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CYTOLOGY: Sylabus for students

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CELL STRUCTURE

A. NUCLEUS

B. CYTOPLASM - hyaloplasm with cell organelles

- paraplasm with cytoplasmic inclusions

1. Cell organelles

> membrane limited: mitochondria, endoplasmic reticulum (rough and smooth), Golgi complex, lysosomes, secretory granules, peroxisomes

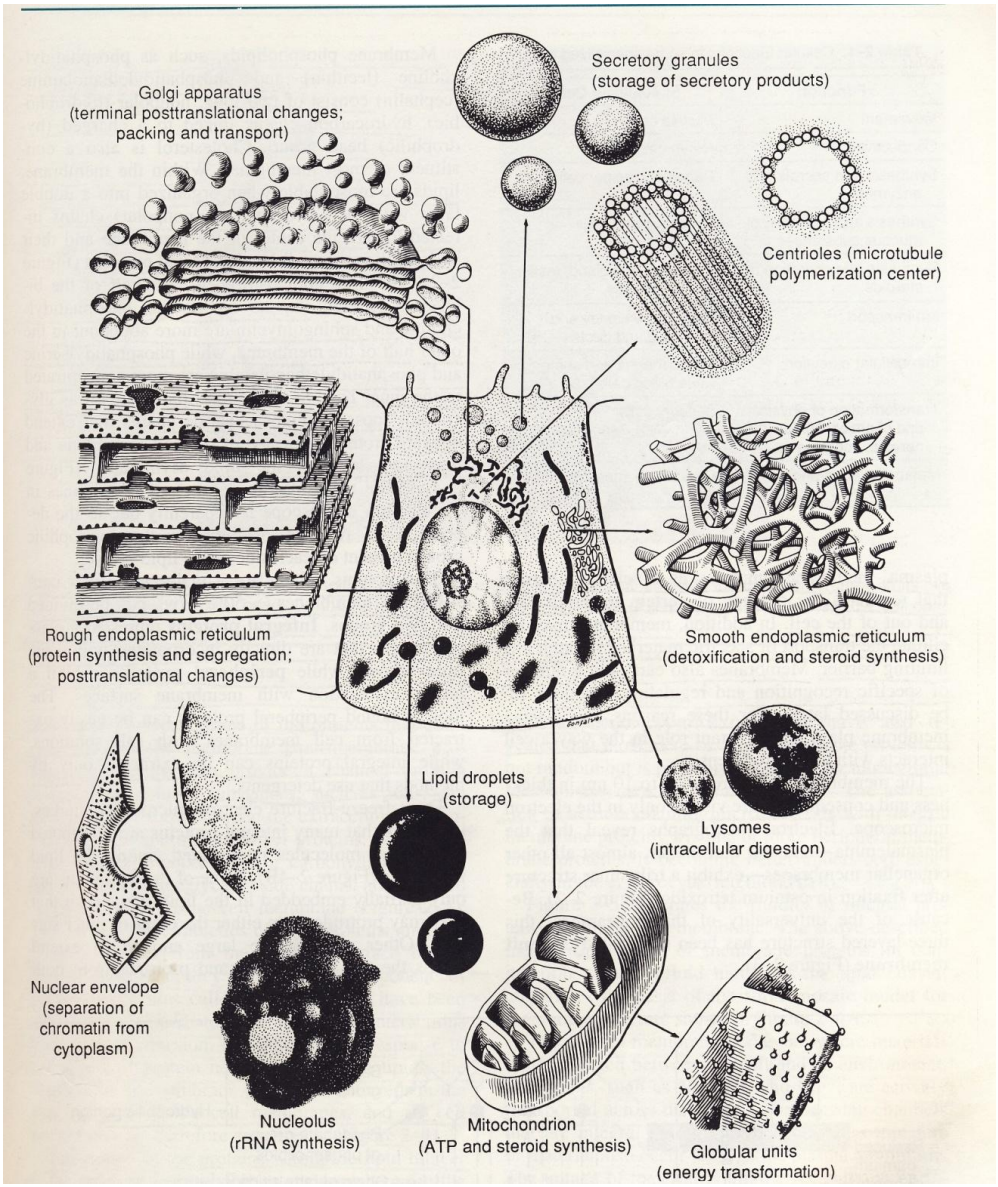
> membrane unlimited: ribosomes, centrioles; nucleolus in nucleus, and

cytoskeleton: microtubules, microfilaments, intermediate filaments

2. Cytoplasmic inclusions: glycogen granules, lipids, pigments

3. Cytoplasmic matrix = cytosol – soluble ground substance: water, ions, metabolites, soluble enzymes, saccharides

Cytoplasm is usually acidophilic structure /contains proteins/



Structure of organells

Cell Membrane (CM) – Plasmalemma

- limiting membrane of eukaryotic cells
- selective barrier that regulates the passage of material into and out of the cell
- recognition and regulatory functions
- plays an important role in the way the cell interacts with its environment

Molecular structure:

Lipids, proteins, saccharides, ions

1. <u>bimolecular phospholipid layer</u>

- two *hydrophilic* portion of phospholipid heads
- linked to long nonpolar *hydrophobic* fatty acid chains and cholesterol

2, *proteins* – 50%

- **peripheral proteins** – looser association with CM
- **integral proteins** – incorporated within the lipid bilayer
- **transmembrane proteins** (belong to integral proteins) completely cross CM and form **ion channels**

***"Fluid mosaic model"* – integral proteins within the CM can change their position**

3. saccharides - oligosacharides chains linked to lipid part = **glycoplipids** or to protein part = **glycoproteins** constitute specific molecules on the outer surface of CM named **glycocalyx;** Function: receptors

