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AGRICULTURE BIOTECHNOLOGY

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AGRICULTURE BIOTECHNOLOGY

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PREFACE

It is always a dream of the student to enter in IITs and other prestigious institutes, universities for better future in Research and industrial world. Every year ICAR conduct the national exam as ICAR-ARS-NET entrance in the field of Life Science, Biotechnology and Food technology. This textbook has the principal objective to understand the basics of biology and applied biology for competitive Entrance exams and other National exams as well. We have tried to provide an astonishingly great amount of information from the enormous and ever-growing field in an easily understanding form. It is transcribed in vibrant and crisp language to enhance self-motivation and strategic learning skill of the students and allowing them with a tool to measure and analyse their abilities and the confidence of winning in competitive exams. We have also tried to cover the updated knowledge for better understanding of Life Science in depth without the burden. The most noteworthy feature of this book is its crystal clear, up-to-date and extra information from which ICAR may ask the questions. We have tried to resist the temptation to include more and more information which may add the information but not increasing understanding of the basic concepts and critical thinking. We hope that this text book will be beneficial both to teachers and students.

ACKNOWLEDGMENT

We wish to acknowledge our heartfelt gratitude to our hardworking writer of this book Mr. Manpreet Sharma and Krisha Sharma for their patience and constant support during writing the book. We wish to acknowledge our heartfelt gratitude to our faculties, friends and colleagues Dr. Sandeep Chaudhary (PhD from INMAS-DRDO), Dr. Sandeep Kundu and Dr. Ayush Attery (PhD from NII, Delhi) for their professional guide and encouragement for undertaking this book. We also very thankful to Er. Sachin Saini (Scientist at SSPL-DRDO) for his sustained and technical support.

We also wish to acknowledge to Dr. Parmod Singh (Lecturer HBSE, Haryana) and our beloved KapMan family for their precious moral support.

At the end we want to say that students are our inspiration for writing this book in concise and precise manner. We look forward for your comments and feedback for improvement of this book.

Delhi, 2022

KapMan Academy

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Ms. Krisha Sharma (CSIR-NET-JRF, IIT-JAM, JNU Biotechnology) is the founder of KapMan Life Science Academy who is the editor and reviewer of this book. She has done her graduation from ANDC Delhi university and M.Sc. from Madurai Kamaraj University, Madurai, Tamil Nādu. Her research work was on plant molecular biology and plant biotechnology and now she is the part of KapMan Life Science Academy. She is ardent about Biological Science and wants to provide best quality books for national competitive exams.

Mr. Manpreet Sharma

Mr. Manpreet Sharma (CSIR-UGC-NET-JRF 2011/2013/2014, BARC, DRDO, DU Exam qualified) is the Co-founder of KapMan Life Science Academy who have also done His Research work in CSIR, DRDO-INMAS and Delhi University. His research work was on Cancer biology and Radiation Biology. Mr Manpreet is passionate about Life Science and Research world and is eager to provide best quality class books for CSIR UGC NET JRF, IIT JAM and GATE in the field of Bio-science, Biotechnology and Life Science.

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Unit 2: Biomolecules and Metabolism

Structure and function of carbohydrates, lipids, proteins and nucleic acids, Synthesis of carbohydrate, glycolysis, HMP, Citric acid cycle and metabolic regulation, Oxidative phosphorylation and substrate level phosphorylation, Vitamins, plant and animal hormones. Functional molecules, antioxidants, nutrient precursor, HSPs, anti-viral compounds.

Unit 3: Enzymology

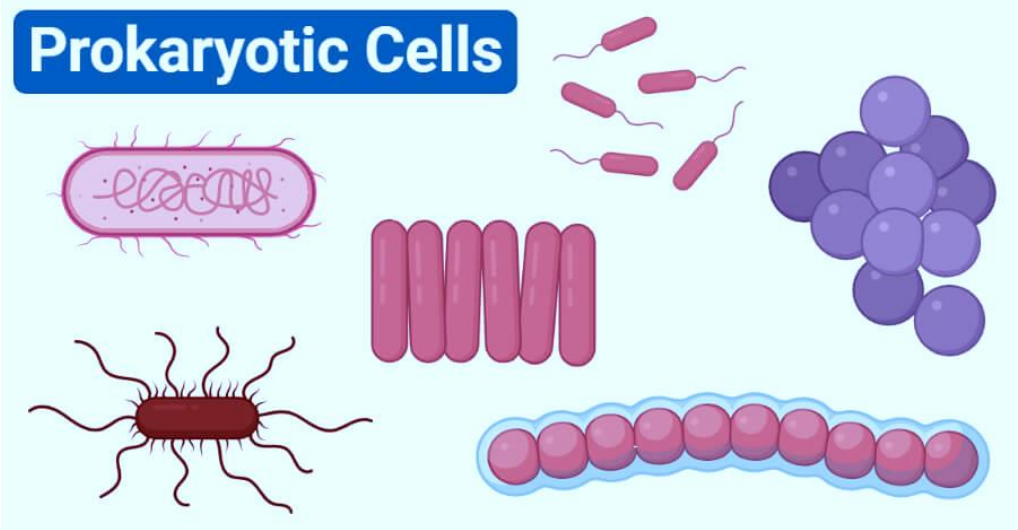
Enzymes, structure conformation, classification, assay, isolation, purification and characterization, catalysis specificity, mechanism of action, active site, regulation of enzyme activity, multienzyme complexes, immobilized enzymes and protein engineering, immobilized enzymes and their application.

