

Eukaryotic Cell Structure and Functions

Learning Objectives

- Classify living cells
- Describe molecular and functional organization of a cell and its subcellular components
- Describe structure and functions of cytoskeleton
- Describe cell fractionation and marker enzymes for different organelles

Classification Of Living Cells

- All organisms are grouped into one of three major domains: **archaea**, **bacteria** and **eukaryotes**.
- Archaea and Bacteria (single-cell microorganism) can be distinguished on genetic and biochemical grounds.

- **Archaea** lives in extreme environments: salt lakes hot springs, highly acidic bogs, and the ocean depths.
Archaea may be the most primitive of the groups.
- **Bacteria** live in soils, surface waters, and the tissues of other living or decaying organisms. Bacteria are classified as **prokaryotes**.
- **Eukaryote** include single cell organism such as yeast, fungi and multicellular plants and animals.

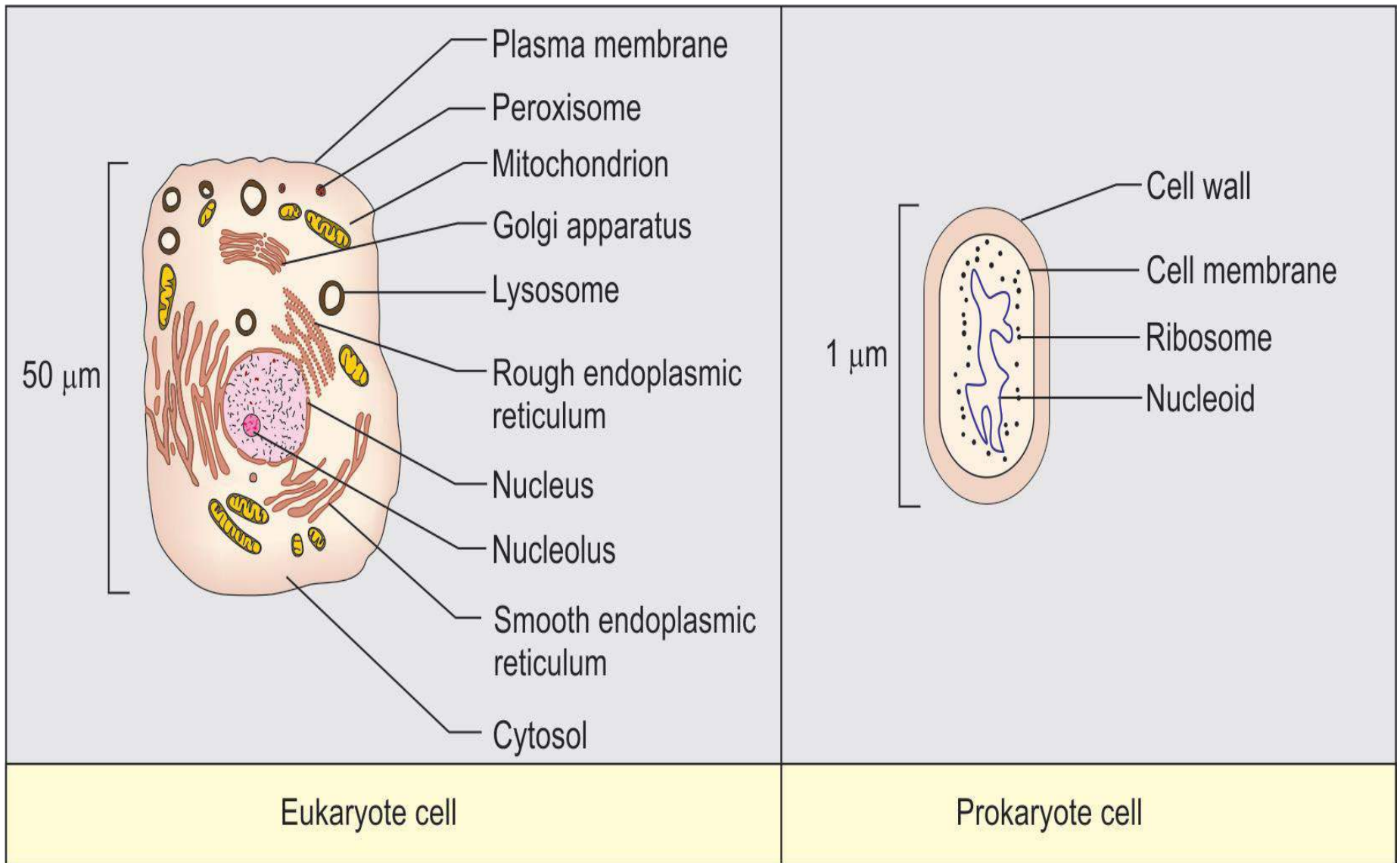


Figure 1.1: Cell structure of eukaryotic and prokaryotic cell.

TABLE 1.1: Structural features of prokaryotes and eukaryotes.

<i>Organelles</i>	<i>Eukaryotes</i>	<i>Prokaryotes</i>
Nucleus	Present	No defined nucleus. DNA present but not separated from rest of the cell
Plasma membrane	Present	Present
Mitochondria	Present	Absent. Enzymes for oxidation reactions are located on plasma membrane
Endoplasmic reticulum	Present	Absent
Ribosomes	Present	Present
Chromosomes	Linear	Circular
Cytoplasm	Contains various membrane-bound organelles, such as mitochondria, lysosomes, peroxisomes, and Golgi apparatus	Undifferentiated
Reproduction	Mitosis	By binary division

What Are viruses?

- **Viruses** are not living organism in the sense that cells are. They are incapable of replicate themselves outside their host cells and have virtually no biochemical activities of their own.
- Viruses are supramolecular complexes of nucleic acid, either DNA or RNA encapsulated in a protein coat, and in some instances, surrounded by a membrane envelope.

- Viruses are not alive; they are not even cellular. Instead, they are packaged bits of genetic material that can parasitize in order to reproduce.
- Viruses infecting bacteria are called **bacteriophages** (“bacteria eaters”): different viruses infect animal cells and plants cells.

Molecular And Functional Organization Of A Cell

And Its Subcellular Components

A cell has three major components.

1. Plasma membrane (cell membrane)

2. Cytoplasm with its organelles

— Endoplasmic reticulum

— Golgi apparatus

— Mitochondria

— Lysosomes

— Peroxisomes.

3. Nucleus

Cell Membrane (Plasma Membrane)

- Most membranes composed primarily of **lipids** and **proteins**.
- The basic structure of the cell membrane is a lipid bilayer (two layers of lipids).
- Large globular protein molecules are interspread in this lipid bilayer.

