

# Histology and Embryology

## Lecturers:

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Brno, 2022

# 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?

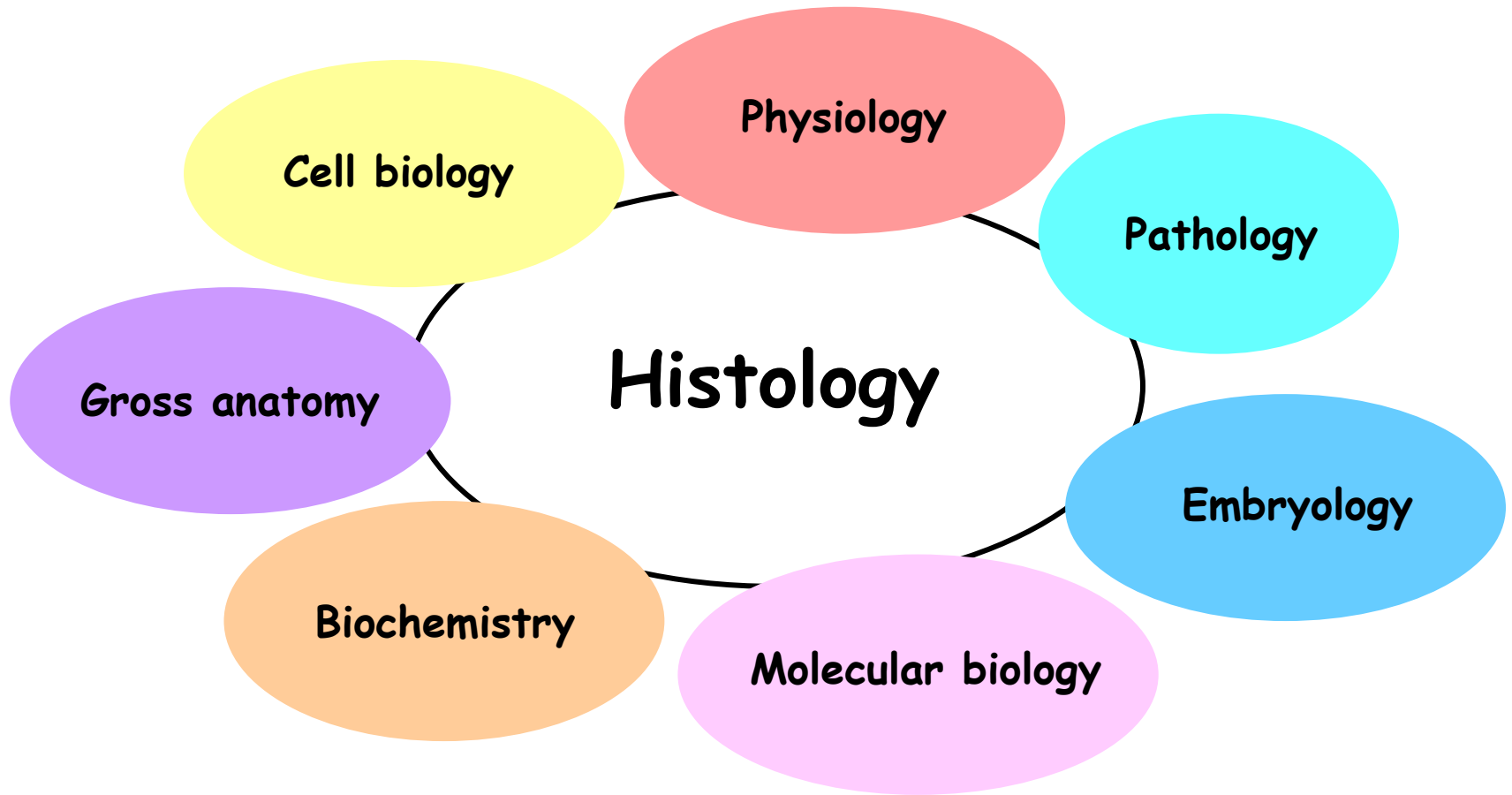
### Microscopic anatomy

Composition and structure of organ systems & individual organs

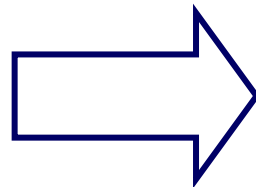
Which tissue types and how organized?  
Which special cell types?  
Which special structures? (e.g. tubules)  
How does it all work?

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

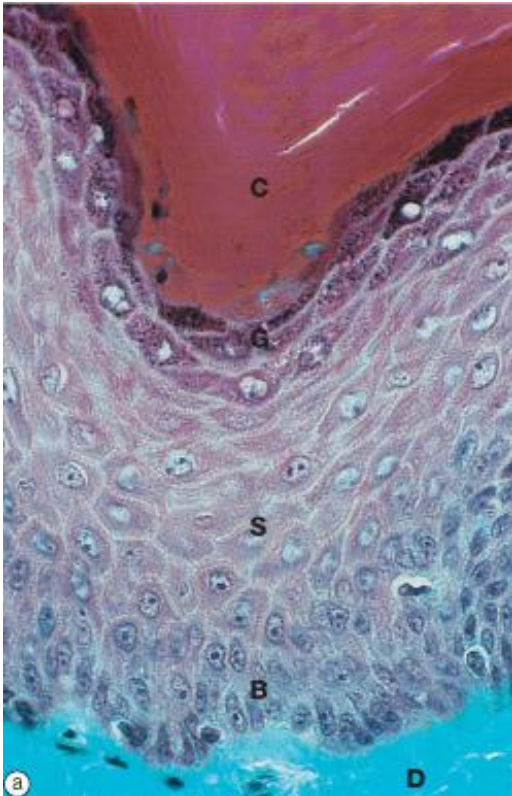
Italian physician

Founder of microscopic anatomy and the first histologist

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



MARCELLO MALPIGHI.  
From an engraving of the self-portrait by A. M. Telfer, 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

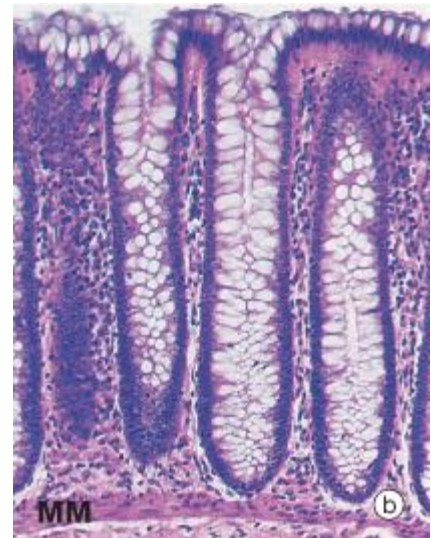
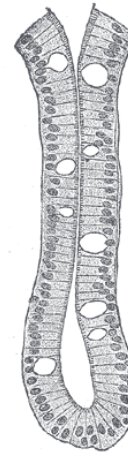
# Johan Nathanael Lieberkuhn

## 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)

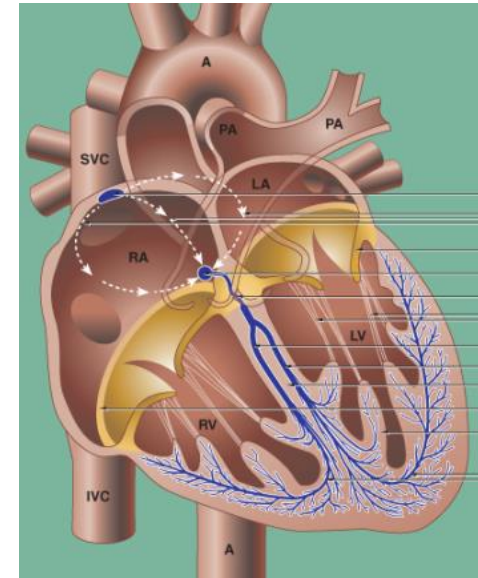
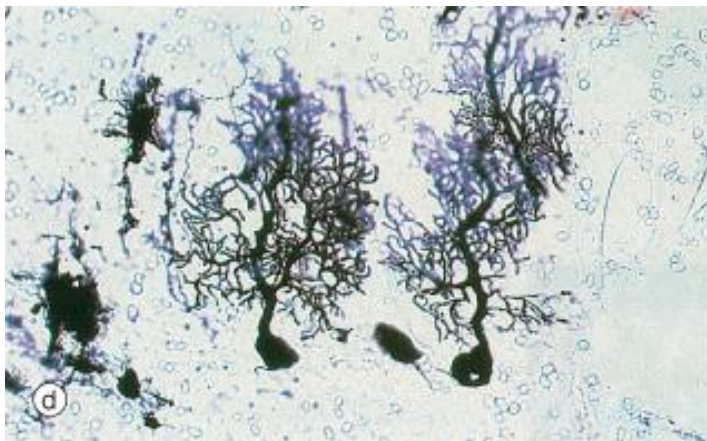
# Jan Evangelista Purkyně

## 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

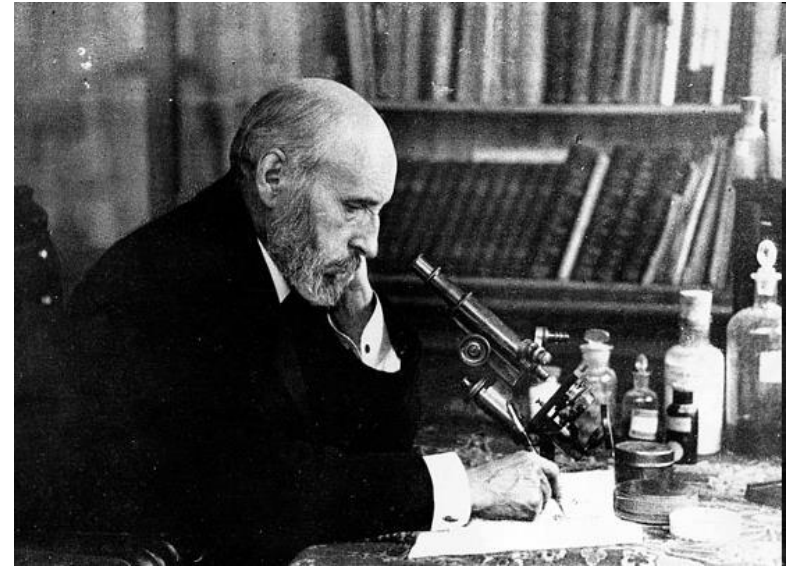
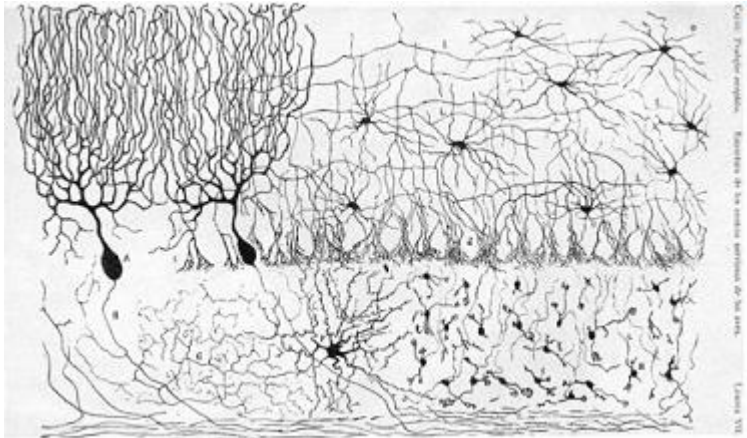




# Santiago Ramón Y Cajal

## 1852 - 1934

Spanish physician and anatomist



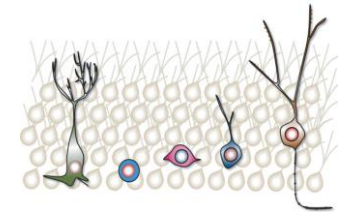
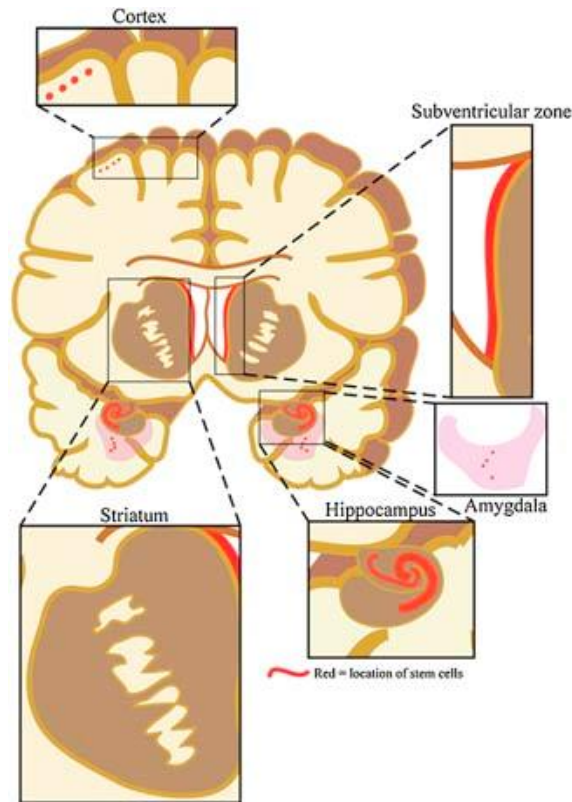
He established the **neuron** as the primary structural and functional unit of the nervous system.  
Nobel Prize in 1906

“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:

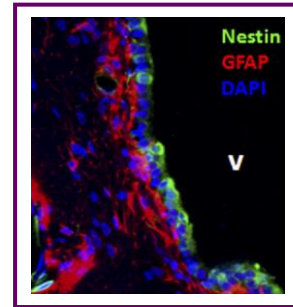
- Cortex ?
- Amygdala ?

