Section

I

Introduction to Histology

1. Microscope
2. Orientation to Histological Techniques
3. Cell and Tissue

Chapter

1

Microscope[1]

eSmartQuiz

Chapter Outline

- Introduction
- Compound light microscope
  - Optical parts
  - Nonoptical parts
- Objective lenses

INTRODUCTION

- Microscopy is an instrument used for magnification of objects.
- Zacharias Janssen and Hans Janssen (Dutch) developed the first microscope (1590). Giovanni Faber coined the term microscope (1625). *Antonie van Leeuwenhoek* (1675) invented a simple light microscope and he is also called '*father of microscope*'.
- Microscopes are classified into three groups as follows:
  1. Optical microscope that utilizes light (photons) for image formation.
  2. Electron microscope that utilizes electrons for image formation.
  3. Scanning probe microscope that utilizes physical probe for the formation of surface images.
- Optical microscopes are classified as follows:
  A. *Simple microscope:* It consists of a single convex lens or a single set of lenses for magnifying objects.
  B. *Compound light microscope:* It consists of two sets of lenses, one near the sample (objective lens) and the second lens near the eye (eyepiece). It is the most commonly used microscope.
- Compound light microscopes are of the following types:
  1. *Bright-field microscope:* In this microscope, the specimen is placed between the light source and the optical system. The specimen looks dark (colorful if stained) and background looks bright (white).
  2. *Dark-field microscope:* It has a special condenser that scatters the light rays. Only the rays that hit specimen form the bright image against a dark background. It is useful for observing unstained samples.
  3. *Phase-contrast microscope:* It is useful for visualization of live, unstained organism and living cells. This microscopy has phase annuls in condenser and phase plate in the objective. It is used to enhance the contrast of images of transparent and colorless specimens against a dark background.
  4. *Fluorescence microscope:* It uses the fluorescence property of the stained specimen. A *fluorophore* is a chemical that changes the wavelength (color) of the light. This microscopy is useful for immunoistochemistry (identification of specific antigen/protein), in situ hybridization, and so on.
  5. *Polarized light microscope:* It is useful for observing birefringent substances such as bone, teeth, striated muscles, and so on. Birefringent substance can produce double refraction of light.

COMPOUND LIGHT MICROSCOPE

- Compound light microscope is the most commonly used microscope.

- It consists of the following parts (Fig. 1.1):
  1. Optical parts: Sources of light, condenser, objective lenses, and eyepiece.
  2. Nonoptical parts: Arm, coarse and fine adjustment, nosepiece, stage, microscopic tube, and base.

Optical Parts

- The quality of image, resolution, and magnification mostly depend on the optical parts and the microscope.
- Optical parts include source of light, condenser, objective lenses, and eyepiece.

Source of Light

- Microscopes are provided with mirror for reflecting light or tungsten/halogen/LED light.
- Plane side of the mirror is useful in bright daylight, whereas concave mirror is useful for artificial light source. *Practical guide*

Substage Condenser

- Substage condenser gathers light and focuses it to the sample to be viewed.
- Aperture/iris diaphragm controls the amount of light passing through the condenser.
- Position of condenser can be adjusted (up and down movements) to focus the light critically.

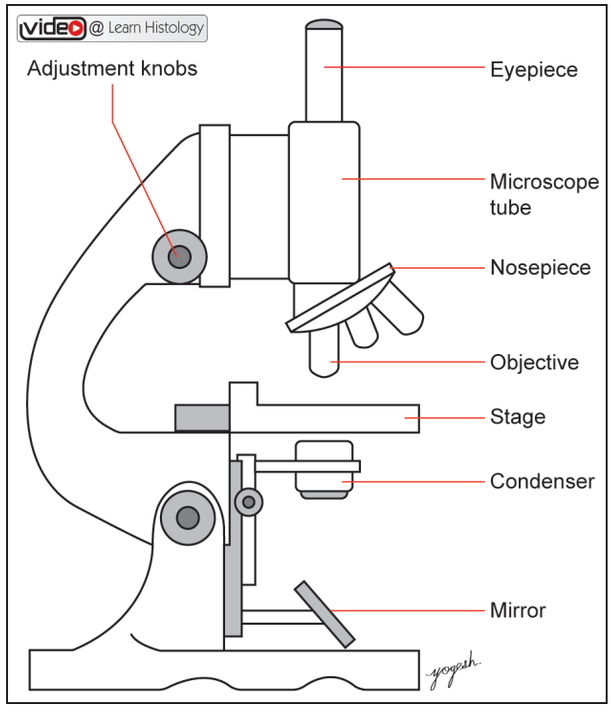


Fig. 1.1: Practice figure: Parts of microscope.

Objective Lens

- The lenses that receive light from the sample (lies near the sample) are called objective lenses.
- Objective lenses have different magnification powers, such as 4X (scanning objective), 10X (low power), 40X (high power), 100X (mostly called oil immersion). *Practical guide*
- Numerical aperture (NA) denotes light absorbing power and resolving power of objective.

Eyepiece (Ocular)

- The observer sees the image through the eyepiece.
- Eyepiece may be 10X (5X or 20X) and may be widefield.
- Monocular microscopes have only one eyepiece, whereas binocular microscopes have two eyepieces.
- Eyepiece produces a magnified virtual image.

Nonoptical Parts

- Nonoptical parts of microscope help in holding and moving the optical parts and specimen (glass slide).
- Nonoptical parts include arm, fine and coarse adjustment, nosepiece, stage, and base/stand.

Base/Stand

It is a heavy metallic part that keeps the entire microscope steady.

Arm

- Arm connects base of the microscope with optical tube.
- Arm is useful to hold the microscope during shifting of microscope from one place to another.

Adjustment Knobs

- Each microscope is filled with coarse and fine adjustment knobs.
- These knobs are useful for moving the sample upward and downward to bring it into focus.

Stage

- It is a platform for holding the sample.
- Stage is filled with
  - Clips to hold the glass slide
  - Mechanical stage to move the specimen in X and Y axis. Mechanical stage is provided with Vernier graduation markings.

Microscope Tube

- It is a tube that extends from objective lens to the eyepiece.
- Microscope tube is fitted with prism that changes the path of light.

Nosepiece

- Nosepiece links the objective lenses with the microscope tube and should be used for rotating the objective lenses.

Some Interesting Facts

Focal length

- It is a distance between the center of lens and point at which the light rays converge to form a clear image of the specimen (Fig. 1.2).
- It is an indicator of capability of the lens to magnify or diverge parallel rays.

Magnification

- A lens magnifies the image. For a microscope, magnification is the multiplication of eyepiece magnification and objective magnification.
- For example, while using a microscope of 40X objective and 10X eyepiece, a sample is magnified 400 times (40 × 10).

Numerical aperture

- The numerical aperture denotes light gathering capacity of a lens (Fig. 1.2).
- NA = n sin θ
- Here, n = refractive index of the medium between objective lens and specimen and theta = half-angle of the maximum cone of light.

Resolution

- Resolution is the ability of microscope to distinguish between closest two points as a separate entity.
- Resolution = 0.6λ/NA
  Here, λ = wavelength of light
  NA = Numerical aperture
- Higher NA of objective and condenser and shorter wavelength of light give better resolution.

Aberrations

- A defect in expected functioning of the lens or optical system is called an optical aberration.
- There are two types of axial aberrations: chromatic and spherical.
- *Chromatic aberration*: If a lens reflects the light according to wavelength (color), it is called chromatic aberration. Blue light gets refracted more than green and red. This phenomenon produces color halos around the magnified image (Fig. 1.3).
- *Spherical aberration*
  In spherical aberration, parallel rays passing from center of lens and that passing from peripheral part of lens are focused at different points (Fig. 1.4).
- Due to spherical aberration, blurred image is formed in the peripheral part.
- Other spherical aberrations include coma, astigmatism, field curvature, barrel distortion, pincushion distortion (For details, *see* Principles of Histological Techniques by Yogesh Sontakke).

Kohlör Illumination

- August Kohlör (1893) described method for illumination in microscopy.
- It describes the steps (settings) by that source of light will not be visible in an image formed in microscopy and provide even illumination, high contrast, less specimen heating, and no glare.[2]

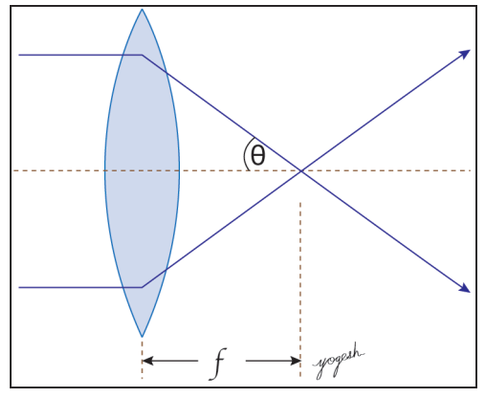


Fig. 1.2: Focal length (f) and numerical aperture (θ).

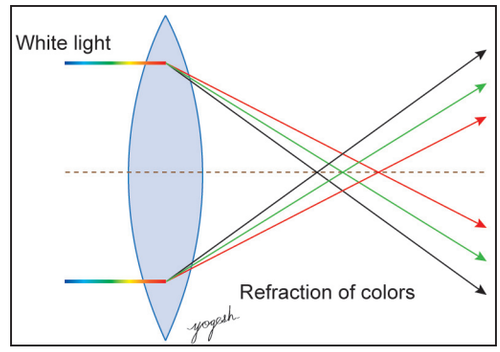


Fig. 1.3: Chromatic aberration.

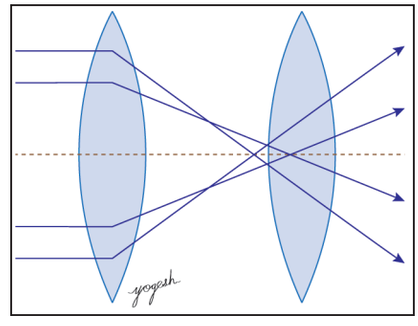


Fig. 1.4: Spherical aberration.

OBJECTIVE LENSES

- Objective lenses are of various types. According to their correction for specific aberrations, they are as follows:
  1. *Achromatic objectives:* These are corrected for axial chromatic aberration for two colors: red and blue.
  2. Fluorites or *semi-apochromatic objectives:* These are constructed with fluorite and glass. They are corrected for 2–3 colors and field curvature aberrations. These are suitable for immunofluorescence microscopy, polarization, and differential interference contrast microscopy.
  3. *Apochromatic objectives:* These are corrected for all color aberrations and spherical aberration. These are suitable for confocal microscopy.
  4. *Plan objectives:* These are very costly lenses. These are corrected for curvature of field. For example, *plan-apochromatic objective* (corrected for maximum aberrations).[MCQ]

Common markings on the objective lens are shown in Fig. 1.5.

