KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed University Established Under Section 3 of UGC Act 1956)
Coimbatore - 641021.
(For the candidates admitted from 2017 onwards)
DEPARTMENT OF BIOCHEMISTRY

STAFF NAME: Ms.P.LOGANAYAKI **SEMESTER : III**
SUBJECT : CELLULAR BIOCHEMISTRY **SUBJECT CODE: 18BCP104**
CLASS: I M.Sc.(BC)

Course objectives

- To enable the students to understanding the molecules within cells and interactions between cells that allows construction of multi cellular organisms.
- To understand the molecular machinery of living cells.
- To understand the membrane transport mechanisms.

Course outcome

- Upon successful completion of this course, participants will be able to:
- Describe the general principles of gene organization and expression in both prokaryotic and eukaryotic organisms.
- Describe the structure and function of biological membranes including the roles of gradients in energy transduction.
- Explain the basic pathways and mechanisms in biological energy transduction from oxidation of metabolites to synthesis of ATP.
- Explain various levels of gene regulation and protein function including signal transduction and cell cycle control.
- Relate properties of cancerous cells to mutational changes in gene function.

UNIT I

Membrane: Membrane bilayer- models, Membrane lipids- fluidity, asymmetry, phase transition, Liposomes. Langmuir trough, Metamorphic mosaic model, Techniques for determination of membrane protein topology

Membrane proteins – Types, Orientation, Mobility – Experiments, flippases, proteins of RBC membrane, RBC ghosts, Bacteriorhodopsin, Porins – aquaporin.

solubilisation of proteins, lipid anchored proteins, Carbohydrates – cell surface carbohydrates – Lectins and selectins.

UNIT II

Membrane transport: Passive diffusion, facilitated diffusion in erythrocytes, Carriers and ion channels, Ion concentration gradients.

Uniporter Catalyzed transport, active transport systems. Transport process driven by ATP- Ion pumps: Calcium ATP ase; Na^+ K^+ ATPase; Mechanism, Gastric H^+ K^+ ATPase, ABC superfamily – ATPases that transport peptides and drugs (MDR proteins).

Co-transport by Symporters and antiporters, Group translocation.

Osmosis,receptor mediated endocytosis and its significance.

UNIT III

Mitochondria – Reduction potential, **Free energy and entropy,** electron transport chain – Complexes, Q-cycle, Cyt C oxidase complex, Translocation of protons and the establishment of a proton motive force, machinery for ATP formation and chemi-osmotic mechanism, ATP synthase – Experiments, inhibitors and uncouplers of oxidative phosphorylation.

Microfilaments – Actin – Stuctures, Assembly, Myosin. Microtubules – Organisation and dynamics, kinesin and dynein. Cilia and flagella – Structure and functions, intermediary filaments.

Mitochondrial transport system: ATP/ADP exchange, malate-glycero phosphate shuttle

UNIT IV

Cell – Matrix interaction: Cell – Cell interaction: Extra cellular matrix; Collagen, hyaluronan and proteoglycans, laminin, integrins, Fibrillin, elastin and fibronectins.

Cell – Cell adhesion: Specialised junctions – Desmosomes, Gap junctions, Tight junctions. Adhesion molecules – Cadherins (E and N), Connexins.

Cell – Cell signaling – Role of Signaling molecules and their receptors; functions of cell surface receptors, pathways of intracellular signal transduction, second messengers, G-protein coupled receptors, receptor tyrosine kinases, Ras, MAP kinases in cellular growth and functions.

UNIT V

Cell cycle and cancer: Cell cycle and its control, Cell cycle control in mammalian cells, checkpoints in cell cycle regulation.

Cancer: Properties of tumour cells and genetic basis and onset of cancer.

Tumour viruses – DNA & RNA Viruses as transforming agents – mechanism.

Tumour suppressor genes and functions of their products. Carcinogenic and anticarcinogenic effect of chemicals and radiation. Apoptosis (Programmed cell death) – pathways, regulators and effectors on apoptosis and necrosis.

TEXT BOOKS

Paul, A., (2009). Text Book of Cell and Molecular Biology,1st edition. Books and Allied (P) Ltd, Kolkata.

Cooper, G.M., and Hausman, R.E., (2013).Cell-A Molecular Approach, 6th Edition.. Sinauer Associates. USA.

Gerald, K., 2013. Cell and Molecular Biology, 7th edition. John Wiley and Sons, Inc, Hoboken, United States.

Nelson, D.L., and Cox, M.M., (2012). Lehninger's Principles of Biochemistry, 6th edition. W.H.Freeman and company, New York.

REFERENCES

Lodish, H., Berk, A., Kaiser, C.A., and Krieger, M., (2012). Molecular Cell Biology, 7th edition. W.H. Freeman & Company, London.

Garrette & Grisham, (2004). Principles of biochemistry, 4th edition. Saunders college publisher, Philadelphia,United States.

Alberts, B.,, Johnson, A., Lewis, J., and Raff, M.,. (2007). Molecular Biology of the Cell, 5th edition. Garland Publishing Co.New York.

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SEMESTER : I
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LECTURE PLAN
DEPARTMENT OF BIOCHEMISTRY

Sl. No	Duration of Period	Topics to be Covered	Page No	Books referred	Web page referred
Unit I: Membrane					
1	1	Membrane bilayer- models, Membrane lipids- fluidity,	369-372	R1	
2	1	Asymmetry, phase transition, Liposomes			
3	1	Membrane proteins – Types, Orientation,	373-375	R1	
4	1	Mobility – Experiments, flippases,	431-432	T1	
5	1	proteins of RBC membrane, RBC ghosts,			W1
6	1	Bacteriorhodopsin, Porins – aquaporin. solubilisation of proteins,	421-424	T1	
7	1	lipid anchored proteins,	424-426	T1	
8	1	Carbohydrates – cell surface carbohydrates – Lectins and selectins.	264-267	T1	
9	1	Class test 1			
Total Hours: 9					
Unit II: Membrane transport					
1	1	Passive diffusion, facilitated diffusion in erythrocytes, Carriers and ion channels.	24-26	T2	
2	1	Ion concentration gradients.			
3	1	Uniporter Catalyzed transport, active transport systems.	26-28	T2	
4	2	Transport process driven by ATP- Ion pumps: Calcium ATP ase; Na^+ K^+	29-30	T2	

		ATPase			
5	1	Mechanism, Gastric H^+ K^+ ATPase,	30-31	T2	
6	1	ABC superfamily – ATPases that transport peptides and drugs (MDR proteins).	31-35	T2	
7	1	Co-transport by Symporters and antiporters, Group translocation.			W2
8	1	Osmosis,receptor mediated endocytosis and its significance.	R1: 56-58, 824-826	R1	
Total Hours: 9					
Unit III: Mitochondria					
1	1	Mitochondria – Reduction potential, electron transport chain – Complexes	696-698	R1	
2	1	Q-cycle, Cyt C oxidase complex, Translocation of protons	698-704	R1	
3	1	Establishment of a proton motive force, machinery for ATP formation	704-712	R1	
4	1	Chemi-osmotic mechanism, ATP synthase.	691-696	R1	
5	1	Experiments, inhibitors and uncouplers of oxidative phosphorylation.			
6	1	Microfilaments – Actin – Stuctures, Assembly, Myosin.	182-184	R1	
7	1	Microtubules – Organisation and dynamics, kinesin and dynein.	184-186	R1	
8	1	Cilia and flagella – Structure and functions, intermediary filaments.	795-798	T1	
9	1	Revision			
Total Hours: 9					
Unit IV: Cell – Matrix interaction					
1	1	Cell – Cell interaction: Extra cellular matrix	807-812	T1	
2	1	Hyaluronan and proteoglycans, laminin, integrins and fibronectins.	827-832	T1	
3	1	Specialised junctions – Desmosomes, Gap junctions, Tight junctions	530-532	T2	
4	1	Adhesion molecules – Cadherins (E and N), Connexins.	525-529	T2	
5	1	Cell – Cell signaling – Role of Signaling molecules and their receptors; functions of cell surface receptors	421-425	R1	
6	1	Pathways of intracellular signal transduction.	435-445	R1	

7	1	second messengers, G-protein coupled receptors			
8	1	receptor tyrosine kinases, Ras, MAP kinases in cellular growth and functions.	429-433	R1	
9	1	Revision			
Total Hours: 9					
Unit V: Cell cycle and cancer					
1	1	Cell cycle and its control, Cell cycle control in mammalian cells	848-859	T1	
2	2	Checkpoints in cell cycle regulation	884-888	T1	
3	1	Cancer: Properties of tumour cells and genetic basis and onset of cancer.	1107-1113	T1	
4	1	Tumour viruses – DNA & RNA Viruses as transforming agents – mechanism	158-159	T1	
5	1	Tumour suppressor genes and functions of their products	701-703	T2	
6	1	Carcinogenic and anticarcinogenic effect of chemicals and radiation.	706-712	T2	
7	2	Apoptosis (Programmed cell death) – pathways, regulators and effectors on apoptosis and necrosis.		R1: 473-474, T1: 936-944	
8	1	Revision			
Total Hours: 10					
Previous year end semester examinations question paper discussion					
1	1	Previous year ESE question paper Discussion			
2	1	Previous year ESE question paper Discussion			
Total Hours: 2					
Grand Total: 48					

REFERNCE BOOKS:

R1: Nelson, D.L. and Cox, M.M., (2013). Lehninger: Principles of Biochemistry 6th ed., W.H. Freeman and Company (New York), ISBN:13:978-1-4641-0962-1 / ISBN:10:1-4641-0962.

TEXT BOOKS:

T1: Lodish, H., Berk, A., Kaiser, C.A., and Krieger, M., (2012). Molecular Cell Biology, 7th edition. W.H. Freeman & Company, London.

T2: Paul, A., (2009). Text Book of Cell and Molecular Biology,1st edition. Books and Allied (P) Ltd, Kolkata.

WEB PAGE REFERANCE:

W1: www.acbd.monash.org/does/red-cell-membrane.pdf
W2: www.ncbi.gov/books/NBK21681/

UNIT-I-SYLLABUS

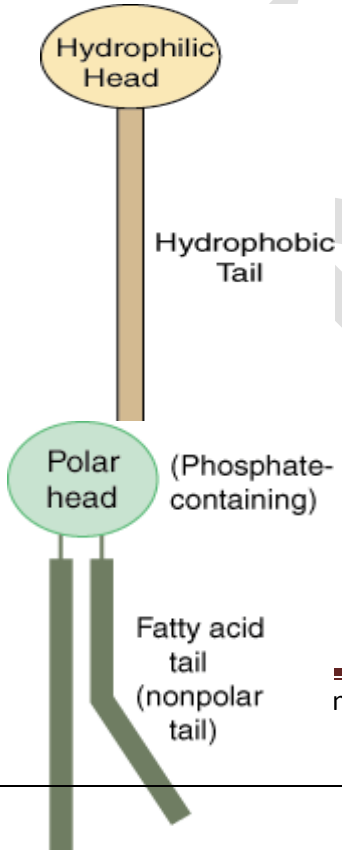
Membrane: Membrane bilayer- models, Membrane lipids- fluidity, asymmetry, phase transition, Liposomes. Langmuir trough, Metamorphic mosaic model, Techniques for determination of membrane protein topology
Membrane proteins – Types, Orientation, Mobility – Experiments, flippases, proteins of RBC membrane, RBC ghosts, Bacteriorhodopsin, Porins – aquaporin.
solubilisation of proteins, lipid anchored proteins, Carbohydrates – cell surface carbohydrates – Lectins and selectins.

Membrane bilayer

- The lipid bilayer is a universal component of all cell membranes. Its role is critical because its structural components provide the barrier that marks the boundaries of a cell.
- The structure is called a "lipid bilayer" because it is composed of *two* layers of *fat* cells organized in two sheets. The lipid bilayer is typically about five nanometers thick and surrounds all cells providing the cell membrane structure.

Lipids and Phospholipids

- The structure of the lipid bilayer explains its function as a barrier. Lipids are fats, like oil, that are insoluble in water.



- There are two important regions of a lipid that provide the structure of the lipid bilayer.
- Each lipid molecule contains a hydrophilic region, also called a polar head region, and a hydrophobic, or nonpolar tail region.

Basic Lipid Structure

- The hydrophilic region is attracted to aqueous water conditions while the hydrophobic region is repelled from such conditions. Since a lipid molecule contains regions that are both polar and nonpolar, they are called amphipathic molecules.
- The most abundant class of lipid molecule found in cell membranes is the phospholipid. The phospholipid molecule's

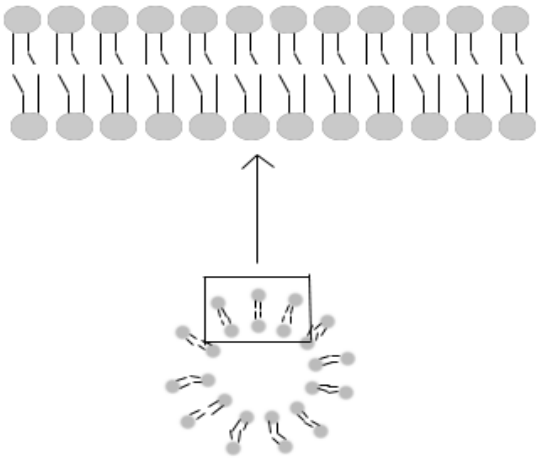
polar head group contains a phosphate group. It also sports two nonpolar fatty acid chain groups as its tail.

Phospholipid Structure

- The fatty acid tail is composed of a string of carbons and hydrogens. It has a kink in one of the chains because of its double-bond structure.

The Bilayer

- The phospholipids organize themselves in a bilayer to hide their hydrophobic tail regions and expose the hydrophilic regions to water.
- This organization is spontaneous, meaning it is a natural process and does not require energy. This structure forms the layer that is the wall between the inside and outside of the cell.



Lipid Bilayer

Properties of the Lipid Bilayer

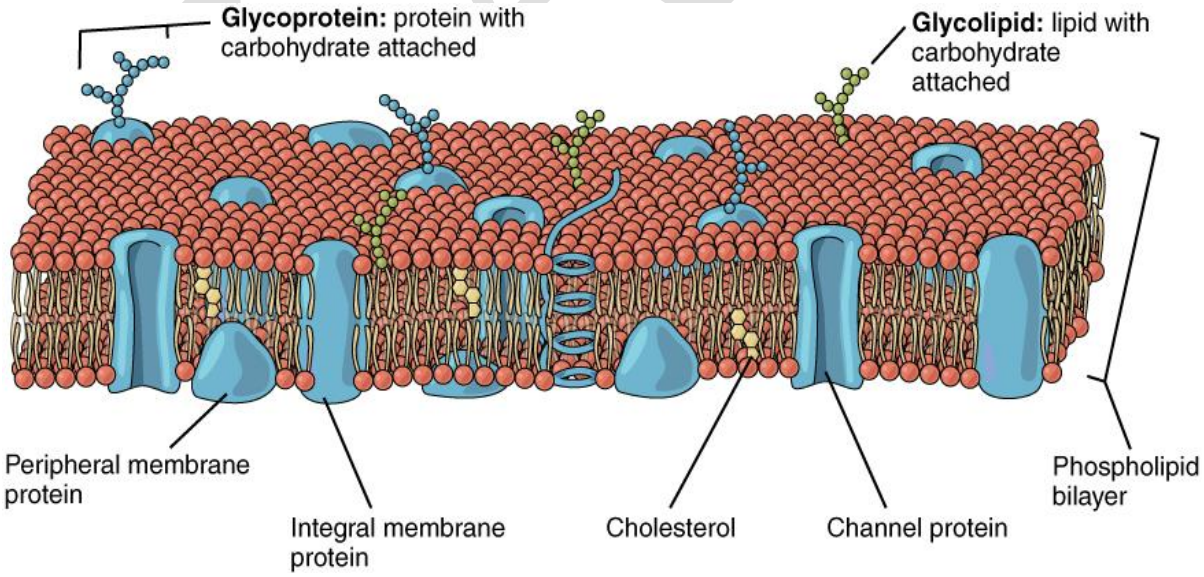
- As we have already mentioned, the most important property of the lipid bilayer is that it is a highly impermeable structure.
- Impermeable simply means that it does not allow molecules to freely pass across it. Only water and gases can easily pass through the bilayer.
- This property means that large molecules and small polar molecules cannot cross the bilayer, and thus the cell membrane, without the assistance of other structures.
- Another important property of the lipid bilayer is its fluidity. The lipid bilayer contains lipid molecules, and, as we will discuss later, it also contains proteins.
- The bilayer's fluidity allows these structures mobility within the lipid bilayer. This fluidity is biologically important, influencing membrane transport.

- Fluidity is dependent on both the specific structure of the fatty acid chains and temperature (fluidity increases at lower temperatures). Structurally, the lipid bilayer is asymmetrical: the lipid and protein composition in each of the two layers is different.

Fluid mosaic model

- Plasma membranes are subcellular structures, approximately 10nm thick, that form a protective boundary around the cell as well as the cell's organelles.
- They serve to both impede foreign material from entering the cell, and prevent the cellular contents from leaking out.
- With the structural makeup of the lipid bilayer conferring membranes unique physical and chemical properties, these structures also contribute to diverse and critical cellular functions.

Membranes are composed of lipids and proteins, balanced in equal proportions by mass. The current views on membrane structure are derived from the Fluid-Mosaic Membrane Model (F-MMM), which depicts them as two-dimensional fluids made up of lipid-bilayers interspersed with proteins. The fluidic nature of membranes is due to the constant rotational or lateral motion of both lipids and proteins. While lipids provide the basic structure of membranes, the proteins carry out a vast array of specialized functions, from ion and small molecule transport to the regulation of signalling
pathw



Types of membrane lipids

- Membrane lipids are amphipathic, which means that they have a polar or hydrophilic end and a non-polar or hydrophobic end. In aqueous mediums, membrane lipids spontaneously organize into bilayers with the polar ends oriented towards, and the non-polar ends oriented away from, the solution.
- The bilayer closes in on itself to avoid free edges with water. These basic structural properties of plasma membranes enable them to carry out their fundamental functions.
- For example, the propensity of membrane lipids to form a thermodynamically stable, closed lipid bilayer structures renders them stability and encourages the formation of closed subcellular compartments.
- It also enables the spontaneous repair of small tears in the membrane, which prevents material leaking out of the cell or organelles. Furthermore, the hydrophobic interior of membranes serves as a barrier to water soluble molecules but allows certain lipid soluble molecules to passively diffuse through.
- Membranes are therefore selectively permeable structures; a property that helps to prevent leakage and protect the cell from the passive entry of many toxins.

Membrane lipids are highly diverse, with a typical membrane containing more than 100 species of lipids. These lipids vary in their structure and extent of saturation of the fatty acyl chains.

There are three major classes of membrane lipids – the phosphoglycerides, sphingolipids and sterols.

The phosphoglycerides and sphingolipids can be combined as one class, the phospholipids. These are the classical membrane lipid, formed of a polar head group and two hydrophobic fatty acid tails. The fatty acid tails typically contain between 14-24 carbon atoms. One of the two tails is unsaturated and therefore contains one or more cis-double bonds, which creates a small kink in the tail. The other tail is saturated, without any cis-double bonds and remains straight. Variations in the length and saturation of the fatty acid tail affect how tightly phospholipids are able to pack against each other, leading to altered membrane fluidity. Linking the polar head group to the fatty acid tail is a backbone made up of either glycerol or sphingosine. The different backbone molecule differentiates between the classes of phospholipid.

Phosphoglycerides have a polar head group esterified to one of three glycerol hydroxyl groups, and two hydrophobic fatty acid tails esterified to the remaining two hydroxyl groups of the glycerol backbone. The polar head group is composed of a phosphate group linked to choline, ethanolamine, serine or inositol. These form phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl serine (PS) and phosphatidylinositol (PI) respectively.

Sphingolipids, on the other hand, have a backbone formed from sphingosine, an amino alcohol with a long hydrocarbon chain; a less abundant class of membrane lipids. Ceramide is a simple sphingolipid which has a hydrophobic fatty acid tail linked to the amino group of the sphingosine. The esterification of additional groups to the terminal hydroxyl group of the sphingosine backbone gives rise to other types of sphingolipids. For instance, sphingomyelin has a polar phosphoryl choline head group and glycolipids have a carbohydrate group. The carbohydrate group of glycolipids can be a simple sugar or an oligosaccharide forming cerebrosides and gangliosides respectively.

Sterols are smaller than phospholipids. They have a single polar hydroxyl head group attached to a rigid steroid ring structure and a short non-polar hydrocarbon tail. Cholesterol is the major sterol component of animal cell membranes. Different sterols are found in other eukaryotic cell membranes. Yeast and fungi use ergosterol, while plants use sitosterol and stigmasterol. However, prokaryotic cell membranes essentially contain no sterols. Sterols insert into the lipid bilayer with their hydroxyl head groups oriented with the phospholipid polar groups. This aligns the rigid ring structure of the sterol with the phospholipid hydrocarbon tail, which decreases phospholipid mobility. This stiffening effect also reduces the water-soluble permeability of the bilayer but does not affect membrane fluidity.

Membrane Proteins

While membrane lipids form the basic structure of the lipid bilayer, the active functions of the membrane are dependent on the proteins. Cell adhesion, energy transduction, signaling, cell recognition and transport are just some of the important biological processes carried out by membrane proteins.

Proteins can associate with the membrane in one of three ways. Intrinsic or integral membrane proteins embed in the hydrophobic region of the lipid bilayer. Experimentally, these proteins can only be isolated by physically disrupting the membrane with detergent or other non-polar solvent. Monotopic proteins insert in one leaflet but do not span the membrane. Transmembrane proteins are the classic examples of intrinsic membrane proteins. These span the membrane, typically in an α-helix conformation and can span the membrane multiple times. Some intergral

membrane proteins use β-barrels to cross the membrane. These structures are typically large and form water filled channels. Extrinsic or peripheral membrane proteins associate loosely with the hydrophilic surfaces of the lipid bilayer or intrinsic membrane proteins. They form weak hydrophobic, electrostatic or non-covalent bonds, but do not embed with the hydrophobic core of the membrane. These proteins can be dissociated from the membrane without disrupting it through application of polar reagents or high pH solutions. Extrinsic membrane proteins may interact with the inner or outer leaflet.

Movement of Bilayer Components

One of the tenets of the Fluid-Mosaic membrane model is that the components of the bilayers are free to move. Before describing the differences between lipid and protein movement in the bilayer, it is important to consider the types of movement possible. Using a phospholipid as an example, the first type of movement is rotational. Here the phospholipid rotates on its axis to interact with its immediate neighbours. The second type of movement is lateral, where the phospholipid moves around in one leaflet. Finally, it is possible for phospholipids to move between both leaflets of the bilayer in transverse movement, in a "flip-flop" manner.

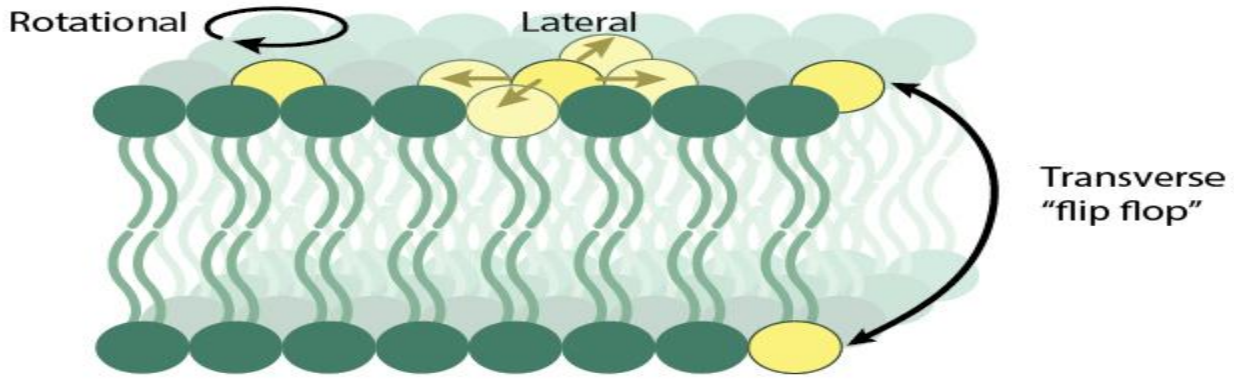


Figure. Phospholipid bilayer movement: Phospholipids in the lipid bilayer can either move rotationally, laterally in one bilayer, or undergo transverse movement between bilayers.

Lateral movement is what provides the membrane with a fluid structure. By labelling single particles and following their movement via high speed video, researchers were able to discover that phospholipids did not move via Brownian motion but rather by "hop diffusion". Phospholipids stay in one region for a short while before hopping to another location. This compartmentalization of lateral movement appears to be linked to contacts between the actin cytoskeleton and the membrane which form the regions that the phospholipids hop between.

As described above, membrane asymmetry is critical for membrane functions. Transverse movement is what allows the asymmetry to be maintained. Uncatalysed movement of phospholipids between the bilayers is possible, but this is slow and cannot be relied upon to maintain the asymmetry equilibrium. Instead, lipid translocator proteins catalyse phospholipid movement between the bilayers. **Flippases** move phospholipids from the outer leaflet to the inner leaflet. In order to maintain the charge gradient across the membrane, flippases predominantly transport phosphatidylserine and to a lesser extent phosphatidylethanolamine. **Floppases** move phospholipids in the opposite direction, particularly the choline derived phospholipids phospatidylcholine and sphingomyelin. Floppases also mediate cholesterol transport from the intracellular monolayer to the extracellular monolayer. These catalyzed movements are typically dependent on ATP hydrolysis. A third class of protein are the **scramblases**, which exchange phospholipids between the two leaflets in a calcium activated, ATP-independent process.

In the case of membrane proteins, they are able to undergo rotational and lateral movement. However, there is no transverse movement of proteins between the leaflets. Intrinsic membrane proteins are tightly embedded in the hydrophobic core, whereas extrinsic membrane proteins associate with their required leaflet. The energy requirements to move either type of membrane protein across the bilayer would be excessive.

Membrane lipid asymmetry

The human red blood cell is functionally specialized for transporting oxygen. In order to maximize oxygen capacity, it has no nucleus or organelles, consisting primarily of plasma membrane and hemoglobin. This made it an ideal candidate for membrane studies. Interestingly, when scientists looked at the lipid bilayer of the red blood cell, they found that the phospholipid composition of the individual monolayers was quite different. The monolayers exhibited lateral heterogeneity, where specific lipids and proteins cluster together in a patchwork fashion. This diversity was enhanced by the observation of transverse asymmetry of lipids and proteins in the two leaflets of the bilayer.

The outer monolayer contained phospholipids with choline in their polar head group such as phosphatidylcholine and sphingomyelin. Conversely, inner monolayer phospholipids were those with a terminal primary amino group, namely phospatidylserine, and phosphatidylethanolamine. The phosphatidyl inositols are also located on the cytosolic side of the bilayer. Cholesterol is distributed evenly throughout the two monolayers.

Although most phospholipids are neutral at physiologic pH, phosphatidylserine and phosphatidylinositol have a net negative charge at physiologic pH. Being present predominately in the inner leaflet, these two lipids generate a significant difference in charge between the two leaflets of the lipid bilayer. This generates a functionally relevant asymmetry in the membrane. In particular, membrane lipid asymmetry is important for signal transduction. Phosphatidyl serine is a binding partner for signaling proteins such as protein kinase C. However, the appearance of phosphatidyl serine on the outer leaflet of the cell membrane is an indication of a loss of membrane integrity. Extracellular expression of phosphatidyl serine targets the cell for engulfment by macrophages and is widely used as a diagnostic marker for apoptosis. Maintaining membrane lipid asymmetry is therefore highly important for cell homeostasis.

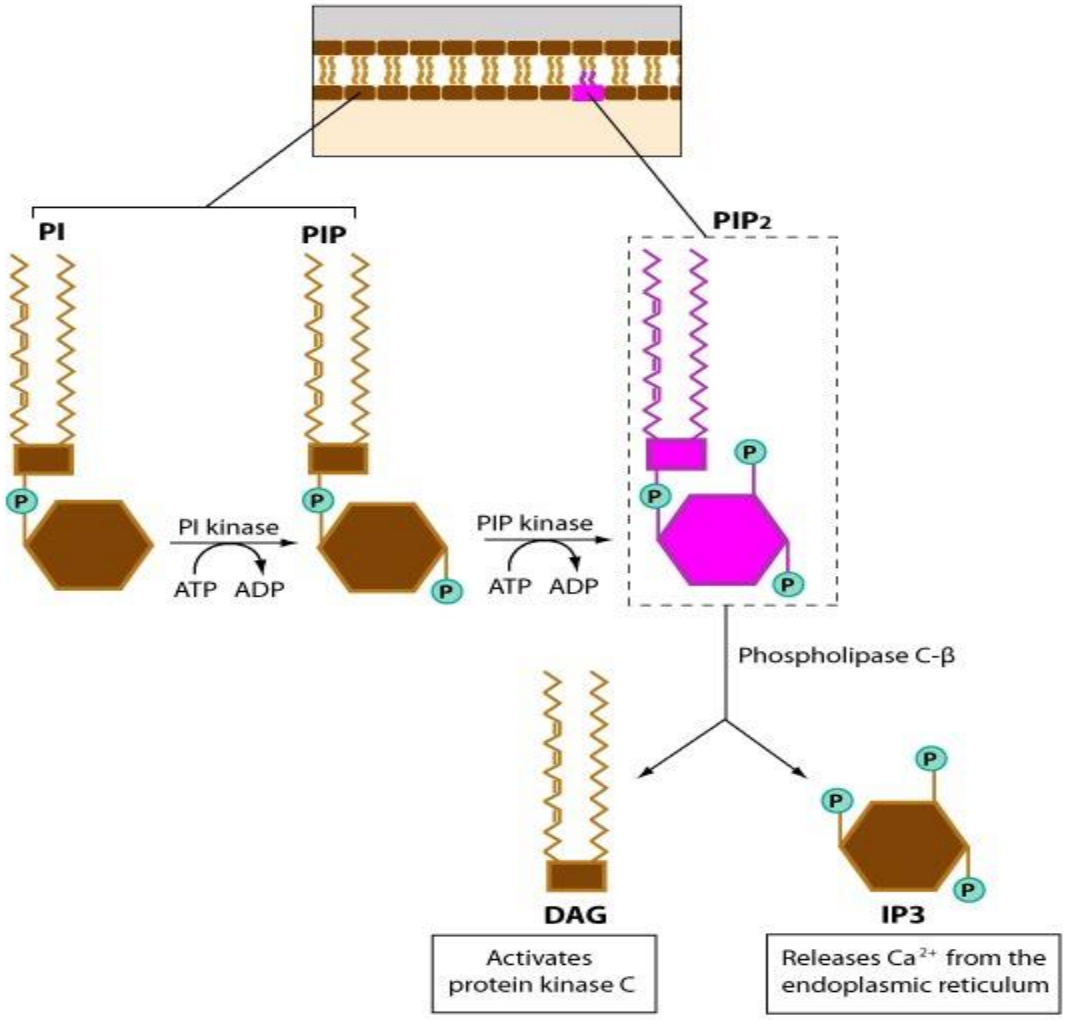


Figure. Phosphoinositides involved in cell signaling: This figure gives an example of how

different phosphoinositides are produced from phosphatidylinositol by the action of two kinases (e.g., PI kinase and PIP kinase) and it shows how signaling molecules (e.g. PIP2, DAG, IP3) are generated. It should be noted that a number variations exist for which hydroxyl groups are phosphorylated, and not surprisingly, a number of kinases are involved in these phosphorylation events. DAG= diacylglycerol; IP3=inositol triphosphate.

Three main types of phosphoinositides have important roles in intracellular signaling, lipid signaling, and membrane trafficking; these phosphoinositides differ solely in the number of phosphate groups that are attached by phosphoinositol kinases to the inositol ring

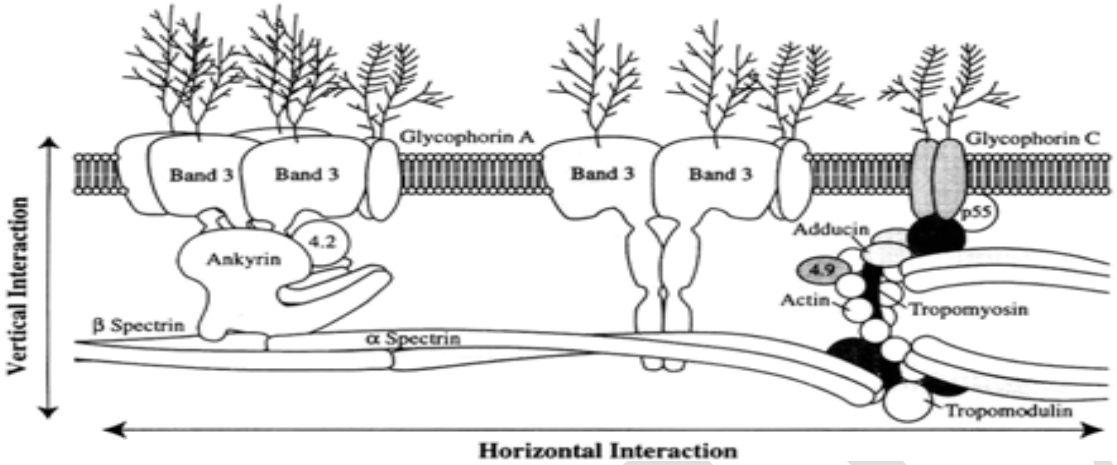
• Phosphatidylinositol-4-phosphate (PIP) – increased levels of PIP in the plasma membrane greatly reduces the F-**actin** binding and depolymerizing activity of **ADF** (actin depolymerizing factor).

• Phosphatidylinositol-4,5-bis-phosphate (PIP2) – increased levels of PIP2 in the plasma membrane inhibits actin filament capping by **capping protein** and greatly reduces the F-actin binding and depolymerizing activity of ADF.

• Phosphatidylinositol-3,4,5-trisphosphate (PIP3) – **Phosphatidylinositol-3-kinase** (PI3K) and **PTEN** (Phosphatase and tensin homolog) signal transduction pathways regulate the level of PIP3 in response to extracellular guidance cues during filopodia motility. The accumulation of PIP3 in filopodia is suggested to cause actin polymerization and increased cellular movement.

The membrane asymmetry in lipid and protein composition led to the proposal of the bilayer couple hypothesis. This states that the two monolayers of the membrane bilayer may respond differently to various forces while remaining coupled to each other. This hypothesis is the basis for the possible shape changes observed in membranes.

Proteins of RBC membrane



The red blood cell membrane consists three basic components: a lipid bilayer, transmembrane (integral) proteins and a cytoskeletal network.

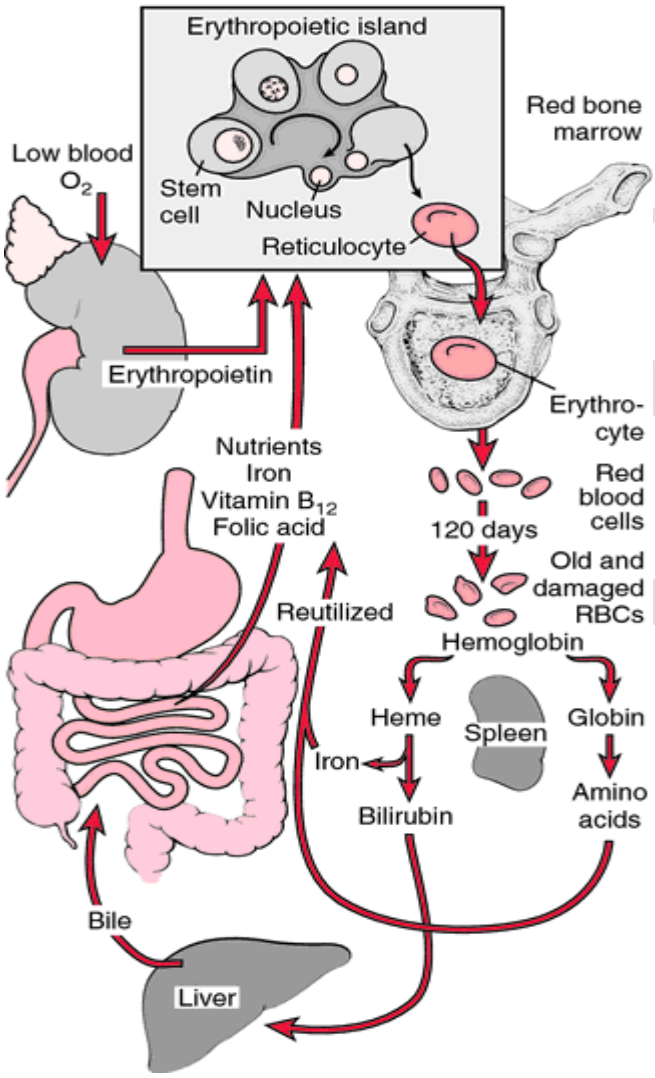
Lipid Bilayer

The lipid bilayer is a semipermeable, incompressible, two dimensional liquid crystal which is asymmetric in composition and separates the cytoplasm, within the cell, from the extracellular medium. Phosphatidylcholine (PC), sphingomyelin and the sterol cholesterol are the dominant extra leaflet components while phosphatidylserine (PS) and phosphatidylethanolamine (PE) are the dominant inner leaflet components. Interestingly enough there are proteins in the bilayer called flippases which maintain the correct lipid asymmetry.

Transmembrane Proteins

Transmembrane proteins are solutes in a two dimensional fluid, the bilayer, and thus have varying degrees of lateral mobility in the plane of the membrane. Dynamics. They have a transbilayer domain and either or both an extrafacial domain, which contributes to the glycocalyx and a cytoplasmic domain. The major transmembrane proteins are glygoproteins, band 3 and glygophorin. Band 3 is a multispanning ion transport channel and exists in a dimer / tetramer equilibrium. It is structurally important because band 3 tetramers, rather than dimers, tether the bilayer to the skeleton via an interaction between its cytoplasmic domain and ankyrin which is associated with spectrin. Glycophorins have a single spanning alpha helix and are a general class of proteins which contribute the major portion of glycosylation (sugar) at the extracellular domain. Glycophorin C (GPC) is another bilayer / skeleton tethering point via its interaction with protein 4.1 within the junctional complex. Glycophorin A (GPA) is partially associated with band 3. There are a host of other transmembrane proteins. Aquaporin, as the name suggests, is a

water channel protein existing as a homotetramer. Rh is a protein complex thought to associate with band 3. One class of proteins, which are not strictly transbilayer, are Glycosylphosphatidylinositol (GPI)-linked proteins. GPI is a lipid analog which links an extrafacial protein, for example, CD59.



The Cytoskeleton

The cytoskeleton is an irregular hexagonal lattice of polymeric spectrin molecules which are tied together by actin, 4.1 and other numerous proteins at nodes called junctional complexes. The skeleton makes a two dimensional network which is very flexible, compressible with obvious structural importance.

RBC ghosts

The functions of erythrocytes include transportation of oxygen and carbon dioxide. They owe their oxygen-carrying ability to hemoglobin, a combination of an iron-containing prosthetic group (heme) with a protein (globin). Hemoglobin attracts and forms a loose connection with free oxygen, and its presence enables blood to absorb some 60 times the amount of oxygen that the plasma by itself absorbs. Oxyhemoglobin is red, which gives oxygenated blood its red color. Erythrocytes are stored in the spleen, which acts as a reservoir for the blood system and discharges the cells into the blood as required. The spleen may discharge extra erythrocytes into the blood during emergencies such as hemorrhage or shock.

Erythrocytes also are important in the maintenance of a normal acid-base balance, and, since they help determine the viscosity of the blood, they also influence its specific gravity. Their average life span is 120 days. They are subjected to much wear and tear in circulation and

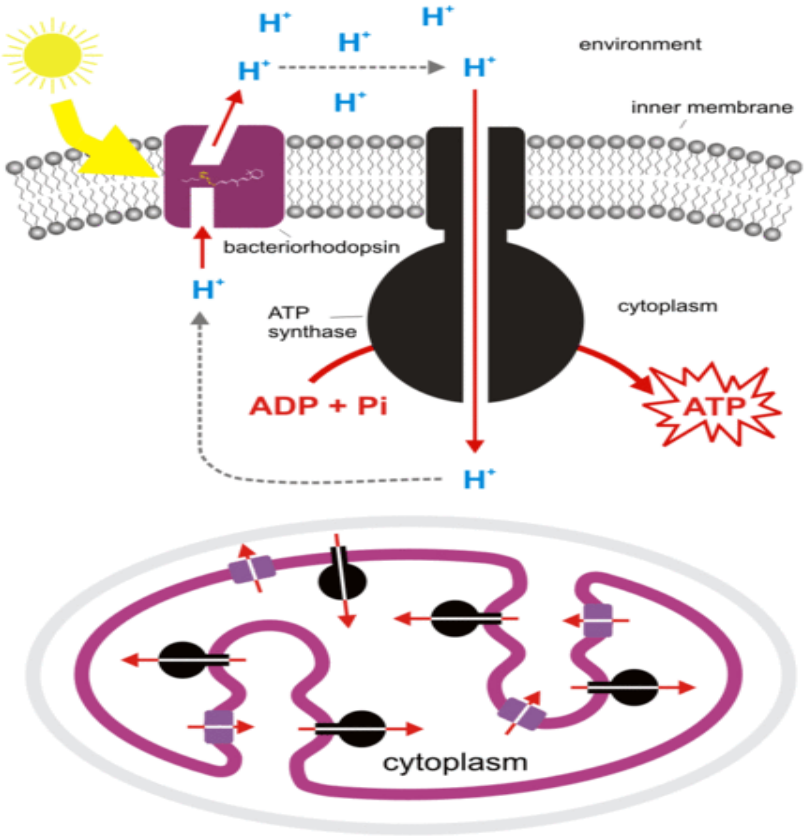
eventually are removed by cells of the reticuloendothelial system, particularly in the liver, bone marrow, and spleen. In spite of this constant destruction and production of erythrocytes, the body maintains a fairly constant number, between 4 and 5 million per mm3 of blood in women and 5 to 6 million per mm3 in men. A decreased number constitutes one form of anemia.

Erythrocytes are destroyed whenever they are exposed to solutions that are not isotonic to blood plasma. If they are placed in a solution that is more dilute than plasma (distilled water for example) the cells will swell until osmotic pressure bursts the cell membrane. If they are placed in a solution more concentrated than plasma, the cells will lose water and shrivel or crenate. It is for this reason that solutions to be given intravenously must be isotonic to plasma.

Aged red cells are ingested by macrophages in the spleen and liver. The iron is transported by the plasma protein transferrin to the bone marrow, where it is incorporated into new red cells. The heme group is converted to bilirubin, a bile pigment secreted by the liver. About 180 million red blood cells are destroyed every minute. Since the number of cells in the blood remains more or less constant, this means that about 180 million red blood cells are manufactured every minute.

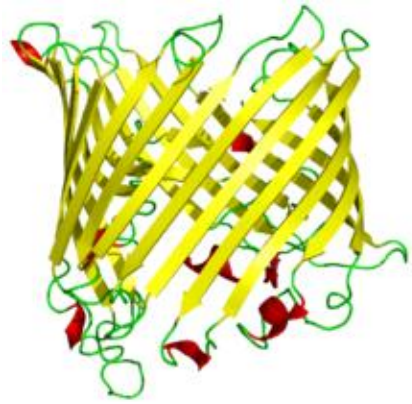
Determination of the red blood cell volume is usually done as a preliminary step in determination of the total blood volume. A radioactive substance, usually chromium, is used to "tag" cells of a sample of blood drawn from the patient. The sample is then reintroduced into the circulating blood and subsequent samples are taken to be evaluated for degree of radioactivity. The degree of dilution is used to calculate total blood volume.

Bacteriorhodopsin is a protein used by Archaea, most notably by Halobacteria, a class of the Euryarchaeota. It acts as a proton pump; that is, it captures light energy and uses it to move protons across the membrane out of the cell. The resulting proton gradient is subsequently converted into chemical energy. **Purple membrane** (PM) is a part of cytoplasmic **membrane** in certain extreme halophilic microorganisms belonging to Domain Archaea. It transduces light energy to generate proton gradient for ATP synthesis in the microorganisms.



Porins

Porins are beta barrel proteins that cross a cellular membrane and act as a pore, through which molecules can diffuse. Unlike other membrane transport proteins,**porins** are large enough to allow passive diffusion, i.e., they act as channels that are specific to different types of molecules.



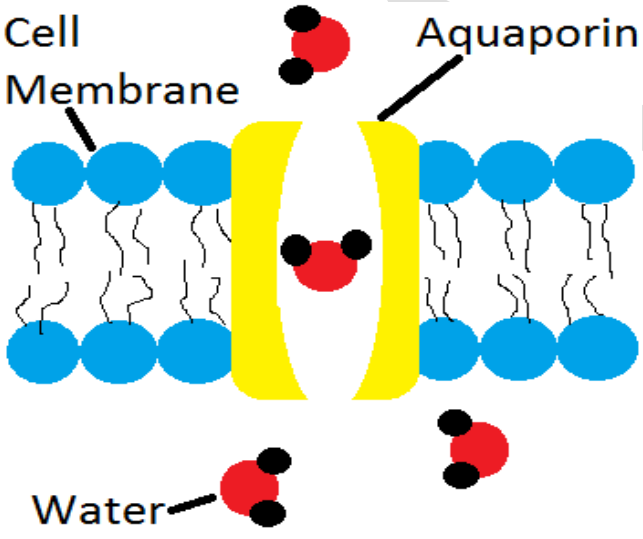
Porins are composed of β strands, which are, in general, linked together by beta turns on the cytoplasmic side and long loops of amino acidson the other. The β strands lie in an antiparallel fashion and form a cylindrical tube, called a β barrel. The amino acid composition of the porin β strands are unique in that polar and nonpolar residues alternate along them. This means that the nonpolar residues face outward so as to interact with the nonpolar lipids of outer membrane,

whereas the polar residues face inwards into the center of the beta barrel to create the aqueous channel.

The porin channel is partially blocked by a loop, called the eyelet, which projects into the cavity. In general, it is found between strands 5 and 6 of each barrel, and it defines the size of solute that can traverse the channel. It is lined almost exclusively with charged amino acids arranged on opposite sides of the channel, creating a transversal electric field across the pore. The eyelet has a local surplus of negative charges from four glutamic acid and seven aspartic acid residues (in contrast to one histidine, two lysine and three argininesidues) is partially compensated for by two bound calcium atoms, and this asymmetric arrangement of molecules is thought to have an influence in the selection of molecules that can pass through the channel.

Aquaporins

Aquaporins (AQP) are integral membrane proteins that serve as channels in the transfer of water, and in some cases, small solutes across the membrane. They are conserved in bacteria, plants, and animals. Structural analyses of the molecules have revealed the presence of a pore in the center of each **aquaporin** molecule.



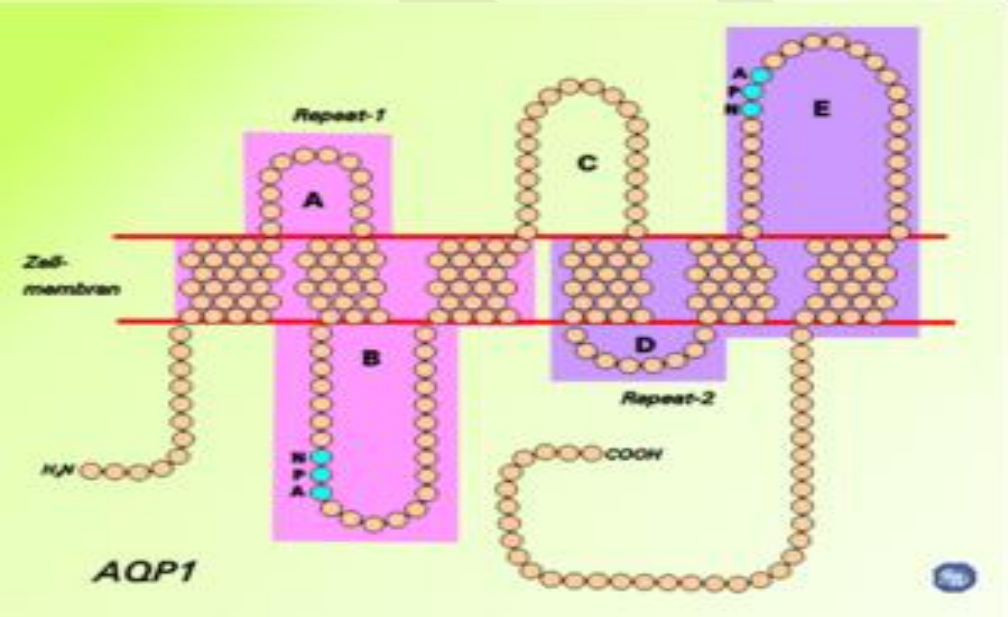
Aquaporin proteins are composed of a bundle of six transmembrane α-helices. They are embedded in the cell membrane. The amino and carboxyl ends face the inside of the cell. The amino and carboxyl halves resemble each other, apparently repeating a pattern of nucleotides. Some researchers believe that this was created by the doubling of a formerly half-sized gene.

Between the helices are five regions (A – E) that loop into or out of the cell membrane, two of them hydrophobic (B, E), with an asparagine–proline–alanine ("NPA motif") pattern. They create a distinctive hourglass shape, making the water channel narrow in the middle and wider at each end.

Another and even narrower place in the channel is the "ar/R selectivity filter", a cluster of amino acids enabling the aquaporin to selectively let through or block the passage of different molecules.

Aquaporins form four part clusters in the cell membrane, with each of the four monomers acting as a water channel. Different aquaporins have different sized water channels, the smallest types allowing nothing but water through.

X-ray profiles show that aquaporins have two conical entrances. This hourglass shape could be the result of a natural selection process toward optimal permeability. It has been shown that conical entrances with suitable opening angle can indeed provide a large increase of the hydrodynamic channel permeability.



Protein solubilization is the process of breaking interactions involved in protein aggregation, which include disulfide bonds, hydrogen bonds, van der Waals forces, ionic interactions, and

hydrophobic interactions. If these interactions are not prevented, proteins can aggregate or precipitate, resulting in artifacts or sample loss. For successful electrophoretic separation, proteins must be well solubilized. This section describes various agents used for protein solubilization, their method of action, and their compatibility to different types of samples. It also provides troubleshooting tips.

