CELL ORGANELLES

Introduction

- Early electron micrographs of cells in 1945 showed certain tubules that did not reach the periphery and were named as endoplasmic reticulum.
- Endoplasmic reticulum (ER) is the major component of cytoplasmic vacuolar system also called endomembrane system that includes the nuclear envelope, ER and the Golgi complex.
- ER with ribosomes is called rough/granular ER.
- ER without ribosomes is called smooth/agranular ER
- Similar to the plasma membranes, ER also has a membrane made up of lipid bilayers of 5-6 nm in thickness with peripheral and integral proteins.
- The size of ER differs with the differentiation status of cells
- In undifferentiated cells such as eggs or embryonic cells ER is small.

Rough Endoplasmic Reticulum (RER)

- RER is well developed in cells involved in protein synthesis on a large scale.
- Like plasma membrane ER also has an inner and outer membrane and a space in between.
- The outer surface is attached with ribosomes present as polysomes in attachment with mRNA.
- 60S subunit of ribosomes is attached with RER but can freely exchange the subunit present in the cytosol as well during protein synthesis.
- RER contains ribophorins I (mol. Wt. 65,000) and II (mol. Wt. 64,000) that are not present in the smooth endoplasmic reticulum (SER)
- Ribophorins interact with each other and mediate the attachment of ribosomes to the RER

Smooth Endoplasmic Reticulum (SER)

- SER lacks ribosome attachment and is present in regions rich in glycogen appearing as dense particles
- The glycogen particles or glycosomes are about 50-200 nm in size and are made up of smaller particles constituting an overall spheroidal structure.

Microsomes

- Microsomes are not typical organelles present in a cell but are actually obtained as a fraction isolated by differential centrifugation after the separation of mitochondria and nucleus from a cell
- They contain fragments of plasma membrane, RER, SER and the Golgi complex
- Microsomes contain NADH-cytochrome-c-reductase and NADH-cytochrome-b5-resuctase and the hemoproteins, cytochrome b5 and cytochrome P-450
- Cytochrome P450 is a terminal oxidase that detoxifies or inactivates many drugs by oxidation and hydroxylates steroid hormones
- Other important enzymes of this fraction include Mg^{2+}-activated ATPase and glucose-6-phospatase

ER formation and its functions

- ER is thought to be formed be evagination from the nuclear envelope
- During cell division at telophase the nuclear envelope is formed again by vesicles of ER
- A multi-step mechanism is involved in the formation of ER
- After the membrane of lipids and proteins is formed, other components such as enzymes and sugars are added resembling the process of differentiation
- In the ER formation there is a notable and rapid distribution of phospholipids between the monolayers and proteins are inserted independent of lipids
- ER functions like a circulatory system carrying various substances such as RNA and nucleoproteins
- ER also provides a mechanical support for the colloidal structure of the cytoplasm
- Carriers and permeases help in the transport of substances across the endomembrane
- Striated muscle fibers contain a special form of ER called sarcoplasmic reticulum that acts as an intracellular conducting system

- Detoxification of many endogenous and exogenous compounds is an important function of SER
- SER is also involved in glucogenolysis through the action of glucose-6- phosphatase
- The synthesis of proteins is one of the major functions of RER and the synthesis of lipids and lipoproteins is an important function for both RER and SER.

Signal Hypothesis

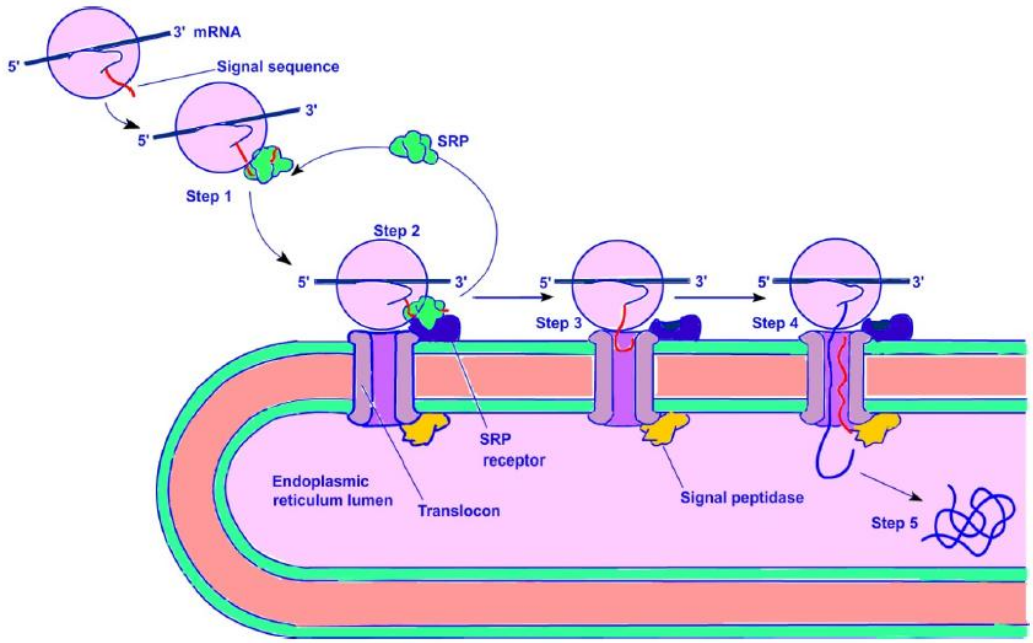
- Free and membrane bound ribosomes are continuously interchanged
- Signal Hypothesis postulates that a signal resides at the N-terminus of polypeptides that directs the attachment of ribosomes to the ER membrane
- The signal peptide interacts with a specific receptor on the ER membrane and the polypeptide can now penetrate into the ER lumen through a channel
- Free ribosomes can be used to translate mRNAs into large peptides called preproteins that contain the signal peptides
- Signal peptides generally have one or two charged groups near the amino terminal and 10-14 hydrophobic amino acids in the middle region
- Signal peptide is removed by the action of signal peptidase in most cases although there are exceptions (signal peptide for ovalbumin)

