KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed University)
(Established Under Section 3 of UGC Act 1956)
Coimbatore - 641021.
(For the candidates admitted from 2019 onwards)
DEPARTMENT OF BIOCHEMISTRY

STAFF NAME : Dr.K.DEVAKI
SUBJECT : CELL BIOLOGY

SEMESTER : I
SUBJECT CODE : 19BCU102 **CLASS : I B.Sc.(BC)**

Course objectives

- To understand the basic structure of cell and model organism for its study
- To know the tools required for studying cell morphology
- To enlight the students with structure and functions of various organelle
- To understand the cytoskeletal network and extracellular matrix
- To introduce the cell cycle, cell division and cell death process

Course outcomes (CO's)

1. Differenttiate the prokaryotic and eukaryotic cell
2. Understand the pricnciple behind studying the cell morphology using various microscope
3. Identify the structure and functions of each organelle in cell
4. Recognise the mechanism behind the protein sorting and transport to their destinations like lysosome, mitochondria and chloroplast
5. Maintenance of cytoskeleton structure and function of micro, macro and intermediary filaments
6. Identify the proteins involved in cell cell interaction
7. Enumerate the phases of cell cycle, events in cell division and mechanism of cell death

Unit I: 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 II: Structure of different cell organelles

Structure of nuclear envelope, nucleao 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 III: 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 IV: 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 V: 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.

SUGGESTED READING

1. Paul, A., (2007). Text Book of Cell and Molecular Biology,1st edition. Books and Allied (P) Ltd, Kolkata.

2. Verma, P.S., and.Agarwal, V.K., (2005). Cell Biology Molecular Biology and Genetics, VII Edition, S.Chand and company Ltd, New Delhi.

3. Shukla, R.M., (2013). A textbook of Cell Biology, Dominant Publishers and Distributors.

4. Powar, C.B., (2001). Cell Biology, 3rd edition, Himalaya Publishing House, New Delhi

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

6. Garret, R. H. and Grisham, C.M., Biochemistry (2010) 4th ed., Cengage Learning (Boston), ISBN-13: 978-0-495-11464-2.

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

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

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

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Coimbatore – 641 021.

LECTURE PLAN
DEPARTMENT OF BIOCHEMISTRY

STAFF NAME : Dr.K.DEVAKI
SUBJECT NAME : Cell biology SUB.CODE:19BCU102
SEMESTER : I CLASS: I B.Sc (BC)

S. No	Duration of period	Topics covered	Books referred	Page No	Web page referred
UNIT-I					
1	1	Introduction to cell biology Prokaryotic cell (*Archae and Eubacteria*)	S7 S8	3-8 7-16	-
2	1	Eukaryotic cell (animal and plant cells),	S9	12-21	
3	1	Cells as experimental models.	S7	15-19	-
4	1	Plasma membrane: Composition, Fluid mosaic model	S5 S8	152-156 122-123	-
5	1	Light microscopy, phase contrast microscopy	S7	20-21	-
6	1	Fluorescence microscopy, confocal microscopy	S7	21-22	-
7	1	Electron microscopy-SEM,TEM	S5	186-188 190-192	W1
8	1	FACS, Centrifugation for sub-cellular fractionation	S7	23-32	-
9	1	Revision and QP discussion			
		Total No of Hours Planned For Unit I : 09			
UNIT-II					
1	1	Structure of nuclear envelope, nuclear pore complex	S7	315-320	-
2	1	Selective transport of proteins to and from the nucleus.	S9	561-568	-
3	1	Regulation of nuclear protein import and export	S9 S7	568-571 321-325	-
4	1	ER structure. Targeting proteins to ER,	S5	659-665	-
5	1	Smooth ER and lipid synthesis.	S7	359-363	-
6	1	Export of proteins and lipids from ER and into ER.	S7	352-359	-
7	1	Protein folding in ER	S7	363-365	
8	1	Peroxisomes and Zellweger syndrome	S7	415-418	-

9	1	Revision and QP discussion			
		Total No of Hours Planned For Unit II: 09			
		UNIT-III			
1	1	Organization of Golgi.	S9 S7	821-834 365-366	
2	1	Lipid and polysaccharide metabolism in Golgi.	S7	366-370	
3	1	Protein sorting and export from Golgi.	S5 S8	168-170 302-306	
4	1	N and O-linked glycosylation	S7	370-372	
5	1	Lysosome. – Acid hydrolases, phagocytosis and autophagy	S7	379-384	
6	1	Mitochondria-Structure and functions,	S9	610-618	
7	1	protein import and mitochondrial assembly protein export from mitochondrial matrix	S7	392-397	
8	1	Chloroplasts- Import and sorting of chloroplast proteins.			
9	1	Revision and discussion of possible question	S7	407-409	
		Total No of Hours Planned For Unit IV: 09			
		UNIT-IV			
1	1	**Cytoskeletal proteins** : Structure and organization of actin filaments.	S9	821-824	
2	1	Treadmilling and role of ATP in microfilament polymerization,	S7 S5	423-426 779-782	
3	1	organization of actin filaments.	S5	784-789	
4	1	Non-muscle myosin.	S7	435-437	
5	1	Intermediate filament protein	S5	805-811	
6	1	Assembly and intracellular organization of intermediate filament proteins	S7	442-447	
7	1	Assembly, organization and movement of cilia	S8	338-350	
8	1	Assembly, organization and movement of flagella.	S8	350-359	
9	9	Revision and discussion of possible question			
		Total No of Hours Planned For Unit IV: 09			
		UNIT-V			
1	1	**Cell wall and extracellular matrix** :Prokaryotic cell wall	S8	279-286	-
2	1	Eukaryotic cell wall	S9	1006-1009	
3	1	Cell matrix proteins.	S7	504-509	-
4	1	Cell-matrix interactions and cell-cell interactions. Adherence junctions, tight junctions, gap junctions,	S9 S8	971-984 266-267	-

5	1	Desmosomes, hemidesmosomes,	S7	445-513	-
6	1	focal adhesions and plasmodesmata.	S8	272-274	
7	1	**Cell cycle, cell death and cell renewal :** Eukaryotic cell cycle, restriction point, and checkpoints.	S5	854-859 886-889	-
8	1	Cell division.	S7	583-587	-
9	1	Apoptosis and necrosis - brief outline.	S7	592-593	
10	1	Salient features of a transformed cell.	S5	935-941	-
					-
		Total No of Hours Planned For Unit V : 10			
1	1	Previous year End Semester Exam- QP discussion	-	-	-
2	1	Previous year End Semester Exam- QP discussion	-	-	-
Total	**02**	**Hours planned for QP discussion : 02**			
Total No of Hours planned for this syllabi : 48					

SUGGESTED READING

1. Paul, A., (2007). Text Book of Cell and Molecular Biology,1st edition. Books and Allied (P) Ltd, Kolkata. **(S1)**

2. Verma, P.S., and.Agarwal, V.K., (2005). Cell Biology Molecular Biology and Genetics, VII Edition, S.Chand and company Ltd, New Delhi. **(S2)**

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4. Powar, C.B., (2001). Cell Biology, 3rd edition, Himalaya Publishing House, New Delhi **(S4)**

5. Lodish, H., Berk, A., Kaiser, C.A., and Krieger, M., (2012). Molecular Cell Biology, 7th edition. W.H. Freeman & Company, London. **(S5)**

6. Garret, R. H. and Grisham, C.M., Biochemistry (2010) 4th ed., Cengage Learning (Boston), ISBN-13: 978-0-495-11464-2. **(S6)**

7. Cooper, G.M., and Hausman, R.E., (2013). Cell-A Molecular Approach, 6th Edition.. Sinauer Associates. USA **(S7)**

8. Karp, G., (2013). Cell and Molecular Biology, 7th edition. John Wiley and Sons, Inc, Hoboken, United States. **(S8)**

9. Alberts, B., Johnson,A., Lewis, J., andEnlarge, M., Molecular Biology of the Cell (2008) 5th ed., Garland Science (Princeton), ISBN: **(S9)**

WEBSITES

http://www.formatex.org/microscopy3/pdf/pp122-131.pdf

UNIT-I

Syllabus

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 organelles

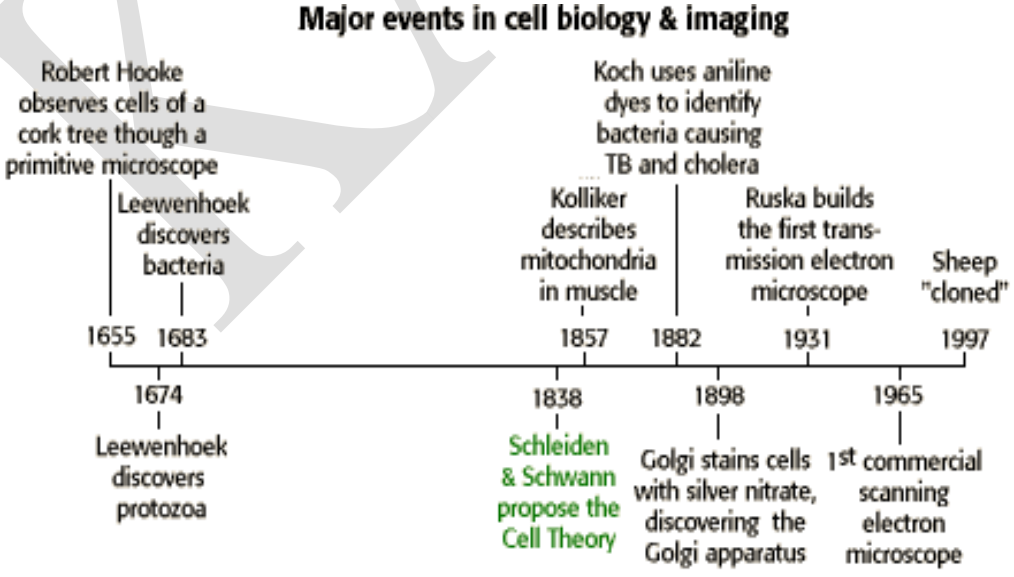
INTRODUCTION TO CELL BIOLOGY

CELL:

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:

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



Each cell is an amazing world unto itself: it can take in nutrients, convert these nutrients into energy, carry out specialized functions, and reproduce as necessary.

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.

The terms prokaryotic and eukaryotic were suggested by Hans Ris in the 1960's. This classification is based on their complexcity. Further based on the kingdom into which they may fall i.e the plant or the animal kingdom, plant and animal cells bear many differences

THE ORIGIN AND EVOLUTION OF CELLS

- It appears that life first emerged at least 3.8 billion years ago, approximately 750 million years after Earth was formed.
- Origin of life and evolution of first cell is a matter of speculation, since these events cannot be reproduced in the laboratory.
- Several types of experiments were carried out to provide important evidence bearing on some steps of the process.

Time scale of evolution:

The following scale indicates the approximate times at which some of the major events in the evolution of cells are thought to have occurred.

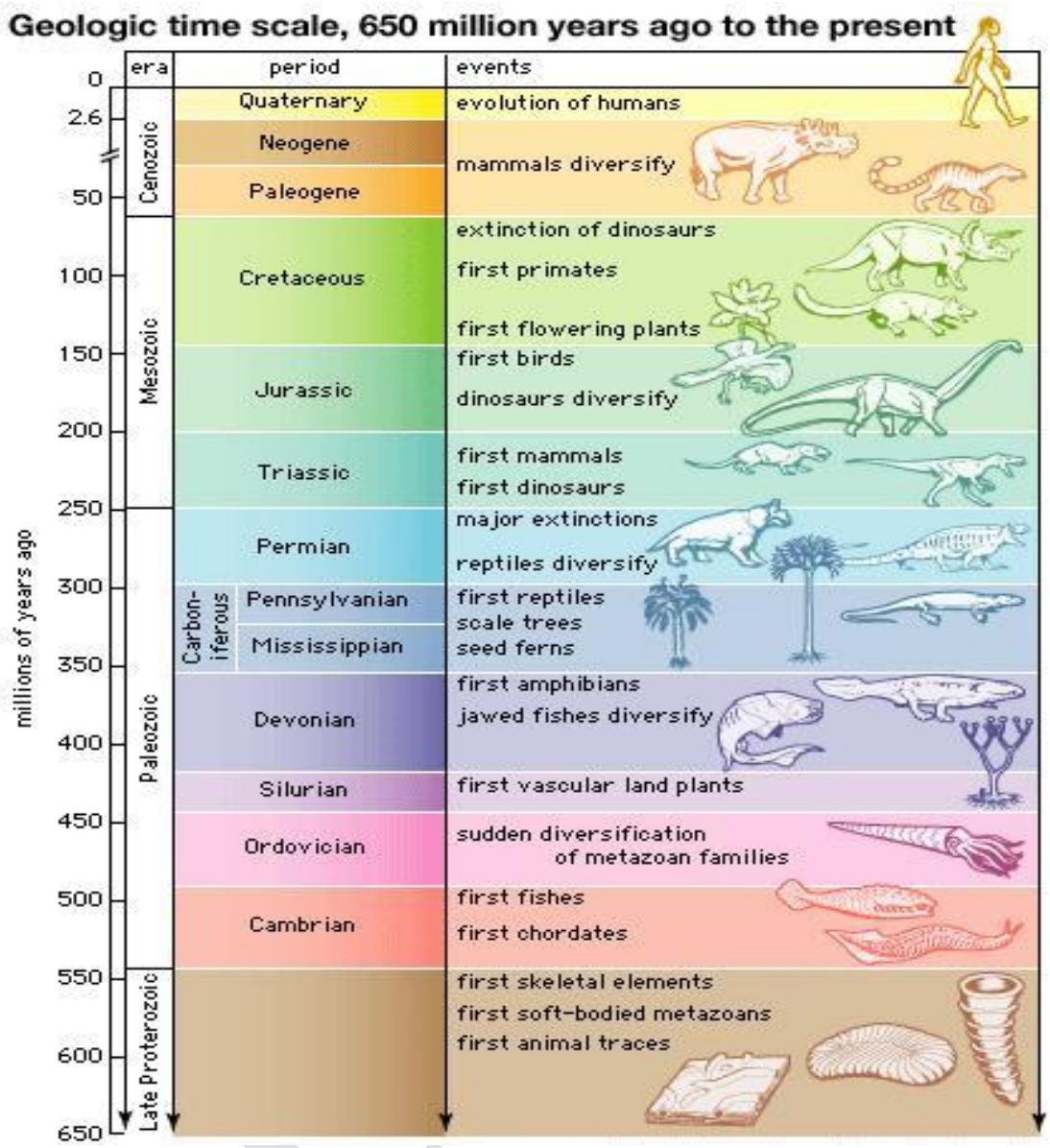


Figure : Time scale of evolution

Experiments for evoltuion

1. Miller's experiment

At the time life arose, the atmosphere of Earth is thought to have contained little or no free oxygen, instead consisting principally of CO_2 and N_2 in addition to smaller amounts of gases such as H_2, H_2S, and CO. Such an atmosphere provides reducing conditions in which organic molecules, given a source of energy such as sunlight or electrical discharge, can form spontaneously. The spontaneous formation of organic molecules was first demonstrated

experimentally in the 1950s, when **Stanley Miller** showed that the **discharge of electric sparks into a mixture of H_2, CH_4, and NH_3,** in the presence of water, **led to the formation of a variety of organic molecules**, including several amino acids.

2. Formation of macromolecules

The next step in evolution was the formation of macromolecules. The monomeric building blocks of macromolecules have been demonstrated to polymerize spontaneously under plausible prebiotic conditions. Heating dry mixtures of amino acids, results in their polymerization to form polypeptides.

A critical step in understanding molecular evolution was thus reached in the early 1980s, when it was discovered in the laboratories of **Sid Altman and Tom Cech** that **RNA is capable of catalyzing a number of chemical reactions**, including the polymerization of nucleotides. RNA is thus uniquely able both to serve as a template for and to catalyze its own replication. Ordered interactions between RNA and amino acids then evolved into the present-day genetic code, and DNA eventually replaced RNA as the genetic material.

PROKARYOTIC CELL

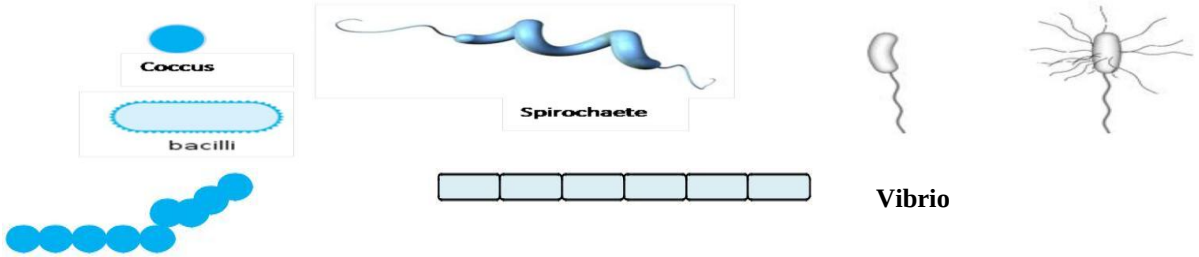
Prokaryote means before nucleus in Greek. They include all cells which lack nucleus and other membrane bound organelles. Mycoplasma, virus, bacteria and cyanobacteria or blue-green algae are prokaryotes.

Bacteria are example of the prokaryotic cell type. Besides bacteria, **cyano bacteria** are major group of 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*) (Figure).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.

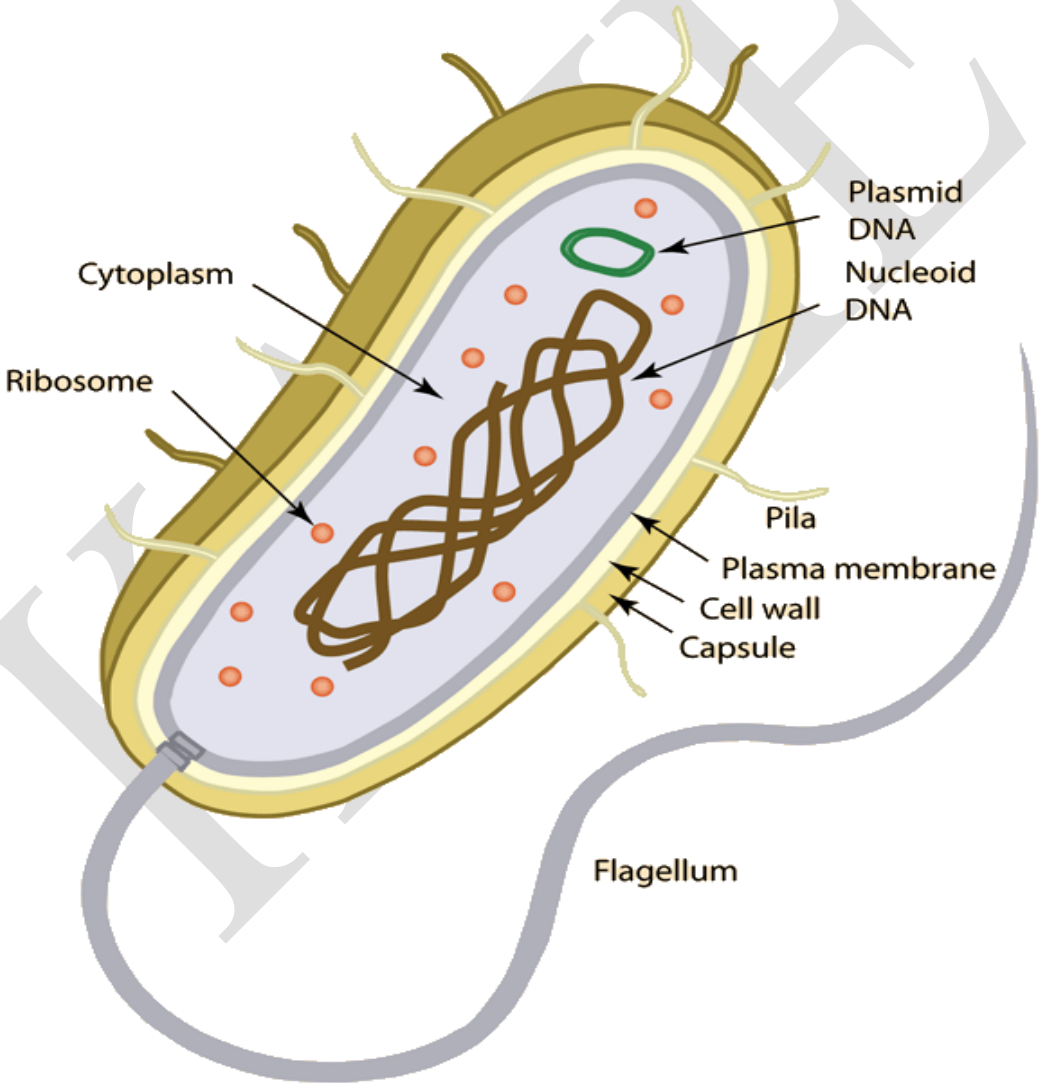


Fig : Structure of *E.coli*

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).

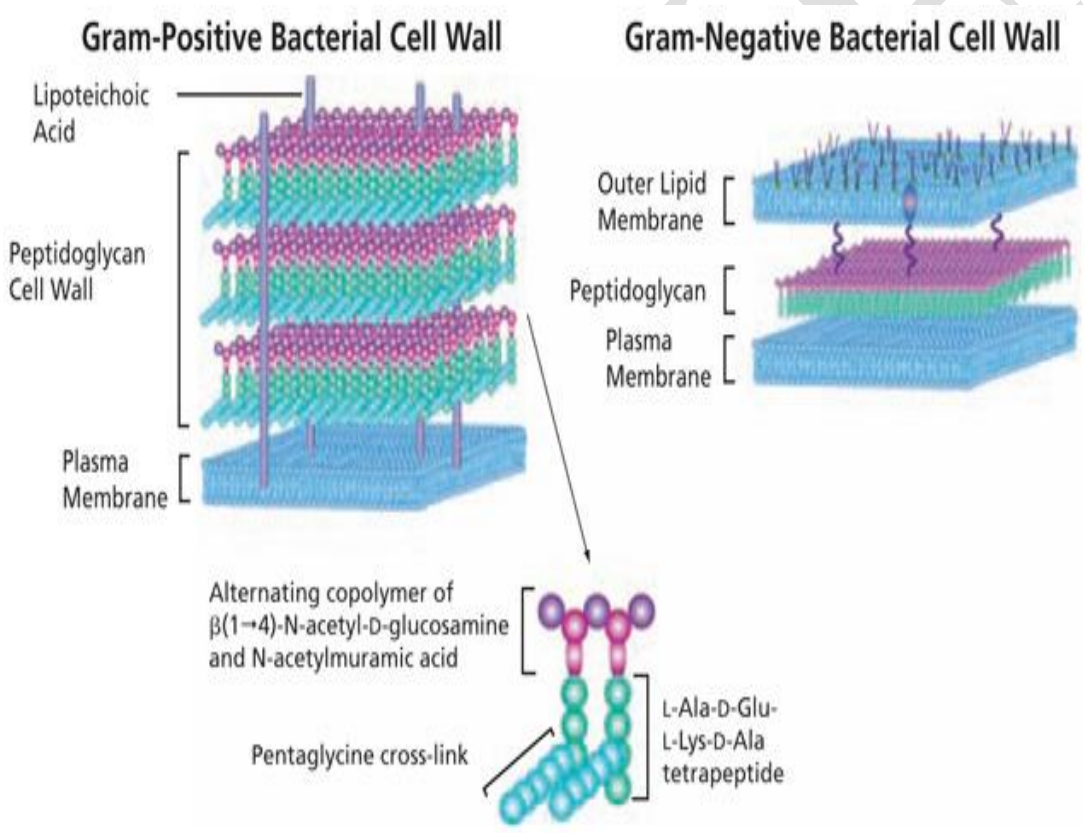


Fig: Bacterial Cell Wall

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

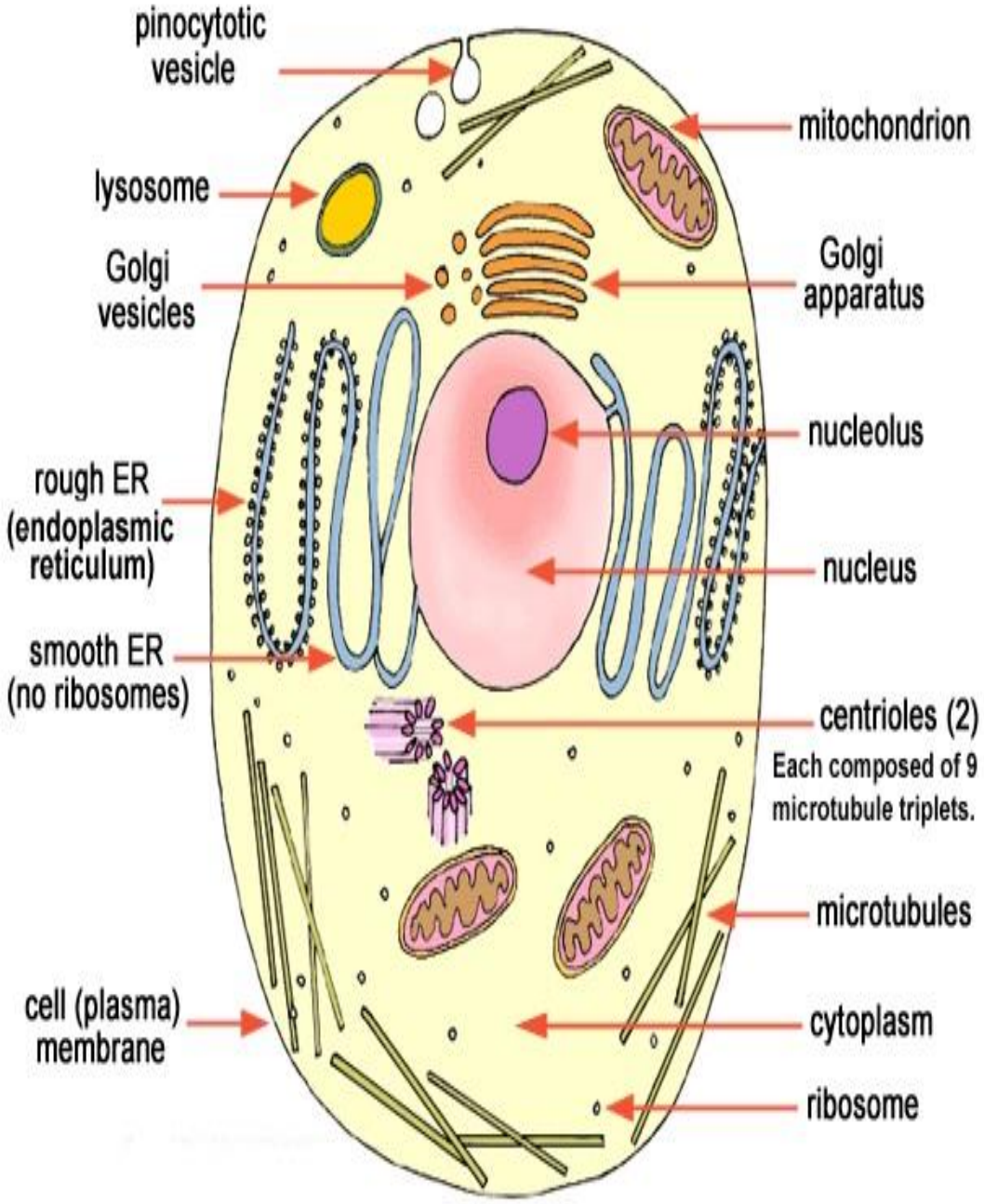
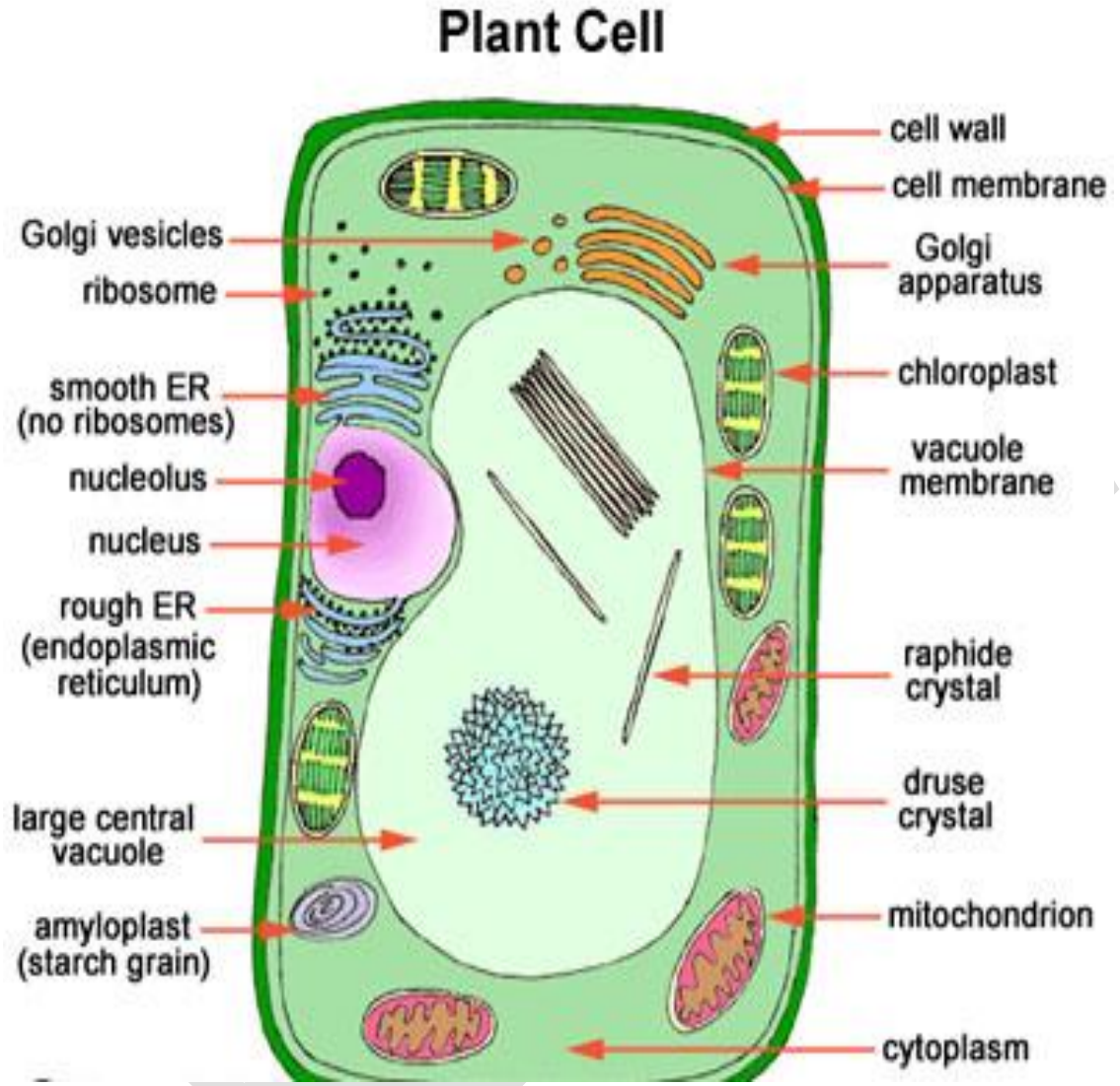


Fig:Animal cell structure



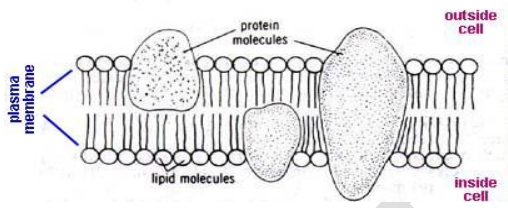
CELL ORGANELLES:

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

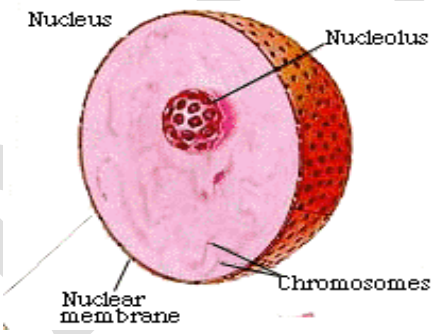
It is jelly-like substance composed of mainly water and found between the cell membrane and nucleus. The cytoplasm makes up most of the "body" of a cell and is constantly streaming.

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.



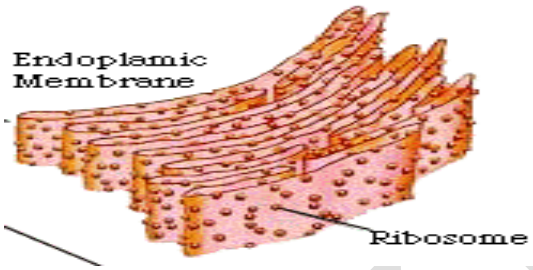
THE FOLLOWING ORGANELLES ARE FOUND IN BOTH PLANT AND ANIMAL CELLS.

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

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

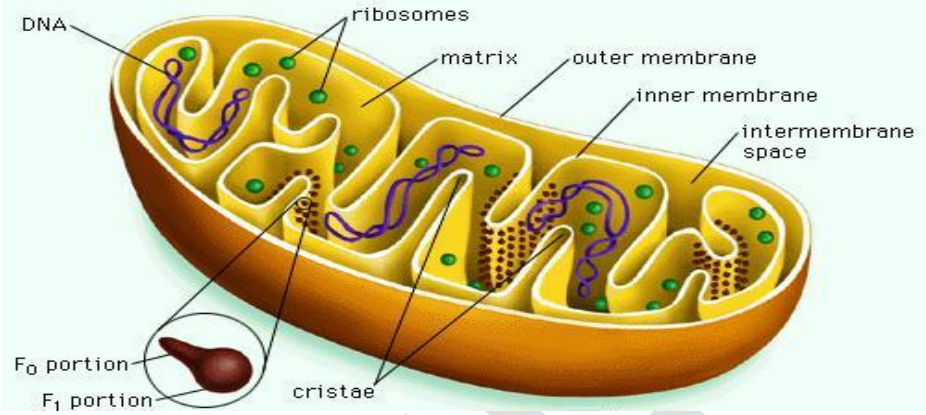


Fig: Mitochondria

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.

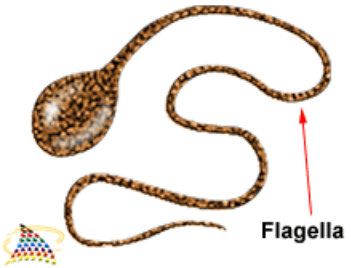
8.PEROXISOME

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

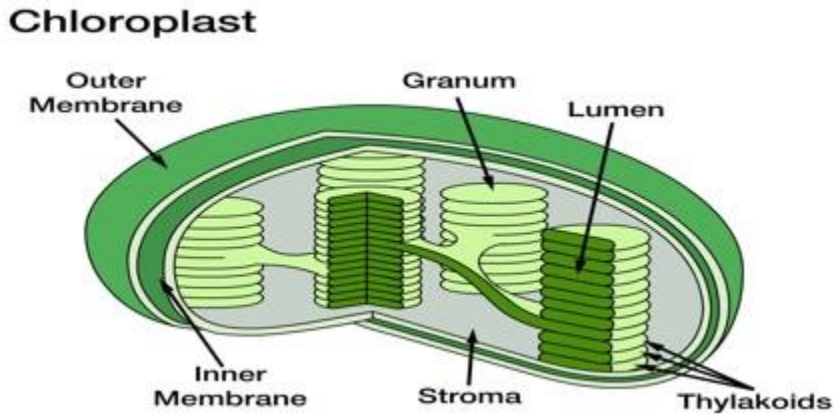
Pores in the primary cell wall through which the plasmalemma and endoplasmic reticulum of adjacent cells are continuous.

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.

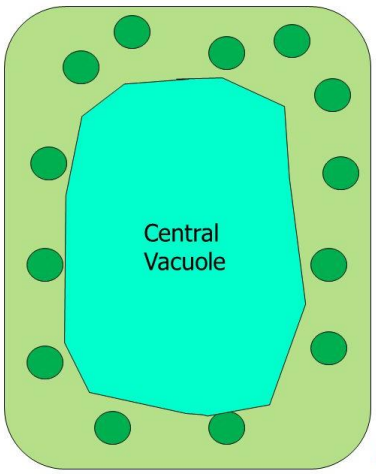


The other plastids is elaioplasts specialized for fat storage

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

	Eukaryotic Cell	Prokaryotic Cell
Nucleus:	Present	Absent
Number of chromosomes:	More than one	One--but not true chromosome: Plasmids
Cell Type:	Usually multicellular	Usually unicellular (some cyanobacteria may be multicellular)
True Membrane bound Nucleus:	Present	Absent
Example:	Animals and Plants	Bacteria and Archaea
Genetic Recombination:	Meiosis and fusion of gametes	Partial, undirectional transfers DNA
Lysosomes and peroxisomes:	Present	Absent
Microtubules:	Present	Absent or rare
Endoplasmic reticulum:	Present	Absent
Mitochondria:	Present	Absent
Cytoskeleton:	Present	May be absent
DNA wrapping on proteins.:	Eukaryotes wrap their DNA around proteins called histones.	Multiple proteins act together to fold and condense prokaryotic DNA. Folded DNA is then organized into a variety of conformations that are supercoiled and wound around tetramers of the HU protein.
Ribosomes:	Larger	Smaller
Vesicles:	Present	Present

Golgi apparatus:	Present	Absent
Chloroplasts:	Present (in plants)	Absent; chlorophyll scattered in the cytoplasm
Flagella:	Microscopic in size; membrane bound; usually arranged as nine doublets surrounding two singlets	Submicroscopic in size, composed of only one fiber
Permeability of Nuclear Membrane:	Selective	not present
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

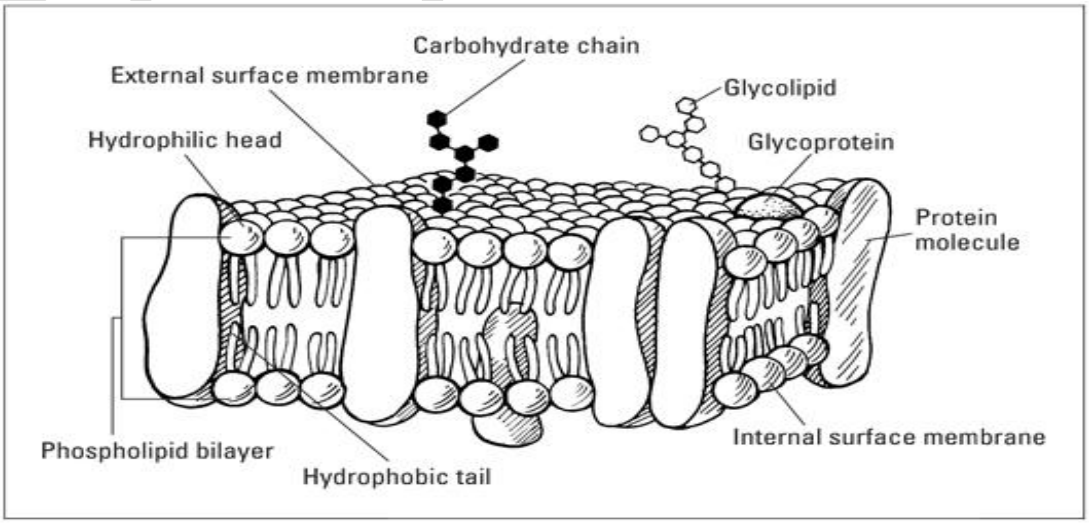


Figure: Plasma membrane

MEMBRANE LIPIDS

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

1. MEMBRANE LIPIDS

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

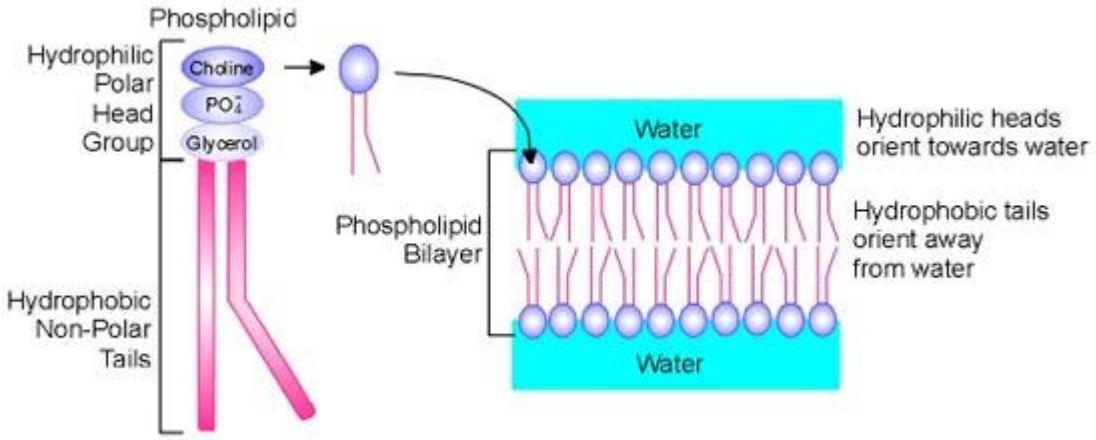


Fig: Phospholipids in membrane

(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.

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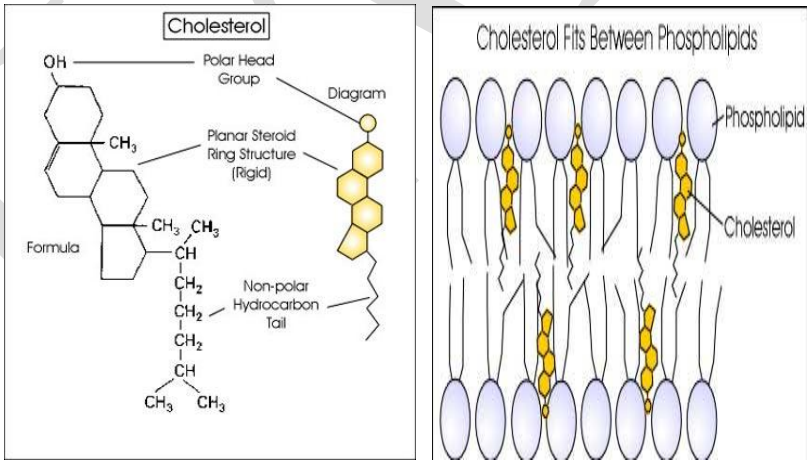


Fig: Amphipathic cholesterol in membrane

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).

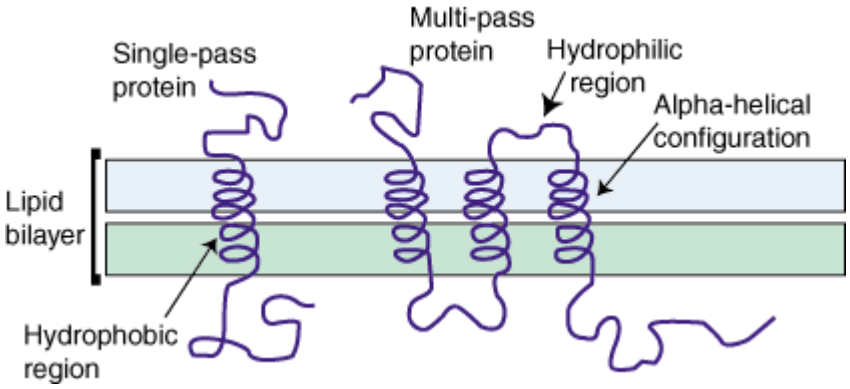


Figure: Membrane Proteins

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

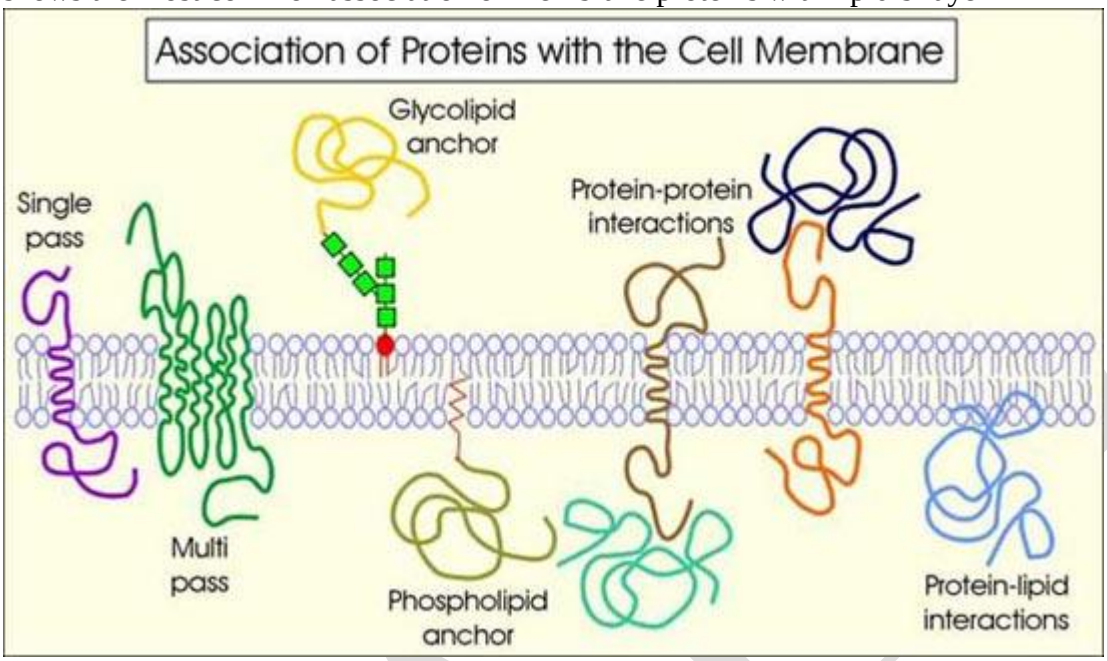
1.Channel Proteins - form small openings for molecules to difuse through
2. Carrier Proteins- binding site on protein surface "grabs" certain molecules and pulls them into the cell
3. Receptor Proteins - molecular triggers that set off cell responses (such as release of hormones or opening of channel 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.

Association of Proteins with the Cell Membrane

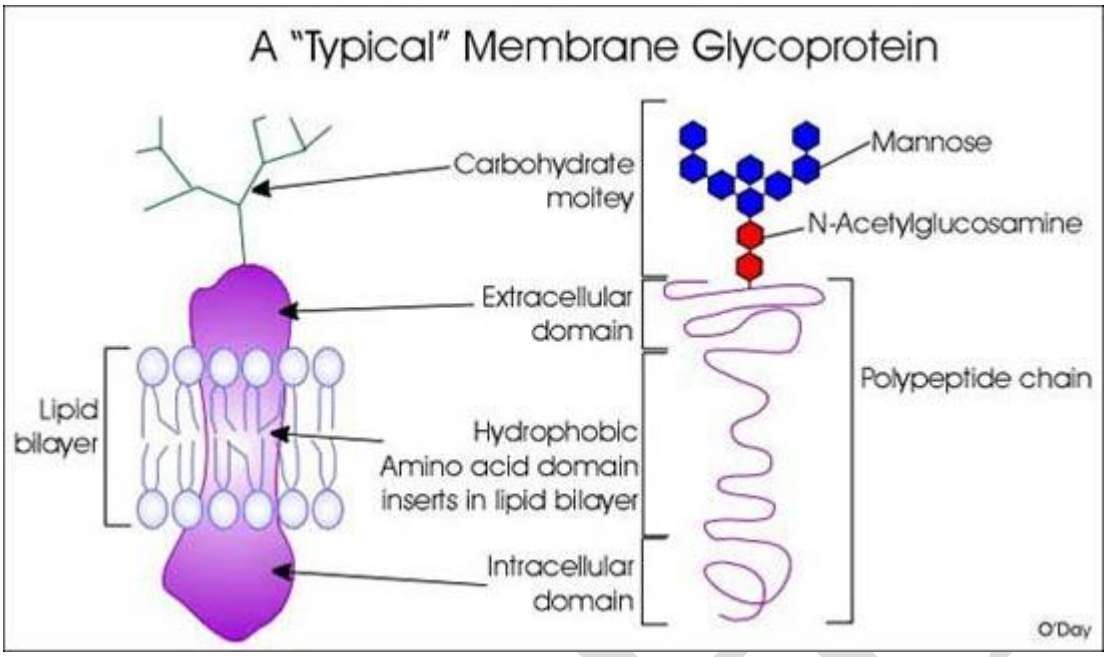
Membrane proteins associate with the lipid bilayer in many different ways. The figure below shows the most common association of membrane proteins with lipid bilayer



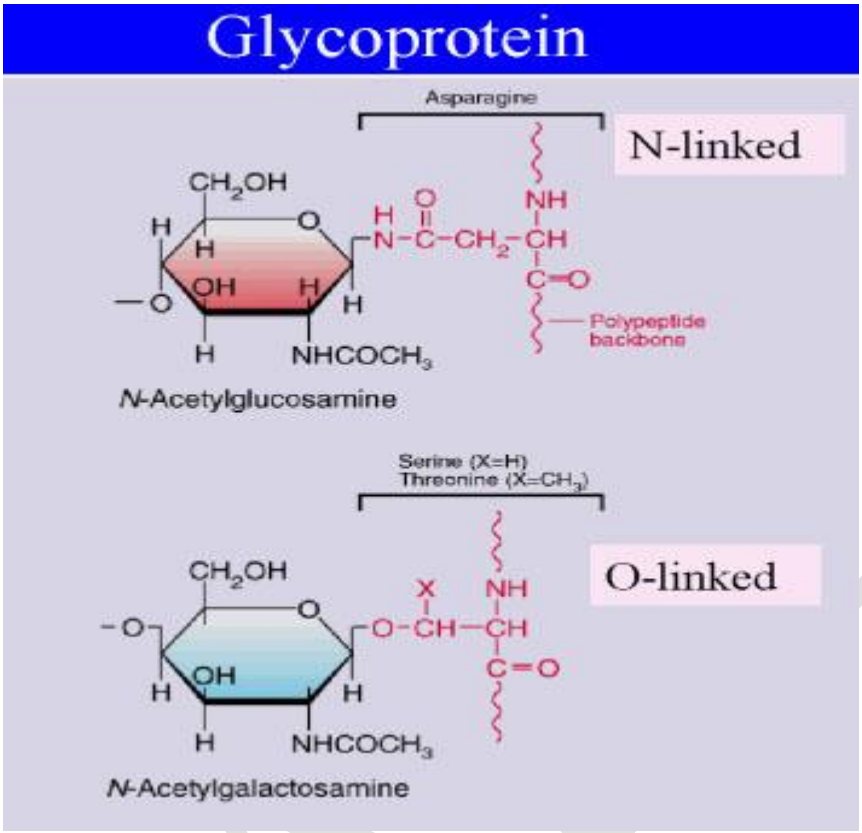
3. CARBOHYDRATES

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.

