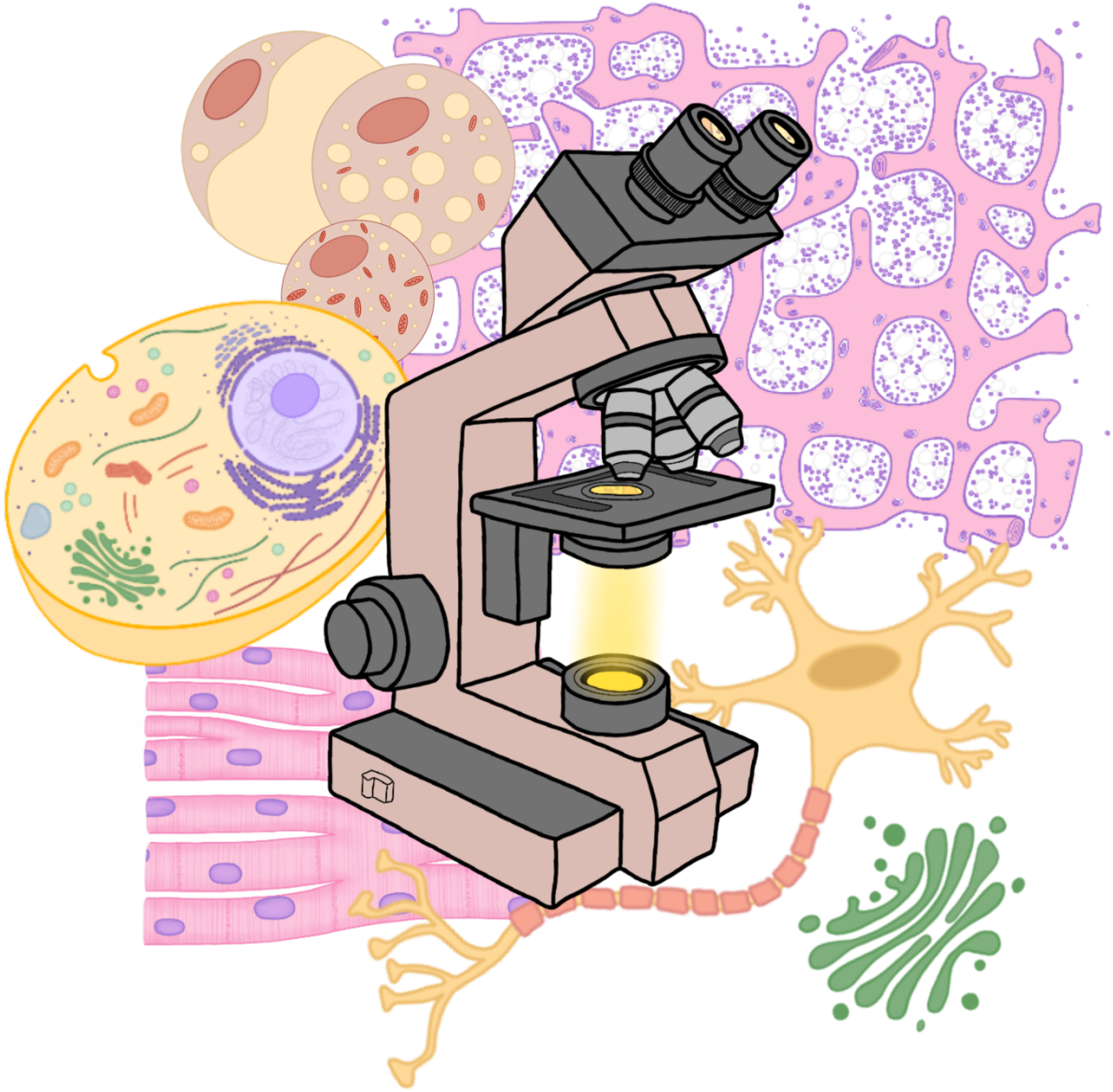

Histology:
Cells and Tissues

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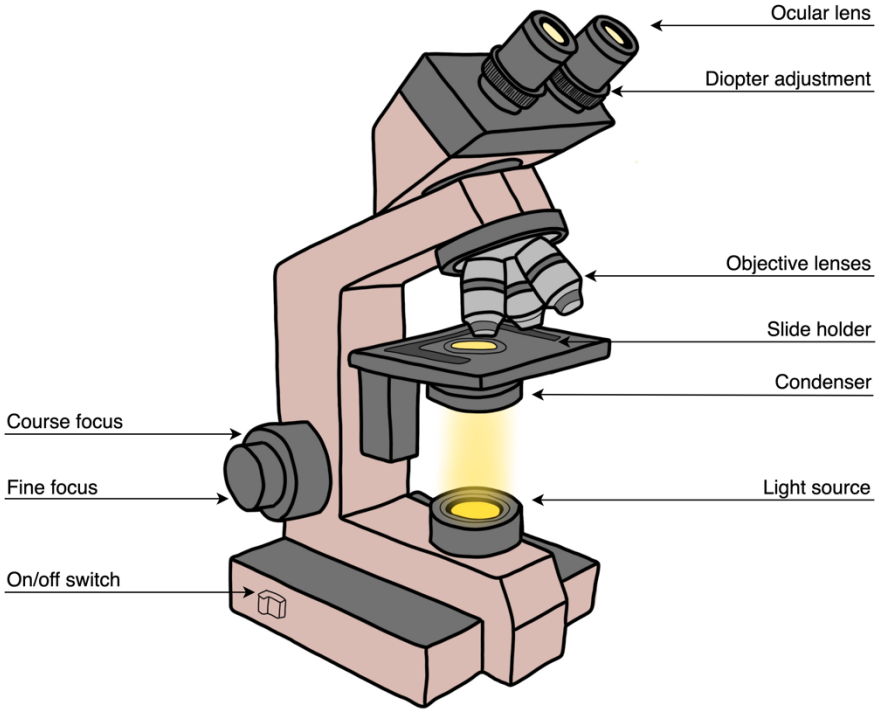
Practical Histology

I. Preparing tissues for investigation

Chemical fixation	Dehydration	Embedding	Sectioning	Mounting	Staining
Preserve tissue by destroying it and preventing cell lysis e.g.: formalin	With alcohol, prepares it for embedding	In paraffin wax or epoxy resin to allow for cutting in it	Specimens are cut into very thin slices	Sections are placed on glass slides	Reveals specific cell components Most common dye: hematoxylin and eosin

II. Light microscopy

- *Bright field microscopy*: normal light is used to investigate specimens. The colors are produced by dye added to the specimens. Most common method used by students and pathologists.



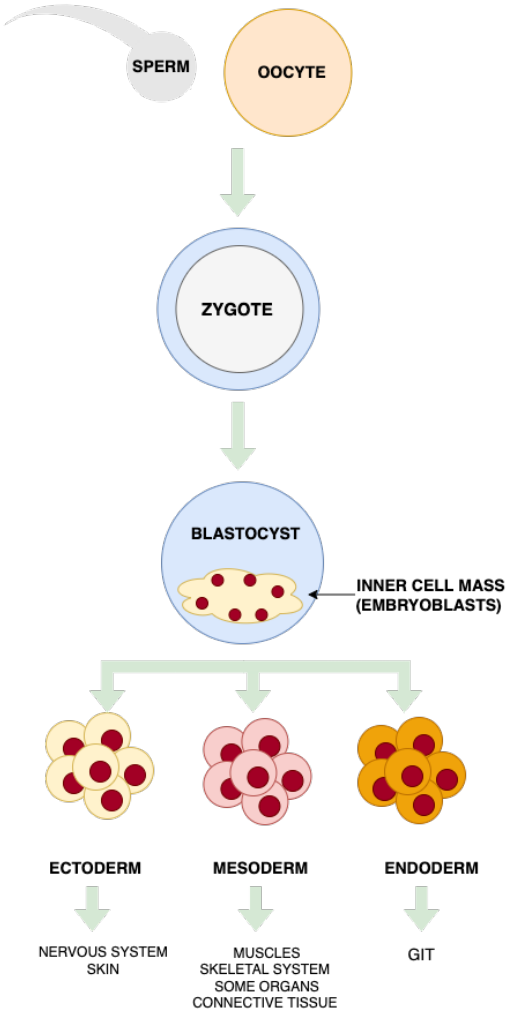
- *Fluorescence microscopy*: allows us to find very specific molecules by using ultraviolet light and fluorescent probes.

III. Electron microscopy

- Uses a beam of electrons as the light source. The electrons have a shorter wavelength than normal light giving us even higher resolution photos! To illustrate: electron microscopes can magnify an object 10 000 000x while a light microscope is only useful under 2000x magnification.

Section 1 – The Cell

1.1 – Cell Structure
1.2 – The Biological Membrane
1.3 – Transport Across the Cell Membrane
1.4 – Cytoplasmic Organelles
1.5 – The Cytoskeleton
1.6 – Inclusions



I. Cell differentiation

- All cells in the human body are derived from the zygote. The first divisions in the zygote produces blastomeres, and all tissues of the fetus are derived from these blastomeres.
- Cell differentiation is the process where cells become tissue-specific with different functions and shapes. This happens by increasing production of specific proteins that are helpful for their specific purpose.

II. Stem Cells

- Stem cells have the potential of dividing into more specialized cells. This happens through asymmetric divisions, where one daughter cell remains a stem cell and one differentiates into a more specialized cell.
- The stem cells have different levels of differentiation potential. Some can produce all types of cells, and others can only produce certain types of cells
- Renewal of stem cells happens through divisions without differentiation.

Types of stem cells

	Totipotent	Pluripotent	Multipotent	Monopotent
Differentiation potential	All cells of the body	Cells from endoderm, ectoderm, and mesoderm layers	Cells from only one embryonic layer Either endoderm, ectoderm or mesoderm	One specific type of cell
Location	The zygote	Cells of the inner cell mass in the blastocyst – embryoblasts	Mesenchymal, ectodermal, or endodermal stem cell	Organ/tissue specific stem cells i.e. epithelial or cardiac stem cells

III. Stem cell niche

- Stem cell niches are specific locations in tissues where stem cells reside due to a favorable microenvironment
- The activity of the stems cells is regulated by a signaling network controlled by the niche
- Components of the niche:
    1. Differentiated cells → paracrine and contact signaling
    2. Blood vessels → endocrine signaling
    3. Nerve terminals → neural signaling
    4. Extracellular substance → contact signaling

IV. Apoptosis

- Cell death: defective cells are eliminated without causing local inflammation
- Apoptosis is induced by DNA damage, deficit of growth factors or nutrients or signaling molecules activating specific "death receptors"
- Mechanism of action
    1. Degradation of proteins and cytoskeleton is done by the proteolytic enzyme caspase
    2. Degradation of nuclear DNA is done by endonucleases
    3. Cytosol is condensed. Cell and nuclear volume rapidly decrease
    4. Late in the process the cell splits into several apoptotic bodies that are phagocytosed by neighboring cells
- As the cell membrane is intact during apoptosis, little or no proteins are released into the extracellular space, and no local inflammation is induced.

V. Necrosis

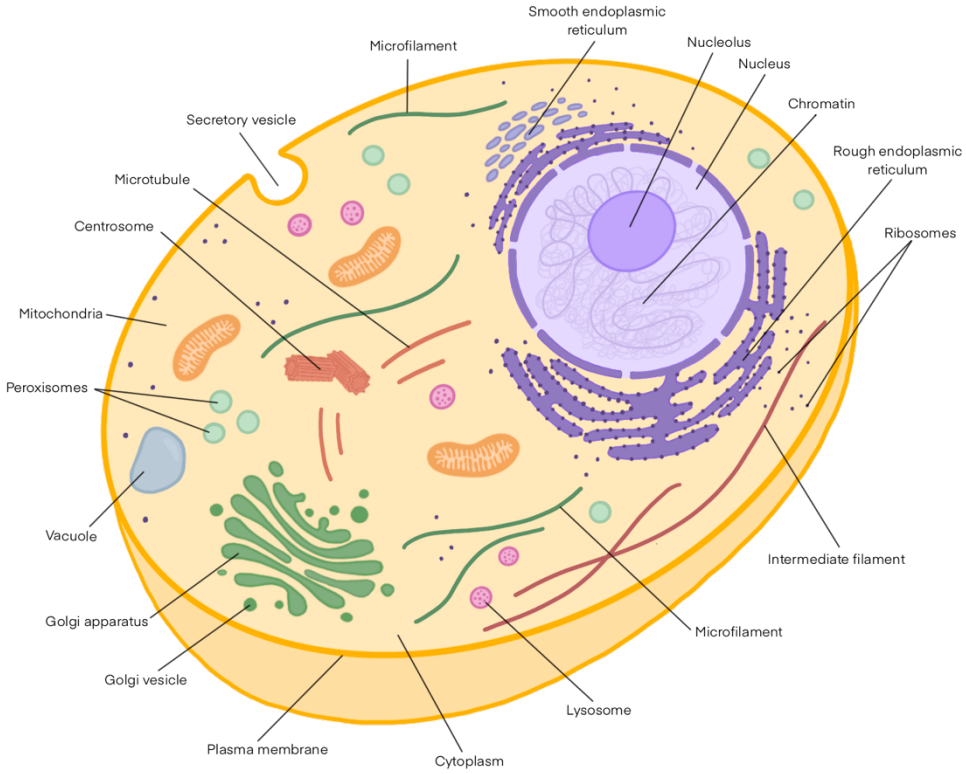
- Cell death of cell due to injury
- The cell membrane is ruptured or perforated, and intracellular materials are released into the extracellular space
- Local inflammation is triggered by the release of intracellular materials

VI. Autophagy

- Rare mechanism of cell death
- Autophagy is a process where nonfunctional organelles or cytosolic materials are digested
- Severe autophagy can lead to cell death – for example during starvation
- Physiological cell death due to autophagy together with apoptosis occurs
    1. In secretory alveoli, where it is responsible for involution after lactation
    2. In the ovary when the corpus luteum is involuted (luteolysis), if the oocyte is not fertilized
    3. During chemotherapy in cancer cells

1.1 – Cell Structure

- Most structures within the cell are made from biological membrane: lipids and proteins



Cell membrane	Phospholipids surrounding the cell
Cytoplasm	All content within the cell membrane
Cytosol	The fluid within the cytoplasm Contains all the enzymes, metabolites, electrolytes etc. that is used or produced in the cell
Organelles	The "organs" of the cell - Endoplasmic reticulum - Golgi apparatus - Mitochondria - Endosomes - Lysosomes - Peroxisomes - Ribosomes - Centrioles
Cytoskeleton	The "skeleton" inside the cell Determines shape and motility of the cell
Inclusions	Deposits of lipids, carbohydrates or pigments
Nucleus	Contains all genetic information

1.2 – The Biological Membrane

- The same type of membrane surrounds most intracellular structures as well as the cell itself.
- Consists of lipids and proteins: the lipids provide integrity to the membrane and have proteins "floating" in it, performing different tasks

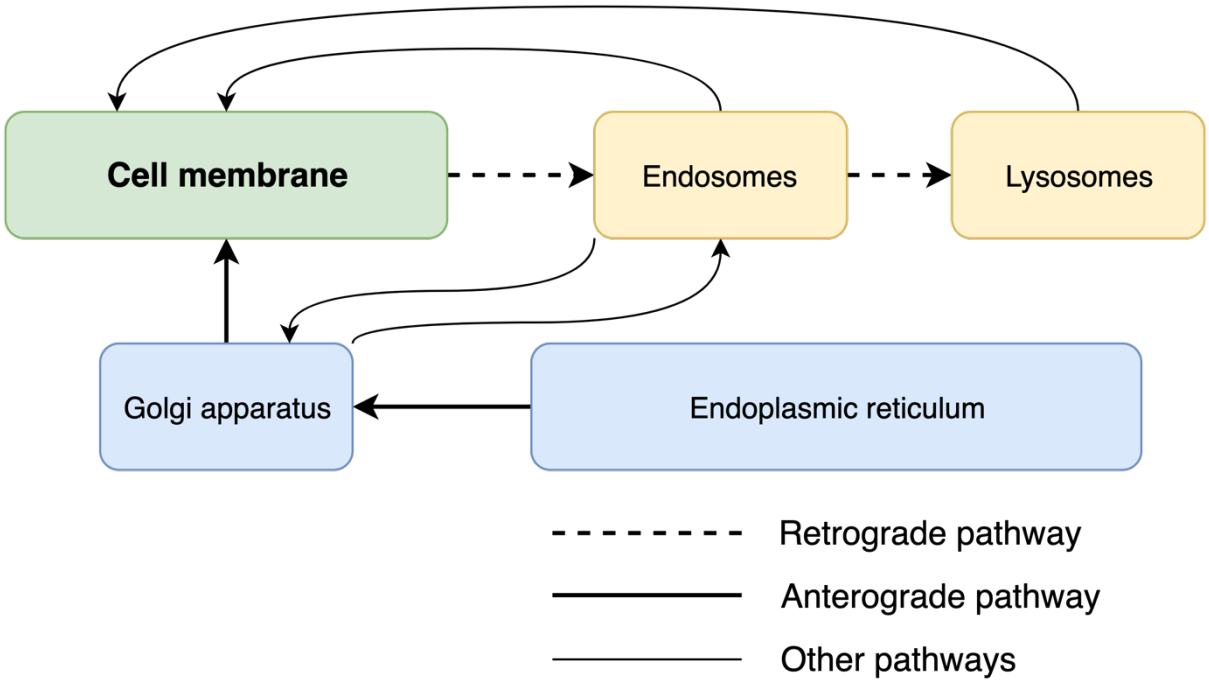
1.2.1 – Growth and Production of the Biological Membrane

- The biological membrane cannot be produced from scratch – it can only grow by adding more proteins and lipids

Protein	Lipids
Endoplasmic reticulum: added *during* translation Mitochondria and peroxisomes: added *after* translation	Incorporated either directly to ER membrane during their synthesis or via lipid transfer proteins into other membranes

- Due to membrane trafficking, the plasma membrane and membranes of the Golgi apparatus, endosomes and lysosomes are continuously renewed.
- Membranes of mitochondria and peroxisomes *does not* have membrane trafficking

I. Membrane Trafficking



1.2.2 – The Plasma Membrane

- Also called plasmalemma
- A specialized form of the biological membrane
- Thick physical barrier (7,5 nm) that separates and protects the inside of the cell from the outside, contain transport proteins and adhesion molecules that make contact with other cells and extracellular substance.
- Trilaminar in the electron microscope: looks like three layers
- Main function: controlled transport across the membrane
    1. Selective permeability: regulates and facilitates transport of ions, nutrients and waste products in and out of the cell
    2. Establishing and maintaining an electrochemical gradient across the plasma membrane: resting potential
    3. Recognition and response to molecular signals via receptors

I. Cell membrane skeleton

- Keeps the membrane stable
- Consists of a web of proteins close to the surface of the cell membrane that is towards the cytosol
- Attaches both to transmembrane proteins and cytoskeleton.

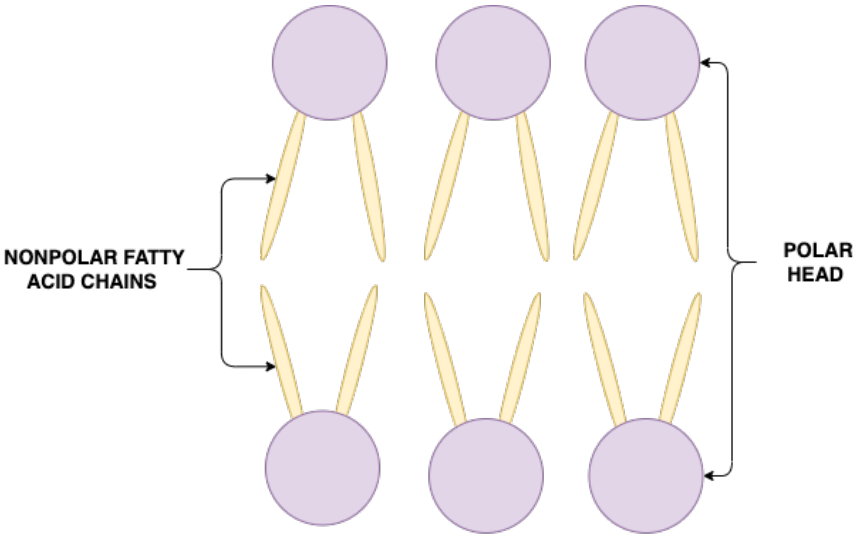
II. Adhesion molecules

- Cadherins: cell-cell adhesion between cells of the same type
- Selectins and immunoglobulin superfamily: cell-cell adhesion between different types of cells
- Integrins: cell-extracellular matrix adhesion
    1. Sometimes cell-cell (different cells)

I. Structure of the plasma membrane

<table>
<tr>
<td>Phospholipids</td>
<td>Asymmetric distributed in a in a double layer, with hydrophobic fatty acid chains facing each other.

Amphipathic: contains both hydrophobic and hydrophilic parts

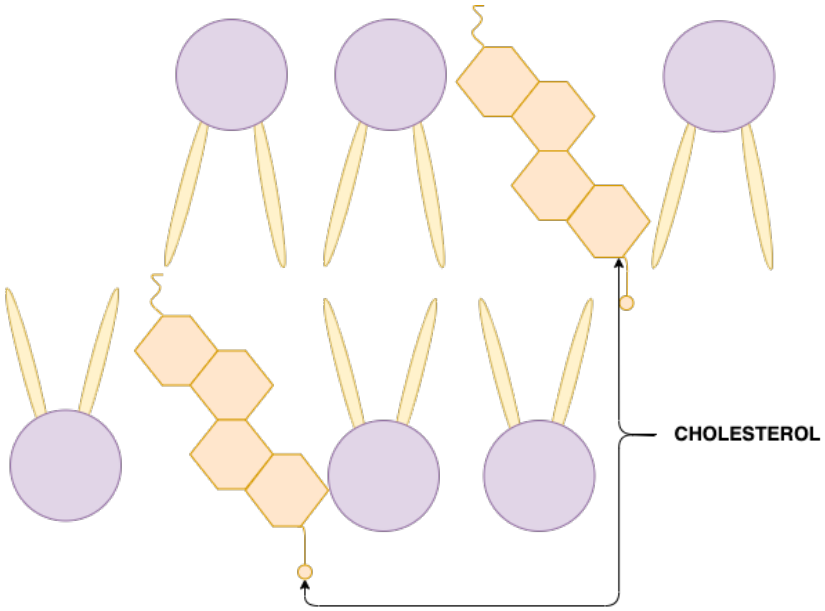
- Hydrophobic: two nonpolar long-chain fatty acids
- Hydrophilic: charged polar head carry a phosphate group</td>
<td> 

Choline phospholipids: phosphatidylcholine = lecithin, sphingomyelin

Amine phospholipids: phosphatidylethanolamine = kephalin, phosphatidylserine

Phosphatidylinositol: Cell-cell signaling</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Inserted between phospholipid tails

Increases the stability of the membrane

Modulates fluidity and movement of membrane components</td>
<td> 
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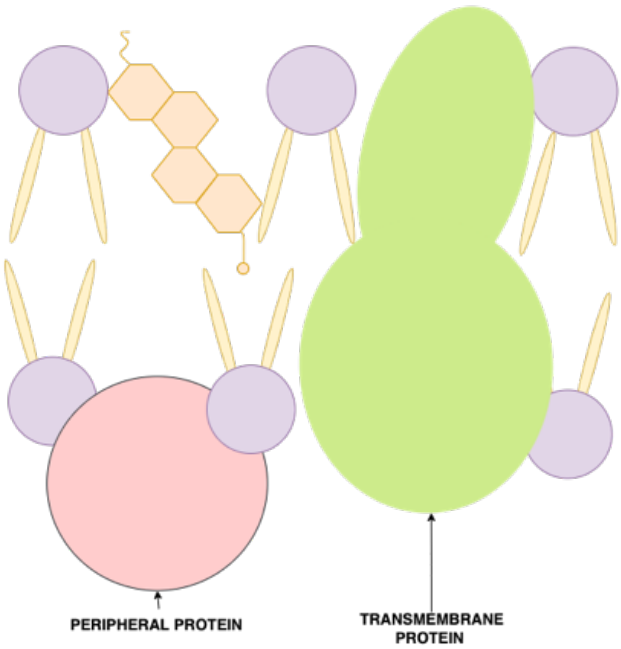
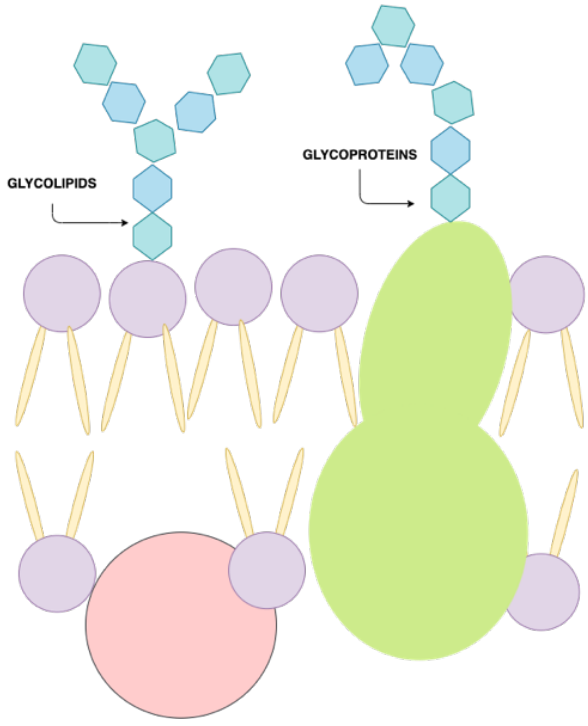
</table>

<table>
<tr><td rowspan="2">Proteins</td><td>Transmembrane (Integral) proteins extend throughout the entire membrane
Single pass = only goes through the membrane once
Multipass = goes back and forth through the membrane</td><td rowspan="2"> 
</td></tr>
<tr><td>Peripheral proteins: loosely connected to phospholipids or to transmembrane proteins</td></tr>
<tr><td>Glycocalyx</td><td>Oligosaccharide chains of glycolipids and glycoproteins that covers the outer side of the membrane
Glycolipids: phospholipids in the outer layer covalently bound to oligosaccharide chains
Glycoproteins: membrane proteins covalently bound to oligosaccharide chains