Signal Recognition Particle

- Recent evidences indicate that in addition to the signal peptide other factors are essential for the translocation of proteins across the membrane
- A large complex consisting of 6 polypeptides and one RNA molecule having 140 nucleotides has been identified and it is called Signal Recognition Particle (SRP)
- SRP helps in decoding the information carried by the signal peptide for secretory, lysosomal and membrane proteins
- SRP arrests the synthesis of proteins as soon as the signal
- peptide comes out from the large ribosomal subunit during protein synthesis

- SRP is free in the cytoplasm or bound with ER. The free SRP binds ribosomes with low affinity but upon translation of signal peptide it binds with high affinity
- When the signal peptide comes out from the ribosome SRP binding arrests the protein synthesis
- SRP receptor is called docking protein (mol. Wt. 72,000)

Signal Hypothesis

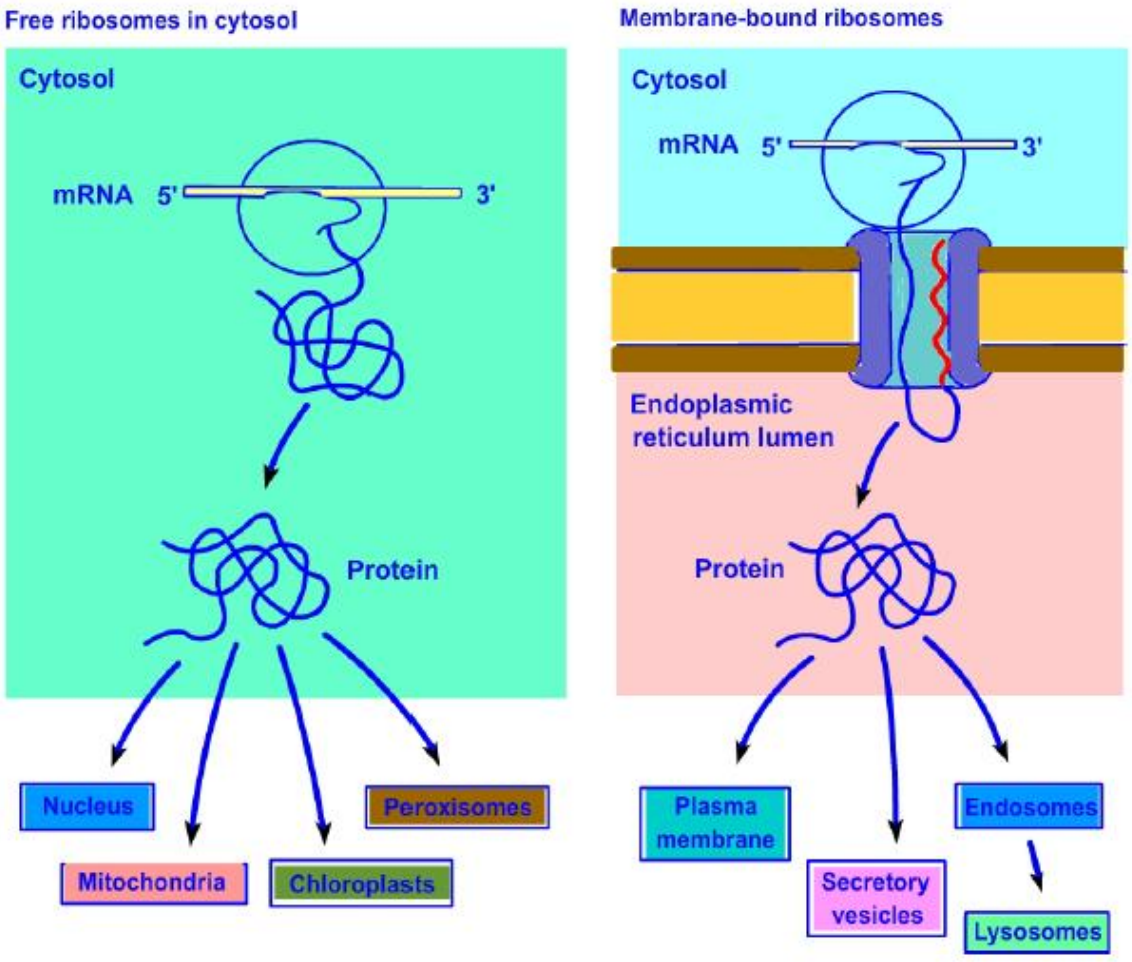


Protein segregation

- Proteins are synthesized on free ribosomes present in the cytosomes or on those attached with ER
- After synthesis, they have to be secreted out or incorporated into the intracellular compartments or integrated with plasma or organellar membranes
- Signal peptides help in finding the destination at least for the secretory proteins