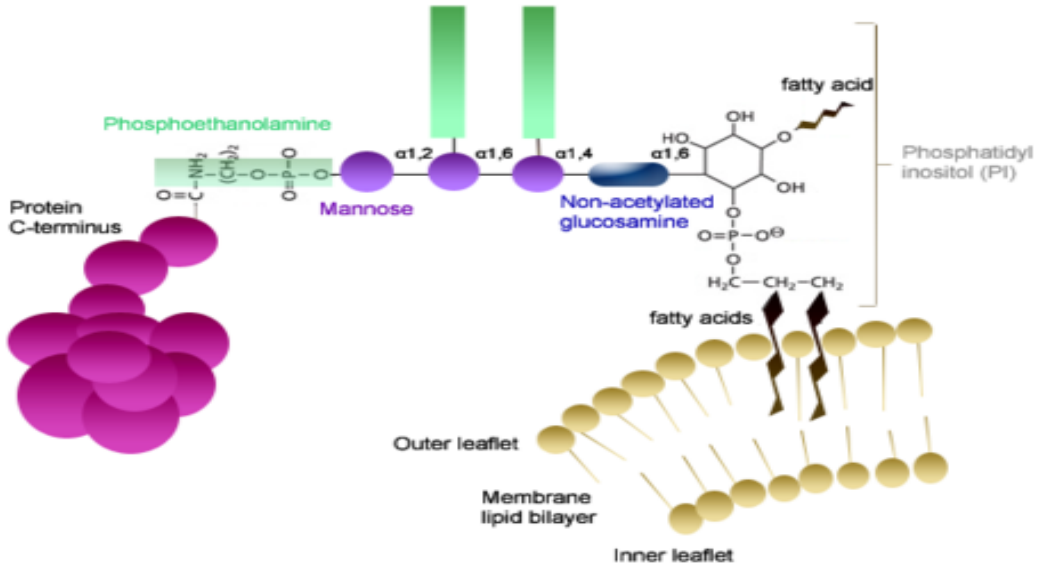
Lipid-anchored proteins (also known as **lipid-linked proteins**) are proteins located on the surface of the cell membrane that are covalently attached to lipids embedded within the cell membrane. These proteins insert and assume a place in the bilayer structure of the membrane alongside the similar fatty acid tails. The lipid-anchored protein can be located on either side of the cell membrane.Thus, the lipid serves to anchor the protein to the cell membrane.

The lipid group plays a role in protein interaction and can contribute to the function of the protein to which it is attached. Furthermore, the lipid serves as a mediator of membrane associations or as a determinant for specific protein-protein interactions.[3] For example, lipid groups can play an important role in increasing molecular hydrophobicity. This allows for the interaction of proteins with cellular membranes and protein domains.

Overall, there are three main types of lipid-anchored proteins which include **prenylated proteins**, **fatty acylated proteins** and **glycosylphosphatidylinositol-linked proteins (GPI)**. A protein can have multiple lipid groups covalently attached to it, but the site where the lipid binds to the protein depends both on the lipid group and protein.

GPI Proteins

Glycosylphosphatidylinositols (GPI) proteins are attached to a GPI complex molecular group via an amide linkage to the protein's C-terminal carboxyl group. This GPI complex consists of several main components that are all interconnected: a phosphoethanolamine, a linear tetrasaccharide (composed of three mannose and a glucosaminyl) and a phosphatidylinositol. The phosphatidylinositol group is glycosidically linked to the non-N-acetylated glucosamine of the tetrasaccharide. A phosphodiester bond is then formed between the mannose at the nonreducingend (of the tetrasaccaride) and the phosphoethanolamine. The phosphoethanolamine is then amide linked to the C-terminal of the carboxyl group of the respective protein. The GPI attachment occurs through the action of GPI-transamidase complex. The fatty acid chains of the phosphatidylinositol are inserted into the membrane and thus are what anchor the protein to the membrane.[19] These proteins are only located on the exterior surface of the plasma membrane.



Roles and Function

The sugar residues in the tetrasaccaride and the fatty acid residues in the phosphatidylinositolgroup vary depending on the protein. This great diversity is what allows the GPI proteins to have a wide range of functions including acting as hydrolytic enzymes, adhesion molecule, receptors, protease inhibitor and complement regulatory proteins. Furthermore, GPI proteins play an important in embryogenesis, development, neurogenesis, the immune system and fertilization. More specifically, the GPI protein IZUMO1R/JUNO (named after the Roman goddess of fertility) on the egg plasma has an essential role in sperm-egg fusion. Releasing the IZUMO1R/JUNO GPI protein from the egg plasma membrane does not allow for sperm to fuse with the egg and it is suggested that this mechanism may contribute to the polyspermy block at the plasma membrane in eggs. Other roles that GPI modification allows for is in the association with membrane microdomains, transient homodimerization or in apical sorting in polarized cells.

Lectins and selectins

Lectins are proteins that recognize and bind specific carbohydrates found on the surfaces of cells. They play a role in interactions and communication between cells typically for recognition. Carbohydrates on the surface of one cell bind to the binding sites of lectins on the surface of another cell. Binding results from numerous weak interactions which come together to form a strong attraction. A lectin usually contains two or more binding sites for carbohydrate units. In

addition, the carbohydrate-binding specificity of a certain lectin is determined by the amino acid residues that bind the carbohydrate. Lectins are specific carbohydrate-binding proteins: - Enormous diversity of carbohydrates have biological significance: Different monosaccharides can be joined to one another through any several -OH groups. Extensive branching is possible. Many more different oligosaccharides can be formed from 4sugars than oligopeptides from 4 amino acids - Lectins promote interactions between cells: Lectin is to facilitate cell-cell contact Lectin and carbohydrates are linked by a number of weak non-covalent interactions C-type(calcium required): calcium ion on the protein acts a bridge between protein and sugar through direct interactions with sugar -OH groups Carbohydrates-binding specificity of a particular lectin is determined by the amino acid residues that bind the carbohydrates. - Influenza virus binds to Sialic acid residues: Influenza virus recognizes sialic acid residues linked to galactose residues that are present on cell-surface glycoproteins. These carbohydrates are bounded to hemagglutinin, viral protein. (virus is engulfed by the cell and starts to replicate) Neuraminidase, enzyme proteins that cleaves the glycosidic bonds to sialic acid residues of hemogglutin, free virus to infect new cells and spreading the infection.

Lectin Binding

Lectins are capable of binding to many different types of carbohydrates. Because of this capability, the way that a lectin binds to carbohydrates, the materials necessary for binding, and the strength of the bond varies. Some of the various forms of binding are discussed below.

-Monosaccharides and disaccharides have shallow grooves to which lectins bind, making the affinity of the bond low. Because of the difficulty that lectins face when binding to these carbohydrates, a subsite multivalency (which is a spatial extension of the grooves) is necessary to achieve binding. This extension makes it so that the contact site on the carbohydrate is embedded into a more complex contact region. This type of binding works most efficiently with small lectins, as evidenced by the lectin, hevein, which is only 43 amino acids long. Rapid binding kinetics also facilitate the binding of lectins to carbohydrates. An example of this is the binding of sialyl Lewisx (a tetrasaccharide) to P-selectin. Rapid binding kinetics allows for spatial complementarity to be reached between a low-energy conformation of the carbohydrate and the prearranged binding site of the lectin.

-The shape of the binding sites in carbohydrates plays a factor in its bondage to lectins. An example of this is the case of galectin-1 binding to ganglioside GM1 (a pentasaccharide). Nuclear Magnetic Resonance and other molecular modeling techniques were used to analyze the bond between these two molecules. The images found showed that two branches of the

carbohydrate are bonded to the lectin. The α2, 3-sialylgalactose linkage is able to adopt three different, low-energy conformers. One of these conformers is energetically favorable for the binding of galectin- to ganglioside GM1. This process is evidence that lectins prefer certain conformations (shapes) when deciding how to bind to a carbohydrate. This evidence shows that oligosaccharides have limited flexibility. This limited flexibility makes oligosaccharides very favorable ligands, seeing as they avoid entropic penalties.

-Core substitutions have been found to occur in N-glycans. These substitutions are added to specific positions on the carbohydrate during its course to being assembled. These substitutions have been found to prominently affect the properties of glycans. The glycan properties are so affected, that they do not even need to be in the presence of lectins in order to be noticed. These substitutions, resulting in changes of certain parts of the carbohydrate, act as molecular switches governing the shape of glycans.

-Branching also introduces molecular switches. This property is most exemplified in the glycoside cluster effect. Enhancing the numerical valency of a molecule results in an increase in affinity. The type of branching appears to have a significant effect on this increase in affinity.

Importance of Carbohydrates in Cell Communication

Carbohydrates contain abundant information as a result of the various composition and structures that are possible. These diverse compounds result from the many OH groups available for linkage, which further allow for extensive branching. Additionally, the substituent attached to the anomeric carbon can assume either an alpha or beta configuration. The presence of these various carbohydrates on cell surfaces allows for effective cell-to-cell communication.

Functions of Lectins

Lectins are known to be very widespread in nature. They can bind to soluble carbohydrates or carbohydrate functional groups that are a part of a gylcoprotein or glycolipid. Lectins typically bind these carbohydrates with certain animal cells and sometimes results in glycoconjugate precipitation.

In animals, lectins regulate the cell adhesion to glycoprotein synthesis, control protein levels in blood, and bind soluble extracellular and intracellular glycoproteins. Also, in the immune system, lectins recognize carbohydrates found specifically on pathogens, or those that are not recognizable on host cells. Clinically, purified lectins can be used to identify glycolipids and glycoproteins on an individual's red blood cells for blood typing.

C-Type Lectins

C-Type lectins are those that require a calcium ion. The calcium ion helps bind the protein and carbohydrate by interacting with the OH groups found on the carbohydrate. Calcium can also form a linkage between the carbohydrate and glutamates in the lectin. Binding is further strengthened through hydrogen bonds that form between the lectin side chains and the OH groups of the carbohydrate. Carbohydrate recognition and binding is made possible by a homologous domain consisting of 120 amino acids. These amino acids determine the specificity of carbohydrate binding.

C Type lectins carry a wide range of functions such as cell to cell adhesion, immune response to foreign bodies and self-cell destruction. C Type lectins are categorized into various different subgroups specific to the different protein functional domains. These lectins are calcium ion dependent and share linear structural homology in their carbohydrate-recognition domains. Among Eukaryotes and the animal kingdom, this wide range of protein families including endocytic receptors, collectins, and selectins is found most abundantly. The differences in members of the family vary in the different kinds of carbohydrate complexes that are recognized with high polarity and affinity. C type lectins are involved with immune defense mechanisms and help protect an organism against tumorous cells.

P-Type Lectins

P-Type lectins contain a phosphate group. CD-MPR and CI-MPR are the only two members of the P-lectin family, cation-dependent and cation-independent. The main function of P-type lectins in eukaryotic cells involves delivering newly synthesized soluble acid hydrolyses to the lysosome. They do this by binding to mannose 6-phosphate residues found on the N-linked oligosaccharides of the hydrolyses.

MPRs (Mannose-6-phosphate receptors) were discovered when studies on mucolipidosis II, a lysosomal storage disorder, were conducted. Hickman and Neufeld found that fibroblasts from ML II patients were able to absorb lysosomal enzymes excreted by normal cells, whereas fibroblasts from normal patients were not able to absorb the lysosomal enzymes. Hickman and Neufeld hypothesized that the lysosomal enzymes had a recognition tag that allowed for receptor-mediated uptake and transport to lysosomes. These tags later became known as MPRs.

CI-MPR is about 300 kDA and exists as a dimer. The overall folding of CI-MPR is similar to that of CD-MPR, but unlike CD-MPR, CI-MPR is cation-independent. In addition, CI-MPR binds to proteins that have the MPR tag, IFG-II (a peptide hormone), and other non lysosomal

hydrolases. The N-terminal three domains of CI-MPR exists as a monomer, and forms a tri-lobed disk that has significant contact with one another. This attribute of the tri-lobed disk is vital in maintaining the structure of its sugar binding site. Phosphorylated Glycan Microarray demonstrates that CI-MPR shows little disparity between glycans having one or two phosphomonoesters when it comes to binding. This is unlike CD-MPR, which has been shown to have affinity towards glycans with two phosphomonoesters. In addition, CI-MPR binds to ligands at the cell surface, unlike CD-MPR. Overall, all of the ligand binding sites of CI-MPR are located on the odd-numbered domains. Four signature residues in CD-MPR and domain 3 of CI-MPR are conserved, and have been found to react with Man-6-P in the same manner, suggesting that the Man-6-P binding pockets are similar. One difference that has been found is the fact that the pocket in CD-MPR contains Mn 2+, whereas the binding pocket in CI-MPR does not. This could be the reason why CI-MPR is cation-independent.

CD-MPR is a 46 kDA cation-dependent homodimer. Three disulfide linkages formed by six cysteine residues in the extracellular region of CD-MPR are key to the folding of the homodimer. Because the 15 contiguous domains of the extracystolic region are similar in size and amino acid sequence when compared to each other, it is understood that CD-MPR and CI-MPR have similar tertiary structures. In fact, CD-MPR domains 1, 2, 3, 11, 12, 13 and 14 of CI-MPR have the same fold in the extracystolic domain. The overall fold of the CD-MPR monomer consists of a flattened beta barrel consisting of two antiparallel beta sheets, one with four strands, and the other with five strands. The CD-MPR dimer consists of two five stranded antiparallel beta sheets. E133, Y143, Q66, and R111 have been found to be essential in Man-6-P binding via mutagenesis studies of CD-MPR. CD-MPR's binding and unbinding mechanism is similar to that of the oxy-to-deoxy transition of hemoglobin. The overall movement has been described as to be a "scissoring and twisting" motion in between the two subunits of the dimer interface. These two subunits are connected via a salt bridge. Absence of this salt bridge results in a weaker bind with lysosomal enzymes, signaling the importance of ionic interactions between the two subunits in binding.

Selectins

Selectins are a type of C-Type lectins that play a role in the **immune system**. Selectins consist of L, E, and P forms that bind to carbohydrates found on lymph-node vessels, endothelium, and activated blood platelets. They behave analogously to C type lectins in that both have a high affinity for calcium binding and are responsible for immune responses. Selectins are sugar binding polymers that are adhesive among other cells which causes it to be highly effective in targeting an inflammatory response for a localized region. Selectins target only specific kinds of

binding sites, but thus allows it to be effective in conjunction with leukocyte cascading to minimize invasively targeting an infected region.

Examples of Lectins

Embryos are attached to the endometrium of the uterus through L-Selectin. This activates a signal to allow for implantation.

E. coli are able to reside in the gastrointestinal tract by lectins that recognize carbohydrates in the intestines.

The influenza virus contains hemagglutinin which recognizes sialic acid residues on the glycoproteins located on the surface of the host cell. This allows the virus to attach and gain entry into the host cell.

POSSIBLE QUESTIONS

2 marks

1. Write a note on transmembrane domain.
2. Which CHO are abundant in Plasma memberane? Give the importance.
3. Give the importance of lipid anchored proteins.
4. Write about lectins and selectins.
5. What is meant by flippases?
6. Write note on liposomes.

6 marks

1. Discuss in detail about membrane bilayer models.
2. Give an account on orientation of membrane proteins and the mobility experiments.
3. Explain the following,
    i) Membrane lipids
    ii) Liposomes
4. Explain about membrane proteins and their types in detail.
5. Detailed note on proteins of RBC membrane and RBC ghost.

6. Describe about solubilisation of proteins and lipid anchored proteins.
7. Discuss about cell surface carbohydrates.

KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
I-M.Sc., BIOCHEMISTRY
CELLULAR BIOCHEMISTRY (18BCP104)
MULTIPLE CHOICE QUESTIONS

UNIT I

S.No	Questions	Option A	Option B	Option C	Option D	Answer
1						
2	The other name of sandwitch model	Protein lipid protein model	Lock & key model	Fluid mosaic model	Unit membrane model	Protein lipid protein model
3	Aquaporin protein ___ the permeability of cells to water	Increases	Decreases	Stable	None of the above	Increases
4	Uabin is potent inhibitors of _____ transport	Na^+K^+	Na^+Ca^{2+}	K^+Ca^{2+}	Fe^{2+} K^+	Na+K+
5	_____ is found in kidney	Allopurinol	Azaserine	Aquaporin	'E'porin	Aquaporin
6	_____proteins located outside ,but covalently linked to lipid within bilayer	Integral protein	Peripheral protein	Lipid anchored protein	None of the above	Lipid anchored protein
7						
8	____ an integral protein with a single transmembrane domain	Glycoptn	GlycophorinA	GlycophorinB	Glycolipid	GlycophorinA
9	Integral proteins are bound to membrane by _____	Ionic bond	Covalent bond	Hydrogen bond	Disulphide bond	Covalent bond
10	Which CHO are abundant in Plasma memberane?	Glycocerebroside	Ganglioside	Cerebroside	Galactosamide	Ganglioside
11	Blood group antigens are present in ____	Within the cell	Outside the cell	Embedded in cell membrane	Anchor in cell membrane	Embedded in cell membrane
12	How many antigens are present in cell membrane	2	3	4	1	2
13	Cell surface antigens are ____	Phospholipid	Cerebroside	Glycoptn	Sphingomyelin	Glycoptn
14	Which one is differ from A antigen and B antigen	N-Propyl	N-acetyl	N-lactyl	Protein	N-acetyl
15	___ contains galactolipids.	Mitochondria	Chloroplast	Golgi complex	Endoplasmic reticulum	Chloroplast
16	Artificial bilayers can also be formed as spherical vesicles called____	Liposomes	Golgi complex	Plasma membrane	Mitochondria	Liposomes
17	Lipid across open a thin film which spontaneously thins to less than _____	8nm	10nm	5nm	12nm	10nm
18	In Glycoptn, the CHO is present in having fewer than about ____per chain	20 sugars	35 sugars	15 Sugars	10 Sugars	15 Sugars
19	The protein spectrin present only on the inner surface of _____	RBC	WBC	Platelets	None of the above	RBC
20	The lipids conjugated with CHO that present in membrane bilayer is	Glycolipid	Phospholipid	Ceramide	Sphingomyelin	Glycolipid
21	Which protein present only on the inner surface of RBC	Spectrin	Integral protein	Both a&b	None	Spectrin
22	Which Ptns are more stable.	Non-cytosolic ptn	Cytosolic ptn	cysteine residue ptn	None of the above	Non-cytosolic ptn
23	Which organelles have unit membrane structure	Lysosome	Mitochondria	Golgi body	All the above	All the above
24						
25	PLP model are otherwise called as	Fluid mosaic model	Lock and Key model	Unit membrane model.	Sanwitch model	Sanwitch model
26	If water enter into the cell this stage is known as	Exo osmosis	Endo Osmosis	Iso osmaosis	Pseudos osmosis.	Endo Osmosis
27	If water exit, in to the cell, this stage is known as	Exo oxmosis	Endo oxmosis	Iso oxmosis	Pseudo oxmosis	Exo oxmosis
28	_____ protein increase the permibility of cell to water	aquaporin	E porin	Nucleo porin	all the above	aquaporin
29	Aquaporin is found in	Liver	Kidney	heart	Brain	Kidney
30	Which one is the potent inhibitors of Na/K transport	Alopurinol	Uabain	Azaserine	ATP binding cassette	Uabain
31	_____ have also been to play role in certain infectious disease	Glycolipids	Glycoptns	Lipoptn	Phospholipids	Glycolipids
32	The simulatones transport by a single transportor is known as	Co-transport	Na/K/ transport	both a &b	None	Na/K/ transport
33	The mobility of membrane lipids can be measured by	NMR	ESR	MALDI TOF	AAS	ESR
34	Cell membranes are _____ nature.	Hydrophilic	hydrophobic	Both a & b	None	hydrophobic
35	Which lipids are present in cell membranes.	cholesterol	free fatty acid	Tri glycerides	sphinomyelin	cholesterol
36	Chlorophast contains _____ lipids.	Galacto lipid	phopholipid	Glycolipid	All the above	Galacto lipid
37	Which organic solvent is used for preparation of biological membrance	Ethanol	Petroleum ether	chloroform	Methanol	chloroform
38	Which protein acts as anchor in bilayer	Integral protein	Intersinc Protein	Peripheral Protein	both a & b	both a & b
39	Which is used to remove proteins from a membrane	Dye	Radio isotopes	Detergent	Saline	Detergent
40	Name of the eye protein	Rodaxin	Xanthocynine	Both a & b	Opsin	Opsin
41	Which trans membrane protein have beta sheet	Bacterioradoxin	Rodaxin	Opsonin	porins	porins
42	Spectrin is a flexible fibrous protein approximately	100nm	50nm	75nm	90nm	100nm
43	The composed of a n alpha and Beta subunit coiled around called_____	Allopurinol	lectin	Spectrin	Aquaporin	Spectrin
44	The structure of the lipid bilayer depends on the _____	Temperature	Pressure	Viscocity	None of the above	Temperature
45	_____ in the distribution of integral membrane protein	Homogeneity	Microhomogeneity	Heterogeneity	Micro Heterogeneity	Heterogeneity
46	Cells respond to osmotic shock by taking up water and swelling termed as	Hemolysis	Erythropoiesis	Both a and b	None of the above	Hemolysis
47	_____first membrane protein to have its amino acid	Glyophorin a	Glycophorin b	Glycophorin c	Glycophorin D	Glyophorin a
48	The major component of the skeleton is _____	Spectrin	E porin	Aquaporin	Azaserine	Spectrin
49	Aquaporin is found in	Liver	Kidney	heart	Brain	Kidney
50	Which one is the potent inhibitors of Na/K transport	Alopurinol	Uabain	Azaserine	ATP binding cassette	Uabain

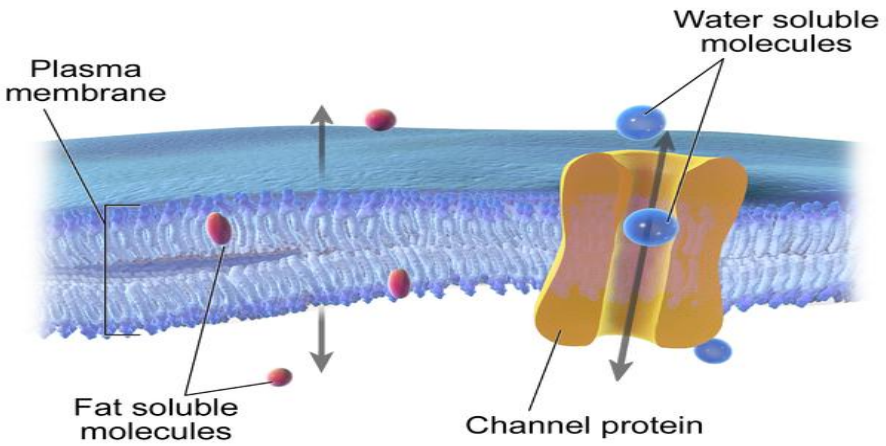
51	Fibronectin is secreted by….	kidney	liver	muscle	all	liver
52	Anchoring junction mechanically attach by….	Cytoskeletons	muscles	nerves	epithelial cells	Cytoskeletons
53	The abchor proteins include…..	tropomyosin	alpha-actin	vinculin	all	all
54	Desmosome consists of proteinaceous ashesion plaques of …….nm	15-25	15-20	20-25	35-40	15-20
55	Major types of cells junction are ….	tight junction	desmosome	gab junction	all the above	all the above
56	Desmosomes are thickened regions of the…..	plasma membrane	mitochondria	cytosol	nucleous	plasma membrane
57	Gap junction cannot synthesis….	d ATP from DNA	GTP from DNA	d GTP from RNA	d ATP fromRNA	d ATP from DNA
58	How many types of desmosomes are recognized…….	Three types	two types	five types	six types.	Three types
59	Adherens junctions also called as…..	Belt desmosomes	spot desmosomes	hemidesmosomes	desmocollin	Belt desmosomes
60	PLP stand for ____	Phospho lipase ptn	Protein lipid ptrotein	Phospho lipo protein	All the above	Protein lipid ptrotein

UNIT-II-SYLLABUS

Membrane transport: Passive diffusion, facilitated diffusion in erythrocytes, Carriers and ion channels, Ion concentration gradients.
Uniporter Catalyzed transport, active transport systems. Transport process driven by ATP- Ion pumps: Calcium ATP ase; Na^+ K^+ ATPase; Mechanism, Gastric H^+ K^+ ATPase, ABC superfamily – ATPases that transport peptides and drugs (MDR proteins).
Co-transport by Symporters and antiporters, Group translocation.
Osmosis,receptor mediated endocytosis and its significance.

Passive diffusion

Passive transport is a movement of ions and other atomic or molecular substances across cell membranes without need of energy input. Unlike active transport, it does not require an input of cellular energy because it is instead driven by the tendency of the system to grow in entropy. The rate of passive transport depends on the permeability of the cell membrane, which, in turn, depends on the organization and characteristics of the membrane lipids and proteins. The four main kinds of passive transport are simple diffusion, facilitated diffusion, filtration, and osmosis.

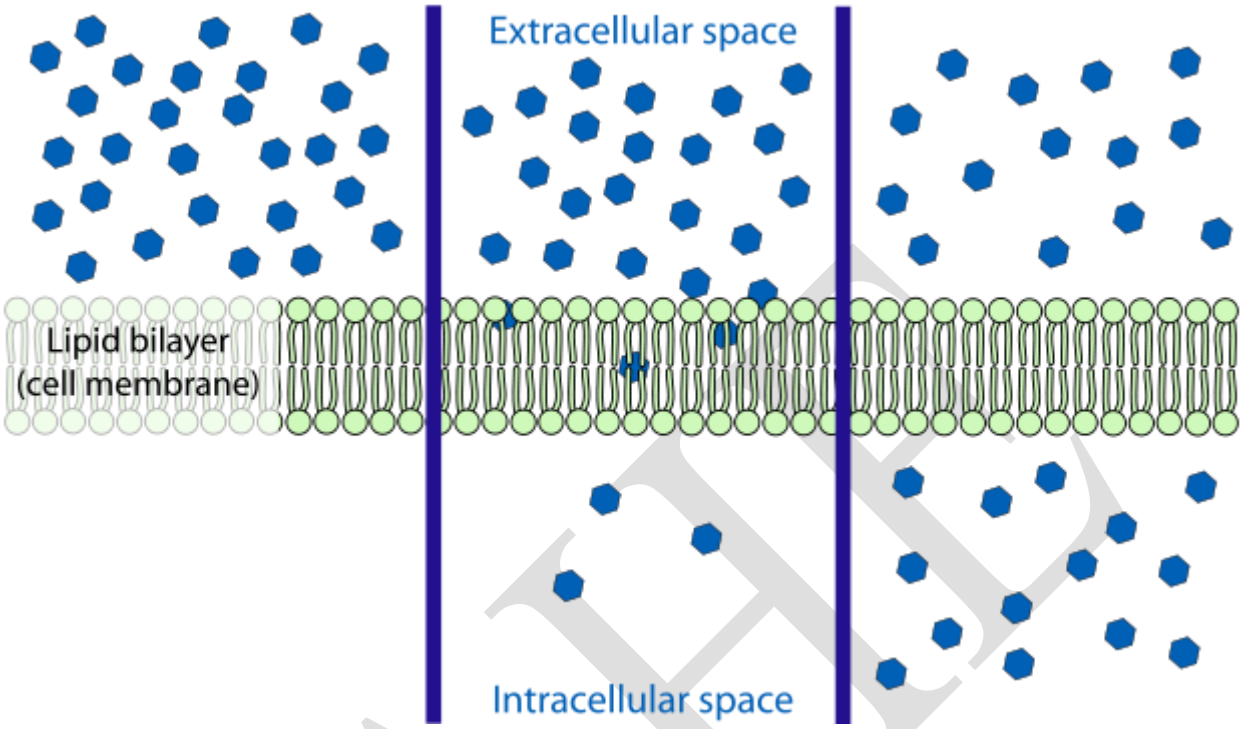


Diffusion Across the Plasma Membrane

Diffusion

Diffusion is the net movement of material from an area of high concentration to an area with lower concentration. The difference of concentration between the two areas is often termed as the *concentration gradient*, and diffusion will continue until this gradient has been eliminated. Since diffusion moves materials from an area of higher concentration to an area of lower concentration, it is described as moving solutes "down the concentration gradient" (compared with active transport, which often moves material from area of low concentration to area of higher concentration, and therefore referred to as moving the material "against the concentration gradient"). However, in many cases (e.g. passive drug transport) the driving force of passive transport can not be simplified to the concentration gradient. If there are different solutions at the two sides of the membrane with different equilibrium solubility of the drug, the difference in degree of saturation is the driving force of passive membrane transport. It is also true for supersaturated solutions which are more and more important owing to the spreading of the application of amorphous solid dispersions for drug bioavailability enhancement.

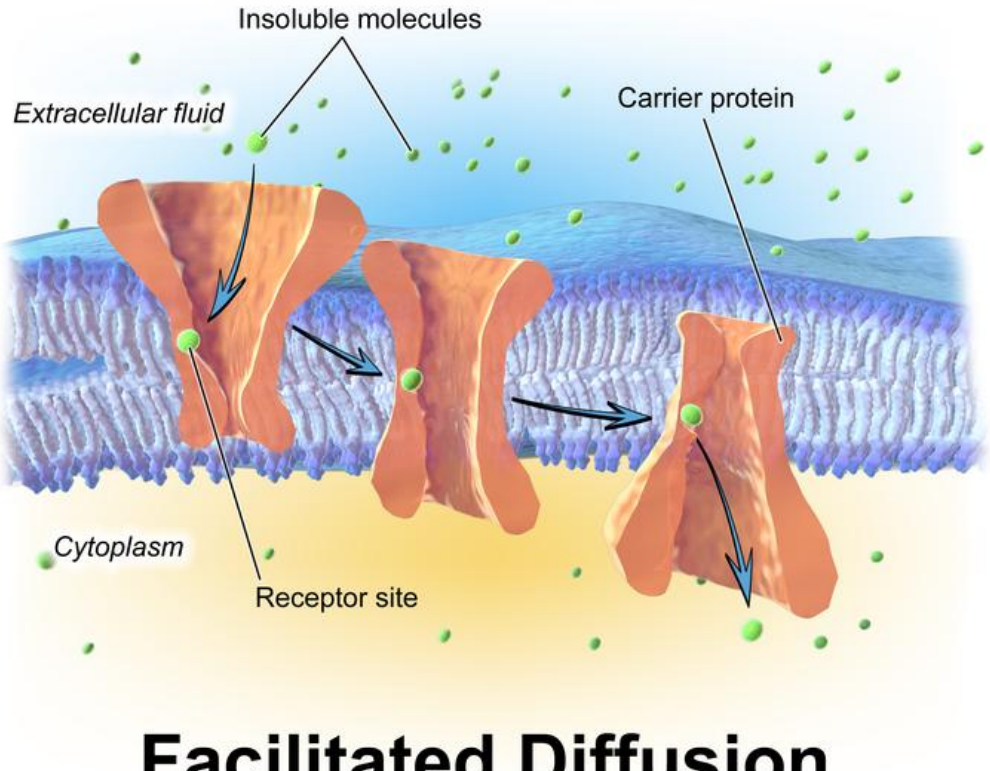
Simple diffusion and osmosis are in some ways similar. Simple diffusion is the passive movement of solute from a high concentration to a lower concentration until the concentration of the solute is uniform throughout and reaches equilibrium. Osmosis is much like simple diffusion but it specifically describes the movement of water (not the solute) across a selectively permeable membrane until there is an equal concentration of water and solute on both sides of the membrane. Simple diffusion and osmosis are both forms of passive transport and require none of the cell's ATP energy.



Facilitated diffusion

Facilitated diffusion, also called carrier-mediated osmosis, is the movement of molecules across the cell membrane via special transport proteins that are embedded within the cellular membrane. Large, insoluble molecules, such as glucose, vesicles and proteins require a carrier molecule to move through the plasma membrane. Therefore, it will bind with its specific carrier proteins, and the complex will then be bonded to a receptor site and moved through the cellular membrane. Facilitated diffusion is a passive process: the solutes move down their concentration gradient and do not require the expenditure of cellular energy for this process. Carrier proteins and channel proteins allow for the diffusion of molecules across the cell membrane. Carrier proteins undergo conformational alterations to allow molecules to pass, while channel proteins form unblocked pores.

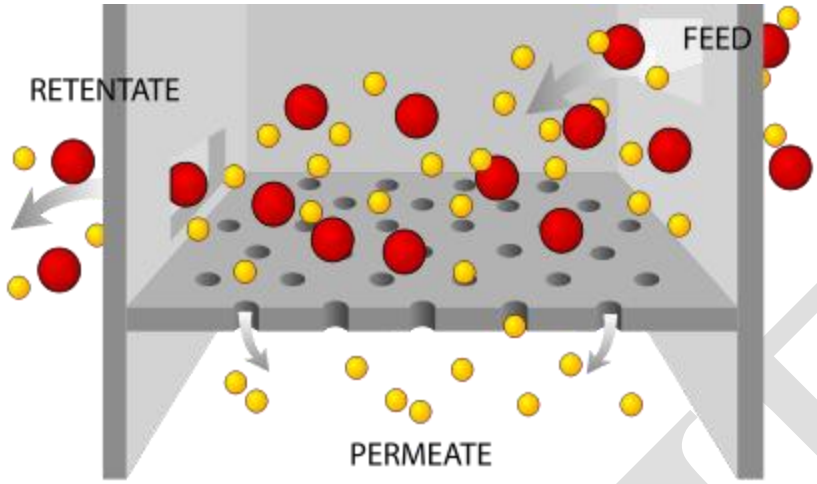
Facilitated diffusion may be achieved as a consequence of charge gradients in addition to concentration gradients. Plant cells create an unequal distribution of charge across their plasma membrane by actively taking up or excluding ions. Active transport of protons by H^+ ATPases alters membrane potential allowing for facilitated passive transport of particular ions such as Potassium down their charge gradient through high affinity transporters and channels.



Facilitated Diffusion

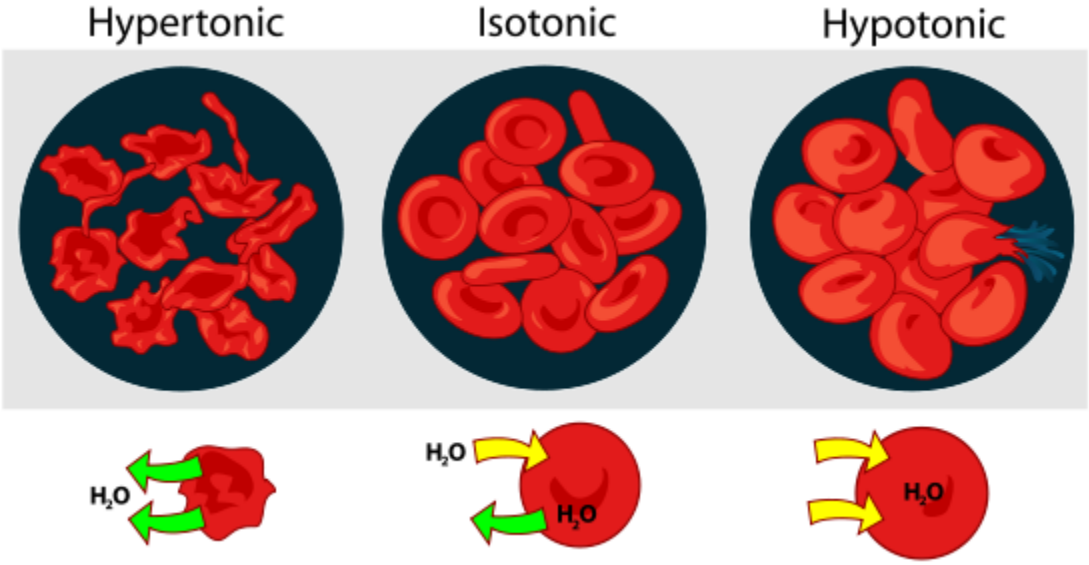
Filtration

Filtration is movement of water and solute molecules across the cell membrane due to hydrostatic pressure generated by the cardiovascular system. Depending on the size of the membrane pores, only solutes of a certain size may pass through it. For example, the membrane pores of the Bowman's capsule in the kidneys are very small, and only albumins, the smallest of the proteins, have any chance of being filtered through. On the other hand, the membrane pores of liver cells are extremely large,but not forgetting cells are extremely small to allow a variety of solutes to pass through and be metabolized.



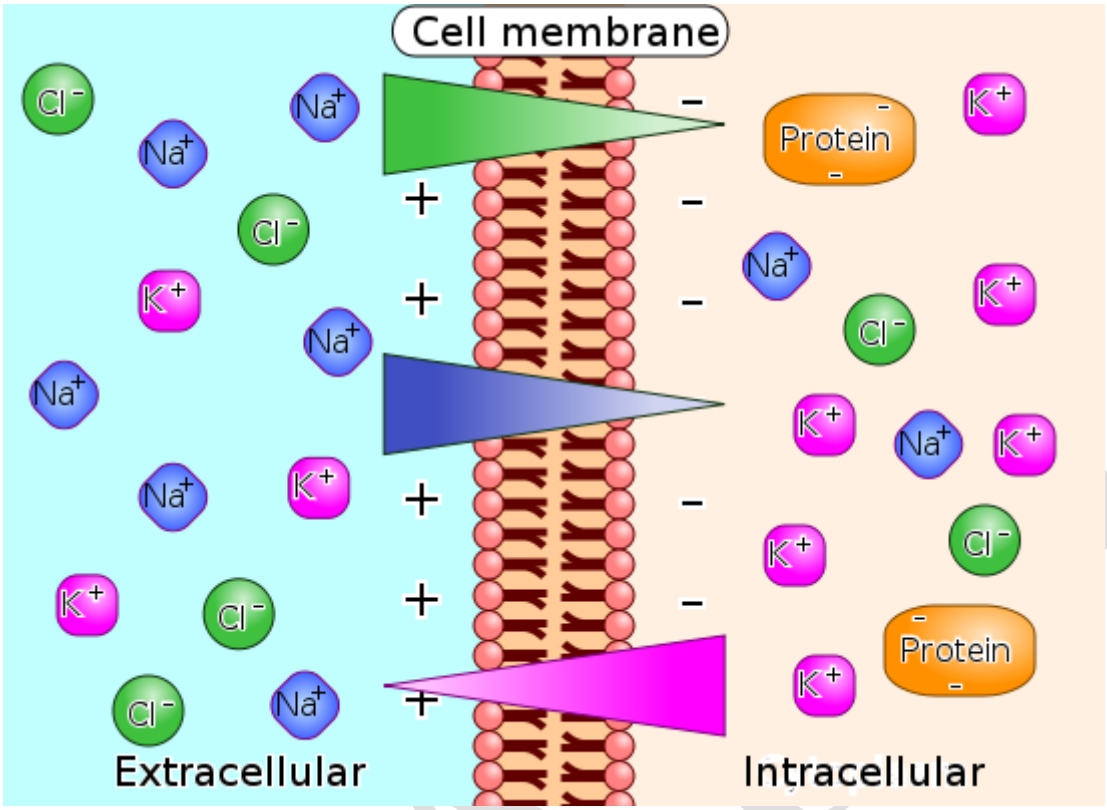
Osmosis

Osmosis is the movement of water molecules across a selectively permeable membrane. The net movement of water molecules through a partially permeable membrane from a solution of high water potential to an area of low water potential. A cell with a less negative water potential will draw in water but this depends on other factors as well such as solute potential (pressure in the cell e.g. solute molecules) and pressure potential (external pressure e.g. cell wall). There are three types of Osmosis solutions: the isotonic solution, hypotonic solution, and hypertonic solution. Isotonic solution is when the extracellular solute concentration is balanced with the concentration inside the cell. In the Isotonic solution, the water molecules still moves between the solutions, but the rates are the same from both directions, thus the water movement is balanced between the inside of the cell as well as the outside of the cell. A hypotonic solution is when the solute concentration outside the cell is lower than the concentration inside the cell. In hypotonic solutions, the water moves into the cell, down its concentration gradient (from higher to lower water concentrations). That can cause the cell to swell. Cells that don't have a cell wall, such as animal cells, could burst In in this solution. A hypertonic solution is when the solute concentration is higher (think of hyper - as high) than the concentration inside the cell. In hypertonic solution, the water will move out, causing the cell to shrink.

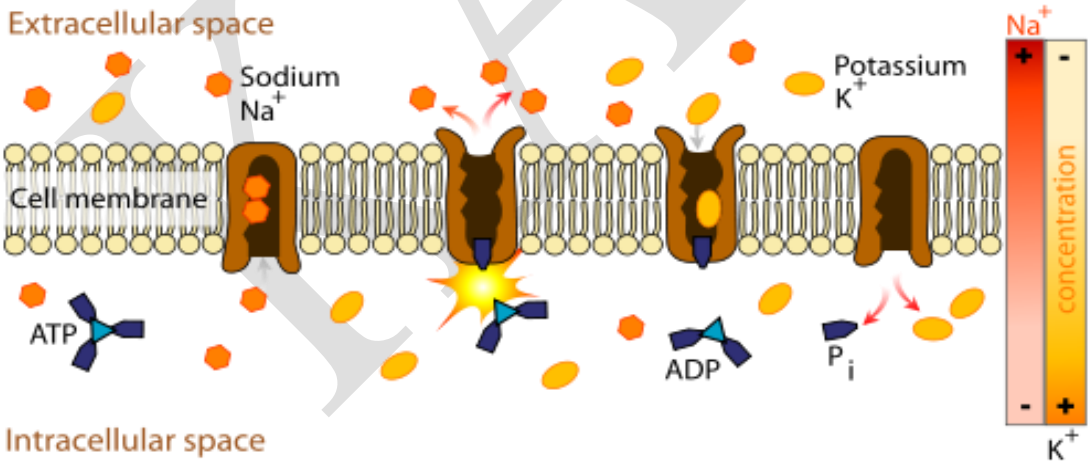


Electrochemical gradient

An electrochemical gradient is a gradient of electrochemical potential, usually for an ion that can move across a membrane. The gradient consists of two parts, the chemical gradient, or difference in solute concentration across a membrane, and the electrical gradient, or difference in charge across a membrane. When there are unequal concentrations of an ion across a permeable membrane, the ion will move across the membrane from the area of higher concentration to the area of lower concentration through simple diffusion. Ions also carry an electric charge that forms an electric potential across a membrane. If there is an unequal distribution of charges across the membrane, then the difference in electric potential generates a force that drives ion diffusion until the charges are balanced on both sides of the membrane.



Ion gradients

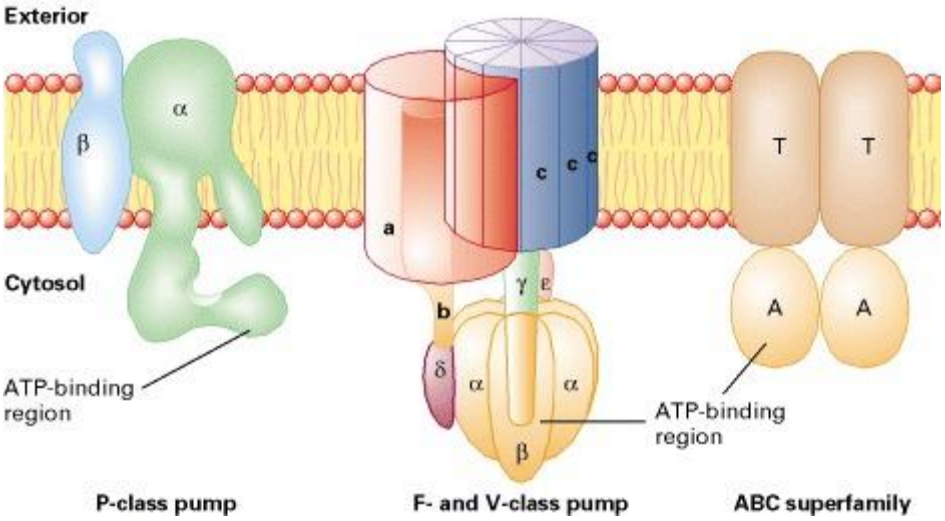


Since the ions are charged, they cannot pass through the membrane via simple diffusion. Two different mechanisms can transport the ions across the membrane: active or passive transport. An example of active transport of ions is the Na^+-K^+-ATPase (NKA). NKA catalyzes

the hydrolysis of ATP into ADP and an inorganic phosphate and for every molecule of ATP hydrolized, three Na^+ are transported outside and two K^+ are transported inside the cell. This makes the inside of the cell more negative than the outside and more specifically generates a V_m of about -60mV. An example of passive transport is ion fluxes through Na^+, K^+, Ca^{2+}, and Cl^- channels. These ions tend to move down their concentration gradient. For example, since there is a high concentration of Na^+ outside the cell, Na^+ will flow through the Na^+ channel into the cell. Since the electric potential inside the cell is negative, the influx of a positive ion depolarizes the membrane which brings the transmembrane electric potential closer to zero. However, Na^+ will continue moving down its concentration gradient as long as the effect of the chemical gradient is greater than the effect of the electrical gradient. Once the effect of both gradients are equal (for Na^+ this at a V_m of about +70mV), the influx of Na^+ stops because the driving force (ΔG) is zero.

Active Transport by ATP-Powered Pumps

We turn now to the ATP-powered pumps that transport ions and various small molecules against their concentration gradients. The general structures of the four principal classes of these transport proteins are depicted in Figures, and their properties are summarized in Table. Note that the P, F, and V classes transport ions only, whereas the ABC superfamily class transports small molecules as well as ions.



P-class pumps are composed of two different polypeptides, α and β, and become phosphorylated as part of the transport cycle. The sequence around the phosphorylated residue, located in the larger α subunits, is homologous among different pumps. F-class and V-class pumps do not form phosphoprotein intermediates. Their structures are similar and contain similar proteins, but none

of their subunits are related to those of P-class pumps. All members of the large ABC superfamily of proteins contain four core domains: two transmembrane (T) domains and two cytosolic ATP-binding (A) domains that couple ATP hydrolysis to solute movement. These core domains are present as separate subunits in some ABC proteins (depicted here), but are fused into a single polypeptide in other ABC proteins.

P-class ion pumps contain a transmembrane catalytic α subunit, which contains an ATP-binding site, and usually a smaller β subunit, which may have regulatory functions. Many of these pumps are tetramers composed of two α and two β subunits. During the transport process, at least one of the α subunits is phosphorylated (hence the label "P"), and the transported ions are thought to move through the phosphorylated subunit. This class includes the Na^+/K^+ ATPase in the plasma membrane, which maintains the Na^+ and K^+ gradients typical of animal cells, and several Ca^{2+} ATPases, which pump Ca^{2+} ions out of the cytosol into the external medium or into the lumen of the sarcoplasmic reticulum (SR) of muscle cells. Another member of the P class, found in acid-secreting cells of the mammalian stomach, transports protons (H^+ ions) out of and K^+ ions into the cell. The H^+ pump that maintains the membrane electric potential in plant, fungal, and bacterial cells also belongs to this class.

The structures of *F-class* and *V-class ion pumps* are similar to each other but unrelated to and more complicated than P-class pumps. F- and V-class pumps contain at least three kinds of transmembrane proteins and five kinds of extrinsic polypeptides that form the cytosolic domain. Several of the transmembrane and extrinsic subunits in F-class and V-class pumps exhibit sequence homology, and each pair of homologous subunits is thought to have evolved from a common polypeptide.

All known V and F pumps transport only protons in a process that does not involve a phosphoprotein intermediate. V-class pumps generally function to maintain the low pH of plant vacuoles and of lysosomes and other acidic vesicles in animal cells by using the energy released by ATP hydrolysis to pump protons from the cytosolic to the exoplasmic face of the membrane against the proton electrochemical gradient. F-class pumps are found in bacterial plasma membranes and in mitochondria and chloroplasts. In contrast to V pumps, they generally function to power the synthesis of ATP from ADP and P_i by movement of protons from the exoplasmic to the cytosolic face of the membrane down the proton electrochemical gradient. Because of their importance in ATP synthesis in chloroplasts and mitochondria, F-class proton pumps are treated separately in the next chapter.

The final class of ATP-powered transport proteins is larger and more diverse than the other classes. Referred to as the *ABC (ATP-binding cassette) superfamily*, this class includes more than

100 different transport proteins found in organisms ranging from bacteria to humans. Each ABC protein is specific for a single substrate or group of related substrates including ions, sugars, peptides, polysaccharides, and even proteins. All ABC transport proteins share a common organization consisting of four "core" domains: two transmembrane (T) domains, forming the passageway through which transported molecules cross the membrane, and two cytosolic ATP-binding (A) domains. In some ABC proteins, the core domains are present in four separate polypeptides; in others, the core domains are fused into one or two multidomain polypeptides.

All classes of ATP-powered pumps have one or more binding sites for ATP, and these are always on the cytosolic faceof the membrane (see Figure 15-10). Although these proteins are often called ATPases, they normally do not hydrolyze ATP into ADP and P_i unless ions or other molecules are simultaneously transported. Because of the tight coupling between ATP hydrolysis and transport, the energy stored in the phosphoanhydride bond is not dissipated. Thus ATP-powered transport proteins are able to collect the free energy released during ATP hydrolysis and use it to move ions or other molecules uphill against a potential or concentration gradient.

The energy expended by cells to maintain the concentration gradients of Na^+, K^+, H^+, and Ca^{2+} across the plasma and intracellular membranes is considerable. In nerve and kidney cells, for example, up to 25 percent of the ATP produced by the cell is used for ion transport; in human erythrocytes, up to 50 percent of the available ATP is used for this purpose. In cells treated with poisons that inhibit the aerobic production of ATP (e.g., 2,4-dinitrophenol), the ion concentration inside the cell gradually approaches that of the exterior environment as the ions move through plasma membrane channels down their electric and concentration gradients. Eventually treated cells die: partly because protein synthesis requires a high concentration of K^+ ions and partly because in the absence of a Na^+ gradient across the cell membrane, a cell cannot import certain nutrients such as amino acids. Studies on the effects of such poisons provided early evidence for the existence of ion pumps. In this section, we discuss in some detail examples of the P, V, and ABC classes of ATP-powered pumps.