Functions:
- Protects the cell from damage: physical and chemical
- Cell-to-cell recognition and contact
- Participates in endocytosis</td><td> 
</td></tr>
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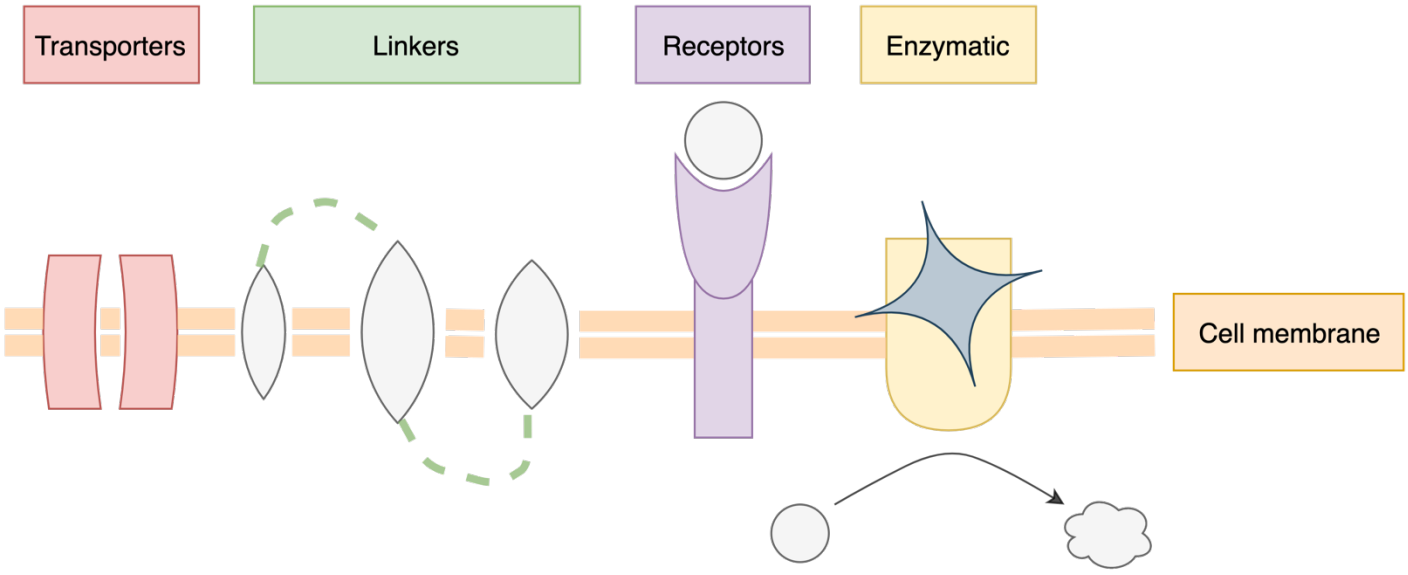
II. Specialized components of the plasma membrane

Lipid rafts	Rigid areas in the lipid bilayer rich in cholesterol and glycolipids Carries proteins that need to stay in close proximity to each other to function
Caveolae	Invagination of the membrane formed by multiple lipid rafts attached via the protein caveolin Participates in pinocytosis Contains some receptors and transporters: Ca^{2+} transport for example
Coated pits	Similar to caveolae, but surrounded by clathrin Participate in receptor mediated endocytosis

1.3 – Transport Across The Cell Membrane

- A membrane protein can often have more than one function, some examples:
    1. Receptor and transporter
    2. Transport and enzymatic activity
    3. Linker and receptor
- Translocons
    1. Transport of large proteins across the membrane in an *unfolded* state
    2. Used for transporting proteins into ER, mitochondria and peroxisomes
    3. ABC transporters can move molecules like peptides and drugs



Types of transport across the cell membrane

Non-energy dependent Direction determined by concentration gradient[1]	**Free diffusion**	Gases and hydrophobic molecules move across the membrane without aid of proteins
	Channels	Facilitated diffusion of *ions* Channels opens depending on different types of signals: - *Voltage gated*: change in electrical potential (nerve cells) - *Ligand gated*: signaling molecules (post-synaptic membranes) - *Mechanically gated*: stress (striated muscle, inner ear) Some channels are constantly open, and regulation of transport depends on removal/insertion of channels: - Potassium leak channels: maintains resting potential - Aquaporins (water channels in renal tubules): final concentration of urine
	Carriers	Facilitated diffusion of *small molecules* Cyclically changes their form to transport the molecules from one side of the membrane to the other
	Osmosis	Diffusion of water across a selectively permeable membrane Direction is determined by solute concentrations and the diffusion continues until equilibrium is reached
Energy dependent Active transport	**Pumps**	Active transport of ions and small molecules Pumps functions as carriers, but the need energy from ATP to pump against the concentration gradient : sodium/potassium, calcium and proton pumps
	Vesicular transport	Transportation of *large* molecules into or out of cells by active participation of the cell membrane: budding and fusion of vesicles. - During transport the membrane and the cargo can be modified - *Membrane trafficking*: all the pathways involved in vesicular transport

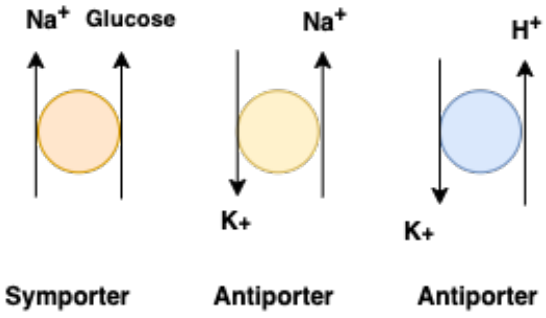
[1] ions diffuse from a location of higher concentration to a location of lower concentration

CLINICAL CORRELATION

Alcohol and peeing

Some aquaporins are dependent on antidiuretic hormone (ADH). Alcohol is one of many substances that inhibits ADH, so when you drink alcohol, some of the aquaporins are removed from the renal tubules. This causes dilution of urine, and increased urine volume. If you ever wonder why you have to pee more while drinking, this is one of the reasons!

- *Pumps* and *carriers* can transport one or two substances:

<table>
<tr><td>Uniport transport</td><td colspan="2">Transporting one substance</td></tr>
<tr><td rowspan="3">Coupled transport</td><td colspan="2">Transporting two substances at the same time</td></tr>
<tr><td>Antiport
The two substances are transported in opposite directions</td><td rowspan="2"> 
</td></tr>
<tr><td>Symport
The two substances are moved in the same direction</td></tr>
</table>

1.3.1 – Exocytosis

- Secretory vesicles inside the cell fuses with plasma membrane and releases substance to the extracellular space

  - Material released into the extracellular space is taken up by other cells, influencing their function.
  - Exocytosis can be either constitutive or regulated
  - Important mechanism for communication and signaling between cells, and regulation of:
    1. Cell differentiation
    2. Inflammation and immune responses
    3. Cell growth (proliferation)

Constitutive	Regulated
Continuous release Slow rate Small vesicles Low density of substance	Release in response to a signal Large volume and fast release High density of substance Large secretory granules

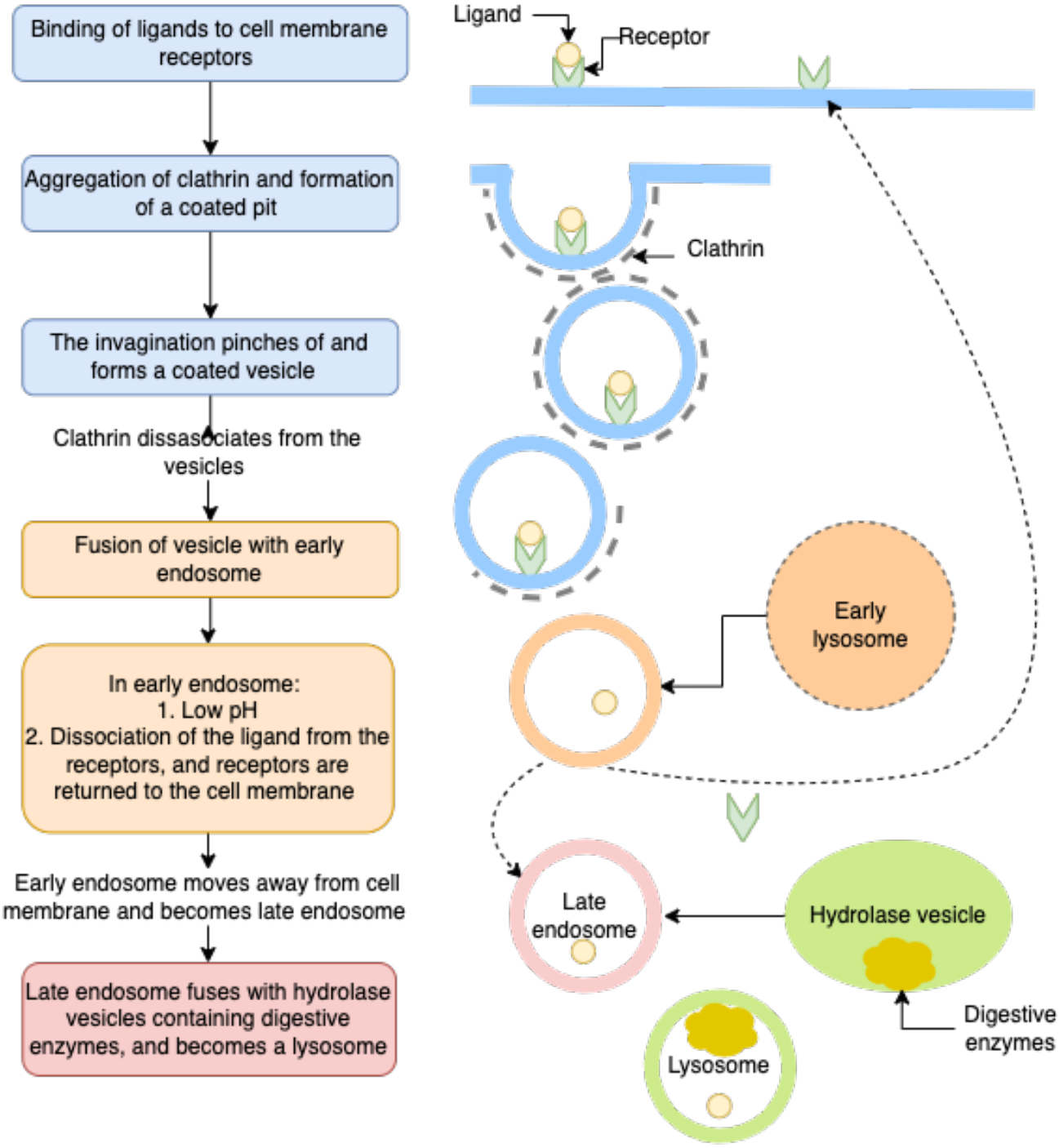
Stages of exocytosis

1. Vesicle migrates to the cell membrane	Moving inside the cytoplasm
2. Fusion with membrane	The fusion of two biological membrane are dependent on either 1. SNARE proteins recognize, bind and begin the fusion 2. Similarity between the two membranes
3. Content of the vesicle is released into the extracellular space	Two types of vesicles are released from the cells into the extracellular space: <u>Microvesicles</u> - Cell membrane encapsulate substances and buds off - Large size, 0.2-1 µm <u>Exosomes</u> - Multivesicular bodies which are large vesicles with numerous small vesicles, release these small vesicles into the extracellular space via - Small size, 30-150 nm

1.3.2 – Endocytosis

Endocytosis	Substances are taken into the cell by formation of vesicles at the plasma membrane
Phagocytosis	"Cell eating": vesicles forms around "particulate substance" Solid substances, even whole cells are taken into the cell The membrane of the cell interacts with the surface and folds around the substance The folded membrane fuses and forms a large vesicle called a phagosome The cytoskeleton is also involved in the formation of the phagosome
Pinocytosis	"Cell drinking": vesicle forms around interstitial fluid The cell membrane forms an invagination that pinches off forming a pinosome The cytoskeleton is *not* involved in this process
Receptor mediated endocytosis	Selective endocytosis. Molecules are only taken into the cell if they are bound to specific receptors Selective uptake of different substances e.g. LDL, transferrin Uptake and inactivation of signal molecules such as hormones, neurotransmitter, and growth factor.

Receptor mediated endocytosis



1.4 – Cytoplasmic Organelles

1.4.1 – Ribosomes

I. Main function

- Produces proteins in the cytoplasm after mRNA templates
- At the presence of mRNA in the cytosol ribosomal subunits come together at the beginning of protein biosynthesis (translation).
- Multiple ribosomes associated with mRNA chain are called polyribosomes

II. RER-bound and cytosolic ribosomes

- Ribosomes are either free, or RER-bound. Depending on location the ribosomes they produce proteins for different parts of the cells

RER-bound ribosomes produce:	Cytosolic (free) ribosomes
1. Trafficking membrane proteins 2. Secretory proteins, 3. Lysosomal proteins	1. Nuclear proteins 2. Mitochondria proteins 3. Peroxisomal proteins 4. Cytoskeletal proteins 5. Cytosolic proteins

III. Amino acid signaling sequences

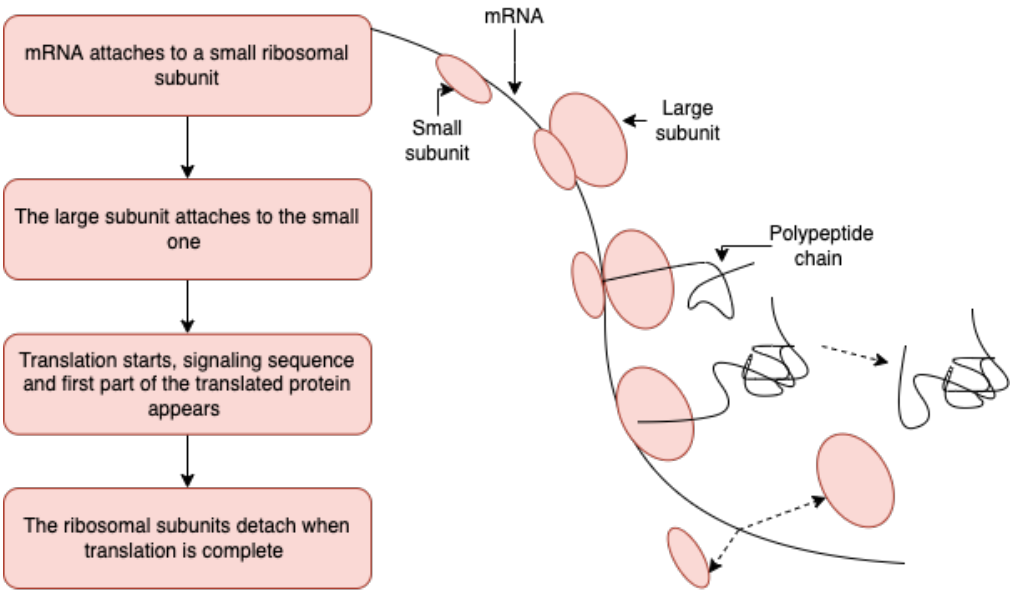
- The destination of a protein is determined by a signal sequence in the amino acid chain
- Each location has different signal sequences
- Receptors on the target organelle recognizes the signaling sequences, and when the proteins binds with the receptors they are incorporated through various mechanisms:

Nuclear proteins	Through the nuclear pore complex
ER, mitochondrial and peroxisomal proteins	Through translocons
Membrane proteins	Has an additional hydrophobic “stop” sequence and is stopped in the membrane

- ER proteins are incorporated during translation, in the other organelles the proteins are incorporated post translationally

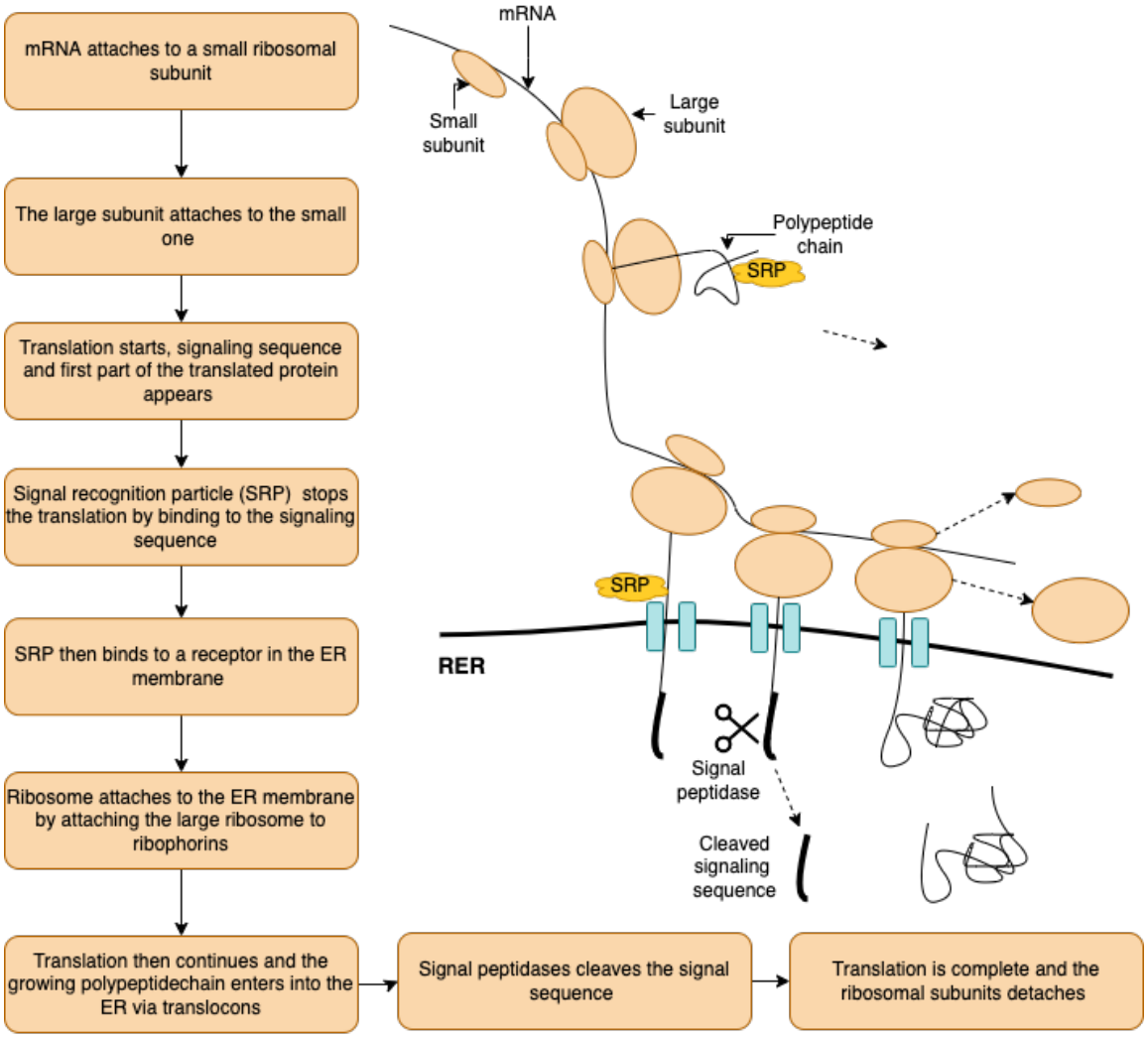
I. Cytosolic ribosomes

- Protein synthesis on cytosolic (free ribosomes)



II. Ribosomes attached to RER

- Protein translation on ribosomes attached to the RER



1.4.2 – Endoplasmic Reticulum

I. Structure and content of ER

- A folded, continuous, membranous network, with a surface area of up to 30x times that of the cell membrane
- The membrane forms multiple cisternae, which is intercommunicating channels and sacs
- Extends from the surface of the nucleus to the cell membrane
- Is divided into rough endoplasmic reticulum and smooth endoplasmic reticulum, which perform different task within the cell

FUN FACT

The rough endoplasmic reticulum is given its name due to the appearance of a "rough surface". This is due to the attachment of ribosomes. The SER that does not attach ribosomes and appears smooth, thereby the name smooth endoplasmic reticulum.

I. Rough endoplasmic reticulum and Smooth endoplasmic reticulum

Type of ER	RER	SER
Content	Flat cisternae SRP receptors and translocons Ribophorins (ribosome-attaching proteins) Ribosomes	Interconnected tubules (Tubular cisternae)
Main functions	Protein synthesis[1] Initial folding of proteins	Lipid synthesis Glycogen metabolism (mainly in liver cells) Detoxification of drugs and alcohol (xenobiotics) Storage of Ca^{2+}

[1]See section 1.4.0 for how the RER participates in protein synthesis

1.4.3 – Golgi Apparatus

I. Main function

- Completes posttranslational modifications of proteins synthesized in the ER; glycosylation, phosphorylation, and sulfation.
- Packs proteins into vesicles and transports to the right destination
- Membrane remodeling – Vesicle membrane is remodeled from ER type to cell membrane type

II. Importance of the Golgi apparatus

- Golgi apparatus is an important part of
    1. Renewal of the cell membrane and production of glycocalyx
    2. Secretion out of the cell
    3. Hydrolase vesicles production (important for formation of lysosomes)

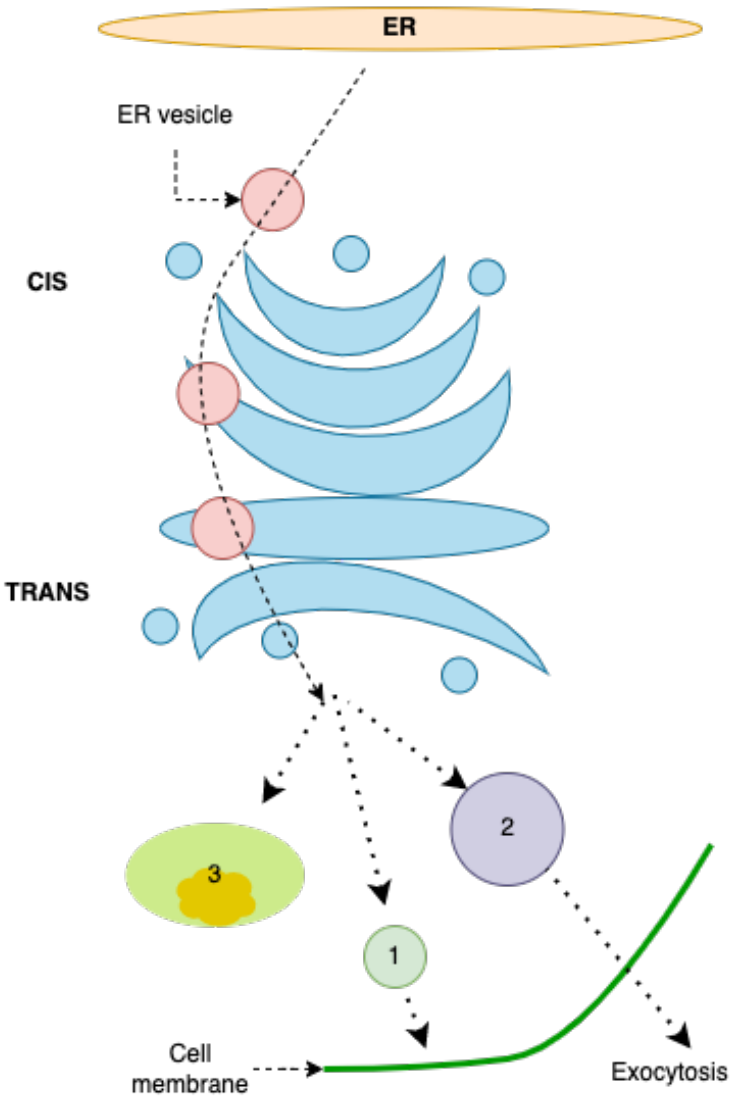
III. Structure

- Composed of smooth membranous small sacs containing enzymes
- Divided into 2 sides, structurally and functionally

CIS – receiving region	TRANS – shipping region
- Convex - 5 nm membrane - Membrane proteins like the ER	- Concave - 7.5 nm membrane - Membrane proteins similar to cell membrane- - Numerous sugar residues bound to the inner aspect of the membrane

IV. Mechanism of action

- Continuous transport vesicles from the ER through the Golgi apparatus
- Vesicles from ER are transported through the apparatus from CIS to TRANS, while undergoing chemical modifications.
- The vesicle membrane is remodeled from ER-type to cell membrane type
- Three types of vesicles are released from the trans region



Transport vesicles are budded of from the ER

The vesicle fuses with the CGN.
Here mistransported proteins can be returned

Vesicles from CGN fuses with the cis region

During transport from cis to trans the content of the vesicle and the membrane proteins are modified. Mainly by glycosylation, but also phosphorylation and sulphatation

Vesicles moves out from the trans region into TGN where the cargo is sorted into larger vesicles and headed for their destinations.

3 types of vesicles are released:
1. Small vesicles containing new materials for the cell membrane
2. Larger vesicles with secretory material for exocytosis – to be released out of the cells
3. Hydrolase vesicles containing digestive enzymes (precursor of lysosomes)[1]

1.4.4 – Lysosomes

- Intracellular digestion of endogenous and exogenous substances

I. Formation of hydrolase vesicles

- Hydrolase vesicles are formed through various mechanisms in different organelles

1	**RER**	Production of lysosomal enzymes
2	**RER and Golgi**	Glycosylation of enzymes
3	**Golgi**	Phosphorylation of mannose residues Mannose-6-phosphate (Man-6-P) is a marker of lysosomal enzymes
4	**Trans Golgi Network (TGN)**	Receptors recognizes and binds to Man-6-P
5		Forms clathrin coated vesicles with the lysosomal enzymes inside

II. Formation of lysosome

- Lysosomes are formed by fusion of hydrolase vesicles, which contain digestive enzymes, with vesicles containing substances that should be digested
- These vesicles are either
    1. Phagosome or late endosomes - products formed in endocytosis
    2. Autophagosomes – intracellular organelles surrounded by ER derived membrane

Heterolysosome	Hydrolase vesicle + late endosome/phagosome	Digest exogenous substances
Autolysosome	Hydrolase vesicle + autophagosome	Digest endogenous substances – e.g. damaged organelles

- In special cells the hydrolase vesicles can fuse with the cell membrane, releasing lysosomal enzymes into to extracellular space for extracellular digestion

III. Mechanism of action

- Lysosomal enzymes inactive in hydrolase vesicles while they are bound to Man-6-P receptors
- When hydrolase vesicles fuses with phagosomes, late endosomes or autophagosomes proton pumps in the membrane are activated and acidify the content. In this acidic environment the enzymes detach from the receptors and become active. This acidic environment is optimal for enzyme activity.
- All types of compound substances can be digested by the lysosomal enzymes (acid hydrolases). Remnants of digestion are either
    1. Simple substances that are released through the membrane and recycled in the cytosol
    2. Indigestible materials that are contained in a small vacuole called residual body
    3. Receptors of Man-6-P that is transported back to TGN via vesicular transport

CLINICAL CORRELATION

Lysosomal storage disease

Lysosomal storge disease is when there are defects in one or more of the digestive enzymes in lysosomes. The cells that need these particular enzymes to break down substrate are no longer able to. This leads to accumulation of large residual bodies with indigestible substrates. Eventually this accumulation may interfere with normal cell or tissue function. This can present with various symptoms of disease, depending on what organs and structures that are affected.

1.4.5 – Proteasomes

I. Main function

- Digestion of cytosolic proteins – this is not done by lysosomes
    1. They degrade denatured or nonfunctional polypeptides
    2. Remove proteins no longer needed by the cell – helpful for restricting protein activity

II. Structure

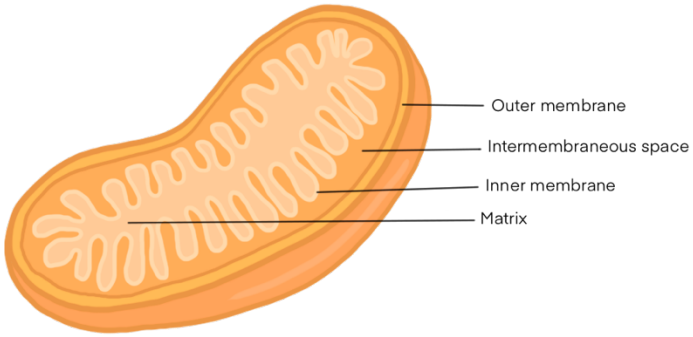
- Small protein complexes abundant in the cell. Cylindrical structure made of 4 rings composed of 7 proteins, at each end the is a regulatory particle that recognizes proteins with ubiquitin
- No membrane

III. Mechanism of action

- Proteins meant for destruction are marked by attachment of ubiquitin
- Proteasome recognizes the ubiquitinated protein
- The protein is then unfolded with the use of ATP
- The protein is moved into the cylindrical shape of the proteasome and degraded into short peptides

- The short peptides are transferred into cytosol where they can be used as they are, or further broken down into amino acids. Ubiquitin is recycled.

