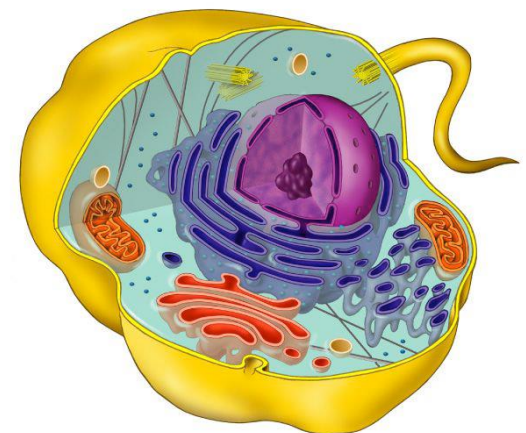


Cytology 2

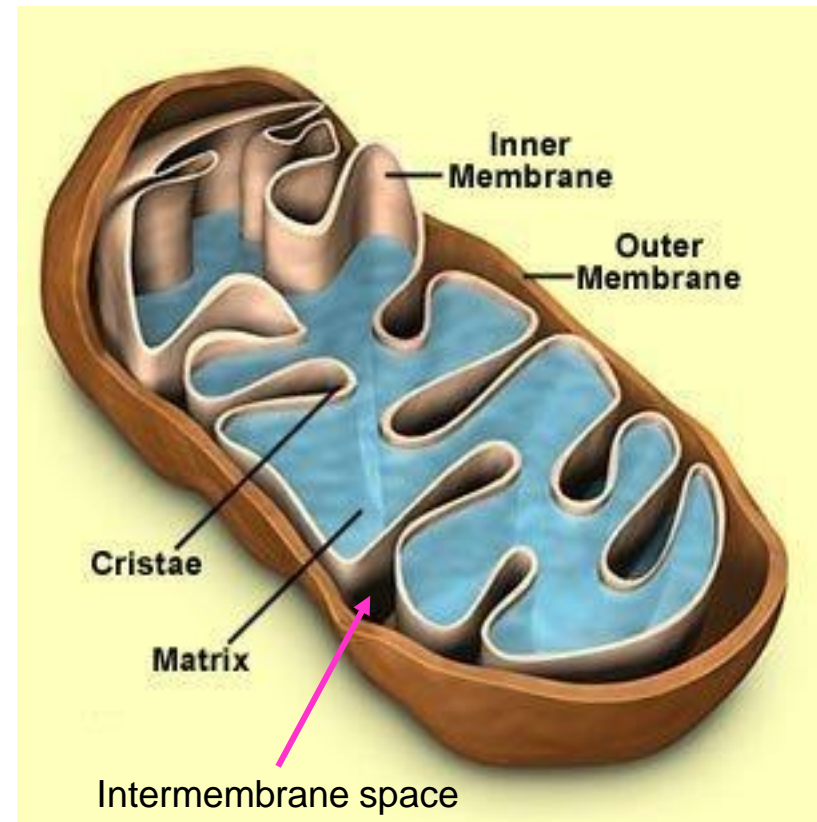
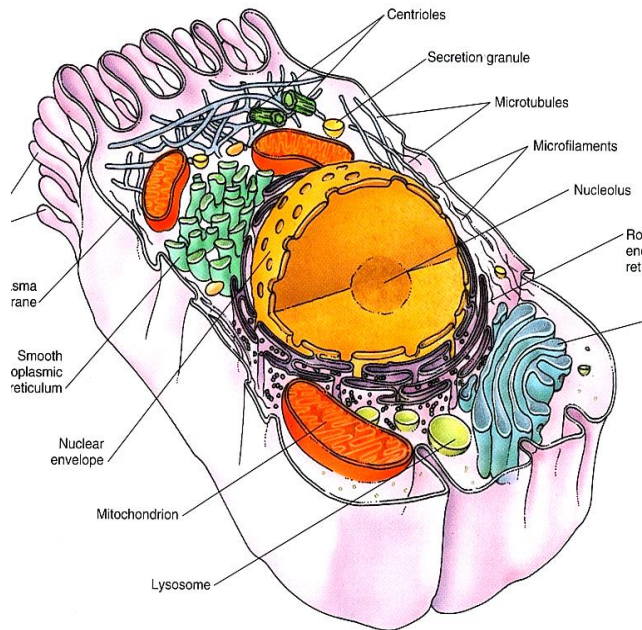
Aleš Hampl

2019



- Mitochondria
- Lysosomes + Peroxisomes
- Cytoplasmic inclusions
- Cytoskeleton
- Cell surface specialisations
- Cell cycle, cell division, cell differentiation

Mitochondria 1



- all cells except erythrocytes
- double membrane
- diameter cca 0,5 μm
- length up to 50 (100) μm
- oxidative metabolism (glucose – ATP + CO_2 + H_2O)
- cytochrome c – activation of apoptotic pathway
- origin in oocyte
- mtDNA (circular)
- brown fat thermogenesis

- both membranes with low fluidity
- both membranes equipped with many protein molecules
- growth and division of mitochondria

Mitochondria 2

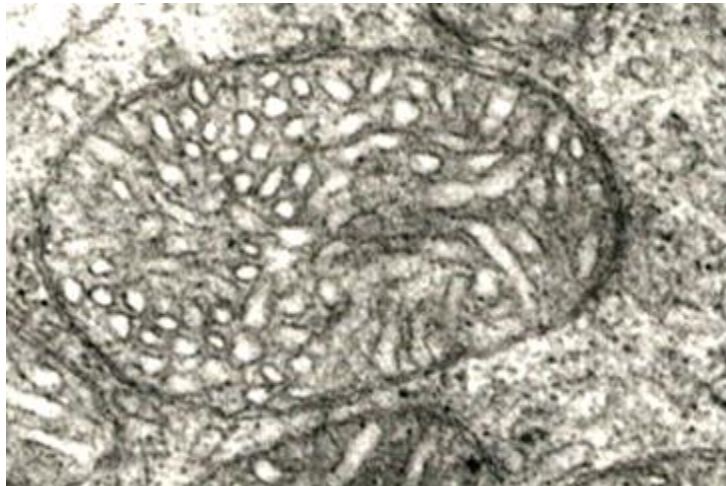


Mitochondria 3

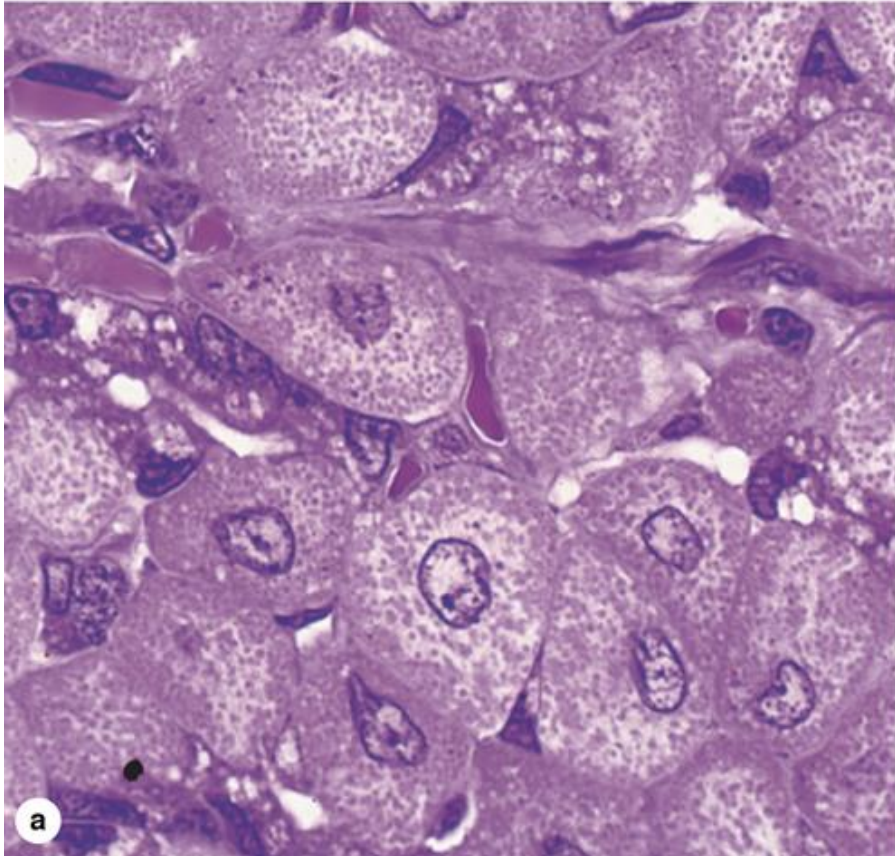
with cristae



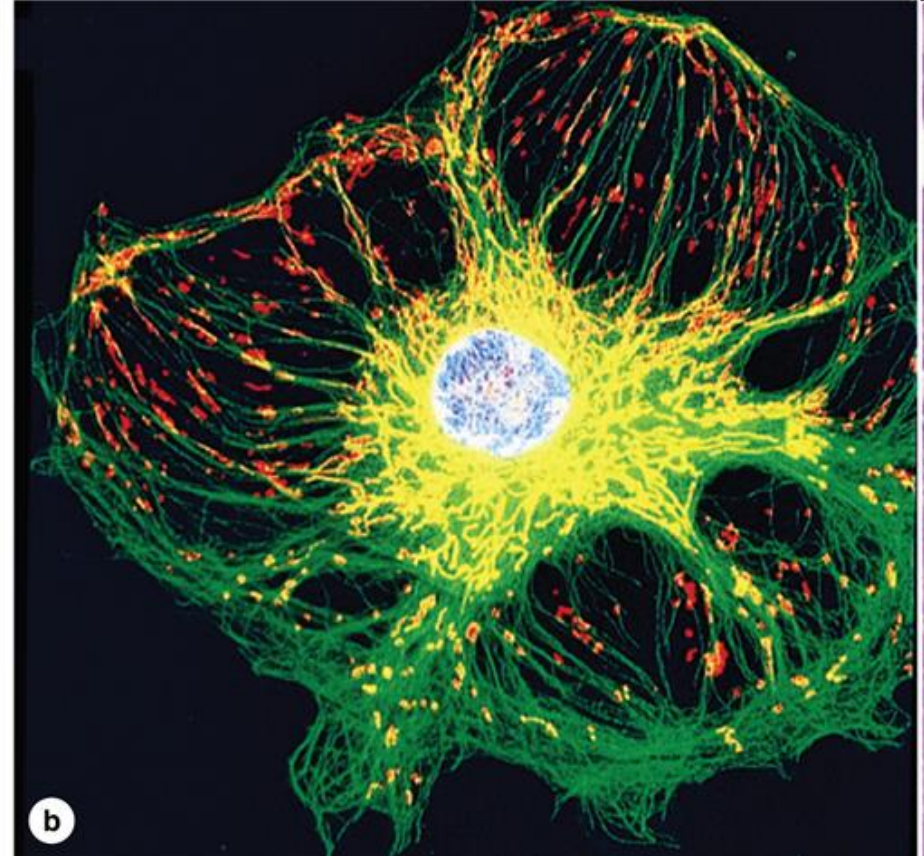
with tubuli (in steroid producing cells)



Mitochondria 4



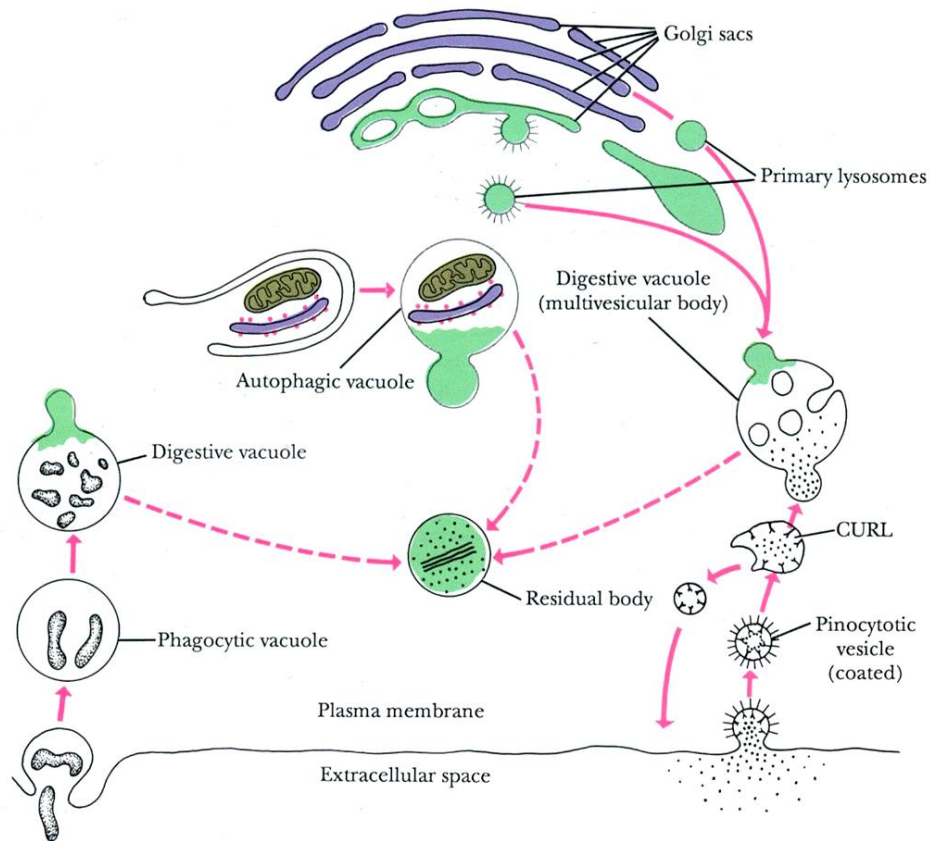
a
mitochondrial eosinophilia



b
mitochondria
microtubuli

Lysosomes 1

endosome-lysosome system

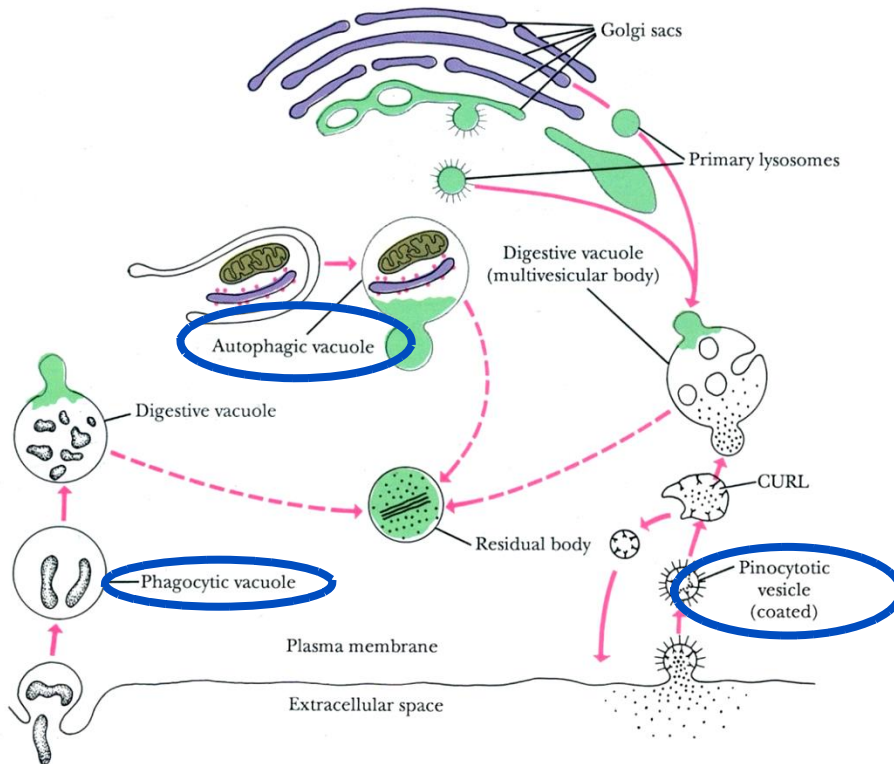


- in all cells except for erythrocytes
- vesicles about 0,05 – 0,5 μm
- membrane-bound
- highly acidic internal space (cca pH 5)
- hydrolytic enzymes inside (min. 50 types)
- tagging by mannose-6-fosphate

Figure 2.17. Origins of primarily lysosomes from the Golgi and trans-Golgi network. Primary lysosomes fuse with and discharge hydrolytic enzymes into autophagic, pinocytotic (or endosome), and phagocytic vacuoles to form secondary lysosomes (digestive vacuoles). Residual bodies contain undigested residue. Endosomes fuse to form a compartment where uncoupling of the ligands and surface receptors occurs (CURL, see text for explanation). The compartment containing the free ligands subsequently fuses with the lysosome; the receptors remain bound to the membrane of vesicles which is partitioned off from the CURL and recycle to the plasma membrane. (Modified from Novikoff AB, Holtzman E: *Cells and Organelles*, 2nd ed. New York, Holt, Rinehart and Winston, 1976.)

Lysosomes 2

primary x secondary

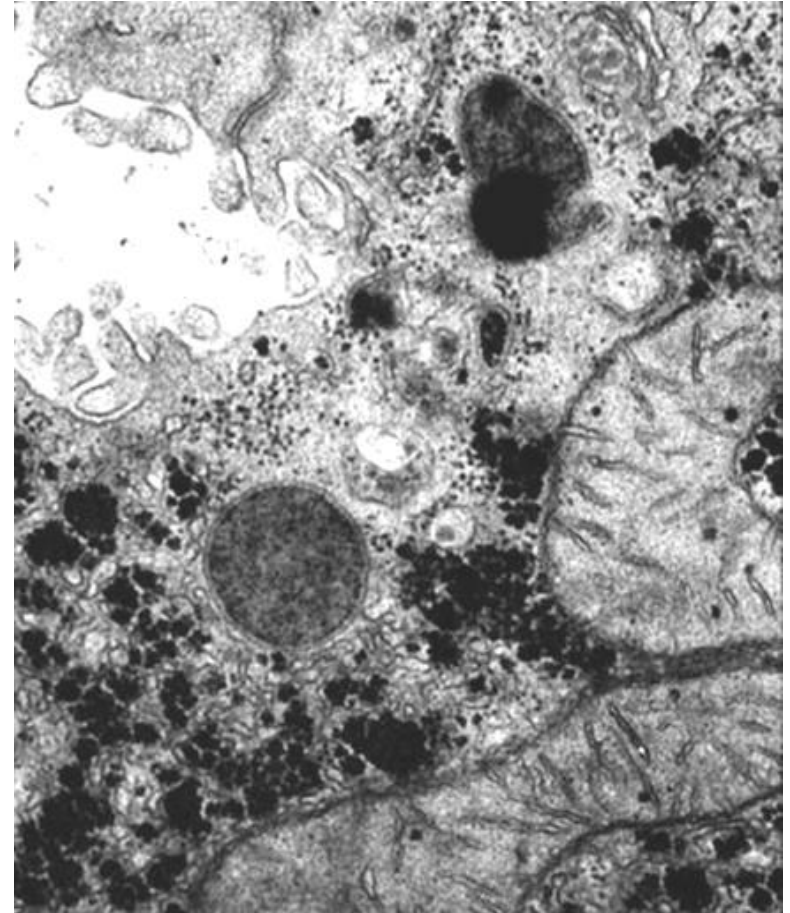
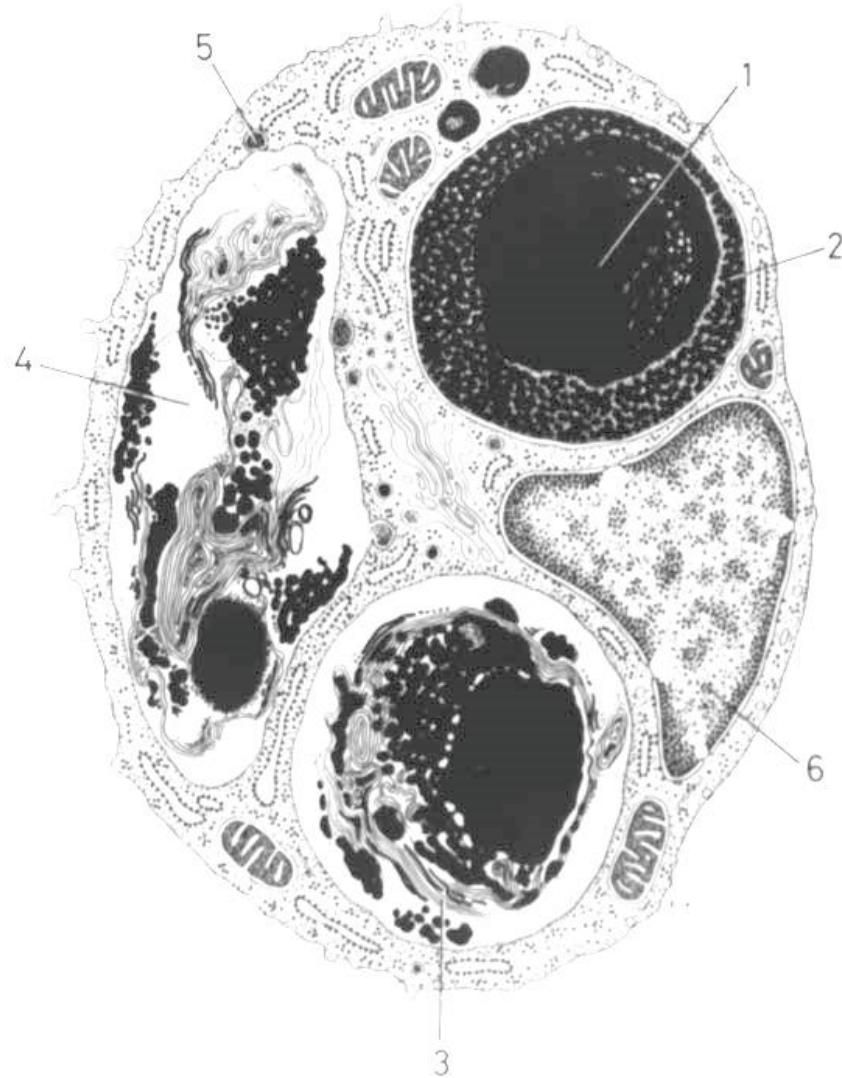


- primary lysosomes
- secondary lysosomes (fagolysosomes)
- residual bodies (lipofuscin)

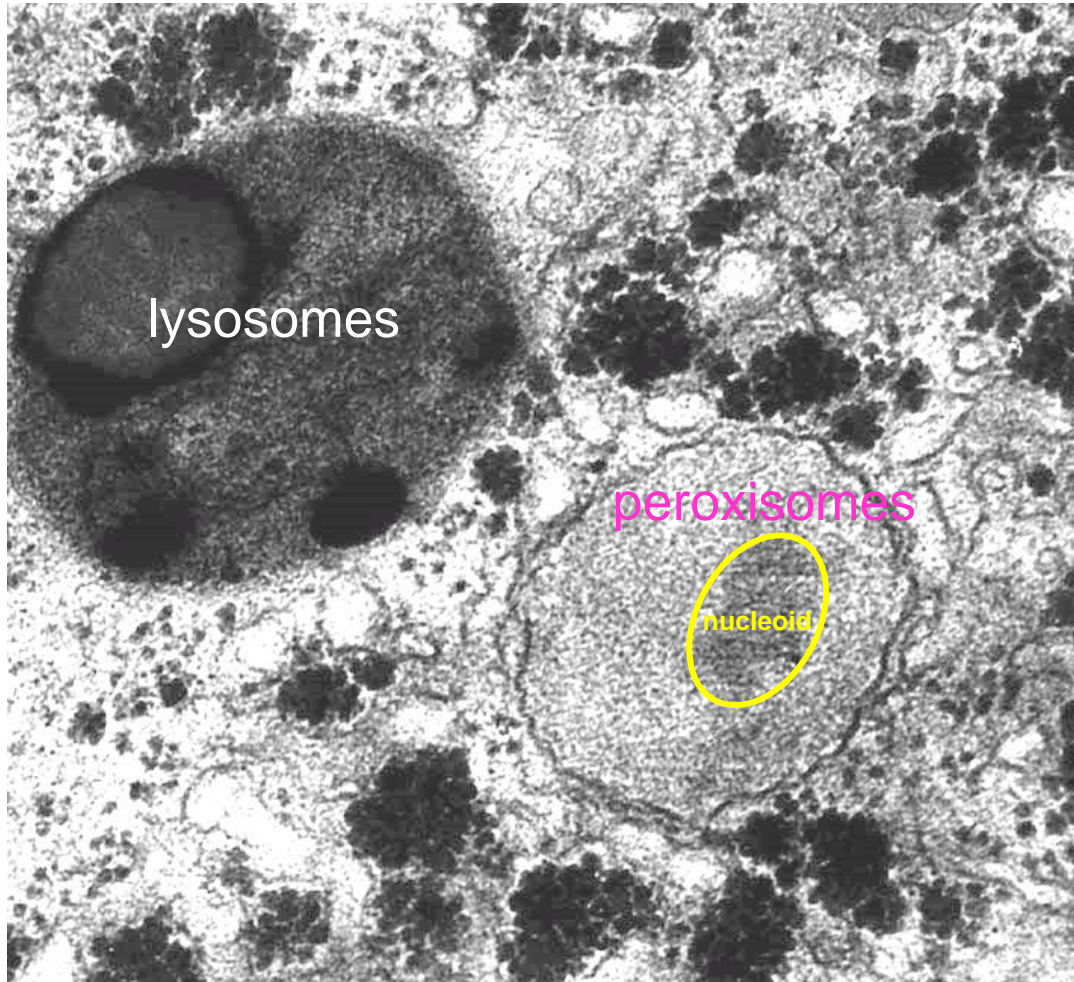
Figure 2.17. Origins of primary lysosomes from the Golgi and trans-Golgi network. Primary lysosomes fuse with and discharge hydrolytic enzymes into autophagic, pinocytotic (or endosome), and phagocytic vacuoles to form secondary lysosomes (digestive vacuoles). Residual bodies contain undigested residue. Endosomes fuse to form a compartment where uncoupling of the ligands and surface receptors occurs (CURL, see text for explanation). The compartment containing the free ligands subsequently fuses with the lysosome; the receptors remain bound to the membrane of vesicles which is partitioned off from the CURL and recycle to the plasma membrane. (Modified from Novikoff AB, Holtzman E: *Cells and Organelles*, 2nd ed. New York, Holt, Rinehart and Winston, 1976.)

