BIO203: Cell Biology and Physiology

Degree Programme	Undergraduate Programme in Biological Sciences, Biochemistry, Biology Education and Microbiology
Semester/ Level	2/200
Credit Unit	3
Class Type/Hours	Lecture/2h per week, Practical course/1 credits or 4 h per week
Lecturers	Mrs M.G. Babakura and Mr M. Bulama Modu
Attendance	75% mandatory for acceptance into examination

Description

From cell to organism: An introduction to structure, organisation, biochemistry and physiology of plant and animals at the cellular level. Biology of cells of higher organisms would be taught these include: Structure, function, and biosynthesis of cellular membranes and organelles; cell growth and oncogenic transformation; transport, receptors, and cell signalling, the cytoskeleton, and cell movements.

Prerequisites

No prerequisites required however, a strong familiarity with basic plant and animal cell structure, and function as taught at the O level, RSP, ND, NCE and 100 level Biology courses is assumed knowledge for this course and are advantageous for students in the course. Some practical experience in the aforementioned level is recommended. If you are having problem(s) with this introductory part of the course, please consult your teaching assistant or one of the course lecturers.

Learning outcomes

On successful completion of this module students should be able to:

- Understand fundamental concepts of cellular function. Describe and distinguish prokaryotic from eukaryotic cells

- Illustrate plant cell from animal cell and explain the functions of their structural components.

- Depict the fluid mosaic structure and biochemistry of biological membranes and describe the mechanisms of transportation of materials across the membranes.

- Explain and describe the physiology and biochemistry of cellular structures and of special cells such as nerve cells, *etc.*

- Describe how activities within the cell are regulated and how normal cells, divide and interact with pathogens.

➢Describe the use of up-to-date methodologies and techniques in cell biology and physiology.

Calendar

The calendar below provides information on the course's lecture (L) and exam (E) sessions.

SES #	Topics
L1	*Road map of course, what is and what is not cell biology, properties and behaviours of cells *Structure of biological membranes, lipids and lipid modification, membrane proteins
L2	*Transport across membranes: pumps, channels, transporters *Receptors, basics of signal transduction (Note: Reprise and extension in lecture 7)
L3	*Intracellular compartments Protein secretion, biogenesis of membrane proteins *Regulation of the cell division cycle
L4	*Regulation of DNA replication *The microtubule cytoskeleton *Regulation of mitosis *Meiosis *Cell cycle checkpoints
L5	*Signal transduction II : Detailed molecular mechanisms *Nerve cells, ion channels, synapse, Ca^{++} regulated events
L6	*Immunity and host pathogen interactions *Cancerous Cells *Stem cells and cloning
L7	*Techniques in Cell Biology and Physiology
E	* Test and preparations for exams and tutorials

Reading Materials

The recommended reading materials for this course:

Griffiths, et al. *An Introduction to Genetic Analysis*. 5th ed. New York, NY: W.H. Freeman and Company, 1993. ISBN: 0-7167-2285-2.

Lodish, et al. *Molecular Cell Biology*. 5th ed. New York, NY: W.H. Freeman and Company, 2003. ISBN: 9780716743668.

Taylor, et al. *Biological Science*. 3rd ed. United Kingdom, UK: Cambridge University Press, 2002. ISBN: 0 521 63923 9.

*Series of Hand-outs covering the main points of the Lectures could be obtained at the NABSUM Business Centre/Secretariat.

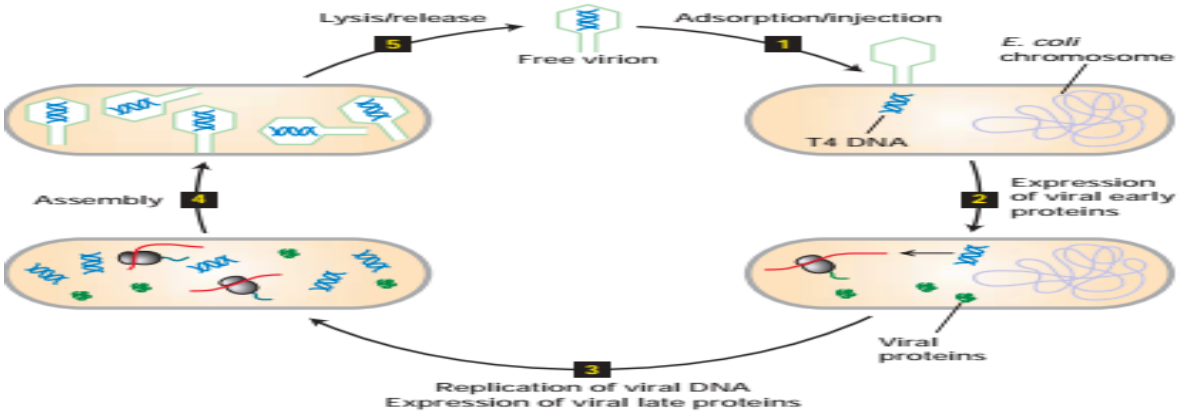
BIO 203: CELL BIOLOGY AND PHYSIOLOGY

L1: ROAD MAP OF THE COURSE

Cell biology is branch of biology that is devoted to the study of structures and functions of specialized cells. The results of these studies are used to formulate the generalization applied to almost all cells as well as to provide the basic understanding of how a particular cell type carries out its specific functions.

Cell is the basic structural and functional unit of organization of all living matter (see Figure1). It is surrounded by plasma membrane. The subunits of cell with specific function necessary to permit its growth and reproduction are referred as organelles. An organelle could be non-membrane bound or membrane-bound. The non-membrane bound organelles, also known as large biomolecular complexes include nucleolus, centriole, ribosome, cytoskeleton, cilium, etc. (see Table 1). While the membrane-bound organelles include nucleus, mitochondria, chloroplast, vacuole, etc. (see Table 2). The largest organelle in the cell is the nucleus. The part of the cell outside the nucleus is termed cytoplasm whose aqueous part *i.e.* the cytosol in which all other organelles are embedded. The dense network of three classes of protein filaments that permeate the cytosol and mechanically support cellular membranes is known as the cytoskeleton (see Figure 2).

It has always been quite difficult to define a cell. Different cell biologists have defined the cell differently as follows: A.G. Loewy and P. Siekevitz (1963) have defined a cell as "*a unit of biological activity delimited by a semipermeable membrane and capable of self-reproduction in a medium free of other living systems*". Wilson and Morrison (1966) have defined the cell as "*an integrated and continuously changing system*." John Paul (1970) has defined the cell as "*the simplest integrated organization in living systems, capable of independent survival*." All these definitions have excluded the viruses. A virus is neither an organism nor a cell, yet it consists of a core of nucleic acid (DNA or RNA) enclosed in an external mantle of protein (Figure 1). In free state, viruses are quite inert. They become activated only when they infect a living host cell and in the process only the nucleic acid core enter the host's cell. The nucleic acid which is the genetic substance, takes over the metabolic activity of the host cell and utilises the cell machinery for the formation of more viruses, ultimately killing the host cell. Thus, viruses are cellular parasites that cannot reproduce by themselves. They could therefore, be considered as **acellular** (*i.e.* a non-cellular primitive and simple unit of life). **Figure 1:** Lytic replication cycle of *E. coli* bacteriophage T4, a virus.



CELLS OF CELLULAR ORGANISMS

The body of all living organisms (bacteria, blue green algae, plants and animals) except viruses has cellular organization and may contain one or many cells. The organisms with only one cell in their body are called unicellular organisms (*e.g.*, bacteria, blue green algae, some algae, Protozoa, *etc*.). The organisms having many cells in their body are called multicellular organisms (*e.g.*, most plants and animals). Any cellular organism may contain only one type of cell from the following types of cells:

A. Prokaryotic cells; B. Eukaryotic cells.

The terms prokaryotic and eukaryotic were suggested by Hans Ris in the 1960's. The prokaryotic (Gr., *pro* = primitive or before; *karyon* = nucleus) cells are small, simple and morphologically most primitive. The eukaryotic (Gr., *eu* = well or true; *karyon* = nucleus) cells have evolved from the prokaryotic cells and the first eukaryotic (nucleated) cells may have arisen 1.4 billion years ago (Vidal, 1983). The **prokaryotic cells** are the most primitive cells from the morphological point of view. They occur in the bacteria (*i.e.*, mycoplasma, bacteria and cyanobacteria or blue-green algae). A prokaryotic cell is essentially a one-envelope system organized in depth. It consists of central nuclear components (*viz*., DNA molecule, RNA molecules and nuclear proteins) surrounded by cytoplasmic ground substance, with the whole enveloped by a plasma membrane. Neither the nuclear apparatus nor the respiratory enzyme system are separately enclosed by membranes, although the inner surface of the plasma membrane itself may serve for enzyme attachment. The cytoplasm of a prokaryotic cell lacks in well-defined cytoplasmic organelles such as endoplasmic reticulum, Golgi apparatus, mitochondria, centrioles, etc. In the nutshell, the prokaryotic cells are distinguished from the eukaryotic cells primarily on the basis of what they lack, *i.e.*, prokaryotes lack in the nuclear envelope, and any other cytoplasmic membrane. They also do not contain nucleoli, cytoskeleton (microfilaments and microtubules), centrioles and basal bodies. Opposite of all above described feature could be found in the **eukaryotic cells** (see Table 1; Figure 3).

Table 1: Differences between Prokaryotic and Eukaryotic Cells

Cellular Characteristic	**Prokaryotic cells**	**Eukaryotic Cells**
Nucleus	Absent	Present
Organelles wit membrane	Absent	Present
Cell diameter	Relatively small, 0.5 – 10µm	Relatively small, 10 – 100µm
Genome	Circular DNA molecule	Multiple linear DNA molecule
Amount of DNA	Relatively small	Relatively large
Cytoskeleton	Absent	Present
Multicellular form	Rare	Common with tissue formation
Cell wall	Present in most cell	Present in plant and fungal cell only
Nucleoli+mitotic apparati	Absent	Present
Plasmid	Commonly present	Rare

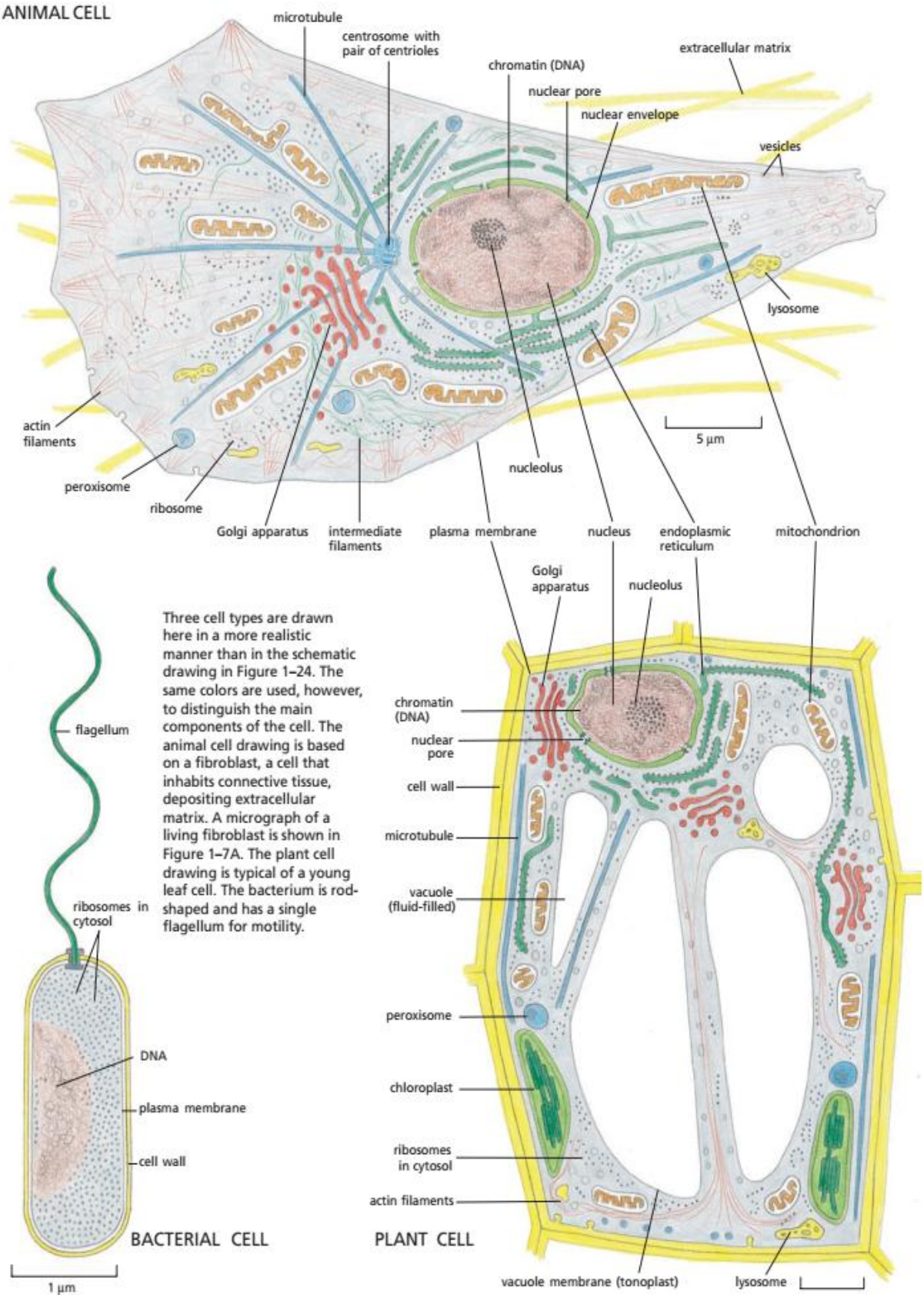


Figure 3: (A) Prokaryotic Cell (Bacterial cell) (B) Eukaryotic Cells (Plant and Animal cell)

In this course however, we would focus mainly on eukaryotic cells (of plants and animals) in view of utilizing such knowledge to formulate the generalisation could be applied to almost all cells as well as to provide the basic understanding of how a particular cell type carries out its specific functions and its structures, physiology and biochemistry, rather than prokaryotic cells which would be covered in BIO 305 and 307, BCH and MCB courses.

The Eukaryotic cells, in general, are bigger and more elaborate than prokaryotic cells (bacteria and archaea). Some live independent lives as single-celled organisms, such as amoebae and yeasts; others live in multicellular assemblies. All of the more complex multicellular organisms - including plants, animals, and fungi - are formed from eukaryotic cells. By definition, all eukaryotic cells have a nucleus. But possession of a nucleus goes hand-in-hand with possession of a variety of other organelles, subcellular structures that perform specialized functions. Most of these are likewise common to all eukaryotic organisms. We will now take a look at the main organelles found in eukaryotic cells from the point of view of their functions (Table 1 and 2).

CELL WALL

Cell wall is a rigid and protective layer around the plasma membrane which provides the mechanical support to the cells of plants, fungi and bacteria. The cell wall also determines the shape of cells of organisms in which they are found. It is due to the shape of cell walls many types of plant cell as the parenchymatous, collenchymatous, etc., have been recognised. The cell wall is a non-living structure which is formed by the living protoplast (A plant cell without its cell wall is called a **protoplast** ; Alberts *et al*.,1989). In most of the plant cells, the cell wall is made up of cellulose, hemicellulose, pectin and protein. In many fungi, the cell wall is formed of chitin and in bacteria; the cell wall contains protein-lipid-polysaccharide complexes.

Chemical Composition of Cell Wall

Chemically speaking, the plant cell wall is composed of a variety of polysaccharides (carbohydrates), lipids, proteins and mineral deposits, all exhibiting distinct staining reactions (see the table below).

Chemical composition of plant cell wall

Substance	**Unit**
Cellulose	Glucose
Hemicellulose	Arabinose, xylose, mannose, glucose and galactose
Pectin	Glucuronic and galacturonic acid
Lignin	Coniferyl alcohol (*e.g*., hydroxy- phenyl propane)
Cuticular substances	Fatty acids
Mineral deposits	Calcium and magnesium as carbonates and silicate

Table 2: Membrane bound (MB) organelles, their description, functions and organisms in which they are found

Mb organelle	**Organism**	**Description**	**Function**
Cell wall	Eubacteria, fungi plants	*outermost layer *rigid, strong, stiff *made of cellulose(plants), chitin(fungi), murein (bacteria) *freely permeable	*support *protection *allows H_2O, O_2, CO_2 to pass into and out of cell freely
Cell surface membrane	both plant/animal	*2 layers of membrane *plant - inside cell wall *animal - outer layer; *made of phospholipid *selectively permeable	*support *protection *controls movement of materials into and out of cell *barrier between cell and its environment *maintains homeostasis
Nucleus	both plant/animal	*largest organelle, oval *contains chromatin= extended chromosome *nucleolus	*controls cell activities *chromosome containsDNA *nucleolus produces ribosomes.
*Nuclear Membrane	both plant/animal	*2 layers surrounding nucleus *Nuclear pores present *selectively permeable	*Controls movement of materials into and out of the nucleus
*Cytoplasm	both plant/animal	*clear, thick, jellylike material and contains other organelles	*supports /protects cell organelles
Endoplasmic Reticulum (E.R.)	both plant/animal	*network of tubes (cistanae) *1 layer membranes	*carries materials through cell
Mitochondrion	both plant/animal	*capsulate, spherical *2 layers of membrane *inner = cristae *matrix present	*centre for aerobic resp. *cristae is site of oxidative phosphorylation *matrix is site resp. enzymes
Vacuole	plant - few/large animal – small	*fluid-filled sacs	*store food, water, waste (plants need to store large amounts of food)
Lysosome	plant - uncommon animal – common	*small, round, with a *1 layer of membrane	*breaks down larger food molecules into smaller molecules *digests old cell parts
Chloroplast	plant, not animal	*green, oval usually containing chlorophyll (green pigment)	*uses energy from sun to make food for the plant (photosynthesis)

Golgi apparatus	both plant/animal	*1 layer of membrane forming flattened sacs called cristae which buds to form vesicles	*transportation of materials *produces lysosomes

Table 3: Non membrane bound (NMB) organelles, their description, functions and organisms in which they are found

NMB organelle	**Organism**	**Description**	**Function**
Nucleolus	Most Eukaryotes	Protein-DNA-RNA	pre-ribosome production
Centriole	Animal	Microtubule protein	anchor for cytoskeleton, organizes cell division by forming spindle fibres
Cytoskeleton	Both plants and Animals	Dense network of protein filament in the cytoplasm	mechanically support cellular membranes
Cilia	Animals, protists, few plants	*Microtubule protein	movement in or of external medium
Ribosome	All eukaryotes	*small bodies free or attached to E.R.	Translation of RNA into protein *i.e.* produces proteins

Middle lamella: The cells of plant tissues generally remain cemented together by an intercellular matrix known as the middle lamella. The middle lamella is mainly composed of the pectin, lignin and some proteins.

Plasmodesmata: Every living cell in a higher plant is connected to its living neighbours by fine cytoplasmic channels, each of which is called a **plasmodesma** (Gr., *desmos* = ribbon, ligament; plural, **plasmodesmata**) which pass through the intervening cell walls. The plasma membrane of one cell is continuous with that of its neighbour at each plasmodesma. A plasmodesma is a roughly cylindrical, membrane-lined channel with a diameter of 20 to 40 nm. Running from cell to cell through the centre of most plasmodesmata is a narrower cylindrical structure, the **desmotubule**, which remains, continuous with elements of the SER membranes of each of the connected cells. Plasmodesmata are formed around the elements of smooth endoplasmic reticulum that become trapped during cytokinesis within the new cell wall that will bisect the parental cell. Plasmodesmata function in intercellular communication, *i.e*., they allow molecules to pass directly from cell to cell.