UNIT 1:

CELL STRUCTURE AND FUNCTION

Prokaryotic cells

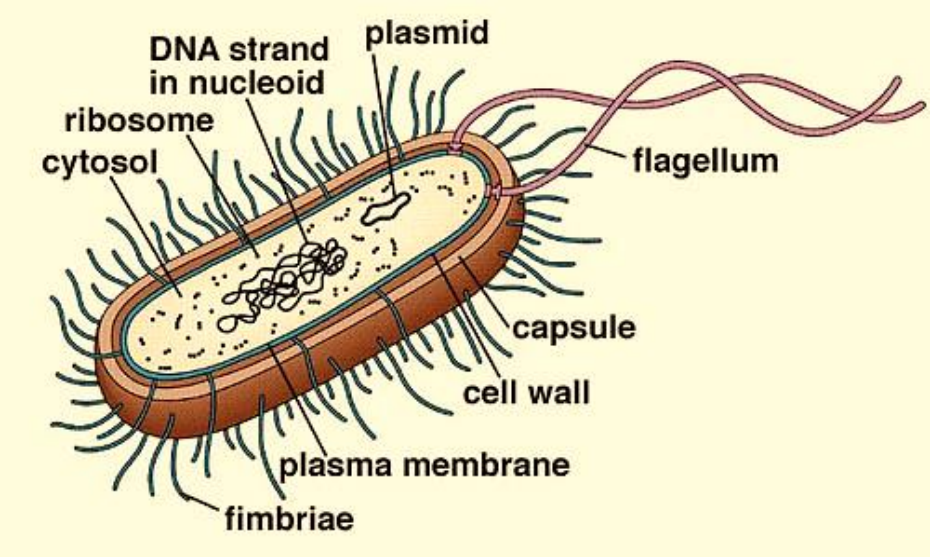
Prokaryotic cells are single-celled entities that are primitive in structure and function as they lack a membrane-bound nucleus and other organelles. The term “prokaryote” is derived from two Greek words, ‘pro’ meaning ‘before’ and ‘karyon’ meaning ‘nucleus’. Prokaryotes are considered to be the first living organisms of the earth as they are the simplest form of life.



Characteristics of prokaryotic cells

The general characteristics of prokaryotic cells are listed below:

- In general, prokaryotic cells range in size from 0.1 to 5.0 µm and are considerably smaller than eukaryotic cells.
- The shape of prokaryotic cells ranges from cocci, bacilli, spirilla, and vibrio. However, prokaryotic cells with modifications of these shapes are also found in nature.
- The cellular organization of prokaryotic cells is primitive as they lack a membrane-bound nucleus and other membrane-bound cell organelles.
- The genetic material of prokaryotic cells in a single chromosome is made up of a single strand of DNA.
- A critical protein, histone protein, that is found bound in the chromosomes of eukaryotes is absent in prokaryotic cells.
- Prokaryotic cells also lack the nucleolus and the mitotic apparatus.
- The cell wall of prokaryotic cells is non-cellulosic and is made up of carbohydrates and lipids.
- Prokaryotic cells are asexual and thus, reproduce via asexual means without the formation of gametes.



Structure (Components/ Parts) of a prokaryotic cell

The structure of a prokaryotic cell is not as complex as eukaryotic cells as they have primitive cell organelles. Generally, most prokaryotic cells have the following components/ parts:

1. Capsule

- This is an additional outer covering in some prokaryotic cells that serve to protect the cell against foreign invaders.
- The capsule is made up of polysaccharides, that allows the cells to cling to various surfaces and preserves the moisture in the cell.