1.4.6 – Mitochondria



I. Shape and structure

- Usually rod-shaped, between 1-3 µm
- Mitochondrial membrane is unique, and is not able to fuse with other membranes inside the cell
- Consists of 4 compartments:

The outer membrane	The intermembrane space	The inner membrane	The matrix
Protein/lipid ratio 1:1 Porins with nonselective permeability Translocons for transport of proteins	Nucleotide kinases (e.g. ADP synthesis) Contact sites for translocon coupling)	Folded to create infoldings called *cristae* Protein/lipid ratio 4:1 Specific lipid- cardiolipin Multiple transport proteins Translocons for transport of proteins Electron transport chain ATP synthase complexes	Genetic apparatus Enzymes that participate in the Krebs cycle and fatty acid β-oxidation Dense bodies - Deposits of calcium phosphate

II. Biogenesis

- New mitochondria are created by growth and division of preexisting mitochondria, they cannot be formed de novo.
- Mitochondria can grow by incorporation of lipids and proteins from internal or external sources

Lipids	Can be incorporated to the mitochondrial membrane through lipid-carrier proteins
Proteins	Can incorporate proteins produced on their own ribosomes
	Can incorporate proteins produced on cytoplasmic ribosomes post translationally These proteins have signal sequences that is recognized by translocons in the mitochondrial membrane

III. Main function

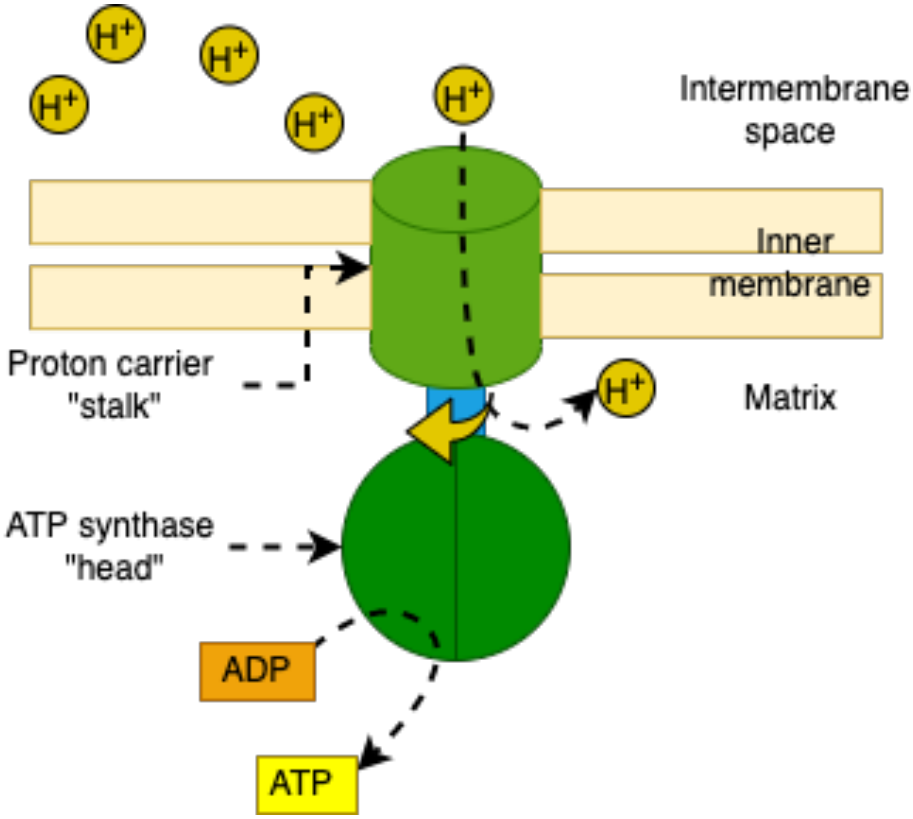
- Main function of the mitochondria is production of ATP through a process combining the Krebs cycle, electron transport chain and ATP synthase complexes
- Other functions of mitochondria:
    1. Beta-oxidation of short- and medium-chain fatty acids
    2. Participation in synthesis of steroid hormones
    3. Storage of calcium ions
    4. Generation of heat (in brown adipose tissue cells)
    5. Involvement in apoptosis

IV. Electron transport chain

- Three main components of the electron transport chain, NADH dehydrogenase complex, Cytochrome b-c_1 complex and cytochrome oxidase complex create a proton concentration gradient.
- They pump protons from the matrix to the intermembrane space through the inner membrane, which is impermeable to protons (H^+), thereby creating a higher proton concentration in the intermembrane space than in the matrix.
- ATP synthase complex uses this gradient to produce ATP
- This proton gradient is also used for other energy-dependent process in the mitochondria
    1. Active transport of molecules
    2. Incorporation of proteins

V. ATP synthase complex

- ATP synthase complex is composed of membrane associated proteins forming
  1. “Stalk” - proton carrier
  2. “Head” - ATP synthase



- Through the complex there is a channel that allows protons to flow down the electrochemical gradient. This flow causes spinning of the stalk against the immobile head.
- This converts the energy of the proton flow into mechanical energy. This energy is stored in the phosphate bond of ATP by binding ADP with inorganic phosphate (Pi).
- A continuous steady flow of protons makes it possible to produce more than 100 molecules of ATP per second.

VI. Genetic apparatus

- Mitochondria are partly autonomous (semiautonomous)
- Possess a complete genetic apparatus with
  1. DNA
  2. mRNA, tRNA
  3. Ribosomes
  4. Enzymes necessary for replication, transcription and translation
- DNA does not encode for all mitochondrial proteins, most are encoded by DNA in the nucleus and produced on cytosolic ribosome before they are post translationally incorporated into the mitochondria.

1.4.7 – Peroxisomes

- Called peroxisomes due to their production and break down of hydrogen peroxide
- Organelle with a single membrane, 0.1 - 1.0 µm in diameter

I. Functions

- Detoxification: oxidizes substrates to create hydrogen peroxide
- Breakdown of hydrogen peroxide by catalase
- β-oxidation of long-chain fatty acids
- Participation in synthesis of some lipids, (cholesterol, plasmalogens) and bile acids
- Participation in degradation of purines

II. Enzymes

- The enzymes inside the peroxisome is synthesized on free cytosolic polyribosomes with a specific signal sequence that allows them to be incorporated into the peroxisome.
- Peroxisomal oxidases (generate hydrogen peroxide as byproduct)
- Peroxidases catalase (catalyses decomposition of hydrogen peroxide)
- Fatty acid β-oxidation enzymes
- Lipid-synthesizing enzymes
- Aminotransferases

III. Biogenesis

- Peroxisomes are created in one of two ways, either:
- By small vesicles containing some peroxisomal membrane proteins (preperoxisomal vesicles) budding of the ER membrane, and then incorporation of other peroxisomal membrane proteins and enzymes
- Growth and division of preexisting peroxisomes

1.4.8 – Inclusions

- Cytoplasmic structures with little or no metabolic activity
- Contain accumulations of metabolites, or other structures with no membrane
- Most inclusions are not a permanent part of the cell, but just present for periods of time in the cytoplasm

I. Important inclusions

Fat droplets	Accumulation of lipid molecules
Glycogen granules	Carbohydrate polymers which stores glucose
Lipofuscin	Residual bodies after lysosomal digestion in long-lived cells
Hemosiderin	Denaturated ferritin with atoms of bound iron – result of phagocytosis of red blood cells

1.5 – The Cytoskeleton

I. Introduction

- The cytoskeleton is an complex collection of protein polymers called microtubules, microfilaments (actin filaments), and intermediate filaments.
- This collection determine the shape and movement of cells, and is important in the movements of organelles and cellular vesicles.

Filament type	Diameter	Protein	Function
Microtubules	25nm	Tubulin	Movement Support
Microfilaments	6 nm	Actin	Movement Support
Intermediate filaments	10nm	Various proteins	Support

- Motility along the cytoskeleton is controlled by motor proteins (enzymes) that uses energy from ATP to use the cytoskeleton filaments are railings. Any structure attached to these motor enzymes will be transported along the filament.

I. Microtubules

- Fine tubular structures built of tubulin. Each microtubule is hollow.
- Microtubules are rigid and help to maintain cell shape. But they are also dynamic structures, capable of both elongating and shortening.
- Microtubules are polarized with a plus and a minus end. Growth of the microtubule occurs faster at the plus end.
- Microtubules can organize into larger structures called axonemes which are the building blocks in cilia and centrioles. In these structures two or three microtubules fuse together to create doublets (2) or triplets (3) that together creates the skeleton.

Cilia	9 doublets of fused microtubules create the peripheral perimeter The center is of 2 single microtubules
Centriole	9 triplets of fused microtubules create the centriole Multiple centrioles are called centrosome

- Two kinds of motor enzymes walks along the microtubules: Kinesin & Dynein
    1. Kinesin – transports structures towards the plus end
    2. Dynein – transports structures towards the minus end
- This cooperation between microtubules and kinesin and dynein is responsible for movement of vesicles, organelles and macromolecular complexes within the cell. As well as movement of chromosomes during cell division.

I. Microfilaments

- Thin polarized polymers built of G-actin monomers that assemble into a double stranded F-Actin.
- More flexible than microtubules
- Also dynamic, as the microtubules. Monomers of G-actin is added at the plus end and removed at the minus end.
- Microfilaments are usually abundant near the cell membrane and cellular extensions
- Movement along the microfilaments is with *myosin motors*, usually towards the plus end. As actin filaments are connected to the cell membrane the cooperation with myosin makes them responsible for cell motility, formation and withdrawal of cytoplasmic processes and contractility of the cell.
    1. Myosin 1 transports vesicles and granules towards to cellular membrane
    2. Myosin 2 aggregates into stable or unstable filaments. Stable filaments can perform forceful contractions in specialized cells – e.g. those in muscles.

II. Intermediate filaments

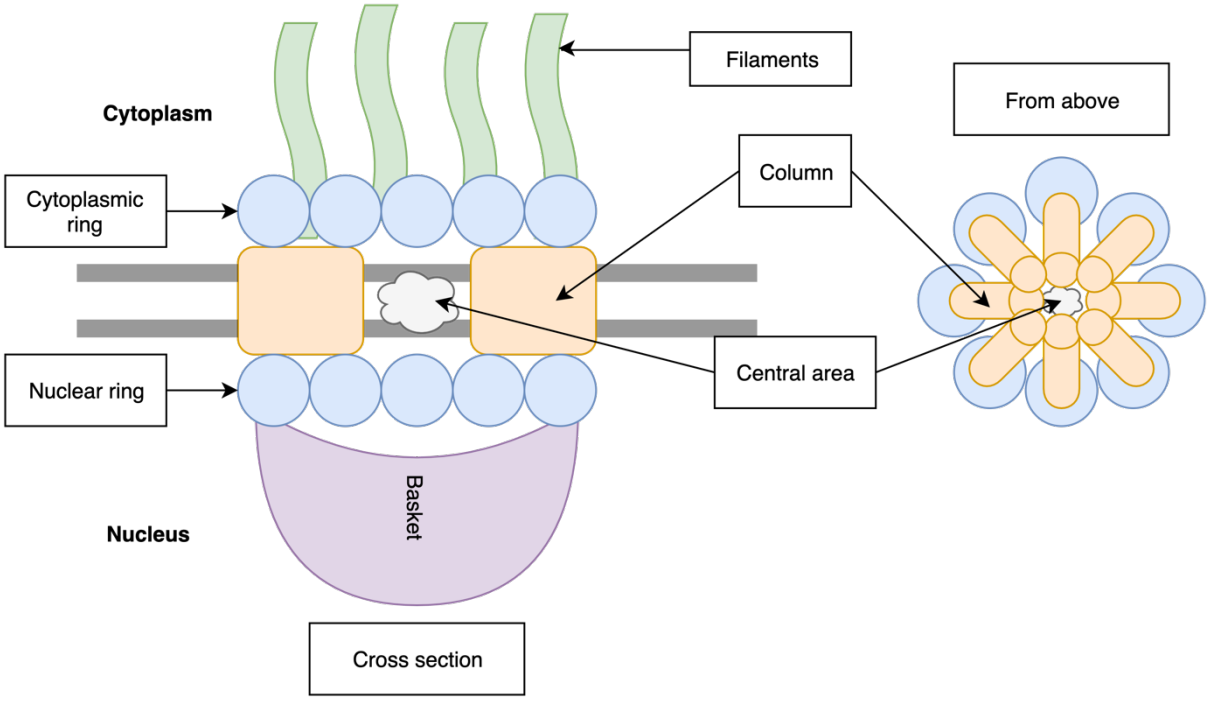
- Built of polypeptide chains
- Resistant and elastic cable like structures, much more stable than the other two
- No polarization
- Does not cooperate with motor enzymes
- Only supports the inner structure of the cell, and participates in cell junctions
- The protein of intermediate filaments is specific to the organ or tissue. Examples of intermediate filaments proteins include:
    1. Keratins – in epithelial cells
    2. Desmin – in muscle cells
    3. Vimentin – in connective tissue cells
    4. Lamins – present in the cell nucleus as a part of the nuclear lamina
    5. Glial fibrillar acidic protein – in neuroglial cells
    6. Neurofilaments – in neurons

Section 2 – The Nucleus

- The nucleus switches on and off genes responsible for synthesis of specific proteins, and through this it controls all cellular processes
- In the nucleolus, ribosomal subunits are produced, and then exported into cytoplasm for assembly into ribosomes.
- Main components
    1. Chromatin
    2. Nucleolus
    3. Nuclear envelope

I. The Nuclear Envelope

- Regarded as a specialized region of ER
- Selectively permeable barrier between the nucleus and the cytosol
- Consists of two membranes, inner and outer membrane, divided by a small perinuclear space.
    1. *Inner membrane* is associated with a meshwork of proteins called nuclear lamina, built of lamins, which stabilizes the envelope
    2. *Outer membrane* is continuous with rough endoplasmic reticulum (RER)
- Nuclear pore complexes: the inner and outer membranes are connected through nuclear pore complexes**.** The pore complex regulates movement of macromolecules between the nucleus and cytoplasm, while ions and small solutes pass through by simple diffusion.

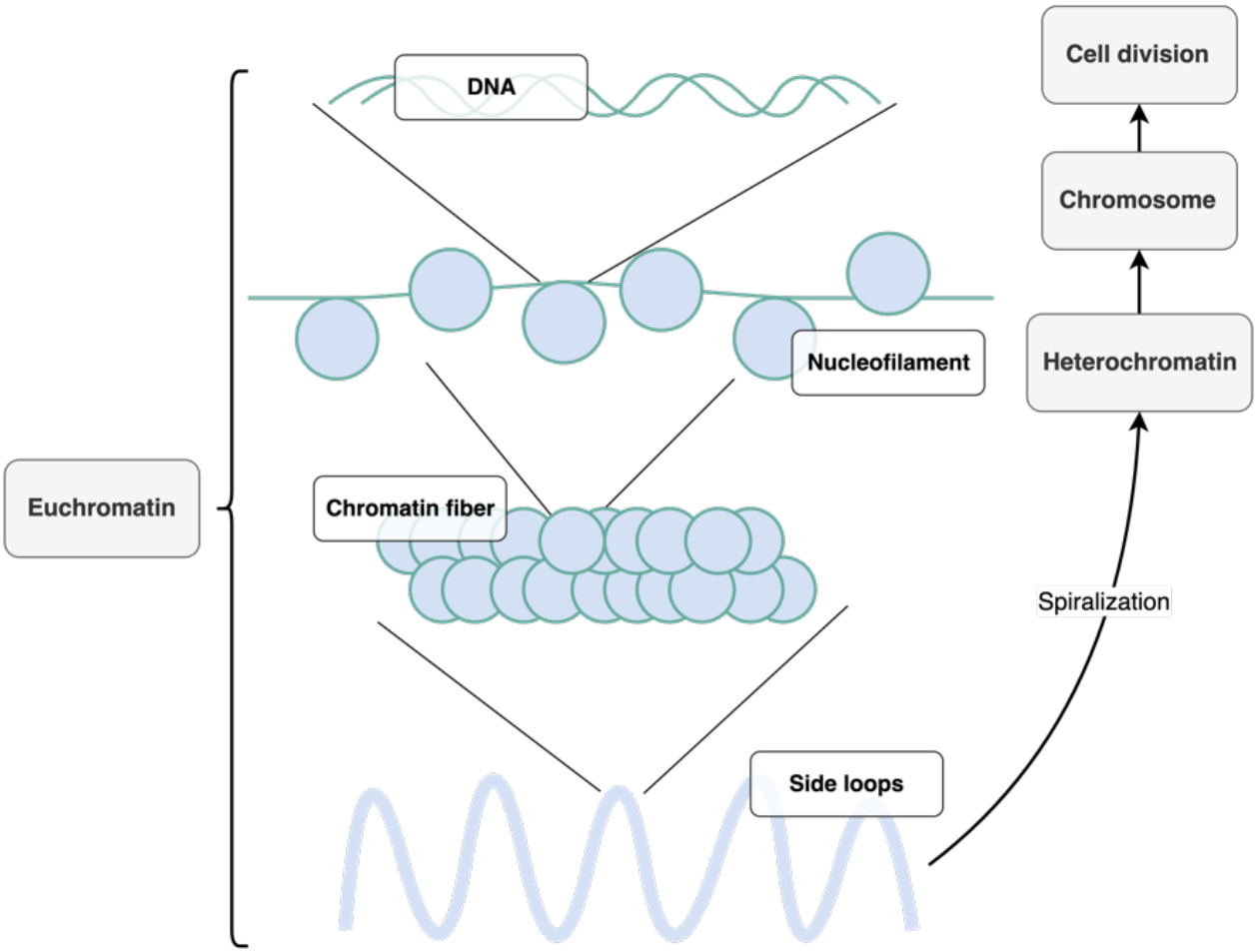


- The movement of large molecules is regulated by receptors:
    1. Molecules are first bound by receptors: cytoplasmic importins (proteins) or nuclear exportins (RNAs and ribosomal subunits)

- Nucleoporin chains transport the molecules complexed with receptors through the nuclear envelope
- Molecules are transported both in and out of the nucleus:
    1. *In*: all nuclear proteins
    2. *Out*: mRNA, tRNA, ribosomal subunits

II. Chromatin

- Composed of
    1. DNA
    2. Histones
    3. Nonhistone proteins
- Two types of chromatin:
    1. *Euchromatin*: pale, loose, transcriptionally *active*
    2. *Heterochromatin*: dark, condensed, transcriptionally *inactive*. Heterochromatin can either be noncoding or permanently inactive (constitutive) or temporarily inactive due to being switched off (facultative)
- Chromatin packaging:



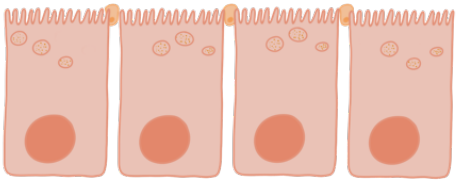
- Chromatin is composed of several subunits called nucleosome.
- Each nucleosome is composed of:
    1. A histone core, an eight protein complex (octamer) of 2x H2A, H2B, H3 and H4
    2. DNA twirled around the octamer – this is a fragment of double helix, consisting 147 base pairs
    3. In between the nucleosomes there is linker DNA fragments connecting neighboring nucleosomes, consisting of 10-90 base pairs and histone H1**.**
       Mark that histone H1 is *not* a part of the octamer.

III. Nucleolus

- In the nucleolus you can find:
    1. Pale fibrillar centers: transcriptionally active rDNA
    2. Dense fibrillar components: freshly transcribed rRNA
    3. Granular components: mainly large ribosomal subunits
- The nucleolus produces ribosomal subunits:
    1. First rDNA is transcribed into pre-rRNA
    2. pre-rRNA is then cut into smaller rRNA pieces
    3. rRNAs are connected with ribosomal proteins imported from cytoplasm
    4. Newly formed small and large ribosomal subunits are then transported into the cytosol

Section 3 – Epithelial Tissue

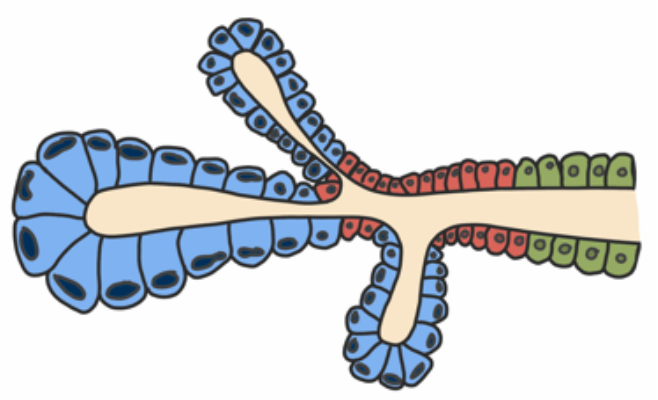
3.1 – Types of Epithelial Tissue



- Linings: covering the outer and inner surfaces of the organism
- Glands: epithelial cells with secretory functions
- It is important to note that epithelia does not contain blood vessels. Blood vessels are located in the layers underneath.

I. Main functions of the epithelia

- Depending on the function, different epithelia is found at different sites. The most important functions the epithelia has is
    1. Protection – e.g. epithelia that lines cavities or the outermost layer of the skin
    2. Absorption – e.g. in organs or structures that needs to absorb nutrients like intestinal epithelia
    3. Secretion – epithelia in glands



    4. Control of transepithelial transport – e.g. epithelia lining blood vessels
    5. Sensation – taste buds and sensory cells of the inner ear
- Epithelia can contain different cell types that perform different function

3.2 – Types of Epithelia

3.2.1 – Classification of epithelia

I. Name of the epithelia include both number of cells and shape of cells.

- In stratified epithelia, the shape of the cells in the outer layer defines the name

Number of cells	Shape of cells
Simple Stratified	Squamous Cuboidal Columnar

II. Different types of epithelia

Name of epithelia	Main function	Examples of localization
Simple Squamous epithelium	Controls transport	Vessels Peritoneum and pleura Pulmonary alveoli Bowman's capsule of renal corpuscle
Simple cuboidal epithelium	Resorption Secretion	Renal tubules Glands and their ducts Lens epithelium Ovary-covering epithelium
Simple columnar epithelium	Resorption Secretion Protection Transport control	Gastrointestinal tract Bile ducts Female genital tract Larger excretory ducts of glands

STUDY TIP

When learning about the types of epithelia, take note of the functions they provide and the examples of where they are located. This can make it easier to remember the types of epithelia that is present in different organs and structures.

For example**:** Simple columnar epithelium can resorb, secrete, protect, and control transport. These are all essential functions in the gastrointestinal (alimentary) tract, so it makes sense that this is the type of epithelia present in the GIT and not simple squamous which only controls transport.

Name of epithelia	Main function	Examples of location	Description
Pseudostratified epithelium	Protection Secretion Sensation	Respiratory tract Ductus epididymis Taste buds	A variant of simple columnar Cells are of different height, with nuclei at different levels. Cell may be of different types The base of the cells always adhere to the basal lamina
Stratified squamous epithelium	Protection	<u>Keratinized</u>: Epidermis <u>Nonkeratinized</u>: Gastrointestinal tract Reproductive tract Cornea	Keratinized: Cells of the upper layers are transformed into thin, hard plates when dying. Losing all internal structures. Nonkeratinized: All cells are still alive and keep all internal structures
Transitional epithelium (urothelium)	Protection	Urinary tract	Flexible, impermeable barrier Dome shaped cells in upper layer, sometimes with two nuclei Cell junctions Apical cell membrane has uroplakin: plaques of protein connected to actin filaments.
Stratified cuboidal	Transport control	Can be found in excretory ducts of sweat glands	Rare
Stratified columnar or cuboidal	Protection Secretion	Conjunctiva Excretory ducts of large glands Male urethra Regions of transition between two types of epithelia – stratified squamous and simple cuboidal: epiglottis and anus	

3.3 – Characteristic Features of Epithelial Cells

- The different surfaces of the epithelial cell have characteristic features important for the function of the epithelium

Apical surface	Lateral surfaces	Basal surface
- Microvilli - Cilia - Transport proteins	- Cell junctions that connects the cell to neighboring cells - intercellular canaliculi	- Junctions between the cell and the extracellular matrix - Transport proteins - Basal cell membrane infoldings

3.4 – The Apical Cell Surface

- Different structures (microvilli, cilia, stereocilia) can be found on the apical surface of epithelial cell. Their main function is either increasing surface area of the epithelium to increase absorption (e.g. gastrointestinal tract), or to move substances along the epithelium (e.g. respiratory tract)

3.4.1 – Microvilli

Main locations	Structure	Function
On cells specialized for absorption i.e. the small intestine	1 µm long and 0.1 µm wide Each villus is formed by bundles of actin filaments bound together by actin binding proteins: fimbrin & fascin. The actin filaments are anchored in the terminal web inside the epithelial cells Microvilli are often relatively stable, but through myosin-based movements they can move to some degree and create optimal conditions for resorption.	High in numbers and forms brush borders which increases the absorptive surface area of the cell Can increase the absorptive area up to 20-30 times

CLINICAL CORRELATION

Celiac disease is a disease in where the patient cannot tolerate gluten. It is a disorder of the small intestine, and one of the first pathologic changes is loss of the brush border (microvilli) on the apical surface of the epithelial cells of the small intestine. This is due to an immune reaction during digestion of gluten. This reaction causes inflammation in the bowel, and changes to the epithelia. Loss of the absorptive surface (brushborder) causes malabsorption, which can present in a patient as failure to thrive in children, weight loss, fatigue or anemia. With removal of gluten from the diet the structural damages

3.4.2 – Stereocilia

Main locations	Structure	Function
Epididymis Proximal part of ductus deferens Sensory cells of inner ear	Long and thick microvilli Less motile than microvilli	Facilitates absorption by increasing surface area of the cell

3.4.3 – Cilia

Main locations	Structure	Function
Respiratory tract Male and female reproductive tract	**1. Cilium proper** The part of the cilia that extends past the cell surface. Core consists of an axoneme, 9 doublets of microtubules around 2 single central microtubules. **2. Basal body (similar to centriole)** Consists of 9 triplets of microtubles, no central microtubules **3. Rootlet** – bundle of protein filaments	Active bending of cilia is due to the movement of dynein arms which coordinate the sliding of microtubules along each other. Elastic nexin is responsible for the passive movement back to the original position. Coordinated movement of several cilia forms a wave like transportation of objects in one direction. Ciliated epithelia can then transport - Mucus in the respiratory tract - Oocytes and spermatozoa in the female and male reproductive tracts

CLINICAL CORRELATION

Primary Ciliary Dyskinesia

Primary Ciliary Dyskinesia is a rare autosomal recessive disorder where cilia are absent or less motile. This is due to a defect in the dynein arm of the microtubules. It will present with symptoms like chronic productive cough, recurrent infections of the upper respiratory tract, infertility in men and reduced

3.4.4 – Primary cilia

- A single, immotile cilia present on many different cells
- Axoneme consists of 9 doublets of microtubules, no central microtubule
- Receptors and proteins of the primary cilia participate in regulation of cell cycle and differentiation
- Acts as mechanosensory in some cells, bending elicits response

3.5 – Transport Across Epithelia

3.5.1 – Cell Junctions

- Intercellular junctions are between all cells of all tissues. Particularly in epithelial tissue
- The membranes of adjacent cells are connected via transmembrane linker proteins

I. Types of junctions

Occluding	Anchoring	Communicating
Tight junctions	Adhesion belt (Zonula adherens) Desmosome (Macula Adherens)	Gap junctions

- The cells are connected by more than one kind of junction. Typically tight junctions at the apical part. Zonula adherens right below. Both forming belts around the cell. Gap junction and desmosomes are patches throughout the cell junction. Hemidesmosomes and focal contact junctions attach the base of the cell to extracellular matrix like the basal lamina.