BIOLOGICAL MEMBRANE (BIOMEMBRANE)

Biological membrane is the ultrathin (~7nm), elastic and selective living transport barrier of all cells and some organelles (see Table 2 above for MB organelles). The biological membrane that encloses the cytoplasm is called cell surface membrane, cytoplasmic membrane, plasma membrane or plasma lemma. All biological membranes including the plasma membrane and internal membranes of eukaryotic cells (*i.e.* membranes bounding endoplasmic reticulum or ER, nucleus, mitochondria, chloroplast, Golgi apparatus, lysosomes, peroxisomes, etc.) are similar in structure (*i.e.*, fluid-mosaic) and selective permeability (allow only water and small molecules such as gases to pass) but differing in other specialised functions (*e.g.* transmission of impulses by nerve cells, etc.). Larger molecules such as glucose, amino acids, fatty acids, glycerol and ions can only diffuse slowly through biomembranes.

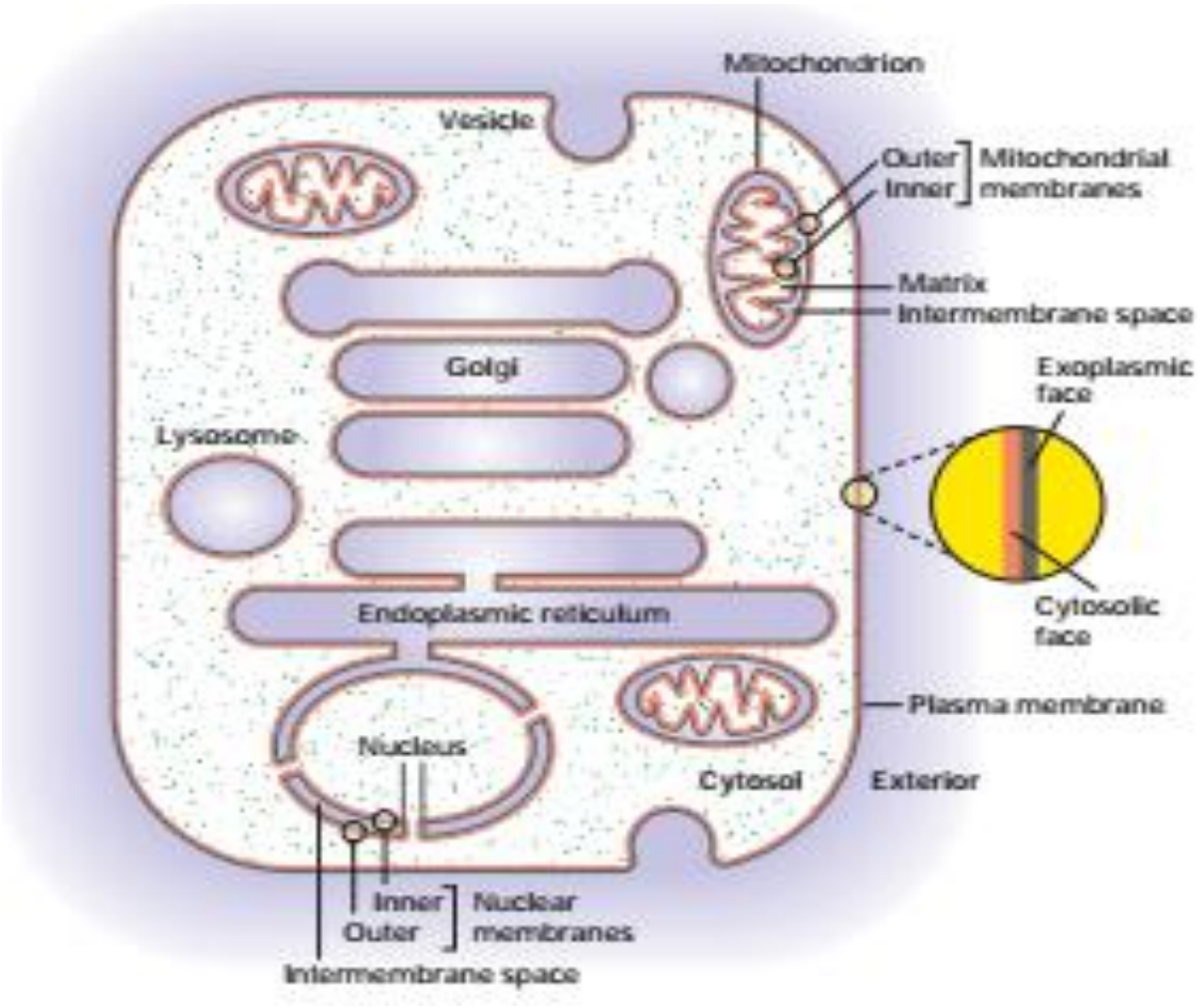


Figure 4: Schematic representation of faces of cellular membranes. Note that three organelles—the nucleus, mitochondrion, and chloroplast (which is not shown)—are enclosed by two membranes separated by a small intermembrane space.

Plasma membrane controls the entry of nutrients and exit of waste products, and generates differences in ion concentration between the interior and exterior of the cell. It also acts as a sensor of external signals (for example, hormonal, immunological, etc.) and allows the cell to react or change in response to environmental signals.

Chemical composition of biomembranes

Plasma membrane and other membranes of different organelles contain variable type and percentage of proteins and lipids (as well as carbohydrates in some cells) see Table 4. For example, in the plasma membrane of human red blood cells proteins represent 52 per cent, lipids 40 per cent and carbohydrates 8 per cent.

Table 4: Chemical composition of some purified membranes (in percentages) -Source: Darnell *et al.* (1986)

Membrane	**Protein (%)**	**Lipid (%)**	**Carbohydrate (%)**
1. Myelin (Nerve cell)	18	79	3
2. Plasma membrane:			
(i) Mouse liver cell	44	52	4
(ii) *Amoeba*	54	42	4
(iii) Human erythrocyte	52	40	8
3. Spinach chloroplast lamellae	70	30	0
4. Mitochondrial inner membrane	76	24	0

Biomenbrane lipids: Lipids are formed by condensation reactions between fatty acid R.COOH and alcohol R.OH. There are four major classes of lipids that are commonly present in the plasma membrane and other membranes: phospholipids (most abundant), sphingolipids, glycolipids and sterols (*e.g.*, cholesterol - only in eukaryotes). All of them are amphipathic molecules, possessing both hydrophilic (water loving – i.e polar=soluble in water) head and hydrophobic (water fearing – non polar) tails as two domains. The relative proportions of these lipids vary in different membranes. The phospholipids being the most abundant of all are the lipids containing a phosphate group $–H_2PO_4$. The commonest type of phospholipid is formed when one of the three –OH group combines with phosphoric acid H_3PO_4 instead of fatty acid R.COOH *e.g* Phosphatidylcholine (a phosphoglyceride derivative) - the most common phospholipid in cell membranes.

The membrane phospholipids of composition present in cells spontaneously form two sheet-like layers known as phospholipid bilayers, which are two molecules thick. This lipid bilayer gives the membrane its basic structure and serves as a permeability barrier to most water-soluble or polar molecules, thus it is a biologically important property in membranes.

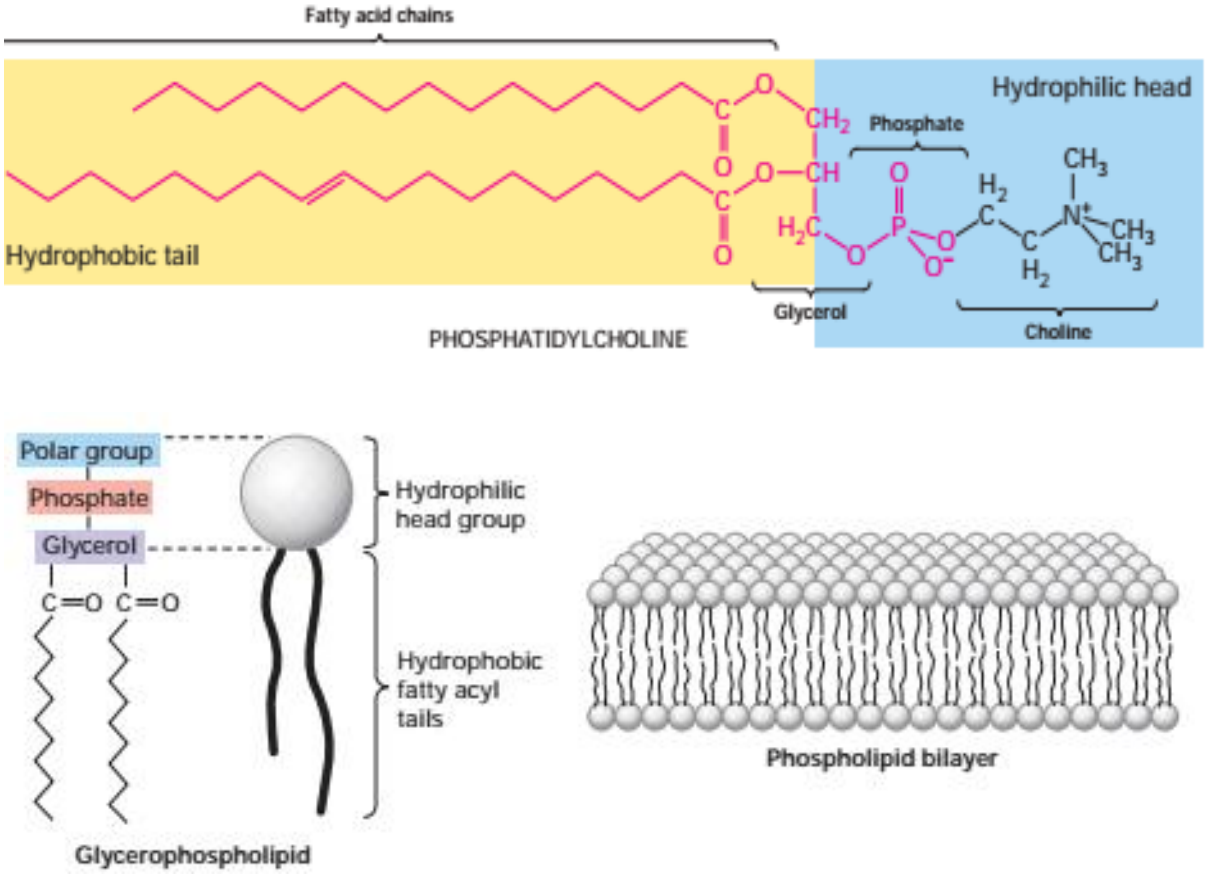


Figure 5: The bilayer structure of biomembranes. (b) Schematic interpretation of the phospholipid bilayer in which polar groups face outward to shield the hydrophobic fatty acyl tails from water. The hydrophobic effect and van der Waals interactions between the fatty acyl tails drive the assembly of the bilayer. [Part (a) courtesy of J. D. Robertson]

Biomembrane proteins: These are proteins that are associated with the phospholipid bilayer of the membranes. The amount of the membrane proteins varies depending on its activity- the more metabolically active the membrane, the more the protein particles found in it *e.g.* the internal membranes of chloroplasts and mitochondria contain ~70% proteins.

Table 5: Some examples of plasma membrane proteins and their functions

Functional class	**Protein example**	**Specific function**
Anchors	Integrins	Link intracellular actin filaments to extracellular matrix proteins
Transporters	Na^+ pump	Anchor for cytoskeleton, organizes cell division by forming spindle fibres
Receptors	Platelet-derived growth factor (PDGF)	binds extracellular PDGF and, as a consequence, generates intracellular signals that cause the cell to grow and divide
Enzymes	Adenylyl cyclase	Catalyses the production of intracellular signalling molecule cyclic AMP in response to extracellular signals

Although the lipid bilayer provides the basic structure of all cell membranes and serves as a permeability barrier to the molecules on either side of it, most membrane functions are carried out by membrane proteins *e.g.* Transporters (Channel proteins and carrier proteins) – In transportation of particular nutrients, metabolites, and ions across the lipid bilayer, Anchors - anchor the membrane to macromolecules on either side. They also function as receptors that detect chemical signals in the cell's environment and relay them to the cell interior, and still others work as enzymes to catalyse specific reactions (See Table 5 above and Figure 6 below).

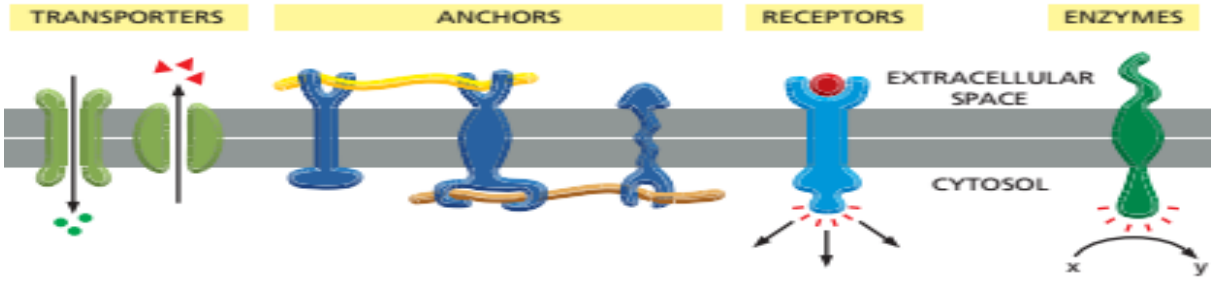


Figure 6: Plasma membrane proteins have a variety of functions

Association of membrane proteins with lipid bilayer

Proteins can be associated with the lipid bilayer of a cell membrane in several ways

1. Many membrane proteins extend through the bilayer, with part of their mass on either side these are called *transmembrane* proteins
2. Other membrane proteins are located entirely in the cytosol, associated with the inner leaflet of the lipid bilayer these called *monolayer-associated* proteins
3. Some proteins lie entirely outside the bilayer, on one side or the other, attached to the membrane only by one or more covalently attached lipid groups - *lipid-linked*
4. Yet other proteins are bound indirectly to one or the other face of the membrane, held in place only by their interactions with other membrane proteins - *protein-linked*

Transmembrane, monolayer associated and lipid-linked proteins are known as *integral membrane proteins*. The remaining membrane proteins are known as *peripheral membrane proteins*.

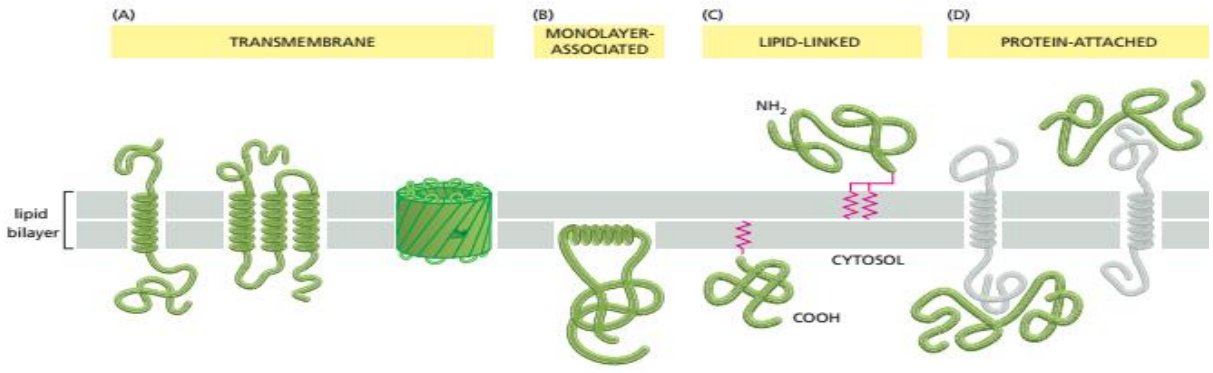
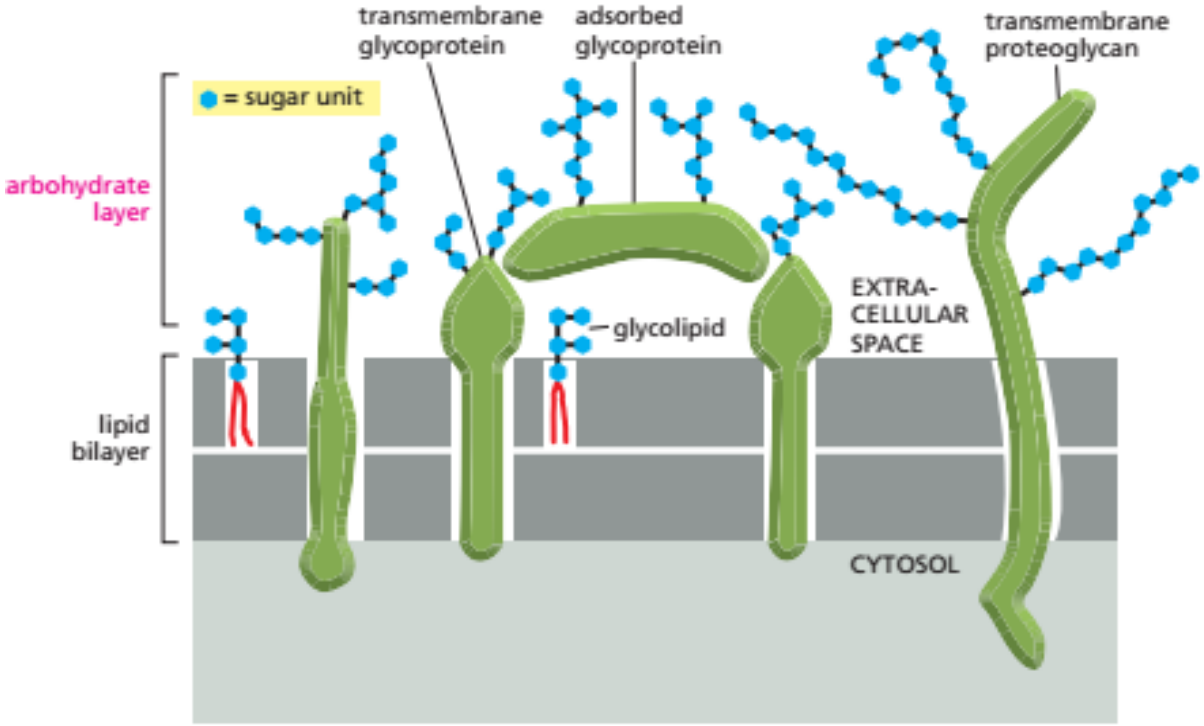


Figure 7: Membrane proteins can associate with the lipid bilayer in several different ways. (a) transmembrane proteins can extend across the bilayer as a single α helix, as multiple β helices, or as a rolled-up β sheet (called a β barrel). (B) Some membrane proteins are anchored to the cytosolic surface by an amphipathic α helix. (C) Others are attached to either side of the bilayer solely by a covalent attachment to a lipid molecule (zigzag lines). (D) Finally, many proteins are attached to the membrane only by relatively weak, noncovalent interactions with other membrane proteins.