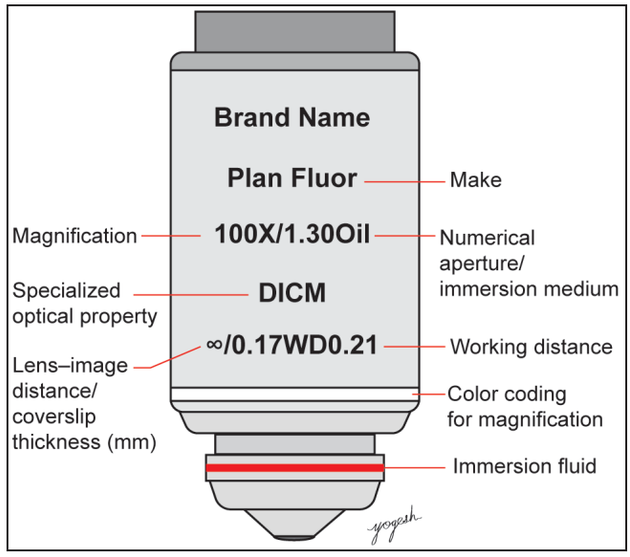


Fig. 1.5: Markings on objective lens.

Some Interesting Facts

Fluorescence microscopy

- Fluorescence microscopy utilizes the property of fluorescence. The sample is excited with high-energy light (shorter wavelength). The sample loses some energy and emits low energy light (higher wavelength).
- This microscope is fitted with mercury vapor lamp or high-power LEDs or LASER light and fluorescence filter cube.
- Fluorescence filter cube consists of excitation filter, dichroic filter and emission filter. The filter cube allows the specific wavelength of light (color) to pass through it (Fig. 1.6).

Confocal microscopy

- Confocal microscopy is modified fluorescence microscopy that forms an image of the sample from one focal plane. For this purpose, it is fitted with *spatial pinhole* that helps to collect images from different focal planes and forms three-dimensional images (Z stacking).
- It is useful for thick specimens that are stained with fluorescence dyes, for construction of *3-D images* of sample, live cell scanning, and colocalization of proteins.

Electron microscope

- Electron microscope utilizes the beam of electrons (instead of light) to illuminate the sample.
- It can detect minute samples such as viruses, and atoms, but are not suitable for living tissue.
- There are various electron microscopes such as transmission electron microscope (TEM), scanning electron microscope (SEM), reflection electron microscope (REM), and so on.
- Electron microscopes utilize electromagnetic lenses, specimen (<100 nm thick) mounted on grid (Fig. 1.7).
- To cut such thin section, *ultra-microtome with glass knife* is required. The specimen is stained with uranyl acetate and lead citrate.

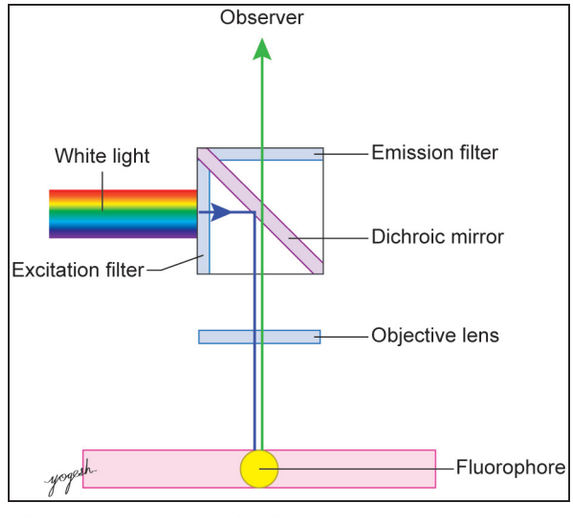


Fig. 1.6: Light path for fluorescence microscopy.

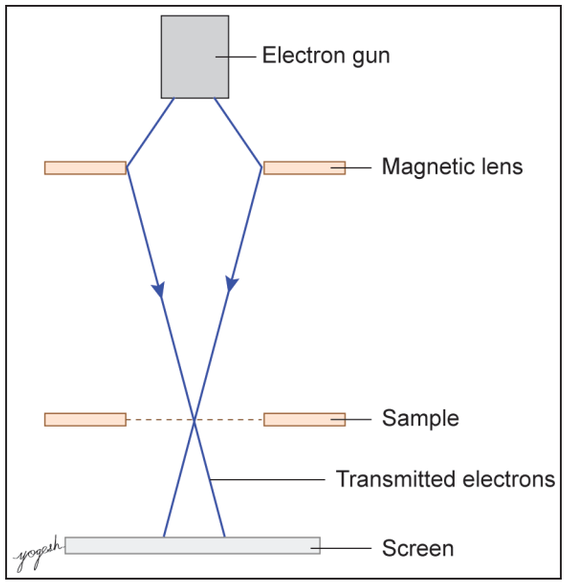


Fig. 1.7: Working principle of a transmission electron microscope.

Atomic force microscopy

- It is powerful, nonoptical microscope. It has cantilever (pointed probe) that touches the surface of the specimen need to be scanned. The movement of the probe is recorded to create magnified surface view. It is useful even for surface view of living cells in cell culture.

Virtual microscopy

- It uses digital process of studying the digital images which are captured using light microscope. These are useful for teaching and diagnostic purposes.

[1.] This chapter is the gist of microscopy section of the book *Principles of Histological Techniques, Immunohistochemistry and Microscopy* by Yogesh Sontakke, Paras Medical Books, Hyderabad, India and reproduced with permission.

[2.] For details, refer the book *Principles of Histological Techniques, Immunohistochemistry and Microscopy* by Yogesh Sontakke, 1st edn., Paras Medical Books Pvt. Ltd., Hyderabad, India.

Chapter

2

eSmartQuiz

Orientation to Histological Techniques

Chapter Outline

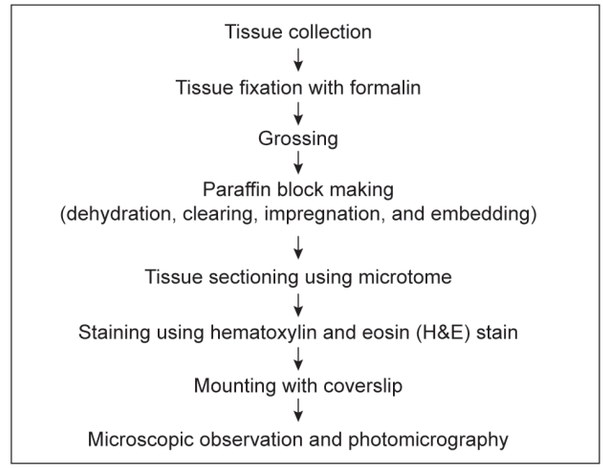
- Tissue processing
- Special stains
  - Hematoxylin and eosin staining
  - Decalcification
- Orientation of sectional plane
  - Interpretation of Sections in Histology
    - *Sections of solid structure*
    - *Sections of tubular structure*

- Microscopic examination is based on thin sectioning of the tissue. AFJK Mayer (1819) coined the term *histology* (*histos* = tissue, *-logy* = science).
- This chapter provides brief overview of the histological techniques that are required for the section/slide preparation of tissue and orientation of sections (relationship of gross structure and microscopic section).

TISSUE PROCESSING

- In routine histology and histopathology, a specimen is sliced into 0.5 cm or so. These slices are processed and finally further sectioned into 5–7 µm thin sections. These sections are mounted and stained on glass slides to make them suitable for microscopic observation. This entire procedure is called *tissue processing.*
- Histological tissue processing involves the following major steps (Flowchart 2.1): Tissue collection, grossing, tissue fixation, dehydration, cleaning, embedding, section cutting, staining, and mounting.

Flowchart 2.1: Steps in tissue processing



1. Tissue collection

- For histological studies, suitable tissue is collected from cadavers, forensic autopsies, surgical procedures, animals (goat, rat, dog, and so on) with permission from authorities (ethics and research committees).

2. Tissue fixation

- Collected specimen is preserved for retaining their biological structure without any significant distortion or decomposition.
- 10% formalin is the most commonly used fixative.[Viva]
- Formalin cross-links proteins (enzymes and others) in the tissue and stop their activity. Other fixatives include glutaraldehyde, mercuric chloride, osmium tetroxide (highly toxic), and so on.

3. Grossing

- Grossing involves observing tissue for gross pathological changes and selecting a suitable part of the tissue for microscopic examination.
- The selected area is cut into ~5 mm thick slices.

4. Paraffin block making

- It is difficult to make thin sections of the tissue (5–7 µm) as most of the cells contain water (50–60%).

Hence, the tissue is processed to replace water with firm material (paraffin wax) and to make the tissue suitable for cutting.

- It involves the following steps:
  - ***Dehydration:*** Tissue slice is treated with ascending grades of alcohols (50%, 70%, 90%, and absolute/100% alcohol) to gradually remove water from the tissue.
  - ***Removal of alcohol/clearing:*** Tissue slice is treated with xylene (clearing agent). Clearing agent makes the tissue clear by changing the refractive index of the tissue.
  - ***Infiltration/removal of clearing agent:*** Tissue slice is kept in melted paraffin that replaces xylene by infiltration in the tissue.
  - ***Embedding/block making:*** Tissue slice is kept fixed in the melted paraffin and allowed to form a solid block on cooling. This solid block makes the tissue suitable for sectioning.

5. Tissue sectioning

- Microtome is an instrument used for thin sectioning of tissue.
- Paraffin block is fitted on microtome and cut into thin sections (5–7 µm). These sections are transferred to glass slides.

6. Staining

- A section on a glass slide is stained with suitable staining.
- Hematoxylin and eosin (*H&E*) are commonly used stains.

7. Mounting

- Stained section is mounted with the help of a coverslip and DPX (glue) to make ready for observation under microscope and preserve. DPX is a mixture of distrene (polystyrene/plastic), plasticizer (tricresyl phosphate) and xylene (solvent); hence, the name DPX.

SPECIAL STAINS

- Many structures or substances cannot be differentiated from each other with the help of routine hematoxylin and eosin staining.
- For the visualization and differentiation of specific structure, special stains are required.
- Some commonly used special stains and their uses are listed in Table 2.1.

