



# Cytology, Histology and Embryology Notes

Citology and Histology (Medical University-Pleven)

# Cytology, Histology and Embryology

## Topic 1: Subject, Purpose and History of Cytology

- Cell theory: Schleiden & Schwann (1838-1839):
  - i) Cell is the unit of structure, physiology, and organization in living things.
  - ii) The cell retains a dual existence as a distinct entity and a building block in the construction of organisms.
  - iii) Cells form by free-cell formation (Spontaneous Generation)
- All living things are made up of cells.
- **Cell:** Structural & Functional Unit of all living things.
- All cells come from pre-existing cells by division (**Spontaneous Generation does not occur**)
- Cells contain **hereditary information** which is passed from cell to cell during cell division.
- Cells have the **same chemical composition**.
- All energy flow of life occurs within cells.

## Topic 2: Introduction in cytology. Methods of the cell study. Principles of cytological, histological, cytochemistry, immunohistochemistry investigations

- **Cell and tissue culture:**
  - Cells can be grown *in vitro* from newly explanted tissues (primary cultures) or as long-established cell lines and can be examined in the living state by phase-contrast light microscopy.
- **Enzyme Histochemistry:**
  - **Histochemical (or cytochemical) techniques** use specific enzymatic activities in lightly fixed or unfixed tissue sections to produce visible products in the specific enzyme locations.
  - Fixation and paraffin embedding denatures most enzymes, so histochemistry usually uses **frozen tissue** section with a **cryostat**.
  - Enzyme classes for which histochemical study is useful include **phosphates, dehydrogenases, and peroxidases**, with peroxidases often conjugated to antibodies used in immunohistochemistry.
- **Visualizing Specific Molecules:**
  - Some substances specifically bind certain targets in cells.
  - **Immunohistochemistry** is based on specific **reactions between an antigen and antibodies labelled with visible markers**, often fluorescent compounds or peroxidase for light microscopy and gold particles for TEM.
  - **Direct immunohistochemistry:** Antigen of interest is detected by directly binding a **labeled primary antibody**.
  - **Indirect immunohistochemistry** uses an unlabeled primary antibody that is detected bound to its antigen with labelled **secondary antibodies**.
  - The indirect immunohistochemical method is more commonly used because the **added level of antibody binding amplifies the signal detected** and provides greater technical flexibility.
  - **In situ hybridization (ISH)** – Specific gene sequences or mRNAs of cells can be

detected microscopically using labelled complementary DNA (**cDNA**) probes.

### Topic 3: Methods of the cell study – Preparation of permanent histological material

- Preparation of Tissues for Study
  - i **Fixation:** Chemical fixatives such as formalin are used to **preserve tissue** structure by cross-linking and **denaturing** proteins, **inactivating** enzymes, and preventing cell **autolysis** or self-digestion.
  - ii **Dehydration:** The fixed tissue is **dehydrated in alcohol** and cleared in organic solvents that prepares it for embedding and sectioning.
  - iii **Infiltration:** Tissue placed in **melted paraffin** until it becomes completely infiltrated.
  - iv **Embedding:** Embedding in **paraffin wax** or epoxy resin allows the tissue to be cut into very thin sections with microtome.
  - v **Mounting:** Sections are mounted on glass for **staining**, which is required to **reveal specific cellular** and **tissue components** with the microscope.
- The most commonly used staining method is a combination of stains **hematoxylin** and **eosin** (H&E), which act as basic and acidic dyes, respectively.
- Cell substances with a net negative charge, such as DNA and RNA, react strongly with hematoxylin and basic stains; such material is said to be '**basophilic**'.
- Cationic substances, such as **collagen** and many **cytoplasmic proteins** react with eosin and other acidic stains and are said to be '**acidophilic**'.
- Interpretation of structures in tissue sections:
  - Many steps in tissue processing, slide preparation, and staining can introduce minor **artefacts** such as spaces and precipitates that are not normally present in the living tissue and must be recognized.
  - Sections of cells tissues are essentially 2-D planes through 3-D structures, and understanding this fact is important for their correct interpretation and study.

### Topic 4: Microscopy and different types of microscopes

- **Resolving Power:** Ability of microscope lens or optical system to produce separate images of closely positioned objects.
- **Light microscope:**
  - **Bright-Field Microscopy:** This method is most commonly used by both students and pathologists, **uses ordinary light and the colours that are imparted by tissue staining**.
    - i) **Mechanical Part:** Stand, Stage, Tube
    - ii) **Optical Part:** Eyepiece lens, Objective lens, Condenser
    - iii) **Illumination Part:** Light bulb, Filter
  - **Fluorescence Microscopy:** **Uses UV light**, under which **only fluorescent molecules are visible**, allowing localization of fluorescent probes which can be much more specific than routine stains.
  - **Phase-Contrast Microscopy:** Uses the differences in **refractive index** of various natural cell and tissue components to produce an image **without staining**, allowing observation of living cells.\*\*\*
  - **Confocal Microscopy:** Involves **scanning** the specimen at successive **focal planes** with a focused light beam, often from a **laser**, and produces a **3-D**

reconstruction from the images.

- **Others:** Dark-field microscope, Interference Microscope and Differential Interference Microscopy (DIC) (used for enhancing contrast of unstained, transparent samples), Polarizing Microscope (specimen viewed between crossed-polarizing elements), Electron Microscope (TEM and SEM: Use beam of electrons in a vacuum chamber), Atomic Force Microscope (measures force between probe and sample)

## Topic 5: The cell – Chemical composition. Hyaloplasm

- Essential elements (atoms): H, C, O, N (Macroelements); Cu, Zn, Mg (Microelements); Hg, Ag, U, Ra (Trace Elements)
- Water- 70-80%; Exogenous & Endogenous\*
- Inorganic Molecules: Ions that are bound with organic molecules
- **Organic compounds:** Carbohydrates, Lipids, Proteins & Nucleic Acids
- **Water:**
  - **Excellent solvent.** **Hydrophilic:** Sugars, Organic Acids, Amino Acids; **Hydrophobic:** Lipids, some Proteins.
  - High Specific Heat Capacity (=1cal/gram)
  - **Aquaporins:** Membrane proteins that form pores in the membrane of the cell, and selectively conduct water molecules into and out of the cell.
- **Carbohydrates:**
  - **Monosaccharides:** Pentoses & Hexoses; General Formula:  $(CH_2O)_n$ ; have 2 or more OH groups; **Aldoses:** contain aldehyde group; **Ketoses:** contain ketone group
  - **Ring formation:** In aqueous solutions, the aldehyde/ketone group react with OH group, forming a ring.
  - **Isomers:** Monosaccharides can differ only in the spatial arrangement of atoms. Minor changes in chemical properties but are recognized by enzymes/proteins thus can have major biological effects.
  - **Sugar Derivatives:** OH groups can be replaced by other functional groups (e.g. -NH<sub>2</sub>)
  - **Disaccharides:** Aldehyde/Ketone group of one monosaccharide reacts with OH group of another. **Condensation Reaction.** **Glycosidic bond** formed.
  - **Oligosaccharides & Polysaccharides:** Repeating sugar subunits; short chains: oligosaccharides; long chains: polysaccharides.
  - **PAS-reaction:** Used to demonstrate polysaccharides in the cell; Demonstration of: **glycogen, glycoproteins, glycosaminoglycan.**
- **Lipids:**
  - **Hydrophobic:** Insoluble in water.
  - Have **high energy content.**
  - Hydrolyzed to give **glycerol** and **fatty acids.**
  - Act as **electrical insulators**, e.g. they insulate nerve axons.
  - Bad conductors of heat.
  - Fatty acids: can be **saturated** or **unsaturated**.
  - **Triglycerides:** Glycerol + 3 Fatty Acids
  - **Phospholipids:** Major components of cell membranes.
  - **Others:** Steroids & Glycolipids.

- Demonstrating lipids in cells: **Lipid soluble dyes & cryostats** are usually used to demonstrate lipids in cells. Examples: Sudan Black, Sudan III (orange), Sudan IV (red).
- **Proteins:**
  - Monomers: Amino acids.
  - Basic Structure of AA: -NH<sub>2</sub>, -COOH, -R, -H
  - Levels of organization:
    - i) **Primary Structure:** Linear sequence of amino acids in the polypeptide chain.
    - ii) **Secondary Structure:** Patterns of H bonds between the main-chain peptide groups. Two main structures: **Alpha Helix & Beta Sheets**.
    - iii) **Tertiary Structure:** 3D structure and folding due to hydrophobic interactions, H bonds and disulphide bonds.
    - iv) **Quaternary Structure:** 3D structure of a multi-subunit protein.
  - **Structural Proteins:** Albumins, Globulins, Histones, Collagen
  - **Compound Proteins:** Glycoproteins, Lipoproteins, Nucleoproteins, Metalloproteins.
  - **Functional Classes:** Structural Proteins, Enzymes, Transport Proteins, Defense Proteins, Contractile Proteins.
  - **Demonstration** of proteins in cells:
    - i) **Specific binding of dye** – Elastic Fibers: Orcein & Weigert's resorcin-fuchsin
    - ii) **Labeled antibody** – Immunohistochemistry & Immunofluorescence.
    - iii) **Enzymes Histochemistry**
- **Nucleic Acids:**
  - **DNA:** Double-stranded, composed of nucleotides, bases present: A, T, C, G
  - **RNA:** rRNA, tRNA, mRNA; single-stranded; composed of nucleotides; bases present: A, U, C, G
  - **Demonstration** of Nucleic Acids:
    - i) Specific Binding of a dye (e.g. H&E)
    - ii) Hybridisation Techniques: \*\*\*
      - I. **Southern Blotting:** Detection of a specific **isolated DNA** sequence on a membrane.
      - II. **Northern Blotting:** Detection of **isolated RNA** fragments
      - III. **Western Blotting:** Detection of specific **proteins** on a membrane
- In situ hybridisation: (ISH)
  - Radiocative in situ hybridization.
  - Nonradioactive in situ hybridization
- ISH ideal for:
  - Determining if a cell has **specific sequence of DNA**, such as a **gene** or part of a gene.
  - Identifying the cells containing **specific mRNAs**.
  - Determining the **localization of a gene** in a specific chromosome.

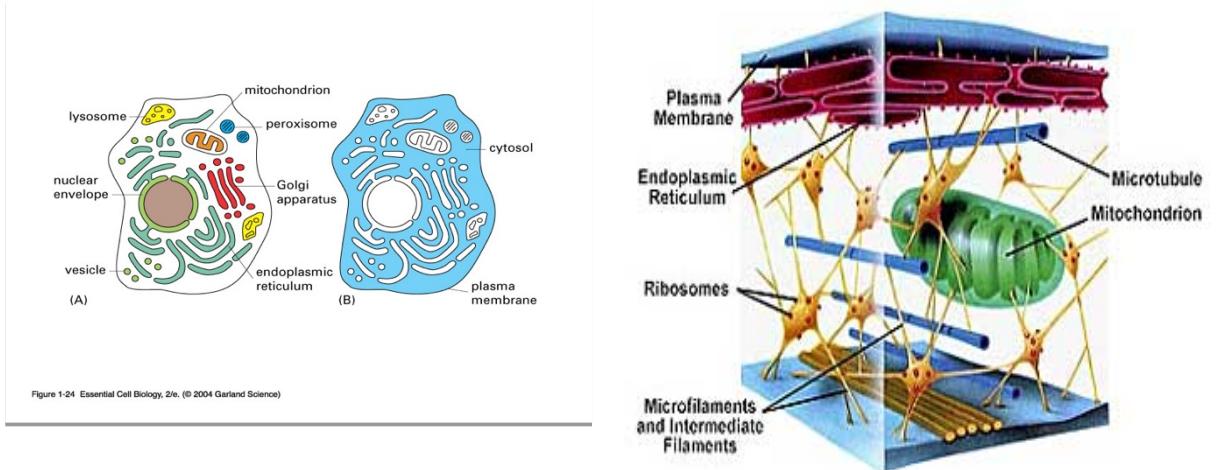
## Topic 6: The Cell – External Morphology

- Cells can have different **size, shape** and **colour**.
- Cell size ranges from 5-200um:

- Small cells are up to 10um
- Medium cells are 10-20um
- Large cells are more than 20um
- Shape of cell is related to the cellular function (e.g. biconcave shape of RBCs):
  - Spherical (Ovum)
  - Spindle-shaped
  - Squamous
  - Cuboidal, etc.
- Colour:
  - Pigmented (RBC)
  - Colourless

## Topic 7: The Cell – Internal Morphology and Organisation

- Cell is composed of the following:
  - **Nucleus** (Controls the cells main cellular activities)
  - **Plasma Membrane** (Controls entry and exit of substances into and out of the cell)



- **Cytoplasm**
  - Organelles:** (Metabolically active structures or complexes, with or without membranes)
    - Universal and specialized organelles.
    - Membranous and non-membranous.
  - Cytoplasmic inclusions:**
    - Carbohydrates, lipids and pigments
  - Cytosol** (Cytoplasmic Matrix)
- **Cytosol:**
  - Cytosol (cytoplasmic matrix) is the liquid found inside cells.
  - It makes up approx. 70% of cell volume.
  - **Translucent fluid**, with high K<sup>+</sup> conc., low Na<sup>+</sup> conc., and pH 6.8
  - Contains:
    - Water (about 90%)
    - Macromolecules** – Proteins, RNA, carbohydrates and lipids.
  - Has a delicate 3D lattice (cytoskeleton) consisting of **intermediate filaments**,

**actin filaments** and **microtubules**.

### Topic 8: The Cell Membrane – Structure and Functions. Glycocalyx (Cell Coat)

- The plasma membrane is the **lipid bilayer** with **embedded proteins** that surrounds a cell and is seen only with the **TEM**.
- The lipid bilayer forms from amphipathic **phospholipids** (hydrophobic and hydrophilic), stabilized by **cholesterol**, and contains many embedded proteins and many peripheral proteins on its cytoplasmic surface.
- Membrane proteins **move laterally** within the lipid bilayer, with less movement in areas referred to as **lipid rafts**, which have **higher concentrations of cholesterol and saturated fatty acids**.
- 2 Types of Lipid Rafts:
  - **Planar:** Contains proteins known as **flotillins**; flotillins participate in recruitment of specific membrane proteins into rafts and involved in cell signaling pathways.
  - **Caveolar:** Represent small invaginations of plasma membrane enriched with integral membrane proteins called **caveolins**.