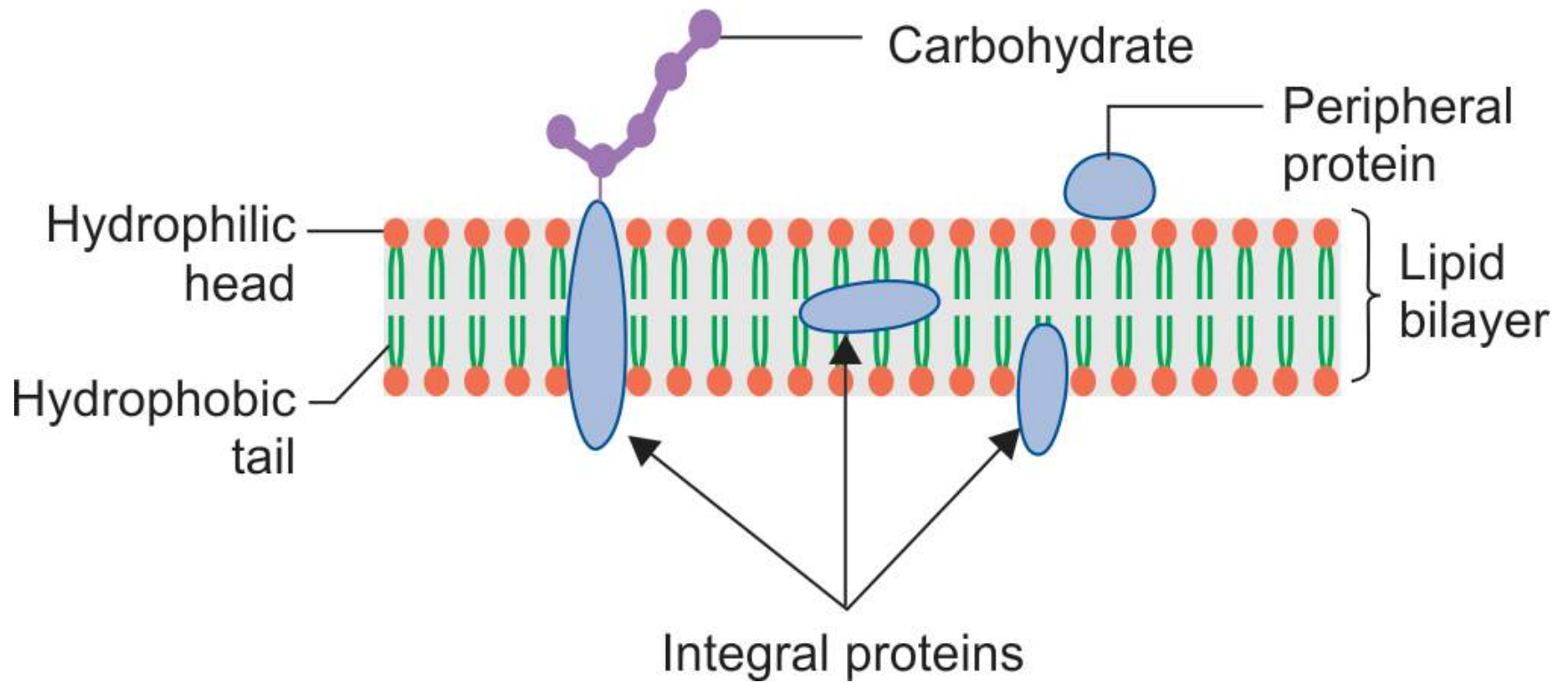


Figure 1.2: The basic organization of biological membrane.

- The membrane is sometimes referred to as a **fluid mosaic**.
- The membrane mosaic is **fluid** because most of the interactions among its components are non-covalent, leaving individual lipid and protein molecules free to move laterally in the plane of the membrane.

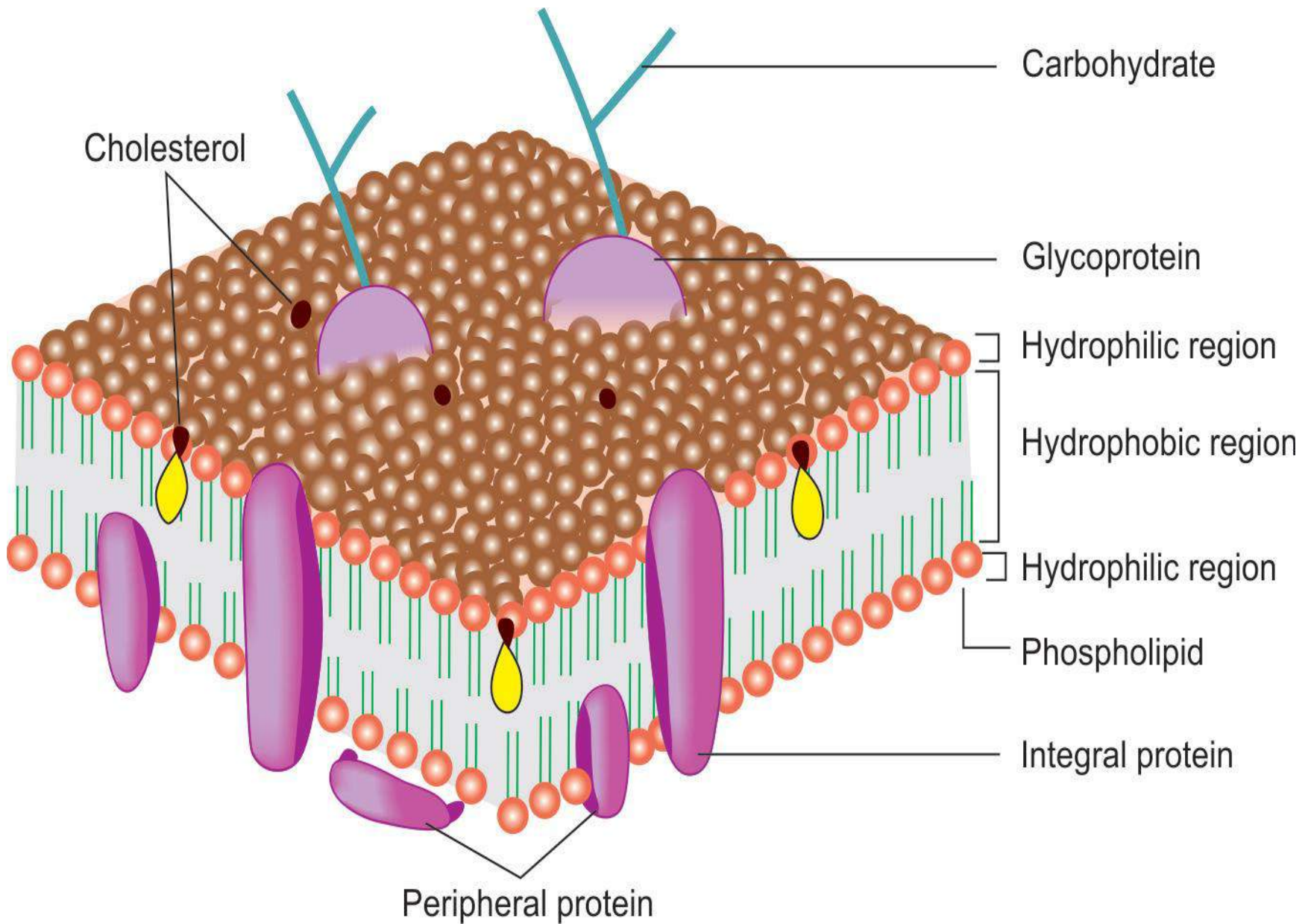


Figure 1.3: The fluid mosaic model of cell membrane.