ORIENTATION OF SECTIONAL PLANE

- In histology, a two-dimensional view of a tissue section is observed under the microscope.
- The observer has to imagine a three-dimensional structure from a two-dimensional image.

Box 2.1: Hematoxylin and eosin staining

- Hematoxylin and eosin stain is most commonly used in routine histology and histopathology.
- *Principle of H&E staining*: The principle of *H&E* stain is the chemical attraction between tissue and dye. Hematoxylin is a basic dye that imparts blue-purple color to the basophilic structures. Eosin is a acidic dye that counterstains the structures with in varying intensities of pink, orange, and red.
- Hematoxylin is a basic dye that is obtained from wood of *Hematoxylon campechianum* tree.
- Hematoxylin stains nuclei, calcium deposits, fibrin, cross striations of muscles, matrix in cartilages, and so on.*Practical guide*
- Eosin is an acidic type that is derived from fluorescein.
- Eosin stains basic or eosinophilic compounds such as cytoplasm, connective tissue (collagen fibers), muscle fibers, red blood cells, and so on.*Practical guide*
- Steps involved in *H&E* staining:
  Deparaffinize the section using xylene, rehydrate the section with descending grades of alcohol (100% or absolute alcohol, 90% alcohol, 70% alcohol, and water), staining with hematoxylin, removal of excess hematoxylin (using acid–alcohol and water wash/bath), staining with eosin and dehydration. Dehydrated slide is preserved by fixing coverslip using DPX (transparent glue).

Box 2.2: Decalcification

- Calcified tissue (bones) cannot be sectioned properly using routine microtome.
- Decalcification is the technique useful for removing deposited minerals from the matrix of the tissue (bone) to facilitate microtome sectioning.
- The following agents are useful for decalcification: strong mineral acids (nitric acid, hydrochloric acid), weak organic acids (formic acids), and calcium chelating agents (EDTA).

For example: If a circular structure is seen, the observer has to decide whether it is a sectional part of a tube or a sphere.

- *Microtome* is an instrument used for section cutting in histology. The knife/blade fitted to the microtome makes thin slices of the tissue.
- Tissue has various structures that have different size, shape, and orientations.
- Some structures are spherical, some are tubular, or solid. Some structures run parallel to the long axis of tissue, some run perpendicularly or obliquely. The resultant section of the tissue gives a two-dimensional image of these structures.

Table 2.1: Special stains and their uses

Structure	Stain	Color
Collagen fibers	Van Gieson technique	Red
	Masson's trichrome stain	Blue/green
Elastic fibers	Verhoeff–van Gieson method	Bluish black
	Weigert's Resorcin–Fuchsin method	Blue-black
	Orcein stain	Dark-brown
Reticular fibers	Gordon–Sweet's method	Black
	Gomori's silver impregnation method	Black
Basement membrane	Periodic acid–Schiff (PAS) method	Magenta
Carbohydrates (glycogen, amyloid, fungi, pancreatic zymogen granules, corpora amylacea in prostate, thyroid colloid)	PAS method	Magenta
Lipids/fat	Oil red-O	Brilliant red
Iron	Perl's Prussian blue reaction	Blue
Hemoglobin	Leuco patent blue V method	Dark blue–green
Bile pigment	Hall's method	Olive green
Melanin	Masson–Fontana method	Black
Lipofuscin	Pearse's staining method	Magenta
Calcium	Von Kossa method	Dark green–black
Uric acid	Hexamine–silver method	Black
Nissl substance in neuron	Cresyl Fast violet stain	Purple–dark blue
Myelin	Luxol fast blue method	Blue
	Osmium tetroxide method	Black
Astrocytes	Cajal's gold chloride method	Reddish-black

Interpretation of Sections in Histology

- To understand the histological section, the following examples are given:
  - Sections of solid structure
  - Sections of tubular structure

Sections of Solid Structure

- A boiled egg has an outer egg white and inner yellow/orange egg yolk.
- The nature of the section depends on orientation of the egg-like solid structure in the tissue (Fig. 2.1).

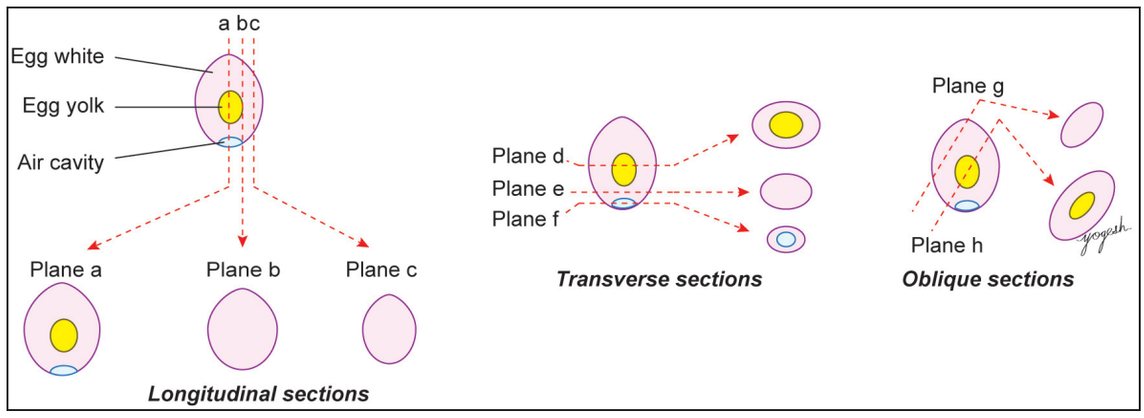


Fig. 2.1: Planes of sections of solid object.

- For example: If an egg is oriented vertically, it will cut in longitudinal plane (*longitudinal section/LS*). If an egg is oriented horizontally, it will cut in transverse plane (*transverse section/TS*). If an egg is oriented obliquely, it will cut in oblique plane.
- In each plane, the egg is sectioned into various oval shapes depending on the depth of the section (from superficial to deep planes). In superficial or tangential planes, egg yolk (similar to cell nucleus) may not be observed. *Practical guide*

Sections of Tubular Structure

- Many tissue structures are tubular, such as, blood vessels, intestine, renal tubules, seminiferous tubules, bronchi, and so on.
- The two-dimensional section of tubular structure depends on
  - Coiling of tubular structure
  - Orientation of the structure in the tissue
  - Plane of section

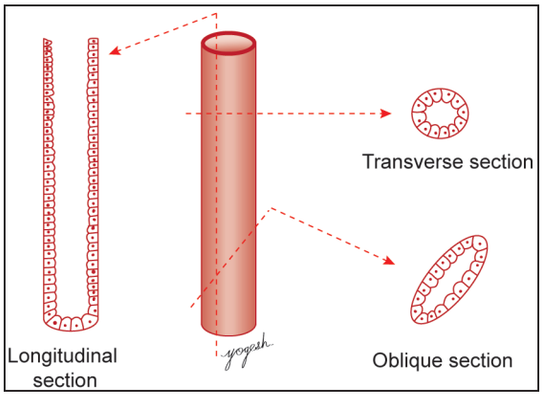


Fig. 2.2: Sections of straight tubular structure.

- If a tubular structure is straight, it produces only one appearance in a section. Based on the plane, it may be longitudinal, transverse or oblique section (Fig. 2.2).
- If a tubular structure is coiled, it may appear multiple time in the same section and its part may show various sections (longitudinal, transverse, oblique, and tangential) (Fig. 2.3).

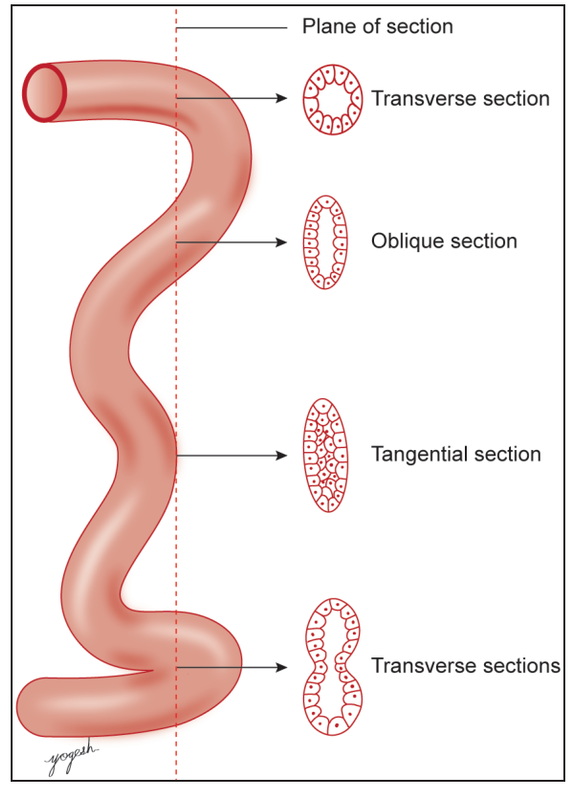


Fig. 2.3: Sections of coiled tubular structure.

Some Interesting Facts

Following points can be concluded with the understanding of plane of section:

- Sweat gland is a simple tubular gland having terminal coiled secretory portion. In a section, it may be seen as multiple oval (oblique) sections and round (transverse) sections. *Practical guide*
- In thyroid gland, some of the follicles may not show colloid, if the section does not pass through the colloid. In addition, it may show cluster of cells in tangential section. *Practical guide*
- In case of epithelium, some cells do not show nuclei, if the section does not pass through the nucleus of the cell.
- In smooth muscle, transverse section of the cell shows round central nucleus, whereas in longitudinal section, spindle of the cell showshaped nucleus. *Practical guide*
- In a longitudinal section of a ground bone, parallel Haversian canals are present, whereas in transverse section round/oval-shaped Haversian canals are present.

