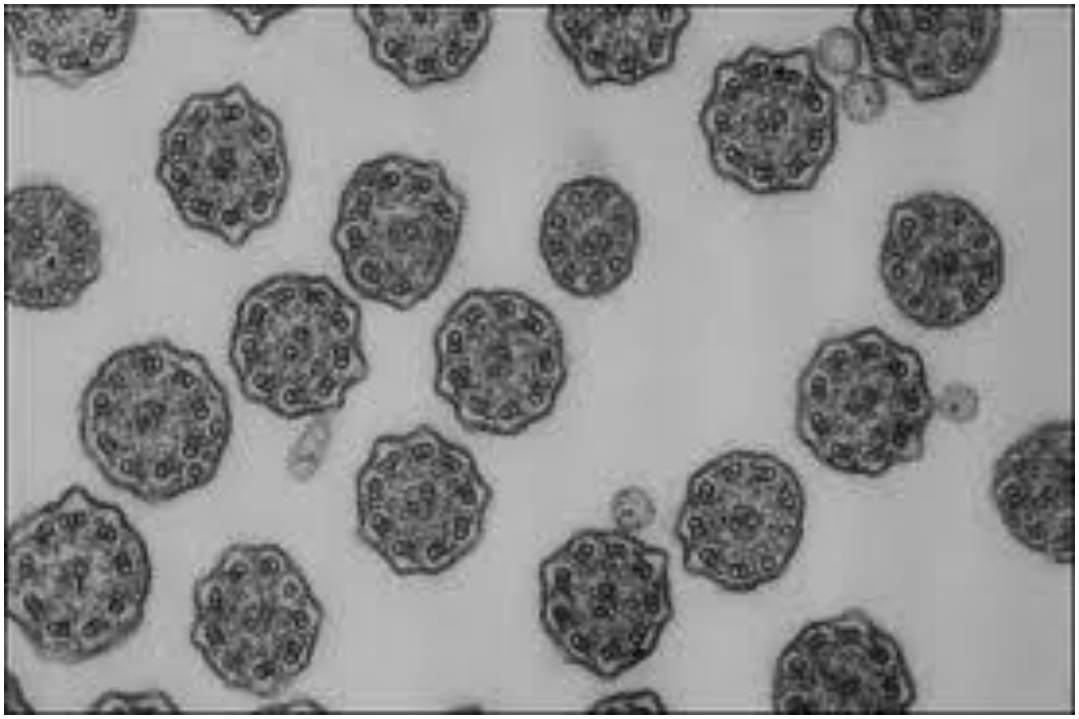
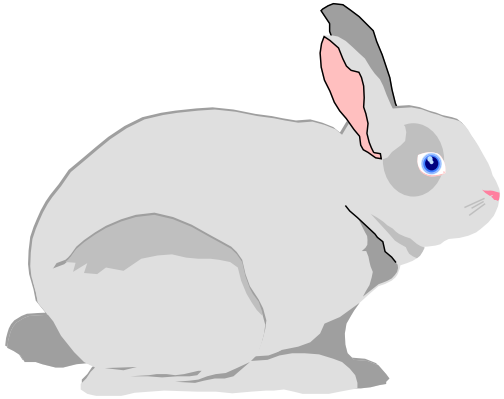
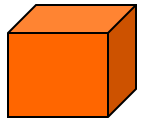


CELL ULTRASTRUCTURE ELECTRON MICROSCOPE

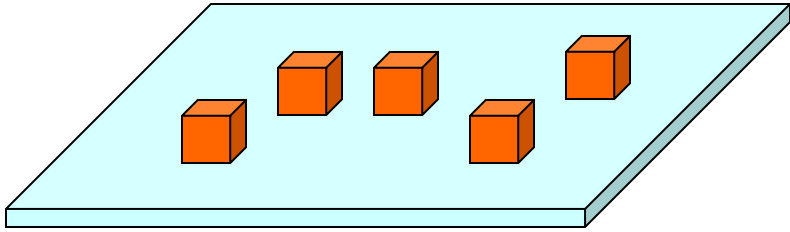
TEM tissue preparation



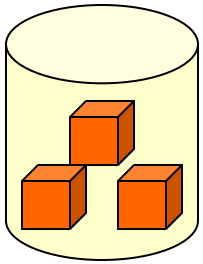
Tissue collection



Tissue trimming

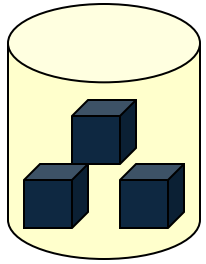


Fixation - **glutaraldehyde**

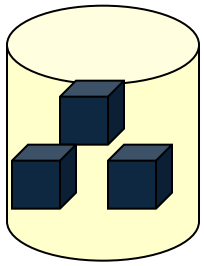


Washing in buffer

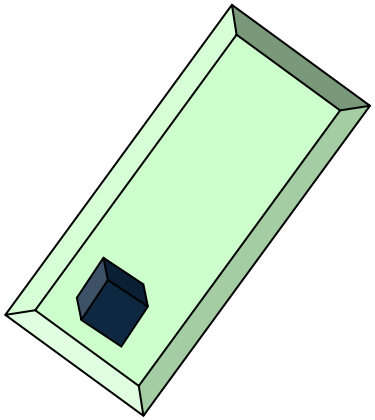
Post-fix in osmium tetroxide

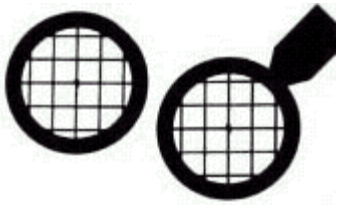


Dehydration in alcohol



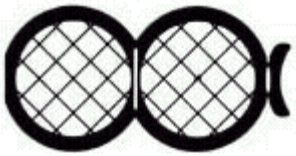
Embedding in Epoxy resins in 60 – 70 ºC



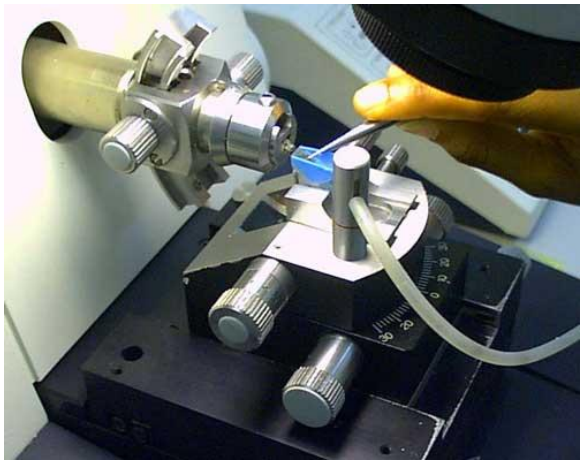


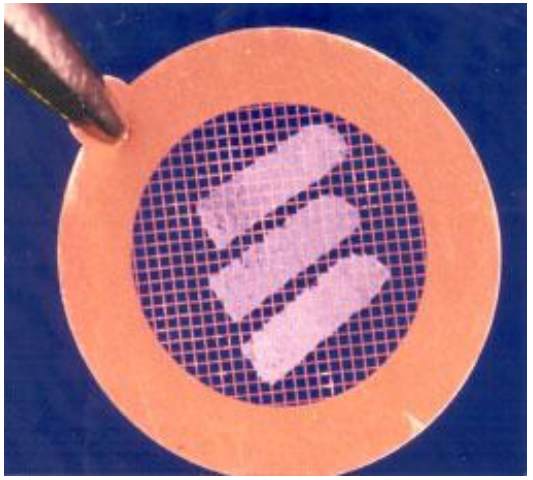
Ultrathin sections at **60-90 nm** thick

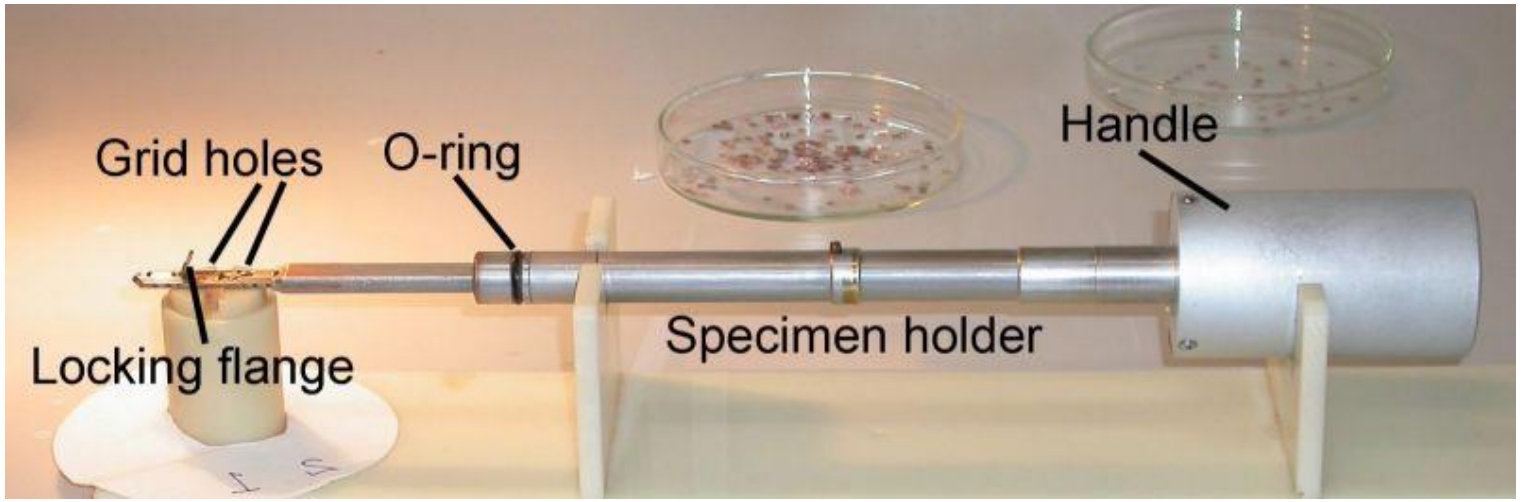
Collecting sections onto grids



Staining grids with uranyl acetate and lead citrate



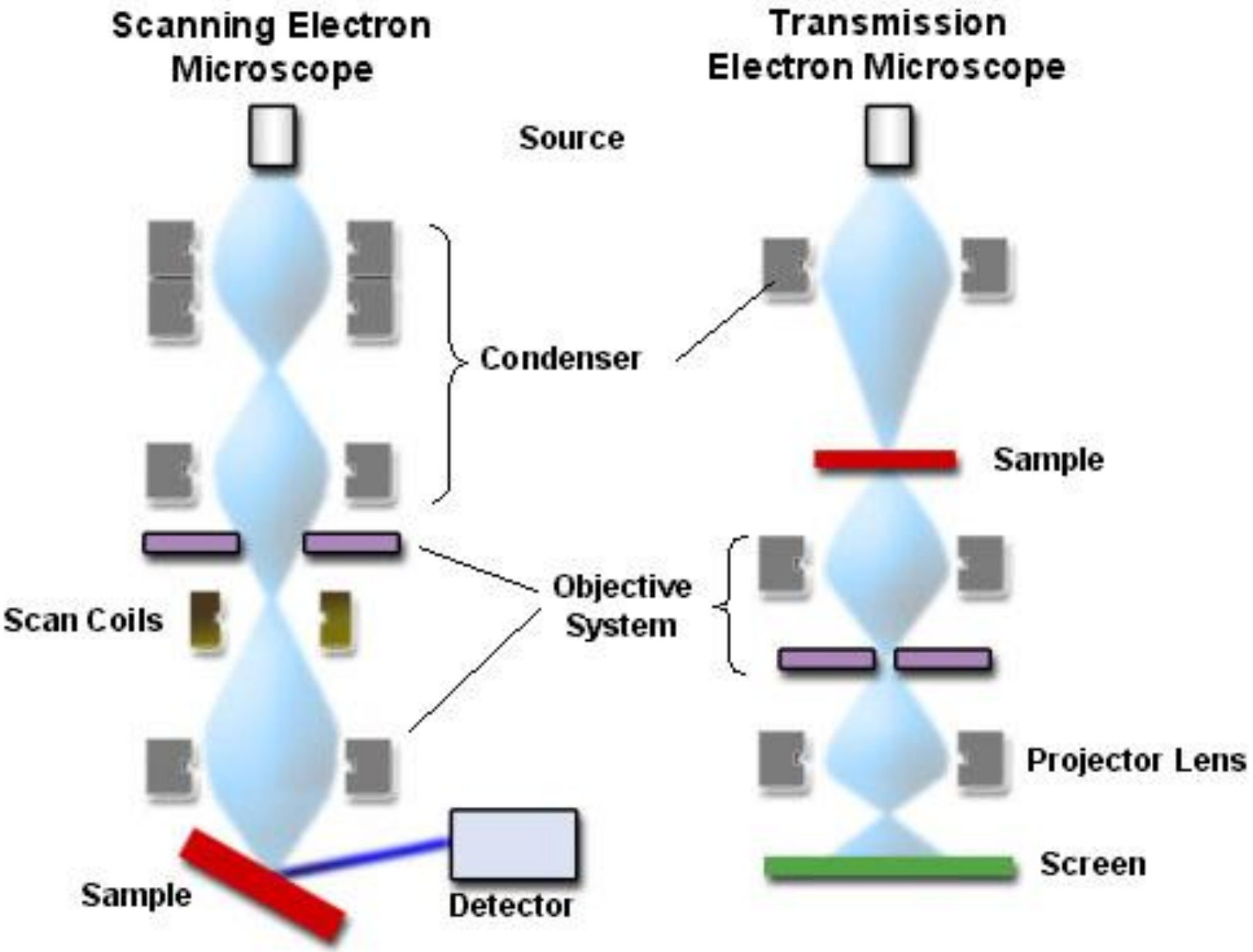



Grid holes
O-ring
Handle
Specimen holder
Locking flange

TEM stains

	Nucleic acids	Proteins	Phospholipids	Polysaccharides	Fat
OsO_4	○	◐	●	○	● Nienasycone +
aldehyde	○	●	○	○	○
Uranyl acetate	●	●	○	○	○
Lead citrate	●	●	●	○ Glikogen +	◐

● Strong ◐ Medium ○ Weak


Scanning Electron Microscope
Transmission Electron Microscope
Source
Condenser
Sample
Objective System
Scan Coils
Projector Lens
Screen
Sample
Detector

Ernst August Friedrich Ruska

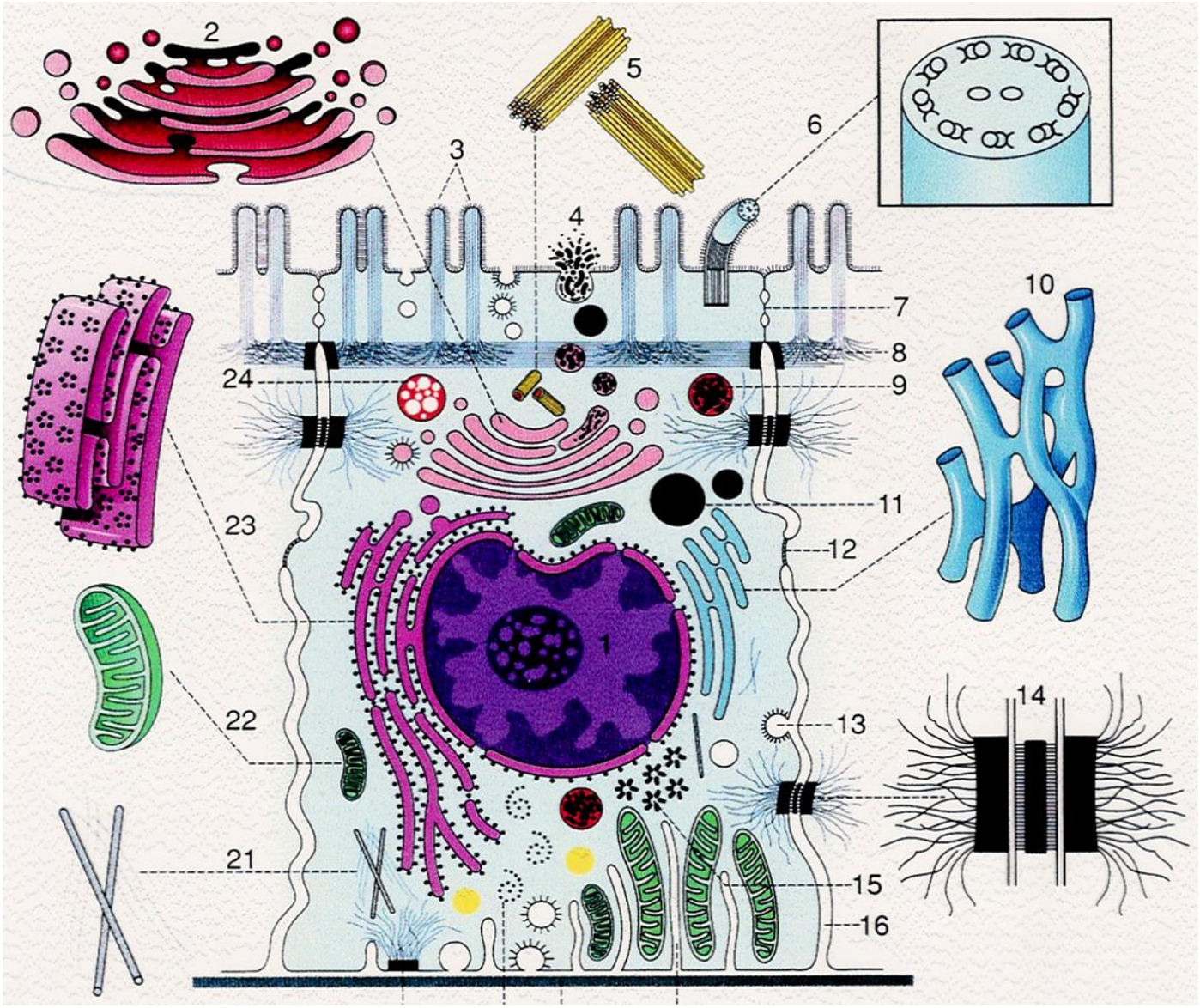
1931 – 1933 – first electron microscope

1939 –Siemens-Reiniger-Werke AG - 'Siemens Super Microscope’

1986 – Nobel prize with Gerd Binnig and Heinrich Rohrer

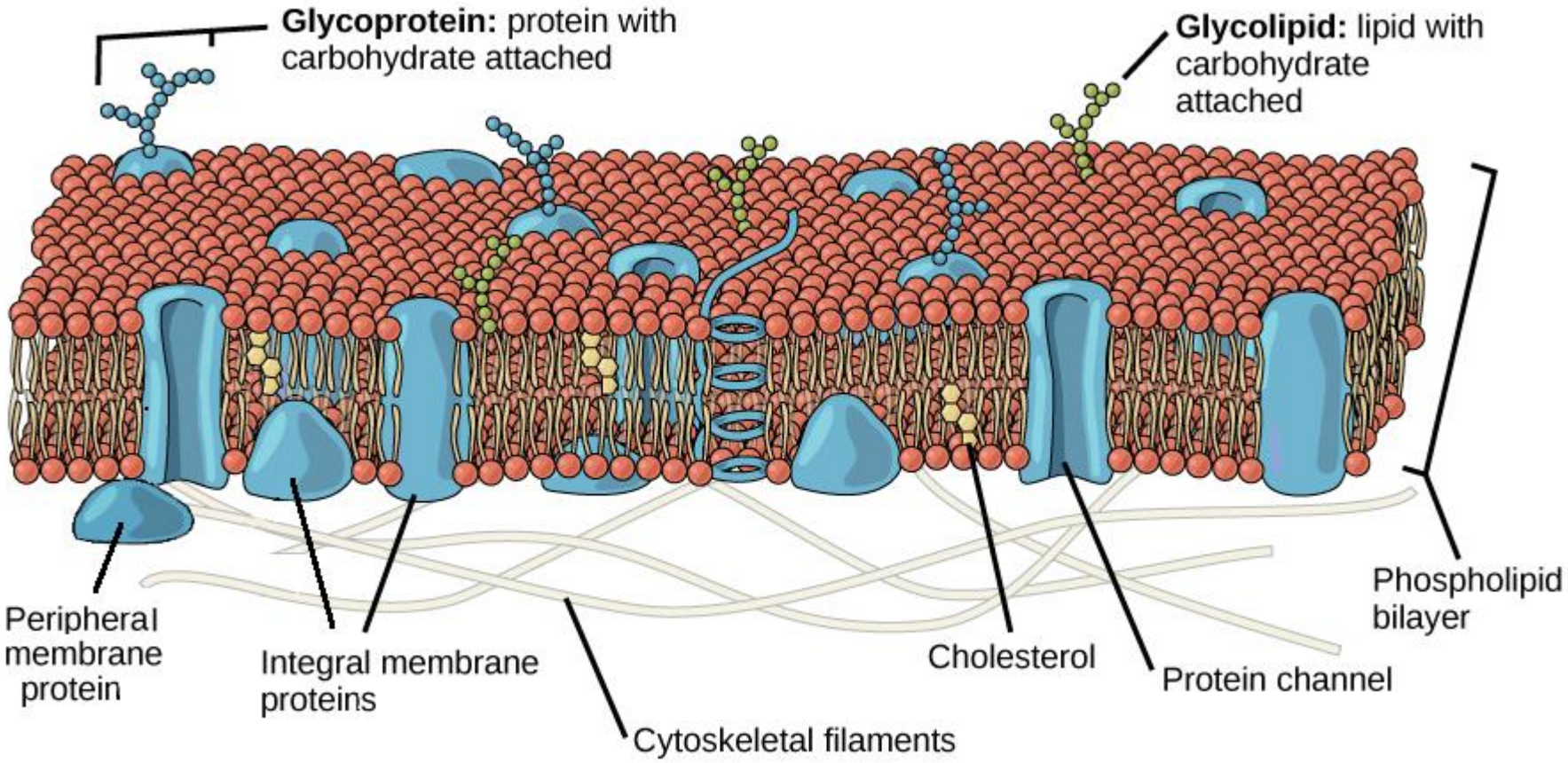
3 MV Ultra-High Voltage Electron Microscope

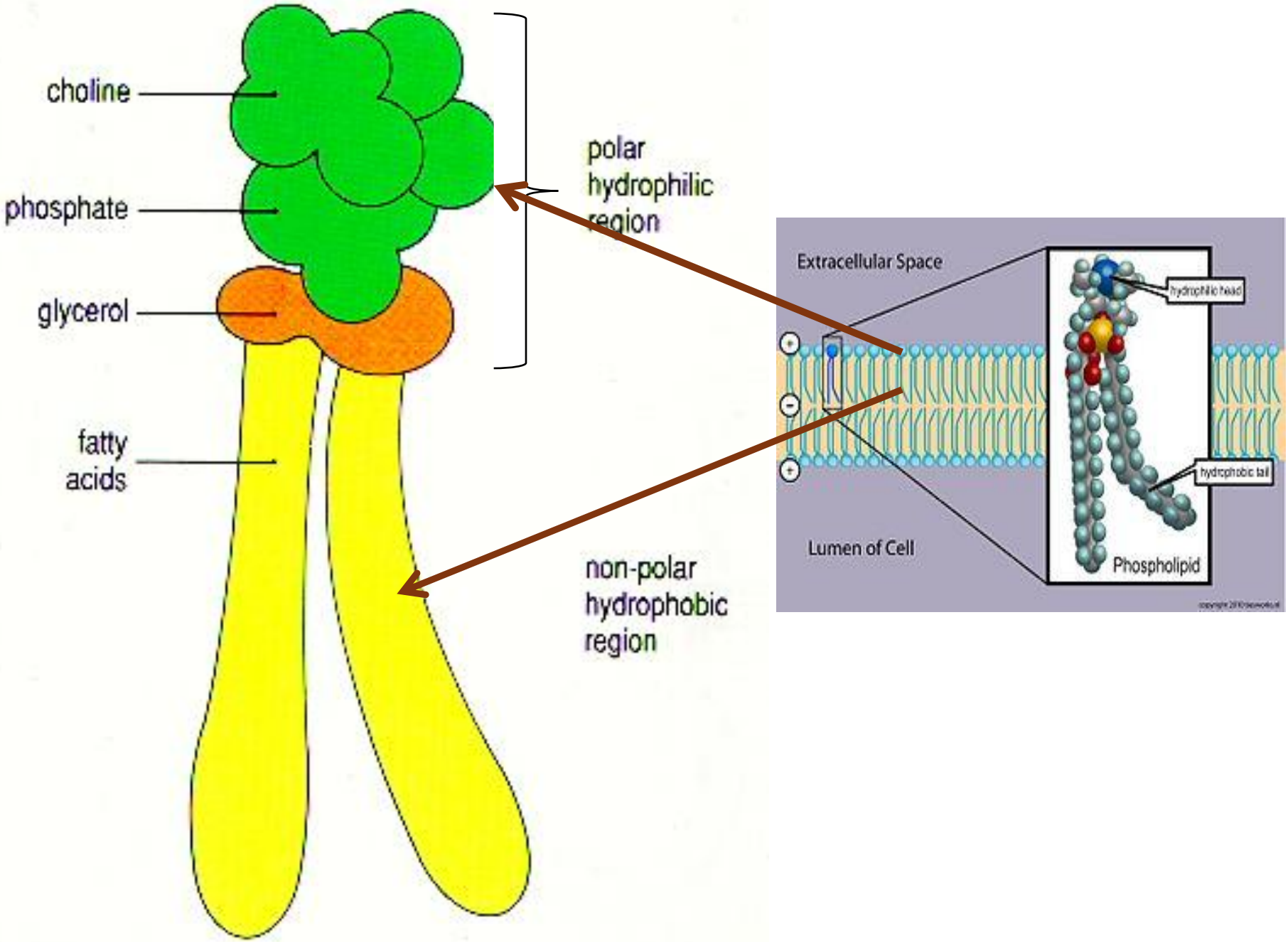
Model : H-3000 Height : 13.5 m Weight : 140 tons


2
5
6
3
4
7
8
24
9
10
23
11
12
1
22
13
14
21
15
16

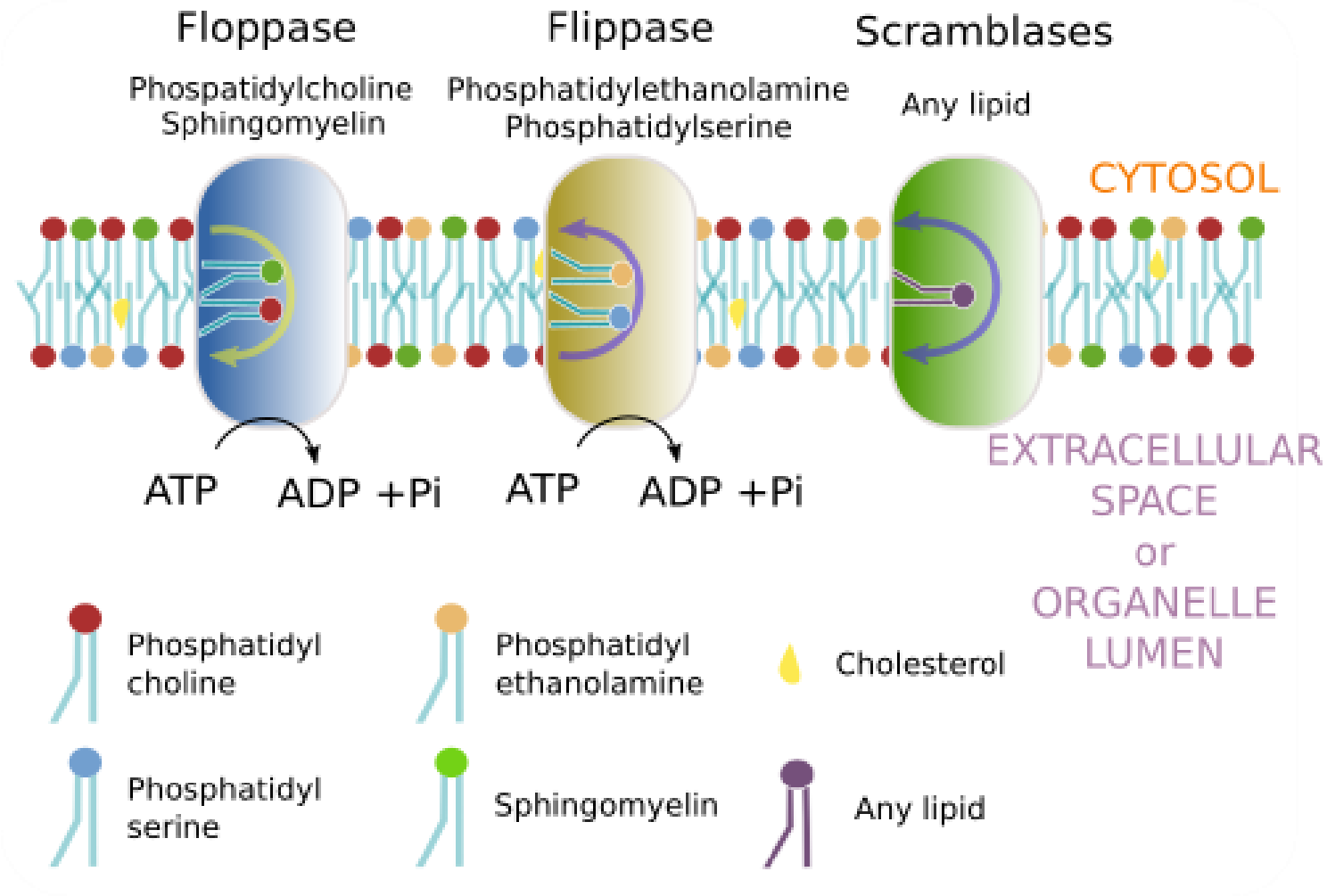
Functions of the cell membrane

- Maintaining the integrity of the cell
- Controlling movements of substances in and out of the cell
- Regulating cell-cell interactions and recognizing of antigens
- Establishing transport system for specific molecules
- Transducing extracellular signals