- In the case of secretory proteins translocation to their destination is coupled with translation

- In the case of mitochondrial proteins, they are translocated after being fully synthesized on the ribosomes and usually a receptor recognition is necessary for the transport
- In the case of proteins integrating with the membrane there are stop sequences that halt and facilitate integration while passing through the hydrophobic portion of the membrane

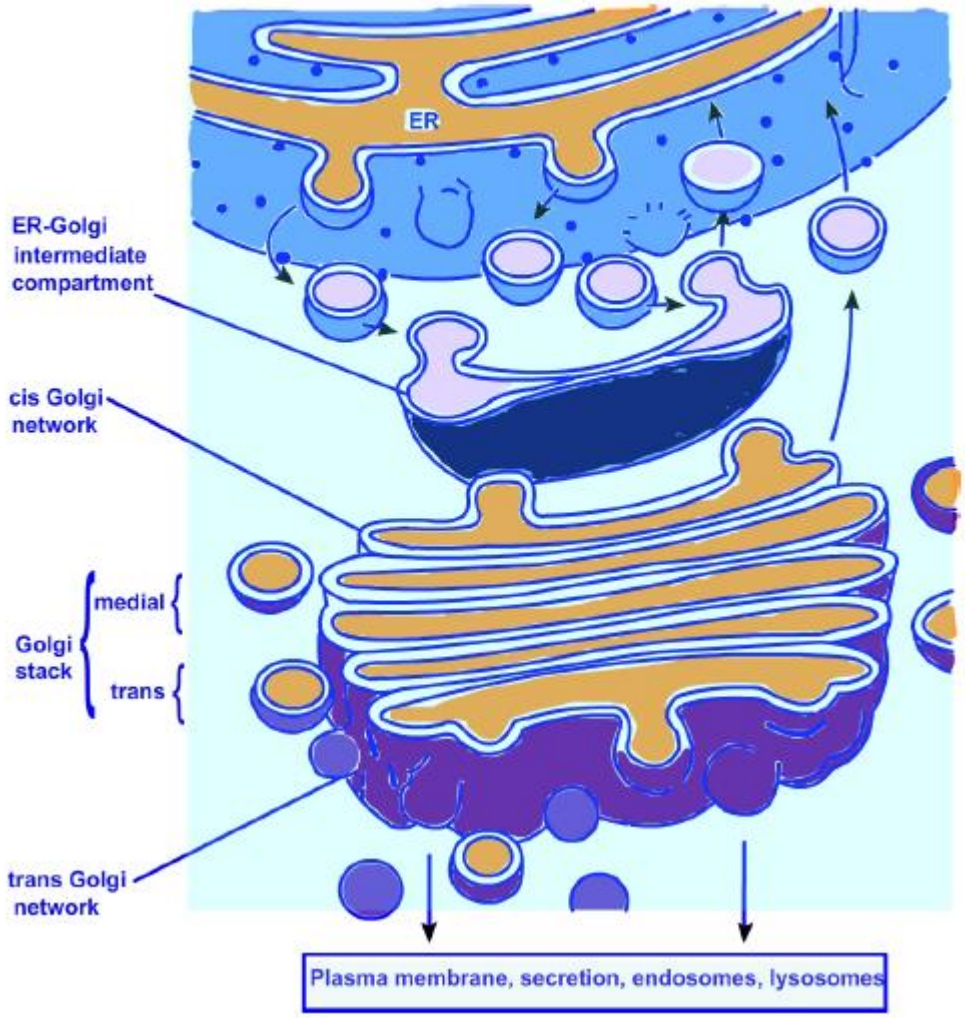
Protein segregation



Golgi Complex

- Golgi complex or Golgi apparatus receives the proteins from ER and processes them further and then helps in sorting them to be transported to their destinations such as endosomes, lysosomes or secretion
- Golgi also serves as a place for the synthesis of glycolipids and sphingomyelin
- In plants the complex polysaccharides of cell wall are synthesized in Golgi
- Golgi is essentially made up of flat membrane-enclosed sacs called cisternae/dictyosome units and associated vesicles
- Proteins from ER enter into its convex face called cis face or entry face oriented towards the nucleus
- Proteins exit through its concave trans/exit face
- There are three regions in Golgi that include cis Golgi network, Golgi stack (medial and trans) and trans Golgi network
- Proteins from ER first enter an intermediate compartment and then into the cis Golgi network