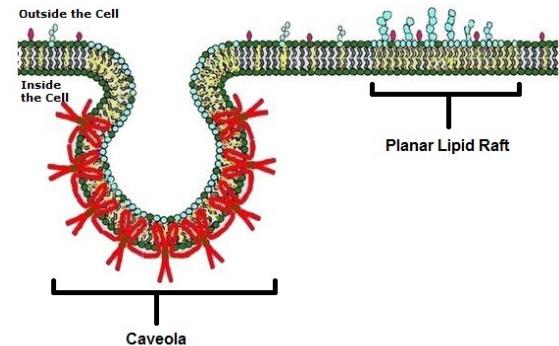
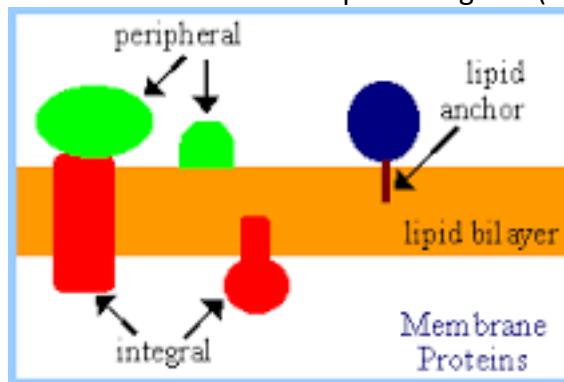


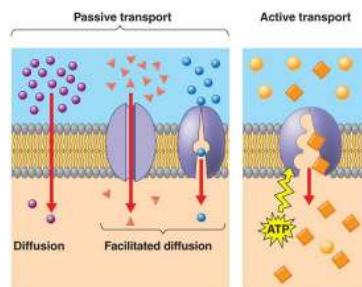
Figure 33

- **Integral membrane proteins:** Portions of these inserted into the bilayer.
- **Peripheral membrane proteins:** Proteins that dissociate from membrane if treated with polar reagents (e.g. acid); not inserted

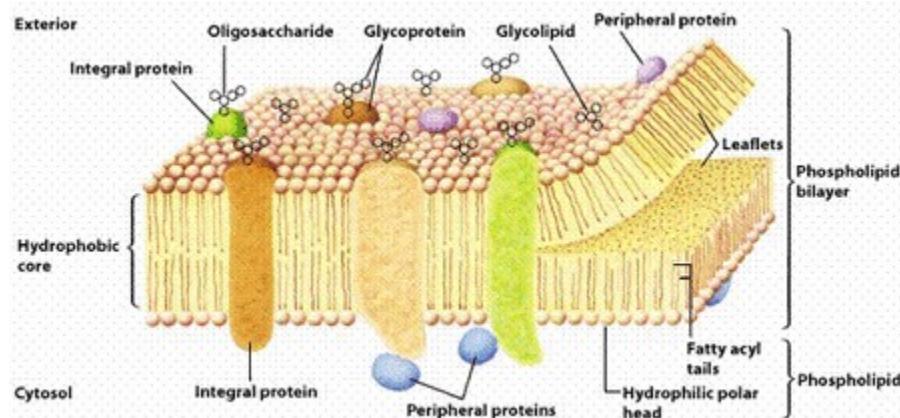


- into the lipid bilayer.
- **Endocytosis** is cellular uptake of **macromolecules** or **fluid** by a plasma membrane engulfment or invagination, followed by the 'pinching off' of a filled membrane vesicle in the cytoplasm.
  - **Functions** of membrane proteins: **Transport** (channels and pumps); **Receptors** (recognition and binding to ligands); **Anchorage** (Link intracellular skeleton to extracellular matrix); **Cell recognition**; **Intercellular joining**; **Enzymatic activity**
  - **Transport Proteins:** Pumps, Carriers, Channels
  -

## Pumps and channels

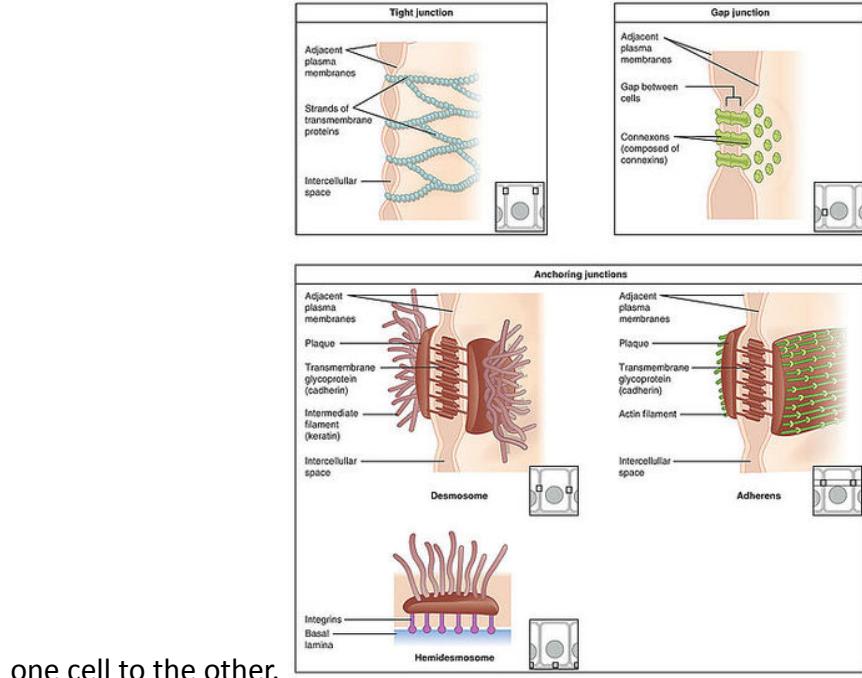


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- **Receptor Proteins:** Neurotransmitter receptors, Hormone receptors, Immune receptors, Endocytosis Receptors
- **Cell Adhesion Molecules (CAMs):** Proteins located on cell surface; composed of three domains: intracellular, transmembrane, extracellular; can be  $\text{Ca}^+$  dependent or independent.
- **Membrane-bound enzymes:** Alkaline Phosphatase, 5'-nucleosidase, ATPases, Digestive enzymes (disaccharides and dipeptidases)
- All types of **cell signaling** use membrane receptor proteins that are often linked to enzymes such as kinases or adenylyl cyclase whose activities initiate intracellular signaling pathways.
- **Glycocalyx (Cell Coat):**
  - Thickness: Up to 100nm; Renewed every 6-8 hours; PAS +
  - Chemical Composition: - Glycolipids (Cerebrosides & Gangliosides)
  - Glycoproteins
  - Functions:
    - i) Aids in **attachment** of some cells to extracellular matrix components.
    - ii) Binds **antigens and enzymes** to the cell surface.
    - iii) Facilitates **cell-cell recognition** and interaction.
    - iv) Protects cells from **injury** by preventing contact with inappropriate substances.
    - v) Assists T cells and antigen-presenting cells in aligning with each other.



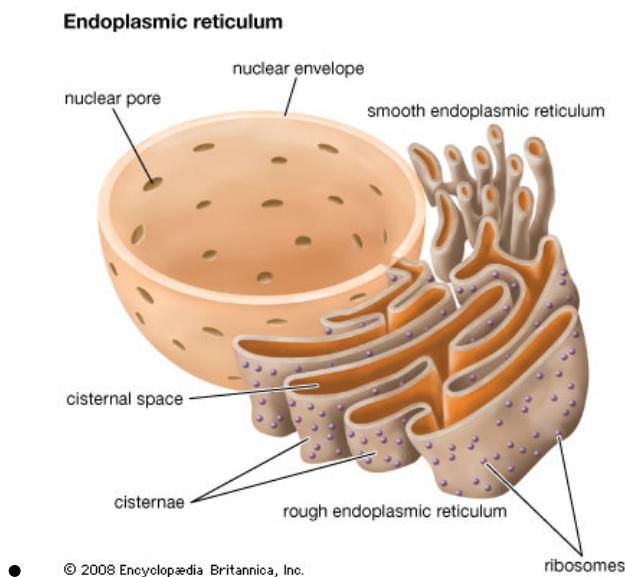
**Topic 9:** The Cell Membrane – specialized structures of the cell membrane, intercellular junctions

- **Microvilli:**
  - Cytoplasmic processes
  - Contains bundle of **actin** filaments.
  - Core bundle of actin filaments inserts into the terminal web of cortical microfilaments at the base of the microvilli.
  - **Brush border** in the small intestine.
- **Stereocilia:**
  - **Non-motile microvilli**
  - Limited to **ductus epididymis** and **sensory hair cells of the inner ear**.
- **Cilia and Flagella:**
  - 3 types of Cilia:
    - Motile Cilia:** Cytoplasmic processes in respiratory epithelia **capable of moving fluid and particles**; possess an internal structure called **axoneme** which consist of specially arranged microtubules in **(9x2)+2 arrangement** and associated **motor proteins**.
    - Primary Cilia:** **Solitary projection** found on almost **all cells** which are **immotile** because of different arrangements of microtubules in the axoneme and **lack of motor proteins**.
    - Nodal Cilia:** Found in **developing embryo** and perform a distinct rotational movement.
  - Flagellum:
    - Much longer**
    - Only 1 flagellum per cell**
    - In human body, present only in **spermatozoa**
- Basal Infoldings:
  - **Increase SA** of basal cell domain
  - Allow **more transport proteins and channels** to be present there
  - Prominent in cells that participate in **active transport** (PCT & DCT of kidney)
  - Mitochondria typically concentrated at this basal site.
- Intercellular Junctions:
  - **Tight or Occluding Junctions:** formed by interacting transmembrane proteins such as **claudin** and **occludin**; linear arrangements of these linked proteins surround their apical ends of the cells and **prevent paracellular passage** of substances (between the cells).
  - **Adherent or Anchoring Junctions:** formed by interacting proteins of the **cadherin** family, are **points of strong attachment holding together cells of the epithelium**.
  - An adherent junction may form **zonula adherens** that encircle epithelial cells just below their tight junctions or scattered, spot-like attachment sites called **desmosomes** or **maculae adherens**, both of which are attached to cytoplasmic keratins.
  - **Hemidesmosomes** composed of transmembrane **integrins** attach cells to proteins of the basal membrane.
  - **Gap or Communicating Junctions:** points of cell contact where both plasma membranes have numerous hexameric complexes of **transmembrane connexons**, each forming a **channel** allowing passage of small molecules from



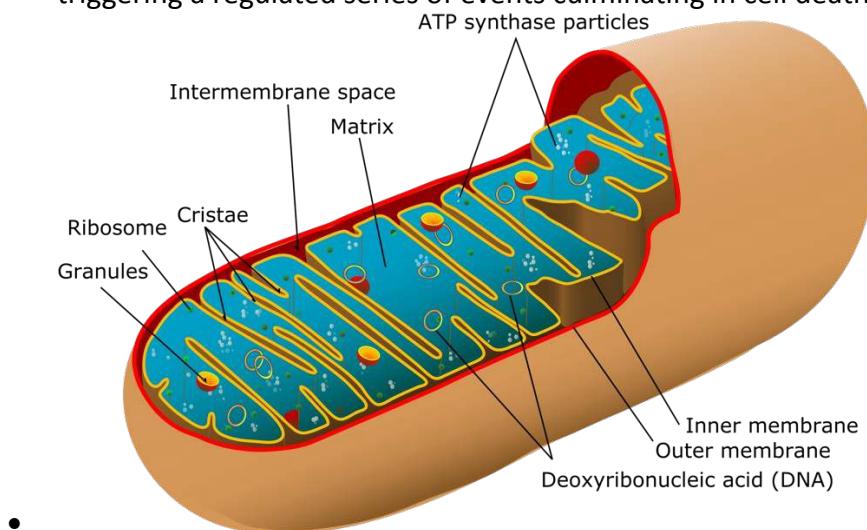
## Topic 10: Membranous Cell Organelles – Endoplasmic Reticulum

- The ER is a convoluted network of membrane enclosing continuous spaces called **cisternae** and extending from the nucleus to the plasma membrane.
- Functions: **Synthesis** (SER=site of **lipid synthesis + carbohydrate metabolism**, RER=  
synthesis of proteins), **Transport, Storage, Detoxification**
- **Rough ER** has a **granular, basophilic cytoplasmic surface** due to the presence of **polysomes** making most membrane proteins, proteins in certain other organelles, or for exocytosis: RER is always well-developed in cells actively secreting proteins.\*
- Proteins to be processed through the RER contain initial **signal peptides** which bind receptors in the ER membrane, localizing them to that organelle.
- After **translocation** across the membrane into the cisterna, the proteins undergo **posttranslational modification and folding** in a process monitored by RER molecular chaperone and enzymes.
- **Smooth ER (SER)** lacks ribosomes, but includes enzymes for **lipid and glycogen metabolism**, for **detoxification reactions**, and for temporary  $\text{Ca}^{2+}$  sequestration.



