Histology and Embryology

Lecturers:

Aleš Hampl, D.V.M., Ph.D., Assoc. Prof., Head of the Dept.
Petr Vaňhara, RNDr., Ph.D., Assoc. Prof.

Brno, 2023

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?

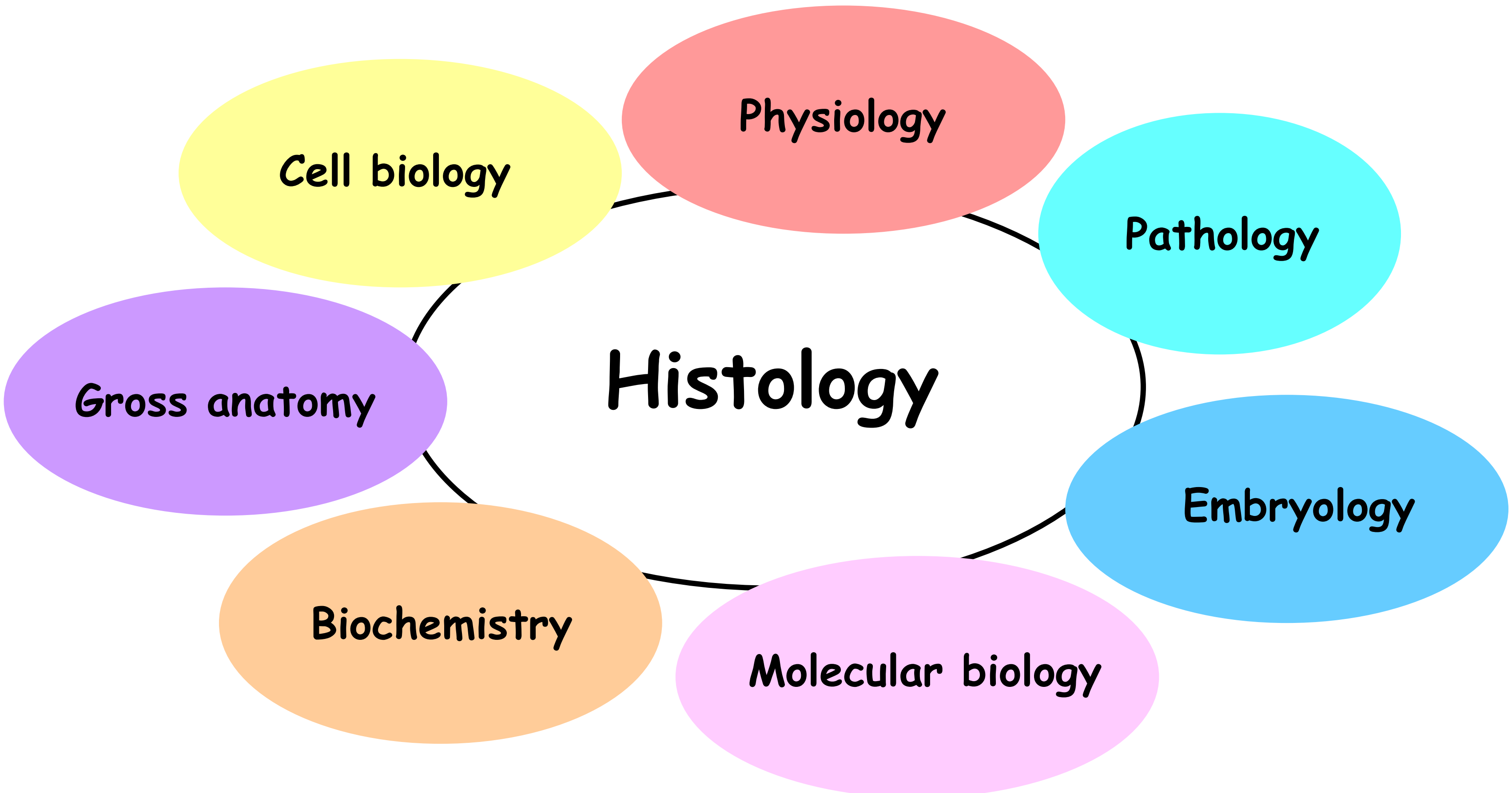
Microscopic anatomy

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 Germans primarily because they made great microscopes.

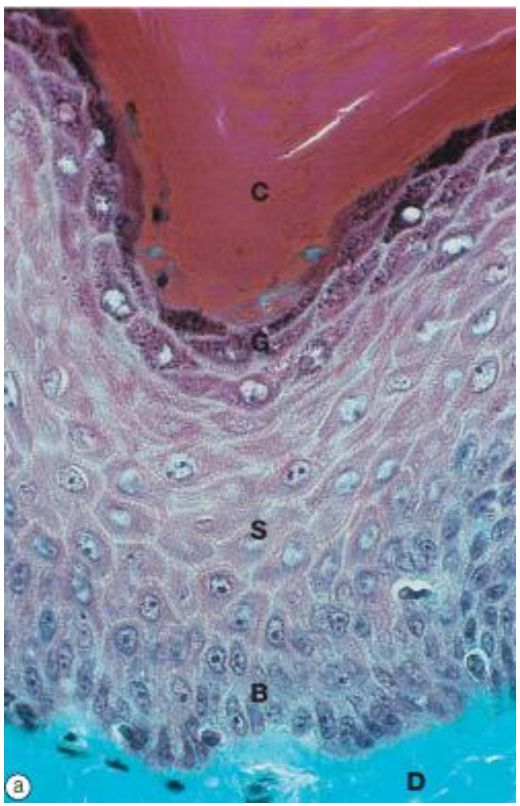
Eponymously theirs…..

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** undermicroscope



Malpighian layer of the skin

Term for basale and spinosum layers of epithelium

Malpighian corpuscles in the kidney & spleen

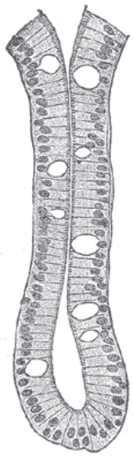
Johan Nathanael Lieberkuhn
1711 - 1756

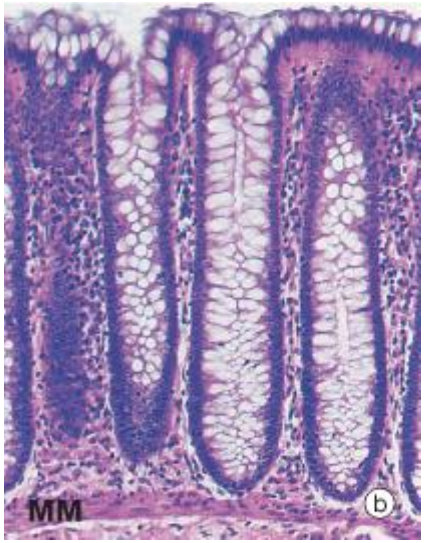
German anatomist and physician

Johann N. Lieberkuhn
(1711-1756)

- 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**





Jan Evangelista Purkyně
1787 - 1869

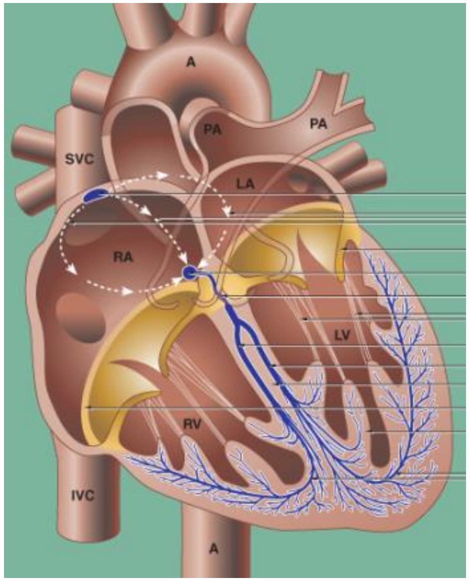
Bohemian physiologist

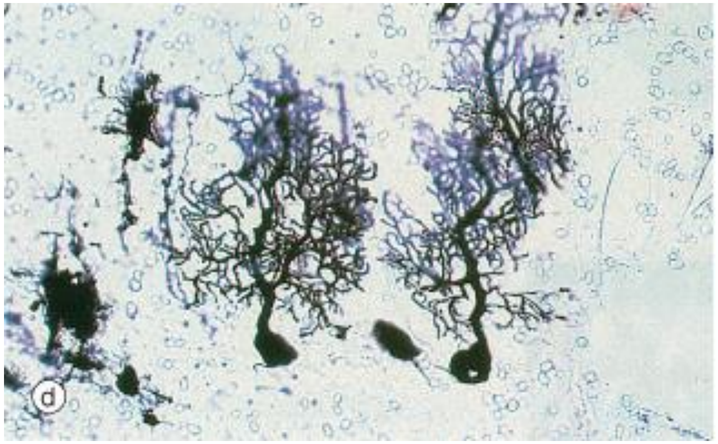
Schwann + Schleiden – 1839 – cell theory

- Pioneer in histological techniques
  First to use something like a **microtome**

- Introduced the term **plasma**

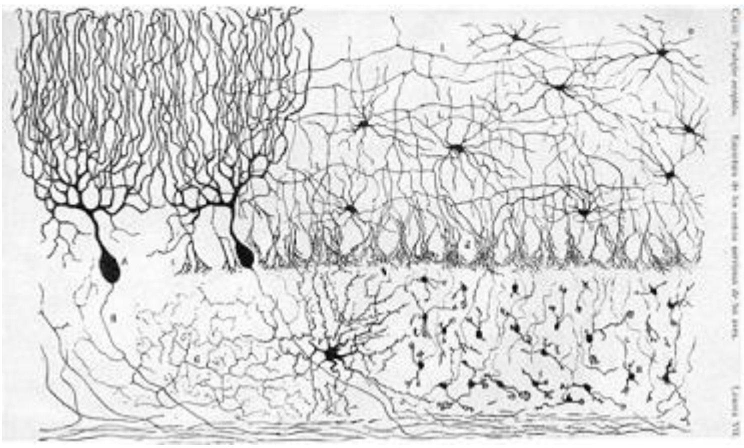
- Found **Purkinje fibers** of the heart
- Found **Purkinje cells** of the cerebellar cortex

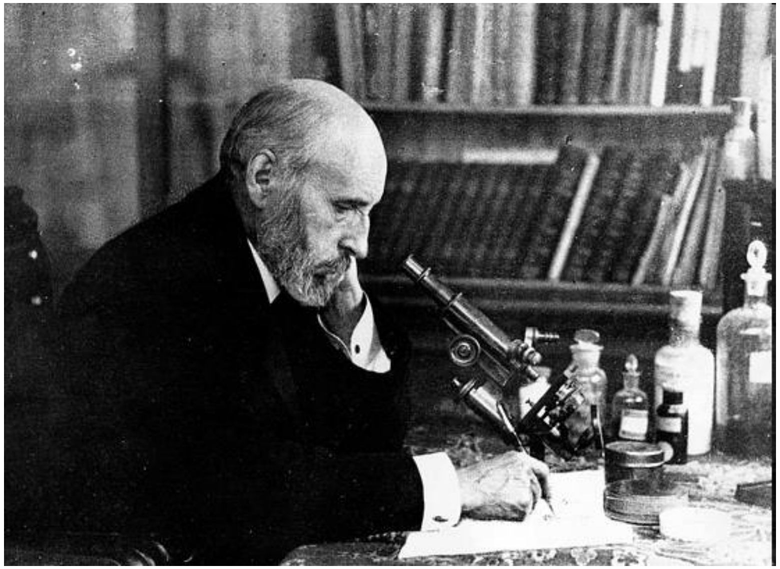




Santiago Ramón Y Cajal
1852 - 1934

Spanish physician and anatomist





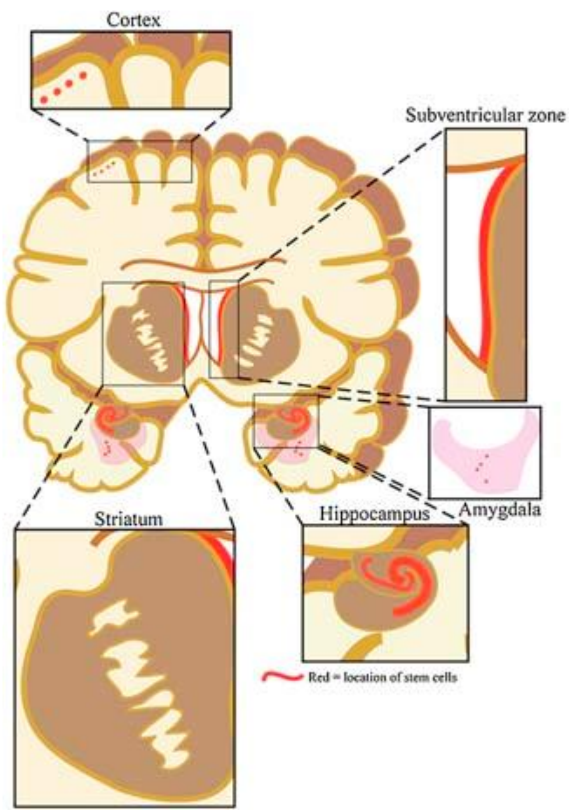
He established the neuron as the primary structural and functional unit of the nervous system.
Nobel Prize in 1906