Chapter

3

eSmartQuiz

Cell and Tissue

Chapter Outline

- Plasma membrane
  - Modified fluid-mosaic model
  - Functions
- Transport across plasma membrane
- Endoplasmic reticulum (ER)
- Golgi complex
- Mitochondria
- Ribosomes
- Lysosomes
- Lysosomal storage diseases
- Peroxisomes
- Endosomes
- Cytoskeleton
  - Microtubules
  - Actin filaments/Microfilaments
  - Intermediate filaments
- Centrioles
- Nucleus
  - Nuclear envelope
  - Nucleoplasm
  - Chromatin
  - Nucleolus
- Tissue
  - Definition
  - Classification

CELL

Introduction

- Cell is basic structural and functional unit of all organisms.
- Human tissue consists of *eukaryotic cells* that have nucleus, cell organelles, and cytoplasm (Note: prokaryotic cells/unicellular organisms do not have membranous nucleus and membranous cell organelles).
- Each cell is bounded by a cell/plasma membrane that encloses *protoplasm.*
- Protoplasm consists of a nucleus and cytoplasm.
- Cytoplasm consists of gel-like matrix called *cytosol/hyaloplasm,* cell organelles, cytoskeleton, and inclusions. Cytoskeleton consists of microtubules, intermediate, and actin filaments.
- Cell organelles are grouped as membranous (membrane-limited) and nonmembranous-bound (Fig. 3.1).
- *Membranous-bound organelles* include plasma/cell membrane, rough endoplasmic reticulum (rER), smooth-surfaced endoplasmic reticulum (sER), Golgi apparatus, endosomes, lysosomes, pinocytic vesicles, endocytic vesicles, mitochondria, and peroxisomes.
- Membranes of intracellular organelles increase intracellular surface area and create intracellular microcompartments for proper physiological functions.
- *Nonmembranous organelles* include microtubules, filaments (actin and intermediate filaments), centrioles, ribosomes, and proteasomes.
- Various cell organelles and their functions are listed in Table 3.1.

PLASMA MEMBRANE (CELL MEMBRANE)

- Plasma membrane is also called cell membrane or *plasmalemma.*
- It separates intracellular compartment from extracellular compartment of the tissue.
- Plasma membrane is a dynamic structure. It consists of an amphipathic lipid bilayer, integral membrane proteins, and peripheral proteins (Fig. 3.2).

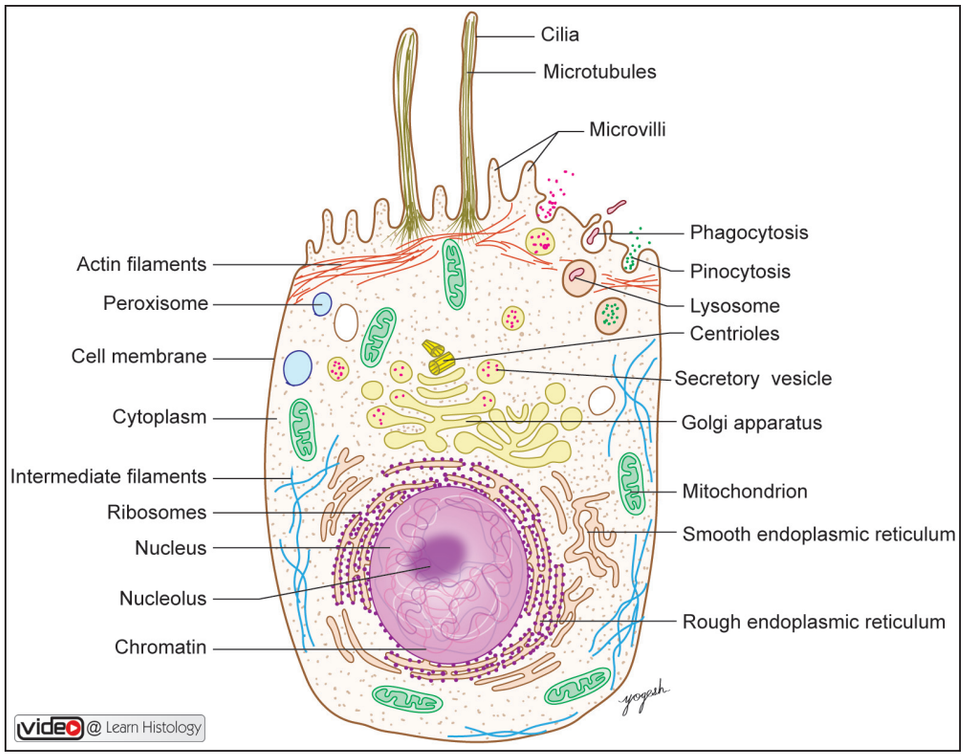


Fig. 3.1: Cell and cell organelles (practice figure).

Table 3.1: Cell organelles and functions *viva*

Cell organelles	Functions	Remarks/characteristics
Plasma membrane	Selective barrier, cell adhesion	Bilipid layer
rER	Synthesis and transfer of proteins to Golgi complex	Flattened sheets with attached ribosomes
sER	Lipid and steroid metabolism	Flattened sheets without ribosomes
Golgi apparatus	Posttranslational modification of proteins	Flattened sheets
Secretory vesicles	Transport and storage of secretory proteins	Small membrane-bound vesicles
Mitochondria	Power house of cell	Outer and inner membranes
		Inner membrane shows cristae (folds)
Lysosomes	Disintegration of phagocytosed material	Membrane-bound vesicles
Peroxisomes	Oxidation of fatty acids, detoxification	Membrane-bound vesicles
Ribosomes	Protein synthesis	Have 40S and 60S subunits

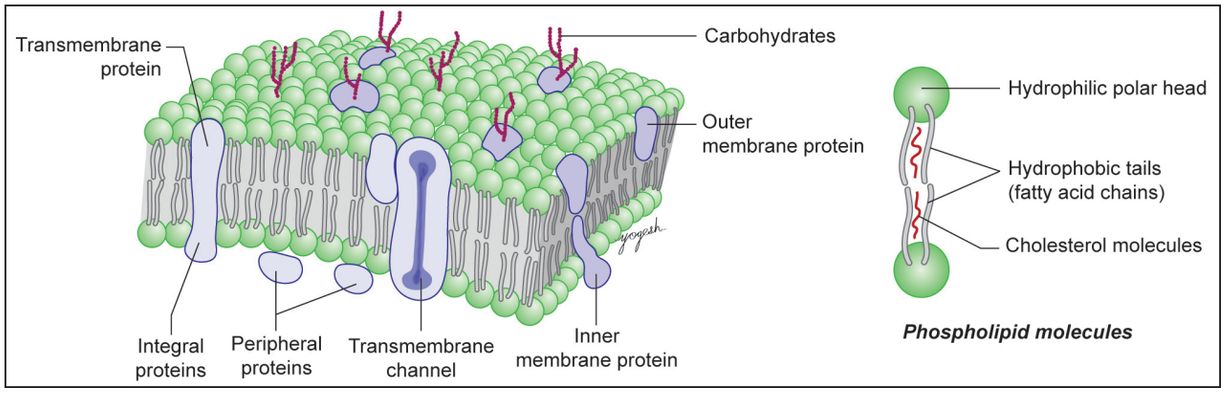


Fig. 3.2: Structure of cell membrane.

Modified Fluid-Mosaic Model
(Singer and Nicolson, 1972):

- Transmission electron microscopy (TEM): On TEM examination, plasma membrane consists of two electron-dense layers separated by middle electron lucent layer (Fig. 3.2).
- Thickness is ~8–10 nm.

Lipids of Plasma Membrane

- Plasma membrane has three types of lipids: phospholipids, cholesterol, and glycolipids.
- *Phospholipid* molecules have polar hydrophilic end/head and nonpolar hydrophobic end/tail. The head consists of choline, phosphate, and glycerol. Nonpolar end consists of two fatty acid chains.
- Hydrophilic ends face toward extracellular and intracellular surfaces.
- *Lipid rafts* are microdomains of the plasma membrane. It consists of high concentration of cholesterol and glycolipids.*Viva*
- Lipid rafts also contain integral and peripheral membrane proteins that are involved in cell signaling.
- *Freeze fracture:* It is a method of tissue processing for electron microscopy. On freeze-fracture, cell membrane shows *E-face* (backed by extracellular compartment) and *P-face* (backed by protoplasm/cytoplasm). This method is useful for identification of integral proteins of cell membrane.

Proteins of Plasma Membrane

- Plasma membrane has two types of proteins: integral membrane proteins and peripheral membrane proteins.
- *Integral proteins* are confined within the plasma membrane and cross the entire or partial thickness of the cell membrane, whereas *peripheral proteins* are confined only on the surfaces of plasma membrane.
- Integral proteins form pumps (Na^+ pump), channels (gap junctions), receptor proteins, linker proteins (anchor cytoskeleton), enzymes (ATPase), and structural proteins.
- Integral proteins can move within the lipid bilayer.

Carbohydrates of Plasma Membrane

- Carbohydrates of plasma membrane form glycoproteins and glycolipids.
- They form *glycocalyx coat* on the outer surface of the plasma membrane.
- They help cell to interact with extracellular environment, cell recognition, cell adhesion, and metabolism.*Functional Correlation*
- Glycocalyx also forms major histocompatibility complexes (MHC) and blood group antigens on RBCs.*Neet*

Functional Correlation of Plasma Membrane

- *Selective barrier*: Plasma membrane limits the mobility of the substances across it.
- *Protection*: Plasma membrane isolates the intracellular environment from extracellular environment.
- *Cell shape/adhesion*: Plasma membrane anchors the cytoskeleton and provides attachment with adjacent cells and basement membrane to provide a particular shape to the cell.
- *Polarity*: Plasma membrane maintains ionic polarization and respond to stimuli by depolarizing.
- *Receptors*: Plasma membrane has receptors for specific molecules (hormones).
- *Transport*: Plasma membrane help in transport across it by endocytosis, exocytosis, pinocytosis, and so on.

TRANSPORT ACROSS PLASMA MEMBRANE

Selective substances can enter or leave the cell through the plasma membrane. These substances follow one of the following modalities of transport (Fig. 3.3).

1. Passive transport
2. Active transport
3. Vesicular transport.

Passive Transport

- For passive transport, energy is not required for transport of substances across plasma membrane. It can take place by simple diffusion or facilitated diffusion (Fig. 3.3).
- *Simple diffusion*
  By simple diffusion, lipid-soluble and uncharged molecules cross plasma membrane from higher to lower concentration.
  For example, oxygen, carbon dioxide, glycerol, and so on.
- *Facilitated diffusion*
  In facilitated diffusion, *channel/carrier proteins* or channels of the plasma membrane help in transport of certain small and water-soluble molecules.
  Carrier proteins move across the plasma membrane and help in the transport of small, water-soluble molecules.
  Channel proteins are transmembrane (integral) proteins. They have pore domain that regulates entry or exit of substances.