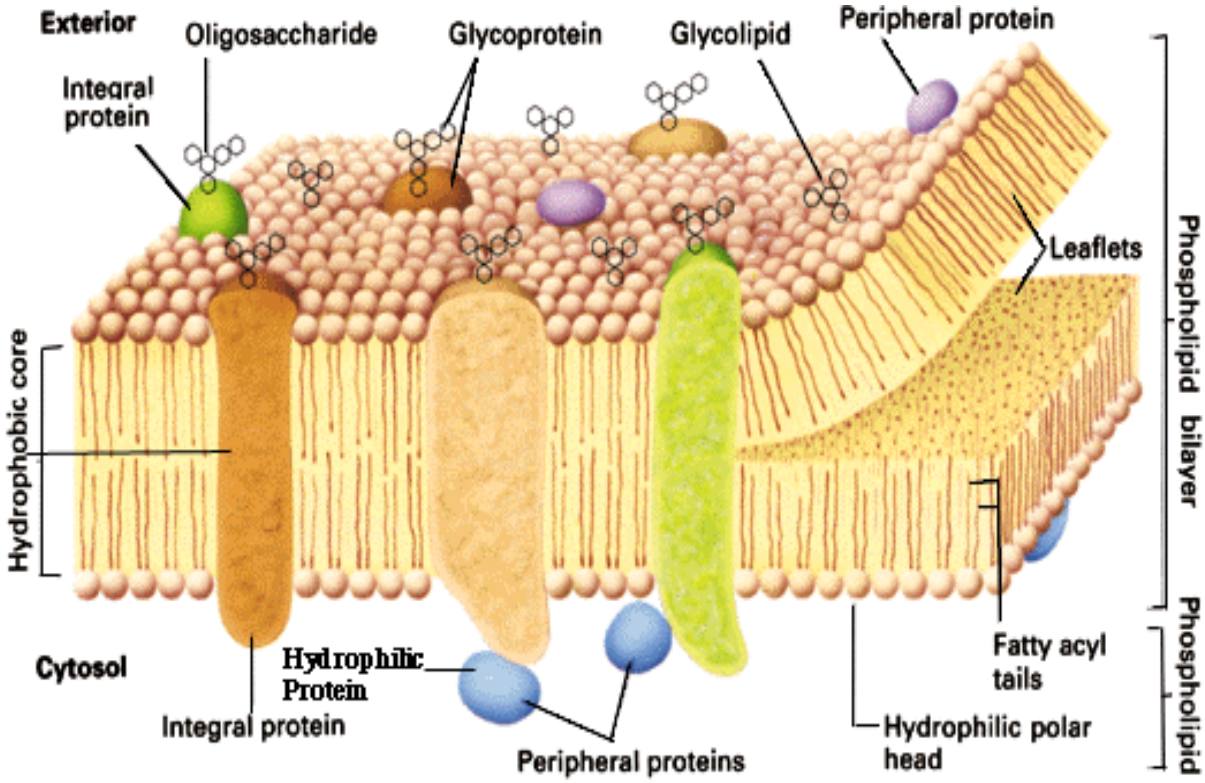


Figure: Fluid Mosaic Model of Membrane Structure

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.

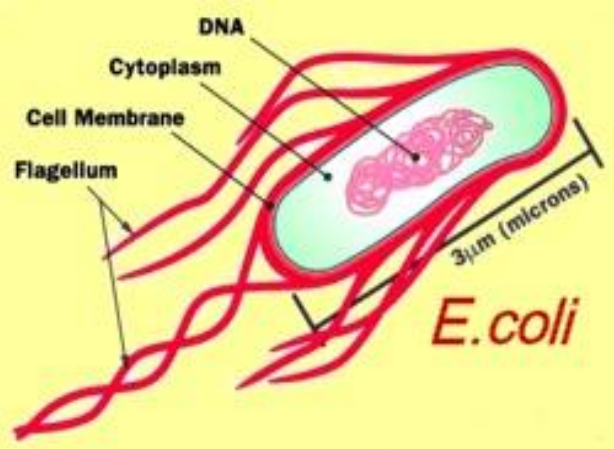


Fig: Typical *E.coli* cell

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.

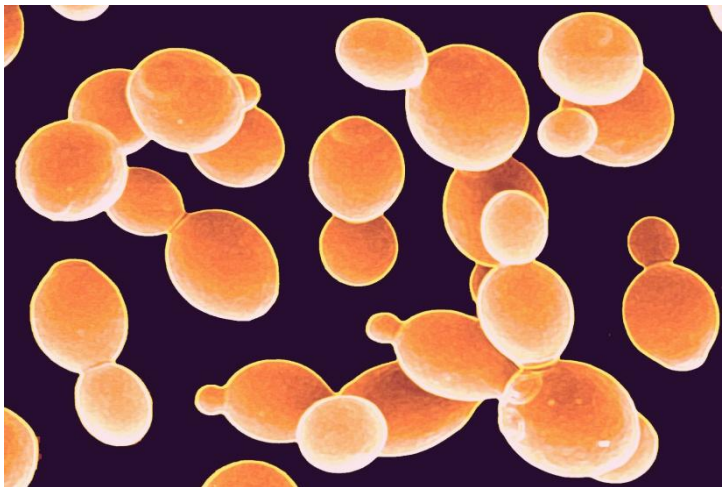


Fig:Electron micrograph (SEM) of a strain of *Saccharomyces cerevisiae*

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.**

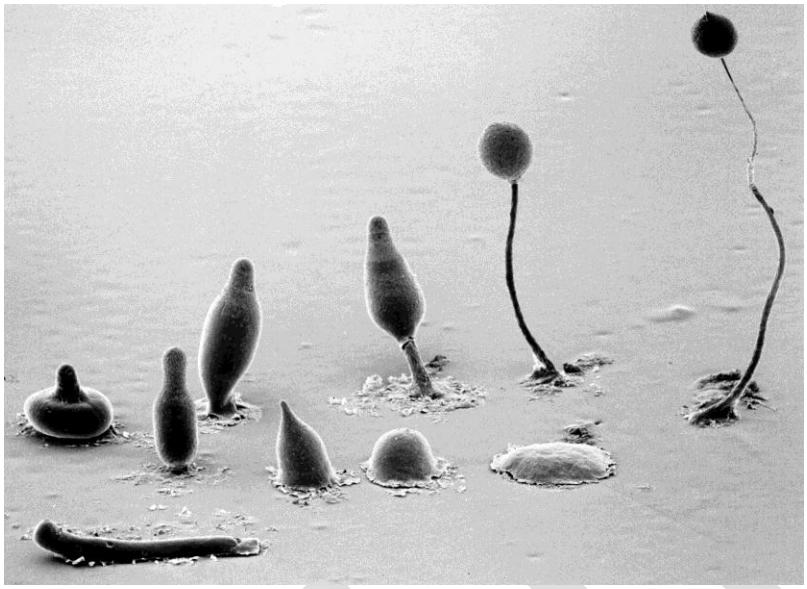


Fig: *Dictyostelium discoidium*, a cellular slime mold

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.

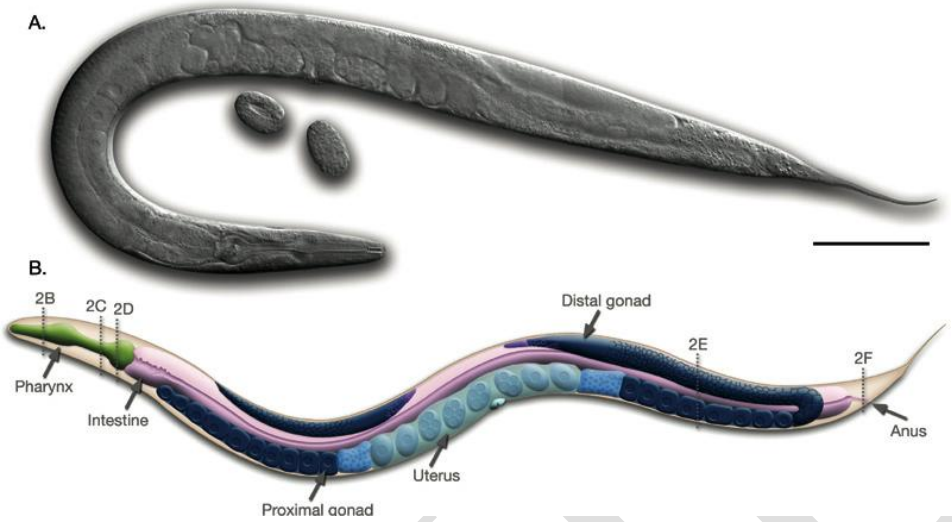


Fig: *C. elegans*

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**.

Fig: *Drosophila melanogaster*, the fruit fly

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**.

Fig: *Arabidopsis thaliana*

7.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**.

Fig: *Xenopus laevis*

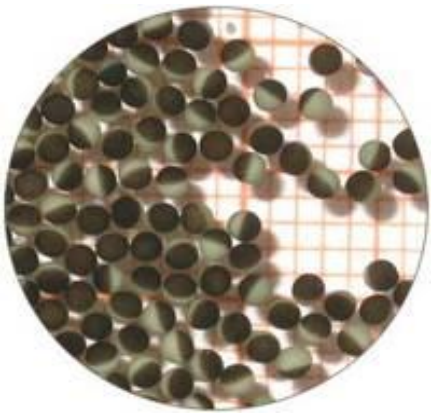


Fig: *Xenopus* eggs

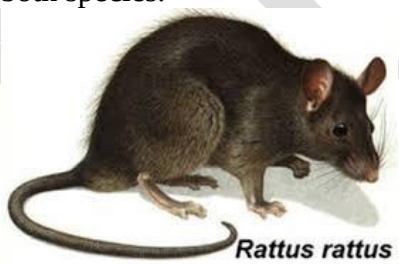
b. Zebrafish

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*.

Fig: Zebra Fish

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.Adjustments 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.

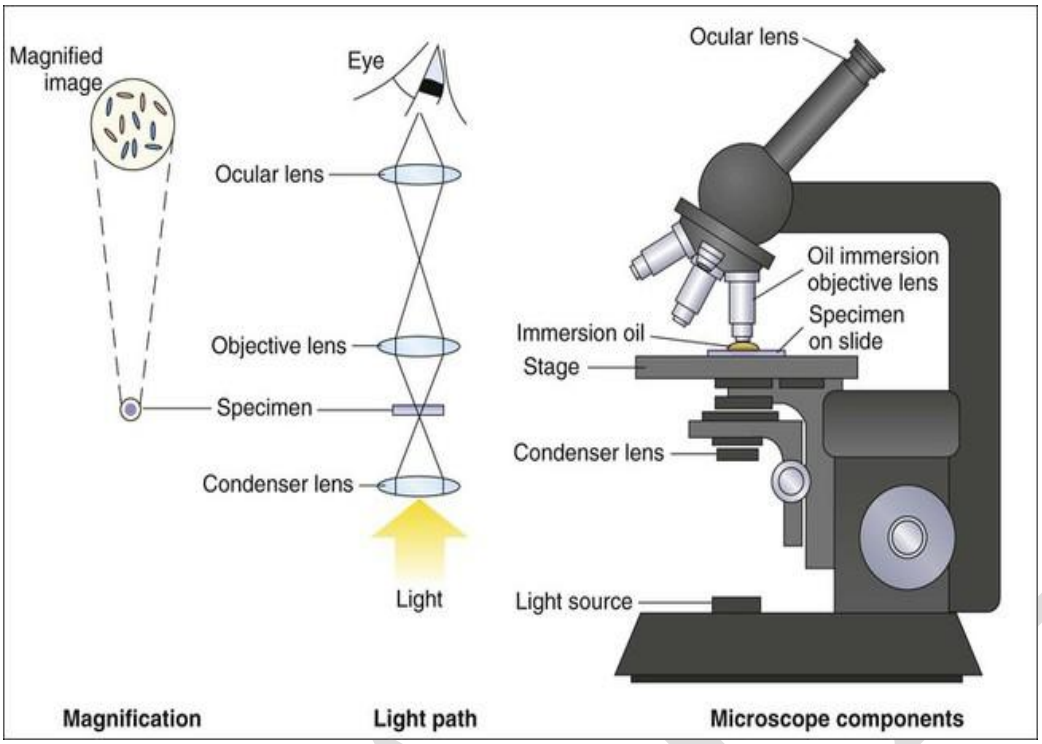


Fig: Light micoscope

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

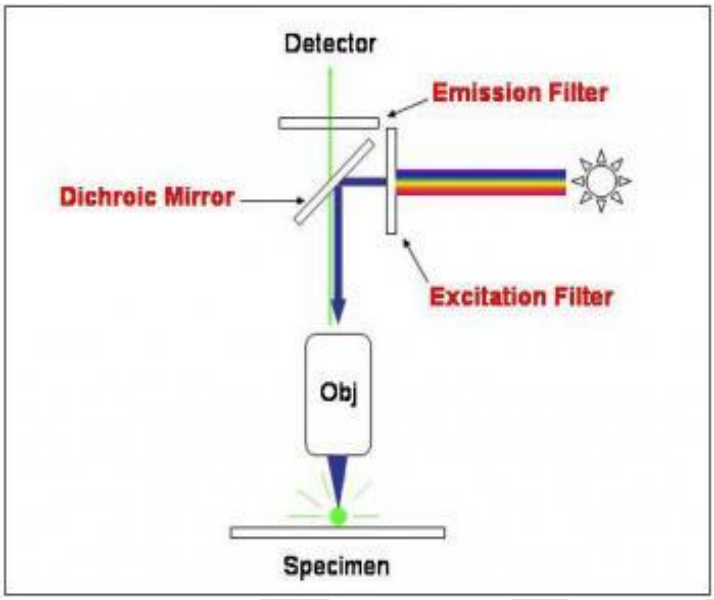


Fig: Flow chart for fluorescent microscope

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

IMMUNOFLUORESCENCE

Immunofluorescence is a technique which uses the highly specific binding of an antibody to its antigen in order to label specific proteins or other molecules within the cell. A sample is treated with a primary antibody specific for the molecule of interest. A fluorophore can be directly conjugated to the primary antibody. Alternatively a secondary antibody, conjugated to a fluorophore, which binds specifically to the first antibody can be used. For example, a primary antibody raised in a mouse which recognises tubulin combined with a secondary anti-mouse antibody derivatised with a fluorophore could be used to label microtubules in a cell.

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.

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.

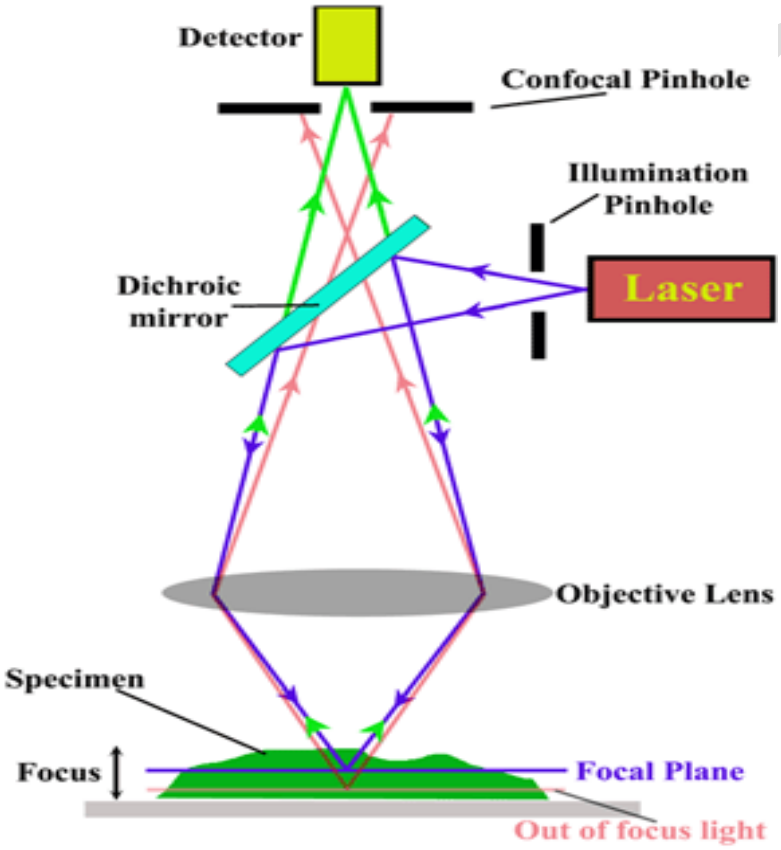


Fig : Schematic repesentation of assembly of confocal microscope

- The emitted/reflected light passing through the detector pinhole is transformed into electrical signals by a photomultiplier and displayed on a computer monitor

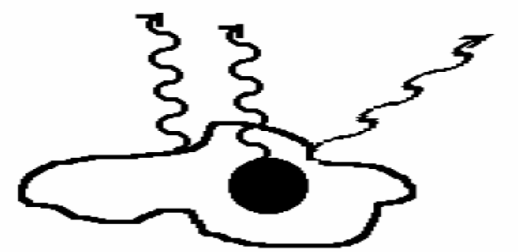
- The wavelength of light, the numerical aperture of the objective and the diameter of the diaphragm (wider detection pinhole reduces the confocal effect) affect the depth of the focal plane. To obtain a full image, the point of light is moved across the specimen by scanning mirrors. Scanning mirrors 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.

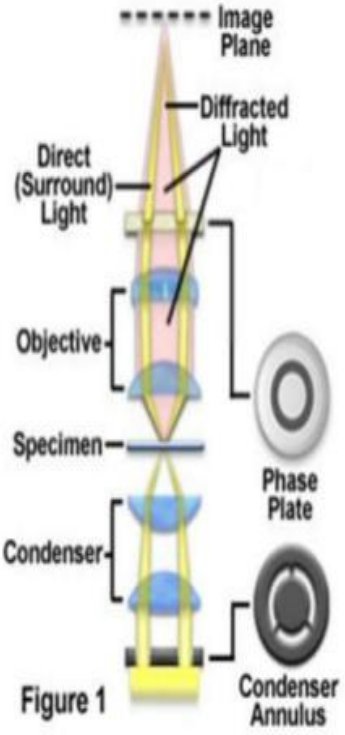


Fig: Instrumentation of phase contrast microscope

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

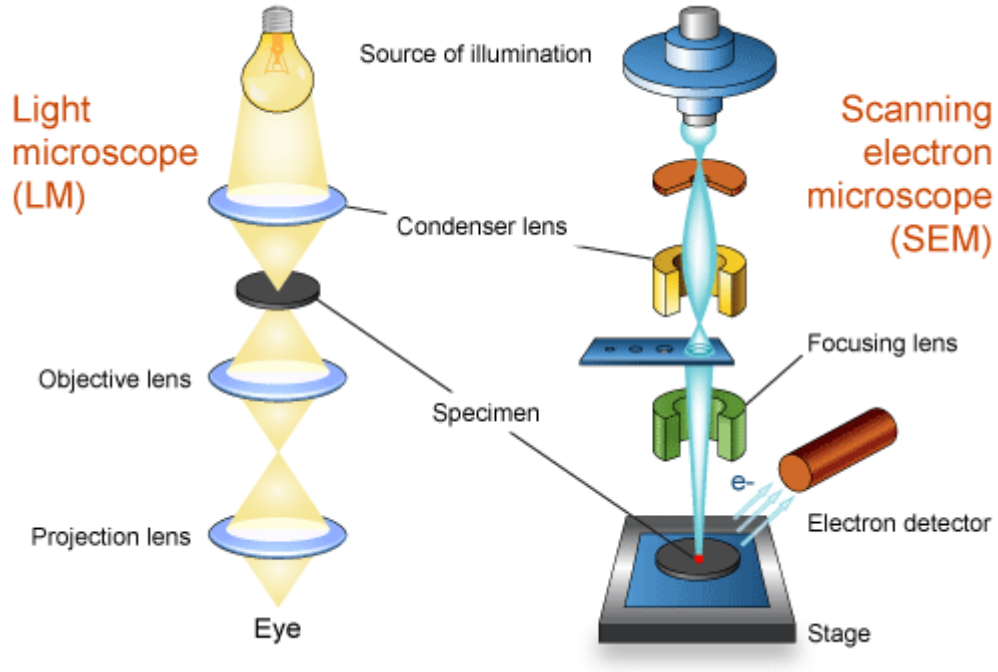


Fig: Comparison of light microcope and electron microscope

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.

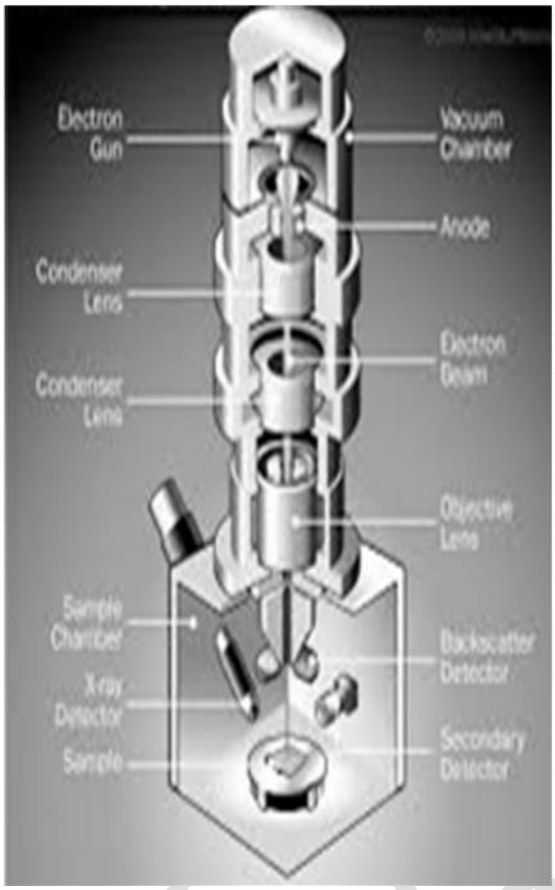


Fig: Scanning electron microscope

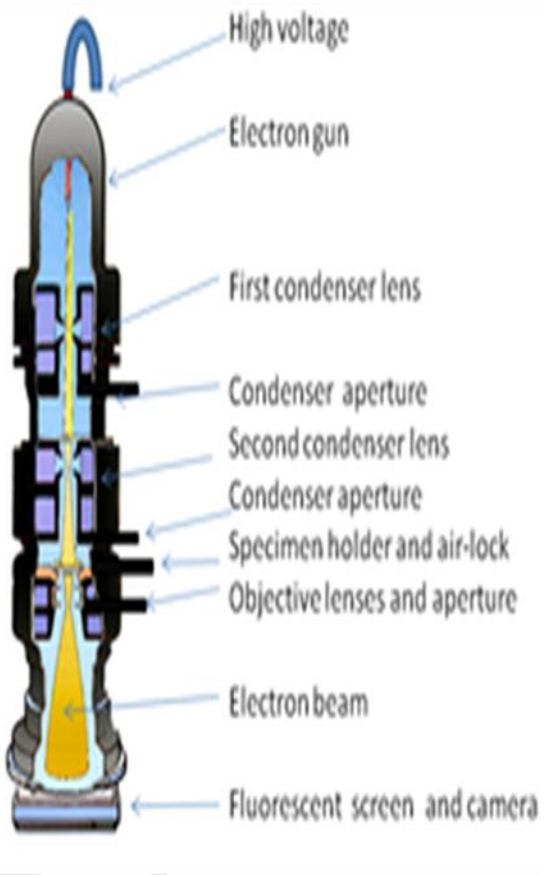


Fig: Transmission electron microscope

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.

Fluorescence Activated Cell Sorter (FACS)

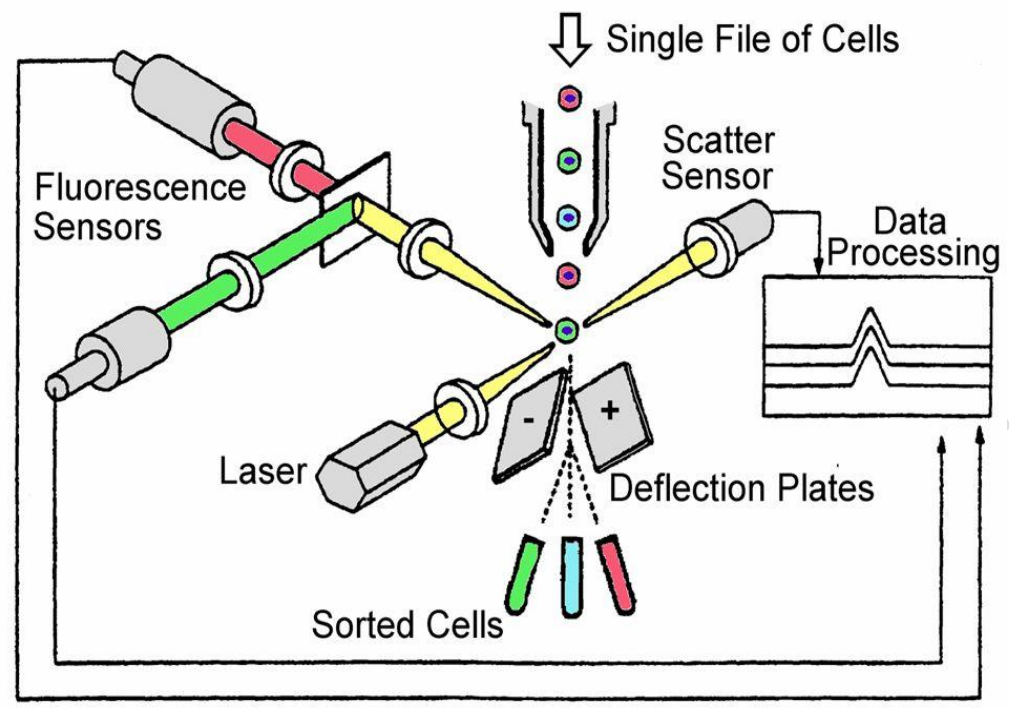


Figure: 1: Flow chart depicting the procedures of FACS

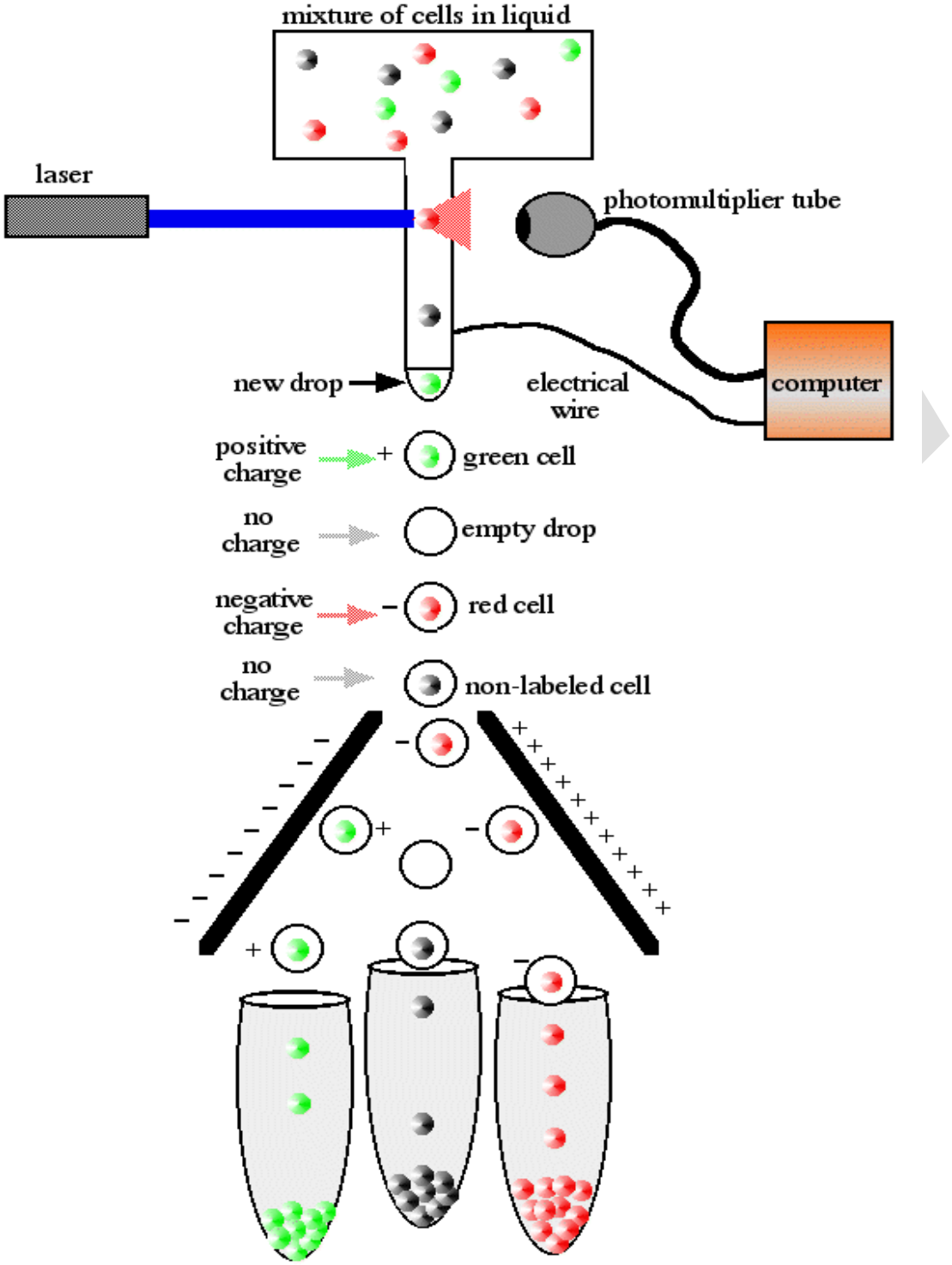


Figure 2: Diagram of FACS machine.

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

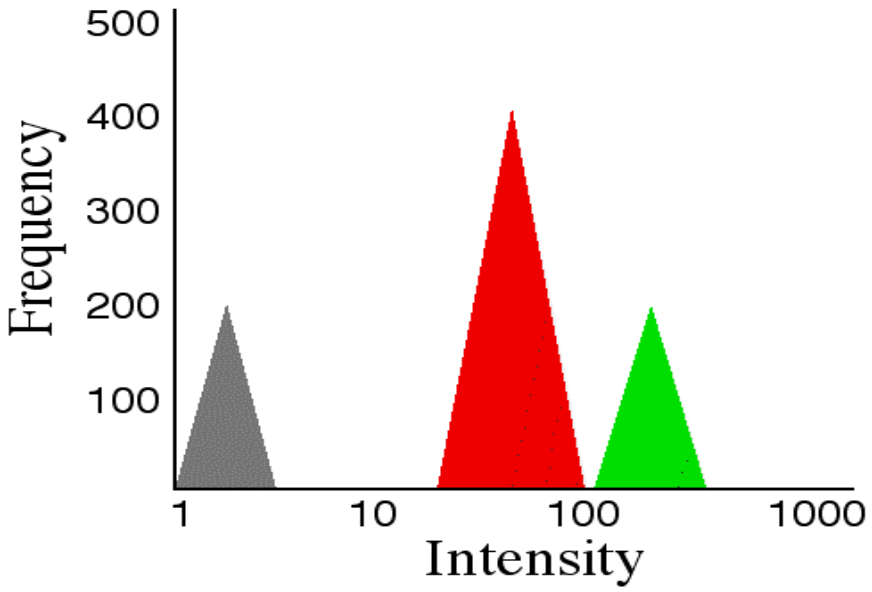


Figure 3. Quantification of FACS data. This graph shows the number of cells (Y-axis) and the level of fluorescence emitted (X-axis) by the labeled cells. Many different colors can be plotted on this graph, but cells should not be labeled by more than one color.

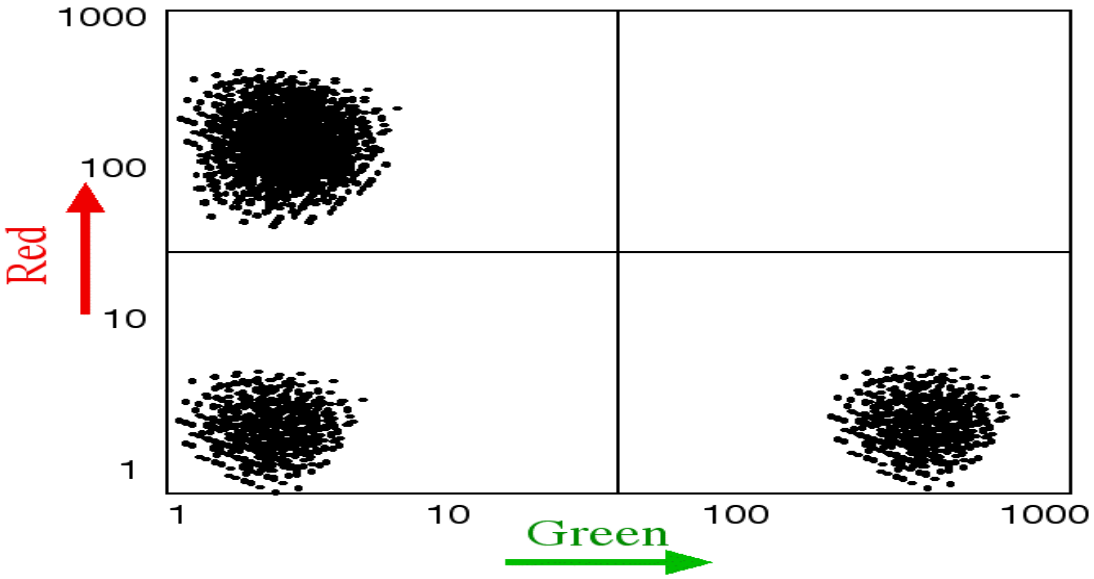


Figure 4. Quantification of FACS data. This graph compares the number of cells labeled by two colors - red (Y-axis) and green (X-axis). The intensity of the emitted light increases as indicated by the arrows. The number of cells at each intensity is shown by the number of dots where each dot represents 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.

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.

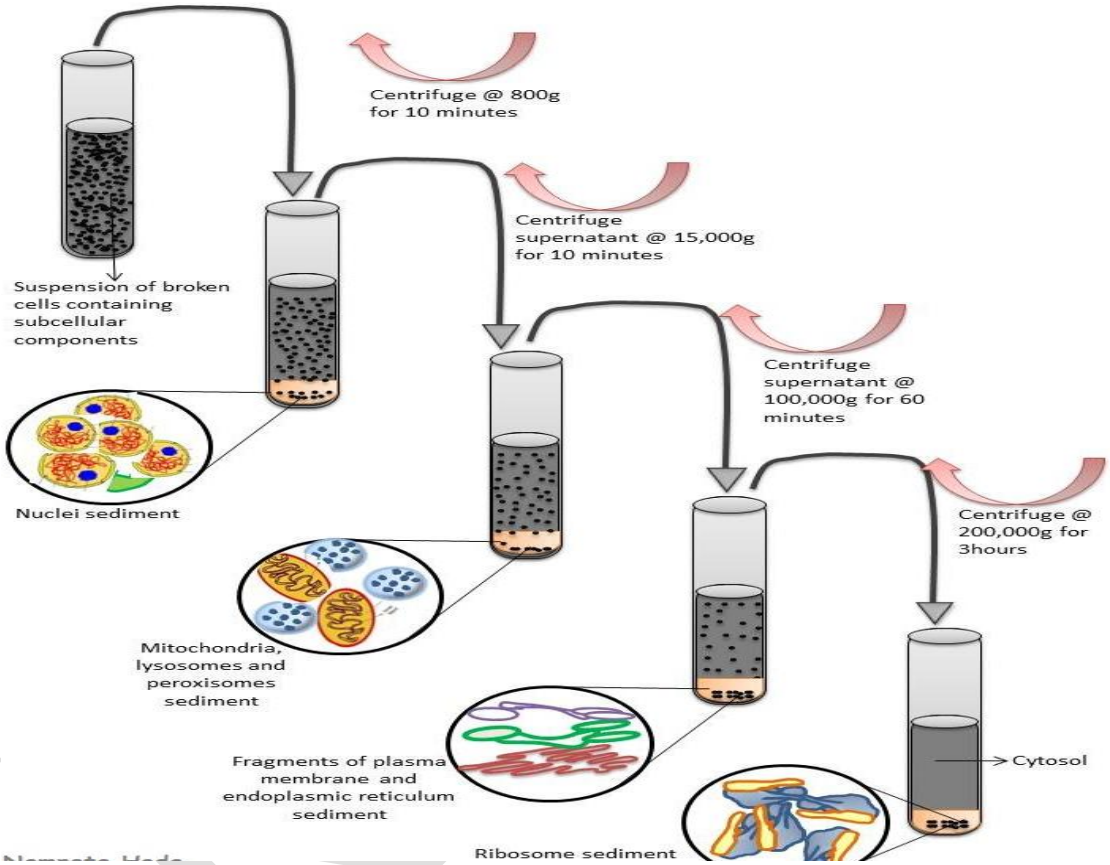


Fig: Differential centrifugation

POSSIBLE QUESTIONS

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 model?
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

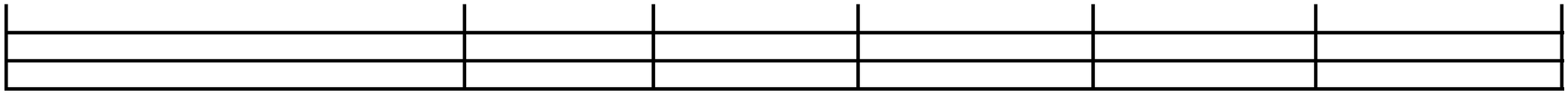
KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
I BSc BIOCHEMISTRY- First Semester
CELL BIOLOGY (19BCU102)
UNIT-I
MULTIPLE CHOICE QUESTIONS

S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	The contribution of Robert Hooke	Coined the term karyokonesis	Coined the term cell membrane	Coined the term cell	Invented microscope	Coined the term cell
2	Schleiden and Schwann proposed cell theory in the year	1949	1839	1769	1689	1839
3	Prokaryotic cells lack	Cell wall	Cytoplasm	DNA	Nucleus	Nucleus
4	----------is the structural and functional unit of all organisms	Cell	DNA	RNA	Gene	Cell
5	Bacteria is Classified in to gram positive and gram negative on the basis of	Cell wall	Nucleus	Mitochondria	Shape	Cell wall
6	The heredity unit of cell is	Genes	Cell membrane	Ribosomes	Nucleus	Genes
7	Important feature of the plant cell is the presence of	Microsome	Cytoplasm	Cell wall	Mitochondria	Cell wall
8	Simple non-nucleated cells are ---------	Prokaryotic cells	Eukaryotic cells	Stem cells	Blood cells	Prokaryotic cells
9	Cell wall is absent in	Mycoplasma	Gram positive bacteria	Gram-negative Bacteria.	plant cell	Mycoplasma
10	In prokaryotes the nuclear body is called -------	Nucleoid	Nucleosome	Plasmid	Nucleus	Nucleoid
11	Cell model for plant species is	C.elegans	A.thaliana	Xenopus oocyte	Zebrafish	A.thaliana
12	Prokaryotic cell does not contain all except	Centriole	DNA	Nuclear envelope	Nuc1eolus	DNA
13	---------- cells does not contain important internal membrane-bound compartments	Prokaryotic	Eukaryotic	Fungi	worms	Prokaryotic
14	Plant cell wall contains	Cellulose	Cytoskeleton	Sucrose	Amylose	Cellulose
15	Animal with 99% homology with human is	Xenopus oocyte	Zebrafish	Mouse	Pig	Mouse
16	Among the following which is eukaryotic unicellular organism	E.coli	S.typhi	S.cerevisiae	C.elegans	S.cerevisiae
17	_____ have also been to play role in certain infectious disease	Glycolipids	Glyco proteins	Lipo proteins	Phospholipids	Glycolipids

18	Integral proteins are bound to membrane by _____	Ionic bond	Covalent bond	Hydrogen bond	Disulphide bond	Covalent bond
19	Which carbohydrate is abundant in Plasma memberane?	Glycocerebroside	Cerebroside	Ganglioside	Galactosamide	Ganglioside
20	Cell surface antigens are ____	Phospholipid	Cerebroside	Glycoproteins	Sphingomyelin	Glycoproteins
21	The fluid model was introduced by	S.J Singer and J.D Robertson	J.N Robertson	Jacob and Monad	S.J Singer and G.Nicolson.	S.J Singer and G.Nicolson.
22	_____ contains galactolipids.	Mitochondria	Chloroplast	Golgi complex	Endoplasmic reticulum	Chloroplast
23	In Glycoprotein, the carbohydrate present is not less than_____ per chain	20 sugars	35 sugars	15 Sugars	10 Sugars	15 Sugars
24	In which ratio the lipids and proteins are bound in membrane bilayer	1:02	1:01	2:01	2:02	2:01
25	The lipids conjugated with carbohydrate that present in membrane bilayer is called	Glycolipid	Phospholipid	Ceramide	Sphingomyelin	Glycolipid
26	Reserve materials of bacteria which are stored in the cytoplasm in granules called+B49	Storage depots	Inclusion bodies	Vacuoles	Plastids	Storage depots
27	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
28	The prokaryotic cells contains	70s ribosomes	80s ribosomes	60s ribosomes	50s ribosomes	70s ribosomes
29	Glycocalyx is	polysaccharide	loose carbohydrate coat of the cell	lipid	carrier protein	loose carbohydrate coat of the cell
30	Cells that are engaged in protein synthesis will have well developed	RER	SER	glyoxysomes	peroxysomes	RER
31	Rough endoplasmic reticulum is embedded with	Proteins	RNA	Ribosomes	Lipids	Ribosomes
32	The golgi apparatus is important for	protein synthesis	DNA synthesis	packaging and secretion of proteins	RNA synthesisis	packaging and secretion of proteins
33	Bilayer fluidity mainly determined by	Phospholipid	protein	Cholesterol	Glycolipid	Cholesterol
34	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
35	Plasma membrane is also called as	Plasmalemma	Periplasm	Cytoplasm	Cell wall	Plasmalemma

36	---------- type of substance not found in cell membrane	phospholipids	glycolipids	steroids	Nucleic acids	Nucleic acids
37	The majority of cases lipid in cell membrane is	phospholipids	glycolipids	steroids	Nucleic acids	phospholipids
38	The entire membrane is held together by---------- interaction of hydrophobic tails	covalent	non covalent	dipeptide	hydroxide	non covalent
39	The large amount of ---------- is responsible for various activities of cell membrane.	proteins	lipids	amino acids	fatty acids	proteins
40	The cell membrane contains ----------"tail" regions	hydrophobic	hydrophilic	thermophilic	mesophilic	hydrophobic
41	The cell membrane contains ----------"head" regions	hydrophobic	hydrophilic	thermophilic	hydrophonic	hydrophilic
42	Cellular components of lipid bilayer is visualized by	SEM	TEM	DFM	EM	TEM
43	Key role of cell membrane is to maintain the	cell function	cell potential	cell organelles	cell structure	cell potential
44	---------- provides the shape of the cell	cytoskeleton	matrix	cytoplasm	organelles	cytoskeleton
45	---------- are described as amphipathic in plasma membrane	lipids	proteins	steroids	carbohydrates	lipids
46	---------- is a common component of animal cell membranes and functions to help stabilize the membrane	Glycerol	Cholesterol	triglycerides	Fat	Cholesterol
47	---------- affects the fluidity of the membrane.	Temperature	pH	Osmolality	Pressure	Temperature
48	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
49	Magnification of light microscope is	1500X	2000X	1000X	2500X	1500X
50	Photograph which is taken from microscope is known as	macrograph	monograph	micrograph	pictograph	micrograph
51	Object can be magnified under electron microscope about	350, 000 times	250, 000 times	300, 000 times	450, 000 times	300, 000 times
52	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

53	Which of the following light is suitable for getting maximum resolution?	Red	Green	Blue	Orange	Blue
54	Which of the following is used to visualize live cells?	SEM	TEM	Phase contrast microscope	Compound microscope	Phase contrast microscope
55	The strength of magnification of oil immersion lens	20X	100X	50X	40X	100X
56	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
57	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
58	The lens that is within the eyepiece of the light microscope is called the	Scanning	Low power	High power	Ocular	Ocular
59	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
60	What is the correct name for the main microscope lens that focuses the image?	Ocular	Binocular	Objective	Condenser	Objective
61	Whicj organism serve as border between prokaryote a	E.coli	S.cerevisiae	D.discoidium	C.elegans	D.discoidium
62	Main organism which helpful to study the body plan formation is	S.cerevisiae	D.discoidium	C.elegans	D.melanogaster	D.melanogaster
63	Among the following organism which is having close proximity with human genome	Frog	Mouse	C.elegans	Zebra fish	Mouse



UNIT II - COURSE MATERIAL

Structure of different cell organelles

Structure of nuclear envelope, nucleao 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.

NUCLEUS

Nucleus is the most important cell organelle which directs and controls all its cellular activities. It occurs in all the eukaryotic cells. The shape and size of the nucleus varies from cell to cell and organism to organism. Usually single nucleus is found in the cells of higher plants and animals.

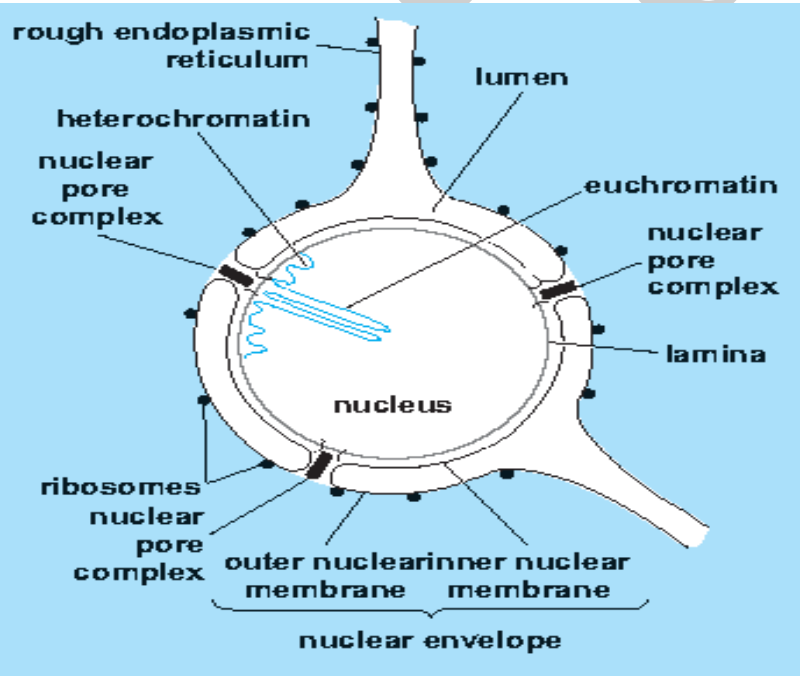


Figure : The schematic representation of nucleus.

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CELL NUCLEUS: ULTRASTRUCTURE

The structure of a cell nucleus consists of a nuclear membrane (nuclear envelope), nucleoplasm, nucleolus, and chromosomes. Nucleoplasm, also known as karyoplasm, is the matrix present inside the nucleus. Following section discusses in brief about the several parts of a cell nucleus.

The ultrastructure of the nucleus reveals that it is made up of

(i) **nuclear membrane** – It encloses the nucleoplasm

(ii) **nucleoplasm** – It is mainly composed of nucleoproteins and a small amount of organic and inorganic substances like nucleic acids, dissolved phosphorus, proteins, enzymes, minerals, ribose sugars and nucleotides. The nuclear reticulum and nucleolus remain suspended in the nucleoplasm

(iii) **chromatin** – It is basophilic in nature. Most of the chromatin material is transformed into a specific number of chromosomes during cell division

(iv) **nucleolus** – It lacks membrane and composed of proteins and nucleic acids found within the nucleus.

Functions of the nucleus

It controls the hereditary characteristics of an organism. This organelle is also responsible for the protein synthesis, cell division, growth, and differentiation. Some important functions carried out by a cell nucleus are:

1. By storing the hereditary material it control gene expression. In nulceus, the genes are present in the form of long and thin DNA (deoxyribonucleic acid) strands, referred to as chromatins.
2. It also stores proteins and RNA (ribonucleic acid) in the nucleolus.
3. Nucleus is the site for transcription in which messenger RNA (mRNA) are produced for the protein synthesis.
4. Exchange of hereditary molecules (DNA and RNA) between the nucleus and rest of the cell.
5. During the cell division, chromatins are arranged into chromosomes in the nucleus.
6. Production of ribosomes (protein factories) in the nucleolus.
7. Selective transportation of regulatory factors and energy molecules through nuclear pores.
8. Cell compartmentalization is the function of the nuclear envelope. It entails separation of the contents of the nucleus from the cytoplasm in order to maintain the identity of the nucleus
9. The nuclear envelope also separates the cytoplasmic process from the nuclear

process and prevents translation of unspliced mRNA, which is a product of the mRNA splicing process.

As the nucleus regulates the integrity of genes and gene expression, it is also referred to as the control center of a cell. Overall, the cell nucleus stores all the chromosomal DNA of an organism.

Other functions

1. Nuclear Transport:
   Nuclear transport is carried out by the pores present in the nuclear envelope. The entry and exit of molecules is controlled by this structure. Cargo proteins are carried from the cytoplasm to the nucleus with the help of exportins while macromolecules like RNA are exported to the cytoplasm in association with importins and karyopherins. Thus, transportation takes place efficiently through the nuclear membrane.
2. The nucleoplasm acts as reservoir of various enzymes and nucleotides
3. Nucleolus is involved in formation of ribosomes, helps in cell division, synthesis of RNA and protein, reservoir of RNA.

NUCLEAR ENVELOPE

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.

1. Underlying the inner nuclear membrane is the nuclear lamina, a fibrous meshwork that provides structural support to the nucleus (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.

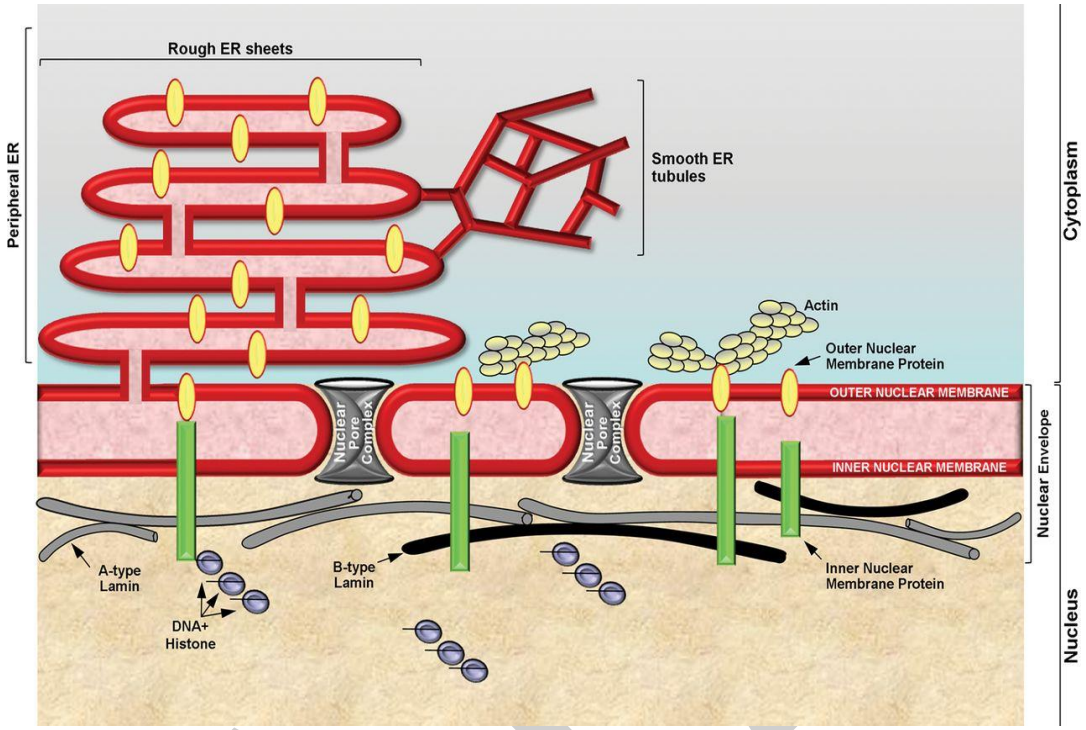


Fig: Structure of nuclear envelope

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.