Plasma-Membrane Ca^{2+} ATPase Exports Ca^{2+} Ions from Cells

Small increases in the concentration of free Ca^{2+} ions in the cytosol trigger a variety of cellular responses. In order for Ca^{2+} to function in intracellular signaling, its cytosolic concentration usually must be kept below 0.1 – 0.2 μM. (Although some cytosolic Ca^{2+} is bound to negatively charged groups, it is the concentration of *free,* unbound Ca^{2+} that is critical to its signaling function.) The plasma membranes of animal, yeast, and probably plant cells contain

Ca^{2+} ATPases that transport Ca^{2+} out of the cell against its electrochemical gradient. These P-class ion pumps help maintain the concentration of free Ca^{2+} ions in the cytosol at a low level.

In addition to a catalytic α subunit containing an ATP-binding site, as found in other P-class pumps, plasmamembrane Ca^{2+} ATPases also contain the Ca^{2+}-binding regulatory protein calmodulin. A rise in cytosolic Ca^{2+} induces the binding of Ca^{2+} ions to calmodulin, which triggers an allosteric activation of the Ca^{2+} ATPase; as a result, the export of Ca^{2+} ions from the cell accelerates, and the original low cytosolic concentration of free Ca^{2+} is restored rapidly.

Muscle Ca^{2+} ATPase Pumps Ca^{2+} Ions from the Cytosol into the Sarcoplasmic Reticulum

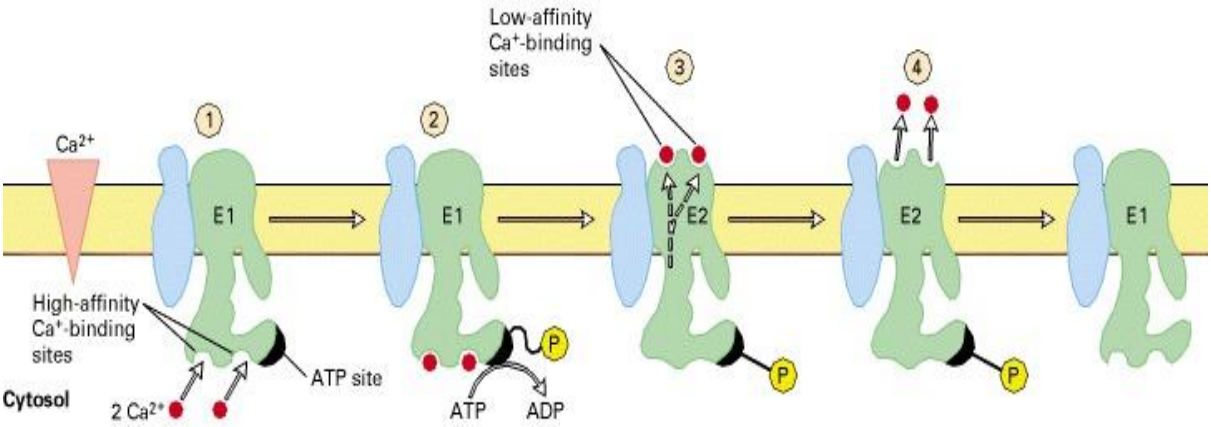
Besides the plasma-membrane Ca^{2+} ATPase, muscle cells contain a second, different Ca^{2+} ATPase that transports Ca^{2+} from the cytosol into the lumen of the sarcoplasmic reticulum (SR), an internal organelle that concentrates and stores Ca^{2+} ions. As discussed in Chapter 18, the SR and its calcium pump (referred to as the *muscle calcium pump*) are critical in muscle contraction and relaxation: release of Ca^{2+} ions from the SR into the muscle cytosol causes contraction, and the rapid removal of Ca^{2+} ions from the cytosol by the muscle calcium pump induces relaxation.

Because the muscle calcium pump constitutes more than 80 percent of the integral protein in SR membranes, it is easily purified and characterized. Each transmembrane catalytic α subunit has a molecular weight of 100,000 and transports two Ca^{2+} ions per ATP hydrolyzed. In the cytosol of muscle cells, the free Ca^{2+} concentration ranges from 10^{-7} M (resting cells) to more than 10^{-6} M (contracting cells), whereas the *total* Ca^{2+} concentration in the SR lumen can be as high as 10^{-2} M. Sites on the cytosolic surface of the muscle calcium pump have a very high affinity for Ca^{2+} ($K_m = 10^{-7}$ M), allowing the pump to transport Ca^{2+} efficiently from the cytosol into the SR against the steep concentration gradient.

The concentration of free Ca^{2+} within the sarcoplasmic reticulum is actually much less than the total concentration of 10^{-2} M. Two soluble proteins in the lumen of SR vesicles bind Ca^{2+} and serve as a reservoir for intracellular Ca^{2+}, thereby reducing the concentration of free Ca^{2+} ions in the SR vesicles, and consequently decreasing the energy needed to pump Ca^{2+} ions into them from the cytosol. The activity of the muscle Ca^{2+} ATPase is so regulated that if the free Ca^{2+} concentration in the cytosol becomes too high, the rate of calcium pumping increases until the cytosolic Ca^{2+} concentration is reduced to less than 1 μM. Thus in muscle cells, the calcium pump in the SR membrane can supplement the activity of the plasma-membrane pump, assuring that the cytosolic concentration of free Ca^{2+} remains below 1 μM.

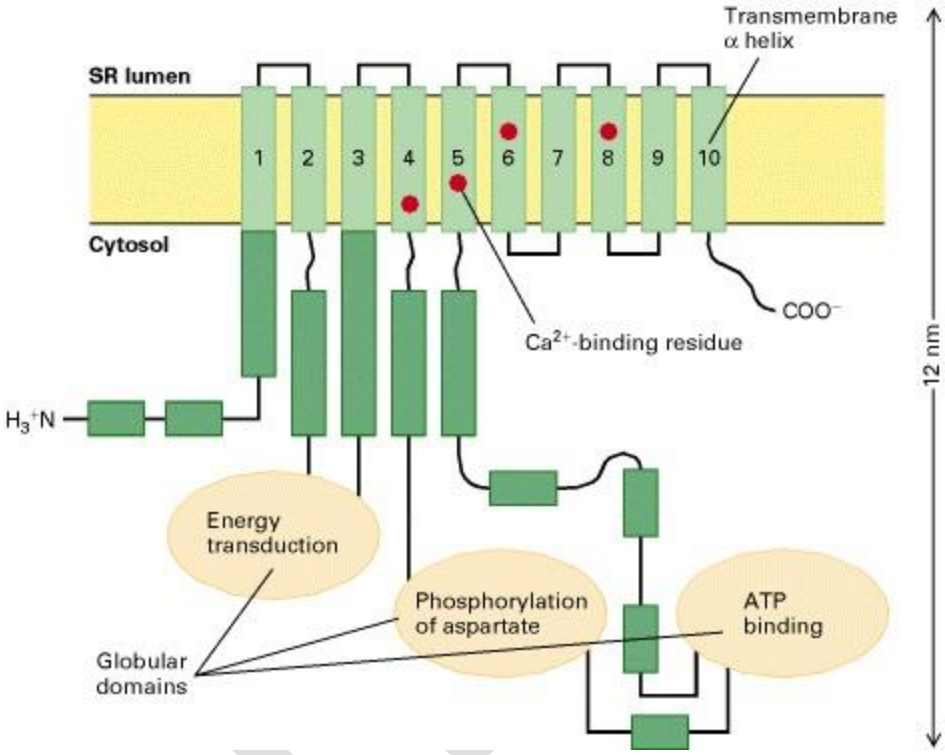
Coupling of ATP hydrolysis with ion pumping involves several steps that must occur in a defined order. When the protein is in one conformation, termed *E1*, two Ca^{2+} ions bind in sequence to high-affinity sites on the cytosolic surface (step 1). Then an ATP binds to its site on the cytosolic surface; in a reaction requiring that a Mg^{2+} ion be tightly complexed to the ATP, the bound ATP is hydrolyzed to ADP and the liberated phosphate is transferred to a specific aspartate residue in the protein, forming a high-energy acyl phosphate bond, denoted by E1~P (step 2). The protein then changes its conformation to E2 – P, generating two lowaffinity Ca^{2+}-binding sites on the exoplasmic surface, which faces the SR lumen; this conformational change simultaneously propels the two Ca^{2+} ions through the protein to these sites (step 3) and inactivates the high-affinity Ca^{2+}-binding sites on the cytosolic face. The Ca^{2+} ions then dissociate from the exoplasmic surface of the protein (step 4). Following this, the aspartyl-phosphate bond in E2 – P is hydrolyzed, causing E2 to revert to E1, a change that inactivates the exoplasmic-facing Ca^{2+}-binding sites and regenerates the cytosolicfacing Ca^{2+}-binding sites (step 5).

Model of the mechanism of action of muscle Ca^{2+} ATPase



Thus phosphorylation of the muscle calcium pump by ATP favors conversion of E1 to E2, and dephosphorylation favors the conversion of E2 to E1. While only E2 – P, not E1~P, is actually hydrolyzed, the free energy of hydrolysisof the aspartyl-phosphate bond in E1~P is greater than that for E2 – P. The reduction in free energy of the aspartyl-phosphate bond in E2 – P, relative to E1~P, can be said to power the E1 → E2 conformational change. The affinity of Ca^{2+} for the cytosolic-facing binding sites in E1 is a thousandfold greater than the affinity of Ca^{2+} for the exoplasmic-facing sites in E2; this difference enables the protein to transport Ca^{2+} unidirectionally from the cytosol, where it binds tightly to the pump, to the exoplasm, where it is released.

Much evidence supports the model depicted in Figure. For instance, the muscle calcium pump has been isolated with phosphate linked to an aspartate residue, and spectroscopic studies have detected slight alterations in proteinconformation during the E1 → E2 conversion. On the basis of the protein's amino acid sequence and various biochemical studies, investigators proposed the structural model for the catalytic α subunit. The membrane-spanning α helices are thought to form the passageway through which Ca^{2+} ions move. The bulk of the subunit consists of cytosolic globular domains that are involved in ATP binding, phosphorylation of aspartate, and energy transduction. These domains are connected by "stalks" to the membrane-embedded domain.



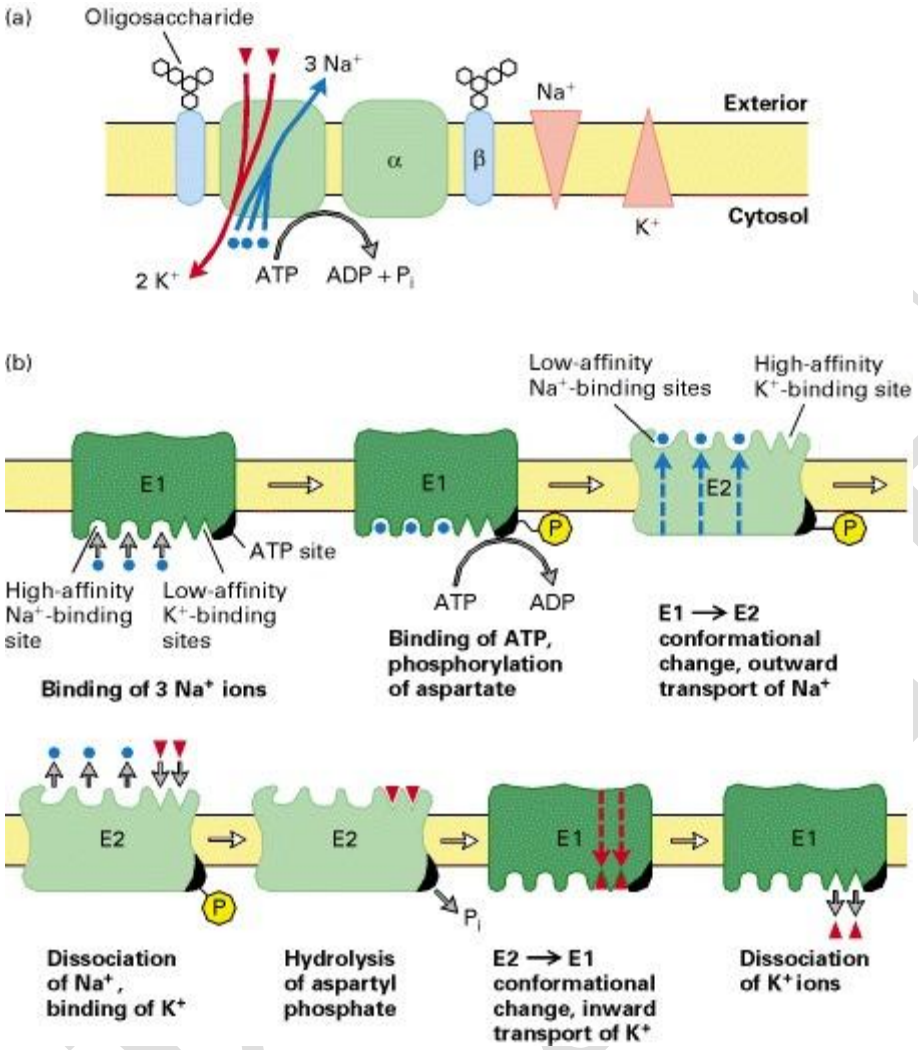
Schematic structural model for the catalytic (α) subunit of muscle Ca^{2+} ATPase

The 10 transmembrane α helices are thought to form a channel through which Ca^{2+} ions move. Site-specific mutagenesis studies have identified four residues (red dots), located in four of the transmembrane helices, that participate in Ca^{2+} binding. Trypsin digestion releases three cytosolic globular domains, which constitute the bulk of the protein. One cytosolic domain functions in ATP binding; a second bears the aspartate that is phosphorylated/ dephosphorylated; and the third is involved in energy.

As noted previously, all P-class ion pumps, regardless of which ion they transport, are phosphorylated during the transport process. The amino acid sequences around the phosphorylated aspartate in the catalytic α subunit are highly conserved in all proteins of this type. Thus the mechanistic model in Figure probably is generally applicable to all these ATP-powered ion pumps. In addition, the α subunits of all the P pumps examined to date have a similar molecular weight and, as deduced from their amino acid sequences derived from cDNA clones, have a similar arrangement of transmembrane α helices. These findings strongly suggest that all these proteins evolved from a common precursor, although they now transport different ions.

Na^+/K^+ ATPase Maintains the Intracellular Na^+ and K^+ Concentrations in Animal Cells

A second P-class ion pump that has been studied in considerable detail is the Na^+/K^+ ATPase present in the plasma membrane of all animal cells. This ion pump is a tetramer of subunit composition $\alpha_2\beta_2$. (Classic Experiment 15.1 describes the discovery of this enzyme.) The β polypeptide is required for newly synthesized α subunits to fold properly in the endoplasmic reticulum but apparently is not involved directly in ion pumping. The α subunit is a 120,000-MW nonglycosylated polypeptide whose amino acid sequence and predicted membrane structure are very similar to those of the muscle Ca^{2+} ATPase. In particular, the Na^+/K^+ ATPase has a stalk on the cytosolic face that links domains containing the ATP-binding site and the phosphorylated aspartate to the membrane-embedded domain. The overall process of transport moves three Na^+ ions out of and two K^+ ions into the cell per ATP molecule split.



Models for the structure and function of the Na^+/K^+ ATPase in the plasma membrane

(a) This P-class pump comprises two copies each of a small glycosylated β subunit and a large α subunit, which performs ion transport. Hydrolysis of one molecule of ATP to ADP and P_i is coupled to export of three Na^+ ions (blue circles) and import of two K^+ ions (dark red triangles) against their concentration gradients (large triangles). It is not known whether only one α subunit, or both, in a single ATPase molecule transports ions. (b) Ion pumping by the Na^+/K^+ ATPase involves a high-energy acyl phosphate intermediate (E1~P) and conformational changes, similar to transport by the muscle Ca^{2+} ATPase. In this case, hydrolysis of the E2 – P intermediate powers transport of a second ion (K^+) inward. Na^+ ions are indicated by blue circles; K^+ ions, by red triangles.

Several lines of evidence indicate that the Na^+/K^+ ATPase is responsible for the coupled movement of K^+ and Na^+into and out of the cell, respectively. For example, the drug *ouabain*, which binds to a specific region on the exoplasmic surface of the protein and specifically inhibits its ATPase activity, also prevents cells from maintaining their Na^+/K^+ balance. Any doubt that the Na^+/K^+ ATPase is responsible for ion movement was dispelled by the demonstration that the enzyme, when purified from the membrane and inserted into liposomes, propels K^+ and Na^+transport in the presence of ATP.

The mechanism of action of the Na^+/K^+ ATPase, outlined in Figure 15-13b, is similar to that of the muscle calcium pump, except that ions are pumped in both directions across the membrane. In its E1 conformation, the Na^+/K^+ATPase has three high-affinity Na^+-binding sites and two low-affinity K^+-binding sites on the cytosolic-facing surface of the protein. The K_m for binding of Na^+ to these cytosolic sites is 0.6 mM, a value considerably lower than the intracellular Na^+ concentration of ≈12 mM; as a result, Na^+ ions normally will fill these sites. Conversely, the affinity of the cytosolic K^+-binding sites is low enough that K^+ ions, transported inward through the protein, dissociate from E1 into the cytosol despite the high intracellular K^+ concentration. During the E1 → E2 transition, the three bound Na^+ ions move outward through the protein. Transition to the E2 conformation also generates two high-affinity K^+sites and three low-affinity Na^+ sites on the exoplasmic face. Because the K_m for K^+ binding to these sites (0.2 mM) is considerably lower than the extracellular K^+ concentration (4 mM), these sites will fill quickly with K^+ ions. In contrast, the three Na^+ ions, transported outward through the protein, will dissociate into the extracellular medium from the low-affinity Na^+ sites on the exoplasmic surface despite the high extracellular Na^+ concentration. Similarly, during the E2 → E1 transition, the two bound K^+ ions are transported inward.

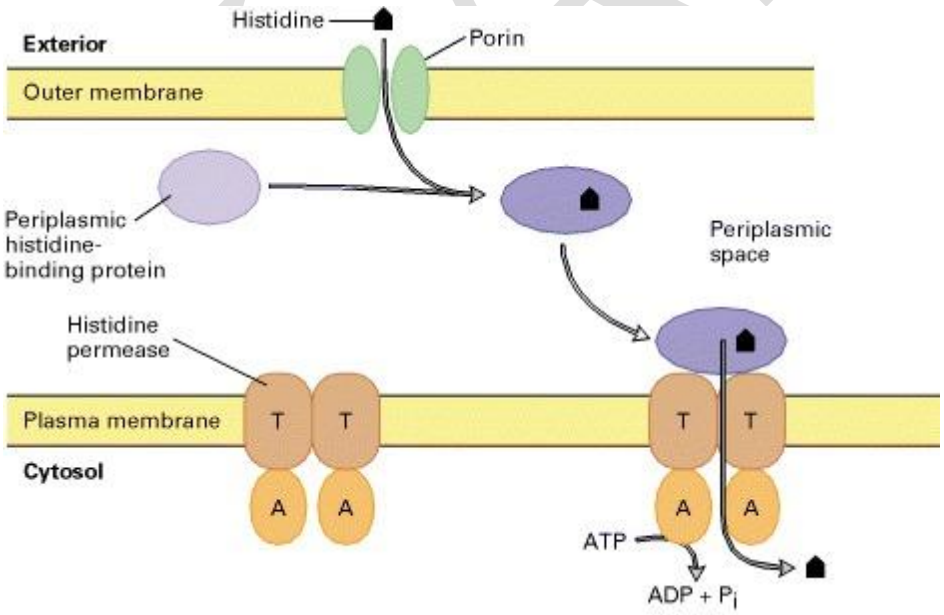
The ABC Superfamily Transports a Wide Variety of Substrates

As noted earlier, all members of the very large and diverse ABC superfamily of transport proteins contain two transmembrane (T) domains and two cytosolic ATP-binding (A) domains. The T domains, each built of six membrane-spanning α helices, form the pathway through which the transported substance (substrate) crosses the membrane and determine the substrate specificity of each ABC protein. The sequence of the A domains is ≈30 to 40 percent homologous in all members of this superfamily, indicating a common evolutionary origin. Some ABC proteins also contain a substrate-binding subunit or regulatory subunit.

Bacterial Plasma-Membrane Permeases

The plasma membrane of many bacteria contain numerous *permeases* that belong to the ABC superfamily. These proteins use the energy released by hydrolysis of ATP to transport specific amino acids, sugars, vitamins, or even peptides into the cell. Since bacteria frequently grow in soil or pond water where the concentration of nutrients is low, these ABC transport proteins allow the cells to concentrate amino acids and other nutrients in the cell against a substantial concentration gradient. Bacterial permeases generally are *inducible;* that is, the quantity of a transport protein in the cell membrane is regulated by both the concentration of the nutrient in the medium and the metabolic needs of the cell.

In *E. coli* histidine permease, a typical bacterial ABC protein, the two transmembrane domains and two cytosolic ATP-binding domains are formed by four separate subunits. In gram-negative bacteria such as *E. coli*, which have an outer membrane, a soluble histidine-binding protein in the periplasmic space assists in transport. This soluble protein binds histidine tightly and directs it to the T subunits, through which histidine crosses the membrane powered by ATP hydrolysis. Mutant *E. coli* cells that are defective in any of the histidine-permease subunits or the soluble binding protein are unable to transport histidine into the cell, but are able to transport other amino acids whose uptake is facilitated by other transport proteins. Such genetic analyses provide strong evidence that histidine permease and similar ABC proteins function to transport solutes into the cell.

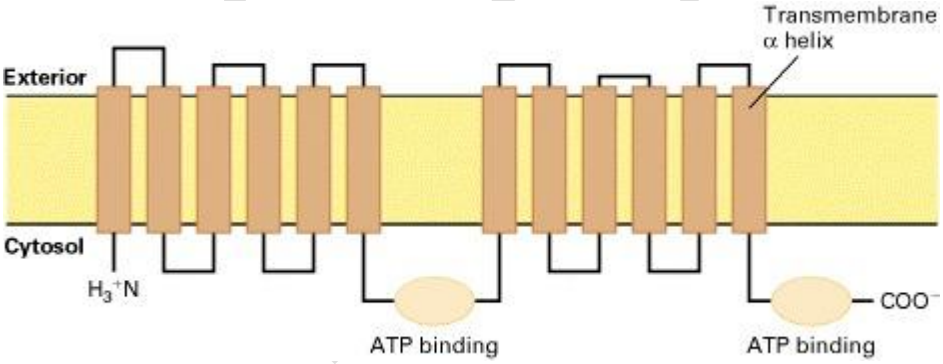


Gram-negative bacteria import many solutes by means of ABC proteins (permeases) that utilize a soluble substrate-binding protein present in the periplasmic space

Depicted here is the import of the amino acid histidine. After diffusing through porins in the outer membrane, histidine is bound by a specific periplasmic histidine-binding protein, which undergoes a conformational change. The histidine-protein complex binds to the exoplasmic surface of a T subunit in histidine permease located in the plasma membrane. Hydrolysis of ATP bound to the A subunit then powers movement of histidine through the protein into the cytosol. The transport process does not appear to involve a phosphoprotein intermediate.

Mammalian MDR Transport Proteins

A series of rather unexpected observations led to discovery of the first eukaryotic ABC protein. Oncologists noted that tumor cells often became simultaneously resistant to several chemotherapeutic drugs with unrelated chemical structures; similarly, cell biologists observed that cultured cells selected for resistance to one toxic substance (e.g., colchicine, a microtubule inhibitor) frequently became resistant to several other drugs, including the anticancer drug adriamycin. Subsequent studies showed that this resistance is due to enhanced expression of a*multidrug-resistance (MDR) transport protein* known as *MDR1*. In this member of the ABC superfamily, all four domains are "fused" into a single 170,000-MW protein. This protein uses the energy derived from ATP hydrolysis to *export* a large variety of drugs from the cytosol to the extracellular medium. The *Mdr1* gene is frequently amplified in multidrug-resistant cells, resulting in a large overproduction of the MDR1 protein.

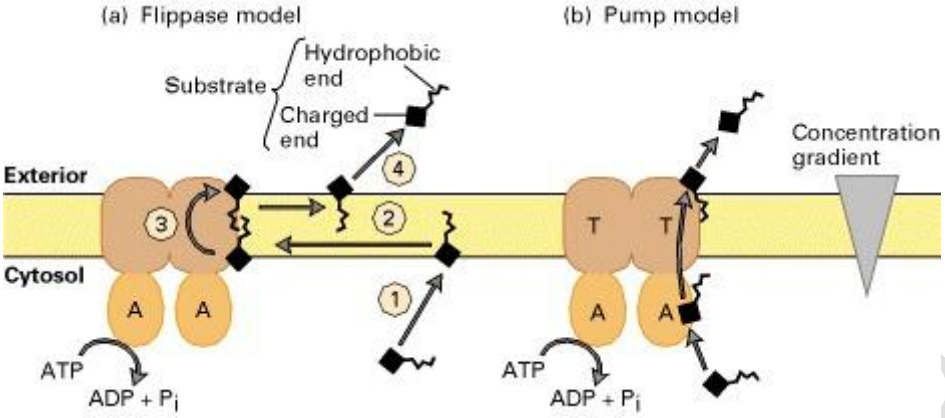


Schematic structural model for mammalian MDR1 protein. In this member of the ABC superfamily, the two transmembrane domains and two cytosolic ATP-binding domains are part of a single polypeptide

Each transmembrane domain contains six α helices. The two halves of this 1280-aa protein have similar amino acid sequences. A variety of lipid-soluble molecules that diffuse across the plasma membrane into the cell are transported outward by MDR1.

Most drugs transported by MDR1 are small hydrophobic molecules, which diffuse from the culture medium across the plasma membrane into the cell. The ATP-powered export of such drugs from the cytosol by MDR1 means a much higher extracellular drug concentration is required to kill cells. That MDR1 is an ATP-powered small-molecule pumphas been demonstrated with liposomes containing the purified protein. The ATPase activity of these liposomes is enhanced by different drugs in a dose-dependent manner corresponding to their ability to be transported by MDR1.

Not only does MDR1 transport a varied group of molecules, but all these substrates compete with one another for transport by MDR1. Although the mechanism of action of MDR1-assisted transport has not been definitively demonstrated, the *flippase model*. Substrates of MDR1 are primarily planar, lipid-soluble molecules with one or more positive charges, and they move spontaneously from the cytosol into the cytosolic-facing leaflet of the plasma membrane. The hydrophobic portion of a substrate molecule is oriented toward the hydrophobic core of the membrane, and the charged portion toward the polar cytosolic face of the membrane and is still in the cytosol. The substrate diffuses laterally until encountering and binding to a site on the MDR1 protein that is within the bilayer. The protein then "flips" the charged substrate molecule into the exoplasmic leaflet, an energetically unfavorable reaction powered by the coupled ATPase activity of MDR1. Once in the exoplasmic face, the substrate diffuses into the aqueous phase on the outside of the cell. Support for the flippase model of transport by MDR1 comes from MDR2, a homologous protein present in the region of the liver cell plasma membrane that faces the bile duct. MDR2 has been shown to flip phospholipids from the cytosolic-facing leaflet of the plasma membrane to the exoplasmic leaflet, thereby generating an excess of phospholipids in the exoplasmic leaflet; these phospholipids peel off into the bile duct and form an essential part of the bile. An alternative *pumpmodel* also has been proposed for MDR1. According to this model, drug molecules in the cytosol bind directly to a single small-molecule binding site on the cytosolic face of the MDR1 protein; subsequent ATP hydrolysis powers movement of the bound drug through the protein to the aqueous phase on the outside of the cell by a mechanism similar to that of other ATP-powered pumps.



Possible mechanisms of action of the MDR1 protein

(a) The flippase model proposes that a lipid-soluble molecule first dissolves in the cytosolic-facing leaflet of the plasma membrane (**1**) and then diffuses in the membrane until binding to a site on the MDR1 protein that is within the bilayer (**2**). Powered by ATP hydrolysis, the substrate molecule flips into the exoplasmic leaflet (**3**), from which it can move directly into the aqueous phase on the outside of the cell (**4**). (b) According to the pump model, MDR1 has a single multisubstrate binding site and transports molecules by a mechanism similar to that of other ATP-powered pumps.

MDR1 protein is expressed in abundance in the liver, intestines, and kidney — sites from which natural toxic products are removed from the body. Thus the natural function of MDR1 may be to transport a variety of natural and metabolic toxins into the bile, intestinal lumen, or forming urine. During the course of its evolution, MDR1 appears to have coincidentally acquired the ability to transport drugs whose structures are similar to those of these toxins. Tumors derived from these cell types, such as hepatomas (liver cancers), frequently are resistant to virtually all chemotherapeutic agents and thus difficult to treat, presumably because the tumors exhibit increased expression of the MDR1 or MDR2 proteins.

Cystic Fibrosis Transmembrane Regulator (CFTR) Protein

Discovery of another ABC transport protein came from studies of cystic fibrosis (CF), the most common lethal autosomal recessive genetic disease of Caucasians. This disease is caused by a mutation in the *CFTR*gene, which encodes a chloride-channel protein that is regulated by cyclic AMP (cAMP), an intracellular second messenger. These Cl^-channels are present in the apical plasma membranes of epithelial cells in the lung, sweat glands, pancreas, and other

tissues. An increase in cAMP stimulates Cl^-transport by such cells from normal individuals, but not from CF individuals who have a defective CFTR protein.

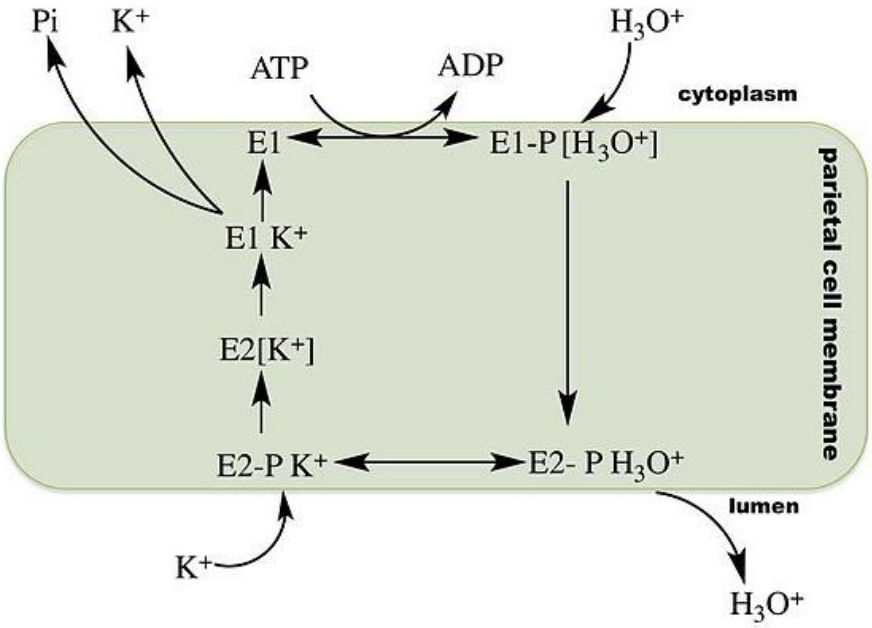
The sequence and predicted structure of the encoded CFTR protein, based on analysis of the cloned gene, are very similar to those of MDR1 protein except for the presence of an additional domain, the regulatory (R) domain, on the cytosolic face. The Cl^--channel activity of CFTR protein clearly is enhanced by binding of ATP. Moreover, as detailed in Chapter 20, cAMP activates a protein kinase that phosphorylates, and thereby activates, CFTR. When purified CFTR protein is incorporated into liposomes, it forms Cl^- channels with properties similar to those in normal epithelial cells. And when the wild-type CFTR protein is expressed by recombinant techniques in cultured epithelial cells from CF patients, the cells recover normal Cl^--channel activity. This latter result raises the possibility that gene therapy might reverse the course of cystic fibrosis.

Since CFTR protein is similar to MDR1 in structure, it may also function as an ATP-powered pump of some as-yet unidentified molecule. In any case, much remains to be learned about this fascinating class of ABC transport proteins.

Enzyme mechanism and activity

H^+/K^+ ATPase is a P_2-type ATPase, a member of the eukaryotic class of P-type ATPases. Like the Ca^{2+} and the Na^+/K^+ ATPases, the H^+/K^+ ATPase functions as an α, β protomer. Unlike other eukaryotic ATPases, the H^+/K^+ ATPase is electroneutral, transporting one proton into the stomach lumen per potassium retrieved from the gastric lumen. As an ion pump the H^+/K^+ ATPase is able to transport ions against a concentration gradient using energy derived from the hydrolysis of ATP. Like all P-type ATPases, a phosphate group is transferred from adenosine triphosphate (ATP) to the H^+/K^+ ATPase during the transport cycle. This phosphate transfer powers a conformational change in the enzyme that helps drive ion transport.

The hydrogen potassium ATPase is activated indirectly by gastrin that causes ECL cells to release histamine. The histamine binds to H2 receptors on the parietal cell, activating a cAMP-dependent pathway which causes the enzyme to move from the cytoplasmic tubular membranes to deeply folded canaliculi of the stimulated parietal cell. Once localized, the enzyme alternates between two conformations, E1 and E2, to transport ions across the membrane.



Mechanism of the H^+/K^+ ATPase demonstrating how E1-E2 conformational change corresponds to ion release.

The E1 conformation binds a phosphate from ATP and hydronium ion on the cytoplasmic side. The enzyme then changes to the E2 conformation, allowing hydronium to be released in the lumen. The E2 conformation binds potassium, and reverts to the E1 conformation to release phosphate and K^+ into the cytoplasm where another ATP can be hydrolyzed to repeat the cycle. The β subunit prevents the E2-P conformation from reverting to the E1-P conformation, making proton pumping unidirectional. The number of ions transported per ATP varies from $2H^+/2K^+$ to $1H^+/1K^+$depending on the pH of the stomach.

Disease relevance and inhibition

Inhibiting the hydrogen potassium pump to decrease stomach acidity has been most common method of treating diseases including gastroesophageal reflux disease (GERD/GORD) and peptic ulcer disease (PUD). Reducing acidity alleviates disease symptoms but does not treat the actual cause of GERD (abnormal relaxation of the esophageal sphincter) or PUD (*Helicobacter pylori* and NSAIDs).

Three drug categories have been used to inhibit H^+/K^+ ATPases. H_2-receptor antagonists, like cimetidine (Tagamet), inhibit the signaling pathway that leads to activation of the ATPase.

This type of inhibitor is effective in treating ulcers but does not prevent them from forming, and patients develop tolerance to them is developed after about one week, leading to a 50% reduction in effect. Proton pump inhibitors were later developed, starting with timoprazole in 1975. PPIs are acid-activated prodrugs that inhibit the hydrogen potassium ATPase by binding covalently to active pumps. Current PPIs like omeprazole have a short half-life of 90 minutes. Acid pump antagonists (APAs) or potassium-competitive acid blockers (PCABs) are a third type of inhibitor that blocks acid secretion by binding to the K^+ active site. APAs provide faster inhibition than PPIs since they do not require acid activation. Revaprazan was the first APA used clinically in east Asia, and other APAs are being developed since they appear to provide better acid control in clinical trials.

Inactivation of the proton pump can also lead to health problems found that a mutation of the pump's α subunit led to achlorhydria which resulted problems with iron absorption, leading to iron deficiency and anemia. The use of PPIs has not been correlated with an elevated risk of anemia, so the H^+/K^+ ATPase is thought to aid iron absorption but is not necessarily required.

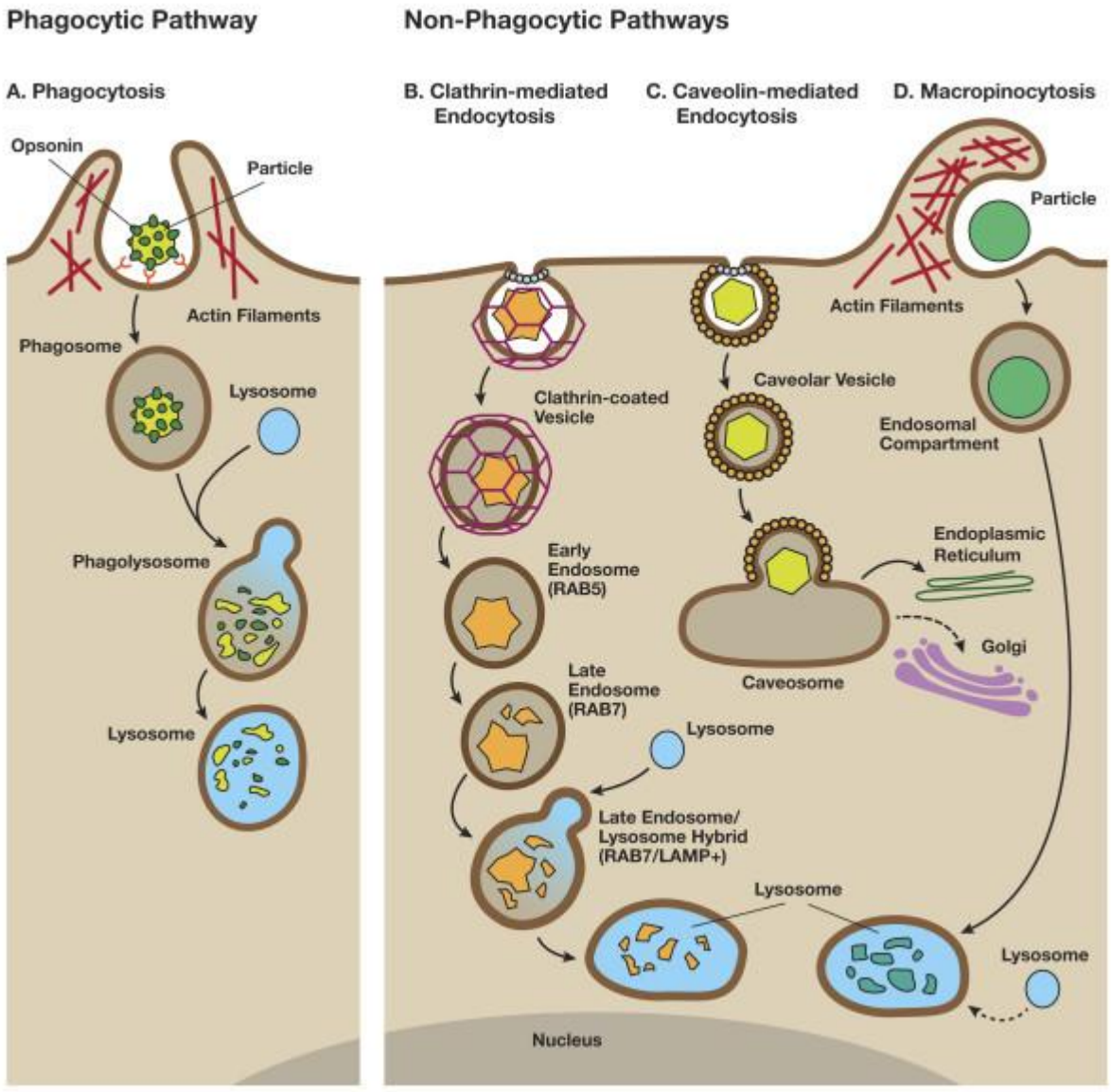
Current association of dementia and PPIs have been documented in Germany and in research articles denoting how benzimidazole derivatives, astemizole (AST) and lansoprazole (LNS) interact with anomalous aggregates of tau protein (neurofibrillary tangles). Current theories include the non-selective blockade of sodium-potassium pumps in the brain causing osmotic imbalances or swelling in the cells. [auth opinion] Interaction of PPIs with other drug affecting the sodium-potassium pump, e.g., digoxin, warfarin etc., has been well documented.[22] Memory has been associated with astrocytes and the alpha3 subunit of adenosine receptor found in hydrogen/Sodium-potassium pumps may be a focal point in dementia. Chronic use of PPIs may cause down regulation of alpha3 subunit increasing damage to astrocytes. Osteopetrosis via TCIRG1 gene has a strong association with pre-senile dementia.

Cotransport by Symporters and Antiporters

Besides ATP-powered pumps, cells have a second, discrete class of proteins that import or export ions and small molecules, such as glucose and amino acids, against a concentration gradient. These proteins use the energy stored in the electrochemical gradient of Na^+ or H^+ ions to power the uphill movement of another substance, a process called cotransport. For instance, the energetically favored movement of a Na^+ ion (the "cotransported" ion) into a cell across the plasma membrane, driven both by its concentration gradient and by the transmembrane voltage gradient, can be coupled obligatorily to movement of the "transported" molecule (e.g., glucose) against its concentration gradient. When the transported molecule and cotransported ion

move in the same direction, the process is called symport; when they move in opposite directions, the process is called antiport.

Receptor mediated endocytosis



Receptor-mediated endocytosis (RME), also called clathrin-mediated endocytosis, is a process by which cells absorb metabolites, hormones, other proteins – and in some cases viruses – by the inward budding of plasma membrane vesicles containing proteins with receptor sites specific to the molecules being absorbed.

POSSIBLE QUESTIONS

2 marks

1. What is meant by diffusion?
2. Differentiate active and passive transport.
3. What is meant by facilitated diffusion?
4. Add note on endocytosis.
5. What is meant by pinocytosis?
6. Short note on transport mechanisms.

6 marks

1. Explain in detail about membrane transport and their types.
2. Discuss about facilitated diffusion in erythrocytes.
3. Describe in detail on ion concentration gradients.
4. Explain the following,

i) Uniporter catalyzed transport

ii) Active transport systems

5. Briefly explain a) Calcium ATPase, Na+K+ ATPase and Gastric H+K+ ATPase mechanisms.
6. Enumerate about ABC super family.
7. Illustrate receptor mediated endocytosis and its significance.

ARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
I-M.Sc., BIOCHEMISTRY
CELLULAR BIOCHEMISTRY (18BCP104)
MULTIPLE CHOICE QUESTIONS

UNIT II

S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	Transport proteins comes under ---------- class	peripheral protein	integral membrane protein	lipid anchored protein	none of the above	ntegral membrane protein
2	_____ is the simplest transport process.	passive diffusion	facilitated diffusion	active transport	none of the above	passive diffusion
3	Glucose transport in erythrocyte is an example of _____	passive diffusion	facilitated diffusion	active transport	none of the above	facilitated diffusion
4	Calcium ion in muscles sequestered inside complex of vesicles called	endoplasmic reticulam	Mitochondria	Golgi bodies	Sarcoplasmic reticulum	Sarcoplasmic reticulum
5	Active transport require	carrier protein	Energy	both (a) & (b)	none of the above	both (a) & (b)
6	Biological transport process which are driven by light energy include	Bacteriorhodopsin	Ca^{2+}ATPase	Halorhodopsin	both (a) & (c)	both (a) & (c)
7	Biological membrane ion & other transports move in opposite direction is	Symport	Antiport	Uniport	none of the above	Antiport
8	For an uncharged molecule passive diffusion is a _____process	Extropic	Entropic	both (a) & (b)	none of the above	Entropic

9	For charged species movement of molecule across membrane depends on _	Electrochemical potential	Energy barrier	Concentration gradient	none of the above	Electrochemical potential
10	For passive diffusion the plot rate versus concentration is _____	Linear	Hyperbolic	Parabolic	Ellipse	Linear
11	The anion transport system is an example of _____	passive diffusion	facilitated diffusion	Uniport	Symport	facilitated diffusion
12	The most common energy input is _____ hydrolysis	ATP	GTP	UTP	ADP	ATP
13	___involves between ET, PT capture of chemical energy in form of ATP	Oxidative phosphorylation	Oxidative dephosphorylation	Substrate level phosphorylation	None	Oxidative phosphorylation
14	Na^+K^+ATPase is an integral protein found in	endoplasmic reticulam	Mitochondria	Golgi bodies	Plasma membrane	Plasma membrane
15	Animal cell maintain cytosolic conc of Na and K of ------- &-------mM	1& 10	10 & 100	5 & 50	10 &30	10 & 100
16	The α-subunit of Na^+ K^+ A contains _____residue.	1055	1013	1016	1033	1016
17	The hydrophobicity of a substance is measured by its _____ coefficient.	Permeability	Partition	Correlation	none of the above	Partition
18	Na^+K^+ATPase is inhibited by _____ glycosides.	Neuroglycosides	Cardiac glycosides	Hepatoglycosides	none of the above	Cardiac glycosides
19	pH of stomach fluid is normally in the range of _____	0.5-1	1-1.5	0.8-1	0.2-0.8	0.8-1
20	The pH of parietal cells of gastric mucosa in mammals is _____	7	6.5	6	7.4	7.4

21	The largest known transmembrane gradient in eukaryotic cells is _____	5.6	6.6	7.8	4.5	6.6
22	H^+ transporting ATPase found in ----- provide Ca^{2+} for nerves & muscles.	Osteoblast	Osteoclasts	Sarcoplasmic reticulam	none of the above	Osteoclasts
23	Once growth is complete, body bal	Osteoblast	Osteoclasts	Sarcoplasmic reti	none of the above	Osteoblast
24	What is the normal composition of bone mineral?	$CaCO_3$& $CaPO_4$	Calcium borate & $CaCO_3$	$CaSO_4$ & $CaPO_4$	$CaCl_2$ & $CaSO_4$	CaCO3& CaPO4
25	H.halobium grows optimally at an NaCl concentration of ____molarity.	3.9	4.3	3.5	5.3	4.3
26	O2 & nutrient defiant conditions ___ colour patches appear on H.halobium.	Purple	Red	Pink	Brown	Purple
27	The purple patches of H.halobium membrane are due to _____ protein.	Bacteriorhodopsin	Halorhodopsin	Keratin	None of the above	Bacteriorhodopsin
28	Bacteriorhodopsin is a transmembrane protein having a molecular weight	20 KD	15KD	30 KD	26 KD	26 KD
29	Halorhodopsin is a transmembrane protein having a molecular weight	27 KD	25 KD	20 KD	15 KD	27 KD
30	Who discovered phosphotransferase system?	Saul Roseman	Johns Hopkins	Hans Kreb	Karry Mullis	Saul Roseman
31	Ability of outer membrane to act as a molecular sieve is due to proteins.	Keratin	Porins	Albumin	Globulin	Porins

32	Porins are also known as _____ proteins.	Glycoproteins	Matrix proteins	Lipoproteins	Nuclear proteins	Matrix proteins
33	Molecular mass of porins generally range from _____ to _____	20-30 KD	30-40 KD	30-50 KD	10-35 KD	30-50 KD
34	Glucose concentration in plasma is _____	5mM	8 mM	10 mM	3 mM	5mM
35	Glucose permease is a _____ protein.	peripheral protein	integral protein	lipid anchored protein	none of the above	integral protein
36	Transporters that carry only one substrate in any direction is	Symport	Antiport	Uniport	none of the above	Uniport
37	In cytoplasm CO_2 AND H_2O react to form bicarbonate in the presence of	Decarboxylase	Carbonic anhydrase	Carboxy peptidase	Carnitine acyltransferase	Carbonic anhydrase
38	Number of molecules in gram mole	Osmolality	Osmolarity	Osmole	None of the above	Osmole
39	Normal osmolality of intracellular fluid in plasma membrane is about ---	100milli osmole/Kg	200milli osmole/Kg	400milli osmole/Kg	300milli osmole/Kg	300milli osmole/Kg
40	Uptake of extracellular material by invagination of the plasma membrane is	Endocytosis	Osmosis	Diffusion	Apoptopsis	Endocytosis
41	The process of cell drinking is also known as	Phagocytosis	Pinocytosis	Transcytosis	Osmosis	Pinocytosis
42	Process by which relatively large pa	Pinocytosis	Transcytosis	Phagocytosis	Osmosis	Phagocytosis
43	Movement of molecule from higher to lower partial pressure is	Osmosis	Diffusion	Endocytosis	Phagocytosis	Osmosis
44	Transporters bind their substrate through _____ interactions	Strong non covalent	Strong Covalent	Weak Non Covalent	Weak Covalent	Weak Non Covalent
45	Commonly the transporters are_____	Proteins	Lipids	carbohydrate	Enzymes	Proteins

46	Transport of 2 solute simultaneously across the membrane is called	Uniport	Symport	Co transport	Antiport	Co transport
47	Chloride- Bicarbonate exchanger in RBC is an example of --------	Symport	Co transport	Uniport	Antiport	Co transport
48	Movement of 2 substance simultaneously in the same direction is called	Symport	Uniport	Cotransport	Antiport	Symport
49	The pH gradient of stomach is maintained by _____	Na/K ATPase	$H^{+}-K^{+}$ ATPase	Ca^{2+} ATPase	None of the above	H+_ K+ ATPase
50	ATP ase is reversibly phosphorylated & dephosphorylated is called	P type ATPase	V type ATPase	F type ATPase	None of the above	P type ATPase
51	Substrate is altered by the enzyme catalyses membrane transport	Osmosis	Group translocation	Diffusion	Endocytosis	Group translocation
52	A chemical reaction that consumes energy is called	Endergonic reaction	Exergonic reaction	Enthalpy	Entropy	Endergonic reaction
53	ATPase responsible for acidifying the intracellular components organism is	P type ATPase	F type ATPase	V Type ATPase	Ca^{2+} ATPase	V Type ATPase
54	F type ATPase is also known as	$Na^{+}K^{+}$ ATPase	Ca^{2+} ATPase	$H^{+}-K^{+}$ ATPase	ATP synthase	ATP synthase
55	Cellulose wall of plant cell is an example of _____	Impermeable membrane	Semi Permeable membrane	Permeable membrane	None of the above	Permeable membrane
56	Osmotic potential of a solution depends upon _____	Number of solute	Amount of solvent	Charge of molecule	None of the above	Number of solute
57	Osmotic potential of cell sap is greater than external solution, it is called	Hypotonic solution	Hypertonic solution	Isotonic solution	none of the these	Hypotonic solution
58	Which are the examples of semipermeable membrane	Cellulose	cell Membrane	Egg shell membrane	Both b and c	Both b and c

59	$H^{+}-K^{+}$ ATPase maintains pH gradient of _____ organ of the body	Stomach	Liver	Kidney	None of the above	Stomach
60	5mM is the concentration of _____ in plasma.	Glucose	Protein	Lipid	None of the above.	Glucose

UNIT-III-SYLLABUS

Mitochondria – Reduction potential, **Free energy and entropy,** electron transport chain – Complexes, Q-cycle, Cyt C oxidase complex, Translocation of protons and the establishment of a proton motive force, machinery for ATP formation and chemi-osmotic mechanism, ATP synthase – Experiments, inhibitors and uncouplers of oxidative phosphorylation.
Microfilaments – Actin – Stuctures, Assembly, Myosin. Microtubules – Organisation and dynamics, kinesin and dynein. Cilia and flagella – Structure and functions, intermediary filaments.