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

# Many questions on NSC remain to be answered

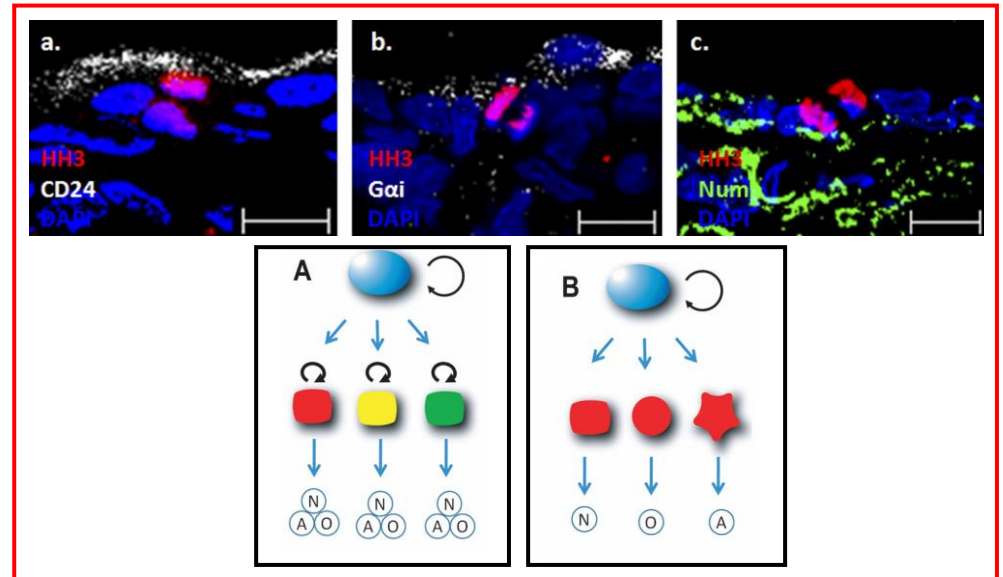
Combination of developmental biology, **histology**, cell biology, and molecular biology approaches is required.

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

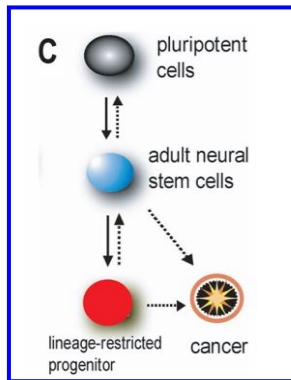


Gleason et al., Neuroscience, 2008.

- developmental potential ?
- involvement in disease development ?

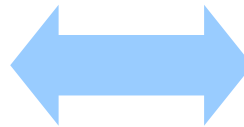


- others

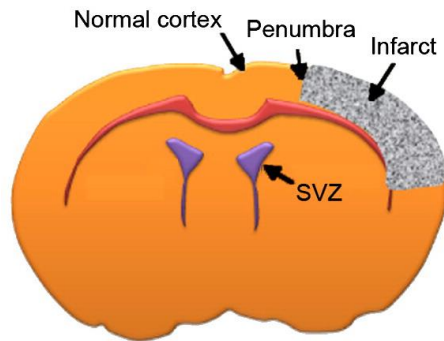
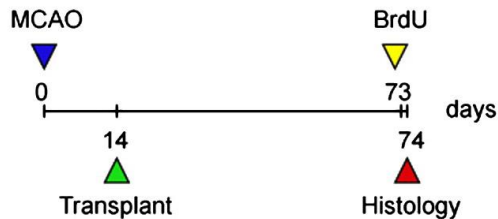


# Any practical use of such discovery ? (1)

Helping brain regenerate after the stroke



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



## Options:

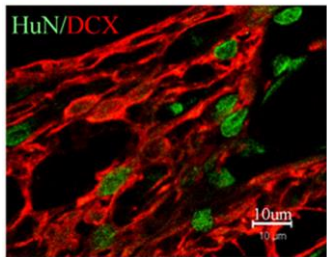
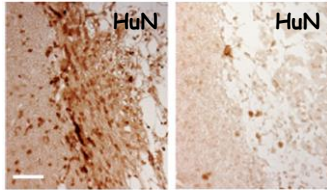
- drugs
- growth factors
- cell implantation

- 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)

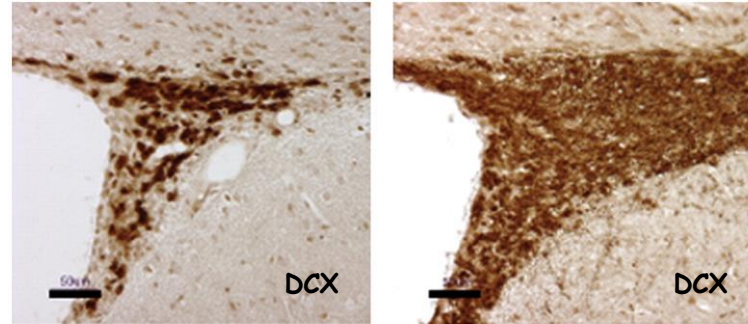
Transplanted human cells  
in the site of infarction

3 months      24 months



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

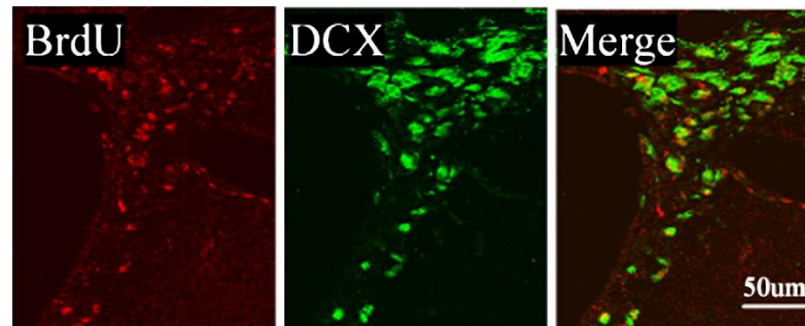
Neurogenesis in SVZ of rat brain becomes stimulated



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  $\beta$  cells  
of pancreatic islets  
of Langerhans

Dysregulated glucose  
metabolism

Diabetes

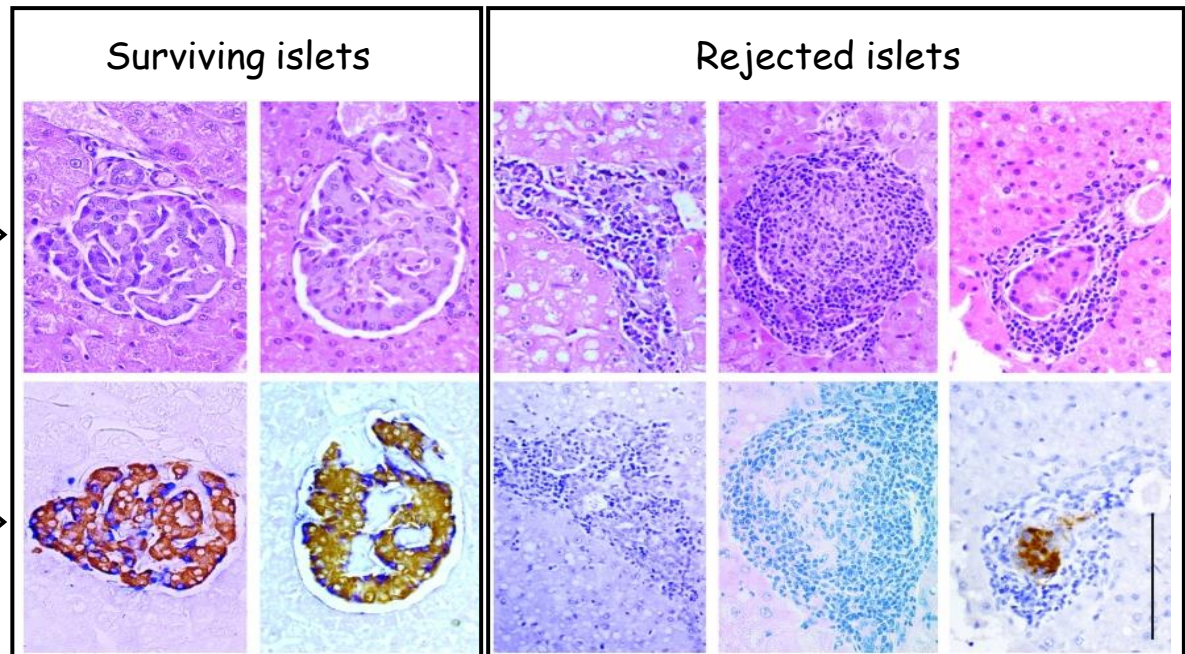
No permanent cure - Transplantation ? - Immunosuppression

Lymphocyte function-associated  
antigen 1 (LFA-1)