Golgi apparatus



Glycosylation in Golgi apparatus

- Golgi stack takes care of the metabolic activities and the modified proteins, carbohydrates and lipids can then enter the trans Golgi network that functions as a sorter and distributor
- Modification of proteins with N-linked glycosylation is the main task at Golgi and involves the removal of four mannose residues followed by the addition of N- acetyl

glucosamine, removal of two mannoses and addition of fucose and two more N-acetyl glucosamines. Next three galactose and three sialic acid residues are added.

- Glycosyltransferases add sugar residues whereas glycosidases remove them.
- Proteins that finally find their way into lysosomes are modified by mannose phosphorylation but initially N-acetylglucosamine phosphates are added to specific mannose residues in the c/sGolgi network
- Phosphorylated mannose residues are recognized by a mannose-6-phosphate receptor in the *trans* Golgi network, which directs the transport of these proteins to endosomes and on to lysosomes
- For some proteins, addition of carbohydrates can be in the side chains of acceptor serine and threonine residues within specific sequences of amino acids (0-linked glycosylation)

Sphingomyelin synthesis

- Sphingomyelin is synthesized by the transfer of a phosphorylcholine group from phosphatidylcholine to ceramide
- Syntheis of sphingomyelin takes place on the lumenal surface of the Golgi
- On the cytosolic portion glucose is added to ceramide
- Glucosylceramide can flip and additional sugar residues can be added on the lumenal side from where they cannot cross the golgi membrane
- In plants cell wall polysaccharides such as cellulose, pectin and hemicellulose are synthesized in Golgi and transported by vesicles to the cell surface

Transport from Golgi apparatus

- Vesicles basically bud out from the trans Golgi network and transport the proteins, lipids and polysaccharides to their destination through the secretory pathway
- Constitutive secretory pathway also operates for some proteins
- Some proteins are transported into lysosomes
- Secretions can also be controlled by specific signals such as hormones, digestive enzymes and neurotransmitters Yeast and in some plant cells that lack lysosomes, vacuoles take over the function of lysosomes

Lysosomes

- The organelles involved in intracellular and extracellular digestion are called lysosomes
- Lysosomes typically contain many digestive enzymes
- More than 50 hydrolases have been identified in lysosomes capable of digesting a variety of biological substances
- Lysosomes are present in protozoa, plants and animals but not in bacteria in which the periplasmatic space between the cell wall and plasma membrane plays a similar role

- Lysosomal enzymes are enclosed by a membrane and thus are not immediately available to their substrates and since the digestion takes place within lysosomes, other parts of the cell are protected from being digested by these enzymes
- Lysosomal enzymes include lysozyme, N-acetyl-beta-glucosaminidase, aryl sulfatase, acid phosphatase, phosphodiesterase, cathepsins, collagenase, elastase and carboxy peptidases
- Intralysosomal pH is about 5 and acidification depends on a proton pump present
  in the lysosomal membrane that acts in an ATP-dependent manner and + accumulates H ions inside the lysosomes

Primary and Secondary Lysosomes

- The primary lysosome is basically a storage granule and the enzymatic proteins are synthesized by ribosomes and handled by ER and Golgi bodies
- The heterophagosomes are essentially digestive vacuoles containing engulfed materials within a membrane and may have association with the primary lysosomes
- If the digestion is incomplete, this results in the formation of residual bodies that may be eliminated by excretion or retained for a long time
- Some times (during starvation) a portion of mitochondria or ER may be present in lysosomes forming autophagic vacuoles in which parts of the cell are digested
- Heterophagosomes, residual bodies and autophagic vacuoles are collectively known as secondary lysosomes
- Secondary lysosomes help in the digestion of proteins resulting in the formation of dipeptides that can cross the membrane
- Carbohydrates are digested to form monosaccharides while some polysaccharides and disaccharies remain undigested