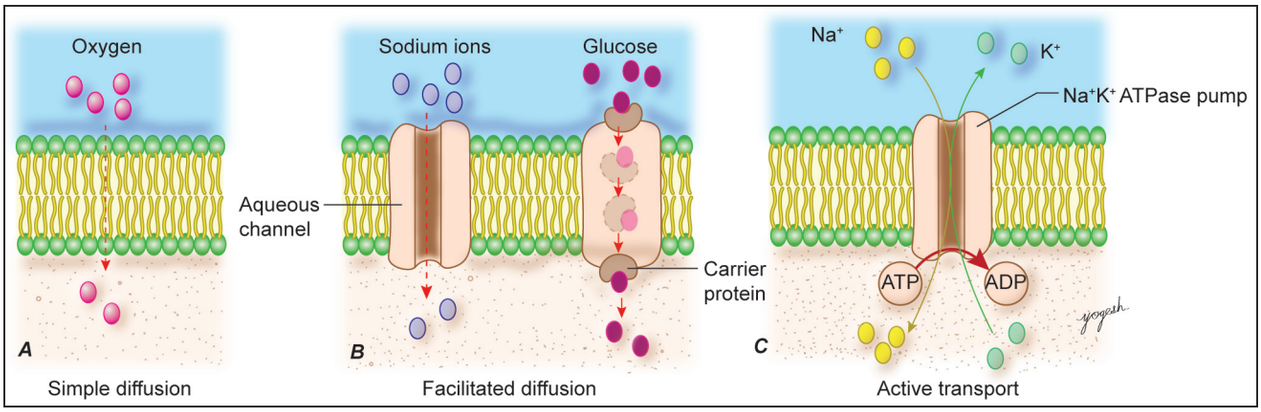


Fig. 3.3: Transport across the cell membrane. (A) Simple diffusion, (B) Facilitated diffusion, (C) Active transport.

Channel proteins are:

- *Voltage-gated ion channels* regulated by membrane potentials
- *Ligand-gated ion channels* regulated by neurotransmitters
- *Mechanical-gated ion channels* regulated by stress (hair cells in internal ear).

Active Transport

- The transport of molecules across the plasma membrane against the concentration gradient requires energy (ATPs). Such transport of molecules is called active transport.
  For example, sodium pump for Na^+ ion transport.

Vesicular Transport

- Large molecules are transported with vesicular transport across the plasma membrane.
- It is of two types: Endocytosis and exocytosis.
- *Endocytosis*
  In endocytosis, the extracellular molecules are brought inside the cell as membranous vesicles.
  Endocytosis may be pinocytosis, phagocytosis, or receptor-mediated endocytosis (Fig. 3.4).
  Pinocytosis (*cell drinking*, in Greek): In pinocytosis, cell ingests liquid and forms pinocytic vesicles.
  Phagocytosis (*cell eating*, in Greek): In phagocytosis, cell ingests large substances such as cell debris, bacteria, and so on and forms large phagocytic vesicles (phagosomes).
  Receptor-mediated endocytosis: Specific molecules (ligand) enter cell with the help of receptor-mediated endocytosis. For example, peptide hormones.
- *Exocytosis*
  In exocytosis, molecules covered in a vesicle is expelled out of the cell through the plasma membrane (Fig. 3.5).

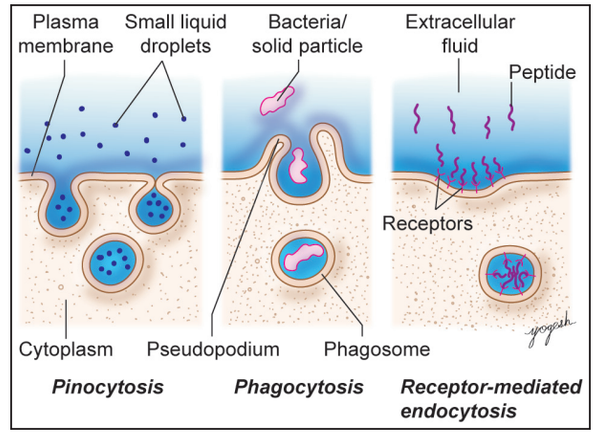


Fig. 3.4: Modalities of endocytosis.

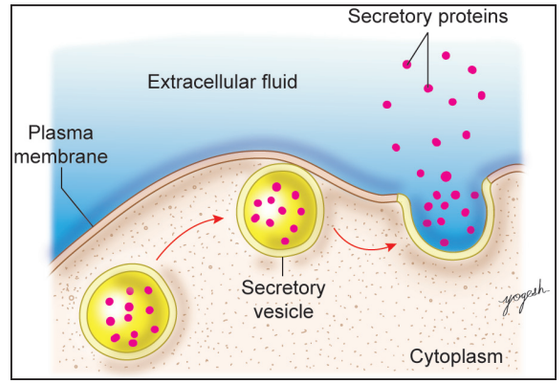


Fig. 3.5: Modalities of exocytosis.

There are two types of exocytosis:

1. Constitutive pathway: In this process, substance is exocytosed immediately after its synthesis. For example, secretion of immunoglobulins.
2. Regulated pathway: In this process, substance is stored temporarily in vesicle after its synthesis in cytoplasm and exocytosed on extracellular signaling. For example, zymogen granules secretion.

CELL ORGANELLES

- Cytoplasm contains numerous structures that perform various functions. These are called cell organelles.
- Cell organelles are grouped as follows:
  1. Membranous cell organelles: Endoplasmic reticulum, Golgi complex, mitochondria, phagosomes, lysosomes, peroxisomes, exocytic vesicles.
  2. Nonmembranous cell organelles: Cytoskeleton elements (microfilaments, microtubules, intermediate filaments).

Endoplasmic Reticulum (ER)

- Endoplasmic reticulum (ER) is a network of interconnecting membranes that form cisternae (Fig. 3.6).
- The cytoplasm enclosed within the cisternae of endoplasmic reticulum is *vaculoplasm*, whereas the rest of the cytoplasm is hyaloplasm/cytosol.
- There are two varieties of ER: Rough-surfaced ER (having coating of ribosomes) and smooth-surfaced ER (without ribosomes).

Rough-surfaced Endoplasmic Reticulum (rER)

- Ribosomes are present on the surface of rough endoplasmic reticulum (Fig. 3.6).
- As ribosomes contain RNA, they give basic (hematoxylin) staining to the cell.[Practical guide] *Ergastoplasm* is the portion of cytoplasm that stains with basic dyes.
- Ribosomes are attached on outer surface of rER by *ribosome docking proteins.*

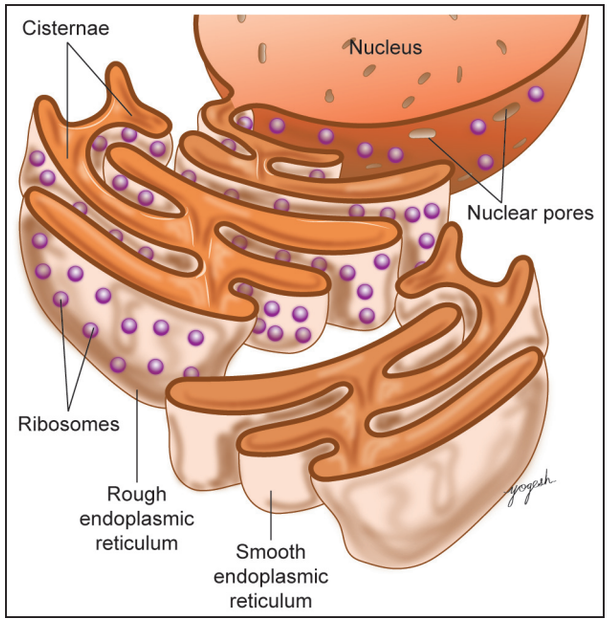


Fig. 3.6: Endoplasmic reticulum.

- Mostly, rER is continuous with outer nuclear membrane.
- The mRNA binds with many ribosomes to form polyribosome complex or *polysome*.
- Newly synthesized proteins have signal sequences/peptides that direct the protein to get transferred to its destination site within the cell.
- Newly synthesized protein enters the lumen/cisternae of rER and undergoes posttransitional modifications such as glycosylation, folding, and so on. Later, modified proteins are delivered to the Golgi apparatus.
- Clinical fact: In *emphysema*, there is an inability of rER to deliver the synthesized enzyme α-1 antitrypsin to Golgi apparatus that results in *α-1 antitrypsin deficiency.*[Neet]
- The rER is predominantly present in active protein secretory cells such as serous cells in the pancreas and salivary glands.
- Note: Free ribosomes synthesize cytoplasmic, structural and functional elements. For example, hemoglobin synthesis in precursor cells of RBCs, contractive protein synthesis in developing muscle.
- The rER and free ribosomes produce Nissl bodies in neurons and cytoplasmic basophilia in other secretory cells.[Practical guide]

Functional Correlation of rER

- *Protein synthesis*: Site for translation (mRNA → proteins)
- *Checkpoint*: rER destroys defective proteins.

Smooth-surfaced Endoplasmic Reticulum (sER)

- Smooth-surfaced endoplasmic reticulum consists of short anastomosing tubules (Fig. 3.6).
- The sER lacks ribosome docking proteins, hence they do not have ribosomes.[Viva]
- As sER is not associated with ribosomes, it gives eosinophilic (pink) color to cytoplasm.[Practical guide]

Functional Correlation of sER

- *Lipid metabolism*: sER is the main site for lipid synthesis. They are abundant in cells of liver, cells of adrenal cortex, and Leydig cells of testis.
- *Sarcoplasmic reticulum*: In smooth and cardiac muscles, sER forms sarcoplasmic reticulum that acts as Ca^{++} ion reservoir.
- *Detoxification*: sER is involved in detoxification of drugs and other chemicals.
- Glycogen metabolism.
- Membrane formation and recycling.