Biomembrane Carbohydrates: Carbohydrates are present only in the plasma membrane. They are present as short, unbranched or branched chains of sugars (oligosaccharides) attached either to exterior ectoproteins (forming glycoproteins) or to the polar ends of phospholipids at the external surface of the plasma membrane (forming glycolipids). No carbohydrate is located at the cytoplasmic or inner surface of the plasma membrane.
All of the carbohydrate on the glycoproteins, proteoglycans, and glycolipids is located on one side of the membrane, the noncytosolic *i.e.* the extracytoplasmic side, where it forms a sugar coating called the carbohydrate layer (Figure 11–35). By forming a layer of material covering the lipid bilayer, the carbohydrate layer helps to protect the cell surface from mechanical and chemical damage. As the oligosaccharides and polysaccharides in the carbohydrate layer adsorb water, they give the cell a slimy surface. This coating helps motile cells such as white blood cells to squeeze through narrow spaces, and it prevents blood cells from sticking to one another or to the walls of blood vessels.



Architecture of Biological Membranes

The structure of the biological membranes in such a way that the protein molecules float about in form of globular molecules and they are dotted about here and there in a mosaic pattern within a fluid phospholipid bilayer is termed 'fluid mosaic' model, this model was suggested by S.J. Singer and G.L. Nicolson (1972). The fluid mosaic model is found to be applied to all biological membranes in general, and it is seen as a dynamic, ever-changing structure. And it can be experimentally proven as a result; it is widely accepted structural depiction of biological membranes. Other models suggested includes trilaminar model, Robertson (1960), Sandwich Model of Danielli and Davson, (1935) *etc.*

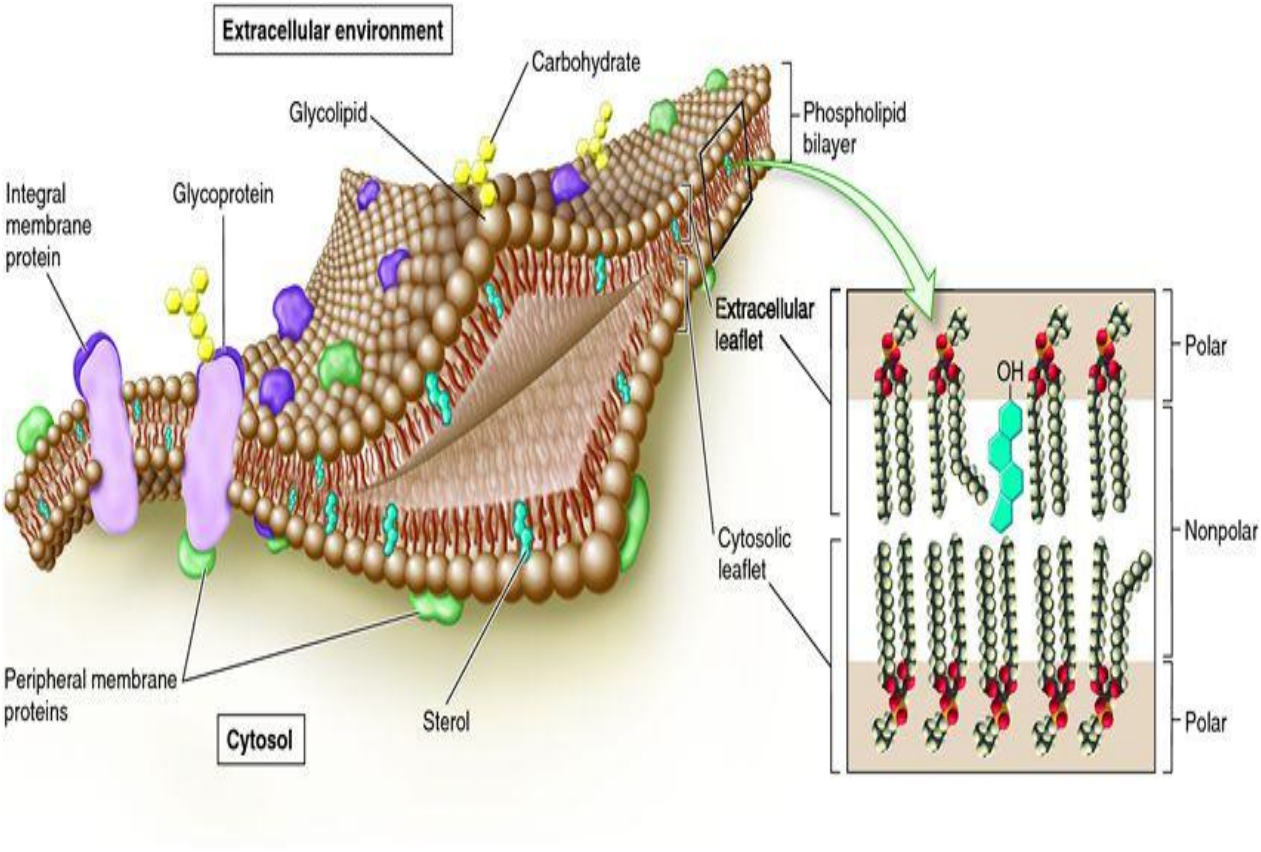


Figure 8: Schematic illustration of fluid membrane structure of biological membrane.

L2: TRANSPORT ACROSS PLASMA MEMBRANE

The plasma membrane acts as a semipermeable barrier between the cell and the extracellular environment. This permeability must be highly selective if it is to ensure that essential molecules such as glucose, amino acids and lipids can readily enter the cell, that these molecules and metabolic intermediates remain in the cell, and that waste compounds leave the cell. In short, the selective permeability of the plasma membrane allows the cell to maintain a constant internal environment (homeostasis).

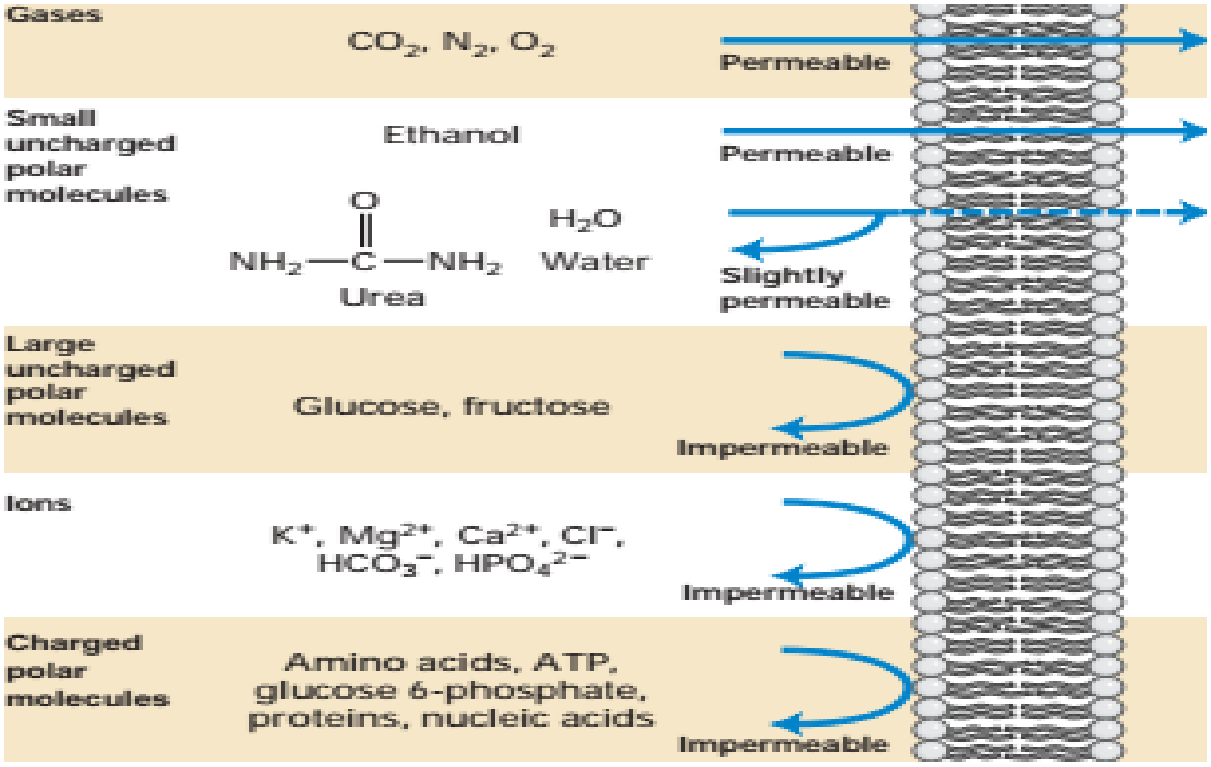
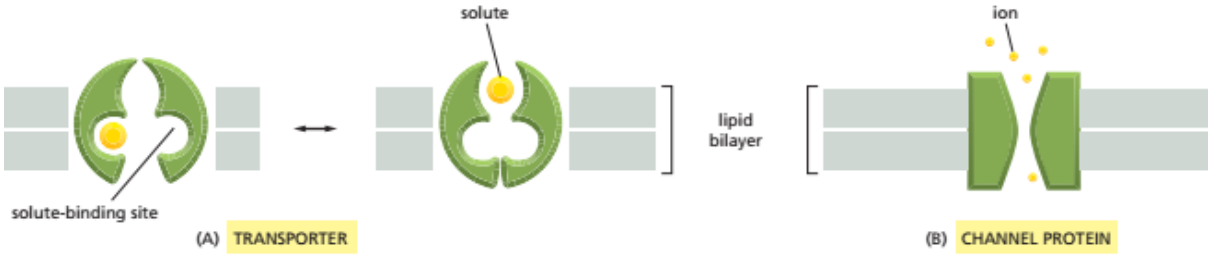
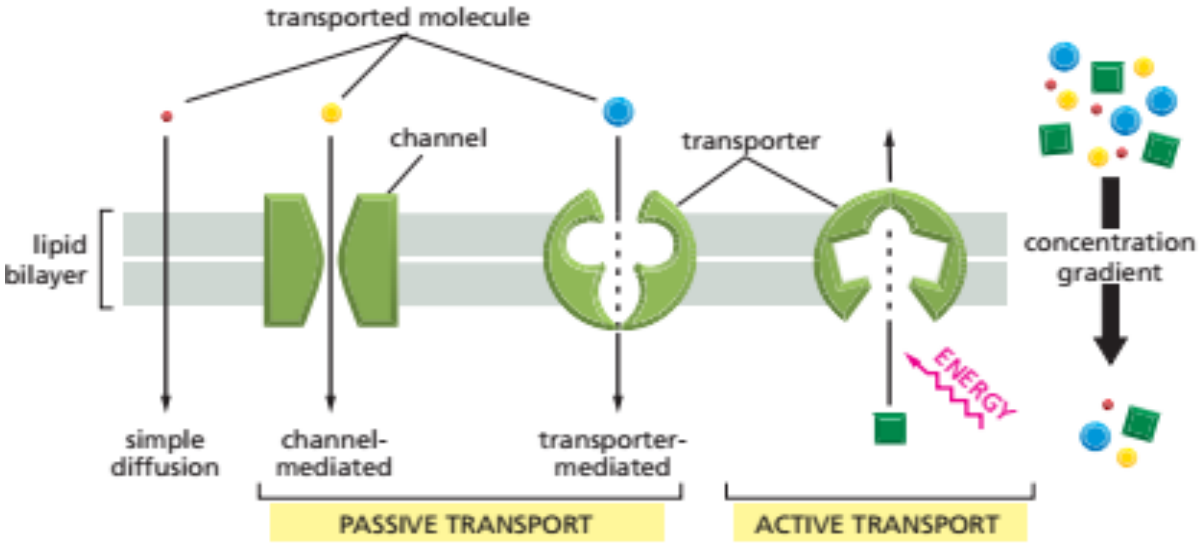


Figure 9: The rate at which a molecule diffuses across a synthetic lipid bilayer depends on its size and solubility. The smaller the molecule and, more importantly, the fewer its favourable interactions with water (that is, the less polar it is), the more rapidly the molecule diffuses across the bilayer. Note that many of the molecules that the cell uses as nutrients are too large and polar to pass through a pure lipid bilayer.

Mechanism: Transport across the membrane may be passive or active. It may occur via the phospholipid bilayer or by the help of specific integral membrane proteins, called permeases or membrane transport proteins (MTP). There are two main classes of membrane proteins that mediate this transfer. A *transporter*, which has moving parts, can shift small molecules from one side of the membrane to the other by changing its shape. Solutes transported in this way can be either small organic molecules or inorganic ions. *Channels*, in contrast, form tiny hydrophilic pores in the membrane through which solutes can pass by diffusion. Most channels let through inorganic ions only and are therefore called *ion channels*.





A. Passive transport it is a type of diffusion in which an ion or molecule crossing membrane moves down its electrochemical or concentration gradient. *No metabolic energy is consumed in passive transport.* Passive transport is of following three types:

1. Osmosis (Gr., *osmos* = pushing) this is a process by which the water molecules pass through a membrane from a region of higher water concentration to the region of lower water concentration. The process in which the water molecules enter into the cell is known as endosmosis, while the reverse process which involves the exit of the water molecules from the cell is known as exosmosis. In plant cells due to excessive exosmosis the cytoplasm along with the plasma membrane shrinks away from the cell wall. This process is known as plasmolysis (Gr., *plasma*=molded, *lysis*=loosing/splitting). The amount of the water inside the cell causes a pressure known as hydrostatic pressure. The hydrostatic pressure which is caused by the osmosis is known as osmotic pressure. The plasma membrane maintains a balance between the osmotic pressure of the intra-cellular and inter-cellular fluids.

2. Simple diffusion. In simple diffusion, transport across the membrane takes place unaided, *i.e.*, molecules of gases such as oxygen and carbon dioxide, nitrogen, benzene and small molecules (*e.g.* ethanol, glycerole) enter the cell by crossing the plasma membrane without the help of any permease. During simple diffusion, a small molecule in aqueous solution dissolves into the phospholipid bilayer, crosses it and then dissolves into the aqueous solution on the opposite side. There is little specificity to the process. The relative rate of diffusion of the molecule across the phospholipid bilayer will be proportional to the concentration gradient across the membrane.

3. Facilitated diffusion. This is a special type of passive transport, in which ions (H^+, Na^+, HCO^{3-}, K^+ Ca^{2+}, CI^-, Mg^{2+}) or molecules (amino acids, glucose, nucleosides, etc)cross the membrane rapidly because specific Membrane Transport Proteins (permeases) in the membrane facilitate their crossing. Membrane Transport Proteins Fall into Two Classes: Channels and Transporters. Channels are MTPs with hydrophilic pore across the bilayer through which specific inorganic ions or in some cases other small molecules can diffuse.

e.g. Ion Channels (K^+ channel, Na^+ channel - such ion channels occur in other types of cells such as muscle, sperm and unfertilized ovum Ca^{2+} channel- essential for release of neurotransmitters).Transporters are the MTPs that allow passage only to those molecules or ions that fit into a binding site on the protein; it then transfers these molecules across the membrane one at a time by changing its own conformation.

B. Active Transport this is movement of ion or molecule against its concentration gradient by the use of specific transporters, called pumps, which use metabolic energy (ATP). There are different types of pumps for the different types of ions or molecules such as calcium pump, proton pump, etc.

C. Bulk transport by the plasma membrane. Cells routinely import and export large molecules across the plasma membrane usually by forming a small membrane enclosed sacs aka *vesicles*. Macromolecules are secreted out from the cell by **exocytosis** and are ingested into the cell from outside through **phagocytosis** and **endocytosis.**

1. Exocytosis. It is also called **emeiocytosis** and **cell vomiting**. In all eukaryotic cells, **secretory vesicles** are continually carrying new plasma membrane and cellular secretions such as proteins, lipids and carbohydrates (*e.g.*, cellulose) from the Golgi apparatus to the plasma membrane or to cell exterior by the process of exocytosis. The proteins to be secreted are synthesized on the rough endoplasmic reticulum (RER). They pass into the lumen of the ER, glycosidated and are transported to the Golgi apparatus by ER-derived **transport vesicles**. In the Golgi apparatus the proteins are modified, concentrated, further glycosidated, sorted and finally packaged into vesicles that pinch off from trans Golgi tubules and migrate to plasma membrane to fuse with it and release the secretion to cell's exterior. In contrast, small molecules to be secreted (*e.g.*, histamine by the mast cells) are actively transported from the cytosol (where they are synthesized on the free ribosomes) into preformed vesicles, where they are complexed to specific macromolecules (*e.g.*, a network of proteoglycans, in case of histamine; **Lawson** *et al.*, 1975), so that, they can be stored at high concentration without generating an excessive osmotic gradient. During exocytosis the vesicle membrane is incorporated into the plasma membrane. The amount of secretory vesicle membrane that is temporarily added to the plasma membrane can be enormous: in a pancreatic acinar cell discharging digestive enzymes, about 900μm^2 of vesicle membrane is inserted into the apical plasma membrane (whose area is only 30 μm3) when the cell is stimulated to secrete.

2. Phagocytosis. Sometimes the large-sized solid food or foreign particles are taken in by the cell through the plasma membrane. The process of ingestion of large-sized solid substances (*e.g.*, bacteria and parts of broken cells) by the cell is known as **phagocytosis** (Gr., *phagein*=to eat, *kytos*=cell or holl vessel).

Occurrence of phagocytosis. The process of phagocytosis occurs in most protozoans and certain cells of multicellular organisms. In multicellular organisms such as mammals, the phagocytosis occurs very actively in granular leucocytes and in the cells of mesoblastic origin. The cells of the mesoblastic origin are collectively known as the cells of **macrophagic** or **reticuloendothelial system**. The cells of macrophagic system are histiocytes of the

connective tissue, the reticular cells of the hemopoietic organs (bone marrow, lymph nodes and spleen) and the endothelial cells which form the lining of capillary sinusoid of the liver, adrenal gland and hypophysis. The cells of macrophagic system can ingest bacteria, Protozoa, cell debris or even colloidal particles by the process of phagocytosis.

3. Endocytosis. In endocytosis, small regions of the plasma membrane fold inwards or **invaginate**, until it has formed new intracellular membrane limited vesicles. In eukaryotes, the following two types of endocytosis can occur: pinocytosis and receptor-mediated endocytosis.

(i) Pinocytosis. Pinocytosis (Gr., *pinein* = to drink; 'cell drinkng') is the non-specific uptake of small droplets of extracellular fluid by **endocytic vesicles** or **pinosomes**, having diameter of about 0.1 µm to 0.2 µm. Any material dissolved in the extracellular fluid is internalized in proportion to its concentration in the fluid. The process of pinocytosis was first of all observed by **Edward** in *Amoeba* and by **Lewis** (1931) in the cultured cells.

The light microscopy has shown that in *Amoeba* tiny **pinocytic channels** are continually being formed at the cell surface by invagination of the plasma membrane. From the inner end of each channel small vacuoles or pinosomes are pinched off, and these move towards the centre of the cell, where they fuse with primary lysosomes, to form **food vacuoles**. Ultimately, ingested contents are digested, small breakdown products such as sugars and amino acids diffuse to cytosol. When this happens within the cell this is called autophagy in this case it will forms autophagic vesicles. The pinocytosis which occurs at submicroscopic level is known as **micropinocytosis**.

