

Cytology

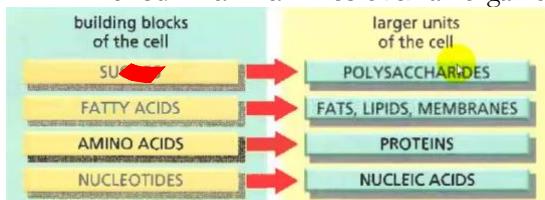
Cell: Introduction

History

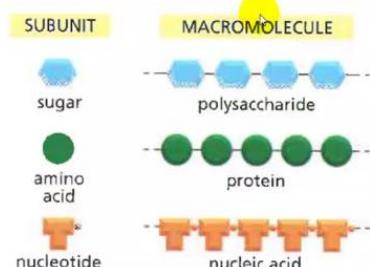
- Robert Hooke introduced the term "cell" when he looked at cork and named his findings cellule / cellula.
- Some of his observations are basic principles of cell theory:
 1. All living cells are comprised of cells
 2. Cells are the smallest "living" unit in an organism
 3. (Cells come from previously existing cells)
- Anton van Leeuwenhoek was a master of microscopy and was the first who observed living cells
- Three German scientists from the early 19 century completed the early observation to a modern cell theory
 - Rudolf Virchow: founder of the modern pathology. "All cells come from preexisting cells"
 - Schleiden (plants) & Schwann (animals) used the work of Virchow to better it

Cell-function

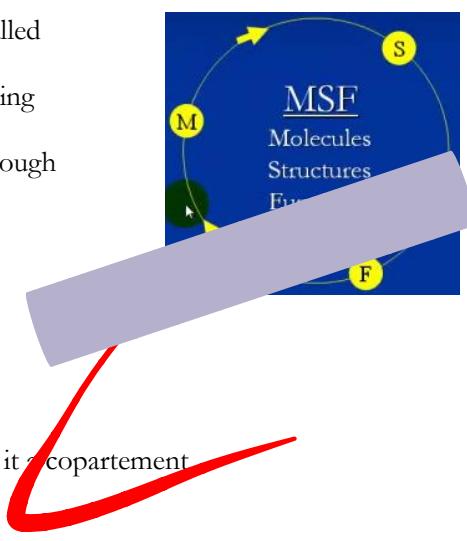
- **Form-follows-function-principle**
- **Heredity:** central definition of life
 - Orderly structures are generated but without the same type of link between the peculiarities of parents and of the offspring
- Vehicle for hereditary information: an aggregate of more than 10^{13} cells generated by cell division from a single cell. The single cell, therefore, is the **vehicle for the hereditary** information
- The **four main families** of small organic molecules in cells:



- Three families of macromolecules:



- Each macromolecule is a polymer formed from small molecules (called monomers) linked together by covalent bond.
- **Macromolecules** are by weight the most abundant carbon-containing molecules in a living cell
- Macromolecules with complementary surfaces can bind tightly through **noncovalent bonds**
- Flow of logic in cell biology (cytology: only structures)
- Cell is composed of two basic parts / compartments:
 - **Cytoplasm**
 - **Nucleus**
- There is a difference between:
 - **Prokaryotic cells** without distinct nucleus
 - **Eukaryotic cells** keep their DNA in a nucleus
- Sometimes the nucleus is called organell, but it's complexity makes it a compartment
- Differences between prokaryotic and eukaryotic cells:



	Prokaryotes	Eukaryotes
Size	Usually 1-2 μm	5-100 μm
Nucleus	Absent	Presence, bounded by nuclear envelope
DNA	Usually a single, circular molecule (chromosome)	Multiple molecules, linear, associated with protein
Cell division	Simple fission	Mitosis & meiosis
Internal membranes	Rare	Complex (nuclear envelope, Golgi apparatus, endoplasmic reticulum, etc)
Ribosome	70S	80S (70S in mitochondria & chloroplasts)
Cytoskeleton	Absent	Microtubules, microfilaments, intermediate filaments
Motility	Rotary motor (drives bacterial flagellum)	Dynein (drives cilia & eukaryote flagellum), kinesin, myosin

- All cells are enclosed in a plasma membrane across which nutrients and waste materials must pass
- Membranes are formed by **amphiphilic phospholipids** which have a **hydrophilic** (water-loving, phosphate) head group and a **hydrophobic** (water-avoiding, hydrocarbon) tail

Cytoplasm

Cytosol - fluid component, in which are contained...

- **Organelles:** metabolically active structures
 - Membranous
 - Non-membranous
- **Cytoskeleton:** determines the shape and motility of eukaryotic cells
- **Inclusions:** deposits of carbohydrates, lipids, or pigments
- Cell types according to Cells:
 - Small (5-10 microm.)
 - Medium (11-30 microm.)
 - Large (31-100 microm.)
 - Giant (101-200 microm.)
- Levels of cellular organization:
 - Biomolecules (building blocks)
 - Macromolecules (nucleic acids, proteins, polysaccharides)
 - Supramolecular complexes (DNA/protein; RNA/protein)
 - Organelles
 - Cell
- Cells can also differ in shape: Squamous, columnar, cuboidal, spherical, pyramidal, stellate, spindle-shaped, polyhedral
- Cells can also differ in color:
 - Most cells are **colorless**
 - Some cells are **pigmented:**
 - Red blood cells, muscle fibers
 - Melanocytes, retinal pigment, epithelium cells
- The cytoskeleton is very important for the complexity of the eukaryotic body, f. e. by allowing skeleton muscles to contract
- The variety of compartments in a cell helps the cell to practice division of labor without interference
- Hepatocyte: cell of the liver, which is very active
- Peroxisomes are a type of organel involved in catabolism of branched chain fatty acids

Cell shapes

- Blood cells have almost the size of a giant cells nucleus
- Squamous is the latin word for flakes. Squamous cells are very flat.
- Spherical cells surround a cell, f. e. the oocyte. This way the latter can have up to 200 micrometer, the size of the eye, so they can be seen under the microscope.
- Osteocytes, the cells of bones, are so called "stellate cells". They can transfer molecules from one to another, because their cytoplasms are connected
- The shape of hepatocytes have almost hexagonal shape.
- Die Dauer und damit die Häufigkeit des Einsatzes von Medikamenten hängt von den Enzymen ab, die die verarbeitenden Zellen enthalten. Je nach Beschaffenheit werden Medikamente schneller oder langsamer abgebaut.

Präsentation: Methods

Thicker things normally have tissues which can be sliced very easily, others do not. Therefore they must be processed: They get fixated, dehydrated (wasser wird durch ethanol ersetzt), cleared (ethanol wird entfernt), infiltrated and embedded. The tissues are now surrounded by paraffin, which can be cutted by a microtome

- Cryomicrotomy freezes the tissue, helping to skip some steps of the histological processing
- H&E (HE) hematoxylin and eosin are the most common basis of all dyes. Hematoxylin stains the DNA, eosin the other cytoplasmic components
- PAS stains help to identify different celltypes

Methods

- Methods of study <> organizational levels:
 - Atoms
 - Molecules
 - Cells
 - Tissues
 - Organs
 - Organ systems
- **Corrosion cast:**
 - Injection of a stained fluid
 - Hardening of the material
 - Processing with concentrated acid or alkaline solution to dissolve tissue content
 - Conservation of the cast
- Preparation of tissues (**histological processing**):
 - Most common procedure: preparation of histological sections or tissue slices that can be studied with the aid of the light microscope
 - Under the light microscope, tissues are examined via light beam
 - Tissues and organs must be sectioned to obtain thin, translucent sections
- Stages of preparation (1-2 days):
 - **Fixation:** object is placed in fixative solutions
 - **Dehydration:** series of increasingly more concentrated alcohol solutions which effectively removes all water from the tissue
 - **Clearing:** alcohol and melted paraffin.
 - **Infiltration:** At 58°, the object becomes completely infiltrated with melted paraffin
 - **Embedding:** Paraffin is allowed to harden. The resulting paraffin block is trimmed to expose the tissue for sectioning.
 - **Slicing:** The object is sectioned in a **Microtome**
- An alternate way is rapid freezing of the object through a **cryostat**. This process is much more faster than the normal embedding (~30 minutes), so it is commonly used within surgeries.
- **Staining:**
 - Microscopically sections must be stained
 - Tissue components with a net negative charge (anionic) stain more readily with basic dyes and are termed **basophilic**
 - Cationic components, such as proteins with many ionized amino groups, have affinity for acidic dyes and are termed **acidophilic**
- The most commonly used dye is a **combination of hematoxylin and eosin (H&E)**
 - **Hematoxylin** stains DNA of the cell nucleus and other acidic structures (such as RNA-rich portions of the cytoplasm and the matrix of cartilage) blue
 - **Eosin** stains other cytoplasmic components and collagen pink
- **Peroxidase-Schiff (PAS)** reaction shows glycoproteins:
 - A ubiquitous free polysaccharide in animal cells is **glycogen**, which can be demonstrated by PAS in liver, striated muscle, and other tissues where it accumulates
 - With PAS, staining is most intense at the cell surface, where projectin microvilli have a prominent layer of glycoproteins (arrow head) and in the mucin-rich secretory granules of goblet cells

Microscopy

- **Light Microscopy (LM)**
 - Based on the interaction of light and tissue components
 - Used to reveal and study tissue features
 - Maximal resolving power of the light microscope is approx. 0,2 microm.; this power permits good images magnified 1000-1500 times. Objects smaller or thinner than 0,2 microm. Cannot be distinguished
- Two types of LM:
 - **Bright-field microscopy** - stained preparations are examined by means of ordinary light that passes through the specimen
 - **Fluorescence microscopy:** tissue sections are irradiated with a certain wavelength of light and the emission is in another wavelength
- **Resolving power R** - the smallest distance between two particles at which they can be seen as separate objects

Maximal resolving power of the light microscope - $\sim 0.2 \mu\text{m}$
R determines the quality of the image

$R = 0.61 \frac{\lambda}{NA}$

λ – the length of the wave
NA – numerical aperture

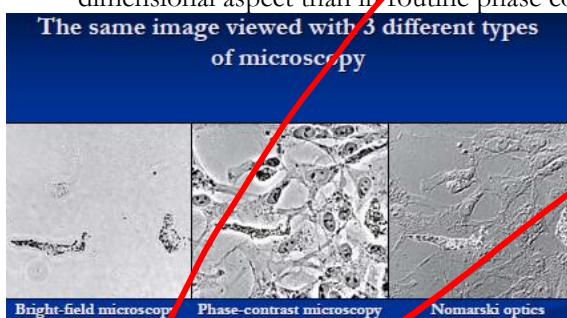
- **Digital imaging techniques**

- Employ computer technology to capture and manipulate histologic images, without the use of film
- Advantages
 - Immediate visualization
 - Digital modification
 - Capability of enhancing

- **Phase-contrast microscopy**

- Lens system that produces visible images from transparent objects
- Based on the principle that light changes speed when passing through structures with different refractive indices
- Structures appear lighter or darker
- Does not require fixation or staining: observation of living cells possible.

- **Differential interference microscopy (Nomarski optics)**: Produces an image with a more apparent three-dimensional aspect than in routine phase contrast microscopy



- **Epifluorescence (conventional fluorescence)**: a method of collecting reflected visible light emitted by UV excitation

Pros and Cons of Fluorescence Microscopy	
Pros	Cons
Allows for multiple labels	Out of focus light blurs image.
Techniques: immunofluorescence, FISH	Must have fluorescent reporter
Sensitive	Bleedthrough
Relatively inexpensive	Difficult with thick sections

- **Confocal microscopy**:

- Avoids stray light and achieves greater resolution by using
 - Small point of high intensity light provided by a laser
 - A plate with a pinhole aperture in front of the image detector
- Creating optical sections at series of focal planes through the specimen allows them to be digitally reconstructed into a 3D image

Pros and Cons of Confocal Microscopy	
Pros	Cons
Allows for higher resolution	Limited EX peaks on lasers
Allow collection of stacks of image planes and 3D reconstruction	Phototoxicity (up to a 40 degree C temp jump at focal point)
Laser penetrates somewhat thick sections	Loss of image intensity
Better control for bleedthrough/autofluorescence	Fairly expensive
Faster than deconvolution	Prone to Photobleaching
Precise Laser Positioning (FRAP)	

- **Deconvolution**: looks at each voxel (3D pixel) and determines its relationship with the voxels around it. It then either subtracts out what it thinks is blur from nearby bright voxels (easy way) or moves out-of-focus light back to its voxel of origin (hard way)

Pros and Cons of Deconvolution Microscopy	
Pros	Cons
Allows for higher resolution	Computationally intense if iterative
Allow collection of stacks of image planes	Minimal penetration of thick sections
Hg Bulb allows for variety of filter combinations	Sensitive to spherical aberrations
Can get superior sensitivity	Can amplify noise, produce artifacts
Cheaper than confocal	Requires 3D dataset

- **Green Fluorescent Protein (GFP)**
 - GFP found in Aequorea victoria, a jellyfish
 - Calcium ions bind aequorin, which then emits blue light. This blue light is absorbed by GFP which then emits green light.
 - We can insert the gene for GFP into transfection constructs
 - Can be used to tag proteins within a cell or visualize specific cell types in an organism
 - GFP is now widely used for *in vivo* studies
- **Polarizing microscopy:**
 - Allows recognition of structures made of highly organized molecules
 - Specifically useful for complex structures like muscle tissues
 - Structures appear bright against a dark background
- **Electron microscopy**
 - Based on the interaction of electrons and tissue components (not photons like in LM)
 - The wavelength in the electron beam is much shorter than of light, allowing a thousand-fold increase in resolution
 - Major types of EM:
 - TEM (transmission EM) - 2D visualization - up to 500,000x (usually 100,000x); resolution approx. 0.2 nm
 - SEM (scanning EM) - Pseudo-3D visualization - usually 20-30,000x; resolution approx. 10nm
- Freezing Techniques combined with EM:
 - Useful for examining membrane structure which can be examined by SEM
- **Diffraction barrier of light microscopy:** max. 0.2 micrometers. Diffraction barrier was postulated by Ernst Abbe 140 years ago
- **STED (Stimulated Emission Depletion)** microscopy breaks the diffraction barrier
 - Combining confocal principles with lasers but doing some tricks to the tissue to increase resolution

Histochemistry

- Localizing cellular structures in tissue sections using unique enzymatic activity present in those structures
- Basic steps:
 - Tissue sections are immersed in a solution that contains the substrate of the enzyme to be localized
 - The enzyme is allowed to act on its substrate
 - The section is put in contact with a marker compound
 - This compound reacts with a marker compound
 - The final reaction product, which must be insoluble and which is visible by light or electron microscopy only if it is colored or electron-dense, precipitates over the site that contains the enzyme
- **Immunohistochemistry:** requires developing an antibody against the particular macromolecule to be localized and labeling the antibody with a dye non fluorescent or fluorescent
- Many pathologic conditions are diagnosed by localizing specific markers of the disorder using antibodies against those antigens in immuno-histochemical staining

Other methods

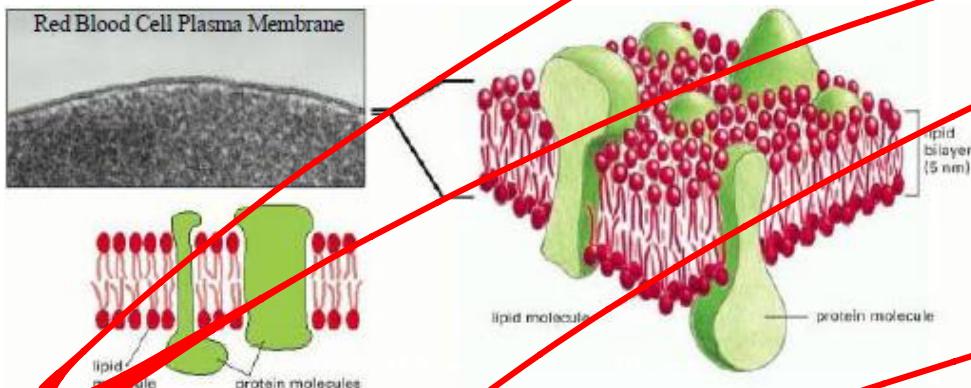
- **In situ hybridization (ISH)**
 - Hybridization is the binding between single strands of nucleic acids
 - Allows specific identification of sequences
 - ISH - solution of nucleic acids is applied directly to cells and tissue sections
- **Autoradiography** - investigating a specific temporal sequence of events by using the incorporation of radioactive isotopes into macromolecules, which are then visualized by the use of an overlay of film emulsion
- **Making tissues transparent** increases 3D-imaging
- Cell & tissue culture:
 - Live cells and tissues can be maintained and studied outside the body
 - Many experiments that cannot be performed in the living animal can be accomplished **in vitro**
 - **Primary cell culture:** directly from the tissue; lives for some time
 - **Cell line:** immortalized cells; live permanently

Plasma Membrane, ER, Ribosomes

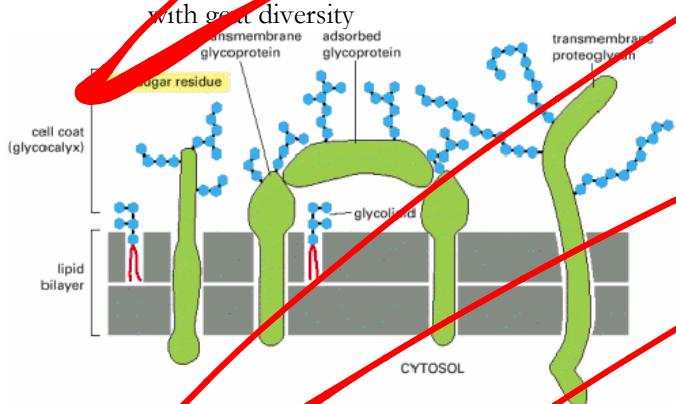
- Organelles are "little organs" in the cytoplasm

Cell membrane (Plasmalemma)

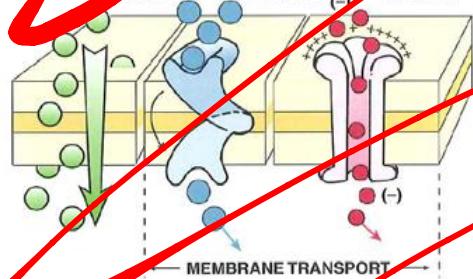
- Consists of:
 - Lipids (mostly phospholipids, e.g. cholesterol)
 - Proteins (mostly glycoproteins)
- Membrane Lipids. Two parts: one hydrophobic or nonpolar part consisting of fatty acids; polar or hydrophilic part consisting of Glycerol, Phosphate & Choline. This constitutes the **hydrophilic head & hydrophobic tail**. Together it is an **amphipathic** molecule.
- Cholesterol is particularly common, Phosphatidylholine, Sphingomyelin & Glycolipids are quite common, too
- Two organizations:
 - Liquid-disordered state - **fluid-mosaic model**: Phospholipids are loosely packed and capable of rapid lateral diffusion



- Liquid-ordered state - **membrane rafts**: microdomains with confined movement of lipids
- Lipid bilayer is approximately 2 nm broad
- Membrane Proteins:
 - Transmembrane**: single-pass & multi-pass
 - Peripheral**: ectoperipheral & endoperipheral
- Cell coat - glycocalyx**
 - Carbohydrate-rich zone on the cell surface**, composed of: glycoproteins, glycolipids, proteoglycans
 - Typically less than 15 oligosaccharides: branched (hundreds of combinations), linked by a variety of bonds, with great diversity



- Simple diffusion vs. carrier proteins (by change of form) using energy & channel proteins



- Functions of membrane proteins:
 - Structural support**
 - Transfer of signals**
 - receptors/receptozymes

cell adhesion molecules (CAM) - integrins, selectins, etc.

Transport of large molecules:

- Endocytosis

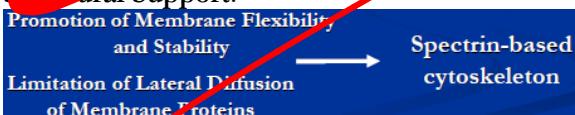
- Exocytosis

Transport of small molecules

- Ion channels: Na, Ca, K, etc.
- Ion pumps: Na/K, H/K, Na/Ca, etc.
- ABC transporters: P-glycoprotein
- Aquaporins 0-9
- Glucose transporters
- Neurotransmitter transporters

Source of inflammatory lipid mediators

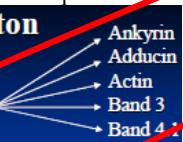
Structural Support:



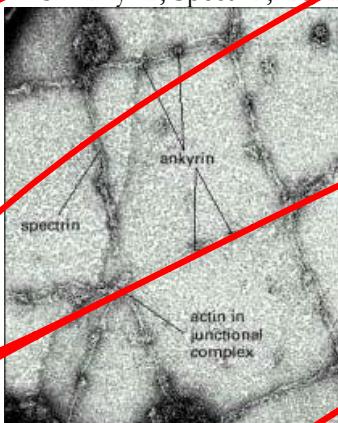
- Spectrin molecule: two chains mingled to a stable structure, with 100 nm pretty large
- Spectrin dimers interact with specific proteins (junctional complexes)

Spectrin-based Cytoskeleton

- spectrin dimers
- junctional complexes



- ASA links: Ankyrin, Spectrin, Actin



- Three layers:
 - Transmembrane: Glycophorins, Band 3
 - Endoperipheral: Ankyrin, Adducin, Band 4.1
 - Intracellular: Spectrin, Actin

Clinical correlates of the RBC (Red blood cells) Cytoskeleton:

- Glycophorin A - entry of Influenza & Hepatitis virus, *P. falciparum*
- Band 4.1 - MN blood groups

- Band 3 } Hereditary spherocytosis (HS)
Ankyrin } Hereditary elliptocytosis (HE)
Spectrin }

- Band 3 ← Southeast Asian ovalocytosis (SAO)
Distal renal tubular acidosis (dRTA)

Mutations in cytoskeletal genes

↓

Weakened interactions among cell membrane proteins

↓

Weakened structure of RBC membrane

↓

Spherical RBC

↓

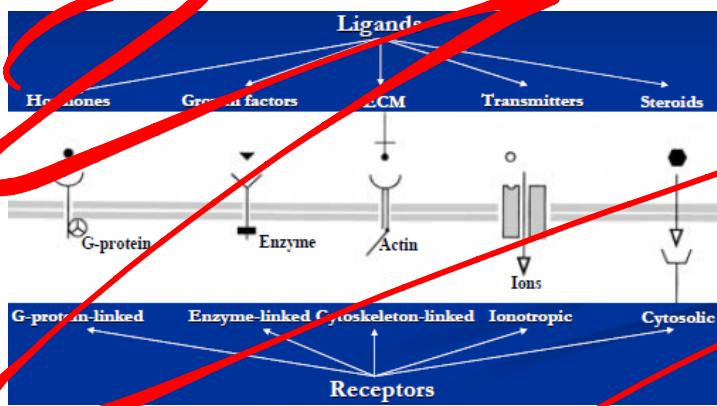
Increased RBC destruction in the spleen

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Hemolytic anemia

Jaundice

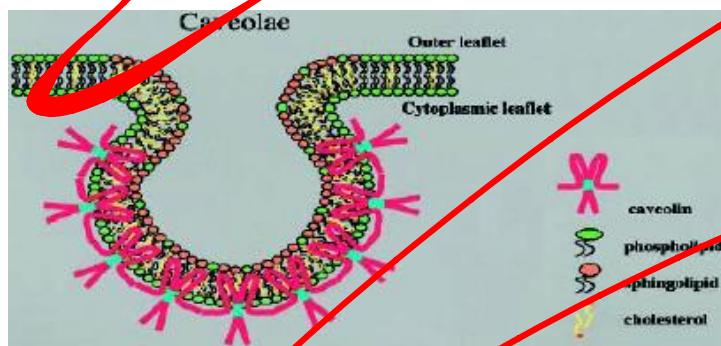
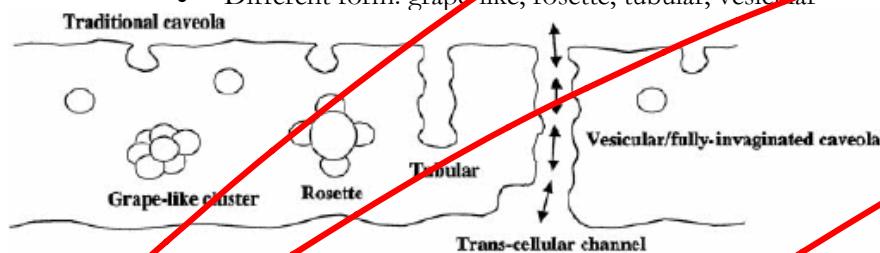
- Signal transduction: Conversion of extracellular signals into intracellular ones



Signal Transduction may take place via **lipid rafts** (microdomains with confined movement of lipids)

- Microdomains** - ~50-70 nm in diameter \rightarrow 300 sphingolipids \rightarrow 10-30 proteins
- Rich in:**
 - cholesterol
 - sphingolipids (glycosphingolipids and sphingomyelin)
- Tend to accumulate certain membrane proteins
 - glycosylphosphatidylinositol (GPI)-anchored proteins
 - doubly acylated proteins (Src-family, G_α-proteins)
 - cholesterol-linked proteins (Hedgehog)
 - transmembrane proteins
- Role**
 - endocytosis - caveolae
 - signal transduction

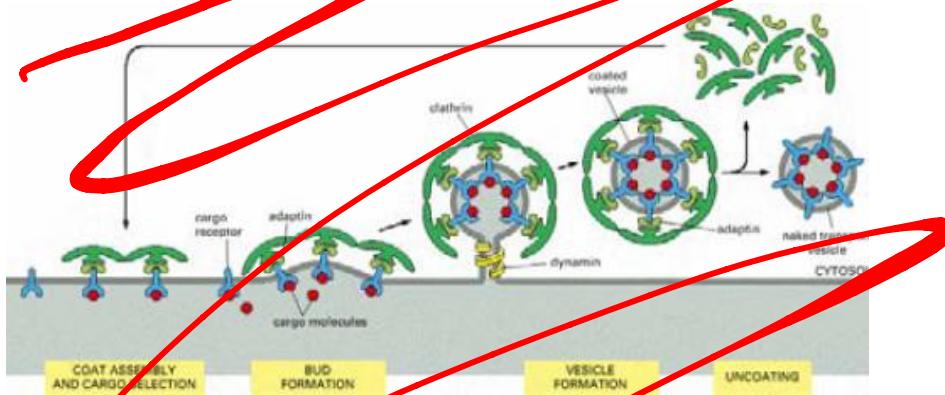
- Transport vesicles:** Clathrin-coated & Caveolae
 - Caveolae:** Membrane invaginations (50-100 nm)
 - Consists of lipid rafts and specific proteins: caveolins
 - Different form: grape-like, rosette, tubular, vesicular



- Vesicular transport:**
 - Transcytosis: endothelial cells
 - Endocytosis: viruses, fungi, bacteria, parasites, prion
- Cholesterol homeostasis:** HDL metabolism
- Signal transduction**
- Diseases:**
 - Tumorigenesis: caveolin-1 (target of oncogenes and tumor suppressor)
 - Diabetes: caveolae are highly enriched in adipocytes; glucose transporters are associated with caveolae; insulin & leptin signaling involves caveolae
 - Vascular abnormalities
 - Cardiomyopathy
 - Increased vascular relaxation due to increased NO
 - Impaired angiogenesis

- Adipogenesis: loss of caveolin-1 is protective

- Clathrin-coated vesicles



Transport via transmembrane channels

- **Aquaporins:** different aquaporins do different transmission in different organelles

- AQP0 – first member identified
 - MIP in fiber cells of the lens
 - defective in congenital cataract
- AQP1 (CHIP28 – channel-forming integral membrane protein, 28kDa)
 - expressed in kidney tubule epithelial cells, RBC, lung
 - defective in polycystic kidney disease
- AQP2
 - expressed in kidney tubule cells
 - defective in congenital nephrotic diabetes insipidus
 - defective in acquired nephrotic diabetes insipidus induced by Li
- AQP4
 - expressed in renal and non-renal cells
 - involved in hypothalamic sensor of blood osmolarity
 - involved in hereditary central diabetes insipidus?
- AQP5
 - expressed in salivary and lacrimal gland epithelial cells
 - involved in Sjogren's syndrome – autoimmune disease,

AQP6-10 – various organs

- Glucose Transporters (GLUT):

- A family of 12 TM glycoproteins
- Function
 - import of glucose and small molecules
- Classification
 - a dozen members
 - GLUT4 – the major isoform; in
 - skeletal muscle
 - adipose tissue
 - heart

Responsible for the glucose influx in response to insulin

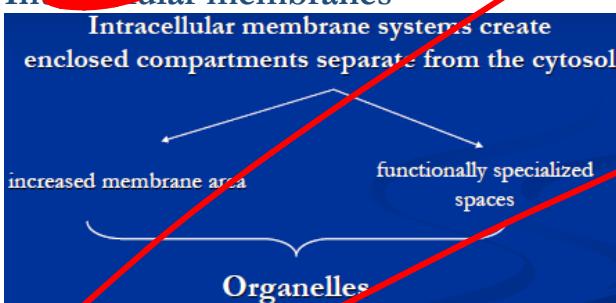
- ABC Transporters (ATP-Binding Cassette transporters): A family of 12 or 17 TM proteins
 - Function:
 - Unidirectional translocation across membranes
 - Chemically diverse substrates
 - Classification:
 - 7 subfamilies (ABCA – ABCG)
 - At least 48 members
 - Ubiquitous expression
- P-glycoprotein:
 - 12 TM domains
 - 170 kDa
 - Contributes to 90% of cancer deaths (500,000/year in USA)
 - Exports a variety of anticancer drugs

- Clinical correlates:

Molecule	Disease
ASA link proteins	Hereditary spheroctosis Hereditary elliptocytosis
Caveolin	Tumorigenesis Diabetes Vascular abnormalities
Aquaporin	Diabetes insipidus
AChR (ion channel)	Myasthenia gravis
CFTR	Cystic fibrosis
ABC transporters (Pgp)	Cancer
GLUT	Diabetes mellitus

- Formation and maturation of cell membrane proteins: synthesized in the rough endoplasm reticulum, modified and completed in the Golgi apparatus, transported in vesicles to the cell surface

Intracellular membranes



ER

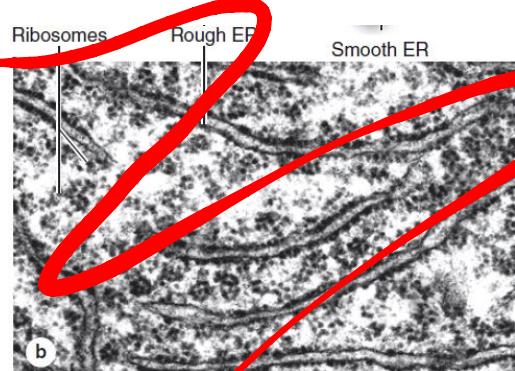
- Endoplasmic Reticulum (ER): present in all eukaryotic cells. Consists of **rough** (coated with ribosomes) and **smooth** (no ribosomes) ER. The ER is constantly being reorganized
 - ER membrane ≥ 50% total cellular membrane
 - ER lumen ≤ 10% of the volume of the cell
- The ER network is continually reorganizing with some connections being broken while new ones are formed.
- Motor proteins moving along microtubules pull out sections of ER membranes to form extended tubules that then fuse to form a network.
- **RER:** synthesis of transmembrane proteins for ER, plasmalemma, and the membranes of Golgi complex, lysosomes, secretory granules. Glycosylation of proteins to form glycoproteins
 - Polyribosome attached membrane enclosed flattened sacs (cisterns) and branching tubules that communicate with each other

Polyribosomes are

- free (unattached to ER membranes)
- attached to ER membranes (guided there by sorting signals of the polypeptide destined for the secretory pathway)

- SER: synthesis of phospholipids and cholesterol for ER, plasmalemma, and the membranes of Golgi complex, lysosomes & secretory granules; Sequestering Ca^{2+}

- **Synthesis of intracellular lipids – all main classes**
 - ❑ cell membrane
 - ❑ nuclear envelope membranes
 - ❑ organelar membranes
- **Synthesis of extracellular lipids – hepatocyte**
 - ❑ synthesis of cholesterol for lipoproteins
 - ❑ detoxification – cytochrome P450 complex
- **Sequestering Ca^{2+} from the cytosol – myocyte**
 - ❑ Ca^{2+} is stored in the smooth ER
 - ❑ Release and reuptake of Ca^{2+} are strictly regulated



Signal Hypothesis (Günter Blobel)

- Theory: how a protein identifies its goal:
 - Signal sequence of polypeptide binds on a Signal Recognition Particle (SRP)
 - Together they bind to a SRP receptor in the Lumen
 - The receptor opens a channel, through that the polypeptide is let in
 - Finally, the protein can be transmitted in the cell
- Free polyribosomes (polysomes)**
 - Initial translation of mRNA (up to 70-80 amino-acid containing a sorting signal)
 - Binding of polypeptide-ribosome complex to SRP
- RER**
 - SRP receptor binds polypeptide-ribosome-SRP complex
 - Polypeptide passage through a RER transmembrane translocator pore into the RER lumen
 - Signal peptidase cleaves the sorting signal
 - Elongation of the polypeptide chain
 - Polypeptide release into the RER lumen or insertion into the RER membrane

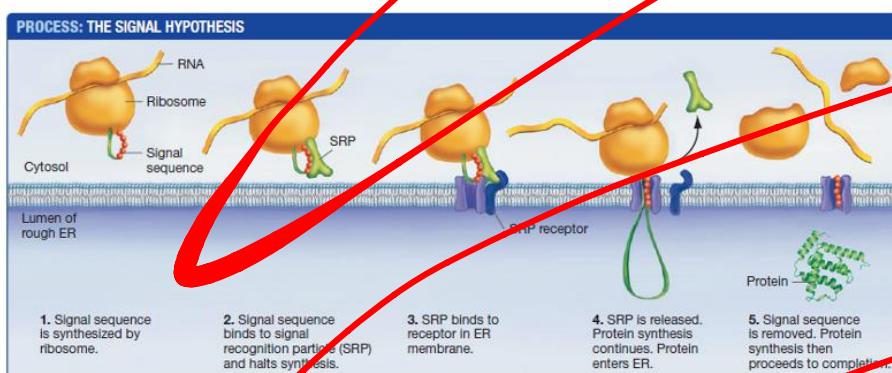
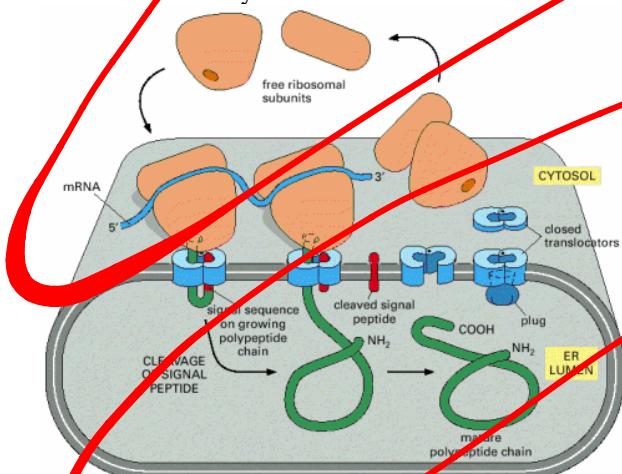


FIGURE 7.21 The Signal Hypothesis Explains How Proteins Destined for Secretion Enter the Endomembrane System. According to the signal hypothesis, proteins destined for secretion contain a short stretch of amino acids that interact with a signal recognition particle (SRP) in the cytoplasm. This interaction directs the synthesis of the remaining protein into the ER.

- The ribosomal cycle:**



- Signal Hypothesis (cont'd):**
 - Translocation of polypeptides into the RER lumen through a translocator pore in the ER membrane
 - Cleavage of signal sequence: signal peptidase
 - Growth (elongation) of polypeptide chains
 - Posttranslation: RER, Golgi complex
 - Vesicular transport: RER to cis-Golgi and vice versa

- Storage in secretory granules, lysosomes or synaptic vesicles, and insertion in the plasmalemma
- Exocytosis: final step of secretory pathway

Exocytosis – the final step of the secretory pathway:

- paracrine
- exocrine → secretion
- endocrin

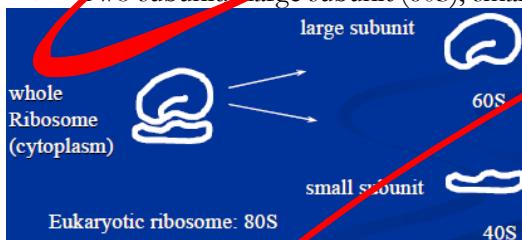
- Why are polyribosomes bound to the ER, but not to any other organelle?

The translocation of polypeptide chain through the pore of the translocator (transmembrane protein complex of RER) occurs usually during translation of mRNA, i.e. **co-translationally**

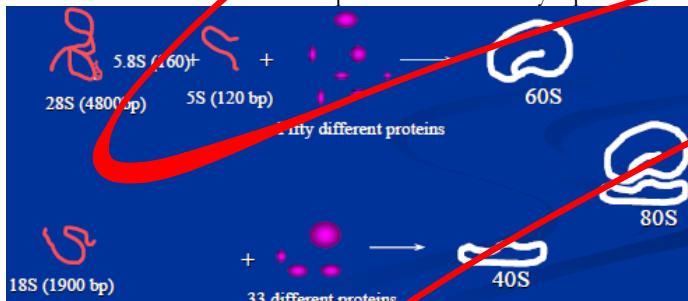
Note: translocation of proteins into mitochondria and peroxisomes occurs **post-translationally**, after protein synthesis and release in the cytosol. This explains why polyribosomes are bound to the ER, but not any other organelle.

Ribosomes

- Function: protein translation
- Structure: ribosomes are made of rRNA and proteins.
 - Eukaryotic ribosome structure:
 - Four rRNAs 28S, 18S, 5.8S, 5S
 - Plus associated proteins (> 50)
 - Prokaryotic structure is very similar, but lacks 5.8S rRNA
 - 4.2 MDa
- Two subunits: large subunit (60S), small subunit (40S)



- Assembled in nucleus
- Proteins have to be imported from the cytoplasm into the nucleus



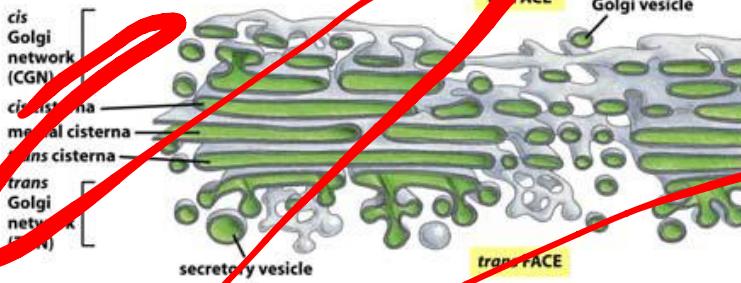
- Ribosome function is basically a ribozyme (enzymatic)
- Lots of internal structure through base-pairing
- Many bases are post-transcriptionally modified
- **Ribosomal Subunits:**
 - The genes responsible for making the rRNAs primary transcript is cut and chemically modified
 - Large 45S rRNA is copied from the DNA. The source of 3 of 4 rRNAs 28S, 18S, 5.8S
 - The 5S gene is outside the nucleolus

Ribosome subunit assembly takes place in the nucleolus, bringing together the genes, the 45S primary transcript and the processing enzymes and the proteins. Finished small and large subunits are sent to the cytoplasm separately to do their work: i.e. **translation**

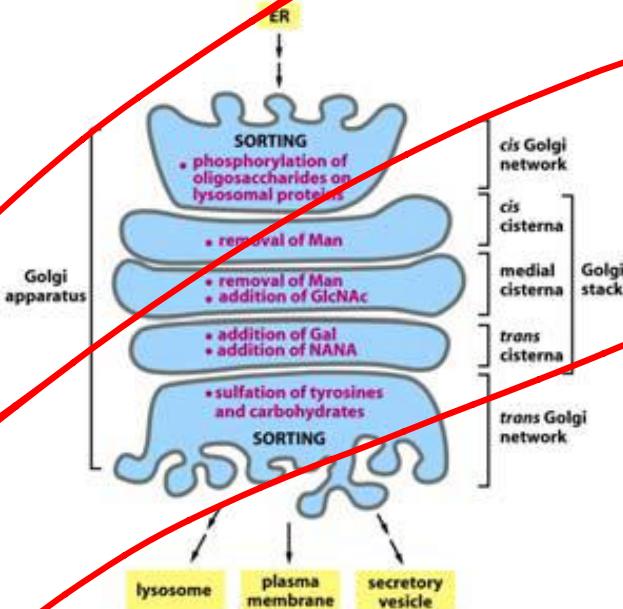
Golgi complex, Lysosomes, Peroxisomes, Mitochondria

Golgi Apparatus

- Parts of the Golgi complex:
 - **Cis Golgi network (CGN)** - network of interconnected tubular and cisternal structures - protein sorting (secretory vs. retrieval proteins)
 - Collection of 4-6 membrane-enclosed flattened cisternae linked by tubules (**dictyosomes**): cis-Golgi, medial-Golgi & trans-golgi compartment
 - **Trans Golgi network (TGN)**: network of interconnected tubular and cisternal structures for protein sorting. Golgi matrix proteins involved in the function of the organelle



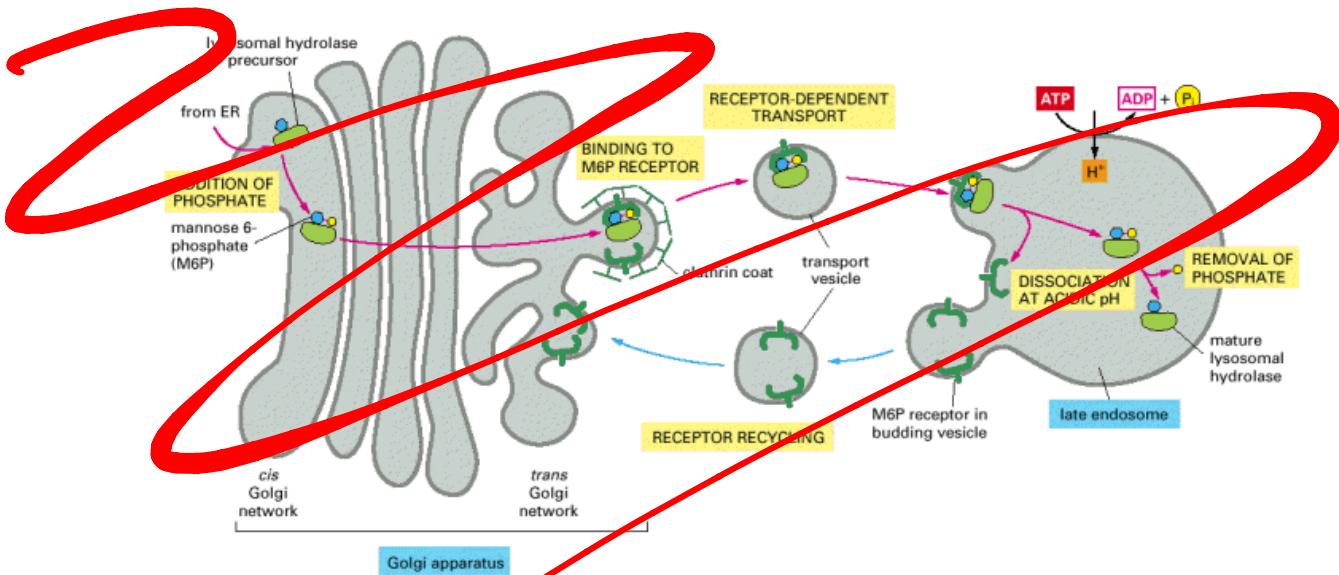
- Functions of Golgi
 - Sorting (CGN): phosphorylation of oligosaccharides on lysosomal proteins
 - Cisternae (Golgi stack) Some sugars get removed and replaced by N-acetylneurameric acid. It is negatively charged and will therefore attract positive charged proteins
 - Sorting (TGN): Decision where the protein is sent to, then release



- Two hypotheses explaining the transport in Golgi Apparatus:
 - Vesicular Transport model: Vesicles move between the cisterns
 - Cisternal Maturation model: the cisternas themselves move
- Microtubules serve as a railway whereon the vesicles are moved to the Golgi apparatus

Lysosomes

- Membrane-enclosed structures filled with different types of acid hydrolases (e.g. proteases, nucleases, lipases). The lysosomes limiting membrane and the intralysosomal pH (around 5.0) protect the cytoplasm and other organelles against digestive attack of the lysosomal enzymes (cytoplasmic pH around 7.2)
- Function: Digestion
 - Digestion of macromolecules, phagocytosed microorganisms, cellular debris
 - Digestion of senescent organelles, such as mitochondria and RER
 - End products are transported into cytosol and are either reused or exported
- Acid Hydrolases:
 - Nucleases
 - Proteases
 - Glycosidases
 - Lipases
 - Phosphatases
 - Sulfatases
 - Sulfatases
 - Phospholipases
- The low pH of a Lysosome is achieved by a H⁺-pump into the cell by using ATP
- Formation of lysosomes:
 - Receive their hydrolytic enzymes as well as their membranes from the TGN
 - Membranes arrive as clathrin-coated vesicles, which fuse with the lysosomal membranes
 - Hydrolytic enzymes possess mannose-6-phosphate receptors, to which these enzymes are bound. IN the acidic environment of the late endosome, lysosomal enzymes dissociate from their receptors



- Formation of lysosomes via late endosomes:
 - Late endosomes contain material received from both the plasma membrane by endocytosis and newly synthesized lysosomal hydrolases, and they therefore already bear a resemblance to lysosomes
 - There is no real distinction between late endosomes and lysosomes
- Lysosomes play an important role in the metabolism of several substances in the human body, and consequently many diseases have been ascribed to deficiencies of lysosomal enzymes (**lysosomal storage diseases** or **mucopolysaccharidoses**)
- Lipofuscin**: insoluble brownish yellow granular intracellular material that accumulates as a function of age or atrophy. Not injurious to the cell but important as a marker of past free-radical injury.

Peroxisomes

- Found in all eukaryotic cells
- Membrane-enclosed organelles
- Self-replicating
- Exist without a genome of its own, must import all proteins
- Peroxisomes are not only involved in metabolic processes such as **hydrogen peroxide detoxification**, but also in **signaling pathways** that promote developmental decisions and cell differentiation in the brain, adipose tissue, placenta, etc.
- Small (0.2-1.0 micrometers in diameter) spherical to ovoid, membranous organelles
- Contain more than 40 oxidative enzymes (especially urate oxidase, catalase, and d-amino acid oxidase)
- Defects of Peroxisomes: **Peroxisome Biogenesis Disorders (PBD)** or **Peroxisome Single Enzyme Disorders (PSED)**
 - Defects in Pex genes responsible for import of proteins
 - Defective peroxisomal enzyme import
 - Clinical manifestations related to the nature of the deficiency

Zellweger (cerebro-hepatorenal) syndrome
 - inherited disease due to Pex gene mutations
 - characterized by facial-cranial and brain abnormalities, liver and kidney affected
 - patients die soon after birth

- Functions:
 - Lipid β-oxidation (fatty acids are shortened to convert them into acetyl CoA)
 - Detoxification (removal of H atoms from certain organic substrates)
 - $\square \text{RH}_2 + \text{O}_2 \rightarrow \text{R} + \text{H}_2\text{O}_2$
 - $\square 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$
 - Define the steady-state levels of signaling lipids outside peroxisomes
 - \square retinoic acid (RA)
 - \square long-chain fatty acids
 - \square signaling lipids activate nuclear receptors (RA receptors) or peroxisome proliferator-activated receptors (PPAR)
 - Biosynthesis of plasmalogens - the most abundant class of phospholipids in myelin → neural symptoms in PBD
 - Viral life cycle (HIV and rotavirus) involves the sorting of some viral proteins to peroxisomes

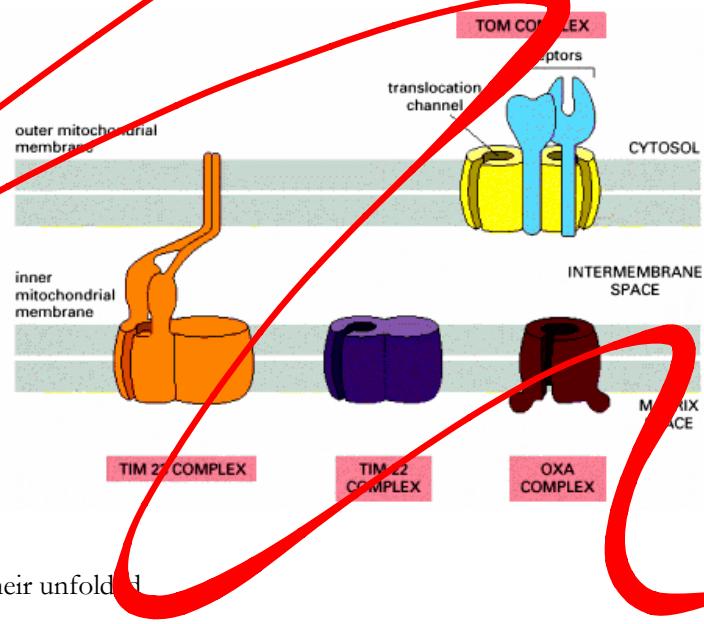
- Biogenesis by **peroxisomal targeting signals (PTS1 or PTS2)**. PTS1 is a tripeptide and is used by more than 90% of human peroxisomal proteins
- PTS-containing proteins are recognized by PTS cytosolic (soluble) receptors, which belong to the Pex family of proteins. Next steps are: (i) docking of PTS proteins to peroxisomal membrane; (ii) dissociation of the receptor-ligand complexes; (iii) import of ligand (peroxisomal matrix proteins) into the peroxisome; (iv) recycling of receptors in cytosol.

Proteasomes

- Not membrane-surrounded
- Translation of mRNA into amino acid sequence is not the end of protein formation. For its proper function, proteins need to be folded correctly into 3D configuration, and assemble with its particular subunits chains.
- Protein folding is mainly done by a proteasome called **chaperone**
- Misfolded Proteins are exported from RER and degraded in cytosol by proteasomes
- The retrotranslocation occurs through the same translocator, through which the proteins enter the RER
- **Ubiquitin-Proteasome System (UPS):**
 - Proteasomes are non-membrane-bound organelles consisting of ATP-dependent proteases that form a central cylinder (proteolytic chamber) supplemented by a cap (gate) at each end
 - Proteasomes recognize misfolded proteins destined for degradation by their linkage to a small molecule: **ubiquitin**. Ubiquitin is recognized by a specific receptor in the cap.
- Misfolded protease-resistant proteins can aggregate causing neurodegenerative disorders
 - Alzheimer
 - Huntington
 - Parkinson
 - Prion diseases

Mitochondria

- Double-membrane-enclosed organelles, most probably evolved from bacteria by endocytosis
 - **Outer membrane**, which contains a large number of transport proteins named **porins**
 - **Inner membrane**, which forms many invaginations (**cristae**) that are rich in enzymes of the respiratory chain/oxidative phosphorylation. They generate ATP (100 molecules/sec) via the ATP synthase
 - **Intermembrane space**
 - **Matrix**, which contains genomic apparatus and proteins
- Functions:
 - Generation of ATP
 - Citric acid (Krebs) cycle
 - Haem biosynthesis
 - β -oxidation of fatty acids
 - Apoptosis (programmed cell death)
 - Calcium storage and signaling
 - Generation and detoxification of reactive oxygen species
- **ATP hydrolysis (ADP + P_i)** drives a large number of cell's energy-requiring processes. Generation of ATP is realized through oxidative phosphorylation, proton gradient in inner membrane, and repeated changes in ATP synthase's conformation that convert mechanical energy into chemical bond energy. Next, ATP is released from mitochondrial matrix into cytosol to be used, while ADP & P_i enter the matrix for ATP re-synthesis
- Parts of the mitochondrial complex:
 - **TOM complex** (translocator of outer membrane): mediates import of all nucleus-encoded mitochondrial proteins
 - **TIM complex** (translocator of inner membrane): mediates import of all nucleus-encoded mitochondrial proteins
 - **OXA complex**: localized in inner membrane, and mediates insertion of inner membrane proteins synthesized in cytosol or mitochondria
 - **SAM complex** (sorting and assembly machinery): localized in outer membrane, mediates insertion of outer membrane proteins
- Transport of nucleus-encoded proteins into mitochondria:
 - Synthesis on cytosolic polyribosomes
 - Binding to cytosolic hsp70/hsp90 to keep their unfolded



- Configuration
- Signal sequence* recognizes import receptor that is component of TOM complex
- Opening of the pores of TOM and TIM23 at contact sites of inner/outer membranes
- Cleavage of signal sequence by signal peptidase
- “Pulling” the protein into matrix* by mitochondrial hsp70
- Removal of hsp70 by ATP hydrolysis allowing imported proteins to fold
- Mitochondria and Apoptosis: Damaged cells commit suicide via apoptosis. This process depends on a family of proteases that have cysteine at their active site at aspartic acids; hence **caspases**. In apoptosis, mitochondria release the inner membrane protein **cytochrome c** into the cytosol. Cytochrome c binds/activates an adaptor protein resulting in activation of caspase cascade, which cleaves key cellular proteins leading to apoptosis. Mitochondria also release a protein that blocks apoptosis-inhibiting proteins; this further increases the efficiency of apoptosis

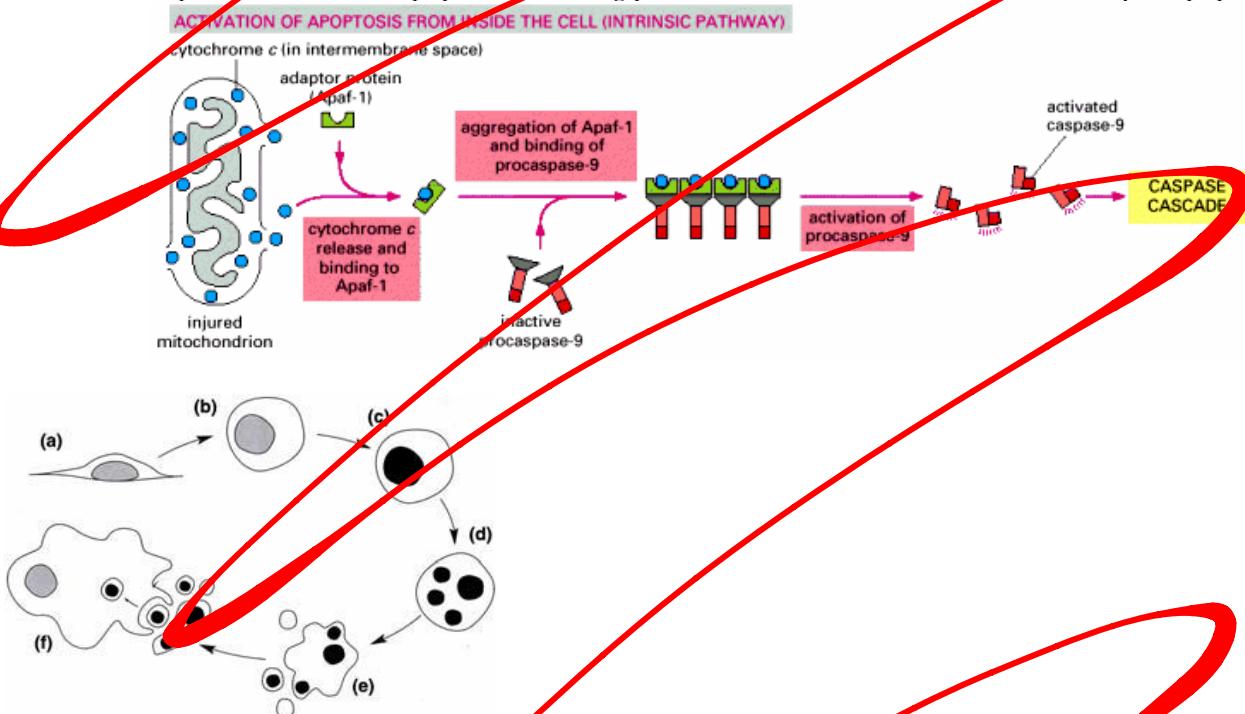
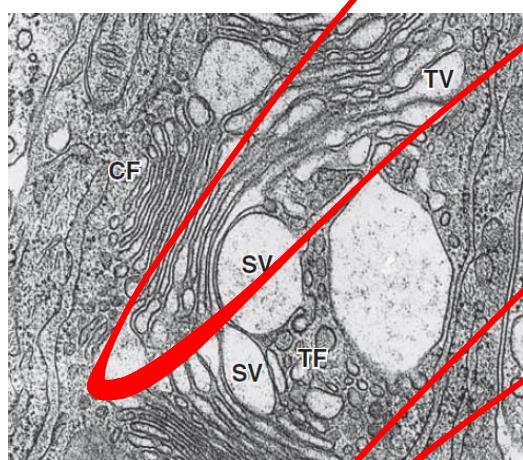
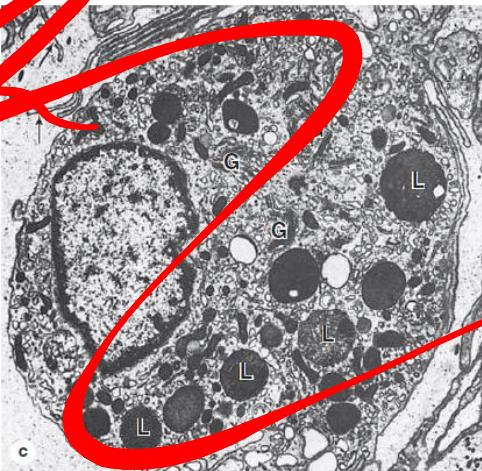


Figure 1
Schematic representation of the process of apoptosis. Upon receipt of a signal to undergo apoptosis, an adherent cell (a) rounds up (b) and rapidly condenses its DNA (c), thought to be a consequence of DNA fragmentation. This is rapidly followed by separation of the nucleus into discrete masses of condensed chromatin (d) and, finally, fragmentation of the cell into several membrane-bound vesicles (apoptotic bodies) (e), which are rapidly recognized and phagocytosed by macrophages or neighbouring cells (f).

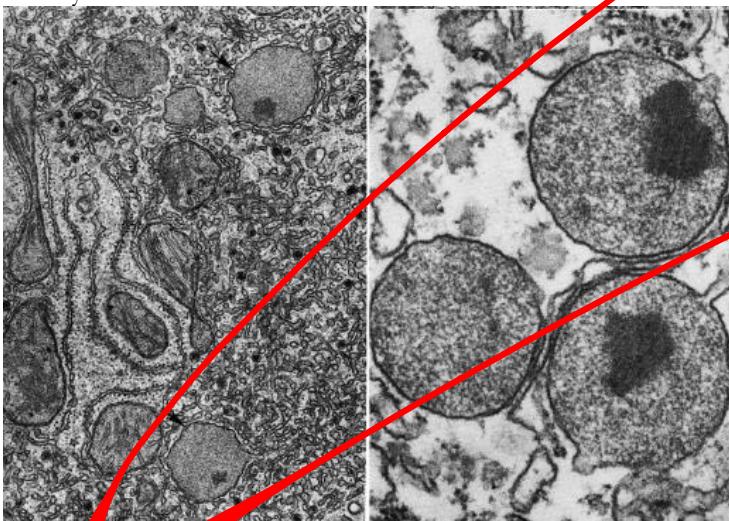


CF = *cis* face
 TV = transport vesicles
 SV = secretory vesicles
 TF = Trans face

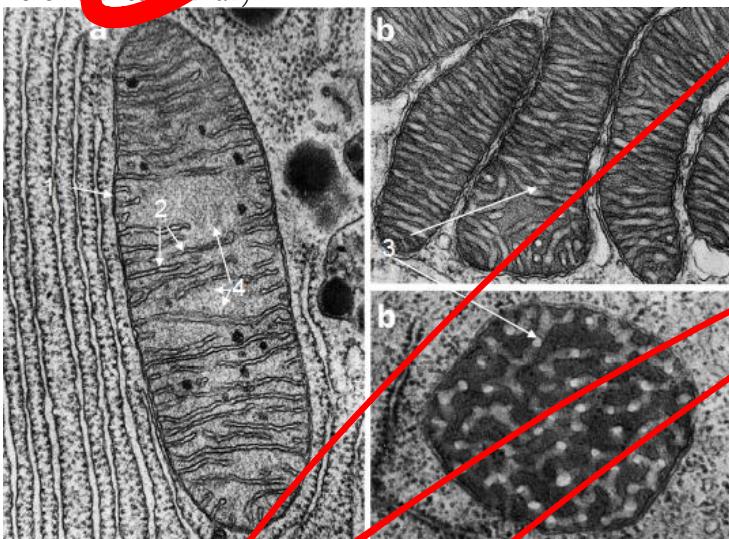


G = Golgi apparatus

L = Lysosomes



Peroxisomes (Human)

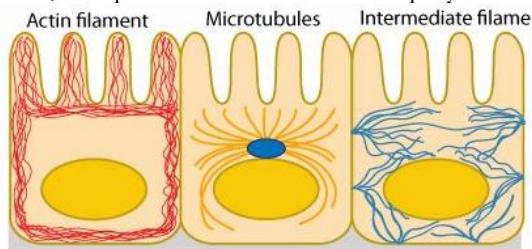


1. Outer Membrane
2. Lamellar cristae of the inner membrane
3. Tubular cristae of the inner membrane
4. Mitochondrial ribosomes

Cytoskeleton

- An intricate cytoplasmic 3D meshwork of protein filaments that are responsible for the cell maintenance, f. e. for:
 - Cellular morphology
 - Cellular motion (the entire cell / organelles within the cell)
- Components:

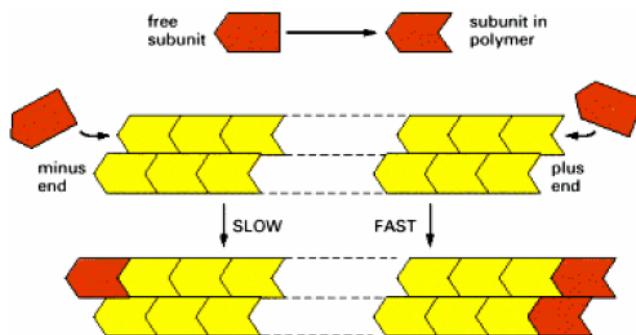
- **Actin-containing filaments** (microfilaments): 5-9 nm, composed of actin and actin-associated proteins, two-stranded helical polymers; organized into variety of linear bundles, two-dimensional networks & three-dimensional gels
- **Microtubules**: 25nm, composed of tubulin & microtubule-associated proteins
- **Intermediate filaments**: 10nm, composed of homo- or heteropolymers of specific proteins



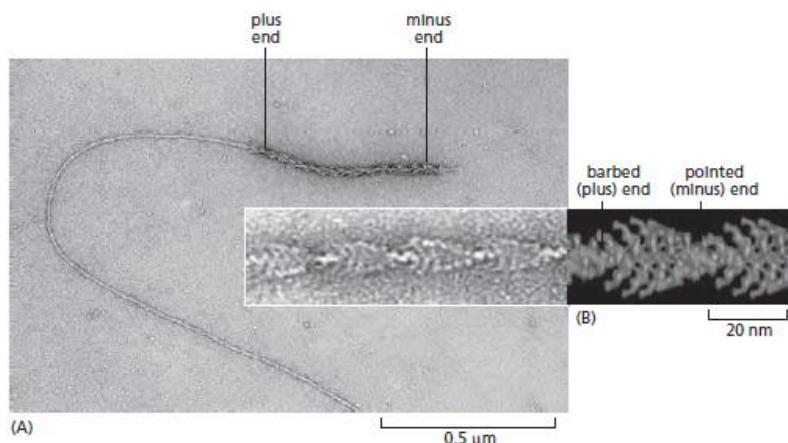
Actin filaments

- **Actin filaments** (also known as *microfilaments*) are **two-stranded helical polymers** of the protein **actin**. They appear as flexible structures, with a **diameter of 5-9 nm**, and they are organized into a variety of linear bundles, two-dimensional networks, and three-dimensional gels.
- Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the **cortex**, just beneath the plasma membrane.
- Actin filaments are continuously assembled and disassembled from **G-Actin** to **F-Actin**
- Type of Actin and their distribution
 - α - muscle-specific
 - β - ubiquitous
 - γ - ubiquitous
- The two ends of an actin filament polymerize at different rates:
 - The fast-growing end is called the **plus end**
 - The slow-growing end is called the **minus-end**

The difference in the rates of growth is made possible by **changes in the conformation** of each subunit **as it enters the polymer**

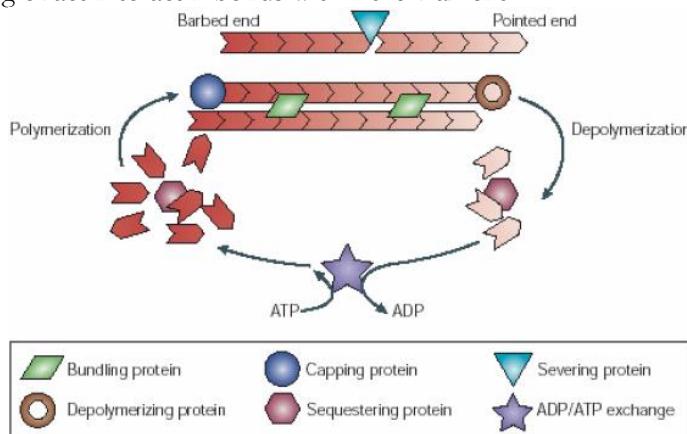


- Actin filaments can dynamically assemble. If a signal comes in, such as a nutrient source, the actin filaments can disassemble and rapidly diffuse into subunits, then reassemble at a new site
- **Glossary:**
 - **G-actin:** monomeric, globular in configuration (42 kDa)
 - **F-actin:** filamentous double-helical assembled G-actin with two ends (+ (growing, barbed) & - (shrinking, pointing))

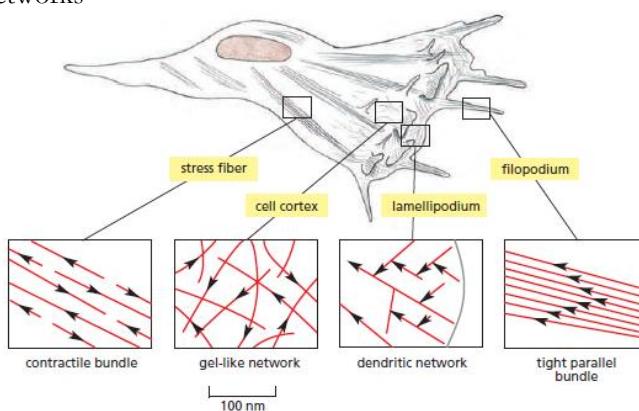


- **Capping:** binding of actin-associated protein to the +End of F-actin that prevents further assembly of G-actin
- **Nucleation:** assembly of G-actin subunits into small oligomers

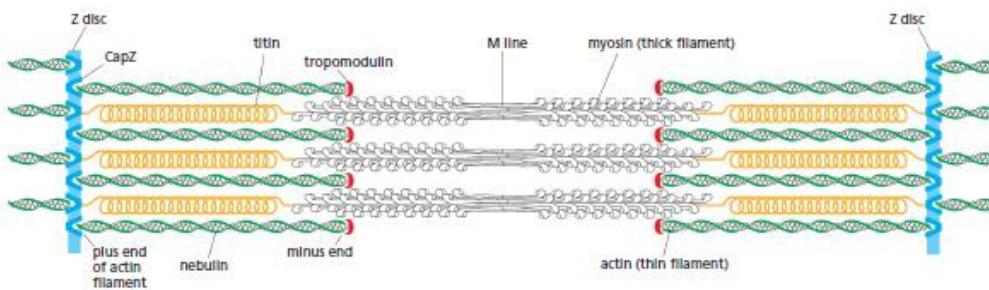
- **Assembly:** non-covalent binding of G-actin to form F-actin
- **Sequestration:** binding of G-actin with actin-associated protein
- **Severing:** breaking of actin-to-actin-bonds within the filament



- Actin-associated Proteins:
 - **Actin-sequestering proteins:**
 - Profilin
 - Thymosin $\beta 4$
 - Gc protein (group-specific component or vit.-D-binding protein)
 - Gelsolin (brevin)
 - **Actin-severing proteins**
 - Gelsolin
 - Fragmin
 - Severin
 - **Actin-crosslinking proteins**
 - Myosin
 - α -actinin
 - Dystrophin
 - Filamin, fimbrin
 - Villin
 - ERM proteins (ezrin, radixin, moesin)
- $AF = A + AAP \rightarrow$ Actin filaments = Actin + Actin-associated proteins
- Actin filaments in animal cells are organized into several **types of arrays:**
 - Dendritic networks
 - Bundles
 - Weblike (gel-like) networks



- Some actin filament structures are assembled and maintained by two classes of proteins: **bundling proteins**, which cross-link actin filaments into a parallel array, and **gel-forming proteins**, which hold two actin filaments together at a large angle to each other, thereby creating a looser meshwork.



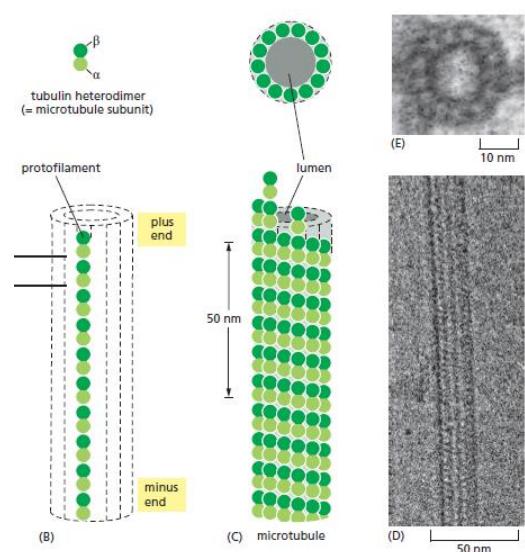
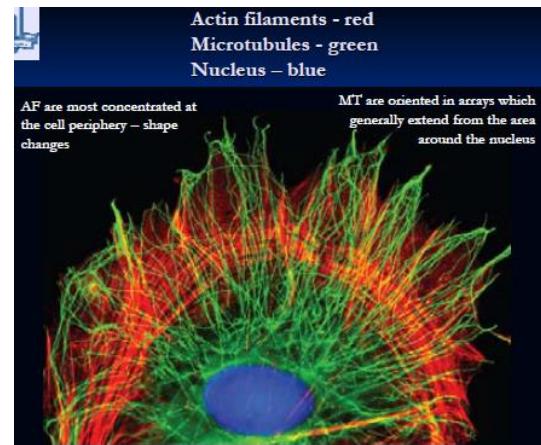
	Distribution	Function
Myosin I	microvilli	cross-linking AF with plasmalemma
Myosin II	muscle sarcomere filopodia, lamellipodia	contractile activity cell movement, cytokinesis
Myosin V	cytoplasm perinuclear	intracellular transport of membrane-bound organelles
Myosin VII	stereocilia	hearing; mutations result in deafness

- **Actin-specific drugs:**
 - Phalloidin: binds and stabilizes filaments
 - Cytochalasin: caps filament plus ends
- A link between AF and ECM is mediated by the protein **dystrophin**. It connects the extra- and intracellular protectin layer (Glycoprotein complex with Actin cytoskeleton)
- Dystrophin-associated proteins and **human muscular dystrophy (MD)**:
 - Dystrophin: Duchenne & Becker MD (DMD, BMD)
 - Sarcoglycan complex: Limb-girdle MD (LGMD)
 - Laminin: Congenital MD (CMD)

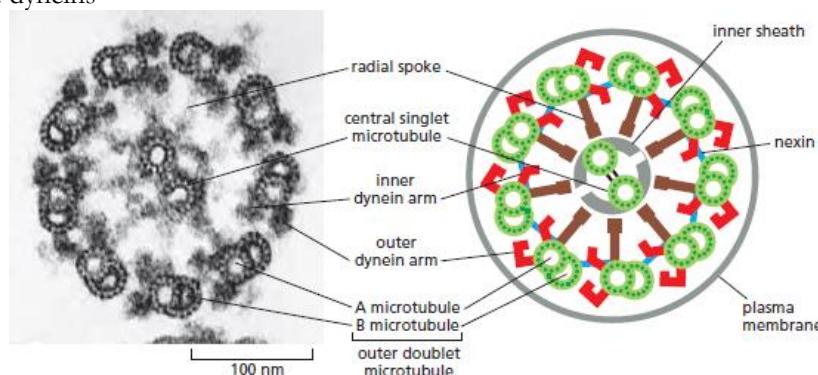
Microtubules

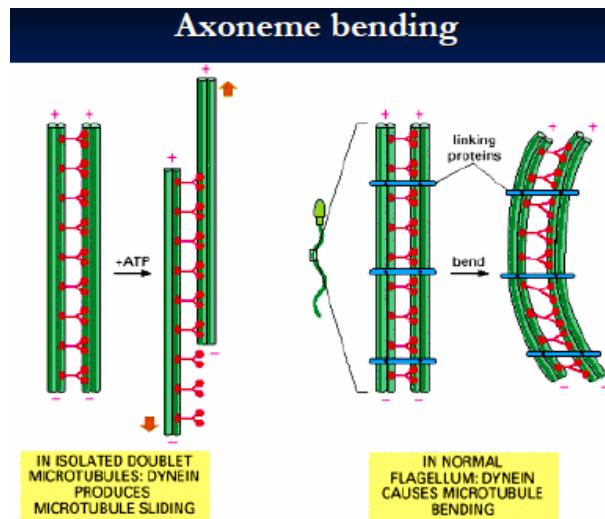
- Microtubules are structurally more complex than actin filaments, but they are also highly dynamic and play comparably diverse and important roles in the cell. Microtubules are polymers of the protein **tubulin**.
- Long, straight, rigid tubular-appearing structures that act as **intracellular "railways"**.
- With an outer **diameter of 25 nm**, they are much more rigid than actin filaments
- Microtubules are long and straight and typically have one end attached to a single **microtubule-organizing center (MTOC)** called a **centrosome**
- The subunit of each **protofilament** is a tubulin heterodimer, formed from a tightly linked pair of **α - and β -tubulin monomers**. Each protofilament consists of many adjacent subunits with the same orientation. The microtubule is a stiff hollow tube formed from 13 protofilaments aligned in parallel.

