Chapter

1

Cell Physiology: Structure and Functions of Cell Organelle

Competency achievement: The student should be able to:
PY1.1: Describe the structure and functions of a mammalian cell.
PY1.9: Describe and discuss the functions of cell products (organelles), its communications and their application in clinical care and research.

INTRODUCTION

The word cell (*L. cella*—a storeroom, a chamber) was first introduced in the biology by Robert Hooke (1635–1703). *The cell is the structural and functional unit of the living matter and is capable of carrying on the processes of life independently*. In the unicellular organism, a single cell is capable of multiple functions. But in the multicellular organisms, all these properties of protoplasm are divided and delegated to specific cells organelles and their protoplasms as well.

CELL STRUCTURE

In multicellular organs, the cell constituents can be broadly divided into three principal units (Fig. 1.1):

i. Cell membrane
ii. Cytoplasm and its organelles
iii. Nucleus and its contents.

Cell Membrane

The plasma membrane or cell membrane is the outer covering of the cell and is a flexible, responsive and a dynamic structure. As seen under electron microscope the cell membrane is 7.2 to 8 nm thick. **The fluid mosaic model** (Fig. 1.2) **was proposed by** SJ Singer and GL Nicolson describing the characteristic structure of cell membrane in 1972. The fluid mosaic model states that: Cell membrane comprises approximately 55% of proteins, 25% phospholipids, 13% cholesterol, 4% of other lipids and around 3% carbohydrates. The cell membranes are composed of a phospholipid bilayer with admixed protein molecules freely floating around it. It is called fluid because individual phospholipids and proteins move side-to-side within the layer like it is a liquid; and is termed mosaic because of the topographic pattern produced by the scattered protein molecules.

Lipid bilayer: The lipid bilayer consists of phospholipid molecules. The fatty acid portion of phospholipid is hydrophobic and faces the interior of the membrane while phosphate end is hydrophilic in nature and this faces the exterior of the cell to the ECF on one side and the ICF on the other side. Cholesterol which is lipid in nature and part of the cell membrane determines the fluidity of the membrane.

Membrane protein: The proteins of cell membrane are glycoprotein and lipoprotein. The glycoprotein acts as receptors for hormones and neurotransmitters while lipoprotein functions as ion channels and enzymes. The cell membrane proteins are intrinsic or extrinsic. The intrinsic proteins are either channel proteins which allow the movement of molecules across the cell membrane or transport proteins (carrier proteins) who act as carriers. The extrinsic proteins serve as enzymes and coenzymes in order to carry out metabolic reactions.

Membrane carbohydrate: The membrane carbohydrates are in the form of glycoprotein or glycolipid. The glycol part of carbohydrate molecules protrude to outside of the cell. Proteoglycans which are carbohydrate substance bind to small protein core and are loosely attached to the cell membrane. The **lose** carbohydrate coat over cell membrane forms the glycocalyx. Both glycolipids and glycoproteins can act as cell receptor sites. Hormones may bind to them, as may drugs, to instigate a response within the cell. They are also involved in cell signalling in the immune system.

Functions of Cell Membrane

1. It facilitates the transport of materials across it. It is selectively permeable to certain substance and helps transports of substances needed for survival. Oxygen, carbon dioxide, small molecules, etc. are transported across cell membrane by diffusion and water is transported by passive osmosis.
2. Helps in the protection of cell. It surrounds cytoplasm of cell and forms a physical barrier between intracellular component and extracellular compartment.