"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 postantal and adult nervous system

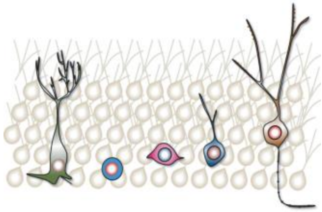


DEFINITELY IN:

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

POSSIBLY IN:

- Cortex ?
- Amygdala ?

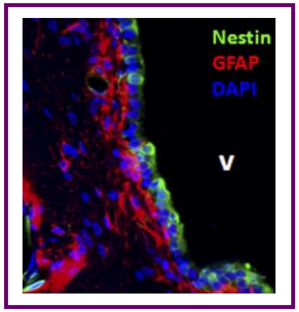


Our view on the organization of the central nervous system has been dramatically changed !!!

Many questions on NSC remain to be answered

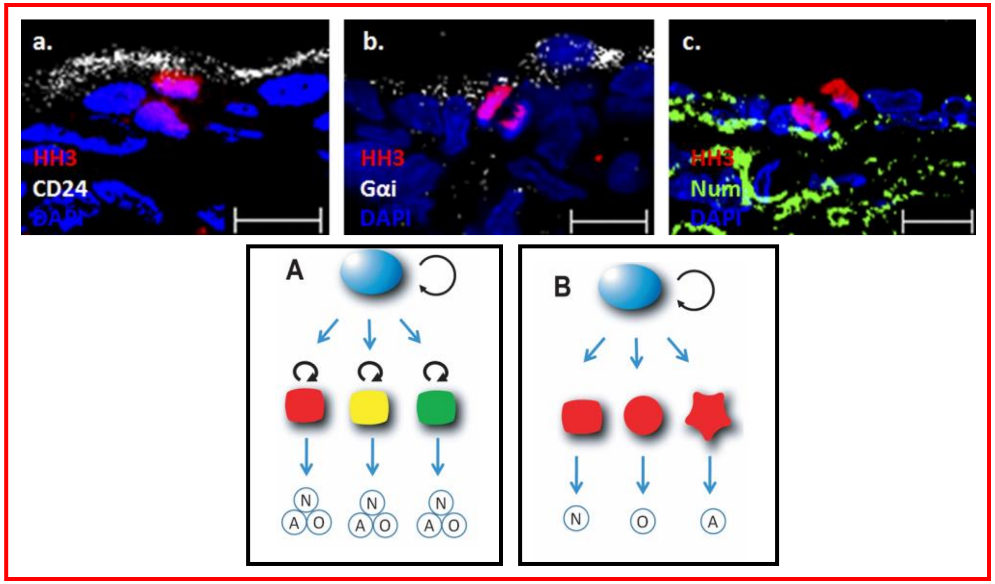
Combination of developmental biology, **histology**, cell biology, and molecular biology approaches is required.

- exact position in the tissue ?

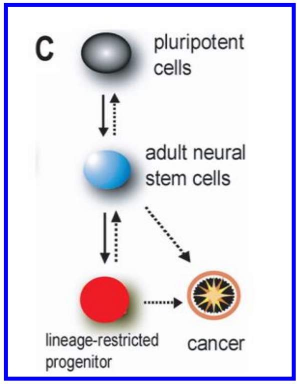


Gleason et al., Neuroscience, 2008.

- proliferative activity and migration ?
- developmental potential ?



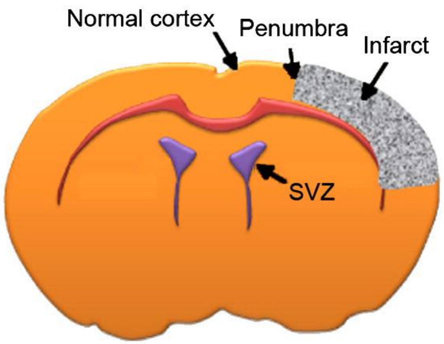
- involvement in disease developement ?



- 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

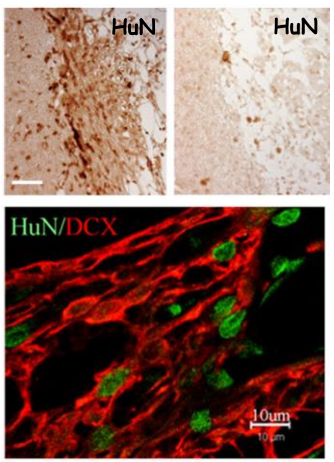
- experiment on rats
- MCAO – middle cerebral artery occlusion to induce infarction
- human neural precursors tranplanted into the site of infarction
- **histologically evaluated**

Jin et al., Brain Research, 2011.

Any practical use of such discovery ? (2)

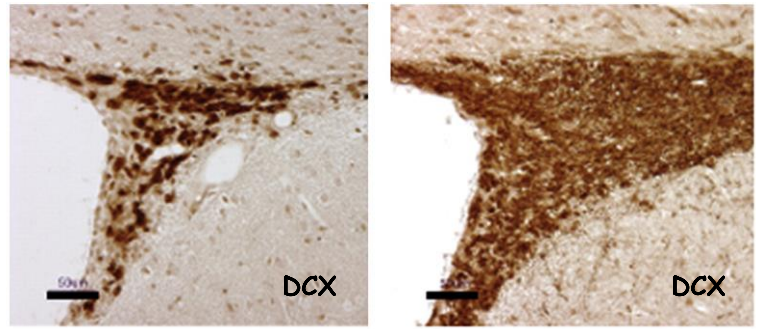
Transplanted human cells in the site of infarction

3 months 24 months



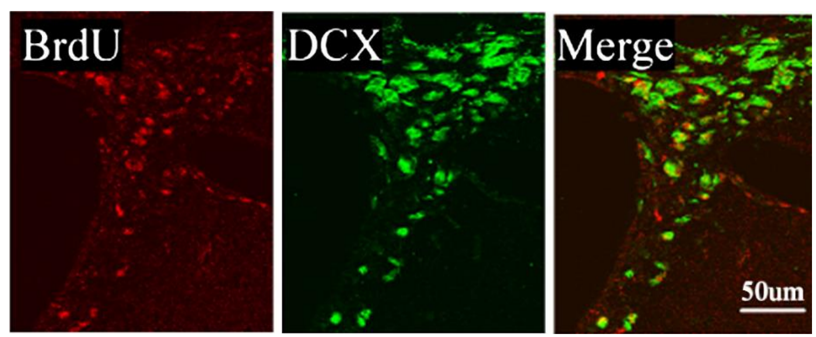
HuN – human nuclei
DCX – doublecortin (marker of early neuronal lineage)

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

Jin et al., Brain Research, 2011.

Tissue & Cell transplantation

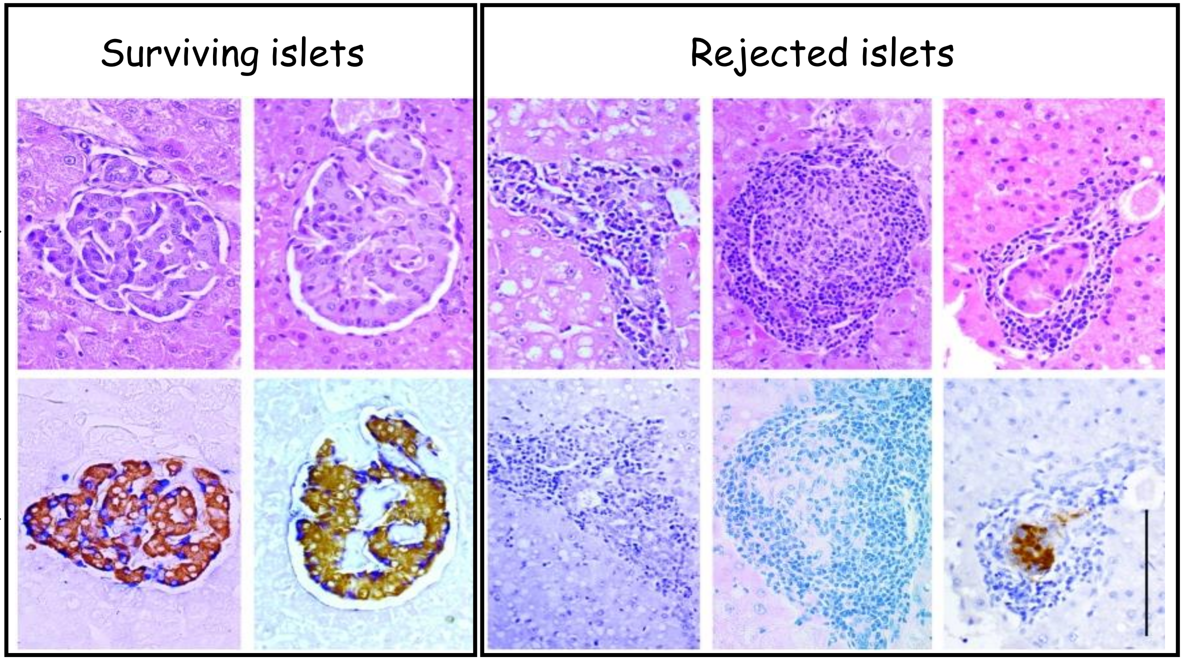
Damage to β cells of pancreatic islets of Langerhans → Dysregulated glucose metabolism → **Diabetes**

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



Badell et al., J. Clin. Invest., 2010

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

Egyptian mummy

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)