(ii) Receptor-mediated endocytosis. In this type of endocytosis, a specific receptor on the surface of the plasma membrane "recognizes" an extracellular macromolecule and binds with it. The substance bound with the receptor is called the **ligand**. Examples of ligands may include viruses, small proteins (*e.g.*, insulin, immunoglobin *etc.*), vitamin B12, cholesterol containing LDL or low density lipoprotein, oligosaccharide, etc. The region of plasma membrane containing the receptor-ligand complex undergoes endocytosis.

SIGNAL TRANSDUCTION I

Cell either of a unicellular or multicellular organism need to sense and response to its environment e.g. to track nutrients, tell the difference between light and dark, and avoid poisons and predators. In cell biology, material that triggers a response of a cell to external conditions or the behaviour of other cells is known as a signal. It is through signals that cells communicate among themselves.

In a typical communication between cells, the *signalling cell* produces a particular type of *signalling molecule* (e.g. hormones, growth factors :proteins, peptides, amino acids, nucleotides, steroids, fatty acid derivatives, or even dissolved gases) that is detected by the *target cell*. As in human conversation, most animal cells both send and receive signals, and they can therefore act as both signalling cells and target cells. Target cells possess *receptor proteins* that recognize and respond specifically to the signal molecule. The process of converting the signal produced by activation of a cell surface receptor into intracellular biochemical signals and hence into an intracellular response is called *signal transduction*. **Signal transduction begins when the receptor protein on a target cell receives an incoming extracellular signal and converts it to the intracellular signals that alter cell behaviour.**

During animal development, for example, cells in the embryo exchange signals to determine which specialized role each cell will adopt, what position it will occupy in the animal, and whether it will survive, divide, or die. Later in life, a large variety of signals coordinate the animal's growth and its day-to-day physiology and behaviour. In plants as well, cells are in constant communication with one another. These cell–cell interactions allow a plant to respond to the conditions of light, dark, and temperature that guide the cycles of growth, flowering, and fruiting (remember your BIO 101: tropism, taxism and nastism,); they also allow the plant to coordinate what happens in its roots, stems, and leaves. In multicellular organisms, the most 'public' style of communication involves broadcasting the signal throughout the whole body by secreting it into the bloodstream (in an animal) or the sap (in a plant). Signal molecules used in this way are called *hormones*, and, in animals, the cells that produce hormones are called *endocrine* cells. Part of the pancreas, for example, is an endocrine gland that produces the hormone insulin, which regulates glucose uptake in cells all over the body. Somewhat less public is the process known as *paracrine signalling*. In this case, rather than entering the bloodstream, the signal molecules diffuse locally through the extracellular fluid, remaining in the neighbourhood of the cell that secretes them. Thus, they act as local mediators on nearby cells (Figure 16–3B). Many of the signal molecules that regulate inflammation at the site of an infection or that control cell proliferation in a healing wound function in this way. In some cases, cells can respond to the local mediators that they themselves produce, a form of paracrine communication called *autocrine signalling*; cancer cells sometimes promote their own survival or proliferation in this way.

Neuronal signalling is a third form of cell communication. Like endocrine cells, nerve cells (neurons) can deliver messages over long distances. In the case of neuronal signalling, however, a message is not broadcast widely but is instead delivered quickly and specifically

to individual target cells through private lines. As described in Chapter 12, the axon of a neuron terminates at specialized junctions (*synapses*) on target cells that can lie far from the neuronal cell body (Figure 10C). The axons that extend from the spinal cord to the big toe, for example, can be more than 1 m in length. When activated by signals from the environment or from other nerve cells, a neuron sends electrical impulses racing along its axon at speeds of up to 100 m/sec. On reaching the axon terminal, these electrical signals are converted into a chemical form: each electrical impulse stimulates the nerve terminal to release a pulse of an *extracellular signal molecule* called a neurotransmitter. The neurotransmitter then diffuses across the narrow (< 100 nm) gap between the axon-terminal membrane and the membrane of the target cell, reaching the target cell receptors in less than 1 msec.

A fourth style of signal-mediated cell–cell communication—the most intimate and short-range of all—does not require the release of a secreted molecule. Instead, the cells make direct physical contact through signal molecules lodged in the plasma membrane of the signaling cell and receptor proteins embedded in the plasma membrane of the target cell. During embryonic development, for example, such *contact-dependent signalling* allows adjacent cells that are initially similar to become specialized to form different cell types (see Figure 10D).

To relate these different signalling styles, imagine trying to advertise a potentially stimulating lecture—or a concert or football game. An endocrine signal would be akin to broadcasting the information over Kanem FM radio station. A localized paracrine signal would be the equivalent of posting a flyer on your selected notice boards. Neuronal signals—long-distance but personal—would be similar to your phone call or an e-mail, and contact dependent signalling would be like a good old-fashioned face-to-face conversation. In autocrine signalling, you might write a note to remind yourself to attend. Details on Signal transduction in nerve cells would be discussed under the subtopic Signal Transduction II.

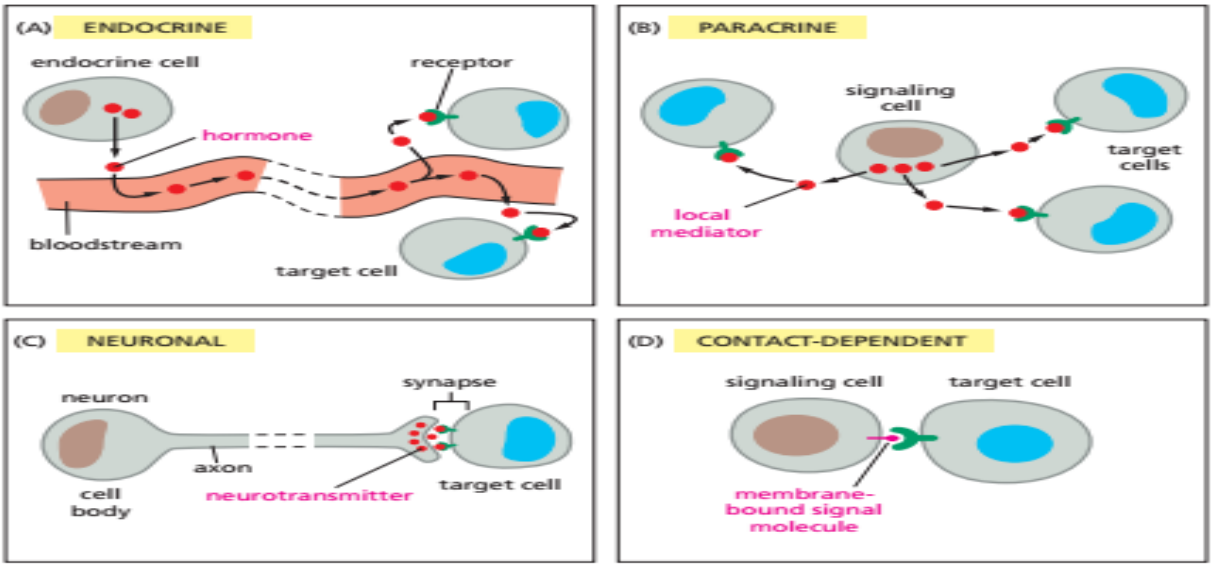


Figure 10: Animal cells can signal to one another in various ways.

L3: INTRACELLULAR COMPARTMENTS, PROTEIN BIOGENESIS AND SORTING

Generally, prokaryotic cells consist of only single compartment, the *cytosol*. In addition to cytosol, eukaryotic cells of plants and animals are elaborately subdivided by internal membranes each with distinct shape but varying number small and large with specialised function. These membranes create enclosed compartments (forming the membrane bound organelles) in which sets of enzymes can operate without interference from reactions occurring in other compartments (Table below). Many of the membrane-enclosed organelles, including the ER, Golgi apparatus, mitochondria, and chloroplasts, are held in their relative locations in the cell by attachment to the cytoskeleton, especially to microtubules.

The main function, % of total cell volume and number per cell of the membrane bound compartments of the eukaryotic cells

Compartment	**Main Function**	**Volume (%)**	**Number/Cell**
Cytosol	contains many metabolic pathways; protein synthesis	54	1
Nucleus	contains main genome; DNA and RNA synthesis	6	1
Endoplasmic reticulum (ER)	synthesis of most lipids for new membrane; synthesis of proteins for distribution to many organelles and to the plasma membrane	12	1
Lysosome	Contains digestive enzymes for intracellular degradation	1	300
Golgi apparatus	Modification, sorting, and packaging of proteins and lipids for either secretion or delivery to another organelle (this chapter)	3	1
Endosomes	sorting of endocytosed material	1	200
Mitochondria	ATP synthesis by oxidative phosphorylation	22	1700
Chloroplasts (in plant cells)	ATP synthesis and carbon fixation by photosynthesis	Na	Na
Peroxisomes	oxidation of toxic molecules	1	400

Origin: Membrane-enclosed organelles are thought to have arisen in evolution in at least two ways. The nuclear membranes and the membranes of the ER, Golgi apparatus, endosomes, and lysosomes are believed to have originated by invagination of the plasma membrane (Figure 11). These membranes, and the organelles they enclose, are all part of what is collectively called the *endomembrane system*. The interiors of these organelles (with the exception of the nucleus) communicate extensively with one another and with the outside of the cell by means of small *vesicles* that bud off from one of these organelles and fuse with another.

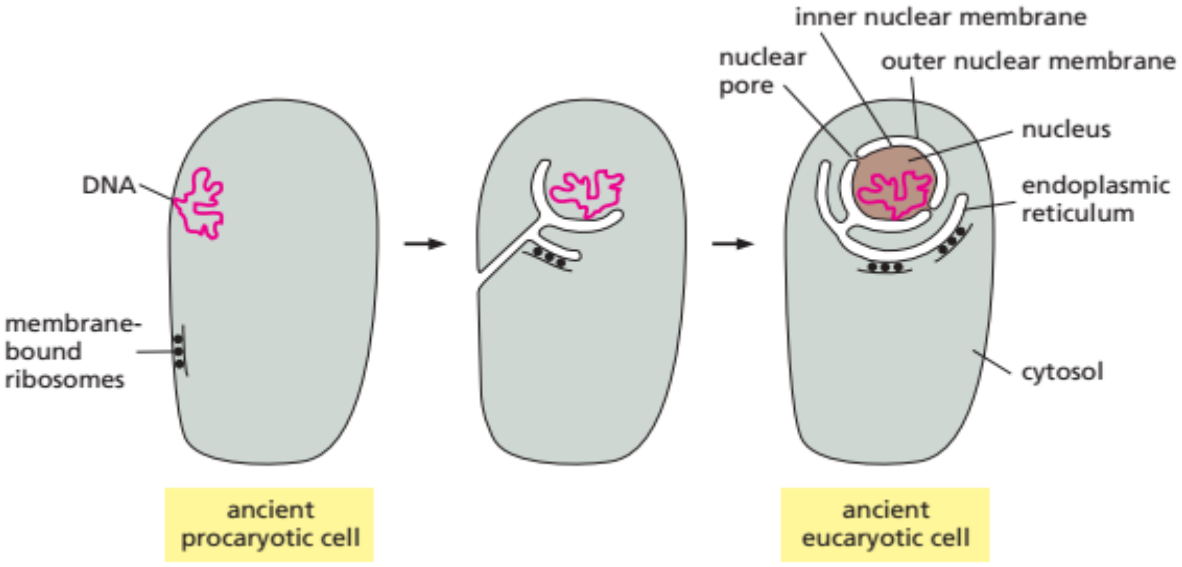


Figure 11: Nuclear membranes and the ER may have evolved through invagination of the plasma membrane.

Mitochondria and chloroplasts are thought to have originated in a different way. They differ from all other organelles in that they possess their own small genomes and can make some of their own proteins. The similarity of these genomes to those of bacteria and the close resemblance of some of their proteins to bacterial proteins strongly suggest that mitochondria and chloroplasts evolved from bacteria that were engulfed by primitive eukaryotic cells with which they initially lived in symbiosis (Figure 12). As might be expected from their origins, mitochondria and chloroplasts remain isolated from the extensive vesicular traffic that connects the interiors of most of the other membrane-enclosed organelles to one another and to the outside of the cell.

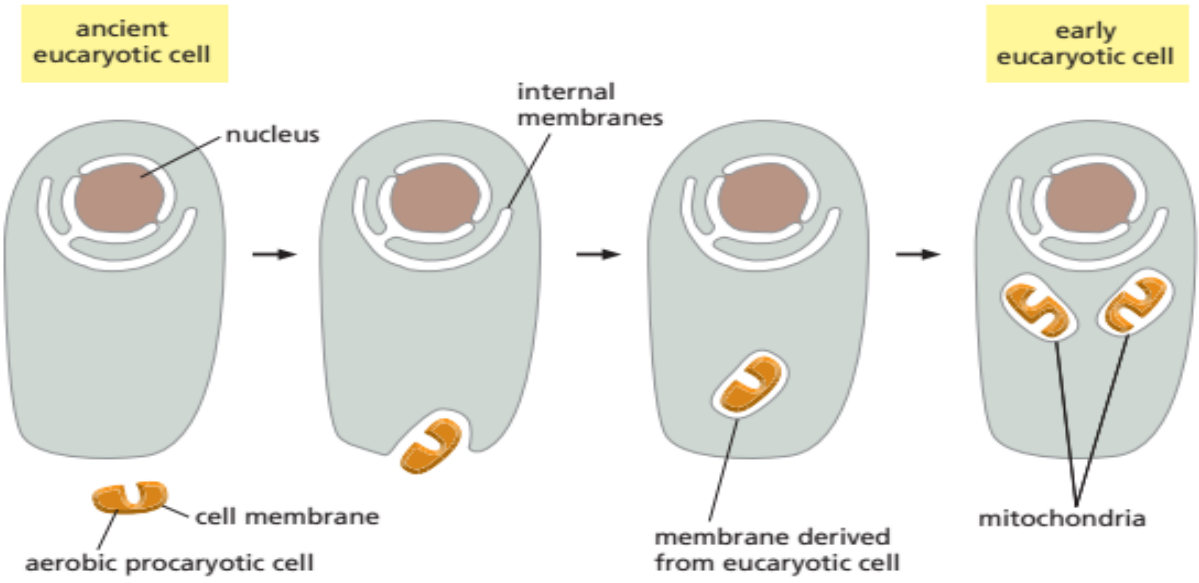


Figure 12: Mitochondria are thought to have originated when a prokaryote was engulfed by a larger eukaryotic cell.

Protein Sorting: Each compartment contains a unique set of proteins that have to be transferred selectively from the cytosol, where they are made, to the compartment in which they are used. This transfer process, called *protein sorting*, depends on signals built into the amino acid sequence of the proteins. For some organelles, including the mitochondria, chloroplasts, and the interior of the nucleus, proteins are delivered directly from the cytosol. For others, including the Golgi apparatus, lysosomes, endosomes, and the nuclear membranes, proteins and lipids are delivered indirectly via the ER, which is itself a major site of lipid and protein synthesis. Proteins enter the ER directly from the cytosol: some are retained there, but most are transported by vesicles to the Golgi apparatus and then onward to other organelles or the plasma membrane. **Proteins made in the cytosol are dispatched to different locations in the cell according to specific address labels that they contain in their *amino acid sequence***. These specific address labels are termed *sorting signals*. Once at the correct address, the protein enters the organelle.

The above task is accomplished in three (3) different ways for different organelles:

1. Proteins moving from the cytosol into the nucleus are transported through the nuclear pores that penetrate the inner and outer nuclear membranes. The pores function as selective gates that actively transport specific macromolecules but also allow free diffusion of smaller molecules (mechanism 1 in Figure 13)
2. Proteins moving from the cytosol into the ER, mitochondria, or chloroplasts are transported across the organelle membrane by *protein translocators* located in the membrane. Unlike transport through nuclear pores, the transported protein molecule must usually unfold in order to snake through the membrane (mechanism 2 in Figure 13). Bacteria have similar protein translocators in their plasma membrane, which they use to export proteins from their cytosol.
3. Proteins moving from the ER onward and from one compartment of the endomembrane system to another are transported by a mechanism that is fundamentally different from the other two. These proteins are ferried by *transport vesicles*, which become loaded with a cargo of proteins from the interior space, or *lumen*, of one compartment, as they pinch off from its membrane. The vesicles subsequently discharge their cargo into a second compartment by fusing with its membrane (mechanism 3 in Figure 13). In the process, membrane lipids and membrane proteins are also delivered from the first compartment to the second.

All of these processes require energy. The protein remains folded during the transport steps in mechanisms 1 and 3 but usually has to be unfolded in mechanism 2.

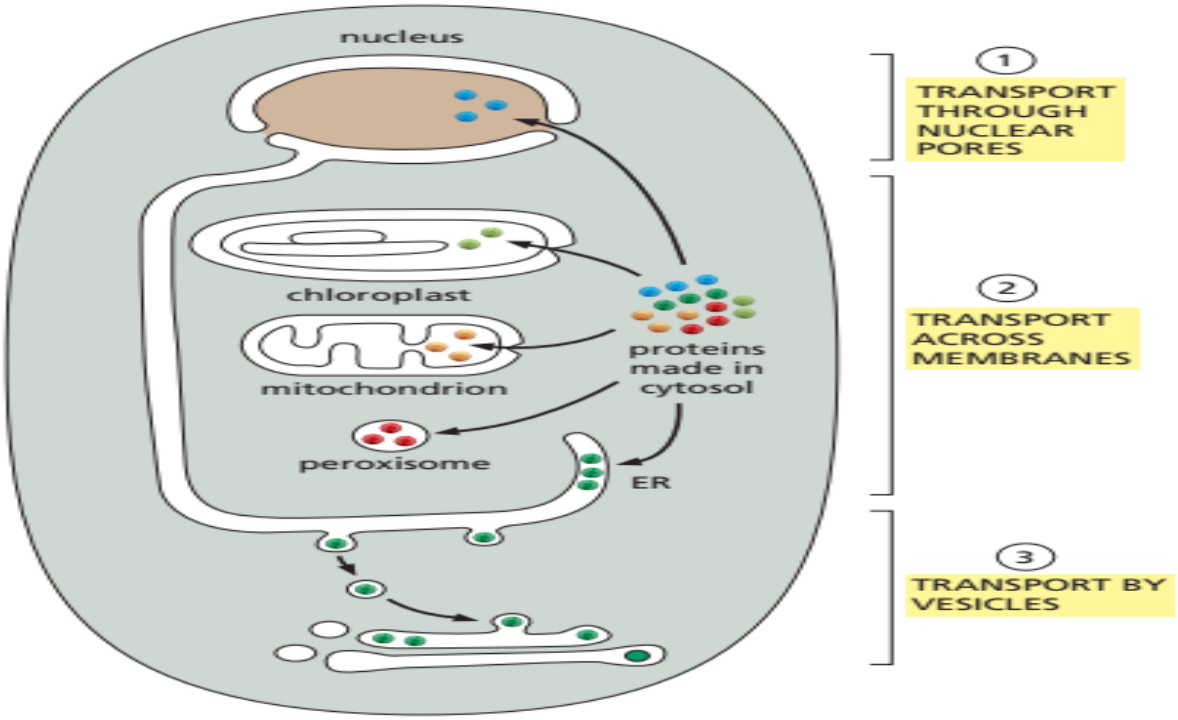


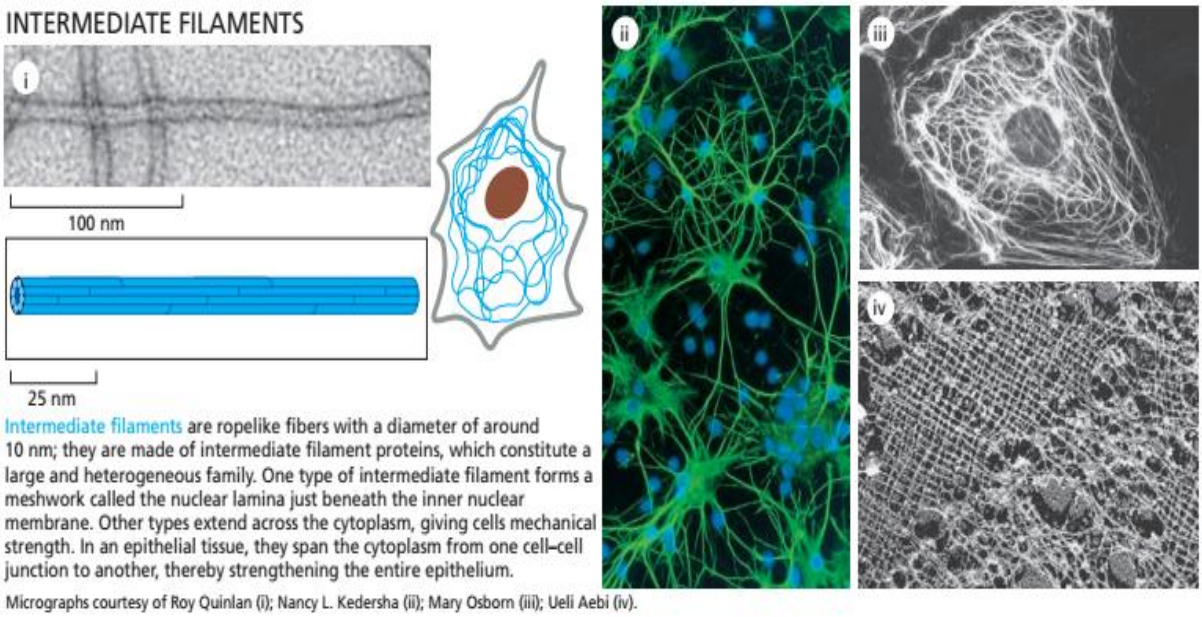
Figure 13: Membrane-enclosed organelles import proteins by one of three mechanisms.