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

Haematoxylin  
&  
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



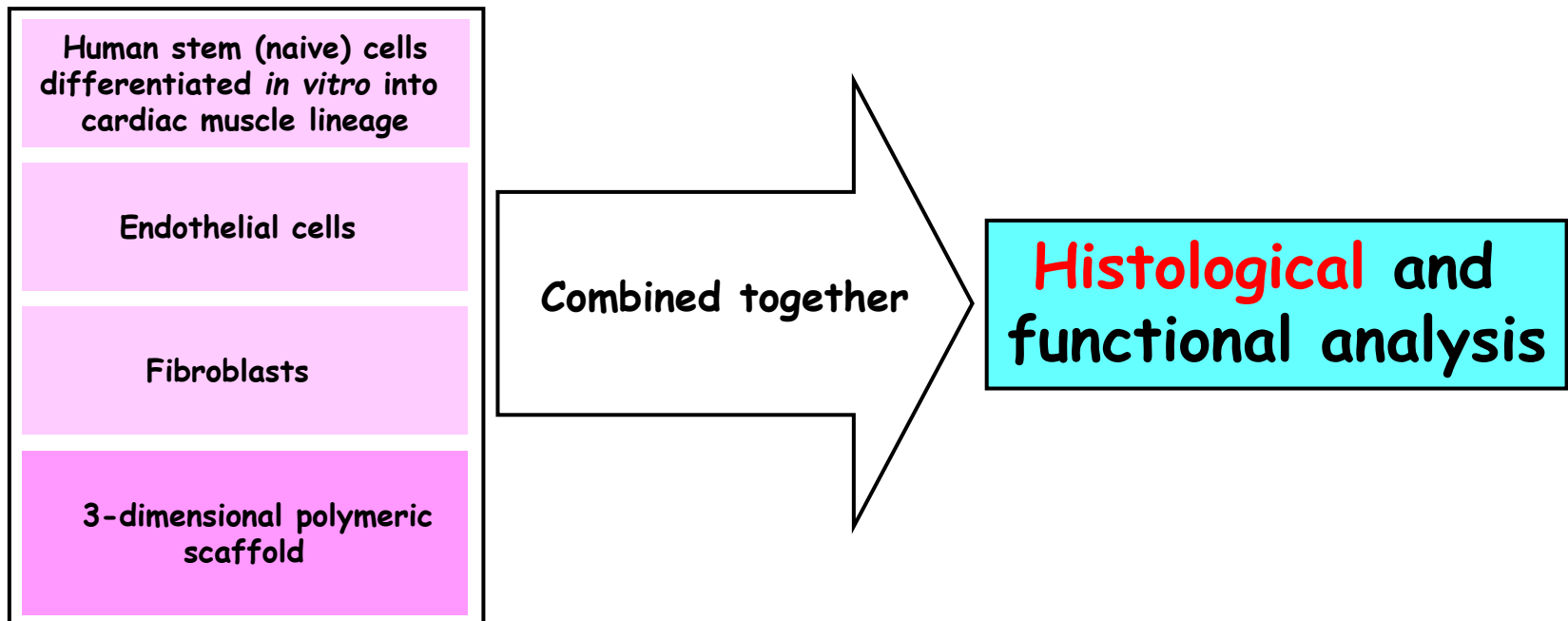
Egyptian mummy

# 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)

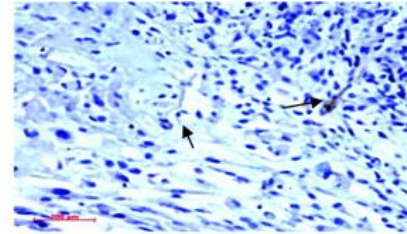
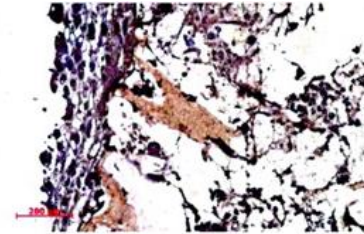
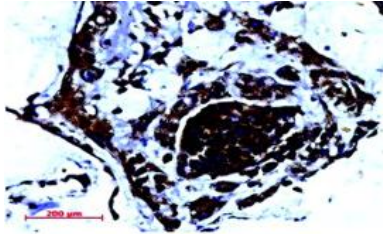
The first report of the construction of 3D vascularized human cardiac tissue that may have unique applications for studies of cardiac development, function, and tissue replacement therapy



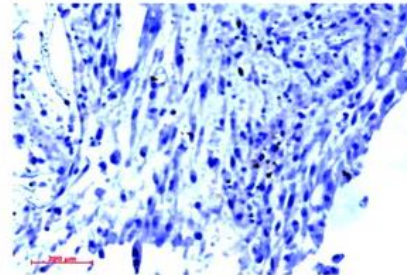
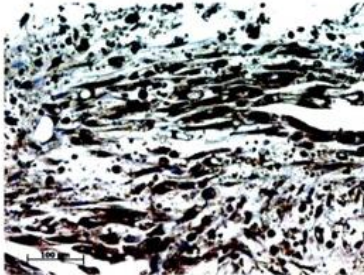
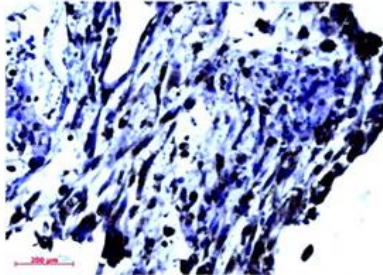


# Tissue engineering 2

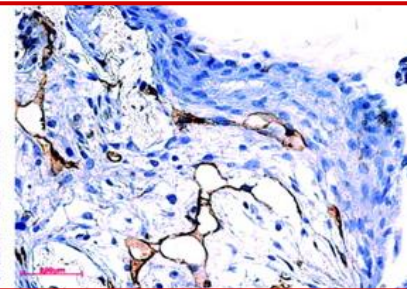
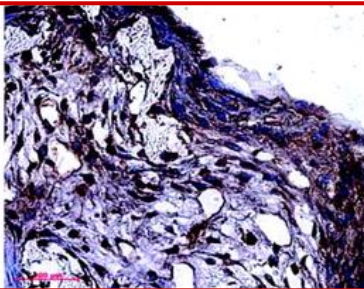
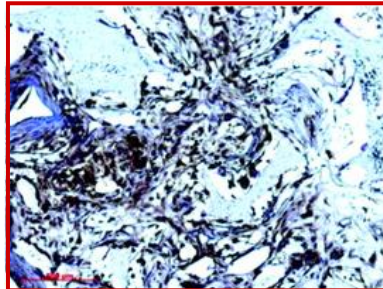
Cardiomyocytes



Cardiomyocytes  
+  
Endothelia



Cardiomyocytes  
+  
Endothelia  
+  
Fibroblasts



Troponin I

Sarcomeric actinin

CD 31

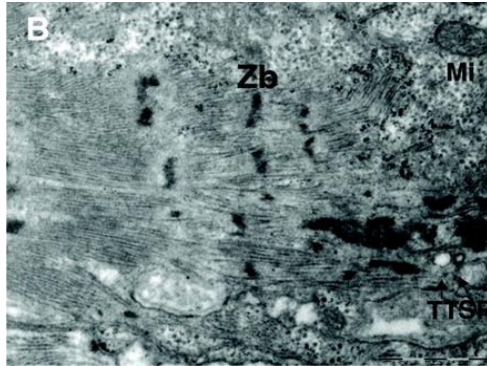
Markers of **cardiac muscle**

Markers of **cardiac endothelia**

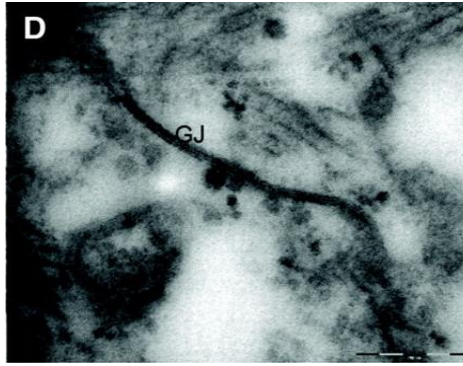
or

# Tissue engineering 3

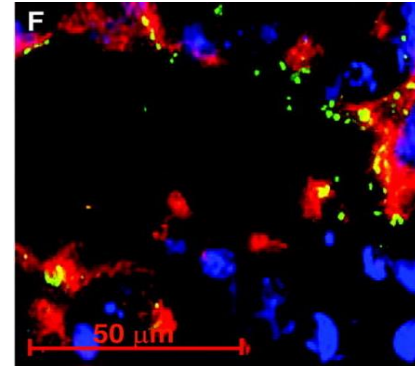
## Ultrastructural characteristics of the engineered cardiac tissue



Myofibrils  
Z bands  
T tubules  
Sarcoplasmic reticulum

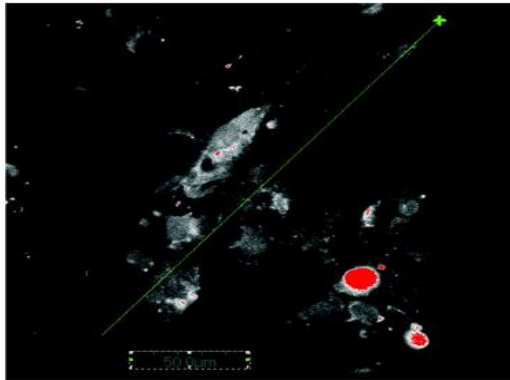


Gap junctions

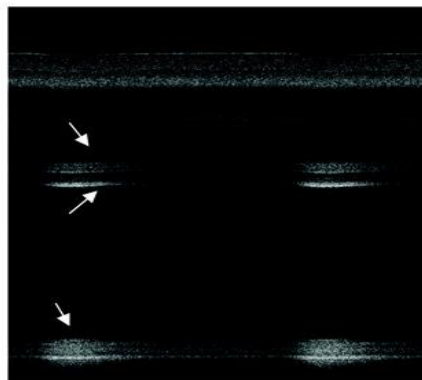


Conexin - Gap junctions  
Troponin - cardiomyocytes

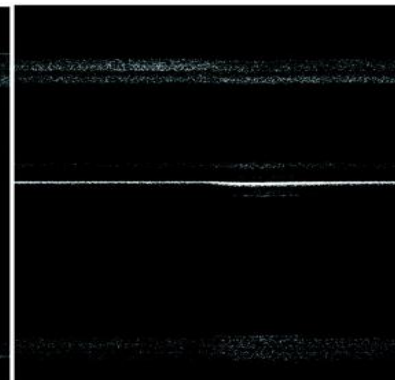
## Engineered cardiac tissue propagates synchronous surges of $Ca^{2+}$



Laser scanning cofocal microscopy



Baseline



1-Heptanol  
(Gap junctions 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  $\mu\text{m}$**

- 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



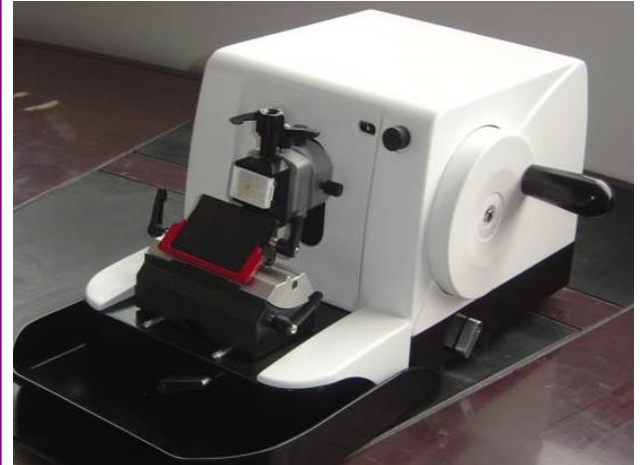
# Methodologies to study cells and tissues 2

## 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“

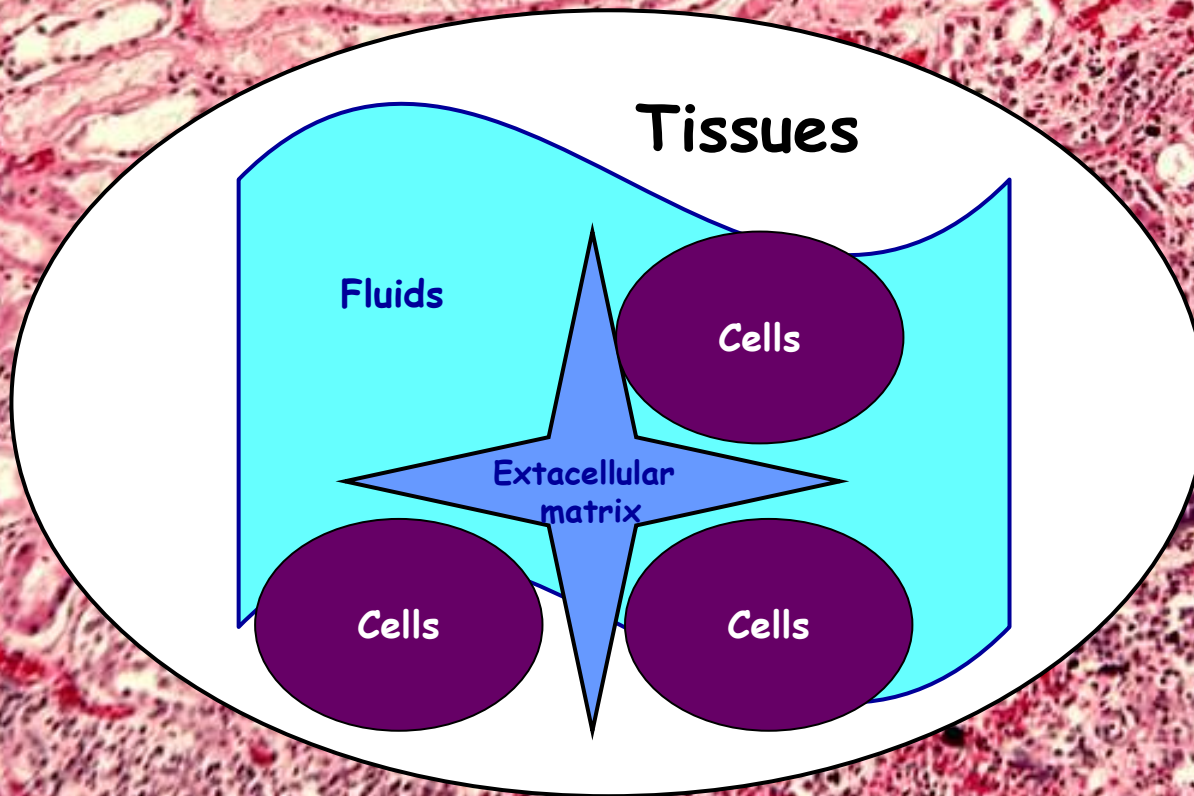
**Chemical stains** (H+E, Azan, van Gieson, ...)