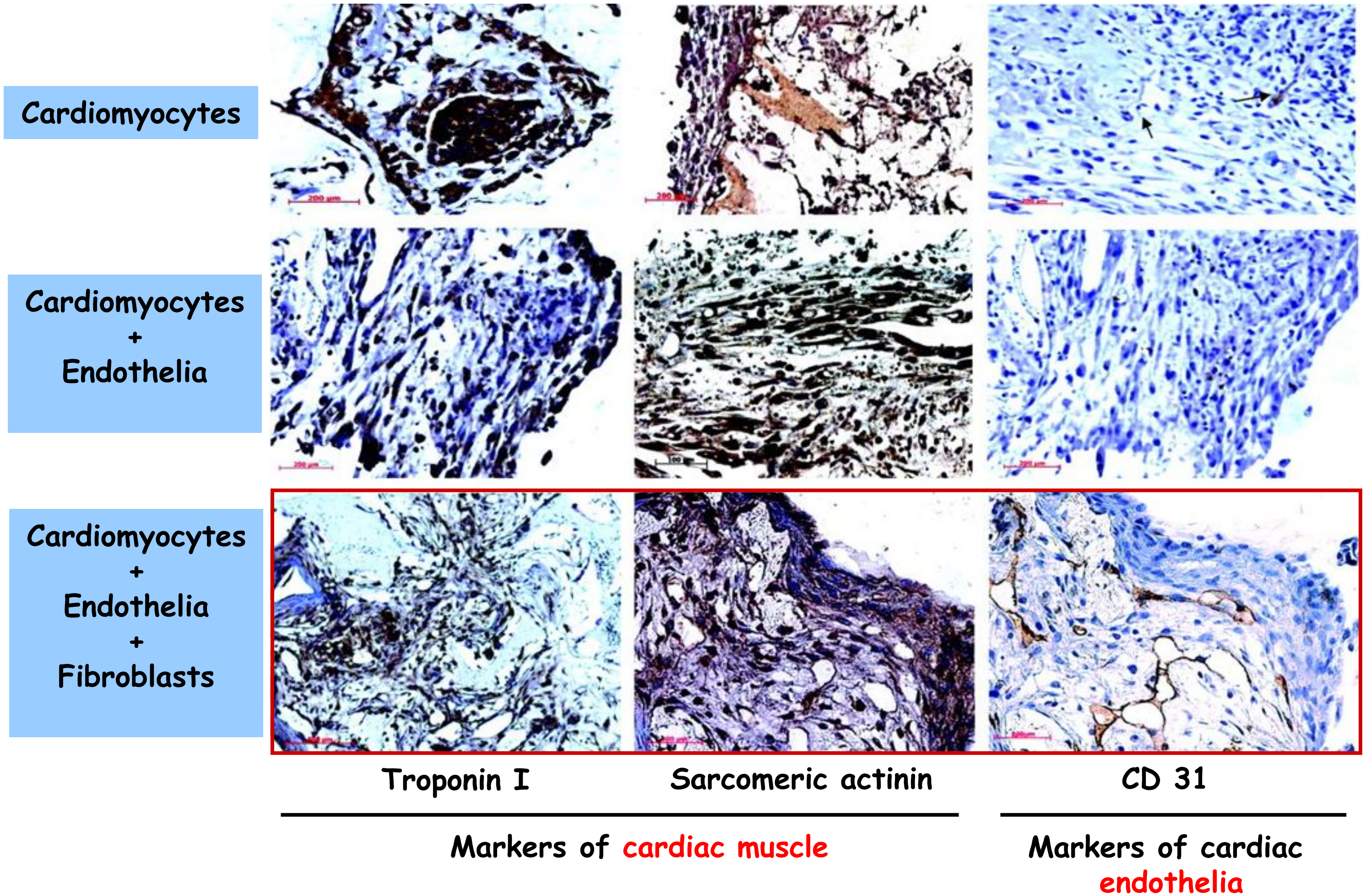
The first report of the construction of 3D vascularized human cardiac tissue that may have unique applications for studies of cardiac development, function, and tissue replacement therapy

- Human stem (naive) cells differentiated *in vitro* into cardiac muscle lineage
- Endothelial cells
- Fibroblasts
- 3-dimensional polymeric scaffold

Combined together →

Histological and functional analysis

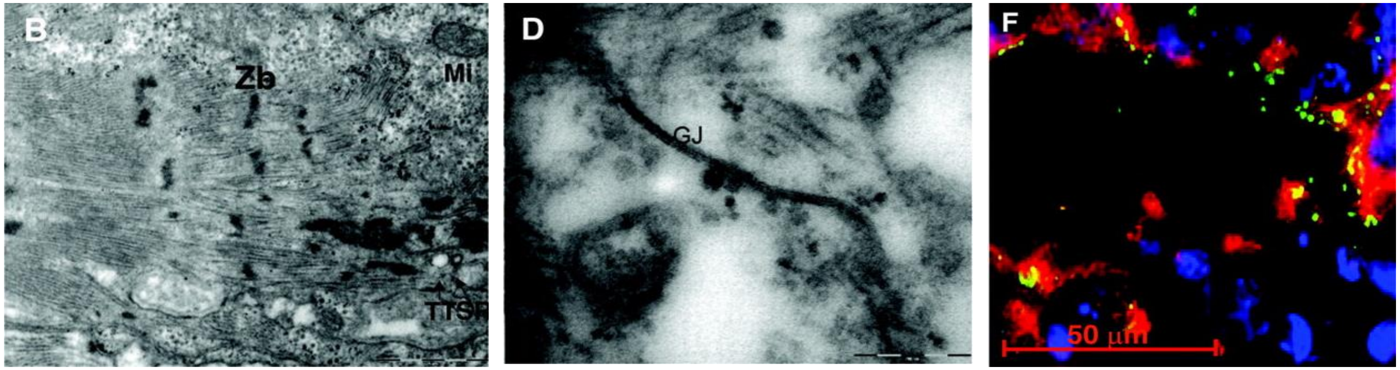
Tissue engineering 2



Caspi et al., Circulation Research, 2007

Tissue engineering 3

Ultrastructural characteristics of the engineered cardiac tissue

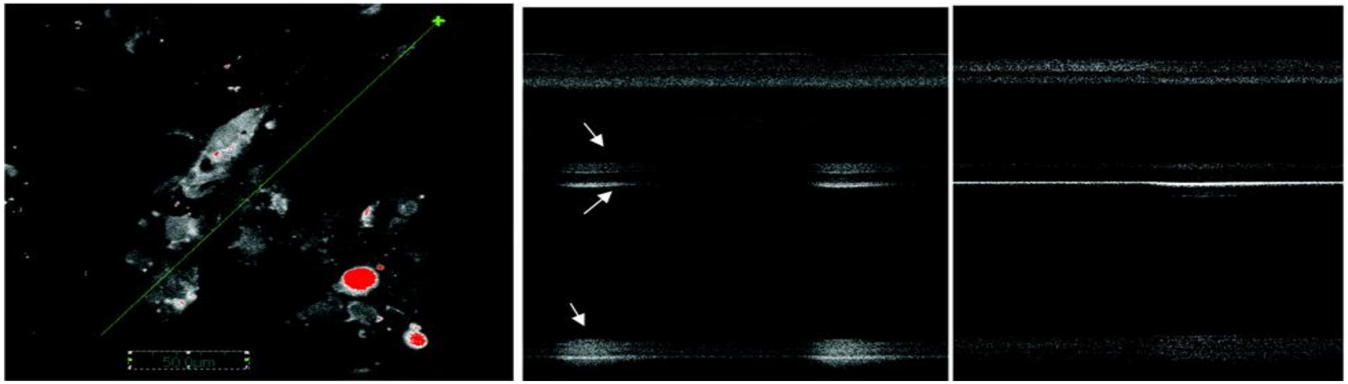


Myofibrils
Z bands
T tubules
Sarcoplasmic reticulum

Gap junctions

Conexin – Gap junctions
Troponin - cardimyocytes

Engineered cardiac tissue propagates synchronous surges of Ca2+



Laser scanning cofocal microscopy

Baseline

1-Heptanol
(Gap junctions uncoupler)

Caspi et al., Circulation Research, 2007

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

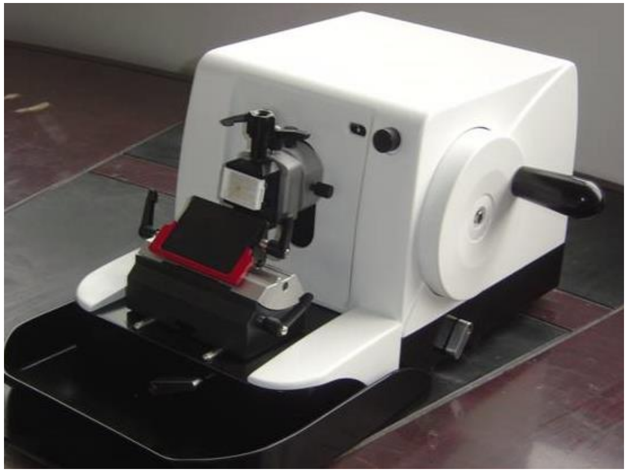
Methodologies to study cells and tissues 2

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“

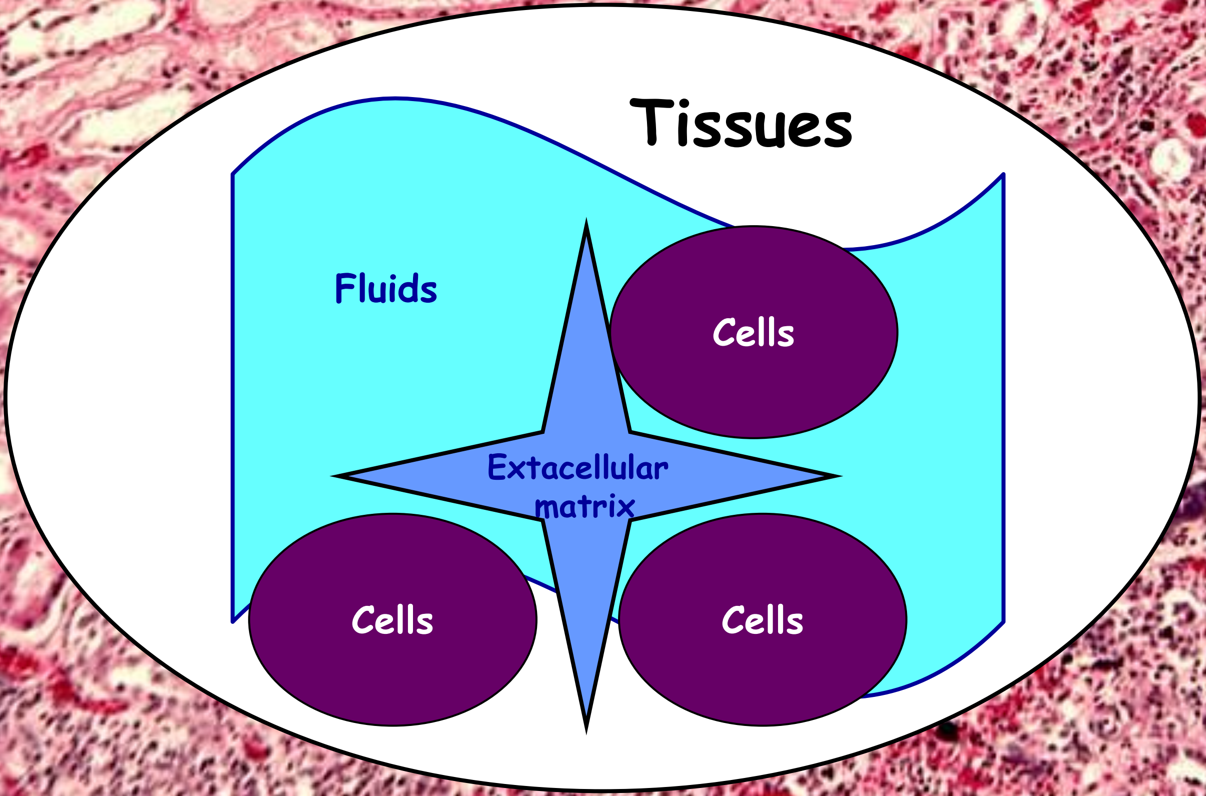
Chemical stains (H+E, Azan, van Gieson, …)

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
- Intersticial 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)

1839

Matthias Schleiden

Theodor Schwann

All organisms are composed
of one or more cells

Rudolph Virchow
1855

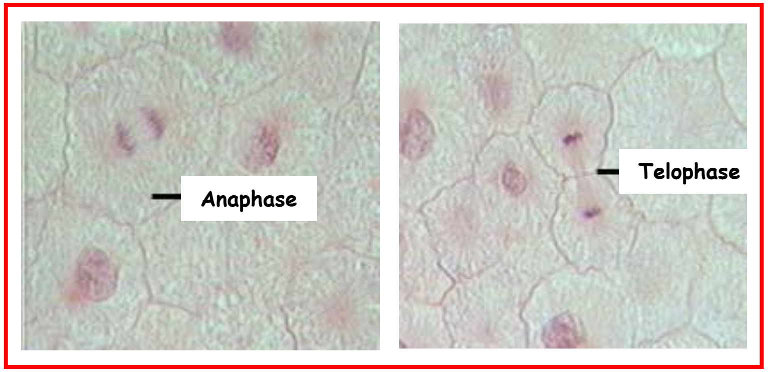
Cell can develop only from preexisting cells
„Omnis cellula e cellula“