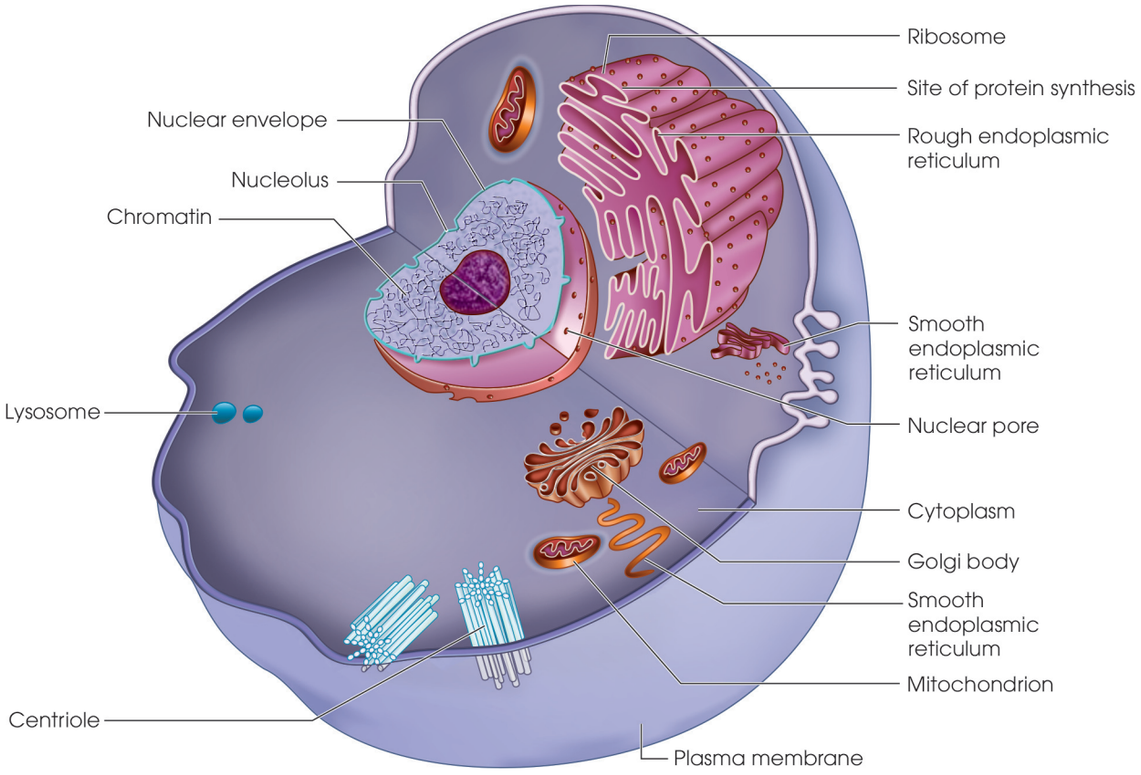


Fig. 1.1: Structure of animal cell

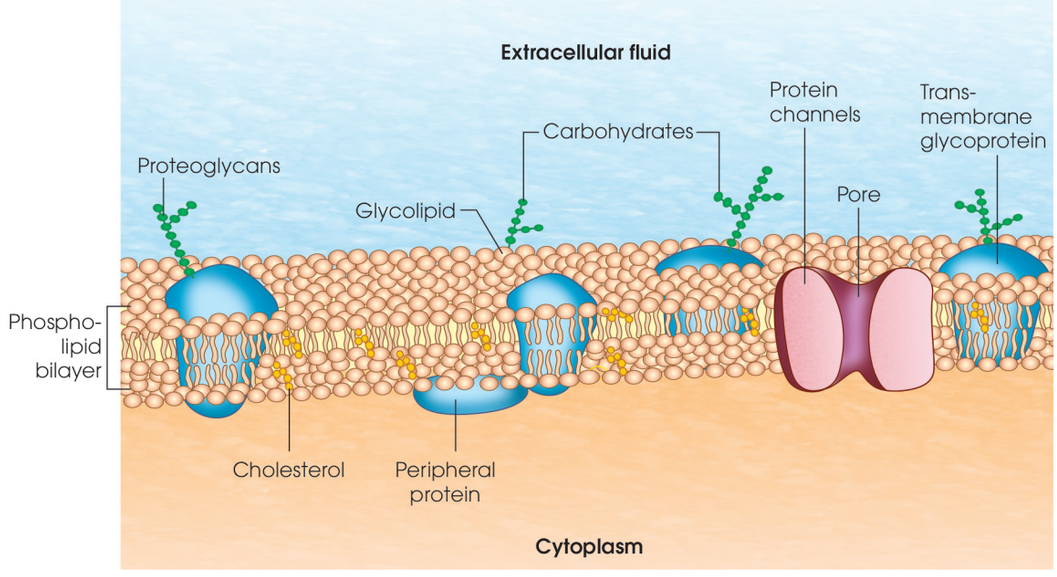


Fig. 1.2: Fluid mosaic model: Structure of cell membrane

3. It anchors to the cytoskeleton to the extracellular matrix and thereby provides shape to the cell and maintains its structural integrity.
4. Receives stimuli from the outside. The protein component of cell membrane acts as ligand receptors. The cell membrane contain receptor site for some hormones, immune proteins and neurotransmitters, thus the cell recognizes and process these signals.
5. Takes in food and excretes waste products.
6. They aid in cell recognition (identifiers).
   Example: Glycoproteins (e.g. major histocompatibility complex, and ABO blood group antigens). The surface protein markers which are embedded in the cell identify the cells, thus helps neighbouring cell to communicate with each other.
7. The proteins in cell membrane act as enzymes and catalyze reactions and thus involved in metabolic process.

Cytoplasm and its Organelles

The cytoplasm is the protoplasm which surrounds the nucleus and is bounded peripherally by the cell membrane. Cytoplasm may be homogeneous, vacuolated, granular, reticular or fibrillar. It consists of number of bodies and structures, vacuoles, etc. In the light microscope, the cytoplasm can be classified into two groups (Flowchart 1.1): Cytoplasmic organelles and cytoplasmic inclusions.

Endoplasmic Reticulum (Ergastoplasm)

It consists of network of canals (tubules) and vesicles (cisternae). These are three-dimensional and bounded by membrane of about 80 Å in thickness. The elements of the endoplasmic reticulum may connect intermittently with the plasma membrane at one hand and on the other hand with the outer nuclear membrane. Two types of endoplasmic reticulum have been recognized:

Rough-surfaced endoplasmic reticulum: This reticulum is studded with osmiophilic granules. The ribosomes lie in rows in contact with the membranes of the endoplasmic reticulum (Fig. 1.3). The roughness of the membrane is due to the presence of these granules (Palade granules).

Smooth-surfaced endoplasmic reticulum: This type of endoplasmic reticulum does not possess osmiophilic granules. The ribosomes lie at the outer border of the membrane. This is why it is smooth.

Functions of endoplasmic reticulum

1. The rough endoplasmic reticulum which is abundant in liver cells is site of protein synthesis.
2. The smooth endoplasmic reticulum (ER) is responsible for synthesis of lipids such as phospholipids and cholesterol. It is involved in production and secretion of steroid hormone.
3. The smooth ER stores and releases calcium ions which play important role in muscle contractor and nervous system activities.

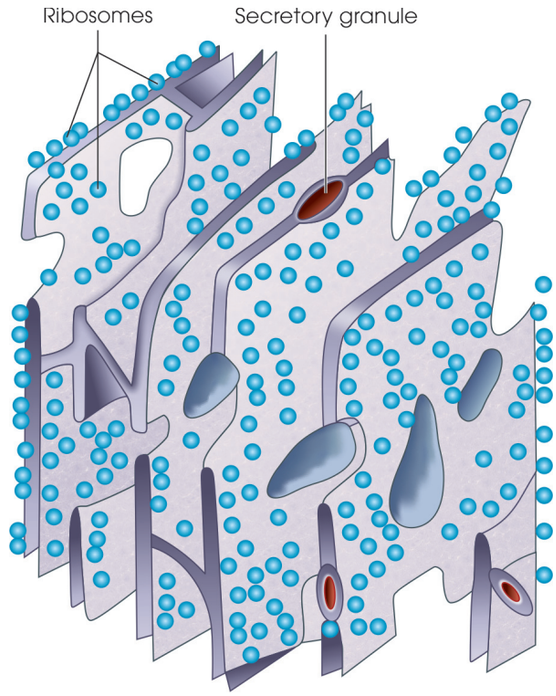


Fig. 1.3: Three-dimensional structural representation of endoplasmic reticulum (rough) showing embedded ribosomes and secretory granules on the wall (diagrammatic representation)

Golgi Apparatus (Golgi Complex)

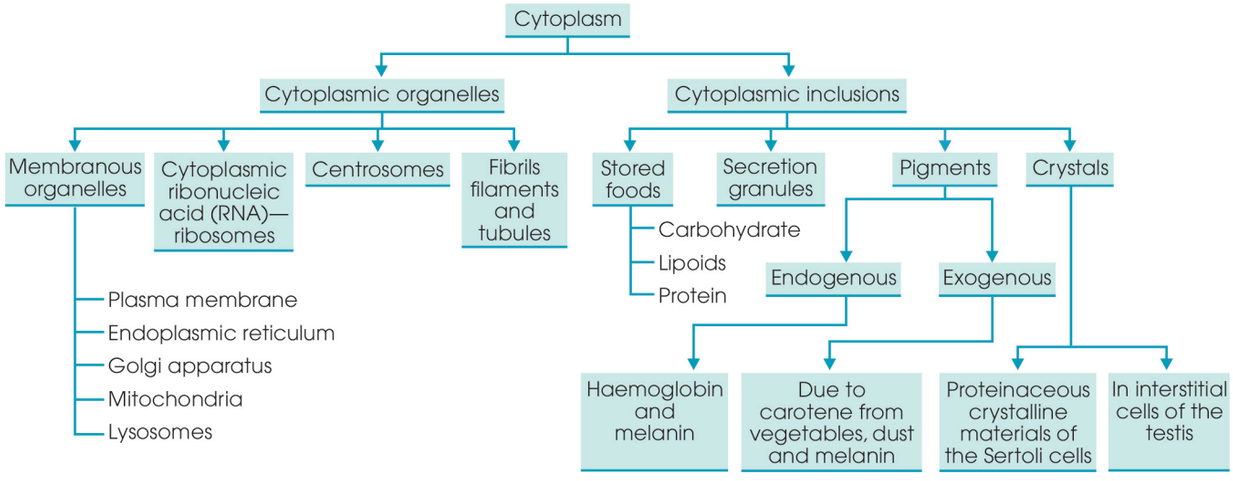
The Golgi apparatus was first discovered in 1898 by Camillo Golgi. The structure (Fig. 1.4) looks like a network of fine threads (Golgi network) or irregular granular material. Following main structures can be observed in Golgi apparatus under electron microscope.