Golgi Complex (Camillo Golgi, 1843–1926)

- It is made up of 3–20 flattened curved membranous cisternae (sacs) that forms a shallow cup-like structure.
- It has convex/forming face (*cis-face*) and concave/maturing face (*trans-face*) (Fig. 3.7).
- Its *cis-face* faces toward rER and nucleus, whereas *trans-face* faces toward cell membrane.
- Middle part of Golgi apparatus is called media-*Golgi network.*

Note: *Glogi complex do not stain with H&E stain.* Practical guide

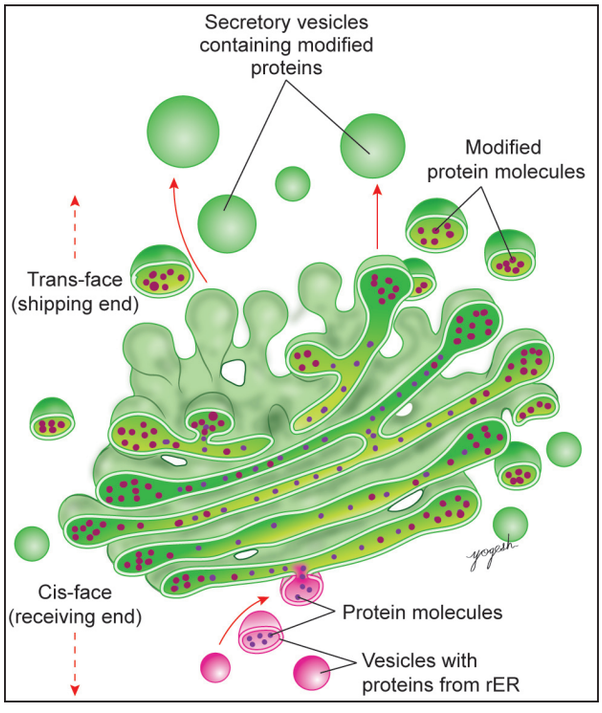


Fig. 3.7: Golgi apparatus.

Functional Correlation of Golgi Complex

- *Posttranslational modification of proteins*: Freshly synthesized proteins are transferred from rER to the Golgi apparatus. These proteins are modified by the Golgi apparatus.
- *Formation of secretory vesicles*: Modified proteins are wrapped around by the membrane of Golgi apparatus and get separated to form membrane-bound secretory vesicles or endosomes or lysosomes.

- Location: Usually, Golgi apparatus is located toward secretory portion (apical portion) of the cell membrane.

Mitochondria

- Mitochondria are *power houses* that generates energy (ATPs).
- Mitochondria are absent in RBCs and terminal keratinocytes of skin. MCQ
- Size: 0.5–2 µm, elliptical-shaped.
- It is bounded by bilaminar membrane (similar to plasma membrane) with intermembranous space and matrix (Fig. 3.8).
- *Outer mitochondrial membrane* is smooth and has voltage-dependent anion channels called *mitochondrial porins*.
- Inner mitochondrial membrane shows folding called *cristae* (for increasing surface area).
- Inner mitochondrial membrane is impermeable to ions due to its cardiolipin phospholipids. It is a site for oxidation reactions, respiratory electron transport chain, and ATP synthesis. It has tennis racket-shaped elementary (F1) particles. Heads of these particles carry out oxidative phosporylation to generate ATP.

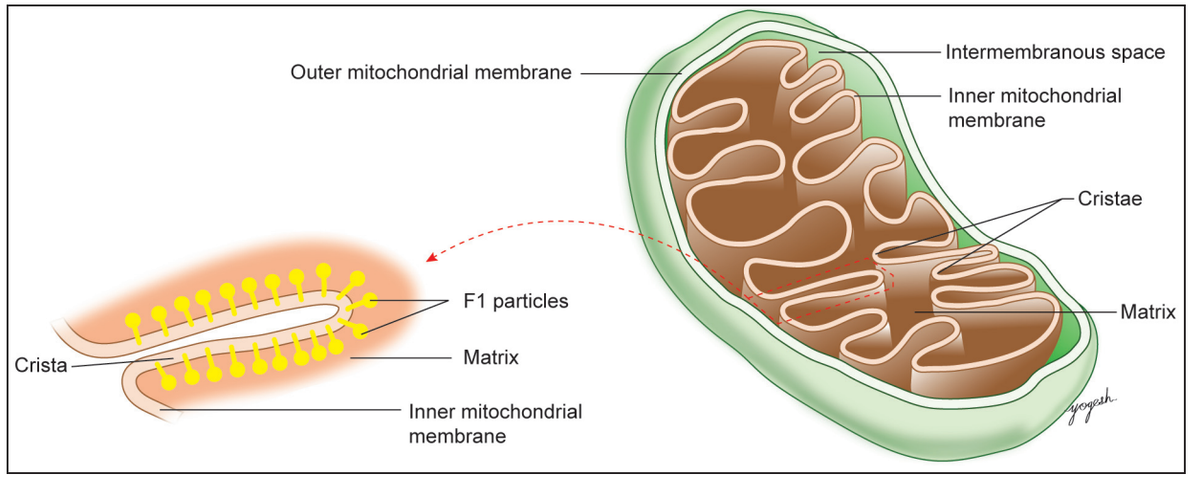


Fig. 3.8: Mitochondrion.

- *Mitochondrial matrix* contains enzymes of Krebs cycle and fatty acid β-oxidation, Ca^{++} storing matrix granules, mitochondrial DNA, and so on.
- *Mitochondrial DNA* is a small circular double helix DNA that contains 37 genes.[MCQ]
- Mitochondrial DNA is inherited from mother (ooplasm of ovum), as cytoplasm of sperm do not contribute to zygote.[Neet]
- Due to mitochondrial DNA, mitochondria are self-replicating.
- Life span: ~10 days.

Functional Correlation of Mitochondria

1. Powerhouse of cell: Mitochondria produces ATP by aerobic respiration.
2. Self-replication: Mitochondrial DNA helps in certain protein synthesis and replication of mitochondria.
3. Apoptosis (programmed cell death): Mitochondria sense cellular stress and release cytochrome C from intermembranous space into the cytoplasm. This cytochrome C initiates programmed cell death (apoptosis).

- *Mitochondrial cytopathy syndrome*: It is produced by defective mitochondrial DNA. It produces muscle weakness, neurological degenerative lesions, and lactic acidosis.[Neet]
- *Myoclonic epilepsy with ragged red fibers* (MERRF) is produced because of defective enzymes of ATP synthesis. It produces muscle weakness, ataxia, seizures, and cardiac and respiratory failures. Red muscle fibers show accumulation of abnormal mitochondria giving ragged-appearance on microscopic examination.

Ribosomes

[Discovered by *George Emil Palade, 1955*]

- Ribosomes are small cytoplasmic particles (15–20 nm)
- Ribosome consists of two subunits: Small (40S) and large (60S) (Fig. 3.9).

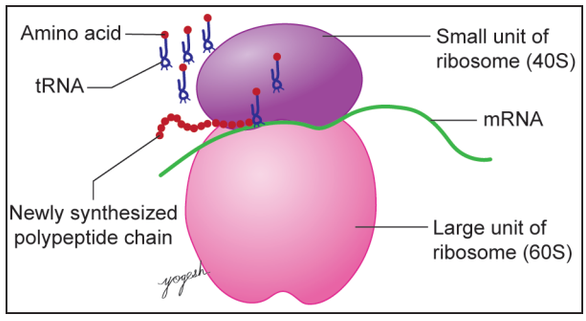


Fig. 3.9: Ribosome.

- Ribosomes lie in association with rER or in freeform in the cytoplasm.
- Polyribosome: It is a cluster of ribosomes bound to a single strand of messenger RNA.
- Ribosome synthesis is controlled by nucleolus (site of rRNA synthesis).
- Nissl bodies consists of both rER and free ribosomes.[MCQ]

Functional Correlation of Ribosomes

Ribosomes synthesize proteins as follows:

- Free ribosomes produce structural proteins of a cell
- Membranous ribosomes (rER) produce secretory proteins.

Lysosomes

- Christian de Duve, 1955 discovered lysosomes (He termed them as *suicide bags*).
- Lysosomes are membranous spherical cytoplasmic vesicles (0.2–0.8 μm in diameter).
- Electron microscopy: Lysosomes are electron-dense bodies.
- Formation: Lysosomes are derived from Golgi apparatus as primary lysosomes (Fig. 3.10).

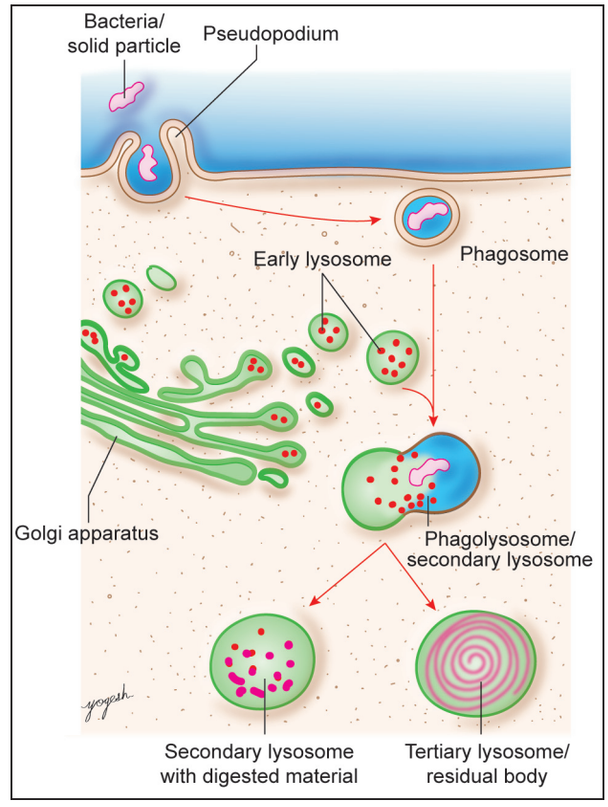


Fig. 3.10: Role of lysosome.

- Primary lysosome fuses with endocytic vesicle that contains material for digestion/destruction and forms secondary lysosome.
- Content: Lysosomes contain hydrolytic enzymes such as proteases, nucleases, glycosidases, lipases, and phospholipases.
- *New concept:* Endosomes receive hydrolytic enzymes from Golgi apparatus to form lysosomes.[Neet]
- Note: Proton (H^+ ion) pumps in lysosomal membrane make the content of lysosome highly acidic (<5 pH).
- Lysosomal membrane has unique lipid lysophosphatidic acid and other proteins. The inner surface of these lipids and proteins are covered by sugar molecules that protect lysosome from its own digestion by lysosomal enzymes.[Viva]
- Recognizable lysosomes in histology[Neet]
  1. Azurophilic granules in neutrophils.
  2. Lipofuscin granules (age pigment) are residual bodies in old cells. They are brown-pigmented granules developed due to lysosomal degradation.[Viva]

Functional Correlation of Lysosomes

- Digestion of foreign material (*Heterophagy*): Lysosomes digest material (bacteria) that entered in to the cell by endocytosis.
- *Autophagy* (removal of old cell organelles): Lysosome removes worn-out organelles of cytosol.
- *Autolysis:* In case of diseases/lack of oxygen supply to the cell, lysosomal enzymes destroy own cells (autolysis).
- Inflammation: Neutrophil releases lysosomal enzymes in extracellular space that digest extracellular matrix and initiates acute inflammation.