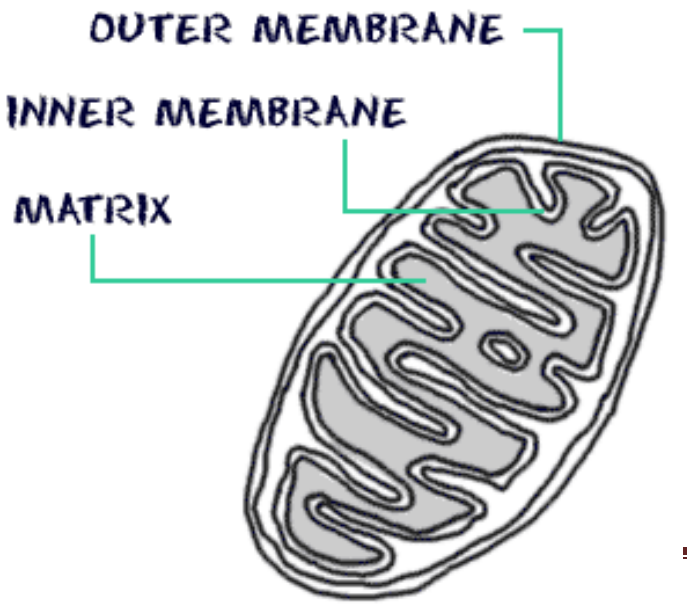
Mitochondria - Turning on the Powerhouse

Mitochondria are known as the powerhouses of the cell. They are organelles that act like a digestive system which takes in nutrients, breaks them down, and creates energy rich molecules for the cell. The biochemical processes of the cell are known as cellular respiration. Many of the reactions involved in cellular respiration happen in the mitochondria. Mitochondria are the working organelles that keep the cell full of energy.

Mitochondria are small organelles floating free throughout the cell. Some cells have several thousand mitochondria while others have none. Muscle cells need a lot of energy so they have loads of mitochondria. Neurons (cells that transmit nerve impulses) don't need as many. If a cell feels it is not getting enough energy to survive, more mitochondria can be created. Sometimes a mitochondria can grow larger or combine with other mitochondria. It all depends on the needs of the cell.

Mitochondria Structure

Mitochondria are shaped perfectly to maximize their productivity. They are made of two membranes. The **outer membrane** covers the organelle and contains it like a skin. The **inner membrane** folds over many times and creates layered structures called **cristae**.



The fluid contained in the mitochondria is called the matrix.

The folding of the inner membrane increases the surface area inside the

organelle. Since many of the chemical reactions happen on the inner membrane, the increased surface area creates more space for reactions to occur. Similar surface area strategies are used by **microvilli** in your intestines.

Using Oxygen to Release Energy

The matrix is filled with water and proteins (**enzymes**). Those proteins take organic molecules, such as **pyruvate** and **acetyl CoA**, and chemically digest them. Proteins embedded in the inner membrane and enzymes involved in the **citric acid cycle** ultimately release water (H_2O) and carbon dioxide (CO_2) molecules from the breakdown of oxygen (O_2) and glucose ($C_6H_{12}O_6$). The mitochondria are the only places in the cell where oxygen is reduced and eventually broken down into water.

Mitochondria are also involved in controlling the concentration of calcium (Ca^{2+}) ions within the cell. They work very closely with the endoplasmic reticulum to limit the amount of calcium in the cytosol.

Reduction potential

Reduction potential (also known as redox potential, oxidation / reduction potential, ORP, pE, ε, or {\displaystyle E_{h}} E_{{h}}) is a measure of the tendency of a chemical species to acquire electrons and thereby be reduced. Reduction potential is measured in volts (V), or millivolts (mV). Each species has its own intrinsic reduction potential; the more positive the potential, the greater the species' affinity for electrons and tendency to be reduced. ORP is a common measurement for water quality.

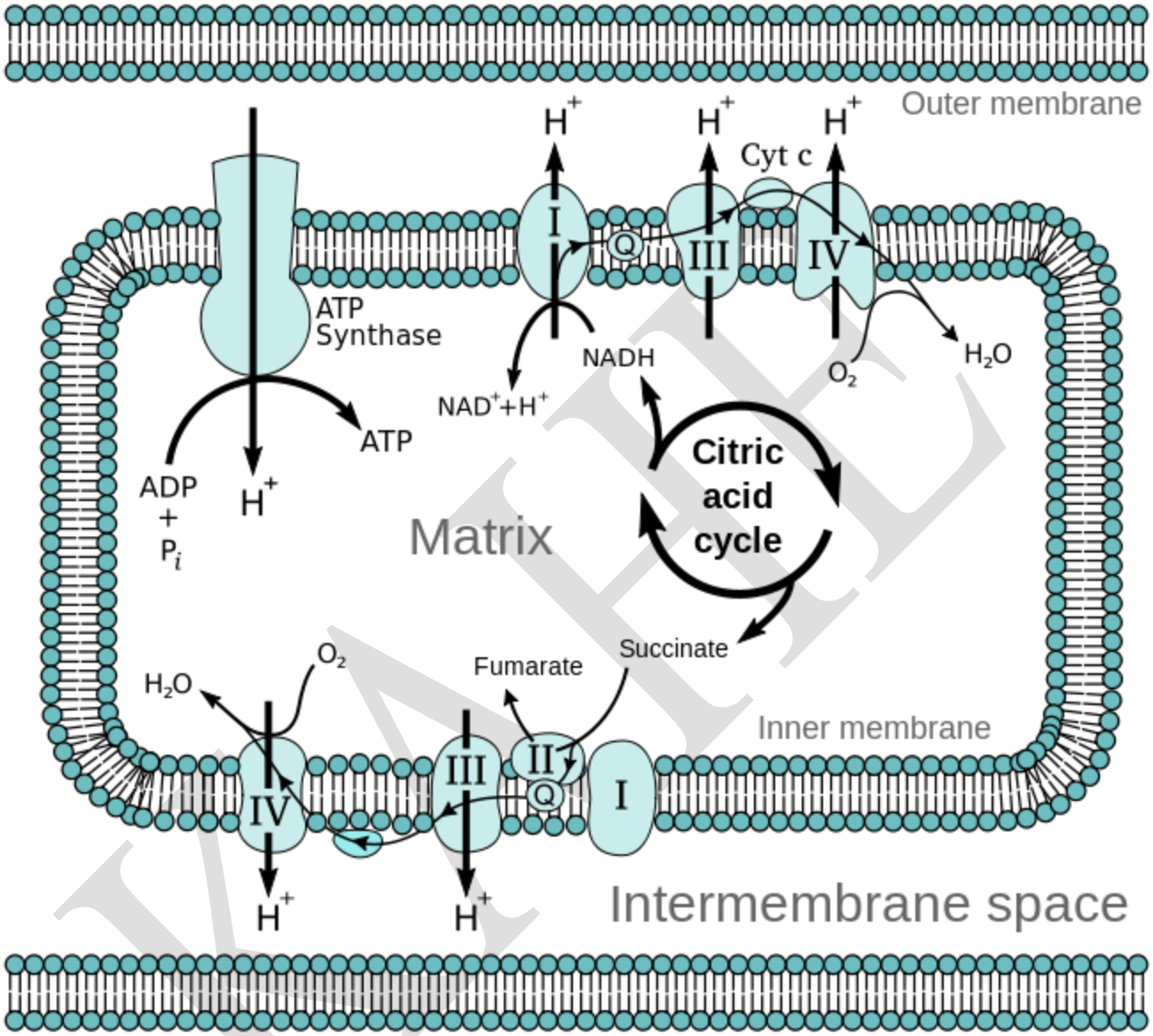
Electron transport chain

An electron transport chain (ETC) is a series of complexes that transfer electrons from electron donors to electron acceptors via redox (both reduction and oxidation occurring simultaneously) reactions, and couples this electron transfer with the transfer of protons (H+ ions) across a membrane. This creates an electrochemical proton gradient that drives the synthesis of adenosine triphosphate (ATP), a molecule that stores energy chemically in the form of highly strained bonds. The molecules of the chain include peptides, enzymes (which are proteins or protein complexes), and others. The final acceptor of electrons in the electron transport chain during aerobic respiration is molecular oxygen although a variety of acceptors other than oxygen such as sulfate exist in anaerobic respiration.

Electron transport chains are used for extracting energy via redox reactions from sunlight in photosynthesis or, such as in the case of the oxidation of sugars, cellular respiration. In eukaryotes, an important electron transport chain is found in the inner mitochondrial membrane where it serves as the site of oxidative phosphorylationthrough the use of ATP synthase. It is also found in the thylakoid membrane of the chloroplast in photosynthetic eukaryotes. In bacteria, the electron transport chain is located in their cell membrane.

In chloroplasts, light drives the conversion of water to oxygen and NADP+ to NADPH with transfer of H+ ions across chloroplast membranes. In mitochondria, it is the conversion of oxygen to water, NADH to NAD+ and succinate to fumarate that are required to generate the proton gradient.

Electron transport chains are major sites of premature electron leakage to oxygen, generating superoxide and potentially resulting in increased oxidative stress.



The electron transport chain in the mitochondrion is the site of oxidative phosphorylation in eukaryotes. The NADH and succinate generated in the citric acid cycle are oxidized, providing energy to power ATP synthase.

Most eukaryotic cells have mitochondria, which produce ATP from products of the citric acid cycle, fatty acid oxidation, and amino acid oxidation. At the mitochondrial inner membrane, electrons from NADH and FADH2 pass through the electron transport chain to oxygen, which is reduced to water. The electron transport chain comprises an enzymaticseries of electron donors

and acceptors. Each electron donor will pass electrons to a more electronegative acceptor, which in turn donates these electrons to another acceptor, a process that continues down the series until electrons are passed to oxygen, the most electronegative and terminal electron acceptor in the chain. Passage of electrons between donor and acceptor releases energy, which is used to generate a proton gradient across the mitochondrial membrane by actively "pumping" protons into the intermembrane space, producing a thermodynamic state that has the potential to do work. The entire process is called oxidative phosphorylation, since ADP is phosphorylated to ATP using the energy of hydrogen oxidation in many steps.

A small percentage of electrons do not complete the whole series and instead directly leak to oxygen, resulting in the formation of the free-radical superoxide, a highly reactive molecule that contributes to oxidative stress and has been implicated in a number of diseases and aging.

Mitochondrial redox carriers

Energy obtained through the transfer of electrons down the ETC is used to pump protons from the mitochondrial matrix into the intermembrane space, creating an electrochemical proton gradient (ΔpH) across the inner mitochondrial membrane (IMM). This proton gradient is largely but not exclusively responsible for the mitochondrial membrane potential ($\Delta\Psi_M$). It allows ATP synthase to use the flow of H^+ through the enzyme back into the matrix to generate ATP from adenosine diphosphate (ADP) and inorganic phosphate. Complex I (NADH coenzyme Q reductase; labeled I) accepts electrons from the Krebs cycle electron carrier nicotinamide adenine dinucleotide (NADH), and passes them to coenzyme Q (ubiquinone; labeled Q), which also receives electrons from complex II (succinate dehydrogenase; labeled II). Q passes electrons to complex III (cytochrome bc_1 complex; labeled III), which passes them to cytochrome *c* (cyt *c*). Cyt *c* passes electrons to Complex IV (cytochrome *c* oxidase; labeled IV), which uses the electrons and hydrogen ions to reduce molecular oxygen to water.

Four membrane-bound complexes have been identified in mitochondria. Each is an extremely complex transmembrane structure that is embedded in the inner membrane. Three of them are proton pumps. The structures are electrically connected by lipid-soluble electron carriers and water-soluble electron carriers. The overall electron transport chain:

$NADH+H^+$ → *Complex I* → Q → *Complex III* → cytochrome *c* → *Complex IV* → H_2O

↑

Complex II

↑

Succinate

Complex I

In *Complex I* (NADH:ubiquinone oxidoreductase, NADH-CoQ reductase, or NADH dehydrogenase; EC 1.6.5.3), two electrons are removed from NADH and ultimately transferred to a lipid-soluble carrier, ubiquinone (Q). The reduced product, ubiquinol (QH_2), freely diffuses within the membrane, and Complex I translocates four protons (H^+) across the membrane, thus producing a proton gradient. Complex I is one of the main sites at which premature electron leakage to oxygen occurs, thus being one of the main sites of production of superoxide.

The pathway of electrons is as follows:

NADH is oxidized to NAD^+, by reducing Flavin mononucleotide to $FMNH_2$ in one two-electron step. $FMNH_2$ is then oxidized in two one-electron steps, through a semiquinoneintermediate. Each electron thus transfers from the $FMNH_2$ to an Fe-S cluster, from the Fe-S cluster to ubiquinone (Q). Transfer of the first electron results in the free-radical (semiquinone) form of Q, and transfer of the second electron reduces the semiquinone form to the ubiquinol form, QH_2. During this process, four protons are translocated from the mitochondrial matrix to the intermembrane space. As the electrons become continuously oxidized and reduced throughout the complex an electron current is produced along the 180 Angstrom width of the complex within the membrane. This current powers the active transport of four protons to the intermembrane space per two electrons from NADH.

Complex II

In *Complex II* (succinate dehydrogenase or succinate-CoQ reductase; EC 1.3.5.1) additional electrons are delivered into the quinone pool (Q) originating from succinate and transferred (via flavin adenine dinucleotide (FAD)) to Q. Complex II consists of four protein subunits: succinate dehydrogenase, (SDHA); succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial, (SDHB); succinate dehydrogenase complex subunit C, (SDHC) and succinate dehydrogenase complex, subunit D, (SDHD). Other electron donors (e.g., fatty acids and glycerol 3-phosphate) also direct electrons into Q (via FAD). Complex 2 is a parallel electron transport pathway to complex 1, but unlike complex 1, no protons are transported to the intermembrane space in this pathway. Therefore, the pathway through complex 2 contributes less energy to the overall electron transport chain process.

Complex III

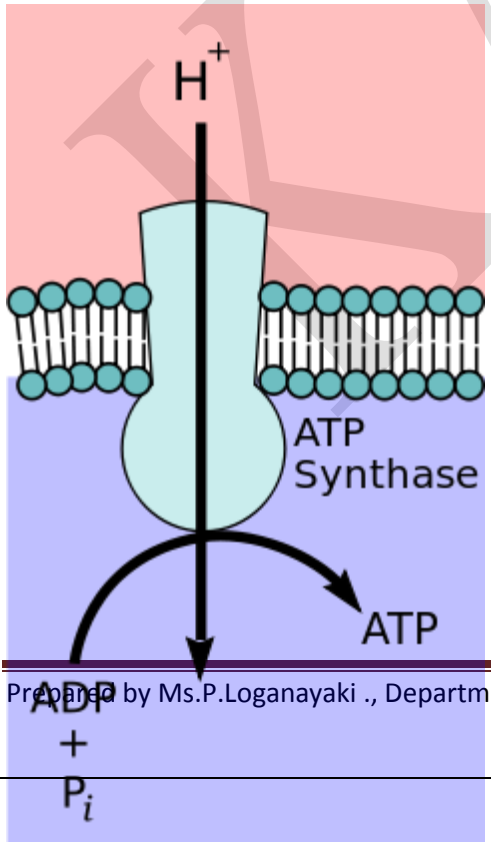
In *Complex III* (cytochrome bc_1 complex or $CoQH_2$-cytochrome *c* reductase; EC 1.10.2.2), the Q-cycle contributes to the proton gradient by an asymmetric absorption/release of protons. Two electrons are removed from QH_2 at the Q_O site and sequentially transferred to two molecules of cytochrome *c*, a water-soluble electron carrier located within the intermembrane space. The two other electrons sequentially pass across the protein to the Q_i site where the quinone part of ubiquinone is reduced to quinol. A proton gradient is formed by one quinol (2H+2e-) oxidations at the Q_o site to form one quinone (2H+2e-) at the Q_i site. (in total four protons are translocated: two protons reduce quinone to quinol and two protons are released from two ubiquinol molecules).

QH2 + 2 cytochrome c (FeIII) + 2 H+in → Q + 2 cytochrome c (FeII) + 4 H+out

When electron transfer is reduced (by a high membrane potential or respiratory inhibitors such as antimycin A), Complex III may leak electrons to molecular oxygen, resulting in superoxide formation.

Complex IV

In *Complex IV* (cytochrome *c* oxidase; EC 1.9.3.1), sometimes called cytochrome AA3, four electrons are removed from four molecules of cytochrome *c* and transferred to molecular oxygen (O_2), producing two molecules of water. At the same time, eight protons are removed from the mitochondrial matrix (although only four are translocated across the membrane), contributing to the proton gradient. The activity of cytochrome c oxidase is inhibited by cyanide.



Coupling with oxidative phosphorylation

Depiction of ATP synthase, the site of oxidative phosphorylation to generate ATP.

According to the chemiosmotic coupling hypothesis, proposed by Nobel Prize in Chemistry winner Peter D. Mitchell, the electron transport chain and oxidative phosphorylation are coupled by a proton gradient across the inner mitochondrial membrane. The efflux of protons from the mitochondrial matrix creates an electrochemical

gradient (proton gradient). This gradient is used by the F_OF_1 ATP synthasecomplex to make ATP via oxidative phosphorylation. ATP synthase is sometimes described as *Complex V* of the electron transport chain.[6] The F_O component of ATP synthase acts as an ion channel that provides for a proton flux back into the mitochondrial matrix. It is composed of a, b and c subunits. Protons in the inter-membranous space of mitochondria first enters the ATP synthase complex through *a* subunit channel. Then protons move to the c subunits. The number of c subunits it has determines how many protons it will require to make the F_O turn one full revolution. For example, in humans, there are 8 c subunits, thus 8 protons are required. After *c* subunits, protons finally enters matrix using *a* subunit channel that opens into the mitochondrial matrix.[7] This reflux releases free energy produced during the generation of the oxidized forms of the electron carriers (NAD^+ and Q). The free energy is used to drive ATP synthesis, catalyzed by the F_1 component of the complex. Coupling with oxidative phosphorylation is a key step for ATP production. However, in specific cases, uncoupling the two processes may be biologically useful. The uncoupling protein, thermogenin—present in the inner mitochondrial membrane of brown adipose tissue—provides for an alternative flow of protons back to the inner mitochondrial matrix. This alternative flow results in thermogenesis rather than ATP production. Synthetic uncouplers (e.g., 2,4-dinitrophenol) also exist, and, at high doses, are lethal.

The **Q cycle** (named for CoQ10) describes a series of reactions that describe how the sequential oxidation and reduction of the lipophilic electron carrier, Coenzyme Q10 (CoQ10), between the ubiquinol and ubiquinone forms, can result in the net movement of protons across a lipid bilayer (in the case of the mitochondria, the inner mitochondrial membrane).

The Q cycle was first proposed by Peter D. Mitchell, though a modified version of Mitchell's original scheme is now accepted as the mechanism by which Complex III moves protons (i.e. how complex III contributes to the biochemical generation of the proton or pH, gradient, which is used for the biochemical generation of ATP).

To summarize, the first reaction of Q cycle is:

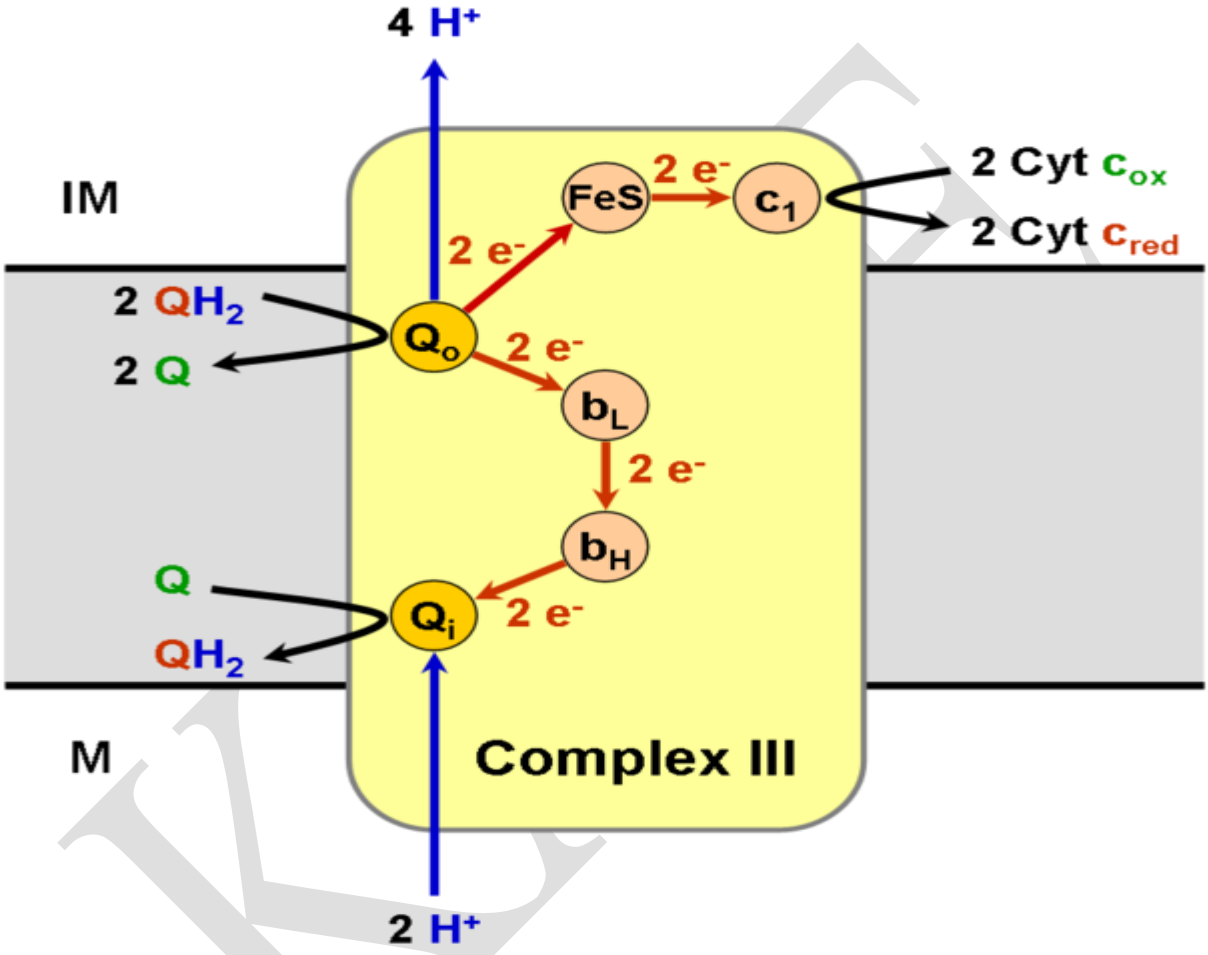
$CoQH_2$ + cytochrome c_1 (Fe^{3+}) → $CoQ^{-\bullet}$ + cytochrome c_1 (Fe^{2+}) + 2 H^+ (intermembrane)

Then the second reaction of the cycle involves the reduction of the transient semiquinone by another electron to give $CoQH_2$:

$CoQH_2$ + $CoQ^{-\bullet}$ + cytochrome c_1 (Fe^{3+}) + 2 H^+ (matrix) → CoQ + $CoQH_2$ + cytochrome c_1 (Fe^{2+}) + 2 H^+ (intermembrane)

Combining the two equations, we have the overall reaction of Q cycle:

$CoQH_2$ + 2 cytochrome c_1 (Fe^{3+}) + 2 H^+ (matrix) → CoQ + 2 cytochrome c_1 (Fe^{2+}) + 4 H^+ (intermembrane)



Uncouplers and Inhibitors

Much of our knowledge of mitochondrial function results from the study of toxic compounds. Specific inhibitors were used to distinguish the electron transport system from the phosphorylation system and helped to define the sequence of redox carriers along the respiratory chain. If the chain is blocked then all the intermediates on the substrate side of the block become more reduced, while all those on the oxygen side become more oxidised. It is easy to see what has happened because the oxidised and reduced carriers often differ in their spectral

properties. If a variety of different inhibitors are available then many of the respiratory carriers can be placed in the correct order.

There are six distinct types of poison which may affect mitochondrial function:

1) **Respiratory chain inhibitors** (e.g. cyanide, antimycin, rotenone & TTFA) block respiration in the presence of either ADP or uncouplers.

2) **Phosphorylation inhibitors** (e.g. oligomycin) abolish the burst of oxygen consumption after adding ADP, but have no effect on uncoupler-stimulated respiration.

3) **Uncoupling agents** (e.g. dinitrophenol, CCCP, FCCP) abolish the obligatory linkage between the respiratory chain and the phosphorylation system which is observed with intact mitochondria.

4) **Transport inhibitors** (e.g. atractyloside, bongkrekic acid, NEM) either prevent the export of ATP, or the import of raw materials across the the mitochondrial inner membrane.

5) **Ionophores** (e.g. valinomycin, nigericin) make the inner membrane permeable to compounds which are ordinarily unable to cross.

6) **Krebs cycle inhibitors** (e.g. arsenite, aminooxyacetate) which block one or more of the TCA cycle enzymes, or an ancillary reation.

Microfilaments

Common to all eukaryotic cells, these filaments are primarily structural in function and are an important component of the cytoskeleton, along with microtubules and often the intermediate filaments. Microfilaments range from 5 to 9 nanometers in diameter and are designed to bear large amounts of tension. In association with **myosin**, microfilaments help to generate the forces used in cellular contraction and basic cell movements. The filaments also enable a dividing cell to pinch off into two cells and are involved in amoeboid movements of certain types of cells.

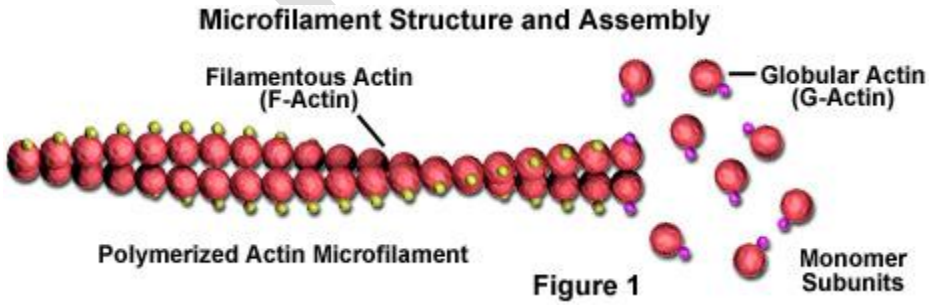


Figure 1

Microfilaments are solid rods made of a protein known as **actin**. When it is first produced by the cell, actin appears in a globular form (**G-actin**; see Figure 1). In microfilaments, however, which are also often referred to as actin filaments, long polymerized chains of the molecules are intertwined in a helix, creating a filamentous form of the protein (**F-actin**). All of the subunits that compose a microfilament are connected in such a way that they have the same orientation. Due to this fact, each microfilament exhibits **polarity**, the two ends of the filament being distinctly different. This polarity affects the growth rate of microfilaments, one end (termed the plus end) typically assembling and disassembling faster than the other (the minus end).

Unlike microtubules, which typically extend out from the centrosome of a cell, microfilaments are typically nucleated at the plasma membrane. Therefore, the periphery (edges) of a cell generally contains the highest concentration of microfilaments. A number of external factors and a group of special proteins influence microfilament characteristics, however, and enable them to make rapid changes if needed, even if the filaments must be completely disassembled in one region of the cell and reassembled somewhere else. When found directly beneath the plasma membrane, microfilaments are considered part of the cell **cortex**, which regulates the shape and movement of the cell's surface. Consequently, microfilaments play a key role in development of various cell surface projections (as illustrated in Figure 2), including filopodia, lamellipodia, and stereocilia.

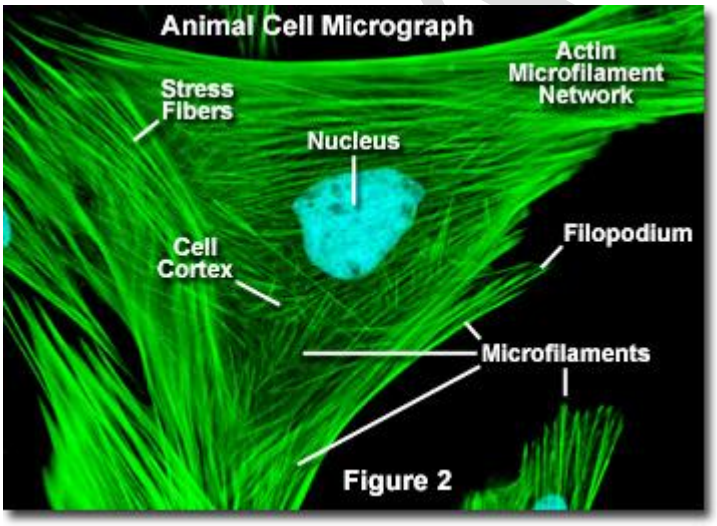


Figure 2

Illustrated in Figure 2 is a fluorescence digital image of an Indian Muntjac deer skin fibroblast cell stained with fluorescent probes targeting the nucleus (blue) and the actin cytoskeletal network (green). Individually, microfilaments are relatively flexible. In the cells of living organisms, however, the actin filaments are usually organized into larger, much stronger

structures by various accessory proteins. The exact structural form that a group of microfilaments assumes depends on their primary function and the particular proteins that bind them together. For instance, in the core of surface protrusions called **microspikes**, microfilaments are organized into tight parallel bundles by the bundling protein **fimbrin**. Bundles of the filaments are less tightly packed together, however, when they are bound by ***alpha***-actinin or are associated with fibroblast stress fibers (the parallel green fibers in Figure 2). Notably, the microfilament connections created by some cross-linking proteins result in a web-like network or gel form rather than filament bundles.

Structure and Organization of Actin Filaments

The major cytoskeletal protein of most cells is actin, which polymerizes to form actin filaments—thin, flexible fibers approximately 7 nm in diameter and up to several micrometers in length (Figure). Within the cell, actin filaments (also called microfilaments) are organized into higher-order structures, forming bundles or three-dimensional networks with the properties of semisolid gels. The assembly and disassembly of actin filaments, their crosslinking into bundles and networks, and their association with other cell structures (such as the plasma membrane) are regulated by a variety of actin-binding proteins, which are critical components of the actin cytoskeleton. Actin filaments are particularly abundant beneath the plasma membrane, where they form a network that provides mechanical support, determines cell shape, and allows movement of the cell surface, thereby enabling cells to migrate, engulf particles, and divide.

Assembly and disassembly of actin filaments

Actin was first isolated from muscle cells, in which it constitutes approximately 20% of total cell protein.. Although actin was initially thought to be uniquely involved in muscle contraction, it is now known to be an extremely abundant protein (typically 5 to 10% of total protein) in all types of eukaryotic cells. Mammals, for example, have at least six distinct actin genes: Four are expressed in different types of muscle and two are expressed in nonmuscle cells. All of the actins, however, are very similar in amino acid sequence and have been highly conserved throughout the evolution of eukaryotes.

The three-dimensional structures of both individual actin molecules and actin filaments were determined in 1990 by Kenneth Holmes, Wolfgang Kabsch, and their colleagues. Individual actin molecules are globular proteins of 375 amino acids (43 kd). Each actin monomer (**globular [G] actin**) has tight binding sites that mediate head-to-tail interactions with two other actin monomers, so actin monomers polymerize to form filaments **(filamentous [F] actin**) (Figure). Each monomer is rotated by 166^{o} in the filaments, which therefore have the appearance of a

double-stranded helix. Because all the actin monomers are oriented in the same direction, actin filaments have a distinct polarity and their ends (called the plus and minus ends) are distinguishable from one another. This polarity of actin filaments is important both in their assembly and in establishing a unique direction of myosin movement relative to actin.

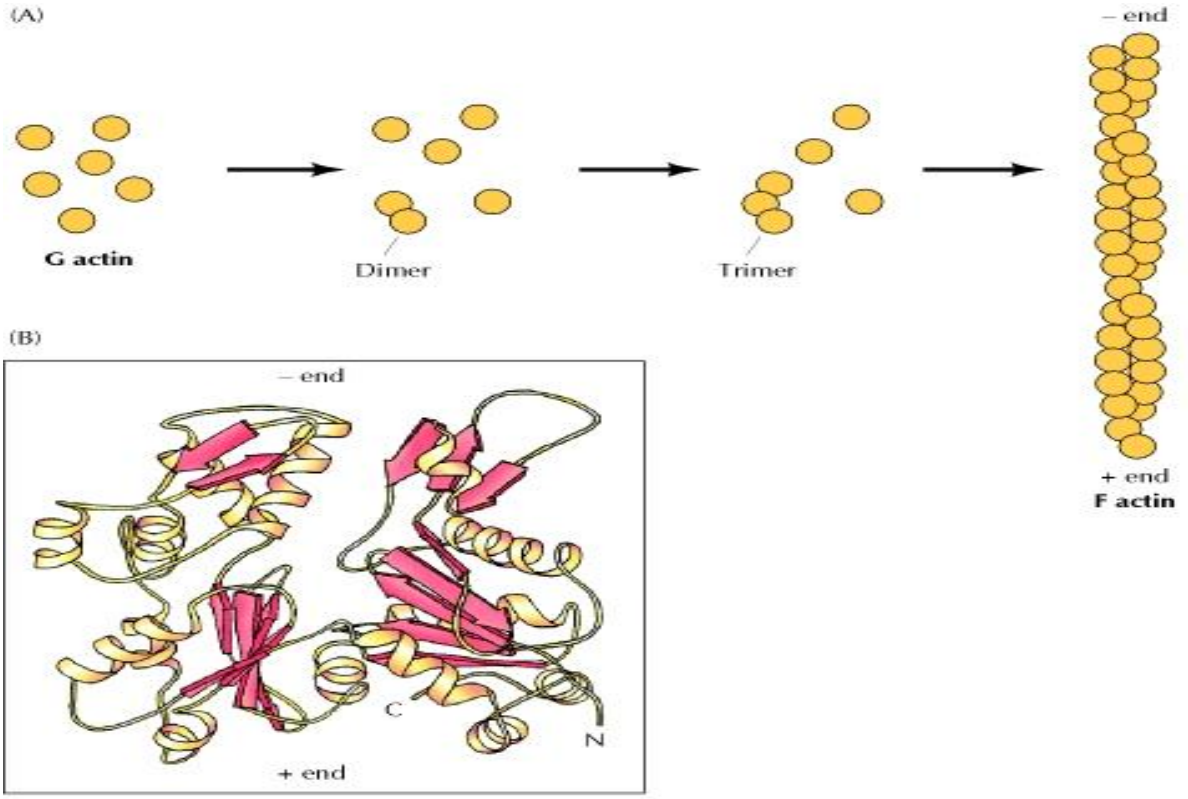


Figure: Assembly and structure of actin filaments:

Fig:(A) Actin monomers (G actin) polymerize to form actin filaments (F actin). The first step is the formation of dimers and trimers, which then grow by the addition of monomers to both ends. (B) Structure of an actin monomer. (C) Space-filling model of F actin. Nine actin monomers are represented in different colors. (C, courtesy of Dan Richardson.)

The assembly of actin filaments can be studied *in vitro* by regulation of the ionic strength of actin solutions. In solutions of low ionic strength, actin filaments depolymerize to monomers. Actin then polymerizes spontaneously if the ionic strength is increased to physiological levels.

Actin polymerization and depolymerization

The first step in actin polymerization (called nucleation) is the formation of a small aggregate consisting of three actin monomers. Actin filaments are then able to grow by the reversible addition of monomers to both ends, but one end (the plus end) elongates five to ten times faster than the minus end. The actin monomers also bind ATP, which is hydrolyzed to ADP following filament assembly. Although ATP is not required for polymerization, actin monomers to which ATP is bound polymerize more readily than those to which ADP is bound. ATP binding and hydrolysis play a key role in regulating the assembly and dynamic behavior of actin filaments.

Because actin polymerization is reversible, filaments can depolymerize by the dissociation of actin subunits, allowing actin filaments to be broken down when necessary (Figure). Thus, an apparent equilibrium exists between actin monomers and filaments, which is dependent on the concentration of free monomers. **The rate at which actin monomers are incorporated into filaments is proportional to their concentration, so there is a critical concentration of actin monomers at which the rate of their polymerization into filaments equals the rate of dissociation.** At this critical concentration, monomers and filaments are in apparent equilibrium.

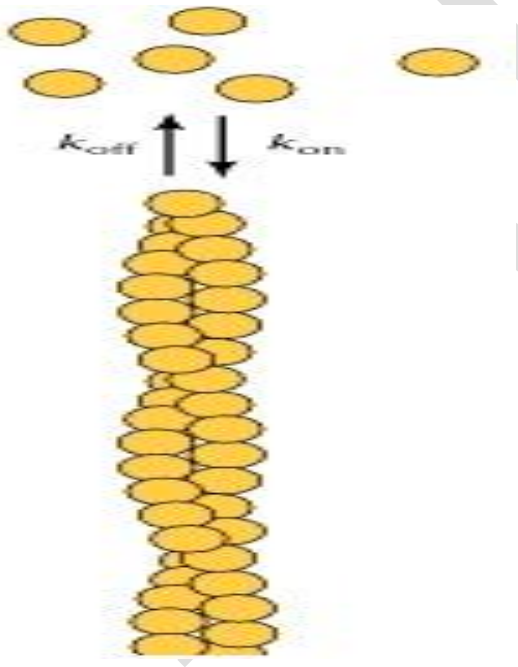


Figure : Reversible polymerization of actin monomers

Fig: Actin polymerization is a reversible process, in which monomers both associate with and dissociate from the ends of actin filaments. The rate of subunit dissociation (k_{off}) is independent of monomer concentration, while the rate of subunit association is proportional to the

concentration of free monomers and given by $C \times k_{on}$ (C = concentration of free monomers). An apparent equilibrium is reached at the critical concentration of monomers (C_c), where $k_{off} = C_c \times k_{on}$.

Treadmilling,

As noted earlier, the two ends of an actin filament grow at different rates, with monomers being added to the fast-growing end (the plus end) five to ten times faster than to the slow-growing (minus) end. Because ATP-actin dissociates less readily than ADP-actin, this results in a difference in the critical concentration of monomers needed for polymerization at the two ends. This difference can result in the phenomenon known as **treadmilling**, which illustrates the dynamic behavior of actin filaments (Figure). For the system to be at an overall steady state, the concentration of free actin monomers must be intermediate between the critical concentrations required for polymerization at the plus and minus ends of the actin filaments. Under these conditions, there is a net loss of monomers from the minus end, which is balanced by a net addition to the plus end. Treadmilling requires ATP, with ATP-actin polymerizing at the plus end of filaments while ADP-actin dissociates from the minus end. Although the role of treadmilling in the cell is unclear, it may reflect the dynamic assembly and disassembly of actin filaments **required for cells to move and change shape.**

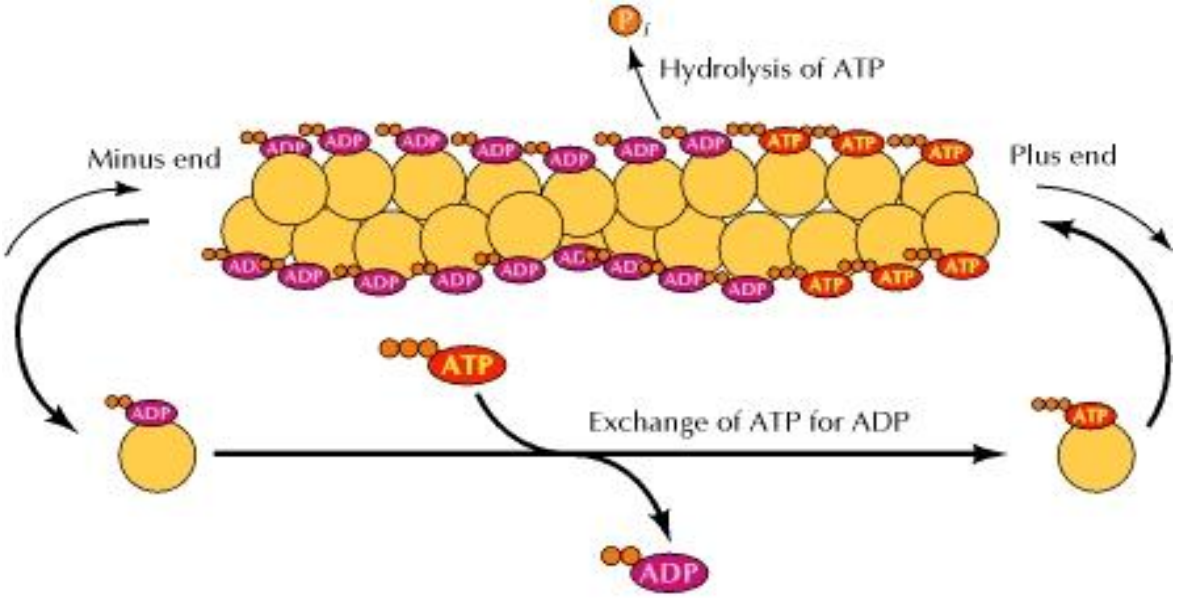


Figure: Treadmilling

It is noteworthy that several **drug**s useful in cell biology act by binding to actin and affecting its polymerization. For example, the **cytochalasins** bind to the plus ends of actin filaments and block their elongation. This results in changes in cell shape as well as inhibition of some types of cell movements (e.g., cell division following mitosis), indicating that actin polymerization is required for these processes. Another drug, phalloidin, binds tightly to actin filaments and prevents their dissociation into individual actin molecules. **Phalloidin** labeled with a fluorescent dye is frequently used to visualize actin filaments by fluorescence microscopy.

Regulation of actin assembly and disassembly

Within the cell, both the assembly and disassembly of actin filaments are regulated by **actin-binding proteins** (Figure). The turnover of actin filaments is about 100 times faster within the cell than it is *in vitro*, and this rapid turnover of actin plays a critical role in a variety of cell movements. The key protein responsible for actin filament disassembly within the cell is **cofilin**, which binds to actin filaments and enhances the rate of dissociation of actin monomers from the minus end. In addition, cofilin can sever actin filaments, generating more ends and further enhancing filament disassembly.

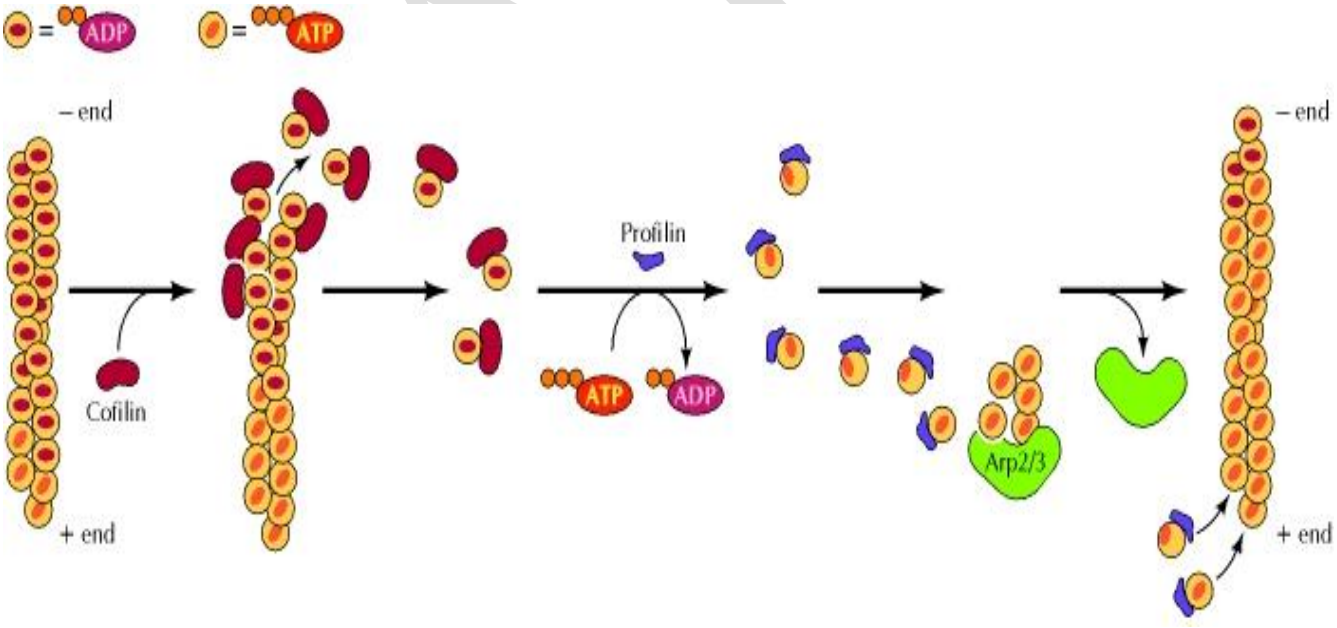


Figure : Effects of actin-binding proteins on filament turnover

However, another actin-binding protein, **profilin**, can reverse this effect of cofilin and stimulate the incorporation of actin monomers into filaments. Profilin acts by stimulating the exchange of bound ADP for ATP, resulting in the formation of ATP-actin monomers, which dissociate from cofilin and are then available for assembly into filaments. Other proteins (**Arp2/3 proteins**) can serve as nucleation sites to initiate the assembly of new filaments, so cofilin, profilin, and the Arp2/3 proteins (as well as other actin-binding proteins) can act together to promote the rapid turnover of actin filaments and remodeling of the actin cytoskeleton which is required for a variety of cell movements and changes in cell shape.

ORGANISATION OF ACTIN FILAMENTS

Individual actin filaments are assembled into two general types of structures, called actin bundles and actin networks, which play different roles in the cell (Figure). In bundles, the actin filaments are crosslinked into closely packed parallel arrays. In networks, the actin filaments are loosely crosslinked in orthogonal arrays that form three-dimensional meshworks with the properties of semisolid gels. The formation of these structures is governed by a variety of actin-binding proteins that crosslink actin filaments in distinct patterns.

Actin bundles

All of the actin-binding proteins involved in crosslinking contain at least two domains that bind actin, allowing them to bind and crosslink two different actin filaments. The proteins that crosslink actin filaments into bundles (called **actin-bundling proteins**) usually are small rigid proteins that force the filaments to align closely with one another.

There are two structurally and functionally distinct types of actin bundles, involving different actin-bundling proteins(Figure). The first type of bundle, containing closely spaced actin filaments aligned in parallel, supports projections of the plasma membrane, such as microvilli. In these bundles, all the filaments have the same polarity, with their plus ends adjacent to the plasma membrane. An example of a bundling protein involved in the formation of these structures is **fimbrin**, which was first isolated from intestinal microvilli and later found in surface projections of a wide variety of cell types. Fimbrin is a 68-kd protein, containing two adjacent actin-binding domains. It binds to actin filaments as a monomer, holding two parallel filaments close together.

Actin filaments are associated into two types of bundles by different actin-bundling proteins. Fimbrin has two adjacent actin-binding domains (ABD) and crosslinks actin filaments into closely packed parallel bundles in which the filaments are approximately 14 nm apart. In

contrast, the two separated actin-binding domains of α-actinin dimers crosslink filaments into more loosely spaced contractile bundles in which the filaments are separated by 40 nm. Both fimbrin and α-actinin contain two related Ca^{2+}-binding domains, and α-actinin contains four repeated α-helical spacer domains.