Lysosomes 3

secondary lysosomes



Peroxisomes



- structurally similar to lysosoms
- functionally similar to mitochondria
- „nucleus“ = nucleoid
- degradation of fatty acids (H_2O_2 , H_2O , O_2)
- detoxification (complement SER)
- origin: growth from ER or division

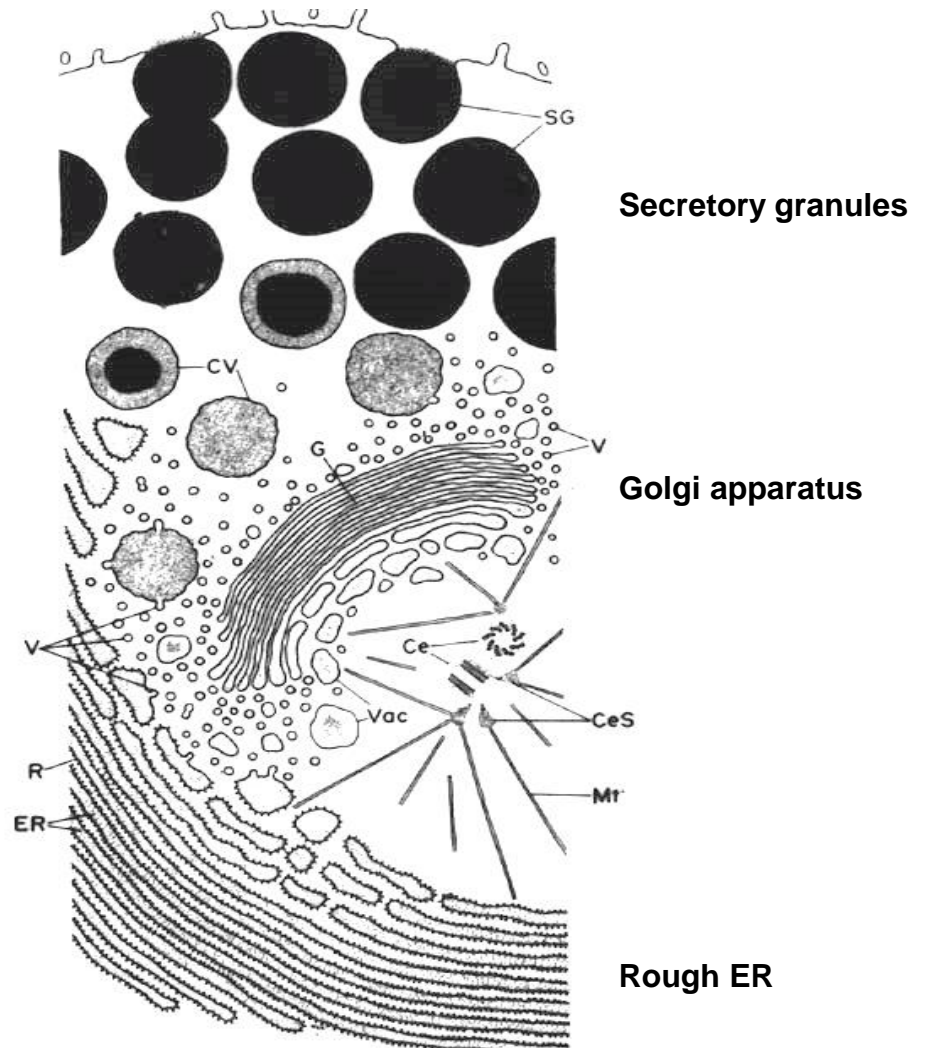
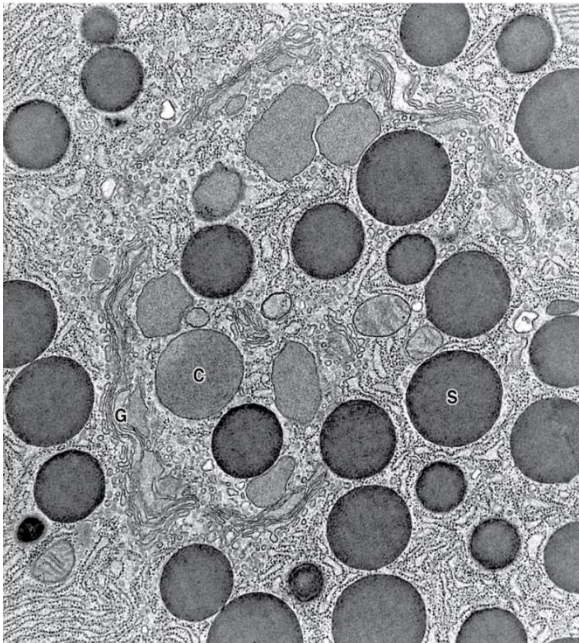
Cytoplasmic inclusions 1

(no or only little metabolic activity on themselves)

- **secretory granules**
- **storage compounds:** sugars (glycogen), lipids
- **crystals** (proteins)
- **pigments:** endogenous (autogenic and hematogenic) + exogenous

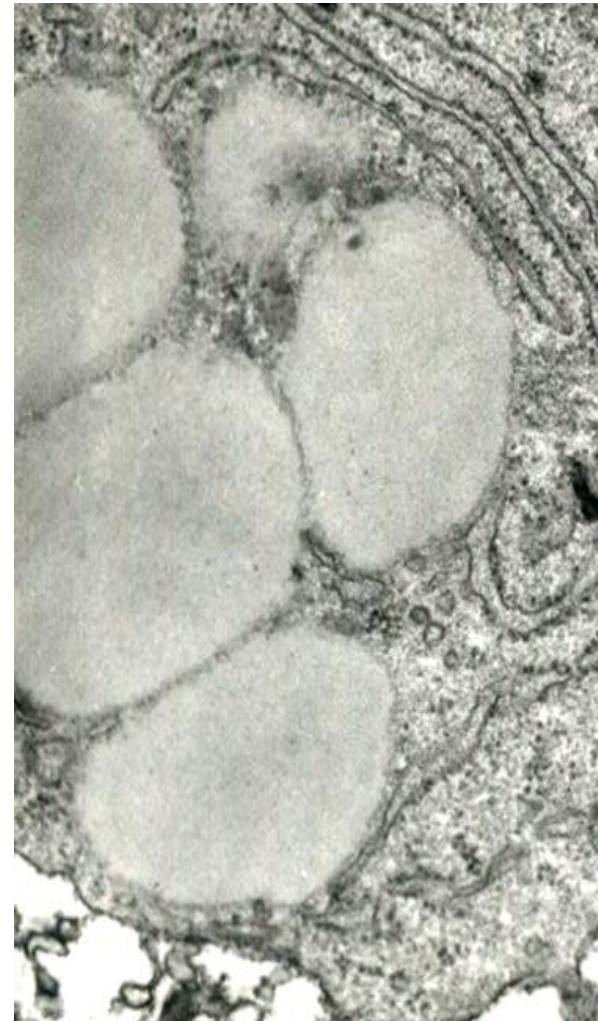
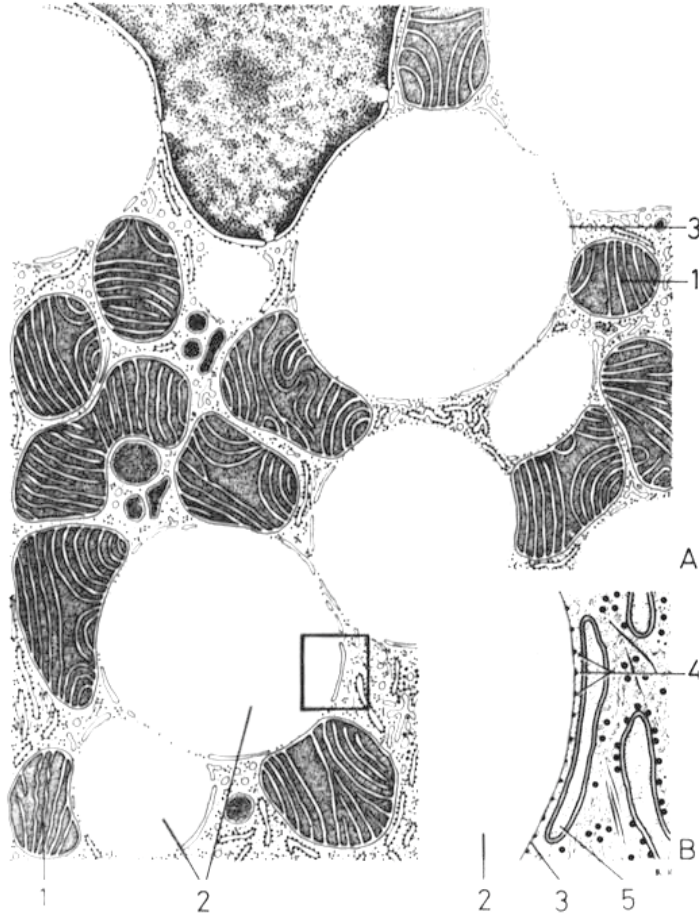
Cytoplasmic inclusions 2

Secretory granules



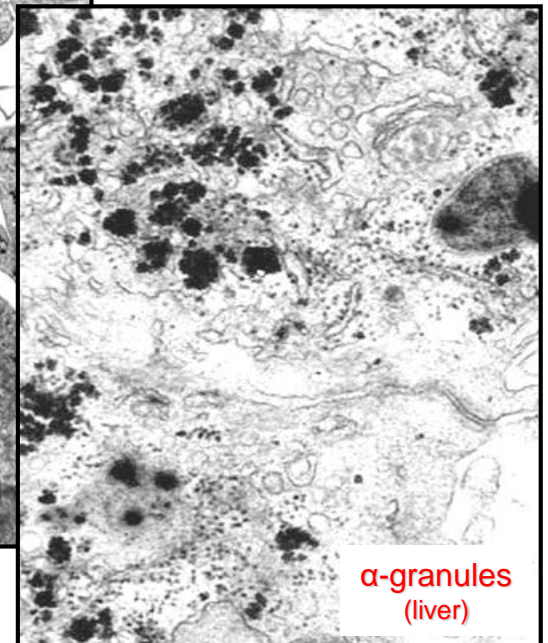
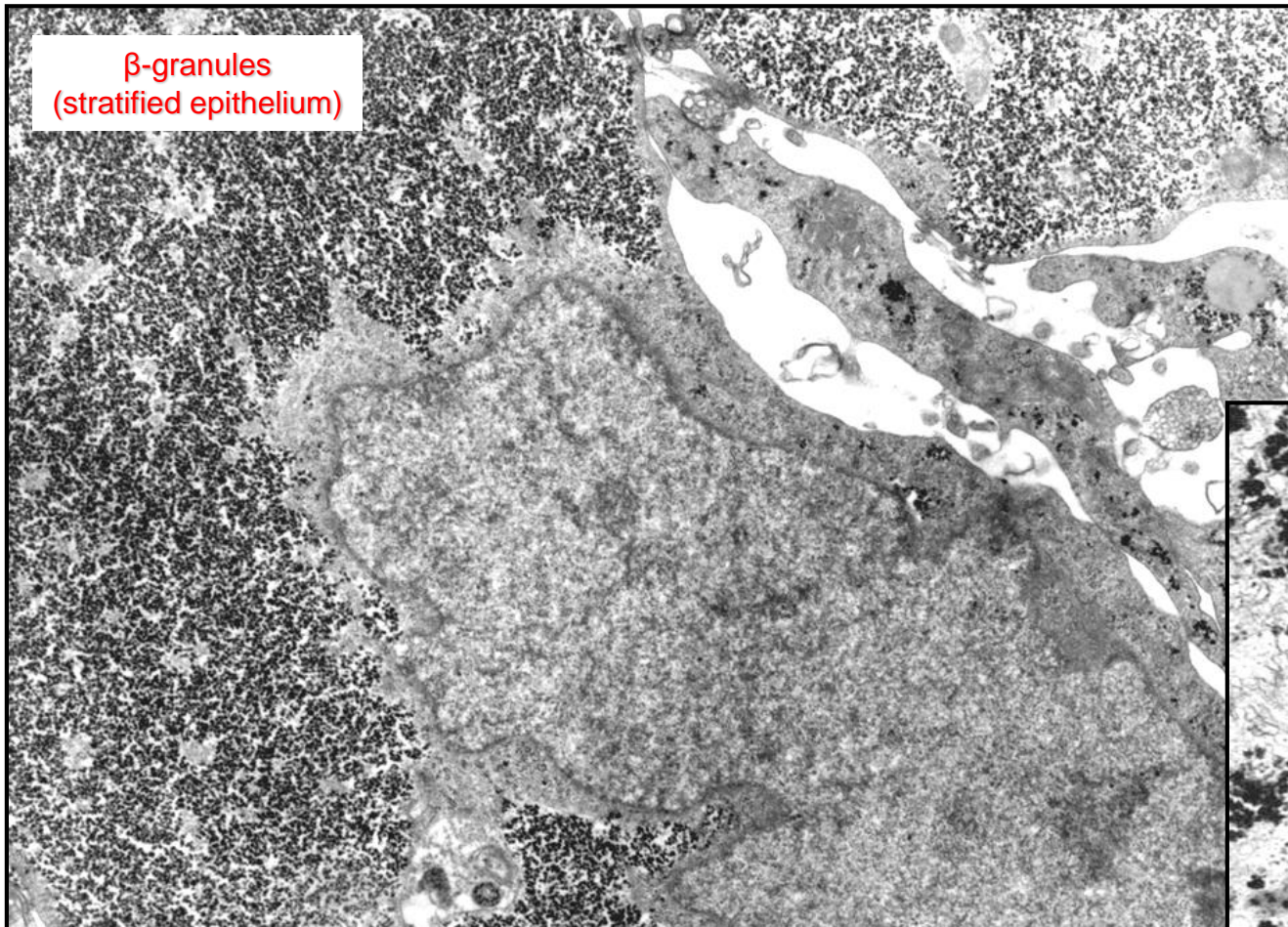
Cytoplasmic inclusions 3

Lipid inclusions



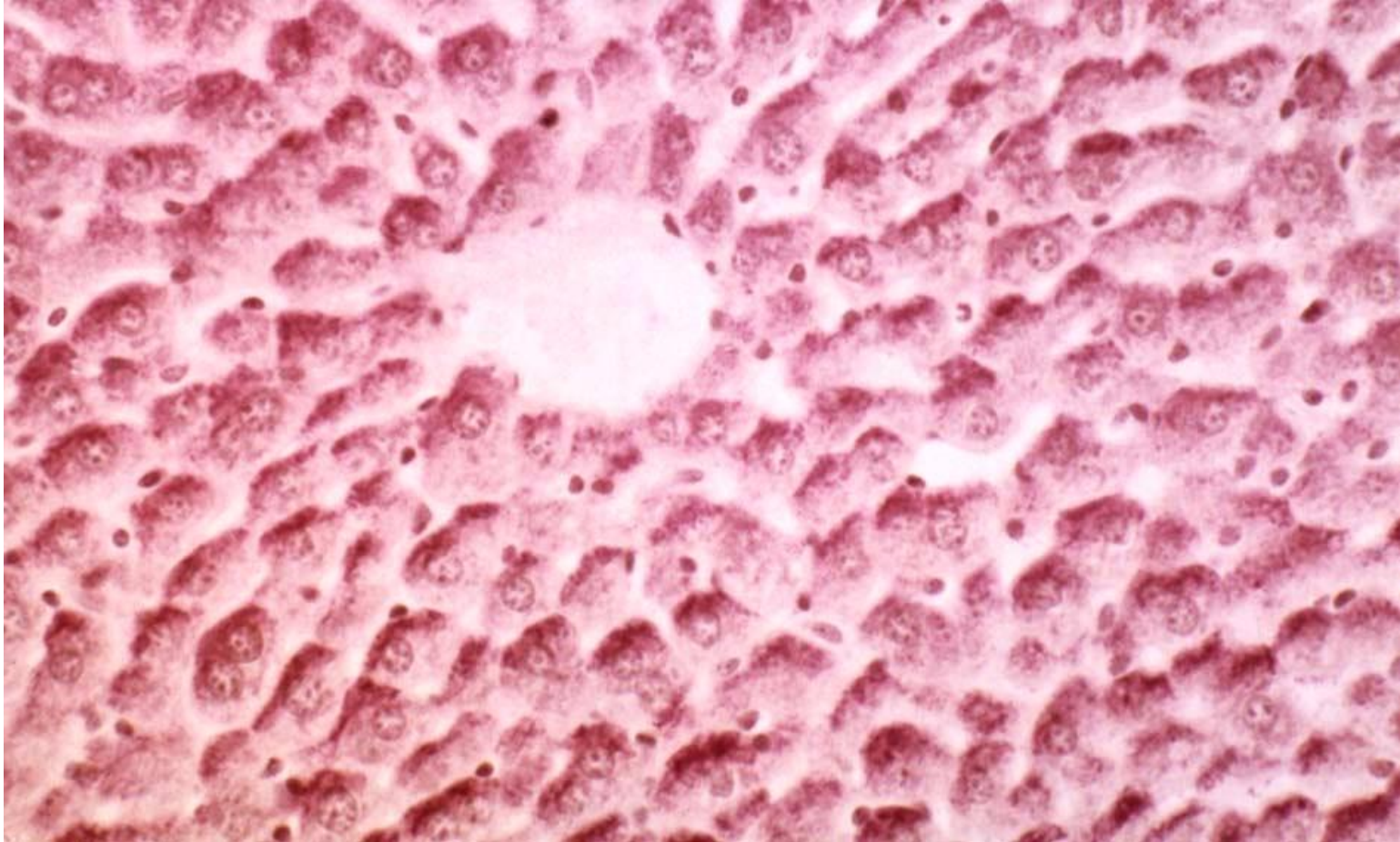
Cytoplasmic inclusions 4

Glycogen



Cytoplasmic inclusions 5

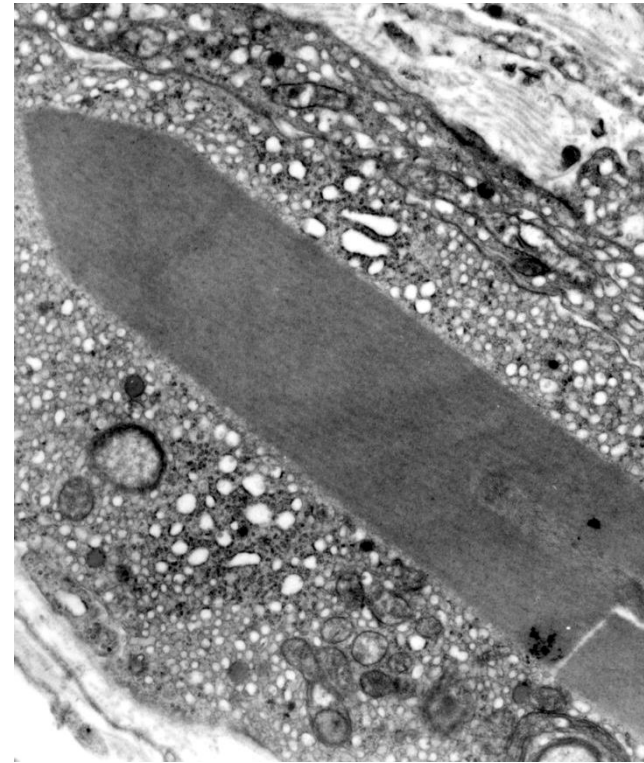
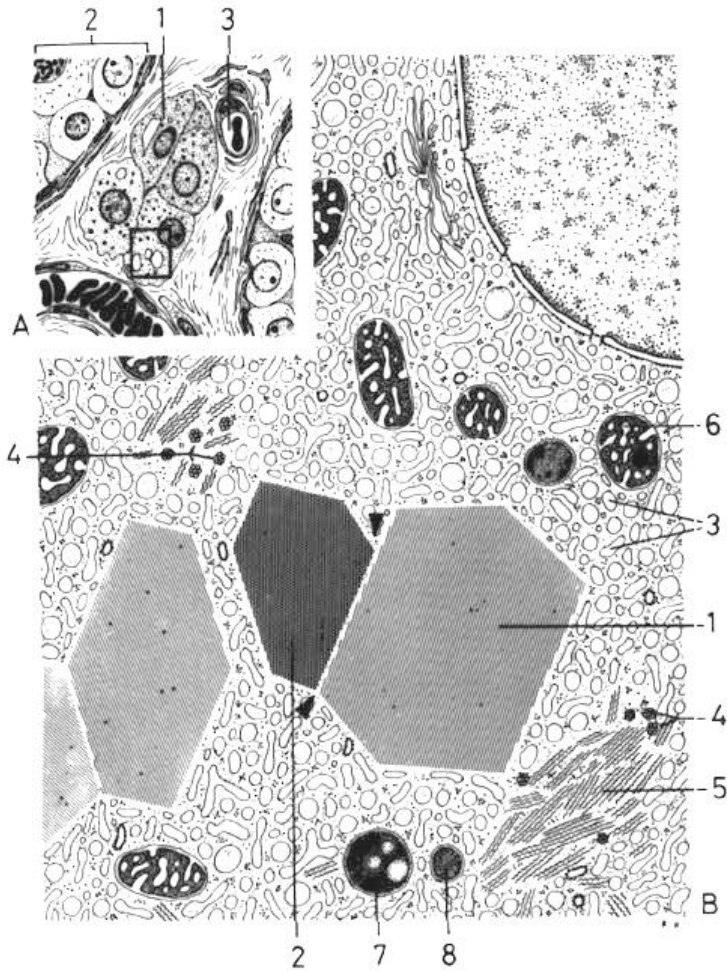
Glycogen



Glycogen in liver cells (light microscope; PAS reaction)