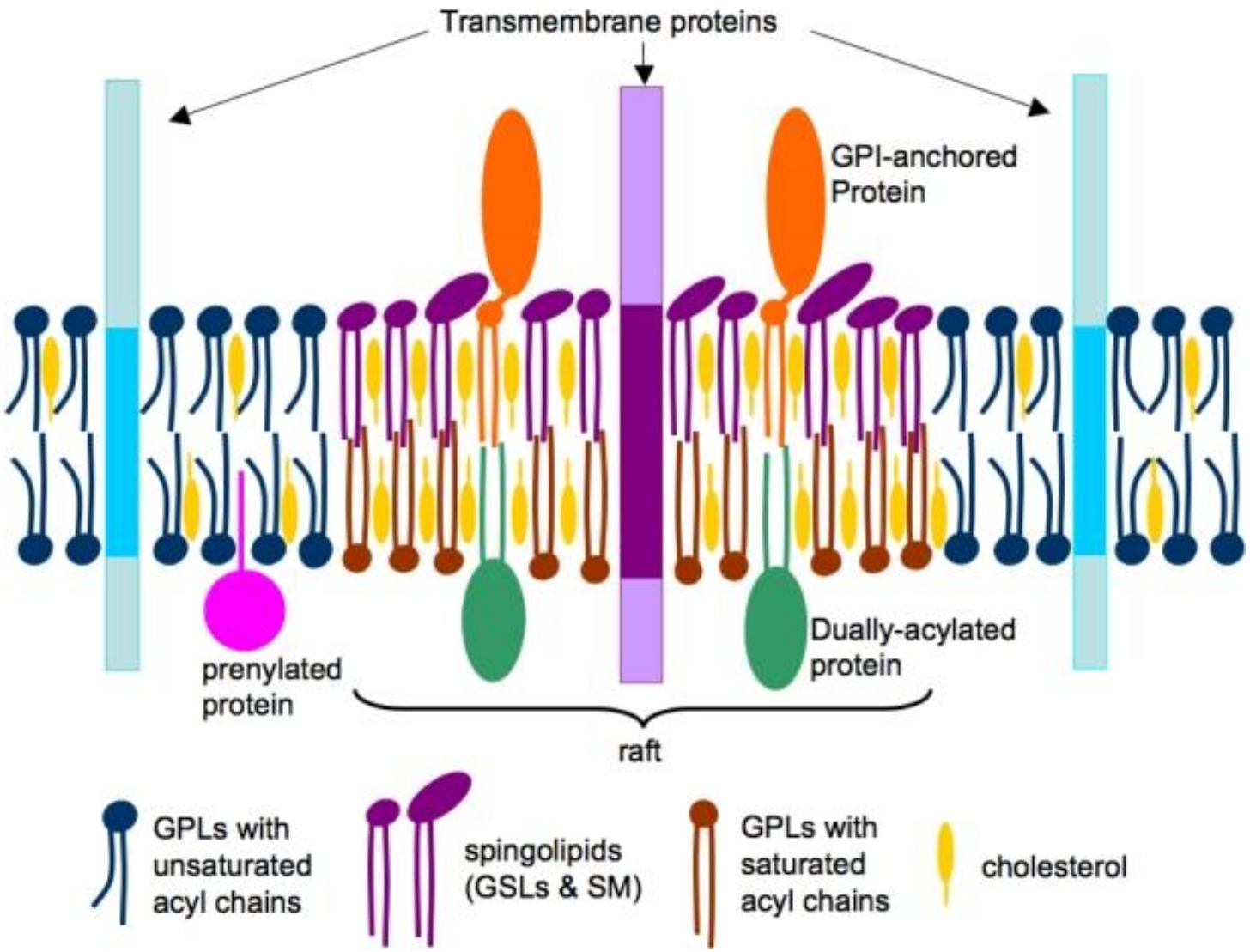



choline
phosphate
glycerol
fatty acids
polar hydrophilic region
non-polar hydrophobic region
Extracellular Space
Lumen of Cell
hydrophilic head
hydrophobic tail
Phospholipid

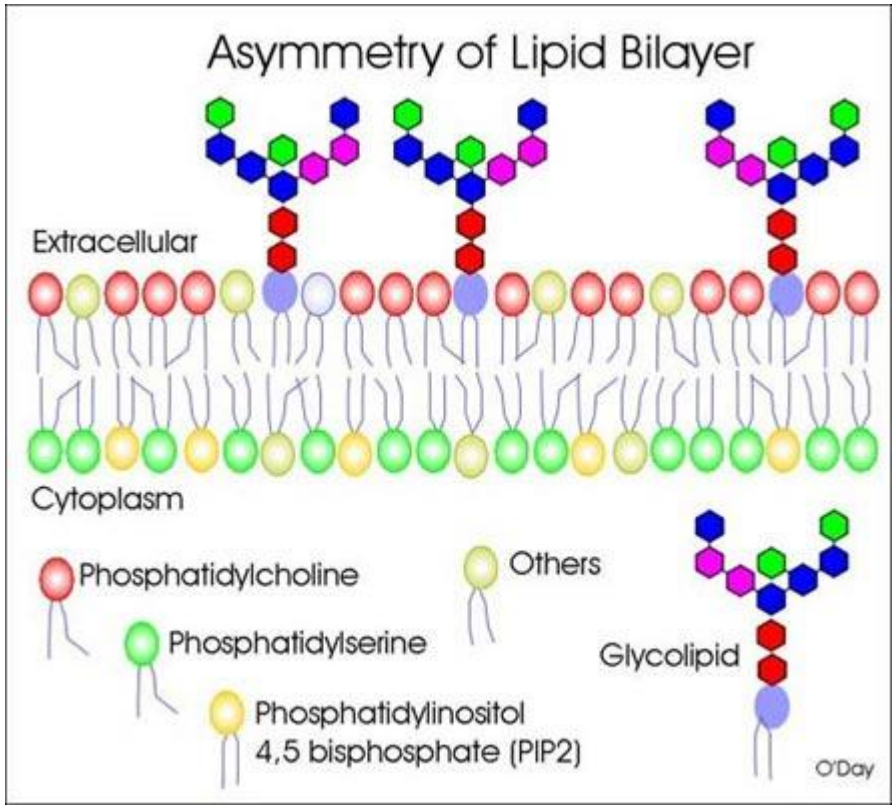
MEMBRANE ASSYMETRY

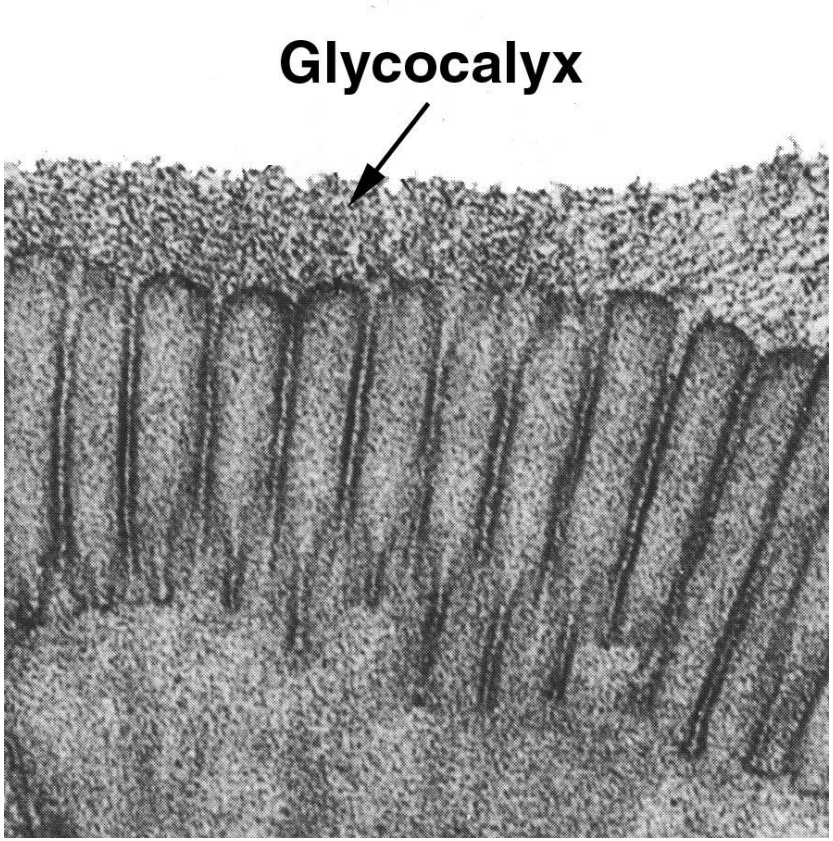


CHOLESTEROL AND LIPID RAFTS

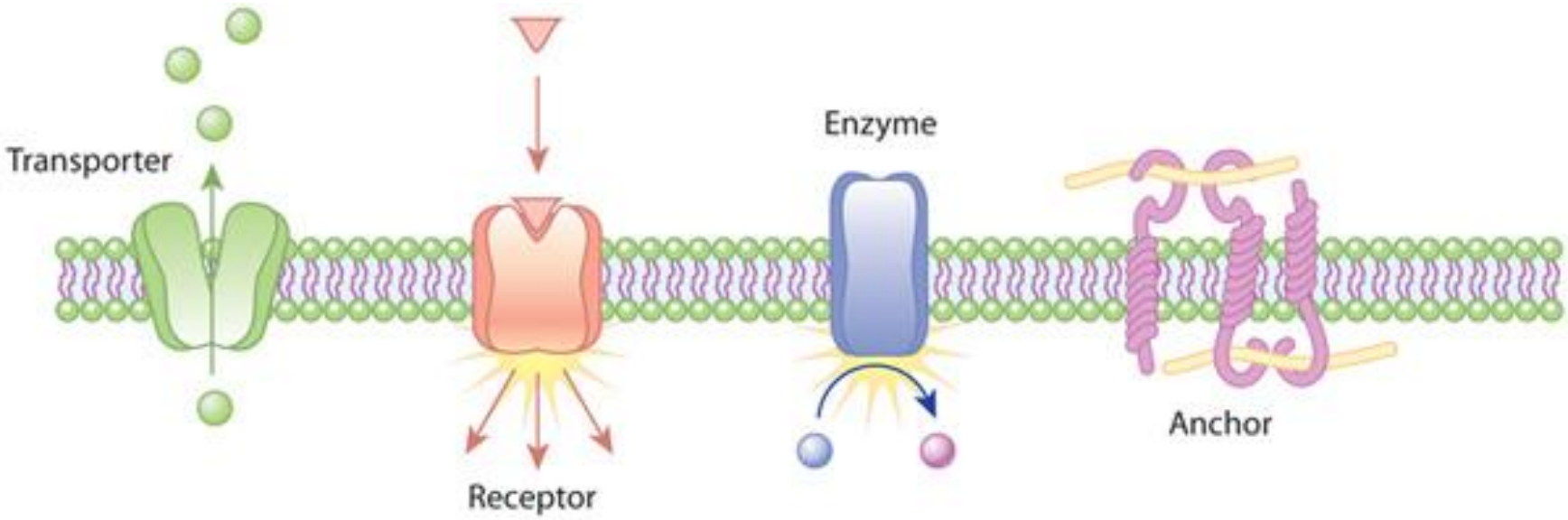


GLYCOLIPIDS

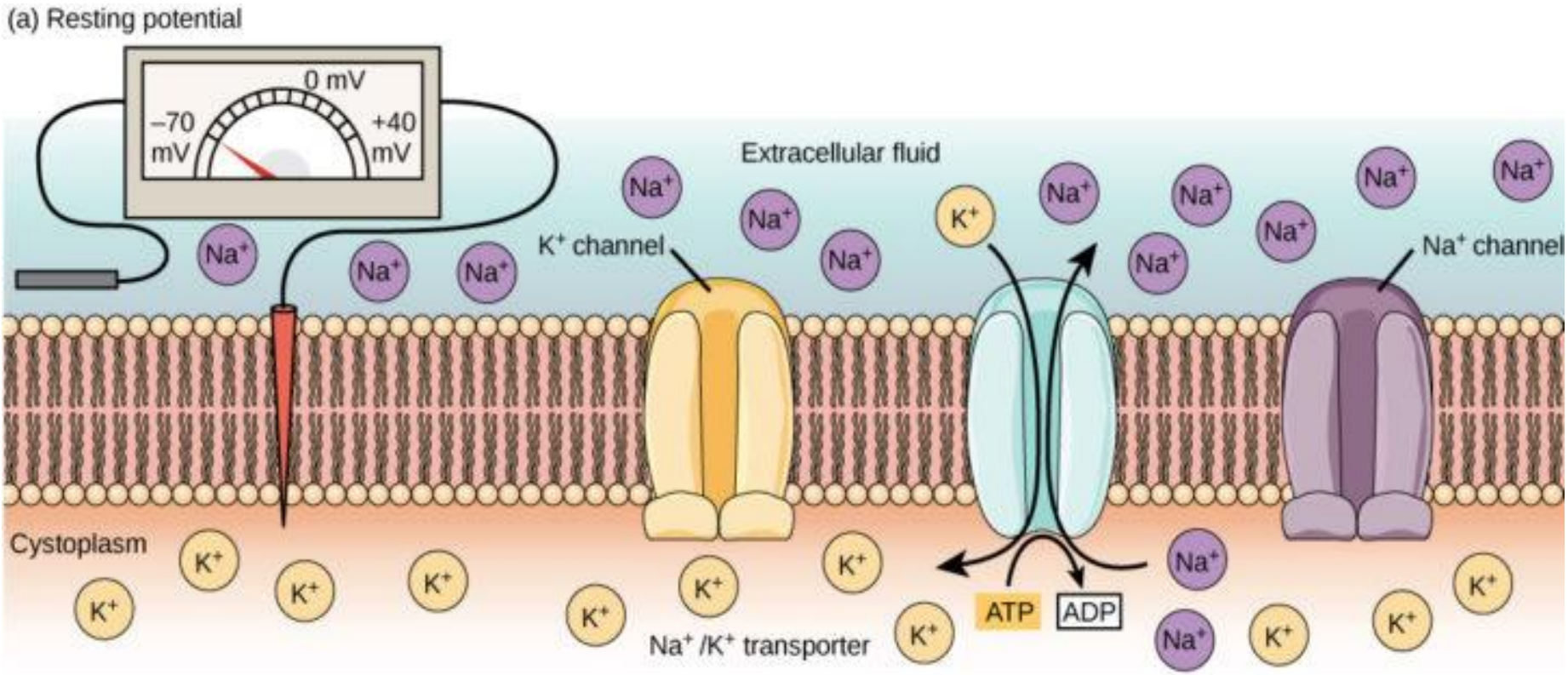




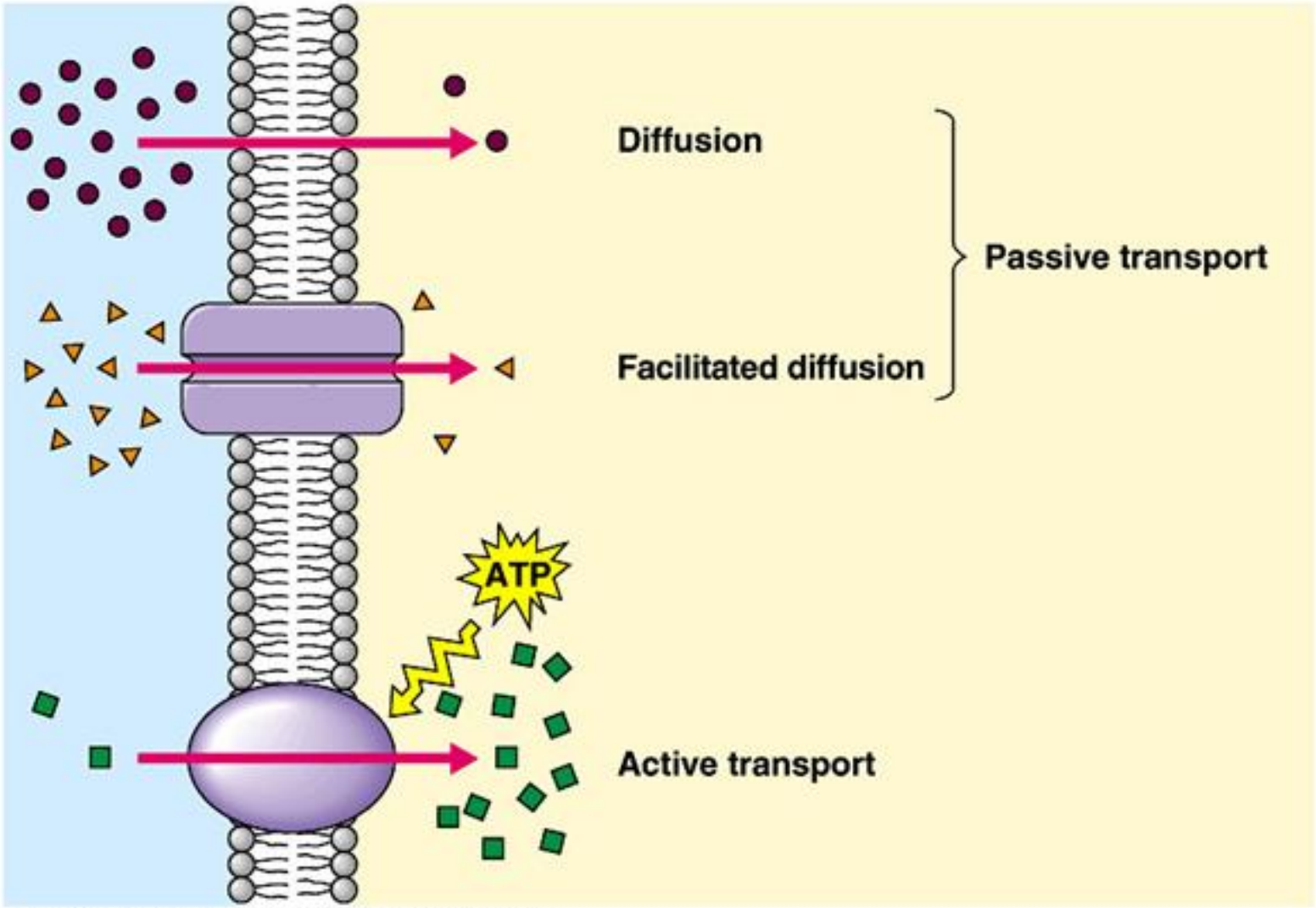
FUNCTION OF TRANSMEMBRANE PROTEINS



MEMBRANE POTENTIAL



At the resting potential, all voltage-gated Na^+ channels and most voltage-gated K^+ channels are closed. The Na^+/K^+ transporter pumps K^+ ions into the cell and Na^+ ions out.



Small hydrophobic

O_2
CO_2
N_2

Steroid hormones, vitamines

Small polar Particles – no charge

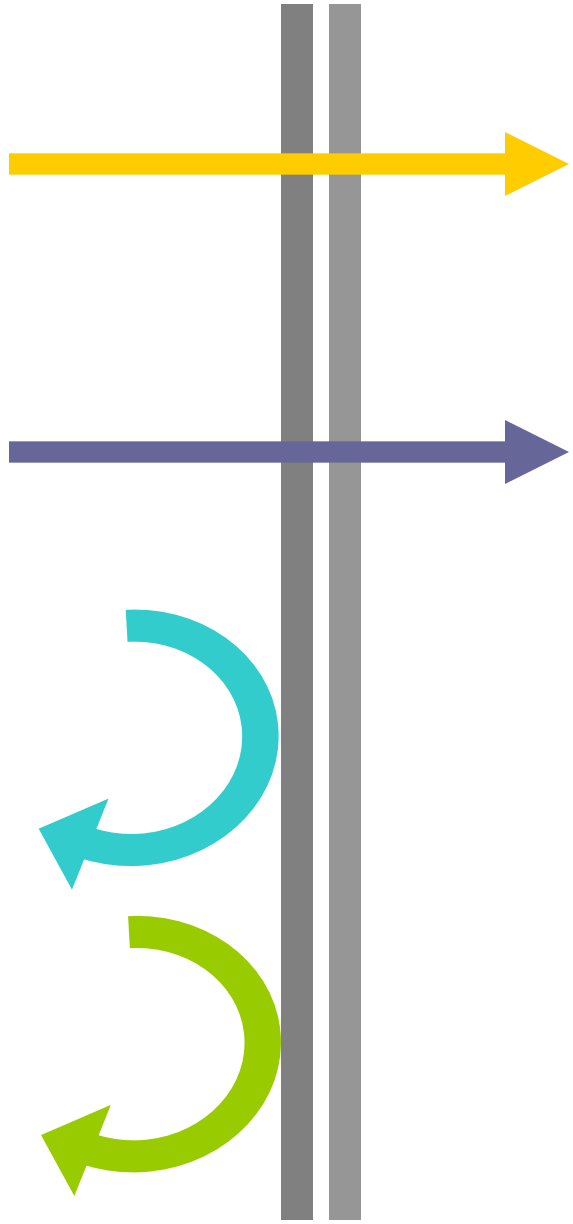
H_2O
glicerol
etanol

Big polar Particles – no charge

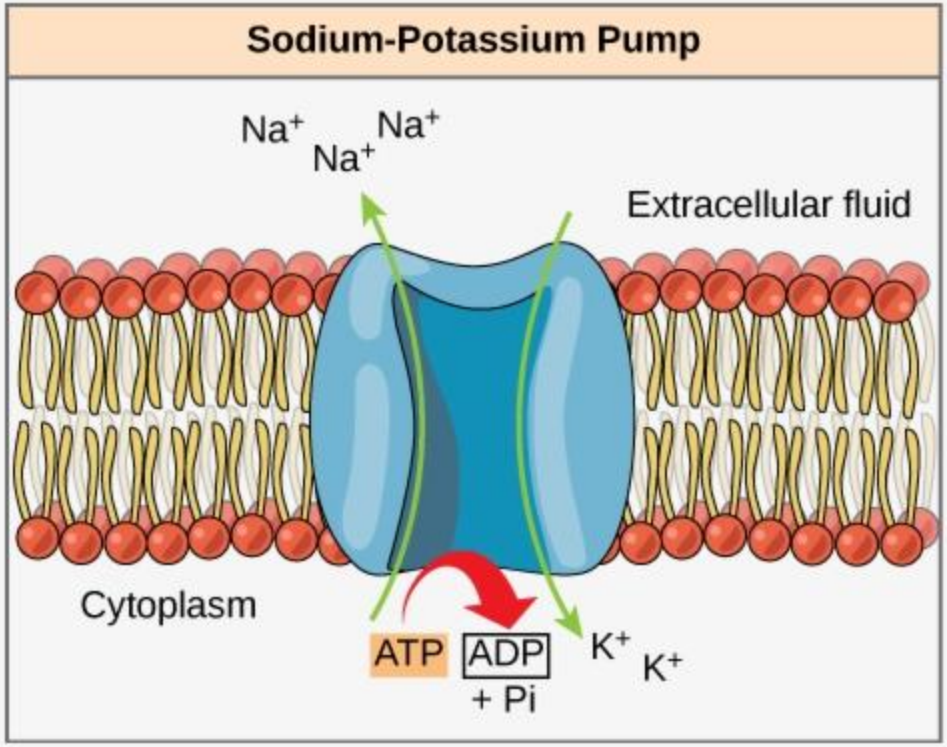
aminokwasy
glukoza
nukleotydy

Ions

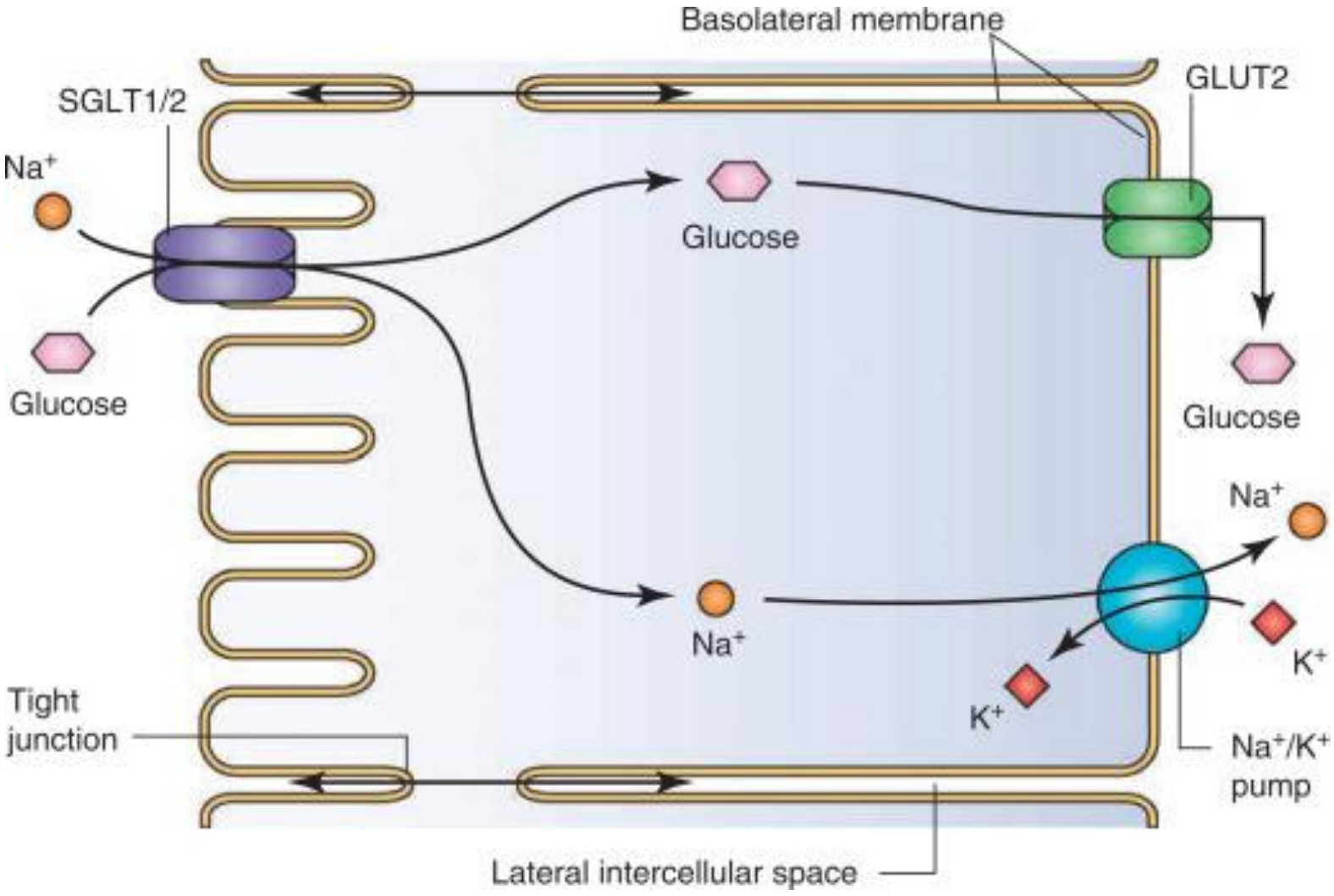
H^+, Na^+
HCO_3^-, K^+
Ca^{2+}, Cl^-
Mg^{2+}