Flattened (distended) vesicles, secretory vesicles and microvesicles.

Functions

1. The secretory substance which is synthesised by the endoplasmic reticulum, passes to the Golgi apparatus for wrapping and packaging.

Flowchart 1.1: Classification of cytoplasm under light microscope



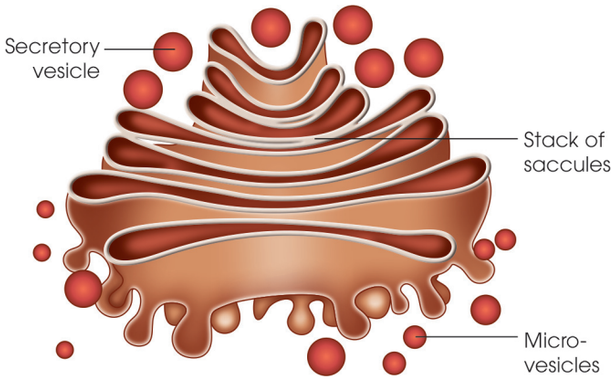


Fig. 1.4: Structure of Golgi apparatus (diagrammatic representation)

2. In addition, Golgi apparatus independently synthesises polysaccharide part of glucoprotein secretion.
3. It is sites of formation of lysosomes.
4. It produces secretory granules which store hormones and enzymes.

Mitochondria

These are relatively solid bodies, granular, rod-shaped or filamentous in form and remain scattered throughout the cytoplasm (dimensions varying from 0.5 to 5.0 microns). They are surrounded by a trilaminar double membrane, the inner one of which remains folded and forms a number of partitions, the cristae mitochondriales. These cristae may be *complete*, *septate* or *incomplete*.

Numerous projecting particles known as elementary particles are present on the inner mitochondrial membrane and cristae. The fluid of the intra-mitochondrial space is called *matrix*. The matrix may contain small dense granules. Most of the enzymes of the mitochondria are present on the elementary particles, the coenzymes in the matrix, and inorganic ions like calcium and magnesium in the granules (Fig. 1.5).

Functions

1. **Oxidative phosphorylation and ATP formation:** The enzymes present in the mitochondria help in oxidative phosphorylation and are the site for formation of adenosine triphosphate (ATP). The mitochondria supply 95% of cell's energy and are called *powerhouse* or *power plant* of the cell.
2. **Cell replication:** Mitochondria possess some amount of deoxyribonucleic acid (DNA). This DNA controls the replication of cells of mitochondrion.
3. It plays vital role in apoptosis.

Lysosomes

The lysosome has been discovered and recognised as a separate cytoplasmic organelle. Its size varies from 0.25 to 0.50 micron. These are membranous vesicles having a spherical and bag-like structure and are filled with hydrolytic enzymes capable of demolishing large molecules (protein, carbohydrate, lipids and nucleic acids) into fragments which may then be oxidised by the mitochondria.

Functions

1. **Digestion:** The general function of the lysosome is the intracellular digestion and for this reason it is sometimes described as *digestive apparatus* of the cell (Fig. 1.6). *Hydrolysing enzymes* of the lysosome digest the food particle.
2. **Cell necrosis or autolysis:** When the cell is damaged, the lysosomal digestive enzymes are released and digest off cellular elements.
3. **Phagocytosis** is also a remarkable function of lysosome. The lysosomes engulf exogenous substances, e.g. bacteria and are degraded by its enzyme. Lysosomes cause autolysis of the remnant, hence they are referred to as suicidal bags.

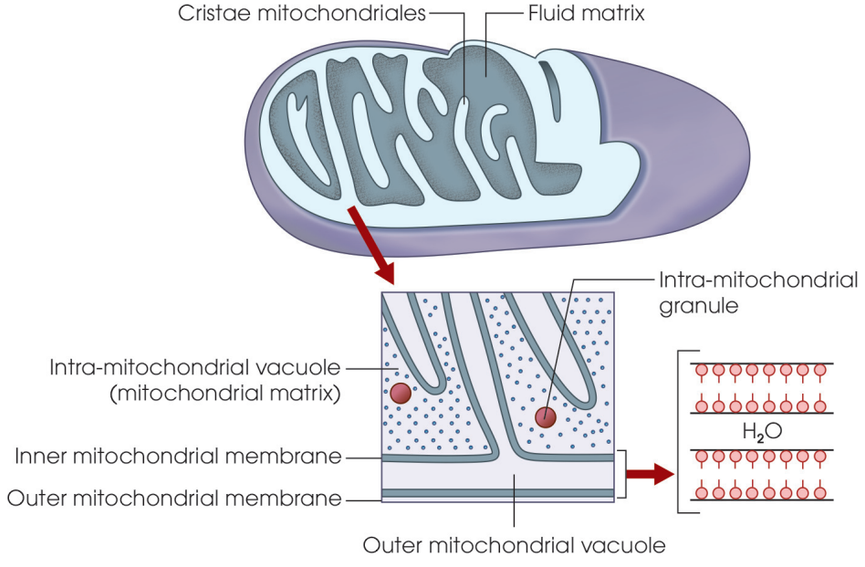


Fig. 1.5: Mitochondrion showing internal structures (diagrammatic representation)