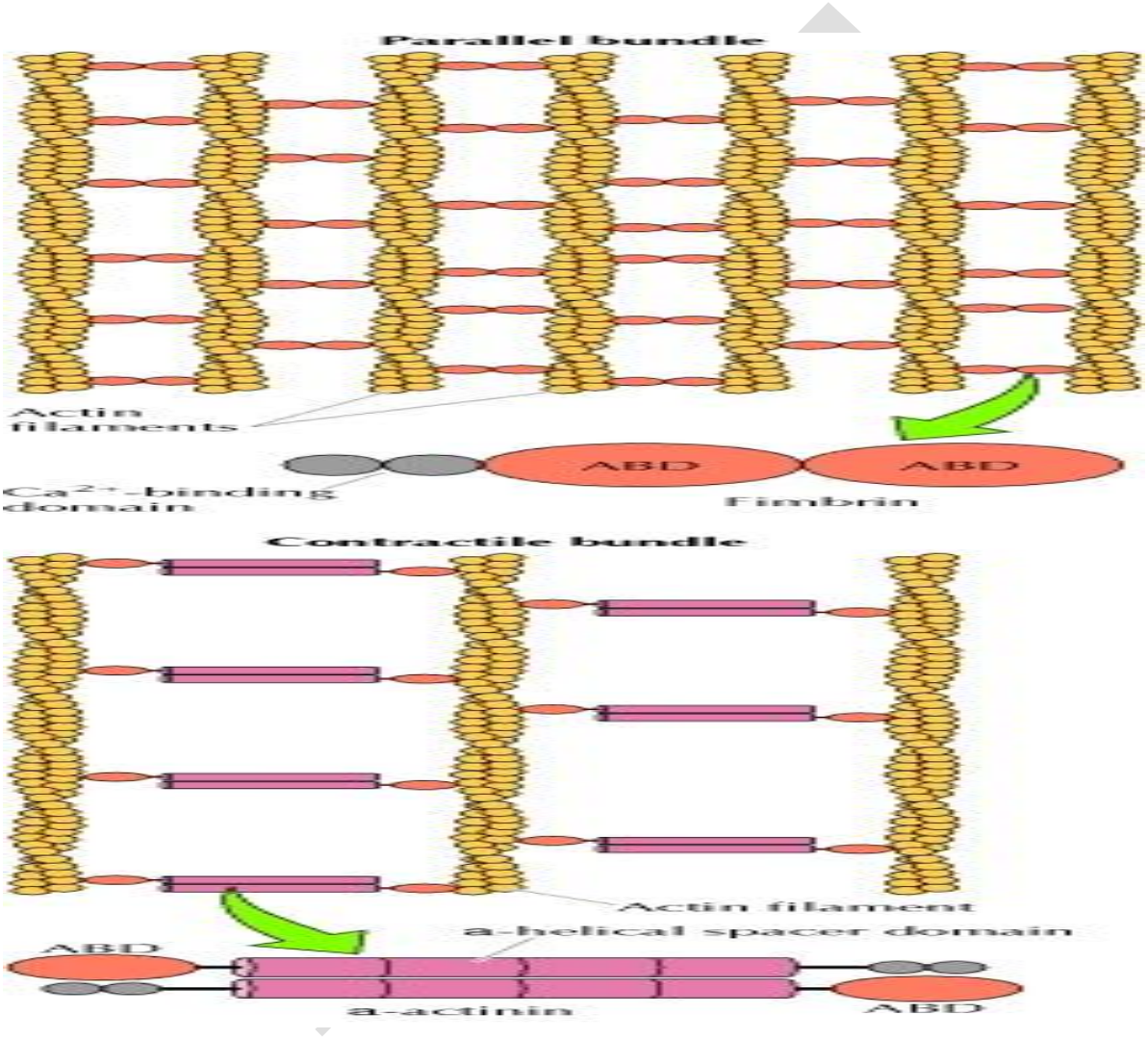


Figure : Actin-bundling proteins

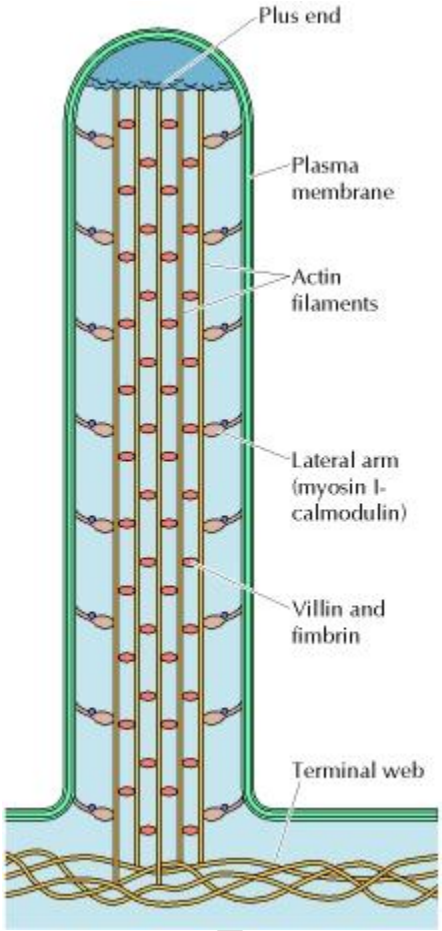


Figure:Organization of microvilli -The core actin filaments of microvilli are crosslinked into closely packed bundles by fimbrin and villin. They are attached to the plasma membrane along their length by lateral arms, consisting of myosin I and calmodulin. The plus ends of the actin filaments are embedded in a cap of unidentified proteins at the tip of the microvillus.

Actin network:

The second type of actin bundle is composed of filaments that are more loosely spaced and are capable of contraction, such as the actin bundles of the contractile ring that divides cells in two following mitosis. The looser structure of these bundles (which are called **contractile bundles**) reflects the properties of the crosslinking protein **α-actinin**. In contrast to fimbrin, α-actinin binds to actin as a dimer, each subunit of which is a 102-kd protein containing a single actin-binding site. Filaments crosslinked by α-actinin are consequently separated by a greater distance than those crosslinked by fimbrin (40 nm apart instead of 14 nm). The increased spacing between

filaments allows the motor protein myosin to interact with the actin filaments in these bundles, which (as discussed later) enables them to contract.

The actin filaments in networks are held together by large actin-binding proteins, such as **filamin** (Fig). Filamin (also called actin-binding protein or ABP-280) binds actin as a dimer of two 280-kd subunits. The actin-bindingdomains and dimerization domains are at opposite ends of each subunit, so the filamin dimer is a flexible V-shaped molecule with actin-binding domains at the ends of each arm. As a result, filamin forms cross-links between orthogonal actin filaments, creating a loose three-dimensional meshwork. As discussed in the next section, such networks of actin filaments underlie the plasma membrane and support the surface of the cell.

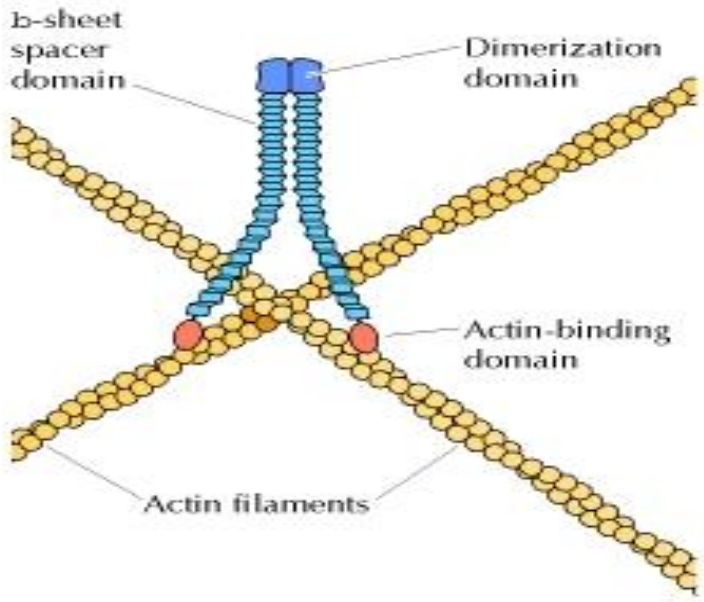


Figure: Actin networks and filamin

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Actin, Myosin, and Cell Movement

Actin filaments, usually in association with myosin, are responsible for many types of cell movements. Myosin is the prototype of a molecular motor—a protein that converts chemical energy in the form of ATP to mechanical energy, thus generating force and movement. The most striking variety of such movement is muscle contraction, which has provided the model for understanding actin-myosin interactions and the motor activity of myosin molecules. However, interactions of actin and myosin are responsible not only for muscle contraction but also for a

variety of movements of nonmuscle cells, including cell division, so these interactions play a central role in cell biology. Moreover, the actincytoskeleton is responsible for the crawling movements of cells across a surface, which appear to be driven directly by actin polymerization as well as actin-myosin interactions.

Muscle contraction

Muscle cells are highly specialized for a single task, contraction, and it is this specialization in structure and function that has made muscle the prototype for studying movement at the cellular and molecular levels. There are three distinct types of muscle cells in vertebrates: skeletal muscle, which is responsible for all voluntary movements; cardiac muscle, which pumps blood from the heart; and smooth muscle, which is responsible for involuntary movements of organs such as the stomach, intestine, uterus, and blood vessels. In both skeletal and cardiac muscle, the contractile elements of thecytoskeleton are present in highly organized arrays that give rise to characteristic patterns of cross-striations. It is the characterization of these structures in skeletal muscle that has led to our current understanding of muscle contraction, and other actin-based cell movements, at the molecular level.

Skeletal muscles are bundles of **muscle fibers**, which are single large cells (approximately 50 μm in diameter and up to several centimeters in length) formed by the fusion of many individual cells during development (Figure). Most of the cytoplasm consists of **myofibrils**, which are cylindrical bundles of two types of filaments: thick filaments of myosin(about 15 nm in diameter) and thin filaments of actin (about 7 nm in diameter). Each myofibril is organized as a chain of contractile units called sarcomeres, which are responsible for the striated appearance of skeletal and cardiac muscle.

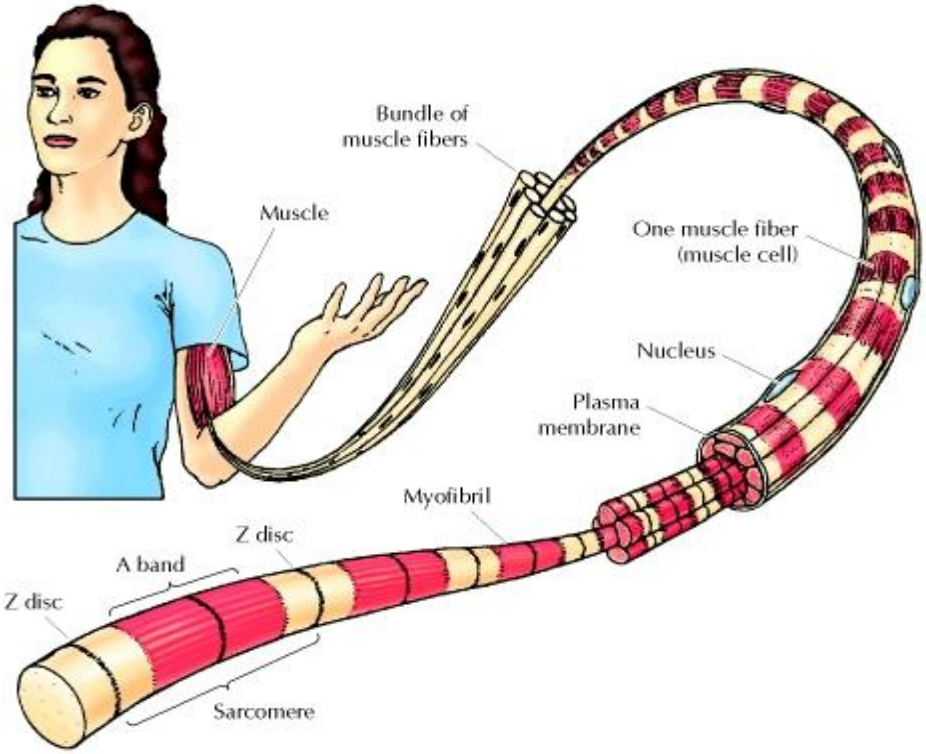


Figure ; Structure of muscle cells

Muscles are composed of bundles of single large cells (called muscle fibers) that form by cell fusion and contain multiple nuclei. Each muscle fiber contains many myofibrils, which are bundles of actin and myosin filaments organized into a chain of repeating units called sarcomeres.

The sarcomeres (which are approximately 2.3 μm long) consist of several distinct regions, discernible by electron microscopy, which provided critical insights into the mechanism of muscle contraction (Figure 11.19). The ends of eachsarcomere are defined by the Z disc. Within each sarcomere, dark bands (called A bands because they are *a*nisotropic when viewed with polarized light) alternate with light bands (called I bands for *i*sotropic). These bands correspond to the presence or absence of myosin filaments. The I bands contain only thin (actin) filaments, whereas the A bands contain thick (myosin) filaments. The myosin and actin filaments overlap in peripheral regions of the A band, whereas a middle region (called the H zone) contains only myosin. The actin filaments are attached at their plus ends to the Z disc, which includes the crosslinking protein α-actinin. The myosin filaments are anchored at the M line in the middle of the sarcomere.

Two additional proteins (**titin** and **nebulin**) also contribute to sarcomere structure and stability (Figure). Titin is an extremely large protein (3000 kd), and single titin molecules extend from the M line to the Z disc. These long molecules of titin are thought to act like springs that keep the myosin filaments centered in the sarcomere and maintain the resting tension that allows a muscle to snap back if overextended. Nebulin filaments are associated with actin and are thought to regulate the assembly of actin filaments by acting as rulers that determine their length.

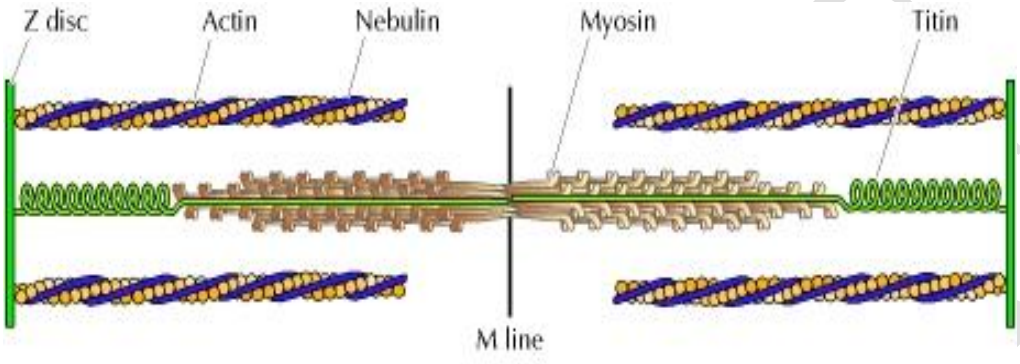


Figure : Titin and nebulin

Fig: Molecules of titin extend from the Z disc to the M line and act as springs to keep myosin filaments centered in the sarcomere. Molecules of nebulin extend from the Z disc and are thought to determine the length of associated actin filaments.

Sliding filament model for muscle contraction

The basis for understanding muscle contraction is the **sliding filament model**, first proposed in 1954 both by Andrew Huxley and Ralph Niedergerke and by Hugh Huxley and Jean Hanson (Figure 11.21). During muscle contraction, eachsarcomere shortens, bringing the Z discs closer together. There is no change in the width of the A band, but both the I bands and the H zone almost completely disappear. These changes are explained by the actin and myosin filaments sliding past one another, so that the actin filaments move into the A band and H zone. Muscle contraction thus results from an interaction between the actin and myosin filaments that generates their movement relative to one another. The molecular basis for this interaction is the binding of myosin to actin filaments, allowing myosin to function as a motor that drives filament sliding.

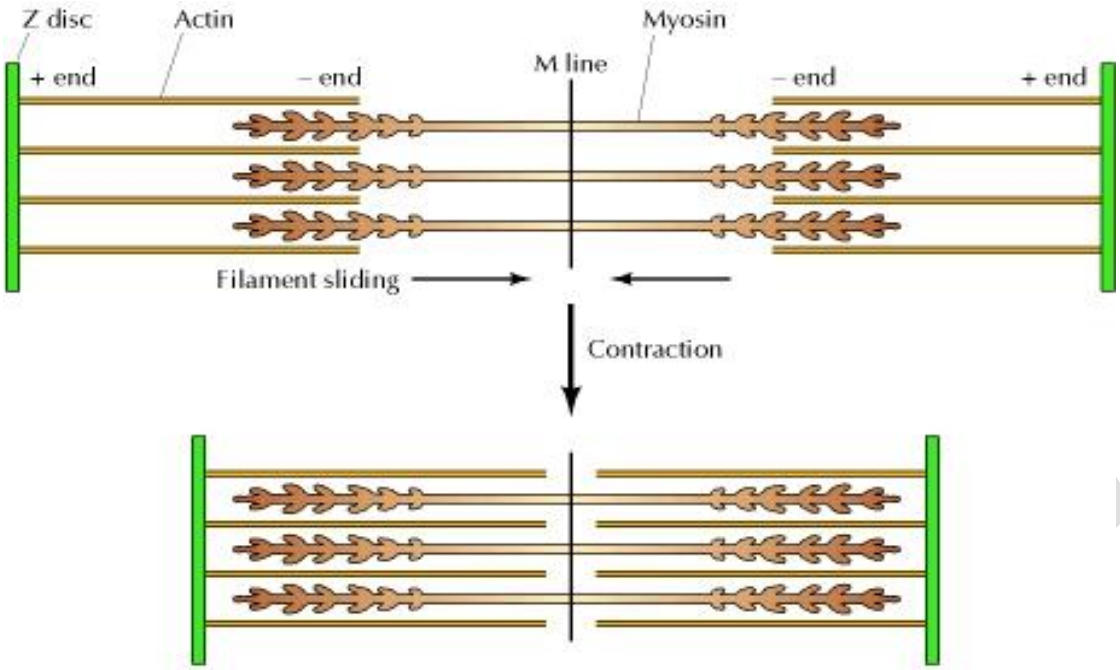


Figure : Sliding-filament model of muscle contraction

Fig; The actin filaments slide past the myosin filaments toward the middle of the sarcomere. The result is shortening of the sarcomere without any change in filament length.

Non muscle myosin

Contractile assemblies of actin and myosin in non muscle cells

Contractile assemblies of actin and myosin, resembling small-scale versions of muscle fibers, are present also in nonmuscle cells. As in muscle, the actin filaments in these contractile assemblies are interdigitated with bipolar filaments of myosin II, consisting of 15 to 20 myosin II molecules, which produce contraction by sliding the actin filaments relative to one another (Figure). The actin filaments in contractile bundles in nonmuscle cells are also associated with tropomyosin, which facilitates their interaction with myosin II, probably by competing with filamin for binding sites on actin.

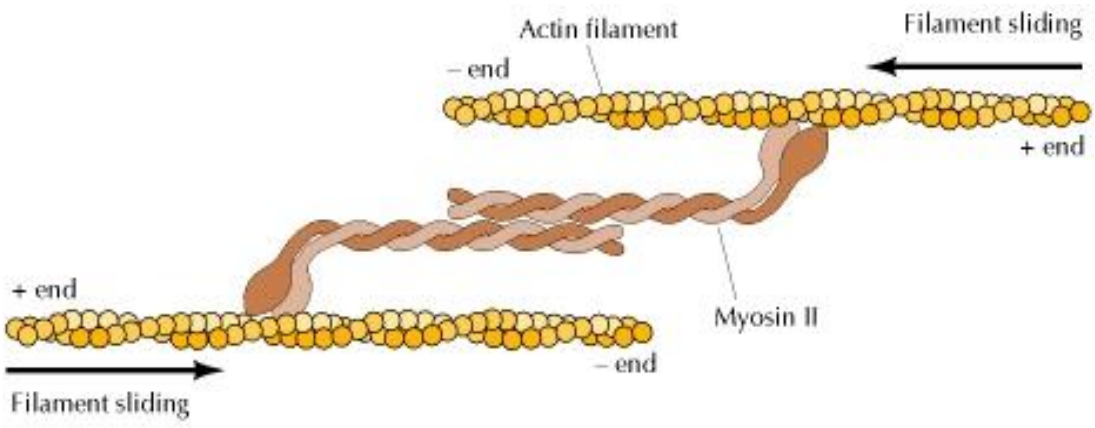


Figure : Contractile assemblies in nonmuscle cells

Bipolar filaments of myosin II produce contraction by sliding actin filaments in opposite directions.

Two examples of contractile assemblies in nonmuscle cells, stress fibers and adhesion belts, were discussed earlier with respect to attachment of the actin cytoskeleton to regions of cell-substrate and cell-cell contacts (see Fig). The contraction of stress fibers produces tension across the cell, allowing the cell to pull on a substrate (e.g., theextracellular matrix) to which it is anchored. The contraction of adhesion belts alters the shape of epithelial cell sheets: a process that is particularly important during embryonic development, when sheets of epithelial cells fold into structures such as tubes.

The most dramatic example of actin-myosin contraction in nonmuscle cells, however, is provided by cytokinesis—the division of a cell into two following mitosis (Figure). Toward the end of mitosis in animal cells, a contractile ringconsisting of actin filaments and myosin II assembles just underneath the plasma membrane. Its contraction pulls the plasma membrane progressively inward, constricting the center of the cell and pinching it in two. Interestingly, the thickness of the contractile ring remains constant as it contracts, implying that actin filaments disassemble as contraction proceeds. The ring then disperses completely following cell division.

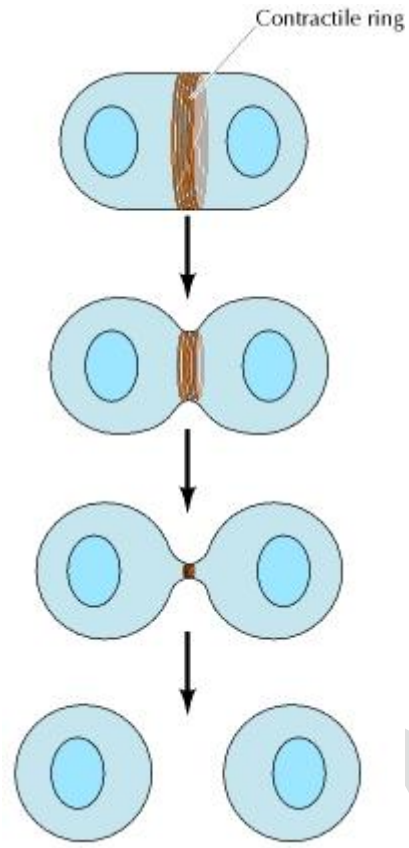


Figure : Cytokinesis

Fig: Following completion of mitosis (nuclear division), a contractile ring consisting of actin filaments and myosin II divides the cell in two.

Intermediate Filaments

Intermediate filaments have a diameter of about 10 nm, which is intermediate between the diameters of the two other principal elements of the cytoskeleton, actin filaments (about 7 nm) and microtubules (about 25 nm). In contrast to actin filaments and microtubules, the intermediate filaments are not directly involved in cell movements. Instead, they appear to play basically a structural role by providing mechanical strength to cells and tissues.

Intermediate Filament Proteins

Whereas actin filaments and microtubules are polymers of single types of proteins (actin and tubulin, respectively), intermediate filaments are composed of a variety of proteins that are

expressed in different types of cells. More than 50 different intermediate filament proteins have been identified and classified into six groups based on similarities between their amino acid sequences (Table). Types I and II consist of two groups of keratins, each consisting of about 15 different proteins, which are expressed in epithelial cells. Each type of epithelial cell synthesizes at least one type I (acidic) and one type II (neutral/basic) keratin, which copolymerize to form filaments. Some type I and II keratins (called**hard keratins**) are used for production of structures such as hair, nails, and horns. The other type I and II keratins (**soft keratins**) are abundant in the cytoplasm of epithelial cells, with different keratins being expressed in various differentiated cell types.

Table: Intermediate Filament Proteins

Type	Protein	Size (kd)	Site of expression
I	Acidic keratins	40–60	Epithelial cells
	(~15 proteins)		
II	Neutral or basic keratins	50–70	Epithelial cells
	(~15 proteins)		
III	Vimentin	54	Fibroblasts, white blood cells, and other cell types
	Desmin	53	Muscle cells
	Glial fibrillary acidic protein	51	Glial cells
	Peripherin	57	Peripheral neurons
IV	Neurofilament proteins		
	NF-L	67	Neurons
	NF-M	150	Neurons

Type	Protein	Size (kd)	Site of expression
	NF-H	200	Neurons
	α-Internexin	66	Neurons
V	Nuclear lamins	60–75	Nuclear lamina of all cell types
VI	Nestin	200	Stem cells of central nervous system

The type III intermediate filament proteins include **vimentin**, which is found in a variety of different kinds of cells, including fibroblasts, smooth muscle cells, and white blood cells. Another type III protein, **desmin**, is specifically expressed in muscle cells, where it connects the Z discs of individual contractile elements. A third type III intermediate filament protein is specifically expressed in glial cells, and a fourth is expressed in neurons of the peripheral nervous system.

The type IV intermediate filament proteins include the three **neurofilament (NF) proteins** (designated NF-L, NF-M, and NF-H for *l*ight, *m*edium, and *h*eavy, respectively). These proteins form the major intermediate filaments of many types of mature neurons. They are particularly abundant in the axons of motor neurons and are thought to play a critical role in supporting these long, thin processes, which can extend more than a meter in length. Another type IV protein (α-internexin) is expressed at an earlier stage of neuron development, prior to expression of the neurofilament proteins. The single type VI intermediate filament protein (nestin) is expressed even earlier during the development of neurons, in stem cells of the central nervous system.

The type V intermediate filament proteins are the nuclear lamins, which are found in most eukaryotic cells. Rather than being part of the cytoskeleton, the nuclear lamins are components of the nuclear envelope (see Figure). They also differ from the other intermediate filament proteins in that they assemble to form an orthogonal meshwork underlying the nuclear membrane.

Structure of intermediate filament

Despite considerable diversity in size and amino acid sequence, the various intermediate filament proteins share a common structural organization (Figure). All of the intermediate filament proteins have a central α-helical rod domain of approximately 310 amino acids (350

amino acids in the nuclear lamins). This central rod domain is flanked by amino- and carboxy-terminal domains, which vary among the different intermediate filament proteins in size, sequence, and secondary structure. As discussed next, the α-helical rod domain plays a central role in filament assembly, while the variable head and tail domains presumably determine the specific functions of the different intermediate filament proteins.

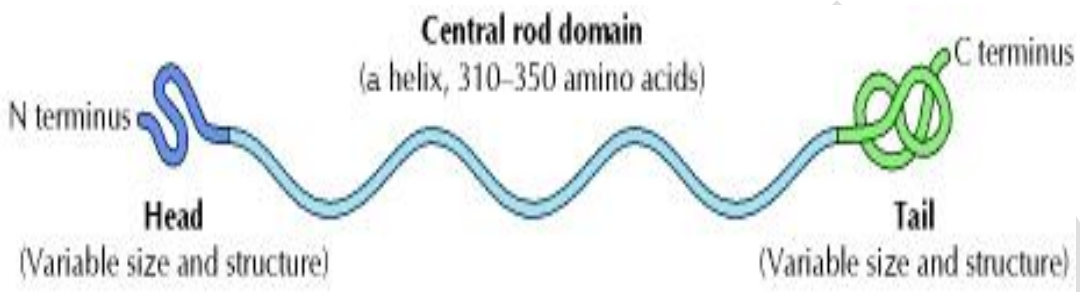


Figure 11.31Structure of intermediate filament proteins

Assembly of Intermediate Filaments

The first stage of filament assembly is the formation of dimers in which the central rod domains of two polypeptidechains are wound around each other in a coiled-coil structure, similar to that formed by myosin II heavy chains (Figure). The dimers then associate in a staggered antiparallel fashion to form tetramers, which can assemble end to end to form protofilaments. The final intermediate filament contains approximately eight protofilaments wound around each other in a ropelike structure. Because they are assembled from antiparallel tetramers, both ends of intermediate filaments are equivalent. Consequently, in contrast to actin filaments and microtubules, intermediate filaments are apolar; they do not have distinct plus and minus ends.

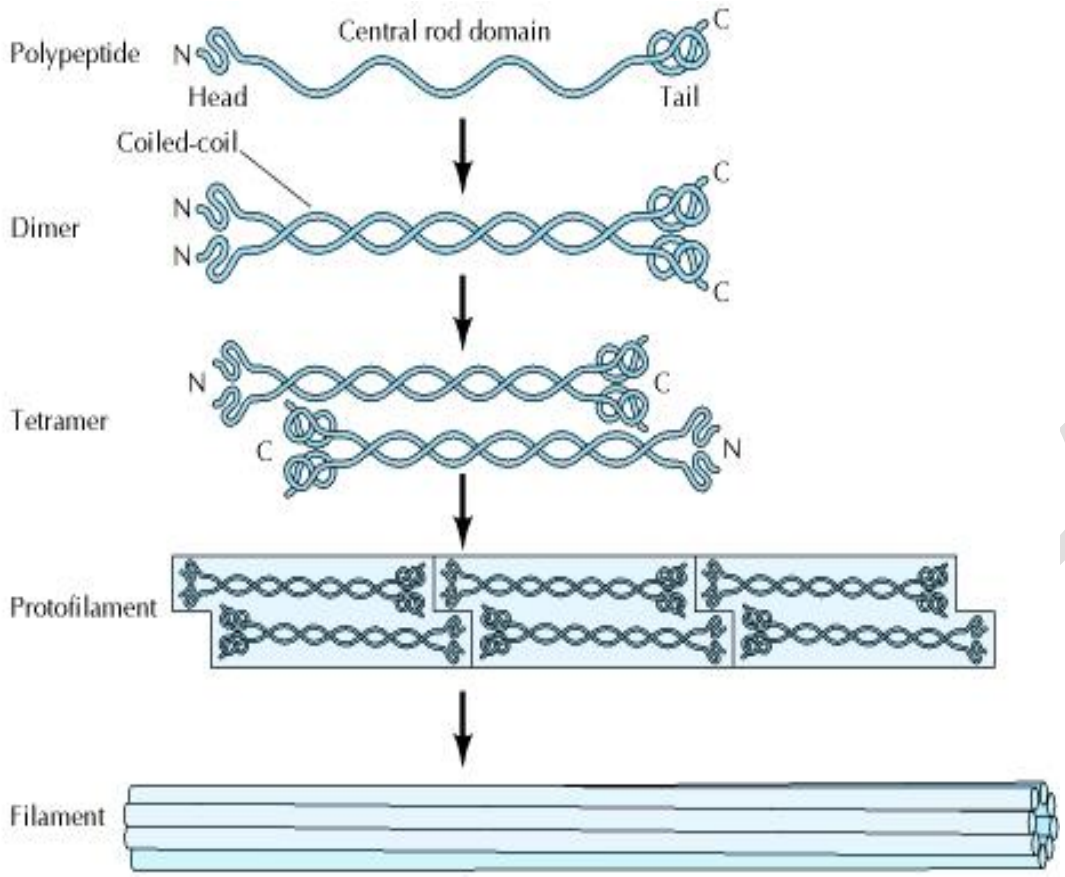


Figure : Assembly of intermediate filaments

Fig: The central rod domains of two polypeptides wind around each other in a coiled-coil structure to form dimers. Dimers then associate in a staggered antiparallel fashion to form tetramers. Tetramers associate end to end to form protofilaments and laterally to form filaments. Each filament contains approximately eight protofilaments wound around each other in a ropelike structure.

Intermediate filaments are generally more stable than actin filaments or microtubules and do not exhibit the dynamic behavior associated with these other elements of the cytoskeleton (e.g., the treadmilling of actin filaments. However, intermediate filament proteins are frequently modified by phosphorylation, which can regulate their assembly and disassembly within the cell. The clearest example is phosphorylation of the nuclear lamins , which results in disassembly of the nuclear lamina and breakdown of the nuclear envelope during mitosis. Cytoplasmic

intermediate filaments, such as vimentin, are also phosphorylated at mitosis, which can lead to their disassembly and reorganization in dividing cells.

Intracellular Organization of Intermediate Filaments

Intermediate filaments form an elaborate network in the cytoplasm of most cells, extending from a ring surrounding the nucleus to the plasma membrane. Both keratin and vimentin filaments attach to the nuclear envelope, apparently serving to position and anchor the nucleus within the cell. In addition, intermediate filaments can associate not only with the plasma membrane but also with the other elements of the cytoskeleton, actin filaments and microtubules. Intermediate filaments thus provide a scaffold that integrates the components of the cytoskeleton and organizes the internal structure of the cell.

The keratin filaments of epithelial cells are tightly anchored to the plasma membrane at two areas of specialized cell contacts, desmosomes and hemidesmosomes (Figure). Desmosomes are junctions between adjacent cells, at which cell-cell contacts are mediated by transmembrane proteins related to the cadherins. On their cytoplasmic side, desmosomes are associated with a characteristic dense plaque of intracellular proteins, to which keratin filaments are attached. These attachments are mediated by desmoplakin, a member of a family of proteins called **plakins** that bind intermediate filaments and link them to other cellular structures. Hemidesmosomes are morphologically similar junctions between epithelial cells and underlying connective tissue, at which keratin filaments are linked by different members of the plakin family (e.g., plectin) to integrins. Desmosomes and hemidesmosomes thus anchor intermediate filaments to regions of cell-cell and cell-substratum contact, respectively, similar to the attachment of the actin cytoskeleton to the plasma membrane at adherens junctions and focal adhesions. It is important to note that the keratin filaments anchored to both sides of desmosomes serve as a mechanical link between adjacent cells in an epithelial layer, thereby providing mechanical stability to the entire tissue.

In addition to linking intermediate filaments to cell junctions, some plakins link intermediate filaments to other elements of the cytoskeleton. Plectin, for example, binds actin filaments and microtubules in addition to intermediate filaments, so it can provide bridges between these cytoskeletal components (Figure). These bridges to intermediate filaments are thought to brace and stabilize actin filaments and microtubules, thereby increasing the mechanical stability of the cell.

Two types of intermediate filaments, desmin and the neurofilaments, play specialized roles in muscle and nerve cells, respectively. Desmin connects the individual actin-myosin assemblies of

muscle cells both to one another and to theplasma membrane, thereby linking the actions of individual contractile elements. Neurofilaments are the major intermediate filaments in most mature neurons. They are particularly abundant in the long axons of motor neurons, where they appear to be anchored to actin filaments and microtubules by neuronal members of the plakin family. Neurofilaments are thought to play an important role in providing mechanical support and stabilizing other elements of the cytoskeleton in these long, thin extensions of nerve cells.

Functions of Keratins and Neurofilaments: Diseases of the Skin and Nervous System

Although intermediate filaments have long been thought to provide structural support to the cell, direct evidence for their function has only recently been obtained. Some cells in culture make no intermediate filament proteins, indicating that these proteins are not required for the growth of cells *in vitro*. Similarly, injection of cultured cells with antibody against vimentin disrupts intermediate filament networks without affecting cell growth or movement. Therefore, it has been thought that intermediate filaments are most needed to strengthen the cytoskeleton of cells in the tissues of multicellular organisms, where they are subjected to a variety of mechanical stresses that do not affect cells in the isolated environment of a culture dish.

Experimental evidence for such an *in vivo* role of intermediate filaments was first provided in 1991 by studies in the laboratory of Elaine Fuchs. These investigators used transgenic mice to investigate the *in vivo* effects of expressing a keratin deletion mutant encoding a truncated polypeptide that disrupted the formation of normal keratin filaments. This mutant keratin gene was introduced into transgenic mice, where it was expressed in basal cells of the epidermis and disrupted formation of a normal keratin cytoskeleton. This resulted in the development of severe skin abnormalities, including blisters due to epidermal cell lysis following mild mechanical trauma, such as rubbing of the skin. The skin abnormalities of these transgenic mice thus provided direct support for the presumed role of keratins in providing mechanical strength to epithelial cells in tissues.

Cilia and Flagella

Cilia and **flagella** are microtubule-based projections of the plasma membrane that are responsible for movement of a variety of eukaryotic cells. Many bacteria also have flagella, but these prokaryotic flagella are quite different from those of eukaryotes. Bacterial flagella (which are not discussed further here) are protein filaments projecting from the cell surface, rather than projections of the plasma membrane supported by microtubules.

Eukaryotic cilia and flagella are very similar structures, each with a diameter of approximately 0.25 μm (Figure . Many cells are covered by numerous cilia, which are about 10 μm in length. Cilia beat in a coordinated back-and-forth motion, which either moves the cell through fluid or moves fluid over the surface of the cell. For example, the cilia of some protozoans (such as *Paramecium*) are responsible both for cell motility and for sweeping food organisms over the cell surface and into the oral cavity. In animals, an important function of cilia is to move fluid or mucus over the surface of epithelial cell sheets. A good example is provided by the ciliated cells lining the respiratory tract, which clear mucus and dust from the respiratory passages. Flagella differ from cilia in their length (they can be as long as 200 μm) and in their wavelike pattern of beating. Cells usually have only one or two flagella, which are responsible for the locomotion of a variety of protozoans and of sperm.

Structure

The fundamental structure of both cilia and flagella is the axoneme, which is composed of microtubules and their associated proteins (Figure 11.51). The microtubules are arranged in a characteristic "9 + 2" pattern in which a central pair of microtubules is surrounded by nine outer microtubule doublets. The two fused microtubules of each outer doublet are distinct: One (called the A tubule) is a complete microtubule consisting of 13 protofilaments; the other (the B tubule) is incomplete, containing only 10 or 11 protofilaments fused to the A tubule. The outer microtubule doublets are connected to the central pair by radial spokes and to each other by links of a protein called nexin. In addition, two arms of dynein are attached to each A tubule, and it is the motor activity of these axonemal dyneins that drives the beating of cilia and flagella.

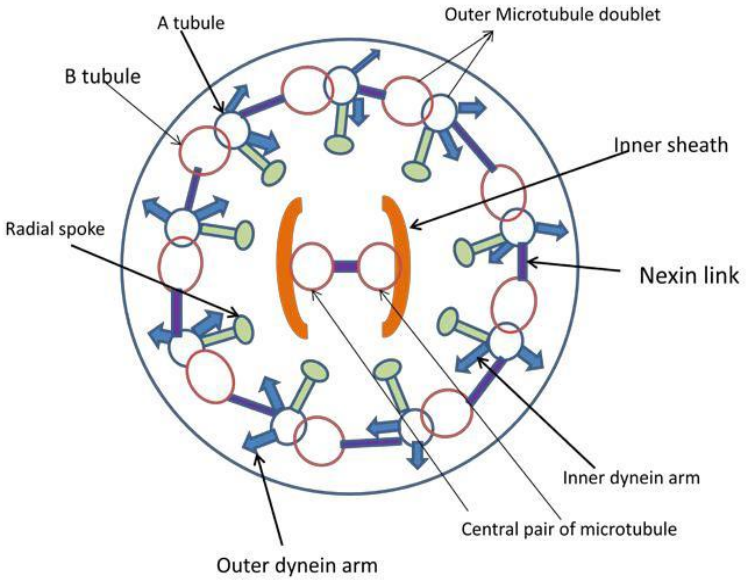


Figure 1: Structure of axoneme of cilia and flagella

The minus ends of the microtubules of cilia and flagella are anchored in a basal body, which is similar in structure to a centriole and contains nine triplets of microtubules (). Centrioles were discussed earlier as components of the centrosome, in which their function is uncertain. Basal bodies, however, play a clear role in organization of theaxoneme microtubules. Namely, each of the outer microtubule doublets of the axoneme is formed by extension of two of the microtubules present in the triplets of the basal body. Basal bodies thus serve to initiate the growth of axonemal microtubules, as well as anchoring cilia and flagella to the surface of the cell.

Mechanism of movement

The movements of cilia and flagella result from the sliding of outer microtubule doublets relative to one another, powered by the motor activity of axonemal dynein (Figure 11.53). The dynein bases bind to the A tubules while the dynein head groups bind to the B tubules of adjacent doublets. Movement of the dynein head group in the minus end direction then causes the A tubule of one doublet to slide toward the basal end of the adjacent B tubule. Because the microtubule doublets in an axoneme are connected by nexin links, the sliding of one doublet along another causes them to bend, forming the basis of the beating movements of cilia and flagella. It is apparent, however, that the activities of dynein molecules in different regions of the axoneme must be carefully regulated to produce the coordinated beating of cilia and the wavelike oscillations of flagella—a process about which little is currently understood.

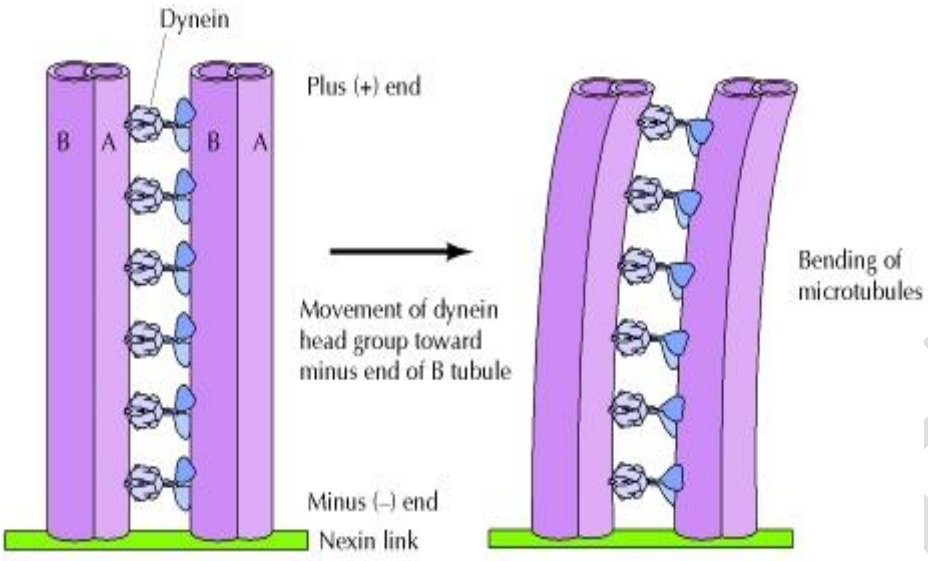


Figure:Movement of microtubules in cilia and flagella

The bases of dynein arms are attached to A tubules, and the motor head groups interact with the B tubules of adjacent doublets. Movement of the dynein head groups in the minus end direction (toward the base of the cilium) then causes the A tubule of one doublet to slide toward the base of the adjacent B tubule. Because both microtubule doublets are connected by nexin links, this sliding movement forces them to bend.

The beating of cilia or flagella is caused by the intraciliary excitation which is followed by the interciliary conduction. Recent studies have shown that cytoplasm is necessary for the ciliary movements. The ATP provides necessary amount of energy for the motion of the cilia and flagella. Four types of ciliary movements have been recognized which are as follows :

1. **The pendulus ciliary movement**: The pendulus type of ciliary movement is carried out in asingle plane. It occurs in the ciliated protozoans which have rigid cilia.

2. **The unciform ciliary movement:** The unciform (hook-like) ciliary movement occurs commonly in the metazoan cells.

3. **The infundibuliform ciliary movement:** The infundibuliform ciliary movement occurs due to the rotary movement of the cilium and flagellum.

4. The undulant movement: The undulant movement is the characteristic of the flagellum. In undulant movement the waves of the contraction proceed from the site of implantation and pass to the border.

Each beat of cilium or flagellum involves the same pattern of microtubule movement. Each cilium moves with a whip-like motion and its beat may be divided into two phases:

1. The fast effective stroke (or forward active stroke or power stroke) in which the cilium is fully extended and beating against the surrounding liquid.

2. The slow recovery stroke, in which the cilium returns to its original position with an unrolling movement that minimizes viscous drag.

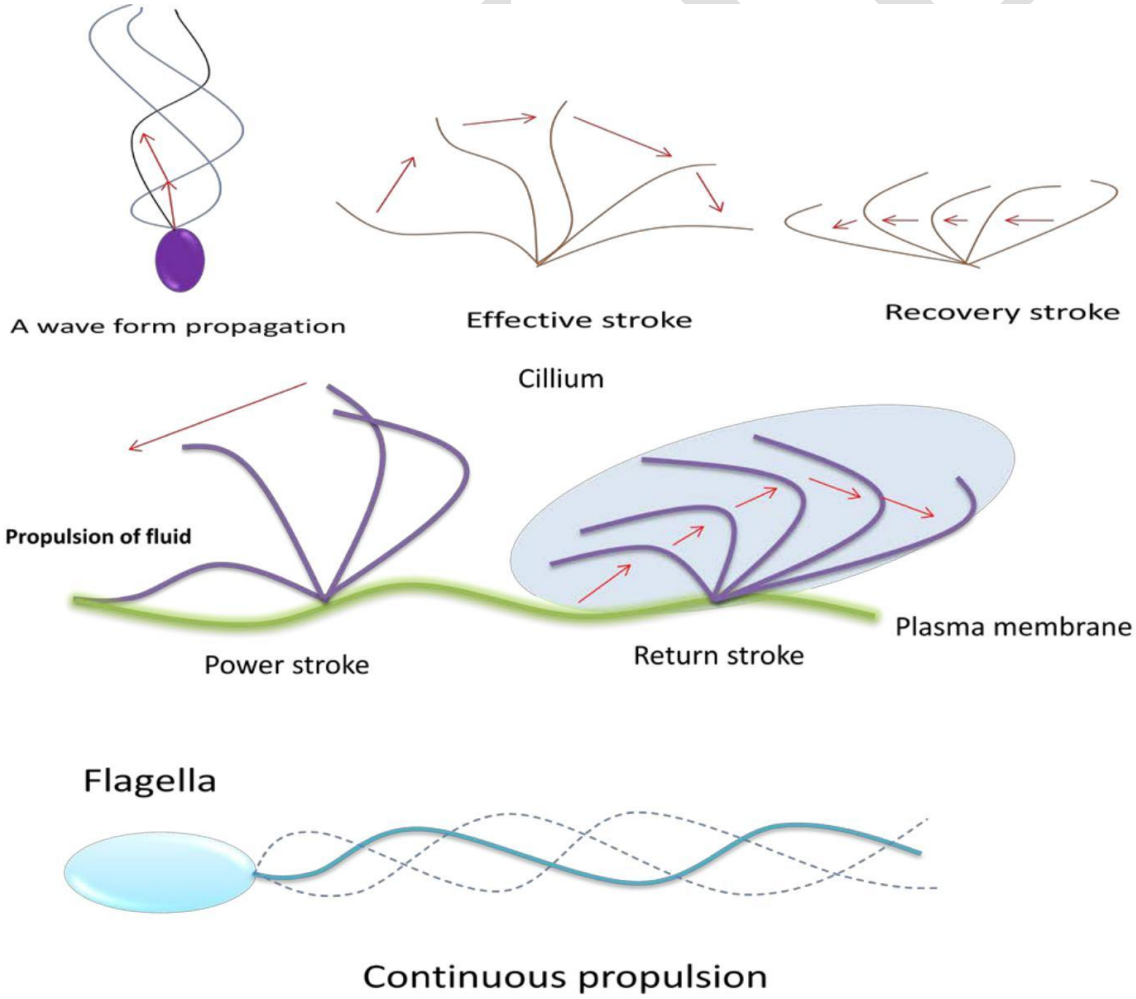


Figure : Ciliary and flagellar movement

Myosin

Myosins comprise a superfamily of ATP-dependent motor proteins and are best known for their role in muscle contraction and their involvement in a wide range of other motility processes in eukaryotes. They are responsible for actin-based motility. The term was originally used to describe a group of similar ATPases found in the cells of both striated muscle tissue and smooth muscle tissue. Following the discovery by Pollard and Korn (1973) of enzymes with myosin-like function in Acanthamoeba castellanii, a large number of divergent myosin genes have been discovered throughout eukaryotes. Thus, although myosin was originally thought to be restricted to muscle cells (hence myo-(s) + -in), there is no single "myosin" but rather a huge superfamily of genes whose protein products share the basic properties of actin binding, ATP hydrolysis (ATPase enzyme activity), and force transduction. Virtually all eukaryotic cells contain myosin isoforms. Some isoforms have specialized functions in certain cell types (such as muscle), while other isoforms are ubiquitous. The structure and function of myosin is strongly conserved across species, to the extent that rabbit muscle myosin II will bind to actin from an amoeba.

Structure and function

Domains

Most myosin molecules are composed of a head, neck, and tail domain.

The head domain binds the filamentous actin, and uses ATP hydrolysis to generate force and to "walk" along the filament towards the barbed (+) end (with the exception of myosin VI, which moves towards the pointed (-) end).

the neck domain acts as a linker and as a lever arm for transducing force generated by the catalytic motor domain. The neck domain can also serve as a binding site for myosin light chains which are distinct proteins that form part of a macromolecular complex and generally have regulatory functions.

The tail domain generally mediates interaction with cargo molecules and/or other myosin subunits. In some cases, the tail domain may play a role in regulating motor activity.

Power stroke

Muscle contraction

Multiple myosin II molecules generate force in skeletal muscle through a power stroke mechanism fuelled by the energy released from ATP hydrolysis. The power stroke occurs at the release of phosphate from the myosin molecule after the ATP hydrolysis while myosin is tightly bound to actin. The effect of this release is a conformational change in the molecule that pulls against the actin. The release of the ADP molecule leads to the so-called rigor state of myosin. The binding of a new ATP molecule will release myosin from actin. ATP hydrolysis within the myosin will cause it to bind to actin again to repeat the cycle. The combined effect of the myriad power strokes causes the muscle to contract.

Nomenclature, evolution, and the family tree

Skeletal muscle myosin, the most conspicuous of the myosin superfamily due to its abundance in muscle fibers, was the first to be discovered. This protein makes up part of the sarcomere and forms macromolecular filaments composed of multiple myosin subunits. Similar filament-forming myosin proteins were found in cardiac muscle, smooth muscle, and nonmuscle cells. However, beginning in the 1970s, researchers began to discover new myosin genes in simple eukaryotes[3] encoding proteins that acted as monomers and were therefore entitled Class I myosins. These new myosins were collectively termed "unconventional myosins"[7] and have been found in many tissues other than muscle. These new superfamily members have been grouped according to phylogenetic relationships derived from a comparison of the amino acid sequences of their head domains, with each class being assigned a Roman numeral[8][9][10][11] (see phylogenetic tree). The unconventional myosins also have divergent tail domains, suggesting unique functions. The now diverse array of myosins likely evolved from an ancestral precursor.

POSSIBLE QUESTIONS

2 marks

1. What is meant by reduction potential?
2. Define oxidative phosphorylation.
3. Write about translocation of protons.
4. What is meant by proton motive force?
5. List some inhibitors of ATP synthesis.
6. Define uncouplers.
7. What is meant by microfilaments?
8. Define microtubules.
9. What is meant by intermediary filaments?

8 marks

1. Explain about electron transport chain complexes and Q cycle.
2. Write the sequence of ETC and the process of electron transport. Add a note on inhibitors.
3. Detailed note on microfilaments and its assembly.
4. Discuss about microtubules.
5. Give the structure and functions of cilia and flagella.
6. Illustrate about intermediary filaments and its importance.

KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
I-M.Sc., BIOCHEMISTRY
CELLULAR BIOCHEMISTRY (18BCP104)
MULTIPLE CHOICE QUESTIONS

UNIT III

S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	……..is called the power house of cell	Golgi complex	nucleus	mitochondria	vacuole	mitochondria
2	……..... is involved in the ETC reaction by oxidative phosphorylation.	Mitochondria	lysosome	ribosome	golgi complex	mitochondria
3	The ETC located in the …….. of matrix.	outer membrane	cristae	inner membrane	matrix	inner membrane
4	UQ transfers electron from …….....	Complex I to III	complex II to III	both	none of the above	both
5	Ubiquinone is a …… soluble benzoquinone with a long isoprenoid side chain.	water soluble	fat soluble	lipid soluble	none of the above	fat soluble
6	The molecular weight of actin is ……	4000 Daltons	2800 Daltons	28000 Daltons	42000 Daltons	42000 Daltons
7	All myosin filament bind reversibly to actin filaments and cont…….	potassium	magnesium	calcium	ATP	calcium
8	The molecular weight of myosin is …….	500000 Daltons	50000 Daltons	5000 Daltons	500 Daltons	500000 Daltons
9	Myosin filament are ……nm.	13-22 nm	20–25 nm	10-15nm	15-20nm	13-22 nm
10	The assembly of actin filaments proceeds in ……sequential phases .	2 phases	1 phases	3 phases	5 phases	3 phases
11	Microtubule after assembling there stability becomes …….dependent.	Density	Gradient	Temperature	Viscosity	Temperature
12	The microtubule assemply depends upon the critical concentration of …….tubulin.	α	β	α - β	none	α - β
13	What is mRNA?	mitosis	mitochondrial	molecular	all the above	mitochondrial
14	The size of mammalian mitochondrial DNA is …….	16s RNA	12sRNA	both a & b	none of the above	both a & b
15	Which protein is used for modifying pepetide chain.?	ubiquitin	protenases	chaperons	protein kinase	chaperons
16	Which enzyme synthesisATP from ADP during oxidative phosphorylation.	ATP synthetase	ATP ase	phosphorylase	ATP synthetase	ATP synthetase
17	Mitoprotein is synthesized from ……	nuclear protein	mitochondrial protein	functional protein	ATP syntheatse	nuclear protein
18	Which transport is called uniport?	symport	antiport	active	passive	symport
19	The role of oxi-3 is ……	coding for cyt b	coding for cyt b oxidase	coding for subunit 1	all the above	coding for subunit 1
20	Mutation of mitochondrial gene that leads to which diseases?	myoclonic epilepsy	ragged red fibre	muscular genetic	both a & b	both a & b
21	During protein synthesis Timtom is transmitted from……..	cytosol to mitochondrial matrix	cytoplasm to nucleus	mitomatrix to cytosol	none of the above	cytosol to mitochondrial matrix
22	Human mitochondria contain……number of genes	13	26	37	45	37
23	Example of inhibitors of Cyt C oxidase.	cyanides	carbonmonoxide	CO2	Azarzerine	carbonmonoxide
24	Retinone is ……	vitamin	protein	protein product	carcinogen	protein product
25	Binary fission is an example for …….	asexual reproduction	sexual reproduction	vertebrate	all the above	asexual reproduction
26	A group of intermediary filament proteins that forms the fibrons on nuclear envelope is known as ……. ….	euchromatin	nuclear laminar	lamins	barr body	lamins
27	How many ATP's formed during pyruvate oxidation?	3	5	7	6	6
28	Protein degradation pathway is otherwise called as ……	Ubiquitin pathway	degradation pathway	a & b	none of the above	Ubiquitin pathway
29	Iron sulphur protein is made up of ……	histidine residues	cystinineresidues	glycine residues	alanine residues	cystinineresidues
30	Which one is used to remove mitochondrial membrane ……	peptidase	digitonin	sucrose	all the above	digitonin
31	The muscle cell contain – Protein	actin	myosin	both a & b	None of the Above	both a & b
32	3 D view of microfilaments is called as ----	microtrabecular lattice	Macrotrabecular lattice	trabecular Lattice	both a & b	microtrabecular lattice
33	Cytoplasmic actin consisting double helical array of ---- molecule	G.actin	G.myosin	Filaments	none of the above	G.actin
34	Name the cross linking protein found in cytoskeleton of RBC	Fodrin	Villin	Spectrin	Vinculin	Spectrin
35	By which protein the actin microfilaments are cross linked is form side by side aggregate	Fodkin	Villin	Spectrin	Vinculin	Fodkin
36	----at high concentration causes fragmentation of actin filaments	Villin	Gelsolin	Acumentin	Fragmin	Villin
37	---- binds to actin & prevents polymerization	Villin	Gelsolin	Acumentin	Profiling	Profiling
38	The 1st phase of actin filament assembly is marked by---	lag phase	stationary phase	both a & b	log phase	lag phase

39	The 2nd phase of actin filament assembly is ----	steady phase	elongation phase	lag phase	log phase	elongation phase
40	------proteins binds to actin filament & by breaking them into shorter filaments.	Gelosin	Cofilin	both a & b	none of the above	both a & b
41	Microtubule filament run the length of the central core of the ---	Connective tissue	Cilia	Flagella	both b & C	Cilia
42	Flagella undulate in a------	oscillation movement	Whiplike manner	both a & b	none of the above	Whiplike manner
43	The beating of ------- is the only mean for locomotion for sperm	Cilia	Flagella	both a & b	none of the above	both a & b
44	Cilia & flagella extend from --- unicellular organism	lysosome	nuclear envelope	golgi bodies	plasmamembrane	plasmamembrane
45	-----protein produces force towards positive end rarely negative end	protease	protein kinase	kinesin	both b & C	kinesin
46	----- Kinesin is localized in ----	golgi	ER	both a & b	none of the above	both a & b
47	--------binds with kinesin and activates its activity during vesicular traffic	peptidase	amylase	protease	kinectin	kinectin
48	---- kinesin protein areresponsible for spindle organization	KAR 3	protease	NCD	both a & C	both a & C
49	Name the subclass of dyneins	Nucleoplasmic	axonemale	cytoplasmic	both b & c	both b & c
50	----- facilates the function of dynein	actins	myosin	myoglobin	dynactins	dynactins
51	When flagella are distributed all around the bacterial cell, the arrangement is called--	polar	random	peritrichous	encapsulated	peritrichous
52	Epithelial that consist of more than 1 layer of cells is formed---	striated	stratified	stipulated	intracalated	stipulated
53	Which of the followings does not describe skeletal muscle tissue fibre	striated	voluntary	multinucleate	branched	branched
54	ATPase of the muscle located in	actinin	troponin	mysin	actin	actin
55	The bacteriu flagellum is made up of –	Protein flagellin	protein flagella	lipid flagellin	lipid flagella	Protein flagellin
56	Cilia is an organelle found in –	prokaryotic cell	eukaryotic cell	pro & Euk cell	none of the above	eukaryotic cell
57	operation of modified Q cycle III results in the reduction of ---	Cyt C	Cyt B	Cyt C P50	both a & b	Cyt C
58	Microtubule are one of the components of the ---	endoskeleton	cytoskeleton	exoskeleton	none of the above	cytoskeleton
59	Microfilaments are found by the --- polymerisation of actin monomers	tail to head	tail only	head only	head to tail	head to tail
60	ETC couples is a reaction between an electron donar & ---	electron acceptor	proton acceptor	proton donar	none of the above	electron acceptor

UNIT-IV-SYLLABUS

Cell – Matrix interaction: Cell – Cell interaction: Extra cellular matrix; Collagen, hyaluronan and proteoglycans, laminin, integrins, Fibrillin, elastin and fibronectins.
Cell – Cell adhesion: Specialised junctions – Desmosomes, Gap junctions, Tight junctions. Adhesion molecules – Cadherins (E and N), Connexins.

Cell – Cell signaling – Role of Signaling molecules and their receptors; functions of cell surface receptors, pathways of intracellular signal transduction, second messengers, G-protein coupled receptors, receptor tyrosine kinases, Ras, MAP kinases in cellular growth and functions.

Extracellular matrix

In biology, the extracellular matrix (ECM) is a collection of extracellular molecules secreted by cells that provides structural and biochemical support to the surrounding cells. Because multicellularity evolved independently in different multicellular lineages, the composition of ECM varies between multicellular structures; however, cell adhesion, cell-to-cell communication and differentiation are common functions of the ECM. The animal extracellular matrix includes the interstitial matrix and the basement membrane. Interstitial matrix is present between various animal cells (i.e., in the intercellular spaces). Gels of polysaccharides and fibrous proteins fill the interstitial space and act as a compression buffer against the stress placed on the ECM. Basement membranes are sheet-like depositions of ECM on which various epithelial cells rest. Each type of connective tissue in animals has a type of ECM: collagen fibers and bone mineral comprise the ECM of bone tissue; reticular fibers and ground substance comprise the ECM of loose connective tissue; and blood plasma is the ECM of blood. The plant ECM includes cell wall components, like cellulose, in addition to more complex signaling molecules. Some singlecelled organisms adopt multicelluar biofilms in which the cells are embedded in an ECM composed primarily of extracellular polymeric substances (EPS).

Role and importance

Due to its diverse nature and composition, the ECM can serve many functions, such as providing support, segregating tissues from one another, and regulating intercellular communication. The extracellular matrix regulates a cell's dynamic behavior. In addition, it sequesters a wide range of cellular growth factors and acts as a local store for them. Changes in physiological conditions can trigger protease activities that cause local release of such stores. This allows the rapid and local growth factor-mediated activation of cellular functions without de novo synthesis. Formation of the extracellular matrix is essential for processes like growth,

wound healing, and fibrosis. An understanding of ECM structure and composition also helps in comprehending the complex dynamics of tumor invasion and metastasis in cancer biology as metastasis often involves the destruction of extracellular matrix by enzymes such as serine proteases, threonine proteases, and matrix metalloproteinases. The stiffness and elasticity of the ECM has important implications in cell migration, gene expression, and differentiation. Cells actively sense ECM rigidity and migrate preferentially towards stiffer surfaces in a phenomenon called durotaxis. They also detect elasticity and adjust their gene expression accordingly which has increasingly become a subject of research because of its impact on differentiation and cancer progression.

Molecular components

Components of the ECM are produced intracellularly by resident cells and secreted into the ECM via exocytosis. Once secreted, they then aggregate with the existing matrix. The ECM is composed of an interlocking mesh of fibrous proteins and glycosaminoglycans (GAGs).
Proteoglycans

Glycosaminoglycans (GAGs) are carbohydrate polymers and are usually attached to extracellular matrix proteins to form proteoglycans (hyaluronic acid is a notable exception, see below). Proteoglycans have a net negative charge that attracts positively charged sodium ions (Na+), which attracts water molecules via osmosis, keeping the ECM and resident cells hydrated. Proteoglycans may also help to trap and store growth factors within the ECM. Described below are the different types of proteoglycan found within the extracellular matrix.

Heparan sulfate

Heparan sulfate (HS) is a linear polysaccharide found in all animal tissues. It occurs as a proteoglycan (PG) in which two or three HS chains are attached in close proximity to cell surface or ECM proteins. It is in this form that HS binds to a variety of protein ligands and regulates a wide variety of biological activities, including developmental processes, angiogenesis, blood coagulation, and tumour metastasis. In the extracellular matrix, especially basement membranes, the multi-domain proteins perlecan, agrin, and collagen XVIII are the main proteins to which heparan sulfate is attached.

Chondroitin sulfate

Chondroitin sulfates contribute to the tensile strength of cartilage, tendons, ligaments, and walls of the aorta. They have also been known to affect neuroplasticity.

Keratan sulfate

Keratan sulfates have a variable sulfate content and, unlike many other GAGs, do not contain uronic acid. They are present in the cornea, cartilage, bones, and the horns of animals.

Non-proteoglycan polysaccharide

Hyaluronic acid

Hyaluronic acid (or "hyaluronan") is a polysaccharide consisting of alternating residues of D-glucuronic acid and N-acetylglucosamine, and unlike other GAGs, is not found as a proteoglycan. Hyaluronic acid in the extracellular space confers upon tissues the ability to resist compression by providing a counteracting turgor (swelling) force by absorbing significant amounts of water. Hyaluronic acid is thus found in abundance in the ECM of load-bearing joints. It is also a chief component of the interstitial gel. Hyaluronic acid is found on the inner surface of the cell membrane and is translocated out of the cell during biosynthesis. Hyaluronic acid acts as an environmental cue that regulates cell behavior during embryonic development, healing processes, inflammation, and tumor development. It interacts with a specific transmembrane receptor, CD44.

Proteins

Collagen

Collagens are the most abundant protein in the ECM. In fact, collagen is the most abundant protein in the human body and accounts for 90% of bone matrix protein content. Collagens are present in the ECM as fibrillar proteins and give structural support to resident cells. Collagen is exocytosed in precursor form (procollagen), which is then cleaved by procollagen proteases to allow extracellular assembly. Disorders such as Ehlers Danlos Syndrome, osteogenesis imperfecta, and epidermolysis bullosa are linked with genetic defects in collagen-encoding genes. The collagen can be divided into several families according to the types of structure they form:

1. Fibrillar (Type I, II, III, V, XI)

2. Facit (Type IX, XII, XIV)

3. Short chain (Type VIII, X)

4. Basement membrane (Type IV)

5. Other (Type VI, VII, XIII).

Elastin

Elastins, in contrast to collagens, give elasticity to tissues, allowing them to stretch when needed and then return to their original state. This is useful in blood vessels, the lungs, in skin, and the ligamentum nuchae, and these tissues contain high amounts of elastins. Elastins are synthesized by fibroblasts and smooth muscle cells. Elastins are highly insoluble, and tropoelastins are secreted inside a chaperone molecule, which releases the precursor molecule upon contact with a fiber of mature elastin. Tropoelastins are then deaminated to become incorporated into the elastin strand. Disorders such as cutis laxa and Williams syndrome are associated with deficient or absent elastin fibers in the ECM.

Extracellular vesicles

In 2016, Huleihel et al., reported the presence of DNA, RNA, and Matrix-bound nanovesicles (MBVs) within ECM bioscaffolds.[26] MBVs shape and size were found to be consistent with previously described exosomes. MBVs cargo includes different protein molecules, lipids, DNA, fragments, and miRNAs. Similar to ECM bioscaffolds, MBVs can modify the activation state of macrophages and alter different cellular properties such as; proliferation, migration and cell cycle. MBVs are now believed to be an integral and functional key component of ECM bioscaffolds.

Other

Fibronectin

Fibronectins are glycoproteins that connect cells with collagen fibers in the ECM, allowing cells to move through the ECM. Fibronectins bind collagen and cell-surface integrins, causing a reorganization of the cell's cytoskeleton to facilitate cell movement. Fibronectins are secreted by cells in an unfolded, inactive form. Binding to integrins unfolds fibronectin molecules, allowing them to form dimers so that they can function properly. Fibronectins also help at the site of tissue injury by binding to platelets during blood clotting and facilitating cell movement to the affected area during wound healing.

Laminin

Laminins are proteins found in the basal laminae of virtually all animals. Rather than forming collagen-like fibers, laminins form networks of web-like structures that resist tensile

forces in the basal lamina. They also assist in cell adhesion. Laminins bind other ECM components such as collagens and nidogens.

Medical applications

Extracellular matrix has been found to cause regrowth and healing of tissue. Although the mechanism of action by which extracellular matrix promotes constructive remodeling of tissue is still unknown, researchers now believe that Matrix-bound nanovesicles (MBVs) are a key player in the healing process. In human fetuses, for example, the extracellular matrix works with stem cells to grow and regrow all parts of the human body, and fetuses can regrow anything that gets damaged in the womb. Scientists have long believed that the matrix stops functioning after full development. It has been used in the past to help horses heal torn ligaments, but it is being researched further as a device for tissue regeneration in humans.

In terms of injury repair and tissue engineering, the extracellular matrix serves two main purposes. First, it prevents the immune system from triggering from the injury and responding with inflammation and scar tissue. Next, it facilitates the surrounding cells to repair the tissue instead of forming scar tissue.

For medical applications, the ECM required is usually extracted from pig bladders, an easily accessible and relatively unused source. It is currently being used regularly to treat ulcers by closing the hole in the tissue that lines the stomach, but further research is currently being done by many universities as well as the U.S. Government for wounded soldier applications. As of early 2007, testing was being carried out on a military base in Texas. Scientists are using a powdered form on Iraq War veterans whose hands were damaged in the war.

Not all ECM devices come from the bladder. Extracellular matrix coming from pig small intestine submucosa are being used to repair "atrial septal defects" (ASD), "patent foramen ovale" (PFO) and inguinal hernia. After one year 95% of the collagen ECM in these patches is replaced by the normal soft tissue of the heart.

Extracellular matrix proteins are commonly used in cell culture systems to maintain stem and precursor cells in an undifferentiated state during cell culture and function to induce differentiation of epithelial, endothelial and smooth muscle cells in vitro. Extracellular matrix proteins can also be used to support 3D cell culture in vitro for modelling tumor development.

A class of biomaterials derived from processing human or animal tissues to retain portions of the extracellular matrix are called ECM Biomaterial.

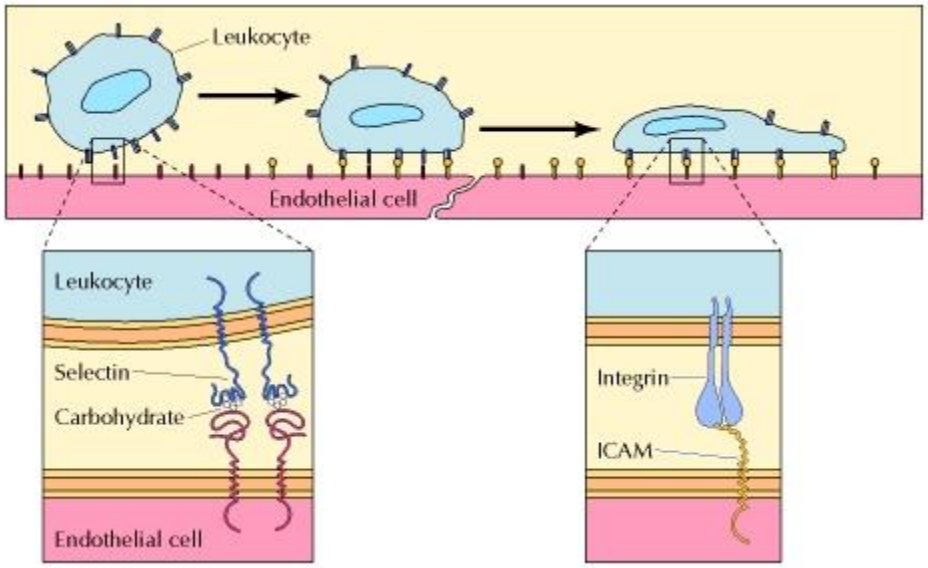
Cell-Cell Interactions

Direct interactions between cells, as well as between cells and the extracellular matrix, are critical to the development and function of multicellular organisms. Some cell-cell interactions are transient, such as the interactions between cells of the immune system and the interactions that direct white blood cells to sites of tissue inflammation. In other cases, stable cell-cell junctions play a key role in the organization of cells in tissues. For example, several different types of stable cell-cell junctions are critical to the maintenance and function of epithelial cell sheets. Plant cells also associate with their neighbors not only by interactions between their cell walls, but also by specialized junctions between their plasma membranes.

Cell Adhesion Proteins

Cell-cell adhesion is a selective process, such that cells adhere only to other cells of specific types. This selectivity was first demonstrated in classical studies of embryo development, which showed that cells from one tissue (e.g., liver) specifically adhere to cells of the same tissue rather than to cells of a different tissue (e.g., brain). Such selective cell-cell adhesion is mediated by transmembrane proteins called cell adhesion molecules, which can be divided into four major groups: the selectins, the integrins, the **immunoglobulin (Ig) superfamily** (so named because they contain structural domains similar to immunoglobulins), and the cadherins. Cell adhesion mediated by the selectins, integrins, and cadherins requires Ca^{2+} or Mg^{2+}, so many adhesive interactions between cells are Ca^{2+}- or Mg^{2+}-dependent.

The selectins mediate transient interactions between leukocytes and endothelial cells or blood platelets. There are three members of the selectin family: L-selectin, which is expressed on leukocytes; E-selectin, which is expressed on endothelial cells; and P-selectin, which is expressed on platelets. As discussed earlier in this chapter, the selectins recognize cell surface carbohydrates. One of their critical roles is to initiate the interactions between leukocytes and endothelial cells during the migration of leukocytes from the circulation to sites of tissue inflammation. The selectins mediate the initial adhesion of leukocytes to endothelial cells. This is followed by the formation of more stable adhesions, in which integrins on the surface of leukocytes bind to intercellular adhesion molecules (ICAMs), which are members of the Ig superfamily expressed on the surface of endothelial cells. The firmly attached leukocytes are then able to penetrate the walls of capillaries and enter the underlying tissue by migrating between endothelial cells.



Adhesion between leukocytes and endothelial cells

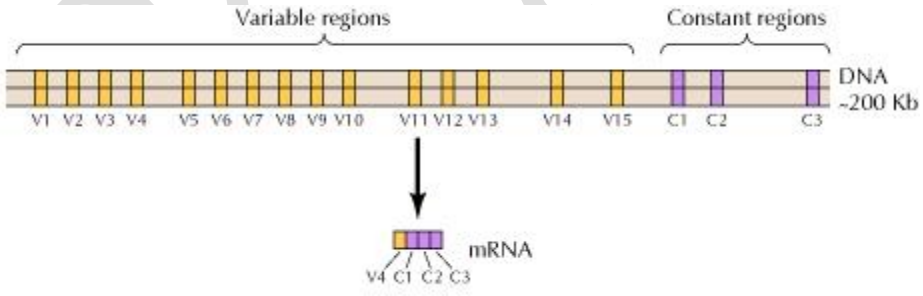
Leukocytes leave the circulation at sites of tissue inflammation by interacting with the endothelial cells of capillary walls. The first step in this interaction is the binding of leukocyte selectins to carbohydrate ligands on the endothelial cell surface. This step is followed by more stable interactions between leukocyte integrins and members of the Ig superfamily (ICAMs) on endothelial cells.

The binding of ICAMs to integrins is an example of a **heterophilic interaction**, in which an adhesion molecule on the surface of one cell (e.g., an ICAM) recognizes a different molecule on the surface of another cell (e.g., an integrin). Other members of the Ig superfamily mediate **homophilic interactions**, in which an adhesion molecule on the surface of one cell binds to the same molecule on the surface of another cell. Such homophilic binding leads to selective adhesion between cells of the same type. For example, nerve cell adhesion molecules (N-CAMs) are members of the Ig superfamily expressed on nerve cells, and homophilic binding between N-CAMs contributes to the formation of selective associations between nerve cells during development. There are more than 100 members of the Ig superfamily, which mediate a variety of cell-cell interactions.

The fourth group of cell adhesion molecules, the cadherins, also display homophilic binding specificities. They are not only involved in selective adhesion between embryonic cells but are also primarily responsible for the formation of stable junctions between cells in tissues. For example, E-cadherin is expressed on epithelial cells, so homophilic interactions between E-

cadherins lead to the selective adhesion of epithelial cells to one another. It is noteworthy that loss of E-cadherin can lead to the development of cancers arising from epithelial cells, illustrating the importance of cell-cell interactions in controlling cell behavior. Different members of the cadherin family, such as N-cadherin (neural cadherin) and P-cadherin (placental cadherin), mediate selective adhesion of other cell types.

About twenty different classic cadherins, such as E-cadherin, have been identified. In addition, a distinct subfamily of cadherins, called protocadherins, are expressed in the central nervous system where they appear to play a role in adhesion between neurons at synapses. Intriguingly, different neurons appear to express different protocadherins, suggesting that the protocadherins may play a role in the establishment of specific connections between neurons. About 50 human protocadherin genes have been identified and shown to be organized into three gene clusters. Each cluster contains multiple exons encoding the N-terminal extracellular and transmembrane protocadherin domains, but only a single set of three exons encoding the C-terminal cytoplasmic domain. The protocadherin gene clusters thus appear to consist of a variable region, encoding multiple extracellular and transmembrane domains, linked to a constant region encoding a single cytoplasmic domain. This organization of protocadherin genes strikingly resembles that of immunoglobulin and T-cell receptor genes, in which multiple variable region exons are joined to a single constant region exon. In the immunoglobulin and T-cell receptor genes, this occurs as a result of DNA rearrangements that generate diversity in the immune system. It remains to be determined whether the variable and constant regions of protocadherins are joined at the DNA or the RNA level (for example, by alternative splicing) and to what extent rearrangements of protocadherin genes might contribute to the establishment of specific synaptic connections in the brain.

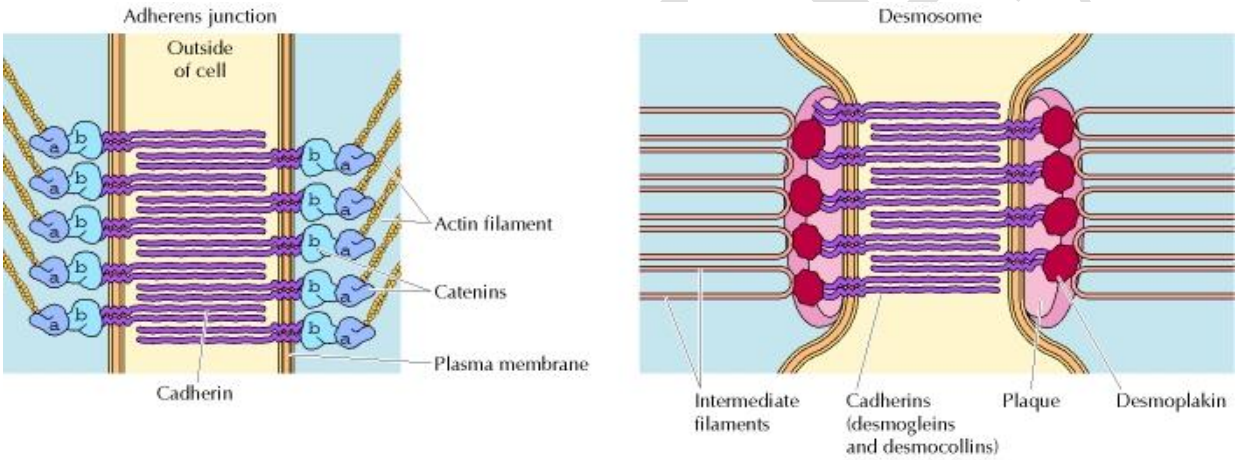


Organization of protocadherin gene clusters

The human protocadherin genes are organized into three clusters. In the cluster illustrated, 15 different variable regions encoding extracellular and transmembrane domains are linked to a

single constant region, consisting of three exons encoding the cytoplasmic domain. Protocadherin mRNAs contain one of the variable region exons linked to the constant region.

In contrast to the stable cell-matrix junctions discussed in the preceding section, the cell-cell interactions mediated by the selectins, integrins, and members of the Ig superfamily are transient adhesions in which the cytoskeletons of adjacent cells are not linked to one another. Stable adhesion junctions involving the cytoskeletons of adjacent cells are instead mediated by the cadherins. The role of the cadherins in linking the cytoskeletons of adjacent cells is thus analogous to that of the integrins in forming stable junctions between cells and the extracellular matrix.



Stable cell-cell junctions mediated by the cadherins

Homophilic interactions between cadherins mediate two types of stable cell-cell adhesions. In adherens junctions, the cadherins are linked to bundles of actin filaments via the catenins. In desmosomes, desmoplakin links members of the cadherin superfamily (desmogleins and desmocollins) to intermediate filaments.

Tight Junctions

In addition to the adhesion junctions mediated by the cadherins, two other types of specialized cell-cell junctions play key roles in animal tissues. Tight junctions, which are usually associated with adherens junctions and desmosomes in a **junctional complex**, are critically important to the function of epithelial cell sheets as barriers between fluid compartments. For example, the intestinal epithelium separates the lumen of the intestine from the underlying connective tissue, which contains blood capillaries. Tight junctions play two roles in allowing

epithelia to fulfill such barrier functions. First, tight junctions form seals that prevent the free passage of molecules (including ions) between the cells of epithelial sheets. Second, tight junctions separate the apical and basolateral domains of the plasma membrane by preventing the free diffusion of lipids and membrane proteins between them. Consequently, specialized transport systems in the apical and basolateral domains are able to control the traffic of molecules between distinct extracellular compartments, such as the transport of glucose between the intestinal lumen and the blood supply.

Tight junctions are the closest known contacts between adjacent cells. They were originally described as sites of apparent fusion between the outer leaflets of the plasma membranes, although it is now clear that the membranes do not fuse. Instead, tight junctions appear to be formed by a network of protein strands that continues around the entire circumference of the cell. Each strand in these networks is thought to be composed of transmembrane proteins (claudins and occludin) that bind to similar proteins on adjacent cells, thereby sealing the space between their plasma membranes.

Gap Junctions

Gap junctions, which are found in most animal tissues, serve as direct connections between the cytoplasms of adjacent cells. They provide open channels through the plasma membrane, allowing ions and small molecules (less than approximately a thousand daltons) to diffuse freely between neighboring cells, but preventing the passage of proteinsand nucleic acids. Consequently, gap junctions couple both the metabolic activities and the electric responses of the cells they connect. Most cells in animal tissues—including epithelial cells, endothelial cells, and the cells of cardiac and smooth muscle—communicate by gap junctions. In electrically excitable cells, such as heart muscle cells, the direct passage of ions through gap junctions couples and synchronizes the contractions of neighboring cells. Gap junctions also allow the passage of some intracellular signaling molecules, such as cAMP and Ca^{2+}, between adjacent cells, potentially coordinating the responses of cells in tissues.

Gap junctions are constructed of transmembrane proteins called **connexins**. Six connexins assemble to form a cylinder with an open aqueous pore in its center. Such an assembly of connexins in the plasma membrane of one cell then aligns with the connexins of an adjacent cell, forming an open channel between the two cytoplasms. The plasma membranes of the two cells are separated by a gap corresponding to the space occupied by the connexin extracellular domains—hence the term "gap junction," which was coined by electron microscopists.

Cell signaling

Cell signaling (cell signalling in British English) is part of any communication process that governs basic activities of cells and coordinates all cell actions. The ability of cells to perceive and correctly respond to their microenvironment is the basis of development, tissue repair, and immunity as well as normal tissue homeostasis. Errors in signaling interactions and cellular information processing are responsible for diseases such as cancer, autoimmunity, and diabetes. By understanding cell signaling, diseases may be treated more effectively and, theoretically, artificial tissues may be created.

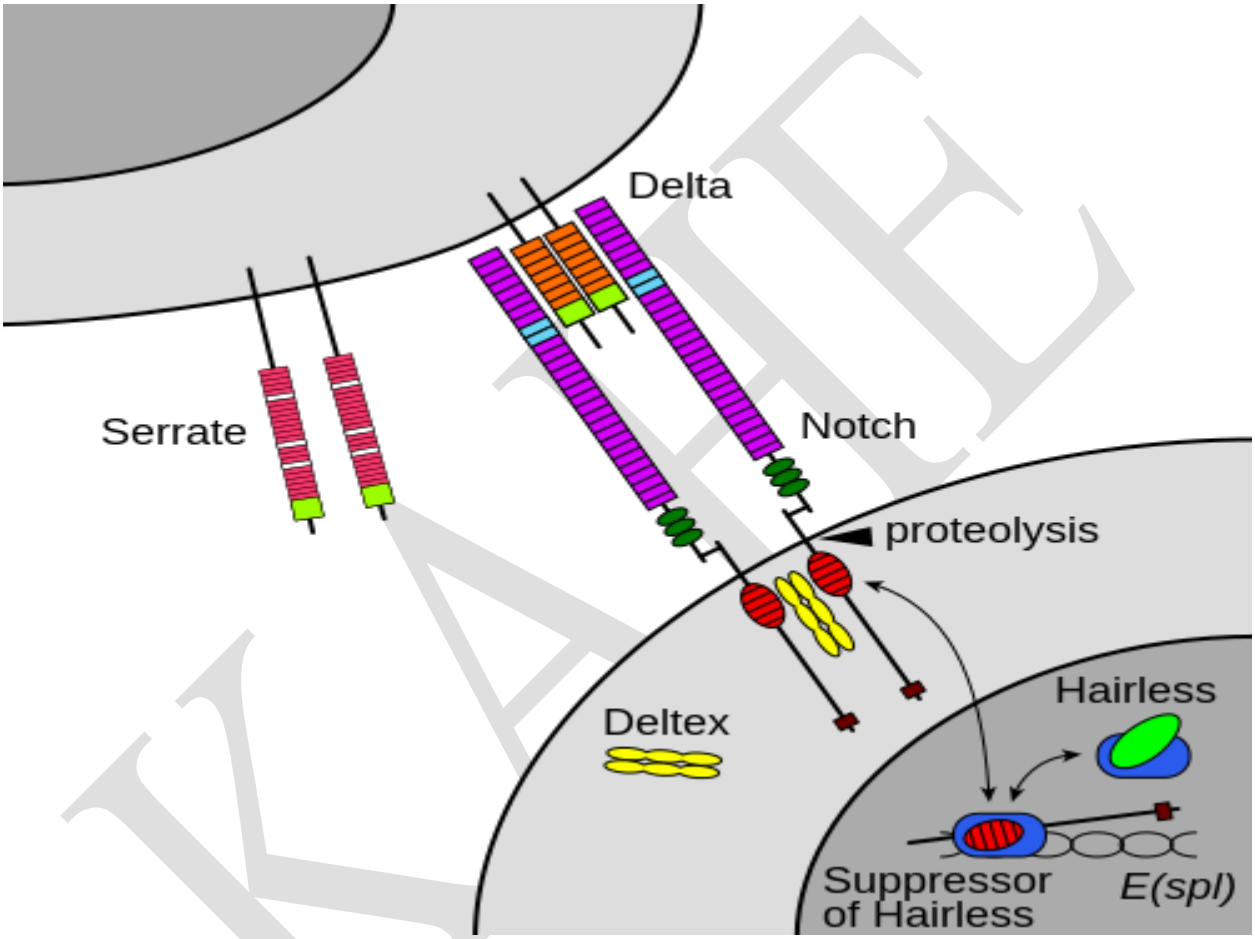
Traditional work in biology has focused on studying individual parts of cell signaling pathways. Systems biology research helps us to understand the underlying structure of cell signaling networks and how changes in these networks may affect the transmission and flow of information (signal transduction). Such networks are complex systems in their organization and may exhibit a number of emergent properties including bistability and ultrasensitivity. Analysis of cell signaling networks requires a combination of experimental and theoretical approaches including the development and analysis of simulations and modeling. Long-range allostery is often a significant component of cell signaling events.

Classification

Cell signaling can be classified to be mechanical and biochemical based on the type of the signal. Mechanical signals are the forces exerted on the cell and the forces produced by the cell. These forces can both be sensed and responded by the cells. Biochemical signals are the biochemical molecules such as proteins, lipids, ions and gases. These signals can be categorized based on the distance between signaling and responder cells. Signaling within, between, and amongst cells is subdivided into the following classifications:

- *Intracrine* signals are produced by the target cell that stay within the target cell.
- *Autocrine* signals are produced by the target cell, are secreted, and affect the target cell itself via receptors. Sometimes autocrine cells can target cells close by if they are the same type of cell as the emitting cell. An example of this are immune cells.
- *Juxtacrine* signals target adjacent (touching) cells. These signals are transmitted along cell membranes via protein or lipid components integral to the membrane and are capable of affecting either the emitting cell or cells immediately adjacent.

- *Paracrine* signals target cells in the vicinity of the emitting cell. Neurotransmitters represent an example.
- *Endocrine* signals target distant cells. Endocrine cells produce hormones that travel through the blood to reach all parts of the body.



Cells communicate with each other via direct contact (juxtacrine signaling), over short distances (paracrine signaling), or over large distances and/or scales (endocrine signaling).

Some cell–cell communication requires direct cell–cell contact. Some cells can form gap junctions that connect their cytoplasm to the cytoplasm of adjacent cells. In cardiac muscle, gap junctions between adjacent cells allows for action potential propagation from the cardiac pacemaker region of the heart to spread and coordinately cause contraction of the heart.

The notch signaling mechanism is an example of juxtacrine signaling (also known as contact-dependent signaling) in which two adjacent cells must make physical contact in order to communicate. This requirement for direct contact allows for very precise control of cell differentiation during embryonic development. In the worm *Caenorhabditis elegans*, two cells of the developing gonad each have an equal chance of terminally differentiating or becoming a uterine precursor cell that continues to divide. The choice of which cell continues to divide is controlled by competition of cell surface signals. One cell will happen to produce more of a cell surface protein that activates the Notch receptor on the adjacent cell. This activates a feedback loop or system that reduces Notch expression in the cell that will differentiate and that increases Notch on the surface of the cell that continues as a stem cell.

Many cell signals are carried by molecules that are released by one cell and move to make contact with another cell. *Endocrine* signals are called hormones. Hormones are produced by endocrine cells and they travel through the blood to reach all parts of the body. Specificity of signaling can be controlled if only some cells can respond to a particular hormone. *Paracrine* signals such as retinoic acid target only cells in the vicinity of the emitting cell. Neurotransmitters represent another example of a paracrine signal. Some signaling molecules can function as both a hormone and a neurotransmitter. For example, epinephrine and norepinephrine can function as hormones when released from the adrenal glandand are transported to the heart by way of the blood stream. Norepinephrine can also be produced by neurons to function as a neurotransmitter within the brain. Estrogen can be released by the ovary and function as a hormone or act locally via paracrine or autocrine signaling. Active species of oxygen and nitric oxide can also act as cellular messengers. This process is dubbed redox signaling.

Cell signaling in multicellular organisms

In a multicellular organism, signaling between cells occurs either through release into the extracellular space, divided in paracrine signaling (over short distances) and endocrinesignaling (over long distances), or by direct contact, known as juxtacrine signaling. Autocrine signaling is a special case of paracrine signaling where the secreting cell has the ability to respond to the secreted signaling molecule. Synaptic signaling is a special case of paracrine signaling (for chemical synapses) or juxtacrine signaling (for electrical synapses) between neurons and target cells. Signaling molecules interact with a target cell as a ligand to cell surface receptors, and/or by entering into the cell through its membraneor endocytosis for intracrine signaling. This generally results in the activation of second messengers, leading to various physiological effects.

A particular molecule is generally used in diverse modes of signaling, and therefore a classification by mode of signaling is not possible. At least three important classes of signaling molecules are widely recognized, although non-exhaustive and with imprecise boundaries, as such membership is non-exclusive and depends on the context:

- Hormones are the major signaling molecules of the endocrine system, though they often regulate each other's secretion via local signaling (e.g. islet of Langerhans cells), and most are also expressed in tissues for local purposes (e.g. angiotensin) or failing that, structurally related molecules are (e.g. PTHrP).
- Neurotransmitters are signaling molecules of the nervous system, also including neuropeptides and neuromodulators. Neurotransmitters like the catecholamines are also secreted by the endocrine system into the systemic circulation.
- Cytokines are signaling molecules of the immune system, with a primary paracrine or juxtacrine role, though they can during significant immune responses have a strong presence in the circulation, with systemic effect (altering iron metabolism or body temperature). Growth factors can be considered as cytokines or a different class.

Signaling molecules can belong to several chemical classes: lipids, phospholipids, amino acids, monoamines, proteins, glycoproteins, or gases. Signaling molecules binding surface receptors are generally large and hydrophilic (e.g. TRH, Vasopressin, Acetylcholine), while those entering the cell are generally small and hydrophobic (e.g. glucocorticoids, thyroid hormones, cholecalciferol, retinoic acid), but important exceptions to both are numerous, and a same molecule can act both via surface receptor or in an intracrine manner to different effects.[16] In intracrine signaling, once inside the cell, a signaling molecule can bind to intracellular receptors, other elements, or stimulate enzyme activity (e.g. gasses). The intracrine action of peptide hormones remains a subject of debate.

Hydrogen sulfide is produced in small amounts by some cells of the human body and has a number of biological signaling functions. Only two other such gases are currently known to act as signaling molecules in the human body: nitric oxide and carbon monoxide.

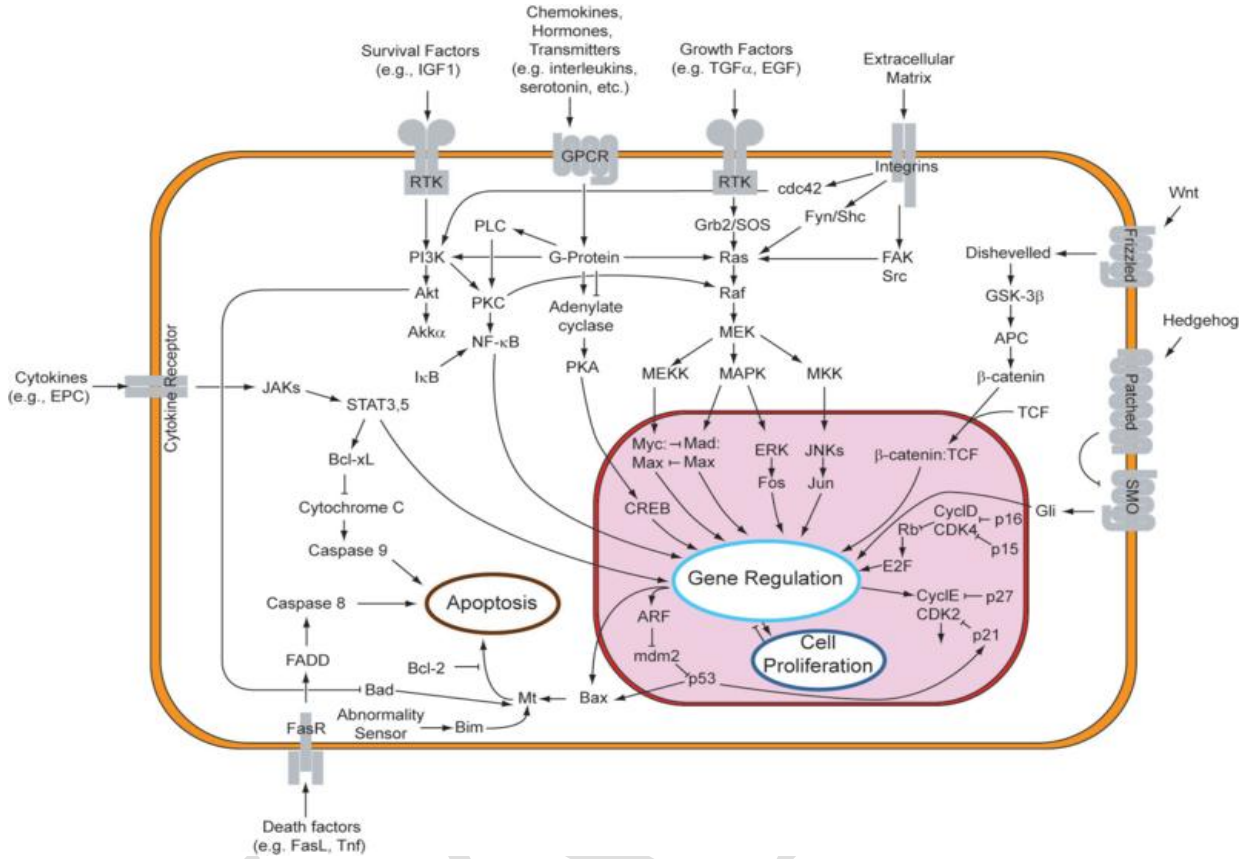
Signaling pathways

In some cases, receptor activation caused by ligand binding to a receptor is directly coupled to the cell's response to the ligand. For example, the neurotransmitter GABA can activate a cell surface receptor that is part of an ion channel. GABA binding to

a $GABA_A$ receptor on a neuron opens a chloride-selective ion channel that is part of the receptor. $GABA_A$ receptor activation allows negatively charged chloride ions to move into the neuron, which inhibits the ability of the neuron to produce action potentials. However, for many cell surface receptors, ligand-receptor interactions are not directly linked to the cell's response. The activated receptor must first interact with other proteins inside the cell before the ultimate physiological effect of the ligand on the cell's behavior is produced. Often, the behavior of a chain of several interacting cell proteins is altered following receptor activation. The entire set of cell changes induced by receptor activation is called a signal transduction mechanism or pathway.

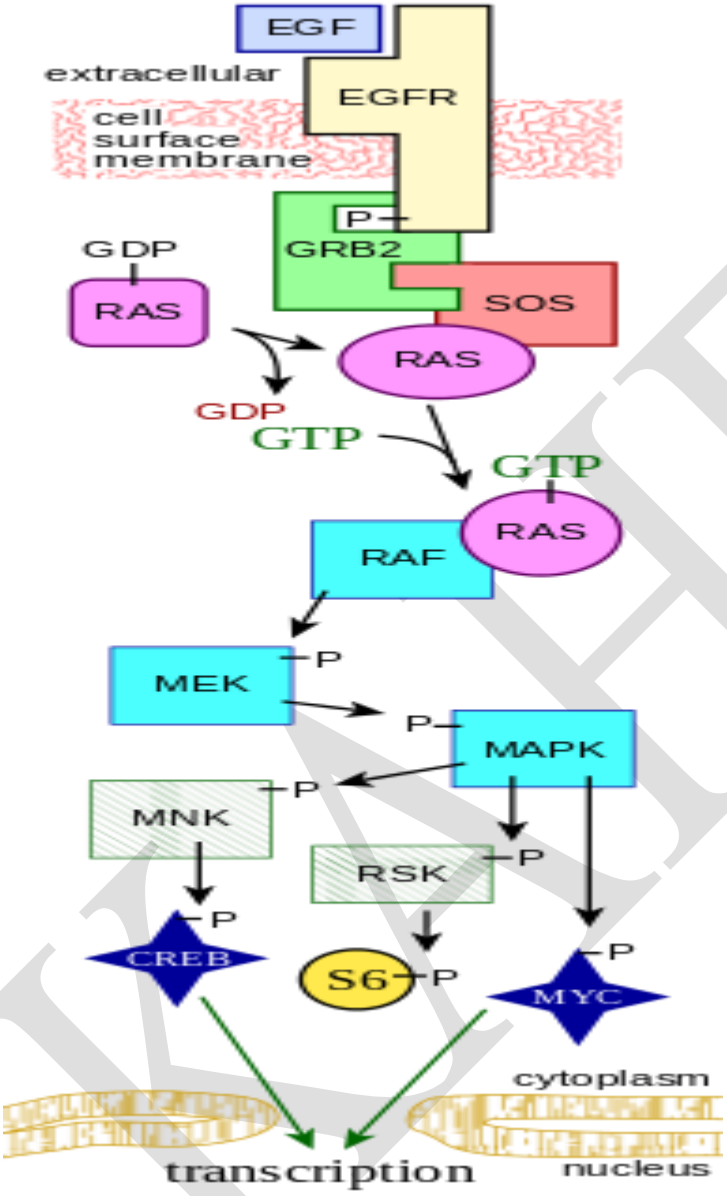
In the case of Notch-mediated signaling, the signal transduction mechanism can be relatively simple. Activation of Notch can cause the Notch protein to be altered by a protease. Part of the Notch protein is released from the cell surface membrane and takes part in gene regulation. Cell signaling research involves studying the spatial and temporal dynamics of both receptors and the components of signaling pathways that are activated by receptors in various cell types.

This pathway involves changes of protein–protein interactions inside the cell, induced by an external signal. Many growth factors bind to receptors at the cell surface and stimulate cells to progress through the cell cycle and divide. Several of these receptors are kinases that start to phosphorylate themselves and other proteins when binding to a ligand. This phosphorylation can generate a binding site for a different protein and thus induce protein–protein interaction. In Figure 3, the ligand (called epidermal growth factor (EGF)) binds to the receptor (called EGFR). This activates the receptor to phosphorylate itself. The phosphorylated receptor binds to an adaptor protein (GRB2), which couples the signal to further downstream signaling processes. For example, one of the signal transduction pathways that are activated is called the mitogen-activated protein kinase (MAPK) pathway. The signal transduction component labeled as "MAPK" in the pathway was originally called "ERK," so the pathway is called the MAPK/ERK pathway. The MAPK protein is an enzyme, a protein kinase that can attach phosphate to target proteins such as the transcription factor MYC and, thus, alter gene transcription and, ultimately, cell cycle progression. Many cellular proteins are activated downstream of the growth factor receptors (such as EGFR) that initiate this signal transduction pathway.



Some signaling transduction pathways respond differently, depending on the amount of signaling received by the cell. For instance, the hedgehog protein activates different genes, depending on the amount of hedgehog protein present.

Complex multi-component signal transduction pathways provide opportunities for feedback, signal amplification, and interactions inside one cell between multiple signals and signaling pathways.



cAMP Transmits Messages Within the Cell

Many of the chemical messengers (eg, neurotransmitters, many hormones, and many mediators of inflammation) that transmit messages from one cell to another never enter the target cell. Instead, these so-called first messengers work by binding to a specific receptor on the cell surface. The receptor then stimulates or inhibits the production of a so-called second messenger within the cell. Because second messengers are small molecules, they can move signaling

information quickly throughout the cytoplasm. The second messenger may then activate an effector within the cell, which then causes some alteration in the cell's metabolism or activities.

Many of the cell surface receptors are G-protein-coupled receptors, whose principal second messenger is cAMP. Typically, when a G-protein-coupled receptor binds to its particular ligand, the receptor either stimulates or inhibits the production of cAMP by the adenyl cyclase associated with that receptor. The adenylyl cyclase can produce many molecules of cAMP, thus amplifying the signal.

cAMP Promotes Immune Homeostasis

Several lines of evidence have shown that cAMP serves as a second messenger in processes that help to maintain immune homeostasis in the skin. Drugs that are known to elevate intracellular cAMP levels tend to reduce the production of proinflammatory mediators and increase the production of anti-inflammatory mediators by cultured human keratinocytes, monocytes, and dendritic cells. Several studies have also suggested that PKA is the downstream effector of cAMP in processes involved in immune homeostasis in the skin.

GPCR signaling

If a receptor in an active state encounters a G protein, it may activate it. Some evidence suggests that receptors and G proteins are actually pre-coupled. For example, binding of G proteins to receptors affects the receptor's affinity for ligands. Activated G proteins are bound to GTP.

Further signal transduction depends on the type of G protein. The enzyme adenylate cyclase is an example of a cellular protein that can be regulated by a G protein, in this case the G protein G_s. Adenylate cyclase activity is activated when it binds to a subunit of the activated G protein. Activation of adenylate cyclase ends when the G protein returns to the GDP-bound state.

Adenylate cyclases (of which 9 membrane-bound and one cytosolic forms are known in humans) may also be activated or inhibited in other ways (e.g., Ca2+/Calmodulin binding), which can modify the activity of these enzymes in an additive or synergistic fashion along with the G proteins.

The signaling pathways activated through a GPCR are limited by the primary sequence and tertiary structure of the GPCR itself but ultimately determined by the particular conformation stabilized by a particular ligand, as well as the availability of transducer molecules. Currently, GPCRs are considered to utilize two primary types of transducers: G-proteins and β-arrestins. Because β-arr's have high affinity only to

the phosphorylated form of most GPCRs (see above or below), the majority of signaling is ultimately dependent upon G-protein activation. However, the possibility for interaction does allow for G-protein-independent signaling to occur.

