KARPAGAM ACADEMY OF HIGHER EDUCATION

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

SUBJECT : CELL BIOLOGY
SEMESTER : I
SUBJECT CODE : 17BCU102 CLASS : I B.Sc.(BC)

Programme Objective: To understand the basic structure and functions of components of prokaryotic and eukaryotic cells especially macromolecules, membranes and organelles and to understand the cell cycle and cell division in depth

Programme learning outcome:
The students after completion of this course will have

- Clear understanding of types of cells and tools used to study cell biology.
- The students can get clear understanding of cell organelles and their functions.
- The students will have a clear knowledge on protein targeting to various organelles.
- The students can have a clear understanding on cytoskeleton proteins.
- Understanding of cell cycle, cell division and transformed cells will kindle the young minds to analyze and apply in future studies.

Unit 1
Introduction to cell biology: Prokaryotic (*archaea and eubacteria*) and eukaryotic cell (animal and plant cells), cells as experimental models.
Plasma membrane: Composition, Fluid mosaic model
Tools of cell biology: Light microscopy, phase contrast microscopy, fluorescence microscopy, confocal microscopy, electron microscopy, FACS. Centrifugation for sub-cellular fractionation.

Unit 2
Structure of different cell organelles
Structure of nuclear envelope, nuclear pore complex. Selective transport of proteins to and from the nucleus. Regulation of nuclear protein import and export.
ER structure. Targeting proteins to ER, smooth ER and lipid synthesis. Export of proteins and lipids from ER and into ER. Protein folding in ER
Peroxisomes and Zellweger syndrome.

Unit 3
Protein trafficking

Organization of Golgi. Lipid and polysaccharide metabolism in Golgi. Protein sorting and export from Golgi. N and O-linked glycosylation.
Lysosome. – Acid hydrolases, phagocytosis and autophagy.
Mitochondria-Structure and functions, protein import and mitochondrial assembly, protein export from mitochondrial matrix.
Chloroplasts- Import and sorting of chloroplast proteins.

Unit 4
Cytoskeletal proteins : Structure and organization of actin filaments. Treadmilling and role of ATP in microfilament polymerization, organization of actin filaments. Non-muscle myosin. Intermediate filament proteins, assembly and intracellular organization. Assembly, organization and movement of cilia and flagella.

Unit 5
Cell wall and extracellular matrix :Prokaryotic and eukaryotic cell wall, cell matrix proteins. Cell-matrix interactions and cell-cell interactions. Adherence junctions, tight junctions, gap junctions, desmosomes, hemidesmosomes, focal adhesions and plasmodesmata.
Cell cycle, cell death and cell renewal : Eukaryotic cell cycle, restriction point, and checkpoints. Cell division. Apoptosis and necrosis - brief outline. Salient features of a transformed cell.

TEXT BOOKS

- Paul, A., (2007). Text Book of Cell and Molecular Biology,1st edition. Books and Allied (P) Ltd, Kolkata.
- Verma, P.S., and Agarwal, V.K., (2005). Cell Biology Molecular Biology and Genetics, VII Edition, S.Chand and company Ltd, New Delhi.
- Shukla, R.M., (2013). A textbook of Cell Biology, Dominant Publishers and Distributors.

REFERENCE BOOKS

- Lodish, H., Berk, A., Kaiser, C.A., and Krieger, M., (2012). Molecular Cell Biology, 7th edition. W.H. Freeman & Company, London.
- Garret, R. H. and Grisham, C.M., Biochemistry (2010) 4th ed., Cengage Learning (Boston), ISBN-13: 978-0-495-11464-2.
- Cooper, G.M., and Hausman, R.E., (2013). Cell-A Molecular Approach, 6th Edition.. Sinauer Associates. USA

- Karp, G., (2013). Cell and Molecular Biology, 7th edition. John Wiley and Sons, Inc, Hoboken, United States.

- Alberts, B., Johnson,A., Lewis, J., and Enlarge, M., Molecular Biology of the Cell (2008) 5th ed., Garland Science (Princeton), ISBN:

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LECTURE PLAN

S.No	Duration of period	Topics covered	Books referred	Page No	Web page referred
Unit I: Introduction and tools of cell biology					
1	1	Introduction to cell biology and Prokaryotic (*Archaea and Eubacteria)* cell	R1 R2 T1	3-8 8-12 2-3	
2	1	Eukaryotic cell (Animal and plant cells)	R1 R2 T1	9-15 3-5 12-15	
3	1	Cells as experimental models.	R1 R2 T1	15-19 120-122 180-181	
4	1	Plasma membrane: Composition, Fluid mosaic model	R1 R2 T1	467-476 716180- 181-717	
5	1	Light microscopy	R1 R2 T1	20-21 717-718 181-183	
6	1	Phase contrast microscopy	R1	20-21	
7	1	fluorescence microscopy	R1 R2 T1	22-23 718-719 181-183	
8	1	Confocal microscopy	R1 R2 T1	22-23 720-723 183-184	
9	1	Electron microscopy, FACS	R1 R2 T1	23-30 723-729 183-184	
10	1	Centrifugation for sub-cellular fractionation.	R1	23-30	

			T1	176-178	
11	1	Revision and Possible questions discussion			
Total no of hours planned for unit I: 11					
Unit II: Structure of different cell organelles					
1	1	Structure of nuclear envelope, nuclear pore complex	R1 T1	315-320 502-505	
2	1	Nuclear pore complex	R1 T2	315-320 502-505	
3	1	Selective transport of proteins to and from the nucleus.	R1 T2	321-325 505-509	
4	1	Regulation of nuclear protein import and export.	R1 T2	321-325 509-512	
5	1	ER structure. Targeting proteins to ER	R1 R2	347-351 273-275	
6	1	Smooth ER and lipid synthesis	R1 R2	359-363 273-275	
7	1	Export of proteins and lipids from ER	R1 R2	352-357 363-365	
8	1	Export of proteins and lipids into ER.	R1 R2	352-357 275-278	
9	1	Protein folding in ER	R1 R2	363-365 275-278	
10	1	Peroxisomes and zellweger syndrome	R1 R2	415-418 201-205	
11	1	Revision and possible questions discussion			
Total no of hours planned for unit II: 11					
		Unit III: Protein trafficking			
1	1	Organization of Golgi	R1 R2	365-366 369-370 284-285	
2	1	Lipid and polysaccharide metabolism in Golgi.	R1 R2	365-366 369-370 284-287	
3	1	Protein sorting and export from Golgi.	R1 R2	370-372 284-287	
4	1	N and O linked glycosylation	R1 R2	370-372 284-287	
5	1	Lysosome. – Acid hydrolases	R1 R2	379-384 291-293	
6	1	Phagocytosis and autophagy	R1 R2	379-384 174-176	
7	1	Mitochondria-Structure and functions	R1 R2	369-390 174-176	
8	1	Protein import and mitochondrial assembly	R1 R2	392-397 175-176	

9	1	Protein export from mitochondrial matrix	R1 R2	392-397 174-176	
10	1	Chloroplast: Import and sorting of chloroplast proteins.	R1 R2	407-409 206-227	
11	1	Revision and Possible questions discussion of unit 3			
Total no of hours planned for unit III: 11					
		Unit IV: Cytoskeletal proteins			
1	1	Structure of actin filaments	R1 R2	423-428 319-321	
2	1	Organization of actin filaments	R1 R2	423-428 319-321	
3	1	Treadmilling	R1 R2	425-426 321-325	
4	1	Role of ATP in microfilament polymerization	R1 R2	425-426 325-328	
5	1	Organization of actin filaments	R1 R2	425-426 325-328	
6	1	Non-muscle myosin.	R1 R2	435-437 260-262	
7	1	Intermediate filament proteins	R1 R2	442-447 560-565	
8	1	Assembly and intracellular organization of intermediate filament protein	R1 R2	442-447 565-569	
9	1	Assembly, organization and movement of cilia.	R1 R2	447-449 569-572	
10	1	Assembly, organization and movement of flagella.	R1 R2	460-462 572-576	
11	1	Revision and Possible questions discussion of unit 4			
Total no of hours planned for unit IV: 11					
		Unit V: Cell wall and extracellular matrix			
1	1	Prokaryotic cell wall	R1 R2	500-504 260-261	
2	1	Eukaryotic cell wall	R1 R2	500-504 262-263	
3	1	Cell matrix proteins. Cell-matrix interactions and cell-cell interactions.	R1 R2	504-509 264-265	
4	1	Adherence junctions	R1 R2	509-513 272-279	
5	1	Tight junctions, gap junctions,	R1 R2	509-513 272-279	
6	1	Desmosomes, hemidesmosomes	R1 R2	445-513 272-279	
7	1	Focal adhesions and plasmodesmata	R1 R2	445-513 272-279	

8	1	Eukaryotic cell cycle, restriction point, and checkpoints	R1 R2	561-583 560-576	
9	1	Cell division, Apoptosis and necrosis - brief outine	R1 R2	583-587 592-593 560-576	
10	1	Salient features of a transformed cell	R1 R2	604-607 560-576	
11	1	Revision and possible questions discussion of unit 5			
Total no of hours planned for unit V: 11					
		Previous year ESE question paper discussion			
1	1	Previous year question paper discussion			
2	1	Previous year question paper discussion			
3	1	Previous year question paper discussion			
4	1	Previous year question paper discussion			
5	1	Previous year question paper discussion			
Total no of hours required to complete the course: 60					

REFERENCE BOOKS

R1- Cooper 2000.Cell-A Molecular Approach,2nd Edition.ASM Press, Washington, United States.

R2- Karp, G., (2013). Cell and Molecular Biology, 7th edition. John Wiley and Sons, Inc, Hoboken, United States.

TEXT BOOKS

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

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UNIT I - COURSE MATERIAL

UNIT 1

Introduction to cell biology: Prokaryotic (*archaea and eubacteria*) and eukaryotic cell (animal and plant cells), cells as experimental models.

Plasma membrane: Composition, Fluid mosaic model

Tools of cell biology: Light microscopy, phase contrast microscopy, fluorescence microscopy, confocal microscopy, electron microscopy, FACS. Centrifugation for sub-cellular fractionation.

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UNIT I – INTRODUCTION AND TOOLS OF CELL BIOLOGY

Introduction to cell biology

The cell was first seen by Robert Hooke in 1665 using a primitive, compound microscope. The cell is smallest unit of a living system and fall in the microscopic range of 1 to 100 µm. They attain various shapes and sizes to attain variety of functions. The understanding of cell is necessary to understand the structure and function of a living organism. One of most important characteristics of cell is ability to divide. The observations of Hooke, Leeuwenhoek, Schleiden, Schwann, Virchow, and others led to the development of the cell theory.

The theory states:

- All living things are made of cells.
- Cells are the basic building units of life.
- 'New cells are created by old cells dividing into two.

Classification of cells:

All living organisms (bacteria, blue green algae, plants and animals) have cellular organization and may contain one or many cells. The organisms with only one cell in their body are called unicellular organisms (bacteria, blue green algae, some algae, Protozoa, etc.). The organisms having many cells in their body are called multicellular organisms (fungi, most plants and animals). Any living organism may contain only one type of cell either

A. Prokaryotic cells;

B. Eukaryotic cells.

PROKARYOTIC CELL

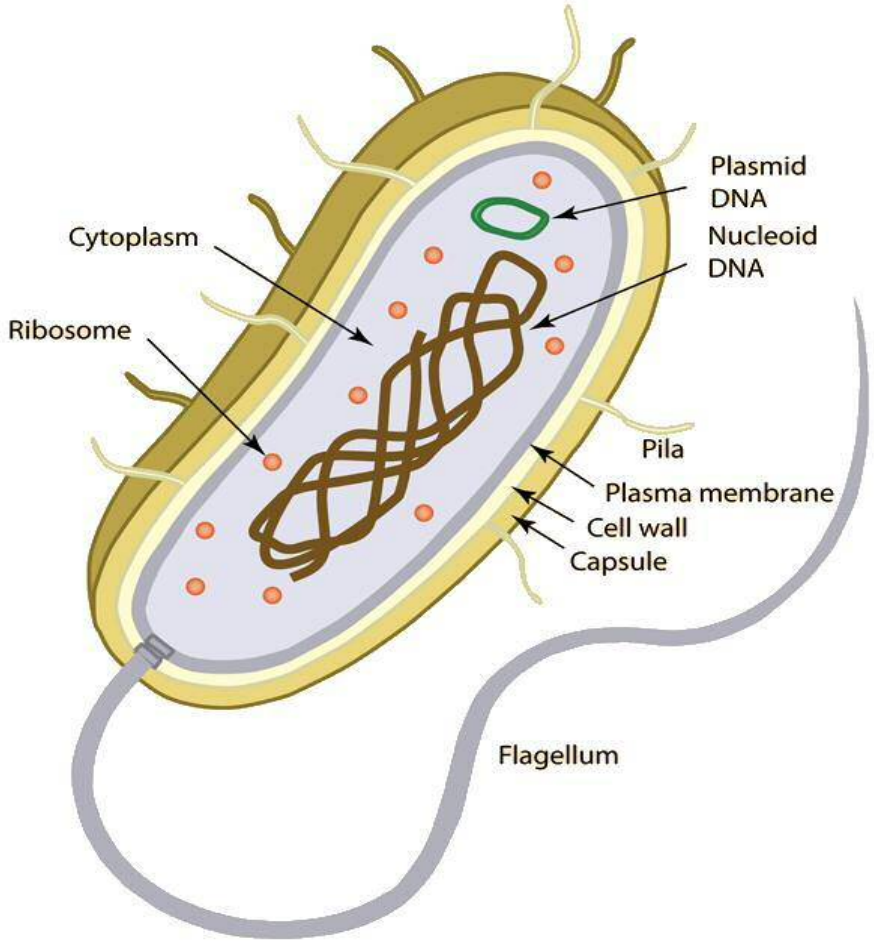
Prokaryote means before nucleus in Greek. They include all cells which lack nucleus and other membrane bound organelles. Mycoplasma, virus, bacteria and cyanobacteria or blue-green algae are prokaryotes. The other domain of the prokaryotes are archae bacteria that live in extreme environment.

The genetic information of prokaryotes is typically in nucleoid of DNA strands, but they have additional DNA in a circular loop called plasmid, Though prokaryotes lack cell organelles they harbor few internal structures, such as the cytoskeletons, ribosomes and vacuoles.

Most bacterial cells are spherical, rod–shaped, or spiral, with diameters of 1 to 10 μm but they can vary in size from 0.2 μm to 750 μm (*Thiomargarita namibiensis*). Their DNA contents range from about 0.6 million to 5 million base pairs, an amount sufficient to encode about 5000 different proteins. The largest and most complex prokaryotes are the cyanobacteria, bacteria in which photosynthesis evolved.

- Cocci –spherical
- Bacilli –rod–shaped
- Spirochaete –spiral–shaped
- Vibrio –comma–shaped

The structure of a typical prokaryotic cell is illustrated by *Escherichia coli* (E. coli) The cell is rod–shaped, about 1 μm in diameter and about 2 μm long. Like most other prokaryotes, *E. coli* is surrounded by a rigid cell wall composed of polysaccharides and peptides. Within the cell wall is the plasma membrane, which is a bilayer of phospholipids and associated proteins. The cell wall is porous and readily penetrated by a variety of molecules, where as the plasma membrane provides the functional separation between the inside of the cell and its external environment. The DNA of *E. coli* is a single circular molecule in the nucleoid, is not surrounded by a membrane separating it from the cytoplasm. The cytoplasm contains approximately 30,000 ribosomes (the sites of protein synthesis), which account for its granular appearance.



Cell wall: Cell wall is the outermost layer of most cells that protects the bacterial cell and gives it shape. One exception is Mycoplasma which lacks cell wall. Bacterial cell walls are made of peptidoglycan which is made from polysaccharide chains cross–linked by unusual peptides containing D–amino acids. Bacterial cell walls are different from the cell walls of plants and fungi which are made of cellulose and chitin, respectively. The cell wall of bacteria is also distinct from that of Archaea, which do not contain peptidoglycan.

The cell wall is essential to the survival of many bacteria. The antibiotic penicillin is able to kill bacteria by preventing the cross–linking of peptidoglycan and this causes the cell wall to weaken and lyse.

There are broadly speaking two different types of cell wall in bacteria, called Gram–positive and Gram–negative (Figure).

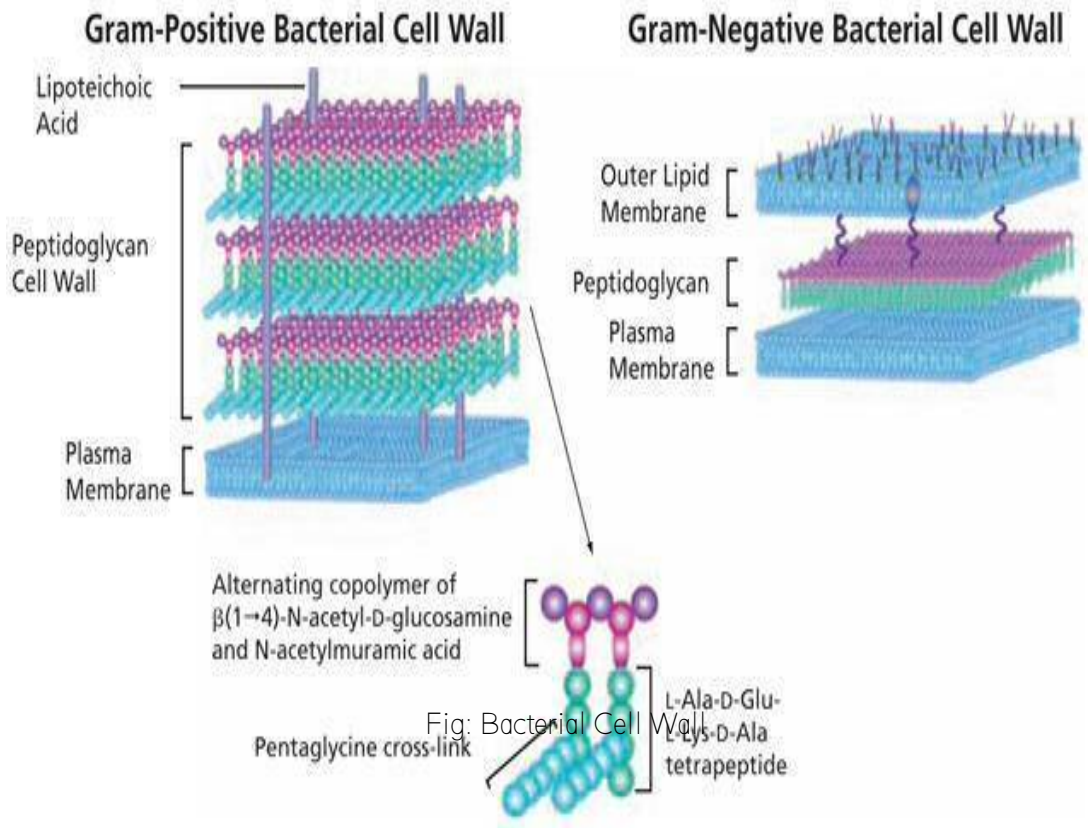


Fig: Bacterial Cell Wall

Plasma membrane: It is a bilayer of phospholipids and associated proteins. This semi–permeable membrane controls the substances moving into and out of the cell. Substances pass through by either active or passive transport.

Cytoplasm: Contains many enzymes used to catalyze chemical reactions of metabolism and it also contains the DNA in a region called the nucleoid. Ribosomes are also found in the cytoplasm.

Ribosomes: They are the site of protein synthesis. Contributes to protein synthesis by translating messenger RNA.

Nucleoid: Region containing naked DNA which stores the hereditary material (genetic information) that controls the cell and will be passed on to daughter cells.

Pili: A pilus is typically 6 to 7 nm in diameter. They help bacteria to adhere with each other for the exchange of genetic material.

Flagella : It is a long, whip–like protrusion found in most prokaryotes that aids in cellular locomotion. Besides its main function of locomotion it also often functions as a sensory organelle, being sensitive to chemicals and temperatures outside the cell..

Plasmids: They are extra chromosomal DNA and are double–stranded, circular structures. Their sizes vary from 1 to over 1,000 kbp. Functionally they carry genes that code for a wide range of metabolic activities, enabling their host bacteria to degrade pollutant compounds, and produce antibacterial proteins. They can also harbour genes for virulence that help to increase pathogenicity of bacteria causing diseases such as plague, dysentry, anthrax and tetanus. They are also responsible for the spread of antibiotic resistance genes that ultimately have an impact on the treatment of diseases. Plasmids are classified into the following types.

1. Fertility F–plasmids–These plasmids contain tra genes and are capable of conjugation.
2. Resistance (R) plasmids: They contain genes that can build a resistance against antibiotics or toxins and help bacteria produce pili.
3. Col plasmids: They contain genes that code for bacteriocins, proteins that can kill other bacteria.
4. Degradative plasmids: Degradative plasmids enable the metabolism of unusual substances, e.g. toluene and salicylic acid.
5. Virulence plasmids: These plasmids enable the bacterium to become pathogenic.

Capsule: The capsule is found in some bacterial cells, this additional outer covering protects the cell when it is engulfed by phagocytes and by viruses, assists in retaining moisture, and helps the cell adhere to surfaces and nutrients. The capsule is found most commonly among Gram–negative bacteria. *Escherichia coli, Klebsiella pneumoniae Haemophilus influenzae, Pseudomonas aeruginosa and Salmonella* are some examples Gram–negative bacteria possessing capsules. Examples of Gram positive bacteria that possess capsules are *Bacillus megaterium, Streptococcus pneumoniae,* Streptococcus *pyogenes*.

EUKARYOTIC CELL

All species of large complex organisms are eukaryotes, including animals, plants and fungi It consists of membrane bound organelles. Eukaryotic cells possess all the characters which prokaryotic cells lack. Thus they are characterized by the presence of definitely organized nucleus with a nuclear membrane and nucleolus and presence of well organized cytoplasmic organelles like mitochondria, plastids, ribosomes, endoplasmic

reticulum, lysosomes, golgi bodies etc. Unlike the eukaryotic cells of plants and fungi, animal cells do not have a cell wall. The lack of a rigid cell wall allowed animals to develop a greater diversity of cell types, tissues, and organs. Most cells, both animal and plant, range in size between 1 and 100 micrometers and are thus visible only with the aid of a microscope. Eukaryotes represent a minority of all living things; even in a human body there are 10 times more microbes than human cells. However, due to their much larger size their collective worldwide biomass is estimated at about equal to that of prokaryotes.

Classification

The eukaryotes are composed of four kingdoms:

- Kingdom Protista
- Kingdom Fungi
- Kingdom Plantae
- Kingdom Animalia

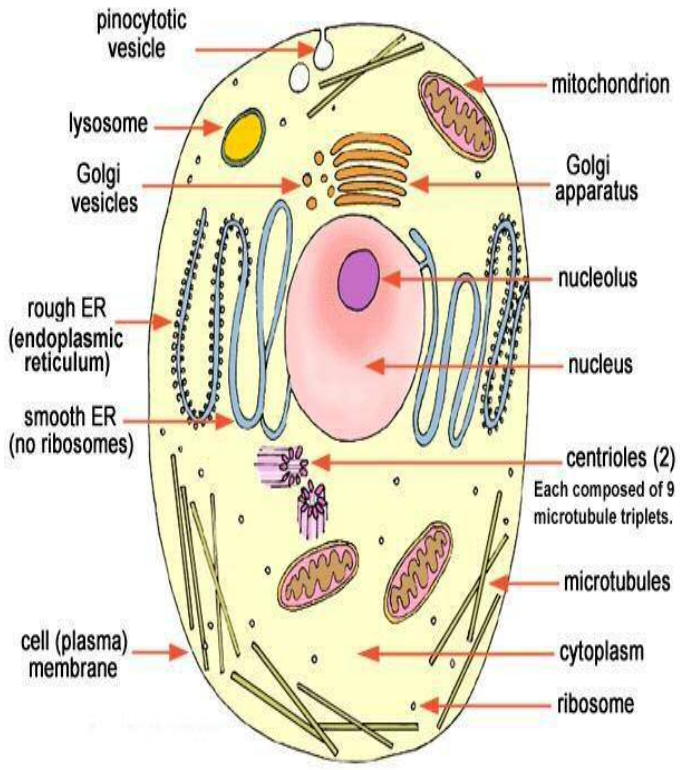
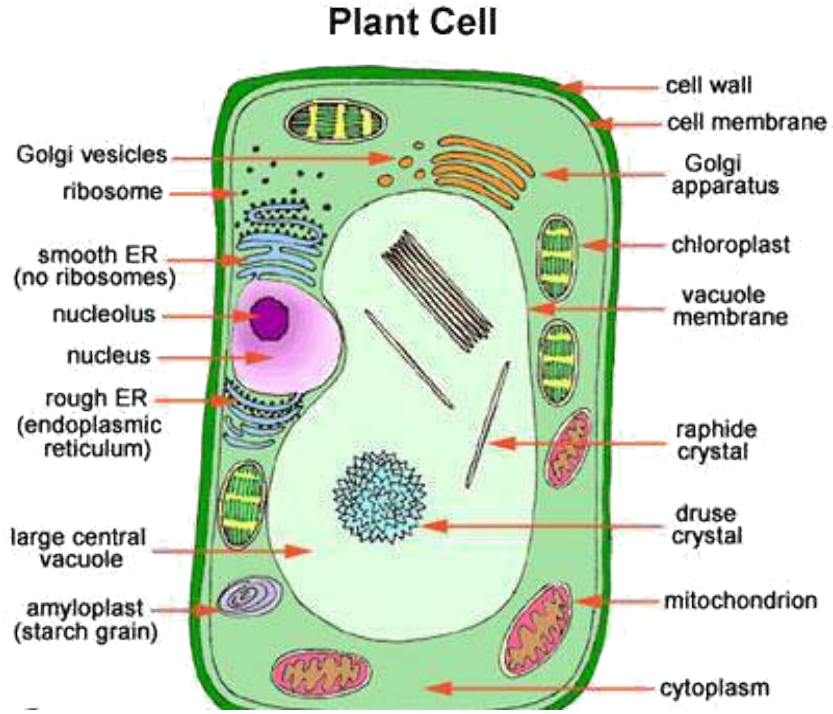


Fig:Animal cell structure

Fig: Animal cell



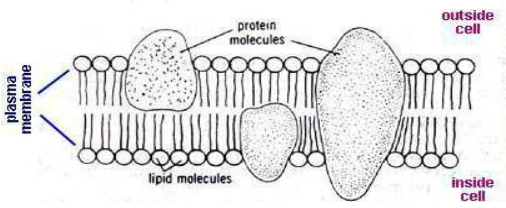
CELL ORGANELLES:

In cell biology, an organelle is a specialized subunit within a cell that has a specific function, and is usually separately enclosed within its own lipid bilayer. Organelles are identified by microscopy, and can also be purified by cell fractionation.

THE THREE MAIN COMPONENTS OF ANY PLANT OR ANIMAL CELL ARE:

1. PLASMA MEMBRANE/ CELL MEMBRANE

Structure–a bilipid membraneous layer composed of proteins and carbohydrates. It is fluid in nature.Proteins are found embedded within the plasma membrane, Carbohydrates are also attached to proteins and lipids on the outer lipid layer.



Function – the cell membrane separates the cell from its external environment, and is selectively permeable allowing selective substances to pass into the cell and blocking others. It protects the cell and provides stability.

2. CYTOPLASM

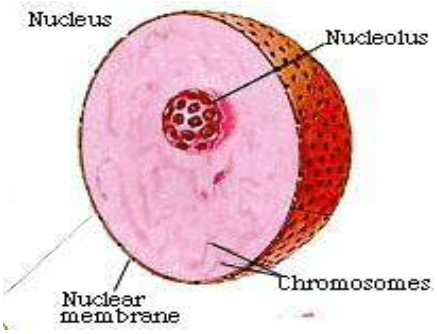
It is jelly-like substance composed of mainly water and found between the cell membrane and nucleus. The cytoplasm makes up most of the "body" of a cell and is constantly streaming.

Function – Organelles are found here and substances like salts may be dissolved in the cytoplasm.

3. NUCLEUS

The largest organelle in the cell. They are spherical body containing many organelles, including the nucleolus and is surrounded by a double membrane called the nuclear envelope/membrane. The nucleus contains genetic information (DNA) on special strands called chromosomes. The nucleolus which is an organelle within the nucleus – it is where ribosomal RNA is produced.

Function – The nucleus is the "control center" of the cell, for cell metabolism and reproduction.



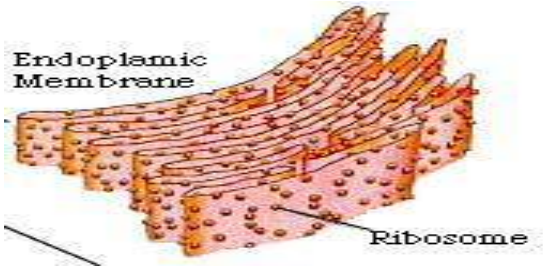
THE FOLLOWING ORGANELLES ARE FOUND IN BOTH PLANT AND ANIMAL CELLS.

1. "ER" OR ENDOPLASMIC RETICULUM

The Endoplasmic Reticulum is a network of membranous canals filled with fluid. They carry materials throughout the cell. The ER is the "transport system" of the cell.

There are two types of ER: rough ER and smooth ER.

Rough Endoplasmic Reticulum is lined with ribosomes and is rough in appearance and smooth endoplasmic reticulum contains no ribosomes and is smooth in appearance.



Rough ER transport materials through the cell and produces proteins.Smooth endoplasmic reticulum contains enzymes that produces and digests lipids (fats)

2. RIBOSOMES

Ribosomes are small particles which are found individually in the cytoplasm and also line the membranes of the rough endoplasmic reticulum. Ribosomes produce protein. They could be thought of as "factories of protein synthesis" in the cell.

3. GOLGI BODY / APPARATUS

Golgi bodies are stacks of flattened, membranous sac–like organelle. It is involved in packaging proteins and carbohydrates into membrane–bound vesicles for export from the cell.

4. LYSOSOMES

Lysosomes are small sac–like structures surrounded by a single membrane and containing hydrolase enzymes which breaks down waste materials and cellular debris , when released can break down and worn out organelles so they are also known as a suicide sac.

5. MITOCHONDRIA

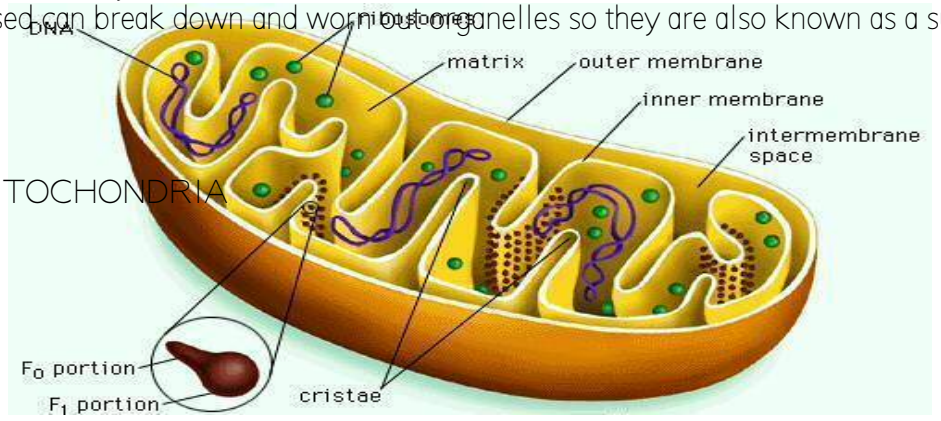


Fig: Mitochondria

These are spherical to rod–shaped organelles with a double membrane. The inner membrane is infolded many times, forming a series of projections called cristae. It releases the energy stored in glucose into ATP (adenosine triphosphate) for the cell. The mitochondria is often referred to as the "powerhouse" of the cell

6. VACUOLES

Vacuoles are fluid filled organelles enclosed by a membrane. They can store materials such as food, water, sugar, minerals and waste products.

7. CENTROSOME

They are small body located near the nucleus and has a dense center and radiating tubules. The centrosomes are the destination where microtubules are made. During

mitosis, the centrosome divides and the two parts move to opposite sides of the dividing cell.

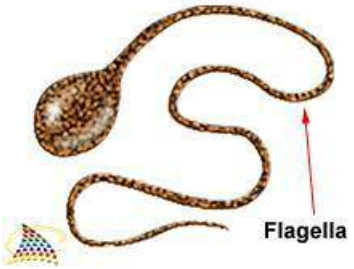
8.PEROXISOME

Peroxisomes are organelles that contain oxidative enzymes, such as D–amino acid oxidase, ureate oxidase, and catalase. They are self replicating, like the mitochondria. Peroxisomes function to rid the body of toxic substances like hydrogen peroxide, or other metabolites. They are a major site of oxygen utilization and are numerous in the liver where toxic by products accumulate.

ANIMAL CELLS ORGANELLES NOT FOUND IN PLANT CELLS:

CILIA AND FLAGELLA

Both cilia and flagella are hair–like organelles which extend from the surface of many animal cells. the structure is identical in both, except that flagella are longer and whiplike and cilia are shorter. There are usually only a few flagella on a cell, while cilia may cover the entire surface of a cell. The function of cilia and flagella ionclude locomotion for one–celled organisms and to move substances over cell surfaces in multi–celled organisms.



An animal cell is a form of eukaryotic cell that makes up many tissues in animals. There are many different cell types. For instance, there are pproximately 210 distinct cell types in the adult human body

ORGANELLES AND OTHER FEATURES FOUND ONLY IN PLANT CELLS:

1. CELL WALL

The cell wall is the extracellular structure surrounding plasma membrane. The cell wall is composed of cellulose, hemicellulose, pectin and in many cases lignin a rigid organelle composed of cellulose and lying just outside the cell membrane. The cell wall gives the plant cell it's box–like shape. it also protects the cell. The cell wall contains pores which allow materials to pass to and from the cell membrane. The cell wall is divided into the primary cell wall and the secondary cell wall. The Primary cell wall: extremely elastic and the secondary cell wall forms around primary cell wall after growth are complete.

2. PLASMODESMATA

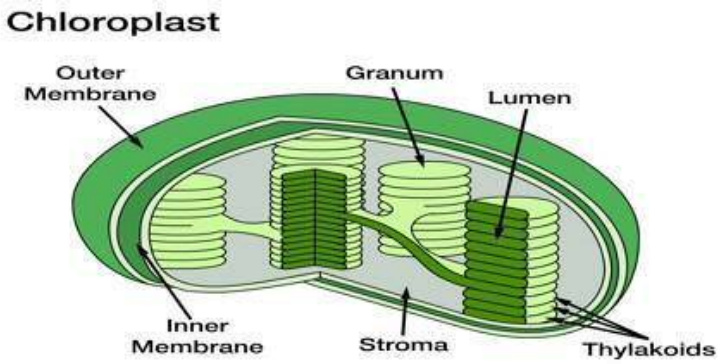
Pores in the primary cell wall through which the plasmalemma and endoplasmic reticulum of adjacent cells are continuous.

3. PLASTIDS

Plastids are double membrane bound organelles. It is in plastids that plants make and store food. Plastids are found in the cytoplasm and there are two main types:

Leucoplasts –colorless organelles which store starch or other plant nutrients. (example – starch stored in a potato)

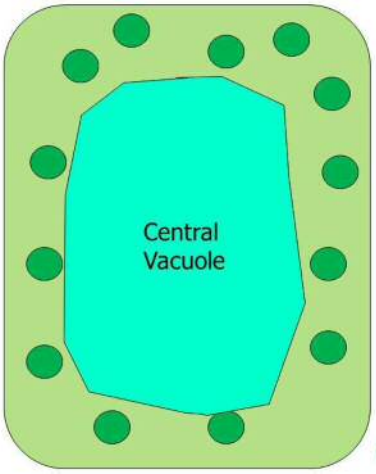
Chromoplasts – contain different colored pigments. The most important type of chromoplast is the chloroplast, which contains the green pigment chlorophyll. This is important in the process of photosynthesis.



The other plastids is elaioplasts specialized for fat storage. As in mitochondria, which have a genome encoding 37 genes, plastids have their own genomes of about 100–120 unique genes.

4. CENTRAL VACUOLE

It is present at the centre and large fluid–filled vacuole found in plants enclosed by a membrane known as the tonoplast. The function is to maintain the cell's turgor, pressure by controlling movement of molecules between the cytosol and sap, stores useful material and digests waste proteins and organelles.



- **Job**: Stores food, water, waste, color pigments
- Plant cells: Large central vacuole

Comparision of prokaryotic and eukaryotic cells.

- Prokaryotic cells have naked DNA which is found in the cytoplasm in a region named the nucleoid. On the other hand, eukaryotes have chromosomes that are made up of DNA and protein. These chromosomes are found in the nucleus enclosed in a nuclear envelope.
- Prokaryotes do not have any mitochondria whereas eukaryotes have MR.
- Prokaryotes have small ribosomes (70S) compared to eukaryotes which have large ribosomes (80S).
- In prokaryotes there are either no or very few organelles bounded by a single membrane in comparison to eukaryotes which have many of them including the Golgi apparatus and the endoplasmic reticulum.
  They are tabulted as follows

Plasma membrane with steroid:	Yes	Usually no
Cell wall:	Only in plant cells and fungi (chemically simpler)	Usually chemically complexed
Vacuoles:	Present	Present
Cell size:	10–100um	1–10um

Difference between prokaryotic and eukaryotic cell:

S.No	Prokaryotic cell	Eukaryotic cell
1	Most primitive, earliest form of life	More complex, evolved organsims
2	Do not have a pre–defined nucleus Chromosomes are dispersed in the Cytoplasm	Contain true nuclei in which chromosomes are compacted as chromatin
3	Contain no membrane–bound Organelles	Contain membrane–boundorganelles
4	Have circular chromosomes and lack histone proteins	Have linear DNA and contain histone proteins
5	Small –typically 1 –5 micrometers in diameter	Larger–typically 10–100 micrometers in diameter
6	Have a primitive cytosketetal structures or don't have a cytoskeleton at all	Have a complex cytosketeton
7	Reproduce sexually by the transfer of DNA fragments through Conjugation	Reproduce sexually with the use of meiosis
8	Contain Smaller (70S) ribosomes	Contain Larger (80S) ribosomes

Differences between plant and animal cells

- Animal cells only have a plasma membrane and no cell wall. Whereas plant cells have a plasma membrane and a cell wall.
- Animal cells do not have chloroplasts whereas plant cells do for the process of photosynthesis.

- Animal cells store glycogen as their carbohydrate resource whereas plants store starch.

- Animal cells do not usually contain any vacuoles and if present they are small or temporary. On the other hand plants have a large vacuole that is always present.

- Animal cells can change shape due to the lack of a cell wall and are usually rounded whereas plant cells have a fixed shape kept by the presence of the cell wall.

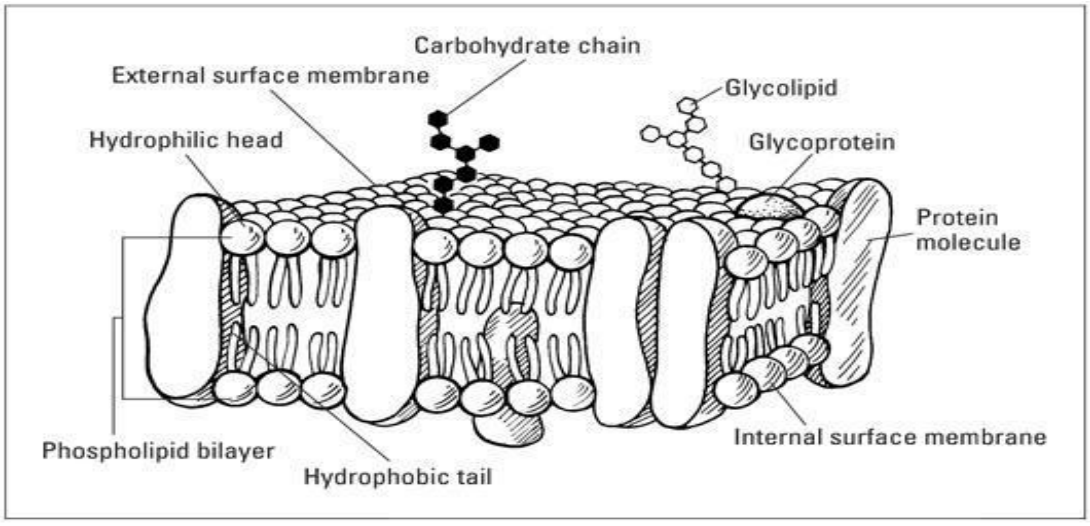
CELL MEMBRANE

PLASMA MEMBRANE:

All living cells possess a cell membrane. These membranes serve to contain and protect cell components from the surroundings as well as regulate the transport of material into and out of the cell. Cell membranes are the selectively permeable lipid bilayers inclusive of membrane proteins which delimits all prokaryotic and eukaryotic cells. In prokaryotes and plants, the plasma membrane is an inner layer of protection bounded to the inner side of a rigid cell wall. Eukaryotes lack this external layer of protection or the cell wall. In eukaryotes the membrane also forms boundary of cell organelles.

Composition of cell membrane is :

- Lipids
- Proteins
- Carbohydrates



MEMBRANE LIPIDS

The cell membrane structure consists of a double layer of lipid molecules in which proteins are embedded

The cell membrane lipids are highly complex comprising of

- Phospholipids
- Cholesterols
- Glycolipids,

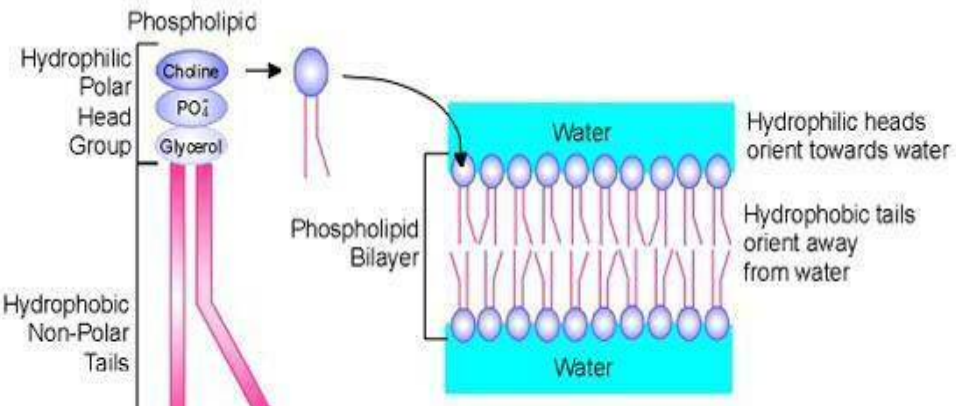
(i)The major membrane lipids are phospholipids. The major membrane phospholipids are

- phosphatidylcholine (PtdCho),
- phosphatidylethanolamine (PtdEtn),
- phosphatidylinositol (PtdIns) and
- phosphatidylserine (PtdSer).

These are amphipathic molecules: one end has a charged region, and the remainder of the molecule, which consists of two long fatty acid chains, is nonpolar. The fatty acids can differ in length (16– and 18–carbon fatty acids are the most common) Fatty acids can be saturated or unsaturated with the double bonds always in *cis* configuration in the later The phospholipids in cell membranes are organized into a bimolecular layer with the nonpolar fatty acid chains in the middle. The polar regions of the phospholipids are oriented toward the surfaces of the membrane as a result of their attraction to the polar water molecules in the extracellular fluid and cytosol. No chemical bonds link the phospholipids to each other or to the membrane proteins, and therefore, each molecule is free to move independently of the others. This results in considerable random lateral movement of both membrane lipids and proteins parallel to the surfaces of the bilayer.

In addition, the long fatty acid chains can bend and wiggle back and forth. Thus, the lipid bilayer has the characteristics of a fluid, much like a thin layer of oil on a water surface, and this makes the membrane quite flexible. This flexibility, along with the fact that cells are filled with fluid, allows cells to undergo considerable changes in shape without disruption of their structural integrity.

Structure of Phospholipids-The Amphipathic Nature of Phospholipids



(ii). The plasma membrane also contains cholesterol (about one molecule of cholesterol for each molecule of phospholipid), whereas intracellular membranes contain very little cholesterol.

Cholesterol, a steroid, is slightly amphipathic because of a single polar hydroxyl group on its nonpolar ring structure. Therefore, cholesterol, like the phospholipids, is inserted into the lipid bilayer with its polar region at a bilayer surface and its nonpolar rings in the interior in association with the fatty acid chains..

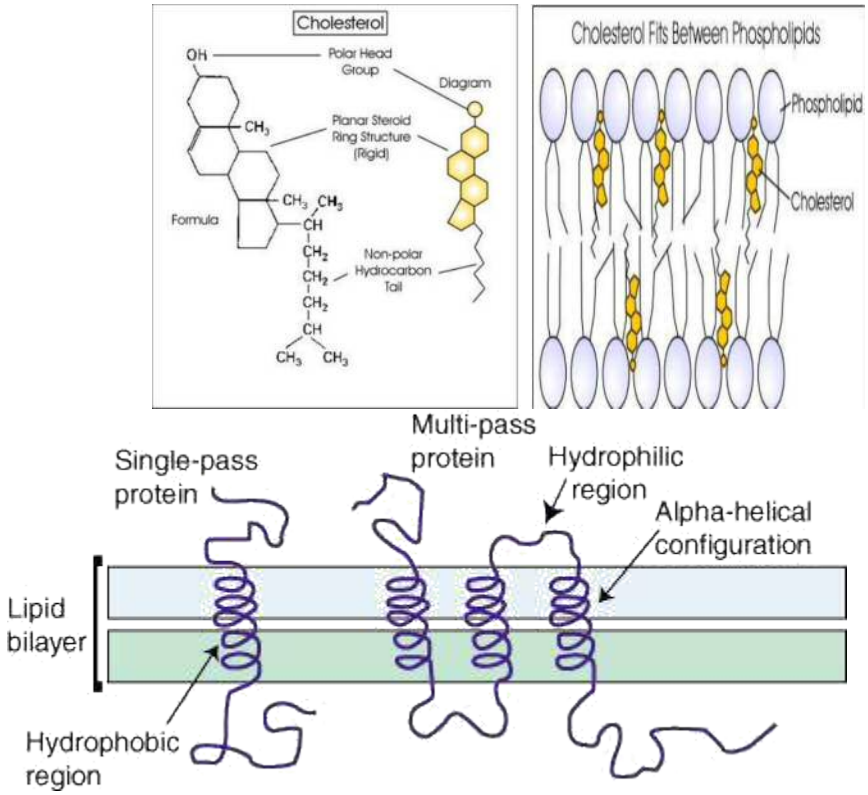


Fig: Amphipathic cholesterol in membrane

Role of Lipid Molecules in Maintaining Fluid Property of membrane

Types of movements of lipid molecules.

In lipid monolayer flip–flop or transbilayer movement occurs once a month for any individual lipid molecule. However, in membranes where lipids are actively synthesized, such as smooth ER, there is a rapid flip–flop of specific lipid molecules across the bilayer and there are present certain membrane–bound enzymes, called phospholipid translocators like flippases to catalyze this activity. The other movement is lateral diffusion. Individual lipid molecules rotate very rapidly about their long axes and their hydrocarbon chains are

flexible, the greatest degree of flexion occurring near the centre of the bilayer and the smallest adjacent to the polar head groups.

Role of unsaturated fats in increasing membrane fluidity.

A synthetic bilayer made from a single type of phospholipid changes from a liquid state to a rigid crystalline state at a characteristic freezing point. This change of state is called a phase transition and the temperature at which it occurs becomes lower if the hydrocarbon chains are short or have double bonds. Double bonds in unsaturated hydrocarbon chains tend to increase the fluidity of a phospholipid bilayer by making it more difficult to pack the chains together. Thus, to maintain fluidity of the membrane, cells of organisms living at low temperatures have high proportions of unsaturated fatty acids in their membranes, than do cells at higher temperatures.

Role of cholesterol in maintaining fluidity of membrane

Eukaryotic plasma membranes are found to contain a large amount of cholesterol; up to one molecule for every phospholipid molecule. Cholesterol inhibits phase transition by preventing hydrocarbon chains from coming together and crystallizing. Cholesterol also tends to decrease the permeability of lipid bilayers to small water–soluble molecules and is thought to enhance both the flexibility and the mechanical stability of the bilayer.

2. MEMBRANE PROTEINS

There are two classification of membrane proteins

- Integral membrane proteins,
- Peripheral membrane proteins,
-

(i). Integral membrane Proteins

Integral proteins are embedded within the lipid bilayer. They cannot easily be removed from the cell membrane without the use of harsh detergents that destroy the lipid bilayer. Integral proteins float rather freely within the bilayer, much like oceans in the sea. In addition, integral proteins are usually transmembrane proteins, extending through the lipid bilayer so that one end contacts the interior of the cell and the other touches the exterior. The stretch of the integral protein within the hydrophobic interior of the bilayer is also hydrophobic, made up of non–polar amino acids. Like the lipid bilayer, the exposed ends of the integral protein are hydrophilic(amphipathic).

Transmembrane proteins can either cross the lipid bilayer one or multiple times. The former are referred to as single-pass proteins and the later as multi-pass proteins. These proteins have polar regions connected by nonpolar segments that associate with the nonpolar regions of the lipids in the membrane interior. The polar regions of transmembrane proteins may extend far beyond the surfaces of the lipid bilayer.

As a result of their structure, transmembrane proteins are the only class of proteins that can perform functions both inside and outside of the cell.

Some transmembrane proteins form channels through which ions or water can cross the membrane, whereas others are associated with the transmission of chemical signals across the membrane or the anchoring of extracellular and intracellular protein filaments to the plasma membrane

Proteins embedded in membrane serve different functions

1.Channel Proteins –form small openings for molecules to difuse through

2. Carrier Proteins–binding site on protein surface "grabs" certain molecules and pulls them into the cell

3. Receptor Proteins –molecular triggers that set off cell responses (such as release of hormones or opening of channel proteins)

4. Cell Recognition Proteins –ID tags, to idenitfy cells to the body's immune system
5. Enzymatic Proteins –carry out metabolic reactions

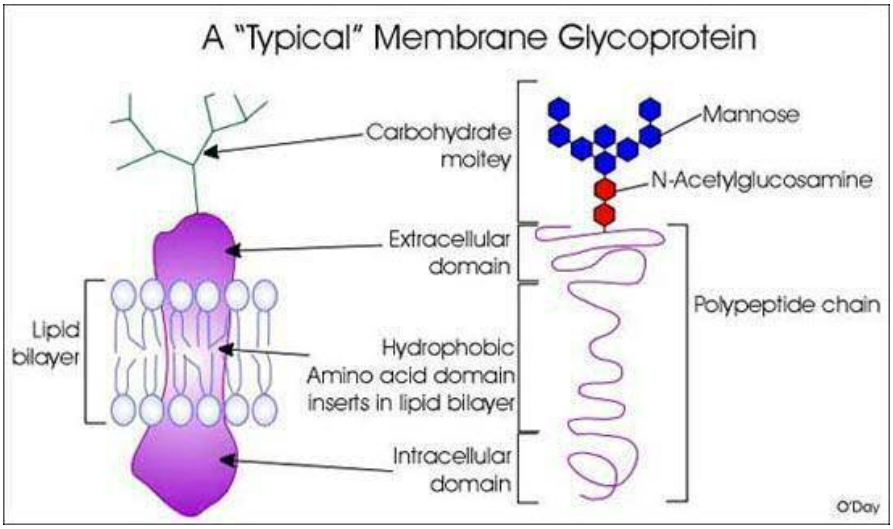
(ii) Peripheral Proteins

Peripheral membrane proteins are not amphipathic and they are attached to the exterior of the lipid bilayer. They are easily separable from the lipid bilayer, able to be removed without harming the bilayer in any way. Peripheral proteins are less mobile within the lipid bilayer. Most of the peripheral proteins are on the cytosolic surface of the plasma membrane where they are associated with cytoskeletal elements that influence cell shape and motility.

3. CARBOHYDRATES

Glycoproteins - Sugar Coat of the Cell

Many of the membrane proteins are covalently linked to sugar residues. They may consist of a few sugars or extend into long carbohydrate moieties. The sugar groups are always oriented towards the external environment, never the cytoplasm. The following shows an example of an integral membrane glycoprotein.



When the carbohydrate component of the glycoprotein is extensive, typically interacting with extracellular matrix components it can be seen in the electron microscope. The extensive "sugar coating" of the intestinal epithelium is called the glycocalyx.There are two types of glycoprotein namely O–linked and N–linked glycoprotein.

MEMBRANE STRUCTURE- FLUID MOSAIC MODEL

Currently, the most accepted model for cell membrane is fluid mosaic model proposed by S.J.Singer and G.L.Nicolson (1972).

According to this model, the plasma membrane contains a bimolecular lipid layer, both surfaces of which are interrupted by protein molecules. Proteins occur in the form of globular molecules and they are dotted about here and there in a mosaic pattern (see Figure). Some proteins are attached at the polar surface of the lipid (i.e., the extrinsic proteins); while others (i.e., integral proteins) either partially penetrate the bilayer or span the membrane entirely to stick out on both sides (called transmembrane proteins). Further, the peripheral proteins and those parts of the integral proteins that stick on the outer surface (i.e., ectoproteins) frequently contain chains of sugar or oligosaccharides (i.e., they are glycoproteins). Likewise, some lipids of outer surface are glycolipids.

The fluid–mosaic membrane is thought to be a far less rigid than was originally supposed. On account of its fluidity and the mosaic arrangement of protein molecules, this model of membrane structure is known as the ―fluid mosaic model‖ (i.e., it describes both properties and organization of the membrane). The fluid mosaic model is found to be applied to all biological membranes in general, and it is seen as a dynamic, ever–changing structure. The proteins are present not to give it strength, but to serve as

enzymes catalysing chemical reactions within the membrane and as pumps moving things across it.

CELLS AS EXPERIMENTAL MODELS

The evolution of present-day cells from a common ancestor has important implications for cell and molecular biology as an experimental science. Because the fundamental properties of all cells have been conserved during evolution, the basic principles learned from experiments performed with one type of cell are generally applicable to other cells. Several different kinds of cells and organisms are commonly used as experimental models to study various aspects of cell and molecular biology.

1.*E.coli*

Because of their comparative simplicity, prokaryotic cells (bacteria) are ideal models for studying many fundamental aspects of biochemistry and molecular biology. The most thoroughly studied species of bacteria is *E. coli*, which has used for investigation of the basic mechanisms of molecular genetics. Most of our present concepts of molecular

biology—including our understanding of DNA replication, the genetic code, gene expression, and protein synthesis—derive from studies of this humble bacterium.

E. coli has been especially useful to molecular biologists because of both its relative simplicity. The genome of *E. coli*, for example, consists of approximately 4.6 million base pairs and encodes about 4000 different proteins. The human genome is nearly a thousand times more complex (approximately 3 billion base pairs) and encodes about 100,000 different proteins

Molecular genetic experiments are further facilitated by the rapid growth of *E. coli* under well-defined laboratory conditions. Depending on the culture conditions, *E. coli* divide every 20 to 60 minutes. Because bacterial colonies containing as many as 10^8 cells can develop overnight, selecting genetic variants of an *E.coli* strain—for example, mutants that are resistant to an antibiotic, such as penicillin—is easy and rapid. The ease with which such mutants can be selected and analyzed was critical to the success of experiments that defined the basic principles of molecular genetics. Thus, the rapid growth and simple nutritional requirements of *E. coli* have greatly facilitated fundamental experiments in both molecular biology and biochemistry.

2.Yeast

Although bacteria have been an invaluable model for studies of many conserved properties of cells, they obviously cannot be used to study aspects of cell structure and function that are unique to eukaryotes. Yeasts, the simplest eukaryotes, have a number of experimental advantages similar to those of *E. coli*. Consequently, yeasts have provided a crucial model for studies of many fundamental aspects of eukaryotic cell biology.

The genome of the most frequently studied yeast, *Saccharomyces cerevisiae,* consists of 12 million base pairs of DNA and contains about 6000 genes. Although the yeast genome is approximately three times larger than that of *E. coli*, it is far more manageable than the genomes of more complex eukaryotes, such as humans. Yet even in its simplicity, the yeast cell exhibits the typical features of eukaryotic cells. (It contains a distinct nucleus surrounded by a nuclear membrane, its genomic DNA is organized as 16 linear chromosomes, and its cytoplasm contains a cytoskeleton and subcellular organelles.

Yeasts can be readily grown in the laboratory and can be studied by many of the same molecular genetic approaches that have proved so successful with *E. coli*. Although yeasts do not replicate as rapidly as bacteria, they still divide as frequently as every 2 hours and can easily be grown as colonies from a single cell. Consequently, yeasts can be used for a variety of genetic manipulations similar to those that can be performed using bacteria.

Yeast mutants have been important in understanding many fundamental processes in

eukaryotes, including DNA replication, transcription, RNA processing, protein sorting, and the regulation of cell division.

3.Dictyostelium discoidium

Dictyostelium is a cellular slime mold, which, like yeast, is a comparatively simple unicellular eukaryote. The genome of *Dictyostelium* is approximately ten times larger than that of *E. coli*—more complex than the yeast genome but considerably simpler than the genomes of higher eukaryotes. Moreover, *Dictyostelium* can be readily grown in the laboratory and is amenable to a variety of genetic manipulations.

It is a highly mobile cell, and this property has made *Dictyostelium* an important model for studying the molecular mechanisms responsible for animal cell movements.

An additional interesting feature of *Dictyostelium* is the ability of single cells to aggregate into multicellular structures. If an adequate supply of food is not available, the cells associate to form wormlike structures called slugs, each consisting of up to 100,000 cells that function as a unit. *Dictyostelium* thus appears to straddle the border between

unicellular and multicellular organisms, providing an important model for studies of cell signaling and cell-cell interactions.

4. Caenorhabditis elegan

The unicellular eukaryotes *Saccharomyces* and *Dictyostelium* are important models for studies of eukaryotic cells, but understanding the development of multicellular organisms requires the experimental analysis of plants and animals, organisms that are more complex. The nematode *Caenorhabditis elegans* possesses several notable features that make it one of the most widely used models for studies of animal development and cell differentiation.

Although the genome of *C. elegans* (approximately 100 million base pairs) is larger than those of unicellular eukaryotes, it is simpler and more manageable than the genomes of most animals. Genome of *C. elegans* contains approximately 19,000 genes—about three times the number of genes in yeast, and one–fifth the number of genes predicted in humans. Biologically, *C. elegans* is also a relatively simple multicellular organism, In addition, *C. elegans* can be easily grown and subjected to genetic manipulations in the laboratory.

Importantly, similar genes have been found to function in complex animals (including humans), making *C. elegans* an important model for studies of animal development.

5. Drosophila melanogaster

Like *C. elegans*, the fruit fly *Drosophila melanogaster* has been a crucial model organism in developmental biology. The genome of *Drosophila* is similar in size to that of *C. elegans*, and *Drosophila* can be easily maintained and bred in the laboratory. Furthermore, the short reproductive cycle of *Drosophila* (about 2 weeks) makes it a very useful organism for genetic experiments. Many fundamental concepts of genetics—such as the relationship between genes and chromosomes—were derived from studies of *Drosophila* early in the twentieth century

Studies of *Drosophila* have led to striking advances in understanding the molecular mechanisms that govern animal development, particularly with respect to formation of the body plan of complex multicellular organisms.

6. Arabidopsis thaliana

Since the genomes of plants cover a range of complexity comparable to that of animal genomes , an optimal model for studies of plant development would be a relatively simple organism with some of the advantageous properties of *C.elegans* and *Drosophila*. The small flowering plant *Arabidopsis thaliana* meets these criteria and is therefore widely used as a model to study the molecular biology of plants.

Arabidopsis is notable for its genome of only about 130 million base pairs—a complexity similar to that of*C. elegans* and *Drosophila*. In addition, *Arabidopsis* is relatively easy to grow in the laboratory, and methods for molecular genetic manipulations of this plant have been developed. These studies have led to the identification of genes involved in various aspects of plant development, such as the development of flowers.

Vertebrate

The most complex animals are the vertebrates, including humans and other mammals. The human genome is approximately 3 billion base pairs—about 30 times larger than the genomes of *C. elegans*, *Drosophila*, or *Arabidopsis*. Moreover, the human body is composed of more than 200 different kinds of specialized cell types. This complexity makes the vertebrates difficult to study from the standpoint of cell and molecular biology, but understanding of many questions of immediate practical importance (e.g., in medicine) must be based directly on studies of human (or closely related) cell types.

One important approach to studying human and other mammalian cells is to grow isolated cells in culture, where they can be manipulated under controlled laboratory conditions. The use of cultured cells has allowed studies of many aspects of mammalian

cell biology, including experiments that have elucidated the mechanisms of DNA replication, gene expression, protein synthesis and processing, and cell division. Moreover, the ability to culture cells in chemically defined media has allowed studies of the signaling mechanisms that normally control cell growth and differentiation within the intact organism.

The specialized properties of some highly differentiated cell types have made them important models for studies of particular aspects of cell biology. Muscle cells, for example, are highly specialized to undergo contraction, producing force and movement. Because of this specialization, muscle cells are a crucial model for studying cell movement at the molecular level.

Another example is provided by nerve cells (neurons), which are specialized to conduct electrochemical signals over long distances. Because of their highly specialized structure and function, these giant neurons have provided important models for studies of ion transport across the plasma membrane, and of the role of the cytoskeleton in the transport of cytoplasmic organelles.

a.*Xenopus laevis*

The frog *Xenopus laevis* is an important model for studies of early vertebrate development. *Xenopus* eggs are unusually large cells, with a diameter of approximately 1. Because those eggs develop outside of the mother, all stages of development from egg to tadpole can be readily studied in the laboratory. In addition, *Xenopus* eggs can be obtained in large numbers, facilitating biochemical analysis. Because of these technical advantages, *Xenopus* has been widely used in studies of developmental biology and has provided important insights into the molecular mechanisms that control development, differentiation, and embryonic cell division.

b. Zebrafish

The zebrafish possesses a number of advantages for genetic studies of vertebrate development. These small fish are easy to maintain in the laboratory and they reproduce rapidly. In addition, the embryos develop outside of the mother and are transparent, so that early stages of development can be easily observed. Powerful methods have been developed to facilitate the isolation of mutations affecting zebrafish development, and several thousand such mutations have now been identified. Because the zebrafish is an easily studied vertebrate, it promises to bridge the gap between humans and the simpler invertebrate systems, such as *C. elegans* and *Drosophila*.

c.Mouse

Among mammals, the mouse is the most suitable for genetic analysis. Although the technical difficulties in studying mouse genetics are formidable, several mutations affecting mouse development have been identified. Most important, recent advances in molecular biology have enabled the production of transgenic mice, in which specific mutant genes have been introduced into the mouse germ line, so that their effects on development or other aspects of cell function can be studied in the context of the whole animal. The suitability of the mouse as a model for human development is illustrated by the fact that mutations in homologous genes result in similar developmental defects in both species.