- Microtubules are very **dynamic** structures; they are continuously assembled and disassembled
- MT = T + MAP; Microtubules = Tubulin + MT-associated proteins
- Spatial and temporal organization of molecules and organelles is ensured by their intracellular transport to various destinations within the cytoplasm
- **Glossary:**
 - **Tracks** (cytoskeletal structures having + and - ends)
 - Microtubules (MT)
 - Actin filaments (AF)
 - **Motor proteins:**
 - **Kinesins** - (+)-end-directed (anterograde) MT motors
 - **Dyneins** - (-)-end-directed (retrograde) MT motors
 - **Myosin Vs** - (+)end-directed (anterograde) AF motors
 - **Cargo**
 - Membrane-bound organelles
 - Non-membranous components: mRNA, IF, viruses



- Human diseases & motor proteins:
 - **Kinesin gene mutations:**
 - Charcot-Marie-Tooth type 2A disease
 - Hereditary spastic paraplegia
 - **Myosin V gene mutations:**
 - Griscelli's syndrome: recessive disorder characterized by skin and neurological symptoms
 - **Dynein:** Kartagener's syndrome
- **Functions of microtubules:**
 - Intracellular vesicular transport
 - Positioning of RER & Golgi complex
 - Structuring of MTOC/centrosome
 - Structuring of cilia & flagella
 - Mitotic spindle
- **Microtubule-Organizing Center (MTOC)**
 - While α - and β -tubulin are building units of MT, γ -tubulin is involved in MT nucleation
 - MT are nucleated from a specific formation known as a MTOC. The centrosome is the major MTOC
 - The centrosome is composed of 2 centrioles surrounded by amorphous matrix containing γ -tubulin ring complexes (γ -TuRC) that serve as templates nucleating MT growth.
 - MT are nucleated at their (-)end with the (+)end growing outward from each MTOC
- **Centrosome:**
 - Cylindrical structures, approx. 0.2 μm in diameter and approx. 0.5 μm in length
 - Each centriole consists of 9 relatively short **microtubular triplets** linked together in a pinwheel-like arrangement
 - In the triplets, microtubule A is complete and consists of 13 protofilaments, whereas microtubules B and C share protofilaments
 - The 2 of centrioles is called **centrosome**
- Cellular structures containing MT:
 - **Intracellular:**
 - MTOC/centrosome
 - Mitotic spindle
 - Basal body
 - **Plasmalemmal:**
 - Cilium
 - Primary (immotile) cilium
 - Flagellum
- **Primary (immotile) cilia:**
 - 9x2 + 0 MTs
 - IFT proteins
 - Motor proteins
 - Key receptors clustered in primary cilia
- **Cilia & flagella** (sing.: cilium, flagellum):
 - Motile processes, covered by cell membrane, with a highly organized microtubule core
 - Cilia are normally many, each about 2-3 μm in length
 - In humans, the spermatozoa are the only cell type with a flagellum
- **Axoneme:** core structure of cilia and flagella
 - 9x2 + 2 microtubules
 - MTs of the peripheral doublets (9x2) are identified as **A** (complete with 13 protofilaments) and **B** (with only 10 protofilaments)
 - Doublets are linked to each other by protein bridges called nexins
 - MT motors: dyneins





- At the base of each cilium or flagellum is a **basal body**, similar to a centriole, which controls the assembly of the axoneme
- Ciliopathies** (diseases of cilia or basal bodies):
 - Joubert syndrome (JBTS)
 - Bardet-Biedl syndrome (BBS)
 - Meckel syndrome (MKS)
 - Ellis Van Ceveld syndrome (EVC)
 - Oro-facial-digital syndrome type 1 (OFD1)
 - Jeune syndrome (JATD)
- Microtubule-specific drugs**:
 - Taxol: binds and stabilizes microtubules
 - Colchicine / Vinblastine / Nocodazole: binds subunits and prevents their polymerization

Intermediate Filaments

- Intermediate filaments are ropelike fibers with a diameter of around 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogenous family.
 - One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane
 - Other types extend across the cytoplasm, giving cells mechanical strength.
 - In epithelial tissue, they span the cytoplasm from one cell-cell junction to another, thereby strengthening the entire epithelium
- Humans have at least 67 genes that encode IF proteins. This gene family is one of the largest in the human genome.
- IF are of five types with either cytoplasmic or nuclear location:
 - Type I-IV - cytoplasmic
 - Type V - nuclear
- IF-Functions:**
 - Cellular stability
 - Cell-to-cell junctions: **desmosomes**
 - Cell-tp-ECM junctions: **hemidesmosomes**
 - Structuring of nucleus: **nuclear lamina**

More than 30 diseases are related IF protein mutations	
Keratinopathies (keratins)	
■ Skin diseases	epidermolysis bullosa simplex epidermolytic hyperkeratosis palmoplantar keratoderma
■ Hair diseases	alopecia fragile hair
■ Gastrointestinal diseases	inflammatory bowel (Crohn's) disease liver cirrhosis hepatitis chronic pancreatitis

Laminopathies (lamins A & C)	
■ Muscular dystrophy (MD)	Emery-Dreifuss MD Limb-girdle MD
■ Cardiovascular diseases	dilated cardiomyopathy
■ Metabolic diseases	familial lipodystrophy lipoatrophy with diabetes
Other diseases	
■ Amyotrophic lateral sclerosis (NF-H, peripherin)	
■ Alexander diseases (GFAP)	
■ Cataract (phakinin, crystallin)	
■ Desmin-related myopathy (desmin)	
■ Charcot-Marie-Tooth disease (NF-L)	

- The combined surface area of AF, MT and IF exceeds by more than 10 times the area of all cellular membranes. This, together with cross-talk among these cytoskeletal systems, demonstrates the significant potential of the cytoskeleton in the regulation of various cellular functions,

including cell shape and motility, intracellular transport, intercellular and cell-matrix junctions, DNA transcription, RNA processing, cell division, and signal transduction

- IF Proteins & Human Disease:

Summary

- All eukaryotic cells contain actin and tubulin. All eukaryotic cells contain actin filaments and microtubules. However, IF (except nuclear lamin-containing IF) are found only in some eukaryotes. Further, most IF proteins are cell-selective, i.e. present in only a certain type of cell.
- Main function of cytoskeletal elements:
 - Actin filaments: movement
 - Microtubules: Transport
 - Intermediate filaments: stability
- Cytoskeletal component cross-linker Proteins:
 - AF cross-linkers:
 - Villin
 - Fimbrin
 - Filamin
 - α -actinin
 - Spectrin
 - MT cross-linkers:
 - MAP1-5
 - Tau
 - IF cross-linkers:
 - Plectin
 - filaggrin

IF vs. AF & MT		
	IF	AF & MT
Diameter	10 nm, intermediate between the diameter of AF and MT	
Number	large number of proteins	actin and tubulin
Distribution	cytoplasm & nucleus	cytoplasm*
Primary structure	rod-shaped tetramers	globular monomers (actin) globular dimers (tubulin)
Polarity	nonpolar	polar (+) and (-) ends
Energy dependence	ATP/GTP-independent	ATP (actin) GTP (tubulin)
Cell selectivity	Yes (except nuclear lamins)	No (ubiquitous)

*some short AF are also found in the nucleus

Summary			
Motor Proteins in Cytoskeleton			
	AF	MT	IF
Motor proteins	myosin V (+) myosin VI (-) myosin II	kinesin (+) dynein (-)	none

Summary	
Cytoskeletal Component Assembly	
Actin Filaments	<ul style="list-style-type: none"> subplasmalemmal nucleation controlled by Arp2/3
Microtubules	<ul style="list-style-type: none"> perinuclear nucleation controlled by γ-TuRC
Intermediate Filaments	<ul style="list-style-type: none"> no nucleation assembly of rope-like tetramers

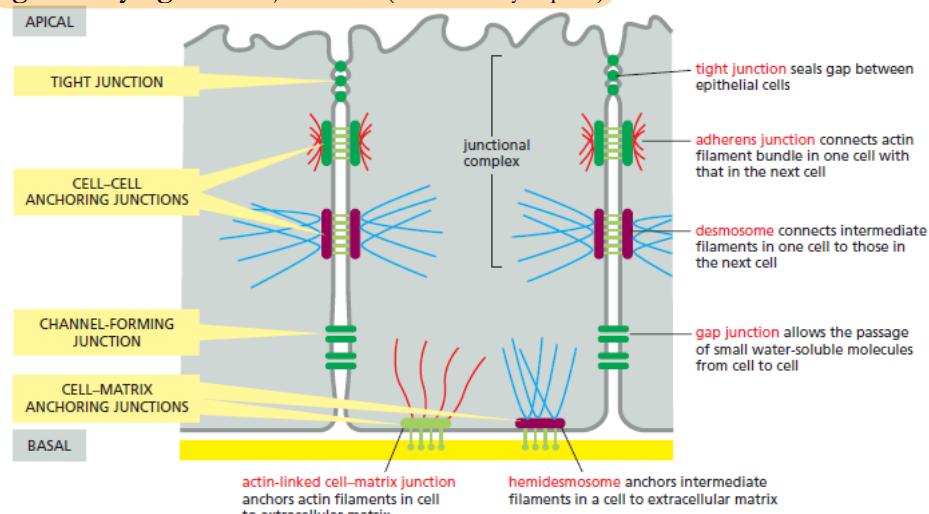
Cell Junctions

- Several membrane-associated structures contribute to adhesion and communication between cells or between cells and ECM. They are present in most tissues but are particularly numerous and prominent in epithelia

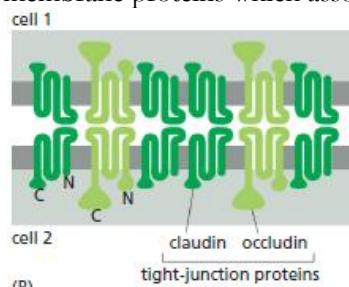
- Cell Junctions:

- Occluding junctions:
 - Tight junctions (**zonula occludens**) - keep neighboring cells together in epithelial tissue in a way that prevents even small molecules to pass through the junction --> apical and basolateral surface in gut epithelial cells
- Anchoring junctions:
 - Actin-filament-associated:**
 - Cell-cell junctions (adherens junctions, zonula adherens)
 - Cell-matrix junctions (focal adhesions)
 - Intermediate filament-associated**
 - Cell-cell junctions (desmosomes)
 - Cell-matrix junctions (hemidesmosomes)
- Communicating junctions:

- **Channel forming** cell-cell junctions (nexus, gap junction)
- **Signal relaying** cell-cell junctions (chemical synapses)

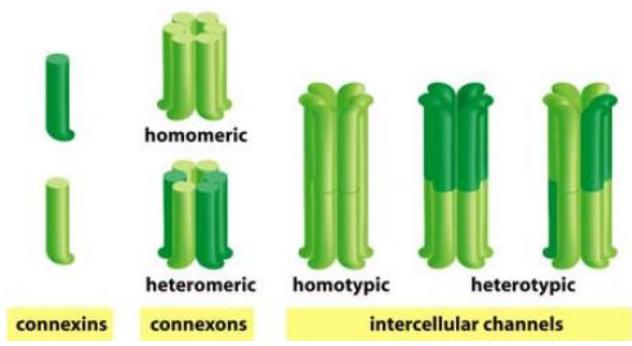


- **Tight junctions (TJ):** Each tight junction is composed of transmembrane adhesion proteins embedded in each of the two interactin plasma membranes. Extracellular domains of these proteins contact directly to one another, thus occlude the intercellular space
 - Major TJ proteins:
 - **Claudins:** transmembrane proteins
 - **Occludins:** transmembrane proteins
 - **ZO proteins** - peripheral membrane proteins which associate with actin filaments



- Transcellular & paracellular pathways of transepithelial transport:
 - **Transcellular** transport requires the cellular uptake of molecules on one side and subsequent release on the opposite side
 - In **paracellular** transport, molecules move extracellularly through parts of tight junctions, whose permeability to small molecules and ions depends on the composition of the junctional components and the physiologic state of the epithelial cells
- **Anchoring junctions:**
 - **Transmembrane** adhesion proteins
 - Cadherins - adhesion junctions & desmosomes
 - Integrins - focal adhesions & hemidesmosomes
 - **Intracellular** anchor proteins - connect the transmembrane proteins with actin or intermediate filaments
 - Anchoring junctions can also contain components of intracellular signaling
- **Anchoring junctions: adherens junctions**
 - Cell-cell junctions
 - Form the **adhesion belt (zonula adherens)** in epithelial cells
 - Molecular composition of zonula adherens:
 - Transmembrane adhesion proteins - **cadherins**
 - **Contractile bundle of AF & myosin** just adjacent to the junction
 - **Intracellular anchor proteins** - catenins, vinculin, α .actinin
 - Cadherins mediate cell-cell-adhesion by a **homophilic** binding
- Cell-cell-adhesion:
 - **Homophilic binding:** molecules on one cell bind the same kind of molecule on the other side
 - **Heterophilic binding:** molecules on one cell bind different kind of molecule on the other side
 - **Linker-dependent** binding - adhesion molecule son adjacent cells are linked by an extracellular molecule
- **Anchoring junctions: desmosomes (= macula adherens)**
 - Cell-cell-junctions
 - Molecular composition:

- Transmembrane adhesion proteins: **desmoglein** & **desmocollin** (cadherin family; disease: pemphigus)
- IF: **keratin** in epithelial cells and **desmin** in cardiomyocytes linked to anchor proteins
- Intraacellular anchor proteins: **plakoglobin** & **desmoplakin** which form dense subplasmalemmal plaques
- **Anchoring Juncions: hemidesmosomes**
 - **Cell-matrix**
 - Morphologically resemble desmosomes
 - Connect parts of the basal surface of epithelial cells to the underlying basal lamina
 - Molecular composition:
 - Transmembrane adhesion proteins: **integrins**
 - IF (**keratin**) linked to anchor protein
 - Intracellular anchor protein: **plectin**
 - Extracellular matrix protein (**laminin**) linked to integrin
- **Anchorin Junctions: focal adhesion**
 - **Cell-matrix**
 - Molecular composition:
 - Transmembrane adhesion proteins: **integrins**
 - AF linked to anchor proteins
 - Intracellular anchor proteins: **α -actinin**, **talin**, **vinculin**, **filamin**
 - Extracellular matrix proteins linked to integrins
- Integrin structure: Cell-Matrix
 - **Cytoplasmic domain** of β -subunit is binding site for the intracellular anchor proteins (talin, filamin, α -actinin) that associate with AF.
 - **Ectodomain** of α - and β -subunits are binding sites for matrix proteins. Hence integrins are "integrators" of matrix and cytoskeleton (**focal adhesions**)
- Anchoring Juncions: Summary of Molecular organization
 - **Cel-cell** junctions:
 - Transmembrane proteins: cadherins
 - Intracellular anchor proteins:
 - α -actinin, catenins, vinculin: adherens junctions
 - Plakoglobin & desmoplakin: desmosomes
 - Cytoskeletal components linked to anchor proteins
 - AF: adherens junctions
 - IF (keratin, desmin): desmosomes
 - **Cell-matrix** junctions:
 - Transmembrane proteins: integrins
 - Intracellular anchor proteins:
 - α -actinin, talin, vinculin, filamin: focal adhesions
 - Plectin: hemidesmosomes
 - Extracellular matrix proteins (laminin, etc.)
- **Gap junction (nexus, communicating junction)**
 - Regions of direct intercellular communication
 - Permit the passage of various small molecules between adjacent cells
 - The intercellular cleft at the gap junction is narrow and constant, about 2 to 4 nm
 - The gap is spanned by intercellular channels composed of 2 **connexons**. Each gap junction contains a cluster of few to many thousands of connexons. Each connexon is formed by 6 **connexins** - 4 pass transmembrane proteins
 - Gap juctions can differ in size
 - **Connexons:**
 - Homomeric: assembled by a single type of connexin
 - Heteromeric: different types of connexins
 - Justictional channels:
 - Homotypic
 - Heterotypic
 - Gap Junctions allow ions and small molecules including signaling molecules to directly pass from cytoplasm of one cell to the other. Examples:
 - Electrically excitable cells
 - Non-electrically excitable cells
 - Blood glucose



- Gap junctional channels can flip between open and closed state. Thus, these channels are dynamic structures changing their conformation in response to intracellular and extracellular signal
- **Signal relaying communicating junctions** (synapse)
- **Membrane specializations:**
 - Lateral membrane specializations - junctional complexes (TJ, ZO, ZA, desmosomes, gap j.)
 - Basal surface specializations - basal lamina, plasma membrane enfoldings, hemidesmosomes
- **Plasma membrane enfoldings:**
 - Increase the surface area available for transport
 - Partition the mitochondria-rich basal cytoplasm (energy for the transport)

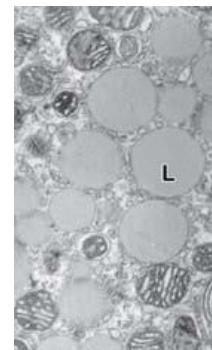
Cell Inclusion

- Materials in the cytoplasm which may or may not be surrounded by a membrane

- Basic features:
 - Not present in all cells
 - Nonmotile and with little or no metabolic activity
 - Filled with stored macromolecules
 - Fat droplets
 - Glycogen granules
 - Pigments
 - Crystals

- **Fat droplets**

- Accumulation of lipid molecules
- Prominent in:
 - Adipocytes (fat cells)
 - Adrenal cortex vells
 - Liver cells



- **Glycogen granules**

- Glycogen is the most common storage form of glucose
- Especially abundant in cells of muscle and liver
- At TEM appears as **clusters (rosettes)** of β -particles (smaller) or α -particles (larger clusters) that resemble ribosomes, located in the vicinity of the SER
- Disorders result in inability to degrade glycogen

- **Glycogen storage disorders:**

- Result of their inability to degrade glycogen
- Major manifestations:
 - Hepatic
 - Myopathic
 - miscellaneous

- **Pigment deposits (PD):**

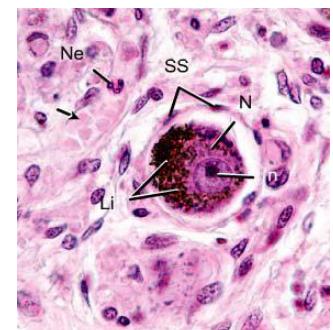
- Occur in many cell types and may contain various complex substances
 - **Lipofuscin:** by-product of lysosomal digestion in long-lived cells
 - **Melanin:** protects cell nuclei from damage to DNA by light
 - **Hemosiderin granules:** containing the protein ferritin, which forms a storage complex for iron

- **Lipofuscin:**

- Insoluble brownish-yellow granular intracellular material that accumulates in a variety of tissues (particularly the heart, liver, and brain) as a function of age or atrophy
- Complexes of lipid and protein that derive from the free radical-catalyzed peroxidation of polyunsaturated lipids of subcellular membranes
- Not injurious to the cell but important as a marker of past free-radical injury

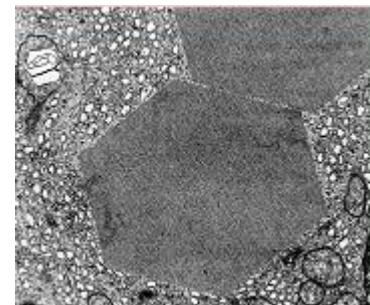
- **Crystals**

- Found in few cells; probably crystalline forms of certain proteins
- Examples:
 - **Sertoli cells** of the testis (crystals of Charcot-Böttcher)
 - **Leydig cell** of the testis (crystals of Reinke)
 - **Macrophages** (sometimes)



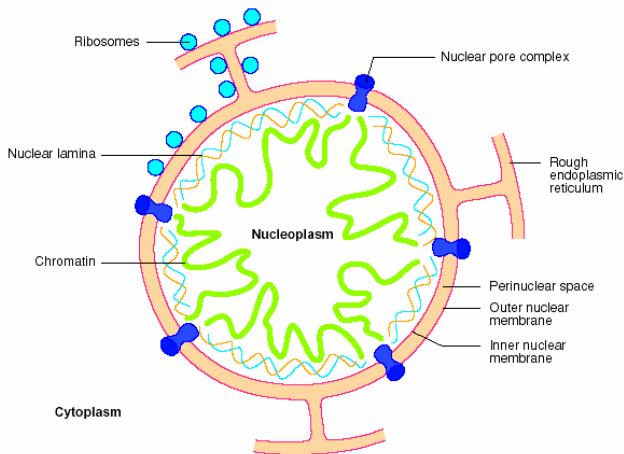
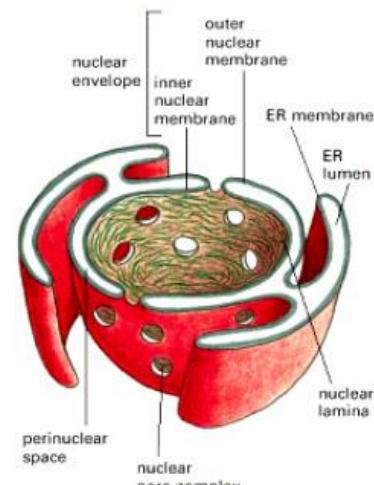
• Reinke's crystals

- Rectangular inclusions (3-20 µm in diameter), composed of protein, in the interstitial cells of the testis (Leydig cells) and hilus cells (analog. to Leydig cells) in the ovary
- They are not found in the Leydig cells of non-primate mammals



Nucleus

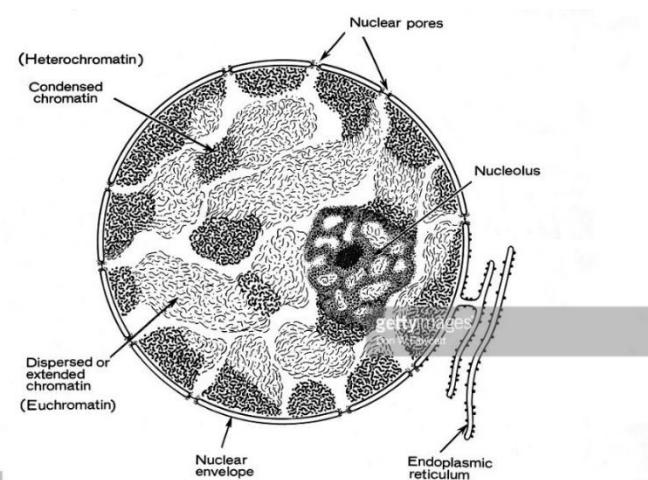
- Cell nucleus: The defining organelle of all eukaryotic cells
- Parts of the nucleus
 - **Inner + outer nuclear membrane (nuclear envelope)**
 - **Perinuclear space**
 - **Nuclear pore complex**
 - **Nucleoplasm**
 - **ER membrane**
 - **ER lumen**



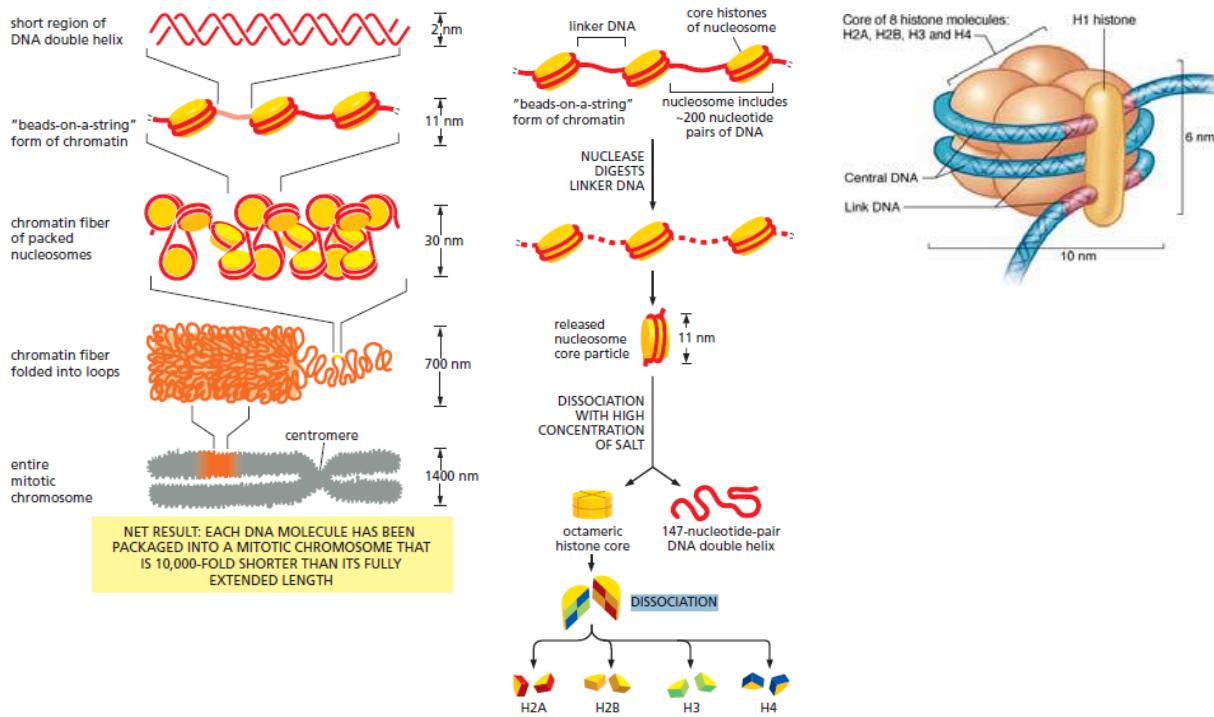
- Functions of the nucleus:
 - **Cellular regulation:** Houses genetic material, which directs all cellular activities and regulates cellular structure
 - **Production:** Produces ribosomal subunits in nucleolus and exports them into cytoplasm for assembly into ribosomes
- Nucleus Constituents:
 - **Nuclear envelope** (separates nucleus from cytoplasm)
 - Inner nuclear membrane (INM)
 - Outer nuclear membrane (ONM)
 - Perinuclear space
 - Nuclear pore complexes (NP)
 - **Nuclear matrix** (nucleoplasm)
 - Chromatin
 - Nuclear bodies - nuclear compartments

Chromatin

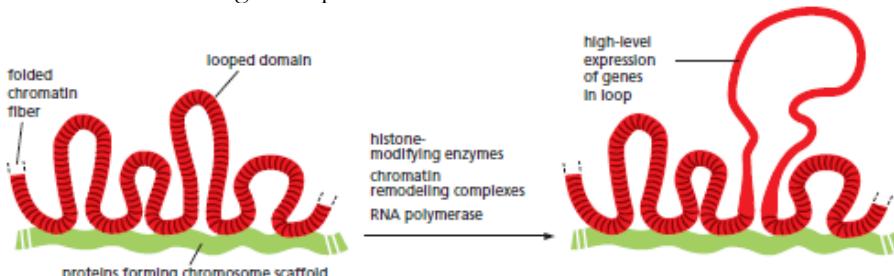
- Complex of DNA, histones, and nonhistone proteins found in the nucleus of a eukaryotic cell. The material of which chromosomes are made.
- **Euchromatin:**
 - 90% of chromatin
 - Composed of 30-nm fibers and looped domains
- **Heterochromatin**
 - 10% of chromatin
 - Involves an additional level of folding of 30-nm fiber and requires many proteins in addition to the histones
 - Transcriptionally inactive during interphase - gene silencing
 - Common in centromeres and telomeres of chromosomes



- Aligned adjacent to the nuclear envelope in association with lamin proteins
- Proteins = histones + non-histone proteins
- Proteins + DNA = Chromatin
- **Nucleosomes** are the basic unit of chromosomes



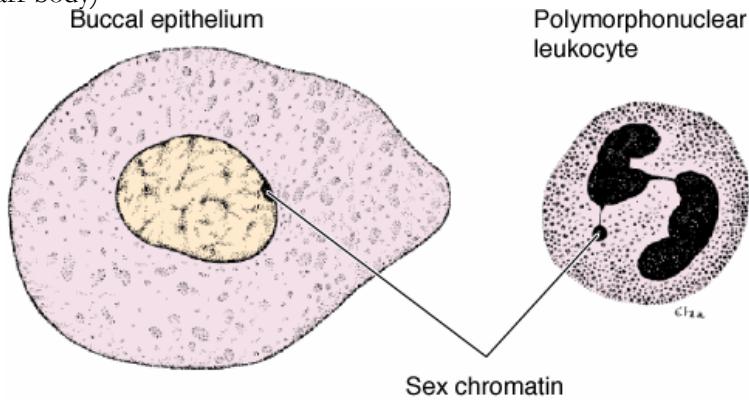
- **Chromosome Compaction (Condensins):** Complex of proteins involved in **chromosome condensation** - a process by which a chromosome becomes packed up into a more compact structure prior to M phase of the cell cycle
- Nucleosomes are modified to allow gene expression:



- Epigenetics: phenotype changes without genotype modifications

Chromosomes

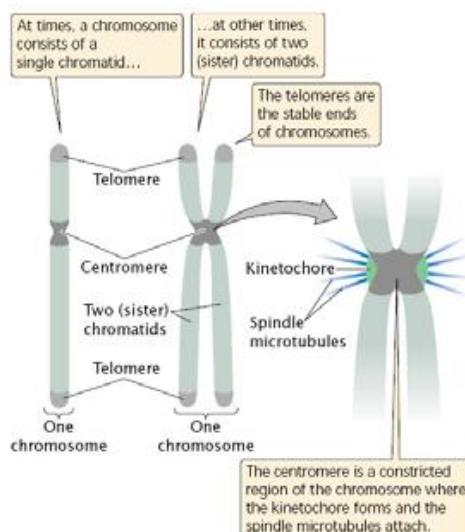
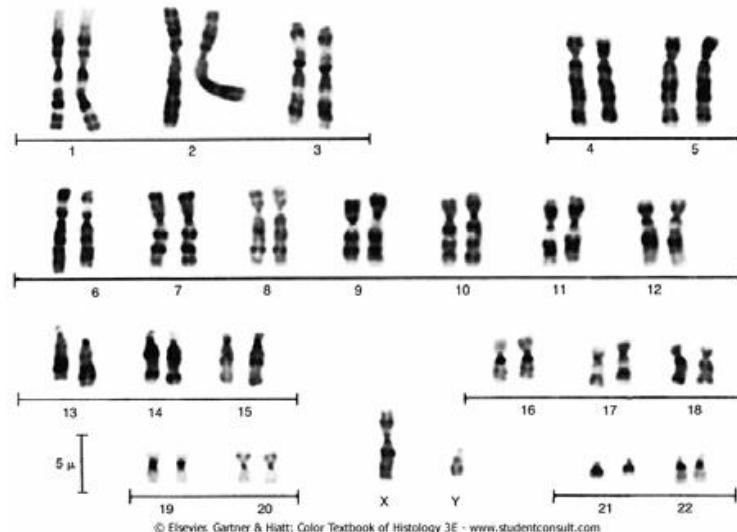
- Chromatin fibers that become so condensed and tightly coiled during mitosis and meiosis that they are visible with the light microscope
- **Genome:** number of chromosomes in somatic cells (specific for the species); the total genetic makeup
- The human genome consists of 46 chromosomes, representing 23 homologous pairs of chromosomes
 - 23 maternal pairs (22 autosomes + 1 sex chromosome: X)
 - 23 paternal pairs (22 autosomes + 1 sex chromosome: Y)
- **Barr body:** Females: XX; males: XY → one of the two X chromosomes in females is compacted (inactive) as heterochromatin (=Barr body)



Source: Mescher AL: Junqueira's Basic Histology: Text and Atlas, 12th Edition: <http://www.accessmedicine.com>

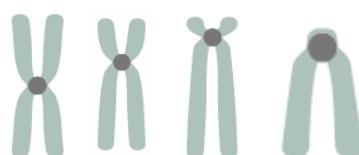
- **Karyotype:**

- The number and characteristics of chromosomes encountered in an individual
- Karyotyping is performed by **chromosome staining**:
 - Cells are grown *in vitro* and mitosis is arrested during metaphase using colchicine (binds tubulin and disrupts microtubules)
 - Arrested cells are then immersed in a hypotonic solution, which causes swelling, stained in various ways, and then flattened between a glass slide and a coverslip
 - The mitotic chromosomes from one nucleus are photographed under the light microscope, cut individually from the photograph, and arranged so that the stained chromosomal bands can be analyzed

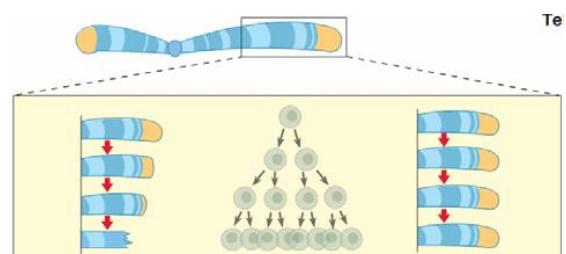


- Major types of eukaryotic chromosomes:

- Metacentric
- Submetacentric
- Acrocentric
- Telocentric

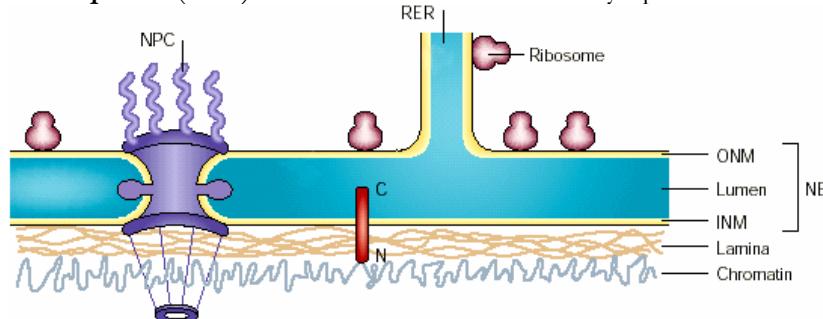


- **Telomeres and telomerase:** Telomerase maintains the telomeres at the ends of the DNA thread. This makes it possible to copy the entire chromosome to its very end each time the cell divides. Without telomerase present, the chromosome is shortened each time the cell divides. Finally, the telomere DNA is eroded and the chromosome is damaged.

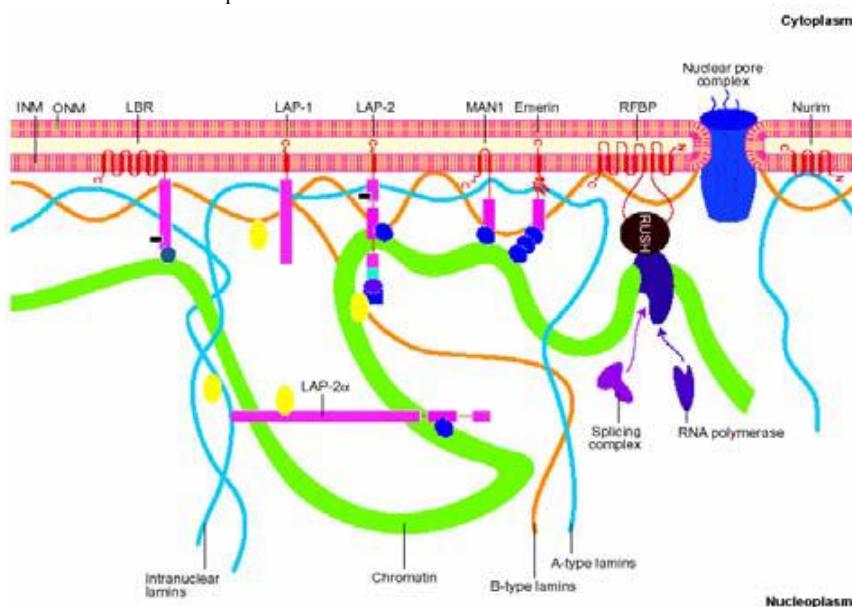


Nuclear envelope

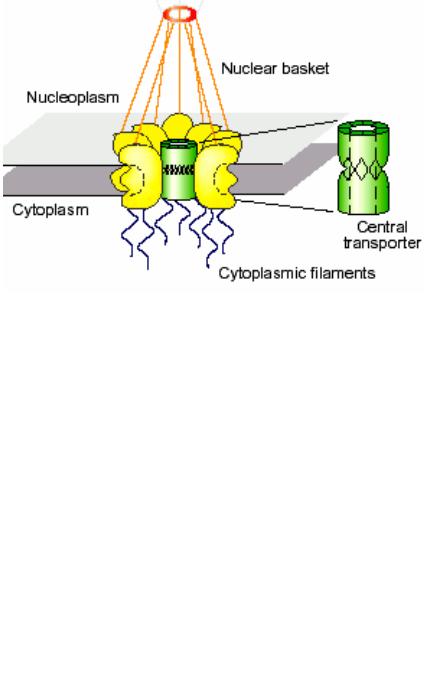
- Consisting of:
 - **Inner nuclear membrane (INM)**: its proteins act as binding sites for
 - Chromatin
 - Nuclear lamina
 - **Outer nuclear membrane (ONM)**:
 - Continuous with the membrane of the ER
 - Studded with ribosomes engaged in protein synthesis
 - **Perinuclear space**: between INM & ONM, continuous with RER
 - **Nuclear pore complexes (NPC)**: channels between nucleus & cytoplasm



- **Nuclear Lamina:**
 - Protein meshwork lining the nuclear surface of INM
 - Thickness: 20-50 nm
 - Maintains extensive interactions with:
 - INM proteins
 - Chromatin
 - Main structural components: type V IF proteins - lamins (A&C, B1&B2)
 - Functions:
 - Provides structural support for the nuclear envelope
 - Provide scaffolds for protein complexes that regulate gene expression
- **Inner Nuclear Membrane (INM) Proteins:**
 - Transmembrane proteins of INM
 - Over 20
 - Representative examples:
 - LAP: lamina-associated polypeptide (1 & 2)
 - LBR: lamin B receptor
 - Emerin (Emery-Dreifuss MD)
 - HP1: heterochromatin protein 1



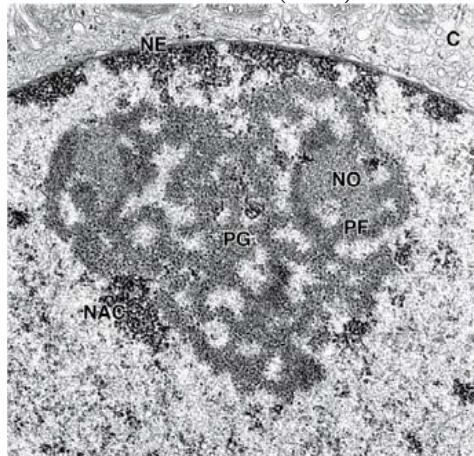
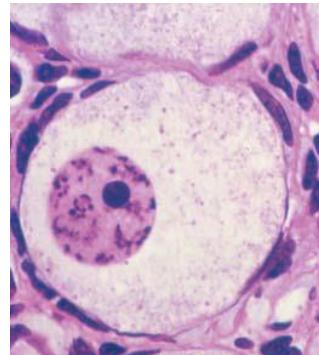
- **Nuclear pores:**
 - Sites where the inner and outer membranes of the nuclear envelope fuse, the resulting lipid-free spaces
 - contain **nuclear pore complexes**: the machinery that regulates transport between nucleus and cytoplasm
 - 3000-4000 *per* nucleus