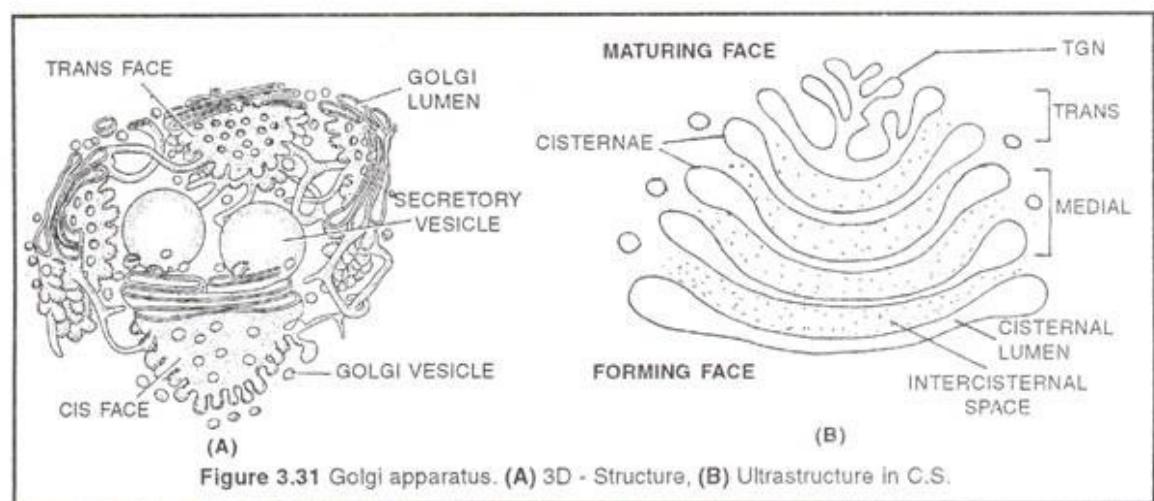
### Topic 11: Membranous Cell Organelles: Mitochondria

- Mitochondria are major **sites of ATP synthesis** and are abundant in cells or cytoplasmic regions where very large amounts of energy are expended.
- Mitochondria are usually elongated organelles and **form by fusion of pre-existing mitochondria**.
- Mitochondria have two membranes: a **porous outer membrane** encloses the intermembrane space and an **inner membrane** with many folds (**cristae**) enclosing a gel-like matrix.
- The mitochondrial matrix contains enzymes **for beta oxidation of fatty acids** and the citric acid cycle (**Krebs Cycle**).
- The inner membrane includes enzyme assemblies of the **Electron Transport Chain** and **ATP synthase**.
- Mitochondria of stressed cells may release **Cytochrome C** from the inner membrane, triggering a regulated series of events culminating in cell death (**Apoptosis**).\*\*



### Topics 12: Membranous Cell Organelles – Golgi Apparatus. Secretory Vesicles, Coated Vesicles.

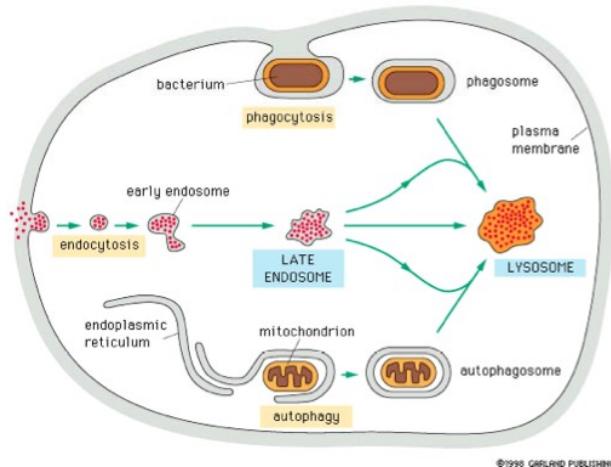
- The **Golgi apparatus** is a dynamic organelle consisting of stacked membranous **cisternae** in which proteins made in RER are **processed** further and **packaged** for secretion or other roles.
- Proteins in **transport vesicles** enter the cis or receiving face of the Golgi, move through medial cisternae of the Golgi network for enzymatic modifications, and are released in other vesicles at the trans face.
- Vesicle movement through the Golgi apparatus is guided by specific **coat proteins** such as COPII and COPI.
- Important protein modifications in the Golgi apparatus include **sulphation** and many **glycosylation** reactions.
- Modified proteins leave the Golgi apparatus after a packaging in vesicles with **coat proteins** that direct movement to lysosomes, the plasma membrane, or secretion by exocytosis.
- Stages:
  - In RER: Newly synthesized proteins **translocated** into ER Cisternae; **MO precursors** added; proteins **folded** by chaperones.
  - In CGN: Coat Protein **COPII** promotes movement from RER to CGN; COPI used in **retrograde movement**; N-linked oligosaccharide trimmed and sugars added.
  - In Medial Layer: **Glycosylation** occurs on some lipid; N-linked oligosaccharides processed further.
  - In Trans and TGN: **Sulphation** of residues; glycoproteins and glycolipids processed into **vesicles** for transport



### Topic 13: Membranous Cell Organelles – Lysosomes & Peroxisomes

- Lysosomes:
  - Two type:
    - Primary Lysosomes** emerge from the Golgi apparatus containing **inactive acid hydrolases** specific for degrading a wide variety of cellular macromolecules.
    - Secondary Lysosomes** are more heterogeneous, having fused with vesicles produced by **endocytosis** that contain material to be digested by the hydrolytic enzymes.
  - During **autophagy**, lysosomes digest unneeded or nonfunctional organelles

- after these are surrounded by membrane that then fuses with a lysosome.
- Products of digestion in secondary lysosomes are released to the cytoplasm for reuse; final condensed vesicles containing any indigestible molecules called **residual bodies**.
- **Autophagosome** (breaks down the lysosome).
- Peroxisomes:
  - **Peroxisomes** are small spherical organelles containing enzymes for various metabolic reactions, notably for **oxidation** and **detoxification**, and **catalase** that breaks down the H<sub>2</sub>O<sub>2</sub> resulting from those reactions.
- Pathways of intracellular digestion:
  - **Phagocytosis**
  - **Pinocytosis and Cell-Mediate Endocytosis**
  - **Autophagy**

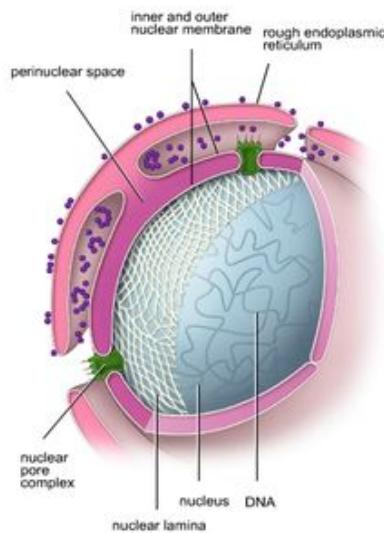


#### Topic 14: Cell Nucleus – Structure of Interphase Nucleus: Chromatin, Nucleolus, Nuclear Matrix

- Chromatin:
  - Combination of DNA and its associated proteins.
  - Made up of 5 basic **histones**: H1, H2A, H2B, H3 and H4, as well as other non-histone proteins.
  - **Euchromatin**: Chromatin with DNA that is **active in transcription** and stains lightly.
  - **Heterochromatin**: Transcriptionally inactive chromatin and stains more darkly. Found in 3 locations as: **Marginal Chromatin**, **Karyosomes**, **Nuclear-Associated Chromatin**
  - The DNA molecule initially wraps around complexes of basic proteins called **histones** to form **nucleosomes**, producing a structure resembling beads on a string.
  - Additional levels of chromatin fiber condensation are less well understood and involve non-histone proteins, including complexes of **condensins**.
  - The extra chromosome in cells of female mammals forms **facultative heterochromatin** and can be seen as the **Barr Body**.
  - **Constitutive Heterochromatin**: Transcriptionally inactive; contains highly **repetitive sequences** of DNA; large amounts found in **centromeres** and

**telomeres.**

- **Facultative Chromatin:** Also transcriptionally inactive and condensed; **not repetitive.**

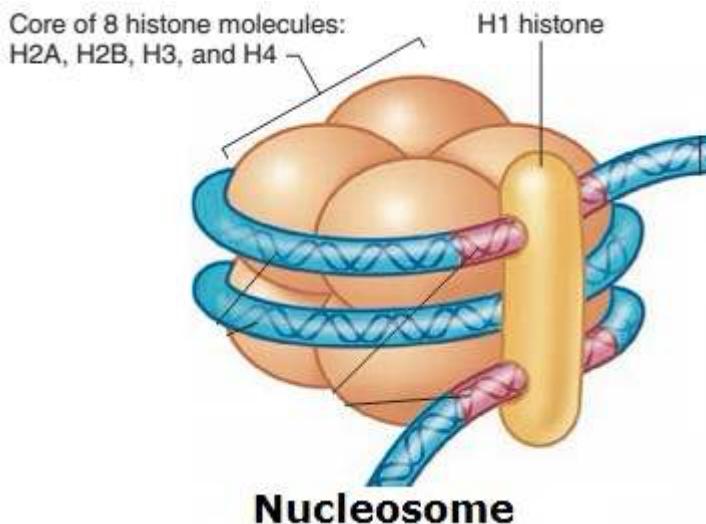


- **Nucleolus:**

- Nucleolus is a very basophilic or electron-dense area of chromatin localized where **rRNA transcription** and **ribosomal subunits assembly** occur.
- By TEM, an active nucleolus is seen to have **fibrous and granular parts** where rRNA forms and ribosomal subunits are assembled, respectively.

- **Levels of Chromatin Organisation:**

- DNA wraps around proteins to form **nucleosome**.



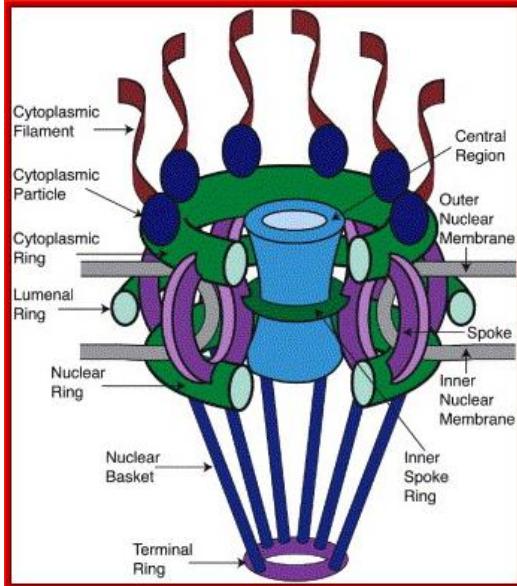
**Nucleosome**

- Strand of nucleosome coiled to produce **chromatin fibril**.
- **Loop domains** are formed.

## Topic 15: Cell Nucleus – Ultrastructural Organization: Structure of the Nuclear Envelope – Nuclear Pores

- Cytoplasm is separated from nucleoplasm by the **nuclear envelope**, a **double set of membranes** with a narrow **perinuclear space**; the outer membrane binds ribosomes and is continuous with the RER.

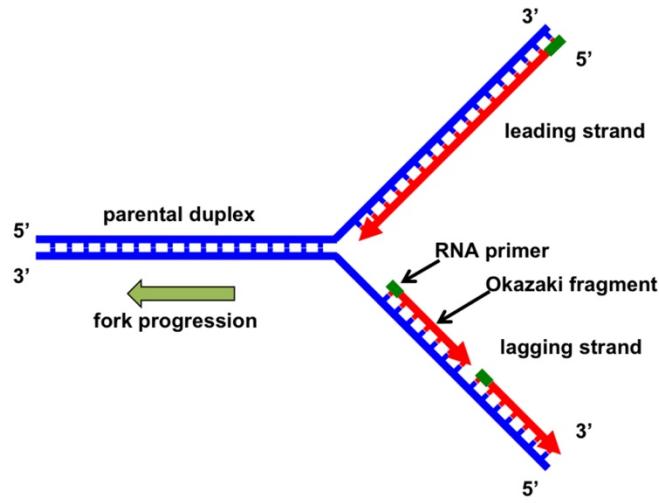
- The nuclear envelope is penetrated by **nuclear pore complexes**, large assemblies of nucleoporins with 8-fold symmetry (cytoplasmic ring composed of **8 protein subunits**) through which proteins and protein-RNA complexes move in both directions.
- The nuclear envelope is supported internally by a meshwork, the **nuclear lamina**, composed of intermediate filament subunits called **lamins**.



### Topic 16: Cell Nucleus – Chromosomes, structure and replication of DNA.

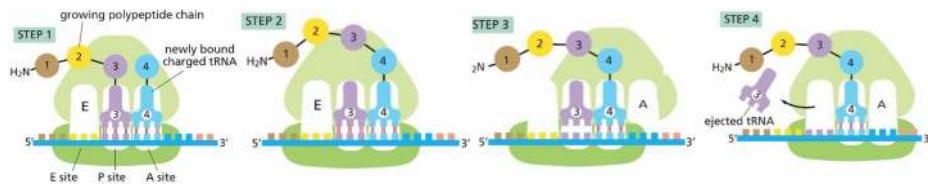
- In dividing cells, **chromatin is condensed and organize into chromosomes**.
- Chromosome:** Single, long linear DNA molecule along with the proteins that fold and pack the fine DNA thread into a more compact structure.
- During mitotic division, **chromatin fibers** undergo condensation to form chromosomes.
- Each chromosome formed by two **chromatids** joined at a point called the **centromere**.
- Area located at each end of the chromosome is called the **telomere**.
- Cohesins** and **Condensin** aid the compaction of chromosomes.
- Centromere:** Constricted region of the chromosome that links sister chromatids.
- Spindle fibers attach to the centromere via the **kinetochore** (proteins found on the centromere).
- P Arm=Short; Q Arm=Long**
- 2 types of centromeres: **Point** (bind to specific proteins that recognize particular DNA sequences) and **Regional** (most centromeres of complex organism).
- Kinetochore:** Protein structure on centromere where the spindle fibers attach during cell division to pull sister chromatids apart.
- 2 regions: **Inner kinetochore** (tightly associated with the centromere DNA) & **Outer Kinetochore** (interacts with microtubules and assembled and function only during cell division)
- Telomere:** Region of repetitive nucleotide sequences at each end of a chromosome, which protects the end of the chromosome from deterioration or from fusion with neighbouring chromosomes.

- **Homologous Chromosomes:** Two copies of each chromosome, maternal and paternal.
- 46 chromosomes (23 pairs).
- 22 pairs of **autosomes**.
- 23<sup>rd</sup> Pair= **Sex chromosome** (X or Y)
- **Karyotyping:** Sorting out of chromosomes according to their **size, shape** and **emitted fluorescent colour**.
- **Chromosomal Abnormalities:** **Numerical** (extra sets of chromosomes) and **Structural** (includes deletions, ring chromosomes, duplications, inversions, insertion, translocation and isochromosomes).
- **Replication:**
  - Semi-conservative replication.
  - Initiated at origins of replication DNA double helix unwound and separated by **DNA Helicase** and **Topoisomerase**.
  - **Replication fork** formed.
  - **Single-strand binding proteins (SSBs)** coat the newly exposed single strands.
  - **DNA Polymerase** starts at 3' end of the **RNA primer**, using original DNA strand as a guide, begins to synthesis a new complementary DNA strand.
  - **Leading + Lagging strand**



### Topic 17: Non-membranous Cell Organelles: Ribosomes & Polyribosomes

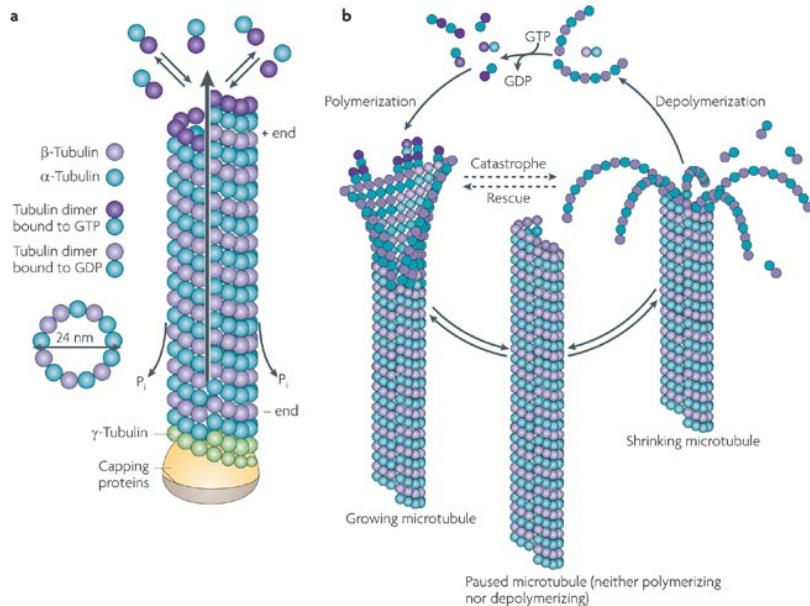
- The two ribosomal subunits, each a complex of rRNA and many proteins, attach to mRNA and translate that message into protein.\*\*
- Ribosomes contain 4 binding sites: 1 for mRNA and the other 3 (A, P and E) for tRNA.
- Function of Ribosomes:
  - **Initiation:** Translation begins with AUG codon (methionine); **Meth-tRNAl complex** binds to small subunit along with **eukaryotic initiation factors**; small subunit binds to 5' end of mRNA.
  - **Elongation:** Each amino acid added to the elongating chain in a cycle of reaction with 4 main steps: i) tRNA binding, ii) Peptide bond formation, iii) Large subunit translocation, iv) Small subunit translocation



- **Termination:** End of protein-coding signaled by presence of stop codons.
- Multiple ribosomes on the same mRNA make up a **polyribosome (polysome)**, and an abundance of these produces basophilic cytoplasm after H&E staining.

