

Histology and Embryology

Lecturers:

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Lecture 1

Introduction

- The object and significance of histology.
- Relevance of histology to other biomedical disciplines.
- History, current state, and future of histology.
- Methodologies to study a structure of cells and tissues.

Cytology

- The cell - definition, characteristics, compartmentalization.
- Cell nucleus - ultrastructure and function, chromosomes, nucleolus.
- Endoplasmic reticulum
- Golgi apparatus
- Centrosome

Histology

Microscopic and submicroscopic structure of the body

(cells, extracellular matrix, fluid substances)

Cytology

General aspects of the structures composing the cells and their functioning

General histology

What are the main types of tissues?
What are their functions?
What cell types these tissues are made of?

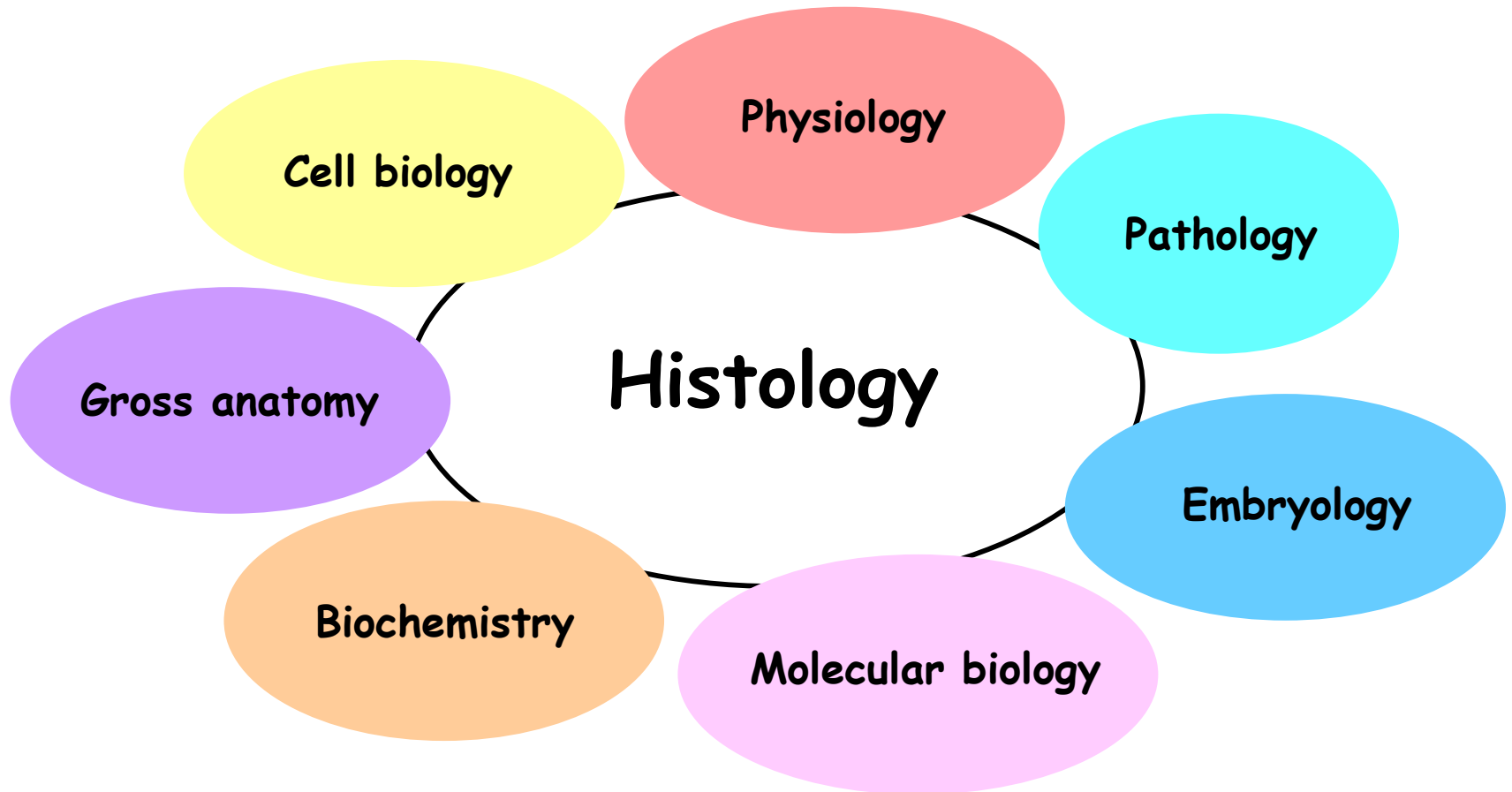
Microscopic anatomy

Composition and structure of organ systems & individual organs

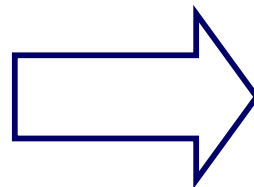
Which tissue types and how organized?
Which special cell types?
Which special structures? (e.g. tubules)
How does it all work?

All this mirrors hierarchical organisation of living organisms

Histology is no longer a static discipline dealing with just the structure !!!



Learn thinking
„histologically“



Have the histology
in action & in motion

Studying histology was first made mandatory for medical students in 1893 by John's Hopkins Medical School !

Most histologists are German primarily because they made great microscopes.

Eponymously theirs.....

Marcello Malpighi

1628 - 1694

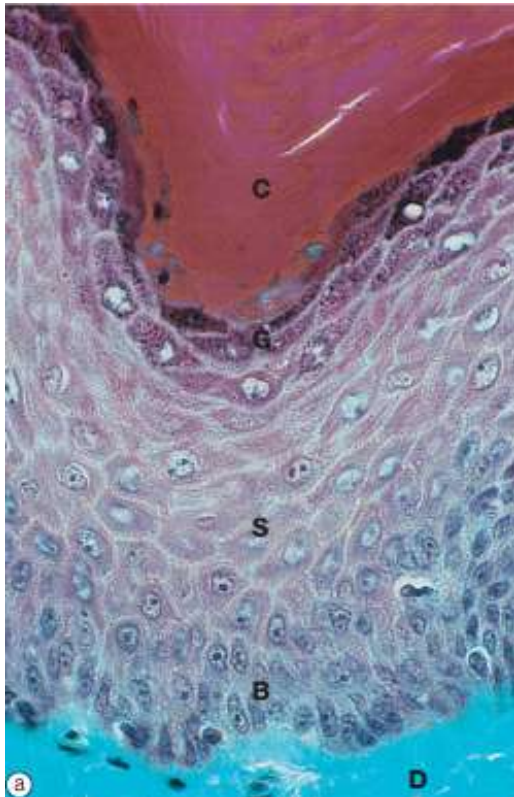
Italian physician

Founder of microscopic anatomy and the first histologist

- Discovered **taste buds**
- Discovered **capillaries**
- Maybe first to see **red blood cells** under microscope



MARCELLO MALPIGHI.
From an engraving of the edifying by A. M. Tolson, presented to the Royal
Society by Malpighi.



← **Malpighian layer** of the skin

Term for basale and spinosum layers of epithelium

Malpighian corpuscles in the kidney & spleen

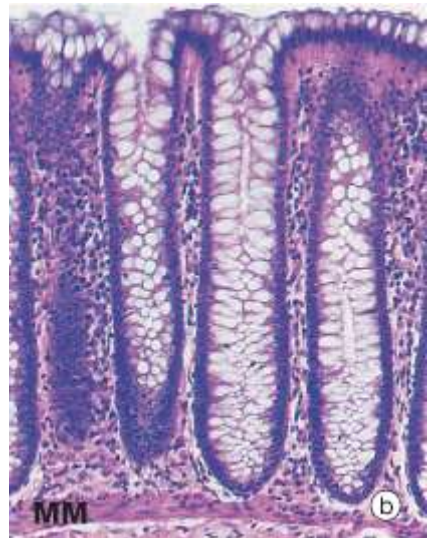
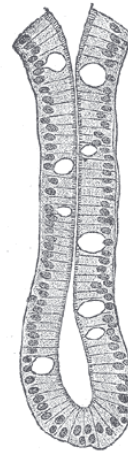
Johan Nathanael Lieberkuhn

1711 - 1756

German anatomist and physician

- Invented the **solar microscope**
- Also invented a **reflector to view opaque specimens easily**

Main histological contribution was discovering the glands of the small intestine and colon-the **crypts of Lieberkuhn**



Johann N. Lieberkuhn
(1711-1756)

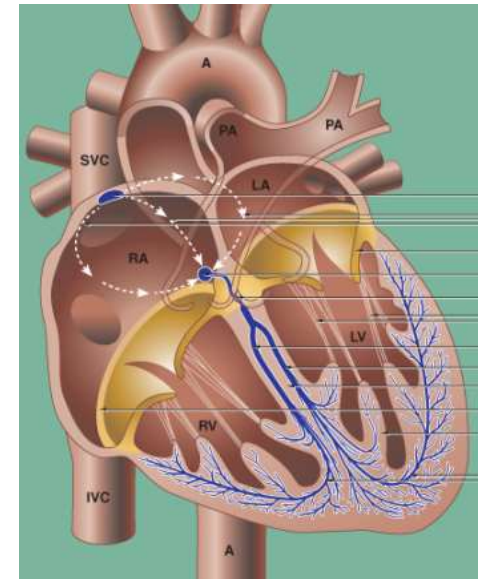
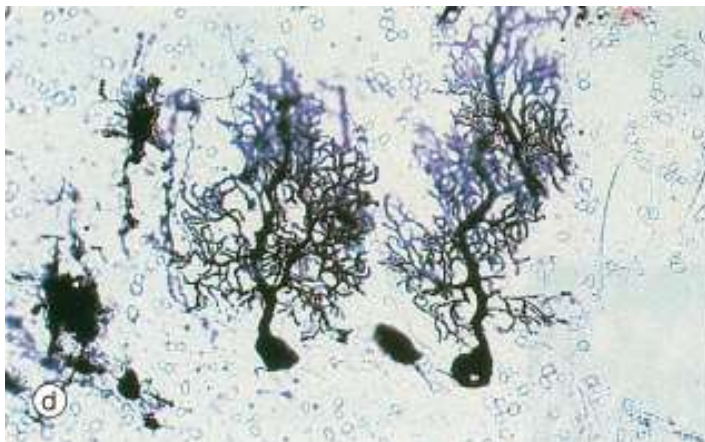
Jan Evangelista Purkyně

1787 - 1869

Bohemian physiologist

Schwann + Schleiden - 1839 - cell theory

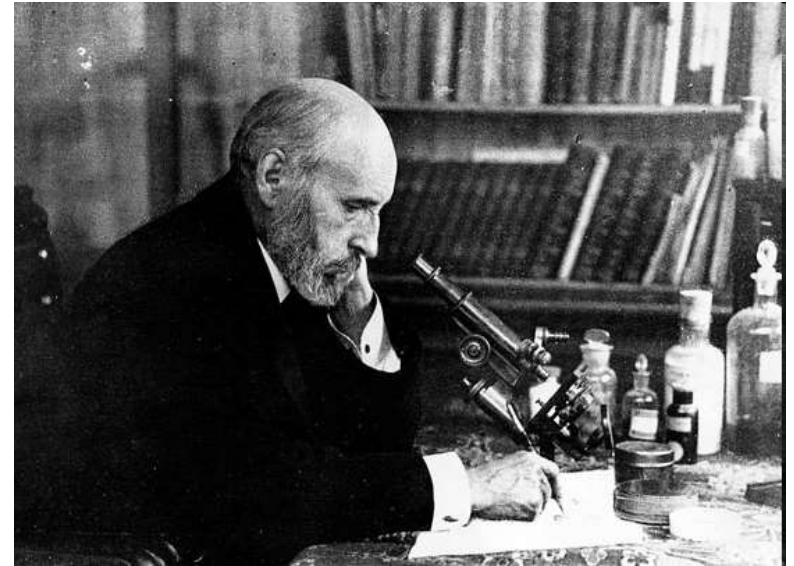
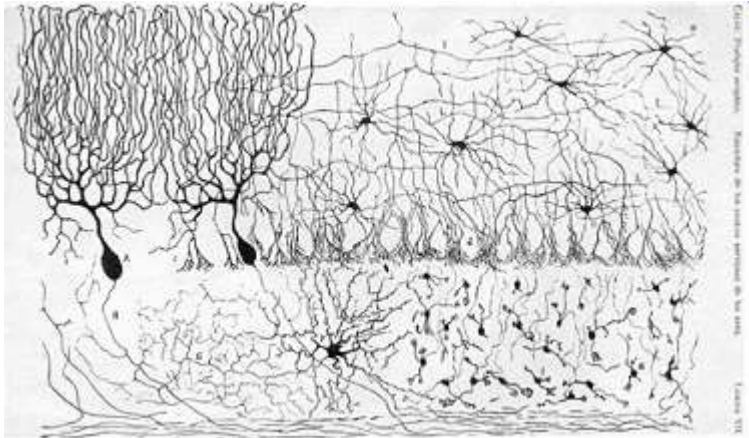
- Pioneer in histological techniques
First to use something like a **microtome**
- Introduced the term **plasma**
- Found **Purkinje fibers** of the heart
- Found **Purkinje cells** of the cerebellar cortex



