologies of living cells such as plant and animal cells
- Studying microbial motility and structures of locomotion
- To detect certain microbial elements such as the bacterial endospores



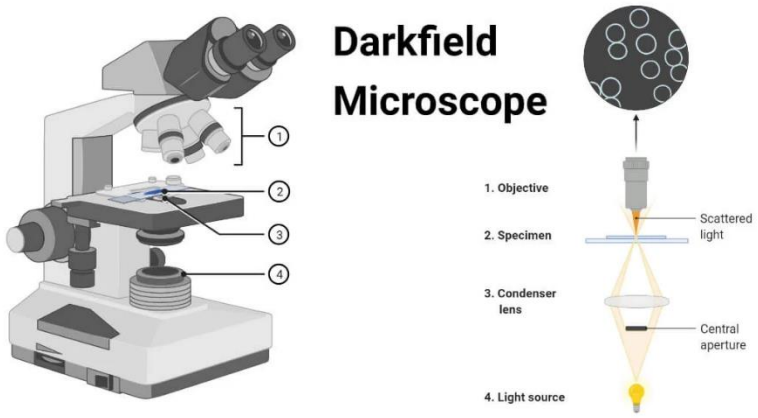
Dark-Field Light Microscope

This is a specialized type of bright field light microscope that has several similarities to the Phase-Contrast Microscope. To make a dark field Microscope, place a darkfield stop underneath and a condenser lens which produces a hollow cone beam of light that enters the objective only, from the specimen.

Darkfield Microscope

This technique is used to visualize living unstained cells. This is affected by the way illumination is done on the specimen in that, when a hollow cone beam of light is transmitted to the specimen, deviated light (unreflected/unrefracted) rays do not pass through the objectives but the undeviated (reflected/refracted) light passes through the objectives to the specimen forming an image.

This makes the surrounding field of the specimen appear black while the specimen will appear illuminated. This is enabled by the dark background this the name, dark-field Microscopy.



Applications of the Dark Field Microscope

- It is used to visualize the internal organs of larger cells such as the eukaryotic cells
- Identification of bacterial cells with distinctive shapes such as *Treponemapallidum*, a causative agent of syphilis.

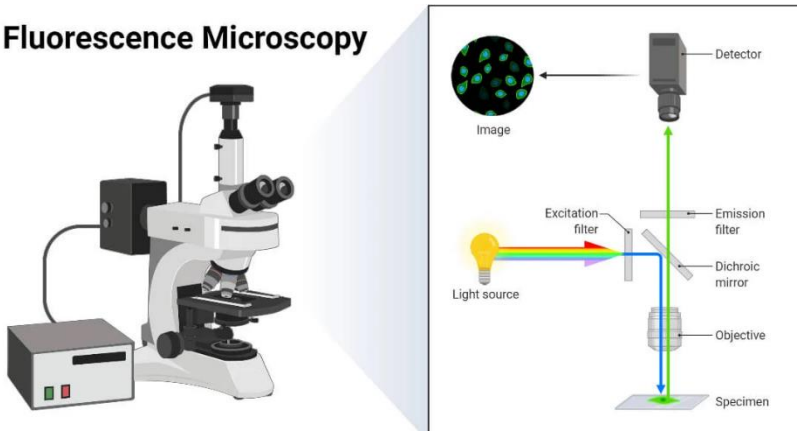
The Fluorescent Microscope

The above-discussed microscopes will normally produce images after a light has been transmitted and passed through the specimen.

In the case of the fluorescent Microscope, the specimen emits light. How? By adding a **dye molecule** to the specimen. This dye molecule will normally become excited when it absorbs light energy, hence it releases any trapped energy as light. The light energy that is released by the excited molecule has a long wavelength compared to its radiating light. The dye molecule is normally a fluorochrome, that fluoresces when exposed to the light of a certain specific wavelength. The image formed is a **fluorochrome-labeled image** from the emitted light

The principle behind this working mechanism is that the fluorescent microscope will expose the specimen to ultra or violet or blue light, which forms an image of the specimen that is emanated by the fluorescent light. They have a mercury vapor arc lamp that produces an intense beam of light that passes through an exciter filter. The exciter filter functions to transmit a specific wavelength to the fluorochrome stained specimen, producing the fluorochrome-labeled image, at the objective.

After the objective, there is a barrier filter that functions primarily to remove any ultraviolet radiation that may be harmful to the viewer's light, thus reducing the contrast of the image.