4. ions – Na^+, K^+, Ca^{2+}

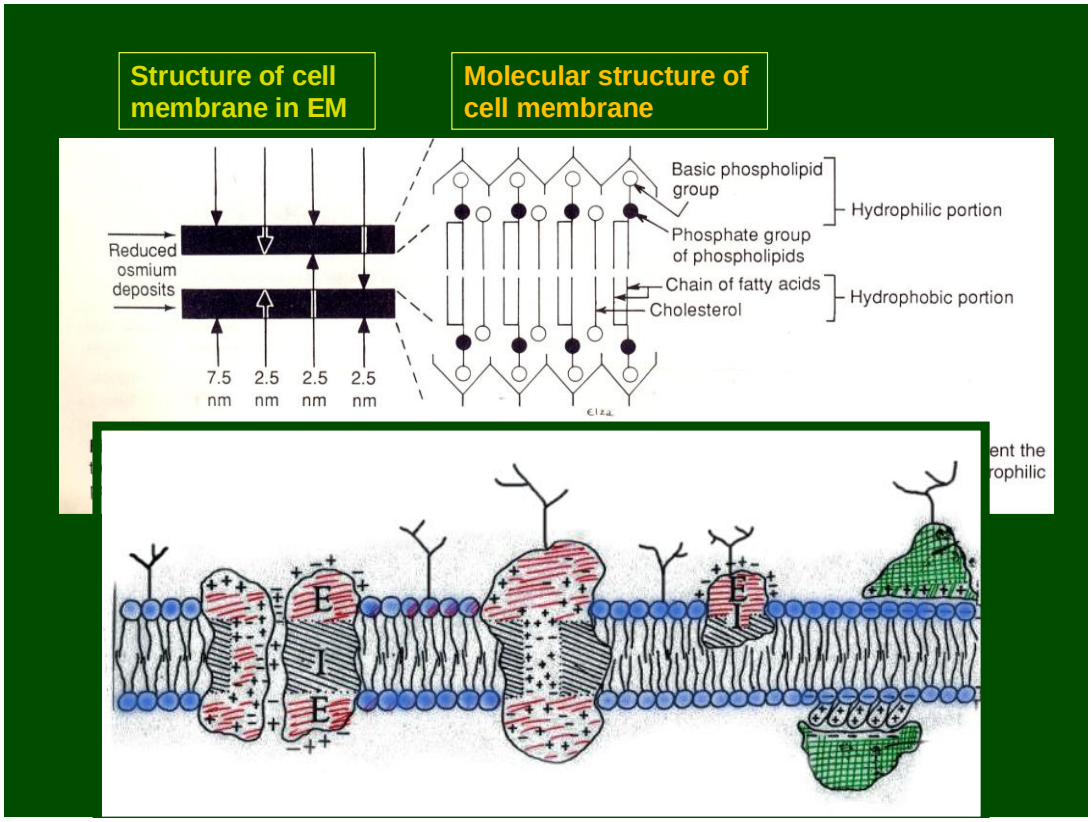
FUNCTION:

Cell membrane is involved in process of:

Endocytosis :

- **pinocytosis –** cell drinking – incorporation of fluid particles to the cell
- **phagocytosis** – cell eating of invading bacteria, protozoa, damaged cells, unneeded extracellular material

Exocytosis: releasing of substances out of the cell – membrane limited **secretory granules**



Ultrastructure of the cell membrane in electron microscope (EM):

- thickness 7.5 – 10 nm
- cell membrane has **trilaminar structure**: in EM is visible like two **electron-dense (dark) layers** and between them is one **electron-lucent (pale) layer**

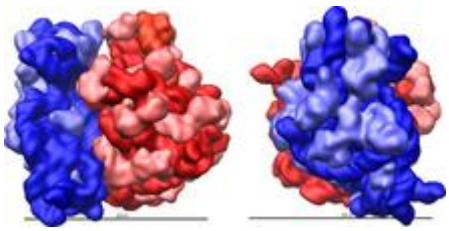
Ribosomes – organel without membrane

EM:

- small **electron dense particles** in the cytoplasm (20x30 nm)
- are composed of **two subunits**:

  large subunit – round in shape

  small subunit – irregular shape



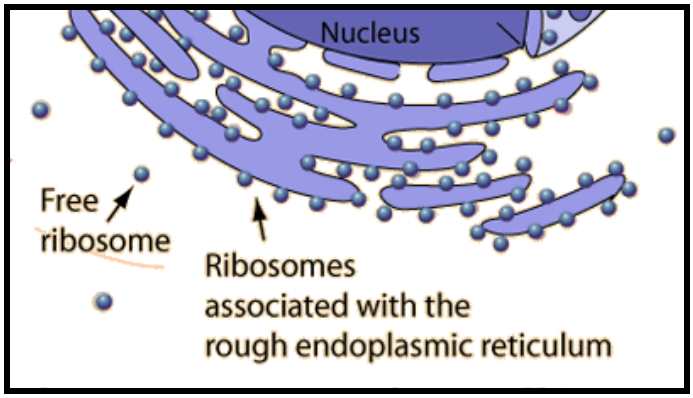
Large (red) and small (blue) subunit fit together

Biochemical composition:

– molecules of rRNA (63 %) and rest part are **proteins (**80 different types**)**

- affinity of RNA to basic dyes (hematoxylin, toluidin blue = basophilic staining)

Ribosomes are present in the cytoplasm in the form of: monosomes, polyribosomes or rough endoplasmic reticulum (rER)



LM:

- basophilic regions in the cytoplasm

Formation of ribosomes :

- basic component of ribosomes is rRNA- **synthesized in nucleolus**

- proteins are syntesized **in the cytoplasm,** proteins are transported to the nucleus through nuclear pores and fuse with molecules of rRNA