Santiago Ramón Y Cajal

1852 - 1934

Spanish physician and anatomist



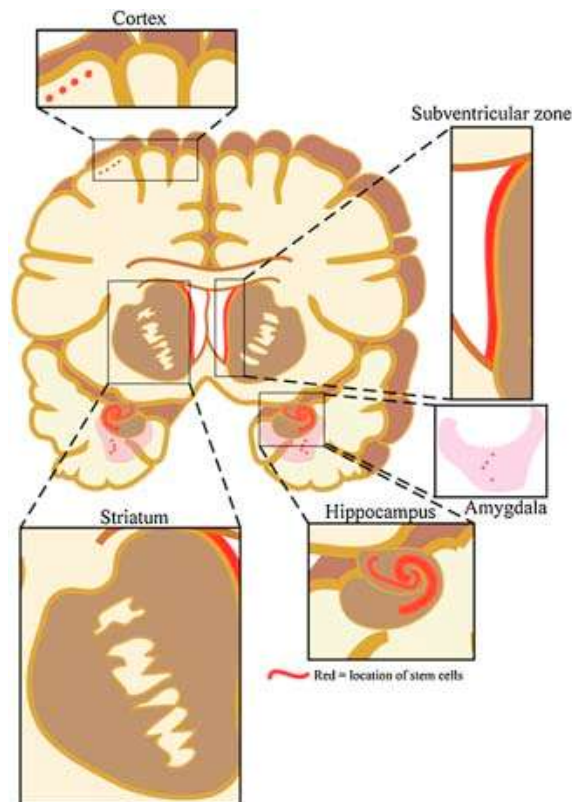
He established the **neuron** as the primary structural and functional unit of the nervous system.
Nobel Prize in 1906

“Once the development was ended, the founts of growth and regeneration of the axons and dendrites dried up irrevocably. In the adult centers, the nerve paths are something fixed, ended, and immutable. Everything may die, nothing may be regenerated. It is for the science of the future to change, if possible, this harsh decree.”

Making unexpected discoveries

(since early 1990s)

The existence of multipotent self-renewing progenitors residing in the postnatal and adult nervous system



DEFINITELY IN:

- Subventricular zone of the lateral ventricle
- Subgranular zone of the dentate gyrus of the hippocampus

POSSIBLY IN:

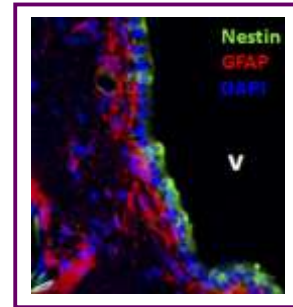
- Cortex ?
- Amygdala ?

Our view on the organization of the central nervous system has been dramatically changed !!!

Many questions on NSC remain to be answered

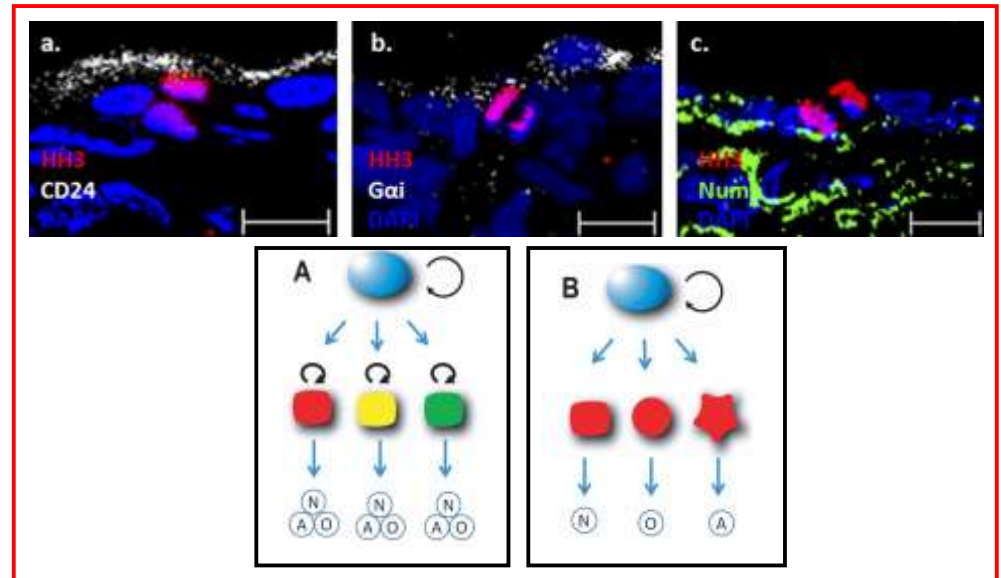
Combination of developmental biology, **histology**, cell biology, and molecular biology approaches is required.

- exact position in the tissue ?
- proliferative activity and migration ?

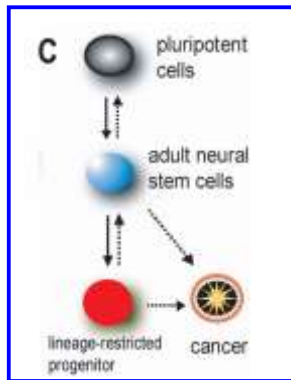


Gleason et al., Neuroscience, 2008.

- developmental potential ?
- involvement in disease development ?



- others

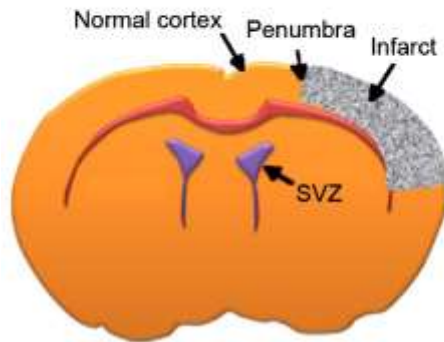


Any practical use of such discovery ? (1)

Helping brain regenerate after the stroke



Promote endogenous neurogenesis and improve **histological structure** and function



Options:

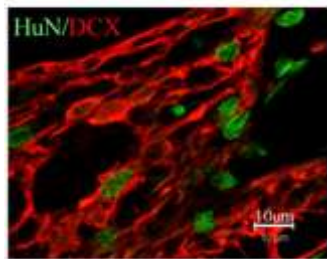
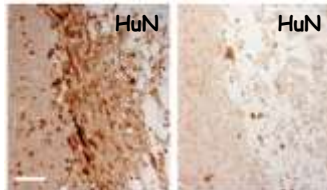
- drugs
- growth factors
- cell implantation

- experiment on rats
- MCAO - middle cerebral artery occlusion to induce infarction
- human neural precursors transplanted into the site of infarction
- **histologically evaluated**

Any practical use of such discovery ? (2)

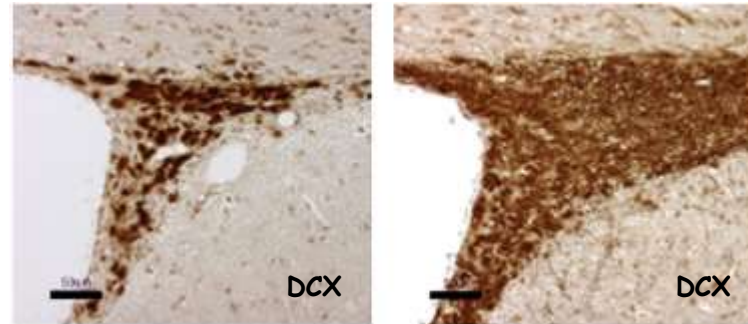
Transplanted human cells
in the site of infarction

3 months 24 months



HuN - human nuclei
DCX - doublecortin (marker of early neuronal lineage)

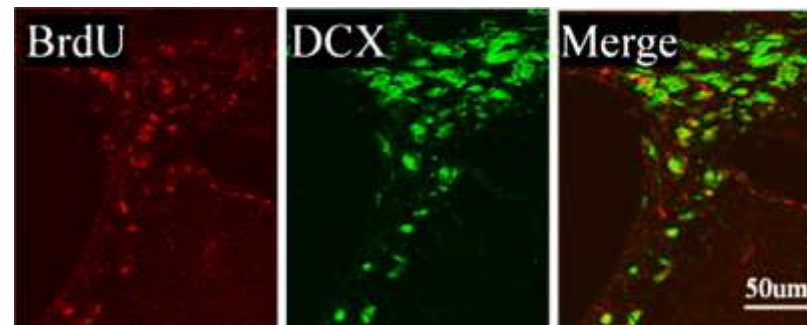
Neurogenesis in SVZ of rat brain becomes stimulated



Non-transplanted

Cells transplanted

Neocytogenesis occurs before day 60 after transplantation



Pulse-labelling with BrdU at day 60 after transplantation

Tissue & Cell transplantation

Damage to β cells
of pancreatic islets
of Langerhans

Dysregulated glucose
metabolism

Diabetes

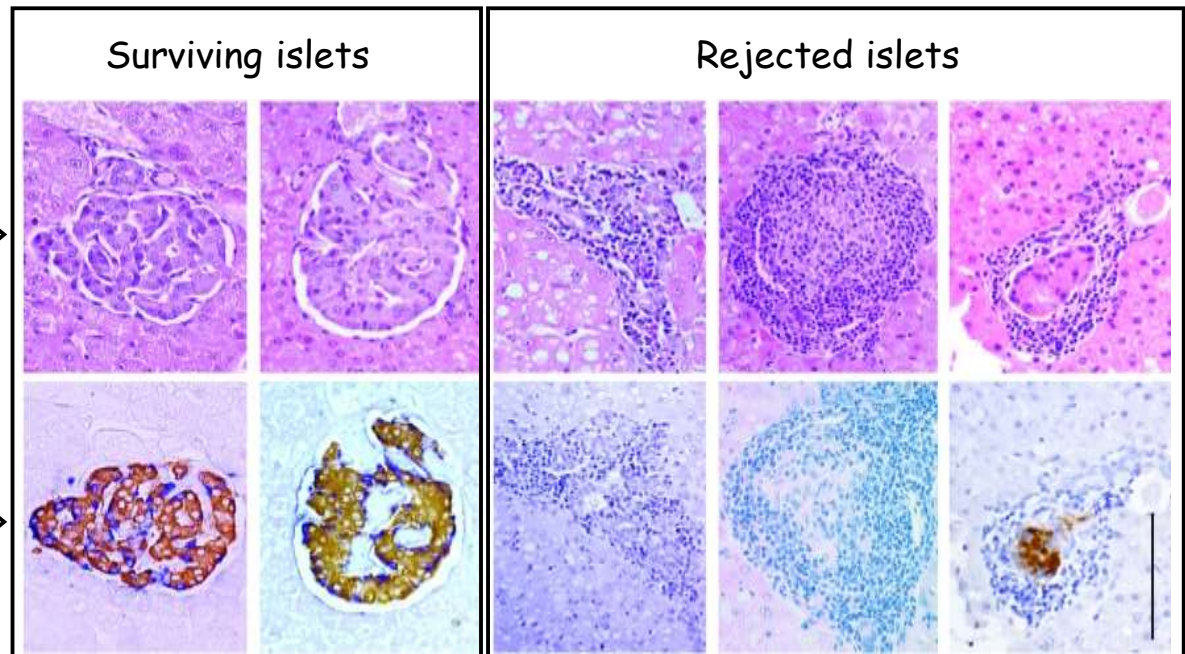
No permanent cure - Transplantation ? - Immunosuppression