Cytoplasmic inclusions 6

Crystals



Protein inclusions in Leydig cells

Cytoplasmic inclusions 7

Pigments (colour inclusions): Exogenous x Endogenous

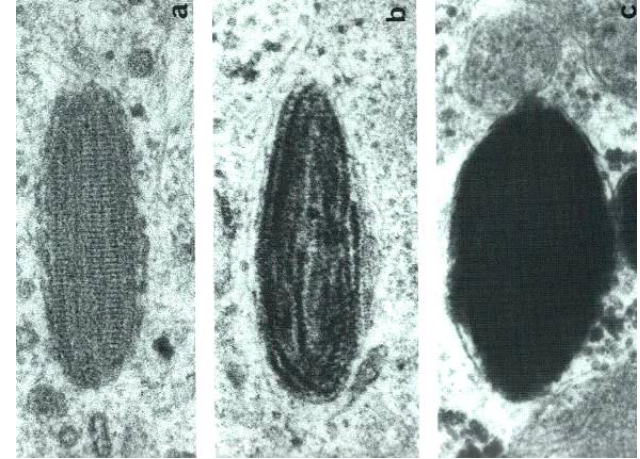
- **Autogenous**

Specific functions – **melanin**



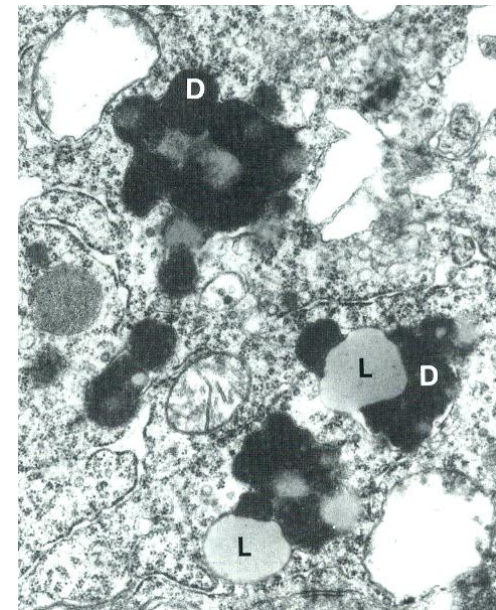
- **Hematogenous**

Hemoglobin decomposition – **hemosiderin, biliverdin, bilirubin**

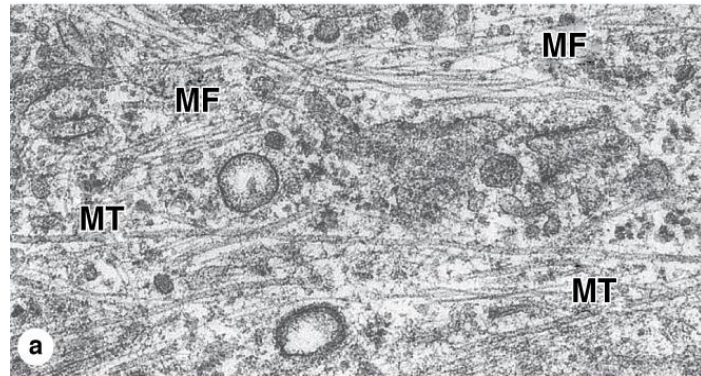
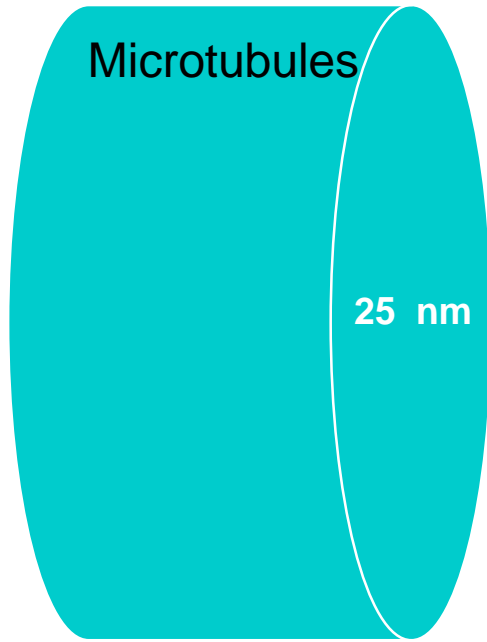


Pigment in aged cells

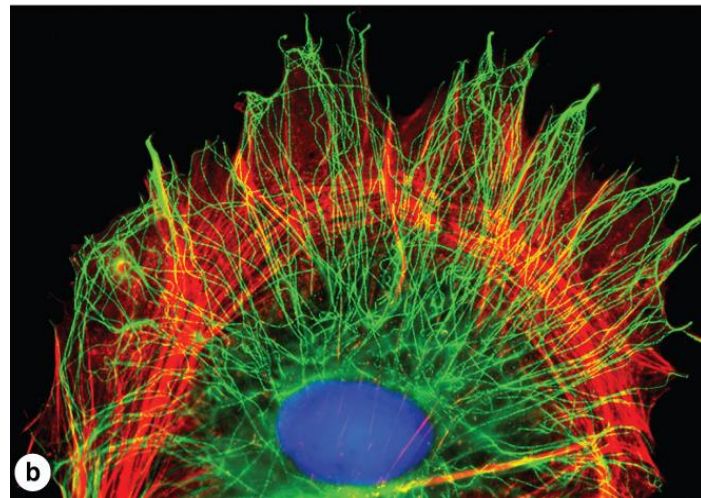
lipofuscin – accumulation of residual bodies in long-lived cells
(neurones, kardiomyocytes)



Cytoskeleton 1

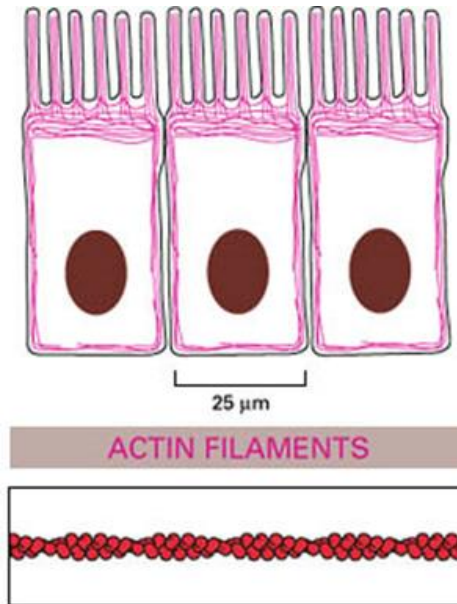
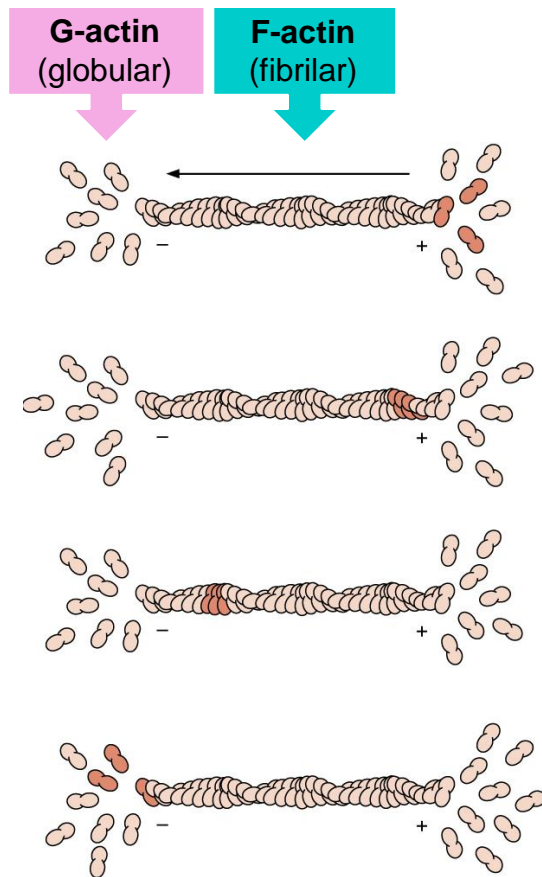


microtubules
microfilaments - actin



Cytoskeleton 2

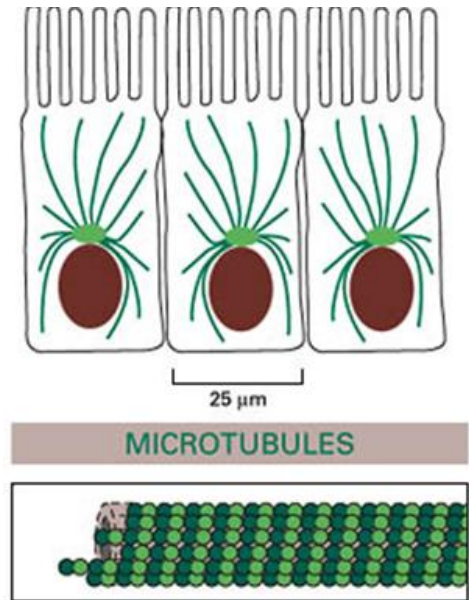
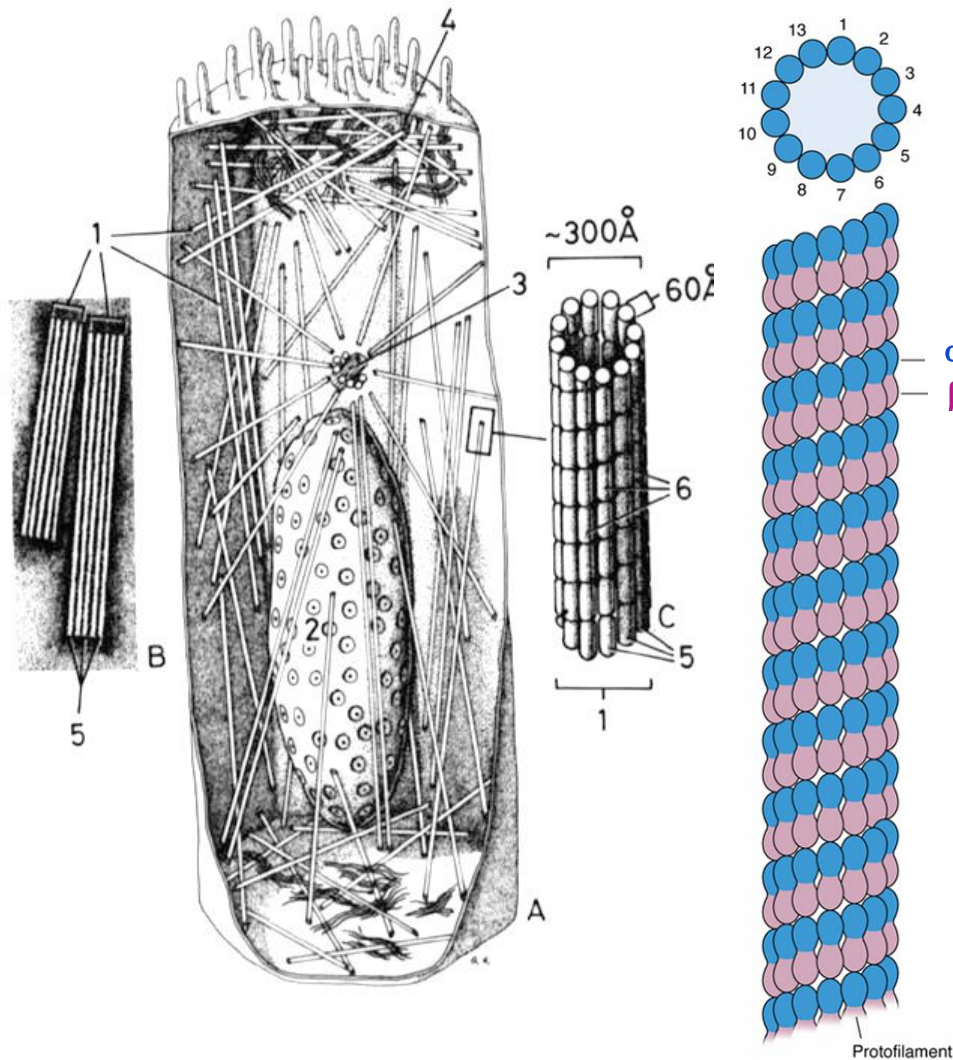
Microfilaments (actin)



- actin isoforms (α , β , γ)
- fast polymerisation and depolymerisation
- polarisation (+ a – ends)
- stabilisation by associated proteins (tropomyosin – myofibrils)
- crosslinking by associated proteins (fimbrin, filamin, ...)
- anchoring to cell membrane (vinculin, tallin, ...)
- cortical actin – membrane skeleton
- myosin motors (*analogous to dynein + kinesin on microtubuli*)

Cytoskeleton 3

Microtubules

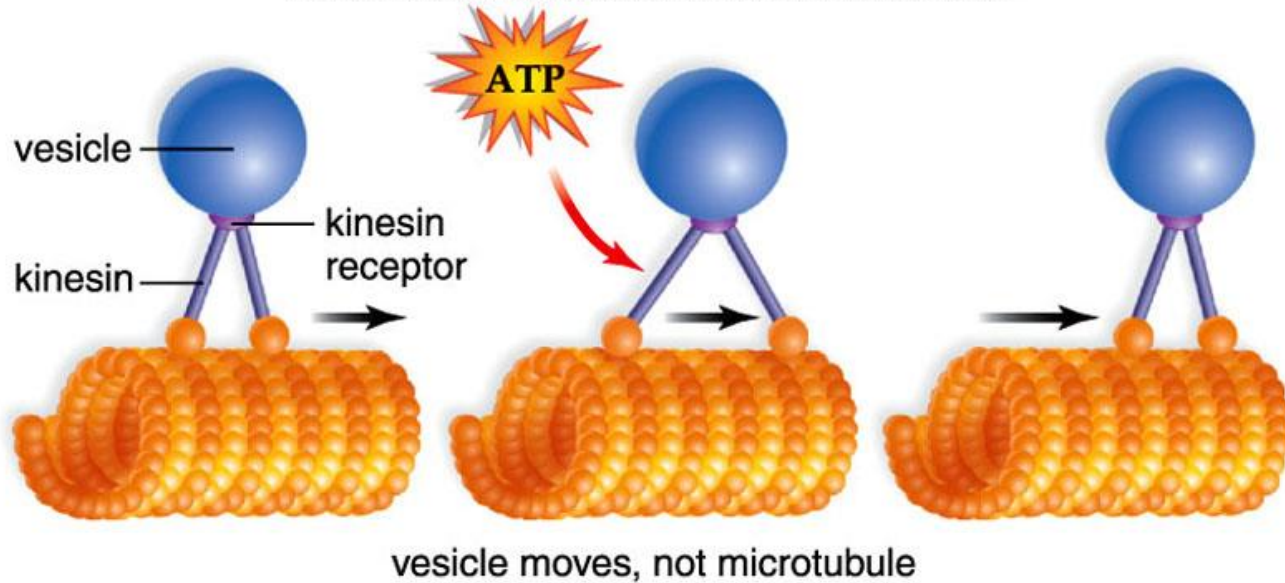


- hollow tubes
- α -tubulin + β -tubulin – dimers
- fast polymerisation and depolymerisation
- polarisation (+ a – ends)
- MAP (proteins associated with microtubuli)
- MTOC – microtubules organizing centre (centrosome; γ -tubulin)
- mechanical support
- intracellular transport
- mitotic spindle
- cilia and flagella
- mitotic poisons (colchicin, taxol, ...)

Cytoskeleton 4

Microtubules - motors

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Kinesins

- move towards „plus“ end of microtubuli
- transport **from** centrosome

Dyneins

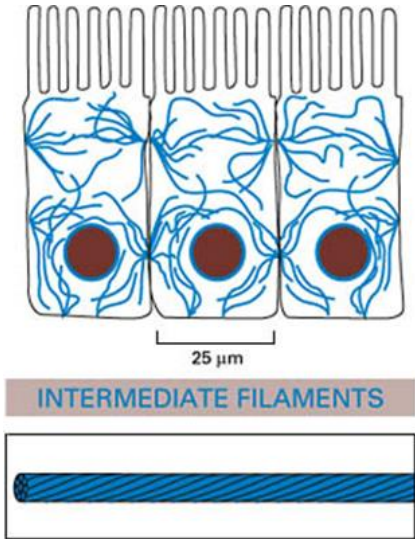
- move towards „minus“ end microtubuli
- transport **towards** centrosome
- axonal transport – long distance

Cytoskeleton 5

Intermediate filaments



Cyokeratin intermediate filaments in stratum basale of epidermis



- „chemically“ highly heterogenous group
- common composition (tetramers) “thread like“
- more stable than actin and tubulin structures
- cell type specific:

Cytokeratins (epithelia)

Vimentin (cells of mesenchymal origin)

Desmin (muscle cells)

Neurofilaments (neurons)

Glial fibrial acidic protein (neuroglia)

Lamins (nuclear envelope)

Cell surfaces 1

Free

- **microvilli** (*irregular, regular* – striated border, brush border)
- **cilia**

Lateral

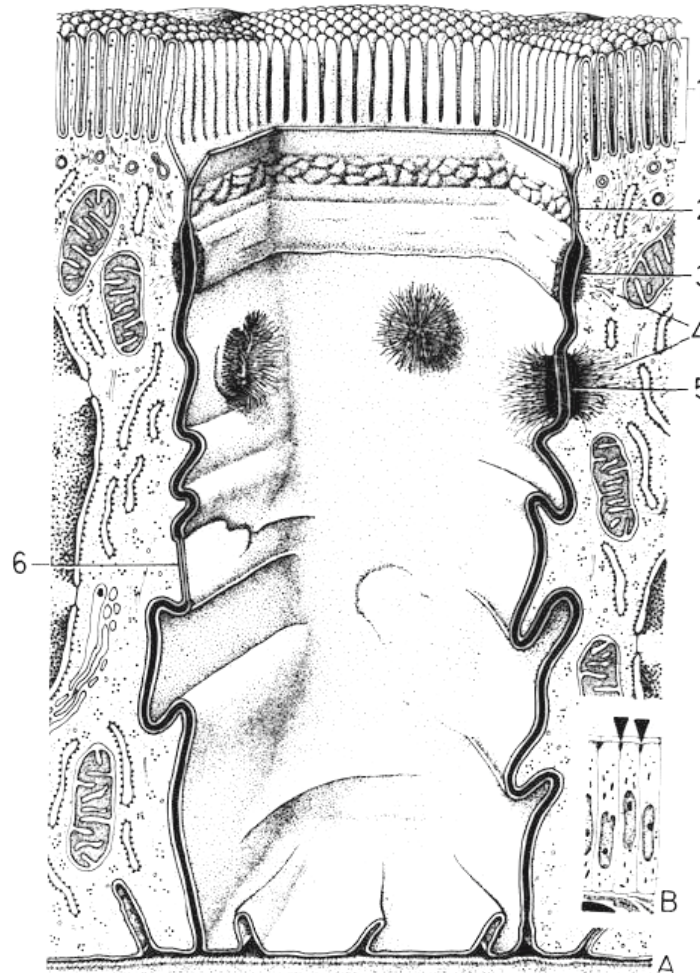
Cell-to-cell junction:

- *sealing*: tight junction=zonula occludens
- *adhesion*: zonula adherens, desmosom
- *communication*: nexus (Gap junction)

Basal

- focal adhesions
- hemidesmosomes
- basal labyrinth

free surface

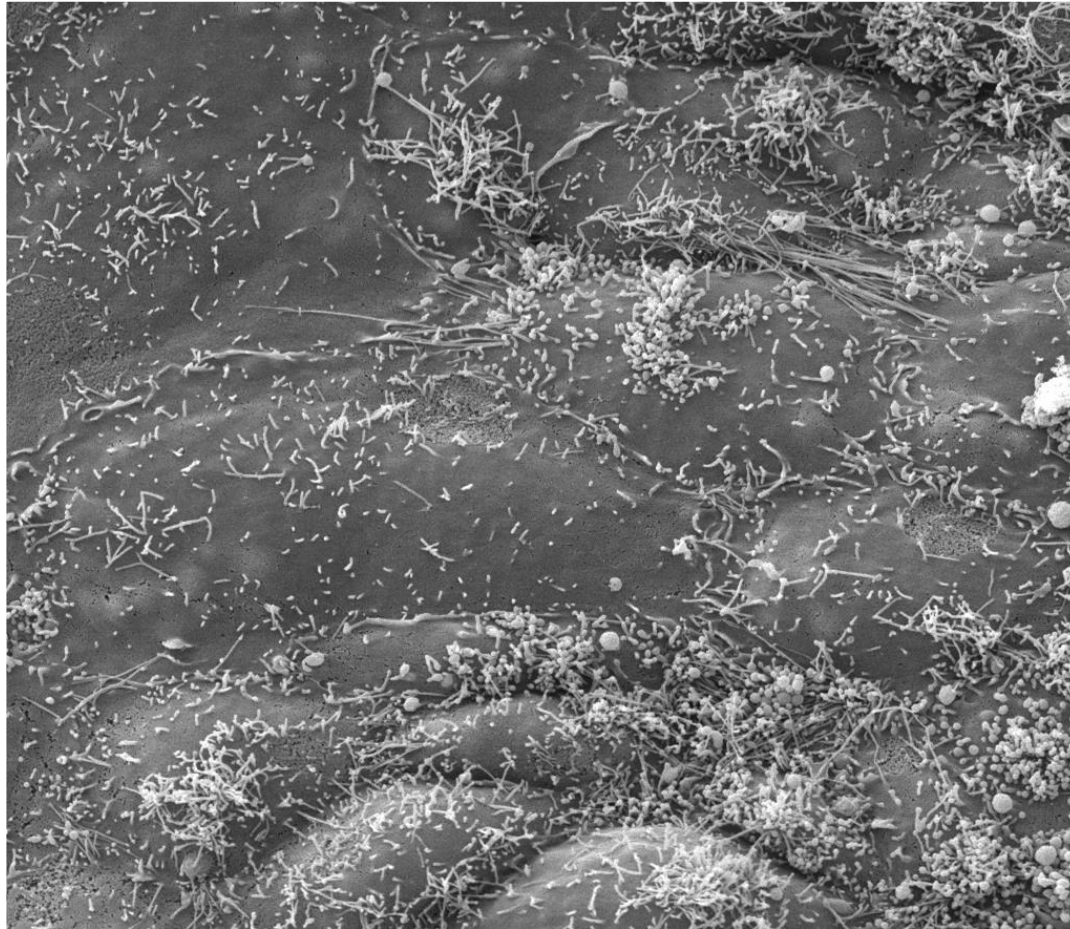


lateral surface

basal surface

Cell surfaces 2

Microvilli



Free surface of cultured human embryonic stem cells

Cell surfaces 3

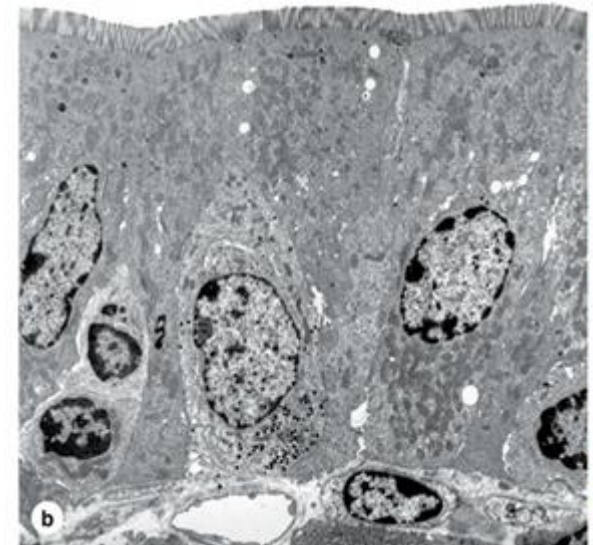
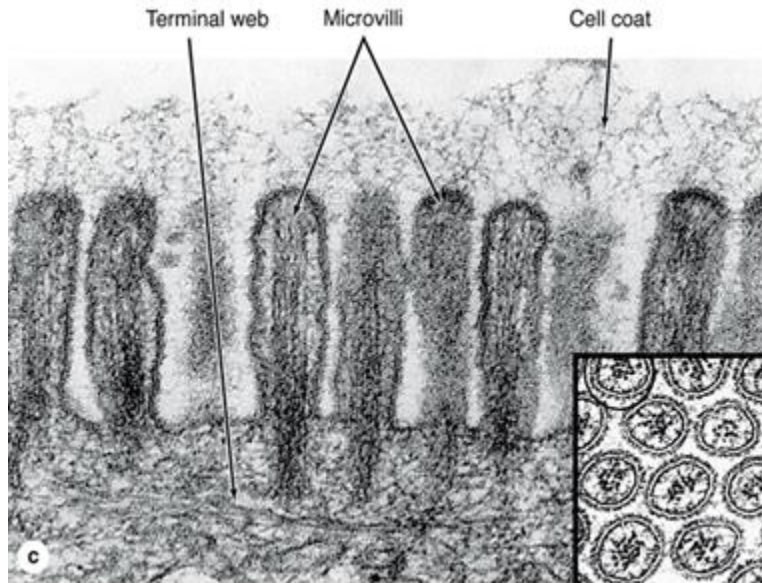
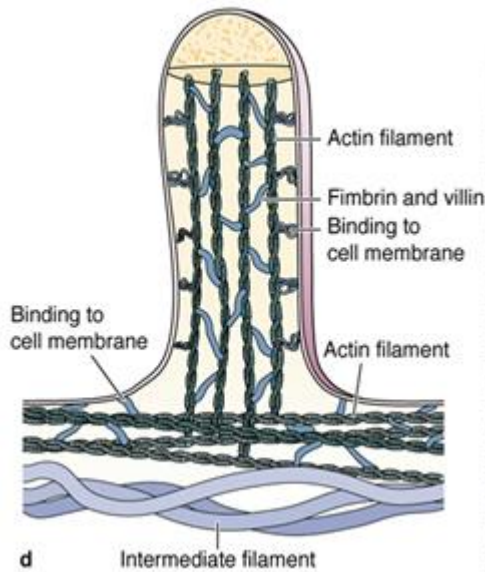
Microvilli

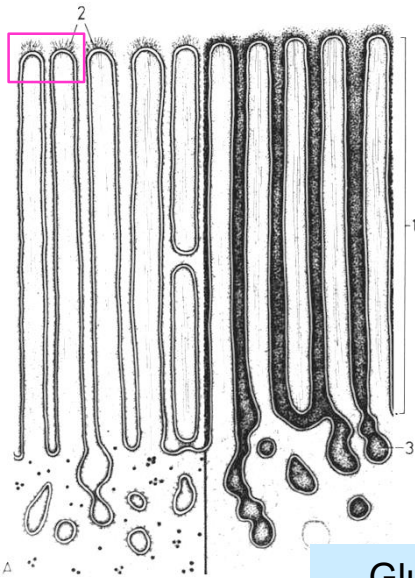
Thickness about $0,1 \mu\text{m}$
Length about $1-6 \mu\text{m}$

