Commentars of cell biology to Junqueira's Basic Histology for the English Medical course in the Semmelweis University by prof. M. Kálmán, Md, PhD, DSc

General notes

These few pages complete your 'official' textbook of cell biology, Junqueira's Basic Histology (by Mescher, 2010), whose 1-64 pages, and later the 65-74, 93-103, 168-175 pages comprises the cell biology. (The other parts of the book are also necessary for your studies of histology!). Essential Cell Biology (by Alberts et al., 2nd ed.2004) Recommended to read, mainly some chapters, which are underrepresented in the JBH. Easy text with a lot of good sketches, but contains however a lot of things which do not belong to our material. Csaba-Madarász: A sejt szerkezete/The structure of the cell/Die Struktur der Zelle is also necessary, an electron microscopic atlas, in 3 langueages.

Abbreviations:

JBH Junqueira's Basic Histology..

ECB Essential Cell Biology

Biochemistry, Physics – initialization with capitals refers to the subject and scholar-book of the course rather than the science.

ECM – extracellular matrix (see pages from 93 to 103).

p, pp – page, pages of JBH unless specified otherwise

Note
- 'Medical application' paragraphs, and tables listing diseases in JBH – to read only!
- Sketches of JBH (but not the photomicrographs and the artistic drawings with 3D effects) are to be reproduced during exams. It is recommended to make them before the questioning.
Note: in JBH figures surpass the text. Therefore they frequently positioned posteriorly to the text they refer to.
- Tables are usually only for illustration, unless specified otherwise. To cite on the exams one or two example from the content is honorable. The same refers to the majority of chemical terms.
- The chapters here are arranged according to JBH, not according to the lectures)

Histotechnics (Lecture 2)

The mechanisms and physical basis of the different microscopes (pp. 4 to 9) are not to be learned (it is a topic of physic, 2nd semester) but their advantages and application fields.

Note to the 'brightfield' microscope: formerly so-called darkfield microscopes were also used. The light entered laterally and small objects were detectable (but not their inner structures) due to light scattering. In the ultraviolet microscope silicon glass lenses were applied. Electron microscope having been introduced these methods went out from the fashion.

Visibility, magnitudes: With eye objects can be detected to 0.1 mm, with light microscope applying immersion oil to 0.1 micrometer, with electron microscope below 1 nanometer. Cells are between 5 and 200 micrometers (but a skeletal muscle fiber can be 35 cm long, some axons are near 1 meter in humans, in some large animals even longer). Cell organelles are usually from 0.1 micrometer to 1-2 micrometer, so they were usually discovered by light microscpic investigations, but to study them requests electron microscope.

Artifacts are resulted of histotechnical procedures: salt precipitation, dye drops, shrinkage, deformations, chatter (parallel lines due to sectioning), rip or fold of the section, etc. Some artifacts are characteristic of some tissues/structures and reveal their features. These are the so-called 'meaningful artifacts'. A meaningful artifact is the 'spiny' form of the cells in the stratum spinosum of the stratified squamosus epithelium (see later).

Notes to electron microscopy: For electron microscopic purposes the so-called ultrathin sections (about 50 nanometer thick) are cut in ultramicrotomes, with glass or diamond knives mounted onto small meshes (grieds), and stained ("contrasted") with uranyl acetate and lead citrate. Negative staining (the background is stained by the vapor of a heavy metal) visualizes even individual molecules of proteins.

Semithin sections (about one micrometer thick) are cut from the tissue samples embedded-for-electron-microscopy, stained with toluidine blue and scanned under light microscope for the area capable for further processing for electron microscopy. Why? Because beside the complex preparation the other handicap of the electron microscopy is the very confined visual field. (A general role is in either the light- or the electron microscopy: the increase of solution diminishes the visual field!!) That's why the area designed to electron microscopy is to be selected by a preliminary light microscopic examination accurately!

Electron microscopic X-ray analysis: The electrons provoke X-rays from the material examined, and the wavelength and intensity of these rays refer to the elements found in the material (but only they atomic weight is over 10. The analysis requests a special equipment.

Serial sections are prepared in both the light- and the electron microscopy to get a 3D-reconstruction of the objects.

Notes to the immunohistochemistry:

When too concentrated reagent is applied and/or its rest is not washed out enough it is adsorbed to every tissue components and results in an intense so-called **background staining**.

If an antibody against an antigen also reacts with another antigen of similar structure it results in a **cross-reaction**.

The molecular groups of a giant molecule, which bind different antibodies are the **epitops.**

Masking is when epitops are covered by other molecules or modified beyond the immunologic recognition. **Exploration** is when these epitops have been prepared to be available for immunohistochemical reaction artificially.

Polyclonal antibody is prepared by the immunization of an animal (horse, goat, ox, pig, rat, rabbit, guinea pig).

Monoclonal antibody is prepared by a clone of an immunized lymphocyte (usually of mouse) in culture.

Double labeling is when two antigens are labeled in the same section. If both antigen localizes in the same structure it is a **co-localization**. To study double fluorescent labeling the best equipment is the **confocal microscope**.