Lymphocyte function-associated
antigen 1 (LFA-1)

Short-term treatment
with the LFA-1-specific Ab

Haematoxilin
&
Eosin

IHC
Insulin - brown
Glucagon - blue



Tissue and organ engineering is not novel in its principle but we develop new approaches based on our understanding of tissue composition

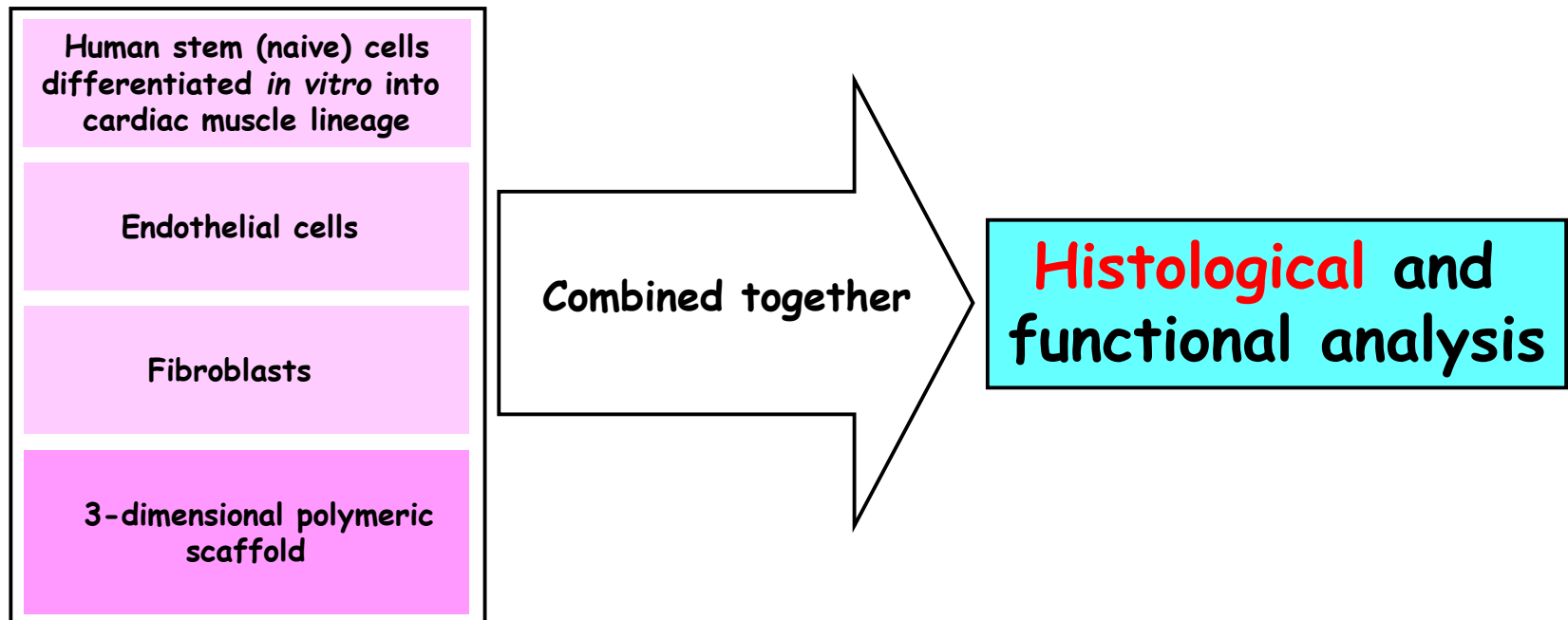


Tissue engineering 1

(stay with the infarction)

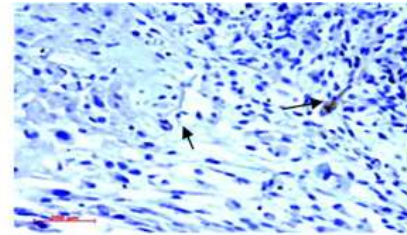
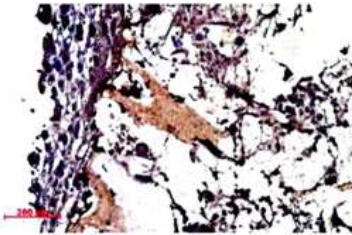
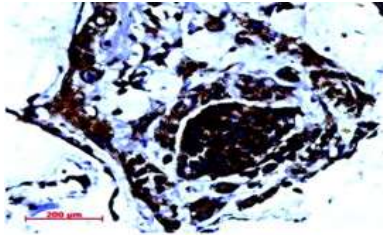
Caspi et al., Tissue Engineering of **Vascularized Cardiac Muscle** From Human Embryonic Stem Cells, *Circulation Research*, 2007 (group of Shulamit Levenberg, Israel)

The first report of the construction of 3D vascularized human cardiac tissue that may have unique applications for studies of cardiac development, function, and tissue replacement therapy

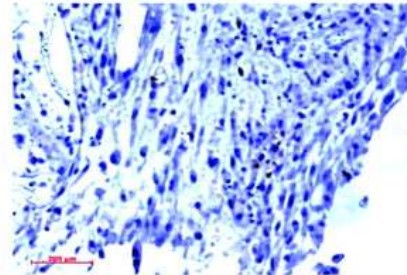
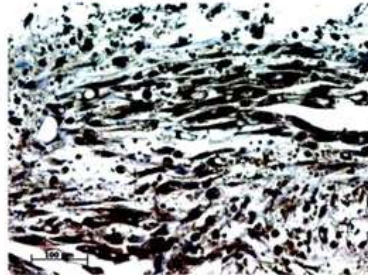
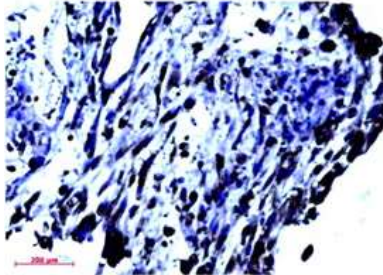


Tissue engineering 2

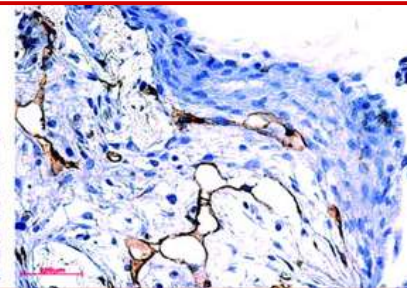
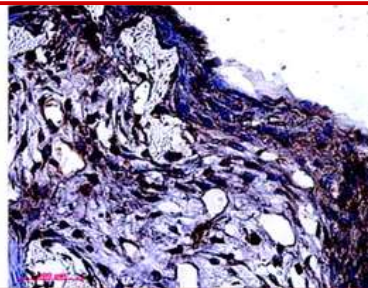
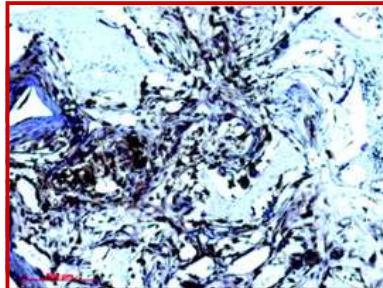
Cardiomyocytes



Cardiomyocytes
+
Endothelia



Cardiomyocytes
+
Endothelia
+
Fibroblasts



Troponin I

Sarcomeric actinin

CD 31

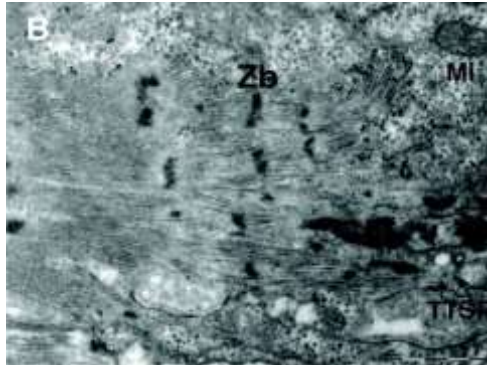
Markers of **cardiac muscle**

Markers of **cardiac endothelia**

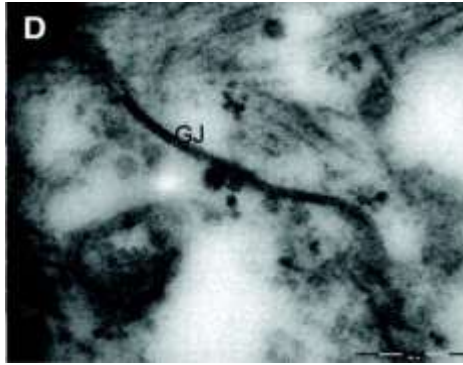
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Tissue engineering 3

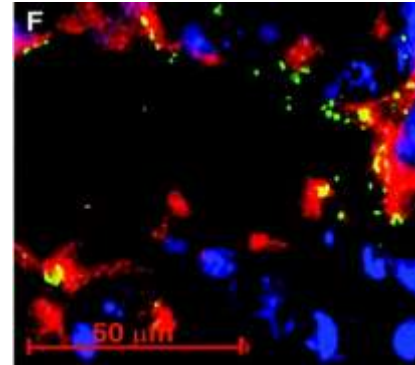
Ultrastructural characteristics of the engineered cardiac tissue



Myofibrils
Z bands
T tubules
Sarcoplasmic reticulum

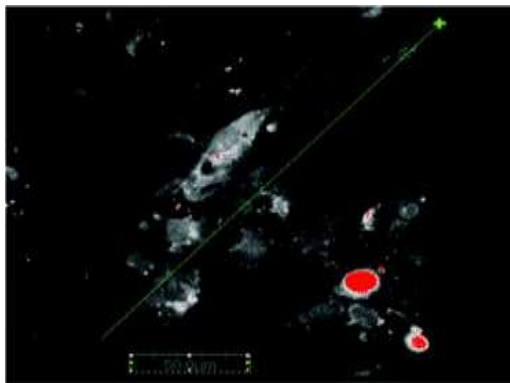


Gap junctions

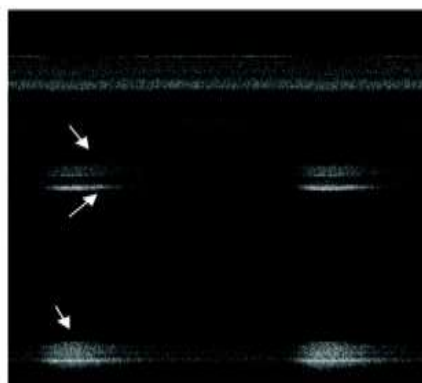


Conexin - Gap junctions
Troponin - cardiomyocytes

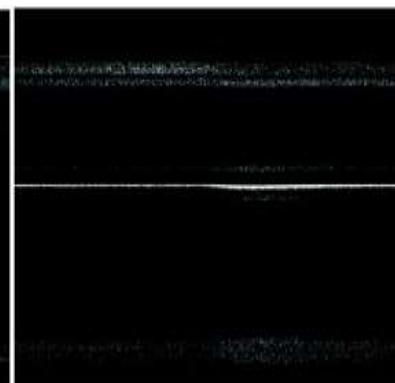
Engineered cardiac tissue propagates synchronous surges of Ca^{2+}



Laser scanning cofocal microscopy



Baseline



1-Heptanol
(Gap junction uncoupler)

Methodologies to study cells and tissues 1

Making it observable



Stabilization of the structure

Fixation

Making the objects smaller -
transmissible for the light

Embedding + Sectioning

Making the structures well visible

„Staining“

Enlargement



Utility of Microscopes



Light (optical) microscopes

(interaction of photons with a matter)

Resolution 0.1 μm

- Equipped for visible light only
- Equipped for fluorescence
- Confocal laser scanning



Electron microscopes

(interaction of electrons with a matter)

Resolution 0.1 nm (in practice 1 nm)

- Transmission
- Scanning



Methodologies to study cells and tissues 2

Fixation (denaturation)

- **Organic solvents** (EtOH, MetOH, Aceton,...)
- **Aldehydes** (form-, paraform-, glutar-aldehyde, ...)
- **Organic acids** (acetic, picric, ...)
- **Heavy metals** (salts of mercury, chrome, osmium, ...)