**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

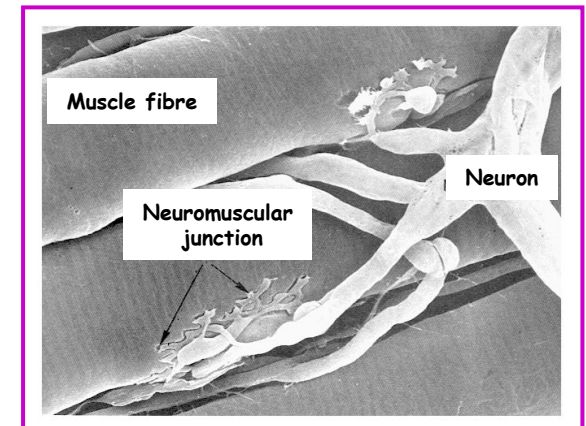
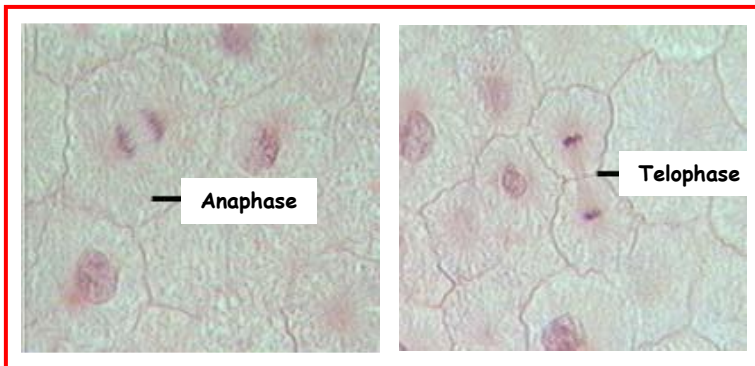
Cell can develop only from preexisting cells  
„Omnis cellula e cellula”

# 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)

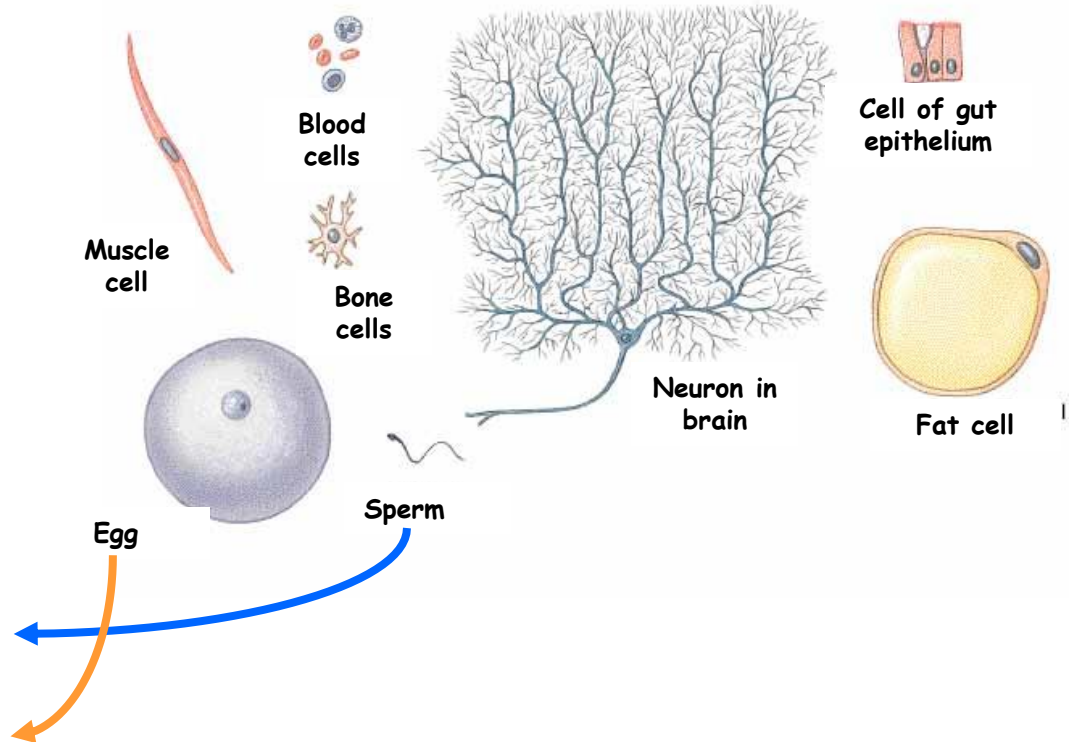
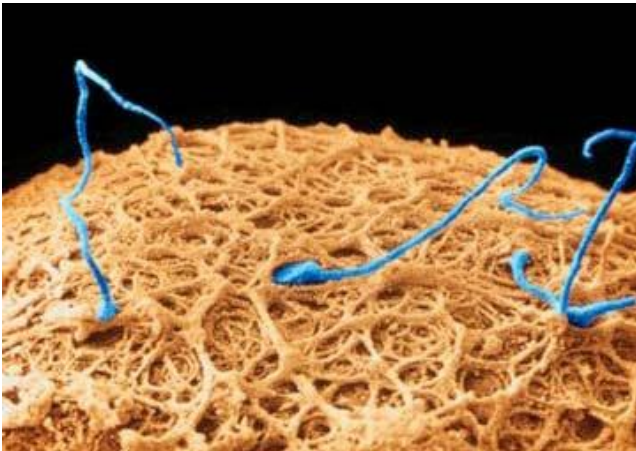


Despite of their common scheme,  
structural and functional  
diversity is a typical feature  
of all eukaryotic cell types



# 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

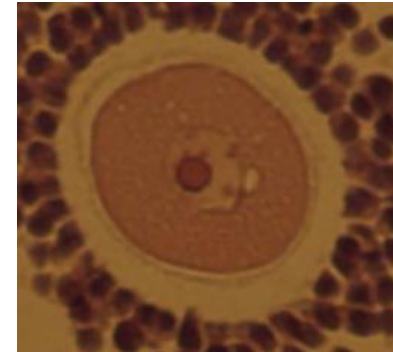
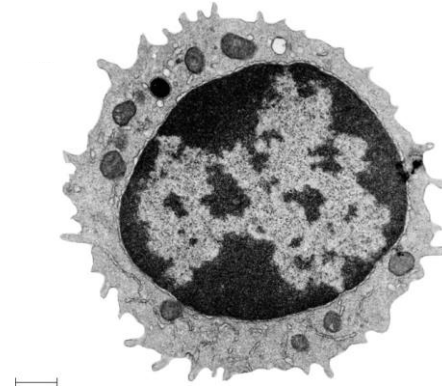
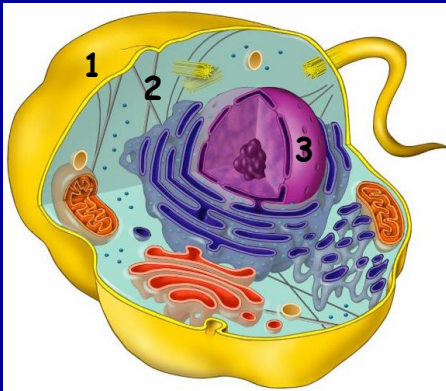


No cell is exactly like all others,  
but cells do have many common  
structural and functional features.

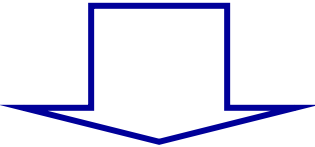
Keep in mind that not all cells contain all the structures we will discuss !

All cells have 3 major parts:

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

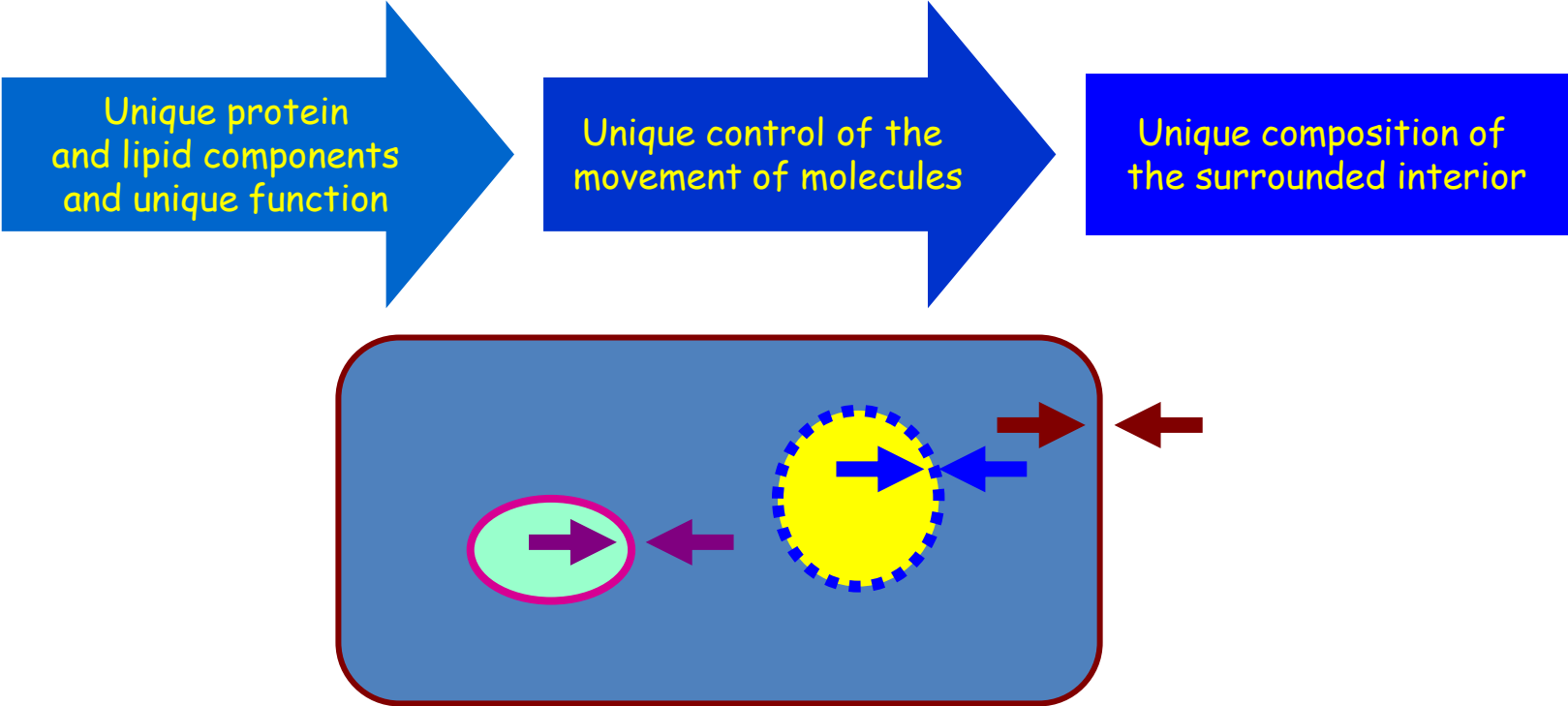


# Cellular organization is based on COMPARTMENTALIZATION



Specialized functions can be carried out in different locations

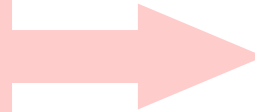
**Membranes** make up boundaries between the compartments