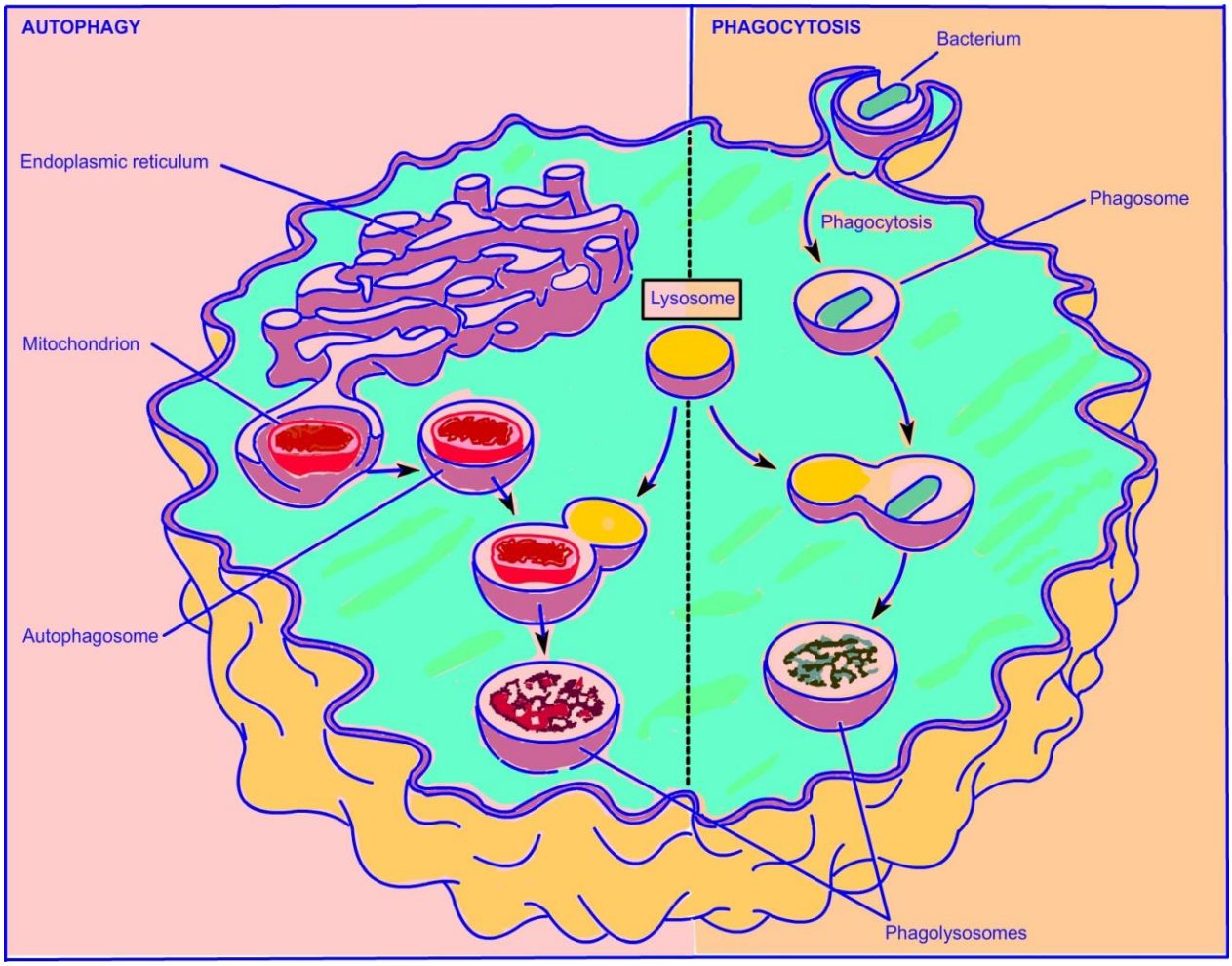
Autophagy

- Autophagy is a cellular mechanism by which parts of the cell are digested and the products are used up again by the cell for survival and this may be stimulated under certain conditions such as starvation
- Lysosomal enzymes discharged into the autophagic vacuoles can digest the cellular parts
- Osteoclasts often release lysosomal enzymes that can degrade bone matrix upon stimulation by parathyroid hormone but this can be inhibited by calcitonin

Endocytosis

- Endocytosis is a process by which solid or fluid materials in bulk are taken up by the cells and thus this is a general term
- Bulk ingestion of solid materials by the cell is called phagocytosis
- Bulk ingestion of fluid materials by the cell is called pinocytosis
- Phagocytosis is a defense mechanism against bacteria, dust and various colloids encountered by the cell
- Particles or bacteria generally first adhere to the cell and then penetrate and finally destroyed by the process of phagocytosis
- It is well developed in macrophages and endothelial cells
- Pinocytosis is induced by proteins, amino acids and certain ions in ameba
- In cells addition of ATP has been shown to increase the rate of endocytosis and can be inhibited by inhibitors of respiration
- Increase in oxygen consumption, glucose uptake and glycogen break down occurs in leukocytes during phagocytosis
- Contraction of microfilaments of actin and myosin facilitates the invagination of the plasma membrane to form the endocytic vacuoles
- Cytochalasin B, known to disrupt microfilaments also interferes with endocytosis
- Endocytic vacuoles move towards the Golgi apparatus where the primary lysosomes attach with them and fuse to liberate their contents forming the secondary lysosomes.

