Cell biology

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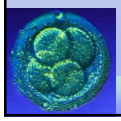


Syllabus

Lectures:

1. differentiation of animal cells in respect to their functions and morphology
2. mechanisms of differentiation and dedifferentiation (regeneration)

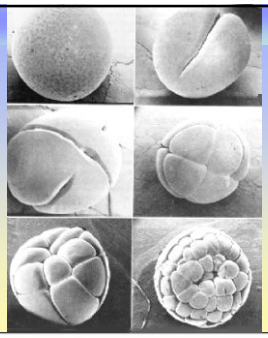
Laboratory courses:

1. how to use pipette in cell culturing techniques
2. types of cell culture and their maintenance

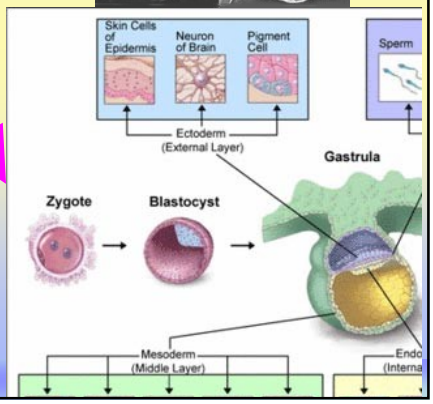
Slides and useful links are on web page of DAFE
https://fns.uniba.sk/pracoviska/biologicka-sekcia/kzf/studenti/bakalarske-studium/

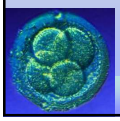
Differentiation

- development and differentiation during **embryogenesis**
- three germ leaf



- development and differentiation **in mature individuals**
  - blood cells
  - epithels





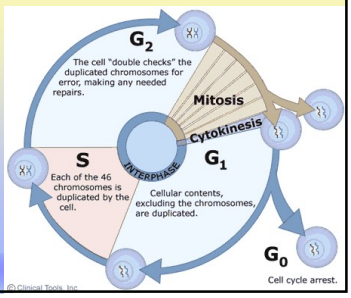
What does happen during the cell cycle?

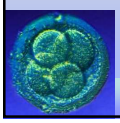
1. generation of new organelles and DNA replication
2. delivery of synthesized compounds into newly formed cells

- all activities must be properly coordinated and timed

- G1(*gap*) phase	- synthesis of mRNA, proteins, organels, preparation for DNA repolication
- S (*synthesis*) phase	- DNA replication
- G2 (*gap*) fáza	- syntéza mRNA, proteínov, organel, príprava na mitózu
- M (*mitosis*) fáza	- mitóza a cytokinéza

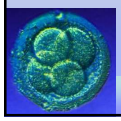
Attention! cell cycle is not mitosis, mitosis in only one part of cell cycle



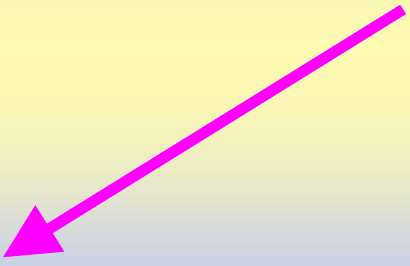


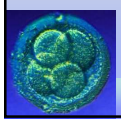
Is the course of cell cycle the same during our lives?

dividing cell

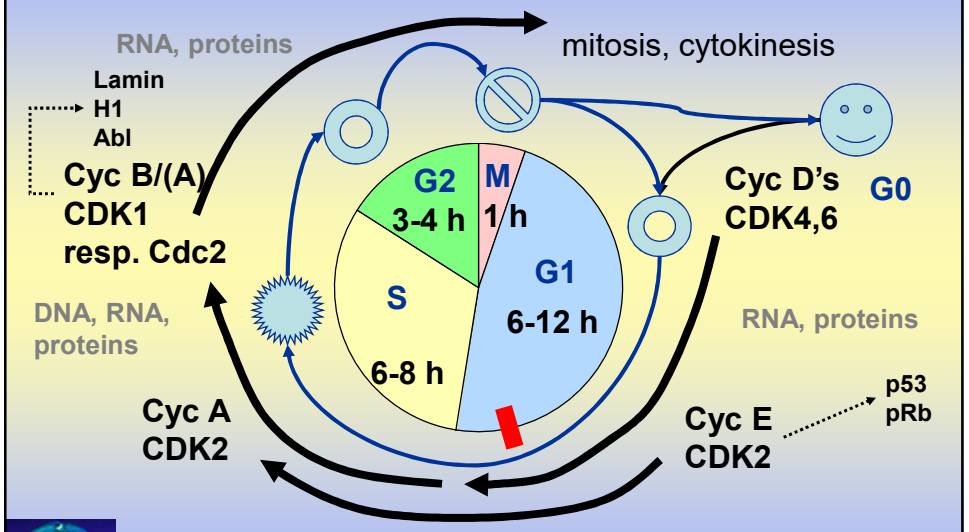


- during embryonic development:
  cell division is synchronous
and the identical cells are arising





Cell cycle control in animal cells



Cell cycle

- regulatory systems of cell cycle has to activate enzymes and proteins necessary for the next step of the cell cycle and deactivate it after they are not needed

- each step of cell cycle must be correctly finished before beginning of the next step

e.g. new DNA replication can not be initiated before cell finis mitosis and acquire necessary size

- regulatory systems has to be responsive to external conditions

Are there some special features of the cell cycle in multicellular organisms?

- in multicellular organisms cell must be responsive to demands of other cells of the body, *e.g. chemicals inducing cell proliferation when growth of tissue is needed*

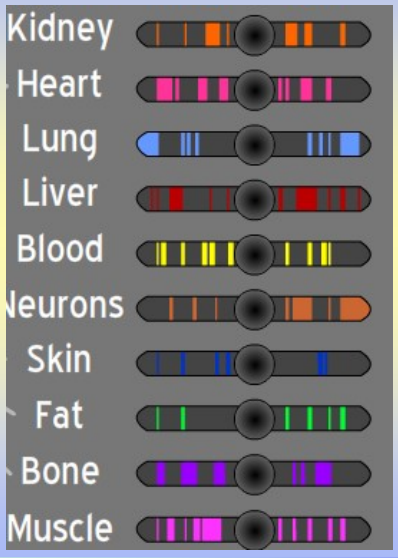
- cell number can be adjusted by programmed cell death (apoptosis)

- if regulation of the cell cycle is not responsive to regulation cancer can develop

- **G1**, S, G2, M phase

http://outreach.mcb.harvard.edu/animations/preloaderStemCells.swf

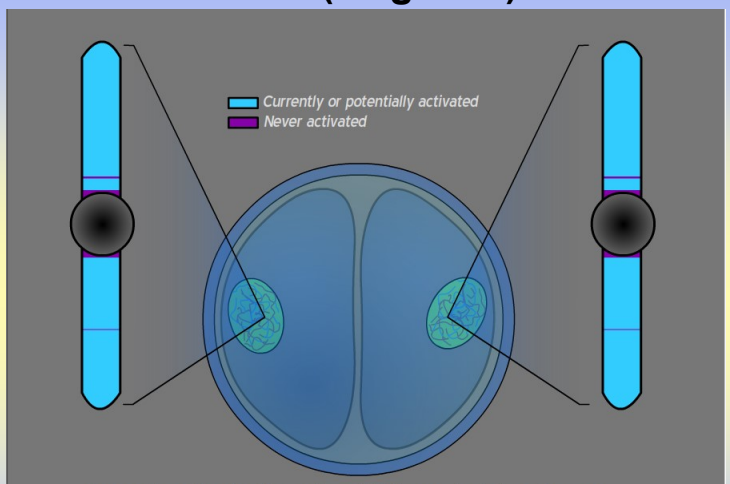
Is DNA (genes) in different tissues the same?



What is a role of cyclines in the cell cycle?

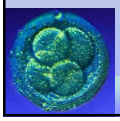
Link 0.5

Is whole DNA (all genes) active?

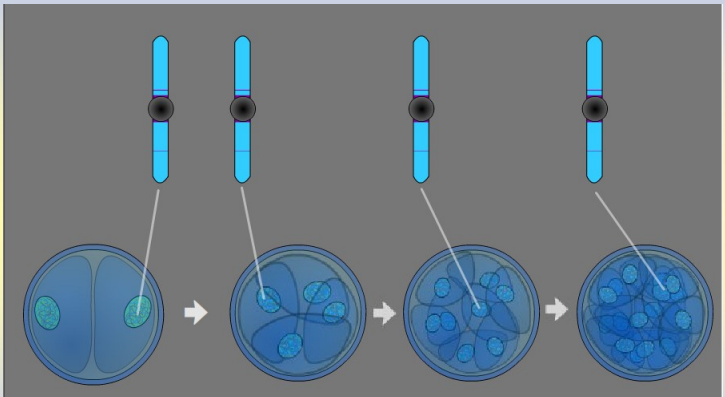


Importantly, both cells still have total potency, and can give rise to all other cells.

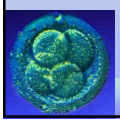
They are called TOTIPOTENT STEM CELLS.



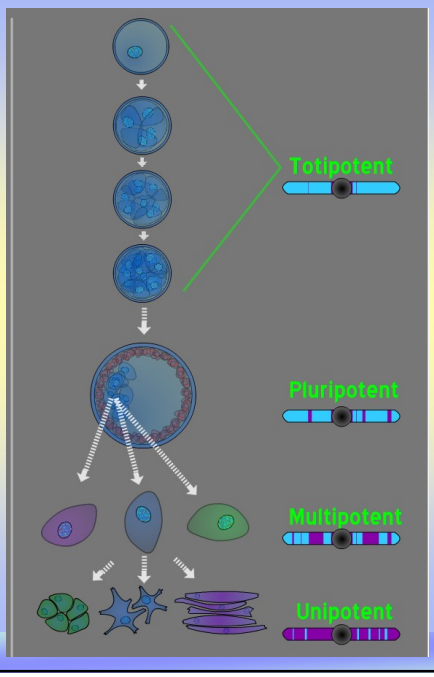
Is amount of used DNA still the same during the differentiation?

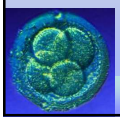


Stem cells remain totipotent through several rounds of division.

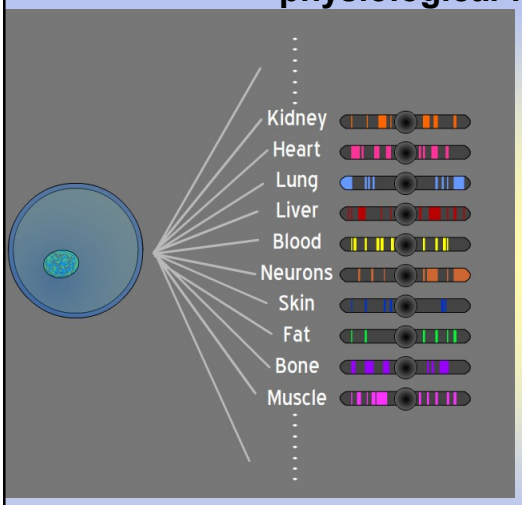


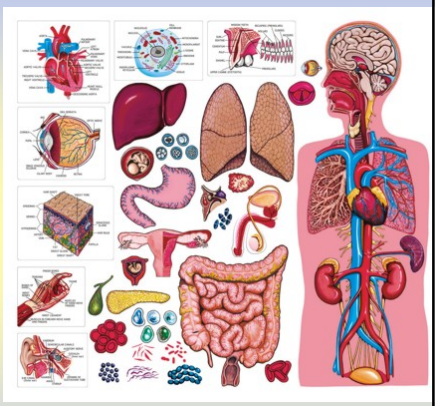
What is the difference between toti- a uni- potent cell?

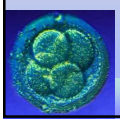




What is relationship between active genes and physiological functions?







http://outreach.mcb.harvard.edu/animations/preloaderStemCells.swf

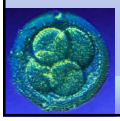
Differentiation - principle

- cells express different morphological, physiological na biochemical properties

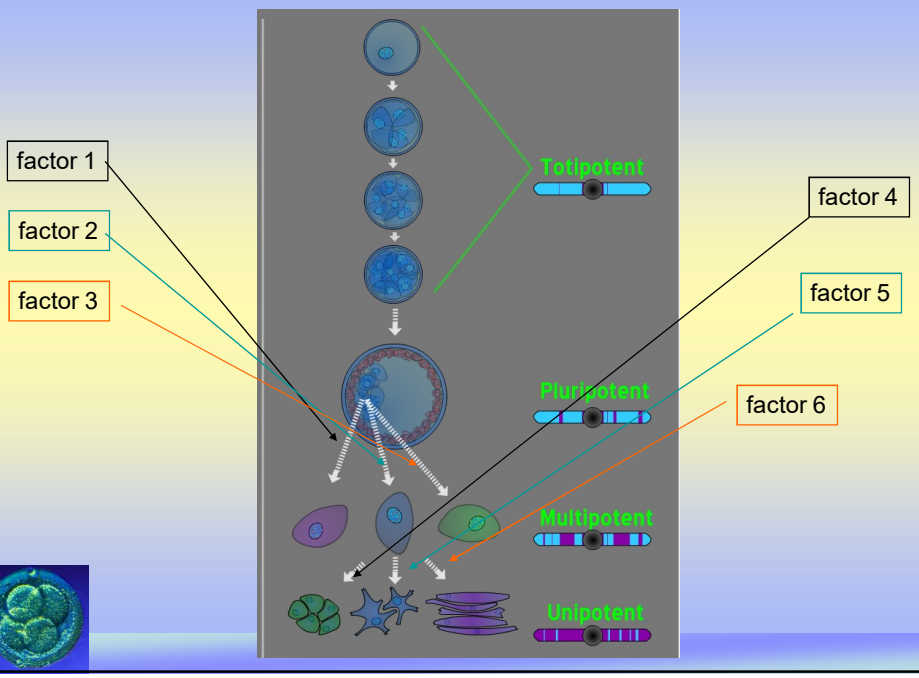
- it is caused by differently expressed genes (specific parts of DNA via corresponding mRNA and proteins exhibit different functions)

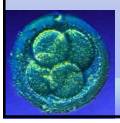
What does cause gene expression differences?

