Lecture 2

General med_2nd semester

Hyaloplasm and cytoskeleton. Cell inclusions.

Cell membrane, cell surfaces and intercellular junctions.

Cell cycle, cell division, and cell differentiation
Textbooks recommended to study:
The Developing Human, 8th Edition - Clinically Oriented Embryology With STUDENT CONSULT Online Access
By Keith L. Moore, BA, MSc, PhD, FIAC, FRSM and T. V. N. Persaud, MD, PhD, DSc, FRC Path(Lond)
536 pages 1805 ills
Trim size 8 1/2 X 10 7/8 in
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http://www.med.muni.cz/histol/histolc.html
Eukaryotic cells consist of:

- **the nucleus** - is the center of cellular activity that plays important role in gene expression, heredity and cell division

- **the cytoplasm** - surrounds the nucleus and is the site of metabolic and synthetic activities of each cell

- **the cell membrane** - plasma membrane, plasmalemma - separates the cell from its environment and maintains its integrity

**Overview of structural components of the fixed animal cell**

**the nucleus (interphase):**
- nuclear envelope
- chromatin
- nucleolus
- nuclear cytoskeleton
  (- nuclear inclusions)
the cytoplasm:

- **hyaloplasm** or cytosol (cytoplasmic ground substance) – as a portion of the cytoplasm surrounding cell organelles and inclusions it contains very fine network - **the cytoskeleton** that can be visualized by electron microscopy and special immunohistochemical methods

- **cell organelles** - are the "little organs" of the cell that possess a distinctive structure and well established function; they are present in most cells in different number
  
  - mitochondria
  - endoplasmic reticulum
  - ribosomes
  - Golgi apparatus
  - lysosomes
  - peroxisomes
  - centrioles

- **cell inclusions** - are lifeless and have temporary character; in most cases they are of a result of the cell activity
  
  - stored foods (proteins, lipids and carbohydrates),
  - crystals,
  - pigments
  - secretory granules
the cell membrane
(plasma membrane, plasmalemma) –

separates cell from the environment and maintains its integrity
The hyaloplasm (cytosol)

Hyaloplasm (cytosol, cytoplasmic ground substance) - is a fluid component of the cytoplasm. It consists of H₂O, macromolecules, low molecular substances (amino acids, mono- and oligosaccharides), ions (K⁺, Na⁺, Mg²⁺, Ca²⁺), phosphate + chloride anions, crystals, pigments, which are within it dissolved and suspended.

In the living cell, it seems to be structureless, viewed by the light microscope is usually homogeneous, but fine granular in TEM.

Cytoskeleton

The three-dimensional structural framework of the cell that maintains its morphologic integrity and is responsible for its dynamic properties. This network extends between the nuclear envelope, the cell membrane, and cell organelles.

Functions: shape of cells, movement of organelles, movement of cells, facilitation of exocytosis and endocytosis, creation of compartments within cells.
cytoskeleton visualized by the fluorescence microscopy
Components of the cytoskeleton

- Intermediate filaments
- Microtubules
- Microfilaments (actin filaments)
Microtubules

diameter 25 nm
are long, hollow-appearing and flexible tubules composed of 13 strands of protofilaments that are formed by linear assemblies of **alpha and beta-tubulin** heterodimers

each microtubule has a growing, **plus end** that is stabilized by a removable **cap** and **minus end** that permits the shortening of the microtubule

the removable cap consists of specific microtubule-associated proteins (MAPs), which prevents the lengthening of microtubule
microtubules are bound to other cytoskeletal elements and cytoplasmic organelles

Function of microtubules:
- they are responsible for organization of the cytoplasm and assist in intracellular transport of organelles and vesicles
- they help to determine cell shape and cell polarity
- they participate in a variety of motile activities (the movement chromosomes during mitosis, the beating of cilia)

Remember!!! microtubules form
- mitotic and meiotic spindle apparatus
- the cores of cilia and flagella
- centrioles and basal bodies

With microtubules are associated special proteins called motor proteins (take participation in transporting processes in cells with utilization of ATP)

(disruption or depolymerisation of microtubules or inhibition of their synthesis stop mitotic division, phagocytosis, processes of releasing of secretory granules, result also in the loss of cell symmetry etc.)
- **cilia and flagella** – 9 sets of microtubules arranged in doublets that surround two central microtubules = axoneme

- **centrioles and kinetosomes** - 9 sets of microtubules arranged in triplets
triplets are turned counterclockwise to each other at a constant angle