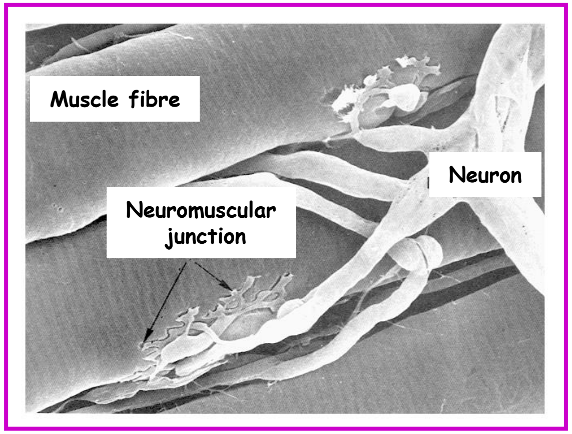
Cell is unifying theme/element of life

(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 bulding 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)

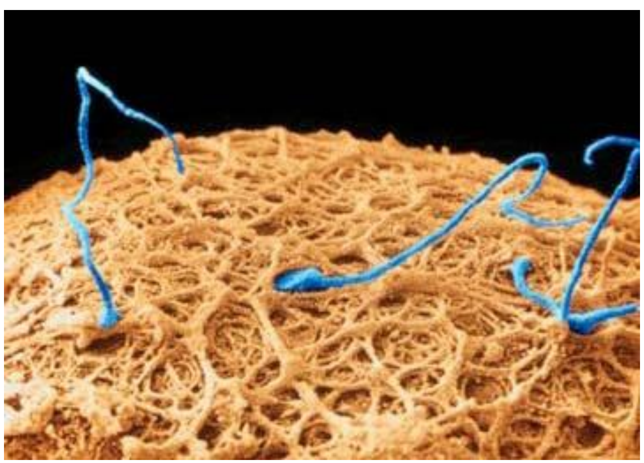


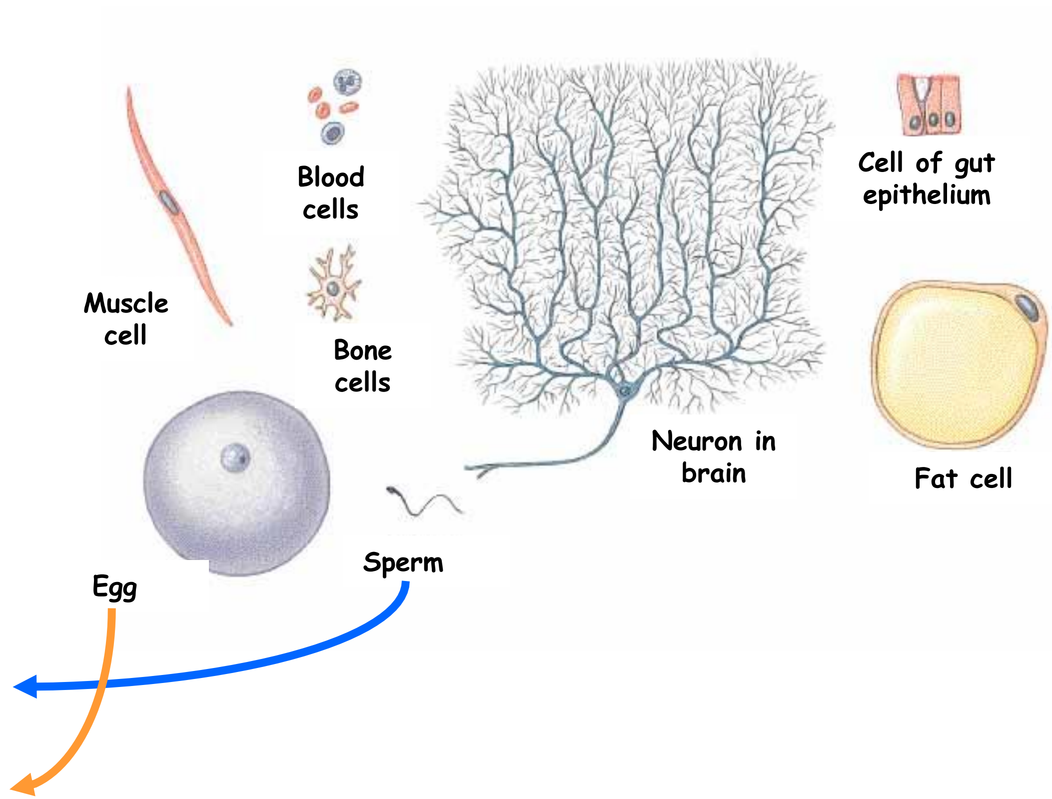


Despite of their common scheme, structural and functional diversity is a typical feature of all eukaryotic cell types

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



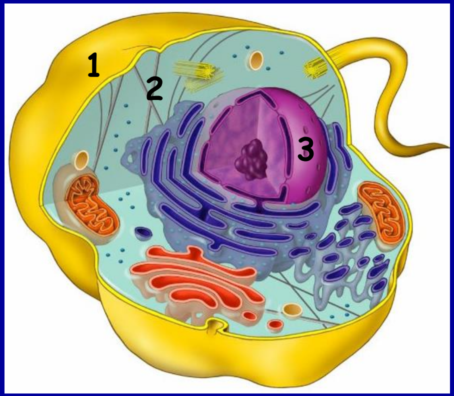


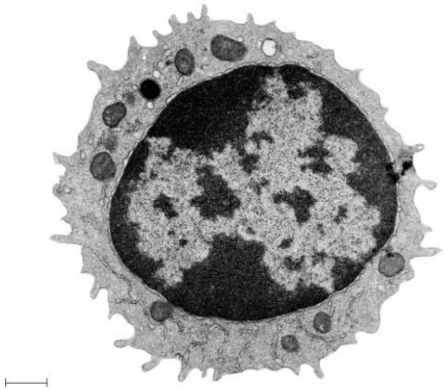
No cell is exactly like all others, but cells do have many common structural and functional features.

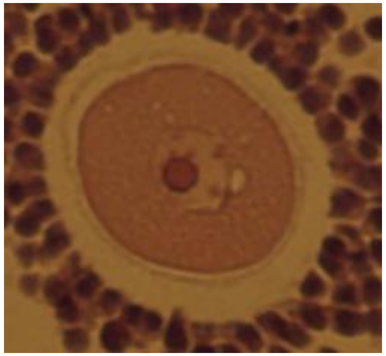
Keep in mind that not all cells contain all the structures we will discuss !

All cells have 3 major parts:

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







Cellular organization is based on COMPARTMENTALIZATION

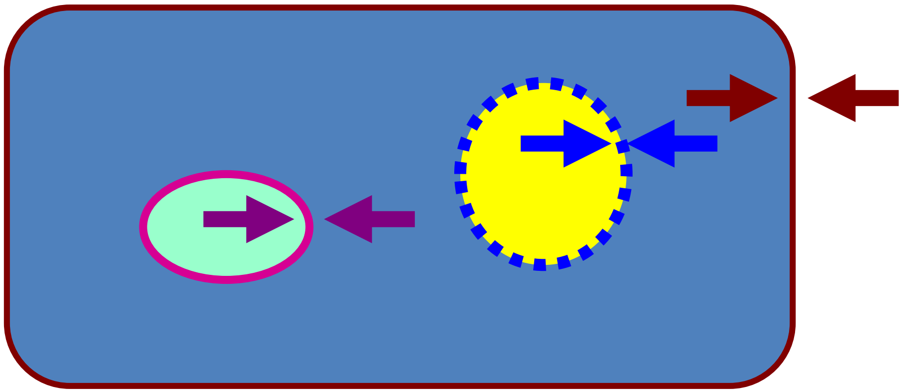
Specialized functions can be carried out in different locations

Membranes make up boundaries between the compartments

Unique protein and lipid components and unique function

Unique control of the movement of molecules

Unique composition of the surrounded interior



Compartments & Membranes

Many small compartments are better

More membrane surface per volume surrounded → More space for:

- **regulation**
- **nutrients exchange**
- **waste removal**

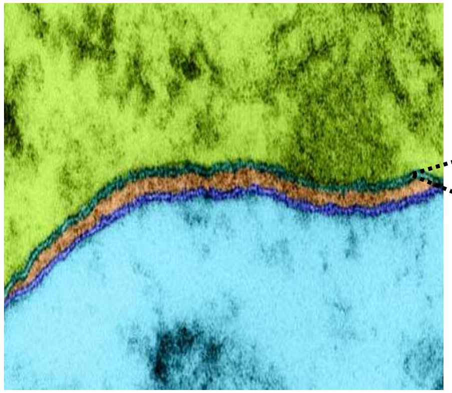
Surface area is proportional to the ***square*** (r^2) of its diameter.
Volume is proportional to the ***cube*** (r^3) of its diameter.

Amplification X Reduction of selected compartments → **Specialization of cells** for different functions

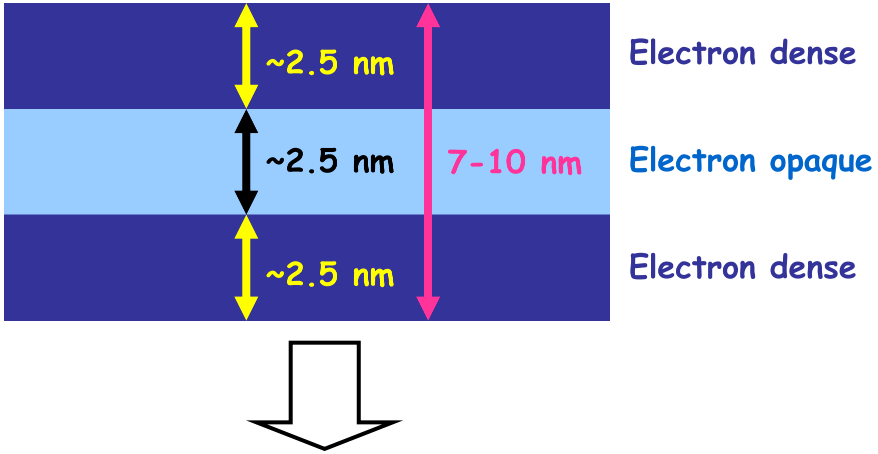
Cell differentiation

Rough ER in secretory cells
Mitochondria in cardiac musle cells

Biological membrane structure 1



Cell membranes seen
in electron microscope
(pseudocolored)