2. Cell wall

- The cell wall is a tough coring of prokaryotic cells present inside the capsule.
- The cell wall of most prokaryotes is made up of polymer of carbohydrates and lipids termed, peptidoglycan.
- In Archaeal cells, however, the cell wall doesn't contain peptidoglycan but some other structure called pseudopeptidoglycan. It Is made up of proteins and other polymers.
- The cell wall provides shape to the cell while protecting the cell organelles present in the cytoplasm of the cell.

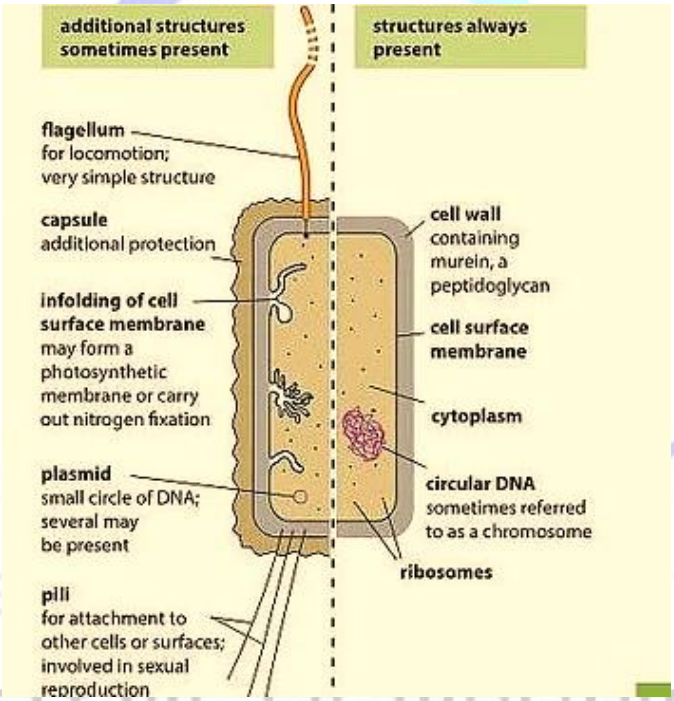
3. Cell membrane/ Plasma membrane/ Cytoplasmic membrane

- Underneath the cell wall is a cell membrane that is made up of phospholipid.
- The phospholipid forms a bilayer consisting of lipid composed of glycerol attached to a hydrophobic phosphate head and two hydrophilic fatty acid tails.

- In archaea, the phospholipid tails are usually connected, forming a monolayer instead of the bilayer structure.
- The plasma membrane in prokaryotic cells provides protection to the cell while allowing the transport of essential molecules in and out of the cell.

4. Cytoplasm

- The cytoplasm is the entire space of cells present inside of the cell membrane.
- It contains a gel-like cytosol and water-based solution that contains minerals and other ions essential for the cell.
- Besides, the cytoplasm also contains other cellular structures like the chromosomes and ribosomes.



Ribosomes

- All prokaryotic cells have 70S ribosomes. The 70S ribosomes are made up of two subunits, 30S, and 50S.
- Here, the 50S subunit contains 23S, and 5S rRNA and the 30S subunit contains 16S rRNA.
- The ribosome is the most commonly observed internal structure in prokaryotic cells.
- The size and number of ribosomes differ in different prokaryotic cells.

- The ribosome is responsible for the formation of polypeptides and in turn, proteins.

6. **Nucleoid region**

- The nucleoid region of cytoplasm in prokaryotic cells contains a single circular chromosome and small rings of extrachromosomal DNA called plasmids.
- The single circular **chromosome** is present as a single copy of genetic material in contrast to the two copies of DNA in eukaryotes.
- The prokaryotic genomes are also smaller in size than the eukaryotic genomes.
- The plasmids, in turn, are copied independently copied outside of the chromosomes. These plasmids might carry some non-essential genes.

7. **Appendages**

- Many prokaryotic cells have cell appendages that protrude out from the cell surface as **flagella**, pili, and fimbriae.
- Flagella are the most common appendages in many prokaryotic cells.
- These are tail-like structures that assist the cell in moving around.
- Fimbriae are thin filamentous structures that are used to stick the cells to various surfaces.
- Pilli, in turn, are longer filaments that have different roles in different cells. One example of this is the sex pilli that holds two cells together as they transfer the DNA molecules by the process of **conjugation.**

Division of prokaryotic cells (Reproduction)

As mentioned earlier, prokaryotic cells reproduce asexually without the formation of gametes. Some asexual modes of reproduction in prokaryotes are:

Binary fission

- Binary fission is a type of asexual reproduction where a single living cell or an organelle grows twice its size and then splits into two identical daughter cells, where each of these daughter cells has the potential to grow into the size of the original cell or organelle.
- Binary fission is the mode of reproduction in many prokaryotes including, archaea, cyanobacteria, and eubacteria.