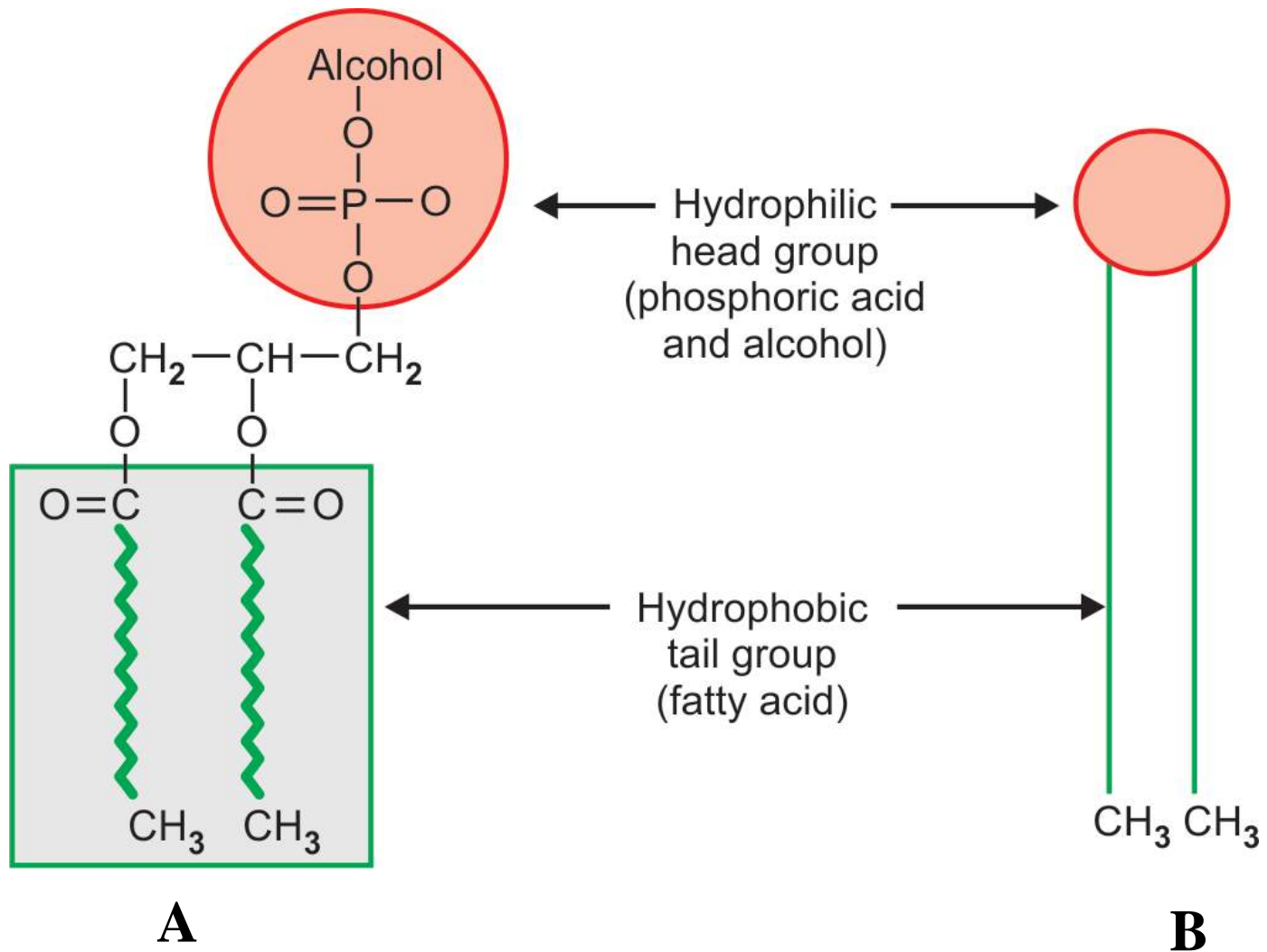
- The lipid of the membrane provides a barrier that obstructs the movements of water and water-soluble substances from one cell compartment to another.
- However, protein molecules in the membrane often do penetrate all the way through the membrane, organized into actual pores, for passage of specific substances through the membrane.
- Also many other membrane proteins are **enzymes** that catalyze different types of chemical reactions.

The approximate composition of cell membrane is:

- Protein: 55%
- Phospholipids: 25%
- Cholesterol: 13%
- Other lipids: 4%
- Carbohydrate: 3%

Lipid of the membrane

- The basic lipid bilayer is composed of phospholipid molecules.
- One end of each phospholipid molecule (head group) is soluble in water that is it is **hydrophilic**.
- The other end (tail group) is soluble only in fats; that is, it is **hydrophobic**.
- The phosphate end of the phospholipid is hydrophilic and the free fatty acid portion is hydrophobic.



Figures 1.4A and B: Structure of phospholipid.

(A) A common glycerophospholipid;

(B) Diagrammatic representation of phospholipid.

The principle phospholipids in the membrane are:

▪ **Glycerophospholipids:**

- phosphatidylcholine,
- phosphatidylethanolamine, and
- phosphatidylserine

▪ **Sphinogophospholipid:** sphingomyelin.

- The lipid composition varies among different cell types, with phosphatidylcholine being the major plasma membrane phospholipid in most cell types.
- Plasma membrane for example, is enriched in **cholesterol** and contains no detectable **cardiolipin**; mitochondrial membrane is very low in cholesterol and sphingolipids but that contain cardiolipin.

Cholesterol

- The cholesterol molecules in the membrane are also lipid in nature.
- Cholesterol, which is interspreaded between the phospholipids, maintains **membrane fluidity**.
- Unsaturated fatty acid chains of the glycerophospholipids bent into the cis conformation form a pocket for cholesterol, which binds with its hydroxyl group in the external hydrophilic region of the membrane and its hydrophobic steroid nucleus in the hydrophobic membrane core.

- The presence of cholesterol and the cis unsaturated fatty acids in the membrane prevent the hydrophobic chains from packing too closely together.
- The changes in membrane fluidity may affect proteins that span the membrane (integral proteins), such as ion channels and receptors for neurotransmitters involved conducting the nerve impulse.

Membrane Proteins

- The protein composition of membrane from different sources varies even more widely than their lipid composition, reflecting functional specialization.
- Two types of membrane proteins differ in their association with the membrane.
- Most of the membrane proteins are **glycoproteins**.

Integral membrane proteins: Integral proteins that are protruding all the way through the membrane. They are very firmly associated with the lipid bilayer.

Peripheral membrane proteins: Peripheral proteins that are attached only to one surface of the membrane and do not penetrate all the way through.

- Peripheral protein molecules are often attached to the integral proteins.
- They are associated with the membrane through **electrostatic interactions** and **hydrogen bonding** with the hydrophilic domains of integral proteins and with polar head groups of membrane lipids

Functions of membrane Proteins

- Integral membrane proteins function primarily as **channels (pores)** through which water molecules and water soluble substances, especially ions, can diffuse between extracellular and intracellular fluids.
- Other integral proteins act as **carrier proteins** transporting substances that otherwise could not penetrate the lipid bilayer.