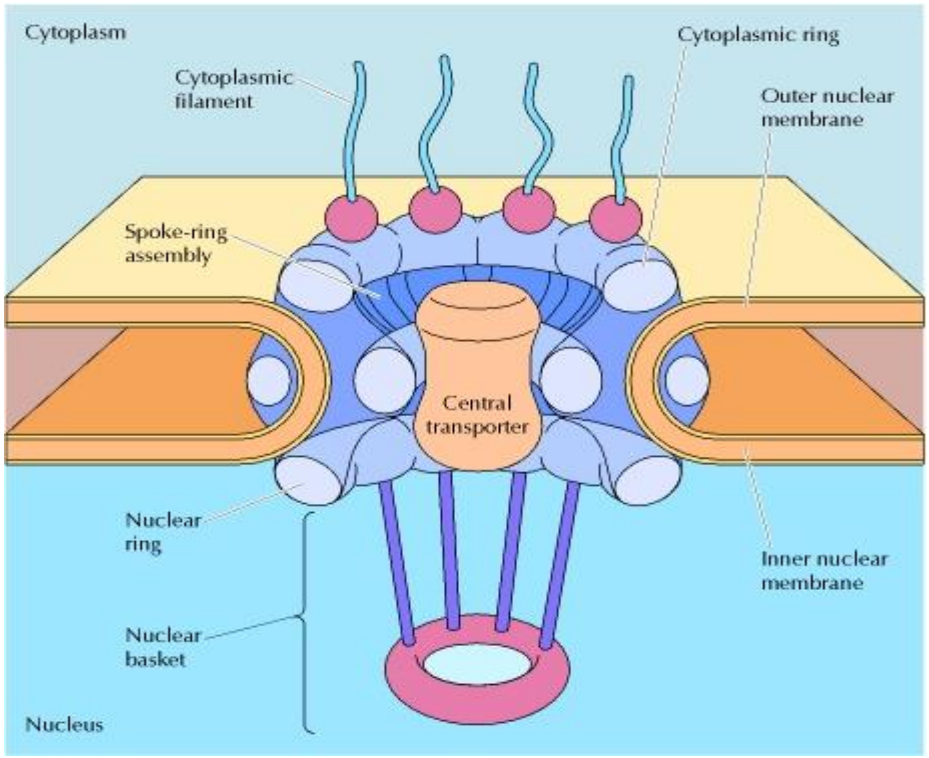


Figure: Model of the nuclear pore complex

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.

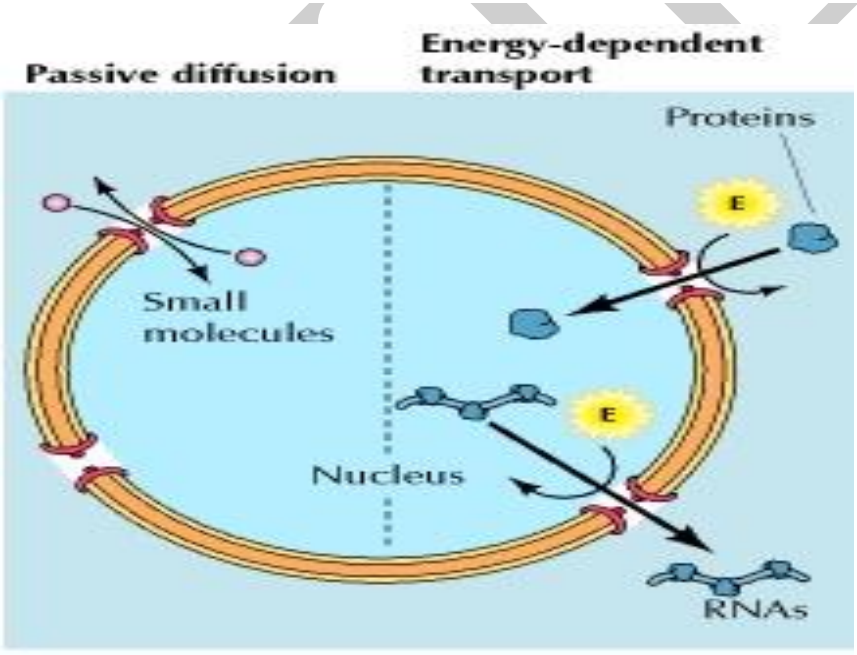


Figure: Molecular traffic through nuclear pore complexes: Small molecules are able to pass rapidly through open channels in the nuclear pore complex by passive diffusion. In contrast, macromolecules are transported by a selective, energy-dependent mechanism that acts predominantly to import proteins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

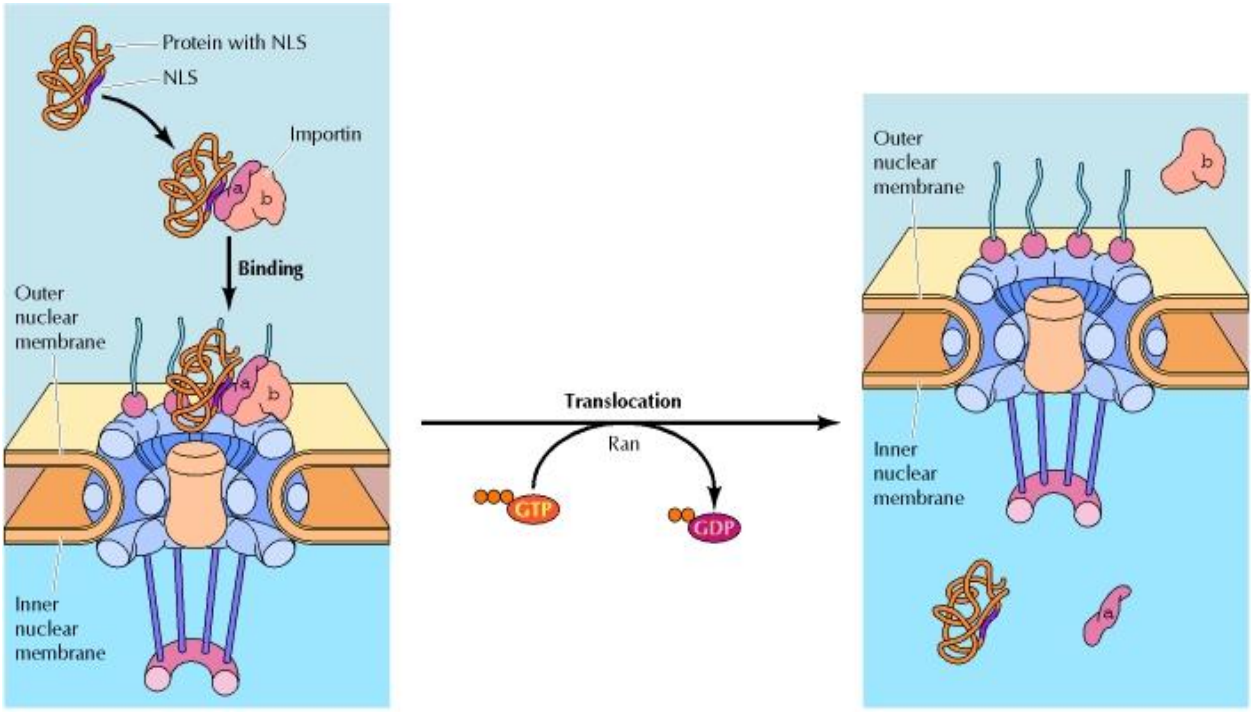


Figure -Protein import through the nuclear pore complex: Proteins are transported through the nuclear pore complex in two steps. In the example shown, a protein with a classical basic amino acid-rich nuclear localization sequence (NLS) is recognized by importin α, which forms a complex with importin 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 with importin 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.

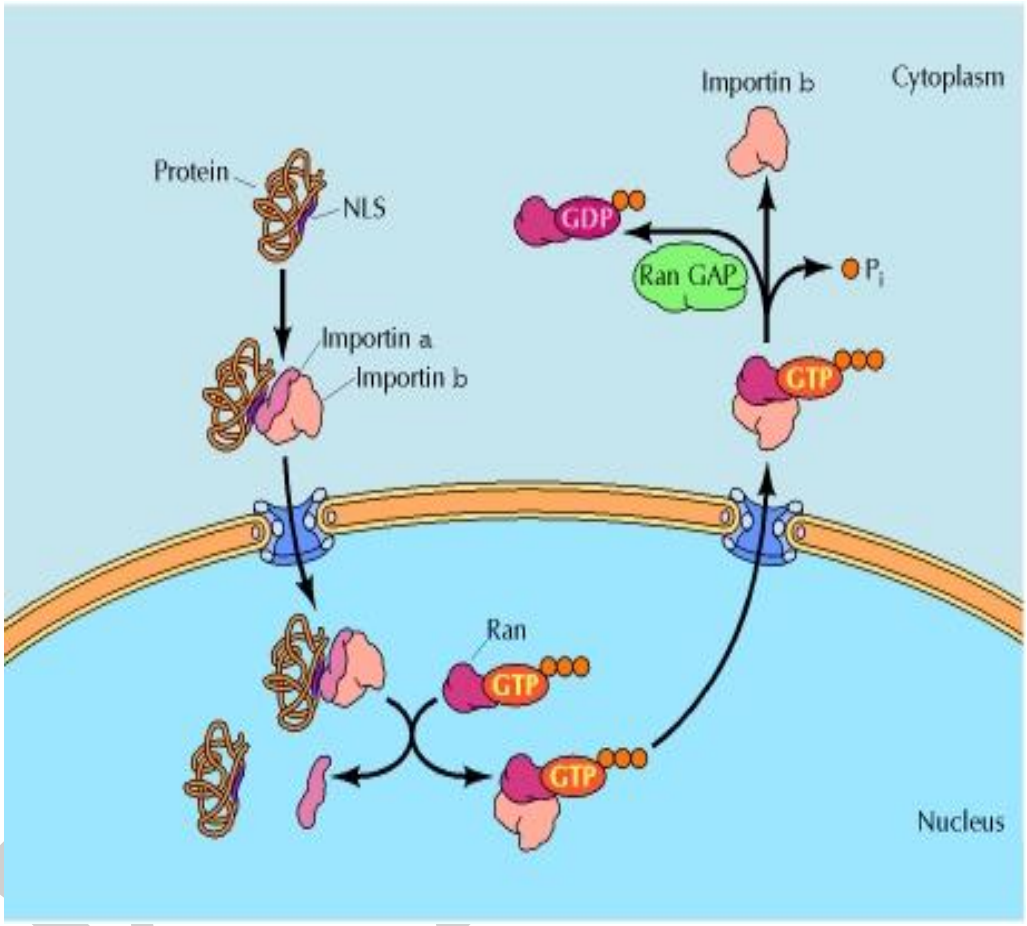


Figure Role of the Ran protein in nuclear import: Transport through the nuclear pore complex is driven by a gradient of Ran/GTP, with a high concentration of Ran/GDP in the cytoplasm and a high concentration of Ran/GTP in the nucleus. Complexes form between target proteins containing nuclear localization 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.

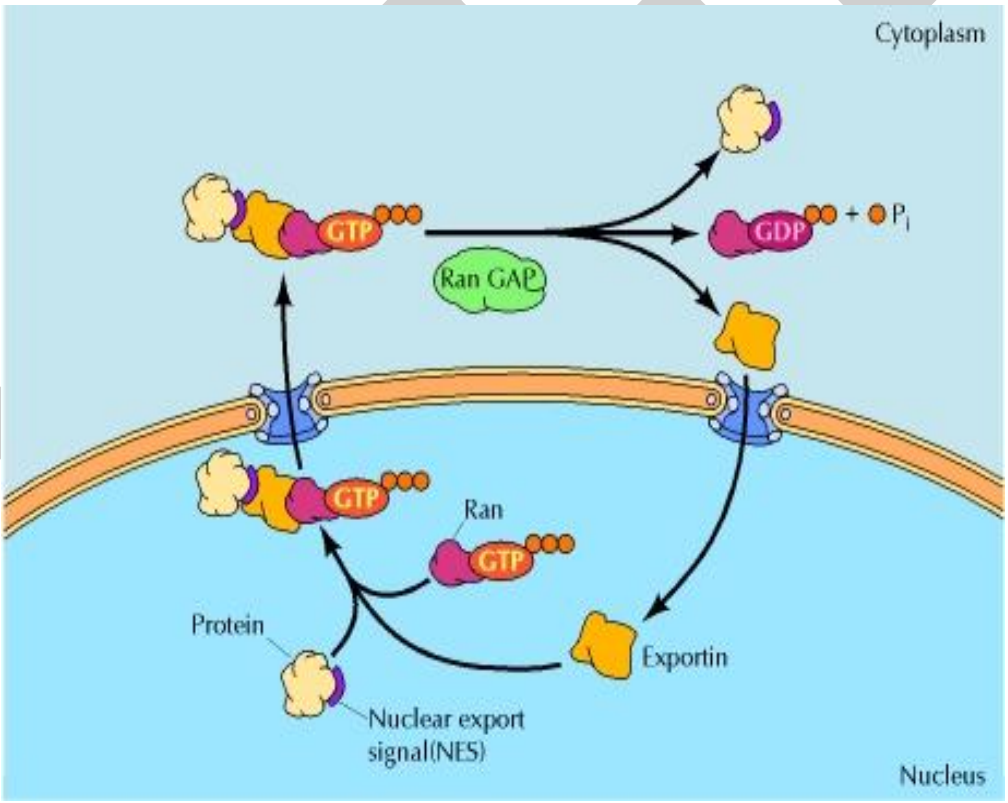


Figure Nuclear export: Complexes between target proteins bearing nuclear export signals (NES), exportins, and Ran/ GTP form in the nucleus. Following transport through thenuclear pore complex, Ran GAP stimulates the hydrolysis of bound GTP, leading to formation of Ran/GDP and release of the target protein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

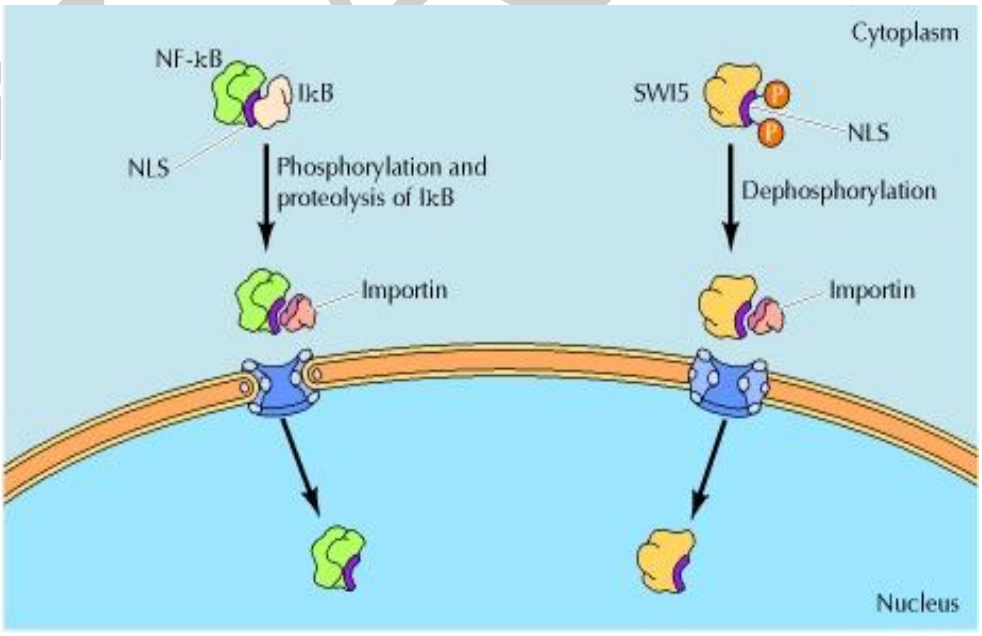


Figure- Regulation of nuclear import of transcription factors: The transcription factor NF-κB is maintained as an inactive complex with IκB, which masks its nuclear localization sequence (NLS), in the cytoplasm. In response to appropriate extracellular signals, IκB is phosphorylated and degraded by proteolysis, 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

Endoplasmic reticulum is a network of interconnected internal membranes generally, the largest membrane in a eukaryotic cell—an extensive network of closed, flattened membrane-bounded sacs called cisternae (Figure 3). The name "endoplasmic reticulum" was coined in 1953 by Porter, who had observed it in electron micrographs of liver cells. The endoplasmic reticulum has a number of functions in the cell but is particularly important in the synthesis of lipids, membrane proteins, and secreted proteins.

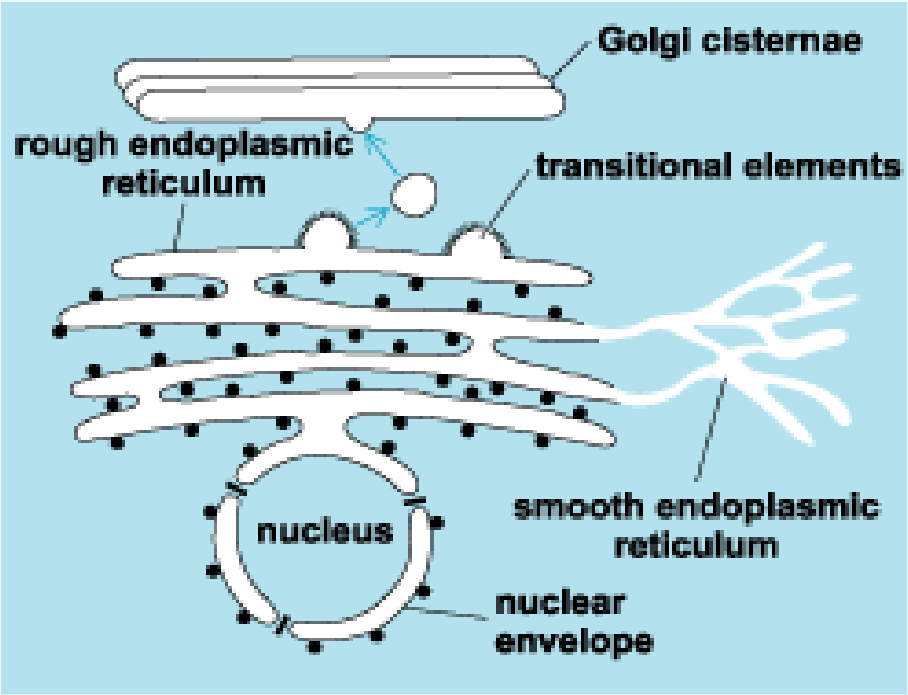


Fig: Ultra structure of endoplasmic reticulum

Occurrence:

The occurrence of the endoplasmic reticulum is in eukaryotic cells with variation in its position from cell to cell. The erythrocytes (RBC), egg and embryonic cells lack in endoplasmic reticulum. ER is poorly developed in certain cells as the RBC which produces only proteins to be retained in the cytoplasmic matrix (haemoglobin), although the cell may contain many ribosomes). The spermatocytes also have poorly developed endoplasmic reticulum.

Morphology:

The endoplasmic reticulum occurs in three forms: 1. Lamellar form or cisternae which is a closed, fluid-filled sac, vesicle or cavity is called cisternae; 2. vesicular form or vesicle and 3. tubular form or tubules.

1. Cisternae: The cisternae are long, flattened, sac-like, unbranched tubules having diameter of 40 to 50 µm. They remain arranged parallely in bundles or stakes. RER mostly exists as cisternae which occur in those cells which have synthetic roles as the cells of pancreas, notochord and brain.

2. Vesicles: The vesicles are oval, membrane-bound vacuolar structures having diameter of 25 to 500 µm. They often remain isolated in the cytoplasm and occur in most cells but especially abundant in the SER.

3. Tubules: The tubules are branched structures forming the reticular system along with the cisternae and vesicles. They usually have the diameter from 50 to 190 µm and occur almost in all the cells. Tubular form of ER is often found in SER and is dynamic in nature, *i.e.*, it is associated with membrane movements, fission and fusion between membranes of cytocavity network.

Ultrastructure:

- The endoplasmic reticulum is a network of folded membranes that form channels. They extend from the cell membrane through the cytoplasm to the nuclear
- The membranes of the ER are concentrated in the inner region of the cell, the endoplasmic region.
- The membranes surround and inner cavity called the lumen. The lumen is basically a compartment for storage of substances that must be kept separate from the cytoplasm.
- It consists of membrane lined channels or spaces. The channels or spaces contain a fluid called endoplasmic matrix. It is quite different from cytoplasmic matrix present out side the reticulum. The membranes of endoplasmic reticulum or 50-60 A° thick.
- Cisternae are flattened, unbranched, saclike elements with a diameter of 40-50 nm. The lie in stacks parallel to one another and the sacs in the stack are interconnected with one another.
- Tubules tube light extensions which may be connected with cisternae or to form a reticular system.

The cavities of cisternae, vesicles and tubules of the endoplasmic reticulum are bounded by a thin membrane of 50 to 60 Aº thickness. The membrane of endoplasmic reticulum is fluid-mosaic like the unit membrane of the plasma membrane, nucleus, Golgi apparatus. The membrane of endoplasmic reticulum remains continuous with the membranes of plasma membrane, nuclear membrane and Golgi apparatus. The cavity of the endoplasmic reticulum is well developed and acts as a passage for the secretory products. Palade in the year 1956 has observed secretory granules in the cavity of endoplasmic reticulum amking it rough in appearance. Sometimes, the cavity of RER is very narrow with two membranes closely apposed and is much distended in certain cells which are actively engaged in protein synthesis (acinar cells, plasma cells and goblet cells). The membranes of the endoplasmic reticulum contains many kinds of enzymes which are needed for various important synthetic activities. Some of the most common enzymes are found to have different transverse distribution in the ER membranes. The most important

enzymes are the stearases, NADH-cytochrome C reductase, NADH diaphorase, glucose-6-phosphotase and Mg++ activated ATPase. Certain enzymes of the endoplasmic reticulum such as nucleotide diphosphate are involved in the biosynthesis of phospholipid, ascorbic acid, glucuronide, steroids and hexose metabolism.

Types of endoplasmic reticulum:

a) Agranular or smooth endoplasmic reticulum:

ER with no studded ribosomes makes it smooth in appearance. The adipose tissues, brown fat cells and adrenocortical cells, interstitial cells of testes and cells of corpus luteum of ovaries, sebaceous cells and retinal pigment cells contain only smooth endoplasmic reticulum (SER). The synthesis of fatty acids and phospholipids takes place in the smooth ER. It is abundant in hepatocytes. Enzymes in the smooth ER of the liver modify or detoxify hydrophobic chemicals such as pesticides and carcinogens by chemically converting them into more water-soluble, conjugated products that can be excreted from the body. High doses of such compounds result in a large proliferation of the smooth ER in liver cells.

b) Granular or rough endoplasmic reticulum:

Ribosomes bound to the endoplasmic reticulum make it appear rough. The rough ER synthesizes certain membrane and organelle proteins and virtually all proteins to be secreted from the cell. A ribosome that fabricates such a protein is bound to the rough ER by the nascent polypeptide chain of the protein. As the growing polypeptide emerges from the ribosome, it passes through the rough ER membrane, with the help of specific proteins in the membrane. Newly made membrane proteins remain associated with the rough ER membrane, and proteins to be secreted accumulate in the lumen of the organelle. All eukaryotic cells contain a discernible amount of rough ER because it is needed for the synthesis of plasma membrane proteins and proteins of the extracellular matrix. Rough ER is particularly abundant in specialized cells that produce an abundance of specific proteins to be secreted. The cells of those organs which are actively engaged in the synthesis of proteins such as acinar cells of pancreas, plasma cells, goblet cells and cells of some endocrine glands are found to contain rough endoplasmic reticulum (RER) which is highly developed.

Rough endoplasmic reticulum and protein secretion:

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.

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 endoplasmic 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.
5. 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.
6. 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)

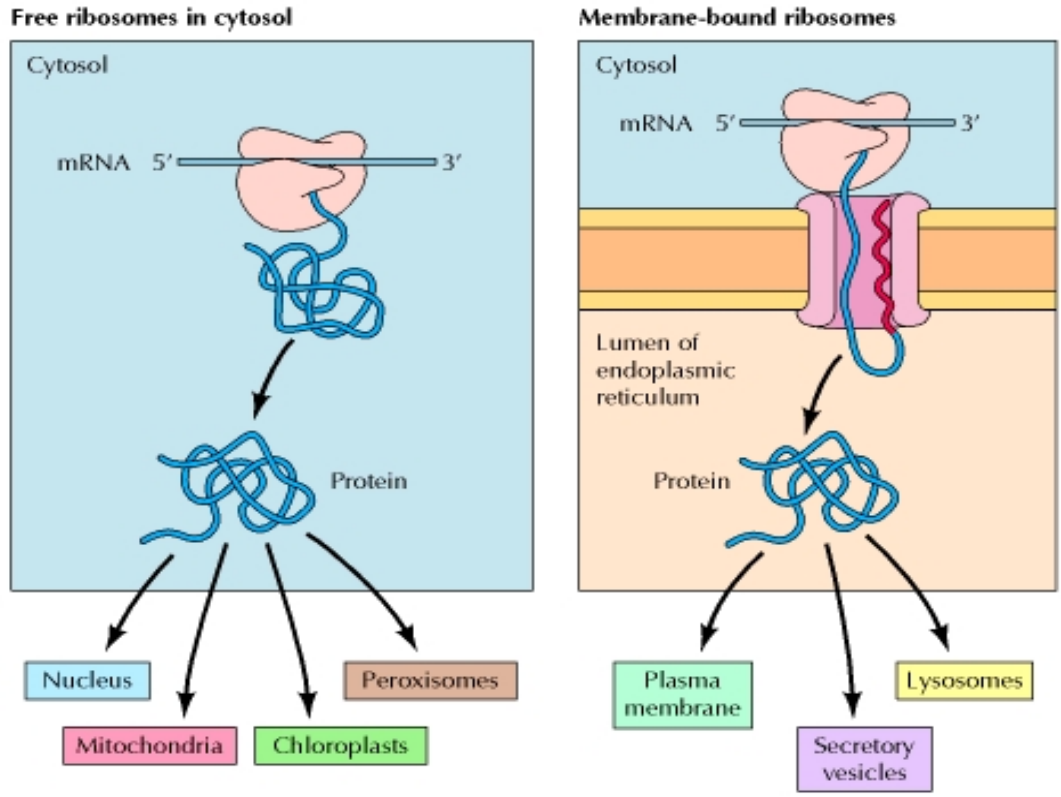
1. It gives mechanical support to the colloidal cytoplasmic matrix
2. It plays a major role in the inter-connection of cells through plasmadesmata
3. It helps in the regulation of entry and exit of materials
4. The ER acts as a circulatory or transporting system for various substances
5. The ER membrane 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
10. It helps in the transportation of genetic material
11. ER membranes provide a site for ATP synthesis in the cel
12. The rough ER play a major role in protein synthesis because of the presence of Ribosomes
13. The smooth ER is involved in the synthesis of lipids, glycogen, polysaccharide metabolism and detoxification process

Endoplasmic reticulum in protein processing and sorting

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.

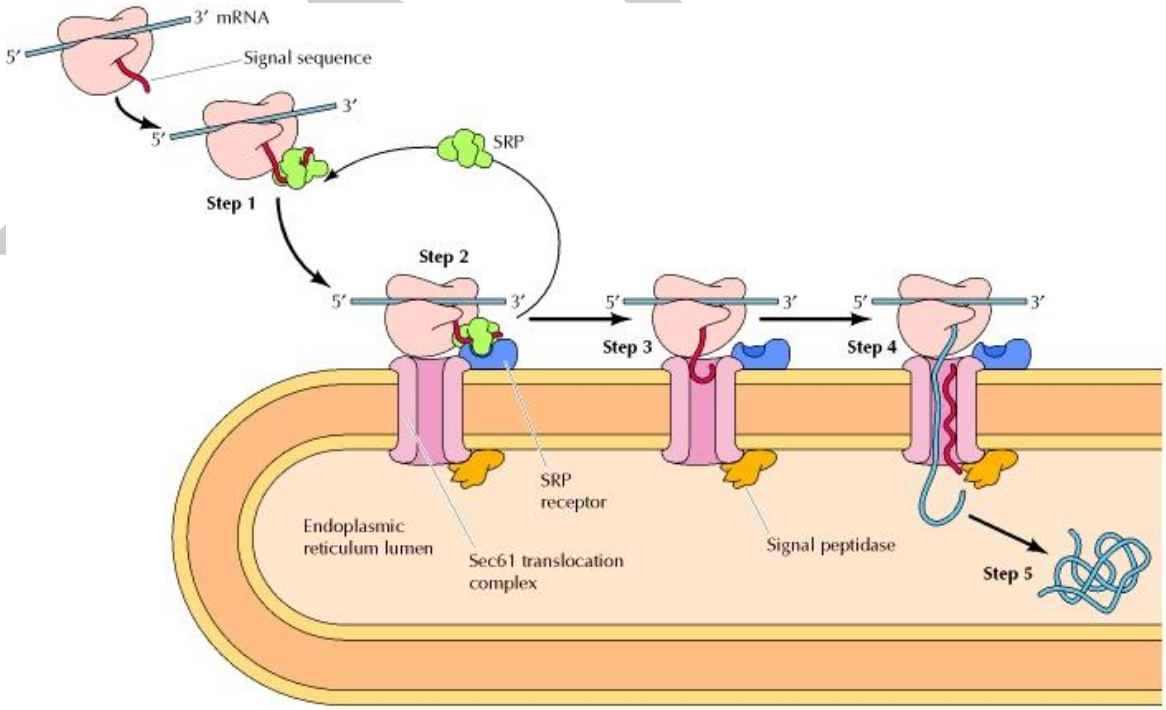


Figure: **Cotranslational targeting of secretory proteins to the ER**

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.

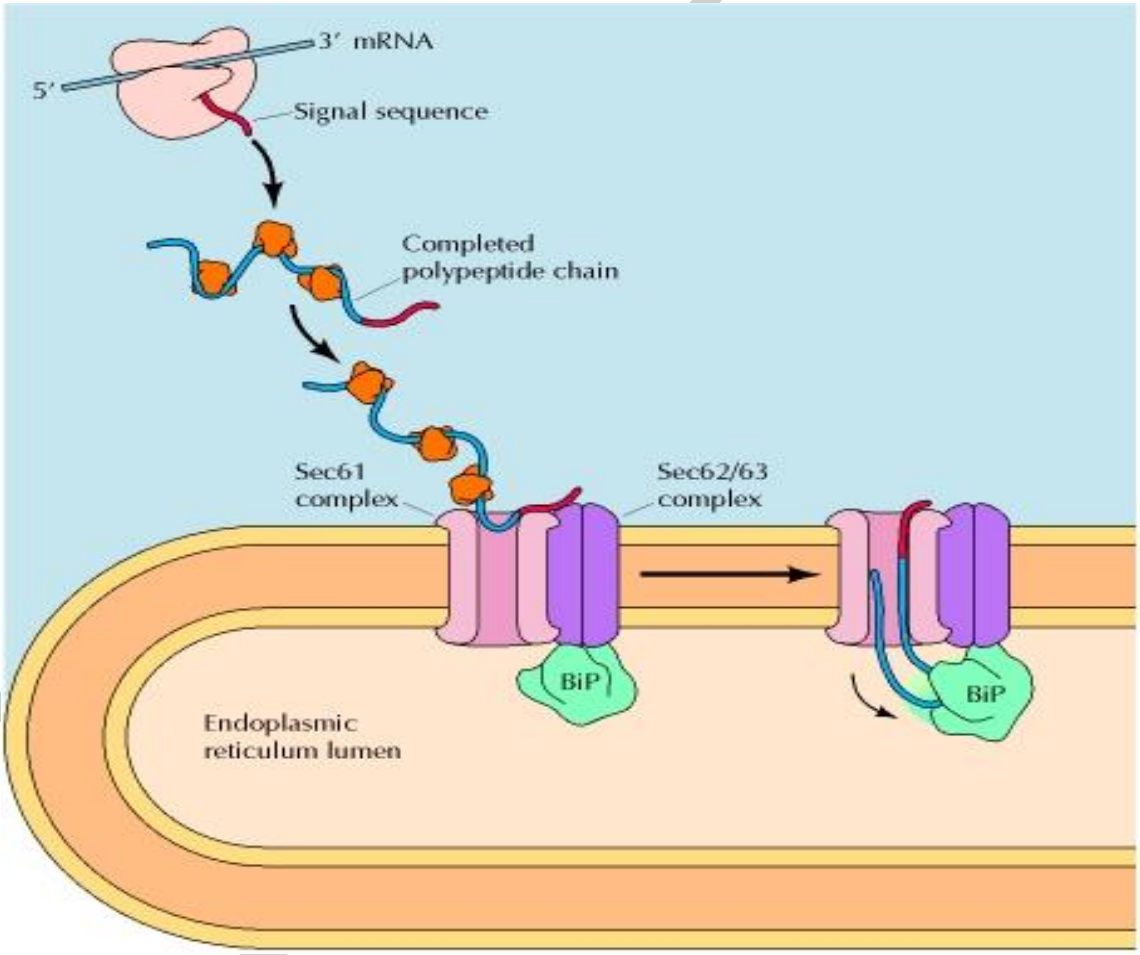


Figure Posttranslational translocation of proteins into the ER: Proteins destined for posttranslational import to the ER are synthesized on free ribosomes and maintained in an unfolded conformation by cytosolic chaperones. Their signal sequences are recognized by the Sec62/63 complex, which is associated 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.

Smooth ER and lipid synthesis

In addition to its activities in the processing of secreted and membrane proteins, the ER is the major site at which membrane lipids are synthesized in eukaryotic cells. Because they are extremely hydrophobic, lipids are synthesized in association with already existing cellular membranes rather than in the aqueous environment of the cytosol. Although some lipids are synthesized in association with other membranes, most are synthesized in the ER. They are then transported from the ER to their ultimate destinations either in vesicles or by carrier proteins.

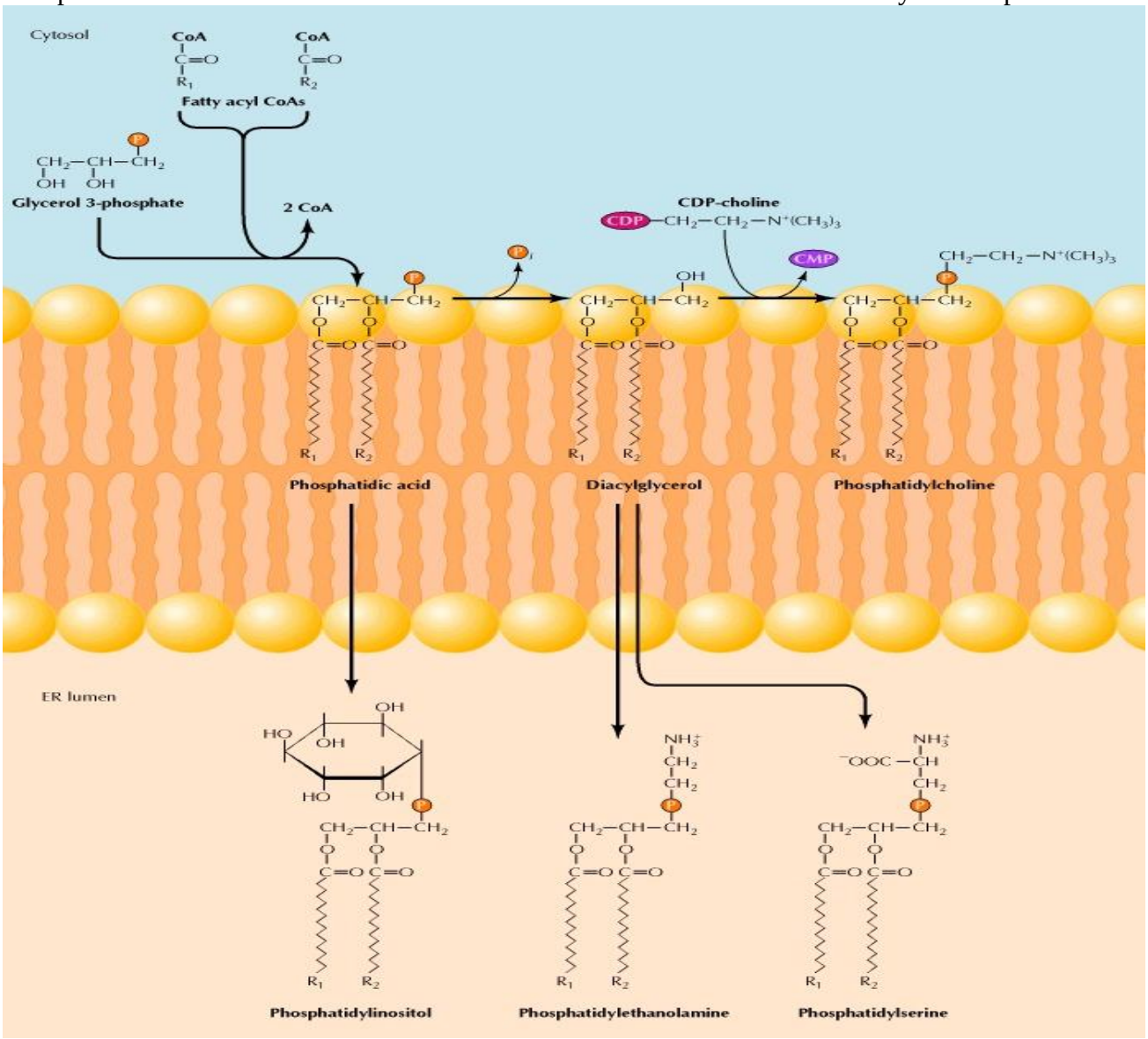


Figure -Synthesis of phospholipids: Glycerol phospholipids are synthesized in the ER membrane from cytosolic precursors. Two fatty acids linked to coenzyme A (CoA) carriers are first joined to glycerol-3-phosphate, yielding phosphatidic acid, which is simultaneously inserted into the membrane. A phosphatase then converts phosphatidic acid to diacylglycerol. The attachment of different polar head groups to diacylglycerol then results in formation of phosphatidylcholine, phosphatidylethanolamine, or phosphatidylserine. Phosphatidylinositol is formed from phosphatidic acid, rather than from diacylglycerol.

The membranes of eukaryotic cells are composed of three main types of lipids: phospholipids, glycolipids, and cholesterol. Most of the phospholipids, which are the basic structural components of the membrane, are derived from glycerol. They are synthesized on the cytosolic side of the ER membrane, from water-soluble cytosolic precursors (Figure). Fatty acids are first transferred from coenzyme A carriers to glycerol-3-phosphate by a membrane-bound enzyme, and the resulting phospholipid (phosphatidic acid) is inserted into the membrane. Enzymes on the cytosolic face of the ER membrane then catalyze the addition of different polar head groups, resulting in formation of phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, or phosphatidylinositol.

The synthesis of these phospholipids on the cytosolic side of the ER membrane allows the hydrophobic fatty acid chains to remain buried in the membrane while membrane-bound enzymes catalyze their reactions with water-soluble precursors (e.g., CDP-choline) in the cytosol. Because of this topography, however, new phospholipids are added only to the cytosolic half of the ER membrane (Figure). To maintain a stable membrane, some of these newly synthesized phospholipids must therefore be transferred to the other (lumenal) half of the ER bilayer. This transfer does not occur spontaneously because it requires the passage of a polar head group through the membrane. Instead, membrane proteins called flippases catalyze the rapid translocation of phospholipids across the ER membrane, resulting in even growth of both halves of the bilayer.

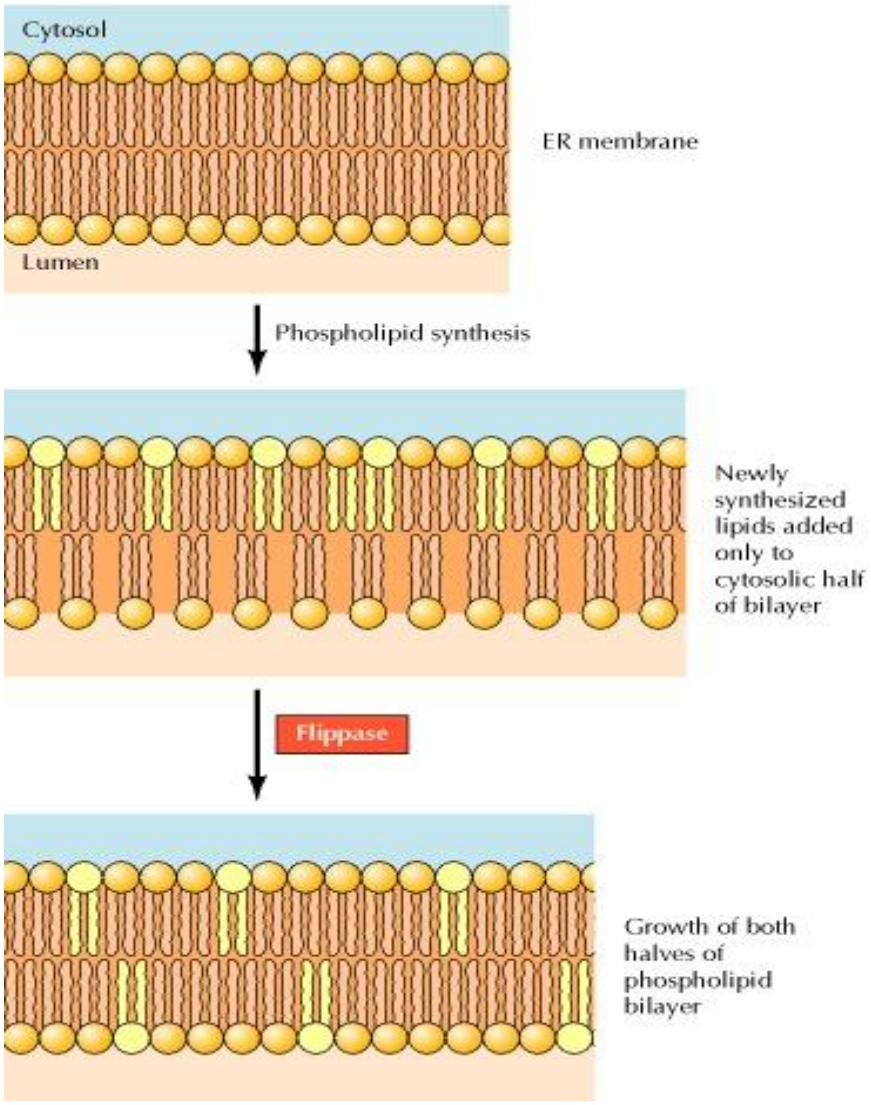


Figure - Translocation of phospholipids across the ER membrane: Because phospholipids are synthesized on the cytosolic side of the ER membrane, they are added only to the cytosolic half of the bilayer. They are then translocated across the membrane by phospholipid flippases, resulting in even growth of both halves of the phospholipid bilayer.

In addition to its role in synthesis of the glycerol phospholipids, the ER also serves as the major site of synthesis of two other membrane lipids: cholesterol and ceramide). As discussed later, ceramide is converted to either glycolipids or sphingomyelin (the only membrane phospholipid not derived from glycerol) in the Golgi apparatus. The ER is thus responsible for synthesis of either the final products or the precursors of all the major lipids of eukaryotic membranes.

Export of proteins and lipids from ER

Both proteins and lipids travel along the secretory pathway in transport vesicles, which bud from the membrane of one organelle and then fuse with the membrane of another. Thus, molecules are exported from the ER in vesicles that bud from the ER and carry their cargo first to the ER-Golgi intermediate compartment and then to the Golgi apparatus(Figure). Subsequent steps in the secretory pathway involve vesicular transport between different compartments of the Golgi and from the Golgi to lysosomes or the plasma membrane. In each case, proteins within the lumen of one organelle are packaged into the budding transport vesicle and then released into the lumen of the recipient organelle following vesicle fusion. Membrane proteins and lipids are transported similarly, and it is noteworthy that their topological orientation is maintained as they travel from one membrane-enclosed organelle to another. For example, the domains of a protein exposed on the cytosolic side of the ER membrane will also be exposed on the cytosolic side of the Golgi and plasma membranes, whereas protein domains exposed on the lumenal side of the ER membrane will be exposed on the lumenal side of the Golgi and on the exterior of the cell.

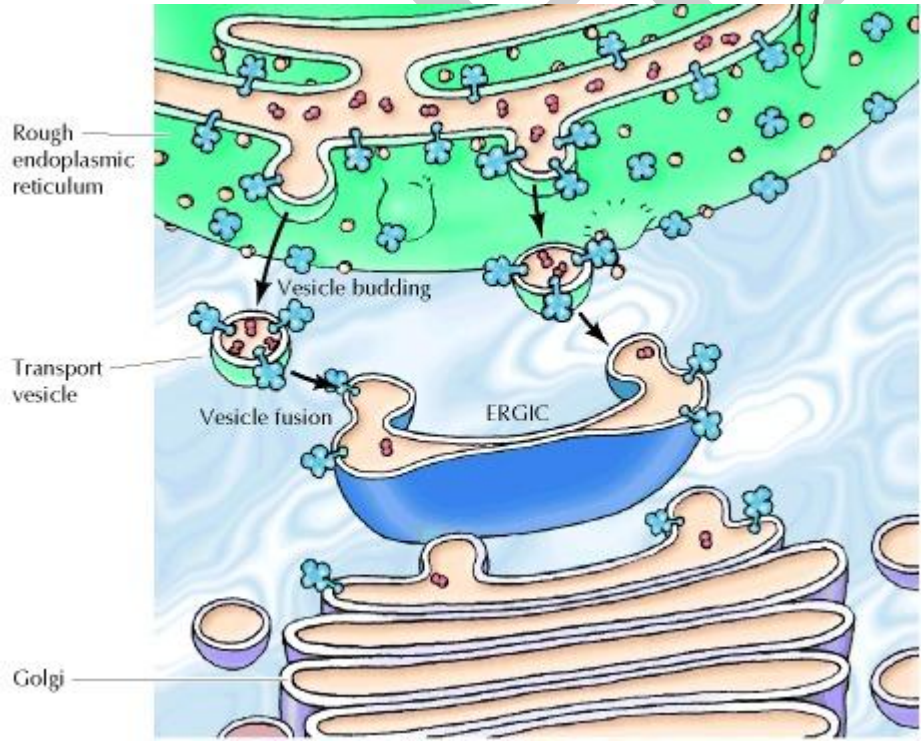


Figure - Vesicular transport from the ER to the Golgi : Proteins and lipids are carried from the ER to the Golgi in transport vesicles that bud from the membrane of the ER and then fuse to form the vesicles and tubules of the ER-Golgi intermediate compartment (ERGIC). Lumenal ER proteins are taken up by the vesicles and released into the lumen of the Golgi. Membrane proteins maintain the same orientation in the Golgi as in the ER

While most proteins travel from the ER to the Golgi, some proteins must be retained within the ER rather than proceeding along the secretory pathway. In particular, proteins that function within the ER (including BiP, signal peptidase, protein disulfide isomerase, and other enzymes discussed earlier) must be retained within that organelle. Export to the Golgi versus retention in the ER is thus the first branch point encountered by proteins being sorted to their correct destinations in the secretory pathway. Similar branch points arise at each subsequent stage of transport, such as retention in the Golgi versus export to lysosomes or the plasma membrane. In each case, specific localization signals target proteins to their correct intracellular destinations.

The distinction between proteins exported from and those retained in the ER appears to be governed by two distinct types of targeting sequences that specifically mark proteins as either (1) destined for transport to the Golgi or (2) destined for retention in the ER. Many proteins are retained in the ER lumen as a result of the presence of the targeting sequence Lys-Asp-Glu-Leu (KDEL, in the single-letter code) at their carboxy terminus. If this sequence is deleted from a protein that is normally retained in the ER (e.g., BiP), the mutated protein is instead transported to the Golgi and secreted from the cell. Conversely, addition of the KDEL sequence to the carboxy terminus of proteins that are normally secreted causes them to be retained in the ER. The retention of some transmembrane proteins in the ER is similarly dictated by short C-terminal sequences that contain two lysine residues (KKXX sequences).

Interestingly, the KDEL and KKXX signals do not prevent soluble ER proteins from being packaged into vesicles and carried to the Golgi. Instead, these signals cause resident ER proteins to be selectively retrieved from the ER-Golgi intermediate compartment or the Golgi complex and returned to the ER via a recycling pathway (Figure). Proteins bearing the KDEL and KKXX sequences appear to bind to specific recycling receptors in the membranes of these compartments and are then selectively transported back to the ER.

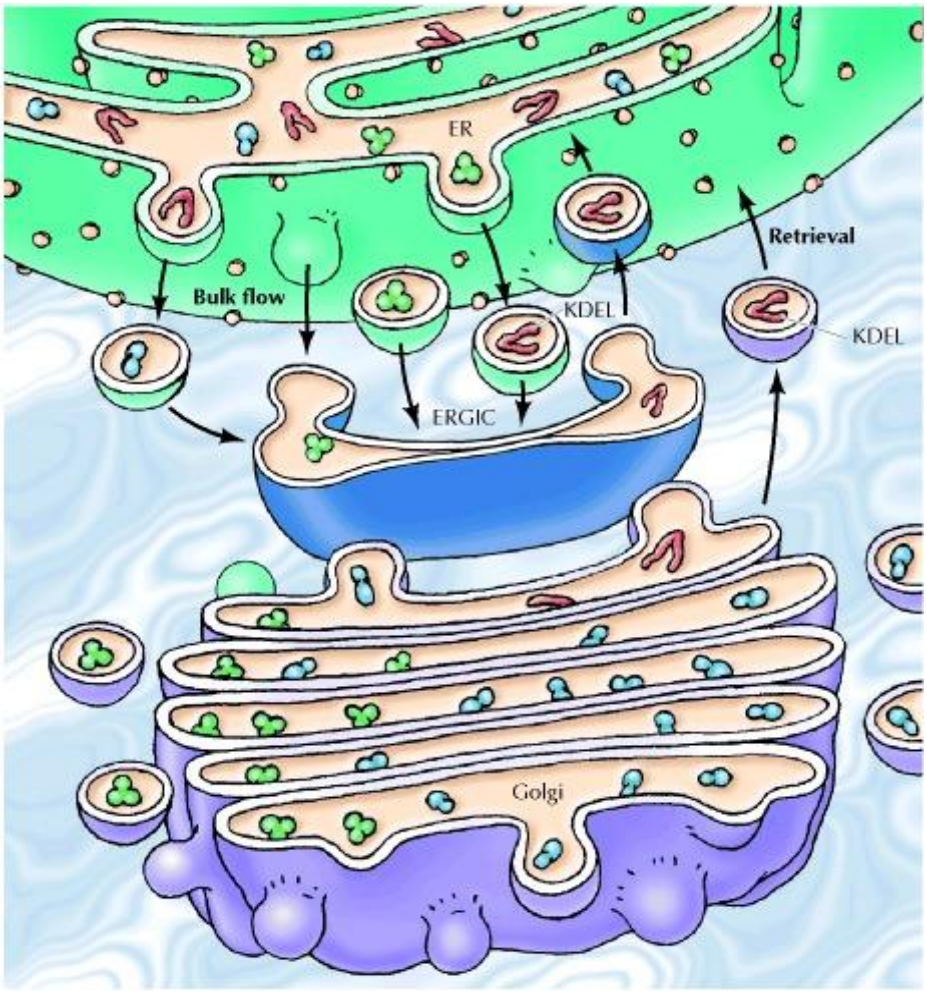


Figure - Retrieval of resident ER proteins: Proteins destined to remain in the lumen of the ER are marked by the sequence Lys-Asp-Glu-Leu (KDEL) at their carboxy terminus. These proteinsare exported from the ER to the Golgi in the nonselective bulk flow of proteins through the secretory pathway, but they are recognized by a receptor in the ER-Golgi intermediate compartment (ERGIC) or the Golgi apparatus and selectively returned to the ER.