L4: CYTOSKELETAL SYSTEM AND REGULATION OF CELL DIVISION

Cytoskeletal System

Membrane-enclosed organelles are distributed throughout the cytoplasm (The cytoplasm is the structureless soup of chemicals and organelles). The rest of the cell, excluding all the membrane-enclosed organelles, is called the **cytosol**. It contains a host of small and large molecules, crowded together so closely that it behaves more like a water-based gel than a liquid solution in which many chemical reactions that are fundamental to the cell's existence take place *e.g*. early breakdown of nutrient molecule, manufacture of protein, *etc*. In addition to other molecules, the cytosol of eukaryotic cells is criss-crossed by long, fine filaments of protein that are seen to be anchored at one end to the plasma membrane or to radiate out from a central site adjacent to the nucleus. This system of filaments is called the **cytoskeleton**. The cytoskeleton is built on a framework of three types of protein filaments: *intermediate filaments*, *microtubules*, and *actin filaments* (figure 14). These three types of filaments, together with other proteins that attach to them, form a system of girders, ropes, and motors that gives the cell its mechanical strength, controls its shape, and drives and guides its movements. Without the cytoskeleton, wounds would never heal, muscles would be useless, and sperm would never reach the egg.

*Intermediate in thickness between actin filaments and microtubules are the *intermediate filaments*, which serve to strengthen the cell mechanically. Intermediate filaments are the toughest and most durable of the three types of cytoskeletal filaments.



Intermediate filaments are ropelike fibers with a diameter of around 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength. In an epithelial tissue, they span the cytoplasm from one cell–cell junction to another, thereby strengthening the entire epithelium.

Micrographs courtesy of Roy Quinlan (i); Nancy L. Kedersha (ii); Mary Osborn (iii); Ueli Aebi (iv).

Intermediate filaments can be grouped into four classes:

(1) *keratin filaments* in epithelial cells of tongue, the cornea, or the lining of the gut, hair, feathers, and claws. Mutation during formation of keratin filament lead to *epidermolysis bullosa simplex* in which the sufferer's skin is highly vulnerable to

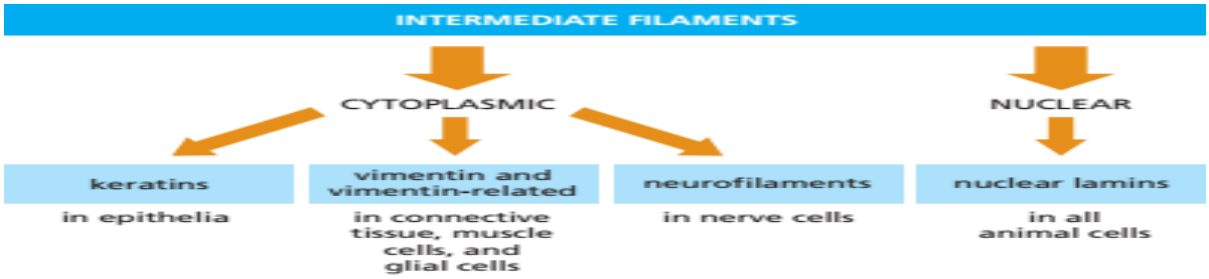
mechanical injury, and even a gentle pressure can rupture its cells, causing the skin to blister.

(2) *vimentin* and *vimentin-related filaments* in connective-tissue cells, muscle cells, and supporting cells of the nervous system (glial cells); disruption cause

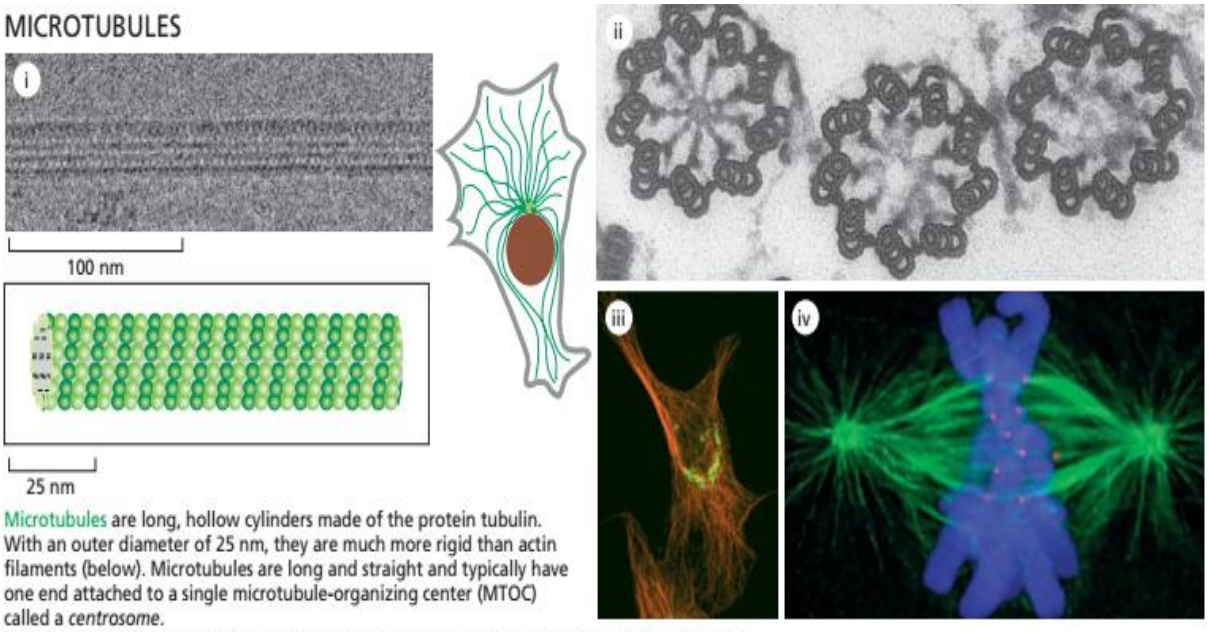
(3) *neurofilaments* in nerve cells their disruption cause neurodegeneration

(4) *nuclear lamins*, which strengthen the nuclear membrane of all animal cells. Defects in a particular nuclear lamins are associated with certain types of *progeria*—rare disorders that cause affected individuals to appear to age prematurely.

The first three intermediate filament types are found in the cytoplasm the fourth in the cell nucleus (See the chart below).



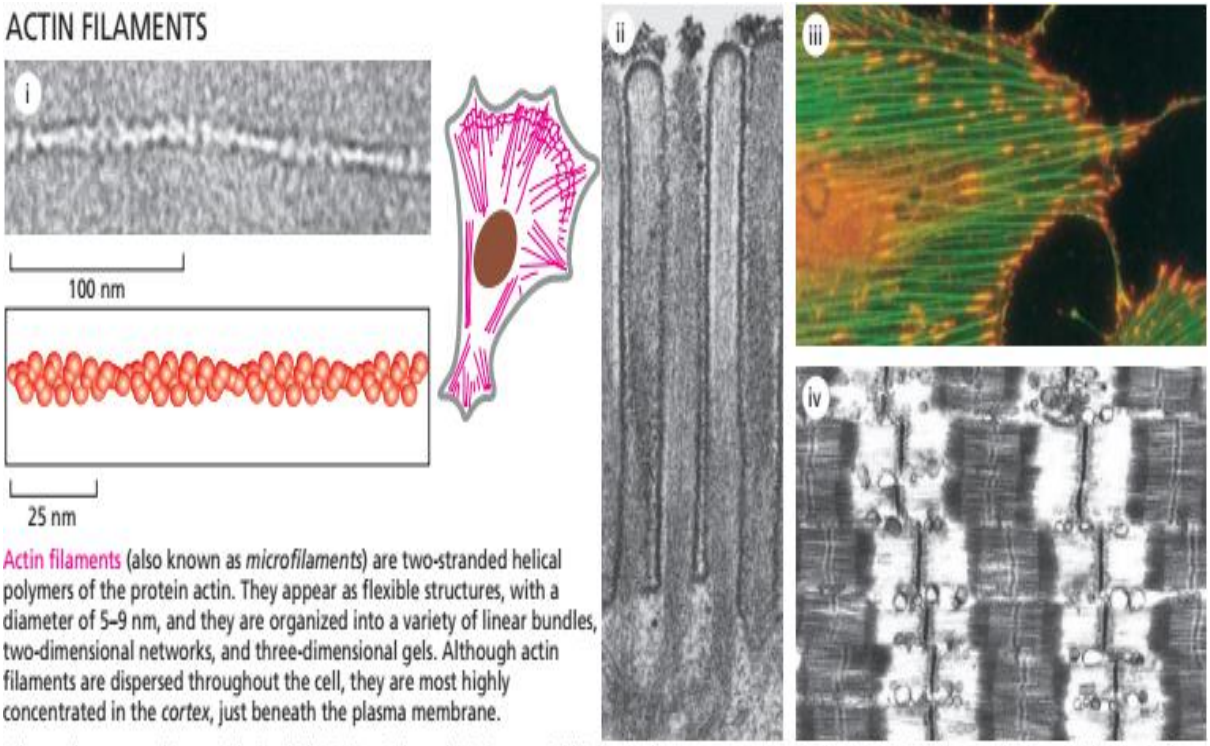
*The thickest filaments are called *microtubules*, because they have the form of minute hollow tubes. In dividing cells they become reorganized into a spectacular array that helps pull the duplicated chromosomes in opposite directions and distribute them equally to the two daughter cells. They are the most important components of cilia l(some protozoa, cells of respiratory tract, oviduct) and flagella (many protozoa and sperm). They are mainly responsible for anchoring membrane-enclosed organelles within the cell and for guiding intracellular transport with the aid of motor protein. In animal cells, microtubules are mostly organised at a region close to the nucleus known as centrosome (a.k.a Microtubule Organizing Centre, MTOC) within which is the centriole. Centriole is absent in plant cells.



Microtubules are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments (below). Microtubules are long and straight and typically have one end attached to a single microtubule-organizing center (MTOC) called a *centrosome*.

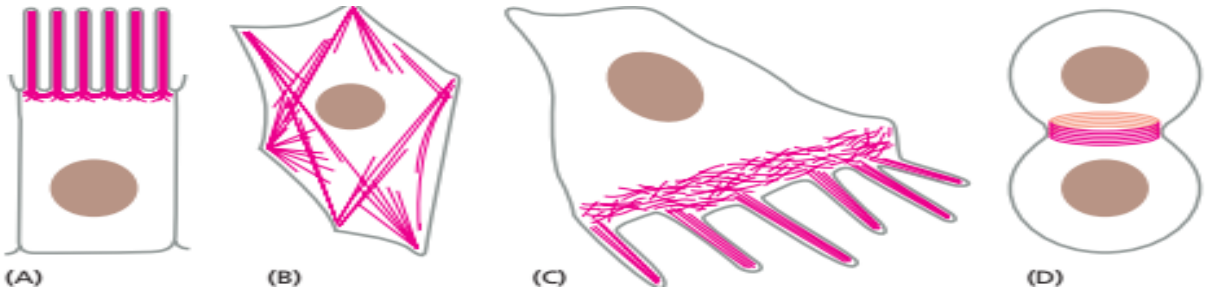
Micrographs courtesy of Richard Wade (i); D.T. Woodrum and R.W. Linck (ii); David Shima (iii); Arshad Desai (iv).

*The thinnest of the filaments are *actin filaments*, which are present in all eukaryotic cells but occur in especially large numbers inside muscle cells, where they serve as part of the machinery that generates contractile forces.



Micrographs courtesy of Roger Craig (i and iv); P.T. Matsudaira and D.R. Burgess, *Cold Spring Harb. Symp. Quant. Biol.* 46:845–854, 1982. With permission from Cold Spring Harbor Laboratory Press (ii); Keith Burridge (iii).

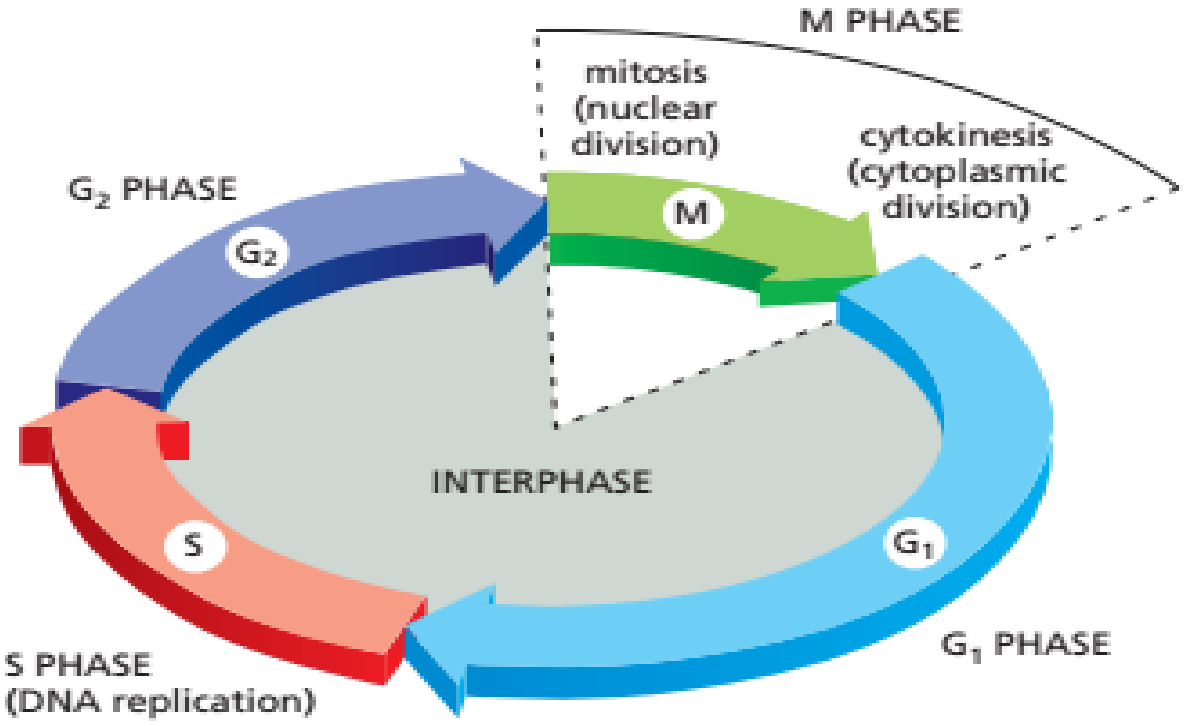
Depending on their association with different proteins, actin filaments can form stiff and relatively permanent structures, such as the *microvilli* on the brush-border cells lining the intestine (Figure 17–28a) or small *contractile bundles* in the cytoplasm that can contract and act like the "muscles" of a cell (Figure 17–28B); they can also form temporary structures, such as the dynamic protrusions formed at the leading edge of a crawling fibroblast (Figure 17–28C) or the *contractile ring* that pinches the cytoplasm in two when an animal cell divides (Figure 17–28D).



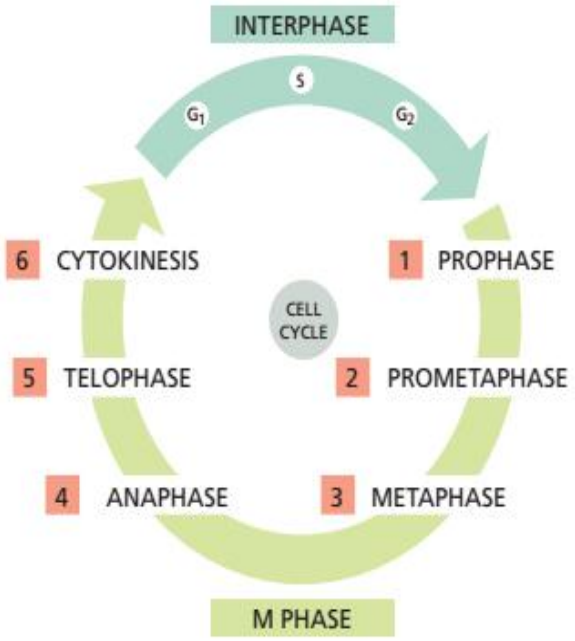
Actin filaments allow eukaryotic cells to adopt a variety of shapes and perform a variety of functions. The figure below illustrates various actin-containing structures are shown in *red:* (A) microvilli; (B) contractile bundles in the cytoplasm; (C) sheetlike *(lamellipodia)* and fingerlike *(flopodia)* protrusions from the leading edge of a moving cell; (D) contractile ring during cell division.

Cell Division Cycle

All living organisms, from a unicellular bacterium to a multicellular mammal, are thought to be products of repeated rounds of cell growth and division extending back in time to the beginnings of life. A cell reproduces by carrying out an orderly sequence of events in which it duplicates its contents and then divides in two. This cycle of duplication and division, known as the *cell cycle*, is the essential mechanism by which all living things reproduce. To produce two genetically identical daughter cells, the DNA in each chromosome must be faithfully replicated, and the replicated chromosomes must then be accurately distributed, or *segregated*, into the two daughter cells, so that each cell receives a complete copy of the entire genome (figure 18–1). Most cells also duplicate their other macromolecules and organelles, and they double in size before they divide; otherwise, each time they divided they would get smaller and smaller. Thus, to maintain their size, dividing cells must coordinate their growth with their division.