- **Nuclear pore complex**
 - Composed of the nuclear pore and its associated glycoproteins
 - Diameter: 100-125 nm; spans INM & ONM
 - Composition
 - 3 ring-like arrays of proteins stacked on top of each other, each ring displaying 8-fold symmetry
 - Cytoplasmic ring
 - Luminal ring
 - Nuclear ring
 - Vertical spokes connecting the 3 rings
 - Cytoplasmic fibers
 - 1 transporter: connected to the luminal ring
 - 1 nuclear basket
- 
- © Elsevier: Gartner & Hiatt: Color Textbook of Histology 3E - www.studentconsult.com
- NPC is composed of **nucleoporins (NUP)**:
 - Nucleocytoplasmonic transport:
- ```

graph TD
 A[nuclear localization signal] --> B[nuclear export signal]
 A --> C[nuclear transport receptors]
 B --> C
 C --> D[binding of complex to nucleoporins]
 D --> E[translocation across NPC]

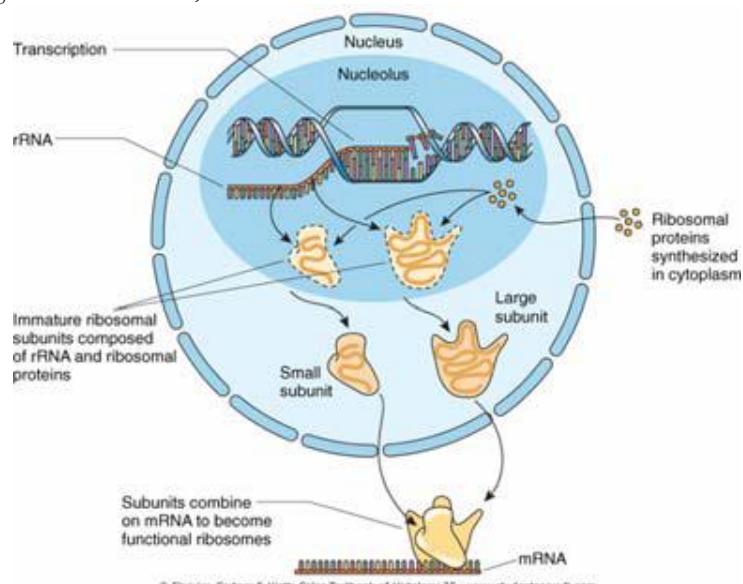
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- **Nuclear bodies** (nuclear compartments):
    - Nucleolus
    - Cajal body (CB)
    - Splicing-factor compartments (SFCs) = nuclear speckles
    - Paraspeckles: subnuclear structures distinct from speckles, often located adjacent to speckles
    - Promyelocytic leukaemia oncprotein (PML) body
    - Perichromatin fibrils (nascent mRNA)
  - **Nucleolus**:
    - Most prominent nuclear substructure
      - 0.5-5.0 μm in diameter
      - 1-5 per cell
      - No membrane: supramolecular complex
    - Assembled around nucleolar-organizing regions (NOR)
      - Clusters of tandemly repeated rDNA genes
      - Target processing and assembly components required for ribosome biosynthesis
    - Structure:
      - FC (fibrillar centers): chromatin-containing rDNA genes
      - DFC (dense fibrillar components): nascent pre-rRNA transcripts entering from FC (RNA Polymerase I)
      - GC (granular components): late processing of pre-rRNA

- Nucleolus under light microscopy:
  - Usually spherical
  - Highly basophilic (due to densely concentrated rRNA)
  - Present in the nuclei of cells active in protein synthesis
- Nucleolar compartments (TEM)
  - **Nucleolar organizer (NO):** DNA-sequences of bases coding for rRNA
  - **Pars fibrosa (PF):** primary rRNA transcripts
  - **Pars granulosa (PG):** maturing ribosomal subunits
  - **Nucleolus-associated chromatin (NAC):** heterochromatin



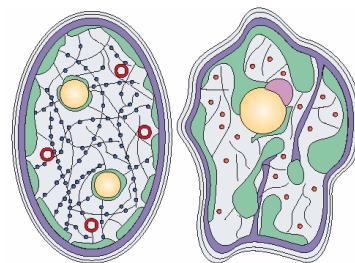
Source: Masteller, Ali. *Anatomical & Basic Pathology - Text and Atlas*, 22nd Edition: <http://www.accessmedicine.com>  
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- Ribosome formation: The nucleolus is a subnuclear structure that performs ribosomal biogenesis and is generated by Pol1-dependent transcription of the nucleolar rRNA genes (rDNA).
  - (a) Ultrastructurally, the nucleolus has a tripartite organization that reflects the spatial separation of various steps of ribosomal biogenesis [2]. That process is initiated in the fibrillar centers (FC), where the 45S rRNA (pre-rRNA) is transcribed by Pol1 from rDNA. Note the 'Christmas tree' structure of the transcribed rDNA loci/elongating pre-rRNA molecules at the edges of the FC. Further steps of ribosomal biogenesis including pre-rRNA modifications and processing to mature rRNAs (18S, 5.8S and 28S), as well as rRNA assembly with ribosomal proteins and the 5S rRNA, occur in the dense fibrillar component (DFC) and granular component (GC) of the nucleolus. The final products of this process, the 40S and 60S ribosome subunits, are exported out of the nucleus, where, following final maturation,



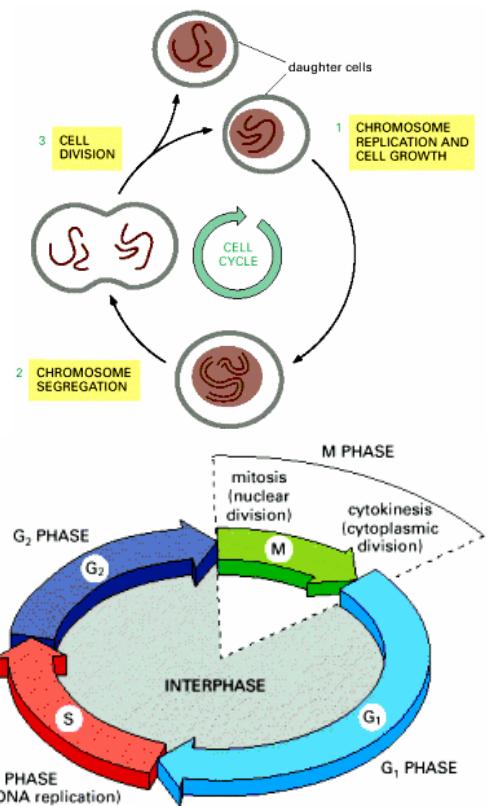
- **Splicing Factor Compartments (SFCs; nuclear speckles)**
  - Irregularly shaped nuclear domains
  - Under EM are known as interchromatin granule clusters (IGC)
  - Usually 25-50 speckles per interphase mammalian nucleus
  - Localization:
    - Nuclear regions that do not contain DNA
    - Out of nucleoli
  - Composed of factors involved in **pre-mRNA-splicing**

- **Pre-mRNA splicing:**
  - **Splicosome:** large complex that consists of 5 **splicing small nuclear ribonucleoprotein particles** (snRNP) as well as numerous protein factors. Mediates the excision of introns from pre-mRNA transcripts and ligates exon ends to produce mature mRNAs
  - SnRNP consists of a tight complex between a short RNA molecule and one or more proteins
- **Cala Body / Coiled Body (CB):**
  - Small spherical nuclear substructure:
    - 0.1-1.0  $\mu\text{m}$  in diameter *per cell*
    - No membrane: supramolecular complex
  - Identified by immunohistochemistry to coilin: **nucleocytoplasmic shuttle protein**
  - Function:
    - Associated with spliceosomal RNPs
    - Associated with nucleolar RNPs
    - Associated with basal transcription factors (RNA Pol II)
- **Promyelocytic leukaemia oncogene (PML) body:**
  - Small spherical nuclear substructure:
    - 0.3-1.0  $\mu\text{m}$  in diameter
    - 10-20 *per cell*
    - No membrane: supramolecular complex
  - Identified by immunohistochemistry to PML oncogene
  - Function:
    - Involved in transcriptional regulation: disruption leads to acute promyelocytic leukemia
    - Targets of viral infection
- Nuclear changes in tumor cells:
  - Nucleolar-organizing regions become larger and more numerous
  - Irregular folded shape
  - Heterochromatin aggregates become frequent
  - Nucleoli enlarged
  - Perinucleolar body is observed
  - PML bodies become speckled



## Cell division & cycle

- **Cell Division:** Separation of a cell into two daughter cells
  - In eukaryotic cells:
    - Division of the nucleus (**mitosis**)
    - Division of the cytoplasm (**cytokinesis**)
  - Importance:
    - During development: produce a functioning organism
    - As adult: replace dead cells
  - Task: pass genetic information onto the next generation of cells
    - Error-proof **replication** of DNA/chromosomes
    - Error-proof **segregation** of replicated chromosomes

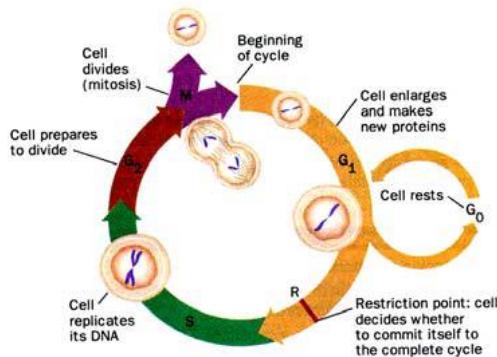


- **Cell cycle:**
  - The orderly sequence of events by which a cell duplicates its contents and divides into two
  - The cyclic alternation between mitosis and interphase
  - Occurs in all tissues with cell turnover
- Major Phases of Cell Cycle:
  - **S (synthesis) phase:** error-proof replication of DNA/chromosomes
  - **M (mitosis) phase:** error-proof segregation of replicated chromosomes

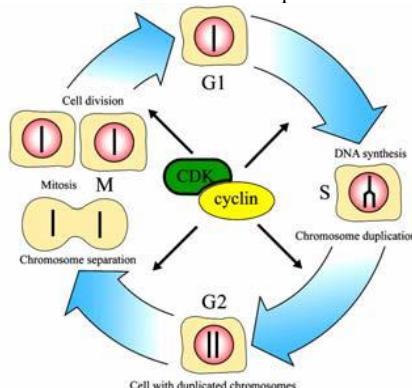
Most cells require much more time to grow and double their mass of proteins and organelles than they require to replicate their DNA and divide. Thus, 2 extra **gap phases** are inserted in most cell types:

- **G1 phase:** between M & S phase
- **G2 phase:** between S & M phase

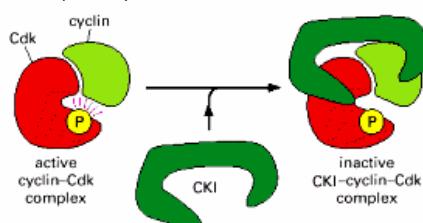
- G1 & G2 have important regulatory functions: If extracellular conditions are favourable and signals to grow and divide are present, cells in early G1 or G0 progress through a commitment point near the end of G1 known as **restriction point**. After passing this point, cells are committed to DNA replication, even if the extracellular signals that stimulate cell growth and division are removed



- Features of the cell cycle control system:
  - **Timer:** provides a fixed amount of time for each cycle
  - **Initiator:** starts the event in the correct order
  - **Non-repeater:** each event is triggered only once per cycle
  - **On/off switches:** trigger events irreversibly
  - **Robustness:** backup mechanisms to ensure that the cycle can work properly even when parts of the system malfunction
  - **Adaptability:** to suit specific cell types or environmental conditions
- Cell Cycle **Checkpoints:** Points at which the cycle can be arrested if previous events have not been completed
  - Progression through G1 and G2: DNA damage
  - Entry into M: DNA replication is not complete
  - Chromosome separation in M: some chromosomes are not properly attached to the mitotic spindle
- The Cell-Cycle Control system is based on cyclically activated protein kinases (**cyclin-dependent kinases**; CDKs); cyclical changes in the phosphorylation of intracellular proteins regulate major events of the cell cycle.
- **Cyclin / CDK complexes** regulate the transition between the phases of the cell cycle



- A **complex of cyclin with CDK** acts as a protein kinase to trigger specific cell-cycle events. Without cyclin, CDK is inactive
- CDK regulation: **CDK Inhibitor Proteins (CKIs)**

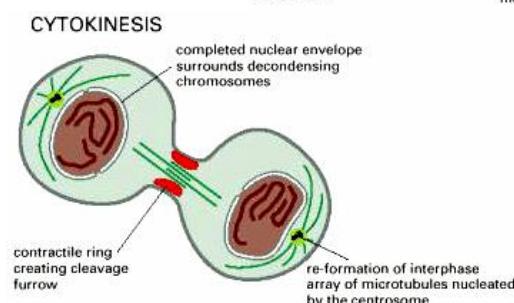
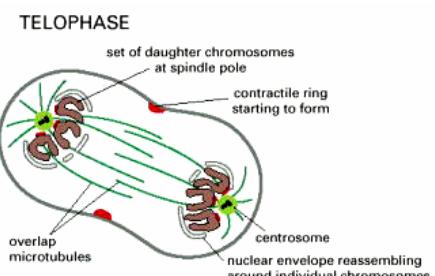
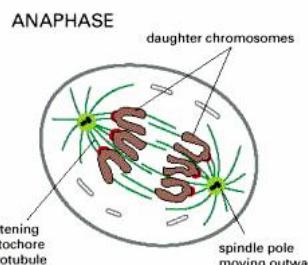
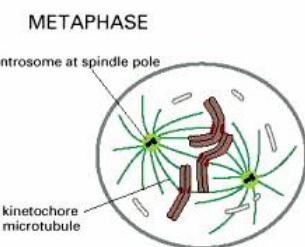


| Cyclin-CDK complex    | Cyclin   | CDK partner |
|-----------------------|----------|-------------|
| G <sub>1</sub> -CDK   | cyclin D | CDK4/6      |
| G <sub>1</sub> /S-CDK | cyclin E | CDK2        |
| S-CDK                 | cyclin A | CDK2        |
| M-CDK                 | cyclin B | CDK1        |

- Cell cycle progression: Progression through the cell cycle can be blocked by **endogenous** and **exogenous inhibitors**

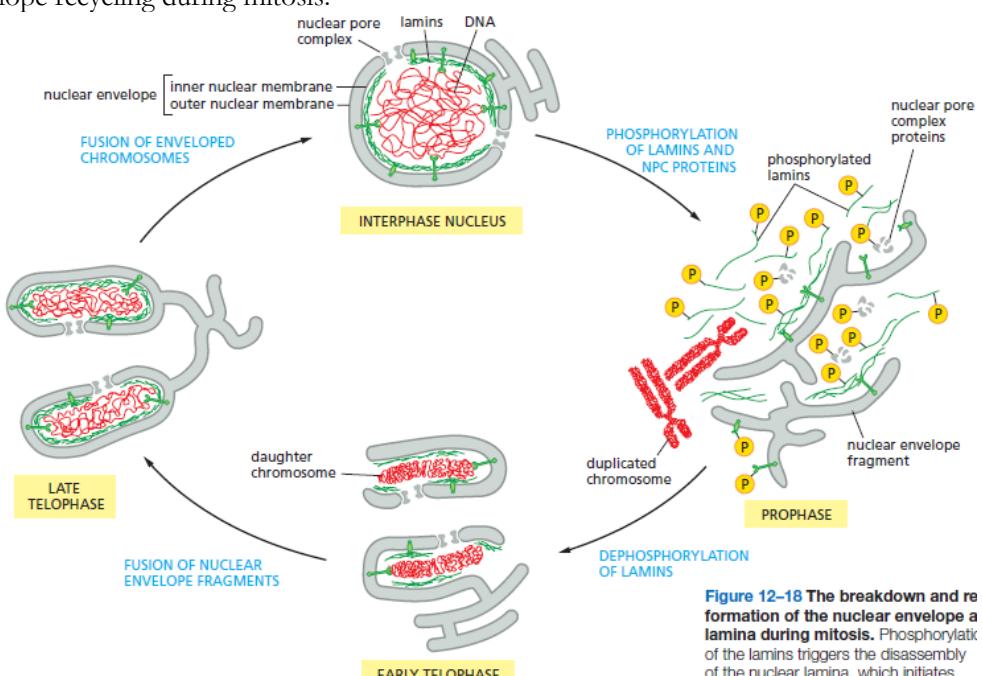
- Cell Division Phases:

- **Metaphase:**
  - Chromosomes at the equator
  - Chromatids attach to the mitotic spindle
- **Anaphase:**
  - Kinetochore MTs shorten
  - Chromosomes separate
- **Telophase:**
  - Chromosomes at the pole
  - Nuclear envelope completed
- **Cytokinesis:**
  - Cleavage furrow
  - Nuclear envelope completed



|                     | <b>Chromosomes</b> | <b>Nuclear envelope</b>           |
|---------------------|--------------------|-----------------------------------|
| <b>Prophase</b>     | condense           | intact<br>(lamin phosphorylation) |
| <b>Prometaphase</b> | attach to MTs      | fragments                         |
| <b>Metaphase</b>    | at spindle equator | absent                            |
| <b>Anaphase</b>     | separation         | absent                            |
| <b>Telophase</b>    | at spindle poles   | reassembles                       |

- Nuclear envelope recycling during mitosis:



**Figure 12–18** The breakdown and reformation of the nuclear envelope a lamina during mitosis. Phosphorylation of the lamins triggers the disassembly of the nuclear lamina, which initiates the nuclear envelope to break up.

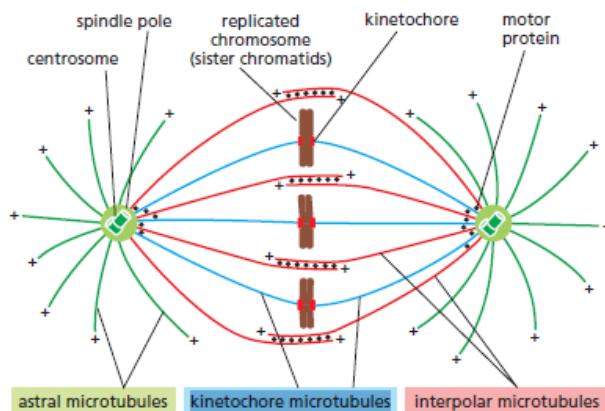
- Two cellular structures duplicate on every cell cycle:

- **Chromosomes** (DNA)
- **Centrosomes** (protein)

Similarities:

- Initiated at the G1 to S transition
- Regulated by CDK2/cyclin A/E
- Require proteolysis of cohesive material to separate their subunits
- Replication is semiconservative - with each newly replicated unit containing both new and old subunits

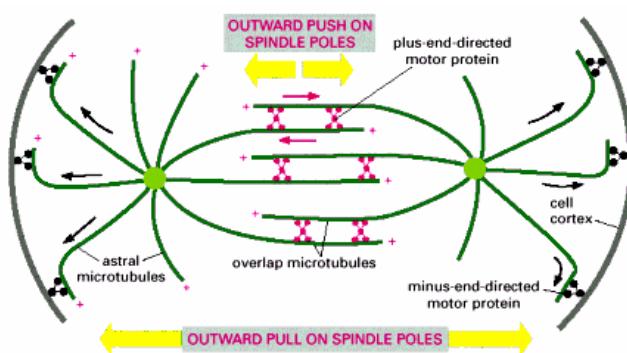
## Mitotic spindle



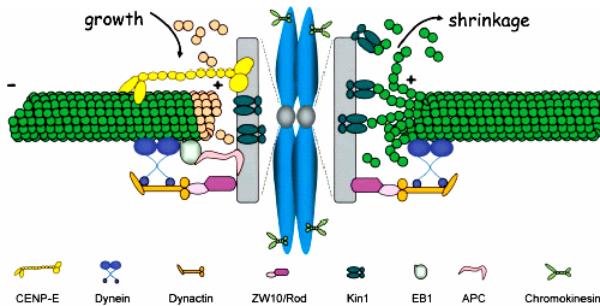
- Microtubules of the mitotic spindle:
  - **Astral**: separate the poles and position the spindle
  - **Kinetochore**: attach to the kinetochore
  - **Overlap**: interdigitate at the equator, responsible for the shape

The assembly and the function of the mitotic spindle depend on **microtubule-dependent motor proteins**:

- Kinesin-related proteins, move toward the plus end of MTs
- Dyneins, which move toward the minus end



- Kinetochore attaches to the kinetochore microtubules:

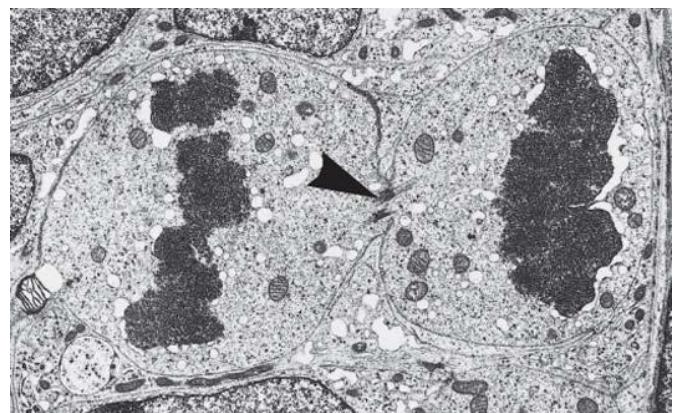


- Role of **cohesin proteins** in chromatid segregation:

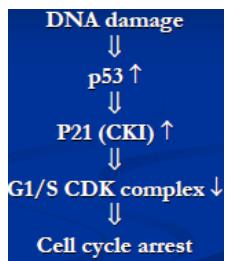
- After chromosome duplication in S phase, the two copies of each replicated chromosome remain tightly bound together as identical sister chromatids.
- They are glued together by multisubunit protein complexes called **cohesins**.
- This cohesion is broken at the start of anaphase to allow the sisters to be pulled apart.

- Cohesin cleavage is mediated by the **anaphase-promoting complex** (APC)

- **Cytokinesis**: Accomplished by the **contractile ring** - a dynamic assembly composed of actin filaments, myosin II filaments, and many structural and regulatory proteins. The ring assembles just beneath the plasma membrane



- The **cleavage furrow** continues to deepen until only the **midbody**, a small bridge of cytoplasm, and remaining polar microtubules connect the two daughter cells
- DNA damage halts cell-cycle progression. If DNA damage is extensive, Apoptosis ensues.
- P53** is a crucial inhibitor of cell cycle progression. A mutant p53 is associated with many cancers.



## Oncogenes

- Cell proliferation and differentiation are controlled by a group of genes called **protooncogenes**; altering the structure or expression of these genes promotes the production of tumors.
- Protooncogenes can transform into **oncogenes** by a mutation in their DNA sequences or other mechanisms

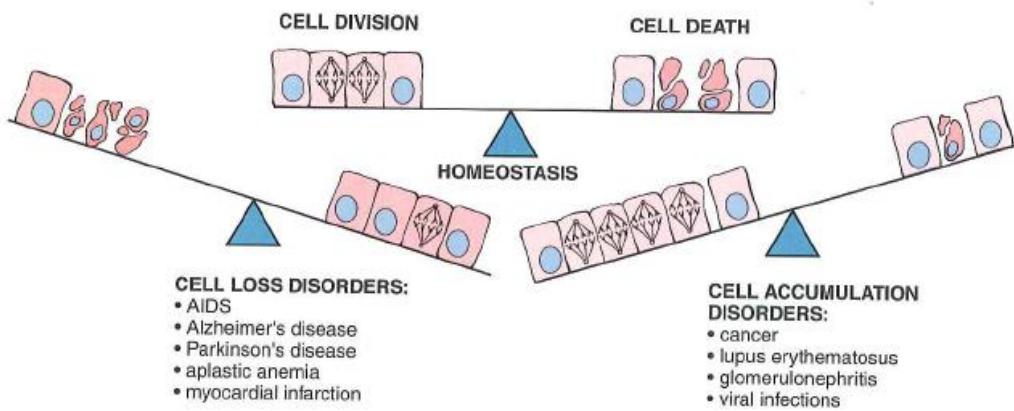
## Stem cells

- A small population of undifferentiated cells whose cycling serves to renew the differentiated cells of the tissues as needed
- Stem cells divide asymmetrically, producing one cell that remains as a stem cell and another which becomes committed to a differentiative pathway - **progenitor** or **transit amplifying cell**
- Progenitor cells divide rapidly by symmetrical mitoses to generate the number of new cells available for the differentiated tissue

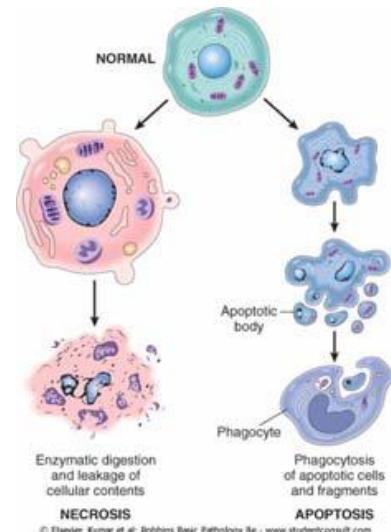
## Endomitosis

- Chromosome duplication without cytokinesis: cells with 2 or more nuclei (e.g. some hepatocytes, megakaryocytes)

## Cell death and cell division



- Major types of cell death:
  - Necrosis:** a series of changes that accompany cell death, largely resulting from the degradative action of enzymes on lethally injured cells. Necrotic cells are unable to maintain membrane integrity, and their contents often leak out
  - Apoptosis:** a pathway of cell death that is induced by a tightly regulated suicide program in which cells destined to die activate enzymes capable of degrading the cells' own nuclear DNA and nuclear and cytoplasmic proteins.
  - Autophagy:** lysosomal digestion of the cell's own components as a survival mechanism in times of nutrient deprivation, such that the starved cell lives by eating its own contents.



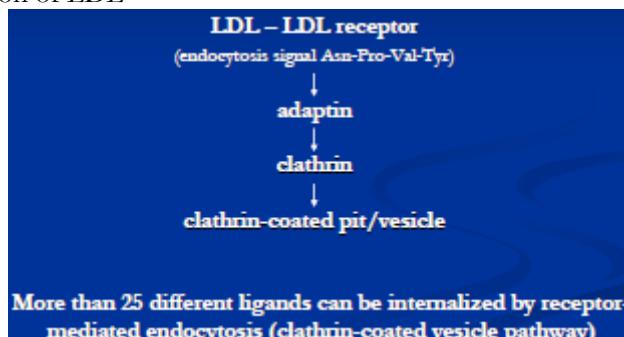
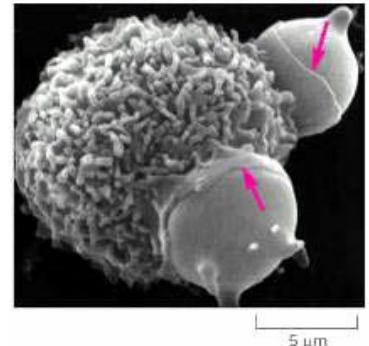
| Feature                        | Necrosis                                                        | Apoptosis                                                                                                                        |
|--------------------------------|-----------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Cell size                      | Enlarged (swelling)                                             | Reduced (shrinkage)                                                                                                              |
| Nucleus                        | Pyknosis → karyorrhexis → karyolysis                            | Fragmentation into nucleosome-size fragments                                                                                     |
| Plasma membrane                | Disrupted                                                       | Intact; altered structure, especially orientation of lipids                                                                      |
| Cellular contents              | Enzymatic digestion; may leak out of cell                       | Intact; may be released in apoptotic bodies                                                                                      |
| Adjacent inflammation          | Frequent                                                        | No                                                                                                                               |
| Physiologic or pathologic role | Invariably pathologic (culmination of irreversible cell injury) | Often physiologic, means of eliminating unwanted cells; may be pathologic after some forms of cell injury, especially DNA damage |

# Cell physiology

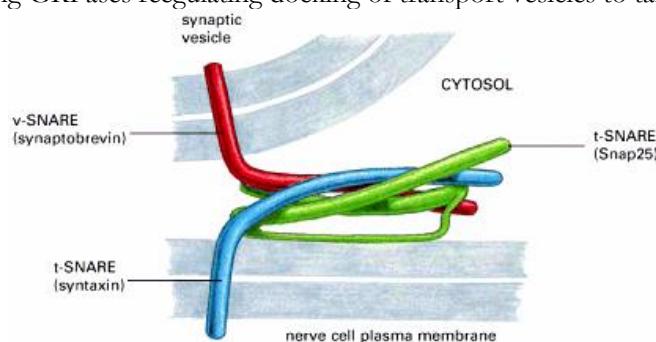
## Endocytosis & Exocytosis

### Endocytosis

- The process whereby a cell ingests macromolecules, particulate matter, and other substances from the extracellular space is referred to as endocytosis
- The endocytosed material is engulfed in a vesicle appropriate for its volume
- Types of Endocytosis:
  - Phagocytosis** ("cellular eating")
    - Ingestion of large particles, such as bacteria
    - Plasmalemma-derived pseudopodia forming large endocytic vesicles (phagosomes,  $>300 \text{ nm}/2R$ )
  - Pinocytosis** ("cellular drinking")
    - Ingestion of fluid/solutes particles
    - Plasmalemma-derived small endocytic vesicles (pinocytosis vesicles - clathrin-coated vesicles, approx.  $150 \text{ nm}/2R$ )
  - Potocytosis** (another type of "cellular drinking")
    - Ingestion of macromolecules
    - Plasmalemma-derived caveolae (Latin, "little cavities", approx.  $50 \text{ nm}/2$ )
  - Receptor-mediated endocytosis** (adsorptive endocytosis)
    - Coated vesicles
    - Caveolae
  - Transcytosis:** involves both endo- and exocytosis of macromolecules from one extracellular space to another (e.g. mother's antibodies/milk/gut epithelium/newborn)
- Phagocytosis:** While phagocytosis in protists is a form of feeding, in most animals it is a form of defense (cellular immunity). Specialized cells (professional phagocytes) - macrophages, neutrophils, dendritic cells - carry out this function. These cells protect the organism against infections by ingesting microorganisms. Macrophages also phagocytose senescent cells as well as cells dying by apoptosis.
- "Don't-eat-me"-Signals:** Macrophages do not phagocytose living animal cells. These cells seem to have a signal presented by the cell surface proteins that inhibit receptors on macrophages.
- Phagocytosis, like many other cell processes, depends on a balance between positive signals that activate the process and negative signals that inhibit the process.
- Pinocytosis:**
  - Constitutive pathway:** fluid-phase endocytosis
  - All eukaryotic cells continually internalize bits of their plasma membrane in the form of clathrin-coated vesicles, which are recycled (returned to it) after uncoating. End effect: endocytic-exocytic-cycle
- Receptor-mediated endocytosis (RME):** This type of pinocytosis provides an efficient pathway for internalization of specific macromolecules that are recognized by cell surface receptors.
  - Clathrin coated pits/vesicles  $\rightarrow$  concentration of receptor (R)-ligand (L) complexes
  - Clathrin-coated vesicles  $\rightarrow$  uncoated vesicles  $\rightarrow$  early endosomes (dissociation of R from L)
    - Recycling endosome (returning R to plasma membrane)
    - Late endosome/lysosome (protein degradation  $\rightarrow$  free cholesterol for membranes and steroidogenesis)
- Low-density Lipoprotein (LDL) particle:** Contains 1500 cholesterol molecules esterified to long-chain fatty acids that is surrounded by lipid monolayer composed of about 800 phospholipid and 500 unesterified cholesterol molecules. A single molecule of a 500 kDa protein organizes the particle and mediates the specific binding of LDL to cell-surface receptor proteins.
- Atherosclerosis:** Internalization of LDL



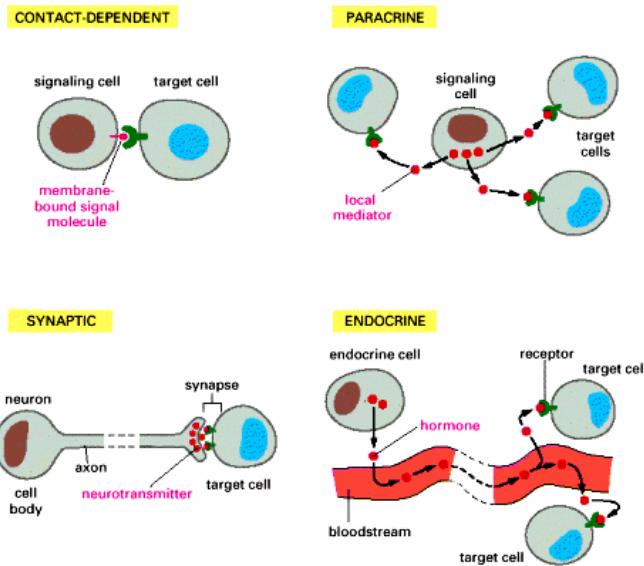
- Caveolae & disease:
  - **Tumorigenesis:**
    - Caveolin-1 is target of oncogenes
    - Caveolin-1 is a tumor suppressor
  - **Diabetes**
    - Caveolae are highly enriched in adipocytes
    - Glucose transporters are associated with caveolae
    - Insulin & leptin signaling involves caveolae
  - **Vascular abnormalities:**
    - Cardiomyopathy
    - Increased vascular relaxation due to increased NO
    - Impaired angiogenesis
    - Atherogenesis: loss of caveolin 1 is protective
- **Transcytosis**
- **Vesicular Transport:** vesicle budding and fusion
  - **Donor compartment:** a membrane-enveloped compartment from where transport vesicles originate
  - **Target compartment:** a membrane-enveloped compartment to which transport vesicles dock
  - **Docking:** 2 membranes come close enough for ectodomains of transmembrane protein to adhere
  - **Fusion:** joining of 2 lipid bilayers that further results in a communication between vesicular lumen and target lumen, thus transferring cargo from one compartment to another
    - **Homotypic fusion**
    - **Heterotypic fusion**
- **SNAREs - Glossary:**
  - **NSF:** N-ethylmaleimide-sensitive factor:
    - Solutee trimeric ATPase arranged in 10x16 nm cylinder
    - Dissociates vSNARE/tSNARE link after a membrane fusion cycle
  - **SNAP - soluble NSF attachment protein**
  - **SNARE - SNAP receptor**
    - Transmembrane proteins, at least 20 in animal cells
    - Each is associated with a particular membrane-bound organelle - mediate membrane docking and fusion (this is best studied for synaptic vesicle release tetanus and botulism toxins interfere with SNARE bindin)
    - vSNARE: vesicle membrane SNARE
    - tSNARE: target membrane SNARE
    - *Trans*-SNARE complex: interacts between vSNARE and tSNARE to lock the 2 membranes together
  - **Rab** proteins - targeting GTPases regulating docking of transport vesicles to target membranes



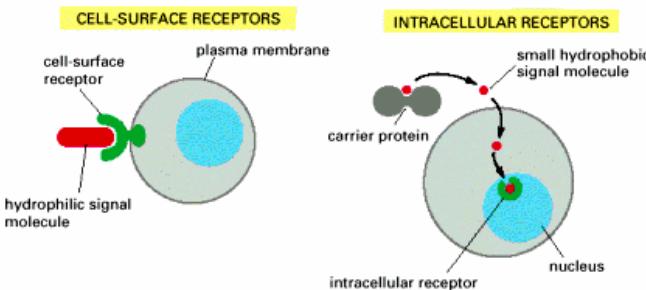
- Protein Sorting in the *trans* golgi Network: **Constitutive** vs. **regulated** pathways
- **Exocytosis:** Fusion of a membrane-limited cytoplasmic vesicle with the plasma membrane, resulting in the release of its contents into the extracellular space without compromising the integrity of the plasma membrane.

## Cell communication

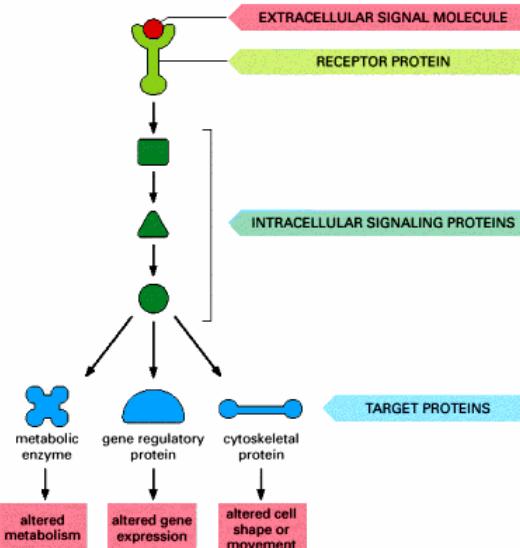
- Mechanisms enabling one cell to influence the behavior of another cell
- Cells can communicate through 3 mechanisms:
  - **Direct interaction** between membrane molecules of the two adjacent cells (**adherens** an **tight junctions**)
  - **Gap junction communications** that form direct cytoplasmic connections between adjacent cells
  - **Secretion** of diffusible factors that can activate specific receptors on the target cells



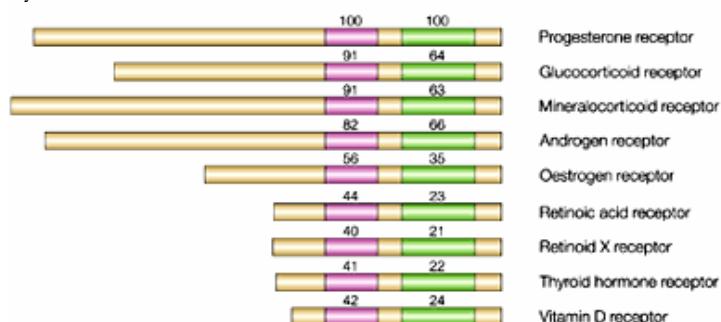
- Types of ligand / receptor molecules:



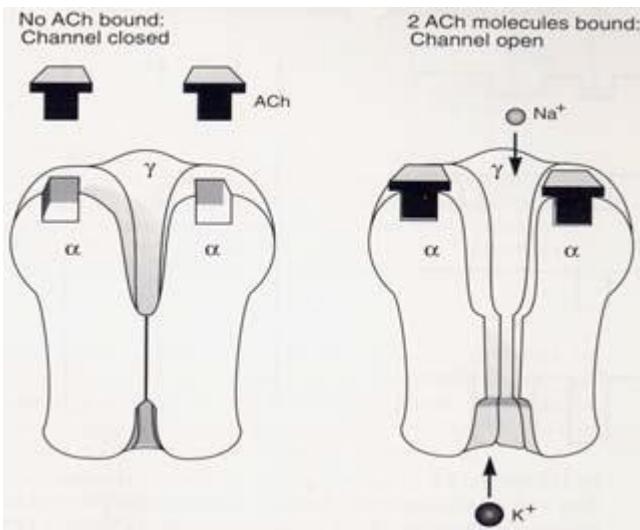
- Specificity:** ligands can interact only with the appropriate specific receptors
- Signal transduction:**



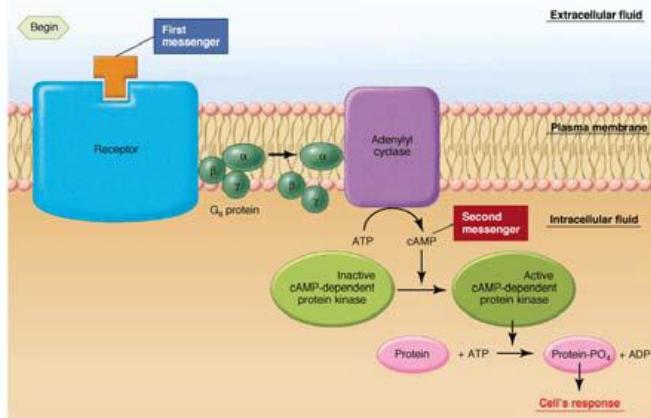
- Nuclear receptor superfamily members



- Regulation of gene expression by hormone nuclear receptors:
- Ionotropic Receptors: **AChR**



- First and second messengers:



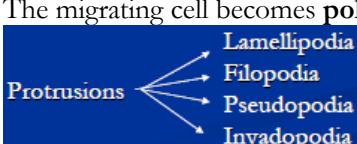
Bonci M, Mascher A. *Amplera's Basic Histology: Text and Atlas*, 22nd Edition. <http://www.accessmedicine.com>. Copyright © The McGraw-Hill Companies, Inc. All rights reserved.

- G-protein-coupled Receptors (GPCRs; 7TM):** An enormous family of over 800 genes encoding receptor proteins that are characterized by a signature seven-transmembrane (7TM) configuration

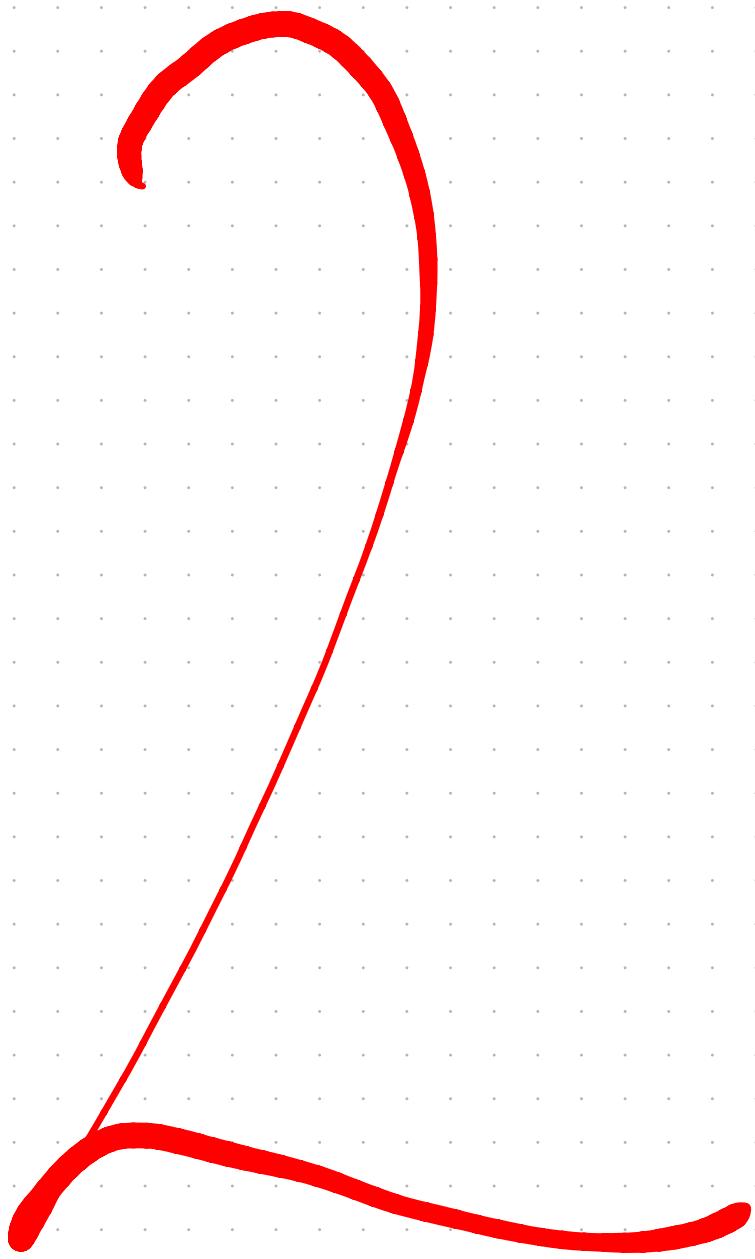


### Cell migration

- To migrate, the cell body must modify its shape and stiffness to interact with the surrounding tissue structures
- The extracellular matrix (ECM) provides the substrate, as well as a barrier towards the advancing cell body.
- Cell migration through tissues results from a continuous cycle of interdependent steps
- The migrating cell becomes **polarized** & forms **protusions**



- Focal adhesions:** Sites of attachment with the substrate. They are a highly specialized type of attachment between actin filaments and the extracellular matrix which allows cells to pull on the substratum to which they are bound



# Histology

## Covering epithelium

- The human body is composed of approx. 200 different types of cells
- The body is composed of 4 basic kinds of tissues:
  - Epithelial tissue**
  - Connective Tissue**
  - Muscle Tissue**
  - Nervous Tissue**
- Tissues: Group of cells which are **similar in structure** and which **perform common or related functions**
- Histology: study of tissues
  - General:** study of the 4 basic tissues
  - Systemic:** system by system study
- Tissues are formed by cells and molecules of **extracellular matrix** (ECM) - an intricate meshwork of proteins and polysaccharides that are secreted by the cell and assembled locally
- Organs are formed by combination of different tissues in variable proportions

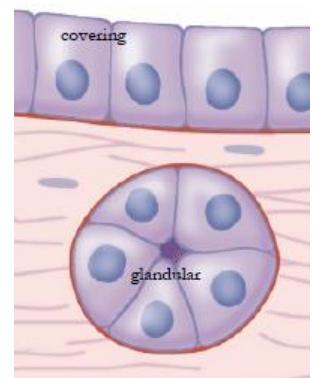
| Main characteristics of the four basic types of tissues |                                            |                 |                                                         |
|---------------------------------------------------------|--------------------------------------------|-----------------|---------------------------------------------------------|
| Tissue                                                  | Cells                                      | ECM             | Main Functions                                          |
| <b>Epithelial</b>                                       | Aggregated polyhedral cells                | Small amount    | Lining of surface or body cavities, glandular secretion |
| <b>Connective</b>                                       | Several types of fixed and wandering cells | Abundant amount | Support and protection                                  |
| <b>Muscle</b>                                           | Elongated contractile cells                | Moderate amount | Movement                                                |
| <b>Nervous</b>                                          | Intertwining elongated processes           | None            | Transmission of nervous impulses                        |

There are also free cells found in body fluids such as **blood** and **lymph**

- Organs can be divided into **parenchyma**, composed of the cells responsible for the organ's main functions, and **stroma**, which is the supporting tissue
- Except in brain and spinal cord, the stroma is always made of connective tissue

## Epithelium

- Epithelium**
  - Composed of **closely aggregated polyhedral cells** with very little extracellular substance
  - Strong adhesion
  - Form cellular sheets that cover the surface of the body and line its cavities
- Principal functions:**
  - Protection** of underlying tissues of the body from abrasion and injury
  - Secretion** of mucus, hormones, enzymes etc. From various glands
  - Absorption** of material from a lumen
  - Detection of **sensations** via taste buds, retina, and specialized hair cells in the ear
  - Contractility**
- Two forms of epithelial tissue
  - Covering** epithelium as sheets of contiguous cells that cover the body on its external surface and line the body on its internal surface
  - Glandular** epithelium - glands, which originate from invaginated epithelial cell
- Epithelium from embryonic germ layers:
  - Ectoderm** gives rise to the oral and nasal mucosae, cornea, epidermis of the skin, and glands of the skin and the mammary glands
  - Endoderm** gives rise to the liver, the pancreas, and the lining of the respiratory and gastrointestinal tract
  - Mesoderm** gives rise to the uriniferous tubules of the kidney, the lining of the male and female reproductive systems, the endothelial lining of the circulatory system, and the mesothelium of the body cavities
- Characteristics:**
  - Polyhedral form**
  - Polarity**
  - Resting on connective tissue:** provided with support and nutrition, because epithelium is **avascular**, and is bound to the underlying structures
  - Basal lamina** at the interface with connective tissue

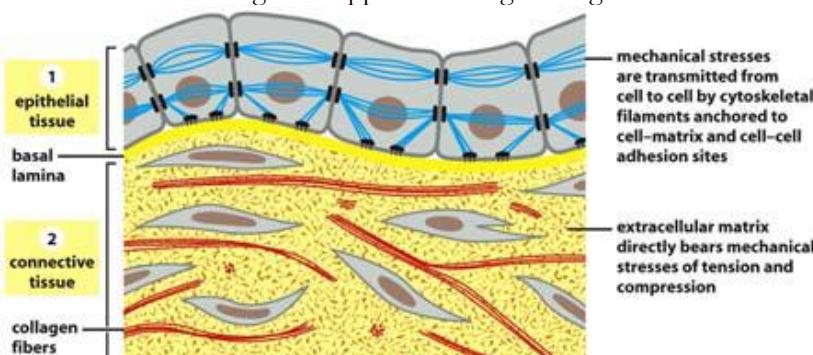


- **Polyhedral form:**
  - Results from their **close juxtaposition** in cellular layers or masses
  - **Nuclear form** often corresponds with the cell shape - the long axis of the nucleus is always parallel to the main axis
    - Cuboidal: spherical nuclei
    - Squamous: flattened nuclei
  - The stained cell nucleus is a clue to the shape and number of cells. Nuclear form is also useful to determine whether the cells are arranged in layers, a primary morphologic criterion for classifying epithelia



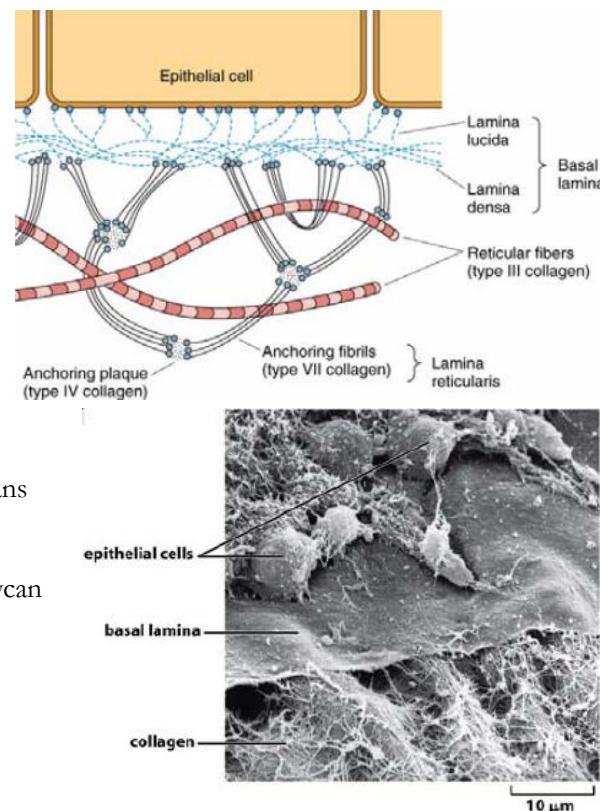
- **Cell polarity:**

- Organelles and membrane proteins are unevenly distributed
- Different regions have different functions
- Glossar:
  - **Basal pole:** region facing the connective tissue
  - **Apical pole:** opposite pole, facing a space
  - **Lateral surfaces:** intervening sides apposed in neighboring cells



## Basal Lamina & Basement membrane

- **Basal Lamina:**
  - Lies at the interface of epithelial cells and connective tissue
  - Nutrients for epithelial cells must diffuse across the lamina
  - Blood capillaries never enter an epithelium across the lamina
  - Nervs enter an epithelium across the lamina
- Under Lightmicroscope, basal lamina is identified as **Basement membrane**, because basal lamina and **reticular lamina** (produced by cytoskeleton cells) are identified as one
- **Basement membrane:**
  - **Lamina lucida:** 50-nm-thick electro-lucent region just beneath the epithelium
    - Transmembrane molecules: integrins & dystroglycans
    - Extracellular glycoproteins: laminin & entactin
  - **Lamina densa:** 50-nm-thick electron-dense region
    - Meshwork of type IV collagen, coated by proteoglycan perlecan
    - Heparan sulfate GAG
  - **Lamina reticularis**
    - Type I & type III collagen
    - Fibronectin
    - Anchoring fibrils (type VII collagen)
    - Microfibrils (fibrillin)



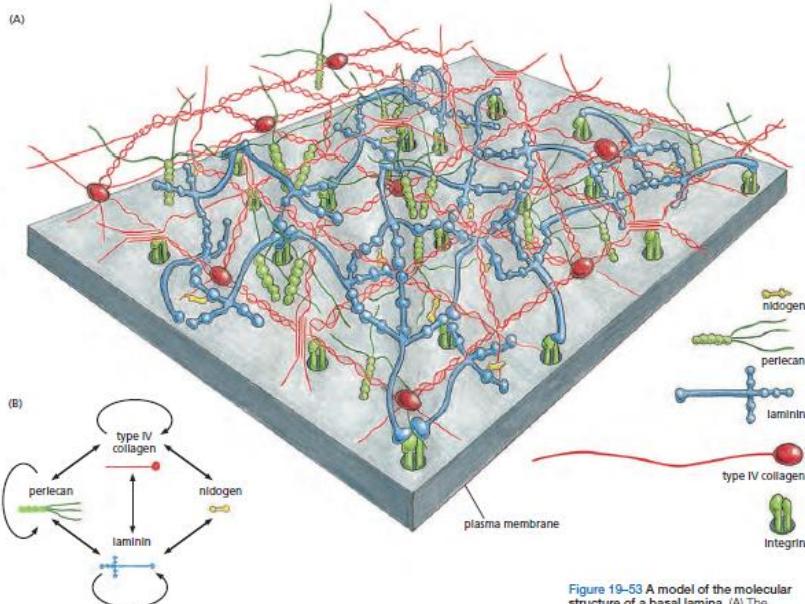


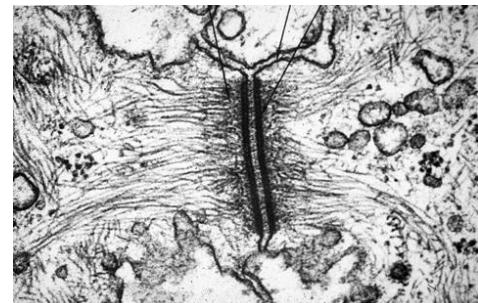
Figure 19-53 A model of the molecular structure of a basal lamina. (A) The

- Functions of the basal lamina:
  - Molecular filter
  - Support of the overlying epithelium
  - Regulation of mitotic activity, cell differentiation, and migration
  - Modulation of cellular metabolism
  - Assisting the establishment of cell polarity
- Basal laminae are also found in muscle, adipocytes, Schwann cells
- Epithelial tissue is **rich in intercellular junctions**

### Plasmalemma of epithelial cells

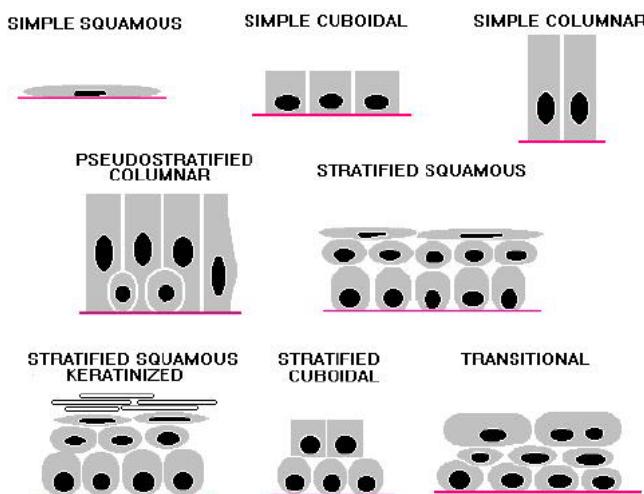
- Specialization of the **apical** cell surface:
  - AF: Microvilli & stereocilia
  - MT: Cilia
- Specializations of the **basolateral** cell surface

TEM of a desmosome with **tonofibrils**

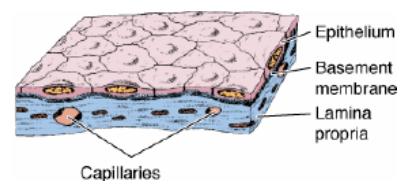


### Types of epithelia in the human body

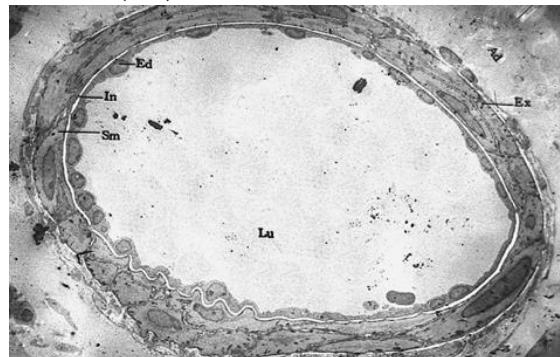
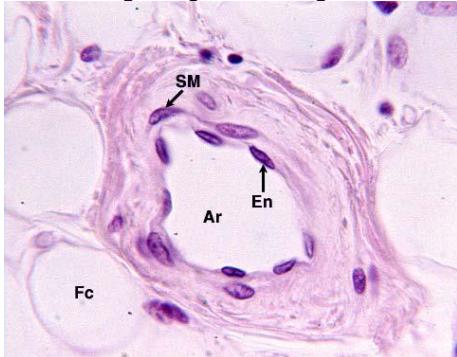
- **Covering** (lining): cells are organized in layers that cover the external surface or line the cavities of the body
- **Glandular** (secretory): formed by cells specialized to secrete proteins, lipids or complexes of carbohydrates and proteins



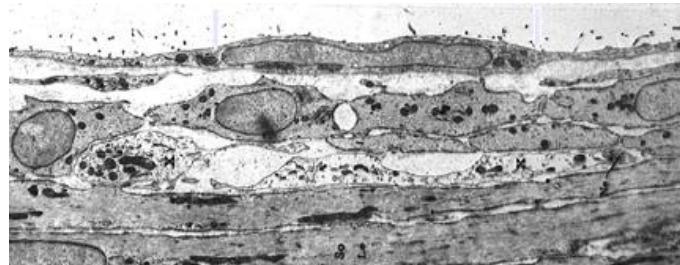
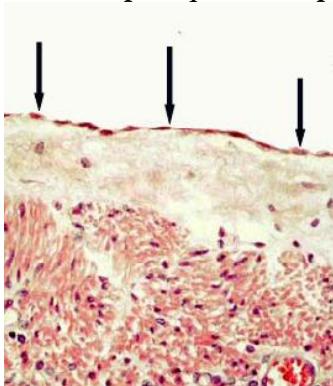
- **Simple squamous epithelium**
  - Simple: One layer
  - Squamous: thin cells
  - Lining of vessels and cavities - often exhibit transcytosis



- Simple squamous epithelium: Endothelium (EN):



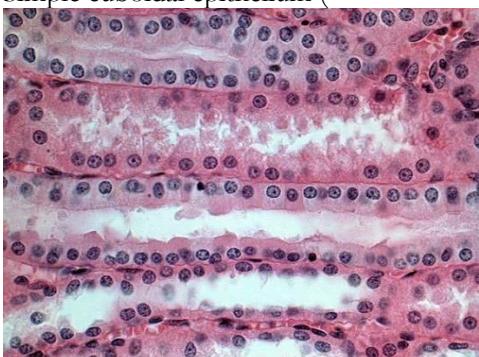
- Simple squamous epithelium: Mesothelium



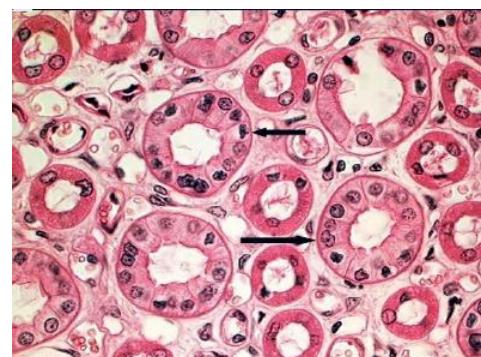
- Simple cuboidal epithelium

- Simple: One layer
- Cuboidal: roughly thick as they're wide
- Greater thickness often includes cytoplasm **rich in mitochondria** providing energy for active transport of substances across the epithelium

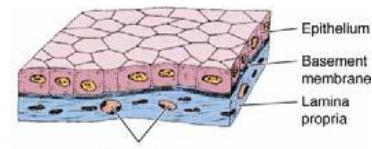
- Simple cuboidal epithelium (



Longitudinal section



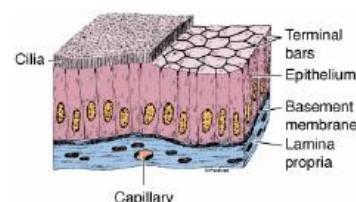
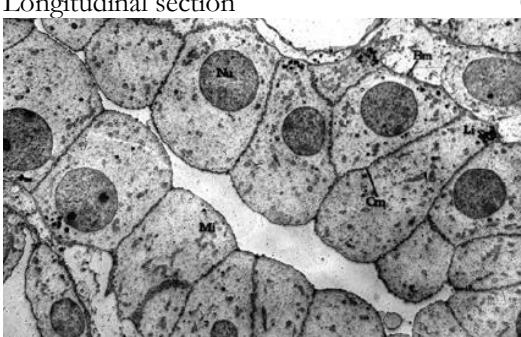
Cross section

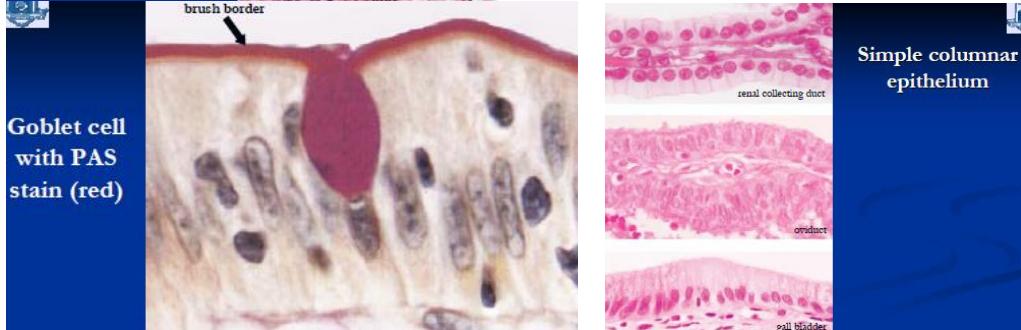
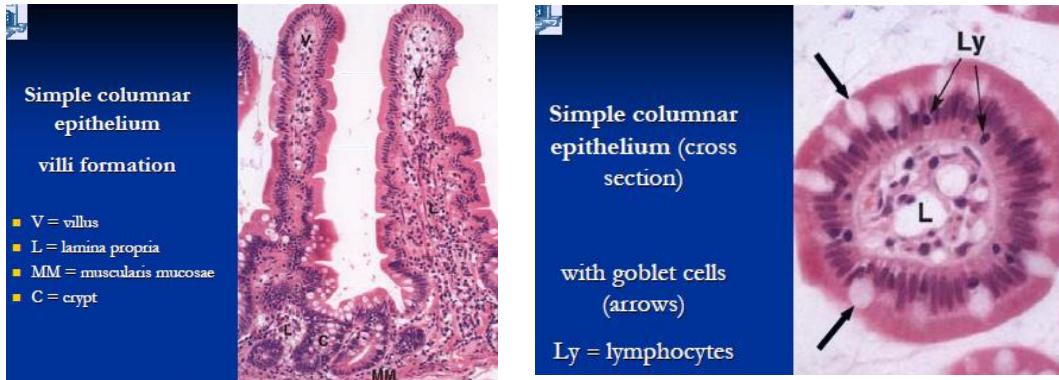


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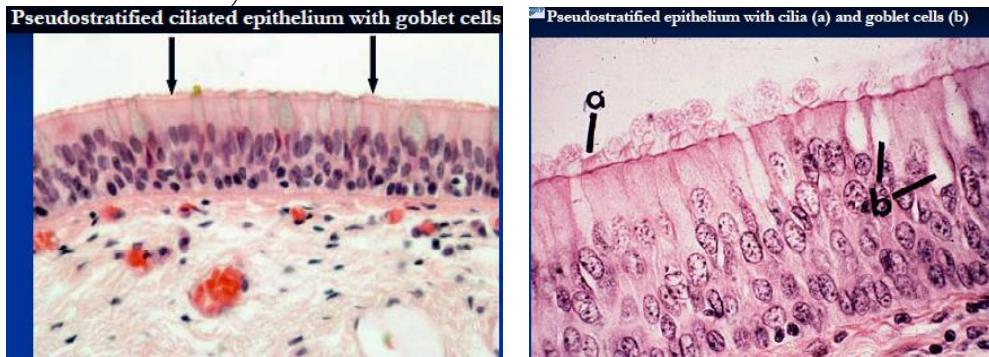
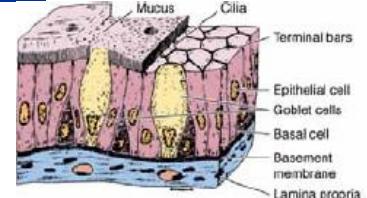
- Simple columnar epithelium:

- Simple: 1 layer
- Columnar: cells are taller than they are wide
- Specialized for **absorption**, with microvilli
- Tight and adherent junctional complexes at the apical surface

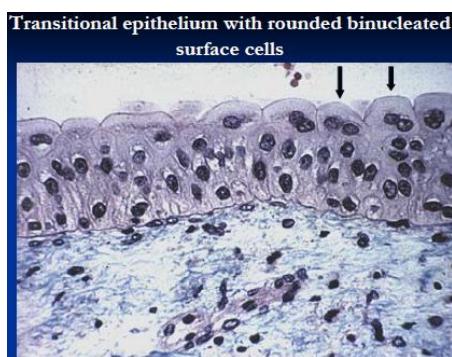
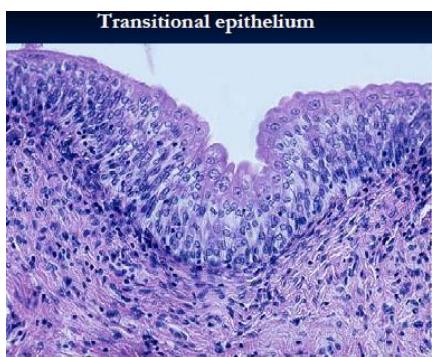
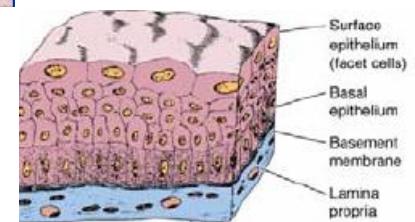




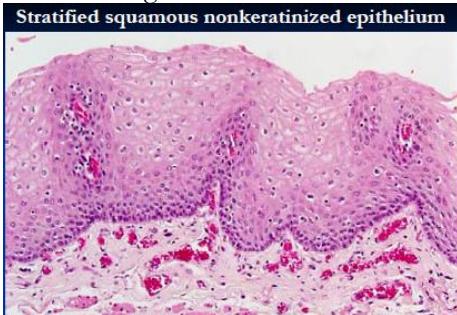
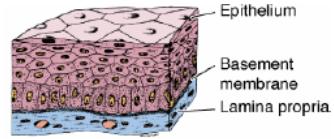
- Pseudostratified columnar epithelium:**
  - Only **appears stratified**; all cells are in contact with the basal lamina
  - Cells are of different heights, their nuclei are located at different levels
  - Can be **ciliated** (e.g. upper respiratory tract) or **non-ciliated** (e.g. male urethra)



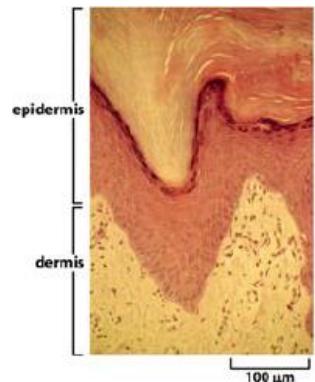
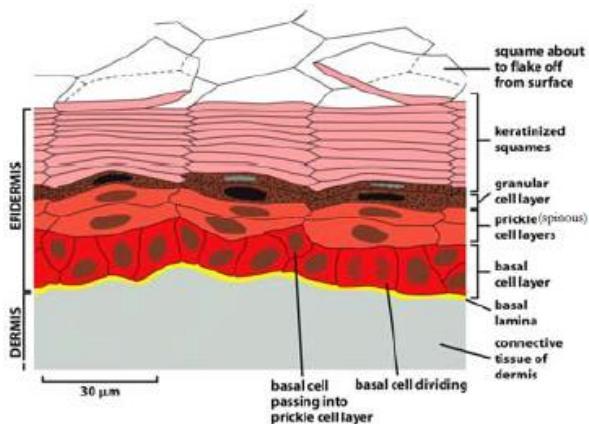
- Transitional epithelium (urothelium)**
  - Lines only the urinary bladder, the ureter, and the upper part of the urethra
  - Composed of many layers of cells
  - Superficial layer of domelike cells that are neither squamous nor columnar (protective against cytotoxic effects of urine)



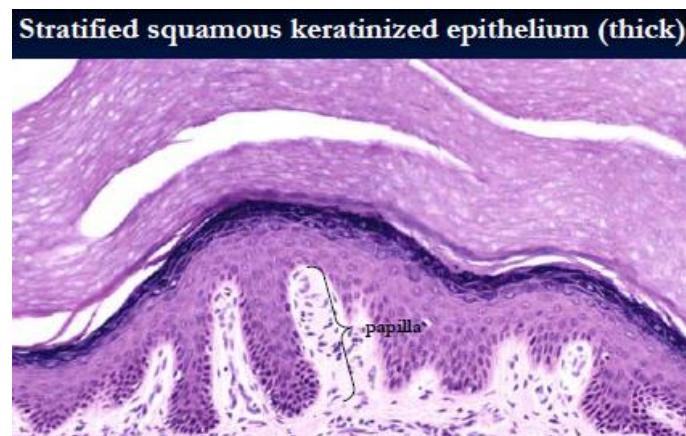
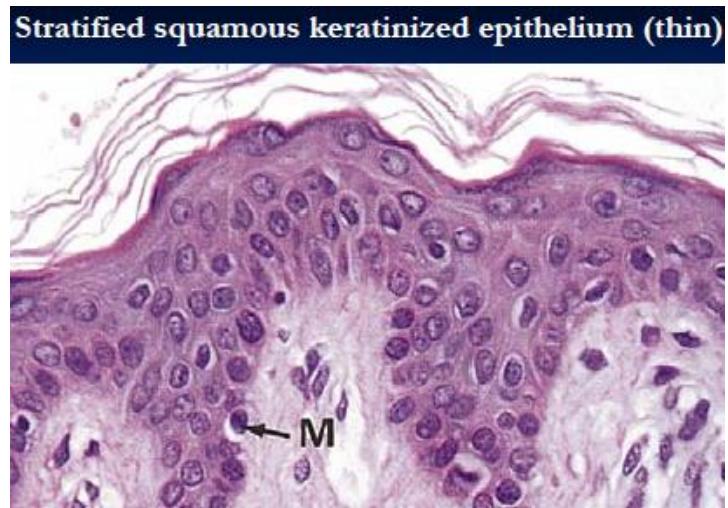
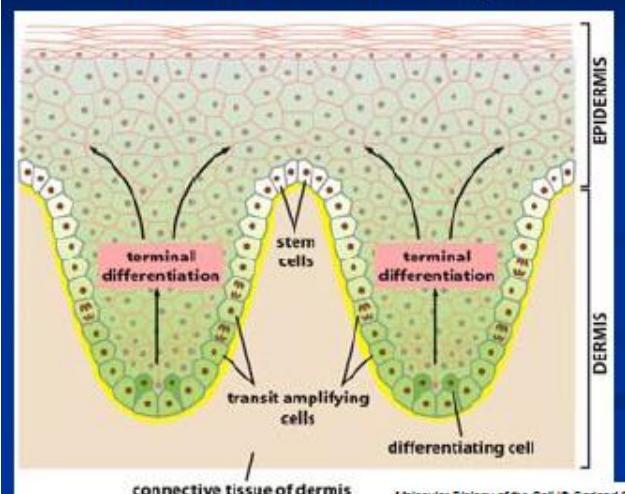
- **Stratified squamous epithelium**
  - **Nonkeratinized:** several layers of cells; the surface-most layer possesses nuclei
  - **Keratinized:** the layer of cells composing the free surface are dead, non-nucleated, and filled with keratin
- **Stratified squamous nonkeratinized epithelium:**
  - Lines **wet cavities** (e.g. mouth, esophagus, and vagina)
  - In such areas where water loss is not a problem, the flattened cells of the epithelial surface layer are living cells containing much less keratin and retaining their nuclei



- **Stratified squamous epithelium**
  - Forms epidermis of the skin
  - Composed of **keratinocytes** (synthesize keratin intermediate filament proteins, which give the epidermis its toughness)
  - Interlocking columns of hexagonal or irregular cells



### Epidermal keratinocytes are renewed by stem cells in the basal layer

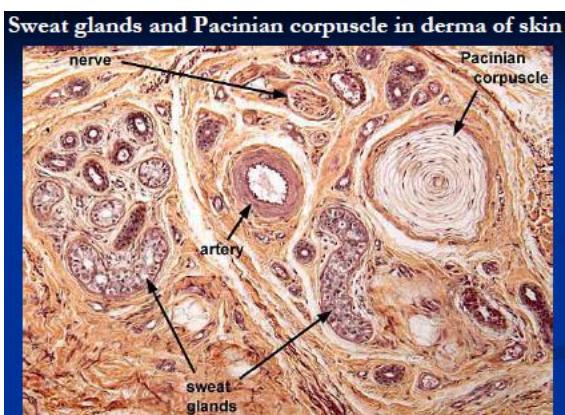
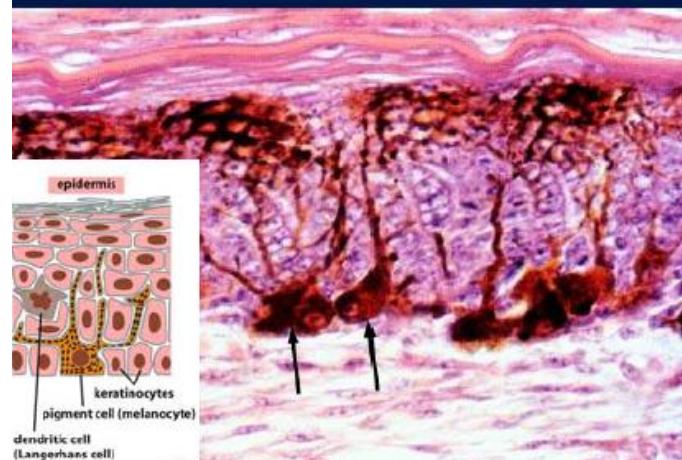


The area of contact between epithelium and connective tissue is increased by irregularities in the connective tissue surface in the form of small evaginations called papillae (L. diminutive of *papilla*, nipple; singular *papilla*). Papillae occur most frequently in epithelial tissues subject to friction, such as the covering of the skin or tongue.

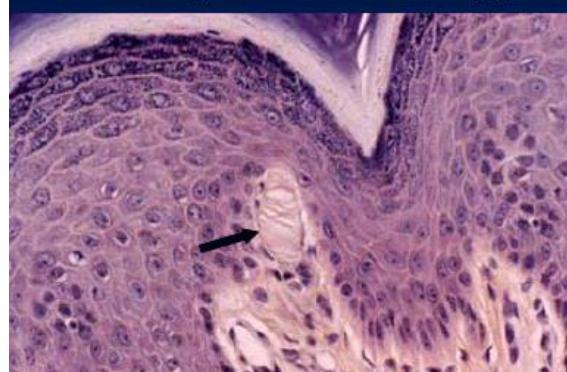
**Stratum spinosum with spiny cell projections (arrow)**



**Thin skin with melanocytes (arrows)**

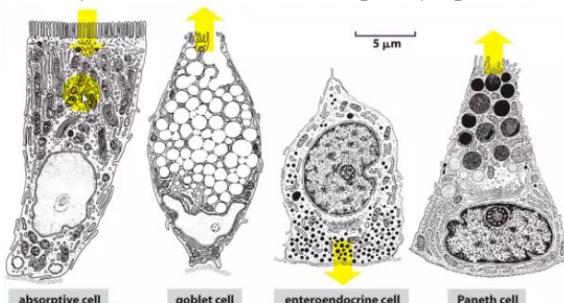


**Meissner's corpuscle within a dermal papilla**



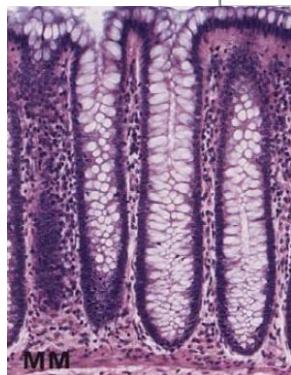
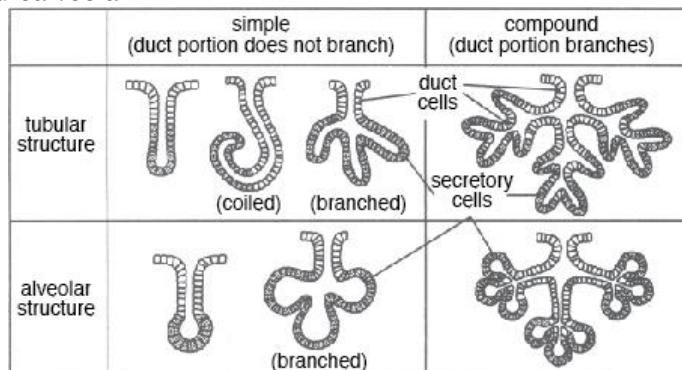
## Glandular epithelium

- Glands, which originate from invaginated epithelial cells
- Glands originate from epithelial cells that leave the surface
- Glandular epithelial cells penetrate into the underlying connective tissue, manufacturing a basal lamina around them
- The secretory units (+ ducts) are the **parenchyme** of the gland, whereas elements of the connective tissue form the **stroma** of the gland
- Glandular epithelia manufacture their product intracellularly by synthesis of macromolecules
  - Products are packaged and stored in vesicles called **secretory granules**
  - The secretory product may be a polypeptide, a waxy substance, a mucinogen, or milk, a combination of protein, lipid, and carbohydrates
- Classification based on the method of product distribution:
  - **Exocrine glands** secrete their products via ducts onto the external or internal epithelial surface from which they originate
  - **Endocrine glands** secrete their products into the blood or lymphatic vessels for distribution
- Exocrine glands:
  - Unicellular or multicellular
  - Secretion: holocrine, merocrine, apocrine
  - Nature of their secretion: mucous, serous, mixed
- **Unicellular exocrine glands (goblet cells):**
  - Simplest form
  - Represented by isolated secretory cells in an epithelium (e. g. intestinal or respiratory)
  - Have basal **stem** and apical theca, filled with membrane-bound secretory droplets (mucinogen granules) - their release is stimulated by chemical irritation and parasympathetic innervation