- During this process, the genetic material of the parent cell is equally divided into two daughter cells. As a result, no genetic variation is observed in the newly formed prokaryotic cells.

Steps of binary fission

1. The DNA of the cell divides to form two identical DNA molecules, both of which are moved towards the cell membrane.
2. The cell then doubles its size, and the cell membrane slowly starts to divide with each having a copy of the DNA.
3. Once the division of the cell membrane is completed, the cell wall is formed between the two strands of DNA dividing the parent cell into two identical daughter cells.

Recombination

- Another asexual mode of reproduction in prokaryotic cells is via recombination.
- In this case, the genetic material of one cell is incorporated into the cell of another prokaryote via transduction, transformation, and conjugation.
- In conjugation, two cells are connected via sex pilli where genes are transferred through the pilli.
- In transformation, the prokaryotic cell takes up the genetic material from the environment and incorporates it into the bacterial chromosome.
- In transduction, the exchange of genes occurs via viral infection. The bacteriophage first infects one bacterium and takes up the targeted gene and transfers it to another cell.

Prokaryotic cell examples

Bacterial cells

- Bacteria are the single-celled organisms that are found in all ecosystems throughout the world.
- The cell wall of the bacterial cell is formed of peptidoglycan that makes it tough and thick.
- Capsules are unique to some bacteria and thus might not be present in other prokaryotic cells.
- The genetic material of bacteria is present in the form of circular coils of chromosomes.
- Examples of bacterial cells are *E. coli*, *Streptomyces* spp, *Pseudomonas* spp, etc.

Archaeal cell (Archaea)

- Archaeal cells are similar to bacterial cells as they too are primitive unicellular organisms.

- Archaeal cells are mostly found in extreme environments like hot springs, oceans, and marshlands.
- The capsule is not present in archaeal cells, and the cell wall is made up of pseudopeptidoglycan, composed of proteins.
- Similarly, the cell membrane of archaeal cells has a monolayer of phospholipid that protects the cell against harsh environments.
- Examples of archaeal cells are *Halobacterium* spp, *Thermoplasma* spp, *Sulfolobus* spp, etc.

Eukaryotic cells

Eukaryotic cells are the cells that are complex in structure and function as they have a membrane-bound well-defined nucleus and other membrane-bound organelles.

- The term "eukaryote" is derived from Greek words, "eu" meaning 'true' and "karyon' meaning 'nucleus.'
- Eukaryotic cells have a more advanced structural composition when compared to prokaryotes.
- By virtue of these advancements, eukaryotic cells are capable of performing more complex functions than **prokaryotic cells.**

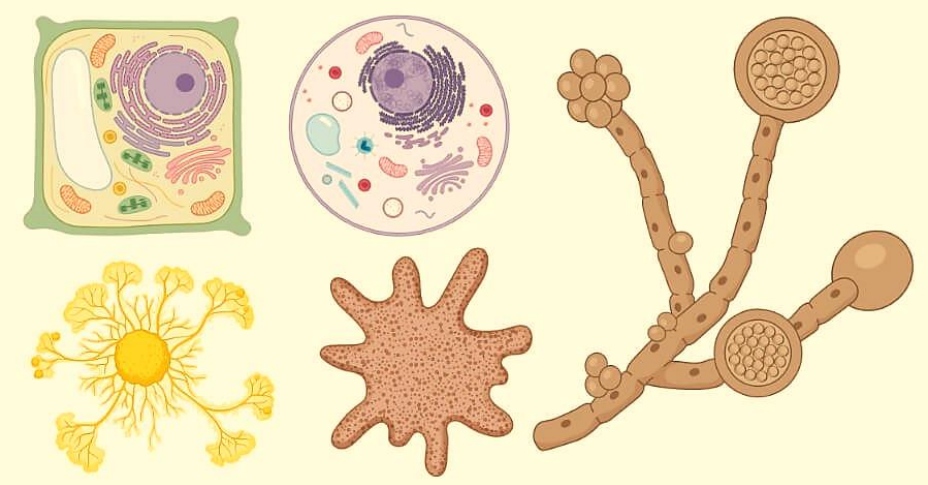
Characteristics of Eukaryotic cells

The general characteristics of eukaryotic cells are listed below:

1. The size of eukaryotic cells is significantly larger than prokaryotic cells as the size ranges from 10-100 μm in diameter.
2. The shape of eukaryotic cells varies significantly with the type of cell. Some cells are pleiomorphic like Amoeba, whereas some have a defined shape like plant cells. The

shape of the cells is highly influenced by environmental factors as well as other functional adaptations.