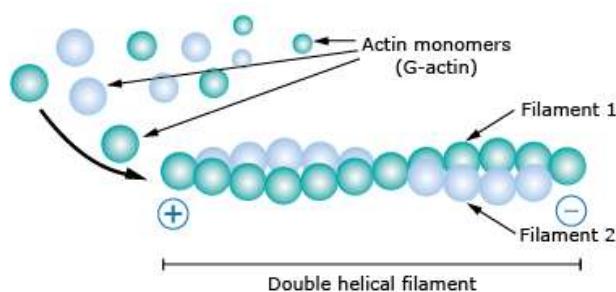
### Topic 18: Non-membranous cell organelles – Microtubules and Cytofilaments

- Cytoskeleton contains 3 types of polymers: i) **Microtubules** (25nm in diameter), ii) **Actin Filaments/Microfilaments** (5-7nm), iii) **Intermediate Filaments** (8-10nm)
- Microtubules are semi-rigid tubular structures with walls composed of **polymerized tubulin** heterodimers; their structure is often very dynamic, with steady addition and dissociation of tubulin.



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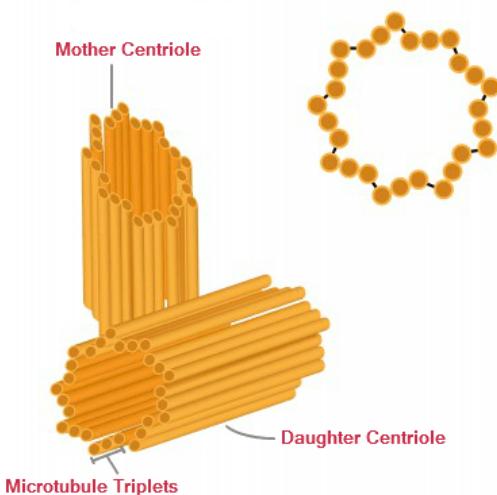
- Microtubules are important in maintaining cell shape and as **tracks for transport of vesicles and organelles** by the **motor proteins kinesin and dynein**.
- Microfilaments are short, flexible, highly dynamic filaments of actin subunits, in which changes in length and interactions with binding proteins regulate cytoplasmic viscosity and movement.
- **Actin:**



- ATP and ADP used in **ACTIN!\*\*\*** (Barbed End, Treadmilling)
- Intermediate filaments are the **most stable** cytoskeletal component, conferring strong mechanical stability to cells.
- Intermediate filaments are composed of various protein subunits in different cells; 6 Classes: **Class I and II**: Keratins; **Class III**: Vimentin; **Class IV**: Neurofilament, **Class V**: Nuclear Lamins; Class VI: **Nestins**
- **Structure:**
  - Monomer
  - Dimer
  - Tetramer

### Topic 19: Non-membranous cell organelles – Cytocenter

- Centrosome:
  - 2 Centrioles
  - Arrangement:  $(9 \times 3) + 0$
  - (Draw Structure and Explain)



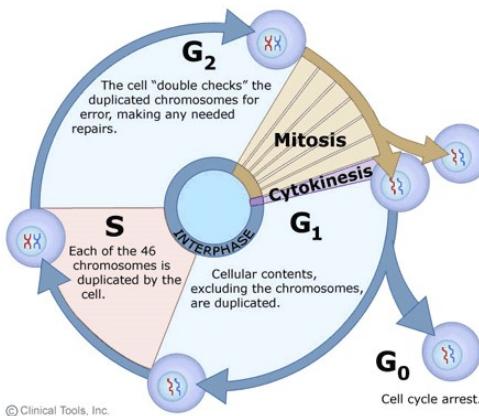
### Topic 20: Specialized cell organelles. Cell Inclusions

- Unlike organelles, inclusions are **not metabolically active** and are primarily **storage sites**, such as **lipid droplets**, **glycogen granules**, **pigment granules**, **crystalline inclusion**, or residual bodies (also called **lipofuscin**).
- **Lipid inclusions:** Lipid-soluble dyes and cryostat sections are used to demonstrate lipids in cells. E.G. Sudan Black, Sudan III (Orange), Sudan IV (Red)

- **Examples:** Lipid droplets in adrenal cortex + Adipocytes in connective tissue
- **Glycogen Granules:** Found in hepatocytes mainly; **PAS** reaction used
- **Pigments:** **Hemosiderin** (Iron-storage complex in liver cells); **Melanin** in malignant melanoma cells; **Keratin** in hair cells; **Lipofuscin** in neurons.
- **Crystalline Inclusions:** **Reinke crystal** in Leydig cell, Crystallloid inclusion bodies of Charcot- Böttcher in the basal cytoplasm of the Sertoli cell.

## Topic 21: Cytophysiology – Viral and mitotic cycle of the cell. Amitosis, Mitosis, Meiosis

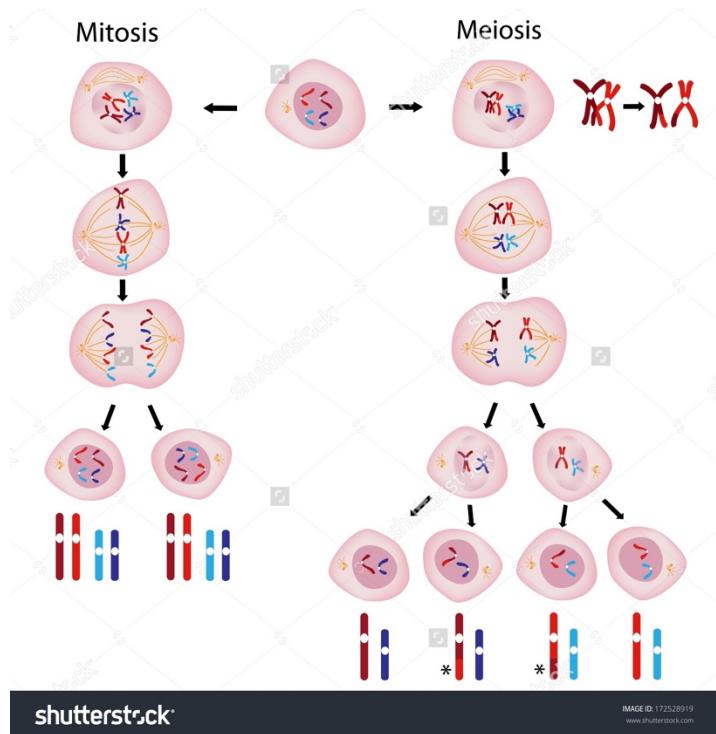
- Cell Cycle:
  - The **cell cycle** is the series of events that take place in a cell leading to duplication of its DNA (DNA replication) and its division to produce two daughter cells.
  - All phases of the cell cycle are strictly controlled and coordinated.
  - Phases:
    - G<sub>1</sub> Phase:** **Longest phase**; cell mass and volume are increases; cell accumulates proteins, enzymes, nucleotides and energy required for DNA replication and cell division.
    - G<sub>0</sub> Phase:** **Non-proliferative** (non-dividing) cells enter this phase and remain quiescent for long periods of time; some cells enter this phase semi-permanently; cells in this phase are **metabolically active**.
    - S Phase:** Initiation of **DNA replication**; nucleus between diploid and tetraploid; **duplication of centrosomes**; newly formed sister chromatids held tightly by the **cohesins**.
    - G<sub>2</sub> Phase:** Cell prepares for cell division; cell examines its replicated DNA; period of cell growth and reorganisation of organelles before mitosis.



- Cell cycling is controlled by the sequential appearance of key cytoplasmic proteins, the **cyclins**, which bind **cyclin-dependent kinases (CDKs)**.
- **CDKs phosphorylate** and **activate** the enzymes and transcription factors whose functions characterize each phase of the cell cycle.
- Progress through the cell cycle stages is monitored at checkpoints, including the **G<sub>1</sub> restriction point**; only when each phase's activities are completed are the cyclins changed to trigger those of the next phase.
- Mitosis:
  - Stages of mitotic cell divisions include **prophase**, when chromosomes condense, the nuclear envelope disassembles, and the microtubular spindle

forms; **metaphase**, when chromosomes are aligned; **anaphase**, when they begin to separate toward the two poles; and **telophase**, when nuclear envelope re-forms around the separated chromosomes.

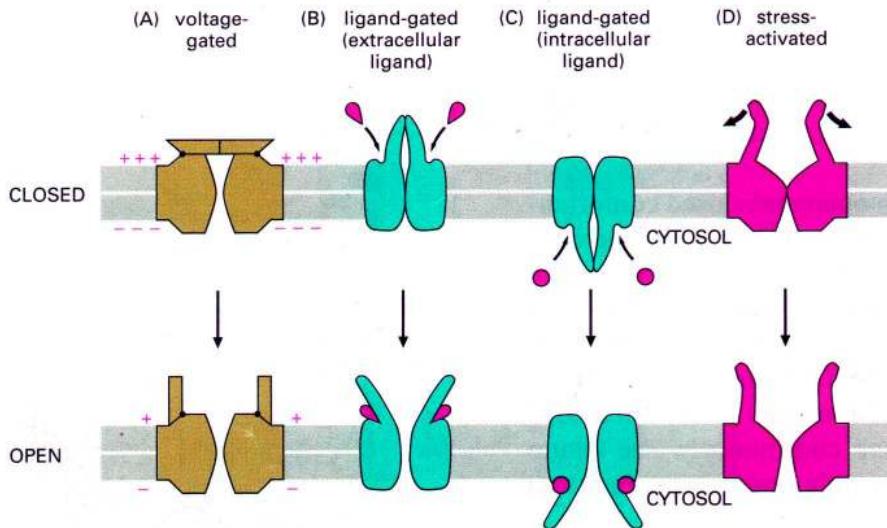
- **Cytokinesis:** Telophase ends with cytokinesis or **cell cleavage** into two daughter cells by a contractile ring of actin filaments and myosin.
- **Prometaphase:** Starts abruptly with the **breakdown of the nuclear envelope**. Chromosomes now attach to **spindle microtubules** via their **kinetochores** and undergo active movement.
- **Mitotic Spindle:** i) Astral Microtubules; ii) Kinetochore Microtubules; iii) Interpolar Microtubules.
- 
- Meiosis:
  - **Meiosis** consists of two successive mitotic divisions without the additional S phase (replication phase) between the two divisions.
  - **Two Divisions: Reductive Division (Meiosis I); Equatorial Division (Meiosis II)**
  - **Meiosis I:** Chromosome number reduced from diploid to haploid, but amount of DNA remains the same.
  - **Meiosis II:** Chromosome number is haploid, but amount of DNA halved.
  - **Prophase 1:**
    - i) **Leptotene: Condensation** of chromatin and appearance of chromosomes; sister chromatids also condense and become connected with each other by meiosis-specific cohesion complexes; pairing of homologous chromosomes initiated.
    - ii) **Zygotene: Synapsis** begins at this stage; process involves the formation of synaptonemal complex.
    - iii) **Pachytene:** Synapsis is complete; **crossing over** occurs.
    - iv) **Diplotene:** Synaptonemal complex dissolves, and chromosomes condense further; homologous chromosomes **separate** and are connected by **chiasmata**.
    - v) **Diakinesis:** Further condensation and **nuclear envelope disintegrates. (pLZPDD) ('Please PDD')**
  - **Metaphase I:** Pairs of homologous chromosomes **arranged** at the metaphase plate; kinetochore microtubules from each pole attach to chromatids.
  - **Anaphase I:** Breakdown of cohesins; homologous chromosomes move toward **opposite poles**; sister chromatids still bound by centromere.
  - **Telophase I:** Chromosomes arrive at the poles; microtubules **disappear**; new nuclear membrane surrounds each haploid set; chromosomes uncoil back into chromatin; **cytokinesis** occurs, creating two daughter cells.
  - **Prophase II:** Spindle apparatus **reforms**;
  - **Metaphase II:** Chromosomes positioned at the metaphase plate; kinetochores of sister chromatids attached to microtubules.
  - **Anaphase II:** Breakdown of proteins at the **centromere**; chromatids separate and move toward opposite poles.
  - **Telophase II & Cytokinesis:** Nuclei form, chromosomes begin to **decondense**, and cytokinesis occurs.