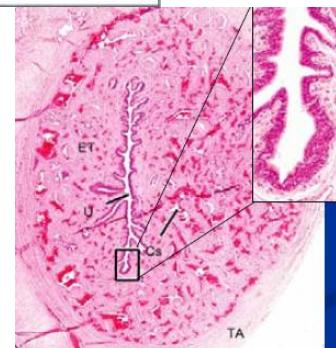


- **Multicellular exocrine glands:**

- Exist as organized clusters of secretory units, arranged in varying degrees of organization
- Together function as secretory organs
- Subclassified according to:
  - Organization of their secretory and duct component:
    - **Simple:** their ducts do not branch
    - **Compound (branched):** their ducts branch
  - Shape of their secretory units:
    - **Tubular**
    - **Acinar (alveolar, resembling a grape)**
    - **Tubuloalveolar**



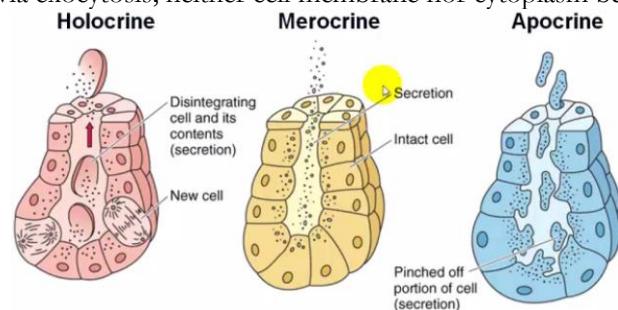
Simple tubular mucus secreting glands: colon



simple acinar mucus secreting glands: male urethra

- Exocrine gland classification on basis of their **mode of secretion:**

- **Holocrine glands:** a secretory cell matures, dies and becomes the secretory product
- **Apocrine glands:** a small portion of the apical cytoplasm is released along with the secretory product
- **Merocrine glands:** via exocytosis; neither cell membrane nor cytoplasm becomes part of the secretion

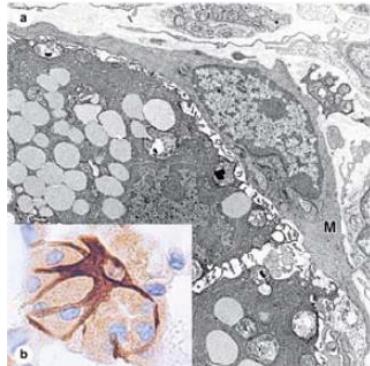
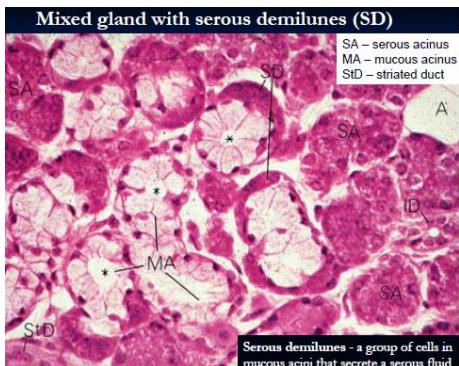
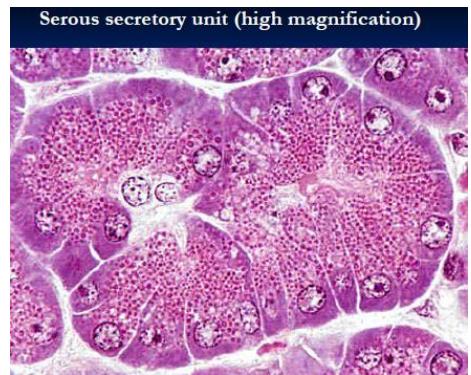
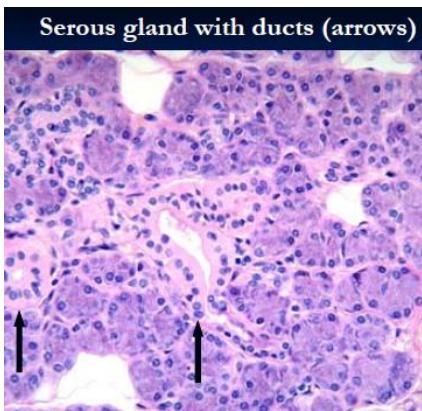
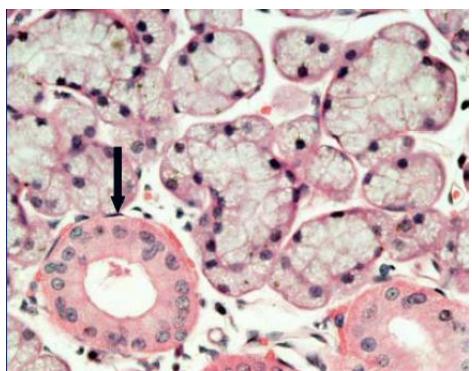


- Larger multicellular glands have additional components:

- Collagenous connective tissue (**Capsule**)
- Capsule sends **septae** (strands of connective tissue) into the gland, subdividing it into smaller compartments known as **lobes** and **lobules**
- Vascular elements, nerves, and ducts utilize the connective tissue septa to enter and exit the gland. In addition, the connective tissue elements provide structural support for the gland.

- **Exocrine glands with merocrine secretion** - Classification based on the nature of secretion

- **Mucous:** secrete **mucinogens** - large glycosated proteins that, upon hydration, swell to become a thick, viscous, gel-like protective lubricant known as **mucin**, a major component of **mucus**
- **Serous:** secrete an enzyme-rich watery fluid
- **Mixed:** mucous and serous components



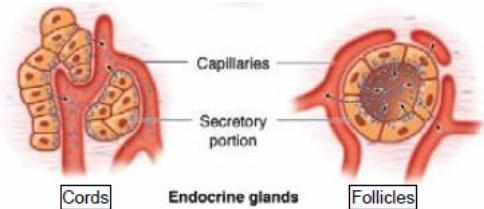
- Organization of a large multicellular gland: a **salivary gland**

- Myoepithelial cells:**

- Stellate or spindle-shaped cells located between the basal lamina and the basal pole of secretory or duct cells in several exocrine glands
- Possess processes which embrace an acinus as an octopus: connected via gap junctions and desmosomes
- Specialized for contraction (contain myosin and a large number of actin filaments): contract around the secretory or conduction portion of the gland and thus help **propel** secretory products into the duct

- Endocrine glands:

- Ductless, and thus their secretory products are released into the bloodstream or the lymphatic system
- Cells can be arranged in **cords** or in **follicles** with lumens for storing the secretory product

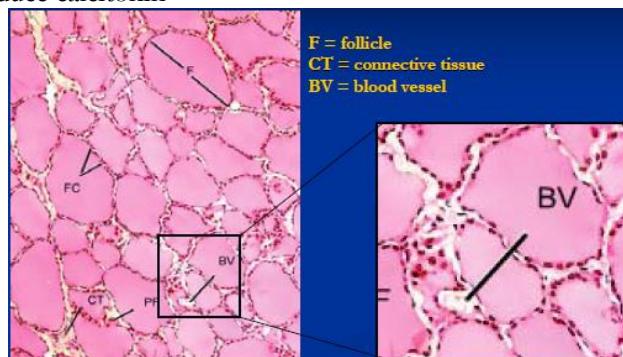


## Endocrine system

- System of ductless glands that secrete hormones
  - Hormones are messenger molecules ("first messenger")
  - Circulate in blood
  - Act on distant target cells
  - Target cells respond to the hormones for which they have receptors
  - The effects are dependent on the programmed response of the target cells
  - Hormones are just molecular triggers
- Basic categories of hormones:
  - Amino acid based: modified, amino acids (or amines), peptides (short chains of amino acids), and proteins (long chains of amino acids)
  - Steroids: lipid molecules derived from cholesterol

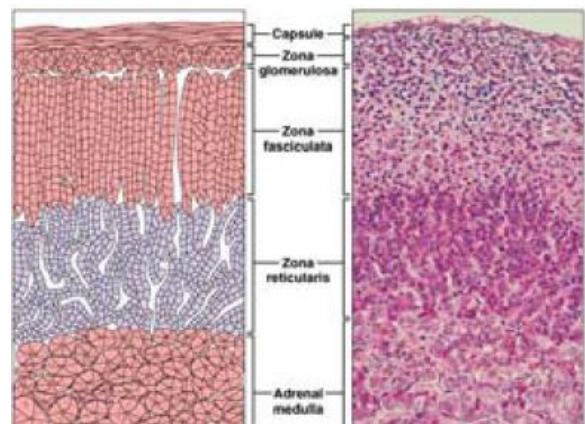
## Thyroid glands

- Follicle cells: produce thyroglobulin, the precursor of thyroid hormone (thyroxin)
- Parafollicular "C" cells: produce calcitonin



## Adrenal (supraarenal) glands

- "suprarenal" means: on top of the kidney
- Each is really two endocrine glands:
  - **Adrenal cortex** (outer)
  - **Adrenal medulla** (inner)
- Unrelated chemicals but all help with extreme situations
- Cords of endocrine cells:



## Pankreas

- **Exocrine and endocrine cells**
  - **Acinar cells** (forming most of the pancreas)
    - **Exocrine** function
    - Secrete digestive enzymes
  - **Islet cells** (of Langerhans)
    - **Endocrine** function

## Connective tissue (CT)

### Tissue = cells + ECM

- Tissues are formed by cells and molecules of the **extracellular matrix (ECM)**
- ECM: intricate meshwork of proteins and polysaccharides that are secreted by the cell and assembled locally
- Organs are formed by combination of different tissues in variable proportions

### Connective tissue

- Connective tissue forms a continuum with epithelial tissue, muscle, and nervous tissue as well as with other components of connective tissues to maintain a functionally integrated body

### Origin

- CT develops from **mesenchyme** (multipotential cells of the embryo)
- Mesenchyme develops mostly from **mesoderm**
- In certain areas of the head and neck, mesenchyme also develops from **neutral crest** cells of the developing embryo

### Functions

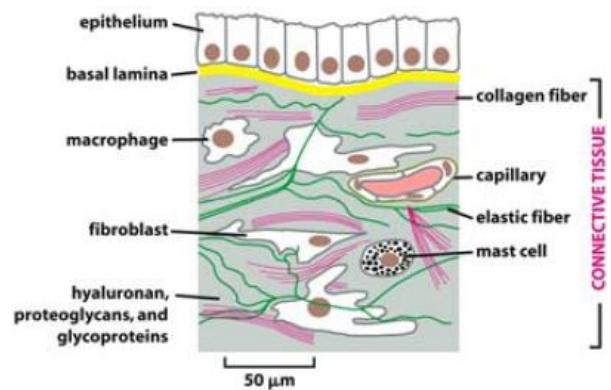
- Support and protection
- Forms a reservoir of factors controlling cell growth and differentiation
- Provides the medium through which nutrients and metabolic wastes are exchanged between cells and their blood supply
- Adipose tissue is the largest endocrine organ in the body

### Structural elements of CT

- **Cells:**
  - Permanent residents: fibroblasts, macrophages, mast cells, adipocytes, mesenchymal cells
  - Transient (wandering) cells: primarily migrating from blood (Neut, Eo, Ly, plasma cells)
- ECM is the major constituent of connective tissue
  - **Protein fibers:** collagen, reticular, and elastic
  - **Ground substance:** anionic macromolecules and multiadhesive glycoproteins that stabilize the ECM by binding to cells and to other ECM components

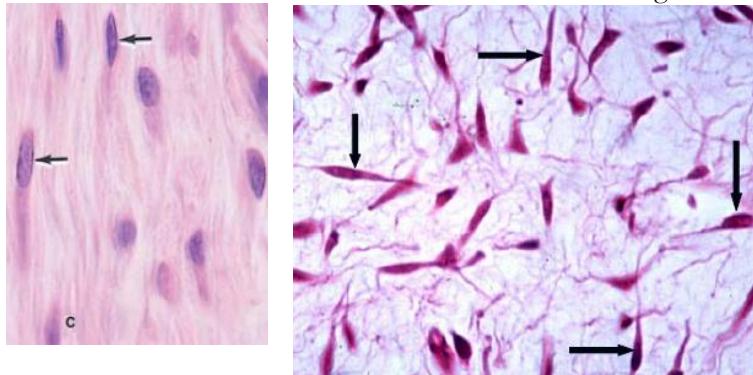
### Cells of C

| Cell Type                                         | Representative Product or Activity                                                                                                              | Representative Function                     |
|---------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------|
| Fibroblast, chondroblast, osteoblast, odontoblast | Production of fibers and ground substance                                                                                                       | Structural                                  |
| Plasma cell                                       | Production of antibodies                                                                                                                        | Immunologic (defense)                       |
| Lymphocyte (several types)                        | Production of immunocompetent cells                                                                                                             | Immunologic (defense)                       |
| Eosinophilic leukocyte                            | Participation in allergic and vasoactive reactions, modulation of mast cell activities and the inflammatory process                             | Immunologic (defense)                       |
| Neutrophilic leukocyte                            | Phagocytosis of foreign substances, bacteria                                                                                                    | Defense                                     |
| Macrophage                                        | Secretion of cytokines and other molecules, phagocytosis of foreign substances and bacteria, antigen processing and presentation to other cells | Defense                                     |
| Mast cell and basophilic leukocyte                | Liberation of pharmacologically active molecules (e.g. histamine)                                                                               | Defense (participate in allergic reactions) |
| Adipocyte                                         | Storage of neutral fats                                                                                                                         | Energy reservoir, heat production           |

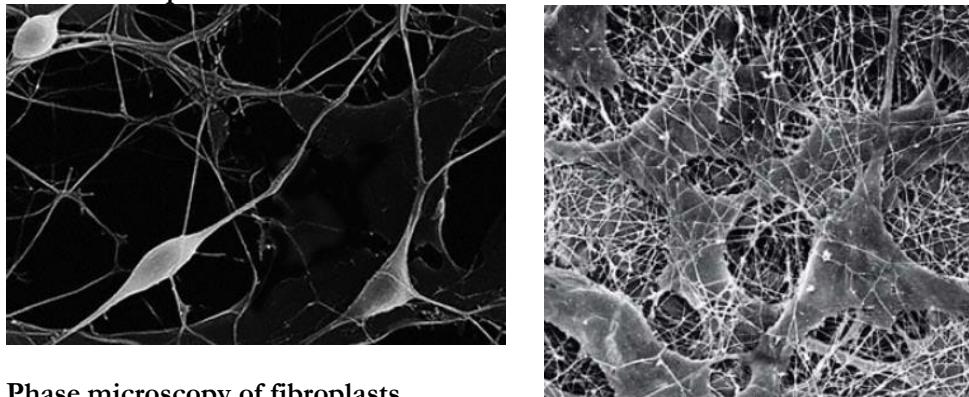


## Fibroblasts

- Most common CT cells
- **Synthesize ECM components:** collagen, elastin, glycosaminoglycans, proteoglycan & multiadhesive glycoproteins
- Cells with intense synthetic activity are called **fibroblasts**, distinct from the quiescent cells that are scattered and have already synthesized called **fibrocytes**
- **LM features:**
  - Large active nuclei and eosinophilic cytoplasm
  - Spindle-shaped nucleus
  - Nuclei are clearly seen, but the cytoplasmic processes resemble the collagen bundles (C) that fill the extracellular matrix and are difficult to distinguish in H&E-stained sections



SEM of fibroblasts:

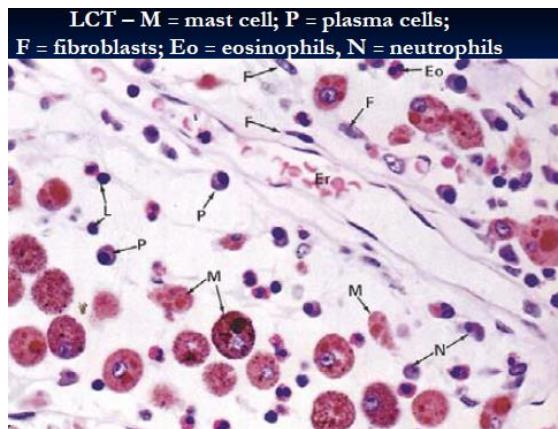


Phase microscopy of fibroblasts

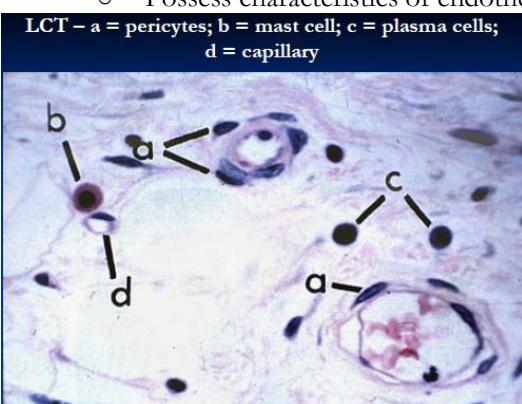
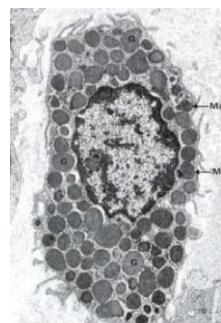
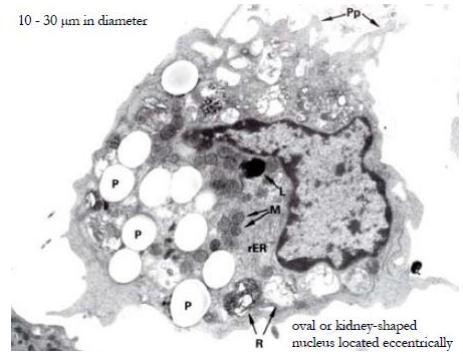


- **Myofibroblasts** are modified fibroblasts that demonstrate characteristics similar to those of both fibroblasts and smooth muscle cells

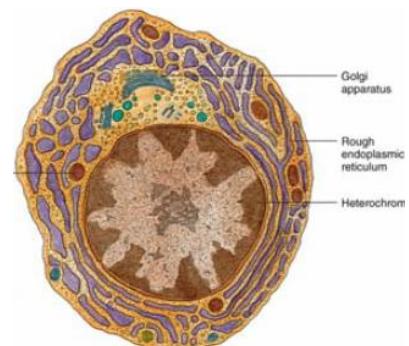
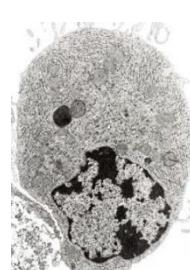
## Defense cells of the CT



- **Macrophages:**
  - Derive from bone marrow precursor cells that divide, producing monocytes which circulate in the blood
  - Monocytes cross the wall of venules and capillaries to penetrate the connective tissue, where they mature and acquire the morphologic features of macrophages (increase in cell size, protein synthesis, number of Golgi complexes and lysosomes)
  - Combined with other monocyte-derived cells they form the family of cells called the **mononuclear phagocyte system**
  - Long-living: may survive for months
- **Mast cells:**
  - Large, oval or round connective tissue cells, 20-40 micrometer in diameter
  - Cytoplasm is filled with basophilic secretory granules:
    - **Heparin:** a sulfated glycosaminoglycan that acts locally as an anticoagulant
    - **Histamine:** promotes increased vascular permeability and smooth muscle contraction
    - **Serine proteases:** activate various mediators of inflammation
    - **Eosinophil and neutrophil chemotactic factors** which attract those leukocytes
    - **Leukotrienes C4, D4, and E4:** trigger smooth muscle contraction
  - Display **metachromasia:** can change the color of some basic dyes (e.g. toluidine blue) from blue to purple or red
  - Especially numerous near small blood vessels in skin and mesenteries (**perivascular mast cells**) and in the mucosa lining digestive and respiratory tract (**muscosal mast cells**)
  - Originate from stem cells in the bone marrow (have a separate progenitor from other blood cells)
  - Mast cells promote allergic reactions known as **immediate hypersensitivity reactions:** occur within a few minutes after penetration by an antigen of an individual previously sensitized to the same antigen
- **Pericytes:**
  - Surround endothelial cells of capillaries and small venules
  - Have their own basal lamina: technically outside the CT compartment
  - Derived from **mesenchymal cells**
  - Possess characteristics of endothelial cells and smooth muscle cells: may function in contraction

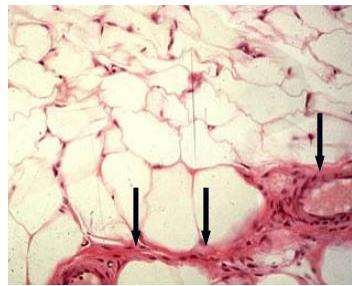
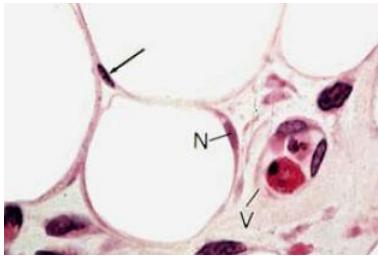


- **Plasma cells:**
  - Antibody-producing cells derived from B cells
  - Large, ovoid cells that have a basophilic cytoplasm due to their richness in rough ER
  - Nucleus: spherical but eccentrically placed



## Adipocytes

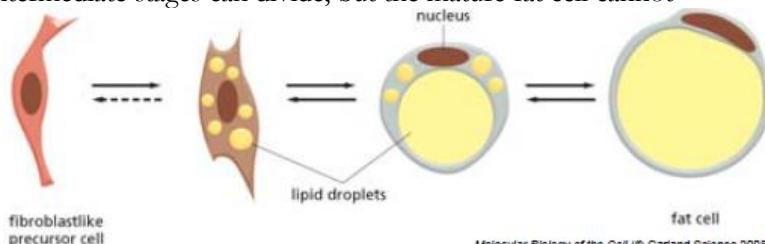
- Also called: **fat cells**



- Adipose tissue:** fat cells + CT septa

- Development:**

- Fibroblastlike precursor cell is converted into a mature fat cell by accumulation and coalescence of lipid droplets
- At least partly reversible
- Cells in early intermediate stages can divide, but the mature fat cell cannot

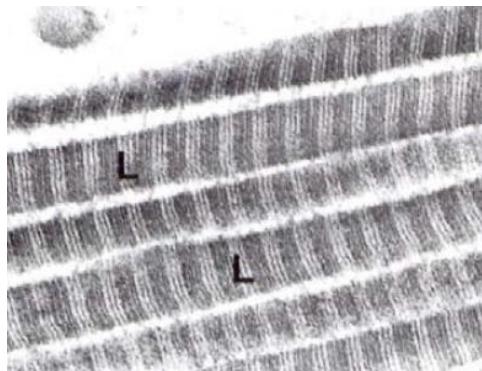
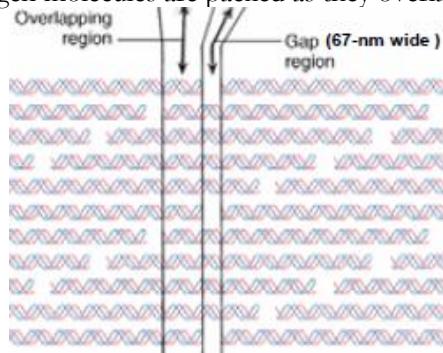


## Mesenchymal cells

- Undifferentiated cells, spindle shaped
- Extend processes which contact other cells: gap junctions

## Extracellular matrix of CT

- Protein fibers:** proteins that polymerize into elongated structures:
  - Collagen fibers
  - Reticular fibers
  - Elastic fibers
- Ground substance:**
  - Highly hydrophilic complex of anionic macromolecules
    - Glycosaminoglycans
    - Proteoglycans
  - Multiadhesive glycoproteins (laminin, fibronectin, and others): stabilize ECM by binding to receptor proteins (integrins) on the surface
- Collagen fibers:**
  - Made up of a family of proteins: **collagens**
  - Collagen is the most abundant protein in the human body, representing 30% of its dry weight
  - Produced by fibroblasts and several other cell types
  - More than 20 types of collagen
  - Triple-helix structure composed of three polypeptide chains ( $\alpha$ -chains)
  - Collagen type I is the most abundant and widespread
  - Six major collagen types:
    - Type I:** connective tissue, bone, dentin, cementum
    - Type II:** hyaline, elastic cartilages
    - Type III:** reticular fibers
    - Type IV:** placenta; associated with type I
    - Type VIII:** attaching basal lamina to lamina reticularis
  - Collagen molecules are packed as they overlap



by 1/4:

- Collagen **fibrillogenesis**: Procollagen -> collagen -> collagen fibrils/fibers
- **Classification:**
  - **Fibril-forming:** types I, II, III, V, XI
  - **Fibril-associated:** IX, XII
  - **Network-forming:** IV (basal lamina), VII (anchoring fibrils/skin)
- **Fibril-associated collagens:**
  - Mediate the interactions of collagen fibrils with one another and with other matrix molecules
  - Type IC molecules bind to type-II-collagen-containing fibrils in cartilage & cornea
  - Type XII molecules bind to type-I-collagen-containing fibrils in tendons and various other tissues
- **Network-forming collagens:**
  - Fibrillar structure is absent in type IV and type VII collagen because the propeptides are not removed from the procollagen molecule
  - Its procollagen molecules assemble into dimers, which then form a meshwork
- **Collagen turnover:**
  - In normal CT: generally very slow
  - To be renewed, collagen must first be degraded
  - Degradation is initiated by specific enzymes called **collagenases**, which are members of an enzyme class called **matrix metalloproteinases** or **MMPs**
- **Collagen diseases:**
  - **Genetic diseases**
    - Osteogenesis imperfecta: Type I
    - Chondrodysplasias: Type II
    - Ehlers-Danlos syndrome: Type III
  - **Non-genetic diseases**
    - Atherosclerosis
    - Liver cirrhosis
    - Glomerulosclerosis

## Fibrogenic cells

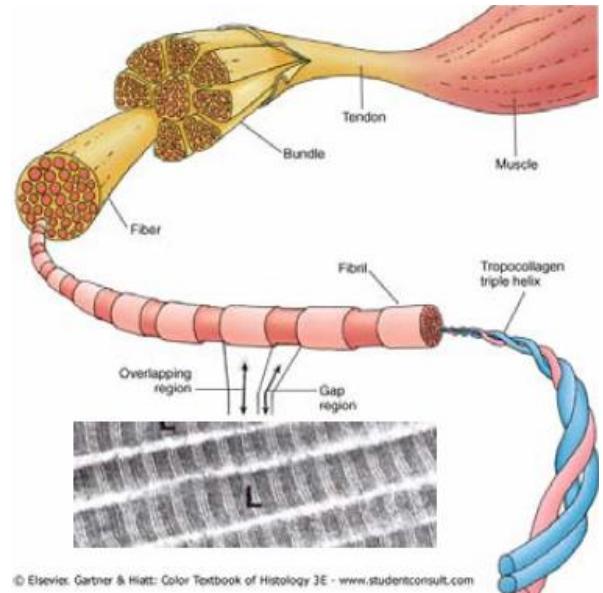
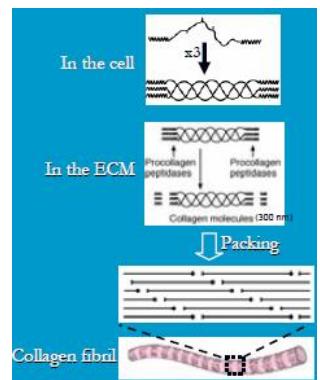
- Cell types:
  - Fibroblasts / myofibroblasts
  - Chondrocytes
  - Osteocytes
  - Odontoblasts
  - Mesangial cells
  - Vascular smooth muscle cells
  - Hepatic stellate cells (Ito cells)

## Reticular fibers

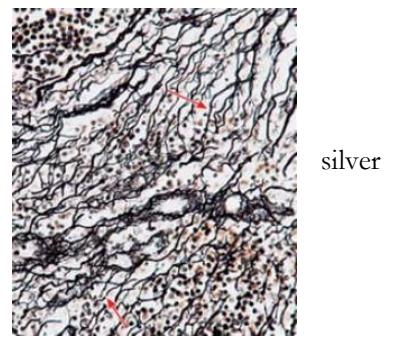
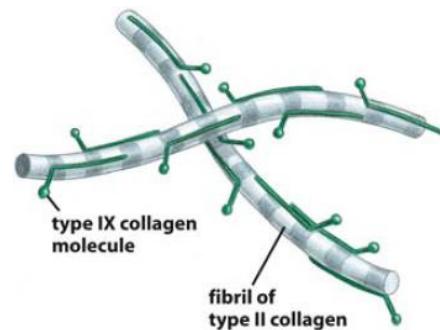
- Consist mainly of collagen type II (in the past thought to be distinct from collagen)
- Form networks of very thin and heavily glycosylated fibers in certain organs
- Not visible in H&E preparations but can be stained black by impregnation with salts
- Particularly abundant in hematopoietic organs (e.g., spleen, lymph nodes, red bone marrow)

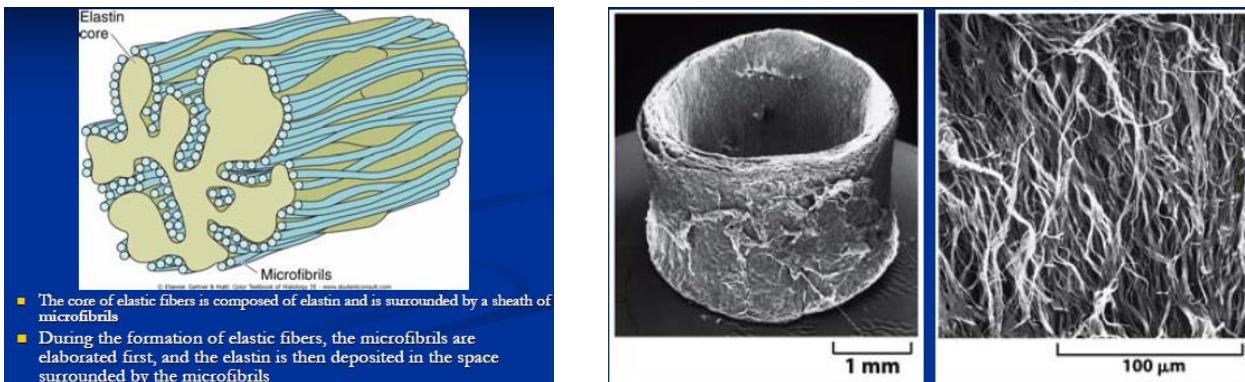
## Elastic fibers

- Thinner than collagen fibers
- Form networks interspersed with collagen bundles in many organs subject to much bending or stretching
- Major components:
  - **Elastin:** synthesized as proelastin (tropoelastin) molecules which assemble to form fibrils / fibers; elastin molecules are globular and are secreted by fibroblasts in connective tissue and by smooth muscle cells in the walls of blood vessels
  - Microfibrils composed of fibrillin and other glycoproteins



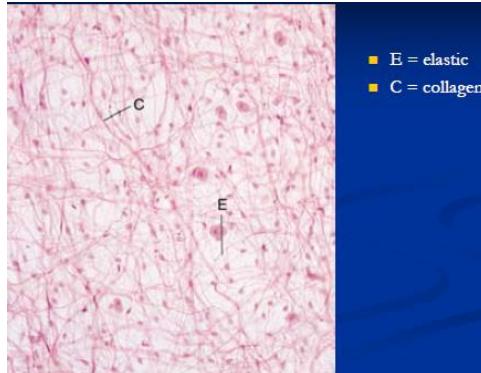
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- Stretching a network of elastin molecules:

- Elastin chains are held together in such a fashion that four lysine molecules, each belonging to a different elastin chain, form covalent bonds with each other to form **desmosine cross-links**
- Highly deformable and they impart a high degree of elasticity to elastic fibers: stretched to about 150% before breaking



## Ground substance of ECM

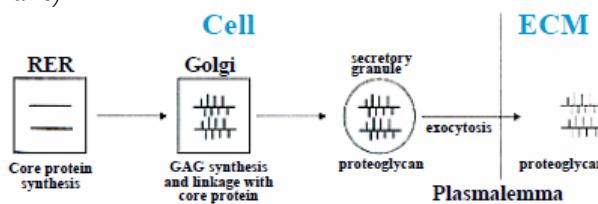
- Highly hydrophilic complex of anionic macromolecules
  - Glycosaminoglycans (GAGs)
  - Proteoglycans (PGs)
- Multiadhesive glycoproteins (laminin, fibronectin, and others): stabilize the ECM by binding to receptor proteins (integrins) on the surface of cells

### Glycosaminoglycans (GAGs) = mucopolysaccharides

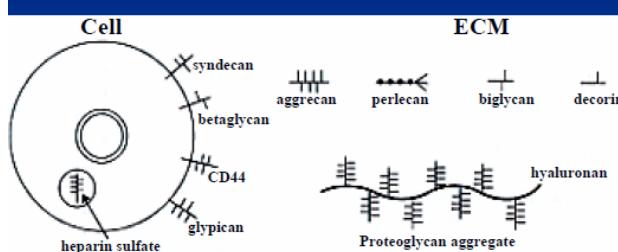
- Unbranched, highly extended conformations of polysaccharide chains composed of **repeating disaccharide units**: one of the two always being an amino sugar (N-acetylglucosamine or N-acetylgalactosamine), hence GAG
- GAGs are highly negatively charged, the most anionic molecules (sulfate and carboxyl groups) produced by animal cells. This attracts many cations, mostly Na<sup>+</sup>, causing a large amount of water to accumulate in the matrix. This creates **swelling pressure (turgor)** that enables ECM to withstand compressive forces

### Proteoglycans structure (PG)

- Consists of a protein core molecule bound with many different types of GAGs
- GAG (except hyaluronan) covalently bound to protein (core protein) form proteoglycans (secreted and integral component of plasma membrane)



### Proteoglycans – Types & Localization

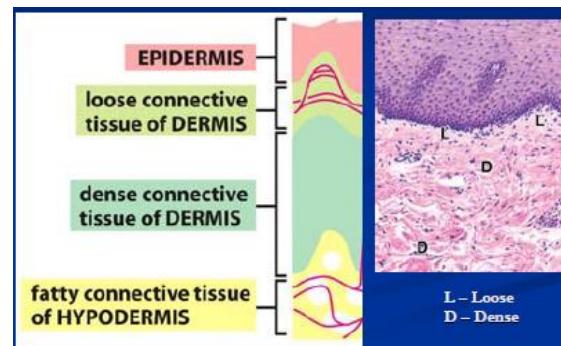
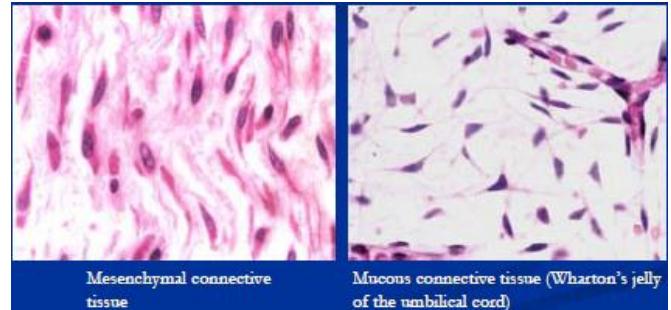


- Functions:
  - Providing hydrated space in the matrix (swelling pressure)
  - Act as co-receptors of growth factors
  - Inhibition of activity of growth factors
  - Bind / regulate activity of matrix enzymes
  - Involvement in collagen fiber formation (decorin)

## Type of connective tissue

### CT classification:

- Embryonic connective tissue
  - Mesenchymal connective tissue
  - Mucous connective tissue
- Connective tissue proper
  - Loose (areolar) connective tissue
  - Dense connective tissue
    - Irregular
    - Regular (collagenous & elastic)
  - Reticular connective tissue
  - Adipose connective tissue
- Specialized connective tissue
  - Cartilage
  - Bone
  - Blood

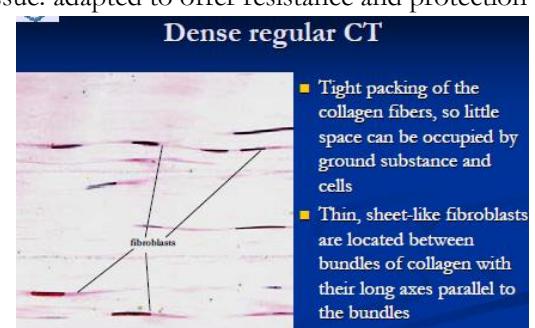


### Loose (areolar) connective tissue

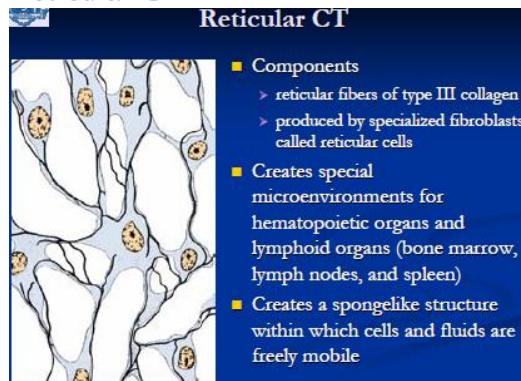
- Fills in the spaces of the body just deep to the skin, lies below the mesothelial lining of the internal body cavity, is associated with the adventitia of blood vessels, and surrounds the parenchyma of glands
- Loose connective tissue of mucous membranes is called **lamina propria**

### Dense connective tissue

- Less flexible and far more resistant to stress than loose connective tissue: adapted to offer resistance and protection
- Types:
  - **Irregular:** arranged in bundles without a definite orientation; often found closely associated with loose connective tissue; found in dermis of skin, sheaths of nerves, and capsules of internal organs
  - **Regular:** densely packed and oriented into parallel cylinders
    - Collagenous: tendons, ligaments, and aponeuroses
    - Elastic: large arteries, lig. flava, suspensory lig. of the penis



### Reticular CT



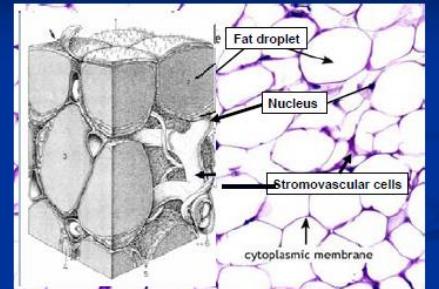
### Adipose tissue

- Specialized type of CT in which **adipocytes** (fat cells) predominate
- Adipocytes are combined with loose or irregular connective tissue, often in large aggregates
- Represents approx. 20% of the body weight of normal persons
- Because of a growing worldwide epidemic of obesity and its associated problems, including diabetes and heart disease

## Functions:

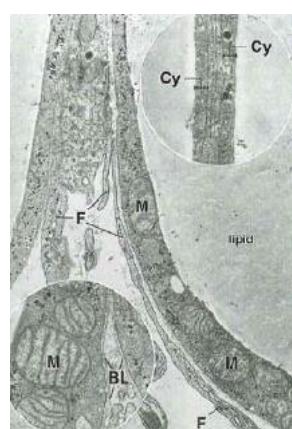
- Largest repository of energy (in the form of triglycerides, the neutral fats): because triglycerides have a higher caloric value than glycogen, adipose tissue has evolved as a very efficient storage tissue
- Adipocytes themselves release hormones and a number of important factors, and adipose tissue is now recognized as a major endocrine and signaling organ
- Poor heat conductor: contributes to the thermal insulation of the body
- Fills up spaces between other tissues and helps to keep some organs in place

The stromal compartment of adipose tissue contains vessels, fibroblasts, macrophages, mast cells, and also has endocrine functions



## Tissue types:

|  |                                                                                                                                                                                                                                                                                                                                                  |
|--|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  | <ul style="list-style-type: none"> <li>■ <b>White (unilocular)</b> <ul style="list-style-type: none"> <li>➢ more common type</li> <li>➢ triglycerides are stored in a single locus</li> <li>➢ contain one large central droplet of whitish-yellow fat in their cytoplasm</li> <li>➢ peripheral nucleus - signet ring cell</li> </ul> </li> </ul> |
|  | <ul style="list-style-type: none"> <li>■ <b>Brown (multilocular)</b> <ul style="list-style-type: none"> <li>➢ less common type</li> <li>➢ multiple lipid droplets interspersed among abundant mitochondria - darker color</li> <li>➢ central oval nucleus</li> </ul> </li> </ul>                                                                 |



**TEM features of white adipocytes**

- M – mitochondria
- F – fibroblast processes
- BL – basal lamina
- Usually also possess small lipid droplets seen by TEM in addition to the single large droplet seen with the light microscope; the droplets are not enveloped by a membrane but show many vimentin intermediate filaments in their periphery

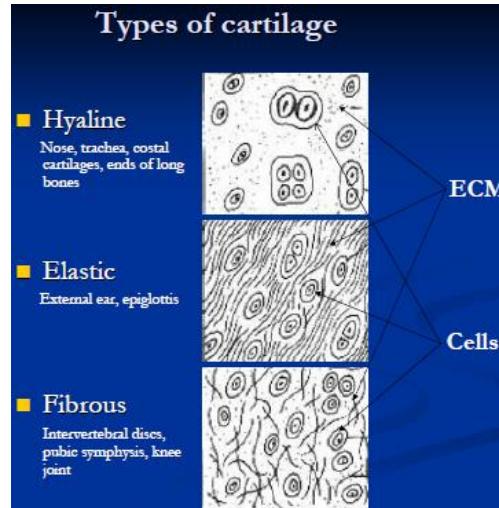
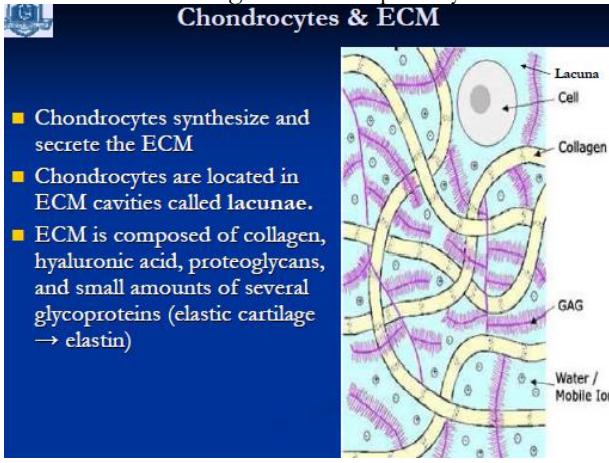
- **Brown adipose tissue:**
  - Numerous mitochondria (containing colored cytochromes)
  - Many small lipid droplets
  - Rich vascular supply

→ heat production
- **Function of brown adipose tissue**
  - Heat production
  - Amount is maximal at birth: newborn is exposed to an environment colder than the mother's uterus
  - In adults it is found only in scattered areas, especially around the kidneys and adrenal glands, the aorta, and mediastinum
- **Development of white and brown fat cells:**
  - Undifferentiated mesenchymal cells differentiate as preadipocytes and are transformed into **lipoblasts** as they accumulate fat and thus give rise to mature fat cells
  - Mesenchymal cells also give rise to a variety of other cell types, including fibroblasts
- **Obesity:** Excessive formation of adipose tissue
  - Adult: involves largely increased size or hypertrophy in existing adipocytes
  - Childhood: can involve both hypertrophy and hyperplasia of preadipocytes from mesenchymal cells
- **Lipoma:** benign tumor of adipose tissue
- **Liposarcoma:** malignant neoplasm of adipocytes

## Cartilage tissue

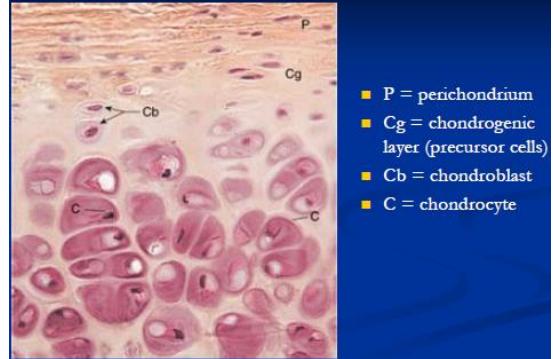
- Cartilage is a **specialized form** of connective tissue:
  - Firm consistency of its ECM allowing to bear mechanical stresses without permanent distortion
  - Supports soft tissues
  - Facilitates bone movements by being the sliding area for joints, because it is smooth
  - Helps the growth of long bones both before and after birth
- **Special features:**
  - Constituents:
    - Cells (5%): **chondrocytes**
    - ECM (95%): fibers + ground substance

- Avascular: cartilage is nourished by the diffusion of nutrients from capillaries in adjacent connective tissue (perichondrium) or by synovial fluid from joint cavities → low metabolism
- No lymphatic vessels or nerves
- Low regeneration capability



### Hyaline cartilage

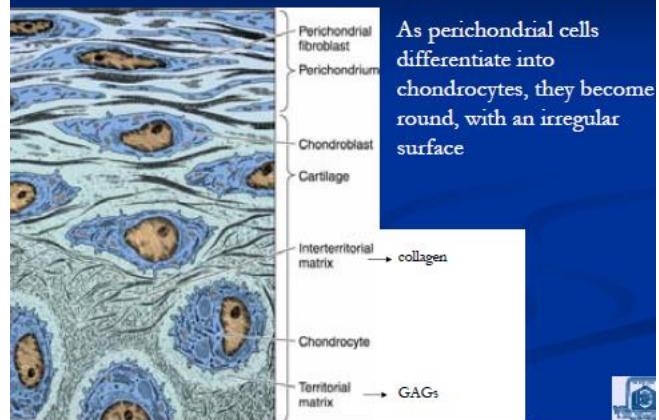
- **Hyaline cartilage** is the most common type of cartilage
  - Embryo: temporary skeleton until it is replaced by bone
  - Adult: articular surfaces of movable joints, respiratory pathways, rib ends
- **Perichondrium**: layer of dense connective tissue, covering the hyaline & elastic cartilage
  - Rich in collagen type I fibers and contains numerous fibroblasts
  - Essential for the growth and maintenance of cartilage
- **Cellular composition** of cartilage
  - **Chondroblasts**:
    - From perichondrium
    - Undifferentiated
    - Generate chondrocytes
  - **Chondrocytes**:
    - 10-30 micrometers in size
    - Secrete the matrix
    - Round and may appear in groups of up to 8 cells originating from mitotic division of a single precursor cell: **isogenous groups** (isos = equal; genos = family)



### Control of matrix production

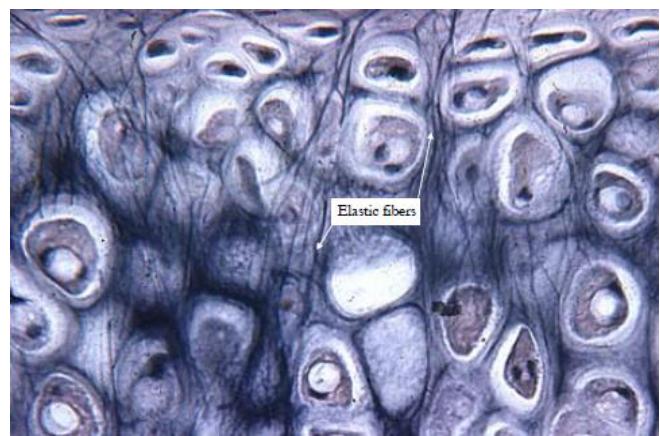
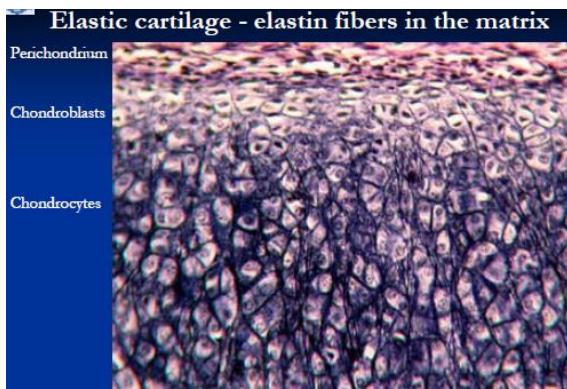
- **Collagen**: Enhanced by growth hormone → somatomedin C (=Insulin-like growth factor I)
- **GAGs** (sulfated):
  - Enhanced by growth hormone, thyroxin, and testosterone, also vit. A, C, D
  - Inhibited by cortisone, hydrocortisone, and estradiol

### Transition between the perichondrium and the hyaline cartilage



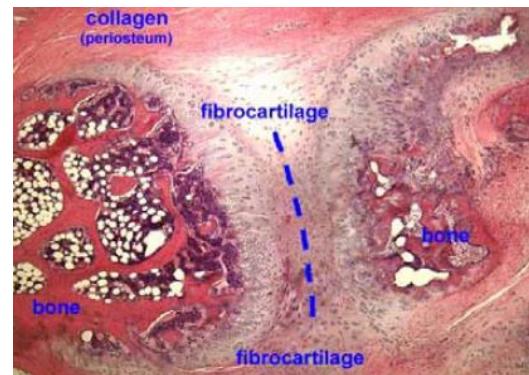
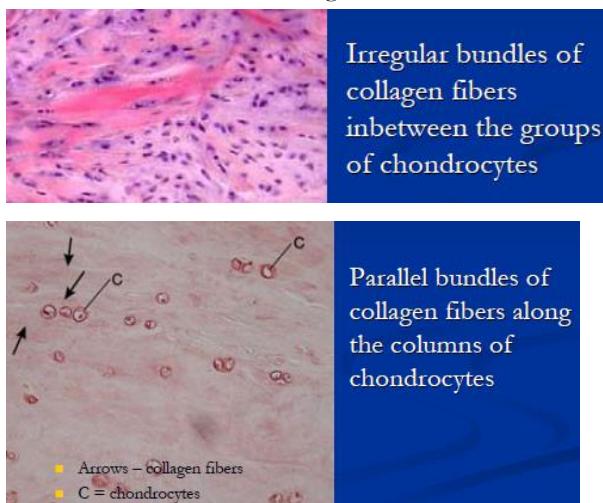
### Elastic cartilage

- Found in the auricle of the ear, the walls of external auditory canals, the auditory tubes, the epiglottis, and the cuneiform cartilage in the larynx
- In addition to the cellular and matrix components of hyaline cartilage → rich in elastic fibers → yellowish color
- Does not calcify



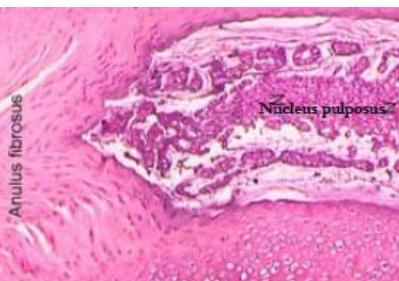
### Fibrocartilage

- Intermediate between dense connective tissue and hyaline cartilage: border areas between these two tissues are not clear-cut
- Chondrocytes, either singly or in isogenous groups
- Collagen type I: matrix is acidophilic
- No perichondrium
- Intervertebral disks, ligament attachment of the cartilaginous surface of bones, symphysis pubis

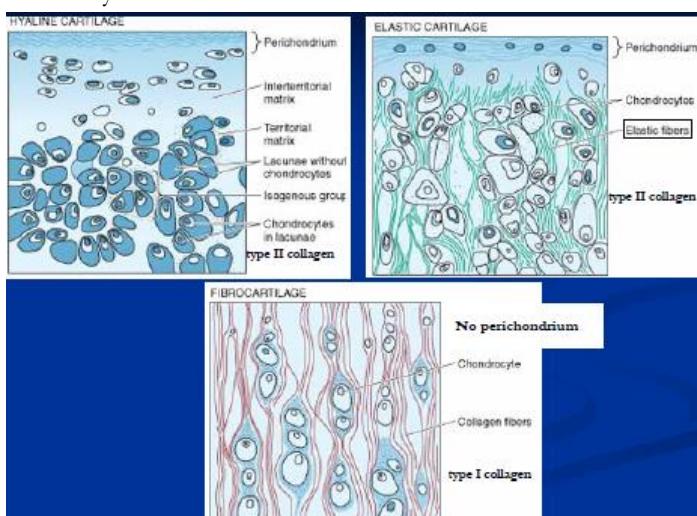


### Intervertebral disk

- **annulus fibrosus:** dense connective tissue + overlapping laminae of fibrocartilage (collagen type I)
- **nucleus pulposus:** a few rounded cells embedded in a viscous matrix rich in hyaluronic acid and type II collagen fibrils

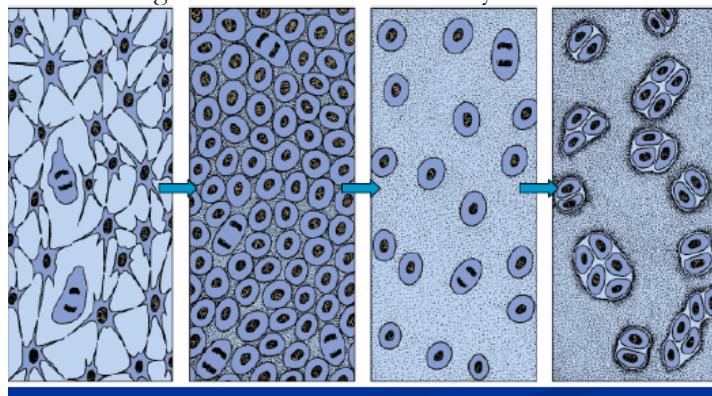


### Summary:

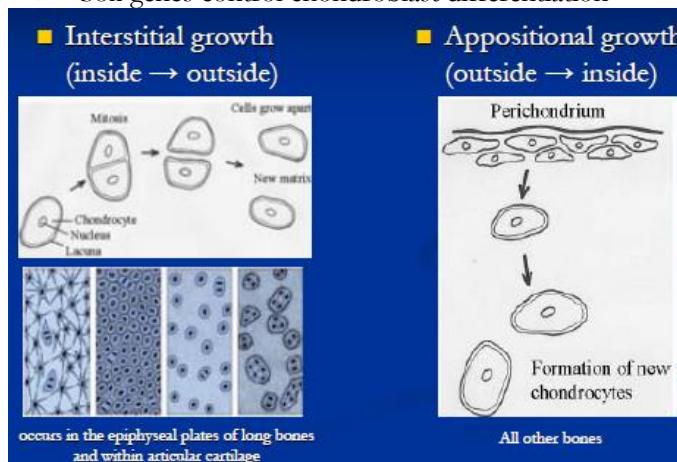


## Histogenesis of cartilage

- Cartilage derives from the mesenchyme



- Sox genes control chondroblast differentiation



## Poor regeneration

- Except in young children, damaged cartilage regenerates with difficulty and often incompletely
- Activity of perichondrium: invades the injured area and generates new cartilage
- In extensively damaged areas the perichondrium produces a scar of dense connective tissue instead of forming new cartilage

## Tumors of cartilage

- Benign: **chondroma**
- Malignant: **chondrosarcoma**

## Bone tissue

- Composed of intercellular calcified material, the **bone matrix**, and three cell types
  - Osteocytes** (osteon = bone, kytos = cell): found in cavities (**lacunae**) within the matrix
  - Osteoblasts** (blastos = germ): synthesize the organic components of the matrix
  - Osteoclasts** (klastos = broken): multinucleated giant cells involved in the resorption and remodeling of bone tissue
- Special features:**
  - Main constituent of the adult skeleton, supports fleshy structures, protects vital organs
  - Serves as reservoir of calcium, phosphate, and other ions (99% of body calcium is in the skeleton)
  - Harbors bone marrow, where blood cells are formed
  - Highly vascularized
  - Metabolically very active
  - Good regeneration capability

## Bone matrix

- Organic component** (34%):
  - Collagen type I (90%)
  - GAGs, proteoglycans
- Non-organic component**
  - Ca phosphate - 85%
  - Ca carbonate - 10%

- The mineral component enables bone to support the weight of the body without breaking

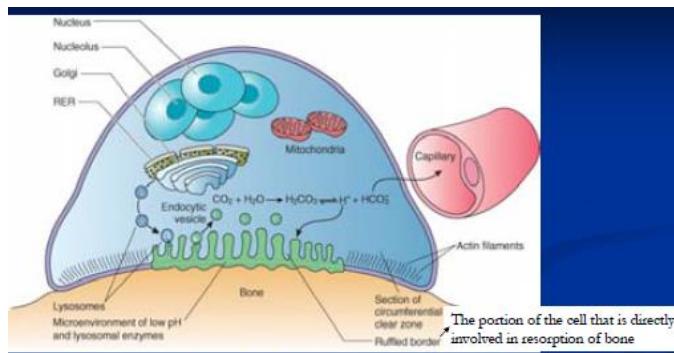
## Osteoblasts

- Derived from osteoprogenitor cells and develop under the influence of the **bone morphogenic protein (BMP)** family and **transforming growth factor- $\beta$**
- Responsible for the synthesis of the organic protein components of the bone matrix, including type I collagen, proteoglycans, and glycoproteins
- Produce **RANKL** (receptor for activation of nuclear factor kappa B), **osteocalcin** (for bone mineralization), **bone sialoprotein** (binding osteoblasts to extracellular matrix), and macrophage colony-stimulating factor (**M-CSF**)

## Osteocysts

- Are differentiated osteoblasts that have become trapped in the matrix they deposited.
- Reside in tiny cavities called **lacunae**, which are connected to each other by slender channels called **canaliculari**
- Each osteocyte has delicate cytoplasmic processes that reach into the canaliculari to meet the processes of neighboring osteocytes
- The processes of neighboring osteocytes are joined by gap junctions, which allow osteocytes to pass nutrients and chemical signals to each other and to transfer wastes to the nearest blood vessels for disposal

## Osteoclasts



Osteoclasts secrete lysosomal hydrolases and metalloproteinases, such as collagenase and gelatinase into the subosteoclastic compartment to degrade the organic components of the decalcified bone matrix.

## Types of bone tissue

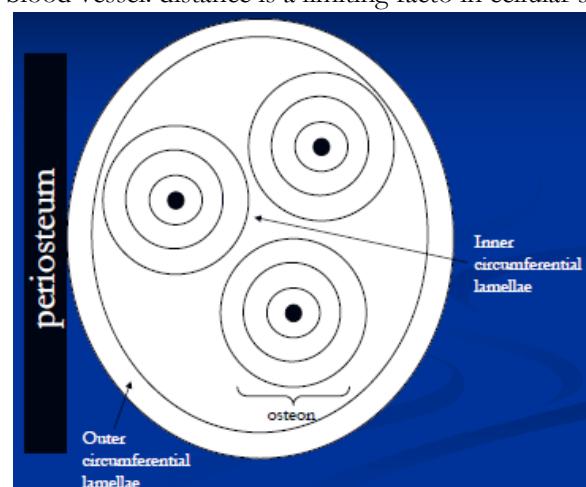
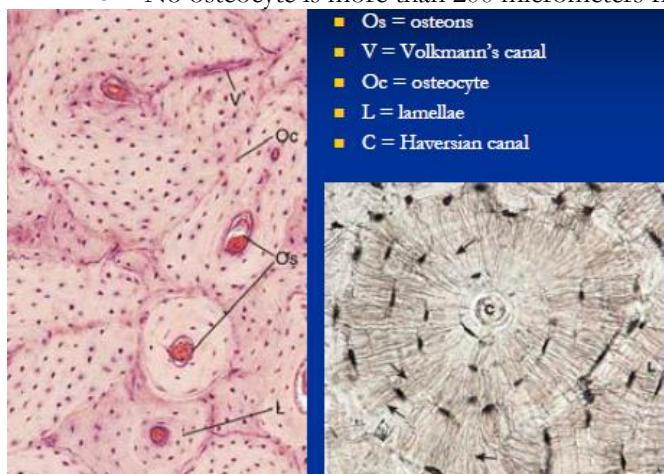
- Primary** (=woven) bone tissue (+ minerals / - cells); temporary; replaced by secondary bone tissue
- Osteoid:** bone matrix with less minerals (i.e. organic)
- Secondary** (lamellar) bone tissue: composed of **osteons** (complexes of concentric lamellae of bone surrounding a canal containing blood vessels, nerves, and loose connective tissue)

## Haversian system (osteon)

- Periosteum**
  - Outer fibrous layer (Sharpey's fibers)
  - Inner cellular layer (osteoprogenitors)
- Endosteum** (osteoprogenitors)

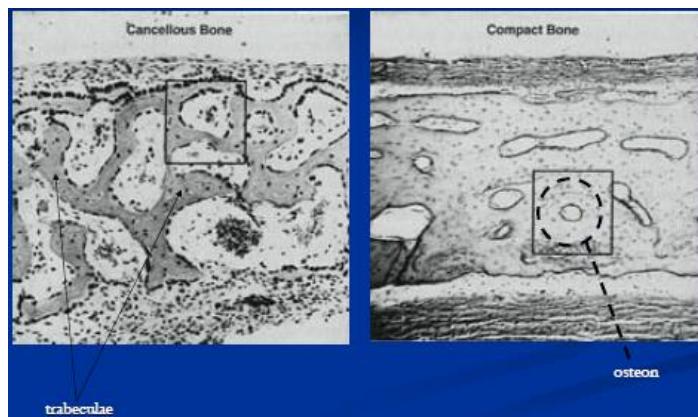
## Bone histology

- Osteons:**
  - 100 to 400 micrometer in diameter
  - Average 30 lamellae
  - About 21 million osteons in adult skeleton
  - Haversian canal mean diameter: 50 micrometer
  - No osteocyte is more than 200 micrometers from a blood vessel: distance is a limiting factor in cellular survival



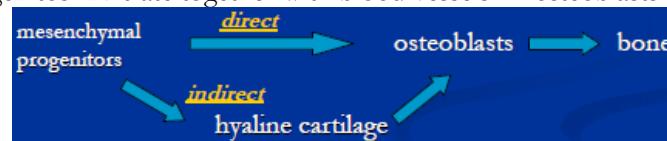
## Types of bone

- Dense areas without cavities: **compact bone**
- Areas with numerous interconnecting cavities: **cancellous (spongy) bone**
- At histological level, both compact bone and the trabeculae separating the cavities of cancellous bone have the same basic structure



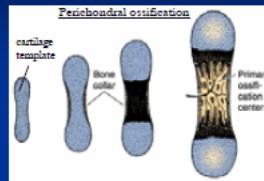
## Osteogenesis

- Osteocytes are derived from osteoblasts which are derived from osteoprogenitors (osteogenic cells)
- Osteoclasts are derived from bone marrow stem cells which are fused: osteoclasts have many nuclei
- Direct = desmal = intramembranous** ossification: Mesenchymal progenitors → osteoblasts → osteocytes (centers of ossification)
- Indirect (chondral)**: Mesenchymal progenitors → chondroblasts → hyaline cartilage → chondrocytes die → matrix is mineralized, osteoprogenitors infiltrate together with blood vessels → osteoblasts



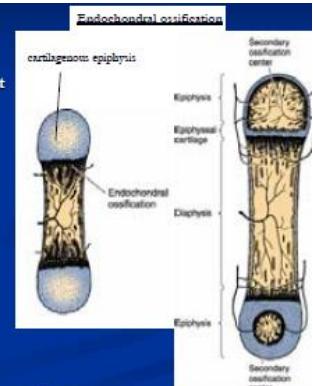
## Chondral ossification (perichondral or endochondral)

- The mesenchyme forms a piece of hyaline cartilage whose shape resembles a small version, or model (template), of the bone to be formed
- The perichondrium of the model ossifies (the chondrogenic cells become osteogenic) → bone collar
- chondrocytes within the core of the cartilage model → the cavity is invaded by blood vessels and osteoprogenitor cells
- The cartilage matrix is mineralized, the osteoprogenitor cells produce the cellular components of the bone → spongy bone



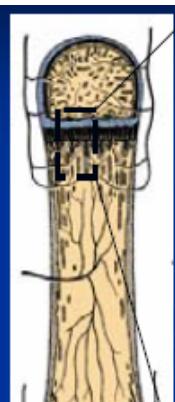
Primary (diaphyseal) ossification center

- At the extremity of the template (=epiphysis) cartilage remains in:
  - articular cartilage, which persists throughout adult life and does not contribute to bone growth in length
  - epiphyseal cartilage, also called the epiphyseal plate (metaphysis), which connects the two epiphyses to the diaphysis
- Ossification occurs similarly to primary centers but without bone collar
- Mostly occurs postnatally



Secondary (epiphyseal) ossification center

- The closure of the epiphyses follows a chronological order according to each bone and is complete at about 20 years of age
- Once the epiphyses have closed, growth in length of bones becomes impossible
- Grow on one side and become replaced by bone on the other side → become progressively separated → diaphyses grow
- At the end of bone growth, cartilage of epiphyseal plate ceases proliferation; bone development continues to unite the diaphysis and epiphysis



## Bone remodeling

- Osteoclasts excavate a tunnel through the old bone: rate approx. 50 micrometers per day
- Osteoblasts enter the tunnel behind them, line its walls, and begin to form new bone, rate of 1-2 micrometers per day
- A capillary sprouts down the center of the tunnel
- Tunnel becomes filled with concentric layers of new bone, with only a narrow central canal remaining
- About 5-10% of the bone in a healthy adult mammal is replaced in this way each year

**Bone remodeling** – the combination of bone synthesis and removal; occurs not only in growing bones but also throughout adult life (fracture repair)

- Fracture → bone matrix destroyed, bone cells adjoining the fracture die
- The blood clot is invaded by small capillaries and fibroblasts from the surrounding connective tissue, forming granulation tissue
- Osteoprogenitors of periosteum and endosteum proliferate → form primary bone (bone callus)
- The primary bone tissue of the callus is gradually resorbed and replaced by secondary tissue

## Osteoporosis

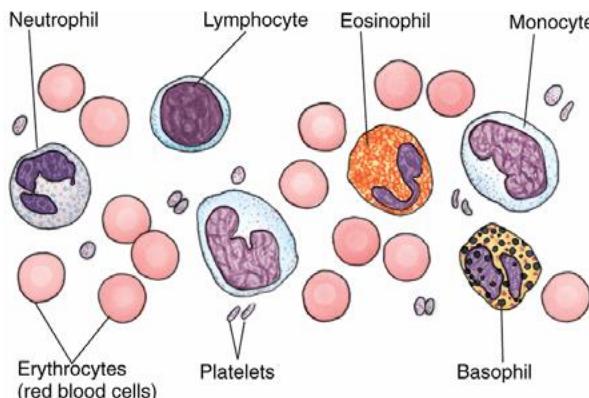
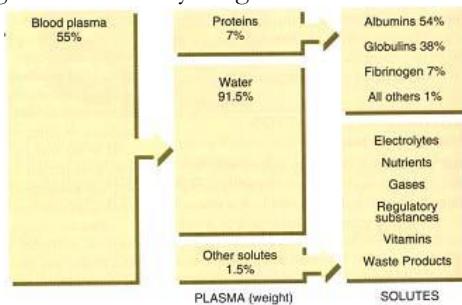
- Loss of matrix and minerals, cells are normal
- Affects spongy bone in particular, since this is the most metabolically active type

## Tumors of bone

- Benign: **osteoma**
- Malignant: **osteosarcoma**

## Blood

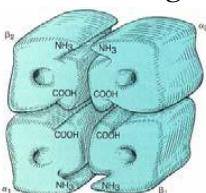
- If blood is removed from the circulatory system, it will clot. This clot contains **formed elements** and a clear yellow liquid called **serum**, which separates from the coagulum.
- The **hematocrit** (volume of erythrocytes per unit volume of blood): 40-50% in men and 35-45% in women
- Humans contain about 5 liters of blood, accounting for 7% of body weight
- Cells of the peripheral blood**
  - Neutrophil
  - Lymphocyte
  - Eosinophil
  - Monocyte
  - Basophil
  - Platelets
  - Erythrocytes



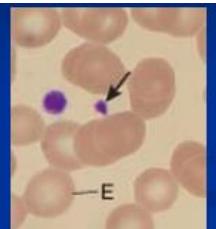
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## Erythrocytes (red blood cells, RBC)

- Erythrocytes are **biconcave disks** without nuclei
- Types:
  - Macrocyte**: diameter greater than 9 micrometers
  - Microcytes**: diameter less than 6 micrometers
  - Anisocytosis** (aniso = uneven): high percentage of erythrocytes with great variations in size
- Human erythrocytes survive for about 120 days. Worn-out erythrocytes are removed mainly by macrophages of the spleen and bone marrow
- Hemoglobin** molecule:



- A large protein composed of four polypeptide chains, each of which is covalently bound to a **heme group**
- Fetal hemoglobin (HbF)** is composed of two  $\alpha$ - and two  $\gamma$ -chains ( $\alpha_2\gamma_2$ )
- 96% of **adult hemoglobin** is HbA1 ( $\alpha_2\beta_2$ )



- E = erythrocyte (RBC)
- Arrow = platelet

- The RBC got a **spectrin-based cytoskeleton**

### Clinical correlates of the RBC cytoskeleton

- Glycophorin A – entry of Influenza & Hepatitis virus, P. falciparum