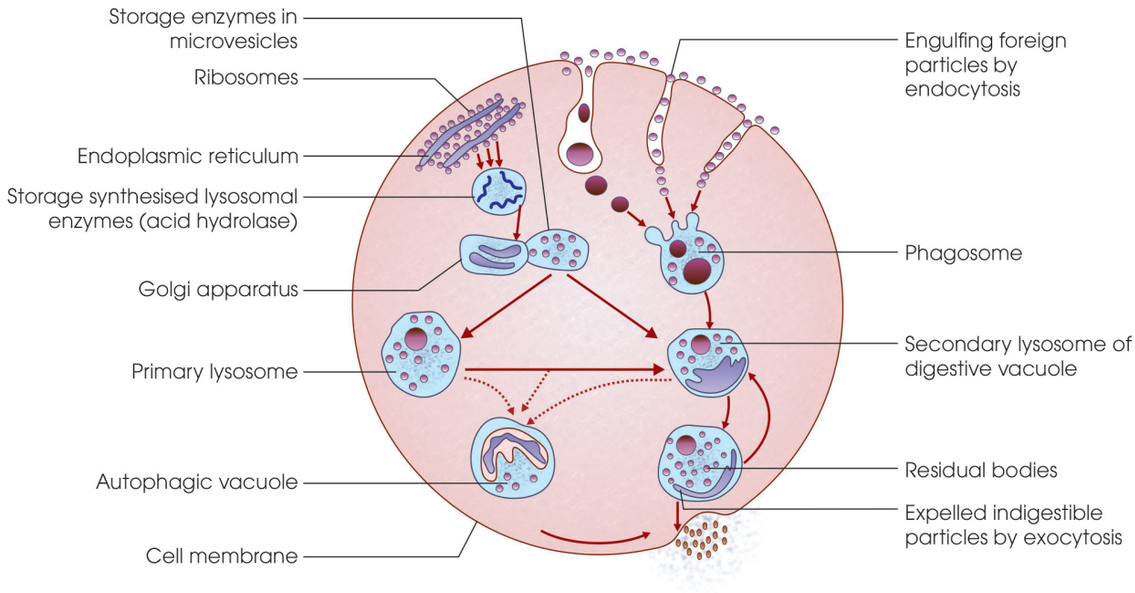


Fig. 1.6: Digestive function of lysosomes (diagrammatic representation)

Ribosomes or Claude's Particles

They are ribonucleoprotein in nature and are found scattered throughout the cytoplasm either singly or in groups (*polyribosomes* or *polysomes*) and range in size from 100 to 150 Å in diameter. They are so rich in RNA that they may contain as much as 60% of total RNA in the entire cell.

Functions: Being attached to the rough-surfaced endoplasmic reticulum ribosomes (Fig. 1.6) synthesise protein and the canals of the reticulum work as passageways through which proteins move on way to Golgi apparatus. So ribosomes are *protein factories*.

Centrosome

It consists of another specialised part of clear cytoplasm, the *centrosphere*, containing in its interior two or more deeply staining particles—the *centriole* (generally arranged in pairs, i.e. *diplosome*) lying close to the nucleus in the resting cell.

Functions: Centrioles control polarisation of spindle fibres cell division during mitosis.

Peroxisomes

They are structurally identical to lysosomes but they contain enzyme oxidases instead of hydroxylase.

Function: They destroy the product substances which are formed from oxygen. *Example*: Hydrogen peroxide.

Microtubules and Microfilament

The microtubules are hollow, unbranched tubes which act as structural tract along which mitochondria, chromosomes and secretory granules more from one part of the cell cytoplasm to another. Microfilaments are contractile protein of 4–6 nm diameter and are present in periphery of cell and they aid in cell motion.

Nucleus

The nucleus (Fig. 1.7) is generally a spherical body occupying the centre of the cell. Its shape, size, position and number vary. The nucleus may contain many lobes.

Nucleolus

Inside a nucleus there is usually single or may be from two to five smaller bodies known as nucleolus or nucleoli.

Functions of Nucleus

1. The genetic material DNA of nucleus acts as a template for RNA synthesis. This RNA regulates the protein synthesis in the cytoplasm.
2. It is responsible for development of chromosomal thread from the network of chromatin initiating cell division and thus play important role in cell reproduction and multiplication.
3. It contains genetic unit, i.e. genes which determine the individuals genetic character.

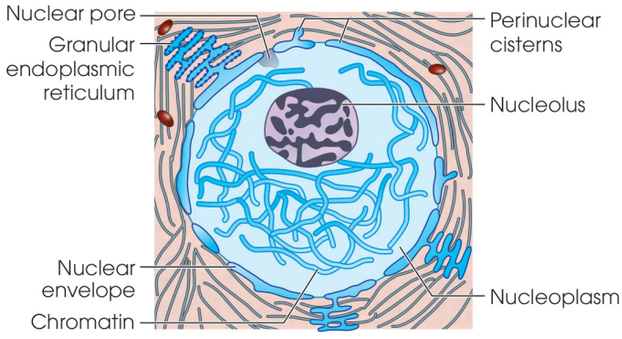


Fig. 1.7: Structure of nucleus

CHROMATIN

Chromatin contains different genes which determine the heredity of the cell, and again the reassembly of different chromatins form chromosome.

Chromosomes (Fig. 1.8)

The predominant component in the chromosome is DNA molecule.

The genes are located in chromosome of the nucleus and can be called the discrete unit of transmission of hereditary character, because it is the specific locus or spot on a chromosome carrying the genetic material or information for a specific character. The gene is a part of the DNA molecule.

Autosomes and Sex Chromosomes

- In human being, there are 46 chromosomes, arranged in pairs, in the nucleus of each cell. In each individual somatic cell nucleus, there are 22 pairs of somatic chromosomes, also called autosomes, which are homologous and concerned with the transmission of ordinary hereditary characteristics and the remaining pair is concerned with the determination of sex.
- In the female, the sex chromosomes consist of a pair of identical large X chromosomes, whereas in the male, the pair consists of an X chromosome and a Y chromosome which is small and has influence on sex determination.

Applied Physiology

Example of sex-linked inheritance of haemophilia. In this disease which is due to hereditary disorder, the blood does not coagulate extravascularly. This disease is transmitted by 'sex-linked' transmission. The haemophilic gene is linked with recessive X chromosome in female which only acts as carrier of the disease but does not suffer from it. This is due to the other X chromosome in female being dominant. If any female carrier (xX) marries a normal male (XY), some of their male offspring (xY) will suffer from the disease and again some of the female offspring (xX) will act as carrier in the first generation (Fig. 1.9A). In case of marriage of male sufferer (xY) with a normal female (XX), male offspring of the first generation will have no abnormalities (XY). But the female offspring will be the carrier (xX) (Fig. 1.9B). In case of marriage between female carrier (xX) and male sufferer (xY), a certain percentage of both male and female offspring the first generation will suffer from the disease and some of the female will be carrier only. According to Mendel's law of heredity, dominant gene always characterizes over the recessive sex-lined gene. The female carrier (xX) does not suffer only for having dominant gene 'X' from the male. On the same ground the female may suffer when the individual possesses the recessive sex-linked gene (xx) (Fig. 1.9C).