Autophagy and Phagocytosis



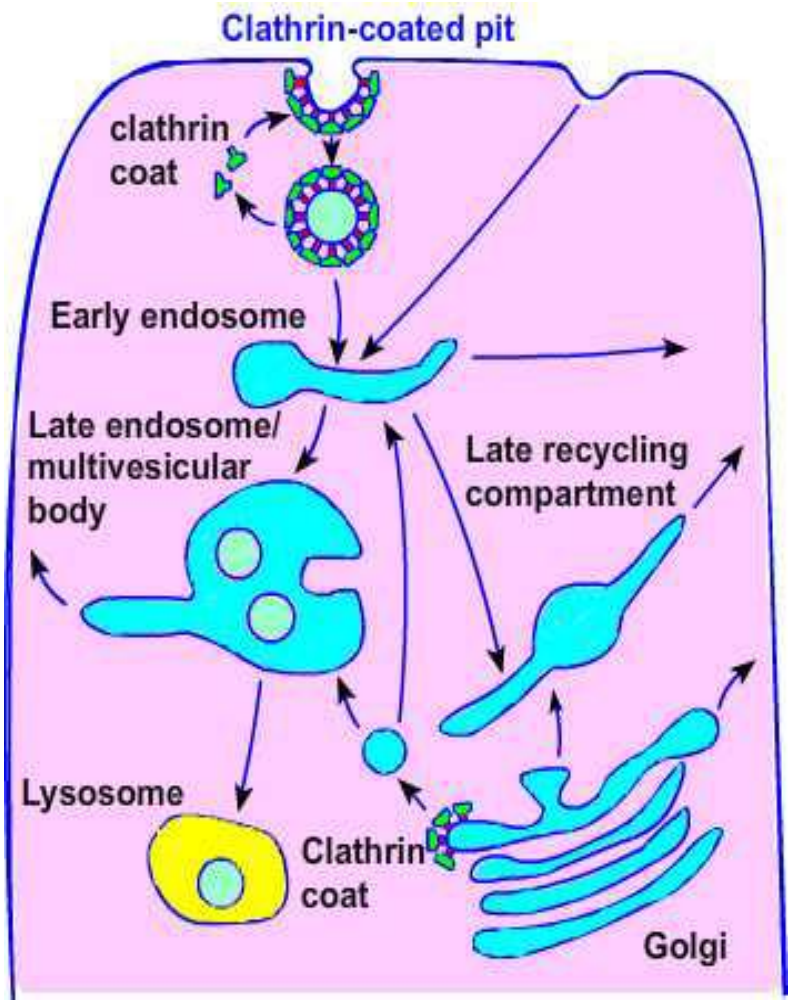
Endocytosis through coated vesicles

- Invaginating regions of the plasma membranes are called coated-pits
- Coats are mainly made up of a protein, Clathrin (mol. wt. 180,000 daltons) that forms a cage or basket like structure needed for the coated vesicles
- Coated pit has hexagonal shape that can transform into a pentagon upon invagination and the unit of assembly is a trimer
- Clathrin can assemble and disassemble rapidly and can bind different cellular membranes
- Coated vesicles can transport bits and pieces of membranes and macromolecules from the membrane into the cytoplasm and vice versa

They can also transport between intracellular compartments such as ER and Golgi apparatus

- They are highly selective to some membrane regions for transport as the coated vesicle system depends on special receptors
- They are involved in the uptake of yolk, transfer of immunoglobulins, growth factors, hormones, low density lipoproteins and recycling of membranes
- They are also important for the transport between endomembranes
- This transport system by coated vesicles is energy dependent