# 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**

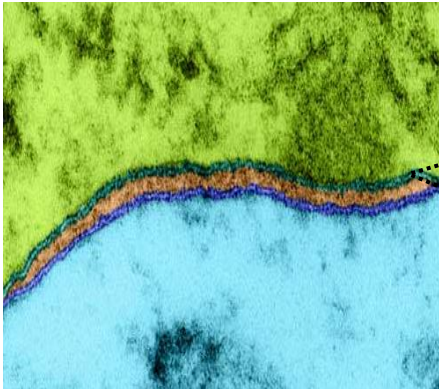


**Specialization of cells  
for different functions**

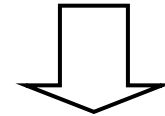
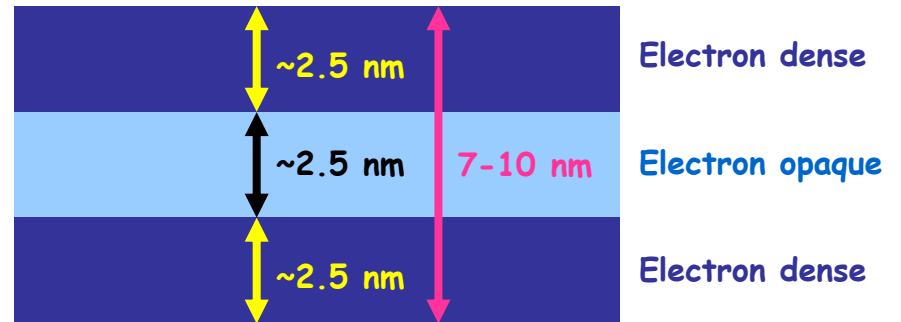
Cell differentiation

Rough ER in secretory cells  
Mitochondria in cardiac muscle cells

# Biological membrane structure 1



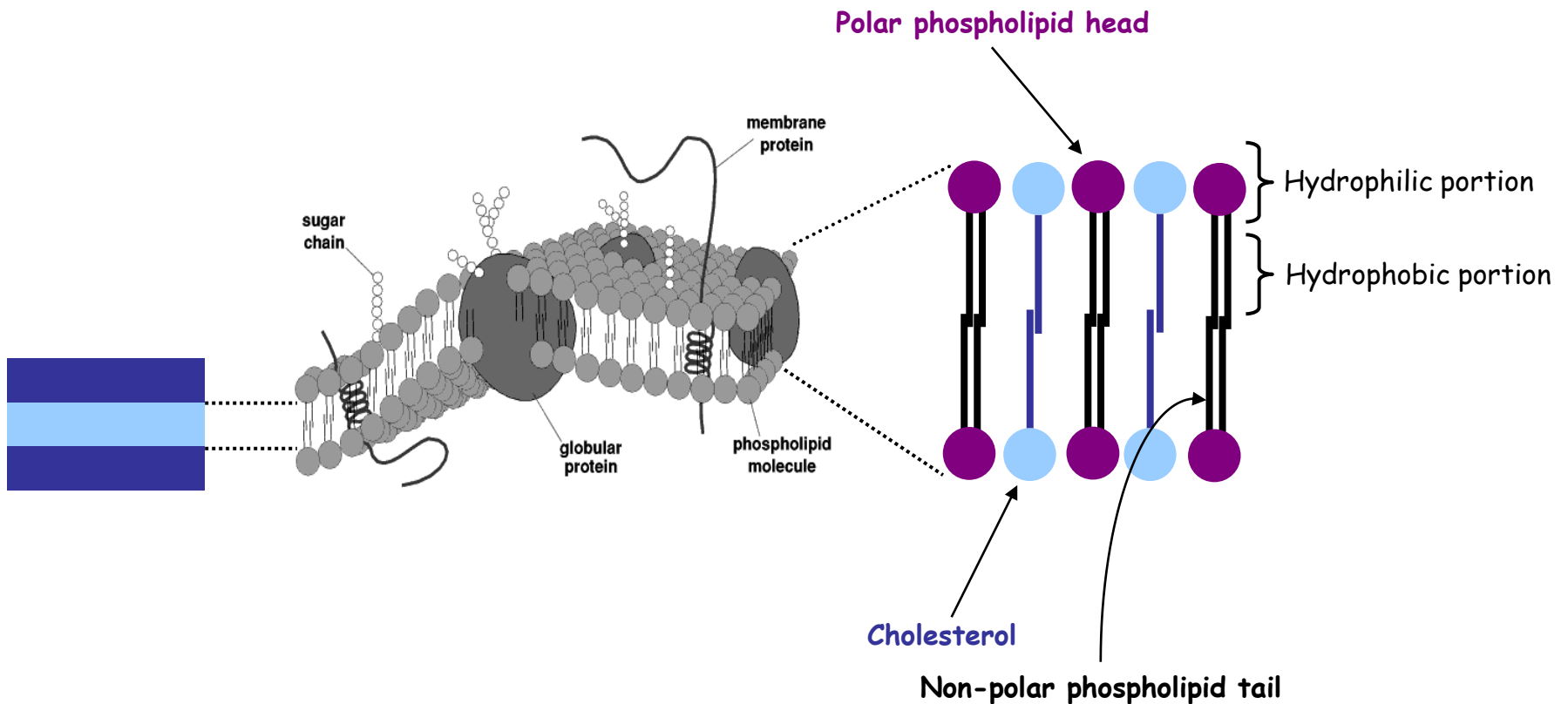
Cell membranes seen  
in electron microscope  
(pseudocolored)



**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

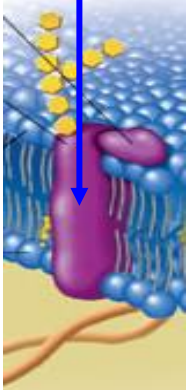
Make up 90-99% of molecules in membrane (in numbers).

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

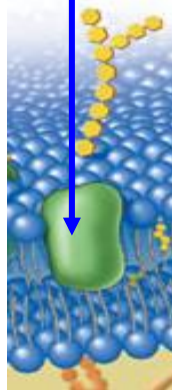
## Membrane proteins

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

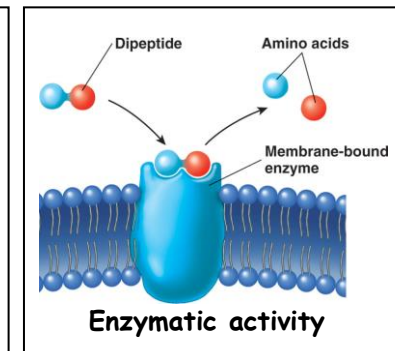
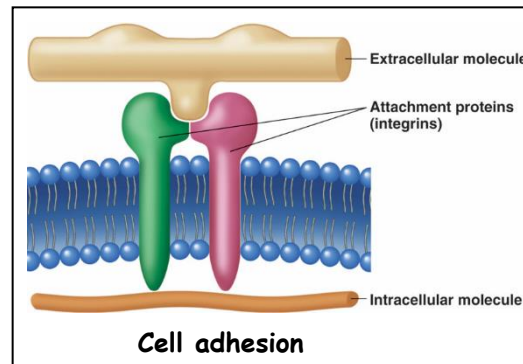
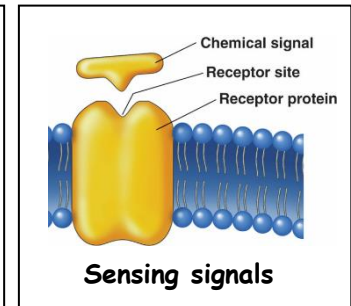
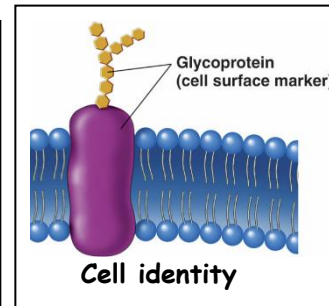
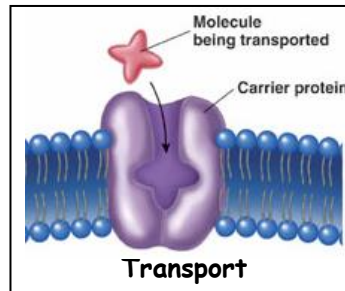
**Integral**



**Peripheral**



+



# Organelles

Specialized internal structures with specialized functions

## Membranous

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

## Non-membranous

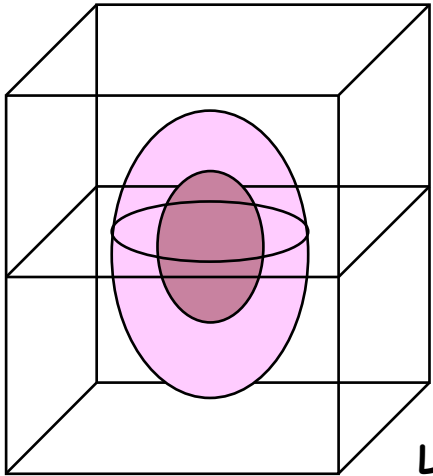
- Ribosomes
- Centrosomes
- Centrioles
- Basal bodies

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



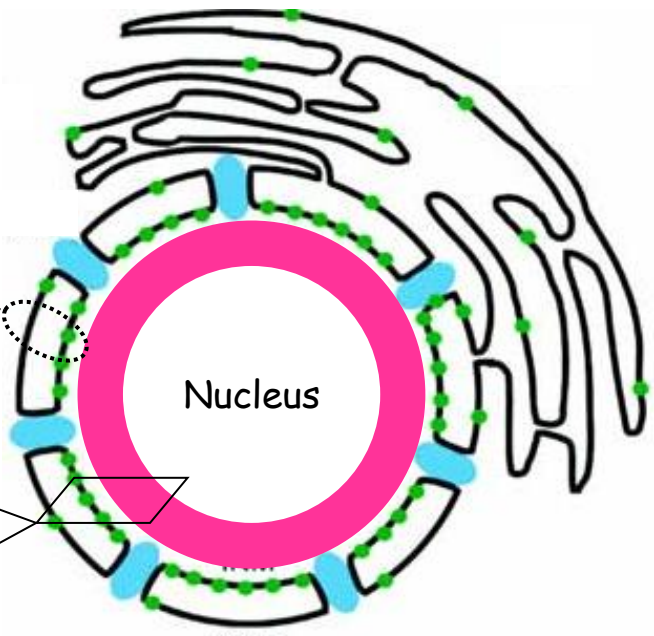
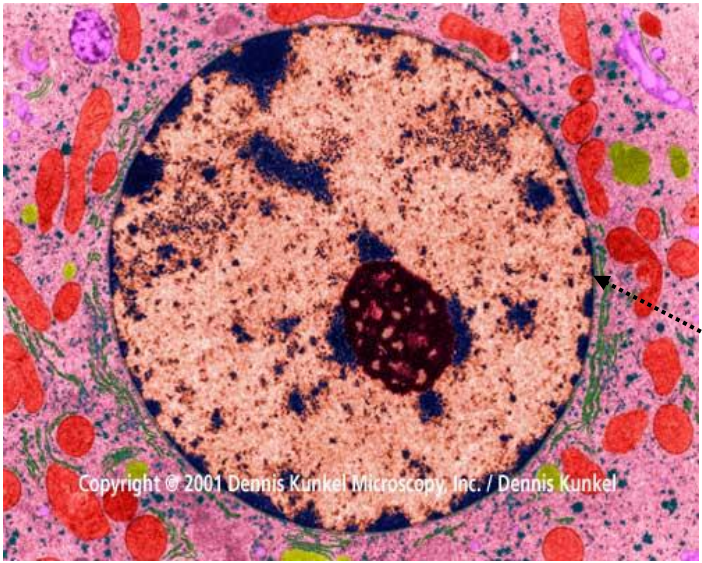
# Nucleus 1