- **Gene:** Region of DNA that encodes a functional RNA or protein, and is the molecular unit of heredity.
- **Genome:** Genetic material of an organism. Includes both the genes and the non-coding sequences of the DNA.
- **Phenotype:** Composite of an organism's observable characteristics or traits.
- **Locus:** Specific location or position of a gene on a chromosome.
- **Allele:** Alternative forms of the same gene.
- **Homozygous:** Identical alleles present on both homologous chromosomes.
- **Heterozygous:** Different alleles present on both homologous chromosomes.
- **Haploid:** Single set of chromosomes.
- **Diploid:** Two homologous copies of each chromosome.
- **Amitosis:** Mitosis for osteoblasts\*\*\*

## Topic 22: Cytophysiology – Cellular metabolism. Transmembrane Transport

- **Cell Metabolism:** Totality of an organism's chemical reactions
- **Permeability of Lipid Bilayer:**
  - Non-polar molecules dissolve in lipid bilayer and cross easily.
  - Polar molecules (e.g. glucose) pass slowly through the layer.
  - Charged particles more difficult to pass through.
- **Passive transport:** Diffusion of substance with no energy investment.
- **Osmosis:** Diffusion of water across a selectively permeable membrane.
- **Facilitated Diffusion:** Passive transport aided by proteins.
- **Active Transport:** Uses energy to move solutes against their concentration gradients.
- Types of **transporter systems:** Uniport + Cotransport (Symport + Antiport)
- **Properties of ion channels:** Composed of transmembrane subunits; **highly selective** for one ion; allow impermeable ions to cross membranes at rates approaching diffusion limits; main types: **voltage-gated, ligand-gated, mechanically-gated.**

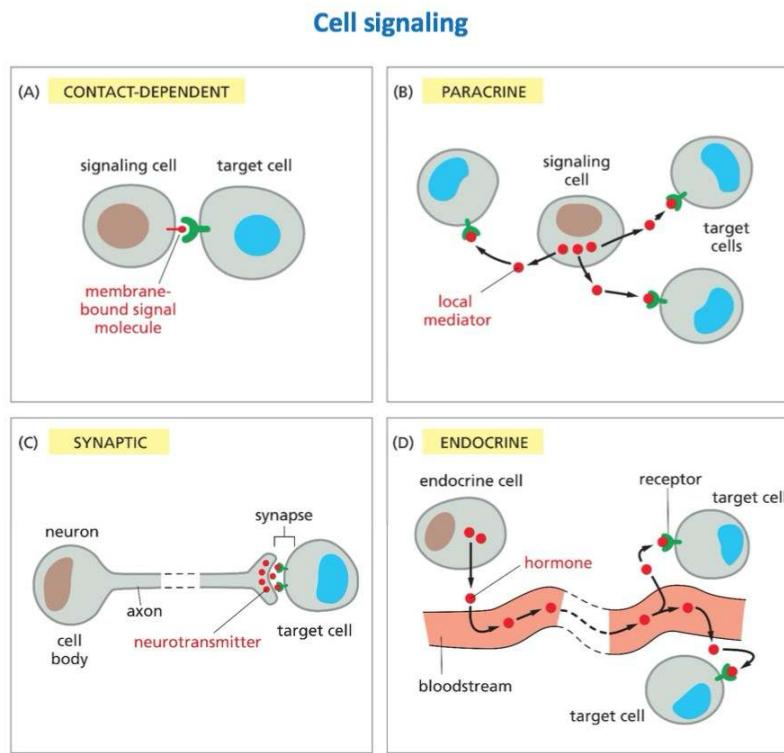


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- **Active transport:** Carry out transport in 3 ways: i) Coupled transporters; ii) ATP-driven pumps; iii) Redox-driven pumps
- **Vesicular transport:** Maintains integrity of the plasma membrane and provides for the transfer of molecules between different cellular compartments; **vesicle budding:** major mechanism.
- **Endocytosis:** Substances enter the cell; plays key roles in nutrient uptake, cell signaling and cell shape changes.
- **Exocytosis:** Substances leave the cell.
- **Pinocytosis:** Non-specific **ingestion of fluid** and small protein molecules via **vesicles**; constitutive (performed by virtually every cell in the organism); **caveolin** and **flotillin** proteins involved in the mechanism.
- **Phagocytosis: Ingestion of macromolecules;** particles engulfed to form **phagosome**; performed mainly by **mononuclear phagocytic system (MPS)**; (Mechanism: Recognition and attachment of antigen by receptors; Antigen engulfed by pseudopods; pseudopods come together and fuse, and antigen is internalized; **Phagosome** formed; Digestive process occurs within the **phagolysosome**)
- **Receptor-Mediated Endocytosis:** Receptors are usually integral membrane proteins; receptors associate with **clathrin** and begin invagination as **coated pits**; coated pits pinched off in cytoplasm as **coated vesicles**; **clathrin-dependent endocytosis**.
- Exocytosis – 2 Pathways:
  - **Constitutive:** Substances designated for export are continuously delivered in transport vesicles to the plasma membrane.
  - **Regulated Secretory Pathway:** Present in specialized cells (e.g. endocrine/exocrine cells and neurons); they concentrate secretory proteins and transiently store them in secretory vesicles within the cytoplasm.

### Topic 23: Cytophysiology – Cellular Signaling, Cellular Reactivity and Motility

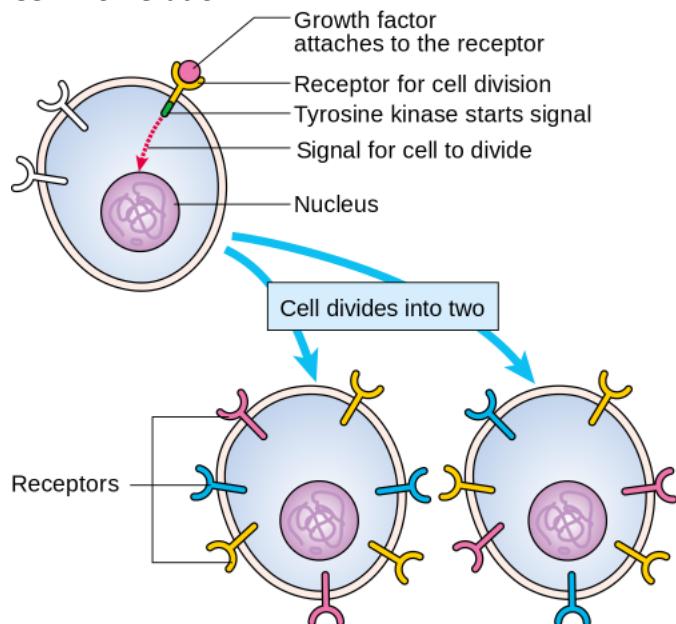
- Cell-to-cell communication allows cells to communicate with each other and coordinate activities with each other.
- 2 Main Types of Signalling:
  - Gap Junctions
  - **Ligands** and physical stimuli detected by various **receptors** on **target cells**.

- Stages of Cell Signaling: i) Reception, ii) Transduction, iii) Response
- 4 Types of Cell Signaling:

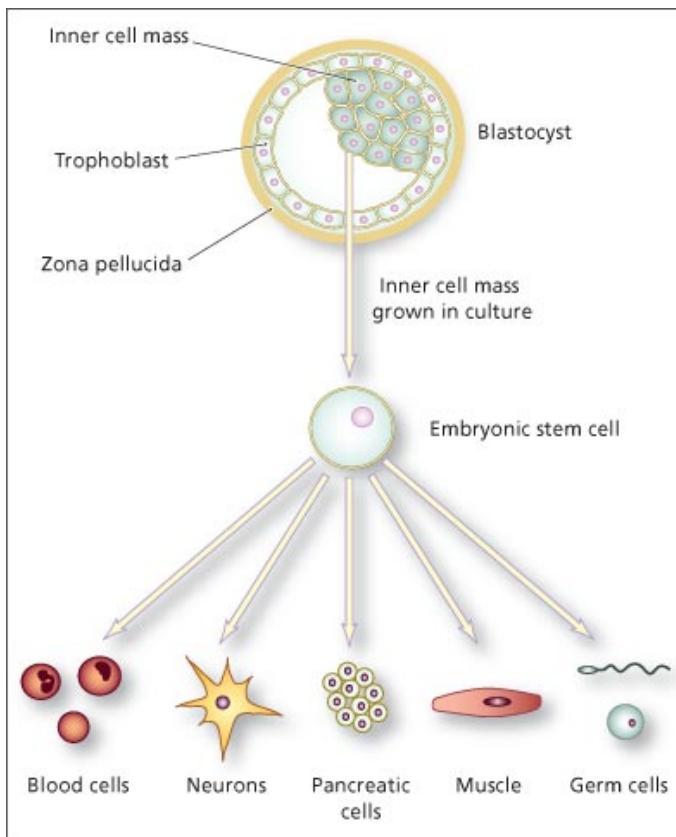


## Topic 24: Cytophysiology – Cellular Differentiation, growth, ageing and death

- **Cell Proliferation:**



- **Cell Differentiation:**



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- Cell Death:**

- **Necrosis: Accidental Cell Death** – Pathological process; damage to plasma membrane, rapid cell swelling and **lysis**.
- **Apoptosis: Programmed Cell Death** – Physiological process; cells initiate their own death; characterized by controlled **autodigestion**. Steps: **DNA Fragmentation**; **Decrease in cell volume**; **Loss of mitochondrial function**; **Membrane blebbing**; **Formation of apoptotic bodies**.

**Topic 25:** General Histology – Introduction. Tissues – definition, general features, classification.

- Classification of Tissues:
  - **Tissue:** Aggregates/Groups of similar cells from the same origin organized to perform one or more specific functions.
  - **4 Types:** Epithelial, Connective, Muscle, Nervous.
- Properties of tissues:
  - **Regeneration** (regrowth of lost tissue)
  - **Degeneration** (change in structure of tissue + deterioration and loss of function of the tissue)
  - **Hyperplasia** (increase in amount of tissue due to proliferation)
  - **Hypertrophy** (increase in volume of tissue/organ due to enlargement of its cells)
  - **Atrophy** (Reduction in cell volume)
  - **Hypoplasia** (underdevelopment of tissue)
  - **Aplasia** (congenital absence of tissue/organ)

- **Metaplasia** (reversible conversion of one mature cell into another)
- **Neoplasia** (New tissue formation)
- Basement Membrane:
  - The **basement membrane** of all epithelia is a thin extracellular layer of specialized proteins usually having **two parts**: a **basal lamina** and a more **fibrous reticular lamina**.
  - The **basal lamina** is a thin meshwork of **type IV collagen** and **laminin** produced by the epithelial cells.
  - The **reticular lamina** contains **type III collagen** and anchoring fibrils of VII collagen, all secreted by cells of the immediately adjacent connective tissue.
  - Together, these components **attach** epithelia to connective tissue, regulate (**filter**) substances passing from connective tissue into epithelia, provide a guide or **scaffold** during tissue regeneration after injury, and **compartmentalize** epithelial cells from one another.
- **Intercellular Junctions** (See topic 9)
- **Basal Infoldings: Infoldings** at the **basal cell surface**; significantly increase the SA of the basal cell domain, allowing for more transport proteins and channels to be present.

## **Topic 26: Epithelial Tissue – General Features, Types of Epithelial Tissue**

- **Features:**
  - An **epithelium** is a tissue in which cells are bound tightly together structurally and functionally to form a **sheetlike** or **tubular structure** with **little extracellular material between the cells**.
  - Cells in epithelia each have an **apical side** facing the sheet's free surface and a **basal side** facing a basement membrane and underlying connective tissue.
  - Epithelia are often specialized for absorption or **transcytosis, pinocytosis** of material at the apical side and **exocytosis** at the basolateral side (or vice versa).
- **Types of Epithelia:**
  - An epithelium in which the basement membrane has **one cell layer** is **simple**, the cells of different simple epithelia range widely in height, from very thin or **squamous**, to roughly **cuboidal**, to very tall or **columnar**.
  - Epithelia with **two or more layers** of cells are **stratified** and almost all such epithelia are stratified squamous, in which the **outer cell layers are thin and flattened**.
  - Cells of stratified squamous epithelia move gradually from the basal to the surface layers, changing shape and becoming filled with **keratin** intermediate filaments.
  - **Stratified squamous epithelia** such as the epidermis cover the body surface, **protecting** underlying tissues from excess dehydration and microbial invasion.
  - **Pseudostratified epithelia** are thick and appear to have several cell layers; all cells attach to the basal lamina but not all extend to the free epithelial surface.
  - **Transitional epithelium or urothelium**, found only in the lining of the urinary system, is stratified, with large rounded surface cells protective against urine.

- **MAIN TYPES:**
- **Simple Squamous:** **Location:** Vascular system, body cavities, Bowman's capsule. **Function:** Exchange, barrier in CNS, exchange and lubrication.
- **Simple Cuboidal:** **Location:** Small ducts of exocrine glands, surface of ovary, kidney tubules. **Function:** Absorption, barrier, absorption and secretion.
- **Simple Columnar:** **Location:** Small and large intestine, stomach lining and gastric glands, gall bladder. **Function:** Absorption and secretion
- **Pseudostratified:** **Location:** Trachea and bronchial tree, Ductus deferens, efferent ductules of epididymis. **Function:** Secretion and conduit, absorption and conduit.
- **Stratified Cuboidal:** **Location:** Sweat gland ducts, large ducts of exocrine glands, anorectal junction.
- **Stratified Columnar:** **Location:** Largest ducts of exocrine glands, anorectal glands.
- **Transitional (Urothelium):** Renal calyces, ureters, bladder, urethra.
- **Stratified Squamous Nonkeratinized:** Cornea, oral cavity, part of the pharynx.
- **Stratified Squamous Keratinized:** Epidermis of skin.

### **Topic 27: Surface Epithelium**

- **Microvilli:** Small membrane projections with cores of **actin filaments** that generally function to increase epithelial cell's apical surface area for **absorption**.
  - **Stereocilia: Non-motile!** Long microvilli with specialized mechanosensory function in cells of the **inner ear** and for absorption in tissues of the **male reproductive tract**.
  - **Cilia:** (3 Types)
    - **Motile: Motile:** Has **Motor Proteins**. Has the **axoneme** in **(9x2)+2** microtubular arrangement; Moves fluids and substances across.
    - **Primary: Non-motile; No motor proteins; Passive movement**
    - **Nodal:** Found in **developing embryo**
  - **Monocilia:** These are **non-motile** and contain a **(9x2)+0** microtubular arrangement. They **passively** bend by the flow of the fluid. They lack microtubules-associated motor proteins needed to generate motile force.
  - **Primary Ciliary Dyskinesia:** Kartagener's syndrome caused by structural abnormality that results due to the absence of dynein arms. Males become **sterile**.
- .....