Peroxidase technique: Beside the fluorescent labeling the histochemical reaction of peroxidase, which results in a dark brown precipitation, is also frequently applied, e.g. in the case of the avidin-biotin method mentioned in the text. Tha avidin-biotin refers to that any proteins – e.g. secondary antibodies and peroxidase can bind biotin which similar to amino acids, and avidin interconnects the molecules bearing biotin.

Lectins are immunoglobulin-like substances in plants which react specifically with some characteristic polysaccharide components, e.g. in the glycocalyx of a cell. Fluorescent- or peroxidase –labeled lectins can be applied to detect these polysaccharids (i.e. the cells bearing them).

Electron-microscopic immunohistochemistry: immunohistochemical reaction can be performed before or following the resin-embedding and sectioning the tissue sample (pre- or postembedding technique). The former method usually applies peroxidase reaction, which results in electrondense precipitation, in the latter one gold-grain-labeled antibodies are applied. Fluorescent labeling, of course, is unable for the electron microscopy.

Histochemistry, autoradiography, immunohistochemistry, in situ hybridization: all these methods can be combined with each other, and are capable of double or triple labeling (except the autoradiography!), i.e. to detect two or three substances together in same tissue sample.

Careful studying of Fig. 1-15. is highly recommended!!!

To read ECB 1-37 is recommended for a better understanding the basic terms and phenomena!

The cytoplasm, (including cell membrane, organelles(Lectures 3 and 4)

Definition of the cell (p. 17) : functional and structural units of the living organisms!
Eucaryotic cells were formed from procaryotic ones during evolution.

a) infolding of the outer membrane formed intracellular membrane systems,
b) surrounding the chromatin, they formed a membrane-enveloped nucleus,
c) incorporation of other prokaryotes resulted the formation of some organelles (mitochondria, chloroplasts in plants, probably peroxysomes, centriolum.

Note (p. 17): protoplasm comprises nucleus and cytoplasm (including cell membrane)

The paragraph on the top of p. 19 is only for reading. The chemical names are not to be memorized (see later in Biochemistry). Just remember:

a) the two layers of the unit membranes are different (asymmetric);
b) differences in the lipid composition results local differences functionally important,
c) outer layer is distinguished among others by glycoproteins and glycolipids (see glycocalyx).

Flip-flop means when a lipoprotein particle ’jumps’ from one lipoprotein layer of the membrane to the other. A very slow process spontaneously, but is helped by the flippase enzyme. Its importance:

a) the new lipoproteins always build in the same layer of the membrane, therefore it is necessary to transfer some components to the other layer;
b) to fold, wrap or engulf the membrane on layer (on the convex side) is to be prolonged (widened), it needs more and more ’plus’ lipoprotein.

Note (Figs. 2-2, 2-3): every transmembrane protein is integral protein but not *vice versa*!

Note (p. 20 top) receptors have different functions

a) signal perception (not only protein hormones, but any signal molecules, light, etc.)
b) cell or extracellular matrix adhesion;
c) cell recognition (tissue formation, immunoreactions).

But: the cell-to-cell contacts (and any contacts!) are usually signals affecting on the cell, too!

Glycocalyx (p. 21, top right) consists of glycosaminoglycans **(**GAGs, see also p.100 to 103**)** bound to integral or (outer) peripheral proteins of the cell membrane.

Membrane transports (p. 21, top right): There are two types: passive but facilitated. They use carriers (transporters) or channels. The difference between these forms:

- the carrier catches the molecule-to-be-transported, transports it to the other side of the membrane and releases it;
- the channel is a transmembrane protein along which molecules pass the membrane.

Both types are dynamical: a) their expression in the membrane changes on demands; b) their activities are regulated, mainly that of channels, e.g. by humoral agents.

Aquaporins are water-channels in the cell membrane. Numbered from zero to ten, they are specific for they occurrence.

For the finer details of the transports see Biochemistry and Physiology. In brief: the passive transports are driven by: a) concentration gradient; b) in the case of ions to the opposite charge, e.g. positive toward the negative. Their derivative is the **electrochemical gradient.** Against this the transport requests energy (e.g. of ATP), therefore it is active. Some molecules are transported together with another molecule (co-transport), in the same direction (synport) or oppositely (antiport).

Due to active transports and the low permeability of the membrane, the intra- and extracellular ion concentrations are different: Na+, Ca2+, Cl- outside, K+, Mg2+, phosphate, bicarbonate, protein (anionic!) inside predominate. However, outer and inner summarized ion concentrations (osmolarities) are equal. If not, through the cell membrane water diffuses toward the side of higher osmolarity, i.e. in hyposmolar environment cells swell, in hyperosmolar one they shrink. The active Na+ ex- and K+ import is done by the N+K+ pump. It is supposed that there is no free Ca2+ in the cytoplasm. The former results detecting considerable values were probably false measuring the Ca2+ contents of the intracellular Ca2+ sequesters which release their contents only in special cases (see intracellular signal pathways, sarcoplasmic reticulum). The voltage is unequal:-90mV intracellularly. The pH is 7.4 (i.e. very weakly basic) outside, and 6.9 inside the cell.