- They can also serve as **receptors** for hormones and neurotransmitter.
- Integral proteins spanning the cell membrane provide a means of conveying information about the environment to the cell interior.
- Peripheral proteins function almost entirely as enzymes or as controllers of transport of substances through the cell membrane “pores”.

Membrane carbohydrates

- Membrane carbohydrates occur in combination with proteins or lipids in the form of **glycoproteins** or **glycolipids**.
- Some of the proteins and lipids on the external surface of the membrane contain short chains of **carbohydrate (oligosaccharides)** that extend into the aqueous medium.

- As well as many other carbohydrate compounds called **proteoglycans** are loosely attached to the outer surface of the cell.
- Thus the entire outside surface of the cell often has a loose carbohydrate coat called the **glycocalaxy**.
- Carbohydrate constitutes 2% to 10% of the weight of cell membrane.

Functions of Membrane carbohydrates

- Many of the carbohydrates have a negative electrical charge, which gives most cells an overall negative surface charge that repels other negative objects and restricts the uptake of hydrophobic compounds.
- The glycocalaxy of some cell attaches to the glycocalaxy of other cells, thus attaching cells to one another.
- Many carbohydrate act as **hormone receptor** such as insulin
- Some carbohydrate moieties involved into immune reactions.

Functions of Plasma Membrane

- The plasma membrane maintains the physical **integrity of the cell** by preventing the contents of the cell from leaking into the outside fluid environment and at the same time facilitating the **entry of nutrients**, inorganic ions and most other charged or polar compounds from the outside.

- The functions of the plasma membrane are coordinated by specialized adhesion receptors called **integrins**. Integrins are integral transmembrane proteins. Integrins represent important cell receptors that regulate fundamental cellular process; such as attachment, movement, growth and differentiation.

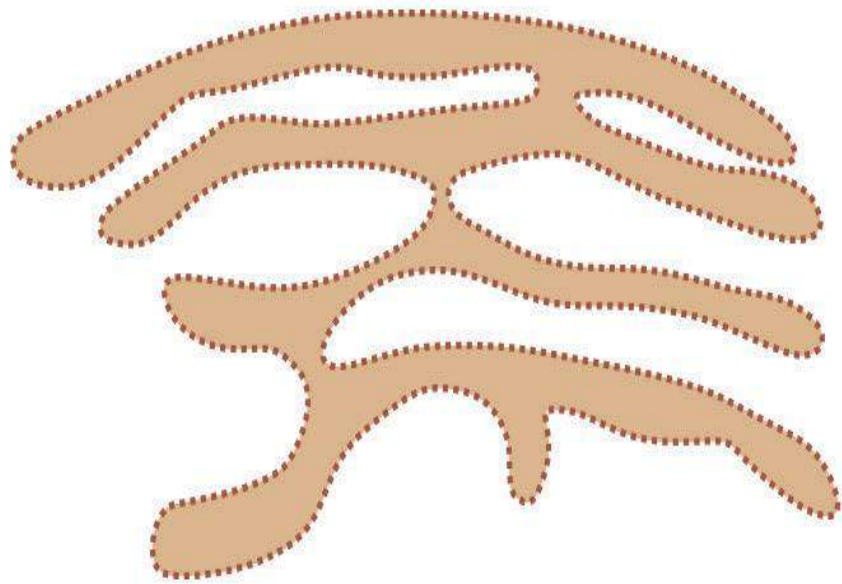
Cytoplasm and its Organelles

Five important organelles that are suspended in the cytosol are.

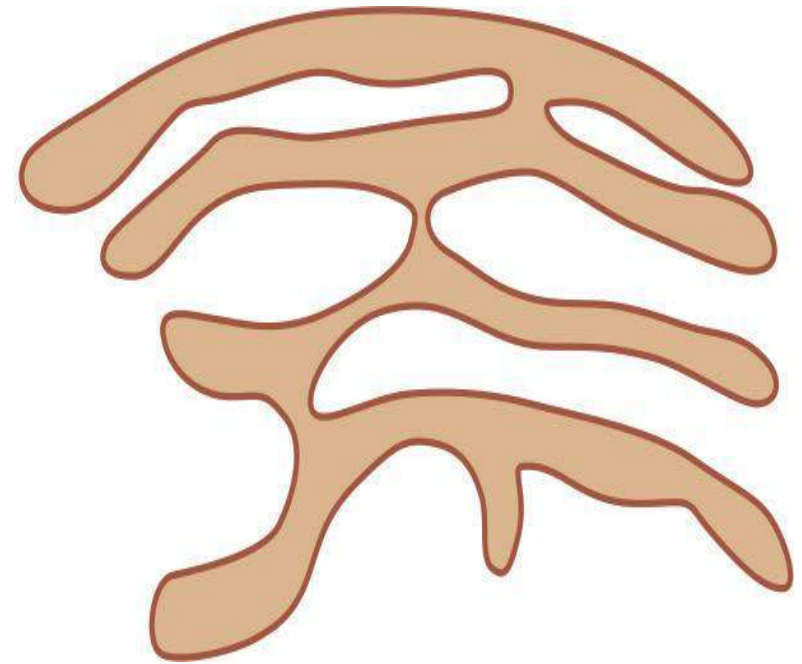
1. Endoplasmic reticulum
2. Golgi apparatus
3. Mitochondria
4. Lysosomes
5. Peroxisomes

Endoplasmic Reticulum

- The endoplasmic reticulum (ER) is the interconnected folded network of tubular structures in the cytoplasm.
- A portion of the endoplasmic reticulum has **ribosomes** bound to it, which give it a rough appearance in contrast with smooth endoplasmic reticulum which is devoid of ribosomes.
- Endoplasmic reticulum and Golgi apparatus are involved in formation of other cellular organelles such as **lysosomes** and **peroxisomes**



A



B

Figures 1.5A and B: Structure of endoplasmic reticulum.
(A) Rough or granular endoplasmic reticulum; and
(B) Smooth or a granular endoplasmic reticulum.

Functions of Endoplasmic Reticulum

- The rough endoplasmic reticulum is the site for **synthesis of proteins** .
- The endoplasmic reticulum also has mechanisms for maintaining the quality of the proteins synthesized.
The endoplasmic reticulum has three different sensor molecules that monitor the amounts of improperly folded proteins that accumulate.