The action of the KDEL and KKXX sequences as retention/retrieval signals indicates that there is a nonselective bulk flow of proteins through the secretory pathway leading from the ER to the cell surface. This bulk flow from the ER to the Golgi may be responsible for the export of many proteins from the ER. However, it also appears that some proteins destined for secretion are marked by signals that actively direct their export from the ER. Protein export from the ER can thus take place not only by bulk flow, but also by a regulated pathway that specifically recognizes targeting signals that mediate selective transport of proteins to the Golgi apparatus

Protein folding in ER

For proteins that enter the secretory pathway, many of these events occur either during translocation across the ERmembrane or within the ER lumen. One such processing event is the proteolytic cleavage of the signal peptide as the polypeptide chain is translocated across the ER membrane. The ER is also the site of protein folding, assembly of multisubunit proteins, disulfide bond formation, the initial stages of glycosylation, and the addition of glycolipidanchors to some plasma membrane proteins. Indeed, the primary role of lumenal ER proteins is to catalyze the folding and assembly of newly translocated polypeptides.

As already discussed, proteins are translocated across the ER membrane as unfolded polypeptide chains while their translation is still in progress. These polypeptides, therefore, fold into their three-dimensional conformations within the ER, assisted by the molecular chaperones that facilitate the folding of polypeptide chains. For example, one of the major proteins within the ER lumen is a member of the Hsp70 family of chaperones called BiP. BiP is thought to bind to the unfolded polypeptide chain as it crosses the membrane and then mediates protein folding and the assembly of multisubunit proteins within the ER (Figure). Correctly assembled proteins are released from BiP and are available for transport to the Golgi apparatus. Abnormally folded or improperly assembled proteins, however, remain bound to BiP and are consequently retained within the ER or degraded, rather than being transported farther along the secretory pathway.

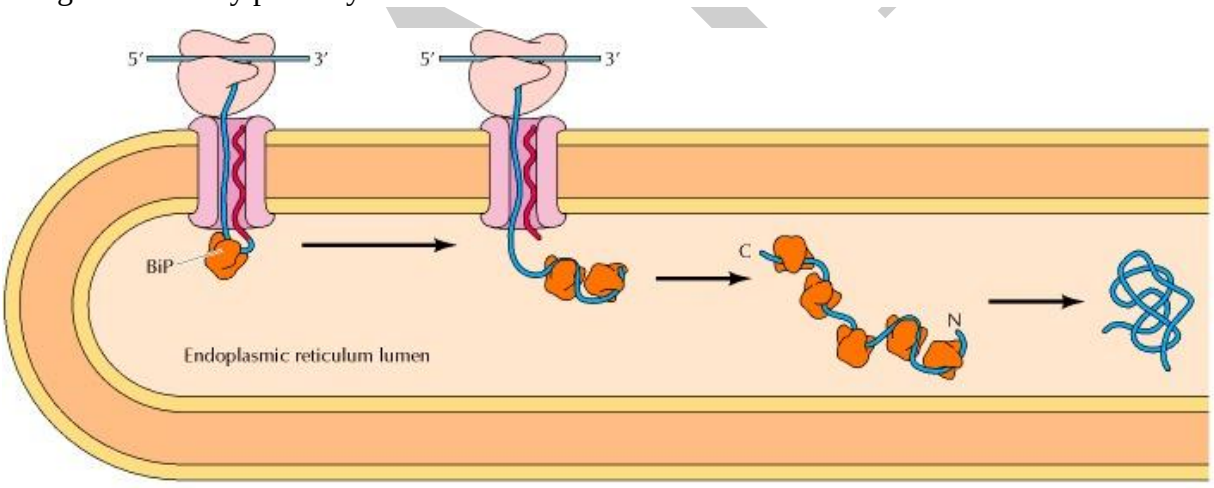


Figure- Protein folding in the ER**: The molecular chaperone BiP binds to polypeptide chains as they cross the ER membrane and facilitates protein folding and assembly within the ER**

The formation of disulfide bonds between the side chains of cysteine residues is an important aspect of protein folding and assembly within the ER. These bonds do not form in the cytosol, which is characterized by a reducing environment that maintains cysteine residues in their reduced (—SH) state. In the ER, however, an oxidizing environment promotes disulfide (S—S)

bond formation, and disulfide bonds formed in the ER play important roles in the structure of secreted and cell surface proteins. Disulfide bond formation is facilitated by the enzyme protein disulfide isomerase which is located in the ER lumen.

Proteins are also glycosylated on specific asparagine residues (N-linked glycosylation) within the ER while their translation is still in process (Figure). Oligosaccharide units consisting of 14 sugar residues are added to acceptor asparagine residues of growing polypeptide chains as they are translocated into the ER. The oligosaccharide is synthesized on a lipid (dolichol) carrier anchored in the ER membrane. It is then transferred as a unit to acceptor asparagine residues in the consensus sequence Asn-X-Ser/Thr by a membrane-bound enzyme called oligosaccharyl transferase. Four sugar residues (three glucose and one mannose) are removed while the protein is still within the ER, and the protein is modified further after being transported to the Golgi apparatus

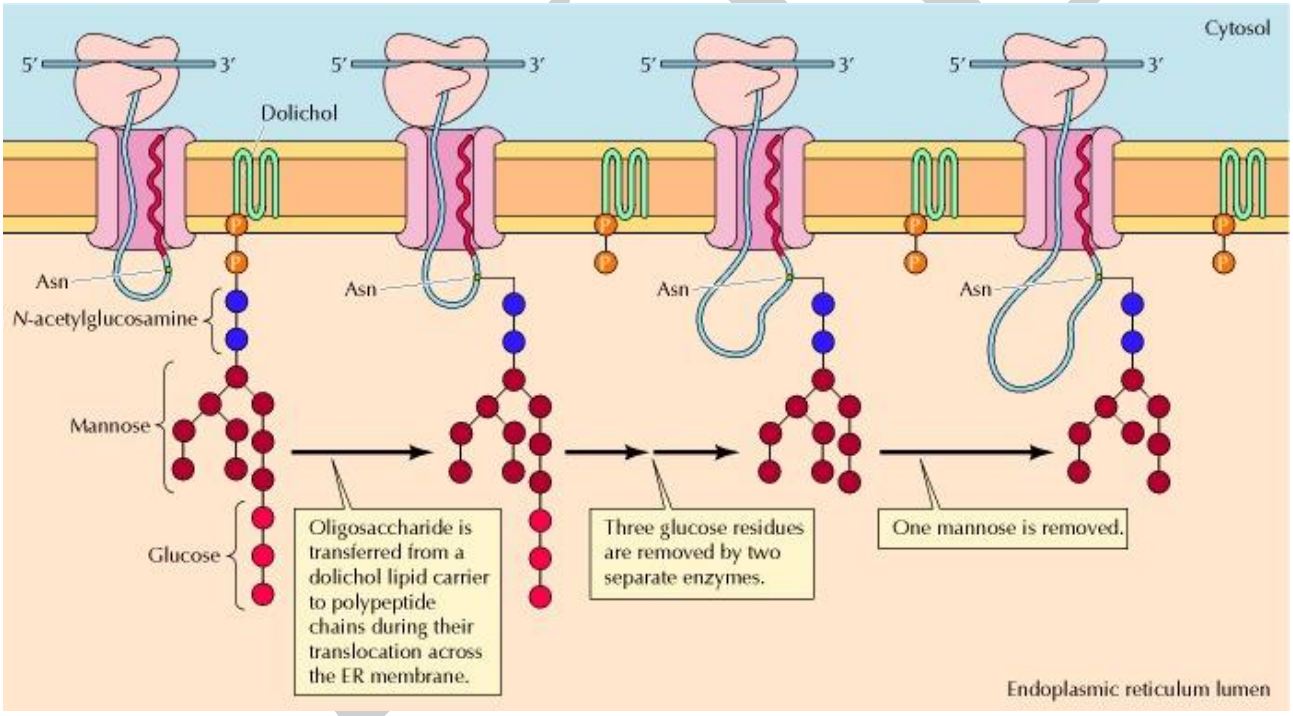


Figure - Protein glycosylation in the ER

Some proteins are anchored in the plasma membrane by glycolipids rather than by membrane-spanning regions of the polypeptide chain. Because these membrane-anchoring glycolipids contain phosphatidylinositol, they are called glycosylphosphatidylinositol (GPI) anchors. The GPI anchors are assembled in the ER membrane. They are then added immediately after

completion of protein synthesis to the carboxy terminus of some proteins anchored in the membrane by a C-terminal membrane-spanning region (Figure). The transmembrane region of the protein is exchanged for the GPI anchor, so these proteins remain attached to the membrane only by their associated glycolipid. Like transmembrane proteins, they are transported to the cell surface as membrane components via the secretory pathway. Their orientation within the ER dictates that GPI-anchored proteins are exposed on the outside of the cell, with the GPI anchor mediating their attachment to the plasma membrane.

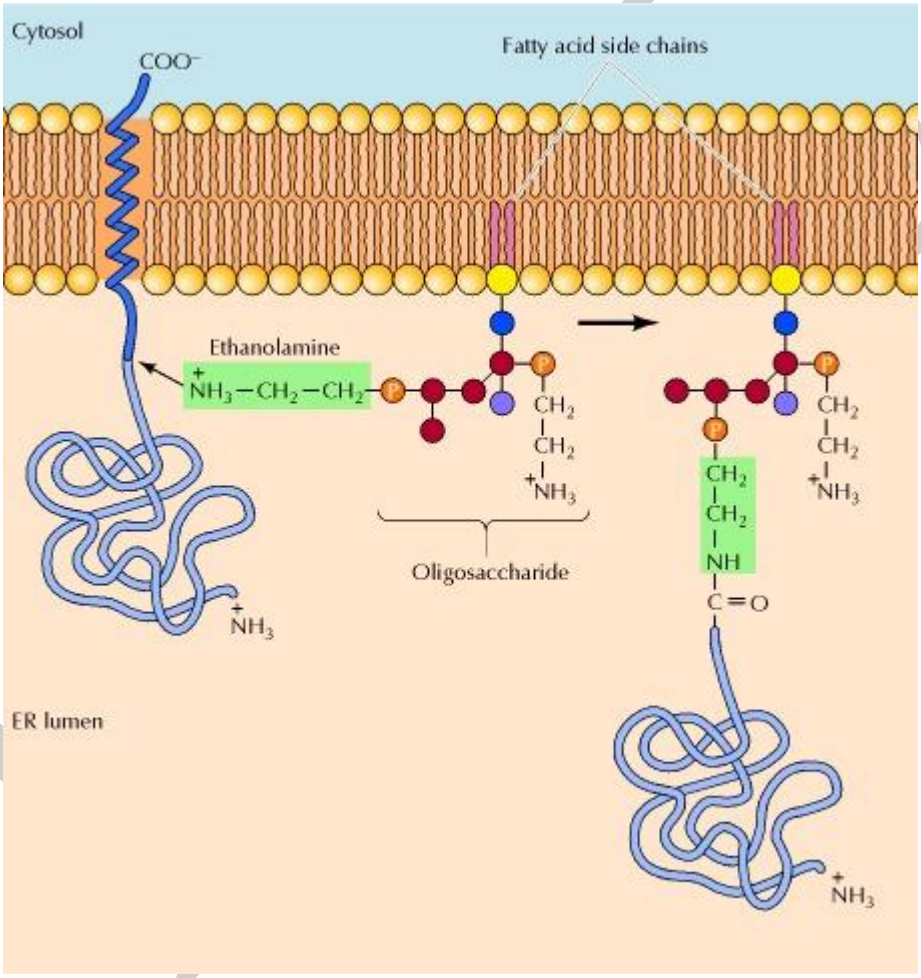


Figure- Addition of GPI anchors: Glycosylphosphatidylinositol (GPI) anchors contain two fatty acid chains, an oligosaccharide portion consisting of inositol and other sugars, and ethanolamine. The GPI anchors are assembled in the ER and added to polypeptides anchored in the membrane by a carboxy-terminal membrane- spanning region. The membranespanning region is cleaved, and the new carboxy terminus is joined to the NH_2 group of ethanolamine immediately after translation is completed, leaving the protein attached to the membrane by the GPI anchor.

PEROXISOMES

Peroxisomes are organelles that contain oxidative enzymes, such as D-amino acid oxidase, ureate oxidase, and catalase. They may resemble a lysosome, however, they are not formed in the golgi complex. Peroxisomes are distinguished by a crystalline structure inside a sac which also contains amorphous gray material. They are self replicating, like the mitochondria. Components accumulate at a given site and they can be assembled into a peroxisome. They may look like storage granules, however, they are not formed in the same way as storage granules. They also enlarge and bud to produce new peroxisomes.

Location

All animal cells (except erythrocytes) and most plant cells contain peroxisomes. They are present in all photosynthetic cells of higher plants in etiolated leaf tissue, in coleoptiles and hypocotyls, in tobacco stem and callus, in ripening pear fruits and also in Euglenophyta, Protozoa, brown algae, fungi, liverworts, mosses and ferns. Peroxisomes contain several oxidases.

Structure:

Peroxisomes are variable in size and shape, but usually appear circular in cross section having diameter between 0.2 and 1.5μm. They have a single limiting unit membrane of lipid and protein molecules, which encloses their granular matrix. Like mitochondria and chloroplasts, they acquire their proteins by selective import from the cytosol. Peroxisomes resemble the Endoplasmic reticulum by being self-replicating, membrane-enclosed organelle that exists without a genome of its own.

Peroxisomes are unusually diverse organelles, and even in the various cell types of a single organism they may contain different sets of enzymes. They can also adapt remarkably to changing conditions. Yeast cells grown on sugar, for example, have small peroxisomes. But when some yeasts are grown on methanol, they develop large peroxisomes that oxidize methanol; and when grown on fatty acids, they develop large peroxisomes that break down fatty

acids to acetyl CoA by β oxidation. Peroxisomes are also important in plants. Two different types have been studied extensively. One type is present in leaves, where it catalyzes the oxidation of a side product of the crucial reaction that fixes CO2 in carbohydrate. This process is called photorespiration because it uses up O2 and liberates CO2. The other type of peroxisome is present in germinating seeds, where it has an essential role in converting the fatty acids stored in seed lipids into the sugars needed for the growth of the young plant. Because this conversion of fats to sugars is accomplished by a series of reactions known as the glyoxylate cycle, these peroxisomes are also called glyoxysomes. In the glyoxylate cycle, two molecules of acetyl CoA produced by fatty acid breakdown in the peroxisome are used to make succinic acid, which then leaves the peroxisome and is converted into glucose. The glyoxylate cycle does not occur in animal cells, and animals are therefore unable to convert the fatty acids in fats into carbohydrates. Glyoxysomes occur in the cells of yeast, Neurospora, and oil rich seeds of many higher plants. They resemble with peroxisomes in morphological details, except that, their crystalloid core consists of dense rods of 6.0 μm diameter.

Chemical composition:

Internally peroxisomes contain several oxidases like catalase and urate oxidase-enzymes that use molecular oxygen to oxidize organic substances, in the process forming hydrogen peroxide (H2O2), a corrosive substance. Catalase is present in large amounts and degrades hydrogen peroxide to yield water and oxygen.

A specific sequence of three amino acids located at the C-terminus of many peroxisomal proteins functions as an import signal. Other peroxisomal proteins contain a signal sequence near the N terminus. If either of these sequences is experimentally attached to a cytosolic protein, the protein is imported into peroxisomes. The import process is yet to be understood completely, although it is known to involve soluble receptor proteins in the cytosol that recognize the targeting signals, as well as docking proteins on the cytosolic surface of the peroxisome. At least 23 distinct proteins, called peroxins, participate as components in the process, which is driven by ATP hydrolysis. Oligomeric proteins do not have to unfold to be imported into peroxisomes, indicating that the mechanism is distinct from that used by mitochondria and chloroplasts and at least one soluble import receptor, the peroxin Pex5, accompanies its cargo all the way into peroxisomes and, after cargo release, cycles back out into the cytosol. These aspects of peroxisomal protein import resemble protein transport into the nucleus.

Formation of peroxisomes:

Most peroxisomal membrane proteins are made in the cytosol and then insert into the membrane of pre-existing peroxisomes. Thus, new peroxisomes are thought to arise from pre-existing ones, by organelle growth and fission

Functions:

1. Hydrogen peroxide metabolism and detoxification: Peroxisomes are so-called, because they usually contain one or more enzymes (D-amino acid oxidase and urate oxidase) that use molecular oxygen to remove hydrogen atoms from specific organic substrates (R) in an oxidative reaction that produces hydrogen peroxide (H2O2):

$$RH2+O2 \longrightarrow R + H2O2$$

This type of oxidative reaction is particularly important in liver and kidney cells, whose peroxisomes detoxify various toxic molecules that enter the blood stream. Almost half of alcohol one drinks is oxidized to acetaldehyde in this way. However, when excess H2O2 accumulates in the cell, catalase converts H2O2 to H2O :

$$2H2O2 \longrightarrow 2H2O + O2$$

Catalase also utilizes the H2O2 generated by other enzymes in the organelle to oxidize a variety of other substrates like phenols, formic acid, formaldehyde, and alcohol. This type oxidative reaction occurs in liver and kidney cells, where the peroxisomes detoxify various toxic molecules that enter the bloodstream.

2. Photorespiration: In green leaves, there are peroxisomes that carry out a process called photorespiration which is a light-stimulated production of CO2 that is different from the generation of CO2 by mitochondria in the dark. In photorespiration, glycolic acid a two-carbon product of photosynthesis is released from chloroplasts and oxidized into glyoxylate and H2O2 by a peroxisomal enzyme called glycolic acid oxidase. Later on, glyoxylate is oxidized into CO2 and formate:

$$CH2OH.\ COOH + O2 \longrightarrow CHO - COOH + H2O2$$

$$CHO — COOH + H2O2 \longrightarrow HCOOH + CO2 + H2O$$

3. Fatty acid oxidation: A major function of the oxidative reactions performed in peroxisomes is the breakdown of fatty acid molecules. In mammalian cells, β oxidation occurs in both mitochondria and peroxisomes; in yeast and plant cells, however, this essential reaction occurs

exclusively in peroxisomes. Peroxisomal oxidation of fatty acids yield acetyl groups and is not linked to ATP formation. The energy released during peroxisomal oxidation is converted into heat, and the acetyl groups are transported into the cytosol, where they are used in the synthesis of cholesterol and other metabolites. In most eukaryotic cells, the peroxisome is the principal organelle in which fatty acids are oxidized, thereby generating precursors for important biosynthetic pathways. In contrast with the oxidation of fatty acids in mitochondria, which produces CO2 and is coupled to the generation of ATP, peroxisomal oxidation of fatty acids yield acetyl groups and is not linked to ATP formation. The energy released during peroxisomal oxidation is converted into heat, and the acetyl groups are transported into the cytosol, where they are used in the synthesis of cholesterol and other metabolites.

4. Peroxisomes play two particularly important roles in plants. First, peroxisomes in seeds are responsible for the **conversion of stored fatty acids to carbohydrates**, which is critical to providing energy and raw materials for growth of the germinating plant. This occurs via a series of reactions termed the **glyoxylate cycle.**

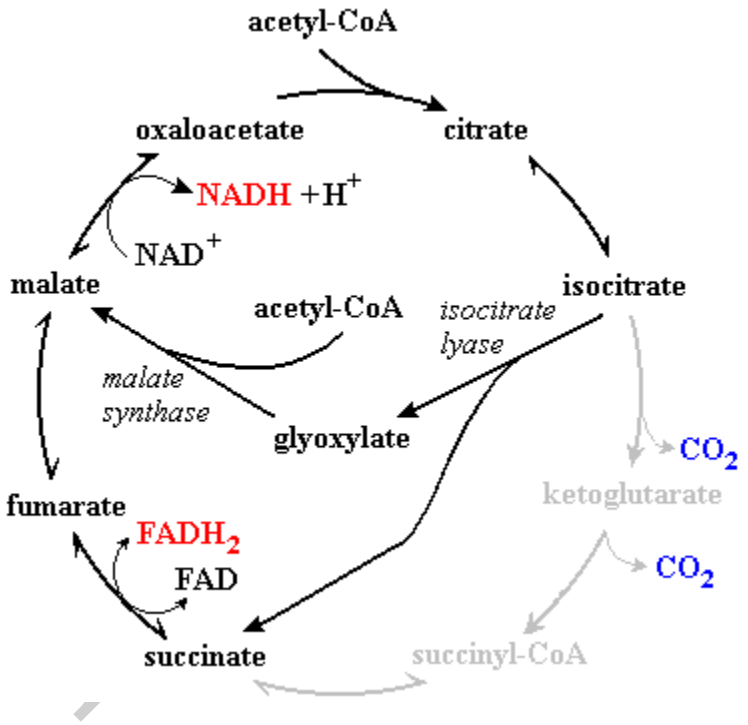


Fig: Glyoxalate cycle

5. Formation of plasmalogens: An essential biosynthetic function of animal peroxisomes is to catalyze the first reactions in the formation of plasmalogens, which are the most abundant class of phospholipids in myelin **(Figure)**. Deficiency of plasmalogens causes profound abnormalities

in the myelination of nerve cells, which is one reason why many peroxisomal disorders lead to neurological disease.

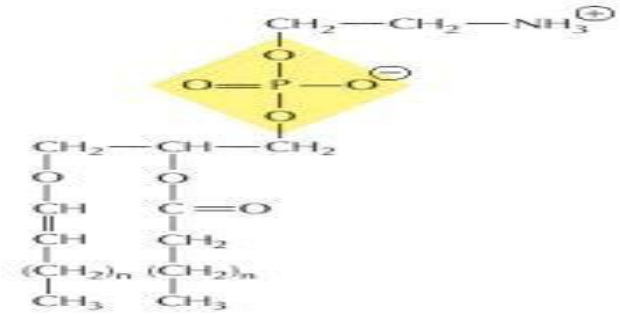


Fig : The structure of plasmalogen.

Peroxisome and diseases:

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 *ADL*gene 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.

POSSIBLE QUESTIONS

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

KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
I BSc BIOCHEMISTRY-First Semester
CELL BIOLOGY (19BCU102)
MULTIPLE CHOICE QUESTIONS

UNIT-II

S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	Presence of nucleus allows the primary regulation of	Reverse transcription	Post translational modification	Post transcriptional modification	Translation	Post transcriptional modification
2	The channel on nucleopore is calles as	Nucleolus	Nucleoplasm	Nucleopore complex	Nuclei	Nucleopore complex
3	Nuclear laminin is located at _____ nuclear membrane	Outer surface	inner surface	Interspace	Cytoplasmic space	inner surface
4	Structural support to nucleus is provided by	Spectrin	Band 3 Protein	Nuclear laminin	Glycoporin	Nuclear laminin
5	Nuclear laminin is_____filament	Micro	Macro	Intermediate	Microsome	Intermediate
6	Which is not transported through nuclear pore complex	Small molecules	Proteins	RNA	DNA	DNA
7	Nucleolus is rich in'	Protein	DNA	RNA	Glycoprotein	RNA
8	The weight of small molecule that transported through nuclearpore complex is_____KD	20	40	10	50	20
9	The diameter of nucleaopore complex is	40nm	10nm	12nm	15nm	40nm
10	RNA and protein are transported through the nuclearpore complex by	Facilitated diffusion	Simple Diffusion	Active transport	Osmosis	Active transport
11	A special channel found in nuclearpore complex is	Central vacuole	Central plug	Centriole	Centrisole	Central plug
12	The signal that target proteins to nucleus is	Signal peptide	NLS	KDEL	KKXX	NLS
13	The cytoso;ic protein that bind the signal with NLS is	Selectin	Importin	Cadherin	intergrin	Importin
14	RNA is transported through nuclear membrane as	Ribonucleo protein	Phospho protein	Glycoprotein	Sphingo lipid	Ribonucleo protein
15	SnRNP is a combination of	RNA and Protein	RNA and DNA	Protein and lipid	RNA and lipid	RNA and Protein
16	Among the following whose protein is not synthesized in membrane bound ribosomes+B19	Plasma Membrane	Secretory vesicle	Lysosomes	Chloroplast	Chloroplast
17	Among the following which is not synthesized in membrane free ribosomes	Nucleus	Mitochondria	Lysosomes	Peroxisomes	Lysosomes
18	ER breaks into	Microsomes	Micro bodies	Microtubules	Microfilaments	Microsomes
19	Peroxisomes and glyoxysomes are	Microfilaments	Microtubules	Microbodies	Intemediary filament	Microbodies
20	SRP contains _____polypeptides	4	6	8	2	6
21	The most favored conformation for hydrophobic amino acids in membrane is	α helix	β sheet	Trans	Cis	α helix
22	Lipids are synthesized on _____ side of SER	Extracellular	Cytosolic	Integral membrane	Transcellular	Cytosolic
23	Thylakoid membrane is found in	Nucleus	Mitochondria	Lysosomes	Chloroplast	Chloroplast
24	H2O2 is destroyed in----------	glyoxysomes	lysosomes	peroxisomes	mesosomes.	peroxisomes
25	Peroxisomes produce hydrogen peroxide which is destroyed by	cytosomes	glyoxisomes	phargmosomes	catalase	catalase
26	Peroxisomes help to detoxify	H_2O_2	Cations	Anions	Xenochemicals	H_2O_2
27	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
28	Among the following which organelle have its own DNA	glyoxysomes	lysosomes	peroxisomes	chloroplast	chloroplast
29	Among the following which organelle have its own DNA	glyoxysomes	lysosomes	peroxisomes	Mitochondria	Mitochondria
30	The folding in mitochondria are called as	Cisternae	Cristae	Cristernae	Chastae	Cristae
31	Mitochondrial proteins are synthesized on	Bound ribosomes	Free ribosomes	SER	RER	Free ribosomes
32	TCA cycle operates at	Cytosol	Mitochondrail matrix	Mitochodrial inner membrane	Mitochondrail outer membrane	Mitochondrail matrix
33	Fatty acid oxidation takes place at	Cytosol	Mitochondrail matrix	Mitochodrial inner membrane	Mitochondrail outer membrane	Mitochondrail matrix
34	Oxidative break down of carbohydrates and lipid is taken care by _____ organelle	Mitochodria	Lysosomes	Golgi	Endoplasmic reticulum	Mitochodria
35	Final electron acceptor in ETC is	Cytochrome C oxidase	Cytochrome C reductaase	Cytochrome	Molecular oxygen	Molecular oxygen
36	Electron transfer in ETC creates _____ gradient	Anion	Cation	Proton	Electron	Proton
37	The number of tRNA encoded by mitochondrial genome is	64	54	42	22	22
38	Protein folding in mitochondria is facilitated by	HSP 70	HSP60	HSP 40	HSP50	HSP 70
39	Major difference between mitochondria and chloroplast in terms of structure is	Outer membrane	Inner membrane	Thylakoid membrane	Inter membrane space	Thylakoid membrane

40	Electron transfer system of chloroplast located at	Outer membrane	Inner membrane	Thylakoid membrane	Inter membrane space	Thylakoid membrane
41	The chloroplast genome range lies between_____kd	150-180	120-160	100-140	80-120	120-160
42	The number of tRNA coded by chloroplast genome is	22	62	30	4029	30
43	Amyloplast store _____	Lipid	Protein	Starch	Peptidoglycans	Starch
44	Elaioplast store _____	Lipid	Protein	Starch	Peptidoglycans	Lipid
45	Chloropast contains	Lipid	Protein	Starch	Carotenoids	Carotenoids
46	Peroxisomes contains enzymes necessary for the synthesis of	peptodo glycans	phospholipids	Plasmologens	Glycolipids	Plasmologens
47	Glyoxylat cycle is operating in _____ organelle	Chloroplast	Glyoxysomes	Golgi	Endoplasmic reticulum	Glyoxysomes
48	In glyoxylat cycle, the stored fat is converted into	Carbohydrates	Lipid	Protein	Nucleic acids	Carbohydrates
49	The organelle which is not involved with photorespiration is	Chloroplast	Peroxisomes	Mitochondria	Endoplasmic reticulum	Endoplasmic reticulum
50	Photorespiration takes place at plant	Leaves	Seed	root	Flowers	Leaves
51	Peroxisomal protein import defects result in	Kleinfelder syndrome	Down syndrome	Zellweger syndrome	Metabolic syndrome	Zellweger syndrome
52	Subunits of ribosomes are manufactured in the	cytoskeleton.	endoplasmic reticulum.	Golgi apparatus.	nucleolus	nucleolus
53	Which of these organelles contains DNA?	Golgi apparatus	lysosomes	mitochondria	rough endoplasmic reticulum	mitochondria
54	The cell process which uses ATP to bring substances into the cell is	osmosis	diffusion	active transport	facilitated transport.	active transport
55	Which of the following aids the movement of glucose across a cell membrane?	Protein	Phosphate	Glycolipid	Cholesterol.	Protein
56	The inner membrane of the mitochondria is usually, highly convoluted forming a series of infolding known as	thylakoids	lamellae	cristae	grana	cristae
57	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
58	In mitochondria , cristae act as sites for	protein synthesis	phosphoryaltion of flavoproteins	breakdown of macromolecules	Oxidation reduction reaction	Oxidation reduction reaction
59	Mitochondrial inner membrane is rich in phospholipid	Cardiolipin	Phosphatidyl inositol	Phosphatidyl serine	Phosphatidyl choline	Cardiolipin
60	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-III

SYLLABUS

Protein trafficking

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

Lysosome- Acid hydrolases, phagocytosis and autophagy

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

Chloroplasts- Import and sorting of chloroplast proteins.

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, occurs in the Golgi complex or Golgi apparatus located in the cytoplasm of animal and plant cells.

Occurrence:

The Golgi apparatus occurs in all eukaryotic cells. The exceptions are the prokaryotic cells (mycoplasmas, bacteria and blue green algae) and eukaryotic cells of certain fungi, sperm cells of bryophytes and pteridiophytes, cells of mature sieve tubes of plants and mature sperm and red blood cells of animals. 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. In higher plants, Golgi apparatuses are particularly common in secretory cells and in young rapidly growing cells. In animal cells, there usually occurs a single Golgi apparatus, however, its number may vary from animal to animal and from cell to cell. *Paramoeba* species has two golgi apparatuses and nerve cells, liver cells and chordate oocytes have multiple golgi apparatuses, there being about 50 of them in the liver cells.

Morphology

The Golgi apparatus is morphologically very similar in both plant and animal cells. However, it is extremely pleomorphic: in some cell types it appears compact and limited, in others spread out and reticular (net-like). Its shape and form may vary depending on cell type. It appears as a complex array of interconnecting tubules, vesicles and cisternae. There has been much debate concerning the terminology of the Golgi's parts. The simplest unit of the Golgi apparatus is the cisterna. This is a membrane bound space in which various materials and secretions may accumulate. Numerous cisternae are associated with each other and appear in a stack-like (lamellar) aggregation. A group of these cisternae is called the dictyosome, and a group of dictyosomes makes up theGolgi apparatus. All dictyosomes of a cell have a common function. The detailed structure of three basic components of the Golgi apparatus are as follows:

1. Flattened Sac or Cisternae

Cisternae of the golgi apparatus are about 1 μm in diameter, flattened, plate-like or saucer-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 which may contain rod-like elements or fibres. Each stack of cisternae forms a dictyosome which may contain 5 to 6 Golgi cisternae in animal cells or 20 or more cisternae in plant cells. Each cisterna is bounded by a smooth unit membrane (7.5 nm thick), having a lumen varying in width from about 500 to 1000 nm. Polarity. The margins of each cisterna are gently curved so that the entire dictyosome of Golgi apparatus takes on a bow-like appearance. The cisternae at the convex end of the dictyosome comprise proximal, forming or cis-face and the cisternae at the concave end of the dictyosome comprise the distal, maturing or trans-face. The forming or cis face of Golgi is located next to either the nucleus or a specialized portion of rough ER that lacks bound ribosomes and is called "transitional" ER. Trans face of Golgi is located near the plasma membrane. This polarization is called cis-trans axis of the Golgi apparatus.

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 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 coalasce 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.

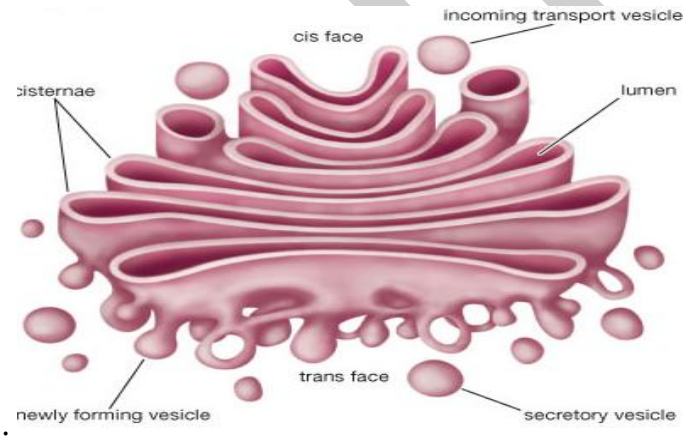


Fig: Golgi apparatus

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. Proteins are modified by enzymes in cisternae by glycosylation and phosphorylation by identifying the signal sequence of the protein in question. For example, the Golgi apparatus adds a mannose-6-phosphate label to proteins destined for lysosomes. One molecule that is phosphorylated in the Golgi is Apolipoprotein, which forms a molecule known as VLDL that is a constituent of blood serum. The phosphorylation of these molecules is important to help aid in their sorting for secretion into the blood serum.

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

Common Functions:

1. Formation of secretary vesicles or primary lysosomes
2. Intracellular transport
3. Production of hormones
4. Cell-plate formation during mitosis
5. Storage of proteins and lipids
6. Activation of mitochondria
7. Synthesis of carbohydrates
8. Regeneration of membranes
9. To check the oxidation of synthetic material
10. Secretion of enzymes and hormones and modification of proteins

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.

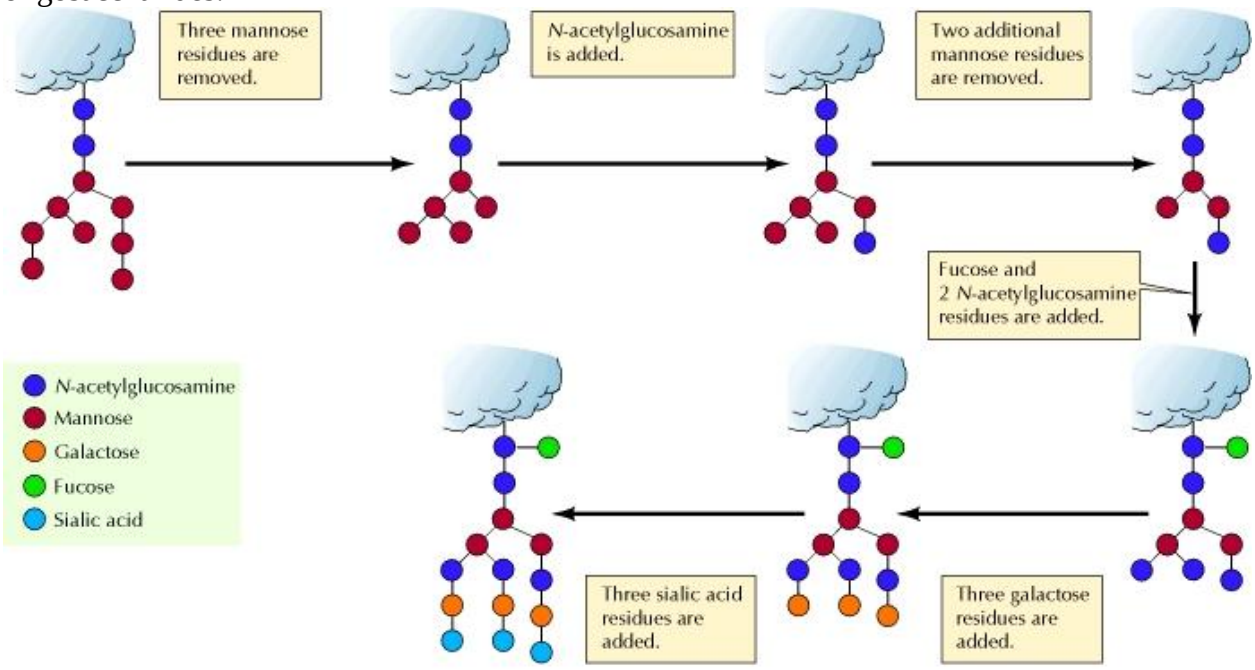


Figure: Processing of *N*-linked oligosaccharides in the Golgi

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.

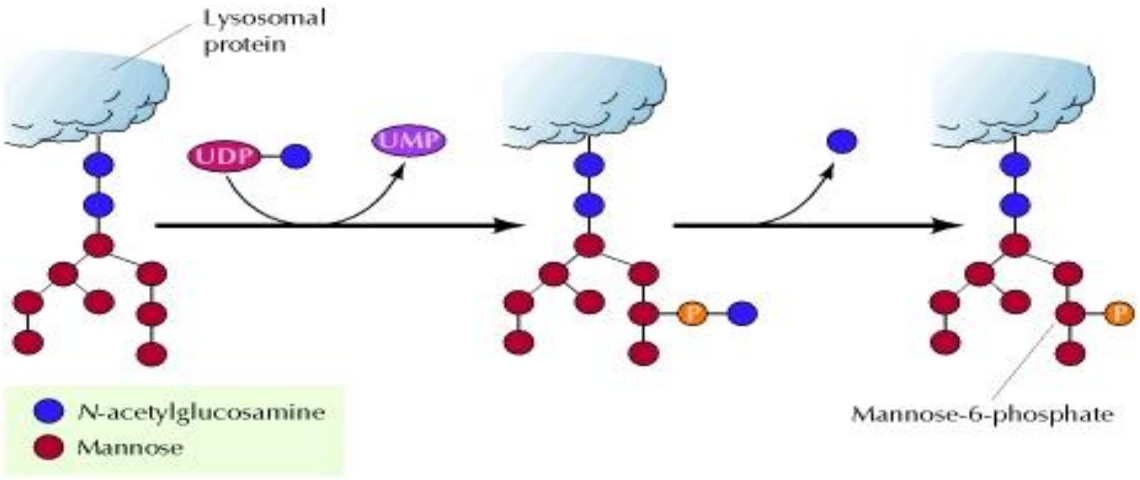


Figure : Targeting of lysosomal proteins by phosphorylation of mannose residues

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).

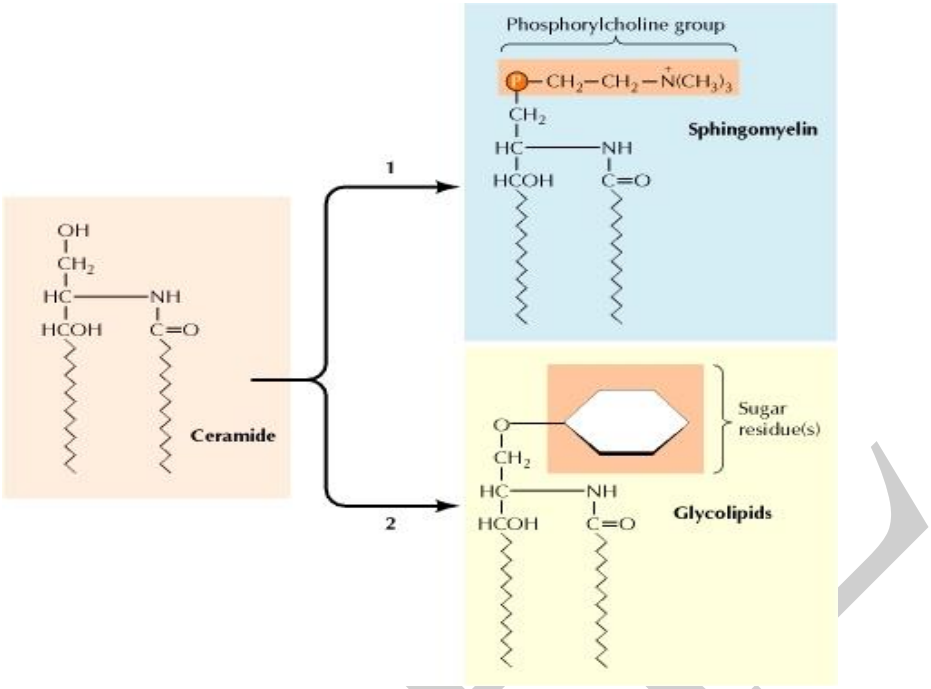


Figure: Synthesis of sphingomyelin and glycolipids in Golgi

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.

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.

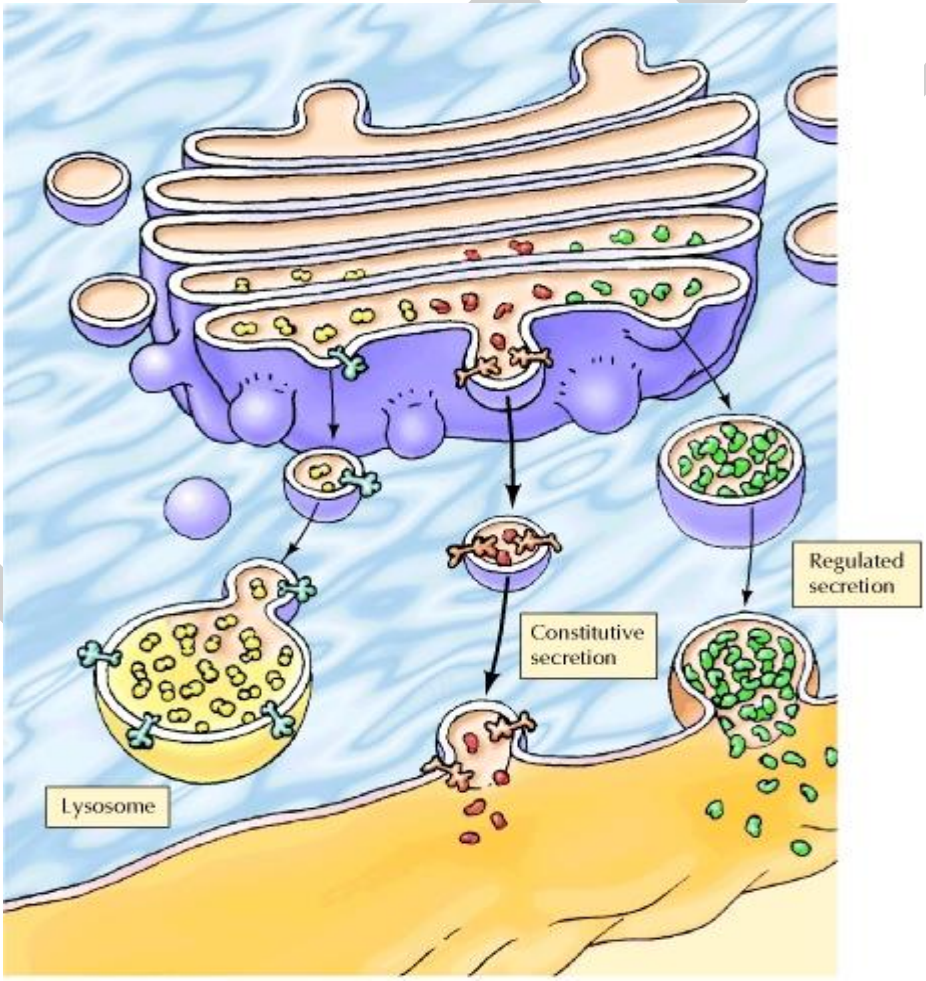


Figure :Transport from the Golgi apparatus

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.

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.

LYSOSOMES

Lysosomes are cellular organelles that contain acid hydrolase enzymes to break down waste materials and cellular debris. They can be described as the stomach of the cell. They are found in animal cells, while in yeast and plants the same roles are performed by lytic vacuoles. Lysosomes digest excess or worn-out organelles, food particles, and engulf viruses or bacteria. The membrane around a lysosome allows the digestive enzymes to work at the 4.5 pH they require. Lysosomes fuse with vacuoles and dispense their enzymes into the vacuoles, digesting their contents. They are created by the addition of hydrolytic enzymes to early endosomes from

the golgi apparatus. The name *lysosome* derives from the Greek words lysis, *to separate*, and soma, *body*. They are frequently nicknamed "suicide-bags" or "suicide-sacs" by cell biologists due to their autolysis. C. de Duve, in 1955, named these organelles as 'lysosomes'.

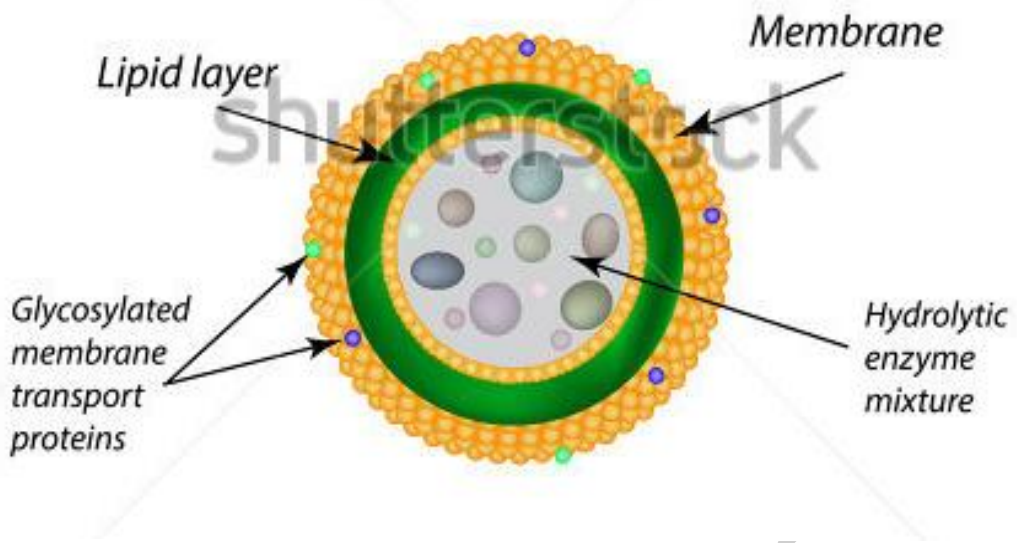


Fig: Lysosome structure

Lysosomes are the cell's waste disposal system and can digest some compounds. They are used for the digestion of macromolecules from phagocytosis (ingestion of other dying cells or larger extracellular material, like foreign invading microbes), endocytosis (where receptor proteins are recycled from the cell surface), and autophagy (where in old or unneeded organelles or proteins, or microbes that have invaded the cytoplasm are delivered to the lysosome). **Autophagy** may also lead to autophagic cell death, a form of programmed self-destruction, or autolysis, of the cell, which means that the cell is digesting itself.

Biogenesis

Lysosomes are often budded from the membrane of the Golgi apparatus, but in some cases they develop gradually from late endosomes, which are vesicles that carry materials brought into the cell by a process known as endocytosis. The biogenesis of the lysosomes requires the synthesis of specialized lysosomal hydrolases and membrane proteins. Both classes of proteins are synthesized in the ER and transported through the Golgi apparatus, then transported from the trans Golgi network to an intermediate compartment (an endolysosome) by means of transport vesicles (which are coated by clathrin protein).

Occurrence:

The lysosomes occur in most animal and few plant cells. They are absent in bacteria and mature mammalian erythrocytes. Few lysosomes occur in muscle cells or in acinar cells of the pancreas. Leucocytes, especially granulocytes are a particularly rich source of lysosomes. Their lysosomes

are so large-sized that they can be observed under the light microscope. They are also numerous in epithelial cells of absorptive, secretory and excretory organs (intestine, liver, and kidney). They occur in abundance in the epithelial cells of lungs and uterus. Phagocytic cells and cells of reticuloendothelial system (bone marrow, spleen and liver) are also rich in lysosomes.

Structure:

The lysosomes are round vacuolar structures bounded by single unit membrane. Their shape and density vary greatly. Lysosomes are 0.2 to 0.5μm in size. Since, size and shape of lysosomes vary from cell to cell and time to time (they are polymorphic), their identification becomes difficult.

C**hemical composition:**

Lysosomes are very delicate and fragile organelles. Lysosomal fractions have been isolated by sucrose-density centrifugation (Isopycnic centrifugation) after mild methods of homogenization. The location of the lysosomes in the cell can also be pinpointed by various histochemical or cytochemical methods. For example, lysosomes give a positive test for acid Schiff reaction. Certain lysosomal enzymes are good histochemical markers. For example, acid phosphatase is the principal enzyme which is used as a marker for the lysosomes by the use of Gomori'staining technique.

Lysosomal Membrane:

The lysosomal membrane is slightly thicker than that of mitochondria. It contains substantial amounts of carbohydrate material, particularly sialic acid. In fact, most lysosomal membrane proteins are unusually highly glycosylated, which may help protect them from the lysosomal proteases in the lumen. The lysosomal membrane has another unique property of fusing with other membranes of the cell. This property of fusion has been attributed to the high proportion of membrane lipids present in the micellar configuration. Surface active agents such as liposoluble vitamins (A,K,D and E) and steroid sex hormones have a destabilizing influence, causing release of lysosomal enzymes due to rupture of lysosomal membranes. Drugs like cortisone, hydrocortisone and others tend to stabilize the lysosomal membrane and have an anti-inflammatory effect on the tissue. The entire process of digestion is carried out within the lysosome. Most lysosomal enzymes act in an acid medium. Acidification of lysosomal contents depends on an ATP-dependent proton pump which is present in the membrane of the lysosome and accumulates H+ inside the organelle. Lysosomal membrane also contains transport proteins that allow the final products of digestion of macromolecules to escape so that they can be either excreted or reutilized by the cell.

Functions:

1. Lysosomes serve as digestion compartments for cellular materials that have exceeded their lifetime or are otherwise no longer useful by autophagy. When a cell dies, the lysosome membrane ruptures and enzymes are liberated. These enzymes digest the dead cells. In the process of metamorphosis of amphibians and tunicates many embryonic tissues,
e.g., gills, fins, tail, etc., are digested by the lysosomes and utilized by the other cells.
2. Lysosomes break down cellular waste products, fats, carbohydrates, proteins, and other macromolecules into simple compounds, which are then transferred back into the cytoplasm as

new cell-building materials. To accomplish the tasks associated with digestion, the lysosomes utilize about 40 different types of hydrolytic enzymes, all of which are manufactured in the endoplasmic reticulum and modified in the Golgi apparatus.

3. Digestion of large extracellular particles: The lysosomes digest the food contents of the phagosomes or pinosomes. The lysosomes of leucocytes enable the latter to devour the foreign proteins, bacteria and viruses.