**Function of centrioles:**

- **mitosis** – induce the organisation of mitotic spindle
- **ciliogenesis** – centrioles give rise to kinetosomes, from which cilia originate (kinetosomes show the same structure as centriole)
Microfilaments (actin filaments) have diameter 5-7 nm.

are composed of **G-actin** monomers that have assembled (if ATP is at disposal) into two chains of **F-actin filaments** coiled around each other (a double-stranded helix)

similar to microtubules, microfilaments have a **plus end** and **minus end**

the lengthening of filament starts at the plus end

when the filamet achieves its required length, the two ends are capped by **capping proteins** - such as **gelsolin**

microfilaments are very dynamic structures that are continually dissociated and reassembled
distribution of microfilaments:

- may be attached to the plasma membrane - are involved in defining the surface morphology of the cell - C
- may penetrate the cytoplasm and be intimately associated with several cell organelles, vesicles or granules - B cytoplasmic streaming
- may support microvilli as terminal web and maintain their shape - A
- may be organized in constriction ring - D
in muscle cells – rhabomyocytes and cardiomyocytes, microfilaments are associated with myosin filaments and form stable structures called as **myofibrils**
Intermediate filaments

average diameter 10-12 nm

are of proteinaceous character and tissue specific

are non-contractile and maintain cells resistant to the traction and pressure forces

can be visualized with the use of immunocytochemical methods and TEM

recently, the microscopic visualization of filaments (their proteins) is explored in human pathology in diagnosis of tumours

filaments are classified into 5 groups
## Intermediate filaments

<table>
<thead>
<tr>
<th>Type</th>
<th>Thickness</th>
<th>Protein</th>
<th>Cell Type</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Keratin filaments</strong>&lt;br&gt;tonofilaments</td>
<td>8-11 nm</td>
<td>Cytokeratin (about 20 kinds)</td>
<td>Epithelial cells</td>
<td>TEM, immun</td>
</tr>
<tr>
<td><strong>Vimentin filaments</strong></td>
<td>8-11 nm</td>
<td>Vimetin</td>
<td>Mesenchymal cells (fibroblasts, chondroblasts, endothelial cells, vascular smooth muscle, macrophages)</td>
<td>TEM, immun</td>
</tr>
<tr>
<td><strong>Desmin filaments</strong></td>
<td>8-11 nm</td>
<td>Desmin</td>
<td>Striated + smooth muscle cells (except vascular smooth muscle)</td>
<td>TEM, immun</td>
</tr>
<tr>
<td><strong>Glial filaments</strong></td>
<td>8-11 nm</td>
<td>Glial fibrillary acidic protein (GFAP)</td>
<td>Astrocytes</td>
<td>TEM, immun</td>
</tr>
<tr>
<td><strong>Neurofilaments</strong></td>
<td>8-11 nm</td>
<td>Neurofilament triplet (NT) protein</td>
<td>Neurons</td>
<td>TEM, impreg</td>
</tr>
</tbody>
</table>
Cell inclusions

Cytoplasmic deposits

are lifeless structures in the cytoplasm of temporary occurrence that are not membrane bound

in most cases they are of a result of the cell activity

- by accumulation of metabolites or substances with stored function
- by phagocytosis of material from the cell surrounding or even external environment

- stored foods
- crystals
- pigments
- secretory granules

**Stored foods**: proteins, lipids and carbohydrates

**Proteins** are found in animal cells rarely – they show crystalic organization

is supposed to be identical with **crystals**

occurrence: in intestinal Leydig cells, Sertoli sells (both in the testis)

**Lipids** - 2 forms: as

- lipid droplets composed mostly of neutral fats
- masked or bound lipids that are contained mainly in membranes
Lipid droplets

variable diameter 100 nm - 10 µm

they serve as a local store of energy and also as a source of short carbon chains

in ordinary histological sections these are likely appear as round clear areas in the cytoplasm, because the lipid is extracted by solvents used in the preparation of the specimen, after osmium tetroxide fixation, lipid droplets transit into insoluble and extraction resistant form and appear as black spherical structures in the light microscope

the same appearance lipid droplets show also in electron micrographs

the degree of blackening or electron density depends upon the degree of unsaturation of the lipid and the nature of the fixative used
Carbohydrates are mostly stored in the form of **glycogen**.

It is not apparent in routine histological sections but may be selectively stained by the **Periodic Acid-Schiff’s reaction (PAS)** (brilliant magenta).

In electron micrographs, the glycogen shows granular appearance (in dependence of fixative used).