Box 3.1: Lysosomal storage diseases

- Many genetic disorders cause lysosomal storage disease because of deficiency of certain lysosomal enzymes.
- Table 3.2 includes some lysosomal storage diseases and responsible enzymes.
- *Tay–Sachs disease* is an inherited lysosomal disorder due to deficiency of *β-hexosaminidase*. It results in accumulation of gangliosides in neurons that cause seizures, muscle rigidity, and death (before 5 years of age).[Next]

Table 3.2: Lysosomal storage diseases[Neet]

Disease	Deficient enzyme
Gaucher disease	Glucocerebrosidase
Tay–Sachs disease	β-hexosaminidase
Krabbe disease	Galactosyl ceramidase
Niemann–Pick disease	Sphingomyelinase
Hurler syndrome	α-L-iduronidase
Hunter syndrome	L-iduronate sulfatase
Maroteaux–Lamy syndrome	Arylsulfatase B
Pompe disease	α-1, 4-glucosidase

Peroxisomes (Microbodies)

- Peroxisomes are membranous organelles.
- Peroxisomes contain oxidative enzymes that are required for the following:
  1. Amino acid oxidation
  2. β-oxidation of fatty acids
- Oxidation of these compounds generate hydrogen peroxide (H_2O_2) that is toxic for cell.
- H_2O_2 is broken down by enzyme *catalase* of peroxisomes and thus, the cell is protected.[MCQ, Functional Correlation]
- Peroxisomes help for detoxification in liver and kidney.[Functional Correlation]
- *Zellweger syndrome/cerebrohepatorenal syndrome:* It is an inherited nonfunctioning peroxisomal disorder and leads to early death. [Hans Ulrich Zellweger, 1909–1990, Swiss–American pediatrician].

Endosomes

- Endosomes are derived from *endocytosis*.
- *Early endosomes:* On endocytosis, the membrane-bound organelle called early endosome is formed.
- *Late endosomes/lysosomes:* Golgi apparatus transfers hydrolytic enzymes and convert early endosomes to late endosomes or lysosomes.
- Transfer of prohydrolase to endosome takes place with the help of mannose-6-phosphate (M-6-P) receptors that are present on endosome surface.
- Mannose-6-phosphate gets separated from prohydrolases by acidic environment to form active hydrolases.

CYTOSKELETON

- Cytoskeleton is a supporting network of protein filaments in cytoplasm.
- Cytoskeleton helps in the following:
  1. Maintaining cellular architecture
  2. Cellular mobility and migration
  3. Movement of cilia, microvilli, tail of sperms

4. Anchoring the cell on basal lamina
5. Form cell junctions
6. Intracellular vesicular transport.

- Components of cytoskeleton:
  1. Microtubules
  2. Microfilaments
  3. Intermediate filaments.

Microtubules

- Microtubules are nonbranching hollow tubules made up of tubulin proteins (α and β tubulin) (Fig. 3.11).
- Locations: Cilia, flagella, centrioles, mitotic spindle, elongating cell processes, and growing axons.
- **Structure**
  - Microtubules are 20–25 nm in diameter with 5 nm thick wall.
  - It consists of 13 protofilaments of dimeric tubulin molecules that have α-tubulin and β-tubulin subunits.
- Centriole has *microtubule organizing center* that gives rise to microtubules.
- Formation of microtubules requires guanosine triphosphate (GTP) and Mg^{++}.
- Microtubule-associated proteins stabilize the microtubules.

Functional Correlation of Microtubules

- Movement of cilia, flagella (tail of sperm)
- Intracellular transport of vesicles
- In cell division, formation of mitotic spindle
- Maintenance of cell shape.

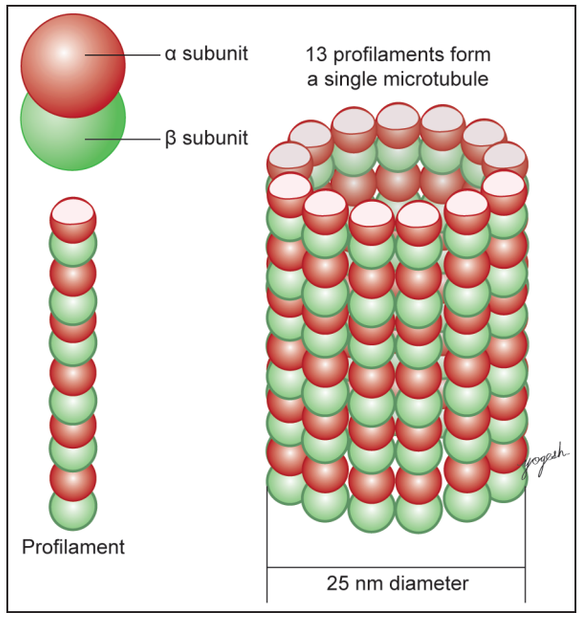


Fig. 3.11: Structure of microtubules.

- Molecular motor proteins help in the movement of cell organelles along the microtubules. For example, dynein (present in cilia and flagella), and kinesins.
- Dynein moves organelles toward the center of cell, whereas kinesins move organelles toward the periphery.

Actin Filaments/Microfilaments

- G-actin molecule assembles to form filamentous actin (F-actin) or microfilaments (Fig. 3.12).
- Microfilament is 6–8 nm in diameter.
- *Actin-binding proteins* (ABPs): They controls polymerization of G-actin, thus determining the length of actin filaments. They cross-link actin filaments to form bundles. For example, fascin, fimbrin in microvilli.
- Actin capping proteins (for example, tropomodulin) block further addition of actin molecules.

Functional Correlation of Actin Filaments

- Anchor the cell membrane by forming cell junctions
- Forms core of microvilli (Chapter 5)
- Forms terminal web (Chapter 5)
- Cell movement by forming extension of plasma membrane called *lamellipodia*.
- *Filopodia* are small spikes/processes on the surface of cell processes (lamellipodia). Filopodia contain actin filaments.

Intermediate Filaments

- The diameter of intermediate filament (8–10 nm) is intermediate between that of microtubules (20–25 nm) and actin filaments (6–8 nm). Hence, these are called intermediate filaments.
- Intermediate filaments are grouped into six major classes based on their protein composition and cellular distribution (Table 3.3).

Functional Correlation of Intermediate Filaments

- To link cells together with basal lamina.
- Keratins form superficial layers of stratified epithelium, core of hair and nails.
- Neurofilaments help maintain shape of nerve processes.
- Lamins maintains shape of nucleus, whereas beaded filaments maintain eye lens integrity.
- Intermediate filament-associated proteins: These include desmoplakins, desmoplakin-like proteins, and plakoglobins. These molecules help in cell to cell and cell to extracellular matrix junctions.

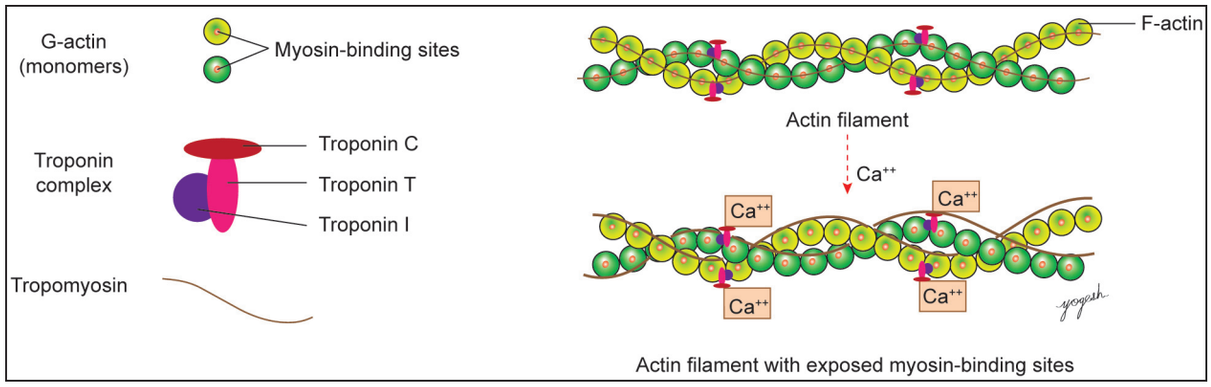


Fig. 3.12: Structure of thin filament. Actin consists of polypeptide chain of G-actin monomers. In relaxed stage, tropomyosin covers the active binding sites of F-actin for myosin. Ca^{++} ions bind with troponin C and results in release of tropomyosin from F-actin. Thus, Ca^{++} exposes myosin-binding sites of actin.

Table 3.3: Types of intermediate filaments

Type of protein	Examples	Location
Classes 1 and 2	Keratins	All epithelial cells
Class 3	Vimentin	Mesenchymal cells
	Desmin	Muscles
	Glial fibrillary acidic protein	Neuroglial cells, Schwann cells
	Peripherin	Peripheral neurons
Class 4	Neurofilaments	Neurons
Class 5	Lamins	Nucleus
Class 6	Phakinins, filensin	Lens (eyeball)

Centrioles

- Centrioles are small spherical area of cytoplasm situated near the nucleus.
- Centrioles are hollow cylindrical structures that are made up of nine microtubule triplets arranged in cylindrical pattern (Fig. 3.13).
- There are two centrioles in a cell. They are arranged at right angle to each other.
- Centrioles are surrounded by *pericentriolar area.*
- Centrioles and pericentriolar area together called as *centrosome or microtubule organizing region.*
- Centrioles are self-replicating organelles.

Functional Correlation of Centrioles

- Centrosome initiate formation of microtubules.
- Centrosome forms mitotic spindle.
- Centrosome provides basal bodies for cilia and flagella.
- Centrioles self-replicate just before cell division.

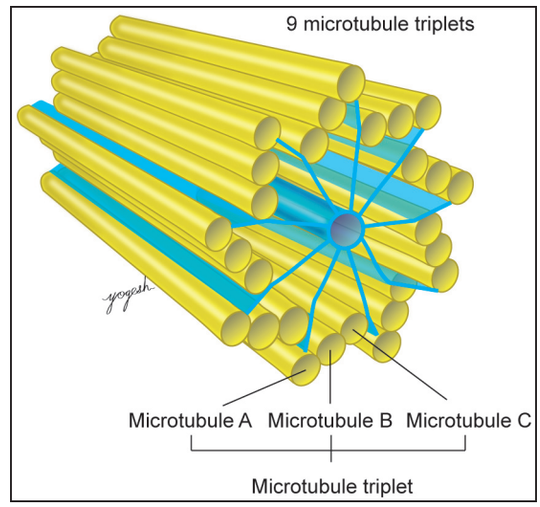


Fig. 3.13: Structure of centriole.