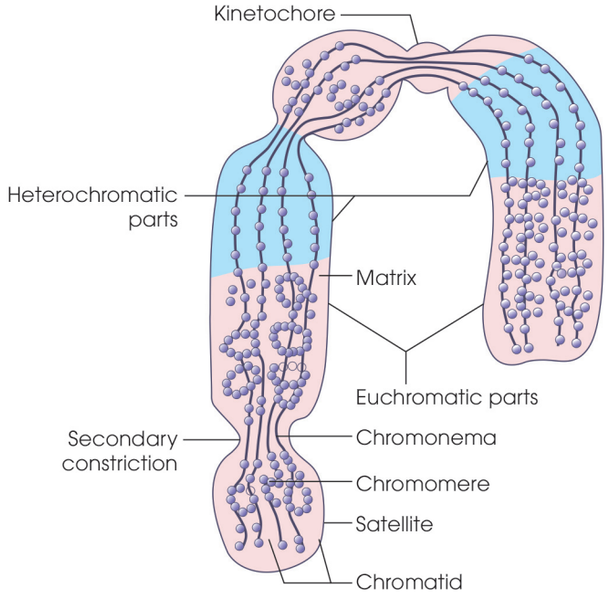


Fig. 1.8: Electron microscopic structure of chromosome (diagrammatic representation)

Competency achievement: The student should be able to:
PY1.3: Describe intercellular communication.

Intercellular Communication and Mode of Communication

The intercellular communication represents and refers to the communication between cells.

The principle mode of intercellular communication is:

1. *Autocrine communication*: The cell secretes an autocrine agent. *Example*: Hormone or chemical messenger that binds to autocrine receptors on same cell. *Example*: Cytokine interleukin 1 in monocytes.
2. *Paracrine communication*: The cell secretes chemical mediators which act immediately on the neighbouring cells. Similarly in autocrine communication the chemical mediator product bind to receptor of the same cell.
3. *Neuronal communication*: It is an electro-chemical event in which neurotransmitters are released at the synaptic junction which acts on post-synaptic membrane receptor and elicit action potential which traverse down the length of axon up to terminal button and further releases neurotransmitter which excite other neurons and neuronal excitability spreads over.
4. *Endocrine communication*: The hormones released from pituitary are carried in circulation to the target organ and acts via second messenger mechanism.

SPECIAL STRUCTURAL FEATURES OF CELL AND INTERCELLULAR COMMUNICATION

Cell Junctions

It is the connection between the neighbouring cells or the contact between the cell and extracellular matrix.

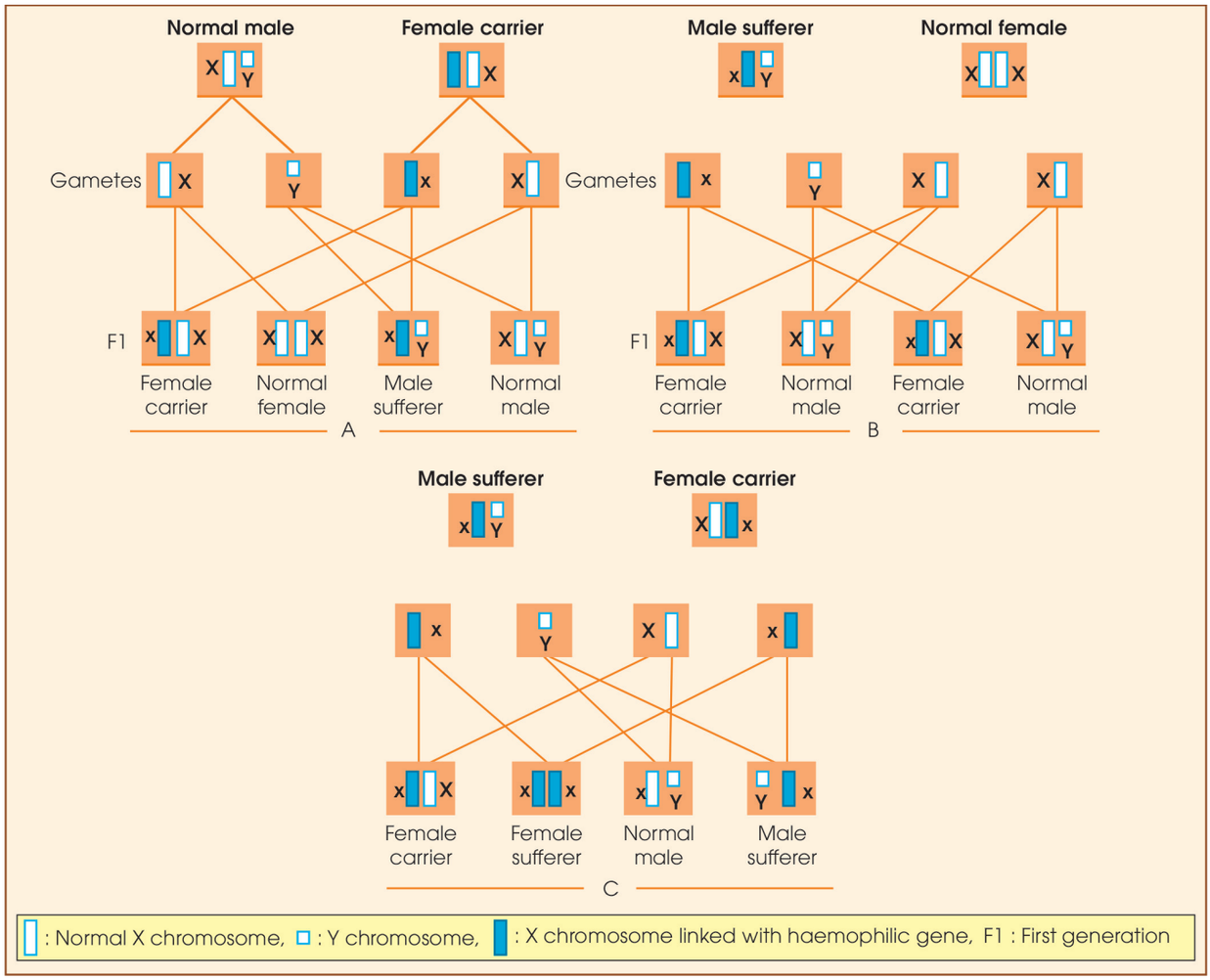


Fig. 1.9A to C: Schematic representation of transmission of haemophilia by union between female carrier and normal male (A), between male sufferer and normal female (B) and between male sufferer and female carrier (C) transmission of haemophilia by union between male sufferer and female carrier

The cell junctions are classified into three types: Occluding junction, communicating junction and anchoring junction.