3. Eukaryotic cells have a more advanced cellular organization with multiple membrane-bound organelles and well-defined nucleus.
4. The genetic material of eukaryotic cells is DNA, and it is linear and has multiple origins of replication.
5. The nucleus of eukaryotic cells is surrounded by a complex nuclear membrane. The chromosomes in the nucleus are complexed with histone protein to form linear chromosomes as opposed to circular chromosomes of prokaryotes.
6. The cell wall that is present in some eukaryotic cells is made up of cellulose or other carbohydrates.
7. Some eukaryotic cells like yeast cells reproduce asexually via mitosis or fission, whereas other cells reproduce sexually.



Structure (components/ parts) of Eukaryotic cell

Eukaryotic cells are much larger in size when compared with prokaryotic cells, having the volume about 10,000 times higher than prokaryotic cells. Eukaryotic cells are formed of a number of membrane-bound and membrane-less organelles that all perform together to support the cell's organization and function. The common component/ parts in eukaryotic cells are as follows:

Cell wall

- The cell wall is present in some eukaryotic cells like some protists, fungal and plant cells.
- The cell wall in plants and some protists is made up of cellulose microfibrils and a network of glycans embedded in the matrix of pectin polysaccharides.
- The composition of the cell wall in fungal cells is different as in fungal cells, the cell wall is composed of a different polysaccharide, chitin.
- The function of the cell wall, however, is similar in eukaryotic cells. The cell wall provides support and shape to the eukaryotic cells.

Cell membrane/ Plasma membrane/ Cytoplasmic membrane

- The cell membrane in eukaryotic cells is present inside the cell wall.
- In cells without the cell wall, the cell membrane functions as the outermost covering that separates the internal contents of the cell from the outside environment.
- The plasma membrane is made up of phospholipid bilayer with integral proteins embedded between the two layers.
- The composition of the cell membrane is similar in eukaryotes and prokaryotes.

Cytoplasm

- The cytoplasm of the eukaryotic cell is a fluid-filled space that accommodates all internal cell organelles and other molecules.
- The cytoplasm consists of a jelly-like cytosol and a water-soluble solution containing minerals, ions and other molecules.
- The amount of cytoplasm is higher in eukaryotic cells as compared to prokaryotic cells as the cell volume is more abundant in eukaryotic cells.

Nucleus

- The nucleus is an organelle present in the cytoplasm of a eukaryotic cell.
- It is more complicated than the prokaryotic nucleus as the nucleus is surrounded by a nuclear membrane having a composition similar to the plasma membrane.
- The genome of a eukaryotic cell is present inside the nucleus where it remains coupled with various proteins like the histone protein.
- Inside the nucleus, the DNA molecules are arranged in chromosomes which are linear and more organized.
- Additionally, the nucleus also houses a nucleolus that is not surrounded by a membrane but has proteins that make up the ribosomes and rRNA.

Ribosomes

- In eukaryotic cells, the ribosomes are 80S type containing 60S and 40S subunits.
- The larger subunit is further composed of 5S RNA, 28S RNA, and proteins, whereas the smaller subunit is composed of 18S RNA and 33 proteins.
- The ribosomes in eukaryotic cells are found either attached to the endoplasmic reticulum or are found free in the cytoplasm.

Mitochondria and Plastids

- Mitochondria and plastids are membrane-bound organelles found in the cytoplasm of eukaryotic cells.
- Both mitochondria and plastids have an extrachromosomal DNA that regulates the functions of the organelles.
- In mitochondria, the outer membrane is made up of phospholipid bilayer, whereas the inner layer is folded into cristae where the major physiological function of the cell takes place.
- Plastids are found in eukaryotic cells of plants and algae that provide color to the cell. Additionally, plastids also have a green pigment, chlorophyll, which is required for photosynthesis.

Cytoskeletal structures

- Many eukaryotic cells have cytoplasmic projections like flagella and cilia that are involved in movement, feeding, and sensation of these cells.
- These structures are mainly composed of tubulin proteins supported by microfilaments and microtubules.
- Cytoskeletal structures are also present in the cytoplasm that provides shape and support to the cell.

Division of Eukaryotic cells (Reproduction)

Some eukaryotic cells can divide only by asexual means while other eukaryotic cells divide both sexually as well as asexually.