Embedding + Sectioning

- **Paraffine wax**
- **Celloidine** (=cellulose nitrate)
- **Durcupan** (synthetic polymer)
- **LR White** (synthetic polymer)
- **others**



„Staining“

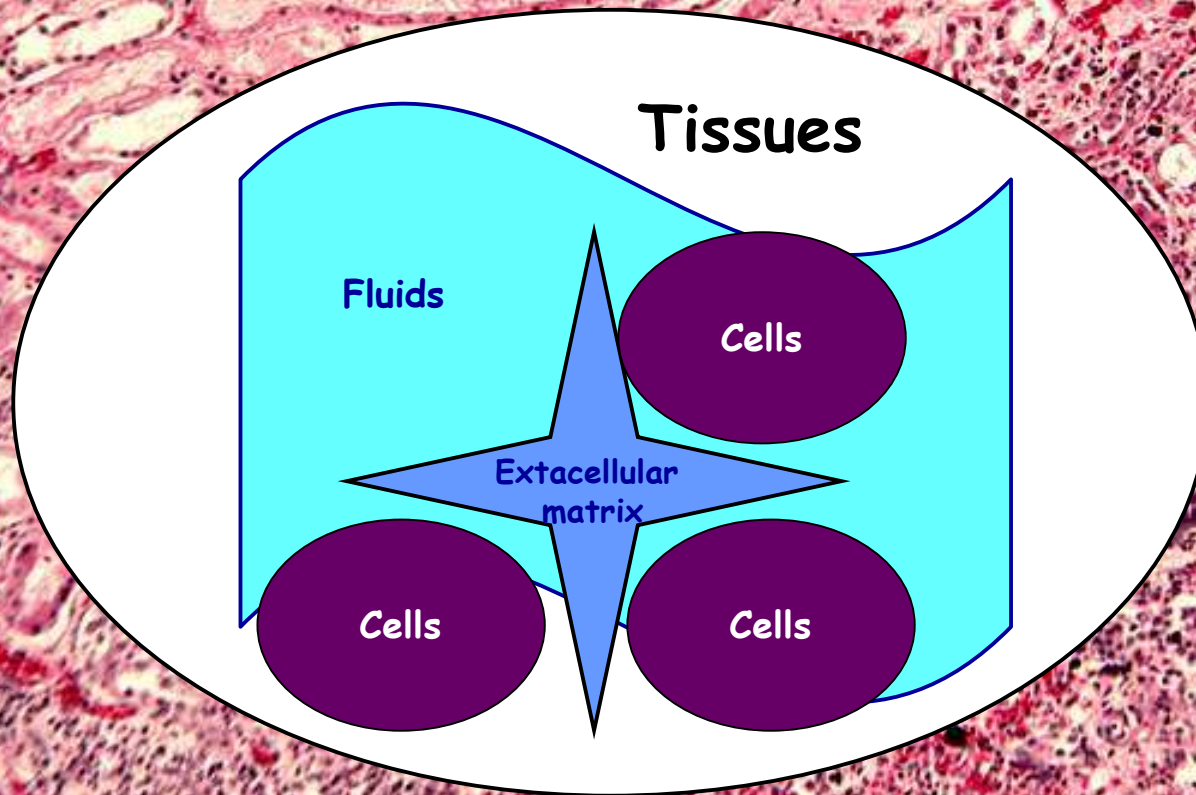
Chemical stains (H+E, Azan, van Gieson, ...)

Histochemical stains (for proteins/enzymes, sugars, lipids, ...)

Immunochemical visualization (labeled antibodies)

Heavy metals (for TEM - salts of uranium, lead, wolfram, ...)

Understanding the complex systems can only be built on understanding its components



Fluids

- Interstitial fluid
- Plasma (in blood)
- Lymph (in lymph vessels)
- Cerebrospinal fluid
- Intracellular fluid (cytosol)

The cells make it all !

Living organisms are composed of cells

Long way to this discovery:



Robert Hooke
1665

He for the first time observed
the structure of cork - cell



Antonie van Leeuwenhoek
1678

He for the first time observed
microscopical organisms
(bacteria, protozoa)



Matthias Schleiden

1839



Theodor Schwann

All organisms are composed
of one or more cells



Rudolph Virchow
1855

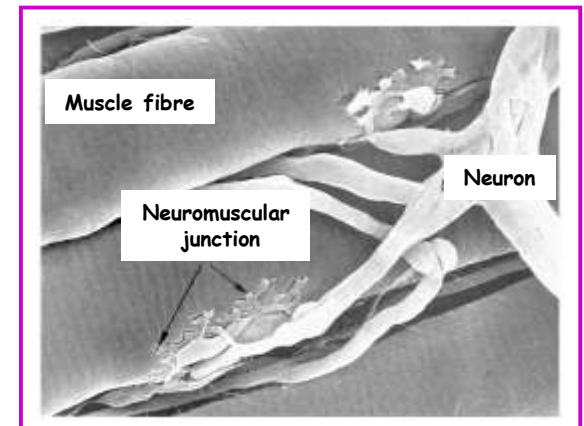
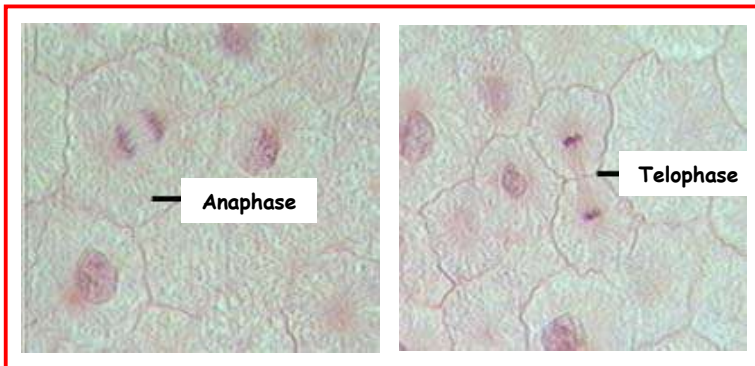
Cell can develop only from preexisting cells
„Omnis cellula e cellula”

Cell is unifying theme/element of life

(cells are very similar among each other: small + specialized functions)

Current cell theory - 6 principles on which it is built

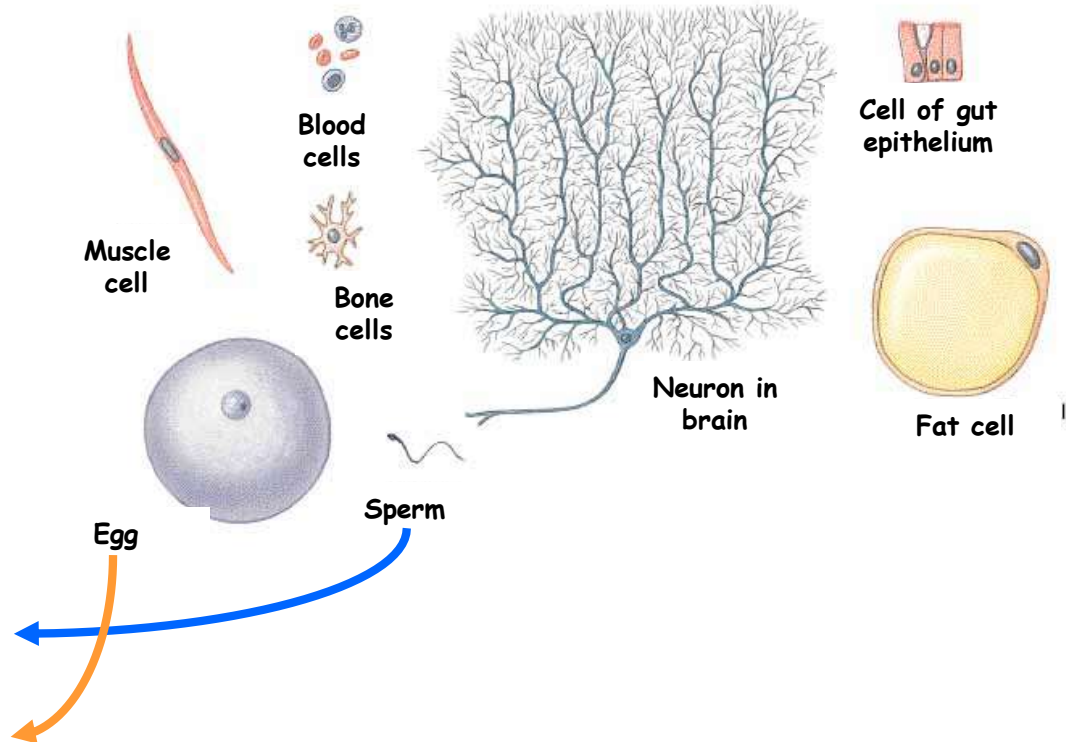
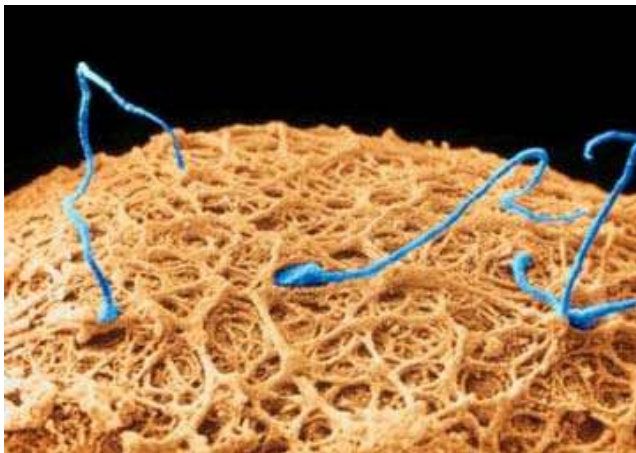
- Cell is the smallest structural and functional unit capable of life functions
- Function of each cell is given by its specific structure
- Cells are building units of all multicellular organisms - cells are responsible for all processes taking place in the organisms
- Structure and function of all organisms is based on structural and functional properties of cells from which they are composed
- All new cells originate from preexisting cells
- Thanks to the continuity of life on the Earth, all cells are in principle the same (universal genetic code and its expression)



Despite of their common scheme,
structural and functional
diversity is a typical feature
of all eukaryotic cell types

The cells of human tissues and organs are also structurally and functionally very diverse

Such diversity is critical for an ability of cells to serve various functions in human body

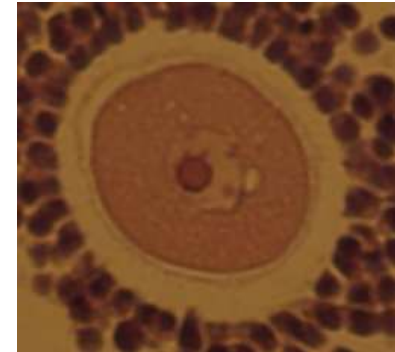
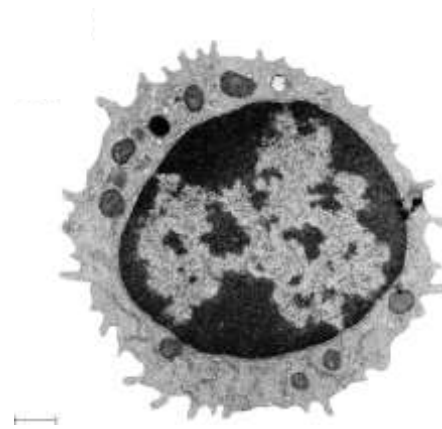
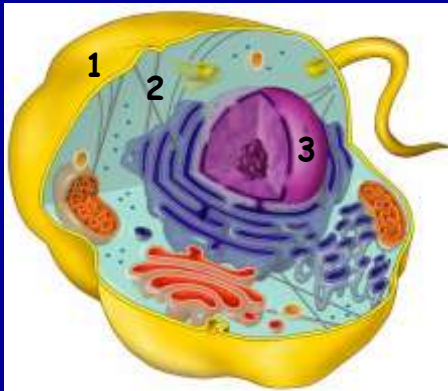


No cell is exactly like all others,
but cells do have many common
structural and functional features.