Endocytosis (pp. 21-23): A large group is the clathrin-assisted endocytosis, in which the inner side of the endocytosis-designed membrane (a kind of rafts) is covered by clathrin particles promoting the pinching-off (intracellular vesicle pinching off may have a similar mechanism). Another type of endocytosis occurs with caveoles formed by caveolin molecules.

Beside cell-feeding, endocytosis have other functions.

- internalizing signal molecules when they affect on intracellular organelles or the nucleus;
- uptake of important macromolecules, e.g. protein-bound iron, special lipoproteins;
- removal of unnecessary receptors, carriers, channel molecules from the cell surface,
- to decrease of the cell surface, to balance the surface-increasing exocytosis.

This latter cases belong to the constitutive endocytosis, the former ones are performed by regulated (signal-evoked) endocytosis.

Transcytosis: the endocytotic vesicle with its content pass the cell and release its content to the environment on the other side. A typical transcellular transport through endothelial cells.

Exocytosis (pp. 23) is also constitutive or regulated. Its result is, beside the secretion of hormones or enzymes, and other products (e.g. components of ECM, immunoglobulins) from the proper cells, receptor expression to the surface, to build in membrane-pieces to increase the surface. Both the endo- and exocytosis are followed by the recycling of some components for re-use.

The ruffle border consists of numerous small processes which can serve an intense endocytosis or exocytosis or both. The best examples in human tissues are the osteoclasts (see in histology).

Cell shape can are also influenced by the exo- and endocytosis, which are usually in balance. See e.g. the urothelium below. The free cells are globular due to the surface tension. If the cells are attached to other cells around (e.g. in glands) they become polygonal, in simple covering epithelia hexagonal to surround maximal volume by minimal surface, even to have flat surfaces to attach to each other. On flat surface the cells are flat. To change shape and to produce and support special forms (microvilli, pseudopodia, long processes) is the task of cytoskeleton.

Umbrella cells of urothelium (transitory epithelium) (see p. 40) can prompt change shape due to their rafts specialized to exocytosis (surface grows, cell flattens) and endocytosis (surface decreases, cell rounds).

Signal reception and transduction

Note (p. 23-24): there is also a **matricicrine** signaling, when the signaling molecules (frequently growth factors) are adsorbed on the ECM. The **cytocrinia** means a direct transport from cell to contact cell (e.g. the pigment in the skin epithelium). It is to be memorized that there are signals affecting on cell membrane receptors, and others, affecting on nuclear receptors, and the latter ones need a transport through the cell. The mechanisms ("One the best studied…. ….(IP3)") are only to read, and Figs. 2-9 and 2-10 are not to be reproduced. Even it is to be noted that calcium ions and prostaglandins are also important part of the intracellular signal transduction as well as the activation of so-called early genes, which non-

specifically help the production of specific proteins, affecting on the transcription by their products. **Cascade mechanism** is when each step enhances the subsequent one.

'Receptor' - this term has different meanings:

At cellular level: a molecule or its detail which can specifically bound another molecules, the ligands (from the latin word ligare – to bind, be careful, ligands is not a group of substances, like lipids, but any molecule bound by a receptor!).

At tissue level: cell or nerve ending specialized for perception of stimuli

At in gross anatomy: receptor organ, i.e. sensory organ)

Note: a cell can be controlled directly by signals (e.g. by a hormone) or indirectly, by the induction/repression of the appearance of receptors (so-called receptor expression) for the same hormone. The receptor-assembly of cells is dynamic, adapted to demands, and permanently renewed (see e.g. the constitutive endo- and exocytosis). See also p. 17 top right.

Mitochondria

The details of their functions belongs to Biochemistry, but summarized in brief, according to their ultrastructural localization:

-Outer membrane:
-Transport functions, most substances are also transported through the inner membrane.

-Inner membrane (other translocons**):**
-Proton-transport and oxydative phosphorylation
- Ca"+ transport (to sequester the the Ca2+ from the cytoplasm)
- Lipid, aminoacid, ADP, phosphate, pyruvate transport inward, ATP outward
- Transports of proteins (mitochondrial enzymes), tRNA (through translocons).

-Matrix:
- Citrate cycle (Krebs- or Szentgyörgyi-Krebs-cycle);
- Lipid- and aminoacid-degradation to acetyl-groups for the citrate cycle;
- Protein- and lipid synthesis (their special lipid is the cardiolipin).
- Ca"+ storage.

Beside these, mitochondria can grow, divide, move (along microtubules), and if their content is released into the cytoplasm, it switches the apoptotic cascade in (see pp. 103-104).

Note (p. 27, second paragraph): the proteins synthesized by mitochondria are mainly their own structural proteins, whereas the enzymes are imported from the rER through the porins. mitochondria have DNA and ribosomes.