- Smooth endoplasmic reticulum is involved in **lipid synthesis** and contains enzymes termed **cytochromes P₄₅₀** that catalyze hydroxylation of a variety of endogenous and exogenous compounds.
- These enzymes are important in **biosynthesis of steroid hormones** and removal of **toxic substances**

Golgi apparatus

- The Golgi (named for its discoverer **Camillo Golgi**) apparatus is a flat, membranous sac.
- In Golgi apparatus proteins are processed, modified and prepared for export from the cell.
- It works in association with endoplasmic reticulum, where proteins for certain destinations are synthesized

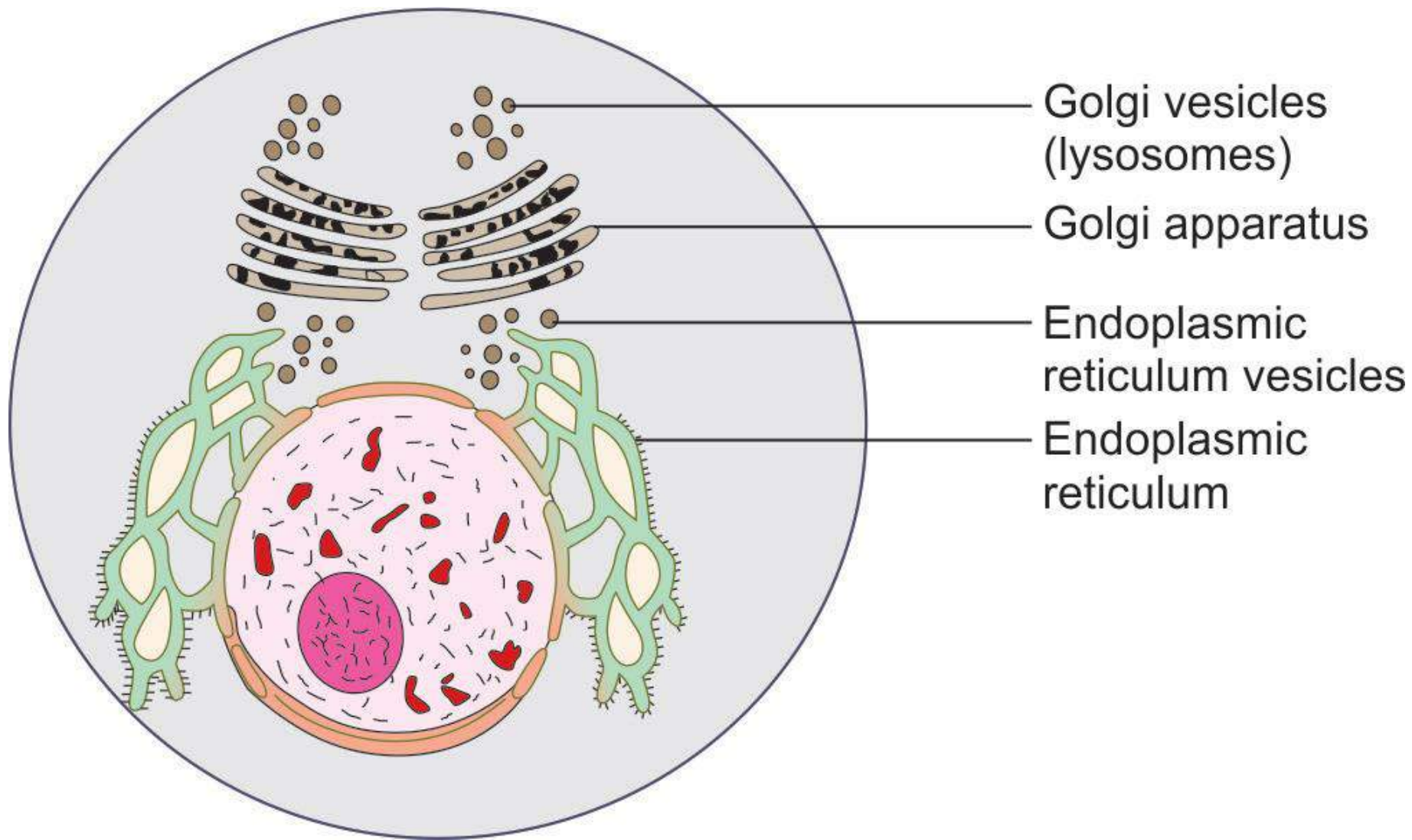


Figure 1.6: A Golgi apparatus and its relationship to the endoplasmic reticulum and nucleus.

Functions of Golgi Apparatus

- Proteins which are synthesized in the endoplasmic reticulum passed through layers of the Golgi apparatus where enzymes in Golgi membranes catalyse transfer of carbohydrate units to proteins to form **glycoproteins** or to lipids to make **glycolipids**, a process that is important in determining the proteins eventual destination.

- The modified proteins are then sorted, packaged and transported to destination inside or outside the cell. Golgi apparatus plays the role of **post office mail sorting room**, the mail in this case being newly synthesized proteins.

Mitochondrion (Power House of Cell)

- Mitochondria are called **Power plant** of the cell, since they generate most of the cell's energy in the form of **ATP**.
- **Erythrocytes** are an exception which derive their ATP from glycolysis due to **lack of mitochondria**.
- Each mitochondrion is bounded by **two membranes**.

- The relatively **porous** smooth **outer membrane** is permeable to most molecules.
- The **inner membrane**, which is **impermeable to ions** and a variety of organic molecules.
- The inner membrane projects inwards into folds that are called **cristae**

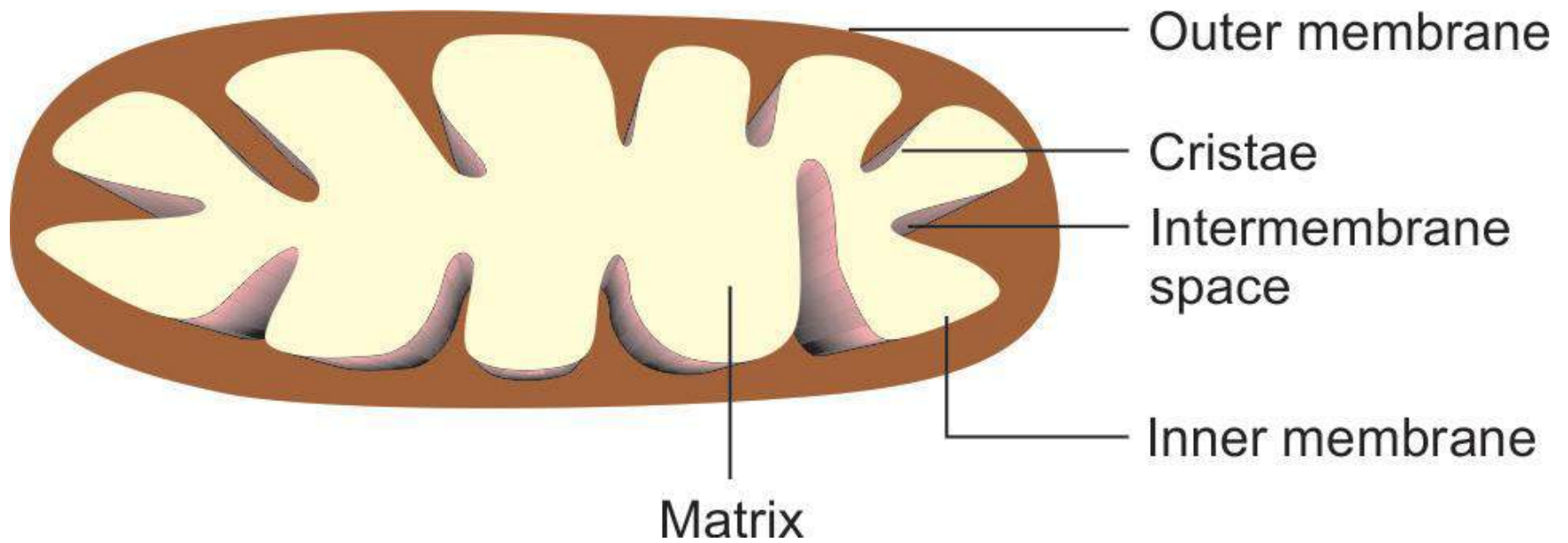


Figure 1.7: Structure of mitochondria.