### **Topic 29: Connective Tissue: General Features & Classification**

- Features:
  - Connective Tissue: **Cells and Extracellular matrix.**
  - Extracellular matrix: **Protein Fibers + Ground Substance**
  - Tissue originates from **embryonic mesenchyme**.
- Functions:
  - Protection and Support
  - Provide Strong Connection
  - Body's Defence System

- Classification:

#### Classification of Connective Tissue

##### Embryonic Connective Tissue

Mesenchyme	Mucous connective tissue		
Connective Tissue Proper			
Loose	Dense	Reticular	Elastic
	regular		
	irregular		

Specialized Connective Tissue			
Cartilage	Bone	Adipose tissue	Blood
Hyaline cartilage	Immature bone		
Elastic cartilage	Mature bone		
Fibrocartilage			

- Resident Cells:** Stable and exhibit little movement:

- **Fibroblasts:** (large euchromatic nucleus, tapers off at both ends)
- **Myofibroblasts:** "
- **Macrophages** (phagocytic cells containing large number of lysosomes; Function: phagocytosis)
- **Adipocytes** (Store fat and produce hormones)
- **Mesenchymal Stem Cell** (found in loose connective tissue)

- Wandering Cells:** Migrated cells to tissue through blood:

- Lymphocytes (
- Plasma Cells (B-Lymphocytes Derived)
- Neutrophils (
- Eosinophils
- Basophils

**Topic 33/34:** Blood and lymph – blood and lymph, plasma: contents, antibodies/Morphology and function of erythrocytes, leukocytes and thrombocytes

- Blood = Specialised Connective Tissue with cells and a fluid extracellular matrix called plasma.
- Functions:
  - **Transport:** O<sub>2</sub> and CO<sub>2</sub>, nutrients, waste substances, hormones.
  - **Protection**
  - **Maintenance of homeostasis** by acting as a buffer
  - Thermoregulation
  - Coagulation
- Plasma: **Albumins** (most abundant), **Globulins** (helps maintain osmotic pressure), **Fibrinogen** (largest plasma protein)
- **Immunoglobulins:** 5 types

- **Erythrocytes:**
  - No nucleus
  - Biconcave
  - Life Span=120 days
  - Haemoglobin:
    - i) 4 polypeptide chains
    - ii) Specialised for O<sub>2</sub> and CO<sub>2</sub>
  - Sickle Cell Disease:
    - i) Point mutation
    - ii) HbS formed from normal Hb
- **Leukocytes:**
  - **Granulocytes**
    - i) **Neutrophils** – Multinucleus, Short-lived, Contains 2 types of granules (primary and secondary)
    - ii) **Eosinophils** – Bilobed nucleus, Pink Granules present, Short-Life Span
    - iii) **Basophils**
  - **Agranulocytes**
    - i) **Monocytes**: Nucleus has a C shape, Contains Primary Granules, Remains in blood for 16 hours.
    - ii) **Lymphocytes**: Small, Medium and Large Lymphocyte; Found in blood and lymph are found to represent immunocompetent cells; 2 Types: T-lymphocytes (Helper T and Cytotoxic T) for Cell-Mediated Immunity; and B-lymphocytes for Humoral Immunity
- **Thrombocytes** (Platelets):
  - Non-nucleated
  - Contains 2 zones
  - Half-life=10 days

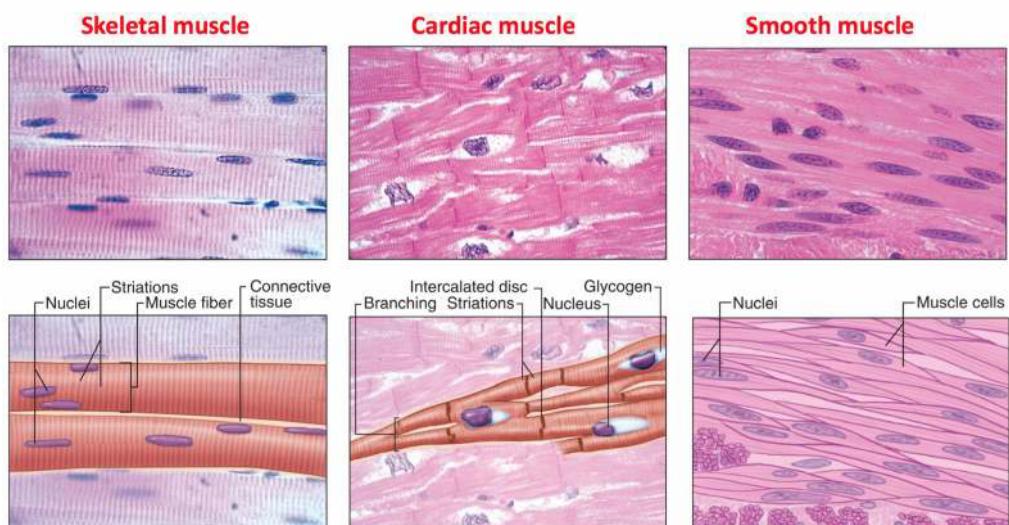
**Topics 35, 36, 37:** Erythropoiesis – Formation of Erythrocytes/Granulocytes & Agranulocytes/Thrombocytes

- **Haemopoiesis:** Making of new RBC;
- Locations and Phases:
  - **Yolk Sac phase:** Formation of blood islands on the wall of the yolk sac of the embryo.
  - **Hepatic Phase:** When the hemopoietic centers appear on the liver
  - **Bone Marrow Phase:** Develops into their medullary cavities
- **Bone Marrow:** 2 types: Red + Yellow
- **Erythropoiesis:** (6 stages)
  - Progenitor Cell
  - Proerythroblast
  - Basophilic Erythrocyte
  - Polychromatophilic Erythrocyte
  - Orthochromatophilic Erythrocyte
  - Reticulocyte
- **Granulocytes & Agranulocytes:** (5 steps)
  - Granulocyte:
    - i) Myeloblast

- ii) Promyelocyte
- iii) Metamyelocyte
- iv) Band Neutrophil
- v) Neutrophil/Eosinophil/Basophil
- **Monocytopoiesis:**
  - i) Monocytes produced in bone marrow
  - ii) Monoblast are progenitor cells and have same morphology as the myeloblast.
  - iii) Differentiation leads to promonocyte
  - iv) Promonocyte divides twice then produce monocytes
- **Lymphopoiesis:**
  - i) **Lymphocyte progenitor cells** (Can differentiate to form B or T lymphocytes)
  - ii) **Lymphoblast**
  - iii) **Lymphocytes**

### **Topic 38, 39, 40: Muscle Tissue: General Features, Types of Muscle Tissue/Skeletal Muscle Tissue, Muscle Contraction/Smooth and Cardiac Muscle Tissue**

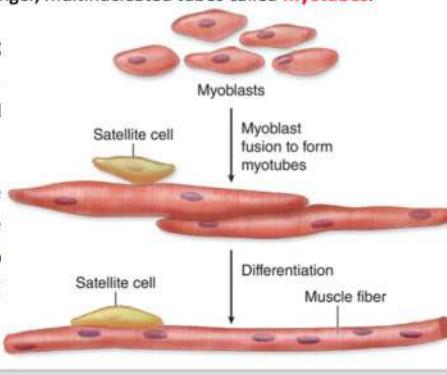
- **Features:**
  - **Muscle tissue** is responsible for movement of the body and its parts and for changes in the size and shape of internal organs.
  - It is characterized by aggregates of specialized, elongated **cells** arranged in parallel array that have the primary role of **contraction**.
  - In all types of muscle, contraction is caused by the sliding interaction of **thick myosin** filaments along **thin actin filaments**.
  - The two types of myofilaments occupy the bulk of the cytoplasm, which in muscle cells is also called **sarcoplasm** (Gr. sarcos, flesh; plasma, thing). The sER is the **sarcoplasmic reticulum**, and the muscle cell membrane and its external lamina are the **sarcolemma** (sarkos + Gr. lemma, husk).
  - Muscle cells are of **mesodermal origin**.
- **Types:**



- **Skeletal Muscle Tissue:**

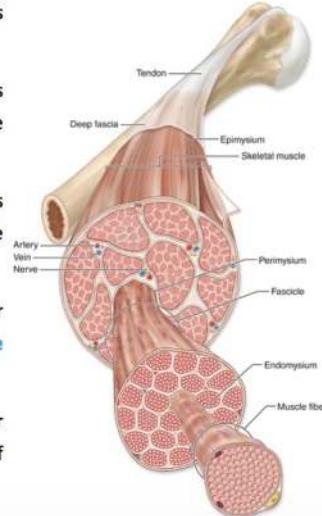
### Organization of Skeletal Muscle

- Skeletal muscle consists of **muscle fibers**, which are long, cylindrical multinucleated cells with diameters of 10 to 100  $\mu\text{m}$ .
- Skeletal muscle begins to differentiate when **mesenchymal cells**, called **myoblasts**, align and fuse together to make longer, multinucleated tubes called **myotubes**.
- Myotubes continue differentiating to form functional myofilaments, and the nuclei are displaced against the sarcolemma.
- A small population of reserve progenitor cells called **muscle satellite cells** remains adjacent to most fibers of differentiated skeletal muscle.



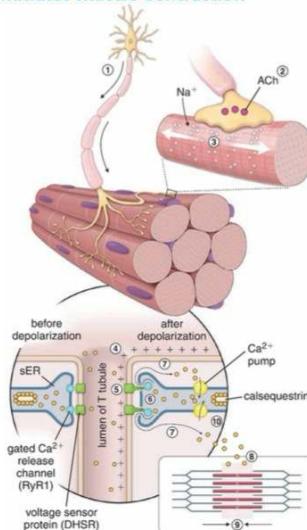
### Organization of Skeletal Muscle

- A skeletal muscle consists of striated muscle fibers held together by connective tissue.
- The connective tissue associated with muscle is named according to its relationship with the muscle fibers:
  - ✓ **Endomysium** is the delicate layer of reticular fibers that immediately surrounds individual muscle fibers.
  - ✓ **Perimysium** is a thicker connective tissue layer that surrounds a group of fibers to form a **bundle** or **fascicle**.
  - ✓ **Epimysium** is the sheath of dense irregular connective tissue that surrounds a collection of fascicles that constitutes the muscle.



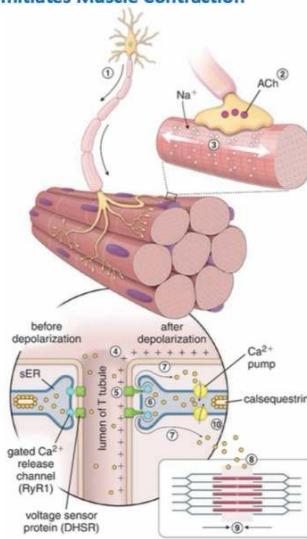
### A Sudden Rise in Cytosolic $\text{Ca}^{2+}$ Concentration Initiates Muscle Contraction

1. The contraction is initiated when a **nerve impulse** arrives at the neuromuscular junction.
2. It prompts the release of acetylcholine into the synaptic cleft that binds into **ACh-gated  $\text{Na}^+$  channels** causing local depolarization of sarcolemma.
3. **Voltage-gated  $\text{Na}^+$  channels** open, and  $\text{Na}^+$  enters the cell.
4. **General depolarization** spreads over the plasma membrane of the muscle cell and continues via membranes of the T tubules.
5. **Voltage sensor proteins** in the plasma membrane of T tubules change their conformation.



### A Sudden Rise in Cytosolic $\text{Ca}^{2+}$ Concentration Initiates Muscle Contraction

6. At the muscle cell triads, the T tubules are in close contact with the lateral enlargements of the sarcoplasmic reticulum, where gated  **$\text{Ca}^{2+}$ -release channels** are activated by conformational changes of voltage sensor proteins.
7.  $\text{Ca}^{2+}$  is rapidly released from the **sarcoplasmic reticulum** into the sarcoplasm.
8. Accumulated  $\text{Ca}^{2+}$  binds to the **TnC** portion of the troponin complex.
9. The **actomyosin cross-bridge cycle** is initiated.
10.  $\text{Ca}^{2+}$  is returned to the terminal cisternae of the sarcoplasmic reticulum, where it is concentrated and captured by **calsequestrin**.



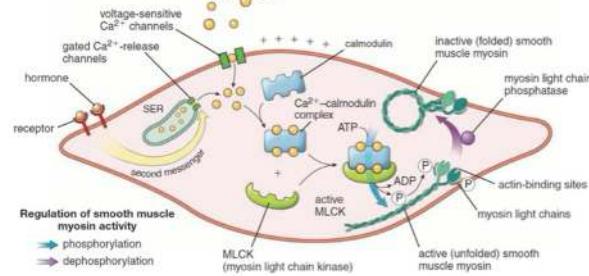
- **Smooth Tissue:**

#### Smooth Muscle

- **Smooth muscle** is specialized for slow, steady contraction and is controlled by a variety of involuntary mechanisms.
- It generally occurs as bundles or sheets of elongated fusiform cells with finely tapered ends.
- Each cell is enclosed by a thin **external lamina** and a network of **type I collagen fibers** and **reticular fibers**, comprising the **endomysium**.
- The smooth muscle cells, also called fibers, lack the striated pattern found in skeletal and cardiac muscle.
- They range in length from 20  $\mu\text{m}$  in the walls of small blood vessels to about 200  $\mu\text{m}$  in the wall of the intestine. They may be as large as 500  $\mu\text{m}$  in the wall of the uterus during pregnancy.
- Smooth muscle cells are interconnected by **gap junctions**.

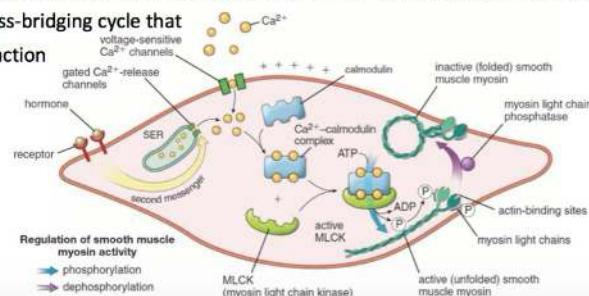
### Smooth Muscle Contraction

- Smooth muscle cells lack a T system.
- Invaginations of the cell membrane (**caveolae**) and the underlying **vesicles** along with the **sER** function in a manner analogous to the T system of striated muscle to deliver  $\text{Ca}^{2+}$  to the cytoplasm.
- An elevation of intracellular  $\text{Ca}^{2+}$  is achieved either by depolarization of the cell membrane with subsequent activation of voltage-sensitive  $\text{Ca}^{2+}$  channels or by direct activation of gated  $\text{Ca}^{2+}$ -release channels in the sER by a second-messenger, most commonly IP3.



### Smooth Muscle Contraction

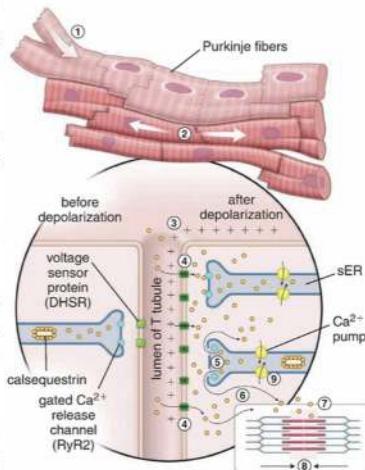
- The  $\text{Ca}^{2+}$  then binds to **calmodulin**, which stimulates **myosin light chain kinase** to phosphorylate one of the two the **regulatory chains** of smooth muscle myosin.
- When the light chain is phosphorylated, smooth muscle myosin changes its conformation from inactive (folded) to active (unfolded) configuration that can assemble into side-polar myosin filaments.
- In the presence of ATP, the myosin head bends, producing contraction.
- Smooth muscle myosin hydrolyzes ATP at about 10% of the rate of skeletal muscle, producing a slow cross-bridging cycle that results in slow contraction of these cells.