The paragraph „Formation the ATP…. ….per second" is only to read, and Fig. 2-13 is not to be reproduced.

Cell fractioning

It promotes the studies on the chemical composition and the activity of the cell organelles when they are isolated and concentrated in a cell fraction. Before fractioning the cells (or the tissue, or organ) is to be homogenized into a suspension. During centrifugation the organelles have a characteristic sedimentation that's why they can be separated from the different organelles and collected. The sedimentation depends on the specific weight but also the shape and surface of the particle (see viscosity, Biophysics). The four major fractions are: nuclear, crude mitochondrial (contains other particles beside mitochondria), microsome (vesicles, fragments of the endoplasmic reticulum and Golgi), and cytosol. The first two ones can be

separated by simple lab centrifuges but the others only by ultracentrifuge. For the latter there are two methods: speed-cf and balance-cf. The first is based on the different distances overcome by different particles during the same time. In the latter a gradient of solutions of different densities (i.e. specific weight) is to be prepared, each particle remains in that fraction which has the same density like the particle itself.

The measure of te sedimentation is the svedberg (named after the constructor of the first ultracentrifuge) $S=(dt/dx)/(\omega^2 x)$, dx/dt is the sedimentation speed, ω is the angular speed, x is the distance from the rotation axis (see Phisics).

A summary of the protein- and other transports which are mentioned at different pages (Part of Lecture 4)

Common features

a) Every protein has marks signaling its destination. Mark can be a short aminoacid sequence, a group of aminoacids formed by the tertiary structure (signal spot), or a shorter or longer saccharide group (it is longer mainly in the proteins destined for exocytosis)
b) Every protein at every station of its carrier is subjected to checking and (if necessary) to repair or elimination (degradation). The proteins repairing other proteins **are the chaperons.**
c) Following arrival into the destination point, signal is removed

There are two main possibilities:
a) proteins synthesized on free ribosomes;
b) proteins synthesized on bound ribosomes (rough endoplasmic reticulum).
The latter one comprises the proteins becoming parts of the membrane system or exported from the cell. The process, how ribosomes get bound and how the protein synthesized on them enters into the endoplasmic reticulum, is only to read, not to memorize in details, as well as Figs. 2-17. The last paragraph, however, the list of the "Proteins synthesized in the RER…" is necessary, except the reproduction of Fig. 2-18. Fig. 2-22 is, however, important!

a) These proteins remain in the cytosol, as cytosol proteins, or may enter some organelles, e.g. mitochondria (by translocons, porins), or the nucleus through its pores, or built into the cytoskeleton. They are degraded when necessary, by the proteasomes (p. 36, the first paragraph is enough).
b) These proteins may remain in the endoplasmic reticulum, or transported in vesicles moved by microtubules to the Golgi apparatus. The main function of the Golgi apparatus is the "sorting" and "re-labeling" of the proteins according to their final targets, mainly by modifying their saccharide components. Lysosomal enzymes transported into the lysosomes, to-be-secreted ones into secretory granules. The membrane-bound proteins degraded by autophagy (see p.34 top left)

Vesicular transport carries also the pino- or phagocytotic vesicles, and the secretory granules. When functions of endoplasmic reticulum, Golgi apparatus and lysosomal system are studied, endo- and exocytoses are to be repeated, and the common points are to be marked (mentally).

The common steps in the vesicular transports are

a) vesicles are labeled according to destination,
b) their pinching-off from the 'mother' membrane system is not spontaneous, but needs special proteins;
c) they moved by and along microtubules;
d) they recognize their destination point and 'docking' by special proteins;
e) they merge with the membrane the target;

f) after delivery the content, the vesicle-material recycles to its maternal system (or degrades).

The latter step is not only to save membrane-material. If the vesicular membranes were permanently integrated into the target organelle, then this latter one would get always bigger and bigger, and – considering that the different membranes have different composition – the original membrane of the target would be mixed with alien components

The cytoskeleton (Lecture 31)

It is a part of the cytosol, but lectured separately. It correlates with some parts of the chapter Muscle Tissue (actin, etc.) 168-175.

For all the three main types of cytoskeleton it is true:

1) They are built up from subunits.
2) They are dynamic, building up and down according to demands.
3) These processes based on protein phosphorylation-phosphatation.
4) The subunits are polarized.
5) They need associated proteins.

Only actin and microtubules have motor functions and remain polarized after polymerization. They are almost uniform in the different cell types.

The units of the cytoskeleton are interconnected with each other, with the connecting points of the cell and the cell organelles. It is the function of some associated proteins. An important protein interconnecting different cytoskeleton elements with each other and with the cell junction points is the plectin. See pp. 67-69. for the cell junctions.

Intermediate filaments: are called so, because their thickness is between the actins's and the microtubules'. For classification, see the table enclosed here. Although their subunits are polarized (head, rod, tail), it is neutralized (in contrast to actin and microtubules) by the tail-to head (69-like) lateral connection to the subunits into di- and tetramers before the further polymerization. The rod is nonspecific, the head and tail are characteristic for the tissue in which the intermediate filament occurs.