Functions of Mitochondria

- The inter-membrane space contains several **enzymes** involved in **nucleotide metabolism**.
- Whereas, the gel-like matrix (mitosol) consists of high concentration of enzymes required for the metabolic pathways of **oxidation of pyruvate**.
- The mitochondrial matrix is the site of most of the reactions of the **citric acid cycle** and **fatty acid oxidation**.

- Components of **electron transport** system and **oxidative phosphorylation** that are responsible for the synthesis of **ATP** are embedded in inner membrane.
- Mitochondria also have a requisite machinery to catalyze **protein synthesis**.
- Mitochondria contain their own DNA, (mtDNA), which in human encodes 13 respiratory chain proteins, as well as small and large ribosomal RNAs and enough tRNAs to translate all codons

- In recent years mitochondria have also been recognized as key regulators of apoptosis.
- Mitochondria have a key role in aging; cytochrome c, a component of the mitochondrial electron transport chain, is an initiator of **apoptosis**.

Lysosomes

- Lysosomes are organelles formed from Golgi apparatus and dispersed throughout the cytoplasm.
- The lysosomes are membrane bounded sacs containing **hydrolytic enzymes** like **proteases, nucleases, glycosidases, lipases, phosphatases and sulfatases**.
- All these enzymes function at acidic pH, so pH of lysosome matrix is maintain at about 5.

Functions of Lysosomes

- Lysosomes are involved in digestion of intra- and extra-cellular substances that must be removed.
- Substances destined to be degraded are identified and taken up by lysosomes through endocytosis.
- During development lysosomes play an important role in the formation of specialized tissues such as fingers and toes.
- For example, lysosomes digest the webbed tissues that join fingers and toes in the embryo.

Peroxisomes

- Peroxisomes (organelles having ability to produce or utilize hydrogen peroxide) are similar to lysosome in that they are membranous sacs containing enzymes.
- The enzyme content of cellular peroxisome varies according to the need of the tissue.
- Liver peroxisomes contain three important detoxification enzymes; **catalase, uric acid oxidase and D-amino acid oxidase.**

Functions of Peroxisomes

- Peroxisomes contain enzymes that are used for **detoxification** rather than for hydrolysis.
- Peroxisomes also participate in degradation of very long chain fatty acids and synthesis of **glycerolipids**, **plasmalogens** and **isoprenoids**.

- In peroxisomes a number of molecules which are not metabolized elsewhere are oxidized by enzymes by using molecular oxygen directly and produce hydrogen peroxide (H_2O_2).
- Hydrogen peroxide is destroyed further by catalase and peroxidases.

Nucleus

- Nucleus is the control center of the cell; it contains the DNA organized into chromosomes which carry genetic information.
- The nucleus is surrounded by a double membrane called **nuclear envelope**.
- The outer membrane is fused with the endoplasmic reticulum at multiple sites.
- **Nuclear pores** (multiprotein complexes) occur at points where the outer and inner membranes are connected

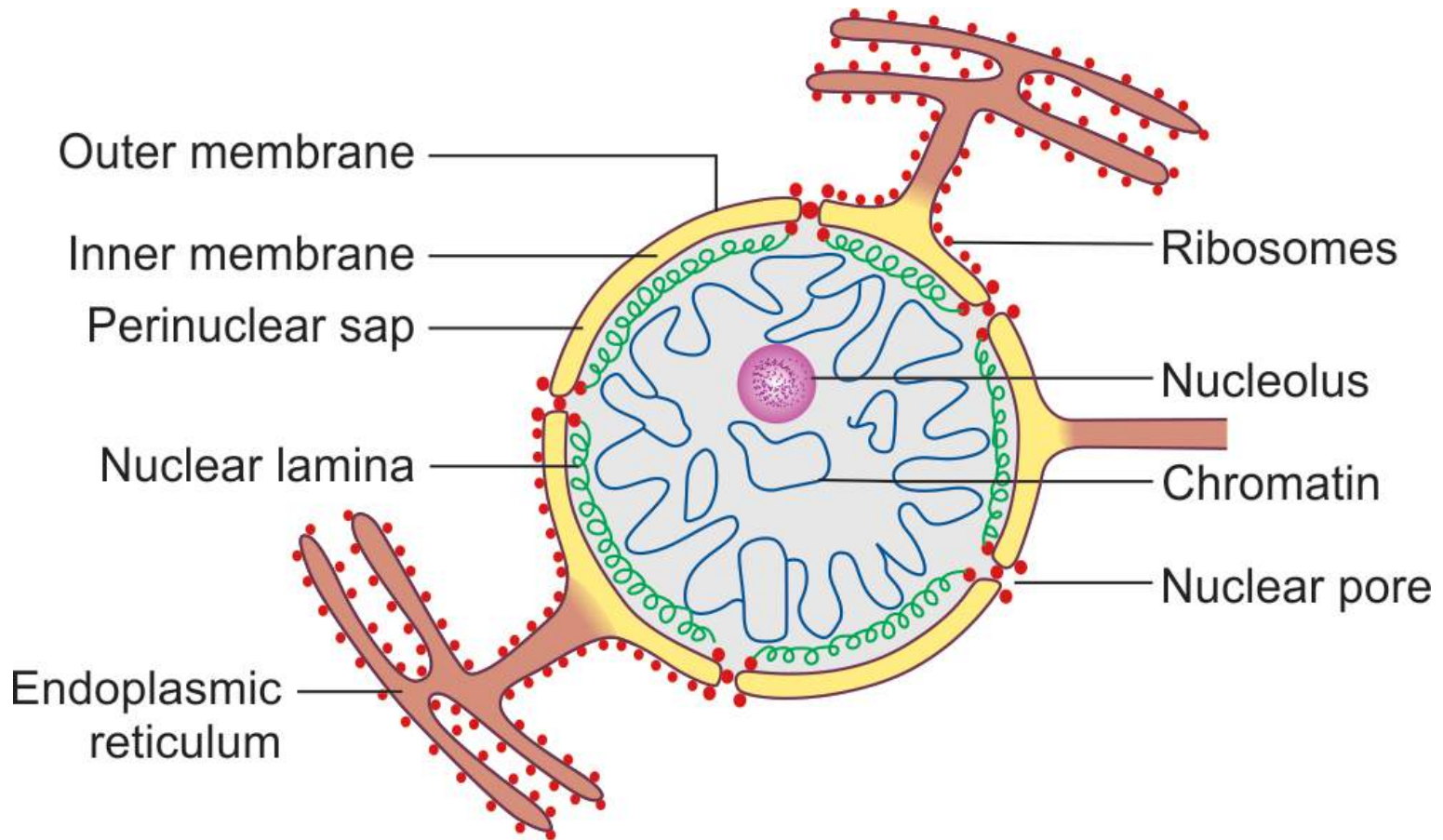


Figure 1.8: General structure of nucleus.