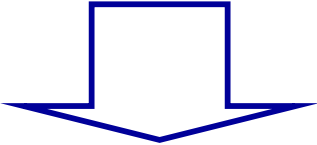
Keep in mind that not all cells contain all the structures we will discuss !

All cells have 3 major parts:

1. PLASMA MEMBRANE
2. CYTOPLASM
3. NUCLEUS (eukaryotic)

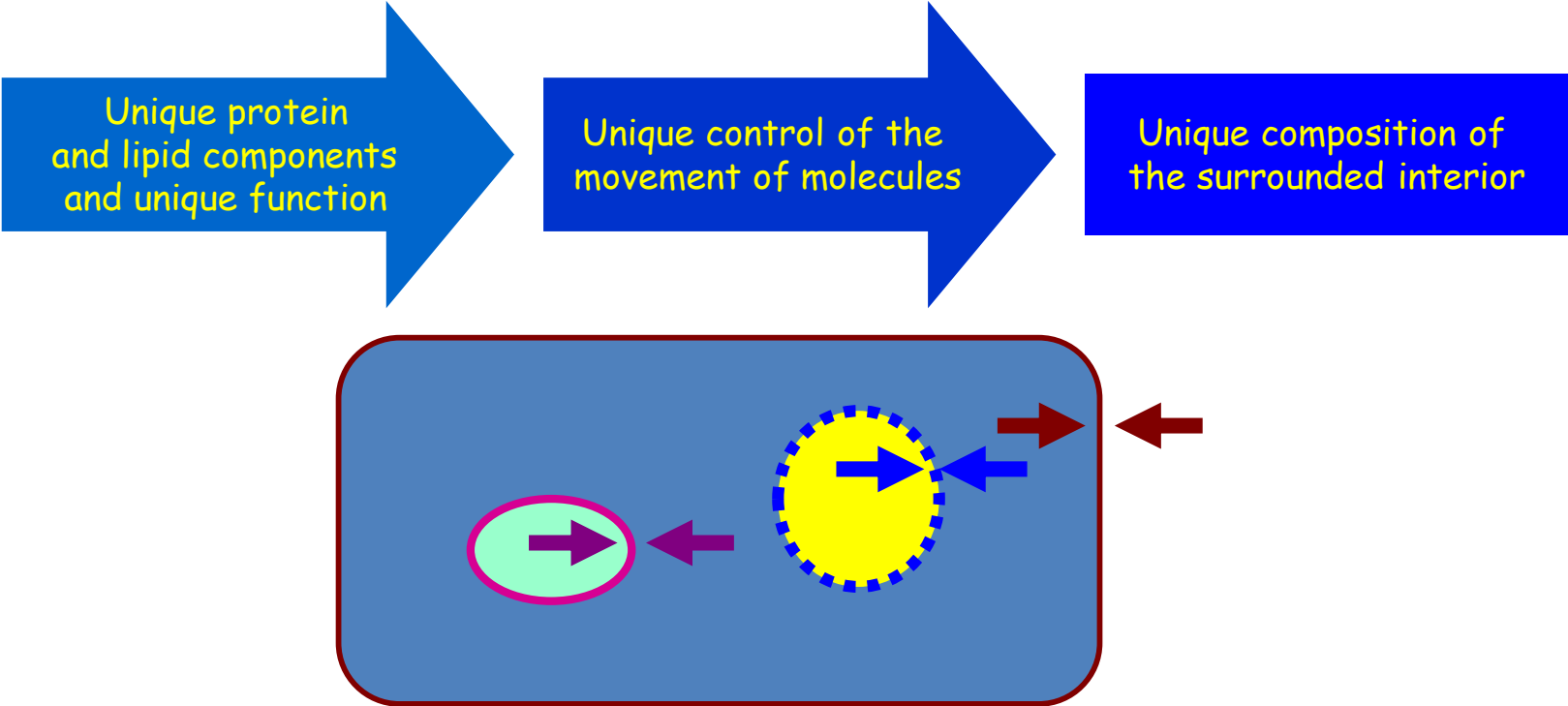


Cellular organization is based on COMPARTMENTALIZATION



Specialized functions can be carried out in different locations

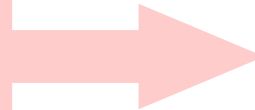
Membranes make up boundaries between the compartments



Compartments & Membranes

Many small compartments are better

More membrane surface
per volume surrounded



More space for:

- regulation
- nutrients exchange
- waste removal

Surface area is proportional to the *square* (r^2) of its diameter.
Volume is proportional to the *cube* (r^3) of its diameter.

Amplification X Reduction
of selected compartments

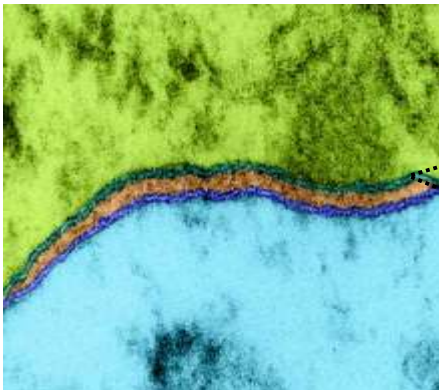


Specialization of cells
for different functions

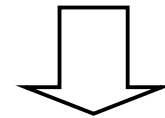
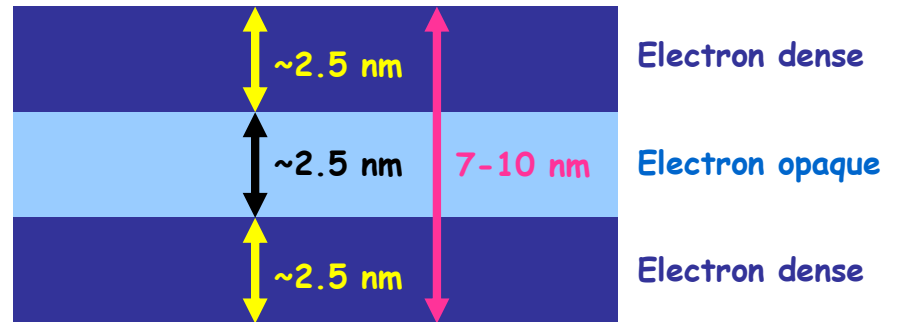
Cell differentiation

Rough ER in secretory cells
Mitochondria in cardiac muscle cells

Biological membrane structure 1



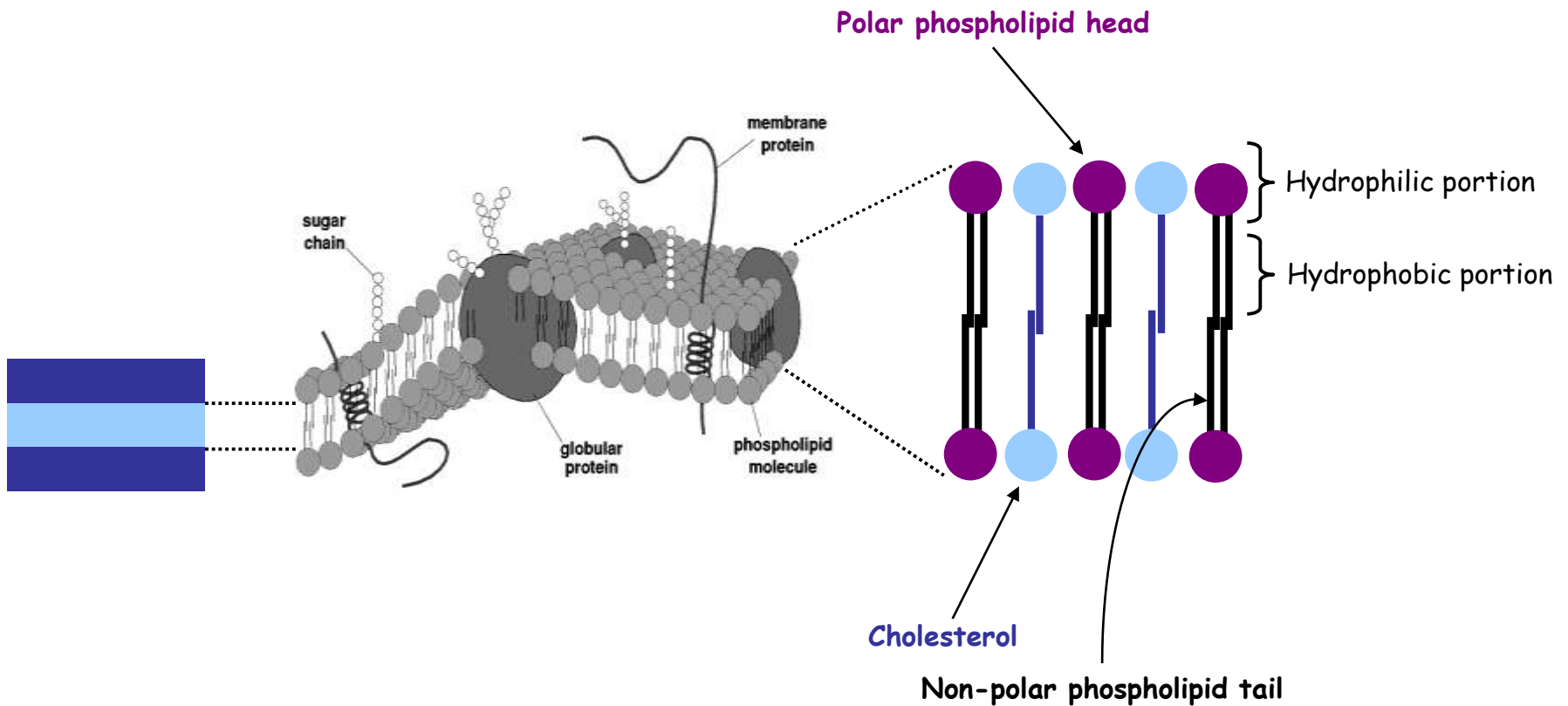
Cell membranes seen
in electron microscope
(pseudocolored)



Unit membrane
common to all membranes

Biological membrane structure 2

Fluid mosaic - A bilayer of lipids with mobile globular proteins



Membrane structure 3

Membrane lipids

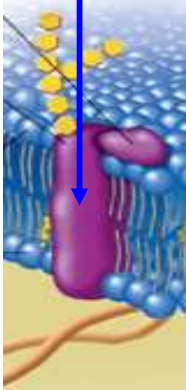
Make up 90-99% of molecules in membrane (in numbers).

- **Phospholipids** - 75% of lipids
- **Cholesterol** - 20%
- **Glycolipids** - 5% - only on cytoplasmic membrane - *GLYCOCALYX*

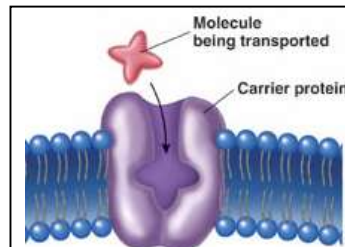
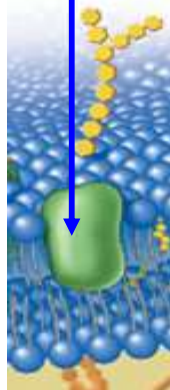
Membrane proteins

Constitute 1-10% of total molecules but 50% of the weight because of their larger size.