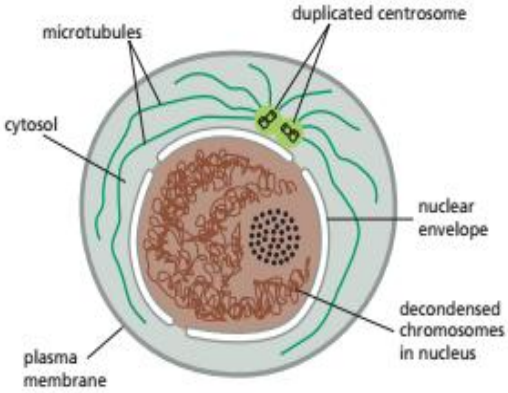


CELL DIVISION AND THE CELL CYCLE



The division of a cell into two daughters occurs in the M phase of the cell cycle. M phase consists of nuclear division, or mitosis, and cytoplasmic division, or cytokinesis. In this figure, M phase has been expanded for clarity. Mitosis is itself divided into five stages, and these, together with cytokinesis, are described in this panel.

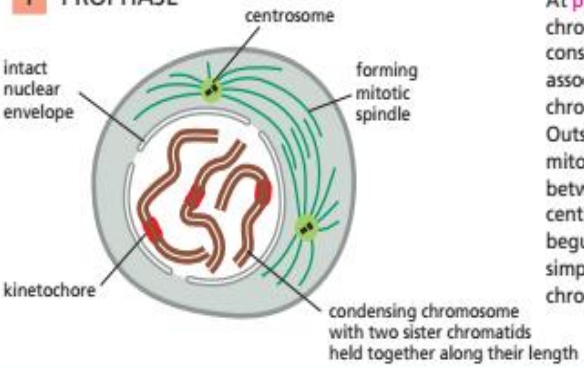
INTERPHASE



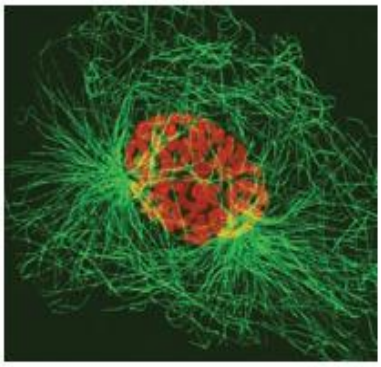
During interphase, the cell increases in size. The DNA of the chromosomes is replicated, and the centrosome is duplicated.

In the light micrographs of dividing animal cells shown in this panel, chromosomes are stained *orange* and microtubules are *green*. (Micrographs courtesy of Julie Canman and Ted Salmon; "Metaphase" from cover of *J. Cell. Sci.* 115(9), 2002, with permission from The Company of Biologists Ltd; "Telophase" from J.C. Canman et al., *Nature* 424:1074–1078, 2003, with permission from Macmillan Publishers Ltd.)

1 PROPHASE



At prophase, the replicated chromosomes, each consisting of two closely associated sister chromatids, condense. Outside the nucleus, the mitotic spindle assembles between the two centrosomes, which have begun to move apart. For simplicity, only three chromosomes are drawn.



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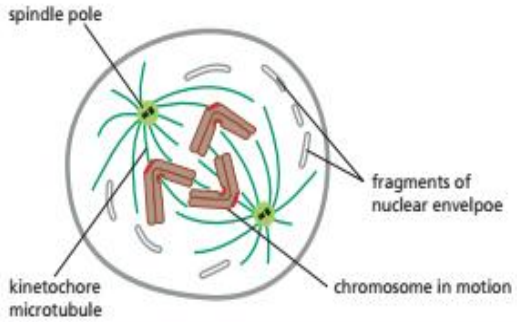
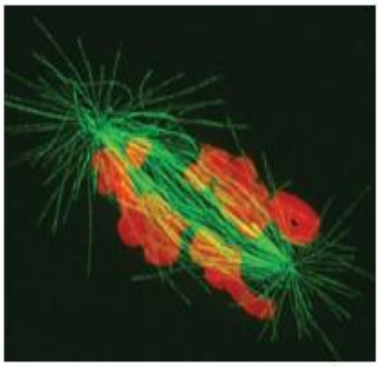
2 PROMETAPHASE

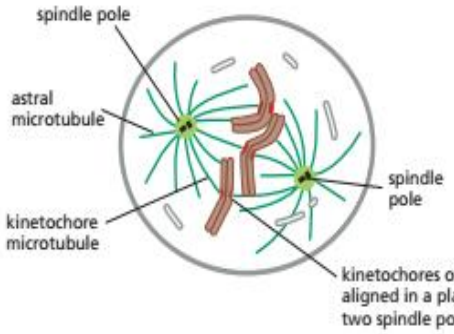


Prometaphase starts abruptly with the breakdown of the nuclear envelope. Chromosomes can now attach to spindle microtubules via their kinetochores and undergo active movement.

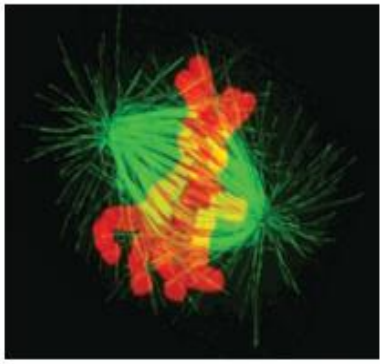


time = 79 min

3 METAPHASE

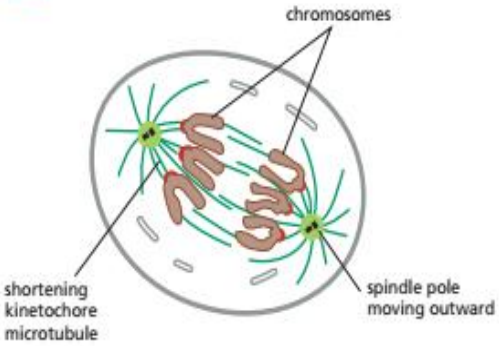


At metaphase, the chromosomes are aligned at the equator of the spindle, midway between the spindle poles. The paired kinetochore microtubules on each chromosome attach to opposite poles of the spindle.

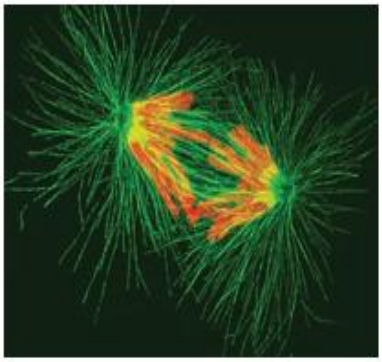


time = 250 min

4 ANAPHASE

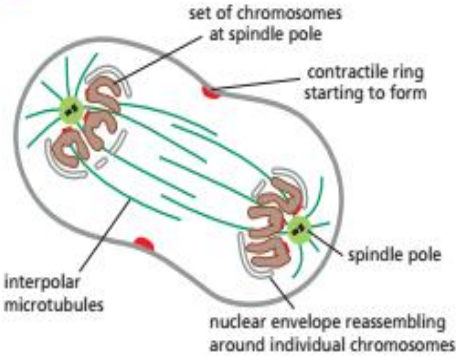


At anaphase, the sister chromatids synchronously separate, and each is pulled slowly toward the spindle pole it is attached to. The kinetochore microtubules get shorter, and the spindle poles also move apart, both contributing to chromosome segregation.

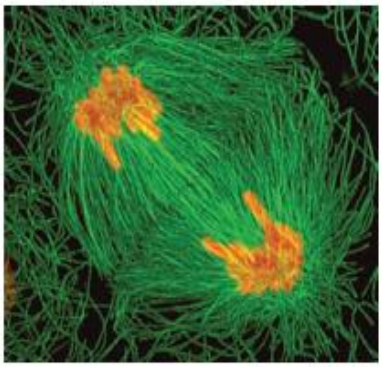


time = 279 min

5 TELOPHASE

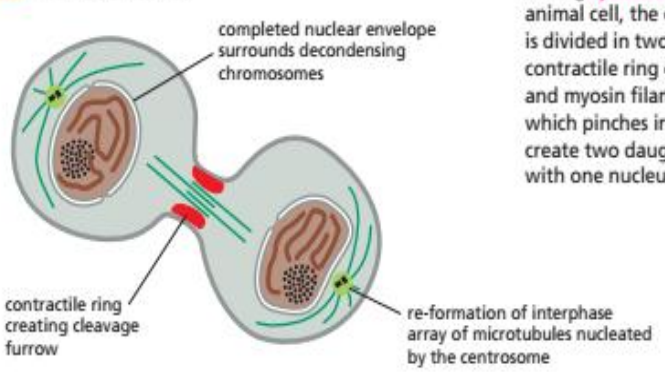


During telophase, the two sets of chromosomes arrive at the poles of the spindle. A new nuclear envelope reassembles around each set, completing the formation of two nuclei and marking the end of mitosis. The division of the cytoplasm begins with the assembly of the contractile ring.

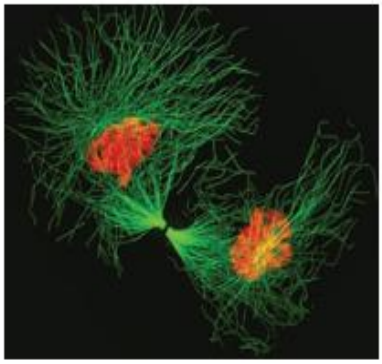


time = 315 min

6 CYTOKINESIS



During cytokinesis of an animal cell, the cytoplasm is divided in two by a contractile ring of actin and myosin filaments, which pinches in the cell to create two daughters, each with one nucleus.



time = 362 min

Phases of Eukaryotic Cell Cycle

The eukaryotic cell cycle is divided into four phases as seen under a microscope, the two most dramatic events in the cycle are when the nucleus divides, a process called *mitosis,* and when the cell later splits in two, a process called *cytokinesis.* These two processes together constitute the M phase of the cell cycle. In a typical mammalian cell, the whole of M phase takes about an hour, which is only a small fraction of the total cell-cycle time. The period between one M phase and the next is called interphase. Under the microscope, it appears, deceptively, as an uneventful interlude during which the cell simply increases in size. Interphase, however, is a very busy time for the cell, and it encompasses the remaining three phases of the cell cycle. During S phase (S = synthesis), the cell replicates its nuclear DNA, an essential prerequisite for cell division. S phase is flanked by two phases in which the cell continues to grow. The G1 phase (G = gap) is the interval between the completion of M phase and the beginning of S phase. The G2 phase is the interval between the end of S phase and the beginning of M phase (figure 18–2). During these gap phases, the cell monitors the internal and external environments to ensure that conditions are suitable and its preparations are complete before it commits itself to the major upheavals of S phase and mitosis. At particular points in G1 and G2, the cell decides whether to proceed to the next phase or pause to allow more time to prepare.

During all of interphase, a cell generally continues to transcribe genes, synthesize proteins, and grow in mass. Together, G1 and G2 phases provide additional time for the cell to grow and duplicate its cytoplasmic organelles: if interphase lasted only long enough for DNA replication, the cell would not have time to double its mass before it divided and would consequently shrink with each division. Indeed, in some special circumstances that is just what happens. In some animal embryos, for example, the first cell divisions after fertilization (called *cleavage divisions)* serve to subdivide a giant egg cell into many smaller cells as quickly as possible.

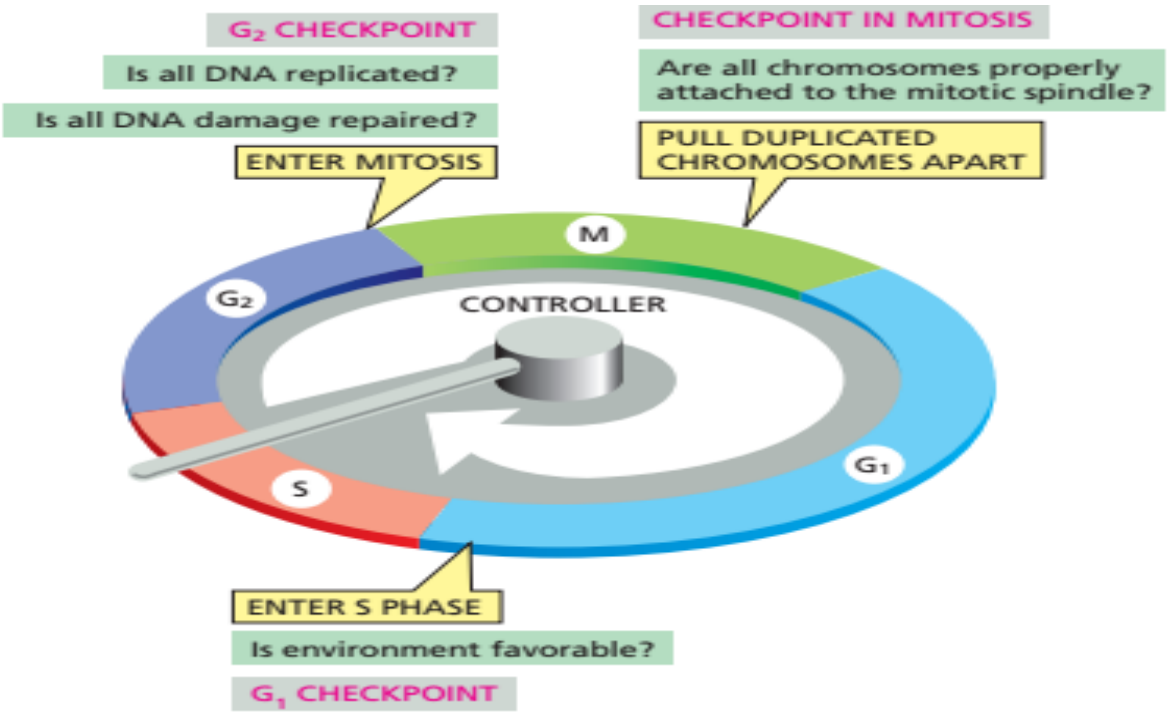
In these embryonic cell cycles, the G1 and G2 phases are drastically shortened, and the cells do not grow before they divide. Following DNA replication in S phase, the two copies of each chromosome remain tightly bound together. The first visible sign that a cell is about to enter M phase is the progressive *condensation* of its chromosomes. As condensation proceeds, the replicated chromosomes first become visible in the light microscope as long threads, which gradually get shorter and thicker. This condensation makes the chromosomes less likely to get entangled, so that they are easier to segregate to the two forming daughter cells during mitosis.

Cell Cycle Control System

To ensure that they replicate all their DNA and organelles, and divide in an orderly manner, eukaryotic cells possess a complex network of regulatory proteins known as the *cell-cycle control system*. The cell-cycle control system achieves all of this by means of molecular brakes that can stop the cycle at various *checkpoints*.

Three checkpoints that control progression through the cell cycle are illustrated in figure 18–3. One checkpoint operates in G1 and allows the cell to confirm that the environment is favourable for cell proliferation before committing to S phase. Cell proliferation in animals requires both sufficient nutrients and specific signal molecules in the extracellular environment; if extracellular conditions are unfavourable, cells can delay progress through G1 and may even enter a specialized resting state known as *G0* (G zero). Many cells, including nerve cells and skeletal muscle cells, remain in G0 for the lifetime of the organism. Another checkpoint operates in G2 and ensures that cells do not enter mitosis until damaged DNA has been repaired and DNA replication is complete. A third checkpoint operates during mitosis and ensures that the replicated chromosomes are properly attached to a cytoskeletal machine, called the *mitotic spindle*, before the spindle pulls the chromosomes apart and distributes them into the two daughter cells.

The checkpoint in G1 is especially important as a point in the cell cycle where the control system can be regulated by signals from other cells. In a multicellular animal, the control system is highly responsive to signals from other cells that stimulate cell division when more cells are needed and block it when they are not. The control system therefore plays a central part in the regulation of cell numbers in the tissues of the body; if the system malfunctions such that cell division is excessive, cancer can result. We will see later how extracellular signals influence the decisions made at this checkpoint.



Extracellular Signals Most of the extracellular signal molecules that influence cell survival, cell growth, and cell division are either soluble proteins secreted by other cells or proteins bound to the surface of other cells or the extracellular matrix. Although most act positively to stimulate one or more of these cell processes, some act negatively to inhibit a particular process. The positively acting signal proteins can be classified, on the basis of their function, into three major categories:

1. *Survival factors* promote cell survival, largely by suppressing apoptosis.
2. *Mitogens* stimulate cell division, primarily by overcoming the intracellular braking mechanisms that tend to block progression through the cell cycle.
3. *Growth factors* stimulate cell growth (an increase in cell size and mass) by promoting the synthesis and inhibiting the degradation of proteins and other macromolecules.