Functions of Nucleus

- DNA, the repository of genetic information is located in the nucleus as a DNA-protein complex, **chromatin**, which is organized in to **chromosomes**.
- The nucleus contains the proteins and enzymes of replication of DNA and for repair of DNA that has been damaged.

- The major functional role of the nucleus is that of **replication**, synthesis of new DNA and **transcription**, synthesis of rRNA, tRNA and mRNA.
- The processing of RNA for assembly of ribosomes, required for protein synthesis in the cytosol, occurs in the nucleolus.

TABLE 1.2: Biochemical functions of subcellular organelles of the eukaryotic cell.

<i>Subcellular organelles</i>	<i>Functions</i>
Plasma membrane	Transport of molecules in and out of cell, receptors for hormones and neurotransmitters
Lysosome	Intracellular digestion of macromolecules and hydrolysis of nucleic acid, protein, glycosaminoglycans, glycolipids, and sphingolipids
Golgi apparatus	Post-transcriptional modification and sorting of proteins and export of proteins
Rough endoplasmic reticulum	Biosynthesis of protein and secretion
Nucleus	Storage of DNA, replication and repair of DNA, transcription, and post-transcriptional processing

Peroxisomes	Metabolism of hydrogen peroxide and oxidation of long chain fatty acids
Nucleolus	Synthesis of rRNA and formation of ribosomes
Mitochondrion	ATP synthesis, site for tricarboxylic acid cycle, fatty acid oxidation, oxidative phosphorylation, part of urea cycle, and part of heme synthesis
Smooth endoplasmic reticulum	Biosynthesis of steroid hormones and phospholipids, metabolism of foreign compounds (cytochrome P450 detoxification)
Cytosol	Site for glycolysis, pentose phosphate pathway, part of gluconeogenesis, urea cycle and heme synthesis, and purine and pyrimidine nucleotide synthesis

CYTOSKELETON

- The cytoplasm of most eukaryotic cells contains network of several types of proteins filaments that interact extensively with each other and with the component of the plasma membrane forming three dimensional meshwork.
- Such an extensive intracellular meshwork of protein has been called **cytoskeleton**.
- Cytoskeleton is not a rigid permanent framework of the cell but is a dynamic, changing structure.

Functions of Cytoskeleton

- The cytoskeleton gives cells their characteristic shape and form, provides attachment points for organelles, fixing their location in cells and also makes communication between parts of the cell possible.
- It is also responsible for the separation of chromosomes during cell division.

- The internal movement of the cell organelles as well as cell locomotion and muscle fiber contraction could not take place without the cytoskeleton.
- It acts as **track** on which cells can move organelles, chromosomes and other things.

Structure of Cytoskeleton

The cytoskeleton is an organized network of three protein filaments differing in width, composition and specific function:

- Microfilaments,
- microtubules and
- intermediate filaments,

- **Microfilaments** consist of long thin strands of protein **actin**, which is also a main component of muscle.
- Actin filament form a meshwork just underlying the plasma membrane of many cells and are referred to **stress fiber** or **cell cortex** which is labile.
- They disappear as cell motility increases or upon **malignant transformation** of cells by chemical or oncogenic viruses.

- **Microtubules** are long, thin tubes composed of the protein **tubulin**.
- They rapidly assemble into tubular structures and disassemble depending on the needs of cells.
- Microtubules comprise the spindle fibers that separate chromosomes prior to cell division.
- Centrioles are composed of microtubules and function as the organizing center for the formation of spindle fibers.

- **Intermediate filaments** are so-called as their diameter is intermediate between that of microfilaments and of microtubules.
- These are formed from fibrous protein which cannot be easily disassembled as either the microtubules or the microfilaments can, except **lamin**.
- Protein structure of intermediate filaments varies with different tissue type. There are major seven classes of intermediate filaments as indicated in

Cell Fractionation And Marker Enzymes For Different Organelles

Investigation of the biochemical properties of organelles requires subcellular fractionation in which the cell is first mechanically homogenized using isotonic 0.25 M sucrose solution to break cells.

- By gently homogenization in an isotonic sucrose solution the cell membrane is ruptured keeping most of the internal organelles intact.
- However, large fragile structures such as the endoplasmic reticulum, is broken into pieces that spontaneously form vesicles called **microsomes**.

- Then homogenate is centrifuged at different speeds.
- Large particles sediment more rapidly than small particles and soluble material does not sediment.
- In differential centrifugation, the homogenate is subjected to a series of centrifugation steps of increasing time and gravitational force