Applications of the Fluorescent Microscope

- Used in the visualization of bacterial agents such as *Mycobacterium tuberculosis.*
- Used to identify specific antibodies produced against bacterial antigens/pathogens in immunofluorescence techniques by labeling the antibodies with fluorochromes.
- Used in ecological studies to identify and observe microorganisms labeled by the fluorochromes
- It can also be used to differentiate between dead and live bacteria by the color they emit when treated with special stains

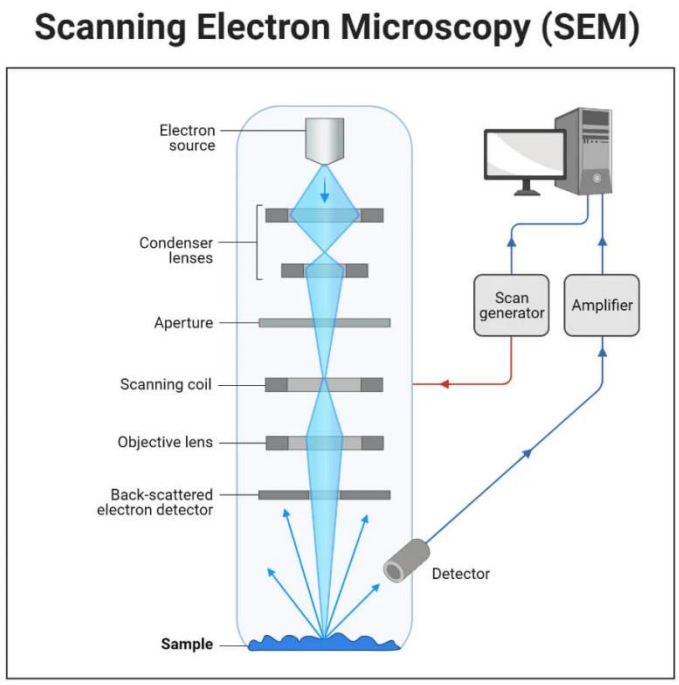
Besides the above-discussed microscopes, there is one not commonly used microscope known as the **Differential Interference Contrast Microscopy.** It is very similar to the phase-contrast microscope whereby the images are formed from the variations in the light either deviated and or undeviated. The difference is, here two beams of light are emitted to the specimen and focused by a prism. One beam passes through the prism to the specimen while another passes through the glass slide clear area without the specimen. The two beams then combine and interfere with each other to form an image. It can be used to view cell structures such as endospores, bacterial cell walls, nuclei, and granules for unstained specimens.

Scanning Electron Microscope (SEM) Definition

Scanning Electron Microscope (SEM) is a type of electron microscope that scans surfaces of microorganisms that uses a beam of electrons moving at low energy to focus and scan specimens. The development of electron microscopes was due to the inefficiency of the wavelength of light microscopes. electron microscopes have very short wavelengths in comparison to the light microscope which enables better resolution power.

Principle of Scanning Electron Microscope (SEM) Unlike the Transmission Electron Microscope which uses transmitted electrons, the scanning electron Microscope uses emitted electrons. The Scanning electron microscope works on the principle of applying kinetic energy to produce signals on the interaction of the electrons. These electrons are secondary electrons, backscattered electrons, and diffracted backscattered electrons which are used to view crystallized elements and photons. Secondary and backscattered electrons are used to produce an image. The secondary electrons are emitted from the

specimen play the primary role of detecting the morphology and topography of the specimen while the backscattered electrons show contrast in the composition of the elements of the specimen.



How does the Scanning Electron Microscope (SEM) work?

1. The source of the electrons and the electromagnetic lenses are from tungsten filament lamps that are placed at the top of the column and it is similar to those of the transmission electron Microscope.
2. The electrons are emitted after thermal energy is applied to the electron source and allowed to move in a fast motion to the anode, which has a positive charge.
3. The beam of electrons activates the emission of primary scattered (Primary) electrons at high energy levels and secondary electrons at low-energy levels from the specimen surface. The beam of electrons interacts with the specimen to produce signals that give information about the surface topography and composition of the specimen.
4. The specimen does not need special treatment for visualization under the SEM, even air-dried samples can be examined directly. However, microbial specimens need fixation, dehydration, and drying in order to maintain the structural features of the cells and to prevent collapsing of the cells when exposed to the high vacuum of the microscope.
5. The samples are mounted and coated with thin layer of heavy metal elements to allow spatial scattering of electric charges on the surface of the specimen allowing better image production, with high clarity.
6. Scanning by this microscope is attained by tapering a beam of electrons back and forth over a thin section of the microscope. When the electrons reach the specimen, the surface releases a tiny staw of electrons known as

secondary electrons which are then trapped by a special detector apparatus.