Asexual reproduction

- Asexual reproduction is common in all eukaryotic cells except for reproductive cells that form the male and female gamete.
- The most common mode of asexual reproduction is mitosis, where the cell grows double its size and then divided to form two identical daughter cells.
- Unicellular fungal cells and protists divide by budding where new cells arise on the surface of dividing cells in the form of a chain.

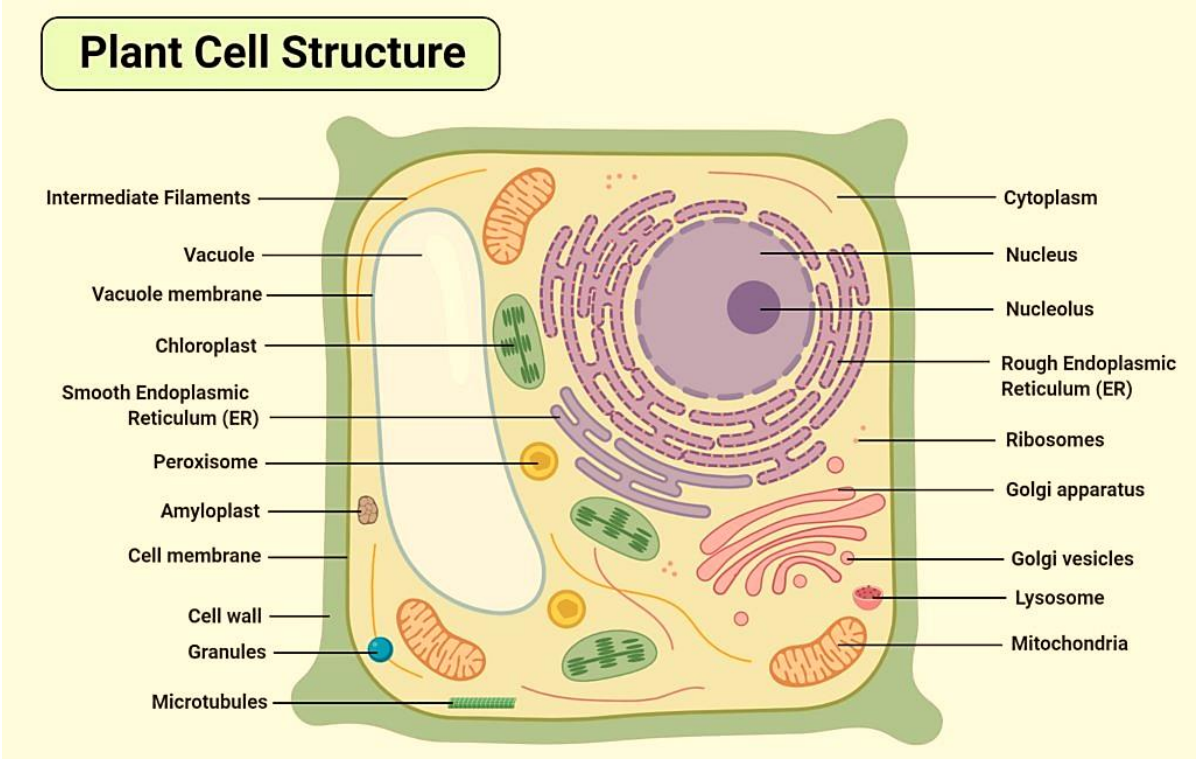
- Processes like binary fission and multiple fission are also observed in cells of primitive eukaryotes.
- Some fungi are also known to divide/ reproduce asexually via sporulation.

Sexual reproduction

- The cells of the reproductive system in plants and animals are divided by the sexual method.
- In this method, the cell divided meiotically to form four daughter cells, each with half the number of chromosomes to their parent cell.
- The sexual reproduction in eukaryotic cells is responsible for the variation in different cells.