- proteins and rRNA form **ribosomal subunits** that are released through nuclear pores into the cytoplasm and give rise to **complete ribosomes connected by mRNA**

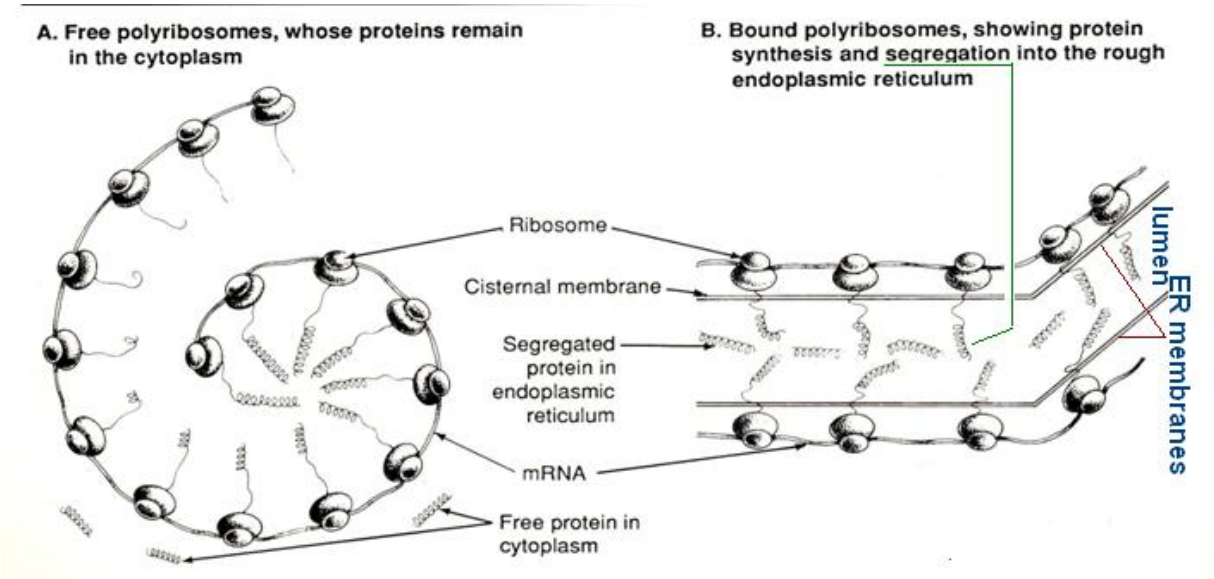
Rough endoplasmic reticulum

The general structure of the endoplasmic reticulum is an membrane network of cisternae (sac-like structures) held together by the cytoskeleton. The phospholipid membrane encloses a space, the cisternal space (or lumen), which is continuous with the perinuclear space of nuclear envelope but separated from the cytosol.

The surface of the rough endoplasmic reticulum (rER) is studded with protein-manufacturing ribosomes giving it a "rough" appearance.
Ribosomes are binded to the ER **only when they begin to synthesize a protein** destined for the secretory pathway.
The membrane of the rER is continuous with the outer layer of the nuclear envelope.
Although there is no continuous membrane between the rER and the Golgi apparatus,
Membrane-bound vesicles shuttle proteins between these two compartments.

Functions:
Protein synthesis, initial glycosylation of proteins

1. Proteins used inside the cells **intracellular proteins** – for building of membranes, enzymes for metabolism, lysosomal enzymes.
2. Proteins released out of the cell - **extracellularly – enzymes for digestion** (gastric glands, salivary glands; ergastoplasm**), immunoglobulins (**released by plasma cells**), material for extracellular matrix in connective tissue** (fibroblasts), **neurotransmitters** (nerve cells; rER=Nissl bodies).



Smooth endoplasmic reticulum (sER)

Structure:

- consists of tubules and vesicles that branch forming a network (like connected channels)
- membranes of sER arise from rER - lacks the associated polyribosomes – smooth surface

Function:

sER contains different types of enzymes

- synthesis of steroid hormones
- lipid synthesis
- detoxification (of drugs, alcohol, poisons – in the liver cells)
- synthesis and breakdown of glycogen in the liver
- synthesis of HCl in stomach (parietal cells)
- function in concentration of calcium ions **in muscle cells** (specialized form of sER = **sarcoplasmic reticulum**)

Smooth ER also contains the enzyme glucose-6-phosphatase, which converts glucose-6-phosphate to glucose, a step in gluconeogenesis

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Golgi apparatus = GA (**Golgi complex**) in LM can be visualised after osmium tetroxide fixation – black colour.

EM Structure:

Golgi apparatus is composed of:

1. 4 or 8 cisternae of membrane-bound structures (cisternae- singular: *cisterna*). An individual stack is sometimes called a dictyosome (from Greek *dictyon*: net + *soma*: body). Each cisterna contains special Golgi enzymes which modify or help to modify proteins that travel from rER to GA.
2. Transported vesicles situated at cis face of GA (they are membrane bounded and contain proteins synthetized in rER)
3. Vacuoles (newly formed vesicles) situated on the lateral sides of GA and trans face (membrane bounded; that contains finally modified proteins, enzymes).