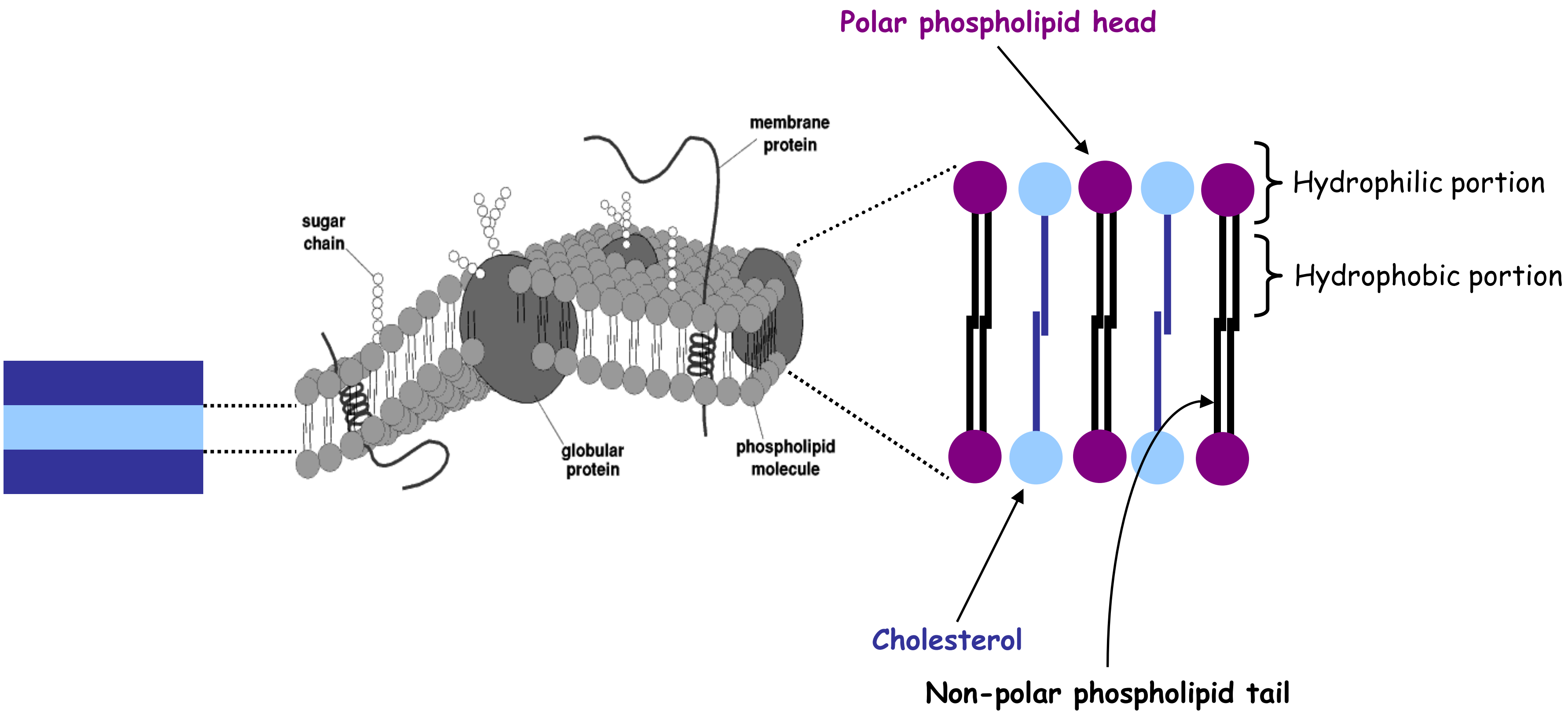


Unit membrane

common to all membranes

Biological membrane structure 2

Fluid mosaic - A bilayer of lipids with mobile globular proteins



Membrane structure 3

Membrane lipids

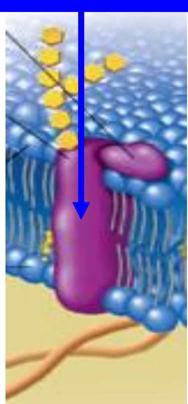
Make up 90-99% of molecules in membrane (in numbers).

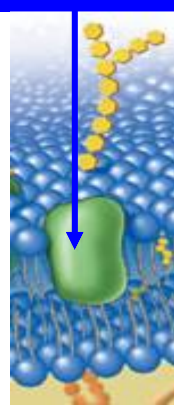
- **Phospholipids** - 75% of lipids
- **Cholesterol** - 20%
- **Glycolipids** - 5% - only on cytoplasmic membrane - GLYCOCALYX

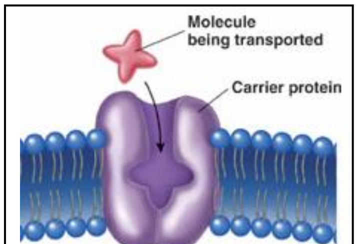
Membrane proteins

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

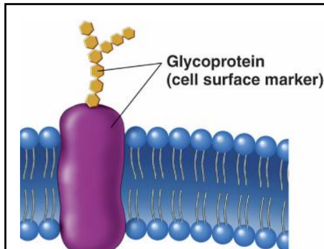
Integral + **Peripheral**



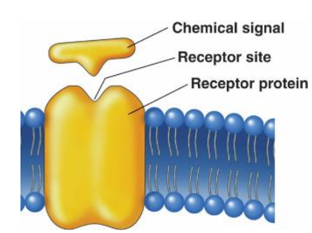




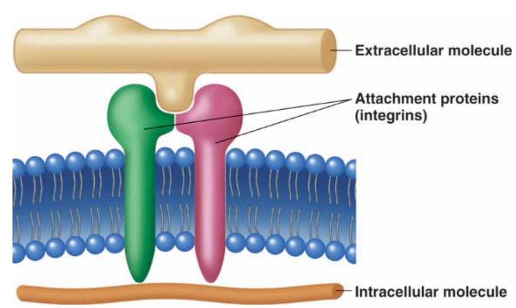
Transport



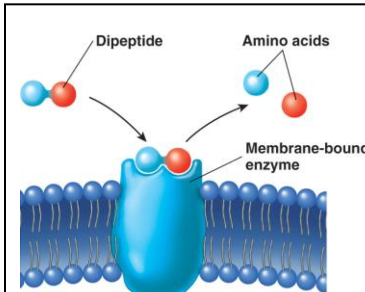
Cell identity



Sensing signals



Cell adhesion



Enzymatic activity

Organelles

Specialized internal structures with specialized functions

Membranous

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

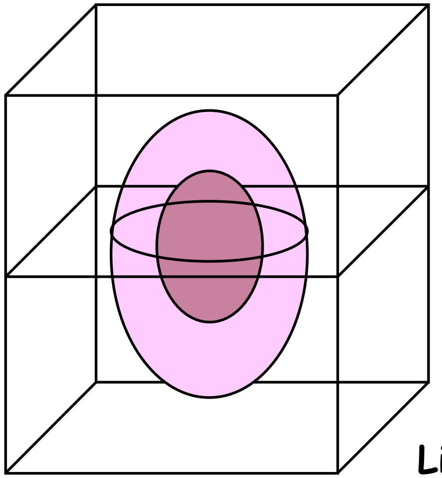
Non-membranous

- Ribosomes
- Centrosomes
- Centrioles
- Basal bodies

Related to specific structure and function of the cell

e.g., much energy needed → many mitochondria

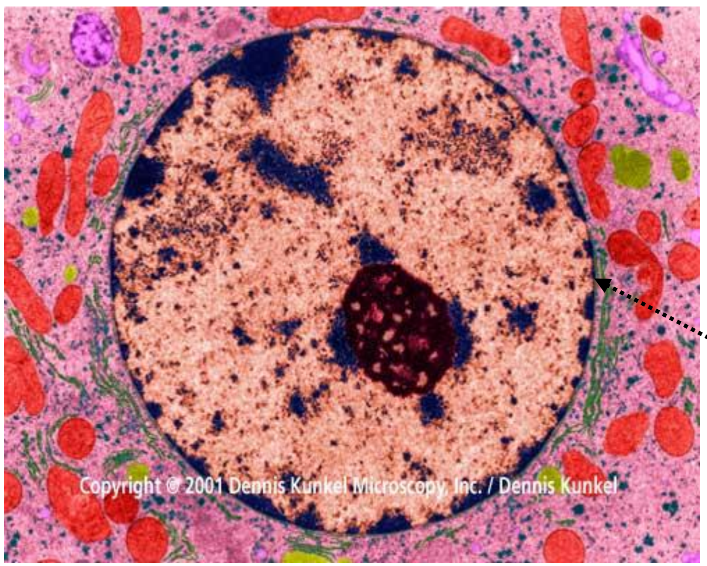
Nucleus 1
Envelop-bounded structure

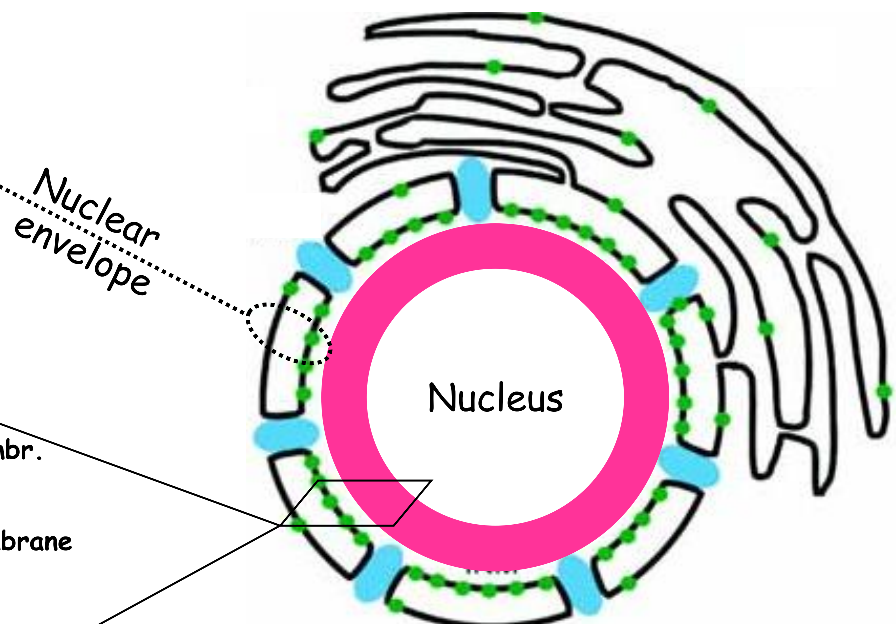


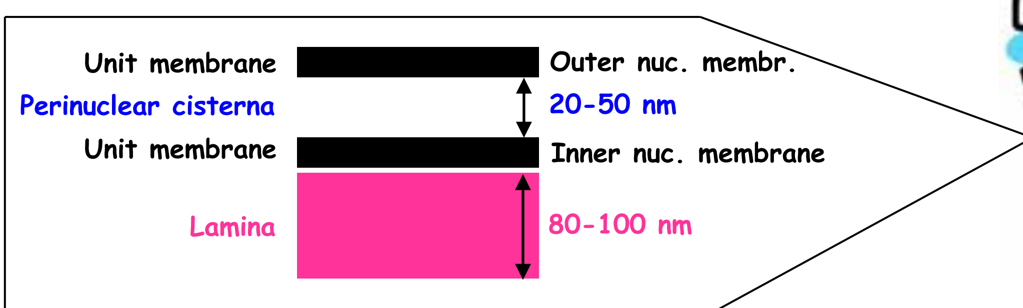
Mostly:

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

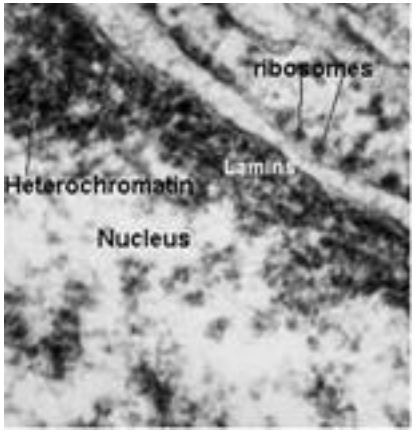
Liver cell nucleus

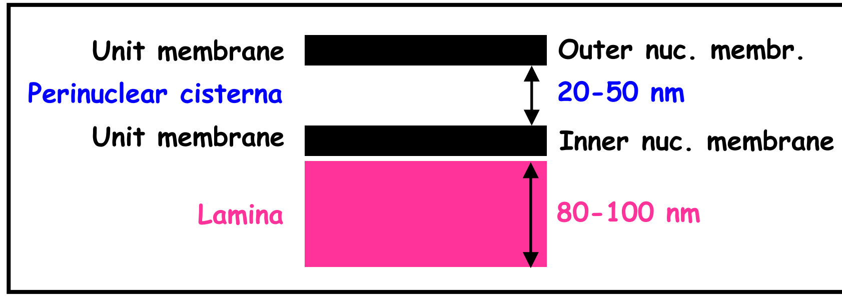


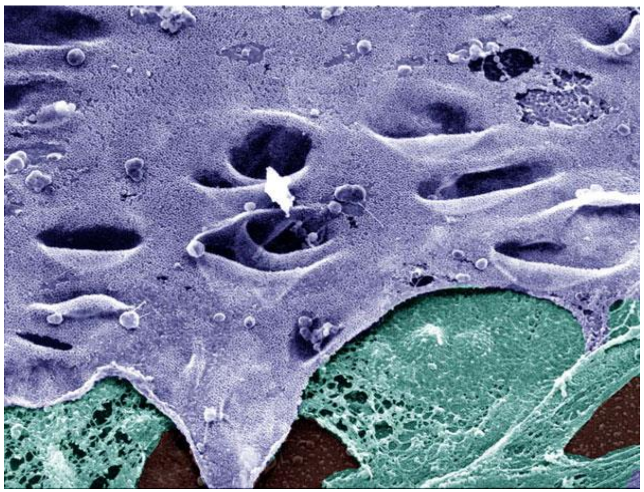




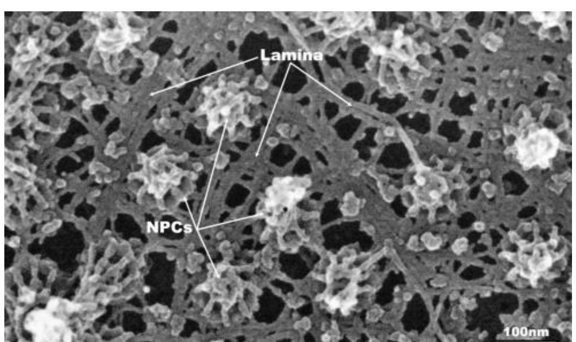
Nucleus 2
Continuation on nuclear envelop







Lamins:
- Intermadiate 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

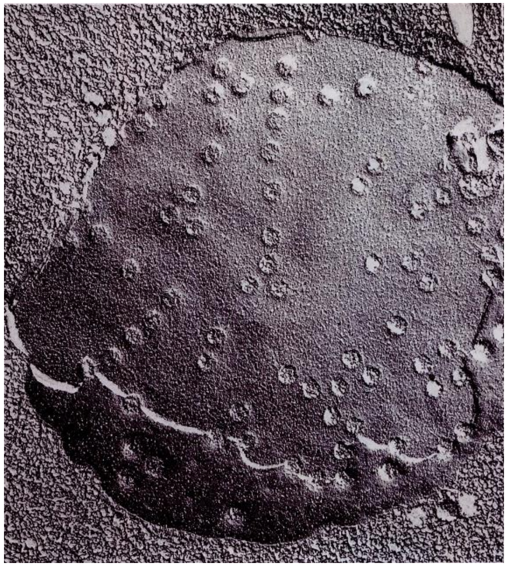


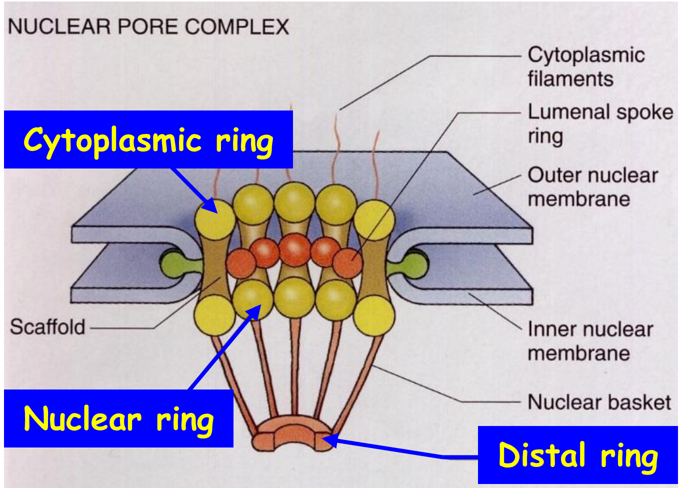
Laminopathies
- Human diseases (at least 13 known)
- Mutations in lamin genes (almost 200 mutations known)
- Deregulated gene expression
- Premature aging

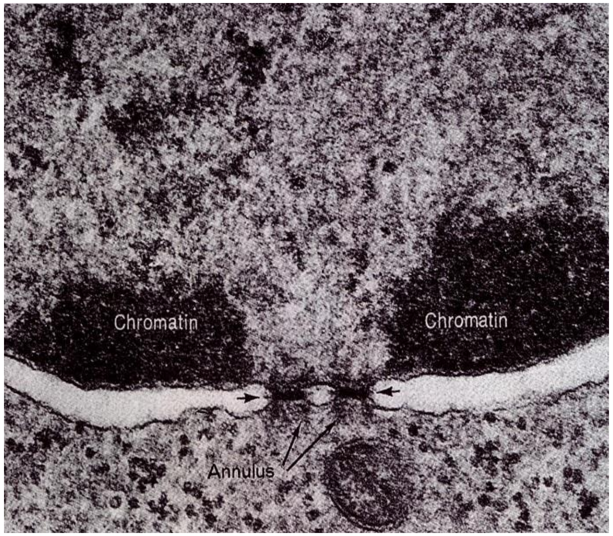
Hutchinson-Gilford progeria

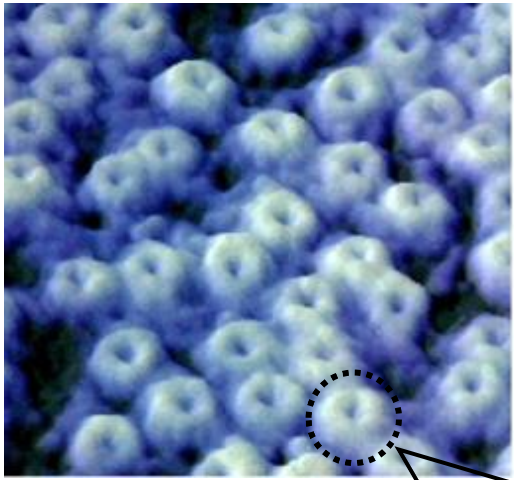
Rare - 1-4 per 8 milion of newborns
Missense mutation in A-type lamin

Nucleus 3
Nuclear pore complex









Diameter ~ 100 – 125 nm

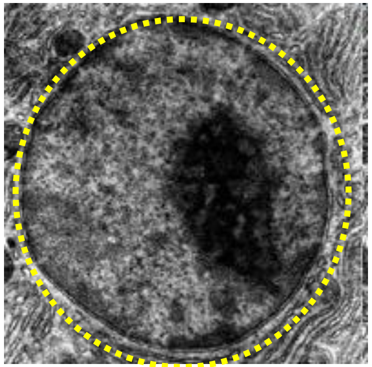
Three rings (8 subunits each)

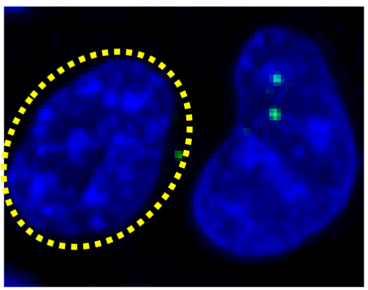
Inner filamentous basket

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





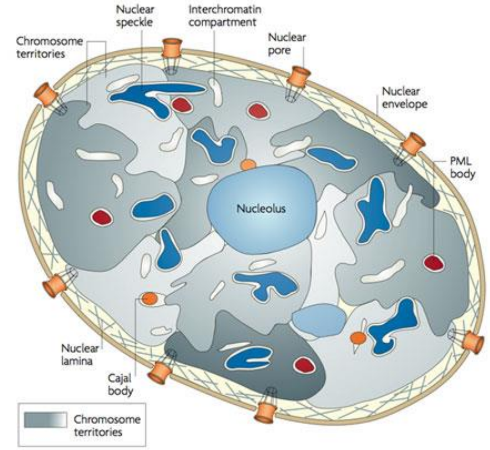
Interphase nucleus

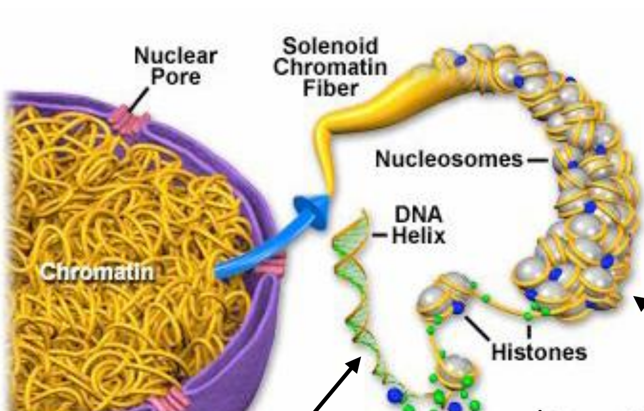
Heterochromatin

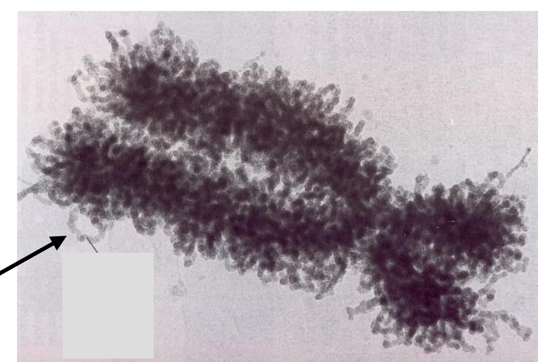
Feulgen positive – dark in light microscope
Dark/dense granular in TEM
Transcriptionally inactive

Euchromatin

Invisible in light microscope
Relaxed uncoiled chromosomes
Transcriptionally active