Actin filaments in microvilli

- 20 in microvilli of epithelial cells
- several hundreds in stereocilia of hair cells

Regularly organised microvilli
= striated border + brush border

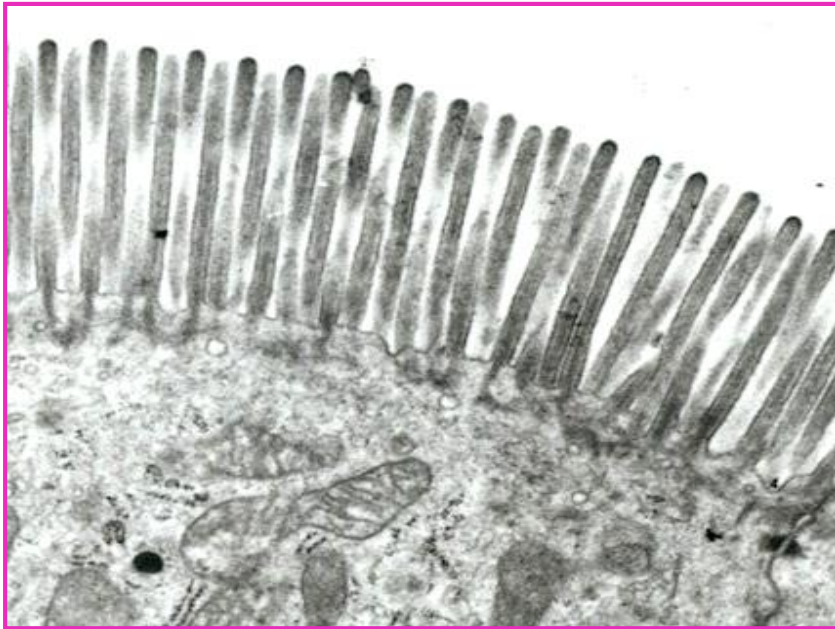




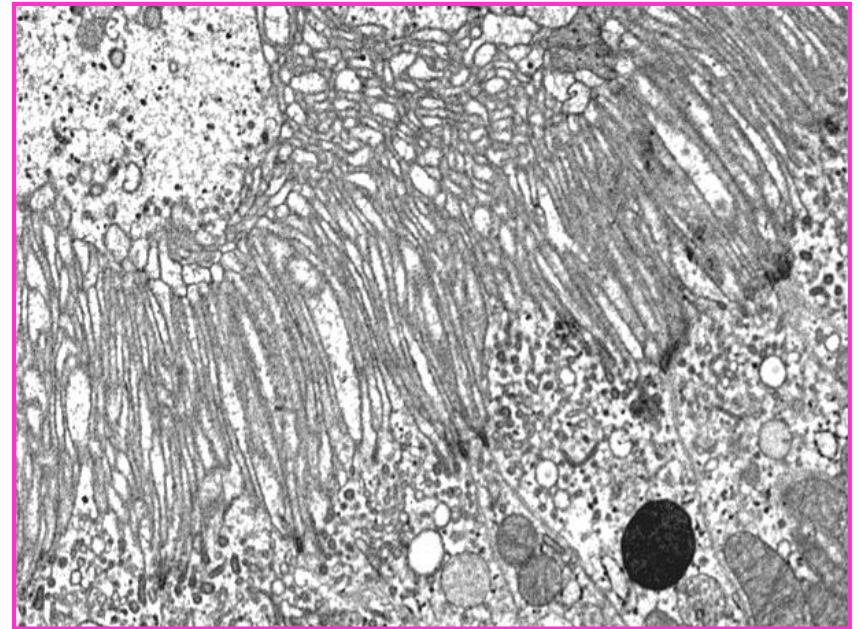
Cell surfaces 4

Microvilli

Gluten – Celiac disease



striated border
(tops of enterocytes)

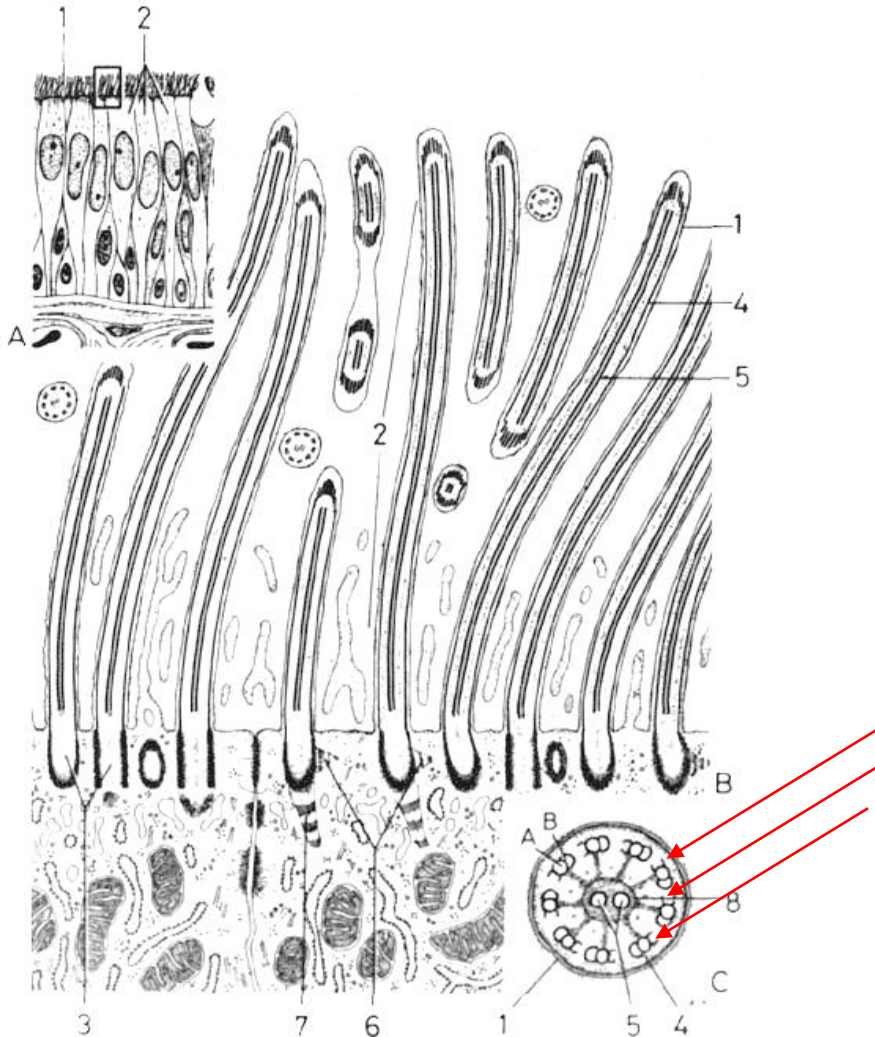


brush border
(proximal tubuli of kidney)

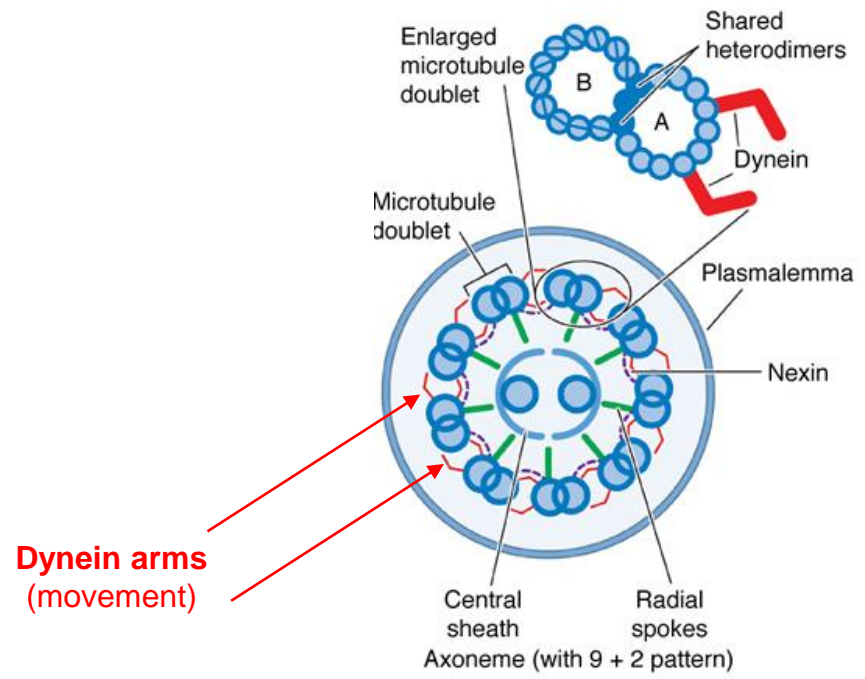
Cell surfaces 5

Cilia + Flagella

Thickness about $0,25 \mu\text{m}$
Length about $7-10 \mu\text{m}$



Axonema 20 microtubuli ($9 \times 2 + 2$)

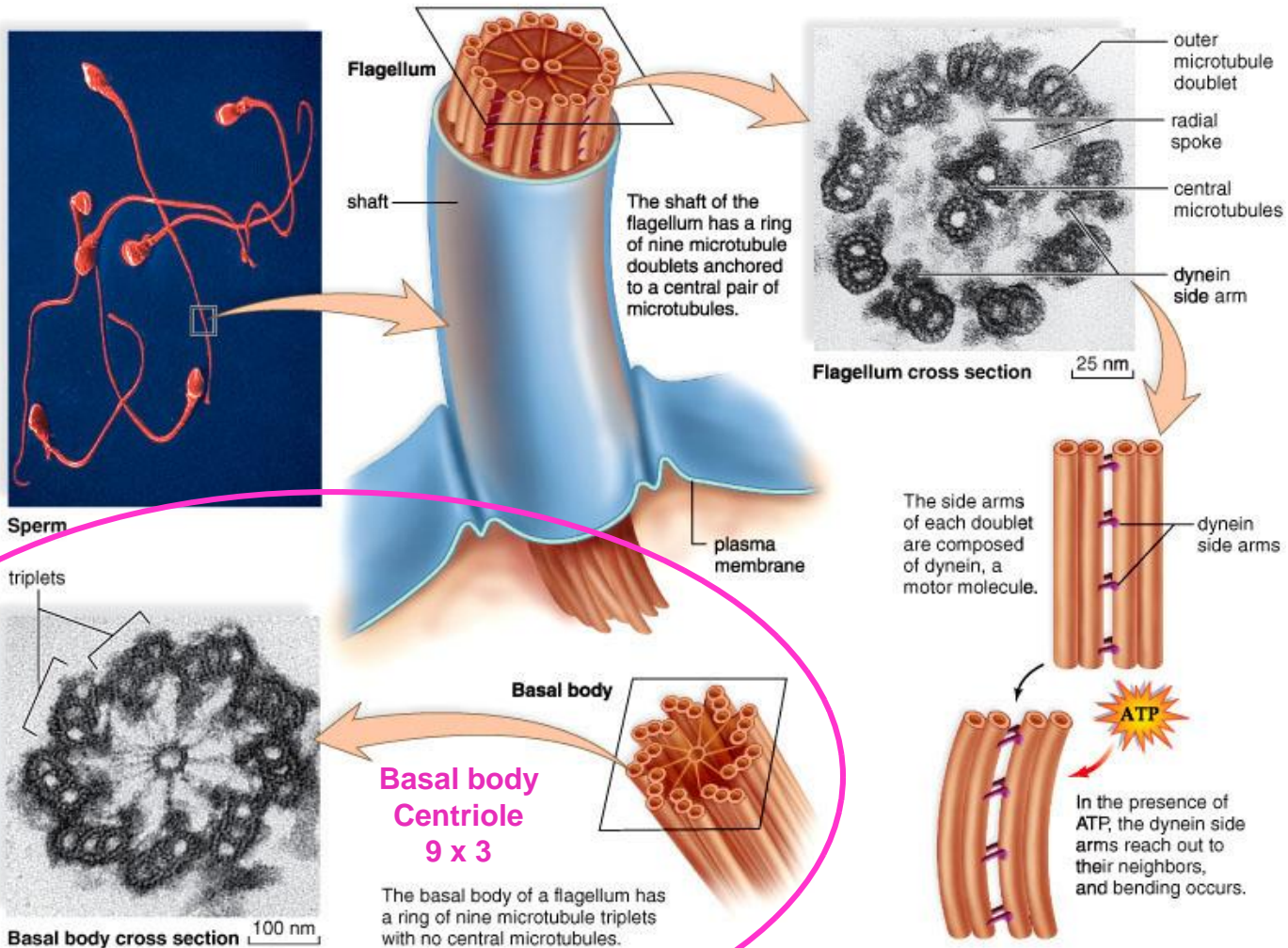


b Cilium

Cell surfaces 6

Cilia + Flagella

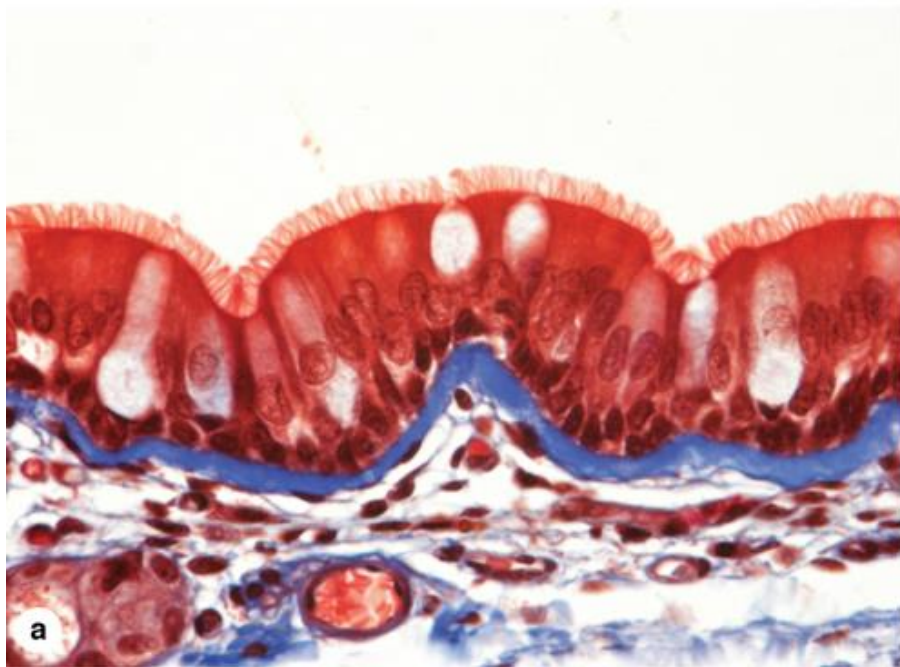
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Cell surfaces 7

Cilia + Flagella

in light microscope

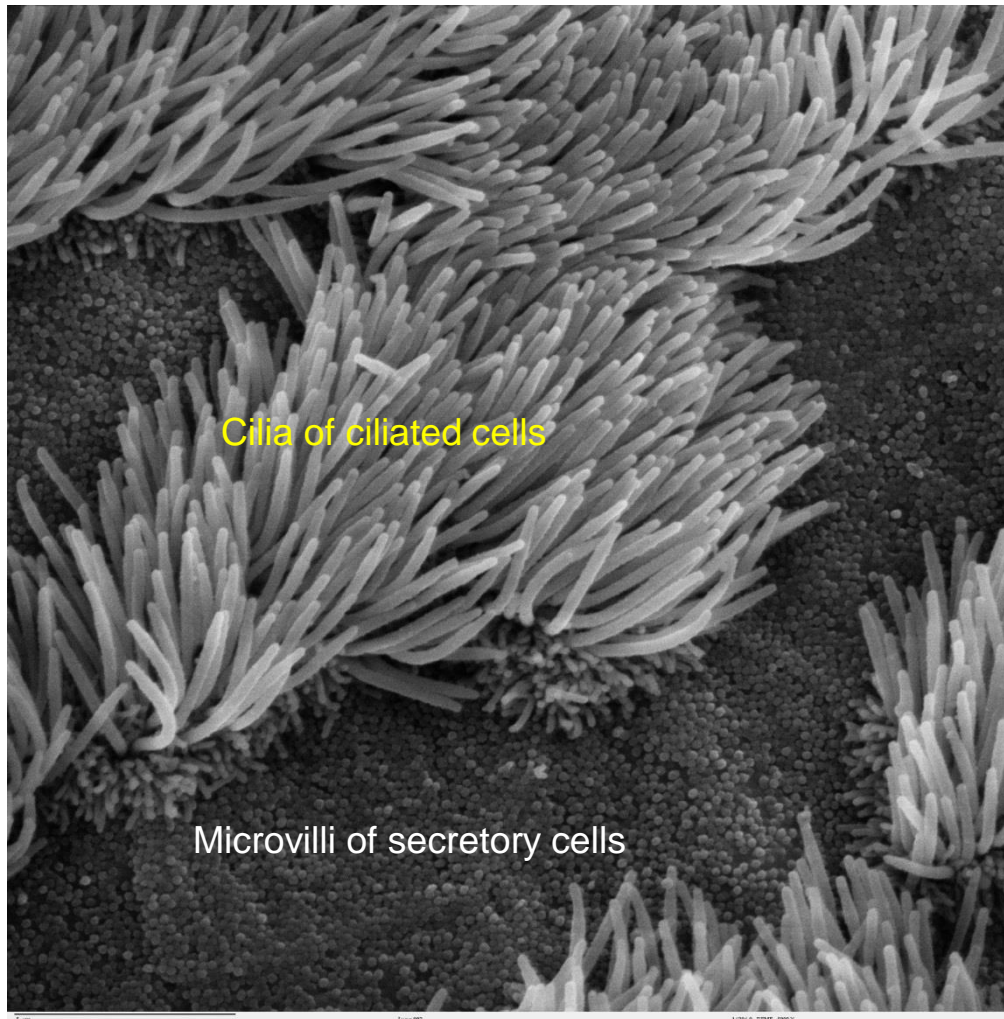


in electron microscope

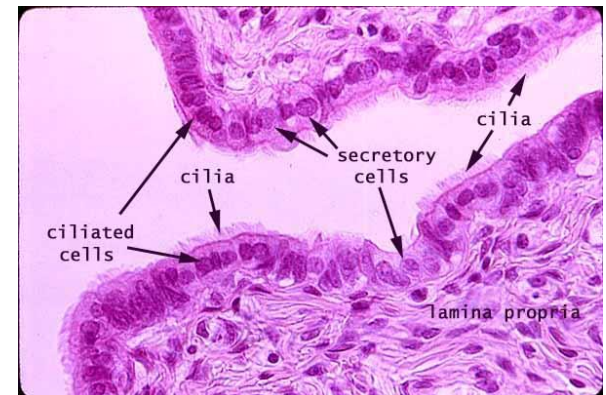


Cell surfaces 8

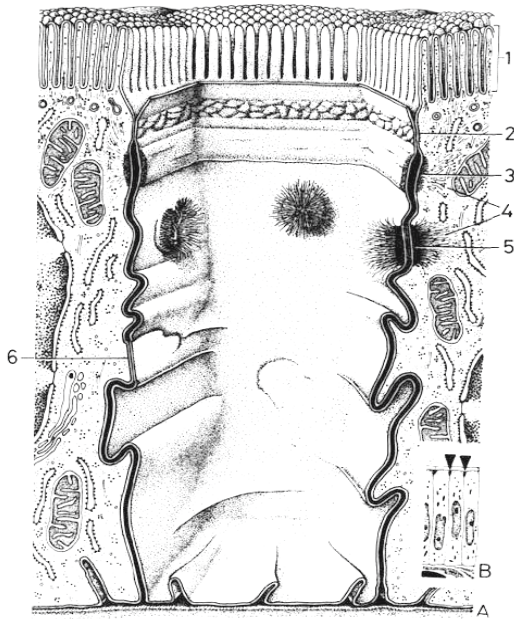
Cilia + Flagella



oviduct

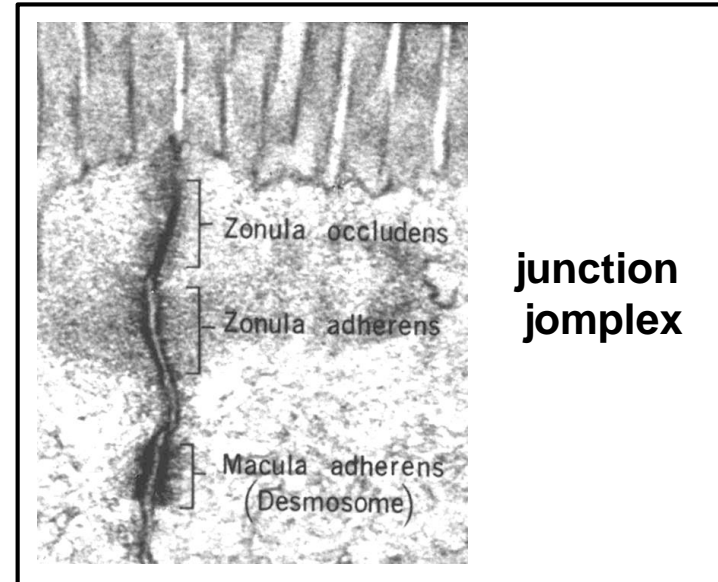


Adhesions and Junctions 1



**lateral
surface**

Basal surface



Adhesion

- **Macula adherens** (desmosome)
- **Zonula adherens**
- **Hemidesmosome**
- **Focal adhesion**

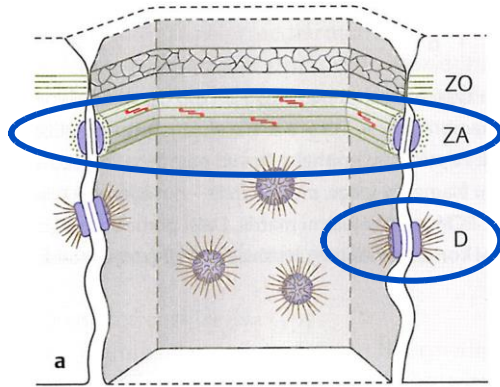
Sealing

- **Zonula occludens** (tight junction)

Communication

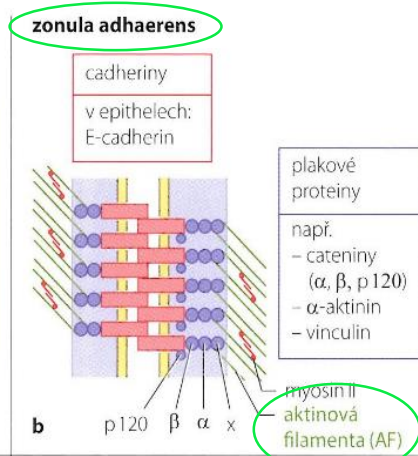
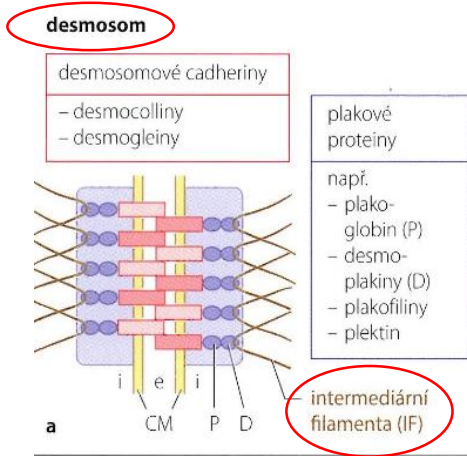
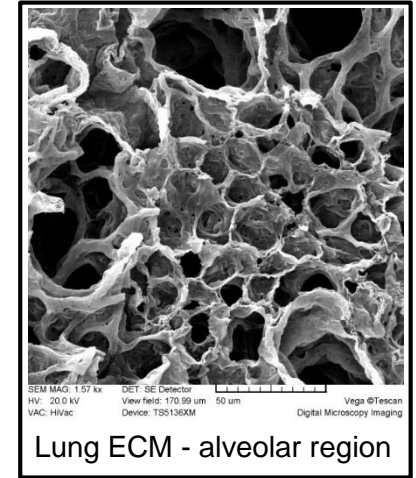
- **Gap junction** (nexus)