Receptor Tyrosine Kinases and Ras

The receptor tyrosine kinases (RTKs) are the second major type of cell-surface receptors that we discuss in detail in this chapter. The ligands for RTKs are soluble or membrane-bound peptide/proteinhormones including nerve growth factor (NGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), and insulin. Binding of a ligand to this type of receptor stimulates the receptor's intrinsic protein-tyrosine kinase activity, which subsequently stimulates a signal-transduction cascade leading to changes in cellular physiology and/or patterns of gene expression. RTK signaling pathways have a wide spectrum of functions including regulation of cell proliferation and differentiation, promotion of cell survival, and modulation of cellular metabolism.

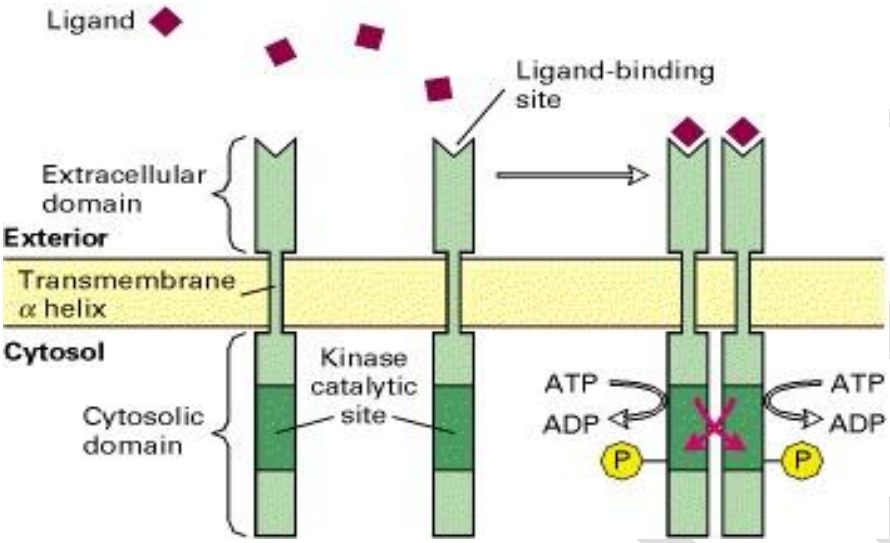
Some RTKs have been identified in studies on human cancers associated with mutant forms of growth-factor receptors, which send a proliferative signal to cells even in the absence of growth factor. One such mutant receptor, encoded at the *neu* locus, contributes to the uncontrolled proliferation of certain human breast cancers. Other RTKs have been uncovered during analysis of developmental mutations that lead to blocks in differentiation of certain cell types in *C. elegans*, *Drosophila*, and the mouse.

In this section we discuss activation of RTKs and how they transmit a hormone signal to Ras, the **GTPase switch protein** that functions in transducing signals from many different RTKs. The second part of RTK-Ras signaling pathways, the transduction of signals downstream from Ras to a common cascade of serine/threonine kinases, is covered in the next section.

Ligand Binding Leads to Autophosphorylation of RTKs

All RTKs comprise an extracellular domain containing a ligand-binding site, a single hydrophobic transmembrane α helix, and a cytosolic domain that includes a region with protein-tyrosine kinase activity. Binding of ligand causes most RTKs to dimerize; the protein kinase of each receptor monomer then phosphorylates a distinct set of tyrosine residues in the cytosolic domain of its dimer partner, a process termed *autophosphorylation*. Autophosphorylation occurs in two stages. First, tyrosine residues in the *phosphorylation lip* near the catalytic site are phosphorylated. This leads to a conformational change that facilitates binding of ATP in some receptors (e.g., the insulin receptor) and binding of protein substrates in

other receptors (e.g., FGF receptor). The receptor kinase activity then phosphorylates other sites in the cytosolic domain; the resulting phosphotyrosines serve as docking sites for other proteins involved in RTK-mediated signal transduction.



General structure and activation of receptor tyrosine kinases (RTKs)

The ligands for some RTKs, such as the receptor for EGF depicted here, are monomeric; ligand binding induces a conformational change in receptor monomers that promotes their dimerization. The ligands for other RTKs are dimeric; their binding brings two receptor monomers together directly. In either case, the kinase activity of each subunit of the dimeric receptor initially phosphorylates tyrosine residues near the catalytic site in the other subunit. Subsequently, tyrosine residues in other parts of the cytosolic domain are autophosphorylated. See text for discussion.

As described later, the subunits of some RTKs, including the insulin receptor, are covalently linked. Although these receptors exist as dimers or tetramers even in the absence of ligand, binding of ligand is required for autophosphorylation to occur. Presumably, ligand binding induces a conformational change that activates the kinase.

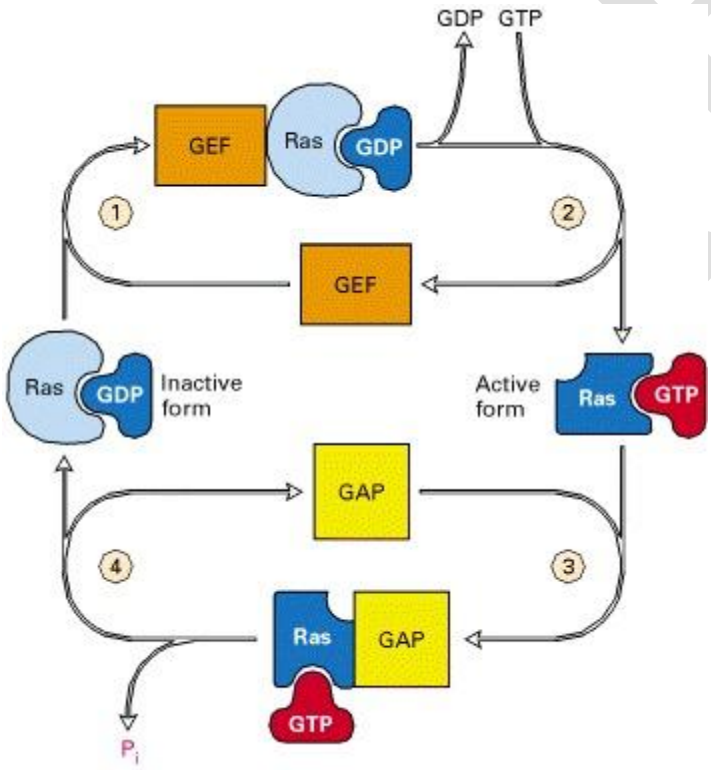
The phosphotyrosine residues in activated RTKs interact with adapter proteins containing SH2 or PTB domains. These proteins couple the activated receptors to other components of the signal-transduction pathway but have no intrinsic signaling properties. Before examining the structure and function of adapter proteins, we discuss the role of Ras, the other key signaling molecule in pathways triggered by activation of RTKs. As we will see later, severalmembrane-associated

enzymes that function in signal transduction also bind to specific phosphotyrosines in activated RTKs.

Ras and G_α Subunits Belong to the GTPase Superfamily of Intracellular Switch Proteins

Ras is a GTP-binding switch protein that, like the G_α subunits in different G proteins, alternates between an active on state with a bound GTP and an inactive off state with a bound GDP. As discussed in the previous section, G_α is directly coupled to GPCRs and transduces signals to various effectors such as adenylyl cyclase. In contrast, Ras is not directly linked to RTKs.

Activation of both Ras and G_α is triggered by hormone binding to an appropriate cell-surface receptor. Ras activation is accelerated by a protein called *guanine nucleotide – exchange factor* (GEF), which binds to the Ras · GDP complex, causing dissociation of the bound GDP. Because GTP is present in cells at a higher concentration than GDP, GTP binds spontaneously to "empty" Ras molecules, with release of GEF. In contrast, formation of an active G_α · GTP complex does not require an exchange factor.



Cycling of the Ras protein between the inactive form with bound GDP and the active form with bound GTP occurs in four steps

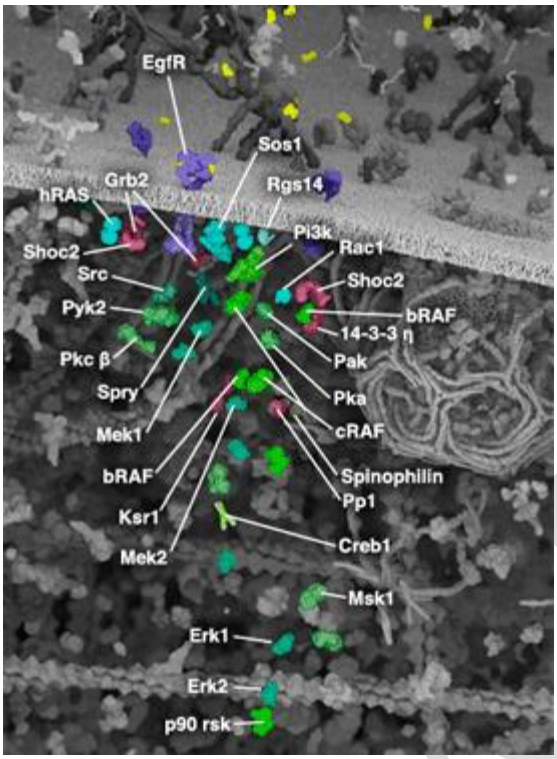
By mechanisms discussed later, binding of certain growth factors to their receptors induces formation of the active Ras · GTP complex. Step **1**: Guanine nucleotide – exchange factor (GEF) facilitates dissociation of GDP from Ras. Step **2**: GTP then binds spontaneously, and GEF dissociates yielding the active Ras · GTP form. Steps **3**and **4**: Hydrolysis of the bound GTP to regenerate the inactive Ras · GDP form is accelerated a hundredfold by GTPase-activating protein (GAP). Unlike G_α, cycling of Ras thus requires two proteins, GEF and GAP; otherwise, G_α and Ras exhibit many common features.

Hydrolysis of the bound GTP deactivates both Ras and G_α. The average lifetime of a GTP bound to Ras is about 1 minute, which is much longer thanthe lifetime of G_α · GTP. The reason for this difference is that the deactivation of Ras, unlike the deactivation of G_α, requires the assistance of another protein: a *GTPase-activating protein* (GAP), which binds to Ras · GTP and accelerates its intrinsic GTPase activity by a hundredfold. Mammalian Ras proteins have been studied in great detail because mutant Ras proteins are associated with many types of human cancer. These mutant proteins, which bind but cannot hydrolyze GTP, are permanently in the "on" state and cause neoplastic transformation.

The differences in the cycling mechanisms of Ras and G_α are reflected in their structures. Ras (≈170 amino acids) is smaller than G_α proteins (≈300 amino acids), but its three-dimensional structure is similar to that of the GTPase domain of G_α. Recent structural and biochemical studies show that G_α also contains another domain, a helical domain that apparently functions like GAP to increase the rate of GTP hydrolysis by G_α. In addition, the direct interaction between an activated receptor and G_α · GDP promotes release of GDP and binding of GTP, so that a separate exchange factor is not required.

Both $G_{s\alpha}$ and Ras are members of a family of intracellular GTP-binding switch proteins collectively referred to as the GTPase superfamily. This family also includes other G_α subunits (e.g., $G_{i\alpha}$), the Rab proteins which regulate fusion of vesicles within cells, and the Rho family proteins which regulate the actin cytoskeleton. The many similarities between the structure and function of Ras and $G_{s\alpha}$, and the identification of both proteins in all eukaryotic cells, indicate that a single type of signaltransducing GTPase originated very early in evolution. The gene encoding this protein subsequently duplicated and evolved to the extent that cells today contain a superfamily of such GTPases, comprising perhaps a hundred different intracellular switch proteins. These related proteins control many aspects of cellular growth and metabolism.

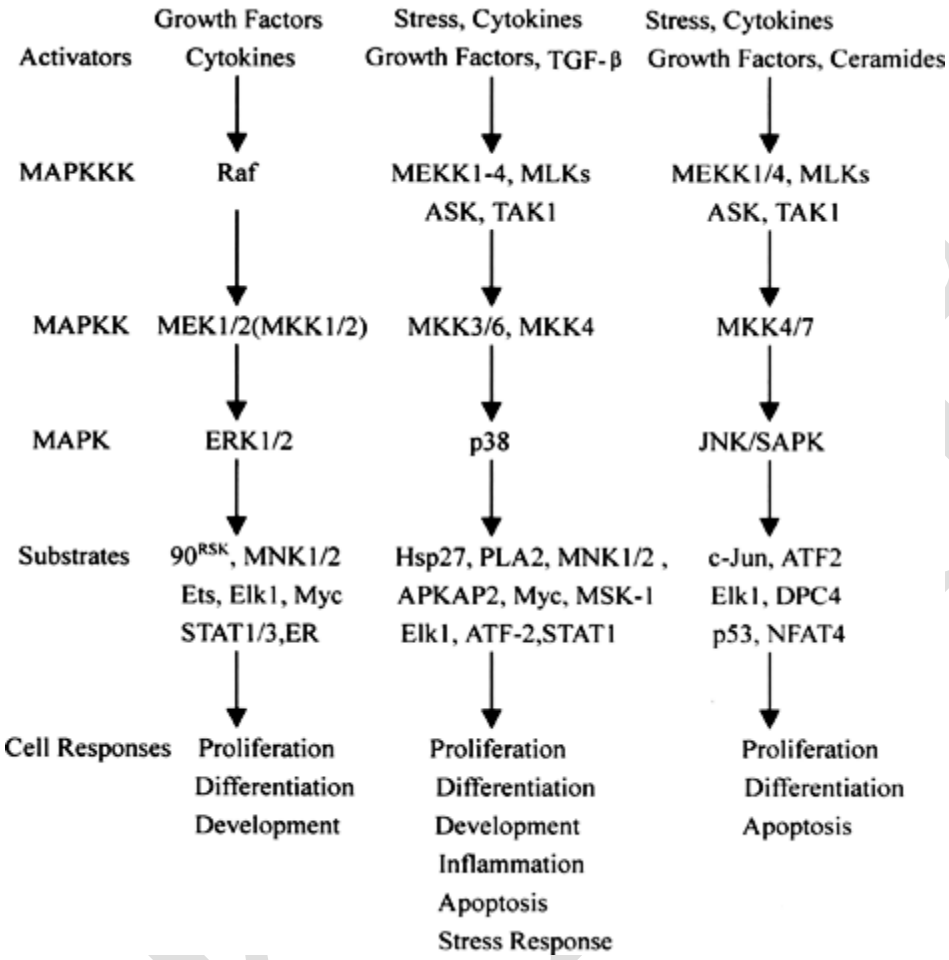
Overview of MAP Kinase Signaling



Mitogen-activated protein kinases (MAPKs) are a highly conserved family of serine/threonine protein kinases involved in a variety of fundamental cellular processes such as proliferation, differentiation, motility, stress response, apoptosis, and survival. Conventional MAPKs include the extracellular signal-regulated kinase 1 and 2 (Erk1/2 or p44/42), the c-Jun N-terminal kinases 1-3 (JNK1-3)/ stress activated protein kinases (SAPK1A, 1B, 1C), the p38 isoforms (p38α, β, γ, and δ), and Erk5. The lesser-studied, atypical MAPKs include Nemo-like kinase (NLK), Erk3/4, and Erk7/8.

A broad range of extracellular stimuli including mitogens, cytokines, growth factors, and environmental stressors stimulate the activation of one or more MAPKK kinases (MAPKKKs) via receptor-dependent and -independent mechanisms. MAPKKKs then phosphorylate and activate a downstream MAPK kinase (MAPKK), which in turn phosphorylates and activates MAPKs. Activation of MAPKs leads to the phosphorylation and activation of specific MAPK-activated protein kinases (MAPKAPKs), such as members of the RSK, MSK, or MNK family, and MK2/3/5. These MAPKAPKs function to amplify the signal and mediate the broad range of biological processes regulated by the different MAPKs. While most MAPKKK, MAPKK, and

MAPKs display a strong preference for one set of substrates, there is significant cross-talk in a stimulus and cell-type dependent manner.



MAP Kinase Pathways

All Ras-linked RTKs in mammalian cells appear to utilize a highly conserved signal-transduction pathway in which the signal induced by ligand binding is carried via GRB2 and Sos to Ras, leading to its activation. Activated Ras then induces a *kinase cascade* that culminates in activation of MAP kinase. This serine/threonine kinase, which can translocate into the nucleus, phosphorylates many different proteins including transcription factors that regulate expression of important cell-cycle and differentiation-specific proteins. In this section, we first examine the components of the kinase cascade downstream from Ras in RTK-Ras signaling pathways in mammalian cells. Then we discuss the linkage of other signaling pathways to similar kinase

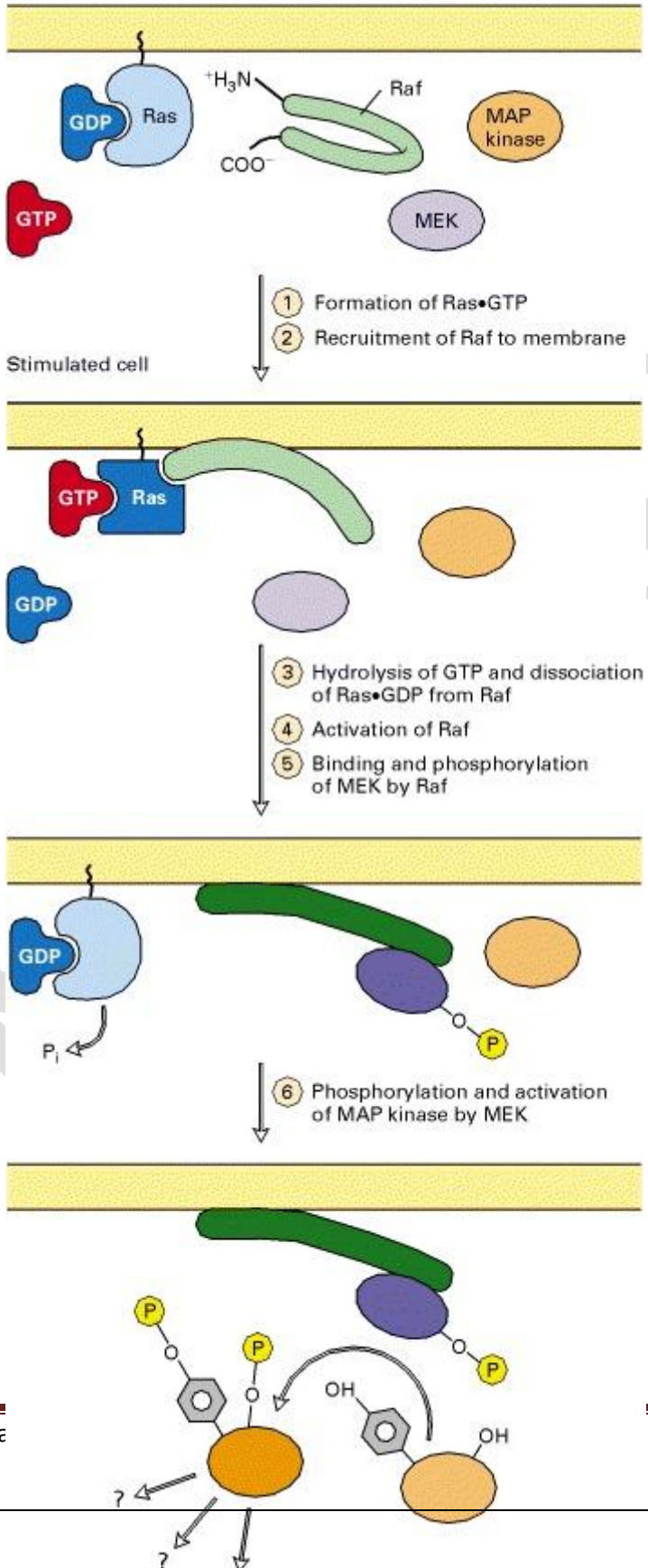
cascades and recent studies indicating that both yeasts and cells of higher eukaryotes contain multiple MAP kinases.

Activation of MAP kinase in two different cells can lead to similar or different cellular responses, as can activation in the same cell by stimulation of different RTKs. The mechanisms controlling the response specificity of MAP kinases are poorly understood and are not considered in this chapter.

Signals Pass from Activated Ras to a Cascade of Protein Kinases

A remarkable convergence of biochemical and genetic studies in yeast, *C. elegans, Drosophila,* and mammals has revealed a highly conserved cascade of protein kinases that operate in sequential fashion downstream from activated Ras as follows:

- Activated Ras binds to the N-terminal domain of Raf, a serine/threonine kinase.
- Raf binds to and phosphorylates MEK, a dual-specificity protein kinase that phosphorylates both tyrosine and serine residues.
- MEK phosphorylates and activates MAP kinase, another serine/threonine kinase.
- MAP kinase phosphorylates many different proteins, including nuclear transcription factors, that mediate cellular responses.


GDP
Ras
$^{+}H_3N$
Raf
COO^{-}
MAP kinase
GTP
MEK
1 Formation of Ras•GTP
2 Recruitment of Raf to membrane
Stimulated cell
GTP
Ras
GDP
3 Hydrolysis of GTP and dissociation of Ras•GDP from Raf
4 Activation of Raf
5 Binding and phosphorylation of MEK by Raf
GDP
P_i
O
P
6 Phosphorylation and activation of MAP kinase by MEK
P
O
P
O
OH
OH
?
?

Kinase cascade that transmits signals downstream from activated Ras protein

In unstimulated cells, most Ras is in the inactive form with bound GDP *(top);* binding of a growth factor to its RTK leads to formation of the active Ras · GTP. A signaling complex then is assembled downstream of Ras, leading to activation of MAP kinase by phosphorylation of threonine and tyrosine residues separated by a single amino acid. Phosphorylation at both sites is necessary for activation of MAP kinase.

POSSIBLE QUESTIONS

2 marks

1. Define cell to cell interaction.
2. Define cell to cell adhesion.
3. Define cell signaling.
4. What are collagens?
5. Define proteoglycans.
6. What is meant by specialized junctions?
7. Define cadherins.

6 marks

1. Discuss about cell-cell interactions in ECM.
2. Explain in detail about collagen, hyalurons and proteoglycans.
3. Illustrate specialized junctions, gap junctions and tight junctions.
4. Give a detailed note on adhesion molecules cadherins and connexins.
5. Describe role of signaling molecules and their receptors.
6. Briefly explain functions of cell surface receptors.
7. Discuss in detail about pathways of intracellular signal transduction.
8. Explain about second messengers and their importance in cellular progression.
9. Detailed note on various proteins in cellular growth and its function.

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CELLULAR BIOCHEMISTRY 18BCP104)
MULTIPLE CHOICE QUESTIONS

UNIT IV

S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	The external environment occur in a selective phenomenon is called ----------	cell-cell adhesion	cell adhesion	cell-junction	cell-interaction	cell adhesion
2	The multicellular organisms by the phenomenon called	cell-cell adhesion	cell adhesion	cell-junction	cell-interaction	cell-cell adhesion
3	In early differentiation ---------- cadherins are mostly expressed	E, P and N	E and N	P and N	none	E, P and N
4	Cadherins are integral ----------	lipoprotein	oncoprotein	glycoprotein	both a & b	glycoprotein
5	P-selection is localized as a vesicle in the ----------	epithelial cell	endothelial cell	exothelial cell	none	endothelial cell
6	Communicating junctions are	gap junctions	chemical synapses	plasmadesmata	all the above	all the above
7	Collagen – right handed helix each with	1000 a.a	1100 a.a	1150 a.a	1050 a.a	1050 a.a
8	Collagen biosynthesis was described by	kivirikko & Risteli	Ramachandran et al.,	both a & b	none of the above	kivirikko & Risteli
9	---------- collagens are flexible type	type I	type II	type III	type IV	type IV
10	Syndecan core proteins range in size from ---------- molecular weight	20000 to 45000	25000 to 40000	100 to 400	2000-4500	20000 to 45000
11	In extracellular signaling by the secreted molecules can be classified in to ---------	1 type	5 types	6 types	3 types	3 types
12	In extracellular signaling the secreted molecules can be classified in to ---------	endocrine	autocrine	exocrine	all the above	all the above

13	Communication by extracellular signals usually involves ---------- step	6 steps	9 steps	10 steps	both b & c	6 steps
14	The signal molecules are also known as	second messenger	first messenger	both a & b	none	first messenger
15	The second messengers are	DAG	IP3	Ca^{++}	all	all
16	The signal molecules are	proteins	aminoacids	fatty acids	all the above	all the above
17	Signal receptors are --------- types	2	5	10	120	2
18	Signal receptor types are	cell surface	intracellular receptor	both a & b	none	both a & b
19	The extracellular signal molecules act over	short distance	long distance	either a & b	none of the above	either a & b
20	Contace dependent signaling are also called as	local effectors	action potential	chemical synapse	none of the above	none of the above
21	Paracrine signaling are also called as	local effector	chemical synapse	action potential	none	local effector
22	The action potential reaches and causes to release some chemical signal molecules called ----------	action potential	local effectors	neurotransmitters	both a & b	neurotransmitters
23	The chemical synapse and new action potential is produced by ----------	denaturing	depolarizing	both a & b	none	depolarizing
24	The cancer cells often use auto urine signaling for ----------	proliferation	excretion	interaction	none	proliferation
25	G-protein couple receptor is also known as	seven-spanning G protein linked receptor	two-spanning G protein liked receptor	cell-cell signalling	all the above	seven-spanning G protein linked receptor
26	Signalling molecules referred as	hormone	pheromone	neurotransmitters	all the above	all the above
27	The aminoacid sequence contains seven stretches of ---------- hydrophobic residues in α-helix	≈20-24	≈24-30	≈22-24	≈20-25	≈22-24
28	Ras protein is a key component of signaling pathways in many ----------	prokaryotes	eukaryotes	both a & b	none	eukaryotes
29	Ras protein belongs to the ---------- family	GTPase	ATPase	UTPase	all the above	GTPase
30	The gap junction always maintains a gap of ---------- wide	2nm-4nm	3nm-4nm	5nm-7nm	4-5nm	2nm-4nm

31	For mating pathway which one is linke to G-protein coupled receptor?	Ras kinase	GPCR	Map kinase	a & b	Map kinase
32	Ras protein contains …….	~ 170 amino acid	~ 270 amino acid	~ 370 amino acid	~ 17 amino acid	~ 170 amino acid
33	Yeast pheromone receptor triggers the dissociation of G – protein in to ……..	G α & G β gamma complex	G α	G β gamma	α & β gamma complex	G α & G β gamma complex
34	Scafffold protein also called ……..	Ste – 5	ste – 6	ste – 7	ste – 11	Ste – 5
35	The hormone receptor complexes act by binding to highly specific DNA sequence are called ……..	MAP kinase	hormone receptor element	none	both a & b	hormone receptor element
36	Collagen is the major class of ……..	In soluble fibrous protein	soluble fibrous protein	both a & b	none	In soluble fibrous protein
37	The basic structural unit of collagen is ……..	Double helix	Trible helix	Single helix	both a &b	Trible helix
38	The principal class of cell matrix adhesion molecule is said to be ……..	Integrin	MAP kinase	Interactions	both a & b	Integrin
39	Hyaluronan is ……..	Positively charged polysaccharide	Negatively charged polysaccharide	Both a& b	None of the above	Negatively charged polysaccharide
40	……. Number of integrin contains in mammals.	22 integrin	30 integrin	25 integrin	32 integrin	22 integrin
41	Talin also binds with ……..	Paxillin	Vinculin	Actin	Zyxin	Vinculin
42	Type – II collagen forms ……	Thin unbanded fibrils	Rough unbanded fibrils	Thin banded fibrils	None of the abive	Thin unbanded fibrils
43	Proteoglycans are …….. found in all connective tissues and extracellular matrices.	Micromolecules	Tetra molecules	Macromolecules	Both a& b	Macromolecules
44	One of the most important extracellular proteoglycans is ……..	Aggeacan	Link protein	core protein	both b & c.	Aggeacan
45	Molecular weight of aggrecan core protein is …….	~ 250000	~ 170000	~ 10000	~ 25000	~ 250000
46	The agyrphilia is dur to the …….	Free hydroxyl groups	hydroxyl groups	banded fibrils	thin fibrils	Free hydroxyl groups
47	The integrins are a large class of…….	G-protein coupled receptor	cell-surface receptor	both a & b	Ras kinase	cell-surface receptor

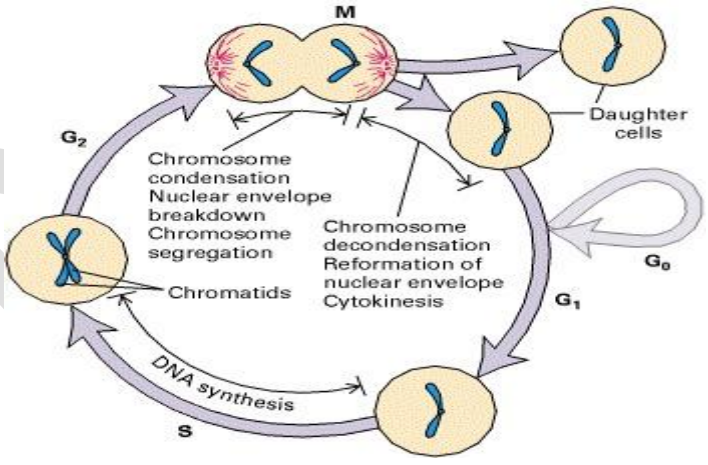
48	The extracellular protein is…..	Pectin	fibronin	anokin	laminin	laminin
49	Fibronectins are another important class of…	Macro molecular	cell-cell-adhesive	multi adhesive matrix proteins	adhesive	multi adhesive matrix proteins
50	Fibronectins are dimmers of two similar poly peptides linked at their c-terminus by….	Two disulphifide bonds	two hydrogen bonds	sulfide bond	hydrogen bond	Two disulphifide bonds
51	fibronectin is secreted by….	kidney	liver	muscle	all	liver
52	Anchoring junction mechanically attach by….	Cytoskeletons	muscles	nerves	epithelial cells	Cytoskeletons
53	The abchor proteins include…..	tropomyosin	alpha-actin	vinculin	all	all
54	desmosome consists of proteinaceous ashesion plaques of …….nm	15-25	15-20	20-25	35-40	15-20
55	Major types of cells junction are ….	tight junction	desmosome	gab junction	all the above	all the above
56	Desmosomes are thickened regions of the…..	plasma membrane	mitochondria	cytosol	nucleous	plasma membrane
57	Gap junction cannot synthesis….	d ATP from DNA	GTP from DNA	d GTP from RNA	d ATP fromRNA	d ATP from DNA
58	How many types of desmosomes are recognized…….	Three types	two types	five types	six types.	Three types
59	Adherens junctions also called as…..	Belt desmosomes	spot desmosomes	hemidesmosomes	desmocollin	Belt desmosomes
60	Movement of numerous proteins contains molecular weight up to……	60kda	70kda	75kda	140kda	70kda

UNIT-V-SYLLABUS

Cell cycle and cancer: Cell cycle and its control, Cell cycle control in mammalian cells, checkpoints in cell cycle regulation.
Cancer: Properties of tumour cells and genetic basis and onset of cancer.
Tumour viruses – DNA & RNA Viruses as transforming agents – mechanism.
Tumour suppressor genes and functions of their products. Carcinogenic and anticarcinogenic effect of chemicals and radiation. Apoptosis (Programmed cell death) – pathways, regulators and effectors on apoptosis and necrosis.

Overview of the Cell Cycle and Its Control

The cell cycle is divided into four major phases. In cycling (replicating) somatic cells, chromosomes are replicated during the S (synthesis) phase. After progressing through the G_2 phase, cells begin the complicated process of mitosis, also called the M phase, which is divided into several stages. Chromosomes condense during the prophase period of mitosis, by tightly folding loops of the 30-nm chromatin fiber attached to the chromosome scaffold. Sister chromatids, produced by DNA replication during the S phase, remain attached at the centromere and multiple points along their length and become aligned in the center of the cell during metaphase. During the anaphase portion of mitosis, sister chromatids separate and move to opposite poles of the mitotic apparatus, or spindle, segregating one of the two sister chromatids to each daughter cell.



The fate of a single parental chromosome throughout the eukaryotic cell cycle

Although chromosomes condense only during mitosis, they are shown in condensed form to emphasize the number of chromosomes at different cell-cycle stages. The nuclear envelope is not depicted. Following mitosis (M), daughter cells contain 2*n* chromosomes in diploid organisms and 1*n* chromosomes in haploid organisms including yeasts maintained in the haploid state. In proliferating cells, G_1 is the period between "birth" of a cell following mitosis and the initiation of DNA synthesis, which marks the beginning of the S phase. At the end of the S phase, cells enter G_2 containing twice the number of chromosomes as G_1 cells (4*n* in diploid organisms). The end of G_2 is marked by the onset of mitosis, during which numerous events leading to cell division occur. The G_1, S, and G_2 phases are collectively referred to asinterphase, the period between one mitosis and the next. Most nonproliferating cells in vertebrates leave the cell cycle in G_1, entering the G_0 state.

In most cells from higher eukaryotes, the nuclear envelope breaks down into multiple small vesicles early in mitosisand re-forms around the segregated chromosomes as they decondense during telophase, the last mitotic stage. The physical division of the cytoplasm, called cytokinesis, then yields two daughter cells. The Golgi complex and endoplasmic reticulum also vesiculate during mitosis and re-form in the two daughter cells after cell division. In yeasts and other fungi, the nuclear envelope does not break down. In these organisms, the mitotic spindle forms within the nuclear envelope, which then pinches off, forming two nuclei at the time of cytokinesis. Following mitosis, cycling cells enter the G_1 phase, the period before DNA synthesis is reinitiated in the S phase.

In vertebrates and diploid yeasts, cells in G_1 have a diploid number of chromosomes (2*n*), one inherited from each parent. In haploid yeasts, cells in G_1 have one of each chromosome (1*n*). Rapidly replicating human cells progress through the full cell cycle in about 24 hours: mitosis takes ≈30 minutes; G_1, 9 hours; the S phase, 10 hours; and G_2, 4.5 hours. In contrast, the full cycle takes only ≈90 minutes in rapidly growing yeast cells.

Postmitotic cells in multicellular organisms can "exit" the cell cycle and remain for days, weeks, or in some cases (e.g., nerve cells and cells of the eye lens) even the lifetime of the organism without proliferating further. Most postmitotic cells in vertebrates exit the cell cycle in G_1, entering a phase called G_0 . G_0 cells returning to the cell cycle enter into the S phase; this reentry is regulated, thereby providing control of cell proliferation.

CELL CYCLE

During development from stem to fully differentiated, cells in the body alternately divide (mitosis) and "appear" to be resting (interphase). This sequence of activities exhibited by cells is called the cell cycle. It can be defined as the entire sequence of events happening from the end of one nuclear division to the beginning of the next division. Cells have the property of division and multiplication and consist of three major phases namely mitosis (M phase) or the nuclear division, cytokinesis or the division of the cell and interphase where replication of genetic material occurs.

MITOSIS OR M PHASE The M phase lasts only for an hour in a period of 24 hour required for a eukaryotic cell to divide.**:** Cell growth and protein production stop at this stage in the cell cycle. All of the cell's energy is focused on the complex and orderly division into two similar daughter cells. Mitosis is much shorter than interphase, lasting perhaps only one to two hours. As in both G1 and G2, there is a Checkpoint in the middle of mitosis (Metaphase Checkpoint) that ensures the cell is ready to complete cell division. Actual stages of mitosis can be viewed at Animal Cell Mitosis.

INTERPHASE: Interphase, is a resting stage between cell divisions, is actually a period of diverse activities. Those interphase activities are indispensible in making the next mitosis possible.

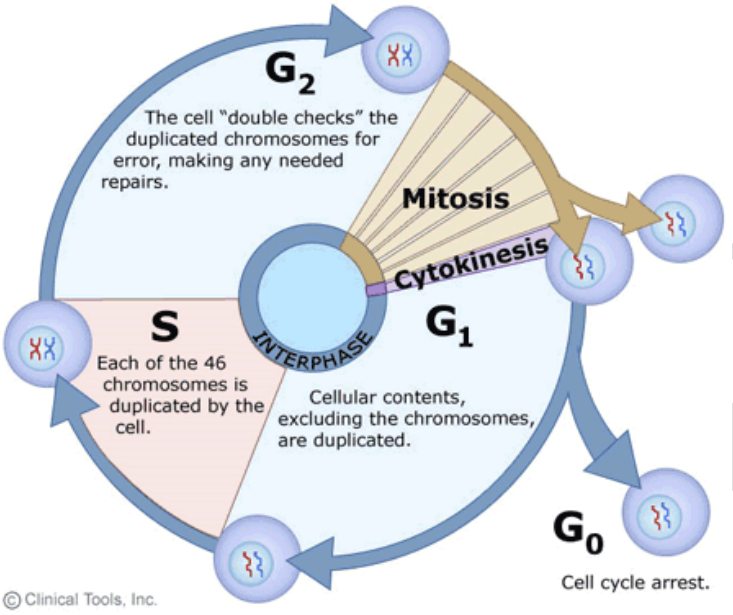
Interphase generally lasts at least 12 to 24 hours in mammalian tissue. During this period, the cell is constantly synthesizing RNA, producing protein and growing in size. By studying molecular events in cells, scientists have determined that interphase can be divided into 4 steps: Gap 0 (G0), Gap 1 (G1), S (synthesis) phase, Gap 2 (G2).

i) Gap 0 (G0): There are times when a cell will leave the cycle and quit dividing. This may be a temporary resting period or more permanent. Cells that are no longer capable of division, whether temporarily or permanently, remain in G0 phase. A cell must receive a growth-promoting signal to proceed from the quiescent stage or G0 into G1 phase and thus reenter the cell cycle. An example of the latter is a cell that has reached an end stage of development and will no longer divide (e.g. neuron).

ii) Gap 1 (G1): Cells increase in size in Gap 1, produce RNA and synthesize protein. An important cell cycle control mechanism activated during this period (G1 Checkpoint) ensures that everything is ready for DNA synthesis.

iii) S Phase: To produce two similar daughter cells, the complete DNA instructions in the cell must be duplicated. DNA replication occurs during this S (synthesis) phase.

iv) Gap 2 (G2): During the gap between DNA synthesis and mitosis, the cell will continue to grow and produce new proteins. At the end of this gap is another control checkpoint (G2 Checkpoint) to determine if the cell can now proceed to enter M (mitosis) and divide.



Phases of cell cycle

Dividing phase: There are two types of cell division possible. Mitosis and meosis. The mitosis (Gr., *mitos*=thread) occurs in the somatic cells and it is meant for the multiplication of cell number during embryogenesis and blastogenesis of plants and animals. Fundamentally, it remains related with the growth of an individual from zygote to adult stage. Mitosis starts at the culmination point of interphase (G2 phase). It is a short period of chromosome condensation, segregation and cytoplasmic division. Mitosis is important for growth of organism, replacement of cells lost to natural friction or attrition, wear and tear and for wound healing. Hence, mitosis is remarkably similar in all animals and plants. It is a smooth continuous process and is divided into different stages or phases.

1.MITOSIS

Mitosis is a process of eukaryotic cell division in which each of two identical daughter cells receives a diploid complements of chromosomes same as the diploid complement of the parent

cell. It is usually followed by cytokinesis in which the cell itself divides to yield two identical daughter cells.

Chromosomes replicated during the S phase are divided in such a way as to ensure that each daughter cell receives a copy of every chromosome. In actively dividing animal cells, the whole process takes about one hour.The replicated chromosomes are attached to a 'mitotic apparatus' that aligns them and then separates the sister chromatids to produce an even partitioning of the genetic material. This separation of the genetic material in a mitotic nuclear division (or **karyokinesis)** is followed by a separation of the cell cytoplasm in a cellular division (or **cytokinesis**) to produce two daughter cells.

In some single-celled organisms mitosis forms the basis of asexual reproduction. In diploid multicellular organisms sexual reproduction involves the fusion of two haploid gametes to produce a diploid zygote. Mitotic divisions of the zygote and daughter cells are then responsible for the subsequent growth and development of the organism. In the adult organism, mitosis plays a role in cell replacement, wound healing and tumour formation.

The basics in mitosis include:

1. Each chromosome is present as a duplicated structure at the beginning of nuclear division (2n).

2. Each chromosome divides longitudinally into identical halves and become separated from each other.

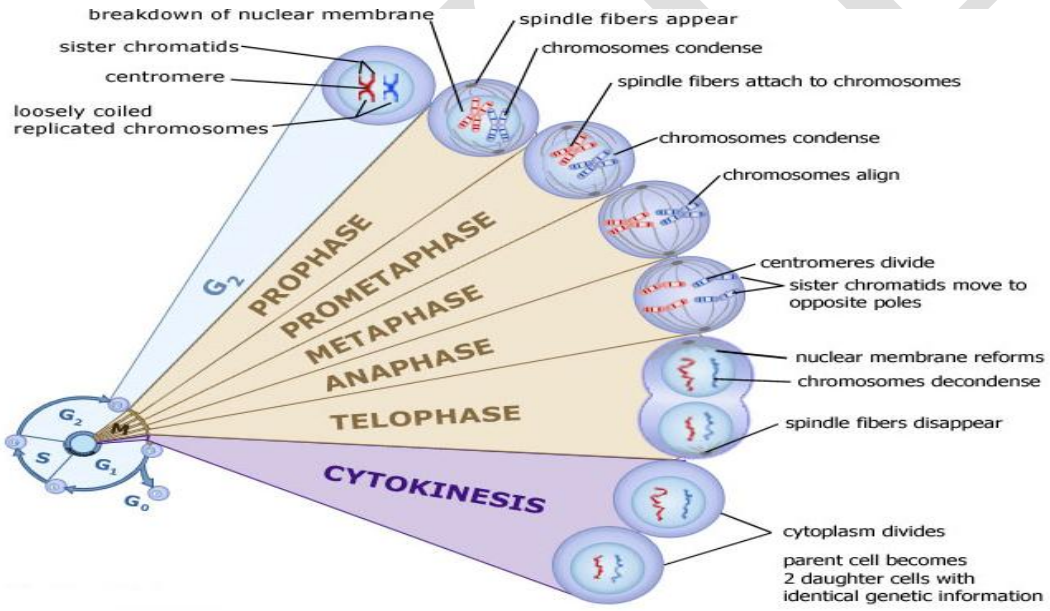
3. The separated chromosome halves move in opposite directions, and each becomes included in one of the two daughter nuclei that are formed.

Mitosis, although a continuous process, is conventionally divided into five stages:

- prophase,
- prometaphase
- metaphase,
- anaphase
- telophase.

Prophase

Prophase occupies over half of mitosis. The nuclear membrane breaks down to form a number of small vesicles and the nucleolus disintegrates. A structure known as the **centrosome**duplicates itself to form two daughter centrosomes that migrate to opposite ends of the cell. The centrosomes organise the production of microtubules that form the spindle fibres that constitute the **mitotic spindle**. The chromosomes condense into compact structures. Each replicated chromosome can now be seen to consist of two identical **chromatids** (or **sister chromatids**) held together by a structure known as the **centromere**.. Each pair of chromatids is the product of the duplication of one chromosome in the S period of interphase. As prophase progresses, the chromosomes become shorter and thicker as a result of intricate coiling. At the end of prophase, the nucleoli disappear and the nuclear envelope, a membrane surrounding the nucleus, abruptly disintegrates.



Phases of Mitosis

Prometaphase

The chromosomes, led by their centromeres, migrate to the equatorial plane in the mid-line of the cell - at right-angles to the axis formed by the centrosomes. This region of the mitotic spindle is known as the **metaphase plate**. The spindle fibres bind to a structure associated with the

centromere of each chromosome called a kinetochore. Individual spindle fibres bind to a **kinetochore** structure on each side of the centromere. The chromosomes continue to condense.

Metaphase

The chromosomes align themselves along the metaphase plate of the spindle apparatus.

At the beginning of metaphase, the mitotic spindle forms which are a bipolar structure and consist of fiber-like bundles of microtubules that extend through the cell between the poles of the spindle. Each chromosome attached to several spindle fibers in the region of the centromere. The structure associated with the centromere to which the spindle fibers attach is known as the kinetochore. After the chromosomes are attached to spindle fibers, they move towards the center of the cell until all the kinetochores lie on an imaginary plane equidistant from the spindle poles. This imaginary plane is called the metaphase plate. Hence the chromosomes reach their maximum contraction and are easiest to count and examine for differences in morphology. The signal for chromosome alignment comes from the kinetochore, and the chemical nature of the signal seems to be the dephosphorylation of certain kinetochore-associated proteins. The role of the kinetochore is demonstrated by the finding that metaphase is not delayed by an unattached chromosome whose kinetochore has been destroyed by a focused laser beam. The role of dephosphorylation is demonstrated through the use of an antibody that reacts specifically with some kinetochore proteins only when they are phosphorylated. Unattached kinetochores combine strongly with the antibody, but attachment to the spindle weakens the reaction. In chromosomes that have been surgically detached from the spindle, the antibody reaction with the kinetochore reappears. Through the signaling mechanism, when all of the kinetochores are under tension and aligned on the metaphase plate, the metaphase checkpoint is passed and the cell continues the process of division.

Anaphase

The shortest stage of mitosis. The centromeres divide, and the sister chromatids of each chromosome are pulled apart - or 'disjoin' - and move to the opposite ends of the cell, pulled by spindle fibres attached to the kinetochore regions. Once the centromere divide, each sister chromatid is treated as a separate chromosome. Chromosome movement results from progressive shortening of the spindle fibers attached to the centromeres, which pulls the chromosomes in opposite directions toward the poles. At the completion of anaphase, the chromosomes lie in two

groups near opposite poles of the spindle. Each group contains the same number of chromosomes that was present in the original interphase nucleus. The separated sister chromatids are now referred to as **daughter chromosomes**. (It is the alignment and separation in metaphase and anaphase that is important in ensuring that each daughter cell receives a copy of every chromosome.)

Telophase

The final stage of mitosis, and a reversal of many of the processes observed during prophase. In telophase, a nuclear envelope forms around each group of chromosomes, nucleoli are formed, and the spindle disappears.

Cell cycle check points

The length of the cell cycle is highly variable, even within the cells of a single organism. In humans, the frequency of cell turnover ranges from a few hours in early embryonic development, to an average of two to five days for epithelial cells, and to an entire human lifetime spent in G_0 by specialized cells, such as cortical neurons or cardiac muscle cells. There is also variation in the time that a cell spends in each phase of the cell cycle. When fast-dividing mammalian cells are grown in culture (outside the body under optimal growing conditions), the length of the cycle is about 24 hours. In rapidly dividing human cells with a 24-hour cell cycle, the G_1 phase lasts approximately nine hours, the S phase lasts 10 hours, the G_2 phase lasts about four and one-half hours, and the M phase lasts approximately one-half hour. In early embryos of fruit flies, the cell cycle is completed in about eight minutes. The timing of events in the cell cycle is controlled by mechanisms that are both internal and external to the cell.

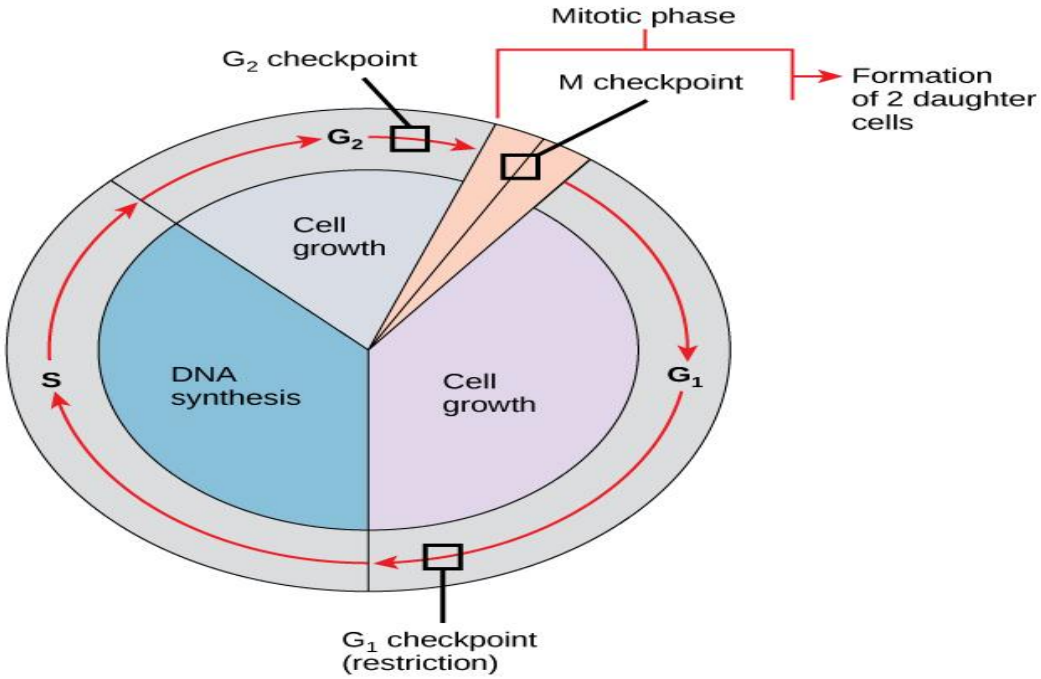
Regulation of the Cell Cycle by External Events

Both the initiation and inhibition of cell division are triggered by events external to the cell when it is about to begin the replication process. An event may be as simple as the death of a nearby cell or as sweeping as the release of growth-promoting hormones, such as human growth hormone (HGH). A lack of HGH can inhibit cell division, resulting in dwarfism, whereas too much HGH can result in gigantism. Crowding of cells can also inhibit cell division. Another factor that can initiate cell division is the size of the cell; as a cell grows, it becomes inefficient due to its decreasing surface-to-volume ratio. The solution to this problem is to divide.

Whatever the source of the message, the cell receives the signal, and a series of events within the cell allows it to proceed into interphase. Moving forward from this initiation point, every parameter required during each cell cycle phase must be met or the cycle cannot progress.