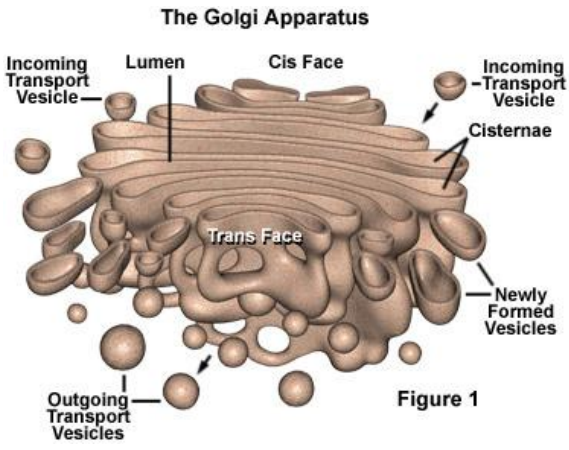
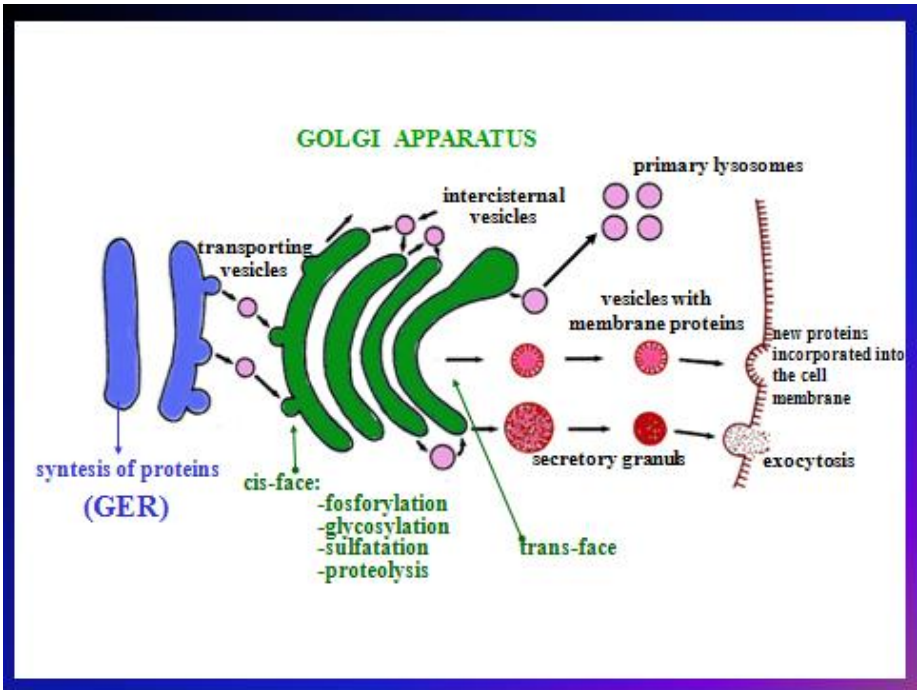


Figure 1

Function:

The vesicles that leave rough endoplasmic reticulum are transported to the *cis* face of the Golgi apparatus, where they fuse with the Golgi membrane and empty their contents into the lumen. Once inside the lumen, the molecules are modified, sorted and shipped towards their final destination.



1. Cells synthesize a large number of different macromolecules. The Golgi apparatus is involved in modifying, sorting, and packaging these macromolecules for cell secretion = exocytosis or use within the cell. It primarily modifies proteins delivered from the rough endoplasmic reticulum
2. involved in the transport of lipids around the cell
3. creates lysosomes

- Enzymes within the cisternae are able to modify the proteins by addition of carbohydrates (glycosylation) and phosphates (phosphorylation).
- site of carbohydrate synthesis
- Golgi involves the sulfation of certain molecules passing through its lumen

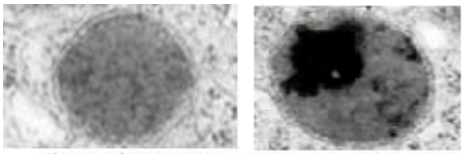
LYSOSOMES

Primary lysosomes

- spherical structures, surrounded by a membrane
- **homogenous** material, electrondense in EM
- contain hydrolytic enzymes

Secondary lysosomes = primary lysosome fuse with phagosome (material for degradation)

- **phagosomes: 1 - autophagosomes** (e.g. old mitochondria)
  2 - heterophagosomes (phagocytosed material)
- **heterogenous** material surrounded by membrane (EM)
- enzymatic degradation takes place here



primary lysosome secondary lysosome

Terciary lysosomes - residual bodies

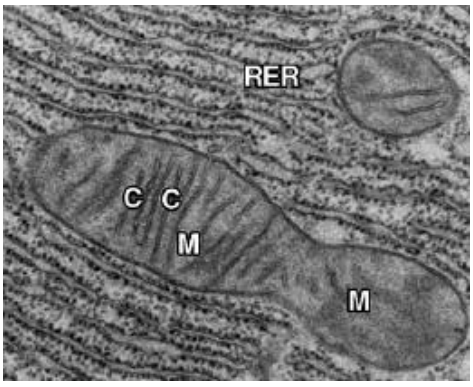
- waste material is stored inside the lysosomes
- are present in long living cells - neurons, cardiomyocytes
- agregations of undigested material (covered by membrane) - **„lipofuscin“ pigment** (yelowish-brown colour in LM)

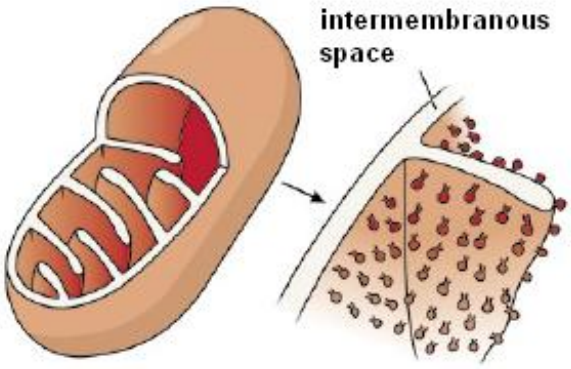
MITOCHONDRIA (M)