- **Cardiac Tissue:**

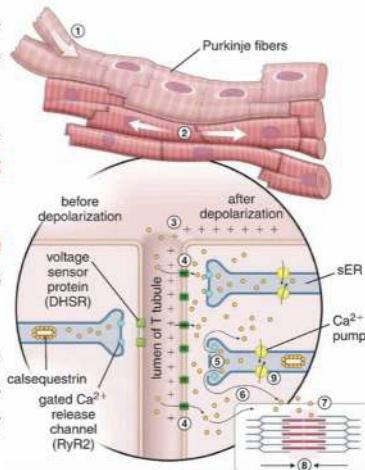
#### The Events Leading to Contraction of Cardiac Muscle

1. Contraction initiates when the cell **membrane depolarization** traveling along **Purkinje fibers** reaches its destination in cardiac myocytes.
2. **General depolarization** spreads over the plasma membrane causing the opening of **voltage-gated Na<sup>+</sup> channels**.
3. General depolarization continues via membranes of the T tubules.
4. **Voltage sensor proteins** in the plasma membrane of T tubules change their conformation into **functional Ca<sup>2+</sup> channels**.
5. Rise in the **cytoplasmic Ca<sup>2+</sup>** concentration opens **gated Ca<sup>2+</sup>-release channels** in the sarcoplasmic reticulum.



#### The Events Leading to Contraction of Cardiac Muscle

6. Ca<sup>2+</sup> is rapidly released from the sarcoplasmic reticulum and increases the pool of Ca<sup>2+</sup> in the sarcoplasm.
7. Accumulated Ca<sup>2+</sup> diffuses to the myofilaments, where it binds to the **TnC** portion of the troponin complex.
8. The **actomyosin cross-bridge cycle** similar to that of skeletal muscle is initiated.
9. Ca<sup>2+</sup> is returned to the terminal cisternae of the sarcoplasmic reticulum, where it is concentrated and captured by **calsequestrin**, a Ca<sup>2+</sup>-binding protein.

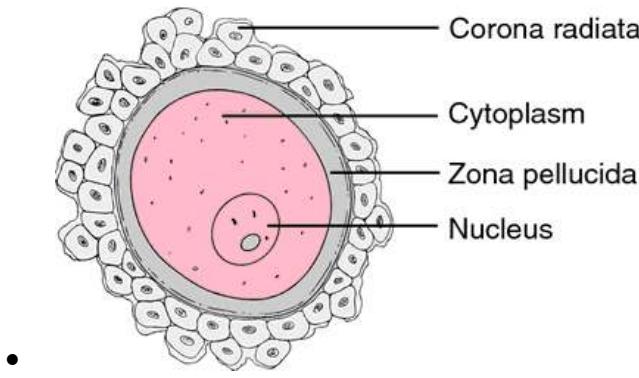


### Topic 46:

- Female sex cells, called **ova** or eggs, are **non-motile** and much larger in comparison to the male gamete
- Female ova are **some of the largest cells** in the body and are **round** in shape
- They are produced in the female **ovaries** and consist of a **nucleus, large cytoplasmic region, the zona pellucida, and the corona radiata**
- The **zona pellucida** is a membrane covering that **surrounds the cell membrane of the ovum**
- The **corona radiata** are outer **protective layers of follicular cells** that surround the zona pellucida
- Human sex cells are produced by a two-part cell division process called **Meiosis**. Through a sequence of steps, the replicated genetic material in a parent cell is distributed among four daughter cells. Meiosis produces gametes with one half the number of chromosomes as the parent cell. Because these cells have one half the

number of chromosomes as the parent cell, they are haploid cells. Human sex cells contain one complete set of 23 chromosomes\*\*\*

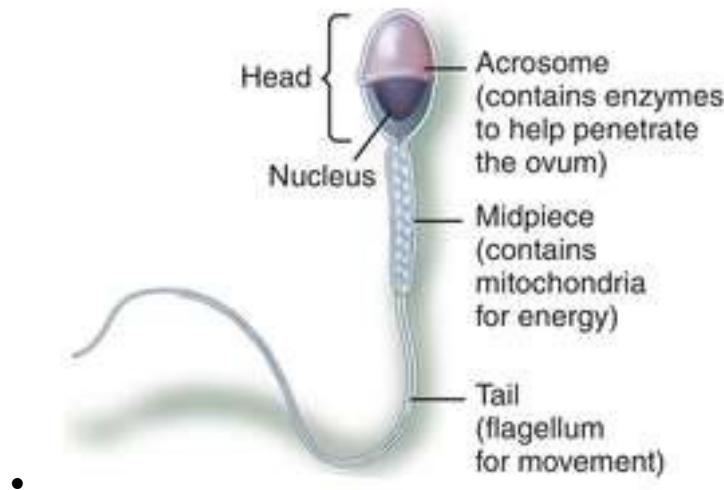
- There are two stages of meiosis: meiosis I and meiosis II. Prior to meiosis, the chromosomes replicate and exist as sister chromatids. At the end of **meiosis I, two daughter cells are produced**. The sister chromatids of each chromosome within the daughter cells are still connected at their centromere. At the end of **meiosis II**, sister chromatids separate and **four daughter cells are produced**. Each cell contains one half the number of chromosomes as the original parent cell
- In oogenesis, or ovum development, the **daughter cells are divided unequally** in meiosis. This asymmetrical cytokinesis results in one large egg cell (oocyte) and smaller cells called polar bodies. The **polar bodies degrade and are not fertilized**. After meiosis I is complete, the egg cell is called a **secondary oocyte**. The secondary oocyte will only complete the second meiotic stage if fertilization begins. Once meiosis II is complete, the cell is called an ovum and can fuse with the sperm cell. When fertilization is complete, the united sperm and ovum become a zygote.
- Female egg cells, however, contain only the X sex chromosome and are therefore **homogametic**.



#### Topic 47:

- Male sperm resemble long, motile projectiles. They are small cells that consist of a **head region, midpiece region, and tail region**. The head region contains a cap-like covering called an **acrosome**. The acrosome contains enzymes that help the sperm cell penetrate the outer membrane of an ovum.
- The **nucleus is located within the head region** of the sperm cell. The **DNA within the nucleus is densely packed** and the cell **does not contain much cytoplasm**.
- The **midpiece region contains several mitochondria** which **provide the energy** for the **motile** cell.
- The tail region consists of a long protrusion called a **flagellum** that aids in cellular **locomotion**.
- The production of sperm cells is known as **spermatogenesis**
- This process occurs **continuously** and takes place within the **male testes**. Hundreds of millions of sperm must be released in order for fertilization to take place. The vast majority of sperm released **never reach the ovum**.

- Male sperm cells in humans and other mammals are **heterogametic** and contain one of two types of sex chromosomes
- They contain either an X or a Y chromosome, and if the fertilization happens between the ovum and an X chromosome, the zygote will be the beginning of a female.
- If the fertilizing sperm contains a Y chromosome, the zygote will then be XY and that is a boy.

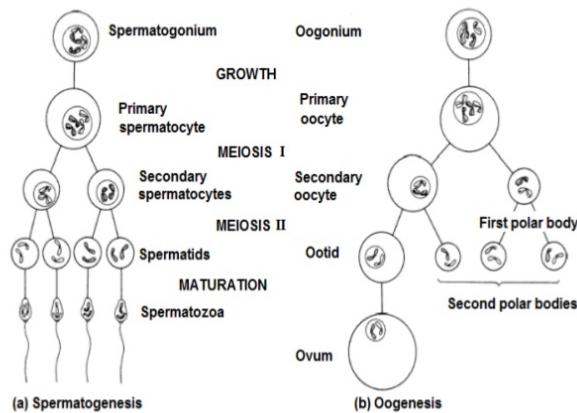


#### Topic 48: Subjects, tasks and methods of general embryology

- Definitions:
  - **Embryology**: Study of Gametes, Fertilization, Embryo & Foetuses
  - **General Embryology**: Cell to establishing organs.
  - **Special Embryology**: Differentiation & Development of Organs/Organ systems
  - **Teratology**: Embryological Origins & Causes of birth defects.
- Period of Human Development:
  - **Prenatal**: **Preimplantation** (0-7 days after fertilization), **Postimplantation** (day 8 to month 3), **Foetus** (month 3 onward)
  - **Postnatal**: After baby is born.
- Phases of human embryology:
  - **Gametogenesis** – Production of gametes from germ cells. First mitosis, followed by meiosis, then differentiation
  - **Fertilisation** – Fusion of ovum and sperm.
  - **Cleavage** – Zygote divides (but doesn't grow) to form **morula** (32-celled)
  - **Gastulation**: 3 **germ layers** formed: Ectoderm, Endoderm, Mesoderm
  - **Organogenesis** – Formation of Organs
- **Gametogenesis**:
  - PGC's (**Primordial Germ Cells**) undergo **mitotic** division within gonads; then undergo gametogenesis (formation of sperm/egg)
  - **Gametogenesis**: Includes **meiosis** then **differentiation** to complete maturation.

## Topic 49: Spermato- and Ovogenesis & Ovulation

- Spermatogenesis: Spermatogonia develops into sperm.
- Three Phases:
  - **Spermatogonial Phase:** Spermatogonium divides into **primary spermocyte** via **Mitosis**.
  - **Spermocyte Phase:** Primary spermatocytes undergo two meiotic divisions to produce spermatids.
  - **Spermatid Phase:** Spermatids **differentiate** into mature sperm cells.
- Other cells: **Serotic** cells (cells around which spermatids develop in the tubes of testes); **Leydig** cells (cells containing lipid droplets).
- Regulation: **Central Regulation**; **Local Regulation**; **External Factors**
- **Oogenesis:**
  - **Primordial Follicle** is the earliest stage of follicular development.
  - **Follicle cells** undergo stratification to form **granulosa** layer of primary follicle.
  - **Secondary Follicle** is then produced and characterized by a fluid containing 'antrum'.
- **Stages:**
  - Oogonium divides mitotically to produce **primary oocyte**.
  - Primary oocyte divides meiotically to produce **secondary oocyte** and polar body.
  - The secondary oocyte does not divide until fertilization occurs.
- **Ovulation:** Secondary oocyte is released from the ovaries, pushed down the fallopian tube and becomes available for fertilization.



## Topic 50: Fertilisation

- **Fertilisation:**
  - Sperm cell fuses with the ovum to produce a zygote.
  - Functions of fertilization:
    - i) Restores **diploid number** of chromosomes.
    - ii) **Activates ovum & stimulates important reactions** that begin

embryonic development.

- **Process:**
  - **Sperm approaches egg.**
  - Sperm comes into contact with **zona pellucida** and **acrosome releases enzymes.**
  - **Digestive enzymes drill a hole** until plasma membrane is reached.
  - Membrane of sperm and egg fuse & **cortical granules** released into zonula pellucida, **preventing** any other sperm from fusing with the ovum.
  - Nucleus of sperm enters the egg and fusion occurs.
- **Fertilisation activates egg:**
  - Fertilisation causes the egg to undergo the **remainder of meiosis II**; diploid cell formed.
  - **Ca<sup>+</sup>** released into the cytoplasm **stimulating metabolic process** (O<sub>2</sub> usage increases + protein synthesis stimulated). Zygote begins **dividing**.

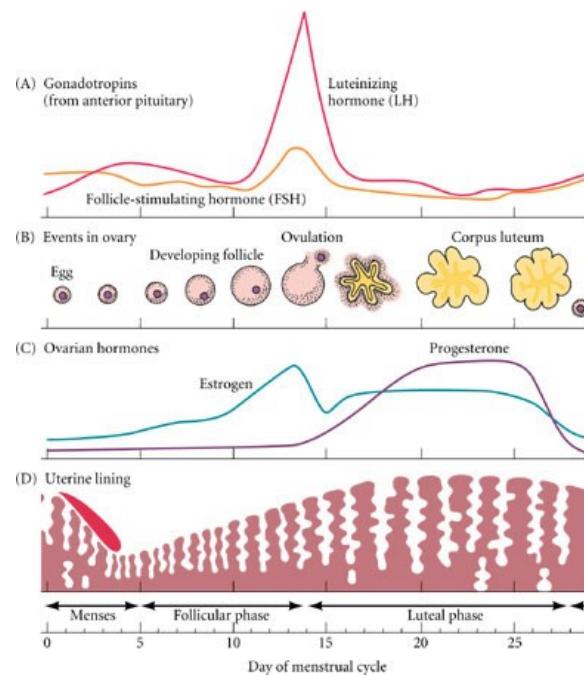
### Topic 51: Segmentation & Blastogenesis

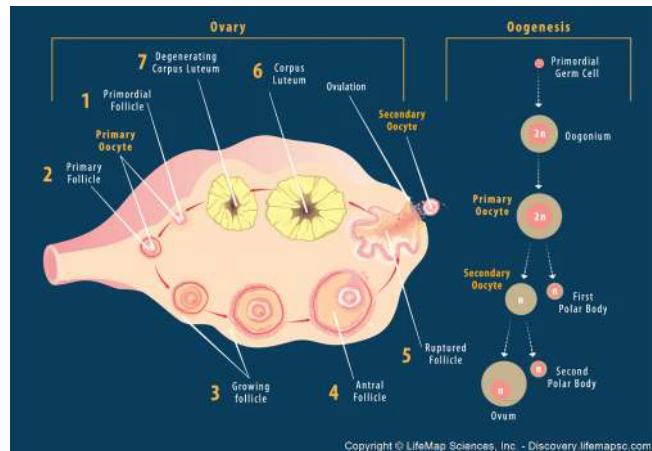
- **Segmentation (Cleavage):**
  - After fertilization, **influx of Ca<sup>+</sup> initiates many metabolic processes** (e.g. protein synthesis).
  - Zygote undergoes **first mitotic division**.
  - Zygote keeps dividing until **morula** (32-celled) formed. (Draw diagram)
  - Important:
    - i) Cells don't grow in size.
    - ii) Zygote portions itself into many identical cells.
- **Blastogenesis (Blastulation):**
  - **Morula** continues to divide until it forms a **blastula**. (Talk about features of blastula)
  - In humans, **Blastula=Blastocyst**
  - Three layers: **Trophoblast, Inner cell mass, Blastocoel**
  - Trophoblast: forms **placenta** and **chorion**
  - Inner cell mass: gives rise to the actual **organism**.
  - Blastocyst implants itself into endometrium in the uterus.