It occurs in 2 forms: as dense, roughly izodiametric, 15 to 30 nm particles, referred to as the **beta particles** or as **rosette-like aggregates** of larger size called **alpha particles**.
Pigments
are inclusions that possess color and they do not have to be stained by histological dyes

- **exogenous** - formed outside the cell and later taken into it, or as
- **endogenous** - formed within the cell cytoplasm

**Exogenous pigments** are **carotin, lipochromes, dusts** (carbon), and **minerals** such as lead and silver (an artificial introducing of some dyes into the deeper layers of the skin is called tattooing)

**Endogenous pigments** involve:
- **Hemoglobin and its breakdown products** (hemosiderin, hematoidin)
- **Melanin** and **lipofuscin**

  **Hemosiderin** is a golden-brown, iron containing pigment that is accumulated in the cytoplasm of the phagocytes (for example in the normal spleen, liver, and bone marrow) Hemosiderin shows staining reactions for iron. In electron micrographs the masses of pigment include large numbers of 9 nm dense particles of the iron-containing protein, called the ferritin

  **Hematoidin** is iron-free pigment that is produced after longer time, mostly located extracellularly, near to blood vessels, especially in extravation sites

  **Melanin** is a brown pigment synthesized by cells called **melanocytes**, in which is present in the form of granules – **melanosomes**

  on the ultrastructural level, melanosomes appear as a homogenous dense granule limited by a membrane unit
**Lipofuscin** ("wear and tear" pigment)
a tan or light brown pigment that fluoresces a golden-brown in ultraviolet light, it occurs in neurons (in cells of the autonomic ganglion)
an amount of lipofuscin is progressively increased with advancing age of cells

recently lipofuscin is thought to be end product of lysosomal activity and is regarded as the indigestible residues of phagocytosed material or degenerated organelles
Cell membrane and its specializations

Cell membrane (plasma membrane, plasmalemma)

A thin limiting membrane that surrounds cell body against the external environment

The plasma membrane is not detectable by the light microscope as viewed with the electron microscope, the plasma membrane is 7 to 10 nm thick and shows trilaminar structure of the unit membrane.

The dense layer closer to the extracellular space is the **E-face**

The dense layer adjacent to the cytoplasm is the **P-face**

In many cells, the outer membrane surface (E-face) is covered by a thin surface coat composed of protein-polysaccharides = **glycocalix**
glycocalix visualized using the ruthenium red staining on apices of cells in the oviduct

functions of the glycocalix:

- stabilizes the cell membrane
- participates in cell adhesion
- is responsible for antigenic properties of the cell
- is also engaged in processes of cell recognizing
Specializations of cell surfaces

Free cells suspended in watery medium communicate with the environment through their whole surface (e.g., blood corpuscles, connective tissue cells), their plasma membrane does not form permanent specializations but only temporary: the most famous are pinocytotic or exocytotic vesicles, than short and irregular microvilli, pseudopodia maintaining the ameboid movement.

In cells that are organized into cell complexes or tissues, the type of cell surface specialization is closely related with cell topography, that is good expressed in cells with a polar orientation.

In such cells are distinguished:

- **an apical cell surface** - it borders a luminal space
- **a lateral cell surface** - is oriented to the adjacent cell
- **a basal cell surface** - it is in contact with the basal lamina
Microvilli

finger-like processes, 0.1 mm in diameter and 0.1 to 0.5 mm in length
projecting from the cell apex either irregularly or regularly and in a great number
microvilli greatly increase the surface area of cells and facilitate the absorption of molecules or substances
are found in cells specialized to absorption and correspond with a
"brush" or "striated" border of the cell detectable by light microscopy

may be smooth
is provided with microvilli, cilia (rarely flagellum),
is often involved in processes of cell internalization of substances e.g. cell drinking or pinocytosis and phagocytosis
Cilia

are larger than microvilli and are motile, they reach 1 μm in diameter and 5-10 μm in length so already well visible by the light microscope

a number of cilia that project from the free apical surface is variable (for instance about 270 cilia are on every ciliated cell in the trachea)
cilium is covered with the plasma membrane and has core called the axoneme

the axoneme consists of a sheaf of 9 doublet microtubules enclosing central pair of microtubules

each cillum terminates within the cell at basal body (kinetosome) with cross-striated rootlet
TEM:

SEM:

cilia beat with frequency 16-20 Hz/min
pseudostratified ciliated columnar epithelium
Pinocytosis (cell drinking)

is a way of an internalization of colloid substances surrounding the cell. The process begins by the binding of colloid molecules to the cell membrane, after which the respective membrane parts start to form small pits or caveolae that then pinch off and give rise to pinocytotic vesicles. These vesicles contain internalized colloid molecules and then pass through the cytoplasm to reach the opposite cell aspect where they fuse with the plasma membrane and release their fluid content.

exocytosis = release of content of pinocytotic vesicles

phagocytosis
Lateral cell surface
may be smooth (rarely) but may also form more or less extensive interdigitations plasma membranes of adjacent cells are separated 10 to 20nm wide space called the intercellular space

Basal cell surface
smooth or in cells specialized for transport of ions it is folded to form basolateral labyrinth
Intercellular junctions

are local specializations of lateral cell membranes of adjacent cells in general, they serve to three functions:

- increase the cellular attachment - adhering (anchoring) junctions
- seal the intercellular space - occluding junctions
- serve for cell-to-cell communication - communicating (gap) junctions

**Adhering junctions:**
form strong bond between adjacent cells or between the basal cell membrane and basal lamina

- **spot desmosome** (macula adherens)
- **belt desmosome** (zonula adherens)
- **hemidesmosome**

for juction is typical presence of calcium dependent transmembrane linker proteins - **cadherins** + **desmoplakin** and **plakoglobin**
Spot desmosome (macula adherens) occurs especially in tissues that are subjected to extreme mechanical stress about 0.1 um in diameter. Intercellular space is 30 nm wide and contains extracellular glycoproteins that promote adhesion of adjacent cells. On both cytoplasmic sides of the macula adherens, there are 20 nm thick electron dense plaques (containing special proteins - desmoplakins I and II), into which tonofilaments insert.
**Belt desmosome** (zonula adherens)

encircles an epithelial cell completely (zone → zonula)

in addition, the intercellular space is about half as wide (15 nm) cytoplasmic plaques are poorly develop

**hemidesmosomes** = junctions between the plasmalema of basal aspect and lamina basalis

**spot-like appearance**
Occluding junction

= sites where plasma membranes are in such close contact that their integral and peripheral proteins are fused

integral proteins that are shared belong to family **occludins** and **claudins**

are called also **tight junctions** or **zonulae occludentes** because they have belt-like structure and encircle epithelial cell completely in a manner similar to that of the belt desmosome

the function of tight junction is to seal the extracellular space between adjacent epithelial cells
they prevent fluid penetration
they also determine the apical and basolateral domains in cells
Communicating junction or gap junction (nexus)

occur between a variety of excitable and non-excitible cells; serve to passage of ions and electrical impulses in the cardiac and smooth muscle

they usually show form of plaques or spot-like regions (0.5 to 1.0 um in d.) in which membranes of adjacent cells run in close apposition intercellular space is retained and reduced to only 2 to 4 nm

are numerous bridges in extent of each gap junction are formed by a special protein, called connexin
**THE CELL CYCLE**

**cell cycle** or **generation time** (individual history of the cell) = time from one mitosis to the beginning of the next, it occurs in all tissues with cell turnover; characterised by many events in both the cell nucleus and the cytoplasm

**cell cycle:** **interphase** and **mitosis**

Interphase is subdivided

- **G₁** - postmitotic
- **S** - DNA synthesis
- **G₂** - premitotic

mitosis (pro-, meta-, ana, telophase)

**G₁** – 8 - 25 h, intense synthesis of RNA and proteins, number of organelles increases, the cell volume of daughter cell is restored to normal size

**S** - 8 h, synthesis of DNA and its duplication, after S phase completion chromosomes composed of 2 identical chromatids, centriol is replicated

**G₂** - (post DNA duplication)- 3 h, cell continues in growth and cumulates energy, tubulin is synthesized, preparation to mitosis,
2 checking (restriction) points included:

- to the end of G\(_1\)
- in the middle G\(_2\)

when the cell passes the first restriction point, it continues through the S, G\(_2\) and mitosis.

the first restriction point stops the cycle under conditions unfavourable to the cell.

in G\(_1\) the cell can leave the cycle and enter a quiescent phase - G\(_0\), from this phase it can return to the cycle.

neurons and muscle cells stay in G\(_0\) for their entire lifetime.

cells are highly metabolic active but have no proliferative potential (capacity).
Mitosis

cca 1 h (40-120 min)
Metaphase
Late anaphase

**constriction ring from** microfilaments is organized in the equatorial plane of the parent cell

Further narrowing of the ring leads to separation of both daughter cells.