Note: When you list the actin-binding proteins (p. 44, top right), do not forget the proteins anchoring actin to the cell junction points (talin, vinculin, paxilin, alpha-actinin, see e.g. p. 101) and to the cell membrane to form the membrane skeleton (e.g spectrin, p. 204, fodrin in neurons, dystrophin in glia, neurons and muscle).

The pseudopodium-formation during amoeboid movement (crawling) or phagocytosis occurs also by actin filaments. They promote formation of 'ruffle border' with a number of small protrusions along the cell border, capable of very intense phagocytosis (e.g. in osteoclasts, see later, in the chapter of ossification). Wide flat thin cytoplasm protrusions are the lamellipodia, from which thin fingerlike processes emerge (filopodia). These are also formed by the participation of actin filaments.

During movements filopodia attach to the surface forming focal contacts and pull (using the motor protein myosin) the rear of cell forward. The stress-filaments are actin-filaments stretched between two points. That's why very important for movement a surface to form contacts with it, but not too strong ones (these stop the movement). The moving cell orient

itself according to the optimal points to form attachments, therefore the ECM orients the cell movement. The crawling is the general way of the cell movements. The mechanism of the growth of neural processes is very similar.

Intermediate filaments

Group	Name	Mol. weight	Subtypes	Occurrence
Type I	Acidic keratins	40-57 kDa	15 <	epithelium
Type II	Basic keratins	53-67 kDa	15 <	epithelium
Type III	Desmin	53 kDa	1	muscle
	GFAP*	50 kDa	4	astroglia
	Vimentin	57 kDa	1	Immature, motile cells
Type IV	Neurofilamentum, light	62 kDa	1	neurons
	Neurofilamentum, medium	102 kDa	1	neurons
	Neurofilamentum, heavy	110 kDa	1	neurons
	Nestin	240 kDa	1	Immature derivatives of neuroepithelium
Type V	Lamin A	70 kDa	1	nucleus
	Lamin B	67 kDa	1	nucleus
	Lamin C	67 kDa	1	nucleus

*glial fibrillary acidic protein

Note: fibrillum refers to a group of intermediate filaments, aggregated artificially or naturally, which is visible under light microscope, whereas detection of filaments requests a high-power electron microscope.

Cell surface specializations: apical side (pp. 70-72, Lecture 10)

An important specialization of the basal part of some cell are the basal folds (basal striae). It consists of parallel deep flat invaginations of the cell membrane. Basal membrane does not intrudes into them. Between the fold there are rows of mitochondria perpendicular to the cell basis. The system increases the basal surface and performs an intense fluid and ion transport (by the usual channels of the cell membrane) from the cells into the extracellular space. It occurs in the tubules of kidney and salivary glands, its role is water and ion resorption from the secrete.

On the adjacent membranes of the adjacent cells frequently interdigitate (form finger-like intrusions). It increases the surface, provides a possibility to increase the number of cell-to-cell contacting elements (see below).

Basal laminae and basement membranes (p. 66, Lecture 17)

Note: The lamina densa contains the major components of the basal lamina whereas in the lamina lucida the laminin molecules are interconnected with their cellular receptor integrins (see p. 101).

The basal lamina is very important in the orientation and arrangement of the cells. E.g. lesion of the cornea heals only with scar, if the basal lamina of its epithelium has been destroyed. Without its destruction, however, the erased epithelium re-grows without any scar.

Note: 1) The principal difference is between basement membrane and basal lamina is that the former one comprises both the basal lamina (of epithelial origin) and the reticular lamina (of connective tissue origin). Light microscopic visibility is only a consequence of the thickening due to the presence of the reticular fiber layer. Even its presence the visibility depend son the thickness and the staining (e.g. azan or PAS is more effective tan HE) On the other hand, basal lamina itself can be visualized for light microscopic investigations, e.g. by the immunohistochemical staining of its components (laminin, etc.).

2) Between epithelium and connective tissue there is a basement membrane whereas between two adjacent epithelia there is a 'composite' basal lamina by the fusion of their proper basal laminae but no reticular lamina.

3) The endomysium and endoneurium around muscle fibers and peripheral neurons/neural fibers, respectively actually types of the basement membrane. The zona pellucida (ovarium) and the lens capsule (eye) are special basal laminae.

Cell junctions (pp. 67-70, Lecture 10)

Note: The junctions can classify according to several points of view. JBH did not extend over of them, but confined to those detectable under electron microscope. Several other cell-to-cell or cell-to-ECM connections are formed by diffuse molecules remaining beyond the electron microscopic observability. Only few of them, however, are integrated into structures detectable with electron microscope. Most probably every junction interconnected with the cytoskeleton system

a) homophylic – heterophylic: between similar or different molecules. The homophylic ones prove the aggregation of similar cells into tissue, the heterophylic promote the connection between different cells, or tissues
b) Ca-dependent – independent
c) Weak-strong: the former helps the migration, the latter inhibits is, and promotes the formation of the final structures (e.g. adult-type tissues).