Clinical Correlation

- *Kartagener syndrome:* It is a defect in the organization of microtubules that results in *defective ciliary movement* in the respiratory tract, defective sperm movement, and defective ciliary movement of fallopian tubes. It results in repeated respiratory infections, and male and female infertility. *Next*
- Colchicine, vinblastine, and vincristine prevent mitotic spindle formation and arrest cell division in mitosis. Colchicine is useful for chromosomal studies in cytogenetics. *Next*
- *Alzheimer's disease:* Defective formation of neurofilaments (intermediate filaments) causes Alzheimer's disease. It results in accumulation of neurofibrillary tangles in neurons.
- In alcoholic liver cirrhosis, keratin filaments get accumulated in hepatocytes and form *Mallory bodies* (inclusions).

- *Duchenne muscular dystrophy* [Duchenne de Boulogne, 1806–1875, French Neurologist]: It is a X-linked recessive disorder that affects only boys. It involves a defective gene for dystrophin protein. Dystrophin is essential in binding contractile assembly to sarcolemma in skeletal muscles.[Next]

NUCLEUS

- Nucleus is an oval or spherical membranous structure.
- Most of the cell contain single nucleus except[MCQ]
  - RBCs and platelets do not have nuclei
  - Striated muscle cells, osteoclasts, and syncytiotrophoblast are multinucleated
  - Few hepatocytes and transitional epithelial cells are binucleated
- Nucleus is present during interphase of the cell.
- It consists of the following components: chromatin, nucleolus, nuclear membrane, and nucleoplasm (Fig. 3.14).

Nuclear Envelope

- Nuclear envelope is bilaminar membrane that separates nucleoplasm from the cytoplasm.
- *Perinuclear cisternal space* lies between two layers of nuclear envelope.
- *Nuclear pores* are intervals in nuclear membrane that transport RNAs and proteins between the nucleus and the cytoplasm.
- Outer membrane of nuclear envelope is continuous with rough endoplasmic reticulum.

Box 3.2: Inclusions

- Inclusions are cytoplasmic or nuclear structures that are products of metabolic activity of cell.
- Inclusions have characteristic staining property.
- Examples: lipofuscin, hemosiderin, glycogen, lipid inclusions, and so on.

Lipofuscin[Viva]

- These are brownish-gold pigments.
- It is visible on *H&E* staining.
- Locations: Neurons, skeletal, and cardiac muscles, macrophages.
- It is a wear and tear pigment.
- Mechanism of formation: Digested bacteria in macrophages, accumulated oxidized lipids in other cells.

Hemosiderin

- These are iron complexes formed by indigestible residues of hemoglobin in phagocytic cells.

Glycogen

- It is a glucose polymer found mostly in hepatocytes and muscle cells.
- Toluidine blue or periodic acid–Schiff staining is useful for detecting glycogen.

Lipid inclusions

- These are fat droplets found in intestinal absorptive cells and adipocytes. They are also found in hepatocytes in lipid storage disease.

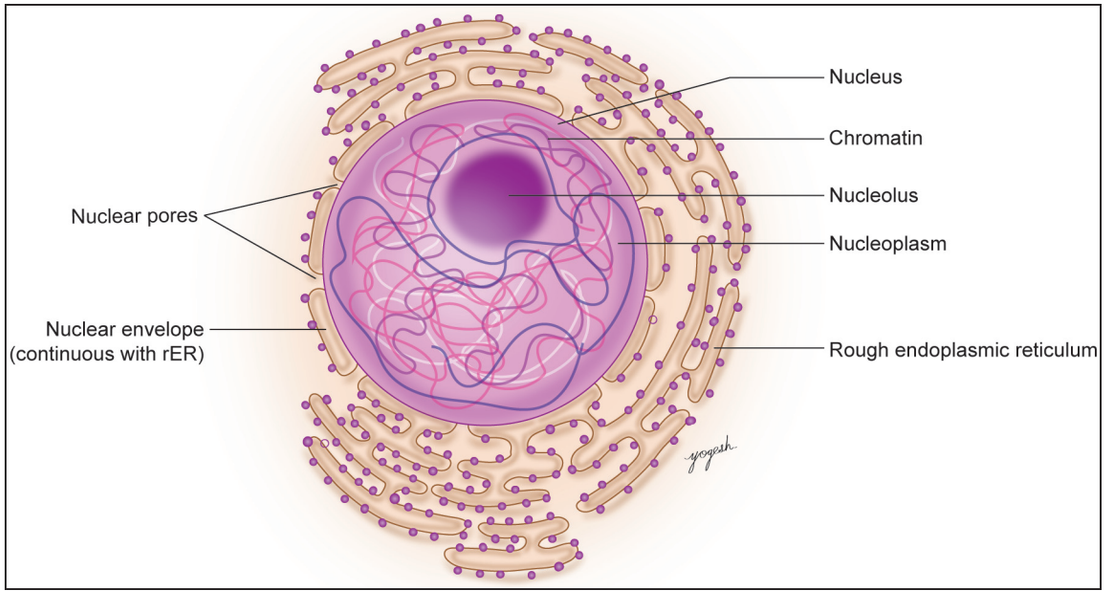


Fig. 3.14: Structure of nucleus.

- Inner nuclear membrane is supported by intermediate filaments (lamin).
- Abnormal lamin proteins are detected in Emery-Dreifuss muscular dystrophy (progressive muscle weakness, *contractures* of tendons and weakening of heart muscle).[Next]
- During cell division, nuclear envelope disappears to allow chromosomal separation. After cell division, nuclear envelope is reassembled.

Nucleoplasm

- Nucleoplasm is a material enclosed by nuclear envelope besides chromatin and nucleoli. It contains various proteins, ions, and inclusions.

Chromatin

- Genetic material of the cell located in the nucleus is in the form of a long thread called *chromatin.*
- Chromatin consists of (Human genome project–2003):
  - 1.8 m long DNA
  - 1000 times longer than the nucleus diameter
  - 46 chromosomes
  - 2.85 billion base pairs of nucleotides
  - 23,000 protein-coding genes
- Chromatin consists of DNA coiled around histone and nonhistone proteins (structural proteins). Presence of DNA and RNA (acids/negative charges) makes the chromatin basophilic (stained with hematoxylin).[Viva]
- Definition of gene: "*Gene is a union of genomic sequences encoding a coherent set of potentially overlapping functional products*" [Histology: A Text and Atlas, Pawlina W, 7th Ed].

Forms of Chromatin

- According to the functional activity, chromatin has coiled DNA and it produces two forms of chromatin as follows (Fig. 3.15):[Functional Correlation]

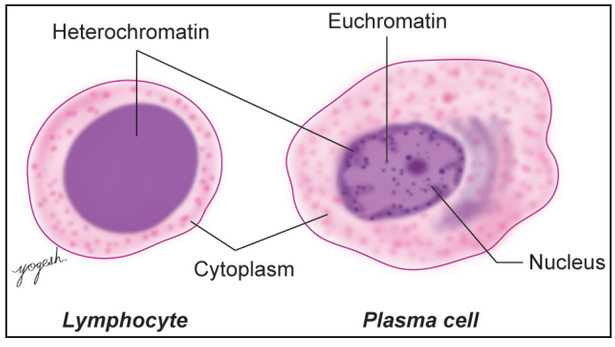


Fig. 3.15: Heterochromatic and euchromatic nuclei.

- *Euchromatin*[Viva]
  It is a partially condensed chromatin.
  It is more active and lightly stained.
  It is expressed during interphase.
- *Heterochromatin*[Viva]
  It is a condensed chromatin.
  It is inactive and darkly stained.
  It does not express during interphase.
  - *Constitutive heterochromatin* remains inactive throughout the cell cycle. For example, around centromere, telomeres, and C-band of chromosomes.
  - *Facultative heterochromatin* remains active in certain phase of the cell cycle. For example, both the X chromosomes remain active during embryogenesis and later one X chromosome becomes inactive. Inactive X chromosome in females form *Barr body* or *drumstick body* in neutrophils.[MCQ]
- Nucleosomes are the smallest units of chromatin. It consists of macromolecular complexes of DNA and histones.

Histone Proteins

- Histone proteins form an octamer having eight molecules of histone proteins. DNA wrapping around histone proteins produce *beads on a string* appearance.
- During cell division, chromatin condenses to form *chromosomes* (*color bodies* in Greek).

Nucleolus

- Nucleolus is a spherical mass of heterochromatin.
- It is a dark staining body, 1–3 µm in diameter.
- Each nucleus shows 1–2 nucleoli (maximum 5–6).[Viva]
- It contains a protein nucleostemin that binds p53 protein and regulates cell cycle and cell differentiation.

Some Interesting Facts

- In degenerative process, the nucleus loses its details and becomes shrunken, and darkly stained. Such nuclei are called *pyknotic nuclei.*[Viva]
- Apoptosis is programmed cell death. It is an active gene-directed process that requires energy.[Viva]
- Necrosis is not a programmed cell death. It may result from various factors such as mechanical chemical injury, infectious agents, toxins, and so on.[Viva]

Functional Correlation of Nucleolus

Ribosomal RNA synthesis and regulation of the cell cycle.

TISSUES

Definition

Tissue is an aggregation of group of cells organized to perform one or more specific functions.[Viva]

Classification

Four basic tissues of the body: The tissues of the body are grouped into four basic types as follows:[MCQ]

1. *Epithelial tissue (epithelium):* The surfaces of the body (inner and outer) and inner surface of tubular structures within the body are covered by a layer of cells that rests on the basement membrane. Such a covering layer is called epithelium. They also form secretory units of glands.
2. *Connective tissue:* It supports the other three basic tissues of the body. It consists of cells, connective tissue fibers and inter cellular matrix.

   Specialized connective tissue include bone (with mineralized matrix), cartilage (with hydrated matrix), and blood (flowing connective tissue).[Next]
3. *Muscle tissue:* It has contractile cells. It is further classified into skeletal, cardiac and smooth muscles.
4. *Nerve tissue:* It consists of cells that have the property of excitability and conduction. Nervous tissue receives information from external and internal environment, interpret the information, and convey it to other organs to control their functions. It consists of nerve cells and supporting neurological cells.