Adhesions and Junctions 2

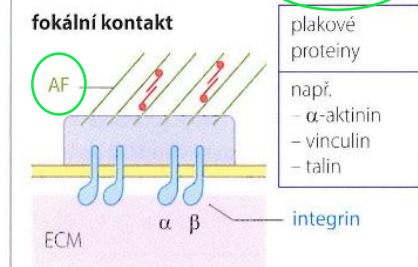
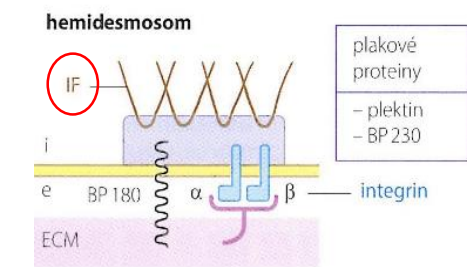


Adhesion

- Macula adherens (desmosom)
- Zonula adherens
- Hemidesmosome
- Focal adhesion



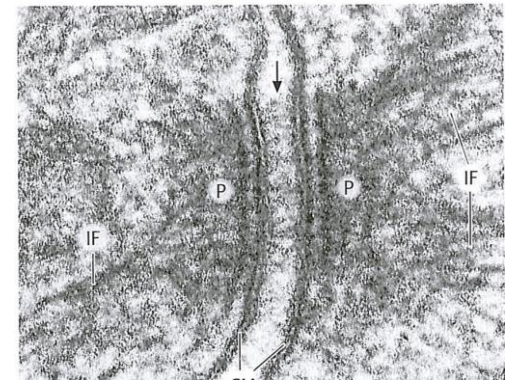
cell-cell



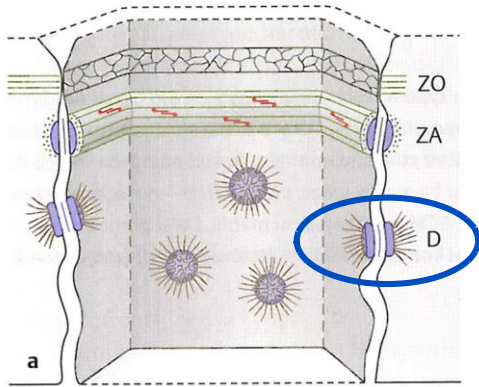
cell-ECM

Unified composition

- Transmembrane proteins (cadherins+ integrins)
- Adaptor (plak) proteins
- Cytoskeletal fibers



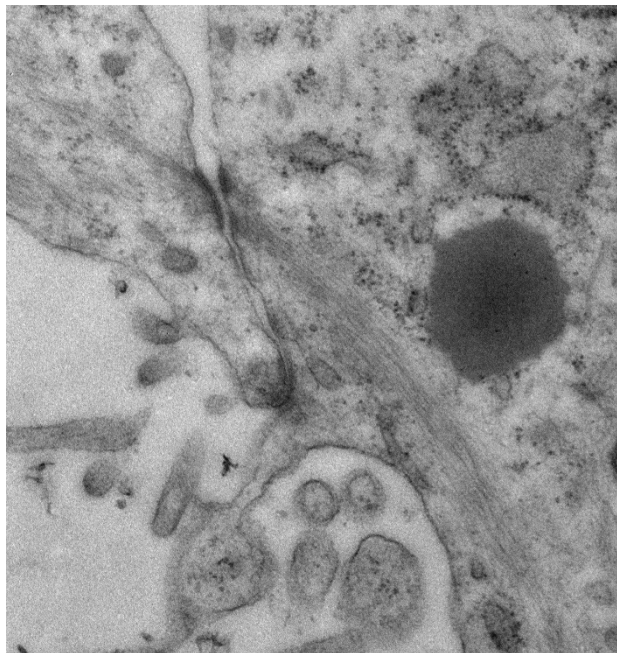
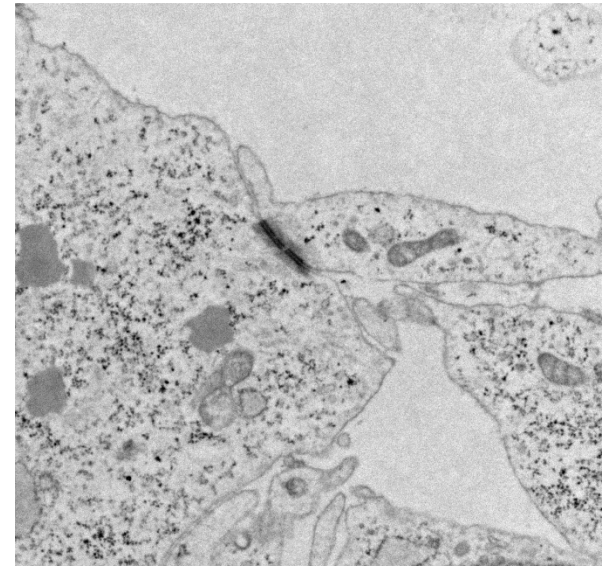
Adhesions and Junctions 3



Adhesion

- **Macula adherens (desmosome)**

Diameter about $0,3 \mu\text{m}$
Distance between membranes about 20-40 nm



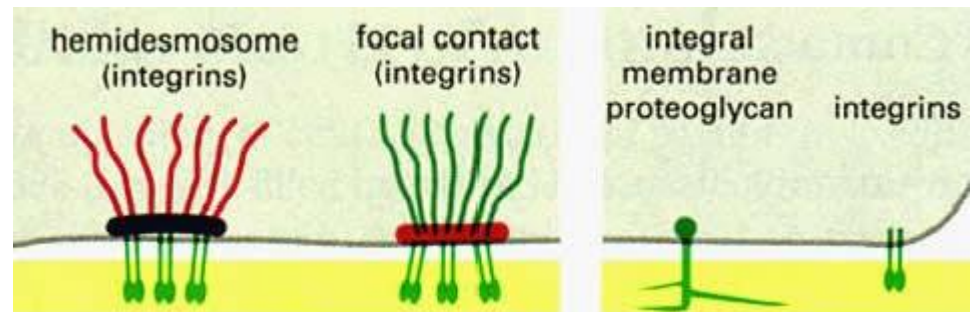
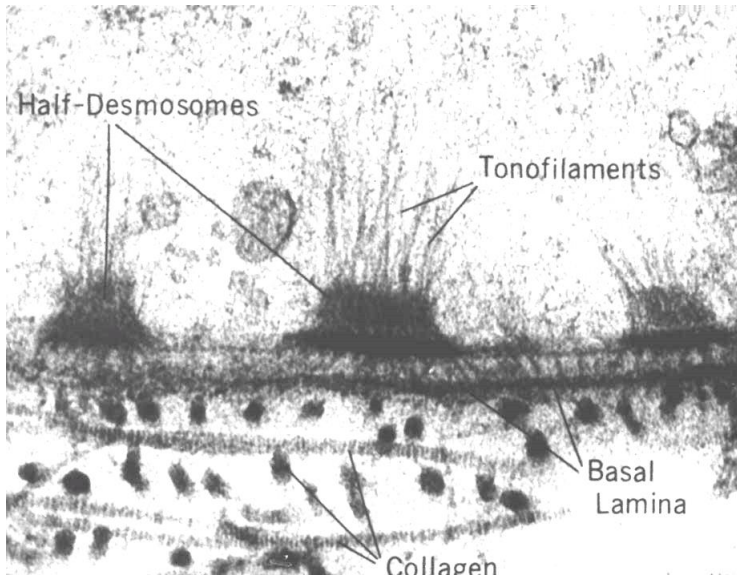
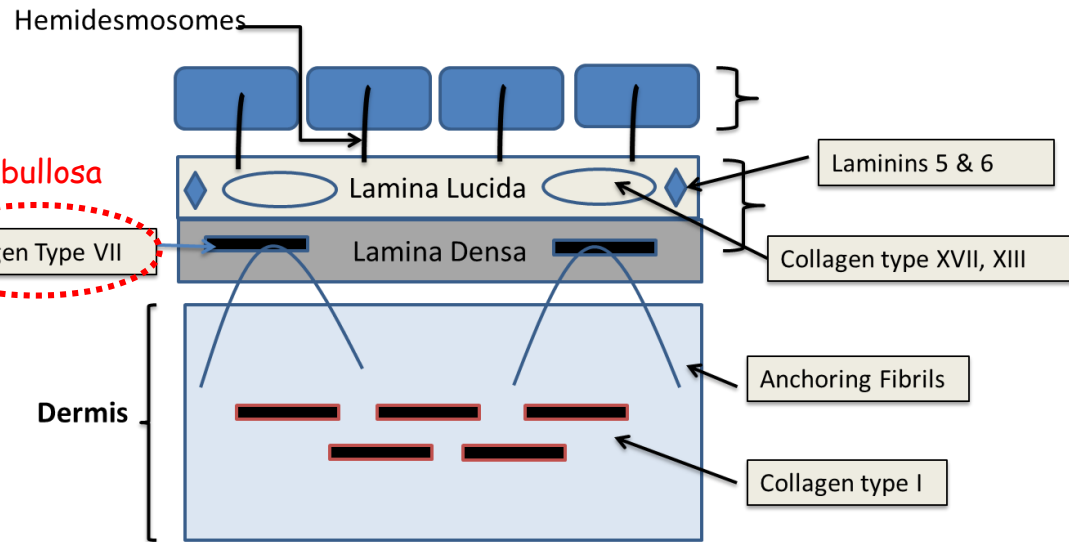
Adhesions and Junctions 4

Adhesion

- Hemidesmosome
- Focal adhesion

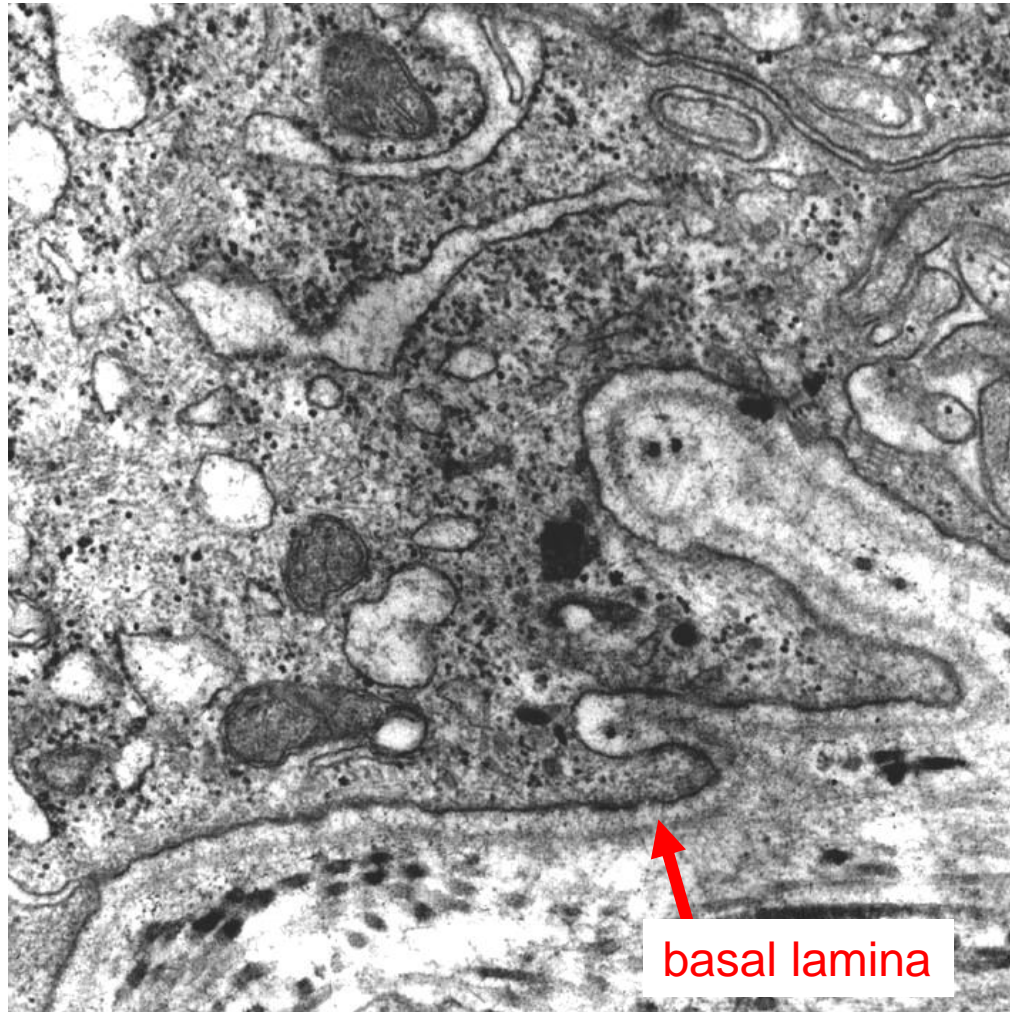
Epidermolysis bullosa

Collagen Type VII



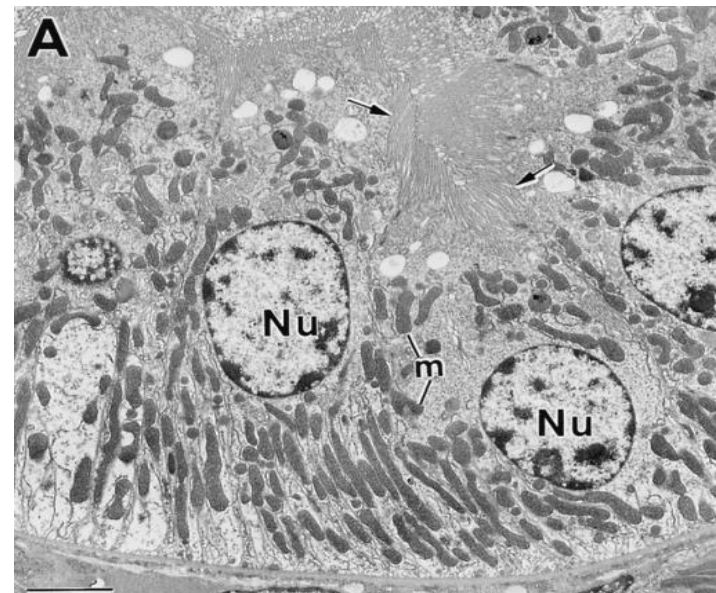
Adhesions and Junctions 5

- Focal adhesion

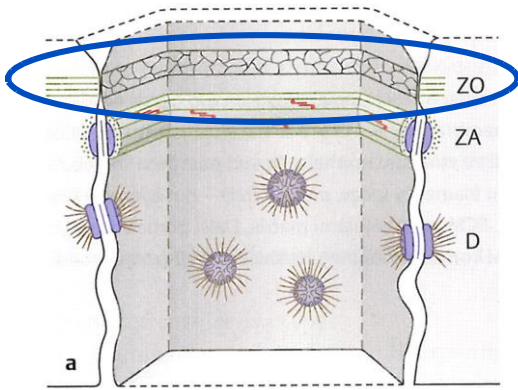


Adhesions and Junctions 6

Basal labyrinth



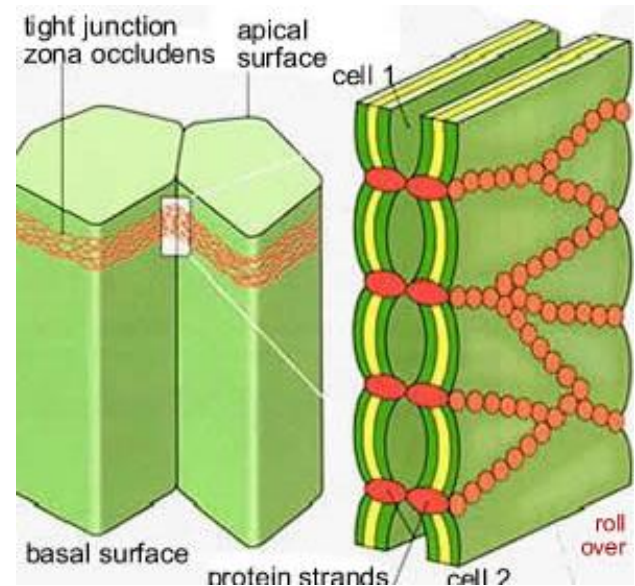
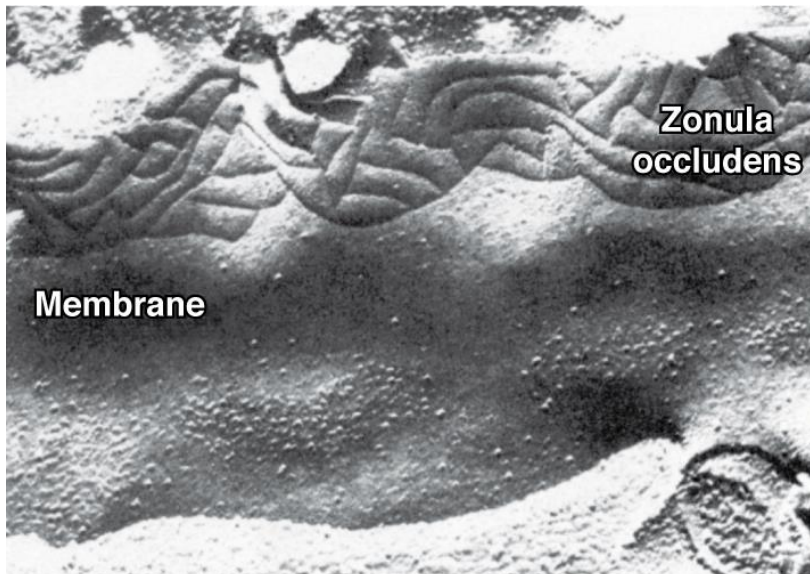
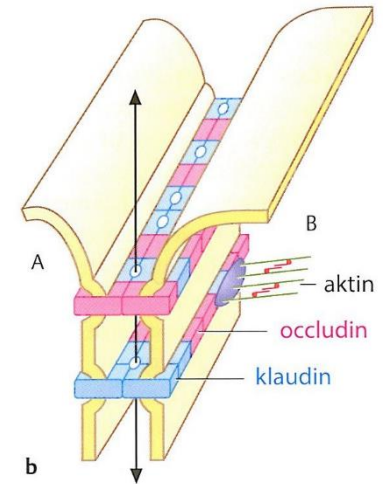
Adhesions and Junctions 7



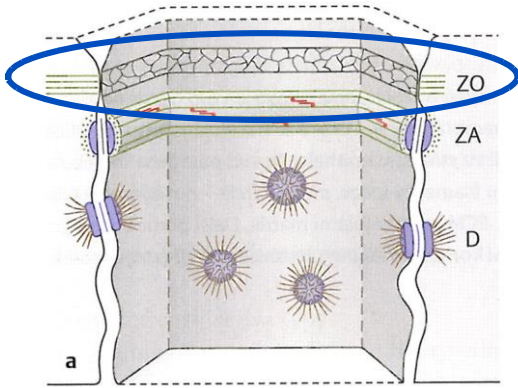
Sealing

- **Zonula occludens (tight junction)**

Damage by:
Clostridium perfringens
Helicobacter pylori (ZO-1)

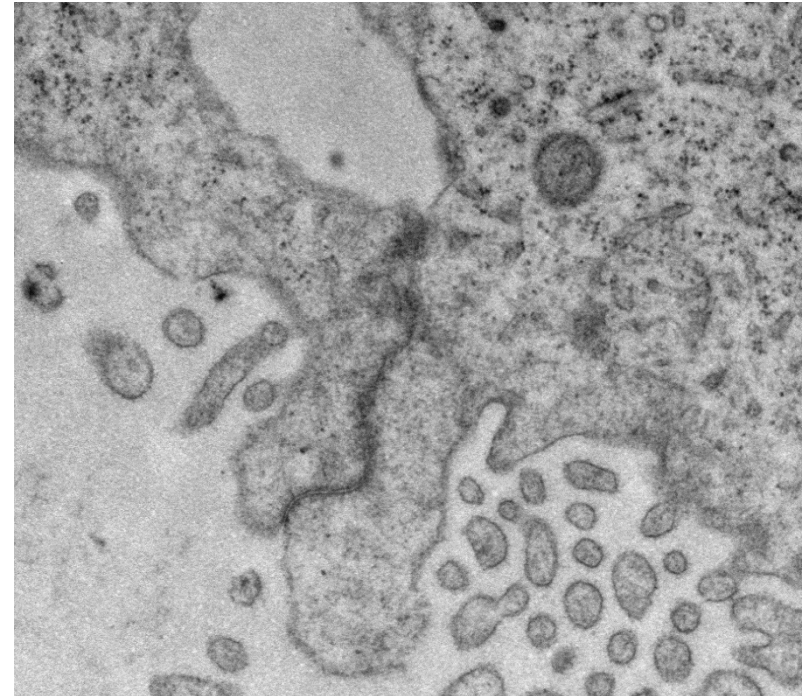
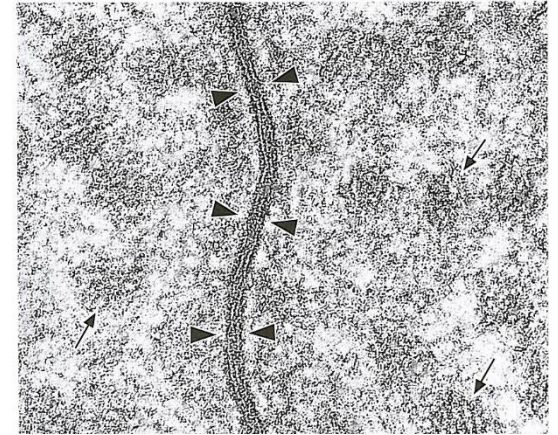


Adhesions and Junctions 8

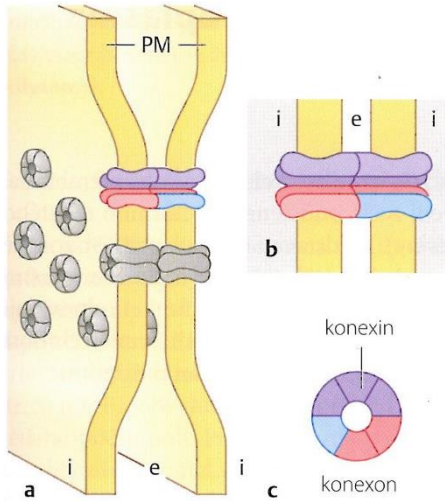


Sealing

- Zonula occludens (tight junction)



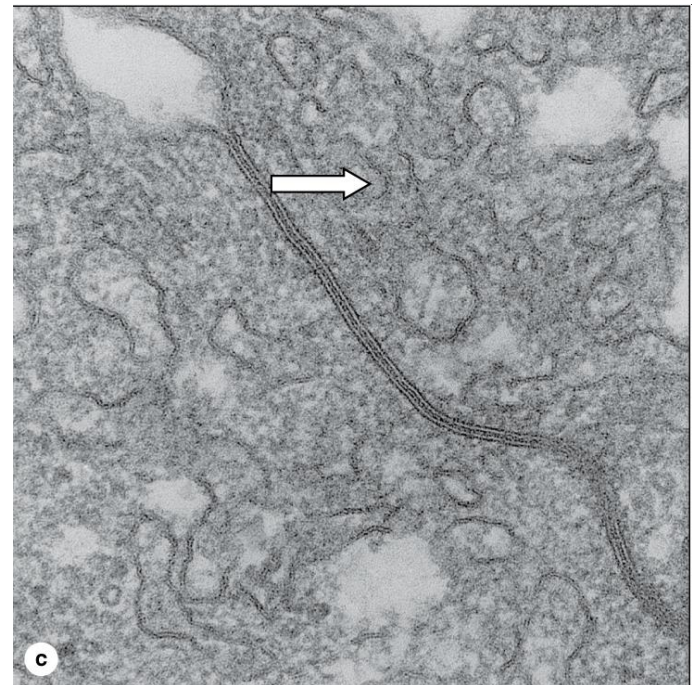
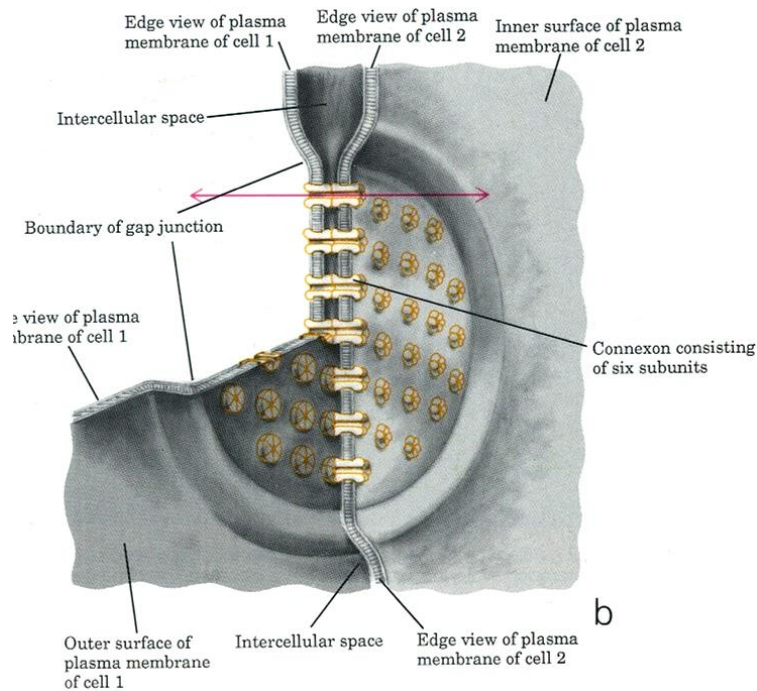
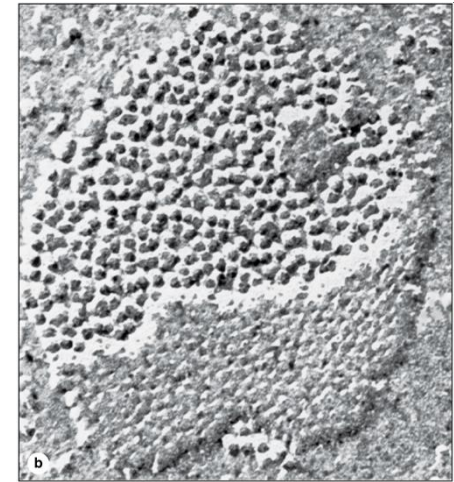
Adhesions and Junctions 9