Endocytosis through coated vesicles

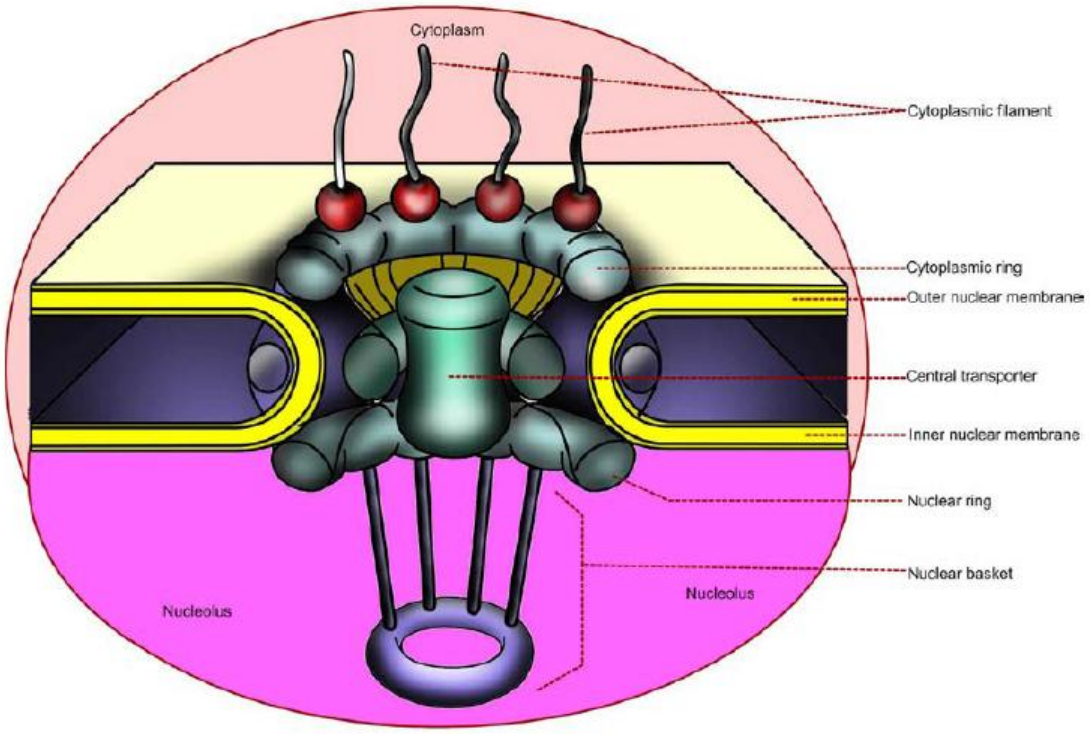


NUCLEUS STRUICTURE

Nuclear envelope

- The nuclear envelope structure is better visualized through electron microscope.
- It has two membranes separated by ***perinuclear space*** of about 10-15 nm in width.
- The membranes have lipid bilayer structure like any other membrane including the plasma membrane.
- The outer surface contains ribosomes and the nuclear membrane directly continues as ER membrane especially the one with ribosomes.
- Nuclear envelope is interrupted with many nuclear pores. A fibrous lamina of proteins (50-80 nm thick) attach with the inner membrane but not with the pores.

Nuclear pore complex



- The NPC with 100-nm diameter is a hollow cylinder embedded in the nuclear envelope.
- It has about 30 different proteins termed nucleoporins (Nups).
- The NPC acts as the gate between the nucleus and the cytoplasm.

- Macromolecules carrying specific import and export signals are only allowed to pass through water and metabolites can freely pass through.
- The central channel is filled and surrounded with FG Nups having many large domains rich in phenylalanine and glycine repeats.
- The nuclear basket has eight filaments that reach into the nucleoplasm, attached to each other by a ring at the end.
- Electron microscopy tomographs have shown that filaments extend from this basket into the nucleus
- The cytoplasmic filaments form highly mobile molecular rods projecting into the cytoplasm.

NLS and NES

- The transport of molecules through the NPC is restricted by size; below a mass of approximately 60 kDa, macromolecules can passively diffuse across the NPC.
- Such small macromolecules below this cut-off also often contain a nuclear localization signal.
- Nuclear localization sequences (NLSs) for import into the nucleus and nuclear export sequences (NESs) for export are required.
- These signals are recognized by transport factors, each with specific signal preferences.

Transport receptors

- Transport receptors are usually from the karyopherin (importin and exportin) families, with a shared a-super helical structure.
- Karyopherins can bind to the NLSs or NESs of their cognate cargoes, to the FG Nups and to the GTPase Ran.
- Import cycle starts with the formation of the cargo-karyopherin complex in the cytoplasm, which is the rate-limiting step *in vivo,* and translocates through the NPC. Disassembling the complex occurs on the nuclear side by the binding of Ran-GTP to the karyopherin.
- Ran cofactors are present in both the nucleoplasm and cytoplasm and a Ran-specific nuclear transport factor (NTF2) maintains a high concentration of nuclear Ran-GTP and of cytoplasmic Ran-GDP.
- NES on a cargo is recognized by a cognate karyopherin-Ran-GTP dimer in the

nucleus and, after translocation across the NPC, the NES-cargo-karyopherin-Ran-GTP complex is disassembled on the cytoplasmic side, through activation of Ran GTPase activity by cytoplasmic RanGAP, achieving directionality.

- Not all transport factors require Ran, nor belong to the karyopherin family; however, notably, all transport receptors can interact directly with FG Nups