TOOLS OF CELL BIOLOGY

Microscope is an optical instrument that uses lens or combination of lens to produce magnified images that are too small to seen by unaided eye. Microscope provides the enlarged view that helps in examining and analyzing the image. Microscope can be separated into optical theory microscopes (Light microscope), electron microscopes (eg.TEM, SEM) and scanning probe microscopes. (eg.AFM, PSTM).

Optical microscopes function on the basis of optical theory of lenses by which it can magnifies the image obtained by the movement of a wave through the sample. The waves used in optical microscopes are electromagnetic and that in electron microscopes are electron beams. Light microscopes can be classified into

- Bright field microscope,
- Phase contrast microscope,
- Dark field microscope and
- Fluorescence microscope.

1. LIGHT MICROSCOPY

Light microscope uses the properties of light to produce an enlarged image. It is the simplest type of microscope. Based on the simplicity of the microscope it may be categorized into:

A) Simple microscope–It is uses only a single lens, e.g.: hand lens

B) Compound microscope–compound microscope used two lenses or lens systems. One of the lens system formed an enlarged image of the object and the second

lens system magnifies the image formed by the first.The total magnification is the product of the magnifications of two lens systems.

Principles of Light Microscopy

For light microscopy, visible light is passed through the specimen and then through a series of lenses that bend the light in a manner that results in magnification of the organisms present in the specimen. The total magnification achieved is the product of the lenses used.

Instrumentation

Parts of a Microscope

It consists of mainly three parts:

1. Mechanical part –base, c–shaped arm and stage.
2. Magnifying part –objective lens and ocular lens.
3. Illuminating part –sub stage condenser, iris diaphragm, light source.

I. Mechanical part

1. Base: It helps in holding the various parts of microscope. It also contains the light source.

2. C-shaped arm: It is used for holding the microscope. And which is connected the eyepiece to the objective lens.

3. Mechanical stage: It is a rigid platform on which specimen to be viewed is placed. It has an aperture at the centre to permit light to reach the object from the bottom. The object on the slide can be moved either sideways or forward and backward with the help of the positioning knobs.

2.Magnifying part
Eyepiece (Ocular lens):

It is the lens where the final image of the object is viewed. Usually; these lenses have a magnification of either 10X or 15X.

Objective lens:

There are three types of objective lens:

10X (Low power objective lens).

40X (High power objective lens).

100X (Oil immersion objective lens).

These objective lenses are fitted on to the revolving nose piece. The working distance of an objective is defined as the distance between the front surface of the lens and the cover glass surface or the specimen when it is in sharp focus.

3.Illuminating part

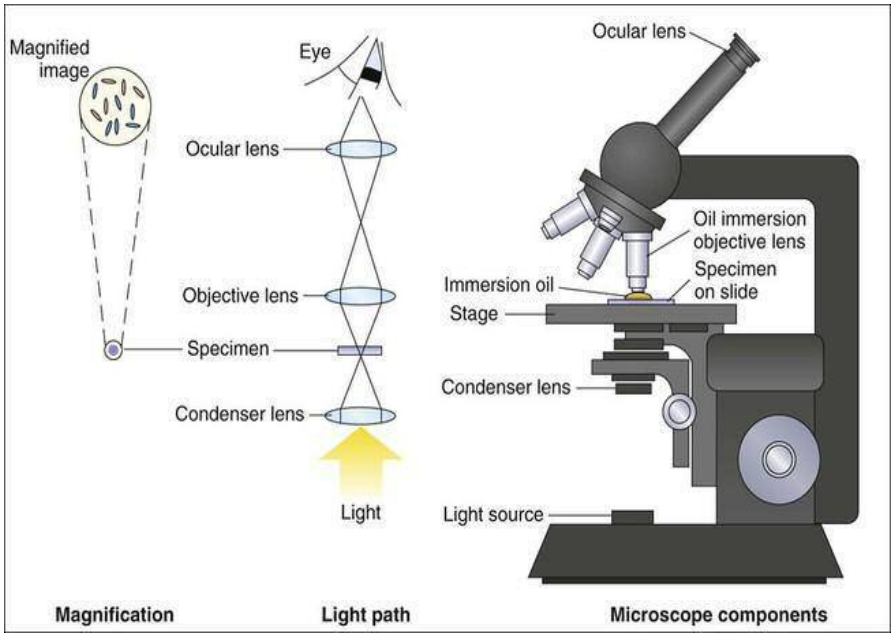
1. Sub stage condenser:

It is seen below the stage and made up of a system of convex lenses which focus light from illuminating sources and is used to condense light towards the object. Lowering the condenser diminishes illumination whereas raising the condenser increases the illumination.

2. Iris diaphragm:

1. It is seen immediately below the condenser and operated by small lenses which protrude to one side. Opening and closing of iris diaphragm controls the light reaching the object.3. Light source:

Light source is situated at the base of the microscope. It is controlled by an ON /OFF switch and a lamp rheostat. Tungsten–halogen lamps are highly reliable light source used in the light microscope. It generates a continuous distribution of light across the visible spectrum.



IV. Adjustment knobs in the microscope

a) Coarse Adjustment Knob:

objective lenses can be moved towards or away from the specimen by using this coarse adjustment knob

b) Fine Adjustment Knob:

It is used to fine tune the focus on the specimen and also used to focus on various parts of the specimen. commonly one uses the coarse focus first to get close and moves to the fine focus knob for fine tuning.

Samples for Light Microscopy Usually Are Fixed, Sectioned, and Stained

Specimens for light microscopy are commonly fixed with a solution containing alcohol or formaldehyde, compounds that denature most proteins and nucleic acids. Formaldehyde also crosslinks amino groups on adjacent molecules; these covalent bonds stabilize protein protein andprotein – nucleic acid interactions and render the molecules insoluble and stable for subsequent procedures.

Usually the sample is then embedded in paraffin or plastic and cut into thin sections of one or a few micrometers thick.Alternatively, the sample can be frozen without prior fixation and then sectioned; this avoids the denaturation of enzymes by fixatives such asformaldehyde.

Since the resolution of the light microscope is ≈0.2 μm and mitochondria and chloroplasts are ≈1 μm long (about the size of bacteria), theoretically one should be able to see these organelles.However, most cellular constituents are not colored and absorb about the same degree of visible light, so that they are hard to distinguish under a light microscope unless the specimen is stained.

Thus the final step in preparing a specimen for light microscopy is to stain it, in order to visualize the main structural features of the cell or tissue. Many chemical stains bind to molecules that have specific features. For example, *hematoxylin* binds to basic amino acids (lysine and arginine) on many different kinds of proteins, whereas *eosin* binds to acidic molecules (such as DNA, and aspartate and glutamate side chains). Because of their different binding properties, these dyes stain various cell types sufficiently differently that they are distinguishable visually. Two other common dyes are *benzidine*, which binds to hemecontaining proteins and nucleic acids, and *fuchsin,* which binds to DNA and is used in Fuelgen staining.

If an enzyme catalyzes a reaction that produces a colored or otherwise visible precipitate from a colorless precursor, the enzyme may be detected in cell sections by their colored reaction products. This technique is called *cytochemical staining*

V.Image Formation

The direct or undeviated light from a specimen is projected by the objective and it spreads evenly across the entire image plane at the diaphragm of the eyepiece.

The light diffracted by the specimen is come to focus at different localized sites on the same image plane, and the diffracted light causes destructive interference.

One of the consequences is the reduction in light intensity resulting the greater or lesser dark areas. The patterns of light and dark that are recognized as an image of the specimen. Because our eyes are very sensitive to variations in brightness, and then the image becomes more or less faithful reconstitution of the original specimen.

The objective lens at first formed a real and inverted magnified image. And then the eye piece further magnifies the same image to virtual magnified image.

Focusing On Microscopic Objects

Start with Clean Lenses:

It is important that microscope lenses be very clean. Before viewing through a microscope, use lens paper to gently clean the lenses.

Begin at Low Power Magnification:

Begin by viewing the object through a low power lens. Depending on how small the object is, start with the scanning or low-power objective.

Using low-power objective lens, get the target object centered in the field-of-view and focus as much as possible, first by using the coarse focus and then fine-tuning the clarity of the image with the fine focus.

Once the object is in focus, switch to the next higher objective power. Do not change the focus or manipulate the focus knobs in any way while changing objectives.

Adjustments for oil immersion objective:

Without changing the adjustment of high power, turn to oil immersion objective. One drop of oil is added into on the slide. The nose piece is turned such that the oil immersion objective touches on the drop of oil. Open the iris diaphragm completely. Use only fine adjustments for focusing.

Advantages and disadvantages

Bright field microscopy is best suited to viewing stained or naturally pigmented specimens such as stained prepared slides of tissue sections or living photosynthetic organisms. It is useless for living specimens of bacteria, and inferior for non–photosynthetic protists or metazoans, or unstained cell suspensions or tissue sections.

2. FLUORESCENT MICROSCOPE

A fluorescence microscope is much the same as a conventional light microscope with added features to enhance its capabilities.

- The conventional microscope uses visible light (400–700 nanometers) to illuminate and produce a magnified image of a sample.
- A fluorescence microscope, on the other hand, uses a much higher intensity light source which excites a fluorescent species in a sample of interest. This fluorescent species in turn emits a lower energy light of a longer wavelength that produces the magnified image instead of the original light source.

Principle

The specimen is illuminated with light of a specific wavelength (or wavelengths) which is absorbed by the fluorophores, causing them to emit light of longer wavelengths (i.e., of a different color than the absorbed light). The illumination light is separated from the much weaker emitted fluorescence through the use of a spectral emission filter.

In most cases the sample of interest is labeled with a fluorescent substance known as a fluorophore and then illuminated through the lens with the higher energy source. The illumination light is absorbed by the fluorophores (now attached to the sample) and causes them to emit a longer lower energy wavelength light. This fluorescent light can be separated from the surrounding radiation with filters designed for that specific wavelength allowing the viewer to see only that which is fluorescing.

Instrumentation

Light Source

Fluorescence microscopy requires intense, near–monochromatic light sources. Four main types of light source are used, including xenon arc lamps or mercury–vapor lamps , lasers, and high–power LEDs. Lasers are most widely used for more complex fluorescence microscopy techniques like confocal microscopy

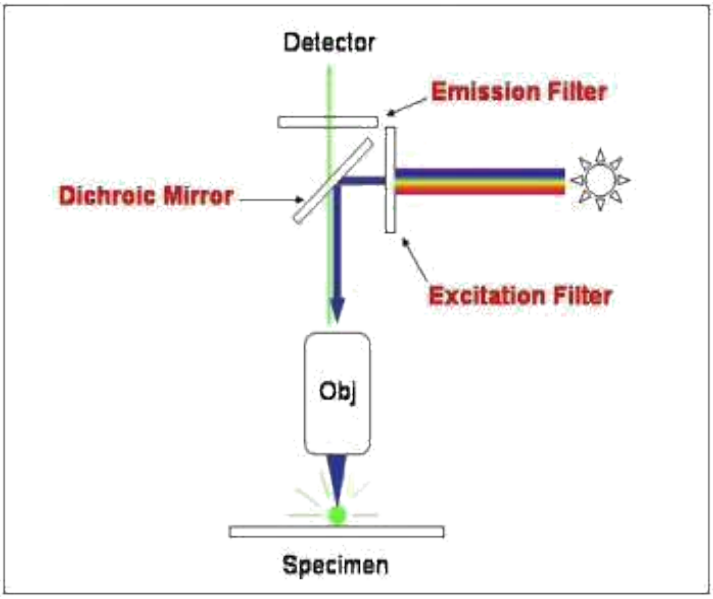


Fig: Flow chart for fluorescent microscope

Dichroic mirror

This is a special mirror that reflects the shorter wave length and passess the longer wave length

Sample preparation

In order for a sample to be suitable for fluorescence microscopy it must be fluorescent. There are several methods of creating a fluorescent sample; the main techniques are labelling with fluorescent stains.

Four very useful dyes for fluorescent staining are rhodamine and Texas red, which emit red light**; Cy3, which emits orange light; and fluorescein**, which emits green light. These dyes have a low, nonspecific affinity for biological molecules, but they can be chemically coupled to purified antibodies specific for almost any desired macromolecule.

Working procedure

The basic task of the fluorescence microscope is to let excitation light radiate the specimen and then sort out the much weaker emitted light from the image.

- First, the Specimen is labeled with fluorescent dye.

- Then the sample is illuminated with high energy source.This microscope has a filter that only lets through radiation with the specific wavelength that matches with the fluorescing material.

- When the radiation collides with the atoms in the specimen thr electrons are excited to a higher energy level. When they relax to a lower level, they emit longer, low wnwergy wave length light.
- To become detectable (visible to the human eye) the fluorescence emitted from the sample is separated from the much brighter excitation light in a second filter. This works because the emitted light is of lower energy and has a longer wavelength than the light that is used for illumination.

- This allows us to visualize the image of a desired organelle or targeted part of a given sample.

(Most of the fluorescence microscopes used in biology today are epi–fluorescence microscopes, meaning that both the excitation and the observation of the fluorescence occur above the sample. Most use a Xenon or Mercury arc–discharge lamp for the more intense light source.)

Applicatrions

These microscopes are often used for –

* Imaging structural components of small specimens, such as cells
* Conducting viability studies on cell populations
* Imaging the genetic material within a cell (DNA and RNA)
* Viewing specific cells within a larger population with techniques such as FISH

3.CONFOCAL MICROSCOPY

Immunofluorescence microscopy has its limitations. The fixatives employed to preserve cell architecture often destroy the *antigenicity* of a protein, that is, its ability to bind to its specific antibody. Also, the method generally gives poor results with thin cell sections, because embedding media often fluoresce themselves, obscuring the specific signal from the antibody. Moreover, in microscopy of whole cells, the fluorescent light comes from molecules above and below the plane of focus (out–of–focus light) result in reduction of image contrast and a decrease in resolution.

Many techniques have been developed to eliminate this out-of-focus light. The most commonly used is confocal microscopy, in which the sample is illuminated by a focused laser beam at a single point in the sample focal plane. Confocal microscopes use a pinhole to exclude the out of focus light.

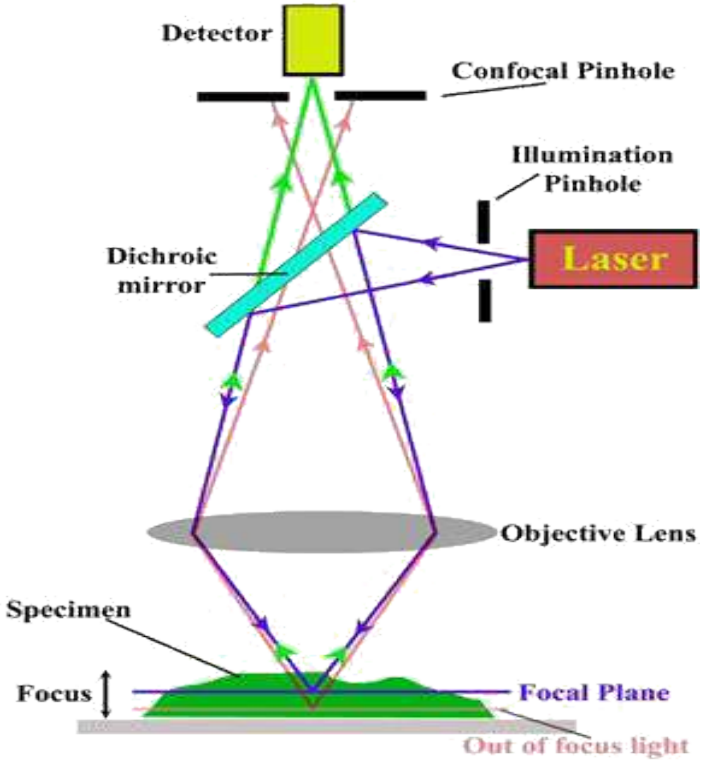


Fig : Schematic repesentation of assembly of confocal microscope

Instrumentation

Working procedure

- In the confocal microscope, all out-of-focus structures are suppressed at image formation. This is obtained by an arrangement of pinhole aperture which, at optically conjugated points of the path of rays, act as a point source(near light) and as a point detector(near detector) respectively.

- Light is passed through a pinhole, such that only light emitted from the focal plane is recorded on the detector. Light from out–of–focus planes is blocked by the pinhole, and so the confocal only records light from the focal plane of the sample. The detection pinhole does not permit rays of light from out–of–focus points to pass through it.

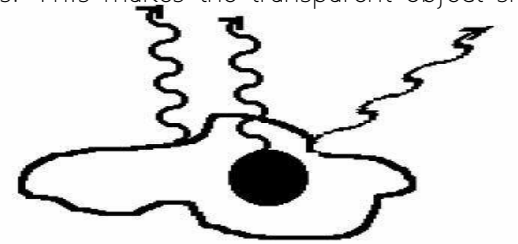
- The emitted/reflected light passing through the detector pinhole is transformed into electrical signals by a photomultiplier and displayed on a computer monitor
  - The wavelength of light, the numerical aperture of the objective and the diameter of the diaphragm (wider detection pinhole reduces the confocal effect) affect the depth of the focal plane. To obtain a full image, the point of light is moved across the specimen by scanning mirrors. Scanning mirrors are used to raster the laser spot across the sample, building up an image point by point.

4.PHASE CONTRAST MICROSCOPY

Phase contrast allow objects that differ slightly in refractive index or thickness to be distinguished within unstained or living cells. Differences in the thickness or refractive index within the specimen result in a differential retardation of light which shifts the phase or deviates the direction of the light (Figure). In phase contrast microscopy the phase differences are converted to intensity differences by special objectives and condensers and leads to an increased resolution without staining.

Principle

When light waves travel through a medium other than vacuum, interaction with the medium causes the wave amplitude and phase to change in a manner dependent on properties of the medium. Changes in amplitude (brightness) arise from the scattering and absorption of light, which is often wavelength–dependent and may give rise to colors. This difference in phase is not visible to the human eye. However, the change in phase can be increased by a transparent phase–plate in the microscope and thereby causing a difference in brightness. This makes the transparent object shine out in contrast to its surroundings.



Phase Shift vs Diffraction

Instrumentation

- Presented in Figure is a cut-away diagram of a modern upright phase contrast microscope, including a schematic illustration of the phase contrast optical train.
- Partially coherent illumination is produced by the tungsten-halogen lamp
- Light is directed through a collector lens and focused on a specialized annulus (labeled **condenser annulus**) positioned in the substage condenser front focal plane.
- Wavefronts passing through the annulus illuminate the specimen and either pass through undeviated or are diffracted and retarded in phase by structures and phase gradients present in the specimen.
- Rays are segregated at the rear focal plane by a **phase plate** and focused at the intermediate image plane to form the final phase contrast image observed in the eyepieces.

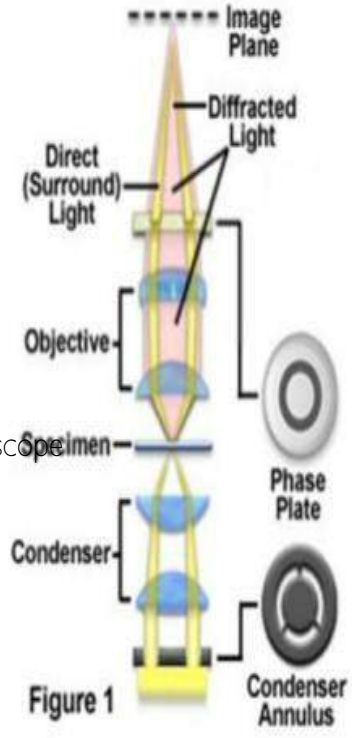


Fig: Instrumentation of phase contrast microscope

Working procedure

The ring–shaped illuminating light that passes the condenser annulus is focused on the specimen by the condenser. Some of the illuminating light is scattered by the specimen. The remaining light is unaffected by the specimen and forms the background light. When observing an unstained biological specimen, the scattered light is weak and typically phase–shifted by −90° (due to both the typical thickness of specimens and the refractive index difference between biological tissue and the surrounding medium) relative to the background light. This leads to the foreground and background having nearly the same intensity, resulting in low image contrast.

In a phase–contrast microscope, image contrast is increased in two ways: First, the background light is phase–shifted by −90° by passing it through a phase–shift ring, which eliminates the phase difference between the background and the scattered light rays. resulting in an increase in the brightness of these areas compared to regions that do not contain the sample

The above describes *negative phase contrast*. In its *positive* form, the background light is instead phase–shifted by +90°. The background light will thus be 180° out of phase relative to the scattered light. This results in that the scattered light will be subtracted from the background light in to form an image where the foreground is darker than the background.

Applications

- Phase contrast enables internal cellular components, such as the membrane, nuclei, mitochondria, spindles, mitotic apparatus, chromosomes, Golgi apparatus, and cytoplasmic granules from both plant and animal cells and tissues to be readily visualized.
- In addition, phase contrast microscopy is widely employed in diagnosis of tumor cells and the growth, dynamics, and behavior of a wide variety of living cells in culture.

5.ELECTRON MICROSCOPY

The fundamental principles of electron microscopy are similar to those of light microscopy; the major difference is that electromagnetic lenses, not optical lenses,focus a high velocity electron beam instead of visible light. Because electrons are absorbed by atoms in air, the entire tube between the electron source and the viewing screen is maintained under an ultrahigh vacuum.

In an electron microscope, these four things are slightly different.

1. The light source is replaced by a beam of very fast moving electrons.
2. The specimen usually has to be specially prepared and held inside a vacuum chamber from which the air has been pumped out (because electrons do not travel very far in air).
3. The lenses are replaced by a series of coil–shaped electromagnets through which the electron beam travels. In an ordinary microscope, the glass lenses bend (or refract) the light beams passing through them to produce magnification. In an electron microscope, the coils bend the electron beams the same way.
4. The image is formed as a photograph (called an electron micrograph) or as an image on a TV screen.

There are actually quite a few different types of electron microscopes and they all work in different ways. The three most familiar types are called transmission electron microscopes (TEMs), scanning electron microscopes (SEMs), and scanning tunneling microscopes (STMs).

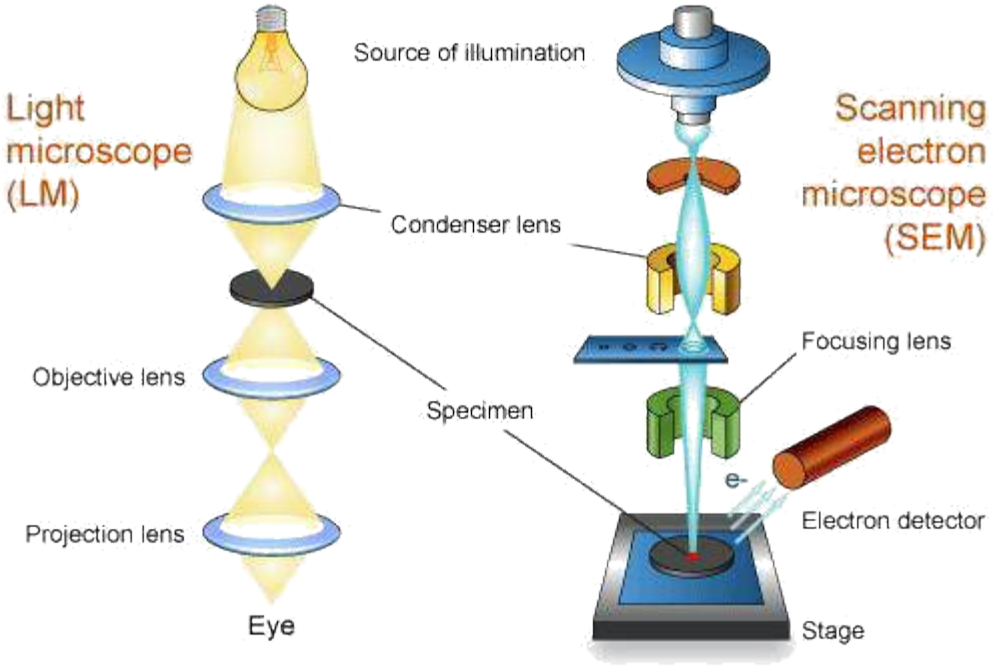


Fig: Electron microscope

Three main types of electron sources are used in electron microscopes: tungsten, lanthanum hexaboride (LaB6 – often called —lab six‖), and field emission gun (FEG). Each represents a different combination of costs and benefits. The choice of source type is an important part of the instrument selection process. Perhaps the single most important characteristic of the source is brightness that ultimately determines the resolution.

Transmission Electron Microscopy Has a Limit of Resolution of 0.1 nm

The *transmission electron microscope* (TEM) directs a beam of electrons through a specimen. Electrons are emitted by a tungsten cathode when it is electrically heated. The electric potential of the cathode is kept at 50,000 – 100,000 volts; that of the anode, near the top of the tube, is zero. This drop in voltage causes the electrons to accelerate as they move toward the anode. A condenser lens focuses the electron beam onto the sample; objective and projector lenses focus

the electrons that pass through the specimen and project them onto a viewing screen or a piece of photographic film

Sample preparation

Like the light microscope, the transmission electron microscope is used to view thin sections of a specimen, but the fixed sections must be much thinner for electron microscopy (only 50 – 100 nm, about 0.2 percent of the thickness of a single cell). Clearly, only a small portion of a cell can be observed in any one section. electron microscopy. Generation of the image depends on differential scattering of the incident electrons by molecules in the preparation. Without staining, the beam of electrons passes through a cell or tissue sample uniformly, so the entire sample appears uniformly bright with little differentiation of components. Staining techniques are therefore used to reveal the location and distribution of specific materials.

Scanning electron microscopy visualizes details on the surfaces of cells and particles

The *scanning electron microscope* allows the investigator to view the surfaces of unsectioned specimens. These cannot be visualized with transmission equipment because the electrons pass through the entire specimen. The sample is fixed, dried, and coated with a thin layer of a heavy metal, such as platinum, by evaporation in a vacuum; in this case, the sample is rotated so that the platinum is deposited uniformly on the surface. An intense electron beam inside the microscope

scans rapidly over the sample. Molecules in the specimen are excited and release secondary electrons that are focused onto a scintillation detector; the resulting signal is displayed on a cathoderay tube. Because the number of secondary electrons produced by any one point on the sample depends on the angle of the electron beam in relation to the surface, the scanning electron micrograph has a threedimensional appearance

The resolving power of scanning electron microscopes, which is limited by the thickness of the metal coating, is only about 10 nm, much less than that of transmission instruments.

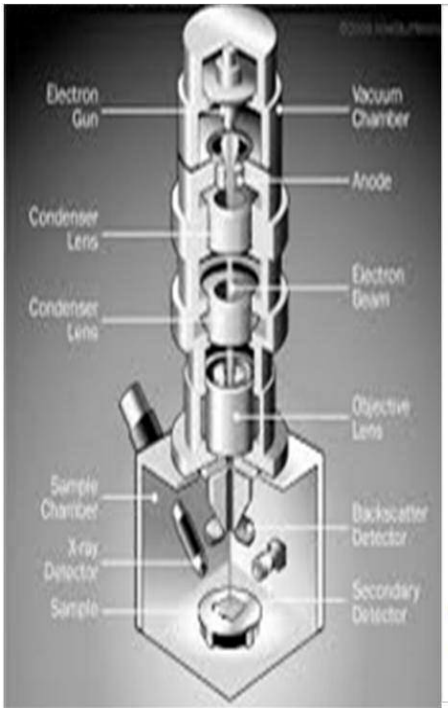


Fig: Scanning electron microscope

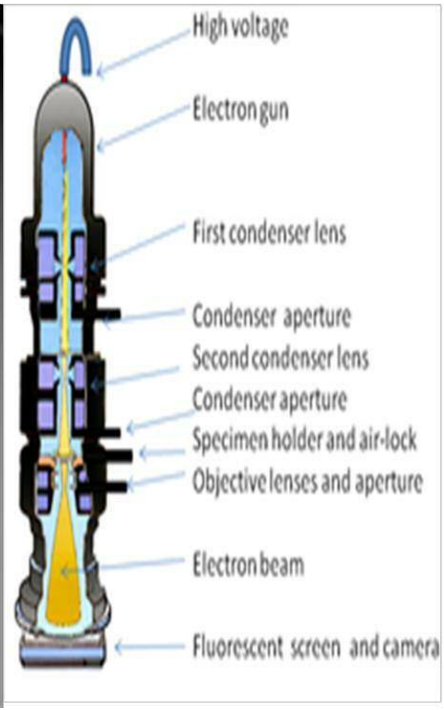


Fig: Transmission electron microscope

Applications

Electron microscopy (EM) gives the highest-resolution and is the only technique with sufficient resolution to localize proteins to small membrane subdomains in the context of the cell.

Electron microscopy also is used to obtain information about the shapes of purified viruses, fibers,enzymes, and other subcellular particles.

Scanning Electron Microscope

1. Scanning Electron Microscope can detect and analyze surface fractures, provide information in microstructures, examine surface contaminations, reveal spatial variations in chemical compositions, provide qualitative chemical analyses and identify crystalline structures.

2. SEMs can be as essential research tool in fields such as life science, biology, gemology, medical and forensic science, metallurgy.
3. In addition, SEMs have practical industrial and technological applications such as semiconductor inspection, production line of miniscule products and assembly of microchips for computers.

Transmission Electron Microscope

1. Transmission Electron Microscope is ideal for a number of different fields such as life sciences, nanotechnology, medical, biological and material research, forensic analysis, gemology and metallurgy as well as industry and education.
2. TEMs provide topographical, morphological, compositional and crystalline information.
3. The images allow researchers to view samples on a molecular level, making it possible to analyze structure and texture.
4. It also has industrial applications where TEMs can be used in semiconductor analysis and production and the manufacturing of computer and silicon chips.
5. Technology companies use TEMs to identify flaws, fractures and damages to micro-sized objects

FACS (Fluorescence activated cell sorter)

In multicellular organisms, all the cells are identical in their DNA but the proteins vary tremendously. Therefore, it would be very useful if we could separate cells that are phenotypically different from each other. In addition, it would be great to know how many cells expressed proteins of interest, and how much of this protein they expressed. Fluorescence Activated Cell Sorting (FACS) is a method that can accomplish all these goals.

Working procedure

The process begins by placing the cells into a flask and forcing the cells to enter a small nozzle one at a time (figure 1). The cells travel down the nozzle which is vibrated at an optimal frequency to produce drops at fixed distance from the nozzle. As the cells flow down the stream of liquid, they are scanned by a laser (blue light in figure 1). Some of the laser light is scattered (red cone emanating from the red cell) by the cells and this is used to count the cells. This scattered light can also be used to measure the size of the cells.

To separate a subpopulation of cells, they can be tagged with an antibody linked to a fluorescent dye. The antibody is bound to a protein that is uniquely expressed in the cells

we want to separate. The laser light excites the dye which emits a color of light that is detected by the photomultiplier tube, or light detector. By collecting the information from the light (scatter and fluorescence) a computer can determine which cells are to be separated and collected.

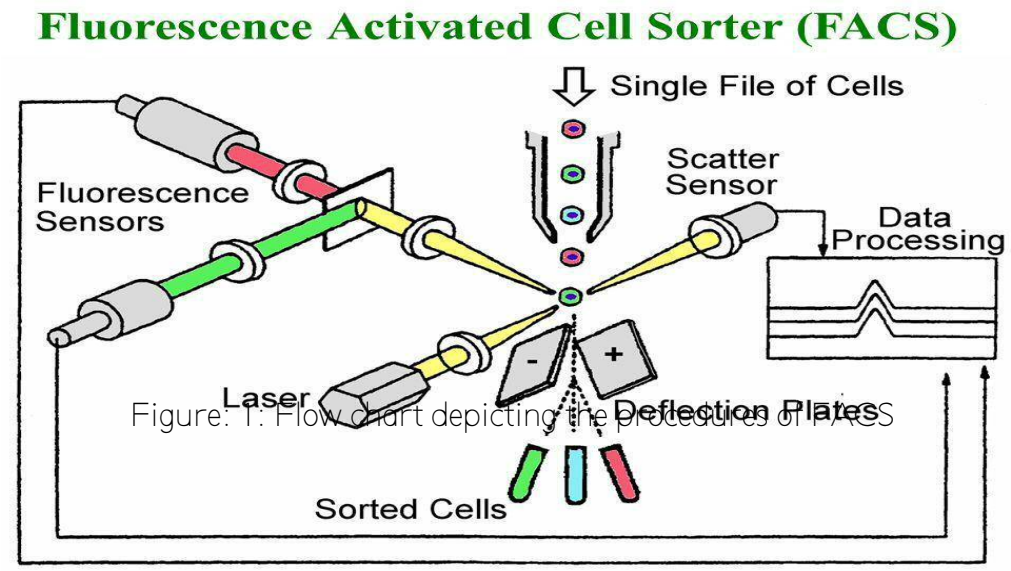


Figure: 1: Flow chart depicting the procedures of FACS

Cells have been fluorescently tagged with either red or green antibodies, though not every cell expresses the epitope and therefore some are not tagged either color.

The final step is sorting the cells which is accomplished by electrical charge. The computer determines how the cells will be sorted before the drop forms at the end of the stream. As the drop forms, an electrical charge is applied to the stream and the newly formed drop will form with a charge. This charged drop is then deflected left or right by

charged electrodes and into waiting sample tubes. Drops that contain no cells are sent into the waste tube. The end result is three tubes with pure subpopulations of cells. The number of cells is each tube is known and the level of fluorescence is also recorded for each cell.

Quantifying FACS Data

FACS data collected by the computer can be displayed in two different ways. What we want to know is how many cells of each color were sorted. In the first example (figure 2), we see the intensity of the green or red fluorescence is plotted on the X–axis and the number of cells with each level of flourescence is plotted on the Y–axis. In this example, there were twice as many red cells sorted as green or unlabeled cells, but the level of light

was greater from the green cells than the red cells. This method is best if all cells are either green, red or unlabeled and no cells are labeled both colors. Figure: 2

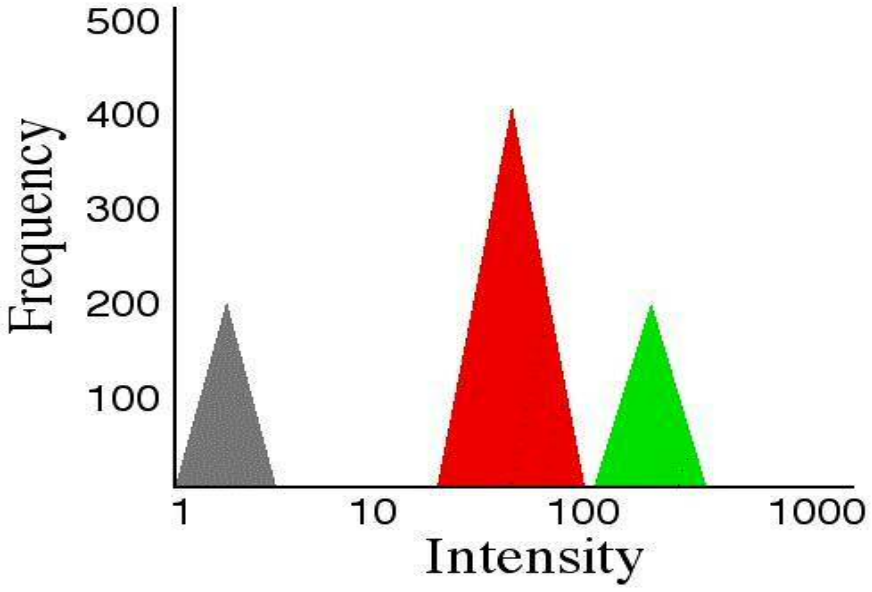


Figure 3. Quantification of FACS data. This graph shows the number of cells (Y-axis) and the level of fluorescence emitted (X-axis) by the labeled cells. Many different colors can be plotted on this graph, but cells should not be labeled by more than one color.

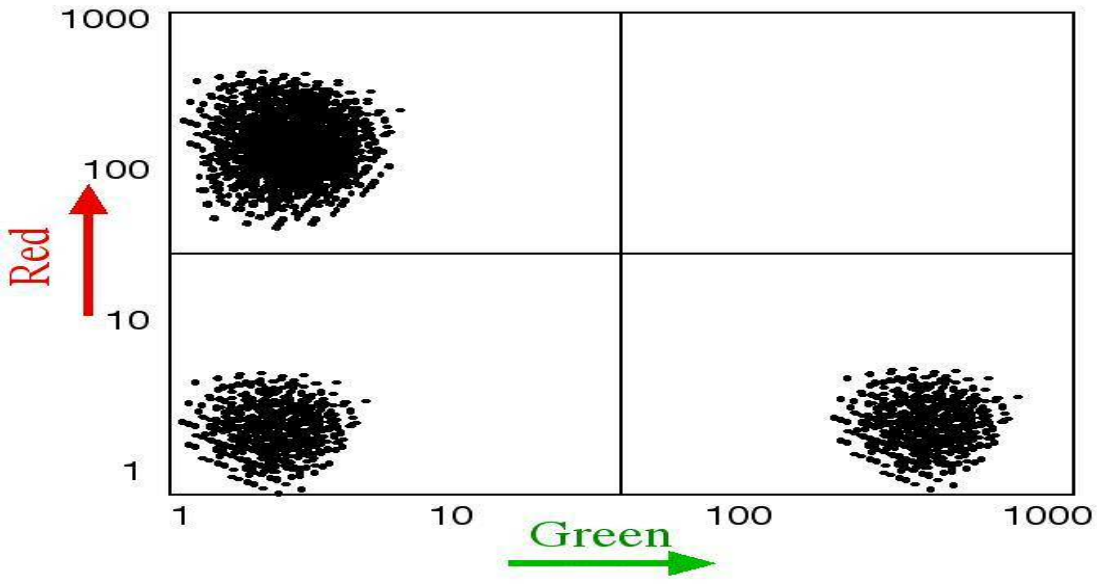


Figure 4. Quantification of FACS data. This graph compares the number of cells labeled by two colors – red (Y-axis) and green (X-axis). The intensity of the emitted light

increases as indicated by the arrows. The number of cells at each intensity is shown by the number of dots where each dot represents a single cell. This graph does not work for more than two colors but it works well when individual cells can be labeled by both colors at the same time.

In figure 4, The X–axis plots the intensity of green fluorescence while the Y–axis plots the intensity of red fluorescence. The individual black dots represent individual cells and. From this graph, we can see there were no cells labeled both red and green (top right) and many cells that were unlabeled (bottom left). The number of green–labeled cells (bottom right) is about the same as the number of unlabeled cells, but the number of red–labeled cells (top left) is about twice that of the other two categories of cells. Again, the level of fluorescence was higher in the green cells than the red ones. This method of graphing the data is especially useful if cells are present that have been labeled both red and green.

Applications of FACS

1.It is routinely used in pharmaceutical and biotechnology companies to isolate cells. Typically, the cells in suspension are ―tagged‖ with fluorescent antibodies. This tag allows for pertinent cells to be identified and isolated into a liquid medium for further analysis.

2.Biological applications for cell sorting include:

Protein Engineering & Development

- Screening of peptide libraries for binding
- Selecting antibody mutants
- Screening for enzymatic activity
- Screening for over–producing cells

Cell Engineering

Disease Identification/Characterization

- Isolating cells to characterize them based on multiple modalities
  - Nucleic acid
  - Protein expression
  - Cellular function

CENTRIFUGATION OF SUBCELLULUAR ORGANELLES

The centrifuge is now firmly established as an indispensable tool in virtually all phases of modern biology. Centrifugation may be used either: (1) to separate a mixture of different substances in suspension or solution and to isolate these substances into suspensions or solutions containing only one kind of particle; or (2) to characterize the size, shape and

density of the particles after they have been separated into purified solutions or suspensions . Consequently, centrifugation can be used in both purification and characterization of a wide range of biological substances. centrifuge invaluable and very widely used in cellular and molecular biology. Because of its practically universal application, an exorbitantly lengthy discussion would be required to review all biological uses of the centrifuge.

Cell fractionation is a procedure for rupturing cells, separation and suspension of cell constituents in isotonic medium in order to study their structure, chemical composition and function. Cell fractionation involves 3 steps: Extraction, Homogenization and Centrifugation.

1. Extraction:

It is the first step toward isolating any subcellular structures. In order to maintain the biological activity of organelles and biomolecules, they must be extracted in mild conditions called cell free systems. For these, the cells or tissues are suspended in a solution of appropriate pH and salt content, usually isotonic sucrose (0.25 mol/L) at – 40°C.

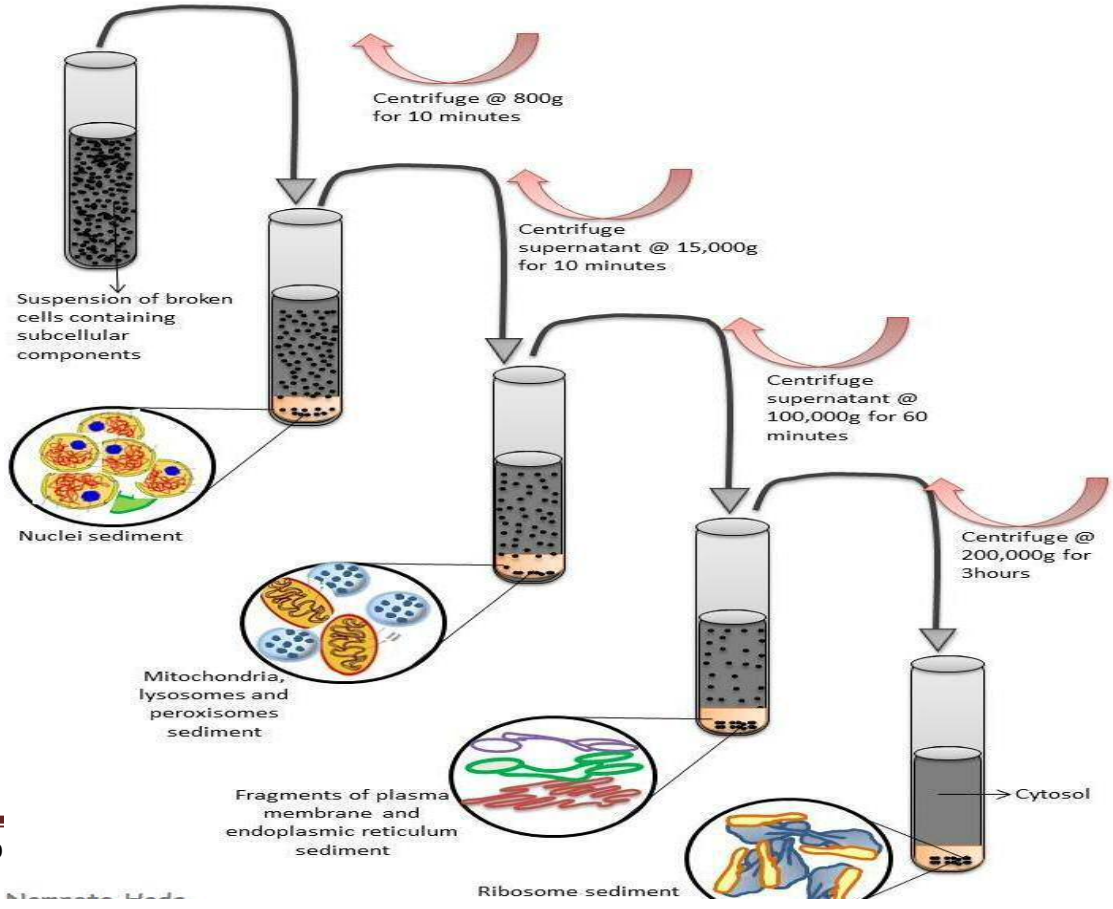


Fig: Diffeential centrifugation

2. Homogenization:

The suspended cells are then disrupted by the process of homogenization.

It is usually done by:

(i) Grinding
(ii) High Pressure (French Press or Nitrogen Bomb),
(iii) Osmotic shock,

(iv) Sonication (ultrasonic vibrations). Grinding is done by pestle and mortar or potter homogenizer (a highspeed blender). The later consists of two cylinders separated by a narrow gap.

The shearing force produced by the movement of cylinders causes the rupture of ceils. Ultrasonic waves are produced by piezoelectric crystal. They are transmitted to a steel rod placed in the suspension containing cells. Ultrasonic waves produce vibrations which rupture the cells. The liquid containing suspension of cell organelles and ether constituents is called homogenate. Sugar or sucrose solution preserves the cell organelles and prevents their clumping.

3. Centrifugation:

The separation (fractionation) of various components of the homogenate is carried out by a series of cemrifugations in an instrument called preparative ultracentrifuge. The ultracentrifuge has a metal rotor containing cylindrical holes to accommodate centrifuge tubes and a motor that spin the rotor at high speed to generate centrifugal forces.

KARPAGAM ACADEMY OF HIGHER EDUCATION
COIMBATORE - 641021
DEPARTMENT OF BIOCHEMISTRY
I-B.Sc., BIOCHEMISTRY - BATCH: 2017 - 2020
PART A (20 X 1 = 20 MARKS) - Online MCQ Questions

SUBJECT: (SUBJECT CODE: 17BCU102

Questions	Option A	Option B	Option C	Option D	Answer
The contribution of Robert Hooke	Coined the term karyokonesis	Coined the term cell membrane	Coined the term cell	Invented microscope	Coined the term cell
Schleiden and Schwann proposed cell theory in the year	1949	1839	1769	1689	1839
Prokaryotic cells lack	Cell wall	Cytoplasm	DNA	Nucleus	Nucleus
----------is the structural and functional unit of all organisms	Cell	DNA	RNA	Gene	Cell
Bacteria is Classified in to gram positive and gram negative on the basis of	Cell wall	Nucleus	Mitochondria	Shape	Cell wall

The heredity unit of cell is	Genes	Cell membrane	Ribosomes	Nucleus	Genes
Important feature of the plant cell is the presence of	Microsome	Cytoplasm	Cell wall	Mitochondria	Cell wall
Simple non-nucleated cells are ---------	Prokaryotic cells	Eukaryotic cells	Stem cells	Blood cells	Prokaryotic cells
Cell wall is absent in	Mycoplasma	Gram positive bacteria	Gram-negative Bacteria.	Gram positive and negative	Mycoplasma
In prokaryotes the nuclear body is called ------	Nucleolus	Nucleosome	Plasmid	Nucleus	Nucleosome
Cell model for plant species is	C.elegans	A.thaliana	Xenopus oocyte	Zebrafish	A.thaliana
Prokaryotic cell Contain	Centriole	DNA	Nuclear envelope	Nuc1eolus	DNA
---------- Cells does not contain important internal membrane-bound compartments	Prokaryotic	Ecukatyotic	Both Prokaryotic and ecukatyotic	Only in Bacteria	Prokaryotic

Plant cell wall contains	Cellulose	Cytoskeleton	Sucrose	Amylose	Cellulose
Animal with 99% homology with human is	Xenopus oocyte	Zebrafish	Mouse	Pig	Mouse
Among the following which is eukaryotic unicellular organism	E.coli	S.typhi	S.cerevisiae	C.elegans	S.cerevisiae
_____ have also been to play role in certain infectious disease	Glycolipids	Glyco proteins	Lipo proteins	Phospholipids	Glycolipids
Integral proteins are bound to membrane by _____	Ionic bond	Covalent bond	Hydrogen bond	Disulphide bond	Covalent bond
Which carbohydrate is abundant in Plasma memberane?	Glycocerebroside	Cerebroside	Gangliosid e	Galactosamid e	Ganglioside
Cell surface antigens are ____	Phospholipid	Cerebroside	Glycoproteins	Sphingomyelin	Glycoproteins

The fluid model was introduced by	S.J Singer and J.D Robertso n	J.N Robertson	Jacob and Monad	S.J Singer and G.Nicolson.	S.J Singer and G.Nicolson.
_____ contains galactolipids.	Mitochon dria	Chloroplast	Golgi complex	Endoplasmic reticulum	Chloroplast
In Glycoprotein, the carbohydrate is present in having fewer than about ____ per chain	20 sugars	35 sugars	15 Sugars	10 Sugars	15 Sugars
In which ratio the lipids and proteins are bound in membrane bilayer	1:02	1:01	2:01	2:02	2:01
The lipids conjugated with carbohydrate that present in membrane bilayer is called	Glycolipi d	Phospholipi d	Ceramide	Sphingomyeli n	Glycolipid

Reserve materials of bacteria are stored in the cytoplasm in granules called	Storage depots	Inclusion bodies	Vacuoles	Plastids	Storage depots
Cholesterol is abundant in the plasma memebrane of mammalian cells but it is	Absent in prokaryotic cells	Small amount is present in prokaryotic cells	Equally present in prokaryotic cells	Present in large amount in prokaryotic cells	Absent in prokaryotic cells
The prokaryotic cells contains	70s ribosomes	80s ribosomes	60s ribosomes	50s ribosomes	70s ribosomes
Glycocalyx is	polysaccharide	loose carbohydrat	lipid	carrier protein	loose carbohydrate
Cells that are engaged in protein synthesis will have well developed	RER	SER	glyoxysomes	peroxysomes	RER
Rough endoplasmic reticulum is embedded with	Proteins	RNA	Ribosomes	Lipids	Ribosomes

The golgi apparatus is important for	protein synthesis	DNA synthesis	packaging and secretion of proteins	RNA synthesisis	packaging and secretion of proteins
Bilayer fluidity mainly determined by	Phospholipid	protein	Cholesterol	Glycolipid	Cholesterol
The size of the prokaryotic cell is	1-10 µm	1µ to 2 mm	10µ to 100 mm	1mm to 3 mm	1-10 µm
Plasma membrane is also called as	Plasmalemma	Periplasm	Cytoplasm	Cell wall	Plasmalemma
---------- type of substance not found in cell membrane	phospholipids	glycolipids	steroids	Nucleic acids	Nucleic acids
The majority of cases lipid in cell membrane is	phospholipids	glycolipids	steroids	Nucleic acids	phospholipids
The entire membrane is held together by---------- interaction of hydrophobic tails	covalent	non covalent	dipeptide	hydroxide	non covalent

The large amount of ---------- is responsible for various activities of cell membrane.	protein	lipids	amino acids	fatty acids	protein
The cell membrane contains ---------- "tail" regions	hydrophobic	hydrophilic	thermophilic	mesophilic	hydrophobic
The cell membrane contains ---------- "head" regions	hydrophobic	hydrophilic	thermophilic	mesophilic	hydrophilic
Cellular components of lipid bilayer is visualized by	SEM	TEM	DFM	EM	TEM
Key role of cell membrane is to maintain the	cell function	cell potential	cell organelles	cell structure	cell potential

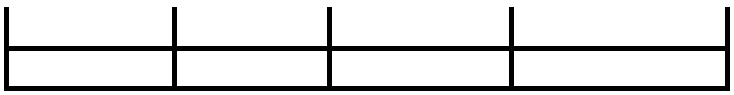
---------- provides the shape of the cell	cytoskeleton	matrix	cytoplasm	organelles	cytoskeleton
---------- are described as amphipathic in plasma membrane	lipids	proteins	steroids	carbohydrates	lipids
---------- is a common component of animal cell membranes and functions to help stabilize the membrane	Glycerol	Cholesterol	triglycerides	Fat	Cholesterol
---------- affects the fluidity of the membrane.	Temperature	pH	Osmolality	Pressure	Temperature

Kind of electron microscope which is used to study internal structure of cells is	scanning electron microscope	transmission electron microscope	light microscope	compound microscope	transmission electron microscope
Magnification of light microscope is	1500X	2000X	1000X	2500X	1500X
Photograph which is taken from microscope is known as	macrograph	monograph	micrograph	pictograph	micrograph
Object can be magnified under electron microscope about	350, 000 times	250, 000 times	300, 000 times	450, 000 times	300, 000 times
When the power of ocular lens is 10 X and objective lens is 20 X, the magnification is	30 times	20 times	200 times	2000 times	200 times

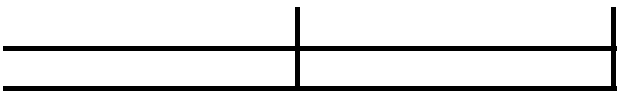
Which of the following light is suitable for getting maximum resolution?	Red	Green	Blue	Orange	Blue
Which of the following is used to visualize live cells?	SEM	TEM	Phase contrast microscope	Compound microscope	Phase contrast microscope
The strength of magnification of oil immersion lens is	20X	100X	50X	40X	100X
Single cells can be isolated using the following technique	Fluorescence activated cell sorting	Polymerase chain reaction	Gel electrophoresis	Agarose gel electrophoresis	Fluorescence activated cell sorting

Which of the following microscopy techniques relies on the specimen interfering with the wavelength of light to produce a high contrast image without the need for dyes or any damage to the sample?	Conventional bright field light microscopy	Phase contrast microscopy	Electron microscopy	Fluorescence microscopy	Phase contrast microscopy
The lens that is within the eyepiece of the light microscope is called the	Scanning	Low power	High power	Ocular	Ocular
What is the condenser on a light microscope used for?	To focus the light source	To diffuse the light source	To provide the light source	To control the light source	To focus the light source

What is the correct name for the main microscope lens that focuses the image?	Ocular	Binocular	Objective	Condenser	Objective



UNIT I



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

SUBJECT : CELL BIOLOGY
SEMESTER : I
SUBJECT CODE : 17BCU102 CLASS : I B.Sc., BC

UNIT II - COURSE MATERIAL

UNIT 2
Structure of different cell organelles
Structure of nuclear envelope, nuclear pore complex. Selective transport of proteins to and from the nucleus. Regulation of nuclear protein import and export.
ER structure. Targeting proteins to ER, smooth ER and lipid synthesis. Export of proteins and lipids from ER and into ER. Protein folding in ER
Peroxisomes and Zellweger syndrome.

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UNIT II - STRUCTURE OF DIFFERENT CELL ORGANELLES

NUCLEAR ENVELOPE AND NUCLEOPORE COMPLEX

The nuclear envelope separates the contents of the nucleus from the cytoplasm and provides the structural framework of the nucleus. The nuclear membranes, acting as barriers that prevent the free passage of molecules between the nucleus and the cytoplasm, maintain the nucleus as a distinct biochemical compartment. The sole channels through the nuclear envelope are provided by the nuclear pore complexes, which allow the regulated exchange of molecules between the nucleus and cytoplasm. The selective traffic of proteins and RNAs through the nuclear pore complexes not only establishes the internal composition of the nucleus, but also plays a critical role in regulating eukaryotic gene expression

Structure of the Nuclear Envelope

The nuclear envelope has a complex structure, consisting of two nuclear membranes, an underlying nuclear lamina, and nuclear pore complexes (Figure). The nucleus is surrounded by a system of two concentric membranes, called the inner and outer nuclear membranes. The outer nuclear membrane is continuous with the endoplasmic reticulum, so the space between the inner and outer nuclear membranes is directly connected with the lumen of the endoplasmic reticulum. In addition, the outer nuclear membrane is functionally similar to the membranes of the endoplasmic reticulum and has ribosomes bound to its cytoplasmic surface. In contrast, the inner nuclear membrane carries unique proteins that are specific to the nucleus.

The critical function of the nuclear membranes is to act as a barrier that separates the contents of the nucleus from the cytoplasm. Like other cell membranes, the nuclear membranes are phospholipid bilayers, which are permeable only to small nonpolar molecules. Other molecules are unable to diffuse through the phospholipid bilayer. The inner and outer nuclear membranes are joined at nuclear pore complexes, the sole channels through which small polar molecules and macromolecules are able to travel through the nuclear envelope (Figure). The nuclear pore complex is a complicated structure that is responsible for the selective traffic of proteins and RNAs between the nucleus and the cytoplasm.

Underlying the inner nuclear membrane is the nuclear lamina, a fibrous meshwork that provides structural support to thenucleus (Figure). The nuclear lamina is composed of one or more related proteins called lamins. Most mammalian cells, for example, contain four different lamins, designated A, B_1, B_2, and C. All the lamins are 60- to 80-kilodalton (kd) fibrous proteins that are related to the intermediate filament proteins of the cytoskeleton. Like other intermediate filament proteins, the lamins associate with each other to form filaments. The first stage of this association is the interaction of two lamins to form a dimer in which the α-helical regions of two polypeptide chains are wound around each other in a structure called a coiled coil. These lamin dimers then associate with each other to form the filaments that make up the nuclear

lamina. The association of lamins with the inner nuclear membrane is facilitated by the posttranslational addition of lipid—in particular, prenylation of C-terminal cysteine residues. In addition, the lamins bind to inner nuclear membrane proteins, which may help organize the lamin filaments into a meshwork and mediate their attachment to the membrane.

NUCLEAR PORE COMPLEXES

The nuclear pore complexes are the only channels through which small polar molecules, ions, and macromolecules (proteins and RNAs) are able to travel between the nucleus and the cytoplasm. The nuclear pore complex is an extremely large structure with a diameter of about 120 nm and an estimated molecular mass of approximately 125 million daltons—about 30 times the size of a ribosome. In vertebrates, the nuclear pore complex is composed of 50 to 100 different proteins. By controlling the traffic of molecules between the nucleus and cytoplasm, the nuclear pore complex plays a fundamental role in the physiology of all eukaryotic cells. RNAs that are synthesized in the nucleus must be efficiently exported to the cytoplasm, where they function in protein synthesis. Conversely, proteins required for nuclear functions (e.g., transcription factors) must be transported into the nucleus from their sites of synthesis in the cytoplasm. In addition, many proteins shuttle continuously between the nucleus and the cytoplasm. The regulated traffic of proteins and RNAs through the nuclear pore complex thus determines the composition of the nucleus and plays a key role in gene expression.

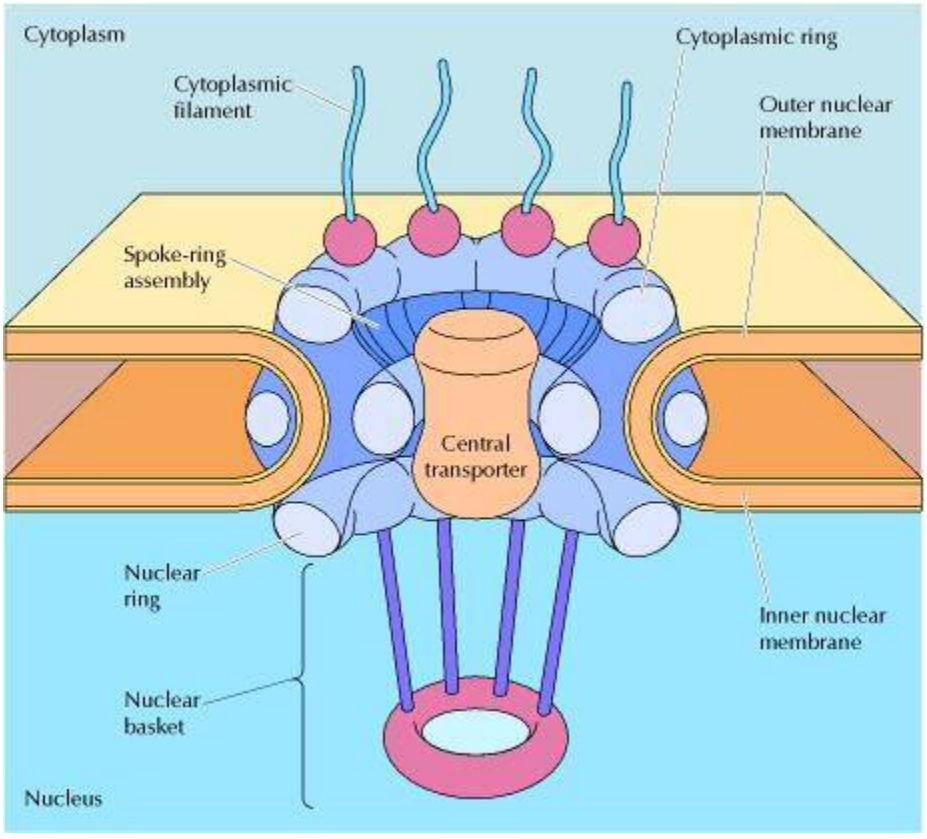


Figure: Model of the nuclear pore complex

Visualization of nuclear pore complexes by electron microscopy reveals a structure with eightfold symmetry organized around a large central channel (Figure), which is the route through which proteins and RNAs cross the nuclear envelope. Detailed structural studies, including computer-based image analysis, have led to the development of three-dimensional models of the nuclear pore complex (Figure). These studies indicate that the nuclear pore complex consists of an assembly of eight spokes arranged around a central channel. The spokes are connected to rings at the nuclear and cytoplasmic surfaces, and the spoke-ring assembly is anchored within the nuclear envelope at sites of fusion between the inner and outer nuclear membranes. Protein filaments extend from both the cytoplasmic and nuclear rings, forming a distinct basketlike structure on the nuclear side. The central channel is approximately 40 nm in diameter, which is wide enough to accommodate the largest particles able to cross the nuclear envelope. It contains a structure called the central transporter, through which the active transport of macromolecules is thought to occur.

Depending on their size, molecules can travel through the nuclear pore complex by one of two different mechanisms (Figure). Small molecules and some proteins with molecular mass less than approximately 50 kd pass freely across the nuclear envelope in either direction: cytoplasm to nucleus or nucleus to cytoplasm. These molecules diffuse passively through open aqueous channels, estimated to have diameters of approximately 9 nm, in the nuclear pore complex. Most proteins and RNAs, however, are unable to pass through these open channels. Instead, these macromolecules pass through the nuclear pore complex by an active process in which appropriate proteins and RNAs are recognized and selectively transported in only one direction (nucleus to cytoplasm or cytoplasm to nucleus). The traffic of these molecules occurs through regulated channels in the nuclear pore complex that, in response to appropriate signals, can open to a diameter of more than 25 nm—a size sufficient to accommodate large ribonucleoprotein complexes, such as ribosomal subunits. It is through these regulated channels that nuclear proteins are selectively imported from the cytoplasm to the nucleus while RNAs are exported from the nucleus to the cytoplasm.