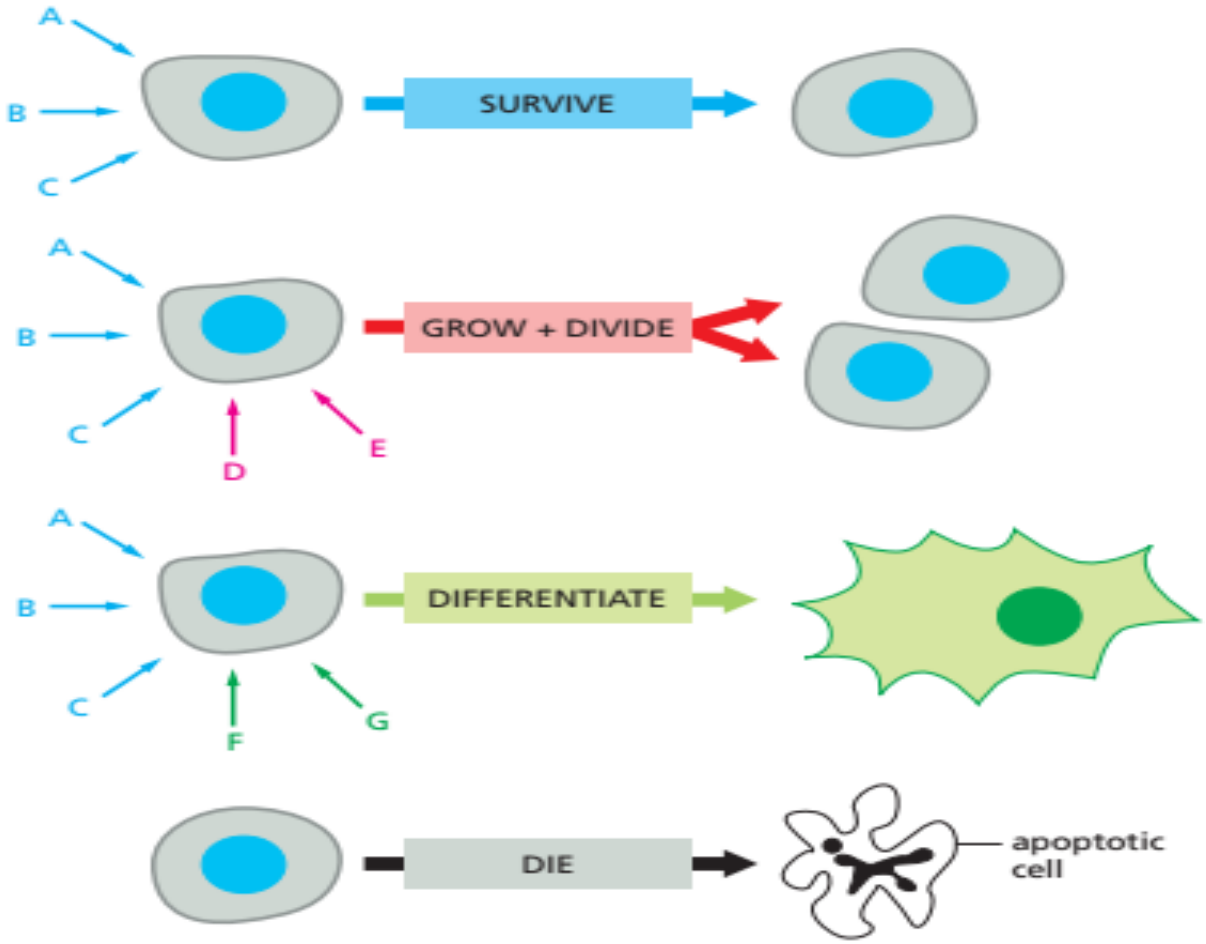


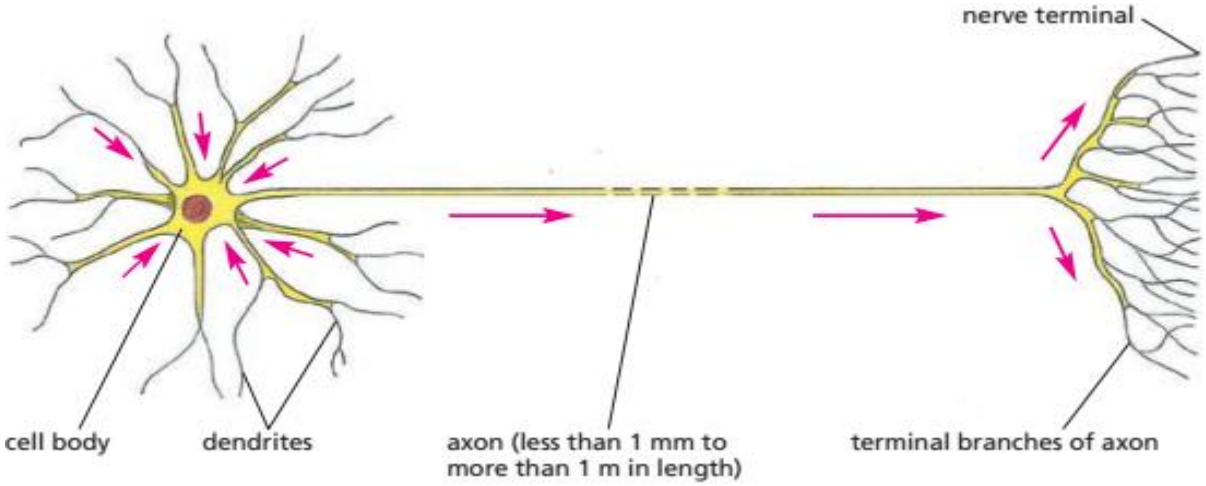
Figure: An animal cell depends on multiple extracellular signals. Every cell type displays a set of receptor proteins that enables it to respond to a specific set of extracellular signal molecules produced by other cells. These signal molecules work in combinations to regulate the behaviour of the cell. As shown here, cells may require multiple signals (*blue* arrows) to survive, additional signals (*red* arrows) to grow and divide, and still other signals (*green* arrows) to differentiate. If deprived of survival signals, most cells undergo a form of cell suicide known as apoptosis.

L5: SIGNAL TRANSDUCTION II: NERVE CELLS, ION CHANNELS, SYNAPSE, Ca^{2+} REGULATED EVENTS

We have examined how different transport proteins work together to absorb nutrients across the intestinal epithelium (see the section on facilitated diffusion) and how different types of extracellular signals trigger intracellular activities (see the section on signal transduction I) The nervous system, however, provides the most striking example of the interplay of various

ion channels, transporters, and ion pumps in carrying out physiological function. The fundamental task of a nerve cell, or neuron, is to **receive**, **conduct**, and **transmit signals**. Neurons carry signals inward from sense organs to the central nervous system—the brain and spinal cord. From the central nervous system, neurons extend processes outward to convey signals for action to muscles and glands. **Neurons** (nerve cells) and certain muscle cells are specialized to generate and conduct a particular type of electric impulse, the **action potential.** This alteration of the electric potential across the cell membrane is caused by the opening and closing of certain voltage-gated ion channels.

All the multicellular organisms perform these functions, neurons are often extremely elongated: the motor neurons in a human that carry signals from the spinal cord to a muscle in the foot, for example, may be a meter long. Although the morphology of various types of neurons differs in some respects, they all contain four distinct regions with differing functions: the cell body, the axon, the axon terminals, and the dendrites (Figure 7- 29). The *cell body* (containing the nucleus) has a number of long, thin extensions radiating outward from it. Usually, a neuron has one long *axon*, which conducts signals away from the cell body toward distant target cells; it also usually has several shorter, branching *dendrites*, which extend from the cell body like antennae and provide an enlarged surface area to receive signals from the axons of other neurons (Figure 12–31). The axon commonly divides at its far end into many branches, each of which ends in a *nerve terminal*, so that the neuron's message can be passed simultaneously to many target cells—either other neurons or muscle or gland cells. Likewise, the branching of the dendrites can be extensive, in some cases sufficient to receive as many as 100,000 inputs on a single neuron. No matter what the meaning of the signal a neuron carries—whether it is visual information from the eye, a motor command to a muscle, or one step in a complex network of neural processing in the brain—the form of the signal is always the same: it consists of *changes in the electrical potential across the neuron's plasma membrane.*



A typical neuron has a cell body, a single axon, and multiple dendrites. The axon conducts signals away from the cell body toward its target cells, while the multiple dendrites receive signals from the axons of other neurons. The *red* arrows indicate the direction in which signals are conveyed.

An action potential originates at the *axon hillock*, the junction of the axon and cell body, and is actively conducted down the axon to the *axon terminals* (*i.e.* the nerve terminals), small branches of the axon that form the synapses, or connections, with other cells. Action potentials move at speeds up to 100 meters per second. In humans, for instance, axons may be more than a meter long, yet it takes only a few milliseconds for an action potential to move along their length. Arrival of an action potential at an axon terminal leads to opening of voltage sensitive Ca^{2+} channels and an influx of Ca^{2+}, causing a localized rise in the cytosolic Ca^{2+} concentration in the axon terminus. The rise in Ca^{2+} in turn triggers fusion of small vesicles containing neurotransmitters with the plasma membrane, releasing neurotransmitters from this *presynaptic cell* into the synaptic cleft, the narrow space separating it from *postsynaptic cells* (Figure 7-31).

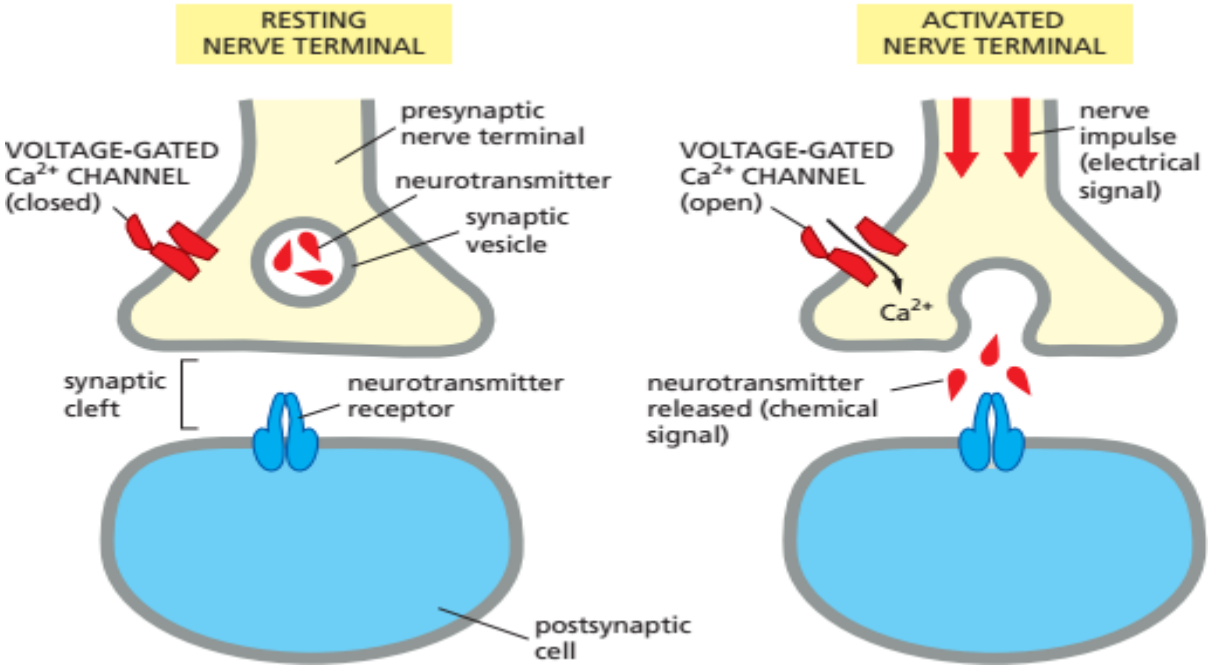


Figure: An electrical signal is converted into a chemical signal at anerve terminal. When an action potential reaches a nerve terminal, it opens voltagegated Ca^{2+} channels in the plasma membrane, allowing Ca^{2+} to flow into the terminal. The increased Ca^{2+} in the nerve terminal stimulates the synaptic vesicles to fuse with the plasma membrane, releasing their neurotransmitter into the synaptic cleft.

The released neurotransmitter rapidly diffuses across the synaptic cleft and binds to *neurotransmitter receptors* concentrated in the postsynaptic membrane of the target cell. The binding of neurotransmitter to its receptors causes a change in the membrane potential of the target cell, which can trigger the cell to fire an action potential. The neurotransmitter is then quickly removed from the synaptic cleft—either by enzymes that destroy it, or by reuptake into the nerve terminals that released it or into neighbouring cells. This rapid removal of the neurotransmitter limits the signal and ensures that, when the presynaptic cell falls quiet, the postsynaptic cell will fall quiet as well.

Neurotransmitter receptors can be of various types; some mediate relatively slow effects in the target cell, whereas others trigger more rapid responses. Rapid responses—on a time scale of milliseconds—depend on receptors that are *transmitter-gated ion channels* (also called ion-channel-coupled receptors). These constitute a subclass of ligand-gated ion channels (see Figure 12–25B), and their function is to convert the *chemical signal* carried by a neurotransmitter back into an *electrical signal*. The channels open transiently in response to the binding of the neurotransmitter, thus changing the ion permeability of the postsynaptic membrane. This in turn causes a change in the membrane potential (Figure 12–42); if the change is big enough, it can trigger an action potential in the postsynaptic cell. A well-studied example of a transmitter-gated ion channel is found at the *neuromuscular junction*—the specialized type of synapse formed between a neuron and a muscle cell. In vertebrates, the neuro transmitter here is *acetylcholine*, and the transmitter-gated ion channel is the *acetylcholine receptor* (Figure 12–43)

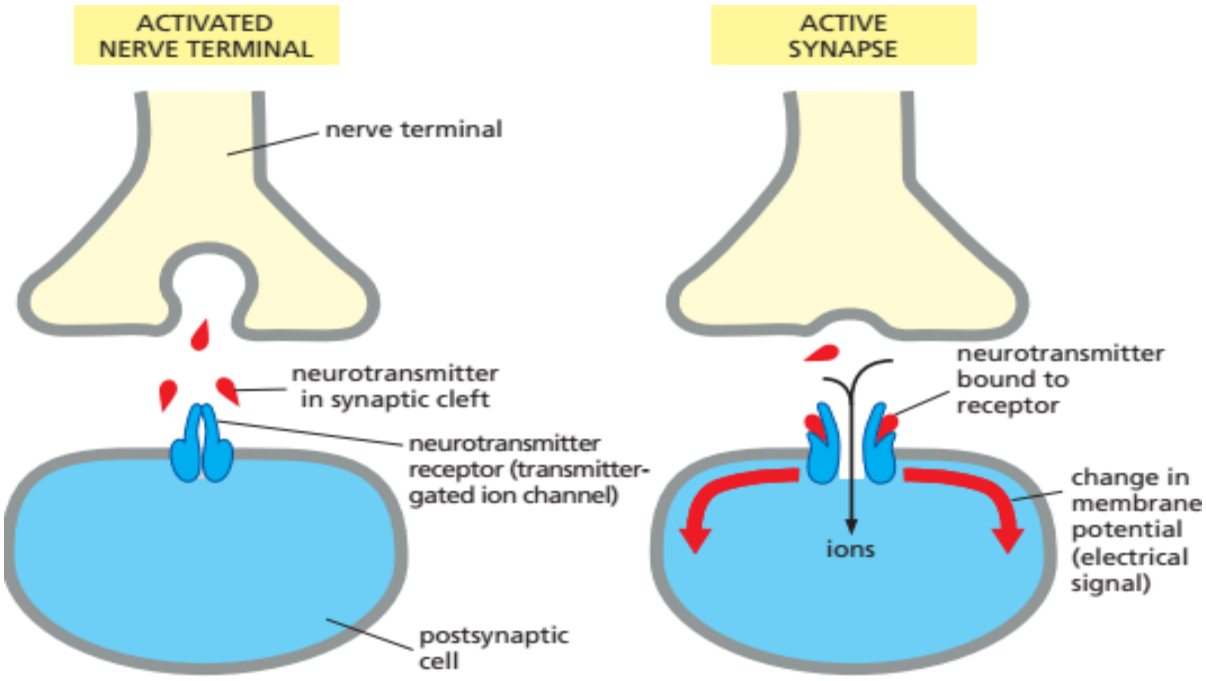
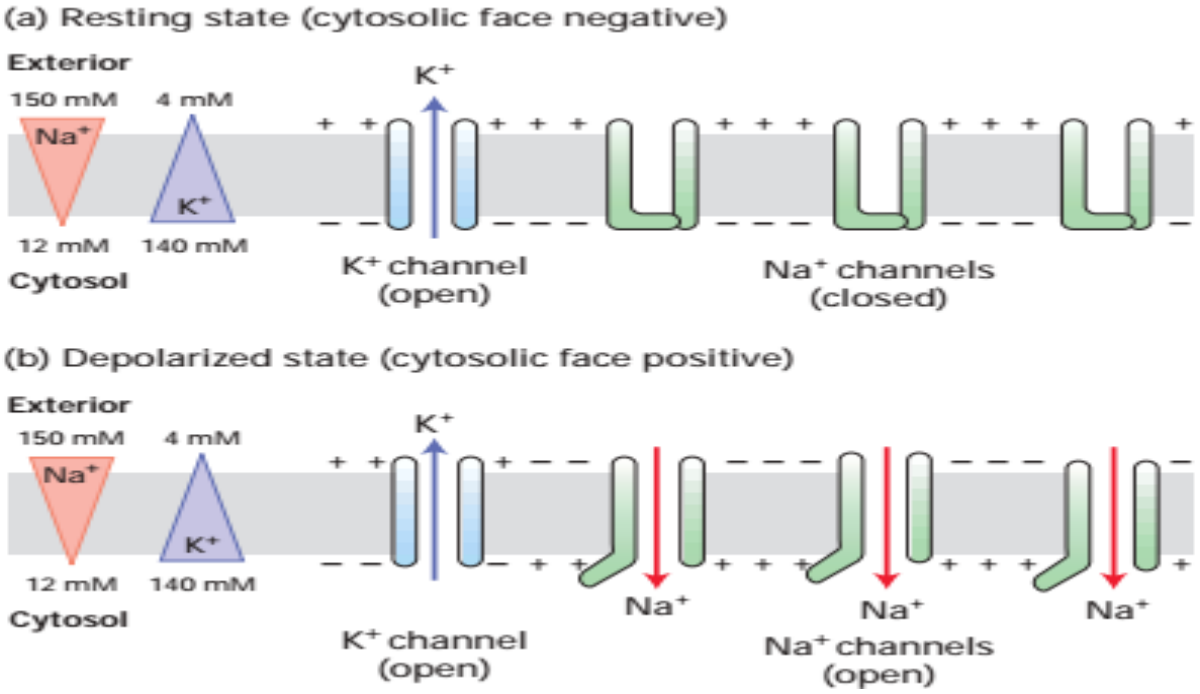


Figure 12–42 a chemical signal is converted into an electrical signal by transmitter-gated ion channels at a synapse. The released neurotransmitter binds to and opens the transmitter-gated ion channels in the plasma membrane of the postsynaptic cell. the resulting ion flows alter the membrane potential of the postsynaptic cell, thereby converting the chemical signal back into an electrical one

It takes about 0.5 millisecond (ms) for neurotransmitters to diffuse across the synaptic cleft and bind to a special type of receptors called *ion-channel–coupled receptors* (also known as transmitter-gated ion channels) on the postsynaptic cells. Binding of neurotransmitter triggers opening or closing of specific ion channels in the plasma membrane of postsynaptic cells, leading to changes in the membrane potential at this point by allowing the flow of specific types of ions, such as Na^+, K^+, Ca^{2+}, or Cl^-. Driven by their electrochemical gradients, the

ions rush into or out of the cell, creating a change in the membrane potential within a millisecond or so. Na/K pump generates a high concentration of K and a low concentration of Na in the cytosol, relative to those in the extracellular medium. The subsequent outward movement of K ions through nongated K channels is driven by the K concentration gradient (cytosol medium), generating the resting membrane potential. The entry of Na ions into the cytosol from the medium also is thermodynamically favored, driven by the Na concentration gradient (medium cytosol) and the inside-negative membrane potential (see Figure 7-20). However, most Na channels in the plasma membrane are closed in resting cells, so little inward movement of Na ions can occur (Figure 7-32a). If enough Na channels open, the resulting influx of Na ions will overwhelm the efflux of K ions though open resting K channels. The result would be a *net* inward movement of cations, generating an excess of positive charges on the cytosolic face and a corresponding excess of negative charges (due to the Cl ions "left behind" in the extracellular medium after influx of Na ions) on the extracellular face (Figure 7-32b). In other words, the plasma membrane is depolarized to such an extent that the inside face becomes positive.



▲ FIGURE 7-32 Depolarization of the plasma membrane due to opening of gated Na channels. (a) In resting neurons, nongated K channels are open, but the more numerous gated Na channels are closed. The movement of K ions outward establishes the inside-negative membrane potential characteristic of most cells. (b) Opening of gated Na channels permits an influx of sufficient Na ions to cause a reversal of the membrane potential. See text for details.

Thus, neurons use changes in the membrane potential, the action potentials, to conduct signals along their length, and small molecules, the neurotransmitters, to send signals from cell to cell.

A single axon in the central nervous system can synapse with many neurons and induce responses in all of them simultaneously. Most neurons have multiple dendrites, which extend outward from the cell body and are specialized to receive chemical signals from the axon termini of other neurons. Dendrites convert these signals into small electric impulses and conduct them toward the cell body.

Neuronal cell bodies can also form synapses and thus receive signals. Particularly in the central nervous system, neurons have extremely long dendrites with complex branches. This allows them to form synapses with and receive signals from a large number of other neurons, perhaps up to a thousand (see Figure 7-29a). Membrane depolarisations or hyperpolarisations generated in the dendrites or cell body spread to the axon hillock. If the membrane depolarization at that point is great enough, an action potential will originate and will be actively conducted down the axon.