7. When the secondary electrons reach and enter the detector, they strike a scintillator (a luminescence material that fluoresces when struck by a charged particle or high-energy photon). This emits flashes of light which get converted into an electric current by a photomultiplier, sending a signal to the cathode ray tube. This produces an image that looks like a television picture that can be viewed and photographed.
8. The quantity of secondary electrons that enter the detector is highly defined by the nature of the specimen i.e raised surfaces to receive high quantities of electrons, entering the detector while depressed surfaces have fewer electrons reaching the surface and hence fewer electrons enter the detector.
9. Therefore raised surfaces will appear brighter on the screen while depressed surfaces appear darker.

The major components of the Scanning Electron Microscope include;

- Electron Source – This is where electrons are produced under thermal heat at a voltage of 1-40kV. the electrons condense into a beam that is used for the creation of an image and analysis. There are three types of electron sources that can be used i. e Tungsten filament, Lanthanum hexaboride, and Field emission gun (FEG)
- Lenses – it has several condenser lenses that focus the beam of electrons from the source through the column forming a narrow beam of electrons that form a spot called a spot size.
- Scanning Coil – they are used to deflect the beam over the specimen surface.
- Detector – It's made up of several detectors that are able to differentiate the secondary electrons, backscattered electrons, and diffracted backscattered electrons. The functioning of the detectors highly depends on the voltage speed, the density of the specimen.
- The display device (data output devices)
- Power supply
- Vacuum system

Transmission Electron Microscope (TEM)

This is a powerful electron microscope that uses a beam of electrons to focus on a specimen producing a highly magnified and detailed image of the specimen.

The magnification power is over 2 million times better than that of the light microscope, producing the image of the specimen which enables easy characterization of the image in its morphological features, compositions and crystallization information is also detailed.

Early discovery of cathode rays like electrons by Louis de Broglie in the early 1920s, paved way into the development of an electron microscope where they used a beam of electrons creating a form of wave motion.

Magnetic fields were used as lenses for the electrons. With these discoveries, the first electron microscope was later developed by Ernst Ruska and Max Knolls in 1931 and modified into a Transmission Electron Microscope (TEM) by Ernst Ruska along with the Sieman's company, in 1933.

This TEM microscope has several advantages compared to the light microscope with its efficiency also being very high.

Among all microscopes both light and electron microscopes, TEM are the most powerful microscopes used in laboratories. It can magnify a mall particle of about 2nm, and therefore they have a resolution limit of 0.2um.

Principle of Transmission Electron Microscope (TEM)

The working principle of the Transmission Electron Microscope (TEM) is similar to the light microscope. The major difference is that light microscopes use light rays to focus and produce an image while the TEM uses a beam of electrons to focus on the specimen, to produce an image.

Electrons have a shorter wavelength in comparison to light which has a long wavelength. The mechanism of a light microscope is that an increase in resolution power decreases the wavelength of the light, but in the TEM, when the electron illuminates the specimen, the resolution power increases increasing the wavelength of the electron transmission. The wavelength of the electrons is about 0.005nm which is 100,000X shorter than that of light, hence TEM has better resolution than that of the light microscope, of about 1000times.

This can accurately be stated that the TEM can be used to detail the internal structures of the smallest particles like a virion particle.

Parts of Transmission Electron Microscope (TEM)

Their working mechanism is enabled by the high-resolution power they produce which allows it to be used in a wide variety of fields. It has three working parts which include:

- Electron gun
- Image producing system
- Image recording system

How does a Transmission Electron Microscope (TEM) work?

From the instrumentation described, the working mechanism is a sequential process of the parts of the TEM mentioned above. To mean:

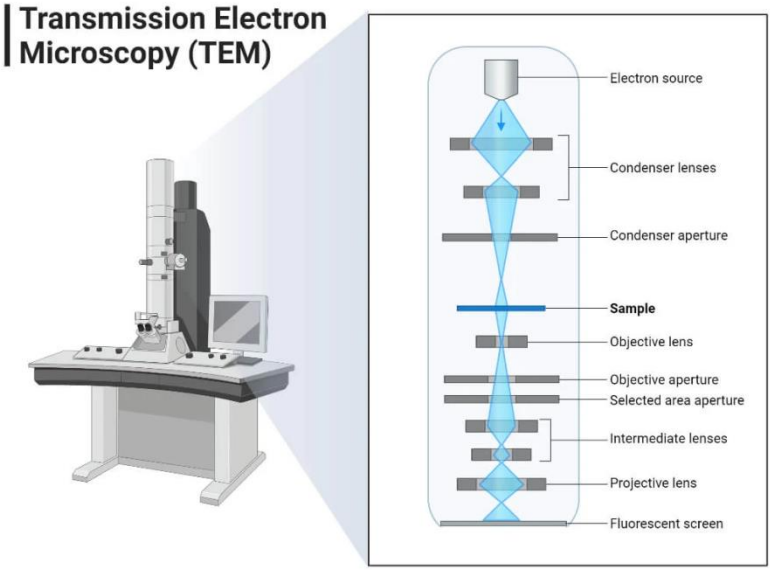
A heated tungsten filament in the electron gun produces electrons that get focus on the specimen by the condenser lenses.