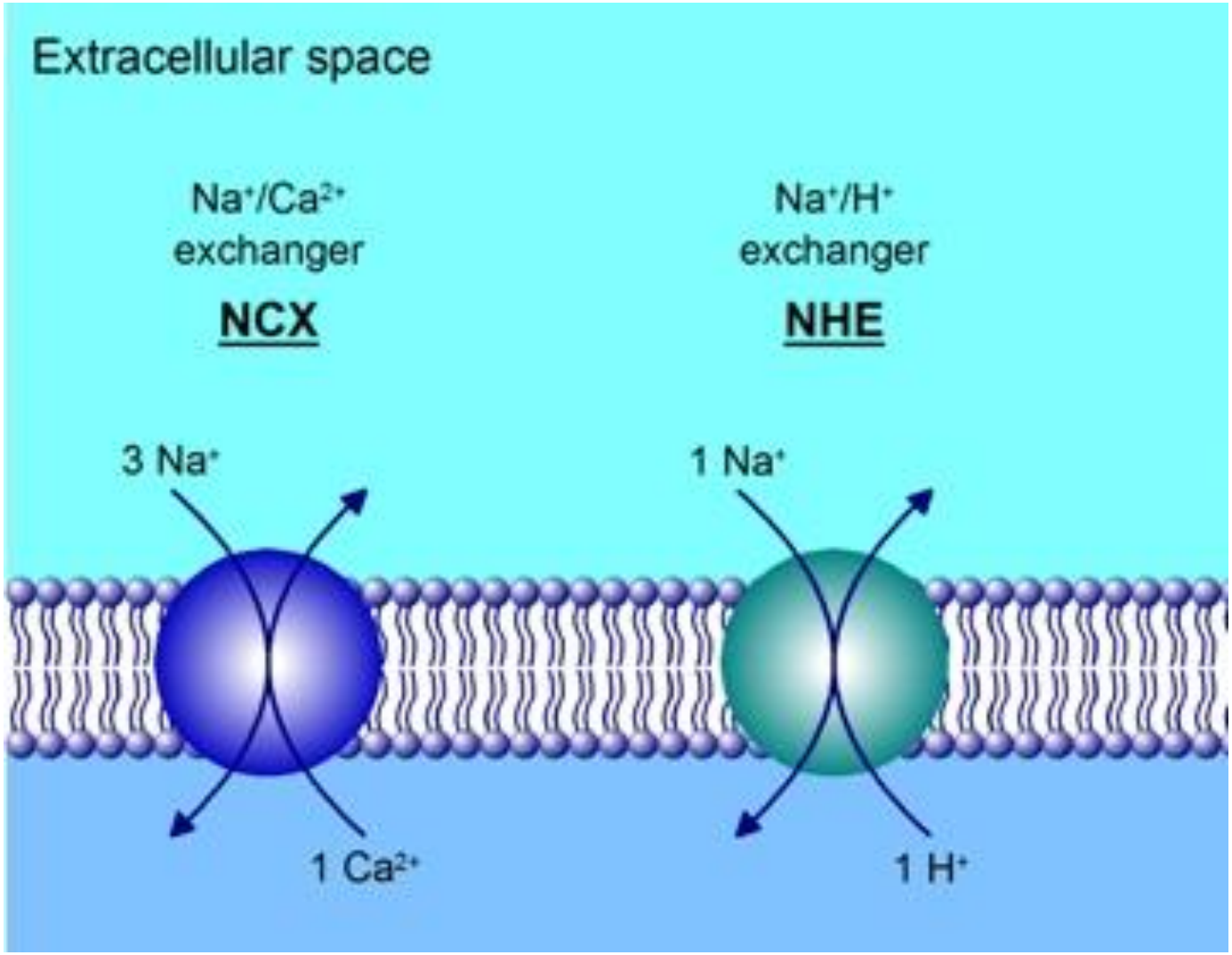
Active primary transport

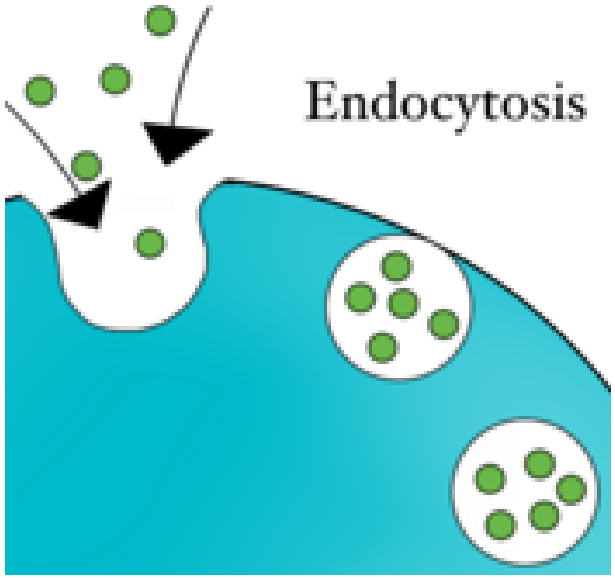


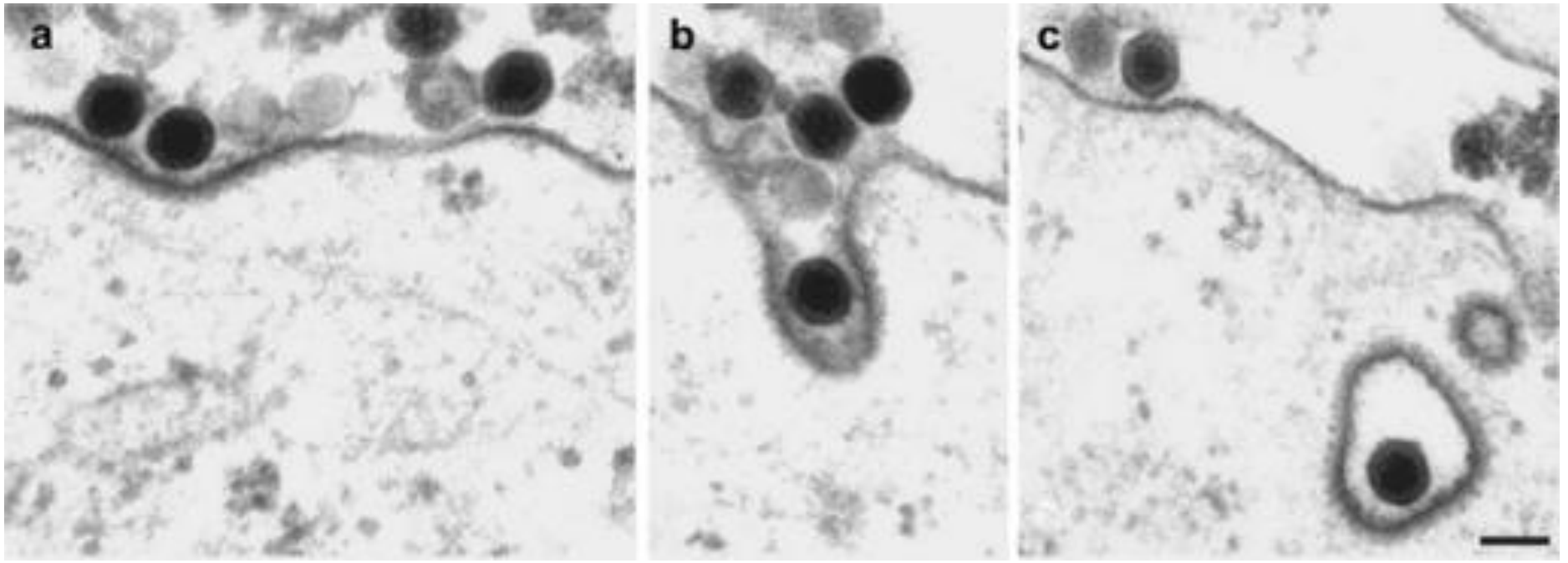
Active secondary transport - symport



Active secondary transport - antyport

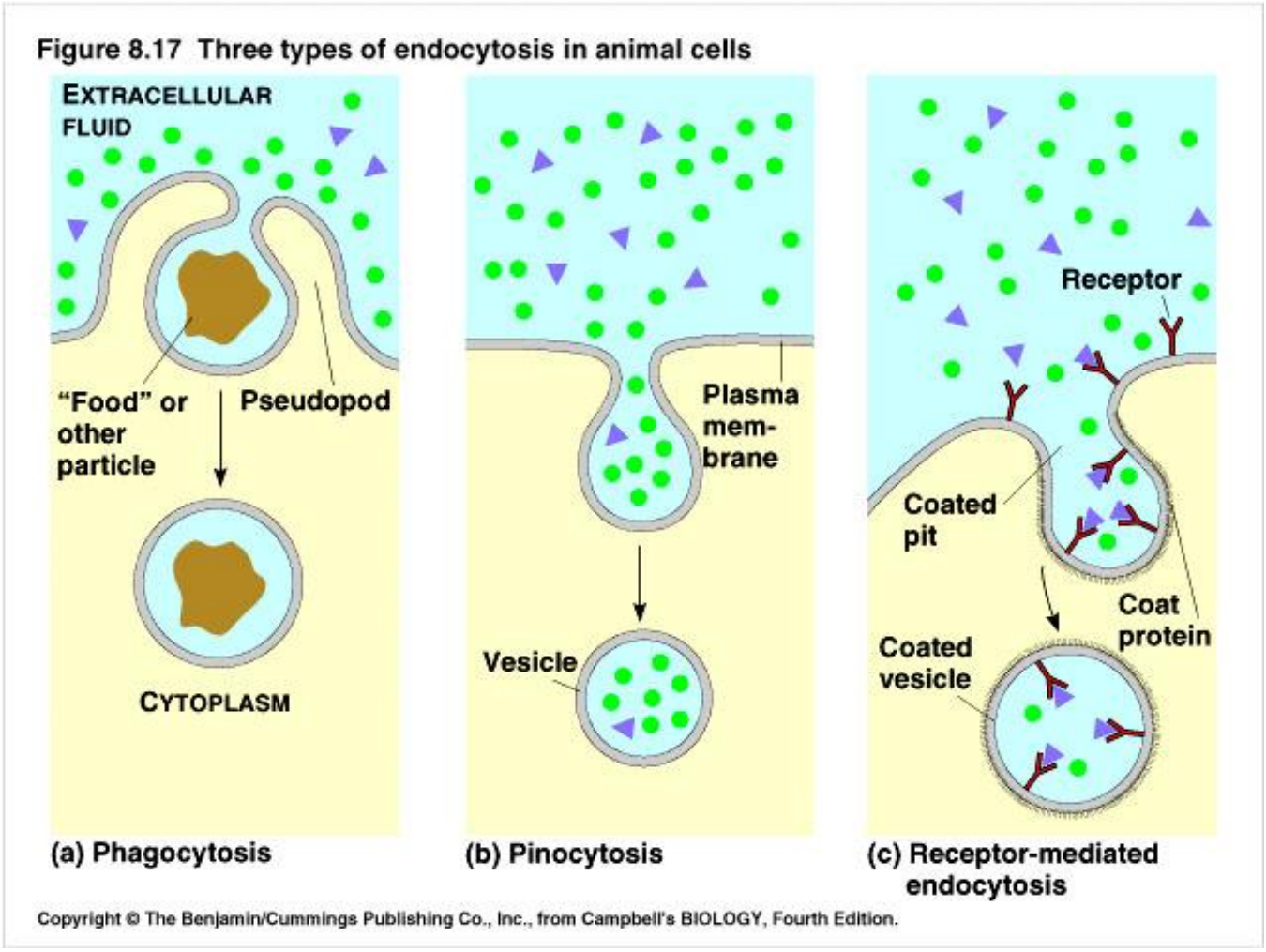



Endocytosis


a
b
c

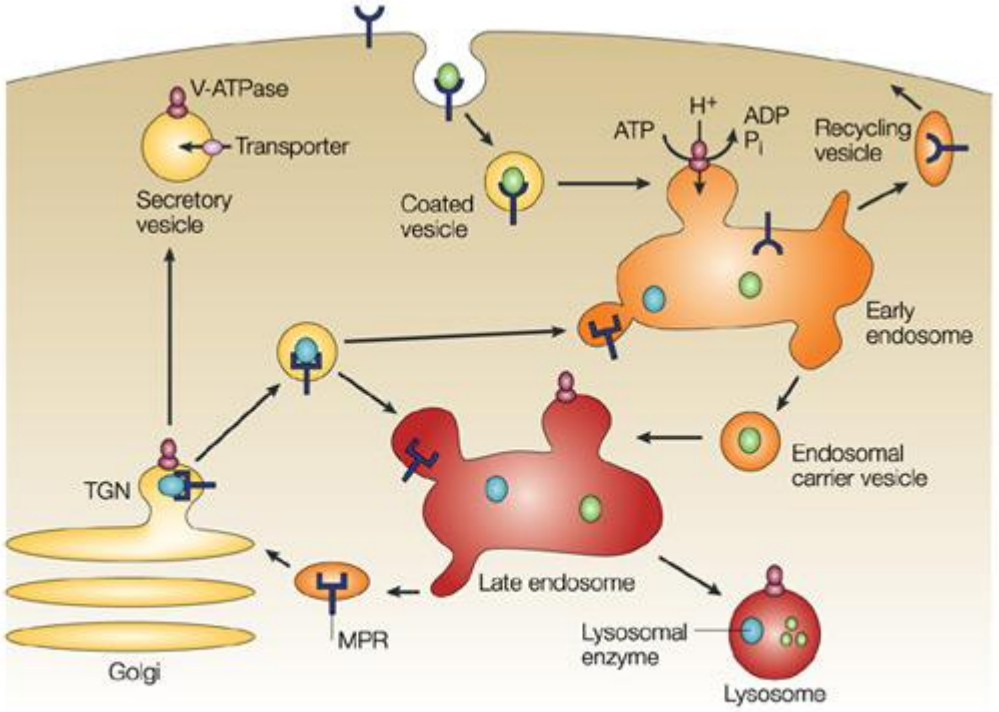
ENDOCYTOSIS - energy-using process by which cells absorb molecules

- Material is engulfed in the vesicle



ENDOSOMES

- early endosomes – near the periphery of the cell
- late endosomes – deeper in the cytoplasm



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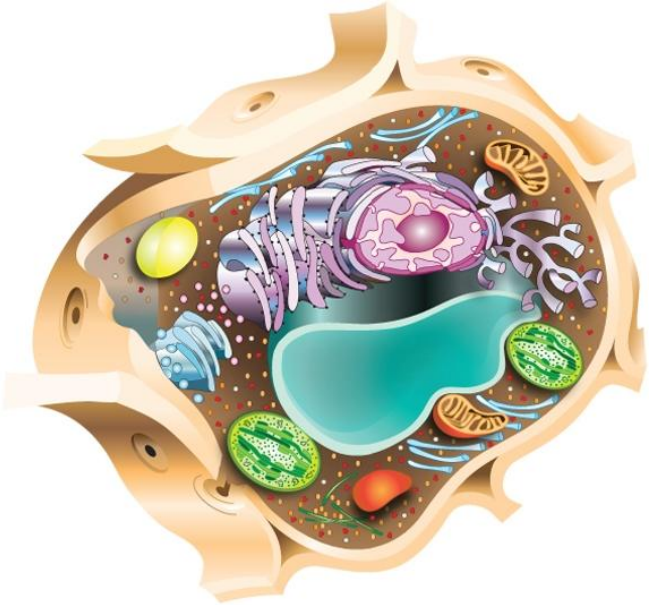
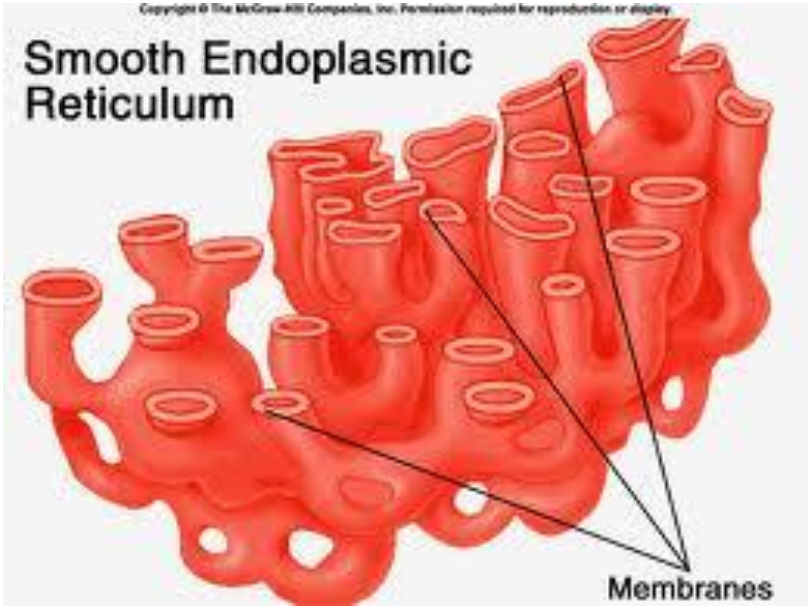
ENDOPLASMIC RETICULUM





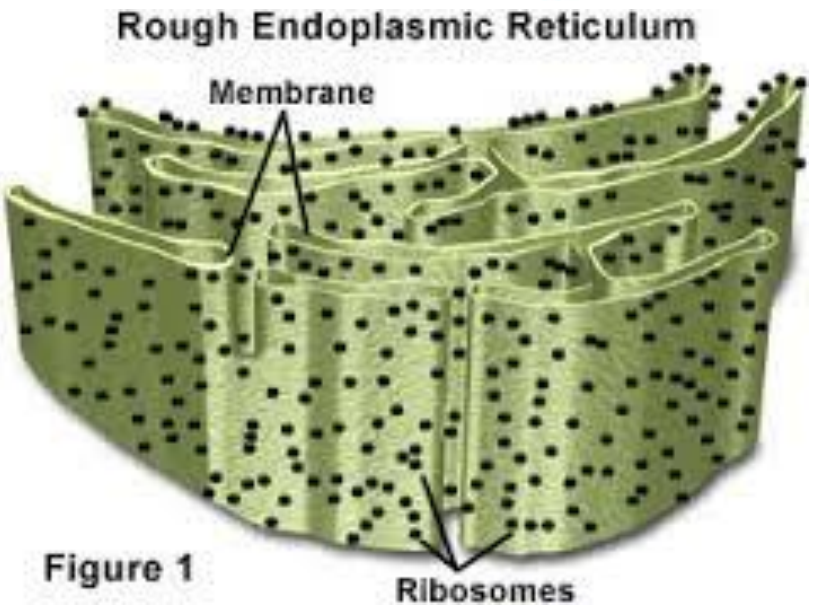
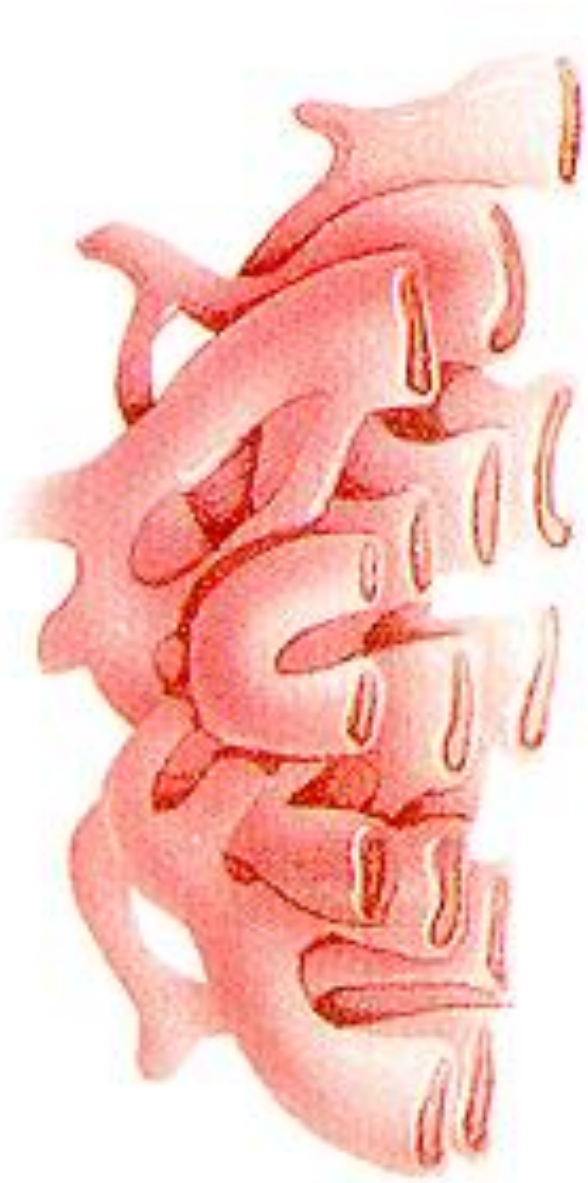
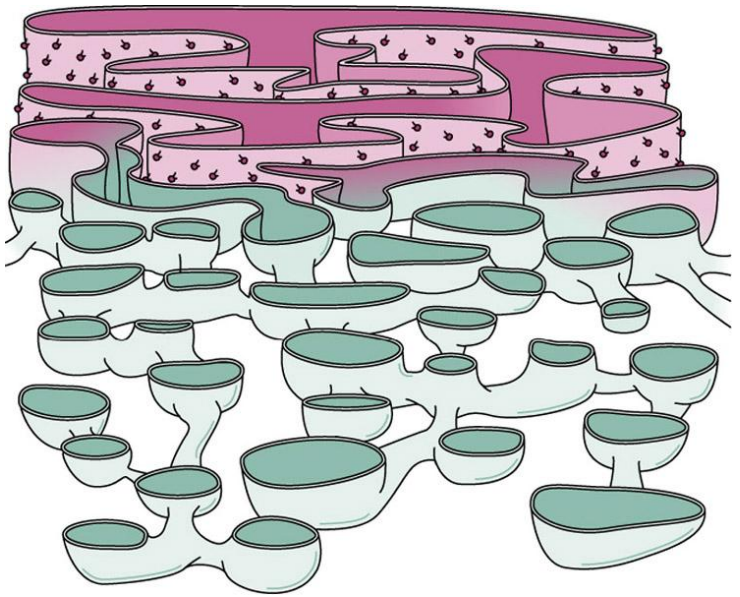


Figure 1

Smooth endoplasmic reticulum (SER)





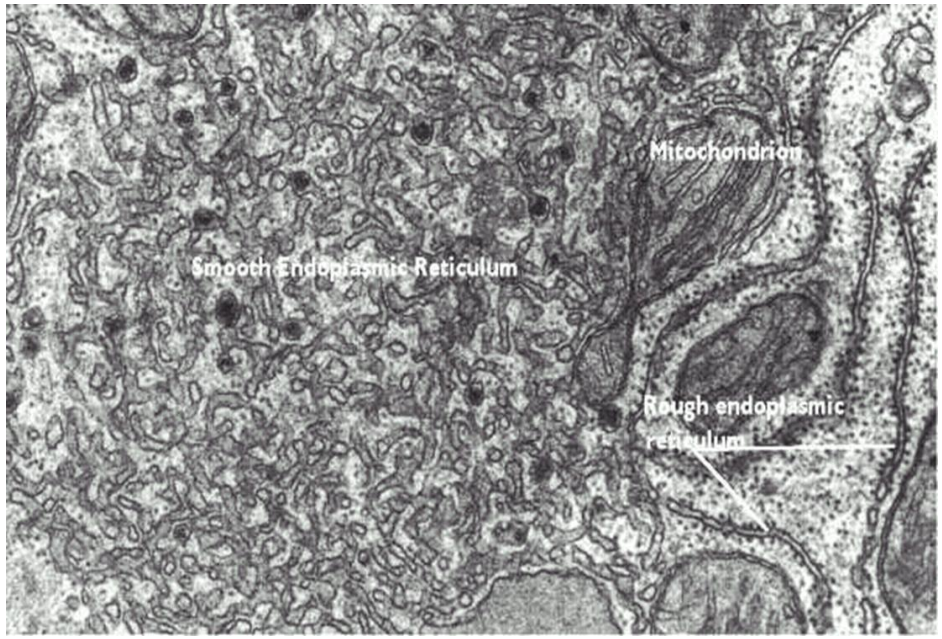
*Synthesis of lipids on the basis of cholesterol (components of cell membrane, steroid hormones)

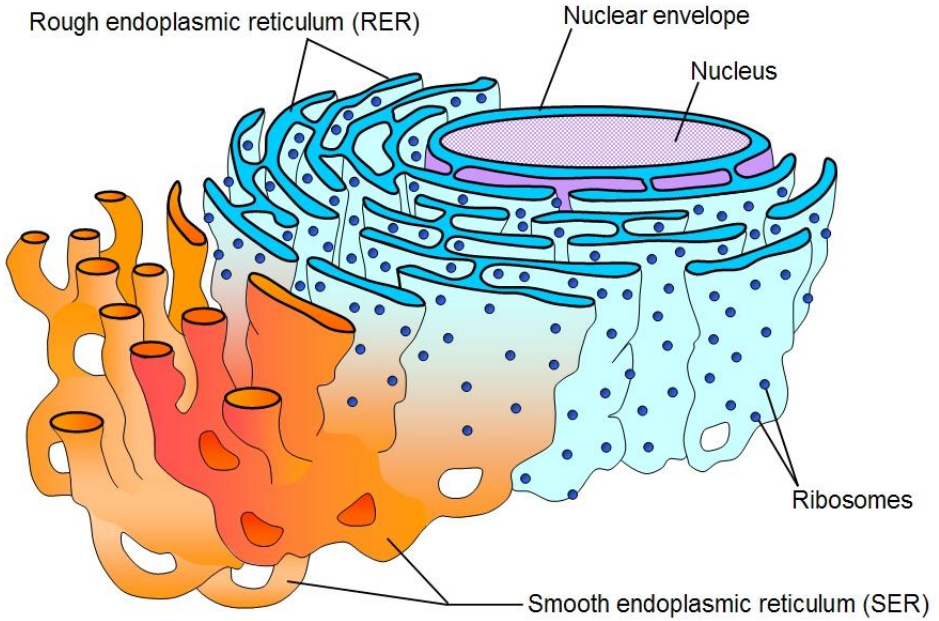
* Detoxification of harmful substances

*Accumulation and storage of calcium

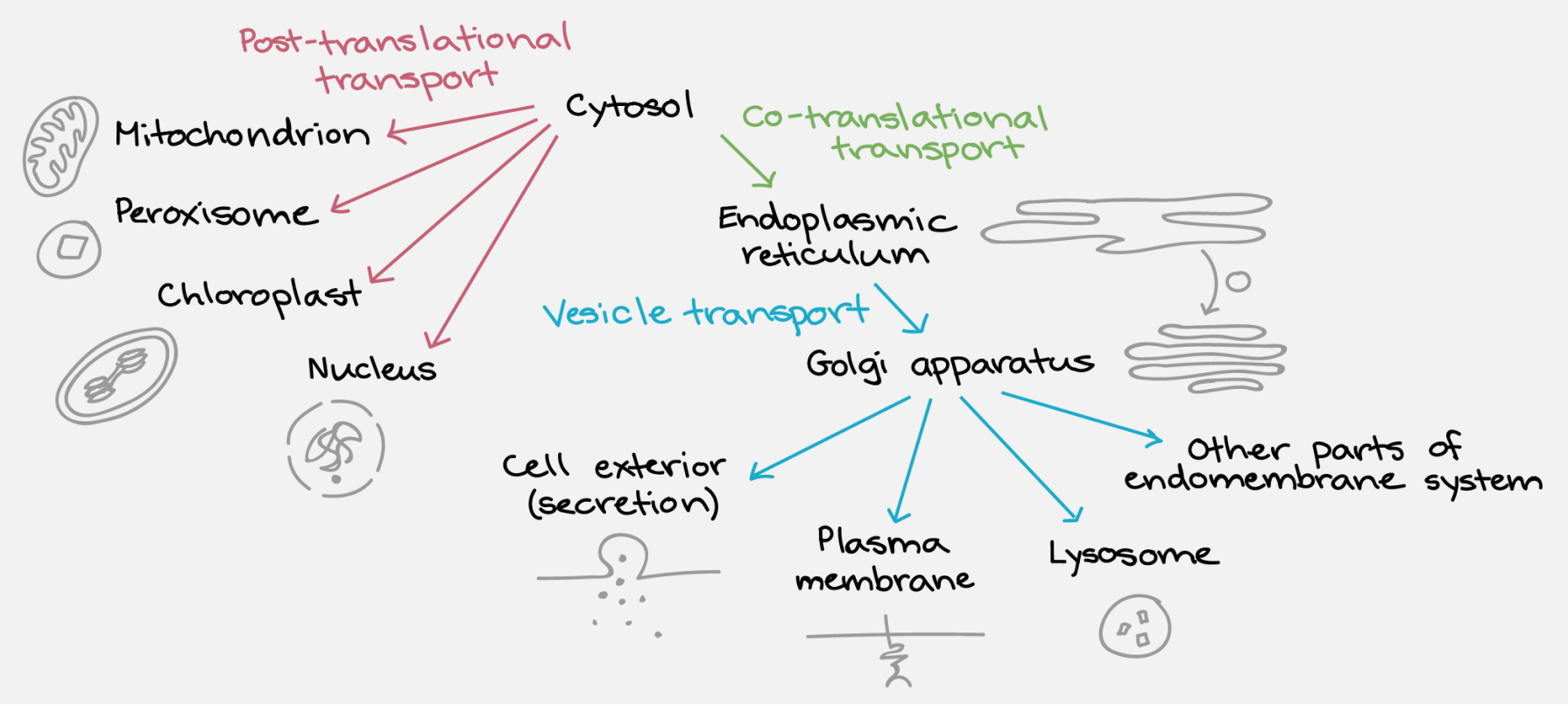
Rough endoplasmic reticulum (RER)

- contains **ribosomes** on the cytosolic face - the sites of protein synthesis
- is the site of synthesis of proteins
- post-translational modifications of proteins

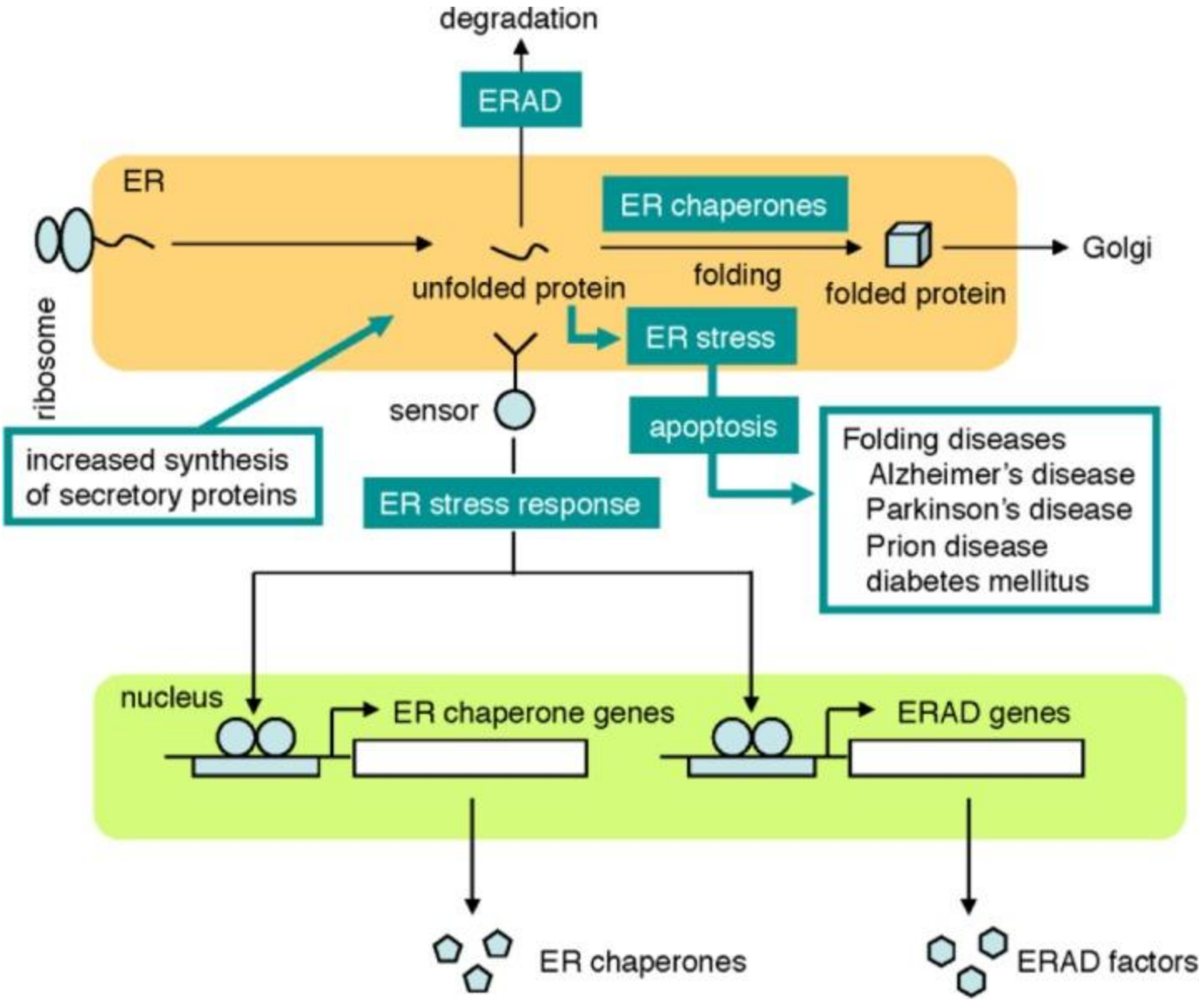




Free rybosomes and membrane bound rybosomes



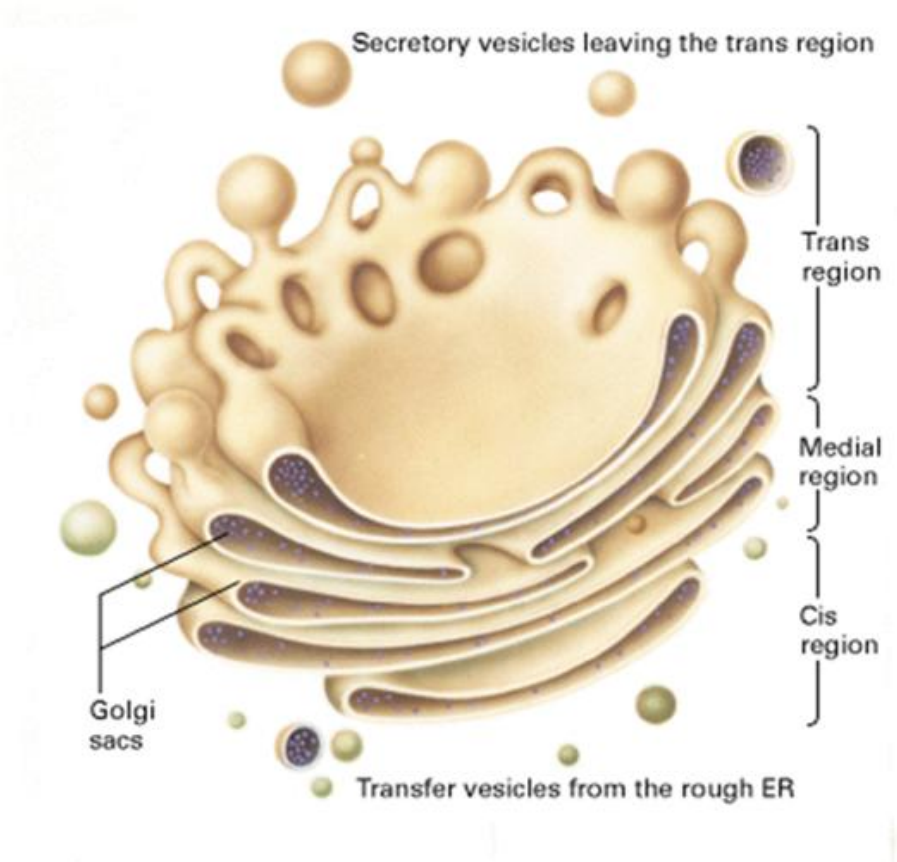
Chaperones

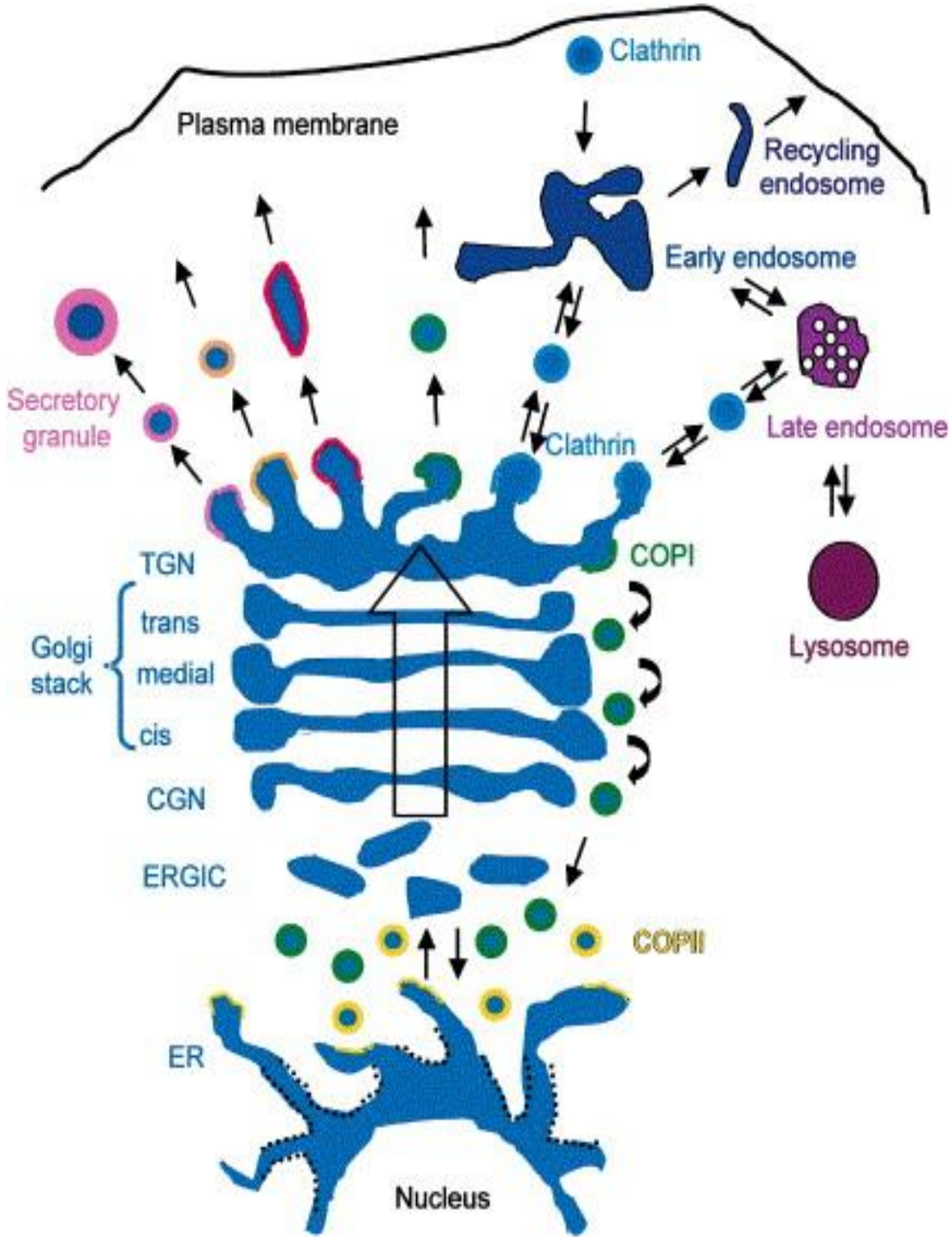

degradation
ERAD
ER
ER chaperones
Golgi
folding
unfolded protein
folded protein
ribosome
ER stress
sensor
apoptosis
increased synthesis
of secretory proteins
Folding diseases
Alzheimer's disease
Parkinson's disease
Prion disease
diabetes mellitus
ER stress response
nucleus
ER chaperone genes
ERAD genes
ER chaperones
ERAD factors

Golgi Apparatus

- flattened membrane-bounded cisternae – Golgi stack
- convex cis-face, closest to the RER (entry face)
- medial face
- concave trans-face (exit face)

Function:
posttranslational modification and packaging of proteins

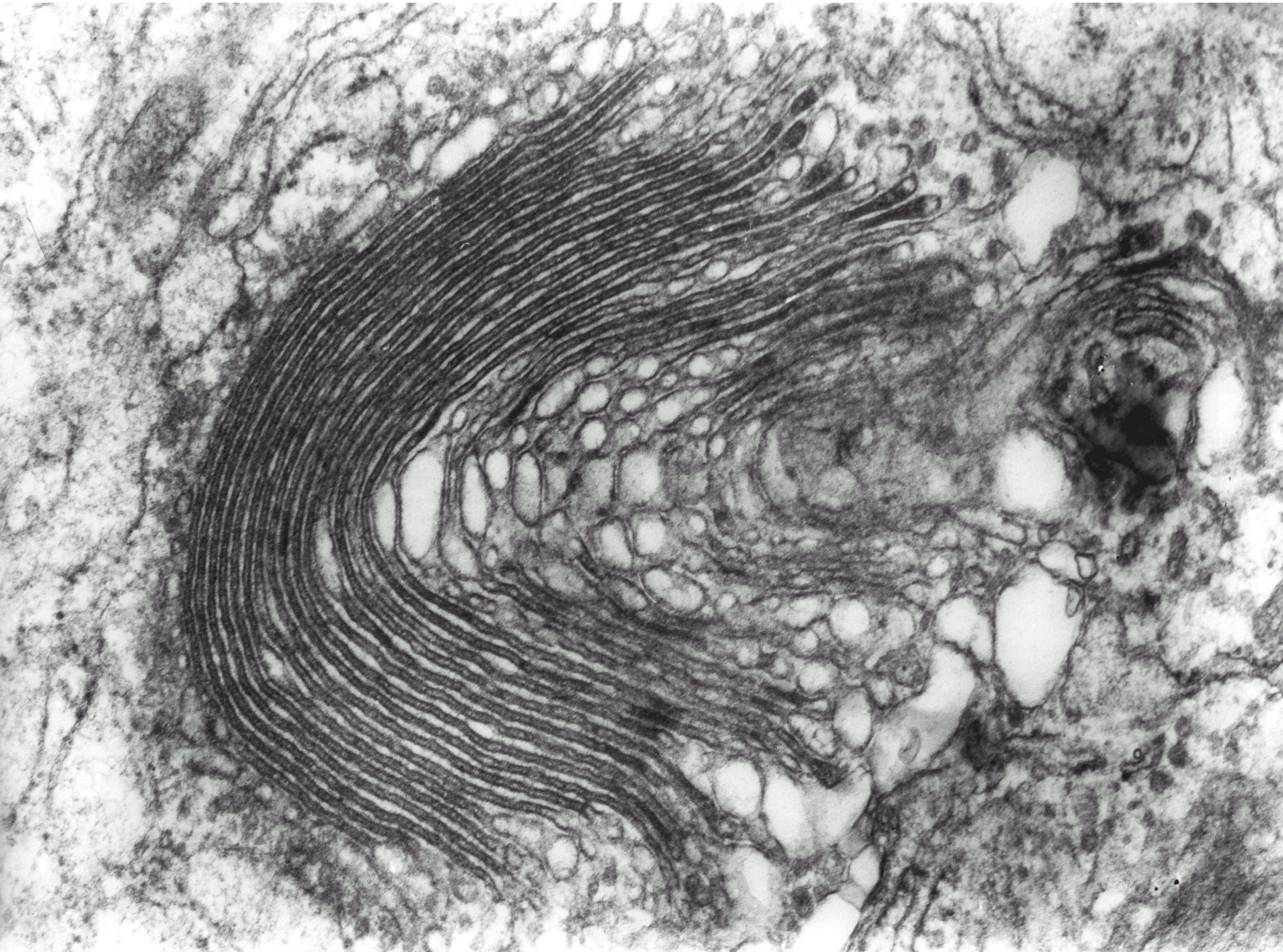




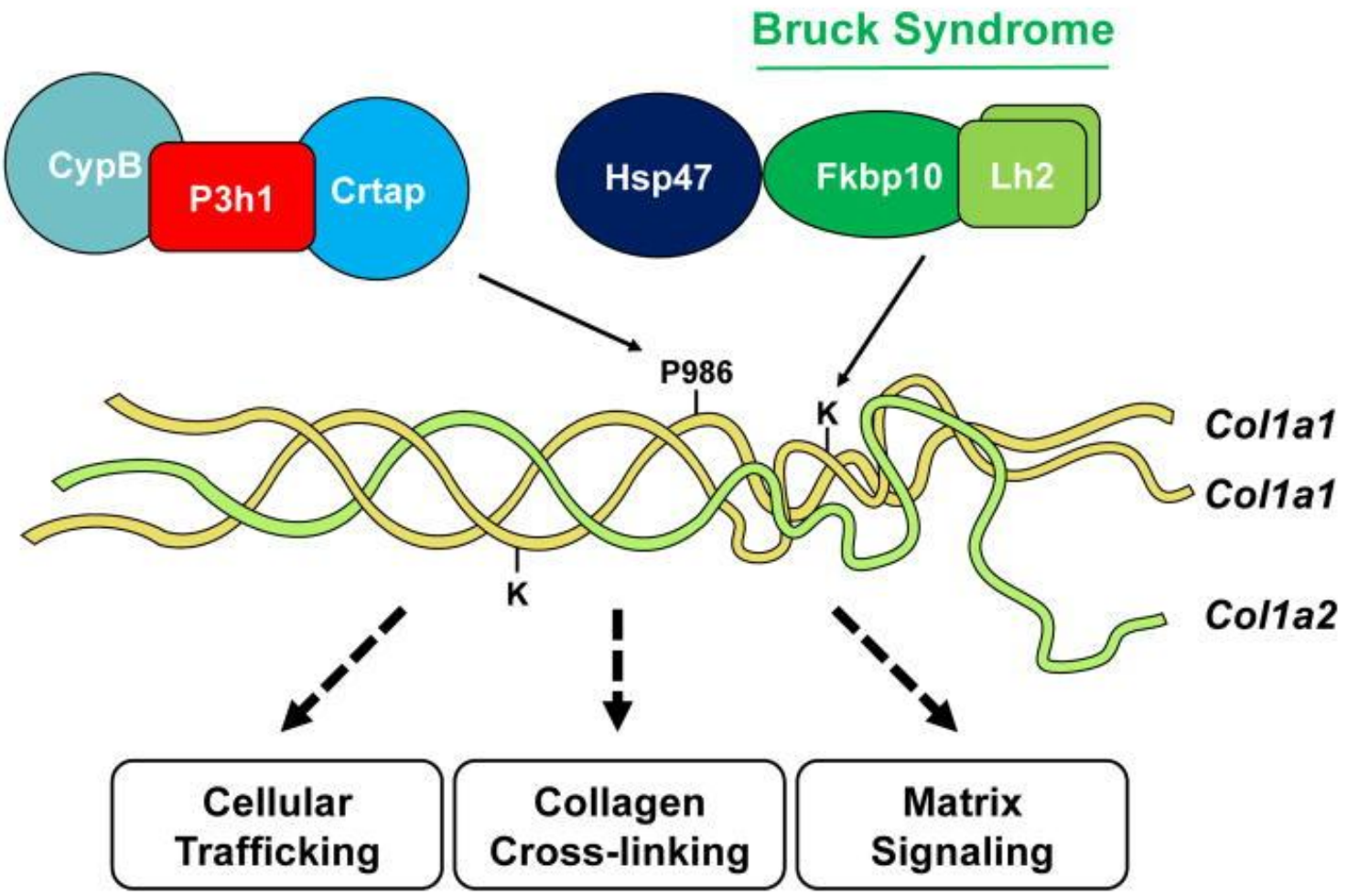
Vesicles that transport proteins are labeled by coat proteins (according to their destination)

Types of coat proteins

- **coatomer I (COP I)** – vesicles that are returned from trans-face
- **coatomer II (COPII)** – vesicles between RER and cis-face
- **clathrin –** vesicles which bud off the trans-face

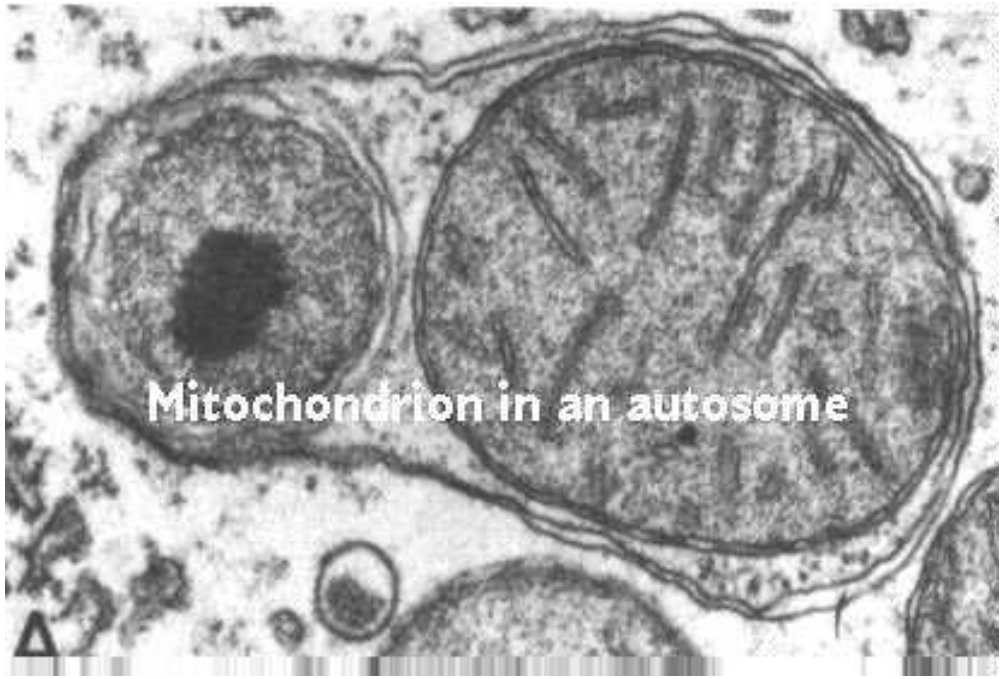


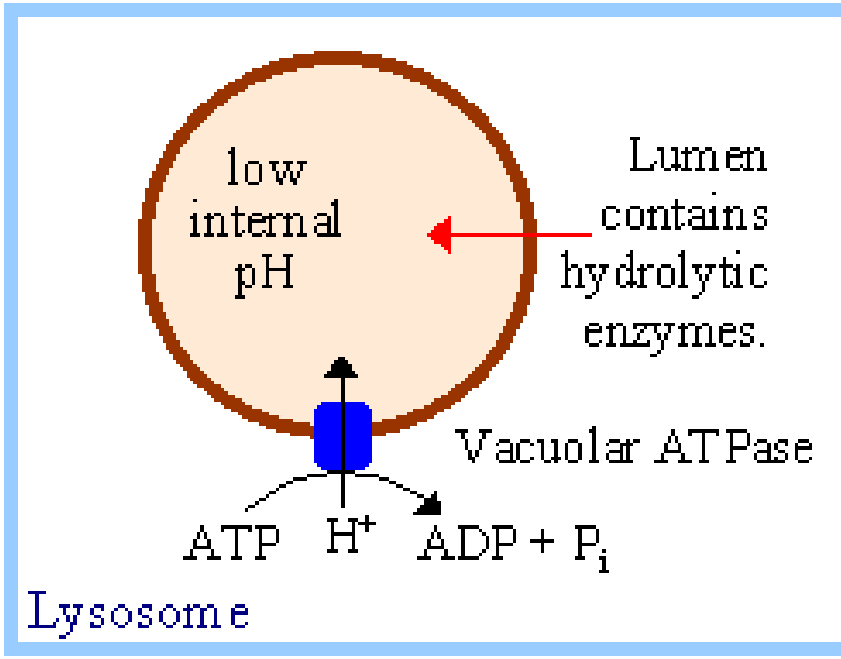
Osteogenesis Imperfecta



LYSOSOMES

- contain acid hydrolases (lipases, proteases, nucleases, sulfatases and glycosidases)
- pH 5.0
- digest excess or worn-out organelles (mitochondria), food particles,
and engulf viruses or bacteria





Transport of substances into lysosomes

- **may be carried out by three ways**:
- Phagocytosis (viruses or bacteria)
- Autophagy (excess or senescent organells)
- Receptor-mediated endocytosis (macromolecules)

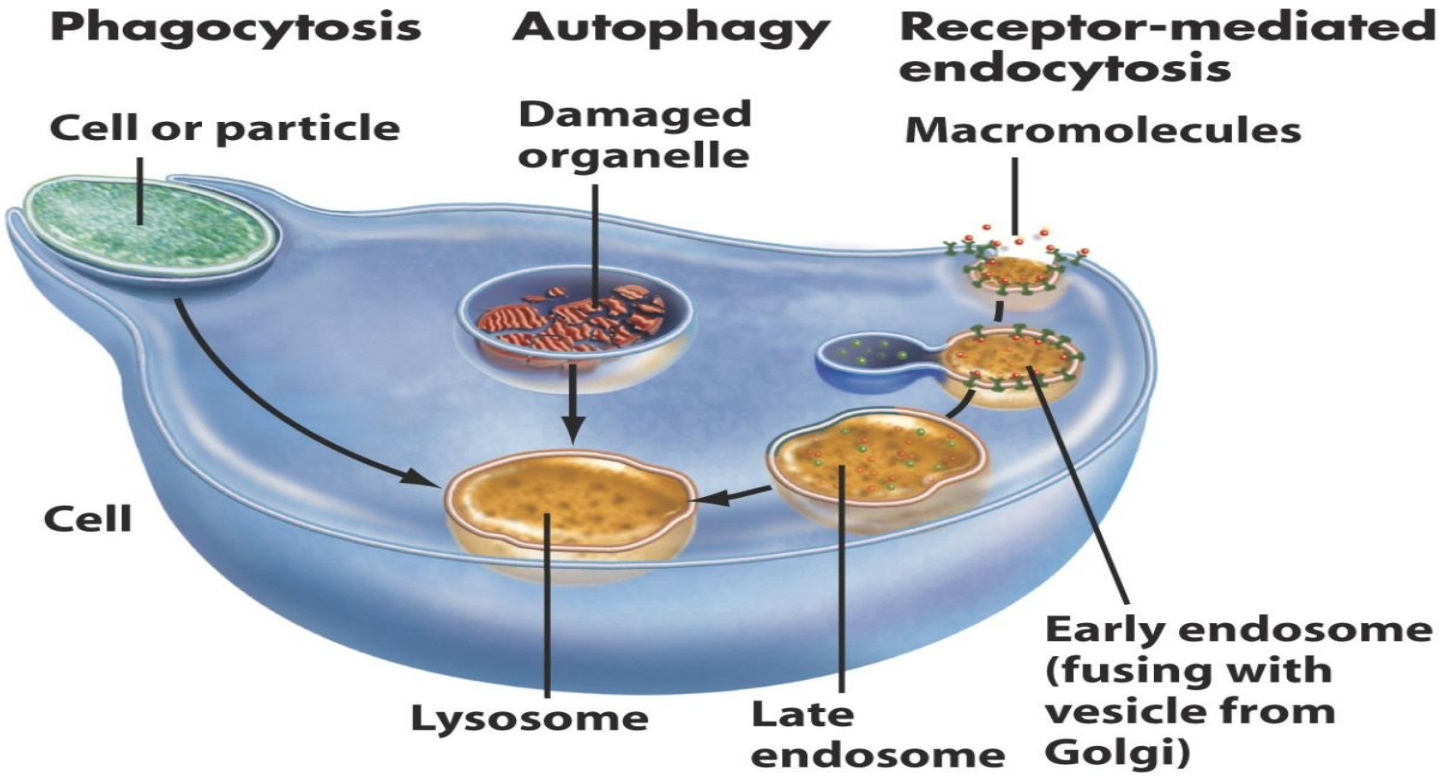
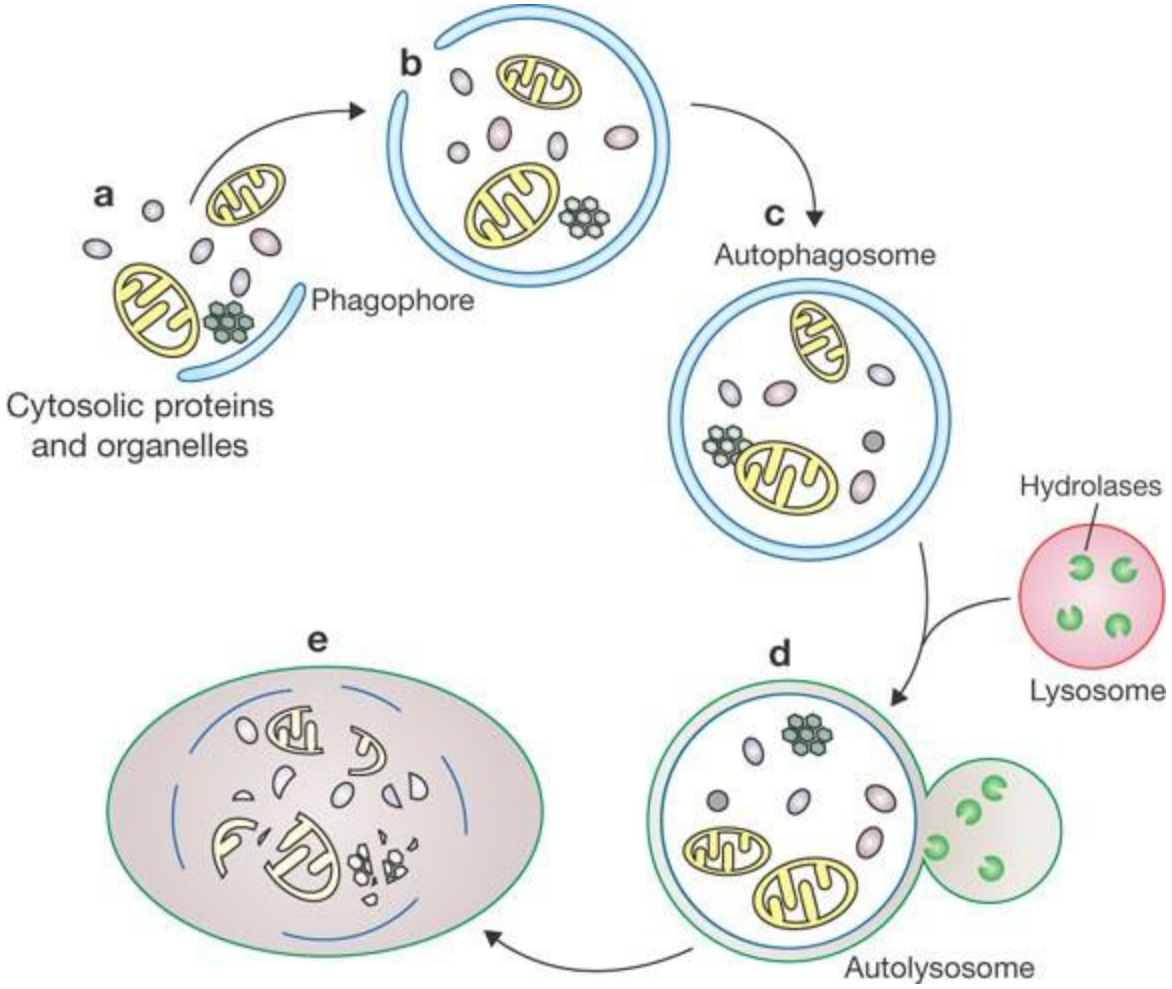
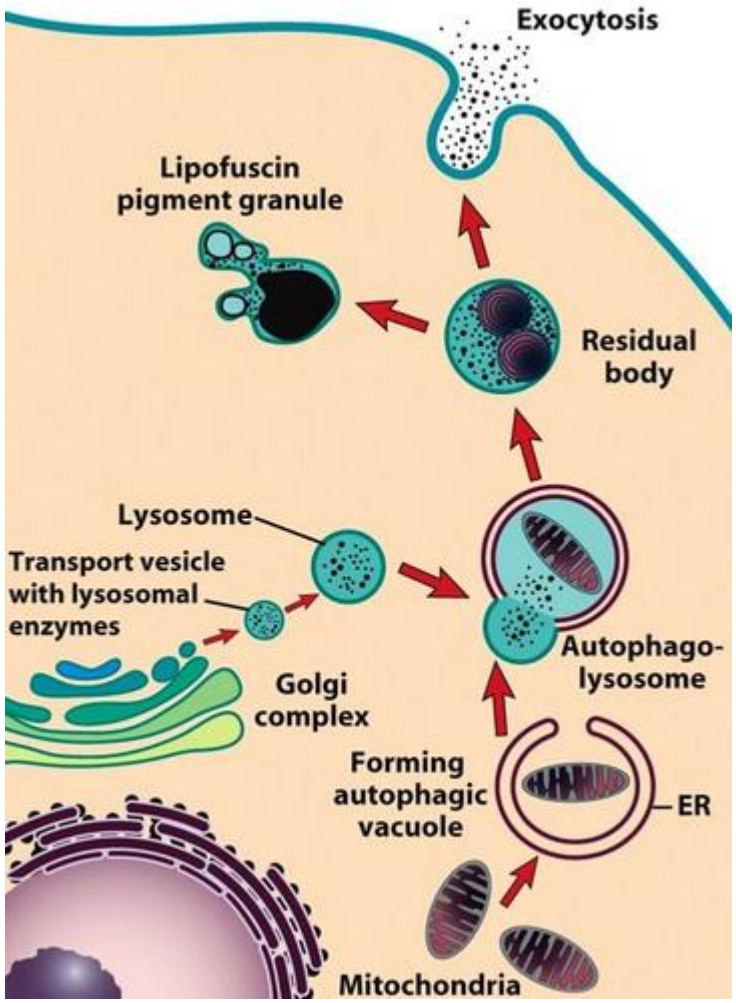