Chemically (the most frequent ones):

Immunoglobulin-like (the similarity between the immune system and the cell-junction system is not occasional!): diffuse, weak, homophylic, Ca-independent. They promote motion and early cell aggregation.

Cadherins: diffuse or aggregated, strong, homophylic, Ca-dependent. Promote the tissue formation and stabilization.

Selectins (selective lectins): diffuse, heterophylic, weak.

Integrins: diffuse or aggregated, heterophylic (cell-basal lamina), strong.

Dystroglycan: diffuse, heterophylic (cell-basal lamina), weak.

Morphologically

diffuse, (immunglobilins, selectins, etc.)

either diffuse or aggregated (cadherins and integrins, which can form zonula adherens, desmosome, focal contact, hemidesmosome, see below)

always aggregated into electron microscopically detectable junctional structure (zonula occludens, gap junctions)

Zonula occludens: further important components are the occludin, the ZO-protein, and the junctional adhesion protein. Note its function in the separation of the apical and basolateral cell membranes which have different compositions and functions.

Zonula and macula adherens: they have several common features, including their electron microscopic appearance. Both of them consist of cadherins (Ca-dependent adhering proteins), catenins, and anchored cytoskeleton elements. The transmembrane cadherins of adjacent cells contact each other in the intracellular slit, forming a dense lamina between. (Cadherins of the macula are called desmoglein, desmocollin). The intracellular peripheral protein catenins form a density in the inner side of the membrane (terminal web, or in the macula adherens: plaque, the plaque forming catenines are the desmoplakin, etc.).

Note: although one of them is called zonula whereas the other is a macula, the principal difference is not the shape (!) but the cytoskeleton elements accompanied to them. The macula adherens (frequently called desmosome) anchors intermediate filaments (i.e., in the epithelia, where it is commonly found, keratin filaments), whereas the zonula integrates actin. Actin-related cell connections may have other forms: fascia adherens, punctum adherens.

Note: The main function of the zonula adherens is to modify the shape of the epithelium. By the contracting the apical poles of the epithelial cells pits, invaginations can be formed. Flat epithelia can form grooves and tubes, e.g. the neural tube in the embryo.

Gap junction (nexus, macula communicans): there are a number of connexins, numbered according to their molecular weight, e.g. Cx46, etc. The type of the connexin is characteristic of the cell type. In general, the connexon is composed from one type of connexin (homomeric connexons), and connexons of same type (homologous connexons) form gap junctions. This way gap junctions are formed between similar cells, which helps the separation and connection of similar cells and forms loose junctions during development. Recently, however, heterologous gap junctions between different connexons of different cells have been discovered, and even heteromeric connexons (formed by different connexins) were found. These, however, form the minority. The pannexins are connexon-like structures which, however, have no counterpart but opens into the extracellular space.

Cell-connective tissue connections.
Epithelium and the extracellular matrix of connective tissue are interconnected by basement lamina. The major connection between the epithelium and its basal lamina is provided by integrins, which are transmembrane laminin-receptor proteins. On the contralateral surface the reticular lamina is connected to the ECM by collagen VII.

Integrins (p. 101) consist of two subunits (α, β) both of them have isoforms labeled by arab numbers. Therefore there are several combinations. The type of the integrin depends on the tissue and varies with the developmental and/or functional states. Simlarly, the three protein chain of laminins (α, β, γ) have isoforms, too, and combined into 15 types of laminins varied according to the tissues and functions. Beside epithelia, integrins occur on any cells contacting lamina.

When integrins are concentrated at a confined area and ’integrate’ intermediate filaments to their intracellular pole, hemidesmosomes (see p. 68) are formed. When the cytoskeleton elements are actin filaments, the structure is the so-called focal contacts. It is important in the looser, transitory cell contacts during movements.

Note: every cell-to-cell or cell-to-matrix contact means a stimulus to the cell like e.g. the humoral effects. The connecting molecules are receptors switch intracellular signaling pathways. Another important function of these receptors is the recognition e.g. in the immunoreactions or during the developmental cell migrations.

Another important factor which interconnects cells and basal lamina is the **dystrophin-dystroglycan complex**. Dystroglycan consists of two subunits (α, β). The β subunit is a transmembrane protein. The α subunit is a extracellular peripheral protein, which connects to laminin and other basal lamina components (perlecan, agrin, biglycan). The intracellular prt of β-subunit binds dystrophin (actually, one of its several types), and other proteins (dystrobrevin, syntrophin). These latter ones fix water-pore aquaporins, ion-channels and signal-receptor systems. Dystrophin also binds actin. The dystroglycan-dystrophin-actin system forms a subcortical actin system (membrane skeleton) which regulates the distributions of channels and receptors, grouping them e.g. the surface where the cell is in contact with laminin. The system is homologous to that found in the erythrocytes (spectrin, ankyrin), and important in muscles, synapses, blood-brain barrier. In the muscles it regulates the formation of the neuromuscular junctions.