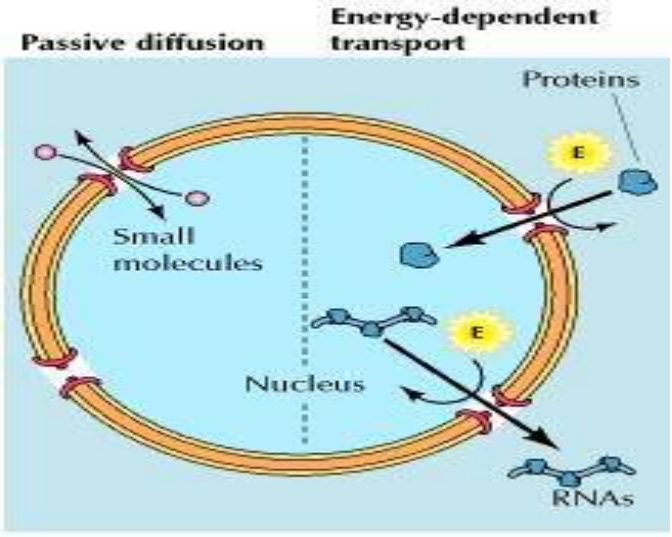


Figure: Molecular traffic through nuclear pore complexes: **Small molecules are able to pass rapidly through open channels in the nuclear pore complex by passive diffusion. In contrast, macromolecules are transported by a selective, energy-dependent mechanism that acts predominantly to import proteins to the nucleus and export RNAs to the cytoplasm.**

Selective Transport of Proteins to and from the Nucleus

The basis for selective traffic across the nuclear envelope is best understood for proteins that are imported from the cytoplasm to the nucleus. Such proteins are responsible for all aspects of genome structure and function; they include histones, DNA polymerases, RNA polymerases, transcription factors, splicing factors, and many others. These proteins are targeted to the nucleus by specific amino acid sequences, called nuclear localization signals, that direct their transport through the nuclear pore complex.

The first nuclear localization signal to be mapped in detail was characterized by Alan Smith and colleagues in 1984. Subsequent studies defined the T antigen nuclear localization signal as the seven-amino-acid sequence Pro-Lys-Lys-Lys-Arg-Lys-Val. Not only was this sequence necessary for the nuclear transport of T antigen.

Both the Lys-Arg and Lys-Lys-Lys-Lys sequences are required for nuclear targeting, but the ten amino acids between these sequences can be mutated without affecting nuclear localization.

Protein import through the nuclear pore complex can be operationally divided into two steps, distinguished by whether they require energy (Figure). In the first step, which does not require energy, proteins that contain nuclear localization signals bind to the nuclear pore complex but do not pass through the pore. In this initial step, nuclear localization signals are recognized by a cytosolic receptor protein, and the receptor-substrate complex binds to the nuclear pore. The prototype receptor, called importin, consists of two subunits. One subunit

(importin α) binds to the basic amino acid-rich nuclear localization signals of proteins. The second subunit (importin β) binds to the cytoplasmic filaments of the nuclear pore complex, bringing the target protein to the nuclear pore.

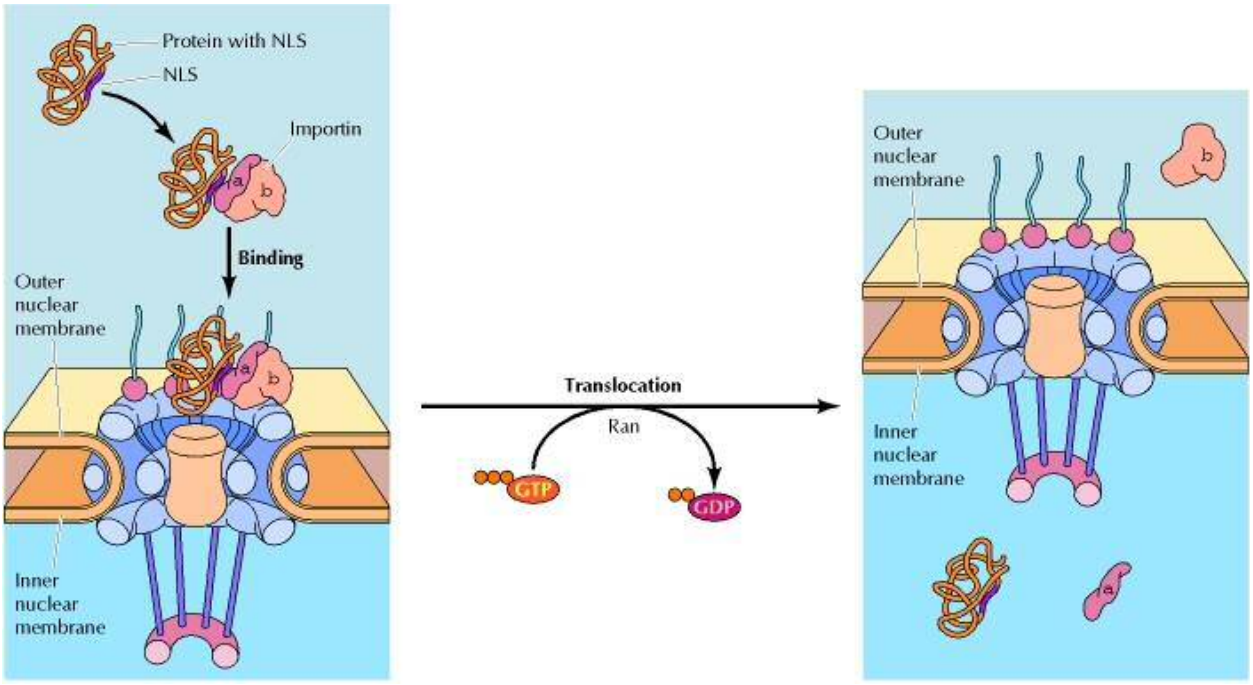


Figure -Protein import through the nuclear pore complex: **Proteins are transported through the nuclear pore complex in two steps. In the example shown, a protein with a classical basic amino acid-rich nuclear localization sequence (NLS) is recognized by importin α, which forms a complex with importin β. Importin β binds to the cytoplasmic filaments of the nuclear pore complex, bringing the target protein to the nuclear pore. The protein and importin α are then translocated through the nuclear pore complex in a second, energy-requiring step, which requires GTP hydrolysis by the Ran protein.**

The second step in nuclear import, translocation through the nuclear pore complex, is an energy-dependent process that requires GTP hydrolysis. A key player in the translocation process is a small GTP-binding protein called Ran, which is related to the Ras proteins (Figure). The conformation and activity of Ran is regulated by GTP binding and hydrolysis. Ran/GTP is thought to determine the directionality of nuclear transport, and GTP hydrolysis by Ran appears to account for most of the energy required for nuclear import. Importin β forms a complex withimportin α and its associated target protein on the cytoplasmic side of the nuclear pore complex, in the presence of a high concentration of Ran/ GDP. This complex is then transported through the nuclear pore to the nucleus, where a high concentration of Ran/GTP is present. At the nuclear side of the pore, Ran/GTP binds to importin β, displacing importin α and the target protein. As a result, the target protein is released within the nucleus. The Ran/GTP-importin β

complex is then exported to the cytosol, where the bound GTP is hydrolyzed to GDP, releasing importin β to participate in another cycle of nuclear import.

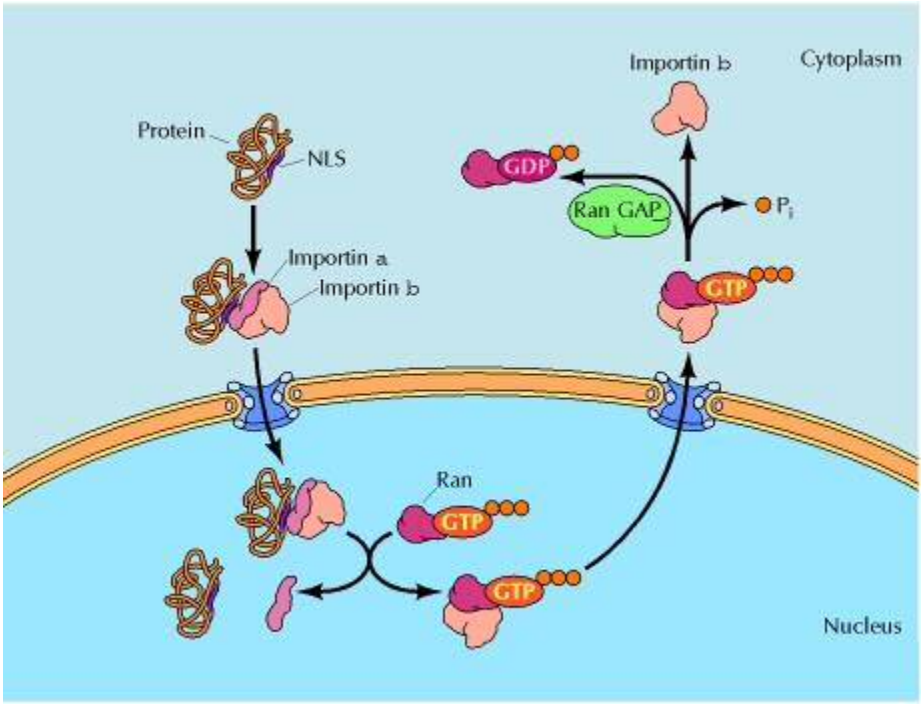


Figure Role of the Ran protein in nuclear import: **Transport through the nuclear pore complex is driven by a gradient of Ran/GTP, with a high concentration of Ran/GDP in the cytoplasm and a high concentration of Ran/GTP in the nucleus. Complexes form between target proteins containing nuclear localization signals (NLS), importin α, and importin β in the cytoplasm where Ran is in the GDP-bound form. Following transport through the nuclear pore complex, Ran/GTP binds to importin β, releasing importin α and the target protein in the nucleus. The Ran/GTP-importin β complex is then transported back to the cytoplasm, where the Ran GTPase-activating protein (Ran GAP) stimulates hydrolysis of the bound GTP to form Ran/GDP. This conversion of Ran/ GTP to Ran/GDP is accompanied by release of importin β.**

Protein Export:

Proteins are targeted for export from the nucleus by specific amino acid sequences, called nuclear export signals. Like nuclear localization signals, nuclear export signals are recognized by receptors within the nucleus that direct protein transport through the nuclear pore complex to the cytoplasm. Interestingly, the nuclear export receptors (called exportins) are related to importin β. Like importin β, the exportins bind to Ran, which is required for nuclear export as well as for nuclear import (Figure). Strikingly, however, Ran/GTP promotes the formation of stable complexes between exportins and their target proteins, whereas it dissociates the complexes between importins and their targets. This effect of Ran/GTP binding on exportins

dictates the movement of proteins containing nuclear export signals from the nucleus to the cytoplasm. Thus, exportins form stable complexes with their target proteins in association with Ran/GTP within the nucleus. Following transport to the cytosolic side of the nuclear envelope, GTP hydrolysis leads to dissociation of the target protein, which is released into the cytoplasm.

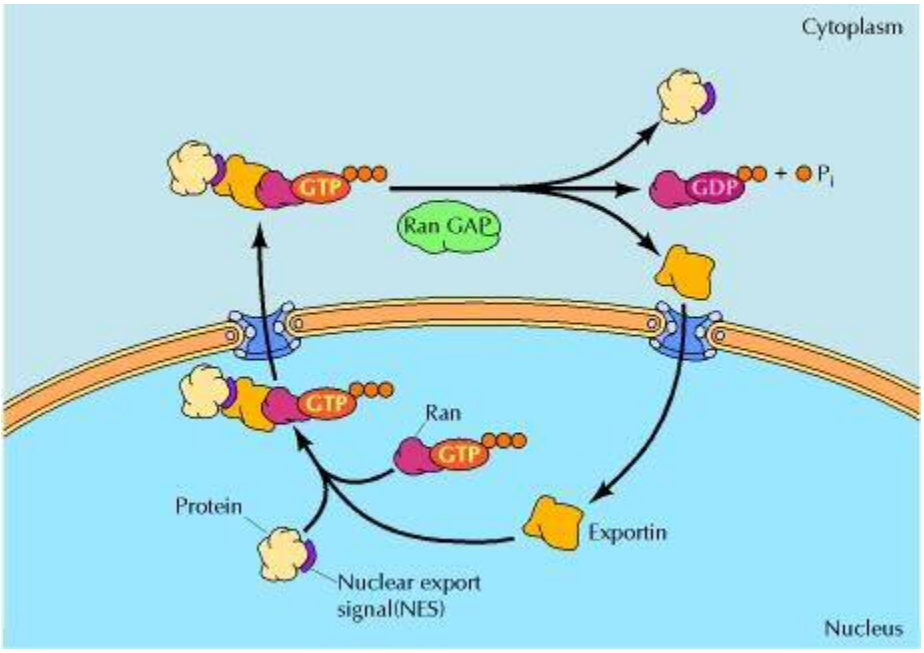


Figure Nuclear export: **Complexes between target proteins bearing nuclear export signals (NES), exportins, and Ran/ GTP form in the nucleus. Following transport through thenuclear pore complex, Ran GAP stimulates the hydrolysis of bound GTP, leading to formation of Ran/GDP and release of the target protein and exportin in the cytoplasm. Exportin is then transported back to the nucleus.**

Regulation of Nuclear Protein Import

The regulated nuclear import of both transcription factors and protein kinases plays an important role in controlling the behavior of cells in response to changes in the environment, because it provides a mechanism by which signals received at the cell surface can be transmitted to the nucleus.

In one mechanism of regulation, transcription factors (or other proteins) associate with cytoplasmic proteins that mask their nuclear localization signals; because their signals are no longer recognizable, these proteins remain in the cytoplasm. A good example is provided by the transcription factor NF-κB, which activates transcription of immunoglobulin-κ light chains in B lymphocytes (Figure). In unstimulated cells, NF-κB is found as an inactive complex with an inhibitory protein (IκB) in the cytoplasm. Binding to IκB appears to mask the NF-κB nuclear localization signal, thus preventing NF-κB from being transported into the nucleus. In stimulated

cells, IκB is phosphorylated and degraded by ubiquitin-mediated proteolysis, allowing NF-κB to enter the nucleus and activate transcription of its target genes.

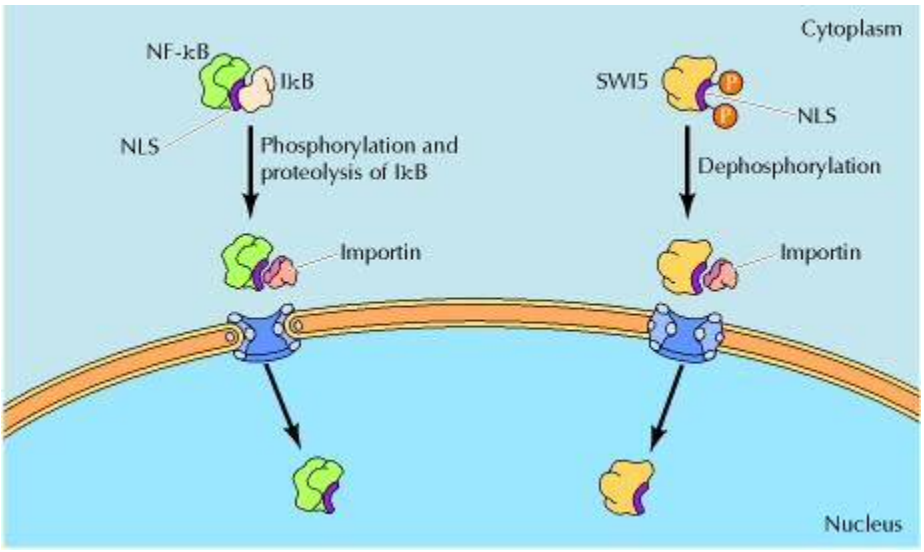


Figure Regulation of nuclear import of transcription factors: **The transcription factor NF-κB is maintained as an inactive complex with IκB, which masks its nuclear localization sequence (NLS), in the cytoplasm. In response to appropriate extracellular signals, IκB is phosphorylated and degraded by proteolysis, allowing the import of NF-κB to the nucleus. In contrast, the yeast transcription factor SWI5 is maintained in the cytoplasm by phosphorylation in the vicinity of its nuclear localization sequence. Regulated dephosphorylation exposes the NLS and allows SWI5 to be transported to the nucleus at the appropriate stage of the cell cycle.**

The nuclear import of other transcription factors is regulated directly by their phosphorylation, rather than by association with inhibitory proteins. For example, the yeast transcription factor SWI5 is imported into the nucleus only at a specific stage of the cell cycle (Figure). Otherwise, SWI5 is retained in the cytoplasm as a result of phosphorylation at serine residues adjacent to its nuclear localization signal, preventing nuclear import. Regulated dephosphorylation of these sites activates SWI5 at the appropriate stage of the cell cycle by permitting its translocation to the nucleus.

ENDOPLASMIC RETICULUM

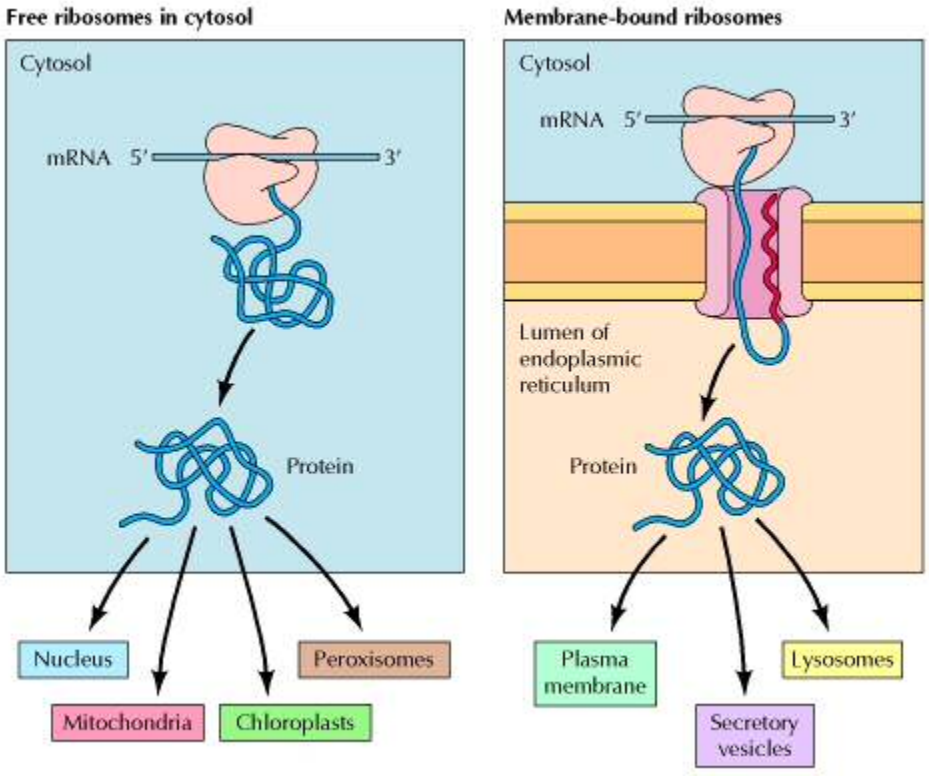
The endoplasmic reticulum (ER) is a network of membrane-enclosed tubules and sacs (cisternae) that extends from the nuclear membrane throughout the cytoplasm (Figure). The entire endoplasmic reticulum is enclosed by a continuous membrane and is the largest organelle of most eukaryotic cells. Its membrane may account for about half of all cell membranes, and the space enclosed by the ER (the lumen, or cisternal space) may represent about 10% of the total cell volume. As discussed below, there are two distinct types of ER that perform different

functions within the cell. The rough ER, which is covered by ribosomes on its outer surface, functions in protein processing. The smooth ER is not associated with ribosomes and is involved in lipid, rather than protein, metabolism.

The role of the endoplasmic reticulum in protein processing and sorting was first demonstrated by George Palade and his colleagues in the 1960s. Palade and coworkers were able to study the pathway taken by secreted proteins simply by labeling newly synthesized proteins with radioactive amino acids.

These experiments defined a pathway taken by secreted proteins, the secretory pathway: rough ER → Golgi →secretory vesicles → cell exterior. Further studies extended these results and demonstrated that this pathway is not restricted to proteins destined for secretion from the cell. Plasma membrane and lysosomal proteins also travel from the rough ER to the Golgi and then to their final destinations. Still other proteins travel through the initial steps of the secretory pathway but are then retained and function within either the ER or the Golgi apparatus.

The entrance of proteins into the ER thus represents a major branch point for the traffic of proteins within eukaryotic cells. Proteins destined for secretion or incorporation into the ER, Golgi apparatus, lysosomes, or plasma membrane are initially targeted to the ER. In mammalian cells, most proteins are transferred into the ER while they are being translated on membrane-bound ribosomes (Figure). In contrast, proteins destined to remain in the cytosol or to be incorporated into the nucleus, mitochondria, chloroplasts, or peroxisomes are synthesized on free ribosomes and released into the cytosol when their translation is complete.



Targeting Proteins to the Endoplasmic Reticulum

Proteins can be translocated into the ER either during their synthesis on membrane-bound ribosomes (cotranslational translocation) or after their translation has been completed on free ribosomes in the cytosol.

The mechanism by which secretory proteins are targeted to the ER during their translation (the cotranslational pathway) is now well understood. The signal sequences span about 20 amino acids, including a stretch of hydrophobic residues, usually at the amino terminus of the polypeptide chain (Figure). As they emerge from the ribosome, signal sequences are recognized and bound by a signal recognition particle (SRP) consisting of six polypeptides and a small cytoplasmic RNA (7SL RNA). SRP binds the ribosome as well as the signal sequence, inhibiting further translation and targeting the entire complex (the SRP, ribosome, and growing polypeptide chain) to the rough ER by binding to the SRP receptor on the ER membrane (Figure). Binding to the receptor releases the SRP from both the ribosome and the signal sequence of the growing polypeptide chain. The ribosome then binds to a protein translocation complex in the ER membrane, and the signal sequence is inserted into a membrane channel. As translocation proceeds, the signal sequence is cleaved by signal peptidase and the polypeptide is released into the lumen of the ER.

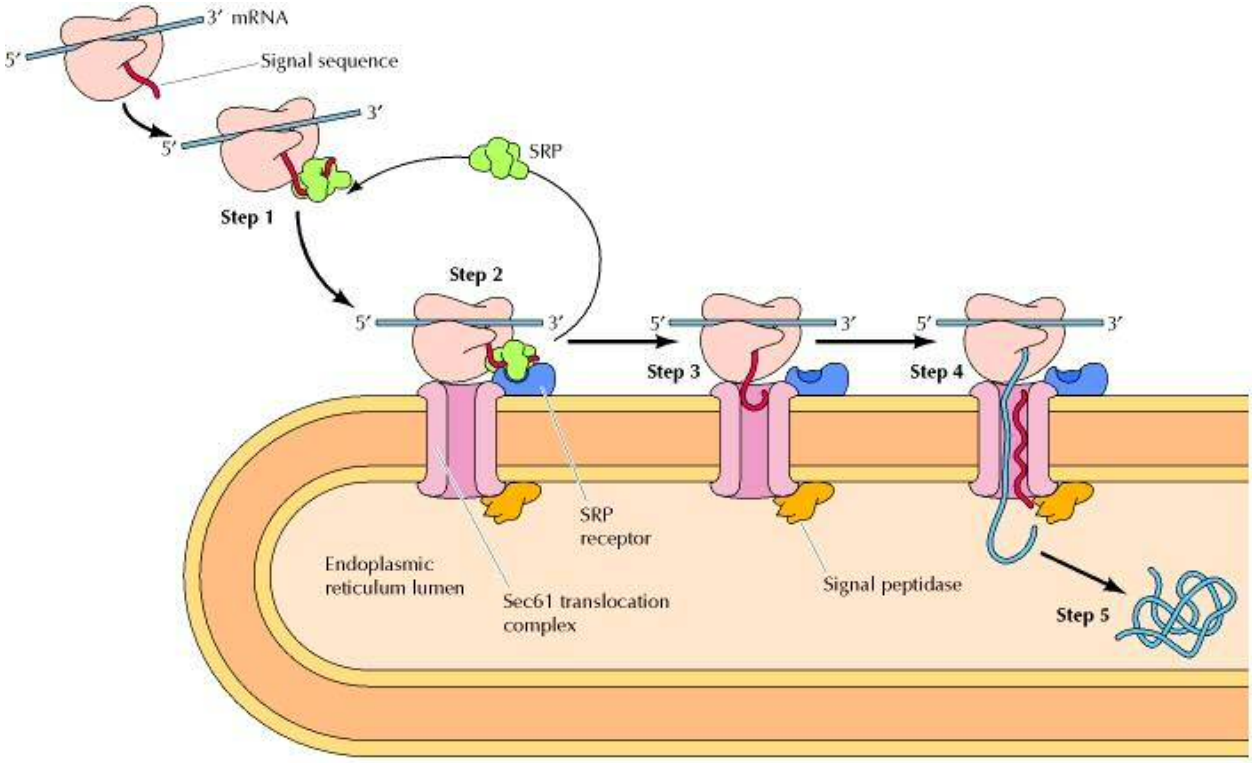


Figure: Cotranslational targeting of secretory proteins to the ER

Step 1: As the signal sequence emerges from the ribosome, it is recognized and bound by the signal recognition particle (SRP). Step 2: The SRP escorts the complex to the ER membrane, where it binds to the SRP receptor. Step 3: The SRP is released, the ribosome binds to a membrane translocation complex of Sec61 proteins, and the signal sequence is inserted into a membrane channel. Step 4: Translation resumes, and the growing polypeptide chain is translocated across the membrane. Step 5: Cleavage of the signal sequence by signal peptidase releases the polypeptide into the lumen of the ER.

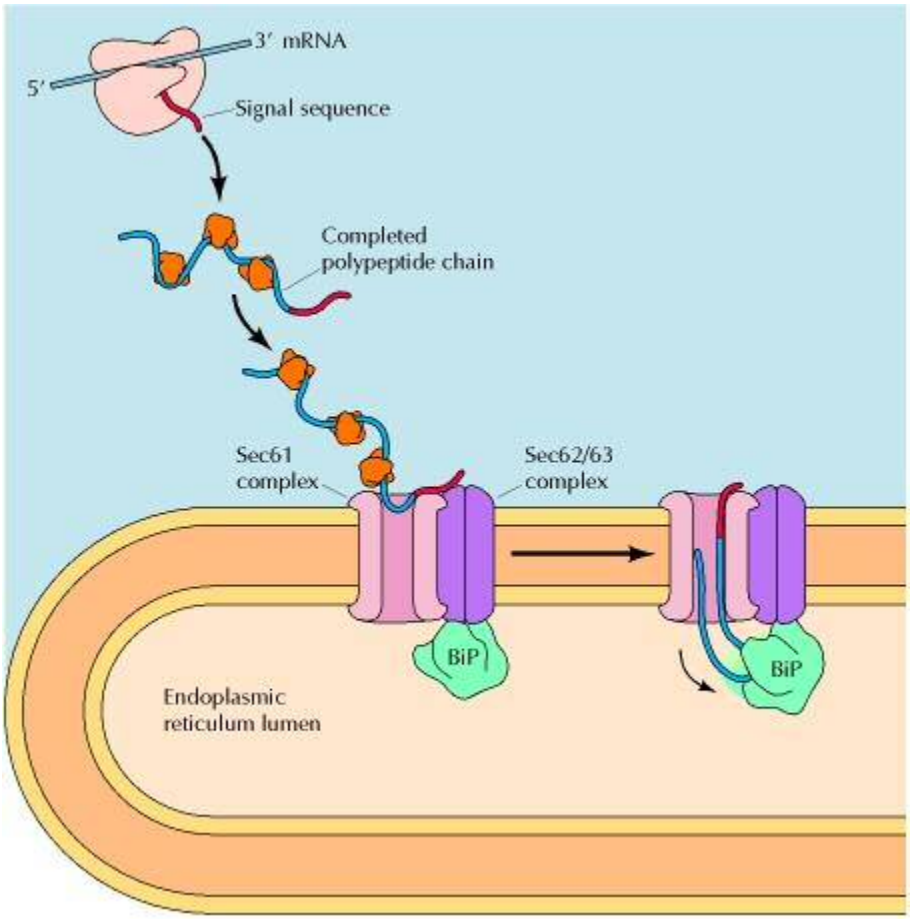


Figure Posttranslational translocation of proteins into the ER: **Proteins destined for posttranslational import to the ER are synthesized on free ribosomes and maintained in an unfolded conformation by cytosolic chaperones. Their signal sequences are recognized by the Sec62/63 complex, which is associated with the Sec61 translocation channel in the ER membrane. The Sec63 protein is also associated with a chaperone protein (BiP), which acts as a molecular ratchet to drive protein translocation into the ER.**

Peroxisomes and Zellweger syndrome

In most eukaryotic cells, the peroxisome is the principal organelle in which fatty acids are oxidized, thereby generating precursors for important biosynthetic pathways. In the human genetic disease X-linked adrenoleukodystrophy (ADL), peroxisomal oxidation of very long chain fatty acids is defective. The ADLgene encodes the peroxisomal membrane protein that transports into peroxisomes an enzyme required for the oxidation of these fatty acids. Persons

with the severe form of ADL are unaffected until midchildhood, when severe neurological disorders appear, followed by death within a few years.

Zellweger syndrome is an inherited human disease, in which a defect in importing proteins into peroxisomes leads to a severe peroxisomal deficiency. These individuals, whose cells contain —empty‖ peroxisomes, have severe abnormalities in their brain, liver, and kidneys, and they die soon after birth. One form of this disease has been shown to be due to a mutation in the gene encoding a peroxisomal integral membrane protein, the peroxin Pex2, involved in protein import. A milder inherited peroxisomal disease is caused by a defective receptor for the N-terminal import signal.

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DEPARTMENT OF BIOCHEMISTRY
I-B.Sc., BIOCHEMISTRY - BATCH: 2017 - 2020
PART A (20 X 1 = 20 MARKS) - Online MCQ Questions

SUBJECT: CEI SUBJECT CODE: 17BCU102

Questions	Option A	Option B	Option C	Option D	Answer
Presence of nucleus allows the primary regulation of	Reverse transcription	Post translational modification	Post transcriptional modification	Translation	Post transcriptional modification
The channel on nucleopore is calles as	Nucleolus	Nucleoplasm	Nucleopore complex	Nuclei	Nucleopore complex
Nuclear laminin is located at _____ nuclear membrane	Outer surface	inner surface	Interspace	Cytoplasmic space	inner surface
Structural support to nucleus is provided by	Spectrin	Band 3 Protein	Nuclear laminin	Glycoporin	Nuclear laminin
Nuclear laminin is_____ filament	Micro	Macro	Intermediate	Microsome	Intermediate

Which is not transported through nuclear pore complex	Small molecules	Proteins	RNA	DNA	DNA
Nucleolus is rich in’	Protein	DNA	RNA	Glycoprotein	RNA
The weight of small molecule that transported through nuclearpore complex is_____KD	20	40	10	50	20
The diameter of nucleaopore complex is	5nm	10nm	12nm	15nm	10nm
RNA and protein are transported through the nuclearpore complex by	Facilitated diffusion	Simple Diffusion	Active transport	Osmosis	Active transport

A special channel found in nuclearpore complex is	Central vacuole	Central plug	Centriole	Centrisole	Central plug
The signal that target proteins to nucleus is	Signal peptide	NLS	KDEL	KKXX	NLS
The cytoso;ic protein that bind the signal with NLS is	Selectin	Importin	Cadherin	intergrin	Importin
RNA is transported through nuclear membrane as	Ribonucle o protein	Phospho protein	Glycoprotei n	Sphingo lipid	Ribonucleo protein
SnRNP is a combination of	RNA and Protein	RNA and DNA	Protein and lipid	RNA and lipid	RNA and Protein
Among the following which is not synthesized in membrane bound ribosomes	Plasma Membrane	Secretory vesicle	Lysosomes	Chloroplast	Chloroplast

Among the following which is not synthesized in membrane free ribosomes	Nucleus	Mitochondria	Lysosomes	Peroxisomes	Lysosomes
ER breaks into	Microsomes	Micro bodies	Microtubules	Microfilaments	Microsomes
Peroxisomes and glyoxysomes are	Microfilaments	Microtubules	Microbodies	Intemediary filament	Microbodies
SRP contains _____polypeptides	4	6	8	2	6
The most favored conformation for hydrophobic amino acids in membrane is	α helix	β sheet	Trans	Cis	α helix
Lipids are synthesized on _____ side of SER	Extracellular	Cytosolic	Integral membrane	Transcellular	Cytosolic

Thylakoid membrane is found in	Nucleus	Mitochondria	Lysosomes	Chloroplast	Chloroplast
H2O2 is destroyed in----------	glyoxysomes	lysosomes	peroxisomes	mesosomes.	peroxisomes
Peroxisomes produce hydrogen peroxide which is destroyed by	cytosomes	glyoxisomes	phargmosomes	catalase	catalase
Peroxisomes help to detoxify	H_2O_2	Cations	Anions	Xenochemicals	H_2O_2
Microbodies are formed by the	cell division process	position of organelles	membrane invaginations	growth and fission of old organelle	growth and fission of old organelle
Among the following which organelle have its own DNA	glyoxysomes	lysosomes	peroxisomes	chloroplast	chloroplast
Among the following which organelle have its own DNA	glyoxysomes	lysosomes	peroxisomes	Mitochondria	Mitochondria

The folding in mitochondria are called as	Cisternae	Cristae	Cristernae	Chastae	Cristae
Mitochondrial proteins are synthesized on	Bound ribosomes	Free ribosomes	SER	RER	Free ribosomes
TCA cycle operates at	Cytosol	Mitochondrail matrix	Mitochodrial inner membrane	Mitochondrail outer membrane	Mitochondrail matrix
Fatty acid oxidation takes place at	Cytosol	Mitochondrail matrix	Mitochodrial inner membrane	Mitochondrail outer membrane	Mitochondrail matrix
Oxidative break down of carbohydrates and lipid is taken care by _____ organelle	Mitochodria	Lysosomes	Golgi	Endoplasmic reticulum	Mitochodria
Final electron acceptor in ETC is	Cytochrome C oxidase	Cytochrome C reductaase	Cytochrome	Molecular oxygen	Molecular oxygen
Electron transfer in ETC creates _____ gradient	Anion	Cation	Proton	Electron	Proton

The number of tRNA encoded by mitochondrial genome is	64	54	42	22	22
Protein folding in mitochondria is facilitated by	HSP 70	HSP60	HSP 40	HSP50	HSP 70
Major difference between mitochondria and chloroplast in terms of structure is	Outer membrane	Inner membrane	Thylakoid membrane	Inter membrane space	Thylakoid membrane
Electron transfer system of chloroplast located at	Outer membrane	Inner membrane	Thylakoid membrane	Inter membrane space	Thylakoid membrane
The chloroplast genome range lies between_____ _____kd	150-180	120-160	100-140	80-120	120-160

The number of tRNA coded by chloroplast genome is	22	62	30	4029	30
Amyloplast store _____	Lipid	Protein	Starch	Peptidoglycans	Starch
Elaioplast store _____	Lipid	Protein	Starch	Peptidoglycans	Lipid
Chloropast contains	Lipid	Protein	Starch	Carotenoids	Carotenoids
Peroxisomes contains enzymes necessary for the synthesis of	peptodo glycans	phospholipids	Plasmologe ns	Glycolipids	Plasmologens
Glyoxylat cycle is operating in _____ organelle	Chloropla st	Glyoxysomes	Golgi	Endoplasmic reticulum	Glyoxysomes
In glyoxylat cycle, the stored fat is converted into	Carbohydr ates	Lipid	Protein	Nucleic acids	Carbohydrates

The organelle which is not involved with photorespiration is	Chloroplast	Peroxisomes	Mitochondria	Endoplasmic reticulum	Endoplasmic reticulum
Photorespiration takes place at plant	Leaves	Seed	root	Flowers	Leaves
Peroxisomal protein import defects result in	Kleinfelder syndrome	Down syndrome	Zellweger syndrome	Metabolic syndrome	Zellweger syndrome
Subunits of ribosomes are manufactured in the	cytoskeleton.	endoplasmic reticulum.	Golgi apparatus.	nucleolus	nucleolus
Which of these organelles contains DNA?	Golgi apparatus	lysosomes	mitochondria	rough endoplasmic reticulum	mitochondria
The cell process which uses ATP to bring substances into the cell is	osmosis	diffusion	active transport	facilitated transport.	active transport

Which of the following aids the movement of glucose across a cell membrane?	Protein	Phosphate	Glycolipid	Cholesterol.	Protein
The inner membrane of the mitochondria is usually, highly convoluted forming a series of infolding known as	thylakoids	lamellae	cristae	grana	cristae
Which of the following statements regarding mitochondrial membrane is not correct?	the outer membrane resembles a sieve	the enzymes of the electron transfer chain are embedded in the outer membrane	The outer membrane is permeable to all kinds of molecules	the outer membrane permeable to all kinds of molecules	the enzymes of the electron transfer chain are embedded in the outer membrane
In mitochondria , cristae act as sites for	protein synthesis	phosphoryaltion of flavoproteins	breakdown of macromolecules	Oxidation reduction reaction	Oxidation reduction reaction

Mitochondrial inner membrane is rich in phospholipid	Cardiolipin	Phosphatidyl inositol	Phosphatidyl serine	Phosphatidyl choline	Cardiolipin
Which of the following is not a function of mitochondrion	electron transport cahin and associated ATP production	glycolysis and associated ATP production	Fatty acid breakdown	nonshivering thermogenesis	glycolysis and associated ATP production

UNIT II

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

SUBJECT : CELL BIOLOGY
SEMESTER : I
SUBJECT CODE : 17BCU102 CLASS : I B.Sc., BC

UNIT III - COURSE MATERIAL

UNIT 3

Protein trafficking

Organization of Golgi. Lipid and polysaccharide metabolism in Golgi. Protein sorting and export from Golgi. N and O-linked glycosylation.

Lysosome. – Acid hydrolases, phagocytosis and autophagy.

Mitochondria-Structure and functions, protein import and mitochondrial assembly, protein export from mitochondrial matrix.

Chloroplasts- Import and sorting of chloroplast proteins.

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- Verma, P.S., and Agarwal, V.K., (2005). Cell Biology Molecular Biology and Genetics, VII Edition, S.Chand and company Ltd, New Delhi.
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UNIT 3 - PROTEIN TRAFFICKING

The Golgi Apparatus

The golgi complex was discovered by Camillo Golgi during an investigation of the nervous system and he named it the "internal reticular apparatus". Functionally it is also known as the post office of the cell. Certain important cellular functions such as biosynthesis of polysaccharides, packaging (compartmentalizing) of cellular synthetic products (proteins), production of exocytotic (secretory) vesicles and differentiation of cellular membranes, and also serves as the site at which the complex polysaccharides of the cell wall are synthesized.

Occurrence:

The Golgi apparatus occurs in all eukaryotic cells. Their number per plant cell can vary from several hundred as in tissues of corn root and algal rhizoids (*i.e.*, more than 25,000 in algal rhizoids, Sievers,1965), to a single organelle in some algae.

Organisation of Golgi

Morphologically the Golgi is composed of three basic components as follows:

1. Flattened membrane-enclosed sacs (cisternae)

2. Tubules

3. vesicles

1. Flattened Sac or Cisternae

Cisternae of the golgi apparatus are about 1 μm in diameter, flattened, plate-like closed compartments which are held in parallel bundles or stacks one above the other. In each stack, cisternae are separated by a space of 20 to 30 nm.

Polarity. A striking feature of the Golgi apparatus is its distinct polarity in both structure and function. Proteins from the ER enter at its *cis* face (entry face), which is convex and usually oriented toward the nucleus. They are then transported through the Golgi and exit from its concave *trans* face (exit face). Trans face of Golgi is located near the plasma membrane .As they pass through the Golgi, proteins are modified and sorted for transport to their eventual destinations within the cell.

2. Tubules

A complex array of associated vesicles and tubules (30 to 50 nm diameter) surround the dictyosome and radiate from it. The peripheral area of dictyosome is fenestrated or lace-like in structure.

3. Vesicles

The vesicles are 60 nm in diameter and are of three types :

(i) Transitional vesicles
(ii) Secretory vesicles
(iii)Clathrin-coated vesicles

Distinct processing and sorting events appear to take place in an ordered sequence within different regions of the Golgi complex, so the Golgi is usually considered to consist of multiple discrete compartments. The modified proteins, lipids, and polysaccharides then move to the *trans* Golgi network, which acts as a sorting and distribution center, directing molecular traffic to lysosomes, the plasma membrane, or the cell exterior.

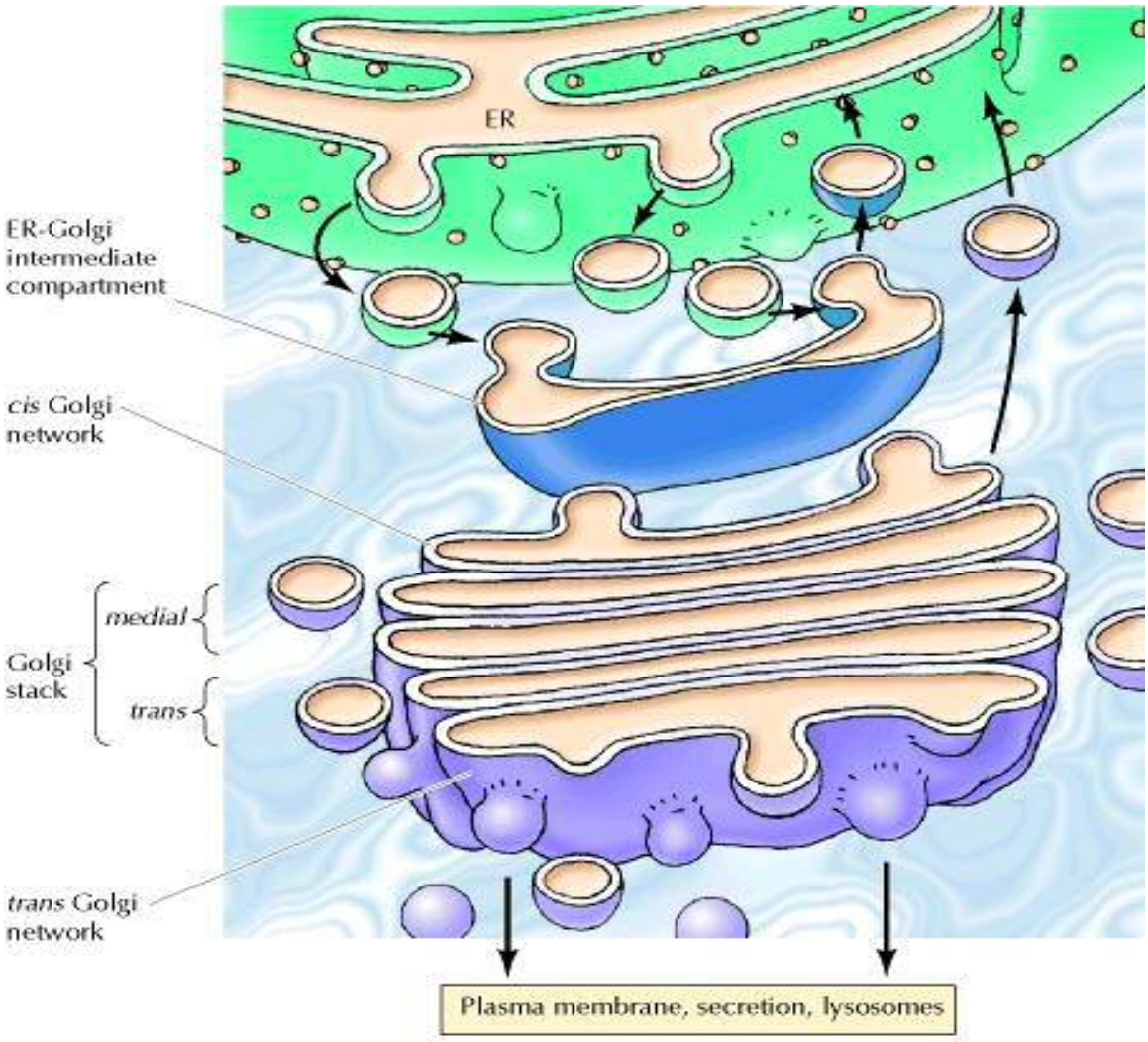


Figure : Organisation of the Golgi apparatus

Fig:Vesicles from the endoplasmic reticulum fuse to form the ER-Golgi intermediate compartment, and proteins from the ER are then transported to the *cis* Golgi network. Resident ER proteins are returned from the ER-Golgi intermediate compartment and the *cis* Golgi network via the recycling pathway. The *medial* and *trans* compartments of the Golgi stack correspond to the cisternae in the middle of the Golgi complex and are the sites of most protein modifications. Proteins are then carried to the *trans* Golgi network, where they are sorted for transport to the plasma membrane secretion, or lysosomes.

Functions:

1. Modifying, sorting, and packaging of macromolecules for cell secretion: The golgi complex is involved in the transport of lipids around the cell, and the creation of lysosomes

2. Proteoglycans and carbohydrate synthesis: This includes the production of glycosaminoglycans (GAGs), long unbranched polysaccharides which the Golgi then attaches to a protein synthesised in the endoplasmic reticulum to form proteoglycans

3. Golgi Functions in Animals:

In animals, Golgi apparatus is involved in the packaging and exocytosis of the following: Zymogen of exocrine pancreatic cells; Mucus (a glycoprotein) secretion by goblet cells of intestine; Lactoprotein (casein) secretion by mammary gland cells (Merocrine secretion); Secretion of compounds (thyroglobulins) of thyroxine hormone by thyroid cells; Secretion of tropocollagen and collagen; Formation of melanin granules and other pigments; and Formation of yolk and vitelline membrane of growing primary oocytes. It is also involved in the formation of certain cellular organelles such as plasma membrane, lysosomes, acrosome of spermatozoa and cortical granules of a variety of oocytes.

4. Golgi Functions in Plants:

In plants, Golgi apparatus is mainly involved in the secretion of materials of primary and secondary cell walls (formation and export of glycoproteins, lipids, pectins and monomers for hemicellulose, cellulose, lignin). During cytokinesis of mitosis or meiosis, the vesicles originating from the periphery of Golgi apparatus, coalesce in the phragmoplast area to form a semisolid layer, called cell plate. The unit membrane of Golgi vesicles fuses during cell plate formation and becomes part of plasma membrane of daughter. The synthesis of these cell wall polysaccharides is a major cellular function, and as much as 80% of the metabolic activity of the Golgi apparatus in plant cells may be devoted to polysaccharide synthesis

PROTEIN GLYCOSYLATION IN GOLGI

Protein processing within the Golgi involves the modification and synthesis of the carbohydrate portions of glycoproteins. One of the major aspects of this processing is the modification of the *N*-linked oligosaccharides that were added to proteins in the ER. Proteins are modified within the ER by the addition of an oligosaccharide consisting of 14 sugar residue. In that, three glucose residues and one mannose are then removed while the polypeptides are still in the ER. Following transport to the Golgi apparatus, the *N*-linked oligosaccharides of these glycoproteins are subject to extensive further modifications.

N-linked oligosaccharides are processed within the Golgi apparatus in an ordered sequence of reactions (Figure). The first modification of proteins destined for secretion or for the plasma membrane is the removal of three additional mannose residues. This is followed by the sequential addition of an *N*-acetylglucosamine, the removal of two more mannoses, and the addition of a fucose and two more *N*-acetylglucosamines. Finally, three galactose and three sialic acid residues are added. As noted earlier, different glycoproteins are modified to different extents during their passage through the Golgi, depending on both the structure of the protein and on the amount of

processing enzymes that are present within the Golgi complexes of different types of cells. Consequently, proteins can emerge from the Golgi with a variety of different *N*-linked oligosaccharides.

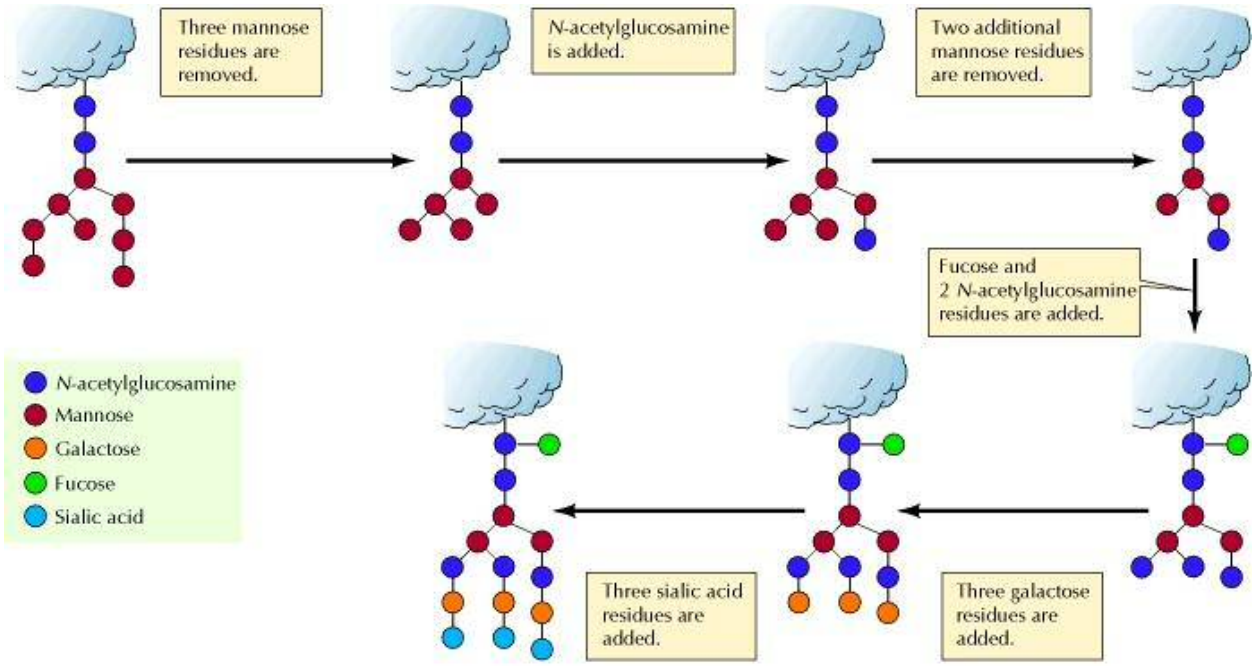


Figure : Processing of *N*-linked oligosaccharides in the Golgi

Targeting of lysosomal proteins

The processing of the *N*-linked oligosaccharide of lysosomal proteins differs from that of secreted and plasma membrane proteins. Rather than the initial removal of three mannose residues, proteins destined for incorporation into lysosomes are modified by mannose phosphorylation. In the first step of this reaction, *N*-acetylglucosamine phosphates are added to specific mannose residues, probably while the protein is still in the *cis* Golgi network (Figure). This is followed by removal of the *N*-acetylglucosamine group, leaving mannose-6-phosphate residues on the *N*-linked oligosaccharide. Because of this modification, these residues are not removed during further processing. Instead, these phosphorylated mannose residues are specifically recognized by a mannose-6-phosphate receptor in the *trans* Golgi network, which directs the transport of these proteins to lysosomes.

The phosphorylation of mannose residues is thus a critical step in sorting lysosomal proteins to their correct intracellular destination. In contrast to the signal sequences that direct protein translocation to the ER, the recognition determinant that leads to mannose phosphorylation, and thus ultimately targets proteins to lysosomes, depends on the three-dimensional conformation of the folded protein. Such determinants are called signal patches, in contrast to the linear targeting signals discussed earlier.

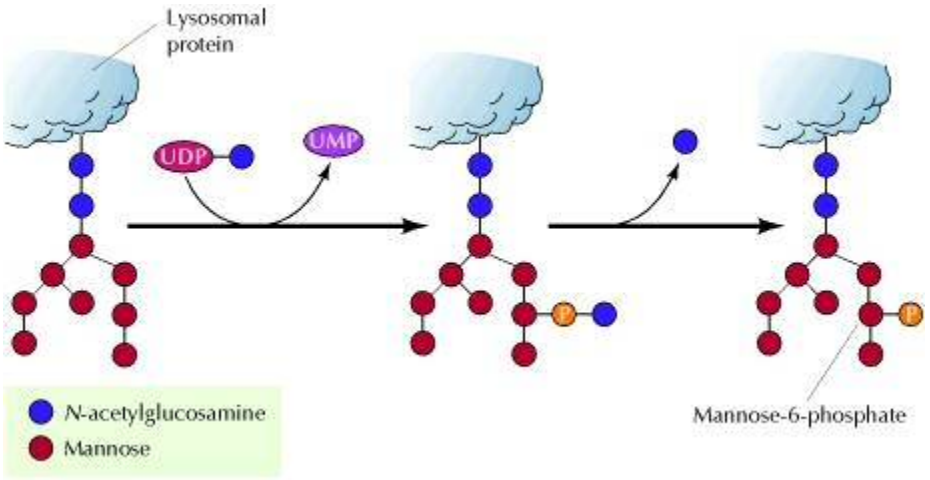


Figure : Targeting of lysosomal proteins by phosphorylation of mannose residues

Proteins can also be modified by the addition of carbohydrates to the side chains of acceptor serine and threonine residues within specific sequences of amino acids (*O*-linked glycosylation. These modifications take place in the golgi apparatus by the sequential addition of single sugar residues. The serine or threonine is usually linked directly to *N*-acetylgalactosamine, to which other sugars can then be added. In some cases, these sugars are further modified by the addition of sulfate groups.

Lipid polysaccharide metabolism in Golgi

In addition to its activities in processing and sorting glycoproteins, the Golgi apparatus functions in lipid metabolism—in particular, in the synthesis of glycolipids and sphingomyelin. As discussed earlier, the glycerol phospholipids, cholesterol, and ceramide are synthesized in the ER. Sphingomyelin and glycolipids are then synthesized from ceramide in the Golgi apparatus (Figure).

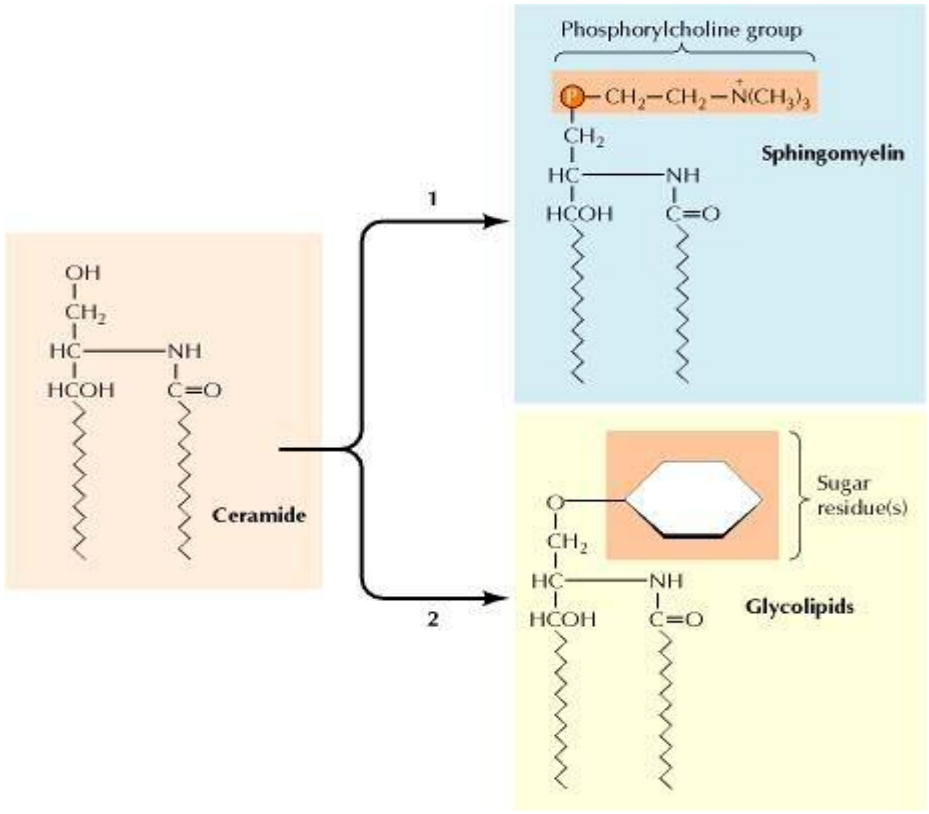


Figure : Synthesis of sphingomyelin and glycolipids in Golgi

Ceramide, which is synthesized in the ER, is converted either to sphingomyelin (a phospholipid) or to glycolipids in the Golgi apparatus. In the first reaction, a phosphorylcholine group is transferred from phosphatidylcholine to ceramide. Alternatively, a variety of different glycolipids can be synthesized by the addition of one or more sugar residues (e.g., glucose).

Sphingomyelin is synthesized on the lumenal surface of the Golgi, but glucose is added to ceramide on the cytosolic side. Glucosylceramide then apparently flips, however, and additional carbohydrates are added on the lumenal side of the membrane. Neither sphingomyelin nor the glycolipids are then able to translocate across the Golgi membrane, so they are found only in the lumenal half of the Golgi bilayer. Following vesicular transport, they are correspondingly localized to the exterior half of the plasma membrane, with their polar head groups exposed on the cell surface. The oligosaccharide portions of glycolipids are important surface markers in cell-cell recognition.

PROTEIN SORTING IN GOLGI

Proteins, as well as lipids and polysaccharides, are transported from the Golgi apparatus to their final destinations through the secretory pathway. This involves the sorting of proteins into different kinds of transport vesicles, which bud from the *trans* Golgi network and deliver their contents to the appropriate cellular locations (Figure). Some proteins are carried from the Golgi to the plasma membrane by a constitutive secretory pathway, which accounts for the incorporation of new proteins

and lipids into the plasma membrane, as well as for the continuous secretion of proteins from the cell. Other proteins are transported to the cell surface by a distinct pathway of regulated secretion or are specifically targeted to other intracellular destinations, such as lysosomes in animal cells or vacuoles in yeast.

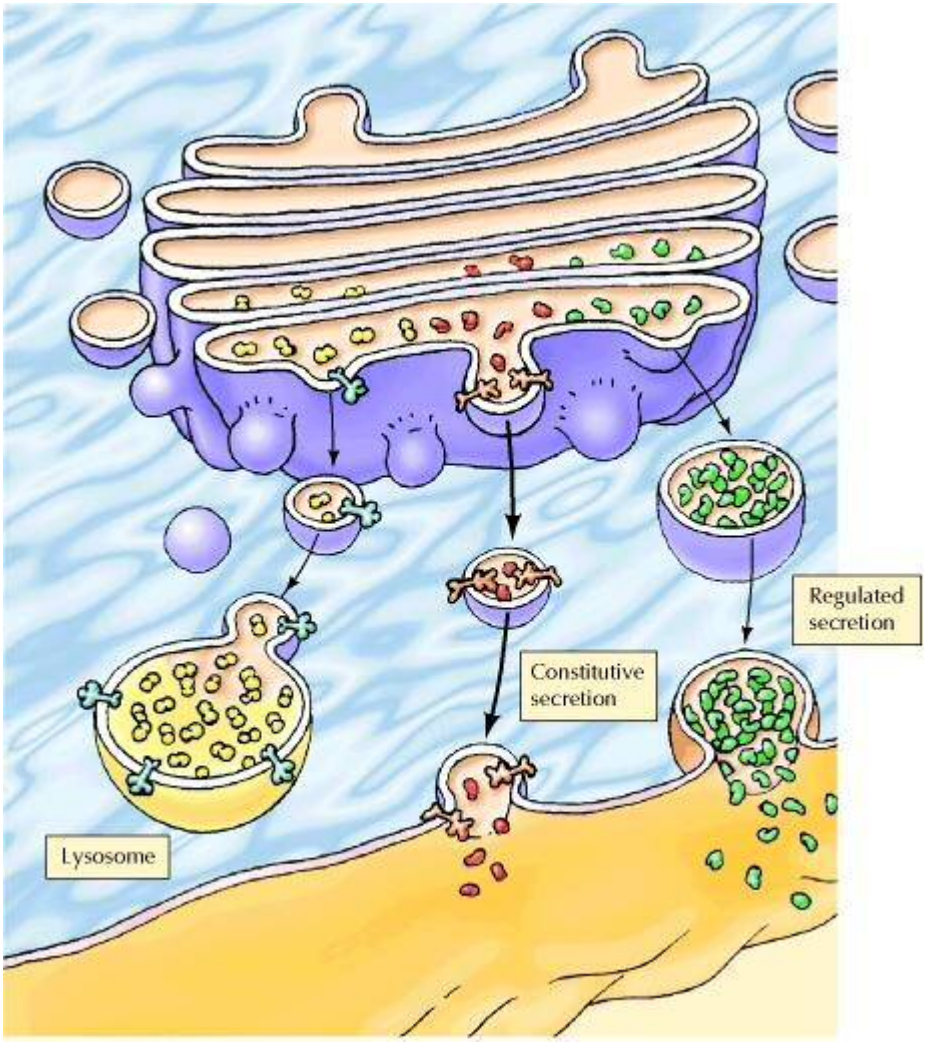


Figure :Transport from the Golgi apparatus

Fig: Proteins are sorted in the *trans* Golgi network and transported in vesicles to their final destinations. In the absence of specific targeting signals,proteins are carried to the plasma membrane by constitutive secretion. The constitutive secretory pathway, which operates in all cells, leads to continual unregulated protein secretion. Alternatively, proteins can be targeted to other destinations, such as lysosomes or regulated secretion from the cells.