- factors influencing gene expression (physical, chemical)
  - **coming from external environment**
  - **coming from other cells**
    - cell signaling – in next lectures

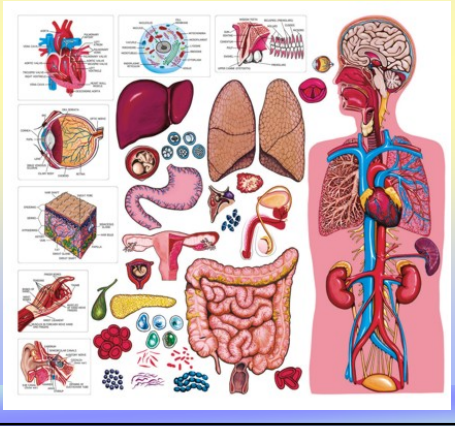


Differentiation - principle





in human body cells differentiate to be able to fulfill their specific physiologic functions
- they can perform their role as single cells
- or as a part of tissue or organ
- in both cases they have to cooperate with all other cells, tissues and organs in the body



Examples of differentiated cells and their physiological function

- cell with high metabolism that live during whole human life

- short time living cells with low metabolism

- short time living cells with high metabolism

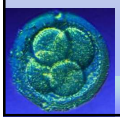
What is differentiation?

Link 1

What kind of cell is typical by high metabolism and lasts for decades?

- the smallest unit of major regulatory and integrating system of the human body
- it is able to quickly and precisely convey information from receptors to central neural system and mediate the response to effectors
- they created complicated network

neurons



The central neural system is the main regulatory and integrative structure of the organism

- regulates or modulates functioning of all organs, coordinates their functioning and relationships in respect to organism needs
- it closely cooperates with endocrine system
- the primary role of NS in respect to ES is given by these features:

- accuracy, speed and capacity of information registration
- prompt new data processing and analysis in context with data in memory storage
- synthesis and output information to maintain homeostasis

Principles of neural system development:

1. functional differentiation of neural cells
2. concentration of neural cells into ganglia
3. centralization, development of brain ganglia, bilateral symmetry
4. increase in number of neural cells

Which type of cell can express more genes?

Link 2

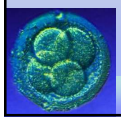
Neuron
structure: cell body (soma), dendrites, axon (neurite), axon hillock, initial segment, myelin sheath

Nucleus (contain DNA, regulated cell development and activity)
Mitochondrion (oxidative metabolism, ATP production)
Ribosomes (usually associated with endoplasmatic reticulum, protein synthesis)
Golgiho system (protein modification, sorting and trafiking, membrane renewal)
Lyzosomes (enzymatic degradation)
Vezicules (endocytosis or exocytosis)
Endoplasmatic reticulum – ER (protein and lipid synthesis)

- ***specific features of neuron organeles***

- very robust rough endoplasmic reticulum called Nissl bodies (they absorb Nissl stain very well)

- many vesicles containing neurotransmitters

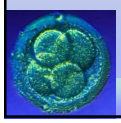


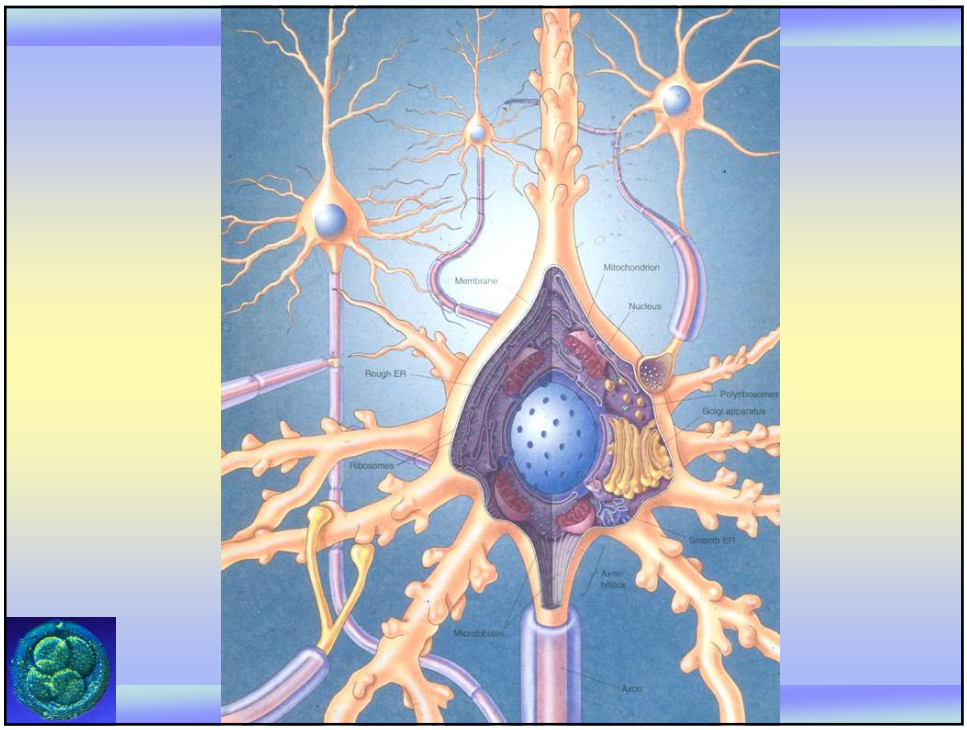
Differentiation of neuron

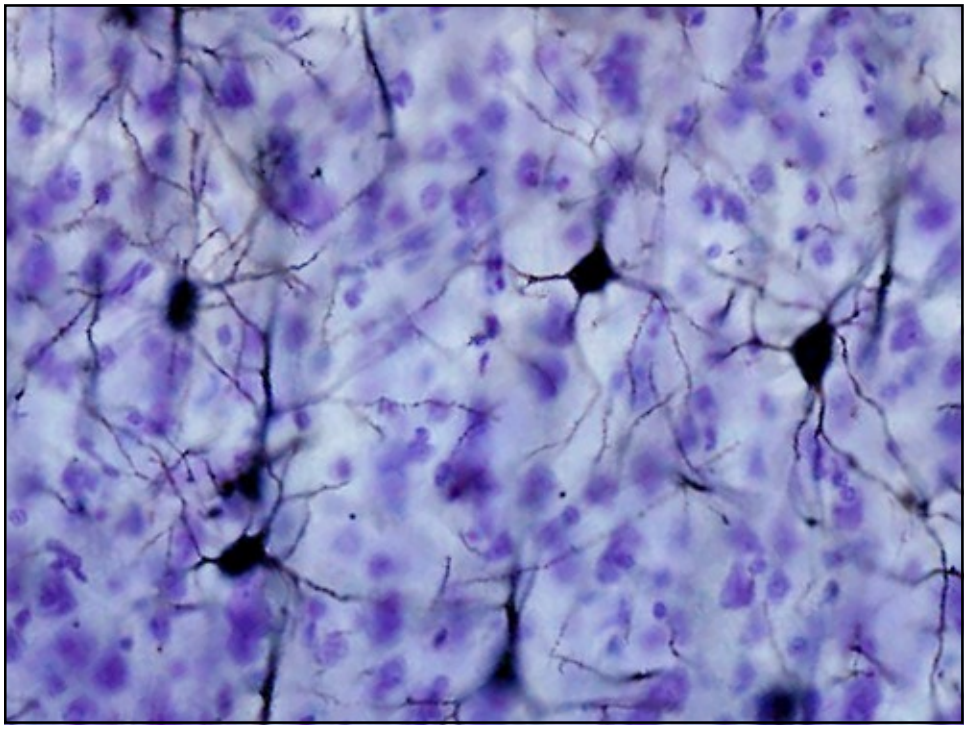
- morphology – cell structures

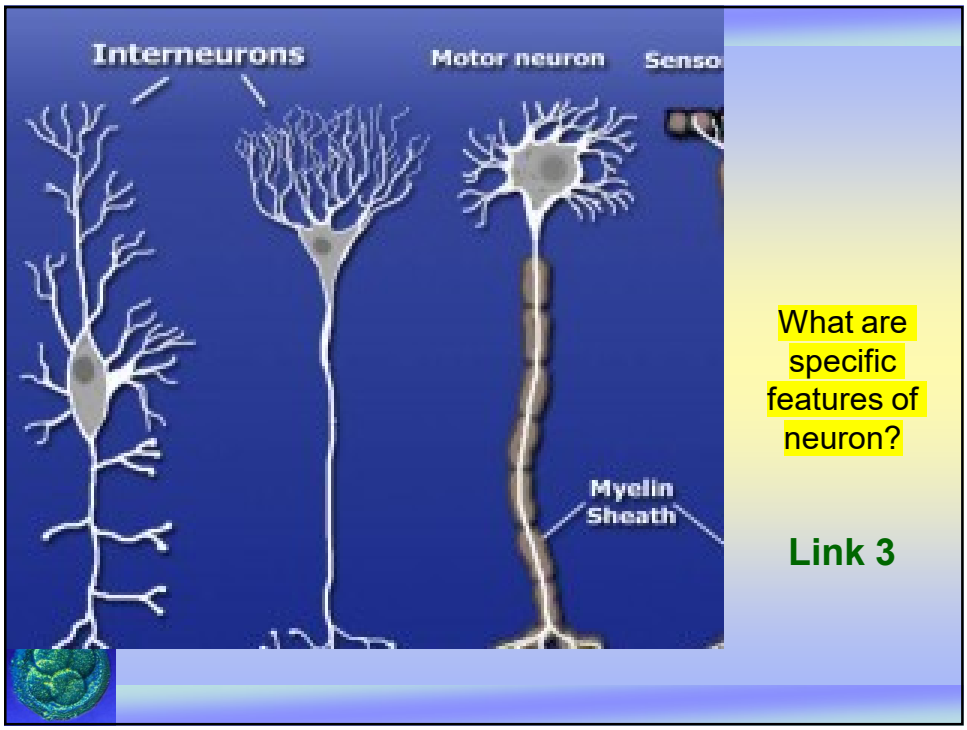
- synthesis of specific neurotransmitters

- cell membrane differs in its features even within single cell



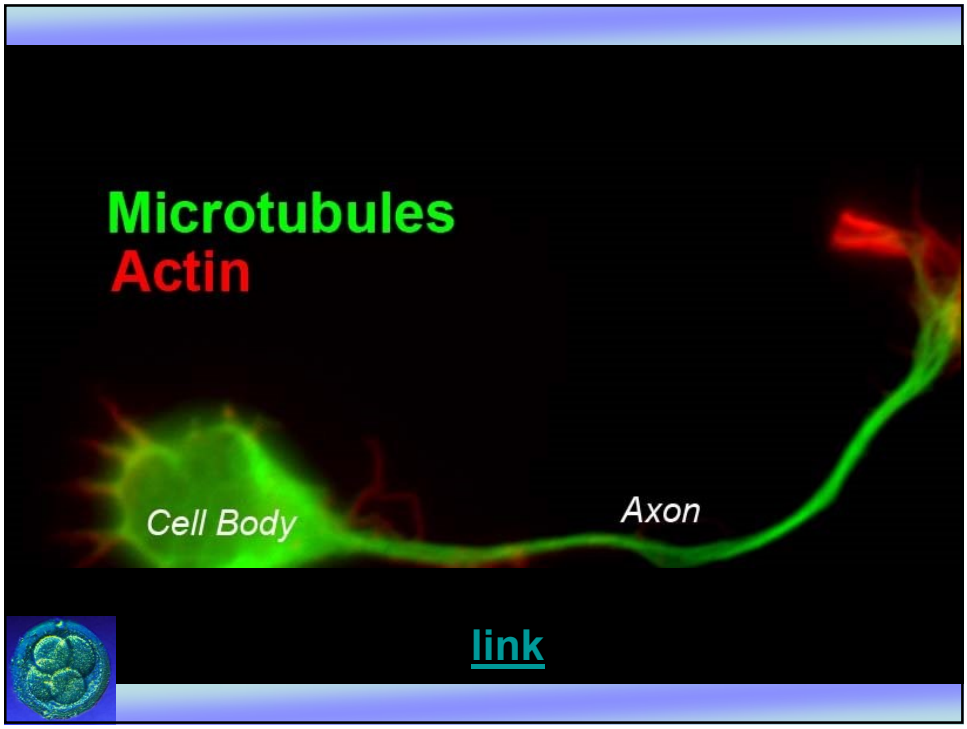

Membrane
Mitochondrion
Nucleus
Rough ER
Polyribosomes
Golgi apparatus
Ribosomes
Smooth ER
Axon hillock
Microtubules
Axon





What are specific features of neuron?