1. **Occluding junctions:** These are cell-to-cell junctions that seal cells together in an epithelium. For example, tight junction.
   Tight junctions: These are also called occluding junctions or zonulaeoccludens. These are the closely associated areas of two cells whose membranes fuse forming an impermeable barrier to fluid. These provide strength and stability to the cell. These are seen in the wall of renal tubules, choroid plexus and along apical margin of intestinal mucosa.
2. **Anchoring junctions:** These are desmosome (connects intermediate filament of one cell with other cells), hemidesmosome and anchoring junction. Desmosomes are also known as macula adherens and is a cell structure specialized for cell-to-cell adhesion. The cell adhesion proteins of the desmosome are members of the cadherin family. The hemidesmosomes look like half-desmosomes that attach cells to the underlying basal lamina. The hemidesmosomes use desmopenetrin cell adhesion proteins which are members of integrin family. The adherens junctions exhibit their nature of anchor through their cytoplasmic actin filaments.
3. **Communicating junctions:** Cell junctions which permit the intercellular exchange of substance are called communicating junctions. These junctions permit the movement of ions and molecules from one cell to another cell. The two types of communicating junctions are gap junction and chemical synapse.
   Gap junctions: These are low resistance intercellular junctions that allow passage of ions and smaller molecules between the cells. It is present in heart, basal part of epithelial cell of intestinal mucosa, etc. The junctional unit is connexons which are array of protein (contains 6 connexins sub-unit). The connexons of one cell align with connexon of other cells. The intercellular space narrows from 25 to 3 nm at gap junction. They act as channel allowing passage

the substance having molecular weight less than 1000, aid in exchange of chemical messenger between cells and are responsible for rapid propagation of action potential from one cell to another cell.

Chemical synapse: It is the junction between a nerve fiber and a muscle fiber or between two nerve fibres; and through which signals get transmitted by the release of chemical transmitter.

Cell adhesion molecules (CAMs): These are proteins which are present on cell membrane and are involved in binding of one cell to another or with extracellular matrix.

These cell adhesion molecules can be divided into 4 major families: Cadherin superfamily, selectins, immunoglobulin superfamily and the integrins.

Cadherins are calcium dependent homophilic glycoprotein and are concentrated at intermediate cell junctions and it links actin filament network via specific linking proteins catenins.

Selectins: They are family of heterophilic cell adhesion molecules which bind fucosylated carbohydrate. The three family members of selectins family are E-selectin (endothelial), P-selectin (platelet) and L-selectin (leukocyte). The P-selectin glycoprotein ligand-1 (PSGL-1) is expressed on all white blood cells.

Immunoglobulin superfamily molecules: They consist of more than 25 molecules. Few of the important immunoglobulin superfamily molecules are intracellular adhesion molecule 1(ICAM1; CD54), intercellular adhesion molecule 2 (ICAM2), platelet endothelial cell adhesion molecule 1 (PECAM 1; CD31), vascular cell adhesion molecule 1 (VCAM1; CD106) and mucosal addressing cell adhesion molecule 1 (MAdCAM1).

Integrins: There are twenty different heterodimeric combinations of integrins (having fifteen different α and eight different β subunits) at cell surfaces. They bind epithelial and muscle cells to laminin in the basal lamina, allow white blood cells and fibroblast to adhere to fibronectin and collagen as they move and also allow platelets to stick to exposed collagen in a damaged blood vessel.

Roles of Cell Adhesion Molecules

1. They promote cell to cell and cell to matrix interactions.
2. They play critical role in many normal biological processes.

   Examples: Embryonic cell migration, immune system functions, wound healing.
3. They participate in intracellular signaling pathways (primarily for cell death/survival, secretion, etc.).

Molecular Motors

These are the biological molecular machine which has a vital role in movement in living organisms. Few examples of biologically active motor molecules are:

1. **Cytoskeleton motors:** These are myosin, kinesin and dynein. Myosin aids in intracellular cargo transport and muscular contraction. Kinesin moves the cargo inside the cell along the microtubules away from the nucleus while dynein transports cargo along microtubules towards the cell nucleus.
2. **Polymerisation motors:** Few polymerisation known molecular motors are actin and dynamin. Actin polymerisation using ATP which generates forces and can be used for propulsion. Dynamin separates clathrin buds from the plasma membrane using GTP.
3. **Nucleic acid motors:** RNA polymerase, DNA polymerase and helicases. The RNA polymerase transcribes RNA from the DNA template, DNA polymerase turns single-stranded DNA into double-stranded DNA and helicase separates double strands of nucleic acids before the transcription or replication.

Competency achievement: The student should be able to:
PY1.4: Describe apoptosis–programmed cell death.

Apoptosis

It is a programmed cell death. The apoptosis is genetically influence and dead cells are removed by phagocytosis. The resorption of the tail of tadpole during metamorphosis into a frog occurs by apoptosis.

Few examples of apoptosis in human are

- The removal of tissue web formation between fingers and toes of the foetus.
- Degeneration of neurons.
- The periodic sloughing off endometrium at time of menstruation.
- Removal of clones of immune cells which are not appropriate.
- Regression of duct system during time of sex differentiation in foetus.

Mechanisms of Apoptosis

Cell commits suicide by apoptosis due to signals arising within the cell; or signal triggered by death activators like tissue necrosis factor-α and lymphotoxin or by reactive oxygen species.

Intrinsic Mechanism of Apoptosis

Details shown in Flowchart 1.2.

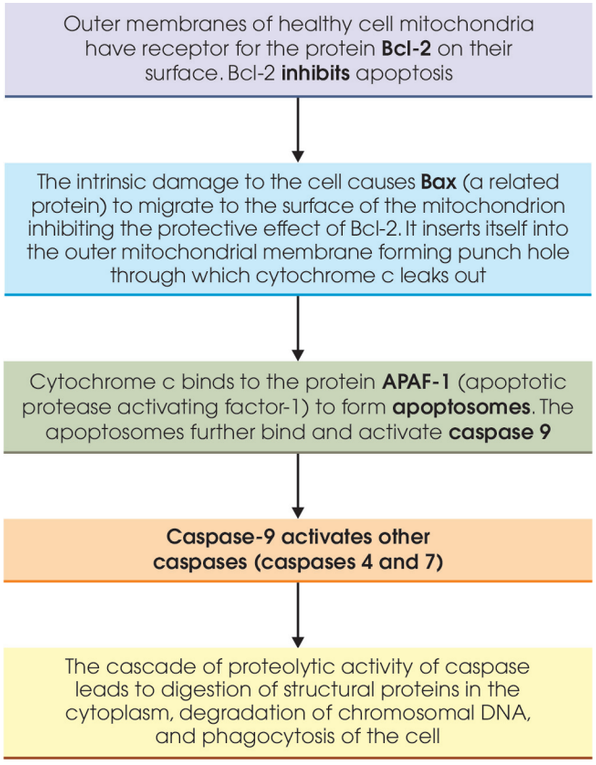
Apoptosis Triggered by External Signals

The Fas (first apoptosis signal) and TNF receptor are integral membrane proteins. The FasL (Fas ligand) and TNF respectively are complementary death activator and they transmit a signal to the cytoplasm that leads to activation of caspase 8. The caspase 8 initiates a cascade of caspase activation leading to phagocytosis of the cell.