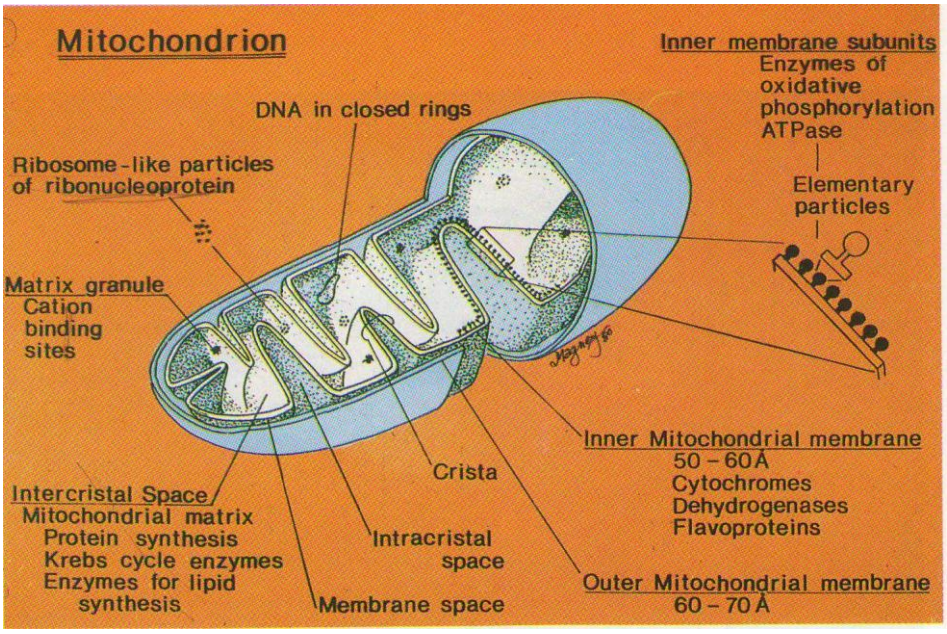
- spherical or oval organelles in diameter 0,5x10 µm visualized by **iron hematoxylin**
- great number in cells with **intensive metabolic activity** – 1000 mitochondria per one liver cell;

Function: transforming of chemical energy into energy easily accessible to the cell (ATP), production and storage of energy

EM-Structure







EM : composed of 2 membranes:

- **outer mitochondrial membrane** is smooth
- **inner mitochondrial membrane**
  a) project folds into the interior of mitochondrion called **cristae** – shelflike cristae = **cristal type of mitochondria**
  b) or inner membrane forms tube-like invaginations = **tubular type of mitochondria** (in steroids secreted cells)

Outer mitochondrial membrane is permeable, contains special transmembrane proteins = **porins** – serve like channels for transport of substances into intermembrane space
Inner mitochondrial membrane is less permeable – contains **elementary particles** = **globular units**, 10 nm, connected with the inner membrane of cristae via cylindrical stalks. Globular units contain enzymes for ***oxidative phosphorylation*** and **ATPase activity**
Intermembrane space – is located between 2 membranes
Intercristal space = mitochondrial matrix contains:

*** ring –like DNA**
*** Mitochondrial ribosomes**
*** Dense granules (Ca2+, Mg 2+)**
In the matrix are enzymes for ***Krebs cycle, β-oxidation of fatty acids***

NUCLEUS

- **contains DNA - genetic information**
- **nucleoprotein (histone proteins and non-histone proteins), RNA**

Sructure in EM:

1. nuclear envelope = karyolemma: **2 parallel unit membranes** separated by space - **perinuclear cisterna**
- nuclear pores – circular gaps (70 nm) in the nuclear envelope; covered by diaphragm
2. chromatin- mainly of coiled strands of DNA bound to basic proteins- histones

- **heterochromatin** - electron-dense in EM, basophilic in LM
  - non-active form
- **euchromatin** - lightly stained areas in LM, electron lucent in EM
  - active form of chromatin

Function: synthesis of precursor of RNA (transcription)

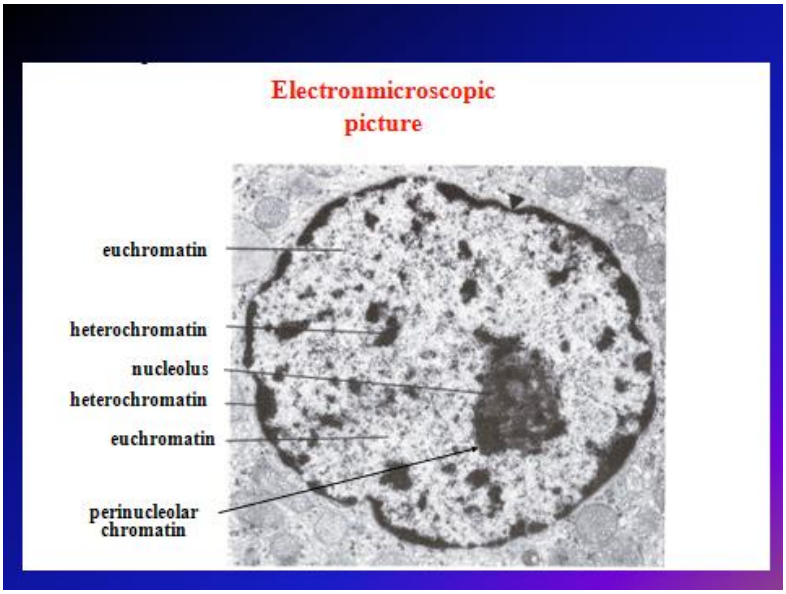
3. nucleolus – basophilic spherical structure (LM)

- electron dense , without membrane (EM)

Function: primary transcription of rRNA

formation of ribosomal subunits

4. nuclear matrix – proteins, matabolites, ions, nucleoskeleton



Nuclear envelope in detail

LM: thin line

EM:

- composed of 2 membranes, between is perinuclear space (cisterna)
- to the inner membrane are attached the **fibrous laminae** composed of polypeptides called **lamins** (ø 80-300 nm)
- 2 membranes fuse together and form nuclear pores covered by **diaphragm**

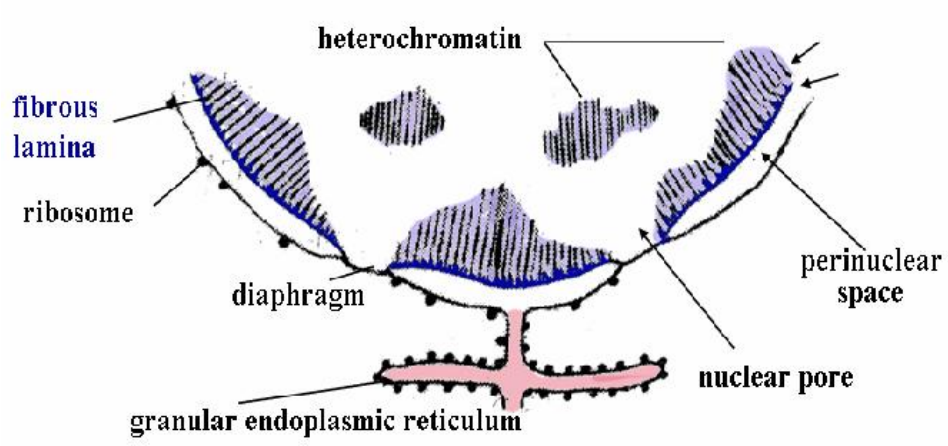
Structure of diaphragm:

- 8 peripheral globular proteins molecules + 1 central globular protein

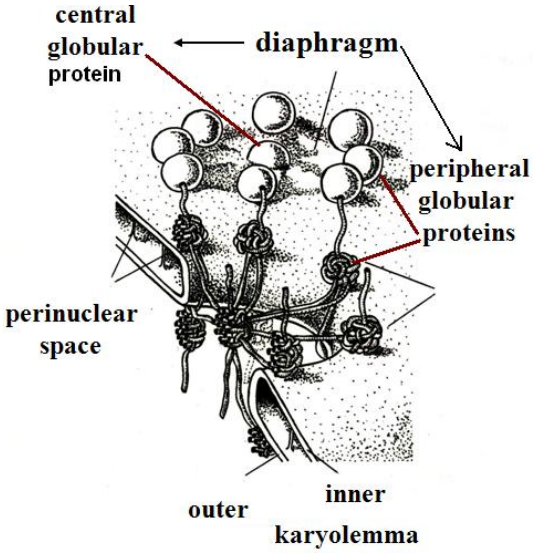
Function:

- passage of macromolecules, mRNA, proteins from the cytoplasm, ions – active transport

Outer membrane of nuclear envelope is covered by ribosomes, perinuclear cisterna is continuous with lumen of rER.



Structure of the nuclear pores:



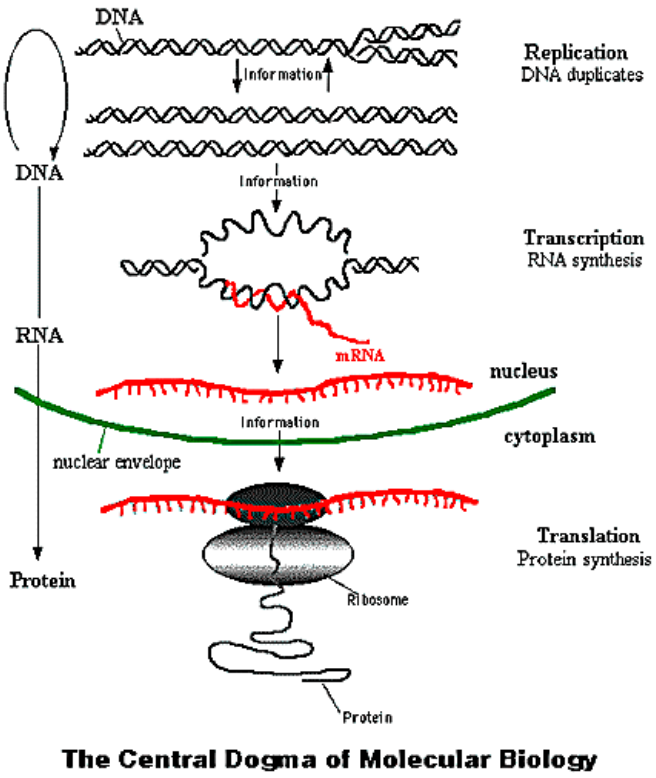
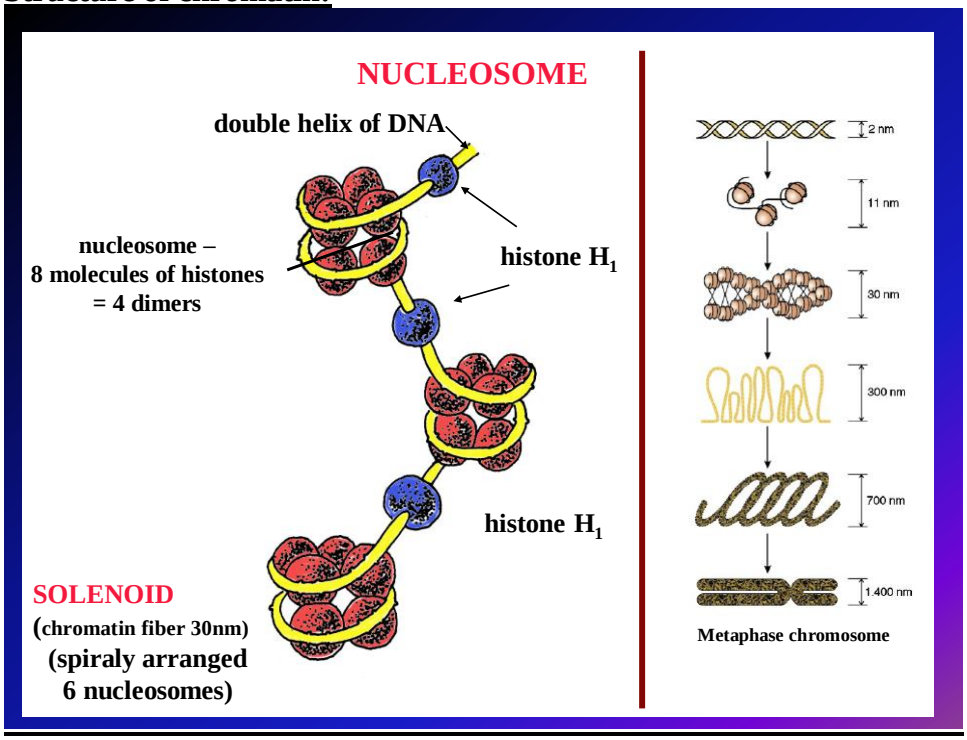