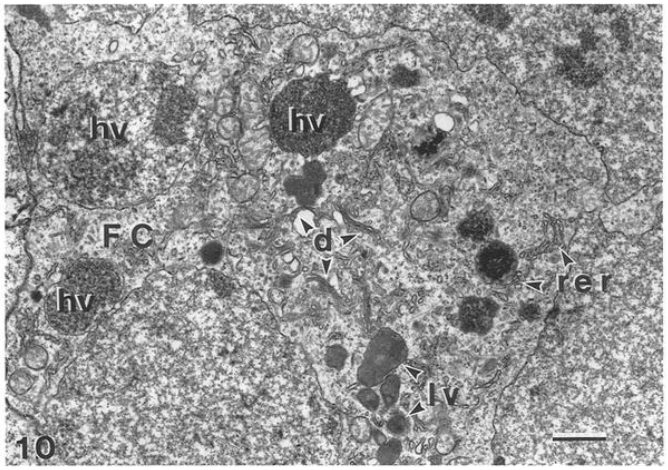
Figure 7-14 Biological Science, 2/e

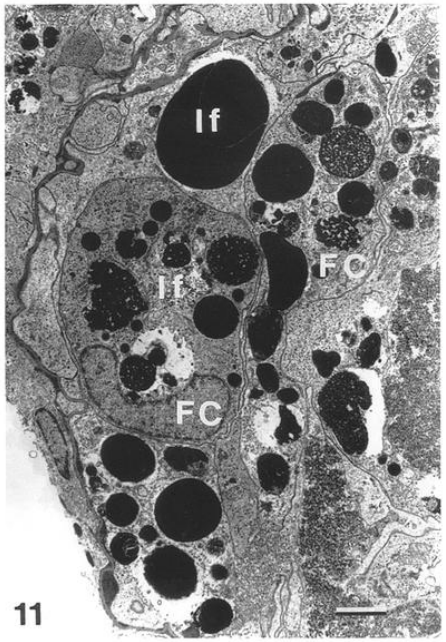
Autofagolysosom

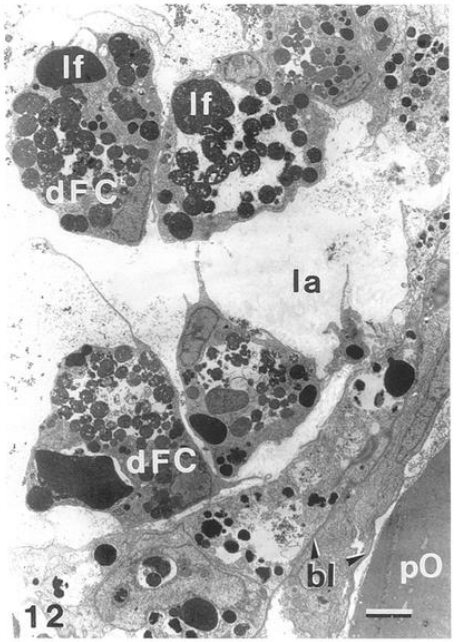


Lipofuscin – residual bodies

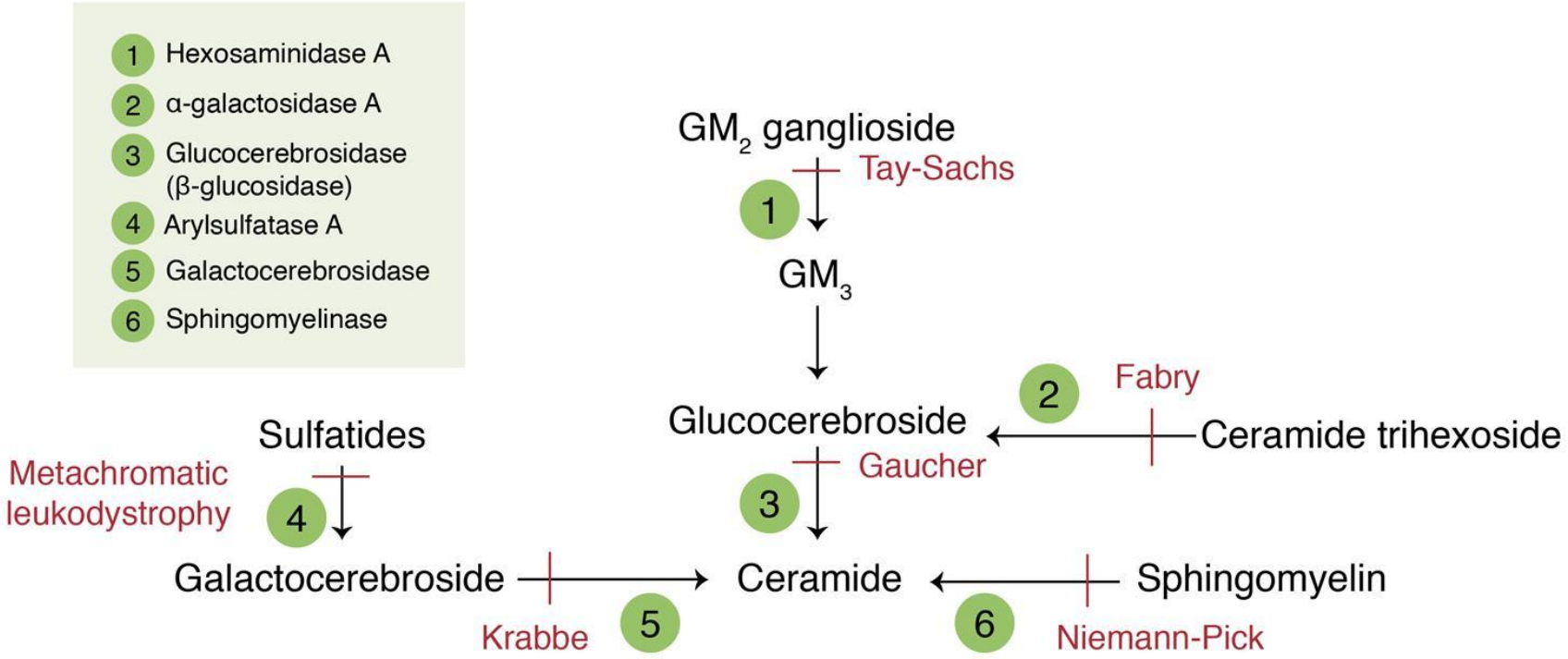








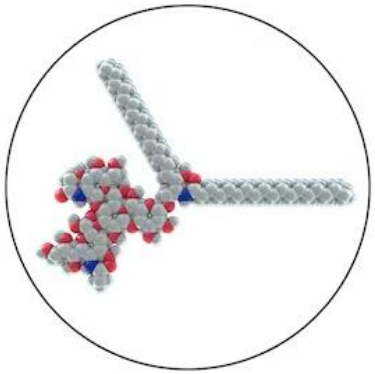
Lysosomal Storage Disorders



Lucy Liu

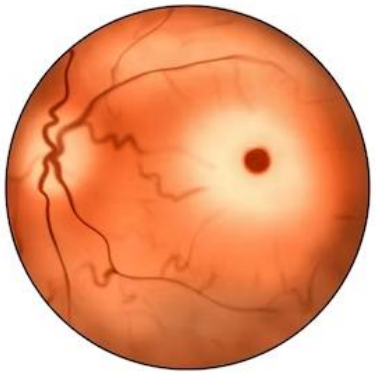
Taya-Sachs disease

HEXA gene mutation

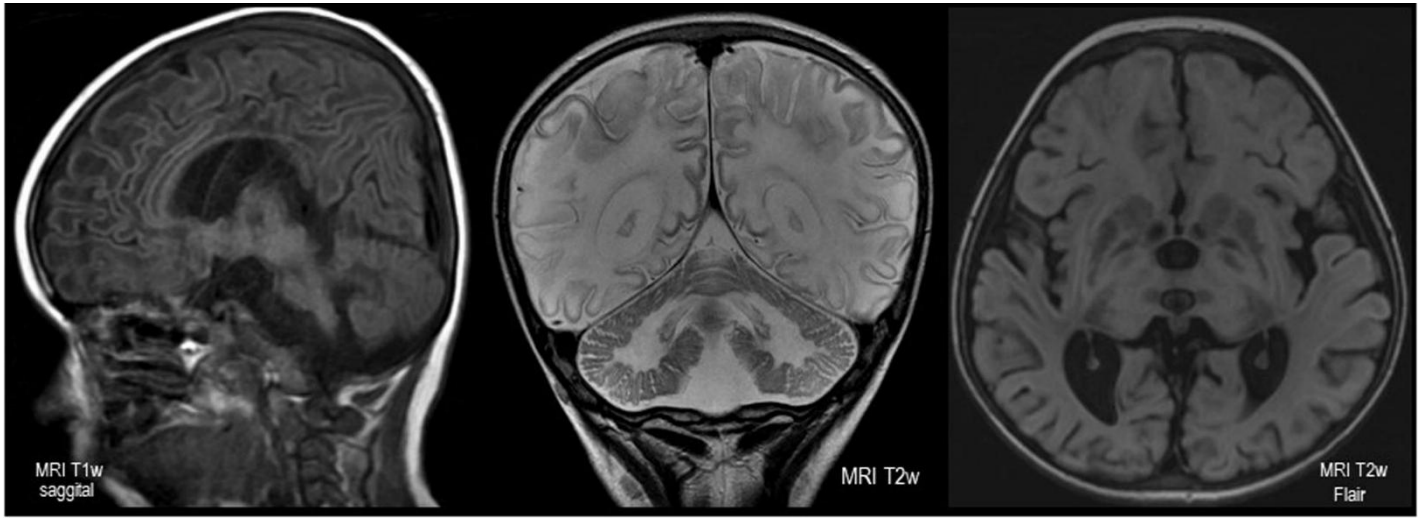


beta-Hexosaminidase A deficiency leading to accumulation of GM2 ganglioside in neurons

Swollen neurons with lamellar inclusions, neuronal degradation



"Cherry red spot" in the macula of the eye and narrowing of the blood vessels



PEROXISOMES

- Membrane-bound organelles.
- Present in all animal cells

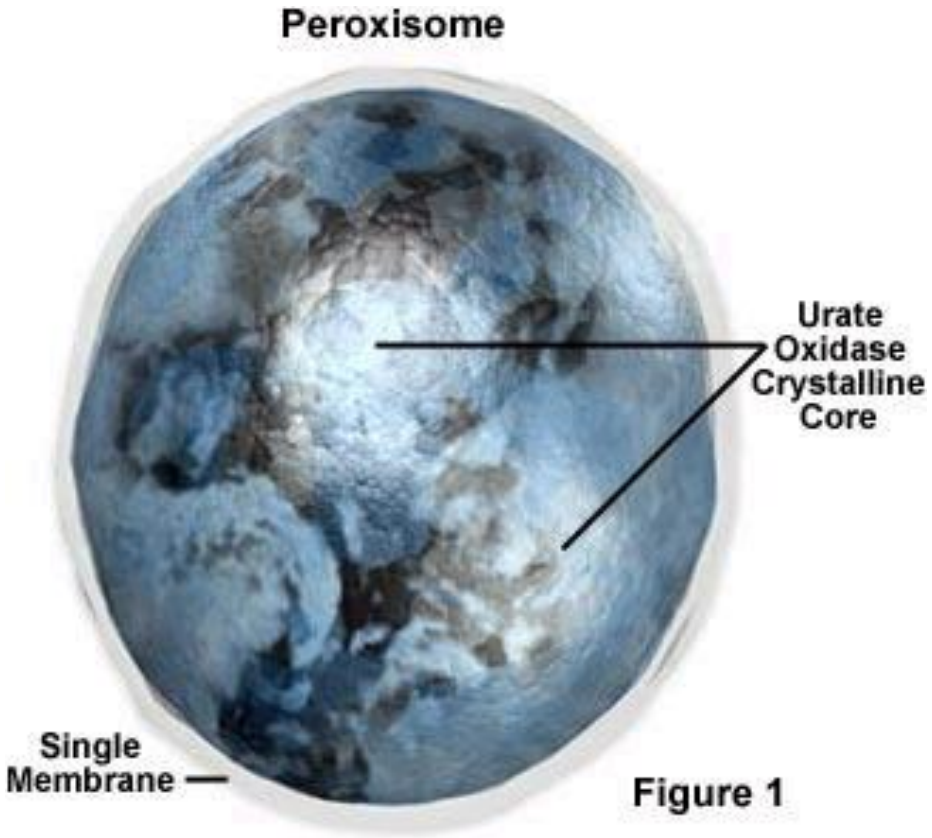
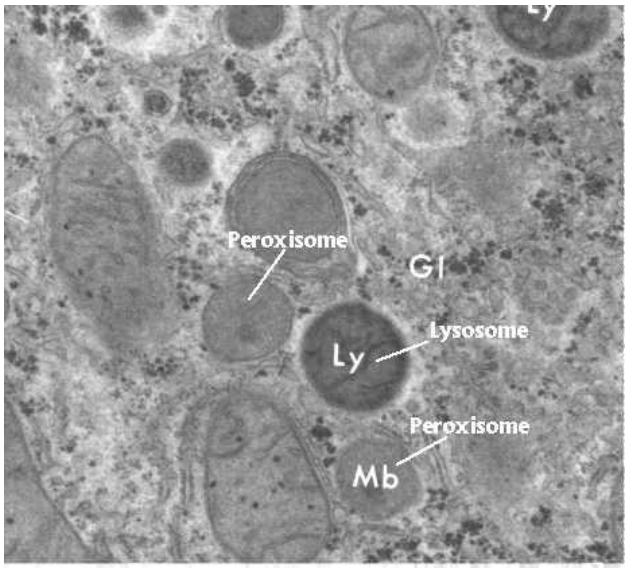
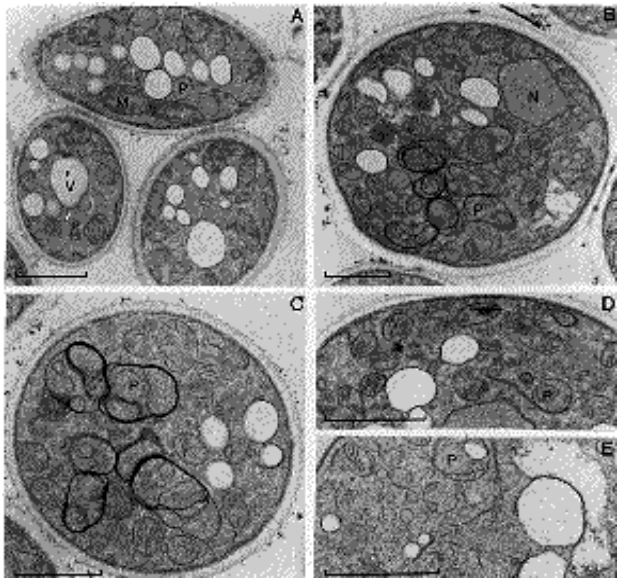


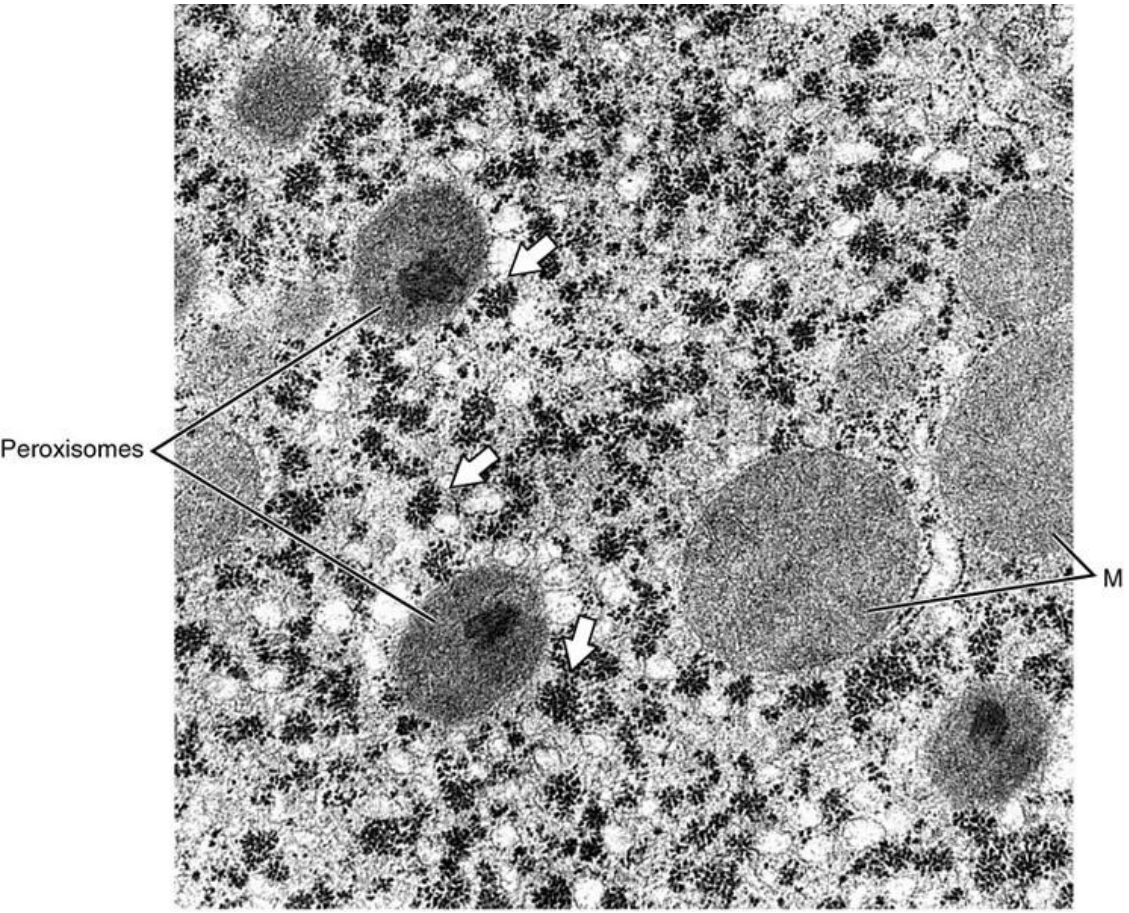
Figure 1





PEROXISOMES contain oxidative enzymes: catalase, D-amino acid oxidase and urate oxidase

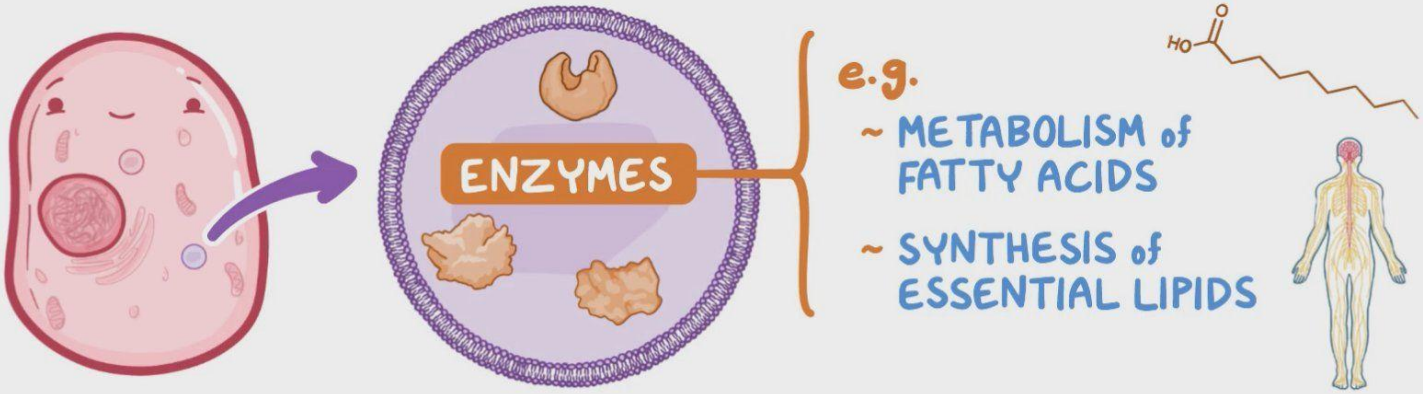
Peroxisomes



- Catabolism of long-chained fatty acids (beta oxidation)
- Forming acetyl coenzyme A (CoA)
- Forming hydrogen peroxide (H_2O_2) – detoxifies various noxious agents and kills microorganisms

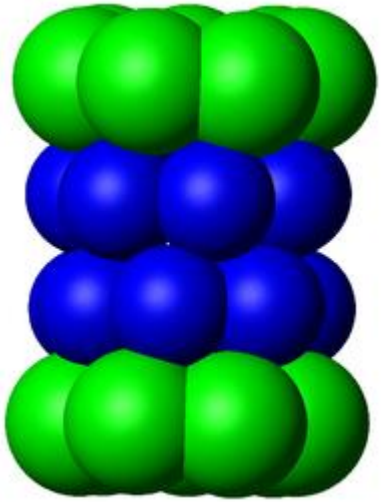
ZELLWEGER SPECTRUM DISORDERS (ZSDs)

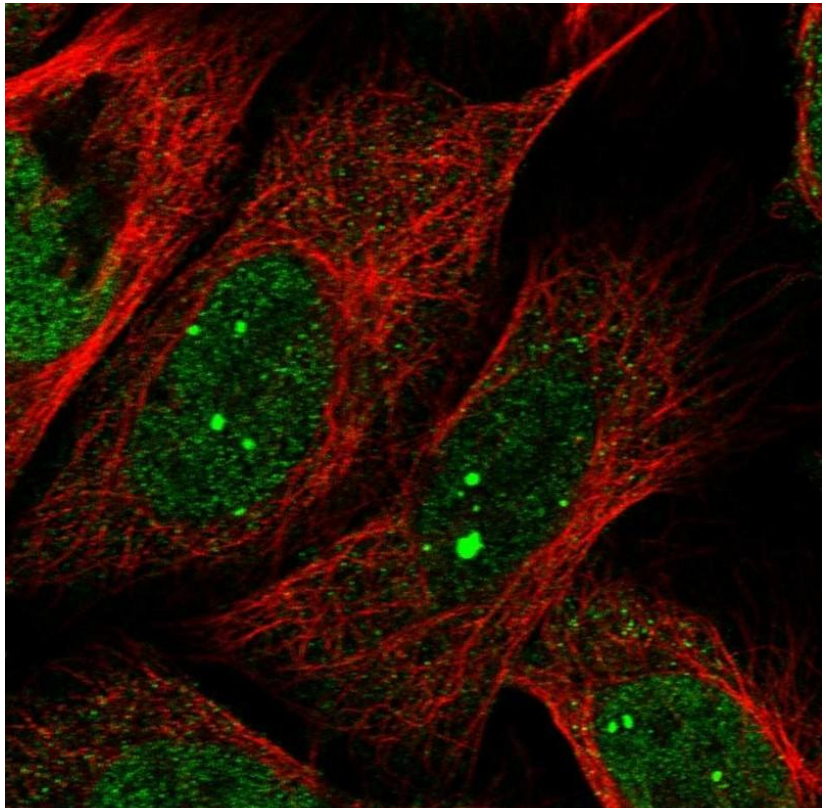
GROUP of RARE GENETIC DISEASES → IMPAIRED DEVELOPMENT of PEROXISOMES



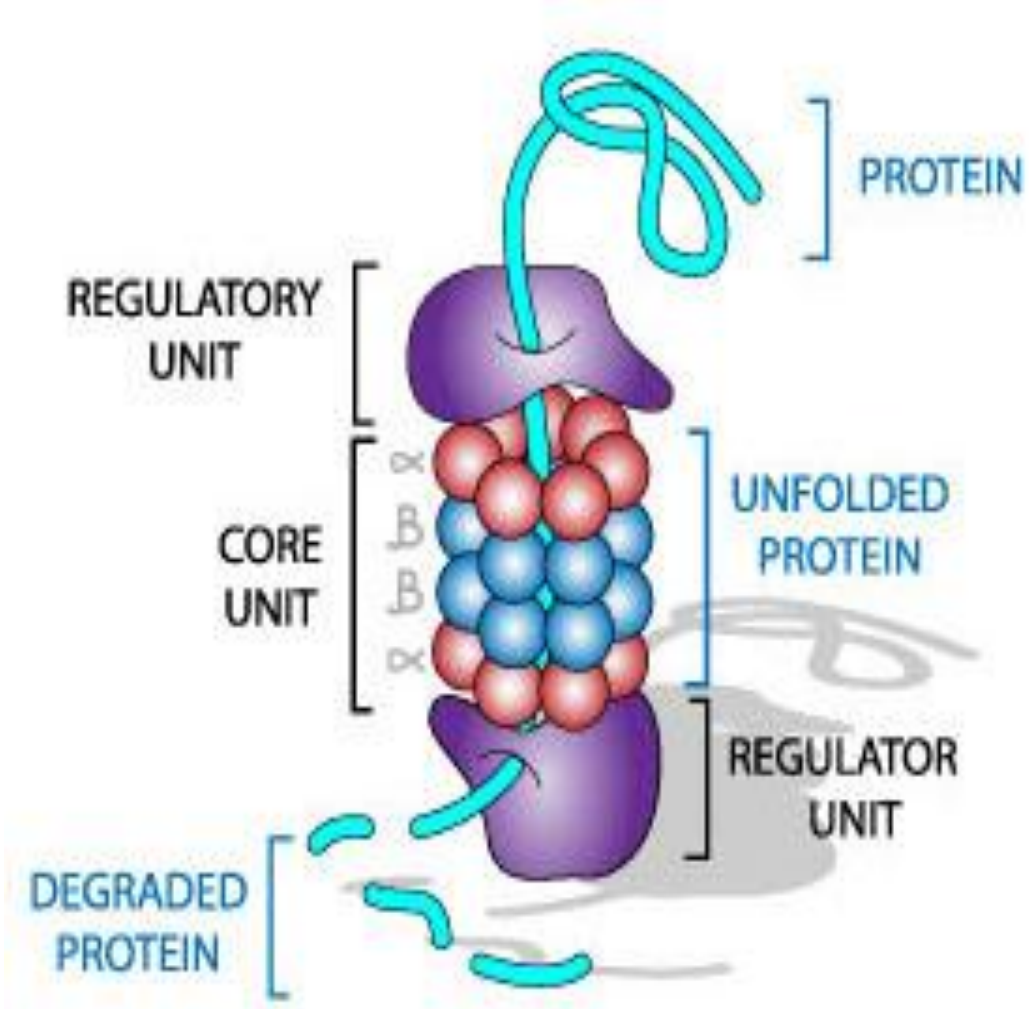
PROTEASOMES

- are protein complexes
- they are located in the nucleus and the cytoplasm
- the main function of the proteasome is to degrade unneeded or damaged proteins by proteolysis





PROTEASOME - STRUCTURE



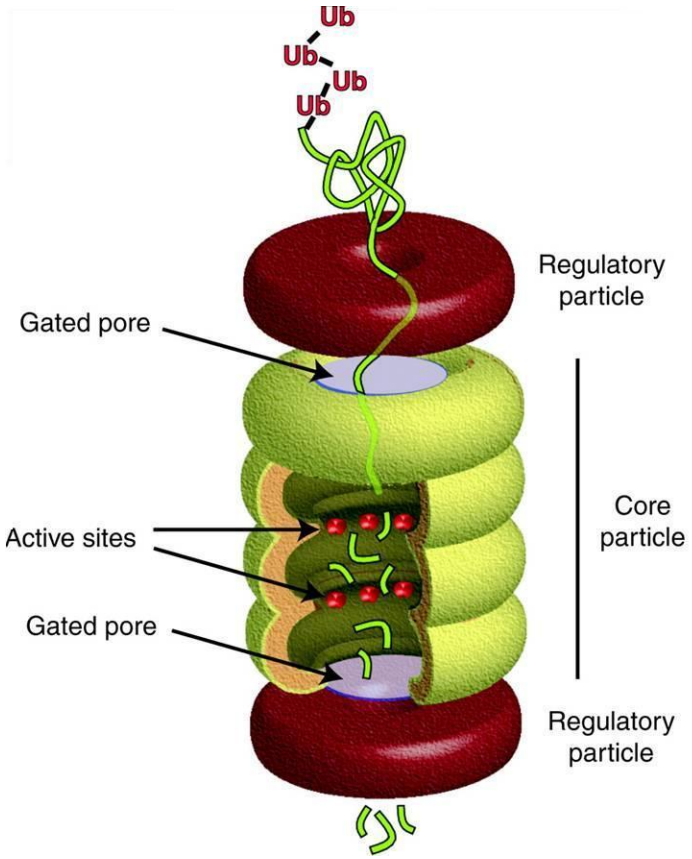
- cylindrical complex containing a „core” of four stacked rings forming a central pore