Applied Physiology

The genetic defects in apoptosis may be seen a mutation in the gene for Fas. It produces autoimmune lymphoproliferative syndrome (ALPS). The features of ALPS include accumulation of lymphocytes in the spleen and lymph nodes (due to which they get enlarged),

Flowchart 1.2: Mechanism of apoptosis



appearance of clones that are auto-reactive producing autoimmune disorders as haemolytic anaemia and thrombocytopenia.

Competency achievement: The student should be able to:
PY1.9: Demonstrate the ability to describe and discuss the methods used to demonstrate the functions of the cells and its products, its communications and their applications in clinical care and research.[5]

Methods used to Demonstrate the Functions of the Cells and its Product

Cell Functions: Investigation and its Application

1. **Microscopy:** Observing cells under microscope to study the characteristics and functions of cells are the most common technique employed in cell biology. The various microscopes used in cell structure studies include compound microscope, electron microscope (higher magnification and better resolving power aids in studying the smaller constituents of cell in finest detail), transmission electron microscopy (magnification to extent of 1000× to 50,000×), scanning electron microscopy (as electrons bounce over object surface a greater depth of field can be visualized), etc. The electron microscopes are very helpful for diagnosing carcinomas, haematological disorders (ex-sideroblastic anemia), staging of neoplasm, histopathological study of any tissue, cell count, sperm count, etc.
2. **Histology and histopathology**
   Application:
   - Histology is used to study the microscopic structure of any biological tissue. Histology has three components and involve study of tissue, study of organs and study of cell (cytology).
   - Histopathology: It is the microscopic study of diseased tissue to study the disease manifestation and prognosis. The pathologist examines biopsy or surgical specimen after processing the specimen and preparation of histopathological slides.

Note

The tissue is stained for microscopic study using various stains. The commonly used stains are haematoxylin and eosin stains, Congo red, periodic acid-Schiff's stain, methyl violet stain, crystal violet stain, etc.

3. **Cell fractionization:** By this method cells are ruptured to obtain pure fraction product of cell organelles such as nucleus, mitochondria, ribosomal units, etc. so that the structure and functions of these organelles can be studied. The steps involved in cell fractionization include extraction (the cells or tissues are suspended in isotonic solution), homogenization (cells are disrupted by grinding, or chemical methods) and centrifugation. The different constituents of cells are separated by differential centrifugation (different size and density or particles get sedimented at variable rates).
4. X-ray crystallography helps in identifying the exact location of atoms in a crystal. In cell biology X-ray crystallography is used to study the structure of protein and DNA.
5. **Techniques of immunofluorescence:** The method employs use of fluorochrome tagged immunoglobulin to identify antigen–antibody interaction. The commonly used fluorochrome in immunological studies is fluorescein isothiocyanate. The direct microscopy and indirect immunofluorescence microscopy are commonly employed methods in antigenic detection.
6. **Pulse labelling technique:** This technique employs determination of rate of synthesis of molecules within a cell with the aid of radioactive isotopes.
   Example: RNA synthesis can be studied using tritium labelled uridine triophosphate.

Note

- *Electron microscopy*: The transmission electron microscopy helps in having a detail cross-section view of a specimen while scanning electron microscopy provide three-dimensional image of a specimen.
- *Fluorescence microscopy*: The fluorescence microscopy employs use of fluorescent material to identify and indicate various structures of a specimen. The fluorescence microscopy may helpful to locate cell molecules such as protein.
- *Confocal and de convolution microscopy*: The confocal and de convolution microscopy helps to override the drawbacks and limitation of fluorescence microscopy such as thick specimens and blurred image formation.

7. **Techniques used in genetic studies:**
   A. *Recombinant DNA technology*: It is a technology or process in which a new DNA piece which is created by transferring DNA fragments from two different sources (two different species). The lab procedure which is employed in creating recombinant DNA is molecular cloning. The product recombinant DNA is formed by DNA replication within a living cell and requires a cloning vector. The vector represents as carrier for transferring the gene from human beings to bacteria. The vector types include plasmid, cosmids, bacteriophages, P-1 vectors, etc. The segments of DNA are combined by various techniques such as by use of enzyme such as restriction endonuclease or DNA ligase enzyme or by Gibson's assembly. The recombinant DNA is introduced into host organism. The vector is chosen depending on the type of host organism and the DNA size required to be cloned. Eventually with molecular cloning the clones are screened with desired DNA inserts and their biological characteristics. The most commonly used application of recombinant DNA technology is for production of hormones and proteins such as recombinant human growth hormone, recombinant human insulin, clotting factors (VIII, IX and tPA), interferons, etc.
   B. *Nucleotide probes*: These probes are radioisotope labelled or even non-radioactive material labelled DNA fragments used to identify a nucleotide sequence which is complimentary to sequence of probe.
   C. *Blotting technique*: This technique is used to identify the specific DNA or RNA fragment from the thousands of contaminating molecules. The commonly used blotting techniques are northern blot, southern blot, western blot and south western blot.
   The northern blot technique is used to quantitate the cells gene expression. The southern blot technique helps to identify the specific nucleotide sequence in a DNA sample. West blot helps in protein identification while DNA binding characteristic of protein is assessed by south western blotting.
   D. *Polymerase chain reaction*: The target DNA is amplified using polymerase chain reaction technique. The various types of PCR include reverse transcriptase (RT-PCR), real time PCR (a fluorescent dye tags the primer and acts as real time reporter for quantitative defection), invert PCR, nested PCR, rapid amplification of cDNA ends (RACE) PCR and multiplex PCR.
   E. *Microarray*: This method helps to detect gene variation or mutation in a DNA sample. Nearly more than thousand gene can be analysed by this method.
8. **Cell culture and its application:** It is the process by which cells are grown under controlled conditions after shifting them from their natural environment. The isolated cells are cultured in suitable media which supply nutrients to the cell and the growth environment in context of pH, temperature and osmotic pressure are controlled.

Cell types: The types of cells used in laboratory for cell culture study are primary cells and established cell lines.

The primary cells are those which are prepared directly from organ or tissue. These cells grow and divide but for finite time only as hayflick limit in reference to telomer length make these cells senescence.

While this drawback is not seen in continuous cell lines and they can be used for long duration research protocol.

Environment for cell growth

a. The condition of cell culture is either adherent culture or suspension culture (free floating).
b. The cells in adherent culture adhere to the vessel with the aid of extracellular matrix. In the suspended culture, cells grow in liquid medium.
c. Growth media: The media in which the cell is cultured consist of vitamins, amino acids, inorganic salts and even fetal bovine serum is added to the media since it is a good source of growth factors and immunocomplexes which get available for the cell growth. It is important to note that different cell lines have different types of media. The pH which is vital for cell growth is maintained between 7.2 and 7.4 by addition of sodium bicarbonate. The temperature of 37°C is required to achieve optimal cell growth (Figs 1.10 and 1.11).
d. Culture vessels: The different shape and size of vessels are utilized in cell culture. The culture plates are coated with collagens, fibronectin and gelatin and they act as an extracellular matrix.