Note: Fibronectin (p. 101) binds mainly fibers to cells. Integrin can bind fibronectin, too. In some tissues fibronectin-relative substances occur (chondronectin in cartilage, hyalonectin in the vitreous body of eye).

Extracellular matrix, ground substance (Lecture17)

Connective tissue = cells + ECM. ECM = fibers + ground substance.

From the Table 5-3. the role of the collagens I-V and VII is to be memorized, no more!

Degradation of ECM (to p. 98, top): not only inflammations, infections, but any re-arrangement, re-building, regeneration, cell movement, growing of nerves or vessels involves the removal of the former ECM.

Note: the GAGs (p. 100-101) usually don’t exist free. Except the hyaluronic acid, all the GAGs occur in the form of proteoglycans, arranged around a core protein (see Fig. 5-17). The main difference between glycoproteins and proteoglycans is the proportion of components (in the former ones the polysaccharide, in the latter one the protein is surpassing).

Not to memorize, just to get the chemical terms familiar (see also Table 5-5):
The ‘uronic acid’ refers to that the last H2COH of the monosaccharide is oxidized into COOH. Galactose, idose, mannose differ from the glycose by the steric position of one or two OH group. The amino-group (NH2) is positioned instead of a terminal OH in the glycosamin, etc., and can be acetylated (it is marked as N-Ac). Te ‘sulfate’ is actually not sulfate (this term refers to salts of the sulfuric acid), but sulfon-esters: an SO3 group is positioned instead of the H of an OH.

These groups help to connect other molecules like a giant 3D-filter (see gel-electrophoresis in chemistry). ECM frequently store snd supply the cells with distributed growth-factors and similar molecules.

The nucleus (Lecture 5)

Karyoplasm (nucleoplasm) comprises the components of the nucleus.

The nuclear pore has a more sophisticated structure than shown in Figs. 3-4, and 3-7. Pictures are found in the internet by typing 'nuclear pore'. However, it is enough that it contains an inner ring (pore for large molecules or aggregates: ribosomes), and an outer ring, between them pores for smaller molecules; the nuclear pore separates the outer and inner laminae of the nuclear envelop (like tight junction separates the apical and basolateral cell membranes)

Karyoskeleton (nucleoskeleton) comprises the skeletal elements within the nucleus.

Karyosol (nucleosol) comprises the fluid material surrounding the chromatin and skeleton.

The two latter ones form the interchromatic material. The main karyoskeleton components are the **lamins**: types A, B, C. A and C bind chromatin (see its territorial arrangement), whereas B binds them to the nuclear membrane.

Chromatin: it comprises DNA and its associated proteins). **Chromatid:** one DNA-chain and its associated proteins. Chromosome consists of densely packed 'transport' form of chromatin, with characteristic shape. However, in functional sense, the term is used for the intermitotic phase, too (see territorial arrangement below).

Euchromatin: the functioning part of chromatin, easy to process (e.g. transcription).

Heterochromatin: the inactive part, two components are to be distinguished: the constitutive heterochromatin, which contains DNS never transcripted (e. g. 'phylogenetic heritage'), and the facultative heterochromatin which is sometimes unfolded into euchromatin and readible according to the functional demands. The constitutive heterochromatin attaches in clumps to the nuclear membrane (correctly: the lamins) and visualizes its contour (otherwise the membrane is too thin to be detected with light microscope.

Territorial arrangement: the chromatin belonging to a chromosome is spread in its proper area. This arrangement is stabilized by anchoring lamins and other skeletal elements.

The parts of the chromosomes**:** The **centromer** is its thinnest part. The k**inetochor** is the part of chromosome, which the mitotic tubule attaches to. The **telomere** regulates mitoses. The **nucleolus organizer** (not in every chromosome!) organizes nucleolus after mitosis.

Pars amorpha: In the nucleolus it is a third region beside the partes fibrosa and granulosa. (see Figs. 3-2. and 3-13.). It contains the rRNA-coding DNA.

Note: the observed compactness of nucleus depends on the thickness of section. The thinner the section is the less compact the nucleus appears. On contrary, the nucleolus dense in sections appears pale in smears (e.g. in the bone marrow).

Mitosis (Lecture 24)

The main purpose of the lecture is to introduce some terms and phenomena necessary to understand the embryologic development. Therefore this chapter of this Commentars is will be completed with another chapter 'Developmental biology'

Note: in the mitosis some descriptions (see e.g. ECB) mention not four but six phases. The events in „late in prophase" (p. 52, right column, second paragraph) are covered by the term 'prometaphase', and the final separation of the cytoplasm belongs to the 'telokinesis' or 'cytokinesis' The nucleoles are dissolved and reorganized in every mitosis.

The centriolum consists of two nine-tubular units. They separate during mitosis, organize the movement of chromosomes (see p. Fig. 3-14) and replicate their pairs after. No proof of the role of DNA in this replication has been found.

The mitotic microtubules can be polar tubules (between the centrioles) or kinetochor tubules (between a centriolum and a kinetochor of a chromosome).