II. Basal Lamina

- The basal lamina mounts the epithelia to underlying tissue. It is the only form of extracellular substance in epithelial tissue.
- Limits the types of molecules passing through the epithelium
- Controls the movement of cells along its plane
- Contains the proteins laminin, collagen IV and entactin and a proteoglycan called perlecan.

III. Basement membrane

- Is found in some epithelia and is the basal lamina + a reticular layer
- The reticular layer is produced by connective tissue and consists of fibrils built of collagen VII, II and fibrillin.

IV. Function and structure of the different junctions

Type of junction	Major transmembrane linker proteins	Cytoskeletal components	Function
Tight junction (Zonula occludens)	Claudin and occludins connects adjacent membranes, while ZO proteins connects them to actin filaments in the cytosol Multiple junctions forms a band around the apical part of the cell	Actin filaments support the junction	Prevents movement between intra- and extracellular environments as well as between adjacent cells. Prevents passage of unregulated substances Maintains polarization of epithelial cells
Adhesion belt (Zonula adherens)	Cadherins, catenin complexes Interact in the presence of Ca^{2+} Cadherins in the intracellular space binds to catenin in the cytosol which is bound to actin filaments. Multiple junctions form the zonula adheres.	Actin filaments Anchoring junctions connect both the cell membranes and cytoskeleton of neighboring cells	Strengthens and stabilizes nearby tight junctions. Provides points of connection between cytoskeleton of adjacent cells.
Desmosome	Cadherin family proteins: desmogleins and desmocollin in the intracellular space attaches to desmosomal plaques (Desmoplakins) in the cytosol that are connected with intermediate filaments.	Intermediate filaments (keratins)	Strengthens the tissue and connects intermediate filaments of adjacent cells
Gap junctions	Connexin	No cytoskeleton involvement	Create a channel (connexon) for small molecules. Each junction has up to hundreds of these connexons and can close if needed. Spread stimuli to adjacent cells Synchronization of developmental and metabolic processes
Hemidesmosome	Integrins	Intermediate filaments	Connects the cell to extracellular matrix Transmembrane integrins binds extracellular laminin and type 4 collagen
Focal contact		Actin filaments	Connects cell to extracellular matrix

CLINICAL CORRELATION

Blistering skin diseases

Pemphigus vulgaris and bullous pemphigoid are examples of autoimmune diseases where antibodies against desmosomes and hemidesmosomes, respectively, are produced. The result is blisters of the skin that can easily rupture (pemphigus vulgaris) or not (bullous pemphigoid).

3.6 – Epithelial glands

3.6.1 – Glands

- Structures with specialized secretory epithelial cells
- The products that are secreted are usually stored in secretory granules within the cells
- Secretory epithelial cells can produce, store and release proteins, lipids and carbohydrate and protein complexes
- Unicellular glands are common in simple cuboidal, simple columnar and pseudostratified epithelia in multiple organs. E.g. in organs that needs mucus for lubrication.
-

3.6.2 – Endocrine Glands

- Secrete hormones directly to extracellular space. There are no ducts in these glands
- Capillaries absorbs the hormone and it is transported in the bloodstream to distant organs

3.6.3 – Exocrine Glands

- Product is secreted via ducts to specific cites
- The gland has secretory portions that each has its own duct

I. Morphologic classification

- Acinar or tubular – refers to the shape of the secretory portion
- Simple branched or unbranched – single duct with branched or unbranched secretory portion
- Compound – branched system of ducts.

II. Location of exocrine glands

- Small exocrine glands can be found e.g. in the wall of respiratory and alimentary tracts
- Large exocrine glands form separate organs
    - Salivary glands
    - Pancreas
    - Liver

3.6.4 – Mode of Secretion

Eccrine (= exocytosis)	Most common method for protein secretion Exocytosis of the material from membrane-bound vesicles
Apocrine	Secretion of lipid droplets Product accumulates at the apical end and forced out with some part of the plasma membrane and cytoplasm
Holocrine	Cells accumulate product, and product is released upon cell disruption together with cell debris. Seen in sebaceous glands

3.7 – Renewal of Epithelial Cells

3.7.1 – Renewal and Regeneration of Epithelial cells

- Epithelia is often renewed and regenerated
- Epithelial stem cells can proliferate and differentiate often to replace nonfunctional epithelial cell

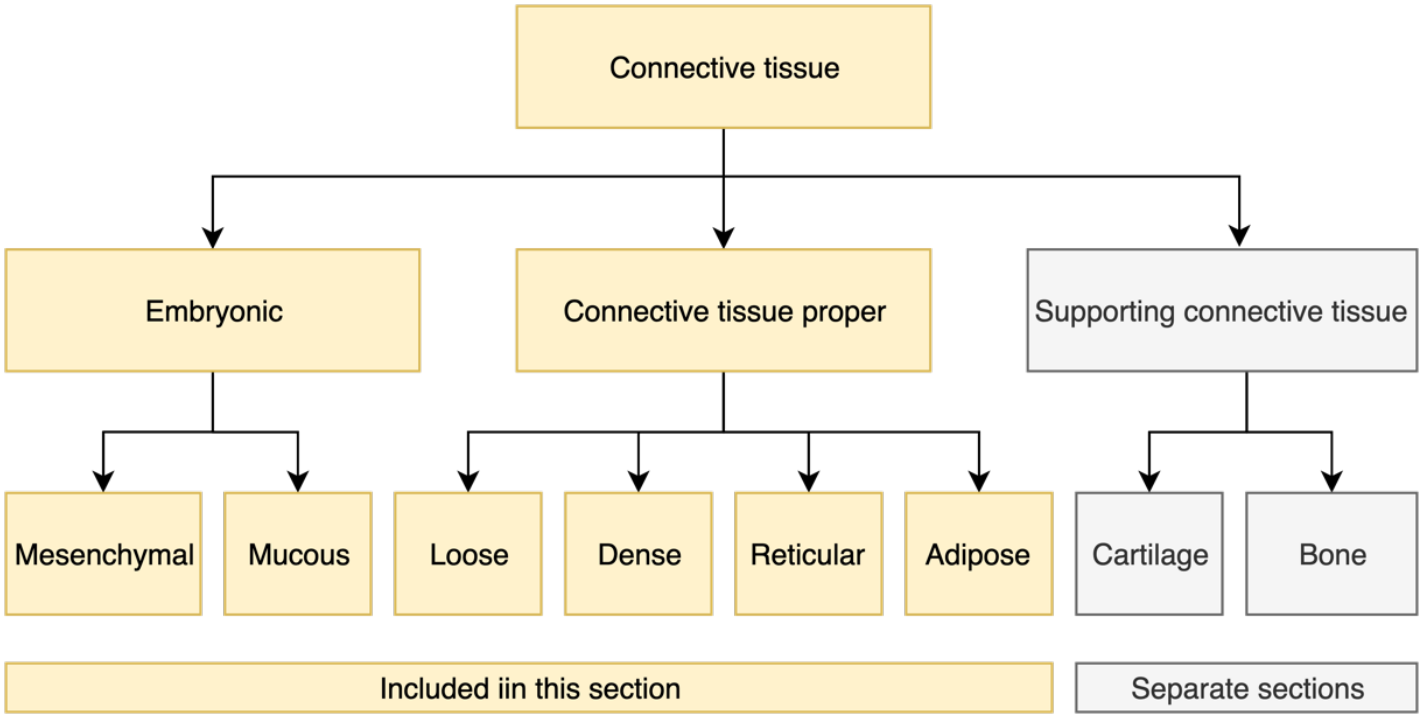
Type of epithelia	Simple epithelia	Stratified epithelia
Mechanism of replacement	Nonfunctional cells die by apoptosis, and the new cells replace them	Cells continuously migrate towards the uppermost layer of the epithelia and there the old cells detach. Just like you can see on the skin.
Location of stem cells	Located on the basal lamina Often called basal cells	Most stem cells are in the basal layer of cells

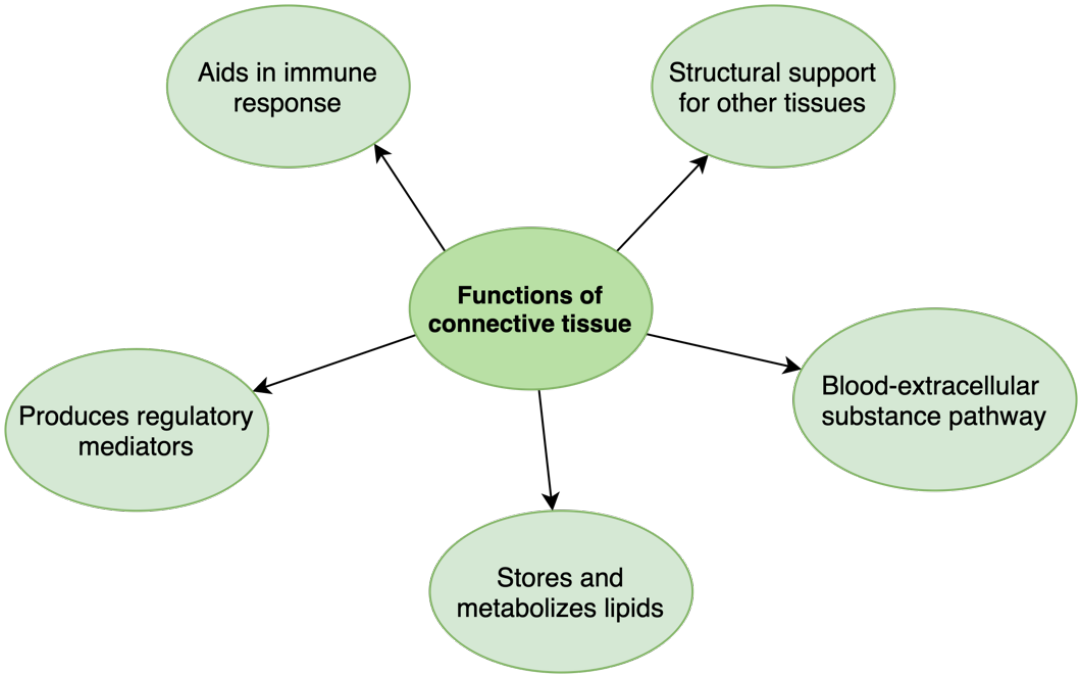
- Sometimes the location of stem cells is in a stem cell niche in the epithelial lining or gland

Section 4 – Connective Tissue

4.1 – Extracellular Substance
4.2 – Cells of Connective Tissue
4.3 – Embryonic Connective Tissue
4.4 – Connective Tissue Proper

- Major difference from other tissues: contains much more extracellular substance than cells
- Main constituents: extracellular substance, fibers and some cells





4.1 – Extracellular Substance

I. Ground substance (matrix)

- Well hydrated, transparent mix of three main kinds of molecules
    1. Glycosaminoglycans (GAGs) = mucopolysaccharides
    2. Proteoglycans
    3. Adhesive glycoproteins
- Acts as a barrier to invaders
- Lubricant in various tissues
- Light microscope (LM): Amorphous, meaning “without shape”
- Electron microscope (EM): electron-dense filaments/granules

Component	Types	Functions
GAGs	Hyaluronic acid	Large and works alone: Lubrication, allows for diffusion
	- Dermatan sulfate - Chondroitin sulfates - Keratan sulfate - Heparan sulfate	- Smaller polyanions bound to proteins as parts of proteoglycans - Binds water and cations - Space filling, cushioning, lubrication
Proteoglycans	- Perlecan - Aggrecan - Decorin	- Protein core serving as a binding site for the smaller GAGs - Perlecan is the main proteoglycan in all basal laminae
Glycoproteins	- Fibronectin - Laminin	- Adhesive molecules with binding sites for integrins and collagen - Provide temporary connections between cells and extracellular matrix needed for cell migration

CLINICAL CORRELATION

Hurler, Hunter, Sanfilippo and Morquio syndromes
Some people lack the specific enzymes required to break down the proteoglycans in connective tissue. The result is accumulation of these substances in lysosomes

Types of proteoglycans

Proteoglycan	Core protein molecular weight	Type of GAG chains	Number of GAG chains	Location	Function
Aggrecan	210 000	Chondroitin and keratan sulphate	100	Cartilage	Form large aggregates with hyaluronic acid
Beta-glycan	36 000	Chondroitin and dermatan sulphate	1	Cell-matrix	Signaling: binds TGF-beta
Decorin	40 000			Connective tissue	Signaling: connects collagen fibrils and TGF-beta
Perlecan	600 000	Heparan sulphate	2-15	Basal laminae	Structural support, filtration
Syndecan	32 000	Chondroitin and heparan sulphate	1-3	Cell surfaces	Cell adhesion, signaling: binds FGF

- The proteoglycan with the most GAG chains bound to it is aggrecan, but the heaviest core protein belongs to perlecan
- Aggrecan and perlecan both have heavy core proteins and are found in supportive structures: cartilage and basal laminae, respectively
- The proteoglycans involved in signaling, beta-glycan, decorin and syndecan, share several features: few GAG chains, core proteins of similar size and the GAG chain chondroitin sulphate.

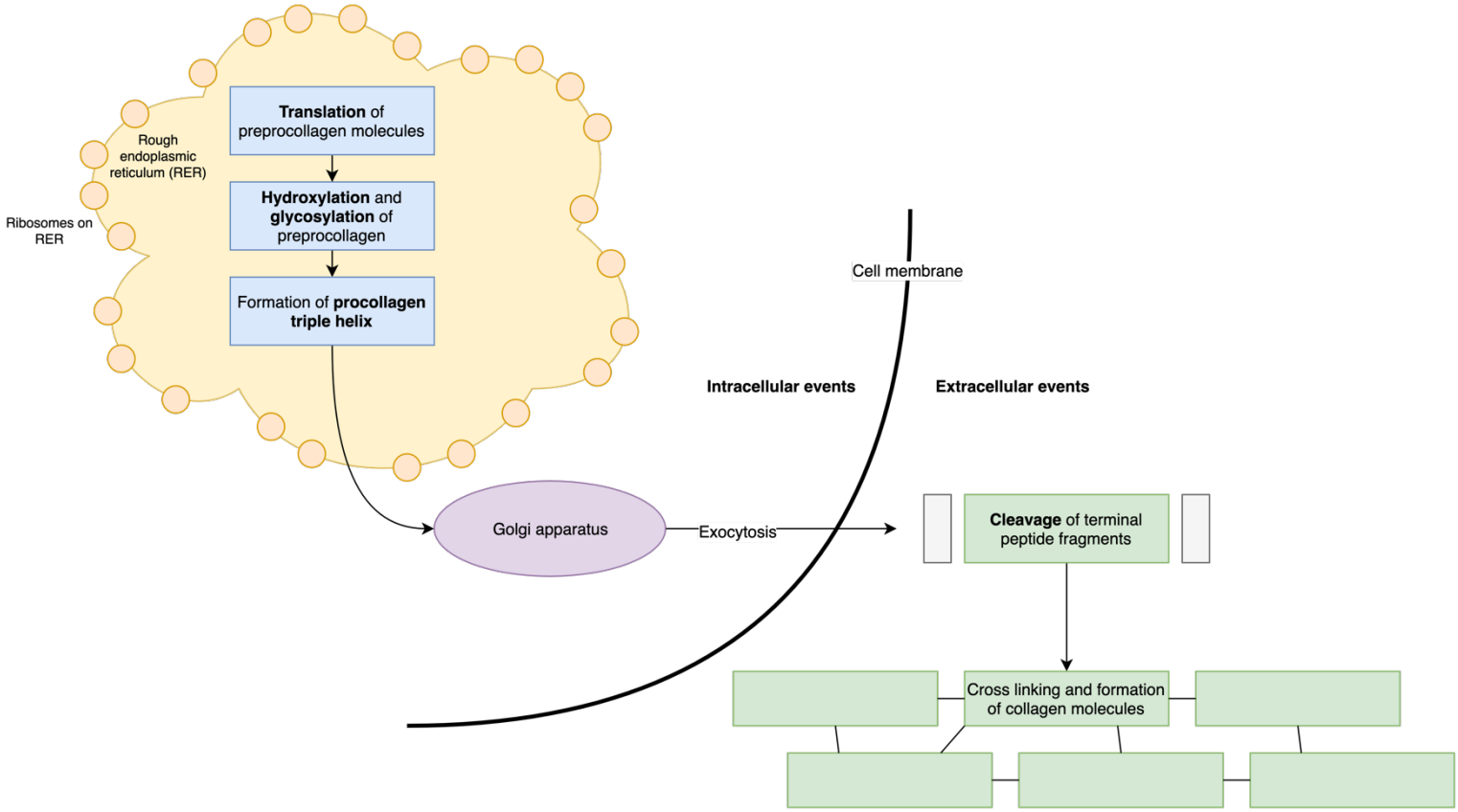
II. Fibers

Fiber	Microscope appearance	Components	Organization	Functional properties
Collagen	LM: Acidophilic EM: Thick striated fibrils, pattern caused by regular arrangement of collagen molecules in the fibrils	See table with collagen types	Form bundles	Resistant to stretch and disruption
Reticular	LM: Stain with silver salts EM: Striated fibrils Thinner than collagen	Collagen type 3	Network with small meshes	Scaffolding for cells and cell groups
Elastic	Require special stains to be seen on LM Thinner than collagen, similar size as reticular fibers	Elastin core and peripheral fibrils built of fibrillin (microfibrillin)	Form networks or fuse into flat sheets called laminae	Very expandable

Types of collagen

Type	Morphology	Main function	Location	Mnemonics
1	Coarse striated collagen fibers	Resistance to tension	Widespread: bone, skin, ligaments and tendons, cornea, dentin	Most important collagen types are type 1, 2, 3 and 4 B**one** and tend**one**, car**two**lage, ar**three**ies (arteries) and type four on the **floor** or **SCAB** 1: **S**kin 2: **C**artilage 3: **A**rteries 4: **B**asal laminae
2	Thin, striated collagen fibers	Resistance to pressure	Cartilage, vitreous body, intervertebral discs	
3	Thin, striated reticular fibers	Structural maintenance in stretchy organs	Blood vessels, skin	
4	Fibrillar networks	Support epithelial cells, filtration	Basal laminae, lens capsule	
5	Thin fibrils	Cooperate with type 1 collagen	Basal laminae in some organs	
7	Thin, short fibrils	Anchoring of basal laminae to underlying reticular laminae	Epithelial basement membranes	
9	Single molecules	Binds proteoglycans, associated with type 2 collagen	Cartilage, vitreous body	

4.1.1 – Assembly of Collagen Fibers



4.1.2 – Mechanical Properties of Elastic Fibers

- Specific features of the elastin molecule allows the elastic properties of elastic fibers:
    1. Coiled elastin molecules are interconnected side-by-side by numerous desmosome cross-links
    2. Under stretch, elastin molecules straighten and integrity of the fiber is maintained by the cross-links
    3. After cessation of stretch elastin molecules coil again

4.2 – Cells of Connective Tissue

Cell type	Origin	Appearance	Function	Secretory products
Fibroblasts	Mesenchymal stem cells	Thin, elongated or multiform Poorly developed organelles	Produce components of the extracellular substance Fibroblast = Active form Fibrocyte = Quiescent form	Collagens Elastin and fibrillin GAGs Proteoglycans Glycoproteins Metalloproteinases
Adipocytes		Large cells with lipid droplets taking up most of the space	Metabolize lipids and cushion organs	Lipid precursors Hormones
Macrophages	Monocytes	Multiform and mobile Numerous lysosomes Marker: acid phosphatase	Make up the mononuclear phagocyte system	Cytokines Antibacterial agents
Plasma cells	B-cells	Clock-face nucleus Basophilic cytoplasm Extensive RER Golgi	Mediate adaptive humoral immune response	Immunoglobulins
Mast cells	Bone marrow	Round or ovoidal Coarse basophilic granules Golgi Scarce microvilli	Main effector cells in allergic reactions	Proinflammatory mediators: Histamine leukotrienes cytokines (and more)
Telocytes		Very long and very thin processes with local dilatations Are interconnected via their processes forming a network	Located in interstitial connective tissue connected to other cells. A signaling network releasing signaling molecules by shedding microvesicles and exosomes Coordinate functions of interstitial connective tissue cells	Signaling molecules
Hematopoietic stem cells		Resemble large lymphocytes	Located in the bone marrow where they are the precursor cells to white and red blood cells	Mostly a target for growth factor secreted by other cells

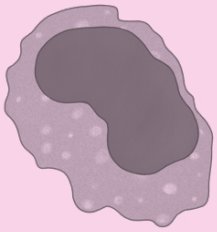
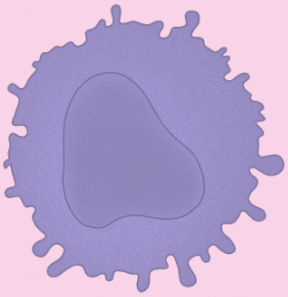
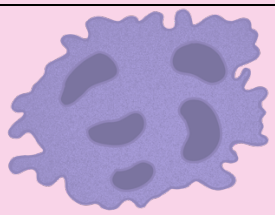
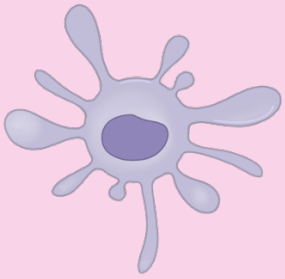
4.2.1 – Mononuclear Phagocyte System

- Macrophages make up the mononuclear phagocyte system along with other monocyte-derived cells.
- Long lived cells that survive in the tissues for months, where they eat (phagocytose) debris and pathogens, as well as present antigens to immune cells.
- Phagocytosis can be non-specific or specific (receptor-mediated)

I. Macrophages

- Secretory products:
    1. Cytokines (interleukins, interferon, TGF, TNF)
    2. Factors controlling proliferation of blood precursors in bone marrow
    3. Antibacterial agents: free radicals, lysozyme
- Functional states of macrophages: resident, elicited (free) and activated
- Two functional types of macrophages: proinflammatory (M1) and anti-inflammatory (M2)

The mononuclear phagocyte system: the macrophage family

Cell type		Location	Function
	**Monocyte**	Blood	Macrophage precursor
	**Macrophage**	Connective tissue, lymphatic organs, lungs, bone marrow, pleural/peritoneal cavities	Production of cytokines, chemotactic factors, and other molecules needed for inflammation Antigen processing and presentation
	Kupffer cells	Liver	
	Microglial cells	CNS	
	Osteoclasts	Bone	Digestion of bone matrix
	**Multinuclear giant cells** (macrophages fused together)	Connective tissue during pathologic processes	Segregating and digestion of foreign bodies
	**Langerhans cells**	Epidermis of skin	Antigen processing and presentation
	Dendritic cells	Lymph nodes Spleen	

4.2.2 – Mast Cells

- Located near blood vessels

I. Main secretory products of mast cells

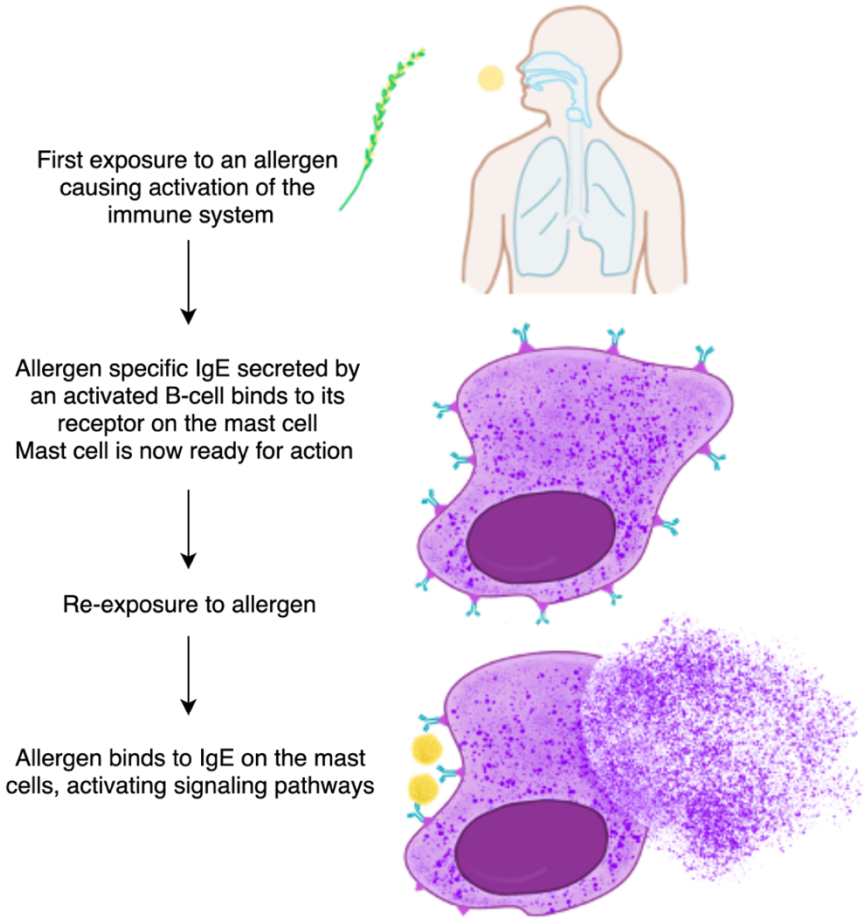
- *Stored in granules*: histamine, heparin, proteases (chymase and tryptase), chondroitin sulfate, chemotactic factors attracting eosinophils and neutrophils
- *Synthesized and/or released after activation*: leukotrienes, cytokines/leukotrienes, platelet-activating factor, free radicals

II. Two types of mast cells

- Connective tissue mast cells: contain both proteases (chymase and tryptase)
- Mucosal mast cells: in the walls of gastrointestinal, respiratory and other tracts.
    1. Smaller and contain only tryptase, not heparin.