- each ring is composed of seven individual proteins

- the inner two rings are made of seven beta subunits that contain protease active sites

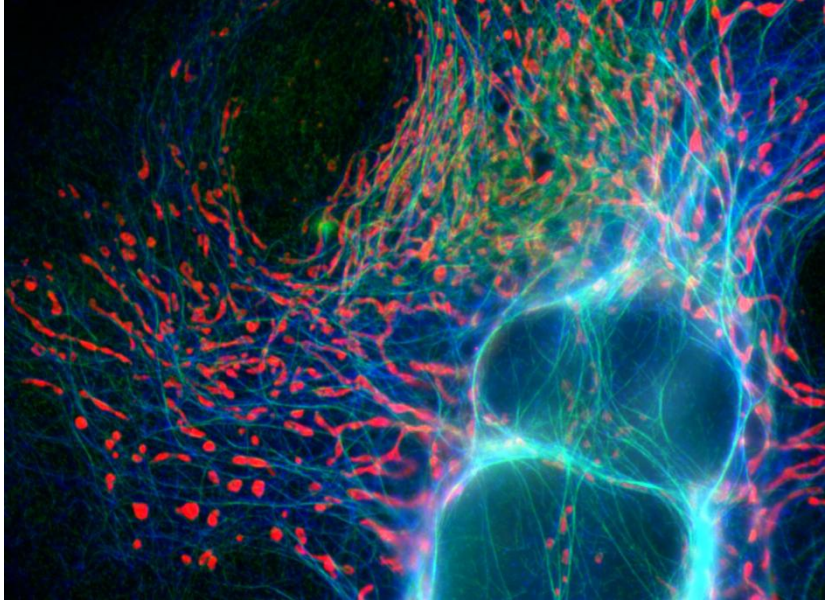
- the outer two rings contain a subunits whose function is to maintain a „gate”

PROTEASOMES

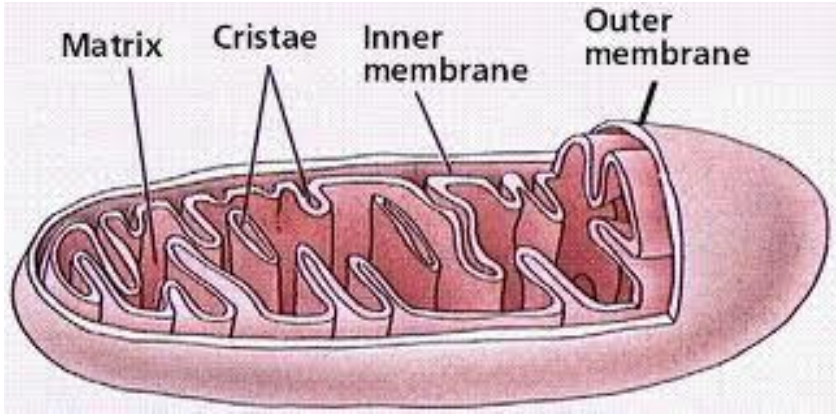


- protein must be ubiquitinated before being degraded (several ubiquitin molecules are attached to a lysine residue of the protein to form polyubiquinated protein)
- protein must be at least partially unfolded before they enter the core

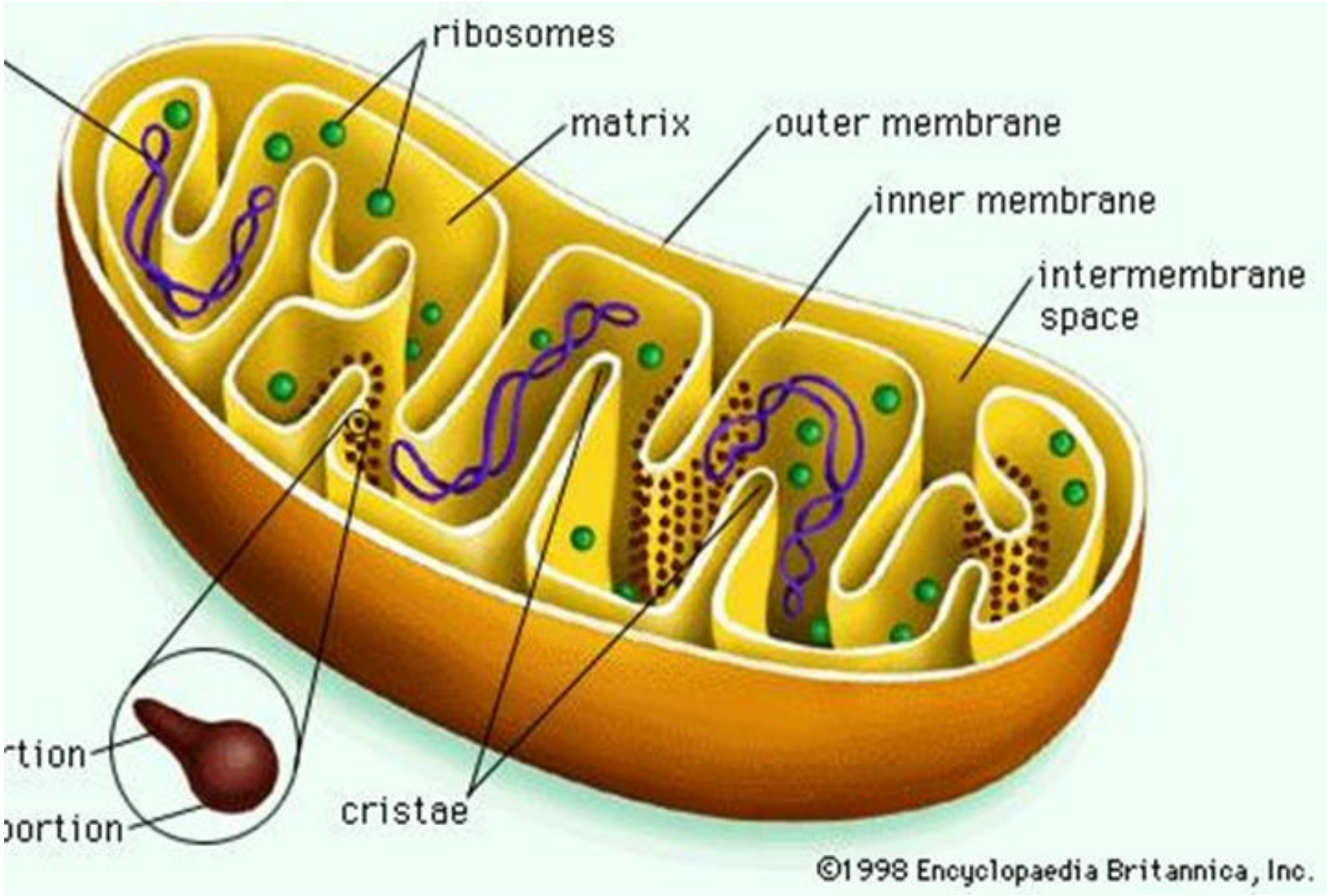
MITOCHONDRIA



- are membrane-bound structures
- they generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy
- the mitochondrion has its own independent genome



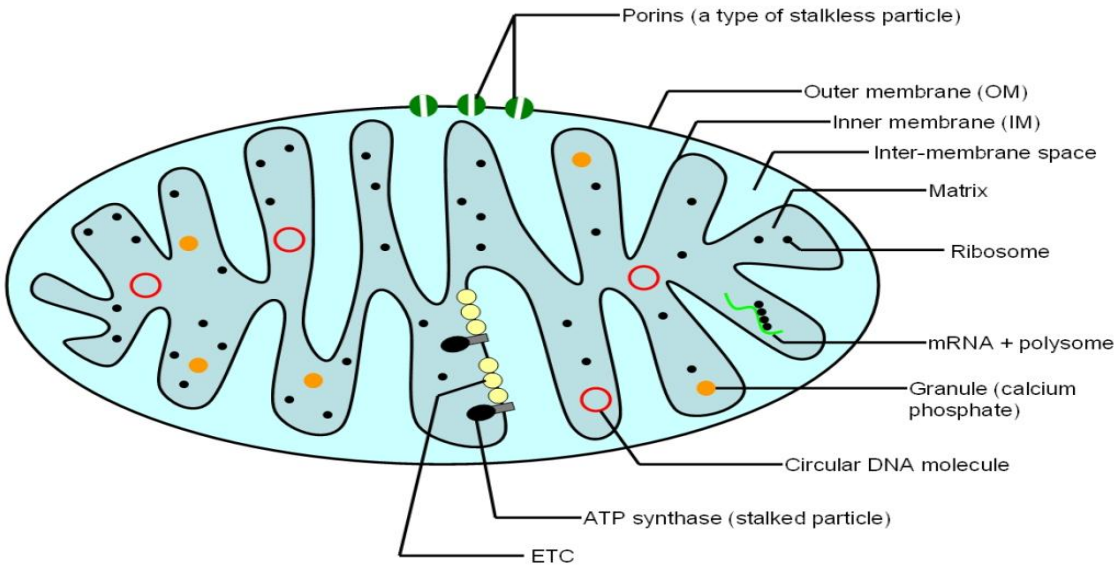
The structure of mitochondria



MITOCHONDRIA - FUNCTION

- **outer mitochondrial membrane**
  - porins - multipass transmembrane proteins – aqueous channels (water-soluble molecules may pass)-intramembrane space resemble cytosol
  - proteins responsible for the formation of mitochondrial lipids
- **inner mitochondrial membrane**
  - cardiolipin – phospholipid (with 4 fatty acyl chains) – impermeable to ions, electrons and protons
  - ATP synthase – protein complex responsible for the generation of ATP from ADP and inorganic phosphate
  - respiratory chains – protein complexes
- **contact sites** – regions in which outer and inner membranes contact each other

Structure of a mitochondrion



MITOCHONDRION - MATRIX

- enzymes responsible for the degradation of fatty acids to the acetyl CoA
- cytric acid cycle (Krebs cycle) – series of chemical reactions used by organisms to generate energy)
- DNA- double-stranded mitochondrial circular deoxyribonucleic acid

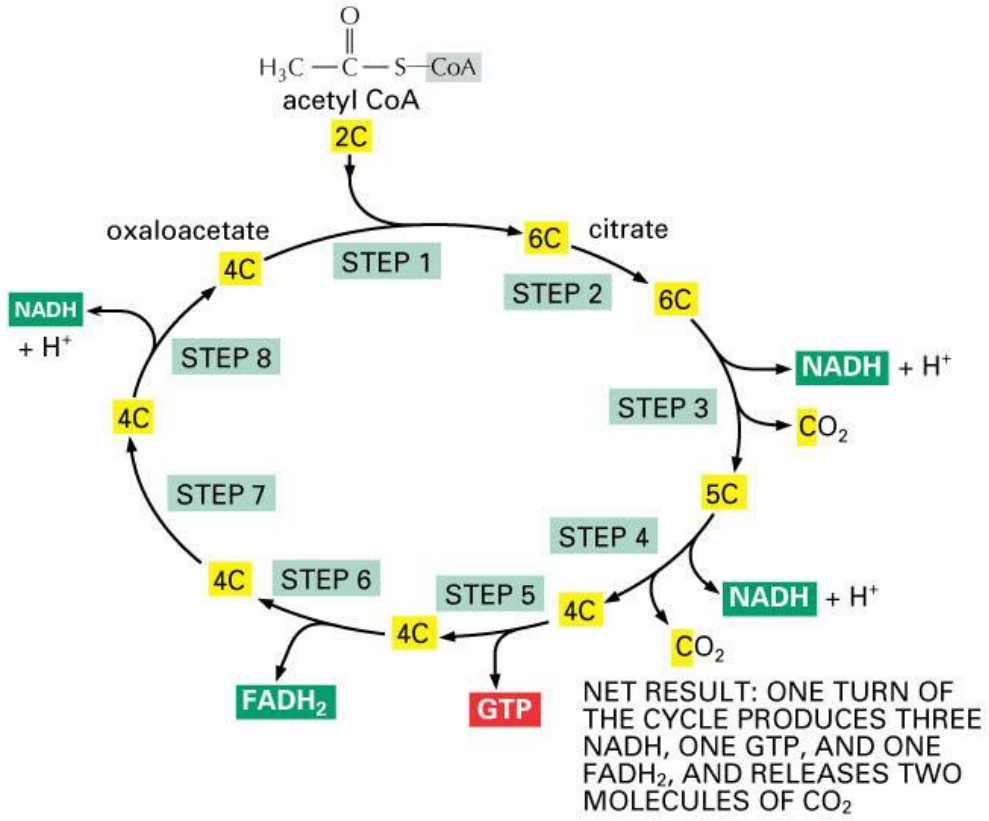
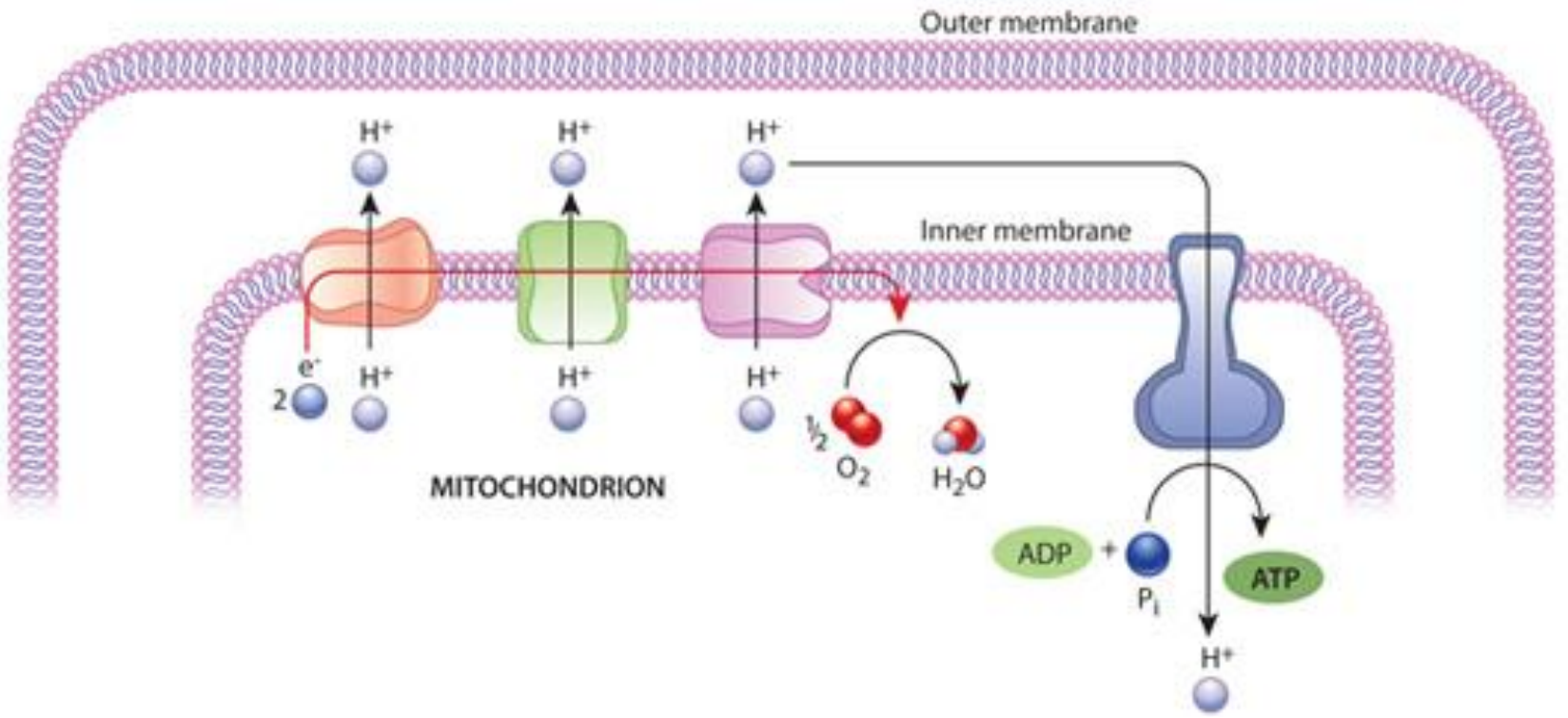
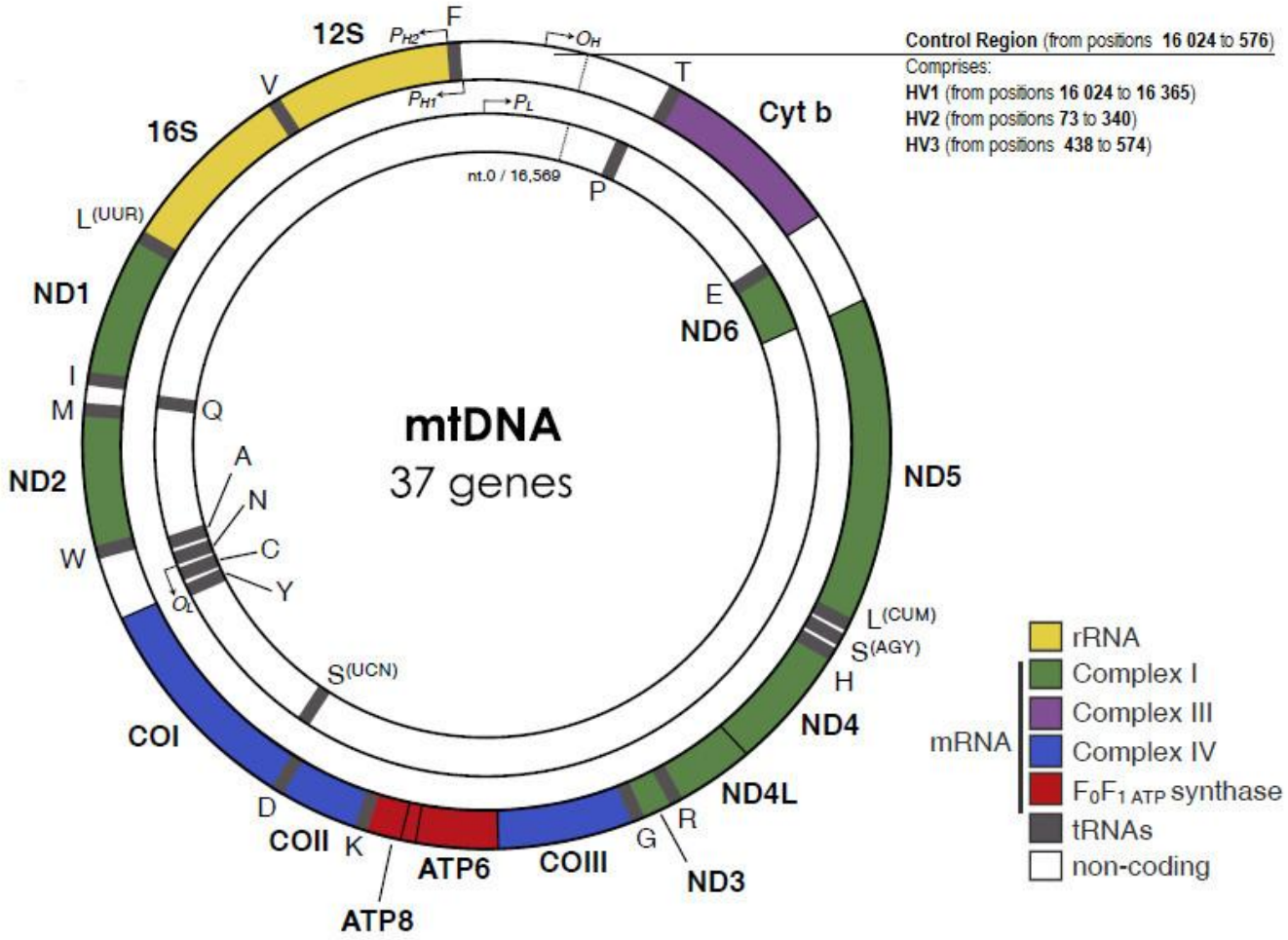


Figure 13-11 Essential Cell Biology, 2/e. (© 2004 Garland Science)


Outer membrane
Inner membrane
H+
H+
H+
e-
2
H+
H+
H+
MITOCHONDRION
½
O2
H2O
ADP
+
Pi
ATP
H+

Mitochondrial DNA



Termogenin (UCP-1) – heat production

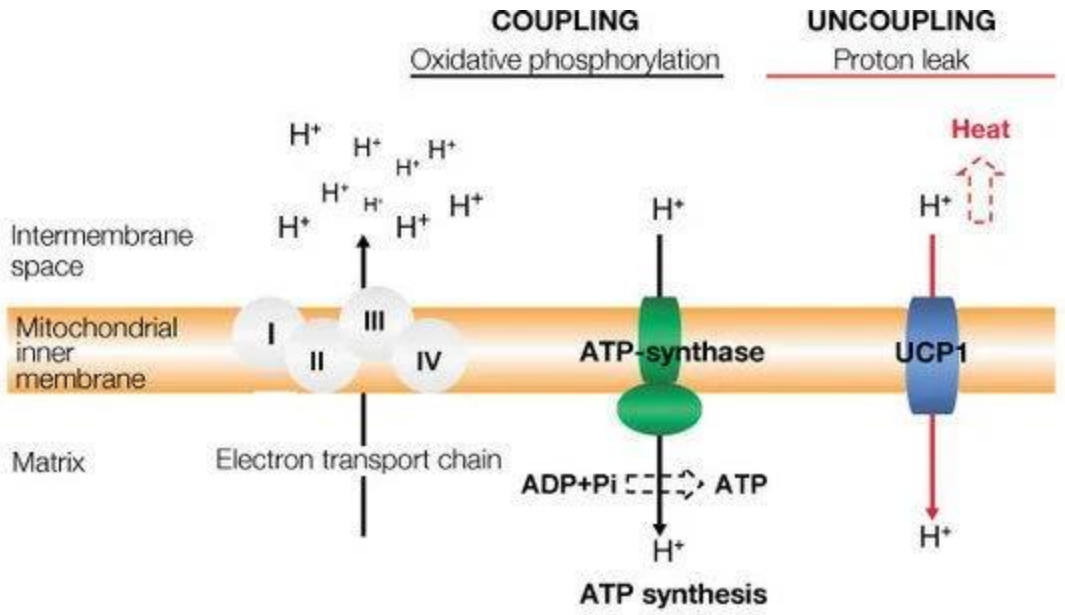
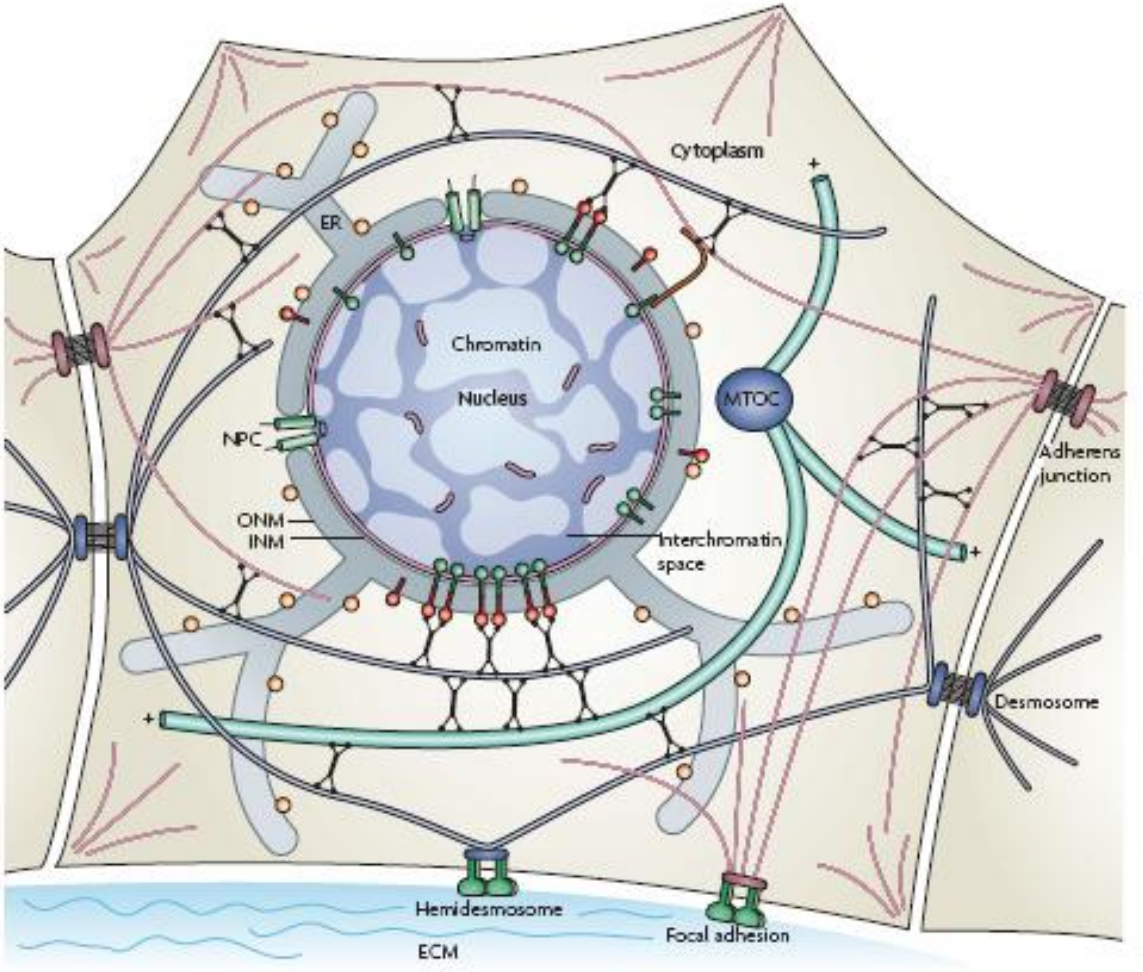


Figure 1. UCP1 location and function in the mitochondrial respiratory chain (MRC). Numbers I-IV corresponds to the MRC complexes. ATP-synthase is the fifth complex of the MRC. During respiration, protons are pumped through the MRC complexes, and a proton gradient is generated. The energy of the proton gradient drives the synthesis of ATP by the ATP-synthase complex. UCP1 catalyzes a regulated re-entry of protons into the matrix, uncoupling the MRC and, consequently, reducing ATP synthesis and generating heat.