- Band 4.1 – MN blood groups
- Band 3 } Ankyrin } Hereditary spherocytosis (HS)  
Spectrin Hereditary elliptocytosis (HE)
- Band 3 <- Southeast Asian ovalocytosis (SAO)  
Distal renal tubular acidosis (dRTA)

- AB0 blood group system:**

- The extracellular surface of the red blood cell plasmalemma has specific inherited carbohydrate chains that act as antigens and determine the blood group of an individual for the purposes of blood transfusion
- The most notable of these are the **A** and **B antigens**, which determine the four primary blood groups, **A, B, AB, and O**
- Other: Rhesus, MNS, Lutheran, Kell, Lewis, Duffy, Kidd, Diego, Cartwright, Colton, Sid, Scianna, Yt, Auberger, Ii, Xg, Indian and Dombrock system

## Granulocytes

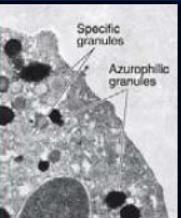
- Contain two types of granules:
  - Specific** granules: specific functions
  - Azurophilic** granules: lysosomes
- Nuclei with two or more lobes
- Nondividing terminal cells with a life span of a few days
- Die by apoptosis in the connective tissue
- Few mitochondria (low energy metabolism); depend mostly on glycolysis

### Neutrophilic granulocyte

- 12-15 micrometers in diameter
- 2-5 lobes linked by fine threads of chromatin
- Short-lived cells with a half-life of 6-7 h in blood and life span of 1-4 days in connective tissue

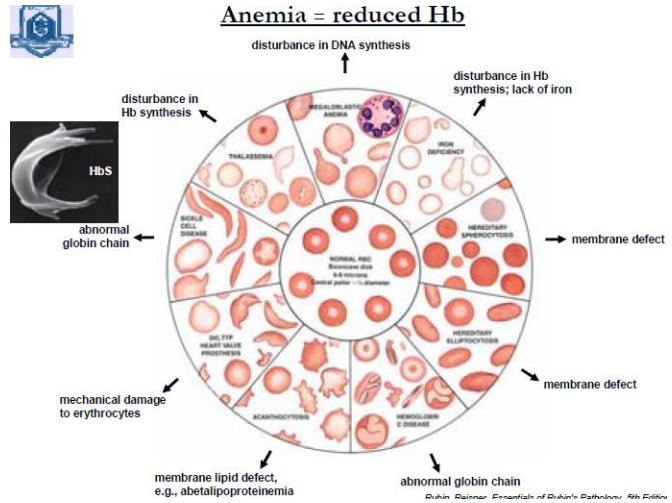
Golgi complex, rough endoplasmic reticulum and mitochondria are not abundant: cell is in the terminal stage of its differentiation

| Specific Granules                                 | Azurophilic Granules            |
|---------------------------------------------------|---------------------------------|
| Alkaline phosphatase                              | Acid phosphatase                |
| Collagenase                                       | -Mannosidase                    |
| Lactoferrin                                       | Arylsulfatase                   |
| Lysozyme                                          | -Galactosidase                  |
| Several nonenzymatic antibacterial basic proteins | -Glucuronidase                  |
|                                                   | Cathepsin                       |
|                                                   | 5'-Nucleotidase                 |
|                                                   | Elastase                        |
|                                                   | Collagenase                     |
|                                                   | Myeloperoxidase                 |
|                                                   | Lysozyme                        |
|                                                   | Cationic antibacterial proteins |

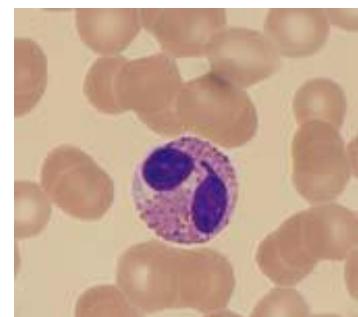
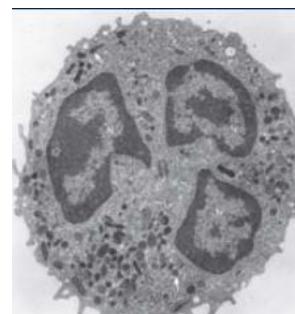
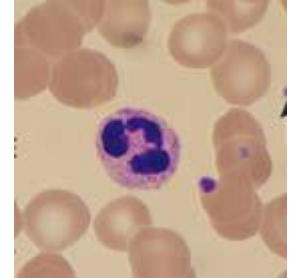


### Eosinophilic granulocyte

- 12-15 micrometers in diameter
- 2 lobes
- Large specific granules stained by eosin
- Found in the connective tissues underlying epithelia
- Participate in anti-parasitic immunity



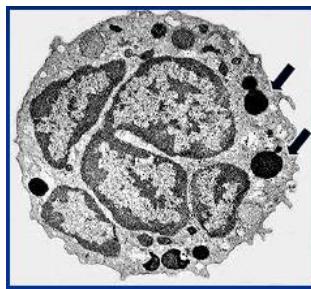
|                            | Group A   | Group B   | Group AB         | Group O |
|----------------------------|-----------|-----------|------------------|---------|
| Red blood cell type        |           |           |                  |         |
| Antibodies in Plasma       |           |           | None             |         |
| Antigens in Red Blood Cell | A antigen | B antigen | A and B antigens | None    |



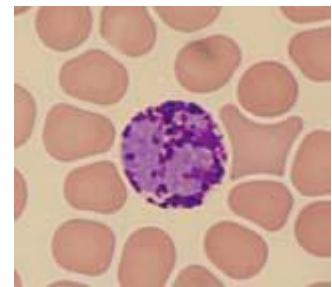
The specific granules have a crystalline core (internum) & outer extremum, or matrix. The internum contains a protein called the **major basic protein** to which the eosinophilia accounts.

## Basophilic granulocyte

- 12-15 micrometers in diameter
- Metachromatic specific granules (heparin, histamine)
- Migrate in the connective tissues, similarly to mast cells

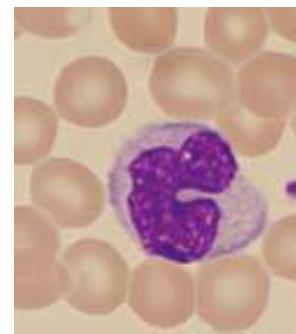
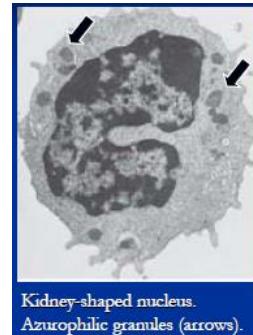
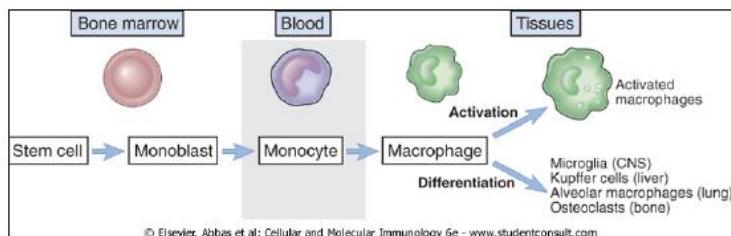


The lobulated nucleus appears as several separated portions. Note the basophilic granule (arrows).

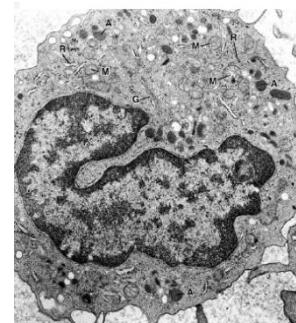


## Monocyte

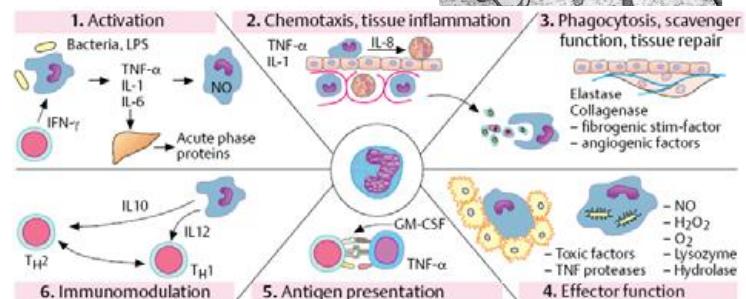
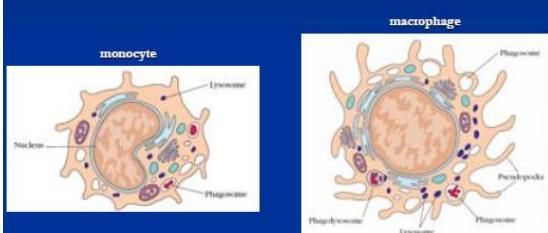
- 12-20 micrometers in diameter (larger than granulocytes)
- Very fine azurophilic granules: lysosomes
- Migrate in the connective tissues to become tissue macrophages
- Precursor cells of the mononuclear phagocytic system



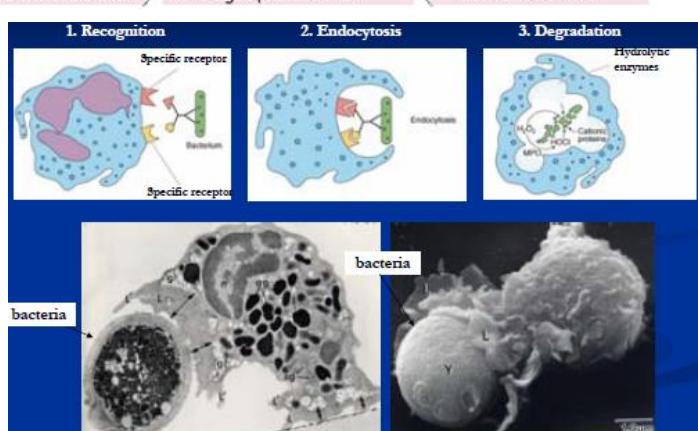
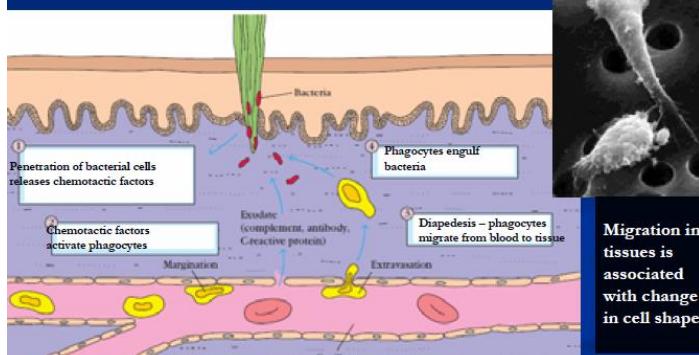
| Cell Type               | Location                                               | Main Function                                               |
|-------------------------|--------------------------------------------------------|-------------------------------------------------------------|
| Monocyte                | Blood                                                  | Precursor of macrophages                                    |
| Macrophage              | Connective tissue, lymphoid organs, lungs, bone marrow | Inflammation (defense), antigen processing and presentation |
| Kupffer cell            | Liver                                                  | Same as macrophages                                         |
| Microglia cell          | Nerve tissue of the central nervous system             | Same as macrophages                                         |
| Langerhans cell         | Skin                                                   | Antigen processing and presentation                         |
| Dendritic cell          | Lymph nodes                                            | Antigen processing and presentation                         |
| Osteoclast              | Bone (fusion of several macrophages)                   | Digestion of bone                                           |
| Multinuclear giant cell | Connective tissue (fusion of several macrophages)      | Segregation and digestion of foreign bodies                 |



## Phagocytes → granulocytes & monocytes

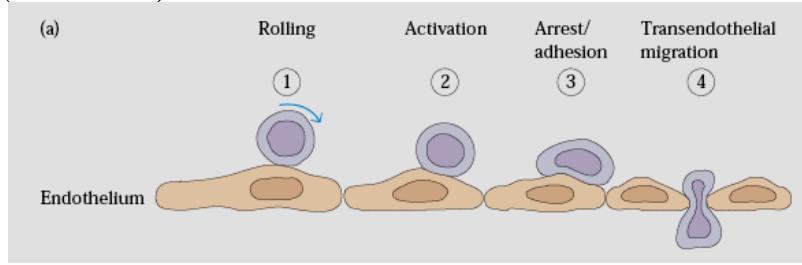


## Phagocytes participate in the anti-bacterial immunity



- Leukocyte Extravasation:
  - Rolling (tethering)
    - Reversible binding of leukocytes to endothelial cells
    - Mediated by selectins and glycosylated ligands
  - Activation:
    - Stimulation of the leukocyte by chemokines
    - Result: increase in integrin  $\alpha 4\beta 1$  (VLA-4) activity
    - Binding of integrin to its endothelial partner (VCAM-1)

- **Adherence** (tight adhesion)
  - Irreversible adhesion mediated by integrins & Ig family)
  - $\alpha\text{L}\beta\text{2}$  (LFA-1) on leukocyte with ICAM-1 on endothelial cell
- **Diapedesis** (extravasation)



- **Leukocyte Adhesion Deficiency (LAD) Syndromes:**

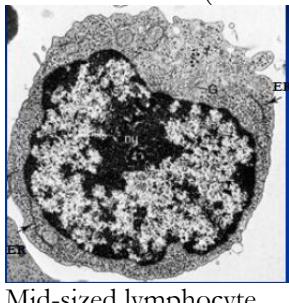
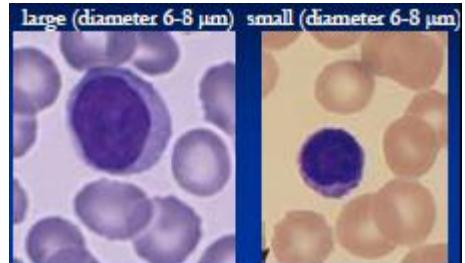
- LAD I syndrome:  $\beta 2$  integrin deficiency
  - Leukocyte adherence deficiency
  - Recurrent severe infections
  - In worst cases patients die by age of 10
- LAD II syndrome: selectin deficiency
  - Leukocyte rolling deficiency
  - Infections rare, developmental abnormalities (growth retardation, neurologic deficits)

### Chemokines are chemotactic cytokines

- Small (8-10 kDa) proteins, over 40 known to date
- Families
  - CXC – the first 2 cysteins are separated by 1 amino acid
  - CC – the first 2 cysteins are separated by 0 amino acids
  - CXXXC – the first 2 cysteins are separated by 3 AAs
  - C – has only 2 cysteins
- Examples
  - CXC – IL-8
  - CC – MIP, MCP
  - CXXXC – fractalkine
  - C – lymphotactin

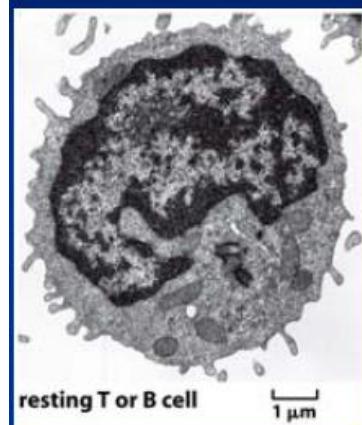
## Lymphocyte

- Larger lymphocytes: cells activated by specific antigens
- Scant cytoplasm, a few azurophilic granules
- Only type of leukocytes that return from the tissues back to the blood
- Variable life span
- 80% of the circulating lymphocytes are T cells, 15% are B cells, and the remainder are null cells (NK or circulating stem cells)



Mid-sized lymphocyte

### Resting & activated lymphocytes



resting T or B cell

little RER

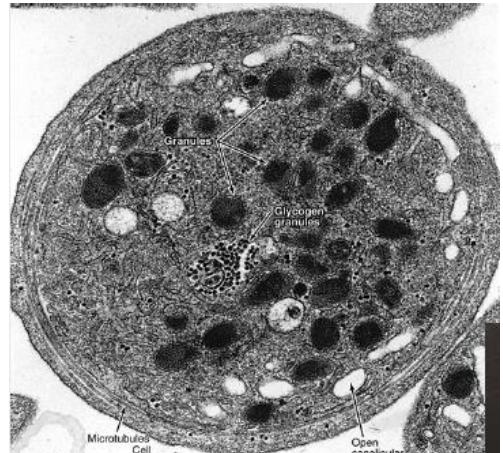
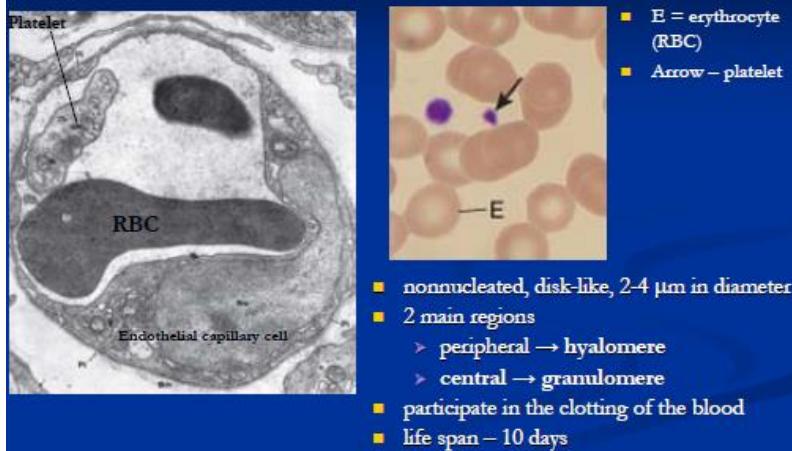


effector B cell (plasma cell)

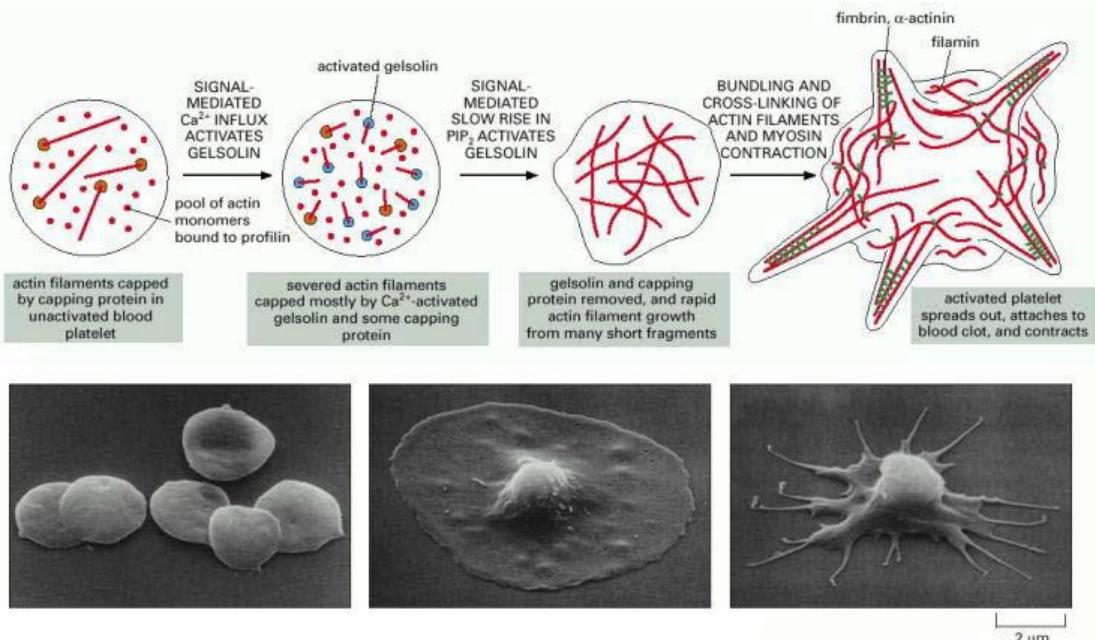
rich RER

→

## Thrombocyte (platelet)



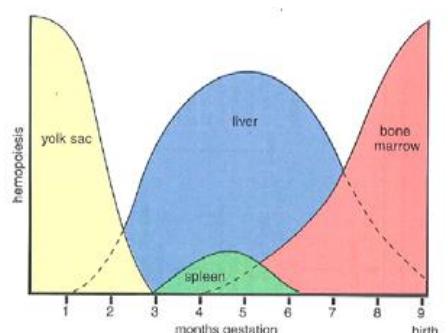
- Platelets & blood clottin

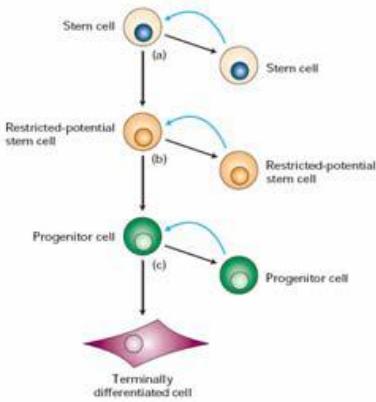
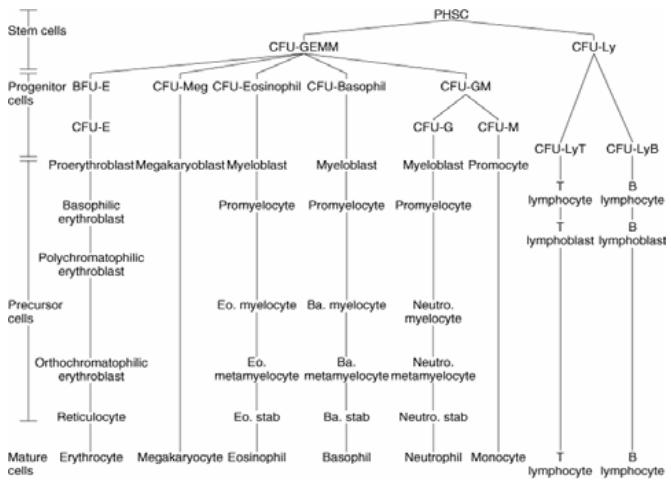


## Hemopoiesis

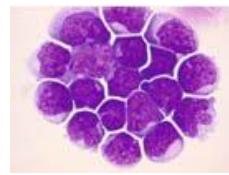
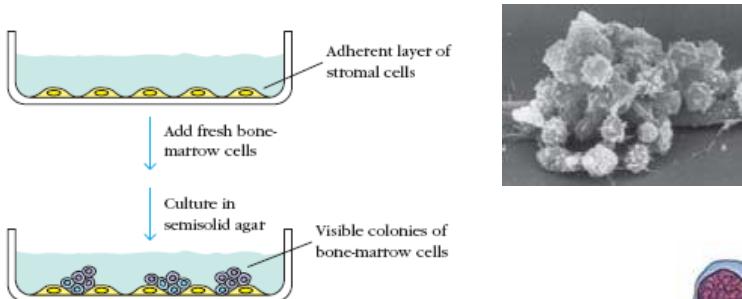
### Hemopoiesis

- **Hemopoiesis** (gr. *Haima*, blood + *poiesis*, making)
- **Prenatal hemopoiesis:**
  - Mesoblastic phase (2 wk after conception): **blood islands** in the mesoderm of the yolk sac, only RBC
  - Hepatic phase (6th week of gestation): leukocytes appear by the 8th week
  - Sponic phase (2nd trimester)
  - Myeloid phase (end of 2nd trimester): bone marrow
- **Postnatal hemopoiesis:** occurs almost exclusively in bone marrow, but the liver and he spleen can revert to forming new blood cells if the need arises
- Stemm cell population in the bone marrow:
  - **Pluripotential hemopoietic stem cells (PHSCs)**
    - About 0.1 % of the nucleated cells in bone marrow
    - Can produce themselves over multipotential hemopoietic stem cells (MHSCs)
  - **Multipotential hemopoietic stem cells (MHSCs):**
    - Responsible for the formation of various progenitor cells
    - CFU-GEMM cells: colony forming unit for granulocyte, erythrocyte, monocyte, megakaryocyte
    - CFU-Ly cells: colony forming unit for lymphocytes
- PHSCs & MHSCs are morphologically indistinguishable, resemble lymphocytes and constitute a small fraction of the null-cell population of circulating blood

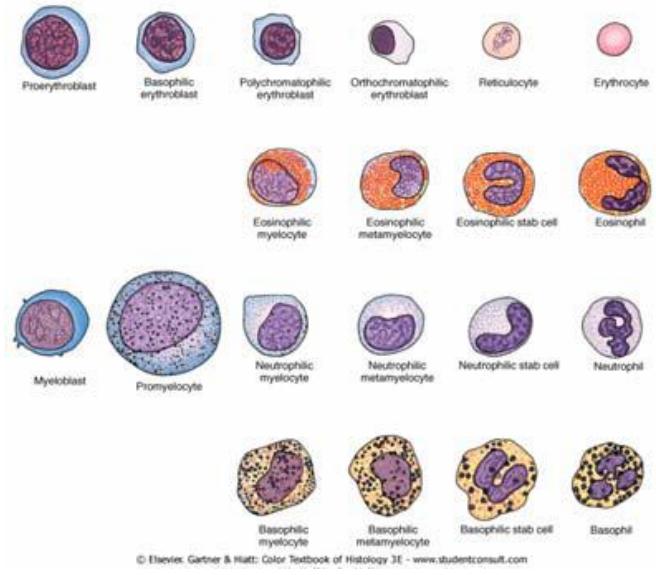




- Stem cells give rise to stem cells and more differentiated cells:
- Researchers studying hemopoiesis have isolated individual lymphocyte-like cells that, under proper conditions, occasionally give rise to groups (**colonies**) of cells composed of granulocytes, erythrocytes, monocytes, lymphocytes, and platelets. Such cells are called **colony forming units (CFUs)**



- **Progenitor cell populations** in the bone marrow
  - Morphologically indistinguishable from stem cells, resemble lymphocytes like stem cells
  - Can be differentiated only by CD expression
  - **Unipotential**: committed to forming a single cell line, responsible for the formation of various progenitor cells
    - CFU-E cells: erythroid lineage
    - CFU-Meg cells: platelet lineage
    - CFU-Eo cells: eosinophil lineage
    - CFU-B cells: basophil lineage
    - CFU-G cells: neutrophil & monocyte lineages
    - CFU-LyT cells: T lymphocytic lineage
    - CFU-LyB cells: B lymphocytic lineage
  - Limited capacity for self-renewal: depend on stem cells for renewal
- **Hemopoietic Growth Factors** (colony-stimulating factors):
  - **Glycoproteins** acting on specific stem cells, progenitor cells, and precursor cells, generally inducing rapid mitosis, differentiation, or both
  - Routes to deliver growth factors to their target cells:
    - Transport via bloodstream (as endocrine hormones)
    - Secretion by stromal cells of the bone marrow near the hemopoietic cells (as paracrine hormones)
    - Direct cell-to-cell contact (as surface signaling molecules)
- **Precursor cell populations** in the bone marrow:
  - Arise from progenitor cells
  - Have **specific morphological characteristics**: permit them to be recognized as the first cell of a particular cell line
    - [cell name]-blast: e.g. erythroblast
    - Pro-[cell name]-cyte
    - [cell name]-cyte
    - Meta-[cell name]-cyte
  - **Incapable of self renewal**

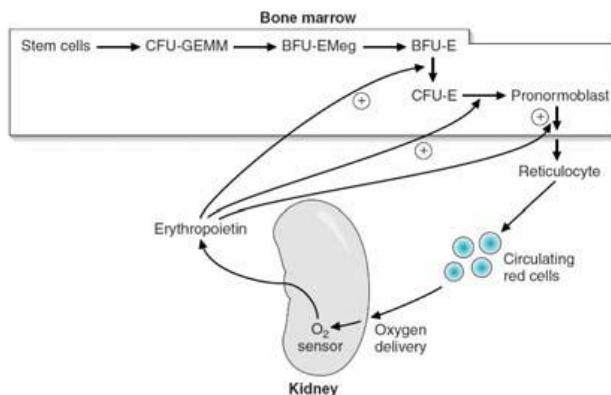
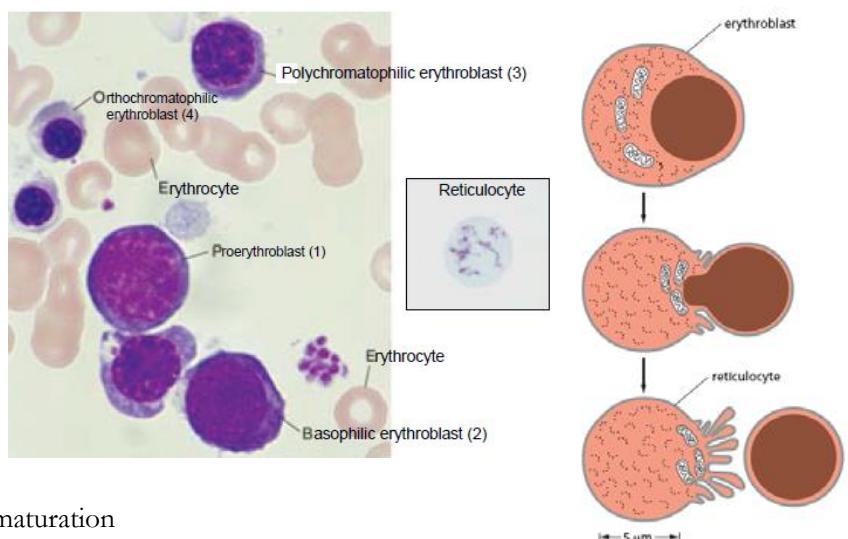


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- Undergo cell division and **differentiation**: give rise to a clone of mature cells
- Succeeding cells become smaller, their nucleoli disappear, their chromatin network becomes denser, and the morphological characteristics of their cytoplasm approximate those of the mature cells

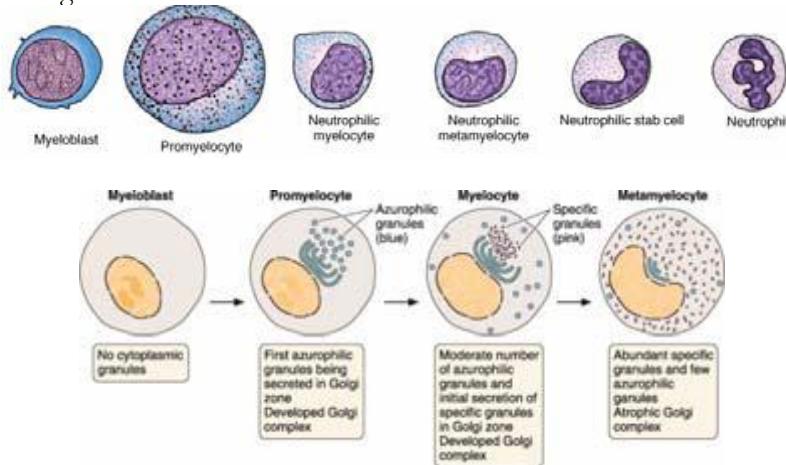
## Development of Erythrocytes

- **Erythrocyte maturation:**
  - Cell volume decreases
  - Nucleoli diminish in size
  - Nuclear diameter decreases
  - Chromatin becomes more dense  
→ pyknotic → extruded from the cell
  - Mitochondria and other organelles gradually disappear
- A developing red blood cell (**erythroblast**) extrudes its nucleus to become an immature erythrocyte (a **reticulocyte**) which then leaves the bone marrow and passes into the bloodstream
- **Erythropoietin** stimulates erythrocyte maturation

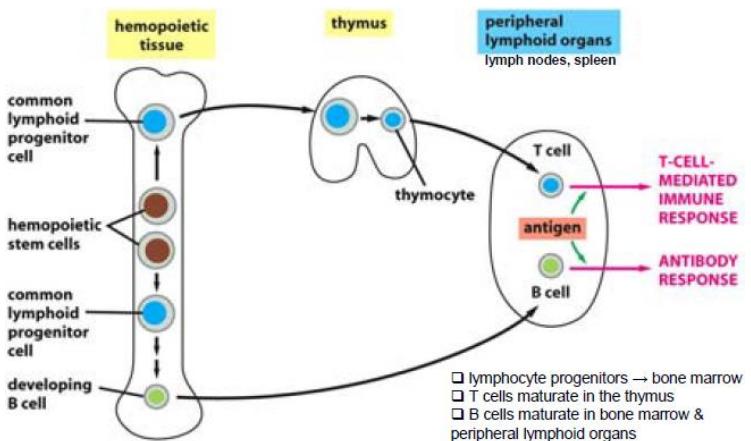


## Development of Neutrophils

- **Neutrophilic lineages**
  - **Myeloblast:** most immature recognizable cell
  - **Myelocyte:** first sign of differentiation



- Each day, the average adult produces approx. 800,000 neutrophils, 170,000 eosinophils, and 60,000 basophils
- **Lymphocyte development:**

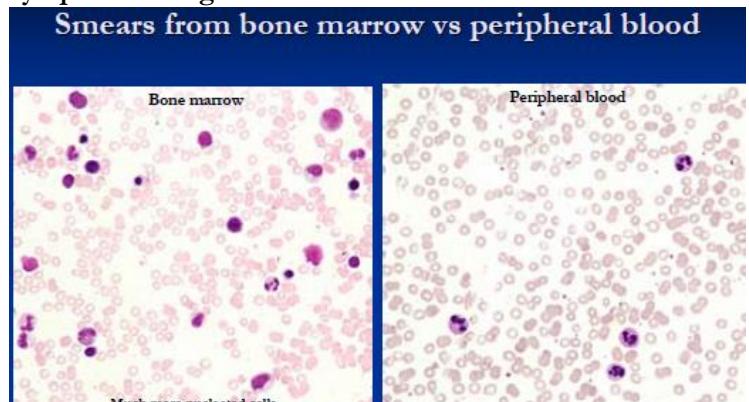
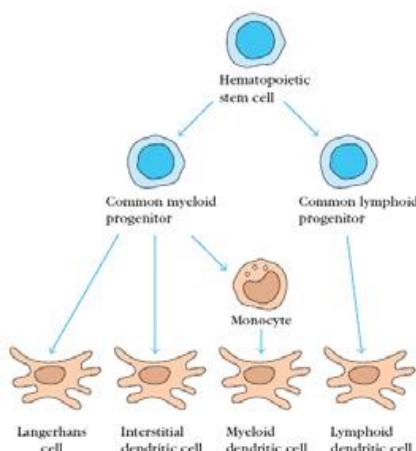


- Lymphocytes can be distinguished only by differential surface marker expression

|        | Fetal liver<br>Bone marrow    | Thymus                                                     |                                                        |                                          |
|--------|-------------------------------|------------------------------------------------------------|--------------------------------------------------------|------------------------------------------|
| Cell   | Pre-thymocytes                | Early thymocyte                                            | General thymocyte                                      | Mature thymocyte                         |
| Marker | TdT enzyme                    | TdT, CD2, CD7 (CD1)                                        | CD1, CD2, CD3, CD5, CD7                                | CD2, CD4, CD5, CD7, CD8, TCR             |
| TCR    | Rearrangement of TCR $\gamma$ | Transcription of TCR $\gamma$ rearrangement of TCR $\beta$ | Cell surface expression of TCR $\gamma, \alpha, \beta$ | Mainly expression of TCR $\alpha, \beta$ |

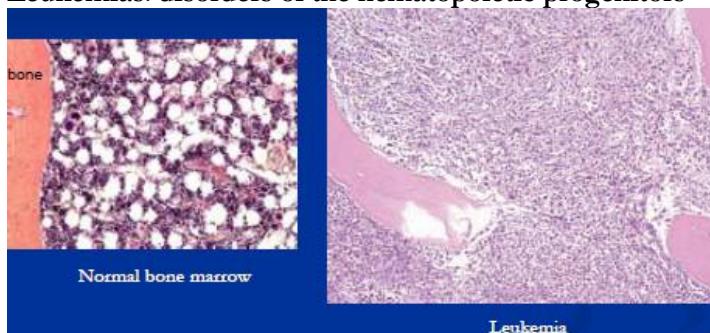
## Development of Dendritic cells

- Dendritic cells arise from both the myeloid and lymphoid lineages:

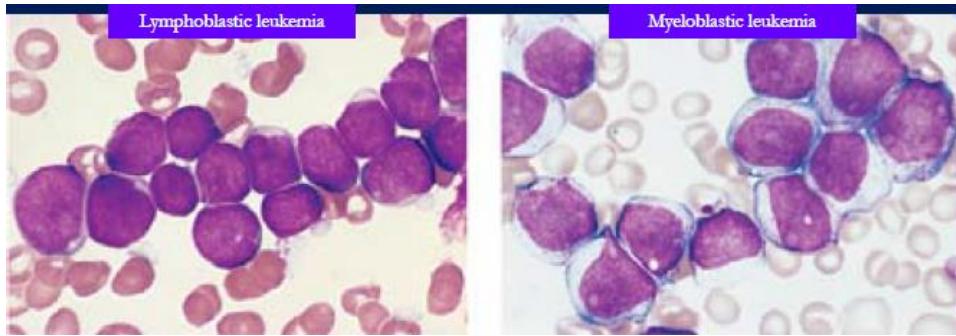


## Leukemias

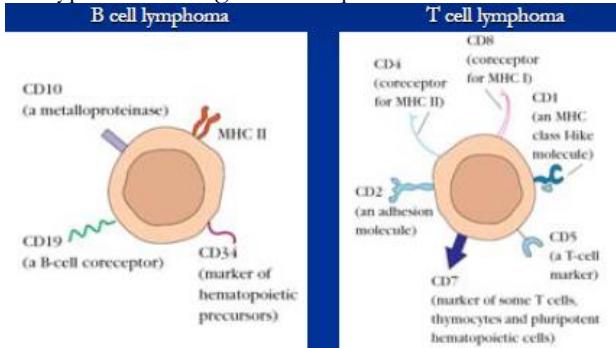
- Leukemias: disorders of the hematopoietic progenitors



- Acute myeloblastic leukemia** results from uncontrolled mitosis of a transformed stem cell whose progeny do not differentiate into mature cells. The cells involved may be the CFU-GM, CFU-Eo, or CFU-Ba, whose differentiation stops at the myeloblast stage



- Leukemia & lymphoma can be typed according to CD expression

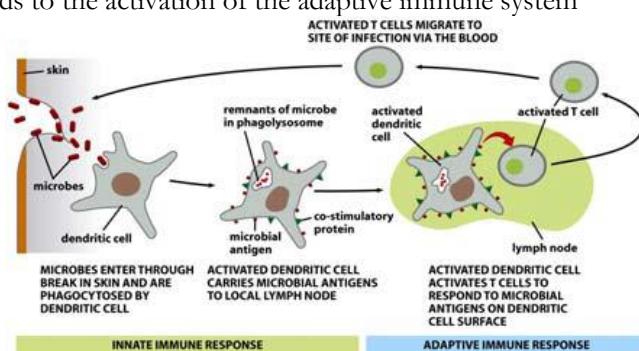
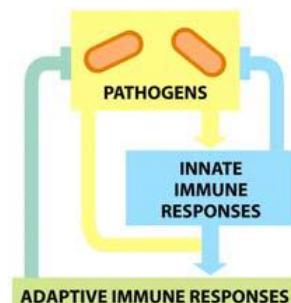
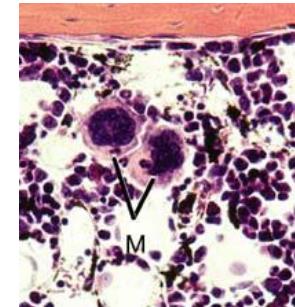


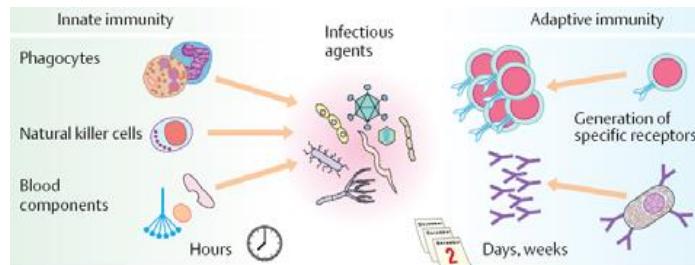
## Development of platelets

- Megakaryoblasts:**
  - 1 nucleus with many lobes & numerous nucleoli
  - Undergo **endomitosis**: up to 64 N (polyploid)
- Megakaryocytes:**
  - Giant cells (35-150  $\mu\text{m}$ )
  - Irregularly lobulated nucleus, coarse chromatin, and no visible nucleoli
  - Numerous invaginations of the plasma membrane: areas that sehd platelets

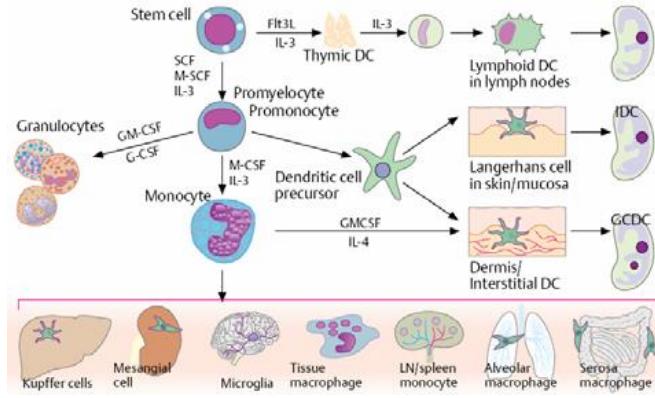
## The immune system

- Innate immunity**
  - Activated immediately after an infection begins
  - Do not depend on the host's prior exposure to the pathogen
  - Present in all multicellular organisms
  - Components: complement, antimicrobial peptides, macrophages, neutrophils, NK cells, Toll-like receptors (TLRs)
- Adaptive immunity**
  - Operat later than the innate response
  - Highly specific for the pathogen
  - Present only in vertebrates
  - Components: T cells, B cells, antigen-presenting cells (APCs), immunoglobulins, cell-mediated immunity components
- Innate immune responses: Protective barriers, toxic molecules and phagocytic cells that ingest and destroy invading microorganisms (microbes) and larger parasites (such as worms)
- Adaptive immune responses: lymphocytes
- Innate immune system leads to the activation of the adaptive immune system





- **Phagocyte system:**

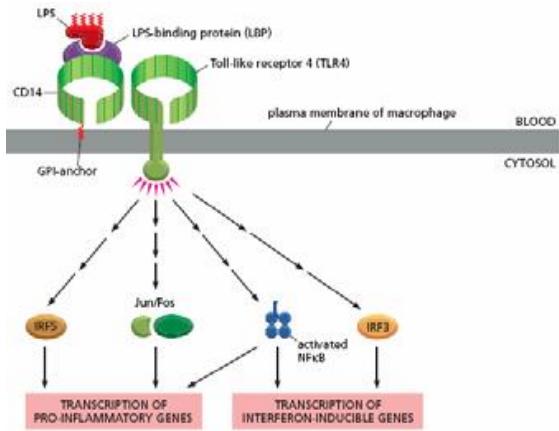


- **Toll-like receptors (TLRs):**

- Highly conserved integral proteins present in the membranes on cells of the innate immune system
- At least 12 in humans
- All TLRs (with the exception of TLR3) associate with and activate the nuclear factor NF- $\kappa$ B pathway
- Lead to release of cytokines and T/B cell activation

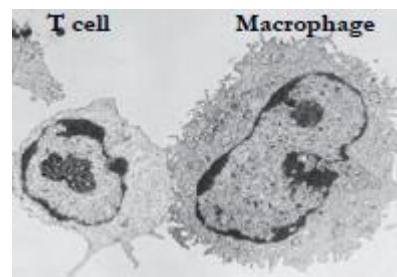
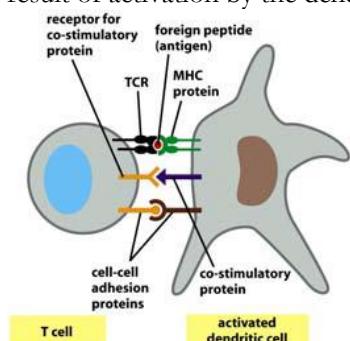
- Features of adaptive immunity:

- Antigenic specificity
- Diversity
- Immunologic memory
- Self/nonself recognition

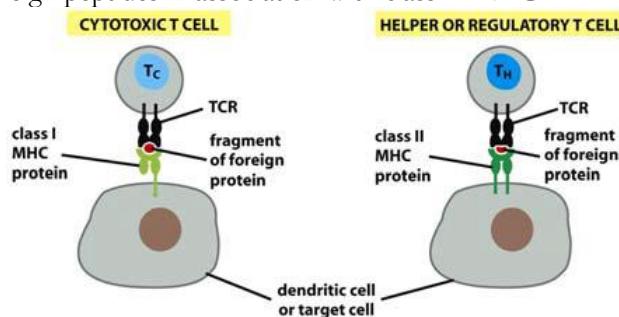


## T cell activation

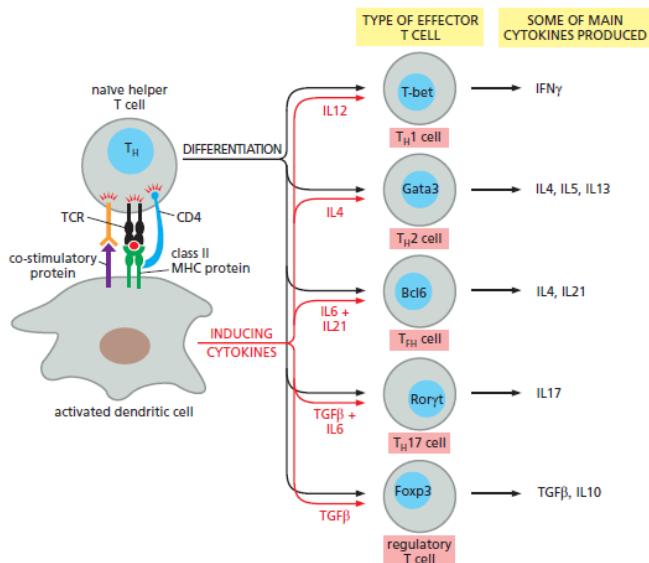
- **T cell activation** by dendritic cell:  $\text{Ca}^{++}$  increases as a result of activation by the dendritic cell.



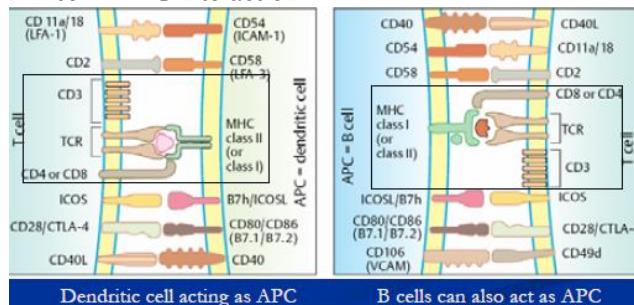
- Cytotoxic T cells recognize foreign peptides in association with class I MHC proteins, whereas helper T cells and regulatory T cells recognize foreign peptides in association with class II MHC



- **$T_{H1}$  vs.  $T_{H2}$  T-helpers:** Differentiation of naive helper T cells into either  $T_{H1}$  or  $T_{H2}$  effector helper cells in a peripheral lymphoid organ

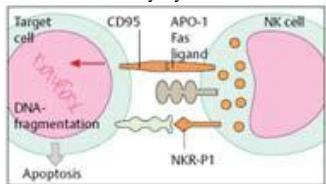


- Many molecules are involved in T cell - APC interaction:

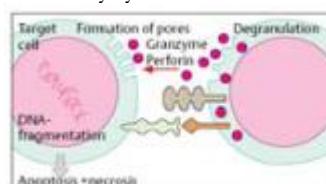


- Cell mediated cytotoxicity:

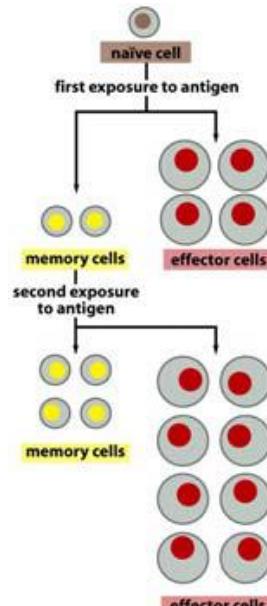
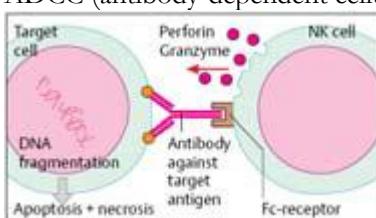
- Nonsecretory lysis



- Secretory lysis



- ADCC (antibody-dependent cellular cytotoxicity)



- Immunological memory:

- When stimulated by their specific antigen, naive cells proliferate and differentiate. Most become effector cells, which function and then usually die, while others become memory cells.
- During a subsequent exposure to the same antigen, the memory cells respond more readily, rapidly, and efficiently than the naive cells did: they proliferate and give rise to effector cells and to more memory cells.

## Muscle tissue

### Muscle

- Special type of cells, which are specialized for contraction, that permits animals to move
- Organisms harness the contraction of muscle cells and the arrangement of the extracellular matrix of muscle to permit locomotion, constriction, pumping and other propulsive movements
- General functions:

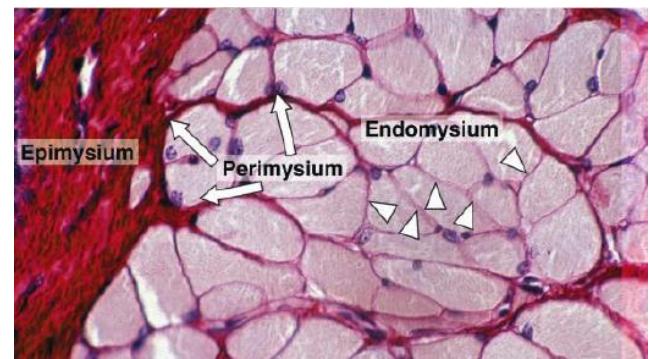
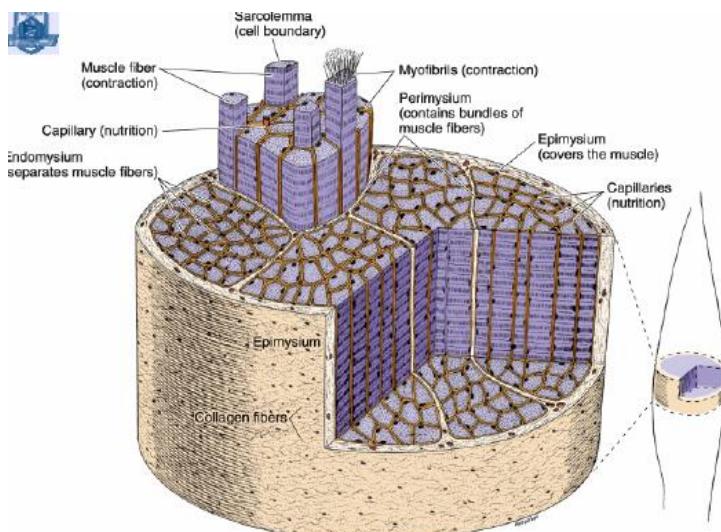
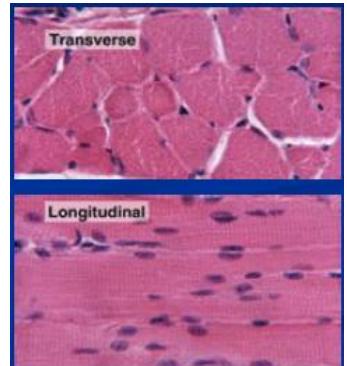
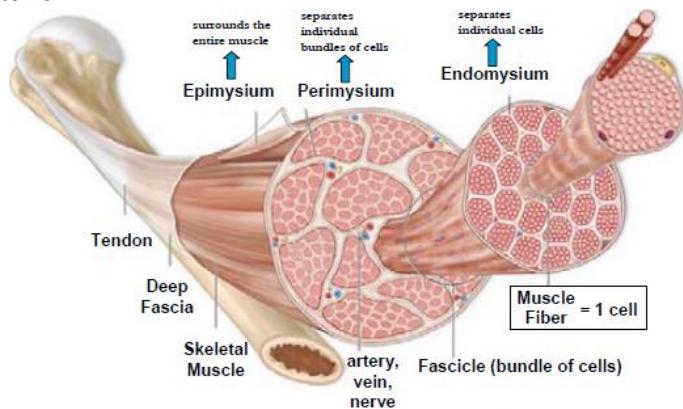
- Movement
- Maintenance of posture
- Joint stabilization
- Heat generation
- **Special functional characteristics:**
  - **Excitability:** chemical signal leads to electrical changes
  - **Conductivity:** Stimulation of muscle fiber triggers a wave of excitation that travels rapidly along the muscle fiber
  - **Contractility:** Shortening, which generate a pulling force
  - **Elasticity:** Recoils passively after being stretched
  - **Extensibility:** Stretch with contraction of an opposing muscle

## Muscle types

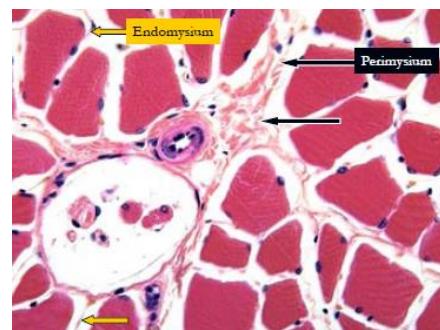
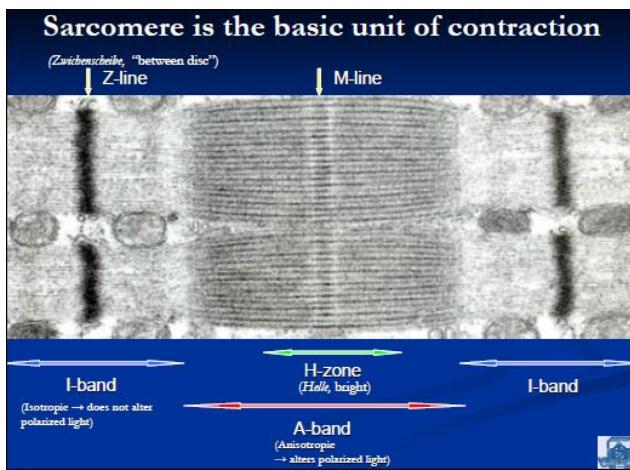
- Muscle tissue: **Myocyte**
- **Smooth:**
  - 1 nucleus
  - Central nucleus
- **Striated**
  - **Skeletal**
    - Many nuclei
    - Peripheral nucleus
  - **Cardiac**
    - 1-2 nuclei
    - Central nucleus
- Some muscle cell organelles have specific names:
  - **Sarcoplasm:** cytoplasm (excluding the myofibrils)
  - **Sarcoplasmic reticulum:** smooth endoplasmic reticulum
  - **Sarcolemma:** cell membrane, plasmalemma
- Similarities:
  - Cells are called **fibers** because they are elongated
  - Contraction depends on **myofilaments:**
    - Actin
    - Myosin
    - Other proteins

## Skeletal muscle

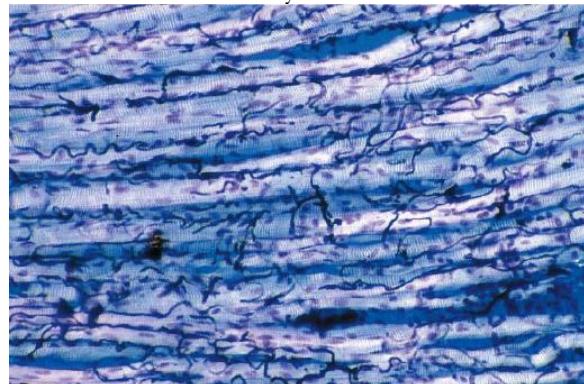
- Attach and move skeleton
- 40% of body weight
- Cells with obvious striations
- Contractions are voluntary
- The connective tissue sheaths of skeletal muscle



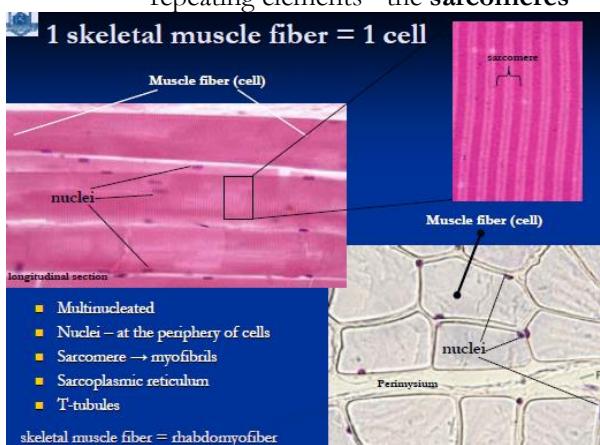
- **Epimysium:** type I collagen
- **Perimysium:** types I & type III collagen
- **Endomysium:** types III & IV collagen



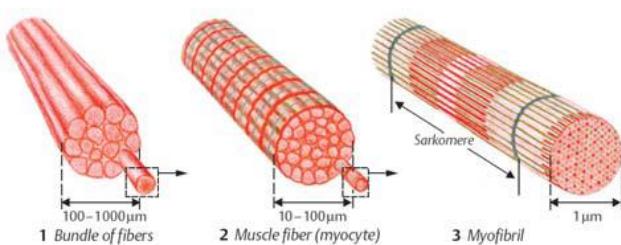
- Skeletal muscle is richly vascularized



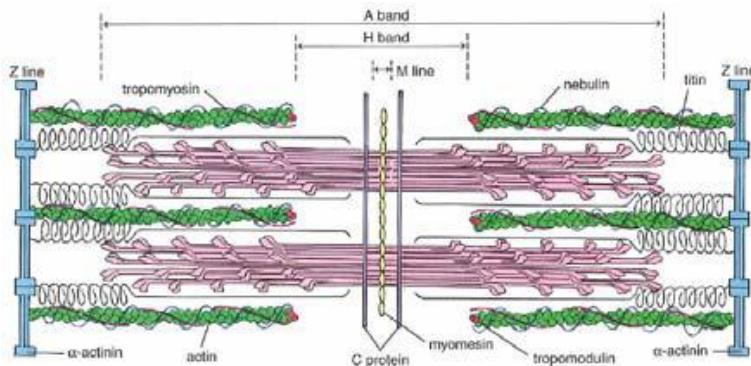
- Cellular units of skeletal muscle are the **muscle fibers / fibres**:
  - Consistent in size within a given muscle, but different in different muscles
    - Diameter: 10 to 100  $\mu\text{m}$
    - Length: up to 30 cm
  - Fibers: multinucleate cells (embryonic cells fuse)
  - Contractile proteins are organized into **cylindrical microfibrils**
  - **Transverse striations**: alignment across the fibre of repeating elements - the **sarcomeres**



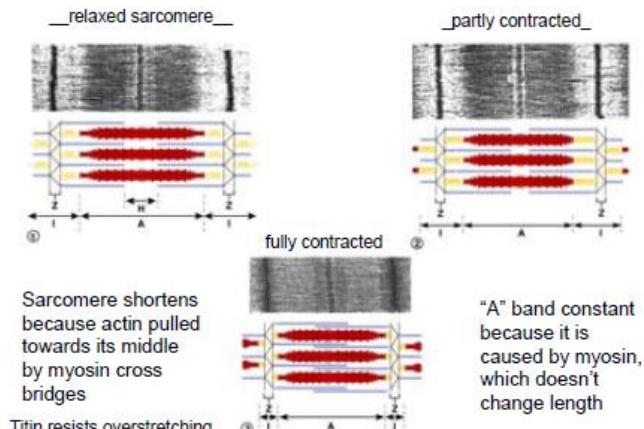
- **Fibers** (each is one cell) have striations
- **Myofibrils** are made up of **myofilaments**
- **Sarcomere**:
  - Basic unit of contraction
  - Myofibrils are long rows of repeating sarcomeres
  - Boundaries: **Z discs** (or lines)



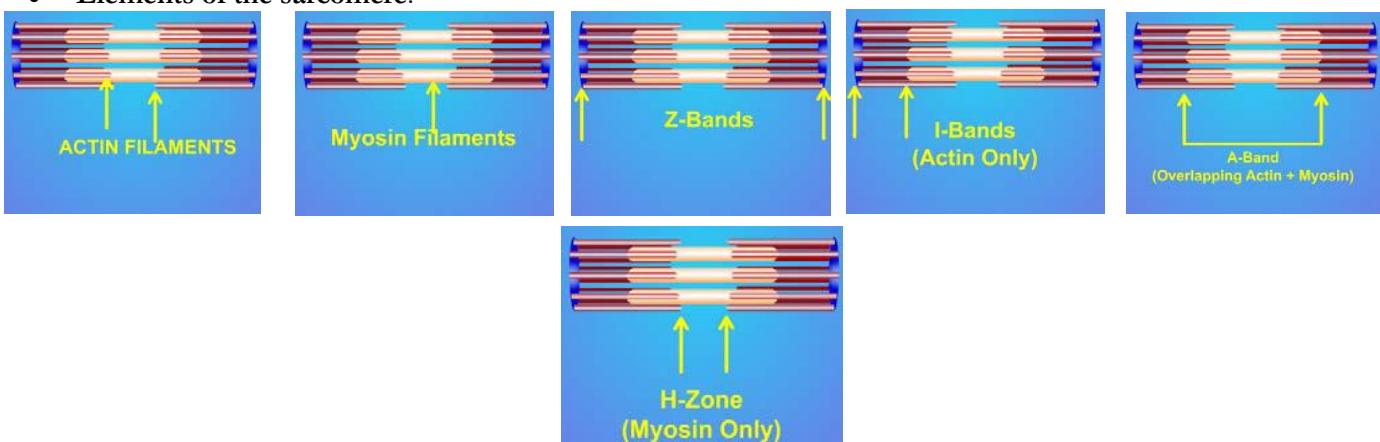
- **Myofilaments**: made of three types of filaments (or myofilaments)
  - **Thick (myosin)**: 1.6  $\mu\text{m}$  long and 15 nm wide
  - **Thin (actin)**: 1  $\mu\text{m}$  long
  - **Elastic (titin)**: 1 nm wide; resists overstretching; emerges from the core of a thick filament and links it to a Z disc



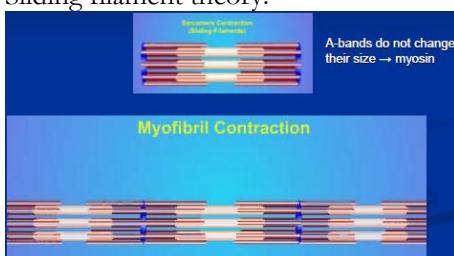
- Sliding filament model:



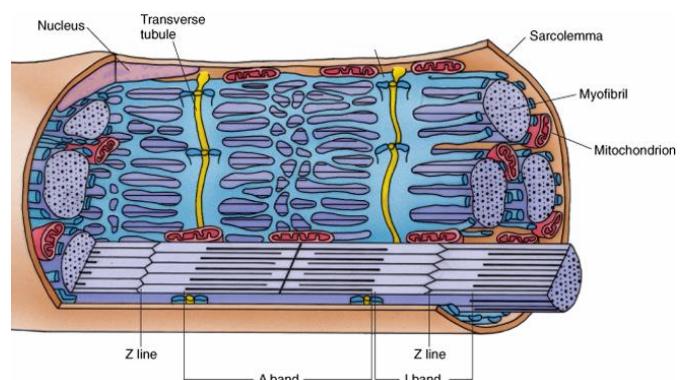
- Elements of the sarcomere:

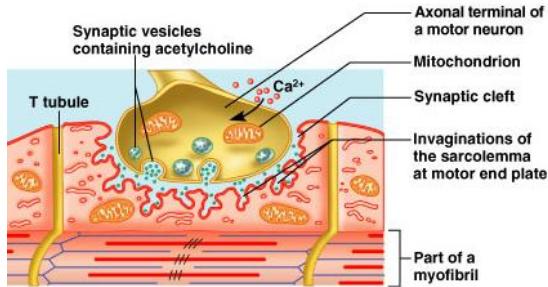


- Sliding filament theory:



- To provide for a uniform contraction, skeletal muscle possesses a system of **transverse (T) tubules**: fingerlike invaginations of the sarcolemma forming a complex anastomosing network of tubules that encircles the boundaries of each sarcomere in every myofibril
- **Acetylcholine** initiates skeletal muscle contraction





- Lateral propagation of the action potential down the T-Tubule:



- The fundamental function  $\text{Ca}^{2+}$  within the myocite **excitation-contraction-relaxation coupling (ECR)**:
  - Membrane channel activation → E
  - SR  $\text{Ca}^{2+}$  channel activation → C
  - $\text{Ca}^{2+}$  reuptake → R

| Component        | Skeletal Muscle                                                                                                                                                                          | Cardiac Muscle                                                                |
|------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| Membrane channel | Dihydropyridine Receptor (DHPR)                                                                                                                                                          | Voltage-gated $\text{Ca}^{2+}$ channels                                       |
| SR channel       | Ryanodine Receptor (RyR1)<br>$\text{IP}_3$ Receptor ( $\text{IP}_3\text{R}$ )                                                                                                            | Ryanodine Receptor (RyR2)<br>$\text{IP}_3$ Receptor ( $\text{IP}_3\text{R}$ ) |
| Reuptake         | SarcoEndoplasmic Reticulum $\text{Ca}^{2+}$ ATPase (SERCA) – 70%<br>Sodium-Calcium exchanger (NCX) – 28%<br>Mitochondrial $\text{Ca}^{2+}$ uptake<br>Sarcolemmal $\text{Ca}^{2+}$ ATPase |                                                                               |

diseases:

- RyR1: Malignant Hyperthermia
- SERCA: Dilated Cardiomyopathy
- **Malignant Hyperthermia (Mh):**
  - Genetic predisposition to anesthetics:
    - Muscle rigidity
    - High fever
    - Life-threatening
  - Defect: skeletal muscle RyR1 and/or related protein
  - Mutations in RyR1 gene
  - Altered RyR1 protein
  - Mutant channel opens more readily and stays open longer
  - High intracellular  $\text{Ca}^{2+}$  stimulates
    - Sustained muscle contraction: rigidity
    - Breakdown of metabolites: increased heat
  - Treatment: dantrolene, an inhibitor of RyR1

## Neuromuscular junction

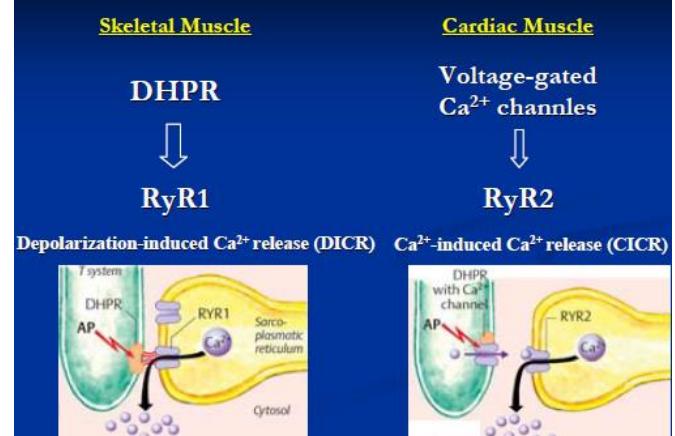


Defects in RyR or SERCA are linked to human

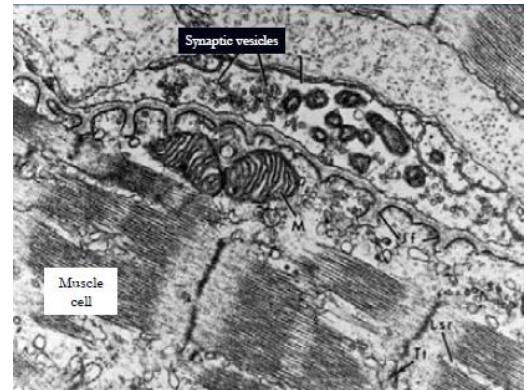
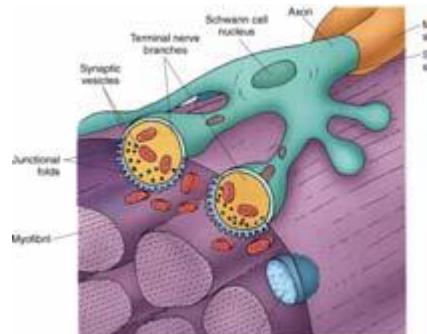
### SR $\text{Ca}^{2+}$ regulation – RyR versus $\text{IP}_3\text{R}$

- Types: 3 for each – RyR1-3,  $\text{IP}_3\text{R}1-3$
- Activated by:
  - RyR – voltage (skeletal muscle) or  $\text{Ca}^{2+}$  (cardiac muscle)
  - $\text{IP}_3\text{R}$  – Inositol-3-phosphate (signal transduction)
- Structure – tetrameric (4 RyR and 4  $\text{IP}_3\text{R}$  subunits)
  - 1 RyR subunit - ~ 600 kDa; total mass ~ 2,4MDa - 10% of the ribosome size
  - 1  $\text{IP}_3\text{R}$  subunit - ~ 300 kDa; total mass ~ 1,2MDa
- Conductance for  $\text{Ca}^{2+}$  – 10 times larger than SL channels
- Distribution
  - RyR1 – skeletal muscle, brain
  - RyR2 – cardiac muscle, brain
  - RyR3 – non-muscle
  - $\text{IP}_3\text{R}1$  – skeletal muscle, brain, ubiquitous
  - $\text{IP}_3\text{R}2$  – cardiac muscle, liver
  - $\text{IP}_3\text{R}3$  – non-excitable tissues

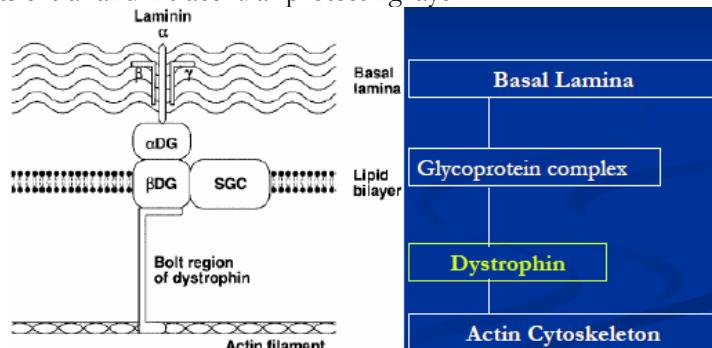
### $\text{Ca}^{2+}$ release – Skeletal versus Cardiac Myocytes



- A link between AF and ECM is mediated by the protein: **dystrophin**: 42 kDa intracellular cytoskeleton protein. Its discovery contributed to both clinical and basic research into the muscular dystrophies (MD) such as Duchenne (DMD) and Becker (BMD)



- Dystrophin "bolt" connects extra- and intracellular protecting layer:



- Dystrophin-Glycoprotein Complex (DGC)**: multisubunit complex that connects the cytoskeleton of a muscle fiber to its surrounding extracellular matrix:

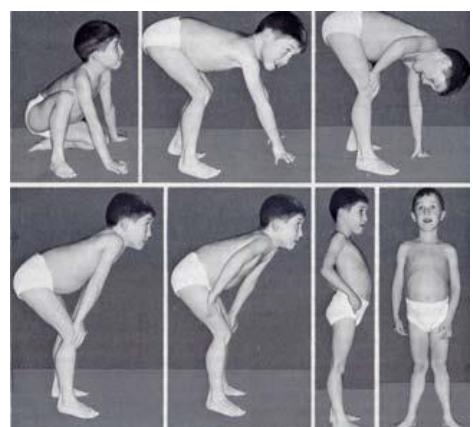
- Dystroglycans (DG)
  - $\alpha$ -DG: extracellular, binds laminin, 156 kDa
  - $\beta$ -DG: transmembrane, binds to dystrophin, 43 kDa
- Sarcoglycans:  $\alpha$ -SG,  $\beta$ -SG,  $\gamma$ -SG,  $\delta$ -SG, 50 kDa
  - All SGs are transmembrane: bind  $\beta$ -DG
  - Increase the affinity of the  $\beta$ -DG to dystrophin and  $\alpha$ -DG
  - Sarcospan: transmembrane protein, function unknown
  - Syntrophins, dystrobrevins
    - Intracellular proteins: bind dystrophin and  $\beta$ -DG
    - Involved in signal transduction (NO, tyrosine kinases)
  - Dystrophin - 2,5 Mbp - the largest human gene

- Duchenne Muscular Dystrophy (DMD):**

- X-linked (mother → son)
- Most common MD: 1/3,500 males
- Clinical symptoms begin at childhood
  - Gower's sign is typical for MD
  - Wheel-chair dependence by the age of 12
- Die in early twenties
  - Respiratory failure: intercostal muscle weakness
  - Cardiac failure: cardiomyopathy
- Becker MD: less severe symptoms

- Utrophin** is a dystrophin homologue

- Gowers' sign**: the use of the child's arms to climb up his body when going from a lying to standing position



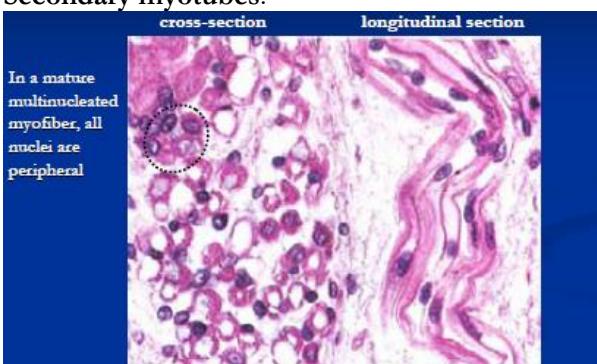
## Musculotendineous

### Musculotendineous junction



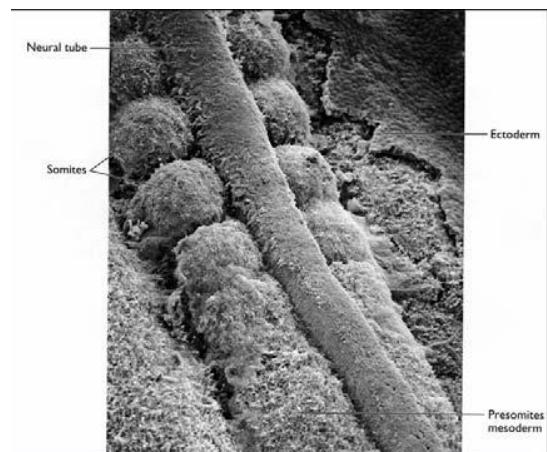
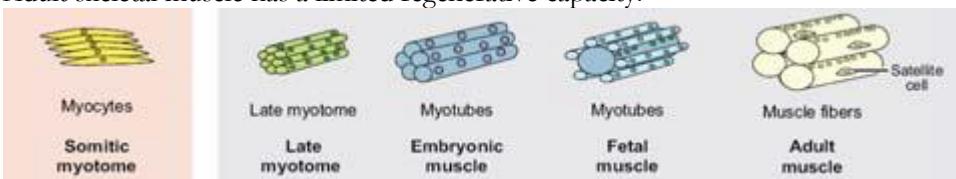
## Genesis

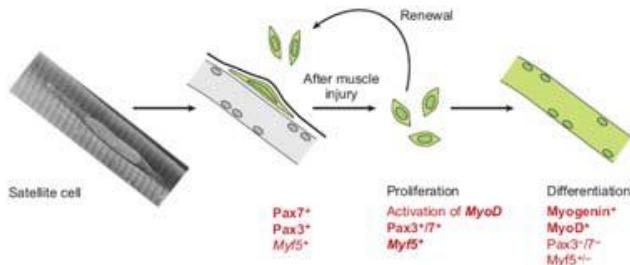
- Skeletal muscle develops embryologically from **somatic mesodermal tissue**:
  - Somites (segmental paired blocks of mesoderm, each side of the notochord) are completely formed by day 30
  - Mesodermal somites separate into the dermatomes and segmental myotomes
  - At the end of the 8th week, the primordia of individual muscles can be appreciated
- 42 to 44 pairs of rounded somites can be found adjacent to the notochord in the midline
- Stages in formation:
  - **Myoblasts:** most immature muscle cells
    - Mononucleate cells with prominent nucleoli
    - No microscopically detectable filaments
  - Myoblasts are the source of **myotubes (primary)**: up to wk 15
    - Chain-like structures formed by fusion of myoblasts
    - Differentiate and start to express myofilaments
  - Late myoblasts produce **secondary myotube** (predominate after wk 20)
    - Have a smaller diameter, increased numbers of nuclei
    - Make contacts with nerve terminals: contractile activity
    - Eventually give rise to muscle fibers
  - During postnatal life muscle fibers continue to increase in length:
    - Existing sarcomeres lengthen
    - New sarcomeres are generated, usually at the myotendinous junctions
- **Secondary myotubes:**



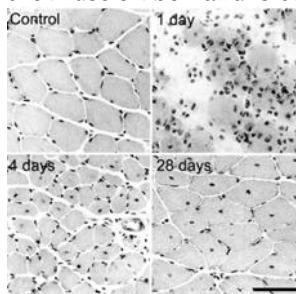
Myotubes have a small diameter and are widely spaced. Nuclei are centrally positioned, but gradually become displaced at the periphery of the cell.

- Adult skeletal muscle has a limited regenerative capacity:



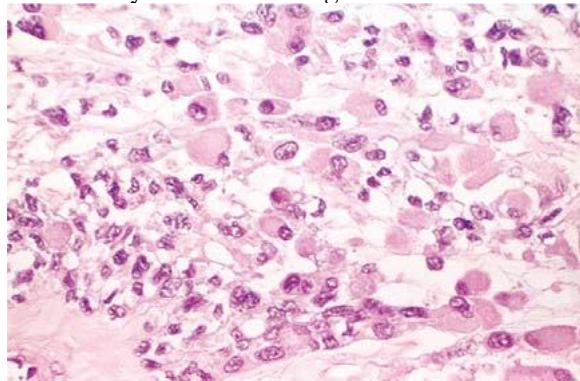


- Satellite cells constitute 3-5% of the nuclei in skeletal muscle: M-cadherin is presented on both the satellite cell and the muscle fiber and is concentrated at the site where their membranes are in contrast



Regeneration of skeletal muscle after experimental injury

- **Rhabdomyosarcoma:** malignant tumor of skeletal muscle



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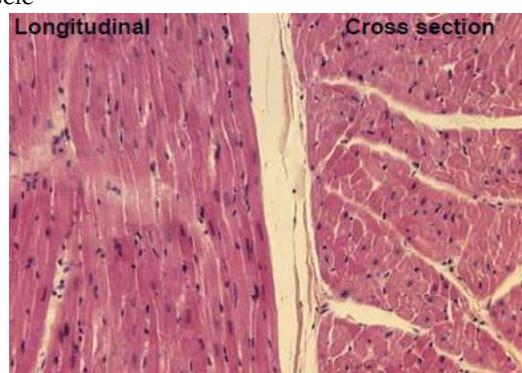
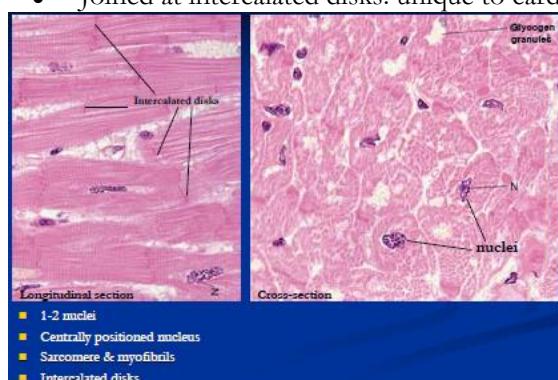
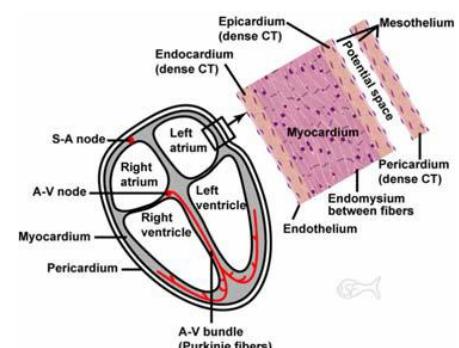
## Cardiac muscle + Smooth muscle

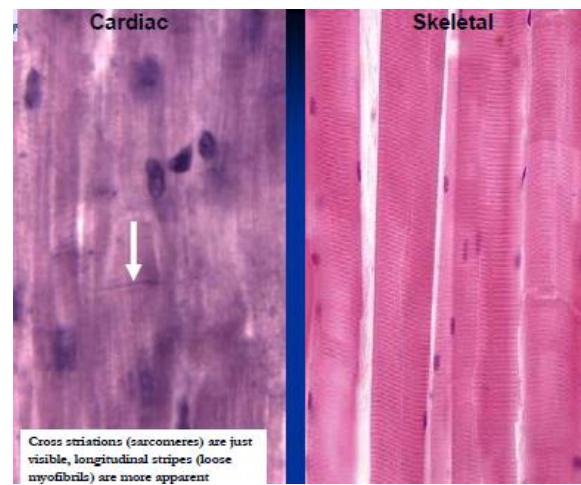
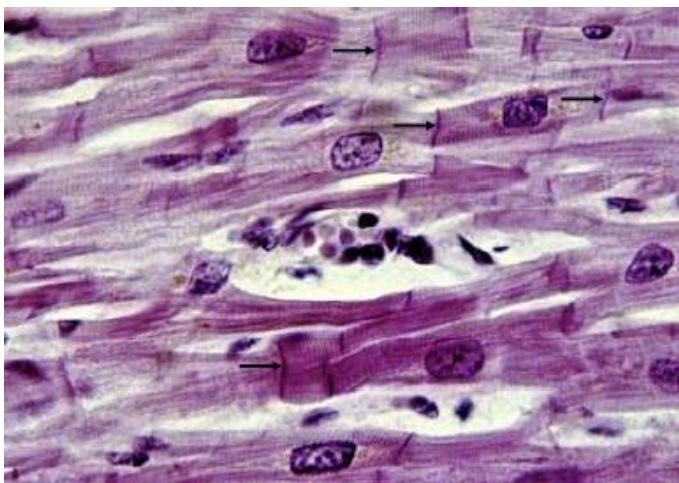
### Cardiac Muscle

- **Nonvoluntary striated** muscle limited to the heart and the proximal portions of the pulmonary veins
- Like skeletal, cardiac muscle cell organelles have specific names:
  - **Sarcoplasm:** cytoplasm, excluding the myofibrils
  - **Sarcoplasmic reticulum:** smooth endoplasmic reticulum
  - **Sarcolemma:** cell membrane, plasmalemma

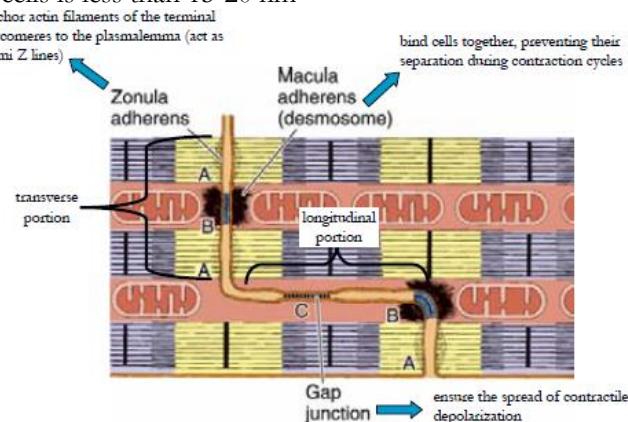
### Cardiac muscle cells: cardiomyocytes

- Exhibit a **cross-striated banding pattern** identical to that of skeletal muscle
- Size 15  $\mu\text{m}$  in diameter, 80-100  $\mu\text{m}$  in length
- Possess only 1 or 2 centrally located nuclei
- Surrounded by a delicate sheath of endomysium
- Joined at intercalated disks: unique to cardiac muscle





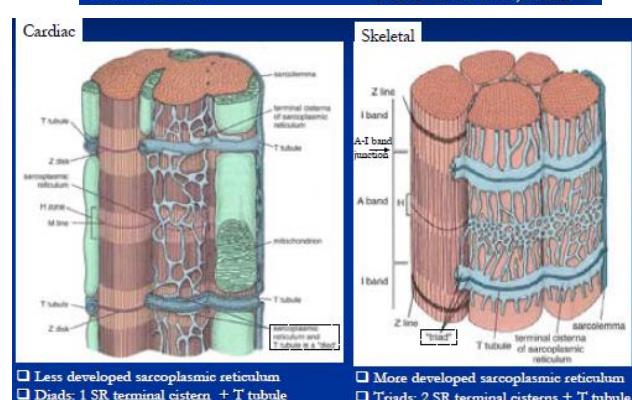
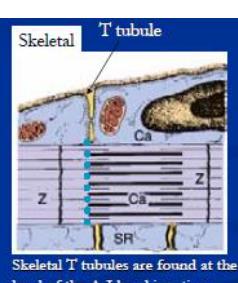
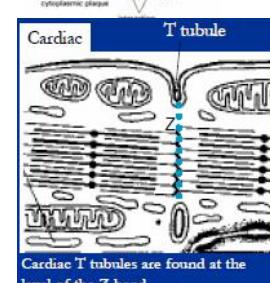
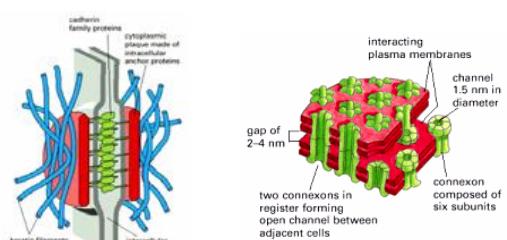
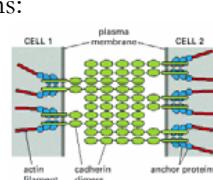
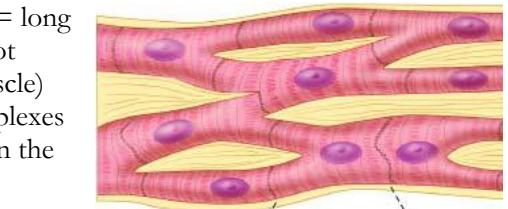
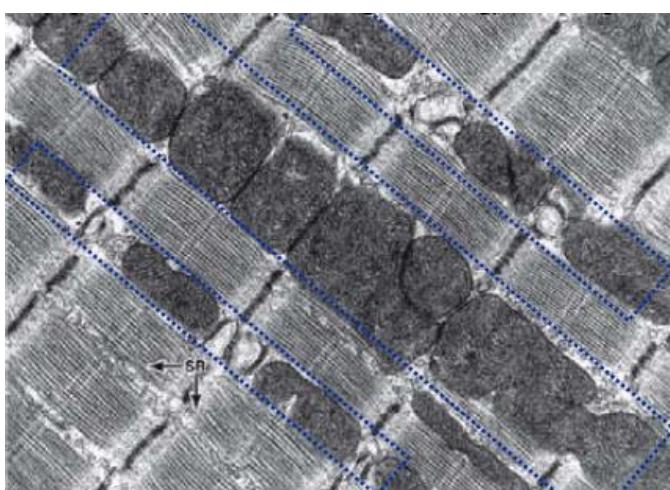
- Cardiomyocytes are branched cells joined to make a fiber: "fiber" = long row of joined cardiac muscle cells; the fibres of cardiac muscle are not single syncytial cells with a common cytoplasm (as in skeletal muscle)
- Intercalated disks:** Cardiomyocytes have specific junctional complexes found at the interface between adjacent cells. The distance between the 2 cells is less than 15-20 nm



- Components of the junctional specializations:
  - Zonula adherens**
  - Desmosome**
  - Gap junctions**

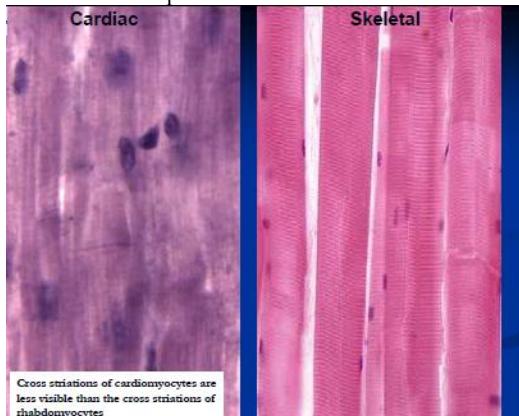
## Differences between cardiac and skeletal muscle

- Cardiac T tubules show differences from skeletal T tubules:
- Cardiac sarcoplasmic reticulum shows differences from skeletal SR
- Almost half of the volume of the cardiac muscle cell is occupied by mitochondria: great energy consumption

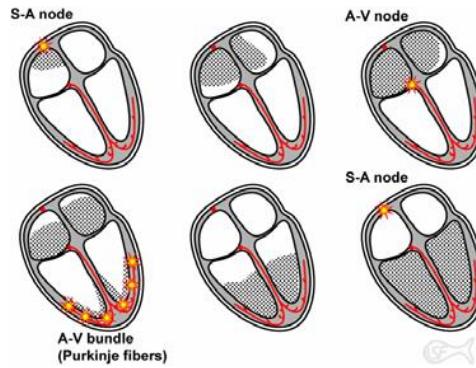


## Cardiac muscle specifics

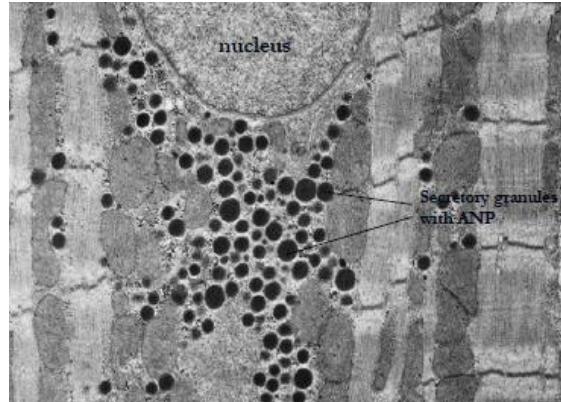
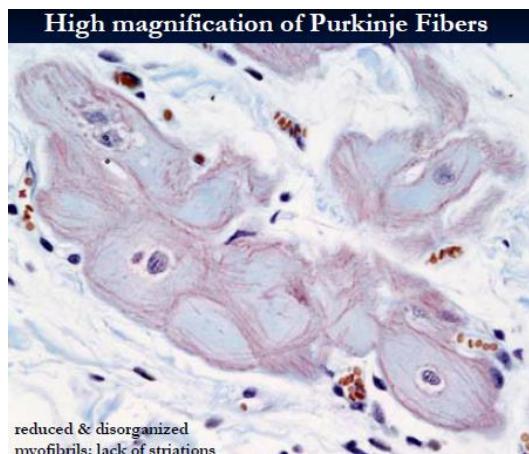
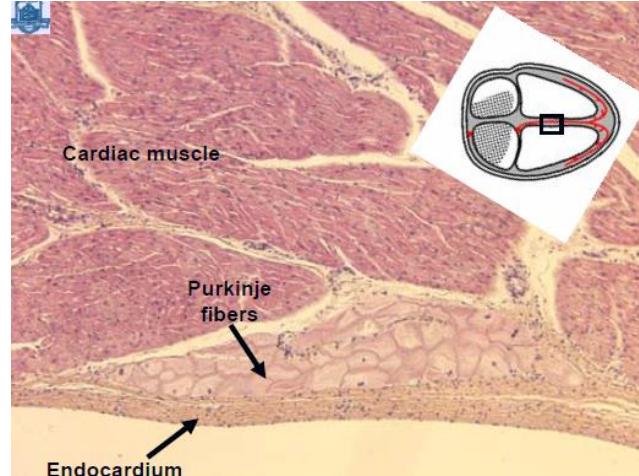
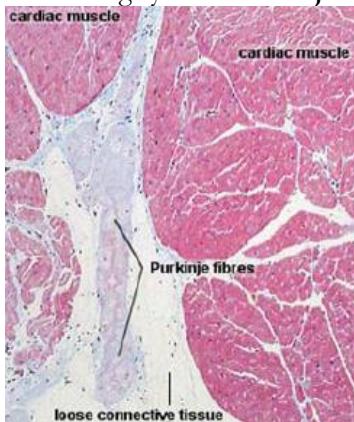
- Because of the contractile apparatus of cardiac muscle lies within an abundant mitochondria-rich sarcoplasm, the cross-striations of cardiac muscle are less conspicuous than those of skeletal muscle



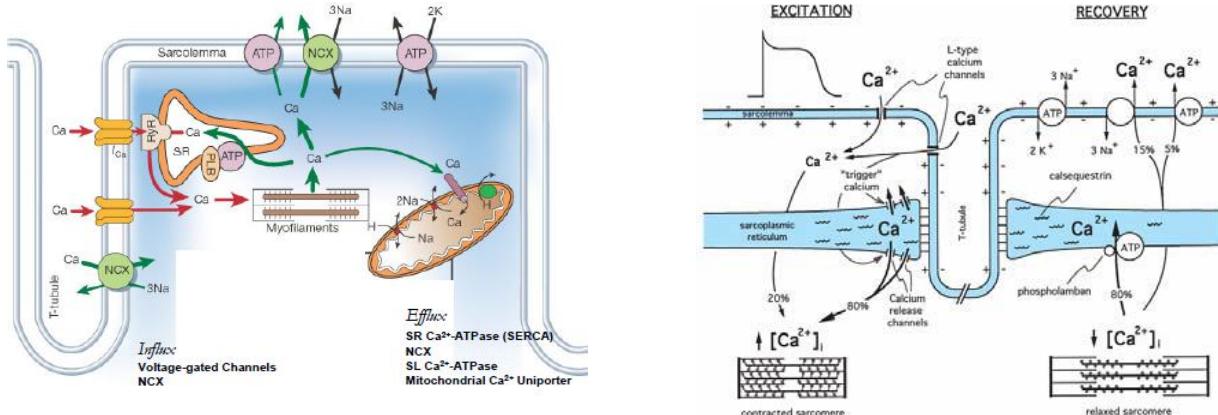
- Cardiomyocytes have an exceptionally rich network of capillaries around the fibres: **very high oxygen demand**
- Cardiac conduction system:**



- Heart conducting system: **Purkinje fibers**



- Some cardiomyocytes have endocrine function: **Atrial Natriuretic Peptide (ANP)**
- Regulation of Cytoplasmic  $\text{Ca}^{2+}$ :



| SR $\text{Ca}^{2+}$ regulation in Skeletal and Cardiac Myocytes |                                                                                                          |                                                                                |
|-----------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Component                                                       | Skeletal Muscle                                                                                          | Cardiac Muscle                                                                 |
| Membrane channel                                                | Dihydropyridine Receptor (DHPR)                                                                          | Voltage-gated $\text{Ca}^{2+}$ channels                                        |
| SR channel                                                      | Ryanodine Receptor (RyR1)<br>$\text{IP}_3$ Receptor ( $\text{IP}_3\text{R1}$ )                           | Ryanodine Receptor (RyR2)<br>$\text{IP}_3$ Receptor ( $\text{IP}_3\text{R2}$ ) |
| Reuptake                                                        | SarcoEndoplasmic Reticulum $\text{Ca}^{2+}$ ATPase (SERCA) – 70%<br>Sodium-Calcium exchanger (NCX) – 28% | Mitochondrial $\text{Ca}^{2+}$ uptake<br>Sarcolemmal $\text{Ca}^{2+}$ ATPase   |

- Anti-arrhythmic drugs act on the ion channels regulating cardiomyocyte contraction:



Arrhythmia



Normal

#### SR $\text{Ca}^{2+}$ regulation – RyR versus $\text{IP}_3\text{R}$

Types: 3 for each – RyR1-3,  $\text{IP}_3\text{R1-3}$

Activated by:

- RyR – voltage (skeletal muscle) or  $\text{Ca}^{2+}$  (cardiac muscle)
- $\text{IP}_3\text{R}$  – Inositol-3-phosphate (signal transduction)

Structure – tetrameric (4 RyR and 4  $\text{IP}_3\text{R}$  subunits)

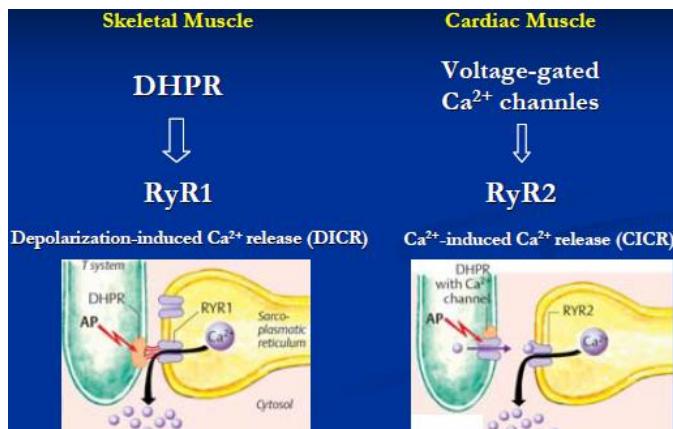
- 1 RyR subunit ~ 600 kDa; total mass ~ 2.4MDa
- 10% of the ribosome size

1  $\text{IP}_3\text{R}$  subunit ~ 300 kDa; total mass ~ 1.2MDa

Conductance for  $\text{Ca}^{2+}$  – 10 times larger than SL channels

Distribution

- RyR1 – skeletal muscle, brain
- RyR2 – cardiac muscle, brain
- RyR3 – non-muscle
- $\text{IP}_3\text{R1}$  – skeletal muscle, brain, ubiquitous
- $\text{IP}_3\text{R2}$  – cardiac muscle, liver
- $\text{IP}_3\text{R3}$  – non-excitable tissues



#### Defects in RyR or SERCA are Linked to Human Diseases

##### RyR1



Malignant Hyperthermia

##### SERCA

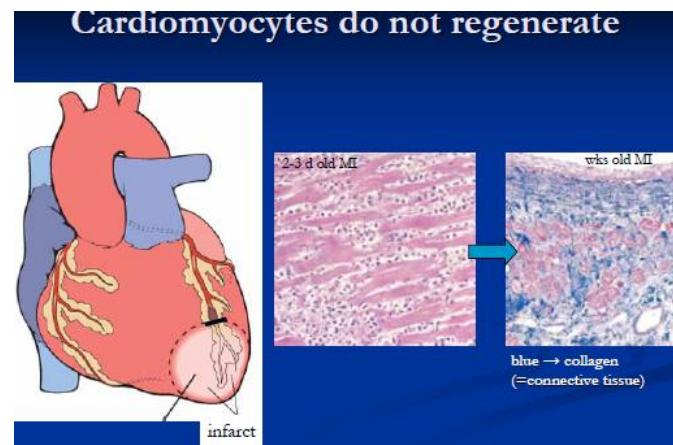
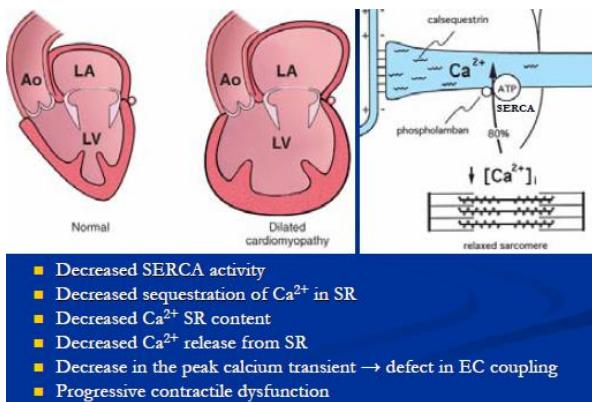


Dilated Cardiomyopathy

- Dilated Cardiomyopathy (DCM):

- Dilated and poorly functioning left ventricle
- Absence of other abnormal conditions (hypertension, ischemic heart)
- Global systolic impairment

DCM is often initiated by environmental stimuli on a background of genetic susceptibility



## Development of cardiac muscle

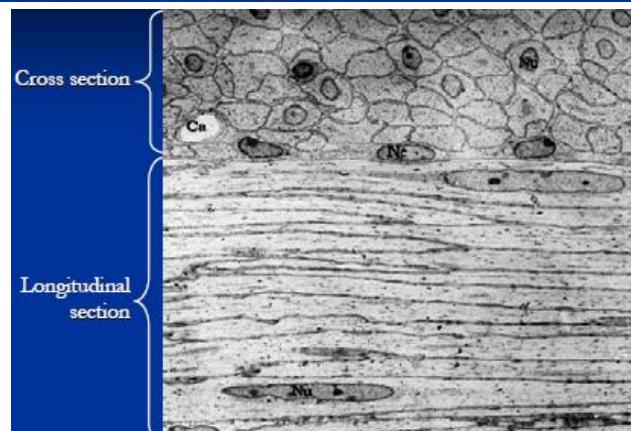
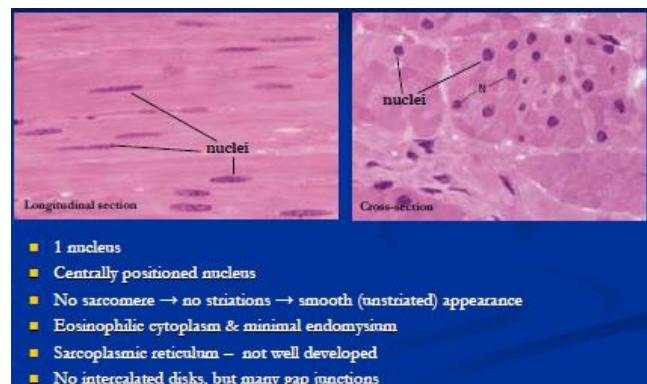
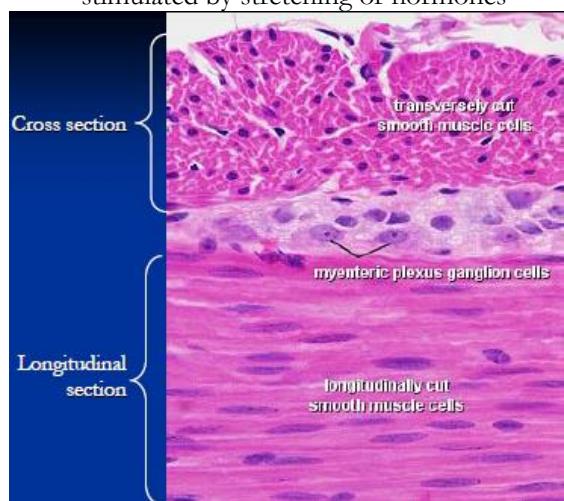
- Cardiac muscle develops from **splanchnic mesoderm** surrounding the endothelial heart tube
- Myoblasts adhere to one another by special attachments that later develop into intercalated discs, but **do not fuse**
- During later development, a few special bundles of muscle cells with irregularly distributed myofibrils become visible. These bundles, the **Purkinje fibers**, form the conducting system of the heart

## Muscle tissue (smooth muscle)

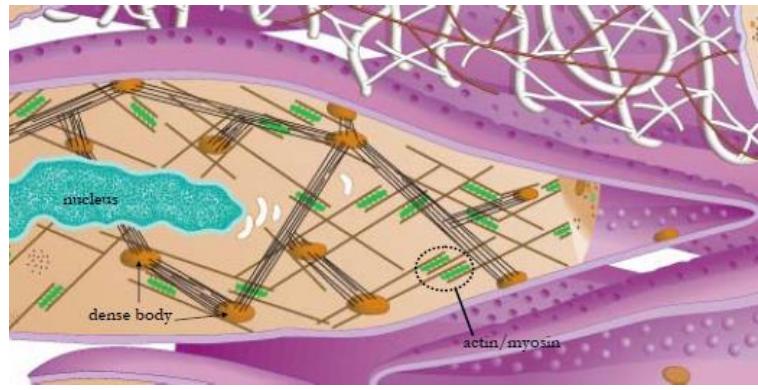
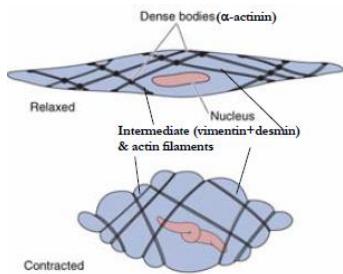
- Smooth muscle is composed of myocytes (fibers) with a **fusiform shape**, which lack **visible striations** (i.e. smooth)
- Major locations:
  - Walls of vessels
  - Respiratory tubes
  - Digestive tubes