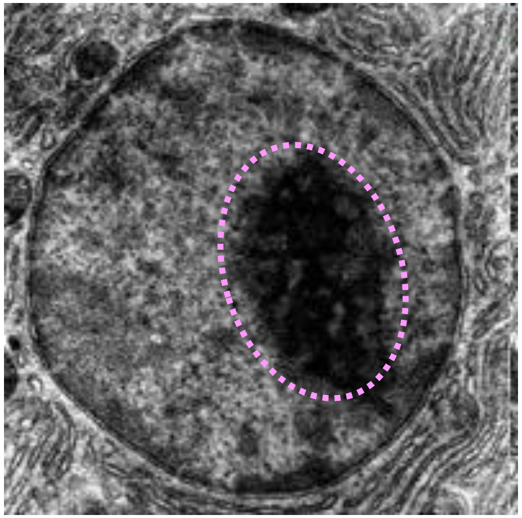




2 nm
DNA double helix

30 nm
Chromatin fiber of packed nucleosomes

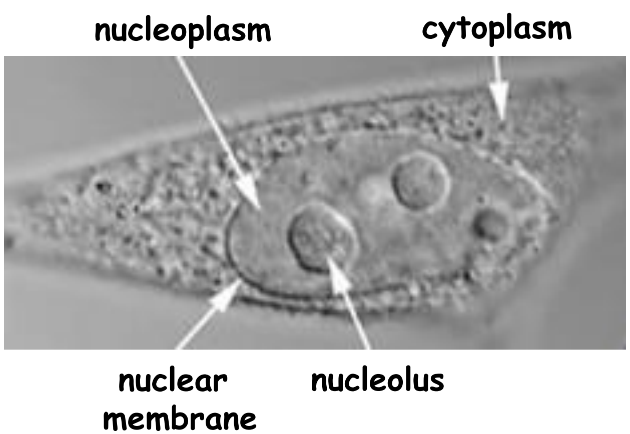
Nucleus 5
Nucleolus

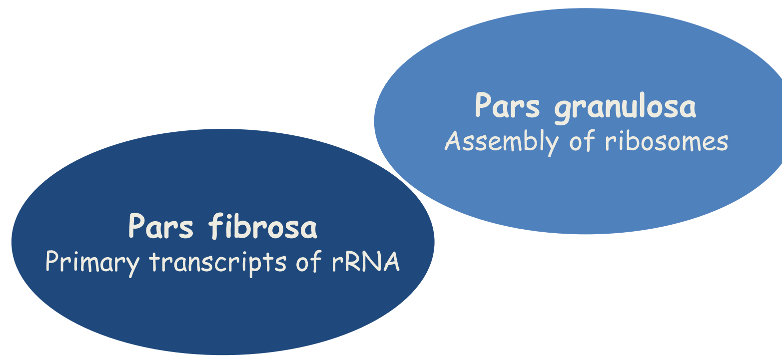


non-membrane-bounded structure

Main functions

Synthesis of rRNA
Assembly of ribosomes





Nucleolar-organizing regions of DNA

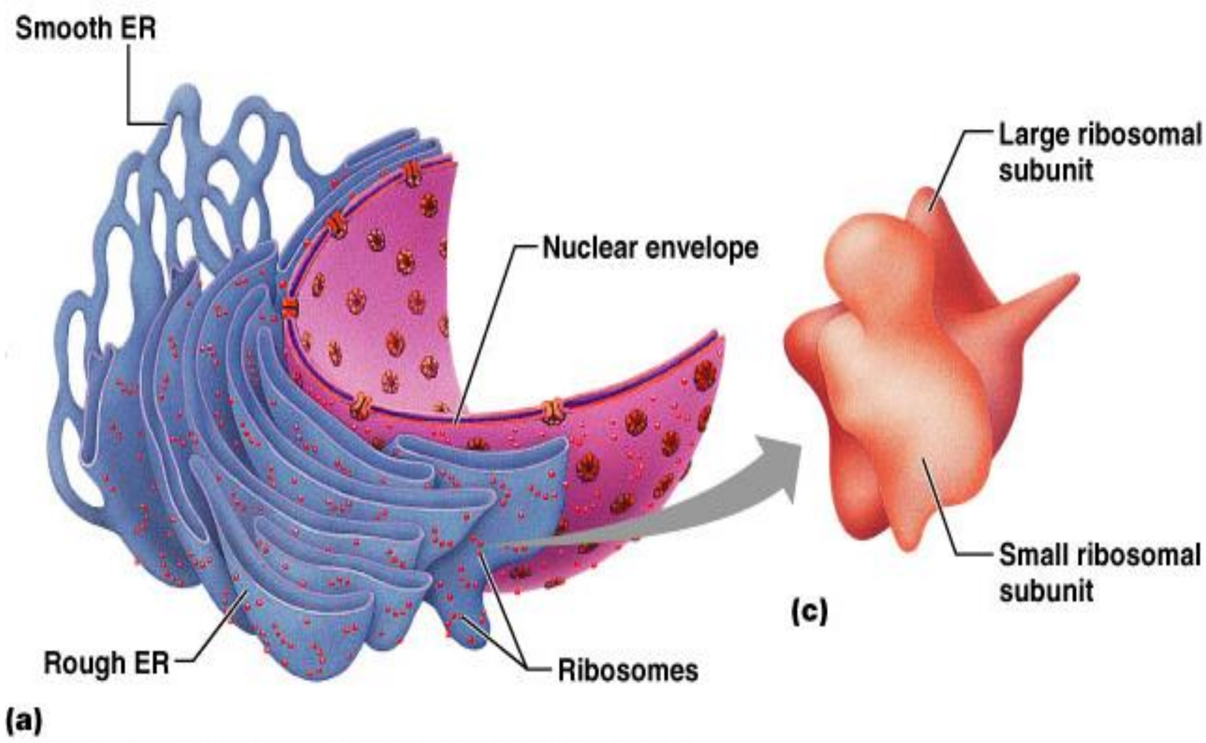
on five chromosomes in human cells
(chrs. 13, 14, 15, 21, 22)

Endoplasmic reticulum 1

„within cell“ „net“

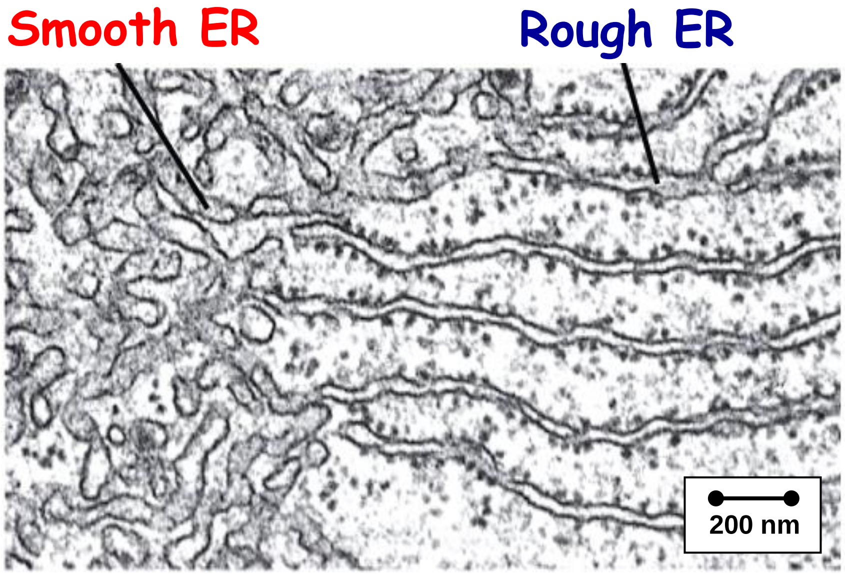
Majority of the membrane within cells.

Interconnected tubuli and vesicles → Cisterns

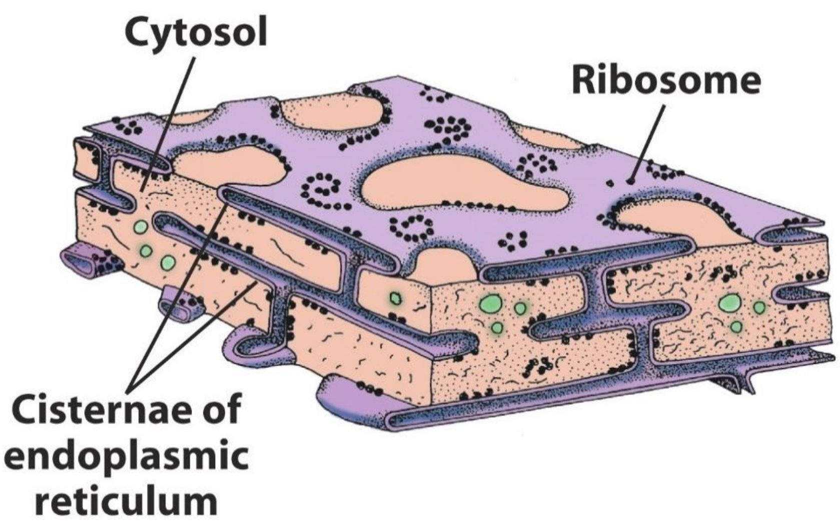


Endoplasmic reticulum 2

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)



External surface **has ribosomes attached**
- Manufactures all secreted proteins
- Synthesizes integral membrane proteins
- Modifies proteins



Ribosomes

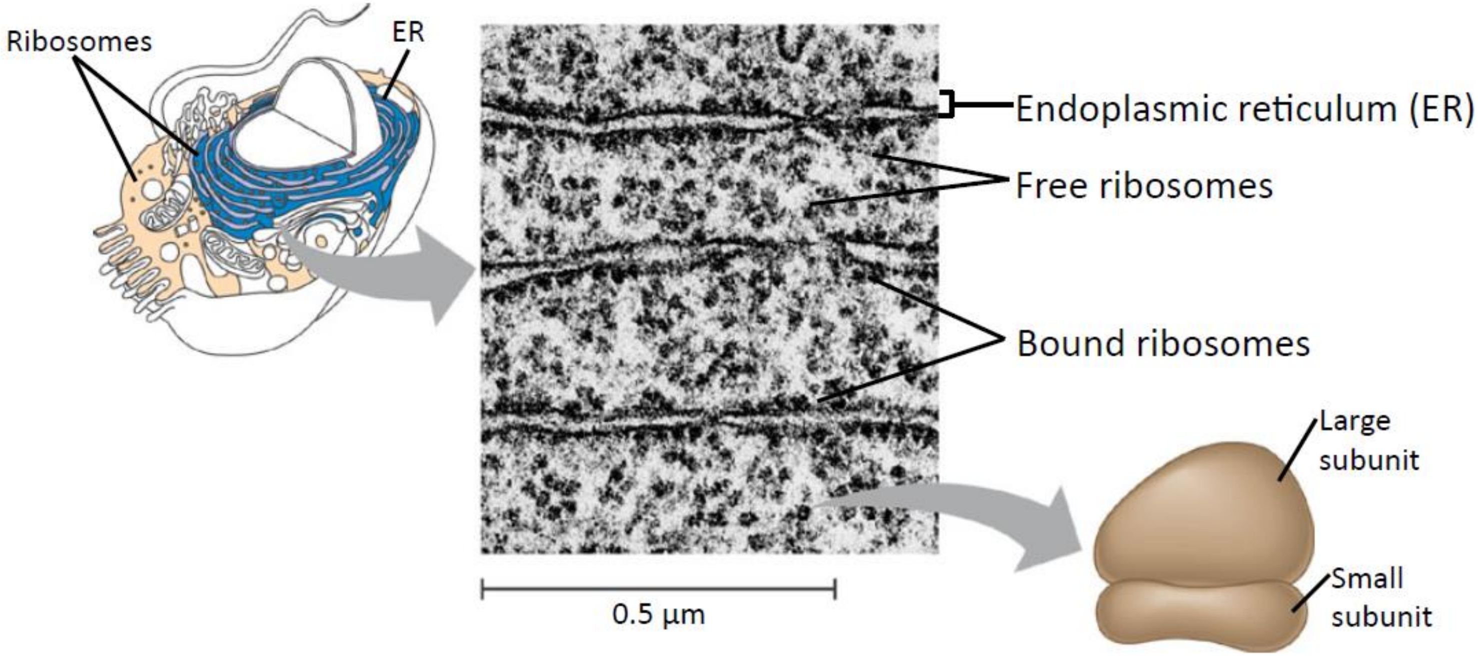
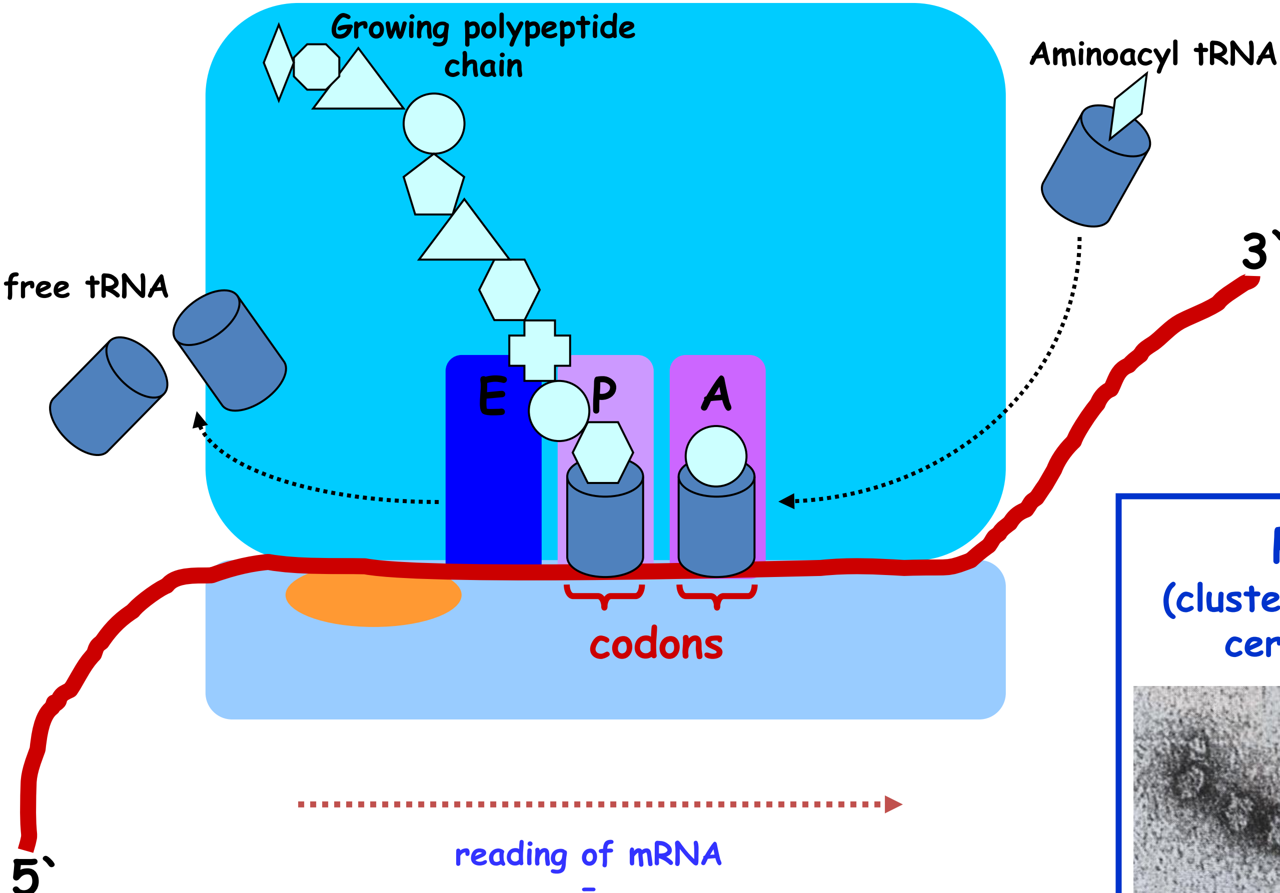