The stem cells: the criterium is that in every divison one of the daughter cells is identical with the mother cell (the other differentiates further as progenitor cell). Therefore the stem cell is renewed and its mitoses are not limited. There are totipotent cells (all the cell types of the organism can develope from them), multipotent cells (several but not any cell types can develope), pluripotent (for few cell types), bi- and even unipotent (e.g. spermatogonium). Not any stem cell is toti- or multipotent!)

The progenitor (transitory amplifier) cells: they divide into two identical daughter cells, but without self-renewing. They have already limited capability of differentiation and division.

Hayflick limit: the cells usually can undergo 'only' 50 mitoses if explanted into cultures (if they divide at all, i.e. can return from G-zero phase to G-one).Probably the number of mitoses is limited by the telomeres (see below).

Not too narrow limit is the above-mentioned 50 division! It means 2^{50} i.e. $1.3x10^{15}$. Let's calculate: 1 microliter blood contains $5.5x10^6$ red blood cells. A man has 5.5 liters blood, i.e. $5.5x10^6$ microliters, what means $3x10^{13}$ cells. Since the red blood cell's lifespan is 120 days, and a man's is 8o years, it means 240 cell generations. Multiplying: $240x3x10^{13} = 7.2x10^{15}$, therefore 6 cells (not stem cells, but only progenitors) would be enough to supply a man with red blood cells for his life! Of course, it is only an example, not a real description of the development of red blood cells.

Telomer is an end-piece of chromosomes, a DNA-segment, which shortens during the subsequent mitoses, and stops mitoses when has shortened to a point. In some cells (following fertilization, in stem cells, in some tumor cells) there is an enzyme, telomerase, which can repair it. Otherwise the non.-dividing cells die. If **telomerase** reconstruct the telomer following the mitosis, the mitotic capability is not limited **(so-called immortalized cells in cultures, stem cells, cells of malignus tumors).** Cell lines which have become free from the mitosis-limiting factors are the **immortalized cell lines (see also p. 10)**.

The paragraph in italics on p. 56 is to be read! At least the definitions of proto-oncogens and oncogens are to be memorized. Try to understand the different 'biological reasons' and mechanisms of tumor-suppressors, proto-oncogens and the telomere-system.

Environmental influences on mitosis: To divide, most cell types have to attach to the surface, with 'focal contacts' to laminin or fibronectin. It means a mitotic signal for the cell. On contrary, most cell types stop mitoses when they have formed a continuous layer, and attach each other ('contact inhibition'). Humoral agents, mainly growth factors are also necessary. Agents promoting cell differentiation usually inhibit mitoses, and *vice versa*.

Amitotic division. Formerly supposed that division can occur without formation of chromosomes and threads (greek: mitos, e.g. the mitotic microtubules). According to the present opinion it results only in a duplication of the nucleus (mainly in the heart, liver, urothelium), without a true cell division.

Plasmodium, syncytium. These terms refer to multinuclear cells, but of different origins. The plasmodium is formed by repeated nuclear divisons without a division of cytoplasm. A typical example is the pathogenic agent of malaria. The syncytium is resulted of the fusion of cells, e.g. the striated muscle (formerly was held as a plasmodium!), the osteo-, chondro-., odontoclasts (this latter degragates the milk-teeth), and the syncytiotrophoblast of the placenta. The megakaryocyte, however, is not multinuclear cell, only polyploidy with a giant nucleus, which seems to be 'multiplex' due to sectioning.

Meiosis

The details belong to the 2nd semester (Anatomy II), to the histology of gonads. In the first semester it is enough that it produces the gametes, it consists of two subsequent mitoses without a DNA replication between, and therefore it reduces the genom from diploid to haploid in the mature gametes.

The prophasis of the first meiotic division consists of 5 sub-phases.

- Leptoten – the chromosomes are visible, and attach to the nuclear envelope by their both ends;
- Zygoten – the homologous chromosomes attach into pairs (synapsis);
- Pachyten – the chromosomes shorten and thicken. It is the period of crossing-over;
- Diploten – the four chromatids are visible, they are attached only at the crossing-overs (this part is the 'chiasma');
- Diakinesis – the pairs separate.

During meiosis (but also even in mitosis) **the chromosomes may be damaged**.
Deletion – a piece has broken off,
Inversion – the broken piece has fit again but in the opposite position.
Translocation - the broken piece has transposed into another chromosome.
They are called **chromosomal mutations**. The '**point mutation'** concerns a small part, usually one nucleotide, and is resulted by some mutagen agent, the **genom-mutation** changes the assembly of chromosomes (number decreases or increases). **Aneuploidia** means that there is/are chromosome/s without pair, it is resulted of the non-dysjunction, i.e. a pair of chromosomes remains together. **Polyploidia** occurs if there are several series of chromosomes, it is regular in megakaryocytes, resulted of DNA-replications, without nuclear mitosis. When during development mutation occurs in a somatic cell (**somatic mutation**) the daughter cells have different genom than the other somatic cells of the body. It is called **genetic mosaicism,** a common example is the different colors of the eyes.