Magnetic lenses are used to focus the beam of electrons of the specimen. By the assistance offered by the column tube of the condenser lens into the vacuum creating a clear image, the vacuum allows electrons to produce a clear image without collision with any air molecules which may deflect them.

On reaching the specimen, the specimen scatters the electrons focusing them on the magnetic lenses forming a large clear image, and if it passes through a fluorescent screen it forms a polychromatic image.

The denser the specimen, the more the electrons are scattered forming a darker image because fewer electron reaches the screen for visualization while thinner, more transparent specimens appear brighter.

NOTE: If the screen is moved aside, a photographic image can be captured in pixels forming a permanent image.



Applications of Transmission Electron Microscope (TEM)

TEM is used in a wide variety of fields From Biology, Microbiology, Nanotechnology, forensic studies, etc. Some of these applications include:

1. To visualize and study cell structures of bacteria, viruses, and fungi
2. To view bacteria flagella and plasmids
3. To view the shapes and sizes of microbial cell organelles
4. To study and differentiate between plant and animal cells.
5. Its also used in nanotechnology to study nanoparticles such as ZnO nanoparticles

6. It is used to detect and identify fractures, damaged microparticles which further enable repair mechanisms of the particles.

Cell Fractionation and Centrifugation

Cell fractionation is a technique used to separate the different components of a cell while maintaining their individual functions. It involves breaking open cells (lysis) and then using centrifugation to isolate organelles based on their size, shape, and density.

Steps in Cell Fractionation:

Homogenization:

Cells are broken open using a homogenizer or blender to form a cell homogenate (a suspension of cell components).

Differential Centrifugation:

The homogenate is spun at different speeds in a centrifuge to separate cellular components based on their sedimentation rate (size and density).

Centrifugation separates components into pellets (the heavier parts that settle at the bottom) and supernatants (the lighter liquid part).

The process is done in a stepwise manner:

Low-speed centrifugation (1,000 g): Separates nuclei.

Medium-speed centrifugation (10,000 g): Separates mitochondria, lysosomes, and peroxisomes.

High-speed centrifugation (100,000 g): Separates microsomes (vesicles derived from the endoplasmic reticulum) and small vesicles.

Ultra-speed centrifugation (>100,000 g): Separates ribosomes, proteins, and small molecules.

Applications of Cell Fractionation:

Isolation and study of specific cell organelles (e.g., mitochondria for metabolic studies).

Biochemical analysis of organelles.

Proteomic and genomic research.

Autoradiography

Autoradiography is a technique used to visualize the distribution of radioactively labeled molecules in a biological sample. It helps to study processes like protein synthesis, DNA

replication, or metabolic activity by tracking the movement of radiolabeled compounds within cells or tissues.

Principle:

A biological specimen (cells, tissues, or organisms) is exposed to a radiolabeled compound (e.g., a radioactive isotope like tritium or carbon-14).

The specimen is then placed in contact with a photographic film or emulsion.

The radiation emitted by the radiolabeled molecules creates an image on the film (like an X-ray).

Steps in Autoradiography:

Radioactive Labeling:

The biological sample is incubated with a radiolabeled precursor, such as a nucleoside for DNA synthesis or an amino acid for protein synthesis.

Exposure to Photographic Emulsion:

After incubation, the sample is fixed on a glass slide and covered with a photographic emulsion sensitive to radiation.

Development of the Film:

After a period of exposure, the film is developed. Areas of the sample where radioactive decay occurs will appear as dark spots on the film.

Analysis:

The dark spots (silver grains) on the film correspond to the location of the radiolabeled molecules within the sample, allowing researchers to determine where specific biological processes are occurring.

Applications of Autoradiography:

DNA and RNA Synthesis: Tracking nucleotide incorporation during replication and transcription.

Protein Synthesis: Identifying where newly synthesized proteins are located within a cell.

Metabolism Studies: Investigating metabolic pathways by tracing the movement of labeled metabolites.

Histology: Studying tissue-specific activity in organs or during development.