Eukaryotic cell examples

Plant cells

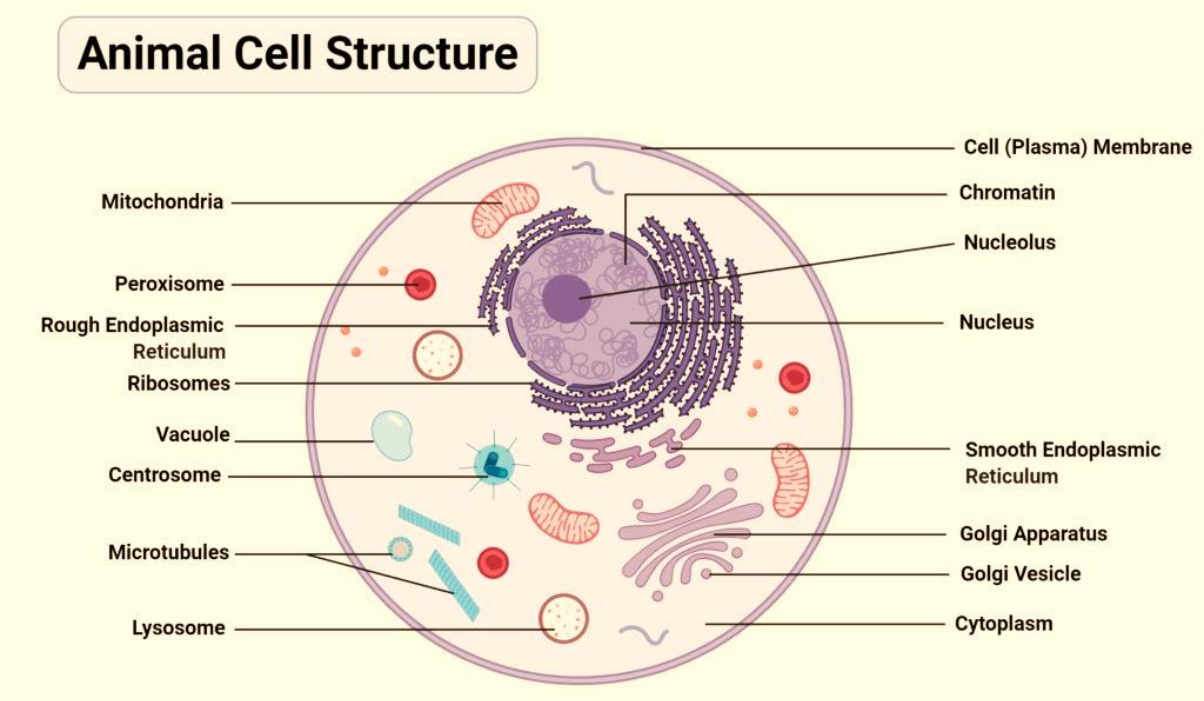


- Plant cells are examples of eukaryotic cells where there is a thick cell wall made up of cellulose that provides the shape and structure to the cell.
- Each plant cell has a larger vacuole in the cytoplasm that maintains the turgor pressure of the cell.

- Additionally, plant cells are unique among eukaryotic cells as they have chloroplasts containing chlorophyll that plays an essential role during the process of photosynthesis.

Animal cells

- Animal cells are another group of eukaryotic cells that do not have a rigid cell wall.
- The lack of cell wall in animals allows the cells to acquire different shapes and assists the process of phagocytosis and pinocytosis.
- Animal cells are different from plant cells as they have a smaller vacuole, and they don't have chloroplasts.
- Animal cells have additional organelles, centriole, that generates the mitotic apparatus required during cell division.



Fungal cells

- Fungal cells are similar to plant cells in that they also have a rigid cell wall.
- However, the cell wall is made up of chitin and not cellulose.

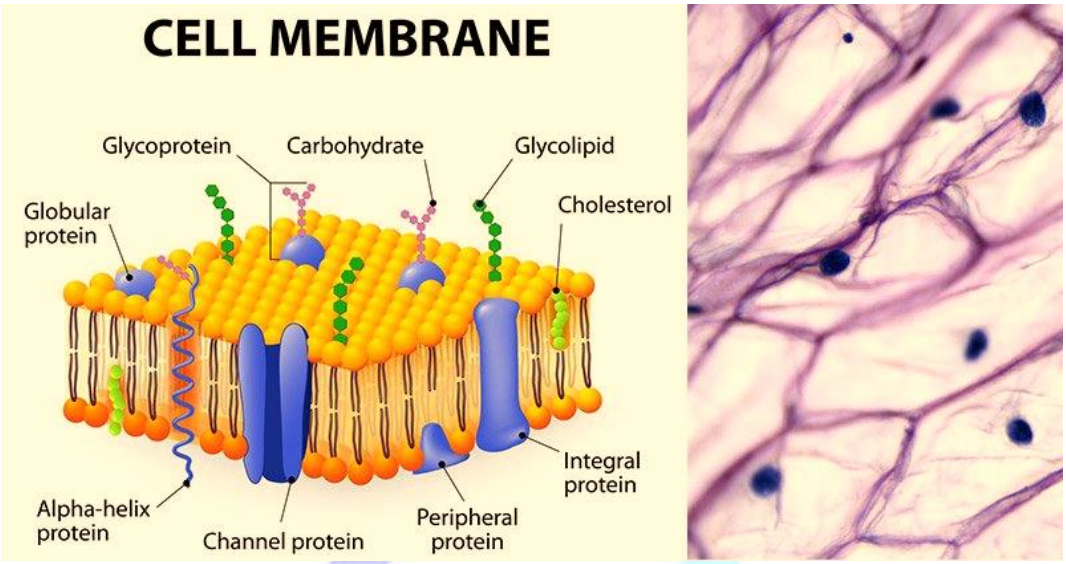
- Some fungi are unicellular like yeasts, which have tiny holes in their cell membrane that allows the cells to exchange cytoplasm and other organelles.