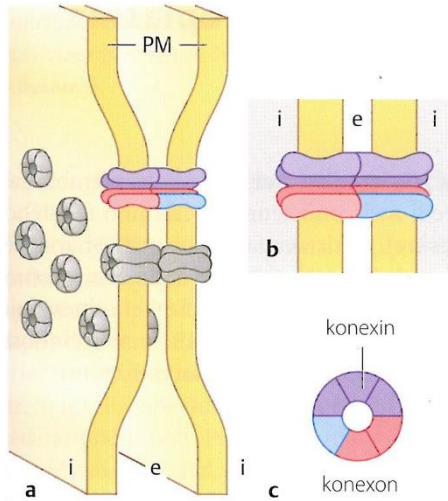
Communication

- Gap junction (nexus)

Diameter about 0,3 μm
 Distance between cell membranes about 3 nm
 Internal diameter of the channel about 2 nm

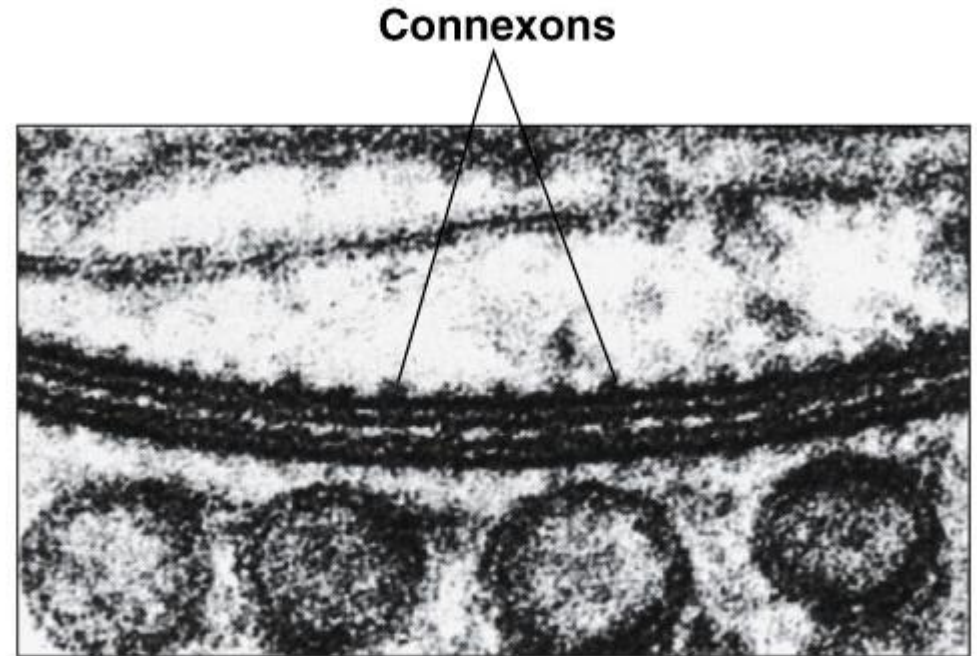
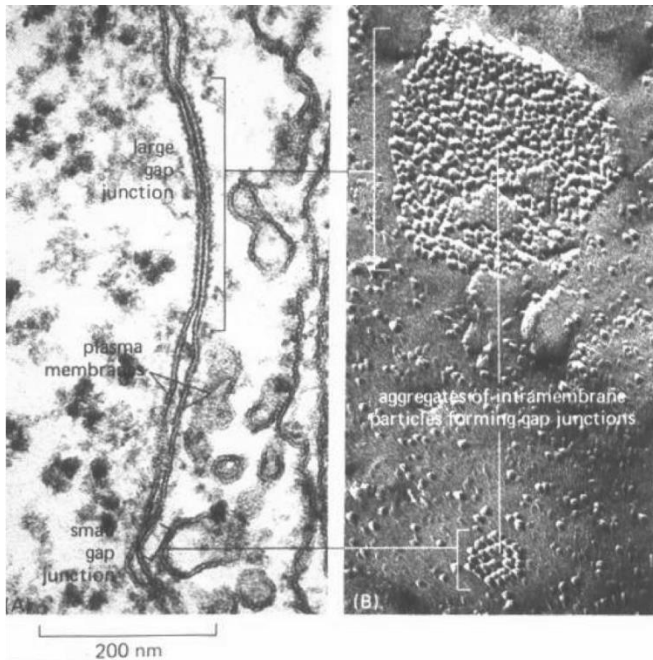


Adhesions and Junctions 10



Communication

- Gap junction (nexus)



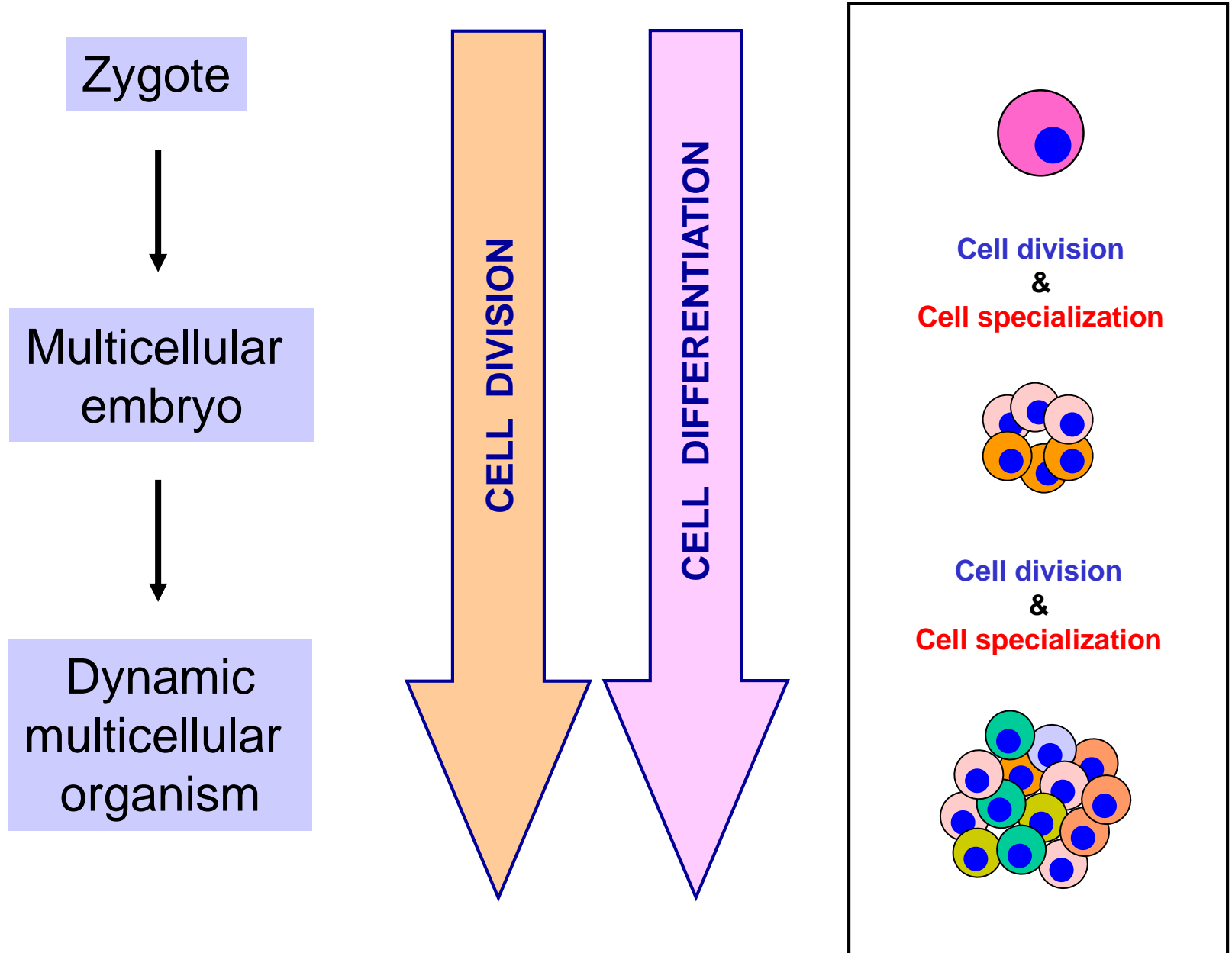
(b) Electron micrograph of a gap junction

0.1 μm

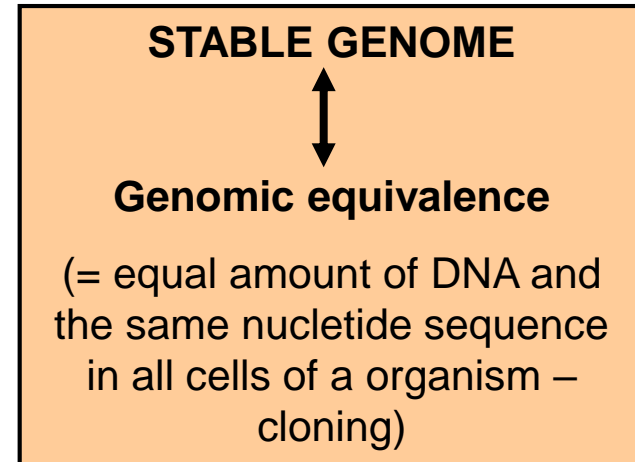
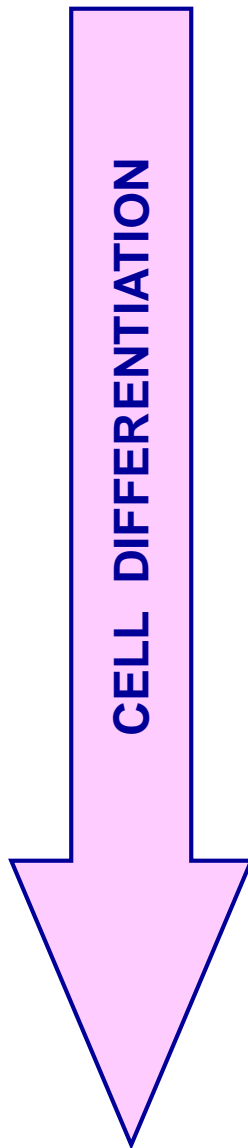
Activities of cells

- **Movement** – intracellular, amoeboid, cilia, flagella
- **Metabolism** – intake, processing, outcome
- **Responsiveness**
- **Growth**
- **Differentiation**
- **Division (amplification)**

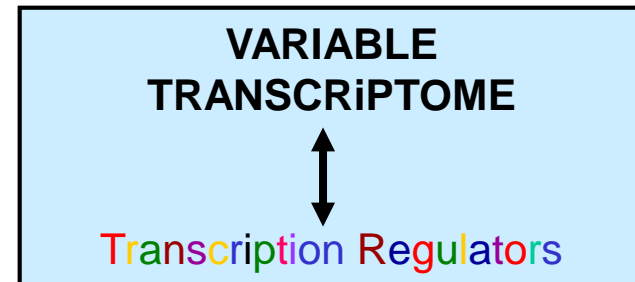
Division x Differentiation of cells 1



Division x Differentiation of cells 2



X



+ other regulations:

- translation
- posttranslational modification

Division x Differentiation of cells 3

Tissue renewal and regeneration

Stem cells

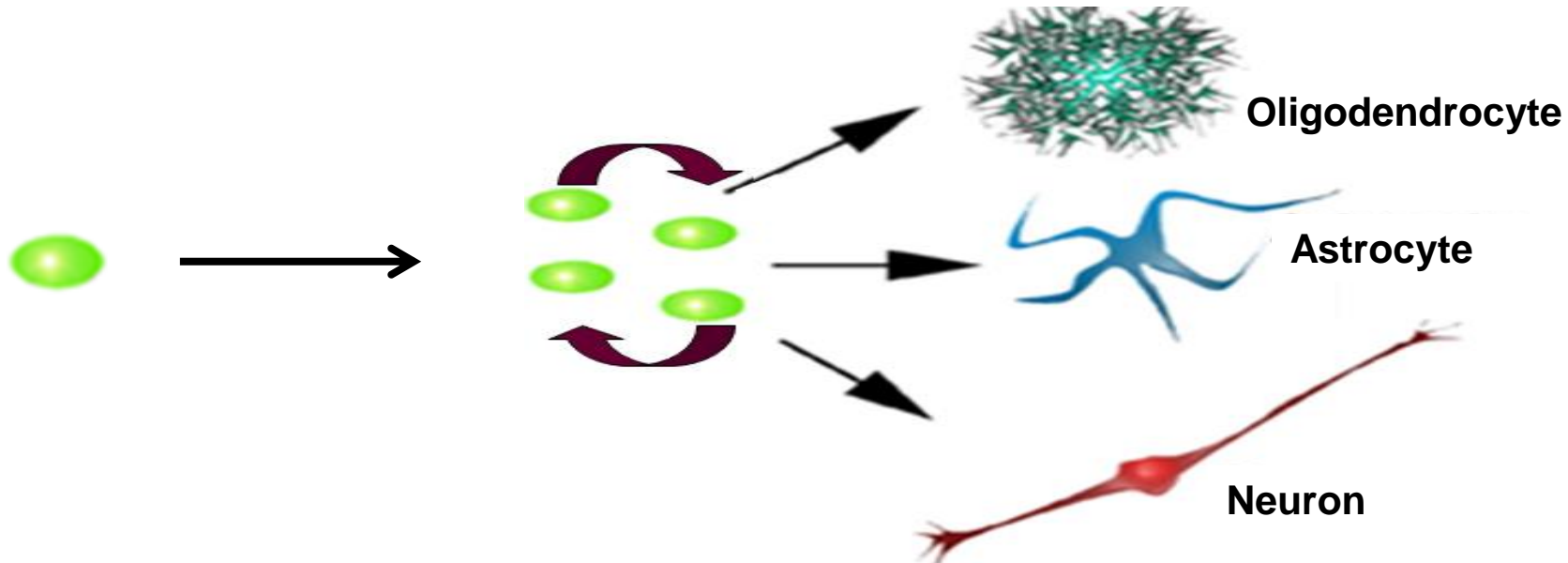
- slowly dividing (usually)
- multipotent

Progenitor cells

- „transit amplifying cells“
- fast proliferation
- multipotent

Terminally differentiated cells

- nondividing



Mother nature and scientists supply us with many

Stem cells generate and regenerate our body

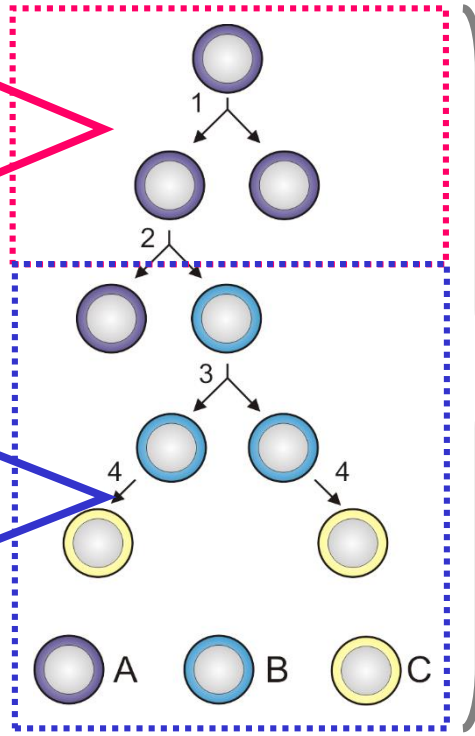
1. Undifferentiated growth

Capability to produce identical copies of itself

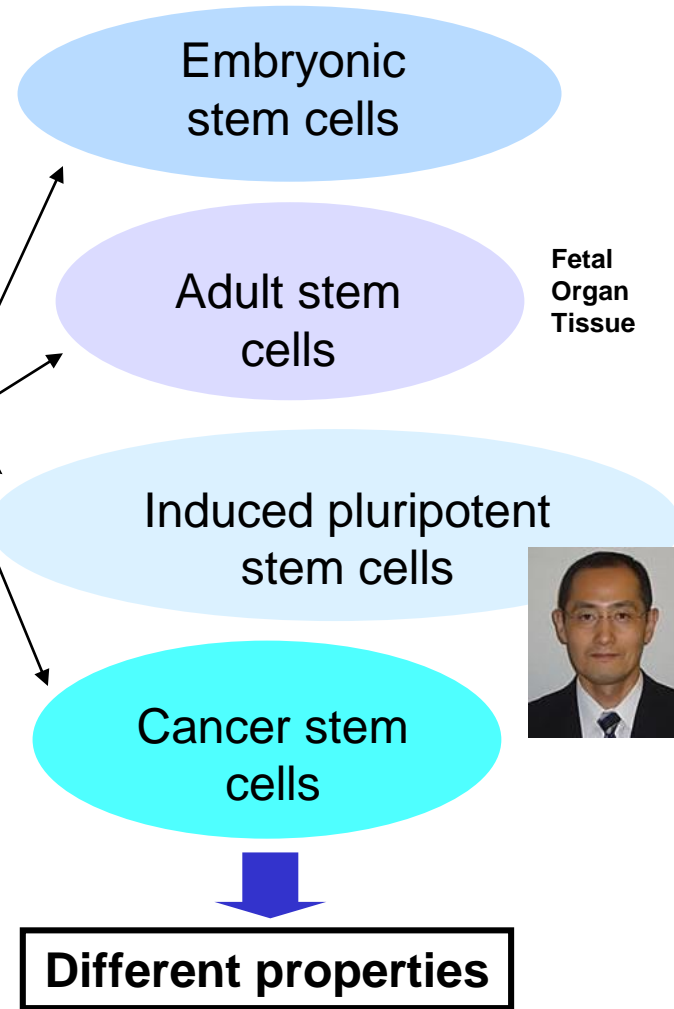
Self-renewal

Capability to differentiate into specialized cell types

Pluripotency

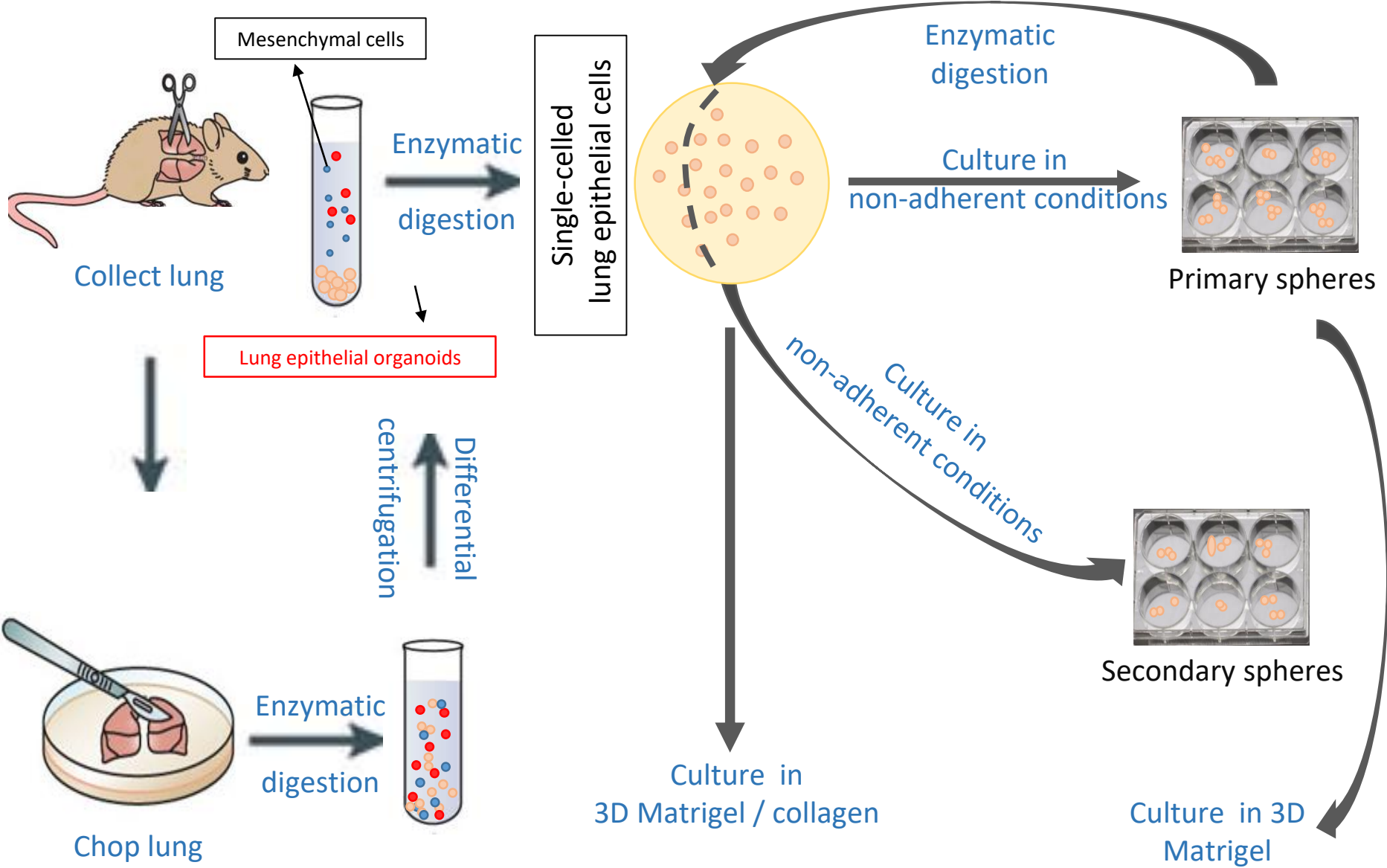


2. Differentiation

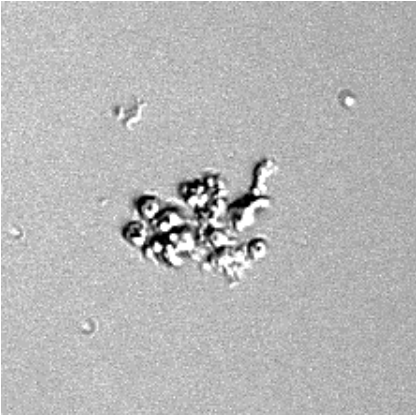


Different properties

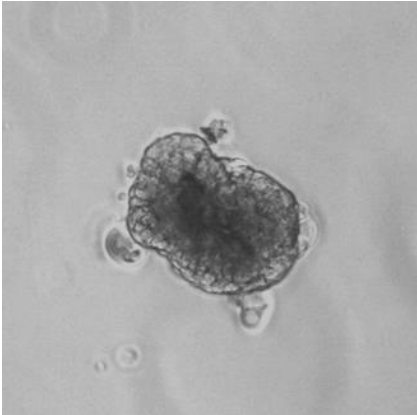
Stem cell can be isolated from tissues and studied in vitro 1