  - Urinary organs
  - Reproductive organs
  - Larger ducts of compound glands
  - Inside the eye
  - Dermis of skin (small bundles)

## Smooth muscle cells - leiomyocytes

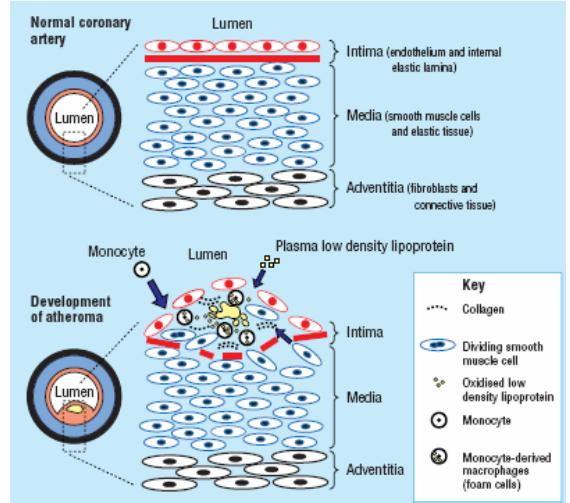
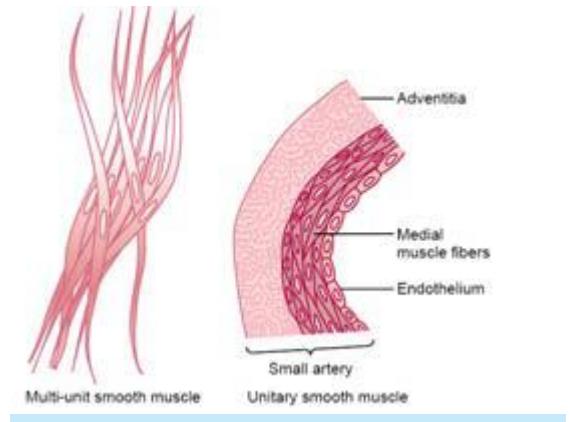
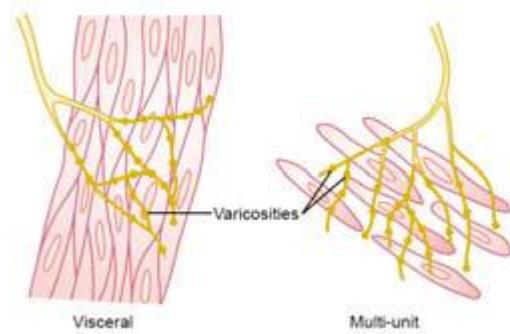
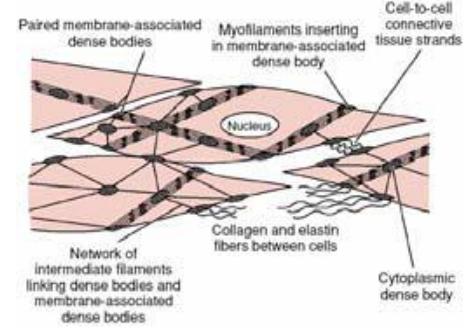
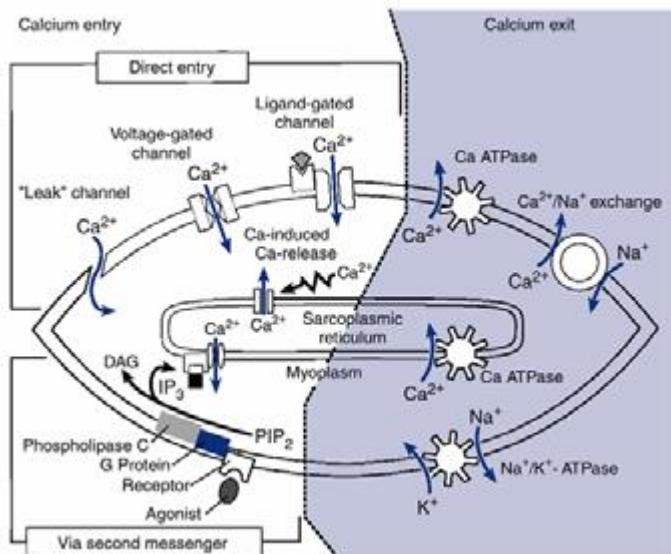
- Size: less than 10 micrometers in diameter, 100-200 micrometers in length
- Process only 1 centrally located nucleus
- Surrounded by a delicate sheath of endomysium
- Do not possess a system of T tubules: have numerous plasma membrane invaginations resembling caveolae
- Contractions are slow, sustained and resistant to fatigue
- Does not always require a nervous signal: can be stimulated by stretching or hormones



- Instead of Z-disks, SM cells have **dense bodies**:
  - Thin filaments: **actin**, caldesmon (blocks the active site of F-actin), and tropomyosin, but no troponin
  - Thick filaments: **myosin II** (same as in skeletal muscle)
  - Intermediate filaments (vimentin & desmin)
  - Dense bodies are small, dark-staining areas, associated with the thin and intermediate filaments.
  - They serve as anchors for thin filaments and to transmit the force of contraction to adjacent cells.

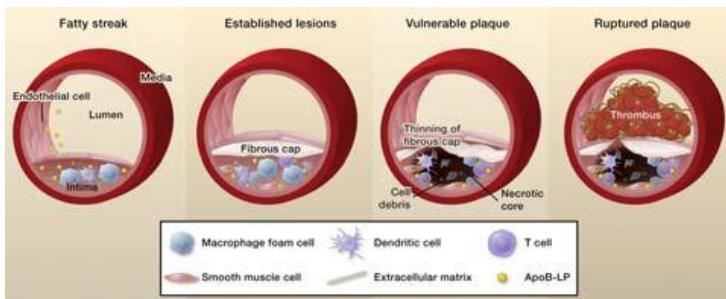


- Force transmission from cell to cell:
  - Membrane-associated dense bodies are opposite one another in adjacent cells and may provide continuity of force transmission between the contractile apparatus in each cell
  - Short strand of connective tissue link adjacent cells
  - Cells are joined to the collagen and elastin fibers running throughout the tissue
- Types of smooth muscle:
  - Multi-unit smooth muscle
    - Each muscle cell receives its own nerve supply
    - Little cell-to-cell communication
    - Locations: iris of the eye, vas deferens
  - Single-unit smooth muscle: functional syncytium via gap junctions:
    - Nerve fibers pass through the tissue without synapsing with any specific muscle cell
    - Axons have many (up to 20,000) beadlike swellings called varicosities along its length; the nerve fiber passes amid several myocytes and stimulates all of them
    - Muscle cells do not have motor end plates or any other specialized area of sarcolemma to bind the neurotransmitter; diffusely distributed receptor
    - Visceral organs
- Calcium entry and exit from the cytoplasm of smooth muscle:



## Atherosclerosis

- Smooth muscle cells are secretory cells in atherosclerosis:

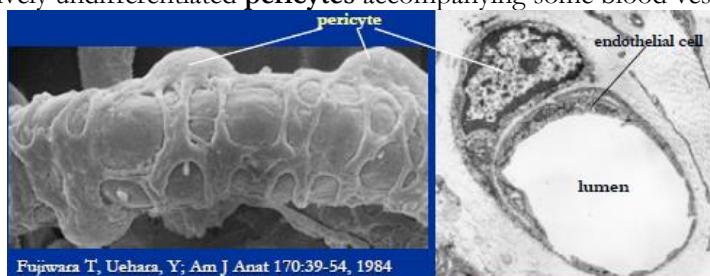


## Development of smooth muscle

- Smooth muscle in the wall of the gut and gut derivates is derived from **splanchnic mesoderm** surrounding the endoderm of these structures
- Vascular smooth muscle differentiates from **mesoderm** adjacent to vascular endothelium
- Sphincter and dilator muscles of the pupil and muscle tissue in the mammary gland and sweat glands originate from **ectoderm**

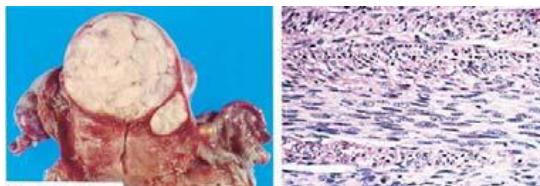
## Regeneration

- Preexisting smooth muscle cells **retain mitotic capability** to form more smooth muscle cells: pregnant uterus
- Differentiation of relatively undifferentiated **pericytes** accompanying some blood vessels



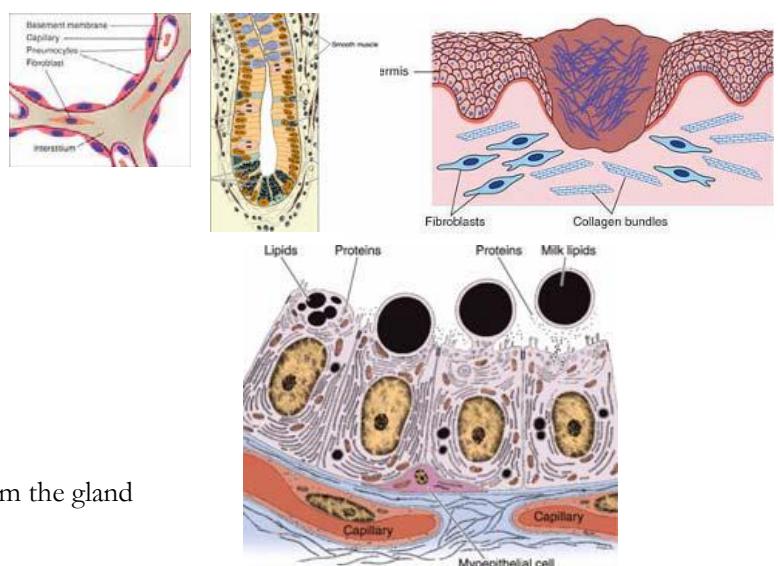
## Leimomyoma

- Leimomyoma: Smooth muscle cell tumor

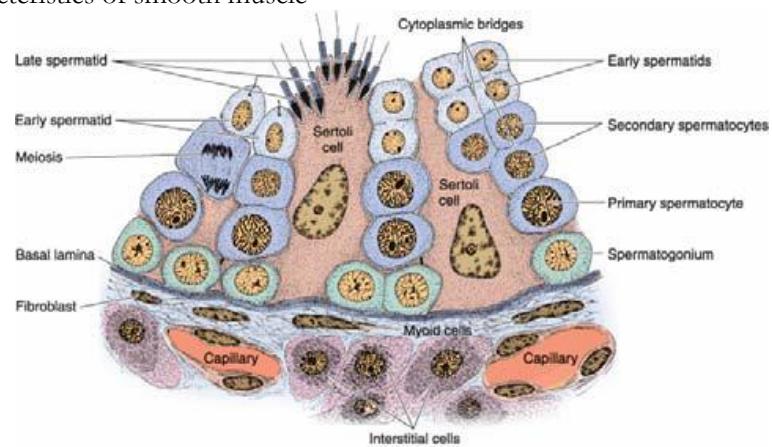


## Specifics

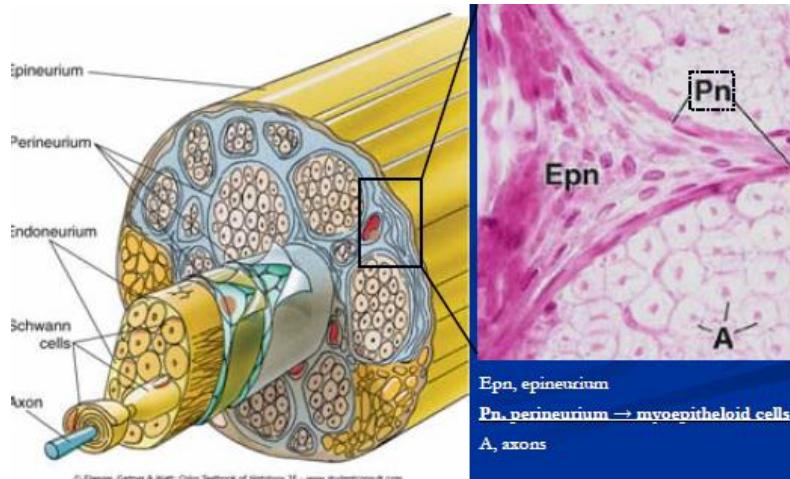
- Dispersed cells with contractile function originate from ectoderm:
  - Myofibroblasts
  - Myoepithelial cells
  - Myoid cells (testis)
  - Cells of perineurium
- Mofibroblasts:**
  - Fibroblasts with contractile functions
    - Retain ECM (e.g. collagen) production
    - Express contractile proteins
  - Location
    - Alveolar septa (lung)
    - Crypts of Lieberkühn (intestine)
    - Healing wounds
- Myoepithelial cells:**
  - Epithelial cells with contractile functions:
    - Retain secretion ability
    - Express contractile proteins
  - Location
    - Sweat glands
    - Salivary glands
    - Mammary glands
  - Function: assist in expressing the fluid from the gland



- **Myoid cells** (testis): The innermost cell layer, adhering to the basal lamina of seminiferous tubules aree glattened **myoid cells** with characteristics of smooth muscle

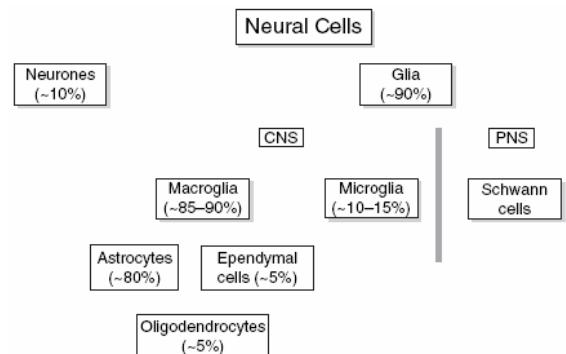


- **Perineurium cells:**



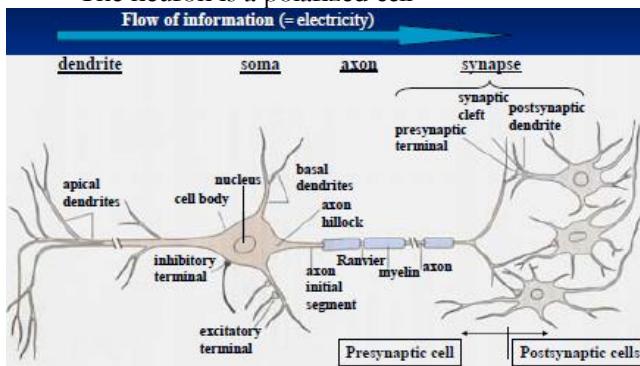
## Nervous tissue

- The human nervous system is the most complex system in the human body
- It is formed by a netwrk of **neurons** and associated **glial cells**
- Nerve tissue is distributed throughout the body as an integrated communications network
- Historical views of the brain:
  - Galen (AD 200): the brain is a gland conveying fluid to the periphery; or a reticulum
  - Golgi & Cajal (1900): The neuron doctrine; the brain is composed of individual cells conveying signals
- The organization of the nervous system:
  - **CNS**: gray matter, white matter
  - **PNS**:
    - Ganglia
      - Cranial
      - Spinal
      - Autonomic
    - Peripheral nerves
    - Nerve endings
      - Afferent
      - Efferent
- Major cell types:
  - **Neurons**: approx.  $10^{11}$  in the human brain (each neuron has, on average, at least 1000 interconnections with other neurons)
  - **Glia**: 10-50 per neuron
    - Macrogia
      - Oligodendrocytes
      - Schwann cells
      - Astrocytes
      - Ependymal cells
    - Microglia



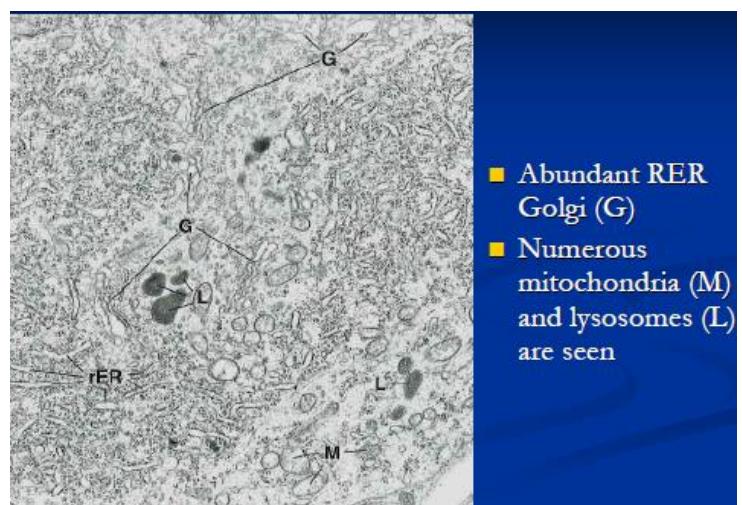
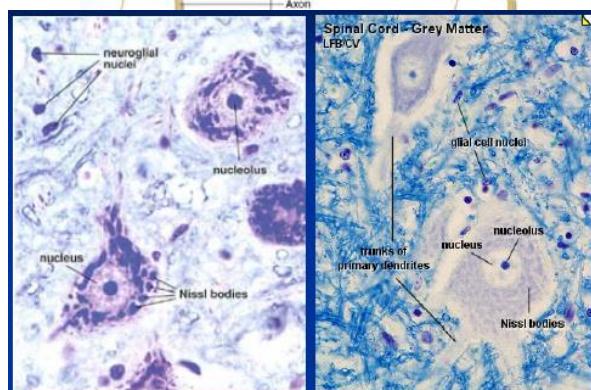
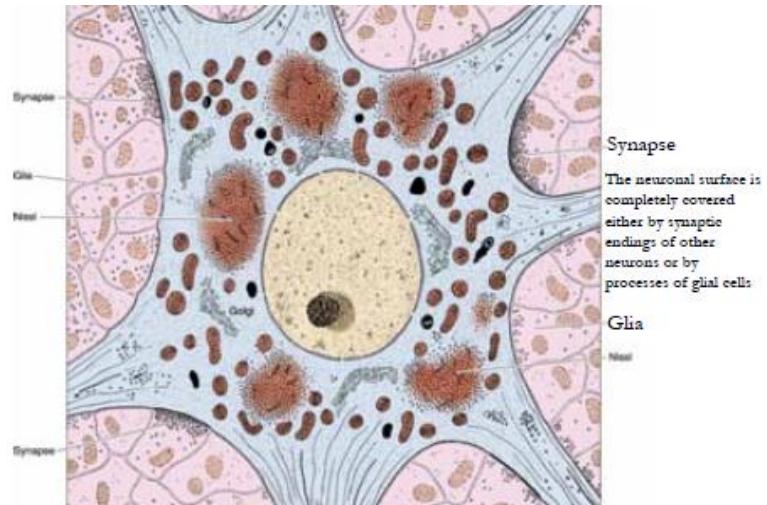
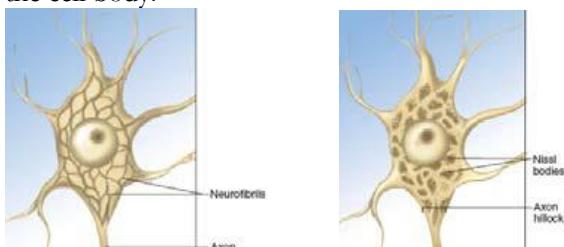
## The neuron

- The neurons is the structural and functional unit of the nervous system
- The neuron is a polarized cell



### Soma = perikaryon = cell body

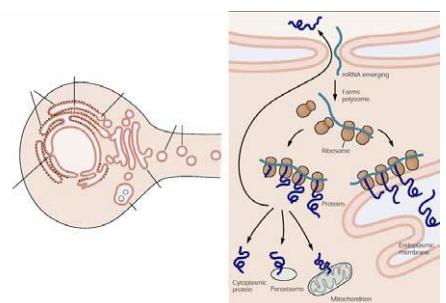
- Dilated region of the neuron, which contains:
  - The neuronal **nucleus**
  - The perinuclear **cytoplasm**: abundant in rough ER, which is seen as **Nissl bodies** under light microscopy
  - Nissl bodies** (stacks of RER), free ribosomes, and Golgi complex extend into the dendrites but not into the axon
  - organelle-free area at the junction between perikaryon & axon: **axon hillock**
- Nissl bodies & neurofibrils (neurofilaments) in the cell body:



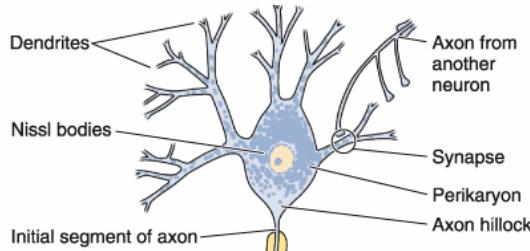
- Neurons are **secretory cells**
- Certain neuronal cell bodies contain pigments:
  - Neuromelanin**: neurons of substantia nigra, locus ceruleus; complexes of excess cytosolic neurotransmitters (e.g. dopamine) that are not accumulated by synaptic vesicles; prevent neuronal damage of substance accumulation
  - Lipofuscin**: many neurons, particularly at older age; products of lysosomal processing

### Dendrites

- Dendrites are receptor processes that receive stimuli from other neurons or from the external environment
- Dendrites are often multibranched (Gr. *dendron*, tree): up to 200,000 axonal terminations establish functional contact with the dendrites of a Purkinje cell of the cerebellum

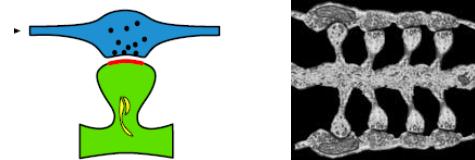


- They are arborized so that they can receive multiple stimuli from many other neurons simultaneously
- The nerve impulses received by the dendrites are then transmitted toward the soma



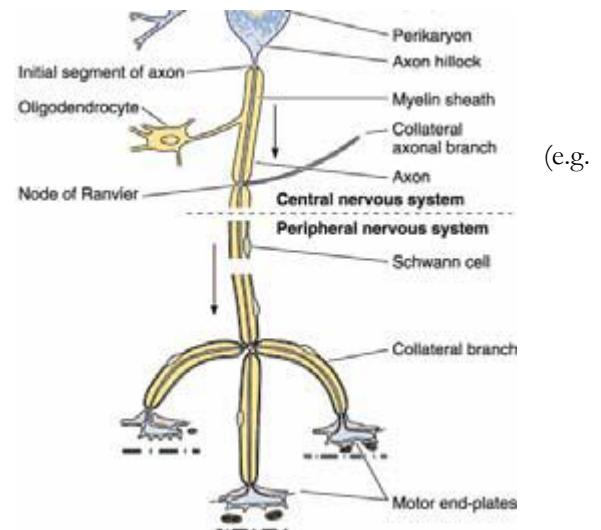
## Synapses

- Most synapses are located in dendritic spines
  - Mushroom-shaped structures (head, neck, shaft)
  - Size: 1-3 μm long, less than 1 μm in diameter
  - Human cerebral cortex: approx.  $10^{14}$  spines



## Axon

- The axon is a single process specialized in generating or conducting nerve impulses to other cells
- Most neurons have only one axon; a very few no axon at all
- Axons are usually very long processes: up to 100 cm in length (motoneuron axons)
- Terminology:
  - **Axon hillock:** initial segment, arises from the perikaryon
  - **Axolemma:** plasma membrane
  - **Axoplasm:** cytoplasm
  - **Initial segment** (in myelinated axons): summation of excitatory & inhibitory stimuli
  - **Collateral branches**
  - **Axon terminals**
- **Axon organelles:**
  - No RER, but some SER: the absence of ribosomes & emphasizes the dependence of the axon on the perikaryon for its maintenance
  - Mitochondria are especially abundant in the axon terminals
  - Abundant in axons (also in soma) are **neurofilaments**: intermediate filaments

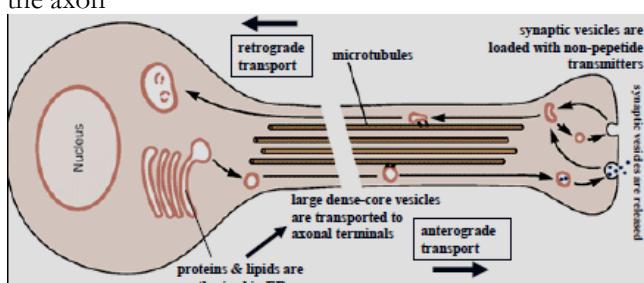


RER

| Intermediate filaments – classification by proteins                                                                                                                                                                                   |                                                          |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------|
| Cytoplasmic                                                                                                                                                                                                                           | Nuclear                                                  |
| Type I<br>keratins 9-20 (epithelial cells)<br>keratins Ha1-Ha8 (hair)                                                                                                                                                                 | Type V<br>lamin A & C<br>lamin B1 & B2<br>nuclear lamina |
| Type II<br>keratins 1-8 (epithelial cells)<br>keratins Hb1-Hb8 (hair)                                                                                                                                                                 |                                                          |
| Type III<br>vimentin (mesenchymal cells)<br>desmin (all muscle cells)<br>glial fibrillary acidic protein (GFAP) – astrocytes, hepatic stellate cells<br>peripherin (peripheral nervous system)<br>syncoilin (skeletal/cardiac muscle) |                                                          |
| Type IV<br>neurofilament proteins (NF-L, -M, -H)<br>α-internexin (central nervous system) – central nervous system<br>nestin (neural stem cells, astrocytes)<br>synenmin (all muscle cells)                                           |                                                          |



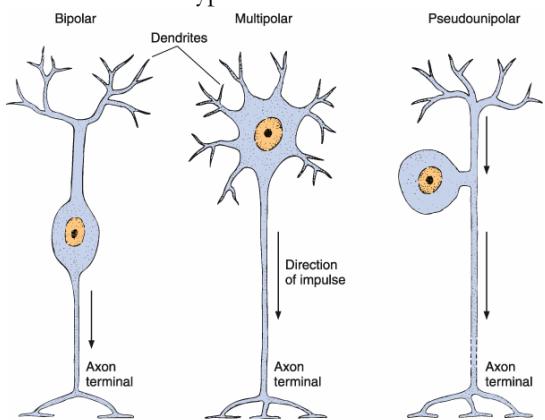
- When impregnated with silver, neurofilaments form neurofibrils that are visible under light microscopy
- There is a lively **bidirectional transport** of small and large molecules along the axon



- Axonal transport is dependent on microtubules; movement along microtubules depends on motor proteins (ATPases):
  - **Dynein:** retrograde flow
  - **Kinesin:** anterograde flow

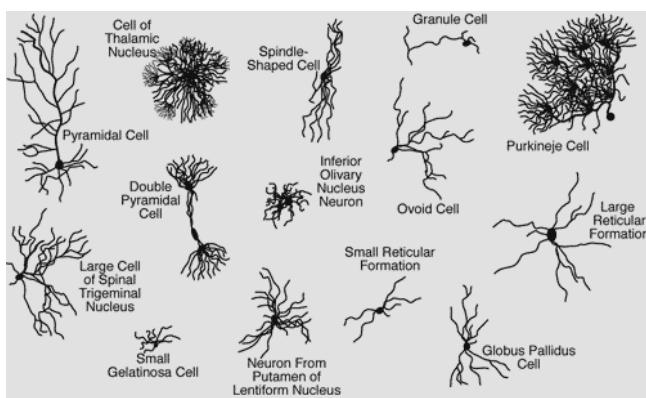
## Classification of neurons

- Neurone types:

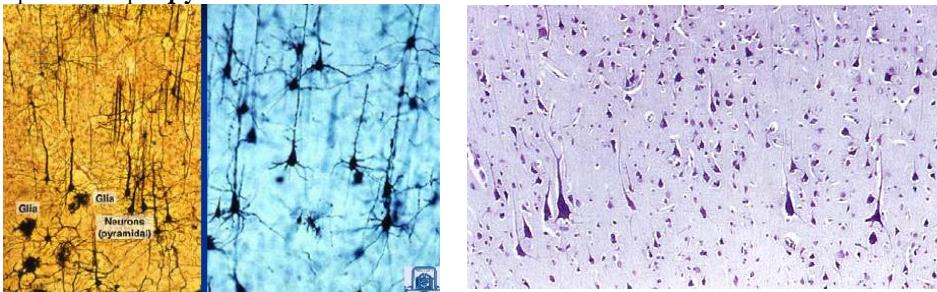


## Types of neurons in the nervous system

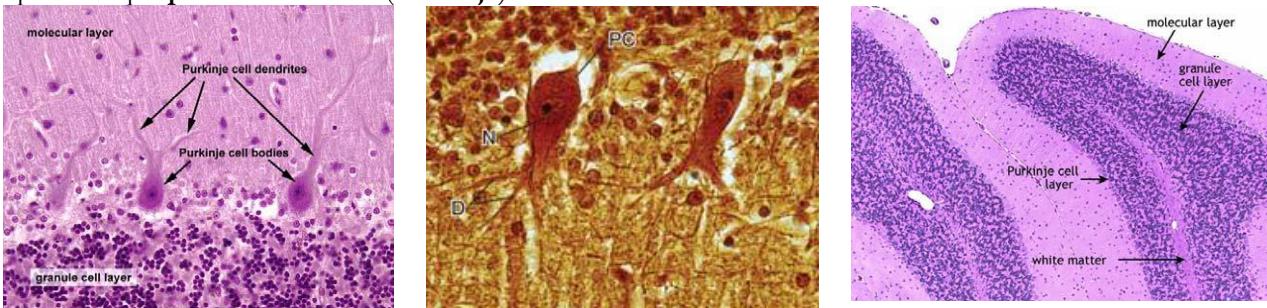
| Neuronal type  | % of neurons | Location                                                                                                                  |
|----------------|--------------|---------------------------------------------------------------------------------------------------------------------------|
| Pseudounipolar | 0.5          | Dorsal root ganglia of spinal cord<br>Cranial nerve ganglia of brain stem<br>Mesencephalic trigeminal nucleus in midbrain |
| Bipolar        | 0.5          | Retina, inner ear, taste buds                                                                                             |
| Multipolar     | 0.1          | Autonomic ganglia                                                                                                         |
| PNS            | 99.8         | Brain and spinal cord                                                                                                     |
| CNS            |              |                                                                                                                           |



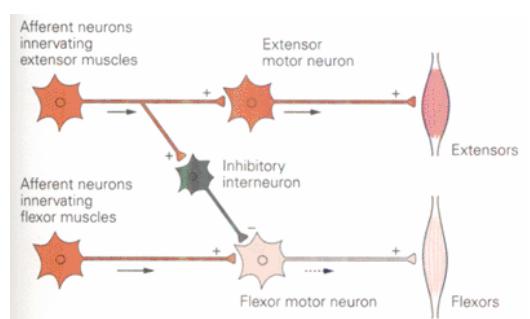
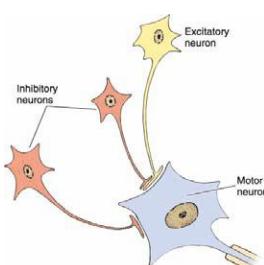
- Special shape: pyramidal neurons



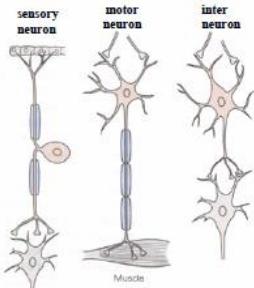
- Special shape: piriform neurons (Purkinje)



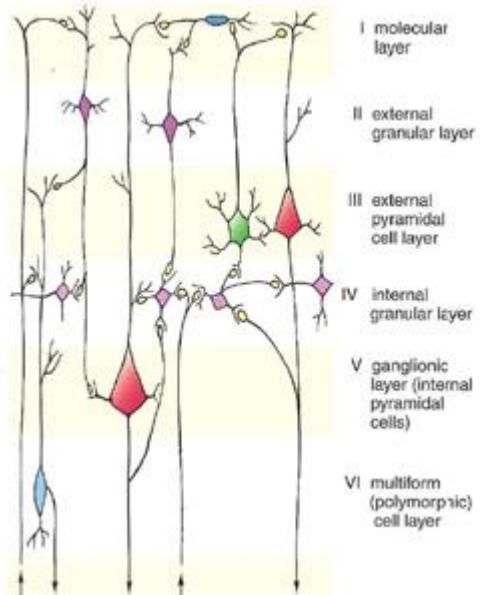
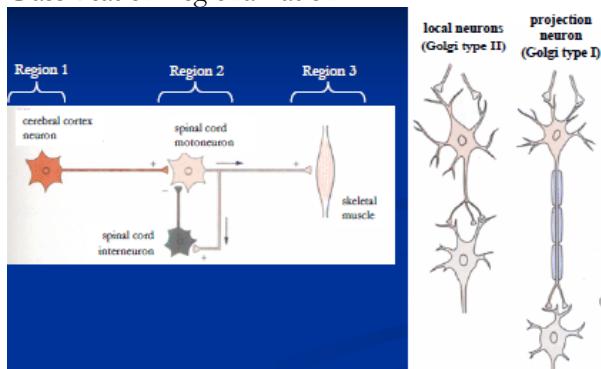
- Classification: effect on other neurons
  - **Excitatory:** glutamate
  - **Inhibitory:** GABA
- Inhibitory interneurons: feed forward inhibition



- Classification: major function

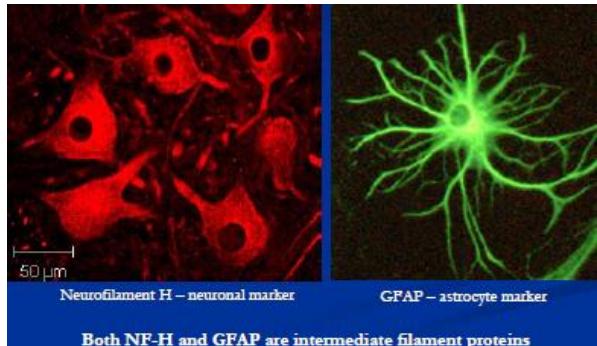
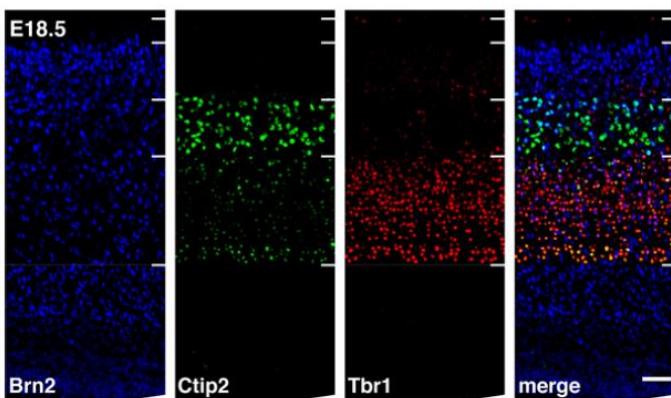


- Classification: regionalization



## Markers

- Cell-selective markers:** Nervous system cellular elements express specific proteins used to distinguish them
  - Neuronal markers
  - Glial markers
- Frequently, markers are **cytoskeletal proteins**
- Molecular markers can differentiate various (sub-sub-...) types of neurons

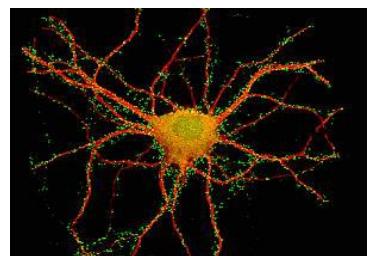


Layer-specific markers in the cerebral cortex

- Numerous layer-specific markers are known today

## Synapses

- Synapses are **specialized junctions** between neurons which facilitate transmission of information
- presynaptic cell** → synapse → **postsynaptic cell**
- Synapses also occur between neurons and target organs (muscles, glands, etc.)
- Classification: mechanisms of information transfer:

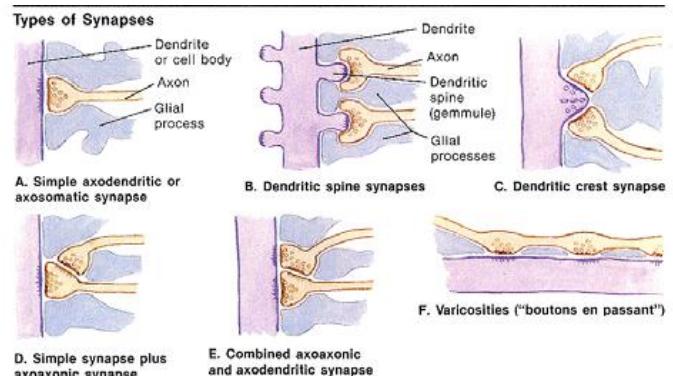
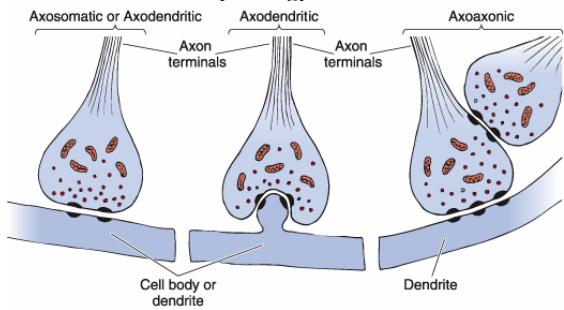


## Challenges to the neuron doctrine

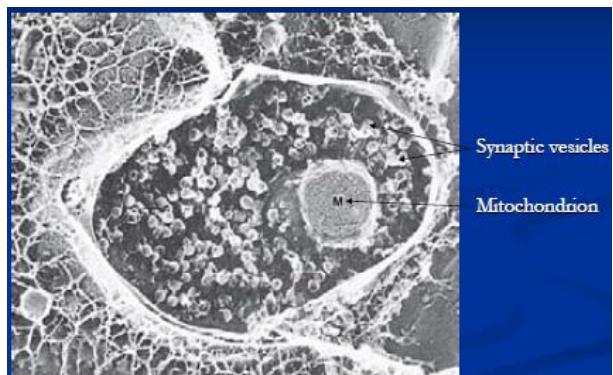
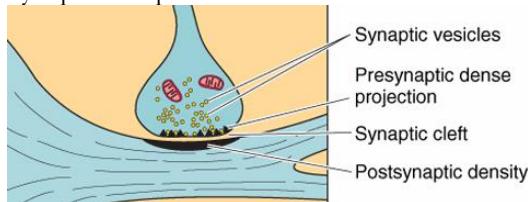
- Dendrites**, like axons, also have voltage gated ion channels and can generate electrical potentials which convey information to and from the soma
- Role of **glia** in processing neural information: neurons may not be the sole information processing cell in the nervous system

| Type of synapse | Distance between pre- and postsynaptic cell membranes | Cytoplasmic continuity between pre- and postsynaptic cells | Ultrastructural components                                    | Agent of transmission | Synaptic delay                                         | Direction of transmission |
|-----------------|-------------------------------------------------------|------------------------------------------------------------|---------------------------------------------------------------|-----------------------|--------------------------------------------------------|---------------------------|
| Electrical      | 3.5 nm                                                | Yes                                                        | Gap-junction channels                                         | Ion current           | Virtually absent                                       | Usually bidirectional     |
| Chemical        | 20–40 nm                                              | No                                                         | Presynaptic vesicles and active zones; postsynaptic receptors | Chemical transmitter  | Significant: at least 0.3 ms, usually 1–5 ms or longer | Unidirectional            |

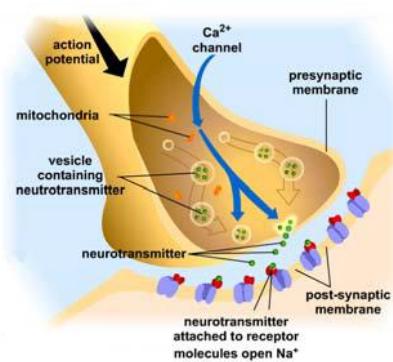
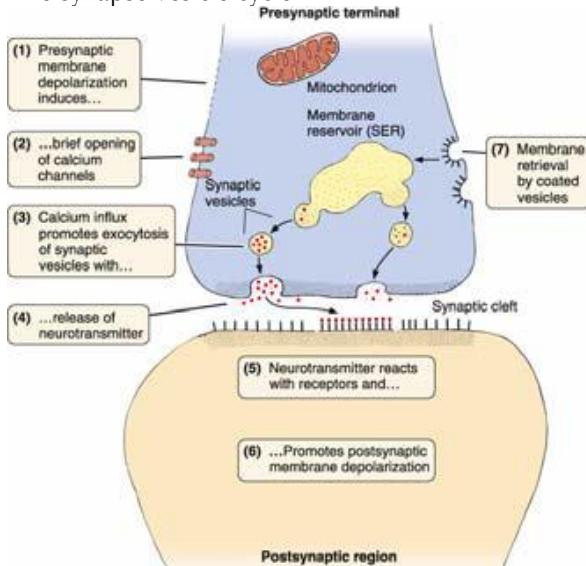
- Classification: morphology



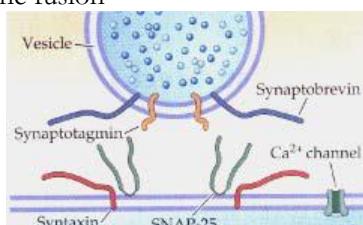
- Synapse composition:



- The synapse vesicle cycle:



- Synaptic vesicle fusion depends on **ligand-receptor-interaction** (vSNARE-tSNARE): calcium influx promotes Syntaxin-SNAP25 interaction → membrane fusion

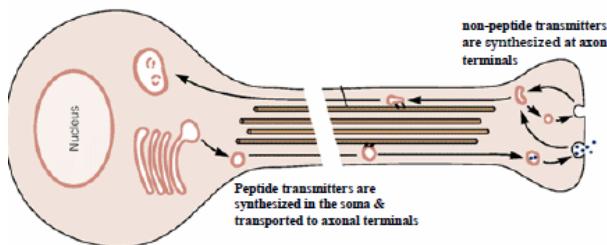


- Bacterial toxins are proteases degrading synaptic membrane proteins:
  - Tetanus toxin:** tetanus
  - Botulinum toxins:** botulism

## Neurotransmitters

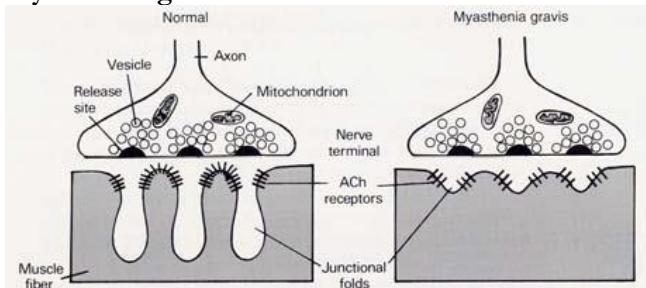
| Neurotransmitter                                                | Postsynaptic effect <sup>a</sup> | Type of vesicle                                          |
|-----------------------------------------------------------------|----------------------------------|----------------------------------------------------------|
| ACh                                                             | Excitatory                       | Small, clear                                             |
| Glutamate                                                       | Excitatory                       | Small, clear                                             |
| GABA                                                            | Inhibitory                       | Small, clear                                             |
| Glycine                                                         | Inhibitory                       | Small, clear                                             |
| Catecholamines<br>(epinephrine,<br>norepinephrine,<br>dopamine) | Excitatory                       | Small dense-core,<br>or large<br>irregular<br>dense-core |
| Serotonin (5-HT)                                                | Excitatory                       | Large,<br>dense-core                                     |
| Histamine                                                       | Excitatory                       | Large,<br>dense-core                                     |
| ATP                                                             | Excitatory                       | Small, clear                                             |
| Neuropeptides                                                   | Excitatory<br>and inhibitory     | Large,<br>dense-core                                     |

- Small-molecule vs. peptide neurotransmitters:



## Diseases

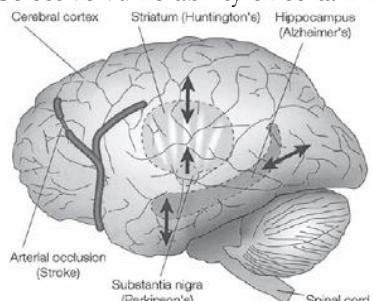
- Myasthenia gravis:



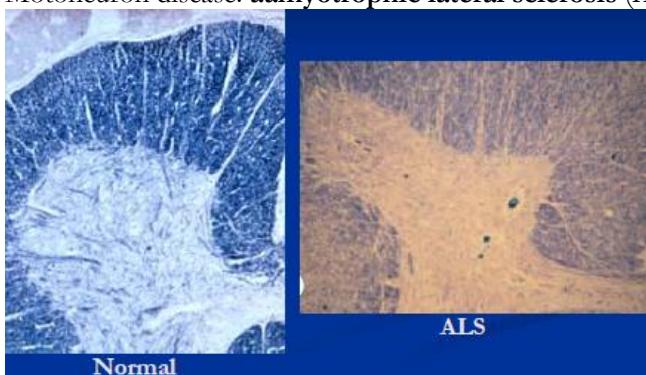
- Ptosis of eyelids



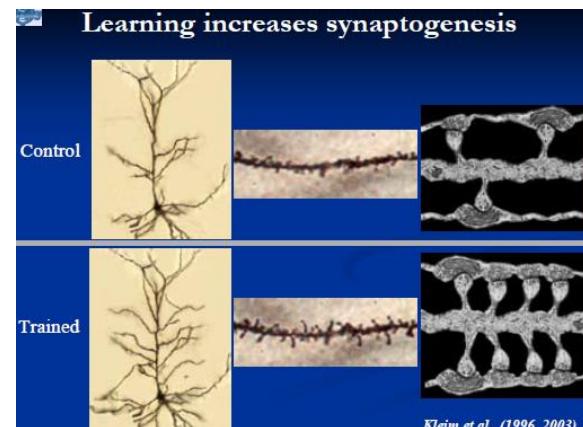
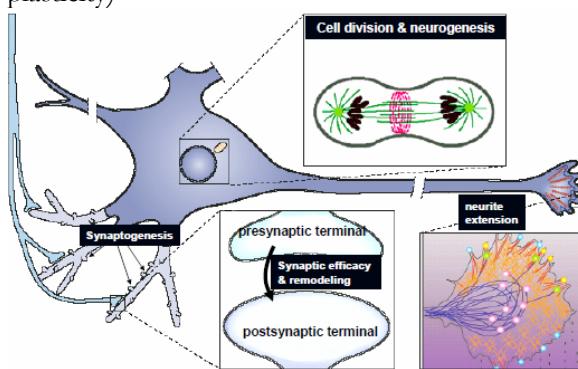
- Selective vulnerability of certain neurons in different neurodegenerative disorders:



- Motoneuron disease: **amyotrophic lateral sclerosis (ALS)**

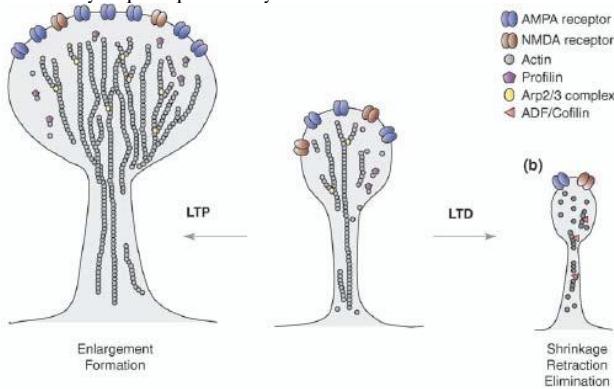


- **Neuroplasticity:**
  - continuous process in reaction to neuronal activity and neuron injury, which involves modulation of structural and functional processes of axons, dendrites, and synapses
  - Both a substrate of learning and memory and a mediator of responses to neuronal injury (compensatory plasticity)



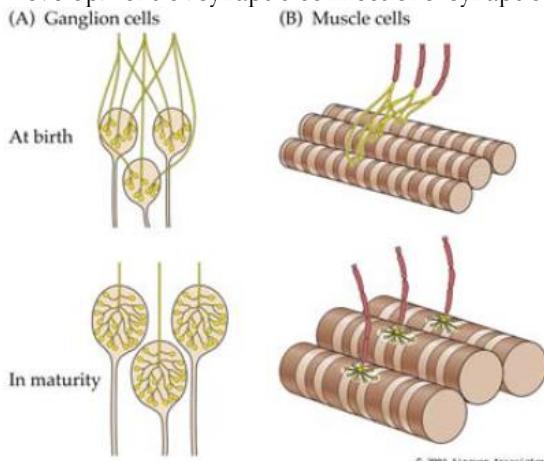
## Neurogenesis

- Synaptic plasticity

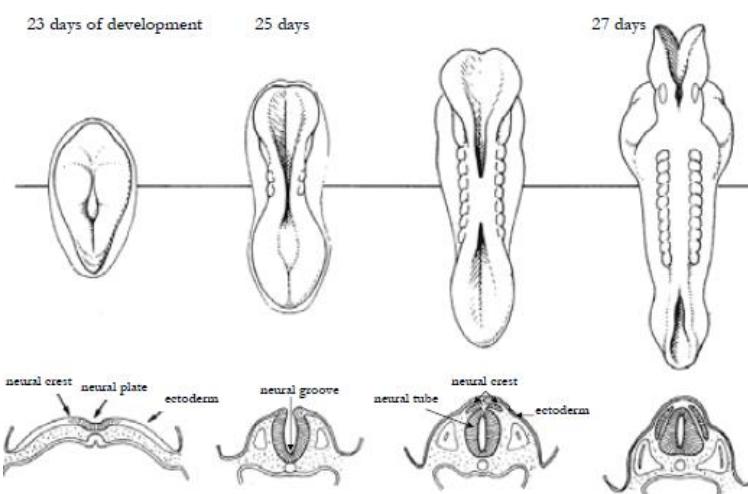


*Current Opinion in Neurobiology*

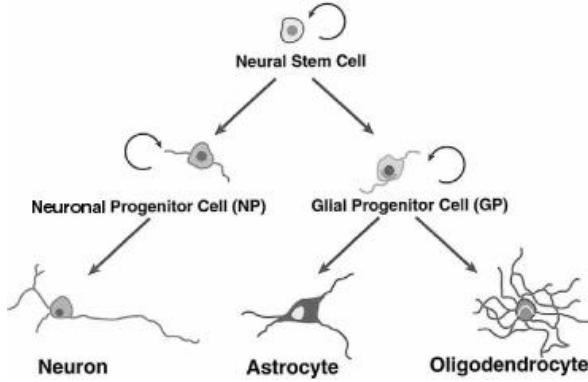
- Development of synaptic connections: synaptic refinement and pruning



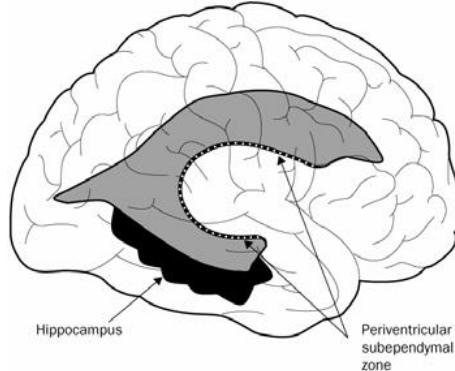
*© 2001 Sinauer Associates.*



- The neural tube is composed of stem cells which generate all neurons & glia
- Neural induction is orchestrated by signals from adjacent tissue
- Neurons cannot undergo mitosis; only their progenitors can!

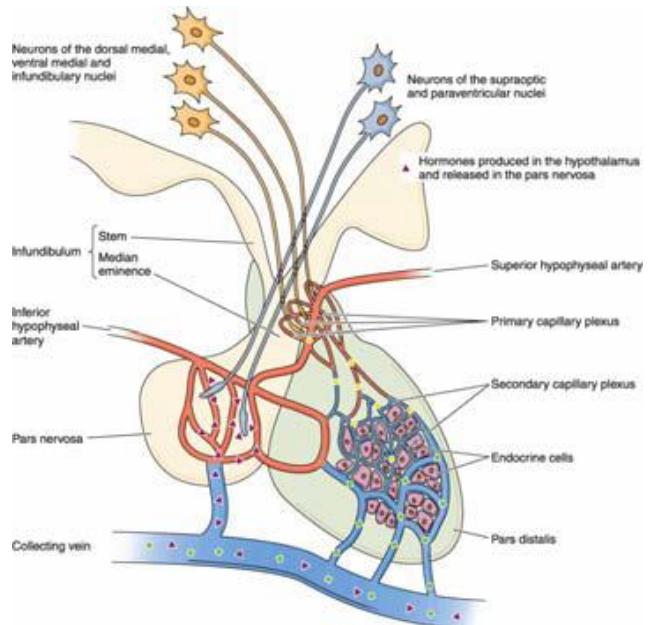


- Stem cells in adult human brain:



## Neuroendocrine cells

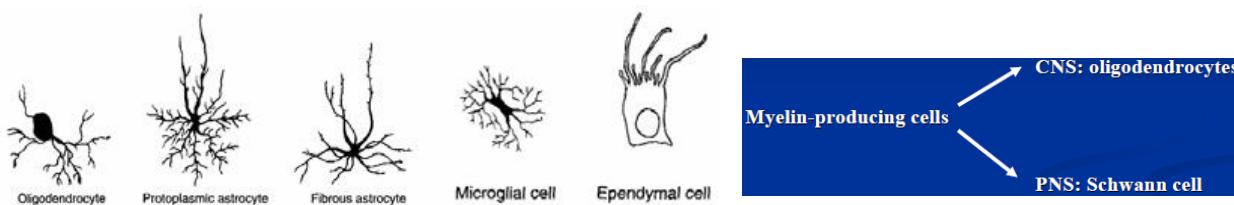
- Also called: neurosecretory cells
- Hormones:
  - Oxytocin
  - Vasopressin
- Diffuse Neuroendocrine System (DNES)**
  - Synonyms: APUD (Amine Precursor Uptake and Decarboxylation) cells; paraneurons
  - Not designated as neurons, but closely related to neurons on the basis of:
    - Fine structure (possession of synaptic vesicle-like granules)
    - Metabolism (production of neurotransmitter-like substances)
    - Origin (neural crest)
  - Apudomas** are tumors derived from polypeptide-secreting cells of the DNES. Clinical symptoms depend on the specific chemical messenger produced



## Glia cells

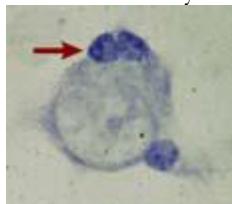
### Neuroglia (glia)

- Numerous cells which surround both cell bodies and processes of neurons and occupy the interneuronal spaces
- Functioning in the physical and metabolic support of neurons
- Major types of glia (Golgi stain):

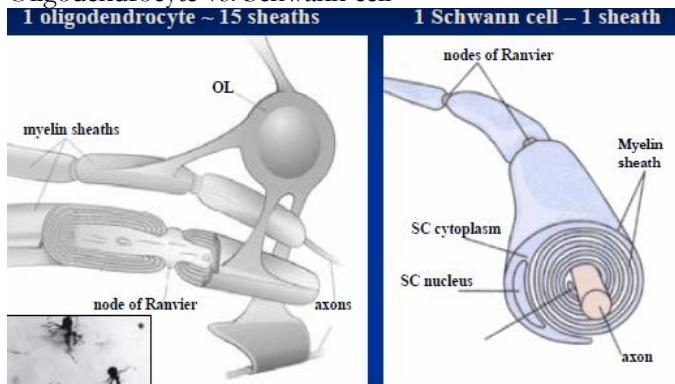


## Oligodendrocytes

- O. function in electrical insulation and in myelin production in the CNS
- **Features:**
  - Contain fewer processes than astrocytes, with sparse branching
  - Smaller than astrocytes
  - Location: CNS gray & white matter
  - Abundant RER, Golgi; MT & Mit also present
  - Types:
    - **Interfascicular:** myelin
    - **Satellite:** closely to cell bodies of large neurons

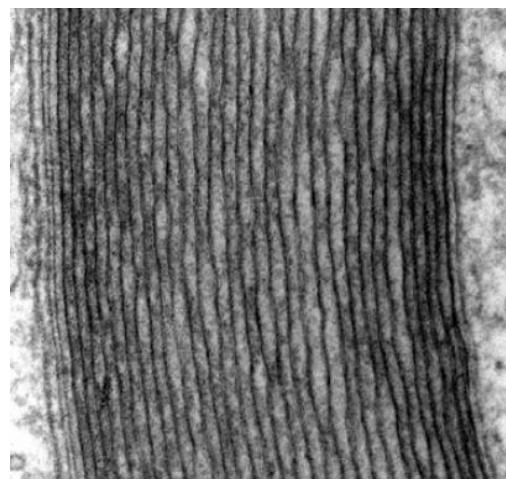
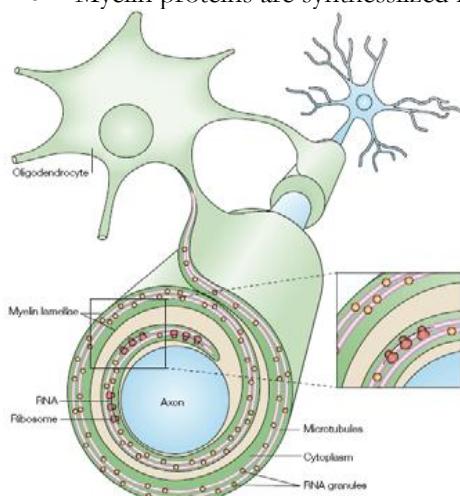


- Oligodendrocyte vs. Schwann cell

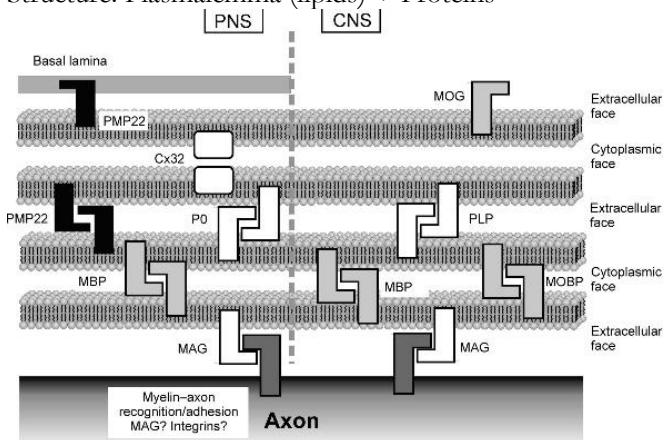


- **Myelin:**

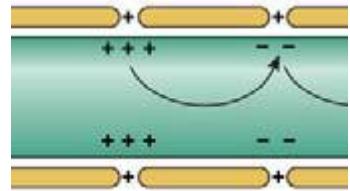
- Plasmalemma of OL organized into a sheath that is wrapped several times (up to 50) around the axon
- Myelin proteins are synthesized locally



- Structure: Plasmalemma (lipids) + Proteins

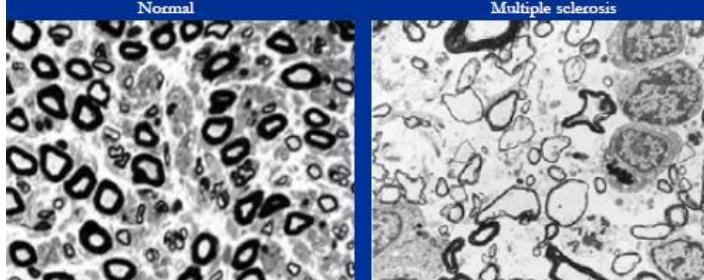
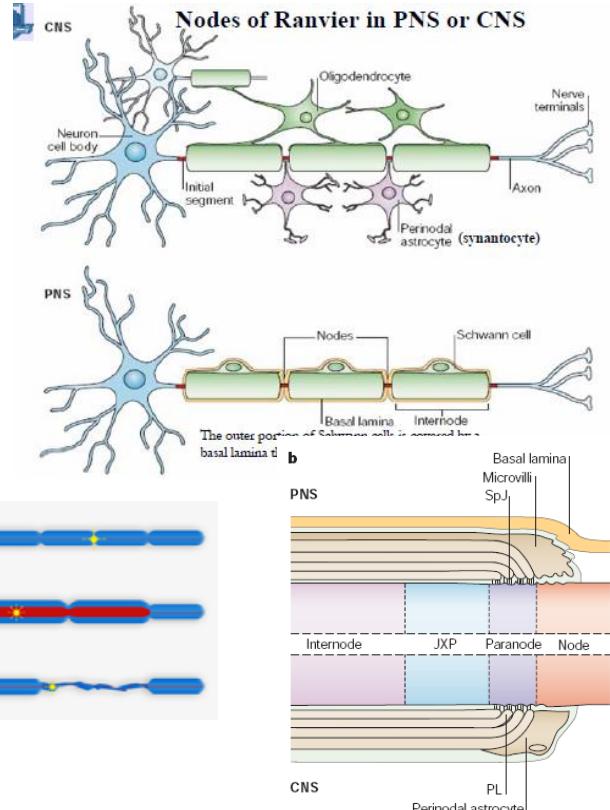


- Advantage of myelination:
  - Unmyelinated axon: **continuous conduction**
  - Myelinated axon: **saltatory conduction**
- Functions:
  - Prevents the outward movement of the excess  $\text{Na}^+$  in the axoplasm associated with the action potential
  - A track for regeneration - in PNS only (unmyelinated axons & CNS axons do not regenerate)



### Nodes of Ranvier

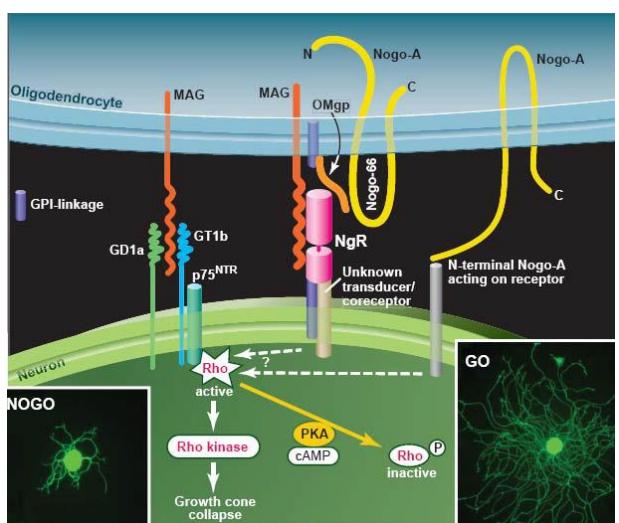
- Interruptions in the myelin sheath at regular intervals along the length of the axon, exposing the axon
- Voltage-gated  $\text{Na}^+$  channels of the axolemma are clustered mostly at the nodes of Ranvier
- The distance between two nodes is called an **internode** and consists of one Schwann cell (in the PNS). The length of the internode varies between 1 and 2 mm
- Myelinated fibers consist of molecular domains:
  - **Internode**
  - **Paranode**
  - **Juxtaparanode**
  - **Node of Ranvier**
- Each molecular domain has a specific molecular "signature"
  - Nodal:  $\text{NaCh}$
  - Paranodal: Caspr (contactin-associated protein)
  - Juxtaparanodal: K
- Defects in molecular domain components lead to diseases (e.g. *Shiverer* mice - MBP mutation)
- **Multiple sclerosis:** demyelination in white matter



- Strategies to promote endogenous remyelination:
  - (a) strategy 1 enhance OPC recruitment into demyelinated area
  - (b) strategy 2 promote OPC differentiation and remyelination
  - (c) strategy 3 remove factors that inhibit remyelination
- Specific markers point the stage of oligodendrocytes development:
  - Oligodendrocyte precursor
  - Differentiation
  - Remyelinated axon
- Oligodendrocytes inhibit axonal growth:

### Schwann cells

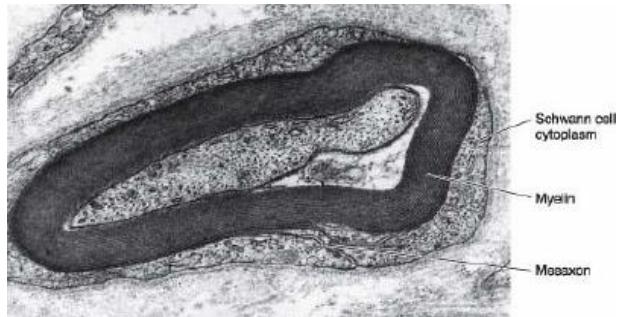
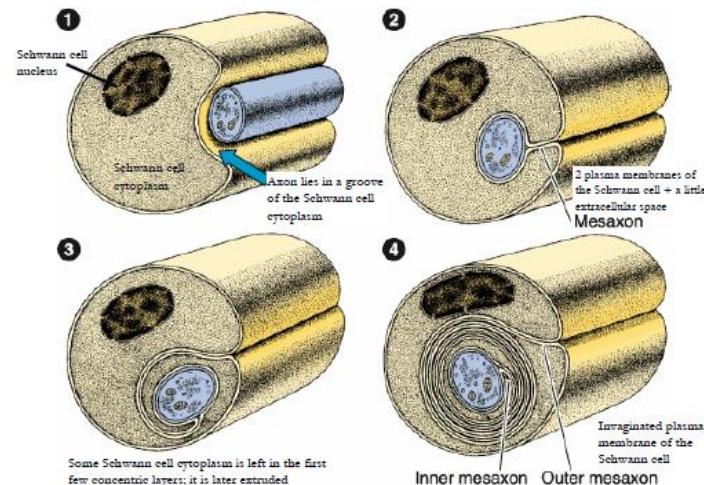
- Myelinate axons of the **peripheral nervous system** (PNS)
- **Neurilemma:** layer of Schwann cell cytoplasm ensheathing the axon
- Schwann cells respond quickly to injury and aid axon regeneration



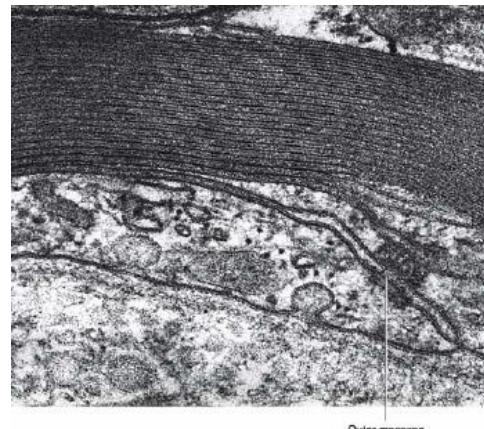
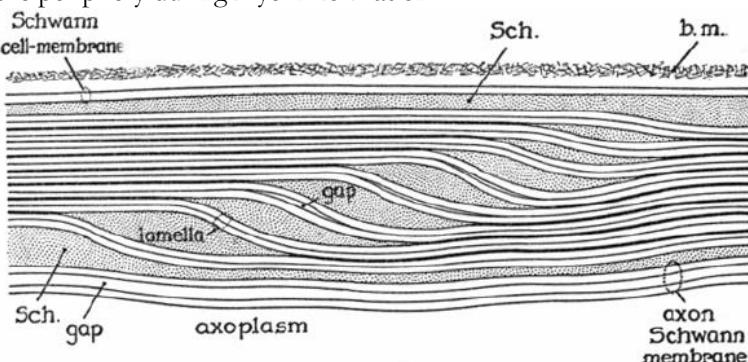
- Schwann cells are associated with a number of demyelinating disorders (Charcot-Marie-Tooth disease and Guillain-Barre Syndrome), infected during leprosy and are responsible for the tumors in both neurofibromatosis type 1 and type 2
- Schwann cell membrane wrapping stages:

### Mesaxon

- A pair of parallel oligodendrocyte / Schwann cell membranes
- Edge-to-edge contact of the oligodendrocyte / Schwann cell membrane



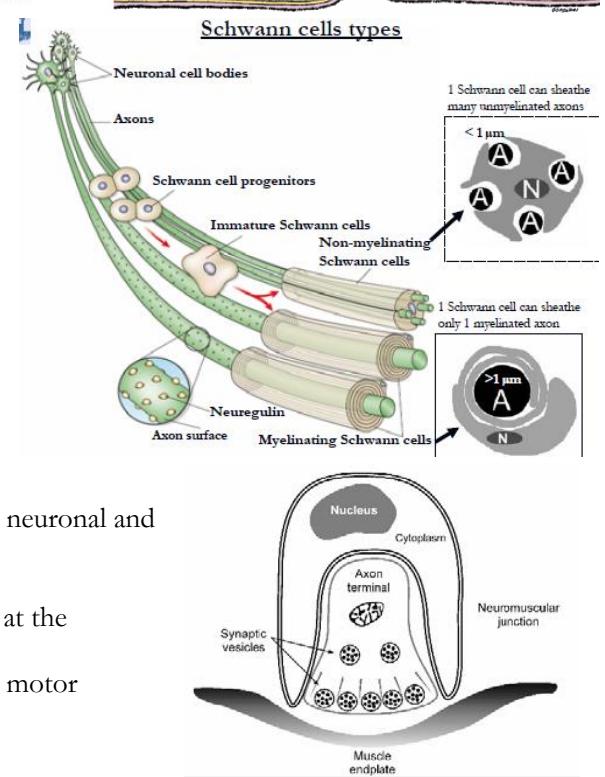
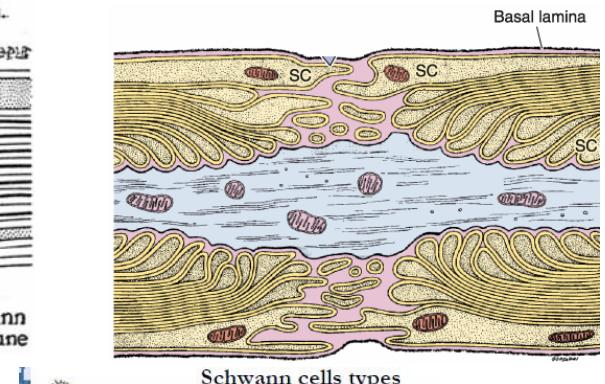
- **Insiures (clefts)** of Schmidt-Lanterman: The clefts formed by oligodendrocyte / Schwann cell cytoplasm that is not displaced to the periphery during myelin formation



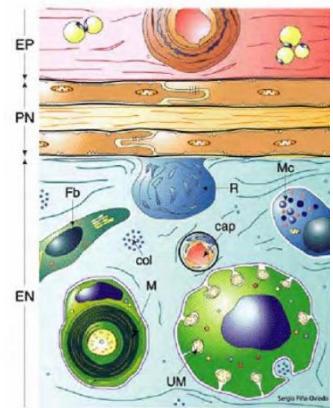
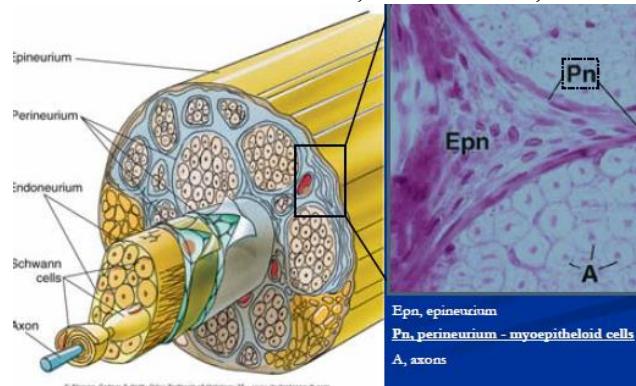
- Schwann cells processes:
  - At the nodes of Ranvier, the Schwann cells' cytoplasm projects interdigitating processes which are in close contact of the axolemma
  - This contact acts as a type of barrier to the movement of material in and out of the periaxonal space

### Nerve fibers

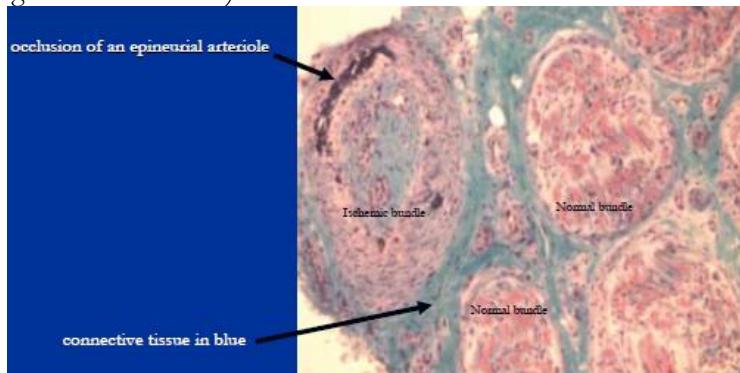
- Nerves are bundles of nerve fibers surrounded by connective tissue sheaths
- Nerve fibers: axons + myelin sheaths
  - Myelinated nerve fibers
  - Unmyelinated nerve fibers - do not have nodes of Ranvier
    - PNS: enveloped within simple clefts of Schwann cells
    - CNS: non-enveloped, i.e. run free among the other neuronal and glial processes
- **Perisynaptic Schwann cells:**
  - Ensheath terminal axonal branches and synaptic boutons at the neuromuscular junction
  - Their basal lamina fuses with that of the muscle fibre and motor endplate



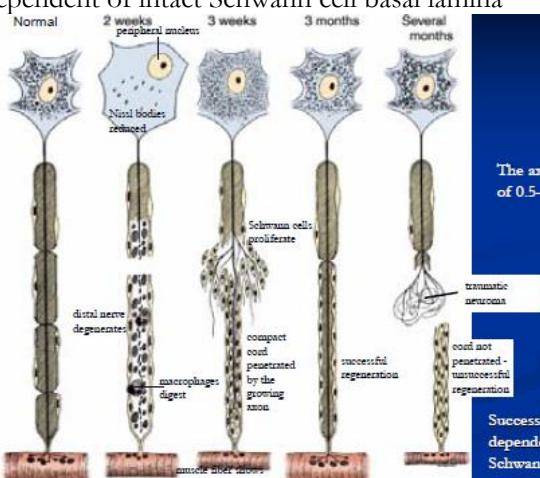
- Cellular elements of peripheral nerve compartments:
  - **Epineurium:** collagen, some adipocytes, vessels
  - **Perineurium:** myoepitheloid cells (TJ - blood-nerve barrier), collagen
  - **Endoneurium:** 90% Schwann cells, 5% fibroblasts, 5% other cells



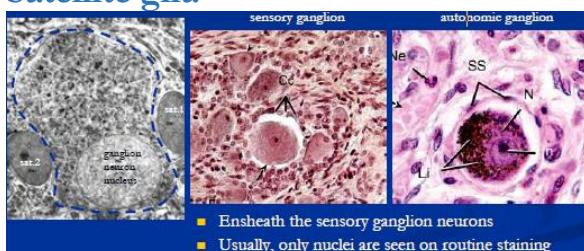
- **Charcot-Marie-Tooth disease (CMT):**
  - One of the most common inherited neurological disorders, affecting approx. 1 in 2,500 people in the United States
  - Comprises a group of disorders caused by gene mutations affecting proteolipid constituent of myelin and associated with Schwann cell dysfunction
  - Onset of symptoms of CMT is most often in adolescence or early adulthood. Progression of symptoms is very gradual and includes muscle weakness and atrophy in the extremities, and the degeneration of sensory nerves results in a reduced ability to feel heat, cold, and pain
- **Ischemic neuropathy** (e.g. diabetes mellitus)



- Response after peripheral nerve fiber injury
  - The axon grows at a rate of 0.5-3 mm/day
  - Successful regeneration is dependent of intact Schwann cell basal lamina

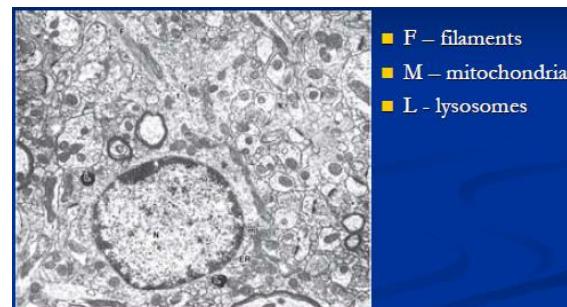
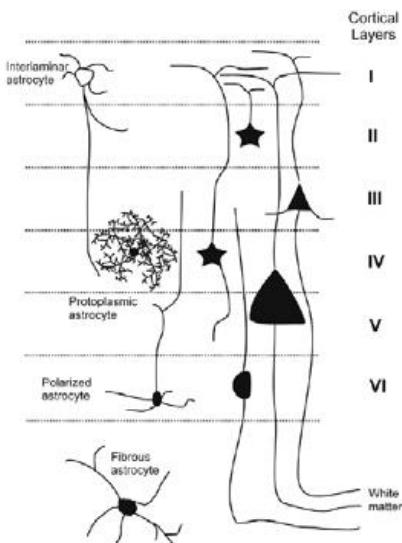
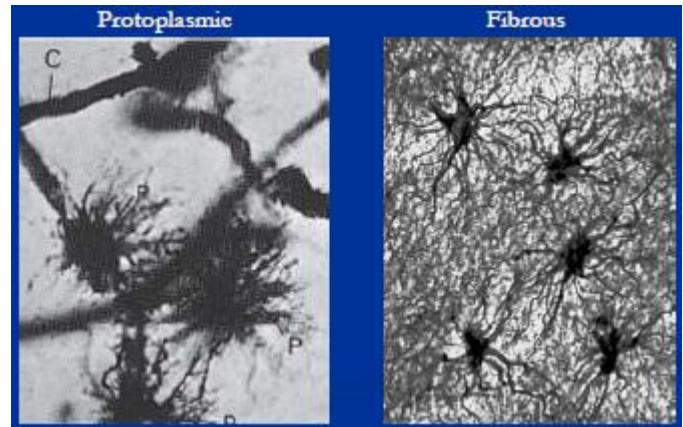
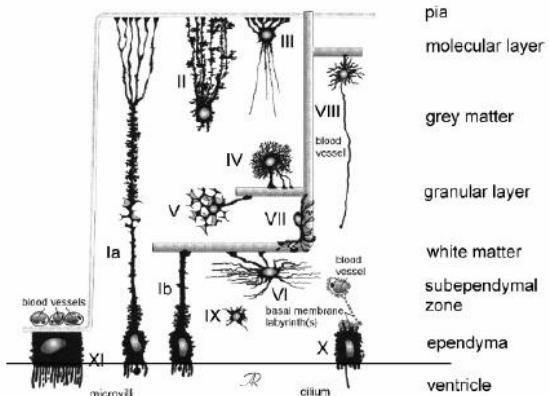


## Satellite glia

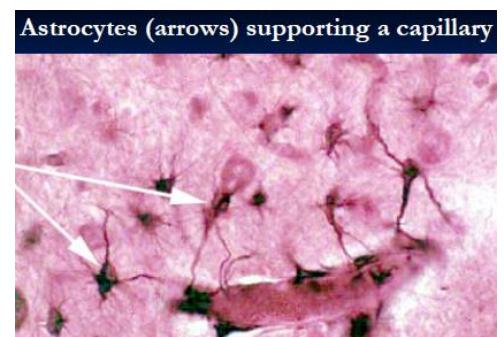
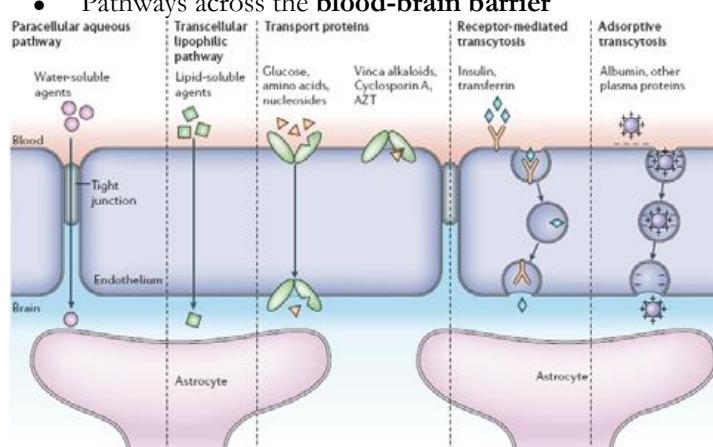


## Astrocytes

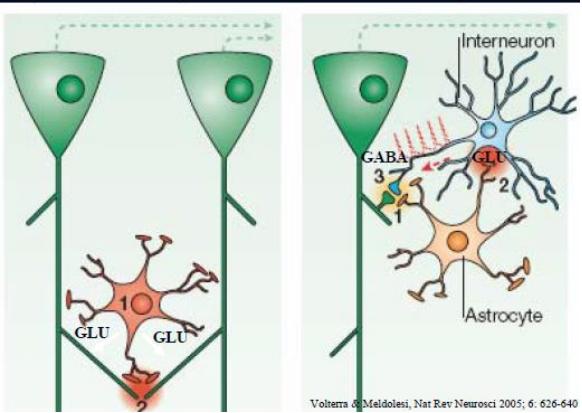
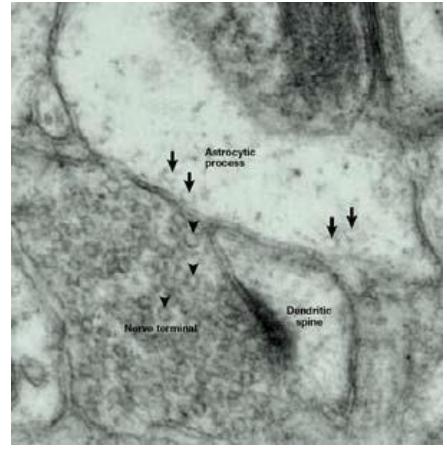
- Glial cells with multiple supportive and barrier in CNS
- Morphological classification:
  - Protoplasmic:** many short-branchend processes
  - Fibrous:** few long processes
- Distribution:
  - Protoplasmic: gray matter
  - Fibrous: white matter
- Morphological types:
  - Ia – pial tanycyte;
  - Ib – vascular tanycyte;
  - II – radial astrocyte (Bergmann glial cell);
  - III – marginal astrocyte;
  - IV – protoplasmic astrocyte;
  - V – velate astrocyte;
  - VI – fibrous astrocyte;
  - VII – perivascular astrocyte;
  - VIII – interlaminar astrocyte;
  - IX – immature astrocyte;
  - X – ependymocyte;
  - XI – choroid plexus cell



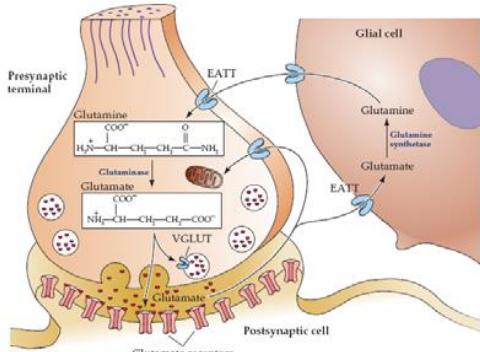
- Glial fibrillary acidic protein (GFAP)** is a marker for astrocytes. It is an intermediate filament protein.
- Functions:**
  - Structural support of the brain
  - Release trophic factors
  - Maintain **neuronal homeostasis**: take up ions & transmitters
  - Participate in blood-brain barrier
  - Modulate **synaptic function**
  - Radial glia**: guide neurons in development and perform the role of progenitors
- There are **tight structural associations** between neurons & astrocytes
- Pathways across the **blood-brain barrier**



- Agents modifying brain endothelial function and BBB tightness:
  - Agents that **impair BBB function**:
    - Bradykinin, histamine, serotonin, glutamate
    - Prine nucleotides: ATP, ADP, AMP
    - Adenosine, platelet-activating factor
    - Phospholipase A2, arachidonic acid, prostaglandins, leukotrienes
    - Interleukins: IL-1 $\alpha$ , IL-1 $\beta$ , IL-6
    - Tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), macrophage-inhibitory proteins
    - Complement-derived polypeptide C3a
    - Free radicals, nitric oxide
  - Agents that cause **BBB tightening** and **improved function**
    - Steroids
    - Elevated intracellular cyclic AMP
    - Adrenomedullin
    - Noradrenergic agents
- Astrocytes contain synaptic-like microvesicles

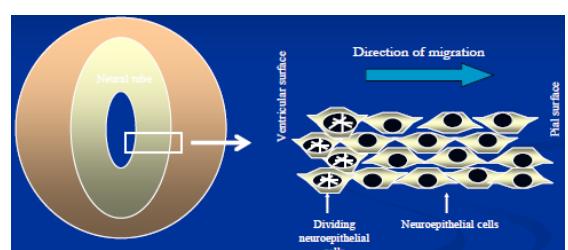


- Astrocytes & glutamate cycle



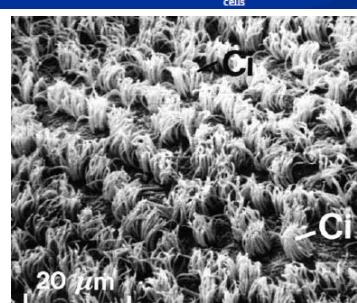
## Genesis of glia

- CNS originates from a single layer of cells: the **neuroepithelium**
- Neurons migrate on a specialized elongated cell: the **radial glial cell** that spans the whole cortical wall fro the ventricular zone to the pial surface. Radial glia are **neuronal precursor cells**.
- Astrocytes get activated after brain injury
- Astrocyte tumor: **Astrocytoma**



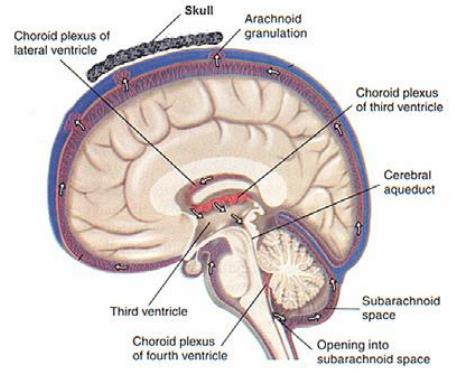
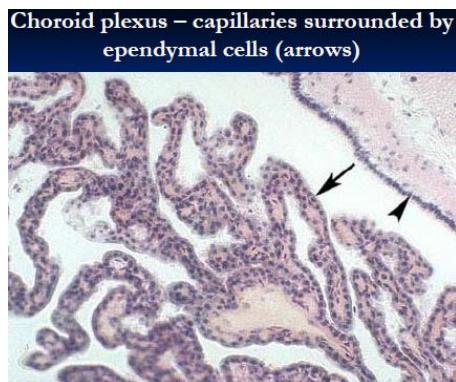
## Ependymal cells (=ependymocytes)

- Epi + endynein (sink into)
- Line the ventricular walls, separate cerebrospinal fluid from white matter
- Ciliated
- Types:
  - "classical"
  - Tanyocyte



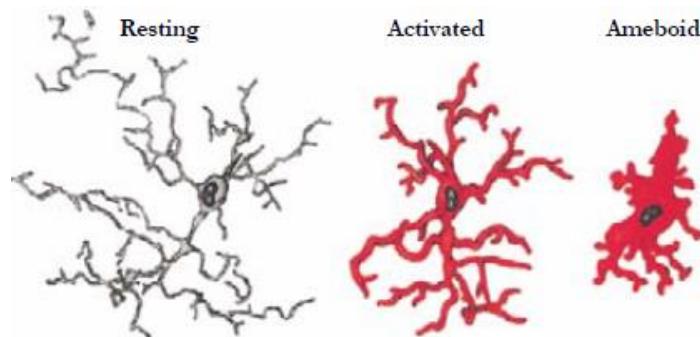
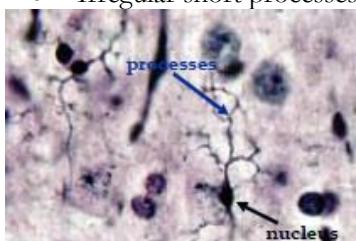
## Choroid plexus

- Invaginated folds of pia mater, rich in dilated fenestrated capillaries, that penetrate the interior of the brain ventricles
- Composed of loose connective tissue (pia mater) covered by cuboidal or columnar epithelium made of ion-transporting cells
- Main function: elaborate cerebrospinal fluid

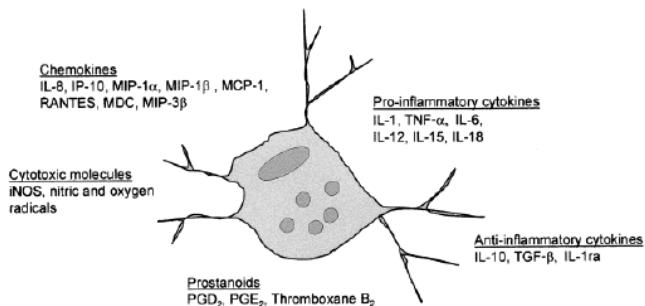


## Microglia

- Characteristics:
  - Scant cytoplasm
  - Oval to triangular nucleus
  - Irregular short processes

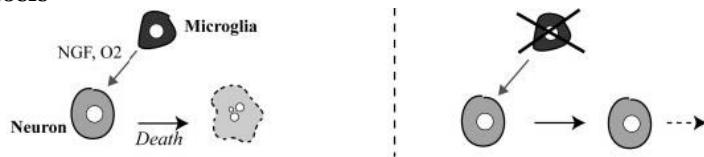


- Inflammatory and immunoregulatory mediators produced by activated microglia

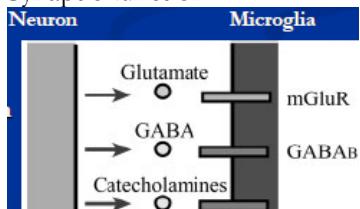


- Additional functions:

- Developmental apoptosis**



- Synaptic function



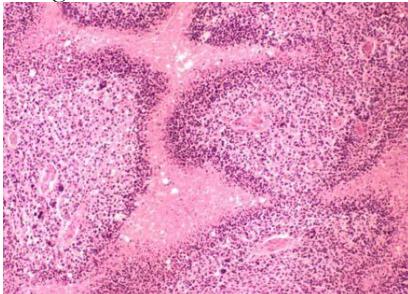
- Derivation of glia

- Neural tube derivatives:**
  - Astrocytes
  - Oligodendrocytes
  - Ependymal cells

- Neural crest derivates
  - Schwann cells
  - Satellite cells of peripheral ganglia
  - Cells of the arachnoid and pia mater
- Generation of glia from stem/progenitor cells. Glial cell can undergo mitosis

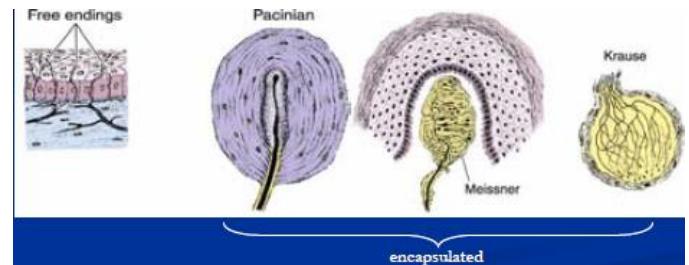


- Malignant tumor of neural stem cells: **Glioblastoma**



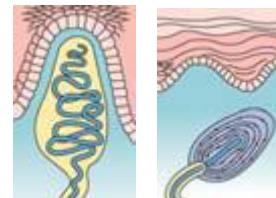
### Sensory skin nerve endings

- Skin is the most sensory receptor
- Types of receptors:
  - **Free nerve endings:** epidermis, hair follicles, and cutaneous glands
  - **Encapsulated** and expanded receptors: dermis and subcutaneous tissue
- Encapsulated corpuscles are not necessary for cutaneous sensation - they act as **mechanoreceptors**
- Free endings:
  - **Thermoreceptors:** respond to temperature differences of about 2° C
    - Warmth receptors
    - Cold receptors
    - Temperature-sensitive nociceptors
  - **Nociceptors:** responsible for pain perception
    - Mechanical stress or damage
    - Extremes in heat or cold
    - Chemical compounds such as bradykinin, serotonin, and histamine

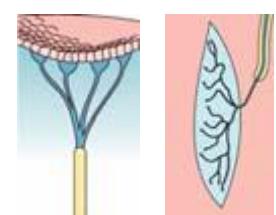


### Skin mechanoreceptors:

- **Meissner's corpuscles**
  - Most mechanoreceptors of hairless skin (40% of hand)
  - Connective tissue capsule + Schwann cell lamellae
  - Low-frequency vibration (30-50 Hz) for rough objects



- **Pacinian corpuscles**
  - Less frequent (20% of hand)
  - Inner core of membrane lamellae → fluid → outer lamella
  - High frequency vibrations (250-350 Hz) - fine textures

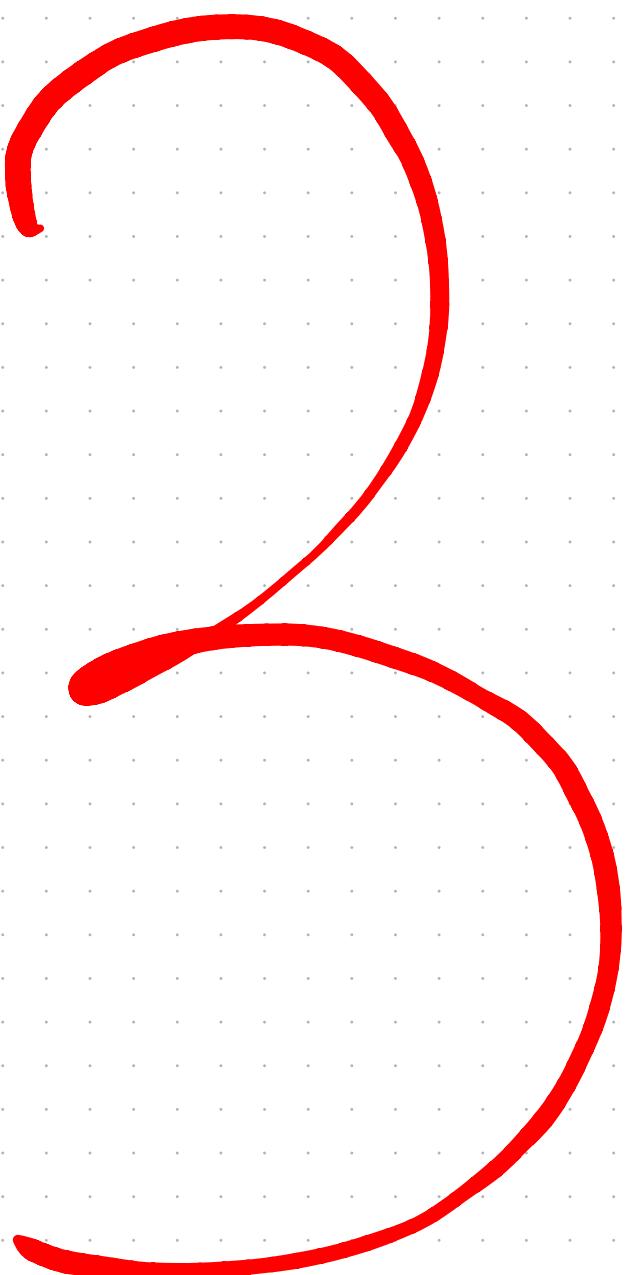


- **Merkel's disks:**
  - Epidermal (20% of hand)
  - Light pressure - discrimination of shapes, edges



- **Ruffini's corpuscles:**
  - Deep in the skin + in ligaments & tendons
  - Sensitive to the cutaneous stretching produced by digit or limb movements





# Embryology

## Meiosis / Gametogenesis

- Gr. "embryon": **unborn**. The sequence of events in the organism from **fertilization to birth**.

### Division of embryology

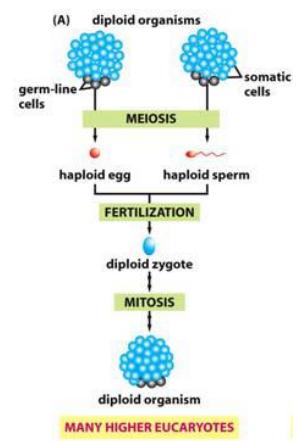
- General embryology:**
  - Gametogenesis:** conversion of **germ cells** into **male and female gametes**
  - 1st week of development: **ovulation** to **implantation**
  - 2nd week of development: **bilaminar germ disc**
  - 3rd week of development: **trilaminar germ disc**
  - 3rd to 8th week: **embryonic period**
  - 3rd month to birth: **fetus** and **placenta**
- Special embryology:**
  - Skeletal system
  - Cardiovascular system
  - Respiratory system
  - Nervous system
  - Etc...

## Meiosis

- Special form of nuclear division involved in **sexual reproduction**
- Genome of 2 parents mix to generate offspring that is genetically distinct from either parents
- Occurs in **diploid** organisms (each cell contains 2 sets of chromosomes, one inherited from each parent)
- Specialized haploid (1 set of chromosomes) cells carry out sexual reproduction
- Finally, both haploid cells fuse and mix their genomes, restoring the **diploid** state.

### Meiosis vs. mitosis

- Meiosis: diploid precursor cell gives rise to haploid progeny cells (**gametes** = eggs (ova) & sperm (spermatozoa);  $2n \rightarrow 4n \rightarrow 2n \rightarrow 1n$ )
- Mitosis:** diploid precursor cell gives rise to diploid progeny cells;  $2n \rightarrow 4n \rightarrow 2n$
- In vertebrates only diploid cells proliferate: haploid gametes exist only briefly, do not divide at all, and are highly specialized for sexual fusion
- Meiosis creates **genetic diversity**:
  - Each individual gamete contains either the **maternal** or **paternal** version of each chromosome: choice of maternal or paternal **occurs independently** and **randomly** for each pair of homologous chromosomes
  - Crossing-over:** maternal & paternal versions of each chromosome undergo genetic recombination during meiosis
  - Sexual reproduction gives organisms a **competitive advantage**
- Nondisjunction:** homologs fail to separate properly
  - Some of the haploid gametes produced lack a particular chromosome, while others have more than one copy of it
  - Cells with correct number of chromosomes: **euploid**
  - Cells with an abnormal number of chromosomes: **aneuploid**; most embryos die
  - Down syndrome:** leading single cause of mental retardation; caused by an extra copy of chromosome 21
  - Klinefelter syndrome:** nondisjunction of the XX homologues; extra X chromosome

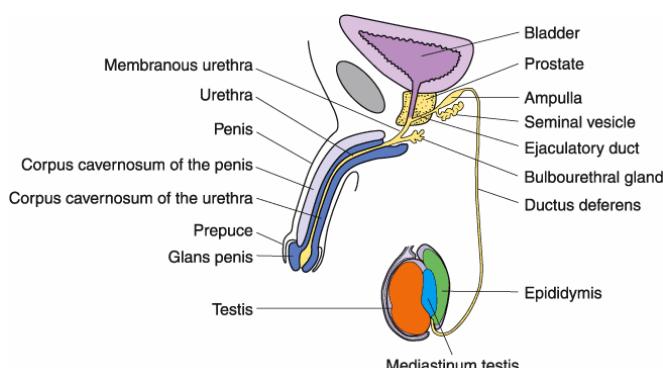


*Basic plan of meiosis:  
1 S-phase + 2 divisions*

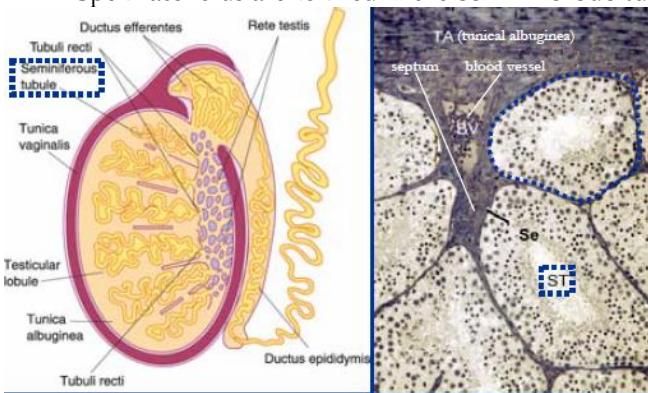
- Meiosis I
  - Prophase I
    - ✓ Leptotene (Gr., thin threads)
    - ✓ Zytotene (Gr., paired threads)
    - ✓ Pachytene (Gr., thick threads)
    - ✓ Diplotene (Gr., two threads)
    - ✓ Diakinesis (Gr., moving through)
  - Metaphase I
  - Anaphase I
  - Telophase I
- Meiosis II
  - Prophase II
  - Metaphase II
  - Anaphase II
  - Telophase II

## Gametogenesis

### Spermatogenesis

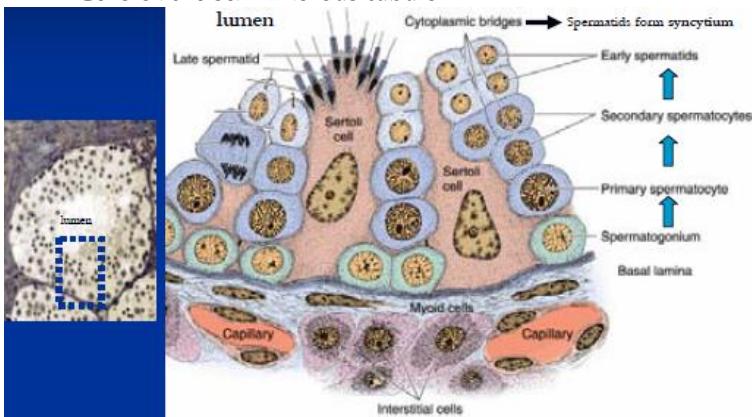


- Process by which spermatozooids are formed
- Spermatozooids are formed in the testis
- Spermatozooids are formed in the **seminiferous tubules** of the testis

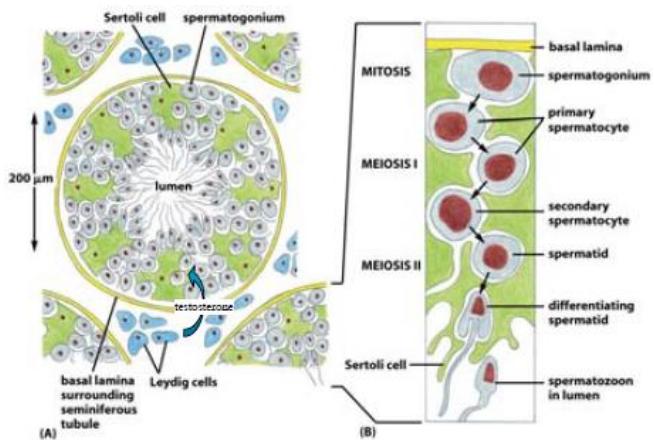


About 1000 seminiferous tubules are present in the two testes, for a total length of nearly 0.5 km

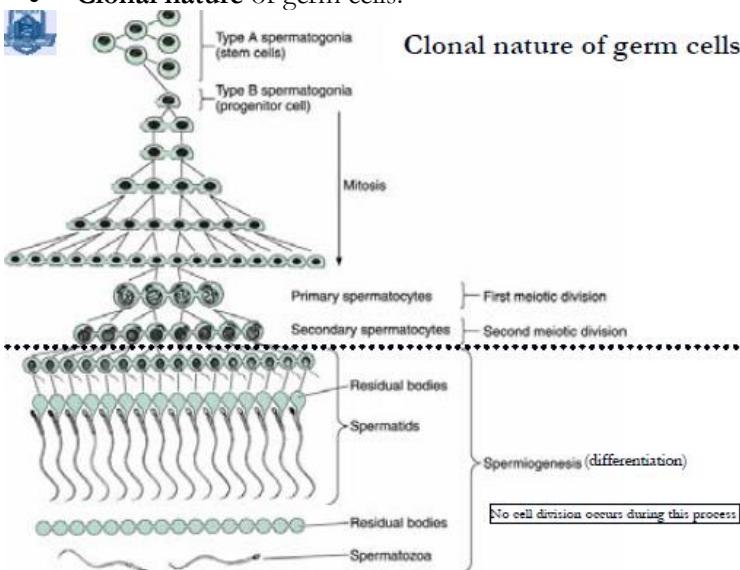
- Cells of the seminiferous tubule:



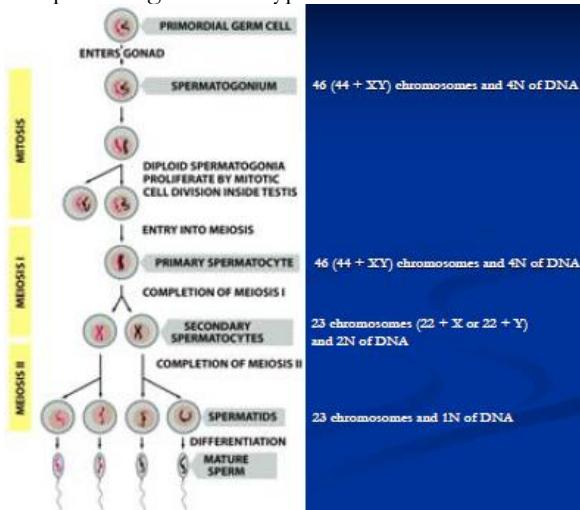
- Cells of the seminiferous tubule



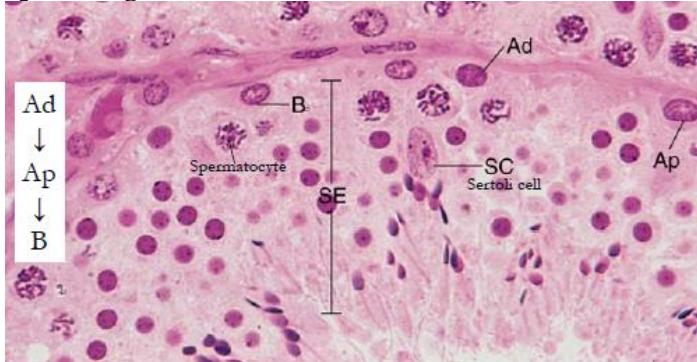
- Clonal nature of germ cells:



- Stages of spermatogenesis & type of division



- Spermatogonia are stem cells:



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- Type A spermatogonia – stem cells
  - Ad (dark) – reserve cells (enter division rarely, replicate Ad & Ap spermatogonia)
  - Ap (pale) – actively dividing stem cells, replicate Ap & B spermatogonia
- Type B spermatogonia – progenitors, give rise to primary spermatocytes

- Spermatocytes are the largest cells of seminiferous tubules:

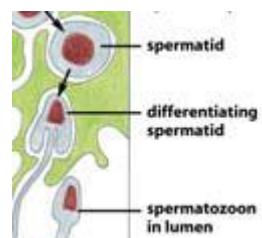
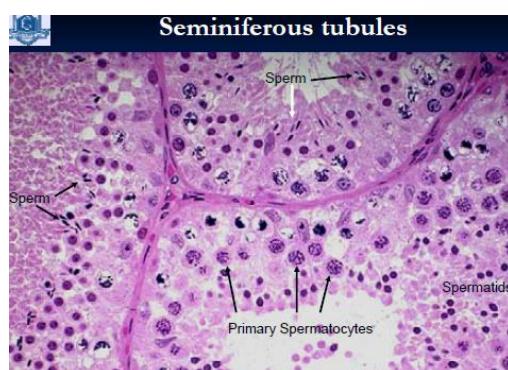
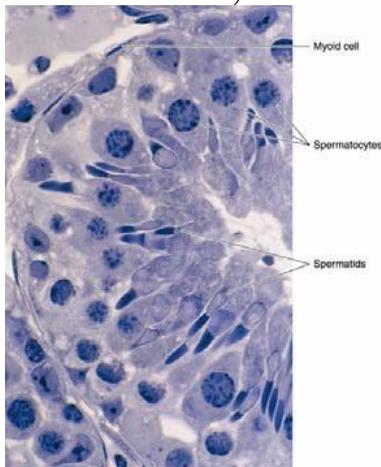
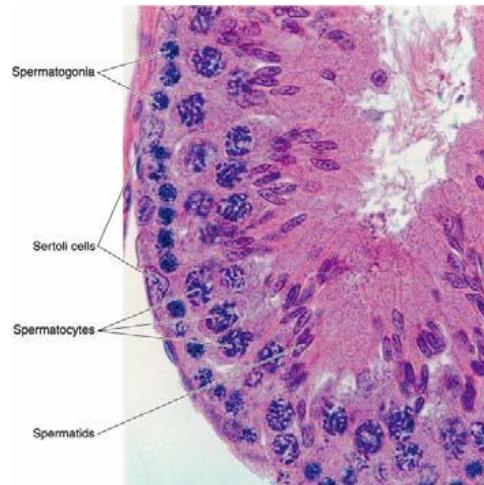
- Migrate from basal into adluminal compartment
- Migrate between adjacent Sertoli cells
- Large nucleus with chromosomes in various stages of condensation
- Primary spermatocytes: meiosis I