## Envelop-bounded structure

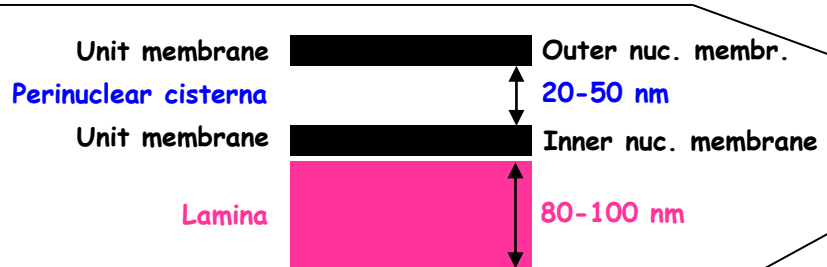


Liver cell nucleus

- Mostly:
- Spherical (5-10  $\mu\text{m}$ ) (lobular, twisted, disk-shaped,...)
  - Located centrally
  - One per cell (osteoclast more, erythrocyte none)

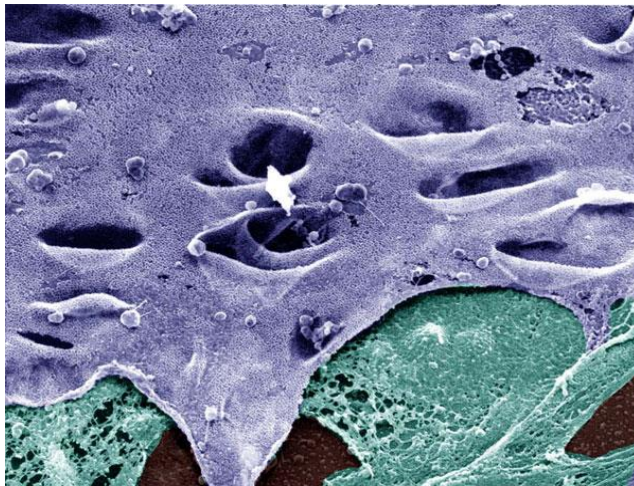
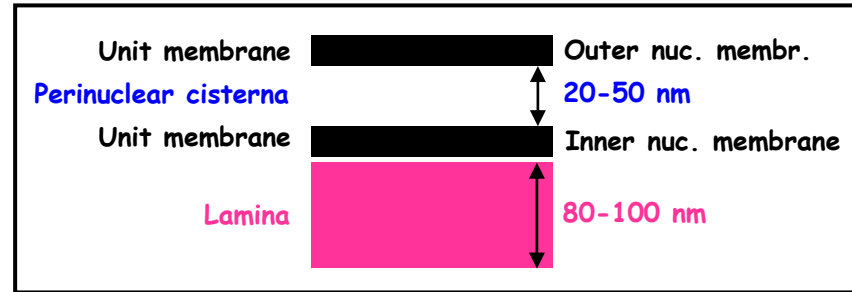
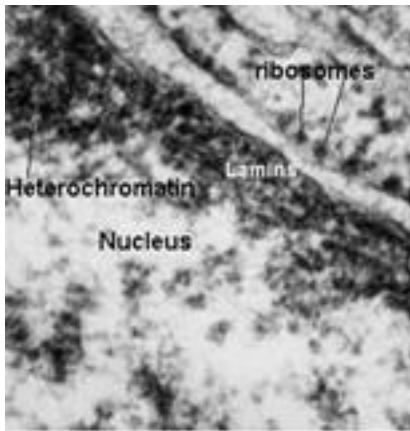


Nuclear envelope

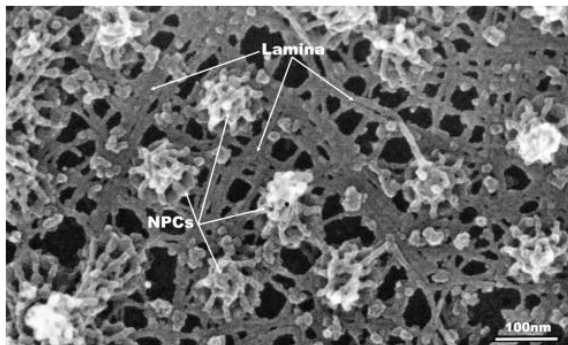


# Nucleus 2

## Continuation on nuclear envelop



- 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

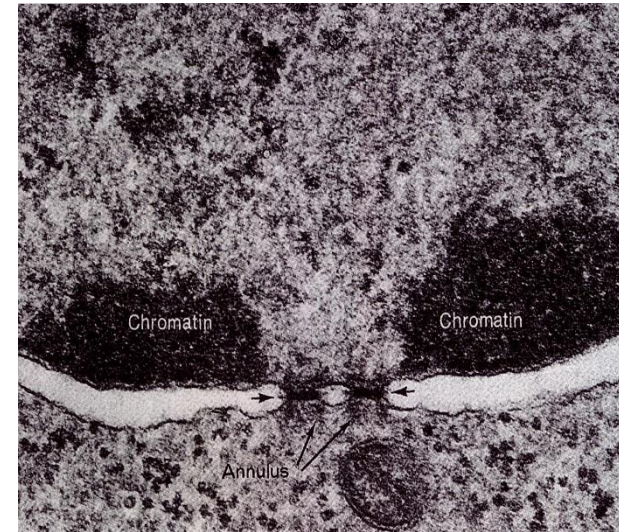
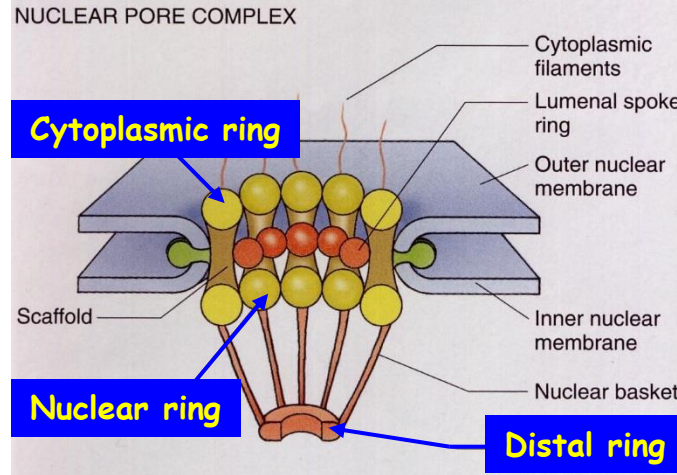
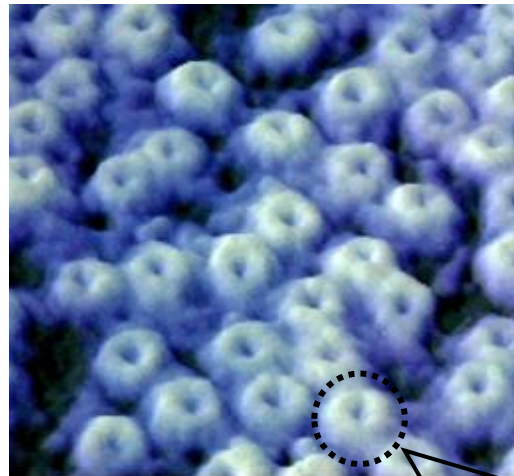


- Laminopathies**
- Human diseases (at least 13 known)
  - Mutations in lamin genes (almost 200 mutations known)
  - Deregulated gene expression
  - Premature aging



# Nucleus 3

## Nuclear pore complex



### Transport via nuclear pores (Nucleocytoplasmic shuttling)

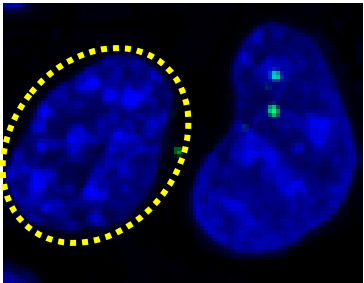
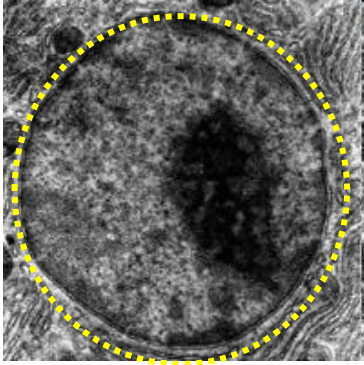
- Proteins, RNAs, ribosome subunits
- Bidirectional
- Needs nuclear localization/export signals
- Helped by importins/exportins
- Regulated by Ran GTPases

Diameter ~ 100 - 125 nm

Three rings (8 subunits each)

Inner filamentous basket

# Nucleus 4 Chromatin



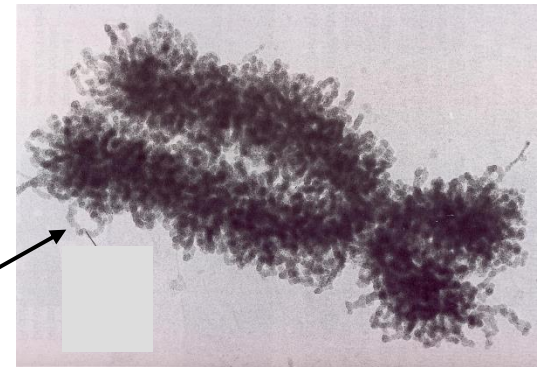
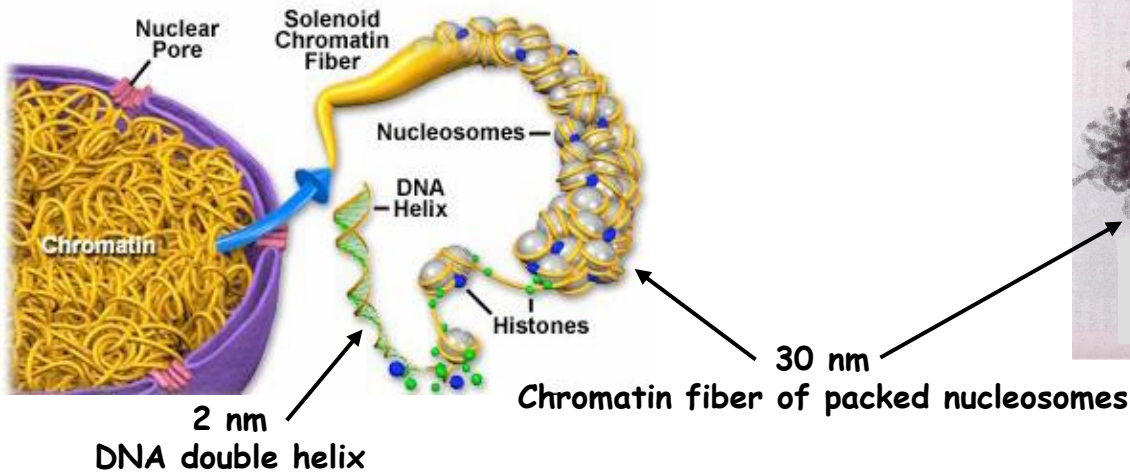
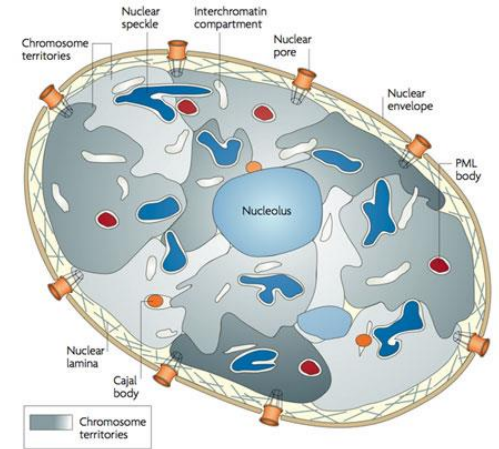
## Interphase nucleus

### Heterochromatin

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

### Euchromatin

Invisible in light microscope  
Relaxed uncoiled chromosomes  
Transcriptionally active