Fig. Explanation of DNA function (replication, transcription – mRNA in **the nucleus;** ribosomal subunits **in the cytoplasm** are attached to mRNA = coding of translation (sequence of aminoacids – translation - protein synthesis

Structure of chromatin:

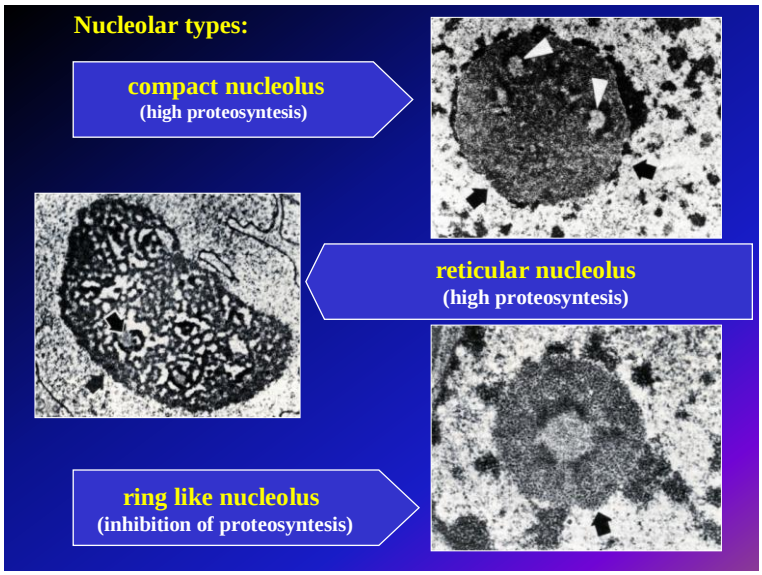


Nucleolus

LM – basophilic, oval structure
EM – electrondense structure without membrane
Nucleolus has 3 distinct regions:

c) **fibrilar centers** - contain DNA genes for rRNA synthesis
d) pars fibrosa - newly formed rRNA
e) pars granulosa - formation of ribosomal subunits contained rRNA (synthesized in the nucleolus) and proteins (synthesized in the cytoplasm).

The network formed by granular and fibrilar parts is called **nucleolonema.**



Function:
rRNA synthesis, formation of ribosomal subunits

Cytoskeleton

A) microfilaments
thin filaments - actin (8 nm)
intermediate filaments (10 nm)
thick filaments - myosin (14 nm)

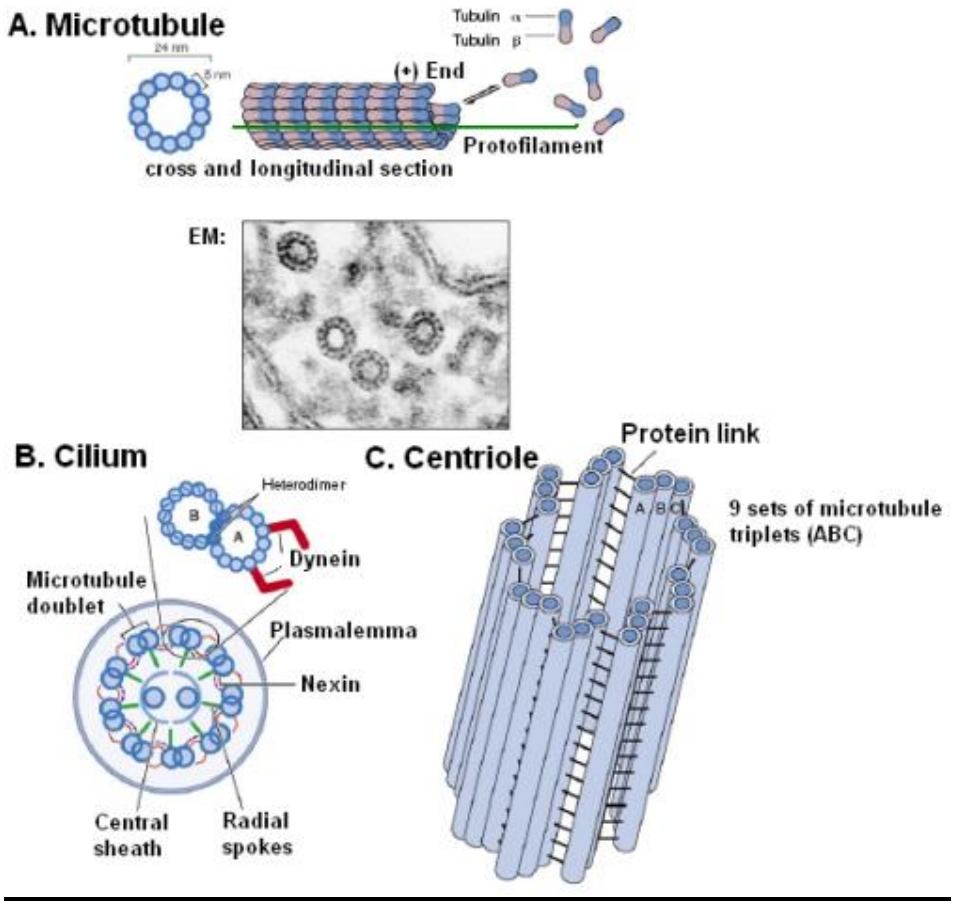
Intermediate filaments:
cytokeratin – in epithelial cells
vimentin – in cells of mesenchymal origin
desmin – in muscle cells
glial – in neuroglial cells (GFAP)
neurofilaments – in neurons