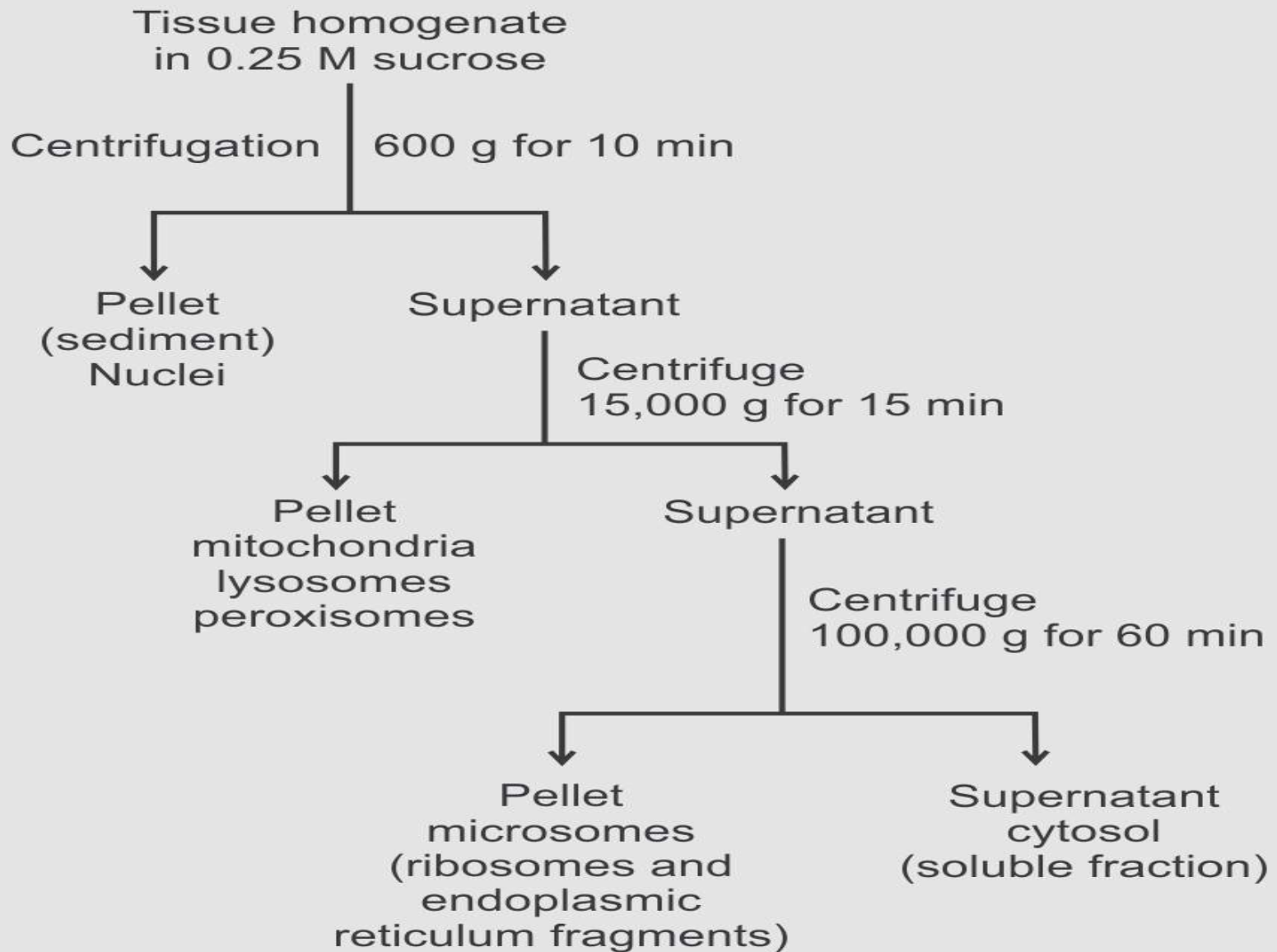


Figure 1.9: Subcellular fractionation of cell by differential centrifugation.

- The subcellular organelles, e.g. nuclei and mitochondria, which differ in size and specific gravity and thus sediment at different rates in a centrifugal field and can then, be isolated from homogenate by differential centrifugation.
- The dense nuclei are sediment first, followed by the mitochondria, and finally the microsomal fraction at the highest forces. After, all the particulate matter has been removed; the soluble remnant is the cytosol.

Isopyknic Centrifugation Technique

- In this technique, a density gradient is set up in a centrifuge tube; i.e. the density of the solution in the tube increases from the top to the bottom.
- Sucrose is often used as a medium.
- Colloidal materials such as **Percoll**, which form density gradients with a low osmotic pressure, are often preferred.

- Particles sediment to an equilibrium position at which their density equals that of the medium at that point in the tube.
- Different organelles are thus separated **according to their density, their size and shape being immaterial.**

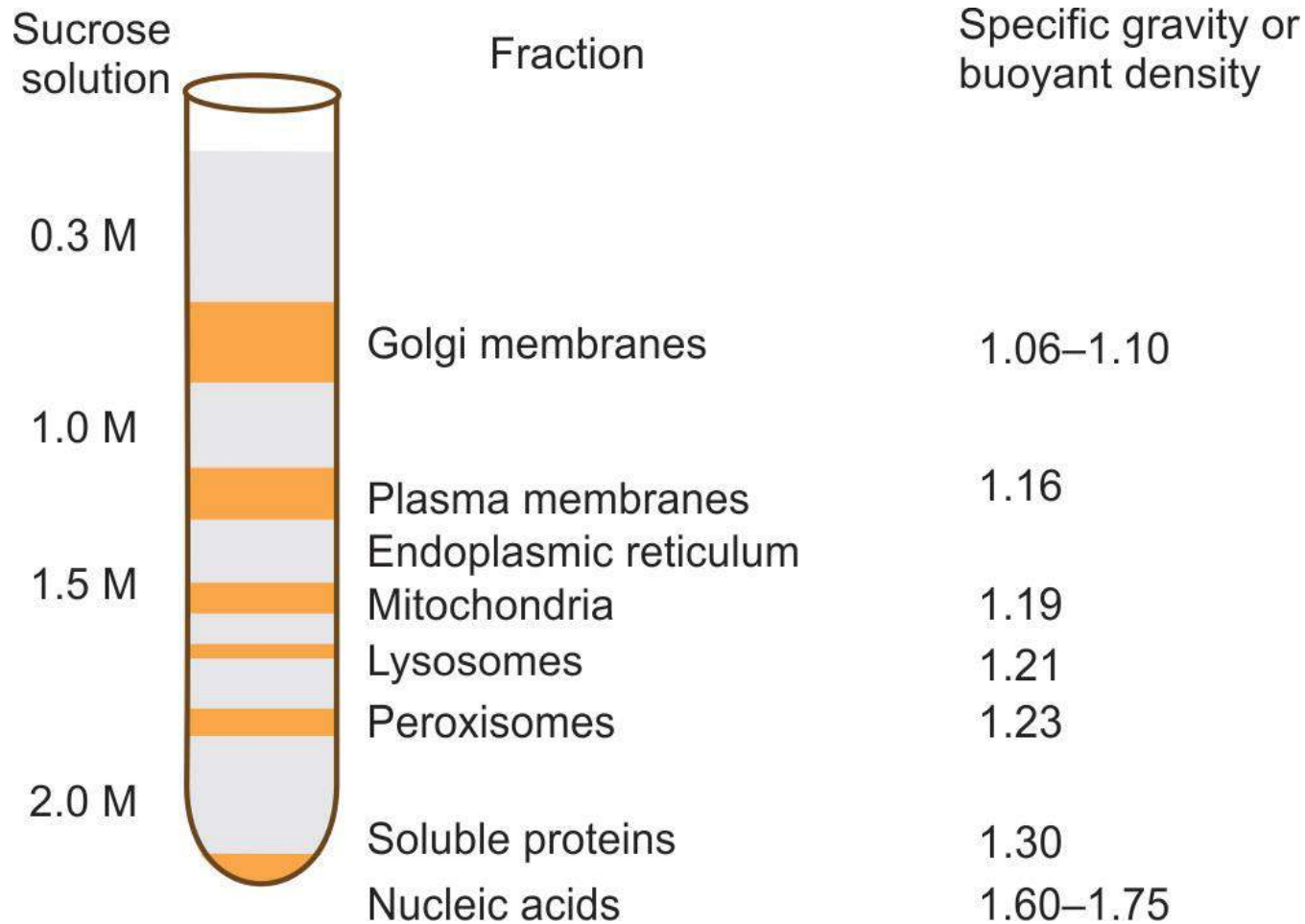


Figure 1.10: Separation of organelles by isopyknic centrifugation technique.

Marker enzymes for different organelles

- The purity of isolated subcellular fraction is assessed by the analysis of **marker enzymes**.
- Marker enzymes are the enzymes that are located exclusively in a particular fraction, and thus become characteristic of that fraction.

- Analysis of marker enzymes confirms the identity of the isolated fraction and indicates the degree of contamination with other organelles.
- For example, isolated mitochondria have a high specific activity of cytochrome oxidase but low catalase and acid phosphatase, the catalase and acid phosphatase activities being due to contamination with peroxisomes and lysosomes respectively.

TABLE 1.5: Marker enzymes of subcellular fractions.

<i>Fractions</i>	<i>Enzymes</i>
Plasma membrane	5'-nucleotidase, Na ⁺ -K ⁺ -ATPase
Nucleus	DNA polymerase RNA polymerase
Endoplasmic reticulum	Glucose-6-phosphatase
Golgi bodies	Galactosyltransferase
Lysosomes	Acid phosphatase β-glucuronidase
Mitochondria	Succinate dehydrogenase Cytochrome c oxidase
Peroxisomes	Catalase
Cytosol	Lactate dehydrogenase Glucose-6-phosphate dehydrogenase

THANK YOU