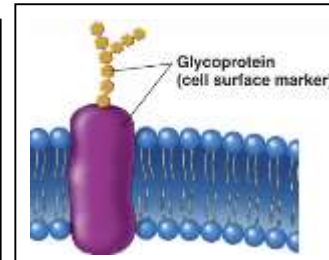
Integral



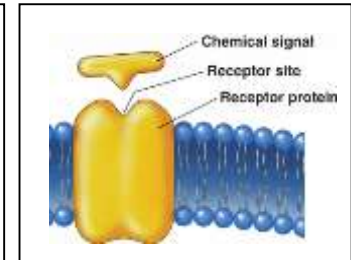
Peripheral



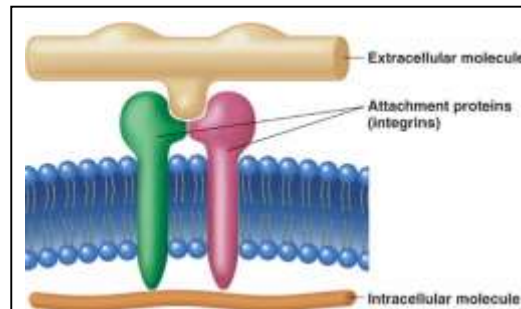
Transport



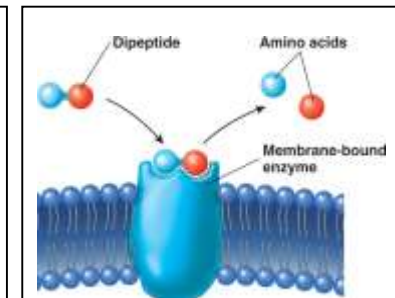
Cell identity



Sensing signals



Cell adhesion



Enzymatic activity

Organelles

Specialized internal structures with specialized functions

Membranous

- Nucleus
- Endoplasmic reticulum
- Golgi apparatus
- Lysosomes
- Endosomes
- Peroxisomes
- Mitochondria

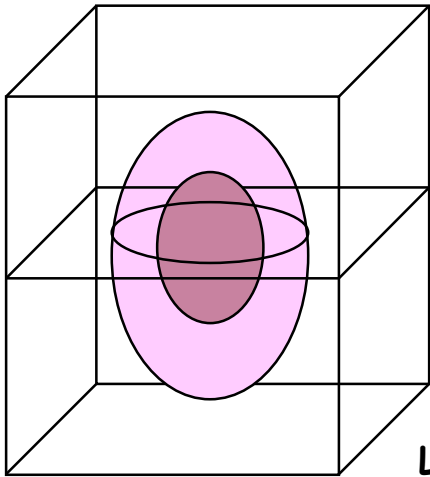
Non-membranous

- Ribosomes
- Centrosomes
- Centrioles
- Basal bodies

Related to specific structure and function of the cell
e.g., much energy needed → many mitochondria

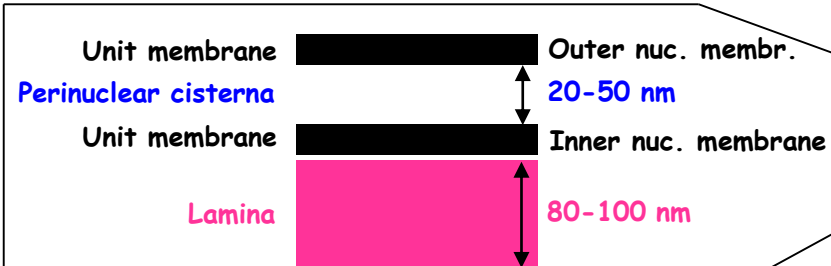
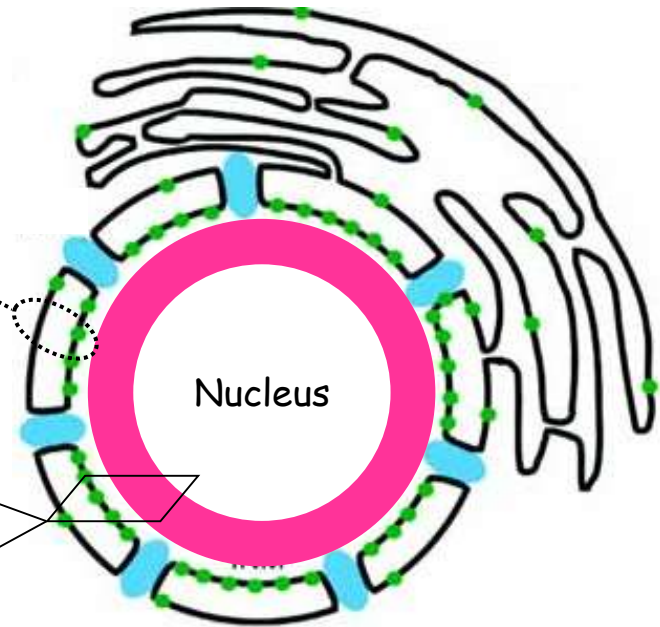
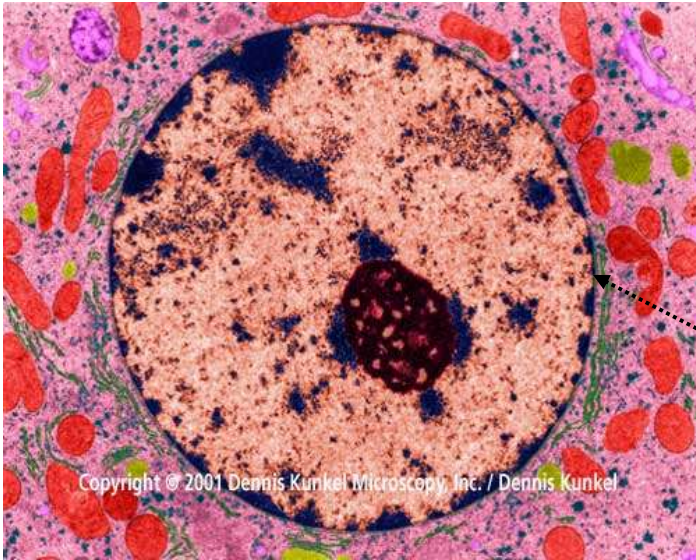
Nucleus 1

membrane-bounded organel



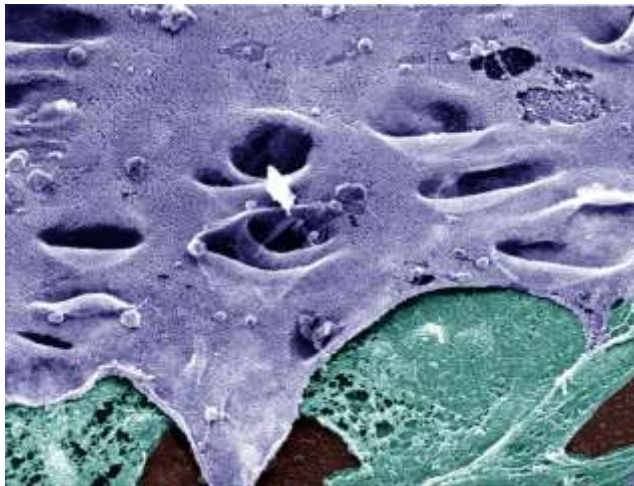
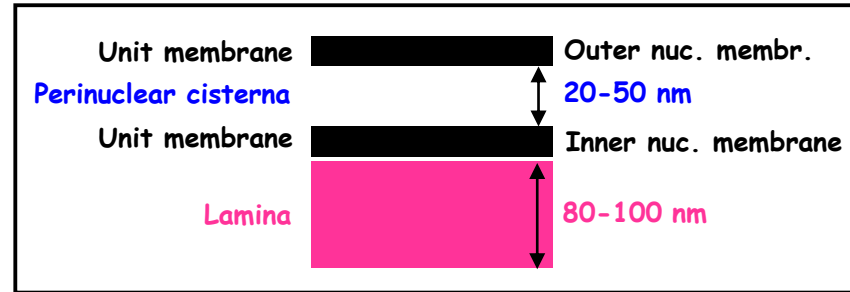
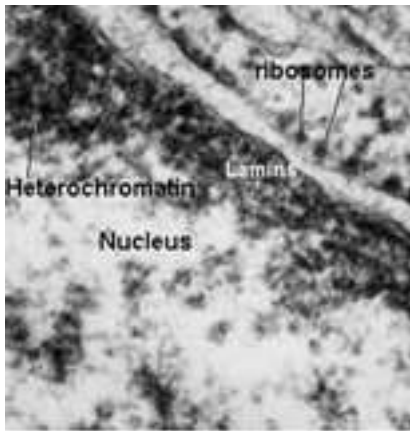
Liver cell nucleus

- Mostly:
- Spherical (5-10 μm) (lobular, twisted, disk-shaped,...)
 - Located centrally
 - One per cell (osteoclast more, erythrocyte none)



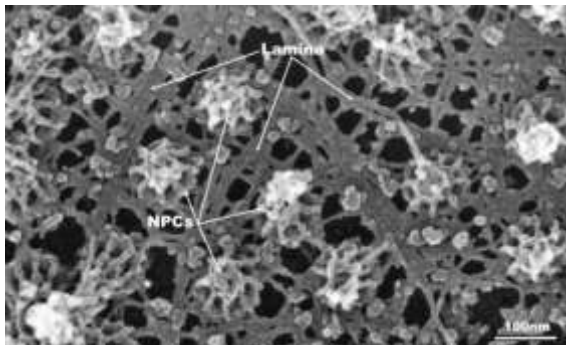
Nucleus 2

Continuation on nuclear membrane



Lamins:

- Intermediate filament proteins (A, B, C)
- Form meshwork inside of INM, some extend into nucleoplasm
- Nuclear strength and architecture
- Anchorage sites for chromatin
- DNA replication and mRNA transcription
- Involved in apoptosis



Laminopathies

- Human diseases (at least 13 known)
- Mutations in lamin genes (almost 200 mutations known)
- Deregulated gene expression
- Premature aging

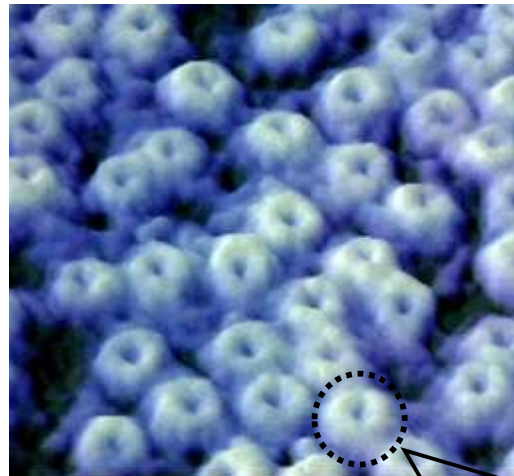
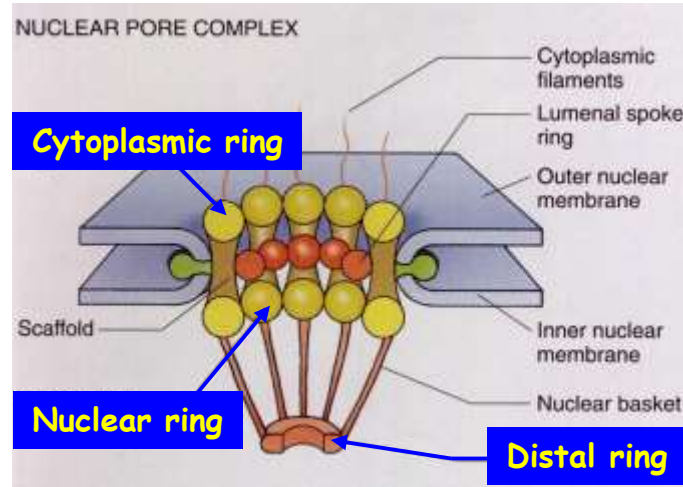
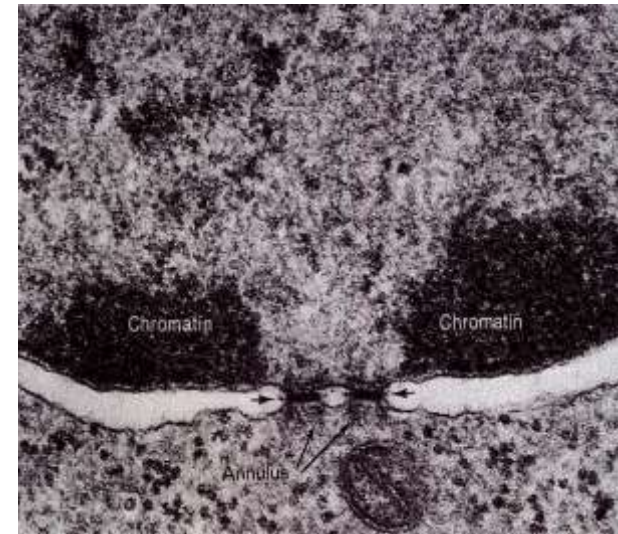