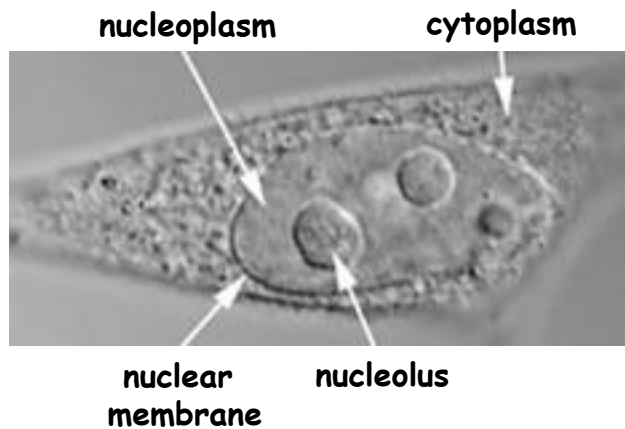
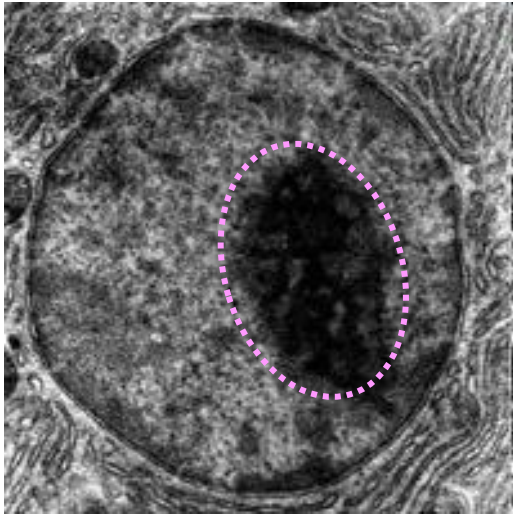


# Nucleus 5 Nucleolus

non-membrane-bounded structure

## Main functions

Synthesis of rRNA  
Assembly of ribosomes



**Pars granulosa**  
Assembly of ribosomes

**Pars fibrosa**  
Primary transcripts of rRNA

## Nucleolar-organizing regions of DNA

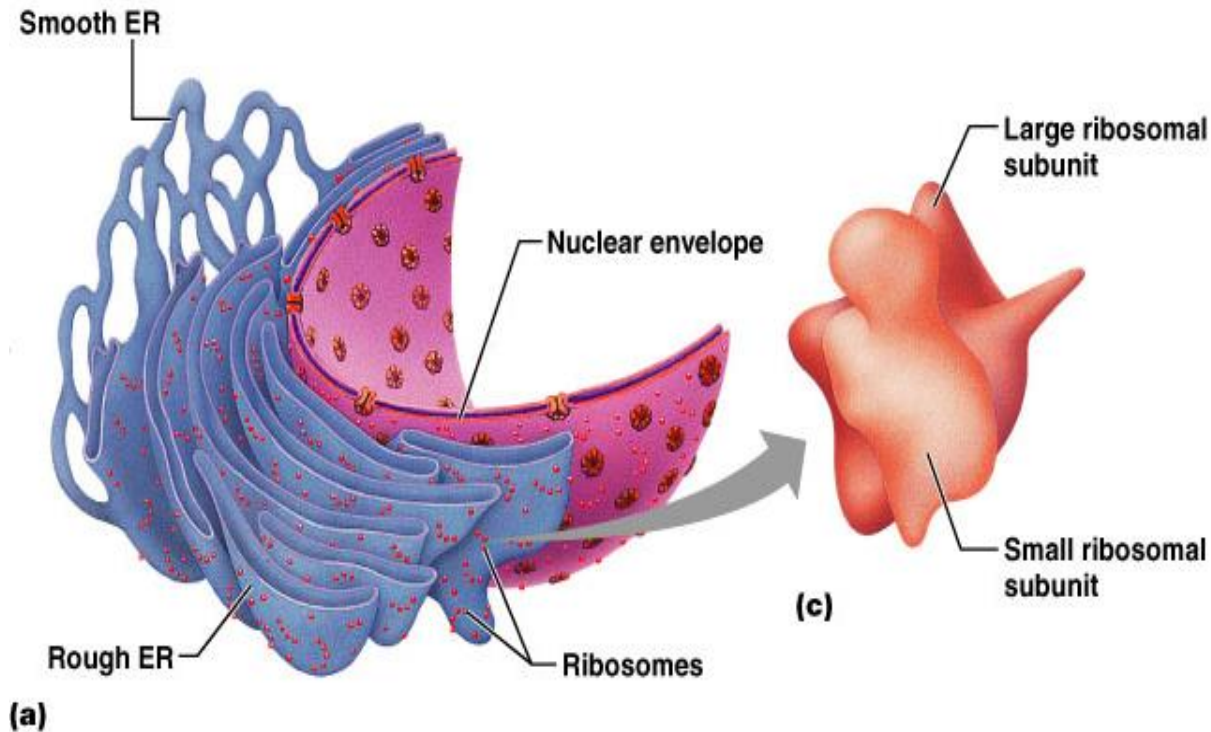
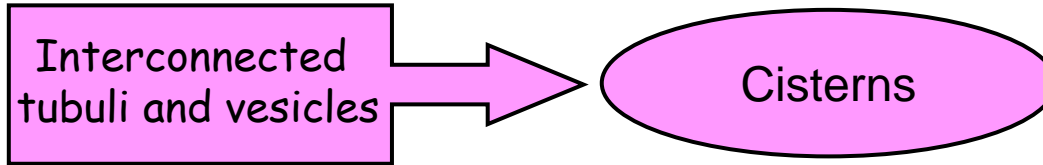
on five chromosomes in human cells  
(chrs. 13, 14, 15, 21, 22)

# Endoplasmic reticulum 1

„within cell“

„net“

Majority of the membrane within cells.



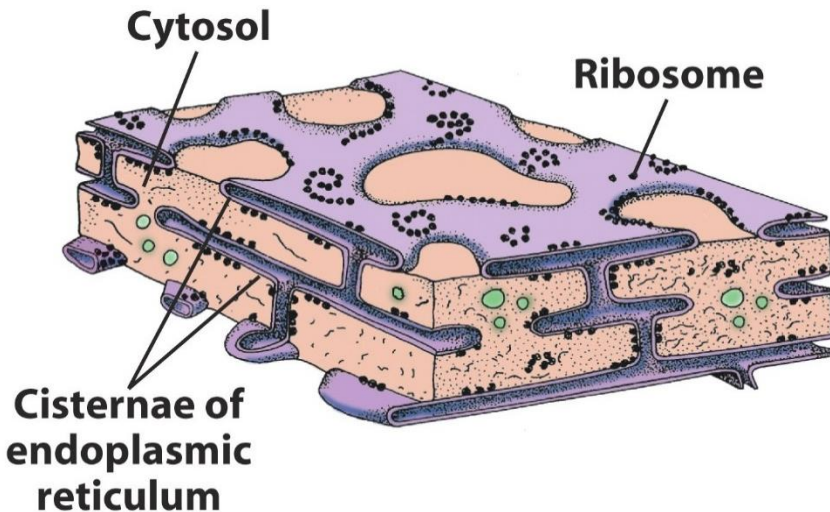
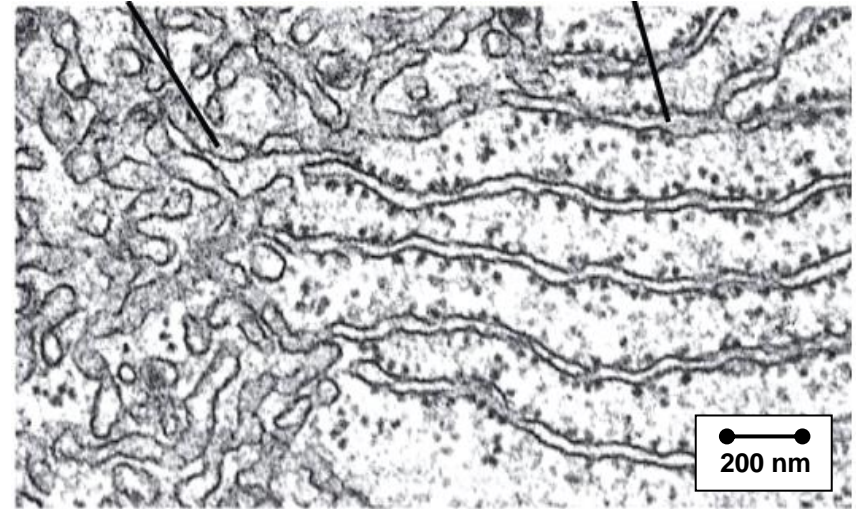
# Endoplasmic reticulum 2

**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

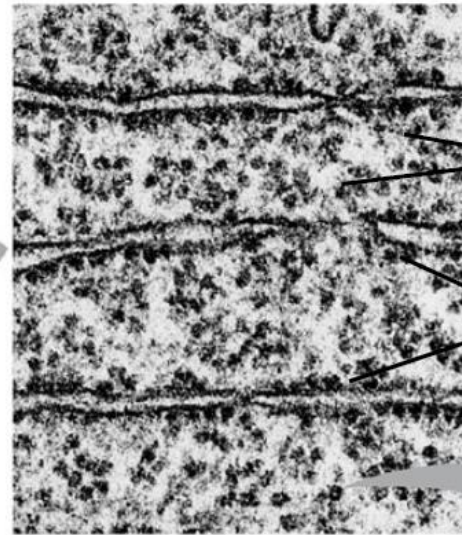
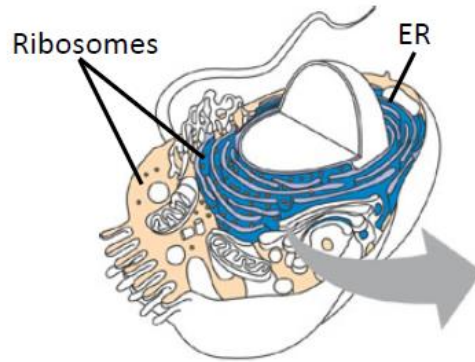
## Rough ER



External surface **has ribosomes attached**

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

# Ribosomes



0.5  $\mu\text{m}$

Endoplasmic reticulum (ER)