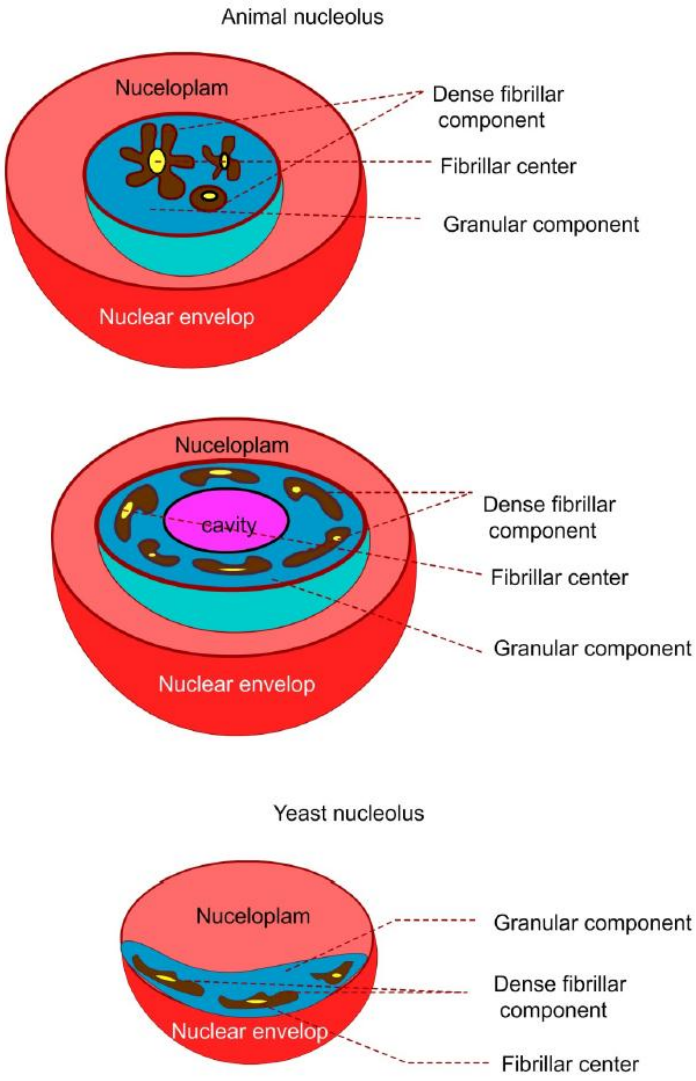
Cargoes

- NPC can be considered an enzyme for transport, in which only the correct substrates (such as transport factors and their cargoes) can bind to the active site and so pass across the nuclear envelope.
- In living cells, this high transport rate is represented by several transport factors carrying many importing and exporting cargoes, including ribonucleoproteins (RNPs).
- ***Import cargoes*** are mostly proteins that have been synthesized in the cytoplasm and are needed in the nucleus. There are also proteins that, once they reach the nucleoplasm, are exported out again by karyopherins such as XPO1 (also known as CRM1).
- ***Export cargoes*** are usually RNAs, as complexes made of RNA and proteins.
- The ribosomal subunits and messenger RNPs (mRNPs) are the most abundant of these export cargoes.
- Protein cargoes (60kDa) are smaller than mRNP cargoes (100 MDa)

Nucleolus

- The nucleolus is involved in the synthesis and processing of ribosomal RNAs (rRNAs) and their assembly into ribosomal subunits
- It is the largest nuclear domain.
- Nucleoli show lightly staining fibrillar centres (FCs) of about 0.1-1.0 ^m in size, surrounded by dense fibrillar component (DFC), which is usually more heavily stained than the rest of the nucleolus.
- Fibrillar centres are the interphase equivalents of the nucleolus organizer regions.
- The other part of the nucleolus is filled with granules (called the granular component—GC).The granules probably represent pre-ribosomal particles in various stages of maturation.

- These filaments help NPCs to reach about 100 nm into the nucleus and cytoplasm.



Nucleolus structure is evolved

- The complex structural organization of the nucleolus differs between anamniotes to amniotes.
- Anamniotes are vertebrates not having an amnion; they lay eggs in water.
- Amniotes are the organisms (reptiles, birds, etc) laying eggs in the environment.
- In this transition the Rdna intergenic region increases.
- Amnion is a protective membrane which covers the embryo in mammals, birds and reptiles.

- It contains the amniotic fluid. This fluid provides the embryo with the required nutrients.

Nucleolus organizer region (NOR)

- Nucleolus is formed in the Nucleolus Organizer Region.
- It is surrounded by filaments called the pars fibrosa (PF).
- The filaments are formed of ribosomal RNA that is newly transcribed.
- After the division of nucleus, this region gets associated with the nucleus. Several copies of genes of ribosomal RNA are present in this region.
- Many tandem copies of the genes of these ribosomal RNAs are found in the NOR.
- Karyotype analysis by means of silver nitrate staining can be used to identify NOR.
- About 10 NORs found in a cell.

Functions of nucleolus

- The major function of nucleolus is the production and assembling of ribosomal subunits.
- 50% of the total production of RNA in cells occurs in the nucleolus.
- Nucleolus is the site for the transcription of rRNA precursor molecule from DNA.
- These RNAs are packaged with certain specific forms of proteins to produce ribosomal units of varying size.
- The ribosomal units are then transported into the cytoplasm for translation and protein synthesis.

Non-ribosomal functions of nucleolus

- Nucleoli have nonstandard functions apart from being ribosome factories
- Nucleolus is also involved in mRNA export or degradation,
- Biosynthesis of the signal recognition particle (SRP),
- Biogenesis of some snRNAs and tRNAs,
- The sequestration of regulatory proteins,
- Control of the cell cycle, Aging, and stress sensing.