Hutchinson-Gilford progeria

Rare - 1-4 per 8 million of newborns
Missense mutation in A-type lamin

Nucleus 3

Nuclear pore complex



Diameter ~ 100 - 125 nm

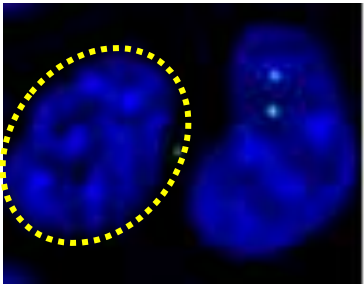
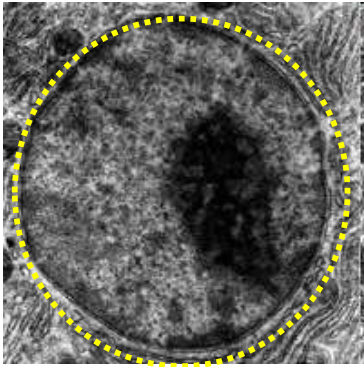
Three rings (8 subunits each)

Inner filamentous basket

Transport via nuclear pores (Nucleocytoplasmic shuttling)

- Proteins, RNAs, ribosome subunits
- Bidirectional
- Needs nuclear localization/export signals
- Helped by importins/exportins
- Regulated by Ran GTPases

Nucleus 4 Chromatin



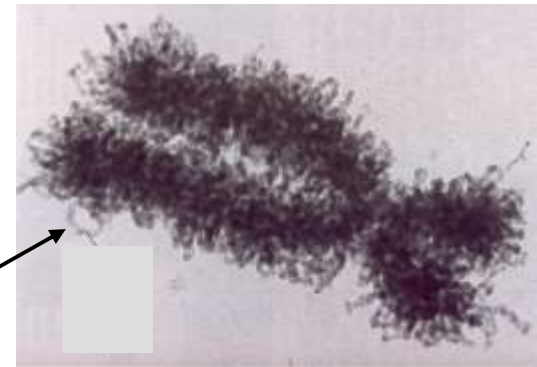
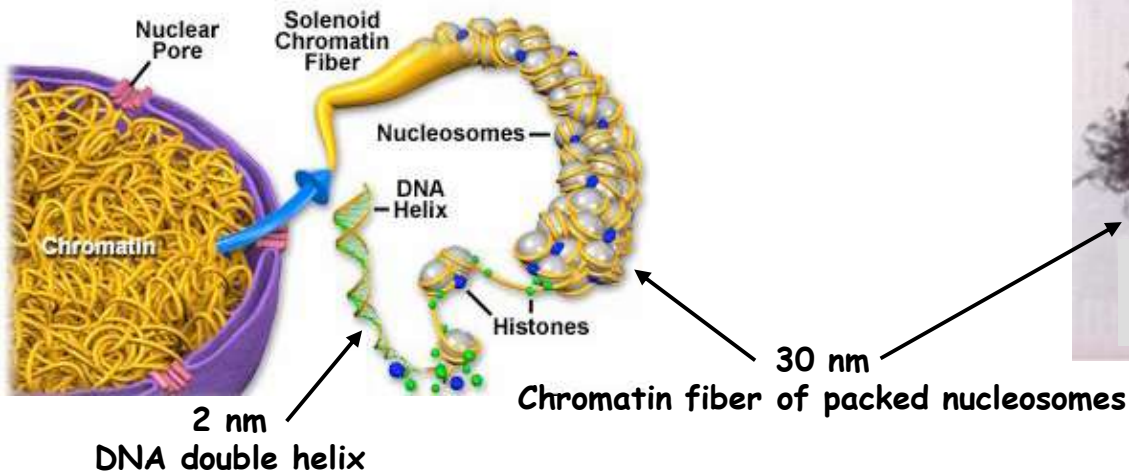
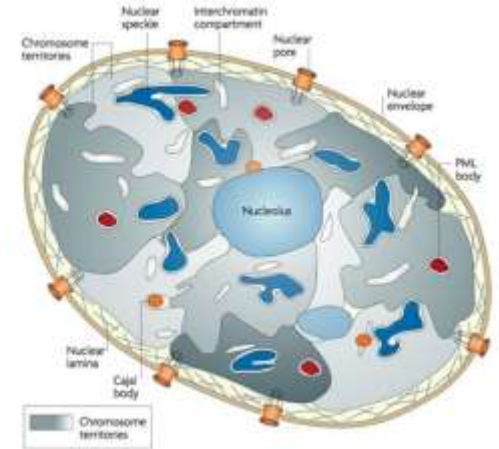
Interphase nucleus

Heterochromatin

Feulgen positive - dark in light microscope
Dark/dense granular in TEM
Transcriptionally inactive

Euchromatin

Invisible in light microscope
Relaxed uncoiled chromosomes
Transcriptionally active

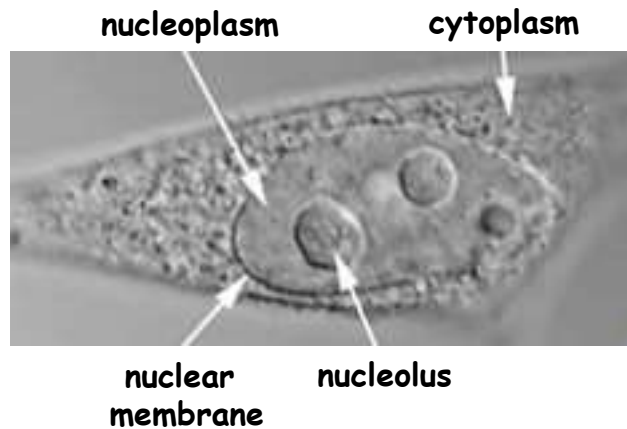
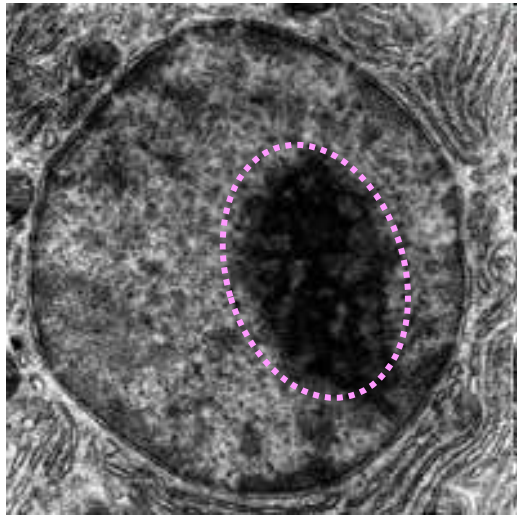


Nucleus 5 Nucleolus

non-membrane-bounded structure

Main functions

Synthesis of rRNA
Assembly of ribosomes



Pars fibrosa
Primary transcripts of rRNA

Pars granulosa
Assembly of ribosomes

Fibrillar centers
Non-transcribed DNA

Nucleolar-organizing regions of DNA

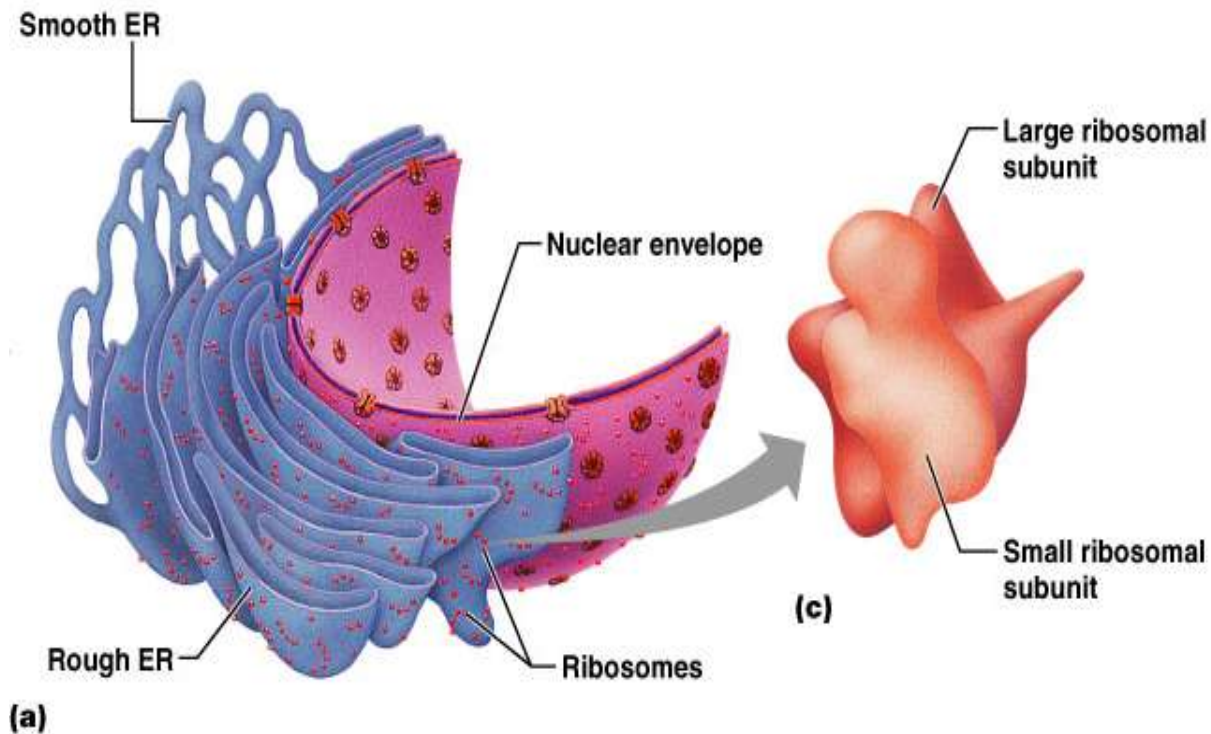
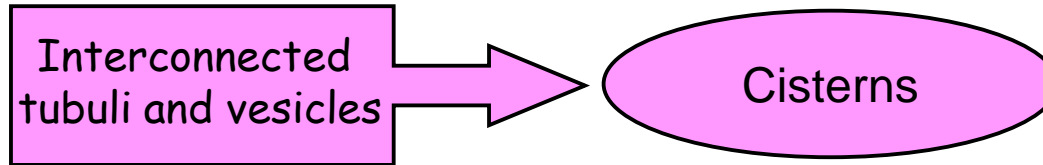
on five chromosomes in human cells
(chrs. 13, 14, 15, 21, 22)

Endoplasmic reticulum 1

„within cell“

„net“

Majority of the membrane within cells.