III. Effects of mast cell activity:

- Local inflammation and cell damage
- Cytokine secretion
- Leukocyte infiltration
- Vasodilation and increased blood vessel permeability: causes the classic redness and swelling inflamed areas
- Smooth muscle contraction: bronchospasm in asthma



4.3 – Embryonic Connective Tissue

I. Mesenchymal Connective Tissue

- Mostly ground substance, almost no fibers or fibrils
- Located in embryonic tissues
- Mesenchymal *tissue*: only found in embryonic and fetal tissues
- Mesenchymal *stem cells:* remain in various regions of the body throughout life
    1. Differentiate into fibroblasts, chondroblasts, osteoblasts, adipocytes and endothelial cells
    2. Can be collected from different sources: bone marrow, peripheral blood, adipose tissue (liposuction), skin and directly after birth from umbilical cord, placenta and amnion.

II. Mucous (Gelatinous) Connective Tissue

- Primitive fibroblasts
- Mostly ground substance
- Very few, delicate collagen fibrils
- Location: umbilical cord and tooth pulp

4.4 – Connective Tissue Proper

I. Loose connective tissue

- Consists of connective tissue cells and leukocytes with fibers and ground substance in equal amounts
- Forms scaffolding in all organs and surrounds larger blood vessels and nerve bundles inside the organs

II. Dense connective tissue

- Mainly quiescent fibroblasts, other cell types are very rare
- Much more fibers than ground substance, mostly collagen
- *Irregular* dense connective tissue: dermis, sclera and capsules of organs
- *Regular* dense connective tissue: tendons, ligaments, cornea
- Structure of tendon: (insert illustration)
    1. Quiescent fibroblasts located between the bundles and forming rows
    2. Small amount of elastic fibers (amortization of stretching force)
    3. Narrow septa built of loose connective tissue, containing blood vessels

III. Reticular connective tissue

- Stellate reticular cells: mainly fibroblasts and macrophages
- Forms scaffolding of lymphoid tissue and bone marrow

4.4.1 – Adipose Tissue

- Adipose tissue = large aggregates of adipose cells, which are the fat storing cells in the body
- Adipose cells are also found in small groups or isolated within other types of connective tissue
- Typically 15-25% of body weight in men, slightly more in women
- Difference from other connective tissue proper: Both types of adipose cells are covered by basal lamina with type 4 collagen

CLINICAL CORRELATION

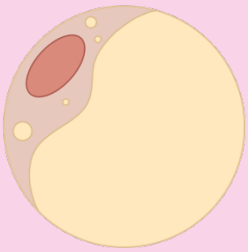
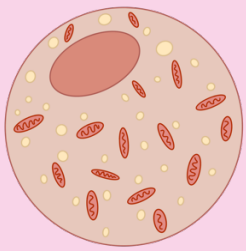
Lipomas

White (unilocular) adipocytes can form lipomas which are common, benign tumors. Malignant liposarcomas can also develop, but much less often.

I. Origin

- White (unilocular) adipocytes: mesenchymal stem cells
- Brown (multilocular) adipocytes: myogenic stem cells
- Beige adipocytes: under specific conditions, mesenchymal stem cells can differentiate into cells similar to multilocular adipocytes

Main features of white vs. brown adipose tissue

 **Yellow adipose tissue**	 **Brown adipose tissue**
Unilocular: one big lipid droplet	Multilocular: many small lipid droplets
Fat storage	Release heat and warm the blood
More common	Most important for neonates
Both cell types are densely packed, surrounded by a small amount of extracellular substance predominately made from reticular fibers and a dense capillary network. Connective tissue separates the cells into lobules	

II. Function of Adipose Tissue

- Storage of lipids, mainly triglycerides from:
    1. Chylomicrons from intestine, triglycerides synthesized in the liver or VLDLs and fatty acids produced by the adipocyte itself
- Metabolism and release of lipids
    1. Lipids are mobilized by hormone sensitive lipase under stimulation by norepinephrine
    2. Lipase facilitates lipid release
    3. Uptake of lipid precursors, synthesis, storage and degradation of lipids
    4. Release of lipid precursors
- Endocrine function
    1. Leptins: released by adipocytes and travels to the hypothalamus to inhibit appetite
    2. Adiponectin: regulation of fatty acid and glucose metabolism
    3. Resistin: regulation of inflammatory processes
- Thermal insulation
- Keep organs in place and protect structures vulnerable to injury

FUN FACT

Endocrine function of adipose tissue

Adipose tissue is considered an endocrine organ due to its important role in homeostasis!

III. Yellow (white) adipose tissue

- Consists of unilocular adipocytes
- Unilocular adipocytes
    1. Large, up to 100 µm
    2. Contains a single lipid droplet that pushes the cytoplasm and organelles to the cell periphery
    3. Lipid droplet – cytoplasm interface is supported by intermediate filaments
- Locations: hypodermis, around intestines and in capsules of some organs

IV. Brown adipose tissue

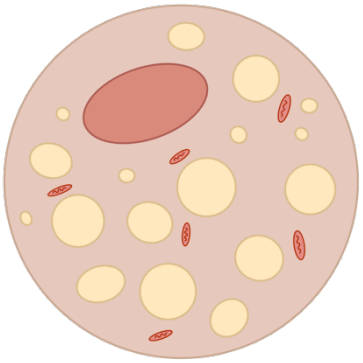
- Consists of multilocular adipocytes
- Multilocular adipocytes
    1. 20-40 µm
    2. Centrally located nucleus
    3. Contain many small lipid droplets
    4. Abundance of mitochondria surrounding the droplets
    5. Highly innervated
- Locations
    1. Neonates: neck, spine, mediastinum, kidneys
        - 5% of neonate body weight
    2. Adults extremely rare: only small cell clusters in the above locations
- Additional function: thermogenesis!
    1. Production of heat: Non-shivering thermogenesis
    2. Uncoupling protein-1 (UCP-1, thermogenin) is unique to the brown adipose tissue because it is the protein that allows the brown adipocytes to metabolize fat for thermogenesis instead of ATP

FUN FACT

Hibernating species
Large amounts of brown adipose tissue allows for heat production during hibernation

V. Beige Adipose Tissue

- Unrelated to age
- Forms small islets in yellow adipose tissue
- Similar brown adipose tissue: contain numerous small lipid droplets and mitochondria with UCP-1, so it produces heat
- Induced by some external factors
    1. Chronic cold exposure
    2. Intense muscular activity – exercise, training
- Reduces the amount of yellow adipose tissue

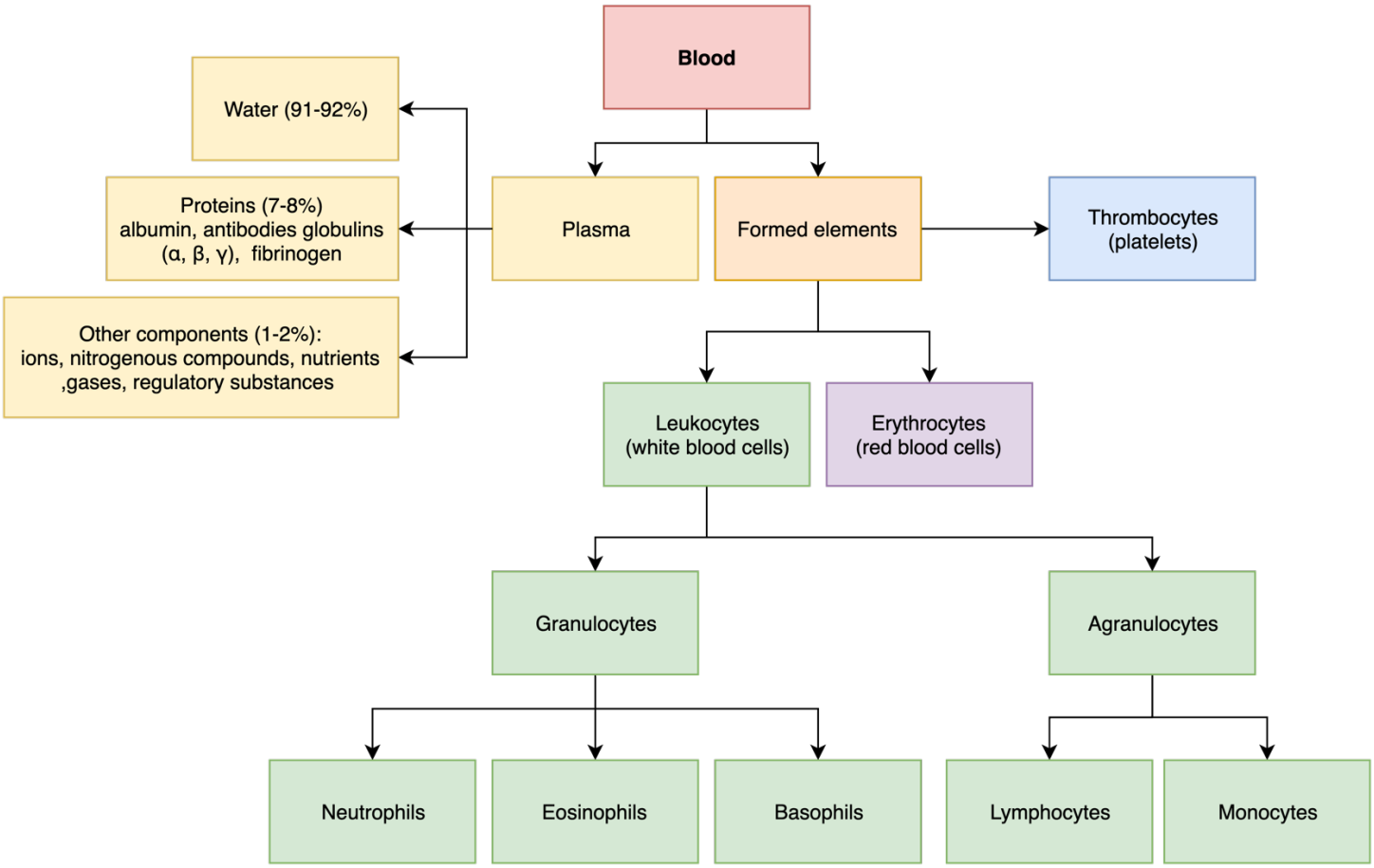


CLINICAL CORRELATION

Obesity treatment
Induction of beige adipose tissue has been proposed as a way of managing obesity and type 2 diabetes. Some agents are being studied, but more research is required in this field.

Section 5 – Blood and Hematopoiesis

5.1 – Erythrocytes (Red Blood Cells)
5.2 – Leukocytes (White Blood Cells)
5.3 – Thrombocytes (Platelets)
5.4 – Red Bone Marrow



- Blood is a liquid tissue because its extracellular substance, plasma, is liquid
- Hematocrit: = formed elements volume/whole blood volume.
    1. Males 0.4 – 0.5, Females 0.35 – 0.45
    2. Used diagnostically to give insight into viscosity of the blood
- γ-globulin fraction of plasma proteins are what we call immunoglobulins, which are antibodies involved in the humoral immune response
- Serum = plasma devoid of fibrinogen and clotting factors
- Blood smear allows us to have a look at the formed blood elements:
    1. Erythrocytes (red blood cells): 4 000 000 - 5 000 000 /mm^3
    2. Thrombocytes (platelets): 200 000 – 400 000 /mm^3
    3. Leukocytes (white blood cells): 6 000 - 9 000/mm^3 (agranulocytes: Lymphocytes 20 - 30% Monocytes 3 - 8%, granulocytes: neutrophils 60-70%, eosinophils 2-4%, basophils 0-1%)

I. Functions of blood

- Only erythrocytes and blood platelets perform their functions in blood. Other blood cells use the blood as transport route, migrate across blood vessel walls and perform their functions in tissues.
- Transport of oxygen and nutrients to cells (via ECS)
- Transport of carbon dioxide and waste materials away from cells (via ECS
- Transport of cells and agents involved in defense reactions
- Delivery of regulatory substances (e.g. hormones) to cells (via ECS)
- Maintenance of body homeostasis (water and ion balance, buffering of body fluids pH, participation in thermoregulation)
- Coagulation

CLINICAL CORRELATION

Cell count with differential and blood smears

A "cell count with differential" is often ordered in the workup of common diseases. We look for elevations of different cell lines, and by knowing the function of each, we get clues to what is going on!

For example: a higher than normal eosinophil count strongly suggest a parasitic or allergic process, reflecting the function of that cell line.

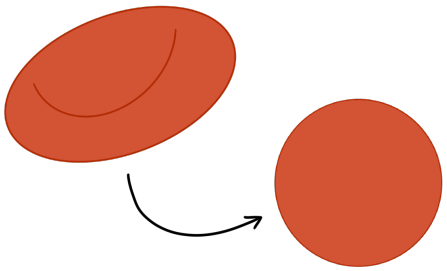
We can also ask for a blood smear to take a look at the actual cells, which is helpful when diagnosing hematological diseases or infections.

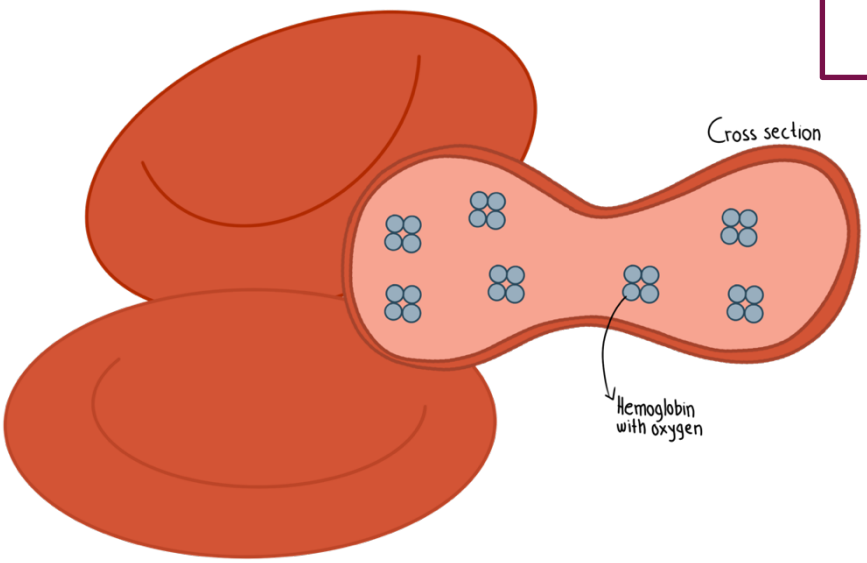
5.1 – Erythrocytes (Red Blood Cells)

- Diameter 7.5 µm
- Lifespan 120 days
- No nucleus or organelles, main constituent is hemoglobin, which transports oxygen and carbon dioxide
- Thick glycocalyx
    1. Sugars present in the glycocalyx act as blood group antigens
- Immature forms of red blood cells are not supposed to be found in peripheral blood, the exception being reticulocytes
    1. 1-2% in peripheral blood is considered normal
- Erythrocytes maintain their biconcave shape due to the presence of subplasmalemmal membrane skeleton built of *spectrin*
- Transporters of the cell membrane:
    1. *Band 3 protein*: anionic transporter
    2. Sodium-potassium pump

CLINICAL CORRELATION

Hereditary spherocytosis
Autosomal dominant disease affecting people of northern European descent. Caused by defects in membrane proteins such as spectrin and band 3 protein, preventing the red blood cells from maintaining their usual shape. Leads to hemolysis and anemia, and the definitive treatment is splenectomy because the spleen is the main site of destruction of these cells!



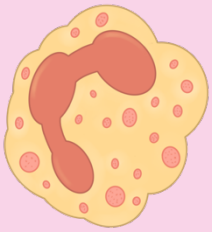
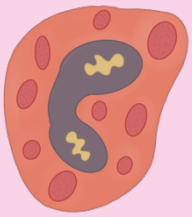


I. Erythrocyte maturation (erythropoiesis)

- Proerythroblast → basophilic erythroblast → polychromatophilic erythroblast → orthochromatophilic erythroblast → reticulocyte (erythrocyte with clusters of ribosomes; the „youngest" form of erythrocyte in the circulation)
- In the bone marrow, differentiating and maturating erythrocytes usually form clusters around macrophages (nurse cells). Macrophages take up and digest cell nuclei extruded from erythroblasts and release factors locally stimulating erythropoiesis.

5.2 – Leukocytes (White Blood Cells)

5.2.1 – Granulocytes

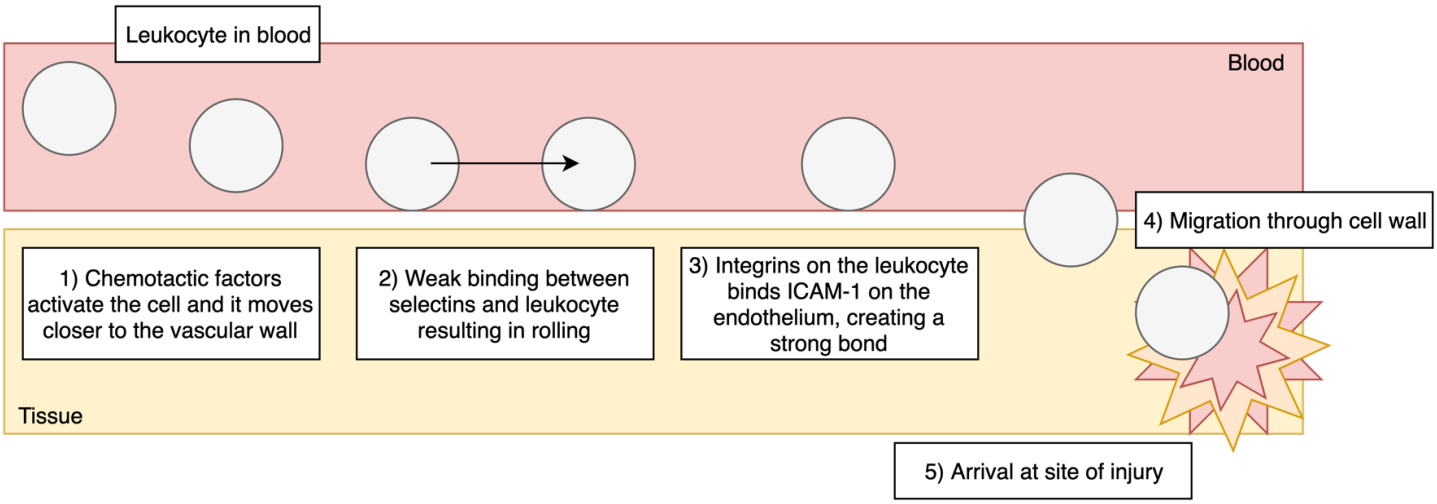
	Neutrophils	Eosinophils	Basophils
			
Function	Phagocytize and kill bacteria Main cell of *acute* inflammation!	- Kills larval parasites by releasing granule content - Cooperation with mast cells and basophils in allergic reactions: regulates release of proinflammatory mediators - Immunoregulation - Bactericidal and tumoricidal properties - Weak phagocytic activity	Morphologically and functionally similar to mast cells, but have a different lineage
Diameter	12 µm	15 µm	10-12 µm
Nucleus morphology	Segmented	Usually 2 segments	non-segmented or segmented
Organelles	Few		
Granule content	*Azurophilic granules:* Myeloperoxidase, defensins, azurocidin, lysozyme, elastase, cathepsin G	Acidophilic specific granules with crystalloid cores: Major basic protein, eosinophil cationic protein, eosinophil-derived neurotoxin, eosinophil peroxidase, hydrolytic enzymes and cytokines	Basophilic specific granules: Histamine, Chondroitin sulphate eosinophil-attracting chemotactic factor Cell membrane contains receptors for IgE
	Specific granules: Cathelicidin, lactoferrin, lysozyme, collagenase		
	Tertiary granules: Gelatinase, metalloproteinases, lysozyme		
	Secretory vesicles: Albumin, alkaline phosphatase, adhesion molecules and receptors for complement to be inserted into cell membrane		

I. Features of granulocytes

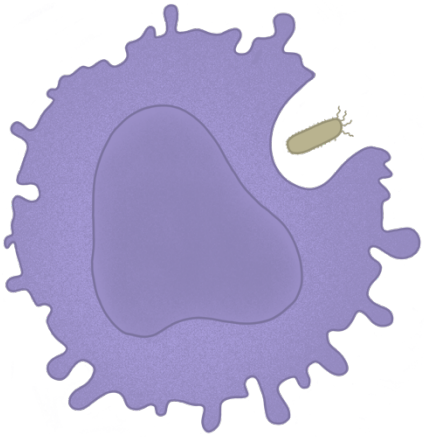
- Do not divide
- Short lifespan (days)
- Contain large amounts of granules:
    1. Azurophilic: modified hydrolase vesicles
    2. Specific: containing specific proteins/enzymes
- Mast cells are similar to basophils, but are considered to be a connective tissue cell rather than a granulocyte

II. Neutrophils

- Migration: when attracted by substances (chemotactic factors, chemoattractants) produced by bacteria and/or cells involved in defense processes, neutrophils (and other leukocytes) pass across the vascular wall and then migrate towards the source of chemoattractants (e.g. bacteria, inflammatory area).
- Migration across the vascular wall depends on interactions between adhesion molecules expressed on the cell membranes of leukocytes and endothelial cells.
    1. Sugars on the leukocytes bind selectins on the endothelial cells
    2. Integrin on the leukocyte binds ICAM-1 on the endothelial cells
- Stages of migration: 1) margination and activation 2) rolling 3) adhesion 4) diapedesis:



III. Phagocytosis



- Phagocytosis is much more effective if bacterial cell is opsonized (coated with antibodies and/or complement) because neutrophils has specific receptors for this
    1. Neutrophils (and some other immune cells) possess pattern-recognition receptors which recognize and bind specific components of bacterial and fungal cell walls, bacterial DNA and viral nucleic acids, allowing these cells to distinguish pathogens from harmless elements or organisms own structures
- Killing and digestion of bacteria
    1. Phagocytosis triggers a manyfold increase in the intensity of oxidative processes (oxidative burst) in the neutrophil, yielding highly aggressive oxygen radicals (e.g. superoxide anions)
    2. Granules fuse with the phagosome and their content kills and digests bacteria
- Neutrophils possess bacteria-killing system: myeloperoxidase interacting with chloride/iodine ions and, superoxide anions stop bacterial metabolism by excessive oxidation, chlorination and iodination.
    1. Lysozyme dissolves bacterial cell wall
    2. Lactoferrin binds ferric (iron) ions necessary for bacterial metabolism
    3. Defensins and cathelicidin make holes in bacterial cell membrane
- Neutrophils kill bacteria even after death with the neutrophil DNA extracellular trap (NET): Dying neutrophils release loosened chromatin with bound antibacterial proteins and enzymes. It forms large extracellular network. Bacteria trapped in this network are killed.

IV. Granulocyte maturation (granulocytopoiesis)

- Myeloblast → promyelocyte (production of azurophilic granules) → myelocytes (production of specific granules) → metamyelocytes (CLINICAL CORRELATION!) (production of tertiary granules in neutrophil lineage)
- Neutrophil – band (CLINICAL CORRELATION) (the „youngest" neutrophil released to blood, with non-segmented nucleus)

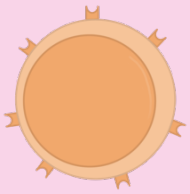
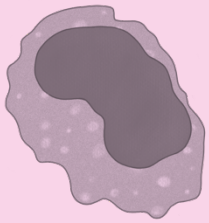
CLINICAL CORRELATION

Chronic granulomatous disease

A defect in an important enzyme involved in the oxidative burst in neutrophils, NADPH oxidase, causes recurrent infections with catalase-positive organisms. Catalase is an enzyme that breaks down toxic metabolites created by the organism itself. When the host neutrophils are not able to create toxic metabolites, and the organism breaks down its own toxins – it survives and cause infection! Examples of catalase-positive organisms: Staphylococcus aureus, Pseudomonas aeruginosa and Aspergillus.