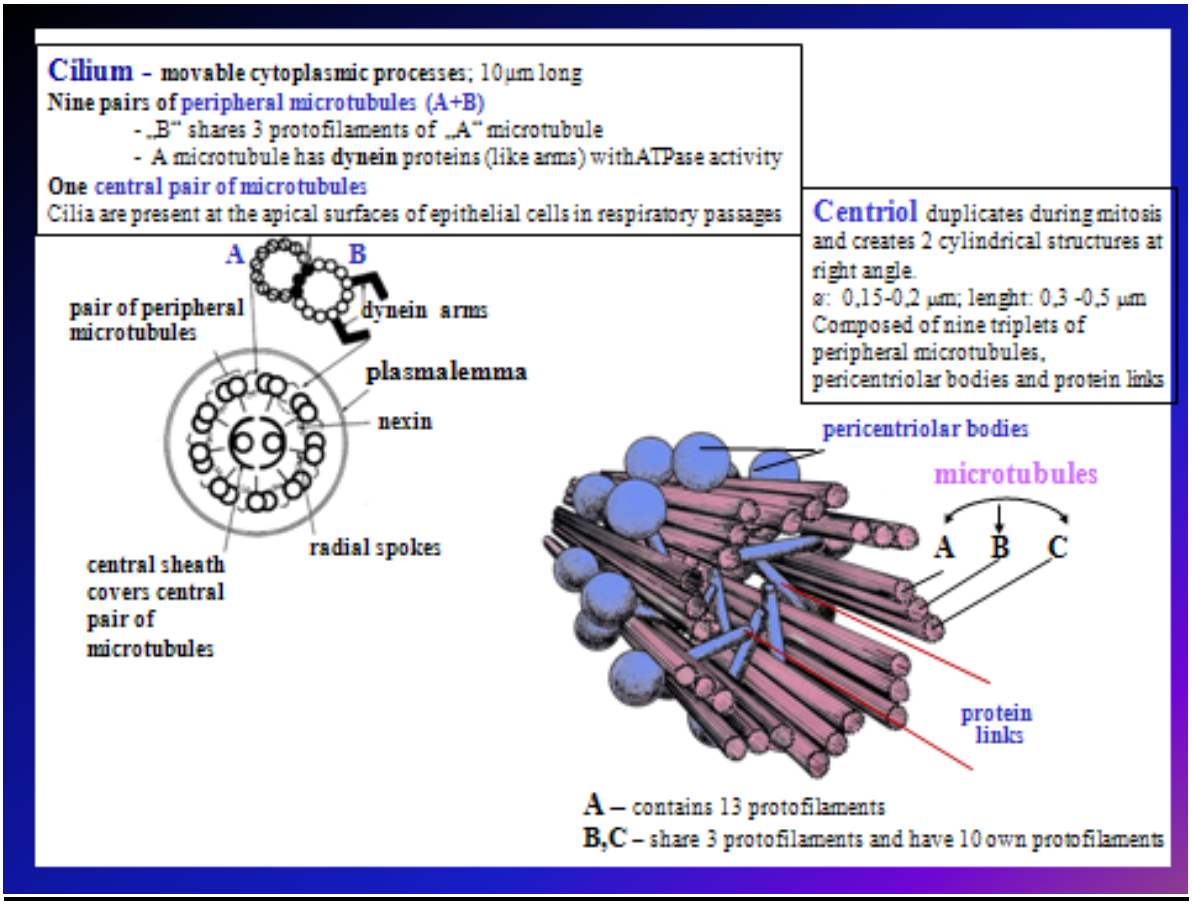
B) microtubules (picture A)

- composed of subunits: ***tubulin a*** & ***tubulin b***
- after polymerization of tubulin heterodimers the **protofilaments** give rise (elongates)
- 13 protofilaments create one microtubule

Function:

- keep the shape of the cell
- cellular transport
- create mitotic spindle
- form **cillia** (apical surface of respiratory epithelium) and **flagella** (spermatozoa)
- **centriol** (nine sets of microtubule triplet)





Cytoplasmic inclusions

- are temporary structures, surrounded or not by membrane

Lipids - dense homogenous lipid droplets
- staining – histochemic reaction with Sudan red colour

Glycogen - EM: electrondense particles - Ø 20 nm
- LM: PAS positive (polysaccharide)

Proteins - like secretory granules with enzymes surrounded by membrane

Pigments: - **exogenous** – dust, carotens, tattoo
- **endogenous** – melanin, lipofuscin, hemoglobin, myoglobin, hemosiderin