Diagram of a ribosome

Ribosomes - Translation



reading of mRNA
=
movement of ribosomes on mRNA

Beginning of translation

Met-tRNA

3` UAC 5`

mRNA 5` — AUG — 3`

START kodon

End of translation

mRNA 5` — UAG — 3`

mRNA 5` — UAA — 3`

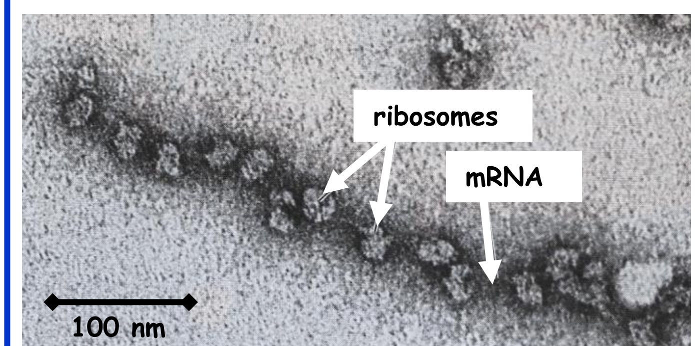
mRNA 5` — UGA — 3`

STOP kodony

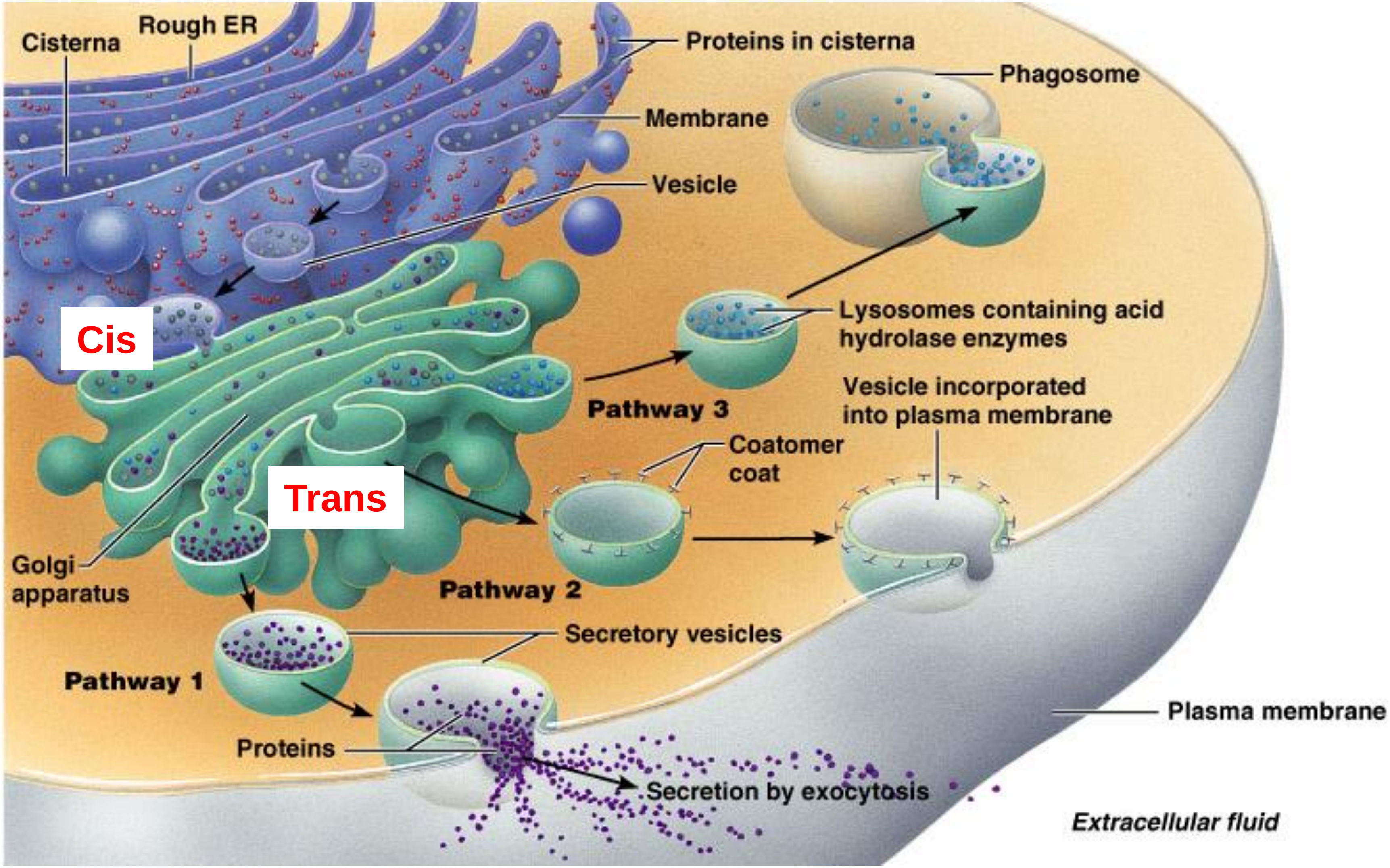
bind „release factor"

POLYRIBOSOME

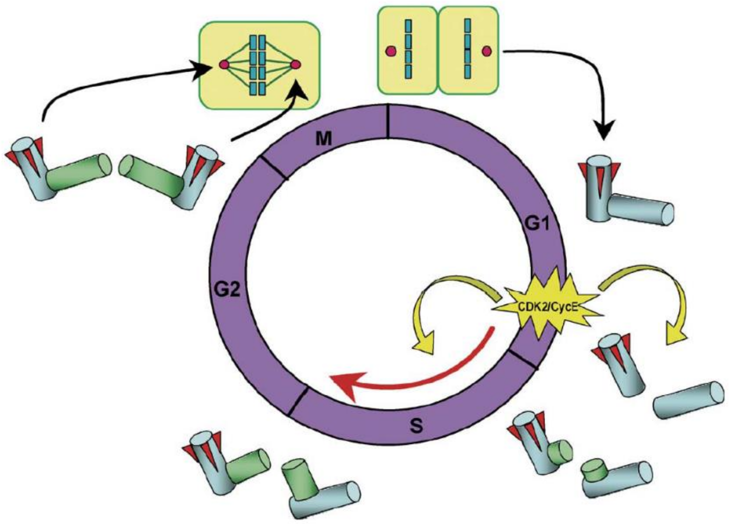
(cluster of ribosomes translating certain segment of mRNA)

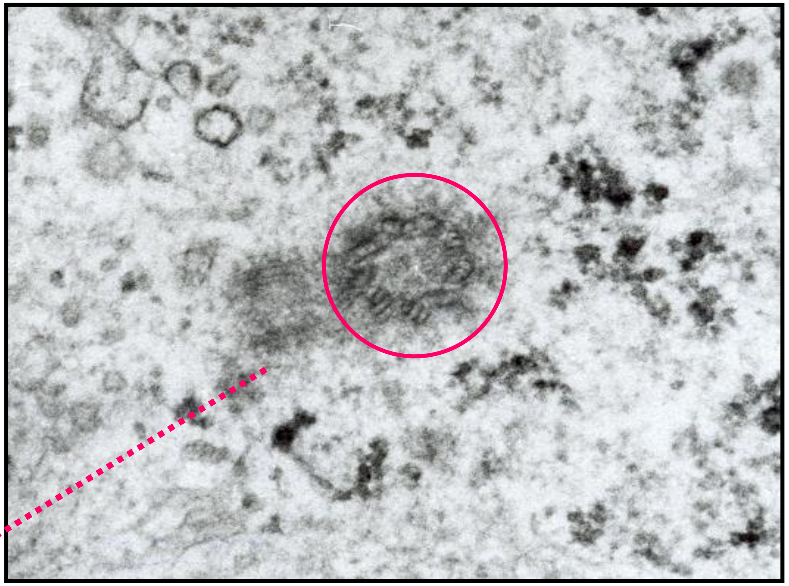


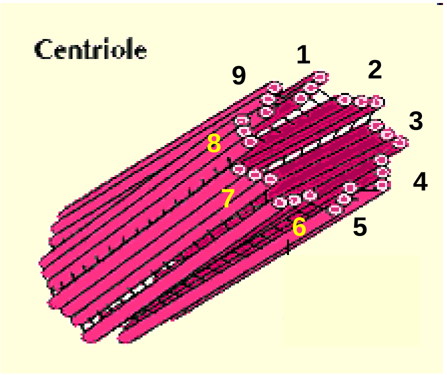
Golgi apparatus – Transgolgi pathway



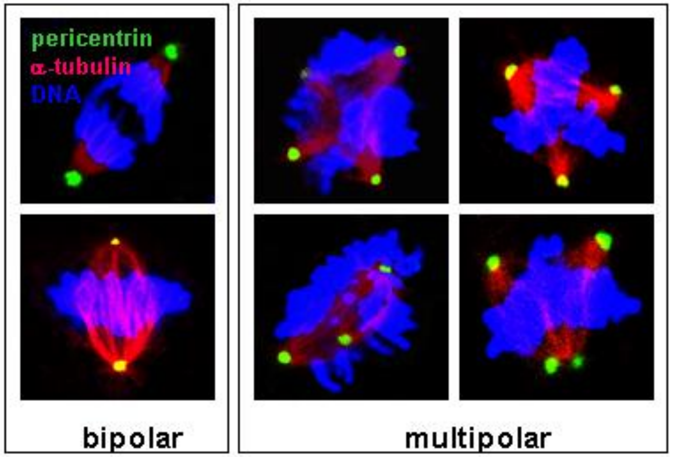
Centrosome







Diameter – 0.2 µm
Length - 0.5 µm



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 !

ahampl@med.muni.cz

Building A1 – 1st floor