Stem cell can be isolated from tissues and studied in vitro 2



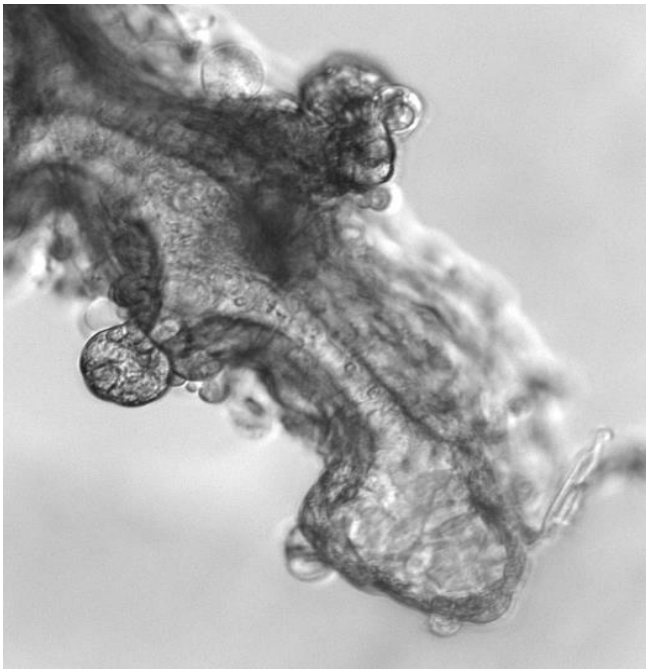
SCs after isolation



Spheroid growing from SC
„lungosphere“



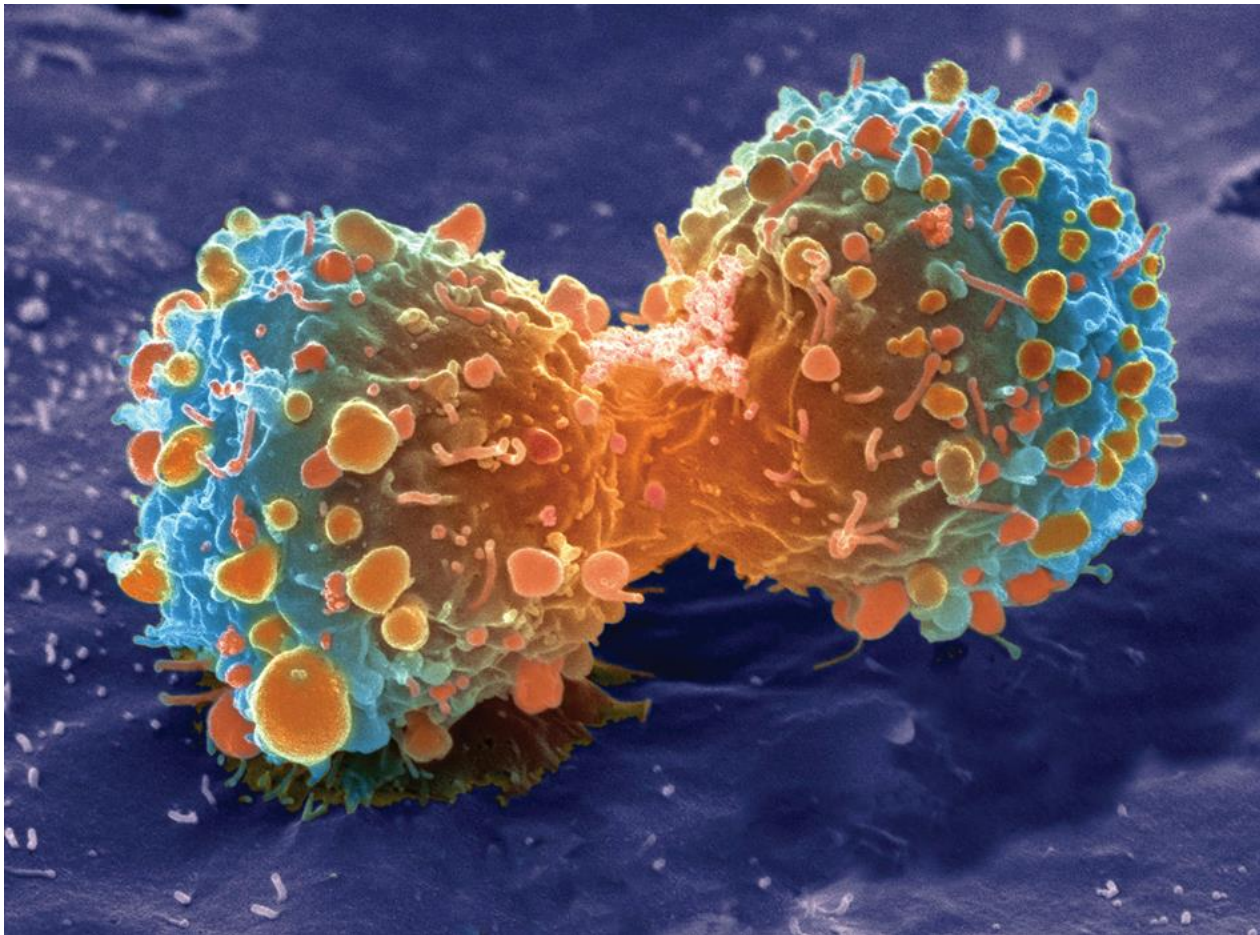
Organoid
Morphogenesis in 3D environment



Cell division 1

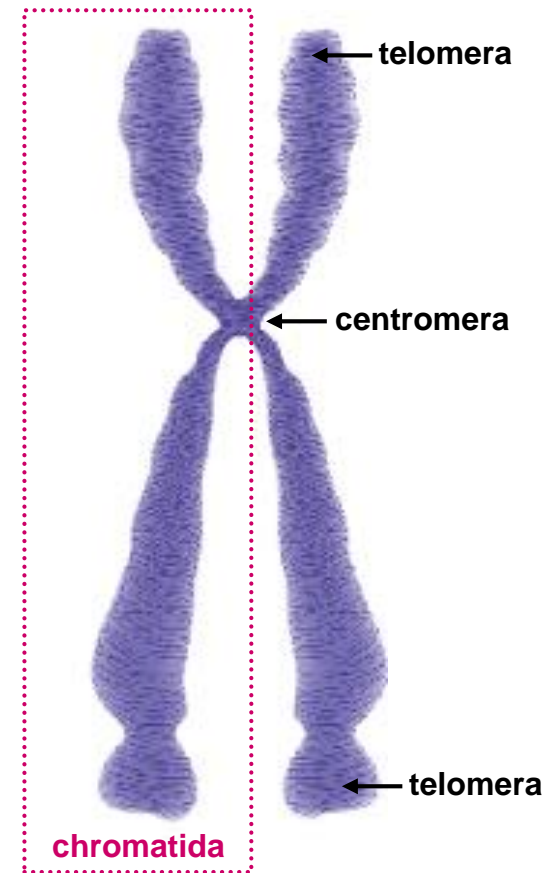
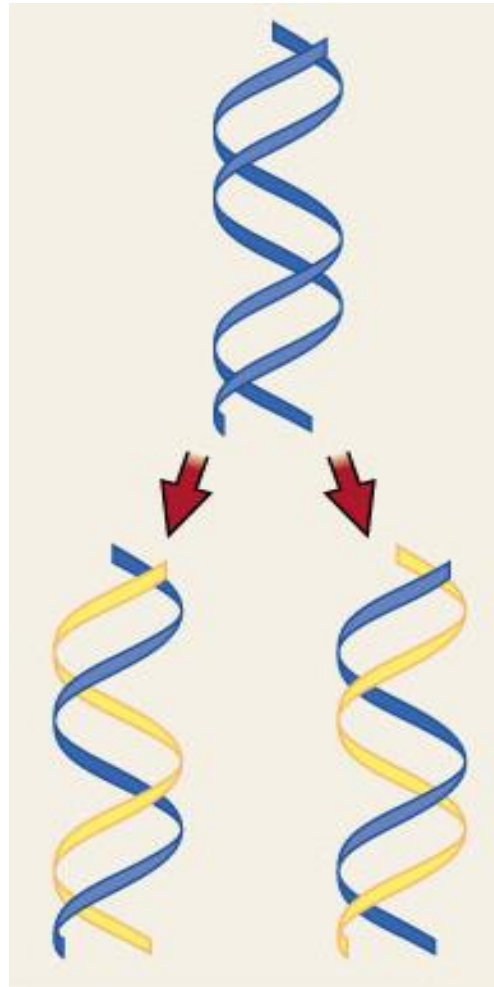
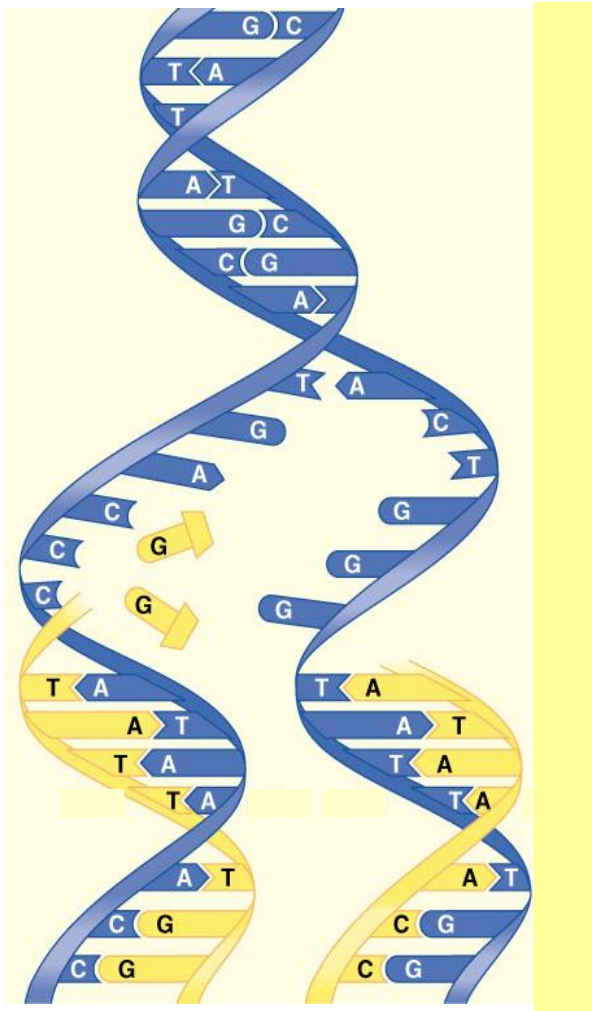
Basic concept 1

MITOSIS and CYTOKINESIS produce genetically identical cells



Cell division 2

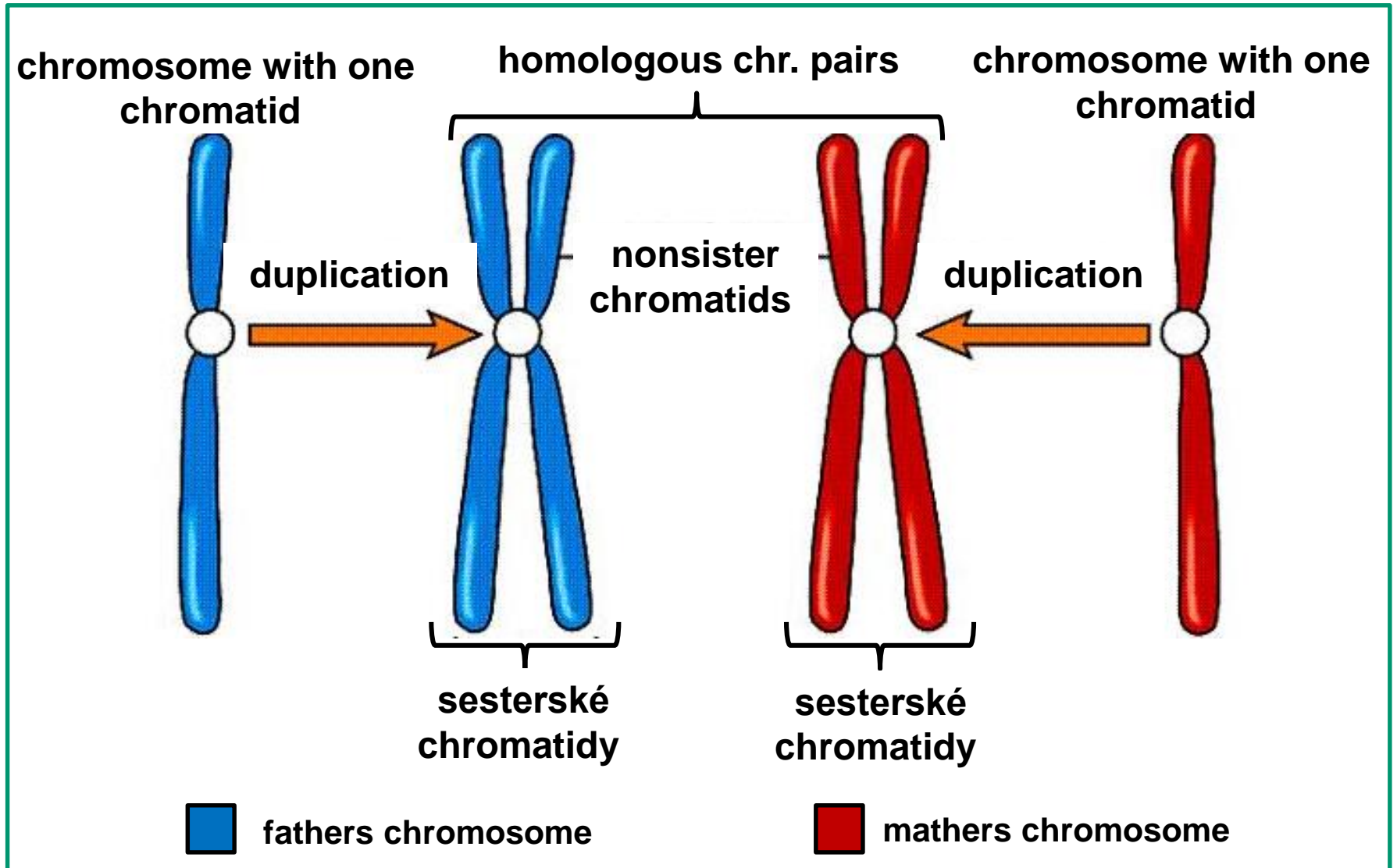
STABLE (non-changing) GENOME
Due to semiconservative duplication of DNA



Kondenzovaný duplikovaný chromozom

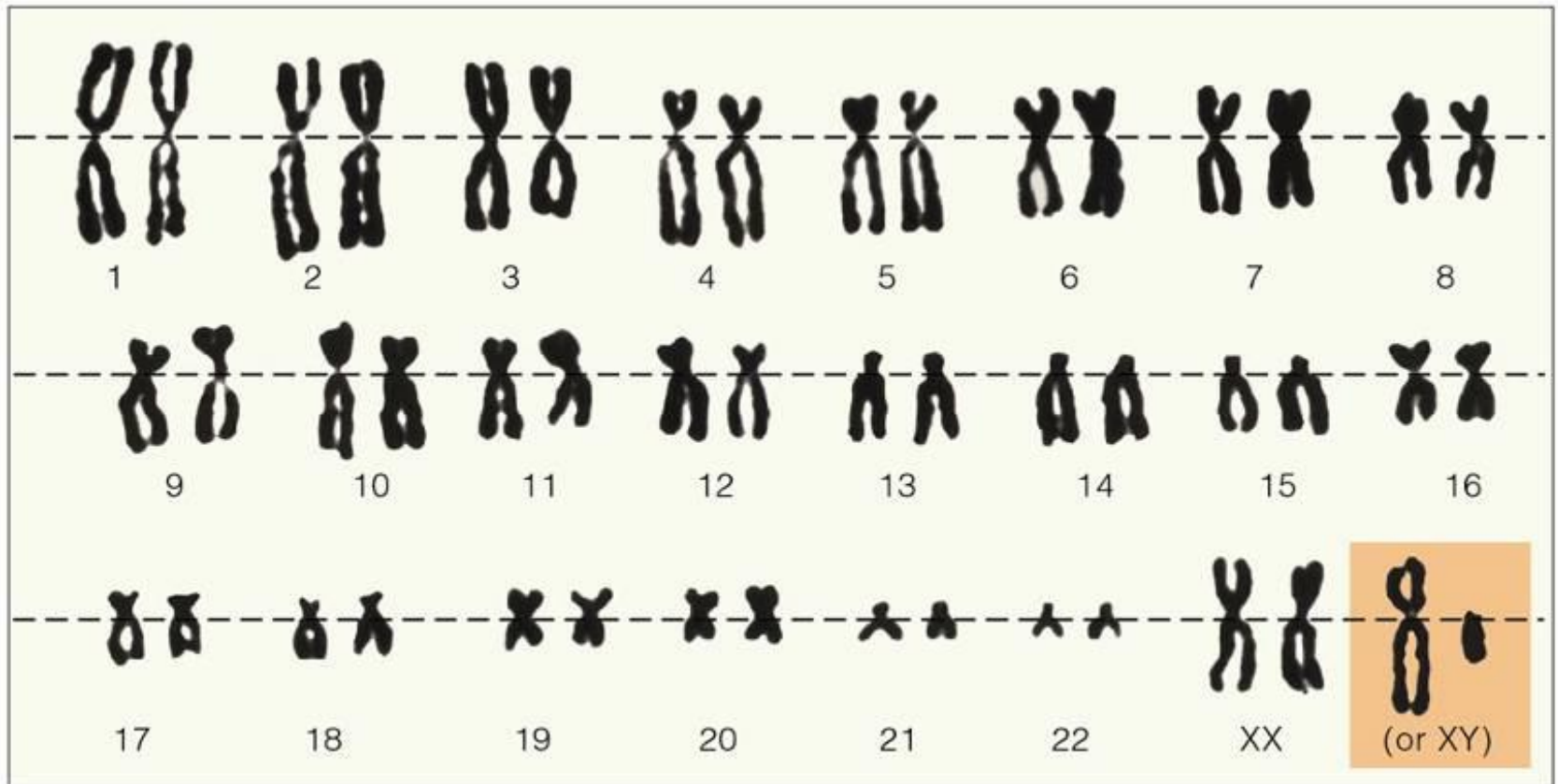
Cell division 3

Metabolism of chromosomes – Homologous chromosomes



Cell division 4

Pairs of homologous chromosomes (2N) organized into so called „KARYOTYPE“



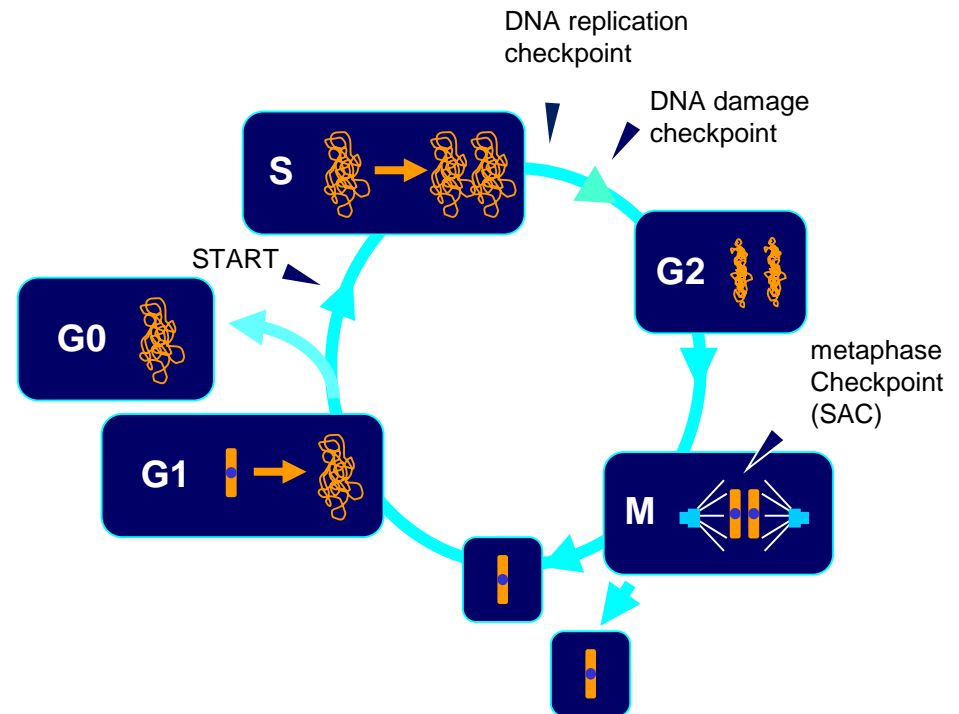
Cell division 5

Basic concept 2

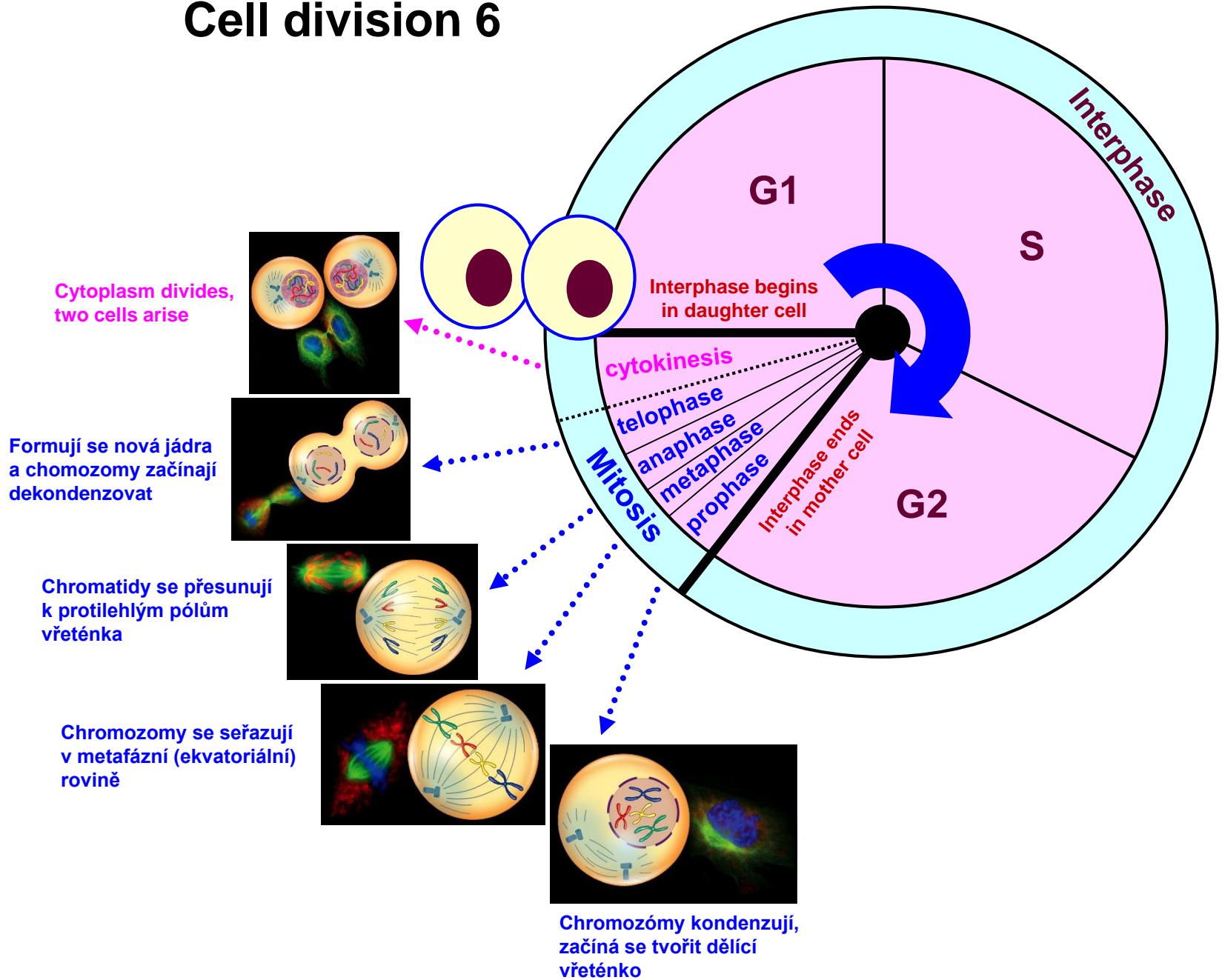
MITOSIS and CYTOKINESIS are parts of cell cycle

CELL CYCLE

- semi-modular character
- equipped with checkpoints
- among cells it is coordinated by signalling molecules