Thus neurons use changes in the membrane potential, the action potentials, to conduct signals along their length, and small molecules, the neurotransmitters, to send signals from cell to cell.

L6: IMMUNITY AND HOST PATHOGEN INTERACTIONS

Immunity is the capacity to recognise the intrusion of materials foreign to the body and to mobilise cells and cell product to help remove that particular sort of foreign material with greater speed and effectiveness

CANCER

The whole cell cycle in any part of the body of either plant or anmal is regulated (see regulation of cell division). In a multicellular animal, the control system is highly responsive to signals from other cells that stimulate cell division when more cells are needed and block it when they are not. The control system therefore plays a central part in the regulation of cell numbers in the tissues of the body. **Cancer** results if the system malfunctions such that cell division is excessive. Because cancer cells are generally less dependent than normal cells on signals from other cells, they can out-survive, outgrow, and out-divide their normal neighbors, producing tumors that can kill their host. Cancer cells sometimes promote their own survival or proliferation by *autocrine signalling*. Disorders of cell growth, proliferation, differentiation, survival, and migration are fundamental to cancer, and abnormalities in signalling via enzyme-coupled receptors have a major role in the development of this class of diseases.

The inactivation or destruction of the mitotic spindle eventually kills dividing cells. Cancer cells, which are dividing with less control than most other cells of the body, can sometimes be killed preferentially by microtubule-stabilizing and microtubule-destabilizing *antimitotic drugs*. Thus, drugs that interfere with microtubule polymerization or depolym- erization, including colchicine, taxol, vincristine, and vinblastine, are used in the clinical treatment of cancer.

STEM CELL

A stem cell *is an undifferentiated cell (i.e. in immature state) in embryo or adult which can undergo unlimited division and can give rise to one or several different cell types*. In adults, an undifferentiated cell from which some renewable tissues, e.g. blood and skin, are formed

Red blood cells, surface epidermal cells, and the absorptive and goblet cells of the gut lining are all of this type. Such cells are referred to as *terminally differentiated*: they lie at the dead end of their developmental pathway.

Replacements for terminally differentiated cells are generated from a stock of *proliferating precursor cells*, which themselves usually derive from small numbers of dividing stem cells. Both stem cells and proliferating precursor cells are retained in the corresponding tissues along with the differentiated cells. Stem cells are not terminally differentiated and can divide without limit (or at least for the lifetime of the animal).

When a stem cell divides, though, each daughter has a choice: either it can remain a stem cell, or it can embark on a course leading irreversibly to terminal differentiation, usually via a series of precursor cell divisions (Figure 20–35). Often, a single type of stem cell gives rise to several types of differentiated progeny: the stem cells of the intestine, for example, produce absorptive cells, goblet cells, and several other secretory cell types. The process of blood-cell formation, or haemopoiesis, provides an extreme example of this phenomenon. All of the different cell types in the blood—both the red blood cells that carry oxygen and the many types of white blood cells that fight infection (Figure 20–38) — ultimately derive from a shared haemopoietic stem cell found in the bone marrow (Figure 20–39).

myeloid stem cell stem cell in bone marrow that gives rise to all blood cells except lymphocytes.

neural stem cells pluripotent cells capable of giving rise to neurons and glial cells. Stem cells persist in certain areas of the adult mammalian brain.

L7: TECHNIQUES IN CELL BIOLOGY AND PHYSIOLOGY

Cells are tiny but complex bodies. It is difficult to see their structure; more difficult to understand their molecular composition and still difficult to find out the function of their various components. What one can learn about cells, depends on the tools at one's disposal and, in fact, major advances in cell biology have frequently taken place with the introduction of new tools and techniques to the study of cell. Thus, to gain divergent types of information regarding cell's structure, molecular organization and function, cell biologists have developed and employed various instruments and techniques. A basic knowledge of some of these methods is earnestly required for proper understanding of BIO203.

MICROSCOPY

In the search for information about the structure and composition of cells, the cell biologists immediately face two limitations : the exceedingly small dimensions of cells and their

component parts and the transparent nature of cells. The diameters of the majority of cells fall within a range of 0.2 and 50 µm. The human eyes have limited distinguishing or resolving power. The ability of an observational instrument such as a human eye or a microscope to reveal details of structure is expressed in terms of ***limit of resolution*** (*l*) which is defined as *the smallest distance that may separate two points on an object and still permit their observation as distinct separate points.* The unaided human eye under optimal conditions in green light (to which it is most sensitive) cannot distinguish between points less than about 0.1 mm or 100 µm apart. Structural details smaller than this, *e.g.*, cell, is unresolved unless some instrument capable of higher resolution is used. **Magnification**, *the increase in size of optical image over the size of the object being viewed,* is of no use unless the observational system can resolve the various parts of the structure being examined. Increased magnification without improved resolution results only in a large blurred image. The human eye has no power of magnification, so magnifying glasses may be used to magnify images up to about 10 times. A light compound microscope in which many lenses are combined together has a useful magnification of about 1,500 times.

The limit of resolution (*l*) of any optical instrument (*i.e.*, eye or microscope) is given approximately by the Abbe's relationships:

Resolution (*l*) = wavelength (λ)/numerical aperture (*n sin* α)

where λ (lambda) is the wavelength ("colour") of the illumination or radiation used to form the image, *n* is refractive index (a function of density) of the material (*i.e.*, mostly air or water) between the specimen and the first lens (or objective lens), and *sin* α is sine of the semi-angle of aperture of the first lens as viewed from the specimen. The quantity "*n sin* α" is often called the **numerical aperture** (NA).

Abbe's relationships make it clear that high resolution in a microscope can only be achieved by manipulating a small number of variables: the wavelength of the illuminating radiation, the refractive index and the aperture. The **aperture** is limited to something less than 90° since that would have the lens and specimen in contact with one another. In fact, 85° is about the limit in good optical microscopes. Such angles require an excellent lens. In most cases, the aperture is less because the edges of the lens introduce distortions and so cannot be used. **Refractive index** is easy to alter, but only within narrow limits. It can be increased by using oils to fill the space between the specimen and the objective lens. Transparent immersion oils used in today's microscopes (*i.e.*, **oil immersion lens**) have *n* up to about 1.6. Still 1.6 is big improvement over air or water (*n*=1). In a microscope, the smallest detectable detail is equal to about one-half the **wavelength** of light with which it is observed.

Thus, a good light microscope, with a numerical aperture of 1.4 and using light of short wavelength (0.4 µm) will resolve two points at about 0.17µm separations. By such a microscope though, one can see considerable details in most cells, there is also a great deal that cannot be seen. For instance, ribosomes and chromatin threads of nucleus are about 0.02 µm in diameter and quite invisible to the light microscope. For them electron microscope is

used. In cell biological studies, the following two types of microscopes are most extensively used:

Light Microscopy

The **compound light microscope** uses visible light for illuminating the object and contains glass lenses that magnify the image of the object and focus the light on the retina of the observer's eye. It consists of two lenses, one at each end of a hollow tube. The lens closer to eye is called **ocular lens** or **eyepiece** and the lens closer to the object being viewed is called **objective lens** (Fig. 2.1). Usually objective lenses of various magnifying powers are mounted on a revolving turret at the lower end of the tube. The object, supported by a glass slide under the objective lens, is illuminated by light beneath it. In ordinary microscopes light is reflected on the object by a mirror having concave and plane surfaces. In some microscopes, a third lens, called **condenser lens**, is located between the object and the light source and serve to focus the light on the object.

In order to make full use of available resolving power of the compound light microscopes, special techniques have been designed to improve contrast. Certain improved types of light microscopes are of the following types:

1. Dark field microscope or Ultramicroscope. This type of light microscope is particularly useful for viewing suspensions of bacteria. In it, the object is viewed only with oblique rays and since one sees only those light rays that are scattered from objects, the images appear bright on a black background. The process is akin to seeing dust particles floating in a sunbeam.

2. Phase contrast microscope. This type of light microscope takes advantage of the fact that different parts of a cell have different densities and, hence, different refractive indices. Regions where the refractive index is changing they tend to bend light rays. In phase contrast microscope these bent rays are used to form patterns of destructive interference, yielding sharp contrasts. This technique is widely used to observe unstained and living cells (especially in mitotically dividing cultured cells). The **interference microscope** is based on the principle of the phase contrast microscope and permits detection of small, continuous changes in refractive index. The variations of phase can be transformed into such vivid colour changes that a living cell looks like a stained preparation.

3. Polarization microscope. This type of light microscope is useful mainly for viewing highly ordered objects such as crystals or bundles of parallel filaments (*i.e.*, microtubules of mitotic spindle.).

Methods of Sample Preparation for Light Microscopy

Cells are transparent and optically homogeneous: so either they are viewed as such by instruments such as phase contrast microscope or to produce necessary contrast, the cells are passed through various steps of slide preparation such as killing, fixation, dehydration, embedding, sectioning, staining and mounting. Thus, superior specimens for microscopic examination can be obtained by **killing** the cells and coagulating or **fixing** the protoplasm by

preservatives, called **fixatives** such as alcohols, formaldehyde, mercuric chloride, picric acid, acetic acid and mixture of these. The process of fixation involves the following events — (1) The proteins and other macromolecules are precipitated. (2) The intracellular hydrolytic enzymes are denatured, preventing autolysis. (3) Cross links are formed between macromolecules, making the preparation more stable and minimizing shrinkage upon drying. (4) Substances are introduced which prevent attack by microorganisms. (5) The tissues become stiffer, making their sectioning easier. (6) The affinity of the tissue for dyes (stains) is increased. Fixation is generally followed by **dehydration** (*i.e.,* gradual removal of water vapours from the tissue) by the organic solvents such as ethanol. The dehydrated specimens are **embedded** *i.e.,* they are infiltrated with molten paraffin which hardens upon cooling and provides enough support to allow thin sections to be cut with a **microtome**. By the microtome, serial sections, 5 to 10µm thick can be cut and placed on slides in the order of cutting and permitting a sequence of specimens for observation. These sections are stained with a non-vital-stain to increase the contrast. **Stains** are the chemicals that can selectively attach to particular molecules of particular cellular structures and make them stand out from other parts of the cell. The non-vital stains fall into two main classses: **acid stains** such as eosin, orange G, aniline blue and fast green, all of which combine with basic molecules such as proteins of the fixed cells; and **basic stains** such as methylene blue, crystal violet, haematoxylin, basic fuchsin, etc., all of which combine with nucleic acids and other acidic molecules of the fixed cells. The cellular structures that stain with acid stains are called acidophilic and those that stain with basic dyes are called **basophilic**.

In addition, there are certain specific stains, called **cytochemical stains** that bind selectively to some specific groups of cellular macromolecules such as proteins, nucleic acids, polysaccharides and lipids. For example, Millon reaction, diazonium reaction and Naphthol Yellow 5 stain are used for the proteins; alkaline fast green is used for histone (basic protein); Feulgen reaction (using Schiff's reagent) is used for DNA; methyl green-pyronine stain (Unna-Pappenheim stain) is used in distin guishing between DNA and RNA and it stains DNA green and RNA red; acetocarmine and acetoorcein stains are used to stain chromosomes of dividing cells; periodic acid-Schiff (PAS) reaction is used for the demonstration of polysaccharide materials such as starch, cellulose, hemicellulose, and pectin in the plant cells and mucoproteins (glycoproteins), hyaluronic acid and chitin in animal cells; and fat soluble dyes such as Sudan Red and Sudan Black B are used for the lipids. The Sudan Black B is a specific stain for phospholipids and is used to stain Golgi apparatus.

Vital stains selectively stain the intracellular structures of living cells without serious alteration of cellular metabolism and function. For example, Janus green B selectively stains mitochondria; neutral red stains plant vacuoles and methylene blue stains Golgi apparatus and also nuclear chromatin of dividing cells.

All these steps often are time-consuming and cause artifacts in the cells. Hence, when speed is important and specimen is required for electron microscopy or for histochemical analysis paraffin embedding may be replaced by fixation by freeze drying. **Freeze drying** is a method that avoids denaturation of enzymes and is particularly useful for histochemical staining. Tissue is frozen rapidly by plunging its small portions into liquid carbon dioxide or liquid

nitrogen and, thus, required rigidity for sectioning by the **freezing microtome** is obtained. Frozen sections are stained and are dehydrated at low temperature (–30 to –400C) in a high vacuum. At such low temperature ice crystals are of minimum size and few distortions or artifacts arise. Chemical composition and physical structure are maintained with little change. Another advantage is that fixation is rapid enough to arrest some cellular functions at their critical junctures which can then be observed and compared.

Electron Microscopy

The electron microscopy (Fig. 2.1) uses the much shorter wavelengths of electrons to achieve resolution as low as 3 A^0, with a usual working range between 5 to 12 A^0. In the electron microscope electromagnetic coils (*i.e.,* magnetic "lenses") are used to control and focus a beam of electrons accelerated from a heated metal wire by high voltages, in the range of 20,000 to 100,000 volts (new instruments are being developed that use 1,000,000 volts). The wavelength of an electron depends on the magnitude of the voltage and may be 0.01 Aº or less. The electrons of the beam are scattered by a specimen placed in the path of the beam. Electrons that do manage to pass through the specimen are focused by an objective coil ('lens') and a final magnified image is produced by a projecter coil or 'lens'. The final image is viewed directly on the fluorescent screen or is recorded on photographic film to produce **electron micrograph**. This type of electron microscope is called **transmission electron microscope** (**TEM**).

Unlike the compound light microscope, in which image formation depends primarily upon differences in light absorption, the electron microscope forms images as a result of differences in the way electrons are scattered by various regions of the object. Electrons have a very low penetrating power, that is, they are easily scattered by objects in their paths. The degree to which electrons are scattered is determined by the thickness and atomic density of the object: regions of high density (possessing atoms of high atomic number) scatter electrons more than regions of lesser density and consequently appear darker in the final image. Because electrons are scattered so easily, the specimen used in electron microscopy must be extremely thin (ultrathin, *i.e.*10 nm to 100 nm thick). If the sections were not extremely thin, most of the electrons would be scattered and a uniform dark image would result. Since, electrons are scattered even by gas molecules and so the electron beam must travel through the electron microscope in a very high vacuum and the samples must be completely dry and otherwise non-volatile. Thus, living cells which are wet cannot be viewed in electron microscope.

Methods of Sample Preparation for Transmission Electron Microscopy

The standard procedure for the preparation of specimen for TEM entails fixation, dehydration, staining and sectioning similar to light microscopy (Table 2-1.). However, the most significant difference being the need for ultra-thin sections. Following techniques of sample preparation are generally used for different types of methods of studying ultrastructure of the cell:

1. Monolayer technique. Macromolecules such as DNA and RNA are studied by monolayer technique in which the macromolecules are extended on the air-water interface before being collected on a film.
2. Thin sectioning. This method uses a cutting device known as ultramicrotome to remove ultrathin (*i.e.,* 10 nm to 100 nm thick) sections from the specimen. To withstand the passage of ultrafine diamond or glass knife without tearing, the specimen is first embedded in a hard plastic such as, epoxy resin (Table 2-1). The resin is allowed to penetrate the sample before it is polymerized. Sections are floated from the knife of ultramicrotome onto the surface of water and picked up by touching them with a fine wire mesh or small circular copper grid (*i.e.,* small discs perforated with numerous openings). Prior to its use mesh or grid is coated with a thin monolayer film (7.5 to 15nm thick) of plastic (such as formvar or collodion) or carbon to provide a support to the sections (or sample) (Fig.2.2). The specimen is visualized through the holes of screen. Sections to be examined with the electron microscope are generally not stained (since no colours are seen with the electron microscope). However, contrast may be improved by "poststaining" with electron stains or electron-dense materials such as urynyl acetate, urynyl citrate, lead citrate, osmium tetroxide, etc. The method of thin sectioning is used to study morphology of cell.
3. **Tracers.** Several biological processes such as pinocytosis, phagocytosis and transport of molecules across plasma membrane can be studied by the use of appropriate tracers (*e.g.,*gold, mercuric sulphide, iron oxide, etc.). These tracers are detected by their electron opacity. An ideal tracer should be non-toxic, physiologically inert, composed of small-sized particles of uniform and known size and preserved *in situ* during the processing of the tissue.

X- RAY DIFFRACTION ANALYSIS

This technique is used to analyze three-dimensional (tertiary) structure of DNA molecule and a variety of proteins such as myoglobin, haemoglobin, collagen, myelin sheath of nerve cells, myofibrils of striated muscles, etc. This method depends on the fact that X-rays are scattered or diffracted by the atoms of a substance. If the material has an ordered crystalline atomic structure, the resulting X-ray diffraction pattern is also ordered and reflects the three-dimensional arrangement of atoms in crystal.

CELL FRACTIONATION

Sometimes it becomes necessary to break up tissues and cells and to isolate various parts of the cell for structural or biochemical analysis. For this purpose, the technique of cell fractionation is employed. Cell fractionation method involves, essentially the homogenisation or destruction of cell boundaries by different mechanical or chemical procedures, followed by the separation of the subcellular fractions according to mass, surface and specific gravity by centrifuges.

In the cell fractionation, the cells are gently broken by grinding a small piece of tissue in a homogeniser having a moving close-fitting glass or plastic pestle within a tube that contains a

medium such as sucrose solution to preserve the cellular organelles (Fig. 2.5). The solution containing homogenised or disrupted cells, is called **homogenate**. The homogenate is subjected to differential centrifugations of increasing velocity. The method depends on the principle that particles of different weight or sizes move at different rates through a solution under the influence of centrifugal force. At each step larger particles form a gelatinous pellet at the bottom of the tube leaving smaller particles in the supernatant solution. By decanting the supernatant and spinning it harder, the next fraction can be brought down. Ultimately one is left with a supernatant solution having only soluble, molecular-sized components. The residual solution is called **cytosol**. The different molecules of cytosol are isolated by a variety of biochemical techniques such as **chromatography**, **dialysis and electrophoresis**.

AUTORADIOGRAPHY

Autoradiography is a technique which is used to locate radioactive isotopes in cells, tissues, organs and whole organisms. A specimen is exposed to a solution containing molecules that have been made radioactive by the incorporation of radioactive isotopes, such as tritium (^{3}H), carbon 14 (^{14}C), phosphorus (^{32}P) and sulphur (^{35}S). The tagged molecules are often precursor molecules used by the cell in the synthesis of other needed molecules. At intervals, samples are removed from the solution; in case of smaller tissues, the samples are sectioned and mounted on glass slides or grids. The sections are then coated with a photographic emulsion and stored in the dark for periods ranging up to several months. When a radioactive atom emits a beta particle (*i.e.*, electron) the photographic emulsion is affected in a manner similar to the exposure of a photographic emulsion to light. Over a period of time sufficient radioactive emissions occur to affect the silver grains of the emulsion. Black spots will appear at those sites when the emulsion is developed. Such spots will mark sites in the tissues where the radioactive atoms have accumulated. These sites can be identified by examining the stained tissue sections under the light microscope.

In the technique of autoradiography, for the study of DNA metabolism ofcell 3H-thymidine is used; for RNA metabolism 3H-uridine is used; for protein synthesis various tritiated (^{3}H-tagged) amino acids are used; and for polysaccharides and glycoproteins tritiated monosaccharides such as ^{3}H-mannose and ^{3}H-fructose are employed.