Free ribosomes

Bound ribosomes

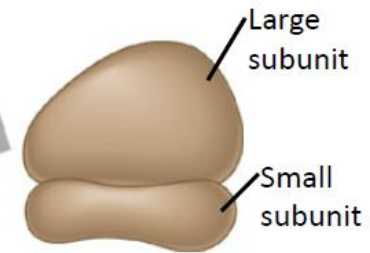
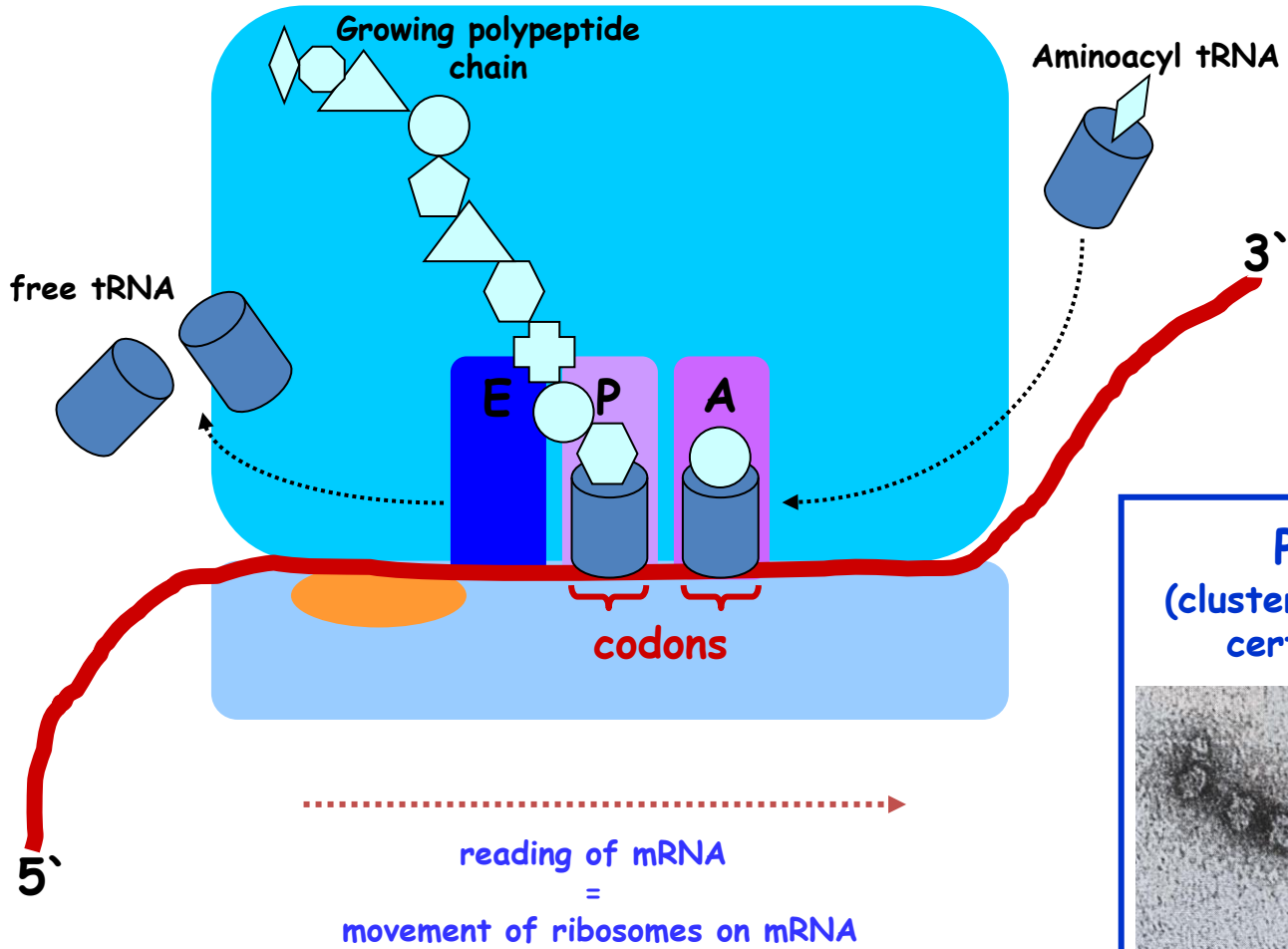


Diagram of a ribosome



# Ribosomes - Translation



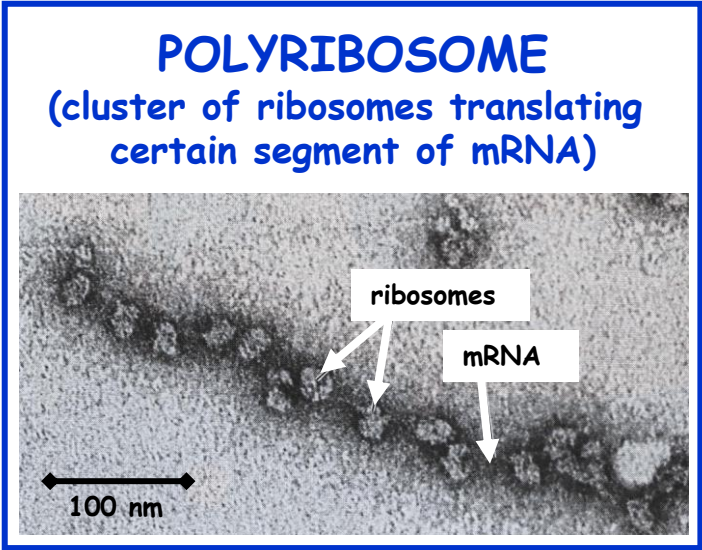
**Beginning of translation**

Met-tRNA

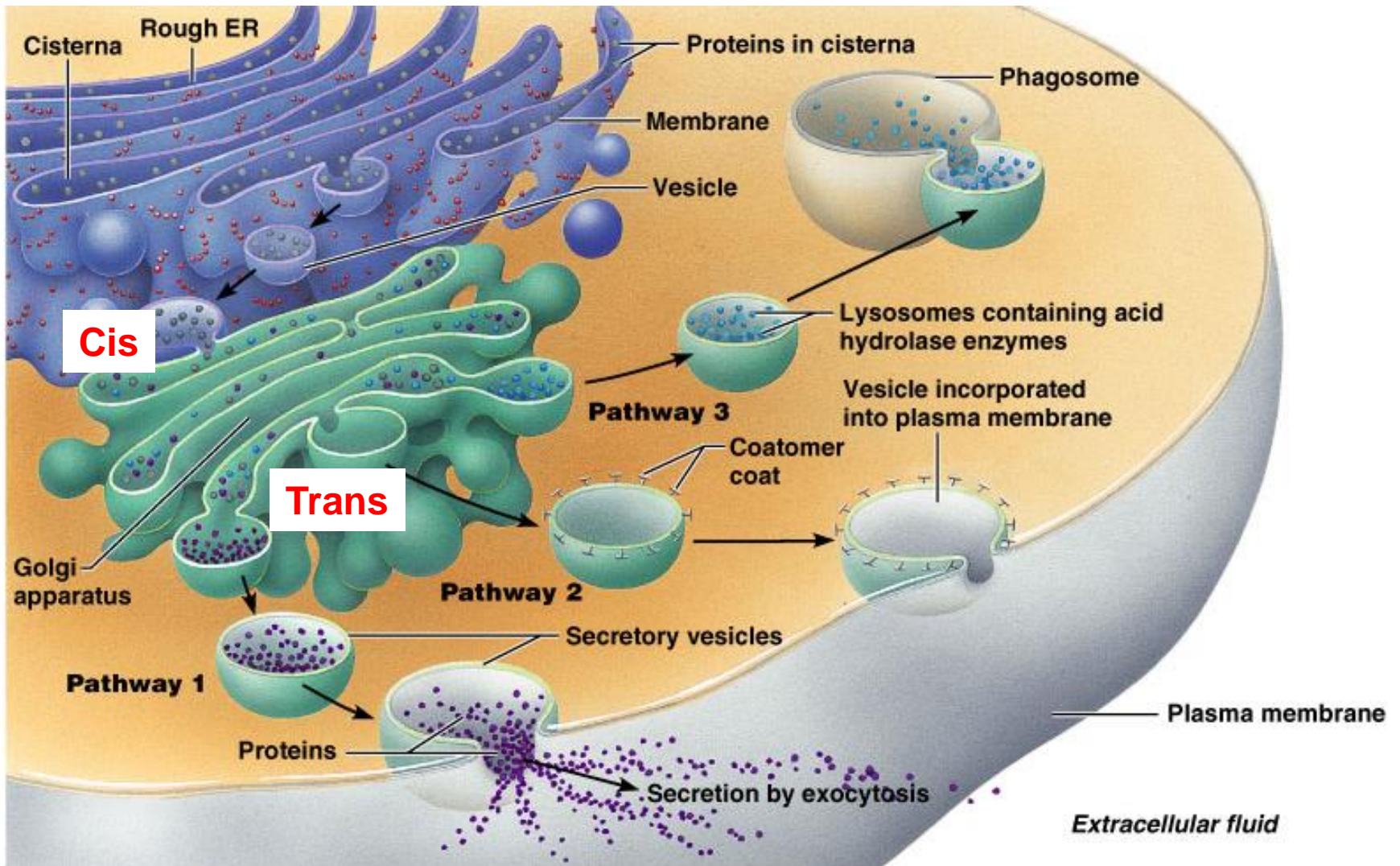
mRNA 5' — **AUG** — 3'  
 3' UAC 5'  
 START kodon

**End of translation**

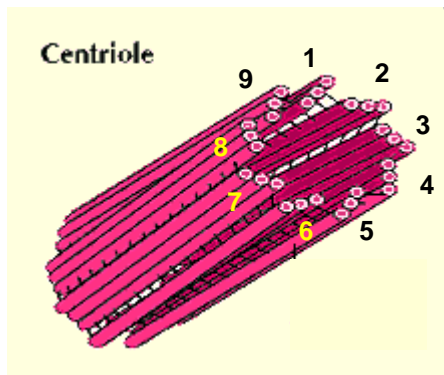
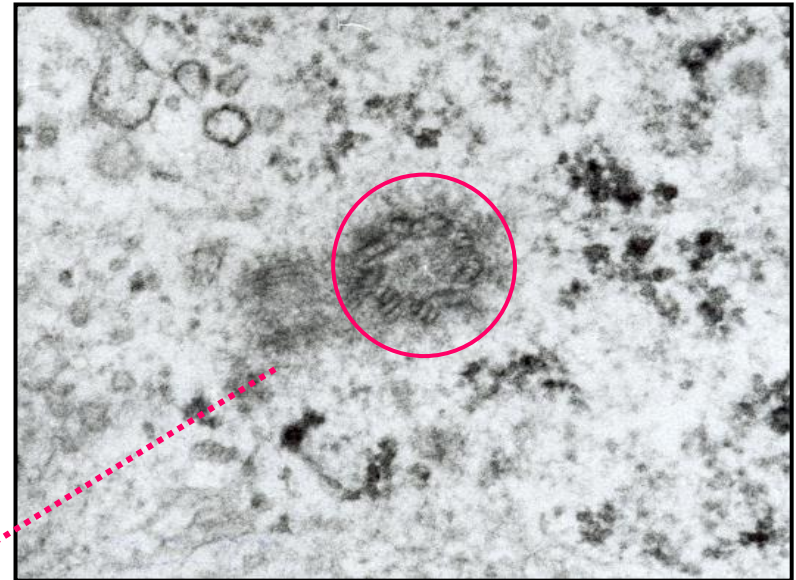
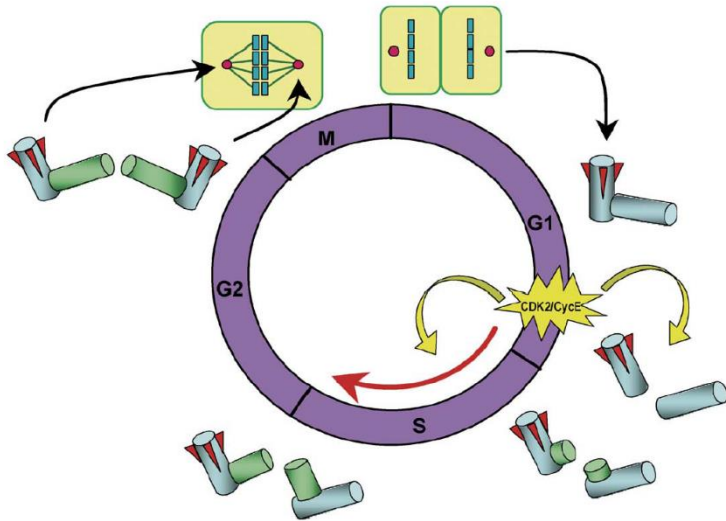
mRNA 5' — UAG — 3'  
 mRNA 5' — UAA — 3'  
 mRNA 5' — UGA — 3'  
 STOP kodony  
 bind „release factor“



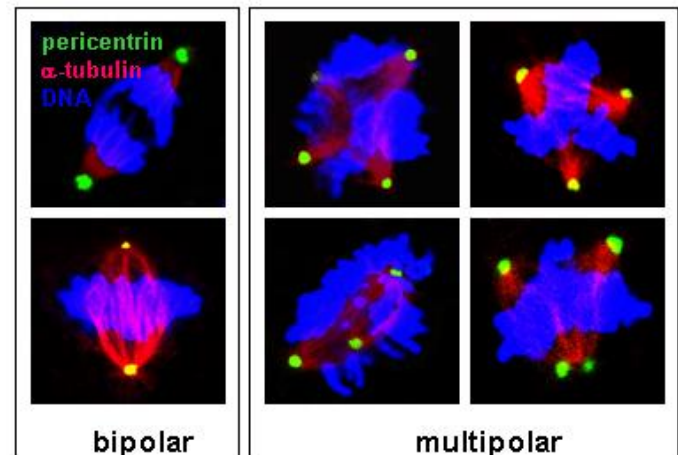
# Golgi apparatus - Transgolgi pathway



# Centrosome



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



# 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 - 1<sup>st</sup> floor