Link 3



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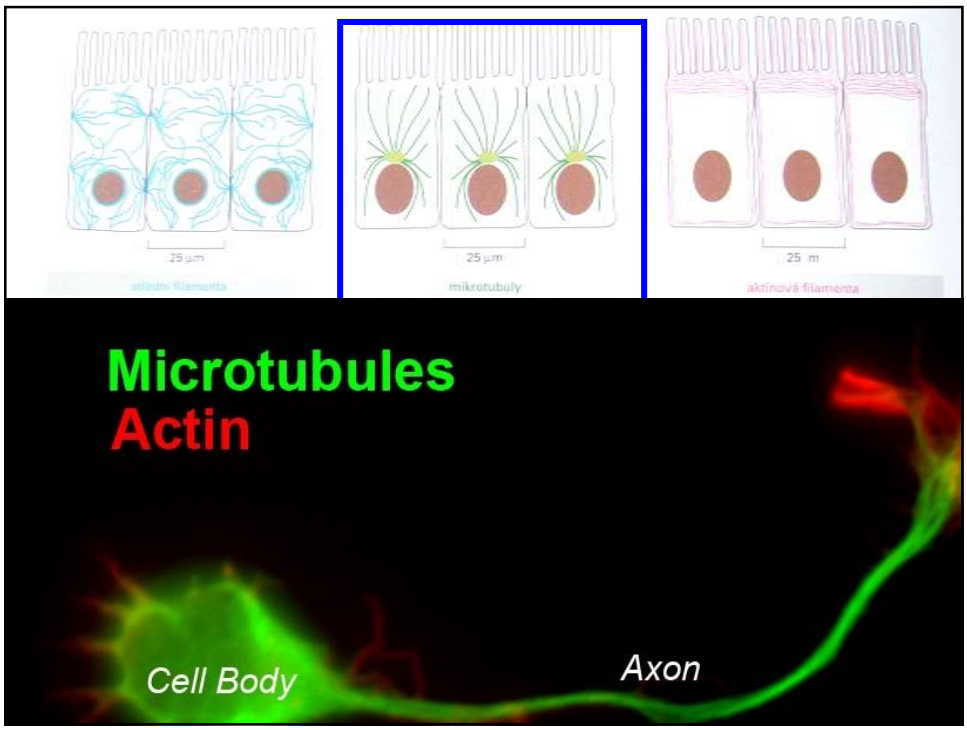
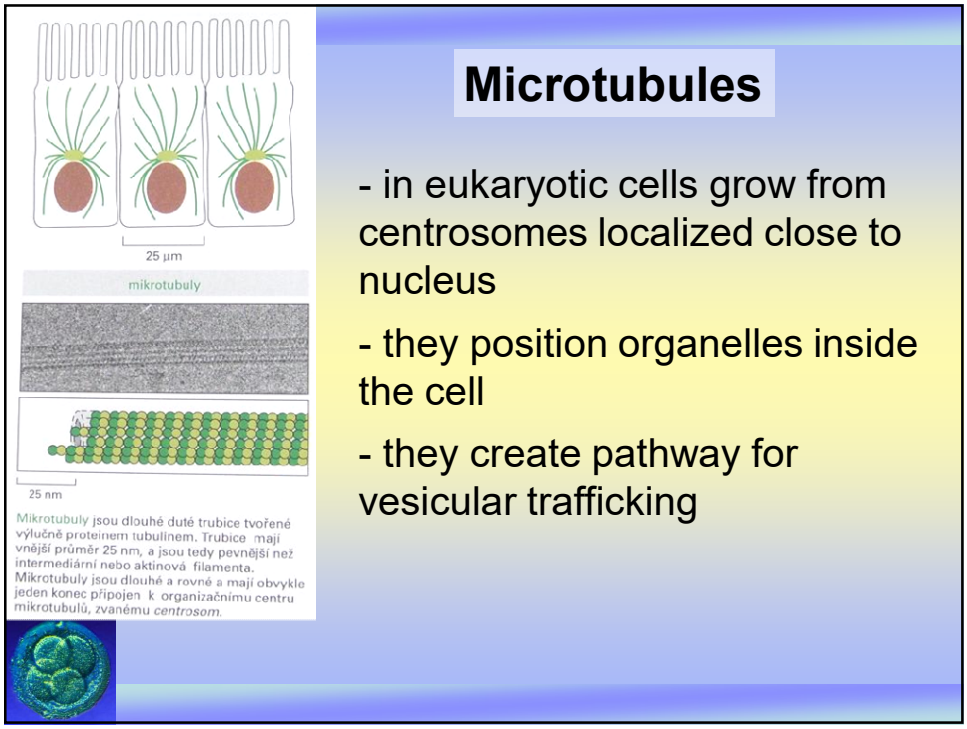
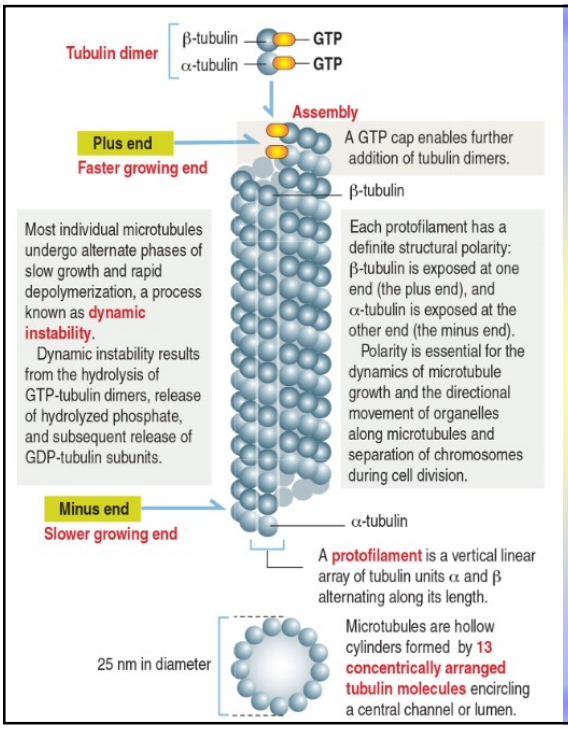

25 µm
mikrotubuly
25 µm
Microtubules
Actin
Cell Body
Axon


25 µm
mikrotubuly
25 nm
Mikrotubuly jsou dlouhé duté trubice tvořené výlučně proteinem tubulinem. Trubice mají vnější průměr 25 nm, a jsou tedy pevnější než intermediární nebo aktinová filamenta. Mikrotubuly jsou dlouhé a rovné a mají obvykle jeden konec připojen k organizačnímu centru mikrotubulů, zvanému centrosom.
Microtubules
- in eukaryotic cells grow from centrosomes localized close to nucleus
- they position organelles inside the cell
- they create pathway for vesicular trafficking



Microtubular structure

- tubulin dimer binding GTP, after GTP hydrolysis tubulin dimers dissociate
- globular proteins: α-tubulin and β-tubulin
- tubulin dimers create protofilament, 13 protofilaments create tubulin cylinder 25nm in diameter

α-tubulin end = minus end

β-tubulin end = plus end (growing)

Axonal transport

Anterograde transport – from cell body to synapse

0.5 mm/days - 400 mm/day

quick transport – up to 410 mm/day, molecules including complex proteins packed into vesicles

intermediate transport - cca 200 mm/day

slow transport – less than 6 mm/day, transport of proteins (not packed in vesicles)

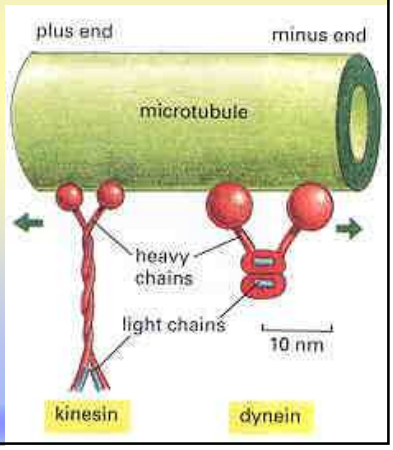
Retrograde transport – transport from periphery to cell body – only material packed into vesicles - 200 mm/days

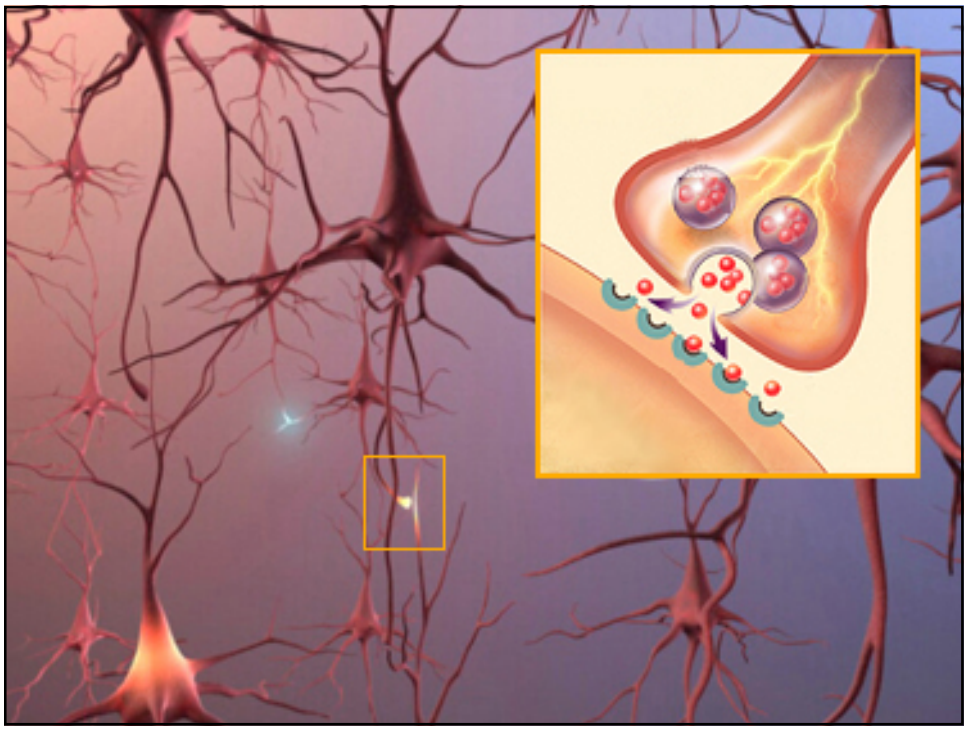
Motor proteins: kinesin discovered first **kinesin (anterograde transport)**:

- two ATP-binding heads and a tail. Energy derives from continuous ATP hydrolysis by ATPases present in the heads. The head domains interact with microtubules, and the tail binds to specific receptor binding sites on the surface of vesicles and organelles.

- hydrolysis of one ATP mol. – conformation change = 1 step
- 1 step = 8nm = 1 tubulin dimer

dynein - retrograde transport





Neurotransmitters

- **Neurotransmitter** is chemical compound released from presynaptic membrane. It is diffusing through synaptic cleft and allow action potential propagation.

- There are excitatory and inhibitory neurotransmitters.

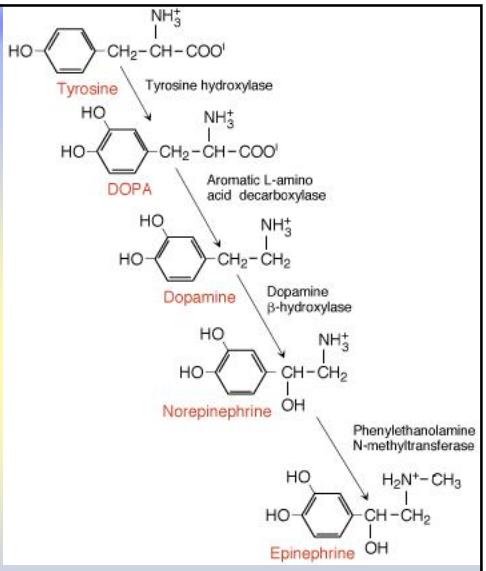
Neurotransmitter definition:

1. It is synthesized in the neuron.
2. It is released in amount sufficient to cause a specific effect.
3. If an exogenous chemical is administered it cause the same effect like endogenous neurotransmitter
4. There is a specific mechanism of neurotransmitter elimination from synaptic cleft

Acetycholine
Biogenic amines:
dopamine
noradrenaline
adrenaline
serotonin
histamine
Aminoacids:
γ-aminobutyric acid (GABA)
glutamate
glycine

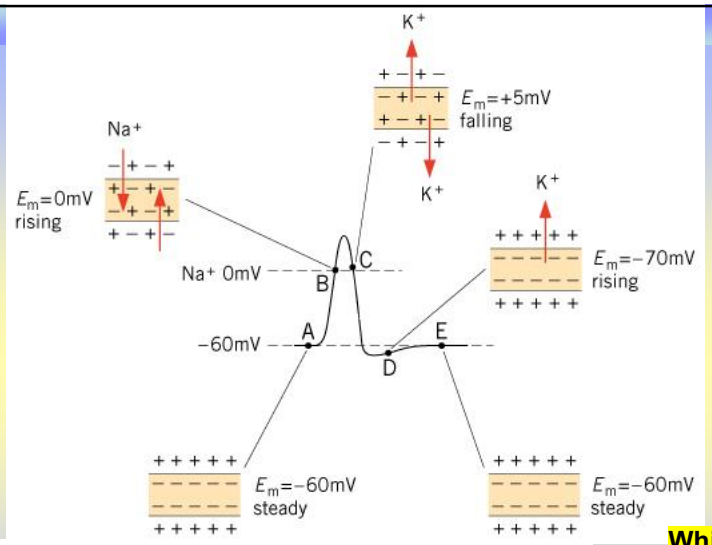
Acetycholine:
• autonomous NS (all preganglionic and parasymphatetic postganglionic connections)
• CNS: spinal cord, medulla oblongata
• neuromuscular connections
receptors:
•***muscarinic type***: in the heart, inhibitor atropine, agonist muscarine
•***nicotinic type***: neuromuscular junction, inhibitor curare, agonist nicotine
it is hydrolyzed by enzyme acetylcholinesterase on postsynaptic membrane

Noradrenalin:
• most of sympathetic postganglionic connections
•CNS: hypothalamus, medulla oblongata, cerebellum
receptors:
•α usually excitatory
•β usually inhibitory
some NA is taken up by glial cells, some is taken up by presynaptic membrane and some is oxidized by MAO or carboxylated by katechol-O-metyltransferase