CELL CYTOSKELETON

Cell cytoskeleton is a network made of biological polymers. They are three main types of polymer namely microtubules, actin (thin) filaments and intermediate filaments

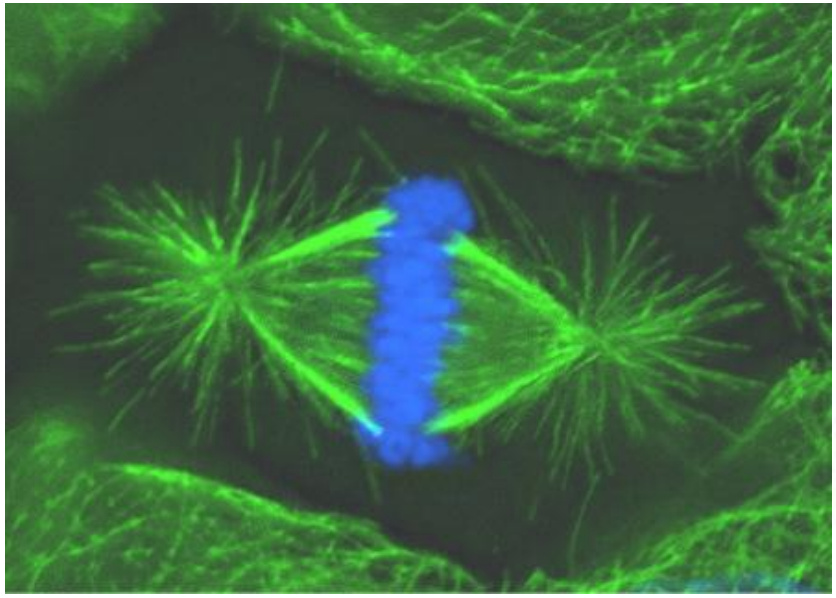


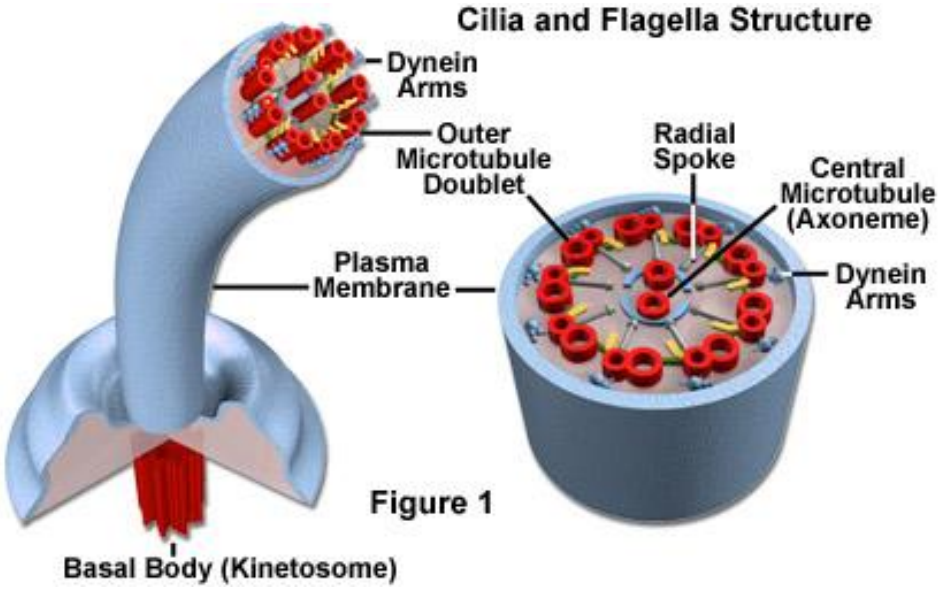
MICROTUBULES

- long, straight, cylindrical structures

They play key roles in:

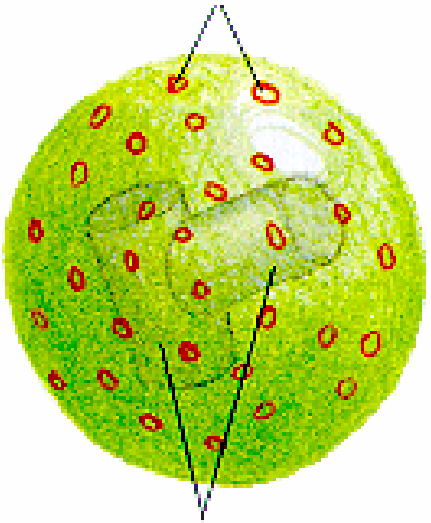
- intracellular transport of secretory vesicles and organelles
- cell division (mitosis and meiosis) including the formation of mitotic spindles
- They also form the internal structure of cilia and flagella



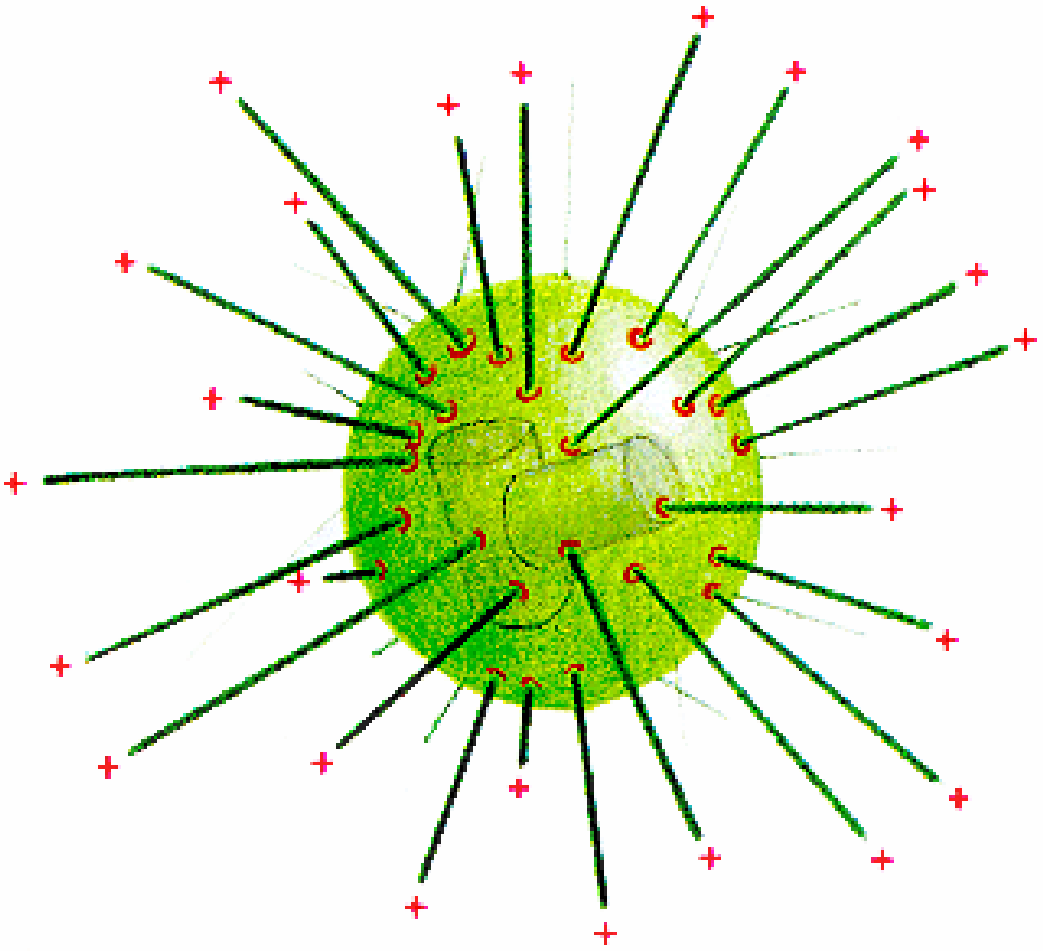


Centrosome

Nucleating sites
(g tubulin ring complexes)



pair of centrioles



Microtubule-Associated Proteins (MAPs)

Motor proteins

- responsible for the intracellular movement of organelles and vesicles
- motor proteins which convert the chemical energy contained

in ATP into the mechanical energy of movement.

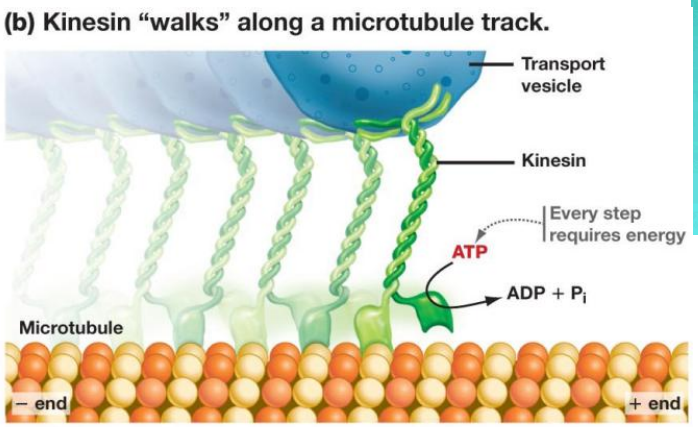
dynein - from plus to minus end

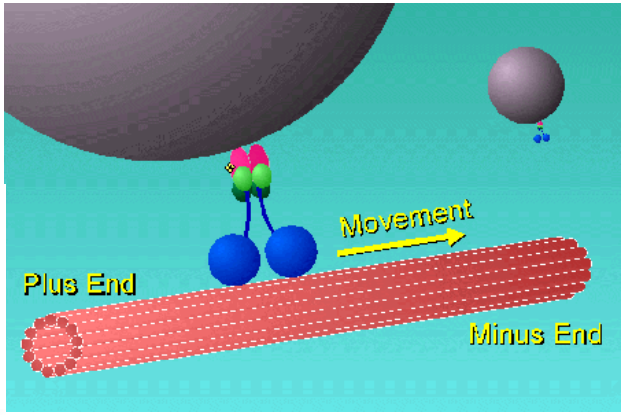
kinesin – from minus to plus end

Structural

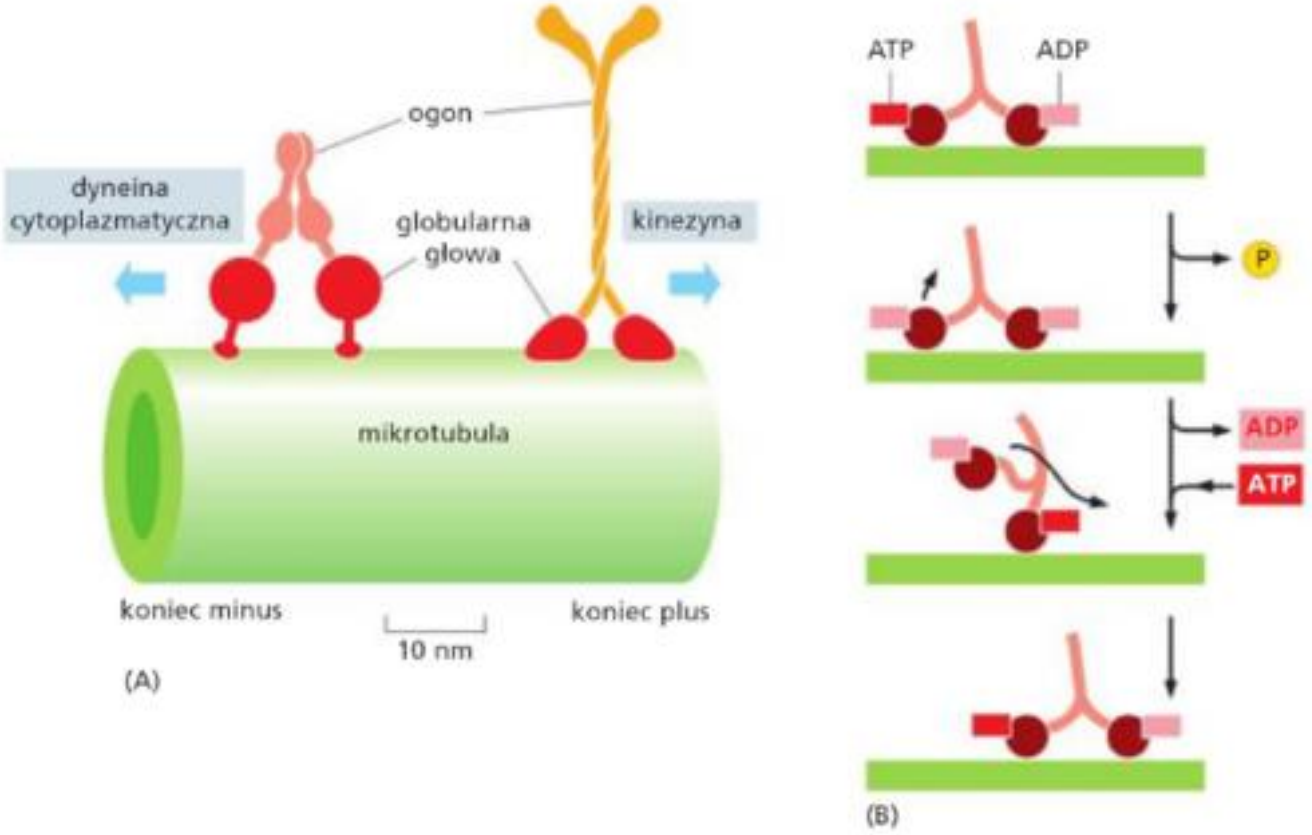
- Tau
- MAP2

Stabilizing

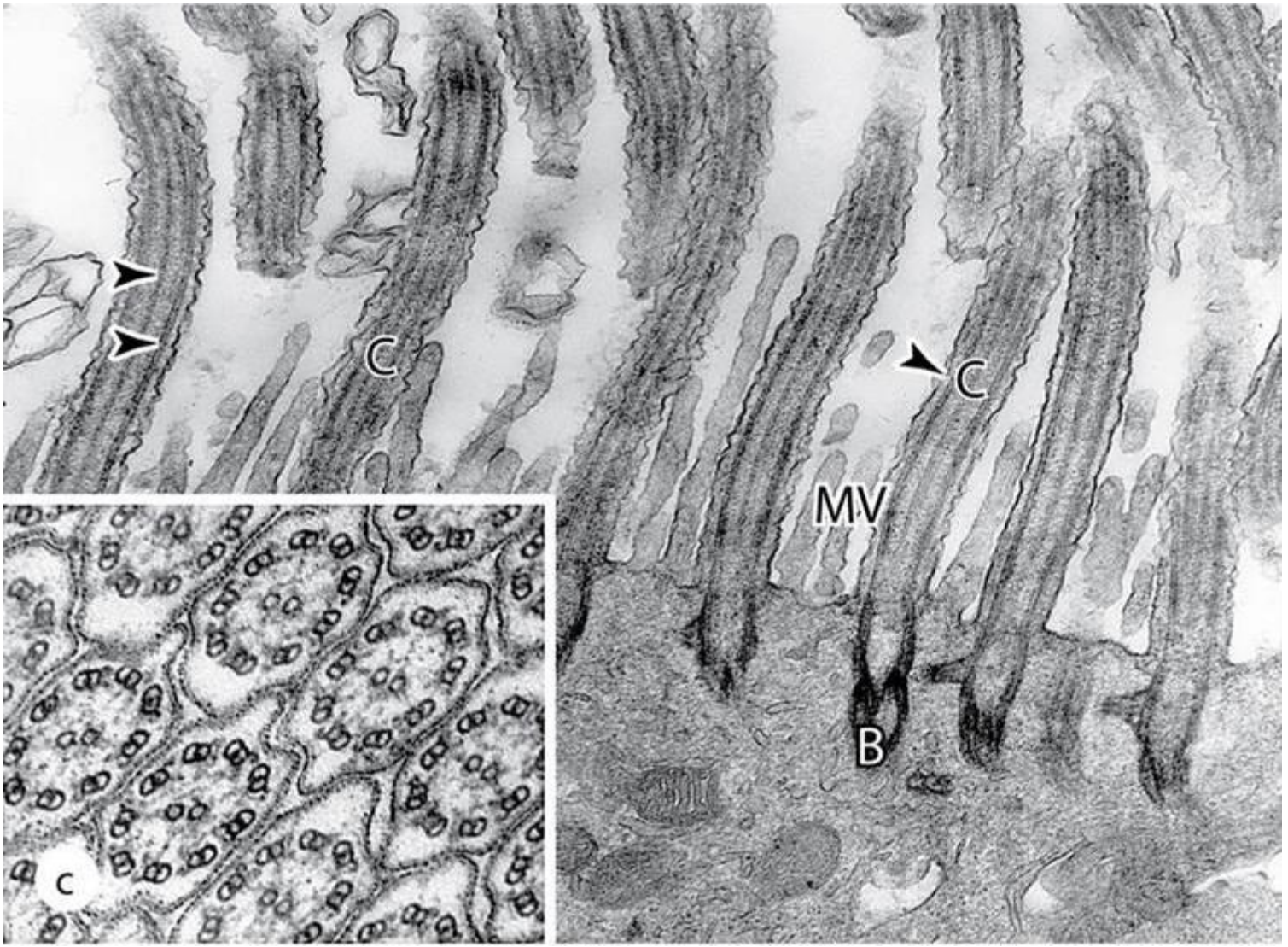




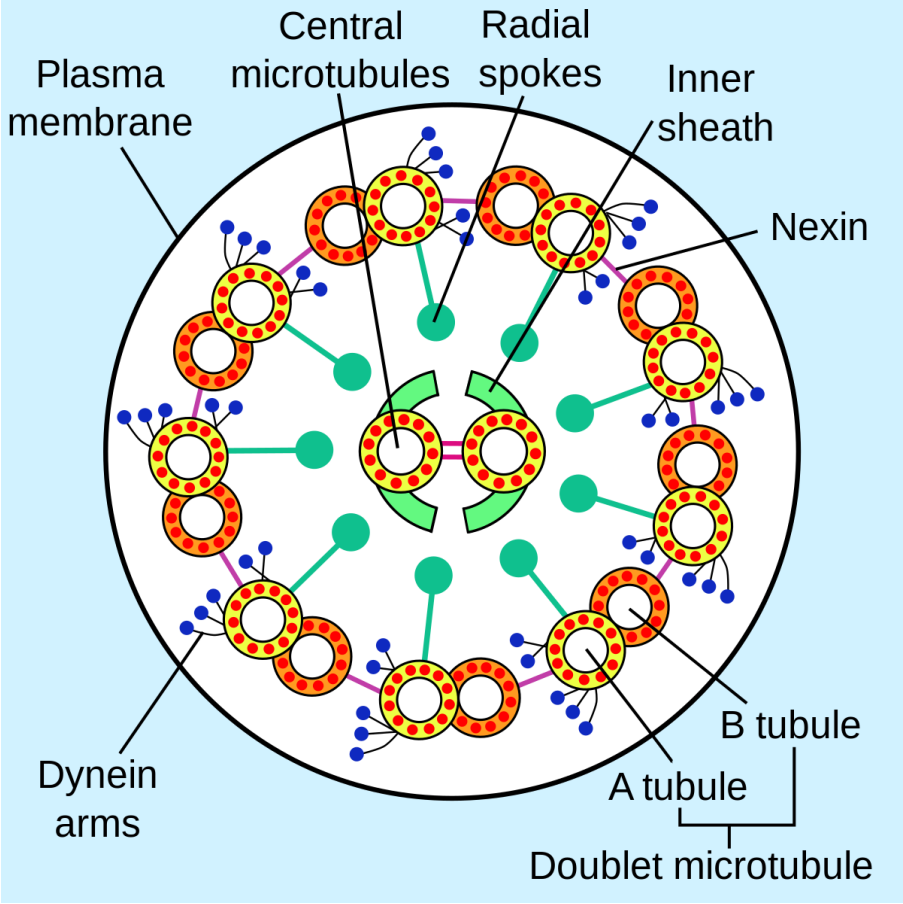
Kinesin and dynein

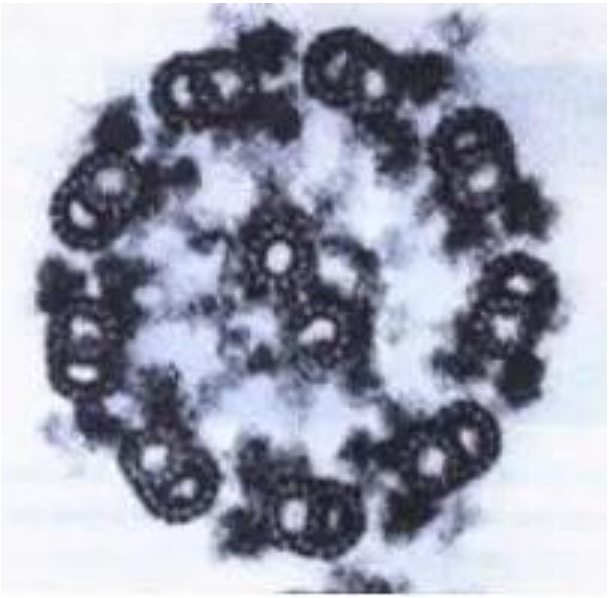


Cilia



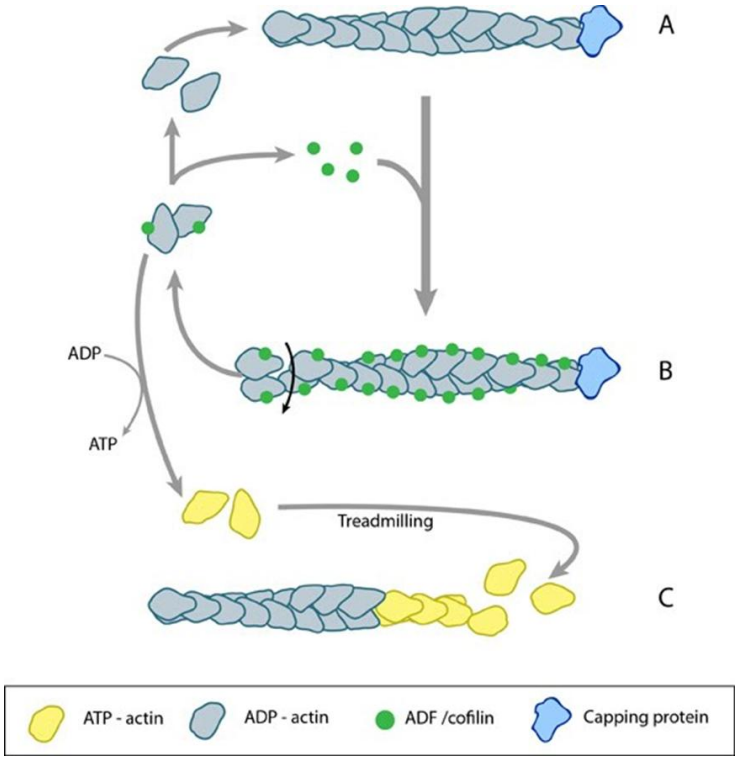
Aksoneme

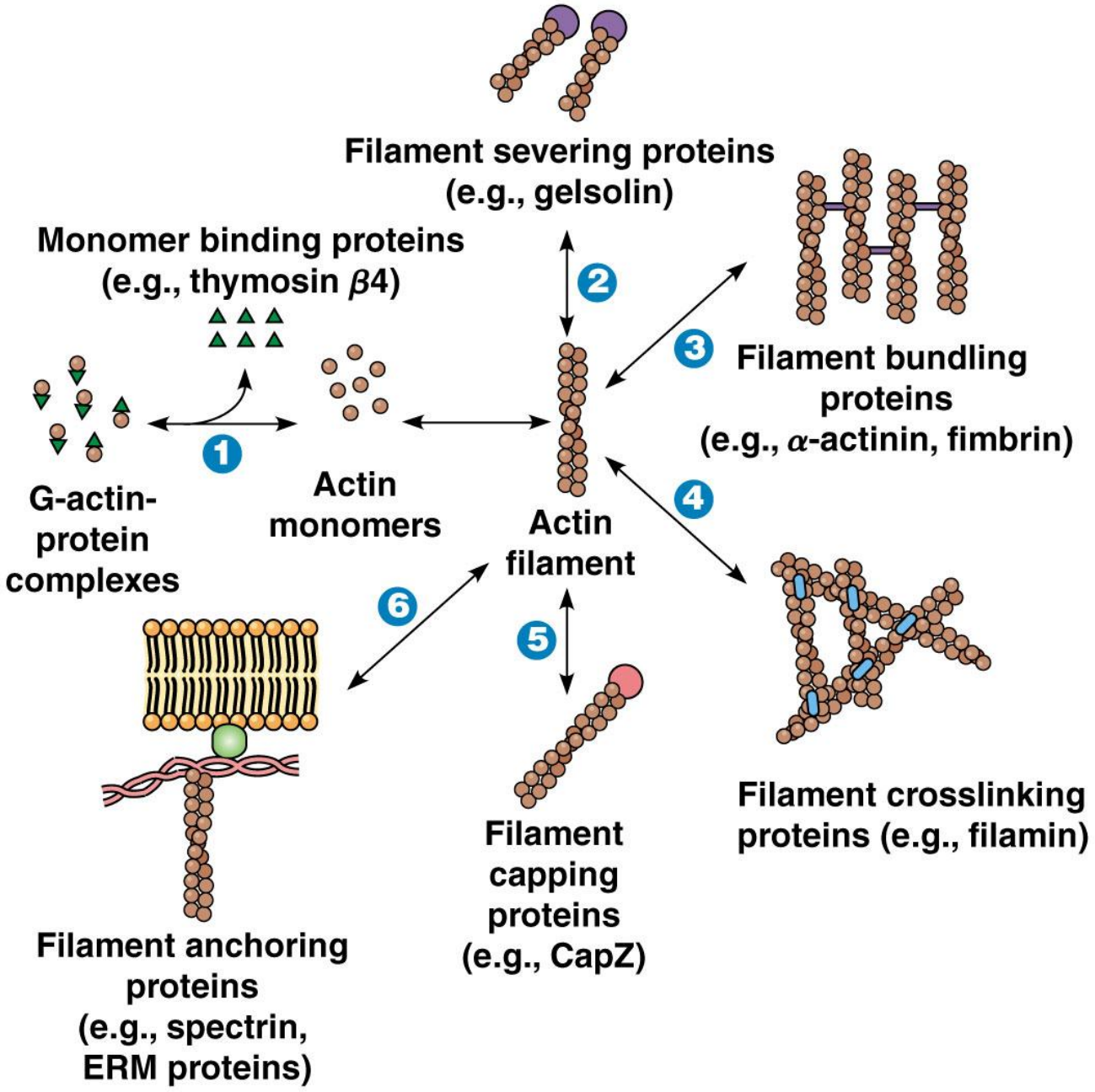

Central microtubules
Radial spokes
Plasma membrane
Inner sheath
Nexin
Dynein arms
B tubule
A tubule
Doublet microtubule