Protists

- Protists are the unicellular eukaryotes that are primitive when compared to plant or animal cells.
- Most protists don't have a cell wall while some might.
- Many protists are known to have chloroplast containing chlorophyll while others might have other photosynthetic pigments.
- Protists are known to have **cilia and flagella** that assist in the movement of the cells.

Cell (Plasma) Membrane

- Membranes are lipid structures that separate the contents of the compartment they surround from its environment.
- Plasma membranes separate the cell from its environment while other membranes define the boundaries of organelles and provide a matrix upon which complex chemical reactions can occur.
- The plasma membrane, also known as the cell surface membrane or plasmalemma, defines the boundary of the cell.
- It is a phospholipid bilayer with embedded proteins that encloses every living cell.
- It regulates the movement of materials into and out of the cell and facilitates electrical signalling between them.
- It is said to be semi-permeable because it allows certain molecules but not others to enter into the cell.
- It serves some specific functions such as controlling the flow of nutrients and ions into and out of the cells, mediating the response of a cell to external stimuli (a process called signal transduction), and interacting with bordering cells.



Structure and Composition

All biological membranes are constructed according to a standard pattern. They consist of a continuous bilayer of amphipathic lipids approximately 5 nm thick, into which proteins are embedded. In addition, some membranes also carry carbohydrates (mono- and oligosaccharides) on their exterior, which are bound to lipids and proteins. The proportions of lipids, proteins, and carbohydrates differ markedly depending on the type of cell and membrane.

- The plasma membrane consists of a **lipid bilayer** containing embedded and peripheral proteins. The major component of membranes is lipids.
- The lipids in the plasma membrane are in the form of **phospholipids**, which contain a polar head group attached to two hydrophobic fatty acid tails; the head group faces the aqueous environment, the fatty acid tails the interior of the bilayer.

1. Glycerol-based lipids contain a glycerol backbone, and consist of phosphatidic acid (PA), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylinositol (PI), and cardiolipin (CL).
2. The one sphingosine-based lipid is sphingomyelin (SM).
3. **Cholesterol** is present in eukaryotic membranes and maintains membrane fluidity at a variety of temperatures. Fluidity is also determined by the content of unsaturated fatty acids in the membrane, which are liquids at room temperature, and the chain length of the fatty acids (shorter chains are more fluid than longer chains).

- **The embedded proteins** in the plasma membrane function as either channels or transporters for the movement of compounds across the membrane, as receptors for the binding of hormones and neurotransmitters, or as structural proteins.
- The **peripheral membrane proteins** provide mechanical support to the membrane through the inner membrane skeleton or the cortical skeleton. An example of this is spectrin in the red blood cell membrane. These can be removed from the membrane by ionic agents.
- The third type of membrane proteins is the glycophosphatidylinositol (GPI) glycan-anchored proteins. One example of a GPI-anchored protein is the prion protein, present in neuronal membranes.
- The plasma membrane **glycocalyx** consists of short chains of carbohydrates attached to proteins and lipids which extend in the aqueous media and both protects the cell from digestion and restricts the uptake of hydrophobic molecules.

Note:

- Membrane lipids are strongly amphipathic molecules with a polar hydrophilic "head group" and a polar hydrophobic "tail." In membranes, they are primarily held together by the hydrophobic effect and weak Van der Waals forces and are therefore mobile relative to each other. This gives membranes a more or less fluid quality.
- Lipids and proteins are mobile within the membrane. If they are not fixed in place by special mechanisms, they float within the lipid layer as if in a two-dimensional liquid; biological membranes are therefore also described as being a **"fluid mosaic".**

Fluid Mosaic Model

- The plasma membrane, also known as the cell surface membrane or plasmalemma, defines the boundary of the cell.
- They are a special type of membranes which are lipid structures that separate the cell from its environment.
- In composition, it is a phospholipid bilayer with embedded proteins that enclosing every living cell.
- It serves some specific functions such as controlling the flow of nutrients and ions into and out of the cells, mediating the response of a cell to external stimuli (a process called signal transduction), and interacting with bordering cells.