- Secondary spermatocytes: meiosis II

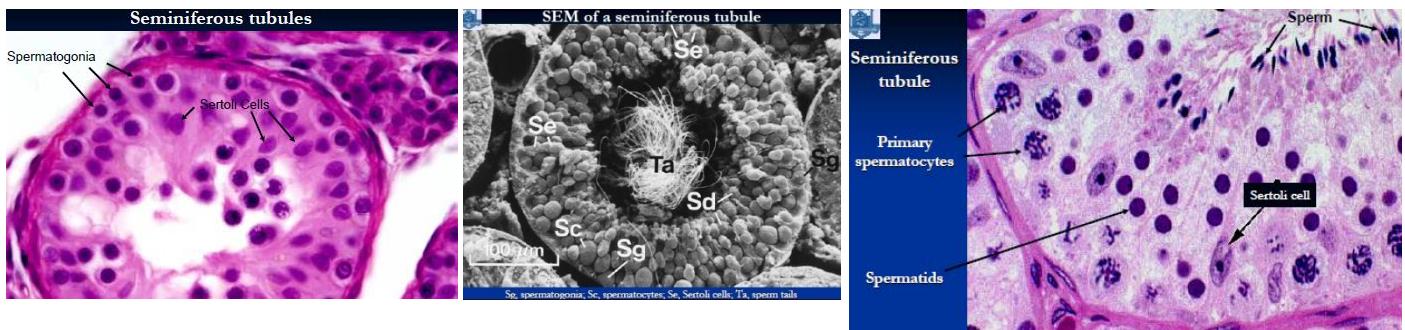
- Primary spermatocytes: **completion of meiosis I**

- Secondary spermatocytes: **completion of meiosis II**

- Spermatids are small cells compared to spermatocytes:

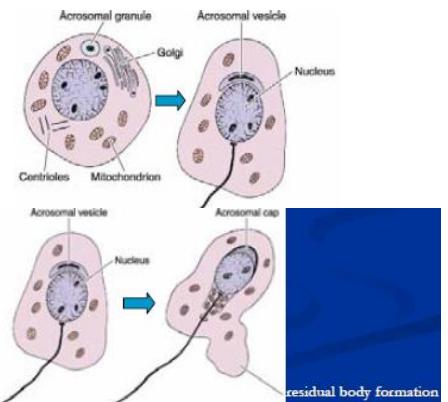
- Large number of spermatids (progeny of a single Ap spermatogonium are connected in a **syncytium** and synchronize their activities)





## Spermiogenesis

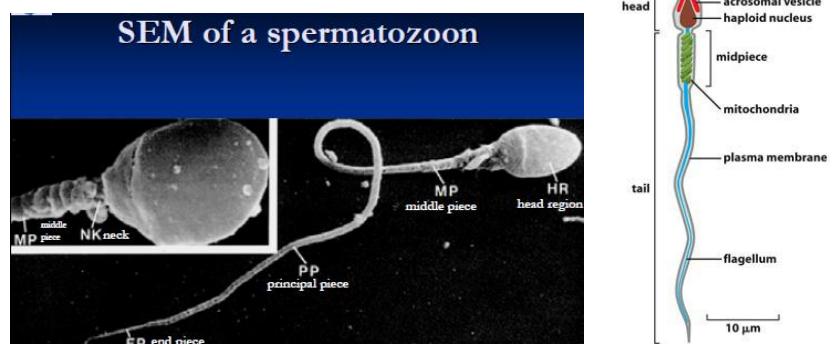
- Transformation of spermatids into spermatozooids
- Phases
  - Golgi phase
  - Cap phase
  - Acrosomal phase
  - Maturation phase
- Golgi phase:**
  - Hydrolytic enzymes are formed (RER) and transported (Golgi) as vesicles: **acrosomal granule** → **acrosomal vesicle**
  - Centrioles form the **flagellar axoneme**



- Cap phase:**
  - Acrosomal vesicle → **acrosome (acrosomal cap)**; (gr. *akron* = extremity + *soma* = body)
  - Nucleus becomes more elongated and condensed
  - Mitochondria start to shift
- Acrosomal phase:**
  - Acrosome** (specialized type of lysosome) is mature (filled with hydrolases & proteases)
  - Chromosomal volume decreases, entire nucleus volume is also reduced; nucleus becomes flattened
  - One of the centrioles grows → forms the **flagellum**
  - Mitochondria aggregate around the proximal part of the flagellum → **middle piece** of spermatozoid
- Maturation phase:**
  - Residual cytoplasm is shed and phagocytosed by Sertoli cells
  - Syncytium is disrupted: **permiation** (spermatozoa are released into the lumen of the tubule)
  - Newly formed spermatozoa are **immotile**: gain motility while passing through the epididymis
  - Spermatozoa **capacitated** (i.e. capable of fertilization) after they enter the female reproductive system



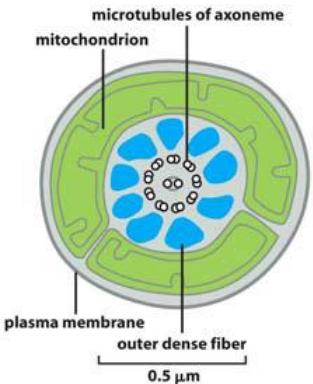
- Structure of a spermatozoon:
  - Head
    - Nucleus: haploid
    - Acrosome: enzymes
  - Tail (= flagellum)
    - Neck: axoneme + 9 dense fibers
    - Middle piece: axoneme + 9 dense fibers
    - Principal piece: axoneme + 7 dense fibers + fibrous sheath
    - End piece: axoneme + plasmalemma
- Flagellum:** axoneme + 9 dense fibers
  - Axoneme:** 2 singlet microtubules + 9 microtubule doublets ( $9 \times 2 + 2$ )



- Dense fibers are stiff and noncontractile: they restrict the flexibility of the flagellum and protect it from shear forces
- Defects in dense fibers lead to abnormal sperm morphology and to infertility

## Male Meiosis

- Begins at puberty
- Goes on continuously, without stop and start mechanisms
- Takes about 24 day for a human spermatocyte to complete meiosis
- Very low error rates: 3-4 %; quality control system arrests meiosis (apoptosis) in case of error
- By the end of meiosis, a sperm has only just begun its differentiation

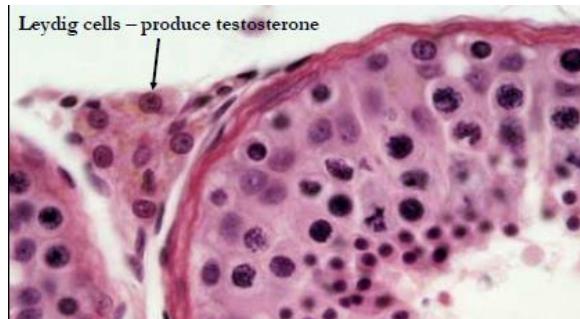
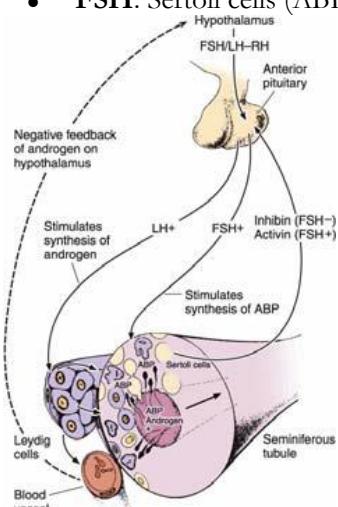


## Sertoli cells

- Support, protection, and nutritional regulation of the developing spermatozoa
- Phagocytosis of residual bodies
- Secretion of androgen-binding protein (binds and concentrates testosterone)
- Secretion of inhibin B: inhibits the production of FSH by the hypophysis
- Blood-testis barrier between blood & testicular fluid

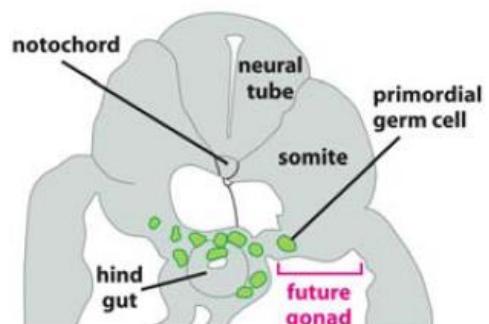
## Hypophyseal control of male reproduction

- LH: Leydig cells (testosterone)
- FSH: Sertoli cells (ABP)

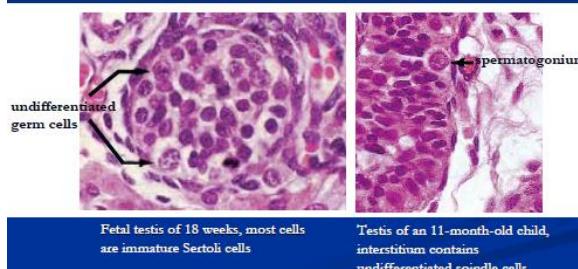


## Germ cells

- Spermatogonia develop from primordial germ cells during embryonic life
  - 2nd month of development: primordial germ cells migrate to the testicular cords
  - 3rd month of birth: gonocytes
  - During early postnatal life gonocytes gradually differentiate to spermatogonia
  - After 6 months of age: only spermatogonia



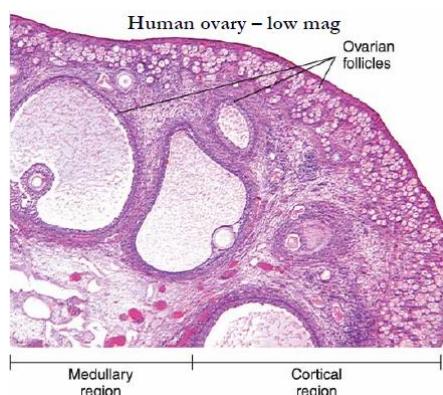
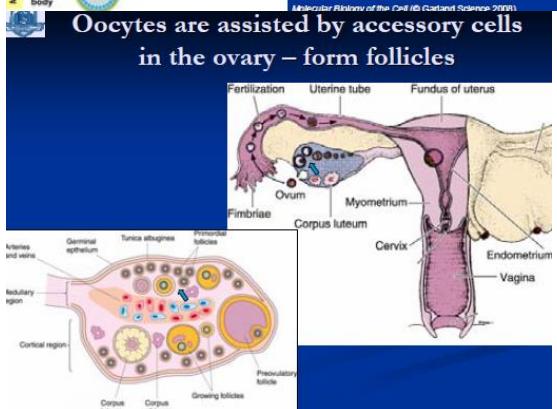
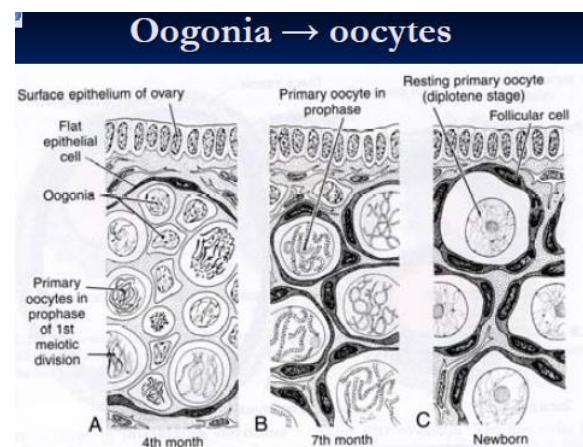
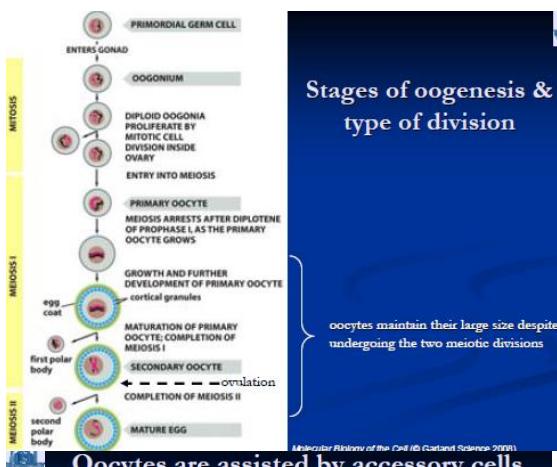
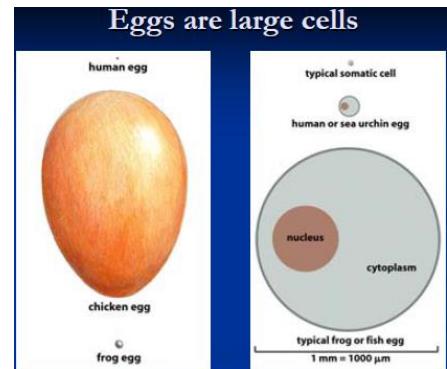
**At birth & postnatal life the germ cells average  
4-5 per tubule cross section**



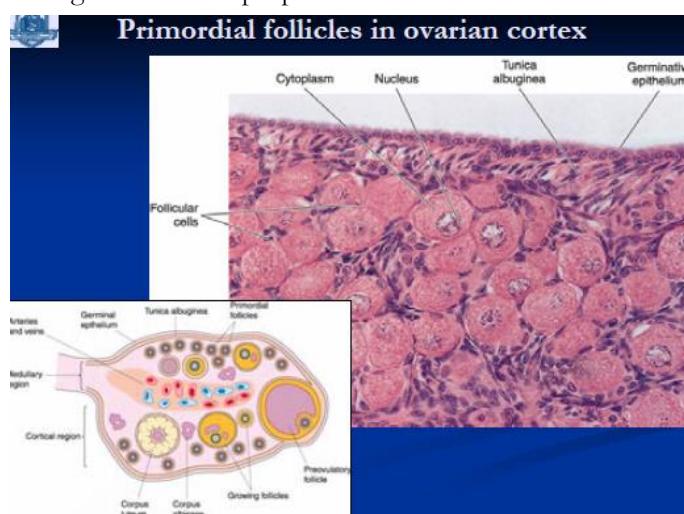
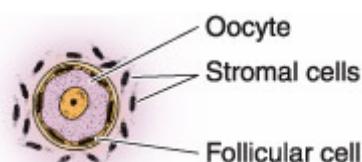
## Oogenesis

- The process by which **oocytes** (female gametes) are formed

- Oogonia develop from primordial germ cells during embryonic life:
  - 1st month of development: oogonia begin to appear
  - Active mitotic division: 2nd month - 600,000 oogonia; 5th month - 5 million oogonia
  - 3rd month: oogonia transform into primary oocytes (enter meiosis I and remain arrested in diplotene)
  - Puberty: 300,000 oocytes
  - During reproductive life 450 oocytes are liberated; all others die by atresia (a specific degenerative process)
  - It is still a puzzle why so many oocytes are formed only to die in the ovaries

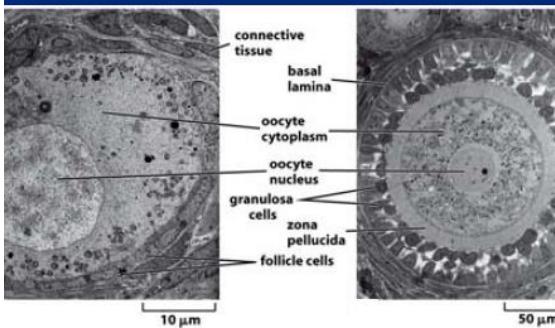


- Ovarian follicles:**
  - 1 follicle = 1 oocyte + 1 more layers of **follicular cells** or **granulosa cells**
- Primordial follicles:**
  - During embryonic life (after 3rd month): until puberty
  - 1 primary oocyte + 1 layer of follicular cells
  - The oocyte is spherical approx. 25  $\mu$ m in diameter; large nucleus with a single nucleolus - prophase I

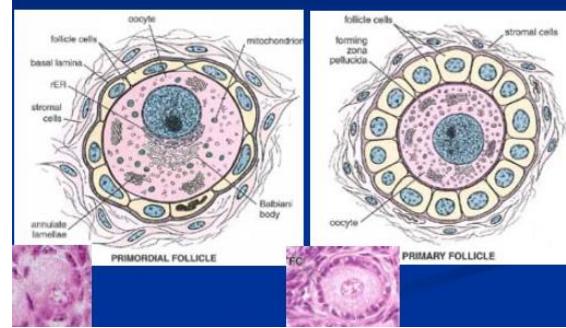


- Follicular growth:
  - From approx. 300,000 primordial follicles at puberty, some start the process of follicular growth
  - Oocyte diameter up to 120 micrometers; enlarged nucleus, mitochondria increase in number; RER hypertrophies, Golgi migrates beneath the cell surface

## Primordial vs primary follicle

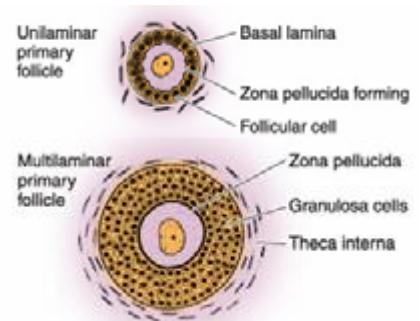


## Primordial vs primary follicle

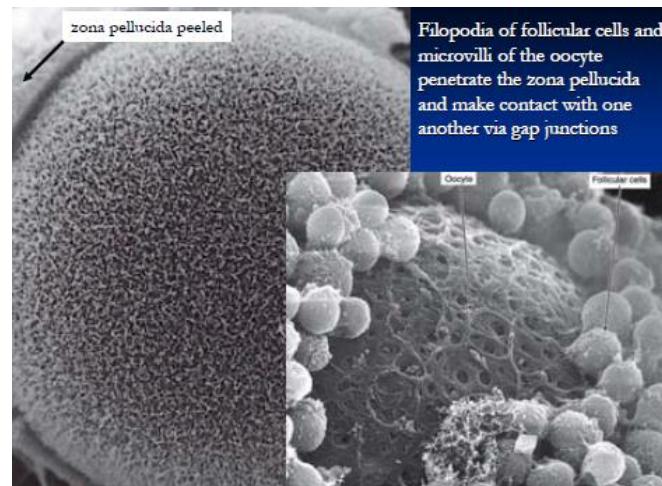
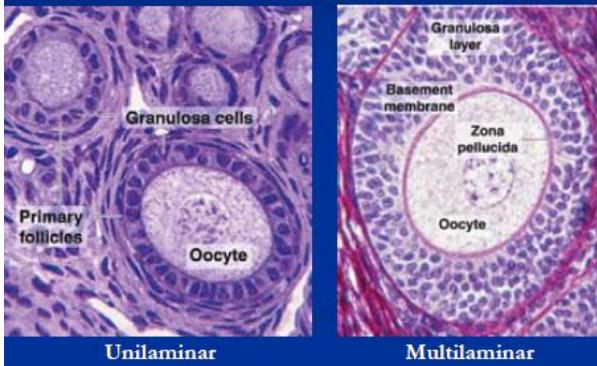


- Primary follicles:

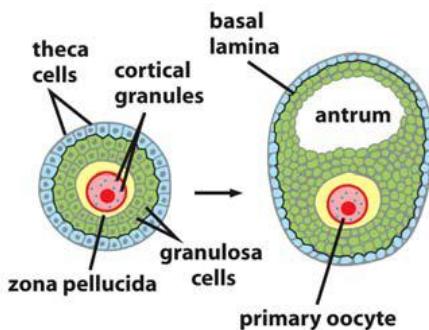
- Follicular cells become cuboidal, divide via mitosis: **granulosa cells**
- **Zona pellucida**: thick amorphous layer of glycoproteins, is secreted by and surrounds the oocyte
- Unilaminar (1 layer) → multilaminar (several layers); **primary follicle**
- Fibroblasts of stroma immediately around the follicle & form the **theca folliculi** (Gr. *Theca*, box); granulosa & theca cells are separated by a basal lamina)



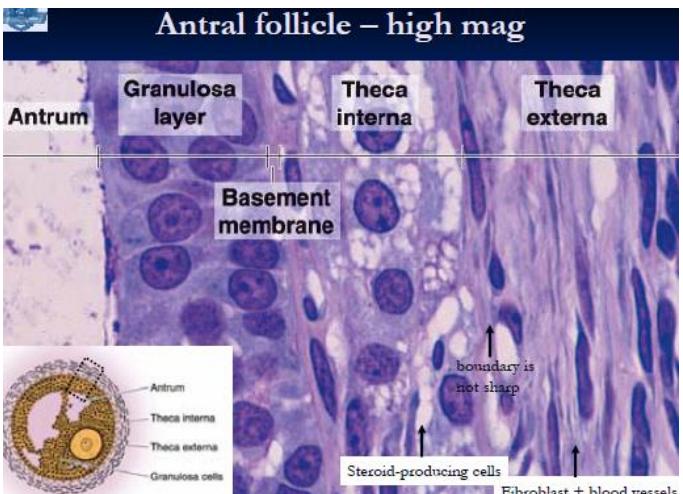
## Unilaminar vs multilaminar primary follicle

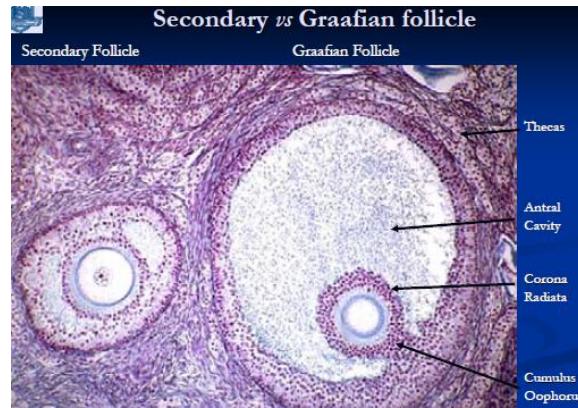
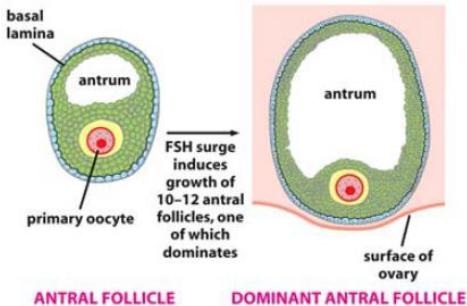


- As follicles grow a liquid (**liquor folliculi**) begins to accumulate between the follicular cells. It forms a cavity (**antrum**)

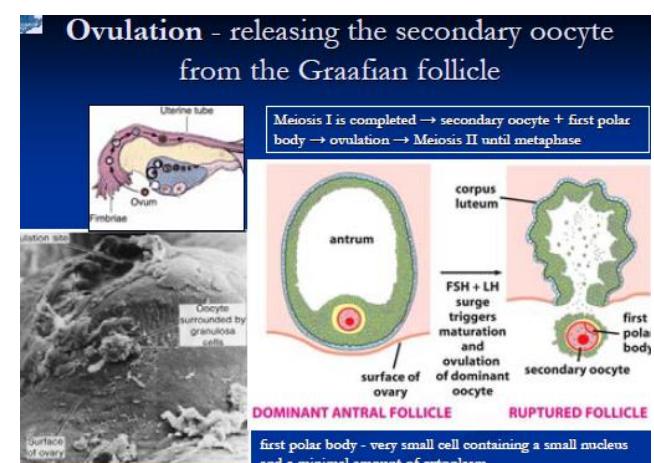
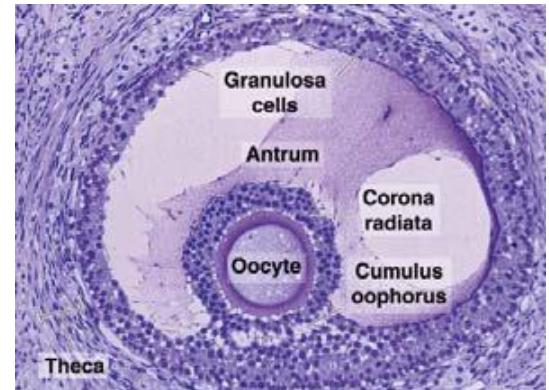


- Once in a month, the pituitary gland accelerates the growth of about 10-12 antral follicles: one is dominant (mature, Graafian) follicle

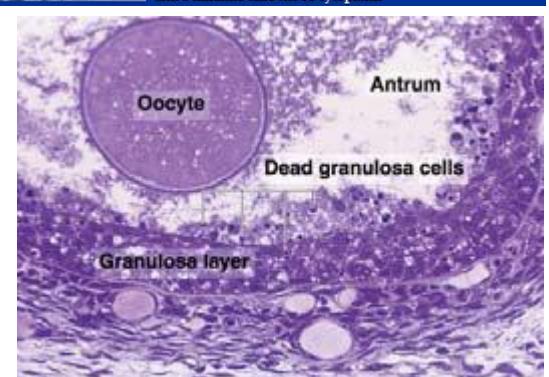


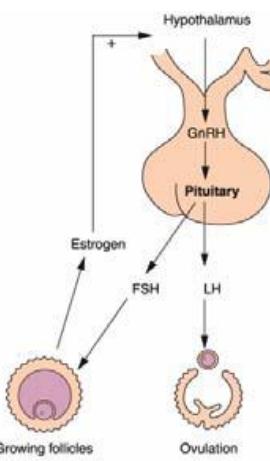
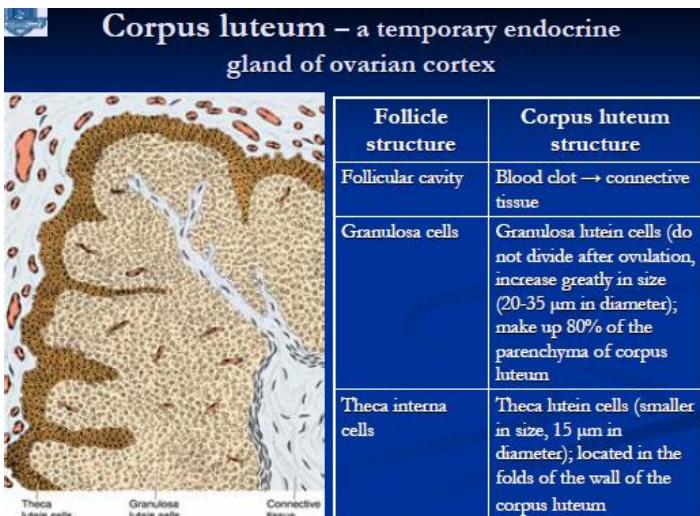
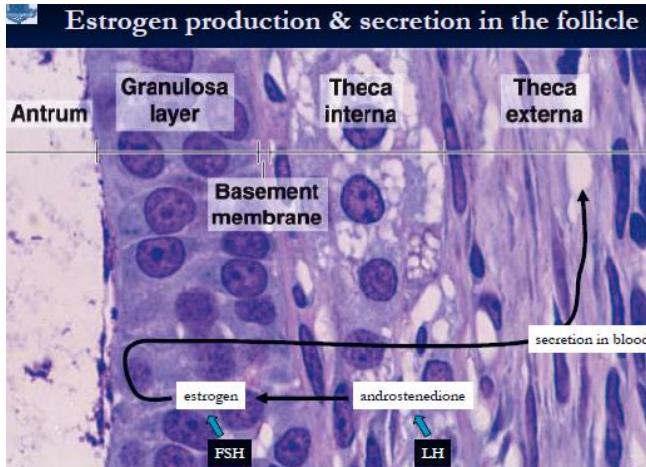


- Graafian follicle:
  - **Cumulus oophorus:**
    - Group of granulosa cells which surround the oocyte
    - Divide to form cells of the membrana granulosa
    - Ovulated along with the oocyte
  - **Corona radiata**
    - Single layer of cells that immediately surround the primary oocyte
- The dominant primary oocyte completes meiosis I, and the resulting secondary oocyte arrests at metaphase II
- Graafian follicle diameter by the time of ovulation may reach as much as 2.5 cm; it can only be observed by **ultrasound**



- Most ovarian follicles undergo **atresia**
  - Follicles at any stage of development may undergo atresia
  - Follicular cells and oocytes die: phagocytic cells
  - Cessation of mitosis in the granulosa cells: detachment from basal lamina
  - Greatly accentuated just after birth, at puberty, during pregnancy
- **Hormonal regulation of ovulation:**
  - FSH stimulates follicle growth & estrogen production by granulosa cells
  - Estrogen stimulates LH pulse via GnRH from hypothalamus
  - LH pulse leads to rapid increase in blood flow through the ovary: **local edema**
  - Area of the follicle wall becomes weak (collagen degradation of the tunica albuginea, ischemia, and death of some cells)

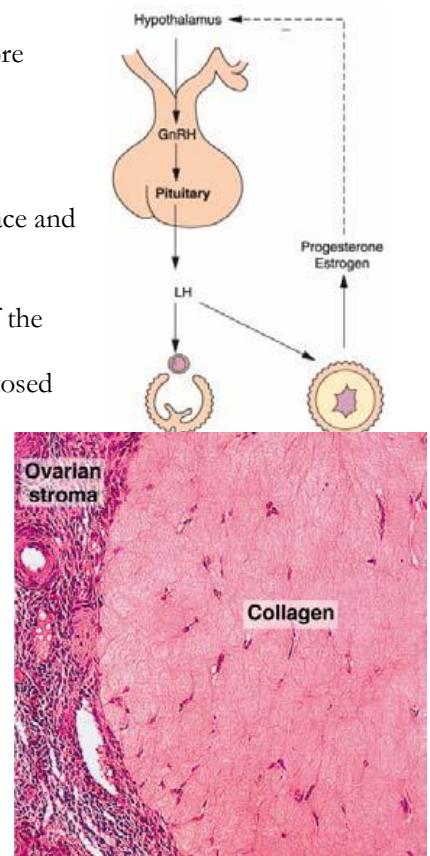


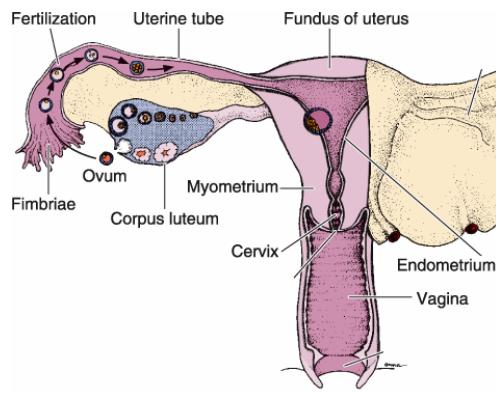


- **Corpus lutein** is formed under influence of LH
  - The development of the corpus luteum result from the LH released before ovulation
  - Under stimulus by LH, the cells of the corpus luteum begin secreting progesterone and estrogens
  - Corpus luteum is programmed to exist for 10-12 days
  - If a pregnancy does not occur, no further hormonal stimulation takes place and the cells of the corpus luteum degenerate by apoptosis
- **Corpus albicans** (white body)
  - If pregnancy does not occur, the absence of LH leads to degeneration of the corpus luteum: **corpus luteum of menstruation**
  - The cellular remnants of the corpus luteum of menstruation are phagocytosed by macrophages
  - Fibroblasts invate the area and produce a scar of dense connective tissue - corpus albicans
- In case **pregnancy** occurs:
  - **Human chorionic gonadotropin (hCG)**, secreted by the placenta, maintains the corpus luteum for 3 months: **corpus luteum of pregnancy**
  - Grows to a diameter of 5 cm and continues to secrete hormones necessary for the maintenance of pregnancy
  - Placenta becomes the main site of production of the various hormones involved in maintaining pregnancy, but corpus luteum continues to form these hormones for several months

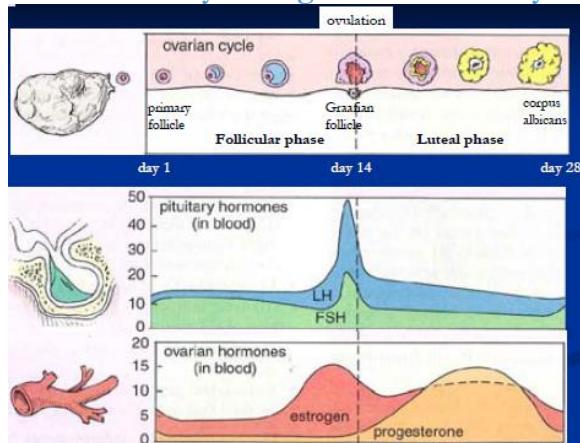
## Menstrual cycle

- Cyclic changes in the female reproductive system
  - **Events in ovary**
  - **Events in endometrium of uterus**

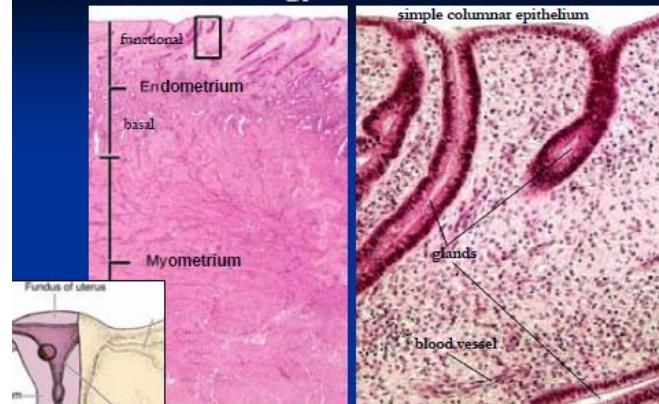




### Events in ovary during the menstrual cycle

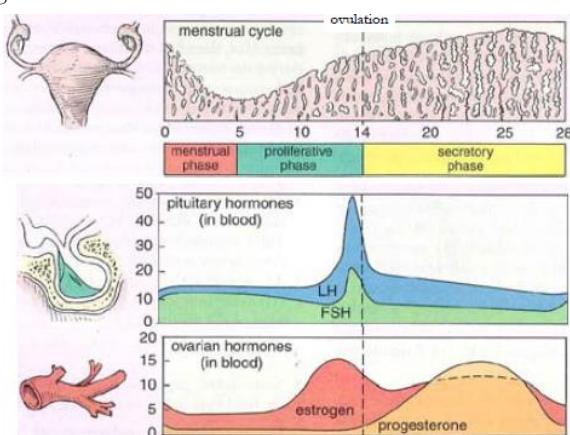


### Endometrial histology (proliferative = follicular phase)



### Events in endometrium during the menstrual cycle

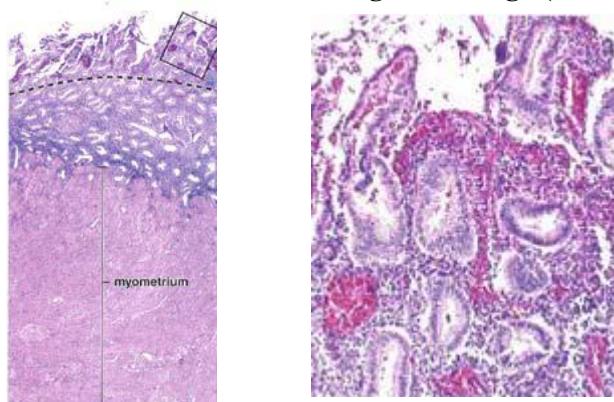
- Phases of menstrual cycle:
  - Menstrual phase:** 1-4 days
  - Proliferative (follicular) phase:** day 4-14
  - Secretory (luteal) phase:** days 15-28
- Menstrual phase:**
  - Desquamation of the functionalis layer of the endometrium
  - Coiled (helical) arterires are intermittently constricted → reduced oxygen → necrosis



Changes in the uterine glands and in the gland cells during the menstrual cycle

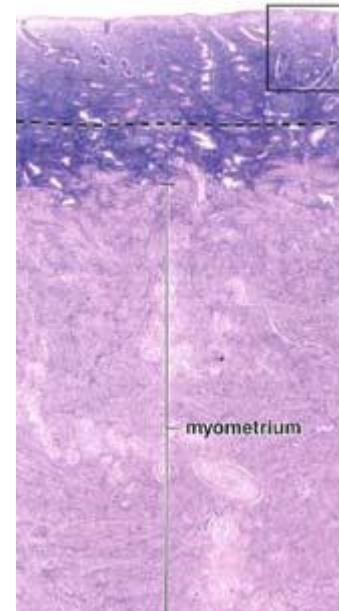


- Coiled arteries dilate again: **hemorrhagic discharge (menses)**



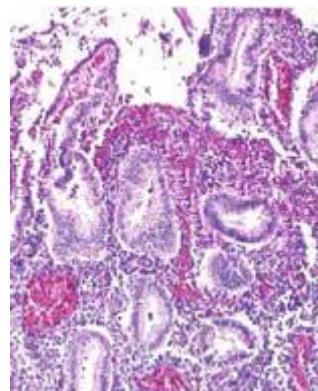
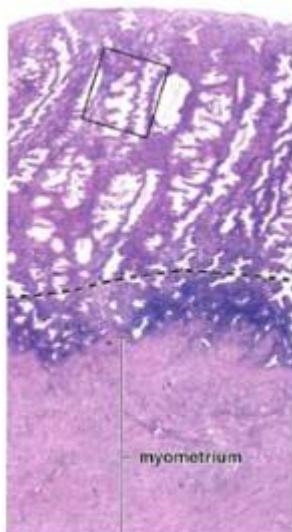
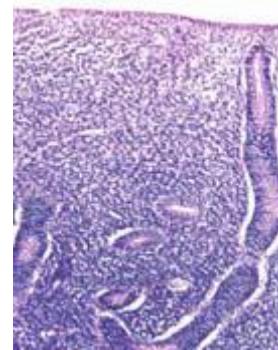
- Proliferative (follicular) phase:**

- Called follicular phase because it occurs at the same time as the development of the ovarian follicles
- Reepithelialization of the lining of the endometrium
- Reconstruction of the glands, connective tissue, and the coiled arteries of the lamina propria
- Endometrium is 2-3 mm thick
- Under influence of estrogens



- Secretory (luteal) phase:**

- Commences after ovulation
- Endometrial glands become highly convoluted and branched
- Endometrial epithelial cells accumulate glycogen
- Endometrium thickens: up to 5 mm
- Under influence by progesterone

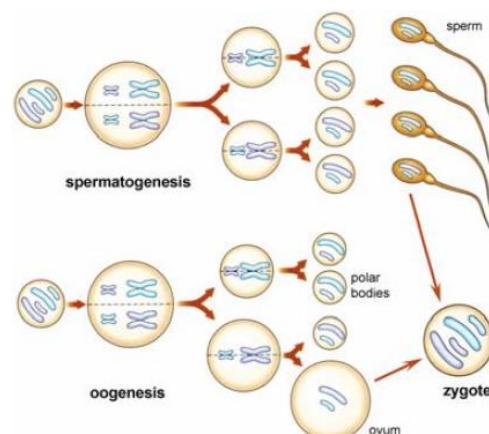


## Female meiosis

- Specific characteristics:

- Begins during embryonic life (1st month)
- Stop and start mechanisms: arrested after diplotene of meiosis I; meiosis I is complete after puberty
  - Meiosis I is completed at the time of ovulation
  - Meiosis II is completed only if the released oocyte is fertilized
- It takes nearly 40 years for some human oocytes to complete meiosis
- High error rates: 20% of aneuploidy; most of these result from nondisjunction in oocytes at meiosis I
- If homologous chromosome segregation fails to occur normally, the cells continue through meiosis and produce aneuploid eggs
- By the end of meiosis, a mammalian oocyte is fully mature
- Estrogen stimulates mitosis in the stratum basale, which becomes thicker and thicker

|                                       | Male                    | Female                    |
|---------------------------------------|-------------------------|---------------------------|
| start                                 | puberty                 | embryo                    |
| stop and start                        | no                      | yes                       |
| time to complete meiosis              | 24 days                 | up to 40 years            |
| differentiation at the end of meiosis | just beginning          | complete                  |
| error rates by nondisjunction         | low                     | high                      |
| quality control system                | very active → apoptosis | low activity → aneuploidy |



# Semen

- The semen
  - is the fluid that is ejaculated at the time of orgasm
  - contains: spermatozoa + secretions of the seminal vesicles, prostate and various glands
  - Abnormally low: <40 million/mL; sterile: <20 million/mL
  - Speed: 3mm/min; sperm cells reach the uterine tubes 30-60 minutes after copulation
- Maturation of sperm in the epididymis:
  - Sperm require several days to pass through the 6-meter-long tubule of the epididymis
  - Sperm removed from the seminiferous tubules and from the early portions of the epididymis are **nonmotile**
  - However, after the sperm have been in the epididymis for some 18 to 24 hours, they develop the **capability of motility**
  - Some inhibitory proteins in the epididymal fluid still prevent final motility until after ejaculation

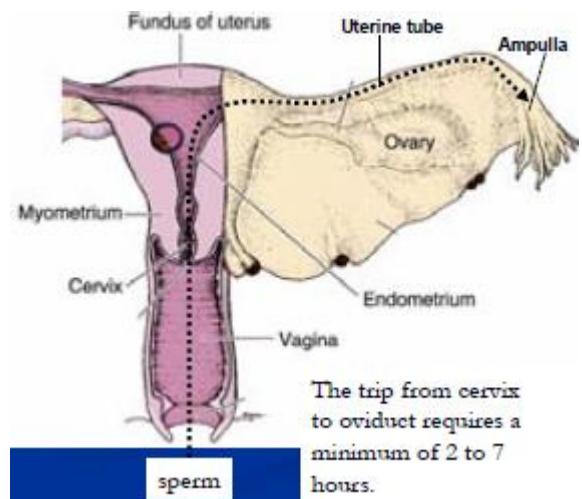
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|--------------------------------------------------------------------------------------|-------------------------------------------------------------------------|
| <b>Color:</b> White, opalescent                                                      |                                                                         |
| <b>pH:</b> 7.35-7.50                                                                 |                                                                         |
| <b>Sperm count:</b> Average about 100 million/mL, with fewer than 20% abnormal forms | The two testes of the human adult form up to 120 million sperm each day |
| <b>Other components:</b>                                                             |                                                                         |
| Fructose (1.5-6.5 mg/mL)                                                             |                                                                         |
| Phosphorylcholine                                                                    | From <b>seminal vesicles</b> (contribute 60% of total volume)           |
| Ergothioneine                                                                        |                                                                         |
| Ascorbic acid                                                                        |                                                                         |
| Flavins                                                                              |                                                                         |
| Prostaglandins                                                                       |                                                                         |
| <b>Spermine</b>                                                                      |                                                                         |
| Citric acid                                                                          |                                                                         |
| Cholesterol, phospholipids                                                           | From <b>prostate</b> (contributes 20% of total volume)                  |
| Fibrinolysin, fibrinogenase                                                          |                                                                         |
| Zinc                                                                                 |                                                                         |
| Acid phosphatase                                                                     |                                                                         |
| <b>Phosphate</b>                                                                     | Buffers                                                                 |
| Bicarbonate                                                                          |                                                                         |
| Hyaluronidase                                                                        |                                                                         |



## Ovulation to Implantation

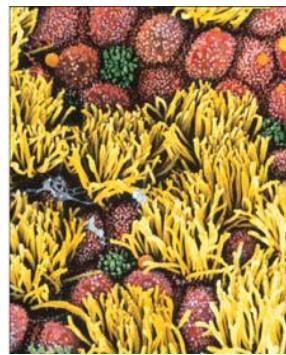
### Insemination

- Deposition of sperm into the vagina → migration to uterus → uterine tubes
  - Natural
  - Artificial
    - Homologous: cryopreservation
    - Heretologous
- Barriers sperm must overcome:
  - Vaginal acid
  - Mucus of the cervical canal
  - Leukocytes in the uterus
  - Going up the wrong uterine tube
- Sperm matures during their passage through the female genital tract
  - Approx. 300 million human sperm are ejaculated during coitus, but only approx. 200 reach the oocyte in the ampulla
  - Capacitation:** modifications of sperm to acquire the capacity to fertilize an egg
  - The modifications take place and are complete only in the female genital tract
  - Duration: 5-6 hours
  - End effects of capacitation:
    - Greatly increased the motility of the flagellum
    - The sperm becomes capable of undergoing the acrosome reaction
  - Capacitation can occur *in vitro* in appropriate culture medium and is usually a required part of *in vitro* fertilization; crucial components are albumin,  $\text{Ca}^{2+}$ , and  $\text{HCO}_3^-$
- Oocyte transport:**
  - Oocytes will degenerate if not fertilized within 24 h
  - Oocytes reach the uterus for 72 h → fertilization in ampulla



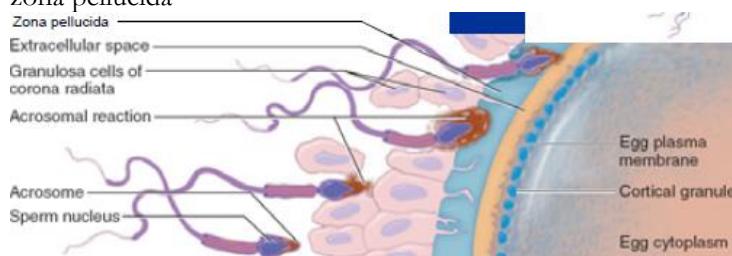
sperm

- Oocyte + some granulosa cells is carried into the tube by sweeping movements of the fimbriae and by motion of cilia on the epithelial lining
- Once in the tube, cumulus cells withdraw their cytoplasmic processes from the zona pellucida and lose contact with the oocyte
- Cilia on the epithelial lining of the uterine tube propel oocytes
- Colorized TEM:
  - Secretory cells: red and green
  - Cilia of the ciliated cells: yellow



## Fertilization

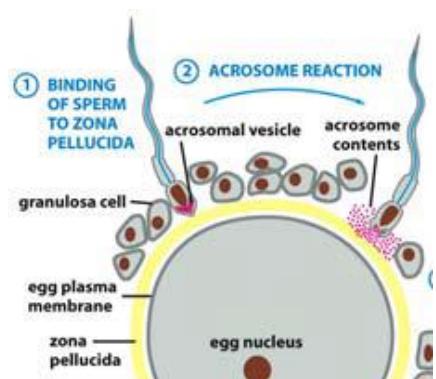
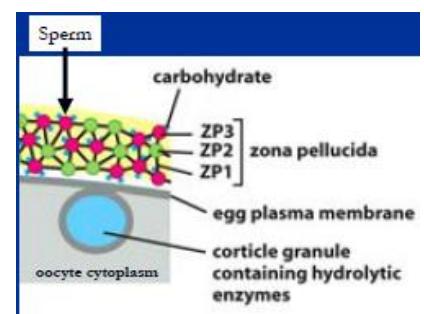
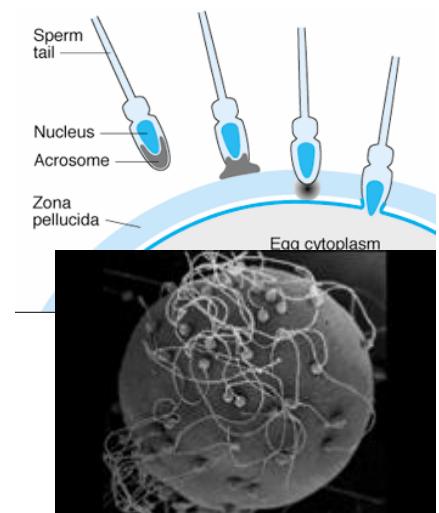
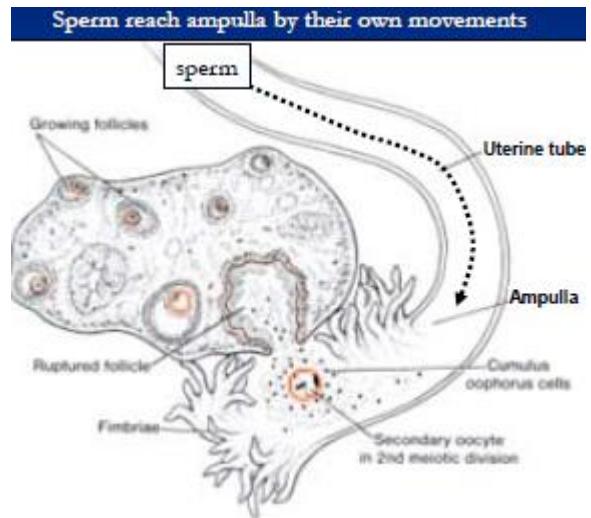
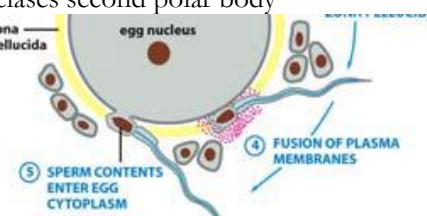
- The process by which male and female gametes fuse
- Basic events during fertilization in mammals
  - Chemoattraction of the sperm by substances produced by the ovum
  - Sperm adherence to the **zona pellucida**
  - Penetration of the zona pellucida and the acrosome reaction
  - Penetration of the zona pellucida and the acrosome reaction
  - Adherence of the sperm head to the oolemma → fusion of the membranes → release of the sperm nucleus into the cytoplasm of the ovum
- Capacitated sperm must penetrate the layers of granulosa cells (hyaluronidase): penetrate the zona pellucida



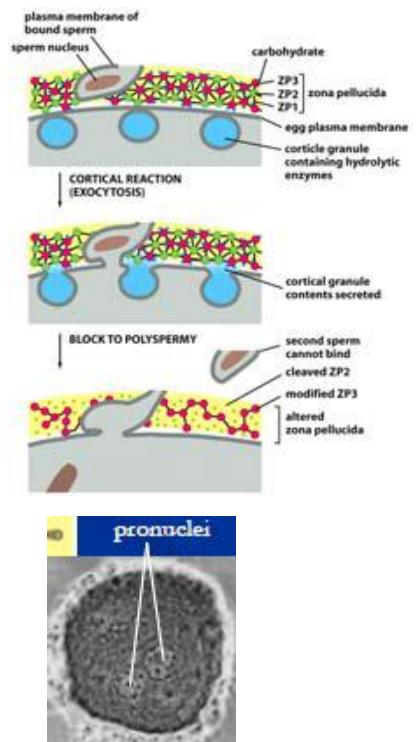
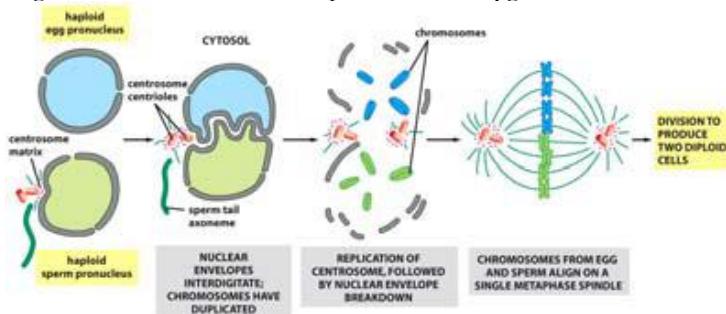
- Zona pellucida proteins:
  - ZP2 & ZP3: long filaments
  - ZP1 cross-links the filaments into a 3D network
  - Sperm use ZP3 to bind to zona pellucida
- Acrosome reaction:
  - Exocytosis of the acrosome contents
  - Trigger: ZP3 binding to a sperm receptor → elevated  $\text{Ca}^{2+}$  in the sperm → exocytosis → enzymes released
- Zona pellucida is a crucial barrier of fertilization; its removal makes fertilization possible
  - Zona pellucida is artificially removed
  - The ability of an individual's sperm to penetrate hamster eggs is used as an assay of male fertility; penetration of more than 10-25% of the eggs is considered to be normal

## Sperm-oocyte fusion

- Sperm-egg binding:
  - Izumo - sperm-specific transmembrane protein
  - CD9 - oocyte-specific glycoprotein
- The sperm binds initially by its tip and then by its side
- Sperm fusion activates the oocyte by increasing  $\text{Ca}^{2+}$  in the cytosol
  - Cortical granules of oocyte are released
  - Oocyte completes meiosis II: releases second polar body

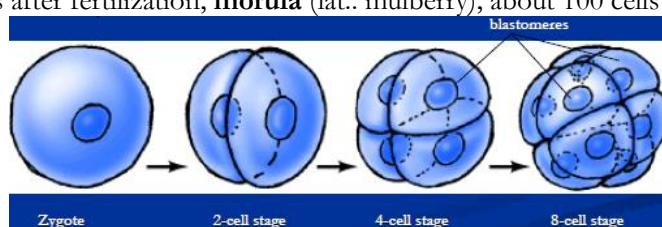


- The **cortical reaction** of the oocyte prevents additional sperm from entering
  - Inactivation of ZP3: it can no longer bind sperm
  - ZP2 is cleaved: zona becomes impenetrable
  - If more than one sperm fuses: **polyspermy** → faulty segregation of chromosomes during the first mitotic cell divisions → aneuploid cells → development stops
- Sperm provides its genome and centrioles to the zygote
  - 2 haploid nuclei (called **pronuclei**): egg + sperm come together and combine their chromosomes into a single diploid nucleus → **zygote**
  - Sperm also donates its centrioles (the oocyte does not have centrioles) → generate the first mitotic spindle of the zygote

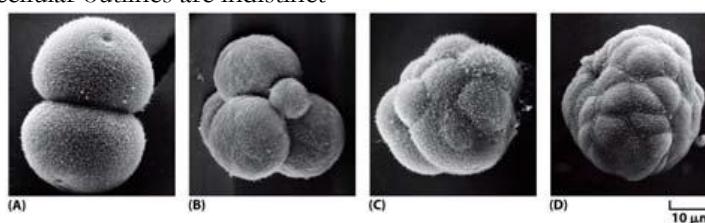


- Stages of **prenatal development**:
  - Preembryonic stage: weeks 1 & 2
  - Embryonic stage: weeks 3 - 8
  - Fetal stage: months 3 - 9
- Preembryonic stage:**
  - The first 2 weeks of development
  - Steps:
    - Cleavage
    - Implantation
    - Embryogenesis
  - Final outcome: embryo

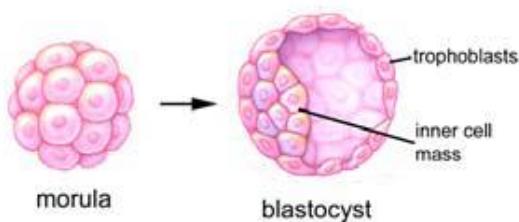
- Cleavage:**
  - Mitotic divisions that occur in the first 3 days
  - 1st cleavage: approx. 30 hours after fertilization
  - 4th cleavage: 72 hours after fertilization; **morula** (lat.: mulberry); about 100 cells after day 4-5



- Morula:**
  - Initially uncompacted (gap junctions) the eight-cell embryos become compacted (tight junctions) → size of individual cells is reduced, while the size of the morula is preserved
  - In the uncompacted state, outlines of each blastomere are distinct, whereas after compaction cell-cell contacts are maximized and cellular outlines are indistinct

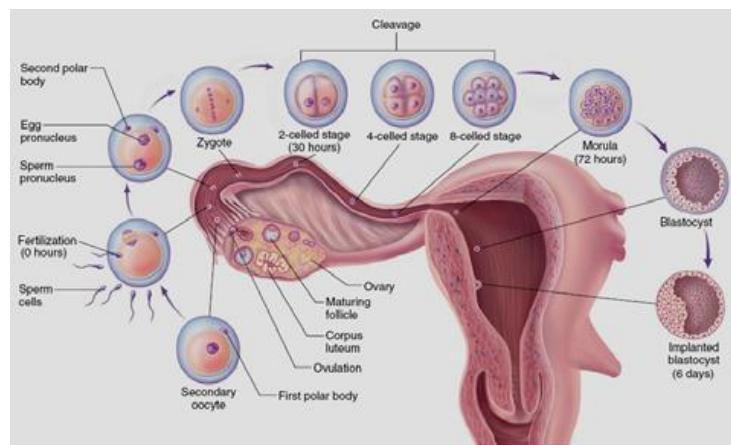
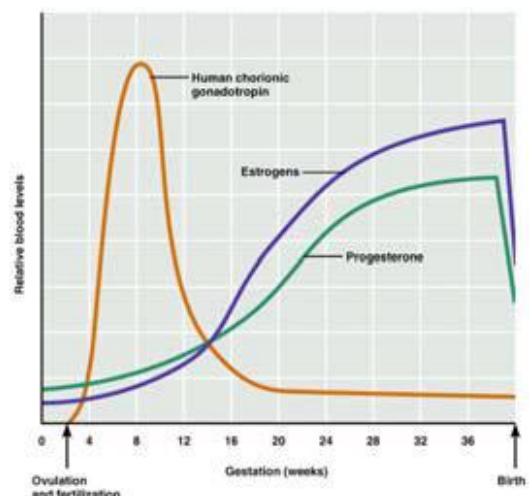
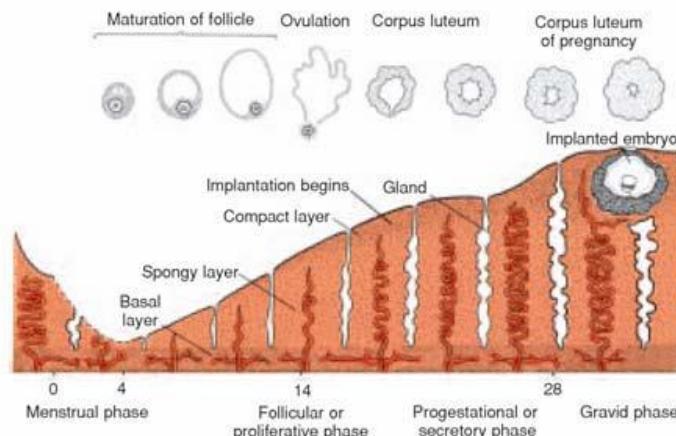
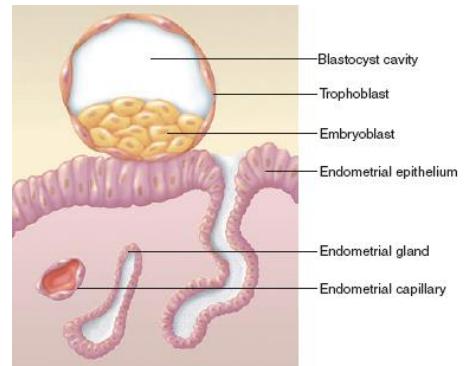
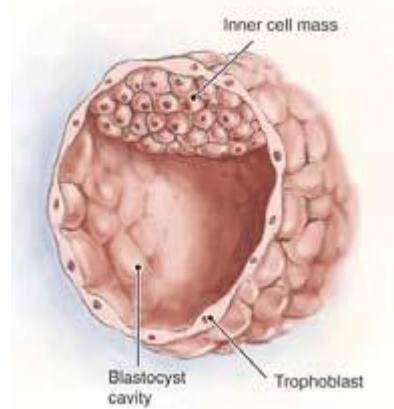


- Morula becomes **blastula**
  - Blastocyst: a fluid-filled hollow sphere composed of:
    - 1 outer layer (= trophoblast): will contribute to the placenta
    - Inner cell mass (= embryoblast): will make the embryo
  - Zona pellucida breaks: fluid enters the center of the morula

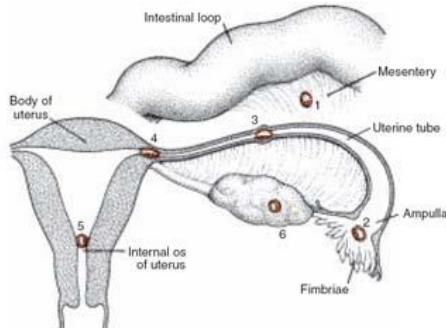


### Implantation

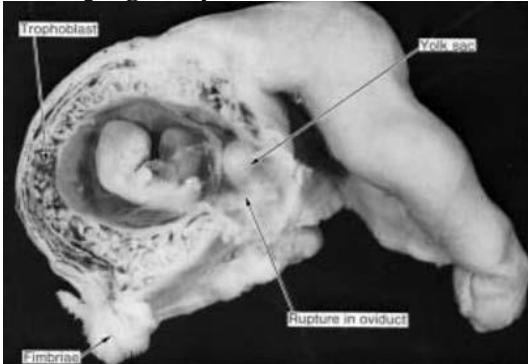
- Process in which the conceptus becomes integrated into the endometrium of uterus
- Starts 6-7 days after fertilization (at the stage of blastocyst)
- Ends at 14 days
- Steps:
  - Dissolution of the zona pellucida
  - Orientation and adhesion of the blastocyst onto the endometrium
  - Trophoblastic penetration into the endometrium
  - Migration of the blastocyst into the endometrium
  - Spread and proliferation of the trophoblast: disruption and invasion of maternal tissues
- Throphoblast cells secrete **human chorionic gonadotropin (hCG)**
  - Stimulates estrogen & progesterone secretion from corpus luteum
  - After 2nd month of pregnancy hCG is no more necessary: chroion produces necessary hormones (ovaries become inactive until end of pregnancy)
  - hCG is used in pregnancy tests
- Changes in uterine mucosa correlate with ovary



- **Ectopic (extrauterine) pregnancy**



- **Tubal pregnancy:**



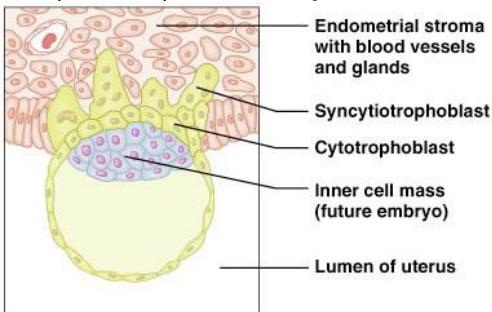
95% of ectopic pregnancies occur in the uterine tube and most of these are in the ampulla

### Bilaminar germ disk

- Events in the 2nd week follow the rule of the "two":
  - Inner cell mass produces 2 layers
  - Trophoblast produces 2 layers
  - 2 cavities are formed that will protect the embryo in the following stages
- The earliest developmental processes in mammalian embryos involve the production of the **extraembryonic** structures, which will support and nourish the embryo during development. Production of these layers begin before implantation is complete.

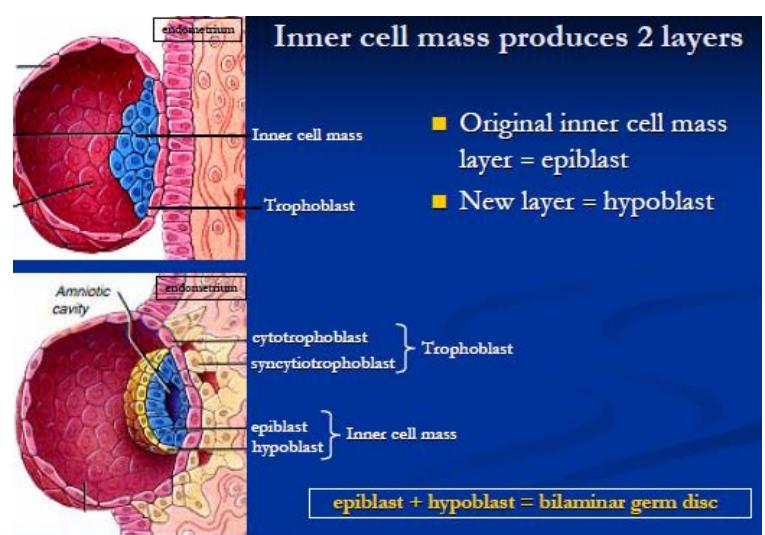
*time*  
**extraembryonic structures → intraembryonic structures**

- The trophoblast produces 2 layers:

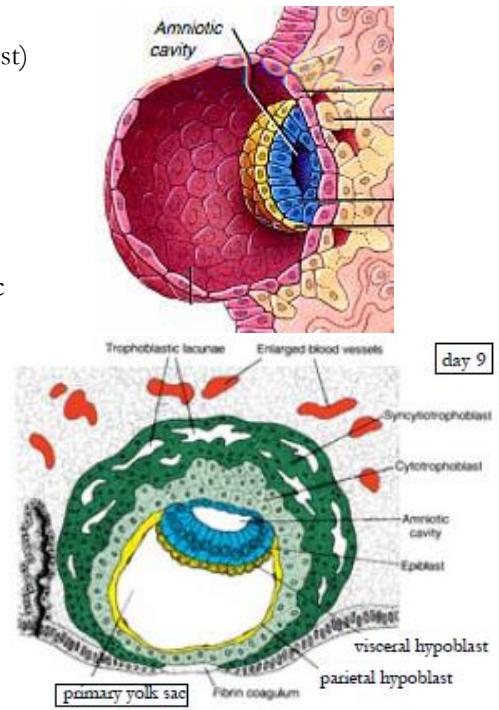


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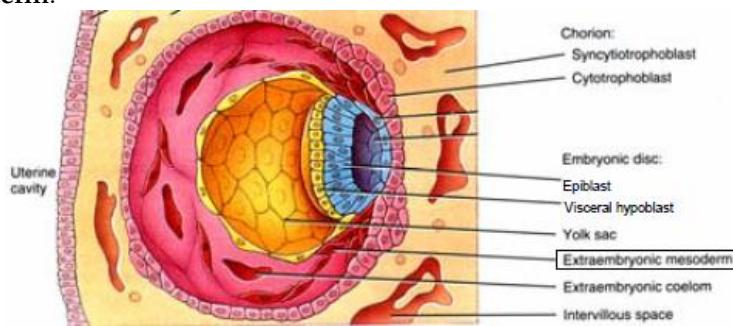
- **Cytotrophoblast:** cells of the inner layer that retain their cell boundaries (plasma membranes)
- **Synctiotrophoblast:** cells in the outer layer that lose their plasma membranes and invade the endometrium; merge into a **syncytium**
- Endometrium reacts to this injury by growing over the trophoblast and eventually enclosing it
- Epiblast & hypoblast form 2 embryonic membranes:
  - **Amnion:** formed by epiblast; filled with amniotic fluid



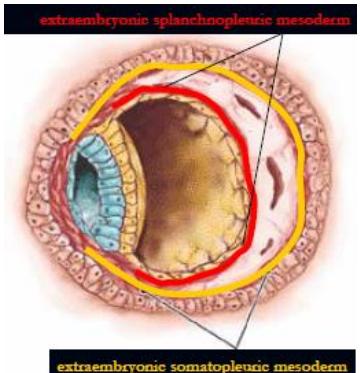
- Provides a protective environment for the embryo
- Helps maintain a constant homeostatic temperature
- Amniotic fluid comes from maternal blood, and later, fetal urine
- **Yolk sac:** hypoblast cells that form a sac on the ventral surface of the embryo
  - Forms part of the digestive tube
  - Produces earliest blood cells and vessels
- Formation of **amniotic cavity:**
  - Within the sphere of epiblast cells, some cells (next to the hypoblast) become more columnar
  - Epiblast sphere becomes flattened
  - Fluid accumulates: amniotic cavity forms
- Formation of the **primary yolk sac**
  - Hypoblast cells migrate and cover the cytotrophoblast
  - 2 hypoblast layers are formed:
    - **Parietal:** in contact with the cytotrophoblast; =exocoelomic (Heuser's) membrane
    - **Visceral:** in contact with epiblast
- **Trophoblastic lacunae:**
  - Cells of the syncytiotrophoblast penetrate deeper into the stroma and erode the endothelial lining of the maternal capillaries: **sinusoids**
  - The syncytial lacunae become continuous with the sinusoids and maternal blood enters the lacunar system



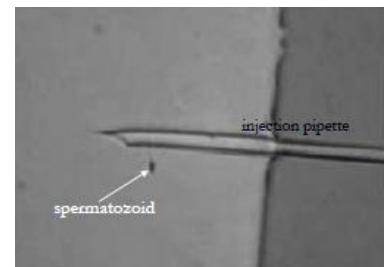
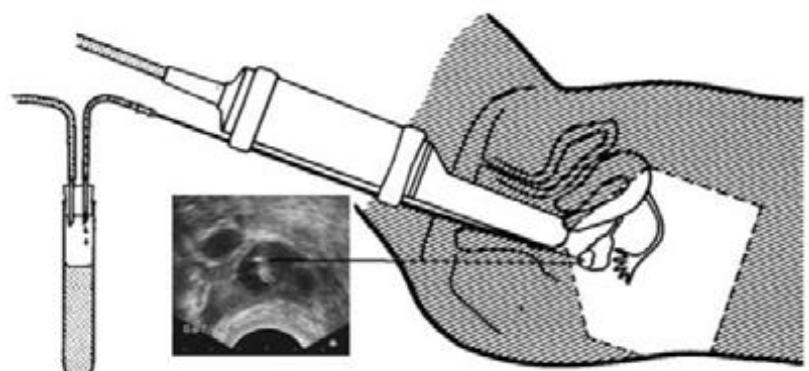
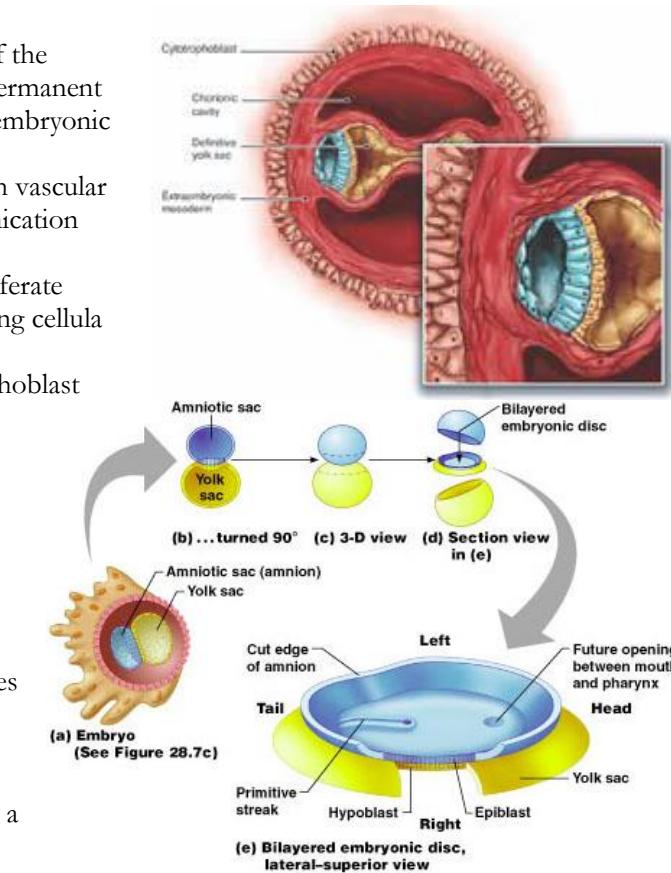
- **Extraembryonic mesoderm:**



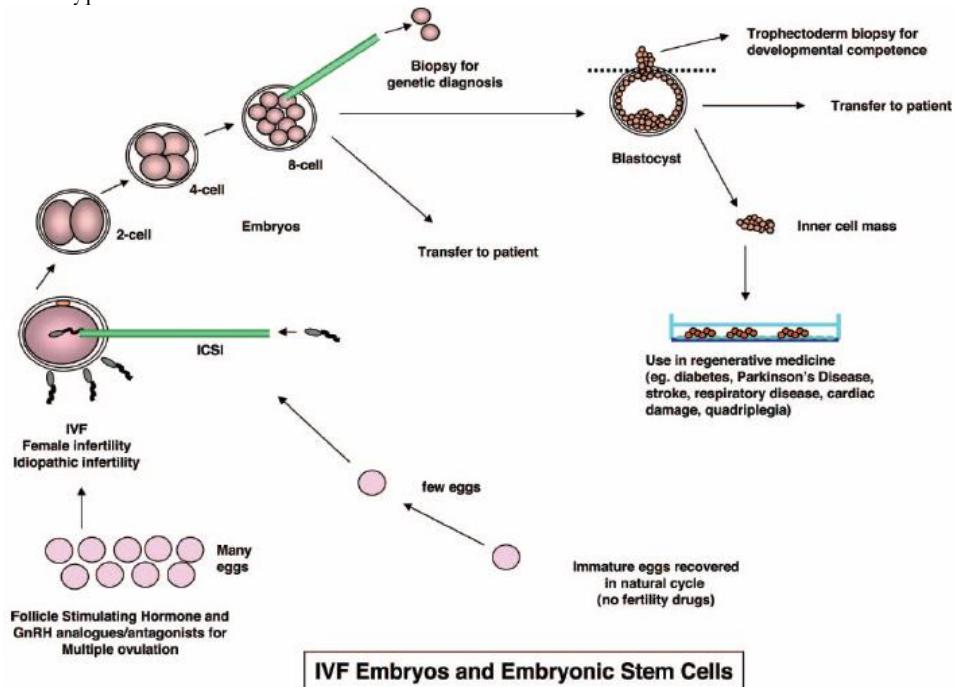
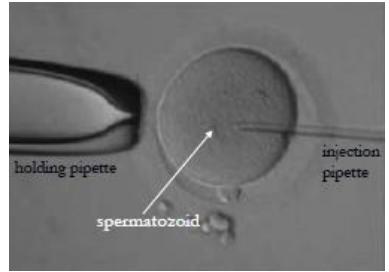
- A new population of cells, derived from epiblast, appears between the cytotrophoblast and parietal hypoblast
- Large cavities develop in the extraembryonic mesoderm → become confluent → extraembryonic coelom (= chorionic cavity)
- Extraembryonic mesoderm forms 2 layers:
  - The extraembryonic mesoderm lining the cytotrophoblast and amnion is called extraembryonic somatopleuric mesoderm
  - The extraembryonic mesoderm lining the yolk sac is called **extraembryonic splanchnopleuric mesoderm**
- Pinching off the primary yolk sac produces **secondary yolk sac**
  - Around day 13 the hypoblast produces additional cells that gradually form a new cavity within the primary yolk sac → **secondary (definitive) yolk sac**
  - Secondary yolk sac is much smaller than primary yolk sac
  - The pinched portions of primary yolk sac: **exocoelomic cysts**
- **Connectin stalk:**



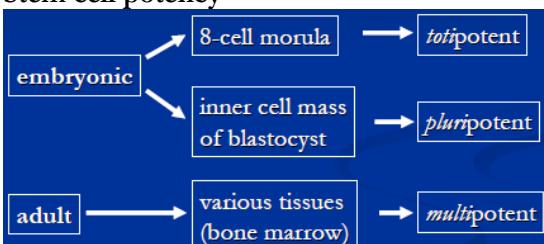
- Continued development and expansion of the extraembryonic coelom restricts the attachment of the embryonic disk to a connecting stalk, which is a permanent connection between the future caudal end of the embryonic disk and the chorion
- The connecting stalk forms a pathway along which vascular anastomoses of embryonic disk establish communication with those of the chorion
- **Primary villi** - day 13: Cells of the cytotrophoblast proliferate locally and penetrate into the syncytiotrophoblast, forming cellular columns surrounded by syncytium
- Bilaminar germ disk formation: Around day 12, the trophoblast continues to erode more and more sinusoids, maternal blood begins to flow through the trophoblastic system, establishing the **uteroplacental circulation**
- **Abnormal blastocysts:**
  - Common: 20-30% of normal pregnancies
  - Major types:
    - Trophoblast hypo/a-plasia
    - Embryoblast hypo/a-plasia
  - **Hydatidiform mole:** trophoblast develops (secretes hCG), embryoblast does not develop
    - May produce benign or malignant (invasive mole, choriocarcinoma) tumors
    - Arise from fertilization of an oocyte lacking a nucleus followed by duplication of the male chromosomes to restore the diploid number: trophoblast is regulated mainly by paternal genes
- Egg to embryo - terminology
  - **Pregnancy:** events that occur from fertilization until the infant is born
  - **Conceptus:** developing offspring
  - **Gestation period:** from the last menstrual period until birth (gestation week = postfertilization week - 2)
  - **Preembryo:** conceptus from fertilization until it is two weeks old
  - **Embryo:** conceptus during the third through the eighth week
  - **Fetus:** conceptus from the ninth week through birth
- **In vitro fertilization (IVF):** a process by which eggs are fertilized by sperm outside the female reproductive tract
  - 10% of human couples have reduced fertility; the female partner fails to become pregnant after 12-18 months of unprotected sex
  - Over 1 million babies have been born via IVF
  - Today, every day 1 IVF baby is born
- Steps in IVF:
  - Evaluation and preparation of the infertile couple
  - Ovarian stimulation: GnRH agonist and FSH
  - Oocyte retrieval: in vitro maturation
  - Assisted fertilization: ICSI
  - Embryo development and assessment of viability
  - Embryo transfer (ET): final step; transcervical route
  - Monitoring IVF outcome
- **ICSI** (Intracytoplasmic sperm injection)
  - A single motile spermatozoon is selected and immobilized by pressing its tail between the microneedle and the bottom of the dish. The sperm cell is then aspirated tail-first into the injection pipette.
  - The sperm cell is delivered into the oocyte with a minimal volume of medium; afterwards, the pipette can be carefully withdrawn



- ICSI has a success rate of better than 50% and has produced more than 100,000 children
- IVF disadvantages
  - IVF costs up to 10,000 per attempt and succeeds only 14% of the time
  - Multiple preembryos are usually introduced to the uterus (insurance against the low probability of implantation) → multiple births: occur in over 30% of cases, compared with about 2% in unassisted pregnancies
- Animal cloning:
  - **Reproductive:** the embryo is transplanted into the uterus of a foster mother
  - **Therapeutic:** the embryo is used to produce ES cells in culture, which can then be used to produce various specialized cell types for the treatment of the individual



- **Stem cell potency**



- Source of **Embryonic Stem (ES) Cells:** Inner cell mass of the blastocyst

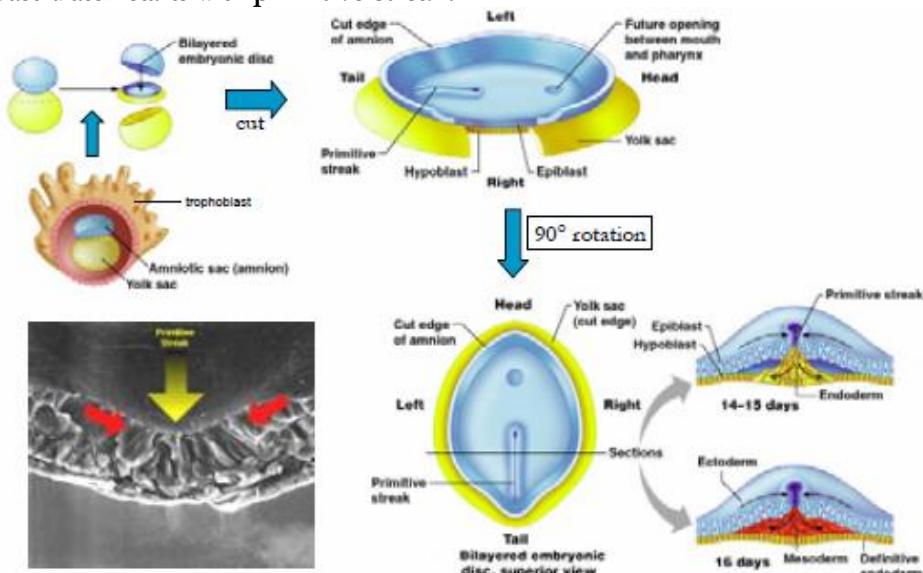


## Trilaminar germ disc & embryonic period

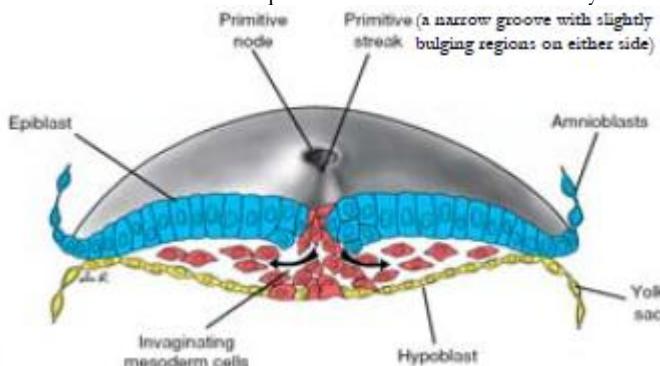
### Gastrulation

- Gastrulation (Gr.: *belly*): Bilaminar disk → trilaminar disk
  - The embryo has reached a point of balance in its effort to
    - Draw nourishment from the mother (trophoblast layers and their derivatives)
    - To be protected against cellular assault (several ensheathing layers & protective cavities)
    - To harbor enough of its own food (secondary yolk sac)
  - Time to move: the inner cell mass derivatives differentiate into the three functional cell layers of life
    - Outer layer: protective and sensitive

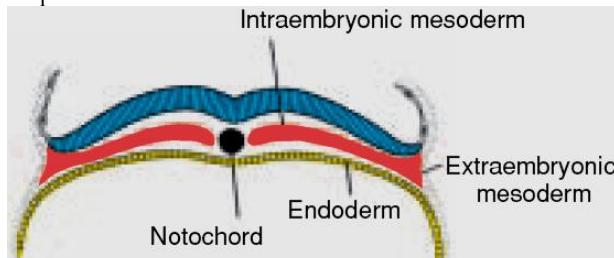
- Inner layer: energy-absorbing
- Connective layer between them: contractile movement
- Gastrulation starts with **primitive streak**:



- Formed by cells of the epiblast which choose one of the two fates:
  - pass deep to the epiblast layer to form the populations of cells within the embryo
    - **Endoderm** (formed by replacement of hypoblast by invading epiblast cells)
    - **Mesoderm** (once definitive endoderm is established, inwardly moving epiblast forms mesoderm)
- remain on the dorsal aspect of the embryo to become the embryonic ectoderm
- Formation of the primitive streak is induced by the underlying visceral hypoblast

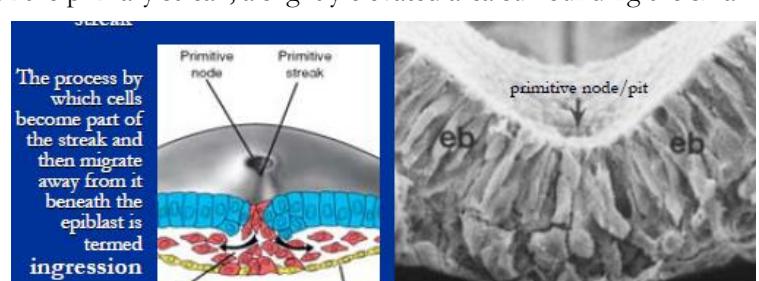


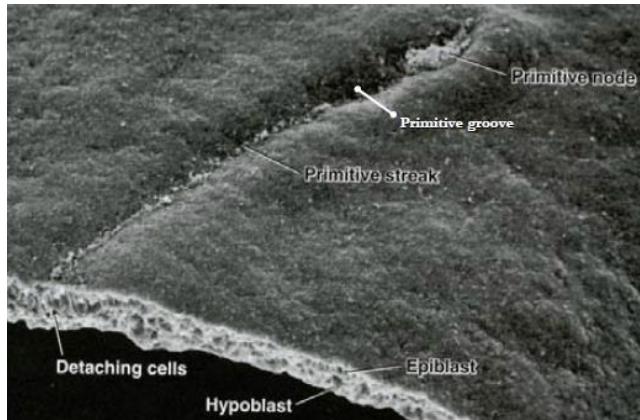
- Cells of the *intraembryonic* mesoderm migrate between ectoderm & endoderm until they establish contact with the *extraembryonic* mesoderm covering yolk sac and amnion
- Mesodermal cells cannot penetrate the adhesion at the head end of the embryo called **buccopharyngeal membrane**, they cannot penetrate the adhesion at the tail end called **cloacal membrane**, and they cannot displace the notochord cells that have streamed out of the primitive streak and remained in the midline



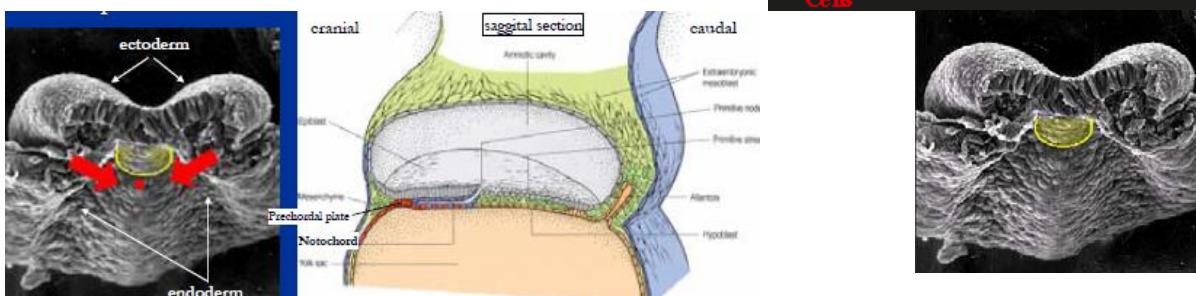
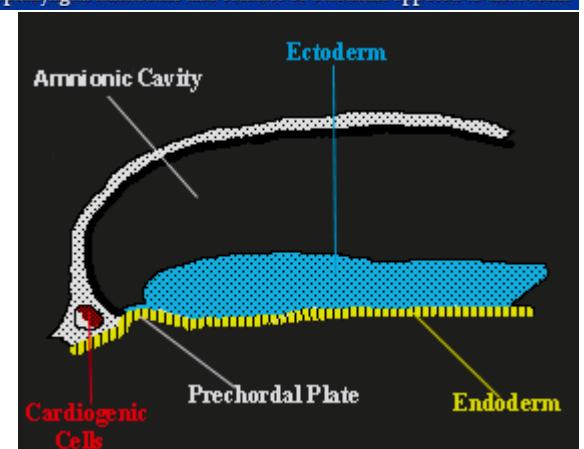
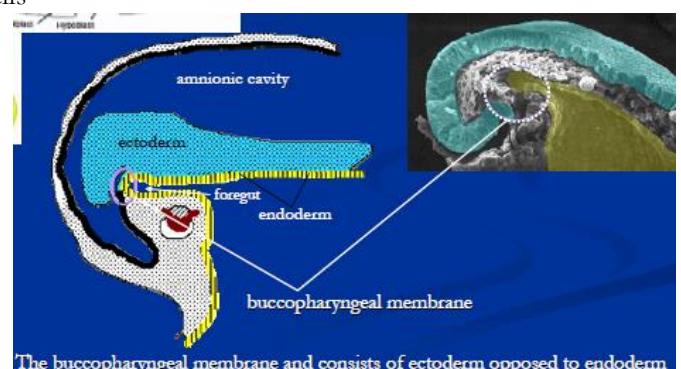
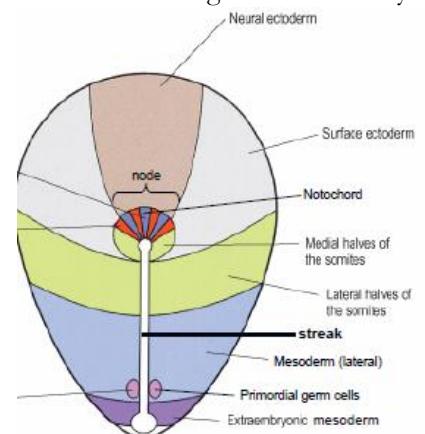
- Structures associated with the primitive streak:

- **Primitive (Hensen's) node:** cephalic end of the primary streak; a slightly elevated area surrounding the small primitive pit
- **Primitive pit:** invagination in the center of the primitive node
- **Primitive groove:** the lower midline portion of the primitive streak

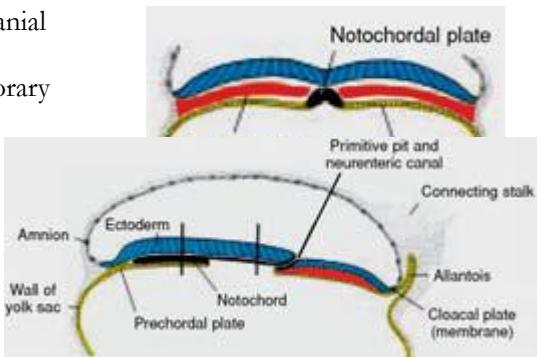




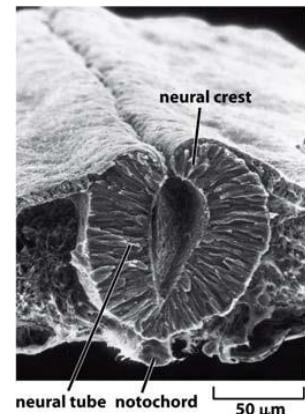
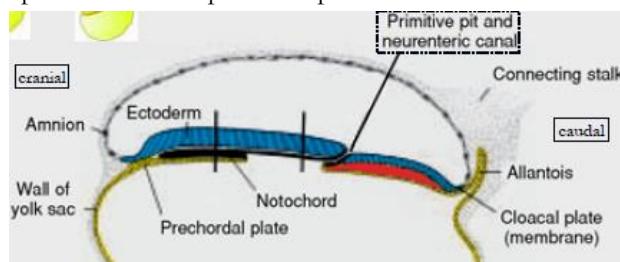
- Epiblast is the **source of all germ layers** and cells in these layers will give rise to all tissues and organs in the embryo
- The position and time of ingression through primary streak directly affects the developmental fate of cells:
  - Cells that ingress through the primitive node give rise to the axial cell lines, the prechordal mesoderm and notochord
  - Primitive streak rostral portion → cell for the lateral halves of the somites
  - Primitive streak middle portion → lateral plate mesoderm
  - Primitive streak caudal portion → primordial germ cells, extraembryonic mesoderm
- Structures in the cranial end of the embryonic disk:
  - Buccopharyngeal membrane:**
    - A small region at the cranial end of the embryonic disc, cranial from the prechordal plate
    - Tightly adherent ectoderm and endoderm cells
    - Represents the future opening of the oral cavity
    - Breaks down in the 4th week, opening the oral cavity to the pharynx
  - Prechordal (prochordal) plate:**
    - Forms between the tip of the notochord and the buccopharyngeal membrane
    - Derived from some of the first cells that migrate through the primitive node in a cephalic direction
    - Important in forebrain induction
    - In the rostral midline, the ectoderm and endoderm are opposed. The endoderm in this location forms the prechordal plate (a thickening of the endoderm)
    - Prechordal plate is located at the anterior end of the notochord, but not reaching the rostral extremity of the embryo
- Primitive streak establishes **cranial / caudal; left / right body axes**
- Notochord (= chorda dorsalis):** specialization of the mesoderm
  - The notochord extends in the midline from the prechordal plate, caudally to the primitive streak



- Prenotochordal cells coming from the primitive pit move in cranial direction until they reach the prechordal plate
- Preenotochordal cells mix with hypoblast cells to form a temporary **notochordal plate**
- As hypoblast is replaced by endoderm, notochordal plate cells detach from the endoderm: **definitive notochord**
- Notochord extends from buccopharyngeal membrane to primitive pit: cranial end forms first, caudal later
- Notochord underlies the **neural tube** and serves as the basis for the **axial skeleton**

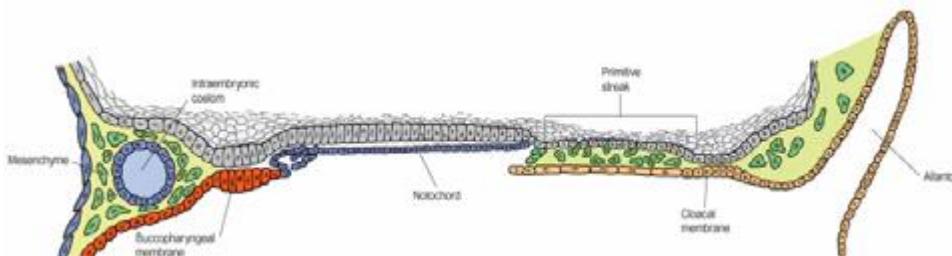


- Neureenteric canal:** Temporary connection between the amniotic and yolk sac cavities at the point where the primitive pit forms and indentation in the epiblast