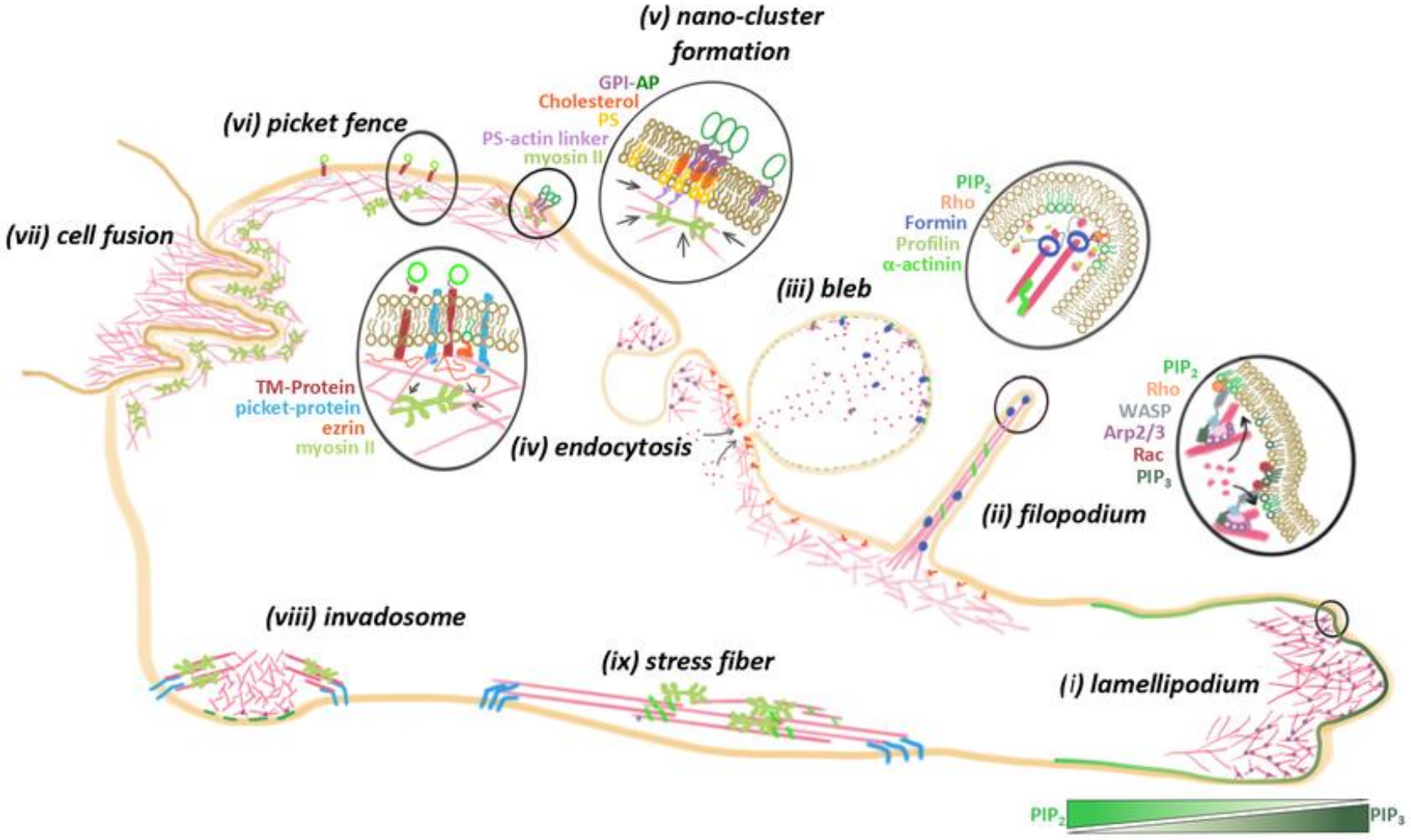


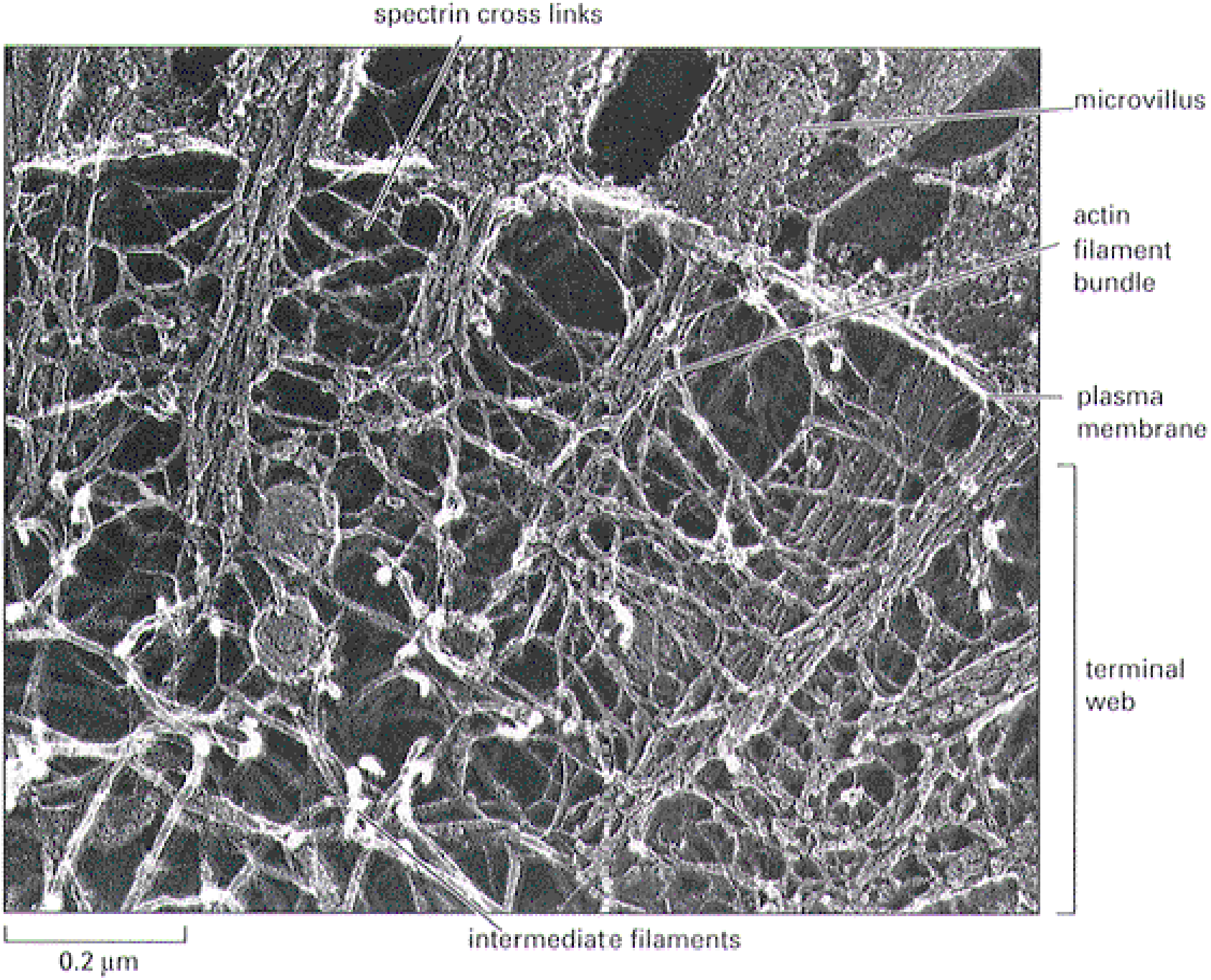
The actin (thin) filaments

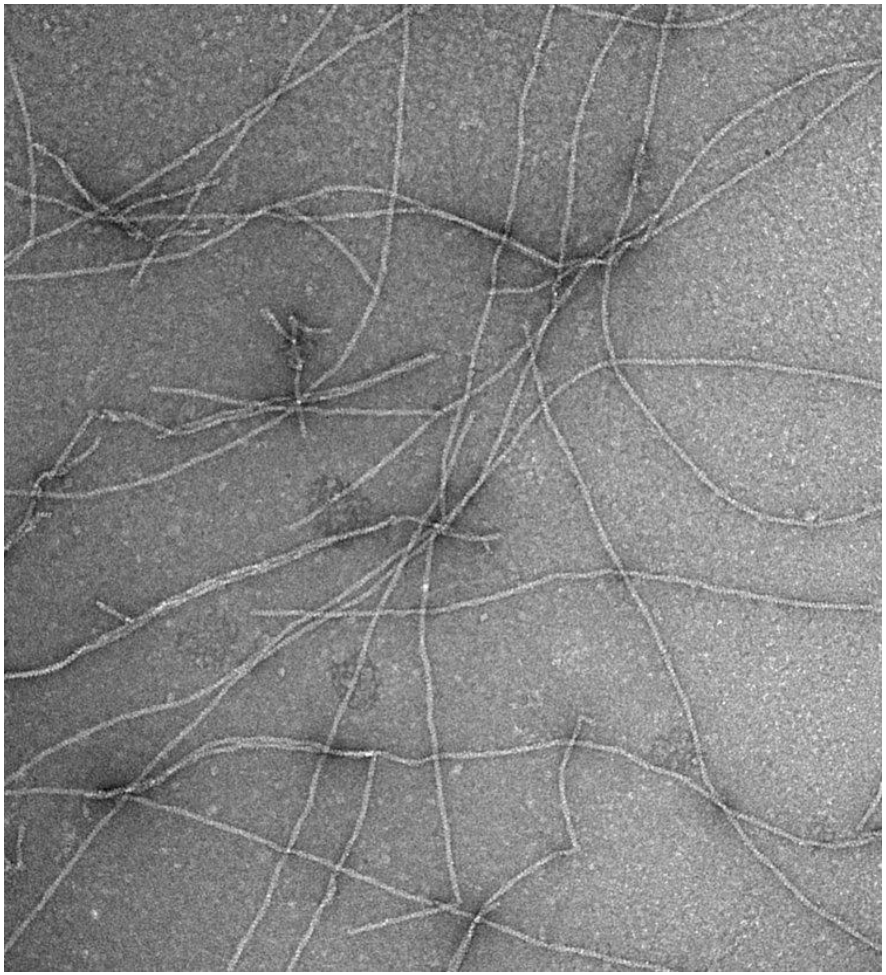
The actin cytoskeleton provides a structural framework for the mechanical stability of eukaryotic cells and is involved in functions including cell adhesion, motility, and division.

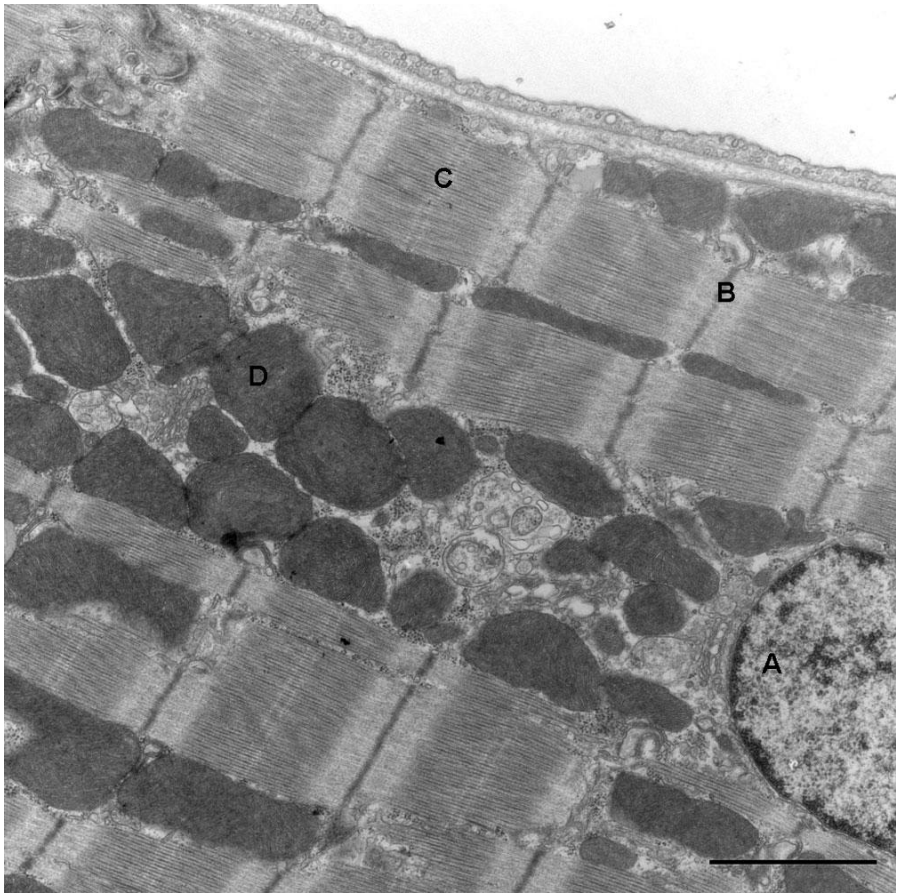




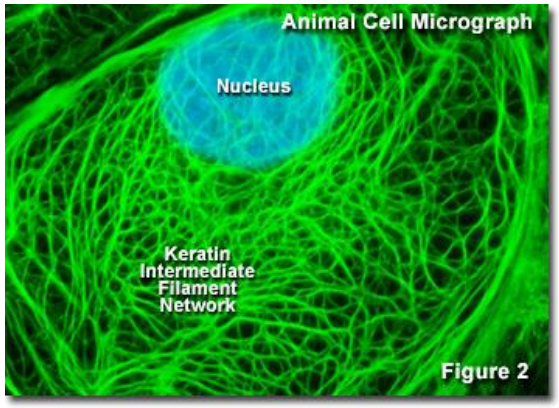

(v) nano-cluster formation
GPI-AP
Cholesterol
PS
PS-actin linker
myosin II
(vi) picket fence
(vii) cell fusion
TM-Protein
picket-protein
ezrin
myosin II
(iv) endocytosis
(iii) bleb
PIP2
Rho
Formin
Profilin
α-actinin
PIP2
Rho
WASP
Arp2/3
Rac
PIP3
(ii) filopodium
(viii) invadosome
(ix) stress fiber
(i) lamellipodium
PIP2
PIP3





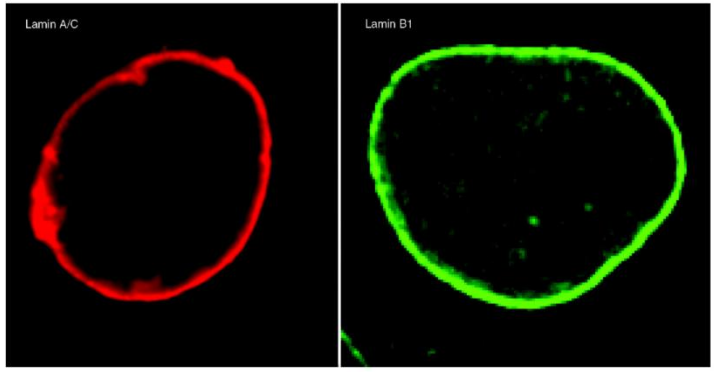

C
B
D
A

INTERMEDIATE FILAMENTS



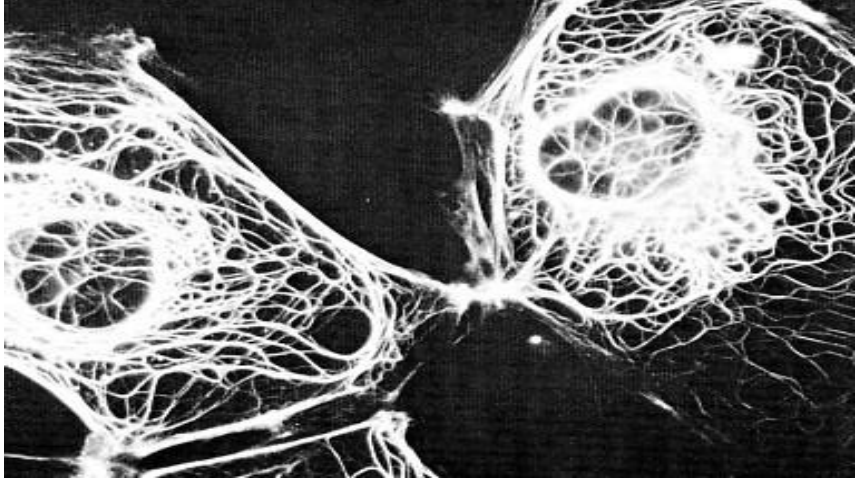
- have an average diameter of 10 nanometers, which is between that of 7 nm actin (microfilaments), and that of 25 nm microtubules

Most types of intermediate filaments are cytoplasmic, but one type, the lamins, are nuclear.



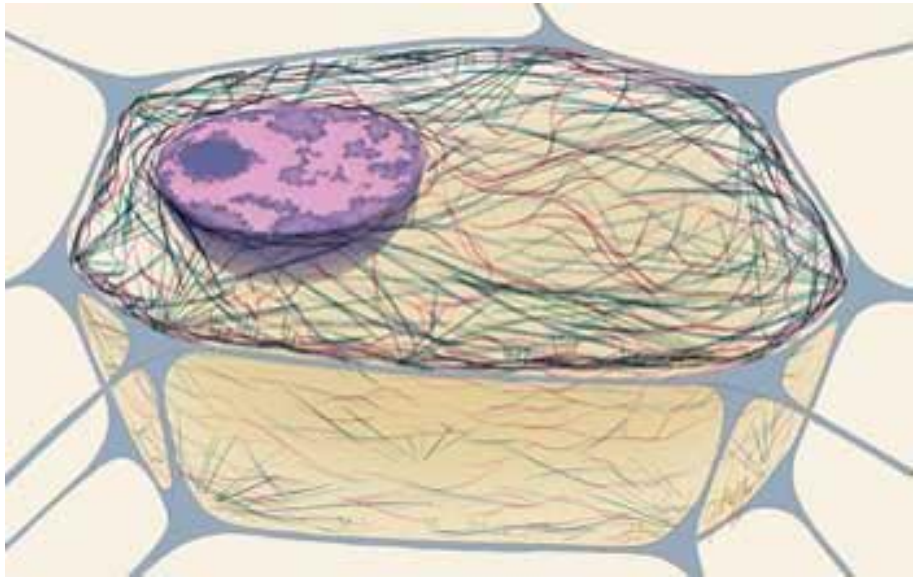
TYPES:

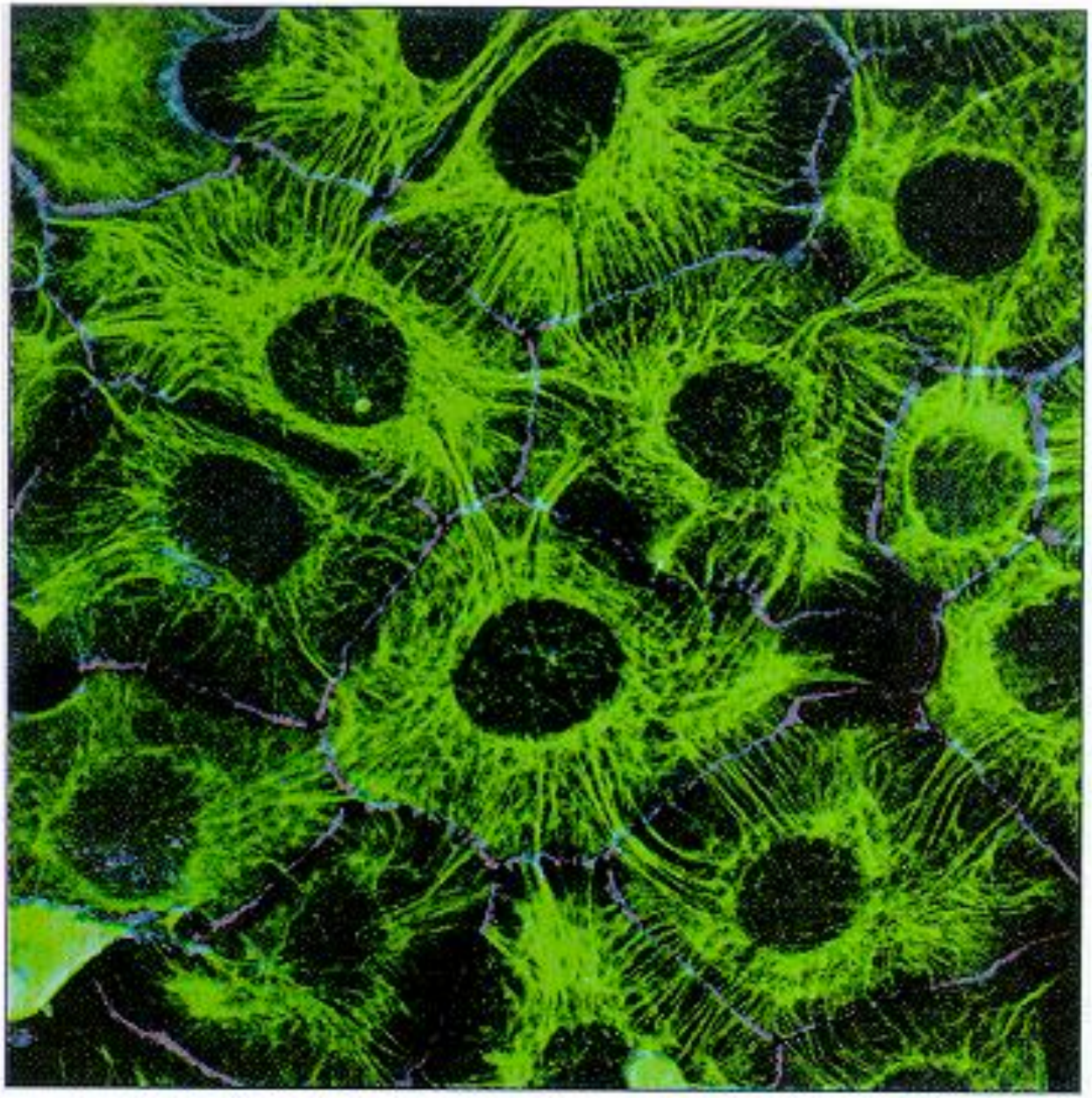
- **cytokeratins** in epithelial cells
- **desmin** in muscle cells
- **vimentin** in many mesodermal tissues
- **GFAP** (glial fibrillary acidic protein) in astrocytes and other glia
- **neurofilaments** in axons of vertebrate neurons
- **lamins** in the cell nucleus



FUNCTION OF INTERMEDIATE FILAMENTS

- provide structural support for the cell (form three-dimensional framework)
- anchor the nucleus in place
- they form the connection between the cell membrane and the cytoskeleton





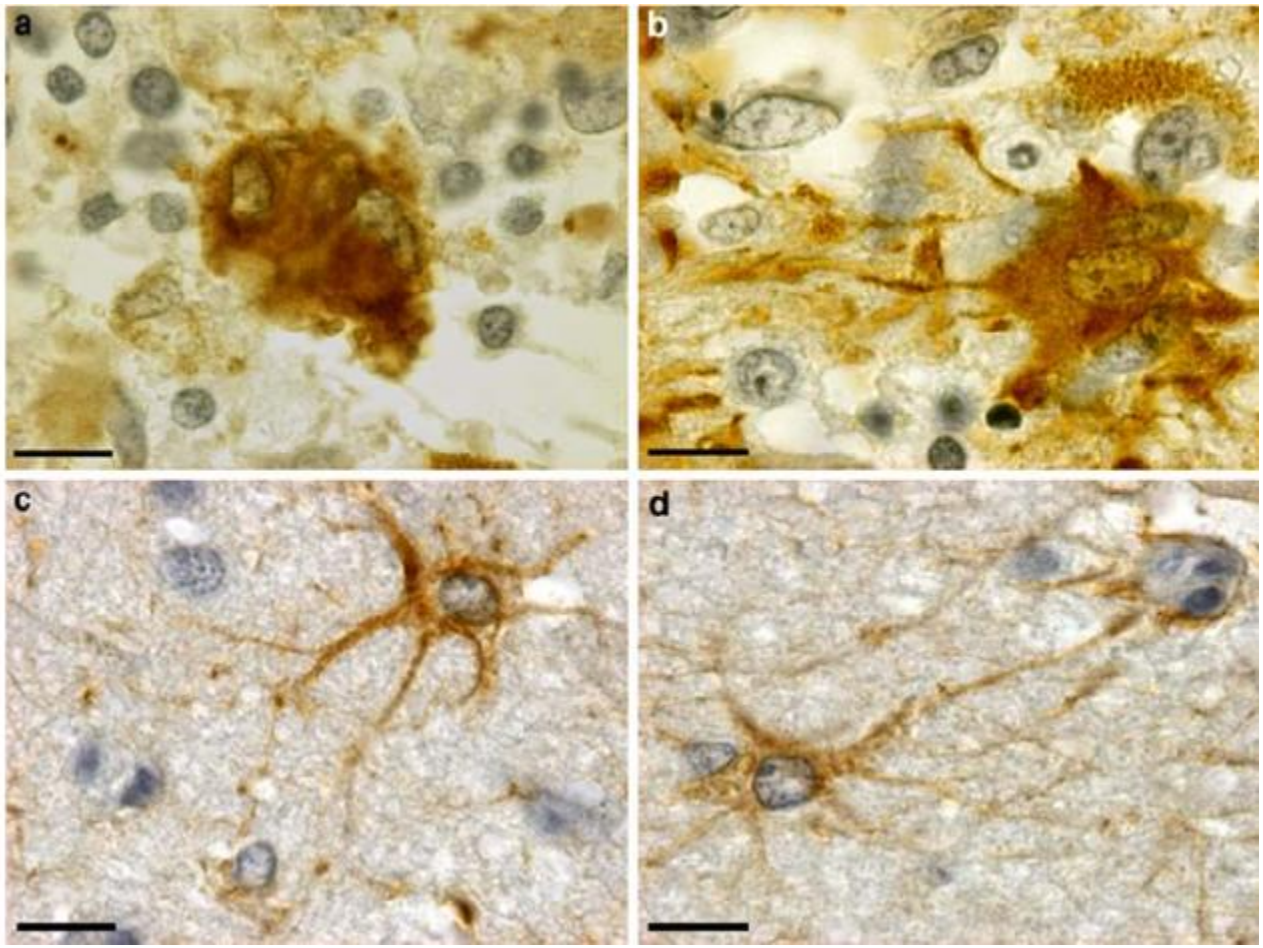
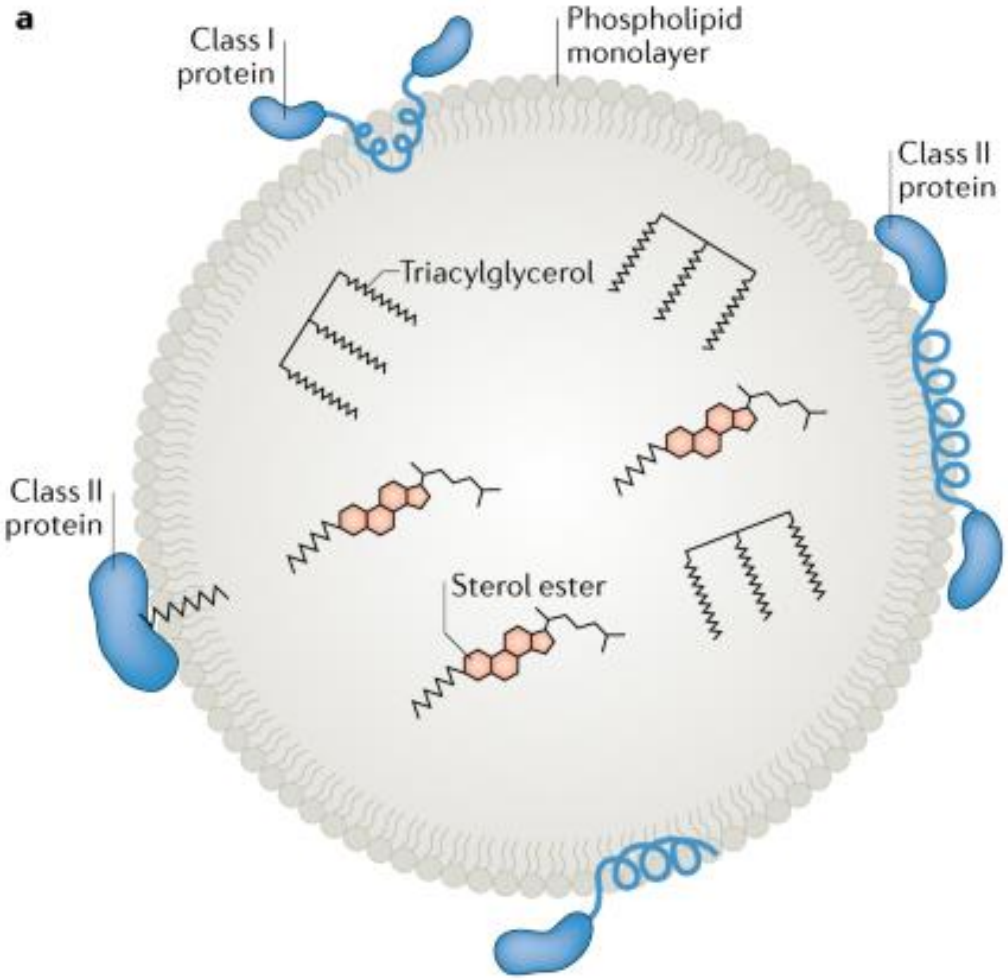
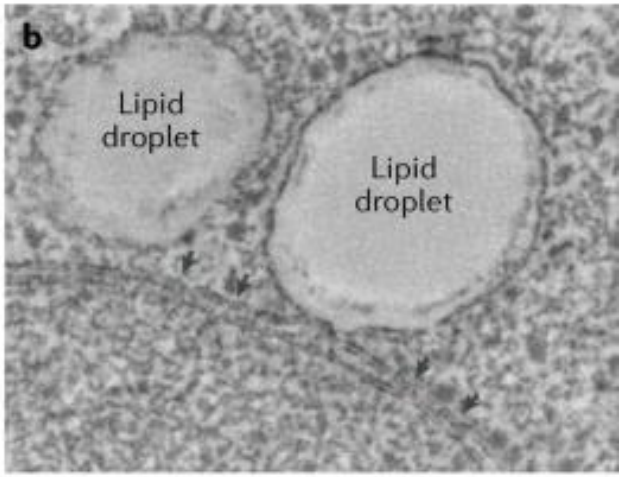
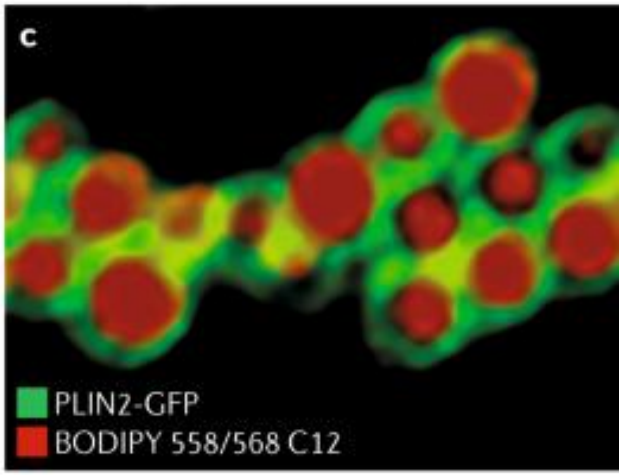


Fig. 5 V_HH immunolabeling of GFAP in astrocytoma. **a**, **b** Abnormal astrocytes in the tumor. **c**, **d** Reactive astrocyte at the periphery of the tumor. *Scale bar* 10 μm

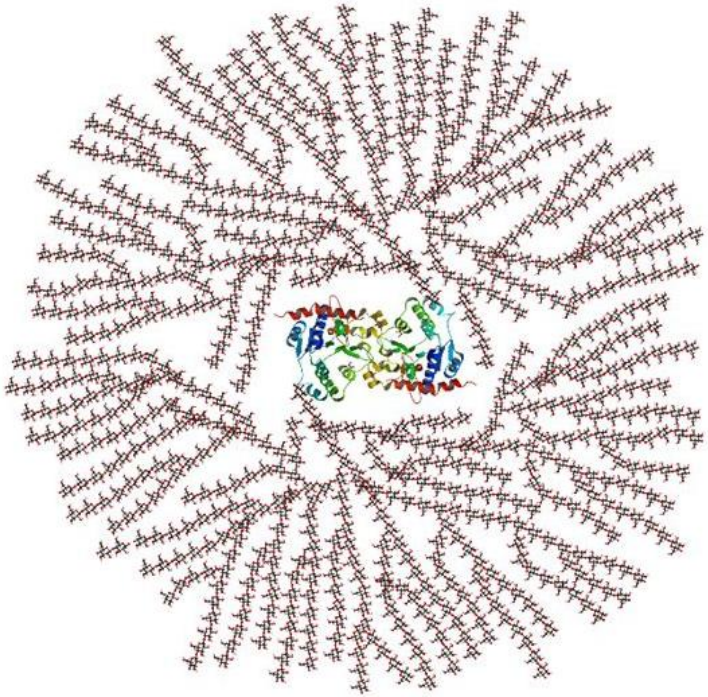
Lipid droplets

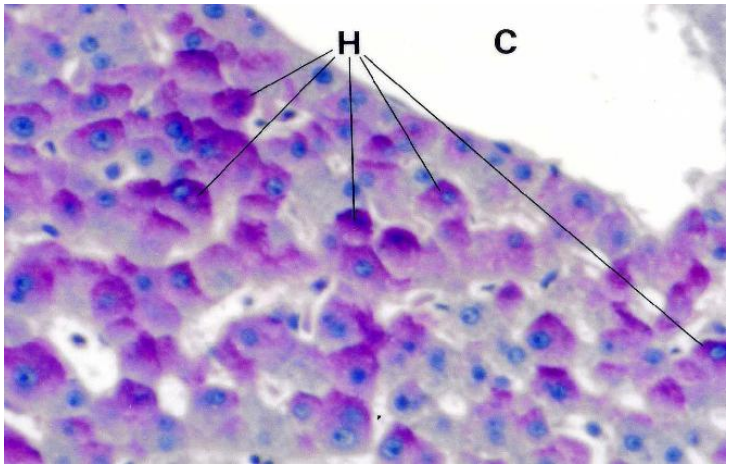






Glycogen granules





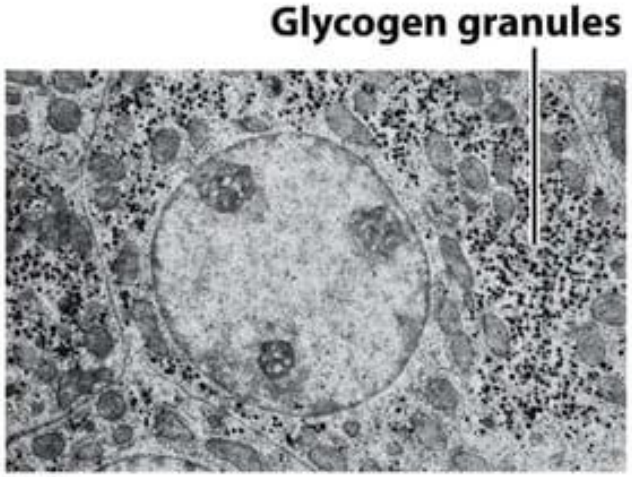


Figure 21.3