4. Extracellular digestion: The lysosomes of certain cells such as sperms discharge their enzymes outside the cell during the process of fertilization. The lysosomal enzymes digest the limiting membranes of the ovum and form penetra path in ovum for the sperms. Acid hydrolases are released from osteoclasts and break down bone for the reabsorption; these cells also secrete lactic acid which makes the local pH enough for optimal enzyme activity. Likewise, preceding ossification (bone formation), fibroblasts release cathepsin D enzyme to break down the connective tissue.

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.

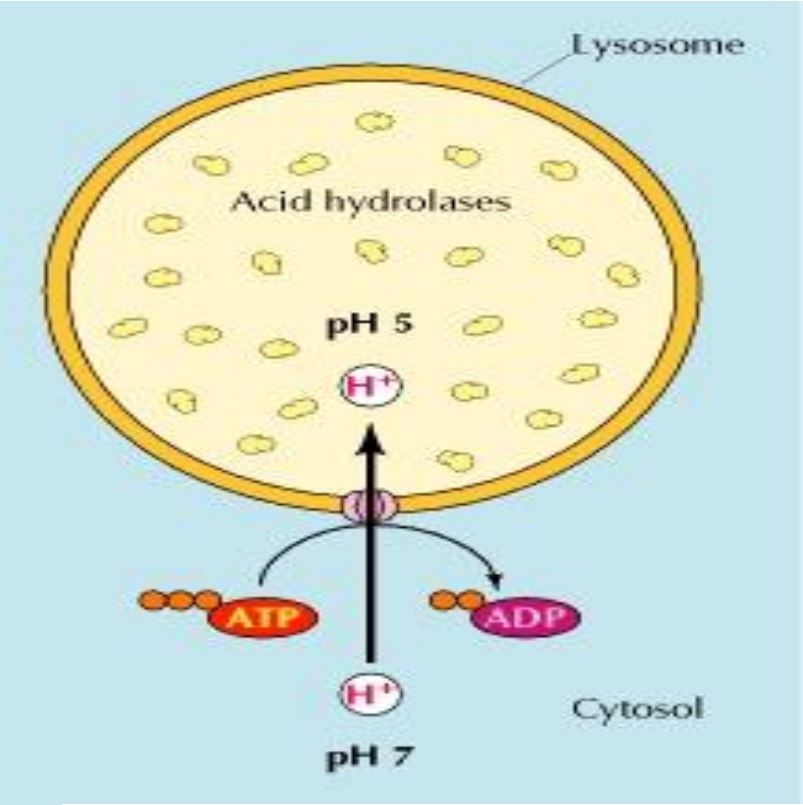


Figure: Proton pump of the lysosome

Table: Lysosomal acid hydrolases

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

Table: Enzymes associated with lysosomal storage diseases (LSD)

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

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).

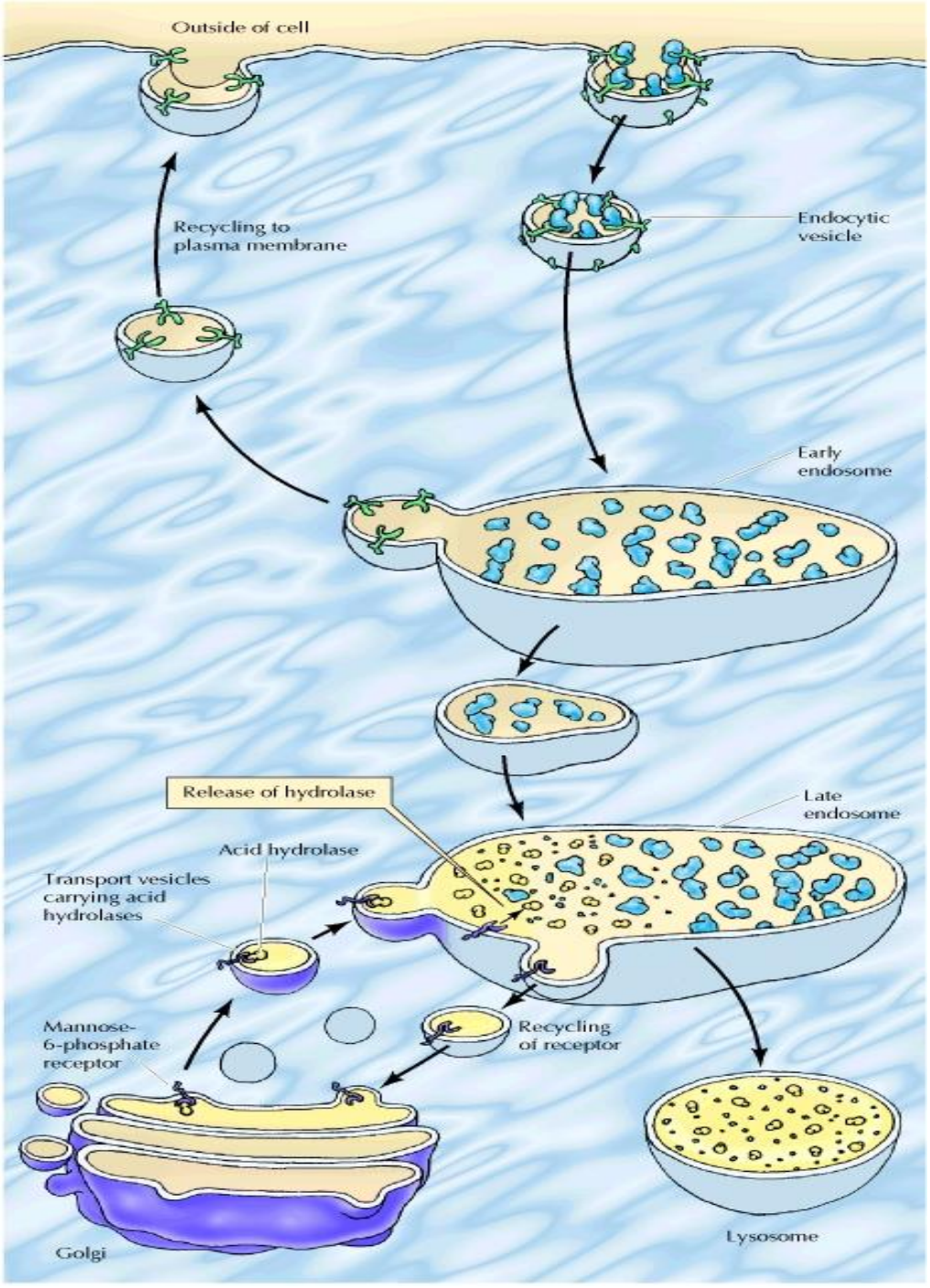


Figure: Endocytosis and lysosome formation

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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 (Figure 9.37). 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.

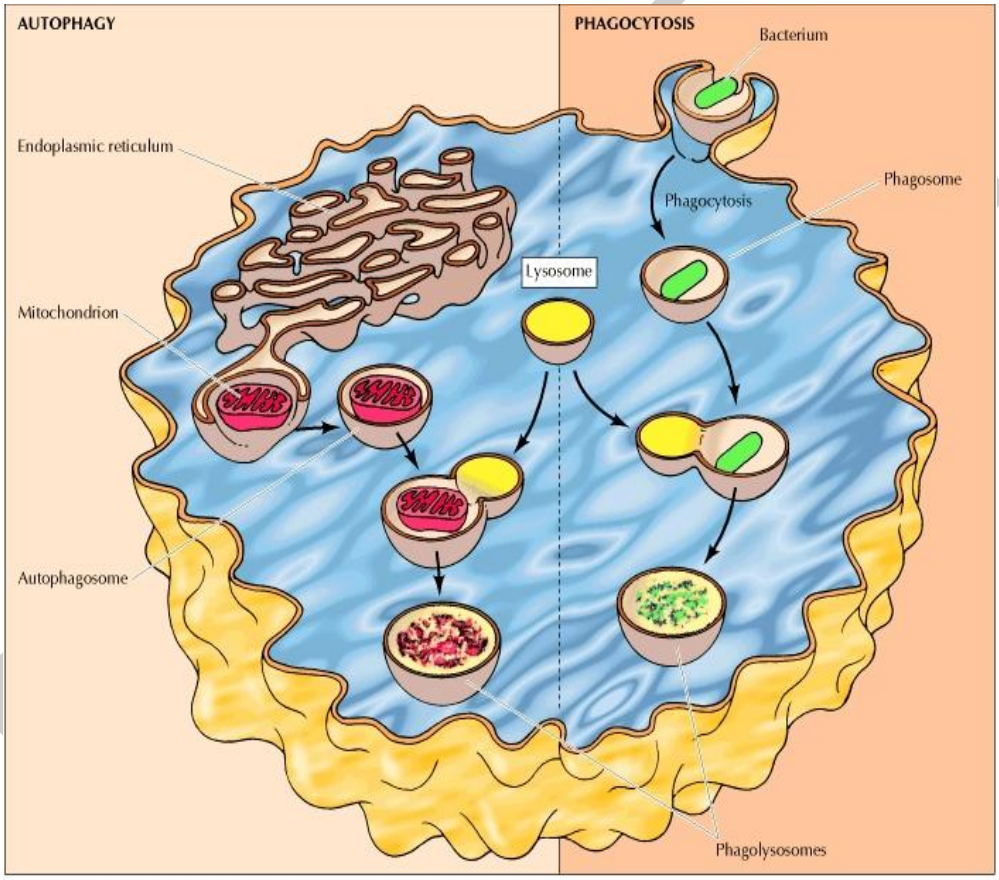


Figure: Lysosomes in phagocytosis and autophagy

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.

MITOCHONDRIA –STRUCTURE AND FUNCTION

Mitochondria is the organelle stated as power house of cell. The **mitochondrion** (plural **mitochondria**) is a double membrane-bound organelle found in all eukaryotic organisms. Some cells in some multicellular organisms may however lack them (for example, mature mammalian red blood cells). Mitochondria are commonly between 0.75 and 3 μm in diameter, but vary considerably in size and structure. The number of mitochondria in a cell can vary widely by organism, tissue, and cell type. For instance, red blood cells have no mitochondria, whereas liver cells can have more than 2000.

Localisation: Mitochondria are present in all eukaryotic cells. The distribution and number of mitochondria can be correlated with type of function the cell performs. Typically mitochondria with many cristae are associated with mechanical and osmotic work situations, where there are sustained demands for ATP *e.g*., between muscle fibres, in the basal infolding of kidney tubule cells, and in a portion of inner segment of rod and cone cells of retina. Myocardial muscle cells have numerous large mitochondria called sarcosomes that reflect the great amount of work done by these cells.

Morphology:

Number: The number of mitochondria in a cell depends on the type and functional state of the cell. It varies from cell to cell and from species to species. Certain cells contain exceptionally large number of the mitochondria, for example the *Amoeba*, *Chaos chaos* contain 50,000; eggs of sea urchin contain 140,000 to 150,000 and oocytes of amphibians contain 300,000 mitochondria. Liver cells of rat contain only 500 to 1600 mitochondria. The cells of green plants contain less number of mitochondria in comparison to animal cells. Some algal cells may contain only one mitochondrion.

Shape: The mitochondria may be filamentous or granular in shape and may change from one form to another depending upon the physiological conditions of the cells. Thus, they may be of club, racket, vesicular, ring or round-shape. Mitochondria are granular in primary spermatocyte or rat, or club-shaped in liver cells. Time-lapse picturisation of living cells shows that mitochondria are remarkably mobile and plastic organelles, constantly changing their shape. They sometimes fuse with one another and then separate again. For example, in certain euglenoid cells, the mitochondria fuse into a reticulate structure during the day and dissociate during darkness. Similar changes have been reported in yeast species, apparently in response to culture conditions.

Size: Normally mitochondria vary in size from 0.5 µm to 2.0 µm and, therefore, are not distinctly visible under the light microscope. Sometimes their length may reach up to 7µm.

Structure

Mitochondria are present in both plant and animal cells. They are rod-shaped structures that are enclosed within two membranes - the outer membrane and the inner membrane. The membranes are made up of phospholipids and proteins. The space in between the two membranes is called the inter-membrane space which has the same composition as the cytoplasm of the cell. However, the protein content in this space differs from that in the cytoplasm.

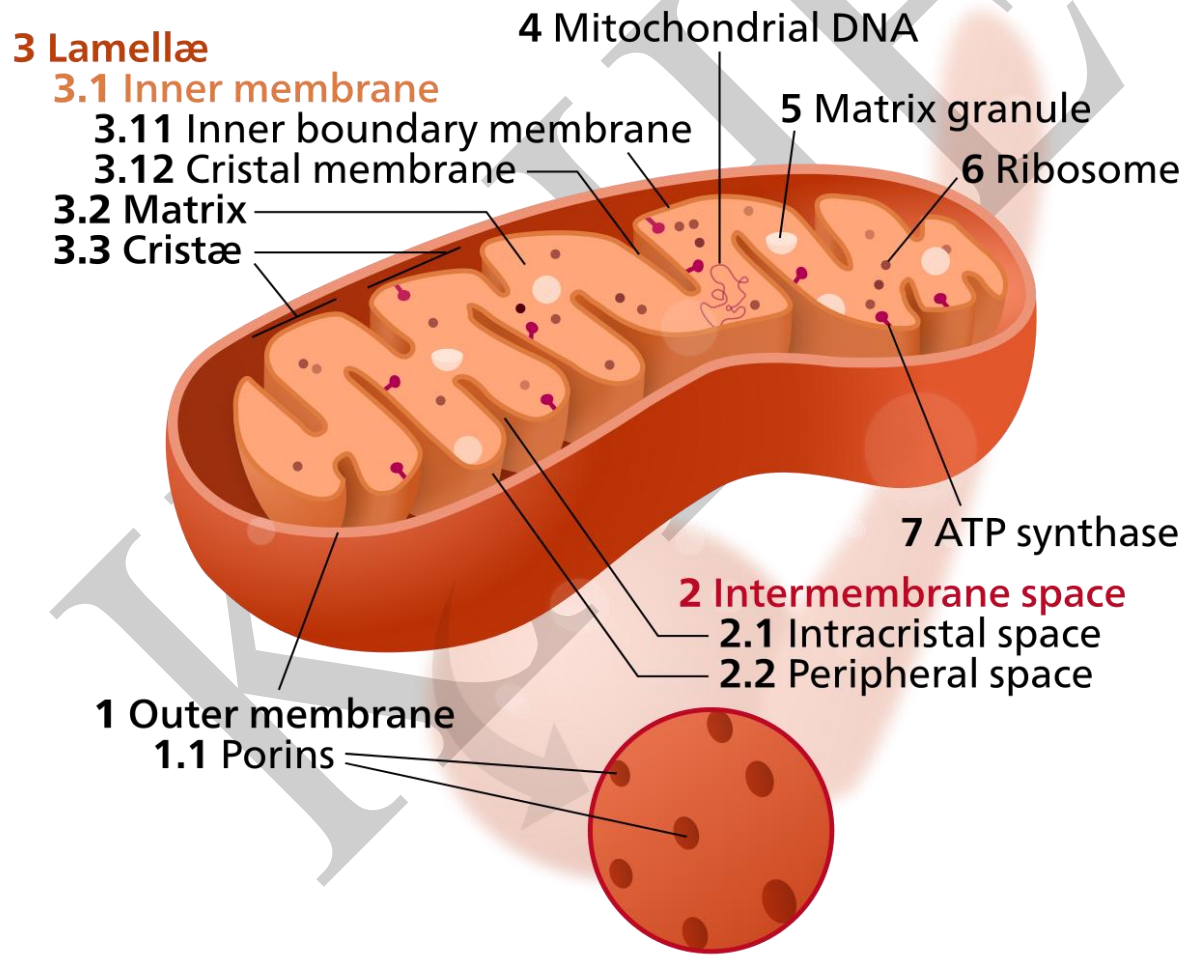


Fig: Ultra structure of mitochondria

The structure of the various components of mitochondria is as follows:

1.Outer Membrane

The outer membrane is smooth unlike the inner membrane and has almost the same amount of phospholipids as proteins. It has a large number of special proteins called *porins*, that allow molecules of 5000 daltons or less in weight to pass through it. The outer membrane is completely permeable to nutrient molecules, ions, ATP and ADP molecules.

2.Intermembrane space

The intermembrane space is the space between the outer membrane and the inner membrane. It is also known as perimitochondrial space. Because the outer membrane is freely permeable to small molecules, the concentrations of small molecules, such as ions and sugars, in the intermembrane space is the same as in the cytosol. However, large proteins must have a specific signaling sequence to be transported across the outer membrane, so the protein composition of this space is different from the protein composition of the cytosol. One protein that is localized to the intermembrane space in this way is cytochrome c.

3.Inner Membrane

The inner membrane is more complex in structure than the outer membrane as it contains the complexes of the electron transport chain and the ATP synthetase complex. It is permeable only to oxygen, carbon dioxide and water. It is made up of a large number of proteins that play an important role in producing ATP, and also helps in regulating transfer of metabolites across the membrane. The inner membrane has infoldings called the *cristae* that increase the surface area for the complexes and proteins that aid in the production of ATP, the energy rich molecules.

The inner mitochondrial membrane contains proteins with five types of functions:

1. Those that perform the redox reactions of oxidative phosphorylation
2. ATP synthase, which generates ATP in the matrix
3. Specific transport proteins that regulate metabolite passage into and out of the matrix
4. Protein import machinery
5. Mitochondrial fusion and fission protein

4.Mitochondrial DNA

Although most of the genetic material of a cell is contained within the nucleus, the mitochondria have their own DNA. They have their own machinery for protein synthesis and reproduce by the process of fission like bacteria do. Due to their independence from the nuclear DNA and similarities with bacteria, it is believed that mitochondria have originated from bacteria by endosymbiosis.

Mitochondria contain their own genome, an indication that they are derived from bacteria through endosymbiosis. The **human** mitochondrial genome is a circular DNA molecule of about 16 kilobases. It encodes 37 genes: 13 for subunits of respiratory complexes I, III, IV and V, 22 for mitochondrial tRNA (for the 20 standard amino acids, plus an extra gene for leucine and serine), and 2 for rRNA. One mitochondrion can contain two to ten copies of its DNA.

5.Cristae

The inner mitochondrial membrane is compartmentalized into numerous cristae, which expand the surface area of the inner mitochondrial membrane, enhancing its ability to produce ATP. For typical liver mitochondria, the area of the inner membrane is about five times as large as the outer membrane. This ratio is variable and mitochondria from cells that have a greater demand for ATP, such as muscle cells, contain even more cristae. These folds are studded with small round bodies known as F_1 particles or oxysomes.

6.Matrix

The matrix is a complex mixture of enzymes that are important for the synthesis of ATP molecules, special mitochondrial ribosomes, tRNAs and the mitochondrial DNA. Besides these, it has oxygen, carbon dioxide and other recyclable intermediates.

Functions

Functions of mitochondria vary according to the cell type in which they are present.

- The most important function of the mitochondria is to produce energy. The food that we eat is broken into simpler molecules like carbohydrates, fats, etc., in our bodies. These are sent to the mitochondrion where they are further processed to produce charged molecules that combine with oxygen and produce ATP molecules. This entire process is known as oxidative phosphorylation.
- It is important to maintain proper concentration of calcium ions within the various compartments of the cell. Mitochondria help the cells to achieve this goal by serving as storage tanks of calcium ions.
- Mitochondria help in the building of certain parts of the blood, and hormones like testosterone and estrogen.
- Mitochondria in the liver cells have enzymes that detoxify ammonia.

Mitochondria play an important role in the process of programmed cell death. Unwanted and excess cells are pruned away during the development of an organism. The process is known as *apoptosis*. Abnormal cell death due to mitochondrial dysfunction can affect the function of the organ

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.

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.

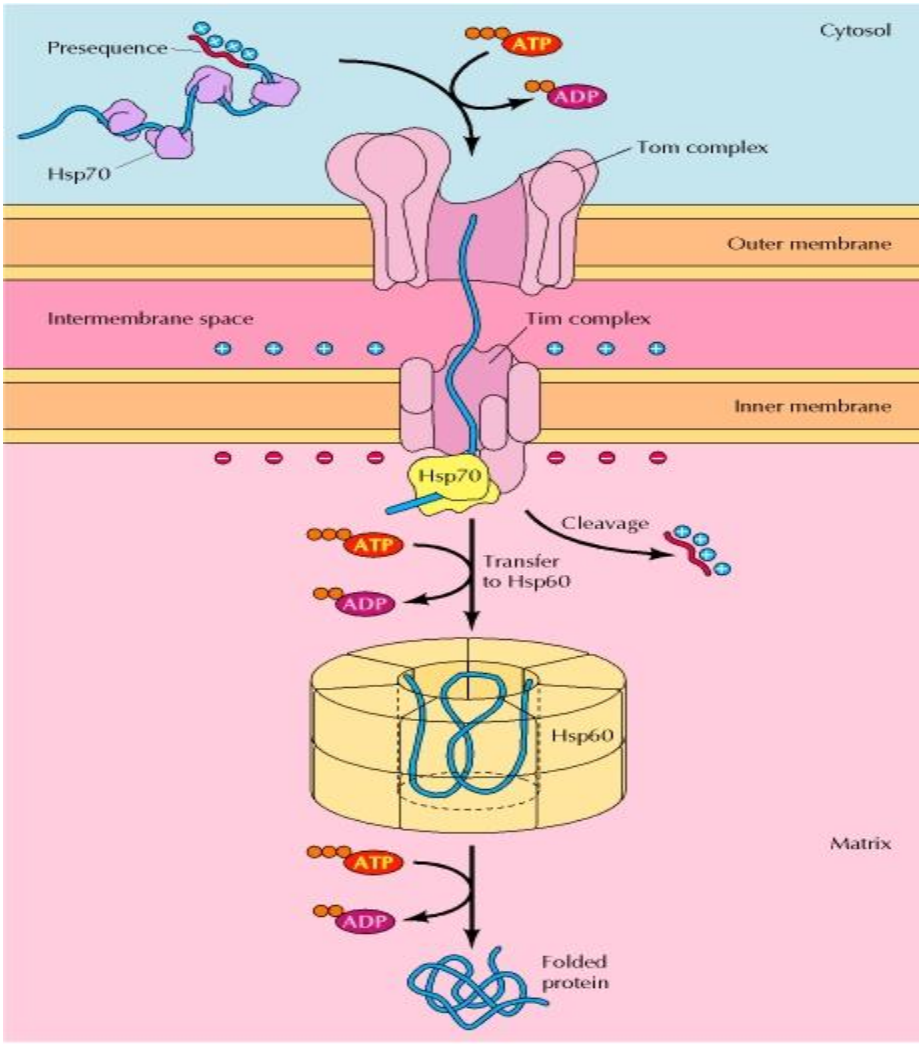


Figure : Import of proteins into mitochondria

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

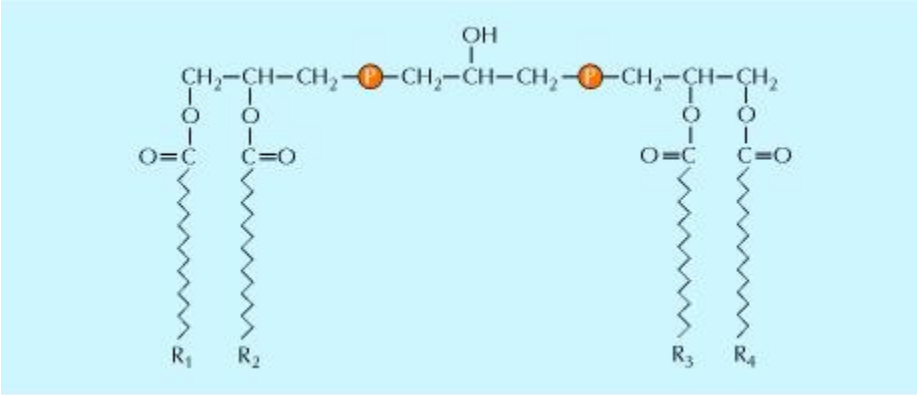


Figure : Structure of cardiolipin

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.

PROTEIN EXPORT FROM MITOCHONDRIA

Assembly of a functional mitochondrion requires import of proteins from the cytosol and export of proteins from the matrix. Most previous studies have focused on the import pathway followed by nucleus-encoded proteins. However, it is now clear that proteins encoded in the nucleus as well as those encoded in the mitochondrion also move from the matrix into and across the inner membrane, a process defined here as export. These exported proteins are found in at least three cellular locations: the inner mitochondrial membrane, the intermembrane space and the cell surface.

Oxa1 belongs to a large protein family with members in mitochondria, Oxa1 proteins are believed to serve as translocation factors that facilitate the transport of hydrophilic protein domains from one side of the membrane to the other.

Mba1 as a second mitochondrial component that is required for efficient protein insertion. Like Oxa1, Mba1 specifically interacts both with mitochondrial translation products and with conservatively sorted, nuclear-encoded proteins during their integration into the inner membrane. Oxa1 and Mba1 overlap in function and substrate specificity, but both can act independently of each other.

Cox2 is synthesized in the matrix as a precursor protein with an N-terminal extension. This presequence is proteolytically removed by the Imp1 protease in the intermembrane space after translocation across the inner membrane. Translocation of the N-terminal domain of Cox2 was found kinetically to precede export of the C terminus, and conditions that block N tail export also prevent translocation of the C terminus

IMPORTING 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.

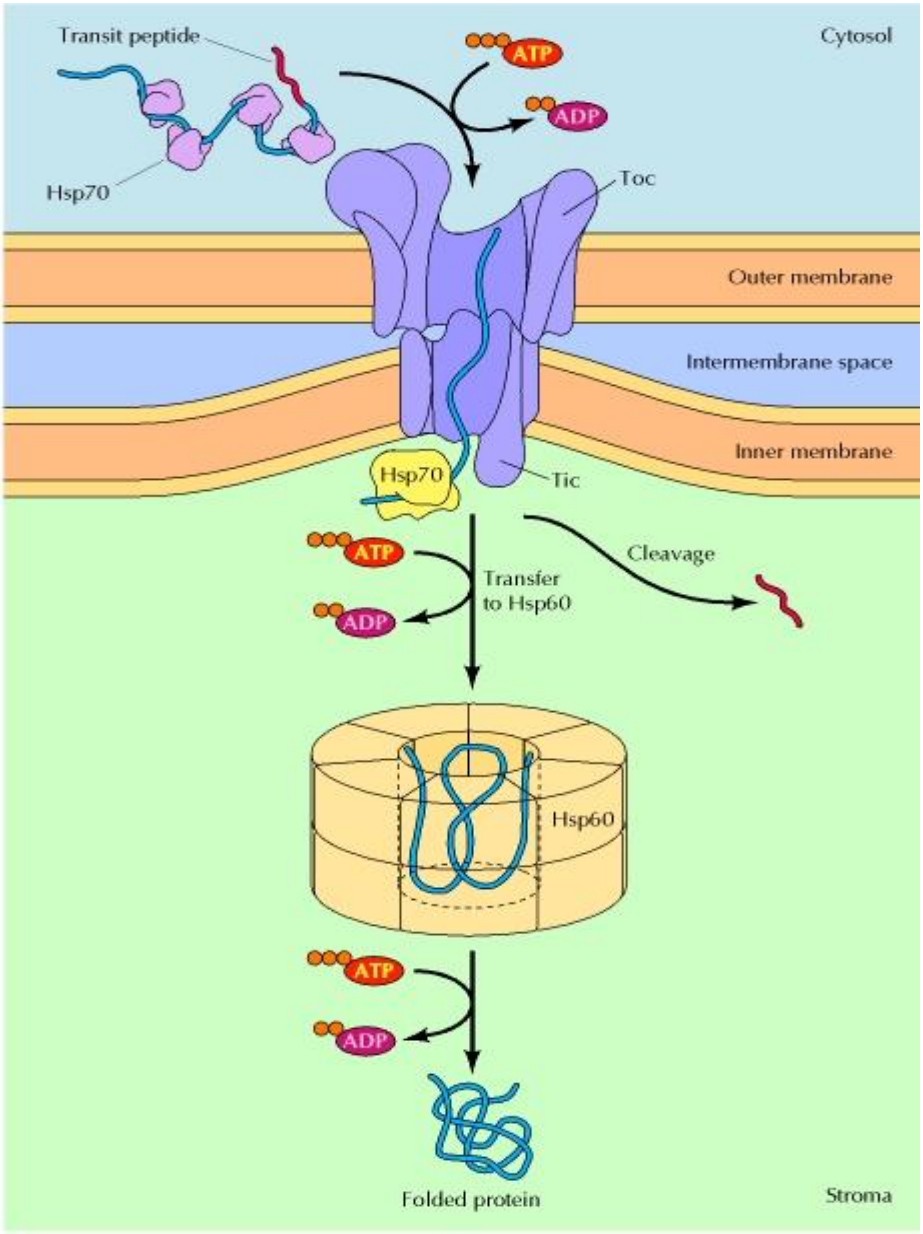


Figure:Protein import into the chloroplast stroma

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.

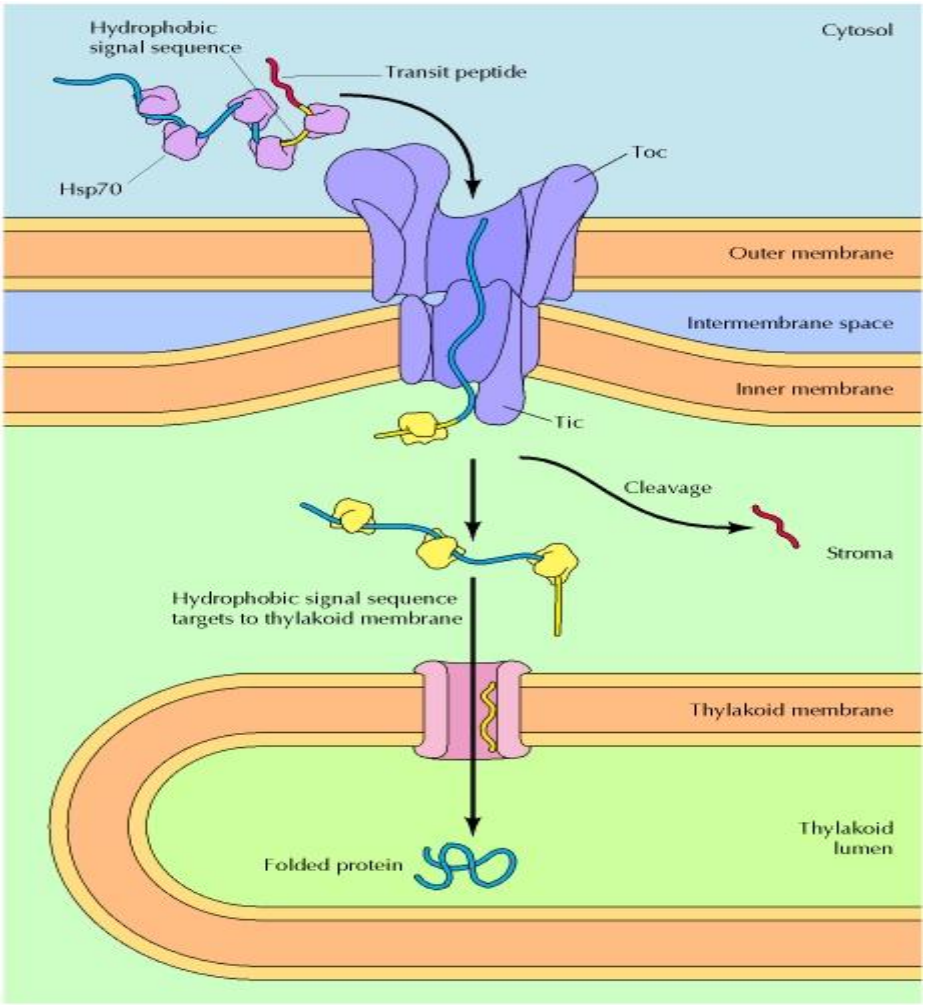


Figure: Import of proteins into the thylakoid lumen

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.

POSSIBLE QUESTIONS

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

KARPAGAM ACADEMY OF HIGHER EDUCATION

DEPARTMENT OF BIOCHEMISTRY

I BSc BIOCHEMISTRY-First Semester

CELL BIOLOGY (19BCU102)

UNIT-III **MULTIPLE CHOICE QUESTIONS**

S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	Suicide bags are otherwise called	Ribosomes	Lysosomes	Golgi apparatus	Endoplasmic reticulam	Lysosomes
2	The respiratory center at the cell is	Nucleus	Cytoplasm	Microtubules	Mitochondria	Mitochondria
3	An interface between the nucleus and cytoplasm is	Nuclear envelope	Nuclear membrane	Nuclear pores	Perinuclear space	Perinuclear space
4	Which of these cellular organelles does not have their own DNA?	Chloroplast	Nucleus	Mitochondrion	Ribosomes	Ribosomes
5	The Golgi apparatus important for	Protein synthesis	DNA synthesis	RN A synthesis	Packaging and secretion of proteins	Packaging and secretion of proteins
6	The prokaryotic cell contain	70s ribosome	60s ribosome	50s ribosome	80s ribosome	50s ribosome
7	Rough endoplasmic reticulum is embedded with	RNA	Ribosome	Protein	Lysosomes	Ribosome
8	ATP is produced within the mitochondria by ---------- process	Reduction	Oxidation	Hydrolysis	Oxidatio and reduction	Oxidation
9	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
10	Electron transport chain is also called as ----------	Respiratory chain	Transport chain	Reproductive chain	Phosphorylation chain	Respiratory chain
11	Rough endoplasmic reticulum is embedded with	RNA	Ribosome	Protein	Lysosomes	Ribosome
12	The golgi apparatus important for	Protein synthesis	DNA synthesis	RNA synthesis	Packaging and secretion of proteins	Packaging and secretion of proteins

13	Which one is important in drug detoxication reaction	RER	Golgi bodies	lysosomes	SER	SER
14	Mitochondria is impermeable from cytosolic side to except	fatty acid	pyruvic acid	Nucleotide	NADH	Nucleotide
15	Secretory vesicles are formed from	ER	golgi bodies	lysosome	Ribosome	golgi bodies
16	The citrice acid cycle is operating in	outer membrane	Inter membrane space	inner membrane	Mitochondrail matrix	inner membrane
17	……..... is involved in the ETC reaction by oxidative phosphorylation.	Mitochondria	lysosome	ribosome	golgi complex	Mitochondria
18	The ETC located in the …….. of matrix.	outer membrane	cristae	inner membrane	matrix	inner membrane
19	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
20	Ubiquinone is a …… soluble benzoquinone with a long isoprenoid side chain.	water soluble	fat soluble	lipid soluble	none of the above	fat soluble
21	Which protein is used for modifying pepetide chain.?	ubiquitin	protenases	chaperons	protein kinase	chaperons
22	Which enzyme synthesisATP from ADP during oxidative phosphorylation.	ATP synthetase	ATP ase	phosphorylase	ATP synthetase	ATP synthetase
23	The genome zize of mitochondria	16kb	20kb	80kb	200kb	16kb
24	ETC is _____ on mitochondrial membrane	Enzyme	RNA	Ribozyme	Abzyme	Enzyme
25	Rough endoplasmic reticulam is involved in	Protein synthesis	photosynthesis	degradation of fatty acids	transport of oxygen	Protein synthesis
26	The foldings of inner membrane of mitochondria are	cisternae	sulci	matrix lines	cristae	cristae
27	The role of mitochondria in oxidative phosphorylation was explained by	lehinger	Embden	krebs	Mayer hoff	lehinger

28	Smooth endoplasmic reticulum is concerned with	protein metabolism	lipid metabolism	carbohydrate metabolism	amino acid metabolism	lipid metabolism
29	The primary lysossomes are called as	Virgin lysosomes	Phagolysososmes	Autolysososmes	Post lysososmes	Virgin lysosomes
30	The basic subunit of microtubules are	protofilaments	tubulin	microfilaments	Actin	tubulin
31	The lysosomal compartment is	more acidic	less acidic	neutral	less basic	more acidic
32	Plasmodesmata are present in	animal cells	mammalian cells	plant cells	bacteria.	plant cells
33	Majority of the enzymes in lysosomes show their catalytic activity in	acidic PH	Alkaline	neutral PH	Osmotic pH	acidic PH
34	The signal that return protein to endoplasmic reticulum itself is	KKLL	KDEL	KKDD	KKXL	KDEL
35	Along with KDEL sequence the other sequence that retain the protein in ER is	KKLL	LKKL	KKDD	KKXX	KKXX
36	Golgi stack close to plasma membrane is	Cis	Trans	Medial	Proximal	Trans
37	Vesicles from endoplasmic reticulum enter into golgi through	Cis	Trans	Medial	Proximal	Cis
38	Among the golgi network which is mainly involved with sorting and distribution of proteins	Cis	Trans	Medial	Proximal	Trans
39	Protein glycosylation is mainly takes place in _____	Mitochodria	Lysosomes	Golgi	Peroxisomes	Golgi
40	Proteins destined for lysosomes are tagged with	Glu-6-phosphate	Fru-6-phosphate	Man-6-phosphate	Gal-6-phosphate	Man-6-phosphate
41	The last residue added in protein glycosylation is	N-acetyl glucosamine	N-acetyl galactosamine	N-acetyl muramic acid	Sialic Acid	Sialic Acid
42	Among the following which is not synthesized in ER	Phospho lipids	Cholesterol	Ceramide	Sphingomyelin	Sphingomyelin
43	Spingomyelin is synthesized by	Mitochodria	Lysosomes	Golgi	Endoplasmic reticulum	Golgi
44	Glycoproteins are synthesized by	Mitochodria	Lysosomes	Golgi	Endoplasmic reticulum	Endoplasmic reticulum

45	Glycolipids are synthesized by	Mitochodria	Lysosomes	Golgi	Endoplasmic reticulum	Golgi
46	In Plant the golgi have additional role in the synthesis of ____	Mitochodria	Lysosomes	Endoplasimc reticulum	Cell wall	Cell wall
47	In Plant, the cell wall is synthesised by	Mitochodria	Lysosomes	Golgi	Endoplasmic reticulum	Golgi
48	Which is not a component of plant cell wall?	Cellulose	Hemi cellulose	Glucose	Pectin	Glucose
49	The cells that lacks golgi, the protein sorting is done by	Mitochodria	Endoplasmic reticulum	Vacuole	Lysosomes	Vacuole
50	Uptake of extracellular molecules from plasma membrane occur through	COP-I	COP-II	COP-III	Clathrin coated vesicles	Clathrin coated vesicles
51	The basket like structure that form lattice is facilitated by _____protein	Spectrin	Ankyrin	Clathrin	Anerin	Clathrin
52	Other name of GTP binding protein involved with budding of vesicle	ARF	ARS	ASR	AFR	ARF
53	Acid hydrolases are synthesized by	Mitochodria	Lysosomes	Golgi	Endoplasmic reticulum	Lysosomes
54	The active pH of acid hydrolases are	7	6.8	5	4	5
55	The internal acidic pH of lysosomes is achieved by _____ pump	Anion	Cation	Proton	Eletron pump	Proton
56	Acid hydrolases are targeted to lysosomes through	Glu-6-phosphate	Fru-6-phosphate	Man-6-phosphate	Gal-6-phosphate	Man-6-phosphate
57	The gradual turnover of cells own compound is achieved by _____process	Phagocytosis	Endocytosis	Autophagy	Exocytosis	Autophagy
58	Uptake of particulate material by cell through plasma membrane is known as	Phagocytosis	Endocytosis	Autophagy	Exocytosis	Phagocytosis
59	Which transporter is not found on mitochondrial membrane?	ATP synthase	Adenine nucleotide translocator	Phosphate- hydroxyl ion translocator	Group translocator	Group translocator
60						

UNIT-IV

SYLLABUS

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

THE CYTOSKELETON AND CELL MOVEMENT

The membrane-enclosed organelles constitute one level of the organizational substructure of eukaryotic cells. A further level of organization is provided by the cytoskeleton, which consists of a network of protein filaments extending throughout the cytoplasm of all eukaryotic cells. It is a dynamic structure that is continually reorganized as cells move and change shape, for example, during cell division.

The cytoskeleton is composed of three principal types of protein filaments:

(1) Micro filaments(actin)
(2) Intermediate filaments
(3) Microtubules,

They are held together and linked to subcellular organelles and the plasma membrane by a variety of accessory proteins.

Functions of cytoskeleton:

- The cytoskeleton provides a **structural framework** for the cell, serving as a scaffold that determines cell shape and the general organization of the cytoplasm.
- It is responsible for **cell movements.** These include not only the movements of entire cells, but also the internal **transport of organelles** and other structures (such as mitotic chromosomes) through the cytoplasm.
- It is also involved **cell division**

1.MICROFILAMENT

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. 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.

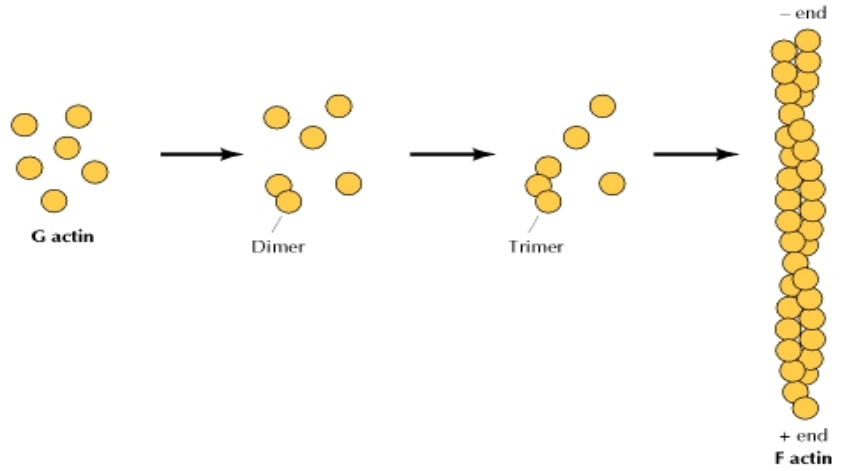
Actin was first isolated from muscle cells, in which it constitutes approximately 20% of total cell protein, in 1942. Although actin was initially thought to be uniquely involved in muscle contraction, it is now known to be an extremely abundant protein (typically 5 to 10% of total protein) in all types of eukaryotic cells.

Assembly and Disassembly of Actin Filaments

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**). Each monomer is rotated by 166^{o}in the filaments, which therefore have the appearance of a double-stranded helix. Because all the actin monomers are oriented in the same direction, actin filaments have a distinct polarity and their ends (called the plus and minus ends) are distinguishable from one another. This polarity of actin filaments is important both in their assembly and in establishing a unique direction of myosin movement relative to actin.

Assembly

- 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.



Disassembly

Because actin polymerization is reversible, filaments can depolymerize by the dissociation of actin subunits, allowing actin filaments to be broken down when necessary. 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.

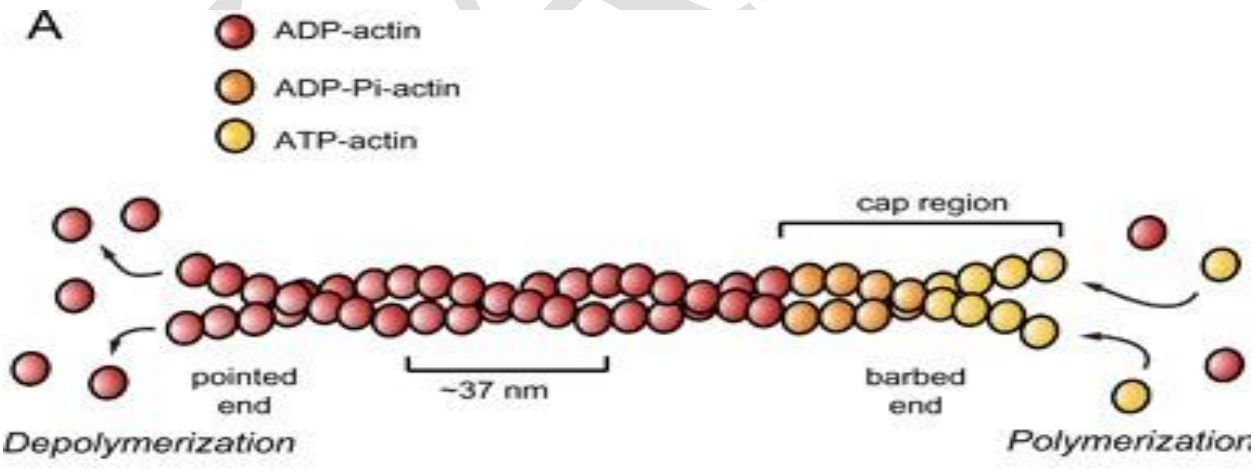


Fig. Assembly And Disassembly Of Actin

Treadmilling

Treadmilling is a phenomenon observed in many cellular cytoskeletal filaments, especially in actin filaments and microtubules. It occurs when one end of a filament grows in length while the other end shrinks resulting in a section of filament seemingly "moving" across a stratum or the cytosol. This is due to the constant removal of the protein subunits from these filaments at one end of the filament while protein subunits are constantly added at the other end.

The two ends of an actin filament differ in their dynamics of subunit addition and removal. They are thus referred to as the *plus end* (with faster dynamics) and the *minus end* (with slower dynamics). This difference results from the fact that subunit addition at the minus end requires a conformational change of the subunits. 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 As a consequence, the actin filaments are structurally polar. 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. Treadmilling required for cells to move and change shape.

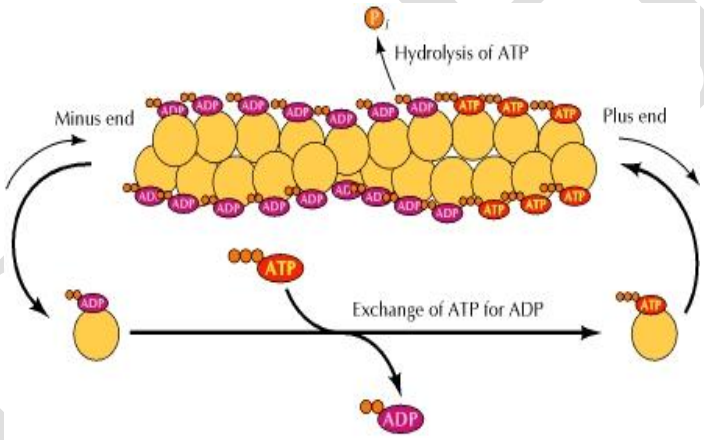


Figure: Tread milling action of actin filaments

Actin-binding proteins:

Within the cell, both the assembly and disassembly of actin filaments are regulated by **actin-binding proteins**. 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. 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.

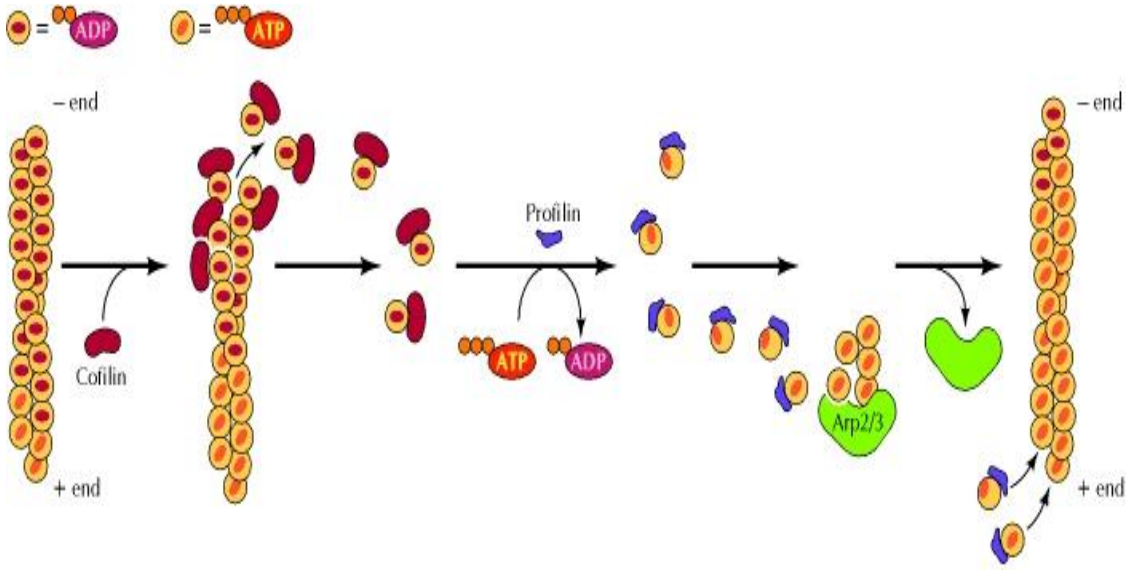


Figure: Actin-binding proteins

ORGANIZATION 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 and networks

All of the actin-binding proteins involved in crosslinking contain at least two domains mfrerthat bind actin, allowing them to bind and crosslink two different actin filaments. The nature of the association between these filaments is then determined by the size and shape of the crosslinking proteins.

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. In contrast, the

proteins that organize actin filaments into networks tend to be large flexible proteins that can crosslink perpendicular filaments

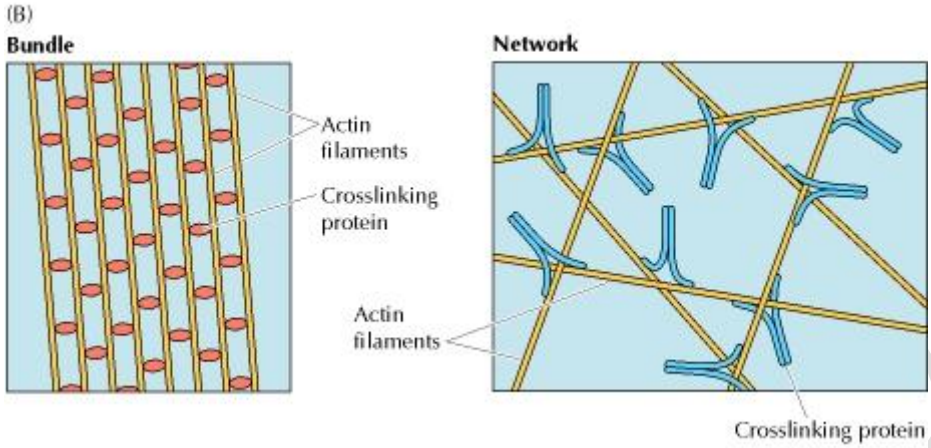


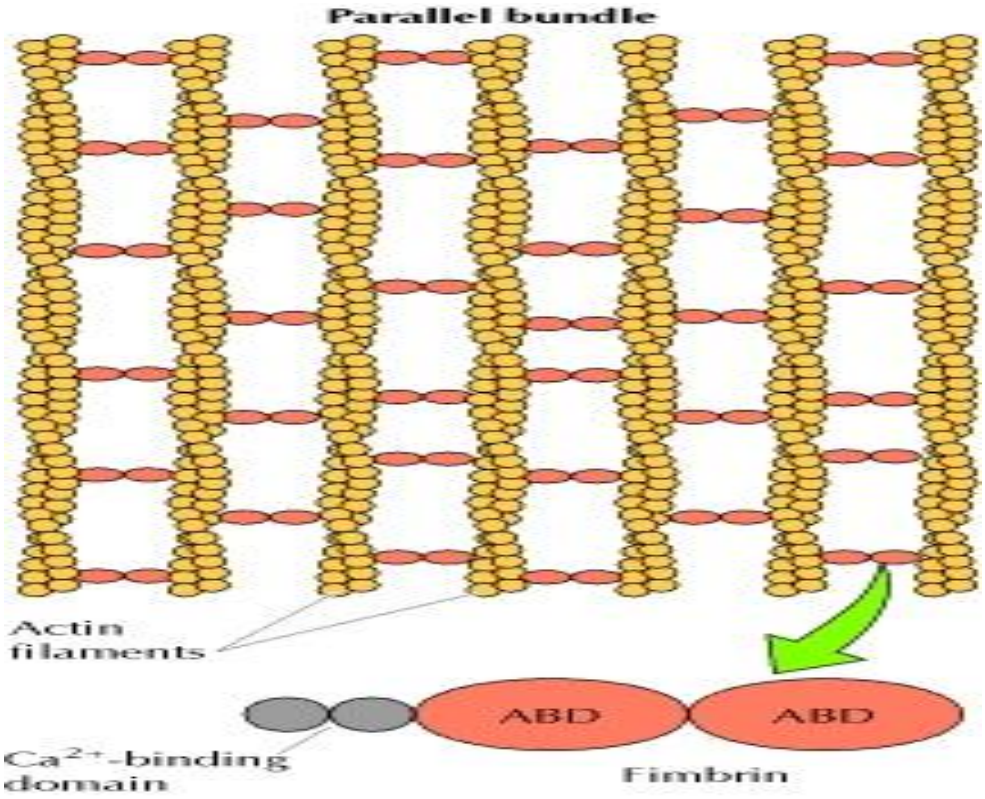
Fig.Organization of Actin

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.