Regulation at Internal Checkpoints

It is essential that the daughter cells produced be exact duplicates of the parent cell. Mistakes in the duplication or distribution of the chromosomes lead to mutations that may be passed forward to every new cell produced from an abnormal cell. To prevent a compromised cell from continuing to divide, there are internal control mechanisms that operate at three main cell cycle checkpoints. A checkpoint is one of several points in the eukaryotic cell cycle at which the progression of a cell to the next stage in the cycle can be halted until conditions are favorable. These checkpoints occur near the end of G_1, at the G_2/M transition, and during metaphase



The G_1 Checkpoint

The G_1 checkpoint determines whether all conditions are favorable for cell division to proceed. The G_1 checkpoint, also called the restriction point (in yeast), is a point at which the cell irreversibly commits to the cell division process. External influences, such as growth factors, play a large role in carrying the cell past the G_1 checkpoint. In addition to adequate reserves and cell size, there is a check for genomic DNA damage at the G_1 checkpoint. A cell that does not

meet all the requirements will not be allowed to progress into the S phase. The cell can halt the cycle and attempt to remedy the problematic condition, or the cell can advance into G_0 and await further signals when conditions improve.

The G_2 Checkpoint

The G_2 checkpoint bars entry into the mitotic phase if certain conditions are not met. As at the G_1 checkpoint, cell size and protein reserves are assessed. However, the most important role of the G_2 checkpoint is to ensure that all of the chromosomes have been replicated and that the replicated DNA is not damaged. If the checkpoint mechanisms detect problems with the DNA, the cell cycle is halted, and the cell attempts to either complete DNA replication or repair the damaged DNA.

The M Checkpoint

The M checkpoint occurs near the end of the metaphase stage of karyokinesis. The M checkpoint is also known as the spindle checkpoint, because it determines whether all the sister chromatids are correctly attached to the spindle microtubules. Because the separation of the sister chromatids during anaphase is an irreversible step, the cycle will not proceed until the kinetochores of each pair of sister chromatids are firmly anchored to at least two spindle fibers arising from opposite poles of the cell.

Properties of Cancer Cells

1) Cancer cells show uncontrolled mitotic divisions causing unorganised growth

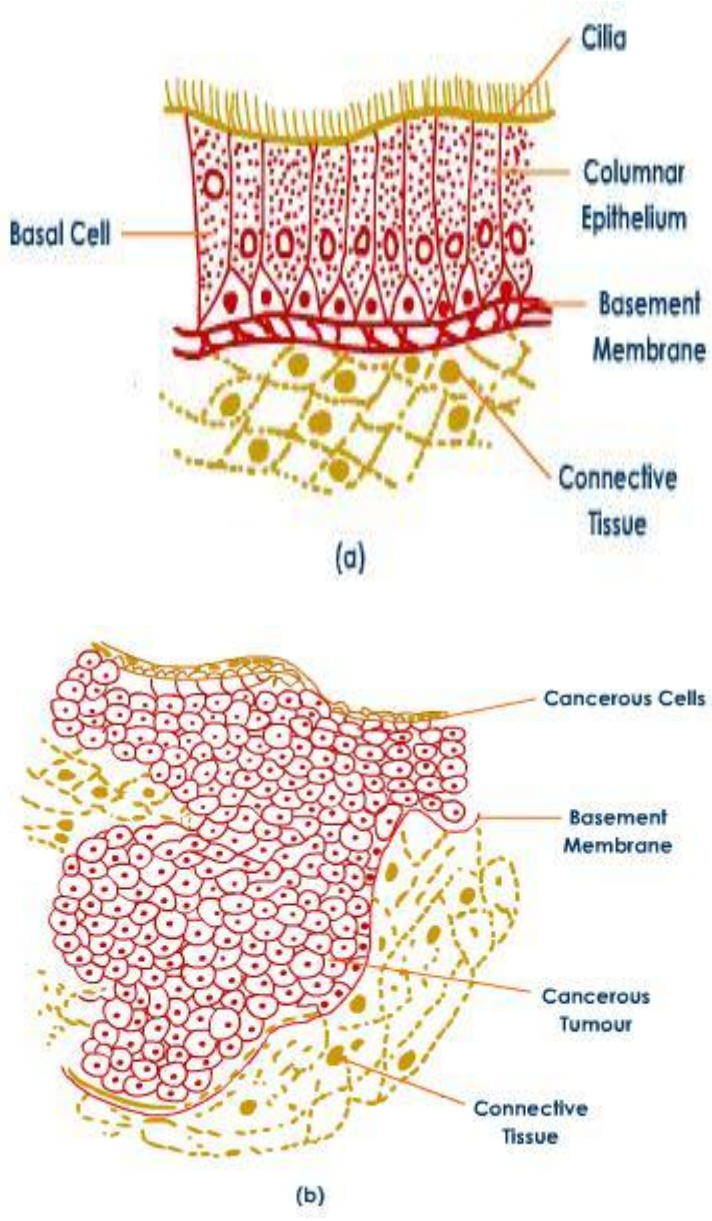
2) Due to uncontrolled growth and division of cells, a tumour (also called Neoplasm is generally formed)

3) Cancer cells are far less adhesive than the normal cells, so these generally wander through the tissues to cause cancerous growth in different parts of the body.

This ability of cancer cells to invade new sites is termed as Metastasis.

4) Cancer cells exhibit a number of alterations on cell surface, in the cytoplasm and in their genes.

5) Cancer cells donot undergo differentiation



Development of cancer (a) Normal Lung epithelium (b) A cancerous tumour in lung epithelium

Tumor Viruses

Members of six distinct families of animal viruses, called **tumor viruses**, are capable of directly causing cancer in either experimental animals or humans (Table). Viruses belonging to five of these families have DNA genomes and are referred to as DNA tumor viruses. Members of the

sixth family of tumor viruses, the retroviruses, have RNAgenomes in virus particles but replicate via synthesis of a DNA provirus in infected cells. The viruses that cause human cancer include hepatitis B virus (liver cancer), papillomaviruses (cervical and other anogenital cancers), Epstein-Barr virus (Burkitt's lymphoma and nasopharyngeal carcinoma), Kaposi's sarcoma-associated herpesvirus (Kaposi's sarcoma), and human T-cell lymphotropic virus (adult T-cell leukemia). In addition, HIV is indirectly responsible for the cancers that develop in AIDS patients as a result of immunodeficiency, and hepatitis C virus (an RNA virus) is an indirect cause of liver cancers resulting from chronic tissue damage.

Virus family	**Human tumors**	**Genome size (kb)**
DNA tumor viruses		
Hepatitis B viruses	Liver cancer	3
SV40 and polyomavirus	None	5
Papillomaviruses	Cervical carcinoma	8
Adenoviruses	None	35
Herpesviruses	Burkitt's lymphoma, nasopharyngeal carcinoma, Kaposi's sarcoma	100–200
RNA tumor viruses		
Retroviruses	Adult T-cell leukemia	9

As already noted, tumor viruses not only are important as causes of human disease but have also played a critical role in cancer research by serving as models for cellular and molecular studies of cell transformation. The small size of their genomes has made tumor viruses readily amenable to molecular analysis, leading to the identification of viral genes responsible for cancer induction and paving the way to our current understanding of cancer at the molecular level.

Hepatitis B Viruses

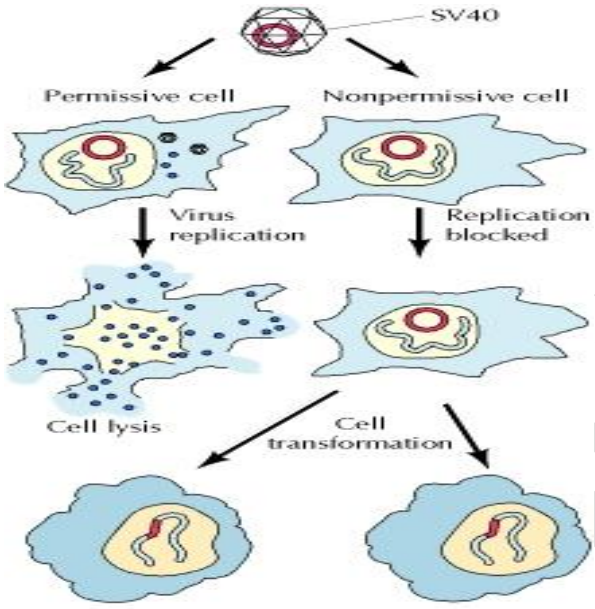
The **hepatitis B viruses**, which have the smallest genomes (approximately 3 kb) of all animal DNA viruses, specifically infect liver cells of several species, including ducks, woodchucks, squirrels, and humans. Infection with hepatitis B virus usually results in acute liver damage. In 5 to 10% of cases, however, the acute infection is not resolved and a chronic infection of the liver develops. Such chronic infection is associated with more than a hundredfold increased risk of liver cancer. Hepatitis B virus infection is particularly common in parts of Asia and Africa, where it is associated with up to a million cases of liver cancer annually (approximately 10% of worldwide cancer incidence).

Cell transformation by hepatitis B virus is mediated by a viral gene (called the *X* gene) that affects expression of a variety of cellular genes that drive abnormal cell proliferation and survival. In addition, the development of cancers induced by hepatitis B virus is driven by the continual proliferation of liver cells that results from chronic tissue damage.

SV40 and Polyomavirus

The best studied DNA tumor viruses, from the standpoint of molecular biology, are probably **simian virus 40** (**SV40**) and **polyomavirus**. Although neither of these viruses is associated with human cancer, they have been critically important as models for understanding the molecular basis of cell transformation. The utility of these viruses in cancer research has stemmed from the availability of good cell culture assays for both virus replication and transformation, as well as from the small size of their genomes (approximately 5 kb).

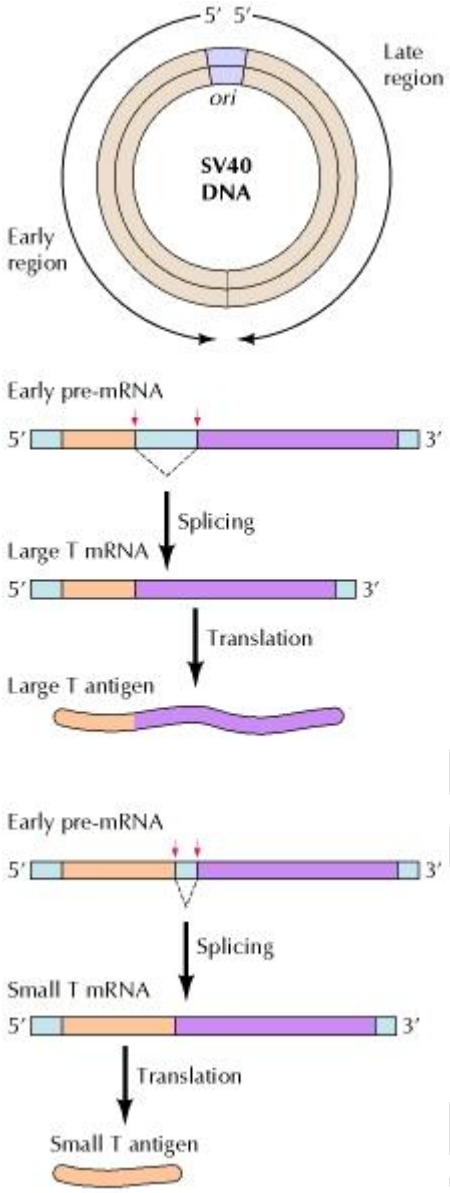
SV40 and polyomavirus do not induce tumors or transform cells of their natural host species—monkeys and mice, respectively. In cells of their natural hosts (permissive cells), infection leads to virus replication, cell lysis, and release of progeny virus particles. Since a permissive cell is killed as a consequence of virus replication, it cannot become transformed. The transforming potential of these viruses is revealed, however, by infection of nonpermissive cells, in which virus replication is blocked. In this case, the viral genome sometimes integrates into cellular DNA, and expression of specific viral genes results in transformation of the infected cell.



SV40 replication and transformation

Infection of a permissive cell results in virus replication, cell lysis, and release of progeny virus particles. In a nonpermissive cell, virus replication is blocked, allowing some cells to become permanently transformed.

The SV40 and polyomavirus genes that lead to cell transformation have been identified by detailed molecular analyses. The viral genomes and mRNAs have been completely sequenced, viral mutants that are unable to induce transformation have been isolated, and the transforming potentials of individual viral genes have been determined by gene transfer assays. Transformation by these viruses has thus been found to result from expression of the same viral genes that function in early stages of lytic infection. The genomes of SV40 and polyomavirus are divided into early and late regions. The early region is expressed immediately after infection and is required for synthesis of viral DNA. The late region is not expressed until after viral DNA replication has begun, and includes genes encoding structural components of the virus particle. The early region of SV40 encodes two proteins, called small and large T antigens, of about 17 kd and 94 kd, respectively. Their mRNAs are generated by alternative splicing of a single early-region primary transcript. Polyomavirus likewise encodes small and large T antigens, as well as a third early-region protein of about 55 kd, designated middle T. Transfection of cells with cDNAs for individual early-region proteins has shown that SV40 large T is sufficient to induce transformation, whereas middle T is primarily responsible for transformation by polyomavirus.



The SV40 genome

The genome is divided into early and late regions. Large and small T antigens are produced by alternative splicing of early-region pre-mRNA.

During lytic infection, these early-region proteins fulfill multiple functions required for virus replication. SV40 T antigen, for example, binds to the SV40 origin and initiates viral DNA replication. In addition, the early-region proteins of SV40 and polyomavirus stimulate

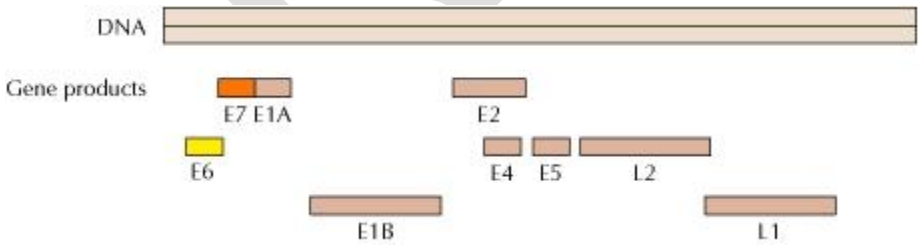
host cell gene expression and DNA synthesis. Since virus replication is dependent on host cell enzymes (e.g., DNA polymerase), such stimulation of the host cell is a critical event in the viral life cycle. Most cells in an animal are nonproliferating, and therefore must be stimulated to divide in order to induce the enzymes needed for viral DNA replication. This stimulation of cell proliferation by the early gene products can lead to transformation if the viral DNA becomes stably integrated and expressed in a nonpermissive cell.

As discussed later in this chapter, both SV40 and polyomavirus early-region proteins induce transformation by interacting with host proteins that regulate cell proliferation. For example, SV40 T antigen binds to and inactivates the host cell tumor suppressor proteins Rb and p53, which are key regulators of cell proliferation and cell cycle progression.

Papillomaviruses

The **papillomaviruses** are small DNA viruses (genomes of approximately 8 kb) that induce both benign and malignant tumors in humans and a variety of other animal species. Approximately 60 different types of human papillomaviruses, which infect epithelial cells of several tissues, have been identified. Some of these viruses cause only benign tumors (such as warts), whereas others are causative agents of malignant carcinomas, particularly cervical and other anogenital cancers. The mortality from cervical cancer is relatively low in the United States, in large part as a result of early detection and curative treatment made possible by the Pap smear. In other parts of the world, however, cervical cancer remains common; it is responsible for 5 to 10% of worldwide cancer incidence.

Cell transformation by human papillomaviruses results from expression of two early-region genes, *E6* and *E7*. The E6 and E7 proteins act analogously to SV40 T antigen by interfering with the function of the cellular Rband p53 proteins. In particular, E7 binds to Rb, and E6 stimulates the degradation of p53 by ubiquitin-mediated proteolysis.



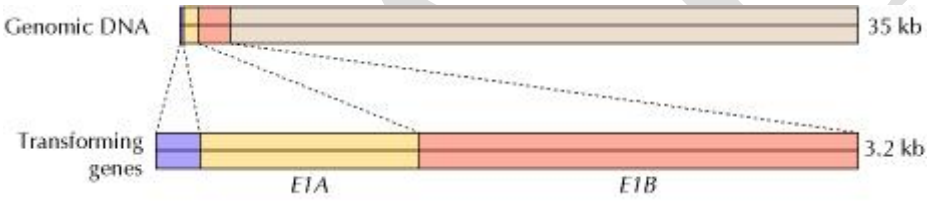
The genome of a human papillomavirus

Gene products are designated E (early) or L (late). Transformation results from the action of E6 and E7.

Adenoviruses

The **adenoviruses** are a large family of DNA viruses with genomes of about 35 kb. In contrast to the papillomaviruses, the adenoviruses are not associated with naturally occurring cancers in either humans or other animals. However, they are widely studied and important models in experimental cancer biology.

Like SV40 and polyomaviruses, the adenoviruses are lytic in cells of their natural host species, but can induce transformation in nonpermissive hosts. Transformation by the adenoviruses results from expression of two early genes, *E1A* and *E1B,* which are required for virus replication in permissive cells. These transforming proteins inactivate the Rb and p53 tumor suppressor proteins, with E1A binding to Rb and E1B binding to p53. It thus appears that SV40, papillomaviruses, and adenoviruses all induce transformation by a common pathway, in which altering regulation of the cell cycle by interfering with the activities of Rb and p53 plays a central role.



The adenovirus genome. Two early-region genes, *E1A* and *E1B,* are responsible for induction of transformation.

Herpesviruses

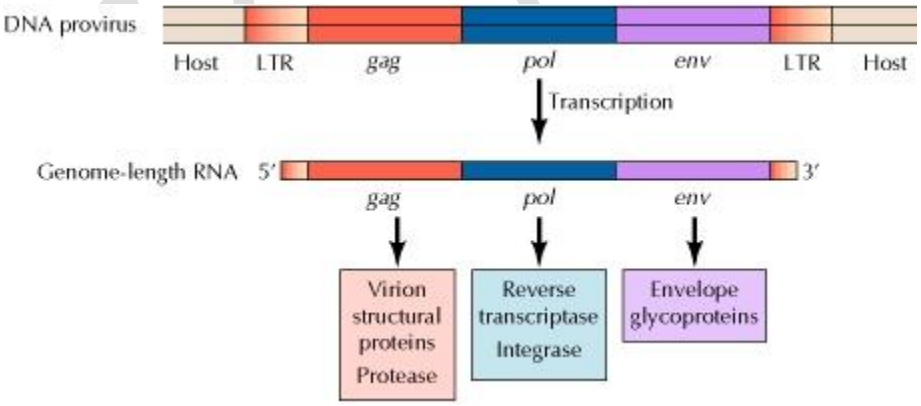
The **herpesviruses** are among the most complex animal viruses, with genomes of 100 to 200 kb. Several herpesviruses induce tumors in animal species, including frogs, chickens, and monkeys. In addition, two members of the herpesvirus family, **Kaposi's sarcoma-associated herpesvirus** and **Epstein-Barr virus**, are associated with human cancers. Kaposi's sarcoma-associated herpesvirus plays a critical role in the development of Kaposi's sarcomas, and Epstein-Barr virus has been implicated in several human malignancies, including Burkitt's lymphomain some regions of Africa, B-cell lymphomas in AIDS patients and other immunosuppressed individuals, and nasopharyngeal carcinoma in China.

In addition to its association with these human malignancies, Epstein-Barr virus is able to transform human B lymphocytes in culture. Partly because of the complexity of the genome, however, the molecular biology of Epstein-Barr virus replication and transformation remains to be fully understood. Several viral genes required to induce transformation of lymphocytes have been identified, but their functions have not been established.

Retroviruses

Members of one family of RNA viruses, the **retroviruses**, cause cancer in a variety of animal species, including humans. One human retrovirus, human T-cell lymphotropic virus type I (HTLV-I), is the causative agent of adult T-cell leukemia, which is common in parts of Japan, the Caribbean, and Africa. Transformation of T lymphocytes by HTLV-I results from expression of the viral gene *tax*, which encodes a regulatory protein affecting expression of several cellular growth control genes. AIDS is caused by another retrovirus, HIV. In contrast to HTLV-I, HIV does not cause cancer by directly converting a normal cell into a tumor cell. However, AIDS patients suffer a high incidence of some malignancies, particularly lymphomas and Kaposi's sarcoma. These cancers, which are also common among other immunosuppressed individuals, apparently develop as a secondary consequence of immunosuppression in AIDS patients.

Different retroviruses differ substantially in their oncogenic potential. Most retroviruses contain only three genes (*gag*, *pol*, and *env*) that are required for virus replication but play no role in cell transformation. Retroviruses of this type induce tumors only rarely, if at all, as a consequence of mutations resulting from the integration of proviral DNA within or adjacent to cellular genes.



A typical retrovirus genome

The DNA provirus, integrated into cellular DNA, is transcribed to yield genome-length RNA. This primary transcript serves as the genomic RNA for progeny virus particles, and as mRNA for the *gag* and *pol* genes. In addition, the full-length RNA is spliced to yield mRNA for *env*. The *gag* gene encodes the viral protease and structural proteins of the virus particle, *pol* encodes reverse transcriptase and integrase, and *env* encodes envelope glycoproteins.

Other retroviruses, however, contain specific genes responsible for induction of cell transformation and are potent carcinogens. The prototype of these highly oncogenic retroviruses is **Rous sarcoma virus (RSV)**, first isolated from a chicken sarcoma by Peyton Rous in 1911. More than 50 years later, studies of RSV led to identification of the first viral oncogene, which has provided a model for understanding many aspects of tumor development at the molecular level.

Oncogenes

Proto-oncogenes are genes that normally help cells grow. When a proto-oncogene mutates (changes) or there are too many copies of it, it becomes a "bad" gene that can become permanently turned on or activated when it is not supposed to be. When this happens, the cell grows out of control, which can lead to cancer. This bad gene is called an oncogene.

It may be helpful to think of a cell as a car. For it to work properly, there need to be ways to control how fast it goes. A proto-oncogene normally functions in a way that is much like a gas pedal. It helps the cell grow and divide. An oncogene could be compared with a gas pedal that is stuck down, which causes the cell to divide out of control.

A few cancer syndromes are caused by inherited mutations of proto-oncogenes that cause the oncogene to be turned on (activated). But most cancer-causing mutations involving oncogenes are acquired, not inherited. They generally activate oncogenes by:

- Chromosome rearrangements: Changes in chromosomes that put one gene next to another, which allows one gene to activate the other
- Gene duplication: Having extra copies of a gene, which can lead to it making too much of a certain protein

Tumor suppressor genes

Tumor suppressor genes are normal genes that slow down cell division, repair DNA mistakes, or tell cells when to die (a process known as *apoptosis* or *programmed cell death*). When tumor suppressor genes don't work properly, cells can grow out of control, which can lead to cancer.

A tumor suppressor gene is like the brake pedal on a car. It normally keeps the cell from dividing too quickly, just as a brake keeps a car from going too fast. When something goes wrong with the gene, such as a mutation, cell division can get out of control.

An important difference between oncogenes and tumor suppressor genes is that oncogenes result from the *activation* (turning on) of proto-oncogenes, but tumor suppressor genes cause cancer when they are *inactivated* (turned off).

Inherited abnormalities of tumor suppressor genes have been found in some family cancer syndromes. They cause certain types of cancer to run in families. But most tumor suppressor gene mutations are acquired, not inherited.

For example, abnormalities of the *TP53* gene (which codes for the p53 protein) have been found in more than half of human cancers. Acquired mutations of this gene appear in a wide range of cancers.

Carcinogenesis

A carcinogen is any substance, radionuclide, or radiation that promotes carcinogenesis, the formation of cancer. This may be due to the ability to damage the genome or to the disruption of cellular metabolic processes. Several radioactive substances are considered carcinogens, but their carcinogenic activity is attributed to the radiation, for example gamma rays and alpha particles, which they emit. Common examples of non-radioactive carcinogens are inhaled asbestos, certain dioxins, and tobacco smoke. Although the public generally associates carcinogenicity with synthetic chemicals, it is equally likely to arise in both natural and synthetic substances. Carcinogens are not necessarily immediately toxic; thus, their effect can be insidious.

Cancer is any disease in which normal cells are damaged and do not undergo programmed cell death as fast as they divide via mitosis. Carcinogens may increase the risk of cancer by altering cellular metabolism or damaging DNA directly in cells, which interferes with biological processes, and induces the uncontrolled, malignant division, ultimately leading to the formation of tumors. Usually, severe DNA damage leads to programmed cell death, but if the programmed cell death pathway is damaged, then the cell cannot prevent itself from becoming a cancer cell.

There are many natural carcinogens. Aflatoxin B1, which is produced by the fungus Aspergillus flavus growing on stored grains, nuts and peanut butter, is an example of a potent, naturally occurring microbial carcinogen. Certain viruses such as hepatitis B and human papilloma virus have been found to cause cancer in humans. The first one shown to cause cancer in animals is Rous sarcoma virus, discovered in 1910 by Peyton Rous. Other infectious organisms which cause cancer in humans include some bacteria (e.g. Helicobacter pylori) and helminths (e.g. Opisthorchis viverrini and Clonorchis sinensis.

Dioxins and dioxin-like compounds, benzene, kepone, EDB, and asbestos have all been classified as carcinogenic. As far back as the 1930s, Industrial smoke and tobacco smoke were identified as sources of dozens of carcinogens, including benzo[a]pyrene, tobacco-specific nitrosamines such as nitrosonornicotine, and reactive aldehydes such as formaldehyde, which is also a hazard in embalming and making plastics. Vinyl chloride, from which PVC is manufactured, is a carcinogen and thus a hazard in PVC production.

Co-carcinogens are chemicals that do not necessarily cause cancer on their own, but promote the activity of other carcinogens in causing cancer.

After the carcinogen enters the body, the body makes an attempt to eliminate it through a process called biotransformation. The purpose of these reactions is to make the carcinogen more water-soluble so that it can be removed from the body. However, in some cases, these reactions can also convert a less toxic carcinogen into a more toxic carcinogen.

DNA is nucleophilic; therefore, soluble carbon electrophiles are carcinogenic, because DNA attacks them. For example, some alkenes are toxicated by human enzymes to produce an electrophilic epoxide. DNA attacks the epoxide, and is bound permanently to it. This is the mechanism behind the carcinogenicity of benzo[a]pyrene in tobacco smoke, other aromatics, aflatoxin and mustard gas.

Radiation

CERCLA identifies all radionuclides as carcinogens, although the nature of the emitted radiation (alpha, beta, gamma, or neutron and the radioactive strength), its consequent capacity to cause ionization in tissues, and the magnitude of radiation exposure, determine the potential hazard. Carcinogenicity of radiation depends on the type of radiation, type of exposure, and penetration. For example, alpha radiation has low penetration and is not a hazard outside the body, but emitters are carcinogenic when inhaled or ingested. For example, Thorotrast, a (incidentally radioactive) suspension previously used as a contrast medium in x-ray diagnostics, is a potent

human carcinogen known because of its retention within various organs and persistent emission of alpha particles. Low-level ionizing radiation may induce irreparable DNA damage (leading to replicational and transcriptional errors needed for neoplasia or may trigger viral interactions) leading to pre-mature aging and cancer.

Not all types of electromagnetic radiation are carcinogenic. Low-energy waves on the electromagnetic spectrum including radio waves, microwaves, infrared radiation and visible light are thought not to be, because they have insufficient energy to break chemical bonds. Evidence for carcinogenic effects of non-ionizing radiation is generally inconclusive, though there are some documented cases of radar technicians with prolonged high exposure experiencing significantly higher cancer incidence.

Higher-energy radiation, including ultraviolet radiation (present in sunlight), x-rays, and gamma radiation, generally is carcinogenic, if received in sufficient doses. For most people, an ultraviolet radiation from sunlight is the most common cause of skin cancer. In Australia, where people with pale skin are often exposed to strong sunlight, melanoma is the most common cancer diagnosed in people aged 15–44 years.

Substances or foods irradiated with electrons or electromagnetic radiation (such as microwave, X-ray or gamma) are not carcinogenic. In contrast, non-electromagnetic neutron radiation produced inside nuclear reactors can produce secondary radiation through nuclear transmutation.

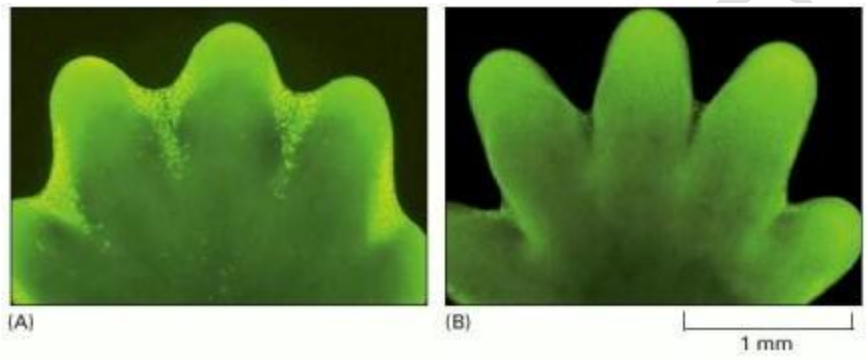
Programmed Cell Death (Apoptosis)

The cells of a multicellular organism are members of a highly organized community. The number of cells in this community is tightly regulated—not simply by controlling the rate of cell division, but also by controlling the rate of cell death. If cells are no longer needed, they commit suicide by activating an intracellular death program. This process is therefore called programmed cell death, although it is more commonly called apoptosis (from a Greek word meaning "falling off," as leaves from a tree).

The amount of apoptosis that occurs in developing and adult animal tissues can be astonishing. In the developing vertebrate nervous system, for example, up to half or more of the nerve cells normally die soon after they are formed. In a healthy adult human, billions of cells die in the bone marrow and intestine every hour. It seems remarkably wasteful for so many cells to die, especially as the vast majority is perfectly healthy at the time they kill themselves.

In some cases, the answers are clear. Mouse paws, for example, are sculpted by cell death during embryonic development: they start out as spadelike structures, and the individual digits

separate only as the cells between them die. In other cases, cells die when the structure they form is no longer needed. When a tadpole changes into a frog, the cells in the tail die, and the tail, which is not needed in the frog, disappears. In many other cases, cell death helps regulate cell numbers. In the developing nervous system, for example, cell death adjusts the number of nerve cells to match the number of target cells that require innervation. In all these cases, the cells die by apoptosis.



Sculpting the digits in the developing mouse paw by apoptosis

(A) The paw in this mouse embryo has been stained with a dye that specifically labels cells that have undergone apoptosis. The apoptotic cells appear as *bright green* dots between the developing digits. (B) This interdigital cell death eliminates the tissue between the developing digits, as seen one day later, when few, if any, apoptotic cells can be seen.

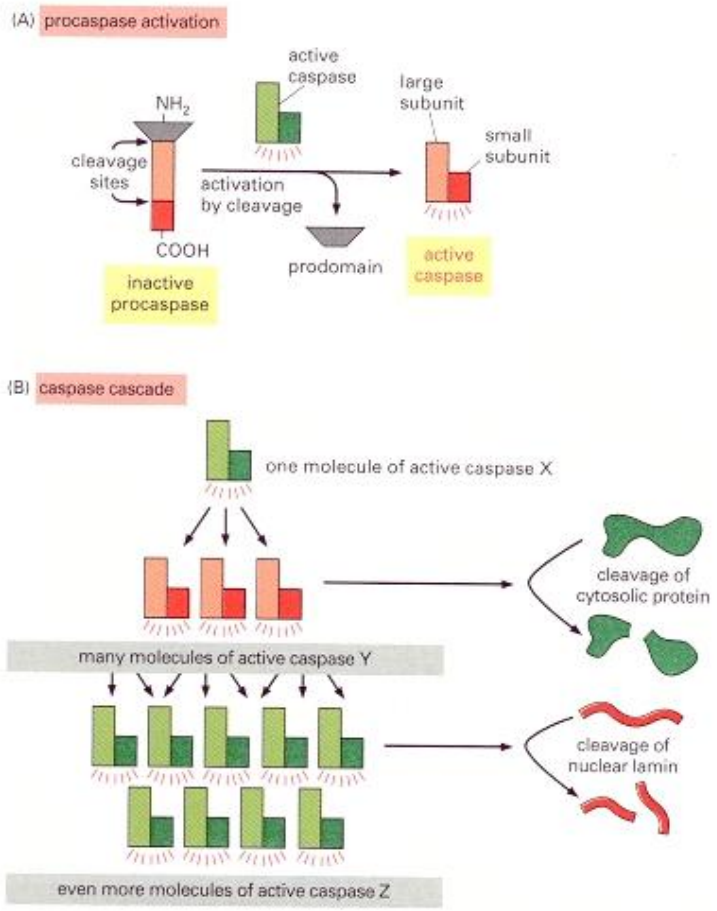
In adult tissues, cell death exactly balances cell division. If this were not so, the tissue would grow or shrink. If part of the liver is removed in an adult rat, for example, liver cell proliferation increases to make up the loss. Conversely, if a rat is treated with the drug phenobarbital—which stimulates liver cell division (and thereby liver enlargement)—and then the phenobarbital treatment is stopped, apoptosis in the liver greatly increases until the liver has returned to its original size, usually within a week or so. Thus, the liver is kept at a constant size through the regulation of both the cell death rate and the cell birth rate.

In this short section, we describe the molecular mechanisms of apoptosis and its control. In the final section, we consider how the extracellular control of cell proliferation and cell death contributes to the regulation of cell numbers in multicellular organisms.

Apoptosis Is Mediated by an Intracellular Proteolytic Cascade

Cells that die as a result of acute injury typically swell and burst. They spill their contents all over their neighbors—a process called cell necrosis—causing a potentially damaging inflammatory response. By contrast, a cell that undergoes apoptosis dies neatly, without damaging its neighbors. The cell shrinks and condenses. The cytoskeleton collapses, the nuclear envelope disassembles, and the nuclear DNA breaks up into fragments. Most importantly, the cell surface is altered, displaying properties that cause the dying cell to be rapidly phagocytosed, either by a neighboring cell or by a macrophage, before any leakage of its contents occurs. This not only avoids the damaging consequences of cell necrosis but also allows the organic components of the dead cell to be recycled by the cell that ingests it.

The intracellular machinery responsible for apoptosis seems to be similar in all animal cells. This machinery depends on a family of proteases that have a cysteine at their active site and cleave their target proteins at specific aspartic acids. They are therefore called caspases. Caspases are synthesized in the cell as inactive precursors, or procaspases, which are usually activated by cleavage at aspartic acids by other caspases. Once activated, caspases cleave, and thereby activate, other procaspases, resulting in an amplifying proteolytic cascade. Some of the activated caspases then cleave other key proteins in the cell. Some cleave the nuclear lamins, for example, causing the irreversible breakdown of the nuclear lamina; another cleaves a protein that normally holds a DNA-degrading enzyme (a DNAse) in an inactive form, freeing the DNAse to cut up the DNA in the cell nucleus. In this way, the cell dismantles itself quickly and neatly, and its corpse is rapidly taken up and digested by another cell.



The caspase cascade involved in apoptosis

(A) Each suicide protease is made as an inactive proenzyme (procaspase), which is usually activated by proteolytic cleavage by another member of the caspase family. As indicated, two of the cleaved fragments associate to form the active site of the caspase. The active enzyme is thought to be a tetramer of two of these units (not shown). (B) Each activated caspase molecule can cleave many procaspase molecules, thereby activating them, and these can then activate even more procaspase molecules. In this way, an initial activation of a small number of procaspase molecules (called initiator caspases) can lead, via an amplifying chain reaction (a cascade), to the explosive activation of a large number of procaspase molecules. Some of the activated caspases (called effector caspases) then cleave a number of key proteins in the cell, including specific cytosolic proteins and nuclear lamins, leading to the controlled death of the cell.

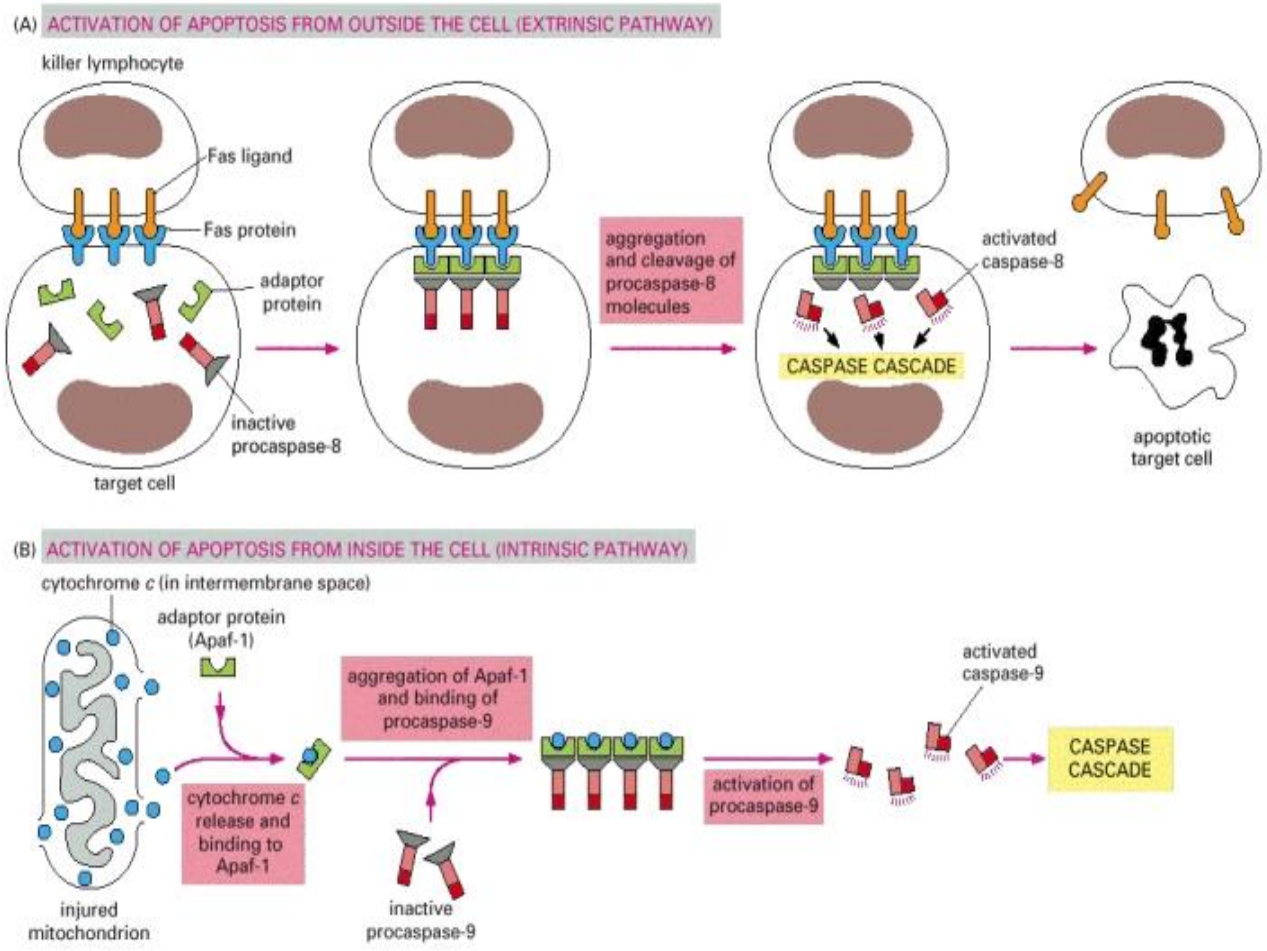
Activation of the intracellular cell death pathway, like entry into a new stage of the cell cycle, is usually triggered in a complete, all-or-none fashion. The protease cascade is not only destructive and self-amplifying but also irreversible, so that once a cell reaches a critical point along the path to destruction, it cannot turn back.

Procaspases Are Activated by Binding to Adaptor Proteins

All nucleated animal cells contain the seeds of their own destruction, in the form of various inactive procaspases that lie waiting for a signal to destroy the cell. It is therefore not surprising that caspase activity is tightly regulated inside the cell to ensure that the death program is held in check until needed.

A general principle is that the activation is triggered by adaptor proteins that bring multiple copies of specific procaspases, known as initiator procaspases, close together in a complex or aggregate. In some cases, the initiator procaspases have a small amount of protease activity, and forcing them together into a complex causes them to cleave each other, triggering their mutual activation. In other cases, the aggregation is thought to cause a conformational change that activates the procaspase. Within moments, the activated caspase at the top of the cascade cleaves downstream procaspases to amplify the death signal and spread it throughout the cell.

Procaspase activation can be triggered from outside the cell by the activation of death receptors on the cell surface. Killer lymphocytes, for example, can induce apoptosis by producing a protein called Fas ligand, which binds to the death receptor protein Fas on the surface of the target cell. The clustered Fas proteins then recruit intracellular adaptor proteins that bind and aggregate procaspase-8 molecules, which cleave and activate one another. The activated caspase-8 molecules then activate downstream procaspases to induce apoptosis. Some stressed or damaged cells kill themselves by producing both the Fas ligand and the Fas protein, thereby triggering an intracellular caspase cascade.



Induction of apoptosis by either extracellular or intracellular stimuli

(A) Extracellular activation. A killer lymphocyte carrying the Fas ligand binds and activates Fas proteins on the surface of the target cell. Adaptor proteins bind to the intracellular region of aggregated Fas proteins, causing the aggregation of procaspase-8 molecules. These then cleave one another to initiate the caspase cascade. (B) Intracellular activation. Mitochondria release cytochrome c, which binds to and causes the aggregation of the adaptor protein Apaf-1. Apaf-1 binds and aggregates procaspase-9 molecules, which leads to the cleavage of these molecules and the triggering of a caspase cascade. Other proteins that contribute to apoptosis are also released from the mitochondrial intermembrane space.

POSSIBLE QUESTIONS

2 marks

1. Define cell cycle.
2. List the properties of tumor cells.
3. Define onset of cancer.
4. Define tumor suppressor genes.
5. Write a note on carcinogenic effect of radiation.
6. Define apoptosis.
7. Define necrosis.
8. Give an account on effectors of apoptosis.

6 marks

1. Describe cell cycle and its control in mammalian cells.
2. Discuss about checkpoints in cell cycle regulation.
3. Explain the properties of cancer cells and its genetic basis.
4. Illustrate in detail about the role of DNA and RNA as transforming agents and its mechanism.
5. Give an account on carcinogenic and anti carcinogenic effect of chemicals and radiation.
6. Brief note on apoptosis.
7. Explain in detail about the regulators and effectors on apoptosis and necrosis

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MULTIPLE CHOICE QUESTIONS

UNIT V

S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	In which phase of cell cycle does chromosome replicate----------	M phase	G1 phase	G2 phase	S phase	S phase
2	The acronym MPF stands for ----------	Mitosis promoting factor	Meiosis promoting factor	Mitosis progressing frequencies	None of the above	Mitosis promoting factor
3	The critical cyclin which is abruptly destroyed at anaphase in early Sea urchin embryos	Cyclin A	Cyclin B	Cyclin D	Cyclin E	Cyclin B
4	Polyubiquitinated proteins are degraded rapidly by multi protein complexes of proteolytic enzymes called------	Lysosomes	Mesosome	Lyosomes	Proteosomes	Proteosomes
5	*S.cervisiae* cells replicate by ----------	Budding	Sporulation	Binary fusion	Conjugation	Budding
6	In which cell cycle phase of action is the association of CDK2 and cyclin D happens	G1	G1-S transition	G2-M transition	S phase	G1
7	Down syndrome is trisomy of chromosome number	2	12	22	21	21
8	Aphidicolin is a specific inhibitor of ----------	DNA polymerase	DNA polymerase	DNA polymerase α	Reverse	DNA polymerase a
9	Which among the following is a microtubule inhibitor?	Tamiflu	Colichicine	Aphidicolin	none	Colichicine
10	DNA is damaged by irradiations or by chemical modifications arrested in ---	G1 phase	G2 phase	S phase	both a and b	both a and b
11	DNA double stranded breaks are repaired when the cell cycle is arrested in --	S phase	M phase	G1 phase	G2 phase	G2 phase

12	Tumors become life threatening if they spread through out the body. Such tumors are called----	Malignant	benign	none	both a and b	Malignant
13	The spread of tumour cells and establishment of secondary areas of growth is called—	Apoptosis	Metástasis	Homeostasis	Erythropoiesis	Metástasis
14	Malignant tumors derived from endoderm or ectoderm is called----	Carcinoma	Sarcoma	Lymphoma	Leucemia	Carcinoma
15	Sarcoma is a tumour derived from ----	Endoderm	mesoderm	Ectoderm	none of the above	mesoderm
16	Massive proliferation of WBC can cause a patients blood to appear milky.	Carcinoma	Lymphoma	Sarcoma	Leucemia	Leucemia
17	The proteins secreted by transformed cells that can stimulate the growth of normal cells	TGF α	TGF β	Both a and b	TGF γ	Both a and b
18	The protease secreted by transformed cells which cleave a peptide bond in plasminogen converting them to plasmin	Plasminogen activator	Fibrinogen activator	TGF	TNF	Plasminogen activator
19	The scientific study of tumours is called	Oncology	Tumour	Cell biology	none of the above	Oncology
20	Cellular genes known to be progenitors of oncogenes are called	Proto oncogenes	Preproto oncogenes	Meta oncogenes	both b and c	Proto oncogenes
21	RB and P53 are prototypes of a class of proteins encoded by	Oncogenes	proto oncogenes	Ras genes	Tumour suppressor genes	Tumour suppressor genes
22	Philadelphia chromosome is --------- translocation	12-Sep	21-22	12-Oct	7-Jan	12-Sep
23	In Burkett’s lymphoma antibody genes are translocated to chromosome------ just at the site of myc gene	9	10	8	22	8
24	Retro viruses are --------	RNA viruses	DNA viruses	Papova virus	none	RNA viruses
25	Tumour viruses are usually -------	RNA viruses	DNA viruses	both	none of the above	both
26	An enzyme which is capable of copying genomic RNA into DNA	DNA α	DNA β	Reverse transcriptase	DNA δ	Reverse transcriptase
27	The Herpes like DNA virus which plays an important role in Burkett’s lymphoma and nasopharyngeal carcinoma	HIV	Epstein Barr virus	Hepatitis B virus	Papiloma virus	Epstein Barr virus
28	Kaposi sarcoma is a tumour of -------- origin	Mesothelial	ectothelial	endothelial	epithelial	endothelial

29	Which of the following tests shows if a compound has the potential to be carcinogenic?	ELISA	RIA	PGEF	AMES	AMES
30	Which of the following genes is involved in DNA REPAIR?	*rec* A	c1p1	*mdm* 2	*src*	*rec* A
31	In which phase of mitosis does chromosome condense	Prophase	metaphase	anaphase	telophase	Prophase
32	In which phase of mitosis does sister chromatins segregated to the opposite poles of mitotic spindle	Prophase	metaphase	anaphase	telophase	anaphase
33	Which of the following cells in multi cellular organisms exist from the cell cycle and does not proliferate in life time?	Skin	nerve	bone	none	nerve
34	Attachment of which protein marks a cell or a protein for degradation	Lyase	ubiquitin	RNase	protease	ubiquitin
35	Nuclear pore complexes which are broken down in to sub pore complexes Nucleomere	Nucleomere	karyomere	cytomere	cytosol	karyomere
36	MPF catalytic subunit is encoded by -------- gene in *S. pombe.*	cdc2+	cdc2D	cdc2-	cdc30	cdc2+
37	Phosphorylation of which amino acid in cdc2 subunits activates MPF.	tyrosine 15	tyrosine 156	tyrosine 161	tyrosine 140	tyrosine 161
38 39	Phosphorylation of which amino acid residue in cdc2 inactivates MPF. tyrosine 15	tyrosine 15	tyrosine 156	tyrosine 161	tyrosine 140	tyrosine 15
40	Protein kinase that phosphorylates the inhibitory tyrosine 15 in cdc2 subunit.	cdc25	CAK	Wee 1^{-}	Wee 1+	Wee 1+
41	------- cells have the ability to metastasis	Normal cells	benign cells	malignant cells	none	malignant cells
42	Characteristic of a tumour cell	Invasiveness and spreading	lack of normal control on cell growth	Alterations in cell to cell interactions	all the above	all the above
43	Malignant tumours are classified as -------- if they derive from endoderm or ectoderm	Sarcoma	carcinoma	lymphoma	none	carcinoma

44	Malignant tumours are classified as -------- if they derive from mesoderm	Sarcoma	carcinoma	lymphoma	none	Sarcoma
45	SV 40 and polyoma are example for-----	Retro virus	papova virus	RNA virus	none	papova virus
46	Controlled cell death is called---------	Apoptosis	metastasis	autophagy	none	Apoptosis
47	The src enzyme is -------- specific protein kinase	Tyrosine	serine	tryptophan	praline	Tyrosine
48	The chromosome which is translocated in all cases of Burkett's lymphoma is	8	9	10	11	8
49	In chronic myelogenous leukemia proto-oncogene *abl transferred* from which chromosome to which chromosome?	8-22	8-14	9-22	9-14	9-22
50	Polyoma gene a and SV40 gene A encode for ------	Protein kinase	T antigen	Ras protein	none	T antigen
51	The migration of tumour cells through lymphatic and blood vessels to distant normal organs to establish secondary tumour masses is called	Neoplasm	metastasis	neuroblastoma	none	metastasis
52	Neuroblastoma is one of the common forms of malignancies in children arising from	Adrenal cortex	adrenal medulla	neurons	fibroblasts	adrenal medulla
53	Solid malignant tumors of lymphoid tissues is called	Carcinoma	sarcoma	leukemia	lymphoma	lymphoma
54	_____is an oncofetal antigen normally synthesized and secreted by fetal liver and yolk sac cells	Alpha fetoprotein	carcinoembryonic antigen	oncofetal antigens	TATA	Alpha fetoprotein
55	_____ is a membrane glycoprotein found on gastrointestinal and liver cells of 2-6month old fetuses	Alpha fetoprotein	carcinoembryonic antigen	oncofetal antigens	TATA	carcinoembryonic antigen
56	Chediak higashi syndrome gives an insight to the importance of _____ cells in tumor immunity	Interferon	TGF	PGDF	TNF α	PGDF
57	Name the tumor specific antigen peptides recognized by human T cells in case of prostrate cancer	PSA	CEA	NAGE 1	HER 2	PSA
58	Papiloma virus causes _____ cancer	Liver	cervical	pancreas	breast	cervical

59	HER 2 is recognized by human T cells in cases of _____ cancer	Breast	ovary	prostrate	both a & b	both a & b
60	Activated macrophages secrete a cytokine called _____ that has antitumor activity	Interferon	TGF	PDGF	TNFα	TNFα