Cell culture techniques

a. Subculturing: As cell grows they become densely populated and this will hinder with normal cell growth and in order to prevent this the cells are subcultured into another container. In case of suspended cells they are transferred into conical tubes, centrifuged and then further suspended in

Fig. 1.10: Cell culture plate adherent type

Flowchart 1.3: Cell structure and functions of organelles

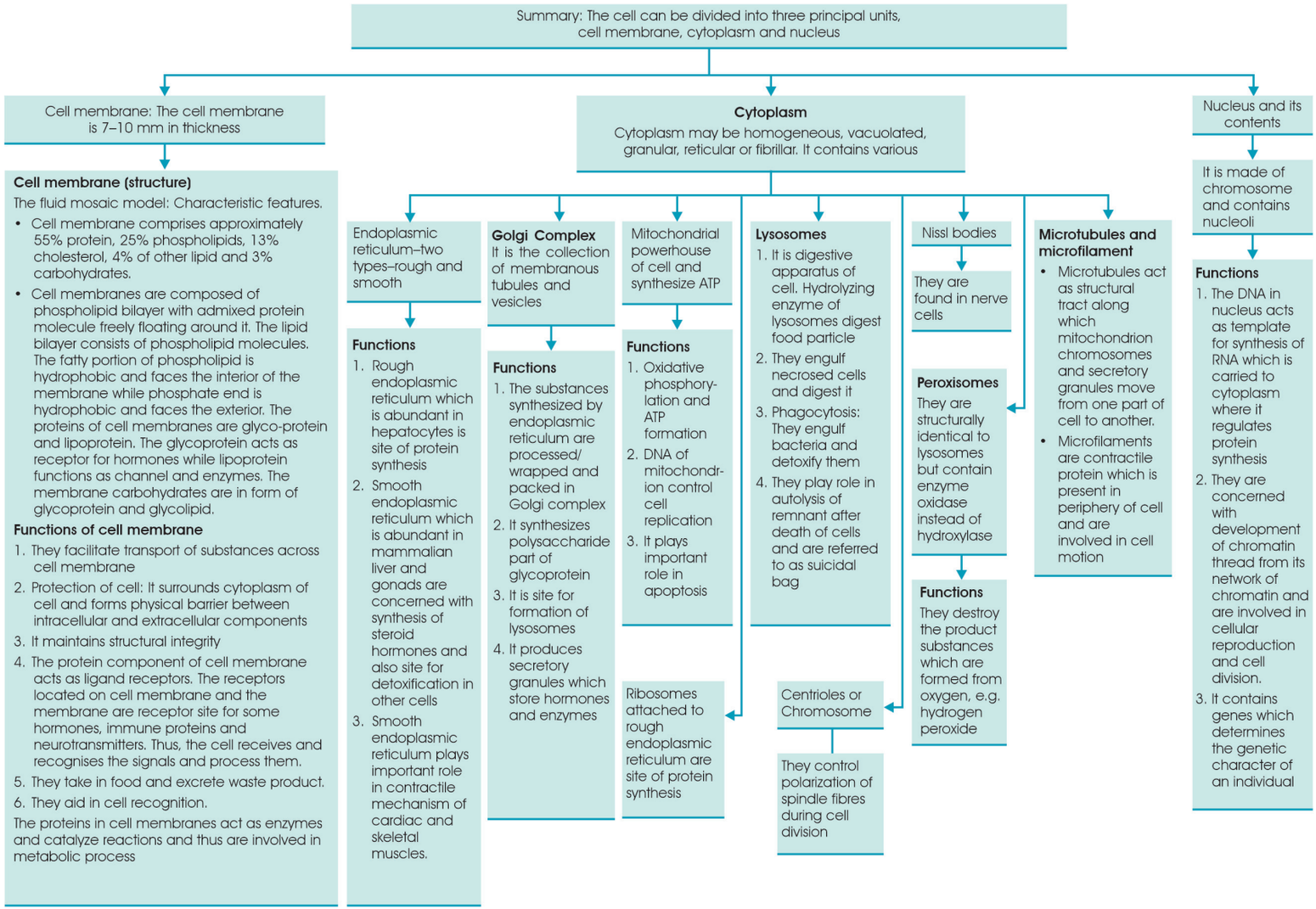


Fig. 1.11: Suspension cell culture flask

fresh media. It can be also shared up in new containers.

b. Cryopreservation: In order to prevent culture lost due to biological contamination or equipment failure, the cells are frozen down for cryopreservation.

Cell viability

The cell viability can be evaluated by staining the cells with trypan blue. The characteristic of trypan blue dye is that it is permeable to non-viable cells. The cells are stained with trypan dye and then it is loaded to haemocytometer. The % of viable cell is calculated as follows:

$$\% \text{ of viable cells} = \frac{\text{Number of unstained cells}}{\text{Total number of cells}} \times 100$$

The common human cell lines are

- HEK-293 Human embryonic kidney
- LNCaP Prostate cancer
- H295R Adrenocortical cancers
- HL60 Leukemia
- MCF-7 Breast cancer
- HeLa Henrietta Lacks

Primate cell lines

- Vero African green monkey kidney epithelial cells
- Cos-7 African green monkey kidney cells

Mouse cell lines

MC3T3 Embryonic calvarium

Application of cell culture

a. It is employed in production of vaccines. As cell growth can be studied by cell culture. A precise therapeutic effects of various drugs and chemicals can be observed over the cell culture and as a result vaccine and other modalities of treatment can be designed.

b. Protein therapeutics: The cell culture technique was a boon and various interferons and antibodies were successfully created with the help of cell culture studies.

c. In cancer research: Preparing cell culture of normal and cancerous cell and designing various therapeutic investigative protocols for observing cell response and behaviour are all helpful to find remedial treatment measures.

9. **Frozen section technique:** A tissue section which is rapidly cooled using cryostat is the frozen section. The frozen tissue is dissected using cryostat to obtain microscopic section. The frozen section are generally stained using dye such as oil red or toluidine blue for quick microscopic analysis so as to ascertain the state of tissue surrounding the tumor for any metastasis or infiltration or whether if any residual carcinoma is present at resection margin. The report is of immense value to oncosurgeon for making quick on the spot decision.

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EXAM-ORIENTED QUESTIONS

Essay

1. Describe the structure and functions of cell membrane.
2. Enlist the organelles in cell. Describe the structure and functions of any two organelles.
3. Describe the structure and functions of mitochondria, endoplasmic reticulum and Golgi apparatus.

Short Notes

1. Fluid mosaic model
2. Structure of nucleus
3. Functions of nucleus
4. Functions of mitochondria
5. Functions of lysosomes
6. Lysosomal storage disease
7. Cell division
8. Mitosis and meiosis
9. Cell adhesion molecules
10. Molecular motor
11. Intercellular cell junction
12. Apoptosis