Neuromodulators:
modulate sensitivity of postsynaptic membrane
endorphíns, enkephalins, P substance, prostaglandins

membrane features differ within one cell in such a way that action potential can be generated only in the axon



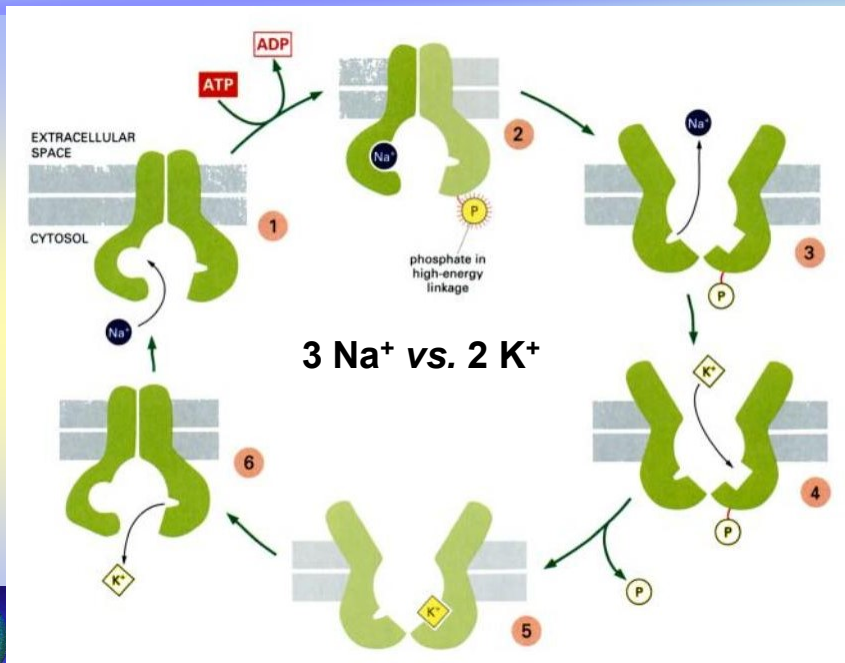
Action potential:

- is basic physiological capacity of neuron
- it is caused by selective change in membrane permeability for ions, which is cause of small local current generation and its propagation and activation of next neuron
- it is generated always in the same way, and it is propagated without decrement
- myelinated axons can propagated action potential more quickly

Which neurotransmiter is inhibitory?

Link 4

link

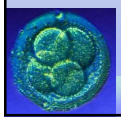


Differentiation

- cell with high metabolism that live during whole human life

- short time living cells with low metabolism

- short time living cells with high metabolism



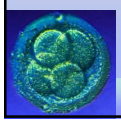
Blood

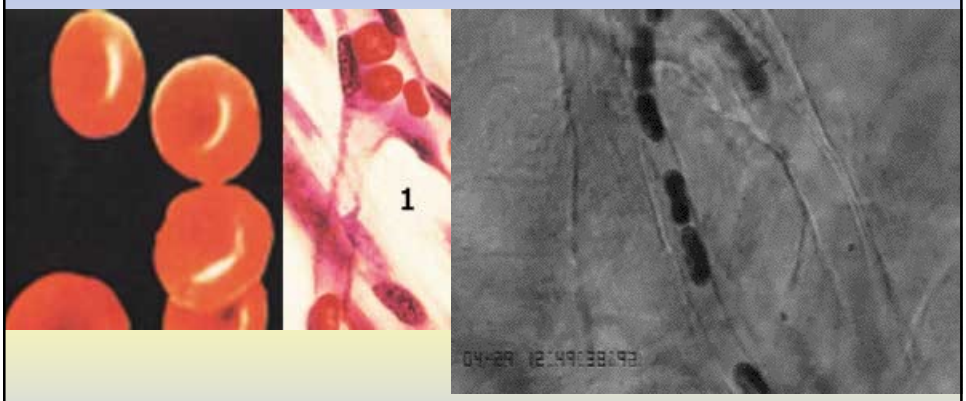
- blood contributes to homeostasis and sustaining of optimal conditions inside the organism significantly

- it is a fluid medium connecting all organs, tissues and cells in the body

How does neuron receive input from other neurons?

Link 5





Red blood cells

Blood functions:

a) Forming internal milieu

b) Delivery of hormones (endocrine regulation) to tissues

c) Transport of O_2 from lungs to tissues and CO_2 from tissues to lungs

d) Transport of metabolites that are absorbed in the intestine and metabolic wastes from tissues.

e) With use of specific antibodies in plasma and white blood cells blood contributes to immune system functioning

f) Thermoregulation (body core temperature and shell)

g) Maintaining optimal pH

h) Strongly influences blood pressure

i) It can protect organism against bleeding by vascular vasoconstriction and activation of blood coagulation

What does happen when neuron fires action potential?

Link 6

Red blood cells (RBC)

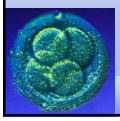
- extremely specialized cell in the human body

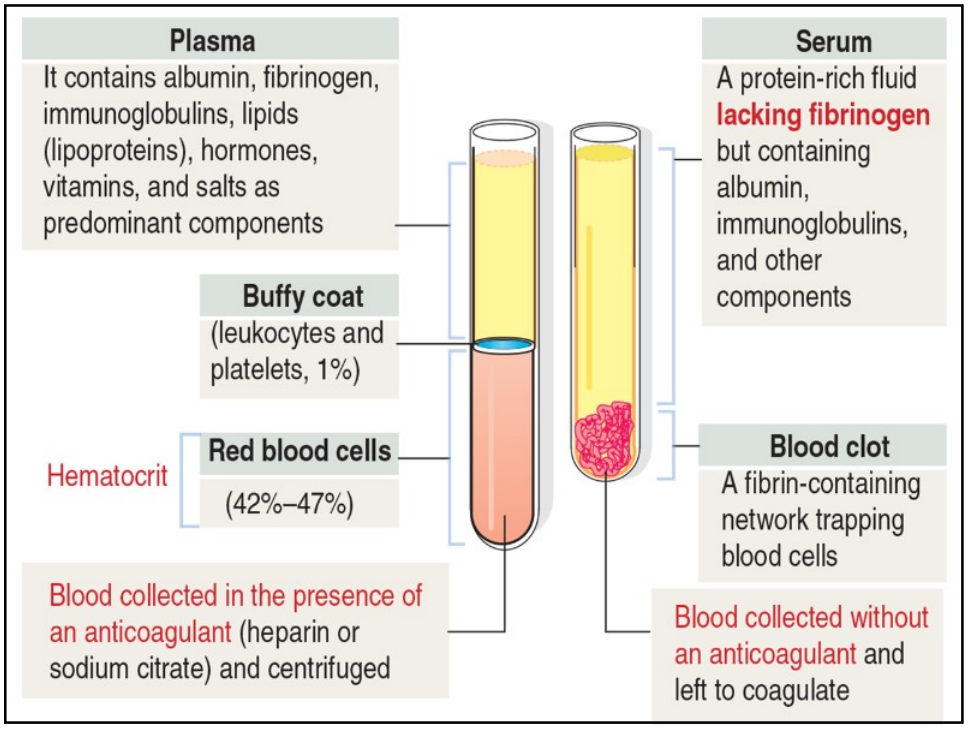
- cell without nucleus and other organelles

- shaped as biconcave disks

- they are present in several forms in the circulation – maturation of RBC

- number of RBC is stable in adult human
  - man 4.3-5.3 *10^{12} per liter
  - women 3.8-4.8 *10^{12} per liter



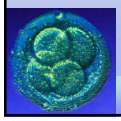


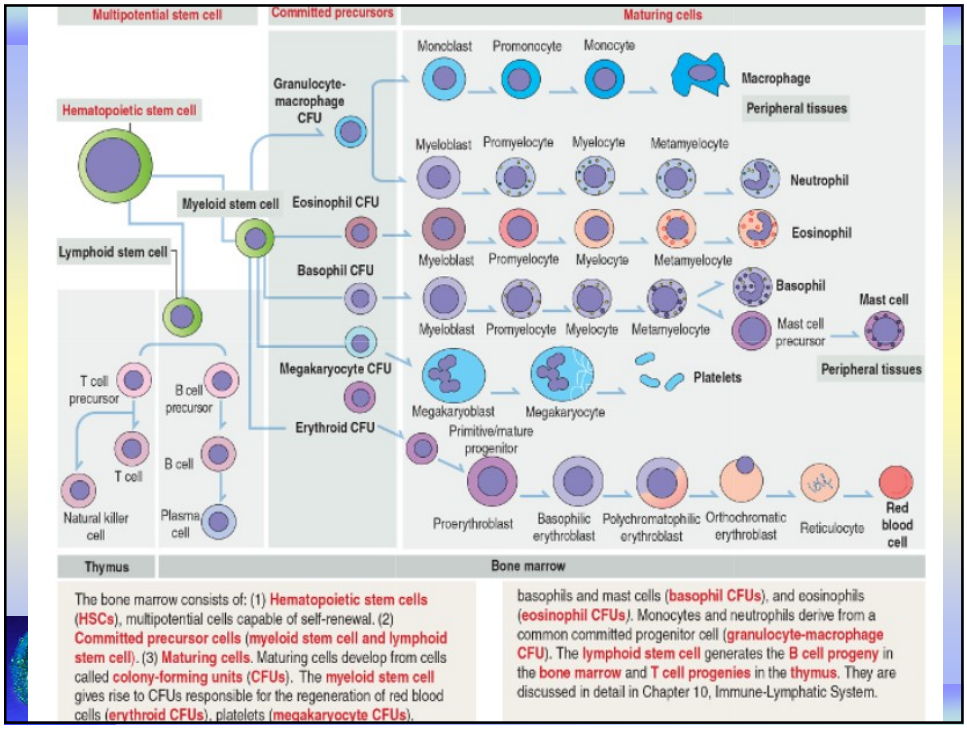
- differentiation is initiated when specific membrane receptors appear on membrane on undifferentiated cell

- these receptors mediate humoral regulatory factors responsible for erythropoiesis initiation (or other kind of differentiation)

- unipotent stem cells emerge, they are already devoted to one specific line of cells

- from specific progenitor cells erythrocytes, megakaryocytes, granulocytes or monocytes can be generated





Blood cell differentiation

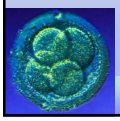
- all blood cells come from **totipotent** stem cell
  - **pluripotent cell for** -
    - ***erythrocytes***
    - ***megakaryocytes***
    - ***granulocytes***
    - ***monocytes***

- a separate stem cell for ***lymphocytes***

- stem cell can differentiate into many different types of cells

What is a function of Na+/K+ ATP dependent pump?

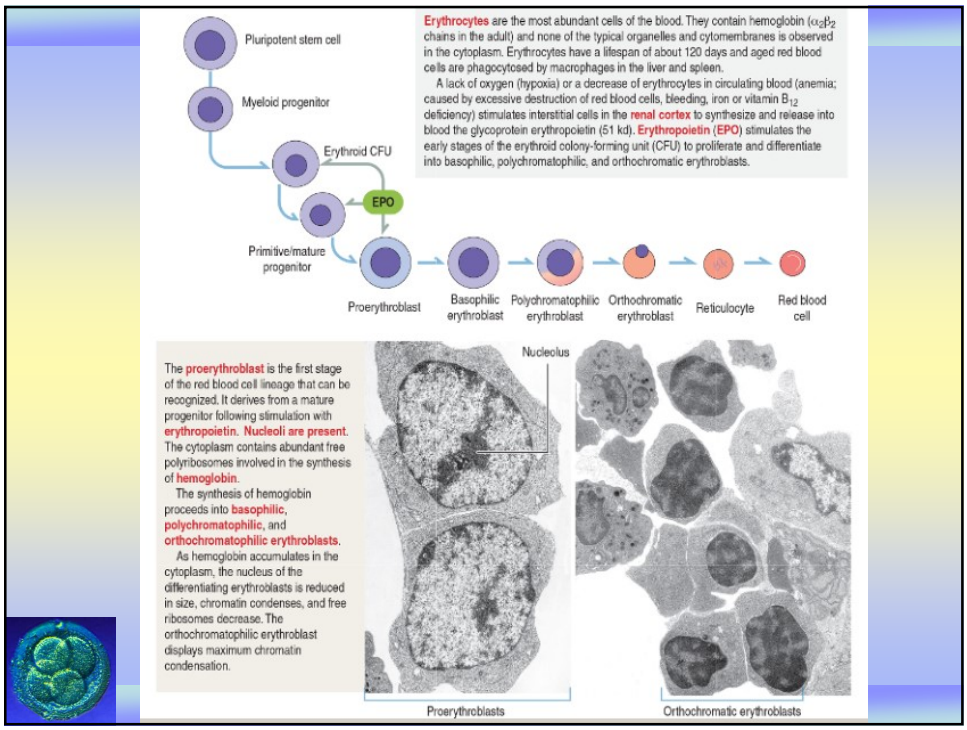
Link 7



Hematopoietic niches

In the **fetus**, hematopoiesis starts during the first trimester in islands of hematopoiesis found in the **yolk sac.** The islands develop from **hemangioblasts**, the progenitors of both hematopoietic and endothelial cells.

Fetal hematopoiesis continues after the second trimester in the **liver** and then in the **spleen**.
During the seventh month of intrauterine life, the **bone marrow** becomes the primary site of hematopoiesis, where it remains during adulthood. In the adult, an approximate volume of 1.7 L of marrow contains 1012 hematopoietic cells, producing about 1 x 109 RBCs and about 1 x 108 leukocytes every hour.



Erythropoesis

- during this process the cell is decreasing its volume and exclusion of the nucleus and other changes in the cytoplasm
- hemoglobine accumulates in the cell, amount of mRNA is slowly decreasing, pH of cytoplasm changes from alkaline to more acidic

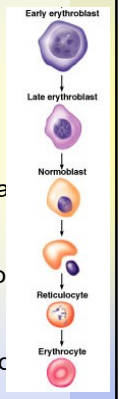
1. big immature cell – **proerythroblast** (pronormoblast)
   diameter15-20 μm, Basophilic staning of cytoplasm

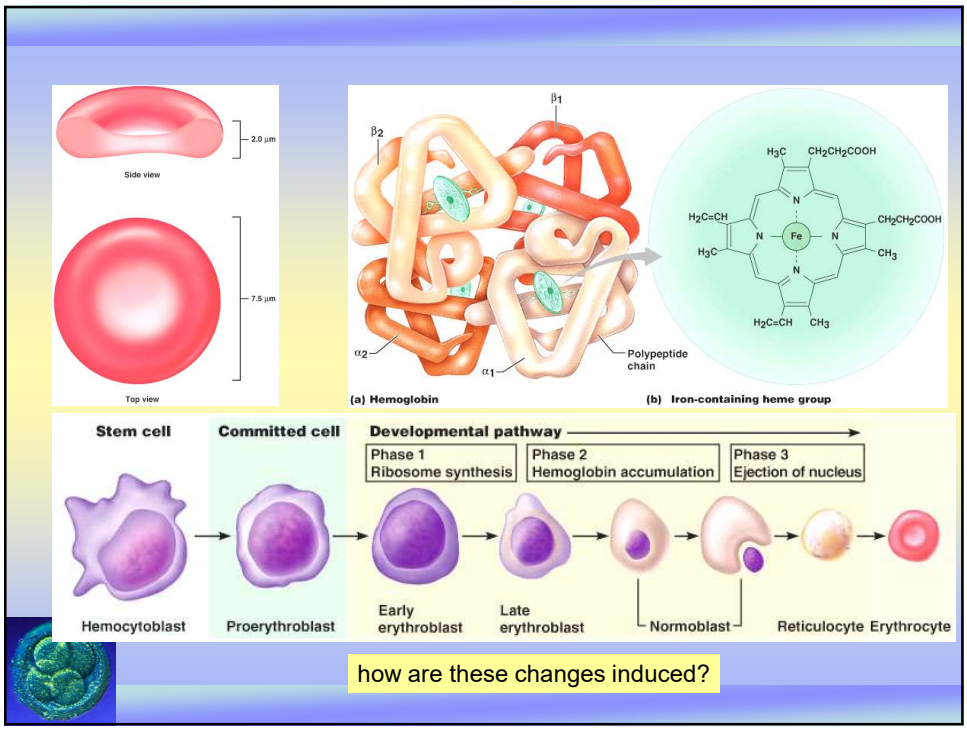
2. **polychromatophylic normoblast** with less alkaline cytoplasm

3. **orthrochrmatic normoblast** with diameter10 μm, acydophilic cytoplasm, a
   stage a nucleus is extruded

4. yang red blood cells – **retikulocytes**, they can generate hemoglobine as lo
   they have some mRNA and ribosomes

5. after 2-3 days retikulocytes are released into circulatation and maturate into
   erythrocytes





how are these changes induced?

Erythropoietin

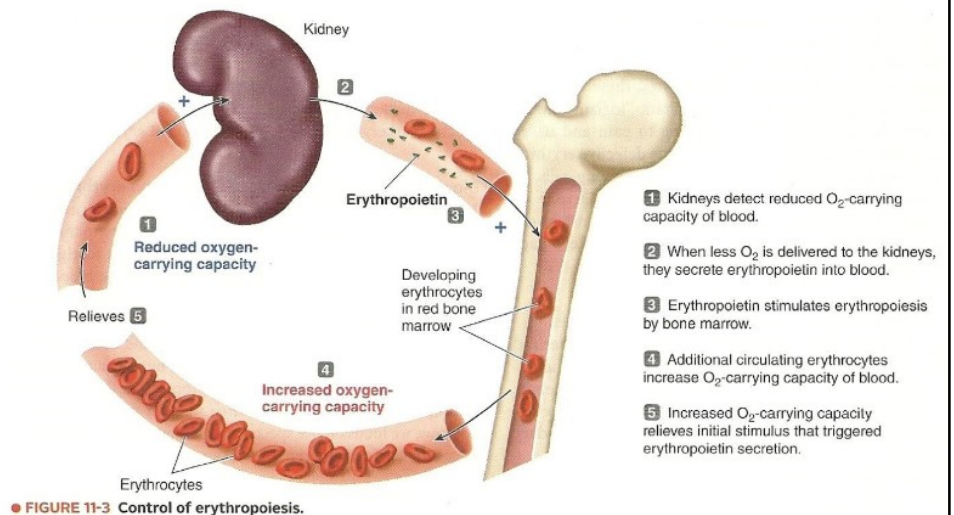
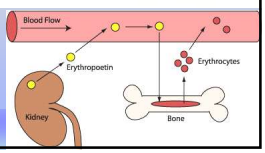
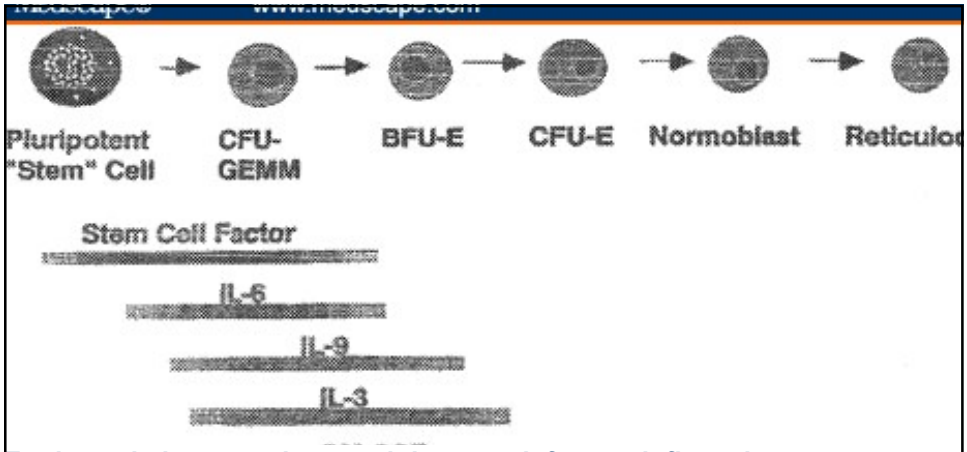


FIGURE 11-3 Control of erythropoiesis.

Why it is useful that erytrocyte does not have nucleus?

Link 8





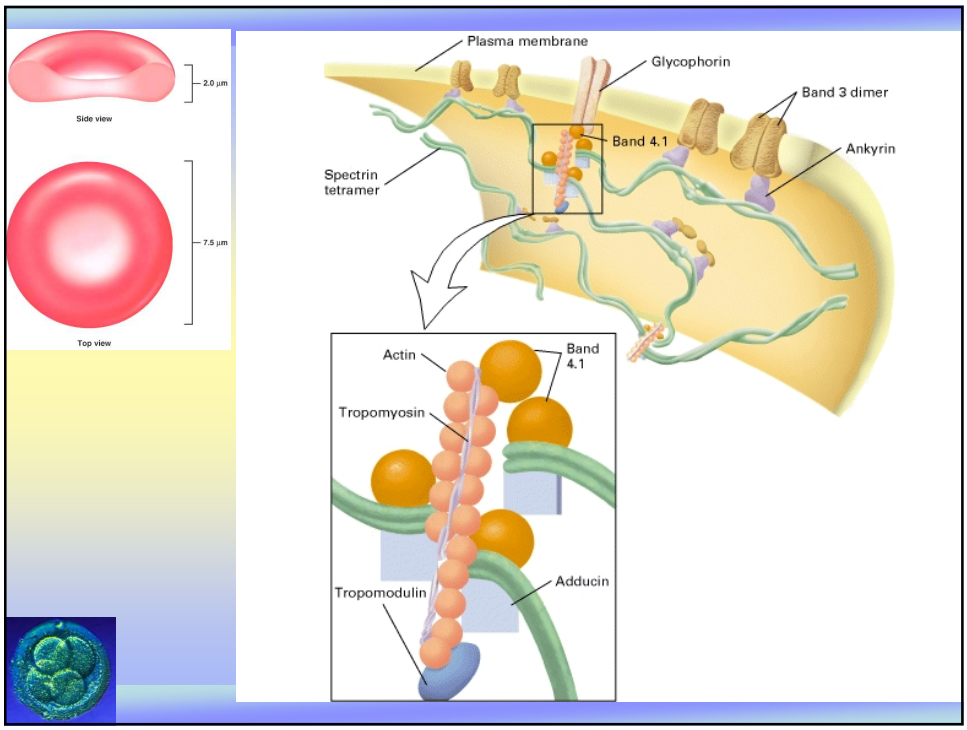
Erythropoietic progenitors and the growth factors influencing erythropoiesis. BFU-E, erythroid burst-forming unit; CFU-E, erythroid colony-forming unit; CFU-GEMM, colony-forming unit-granulocyte, erythrocyte, macrophage, and megakaryocyte; Epo, erythropoietin; GM-CSF, granulocyte macrophage colony stimulating factor; IL, interleukin. From Ohls RK: Developmental erythropoiesis, in Polin RA, Fox WW, Abman SH (eds): Fetal and Neonatal Physiology (ed 3). Philadelphia, PA, Saunders, 2004, p 1400 (Fig 146-2).

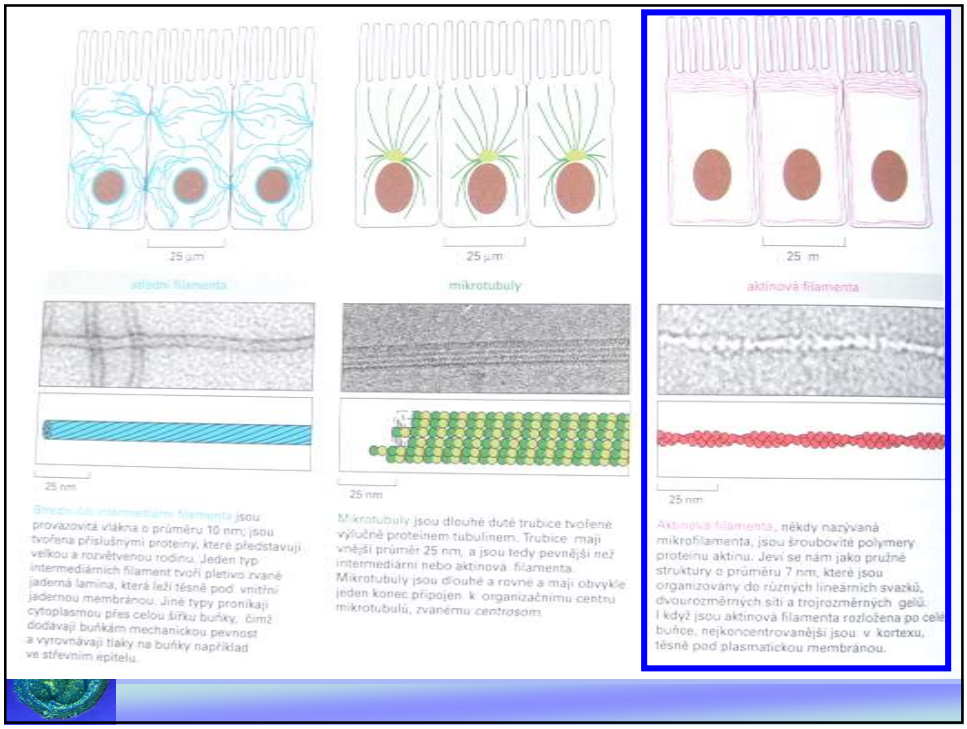
Erythrocyte Cytoskeleton

A red blood cell must squeeze through narrow blood capillaries without rupturing its membrane. The strength and flexibility of the erythrocyte plasma membrane depends on a dense cytoskeletal network that underlies the entire membrane and is attached to it at many points.

The primary component of the erythrocyte cytoskeleton is *spectrin*, a long fibrous protein. Two dimeric subunits of spectrin, each composed of an α and β polypeptide chain, associate to form head-to-head tetramers, which are 200 nm long.

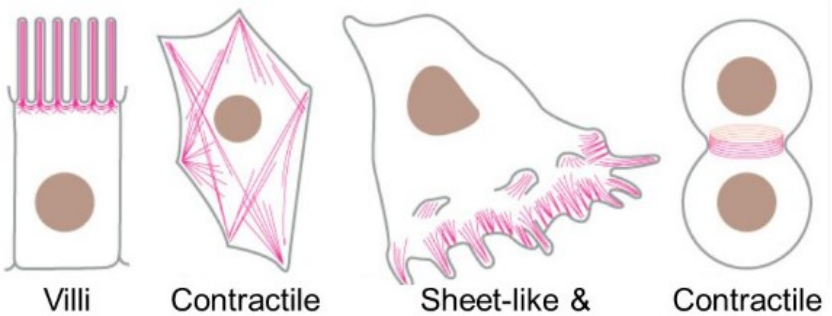
The entire cytoskeleton is arranged in a spoke-and-hub network. Each spectrin tetramer comprises a spoke, extending from and cross-linking a pair of hubs, called *junctional complexes*. Each junctional complex is composed of a short (14-subunit) actin filament plus adducin, tropomyosin, and tropomodulin. The last two proteins strengthen the network by preventing the actin filament from depolymerizing. Because several spectrin molecules can bind the same actin filament, the erythrocyte cytoskeletal network has a spoke-and-hub organization. This polygonal arrangement acts as a lamination of the membrane.


2.0 µm
Side view
7.5 µm
Top view
Plasma membrane
Glycophorin
Band 3 dimer
Band 4.1
Ankyrin
Spectrin tetramer
Actin
Band 4.1
Tropomyosin
Adducin
Tropomodulin


25 µm
střední filamenta
25 µm
mikrotubuly
25 µm
aktinová filamenta
25 nm
25 nm
25 nm
Střední (též intermediární) filamenta jsou provazovitá vlákna o průměru 10 nm; jsou tvořena příslušnými proteiny, které představují velkou a rozvětvenou rodinu. Jeden typ intermediárních filament tvoří pletivo zvané jaderná lamina, která leží těsně pod vnitřní jadernou membránou. Jiné typy pronikají cytoplasmou přes celou šířku buňky, čímž dodávají buňkám mechanickou pevnost a vytrovnávají tlaky na buňky například ve střevním epitelu.
Mikrotubuly jsou dlouhé duté trubice tvořené výlučně proteinem tubulinem. Trubice mají vnější průměr 25 nm, a jsou tedy pevnější než intermediární nebo aktinová filamenta. Mikrotubuly jsou dlouhé a rovné a mají obvykle jeden konec připojen k organizačnímu centru mikrotubulů, zvanému centrosom.
Aktinová filamenta, někdy nazývaná mikrofilamenta, jsou šroubovité polymery proteinu aktinu. Jeví se nám jako pružné struktury o průměru 7 nm, které jsou organizovány do různých lineárních svazků, dvourozměrných sítí a trojrozměrných gelů. I když jsou aktinová filamenta rozložena po celé buňce, nejkoncentrovanější jsou v kortexu, těsně pod plasmatickou membránou.

How actin contributes to plasma membrane plasticity

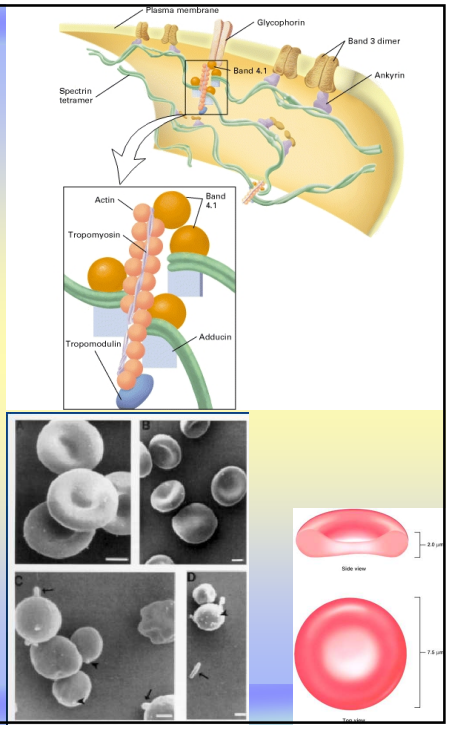
Actin filaments allow cells to adopt different shapes and perform different functions

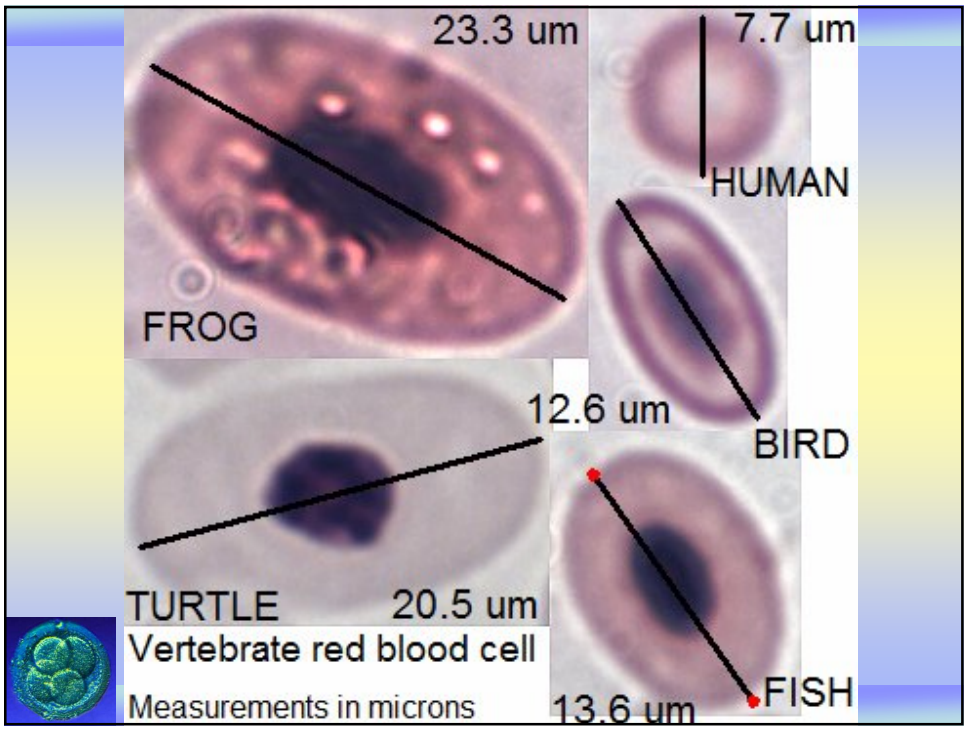


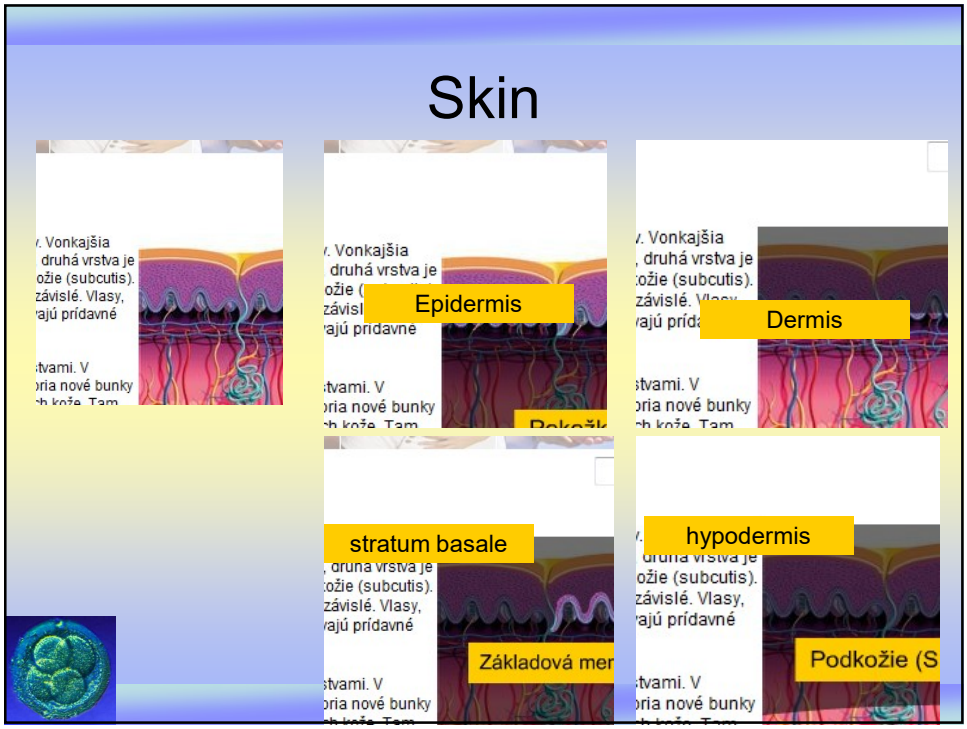
<u>when membrane is broken</u>

Hereditary spherocytosis

- RBCs are spheroidal, less rigid, of variable diameter and subject to destruction in the spleen. This alteration is caused by cytoskeletal abnormalities involving sites of interactions between spectrin alpha and beta and protein 4.1.




23.3 um
7.7 um
HUMAN
FROG
12.6 um
BIRD
TURTLE
20.5 um
Vertebrate red blood cell
Measurements in microns
13.6 um
FISH


Skin
Epidermis
Dermis
stratum basale
hypodermis

The skin consists of three layers firmly attached to one another:

- The outer **epidermis**, derived from ectoderm.
- The deeper **dermis**, derived from mesoderm.
- The **hypodermis** or **subcutaneous layer.**

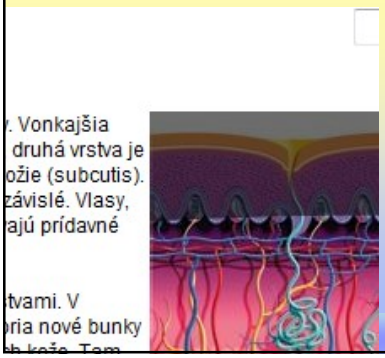
epidermal derivatives: nails, hair, and glands (sweat and sebaceous glands).

Skin – high resistance and regenerative potential
- renewal every **28 days**

the lowest layer = **subcutis**:
Branches of the hypodermic and cutaneous plexuses nourish the adipose tissue of the hypodermis, the sweat glands, and the deeper segment of the hair follicle.

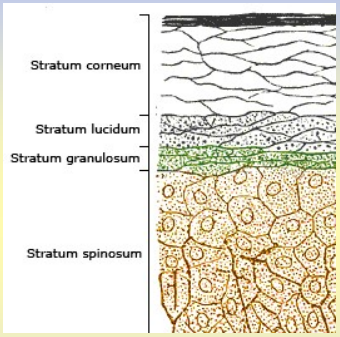
dermis is created by two layers:

1. The **papillary layer**, consisting of numerous dermal papillae split by interpapillary pegs, form the dermal-epidermal junction
2. The **reticular layer**, containing thick bundles of collagen fibers and coarse elastic fibers
- blood vessel – nutrition
- neural fibers,

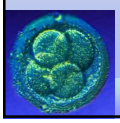


- epidermal derivatives: **air follicles, sweat** and **sebaceous glands** are present at various levels of the dermis

epidermis



- 5 layers
- new cells are generated in *stratum basale*
- they migrate in the direction of *stratum corneum*
- keratinocyte differentiation = the higher corneous skin layer



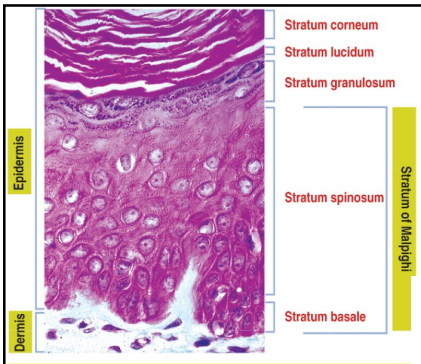
The stratified squamous epithelial layer of the epidermis consists of four distinct cell types:

Keratinocytes - predominant cell type, its major product is **keratin** (an intermediate filament protein).

Melanocytes produce **melanin.**

Langerhans cells are cells of immune system, acting as antigen - trapping cells interacting with CD8+ T cells.

Merkel cells are involved in tactile sensation



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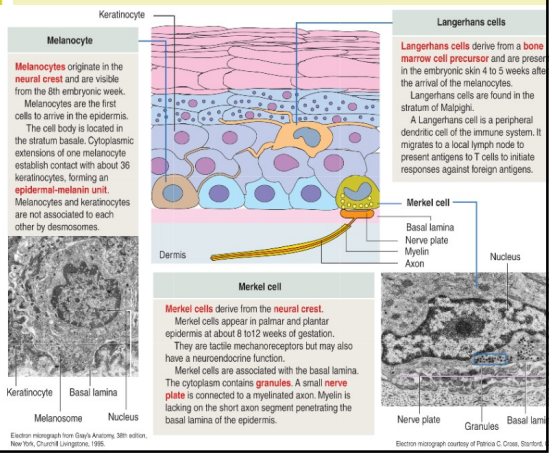
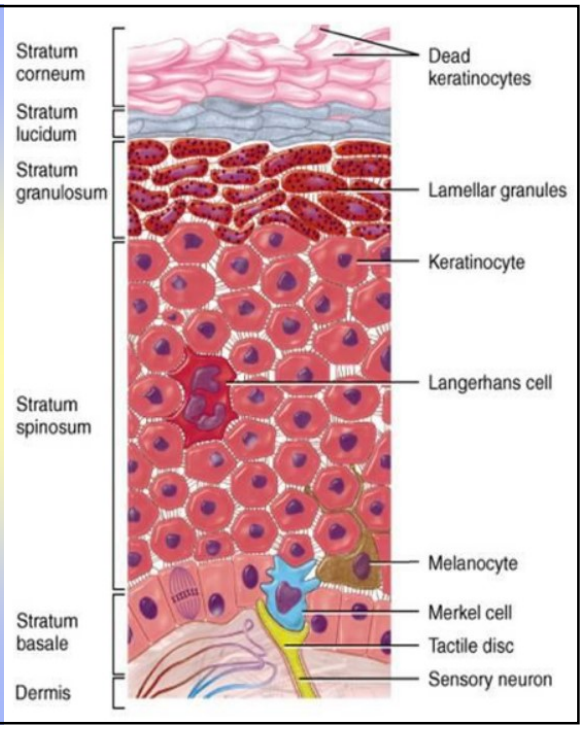
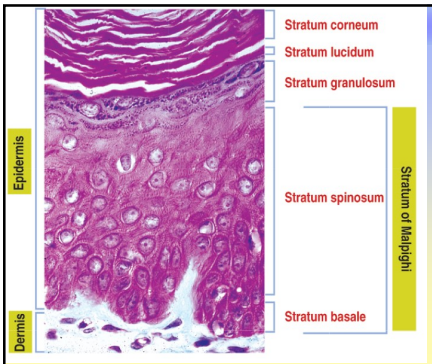


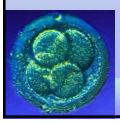
Keratinocytes are the most abundant cells of epidermis =
- 95% of cells epidermis
- produce keratin filaments
- contain melanin produced by melanocytes

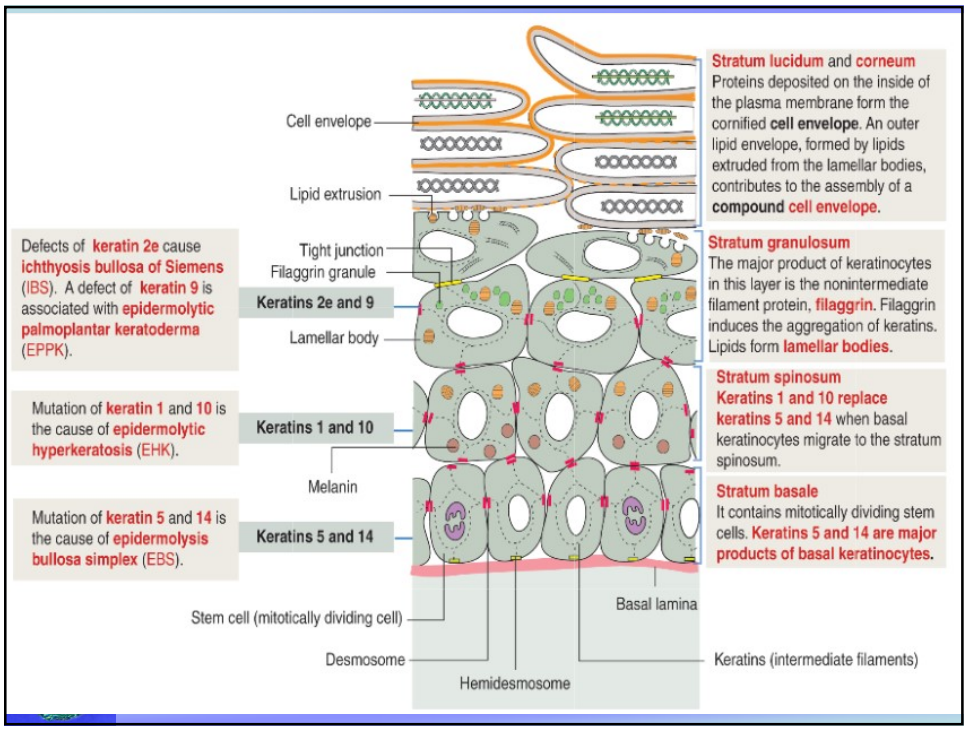




Keratinocytes are arranged in **five layers** or **strata**:

1. The ***stratum basale*** (basal cell layer).
2. The ***stratum spinosum*** (spinous or prickle cell layer).
3. The ***stratum granulosum*** (granular cell layer).
4. The ***stratum lucidum*** (clear cell layer).
5. The ***stratum corneum*** (cornified cell layer).



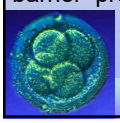


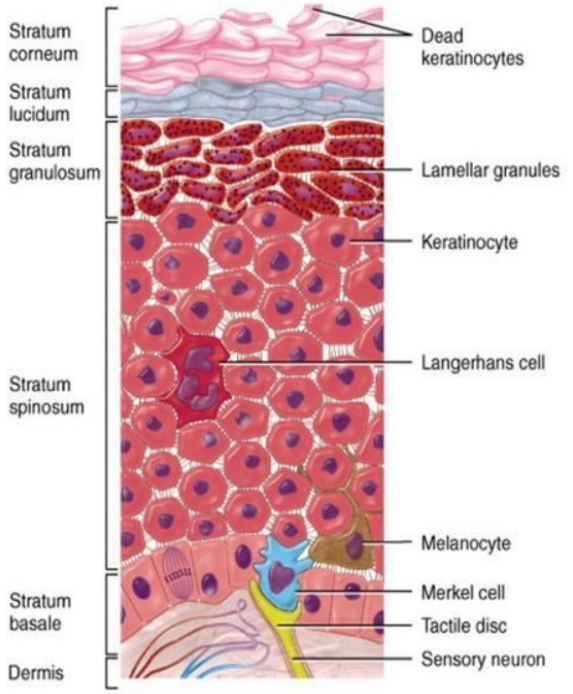
The first cell layers consist of metabolically active cells. The ***stratum basale*** consists of a single layer of columnar or high cuboidal keratinocytes resting on a basement membrane. **Hemidesmosomes** and associated intermediate filaments anchor the basal domain of basal cells to the basement membrane.

The cells of the **stratum basale** have all organelles and maintain the equilibrium between cell differentiation and mitotic cell division and the repair of damage. While some of the dividing cells add to the population of **stem cells** of the **stratum basale**, others migrate into the **stratum spinosum**.

In ***stratum spinosum*** cells change from columnar or high cuboidal to become polygonal in shape and initiate the differentiation process by starting the synthesis of keratins that are distinct from the basal cells. The cytoplasm contains intermediate filaments associated with **desmosomes**. Bundles of intermediate filaments, visible under the light microscope, are called **tonofilaments**.

Keratinocytes in the ***stratum granulosum*** display dark clusters of cytoplasmic material, keratins and lipids. At the transition between this layer and the stratum corneum, cells secrete lamellar bodies (containing lipids and proteins) into the extracellular space. This results in the formation of the hydrophobic lipid envelope responsible for the skin's barrier properties. Concomitantly, cells lose their nuclei and organelles causing the granular cells to become non-viable corneocytes in the stratum corneum.





In ***stratum lucidum*** cells are dead, some organelles are removed, cells are flattened and surrounded by an oily substance that is the result of the exocytosis of lamellar bodies.

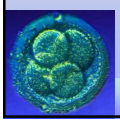
The ***stratum corneum*** consists from terminally maturing dead keratinocytes containing keratin, cholesterol and fatty acids.

This is the outermost layer of the epidemis and responsible for the barrier function of the skin.
The barrier protects against many toxic agents and prevents dehydration.
Cells are shedding from the surface of the *stratum corneum*.

Psoriasis

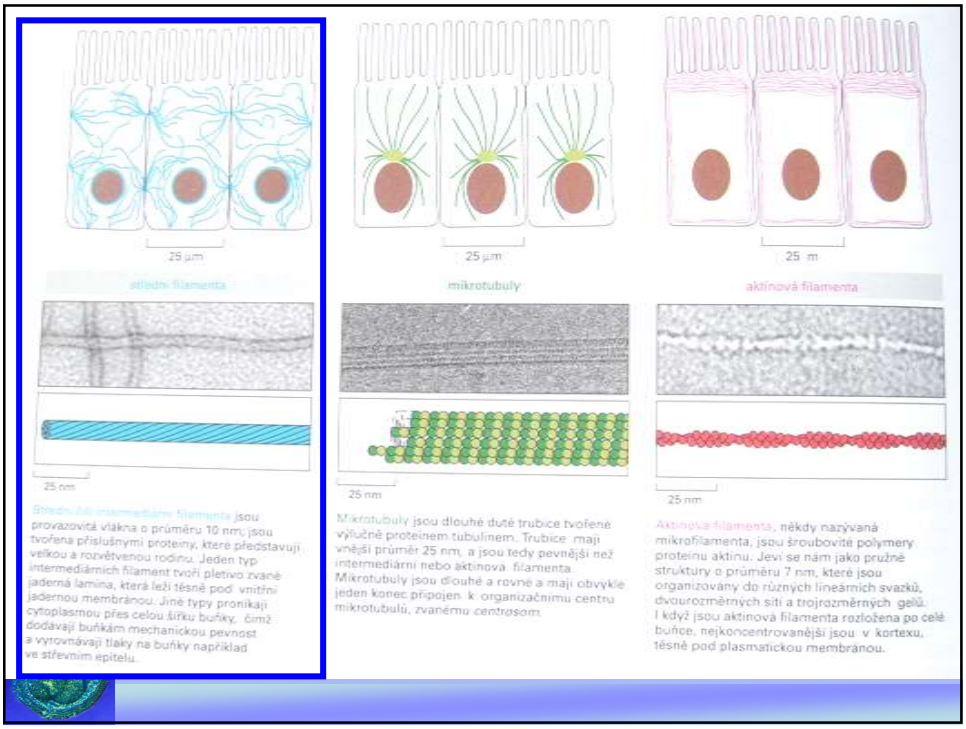
autoimmune disease

Excessive proliferation of epidermal keratinocytes - caused by an accelerated migration of keratinocytes from the stratum basale to the stratum corneum - 3-5 days instead of 28 days
Presence of inflammatory cells.
Elongation of epidermic papillae, and prominent **angiogenesis**



Epidermolysis bullosa simplex

- inherited bullous disorders characterized by blister formation in response to mechanical trauma
- mutations in the genes encoding keratin 5 or keratin 14
- the skin very fragile and to blister easily



Intermediate filaments

- diameter of about 10 nm
- they are not directly involved in cell movements
- they provide structural role by providing mechanical strength to cells and tissues
- keratin filaments are tightly anchored to the plasma membrane at two areas of specialized cell contacts: desmosomes and hemidesmosomes
- desmosomes and hemidesmosomes anchor intermediate filaments in cell-cell and cell-substratum fashion
- they are resistant to concentrated salts and some detergents
- they create nuclear lamina

Differentiation

- cell with high metabolism that live during whole human life – neurons

- short time living cells with low metabolism – red blood cells, keratinocytes

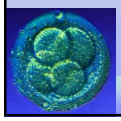
- **short time living cells with high metabolism ???**

link

Gastrointestinal tract

- the main role of GIT is to release and absorb components of food needed by the organism – water, minerals, vitamins, nutrients

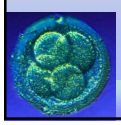
- food must pass through different segments of the gastrointestinal tract to undergo mechanical and enzymatic digestion and resoption



Gastrointestinal tract

Cell of intestinal epithelium

- small intestine:
  - **resorption of nutrients from chyme**
  - **activation of pancreatic enzymes**
  - **neutralization of acid chyme from stomach**
  - enterocytes – cells with brush border
    - renewal every **3-5 days**



1) ***duodenum***

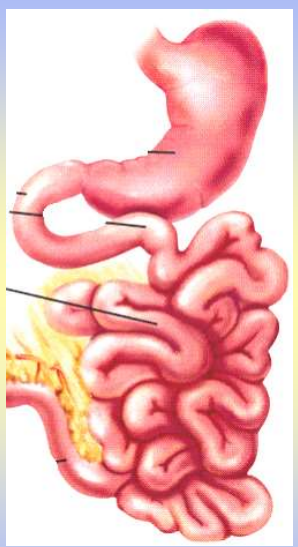
- ~ 25 cm

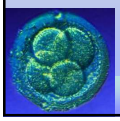
2) ***jejunum***

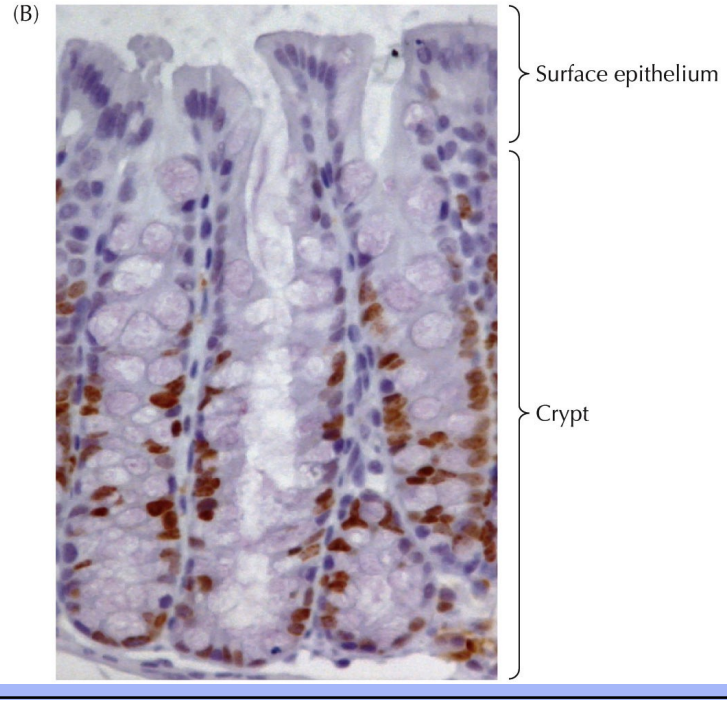
- another 2.5 m

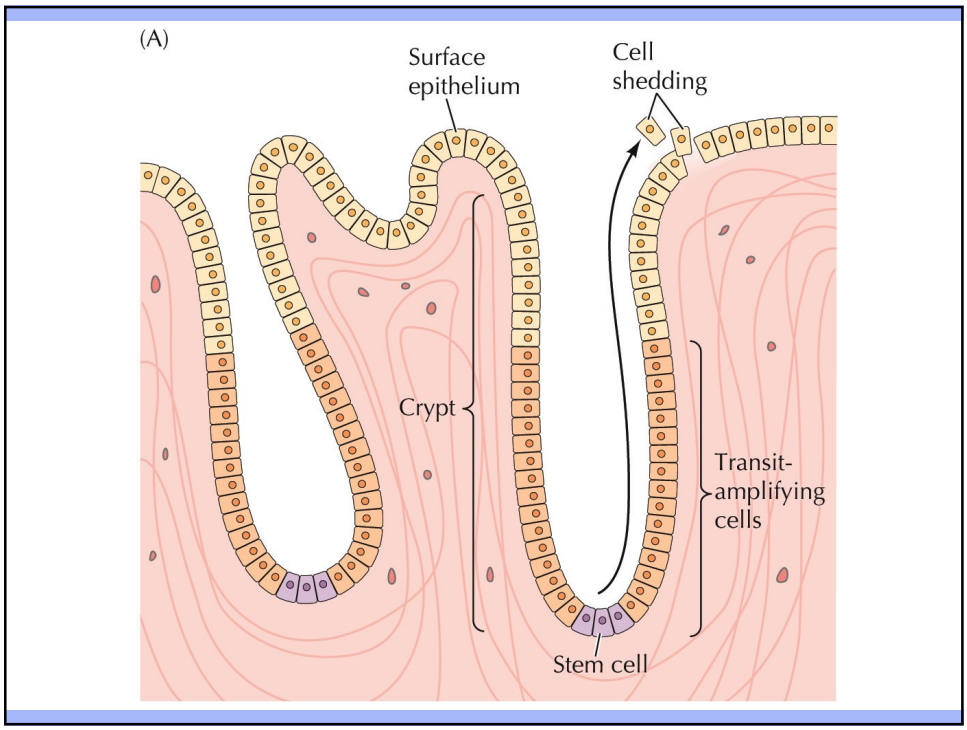
3) ***ileum***

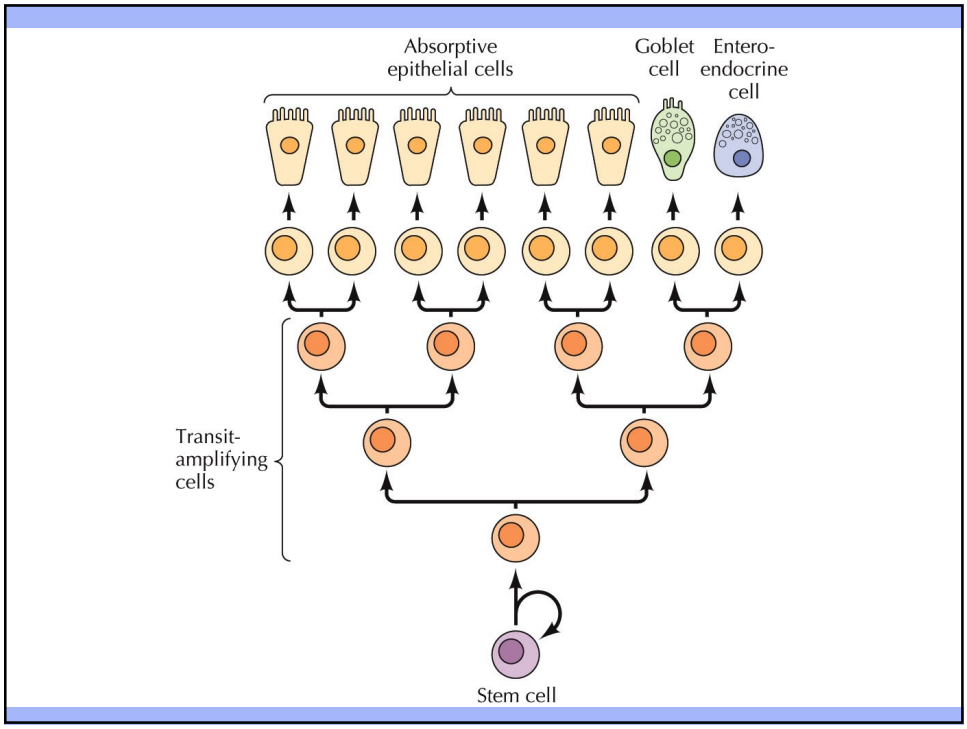
- last 3.6 m








(A)
Surface epithelium
Cell shedding
Crypt
Transit-amplifying cells
Stem cell

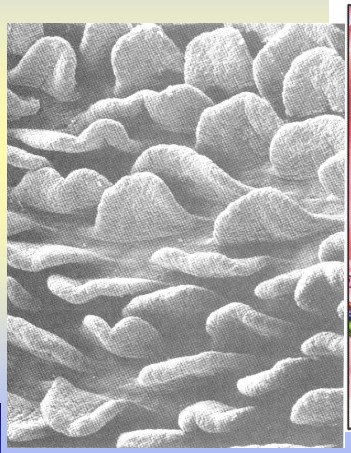

Absorptive epithelial cells
Goblet cell
Entero-endocrine cell
Transit-amplifying cells
Stem cell

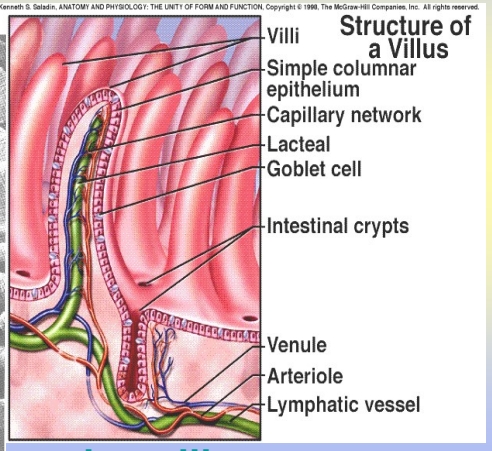
Stem cells and renewal of mature tissues

Epithelial cells create an interface between intestinal lumen and intestinal tissue persist only during several days and than undergo apoptosis.

New epithelial cells arise from continuously dividing stem cells in the epithelial crypts.

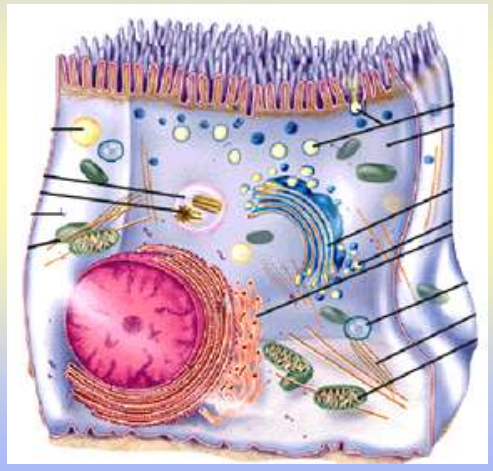
- intestine surface is enlarged by:
- villi 0.5-1 mm
- microvilli – brush border on enterocytes

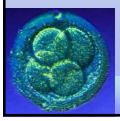


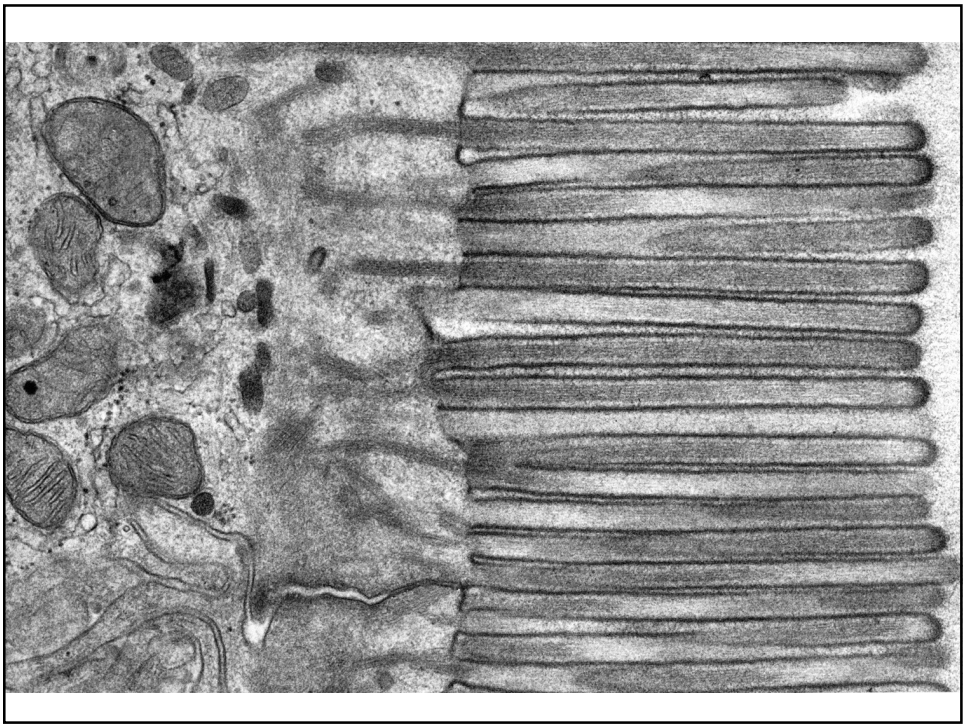


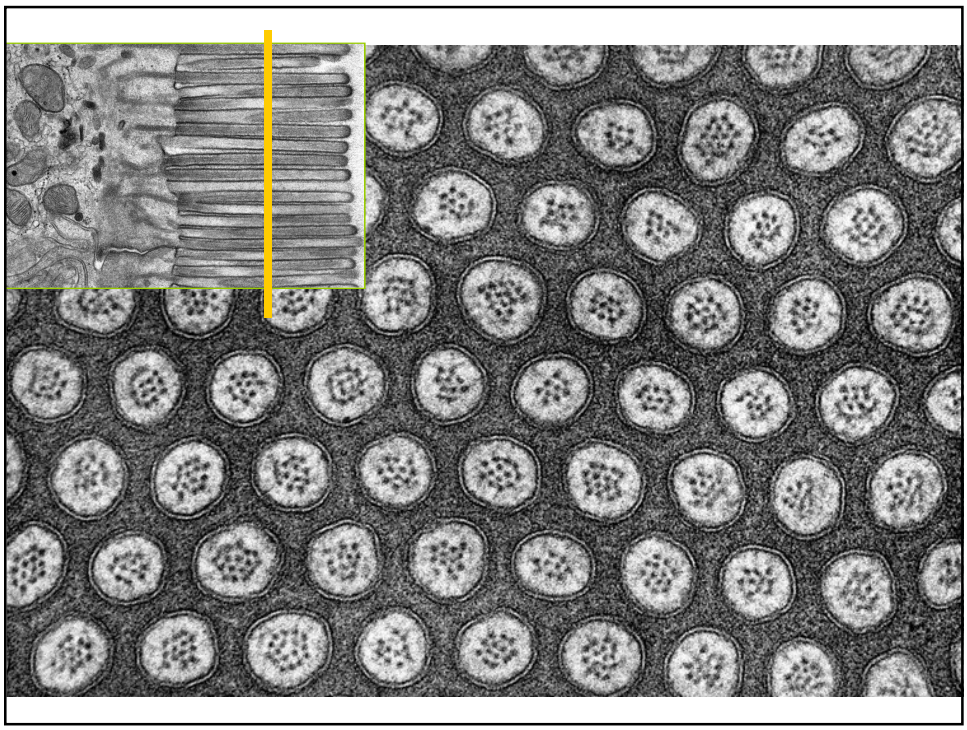
microvili

Microvilli (brush border)









Metabolite transport in the gut:

- from lumen: glucose and galactose by active sodium co-transport
- glucose and galactose is transported into paracellular space by facilitated diffusion
- fructose is absorbed by facilitated diffusion
- concentration gradient of Na^+ is generated by Na^+-K^+ ATPase in basolateral membrane of enterocyte

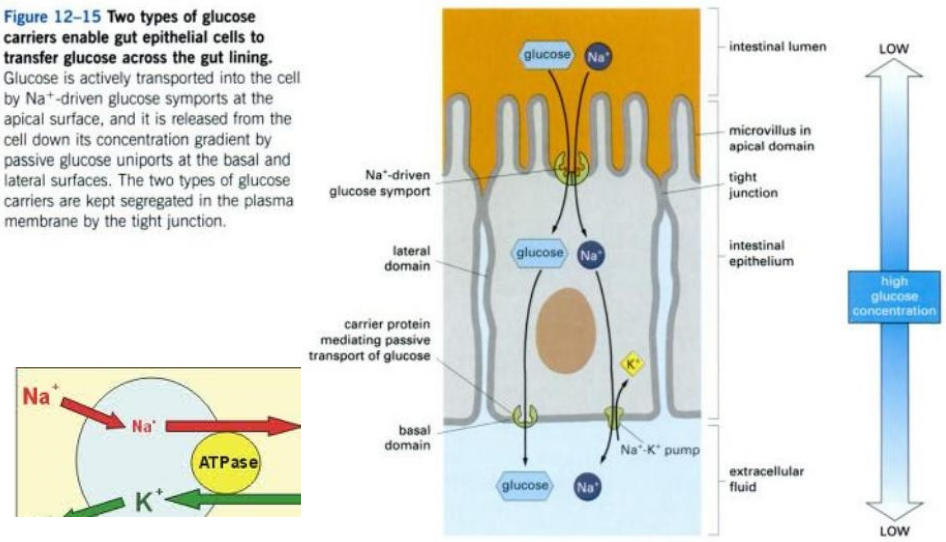


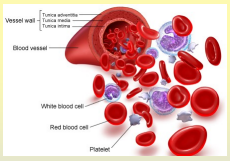
Figure 12–15 Two types of glucose carriers enable gut epithelial cells to transfer glucose across the gut lining. Glucose is actively transported into the cell by Na^+-driven glucose symports at the apical surface, and it is released from the cell down its concentration gradient by passive glucose uniports at the basal and lateral surfaces. The two types of glucose carriers are kept segregated in the plasma membrane by the tight junction.

link

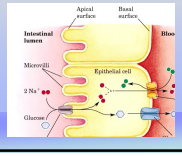
Differentiation

- cell with high metabolism that live during whole human life
– **neurons**

- short time living cells with low metabolism
  - **red blood cells**
  - **epidermal cells**



- short time living cells with high metabolism
– **epithelial cells**



Thank you for your attention