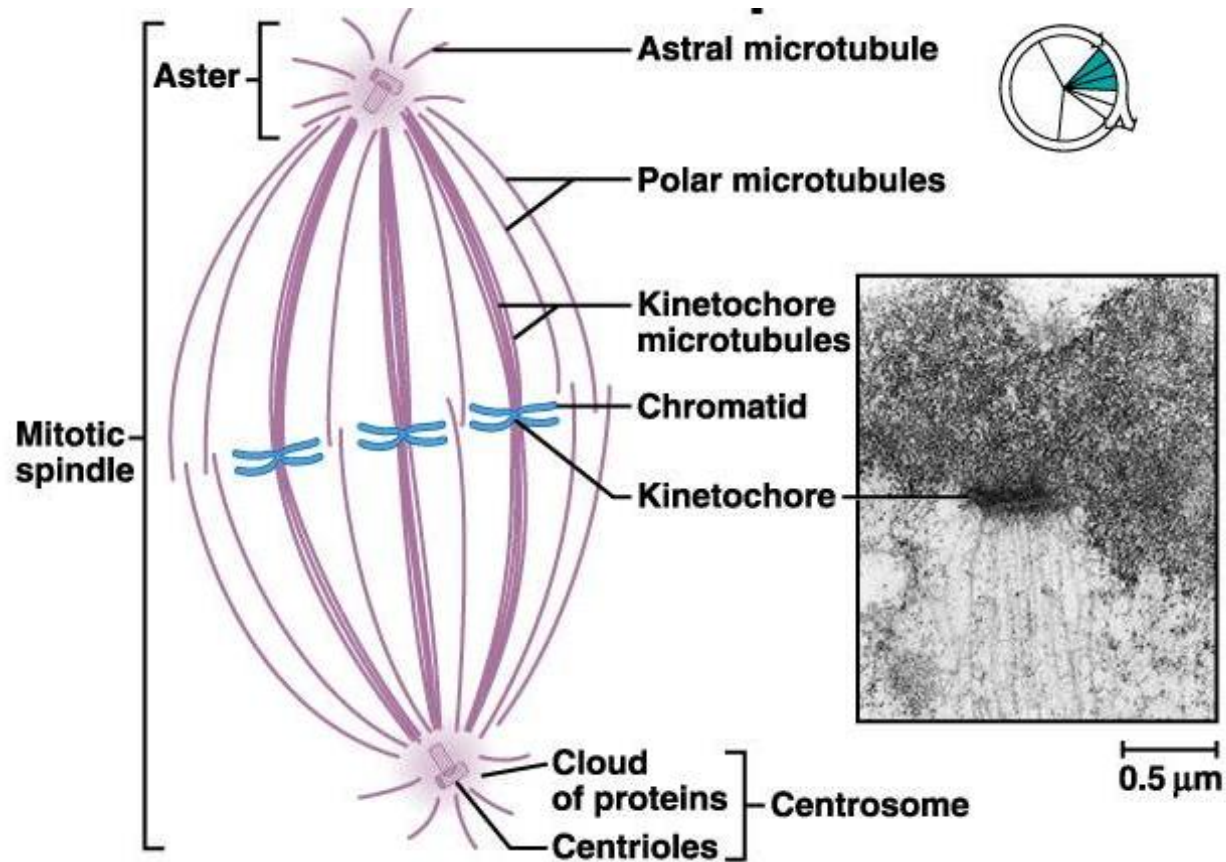


Cell division 6



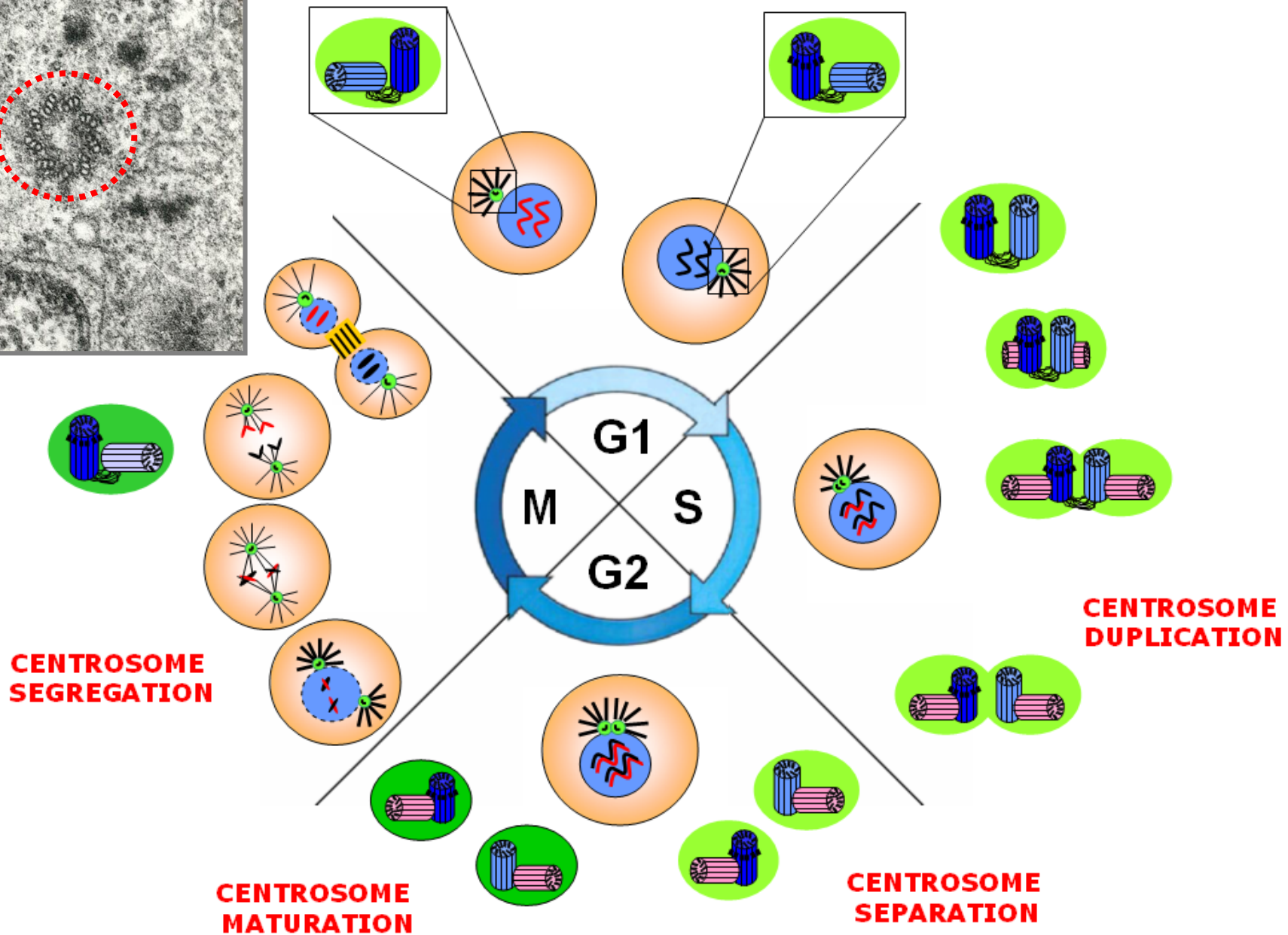
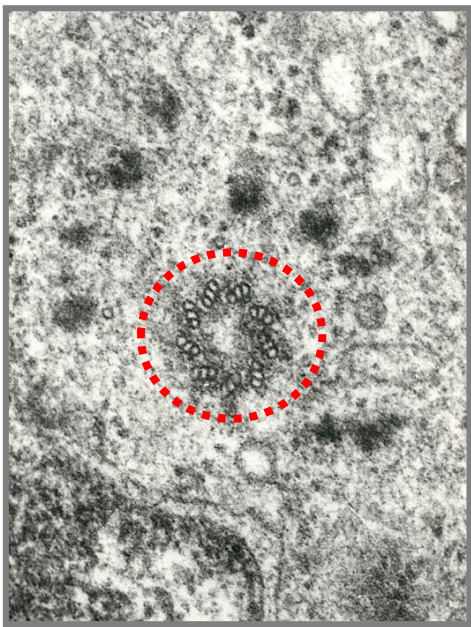
Cell division 7

Mitotic spindle



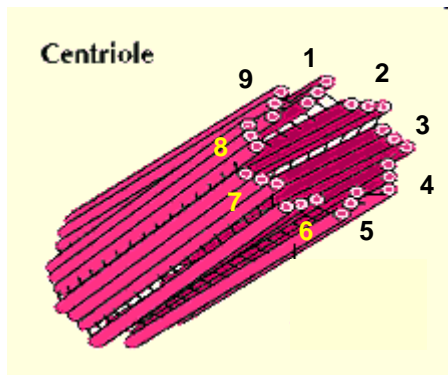
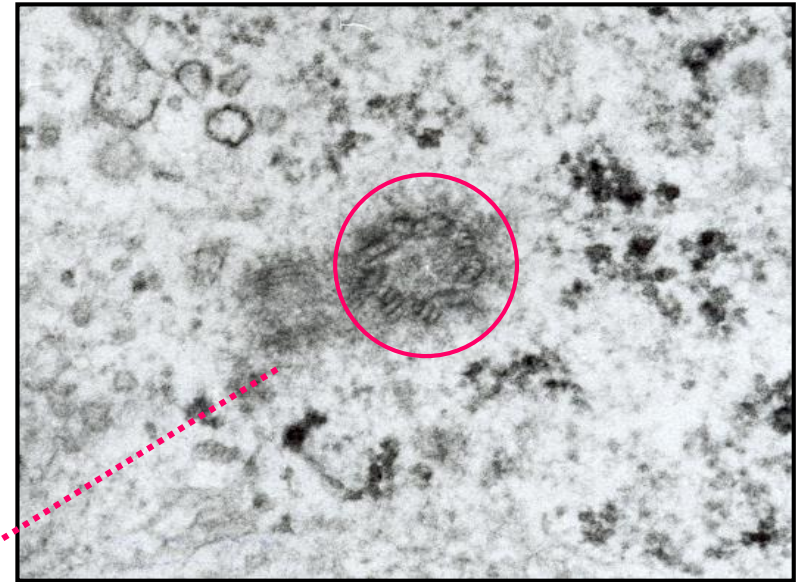
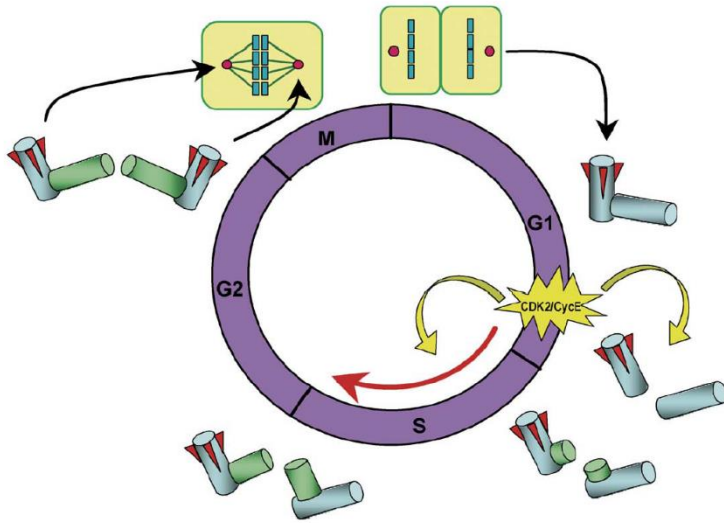
Cell division 8

Centrosomal metabolism
Semiconservative duplication

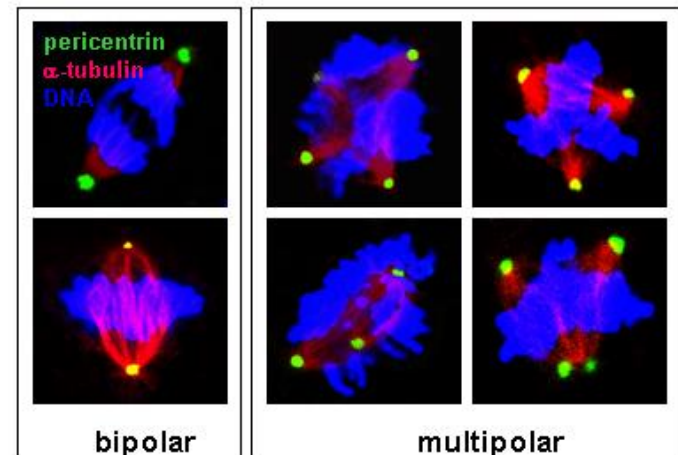


Cell division 9

Centrosome structure



Diameter - 0.2 μm
Length - 0.5 μm



Cell division 10

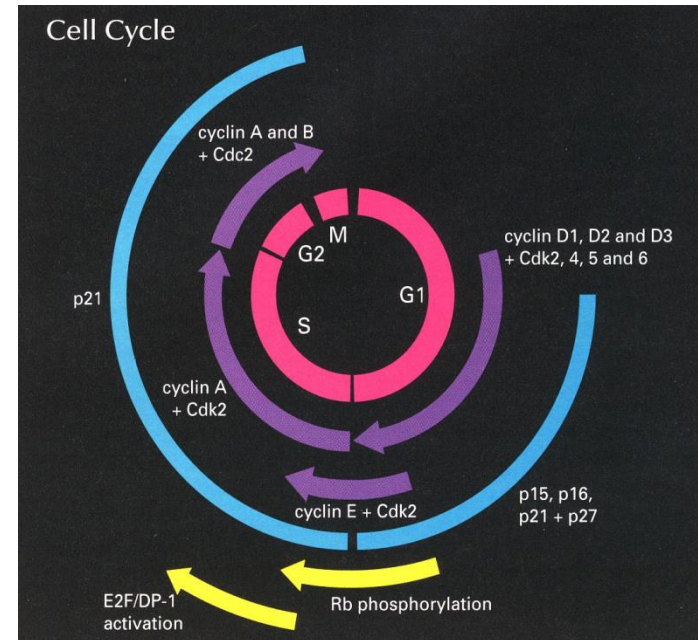
Regulation – Cyclin-Dependent Kinases (CDK) + Cyclins

Cdks and Related Proteins

kinase	PSTAIRE motif	regulatory subunits	putative substrates
Cdc2 p34	PSTAIRE	cyclin A & B	Rb, NF, histone H1
Cdk2	PSTAIRE	cyclin A, E & D	Rb, p27
Cdk3	PSTAIRE	cyclin E	E2F-1/DP-1
Cdk4	PV/ISTVRE	cyclin D1, D2, & D3	Rb
Cdk5	PISSLRE	p35	NF, Tau
Cdk6	PLSTIRE	cyclin D1, D2, & D3	Rb
Cdk7	NRTALRE	cyclin H	Cdc2, Cdk4/6
Cdk8	SACRE	cyclin C	RNA Pol II
Cdk9	PITALRE	cyclin T	Rb, MBP

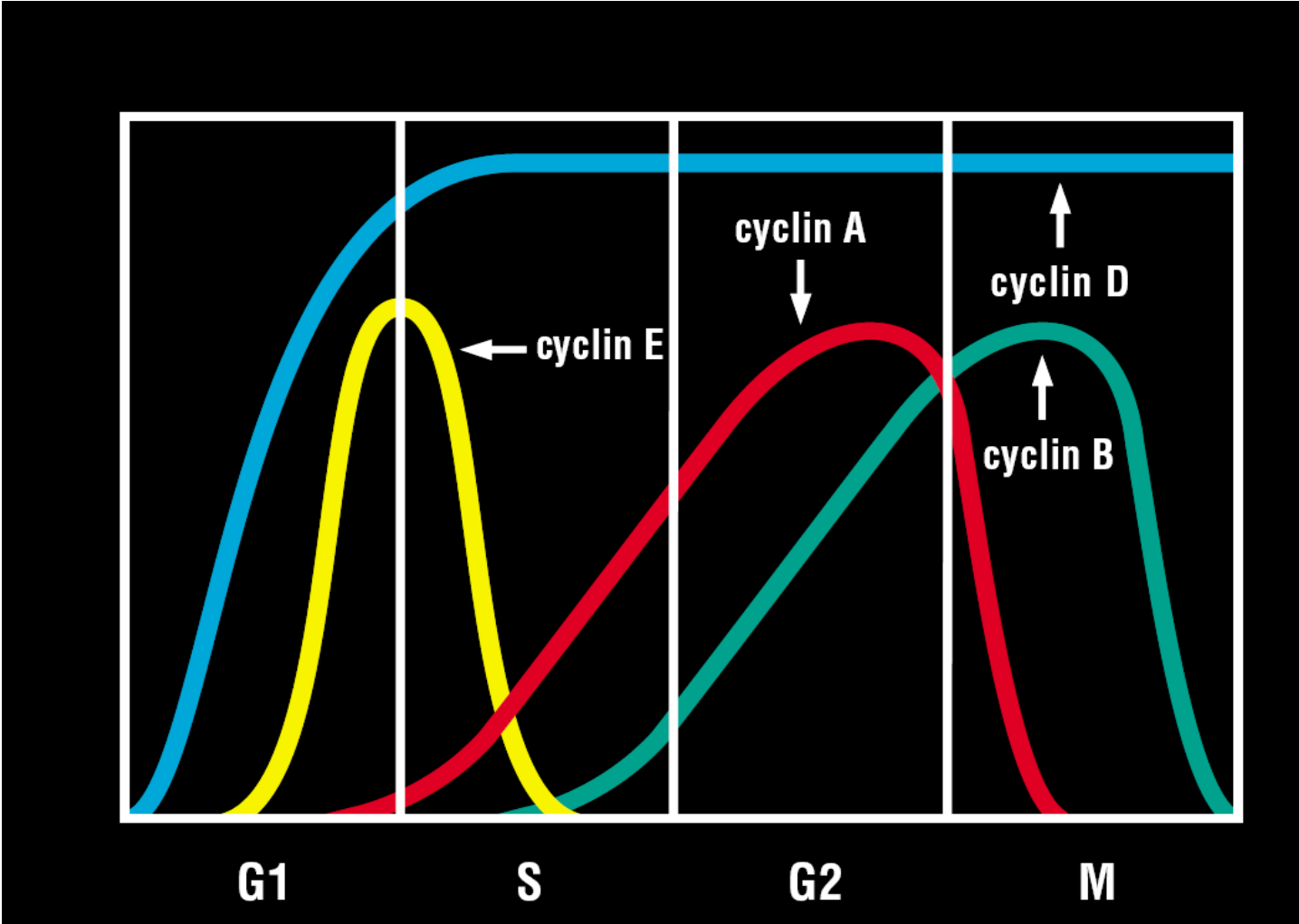
Major Cyclin-Cdk Cell Cycle Complexes

cell cycle stage	cyclin-Cdk complexes	inhibitors						
		p15	p16	p18	p19	p21	p27	p57
G1	cyclin D-Cdk4/6	+	+	+	+	+	+/-	+/-
G1/S	cyclin E-Cdk2	-	-	-	-	+	+	+
S	cyclin A-Cdk2	-	-	-	-	+	-	+
G2/M	cyclin B-Cdc2	-	-	-	-	+	-	-

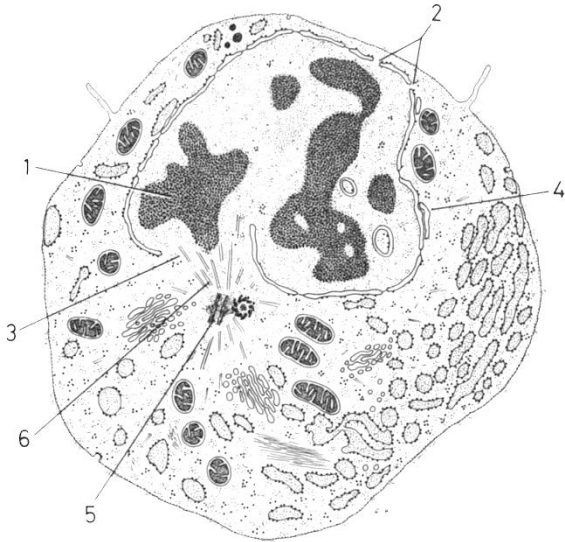


Cell division 11

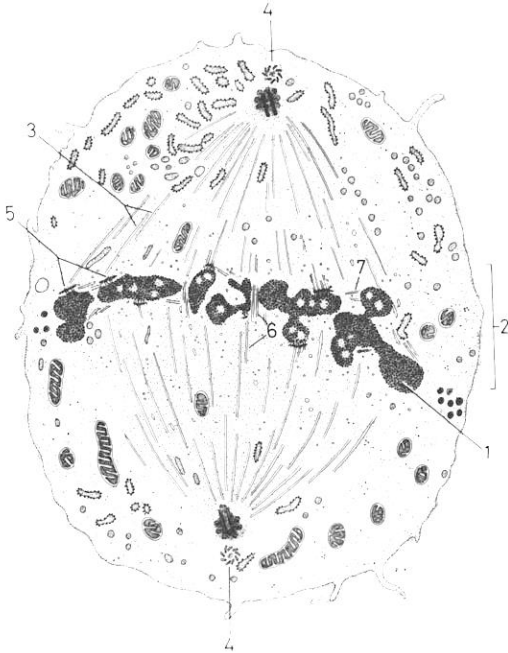
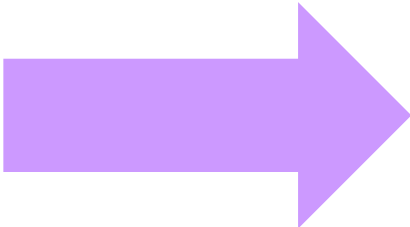
Periodicity of cyclin expression



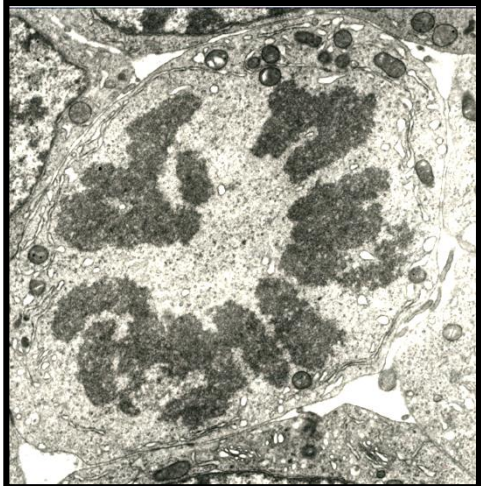
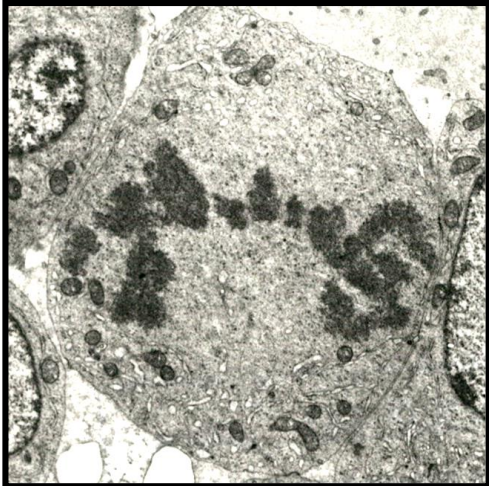
Cell division 12



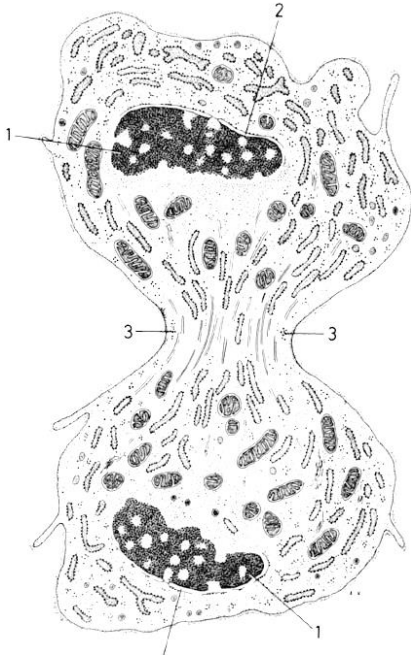
prophase



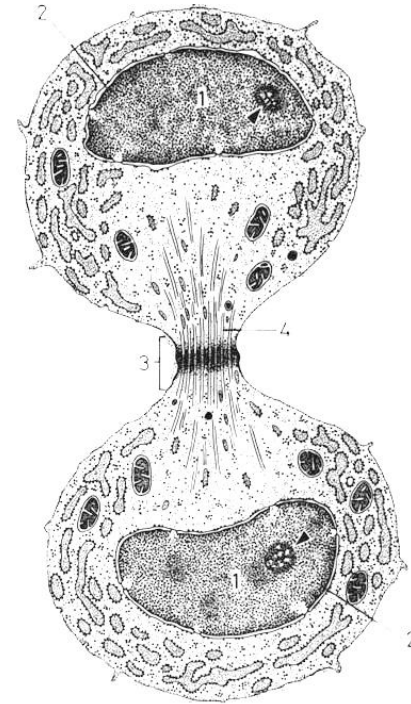
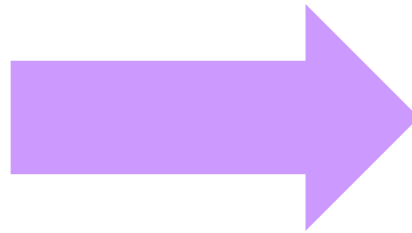
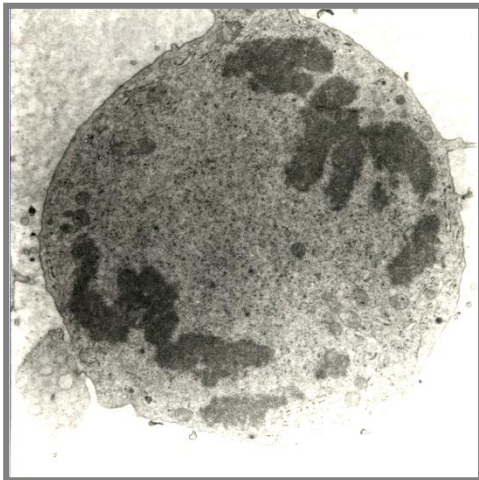
metaphase



Cell division 13



anaphase - telophase



telophase

Thank you for your attention !