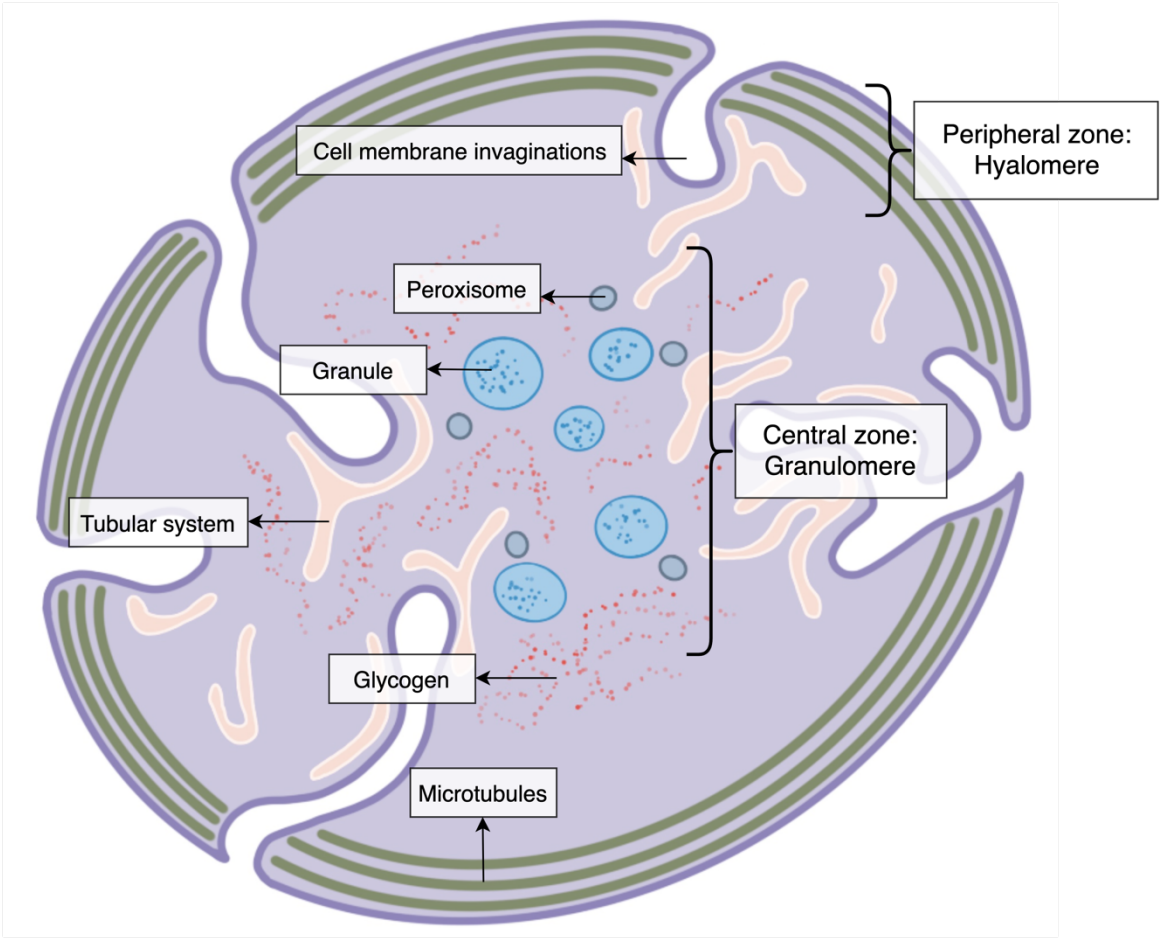
5.2.2 – Agranulocytes

- Long lifespan (weeks - years)
- Contain small amount of azurophilic granules
- Non-segmented nucleus
- Can divide and differentiate

	 **Lymphocytes**	 **Monocytes**
Function	Immune response: B-cells → humoral immune response T- cells → cell-mediated immune response NK-cells → kills virus infected cells and tumor cells	Migrate from blood to tissues, where they become macrophages, antigen-presenting (dendritic) cells or osteoclasts. Has phagocytic activity
Diameter	8-15 µm	15-20 µm
Morphology	large spherical nucleus with a thin layer of cytoplasm	Kidney-shaped nucleus
Organelles	Few	Well developed

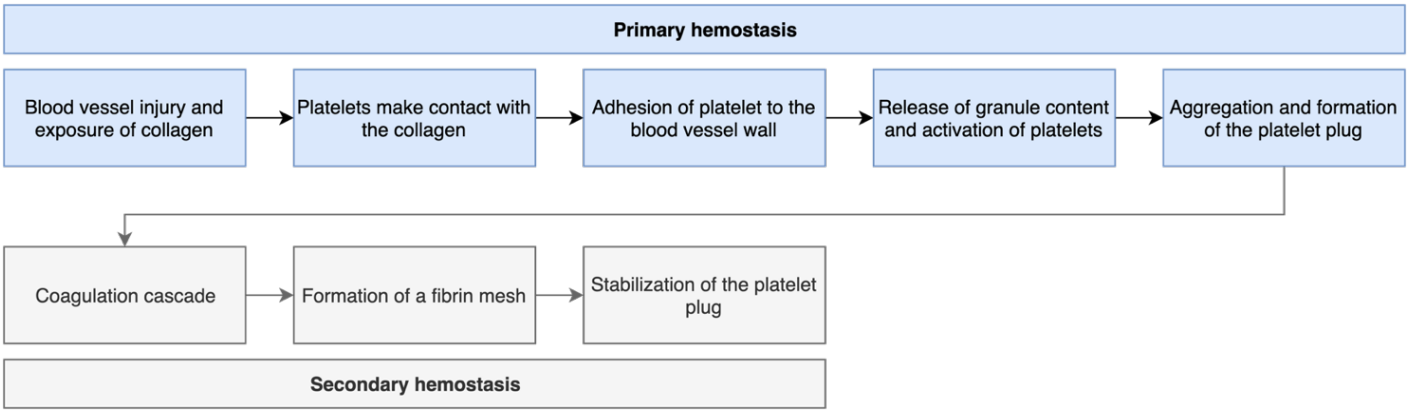
5.3 – Thrombocytes (Platelets)

- Cytoplasmic fragments of the very large precursor cell called megakaryocyte.
  1. Diameter 4 µm
  2. No nucleus
- Peripheral zone: hyalomere
  1. Ring of microtubules and actin filaments
  2. Open canalicular system: branched invaginations of the cell membrane
  3. Tubular system: calcium ion storage
- Central zone: granulomere
  1. Mitochondria, hydrolase vesicles, peroxisomes, granules, glycogen



I. Primary hemostasis

- Platelets initiate the blood coagulation (clotting) process: primary hemostasis
- Factors released from platelets, together with other coagulation factors (from plasma and tissues) induce formation of blood clot



II. Platelet formation

- Megakaryoblast → multiple endomitoses → megakaryocyte
    1. Endomitoses = multiple DNA replications without cell division in between.
- Megakaryoblasts
    1. Giant cell up to 100 µm
    2. Polyploidy up to 64N
    3. Lobated nucleus
- Mature megakaryocytes have invaginations in their cell membrane called demarcation channels, or demarcation membranes. These surround the cytoplasm with the future platelets and facilitates rapid release of new platelets.

5.4 – Red Bone Marrow

- Site of hematopoiesis (production of blood cells)
- Compartments: vascular and hematopoietic

I. Vascular compartment

- The vascular compartment:
    1. Thin-walled sinusoids (variant of capillary vessels): endothelium, adventitial reticular cells (modified pericytes)
- Endothelial cells of sinusoids form transient migration pores for cells passing to blood
- Adventitial cells control migration by locally uncovering endothelial cells
- Only mature cells can pass across the vascular wall in bone marrow

II. Hematopoietic compartment

- Scaffolding built of reticular connective tissue: reticular fibers and stromal cells: fibroblasts, macrophages, mesenchymal stem cells
- Clusters of differentiating hematopoietic cells and scarce adipocytes
- Stem cells and progenitor cells look like large lymphocytes, we can only see a separate their stage of differentiation later in the process.
- Bone marrow stem cells can be obtained from bone marrow and peripheral blood
    1. Hematopoietic stem cells, stromal (mesenchymal) stem cells
- Hematopoiesis can be examined by microscopic inspection of bone marrow smear
    1. Erythropoietic lineage: 20%
    2. Granulopoietic lineage: 65%
    3. Other lineages: 15%

Section 6 – Bone and Cartilage

6.1 – Composition and Structure

- Bone contains both mineralized, inorganic and non-mineralized, organic tissue.
- The organic portion of bone includes cells which secrete the bone matrix and carry out important metabolic functions.

6.1.1 – Extracellular Substance

<table>
<tr><th>Component</th><th colspan="2">Description</th><th>Function</th></tr>
<tr><td>Hydroxyapatite</td><td colspan="2">Mineral containing calcium and phosphate
Makes up 70% of bone mass</td><td>Makes bones hard
Store of calcium and phosphate ions</td></tr>
<tr><td>Type I collagen</td><td colspan="2">Fibrous protein which predominates in bone tissue</td><td>Provides a framework for hydroxyapatite crystals to grow in</td></tr>
<tr><td>Enzymes</td><td colspan="2">Proteases, alkaline phosphatase</td><td>Participate in mineralization and bone remodeling</td></tr>
<tr><td rowspan="4">Osteoid
(ground substance)</td><td colspan="2">Non-mineralized tissue made from glycoproteins, proteoglycans and collagen.</td><td>Undergoes mineralization to form bone tissue</td></tr>
<tr><td>Glycoprotein</td><td colspan="2">Function</td></tr>
<tr><td>Osteonectin
Osteocalcin</td><td colspan="2">Involved in bone mineralization:
Osteonectin: connects hydroxyapatite crystals to collagen
Osteocalcin: binds calcium</td></tr>
<tr><td>Osteopontin
(Bone sialoprotein 1)</td><td colspan="2">Acts as a mineralization inhibitor by binding calcium, thereby regulating the mineralization
Also connects osteoclasts to bone surfaces, playing a role in bone remodeling</td></tr>
<tr><td>Bone sialoprotein 2</td><td colspan="2">Stimulates bone mineralization</td></tr>
</table>

7.1.2 – Cells

- The different cell types mediate a delicate balance between bone formation and bone resorption, which releases Ca^{2+} into the bloodstream.

Cell	Morphology	Function
Osteoprogenitor cells Stem cells	Spindle-shaped, with pale cytoplasm	Active during fetal bone development, remodeling and repair of mature bone In mature bone: resting in the periosteum, vascular canals and endosteum Can differentiate into osteoblasts if stimulated
Osteoblasts Bone builders	Cuboidal, with dark basophilic cytoplasm	Produce osteoid and control its mineralization Active during fetal bone development, as well as remodeling and repair of mature bone Turn into osteocytes after cessation of activity
Osteocytes Bone builders on holiday	Flattened, large nucleus, long processes connected by gap junctions	Predominant cells of mature bone Occupy fluid-filled lacunae and canaliculi that openins up to a space with blood vessels, which is how the osteocytes exchange nutrients
Osteoclasts Bone macrophages	Large and multinucleated Ruffled border Many vesicles and lysosomes	Monocytes expressing RANK receptor are stimulated by RANK ligand on osteoblasts, which induces differentiation to osteoclasts Break down bone, secretes acid and proteolytic enzymes called matrix metalloproteinases Combines intra- and extracellular digestion

I. Response to mechanical stress

- With a primary cilium, a mechanoreceptor, osteocytes sense movement and change in pressure. They respond by changing their functional state and sending signals to other osteocytes, osteoblasts and osteoclasts via gap junctions in their cell processes
    1. Result: bone remodeling aimed at adapting to the environmental forces on bone
- Functional states of osteocytes
    1. *Quiescent (majority):* few organelles, little activity
    2. *Formative:* more RER, Golgi for production of osteoid
    3. *Resorptive:* some RER, Golgi, lysosomes for breaking down surrounding extracellular matrix. Liberate calcium ions by releasing metalloproteinases

CLINICAL CORRELATION

Cleidocranial dysplasia

Genetic disease caused by faulty osteoblast differentiation leading to weak and sometimes absent bones. The classic example is the absence of collar bones.

Maybe you've read about the actor Gaten Matarazzo who has this disease?

II. Differentiation and recruitment of osteoclasts

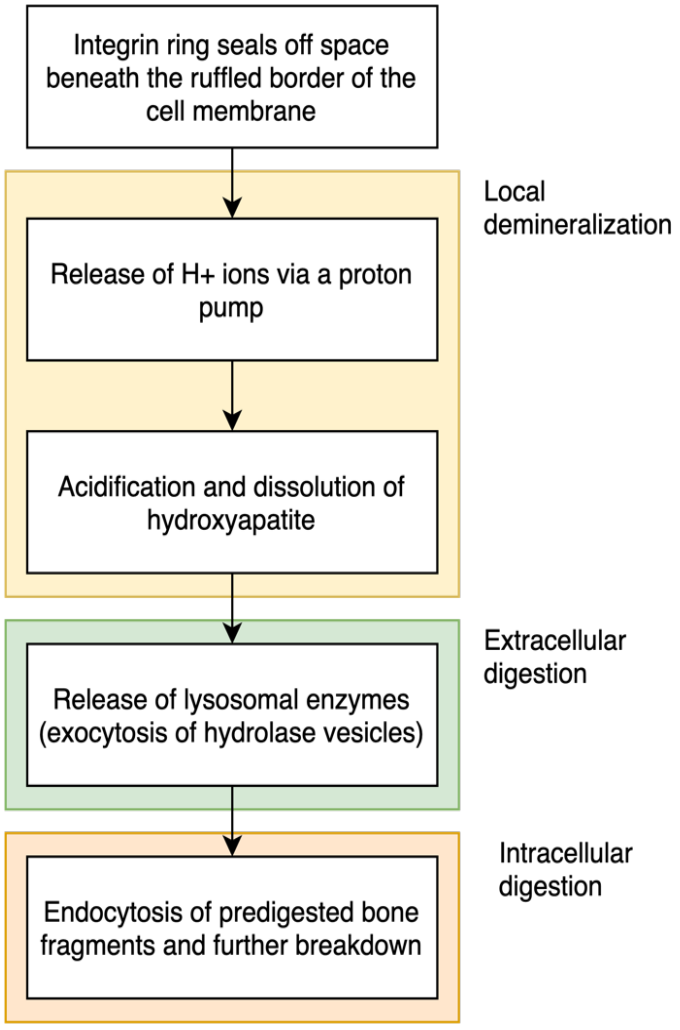
- M-CSF secreted by bone marrow stromal cells induces expression of RANK receptor in mononuclear osteoclast precursors
- Osteoblasts expresses the RANK ligand, which binds to the RANK receptor. This induces differentiation and fusion of precursors into multinucleated osteoclasts
- Osteoproteregrin secreted by osteoblasts inhibits the RANK-RANKL interaction, thereby regulating bone remodeling

CLINICAL CORRELATION

Osteoporosis

Estrogen induces osteoproteregrin, thereby inhibiting the RANK-RANKL interaction and bone resorption. Low estrogen → less osteoproteregrin → more RANK-RANKL binding → bone loss and osteoporosis

I. Osteoclast-mediated bone digestion



CLINICAL CORRELATION

Hyperparathyroidism

Parathyroid hormone (PTH) stimulates osteoclast activity, i.e. it increases bone resorption. This is why hyperparathyroidism presents with hypercalcemia (more calcium being released from bone matrix) and increased susceptibility to fractures.

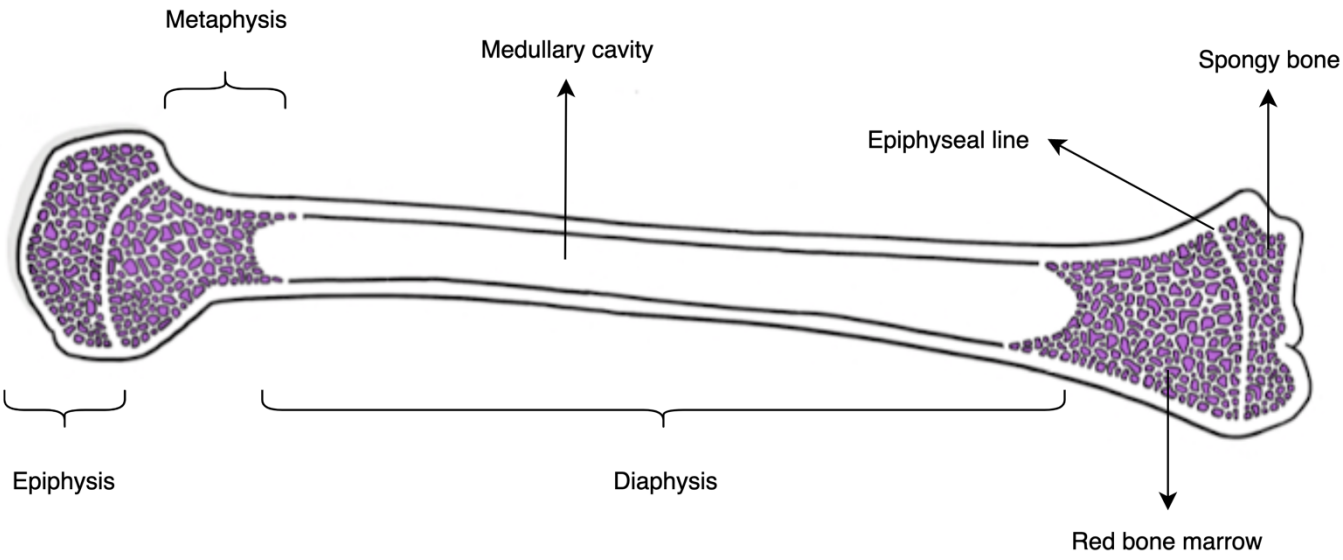
7.1.3 – Bone Structure

I. Types of bone slides prepared for microscopic evaluation

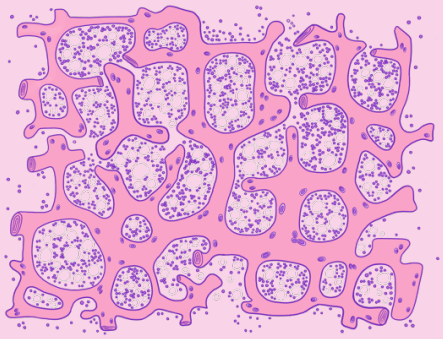
- Decalcified sections
    1. Demineralization
    2. Sectioning and staining
    3. Visible on the slide: acidophilic collagen fibers, osteocytes and blood vessels
- Ground sections
    1. Removal of „soft" components (cells, blood vessels), only mineralized components are preserved
    2. Grinding to produce very thin plates
    3. Immersion in stain
    4. All „empty" (nonmineralized) spaces visible on the slide: lacunae, canaliculi, vascular canals

II. Types of bone

- Immature bone = primary bone = woven bone
    1. High turnover rate and low mineral content
    2. Collagen fibers: random and irregular arranged = mechanically weak
    3. Seen *physiologically* during fetal bone development and adult tooth sockets, otherwise *pathological* in adults (e.g. post-fracture repair, Paget disease)
- Mature bone = secondary bone = lamellar bone
    1. Low turnover rate and high mineral content
    2. Mechanically strong
    3. Structural unit: bone lamella consisting of collagen fibers in parallel arrangement around osteocytes, with lacunae around their cell bodies and canaliculi around their cell processes (insert illustration)
    4. Two types: compact and spongy bone



Sub-types of *mature* bone

	Compact = Haversian = Cortical bone	**Spongy = Cancellous = Trabecular bone**
		
Structural organization	Osteons[1] form dense, parallel columns. Each osteon has a central *Haversian canal* surrounding a blood vessel. Haversian canals are connected by larger *Volkmann's canals* contain larger blood vessels coming from periosteum or bone marrow, and run perpendicularly.	Trabeculae lined with osteoblasts form a meshwork filled with bone marrow
Blood supply	From the bone marrow and vessels in the periosteum	Bone marrow: contains blood vessels and hematopoietic tissue (red) with varying amounts of adipose tissue (yellow)
Locations	Diaphysis of long bones,[3] e.g. femur Cortex of *all* bones	Epiphysis and medullary cavity of long bones (**insert illustration**) Flat and irregular bones

[1] Osteon = Haversian system

Layers of the bone: superficial → deep

Inside <---------- Outside	**Layer**	**Description**
	Periosteum	Outer fibrous layer: dense connective tissue anchored by Sharpey fibers
		Inner cellular layer: contains osteoprogenitor cells, osteoblasts, and osteoclasts
	Compact bone	Outer circumferential lamellae
		Network of Haversian systems
		Inner circumferential lamellae
	Spongy bone	Trabeculae extending into marrow cavity
	Endosteum	Single layer of flat cells - inactive osteoblasts
	Bone marrow	Hematopoietic and adipose tissue

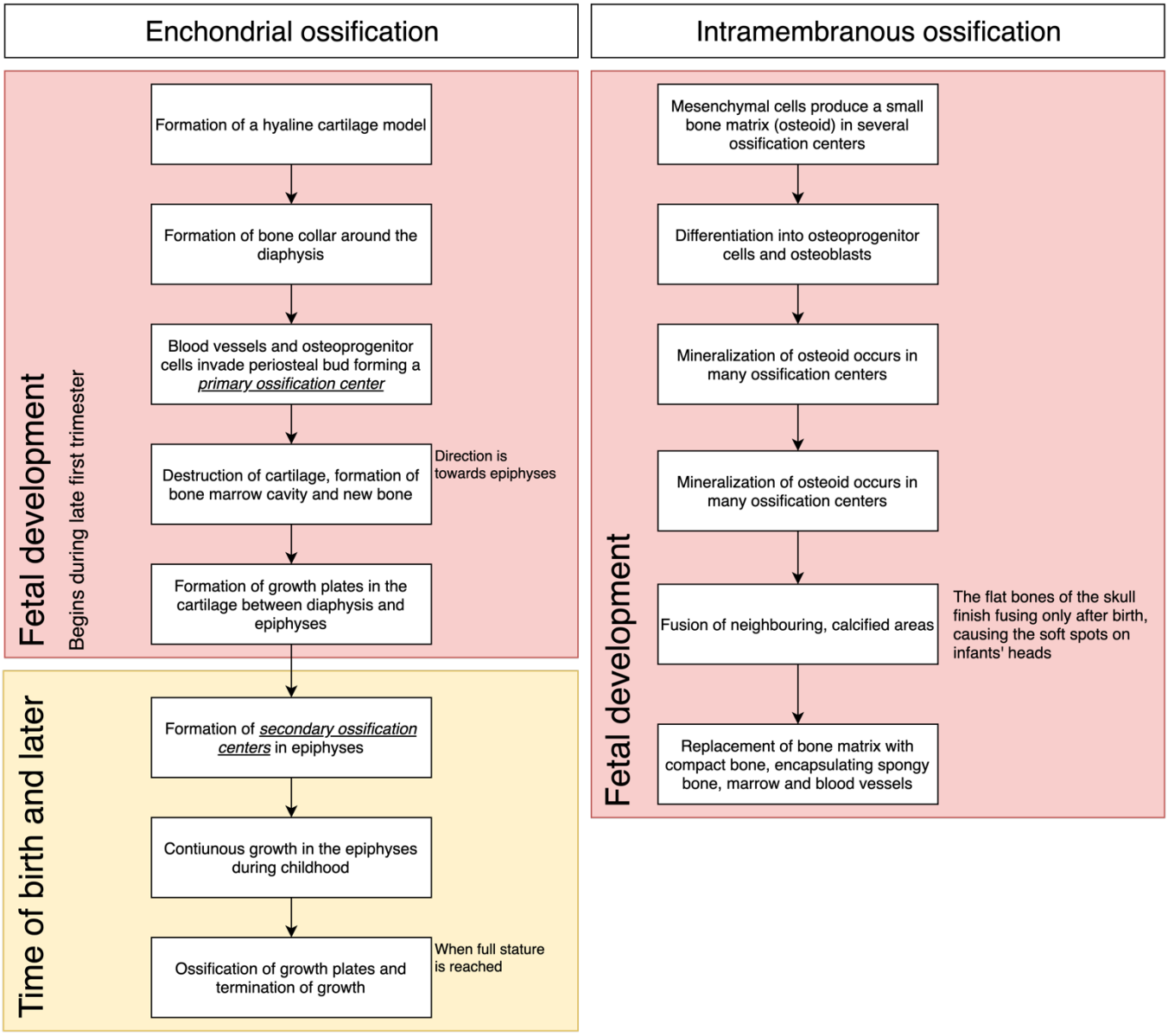
6.2 – Bone Formation, Remodeling and Repair

6.2.1 – Bone Formation

- Bone is formed by 2 types of ossification. Both are important during fetal bone development and in adult bone repair

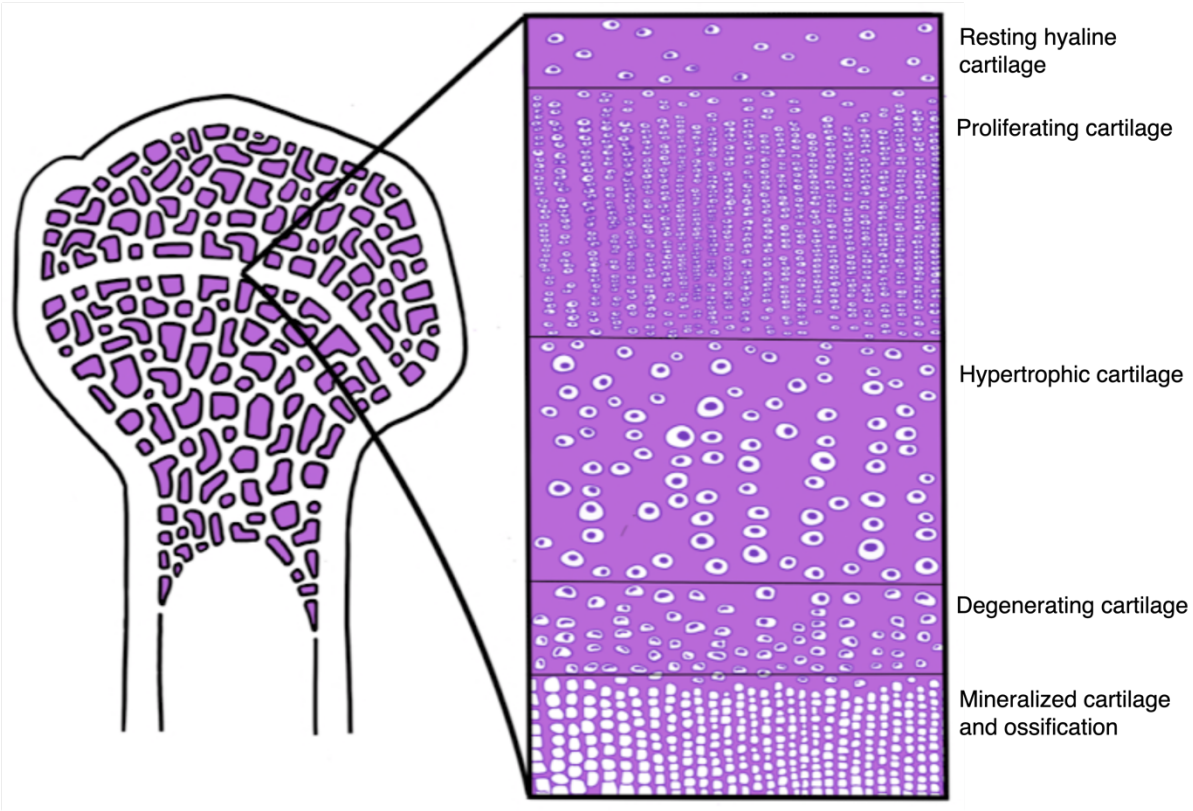
Ossification types

	Endochondral	Intramembranous
Hyaline cartilage involvement	Yes	No
Type of bone formed	Compact bone (long bones)	Spongy bone (long, flat & irregular bones)



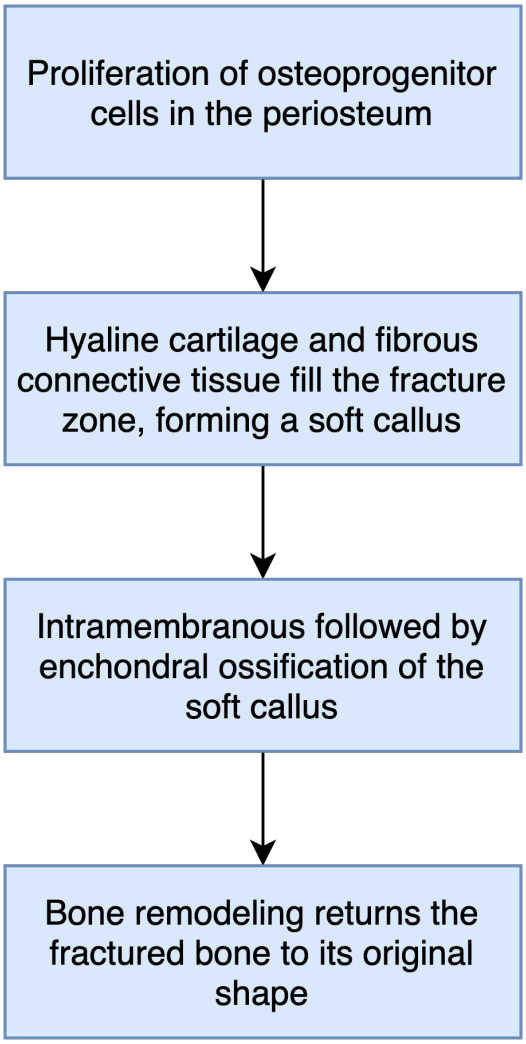
I. The epiphyseal plate

- Mineralization of the extracellular substance begins with the formation of „matrix vesicles” released by degenerating chondrocytes and by osteoblasts and containing high concentrations of calcium and phosphate ions.
  First crystals of hydroxyapatite grow inside such vesicles and after piercing the membrane they become centers (nidi) of crystallization. Further crystals grow and fuse, invading the extracellular substance.
- The zones of the epiphyseal plates reflects the stages of converting cartilage into bone



6.2.2 – Bone Remodeling and Repair

- The skeleton adapts to mechanical loads and metabolic needs by remodeling. This occurs through a combination of bone formation and resorption which alters the bone structure:



6.3 – Joints

- Articular cartilage (hyaline) is not covered by perichondrium

I. Joint capsule

- Fibrous layer: dense connective tissue
- The synovial membrane: loose connective tissue
    1. contains macrophages (A synoviocytes) and fibroblasts (B synoviocytes), which secrete synovial fluid
- Blood vessels

CLINICAL CORRELATION

Arthritis

Osteoarthritis: a degenerative joint disease caused by chronic overload, with micro-injuries leading to destruction of the articular cartilage and underlying bone

Rheumatoid arthritis: auto-immune destruction of articular cartilage and connective tissue

6.4 – Cartilage

- The mechanical resistance of the supporting tissues depend on the composition and properties of the extracellular substance
- Cells producing components of the extracellular substance in cartilage (chondroblasts and chondrocytes) and bone (osteoblasts and osteocytes) are specialized variants of active and quiescent fibroblasts

I. Characteristics common for all cartilage types

- No blood vessels
- Ground substance rich in chondroitin sulphates
- Chondrocytes residing in lacunae: singly or in small groups
- Lacunae surrounded by condensed ground substance (matrix) are called chondral territories (isogenous groups)

II. Extracellular substance

- Collagen fibers
- Aggregates of proteoglycans
- Rich in chondroitin sulphates
- Glycoproteins (anchorin, tenascin, fibronectin, chondronectin)

III. Cells

- *Chondrogenic cells*: stem cells for cartilage, can transform into chondroblasts
- *Chondroblasts*: actively produce components of the extracellular substance during cartilage formation, and later transform into chondrocytes
- *Chondrocytes*: cells of mature cartilage, continue to produce components of the extracellular substance (in lesser amounts)
- Chondrogenic cells → chondroblasts → chondrocytes

IV. Perichondrium

- Blood vessels supplying oxygen and nutrients are present in perichondrium - a layer of dense connective tissue covering the cartilage surface
- Has an outer fibrous layer and an inner cellular layer (blood vessels) and chondrogenic cells
- Growth of the perichondrium:
    1. Growth by apposition: the inner layer of perichondrium contains chondrogenic cells that can be activated, becoming chondroblasts that produce extracellular substance and enclose themselves in lacunae.
    2. Interstitial growth: growth „from inside” by divisions of chondrocytes in lacunae and their activity in ECS production.
    3. In spite of these possibilities, mature cartilage has very weak regenerative capabilities in young persons and none in elderly persons

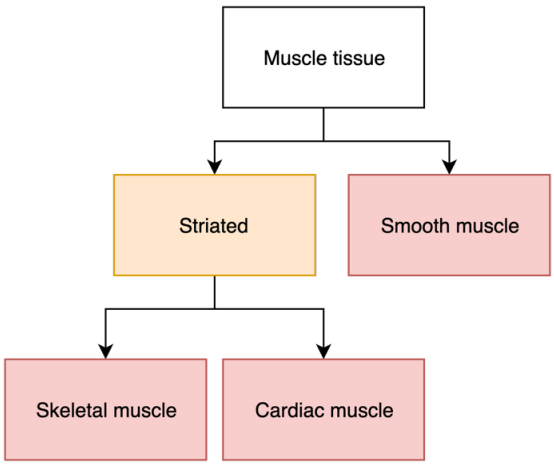
Types of Cartilage

Type	Organization	Mechanical properties	Locations
Hyaline	Chondral territories Meshwork of fibers of collagen type 2 surrounding the chondral territories Ground substance rich in the large aggrecan proteoglycan	Resistance to compression	Articular surfaces Nose, larynx, trachea & bronchi In fetus: „models" of long bones
Elastic	Chondral territories Meshwork of thin fibers of collagen type 2 Network of elastic fibers Ground substance	Elasticity	Auricle and auditory tube Larynx (e.g. epiglottis)
Fibrous	Few chondral territories Parallel bundles of collagen type 1 Small amount of ground substance No perichondrium	Resistance to stretch	Temporomandibular joint Intervertebral disks Pubic symphysis Some attachments to bone (continuous with tendons)

II. Hyaline cartilage: how is it so resistant to compression?

- Due to the properties of the highly hydrated proteoglycan aggrecan: under pressure water molecules are pushed out from spaces between GAG chains, negatively charged sulphate residues of GAG chains repel each other, resisting collapse of the tissue.

Section 7 – Muscle Tissue

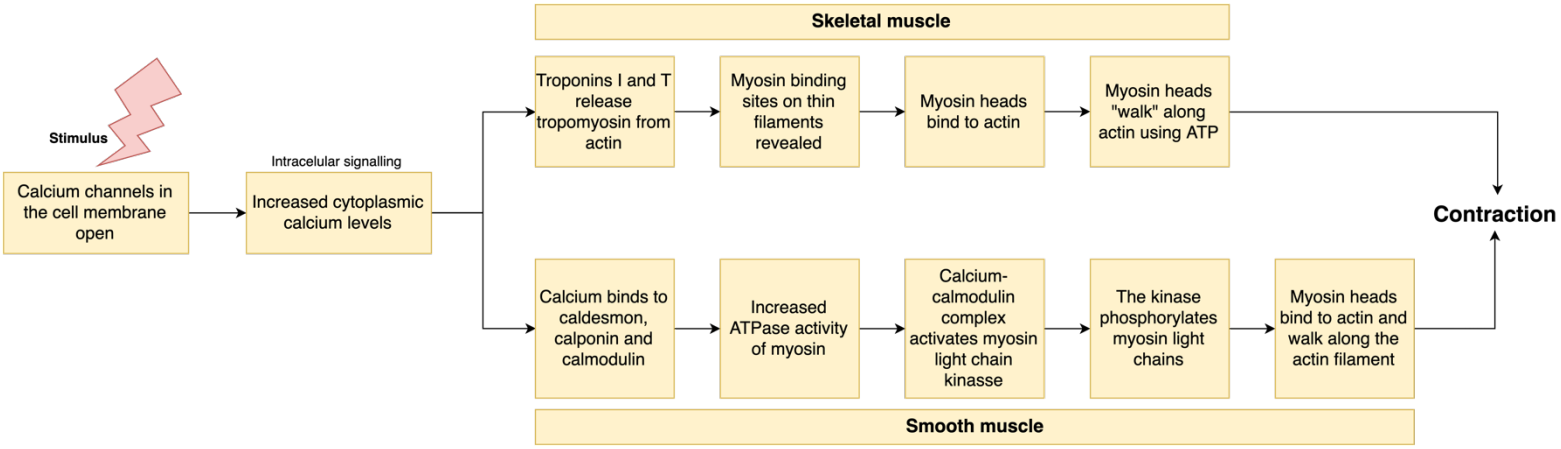


- Sarcolemma = muscle cell membrane + basal lamina
- Sarcoplasm = muscle cell cytoplasm

I. Contractile apparatus

- Thin myofilaments: actin + accessory proteins
    1. The types of accessory proteins vary among the different types of muscle
- Thick myofilaments: myosin 2

7.1 – Molecular Mechanism of Muscle Contraction



7.2 – Smooth Muscle

- Irregularly organized contractile apparatus
- Not under voluntary control
- Slow but durable contraction
- Cells produce their own basal laminae and other components of extracellular substance (elastic and reticular fibers)
- Smooth muscle responds to various kinds of signals:

Type of signal	Location
Nerve impulse: autonomic nervous system	Vascular smooth muscle: arteries
Mechanical signal: myogenic stretch	Visceral smooth muscle: intestines
Hormones like oxytocin	Uterus

7.2.1 – Structure

- Smooth muscle cells are grouped in layers or bundles and interconnected by gap junctions which allow propagation of stimuli
    1. Layers: respiratory tract, gastrointestinal (alimentary) system, urinary tract, blood vessels, reproductive system
    2. Bundles: Hair follicles and iris
- Thin myofilaments are much more abundant than thick ones, forming an elongated network
- Dense bodies with actin-binding proteins are located at sites where thin myofilaments are attached to each other and to the cell membrane

7.2.2 – Smooth Muscle Cell

- Elongated, fusiform cells surrounded by basal lamina with elongated nucleus
- Organelles clustered near poles of the nucleus with the remaining cytoplasm occupied by contractile apparatus

7.2.3 – Non-Muscle Contractile Cells

- Cells of different origin with contractile apparatus similar to that of smooth muscle

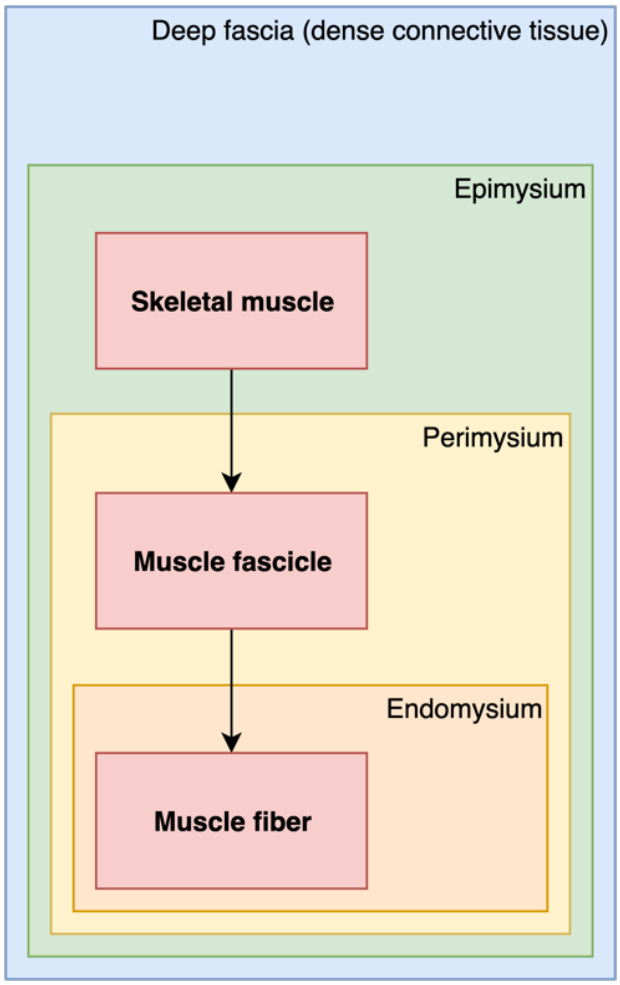
	Origin	Location	Function
Myoepithelial cells	Epithelial	Some glands	Helps push secretions through the ducts
Myofibroblasts (fibrocytes)	- Mesenchymal stem cells - Bone marrow	Skin and some organs	- Wound closure - Produce collagen fibers
Myoid cells	Mesenchymal	Testes	Push the spermatozoa
Pericytes		Capillaries	Change lumen size

7.3 – Skeletal Muscle

- Progenitor cell: myoblasts
- Very regularly organized contractile apparatus
- Responds only to neural stimuli
- Under voluntary control
- Fast contraction but prone to fatigue
- Muscle fibers produce their own basal laminae

7.3.1 – Structure

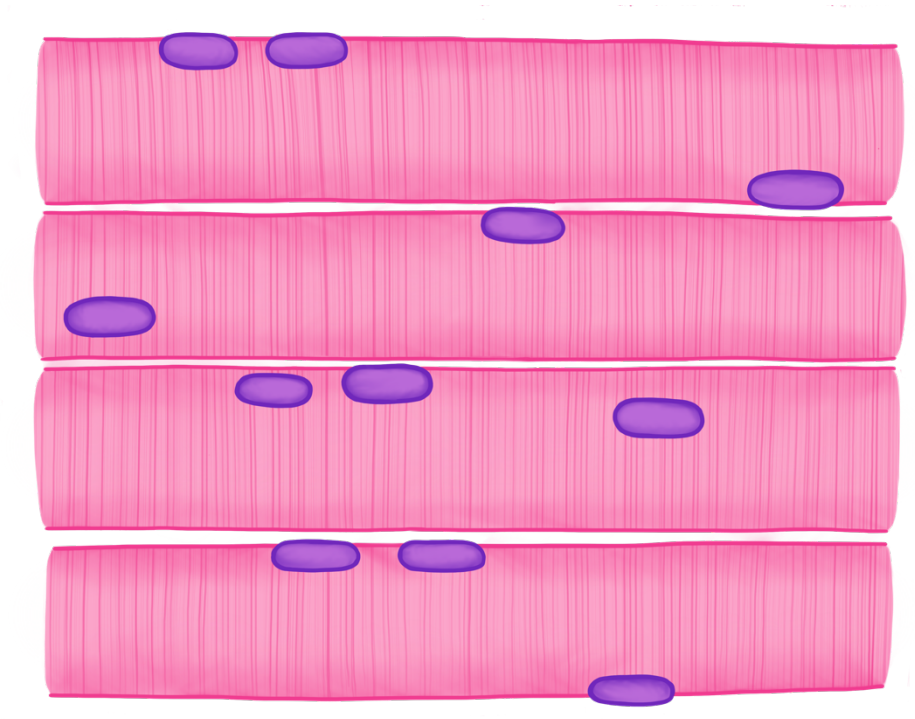
- Composed of skeletal muscle fibers and connective tissue investments:
    1. Epimysium surrounds the muscle
    2. Perimysium surrounds bundles of muscle fibers
    3. Endomysium fills spaces between muscle fibers.
- These connective tissue investments are continuous with the *myotendinous junction*, which is where each muscle fiber is firmly anchored to tendons via integrins



7.3.2 – Skeletal Muscle Cells: Muscle Fibers

- Skeletal muscle fiber is a syncytium formed by fusion of myoblasts
    1. Syncytium = cell with multiple nuclei, formed by fusion of multiple cells or division of the nucleus of a single cell
- *Muscle fiber periphery*: sarcoplasm with nuclei and some organelles like rough endoplasmic reticulum and Golgi
- *Muscle fiber center*: the contractile apparatus
    1. Consists of striated myofibrils that runs parallel to the long axis of the fiber
- Spaces between myofibrils: mitochondria, T tubules, sarcoplasmic reticulum and abundant glycogen and myoglobin

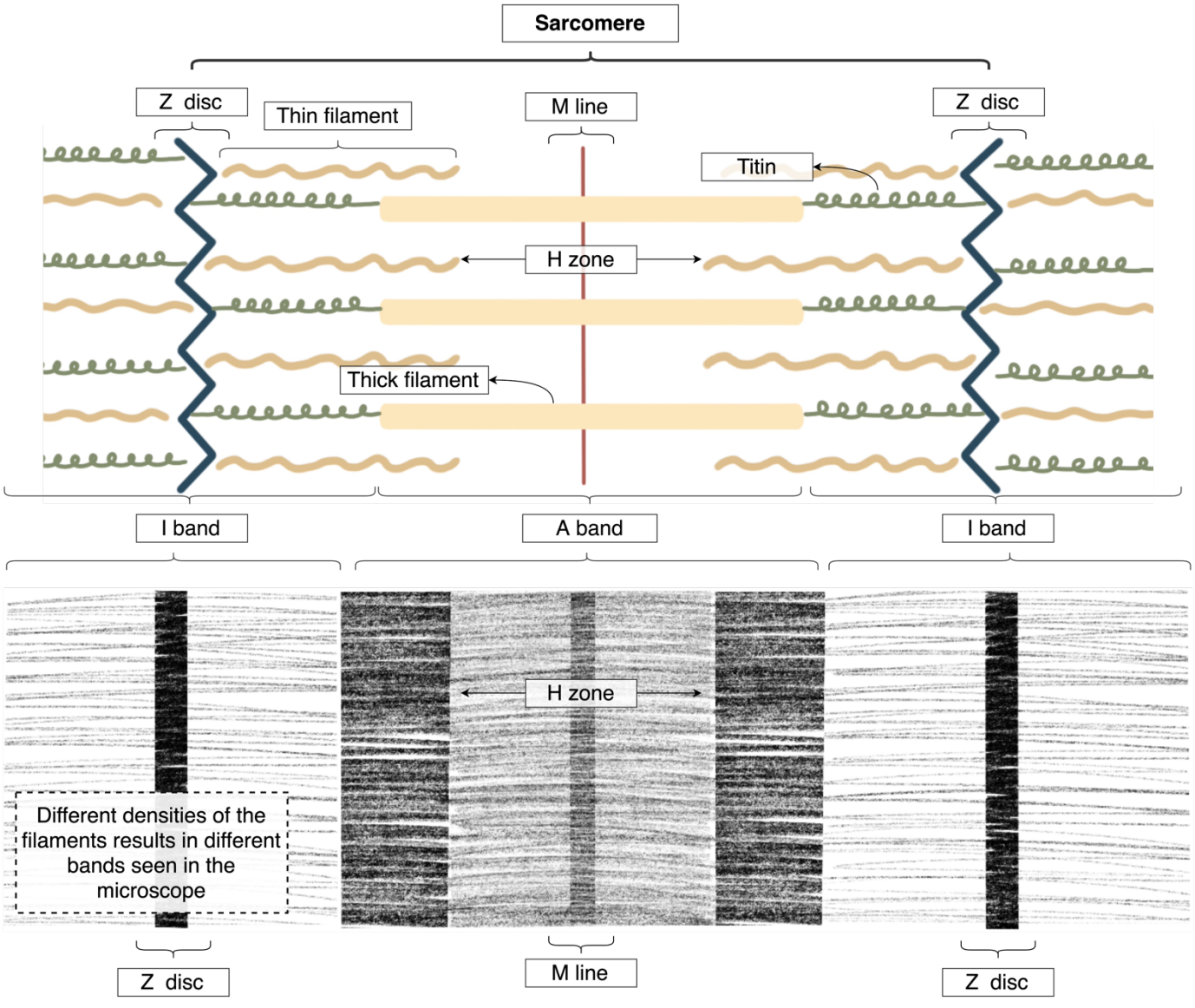
Sarcolemma	Plasma membrane + basal lamina of the muscle cell	Site of neuromuscular junction and propagation of signals
T-tubules	Invaginations of the sarcolemma that run perpendicular to myofibrils, surrounding them at the level og the I/A band border	Introduce the stimulus inside the muscle fiber
Sarcoplasmic reticulum	Specialized form of smooth endoplasmic reticulum. Forms a meshwork around each myofibril *with terminal cisternae* adjacent to T-tubules.	Stores and releases Ca^{2+} ions
Muscular triad	T-tubule + 2 terminal cisternae	Contraction propagation



The sarcomere

Component	Properties	Notes
A-band	Regular arrangement of thick and thin myofibrils	Thick, myosin filaments
I-band		The thin, actin filaments that *do not overlap* with thick filaments
Z-disc	α-actinin interconnecting positive ends of thin actin filaments	Maintains the regular structure of the sarcomeres in the muscle fiber
M-line	Myomesin forms lateral bridges interconnecting thick myofilaments	Also contains creatine kinase that help supply ATP for muscle contraction

- The striations of the muscle fibers are due to successive sarcomeres. The different bands on the sarcomeres we see in the microscope are created by different density of the myofilaments in each band



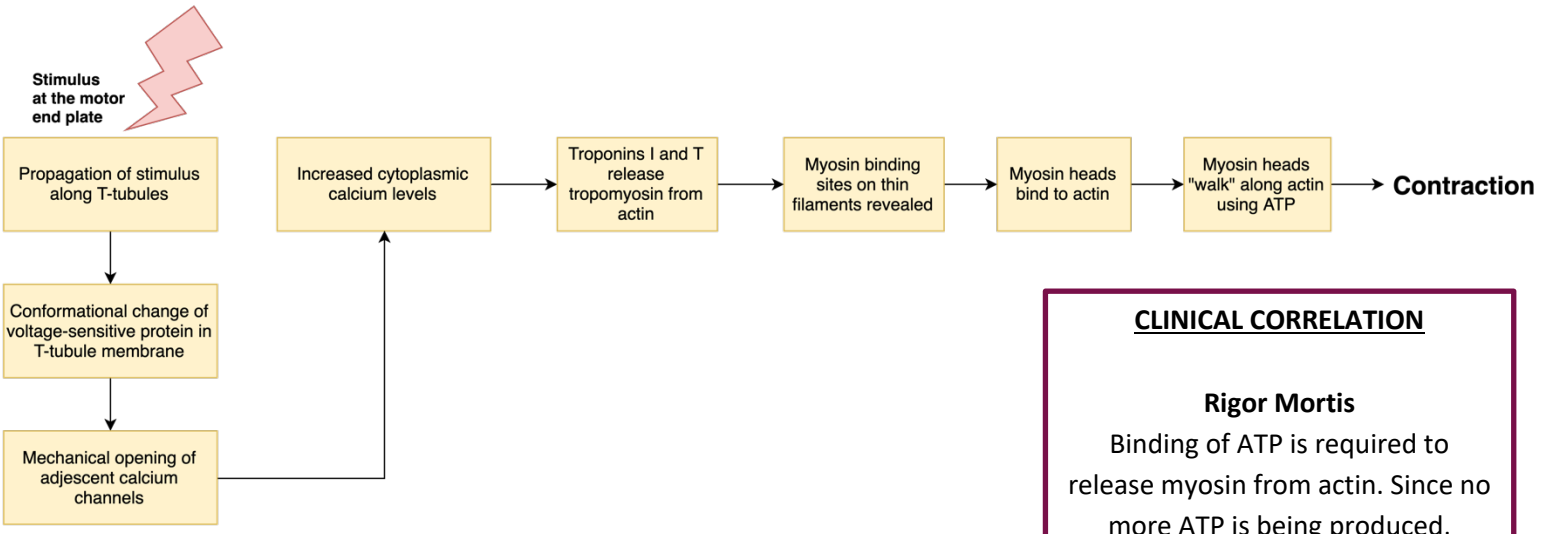
Accessory proteins of skeletal muscle		
Titin	I-bands	Connects the thick filaments to the Z-disc and *prevents excessive stretching*
Nebulin		Anchors actin filaments to α-actinin and *provides stabilization*
Desmin	Z-lines	Transverse intermediate filaments that hold adjacent sarcomeres in place by binding them to the Z-line. Creates the striated appearance.
Dystrophin	Anchors peripheral actin filaments to the cell membrane	
Tropomodulin	prevents elongation of thin myofilaments	

7.3.3 – Skeletal Muscle Contraction

FUN FACT

Why are they called A, Z , I and M bands?
A: *An*isotropic = birefringent in polarized light
I: Isotropic = does not alter polarized light
Z: Zwishen, German for "between"
M: Mitte, German for "middle"

- The neuromuscular synapse: every muscle fiber is stimulated by their own nerve terminal at the motor end plate
- Components of the motor end plate
    1. Sarcolemmal folds (subneural clefts)
    2. Sodium channels
    3. Neurotransmitter: acetylcholine
- Sequence of events from excitation to intracellular rise of Ca^{2+}
    1. Nerve stimulus at the motor end plate
    2. Propagation of stimulus along the cell membrane and T-tubule membranes
    3. Conformational change of voltage-sensitive protein in T-tubule membrane
    4. Mechanical opening of the adjacent calcium channels
- The contraction wave: propagation of stimulus along the cell membrane of skeletal muscle fiber and activation of successive triads is responsible for creation of „contraction wave" moving away from the motor end plate.



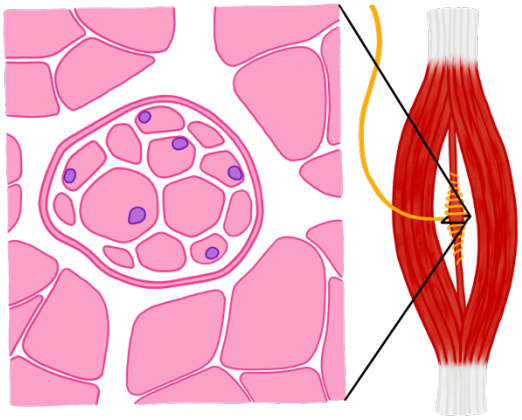
CLINICAL CORRELATION

Rigor Mortis
Binding of ATP is required to release myosin from actin. Since no more ATP is being produced, myosin remains bound to actin and muscles stay in a contracted state.

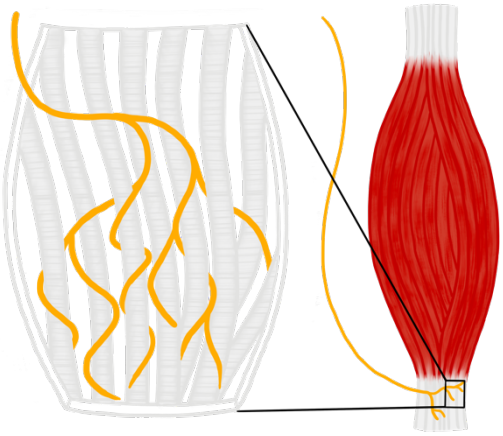
I. Regulation and coordination of movement

- Muscle spindles and Golgi tendon organs are mechanoreceptors monitoring muscle shortening and tendon tension, participating in coordination of movement
    1. Muscle spindle: stretch detectors
    2. Golgi tendon organs: tension detectors within tendons

Muscle spindle



Golgi tendon organ



7.3.4 – Skeletal Muscle Fiber Types

- Skeletal muscle fibers can be classified as red (slow), white (fast), or intermediate depending on their morphologic and metabolic characteristics.
- A single muscle may contain all three types of fibers, and they may even change from one type to another depending on their innervation.

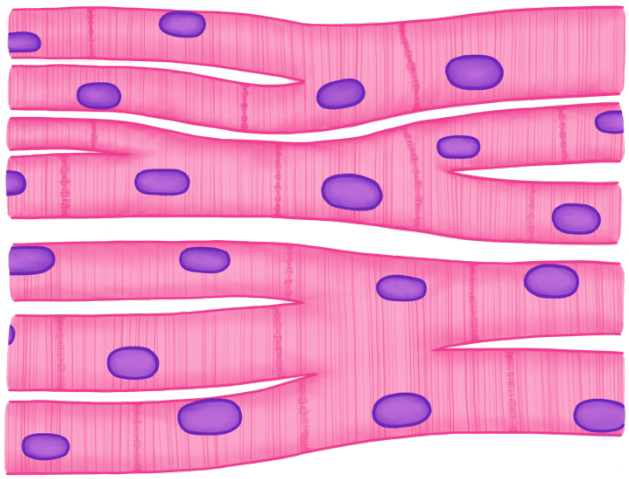
	Type 1: red fibers	Type 2a: red fibers	Type 2b: white fibers
Diameter	Small	Medium	Large
Speed	Slow	Rapid	
Z-lines	Wider	Intermediate	Narrower
Endurance	Good		Poor
Organelles	More myoglobin and mitochondria (produces the red color of the fibers)		Less myoglobin and mitochondria
Energy source	Lipid oxidation (oxidative phosphorylation)		Anaerobic glucose metabolism using glycogen
Location	Postural muscles of the back	Major muscles of legs	Extraocular muscles
Mnemonic	1 red, slow ox	–	2 white, fast antelopes

I. Satellite cells

- Satellite cells are skeletal muscle stem cells located on the surface of skeletal muscle fibers basal lamina apposed cell membranes
- Location under basal lamina
- Can divide and either incorporate into the existing fibers or fuse to form new fibers
- Are responsible for hypertrophy, remodeling and regeneration of skeletal muscles

7.4 – Cardiac Muscle

- Built of separate cells with regularly organized contractile apparatus: sarcomeres
- Responds to its own self-excitable cells: conduction system
- Permanent rhythmic contraction
- Spatial contraction: because of spatial character of contraction, cardiac muscle cells are branched and form a 3-dimensional network, as also does their contractile apparatus
- Numerous capillary vessels are located between cardiac muscle cells



7.4.1 – Cardiac Muscle Cells

- Called cardiomyocytes
- 1-2 centrally located nuclei with organelles surrounding it
- Branched bundles of myofilaments organized into sarcomeres, between numerous mitochondria
- Cannot regenerate. After damage they are replaced by fibrous connective tissue, leading to heart failure
- Cardiac muscle cells are interconnected end-to-end by multiple junctional complexes, intercalated discs, containing desmosomes, adhesion junctions and gap junctions
- Atrial cells are smaller than ventricular cells, have no T-tubules and some of them produce a hormone (atrial natriuretic peptide)

I. T-tubules and sarcoplasmic reticulum

- Same function as in the skeletal muscle, but differ slightly in morphology and mechanism of action
    1. Wider, glycocalyx-lined T-tubules at the levels of Z-line
    2. Smaller terminal cisternae of SR
    3. Diads instead of triads (single cisterna adjacent to T-tubule)
    4. Voltage-gated calcium channels in T tubules
    5. Amplification: initial increase in Ca level opens other Ca-gated calcium channels in SR

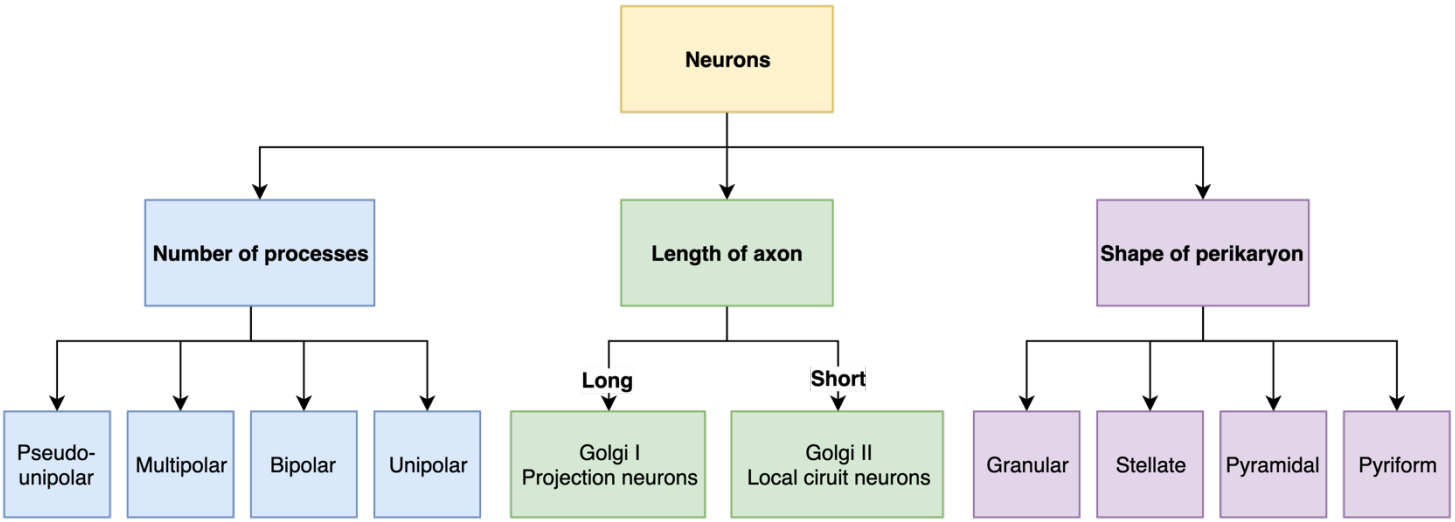
II. Cardiomyocytes of the conduction system

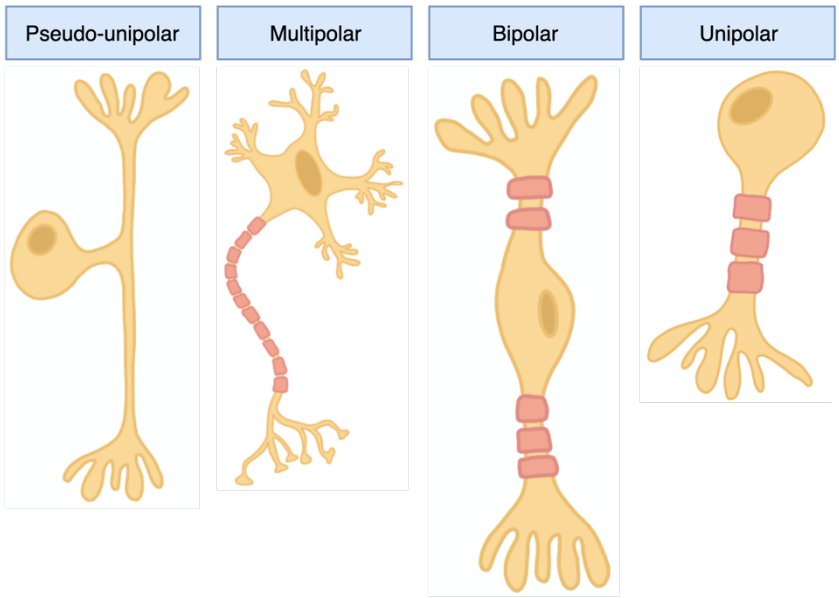
- Cells of the conduction system are primitive cardiomyocytes with poor contractile apparatus and no T tubules
- Numerous gap junctions
- Sinoatrial node, atrioventricular node: spontaneous, rhythmic depolarization
- Bundle of His, Purkinje fibers: bundles of cells connected by gap junctions with each other and with working cardiomyocytes (propagation of stimuli)

Section 8 – Nerve Tissue

- Neurons (nerve cells): signaling and neurosecretion
- Neuroglial cells: protection and support
- Extracellular substance: almost none, basal laminae around some neuroglial cells

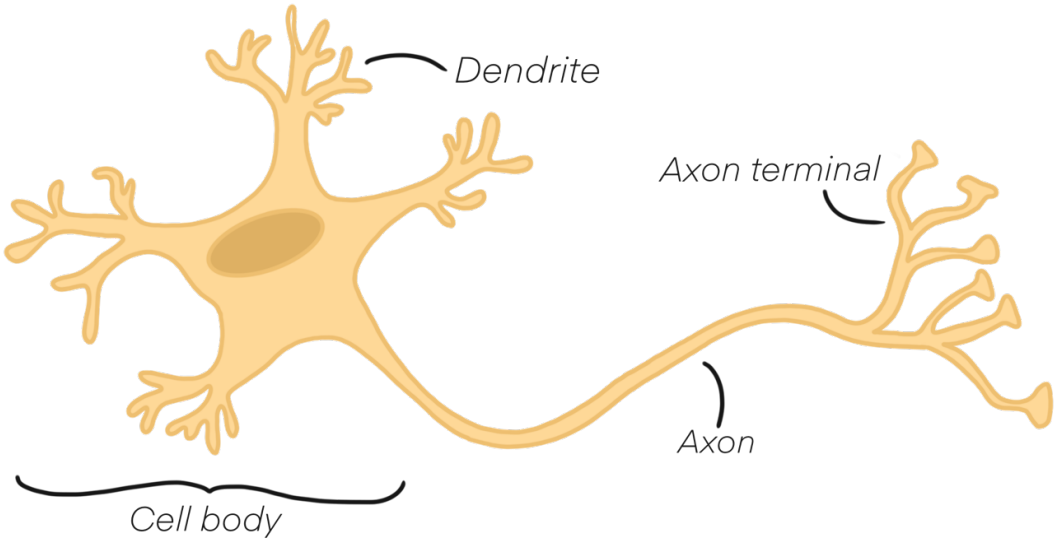
8.1 – Neurons





I. Structure

- Consist of 2 main parts: the cell body (perikaryon) and processes (dendrites and axon)
- Dendrites: a variable number of shorter, branched processes.
    1. Contain Nissl bodies
    2. Do not have voltage-gated sodium channels
    3. Centripetal conduction: the signals moves *towards* the center (i.e the cell body)
- Axon: A single, longer, less branched process
    1. Does not contain Nissl bodies
    2. Has voltage-gated sodium channels
    3. Covered by myelin sheaths
    4. Centrifugal conduction: signals move *away* from the center (cell body)
- Organelles: Nissl bodies, Golgi, mitochondria and lysosomes
- Pigment granules: Neuromelanin and lipofuscin



II. Nissl bodies

- Extensive protein-producing machinery required for turnover of membrane and cytoplasmic proteins
- Located in the cell body and dendrites
    1. Never in axons
- Light microscope: basophilic clumps
- Electron microscope: aggregates of RER and free ribosomes.

III. Neurofibrils

- Bundles of two cytoskeletal structures:
    1. Neurofilaments = intermediate filaments
    2. Neurotubules = microtubules
- Neurofilaments play a supporting role
- Neurotubules: cooperates with motor enzymes, govern the transport of organelles, vesicles and large molecules in the perikaryon and inside processes.
    1. In the axon, it is called axonal transport: anterograde with kinesin and retrograde with dynein

8.2 – Nerve Signals

8.2.1 – Synapses

- The synaptic junctions: connection between a nerve ending and
    1. Another nerve at different locations: axodendritic, axosomatic, axoaxonic
    2. Muscle fibers: the motor end plate

Part of synapse	Content	Synaptic transmission
Presynaptic part	Synaptic vesicles with neurotransmitters Mitochondria Voltage-gated calcium channels Active zone = the area close to presynaptic membrane	1) Action potential arrives at the presynaptic part 2) Voltage-gated calcium channels open 3) Increase in cytoplasmic Ca^{2+} concentration triggers massive exocytosis of synaptic vesicles
Synaptic cleft	Cadherins connecting pre- and postsynaptic membrane	4) Neurotransmitter molecules diffuse across synaptic cleft and bind to postsynaptic membrane receptors
Postsynaptic part	Postsynaptic membrane with receptors for neurotransmitters Postsynaptic density: protein network, a special variant of membrane skeleton	5) Ion channels open in the postsynaptic membrane

- Neurotransmitter molecules released to the cleft but not bound by the receptors are mostly reabsorbed to the presynaptic part by receptor-mediated endocytosis
- Signals transmitted via synapses are conducted by dendrites as weak electric currents (small postsynaptic potentials)
    1. They are summed in the perikaryon: great postsynaptic potential (GPP)
    2. If GPP is large enough, it triggers an action potential in the initial segment of the axon
- Nerve terminals do not make synapses with smooth muscle and glandular cells. Signals are transmitted in a paracrine way: neurotransmitters diffuse through the extracellular substance

CLINICAL CORRELATION

Botulism

Botulinum toxin released by the bacterium Clostridium botulinum cleaves the presynaptic SNARE proteins needed to release the presynaptic vesicle. The result is flaccid paralysis that can cause death from paralysis of the respiratory muscles. Patients can remain in the hospital for months for supportive treatment in severe cases!

I. Control of neurotransmitter release

- Exocytosis of neurotransmitters is controlled by numerous proteins, some in the presynaptic membrane and some in the vesicle membrane
- Presynaptic membrane: fusion proteins from SNARE family: syntaxin and SNAP-25
- Vesicle membrane:
    1. Proteins linking vesicles to actin filaments and facilitating their aggregation: synapsins and rab3
    2. Proteins docking vesicles to presynaptic membrane: synaptophysin and synaptotagmin
    3. Fusion proteins from SNARE family: synaptobrevin

II. Type of synapse

- Type of synapse depends on character of postsynaptic receptors and their mode of action

Synapse type	Channels that open	Effect
Excitatory	Cationic: Na^+, Ca^{2+}	Postsynaptic membrane depolarization
Inhibitory	Anionic: Cl^-	Postsynaptic membrane hyperpolarization

- The type of receptors in the postsynaptic membrane determines what type of response the postsynaptic cell elicits:
    1. Ionotropic receptors: neurotransmitter-gated ion channels that leads to an *immediate* response
    2. Metabotropic receptors: stimulate metabolic reactions in the postsynaptic cell, also leading to opening of ion channels but after a slight delay
- Ribbon synapses: continuously release neurotransmitters for a long time
    1. Large presynaptic part with numerous synaptic vesicles and intense reabsorption of neurotransmitters
    2. Protein strands in the presynaptic membrane bind vesicles and keeps them in the active zone: *synaptic ribbons*
    3. Rare: located in retina and in sensory areas of the inner ear
- Electrical synapses (very rare in mammals)
    1. Gap junctions between nerve cells directly transmit depolarization from the presynaptic to the postsynaptic membrane: avoids the 0.5 ms delay characteristic for chemical (neurotransmitter-mediated) synapses
    2. Located in the retina

III. Neurotransmitters

- Acetylcholine is the main neurotransmitter at the neuromuscular junction
- In the CNS the most important neurotransmitters are:
    1. Gamma-aminobutyrate (GABA): main inhibitory neurotransmitter in the brain
    2. Glycine: main inhibitory neurotransmitter in the spinal cord
    3. Glutamate: main excitatory neurotransmitter in all of CNS
    4. Dopamine, serotonin, opioids (endorphins) etc. have a wide range of CNS effects

<table>
<tr><th>Chemical group</th><th>Neurotransmitter</th><th>Effect</th><th>Type of postsynaptic receptors</th></tr>
<tr><td>Choline compound</td><td>Acetylcholine</td><td rowspan="3">Excitatory</td><td rowspan="2">Metabotropic and ionotropic</td></tr>
<tr><td rowspan="3">Biogenic amines</td><td>Serotonin</td></tr>
<tr><td>Noradrenaline</td><td rowspan="2">Metabotropic</td></tr>
<tr><td>Dopamine</td><td rowspan="3">Inhibitory</td></tr>
<tr><td rowspan="3">Amino acids</td><td>Glycine</td><td>Ionotropic</td></tr>
<tr><td>GABA</td><td rowspan="2">Metabotropic and ionotropic</td></tr>
<tr><td>Glutamate</td><td rowspan="3">Excitatory</td></tr>
<tr><td>Purines</td><td>ATP and GTP</td></tr>
<tr><td>Gases</td><td>Nitric oxide</td><td rowspan="4">Metabotropic</td></tr>
<tr><td rowspan="3">Peptides</td><td>Opioids</td><td>Inhibitory</td></tr>
<tr><td>VIP</td><td rowspan="2">Various</td></tr>
<tr><td>Substance P</td></tr>
</table>

CLINICAL CORRELATION

Depression and anxiety

The levels of neurotransmitters in our brains changes in relation to different illnesses. For example, in anxiety there is elevated levels of the excitatory neurotransmitter noradrenaline and decreased levels of the inhibitory neurotransmitter GABA. In depression, there is decreased levels of serotonin, dopamine and noradrenaline.

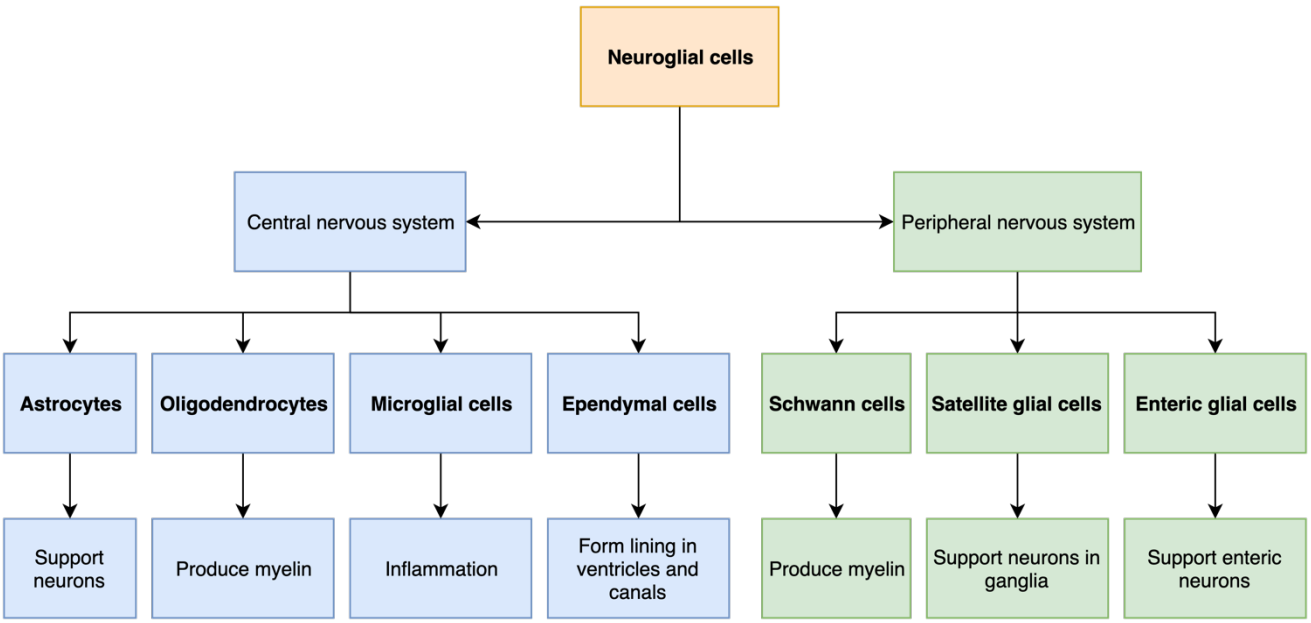
8.2.2 – Action Potential

- Unequal distribution of ions across the nerve cell membrane results in the resting potential (-90 mV) on the cytoplasmic side
    1. Potassium leak channels: open
    2. Sodium channels: closed
    3. Sodium-potassium pump: active
- Successive opening of voltage-gated sodium channels results in the reversal of the membrane potential, called depolarization of the membrane or action potential that moves along the membrane in one direction with the velocity up to 3 m/s
- Unidirectional conduction results from successive states of voltage-gated sodium channel: closed → open → closed and inactivated → closed

(insert curve with 1. Resting potential (Threshold) 2. Depolarization 3. Repolarization)

- Action potential is generated in the initial segment of the axon, where numerous voltage-gated ion channels are located

8.3 – Neuroglial Cells



I. Schwann cells

- Produce myelin sheaths around axons in the *peripheral* nervous system
- Highly elongated as segments of sheaths
- In other locations (e.g. in sensory corpuscles of the skin) can be multiform
- Well-developed organelles (exception: segments of myelin sheath)
- Produce their own basal lamina

II. Astrocytes

- Has many processes that surround blood vessels and neurons
- Resorb ions and neurotransmitters and release glucose, regulatory and trophic factors
- Proliferate and replace damaged tissue at sites of CNS injury
- Protoplasmic (in grey matter) or fibrous (in white matter)

III. Oligodendrocytes

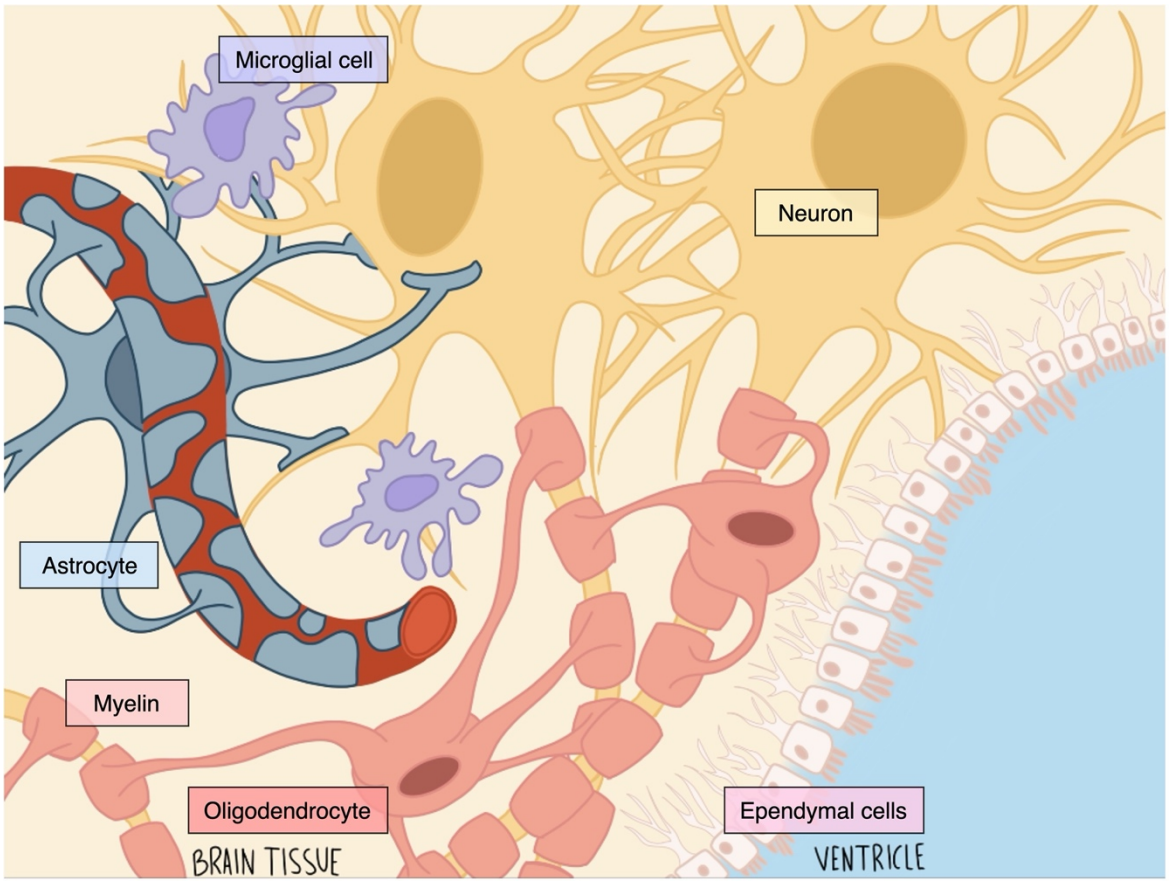
- Produce myelin sheaths around axons in the *central* nervous system
- Few processes
- Well-developed organelles
- A single oligodendrocyte can produce several segments of myelin sheath around several axons located nearby

IV. Microglial cells

- Macrophage-related cells residing in CNS
- Originate from embryonic hematopoietic islets: microglia precursors migrate to the CNS
- When activated, microglial cells change their shape, migrate to the target site, develop intense phagocytotic activity and produce cytokines

V. Ependymal cells

- Form columnar or cuboidal epithelia-like linings of CNS canals and ventricles
- Similar to epithelial cells: may have microvilli, cilia and abundant intercellular junctions, but they mostly do not produce basal lamina
- Specialized ependymal cells form the choroid plexus, which produce cerebrospinal fluid



8.4 – Nerve Fibers

- Nerve fiber = axon surrounded by a sheath
- The sheaths are produced by neuroglial cells: in PNS by Schwann cells and in CNS by oligodendrocytes and astrocytes
- Since there are two types of sheaths, nerve fibres can be:
    1. Myelinated (axons have myelin sheath)
    2. Unmyelinated (axons have a simple cytoplasmic sheath, or – rarely – no sheath)
- In unmyelinated peripheral nerve fiber, the axon is located in the invagination of Schwann cell membrane. A single Schwann cell can form such invaginations for many axons
- Unmyelinated axons have regularly distributed voltage-gated sodium channels and conduct the signals by depolarization wave (continuous conduction, 1-3 m/s)
- In myelinated nerve fibre, the axon is surrounded by a specialized myelin sheath, also produced by Schwann cell
- Myelinated axons have irregularly distributed voltage-gated sodium channels and conduct the signals by a combination of membrane depolarization and electric current (saltatory conduction, up to 120 m/s)

I. Myelinization

- Invaginations of Schwann cell membrane (mesaxon) wraps many times around the axon leading to formation of a tight, concentric array of multiple phospholipid layers. Most proteins are eliminated during this process.
- One Schwann cell forms one segment of the myelin sheath. The site where the two segments meet is called a Node of Ranvier,
- The axonal membrane (axolemma) contains numerous voltage-gated sodium channels propagating the depolarization
- Nerve signals „jump" from one node to the next one = *saltatory conduction*. The action potential moves through the axonal cytoplasm in the myelinated parts of the axon as a weak electrical current (fast phase) and is restored in the node by axon membrane depolarization (slow phase). Myelin sheath acts as electric insulator.
- There are several types of nerve fibers, with different functions and velocity of conduction. The velocity depends on:
    1. Presence and length of myelin sheath
    2. Thickness of myelin sheath
    3. Thickness of axon
- Three types of nerve fibers:
    1. A: thick myelinated: 15-120 m/s
    2. B: thin myelinated: 3-15 m/s
    3. C: unmyelinated: 1-3 m/s

8.5 – The Nervous System

8.5.1 – Peripheral Nervous System

- Components
    1. Epineurium: dense connective tissue
    2. Perineurium: several layers of flat fibroblasts
    3. Bundles of nerve fibers
- Posterior root ganglion contains pseudounipolar ganglionic cells, satellite glial cells, myelinated nerve fibres and capillaries
- Satellite glial cells resemble astrocytes and perform similar functions
- Autonomic ganglia: contain multipolar nerve cells surrounded by satellite glial cells
    1. Sympathetic ganglia
    2. Parasympathetic ganglia
    3. Intramural ganglia (e.g. in the wall of alimentary canal)

8.5.2 – Central Nervous System

The brain

Grey matter	Contain perikaryons of nerve cells and mostly unmyelinated nerve fibers Astrocytes and numerous blood vessels
White matter	Mostly myelinated nerve fibers and no perikaryons Numerous oligodendrocytes and fibrous astrocytes Less abundant blood vessels
Cerebral cortex	Has 6 layers: 1) Molecular 2) Outer granular 3) Outer pyramidal 4) Inner granular 5) Inner pyramidal 6) Multiform
Cerebellar cortex	Has 3 layers: 1) Molecular: stellate cells and basket cells 2) Ganglionic (Purkinje cells): have immensely branched dendrites and process signals from other cerebellar cells 3) Granular: granule cells and Golgi cells

- Cerebellar cortex: the purkinje layer
    1. Purkinje cells have dendrites immensely branched, but only in one plane
    2. Perikaryon of Purkinje cell is surrounded by „basket” – plexus of nerve fibers
    3. Although there are several nerve cell types in the cerebellar cortex, only axons of Purkinje cells leave the cerebellum

I. Neural stem cells

- Localization in the central nervous system:
- Subventricular zone (around lateral ventricles)
- Subgranular zone (hippocampal dentate gyrus)
- Niche components: stem cells progenitor cells neuroblasts
- Neuroglial cells (ependyme)
- Capillaries
- Extracellular substance
- Neural stem cells in culture differentiate into neurons, astrocytes and oligodendrocytes

II. Blood-brain barrier

- Components:
    1. Endothelial cells of blood vessel
    2. Basal lamina
    3. Layer of astrocyte processes
- Extremely selective permeability of blood vessels in the brain is caused by tight junctions between endothelial cells and selective transport proteins in the endothelial cell membrane

III. The meninges

- Dura mater: dense connective tissue
- Arachnoid: fibroelastic connective tissue forming trabeculae lined with fibroblasts
- Pia mater: delicate loose connective tissue with numerous cells. It is separated from neural tissue by layer of astrocyte processes (glial limiting membrane)
- Folds of pia mater
    1. Contain numerous fenestrated capillaries
    2. Lined with ion-transporting ependymal cells which form simple cuboidal layer
    3. Ependymal cells transport ions (Na, K, HCO_3) from blood to cerebrospinal fluid, water follows ions
- The choroid plexus, made up by modified ependymal cells, produces cerebrospinal fluid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