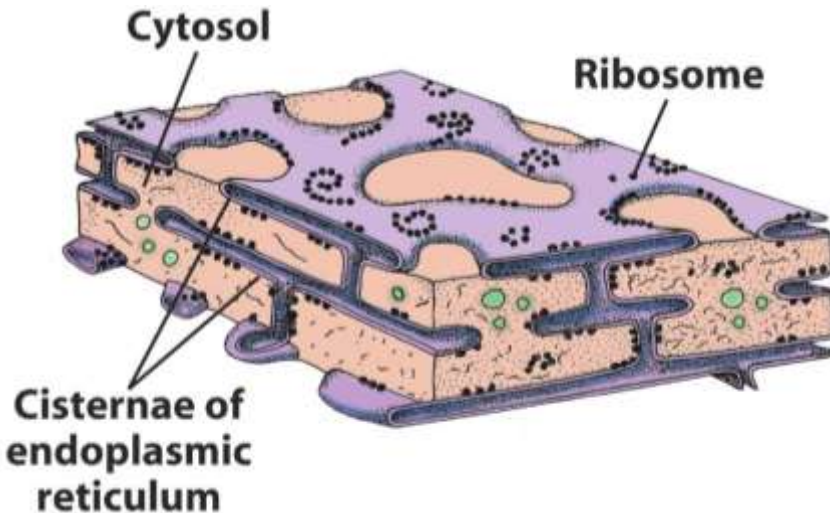
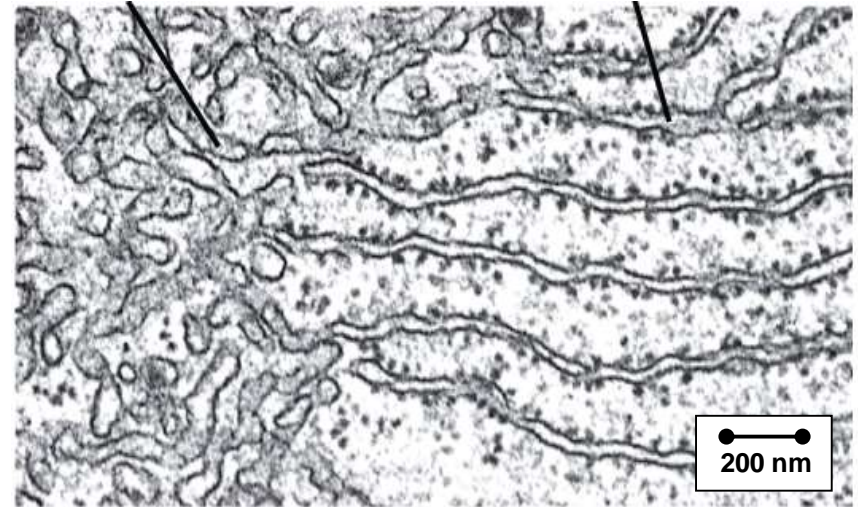
Endoplasmic reticulum 2

NO attached ribosomes → **No** protein-synthesis functions!
Manufactures phospholipids and cholesterol

- **Liver** - lipid and cholesterol metabolism, breakdown of glycogen and, along with the kidneys, detoxification of drugs
- **Testes** - synthesis of steroid-based hormones (testosterone)
- **Intestinal cells** - absorption, synthesis, and transport of lipids
- **Skeletal and cardiac muscle** - storage and release of calcium (sarcoplasmic reticulum)

Smooth ER

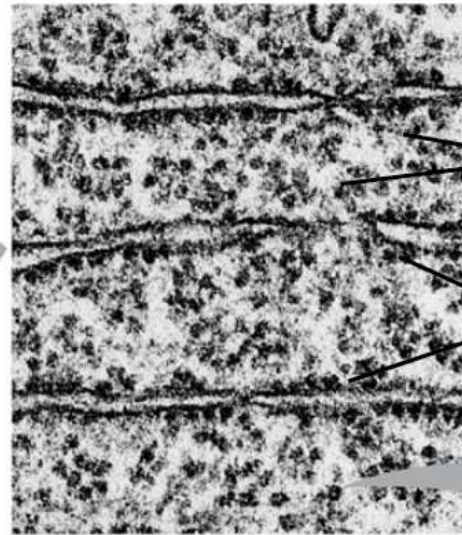
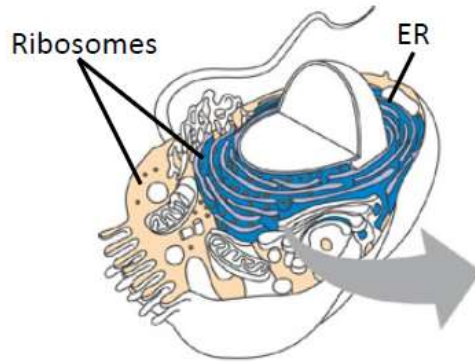
Rough ER



External surface **has ribosomes attached**

- Manufactures all secreted proteins
- Synthesizes integral membrane proteins
- Modifies proteins

Ribosomes



Endoplasmic reticulum (ER)

Free ribosomes

Bound ribosomes

0.5 μm

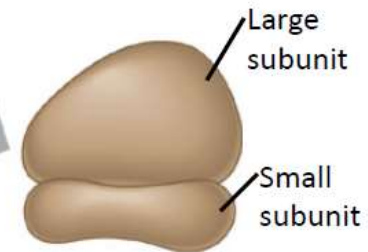
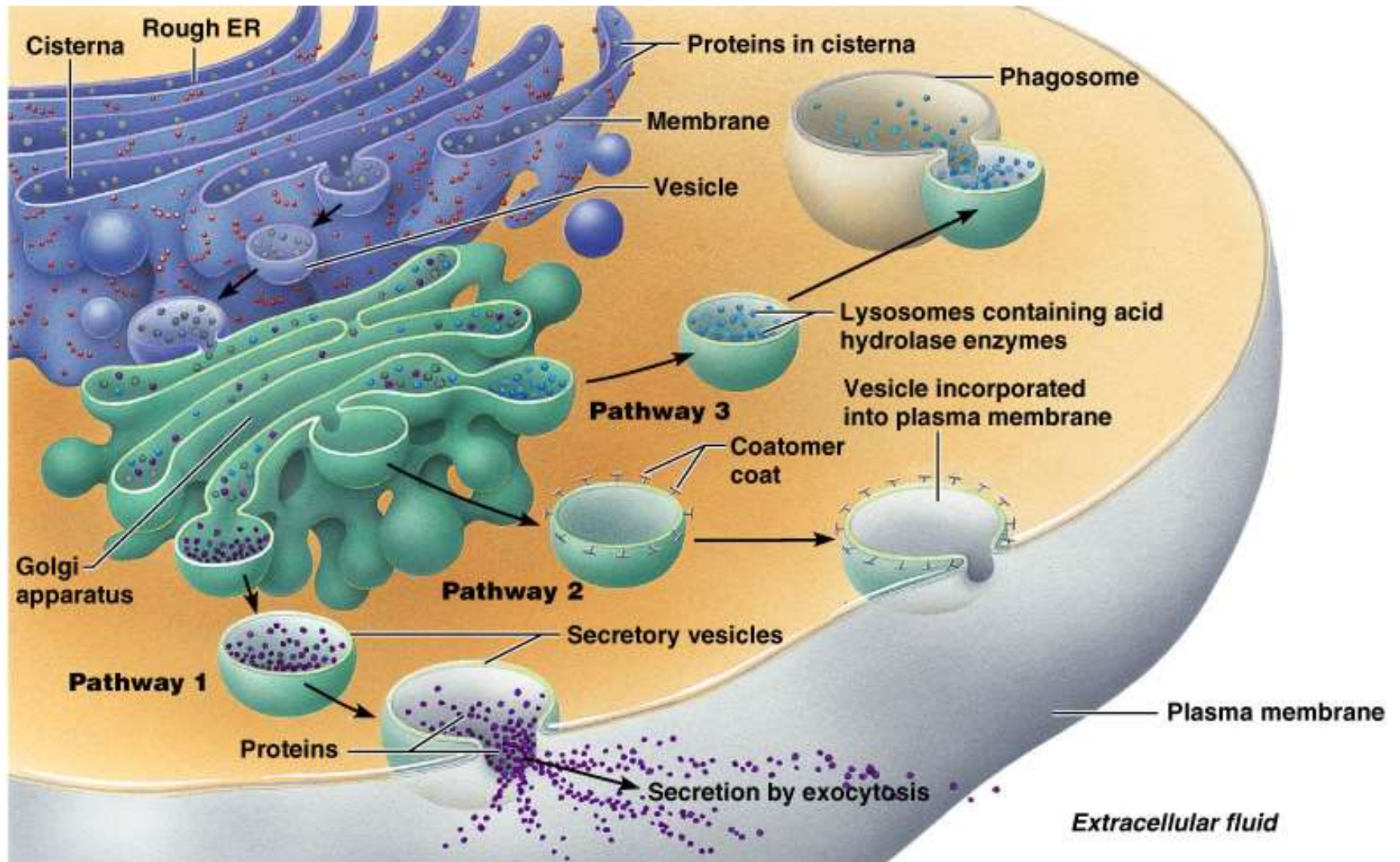
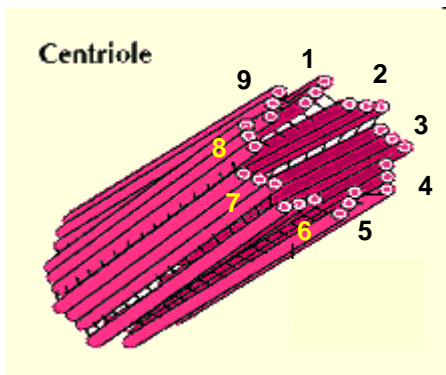
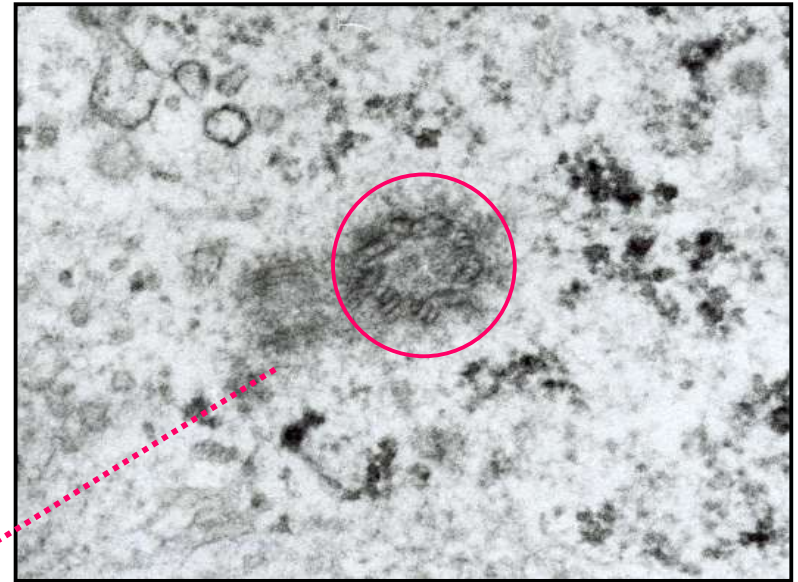
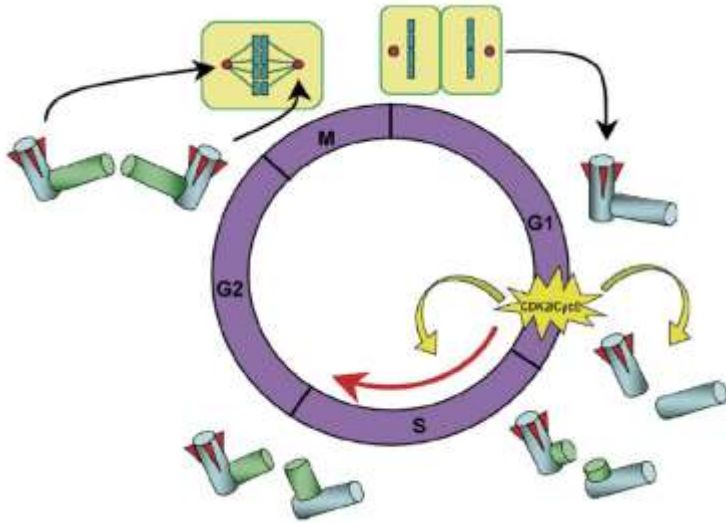


Diagram of a ribosome

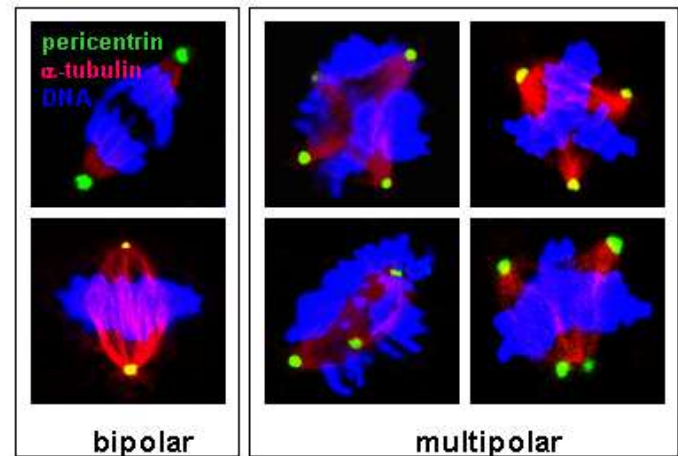
Golgi apparatus - Transgolgi pathway



Centrosome



Diameter - $0.2 \mu\text{m}$
Length - $0.5 \mu\text{m}$



Homework

Independent study:

Mitochondria

Endosomes

Lysosomes

Cytoskeleton

Histology lectures

Key elements of the microscopic structure of tissues and organs and their relevance to the function

Very latest discoveries in the field of tissue structure and maintenance and their relevance to the disease development and therapy

Thank you for your attention !

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Building A1 - 1st floor