Proteins that function within the Golgi apparatus must be retained within that organelle, rather than being transported along the secretory pathway. All of the proteins retained within the Golgi complex are associated with the Golgi membrane rather than being soluble proteins within the lumen. The signals responsible for retention of some proteins within the Golgi have been localized to their transmembrane domains, which retain proteins within the Golgi apparatus by preventing them from being

packaged in the transport vesicles that leave the *trans* Golgi network. In addition, signals in the cytoplasmic tails of some Golgi proteins mediate the retrieval of these proteins from subsequent compartments along the secretory pathway.

However, some cells also possess a distinct regulated secretory pathway in which specific proteins are secreted in response to environmental signals. Examples of regulated secretion include the release of hormones from endocrine cells, the release of neurotransmitters from neurons, and the release of digestive enzymes from the pancreatic acinar cells.

Proteins are sorted into the regulated secretory pathway in the *trans* Golgi network, where they are packaged into specialized secretory vesicles. These secretory vesicles, which are larger than other transport vesicles, store their contents until specific signals direct their fusion with the plasma membrane. For example, the digestive enzymes produced by pancreatic acinar cells are stored in secretory vesicles until the presence of food in the stomach and small intestine triggers their secretion.

Protein transport from Golgi-The Mechanism of Vesicular Transport

Transport vesicles play a central role in the traffic of molecules between different membrane-enclosed compartments of the secretory pathway.Vesicles are similarly involved in the transport of materials taken up at the cell surface. Vesicular transport is thus a major cellular activity, responsible for molecular traffic between a variety of specific membrane-enclosed compartments. Three types of vesicle were found. They are,

(i) Transitional vesicles are small membrane limited vesicles which are form as blebs from the transitional ER to migrate and converge to cis face of Golgi, where they join together to form new cisternae.
(ii) Secretory vesicles are varied-sized membrane-limited vesicles which discharge from margins of cisternae of Golgi. They, often, occur between the maturing face of Golgi and the plasma membrane.
(iii) Clathrin-coated vesicles are spherical protuberances, about 50 μm in diameter and with a rough surface. They are found at the periphery of the organelle, usually at the ends of single tubules, and are morphologically quite distinct from the secretory vesicles. The clathrin-coated vesicles are known to play a role in intra-cellular traffic of membranes and of secretory products.

The selectivity of such transport is therefore key to maintaining the functional organization of the cell. For example, lysosomal enzymes must be transported specifically from the Golgi apparatus to lysosomes—not to the plasma membrane or to the ER. so the specificity of transport is based on the selective packaging of the intended cargo into vesicles that recognize and fuse only with the appropriate target membrane.

EXPERIMENTAL APPROACH TO UNDERSTAND THE VESICULAR TRANSPORT

Progress toward elucidating the molecular mechanisms of vesicular transport has been made by three distinct experimental approaches: (1) isolation of yeast mutants that are defective in protein transport and sorting; (2) reconstitution of vesicular transport in cell-free systems; and (3) biochemical analysis of synaptic vesicles, which are responsible for the regulated secretion of neurotransmitters by neurons.

Golgi stacks prepared from a virus-infected mutant cell line unable to catalyze the addition of *N*-acetylglucosamine to *N*-linked oligosaccharides are mixed with Golgi stacks from a normal cell line. Because the mutant cell line is infected with a virus, the proteins it synthesizes can be specifically detected. Transport of these proteins to normal Golgi stacks is signaled by the addition of *N*-acetylglucosamine.

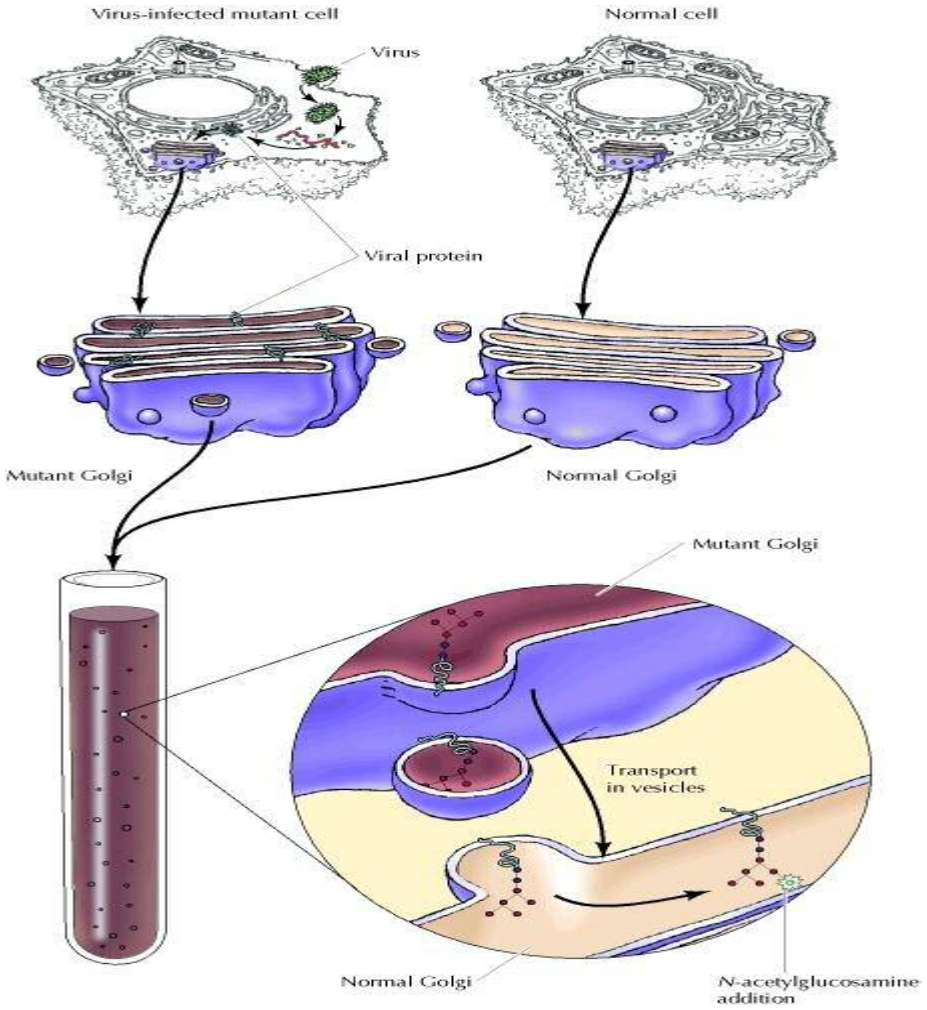


Figure: Reconstituted vesicular transport

COAT PROTEINS AND VESICLE BUDDING

The first step in vesicular transport is the formation of a vesicle by budding from the membrane. The cytoplasmic surfaces of transport vesicles are coated with proteins, and it appears to be the assembly of these protein coats that drives vesicle budding by distorting membrane conformation.

Three kinds of coated vesicles, which appear to function in different types of vesicular transport, have been characterized. The first to be described were the clathrin-coated

vesicles, which are responsible for the uptake of extracellular molecules from the plasma membrane by endocytosis as well as the transport of molecules from the *trans* Golgi network to lysosomes. Two other types of coated vesicles have been identified as budding from the ER and Golgi complex. These vesicles are called nonclathrin-coated or COP-coated vesicles (COP stands for coat protein). One class of these vesicles (COPII-coated vesicles) bud from the ER and carry their cargo forward along the secretory pathway, to the Golgi apparatus. COPI-coated vesicles bud from the ER-Golgi intermediate compartment or the Golgi apparatus and function in the retrieval pathways that serve to retain resident proteins in the Golgi and ER. For example, COPI-coated vesicles transport resident ER proteins marked by the KDEL or KKXX retrieval signals back to the ER from the ER-Golgi intermediate compartment or the *cis* Golgi network.

The coats of clathrin-coated vesicles are composed of two types of protein complexes, clathrin and adaptor proteins, which assemble on the cytosolic side of membranes (Figure). Clathrin plays a structural role by assembling into a basketlike lattice structure that distorts the membrane and drives vesicle budding. The binding of clathrin to membranes is mediated by a second class of proteins, called adaptor proteins. Different adaptor proteins are responsible for the assembly of clathrin-coated vesicles at the plasma membrane and at the *trans* Golgi network, and it is the adaptor proteins that are involved in selecting the specific molecules to be incorporated into the vesicles. For example, the AP-1 adaptor protein involved in budding from the *trans* Golgi network binds to the cytosolic portion of the mannose-6-phosphate receptor, thereby directing proteins destined for lysosomes into clathrin-coated vesicles.

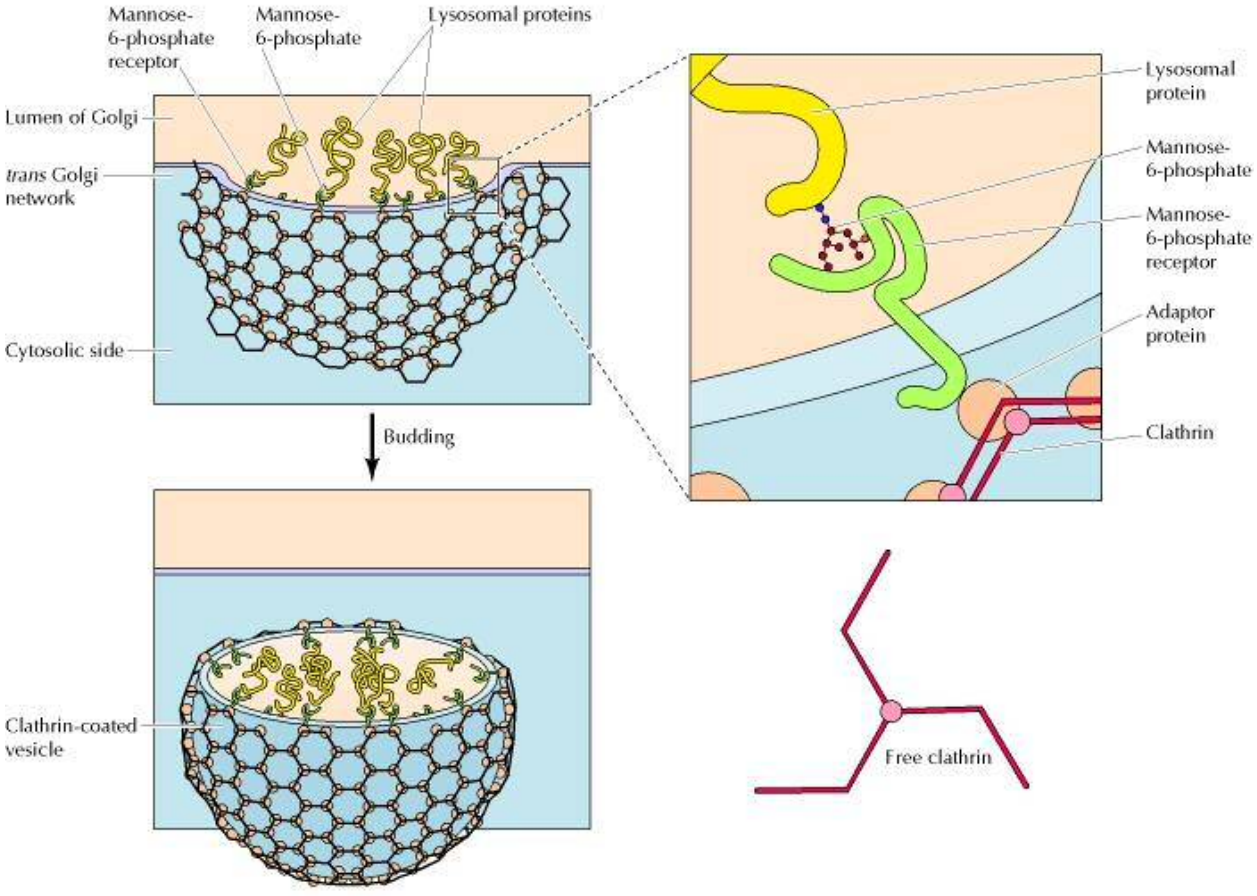
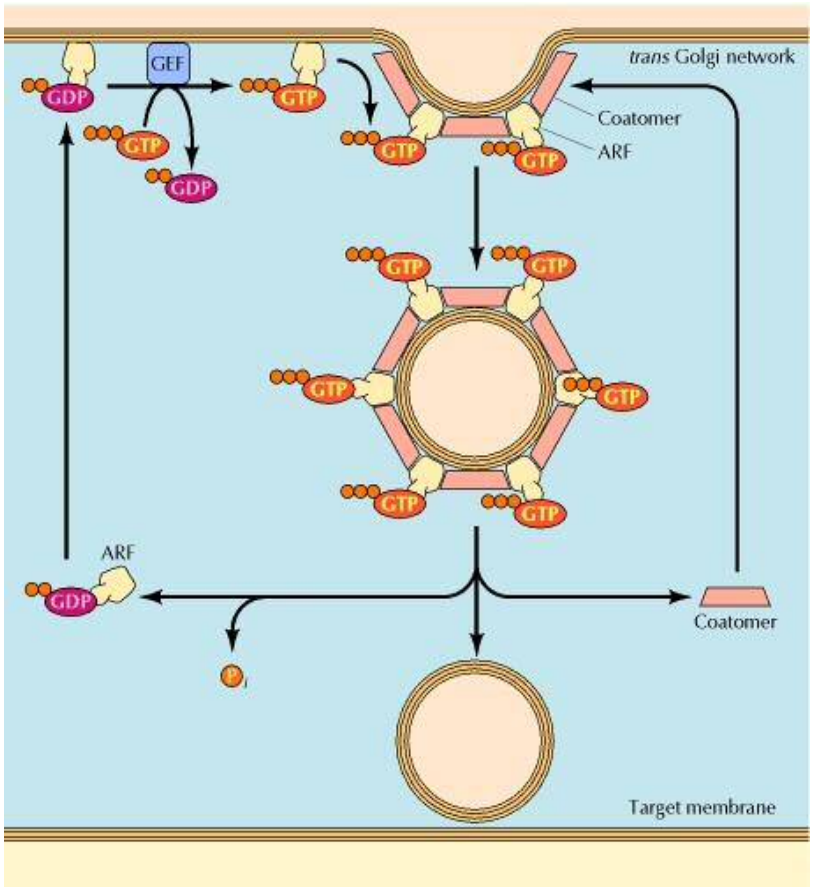


Figure : Incorporation of lysosomal proteins into clathrin-coated vesicles

Proteins targeted for lysosomes are marked by mannose-6-phosphates, which bind to mannose-6-phosphate receptors in the *trans* Golgi network. The mannose-6-phosphate receptors span the Golgi membrane and serve as binding sites for cytosolic adaptor proteins, which in turn bind clathrin. Clathrins consist of three protein chains that associate with each other to form a basketlike lattice that distorts the membrane and drives vesicle budding.

The coats of COPI- and COPII-coated vesicles are composed of distinct protein complexes, which function analogously to clathrin and adaptor proteins in vesicle budding. Interestingly, components of the COPI coat interact with the KKXX motif responsible for the retrieval of endoplasmic reticulum proteins from the Golgi complex consistent with a role for COPI-coated vesicles in recycling from the Golgi to the ER.

The assembly of vesicle coats also requires GTP-binding proteins which appear to regulate the binding of coat proteins to the membrane. The budding of both clathrin coated and COPI-coated vesicles from the Golgi complex requires a GTP-binding protein called ADP-ribosylation factor, while the budding of COPII-coated vesicles from the endoplasmic reticulum requires a distinct GTP-binding protein called Sar1. The role of these proteins is illustrated by the function of ARF in assembly of COPI-coated vesicles. The first step in vesicle formation is the association of ARF bound to GDP with the Golgi membrane. Proteins in the Golgi membrane then stimulate the exchange of the GDP bound to ARF for GTP, and the COPI coat proteins bind to the ARF/GTP complex. Assembly of the coat is then followed by deformation of the membrane and vesicle budding. ARF then hydrolyzes its bound GTP, leading to the conversion of ARF to the GDP-bound state and the dissociation of coat proteins from the vesicle membrane.



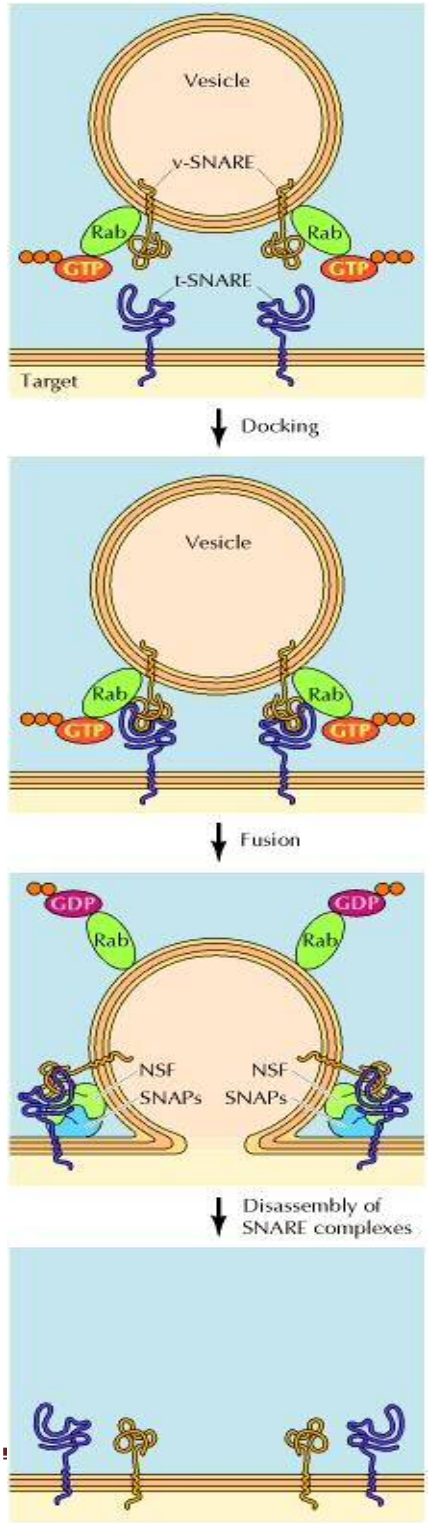


Figure : Role of ARF in the formation of COP-coated vesicles

ARF alternates between GTP-bound and GDP-bound states. When bound to GDP, ARF associates with the membrane of the *trans* Golgi network, where guanine nucleotide exchange factors (GEF) promote the exchange of the bound GDP for GTP. In its GTP-bound state, ARF promotes the binding of COPI coat protein (coatomer), leading to vesicle budding. Hydrolysis of the bound GTP then converts ARF to the GDP-bound state, leading to disassembly of the vesicle coat prior to fusion with the target membrane.

VESICLE FUSION

The fusion of a transport vesicle with its target involves two types of events.

First, the transport vesicle must specifically recognize the correct target membrane; for example, a vesicle carrying lysosomal enzymes has to deliver its cargo only to lysosomes.

Second, the vesicle and target membranes must fuse, thereby delivering the contents of the vesicle to the target organelle.

Analysis of the proteins involved in vesicle fusion in these systems led Rothman and his colleagues to propose a general model, called the SNARE hypothesis, in which vesicle fusion is mediated by interactions between specific pairs of proteins, called SNAREs, on the vesicle and target membranes (v-SNAREs and t-SNAREs, respectively)

Vesicle fusion is mediated by binding between specific pairs of v-SNAREs and t-SNAREs on the vesicle and target membranes, respectively. Rab GTP-binding proteins are required to facilitate formation of v-SNARE/t-SNARE complexes. Following membrane fusion, the NSF/SNAP proteins disassemble the SNARE complex.

In addition to SNAREs, vesicle fusion requires at least two other types of proteins. The Rab proteins are a family of small GTP-binding proteins that are related to the Ras proteins. Following the formation of complexes between complementary SNAREs and membrane fusion, a complex of two additional proteins (the NSF/ SNAP complex) is needed to complete the process of vesicle transport. The NSF/SNAP proteins are recruited to membranes following the formation of v-SNARE/t-SNARE complexes, and they are not required directly for either vesicle/target pairing or for the fusion of paired membranes. Instead, the NSF/SNAP proteins act after membrane fusion to disassemble the SNARE complex, thereby allowing the SNAREs to be reutilized for subsequent rounds of vesicle transport.

LYSOSOMES

Lysosomes are membrane-enclosed organelles that contain an array of enzymes capable of breaking down all types of biological polymers—proteins, nucleic acids, carbohydrates, and lipids. Lysosomes function as the digestive system of the cell, serving both to degrade material taken up from outside the cell and to digest obsolete components of the cell itself. In their simplest form, lysosomes are visualized as dense spherical vacuoles, but they can display considerable variation in size and shape as a result of differences in the materials that have been taken up for digestion. Lysosomes thus represent morphologically diverse organelles defined by the common function of degrading intracellular material.

Lysosomal Acid Hydrolases

Lysosomes contain about 50 different degradative enzymes that can hydrolyze proteins, DNA, RNA, polysaccharides, and lipids. Mutations in the genes that encode these enzymes are responsible for more than 30 different human genetic diseases, which are called lysosomal storage diseases because undegraded material accumulates within the lysosomes of affected individuals. Most of these diseases result from deficiencies in single lysosomal enzymes. For example, **Gaucher's disease (the most** common of these disorders) results from a mutation in the gene that encodes a lysosomal enzyme required for the breakdown of glycolipids.

All of the lysosomal enzymes are acid hydrolases, which are active at the acidic pH (about 5) that is maintained within lysosomes but not at the neutral pH (about 7.2) characteristic of the rest of the cytoplasm (Figure). The requirement of these lysosomal hydrolases for acidic pH provides double protection against uncontrolled digestion of the contents of the cytosol; even if the lysosomal membrane were to break down, the released acid hydrolases would be inactive at the neutral pH of the cytosol. To maintain their acidic internal pH, lysosomes must actively concentrate H^+ ions (protons). This is accomplished by a proton pump in the lysosomal membrane, which actively transports protons into the lysosome from the cytosol. This pumping requires expenditure of energy in the form of ATP hydrolysis, since it maintains approximately a hundredfold higher H^+ concentration inside the lysosome.

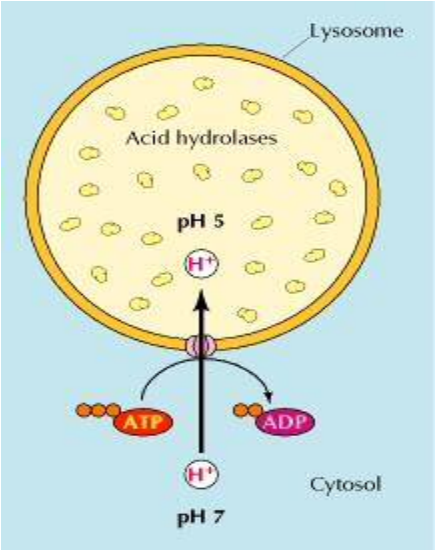


Figure: Organization of the lysosome

TABLE 19–2 SOME ENZYMES PRESENT IN LYSOSOMES

Enzyme	Substrate
Proteases and peptidases	
Cathepsin A, B, C, D, and E	Various proteins and peptides
Collagenase	Collagen
Arylamidase	Amino acid arylamides
Peptidase	Peptides
Nucleases	
Acid ribonuclease	RNA
Acid deoxyribonuclease	DNA
Phosphatases	
Acid phosphatase	Phosphate monoesters
Phosphodiesterase	Oligonucleotides, phosphodiesters
Phosphatidic acid phosphatase	Phosphatidic acids
Enzymes acting on carbohydrate chains of glycoproteins and glycolipids	
β-Galactosidase	β-Galactosides
Acetylhexosaminidase	Acetylhexosaminides, heparin sulfate
β-Glucosidase	β-Glucosides
α-Glucosidase	Glycogen
α-Mannosidase	α-Mannosides
Sialidase	Sialic acid derivatives
Enzymes acting on glycosaminoglycans	
Lysozyme	Mucopolysaccharides, bacterial cell walls
Hyaluronidase	Hyaluronic acid, chondroitin sulfates
β-Glucuronidase	Polysaccharides, mucopolysaccharides
Arylsulfatase A and B	Arylsulfates, cerebroside sulfates, chondroitin sulfate
Enzymes acting on lipids	
Phospholipase	Lecithin, phosphatidyl ethanolamine
Esterase	Fatty acid esters
Sphingomyelinase	Sphingomyelin

Lysosomal Enzyme	**LSD**
β-mannosidase	β-mannosidosis
α-neuraminidase	sialidosis mucolipidosis type I
β-galactoisidase	GM1 gangliosidosis
β-glucosidase/glucocerebrosidase	Gaucher disease
MPS VII, β-glucuronidase	Sly syndrome
hexosaminidase A	GM2 gangliosidosis Tay-Sachs disease
laronidase recombinant human a-L-iduronidase	mucospolysaccharidosis, MPS I
idursulfase	mucopolysaccharidosis II
N-acetylgalactosamine 4-sulfatase galsulfase	mucopolysaccharidosis VI Maroteaux-Lamy syndrome
sphingomyelinase	Niemann-Pick disease
galactocerebrosidase	Globoid cell leukodystrophy Krabbe disease
arylsulfatase A	methachromatic leukodystrophy
glycosaminoglycan cleaving enzymes	mucopolysaccharidoses
glycoprotein cleaving enzymes	glycoproteinoses
α-glucosidase	glycogenosis type II Pompe disease
lysosomal proteases	neuronal ceroid lipofuscinoses

Lysosomal Enzymes, the Substrate on Which these Work and the End Products

Enzymes	*Substrate*	*End Product*
1. Nucleases		
(i) Acid ribonuclease	Polynucleotides of RNA	Nitrogenous bases + Phosphate + ribose sugar
(ii) Acid deoxyribonuclease	Nitrogenous base+DNA	Phosphate + deoxyribose sugar
2. Phosphatases		
(i) Acid phosphatase	Phosphomonoesters	Monophosphates
(ii) Phosphodiesterase	Oligonucleotides, phosphodiesters	Monophosphates
3. Proteases and Peptidases		
(i) Cathepsins (A.B.) and peptidase	Various proteins	Amino acids
(ii) Collagenase	Collagen	Amino acids
(iii) Peptidase	Peptides	Amino acids
4. Glycosidase		
(i) β-galactosidase	β-galactosides	
(ii) α-glucosidase	Glycogens	
(iii) β-glucosidase	β-glucosides	
(iv) α-Mannosidase	α-mannosides	Monosaccharides
(v) β-glucuronidase	Polysaccharides and mucopolysaccharides	
5. Sulphatases	Sulphate esters	Fragments of lipids
6. Lipases	Lipids	Fragments of lipids
7. Esterases	Fatty acids esters	Fragements thereof
8. Lysozyme	Mucopolysaccharides, bacterial cell alls	Fragements thereof
9. Sphingomyelinase	Sphingomyelin	Fragements thereof

ENDOCYTOSIS AND LYSOSOME FORMATION

One of the major functions of lysosomes is the digestion of material taken up from outside the cell by endocytosis. However, the role of lysosomes in the digestion of material taken up by endocytosis relates not only to the function of lysosomes but also to their formation. In particular, lysosomes are formed by the fusion of transport vesicles budded from the *trans* Golgi network with endosomes, which contain molecules taken up by endocytosis at the plasma membrane.

The formation of lysosomes thus represents an intersection between the secretory pathway, through which lysosomalproteins are processed, and the endocytic pathway, through which extracellular molecules are taken up at the cell surface (Figure). Material from outside the cell is taken up in clathrin-coated endocytic vesicles, which bud from the plasma membrane and then fuse with early endosomes. Membrane components are then recycled to the plasma membrane and the early endosomes gradually mature into late endosomes, which are the precursors to lysosomes. One of the important changes

during endosome maturation is the lowering of the internal pH to about 5.5, which plays a key role in the delivery of lysosomal acid hydrolases from the *trans* Golgi network.

As discussed earlier, acid hydrolases are targeted to lysosomes by mannose-6-phosphate residues, which are recognized by mannose-6-phosphate receptors in the *trans* Golgi network and packaged into clathrin-coated vesicles. Following removal of the clathrin coat, these transport vesicles fuse with late endosomes, and the acidic internal pH causes the hydrolases to dissociate from the mannose-6-phosphate receptor. The hydrolases are thus released into the lumen of the endosome, while the receptors remain in the membrane and are eventually recycled to the Golgi. Late endosomes then mature into lysosomes as they acquire a full complement of acid hydrolases, which digest the molecules originally taken up by endocytosis (Figure).

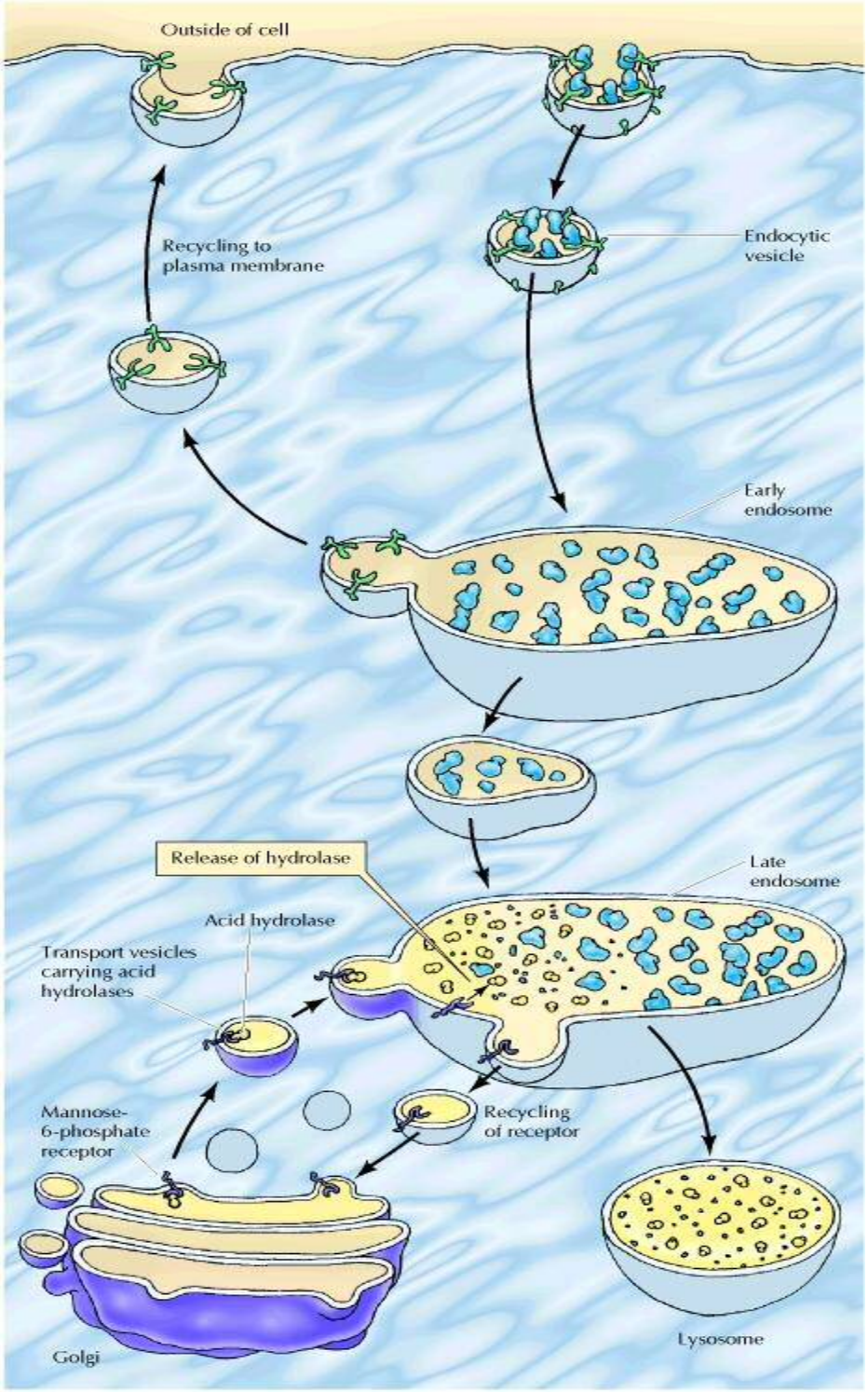


Figure :Endocytosis and lysosome formation

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PHAGOCYTOSIS AND AUTOPHAGY

In addition to degrading molecules taken up by endocytosis, lysosomes digest material derived from two other routes:phagocytosis and autophagy. In phagocytosis specialized cells, such as macrophages, take up and degrade large particles, including bacteria, cell debris, and aged cells that need to be eliminated from the body. Such large particles are taken up in phagocytic vacuoles (phagosomes), which then fuse with lysosomes,

resulting in digestion of their contents. The lysosomes formed in this way (phagolysosomes) can be quite large and heterogeneous, since their size and shape is determined by the content of material that is being digested.

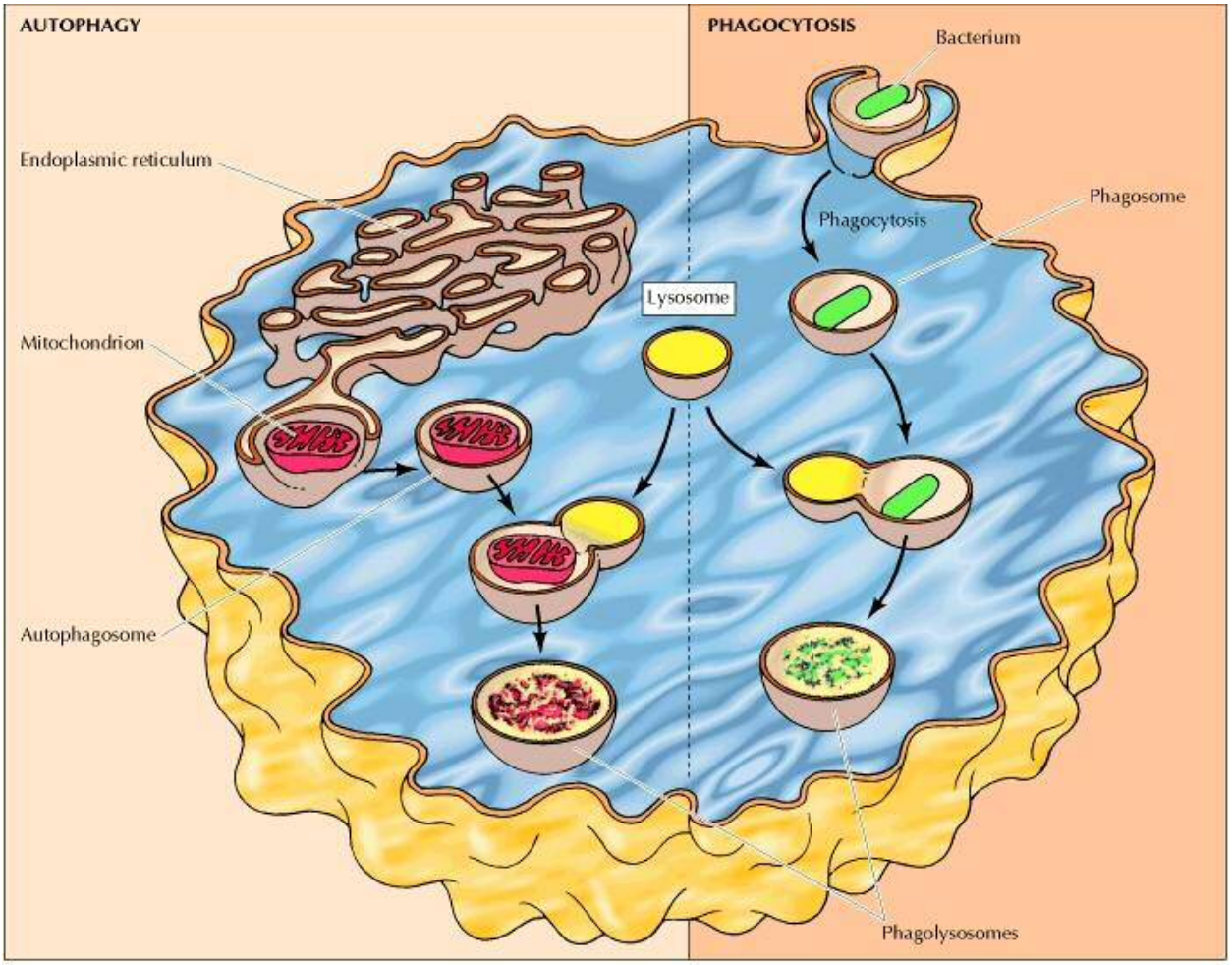

Figure : Lysosomes in phagocytosis and autophagy

In phagocytosis, large particles (such as bacteria) are taken up into phagocytic vacuoles or phagosomes. In autophagy, internal organelles (such as mitochondria) are enclosed by membrane fragments from the ER, forming autophagosomes. Both phagosomes and autophagosomes fuse with lysosomes to form large phagolysosomes, in which their contents are digested.

Lysosomes are also responsible for autophagy, **the gradual turnover of the cell's own** components. The first step ofautophagy appears to be the enclosure of an organelle (e.g., a mitochondrion) in membrane derived from the ER. The resulting vesicle (an autophagosome) then fuses with a lysosome, and its contents are digested (see Figure). Autophagy is responsible for the gradual turnover of cytoplasmic organelles.

MITOCHONDRIA

Mitochondria play a critical role in the generation of metabolic energy in eukaryotic cells. It is responsible for most of the useful energy derived from the breakdown of carbohydrates and fatty acids, which is converted to ATP by the process of oxidative phosphorylation. Most mitochondrial proteins are translated on free cytosolic ribosomes and imported into the organelle by specific targeting signals. In addition, mitochondria are unique among the cytoplasmic organelles already discussed in that they contain their own DNA, which encodes tRNAs, rRNAs, and some mitochondrial proteins. The assembly of mitochondria thus involves proteins encoded by their own genomes and translated within the organelle, as well as proteins encoded by the nuclear genome and imported from the cytosol.

ORGANISATION AND FUNCTIONS OF MITOCHONDRIA

Mitochondria are surrounded by a double-membrane system, consisting of inner and outer mitochondrial membranes separated by an intermembrane space (Figure). The inner membrane forms numerous folds (cristae), which extend into the interior (or matrix) of the organelle. Each of these components plays distinct functional roles, with the matrix and inner membrane representing the major working compartments of mitochondria.

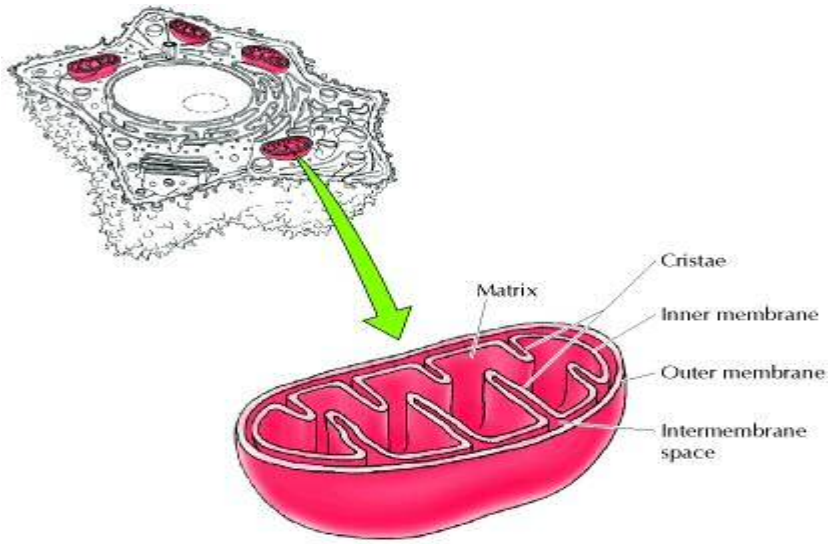

Figure 10.1Structure of a mitochondrion

The matrix contains the mitochondrial genetic system as well as the enzymes responsible for the central reactions of oxidative metabolism (Figure). As discussed eralier, the oxidative breakdown of glucose and fatty acids is the principal source of metabolic energy in animal cells. The initial stages of glucose metabolism (glycolysis) occur in the cytosol, where glucose is converted to pyruvate (see Figure). Pyruvate is then transported into mitochondria, where its complete oxidation to CO_2 yields the bulk of usable energy (ATP) obtained from glucose metabolism. This involves the initial oxidation of pyruvate to acetyl CoA, which is then broken down to CO_2 via the citric acid cycle (seeFigures). The oxidation of fatty acids also yields acetyl CoA (see Figure), which is similarly

metabolized by the citric acid cycle in mitochondria. The enzymes of the citric acid cycle (located in the matrix of mitochondria) thus are central players in the oxidative breakdown of both carbohydrates and fatty acids.

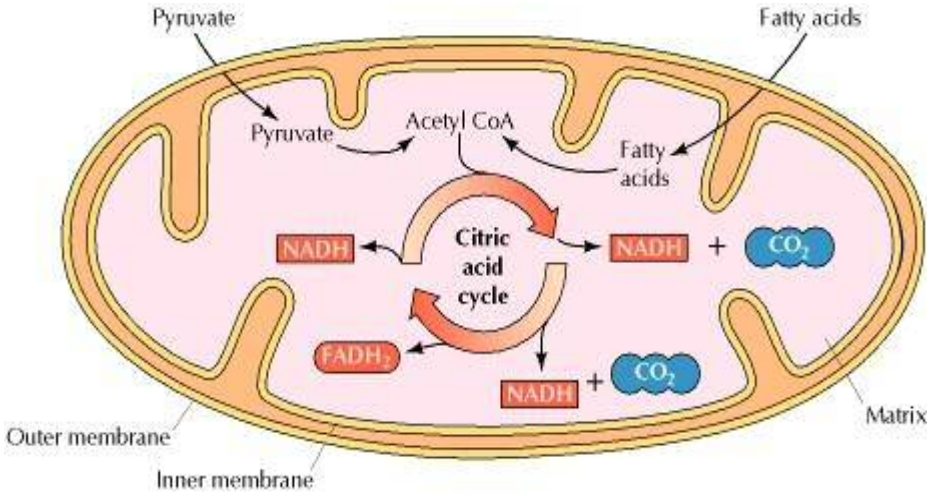

Figure: Metabolism in the matrix of mitochondria

ETC in inner membrane

The oxidation of acetyl CoA to CO_2 is coupled to the reduction of NAD^+ and FAD to NADH and $FADH_2$, respectively. Most of the energy derived from oxidative metabolism is then produced by the process of oxidative phosphorylation, which takes place in the inner mitochondrial membrane. The high-energy electrons from NADH and $FADH_2$ are transferred through a series of carriers in the membrane to molecular oxygen. The energy derived from these electron transfer reactions is converted to potential energy stored in a proton gradient across the membrane, which is then used to drive ATP synthesis. The inner mitochondrial membrane thus represents the principal site of ATP generation, and this critical role is reflected in its structure. First, its surface area is substantially increased by its folding into cristae. In addition, the inner mitochondrial membrane contains an unusually high percentage (greater than 70%) of proteins, which are involved in oxidative phosphorylation as well as in the transport of metabolites (e.g., pyruvate and fatty acids) between the cytosol and mitochondria. Otherwise, the inner membrane is impermeable to most ions and small molecules—a property critical to maintaining the proton gradient that drives oxidative phosphorylation.

In contrast to the inner membrane, the outer mitochondrial membrane is freely permeable to small molecules. This is because it contains proteins called porins, which form channels that allow the free diffusion of molecules smaller than about 6000 daltons. The composition of the intermembrane space is therefore similar to the cytosol with respect to ions and small molecules. Consequently, the inner mitochondrial membrane is the functional barrier to the passage of small molecules between the cytosol and the matrix and maintains the proton gradient that drives oxidative phosphorylation.

GENETIC SYSTEM OF MITOCHONDRIA

Mitochondria contain their own genetic system, which is separate and distinct from the nuclear genome of the cell. Mitochondria are thought to have evolved from bacteria that developed a symbiotic relationship in which they lived within larger cells (endosymbiosis).

Mitochondrial genomes are usually circular DNA molecules, like those of bacteria, which are present in multiple copies per organelle. They vary considerably in size between different species. The genomes of human and most other animal mitochondria are only about 16 kb, but substantially larger mitochondrial genomes are found in yeasts (approximately 80 kb) and plants (more than 200 kb). In addition, mitochondrial genomes encode all of the ribosomal RNAs and most of the transfer RNAs needed for translation of these protein-coding sequences within mitochondria. Other mitochondrial proteins are encoded by nuclear genes, which are thought to have been transferred to the nucleus from the ancestral mitochondrial genome.

The human mitochondrial genome encodes 13 proteins involved in electron transport and oxidative phosphorylation (Figure). In addition, human mitochondrial DNA encodes 16S and 12S rRNAs and 22 tRNAs, which are required for translation of the proteins encoded by the organelle genome. The two rRNAs are the only RNA components of animal and yeast mitochondrial ribosomes, in contrast to the three rRNAs of bacterial ribosomes (23S, 16S, and 5S). Plant mitochondrial DNAs, however, also encode a third rRNA of 5S. The mitochondria of plants and protozoans also differ in importing and utilizing tRNAs encoded by the nuclear as well as the mitochondrial genome, whereas in animal mitochondria, all the tRNAs are encoded by the organelle.

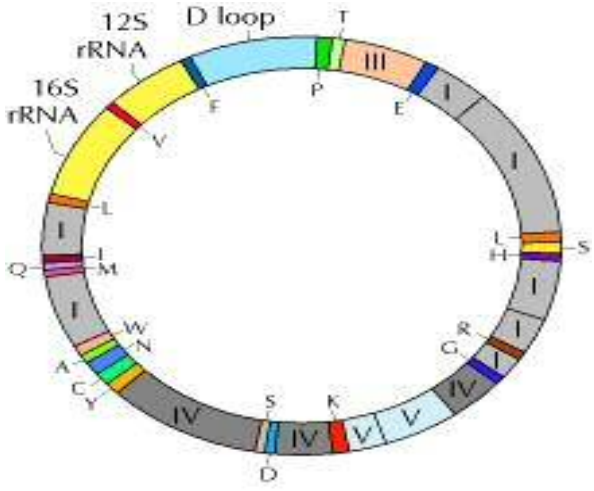

Figure: The human mitochondrial genome

PROTEIN IMPORT AND MITOCHONDRIAL ASSEMBLY

In contrast to the RNA components of the mitochondrial translation apparatus (rRNAs and tRNAs), most mitochondrial genomes do not encode the proteins required for DNA replication, transcription, or translation. Instead, the genes that encode proteins

required for the replication and expression of mitochondrial DNA are contained in the nucleus. In addition, the nucleus contains the genes that encode most of the mitochondrial proteins required for oxidative phosphorylation and all of the enzymes involved in mitochondrial metabolism (e.g., enzymes of the citric acid cycle). The proteins encoded by these genes (more than 95% of mitochondrial proteins) are synthesized on free cytosolic ribosomes and imported into mitochondria as completed polypeptide chains. Because of the double-membrane structure of mitochondria, the import of proteins is considerably more complicated than the transfer of a polypeptide across a single phospholipid bilayer. Proteins targeted to the matrix have to cross both the inner and outer mitochondrial membranes, while other proteins need to be sorted to distinct compartments within the organelle (e.g., the intermembrane space).

The import of proteins to the matrix is the best-understood aspect of mitochondrial protein sorting (Figure). Most proteins are targeted to mitochondria by amino-terminal sequences of 20 to 35 amino acids (called presequences) that are removed by proteolytic cleavage following their import into the organelle. The presequences of mitochondrial proteins, first characterized by Gottfried Schatz, contain multiple positively charged amino acid residues, usually in anamphipathic **α helix**.

- The first step in protein import is the binding of these presequences to receptors on the surface of mitochondria.
- The polypeptide chains are then inserted into a protein complex that directs translocation across the outer membrane (the translocase of the outer membrane or Tom complex).
- The proteins are then transferred to a second protein complex in the inner membrane (the translocase of the inner membrane or Tim complex).
- Continuing protein translocation requires the electrochemical potential established across the inner mitochondrial membrane during electron transport. The transfer of high-energy electrons from NADH and $FADH_2$to molecular oxygen is coupled to the transfer of protons from the mitochondrial matrix to the intermembrane space. Since protons are charged particles, this transfer establishes an electric potential across the inner membrane, with the matrix being negative. During protein import, this electric potential drives translocation of the positively charged presequence.

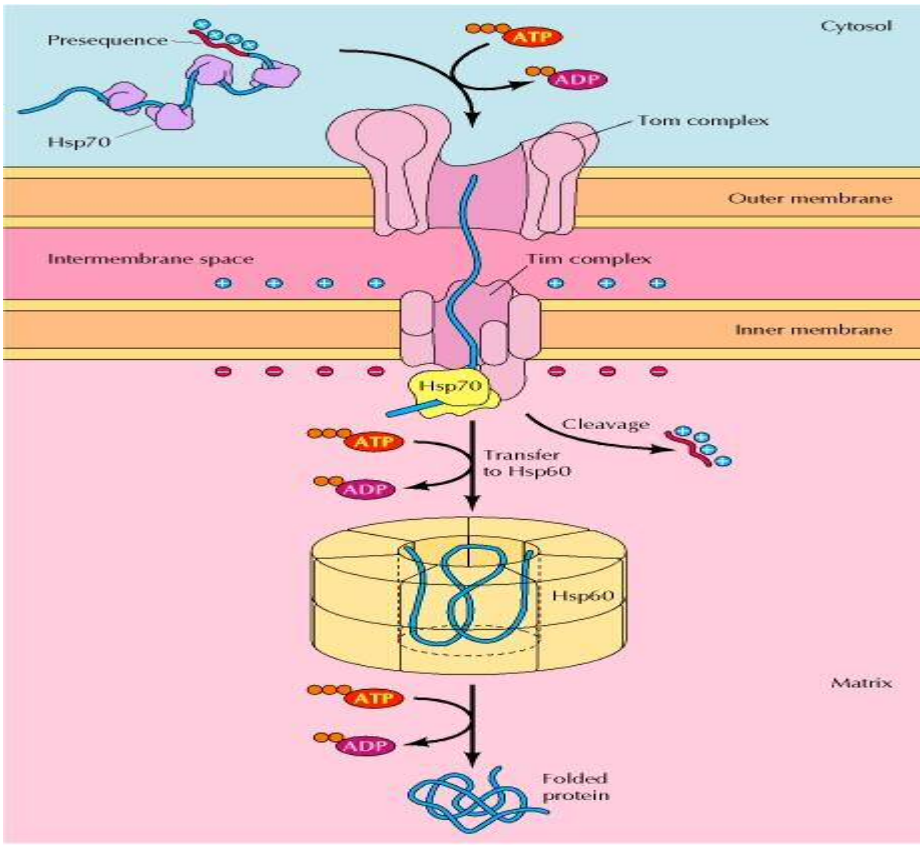

Figure : Import of proteins into mitochondria

Proteins are targeted for mitochondria by an amino-terminal presequence containing positively charged amino acids. Proteins are maintained in a partially unfolded state by association with a cytosolic Hsp70 and are recognized by a receptor on the surface of mitochondria. The unfolded polypeptide chains are then translocated through the Tom complex in the outer membrane and transferred to the Tim complex in the inner membrane. The voltage component of the electrochemical gradient is required for translocation across the inner membrane. The presequence is cleaved by a matrix protease, and a mitochondrial Hsp70 binds the polypeptide chain as it crosses the inner membrane, driving further protein translocation. A mitochondrial Hsp60 then facilitates folding of the imported polypeptide within the matrix.

To be translocated across the mitochondrial membrane, proteins must be at least partially unfolded. Consequently, protein import into mitochondria requires molecular chaperones in addition to the membrane proteins involved in translocation (see Figure). On the cytosolic side, members of the Hsp70 family of chaperones maintain proteins in a partially unfolded state so that they can be inserted into the mitochondrial membrane. As they cross the inner membrane, the unfolded polypeptide chains bind to another member of the Hsp70 family, which is associated with the Tim complex and acts as a motor that drives protein import. The polypeptide is then transferred to a chaperone of the Hsp60 family (achaperonin), within which protein folding takes place. Since these interactions of polypeptide chains with molecular chaperones depend on ATP, protein import requires

ATP both outside and inside the mitochondria, in addition to the electric potential across the inner membrane.

As noted above, some mitochondrial proteins are targeted to the outer membrane, inner membrane, or intermembrane space rather than to the matrix, so additional mechanisms are needed to direct these proteins to the correct submitochondrial compartment. These proteins are targeted to their destinations by a second sorting signal following the positively charged presequence that directs mitochondrial import. The targeting of proteins to the mitochondrial membranes appears to be mediated by hydrophobic stop-transfer sequences that halt translocation of the polypeptidechains through the Tim or Tom complexes, leading to their insertion into the inner or outer mitochondrial membranes, respectively

Not only the proteins, but also the phospholipids of mitochondrial membranes are imported from the cytosol. In animal cells, phosphatidylcholine and phosphatidylethanolamine are synthesized in the ER and carried to mitochondria byphospholipid transfer proteins, which extract single phospholipid molecules from the membrane of the ER. The lipid can then be transported through the aqueous environment of the cytosol, buried in a hydrophobic binding site of the protein, and released when the complex reaches a new membrane, such as that of mitochondria. The mitochondria then synthesize phosphatidylserine from phosphatidylethanolamine, in addition to catalyzing the synthesis of the unusual phospholipid cardiolipin, which contains four fatty acid chains (Figure).

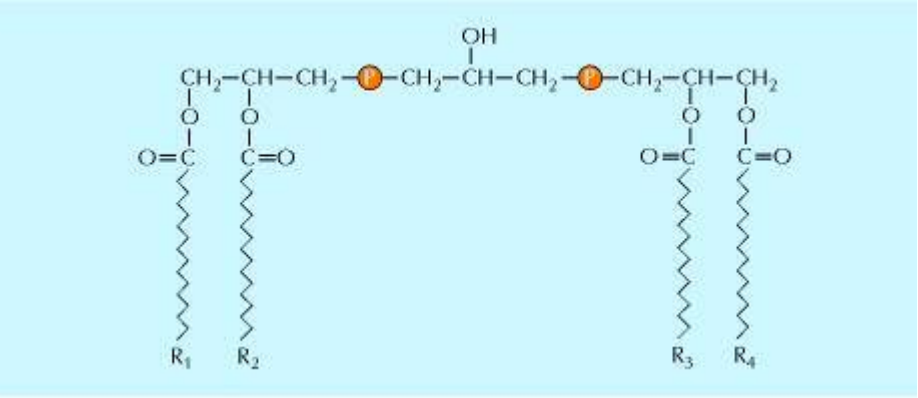

Figure 10.7Structure of cardiolipin

Cardiolipin is an unusual **"double" phospholipid, containing four fatty acid chains,** that is found primarily in the inner mitochondrial membrane.

CHLOROPLASTS AND OTHER PLASTIDS

Chloroplasts, the organelles responsible for photosynthesis, are in many respects similar to mitochondria. Both chloroplasts and mitochondria function to generate metabolic energy, evolved by endosymbiosis, contain their own genetic systems, and replicate by division. However, chloroplasts are larger and more complex than mitochondria, and they perform several critical tasks in addition to the generation of ATP. Most importantly,

chloroplasts are responsible for the photosynthetic conversion of CO_2 to carbohydrates. In addition, chloroplasts synthesize amino acids, fatty acids, and the lipid components of their own membranes. The reduction of nitrite (NO_2^-) to ammonia (NH_3), an essential step in the incorporation of nitrogen into organic compounds, also occurs in chloroplasts. Moreover, chloroplasts are only one of several types of related organelles (plastids) that play a variety of roles in plant cells.

STRUCTURE AND FIUNCTIONS OF CHLOROPLAST

Plant chloroplasts are large organelles (5 to 10 μm long) that, like mitochondria, are bounded by a double membrane called the chloroplast envelope (Figure 10.13). In addition to the inner and outer membranes of the envelope, chloroplasts have a third internal membrane system, called the thylakoid membrane. The thylakoid membrane forms a network of flattened discs called thylakoids, which are frequently arranged in stacks called grana. Because of this three-membrane structure, the internal organization of chloroplasts is more complex than that of mitochondria. In particular, their three membranes divide chloroplasts into three distinct internal compartments: (1) the intermembrane space between the two membranes of the chloroplast envelope; (2) the stroma, which lies inside the envelope but outside the thylakoid membrane; and (3) the thylakoid lumen.

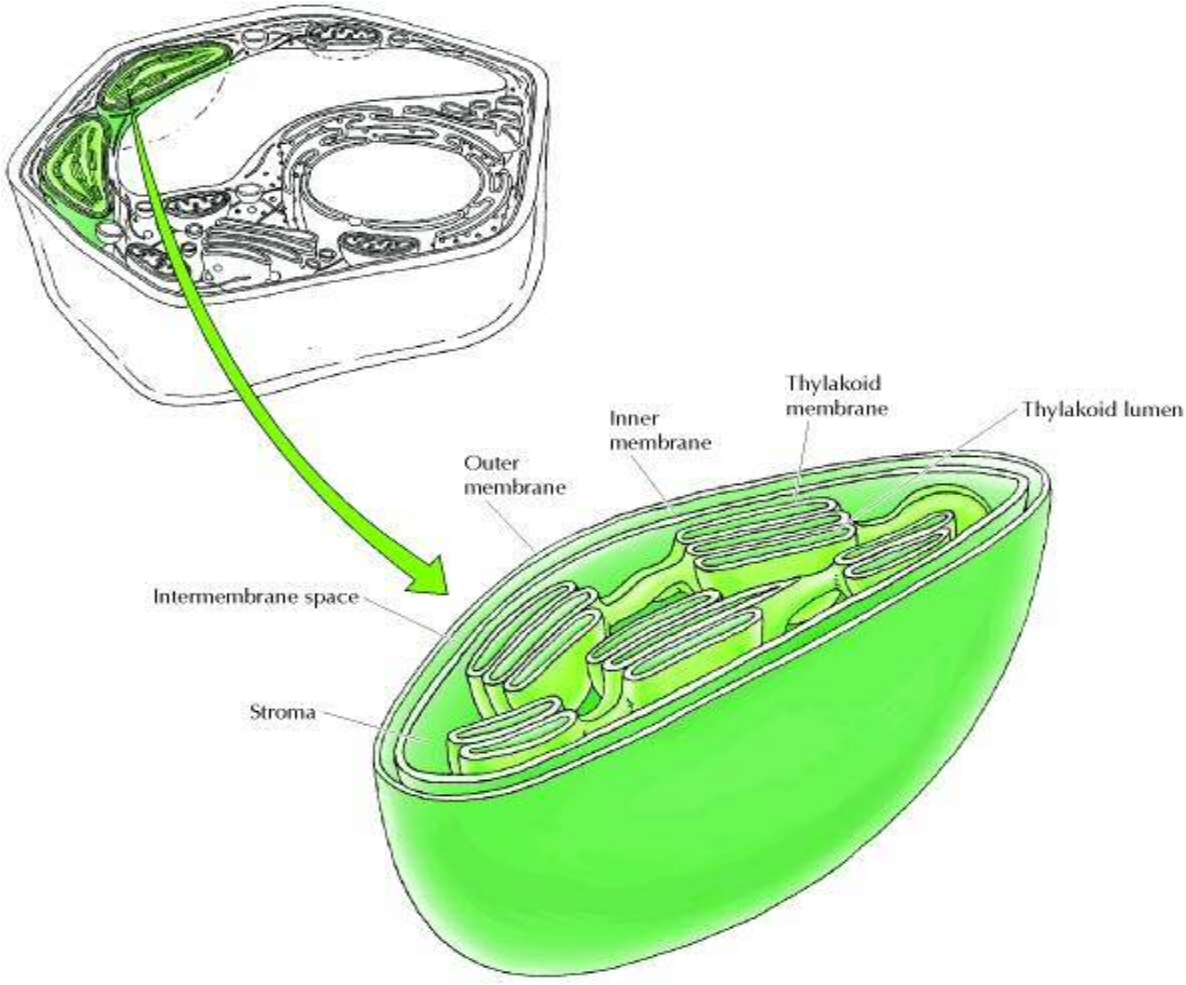

Figure: Structure of a chloroplast

In addition to the inner and outer membranes of the envelope, chloroplasts contain a third internal membrane system: the thylakoid membrane. These membranes divide chloroplasts into three internal compartments.

Despite this greater complexity, the membranes of chloroplasts have clear functional similarities with those ofmitochondria—as expected, given the role of both organelles in the chemiosmotic generation of ATP. The outer membrane of the chloroplast envelope, like that of mitochondria, contains porins and is therefore freely permeable to small molecules. In contrast, the inner membrane is impermeable to ions and metabolites, which are therefore able to enter chloroplasts only via specific membrane transporters. These properties of the inner and outer membranes of the chloroplast envelope are similar to the inner and outer membranes of mitochondria: In both cases the inner membrane restricts the passage of molecules between the cytosol and the interior of the organelle. The chloroplast stroma is also equivalent in function to the mitochondrial matrix: It contains the chloroplast genetic system and a variety of metabolicenzymes, including those responsible for the critical conversion of CO_2 to carbohydrates during photosynthesis.

The major difference between chloroplasts and mitochondria, in terms of both structure and function, is the thylakoid membrane. This membrane is of central importance in chloroplasts, where it fills the role of the inner mitochondrial membrane in electron transport and the chemiosmotic generation of ATP (Figure). The inner membrane of the chloroplast envelope (which is not folded into cristae) does not function in photosynthesis. Instead, the chloroplast electron transport system is located in the thylakoid membrane, and protons are pumped across this membrane from the stroma to the thylakoid lumen. The resulting electrochemical gradient then drives ATP synthesis as protons cross back into the stroma. In terms of its role in generation of metabolic energy, the thylakoid membrane of chloroplasts is thus equivalent to the inner membrane of mitochondria.

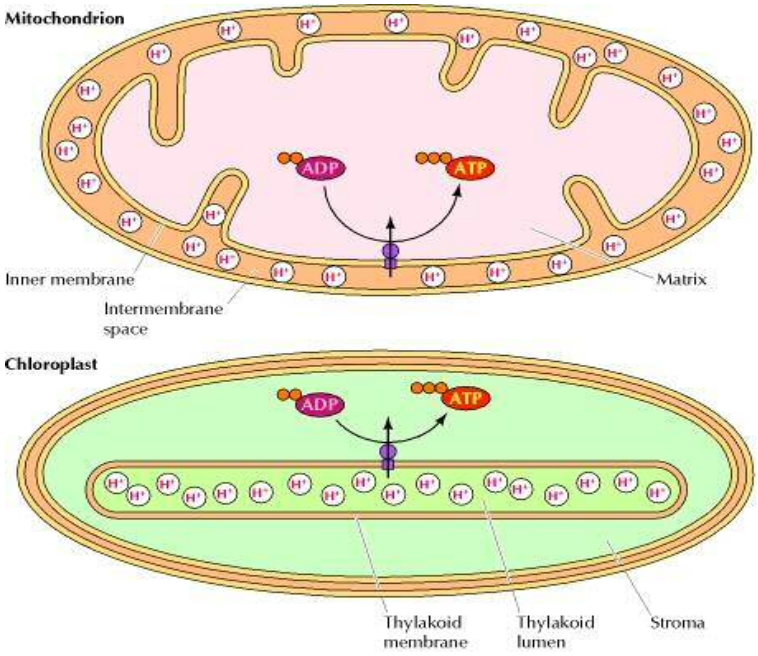

Figure:Generation of ATP in chloroplasts and mitochondria

In mitochondria, electron transport generates a proton gradient across the inner membrane, which is then used to drive ATP synthesis in the matrix. In chloroplasts, the proton gradient is generated across the thylakoid membrane and used to drive ATP synthesis in the stroma.

Chloroplasts Genome

Like mitochondria, chloroplasts contain their own genetic system, reflecting their evolutionary origins from photosynthetic bacteria. The genomes of chloroplasts are similar to those of mitochondria in that they consist of circular DNA molecules present in multiple copies per organelle. However, chloroplast genomes are larger and more complex than those of mitochondria, ranging from 120 to 160 kb and containing approximately 120 genes. These chloroplast genes encode both RNAs and proteins involved in geneexpression, as well as a variety of proteins that function in photosynthesis

These chloroplast genes encode both RNAs and proteins involved in geneexpression, as well as a variety of proteins that function in photosynthesis. In addition to these RNA components of the translation system, the chloroplast genome encodes about 20 ribosomal proteins, which represent approximately a third of the proteins of chloroplast ribosomes. Some subunits ofRNA polymerase are also encoded by chloroplasts, although additional RNA polymerase subunits and other factors needed for chloroplast gene expression are encoded in the nucleus.

IMPORTING AND SORTING OF MITOCHONDRIAL PROTEIN

Although chloroplasts encode more of their own proteins than mitochondria, about 90% of chloroplast proteins are still encoded by nuclear genes. As with mitochondria, these

proteins are synthesized on cytosolic ribosomes and then imported into chloroplasts as completed polypeptide chains. They must then be sorted to their appropriate location within chloroplasts—an even more complicated task than protein sorting in mitochondria, since chloroplasts contain three separate membranes that divide them into three distinct internal compartments.

- Protein import into chloroplasts generally resembles mitochondrial protein import (Figure). Proteins are targeted for import into chloroplasts by N-terminal sequences of 30 to 100 amino acids, called transit peptides, which direct protein translocation across the two membranes of the chloroplast envelope and are then removed by proteolytic cleavage.
- The transit peptides are recognized by the translocation complex of the chloroplast outer member (the Toc complex), and proteins are transported through this complex across the membrane. They are then transferred to the translocation complex of the inner membrane (the Tic complex) and transported across the inner membrane to the stroma.
- As in mitochondria, molecular chaperones on both the cytosolic and stromal sides of the envelope are required for protein import, which requires energy in the form of ATP. In contrast to the presequences of mitochondrial import, however, transit peptides are not positively charged and the translocation of polypeptide chains into chloroplasts does not require an electric potential across the membrane.

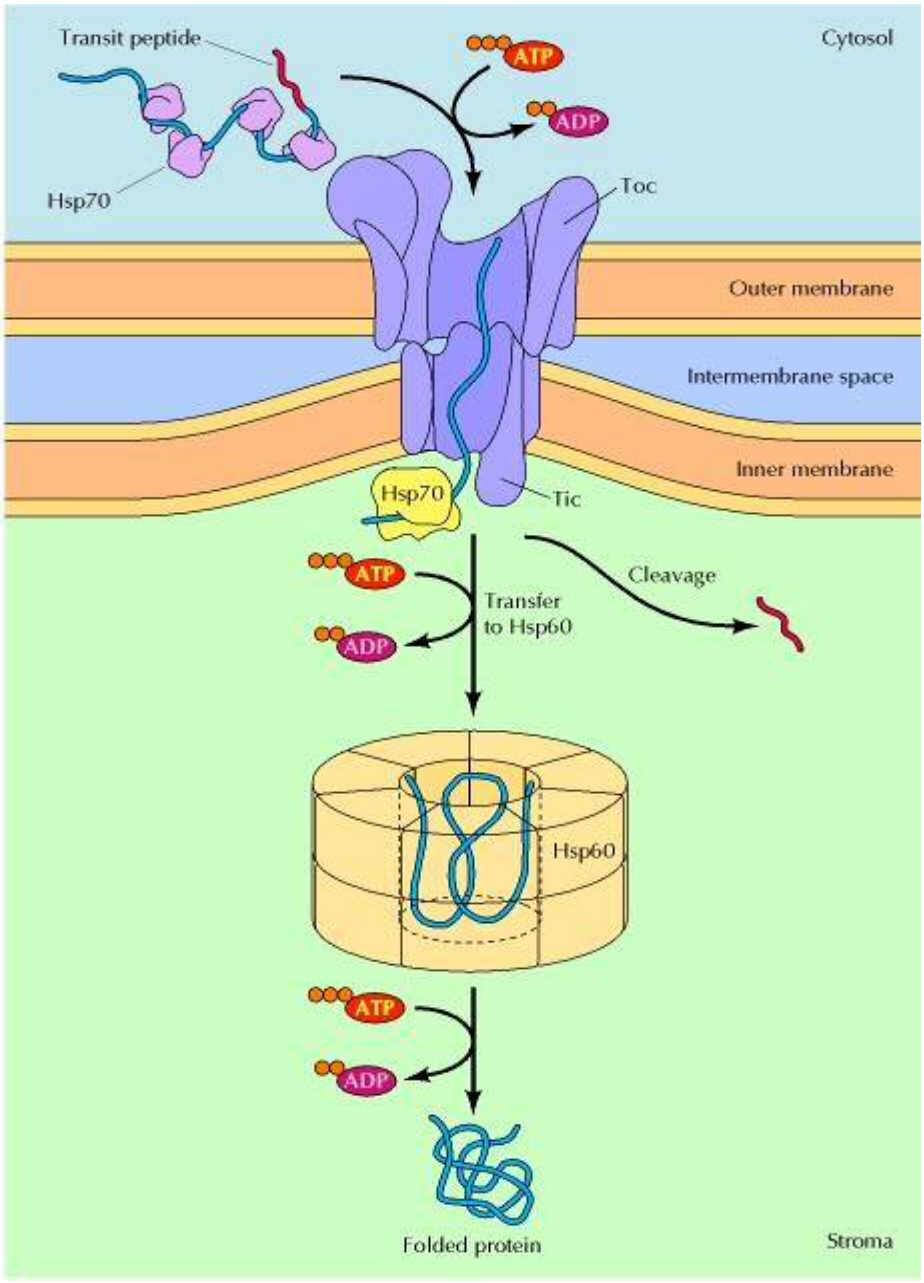

Figure:Protein import into the chloroplast stroma

Proteins are targeted for import into chloroplasts by a transit peptide at their amino terminus. The transit peptide directs polypeptide translocation through the Toc complex in the chloroplast outer membrane and the Tic complex in the chloroplast inner membrane. This peptide is then removed by proteolytic cleavage within the stroma. Both cytosolic and chloroplast chaperones (Hsp60 and Hsp70) are required for protein import.

Proteins incorporated into the thylakoid lumen are transported to their destination in two steps (Figure).

They are first imported into the stroma, as already described, and are then targeted for translocation across the thylakoid membrane by a second hydrophobic signal sequence, which is exposed following cleavage of the transit peptide. The hydrophobic signal

sequence directs translocation of the polypeptide across the thylakoid membrane and is finally removed by a second proteolytic cleavage within the lumen.

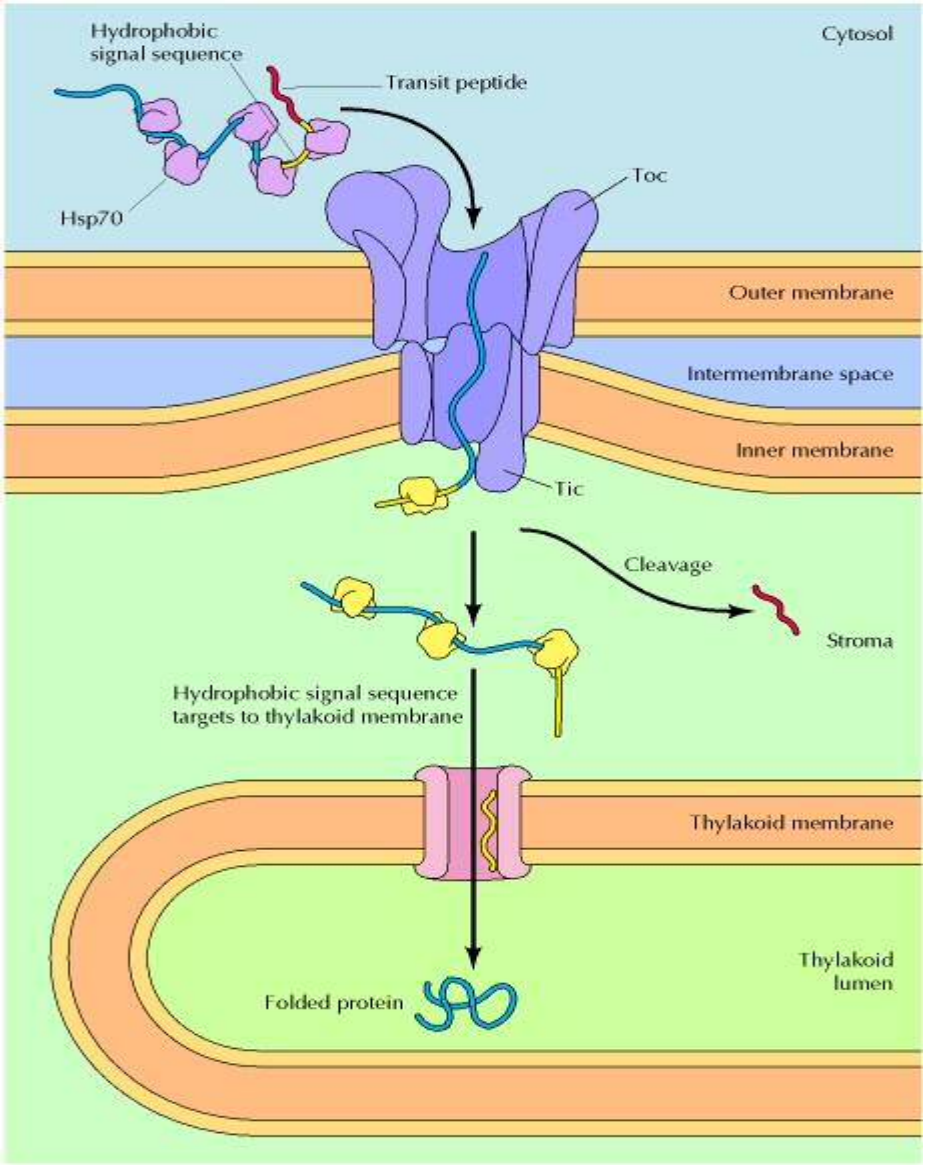

Figure: Import of proteins into the thylakoid lumen

Proteins are imported into the thylakoid lumen in two steps. The first step is import into the chloroplast stroma, as illustrated in Figure . Cleavage of the transit peptide then exposes a second hydrophobic signal sequence, which directs protein translocation across the thylakoid membrane.

The pathways of protein sorting to the other four compartments of chloroplasts—the inner and outer membranes,thylakoid membrane, and intermembrane space—are less well established. As with mitochondria, proteins appear to be inserted directly into the outer membrane of the chloroplast envelope. In contrast, proteins destined for either the thylakoid membrane or the inner membrane of the chloroplast envelope are initially targeted for import into the stroma by N-terminal transit peptides. Following cleavage of the transit peptides, these proteins are then targeted for insertion into the appropriate membrane by other sequences, which are not yet well characterized. Finally, neither the

sequences that target proteins to the intermembrane space nor the pathways by which they travel to that destination have been identified.

KARPAGAM ACADEMY OF HIGHER EDUCATION
COIMBATORE - 641021
DEPARTMENT OF BIOCHEMISTRY
I-B.Sc., BIOCHEMISTRY - BATCH: 2017 - 2020
PART A (20 X 1 = 20 MARKS) - Online MCQ Questions

SUBJECT: CE SUBJECT CODE: 17BCU102

Questions	Option A	Option B	Option C	Option D	Answer
Suicide bags are otherwise called	Ribosomes	Lysosomes	Golgi apparatus	Endoplasmic reticulam	Lysosomes
The respiratory center at the cell is	Nucleus	Cytoplasm	Microtubules	Mitochondria	Mitochondria
An interface between the nucleus and cytoplasm is	Nuclear envelope	Nuclear membrane	Nuclear pores	Perinuclear space	Perinuclear space
Which of these cellular organelles does not have their own DNA?	Chloroplast	Nucleus	Mitochondrion	Ribosomes	Ribosomes
The Golgi apparatus important for	Protein synthesis	DNA synthesis	RN A synthesis	Packaging and secretion of proteins	Packaging and secretion of proteins
The prokaryotic cell contain	70s ribosome	60s ribosome	50s ribosome	80s ribosome	50s ribosome

Rough endoplasmic reticulum is embedded with	RNA	Ribosome	Protein	Lysosomes	Ribosome
ATP may be produced within the mitochondria by ----------	Reduction	Oxidation	Hydrolysis	Oxidatio and reduction	Oxidation
The period in which phosphorylation of ADP and electron transport occur at high rate is -------	Orthodox conformational state	Condensed conformational state	Relaxed conformational state	Reduced conformational state	Condensed conformational state
Electron transport chain is also called as ----------	Respiratory chain	Transport chain	Reproductive chain	Phosphorylation chain	Respiratory chain
Rough endoplasmic reticulum is embedded with	RNA	Ribosome	Protein	Lysosomes	Ribosome

The golgi apparatus important for	Protein synthesis	DNA synthesis	RNA synthesis	Packaging and secretion of proteins	Packaging and secretion of proteins
Which one is important in drug detoxication reaction	RER	Golgi bodies	lysosomes	SER	SER
Mitochondria is impermeable from cytosolic side to except	fatty acid	pyruvic acid	Nucleotide	NADH	Nucleotide
Secretory vesicles are formed from	ER	golgi bodies	lysosome	Ribosome	golgi bodies
The citrice acid cycle is operating in	outer membrane	Inter membrane space	inner membrane	Mitochondra il matrix	inner membrane
……..... is involved in the ETC reaction by oxidative phosphorylation.	Mitochondria	lysosome	ribosome	golgi complex	Mitochondria

The ETC located in the …….. of matrix.	outer membrane	cristae	inner membrane	matrix	inner membrane
UQ transfers electron from …….....	Complex I to III	complex II to III	Complex I to III and Complex II to III	Complex I to III	Complex I to III and Complex II to III
Ubiquinone is a …… soluble benzoquinone with a long isoprenoid side chain.	water soluble	fat soluble	lipid soluble	none of the above	fat soluble
Which protein is used for modifying pepetide chain.?	ubiquitin	protenases	chaperons	protein kinase	chaperons
Which enzyme synthesisATP from ADP during oxidative phosphorylation.	ATP synthetase	ATP ase	phosphorylase	ATP synthetase	ATP synthetase
The genome zize of mitochondria	16kb	20kb	80kb	200kb	16kb

ETC is _____ on mitochondrial membrane	Enzyme	RNA	Ribozyme	Abzyme	Enzyme
Rough endoplasmic reticulam is involved in	Protein synthesis	photosynthesis	degradation of fatty acids	transport of oxygen	Protein synthesis
The foldings of inner membrane of mitochondria are	cisternae	sulci	matrix lines	cristae	cristae
The role of mitochondria in oxidative phosphorylation was explained by	lehinger	Embden	krebs	Mayer hoff	lehinger
Smooth endoplasmic reticulum is concerned with	protein metabolism	lipid metabolism	carbohydrate metabolism	amino acid metabolism	lipid metabolism
The primary lysossomes are called as	Virgin lysosomes	Phagolysososmes	Autolysososmes	Post lysososmes	Virgin lysosomes

The basic subunit of microtubules are	protofilaments	tubulin	microfilaments	Actin	tubulin
The lysosomal compartment is	more acidic	less acidic	neutral	less basic	more acidic
Plasmodesmata are present in	animal cells	mammalian cells	plant cells	bacteria.	plant cells
Majority of the enzymes in lysosomes show their catalytic activity in	acidic PH	Alkaline	neutral PH	Osmotic pH	acidic PH
The signal that return protein to endoplasmic reticulum itself is	KKLL	KDEL	KKDD	KKXL	KDEL

Along with KDEL sequence the other sequence that retain the protein in ER is	KKLL	LKKL	KKDD	KKXX	KKXX
Golgi stack close to plasma membrane is	Cis	Trans	Medial	Proximal	Trans
Vesicles from endoplasmic reticulum enter into golgi through	Cis	Trans	Medial	Proximal	Cis
Among the golgi network which is mainly involved with sorting and distribution of proteins	Cis	Trans	Medial	Proximal	Trans
Protein glycosylation is mainly takes place in _____	Mitochodria	Lysosomes	Golgi	Peroxisomes	Golgi

Proteins destined for lysosomes are tagged with	Glu-6-phosphate	Fru-6-phosphate	Man-6-phosphate	Gal-6-phosphate	Man-6-phosphate
The last residue added in protein glycosylation is	N-acetyl glucosamine	N-acetyl galactosamine	N-acetyl muramic acid	Sialic Acid	Sialic Acid
Among the following which is not synthesized in ER	Phospho lipids	Cholesterol	Ceramide	Sphingomyelin	Sphingomyelin
Spingomyelin is synthesized by	Mitochodria	Lysosomes	Golgi	Endoplasmic reticulum	Golgi
Glycoproteins are synthesized by	Mitochodria	Lysosomes	Golgi	Endoplasmic reticulum	Endoplasmic reticulum
Glycolipids are synthesized by	Mitochodria	Lysosomes	Golgi	Endoplasmic reticulum	Golgi
In Plant the golgi have additional role in the synthesis of ____	Mitochodria	Lysosomes	Endoplasimc reticulum	Cell wall	Cell wall

In Plant, the cell wall is synthesised by	Mitochodria	Lysosomes	Golgi	Endoplasmic reticulum	Golgi
Which is not a component of plant cell wall?	Cellulose	Hemi cellulose	Glucose	Pectin	Glucose
The cells that lacks golgi, the protein sorting is done by	Mitochodria	Endoplasmic reticulum	Vacuole	Lysosomes	Vacuole
Uptake of extracellular molecules from plasma membrane occur through	COP-I	COP-II	COP-III	Clathrin coated vesicles	Clathrin coated vesicles
The basket like structure that form lattice is facilitated by _____protein	Spectrin	Ankyrin	Clathrin	Anerin	Clathrin

Other name of GTP binding protein involved with budding of vesicle	ARF	ARS	ASR	AFR	ARF
Acid hydrolases are synthesized by	Mitochodria	Lysosomes	Golgi	Endoplasmic reticulum	Lysosomes
The active pH of acid hydrolases are	7	6.8	5	4	5
The internal acidic pH of lysosomes is achieved by _____ pump	Anion	Cation	Proton	Eletron pump	Proton
Acid hydrolases are targeted to lysosomes through	Glu-6-phosphate	Fru-6-phosphate	Man-6-phosphate	Gal-6-phosphate	Man-6-phosphate

The gradual turnover of cells own compound is achieved by _____process	Phagocytosis	Endocytosis	Autophagy	Exocytosis	Autophagy
Uptake of particulate material by cell through plasma membrane is known as	Phagocytosis	Endocytosis	Autophagy	Exocytosis	Phagocytosis
Which transporter is not found on mitochondrial membrane?	ATP synthase	Adenine nucleotide translocator	Phosphate-hydroxyl ion translocator	Group translocator	Group translocator
Transit peptide is related to	Chloroplast	Lysosomes	Golgi	Endoplasmic reticulum	Chloroplast

UNIT III

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

SUBJECT : CELL BIOLOGY
SEMESTER : I
SUBJECT CODE : 17BCU102 CLASS : I B.Sc., BC

UNIT IV - COURSE MATERIAL

Unit 4

Cytoskeletal proteins : Structure and organization of actin filaments. Treadmilling and role of ATP in microfilament polymerization, organization of actin filaments. Non–muscle myosin. Intermediate filament proteins, assembly and intracellular organization. Assembly, organization and movement of cilia and flagella.

TEXT BOOKS

- Paul, A., (2007). Text Book of Cell and Molecular Biology,1st edition. Books and Allied (P) Ltd, Kolkata.
- Verma, P.S., and Agarwal, V.K., (2005). Cell Biology Molecular Biology and Genetics, VII Edition, S.Chand and company Ltd, New Delhi.
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- Lodish, H., Berk, A., Kaiser, C.A., and Krieger, M., (2012). Molecular Cell Biology, 7th edition. W.H. Freeman & Company, London.
- Garret, R. H. and Grisham, C.M., Biochemistry (2010) 4th ed., Cengage Learning (Boston), ISBN–13: 978–0–495–11464–2.
- Cooper, G.M., and Hausman, R.E., (2013). Cell–A Molecular Approach, 6th Edition.. Sinauer Associates. USA
- Karp, G., (2013). Cell and Molecular Biology, 7th edition. John Wiley and Sons, Inc, Hoboken, United States.
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UNIT IV - CYTOSKELETAL PROTEINS

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° 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.

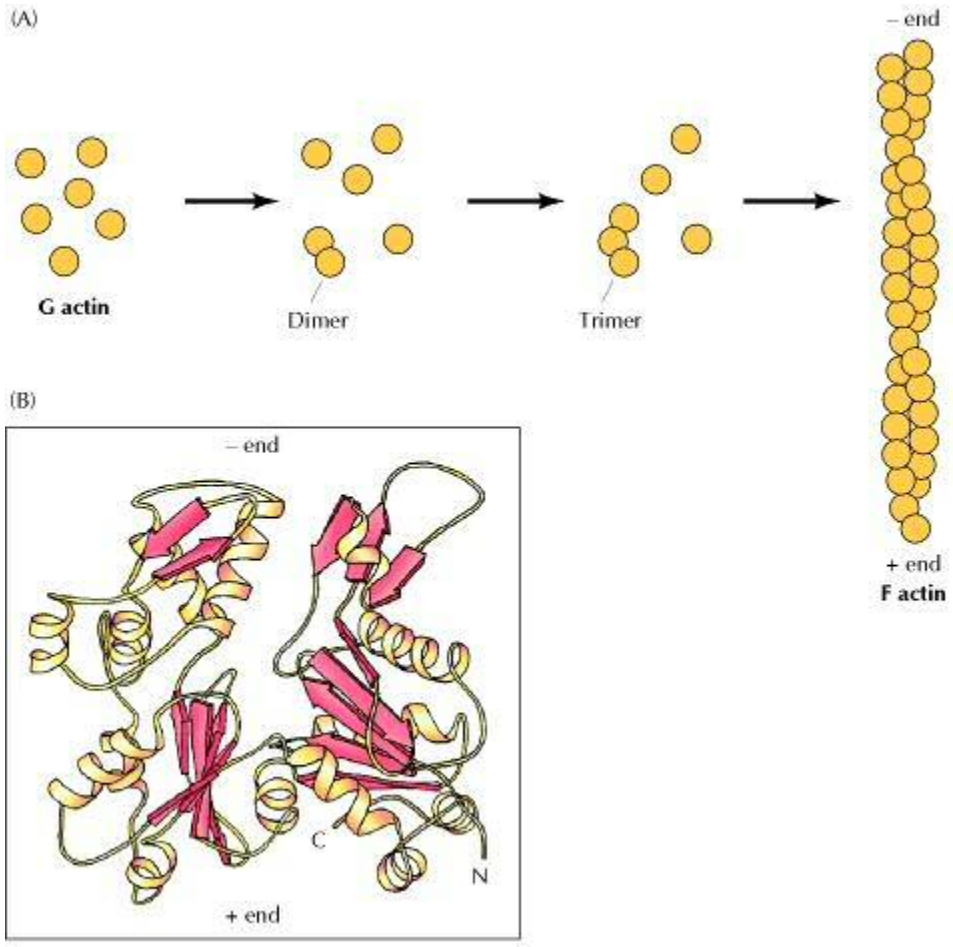

Figure: Assembly and structure of actin filaments:

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.

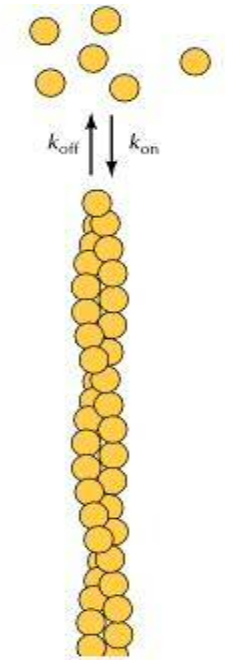

Figure : Reversible polymerization of actin monomers

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.

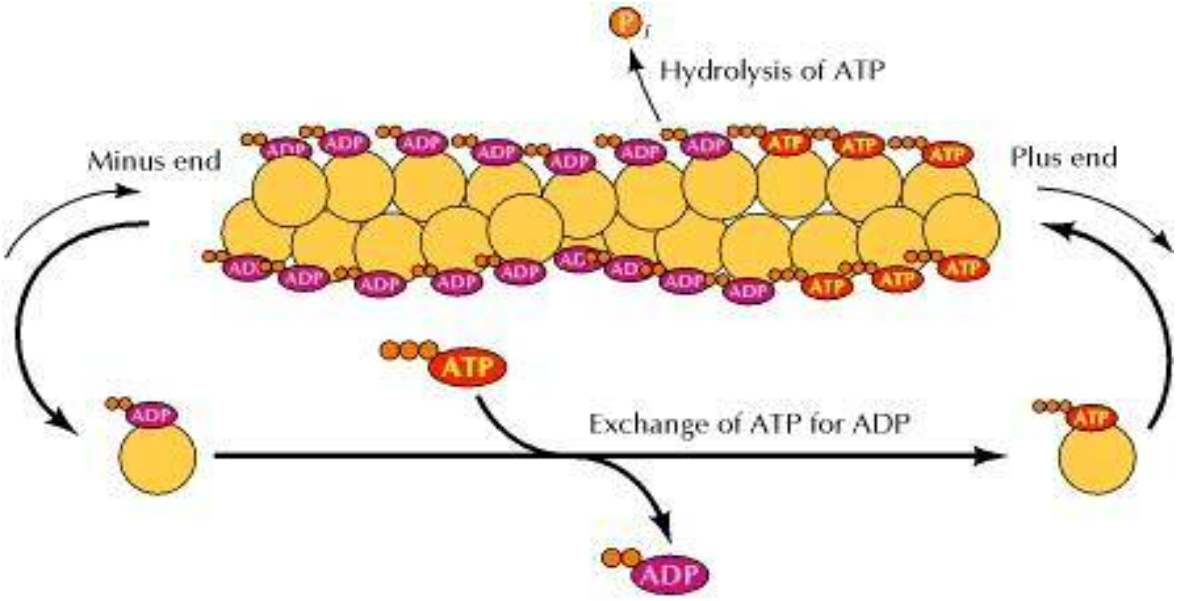

Figure: Treadmilling

It is noteworthy that several drugs 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. 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.

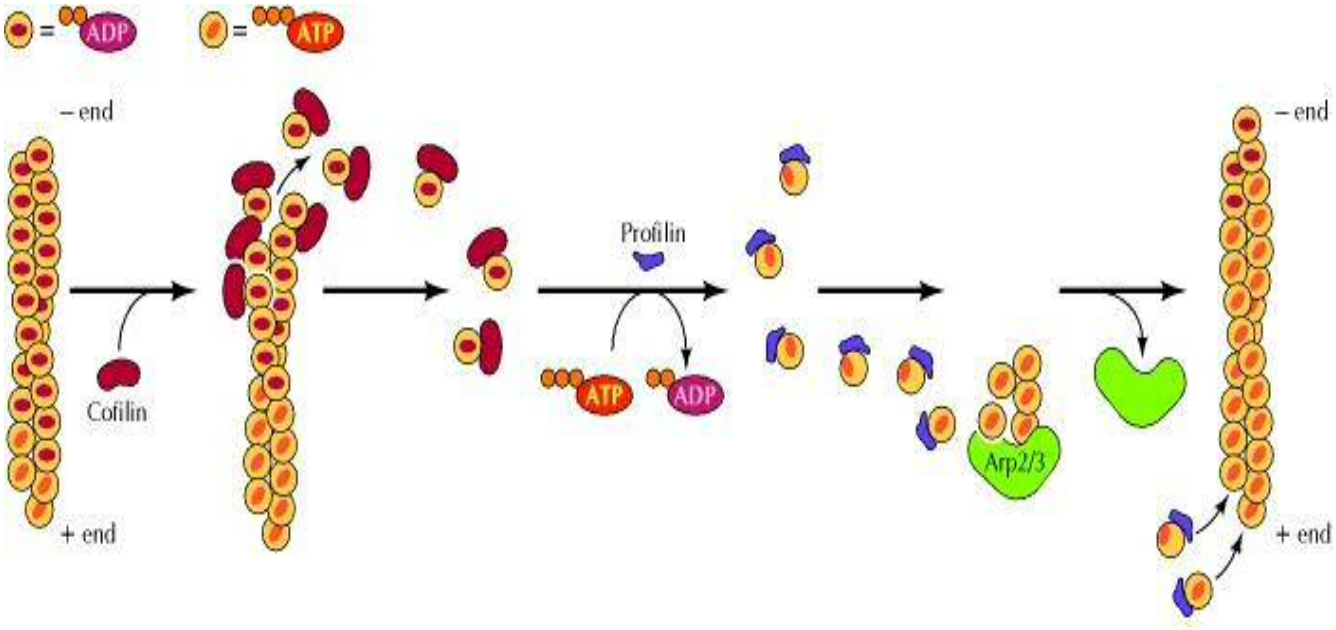

Figure : Effects of actin-binding proteins on filament turnover

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.

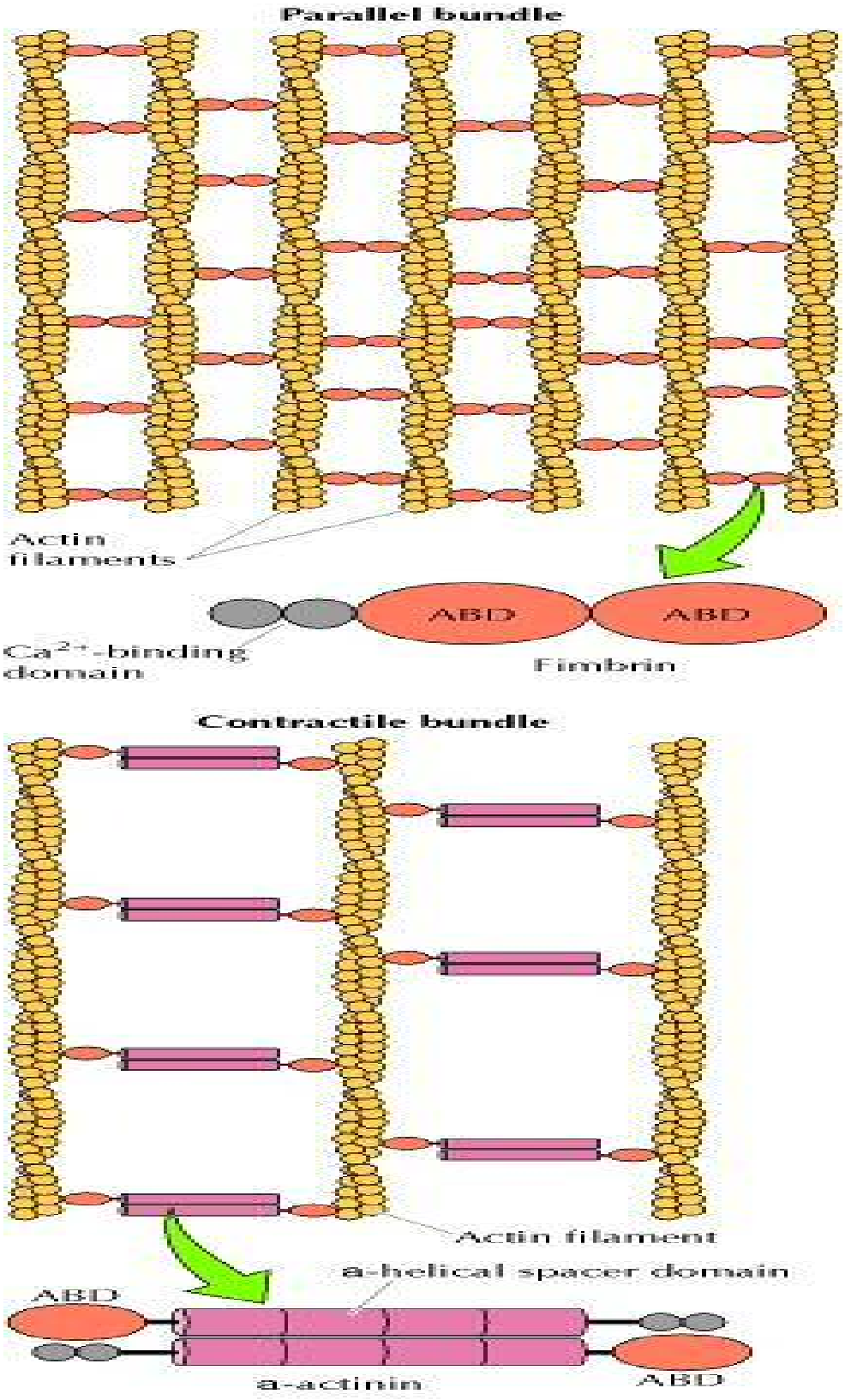

Figure : Actin-bundling proteins

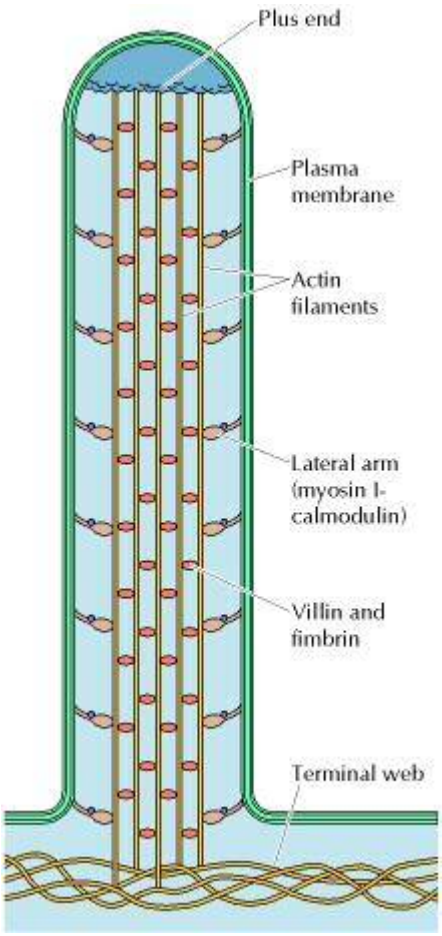

Figure:Organization of microvilli -The core actin filaments of microvilli are crosslinked into closely packed bundles by fimbrin and villin. They are attached to the plasma membrane along their length by lateral arms, consisting of myosinI and calmodulin. The plus ends of the actin filaments are embedded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

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.

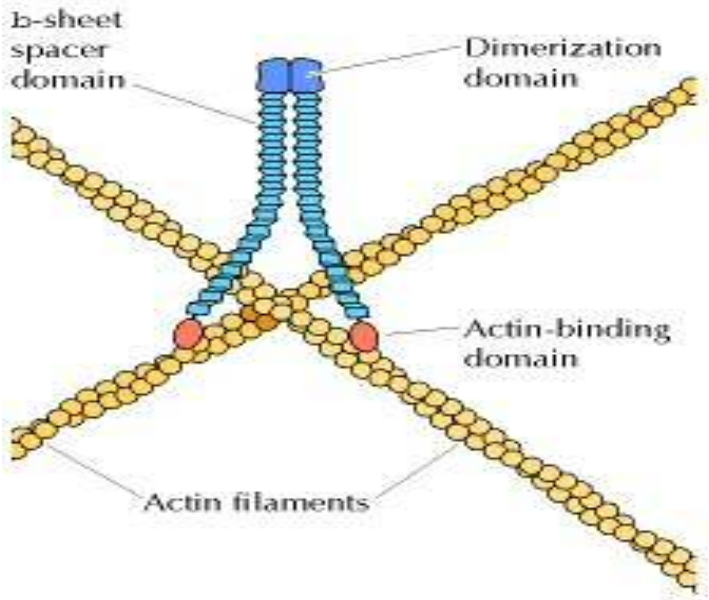

Figure: Actin networks and filamin

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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.

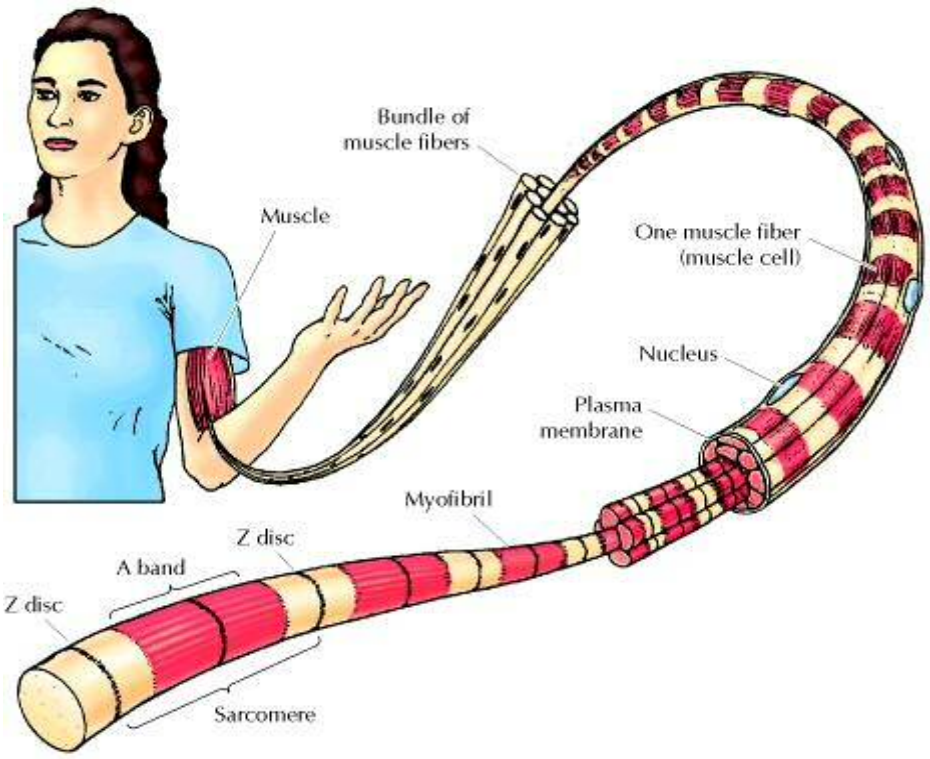

Figure ; Structure of muscle cells

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.

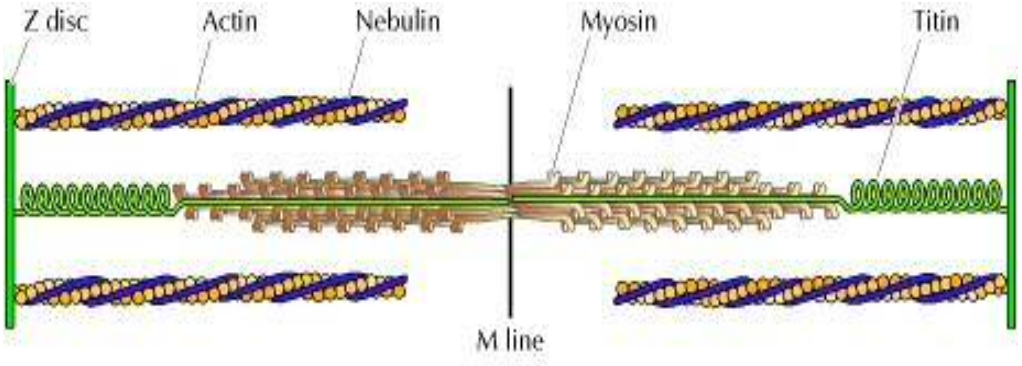

Figure : Titin and nebulin

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.

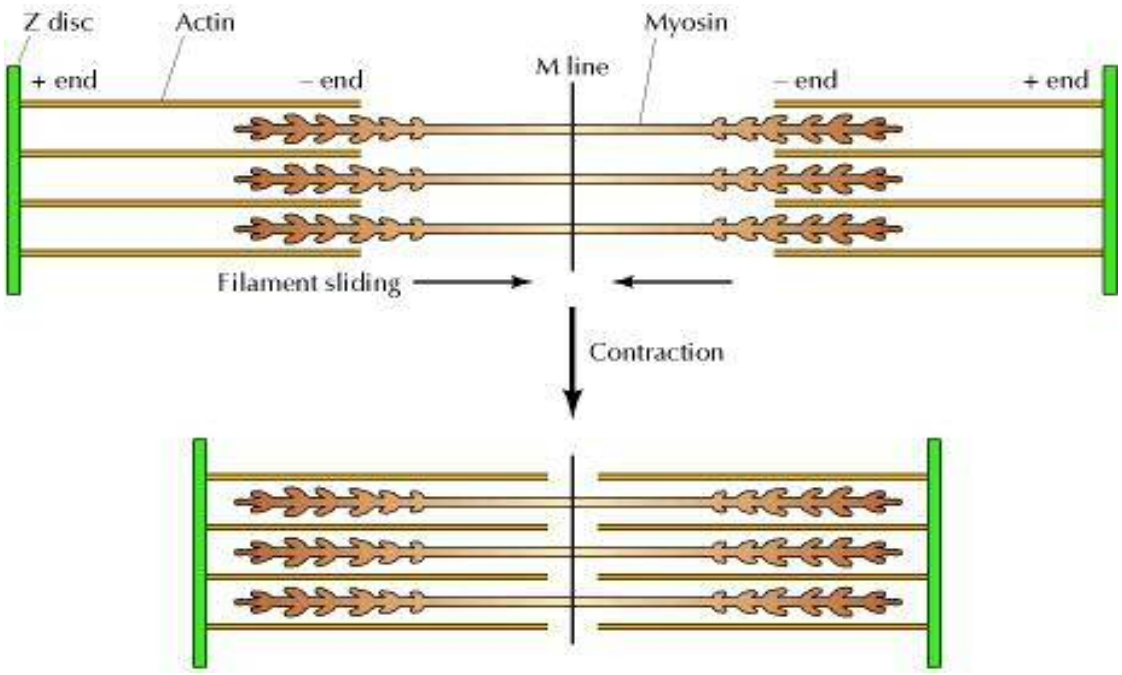

Figure : Sliding-filament model of muscle contraction

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.

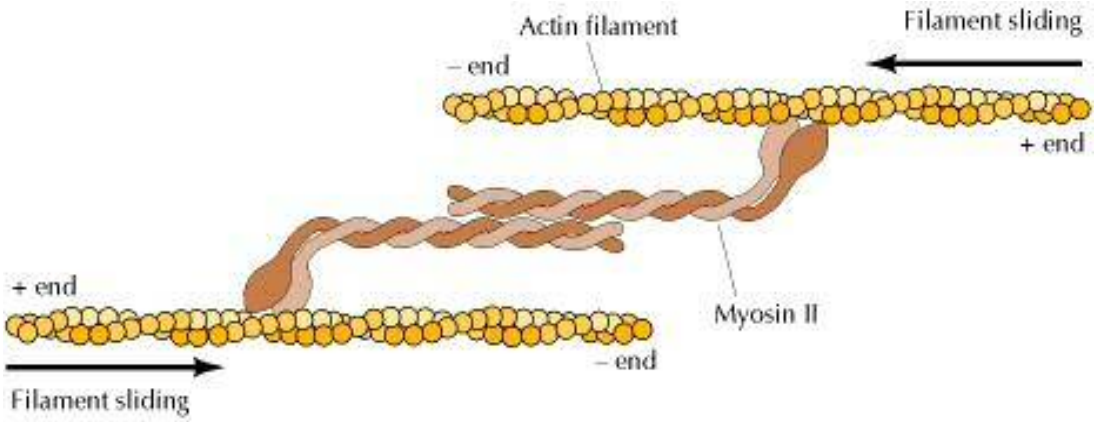

Figure : Contractile assemblies in nonmuscle cells

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.

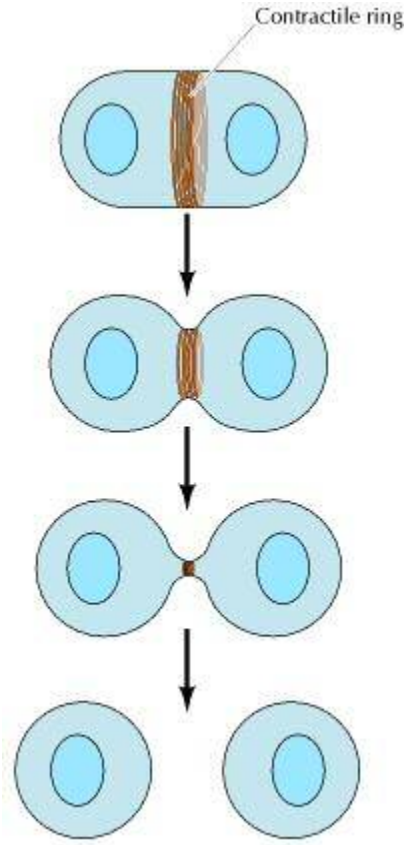

Figure : Cytokinesis

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. 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 (calledhard 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.

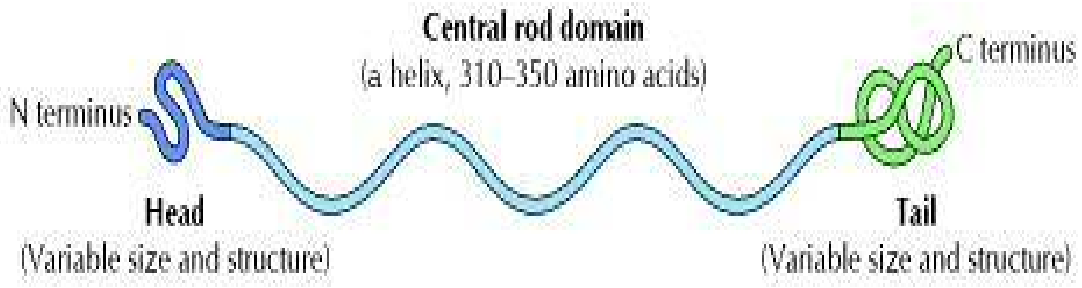

Figure 11.31Structure of intermediate filament proteins

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.

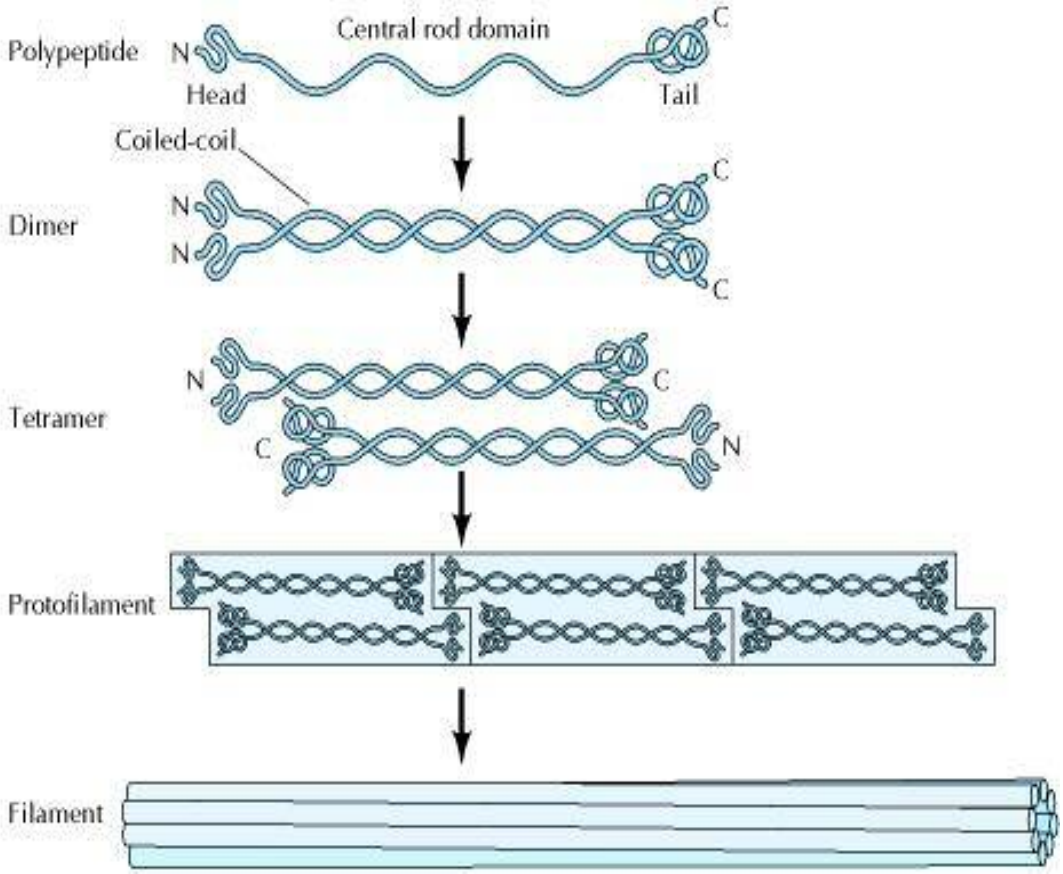

Figure : Assembly of intermediate filaments

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.

Figure

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.

Figure

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.

Figure

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. 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. 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.

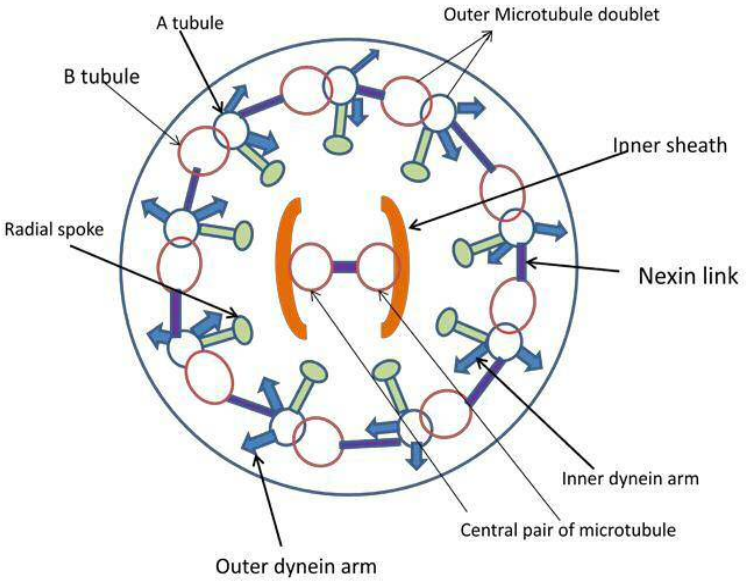

Figure 1: Structure of axoneme of cilia and flagella

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.

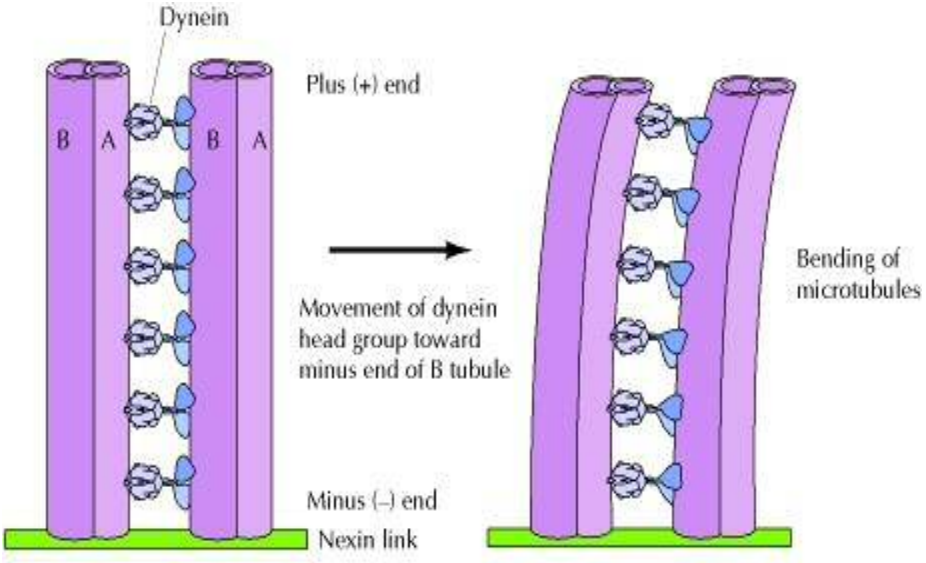

Figure:Movement of microtubules in cilia and flagella

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.

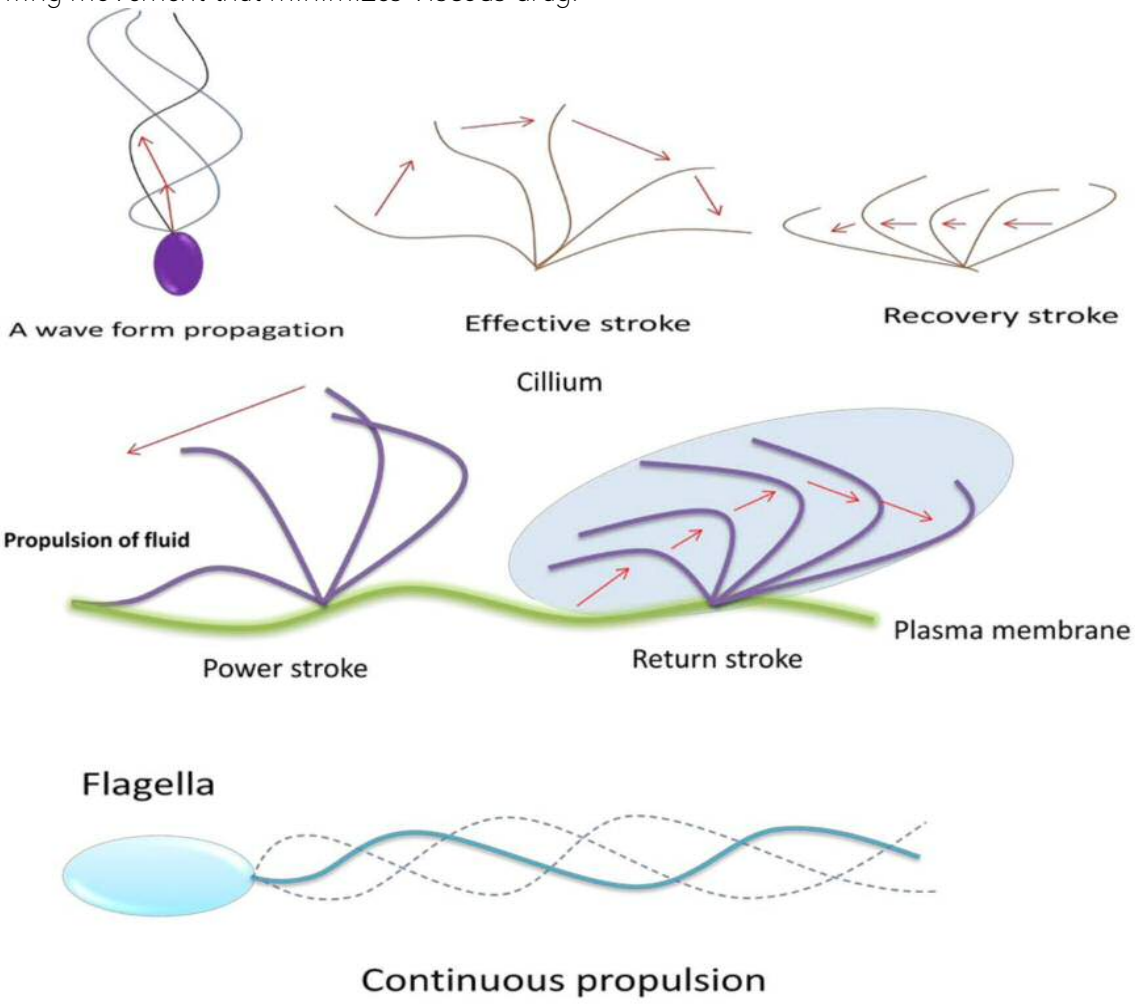

Figure : Ciliary and flagellar movement

The overall structure of bacterial flagella

The bacterial flagellum (Figure 4) is made up of the protein flagellin. Its shape is a 20 nanometer thick hollow tube. It is helical and has a sharp bend just outside the outer membrane which is called the hook. It allows the axis of the helix to point directly away from the cell. A shaft runs between the hook and the basal body, passing through protein rings in the cell's membrane that act as bearings. Gram–positive organisms have 2 of these basal body rings, one in the peptidoglycan layer and one in the plasma membrane. Gram–negative organisms have 4 such rings: the L ring associates with the lipopolysaccharides, the P ring associates with peptidoglycan layer, the M ring is embedded in the plasma membrane, and the S ring is directly attached to the plasma membrane. The filament ends with a capping protein. The bacterial flagellum is driven by a rotary engine (the Mot complex) made up of protein, located at the flagellum's anchor point on the inner cell membrane. The engine is powered by proton motive force, i.e., by the flow of protons (hydrogen ions) across the bacterial cell membrane due to a concentration gradient set up by the cell's metabolism. The rotor transports protons across the membrane, and is turned in the process.

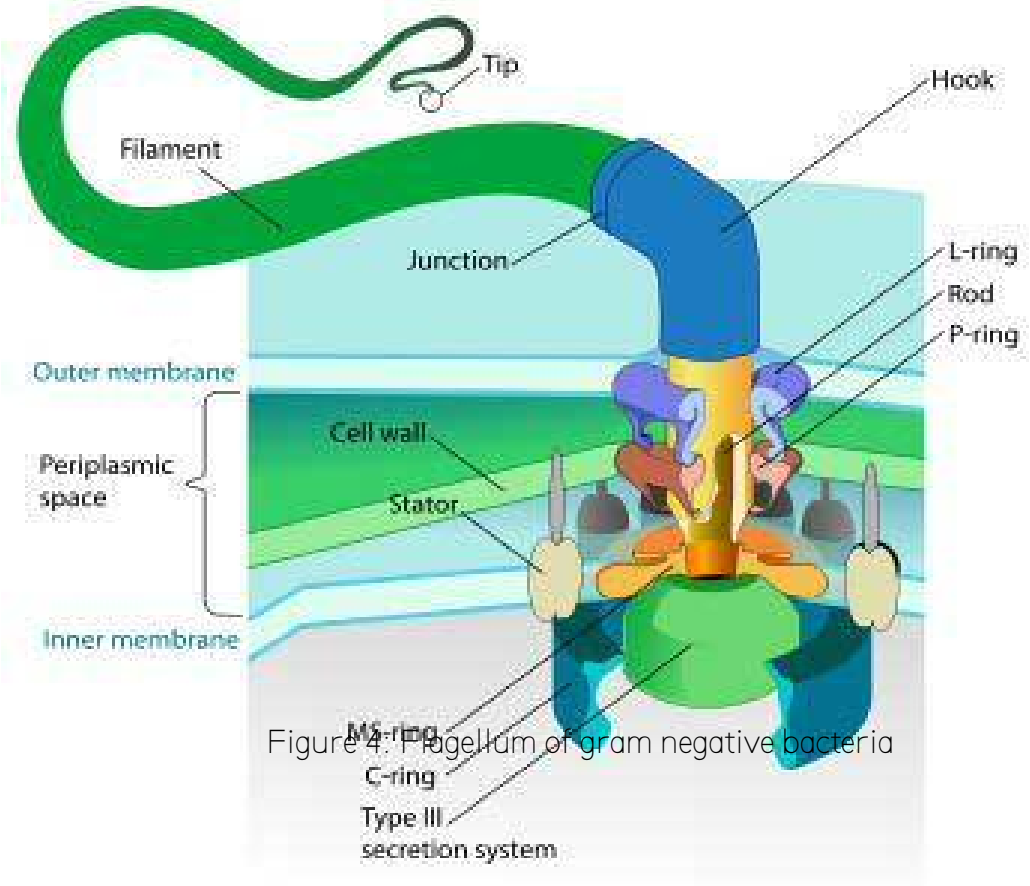

Figure 4: Flagellum of gram negative bacteria

KARPAGAM ACADEMY OF HIGHER EDUCATION
COIMBATORE - 641021
DEPARTMENT OF BIOCHEMISTRY
I-B.Sc., BIOCHEMISTRY - BATCH: 2017 - 2020
PART A (20 X 1 = 20 MARKS) - Online MCQ Questions

SUBJECT: C SUBJECT CODE: 17BCU102

Questions	Option A	Option B	Option C	Option D	Answer
The length of cilia and flagella ranged from	1µ to 2 mm	10µ to 150 µm	1µ to 100 mm	1mm to 3 mm	10µ to 150 µm
The size of the microtubules	10nm in diameter	18nm in diameter	1-3 µm in diameter	24nm in diameter	24nm in diameter
Plasma membrane coated bundle of microtubules present in cilium or flagella are known as	nexin	axoneme	dynein arm	axon.	axoneme
Actin is present	microtubules	microfilaments	glyoxysomes	Actin	microfilaments
Cytoskeleton of the cell is made up of	plasma membrane	microtubules and filaments	endoplasmic reticulum	Golgi complex	microtubules and filaments

The 1st phase of actin filament assembly is marked by---	lag phase	stationary phase	lag and log phase	log phase	lag phase
The 2nd phase of actin filament assembly is ----	steady phase	elongation phase	lag phase	log phase	elongation phase
Microtubule filament run the length of the central core of the ---	Connective tissue	Cilia	Flagella	Ligaments	Cilia
Flagella undulate in a------	oscillation movement	Whiplike manner	Ovoid movement	Zig zag	Whiplike manner
The beating of ---- is the only mean for locomotion for sperm	Cilia	Flagella	Cilia and flagella	Tubules	Cilia and flagella
Cilia & flagella extend from --- unicellular organism	lysosome	nuclear envelope	golgi bodies	plasmamembrane	plasmamembrane

----- facilates the function of dynein	actins	myosin	myoglobin	dynactins	dynactins
When flagella are distributed all around the bacterial cell, the arrangement is called--------?	polar	random	peritrichous	encapsulated	peritrichous
Which of the followings does not describe skeletal muscle tissue fibre?	striated	voluntary	multinucleate	branched	branched
ATPase of the muscle located in	actinin	troponin	myosin	actin	actin
The bacteria flagellum is made up of –	Protein flagellin	protein flagella	lipid flagellin	lipid flagella	Protein flagellin
Cilia is an organelle found in –	prokaryotic cell	eukaryotic cell	prokaryotic & Eukaryotic cell	none of the above	eukaryotic cell

Operation of modified Q cycle III results in the reduction of ---	Cyt C	Cyt B	Cyt C P50	cyt p450	Cyt C
Microtubule are one of the components of the ---	endoskeleton	cytoskeleton	exoskeleton	none of the above	cytoskeleton
Microfilaments are found by the ---polymerisation of actin monomers	tail to head	tail only	head only	head to tail	head to tail
Microtubules are found in all eukaryotic cells except	Brain cells	Human erythrocytes	hepatocytes	kidney cells	Human erythrocytes
---------- is a term applied to the axial basic microtubular structure of cilia and flagella	Cilium	Basal body	Axoneme	Dynein arm	Axoneme

Intermediate filaments are	50-100 um in diameter	5-10 um in diameter	8-10 um in diameter	100-200 um in diameter	8-10 um in diameter
The size of the microtubules	10nm in diameter	18nm in diameter	1-3 μm in diameter	24nm in diameter	24nm in diameter
Hydrolysis of phosphate groups in ATP is an	exergonic process	endergonic process	endothermic process	exergonic and endergonic process	exergonic process
ATP is hydrolyzed in to	ADP	inorganic phosphate	ADP and inorganic phosphate	organic phosphate	ADP and inorganic phosphate
Reaction by which chemical energy that has been stored in high energy phosphoanhydride bonds in ATP is released is called	ATP phosphorylation	ATP dehydrogenation	ATP hydrogenation	ATP hydrolysis	ATP hydrolysis
Which term is based on the Greek root words for "before" and "kernel"?	peptidoglycan.	eukaryote.	nucleolus.	Prokaryote	prokaryote

Which of the following apply to the cytoskeleton?	It occupies the general nucleoplasm as well as occurring in the cytosol.	It is typically composed of three types of fibrous protein systems.	It consists in part of networks of fibrous proteins.	Its protein components have been highly conserved throughout evolution.	It is typically composed of three types of fibrous protein systems.
Which of the following cytoskeleton proteins have an almost all helical secondary structure?	Tubulins	Keratins	Lamins	Actins	Lamins
What is a property that is shared by microtubules and actin filaments?	The polymer is assembled from subunits that are protein monomers	Polymer assembly requires that subunits contain a bound GTP	Polymer assembly requires that subunits contain a bound ATP	Subunit assembly is followed by nucleotide hydrolysis	Subunit assembly is followed by nucleotide hydrolysis
Which of the following is not a correct statement about cytoskeletal motor proteins?	They are all ATPases.	Each of them is capable of movement in only one direction along a cytoskeletal polymer.	Each of them has at least one globular head domain containing a cytoskeleton binding site.	All microtubule associated motor proteins move toward the plus end of the microtubule.	All microtubule associated motor proteins move toward the plus end of the microtubule.

Which of the following motility processes requires the involvement of myosin motor proteins?	Coordinated movement of cilia on ciliated epithelia	Swimming motility of spermatozoa	Mitosis	Cytokinesis	Cytokinesis
All of the following statements about dynein are correct EXCEPT	dynein is necessary for both directions of rapid axoplasmic transport	dynein is an ATPase	dynein is a microtubule-binding protein	dynein is necessary for male fertility	dynein is necessary for both directions of rapid axoplasmic transport
Which of the following are functions of cilia and flagella?	locomotion	body's defence	excretion and body's defence	locomotion, body's defence and excretion	locomotion, body's defence and excretion
The basic microtubular structure of cilia and flagella is called	radial spoke	axoneme	nexin	dyenein	axoneme
Eukaryotic flagella is made up of	dyenien	tubulin	flagellin	vimetin	tubulin

Which of the following is not a characteristic of prokaryotes?	DNA	Cell membrane	cell wall	endoplasmic reticulum	endoplasmic reticulum
Ribosomes are made up of ____ subunits.	0	2	3	4	2
Plants differ from animals in that plants have	an endoplasmic reticulum	a central vacuole	Golgi complexes	vesicles	a central vacuole
The rough ER is so named because it has an abundance of _____ __ on it.	mitochondria	lysosomes	Golgi bodies	ribosomes	ribosomes
Depolymerization of microtubules is inhibited by	kinesin	dyneins	actin	guanosine triphosphate	guanosine triphosphate

Clusters of rRNA where ribosomes are assembled are called	nuclei	cisternae	nucleoli	Golgi complexes	nucleoli
The smooth ER is especially abundant in cells that synthesize extensive amounts of	toxins	proteins	enzymes	lipids	lipids
Synaptic signaling involves	endocrine signals	paracrine signals	autocrine signals	neurotransmitters	neurotransmitters
The ____ surrounds the cell like a belt, preventing the passage of substances between the cells.	gap junction	desmosome	hemidesmosome	tight junction	tight junction

In desmosomes, cadherins link to _____ __ of an adjacent cell.	integrins	connexons	ras proteins	intermediate filaments	intermediate filaments
_____ junctions may protect a damaged cell through chemical gating.	Tight	Gap	Adherens	Occluding	Gap
Narrow gaps between nerve cells through which paracrine signals travel are called	desmosomes	calmodulins	synapses	integrins	synapses
_____ __ is a common second messenger.	cAMP	cGTP	cMHC	cATP	cAMP

Desmosomes are associated with _____ junctions.	adherens	tight	anchoring	communicating	anchoring
One protein kinase cascade begins with the phosphorylation of the	tap protein	gat protein	sat protein	ras protein	ras protein
Gap junctions are formed by	the fusion of plasma membranes to form a single membrane	the insertion of protein complexes that form tunnels between cells	protein hooks that extend into the membrane of adjacent cells	gaps in the cell wall of plants	the insertion of protein complexes that form tunnels between cells
Plasmodesmata occur in	bacterial cells	all eukaryotic cells	plant cells	animal cells	plant cells
A _____ __ is a type of adhering junction between animal cells.	tight junction	plasmodesma	chemical synapse	gap junction	tight junction
--------- are attached to integral membrane protein	Intrinsic protein	Peripheral protein	Lipid anchored Protein	GPI anchored protein	Peripheral protein

Transmembrane proteins are also known as	integral protein	Peripheral protein	Lipid anchored Protein	GPI anchored protein	integral protein
The trigger to initiate the contractile process in skeletal muscle is:	potassium binding to myosin	calcium binding to tropomyosin	ATP binding to the myosin cross bridges	calcium binding to troponin	calcium binding to troponin
The energy for all forms of muscle contraction is provided by:	ATP	ADP	phosphocreatine	oxidative phosphorylation	ATP
Each of the following is true of both cadherins and integrins EXCEPT	both are transmembrane glycoproteins X	both can associate with the actin cytoskeleton X	both represent gene families	both may be found in zonula adherens junctions	both may be found in zonula adherens junctions

UNIT IV

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

SUBJECT : CELL BIOLOGY
SEMESTER : I
SUBJECT CODE : 17BCU102 CLASS : I B.Sc., BC

UNIT V - COURSE MATERIAL

Unit 5

Cell wall and extracellular matrix :Prokaryotic and eukaryotic cell wall, cell matrix proteins. Cell-matrix interactions and cell-cell interactions. Adherence junctions, tight junctions, gap junctions, desmosomes, hemidesmosomes, focal adhesions and plasmodesmata.

Cell cycle, cell death and cell renewal : Eukaryotic cell cycle, restriction point, and checkpoints. Cell division. Apoptosis and necrosis - brief outline. Salient features of a transformed cell.

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UNIT V – CELL WALL AND CELL CYCLE

BACTERIAL CELL WALL

The rigid cell walls of bacteria determine cell shape and prevent the cell from bursting as a result of osmotic pressure. The structure of their cell walls divides bacteria into two broad classes namely Gram-positive bacteria (such as the common human pathogen Staphylococcus aureus) and Gram-negative bacteria (such as E. coli).

Gram-negative bacteria have a dual membrane system, in which the plasma membrane is surrounded by a permeable outer membrane. These bacteria have thin cell walls located between their inner and outer membranes. In contrast, Gram-positive bacteria have only a single plasma membrane, which is surrounded by a much thicker cell wall.

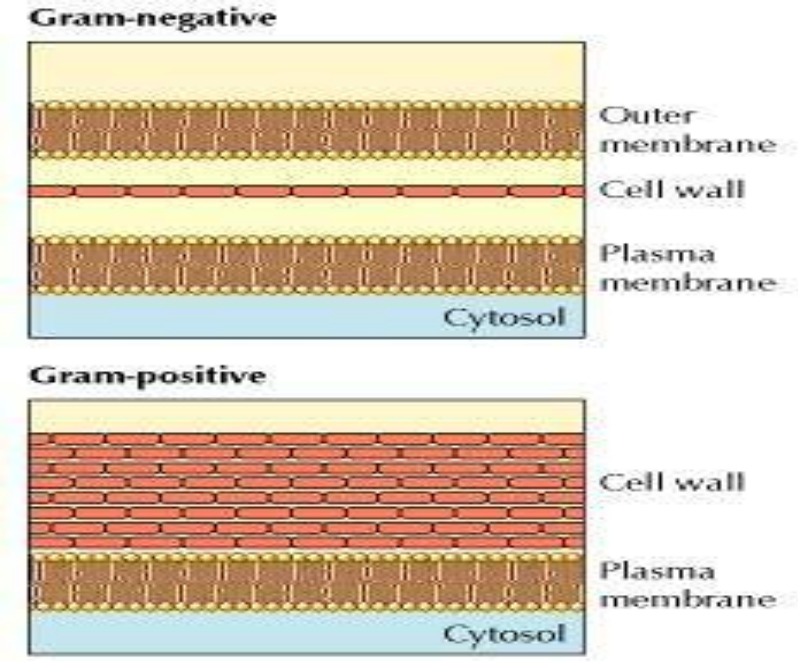


Figure Bacterial cell walls.

Composition

Despite these structural differences, the principal component of the cell walls of both Gram-positive and Gram-negative bacteria is a peptidoglycan (Figure) consisting of linear polysaccharide chains crosslinked by short peptides. Because of this crosslinked structure, the peptidoglycan forms a strong covalent shell around the entire bacterial cell.

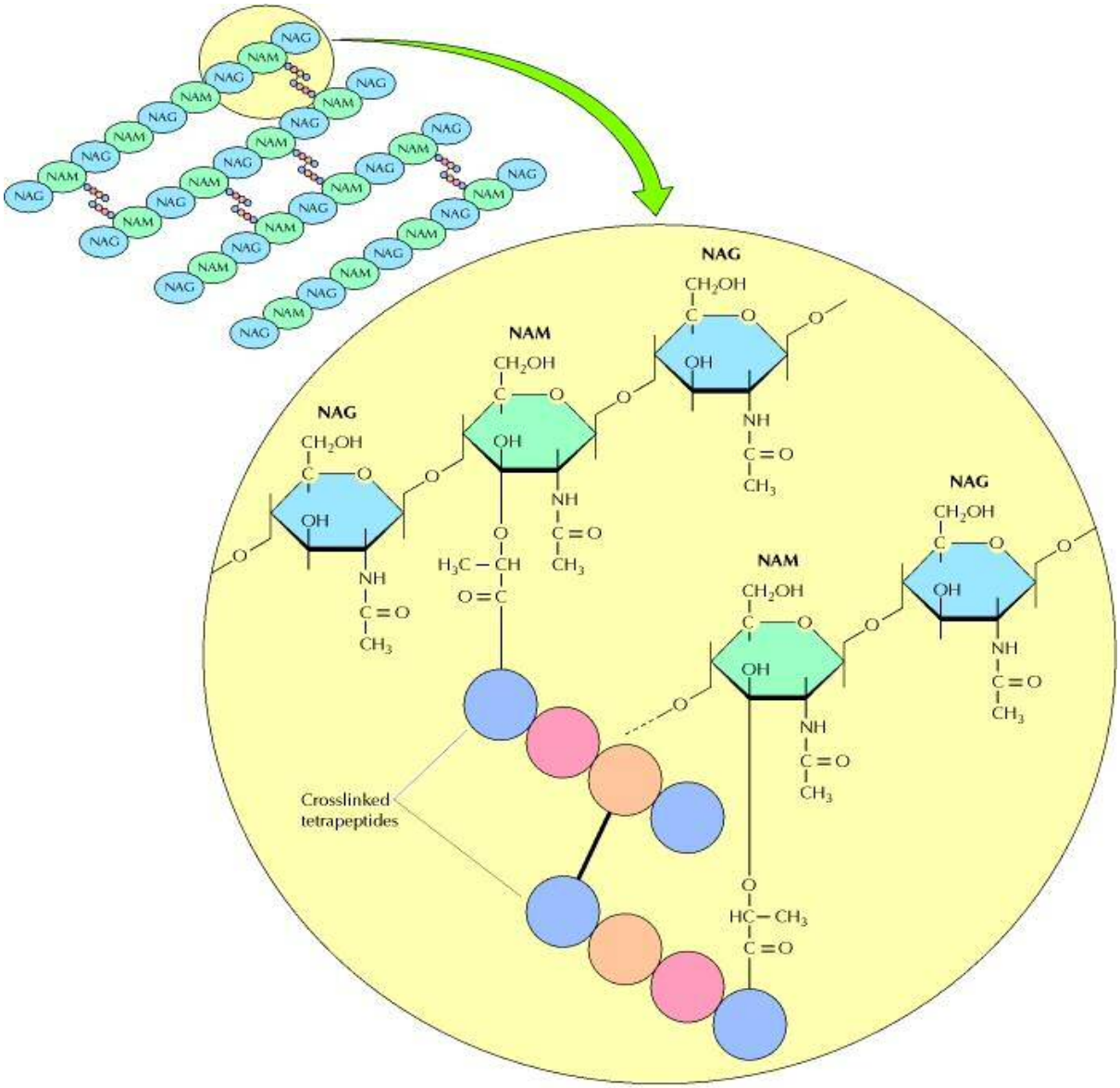


Figure: The peptidoglycan of E.coli. Polysaccharide chains consist of alternating N-acetylglucosamine (NAG) and N-**acetylmuramic acid (NAM) residues joined by β(1→4)** glycosidic bonds. Parallel chains are crosslinked by tetrapeptides

PLANT CELL WALL

In contrast to bacteria, the cell walls of eukaryotes (including fungi, algae, and higher plants) are composed principally of polysaccharides (Figure). The basic structural polysaccharide of fungal cell walls is chitin (a polymer of N-acetylglucosamine residues), which also forms the exoskeleton of arthropods (e.g., the shells of crabs). The cell walls of most algae and higher plants are composed principally of cellulose, which is the single most abundant polymer on Earth. Cellulose is a linear polymer of glucose residues, often containing more than 10,000 glucose monomers. The glucose residues are **joined by β(1→4) linkages, which allow th**e polysaccharide to form long straight chains. Several dozen such chains then associate in parallel with one another to form cellulose microfibrils, which can extend for many micrometers in length.

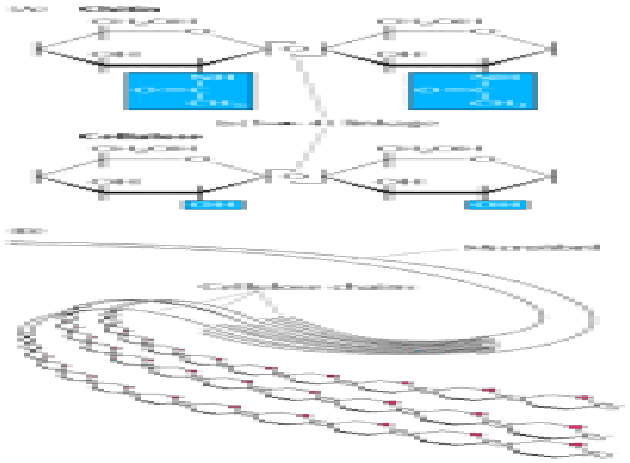


Figure:Polysaccharides of fungal and plant cell walls.

Within the cell wall, cellulose microfibrils are embedded in a matrix consisting of proteins and two other types of polysaccharides: hemicelluloses and pectins. Hemicelluloses are highly branched polysaccharides that are hydrogen-bonded to the surface of cellulose microfibrils. This crosslinks the cellulose microfibrils into a network of tough, fibrous molecules, which is responsible for the mechanical strength of plant cell walls.Pectins are branched polysaccharides containing a large number of negatively charged galacturonic acid residues. Because of these multiple negative charges, pectins bind positively charged ions (such as Ca^{2+}) and trap water molecules to form gels. An illustration of their gel-forming properties is provided by the fact that jams and jellies are produced by the addition of pectins to fruit juice. In the cell wall, the pectins form a gel-like network that is interlocked with the crosslinked cellulose microfibrils. In addition, cell walls contain a variety of glycoproteins that are incorporated into the matrix and are thought to provide further structural support.

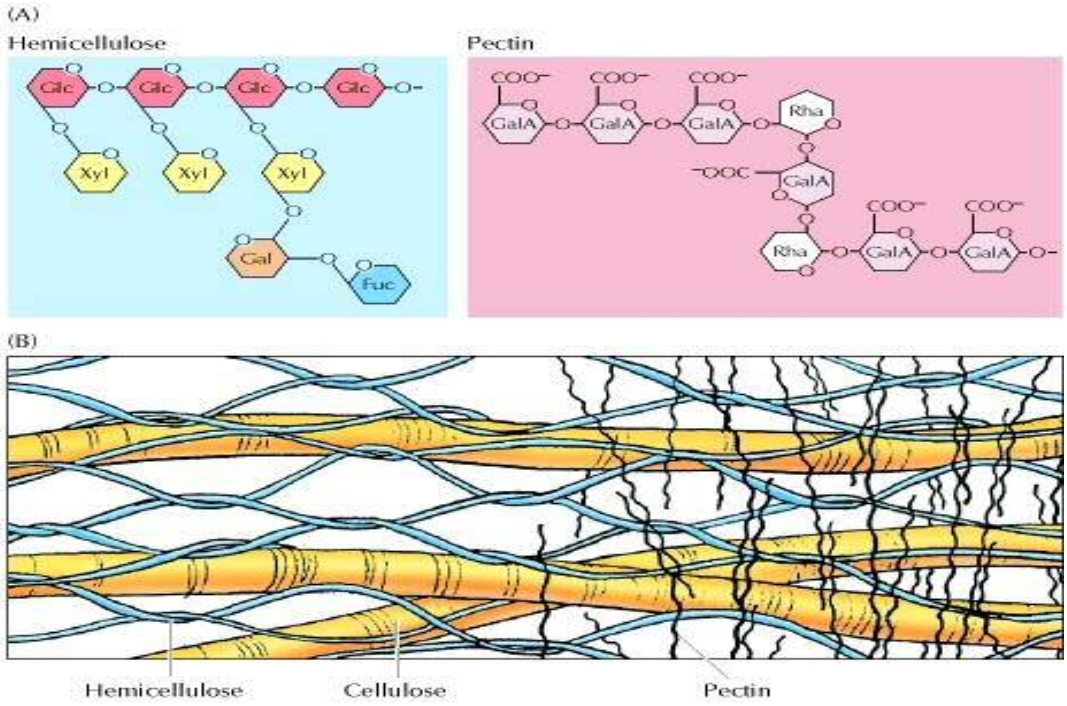


Figure Model of a plant cell wall

Both the structure and function of cell walls change as plant cells develop. The walls of growing plant cells (calledprimary cell walls) are relatively thin and flexible, allowing the cell to expand in size. Once cells have ceased growth, they frequently lay down secondary cell walls between the plasma membrane and the primary cell wall . Such secondary cell walls, which are both thicker and more rigid than primary walls, are particularly important in cell types responsible for conducting water and providing mechanical strength to the plant.

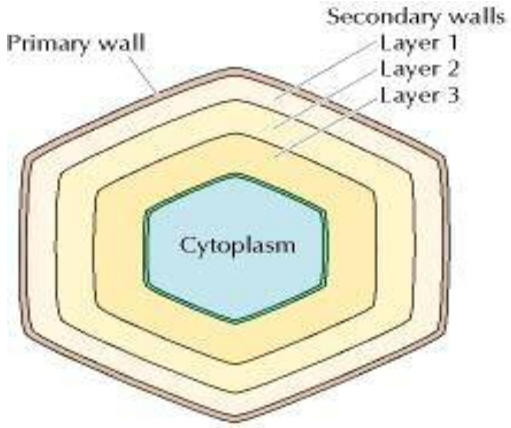


Figure: Primary and secondary cell walls.

Primary and secondary cell walls differ in composition as well as in thickness. Primary cell walls contain approximately equal amounts of cellulose, hemicelluloses, and pectin's. In contrast, the more rigid secondary walls generally lack pectin and contain 50 to 80% cellulose. Many secondary walls are further strengthened by lignin, a complex polymer of phenolic residues that is responsible for much of the strength and density of wood. The orientation of cellulose microfibrils also differs in primary and secondary cell walls. The cellulose fibers of primary walls appear to be randomly arranged, whereas those of secondary walls are highly ordered (see Figure). Secondary walls are frequently laid down in layers in which the cellulose fibers differ in orientation, forming a laminated structure that greatly increases cell wall strength.

One of the critical functions of plant cell walls is to prevent cell swelling as a result of osmotic pressure. Osmotic pressure continually drives the flow of water into the cell. This water influx is tolerated by plant cells because their rigid cell walls prevent swelling and bursting. Instead, an internal hydrostatic pressure (called turgor pressure) builds up within the cell, eventually equalizing the osmotic pressure and preventing the further influx of water.

EXTRACELLULAR MATRIX

Animal cells are surrounded by extracellular matrix beyond the immediate vicinity of their plasma membrane, filling spaces between cells and adhering cells together. Extracellular matrices are of various types consisting of secreted proteins and polysaccharides and are most abundant in connective tissues. One of the examples of extracellular matrix is the basal laminae. It is a continuous sheet of 50 to 200 nm thickness and on top of which a thin layer of epithelial cells rest. Such basal laminae surround muscle cells, adipose cells, and peripheral nerves.
The three major components of extracellular matrix are

- Matrix proteins,
- Matrix polysaccharides

- Matrix adhesion proteins.

The major components of the extracellular matrix have been illustrated in Figure 1.

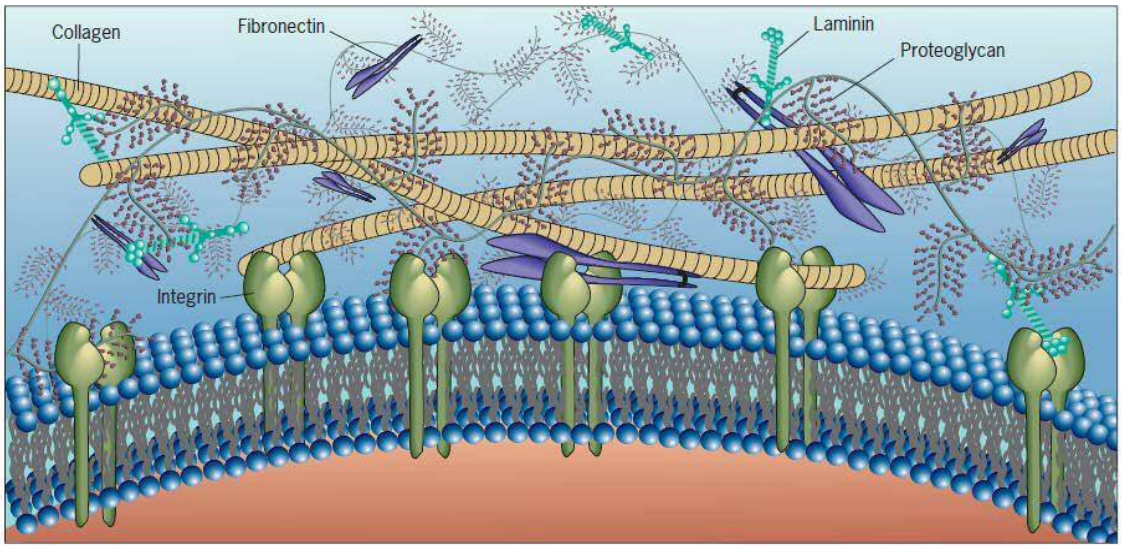


Figure 1: An overview of the extracellular matrix molecular organization. The proteins; fibronectin, collagen, and laminin contain binding sites for one another, as well as binding sites for receptors like integrins that are located at the cell surface. The proteoglycans are huge protein polysaccharide complexes that occupy much of the volume of the extracellular space.

i) Matrix proteins

Matrix proteins are fibrous in nature. The major structural protein is collagen whose secondary structure is a triple helix. The collagens belong to large family of proteins and are characterized by the formation of triple helices in which three polypeptide chains are wound tightly around one another in a ropelike manner. The different collagen polypeptides can assemble into 42 different trimers. The triple helix domains of the collagens consist of repeats of the amino acid sequence Gly-X-Y. Proline is frequently found in the X position and hydroxyproline in the Y position; because of their ring structure, these amino acids stabilize the helical conformations of the polypeptide chains. The most abundant type is collagen type I and is one of the fibril forming collagens that are the basic structural components of connective tissues (Figure 2).

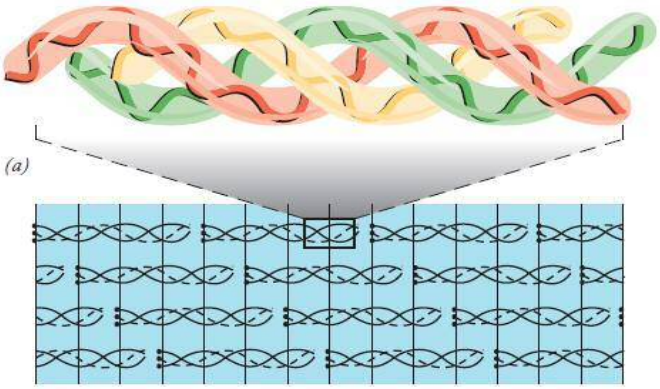


Figure 2: The structure of collagen I. (*a*) The monomer of collagen. (*b*) Collagen I molecules become aligned in and a bundle of collagen I molecules, such as that shown here, form a collagen fibril.

Elastin is another matrix protein, which gives elasticity to tissues, allowing them to stretch when needed and then return to their original state. They are present in blood vessels, the lungs, in skin, and the ligaments. elastin, which is crosslinked into a network by covalent bonds formed between the side chains of lysine residues (similar to those

found in collagen). This network of crosslinked elastin chains behaves like a rubber band, stretching under tension and then snapping back when the tension is released.Elastins are synthesized by fibroblasts and smooth muscle cells.

ii) Matrix polysaccharides

The fibrous structural proteins of the extracellular matrix are embedded in gels formed from polysaccharides called glycosaminoglycans, or GAGs, which consist of repeating units of disaccharides (Figure). One sugar of the disaccharide is either *N*-acetylglucosamine or *N*-acetylgalactosamine and the second is usually acidic (either glucuronic acid or iduronic acid). With the exception of hyaluronan, these sugars are modified by the addition of sulfate groups. Consequently, GAGs are highly negatively charged. Like the pectins of plant cell walls, they bind positively charged ions and trap water molecules to form hydrated gels, thereby providing mechanical support to the extracellular matrix.

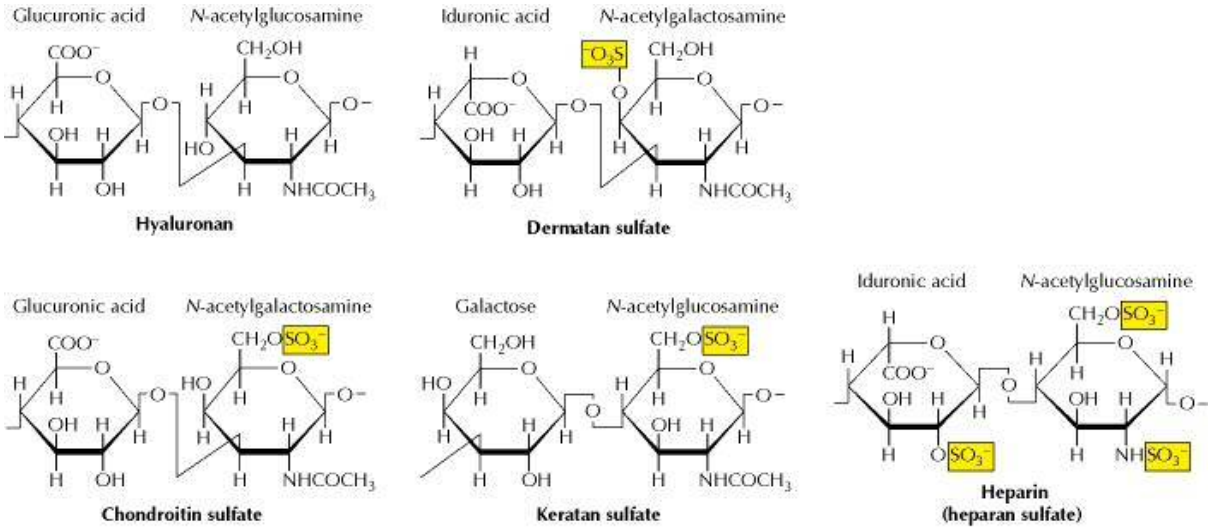


Figure :Major types of glycosaminoglycans.

Hyaluronan is the only GAG that occurs as a single long polysaccharide chain. All of the other GAGs are linked to proteins to form proteoglycans which can consist of up to 95% polysaccharide by weight.

A number of proteoglycans interact with hyaluronan to form large complexes in the extracellular matrix. A well-characterized example is aggrecan, the major proteoglycan of cartilage.

Aggrecan is a large proteoglycan consisting of more than 100 chondroitin sulfate chains joined to a core protein. Multiple aggrecan molecules bind to long chains of hyaluronan, forming large complexes in the extracellular matrix.

Proteoglycans also interact with both collagen and other matrix proteins to form gel-like networks in which the fibrous structural proteins of the extracellular matrix are embedded.

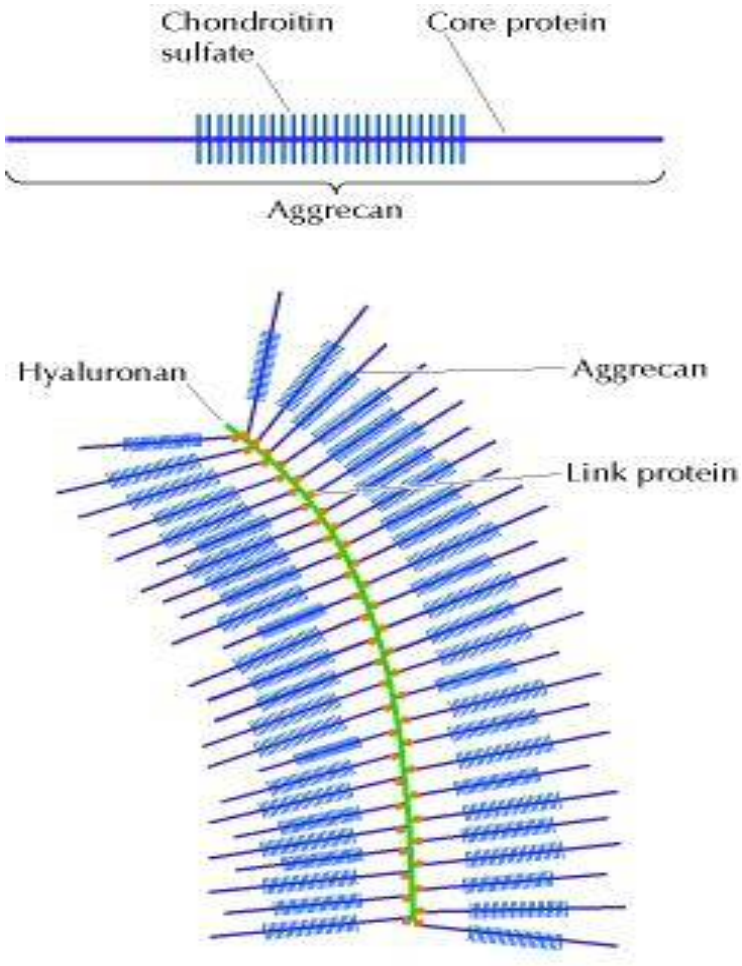


Figure : Complexes of aggrecan and hyaluronan.

iii) Matrix adhesion proteins

Adhesion proteins, the third class of extracellular matrix constituents, are responsible for linking the components of the matrix both to one another and to the surfaces of cells. Fibronectin, is the main adhesion protein of connective tissues Fibronectin is a glycoprotein with two polypeptide chains, of 2500 amino acids. Fibronectin has binding sites for both collagen and GAGs, so it crosslinks these matrix components. A distinct site on the fibronectin molecule is recognized by cell surface receptors and is thus responsible for the attachment of cells to the extracellular matrix.

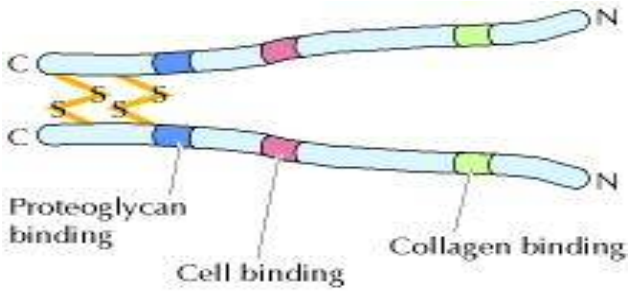


Figure : Structure of fibronectin.

Basal laminae contain a distinct adhesion protein called laminin (Figure). Like type IV collagen, laminins can self-assemble into meshlike polymers. Such laminin networks are the major structural components of the basal laminae. Laminin consists of three polypeptide chains designated A, B1, and B2. Some of the binding sites for entactin, type IV collagen, proteoglycans, and cell surface receptors.The laminins also have binding sites for cell surface receptors, type IV collagen, and perlecan. As a result of these multiple interactions, laminin, entactin, type IV collagen, and perlecan form crosslinked networks in the basal lamina

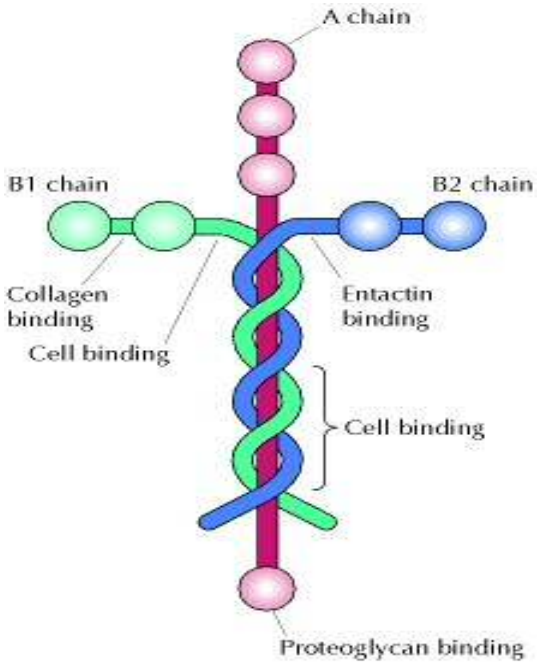


Figure : Structure of laminin.

CELL MATRIX INTERACTION

The major cell surface receptors responsible for the attachment of cells to the extracellular matrix are the integrins. The integrins are heterodimers of two transmembrane subunits, designated α and β. The α subunit binds divalent cations (M^{2+}). The matrix-binding region is composed of portions of both subunits The integrins belong to the family of transmembrane proteins consisting of one α and one β subunits. More than 20 different integrins, formed from combinations of 18 known α subunits and 8 known β subunits, have been identified.The integrins bind to short amino acid sequences present in multiple components of the extracellular matrix, including collagen, fibronectin, and laminin.

In addition to attaching cells to the extracellular matrix the integrins also provide anchors for the cytoskeleton resulting in stability of the cell matrix junctions. Integrins interact with the cytoskeleton at two junctions of the extracellular matrix known as the focal adhesions and hemidesmosomes. (Figure 5).

Focal adhesions attach a variety of cells, including fibroblasts, to the extracellular matrix. The cytoplasmic domains of the β subunits of integrins at these cell-matrix junctions anchor the actin cytoskeleton by associating with bundles of actin filaments. Hemidesmosomes are specialized sites of epithelial cell attachment at which a specific integrin (designated $\alpha_6\beta_4$) interacts with intermediate filaments instead of with actin. The $\alpha_6\beta_4$ integrin binds to laminin, so hemidesmosomes anchor epithelial cells to the basal lamina

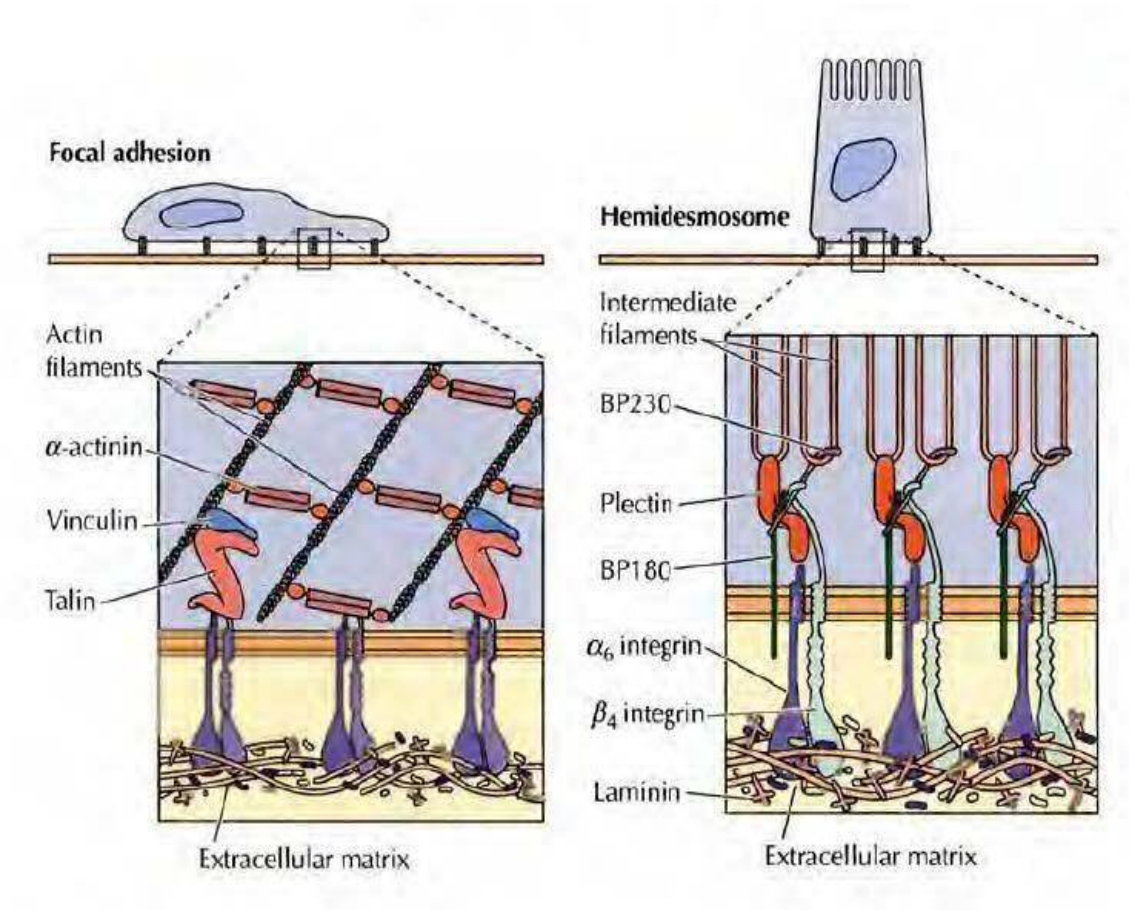
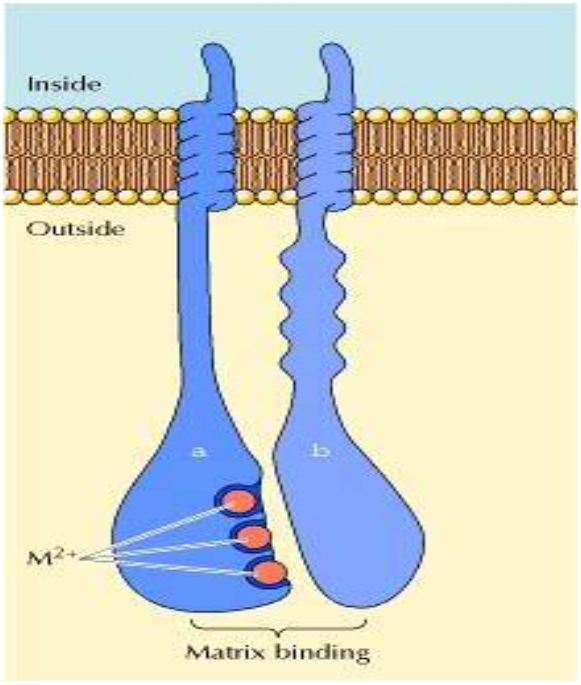


Figure 5: Cell-matrix junctions mediated by integrins. Integrins mediate two types of stable junctions the focal adhesions where bundles of actin filaments are anchored to integrins through associations with a number of other proteins, including **α**-actinin, talin, and vinculin. In hemidesmosomes, integrin links the basal lamina to intermediate filaments via plectin and BP230. BP180 functions in hemidesmosome assembly and stability.

Structure of integrins:

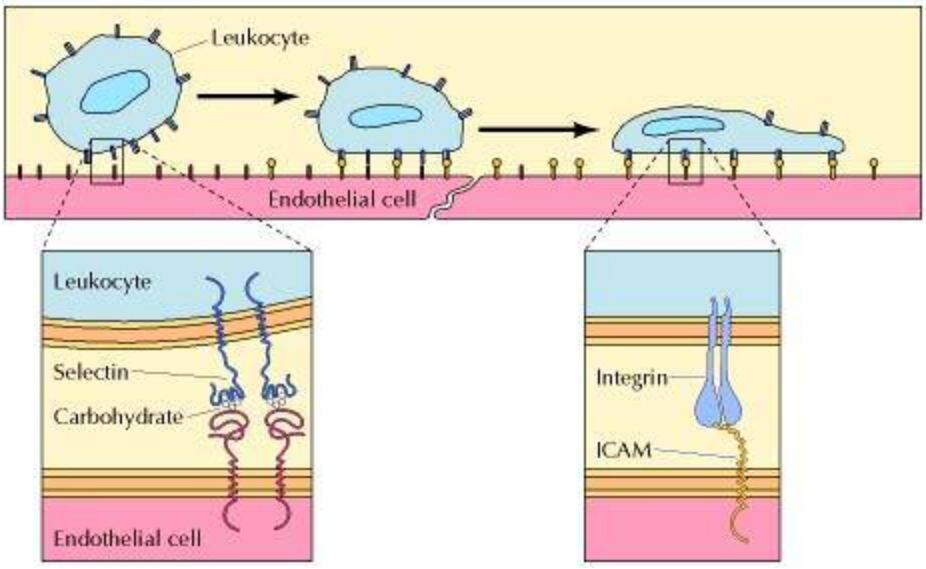


Cell-matrix interaction is a step wise process and occurs through recruitment of specific junctional molecules. Focal adhesions develop from a small cluster of integrins, termed **focal complexes, by the sequential recruitment of talin, vinculin, and α**-actinin. This follows recruitment of formin, which initiates actin bundle formation. Myosin II then comes leads the development of tension at the point of adhesion resulting in cell signaling.

CELL ADHESION PROTEINS

Cell-cell adhesion is a selective process, such that cells adhere only to other cells of specific types. 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.

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. The selectins recognize cell surface carbohydrates (see Figure). 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 (Figure). 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



The fourth group of cell adhesion molecules, are the cadherins. 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. 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.

CELL TO CELL INTERACTION

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.

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.

These cell-cell junctions are of two types: adherens junctions and desmosomes, in which cadherins or related proteins (desmogleins and desmocollins) are linked to actin bundles and intermediate filaments, respectively (Figure). 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.

The plasma membranes of adjacent cells are attached together with four kinds of junctions occur in vertebrates:

- Tight junctions
- Adherens junctions
- Gap junctions
- Desmosomes

In many plant tissues, it turns out that the plasma membrane of each cell is continuous with that of the adjacent cells. The membranes contact each other through openings in the cell wall called

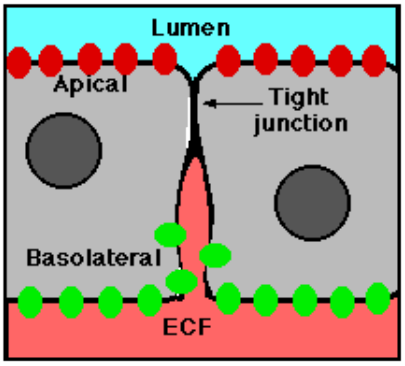
- Plasmodesmata.

1.TIGHT JUNCTIONS

Tight junctions, which are usually associated with adherens junctions and desmosomes in ajunctional complex (Figure 12.65), are critically important to the function of epithelial cell sheets as barriers between fluid compartments.

Epithelia are sheets of cells that provide the interface between masses of cells and a cavity or space (a lumen).

- The portion of the cell exposed to the lumen is called its apical surface.
- The rest of the cell (i.e., its sides and base) make up the basolateral surface.



Tight junctions seal adjacent epithelial cells in a narrow band just beneath their apical surface. They consist of a network of claudins and other proteins.

Tight junctions perform two vital functions:

- They limit the passage of molecules and ions through the space between cells. So most materials must actually enter the cells (by diffusion or active transport) in order to pass through the tissue. This

pathway provides tighter control over what substances are allowed through.

- They block the movement of integral membrane proteins (red and green ovals) between the apical and basolateral surfaces of the cell. Thus the special functions of each surface, for example
  - receptor-mediated endocytosis at the apical surface
  - exocytosis at the basolateral surface

  can be preserved.

Tight junctions appear to be formed by a network of protein strands that continues around the entire circumference of the cell (see Figure). 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.

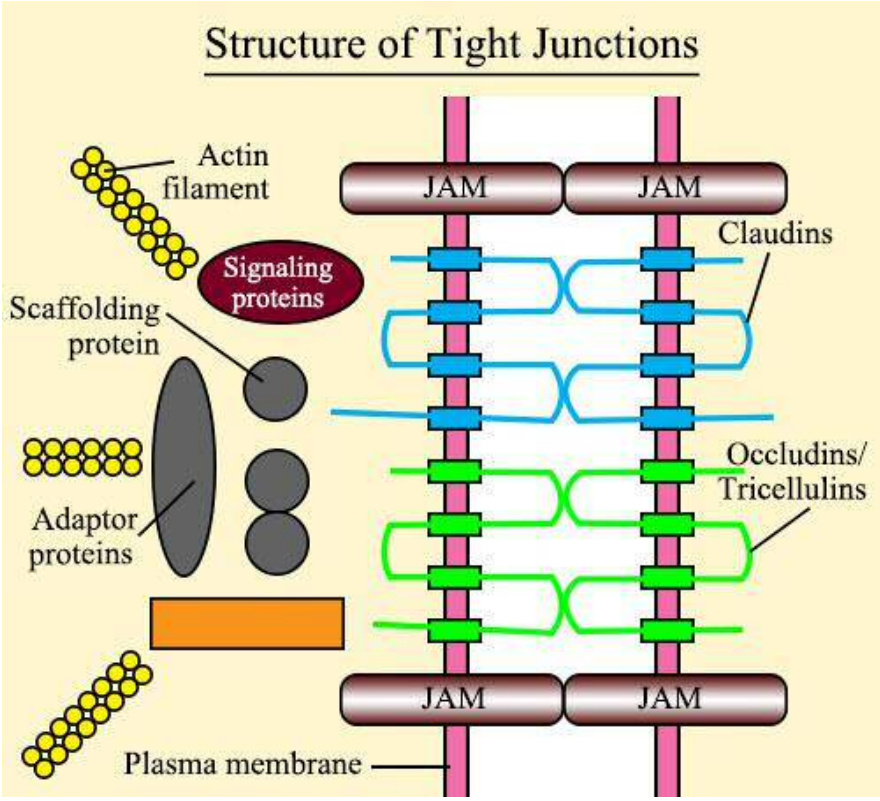
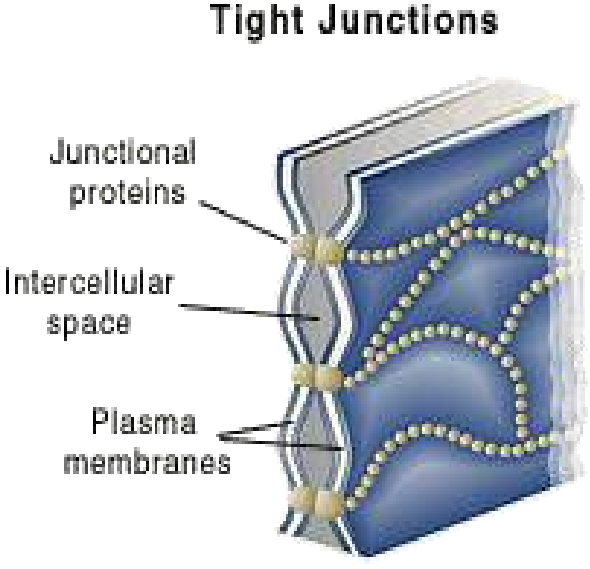


Fig: Tight Junctions



2.ADHERENS JUNCTIONS

Adherens junctions provide strong mechanical attachments between adjacent cells.

- They hold cardiac muscle cells tightly together as the heart expands and contracts.
- They hold epithelial cells together.
- They seem to be responsible for contact inhibition.
- Some adherens junctions are present in narrow bands connecting adjacent cells.
- Others are present in discrete patches holding the cells together.

Adherens junctions are built from:

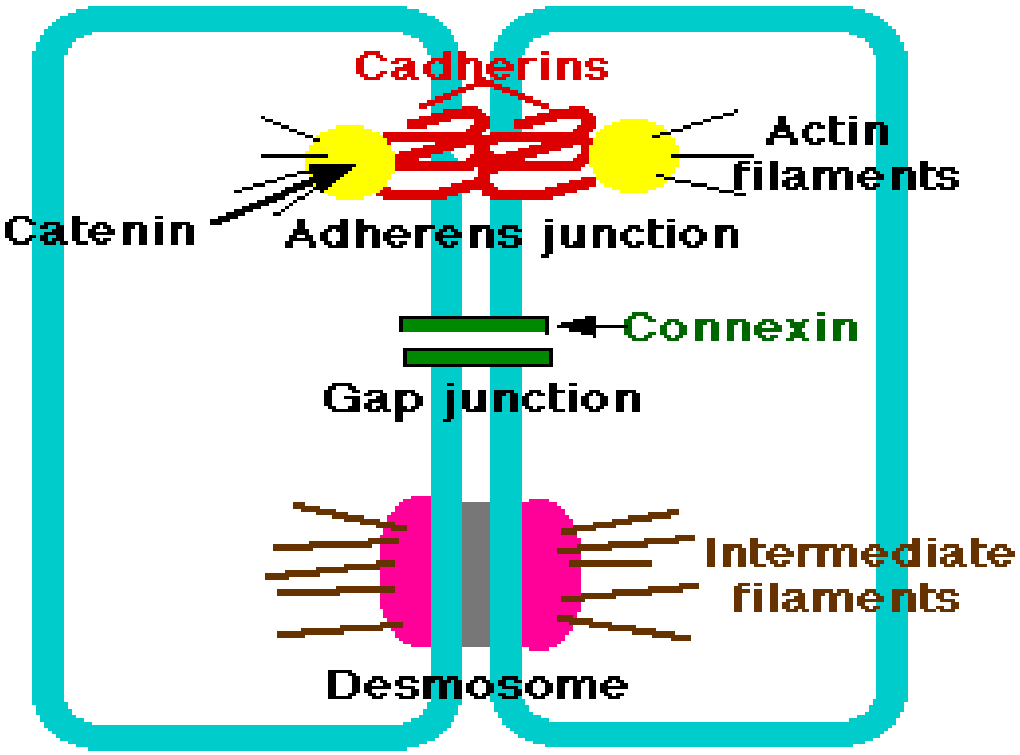
- cadherins — transmembrane proteins (shown in red) whose
  - extracellular segments bind to each other and
  - whose intracellular segments bind to
- catenins (yellow). Catenins are connected to actin filaments

We synthesize some 80 different types of cadherins. In most cases, a cell expressing one type of cadherin will only form adherens junctions with another cell expressing the same type. This is because molecules of cadherin tend to form homodimers not heterodimers.

Inherited mutations in a gene encoding a cadherin can cause stomach cancer. Mutations in a gene (*APC*), whose protein normally interacts with catenins, are a common cause of colon cancer.

Loss of functioning adherens junctions may accelerate

- the edema associated with sepsis;
- tumor metastasis.



3.GAP JUNCTIONS

Gap junctions are intercellular channels some 1.5–2 nm in diameter. These permit the free passage between the cells of ions and small molecules (up to a molecular weight of about 1000 daltons).

Gap junctions are constructed of transmembrane proteins called connexins (Figure). 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.

Because ions can flow through them, gap junctions permit changes in membrane potential to pass from cell to cell.

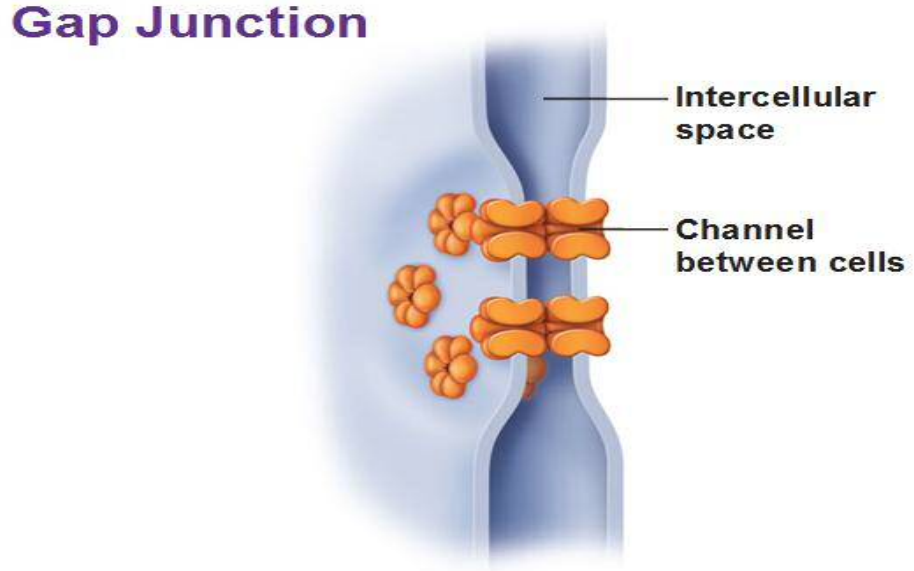
Examples:

- The action potential in heart (cardiac) muscle flows from cell to cell through the heart providing the rhythmic contraction of the heartbeat.
- At some so-called electrical synapses in the brain, gap junctions permit the arrival of an action potential at the synaptic terminals to be transmitted across to the postsynaptic cell without the delay needed for release of a neurotransmitter.
- As the time of birth approaches, gap junctions between the smooth muscle cells of the uterus enable coordinated, powerful contractions to begin.

Several inherited disorders of humans such as

- certain congenital heart defects and
- certain cases of congenital deafness

have been found to be caused by mutant genes encoding connexins.



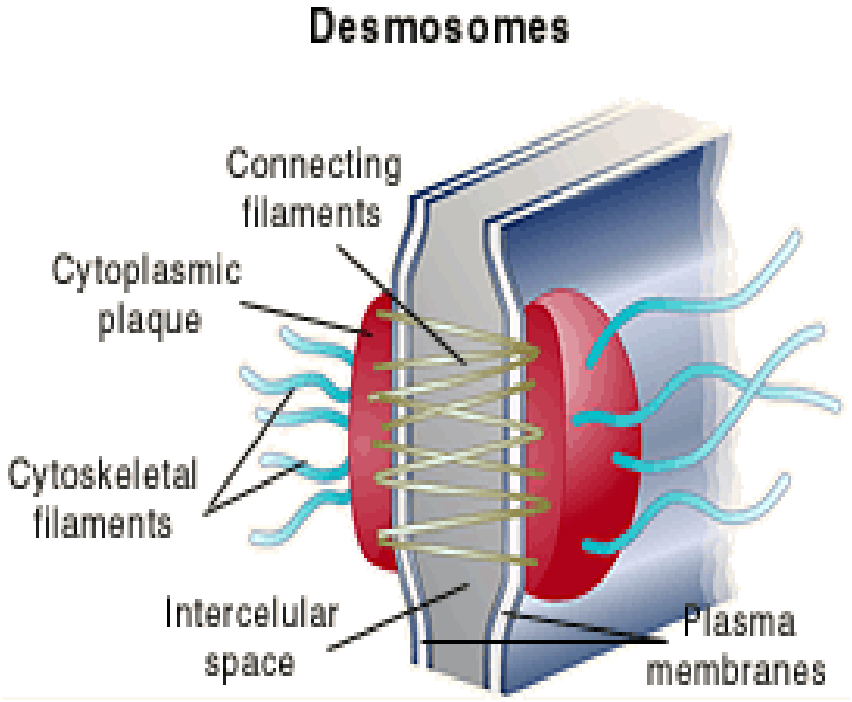
(c) Gap junctions: Communicating junctions allow ions and small molecules to pass from one cell to the next for intercellular communication.

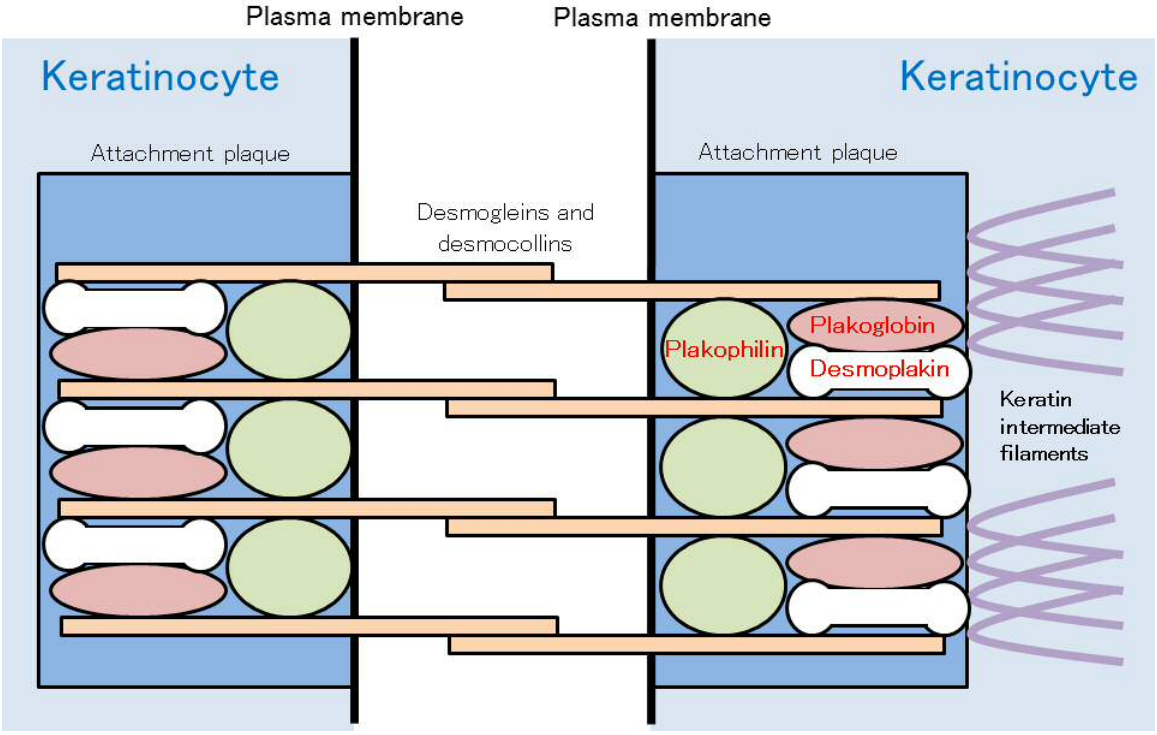
4.DESMOSOMES

Desmosomes are localized patches that hold two cells tightly together. They are common in epithelia (e.g., the skin). Desmosomes are attached to intermediate filaments of keratin in the cytoplasm.

Pemphigus is an autoimmune disease in which the patient has developed antibodies against proteins (cadherins) in desmosomes. The loosening of the adhesion between adjacent epithelial cells causes blistering.

Carcinomas are cancers of epithelia. However, the cells of carcinomas no longer have desmosomes. This may partially account for their ability to metastasize.

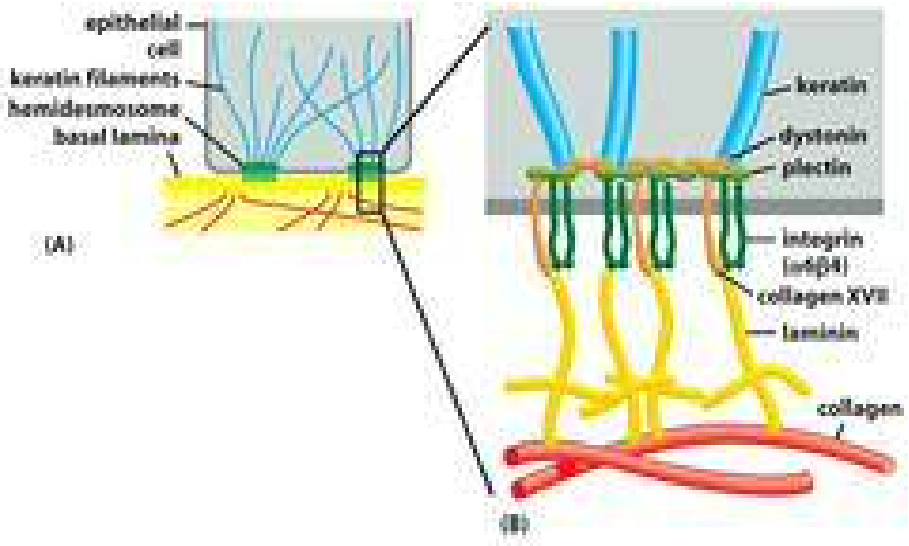




5.HEMIDESMOSOMES

These are similar to desmosomes but attach epithelial cells to the basal lamina ("basement membrane" – View) instead of to each other. Hemidesmosomes are specialized sites of epithelial cell attachment at which a specific integrin (designated $\alpha_6\beta_4$) interacts with intermediate filaments instead of with actin. The $\alpha_6\beta_4$ integrin binds to laminin, so hemidesmosomes anchor epithelial cells to the basal lamina

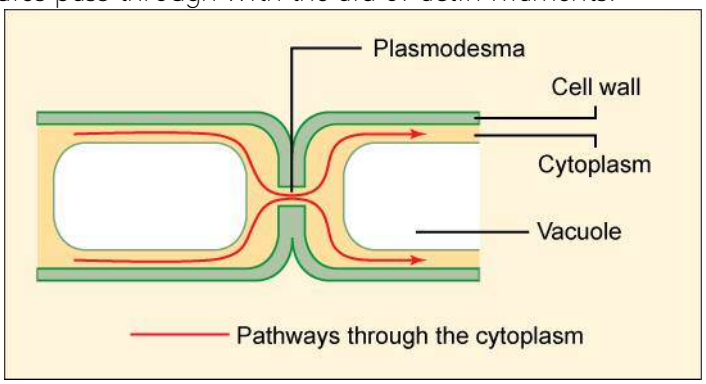
Pemphigoid is an autoimmune disease in which the patient develops antibodies against proteins (integrins) in hemidesmosomes. This, too, causes severe blistering of epithelia.

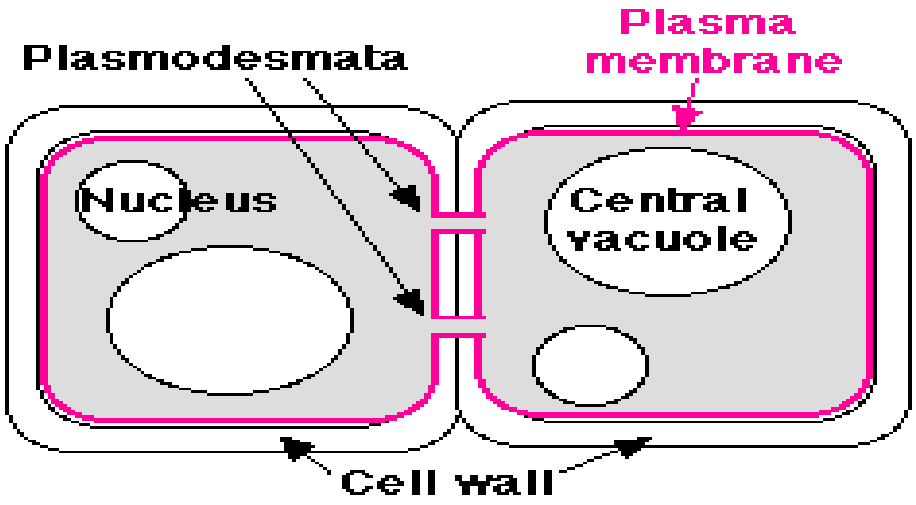


6.PLASMODESMATA

Although each plant cell is encased in a boxlike cell wall, it turns out that communication between cells is just as easy, if not easier, than between animal cells. Fine strands of cytoplasm, called plasmodesmata, extend through pores in the cell wall connecting the cytoplasm of each cell with that of its neighbors.

Plasmodesmata provide an easy route for the movement of ions, small molecules like sugars and amino acids, and even macromolecules like RNA and proteins, between cells. The larger molecules pass through with the aid of actin filaments.





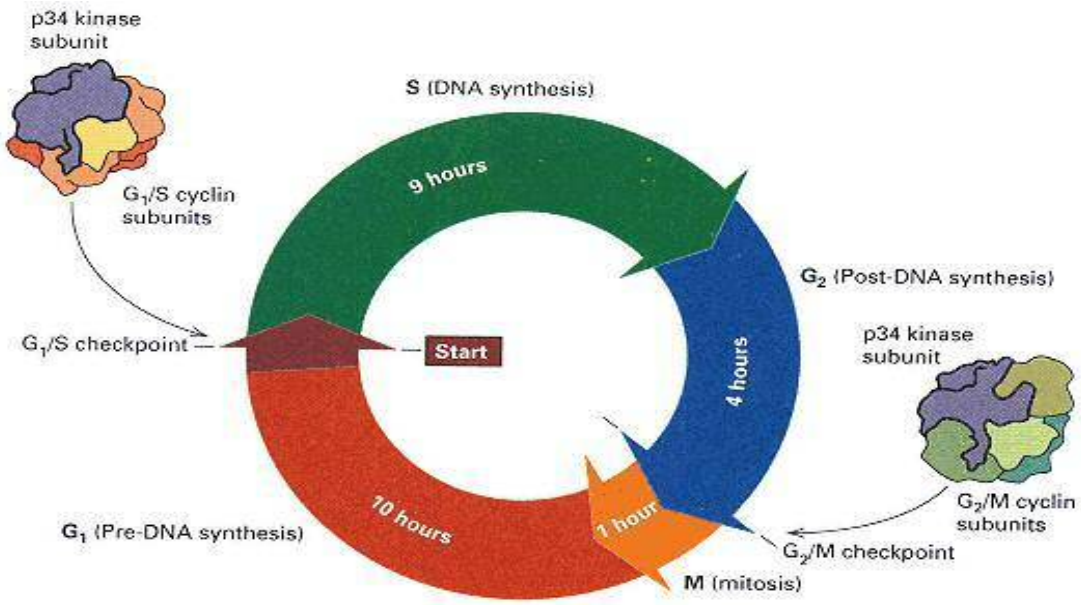
CELL DIVISION and its significance:

Continuity of life depends on cell division. All cells are produced by divisions of pre-existing cell (Please recall our discussion about the cell theory in our first lecture). A cell born after a division, proceeds to grow by macromolecular synthesis, and divides after reaching a species-determined division size. Growth of a cell is an increase in size or mass which is an irreversible process that occurs at all organizational levels.

CELL CYCLE:

Cell cycle 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. The M phase lasts only for an hour in a period of 24 hour required for a eukaryotic cell to divide. The interphase can be further divided into G1 (gap phase 1), S (synthesis) and G2 (gap phase 2) phases (Figure). 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.

Figure 1: The cell cycle



Interphase: During interphase the chromosomes are not visible with a light microscope when the cell is not undergoing mitosis. The genetic material (DNA) in the chromosomes is replicated during the period of interphase to carry out mitosis and is called S phase (S stands for *synthesis* of DNA). DNA replication is accompanied by chromosome duplication. Before and after S, there are two periods, called G1 and G2, respectively, in **which DNA replication does not take place. The order of cell cycle events is G1 → S → G2 → M and then followed by cytokinesis. The G1 phase, S phase and G2 phase together** form the interphase.

Events of Interphase: The interphase is characterized by the following features:

The nuclear envelope remains intact. The chromosomes occur in the form of diffused, long, coiled and indistinctly visible chromatin fibres. The DNA amount becomes double. Due to accumulation of ribosomal RNA (rRNA) and ribosomal proteins in the nucleolus, the size of the latter is greatly increased. In animal cells, a daughter pair of centrioles

originates near the already existing centriole and, thus, an interphase cell has two pairs of centrioles. In animal cells, net membrane biosynthesis increases just before cell division (mitosis). This extra membrane is stored as blebs on the surface of the cells about to divide. Events in interphase takes place in three distinct phases.

G1 Phase: After the M phase of previous cell cycle, the daughter cells begin G1 of interphase of new cell cycle. G1 is a resting phase. It is also called first gap phase, as no DNA synthesis takes place during this stage. It is also known as the first growth phase, since it involves synthesis of RNA, proteins and membranes which leads to the growth of nucleus and cytoplasm of each daughter cell towards their enhancing size. During G1 phase, chromatin is fully extended and not distinguishable as discrete chromosomes with the light microscope.
Thus, it involves transcription of three types of RNAs, namely rRNA, tRNA and mRNA; rRNA synthesis is indicated by the appearance of nucleolus in the interphase (G1 phase) nucleus. Proteins synthesized during G1 phase (a) regulatory proteins which control various events of mitosis (b) enzymes (DNA polymerase) necessary for DNA synthesis of the next stage and (c) tubulin and other mitotic apparatus proteins. G1 phase is most variable as to duration it either occupies 30 to 50 per cent of the total time of the cell cycle. *Terminally differentiated somatic cells (end cells such as neurons and striated muscle cells) that no longer divide, are arrested usually in the G1 stage, such a type of G1 phase is called G0 phase.*

S phase: During the S phase or synthetic phase of interphase, replication of DNA and synthesis of histone proteins occur. New histones are required in massive amounts immediately at the beginning of the S period of DNA synthesis to provide the new DNA with nucleosomes. At the end of S phase, each chromosome has two DNA molecules and a duplicate set of genes. S phase occupies roughly 35 to 45 per cent time of the cell cycle.

G2 phase: This is a second gap or growth phase or resting phase of interphase. During G2 phase, synthesis of RNA and proteins continues which is required for cell growth. It may occupy 10 to 20 per cent time of cell cycle. As the G2 phase draws to a close, the cell enters the M phase.

Dividing phase: There are two types of cell division possible. Mitosis and meiosis. 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.

Cell cycle regulation

Cell cycle is a highly regulated and coordinated process mediated by extracellular signals from the environment, as well as by internal signals. In most cells, this coordination between different phases of the cell cycle is dependent on a series of cell cycle checkpoints that prevent entry into the next phase of the cell cycle until the events of the preceding phase have been completed.

The major cell cycle regulatory check point occurs late in G1 and controls progression from G1 to S. Other check points function to ensure complete genome transmittance to daughter cells. DNA damage checkpoints in G1, S, and G2 lead to cell cycle arrest in response to damaged or unreplicated DNA. Another checkpoint, called the spindle assembly checkpoint, arrests mitosis if the chromosomes are not properly aligned on the mitotic spindle (Figure 1).

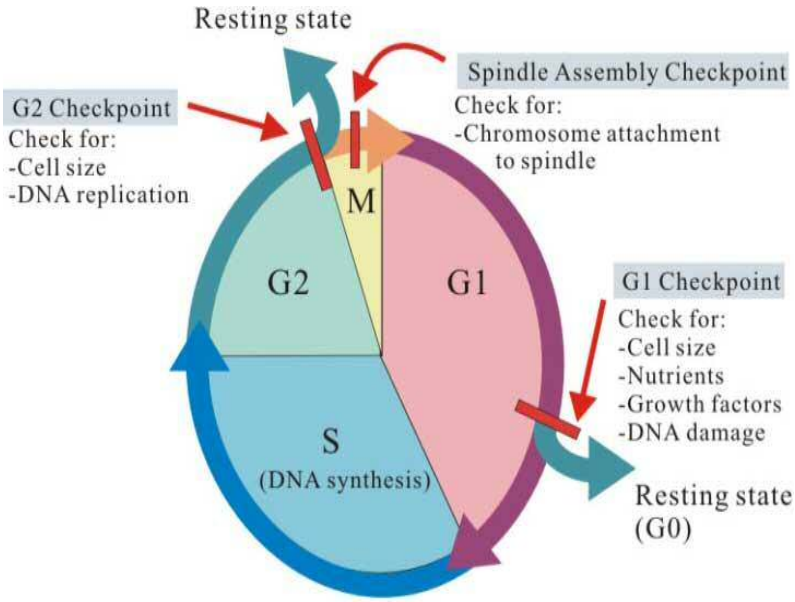


Figure 1:Cell cycle and its check points

To restrict DNA replication once per cell cycle the G2 checkpoint ensures that the genome is replicated only once per cell cycle and that incompletely replicated DNA is not distributed to daughter cells. The molecular mechanism underlying this involves the action of the MCM (minichromosome maintenance complex) helicase that bind to replication origins together with the origin recognition complex (ORC) proteins. The MCM proteins are allowed to bind to replication origins during G1, leading to DNA replication when the cell enters S phase. After initiation the MCM proteins are dissociated from the origin, so replication cannot initiate again until next cell cycle. The association of MCM proteins with DNA during the S, G2 and M phases of the cell cycle is blocked by activity of the protein kinases that regulate cell cycle progression.
The cell cycle itself is under genetic control and the mechanisms of control are identical in all eukaryotes. There are two critical transitions: from G1 into S and from G2 into M. The G1/S and G2/M transitions are called "checkpoints" because the transitions are delayed unless key processes have been completed. For example, at the G1/S checkpoint, either sufficient time must have elapsed since the preceding mitosis or the cells have attained sufficient size for DNA replication to be initiated. Similarly, the G2/M checkpoint requires that DNA replication and repair of any DNA damage be completed for the M phase to commence.

Cyclin dependent kinases (Cdks) are the central components that coordinate activities throughout the cell cycle whose activities in turn are regulated by cyclin binding. The cyclin-Cdk complex causes phosphorylation of proteins that control chromosome condensation, nuclear envelope breakdown and other events that occur at the onset of mitosis. Cyclins can be divided into four classes.

1. G1/S cyclin: They activate Cdks in late G1 and their level fall in S phase.

2. S cyclin: They stimulate DNA replication and their level remains high until mitosis.

3. M cyclin: Activate Cdks that stimulate entry into mitosis at the G2/M checkpoint.

4. G1 cyclins: Governs the activities of G1/S cyclins.

The cyclin protein not only activates Cdks but directs them to specific target proteins phosphorylating a different set of proteins. The different cyclin and Cdks of vertebrates has been presented in Table 1.

Table 1: The major cyclins and Cdks

Cyclin-Cdk complex	Vertebrates	
	Cyclin	Cdk partner
G1-Cdk	D	Cdk4, Cdk6
G1/S	E	Cdk2
S	A	Cdk2
M	B	Cdk1

MITOSIS

Mitosis is a process of 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.
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 is divided into four stages: prophase, metaphase, anaphase and telophase. The stages have the following characteristics:

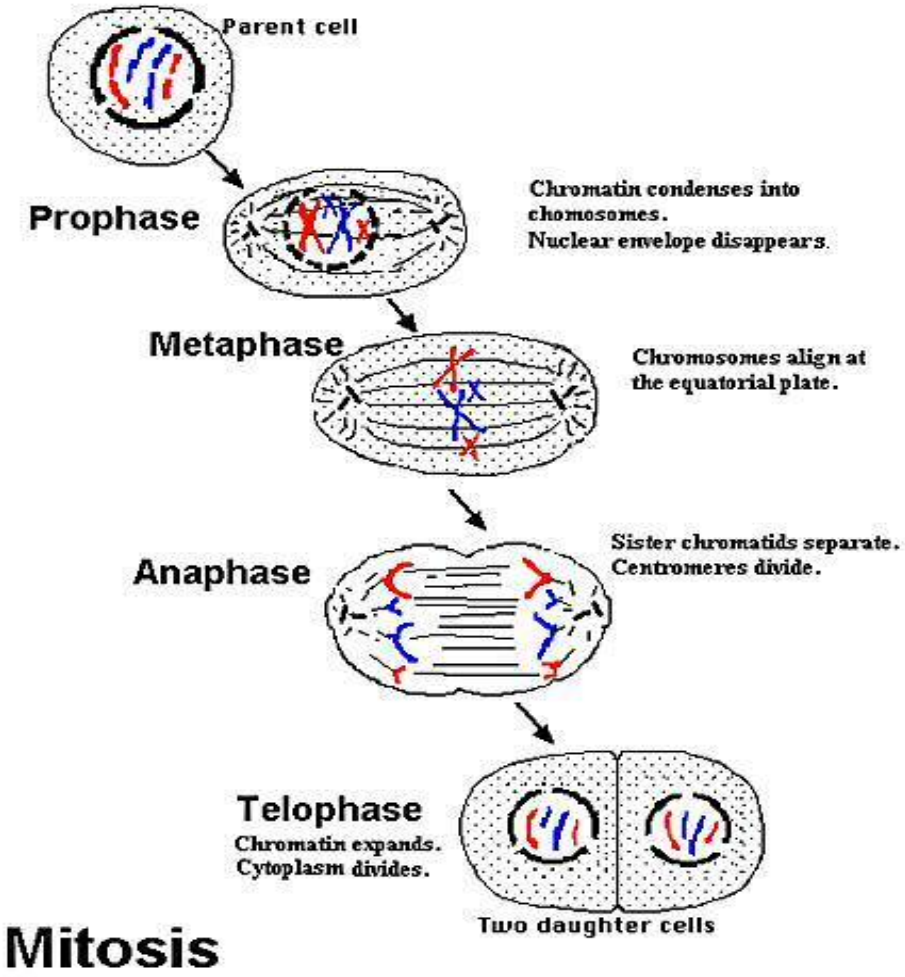


Fig.3: Mitosis cell cycle

1. Prophase:

The chromosomes are in the form of extended filaments and cannot be seen with a light microscope as discrete bodies except for the presence of one or more dark bodies (i.e. nucleoli) in the interphase stage. The beginning of prophase is marked by the condensation of chromosomes to form visibly distinct, thin threads within the nucleus. Each chromosome is already longitudinally double, consisting of two closely associated subunits called chromatids which are held together by 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.

2. Metaphase:

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.

3. Anaphase:

In anaphase, the centromeres divide longitudinally, and the two sister chromatids of each chromosome move toward opposite poles of the spindle. 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.

4. Telophase:

In telophase, a nuclear envelope forms around each group of chromosomes, nucleoli are formed, and the spindle disappears. The chromosomes undergo a reversal of condensation until and unless they are no longer visible as discrete entities. The two daughter nuclei slowly goes to interphase stage the cytoplasm of the cell divides into two by means of a gradually deepening furrow around the periphery.

5. Cytokinesis:

The chromosomes moved close to the spindle pole regions, and the spindle mid-zone begins to clear. In this middle region of the spindle, a thin line of vesicles begins to accumulate. This vesicle aggregation is an indication to the formation of a new cell wall that will be situated midway along the length of the original cell and hence form boundary between the newly separating daughter cells.

MEIOSIS

Meiosis was first described by the German biologist Oscar Hertwig in 1876 in the sea urchin egg. Meiosis is the process of cell division that occurs only in the germ cells of eukaryotes unlike mitosis which takes place in the somatic cells. Unlike mitosis meiosis is only initiated once in the life cycle of eukaryotes (John 1990). The cells produced by meiosis are known as gametes or spores. Meiosis leads to reduction of chromosome number, of a diploid cell (2n) to half (n). Meiosis begins with one diploid cell containing two copies of each chromosome and ultimately produces four haploid cells containing one copy of each chromosome which have undergone recombination, giving rise to genetic diversity in the offspring.

Stages of meiosis

Meiosis can be separated into two phases which are meiosis I and meiosis II and they can be further subdivided into numerous phases which have particular identifiable features. They have been broadly described in the following sections.

Meiosis II

Meiosis II is the second stage of the meiotic process. The overall process is similar to mitosis. The end result is production of four haploid cells. The four main steps of Meiosis II are: Prophase II, Metaphase II, Anaphase II, and Telophase II (see Fig 3).

Prophase II
In prophase II the nucleoli and nuclear envelope disappear. Centrioles move to opposite poles and arrange spindle fibers for the second meiotic division (see Fig 3).

Metaphase II
In metaphase II, the centromeres contain two kinetochores that attach to spindle fibers from the centrosomes (centrioles) at each pole. The new equatorial metaphase plate is rotated by 90 degrees when compared to meiosis I, perpendicular to the previous plate (see Fig 3).

Anaphase II
This is followed by anaphase II, where the centromeres are cleaved, allowing microtubules attached to the kinetochores to pull the sister chromatids apart. The sister chromatids by convention are now called sister chromosomes as they move toward opposing poles (see Fig 3).

Telophase II
The process ends with telophase II, which is similar to telophase I, and is marked by uncoiling and lengthening of the chromosomes and the disappearance of the spindle. Nuclear envelopes reform and cleavage or cell wall formation eventually produces a total of four daughter cells, each with a haploid set of chromosomes. Meiosis is now complete and ends up with four new daughter cells (see Fig 3).

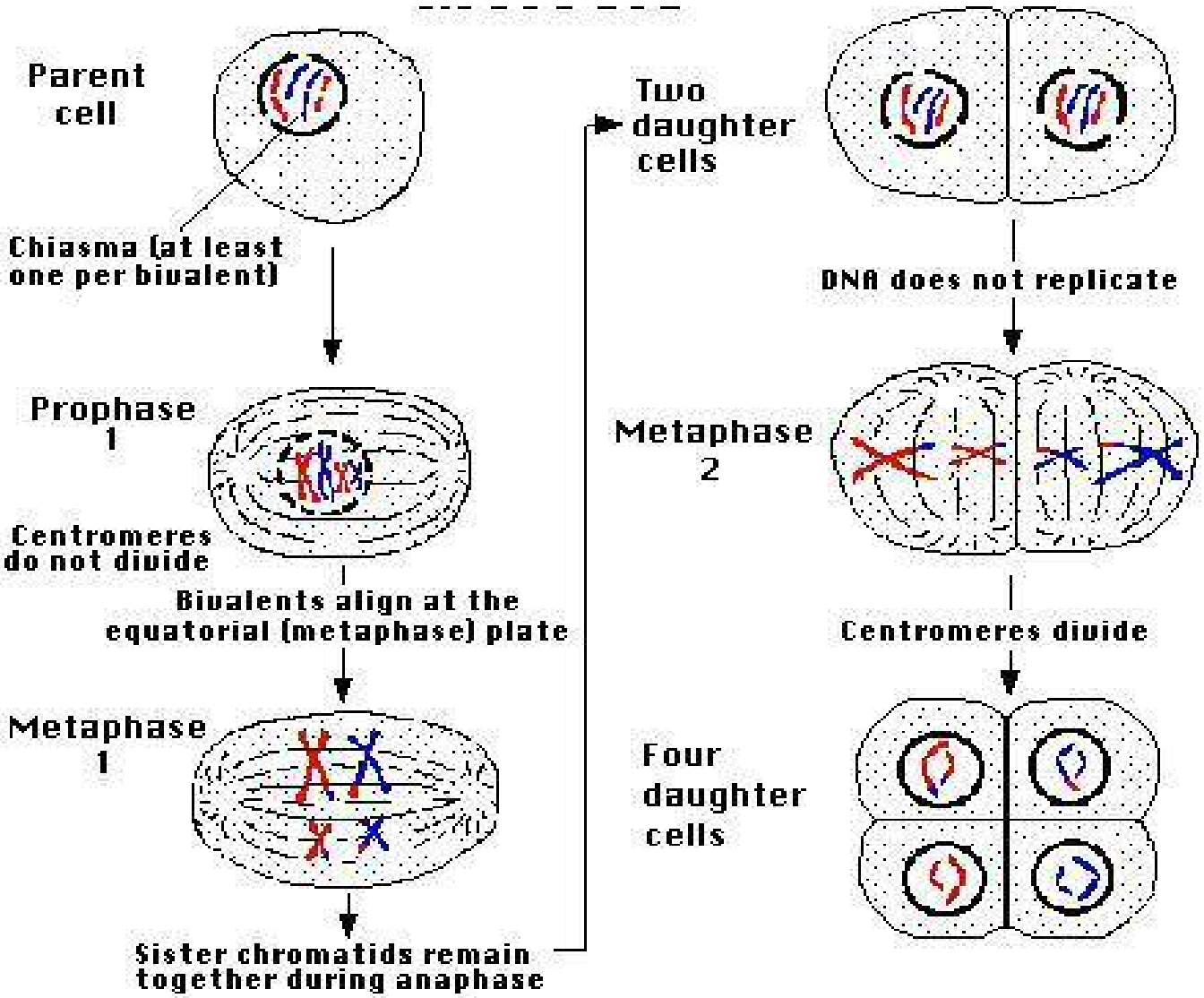


Figure 3: Events in meiosis I and II

The difference between male and female meiosis

There are mainly three differences between male and female meiosis 1.
Male meiosis creates sperm, while female meiosis creates eggs.

2. Male meiosis takes place in the testicles, while female meiosis takes place in the ovaries.
3. A male will generally have one X and one Y sex chromosome, while a female have two X chromosomes, however only one of the two is active and the other is known as a barr body . During meiosis I, the sex chromosomes separate and enter different sperm or egg cells (gametes). Males will end up with one half X sperm and the other half Y sperm, while females will all have X eggs because they had no Y chromosome in the first place.
4. At the end of meiosis, females produce one daughter cells and 3 are discarded as polar bodies where as four progeny cells are produced by male cells.

TRANSFORMATION - ELEMENTARY CONCEPTS

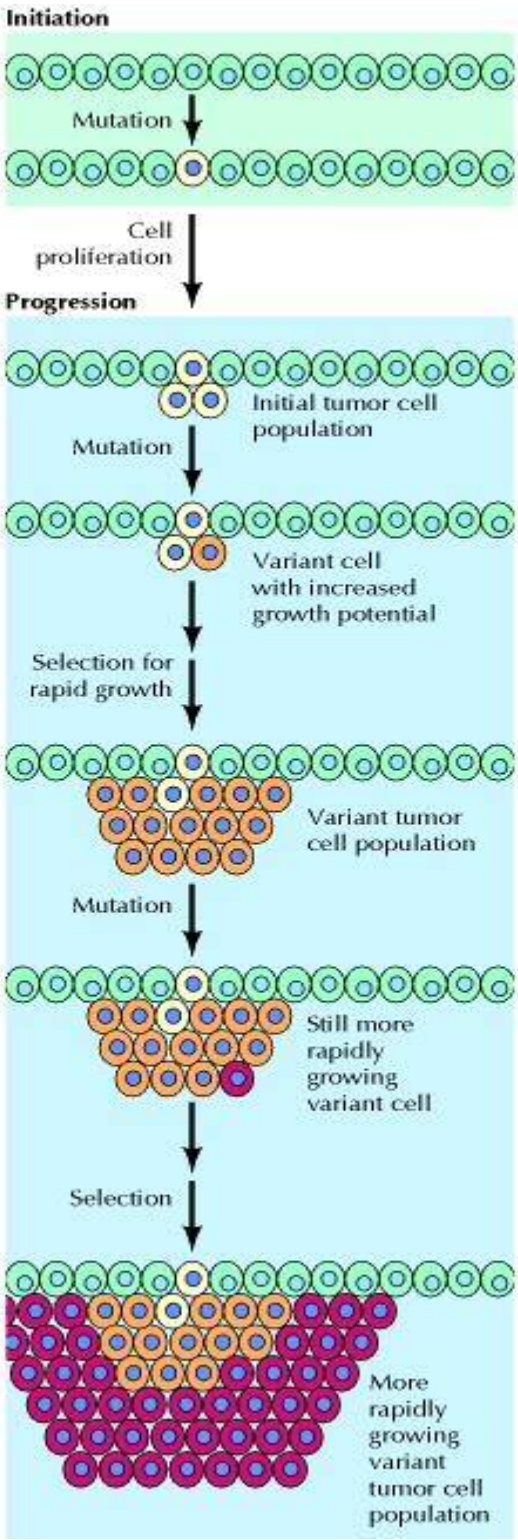
Transformation is process of conversion of normal cell into cancer cell

The Development and Causes of Cancer

The fundamental abnormality resulting in the development of cancer is the continual unregulated proliferation of cancer cells. Rather than responding appropriately to the signals that control normal cell behavior, cancer cells grow and divide in an uncontrolled manner, invading normal tissues and organs and eventually spreading throughout the body. The generalized loss of growth control exhibited by cancer cells is the net result of accumulated abnormalities in multiple cell regulatory systems and is reflected in several aspects of cell behavior that distinguish cancer cells from their normal counterparts.

The Development of Cancer

At the cellular level, the development of cancer is viewed as a multistep process involving mutation and selection for cells with progressively increasing capacity for proliferation, survival, invasion, and metastasis. The first step in the process, tumor initiation, is thought to be the result of a genetic alteration leading to abnormal proliferation of a single cell. Cell proliferation then leads to the outgrowth of a population of clonally derived tumor cells. Tumor progression continues as additional mutations occur within cells of the tumor population. Some of these mutations confer a selective advantage to the cell, such as more rapid growth, and the descendants of a cell bearing such a mutation will consequently become dominant within the tumor population. The process is called clonal selection, since a new clone of tumor cells has evolved on the basis of its increased growth rate or other properties (such as survival, invasion, or metastasis) that confer a selective advantage. Clonal selection continues throughout tumor development, so tumors continuously become more rapid-growing and increasingly malignant.



Stages of tumor development

The development of cancer initiates when a single mutated cell begins to proliferate abnormally. Additional mutations followed by selection for more rapidly growing cells within the population then result in progression of the tumor to increasingly rapid growth and malignancy.

Causes of Cancer

Causes

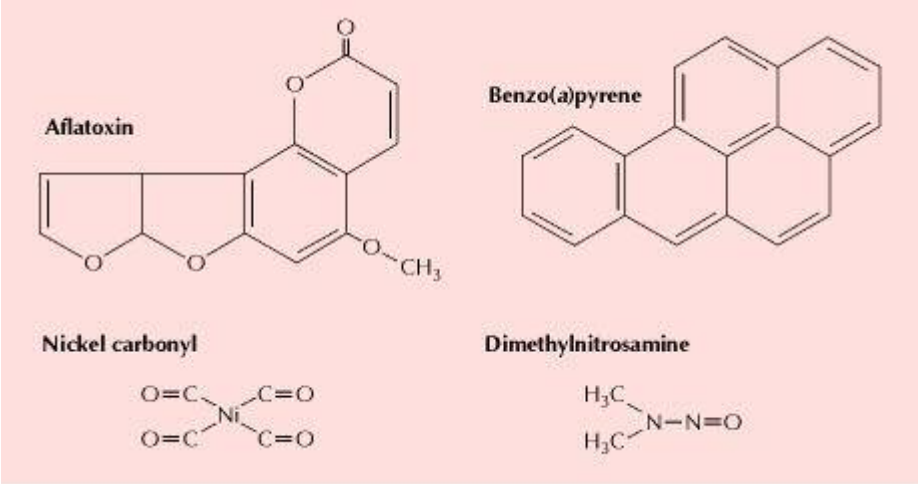
The causes may

i) Physical: In which the X rays and UV rays affect the DNA and form thymine dimer and adducts that result in faulty DNA replication and this result in transformation
ii) Chemical : Aflatoxin, benzo(*a*)pyrene, dimethylnitrosamine
iii) Biological- Tumuor viruses cause mutation and over expression of certain gene and cause cancer

Substances that cause cancer, called carcinogens, have been identified both by studies in experimental animals and by epidemiological analysis of cancer frequencies in human populations (e.g., the high incidence of lung cancer among cigarette smokers). Since the development of malignancy is a complex multistep process, many factors may affect the likelihood that cancer will develop, and it is overly simplistic to speak of single causes of most cancers. Nonetheless, many agents, including radiation, chemicals, and viruses, have been found to induce cancer in both experimental animals and humans.

Radiation and many chemical carcinogens act by damaging DNA and inducing mutations. These carcinogens are generally referred to as initiating agents, since the induction of mutations in key target genes is thought to be the initial event leading to cancer development. Some of the initiating agents that contribute to human cancers include solar ultraviolet radiation (the major cause of skin cancer), carcinogenic chemicals in tobacco smoke, and aflatoxin (a potent liver carcinogen produced by some molds that contaminate improperly stored supplies of peanuts and other grains). The carcinogens in tobacco smoke (including benzo(*a*)pyrene, dimethylnitrosamine, and nickel compounds) are the major identified causes of human cancer. Smoking is the undisputed cause of 80 to 90% of lung cancers, as well as being implicated in cancers of the oral cavity, pharynx, larynx, esophagus, and other sites. In total, it is estimated that smoking is responsible for nearly one-third of all cancer deaths—an impressive toll for a single carcinogenic agent.

Structure of representative chemical carcinogens

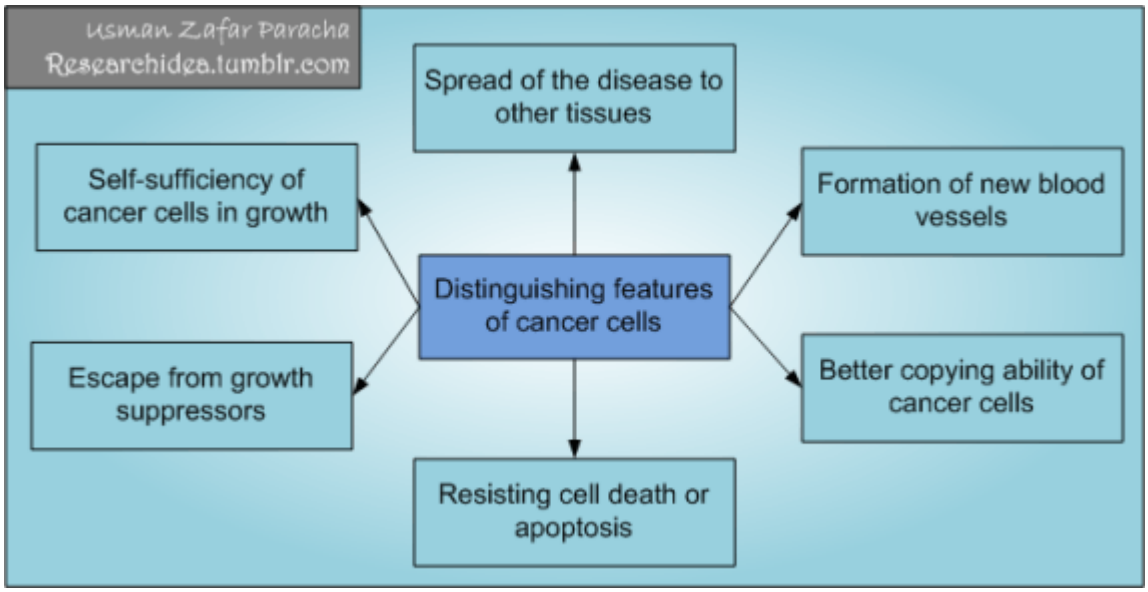


Hormones, particularly estrogens, are important as tumor promoters in the development of some human cancers. The proliferation of cells of the uterine endometrium, for

example, is stimulated by estrogen, and exposure to excess estrogen significantly increases the likelihood that a woman will develop endometrial cancer. The risk of endometrial cancer is therefore substantially increased by long-term postmenopausal estrogen replacement therapy with high doses of estrogen alone. Fortunately, this risk is minimized by administration of progesterone to counteract the stimulatory effect of estrogen on endometrial cell proliferation. However, long-term therapy with combinations of estrogen and progesterone may lead to an increased risk of breast cancer.

In addition to chemicals and radiation, some viruses induce cancer both in experimental animals and in humans. The common human cancers caused by viruses include liver cancer and cervical carcinoma, which together account for 10 to 20% of worldwide cancer incidence. These viruses are important not only as causes of human cancer; as discussed later in this chapter, studies of tumor viruses have played a key role in elucidating the molecular events responsible for the development of cancers induced by both viral and nonviral carcinogens.

Properties of Cancer Cells

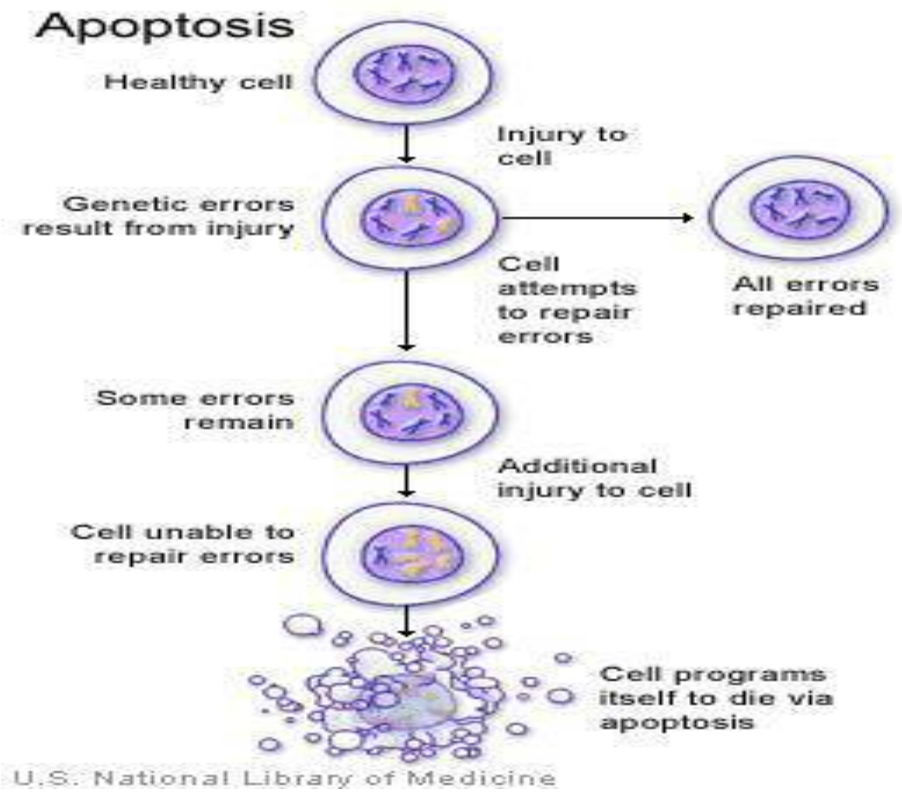


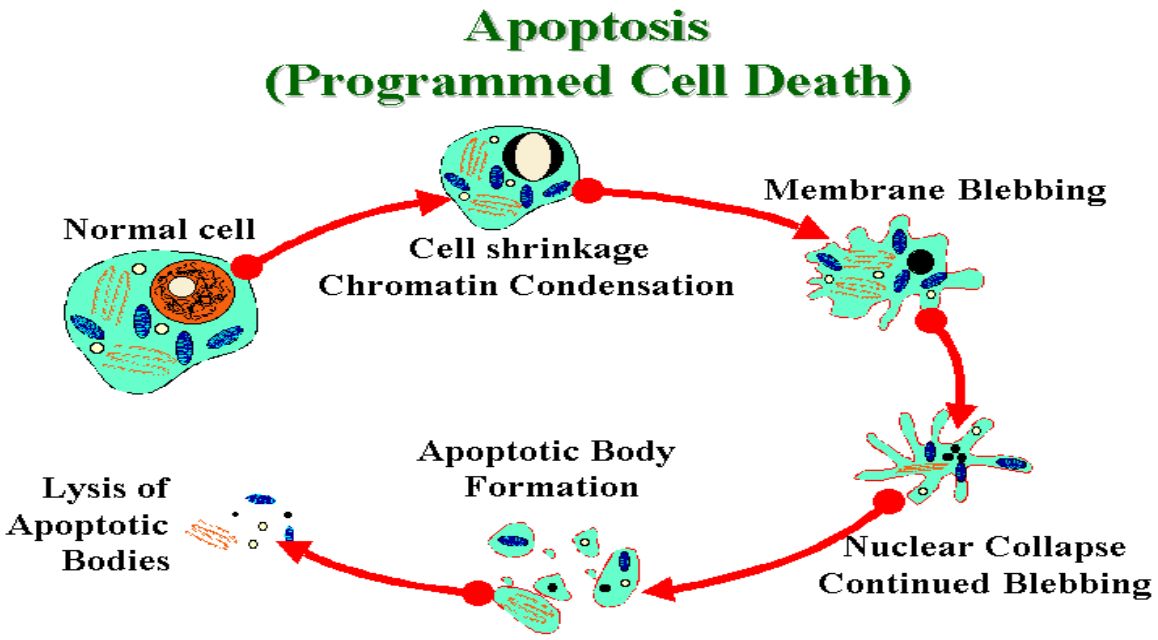
Apoptosis

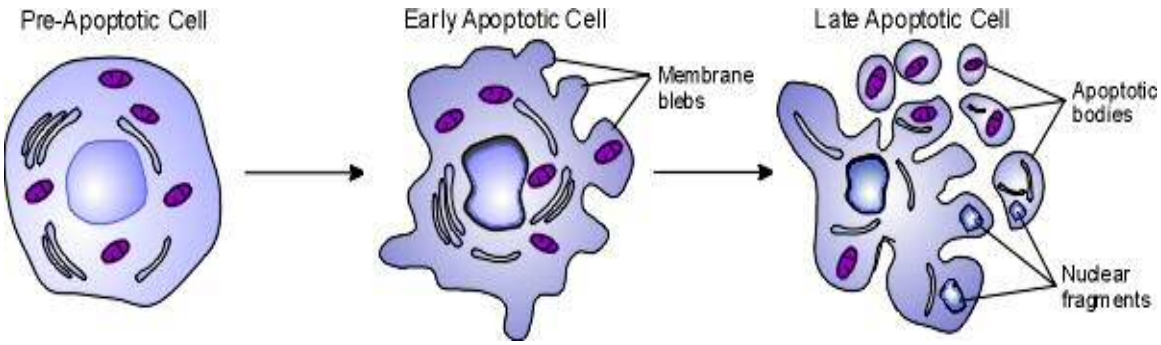
Apoptosis is a process of programmed cell death that occurs in multicellular organisms. Biochemical events lead to characteristic cell changes and death. These changes include blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation, and global mRNA decay. Between 50 and 70 billion cells die each day due to apoptosis in the average human adult.[a] For an average child between the ages of 8 and 14, approximately 20 billion to 30 billion cells die a day.

In contrast to necrosis, which is a form of traumatic cell death that results from acute cellular injury, apoptosis is a highly regulated and controlled process that confers advantages during an organism's lifecycle. For example, the separation of fingers and toes in a developing human embryo occurs because cells between the digits undergo apoptosis. Unlike necrosis, apoptosis produces cell fragments called apoptotic bodies that phagocytic cells are able to engulf and quickly remove before the contents of the cell can spill out onto surrounding cells and cause damage.

Because apoptosis cannot stop once it has begun, it is a highly regulated process. Apoptosis can be initiated through one of two pathways. In the *intrinsic pathway* the cell kills itself because it senses cell stress, while in the *extrinsic pathway* the cell kills itself because of signals from other cells. Both pathways induce cell death by activating caspases, which are proteases, or enzymes that degrade proteins. The two pathways both activate initiator caspases, which then activate executioner caspases, which then kill the cell by degrading proteins indiscriminately.





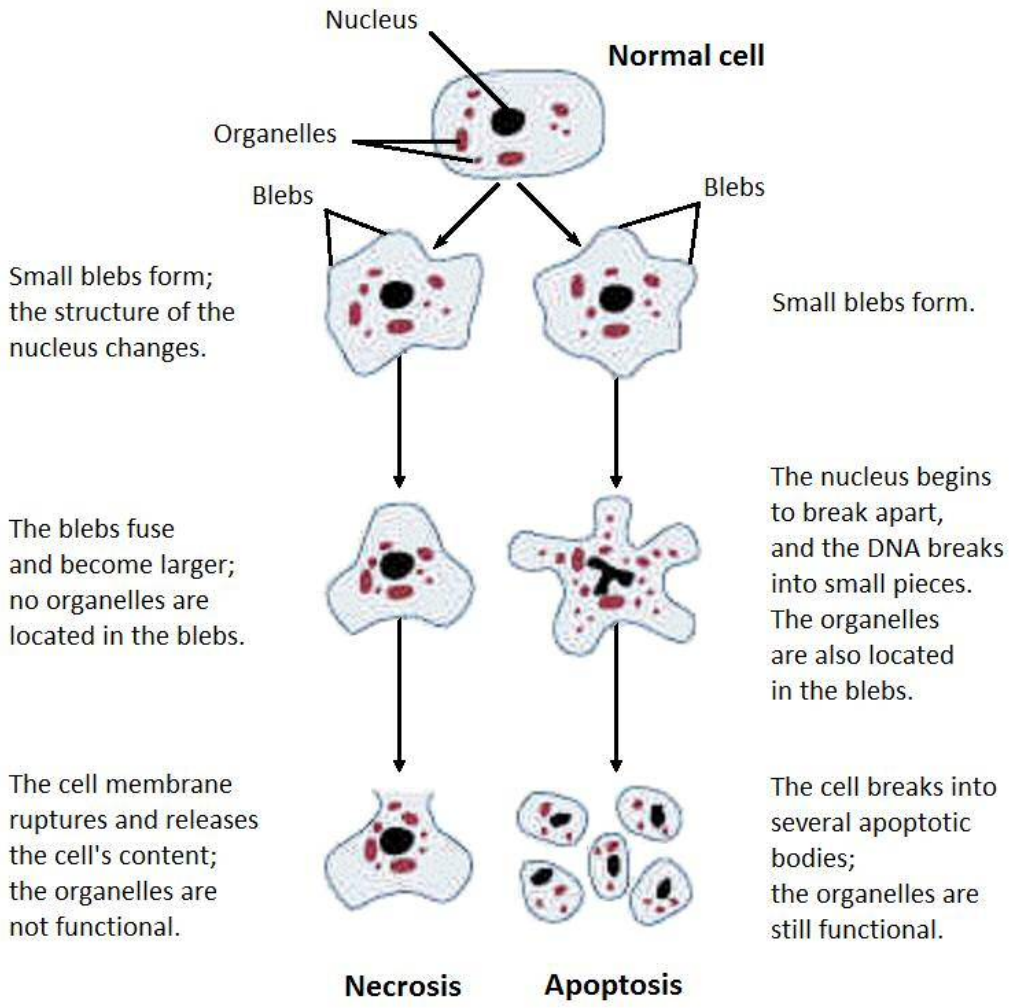


Necrosis, the stage of dying, the act of killing" is a form of cell injury which results in the premature death of cells in living tissue by autolysis.
Necrosis is caused by factors external to the cell or tissue, such as infection, toxins, or trauma which result in the unregulated digestion of cell components.
In contrast, apoptosis is a naturally occurring programmed and targeted cause of cellular death.
While apoptosis often provides beneficial effects to the organism, necrosis is almost always detrimental and can be fatal.
Cellular death due to necrosis does not follow the apoptotic signal transduction pathway, but rather various receptors are activated, and result in the loss of cell membrane integrity and an uncontrolled release of products of cell death into the extracellular.
This initiates in the surrounding tissue an inflammatory response which prevents nearby phagocytes from locating and eliminating the dead cells by phagocytosis. For this reason, it is often necessary to remove necrotic tissue surgically, a procedure known as debridement. Untreated necrosis results in a build-up of decomposing dead tissue and cell debris at or near the site of the cell death. A classic example is gangrene.
Causes
Necrosis may occur due to external or internal factors.
External factors may involve mechanical trauma (physical damage to the body which causes cellular breakdown), damage to blood vessels (which may disrupt blood supply to associated tissue), and ischemia. Thermal effects (extremely high or low temperature) can result in necrosis due to the disruption of cells.
Internal factors causing necrosis include: troponeuronic disorders; injury and paralysis of nerve cells. Pancreatic enzymes (lipases) are the major cause of fat necrosis.
Generally speaking, there are two steps that occur when a cell dies:

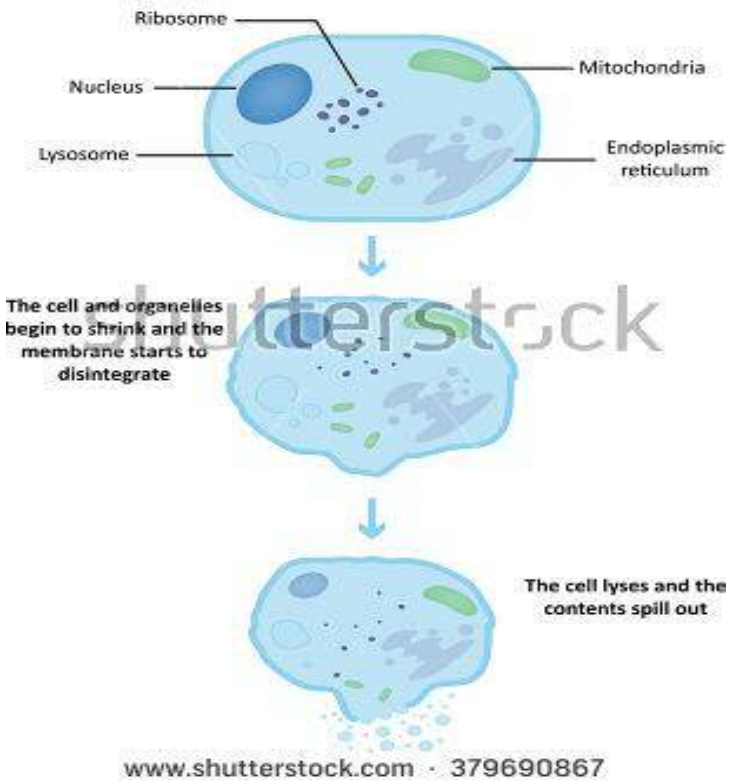
1. Proteins inside the cell break down
2. The body releases enzymes that digest these dead cells

The apoptosis process is a natural part of the cell's life cycle, and the body is ready to carry the dead cell materials away. In necrosis, however, the body isn't prepared to remove the dead cells, and as a result, causes an inflammatory response.
Cells need blood to live, and any interruption to blood flow results in necrosis. Injury, infection, disease, toxins, and many other factors can block blood from getting to a cell and cause unnatural death. Sometimes a dead cell releases chemicals that can affect the nearby cells, spreading necrosis to wide areas in a condition called gangrene, which is when tissue in certain areas, usually the hands and feet, dies. Again, a dead cell can't turn back into a living one, so the only cure for gangrene is to amputate the area.
It is less orderly than apoptosis, which are part of programmed cell death.
In contrast with apoptosis, cleanup of cell debris by phagocytes of the immune system is generally more difficult, as the disorderly death generally does not send cell signals which tell nearby phagocytes to engulf the dying cell.

This lack of signalling makes it harder for the immune system to locate and recycle dead cells which have died through necrosis than if the cell had undergone apoptosis.
The release of intracellular content after cellular membrane damage is the cause of inflammation in necrosis.
There are many causes of necrosis including injury, infection, cancer, infarction, toxins and inflammation.
Severe damage to one essential system in the cell leads to secondary damage to other systems, a so-called "cascade of effects".
Necrosis can arise from lack of proper care to a wound site.



Necrosis



Necrosis is accompanied by the release of special enzymes, that are stored by lysosomes, which are capable of digesting cell components or the entire cell itself.

The injuries received by the cell may compromise the lysosome membrane, or may initiate an unorganized chain reaction which causes the release in enzymes.

Unlike in apoptosis, cells that die by necrosis may release harmful chemicals that damage other cells.

KARPAGAM ACADEMY OF HIGHER EDUCATION
COIMBATORE - 641021
DEPARTMENT OF BIOCHEMISTRY
I-B.Sc., BIOCHEMISTRY - BATCH: 2017 - 2020
PART A (20 X 1 = 20 MARKS) - Online MCQ Questions

SUBJECT: (SUBJECT CODE: 17BCU102

Questions	Option A	Option B	Option C	Option D	Answer
The division of the nucleus separates the duplicated genome into two sets identical to the parent's in -----	Mitosis	Meiosis	Cytokinesis	Interphase	Mitosis
---------- is the division of the cytoplasm, separating the organelles and other cellular components.	Mitosis	Meiosis	Cytokinesis	Prophase	Cytokinesis

---------- is the division of the nucleus in sex cells, making one cell into four sex cells identical to the parent sex cell.	Mitosis	Meiosis	Cytokinesis	Metaphase	Meiosis
In humans cell division eventually halts after -------- divisions	42	52	62	72	52
The sister chromatics will be attached by	a centromere	a telomere	a centriole	golgi bodies	a centromere
Meiosis I is -------	Reduction	Dublication	Maintanance	Division	Reduction
Meiosis produces ---------cells.	4 diploid	4 haploid	2 haploid	2 diploid	4 haploid
Mitosis produces ---------cells	2 haploid	2 diploid	4 diploid	4 haploid	2 diploid

Chromatin condenses into chromosomes, the nucleolus dissolves and nuclear membrane is disassembled, and the spindle apparatus forms in	Prophase	Metaphase	Anaphase	Telophase	Prophase
---------- Tetrads line-up along the equator of the spindle	Metaphase I	Metaphase II	Anaphase I	Anaphase II	Metaphase I
---------- Considered a reduction phase	Meiosis	Mitosis	karyokinesis	Cytokinesis	Meiosis
Chromosomes are seen in the equatorial plane in	Prophase	Metaphase	Anaphase	Telophase	Metaphase

In prokaryotes the nuclear body is called ------	Nucleolus	Nucleosome	Plasmid	Liposome	Nucleosome
Important feature of plant cell is the presence of	Mitochondria	Cell- wall	Microsome	Cytoplasm	Cell- wall
An interphase between nucleus and cytoplasm is	Nuclear envelope	Nuclear membrane	Nuclear pores	Perinuclear space	Perinuclear space
Dyed formation is the Characteristics of -----	Mitosis	Meiosis	Duplication	Reduction	Meiosis
---------Occur in the somatic cells	Meiosis	Mitosis	Maintenance	Only fusion	Mitosis
The cell cycle consists of ----------	Two distinct phases	Three distinct phases	Four distinct phases	Five distinct phases	Four distinct phases

---------- phases collectively known as interphase	Gl, S and G2	GI, M and G2	M, S and GO	Gl, MandS	Gl, S and G2
Which is the breeding method used by human to produce new and improve varieties of plant and animals?	Independent assortment	Crossing over	Artificial selection	Natural selection	Crossing over
Nucleosome is found in which organism	Fungi	Algae	Protozoan	Drosophila	Algae
The first stage in meiotic prophase	leptotene	zygonema	pachynema	diplonema	leptotene
During cytokinesis, the cytoplasm divides by a process called	Cleavage	Separation	Splitting	Joining.	Cleavage

After M phase, the daughter cells each begin ----------of a new cycle	Growth phase	Inter phase	Multiplication phase	Synthesis phase	Inter phase
All of the chromosomes have been replicated in	G0 phase	Gl Phase	M phase	S phase	S phase
DNA synthesis takes place during	Gl Phase	S-Phase	G2 Phase	Interphase	S-Phase
The space between the nuclear envelop and nucleolus in filled by	Nucleic acids	Nucleoplasm	Nucleotides	Nucleoproteins	Nucleoplasm
The cell cycle consists of ----------	Two distinct phases	Three distinct phases	Four distinct phases	Five distinct phases	Four distinct phases

Cells permanently stop division due to age or accumulated DNA damage are called	Cell death	Senescent	Leptotene	Cleavage	Senescent
---------- are regulatory molecules that determine a cell's progress through the cell cycle	Cycline and dehydrogenases	Cyclins and cycline dependent kinases	Cycline and cdhdrines	Cycline and indeherins	Cyclins and cycline dependent kinases
In which phase of mitosis does chromosome condense	Prophase	metaphase	anaphase	telophase	Prophase
In which phase of mitosis does sister chromatins segregated to the opposite poles of mitotic spindle	Prophase	metaphase	anaphase	telophase	anaphase

Which of the following cells in multi cellular organisms exist from the cell cycle and does not proliferate in life time?	Skin	nerve	bone	Muscle	nerve
The attachment of which of the following protein marks a cell or a protein for degradation	Lyase	ubiquitin	RNase	protease	ubiquitin

Nuclear pore complexes which are broken down in to sub pore complexes during prophase reassemble into nuclear membrane around each chromosome forming individual mini nuclei called	Nucleomere	karyomere	cytomere	cytosol	karyomere
The space between the nuclear envelope and the nucleolus is filled by a transparent matrix knowns as	Nucleic acids	Nucleohistons	Nucleoprotamines	Nucleoplasm	Nucleoplasm
Nuclesomes are fundamental units of	DNA	Nucleus	Nucleolus	Chromatin	DNA

Nucleolus is rich in’	protein	DNA	RNA	Glycoprotein	RNA
Tumors become life threatening if they spread throughout the body. Such tumors are called---	Malignant	benign	Acute	Chronic	Malignant
The spread of tumour cells and establishment of secondary areas of growth is called—	Apoptosis	Metástasis	Homeostasis	Erythropoiesis	Metástasis
Malignant tumors derived from endoderm or ectoderm is called----	Carcinoma	Sarcoma	Lymphoma	Leukemia	Carcinoma
Sarcoma is a tumour derived from ----	Endoderm	mesoderm	Ectoderm	Lymphoma	mesoderm

The massive proliferation of WBC can cause a patients blood to appear milky. This feature is seen in which type of malignant tumour	Carcinoma	Lymphoma	Sarcoma	Leukemia	Leukemia
The proteins secreted by transformed cells that can stimulate the growth of normal cells	TGF α	TGF β	TGF α and TGF β	TGF γ	TGF a and TGF b

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
The scientific study of tumours is called	Oncology	Tumour immunology	Cell biology	Physiology	Oncology
Cellular genes known to be progenitors of oncogenes are called	Proto oncogenes	Preproto oncogenes	Meta oncogenes	Premeta oncogenes	Proto oncogenes
RB and P53 are prototypes of a class of proteins encoded by	Oncogenes	proto oncogenes	Ras genes	Tumour suppressor genes	Tumour suppressor genes
Retro viruses are --------	RNA viruses	DNA viruses	Papova virus	Papiloma viruses	RNA viruses

Tumour viruses are usually -------	RNA viruses	DNA viruses	RNA and DNA viruses	Si RNA	RNA and DNA viruses
An enzyme which is capable of copying genomic RNA into DNA	DNA a	DNA b	Reverse transcriptase	DNA d	Reverse transcriptase
-------- cells have the ability to metastasis	Normal cells	benign cells	malignant cells	Viral cells	malignant cells
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
Malignant tumours are classified as ------ if they derive from endoderm or ectoderm	Sarcoma	carcinoma	lymphoma	leukemia	carcinoma

Malignant tumours are classified as ---------- if they derive from mesoderm	Sarcoma	carcinoma	lymphoma	leukemia	Sarcoma
SV 40 and polyoma are example for-----	Retro virus	papova virus	RNA virus	Papilloma virus	papova virus
Controlled cell death is called----------	Apoptosis	metastasis	autophagy	Sarcoma	Apoptosis
The src enzyme is -------- specific protein kinase	Tyrosine	serine	tryptophan	proline	Tyrosine
Solid malignant tumors of lymphoid tissues is called	Carcinoma	sarcoma	leukemia	lymphoma	lymphoma

F+ bacteria can construct which of the following that allow the bacteria to join together to transfer genes?	gap junctions	pili	connecting channels	porins	pili

UNIT V

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed University *established Under Section 3 of UGC Act 1956*)

DEPARTMENT OF BIOCHEMISTRY

I BSc Biochemistry

First Semester

CELL BIOLOGY (17BCU102)

Enable | Enlighten | Enrich
(Deemed to be University)
(Under Section 3 of UGC Act 1956)

POSSIBLE QUESTIONS

Unit I

Two mark questions

1. Draw the structure of prokaryotic
2. Differentiate archaea and eubacteria
3. Draw the structure of animal cell
4. Draw the structure of plant cells
5. How *S,cerevisiae* can acts as cell mode?l
6. Write the composition of plasma membrane:
7. Draw the structure of Fluid mosaic model of plasma membrane
8. Write the principle of Light microscopy
9. What is the principle of Phase contrast microscopy?
10. What is Fluorescence?
11. Note on Confocal microscopy,
12. Give the principle of Electron microscopy,
13. What is the principle for FACS.
14. On what basis cell organelles are separated

Essay type questions

1. Draw the structure of animal and plant cell and brief the role of various organelles

2. Explain the following
   (i) Light microscope
   (ii) Fluid mosaic model of plasma membrane
3. With neat diagram brief the role various organelles of eukaryotic cells
4. Explain the following
   (i) Fluorescence microscopy (ii) FACS
5. Explain the composition and fluid mosaic model of plasma membrane
6. Describe the principle, instrumentation and applications of electron microscope
7. Draw the structure and label the parts of following
   i) Plant cell (iii) Prokaryotic cell
8. Explain the instrumentation of the following
   (i) Light microscope
   (ii) Phase contrast microscope

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POSSIBLE QUESTIONS

Unit II

Two mark questions

1. Draw the structure of nuclear envelope,
2. Illustrate with neat diagram about the nuclear pore complex.
3. Selective transport of proteins to nucleus.
4. Structure of ER
5. Note on signal sequences
6. How lipid synthesis occur in SER?
7. Export of proteins from ER
8. Export of lipids from ER.
9. Structure and functions of mitochondria
10. Structure and functions of chloroplast
11. Give the importance of peroxisomes
12. Regulation of nuclear protein import and export
13. Add note on Zellweger syndrome.

Essay type questions

1. Explain the structure of nuclear envelope and transport across it
2. Describe the process of protein targeting to various organelles
3. Explain the following
   (i)Nuclear envelope (ii) Targeting proteins to ER
4. Explain the following
   (i) Structure of mitochondria (ii) Lipid biosynthesis in SER
5. Describe the protein targeting to endoplasmic reticulum
6. Explain the structure nucleopore complex and transport across it
7. Explain the following with diagram
   i. Struture of nucleopore complex
   ii. Chloroplast struture
8. Describe the process protein targeting to ER with neat diagram
9. With neat diagram explain the nucleopore complex and its role
10. How the transport of proteins to endoplasmic reticulum occurs? Explain with neat diagram

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POSSIBLE QUESTIONS

Unit IV

Two mark questions

1. Note on the structure of actin filaments
2. Explain the assembly of actin filaments
3. Give notes on organization of actin filaments
4. Add note on muscle myosin
5. What is the significance of non muscle myosin
6. Write the classification of intermediary filaments
7. Draw the structure of axoneme
8. Explain the ciliary movement
9. Draw the structure of flagella
10. Add note on treadmilling

Essay type questions

11. Explain the structure, assembly and organization of actin filaments
12. With neat diagram explain the meiotic cell division
13. Describe the structure, assembly and organization of intermediary filaments
14. Describe the
15. Explain the assembly, organization and movement of cilia and flaglla
16. Explain the classification and assembly of intermediary filaments
17. With neat diagram explain the assembly of actin and tubulin
18. Narrate about cilia and flagella
19. Explain the following
    (i) Actin filament- assembly
    (ii) Intermediate filament organisation

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POSSIBLE QUESTIONS

Unit III

Two mark questions

1. Draw the structure of Golgi complex
2. Why Golgi complex is called as traffic police man of cell
3. What is protein sorting?
4. What is the role of chaperon in protein folding
5. Add note on vesicular transport
6. Give short note on Coat proteins
7. Why lysosomes are called as suicide bag of cell?
8. Brief about the acid hydrolases
9. Differentiate phagocytosis and autophagy
10. Write the importance of autophagy

Essay type questions

1. With neat diagram explain the protein sorting and export from golgi.
2. Explain the following
   (i) Lysosomal acid hydrolases
   (ii) Protein export from mitochondria
3. Differentiate the processes phagocytosiis and autophagy with neat diagram
4. With neat diagram explain the import and sorting of chloroplast proteins
5. Describe the acid hydrolases of lysosomes
6. Explain the lipid and polysaccharide metabolism in golgi
7. Explain the following
   (i) Protein import into thylakoid lumen of chloroplast
   (ii) Autophagy
8. Which organelle is stated suicide bag of cell? Why? Narrate its function
9. Explain the following
   (i) Protein import to mitochondria
   (ii) Protein sorting in golgi

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POSSIBLE QUESTIONS

Unit V

Two mark questions

1. Draw the structure of prokaryotic cell wall
2. Write the composition of prokaryotic cell wall
3. Illustrate the structure of plant cell wall with neat diagram
4. Write note on cell matrix proteins
5. Give an example to Cell matrix interaction
6. Illustrate the following with neat diagram
    - Tight junction
    - Gap Junction
    - Adheren Junction
    - Desmosomes
    - Hemisdesmosomes
    - Focal adhesions
    - Plasmadesmata
7. Draw the structure Cell cycle
8. Add note on cell cycle check points
9. Define apoptosis
10. Differentiate apoptosis and necrosis
11. Name the various stages of meiotic cell division
12. Name the various stages of mitotic cell division
13. Illustrate the importance of metaphase
14. Give the significance of anaphase
15. List the features of transformed cell
16. Write the significance of meiotic cell division

Essay type questions

1. Explain the following
    (i) Cell matrix proteins
    (ii) Tight and Gap junctions
2. Explain the following
    (i) Cell cycle
    (ii) Apoptosis
3. Explain the following
    (i) Tight junctions

(ii) Extra cellular matrix proteins

4. Explain the following
   (i) Cell cycle
   (ii) Properties of transformed cell
5. Explain the following
   (i)Prokaryotic cell wall
   (i) Gap junctions
6. Explain the following
   (i) Mitosis
   (ii) Cell cycle
7. Explain the eukaryotic cell wall with neat diagram
8. Explain the following
   (i) Apoptosis
   (ii) Cell cycle check points
9. Explain the following
   (i) Cell matrix proteins
   (ii) Plant cell wall
10. Describe the process cell division
11. Explain the mitotic cell division
12. Explain the meiotic cell division

Reg. No. _____

[17BCU102]

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DEPARTMENT OF BIOCHEMISTRY AND BIOINFORMATICS

I B.Sc., Biochemistry - First Semester
I-Internal Examination, July 2017
17BCU102 – CELL BIOLOGY

Time: 2 hrs **Maximum: 50 marks**

PART A (20 x 1 = 20 marks)
Answer ALL questions

1. Prokaryotic cells lack
a. Cell wall b. cytoplasm
c. DNA d. Nucleus

2. Important feature of the plant cell is the presence of
a. Microsome b. Cytoplasm c. Cell wall d. Mitochondria

3. Simple non-nucleated cells are --------
a. Prokaryotic cells b. Eukaryotic cells c. Stem cells d. Blood cells

4. Integral proteins are bound to membrane by ____
a. Ionic bond b. Covalent bond c. Hydrogen bond d. Disulphide bond

5. The fluid model was introduced by
a, S.J Singer and J.D Robertson b. J.N Robertson
c. Jacob and Monad d. S.J Singer and G.Nicolson.

6. Among the following which is eukaryotic unicellular organism
a. E.coli b. S.typhi c. S.cerevisiae d. C.elegans

7. Glycocalyx is
a. protein coat of the cell b. carbohydrate coat of the cell
c. lipid coat of the cell d. carrier protein cell

8. Cells that are engaged in protein synthesis will have well developed
a. Rough Endoplasmic Reticulum b. Smooth Endoplasmic Reticulum
c. Glyoxysomes d. Peroxysomes

9. The golgi apparatus is important for
a. protein synthesis b. DNA synthesis
c. packaging and secretion of proteins d. RNA synthesisis

10.Bilayer fluidity mainly determined by
a. Phospholipid b. protein c. Cholesterol d. Glycolipid.

11. The size of the prokaryotic cell is
a. 1-10 µm b. 1µ to 2 mm c 10µ to 100 mm d. 1mm to 3 mm

12. ---------- are described as amphipathic in plasma membrane
a. lipids b .proteins c. steroids d. carbohydrates

13. When the power of ocular lens is 10 X and objective lens is 20 X, the magnification is
a. 30 times b. 20 times c. 200 times d. 2000 times

14. Kind of microscope which is used to study internal structure of cells is
a. scanning electron microscope b. transmission electron microscope
c. light microscope d. Fluorescent microscope

15. The strength of magnification of oil immersion lens is
a. 20X b. 100X c. 50X d. 40X

16. Single cells can be isolated using the following technique
a. Fluorescence activated cell sorting b. Polymerase chain reaction
c. Thin layer chromatography d. Agarose gel electrophoresis

17. The channel on nucleopore is calles as
a. Nucleolus b. Nucleoplasm c. Nucleopore complex d. Nuclei

18. Nuclear laminin is located at _____ nuclear membrane
a) a) Outer surface b) inner surface c) Interspace d) Cytoplasmic

19.T he diameter of nucleaopore complex that transport small molecules is
a) 5nm b) 10nm c) 12nm d) 15nm

20. RNA and protein are transported through the nuclearpore complex by
a) Facilitated diffusion b) Simple Diffusion c) Active transport d) Osmosis

Class: I BSc Biochemistry.

Subject: Cell biology.

Subject code: 1TBCH102.

No of copies: 65

K Pradeep

PART B (3 x 2 = 6 marks)
Answer ALL questions

21. Draw the prokaryotic cell structure and label its parts

22. Add notes on composition of plasma membrane

23. Give short note on nuclear envelope

PART C (3 x 8 = 24 marks)
Answer ALL questions

24. a) Describe the eukaryotic cell structure with neat diagram

Or

b) Explain in detail on cells as experimental models

25. a) With neat diagram explain the facts of light microscope

Or

b) Explain the principle, instrumentation and applications of Fluorescent microscopy

26. a) Discuss in detail about targeting of protein to endoplasmic reticulum

Or

b) Draw the structure of nucleopore complex and add note on transport across it

****

Reg. No. _____

[17BCU102]

KARPAGAM ACADEMY OF HIGHER EDUCATION

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DEPARTMENT OF BIOCHEMISTRY

I B.Sc., Biochemistry - First Semester

I-Internal Examination, **August'** 2017

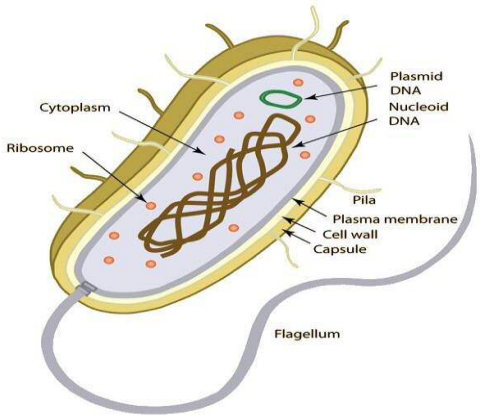
17BCU102 – CELL BIOLOGY

Time: 2 hrs Maximum: 50 marks

1. Nucleus
2. Cell wall
3. Prokaryotic cells
4. Covalent bond
5. S.J Singer and G.Nicolson
6. Chloroplast
7. Protein coat of the cell
8. RER
9. packaging and secretion of proteins
10. Cholesterol
11. 1-**10 μm**
12. Lipids
13. 200 times
14. transmission electron microscope
15. 100X
16. Fluorescence activated cell sorting
17. Nucleopore complex
18. inner surface
19. 10nm
20. Active transport

PART B (3 × 2 = 6)

21.

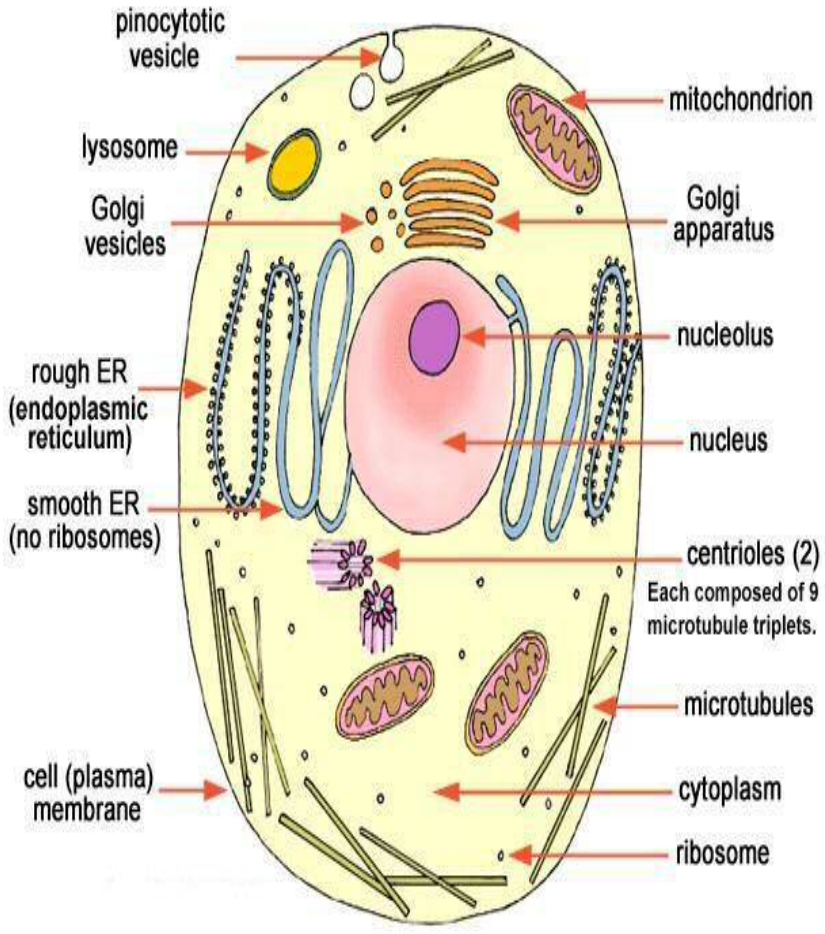


22. The plasma membrane is composed of a phospholipid bilayer, which is two layers of phospholipids back-to-back. Phospholipids are lipids with a phosphate group attached to them. The phospholipids have one head and two tails. The head is polar and hydrophilic, or water-loving. The tails are nonpolar and hydrophobic, or water-fearing.

23 The nuclear envelope (or perinuclear cisterna) encloses the DNA and defines the nuclear compartment of interphase and prophase nuclei.

PART C (3 × 8 = 24)

24. a)



b. The evolution of present-day cells from a common ancestor has important implications for cell and molecular biology as an experimental science. Because the fundamental properties of all cells have been conserved during evolution, the basic principles learned from experiments performed with one type of cell are generally applicable to other cells. Several different kinds of cells and organisms are commonly used as experimental models to study various aspects of cell and molecular biology.

1.*E.coli*

Because of their comparative simplicity, prokaryotic cells (bacteria) are ideal models for studying many fundamental aspects of biochemistry and molecular biology. The most thoroughly studied species of bacteria is *E. coli*, which has used for investigation of the basic mechanisms of molecular genetics. Most of our present concepts of molecular

biology—including our understanding of DNA replication, the genetic

code, gene expression, and protein synthesis—derive from studies of this humble bacterium.

E. coli has been especially useful to molecular biologists because of both its relative simplicity. The genome of *E. coli*, for example, consists of approximately 4.6 million base pairs and encodes about 4000 different proteins. The human genome is nearly a thousand times more complex (approximately 3 billion base pairs) and encodes about 100,000 different proteins

Molecular genetic experiments are further facilitated by the rapid growth of *E. coli* under well-defined laboratory conditions. Depending on the culture conditions, *E. coli* divide every 20 to 60 minutes. Because bacterial colonies containing as many as 10^8 cells can develop overnight, selecting genetic variants of an *E.coli* strain—for example, mutants that are resistant to an antibiotic, such as penicillin—is easy and rapid. The ease with which such mutants can be selected and analyzed was critical to the success of experiments that defined the basic principles of molecular genetics.

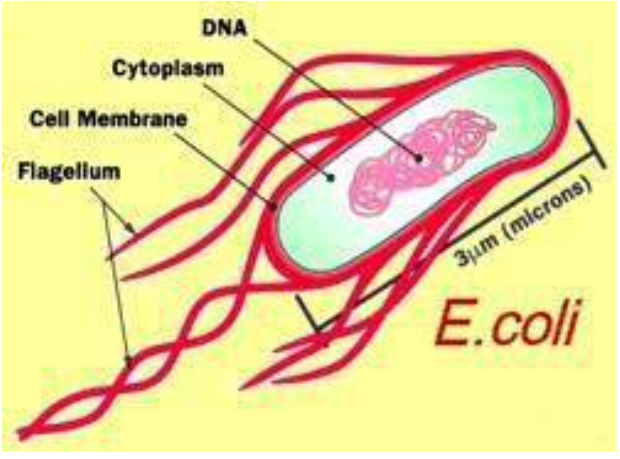
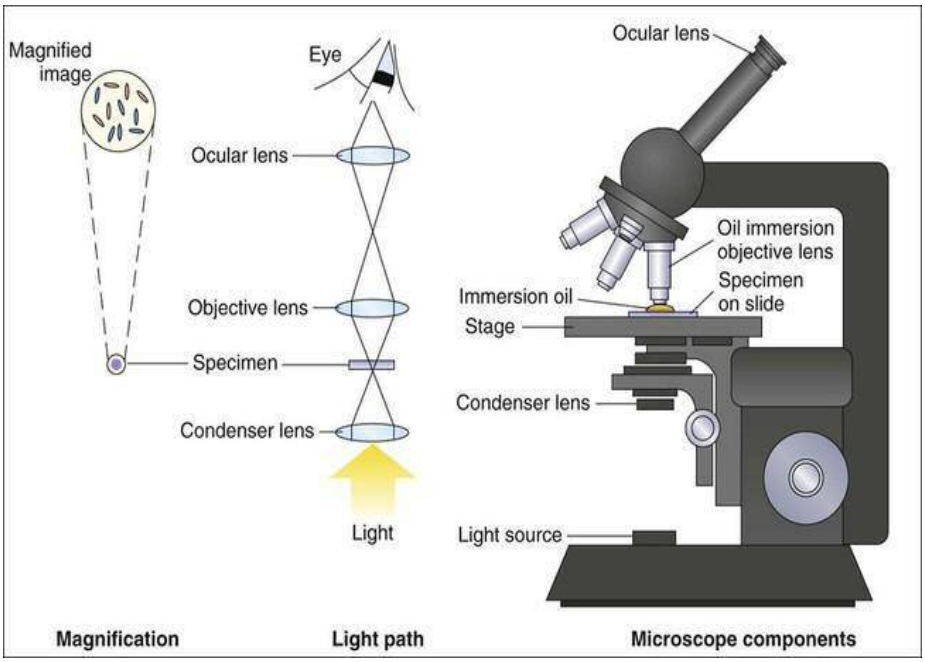


Fig: Typical *E.coli* cell

25. a



Principles of Light Microscopy

For light microscopy, visible light is passed through the specimen and then through a series of lenses that bend the light in a manner that results in magnification of the organisms present in the specimen. The total magnification achieved is the product of the lenses used.

Instrumentation

Parts of a Microscope

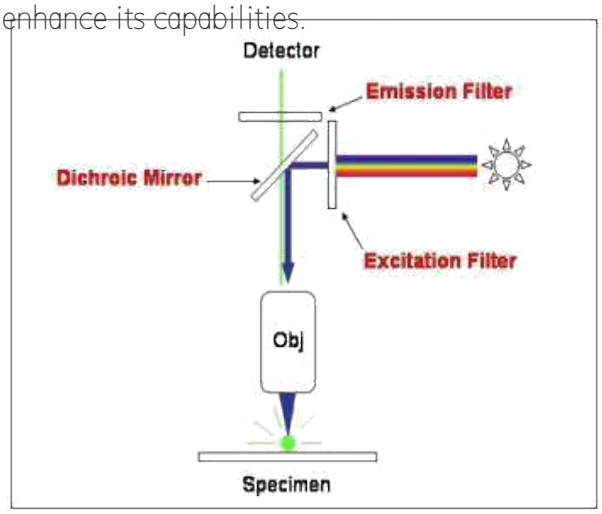
It consists of mainly three parts:

- ✓ Mechanical part - base, c-shaped arm and stage.
- ✓ Magnifying part - objective lens and ocular lens.
- ✓ Illuminating part - sub stage condenser, iris diaphragm, light source

Objective lens:

1. There are three types of objective lens:
2. 10X (Low power objective lens).
3. 40X (High power objective lens).
4. 100X (Oil immersion objective lens).
5. These objective lenses are fitted on to the revolving nose piece. The working distance of an objective is defined as the distance between the front surface of the lens and the cover glass surface or the specimen when it is in sharp focus.

b. Fluorescence microscope is much the same as a conventional light microscope with added features to enhance its capabilities.



- The conventional microscope uses visible light (400-700 nanometers) to illuminate and produce a magnified image of a sample.
- A fluorescence microscope, on the other hand, uses a much higher intensity light source which excites a fluorescent species in a sample of interest. This fluorescent species in turn emits a lower energy light of a longer wavelength that produces the magnified image instead of the original light source.

Principle

The specimen is illuminated with light of a specific wavelength (or wavelengths) which is absorbed by the fluorophores, causing them to emit light of longer wavelengths (i.e., of a different color than the absorbed light). The illumination light is separated from the much weaker emitted fluorescence through the use of a spectral emission filter.

In most cases the sample of interest is labeled with a fluorescent substance known as a fluorophore and then illuminated through the lens with the higher energy source. The illumination light is absorbed by the fluorophores (now attached to the sample) and causes them to emit a longer lower energy wavelength light. This fluorescent light can be separated from the surrounding radiation with filters designed for that specific wavelength allowing the viewer to see only that which is fluorescing.

Instrumentation

Light Source

Fluorescence microscopy requires intense, near-monochromatic light sources. Four main types of light source are used, including xenon arc lamps or mercury-vapor lamps , lasers, and high-power LEDs. Lasers are most widely used for more complex fluorescence microscopy techniques like confocal microscopy

Applicatrions

These microscopes are often used for -

* Imaging structural components of small specimens, such as cells
* Conducting viability studies on cell populations
* Imaging the genetic material within a cell (DNA and RNA)
* Viewing specific cells within a larger population with techniques such as FISH

26. a. George Palade and his colleagues in the 1960s were the first to demonstrate the role of endoplasmic reticulum in protein secretion. The defined pathway taken by secreted protein is: Rough ER - Golgi - secretory vesicles- cell exterior. The entrance of proteins into the ER represents a major branch point for the traffic of proteins within eukaryotic cells. In mammalian cells most proteins are transferred into the ER while they are being translated on membrane bound ribosomes. Proteins that are destined for secretion are then targeted to the endoplasmic reticulum by a signal sequence (short stretch of hydrophobic amino acid residues) at the amino terminus of the growing polypeptide chain. The signal sequence is K/HDEL which is Lys/His-Asp-Glu-Leu. This signal peptide is recognized by a signal recognition particle consisting of six polypeptides and srpRNA. The SRP binds the ribosome as well as the signal sequence, inhibiting further translation and targeting the entire complex (the SRP, ribosome, and growing polypeptide chain) to the rough ER by binding to the SRP receptor on the ER membrane. Binding to the receptor releases the SRP from both the ribosome and the signal sequence of the growing polypeptide chain. The ribosome then binds to a protein translocation complex in the ER membrane, and the signal sequence is inserted into a membrane channel or translocon with the aid of GTP. Transfer of the ribosome mRNA complex from the SRP to the translocon opens the gate on the translocon and allows translation to resume, and the growing polypeptide chain is transferred directly into the translocon channel across the ER membrane as translation proceeds.

Smooth endoplasmic reticulum and lipid synthesis:

Hydrophobic lipids are synthesized in the ER and then they are then transported from the ER to their ultimate destinations either in vesicles or by carrier proteins. Phospholipids are synthesized in the cytosolic side of the ER membrane from water-soluble cytosolic precursors. Other lipids that are synthesized in the ER are cholesterol and ceramide which is further converted to either glycolipids or sphingomyelin in the golgi apparatus. Smooth ER are also the site for the synthesis of the steroid hormones from cholesterol. Thus steroid producing cells in the testis and ovaries are abundant in smooth ER.

Common functions of SER and RER:

1. The endoplasmic reticulum provides an ultrastructural skeletal framework to the cell and gives mechanical support to the colloidal cytoplasmic martix.

2. The exchange of molecules by the process of osmosis, diffusion and active transport occurs through the membranes of endoplasmic reticulum. The ER membrane has permeases and carriers.

3. The endoplasmic membranes contain many enzymes which perform various synthetic and

metabolic activities and provides increased surface for various enzymatic reactions.

4. The
5. reticulum acts as an intracellular circulatory or transporting system. Various secretory products of granular endoplasmic reticulum are transported to various organelles as follows: Granular ER– agranular ER – Golgi membrane–lysosomes, transport vesicles or secretory granules. Membrane flow may also be an important mechanism for carrying particles, molecules and ions into and out of the cells. Export of RNA and nucleoproteins from nucleus to cytoplasm may also occur by this type of flow.

6. The ER membranes are found to conduct intra-cellular impulses. For example, the sarcoplasmic reticulum transmits impulses from the surface membrane into the deep region of the muscle fibres.

7. The sarcoplasmic reticulum plays a role in releasing calcium when the muscle is stimulated and actively transporting calcium back into the sarcoplasmic reticulum when the stimulation stops and the muscle must be relaxed.

Functions: (Concised)

1. It gives mechanical support to the colloidal cytoplasmic matrix
2. It plays a major role in the inter-connection of cells through plasmadesmata
3. It helps in the regulation of entry and exit of materials
4. The ER acts as a circulatory or transporting system for various substances
5. The ER membrane are found to conduct intracellular impulses. Example the sarcoplasmic reticulum transmits impulses from the surface membranes into the deep region of muscle fibres.
6. It determines the plane of cell division
7. It plays an important role in the formation of nuclear envelope after each nuclear Division
8. The ER protects the cell by the toxic effects of various substances by a process of detoxification
9. ER provides increased surface for various enzymatic reactions

b. Nuclear pores appear circular in surface view and have a diameter between 10nm to 100 nm. Previously it was believed that a diaphragm made of amorphous to fibrillar material extends across each pore limiting free transfer of material. Such a diaphragm called annulus has been observed in animal cells, but lack in plant cells. Recent electron microscopic studies have revealed that a nuclear pore has far more complex structure, so it is called nuclear pore complex with an estimated molecular weight of 50 to 100 million daltons. Negative staining techniques have demonstrated that pore complexes have an eight-fold or octagonal symmetry.

Nuclear Pore density: In nuclei of mammals it has been calculated that nuclear pores account for 5 to 15 per cent of the surface area of the nuclear membrane. In amphibian oocytes, certain plant cells and protozoa, the surface occupied by the nuclear pores may be as high as 20 to 36 per cent.

Arrangement of nuclear pores on nuclear envelope: In somatic cells, the nuclear pores are evenly or randomly distributed over the surface of nuclear envelope. However, pore arrangement in other cell types is not random but rather range from rows (spores of *Eqisetum*) to Clusters (oocytes of *Xenopus laevis*) to hexagonal (Malpighian tubules of leaf hoppers) packing order. Nucleo-cytoplasmic traffic: Quite evidently there is considerable trafficking across the nuclear envelope during interphase. Ions, nucleotides and structural, catalytic and regulatory proteins are imported from the cytosol (cytoplasmic matrix); mRNA, tRNA are exported to the cytosol (cytoplasmic matrix). However, one of the main functions of the nuclear envelope is to prevent the entrance of active ribosomes into the nucleus.

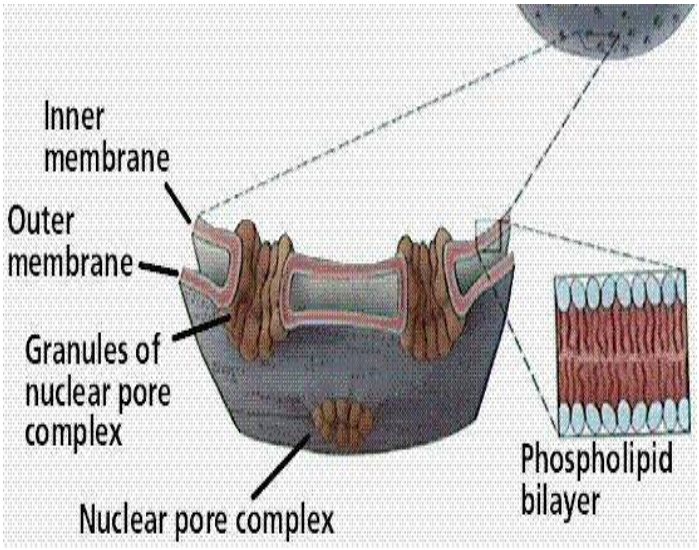


Figure : An illustration of the nuclear membrane

Reg. No. _____

[17BCU102]

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed University *established Under Section 3 of UGC Act 1956*)

DEPARTMENT OF BIOCHEMISTRY

I B.Sc., Biochemistry - First Semester

II-Internal Examination, August' 2017

17BCU102 – CELL BIOLOGY

Date : 19.08.2017

Time: 2 hrs Maximum: 50 marks

PART A (20 x 1 = 20 marks)

Answer ALL questions

1. Microsomes
2. Microbodies
3. Autophagy
4. Man-6-phosphate
5. 22
6. HSP60
7. Thylakoid membrane
8. Plasmologens
9. Carbohydrates
10. Endoplasmic reticulum
11. . Mitochondria
12. H_2O_2
13. Virgin lysosomes
14. tubulin
15. KKXX
16. acidic PH

17. Cristae

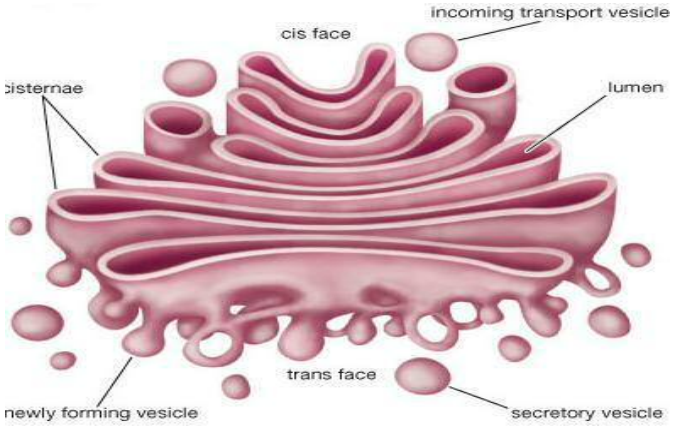
18. Group translocator

19. Chloroplast

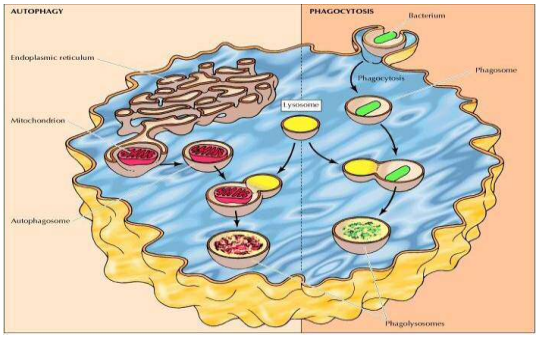
20. Proton

PART B (3 x 2 = 6 marks)
Answer ALL questions

21. Add note on structure and organisation of golgi complex



22. Differentiate autophagy and phagocytosis



23. Add a note on Zellweger syndrome.

Zellweger syndrome is an inherited human disease, in which a defect in importing proteins into peroxisomes leads to a severe peroxisomal deficiency. These individuals, whose cells contain ―empty‖ peroxisomes, have severe abnormalities in their brain, liver, and kidneys, and they die soon after birth. One form of this disease has been shown to be due to a mutation in the gene encoding a peroxisomal integral membrane protein, the peroxin Pex2, involved in protein import. A milder inherited peroxisomal disease is caused by a defective receptor for the N-terminal import signal.

PART C (3 x 8 = 24 marks)
Answer ALL questions

24. a) With neat diagram explain the protein sorting and export from golgi.

PROTEIN SORTING IN GOLGI

Proteins, as well as lipids and polysaccharides, are transported from the Golgi apparatus to their final destinations through the secretory pathway. This involves the sorting of proteins into different kinds of transport vesicles, which bud from the *trans* Golgi network and deliver their contents to the appropriate cellular locations (Figure). Some proteins are carried from the Golgi to the plasma membrane by a constitutive secretory pathway, which accounts for the incorporation of new proteins and lipids into the plasma membrane, as well as for the continuous secretion of proteins from the cell. Other proteins are transported to the cell surface by a distinct pathway of regulated secretion or are specifically targeted to other intracellular destinations, such as lysosomes in animal cells or vacuoles in yeast.

Proteins that function within the Golgi apparatus must be retained within that organelle, rather than being transported along the secretory pathway. All of the proteins retained within the Golgi complex are associated with the Golgi membrane rather than being soluble proteins within the lumen. The signals responsible for retention of some proteins within the Golgi have been localized to their transmembrane domains, which retain proteins within the Golgi apparatus by preventing them from being packaged in the transport vesicles that leave the *trans* Golgi network. In addition, signals in the cytoplasmic tails of some Golgi proteins mediate the retrieval of these proteins from subsequent compartments along the secretory pathway.

However, some cells also possess a distinct regulated secretory pathway in which specific proteins are secreted in response to environmental signals. Examples of regulated secretion include the release of hormones from endocrine cells, the release of neurotransmitters from neurons, and the release of digestive enzymes from the pancreatic acinar cells.

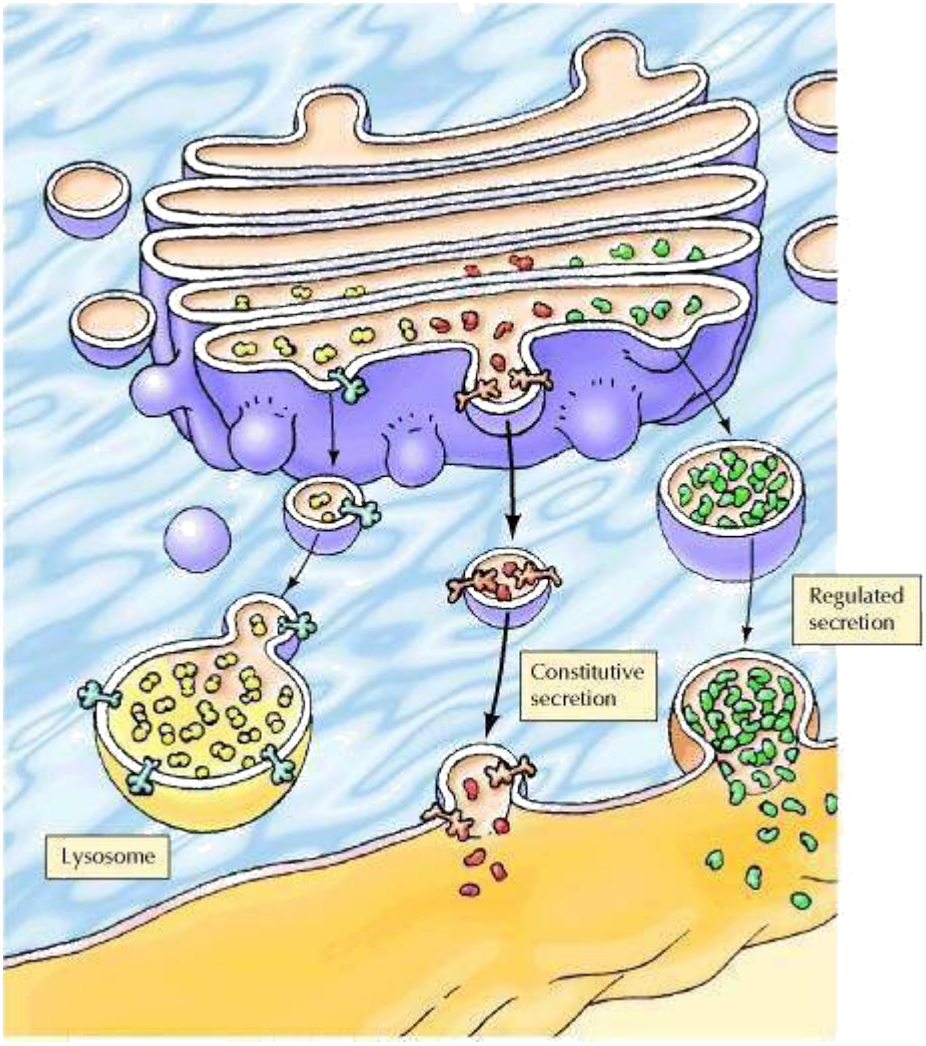


Figure :Transport from the Golgi apparatus

Fig: Proteins are sorted in the *trans* Golgi network and transported in vesicles to their final destinations. In the absence of specific targeting signals, proteins are carried to the plasma membrane by constitutive secretion. The constitutive secretory pathway, which operates in all cells, leads to continual unregulated protein secretion. Alternatively, proteins can be targeted to other destinations, such as lysosomes or regulated secretion from the cells.

Proteins are sorted into the regulated secretory pathway in the *trans* Golgi network, where they are packaged into specialized secretory vesicles. These secretory vesicles, which are larger than other transport vesicles, store their contents until specific signals direct their fusion with the plasma membrane. For example, the digestive enzymes produced by pancreatic acinar cells are stored in secretory vesicles until the presence of food in the stomach and small intestine triggers their secretion.

Or

a) Explain the following mechanism of vesicuar transport in golgi

Protein transport from Golgi - The Mechanism of Vesicular Transport

Transport vesicles play a central role in the traffic of molecules between different membrane-enclosed compartments of the secretory pathway.Vesicles are similarly involved in the transport of materials taken up at the cell surface. Vesicular transport is

thus a major cellular activity, responsible for molecular traffic between a variety of specific membrane-enclosed compartments. Three types of vesicle were found. They are,

(i) Transitional vesicles are small membrane limited vesicles which are form as blebs from the transitional ER to migrate and converge to cis face of Golgi, where they join together to form new cisternae.

(ii) Secretory vesicles are varied-sized membrane-limited vesicles which discharge from margins of cisternae of Golgi. They, often, occur between the maturing face of Golgi and the plasma membrane.

(iii) Clathrin-coated vesicles are spherical protuberances, about 50 μm in diameter and

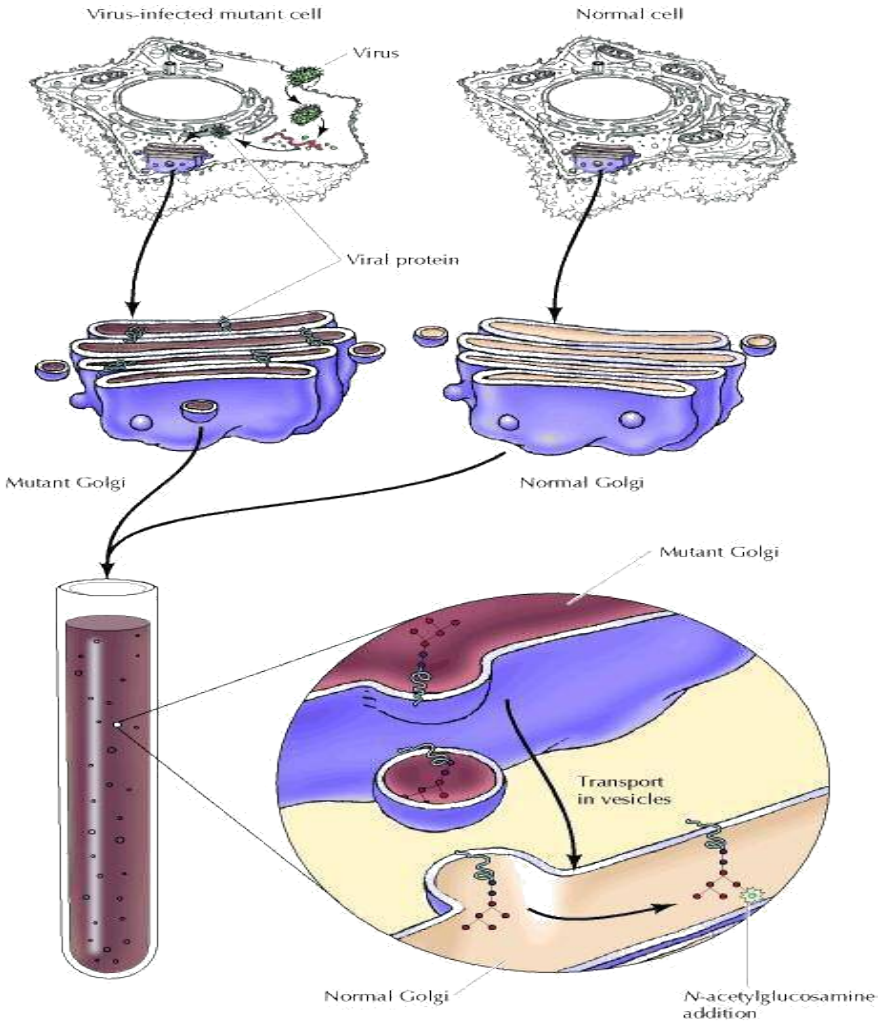
with a rough surface. They are found at the periphery of the organelle, usually at the ends of single tubules, and are morphologically quite distinct from the secretory vesicles. The clathrin-coated vesicles are known to play a role in intra-cellular traffic of membranes and of secretory products.

The selectivity of such transport is therefore key to maintaining the functional organization of the cell. For example, lysosomal enzymes must be transported specifically from the Golgi apparatus to lysosomes—not to the plasma membrane or to the ER. so the specificity of transport is based on the selective packaging of the intended cargo into vesicles that recognize and fuse only with the appropriate target membrane.

EXPERIMENTAL APPROACH TO UNDERSTAND THE VESICULAR TRANSPORT

Progress toward elucidating the molecular mechanisms of vesicular transport has been made by three distinct experimental approaches: (1) isolation of yeast mutants that are defective in protein transport and sorting; (2) reconstitution of vesicular transport in cell-free systems; and (3) biochemical analysis of synaptic vesicles, which are responsible for the regulated secretion of neurotransmitters by neurons.

Golgi stacks prepared from a virus-infected mutant cell line unable to catalyze the addition of *N*-acetylglucosamine to *N*-linked oligosaccharides are mixed with Golgi stacks from a normal cell line. Because the mutant cell line is infected with a virus, the proteins it synthesizes can be specifically detected. Transport of these proteins to normal Golgi stacks is signaled by the addition of *N*-acetylglucosamine.


Virus-infected mutant cell
Normal cell
Virus
Viral protein
Mutant Golgi
Normal Golgi
Mutant Golgi
Transport
in vesicles
Normal Golgi
N-acetylglucosamine
addition

25. a) Explain the import and sorting of chloroplast proteins

Although chloroplasts encode more of their own proteins than mitochondria, about 90% of chloroplast proteins are still encoded by nuclear genes. As with mitochondria, these proteins are synthesized on cytosolic ribosomes and then imported into chloroplasts as completed polypeptide chains. They must then be sorted to their appropriate location within chloroplasts—an even more complicated task than protein sorting in mitochondria, since chloroplasts contain three separate membranes that divide them into three distinct internal compartments.

Protein import into chloroplasts generally resembles mitochondrial protein import (Figure). Proteins are targeted for import into chloroplasts by N-terminal sequences of 30 to 100 amino acids, called transit peptides, which direct protein translocation across the two membranes of the chloroplast envelope and are then removed by proteolytic cleavage.

The transit peptides are recognized by the translocation complex of the chloroplast outer member (the Toc complex), and proteins are transported through this complex across the membrane. They are then transferred to the translocation complex of the inner membrane (the Tic complex) and transported across the inner membrane to the stroma.

As in mitochondria, molecular chaperones on both the cytosolic and stromal sides of the envelope are required for protein import, which requires energy in the form of ATP. In contrast to the presequences of mitochondrial import, however, transit peptides are not positively charged and the translocation of polypeptide chains into chloroplasts does not require an electric potential across the membrane.

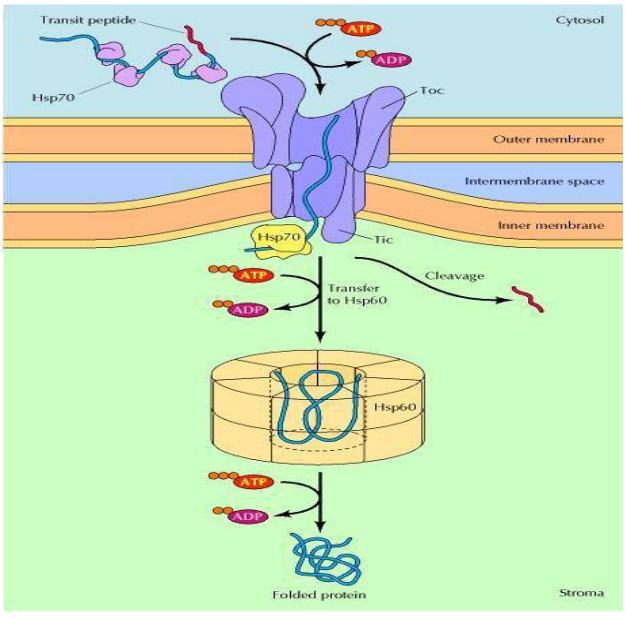


Figure:Protein import into the chloroplast stroma

Proteins are targeted for import into chloroplasts by a transit peptide at their amino terminus. The transit peptide directs polypeptide translocation through the Toc complex in the chloroplast outer membrane and the Tic complex in the chloroplast inner membrane. This peptide is then removed by proteolytic cleavage within the stroma. Both cytosolic and chloroplast chaperones (Hsp60 and Hsp70) are required for protein import.

Proteins incorporated into the thylakoid lumen are transported to their destination in two steps (Figure).

They are first imported into the stroma, as already described, and are then targeted for translocation across the thylakoid membrane by a second hydrophobic signal sequence, which is exposed following cleavage of the transit peptide. The hydrophobic signal sequence directs translocation of the polypeptide across the thylakoid membrane and is finally removed by a second proteolytic cleavage within the lumen.

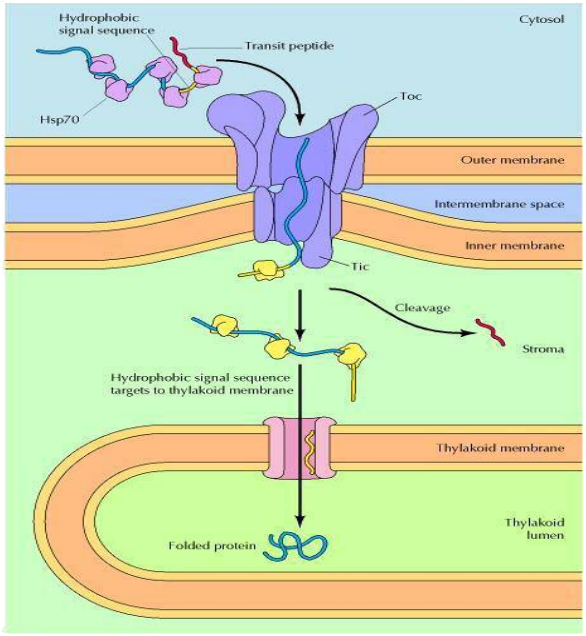


Figure: Import of proteins into the thylakoid lumen

Proteins are imported into the thylakoid lumen in two steps. The first step is import into the chloroplast stroma, as illustrated in Figure . Cleavage of the transit peptide then exposes a second hydrophobic signal sequence, which directs protein translocation across the thylakoid membrane.

The pathways of protein sorting to the other four compartments of chloroplasts—the inner and outer membranes,thylakoid membrane, and intermembrane space—are less well established. As with mitochondria, proteins appear to be inserted directly into the outer membrane of the chloroplast envelope. In contrast, proteins destined for either the thylakoid membrane or the inner membrane of the chloroplast envelope are initially targeted for import into the stroma by N-terminal transit peptides. Following cleavage of the transit peptides, these proteins are then targeted for insertion into the appropriate membrane by other sequences, which are not yet well characterized. Finally, neither the sequences that target proteins to the intermembrane space nor the pathways by which they travel to that destination have been identified.

Or

b) Describe about the lysosomal acid hydrolases

Lysosomes contain about 50 different degradative enzymes that can hydrolyze proteins, DNA, RNA, polysaccharides, and lipids. Mutations in the genes that encode these enzymes are responsible for more than 30 different human genetic diseases, which are called lysosomal storage diseases because undegraded material accumulates within the lysosomes of affected individuals. Most of these diseases result from deficiencies in single lysosomal enzymes. For example, **Gaucher's disease (the most common of these disorders) results from a** mutation in the gene that encodes a lysosomal enzyme required for the breakdown of glycolipids. An intriguing exception is I-cell disease, which is caused by a deficiency in the enzyme that catalyzes the first step in the tagging of lysosomal enzymes with mannose-6-phosphate in the Golgi apparatus. The result is a general failure of lysosomal enzymes to be incorporated into lysosomes.

All of the lysosomal enzymes are acid hydrolases, which are active at the acidic pH (about 5) that is maintained within lysosomes but not at the neutral pH (about 7.2) characteristic of the rest of the cytoplasm. The requirement of these lysosomal hydrolases for acidic pH provides double protection against uncontrolled digestion of the contents of the cytosol; even if the lysosomal membrane were to break down, the released acid hydrolases would be inactive at the neutral pH of the cytosol. The following table gives a clear picturisation types and functions of lysosomal acid hydrolases

To maintain their acidic internal pH, lysosomes must actively concentrate H^+ ions (protons). This is accomplished by a proton pump in the lysosomal membrane, which actively transports protons into the lysosome from the cytosol. This pumping requires

expenditure of energy in the form of ATP hydrolysis, since it maintains approximately a hundredfold higher H^+ concentration inside the lysosome.

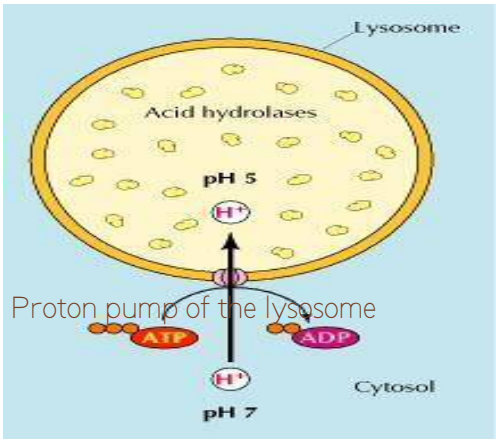


Figure: Proton pump of the lysosome

26. a) Describe the process of export of proteins and lipids from ER and into ER.

George Palade and his colleagues in the 1960s were the first to demonstrate the role of endoplasmic reticulum in protein secretion. The defined pathway taken by secreted protein is: Rough ER - Golgi - secretory vesicles- cell exterior. The entrance of proteins into the ER represents a major branch point for the traffic of proteins within eukaryotic cells. In mammalian cells most proteins are transferred into the ER while they are being translated on membrane bound ribosomes. Proteins that are destined for secretion are then targeted to the endoplasmic reticulum by a signal sequence (short stretch of hydrophobic amino acid residues) at the amino terminus of the growing polypeptide chain. The signal sequence is K/HDEL which is Lys/His-Asp-Glu-Leu. This signal peptide is recognized by a signal recognition particle consisting of six polypeptides and srpRNA. The SRP binds the ribosome as well as the signal sequence, inhibiting further translation and targeting the entire complex (the SRP, ribosome, and growing polypeptide chain) to the rough ER by binding to the SRP receptor on the ER membrane. Binding to the receptor releases the SRP from both the ribosome and the signal sequence of the growing polypeptide chain. The ribosome then binds to a protein translocation complex in the ER membrane, and the signal sequence is inserted into a membrane channel or translocon with the aid of GTP. Transfer of the ribosome mRNA complex from the SRP to the translocon opens the gate on the translocon and allows translation to resume, and the growing polypeptide chain is transferred directly into the translocon channel and

across the ER membrane as translation proceeds. As translocation proceeds, the signal sequence is cleaved by signal peptidase and the polypeptide is released into the lumen of the ER.

Smooth endoplasmic reticulum and lipid synthesis:

Hydrophobic lipids are synthesized in the ER and then they are then transported from the ER to their ultimate destinations either in vesicles or by carrier proteins. Phospholipids are synthesized in the cytosolic side of the ER membrane from water-soluble cytosolic precursors. Other lipids that are synthesized in the ER are cholesterol and ceramide which is further converted to either glycolipids or sphingomyelin in the golgi apparatus. Smooth ER are also the site for the synthesis of the steroid hormones from cholesterol. Thus steroid producing cells in the testis and ovaries are abundant in smooth ER.

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4. The ER acts as a circulatory or transporting system for various substances
5. The ER membrane are found to conduct intracellular impulses. Example the sarcoplasmic reticulum transmits impulses from the surface membranes into the deep region of muscle fibres.
6. It determines the plane of cell division
7. It plays an important role in the formation of nuclear envelope after each nuclear Division
8. The ER protects the cell by the toxic effects of various substances by a process of detoxification
9. ER provides increased surface for various enzymatic reactions

Or

b. Explain the protein import and export from mitochondria

In contrast to the RNA components of the mitochondrial translation apparatus (rRNAs and tRNAs), most mitochondrial genomes do not encode the proteins required for DNA replication, transcription, or translation. Instead, the genes that encode proteins required for the replication and expression of mitochondrial DNA are contained in the nucleus. In addition, the nucleus contains the genes that encode most of the

mitochondrial proteins required for oxidative phosphorylation and all of the enzymes involved in mitochondrial metabolism (e.g., enzymes of the citric acid

cycle). The proteins encoded by these genes (more than 95% of mitochondrial proteins) are synthesized on free cytosolic ribosomes and imported into mitochondria as completed polypeptide chains. Because of the double-membrane structure of mitochondria, the import of proteins is considerably more complicated than the transfer of a polypeptide across a single phospholipid bilayer. Proteins targeted to the matrix have to cross both the inner and outer mitochondrial membranes, while other proteins need to be sorted to distinct compartments within the organelle (e.g., the intermembrane space).

The import of proteins to the matrix is the best-understood aspect of mitochondrial protein sorting (Figure). Most proteins are targeted to mitochondria by amino-terminal sequences of 20 to 35 amino acids (called presequences) that are removed by proteolytic cleavage following their import into the organelle. The presequences of mitochondrial proteins, first characterized by Gottfried Schatz, contain multiple positively charged amino acid residues, usually in anamphipathic **α helix**.

- The first step in protein import is the binding of these presequences to receptors on the surface of mitochondria.

The polypeptide chains are then inserted into a protein complex that directs translocation across the outer membrane (the translocase of the outer membrane orom complex).

- The proteins are then transferred to a second protein complex in the inner membrane (the translocase of the inner membrane or Tim complex).

- Continuing protein translocation requires the electrochemical potential established across the inner mitochondrial membrane during electron transport. The transfer of high-energy electrons from NADH and $FADH_2$to molecular oxygen is coupled to the transfer of protons from the mitochondrial matrix to the intermembrane space. Since protons are charged particles, this transfer establishes an electric potential across the inner membrane, with the matrix being negative. During protein import, this electric potential drives translocation of the positively charged presequence.

Proteins are targeted for mitochondria by an amino-terminal presequence containing positively charged amino acids. Proteins are maintained in a partially unfolded state by association with a cytosolic Hsp70 and are recognized by a receptor on the surface of mitochondria. The unfolded polypeptide chains are then translocated through the Tom complex in the outer membrane and transferred to the Tim complex in the inner membrane. The voltage component of the electrochemical gradient is required for translocation across the inner membrane. The presequence is cleaved by a matrix protease, and a mitochondrial Hsp70 binds the polypeptide chain as it crosses the inner membrane, driving further protein translocation. A mitochondrial Hsp60 then facilitates folding of the imported polypeptide within the matrix.

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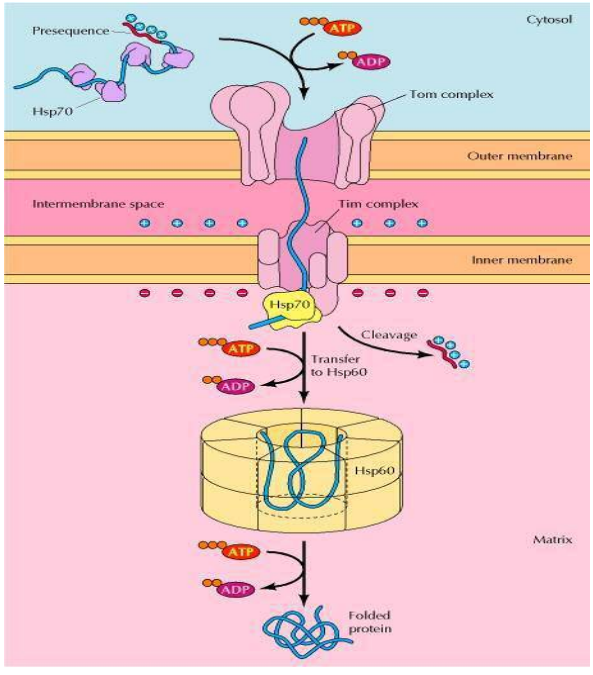
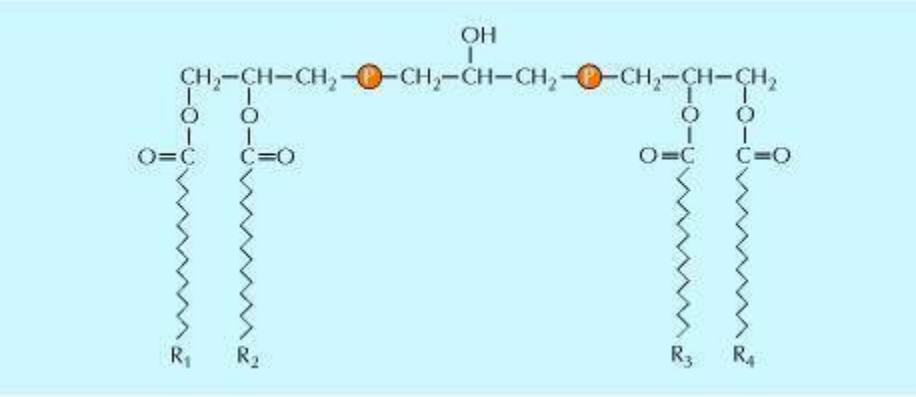


To be translocated across the mitochondrial membrane, proteins must be at least partially unfolded. Consequently, protein import into mitochondria requires molecular chaperones in addition to the membrane proteins involved in translocation (see Figure). On the cytosolic side, members of the Hsp70 family of chaperones maintain proteins in a partially unfolded state so that they can be inserted into the mitochondrial membrane. As they cross the inner membrane, the unfolded polypeptide chains bind to another member of the Hsp70 family, which is associated with the Tim complex and acts as a motor that drives protein import. The polypeptide is then transferred to a chaperone of the Hsp60 family (achaperonin), within which protein folding takes place. Since these interactions of polypeptide chains with molecular chaperones depend on ATP, protein import requires ATP both outside and inside the mitochondria, in addition to the electric potential across the inner membrane.

As noted above, some mitochondrial proteins are targeted to the outer membrane, inner membrane, or intermembrane space rather than to the matrix, so additional mechanisms are needed to direct these proteins to the correct submitochondrial compartment. These proteins are targeted to their destinations by a second sorting signal following the positively charged presequence that directs mitochondrial import. The targeting of proteins to the mitochondrial membranes appears to be mediated by hydrophobic stop- transfer sequences that halt translocation of the polypeptidechains through the Tim or Tom complexes, leading to their insertion into the inner or outer mitochondrial membranes, respec



Not only the proteins, but also the phospholipids of mitochondrial membranes are imported from the cytosol. In animal cells, phosphatidylcholine and

phosphatidylethanolamine are synthesized in the ER and carried to mitochondria byphospholipid transfer proteins, which extract single phospholipid molecules from the membrane of the ER. The lipid can then be transported through the aqueous environment of the cytosol, buried in a hydrophobic binding site of the protein, and released when the complex reaches a new membrane, such as that of mitochondria. The mitochondria then synthesize phosphatidylserine from phosphatidylethanolamine, in addition to catalyzing the synthesis of the unusual phospholipid cardiolipin, which contains four fatty acid chains (Figure).

Cardiolipin is an unusual ―double‖ phospholipid, containing four fatty acid chains, that is found primarily in the inner mitochondrial membrane.

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Reg. No.....

[17BCU102]

KARPAGAM UNIVERSITY

Karpagam Academy of Higher Education
(Established Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2017 onwards)

B.Sc., DEGREE EXAMINATION, NOVEMBER 2017

First Semester

BIOCHEMISTRY

CELL BIOLOGY

Time: 3 hours Maximum : 60 marks

PART – A (20 x 1 = 20 Marks) (30 Minutes)
(Question Nos. 1 to 20 Online Examinations)

PART B (5 x 2 = 10 Marks) (2 ½ Hours)
Answer ALL the Questions

21. Write the working principle of a phase contrast microscope.
22. How are proteins targeted to the ER?
23. List out the functions of mitochondria.
24. Comment on actin treadmilling mechanism.
25. What are desmosomes? Mention their types.

PART C (5 x 6 = 30 Marks)
Answer ALL the Questions

26. a. Explain the fluid mosaic model of plasma membrane. Add a note on the importance of fluid mosaic model.

Or

b. Describe the separation of sub cellular fractions by differential centrifugation.

27. a. Describe the structure of nuclear envelope with a neat labeled diagram.

Or

b. Critically evaluate the regulation of nuclear protein import and export.

28. a. Describe the polymorphic forms of lysosomes. Add a note on the functions of lysosomes.

Or

b. Describe the ultra structure of a mitochondrion.

29. a. Explain how do actin monomers polymerize to form an actin filament?

Or

b. Briefly discuss the biochemical properties and types of intermediate filament proteins.

30. a. Discuss the salient features of cell cycle.

Or

b. Give an illustrative account on Gap junction and tight junction.

Reg. No.....

[12BCU302]

KARPAGAM UNIVERSITY

(Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2012 onwards)

B.Sc. DEGREE EXAMINATION, NOVEMBER 2013

Third Semester

BIOCHEMISTRY

CELL BIOLOGY

Time: 3 hours Maximum : 100 marks

PART – A (15 x 2 = 30 Marks)
Answer ALL the Questions

1. Define cell theory
2. Add note on molecular composition of cell
3. Differentiate the prokaryotic and eukaryotic cell
4. Write notes on membrane lipids
5. Write notes on properties of integral membrane proteins
6. What are glycoproteins?
7. Explain the role of acid hydrolases
8. Traffic police man of cell- golgi complex-Justify
9. Narrate the glyoxalate cycle and its role
10. Narrate the role of microtubules
11. Brief about intermediary filaments
12. In brief explain the cytoskeletal protein of RBC
13. Add notes on mitotic cell division
14. Write notes on chromosomes
15. Differentiate euchromatin and hetero chromatin

PART B (5 X 14= 70 Marks)
Answer ALL the Questions

16. a. Draw the structure of animal and plant cell and brief the role its various organelles

Or

b. Explain the following : (i) Prokaryotic cell (ii) Origin and evolution of cell

17. a. Explain the fluid mosaic model of plasma membrane

Or

b. Describe the various types of transport across the plasma membrane with example

18. a. With neat diagram explain the structure and functions of mitochondria.

Or

b. Describe the structure and functions of endoplasmic reticulum

19. a. Explain the structure, assembly and organization of actin filaments

Or

b. Explain the following: (i) Cilia (ii) Flagella

20. a. What is cell cycle? Describe in detail about the phases of cell cycle

Or

b. With neat diagram explain the meiotic cell division

Reg. No.....

[14BCU302]

KARPAGAM UNIVERSITY

Karpagam Academy of Higher Education
(Established Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2014onwards)

B.Sc., DEGREE EXAMINATION, NOVEMBER 2015

Third Semester

BIOCHEMISTRY

CELL BIOLOGY

Time: 3 hours Maximum : 60 marks

PART – A (20 x 1 = 20 Marks) (30 Minutes)
(Question Nos. 1 to 20 Online Examinations)

PART B (5 x 8 = 40 Marks) (2 ½ Hours)
Answer ALL the Questions

21. a. Illustrate and explain structural organization of prokaryotic cell
Or
b. Briefly describe about molecular composition and function of cell wall.

22. a. Discuss about different models of plasma membrane
Or
b. Give an account on transport mechanisms.

23. a. Lysosomes are suicidal bags- Explain in detail.
Or
b. Give an account on ATP synthesis in mitochondria.

24. a. Write prophase and metaphase stages of mitosis with labeled diagram.
Or
b. Draw and explain the structure of chromosome and its function.

25. a. Write short notes cytoskeleton and its function.
Or
b. Explain the structure and function of the following.
i) Flagella ii) Cilia