Parallel bundle
Actin filaments
ABD
ABD
Ca^{2+}-binding domain
Fimbrin

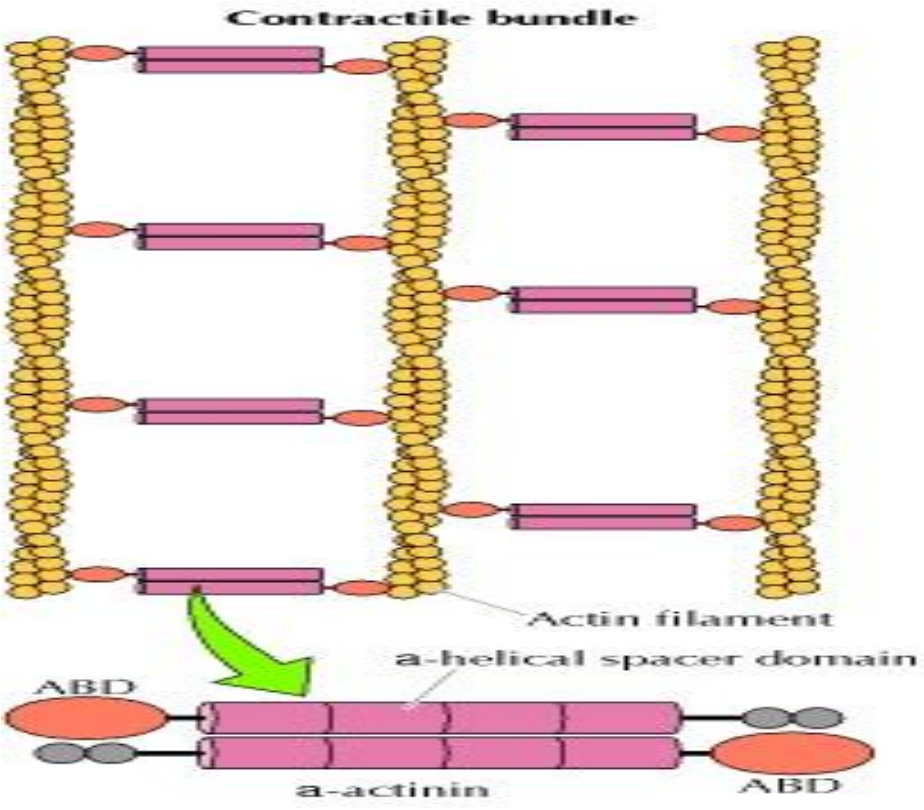

Contractile bundle
Actin filament
a-helical spacer domain
ABD
a-actinin
ABD

Figure : Actin-bundling proteins

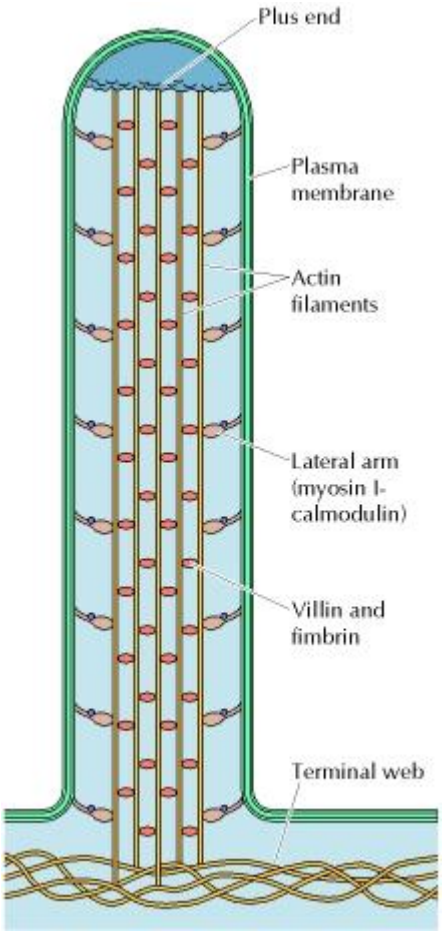


Figure: Organization of microvilli -The core actin filaments of microvilli are crosslinked into closely packed bundles by fimbrin and villin. They are attached to the plasma membrane along their length by lateral arms, consisting of myosin I and calmodulin. The plus ends of the actin filaments are embedded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

Actin network:

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

than those crosslinked by fimbrin (40 nm apart instead of 14 nm). The increased spacing between filaments allows the motor protein myosin to interact with the actin filaments in these bundles, which (as discussed later) enables them to contract.

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

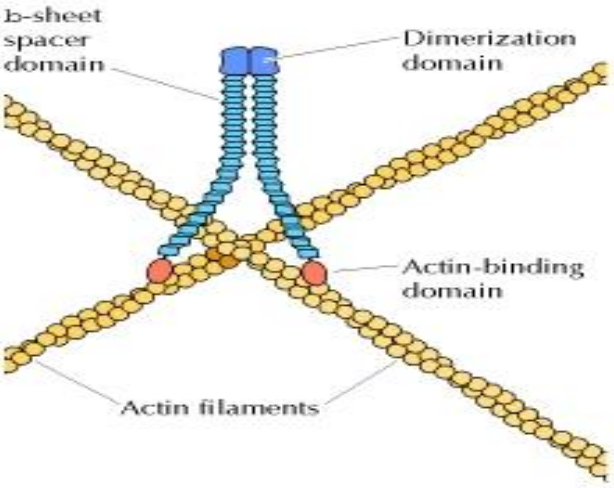


Figure: Actin networks and filamin

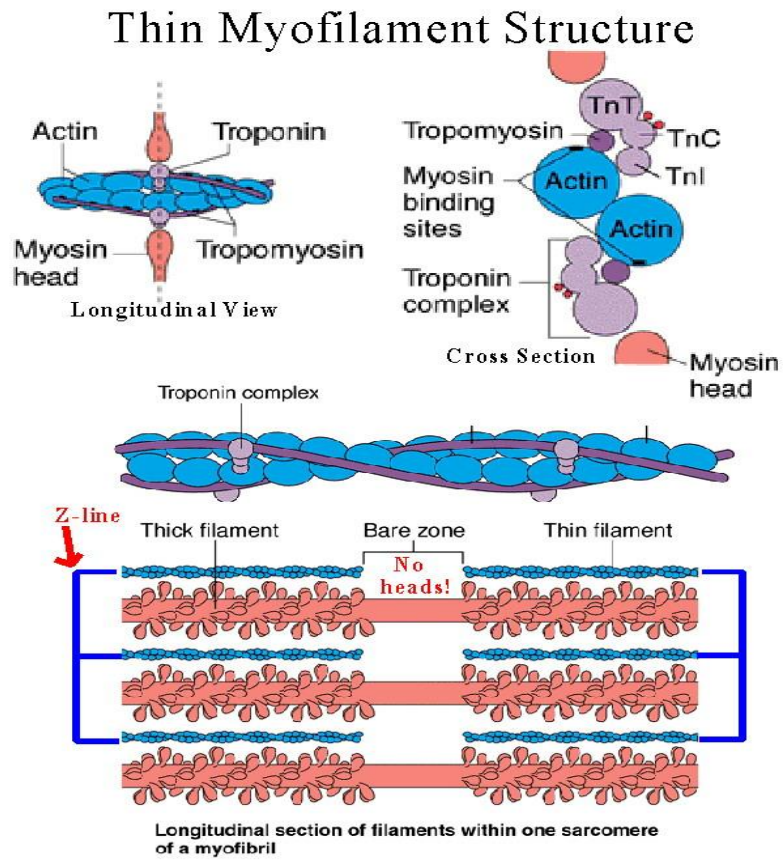
.**Actin, Myosin, and Cell Movement**

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 actin cytoskeleton 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.

Myosin filaments:

Myofibrils are contractile units within the cell which consist of a regular array of protein myofilaments. Each myofilament runs longitudinally with respect to the muscle fiber. There are two types: the thick bands and the thin bands. Thick bands are made of multiple molecules of a protein called myosin. The thin bands are made of multiple molecules of a protein called actin. The thin actin bands are attached to a Z-line or Z-disk of an elastic protein called titin. The titin protein also extends into the myofibril anchoring the other bands in position. From each Z-line to the next is a unit called the sarcomere. The sarcomere is the smallest contractile unit in the myofibril. Sarcomeres contract because the Z-lines move closer together. As the sarcomeres contract the myofibrils contract. As the myofibrils contract the muscle cell contracts. And as the cells contract the entire muscle contracts.

The arrangement of the thick myosin filaments across the myofibrils and the cell causes them to refract light and produce a dark band known as the A Band. In between the A bands is a light area where there are no thick myofilaments, only thin actin filaments. These are called the I Bands. The dark bands are the striations seen with the light microscope.



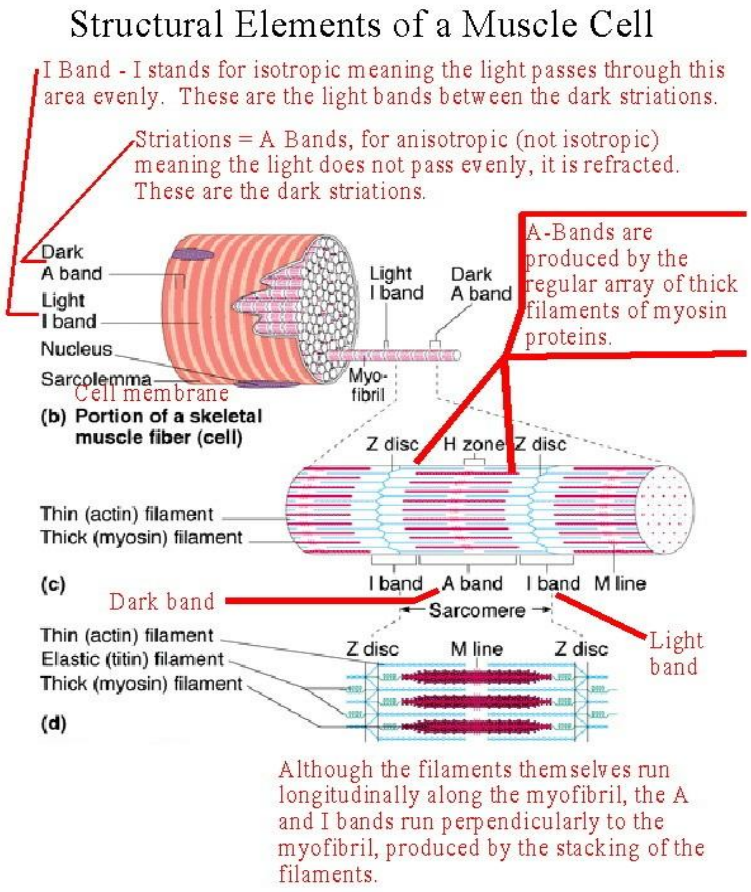


Fig: Structure of myofilament and its elements

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.

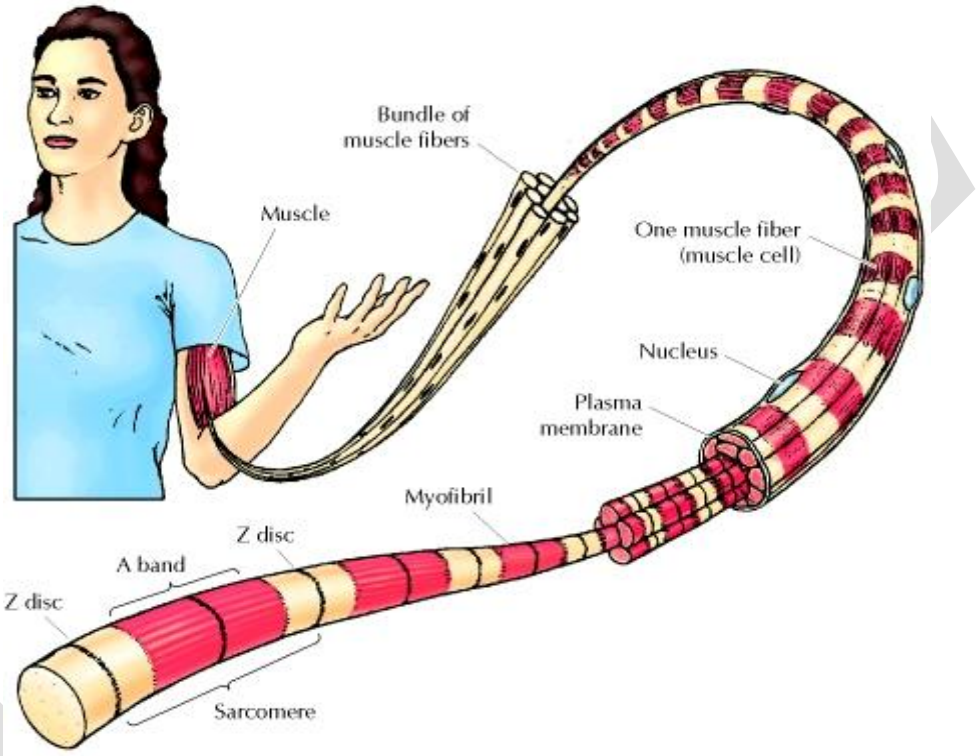


Figure: Structure of muscle cells

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 (fig). 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.

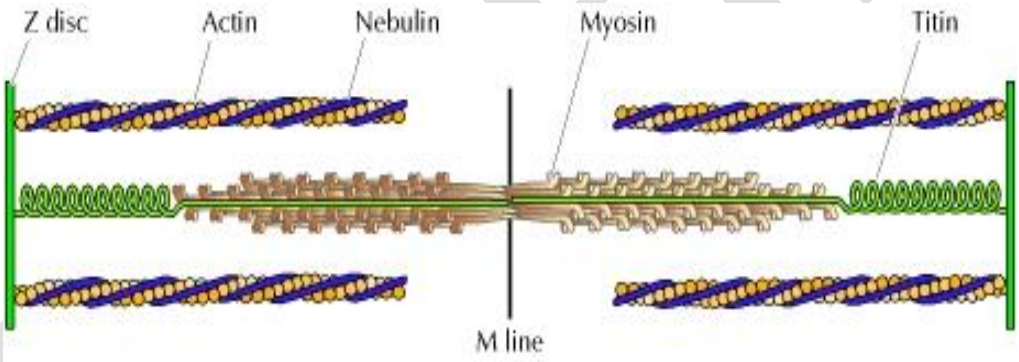


Figure : Titin and nebulin

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 (Fig). 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.

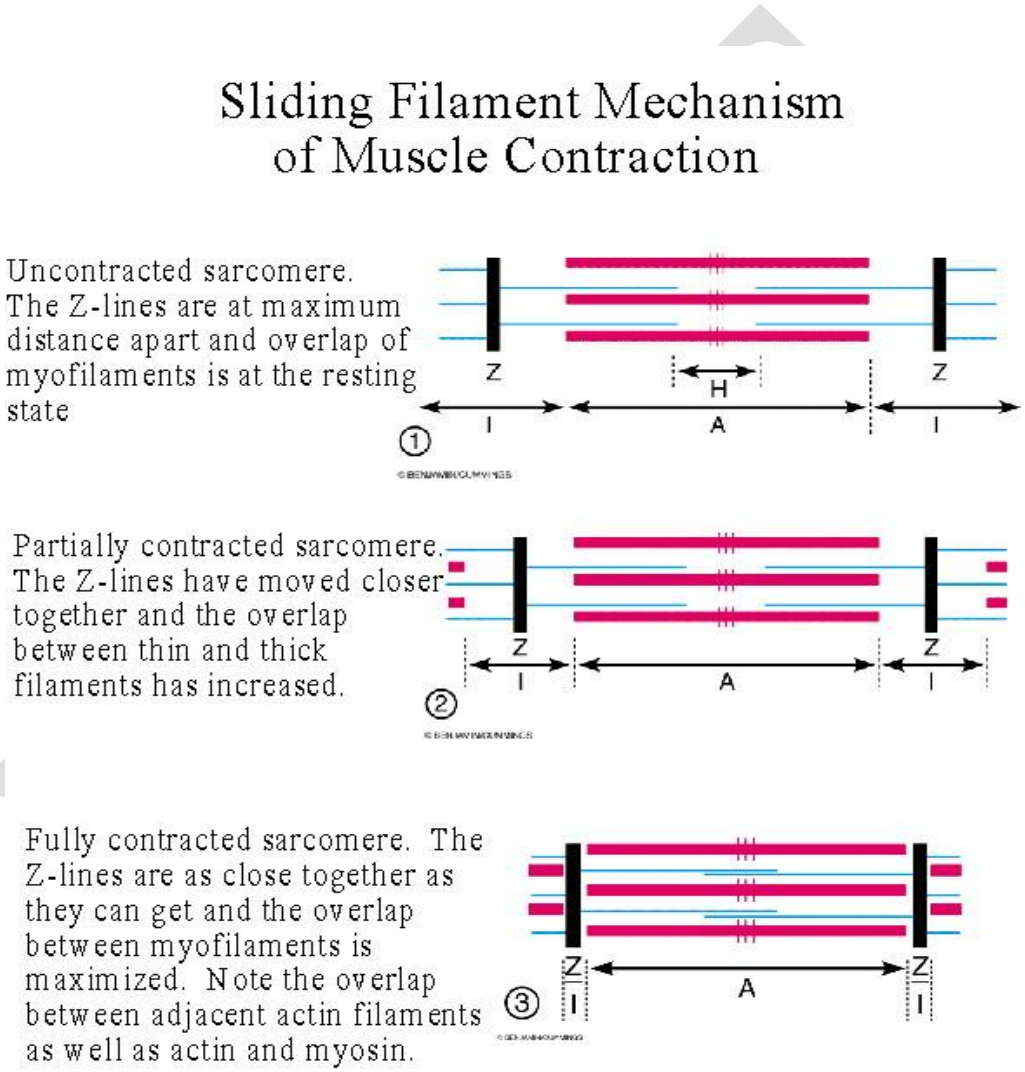
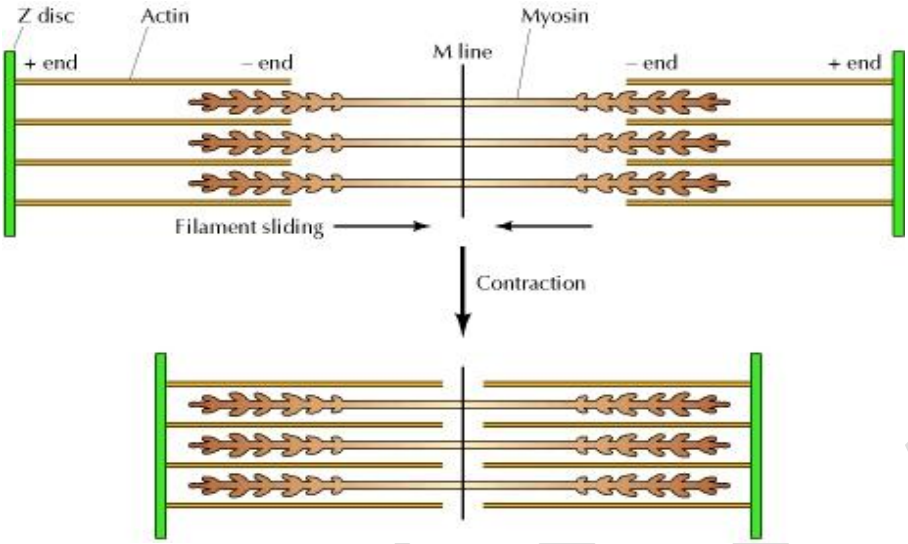


Fig: Sliding Filament Model of muscle contraction



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.

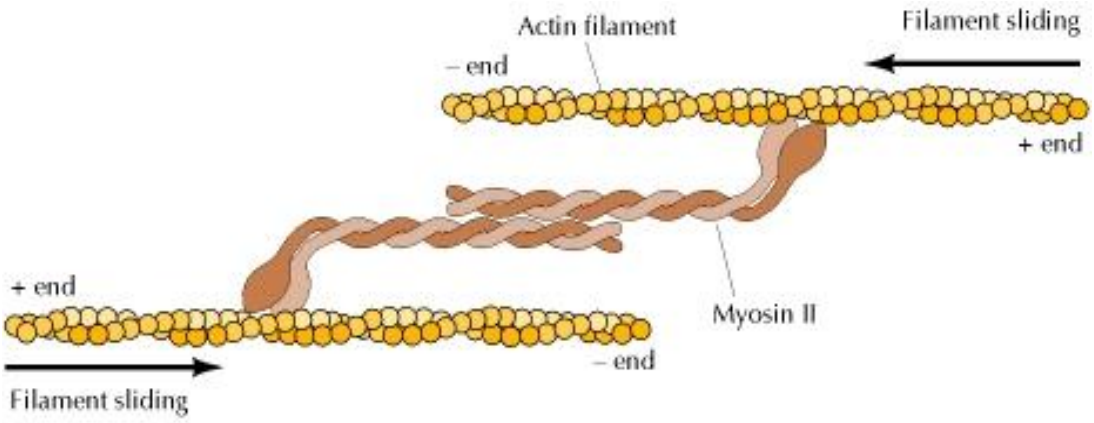


Figure : Contractile assemblies in nonmuscle cells

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.

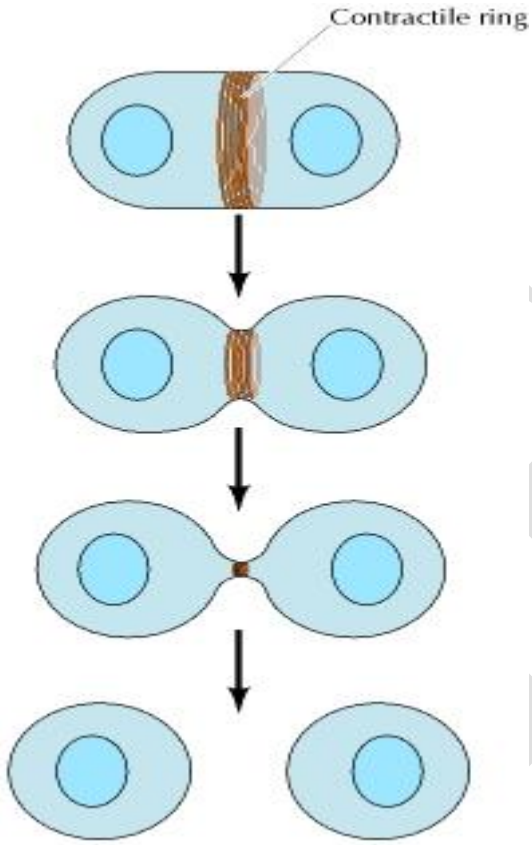


Fig: Following completion of mitosis (nuclear division), a contractile ring consisting of actin filaments and myosin II divides the cell in two.

2.MICROTUBULES:

Microtubules, the third principal component of the cytoskeleton, are rigid hollow rods approximately 25 nm in diameter. Like actin filaments, microtubules are dynamic structures that undergo continual assembly and disassembly within the cell. They function both to determine cell shape and in a variety of cell movements, including some forms of cell locomotion, the intracellular transport of organelles, and the separation of chromosomes during mitosis.There are many proteins that bind to the microtubule, including motor proteins such as kinesin and dynein, severing proteins like katanin, and other proteins important for regulating microtubule dynamics.

Structure:

Microtubules are long, hollow cylinders made up of polymerised α- and β-tubulin dimers. Tubulin dimers polymerize end to end in protofilaments which are the building block for the microtubule structure. 13 protofilaments associate laterally to form a single microtubule and this

structure can then extend by addition of more protofilaments to generate the long, hollow, cylindrical structure of a microtubule. Microtubules can extend in length indefinitely.

Microtubules have a distinct polarity which is important for their biological function. Tubulin polymerizes end to end with the α subunit of one tubulin dimer contacting the β subunit of the next. Therefore, in a protofilament, one end will have the α subunit exposed while the other end will have the β subunit exposed. These ends are designated the (−) and (+) ends, respectively. The protofilaments bundle parallel to one another, so, in a microtubule, there is one end, the (+) end, with only β subunits exposed, while the other end, the (−) end, has only α subunits exposed. Elongation of microtubules typically only occurs from the (+) end.

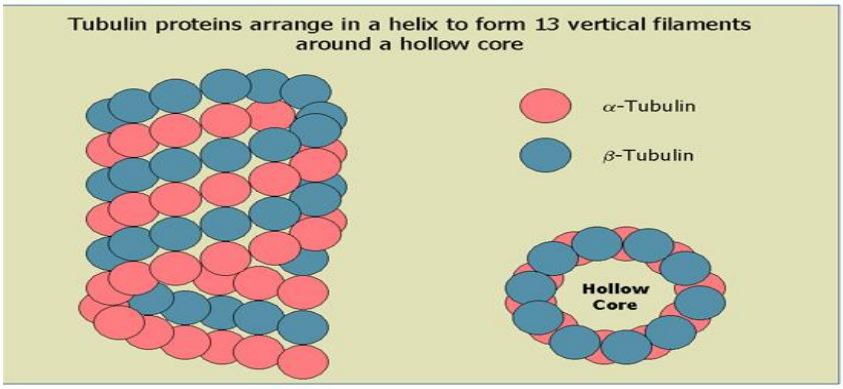


Figure: Structure of tubulin

Assembly and Dynamic Instability of Microtubules:

In contrast to intermediate filaments, which are composed of a variety of different fibrous proteins, microtubules are composed of a single type of globular protein, called tubulin. Tubulin is a dimer consisting of two closely related 55-kd polypeptides, α-tubulin and β-tubulin. Like actin, both α- and β-tubulin are encoded by small families of related genes. In addition, a third type of tubulin (γ-tubulin) is specifically localized to the centrosome, where it plays a critical role in initiating microtubule assembly .

Microtubule structure:

Tubulin dimers polymerize to form microtubules, which generally consist of 13 linear protofilaments assembled around a hollow core. The protofilaments, which are composed of head-to-tail arrays of tubulin dimers, are arranged in parallel. Consequently, microtubules (like actin filaments) are polar structures with two distinct ends: a fast-growing plus end and a slow-growing minus end. This polarity is an important consideration in determining the direction of movement along microtubules, just as the polarity of actin filaments defines the direction of myosin movement.

Assembly of microtubules:

Tubulin dimers can depolymerize as well as polymerize, and microtubules can undergo rapid cycles of assembly and disassembly. Both α- and β-tubulin bind GTP, which functions

analogously to the ATP bound to actin to regulate polymerization. In particular, the GTP bound to β-tubulin (though not that bound to α-tubulin) is hydrolyzed to GDP during or shortly after polymerization. This GTP hydrolysis weakens the binding affinity of tubulin for adjacent molecules, thereby favoring depolymerization and resulting in the dynamic behavior of microtubules. Like actin filaments, microtubules undergo treadmilling, a dynamic behavior in which tubulin molecules bound to GDP are continually lost from the minus end and replaced by the addition of tubulin molecules bound to GTP to the plus end of the same microtubule. In microtubules, GTP hydrolysis also results in the behavior known as dynamic instability, in which individual microtubules alternate between cycles of growth and shrinkage. Whether a microtubule grows or shrinks is determined by the rate of tubulin addition relative to the rate of GTP hydrolysis. As long as new GTP-bound tubulin molecules are added more rapidly than GTP is hydrolyzed, the microtubule retains a GTP cap at its plus end and microtubule growth continues. However, if the rate of polymerization slows, the GTP bound to tubulin at the plus end of the microtubule will be hydrolyzed to GDP. If this occurs, the GDP-bound tubulin will dissociate, resulting in rapid depolymerization and shrinkage of the microtubule.

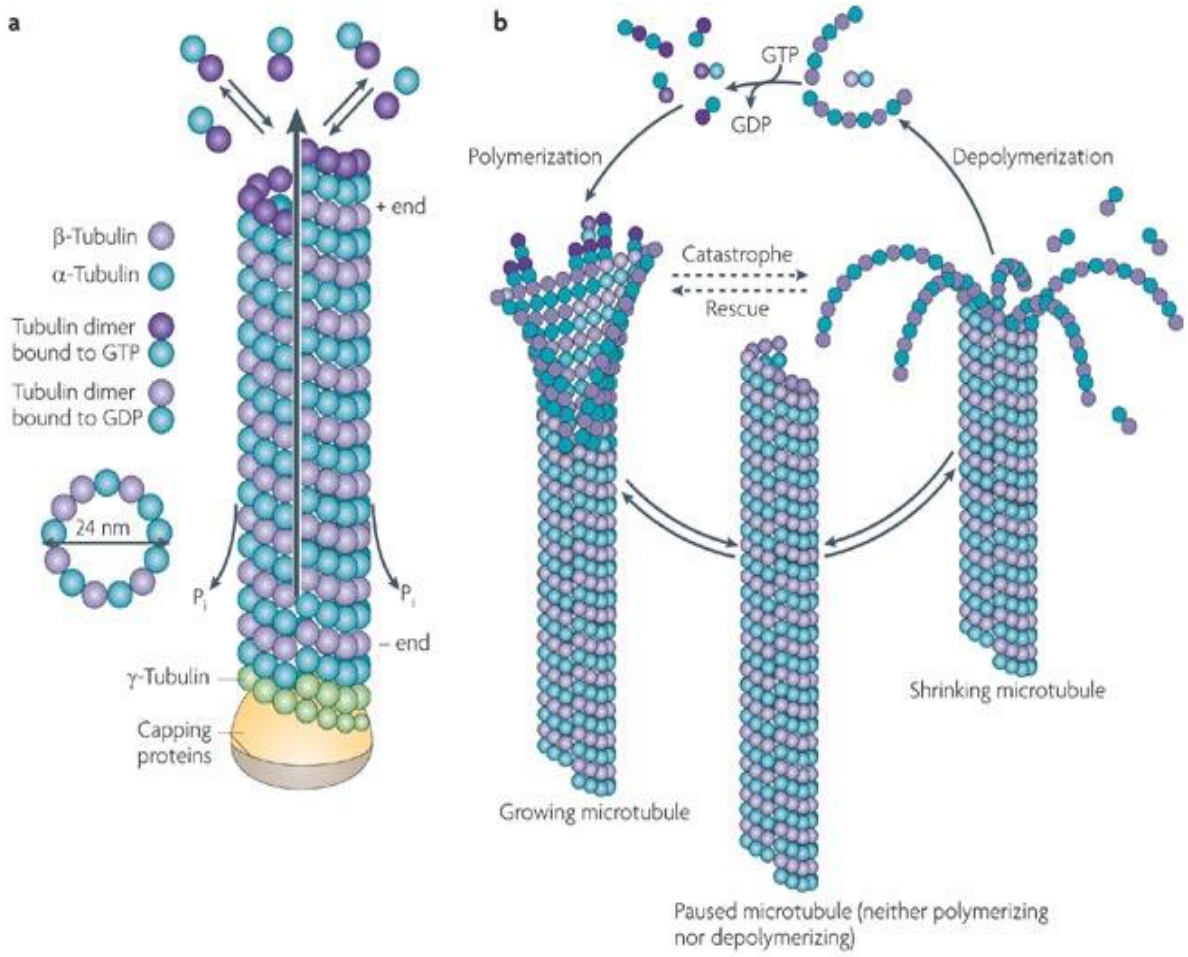


Fig: Assembly of Microtubule

Dynamic instability of microtubules:

Dynamic instability, described by Tim Mitchison and Marc Kirschner in 1984, results in the continual and rapid turnover of most microtubules, which have half-lives of only several minutes within the cell. As discussed later, this rapid turnover of microtubules is particularly critical for the remodeling of the cytoskeleton that occurs during mitosis. Because of the central role of microtubules in mitosis, drugs that affect microtubule assembly are useful not only as experimental tools in cell biology but also in the treatment of cancer. Colchicine and colcemid are examples of commonly used experimental drugs that bind tubulin and inhibit microtubule polymerization, which in turn blocks mitosis. Two related drugs (vincristine and vinblastine) are used in cancer chemotherapy because they selectively inhibit rapidly dividing cells. Another useful drug, taxol, stabilizes microtubules rather than inhibiting their assembly. Such

stabilization also blocks cell division, and taxol is used as an anticancer agent as well as an experimental tool.

Functions:

- γ-Tubulin-mediated assembly of microtubules.
- Reorganization of Microtubules during Mitosis
- Formation of the mitotic spindle
- The centromere of a metaphase chromosome
- Stabilization of Microtubules and Cell Polarity
- Organization of microtubules in nerve cells

3.INTERMEDIARY FILMENTS:

Intermediate filaments (IFs) are a family of related proteins that share common structural and sequence features. Intermediate filaments have an average diameter of 10 nanometers, which is between that of 7 nm actin (microfilaments), and that of 25 nm microtubules, although they were initially designated 'intermediate' because their average diameter is between those of narrower microfilaments (actin) and wider myosinfilaments. Most types of intermediate filaments are cytoplasmic, but one type, the lamins, are nuclear.

Intermediate Filament Proteins:

Whereas actin filaments and microtubules are polymers of single types of proteins (actin and tubulin, respectively), intermediate filaments are composed of a variety of proteins that are expressed in different types of cells. More than 50 different intermediate filament proteins have been identified and classified into six groups based on similarities between their amino acid sequences (Table).

Intermediate filament		Tissue/cell type distribution
Class	Type	
I	Acidic keratin	Epithelium
II	Basic keratin	
III	Desmin	Skeletal smooth muscle, lesser amount in myofibroblasts
	Vimentin	Leukocytes, blood vessels, endothelial cells, astrocytes, some epithelial and mesenchymal cell
	GFAP	Astrocytes and other glial cells
	Peripherin	Peripheral and central neuron
IV	NFP	Mature neurons, adrenal medullary cells
	Alpha-internexin	Neurons
	Nestin	Stem cell marker, primitive neuroepithelium, developing astrocytes, developing neuron and Schwann cells
V	Lamin A/C	Ubiquitous
	Lamin B1/2	Ubiquitous

GFAP - Glial fibrillary acidic protein, NFP - Neurofilament protein

Types I and II consist of two groups of keratins, each consisting of about 15 different proteins, which are expressed in epithelial cells. Each type of epithelial cell synthesizes at least one type I (acidic) and one type II (neutral/basic) keratin, which copolymerize to form filaments. Some type I and II keratins (called hard keratins) are used for production of structures such as hair, nails, and horns. The other type I and II keratins (soft keratins) are abundant in the cytoplasm of epithelial cells, with different keratins being expressed in various differentiated cell types.

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 light, medium, and heavy, 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. 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 proteins:

Despite considerable diversity in size and amino acid sequence, the various intermediate filament proteins share a common structural organization (Fig 6.22). 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.

Fig. Structure of intermediary filament protein

Assembly of Intermediate Filaments:

The first stage of filament assembly is the formation of dimers in which the central rod domains of two polypeptide chains are wound around each other in a coiled-coil structure, similar to that formed by myosin II heavy chains. 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.

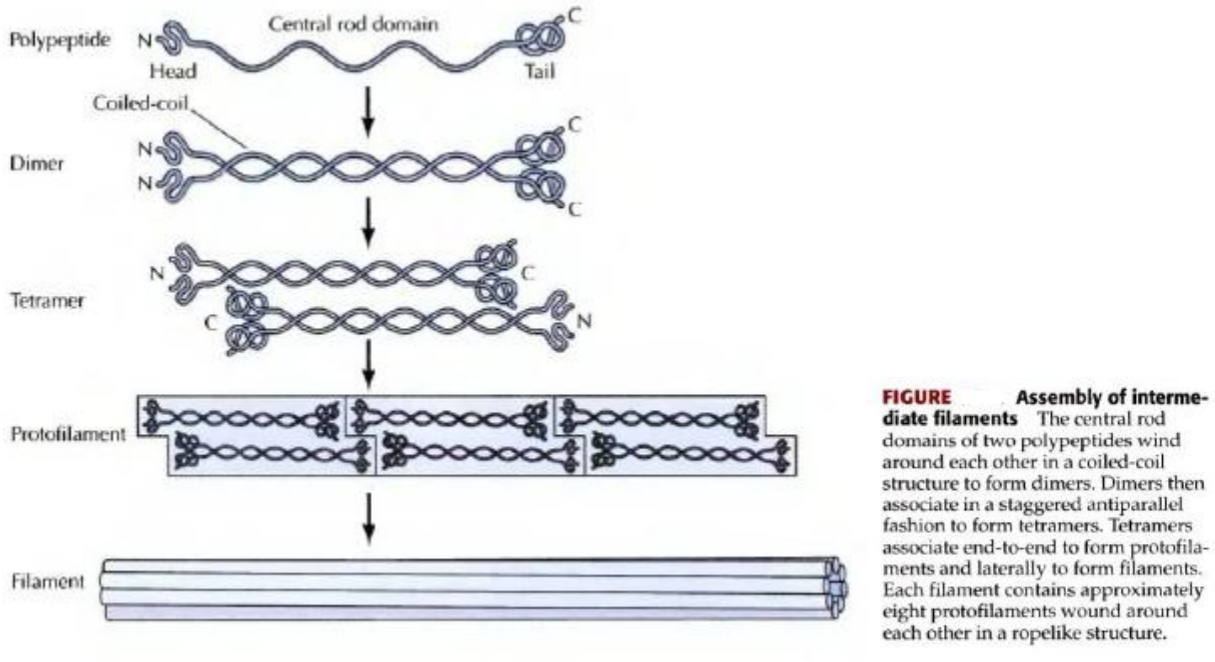


FIGURE Assembly of intermediate filaments The central rod domains of two polypeptides wind around each other in a coiled-coil structure to form dimers. Dimers then associate in a staggered antiparallel fashion to form tetramers. Tetramers associate end-to-end to form protofilaments and laterally to form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

Fig: Intermediate filament assembly

Biochemical properties:IFs are rather deformable proteins that can be stretched several times their initial length. The key to facilitate this large deformation is due to their hierarchical structure, which facilitates a cascaded activation of deformation mechanisms at different levels of strain.

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 ORGANISAION OF INTERMEDIATE FILAMENTS

Intermediate filaments form an elaborate network in the cytoplasm of most cells, extending from a ring surrounding the nucleus to the plasma membrane. Both keratin and vimentin filaments attach to the nuclear envelope, apparently serving to position and anchor the nucleus within the

cell. In addition, intermediate filaments can associate not only with the plasma membrane but also with the other elements of the cytoskeleton, actin filaments and microtubules. Intermediate filaments thus provide a scaffold that integrates the components of the cytoskeleton and organizes the internal structure of the cell.

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

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

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

Functions of Keratins and Neurofilaments

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.

4. 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.

Many bacteria also have flagella, but these prokaryotic flagella are quite different from those of eukaryotes. Bacterial flagella are protein filaments projecting from the cell surface, rather than projections of the plasma membrane supported by microtubules. Cilia are short, usually numerous, hairlike projections that can move in an undulating fashion (e.g., the protzoan Paramecium, the cells lining the human upper respiratory tract). Flagella are longer, usually fewer in number, projections that move in whip-like fashion (e.g., sperm cells). Cilia and flagella grow by the addition of tubulin dimers to their tips.

Eukaryotic cilia and flagella are very similar structures, each with a diameter of approximately 0.25 pm. Many cells are covered by numerous cilia, which are about 10 pm in length. Cilia beat in a coordinated back-and-forth motion. 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 pm) 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.

Occurrence:

The flagella occur in the protozoans of the class Flagellata, choanocyte cells of the sponges, spermatozoa of the Metazoa and among plants in the algae and gamete cells. The cilia occur in the protozoans of the class Ciliata and members of other classes and ciliated epithelium of the Metazoa. The cilia may occur on external body surface and may help in the locomotion of such animals as the larvae of certain Platyhelminthes, Nemertines, Echinodermata, Mollusca and Annelida. The cilia may line the internal cavities or passages of the metazoan bodies as air passage of the respiratory system and reproductive tracts. The nematode worms and arthropods

have no cilia. Except for sperm, the cilia in mammalian systems are not organelles of locomotion. But their effect is the same, that is, to move the environment with respect to the cell surface.

Arrangement:

Different species of bacteria have different numbers and arrangements of flagella. Monotrichous bacteria have a single flagellum. Lophotrichous bacteria have multiple flagella located at the same spot on the bacteria's surfaces. Amphitrichous bacteria have a single flagellum on each of two opposite ends. Peritrichous bacteria have flagella projecting in all directions.

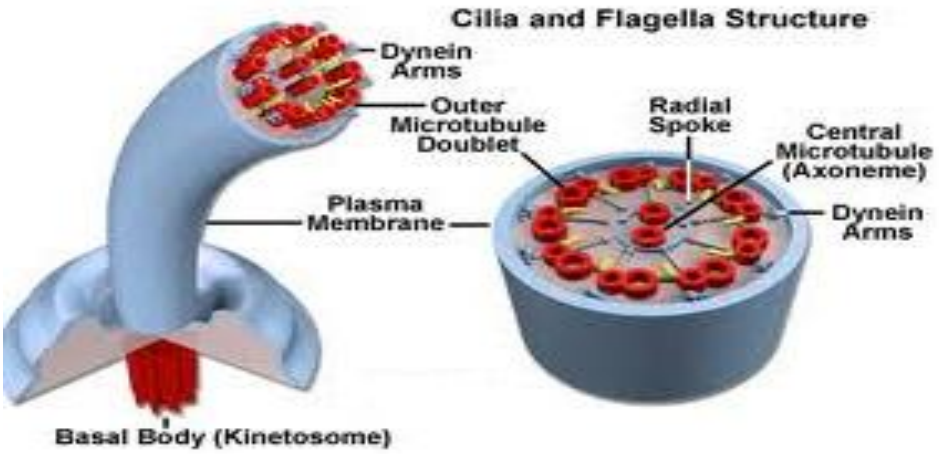


Fig: Cilia and Flagella

Structure of the axoneme of cilia and flagella

Cilia and flagella move because of the interactions of a set of microtubules inside. Collectively, these are called an "axoneme", This figure shows a microtubule (top panel) in surface view and in cross section (lower left hand panel). Two of these microtubules join to form one doublet in the cilia or flagella This is shown in the middle panel. one of the tubules is incomplete. Furthermore, there are important microtubule associated proteins (MAPs) projecting from one of the microtubule subunits.

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.

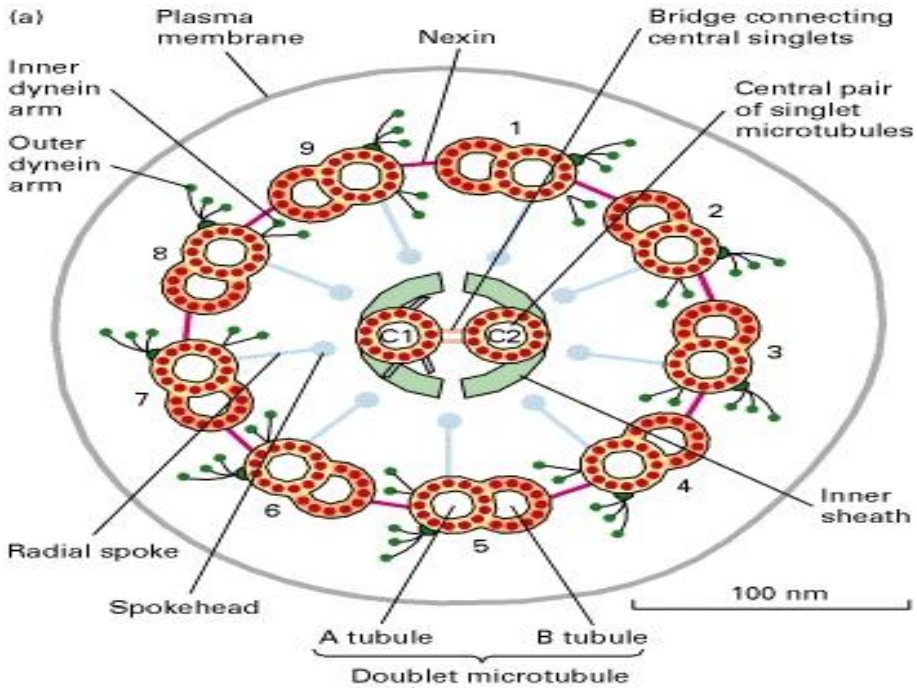
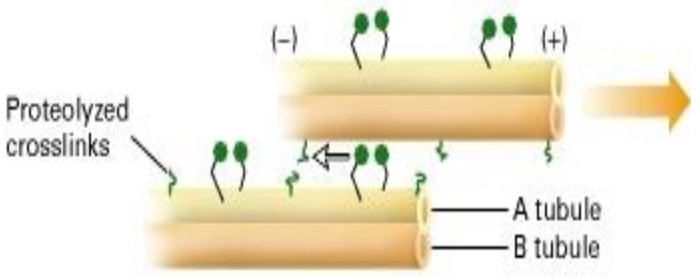


Figure: Structure of the axoneme

Movement of microtubules in 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 the axoneme 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.

Structure of axonemal dynein.



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. 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

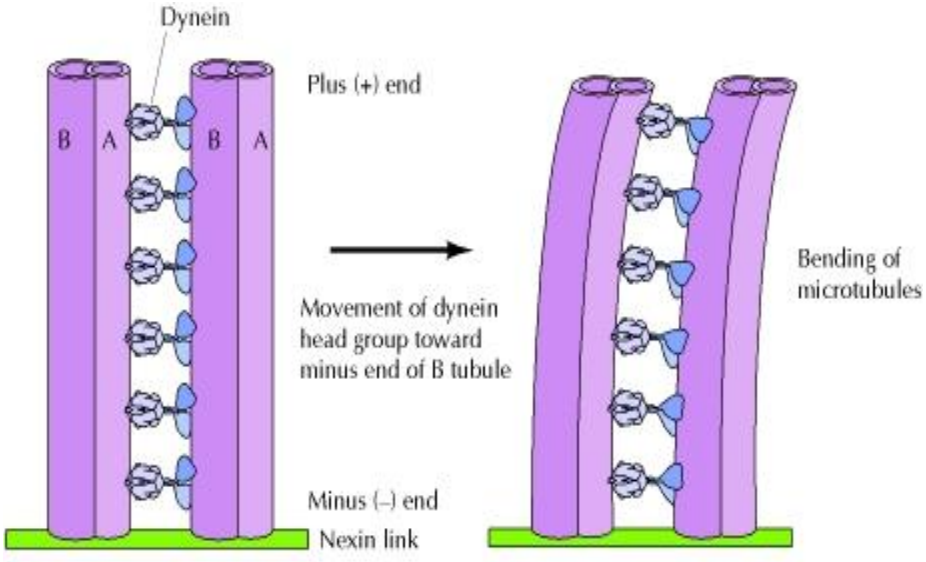


Figure : Model for movement of microtubules

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.

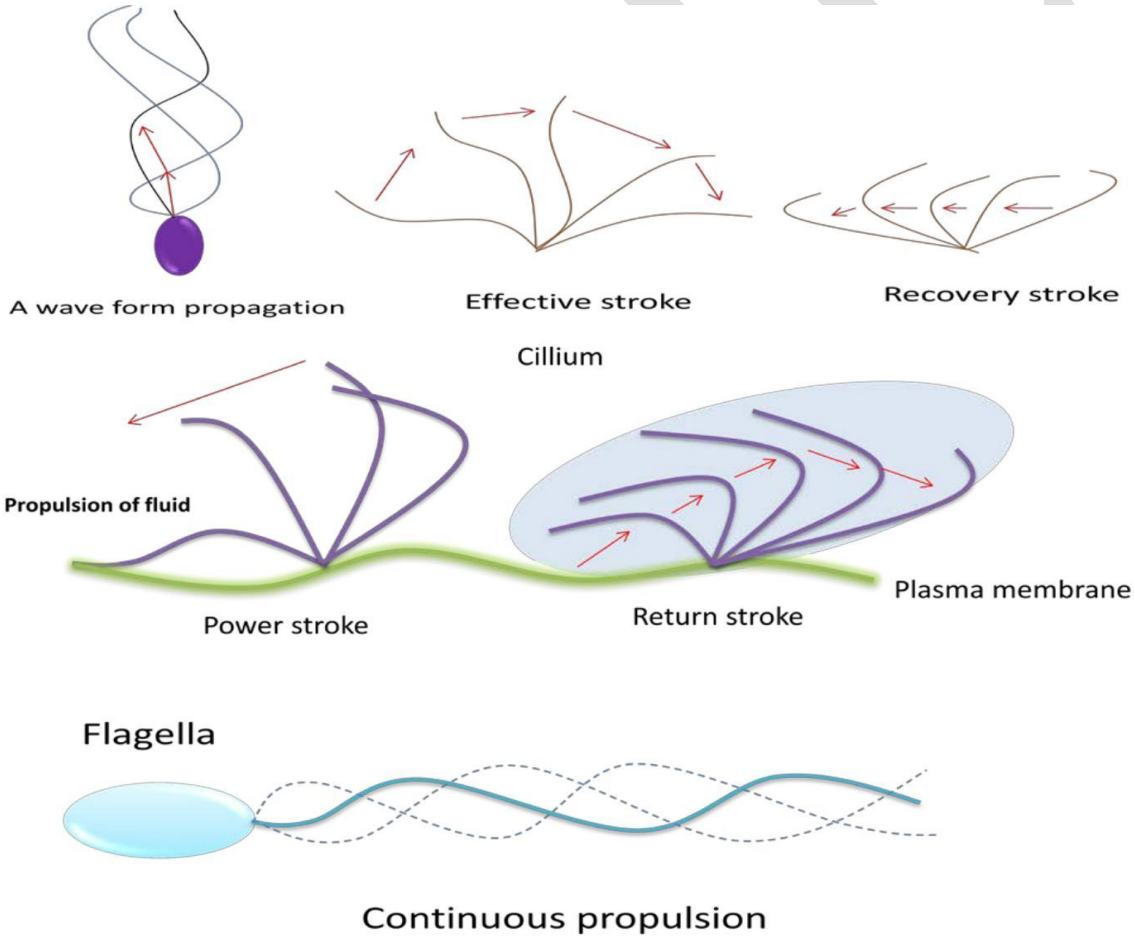


Fig.Movement of cilia and flagella

The overall structure of bacterial flagella

The bacterial flagellum (Figure) 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.

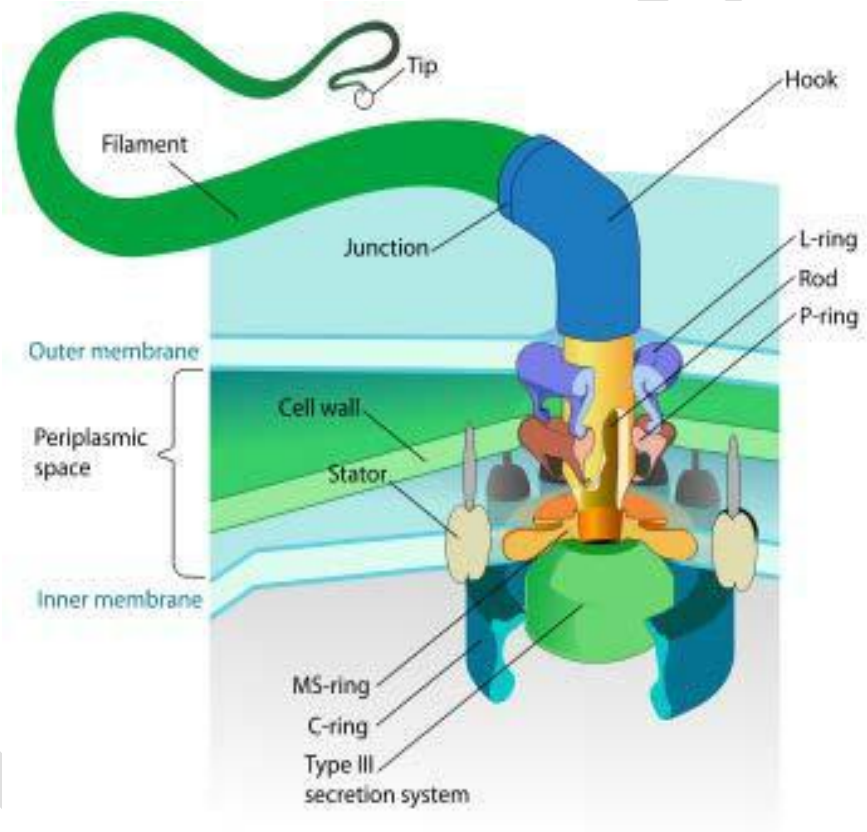


Figure 4: Flagellum of gram negative bacteria

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.

POSSIBLE QUESTIONS

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

KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
I BSc BIOCHEMISTRY-First Semester
CELL BIOLOGY (19BCU102)
MULTIPLE CHOICE QUESTIONS

UNIT-IV

S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	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
2	The size of the microtubules	10nm in diameter	18nm in diameter	1-3 µm in diameter	24nm in diameter	24nm in diameter
3	Plasma membrane coated bundle of microtubules present in cilium or flagella are known as	nexin	axoneme	dynein arm	axon.	axoneme
4	Actin is present	microtubules	microfilaments	glyoxysomes	Actin	microfilaments
5	Cytoskeleton of the cell is made up of	plasma membrane	microtubules and filaments	endoplasmic reticulum	Golgi complex	microtubules and filaments
6	The 1st phase of actin filament assembly is marked by---	lag phase	stationary phase	lag and log phase	log phase	lag phase
7	The 2nd phase of actin filament assembly is ----	steady phase	elongation phase	lag phase	log phase	elongation phase
8	Microtubule filament run the length of the central core of the ---	Connective tissue	Cilia	Flagella	Ligaments	Cilia
9	Flagella undulate in a------	oscillation movement	Whiplike manner	Ovoid movement	Zig zag	Whiplike manner
10	The beating of ------- is the only mean for locomotion for sperm	Cilia	Flagella	Cilia and flagella	Tubules	Cilia and flagella
11	Cilia & flagella extend from --- unicellular organism	lysosome	nuclear envelope	golgi bodies	plasmamembrane	plasmamembrane
12	----- facilates the function of dynein	actins	myosin	myoglobin	dynactins	dynactins
13	When flagella are distributed all around the bacterial cell, the arrangement is called--------?	polar	random	peritrichous	encapsulated	peritrichous
14	Which of the followings does not describe skeletal muscle tissue fibre?	striated	voluntary	multinucleate	branched	branched
15	ATPase of the muscle located in	actinin	troponin	myosin	actin	actin
16	The bacteria flagellum is made up of –	Protein flagellin	protein flagella	lipid flagellin	lipid flagella	Protein flagellin
17	Cilia is an organelle found in –	prokaryotic cell	eukaryotic cell	prokaryotic & Eukaryotic cell	none of the above	eukaryotic cell
18	Operation of modified Q cycle III results in the reduction of ---	Cyt C	Cyt B	Cyt C P50	cyt p450	Cyt C
19	Microtubule are one of the components of the ---	endoskeleton	cytoskeleton	exoskeleton	none of the above	cytoskeleton
20	Microfilaments are found by the --- polymerisation of actin monomers	tail to head	tail only	head only	head to tail	head to tail
21	Microtubules are found in all eukaryotic cells except	Brain cells	Human erythrocytes	hepatocytes	kidney cells	Human erythrocytes
22	---------- is a term applied to the axial basic microtubular structure of cilia and flagella	Cilium	Basal body	Axoneme	Dynein arm	Axoneme
23	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
24	The size of the microtubules	10nm in diameter	18nm in diameter	1-3 µm in diameter	24nm in diameter	24nm in diameter
25	Hydrolysis of phosphate groups in ATP is an	exergonic process	endergonic process	endothermic process	exergonic and endergonic process	exergonic process
26	ATP is hydrolyzed in to	ADP	inorganic phosphate	ADP and inorganic phosphate	organic phosphate	ADP and inorganic phosphate
27	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
28	Which term is based on the Greek root words for "before" and "kernel"?	peptidoglycan.	eukaryote.	nucleolus.	Prokaryote	prokaryote
29	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.
30	Which of the following cytoskeleton proteins have an almost all helical secondary structure?	Tubulins	Keratins	Lamins	Actins	Lamins
3`	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
32	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.
33	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

34	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
35	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
36	The basic microtubular structure of cilia and flagella is called	radial spoke	axoneme	nexin	dyenein	axoneme
37	Eukaryotic flagella is made up of	dyenien	tubulin	flagellin	vimetin	tubulin
38	Which of the following is not a characteristic of prokaryotes?	DNA	Cell membrane	cell wall	endoplasmic reticulum	endoplasmic reticulum
39	Ribosomes are made up of ____ subunits.	0	2	3	4	2
40	Plants differ from animals in that the plants have	an endoplasmic reticulum	a central vacuole	Golgi complexes	vesicles	a central vacuole
41	The rough ER is so named because it has an abundance of _____ on it.	mitochondria	lysosomes	Golgi bodies	ribosomes	ribosomes
42	Depolymerization of microtubules is inhibited by	kinesin	dyneins	actin	guanosine triphosphate	guanosine triphosphate
43	Clusters of rRNA where ribosomes are assembled are called	nuclei	cisternae	nucleoli	Golgi complexes	nucleoli
44	The smooth ER is especially abundant in cells that synthesize extensive amounts of	toxins	proteins	enzymes	lipids	lipids
45	Synaptic signaling involves	endocrine signals	paracrine signals	autocrine signals	neurotransmitters	neurotransmitters
46	The ____ surrounds the cell like a belt, preventing the passage of substances between the cells.	gap junction	desmosome	hemidesmosome	tight junction	tight junction
47	In desmosomes, cadherins link to _____ of an adjacent cell.	integrins	connexons	ras proteins	intermediate filaments	intermediate filaments
48	_____ junctions may protect a damaged cell through chemical gating.	Tight	Gap	Adherens	Occluding	Gap
49	Narrow gaps between nerve cells through which paracrine signals travel are called	desmosomes	calmodulins	synapses	integrins	synapses
50	_____ is a common second messenger.	cAMP	cGTP	cMHC	cATP	cAMP
51	Desmosomes are associated with _____ junctions.	adherens	tight	anchoring	communicating	anchoring
52	One protein kinase cascade begins with the phosphorylation of the	tap protein	gat protein	sat protein	ras protein	ras protein
53	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
54	Plasmodesmata occur in	bacterial cells	all eukaryotic cells	plant cells	animal cells	plant cells
55	A _____ is a type of adhering junction between animal cells.	tight junction	plasmodesma	chemical synapse	gap junction	tight junction
56	--------- are attached to integral membrane protein	Intrinsic protein	Peripheral protein	Lipid anchored Protein	GPI anchored protein	Peripheral protein
57	Transmembrane proteins are also known as	integral protein	Peripheral protein	Lipid anchored Protein	GPI anchored protein	integral protein
58	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
59	The energy for all forms of muscle contraction is provided by:	ATP	ADP	phosphocreatine	oxidative phosphorylation	ATP
60	Each of the following is true of both cadherins and integrins EXCEPT	both are transmembrane glycoproteinsX	both can associate with the actin cytoskeletonX	both represent gene families	both may be found in zonula adherens junctions	both may be found in zonula adherens junctions

UNIT-V

Syllabus

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

PROKARYOTIC AND EUKARYOTIC CELL WALL

BACTERIAL (PROKARYOTIC) 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.

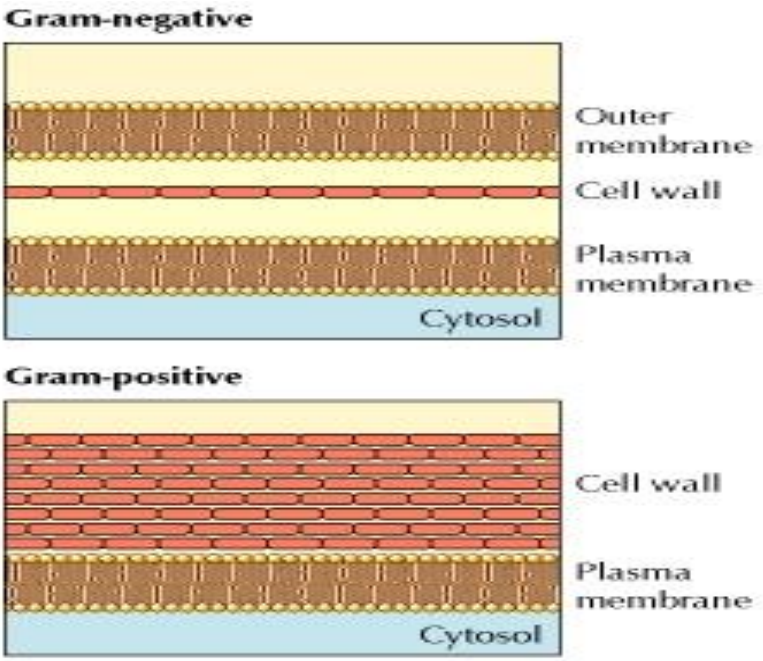


Figure Bacterial cell walls.

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.

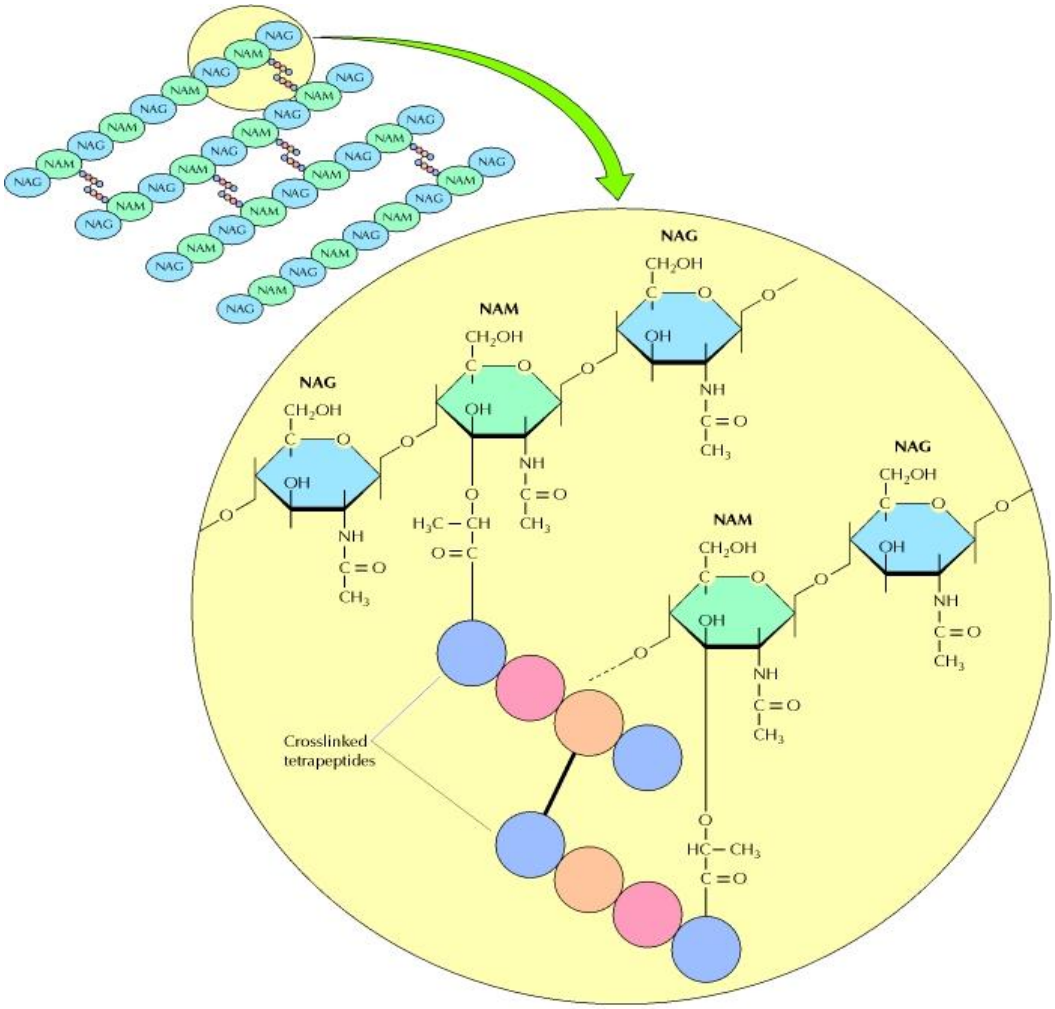


Figure: The peptidoglycan of E.coli. Polysaccharide chains consist of alternating N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) residues joined by β(1→4) glycosidic bonds. Parallel chains are crosslinked by tetrapeptides

PLANT (EUKARYOTIC) 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 the 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.

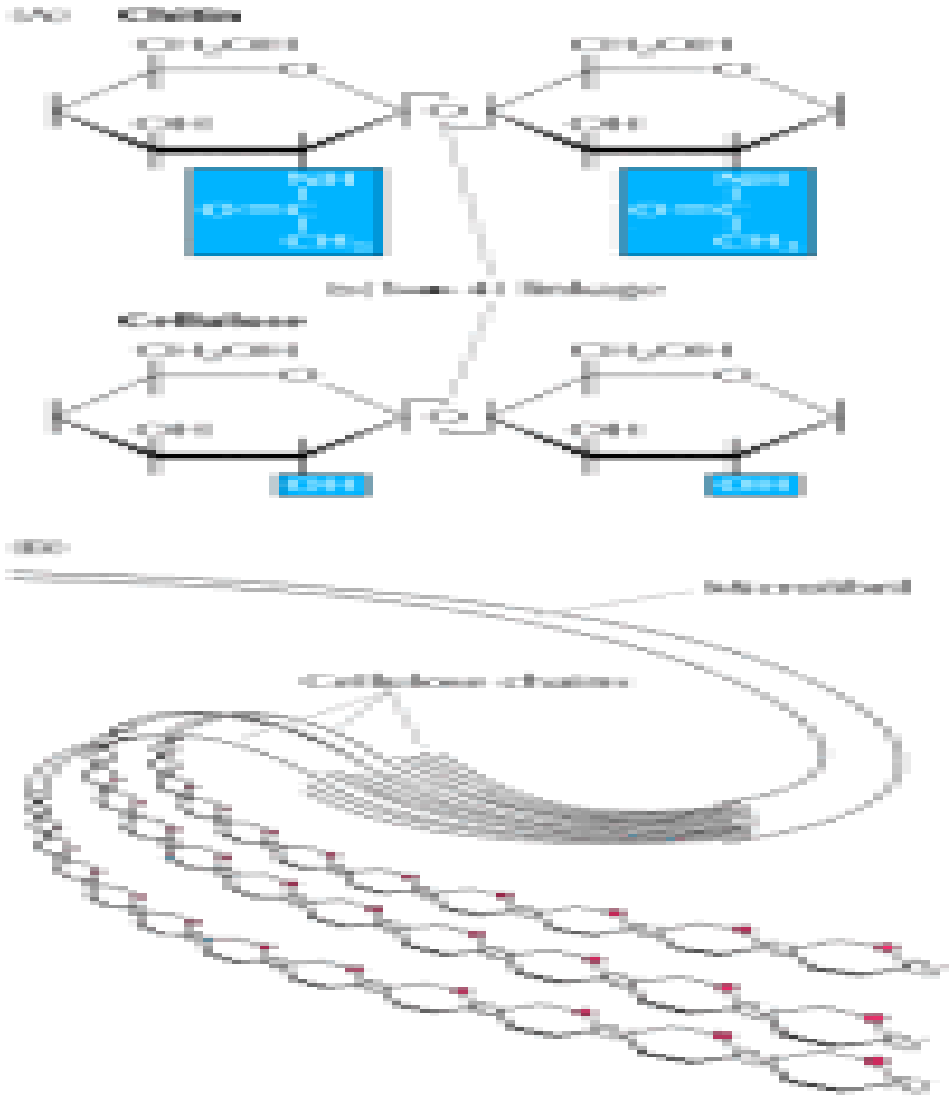


Figure:Polysaccharides of fungal and plant cell walls.

Within the cell wall, cellulose microfibrils are embedded in a matrix consisting of proteins and two other types of polysaccharides: hemicelluloses and pectins (Figure). 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.

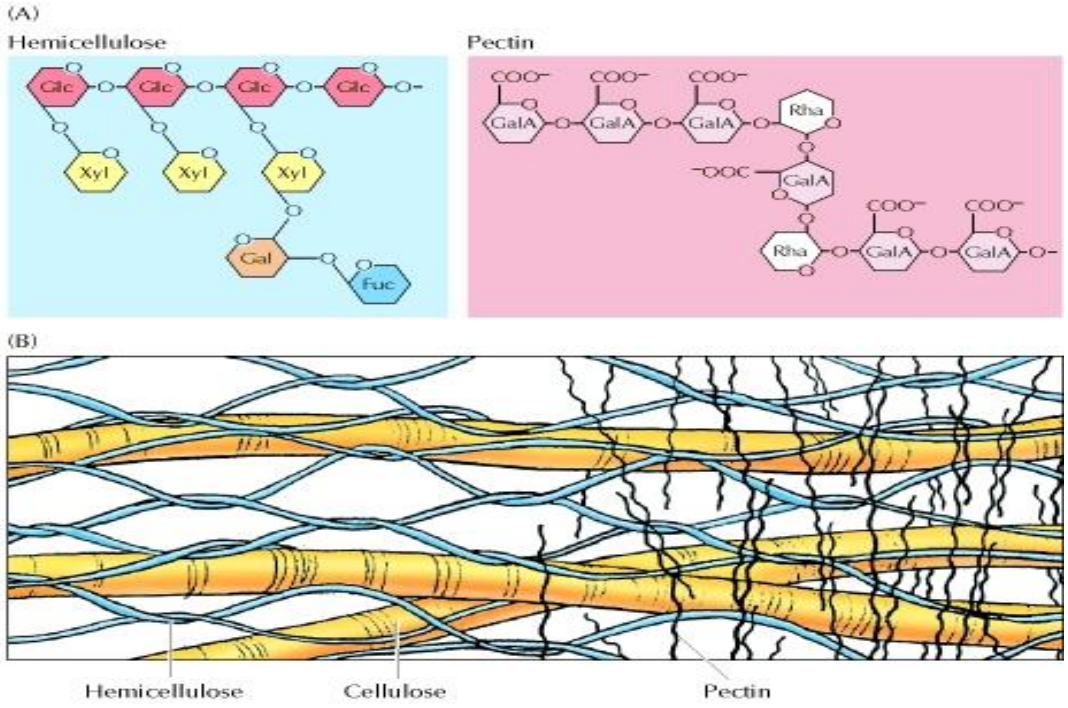


Figure Model of a plant cell wall

Both the structure and function of cell walls change as plant cells develop. The walls of growing plant cells (called**primary 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 (Figure). 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.

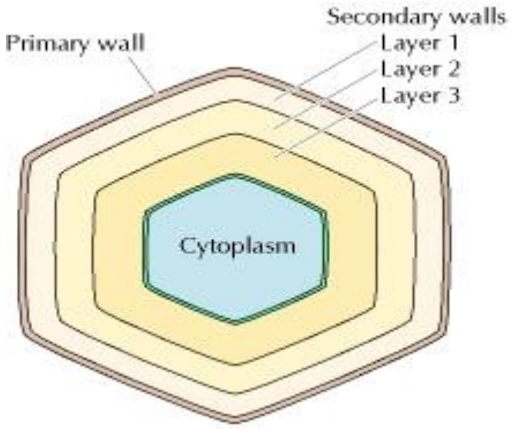


Figure: Primary and secondary cell walls.

Primary and secondary cell walls differ in composition as well as in thickness. Primary cell walls contain approximately equal amounts of cellulose, hemicelluloses, and pectins. In contrast, the more rigid secondary walls generally lack pectinand 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.

CELL MATRIX AND CELL-CELL INTERACTION

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.

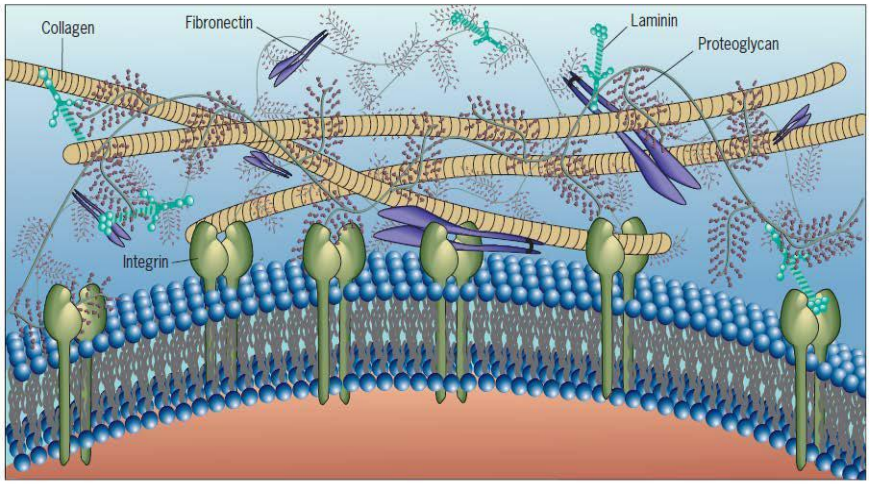


Figure : An overview of the extracellular matrix molecular organization. The proteins; fibronectin, collagen, and laminin contain binding sites for one another, as well as binding sites for receptors like integrins that are located at the cell surface. The proteoglycans are huge protein polysaccharide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

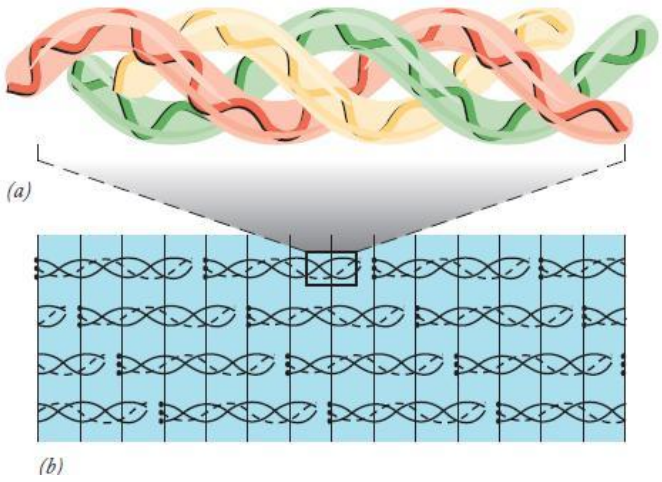


Figure : The structure of collagen I. (*a*) The monomer of collagen. (*b*) Collagen I molecules become aligned in and a bundle of collagen I molecules, such as that shown here, form a collagen fibril.

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.

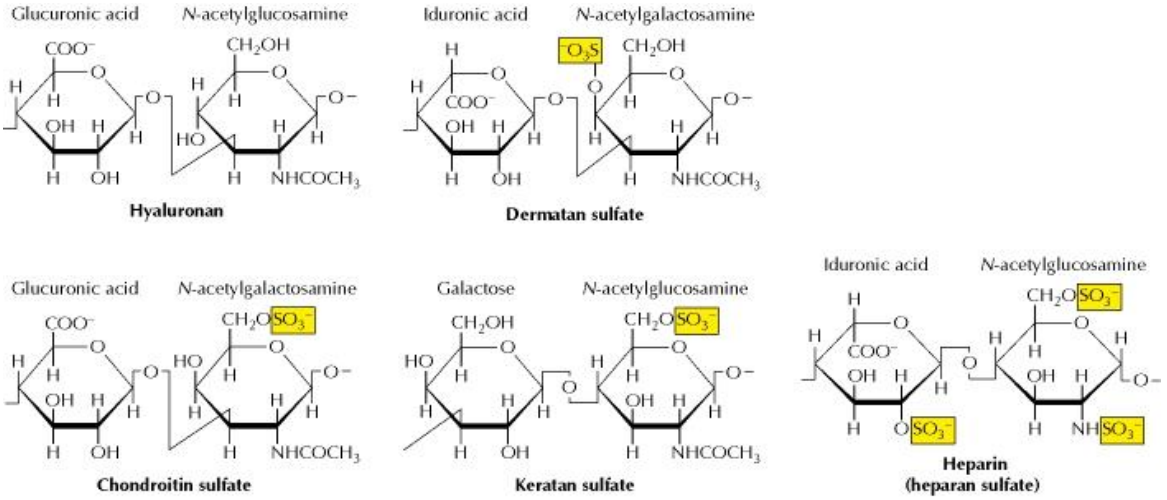


Figure: Major types of glycosaminoglycans

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 (Figure).

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.

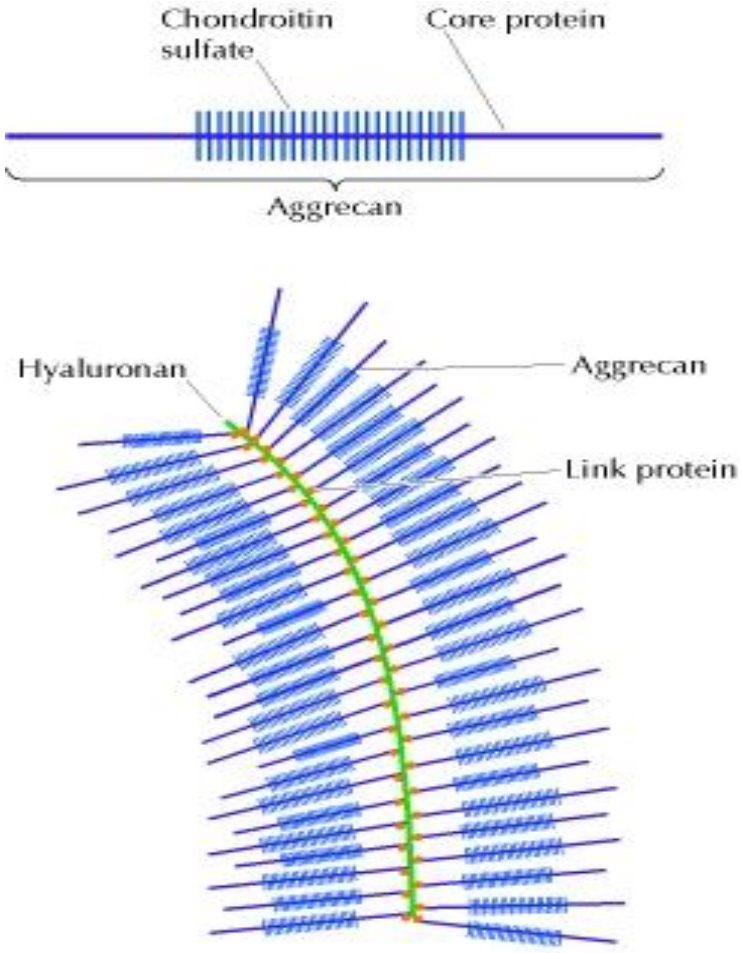


Figure : Complexes of aggrecan and hyaluronan**.**

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.

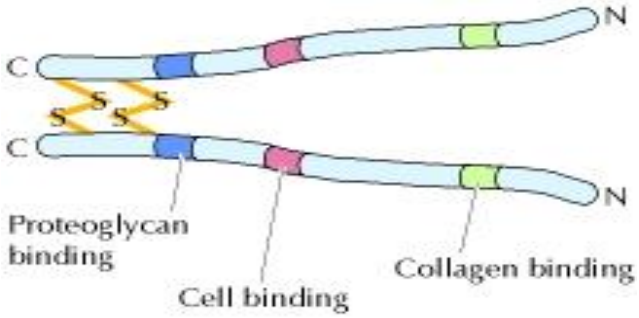


Figure : Structure of fibronectin.

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

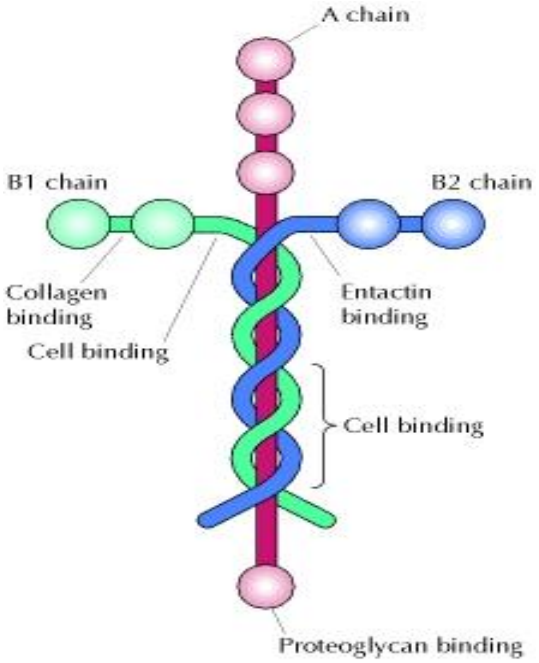


Figure : Structure of laminin.

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).

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

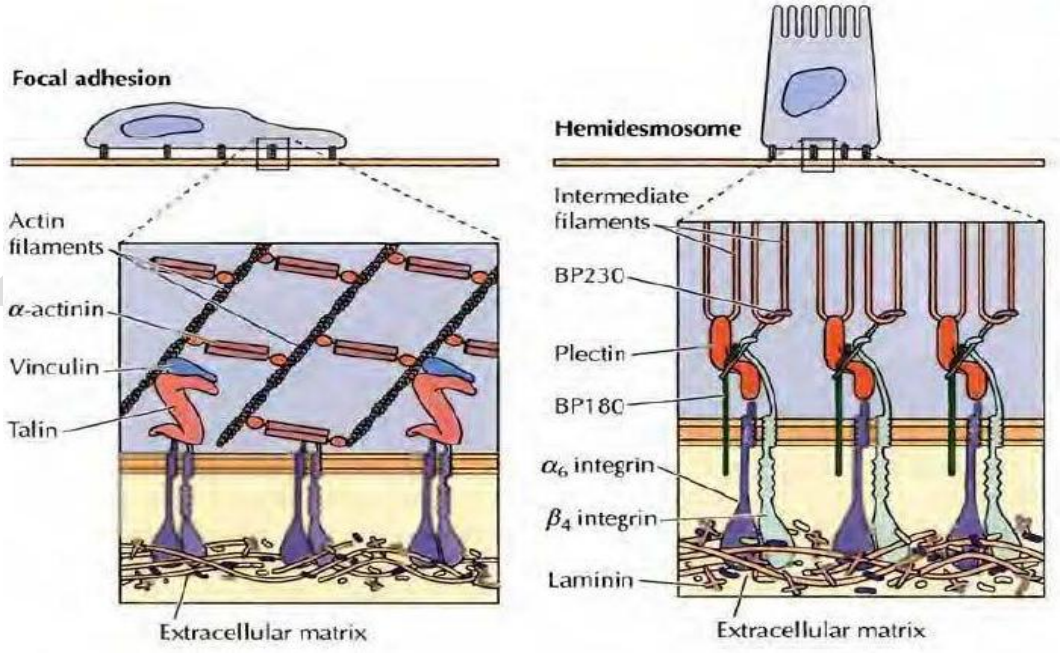


Figure : Cell-matrix junctions mediated by integrins. Integrins mediate two types of stable junctions the focal adhesions where bundles of actin filaments are anchored to integrins through associations with a number of other proteins, including α-actinin, talin, and vinculin. In hemidesmosomes, integrin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

Structure of integrins:

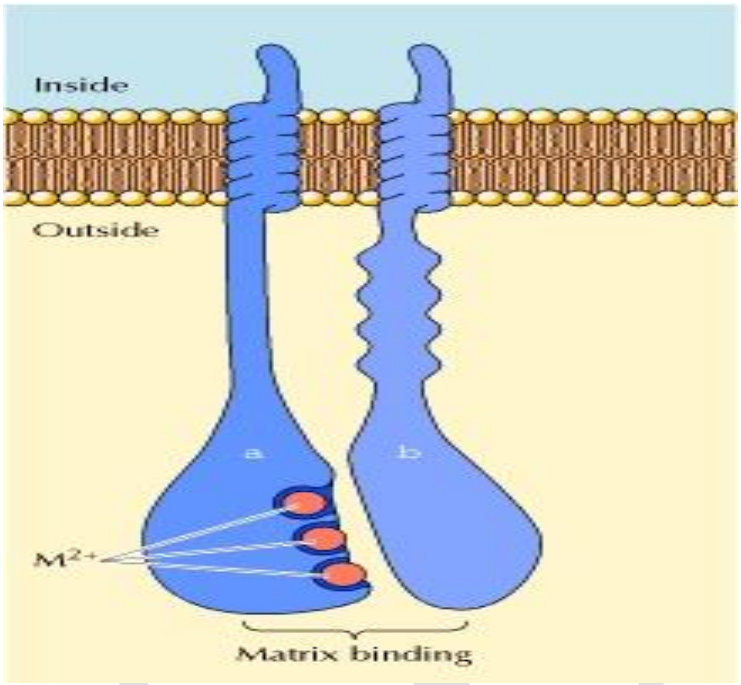


Fig:Integrin association with membrane

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, cadherins.

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

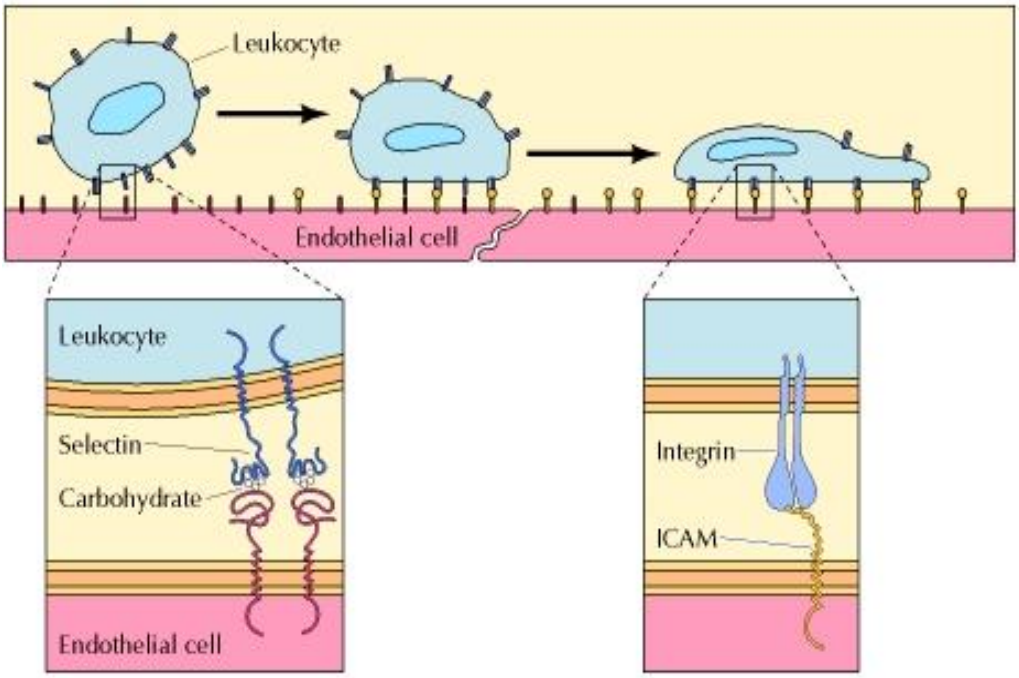


Fig: Extravasation of leucocytes

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-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.

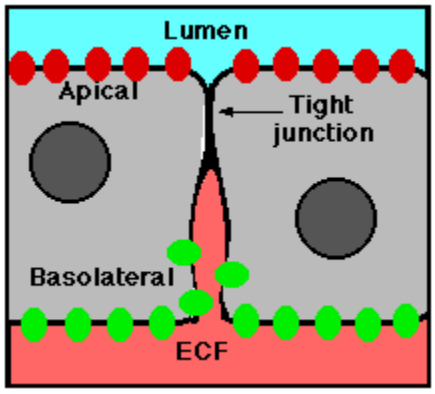
1.TIGHT JUNCTIONS

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.



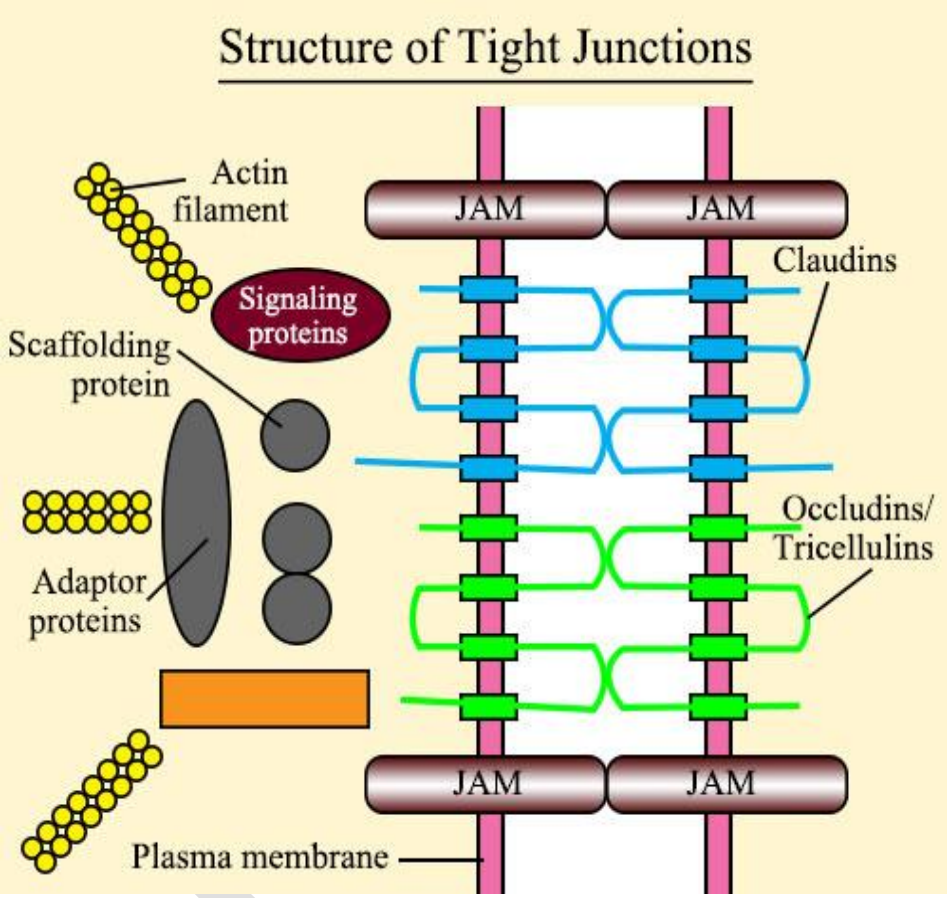
Tight junctions perform two vital functions:

- 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.



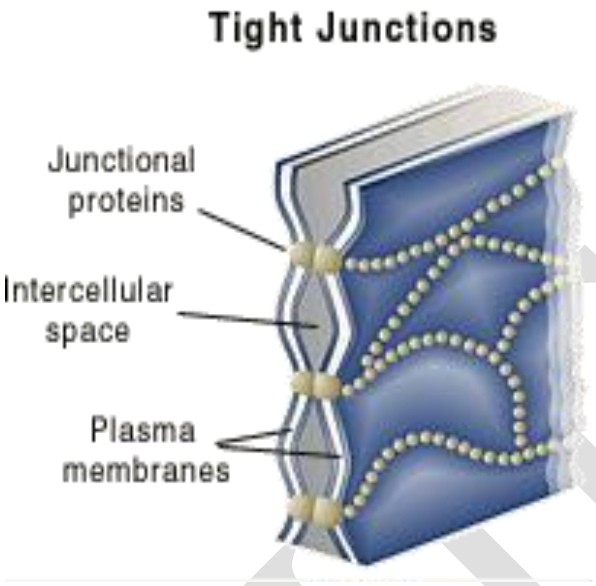


Fig: Tight Junctions

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.

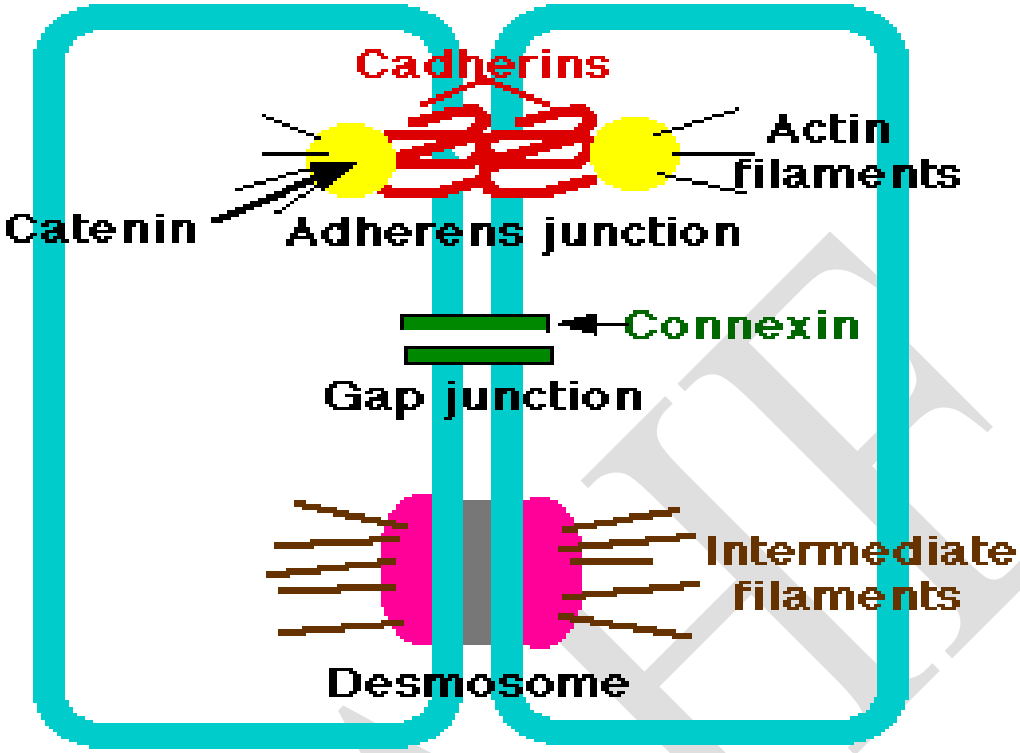


Fig: Illustration of adheren junction, gap junction and desmosomes

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:

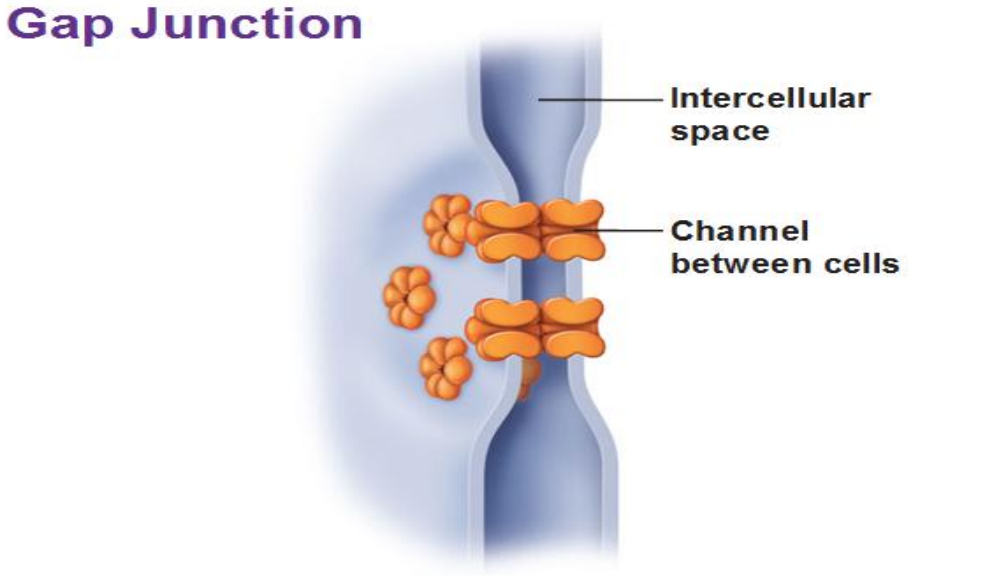
- The action potential in heart (cardiac) muscle flows from cell to cell through the heart providing the rhythmic contraction of the heartbeat.
- At some so-called electrical synapses in the brain, gap junctions permit the arrival of an action potential at the synaptic terminals to be transmitted across 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.

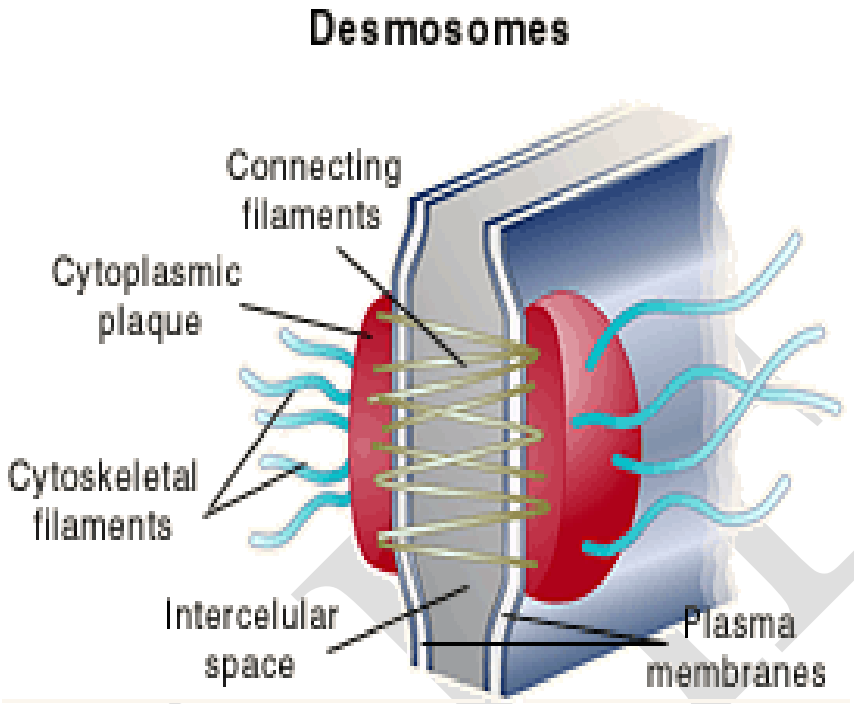
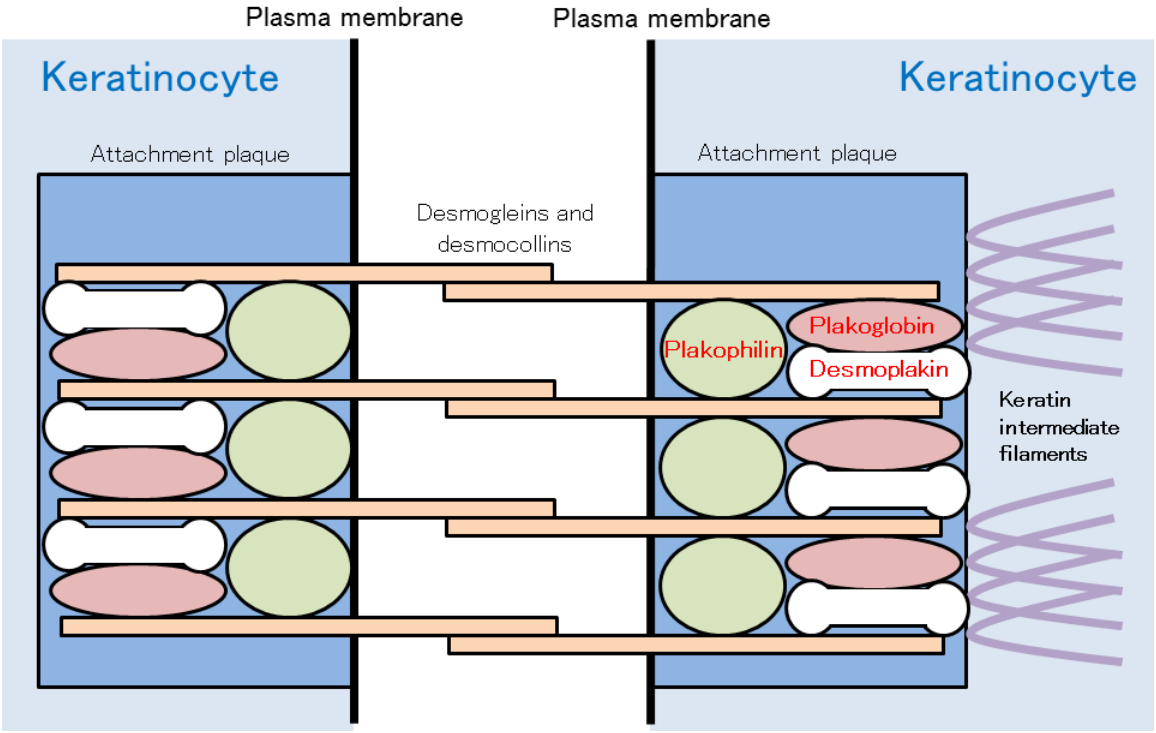


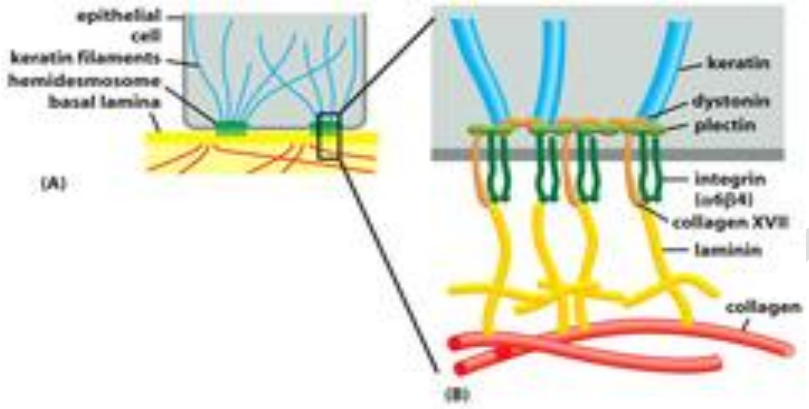
Fig:Desmosome



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.

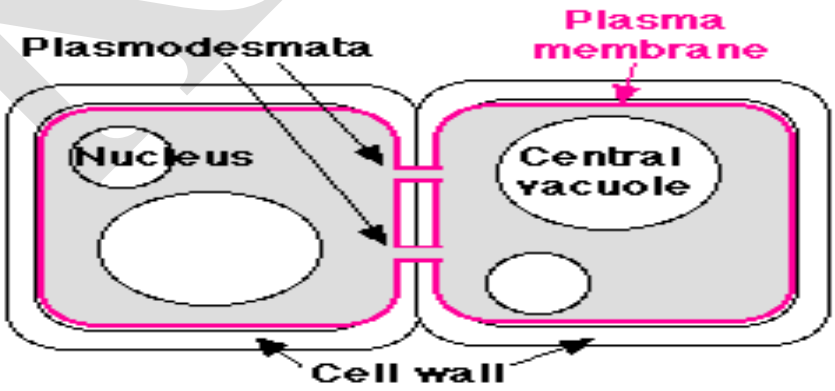


Fig: Plasmadesmata

CELL CYCLE AND CELL DIVISION

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

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

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

INTERPHASE: Interphase, is a resting stage between cell divisions, is actually a period of diverse activities. Those interphase activities are indispensible in making the next mitosis possible.
Interphase generally lasts at least 12 to 24 hours in mammalian tissue. During this period, the cell is constantly synthesizing RNA, producing protein and growing in size. By studying molecular events in cells, scientists have determined that interphase can be divided into 4 steps: Gap 0 (G0), Gap 1 (G1), S (synthesis) phase, Gap 2 (G2).

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

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

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

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

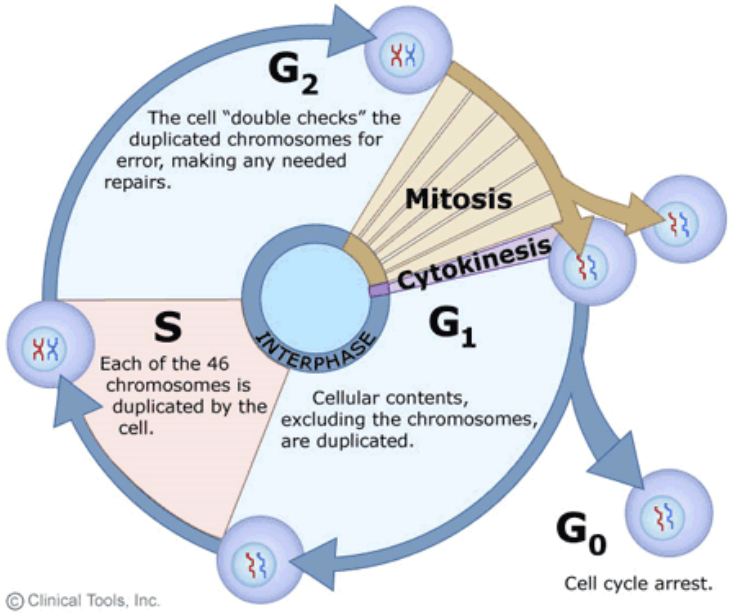


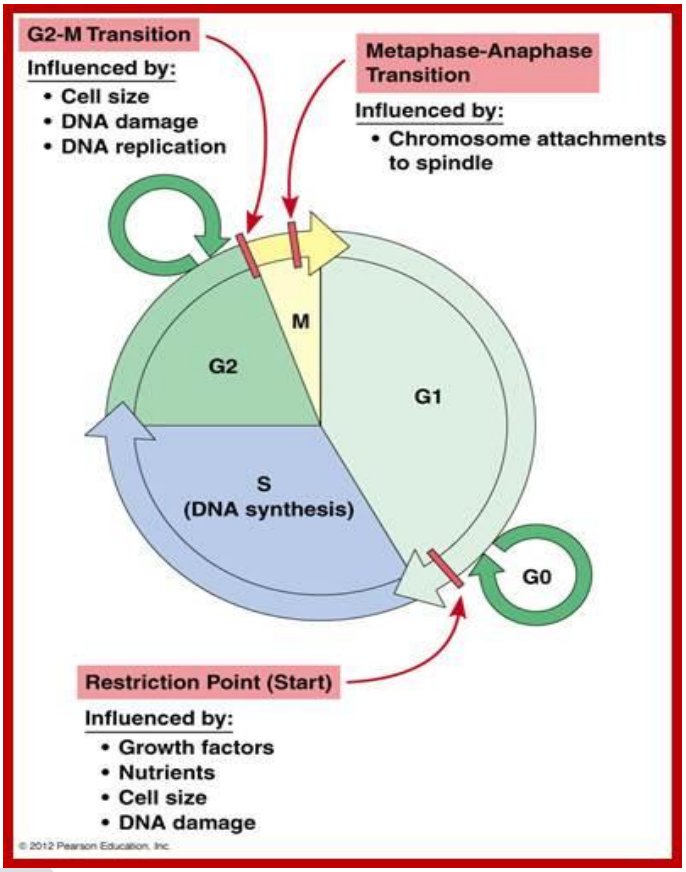
Figure: Phases of cell cycle

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

RESTRICTION POINT (R)

A cell's decision to enter, or reenter, the cell cycle is made before S-phase in G_1 at what is known as the restriction point, and is determined by the combination of promotional and inhibitory

extracellular signals that are received and processed. Before the R-point, a cell requires these extracellular stimulants to begin progressing through the first three sub-phases of G_1 (competence, entry G_{1a}, progression G_{1b}). After the R-point has been passed in G_{1b}, however, extracellular signals are no longer required, and the cell is irreversibly committed to preparing for DNA duplication. Further progression is regulated by intracellular mechanisms. Removal of stimulants before the cell reaches the R-point may result in the cell's reversion to quiescence. Under these conditions, cells are actually set back in the cell cycle, and will require additional time (about 8 hours more than the withdrawal time in culture) after passing the restriction point to enter S phase.



CELL CYCLE CHECK POINTS

The eukaryotic cell cycle is a complex process, eukaryotes have evolved a network of regulatory proteins, known as the cell cycle control system, which monitors and dictates the progression of the cell through the cell cycle. The cell cycle checkpoints play an important role in the control system by sensing defects that occur during essential processes such as DNA replication or chromosome segregation, and inducing a cell cycle arrest in response until the defects are repaired. The main mechanism of action of the cell cycle checkpoints is through the

regulation of the activities of a family of protein kinases known as the cyclin-dependent kinases (CDKs), which bind to different classes of regulator proteins known as cyclins, with specific cyclin-CDK complexes being formed and activated at different phases of the cell cycle. Those complexes, in turn, activate different downstream targets to promote or prevent cell cycle progression.

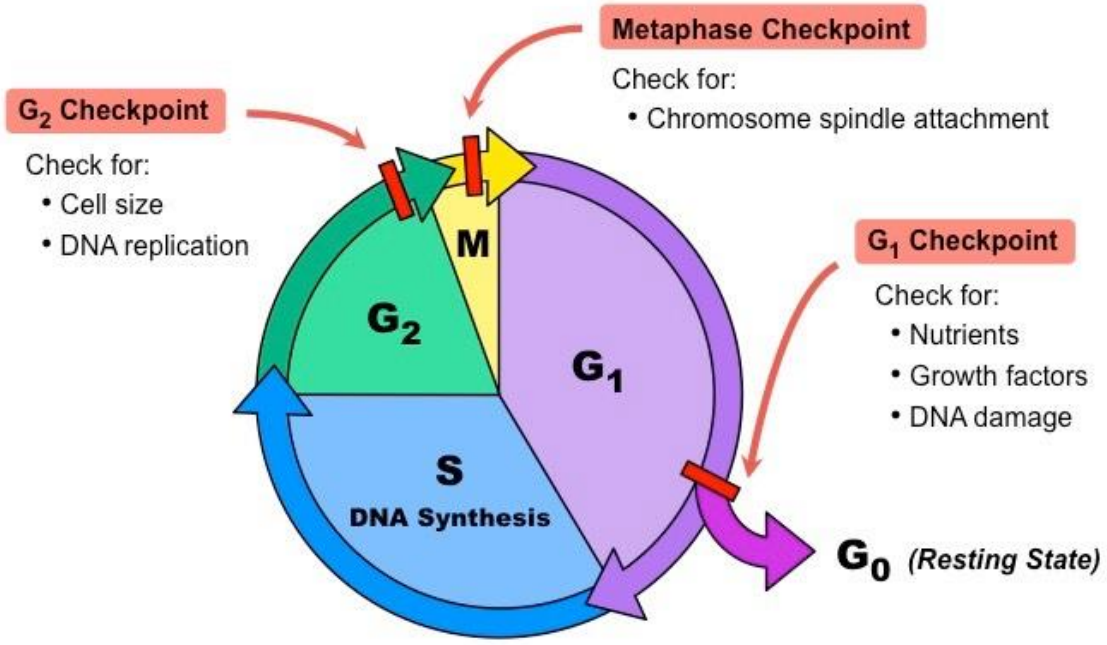


Fig: Cell cycle check points

G1 Check point

G_1 Checkpoint

The G_1 checkpoint determines whether all conditions are favorable for cell division to proceed. The G_1 checkpoint, also called the restriction point (in yeast), is a point at which the cell irreversibly commits to the cell division process. External influences, such as growth factors, play a large role in carrying the cell past the G_1 checkpoint. In addition to adequate reserves and cell size, there is a check for genomic DNA damage at the G_1 checkpoint. A cell that does not meet all the requirements will not be allowed to progress into the S phase. The cell can halt the cycle and attempt to remedy the problematic condition, or the cell can advance into G_0 and await further signals when conditions improve.

The G_2 Checkpoint

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

The M Checkpoint

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

CELL DIVISION

Cell division is broadly classified into

I.Mitosis

II.Meiosis

1.MITOSIS

Mitosis is a process of eukaryotic cell division in which each of two identical daughter cells receives a diploid complements of chromosomes same as the diploid complement of the parent cell. It is usually followed by cytokinesis in which the cell itself divides to yield two identical daughter cells.

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

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

The basics in mitosis include:

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

2. Each chromosome divides longitudinally into identical halves and become separated from each other.
3. The separated chromosome halves move in opposite directions, and each becomes included in one of the two daughter nuclei that are formed.

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

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

Prophase

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

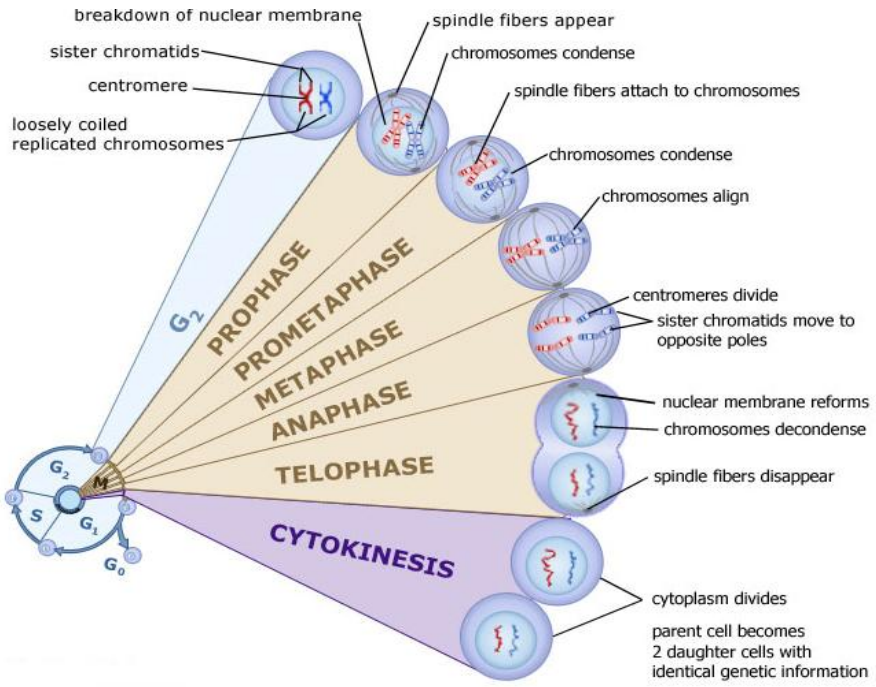


Figure: Phases of Mitosis

Prometaphase

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

Metaphase

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

Anaphase

The shortest stage of mitosis. The centromeres divide, and the sister chromatids of each chromosome are pulled apart - or 'disjoin' - and move to the opposite ends of the cell, pulled by spindle fibres attached to the kinetochore regions. Once the centromere divide, each sister chromatid is treated as a separate chromosome. Chromosome movement results from progressive shortening of the spindle fibers attached to the centromeres, which pulls the chromosomes in opposite directions toward the poles. At the completion of anaphase, the chromosomes lie in two groups near opposite poles of the spindle. Each group contains the same number of chromosomes that was present in the original interphase nucleus. The separated sister chromatids are now referred to as **daughter chromosomes**. (It is the alignment and separation in metaphase

and anaphase that is important in ensuring that each daughter cell receives a copy of every chromosome.)

Telophase

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

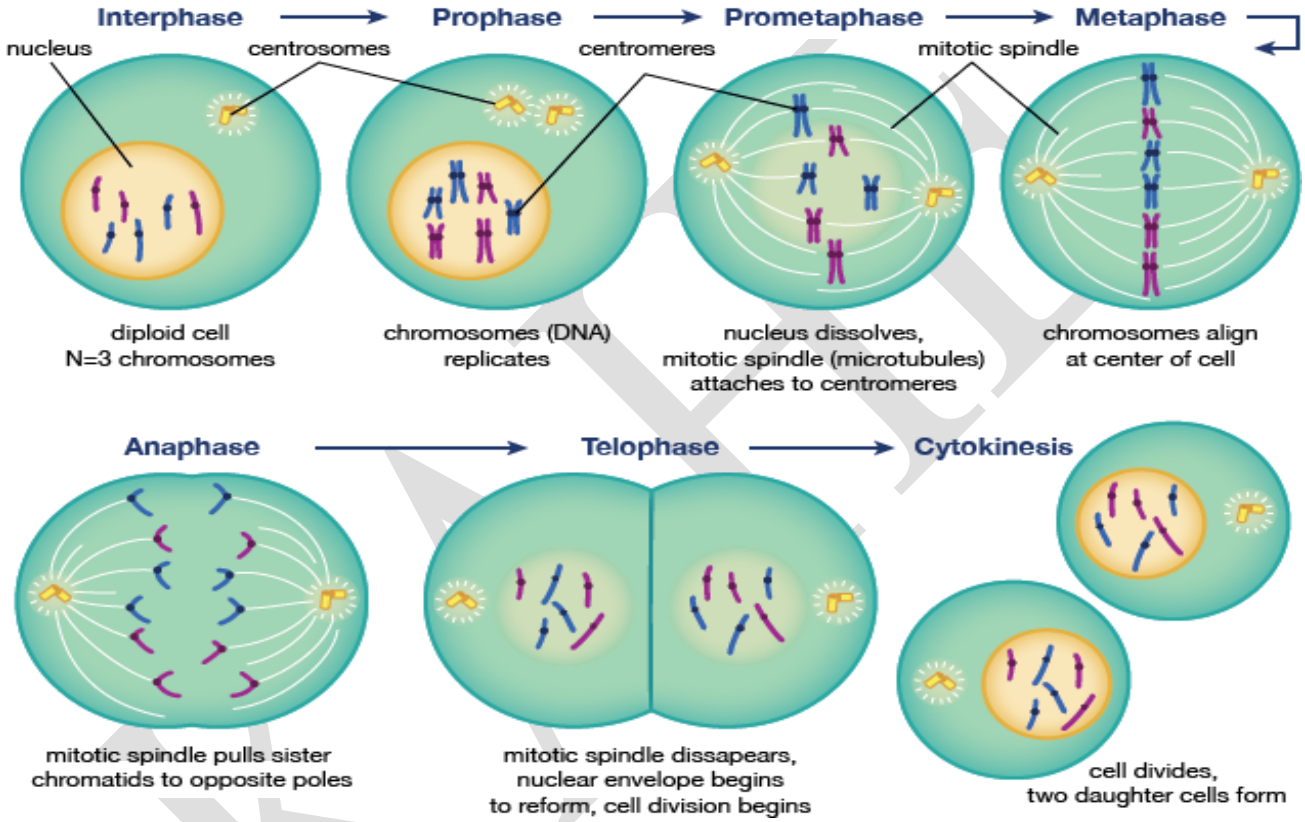


Fig:Events in Mitosis

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

CYTOKINESIS

The final cellular division to form two new cells. In plants a cell plate forms along the line of the metaphase plate; in animals there is a constriction of the cytoplasm. 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

II.MEIOSIS

Meiosis is a type of cell division that reduces the number of chromosomes in the parent cell by half and produces four gamete cells. This process is required to produce egg and sperm cells for sexual reproduction. Meiosis I and II are each divided into prophase, metaphase, anaphase, and telophase stages, similar in purpose to their analogous subphases in the mitotic cell cycle. Therefore, meiosis includes the stages of meiosis I (prophase I, metaphase I, anaphase I, telophase I) and meiosis II (prophase II, metaphase II, anaphase II, telophase II).

Meiosis generates gamete genetic diversity in two ways: (1) Law of Independent Assortment. The independent orientation of homologous chromosome pairs along the metaphase plate during metaphase I & orientation of sister chromatids in metaphase II, this is the subsequent separation of homologs and sister chromatids during anaphase I & II, it allows a random and independent distribution of chromosomes to each daughter cell (and ultimately to gametes) and (2) Crossing Over. The physical exchange of homologous chromosomal regions by homologous recombination during prophase I results in new combinations of DNA within chromosomes

During meiosis, specific genes are more highly transcribed. In addition to strong meiotic stage-specific expression of mRNA, there are also pervasive translational controls (e.g. selective usage of preformed mRNA), regulating the ultimate meiotic stage-specific protein expression of genes during meiosis Thus, both transcriptional and translational controls determine the broad restructuring of meiotic cells needed to carry out meiosis.

Meiosis I

Meiosis I segregates homologous chromosomes, which are joined as tetrads (2n, 4c), producing two haploid cells (n chromosomes, 23 in humans) which each contain chromatid pairs (1n, 2c). Because the ploidy is reduced from diploid to haploid, meiosis I is referred to as a *reductional division*. Meiosis II is an *equational division* analogous to mitosis, in which the sister chromatids are segregated, creating four haploid daughter cells (1n, 1c).

Prophase I

Prophase I is typically the longest phase of meiosis. During prophase I, homologous chromosomes pair and exchange DNA (homologous recombination). This often results in chromosomal crossover. This process is critical for pairing between homologous chromosomes and hence for accurate segregation of the chromosomes at the first meiosis division. The new combinations of DNA created during crossover are a significant source of genetic variation, and result in new combinations of alleles, which may be beneficial. The paired and replicated chromosomes are called bivalents or tetrads, which have two chromosomes and four chromatids, with one chromosome coming from each parent. The process of pairing the homologous chromosomes is called synapsis. At this stage, non-sister chromatids may cross-over at points called chiasmata (plural; singular chiasma). Prophase I has historically been divided into a series of substages which are named according to the appearance of chromosomes.

Leptotene

The first stage of prophase I is the *leptotene* stage, also known as *leptonema*, from Greek words meaning "thin threads". In this stage of prophase I, individual chromosomes—each consisting of two sister chromatids—become "individualized" to form visible strands within the nucleus. The two sister chromatids closely associate and are visually indistinguishable from one another. During leptotene, lateral elements of the synaptonemal complex assemble. Leptotene is of very short duration and progressive condensation and coiling of chromosome fibers takes place.

Zygotene

he *zygotene* stage, also known as *zygonema*, from Greek words meaning "paired threads",occurs as the chromosomes approximately line up with each other into homologous chromosome pairs. In some organisms, this is called the bouquet stage because of the way the telomeres cluster at one end of the nucleus. At this stage, the synapsis of homologous chromosomes takes place, facilitated by assembly of central element of the synaptonemal complex. Pairing is brought about in a zipper-like fashion and may start at the centromere (procentric), at the chromosome ends (proterminal), or at any other portion (intermediate). Individuals of a pair are equal in length and in position of the centromere. Thus pairing is highly specific and exact. The paired chromosomes are called bivalent or tetrad chromosomes.

Pachytene

The *pachytene* stage, also known as *pachynema*, from Greek words meaning "thick threads", At this point a tetrad of the chromosomes has formed known as a bivalent. This is the stage when homologous recombination, including chromosomal crossover (crossing over), occurs. Nonsister chromatids of homologous chromosomes may exchange segments over regions of homology. Sex chromosomes, however, are not wholly identical, and only exchange information over a small region of homology. At the sites where exchange happens, chiasmata form. The exchange of information between the non-sister chromatids results in a recombination of information; each chromosome has the complete set of information it had before, and there are no gaps formed as a result of the process. Because the chromosomes cannot be distinguished in the synaptonemal complex, the actual act of crossing over is not perceivable through the microscope, and chiasmata are not visible until the next stage.

Diplotene

During the *diplotene* stage, also known as *diplonema*, from Greek words meaning "two threads", the synaptonemal complex degrades and homologous chromosomes separate from one another a little. The chromosomes themselves uncoil a bit, allowing some transcription of DNA. However, the homologous chromosomes of each bivalent remain tightly bound at chiasmata, the regions where crossing-over occurred. The chiasmata remain on the chromosomes until they are severed at the transition to anaphase I.

In mammalian and human fetal oogenesis all developing oocytes develop to this stage and are arrested before birth. This suspended state is referred to as the *dictyotene stage* or dictyate. It

lasts until meiosis is resumed to prepare the oocyte for ovulation, which happens at puberty or even later.

Diakinesis

Chromosomes condense further during the *diakinesis* stage, from Greek words meaning "moving through"This is the first point in meiosis where the four parts of the tetrads are actually visible. Sites of crossing over entangle together, effectively overlapping, making chiasmata clearly visible. Other than this observation, the rest of the stage closely resembles prometaphase of mitosis; the nucleoli disappear, the nuclear membrane disintegrates into vesicles, and the meiotic spindle begins to form.

Synchronous processes

During these stages, two centrosomes, containing a pair of centrioles in animal cells, migrate to the two poles of the cell. These centrosomes, which were duplicated during S-phase, function as microtubule organizing centers nucleating microtubules, which are essentially cellular ropes and poles. The microtubules invade the nuclear region after the nuclear envelope disintegrates, attaching to the chromosomes at the kinetochore. The kinetochore functions as a motor, pulling the chromosome along the attached microtubule toward the originating centrosome, like a train on a track. There are four kinetochores on each tetrad, but the pair of kinetochores on each sister chromatid fuses and functions as a unit during meiosis I.

Microtubules that attach to the kinetochores are known as *kinetochore microtubules*. Other microtubules will interact with microtubules from the opposite centrosome: these are called *nonkinetochore microtubules* or *polar microtubules*. A third type of microtubules, the aster microtubules, radiates from the centrosome into the cytoplasm or contacts components of the membrane skeleton.

Metaphase I

Homologous pairs move together along the metaphase plate: As *kinetochore microtubules* from both centrosomes attach to their respective kinetochores, the paired homologous chromosomes align along an equatorial plane that bisects the spindle, due to continuous counterbalancing forces exerted on the bivalents by the microtubules emanating from the two kinetochores of homologous chromosomes. This attachment is referred to as a bipolar attachment. The physical basis of the independent assortment of chromosomes is the random orientation of each bivalent along the metaphase plate, with respect to the orientation of the other bivalents along the same equatorial line.[13] The protein complex cohesinholds sister chromatids together from the time of their replication until anaphase. In mitosis, the force of kinetochore microtubules pulling in opposite directions creates tension. The cell senses this tension and does not progress with anaphase until all the chromosomes are properly bi-oriented. In meiosis, establishing tension requires at least one crossover per chromosome pair in addition to cohesin between sister chromatids.

Anaphase I

Kinetochore microtubules shorten, pulling homologous chromosomes (which consist of a pair of sister chromatids) to opposite poles. Nonkinetochore microtubules lengthen, pushing the centrosomes farther apart. The cell elongates in preparation for division down the center.[13] Unlike in mitosis, only the cohesin from the chromosome arms is degraded while the cohesin surrounding the centromere remains protected. This allows the sister chromatids to remain together while homologs are segregated.

Telophase I

The first meiotic division effectively ends when the chromosomes arrive at the poles. Each daughter cell now has half the number of chromosomes but each chromosome consists of a pair of chromatids. The microtubules that make up the spindle network disappear, and a new nuclear membrane surrounds each haploid set. The chromosomes uncoil back into chromatin. Cytokinesis, the pinching of the cell membrane in animal cells or the formation of the cell wall in plant cells, occurs, completing the creation of two daughter cells. Sister chromatids remain attached during telophase I.

Cells may enter a period of rest known as interkinesis or interphase II. No DNA replication occurs during this stage.

Meiosis II

Meiosis II is the second meiotic division, and usually involves equational segregation, or separation of sister chromatids. Mechanically, the process is similar to mitosis, though its genetic results are fundamentally different. The end result is production of four haploid cells (n chromosomes, 23 in humans) from the two haploid cells (with n chromosomes, each consisting of two sister chromatids) produced in meiosis I. The four main steps of Meiosis II are: Prophase II, Metaphase II, Anaphase II, and Telophase II.

In **prophase II** we see the disappearance of the nucleoli and the nuclear envelope again as well as the shortening and thickening of the chromatids. Centrosomes move to the polar regions and arrange spindle fibers for the second meiotic division.

In **metaphase II**, the centromeres contain two kinetochores that attach to spindle fibers from the centrosomes at opposite poles. The new equatorial metaphase plate is rotated by 90 degrees when compared to meiosis I, perpendicular to the previous plate. This is followed by **anaphase II**, in which the remaining centromeric cohesin is cleaved allowing the sister chromatids to segregate. The sister chromatids by convention are now called sister chromosomes as they move toward opposing poles.

The process ends with **telophase II**, which is similar to telophase I, and is marked by decondensation and lengthening of the chromosomes and the disassembly of the spindle. Nuclear

envelopes reform and cleavage or cell plate 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.

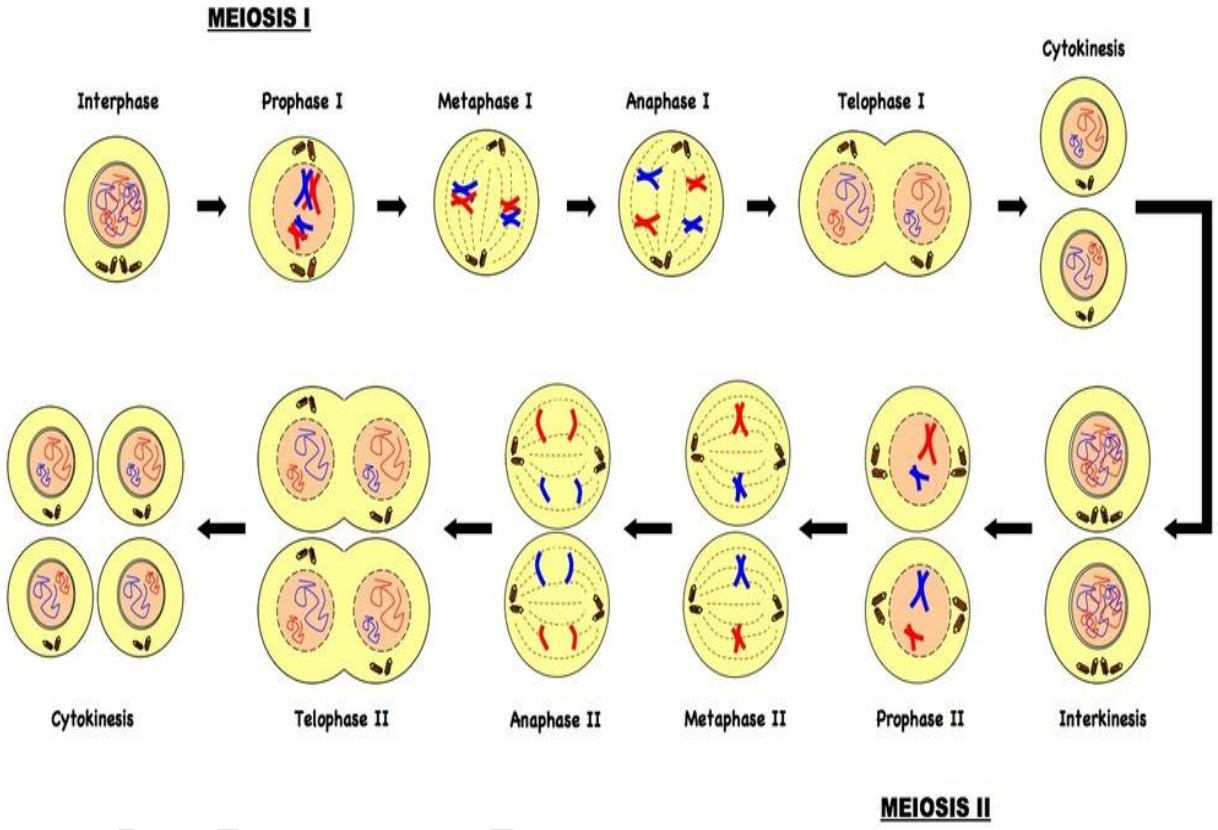


Fig:Events in Meiosis

APOPTOSIS AND NECROSIS - brief outline

wo different ways cells can die: apoptosis (programmed cell death) and necrosis (unplanned cell death). It is easy to tell these two apart morphologically under the microscope.

Necrosis

Necrosis is when cells die accidentally due to, say, trauma (ex. a poisonous spider bite), or lack of nutrients (ex. lack of blood supply). Necrosis begins with cell swelling, the chromatin gets digested, the plasma and organelle membranes are disrupted, the ER vacuolizes, the organelles break down completely and finally the cell lyses, spewing its intracellular content and eliciting an immune response (inflammation).

apoptosis

Apoptosis can constitute cell suicide or cell murder. Cells will commit suicide when they lack any incoming survival signal in the form of trophic factors, or when they detect extensive DNA

damage in their own nucleus. Cells will murder other cells to clear out unneeded cells or to eliminate potentially self-attacking immune cells.

Either of these processes constitutes programmed cell death. During embryonic development, people have webbed hands and feet and tails; the cells that constitute those parts later apoptize. Apoptosis also goes on constantly in many tissues including the intestines.

Major steps of apoptosis:

- Cell shrinks
- Cell fragments
- Cytoskeleton collapses
- Nuclear envelope disassembles
- Cells release apoptotic bodies

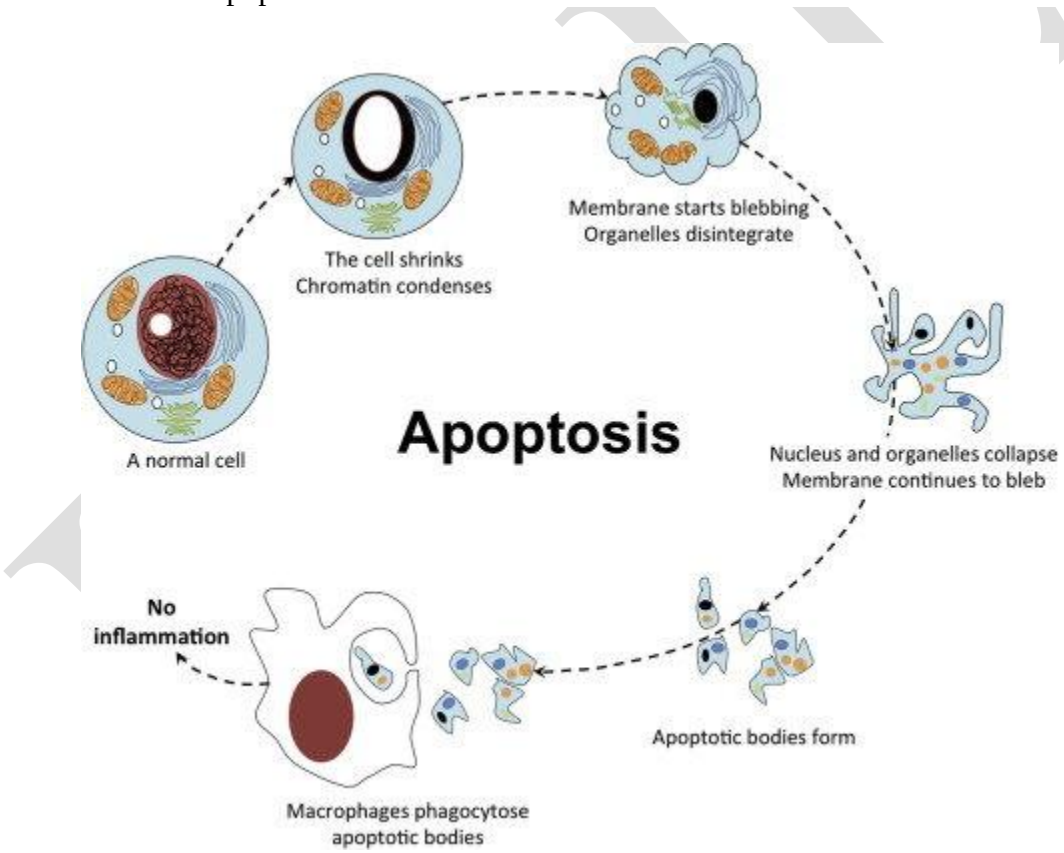


Fig: Events in Apoptosis

Proteins important in apoptosis:

- 'killer proteins': the caspases (discussed in detail below).
- 'destruction proteins' that digest DNA, fragment the cell and break down the cytoskeleton
- 'engulfment proteins' that elicit and promote phagocytosis by other cells

In mammals, apoptosis is governed chiefly by caspases (cysteine-aspartic proteases). The entire caspase pathway is post-translationally regulated: the caspases are always present in inactive form (called procaspases, containing a prodomain, which contains a caspase recruitment domain (CARD)) and can be activated by cleavage. This allows a very quick response if cell suicide is needed. In order for apoptosis to occur, the initiator caspases must be cleaved and dimerize. Thus activated, they must then cleave the effector caspases (aka pro-caspases), triggering a 'caspase cascade'. This amplifies the number of activated caspases in the cell. The effector caspase have many targets including the nuclear lamina and cytoskeleton.

There are both pro-survival and pro-apoptotic caspases, and they share many common domains. Pro-survival caspases have BH1, 2, 3 and 4; pro-apoptosis caspases have either BH1, 2 and 3 or just BH3.

Inhibitor of apoptosis proteins (IAPs) restrain both the initiator and effector caspases. They each have a zinc binding domain that binds directly to caspases, inhibiting their activity.

However, there are also mitochondrial proteins called SMAC and DIABLO which inhibit the inhibitors. Upon mitochondrial injury they are released and will bind IAPs, freeing the caspases to go cause apoptosis. Another collection of mitochondrial proteins called Htra2/Omi, apoptosis-inducing factor (AIF) and endonuclease G can also be released and will cleave IAPs. AIF also causes chromosome condensation and DNA fragmentation independent of caspases.

Indeed, the mitochondria are central regulators of apoptosis. Outer mitochondrial membrane proteins Bcl-2, the BH3-only proteins and Bax are involved: Bax can form a pore in the membrane to allow cytochrome c, normally located in the intermembrane space, out into the cytosol. Bax monomers move from the cytoplasm to the outer mitochondrial membrane, where they oligomerize and permit the influx of ions through the membrane. This has also been shown in in vitro experiments where you can show that vesicles made of outer mitochondrial memrbanes are permeabilized in the presence of Bax. It is not currently known why this influx of ions leads to cytochrome c release.

Bcl-2 prevents release of cytochrome c, thus blocking apoptosis. Bcl-2 was the first mammalian apoptosis gene to be cloned. In some lymphomas, it gets translocated to a position under a stronger promoter, causing overexpression that prevents the cancer cell from apoptosing. See also bad & bid.

Once cytochrome c is released, it binds to Apaf-1 (apoptotic protease activating factor), causing the latter to hydrolyze the ATP to which it is usually bound, thus causing a conformational change that activates Apaf-1 and triggers the caspase cascade. Apaf-1 forms a disc-shaped heptamer called the 'wheel of death' or apoptosomewhich activates caspases

When a trophic factor is present, the receptor activates PI3K, which activates PKB/Akt, which phosphorylates Bad. p-Bad is then retained in the cytosol by 14-3-3, preventing p-Bad from inhibiting Bcl-2. Thus apoptosis is prevented.

Trophic factors are an example of a cell extrinsic signal that promotes survival. There are also extrinsic signals that promote death (this is cell murder). Tumor necrosis factor (TNF-alpha) is released by macrophages to trigger cell death by binding to 'death receptors'. Death receptors

have a single transmembrane domain. They must trimerize in order to activate FADD (Fas-associated death domain). These serve as adapters for caspase-8 and -10 and form a death-inducing signaling complex (DISC) which can initiate the caspase cascade. Though this whole process originates independent of mitochondria, it can also activate (?) t-Bid, leading to a mitochondrial apoptosis signal as well.

Cells can become murder-resistant by expressing decoy receptors which have only the 'death ligand' binding domain and no active cytosolic domain. This occurs sometimes normally in animal cells but is also a trick that some viruses use – they encode decoy receptor proteins to keep their host cells safe from immune attack.

TNF-alpha usually promotes death, but can also promote survival in certain cell types by activating NF-κB. Sometimes cells use decoy receptors to promote an inflammatory response instead of death.

p53 is a key regulator of DNA damage response and can promote DNA repair, apoptosis or cell cycle arrest. It does this by binding to promoters of target genes. It is still not clear what determines when p53 will induce cell cycle arrest versus apoptosis.

Apoptotic cells exhibit a particular chemical signature. One of these is that an endonuclease cleaves DNA into fragments in the linker regions between nucleosomes and the resulting fragments form a ladder when run on a gel. Another is TUNEL (Terminal deoxynucleotide transferase dUTP Nick End Labeling) staining. This involves adding a Tdt enzyme and a BrdU which Tdt will add to the ends of cleaved DNA. After giving it a chance to do this you wash away excess BrdU and then use an antibody against BrdU. Yet another method is that phosphatidylserine (PS) is normally located in the cytosolic leaflet of the plasma membrane; during apoptosis, it flips to the exoplasmic leaflet, where it serves as a signal to request other cells to phagocytose the dying cell. A fluorescently labeled annexin V protein can label PS on the outside of apoptotic cells.

Difference between apoptosis and necrosis

APOPTOSIS	NECROSIS
Role for mitochondria and cytochrome C	No role for mitochondria
No leak of lysosomal enzymes	Leak of lysosomal enzymes
Characteristic nuclear changes	Nuclei lost
Apoptotic bodies form	Do not form
DNA cleavage	No DNA cleavage
Activation of specific proteases	No activation
Regulatable process	Not regulated
Evolutionarily conserved	Not conserved
Dead cells ingested by neighboring cells	Dead cells ingested by neutrophils and macrophages

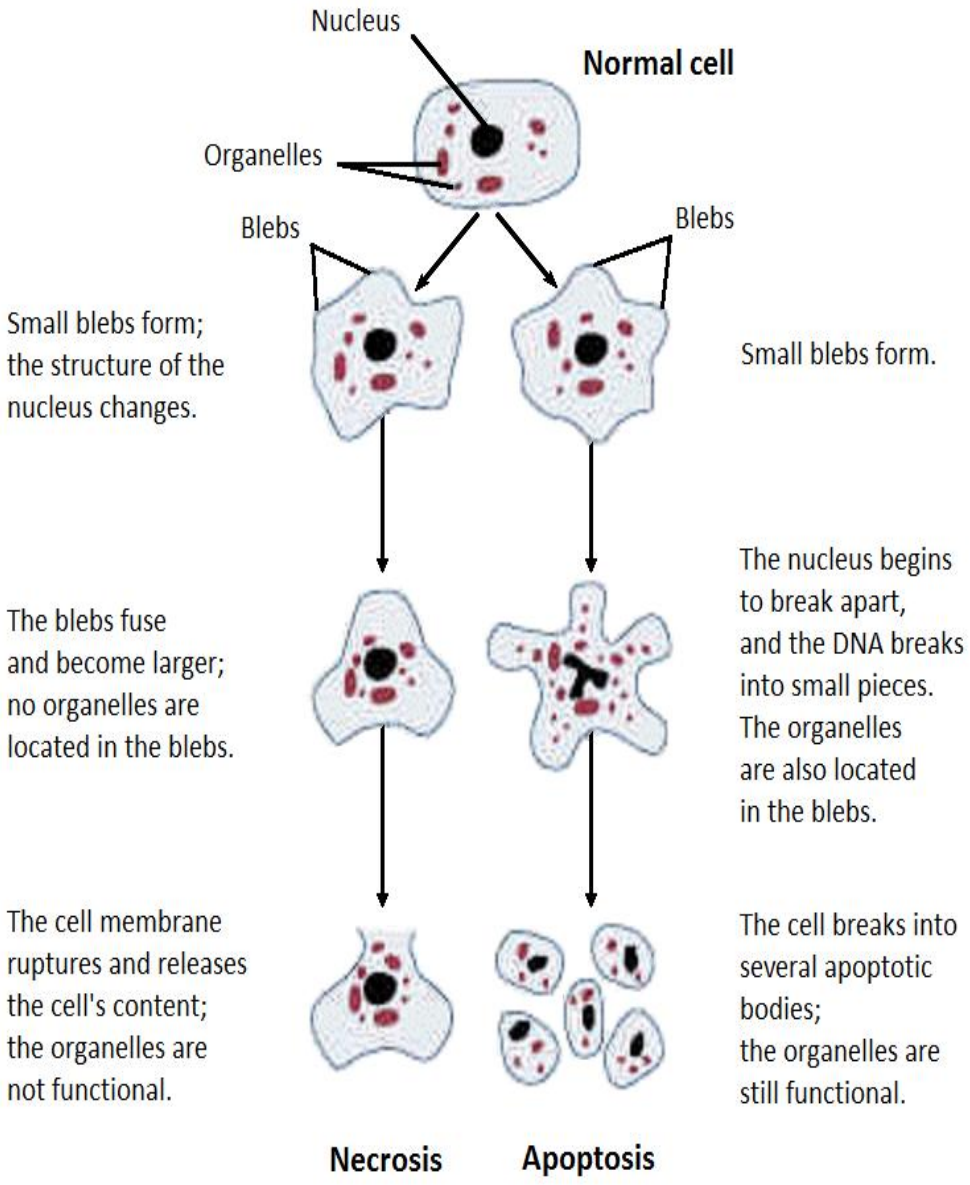


Fig: Difference between apoptosis and Necrosis

SALIENT FEATURES OF A TRANSFORMED CELL.

Transformed cells are created by exposing embryo cells (or fibroblasts) to ionising radiation or chemicals. When viewed on a plate, the transformed cells will tend to pile up with haphazard cell-cell orientation.

The other types of transformed cells are from immortal cell lines which originated from mouse embryos

The four aspects of Cell Transformation are:

(1) Genetic Instability (2) Immortalization (3) Aberrant Growth Control and (4) Tumorigenicity.

Cell transformation due to changes in the genetic material, and cell cloning involving the production of a population single cell are described here.

Transformation of Cells:

Transformation broadly refers to the change in phenotype of a cell due to a new genetic material. As regards the cultured cells, transformation involves spontaneous or induced permanent phenotypic alterations as a result of heritable changes in DNA, and consequently gene expression.

Characteristics of Transformed Cells:

The general characters of transformed cells are given in Table .They are grouped as genetic, structural, growth and neoplastic, and listed.

TABLE 1 General characteristics of transformed cells

Genetic characters
Aneuploid
Heteroploid
High spontaneous mutation rate
Overexpressed oncogenes
Mutated or deleted suppressor genes
Structural characters
Altered cytoskeleton
Changed extracellular matrix
Modified expression of cell adhesion molecules
Disrupted cell polarity
Growth characters
Immortalized cells
Loss of contact inhibition
Anchorage independent
Density limitation of growth reduced
Growth factor independent
Low serum requirement
Shorter population doubling time
Neoplastic characters
Tumorigenic
Invasive
Increased protease secretion

POSSIBLE QUESTIONS

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

KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF BIOCHEMISTRY
I BSc BIOCHEMISTRY-First Semester
CELL BIOLOGY (19BCU102)
UNIT-V **MULTIPLE CHOICE QUESTIONS**

S.No	Questions	Option A	Option B	Option C	Option D	Answer
1	The division of the nucleus separates the duplicated genome into two sets identical to the parent's in -----	Mitosis	Meiosis	Cytokinesis	Interphase	Mitosis
2	---------- is the division of the cytoplasm, separating the organelles and other cellular components.	Mitosis	Meiosis	Cytokinesis	Prophase	Cytokinesis
3	---------- 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
4	In humans cell division eventually halts after --------divisions	42	52	62	72	52
5	The sister chromatics will be attached by	a centromere	a telomere	a centriole	golgi bodies	a centromere
6	Meiosis I is -------	Reduction	Dublication	Maintanance	Division	Reduction
7	Meiosis produces ----------cells.	4 diploid	4 haploid	2 haploid	2 diploid	4 haploid
8	Mitosis produces ----------cells	2 haploid	2 diploid	4 diploid	4 haploid	2 diploid
9	Chromatin condenses into chromosomes, the nucleolus dissolves and nuclear membrane is disassembled, and the spindle apparatus forms in	Prophase	Metaphase	Anaphase	Telophase	Prophase
10	---------- Tetrads line-up along the equator of the spindle	Metaphase I	Metaphase II	Anaphase I	Anaphase II	Metaphase I
11	---------- Considered a reduction phase	Meiosis	Mitosis	karyokinesis	Cytokinesis	Meiosis
12	Chromosomes are seen in the equatorial plane in	Prophase	Metaphase	Anaphase	Telophase	Metaphase
13	In prokaryotes the nuclear body is called -------	Nucleolus	Nucleosome	Plasmid	Liposome	Nucleosome
14	Important feature of plant cell is the presence of	Mitochondria	Cell- wall	Microsome	Cytoplasm	Cell- wall
15	An interphase between nucleus and cytoplasm is	Nuclear envelope	Nuclear membrane	Nuclear pores	Perinuclear space	Perinuclear space
16	Dyed formation is the Characteristics of ----	Mitosis	Meiosis	Duplication	Reduction	Meiosis
17	---------Occur in the somatic cells	Meiosis	Mitosis	Maintenance	Only fusion	Mitosis
18	The cell cycle consists of ----------	Two distinct phases	Three distinct phases	Four distinct phases	Five distinct phases	Four distinct phases
19	----------phases collectively known as interphase	Gl, S and G2	GI, M and G2	M, S and GO	Gl, MandS	Gl, S and G2
20	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
21	Nucleosome is found in which organism	Fungi	Algae	Protozoan	Drosophila	Algae
22	The first stage in meiotic prophase	leptotene	zygonema	pachynema	diplonema	leptotene
23	During cytokinesis, the cytoplasm divides by a process called	Cleavage	Separation	Splitting	Joining.	Cleavage
24	After M phase, the daughter cells each begin ----------of a new cycle	Growth phase	Inter phase	Multiplication phase	Synthesis phase	Inter phase
25	All of the chromosomes have been replicated in	G0 phase	Gl Phase	M phase	S phase	S phase
26	DNA synthesis takes place during	Gl Phase	S-Phase	G2 Phase	Interphase	S-Phase
27	The space between the nuclear envelop and nucleolus in filled by	Nucleic acids	Nucleoplasm	Nucleotides	Nucleoproteins	Nucleoplasm
28	The cell cycle consists of ----------	Two distinct phases	Three distinct phases	Four distinct phases	Five distinct phases	Four distinct phases
29	Cells permanently stop division due to age or accumulated DNA damage are called	Cell death	Senescent	Leptotene	Cleavage	Senescent
30	---------- 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
31	In which phase of mitosis does chromosome condense	Prophase	metaphase	anaphase	telophase	Prophase
32	In which phase of mitosis does sister chromatins segregated to the opposite poles of mitotic spindle	Prophase	metaphase	anaphase	telophase	anaphase
33	Which of the following cells in multi cellular organisms exist from the cell cycle and does not proliferate in life time?	Skin	nerve	bone	Muscle	nerve
34	The attachment of which of the following protein marks a cell or a protein for degradation	Lyase	ubiquitin	RNase	protease	ubiquitin
35	Nuclear pore complexes which are broken down in to sub pore complexes during prophase reassemble into nuclear membrane around each chromosome forming individual mini nuclei called	Nucleomere	karyomere	cytomere	cytosol	karyomere

36	The space between the nuclear envelope and the nucleolus is filled by a transparent matrix knowns as	Nucleic acids	Nucleohistons	Nucleoprotamines	Nucleoplasm	Nucleoplasm
37	Nuclesomes are fundamental units of	DNA	Nucleus	Nucleolus	Chromatin	DNA
38	Nucleolus is rich in’	protein	DNA	RNA	Glycoprotein	RNA
39	Tumors become life threatening if they spread throughout the body. Such tumors are called---	Malignant	benign	Acute	Chronic	Malignant
40	The spread of tumour cells and establishment of secondary areas of growth is called—	Apoptosis	Metástasis	Homeostasis	Erythropoiesis	Metástasis
41	Malignant tumors derived from endoderm or ectoderm is called----	Carcinoma	Sarcoma	Lymphoma	Leukemia	Carcinoma
42	Sarcoma is a tumour derived from ----	Endoderm	mesoderm	Ectoderm	Lymphoma	mesoderm
43	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
44	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
45	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
46	The scientific study of tumours is called	Oncology	Tumour immunology	Cell biology	Physiology	Oncology
47	Cellular genes known to be progenitors of oncogenes are called	Proto oncogenes	Preproto oncogenes	Meta oncogenes	Premeta oncogenes	Proto oncogenes
48	RB and P53 are prototypes of a class of proteins encoded by	Oncogenes	proto oncogenes	Ras genes	Tumour suppressor genes	Tumour suppressor genes
49	Retro viruses are --------	RNA viruses	DNA viruses	Papova virus	Papiloma viruses	RNA viruses
50	Tumour viruses are usually -------	RNA viruses	DNA viruses	RNA and DNA viruses	Si RNA	RNA and DNA viruses
51	An enzyme which is capable of copying genomic RNA into DNA	DNA a	DNA b	Reverse transcriptase	DNA d	Reverse transcriptase
52	-------- cells have the ability to metastasis	Normal cells	benign cells	malignant cells	Viral cells	malignant cells
53	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
54	Malignant tumours are classified as ------ -- if they derive from endoderm or ectoderm	Sarcoma	carcinoma	lymphoma	leukemia	carcinoma
55	Malignant tumours are classified as ------ -- if they derive from mesoderm	Sarcoma	carcinoma	lymphoma	leukemia	Sarcoma
56	SV 40 and polyoma are example for-----	Retro virus	papova virus	RNA virus	Papilloma virus	papova virus
57	Controlled cell death is called---------	Apoptosis	metastasis	autophagy	Sarcoma	Apoptosis
58	The src enzyme is -------- specific protein kinase	Tyrosine	serine	tryptophan	proline	Tyrosine
59	Solid malignant tumors of lymphoid tissues is called	Carcinoma	sarcoma	leukemia	lymphoma	lymphoma
60	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
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