### Topic 52: Cyclic changes in uterine mucosa

- **Menstrual Cycle:**
  - Sequence of events that last around 28 days.
  - Prepares the human for pregnancy.
  - If fertilization doesn't occur, the inner lining of the uterus along with secondary oocyte is discharged. (**Menstruation**)
- **3 Phases:**
  - **Follicular Phase** (Transition from primary follicle into secondary follicle;

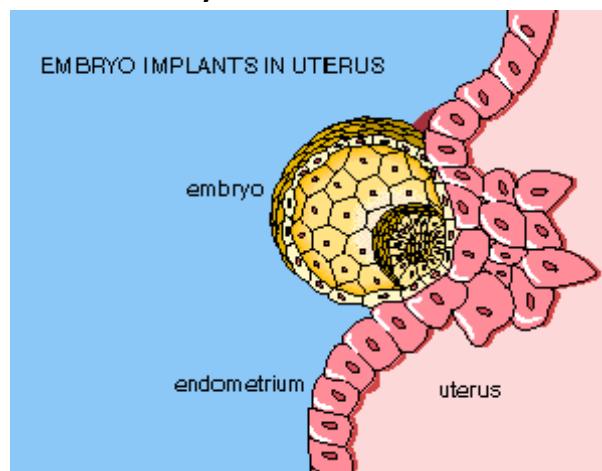
- primary oocyte to secondary oocyte)
- **Ovulation** (Release of egg into fallopian tube)
- **Luteal Phase** (Formation of corpus luteum)\*\*
- **Sequence:**
  - **GnRH** (Gonadotropin Releasing Hormone) causes release of **LH** (Luteinizing Hormone) and **FSH** (Follicle Stimulating Hormone) which act on the **immature follicle** and stimulates it to begin development.
  - Developing follicle begins to release **estrogen** which **stimulates the thickening and formation of the endometrium** in preparation for implantation.
  - **Ovulation** occurs when there is a **rapid surge** of LH, which stimulates secondary follicles to rupture and release the secondary oocyte + Corpus Luteum formed.
  - Corpus Luteum begins producing **progesterone**.
  - **Negative feedback** inhibition of GnRH due to rising levels of progesterone, therefore decreasing levels of LH and FSH.
  - If fertilization does not occur, decreasing levels of LH causes corpus luteum to **disintegrate to form corpus albicans**. Levels of progesterone decreases and endometrium breaks down.





### Topic 53: Implantation

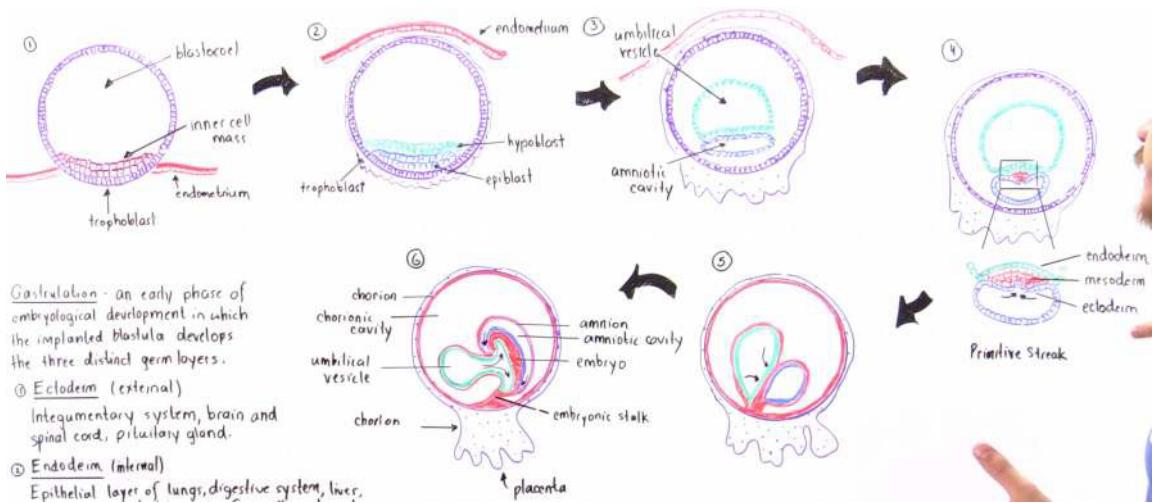
- Stages:
  - **Implantation** begins around 7 days after fertilization.
  - Trophoblast releases **enzymes** that dig a hole into the endometrium to allow embryo to be implanted.
  - Embryo makes it further into **vascular** and **connective tissue** of endometrium.
  - Trophoblast forms **chorion** (membrane for placenta formation) around day 10 after fertilization.
  - Endometrium completely takes in the embryo and is **sealed off**.
  - Digestive enzymes digest into some **maternal blood vessels** creating a temporary source of O<sub>2</sub> and nutrients.
  - **Amniotic cavity** and **umbilical cord** formed.



### Topic 54: Formation of germ layers (Gastrulation) and axial organs

- Gastrulation: Implanted blastula develops 3 distinct **germ layers**:
  - **Ectoderm** (External): Integumentary System, Brain & Spinal Cord, Pituitary Gland.
  - **Endoderm** (Internal): Epithelial layers of lungs, Digestive System, Liver, Pancreas and Bladder, Thyroid & Parathyroid glands

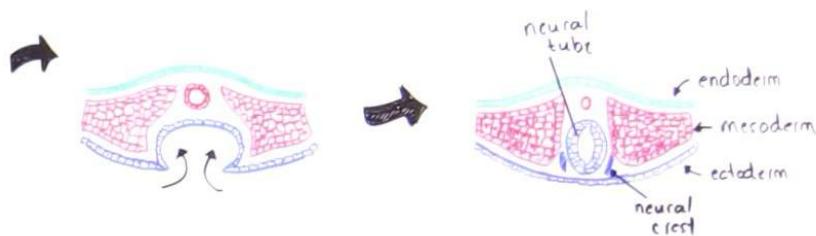
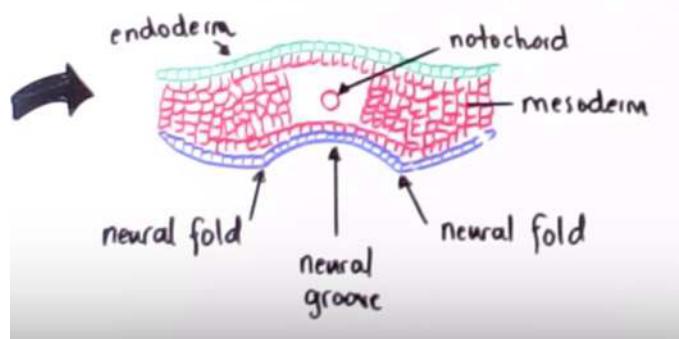
- **Mesoderm (Middle):** Musculoskeletal system, Cardiovascular System, Excretory System, Reproductive System.
- **Stages:**
  - **Implantation;** trophoblast implants itself onto the endometrium; The inner cell mass eventually forms 3 germ layers; trophoblast produce chorion and placenta.
  - Cells of inner cell mass differentiate into **hypoblast** and **epiblast** cells.
  - Hypoblast: forms **Endoderm**
  - Epiblast: forms **Ectoderm**.
  - Mesoderm develops inbetween via **invagination**.
  - Upper portion of hypoblast and epiblast migrate upwards.
  - Amniotic cavity forms in epiblast cells.
  - Mesoderm forms
  - Epiblast invaginate to form **primitive streak**.
  - New ectodermal cells pushed through the primitive streak creating new mesodermal cells.
  - Endodermal layer pushed into the ectodermal layer to form an embryo assembly.
  -



## Topic 55: Derivatives of Germ Layers. Disturbances in Development

- **Neurulation:**
  - Follows formation of germ layers.
  - Process by which **nervous system** is formed.
- **Neural plate and tissue formation:**
  - **Mesoderm cells produce the notochord**
  - Notochord directs and induces thickening of **ectodermal** cells to form **neural plate**.

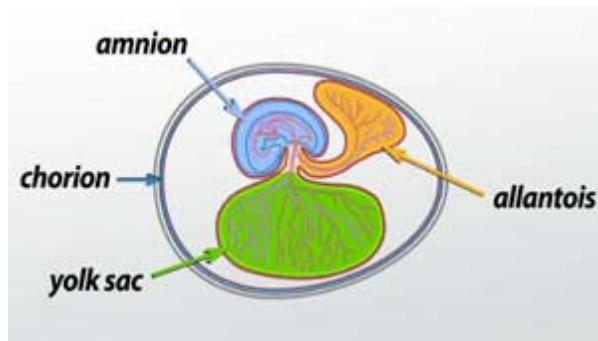
- Cells of neural plate start to invaginate towards the notochord forming the neural groove and neural folds.
- Neural tubes formed.



- Organogenesis:
  - Process by which 3 germ layers form many organs and structures.
  - 3 layers:
    - i) Ectoderm (Integumentary System, Nervous System, Sensory Glands  
e.g. cornea of eye, Epithelium of Mouth and Anus, Adrenal Medulla, Tooth Enamel)
    - ii) Endoderm (Epithelium of Digestive Tract, Organs of Digestive System  
e.g. liver, pancreas, gall bladder, thyroid, parathyroid, thymus, epithelium of reproductive ducts, urethra and bladder)
    - iii) Mesoderm (Muscle, Cardiovascular System, Skeletal System, Lymphatic System, Excretory System, Dermis of Skin, Adrenal Cortex)

## Topic 56: Embryonic Envelope

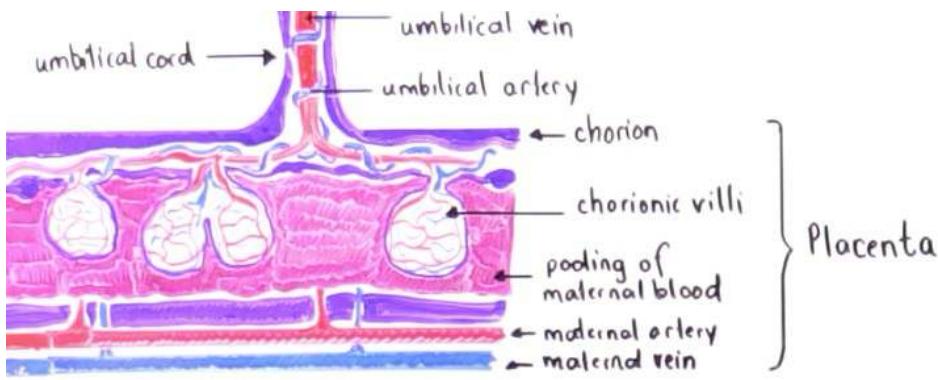
- Embryo forms **extraembryonic membranes**, which are discharged during birth:
- 4 Membranes:
  - Chorion
  - Allantois
  - Yolk Sack (Umbilical Vessel)
  - Amnion



- **Chorion:** Formed from cells that come from trophoblasts and mesodermal germ layers. **Function:** Contains fluid that absorbs shock to embryo; helps form placenta
- **Amnion:** Structure that **directly surrounds the developing embryo**; secretes a watery fluid that fills the **amniotic cavity**; **Functions:** Creates **protective barrier**; prevents embryo from **drying out**; enables the embryo some **freedom of movement**.
- **Yolk sack** (Umbilical Vessel): Helps form blood in early development.
- **Allantois:** Small outgrowth of developing **digestive tract** and is mostly non-functional.

#### Topic 57: Placenta, Structure, Umbilical Cord

- Placenta: Organ of exchange between mother and foetus
- Functions:
  - Provides **nutrients** like water and glucose.
  - Exchange **gases**, e.g. CO<sub>2</sub> and O<sub>2</sub>
  - Waste Product Removal, e.g. urea, uric acid and creatine
  - Endocrine Gland
  - Immune protection
- Placenta Formation:
  - Trophoblast enters endometrium and produces structures called **chorionic villi** (extensions of the chorion).
  - Chorionic villi become populated with embryo **blood vessels**.
  - Blood fills areas of chorionic villi.
  - Umbilical cord formed, and this connects to the chorionic villi.
  - Maternal blood is exchanged in the chorionic villi.\*\*\*
- Placenta: **Chorion** & surrounding **Uterine Tissue**
- Maternal and fetal blood do not mix!



### Topic 58: Embryonal Blood Circulation

- Oxygenated blood from the placenta is carried via the **umbilical vein**. As it travels past the under-developed liver of the fetus, the blood bypasses the liver via a blood duct called the **ductus venosus**.
- This duct connects with the inferior vena cava, which mixes the oxygenated with the deoxygenated blood. The mixed blood then travels through the right atrium of the heart.
- The deoxygenated blood from the superior vena cava is further mixed with the oxygenated blood coming from the inferior vena cava before it enters the right atrium.
- Since the fetal lungs are filled with fluid, they contain high resistance and high pressure. As a result, the blood moves to the lower pressure left atrium via the foramen ovale.
- Some blood however does make it into the right ventricle and into the pulmonary veins. However, some blood is rerouted to move to the **ductus arteriosus**, which connects the pulmonary trunk to the aorta.
- The partially oxygenated blood then travels from the aorta and to the developing organs of the foetus. The deoxygenated blood is eventually returned back to the placenta via the internal **iliac arteries** that connect with the umbilical cord.

### Topic 59: Teratology

- **Teratology:** Science that studies the causes, mechanisms, and patterns of **abnormal development**.
- **Birth defect, congenital malformation, and congenital anomaly** are synonymous terms used to describe structural, behavioral, function and metabolic disorders present at birth.
- Causes of birth defect:
  - Environmental Factors – 15%
  - Genetic Factors – 30%
  - Multifactorial – 55%
- Principles:

- Embryo susceptible to teratogens during **critical sensitive periods** (i.e. during differentiation and morphogenesis).
- Embryonic structure susceptible to a **critical dose** of teratogen.
- Susceptibility to a teratogen depends on the **genetic constitution** of the embryo.
- Anomalies caused by genetic factors:
  - **Chromosomal Aberrations:** i) **Numerical Chromosomal Abnormalities** (Down Syndrome, Turner Syndrome, etc.); ii) **Structural Chromosomal Abnormalities** (translocation, deletions, duplications, inversion, etc.)
  - **Gene Mutations** – Achondroplasia, fragile-X syndrome
- **Environmental Factors:**
  - Infectious Agents (HIV)
  - Physical Agents (Radiation)
  - Chemical Agents (Thalidomide)
  - Hormones
  - Maternal Diseases (Diabetes)
  - Nutritional Deficiencies
- Types of abnormalities:
  - **Malformations** – Results in complete/partial **absence of structure** or **alternations** in its **normal configuration**; occurs during formation of structures (3<sup>rd</sup>-8<sup>th</sup> weeks of gestation).
  - **Disruptions** – Morphological Alternations
  - **Deformations** – Mechanical forces mold a part of the foetus over a **prolonged period**.
  - **Syndrome** – Group of anomalies occurring together that have a specific common cause
  - **Association** – Appearance of two or more anomalies that occur together.
- **Twins:**
  - **Monozygotic (Identical)**: Splitting of a single original embryo; occurs **infrequently** (0.4%)
  - **Dizygotic (Fraternal)**: Two eggs are independently fertilized by two different sperm cells and implanted in the uterus at the **same time**; more common for older mothers.