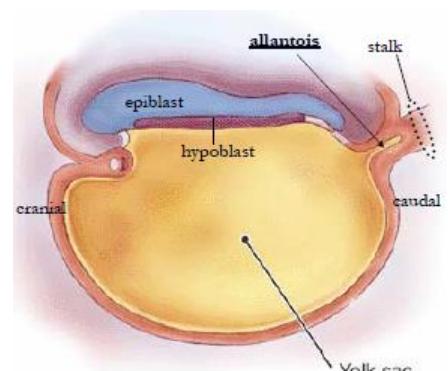
- Cloacal membrane:**

- Formed at the caudal end of the embryonic disc
- Similar structure to the buccopharyngeal membrane → tightly adherent ectoderm and endoderm cells with no intervening mesoderm
- Breaks down in the 7th week → anus & urinary / reproductive systems openings



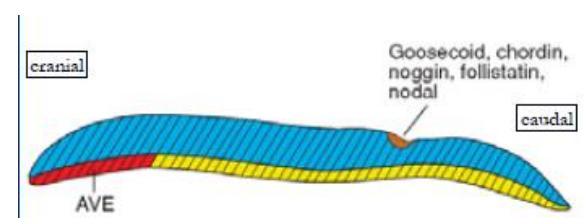
- Allantois:**

- When the cloacal membrane appears (day 16), the posterior wall of the yolk sac forms a small diverticulum that extends into the connecting stalk: **allantois**
- A little out-pocketing of the caudal end of yolk sac trapped in the connecting stalk
- This blind pouch is called the allantois (Gr.: *sausage-shaped*=)
- Importance:
  - Structural base for the umbilical cord
  - Becomes part of the urinary bladder



- Establishment of body axes** takes place before and during the period of gastrulation:

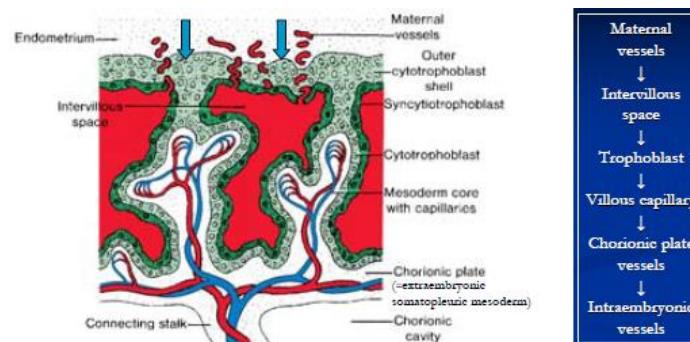
- Body axes:
  - Anteroposterior**
  - Dorsoventral**
  - Left-right**
- Anteroposterior axis is signaled by cells at the anterior (cranial) margin of the embryonic disc: **anterior visceral endoderm (AVE)** expresses genes essential for head formation
- Left-right sidedness and dorsoventral axis formation are orchestrated by genes expressed in the primitive streak and pit



- Growth of the embryonic disc:**

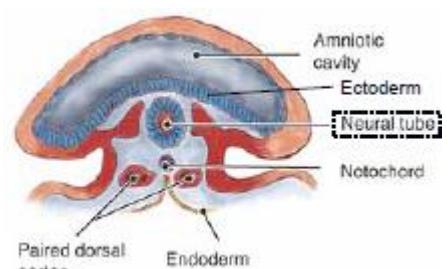
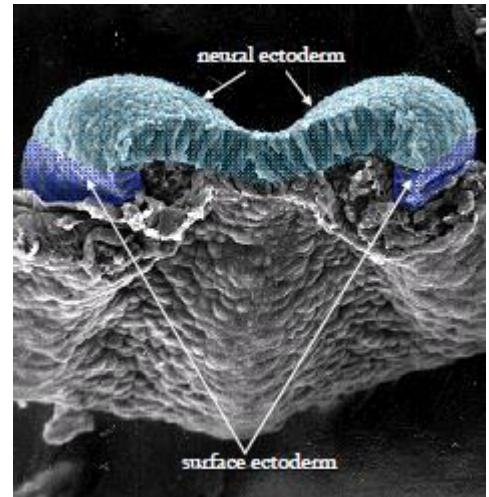
- Initially flat and almost round, embryonic disk gradually becomes elongated with a broad cephalic and a narrow caudal end

- Expansion of the embryonic disc occurs mainly in the cephalic region; the region of the primitive streak remains more or less the same size
- In the cephalic part, germ layers begin their specific differentiation by the middle of the 3rd week, whereas in the caudal part, differentiation begins by the end of the 4th week
- Gastrulation may be disrupted by genetic abnormalities and toxic insults:
  - **Caudal dysgenesis (sirenomelia)**: insufficient mesoderm is formed in the caudal-most region of the embryo
  - Because this mesoderm contributes to formation of the lower limbs, urogenital system, and lumbosacral vertebrae, abnormalities in these structures ensue
- **Secondary villi**: Mesodermal cells penetrate the core of primary villi and grow toward the decidua
- **Tertiary (definitive, placental) villi**: By the end of 3rd week, mesodermal cells in the core of the villus begin to differentiate into blood cells and vessels, forming the villous capillary system
- Development of **trophoblast**: end of 3rd week
  - Cytotrophoblastic cells surround the trophoblast entirely (form outer shell) and are in direct contact with the endometrium
  - The outer shell gradually surrounds the trophoblast entirely and attaches the chorionic sac firmly to the maternal endometrial tissue
  - Embryo is suspended in the chorionic cavity by means of the connecting stalk
  - Maternal vessels penetrate the cytotrophoblastic shell to enter intervillous spaces, which surround the villi
- **Stem vs. terminal tertiary villi**:
  - Stem (anchoring) villi: extend from the chorionic plate to the endometrium
  - **Terminal** (free) villi: branch from the sides of stem villi. Through them, exchange of nutrients will occur

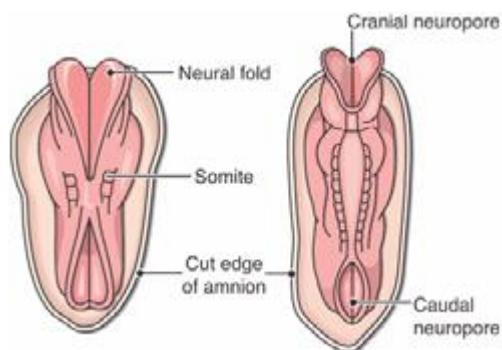
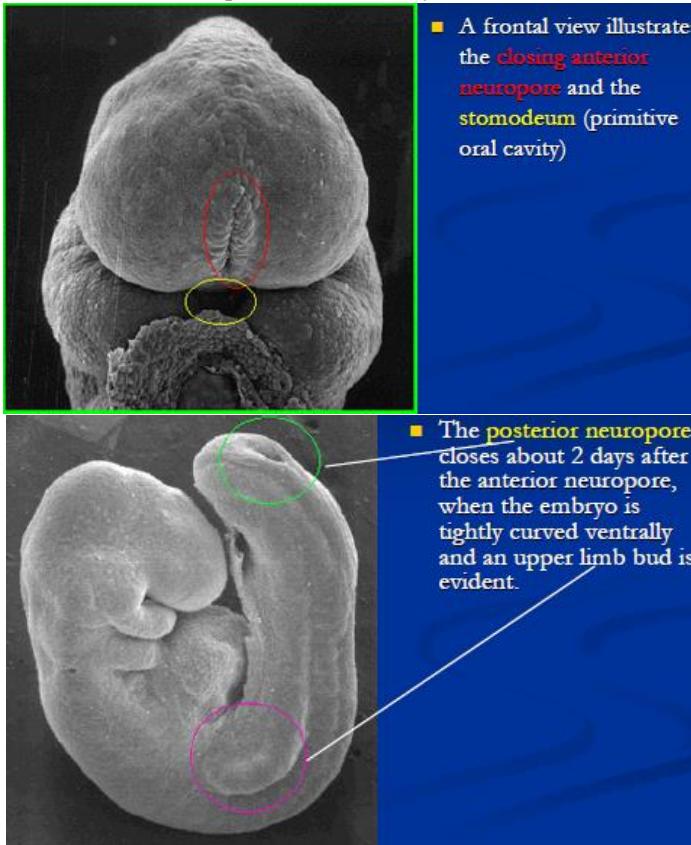


## Embryonic period

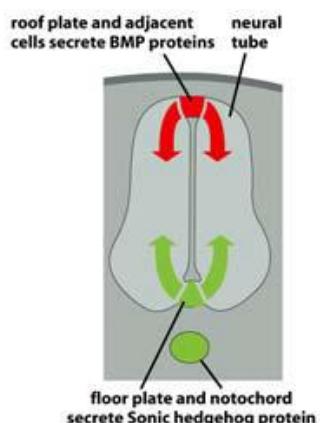
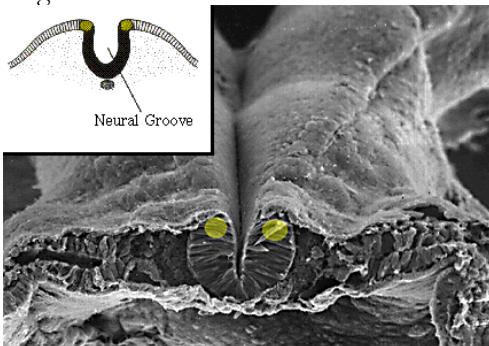
- Embryonic period = period of **organogenesis**
- Each of the 3 germ layers gives rise to a number of specific tissues
- By the end of the 2nd month, the main organ systems have been established: the major features of the external body form are recognizable
- **Ectoderm derivates:**
  - **Surface ectoderm:**
    - Skin (epidermis, hair, nails, glands)
    - Anterior pituitary
    - Ear (receptor apparatus of inner ear)
    - Nose (olfactory epithelium)
  - **Neuroectoderm:**
    - Neural tube: central nervous system, eye (iris, ciliary)
    - Neural crest: cells outside CNS
- **Neurulation:**
  - Differentiation of a subset of neuroectodermal cells into neural precursor cells
    - Neural plate
    - Neural fold
    - Neural tube
  - Key consequence of neurulation
  - Formation of **neural tube**: central nervous system
  - Formation of **neural crest**: all neurons outside of the brain and spinal cord + numerous dispersed cell types
- **Neural plate → neural groove:**
  - Notochord and prechordal mesoderm induces the overlying ectoderm to thicken and form the **neutral plate** (around Day 18)
  - Neural plate gradually expands toward the primitive streak → at the end of 3rd week the primitive streak runs 1/4 of the embryo
  - The edges of the plate (**neural folds**) rise and form a concave area known as the **neural groove** (about day 20)
  - **Neural groove**: The ectoderm can be distinguished as neural ectoderm that comprises the central nervous system, and surface ectoderm that will cover the outside of the body
- **Neural groove → Neural tube:**
  - Neural folds approach each other in the midline where they fuse (about day 21)



- The tube pulls away from the ectoderm above it to become embedded underneath the ectoderm
- Neural folds fusion begins in the cervical region and proceeds cranially and caudally
- Until fusion is complete, the extremities of the neural tube communicate with the amniotic cavity by way of the **cranial and caudal neuropores**
- The **neural tube closure** marks the end of neurulation
  - Cranial neuropore closes on day 25
  - Caudal neuropore closes on day 27

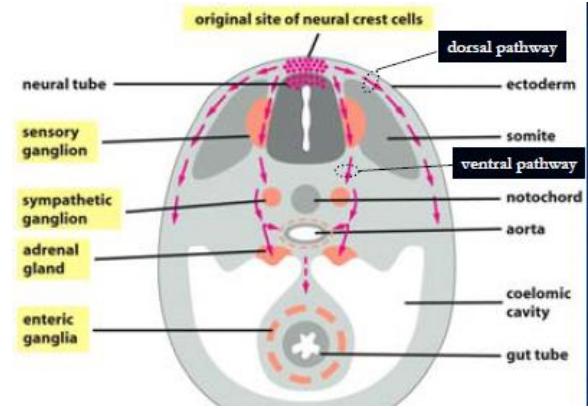


- Disorders of Primary Neurulation: Neural Tube Defects**
  - Craniorachischisis totalis
  - Anencephaly
  - Myeloschisis
  - Encephalocele
  - Myelomeningocele
  - Arnold-Chiari malformation
- Neuronal specifications within the neural tube is orchestrated by signals from notochord & floor plate
- Neural crest:** as the neural folds elevate and fuse, cells at the dorsal border of the neural fold begins to dissociate from their neighbors



- Neural crest cells migrate away from the neural tube:
  - Following the closure of the trunk neural folds, the neural crest cells leave the dorsal aspect of the neural tube
  - Neural crest cells leave the neuroectoderm by active migration and displacement to ender the underlying mesoderm

- Neural crest derivatives:
  - Most of the sensory components of the PNS
  - Sensory neurons of cranial and spinal sensory ganglia
  - Autonomic ganglia and the postganglionic autonomic neurons
  - Much of the mesenchyme of the anterior head and neck
  - Melanocytes of the skin and oral mucosa
  - Odontoblasts (cells responsible for production of dentin)
  - Chromaffin cells of the adrenal medulla
  - Cells of the arachnoid and pia mater
  - Sattelite cells of peripheral ganglia
  - Schwann cells

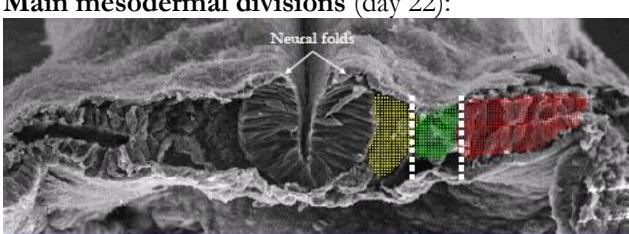


- Ectodermal placodes:

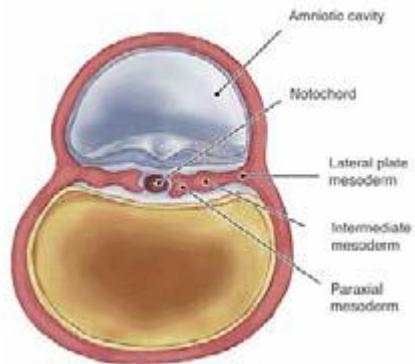
- Small local aggregates of ectoderm remaining within the surface ectoderm → pass deep to the surface ectoderm after neurulation
- Give rise to neural / non neural structures:
  - Optic placodes: lens of the eye
  - Dental placodes: enamel of teeth
  - Otic placodes: labyrinth of the inner ear
  - Adenohypophyseal placode: anterior pituitary
  - Olfactory placode: olfactory epithelial cells

## Mesoderm

- Epiblast cells ingress through the primitive streak between ectoderm & endoderm → become elongated → detach from the epiblast
- Mesoderm derivatives:
  - Mesoderm cells coming from primitive node and rostral primitive streak form the paraxial mesoderm (day 17)
  - Intermediate mesoderm: lateral to the paraial, for a short stretch of the embryo's length
  - Mesoderm cells coming from the middle to caudal streak form the lateral plate mesoderm. Divided into two layers:
    - Somatic (parietal) mesoderm: continuous with mesoderm covering the amnion
    - Splanchnic (visceral) mesoderm: continuous with mesoderm covering the yolk sac
- Main mesodermal divisions (day 22):



■ The intraembryonic mesoderm differentiates into **paraxial**, **intermediate**, and **lateral plate** portions



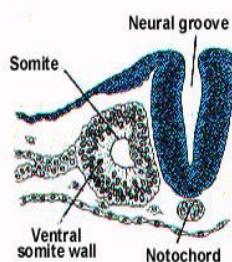
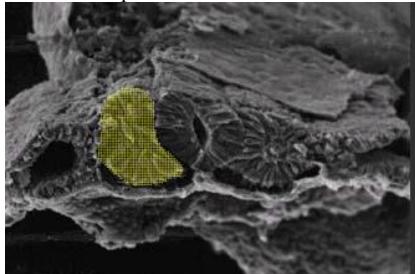
- Mesoderm partition: paraxial
  - Paraxial mesoderm accumulates under the neural plate with thinner mesoderm laterally
  - Forms 2 thickened streaks running the length of the embryonic disc along the rostrocaudal axis
  - During the 3rd week, paraxial mesoderm begins to segment
- Mesoderm partition: intermediate & lateral
  - Intermediate mesoderm connects paraxial and lateral plate mesoderm
  - Lateral plate mesoderm layers line a newly formed cavity, the **intraembryonic cavity (coelom)**, which is continuous with the extraembryonic cavity on each side of the embryo
- Paraxial mesoderm development: segmentation
  - Early 3rd week: paraxial mesoderm forms paired ball-like structures (**segments = somitomeres**). By day 20 they organize into **somites**
  - Somite formation starts cranially:
    - Caudally at a speed of 3 pairs/day
    - At 5th week 42-44 pairs (4 occipital, 8 cervical, 12 thoracic, 5 lumbar, 5 sacral, 8-10 coccygeal pairs)
  - Occipital & coccygeal later disappear

- Somite number correlates to age

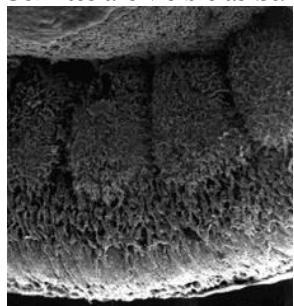
| Approximate Age (days) | No. of Somites |
|------------------------|----------------|
| 20                     | 1-4            |
| 21                     | 4-7            |
| 22                     | 7-10           |
| 23                     | 10-13          |
| 24                     | 13-17          |
| 25                     | 17-20          |
| 26                     | 20-23          |
| 27                     | 23-26          |
| 28                     | 26-29          |
| 30                     | 34-35          |

- **Somite:** day 23

- the paraxial mesodermal cells in the trunk become organized into **somites**

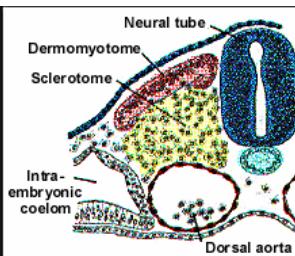
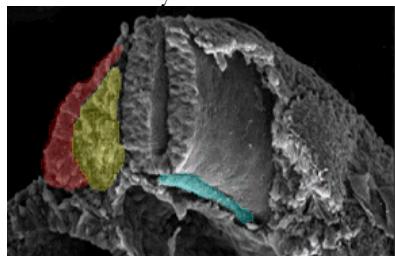


- Somites are visible as **ball-like structures** at day 28



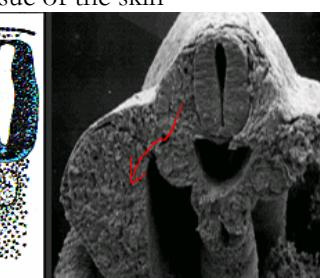
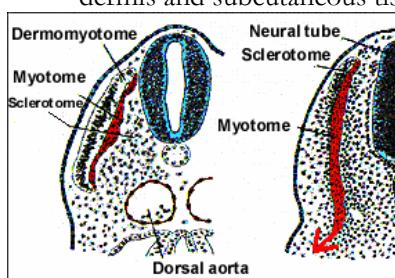
- **Somite differentiation:** day 27

- Each somit differentiates, forming a **dermomyotome** and a **sclerotome**
- Sclerotome cells build the ventral and medial walls of the somite, lose their compact organization, surround the notochord
- Dermomyotome cells build the dorsolateral walls of the somite: divide into dermatome & myotome



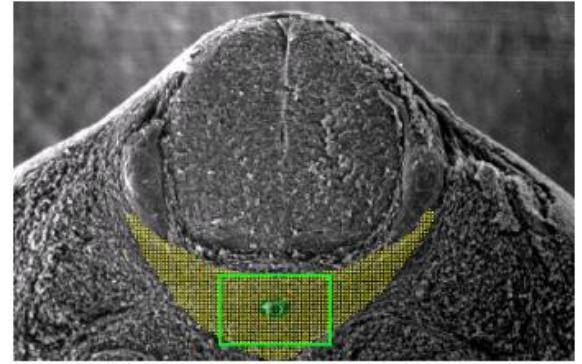
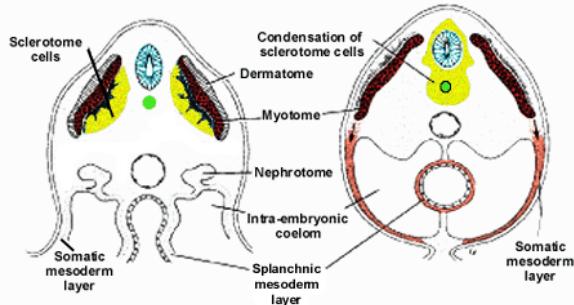
- Dermomyotome cells form dermatome & myotome: day 28

- The myotomes are comprised of muscle cells, some of which migrate into the body wall and limbs
- Dermatome cells lose their epithelial configuration and spread out under the overlying ectoderm: form dermis and subcutaneous tissue of the skin

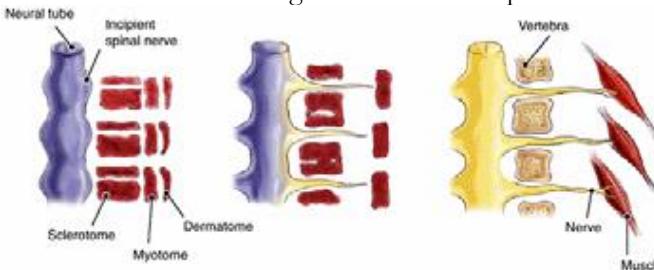


- **Sclerotome differentiation:** day 28

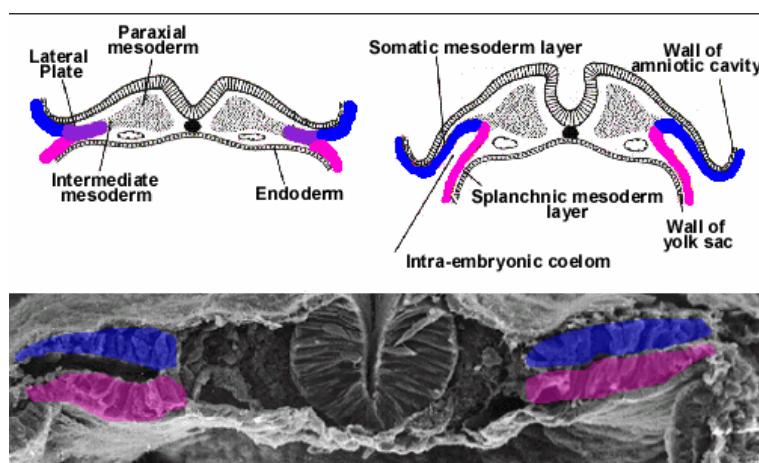
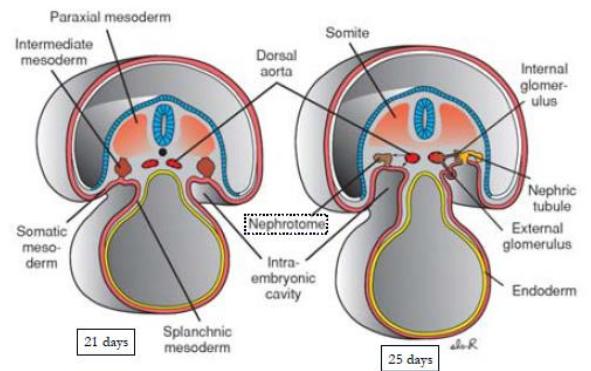
- The **sclerotomes** are comprised of cells that form the vertebrae. In the midline, these cells condense around the notochord

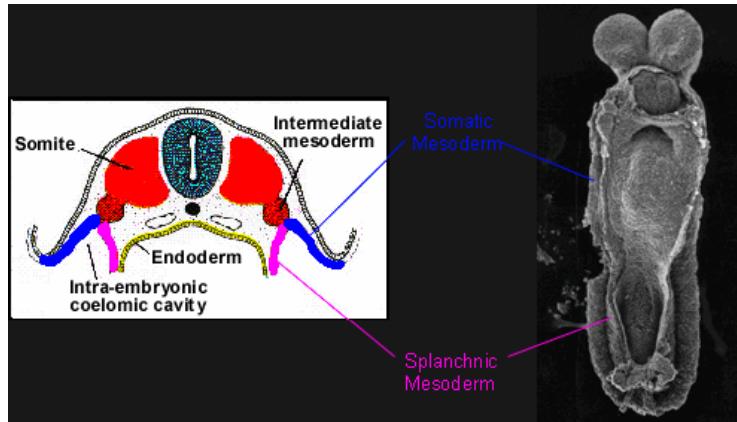


- The **sclerotomes** cells differentiate into cartilage and bone, and surround the notochord
- Within the intervertebral disks that form between the vertebral bodies, the notochord persists as the nucleus pulposus
- Each myotome and dermatome retains its segmental innervation no matter where the cells migrate
  - Each somite forms its own
    - **sclerotome:** cartilage and bone component
    - **Myotome:** segmental muscle component
    - **Dermatome:** segmental skin component
  - Each myotome and dermatome has its own segmental nerve component

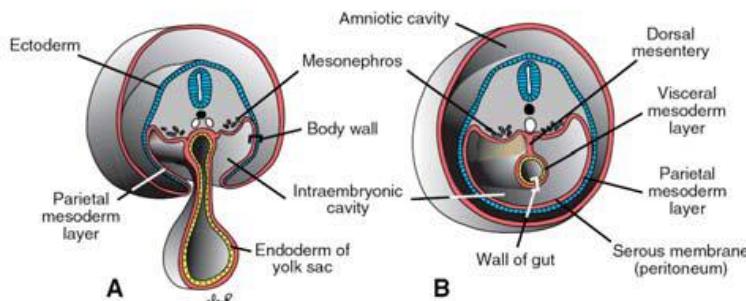


- **Regulation of body plan by Hox genes**
  - **Homeobox** genes are known for their DNA binding motif, the **homeobox**
  - They code for transcription factors that activate cascades of genes regulating phenomena such as segmentation and axis formation
- **Development of intermediate mesoderm:**
  - Temporarily connects paraxial mesoderm with the lateral plate mesoderm
  - Differentiates into urogenital structures
    - Cervical and upper thoracic regions: **nephrotomes** (segmented cell clusters)
    - More caudally: **neurogenic cord** (unsegmented mass of tissue)
- **Development of lateral plate mesoderm:**
  - The lateral plate mesoderm splits into somatic (parietal) and splanchnic (visceral) layers
  - Between them - the intraembryonic coelom
    - **Somatic mesoderm:** ventral & lateral body wall and limbs
    - **Splanchnic mesoderm:** wall of the viscera (heart, hollow organs)

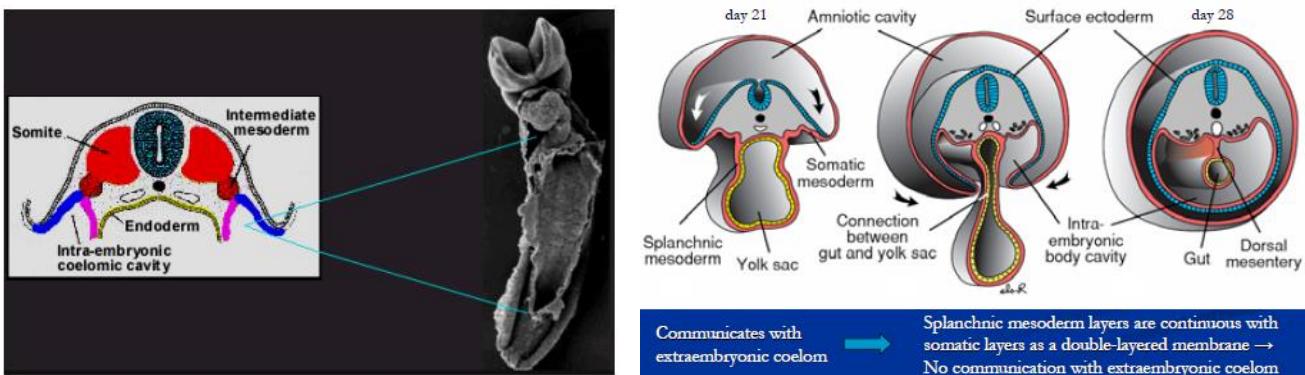




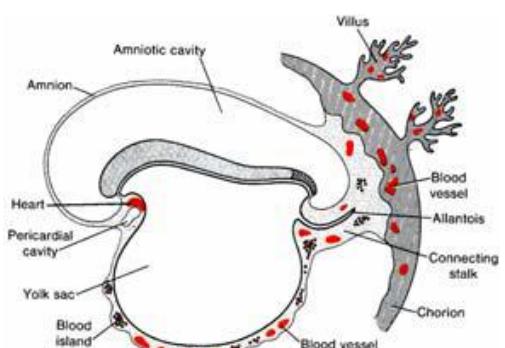
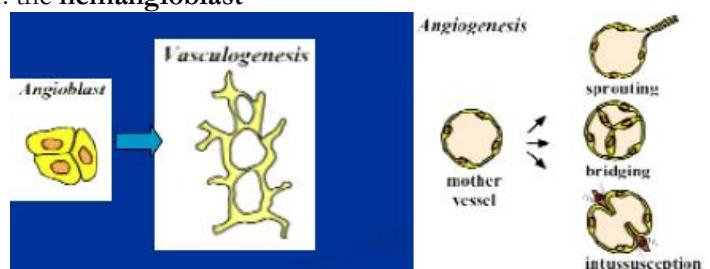
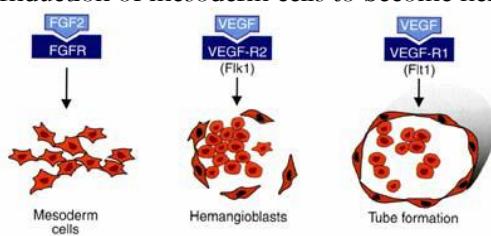
- Lateral plate mesoderm contributes to serous membranes around internal organs
  - **Somatic mesoderm:** peritoneal, pleural, and pericardial cavities
  - **Splanchnic mesoderm:** serous membrane around each organ

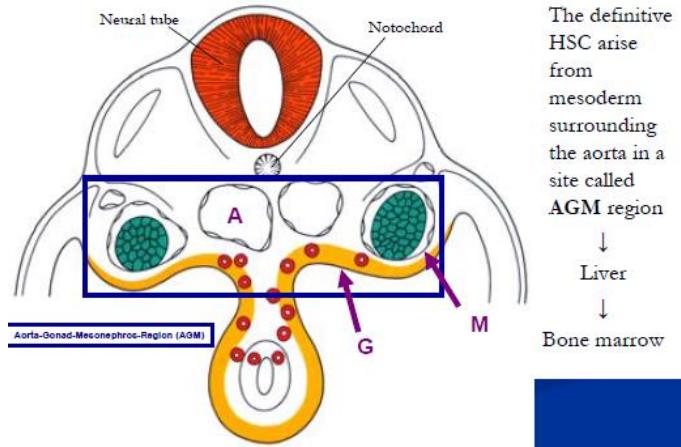


- The space between the two layers of lateral plate mesoderm forms the **intraembryonic coelom**:



- **Mesoderm vs. mesenchyme:**
  - **Mesoderm** refers to cells derived from the epiblast and extraembryonic tissues
  - **Mesenchyme** refers to loosely organized embryonic connective tissue regardless of origin
- Vessel and blood cell from from a commom precursor: the **hemangioblast**
  - Types of blood vessel production:
    - **Vasculogenesis** from hemangioblasts: angioblasts
    - **Angiogenesis** from pre-existing vessels
  - Hemangioblasts appear in mesoderm surrounding the wall of the yolk sac at mid-3 weeks of development:
    - Mesoderm cells form extraembryonic splanchnopleure form blood islands
    - Center of blood islands form HSC: blood cells
    - Periphery of blood islands form angioblasts: blood vessels
  - Induction of mesoderm cells to become hemangioblasts



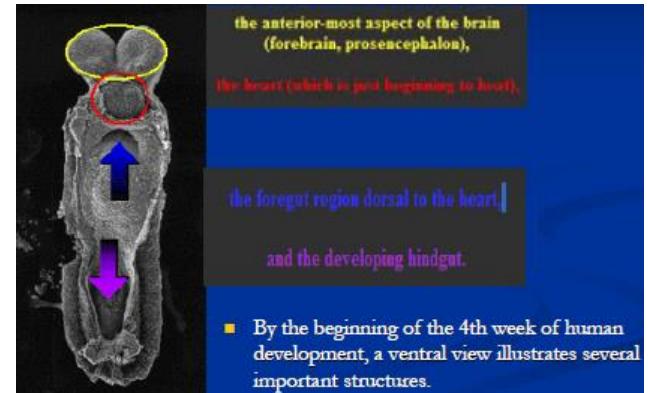
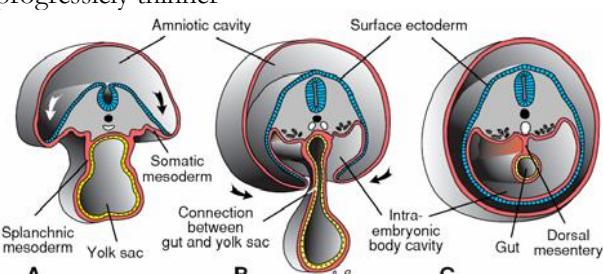


- Formation of the cardiogenic field:

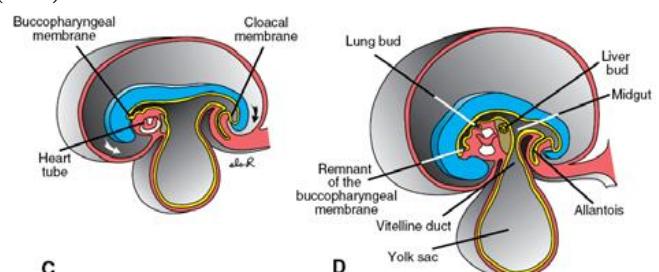
- Cardiac progenitor cells lie in the epiblast, immediately lateral to the primitive streak
- Migrate through the streak: position in front of the neuroectoderm at day 18
- The intraembryonic cavity over the cardiogenic field later develops into the **pericardial cavity**
- Heart starts beating around day 21
- Rapid growth of the developing brain pushes the heart ventrally
  - Rapid growth of the developing brain produces more caudal and ventral positioning of the cardiogenic region and formation of the foregut
  - As the neural cells proliferate and grow forward, the cardiogenic area is folded over and the endoderm-lined foregut is formed

- Endoderm derivatives:

- Covers the ventral surface of the embryo and forms the roof of the yolk sac
- Initially forms the epithelial lining of the primitive gut
  - Foregut:** temporarily bounded by the buccopharyngeal membrane; after 4th week a connection is formed between the foregut and the amniotic cavity
  - Midgut:** temporarily communicates with the yolk sac by way of a broad stalk, the **vitelline duct**
  - Hindgut:** temporarily bounded by the cloacal membrane; after 7th week a connection is formed between the hindgut and the amniotic cavity (anus)
- Progressive **cephalocaudal** folding of the embryo: continuously larger portion of the endoderm-lined cavity is incorporated into the body of the embryo proper
- The passage between midgut / yolk sac becomes progressively thinner

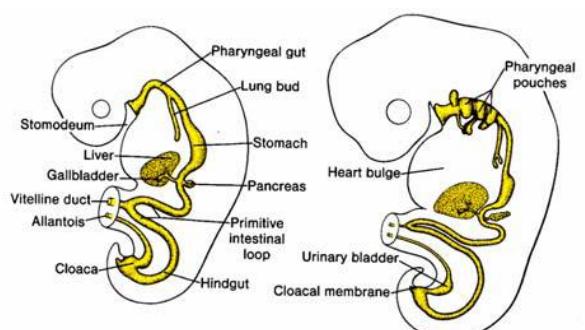


- By the beginning of the 4th week of human development, a ventral view illustrates several important structures.



- Derivatives of the endodermal germ layer:

- Early structures:
  - Epithelial lining of the primitive gut
  - Epithelial lining of the intraembryonic portions of the allantois and vitelline duct
- Later structures:
  - Epithelial lining of the respiratory tract
  - Parenchyma** of the thyroid, parathyroids, liver, and pancreas



- Reticular stroma of the tonsils and thymus
- Epithelial lining of the urinary bladder and urethra
- Epithelial lining of the tympanic cavity and auditory tube
- The embryonic period is divided into **23 Carnegie stages** based on external and internal morphological criteria and not on length or age

| Endoderm                                                                                                                                                                      | Mesoderm                                                                                                                                                                                                                                | Ectoderm                                                                                                                 |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|
| <ul style="list-style-type: none"> <li>◦ lining of digestive system</li> <li>◦ lining of respiratory system</li> <li>◦ liver</li> <li>◦ pancreas</li> <li>◦ glands</li> </ul> | <ul style="list-style-type: none"> <li>◦ muscle</li> <li>◦ outer covering of organs</li> <li>◦ excretory system</li> <li>◦ gonads</li> <li>◦ circulatory system</li> <li>◦ bones and cartilage</li> <li>◦ circulatory system</li> </ul> | <ul style="list-style-type: none"> <li>◦ nervous system and brain</li> <li>◦ epidermis and related structures</li> </ul> |

## External appearance of the embryo & fetus

- **External appearance:** 2nd month (w. 5-8)
  - 5th-8th week period is the tie of organogenesis
  - At the end of the 4th week the main external features are the somites and **pharyngeal arches**
  - In 2nd month the age of the embryo is indicated as **crown-rump length (CRL)** as counting somites becomes difficult
  - **CRL** is the distance from the vertex of the skull to the midpoint between the apices of the buttocks in millimeters
  - At the end of the embryonic period, the embryo has human appearance

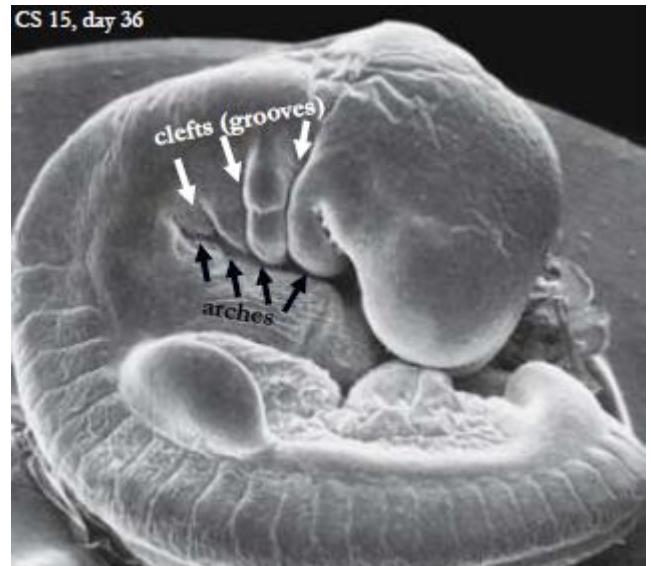
| Carnegie stages | Length (mm) | Age (days) | External features                                                                              |
|-----------------|-------------|------------|------------------------------------------------------------------------------------------------|
| 10              | 2-3.5       | 28         | Neural folds begin to fuse; otic pit develops; 4-12 somites; pharyngeal arches 1 and 2 visible |
| 11              | 2.5-4.5     | 29         | Rostral neuropore closes; 13-20 somites                                                        |
| 12              | 3-5         | 30         | Caudal neuropore closes; 21-29 somites; 4 pharyngeal arches visible; upper limb buds appearing |
| 13              | 4-6         | 32         | Otic vesicle closed; lens disc not yet indented; 30 or more somites; 4 limb buds visible       |
| 14              | 5-7         | 33         | Lens pit appears; upper limb buds elongated                                                    |
| 15              | 7-9         | 36         | Lens pit closed; nasal pit appearing; hand plate forming                                       |

| Carnegie stages | Length (mm) | Age (days) | External features                                                                                    |
|-----------------|-------------|------------|------------------------------------------------------------------------------------------------------|
| 16              | 8-11        | 38         | Retinal pigment visible; nasal sacs face ventrally; auricular hillocks beginning; foot plate appears |
| 17              | 11-14       | 41         | Head relatively larger; trunk straighter; auricular hillocks distinct; finger rays                   |
| 18              | 13-17       | 44         | Body more cuboidal; elbow region and toe rays appearing                                              |
| 19              | 16-18       | 46         | Trunk elongating and straightening                                                                   |
| 20              | 18-22       | 49         | Upper limbs longer and bent at elbows                                                                |
| 21              | 22-24       | 51         | Fingers longer; hands approach each other; feet likewise                                             |
| 22              | 23-28       | 53         | Eyelids and external ear more developed                                                              |
| 23              | 27-31       | 56         | Head more rounded; limbs longer and more developed                                                   |

Week 5

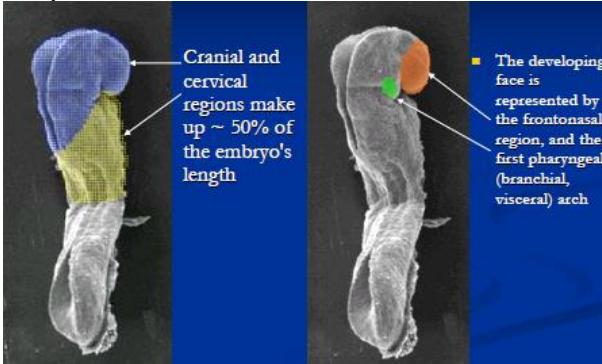
Week 6-8

- Week 9 is the start of the fetal period:
- Size of the fetus:
  - At the junction of trimesters 1 & 2 (=90 days old) → length of 90 mm
  - At the junction of trimesters 2 & 3, the fetus is approx. 250 mm length and weight is approx. 1,000 kg
- **Development of the head & neck:**
  - **Early stages:** transformation of the head (cephalic) folds
    - Neural walls with the eye primordia
    - Otic disc & pharyngeal arches
    - Lens & nasal placodes
  - Development of the human face is usually described as a process of merging of 5 swellings (growth centres) surrounding the stomodeum, termed processes ( prominences )
    - Bilateral maxillary processes
    - Bilateral mandibular processes
    - Frontonasal prominence
- **Pharyngeal arches:**
  - Series of bulges on the lateral surface of the head and neck
  - Separated by clefts (grooves)
  - 4 pairs are visible by day 30
  - More caudally, no clear-cut arrangement, but a 5th and a 6th arch are distinguished

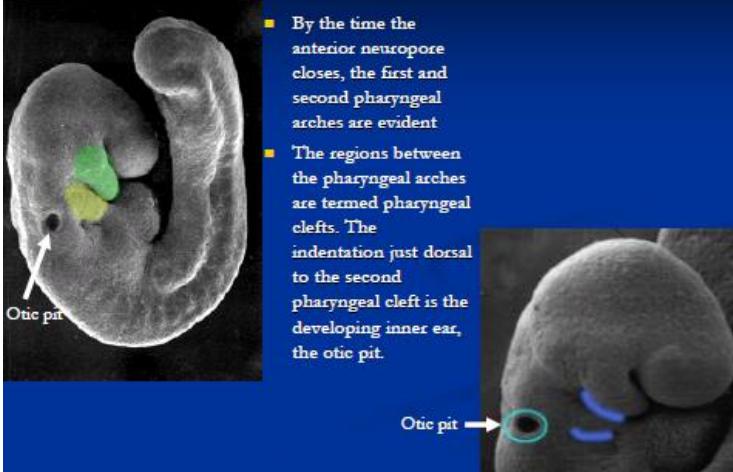


- Clefts have internal counterparts, the **pharyngeal pouches**

- Early 4th week:



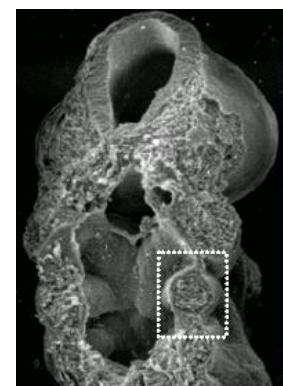
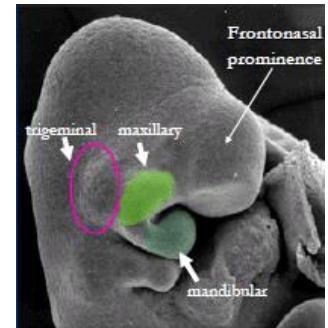
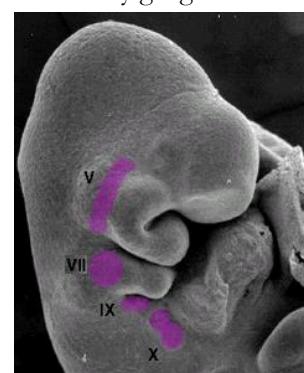
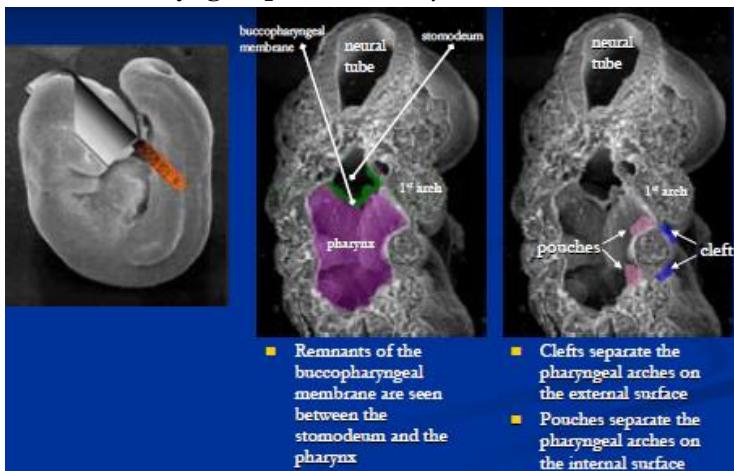
- Late 4th and early 5th week:



- Early 5th week:

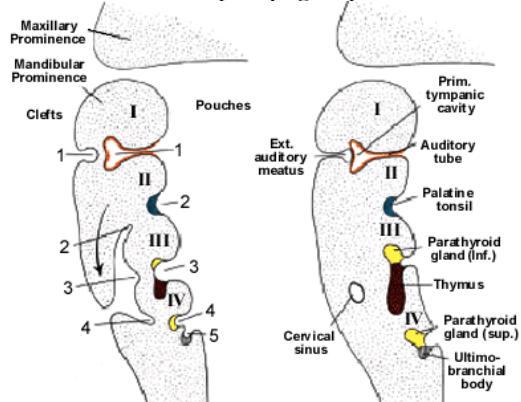
- The **1st pharyngeal arch** has both a **maxillary** and a **mandibular** prominence. Dorsal to the first is an elevation formed by the underlying **trigeminal ganglion** that supplies tissues derived from the first arch
- Epibranchial placodes** are specialized regions of surface ectoderm, the cells of which invaginate to contribute to the formation of the sensory ganglia of cranial nerves V, VII, IX, and X

- Pharyngeal pouches at day 29:

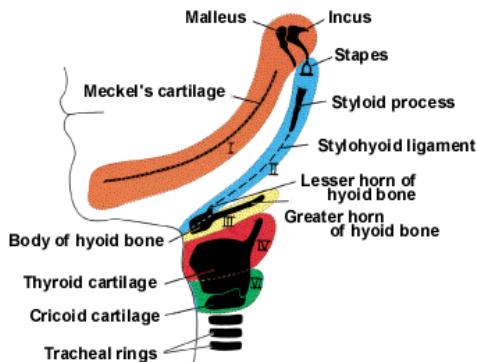


The mesenchyme of the arches is derived in part from neural crest and in part from mesoderm

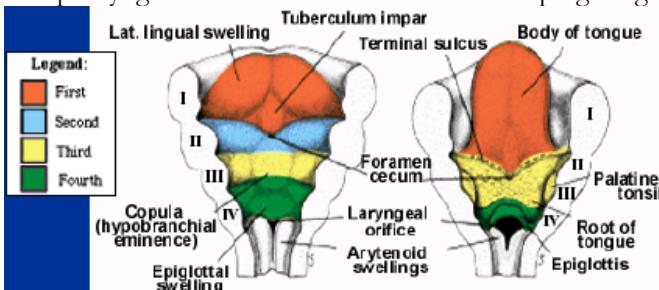
- Derivatives of the pharyngeal pouches:



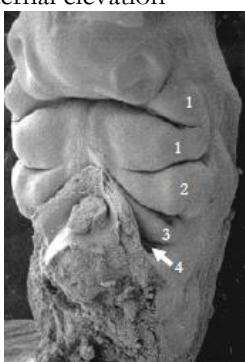
- Some of the neural crest cells in each of the arches become cartilage:



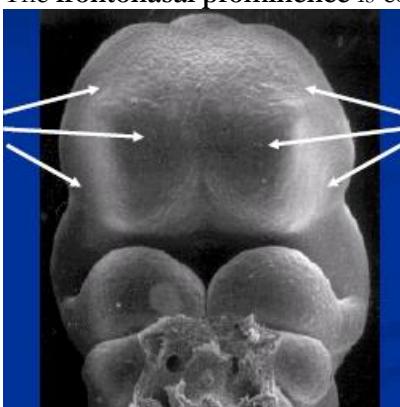
- The pharyngeal arches contribute to the developing tongue and epiglottis



- Pharyngeal arches:** day 35: The 1st, 2nd, 3rd, and 4th arches are visible externally. The sixth arch does not form an external elevation

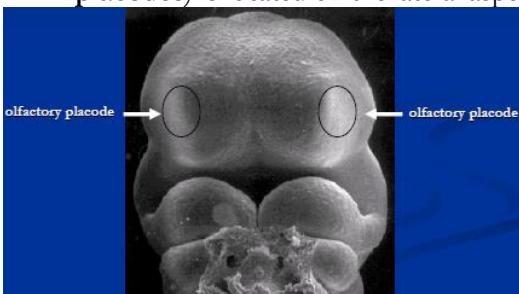


- The frontonasal prominence is composed of the tissue that surrounds the forebrain (day 35)

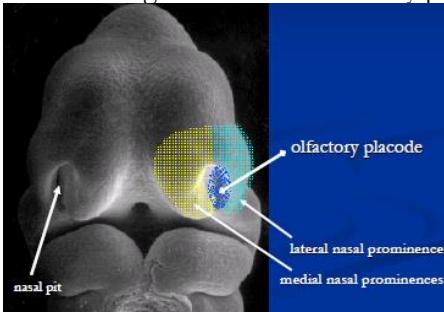


- **Olfactory placodes:**

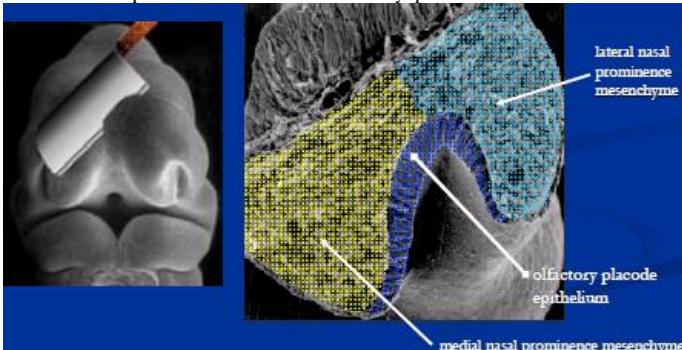
- Following closure of the anterior neuropore, the ectoderm that will line the nasal cavities (**olfactory placodes**) is located on the lateral aspects of the frontonasal prominence (day 35)



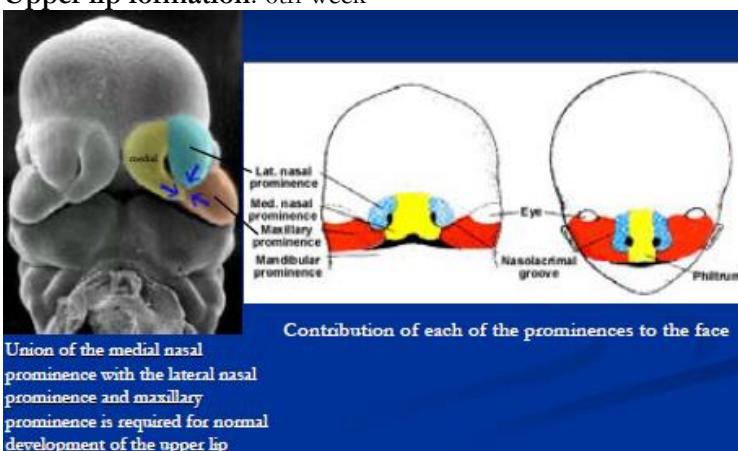
- During 5th week the olfactory placodes line the nasal pits



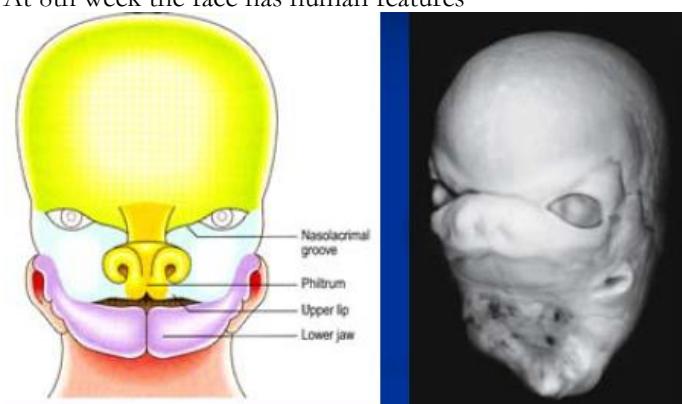
- Composition of the olfactory placode:



- **Upper lip formation: 6th week**



- At 8th week the face has human features

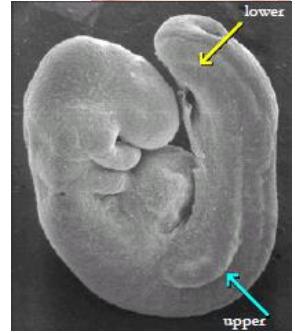


- **Craniofacial defects:** Complete **holoprosencephaly** was diagnosed at 22 weeks of gestation, after which pregnancy was terminated at 24 weeks. At autopsy, the female fetus showed agenesis of both eyes and orbits, arhinia and mirostomia.

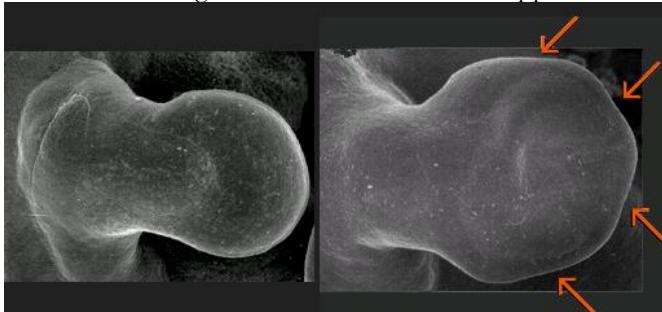


- **Limb development:**

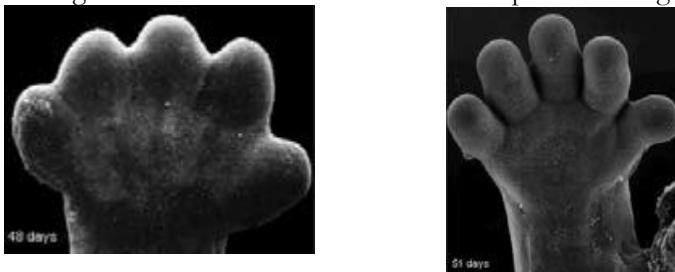
- By the beginning of the 5th week, forelimbs and hindlimbs appear as paddle-shaped buds
- Buds flatten → radial grooves (rays) appear on the distal portion of the buds → digits
- The developing upper limb is evident earlier than the lower limb
- The thickened ectoderm at the distal rim of the limb bud is termed the **apical ectodermal ridge**. Integrity of the apical ectodermal ridge is essential for continued limb outgrowth



- As the limb bud grows, indentations become apparent in the hand (or foot) plate



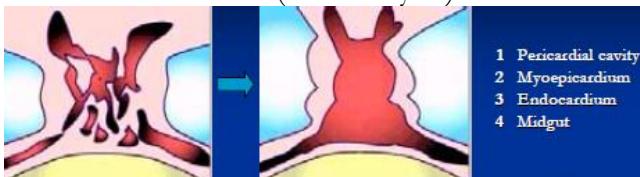
- During the 7th & 8th week of human development the digits of the hand become apparent



- At the beginning of the fetal period, touch pads are prominent features of the hands and feet
- As the hand develops, webs that are present between the outgrowing digits must regress
- Development of the feet is like that of the hands, but in the human it starts approx. 3-4 days later

- **Formation of the cardiogenic field:**

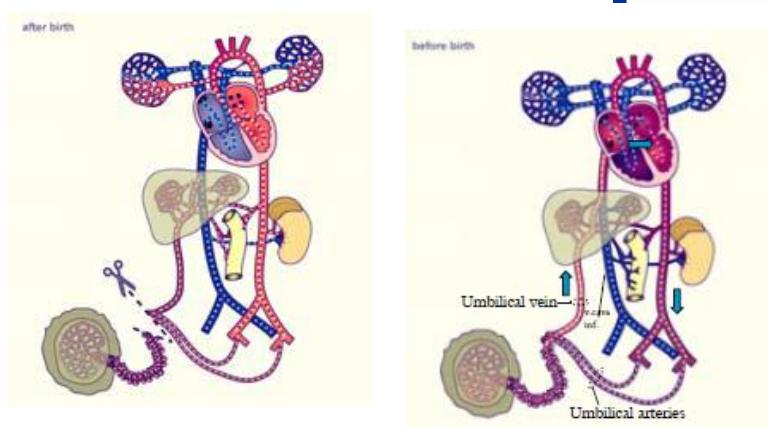
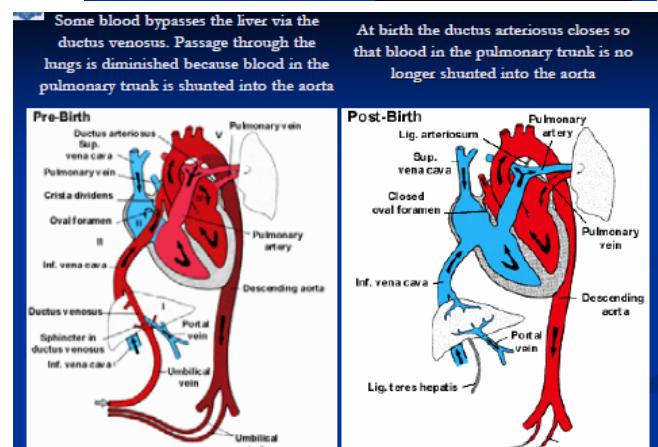
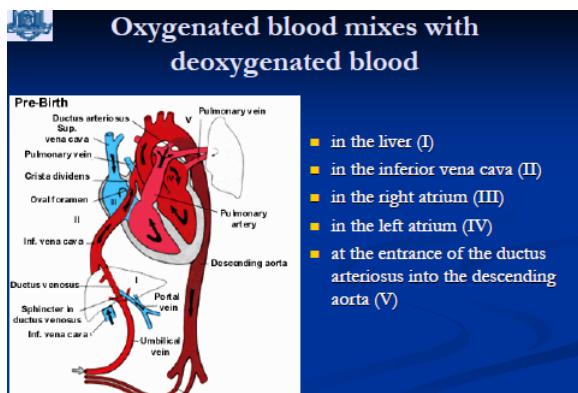
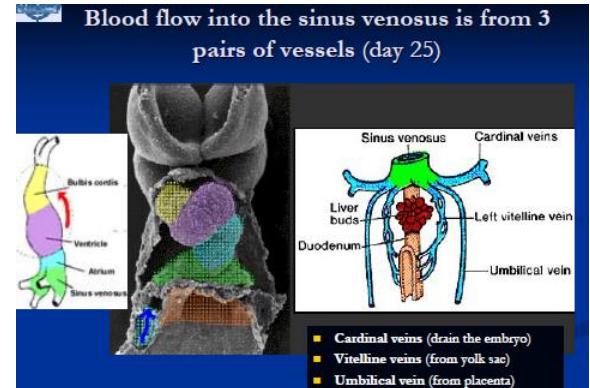
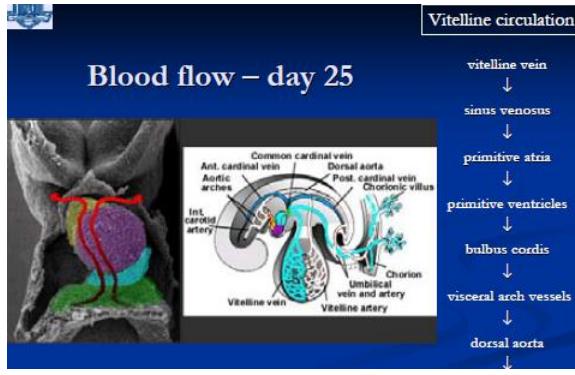
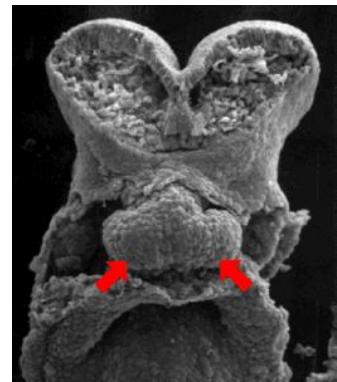
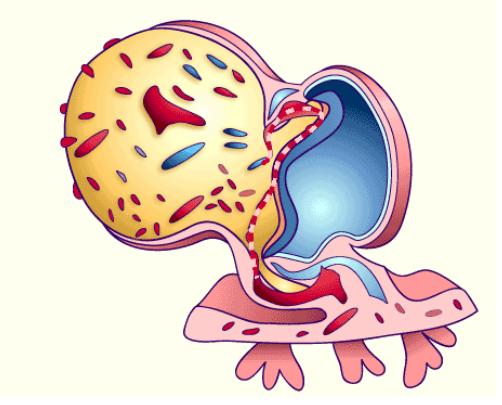
- Cardiac progenitor cells lie in the epiblast, immediately lateral to the primitive streak
- Migrate through the streak → position in front of the neuroectoderm at day 18
- The intraembryonic cavity over the cardiogenic field later develops into the **pericardial cavity**
- **Heart tube formation** (around day 20)



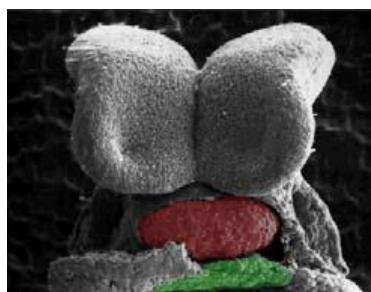
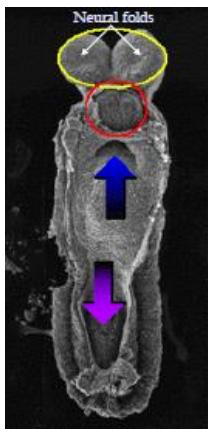
This is a frontal section through the cardiac anlage. The endothelial cells differentiate out of the neighboring mesenchymal cells of the splanchnopleure and flow together to make up numerous vesicles that form the **endocardial plexus**. The myocardial mantle arises out of the material of the cardiogenic plate.



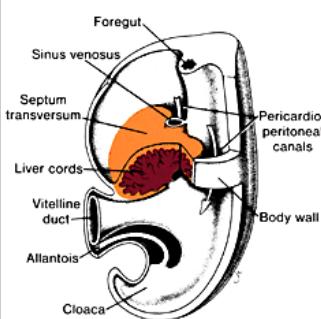
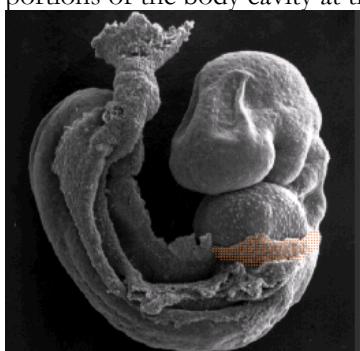
- Mid-4th week (day 24): Blood enters the caudal aspect of the heart tube and leaves in the region of the forming visceral arch
- Embryonic blood circulation:



- Early 4th week, day 22: The **septum transversum** is located just below the developing heart, which at this stage, begins to beat

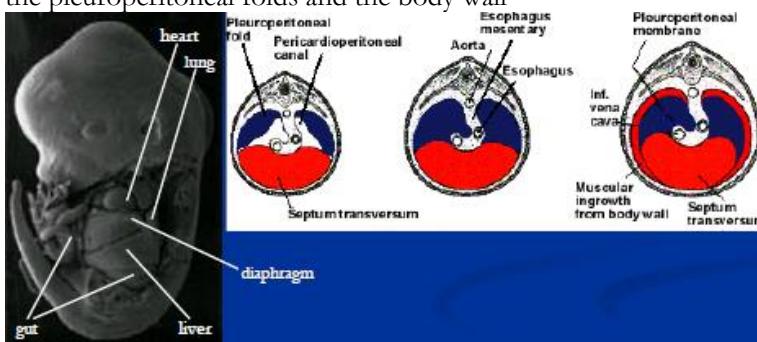


- The body cavity, which forms between the splanchnic and somatic mesoderm, becomes divided by the septum transversum into the pleuroperitoneal cavity and the peritoneal cavity
- The septum transversum is primordium of the diaphragm which is located just rostral to the developing liver. Bilateral passageways (pericardioperitoneal canals) connect the pleuroperitoneal and peritoneal portions of the body cavity at this developmental stage



- Boy cavities (week 7):

- Separation of the space containing the lungs and heart (pleuroperitoneal cavity) and that with the liver and gut (peritoneal cavity) is completed as the diaphragm forms with contributions from the septum transversum, the pleuroperitoneal folds and the body wall



- Embryonic membranes:**

- Amnion
- Chorion
- Yolk (vitelline) sac
- Allantois
- Epiblast & hypoblast form 2 embryonic membranes
  - Amnion:** formed by epiblast; filled with amniotic fluid
    - Provides a protective environment for the embryo
    - Helps maintain a constant homeostatic temperature
    - Amniotic fluid comes from maternal blood, and later, fetal urine
  - Yolk sac:** hypoblast cells that form a sac on the ventral surface of the embryo
    - Forms part of the digestive tube
    - Produces earliest blood cells and vessels
  - Gradually amnion expands while yolk sac disappears

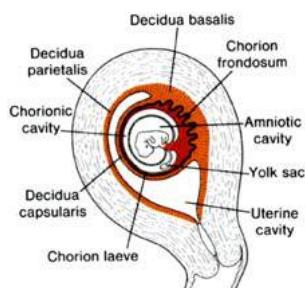


- **Amniotic fluid:**

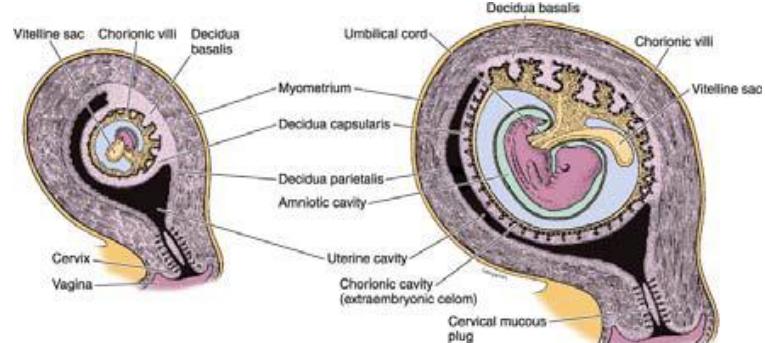
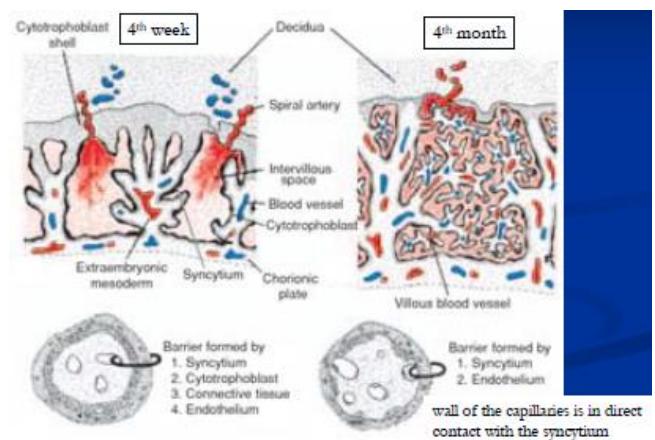
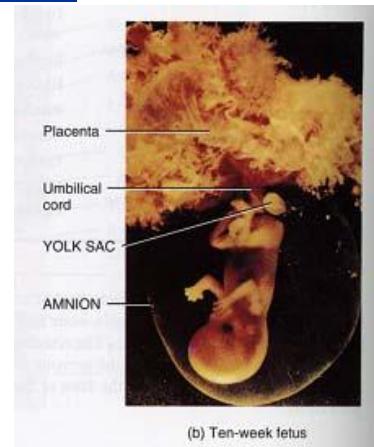
- Clear, watery fluid, derived primarily from maternal blood
- Volume: 30ml (8 w.), 450 ml (29 2.), 800-1000 ml (at birth)
- Functions:
  - Absorbs stress on the embryo / fetus
  - Prevents adherence of the embryo to the amnion
  - Allows for fetal movements
- **Polyhydramnios / oligohydramnios:** increased or decreased volume which leads to birth defects

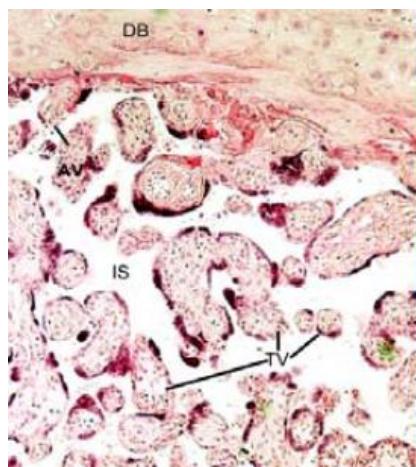
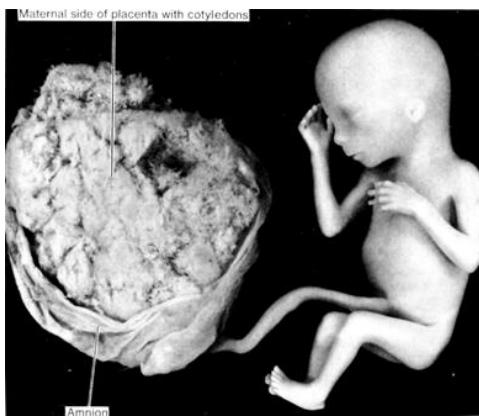
## Development of the placenta

- Stations:
  - Primary villi (day 13)
  - Trophoblast (3rd week)
  - Tertiary villi (3rd week)
  - Placenta is fully formed and functional by the end of the 3rd month
- **Chorion compartments:**
  - Villi on the embryonic pole continue to grow and expand, giving rise to the **chorion frondosum** (bushy)
  - Villi on the abembryonic pole degenerate and by the 3rd month: **chorion laeve** (smooth)



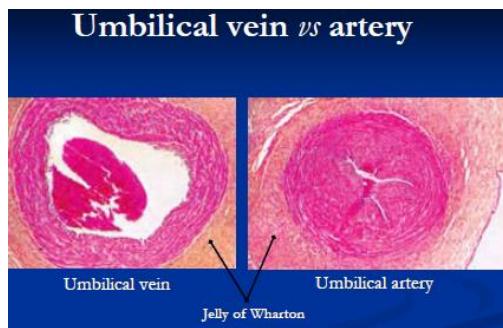
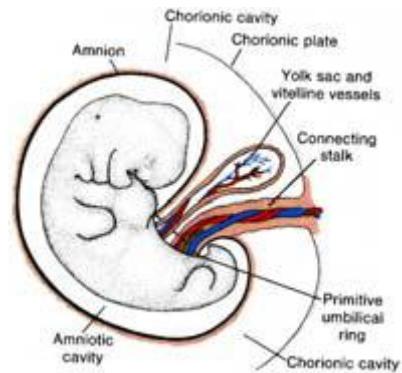
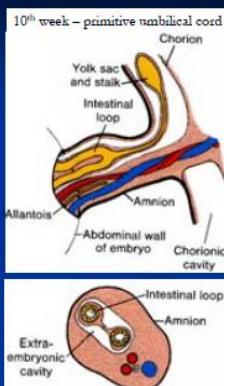
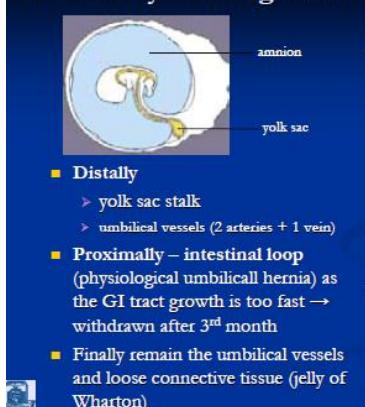
- During pregnancy endometrium is called **decidua**
- Placenta is composed of cells derived from two genetically distinct individuals: Placenta = fetal part (**chorion**) + maternal part (**decidua basalis**)
- **Cotyledon:**
  - In the second half of pregnancy, decidua (maternal) septa form, which project into intervillous spaces but do not reach the chorionic plate
  - Thus, the placenta is divided into a number of compartments: cotyledons
- At full term, the placenta is **discoid**.
- Measures: 15-25 cm diameter; 3 cm thick; 500 to 600 g





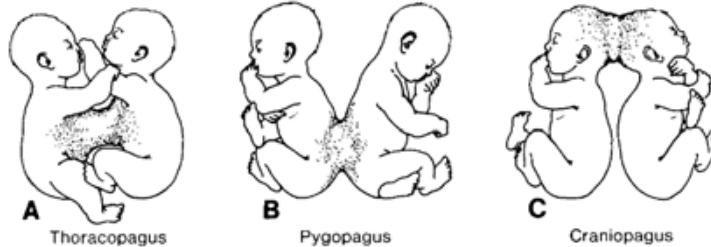
- Functions of placenta:
  - Exchange of gases
  - Exchange of nutrients and electrolytes
  - Transmission of maternal antibodies
  - Hormone production
- Structures passing via the umbilical ring (5th week):
  - Connecting stalk:**
    - Allantois
    - Umbilical vessels (2 arteries, 1 vein)
  - Yolk stalk (vitteline duct):** accompanied by the vitelline vessels
  - Canal** connecting the intraembryonic and extraembryonic cavities

#### After embryo folding & amnion enlargement



- Twins:
  - Dizygotic (fraternal):**
    - 70% of twins; 7-11 / 1000 births
    - Ovulation of 2 oocytes and fertilization by 2 different spermatozoa; may or may not be different sex; no more resemblance than any other brothers or sisters
  - Monozygotic (identical):**
    - 30% of twins; 3-4 / 1000 births

- 1 oocyte & 1 spermatozoon: splitting of the zygote at various stages of development (2-cell stage or blastocyst stage or bilaminar disk stage)
  - Triplets are rare (1 / 7600 births), quadruplets, quintuplets, and so forth is rarer
- **Partial splitting of the primitive node and streak: conjoined (Siamese) twins**

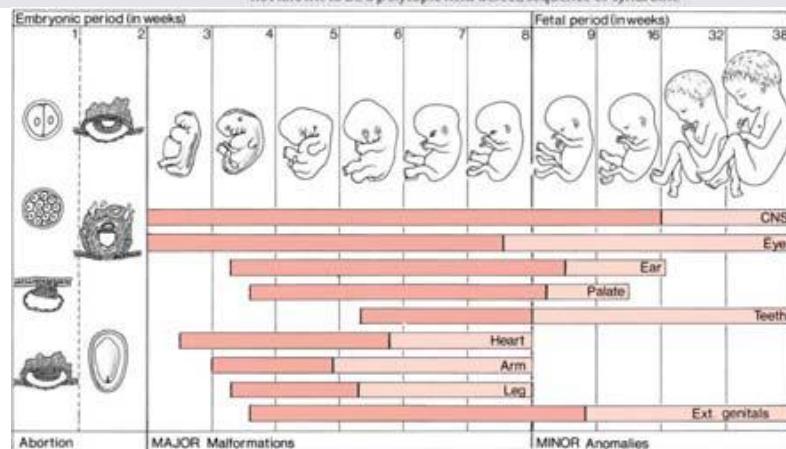


- Half of monochorionic monoamniotic twins are cojoined

### Teratology:

- **Birth defect, congenital malformation, and congenital anomaly** are synonymous terms used to describe structural, behavioral, functional, and metabolic disorders present at birth
- **Teratogens** (Gr. *Teratos*, monster + *gen*, producing)
- Major structural anomalies occur in 2 to 3 % of liveborn infants, and an additional 2 to 3 % are recognized in children by age 5 years (totally: 4-6 %)

| Individual alterations of form and structure |                                                                                                                                                                                                 |
|----------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Malformation                                 | A morphological defect of an organ, part of an organ or a larger region of the body resulting from an intrinsically abnormal developmental process                                              |
| Disruption                                   | A morphological defect of an organ, part of an organ or a larger region of the body resulting from the extrinsic breakdown of, or interference with, an originally normal developmental process |
| Deformation                                  | An abnormal form, shape or position of a part of the body caused by mechanical forces                                                                                                           |
| Dysplasia                                    | An abnormal organization of cells into tissue(s) and its morphological result(s)                                                                                                                |
| General terminology                          |                                                                                                                                                                                                 |
| Hypoplasia, hyperplasia                      | Underdevelopment and overdevelopment of an organism, organ or tissue resulting from a decreased or increased number of cells, respectively                                                      |
| Hypotrophy, hypertrophy                      | A decrease or increase in the size of cells, tissues or organs, respectively                                                                                                                    |
| Agenesis                                     | Absence of a part of the body caused by an absent anlage (primordium)                                                                                                                           |
| Aplasia                                      | Absence of a part of the body resulting from a failure of the anlage to develop                                                                                                                 |
| Atrophy                                      | Decrease of a normally developed mass of tissue(s) or organ(s) because of a decrease in cell size and/or cell number                                                                            |
| Patterns of morphological defects            |                                                                                                                                                                                                 |
| Polytopic field defect                       | A pattern of anomalies derived from the disturbance of a single developmental field                                                                                                             |
| Sequence                                     | A pattern of multiple anomalies derived from a single known or presumed prior anomaly or mechanical factor                                                                                      |
| Syndrome                                     | A pattern of multiple anomalies thought to be pathogenetically related and not known to represent a single sequence or a polytopic field defect                                                 |
| Association                                  | A non-random occurrence in two or more individuals of multiple anomalies not known to be a polytopic field defect, sequence or syndrome                                                         |



- The most sensitive period for inducing birth defects is the **third to eighth weeks** of development, the period of **embryogenesis**
- **Thalidomide:** Taken as a sedative in early pregnancy, thalidomide proved to be a teratogen with severe effects on embryonic limb development
- Invasive sampling tests:

