МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ РЕСПУБЛИКИ БЕЛАРУСЬ

БЕЛОРУССКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ

КАФЕДРА БИОЛОГИИ

В. Э. Бутвиловский, В. В. Григорович, В. В. Давыдов

МЕДИЦИНСКАЯ БИОЛОГИЯ И ОБЩАЯ ГЕНЕТИКА

**для иностранных студентов, обучающихся
по специальности «Стоматология»**

MEDICAL BIOLOGY AND GENERAL GENETICS

**for foreign students
studying in the specialty «Dentistry»**

Курс лекций

Минск БГМУ 2019

Учебное издание

Бутвиловский Валерий Эдуардович
Григорович Виктор Васильевич
Давыдов Владимир Витольдович

МЕДИЦИНСКАЯ БИОЛОГИЯ И ОБЩАЯ ГЕНЕТИКА
для иностранных студентов, обучающихся по специальности «Стоматология»

MEDICAL BIOLOGY AND GENERAL GENETICS
for foreign students studying in the specialty «Dentistry»

Курс лекций
На английском языке

Ответственная за выпуск Е. В. Чаплинская
Переводчик Т. Ф. Данилова
Компьютерная верстка А. В. Янушкевич

Lecture 1. THE FLOW OF SUBSTANCE AND ENERGY THROUGH THE CELL

Outline:
1. Overview of prokaryotes and eukaryotes.
2. The structure of plasma membrane.
3. Transport across the membrane.
4. Organelles of the cell. Anabolic and catabolic systems of the cell.
5. Energy exchange in cells.

1. Overview of prokaryotes and eukaryotes

Basic structure of cells. Any living cell is covered with plasma membrane. This membrane covers the cell's content which is called cytoplasm. Cytoplasm includes viscous solution cytosol and other cell components such as organelles. Cells have DNA which can be located within the nucleus or in the cytoplasm.

Based on structural organization, there are two major kinds of cells: prokaryotes and eukaryotes.

Prokaryotic cell is a cell lacking membrane-bound organelles and a membrane-enclosed nucleus.

- Prokaryotes include archaea and bacteria.
- They are generally unicellular and their cells do not form tissues.
- Prokaryotic cells are commonly much smaller than eukaryotic cells.
- The DNA of prokaryotes is in the cytoplasm of the cell as a membrane-bound nucleus is absent.
- Commonly haploid — contain only one «chromosome». Genomes are generally smaller than those of eukaryotes.
- Membrane-bound organelles are absent.
- Almost all have cell walls.

Eukaryotic cell is a cell with a membrane-enclosed nucleus and membrane-enclosed organelles.

- Examples: protists, fungi, plants, animals.
- Internal membranes subdivide eukaryotic cells into different functional compartments which are called *membrane-bound organelles*.
- DNA is organized with proteins into several or many chromosomes that are located within the nucleus.
- Eukaryotes can be haploid or diploid.
- Some cells have a cell wall outside the plasma membrane. Animal cells lack cell walls.

Though structurally different, eukaryotic and prokaryotic cells have many similarities, especially in their chemical processes.

Origin of eukaryotes. The most popular theory states that membrane-bound cell compartments originated from ingrowings of plasma membrane. Many evi-

dences suggest that mitochondria arose from engulfed aerobic prokaryotes which lost their self-sufficiency. Among those evidences are:

– Mitochondria possess own DNA and ribosomes.

– The mitochondrial ribosomes, proteins and genes have similarities with those of bacteria.

In 2010 sediments from the rift valley in the Arctic Ocean (near Loki's Castle hydrothermal vent), were analyzed. The use of a special technique led to the discovery of the DNA of new archaea. The analysis shown that the new archaea must have some traits typical only for eukaryotes, e. g. components of cytoskeleton, or proteins participating in vesicular transport. This group of new archaea might be the evolutional link between eukaryotes and prokaryotes.

2. THE STRUCTURE OF PLASMA MEMBRANE

Lipid bilayer. Lipid nature of membrane was supposed by *Quincke*. He noted that cells generally form a spherical shape in water and the cell broken in half, forms two smaller spheres. The only known material with such behavior was oil.

Irving Langmuir proposed the idea that a drop of oil-like substance should stop sprawling on the surface of water when reaches the thickness of 1 molecule.

In 1925, *Gorter* and *Grendel*, discovered that the surface area of red blood cells is ½ of the surface area formed by all their membrane lipids. Conclusion: cells are covered by double layer of lipids (lipid bilayer).

Lipids of the membrane arrange themselves into bilayer due to their properties.

• A molecule of a *phospholipid* — the basic lipid of any cell's membrane — consists of *polar* (or *hydrophilic*) *head* and *non-polar* (or *hydrophobic*) *tails*.

• Water molecules are polar and easily dissolve other polar molecules (remember that water is basic component of cell's inside and cell's surroundings).

• Non-polar molecules do not easily dissolve in water.

• As phospholipids consist of two regions with different properties, the *random motion of molecules* in a solution ultimately makes them arrange into phospholipid bilayers which form vesicles.

• The position in a phospholipid bilayer «hides» the non-polar tails inside the bilayer and exposes the polar heads to surrounding water.

• Covalent bonds between lipids are absent as they are not required.

Studies of the membrane's chemical composition shown that, apart from phospholipids, it contains sphingolipids and cholesterol. Other components of the membrane are proteins.

In 1935, Davson and Danielli, proposed *sandwich model* of the plasma membrane: lipid bilayer is locked between two layers of proteins. This model explained some properties of the membrane such as surface tension of lipid bilayers, but did not match to many other properties such as permeability for certain substances.

In 1972, Singer and Nicolson, proposed the *fluid mosaic model* of the membrane: proteins are individually embedded into lipid bilayer. Hydrophilic portions

of proteins are maximally exposed to water while the hydrophobic portions of proteins are in the nonaqueous environment inside the bilayer. Hence, proteins float in a «lipid sea». This model of the membrane is illustrated in the fig. 1.
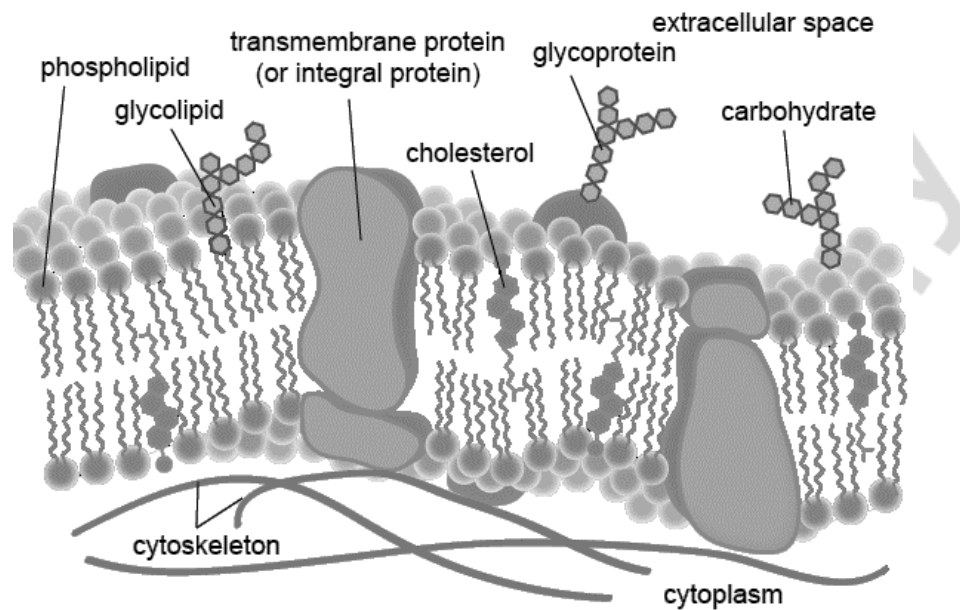

Fig. 1. The fluid mosaic model of plasma membrane

The membrane proteins. The proteins in the membrane vary in both structure and function. They occur in two spatial arrangements:

– *Integral proteins* are generally transmembrane protein with hydrophobic regions that completely span the hydrophobic interior of the membrane. *Integral bitopic* and *polytopic* proteins penetrate phospholipid bilayer one or several times respectively. *Integral monotopic* proteins penetrate only one layer of lipids.

– *Peripheral proteins* are not embedded into lipids but attached to the membrane's surface.

The most common functions of membrane proteins:

– *Transport proteins* pump molecules across the membrane.

– *Receptor proteins* interact with signal molecules and cause the cell to respond.

– *Membrane enzymes* catalyze particular chemical reactions.

– *Cell adhesion molecules* bind with the extracellular matrix or with other cells.

Membrane carbohydrates and cell-cell recognition. *Cell-cell recognition* is the ability of a cell to determine if other cells it encounters are alike or different from itself. Cells recognize other cells by keying on cell markers found on the external surface of the plasma membrane. Such cell markers are membrane carbohydrates. The carbohydrates covalently bonded to lipids are called *glycolipids*, those covalently bonded to proteins are *glycoproteins*.

3. THE TRANSPORT ACROSS THE MEMBRANE

Active and passive transport. The plasma membrane is selectively permeable. The *selective permeability* is the property of biological membranes which allows some substances to cross more easily than others. This allows to regulate the type and rate of molecular traffic into and out of the cell.

Due to selective permeability the concentration of particular substance on the different sides of the membrane can be different. Such difference in substance concentration is called *concentration gradient*.

• If molecules of a substance move from the area of their high concentration to the area of their low concentration (i. e. they move *down the gradient*), the concentration gradient decreases. This occurs if molecules just passively move in random directions in a solution.

• If molecules of a substance move from the area of their low concentration to the area of their high concentration (i. e. they move *against the gradient*), the concentration gradient increases. This is possible only if cell's proteins actively pump these molecules.

Based on these mechanisms, the transport across the membrane can be *passive* or *active*.

Types of passive transport. An example of passive transport is diffusion. *Diffusion* is the passive transport of solutes across the membrane down their concentration gradient. Cell does not need to use its energy for such transport.

• *Simple diffusion* occurs when molecules pass directly through the lipid bilayer by themselves (between lipid molecules). This is possible for lipid-soluble and small nonpolar molecules such as hydrocarbons, O_2, CO_2. This is also possible for small, polar uncharged molecules such as H_2O or ethanol.

• All ions (e. g., Na^+, H^+), larger, polar uncharged molecules (e.g., glucose) will not easily pass through membranes. The diffusion of such molecules across the membrane may occur through transport proteins. Such passive transport is called *facilitated diffusion*.

Another example of passive transport is osmosis.

• *Osmosis* is diffusion of water across a selectively permeable membrane. If two solutions of different concentrations are separated by a selectively permeable membrane that is permeable to water but not to solute, water will diffuse from the solution with the lower solute concentration to the solution with the higher solute concentration.

• If a solution has an equal solute concentration compared to that inside a cell, it is called *isotonic solution.*

• *Hypertonic solution* is the solution with greater solute concentration than that inside a cell. The cells placed in such solution passively lose their water.

• *Hypotonic solution* is a solution with a lower solute concentration compared to that inside a cell. Cells placed in such solution passively uptake water from the outside.

Types of active transport. *Active transport* occurs when transport proteins in a membrane pump a solute through the membrane against its concentration gradient. This process requires source of energy (common energy source in the cell — molecules of ATP). Various transport proteins may pump particular solutes differently:

‒ *Uniport* is the transport of one type of molecule in particular direction.

‒ *Symport* is the transport of two different kinds of molecules or ions together in same direction by a transport protein.

‒ *Antiport* is the transport of two different kinds of molecules or ions in opposite directions by the same a transport protein.

‒ Example of antiport is *sodium-potassium pump*. This transport protein uses ATP to pump 3 Na^+ ions to the outside and take 2 K^+ ions into the cell.

‒ This is associated with normal physiology of many cells (e. g. human cells): Na^+ on the outside must be much more concentrated than in the cytoplasm, while the K^+ ions must be more concentrated inside the cell than on the outside.

Endocytosis and exocytosis. *Exocytosis* is the process of exporting macromolecules from a cell by fusion of vesicles with the plasma membrane.

• Vesicles usually bud from the ER or Golgi apparatus (they are covered with lipid bilayers) and merge with plasma membrane.

• This is how many cell products can be secreted from the cell to the cell's environment.

Endocytosis is the process of importing macromolecules into a cell by forming vesicles derived from the plasma membrane.

• Vesicle forms from a localized region of plasma membrane that sinks inward and pinches off into the cytoplasm.

• The endocytosis of solid particles or other cells is called *phagocytosis*; the endocytosis of liquids is called *pinocytosis*.

• The produced vesicle is called phagosome.

• Phagosomes may fuse with lysosomes (organelles containing enzymes for breakdown of various macromolecules, e. g. proteins). The vesicle formed from a phagosome and lysosome is called phagolysosome. The substances engulfed by endocytosis are digested there.

4. ORGANELLES OF THE CELL. ANABOLIC AND CATABOLIC SYSTEMS OF THE CELL

Organelles of the cell. The cytoplasm of the cell contains various structures performing their own specific functions. Such structures are organelles. The structure of animal cell with its organelles is illustrated in the fig. 2.

Ribosomes. Ribosomes are organelles that synthesize cell's proteins. (Remember that proteins are basic molecules of life. They are «workers» of the cell, and each protein does its own of jobs.)

• Ribosomes are much smaller than other organelles.

• They consist of two subunits (large and small ones).

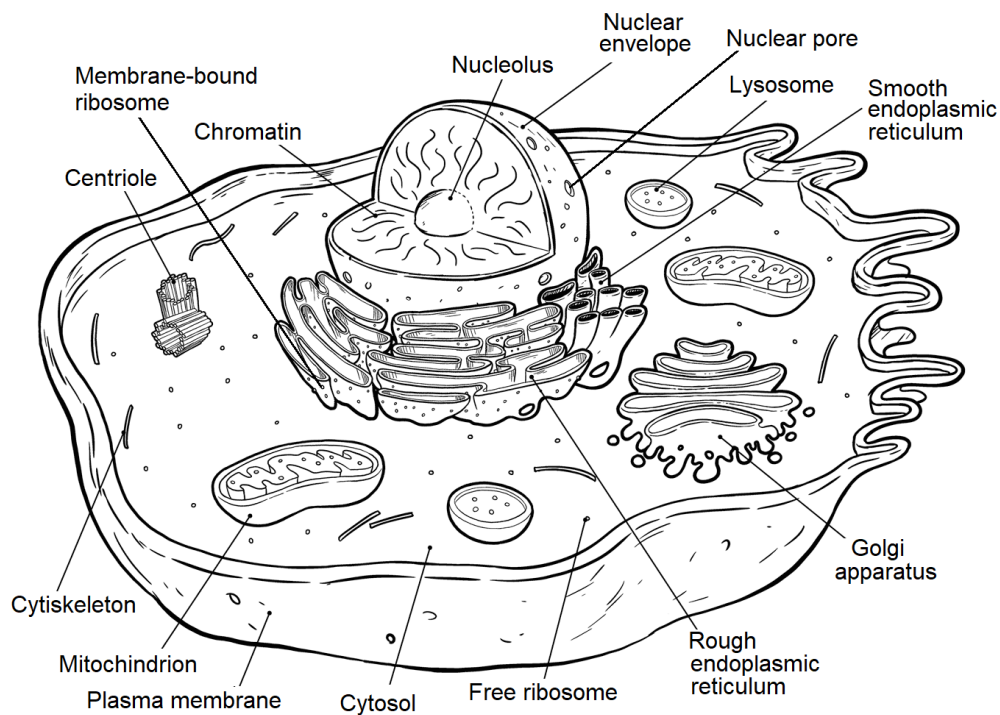• Both subunits consist of RNA and protein and have no membranes.

Fig. 2. The structure of animal cell

- They are produced in the nucleolus in eukaryotic cells.
- Most ribosomes are bound with the membranes of the rough endoplasmic reticulum and outer membrane of the nucleus (*bound ribosomes*) when work. Bound ribosomes generally make proteins that are destined for membrane inclusion or export.
- Some ribosomes are not bound to the ER (*free ribosomes*). Most proteins made by free ribosomes will function in the cytosol.

Endoplasmic reticulum. Endoplasmic reticulum (ER) is membranous network of tubules and sacs (cisternae) in the cytoplasm. These membranes separate *internal lumen* (cisternal space) from the rest of cytosol. ER is connected to the outer membrane of the nucleus.

There are two distinct regions of ER that differ in structure and function: smooth ER and rough ER.

1. Smooth ER:
– Lacks ribosomes.
– Participates in the synthesis of lipids including phospholipids and steroids.
– Participates in carbohydrate metabolism (e. g. in liver cells it contains an embedded enzyme that participates in conversion of glycogen to glucose).
– Detoxifies poisons and drugs (especially in the liver cells) by making them soluble.
– In a muscle cell it stores Ca^{2+} ions necessary for muscle contraction.
2. Rough ER:
– Looks rough under an electron microscope due to ribosomes it contains.

8

– Connected with outer membrane of the nucleus.

– Processes secretory proteins: ribosomes attached to rough ER synthesize proteins immediately transporting their growing amino acid chain to the ER where protein folds into its normal 3-dimentional structure. Enzymes of the ER mark the protein with carbohydrate chains. Then the protein departs to Golgi apparatus in a transport vesicle pinched off from ER.

Golgi apparatus. Golgi apparatus is an organelle made of stacked, flattened membranous sacs (cisternae). It modifies, stores and routes products delivered in vesicles from the endoplasmic reticulum.

• The side of Golgi apparatus, which receives vesicles with the products from the ER is called *cis face*.

• The side from which vesicles containing processed products pinch off to be transported is called *trans face*.

• Each cisterna between the cis and trans face contains unique combinations of enzymes. Those enzymes modify products of the ER as they move through the stack of cisternae from the cis to the trans face.

• Those modifications include alternation of some membrane phospholipids, changes in carbohydrates, addition of other chemical groups.

• The Golgi apparatus sorts products for secretion in vesicles from the trans face.

• The vesicles budding from the Golgi apparatus can secrete their contents to the cell's environment via exocytosis.

Lysosomes. Lysosomes are the vesicle-like organelles which contain hydrolytic enzymes that digest all major classes of macromolecules e. g. lipids, carbohydrates, proteins, nucleic acids.

• Lysosomes bud from the Golgi apparatus.

• Their inner pH is acidic.

• Lysosomes perform intracellular digestion of products contained in phagosomes when fuse with them as described above. This process is called *heterophagy*. This mechanism is used for feeding of many single-cell organisms (e. g. amoeba) and for the destruction of pathogens such as bacteria invading human body in specialized cells of the immune system.

• Lysosomes may engulf damaged or worn out organelles or part of the cytosol and digest them. This process is called *autophagy*.

• Lysosomes participate in programmed cell self-destruction which is called *autolysis*.

Peroxisomes. Peroxisomes are small membrane-enclosed vesicles 0.3–1.5 μm in diameter.

• They are created by the ER.

• Often contain crystalized core.

• Contain enzymes that transfer hydrogen from various substrates to oxygen, producing hydrogen peroxide.

• Functions of peroxisomes include breakdown of fatty acids, detoxification of H_2O_2 by the enzyme catalase.

Mitochondria. Mitochondria are the organelles which perform final steps in breakdown of organic macromolecules into CO_2 and H_2O in order to produce ATP. This conversion requires oxygen. Mitochondria are present in nearly all eukaryotic cells.

• Mitochondria are enclosed by two membranes (the inner and outer ones). The inner membrane has multiple folds called *cristae*.

• The space between the membranes is called intermembrane space. When work, mitochondria pump protons to this space.

• The inner membrane contains embedded enzymes that pump protons and are involved in synthesis of ATP.

• The compartment enclosed by the inner mitochondrial membrane is called mitochondrial matrix. It contains enzymes that help to break organic molecules and collect protons and electrons.

Cytoskeleton. Cytoskeleton is the network of protein filaments and microtubules in the cytoplasm that controls cell shape, maintains intracellular organization, and is involved in cell movement.

Actin microfilaments are involved in cellular contraction, maintaining the cell's shape and in basic cell movements. Their diameter is about 7 nanometers.

Microtubules are hollow cylinders serving as «railways» for special transport proteins. Microtubules are composed of tubulin and are about 25 nm in diameter. Membrane vesicles in the cell are transported along microtubules. Microtubules participate in mitosis (a type of cell division) when pull chromosomes to different sides of dividing cell.

Intermediate filaments include broad class of fibrous proteins ranging in size from 8 to 12 nanometers. The intermediate filaments function as tension-bearing elements to help maintain cell shape and rigidity, and serve to anchor in place several organelles.

Anabolic and catabolic systems of the cell. All the chemical reactions occurring in the body or in a cell are referred to as *metabolism*. It includes anabolic and catabolic reactions.

• *Anabolism* (*assimilation*) is the synthesis of complex molecules from simpler ones. Such reactions commonly consume energy.

• Example: synthesis of glucose by plants as result of photosynthesis.

• Relatively simple molecules (CO_2 and H_2O) are used to build a complex molecule: $6CO_2 + 6H_2O + ENERGY \rightarrow C_6H_{12}O_6 + 6O_2$.

• *Catabolism* (*dissimilation*) includes metabolic pathways performing breakdown of complex compounds into simpler molecules with energy release.

• Example: breakdown of glucose in the cytosol and mitochondria.

• Glucose is broken down into simpler molecules (CO_2 and H_2O): $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + ENERGY$.

5. ENERGY EXCHANGE IN CELLS

Extracting the energy of chemical bonds in animals. As all other animals, human receives energy from the chemical bonds of food molecules. The basic cell's molecule storing chemical energy for various reaction is ATP (adenosine triphosphate). The of ATP production be subdivided into 3 steps.

The first step in the extraction of chemical energy is **digestion** of food in the canal of the digestive system and, partially, in the cells of the digestive tract. This is considered as the *first, preparatory stage of energy exchange.*

- Polysaccharides are broken into monosaccharides,
- Proteins are broken into amino acids,
- Fats are broken into glycerol and fatty acids,
- Nucleic acids are broken into nucleotides and then into smaller molecules.
- The energy released from these chemical reactions is not captured by the cell and dissipates as warmth.

In this lecture, further ATP production is described on the example of glucose breakdown.

The second, anaerobic stage, occurs in the cytoplasm of cells. It is **glycolysis**: a chain of ten reactions in which:

‒ Glucose is broken down into 2 molecules of *pyruvic acid* (they are then used by mitochondria);

‒ 2 *ATP* are created;

‒ 2 NAD are reduced into *2NADH* which is a carrier of electrons. The electrons (and protons) can be used by mitochondria to create more ATP.

The third, aerobic stage is breakdown of pyruvate created during glycolysis into CO_2 and H_2O. This allows to create much more ATP than glycolysis.

The aim of this stage is to pump protons in the intermembrane space and then use their flow down the electrochemical gradient to synthesize ATP:

1. Pyruvic acids are transported into the matrix of mitochondria where it is broken down and the products interact with *coenzyme A* (CoA). The result of this step is conversion of pyruvic acid into *acetyl CoA* and other products. These products include NADH which is used in the next steps.

2. The acetyl CoA participates in *citric acid cycle*, where it is broken into CO_2; more NAD are reduced into NADH.

3. The e^- (electrons) collected during previous reactions (they were collected to the NADH) are delivered to the system of *cellular respiration*. It is a complex of enzymes situated in the inner mitochondrial membrane. Electrons run through this system to one of the most electronegative matter — to oxygen. This process is associated with release of energy that is captured and used for pumping protons into intermembrane space (like the energy of a ball rolling downhill can be used).

When critical concentration of protons in the intermembrane space is reached, protons start to run from the intermembrane space to the matrix through the only way they have — through the enzyme complex *ATP-synthase*. The energy of this flow is used to create ATP (like the energy of water is used in a water-

mill). The protons join the oxygen which received electrons from cellular respiration, i. e. water is formed.

All the reactions of energy exchange in the cells of brain and skeletal muscles result in production of 30 mol of ATP from 1 mol of glucose. Cells of cardiac muscle, liver, kidneys and other organs form 32 mol of ATP from 1 mol of glucose.
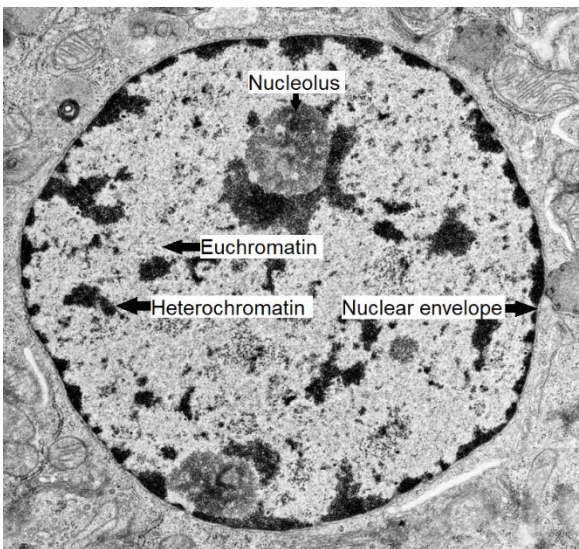
Lecture 2. ARRANGEMENT OF GENETIC MATERIAL

Outline:
1. The structure of nucleus. Chromatin.
2. Chromosomes.
3. Cell cycle. Mitosis and meiosis.
4. The structure and functions of nucleic acids.
5. The mechanism of replication.
6. Protein synthesis.

1. THE STRUCTURE OF NUCLEUS. CHROMATIN AND CHROMOSOMES

Nucleus. Nucleus is a membrane-enclosed compartment of eukaryotic cell where genetic information stored. Its structure is illustrated in the fig. 3.

- It is enclosed by nuclear envelope. The *nuclear envelope* is composed of two membranes. Each membrane is a lipid bilayer which has its own specific proteins

- The gap of about 20 to 40 nm between the membranes is called *intermembrane* (or perinuclear) *space*.

- The outer membrane of the nucleus proceeds into the membranes of the rough endoplasmic reticulum. It also carries bound ribosomes on the outer surface.

- The exchange of materials between the nucleus and cytoplasm occurs through pores. Nuclear pores are a protein-lined channels in the nuclear envelope. The envelope's inner and outer membranes are fused at the lip of each pore.

- The complex of proteins in a nuclear pore is called *nuclear pore complex*. Such complexes regulate the transport through pores into and out of the nucleus.
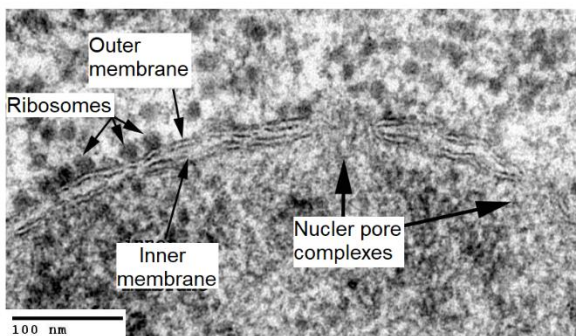

Fig. 3. The structure of the nucleus

12

• There is a network of protein filaments attached to proteins of the inner membrane inside the nucleus. This protein network is called *nuclear lamina*. The lamina stabilizes nuclear shape.

• There is a network of fibers found throughout the inside of the nucleus. It is called the *nuclear matrix*. Its function might be comparable with that of cytoskeleton, though it must aid in organization of chromatin and participates in expression of genes.

Chromatin. Chromatin is a complex of DNA and proteins. Chromatin makes up most of the content inside the nucleus.

• Chromatin is also material of chromosomes.

• In nondividing cells chromatin appears as a mass of loose material filling the nucleus. Separate chromosomes are undistinguishable under the microscope.

• Chromosomes, commonly familiar as X-shaped structures, appear only in dividing cell when chromatin condenses and becomes tightly packaged. After division, chromosomes lose their clear outlines as their chromatin becomes loose and less tightly packaged. Separate chromosomes become undistinguishable again.

• Although chromatin in the nucleus looks like solid mass, it was demonstrated that every chromosome in the nucleus has own chromosome territory. These territories can be separated by interchromatin domains.

• The chromatin regions in the nucleus may have different density. The more condensed chromatin is called *heterochromatin*, the chromatin with lower density is *euchromatin*.

• Under the microscope, the heterochromatin appears as darker clumps in the lighter areas of the nucleus. The inner regions of such clumps contain mostly inactive chromatin the genes of which are not expressed.

Nucleolus. Nucleolus is a roughly spherical region in the nucleus of nondividing cells where ribosomes are created.

• It consists of nucleolar organizers and ribosomes in various stages of production.

• Nucleolar organizers are the specialized regions of some chromosomes, with multiple copies of genes for rRNA (ribosomal RNA) synthesis.

• Ribosomal subunits are assembled from rRNA transcribed in the nucleolus and ribosomal proteins produced in the cytoplasm and imported in the nucleus.

• Ribosomal subunits then pass through nuclear pores to the cytoplasm, where their protein synthesis occurs.

2. CHROMOSOMES

The structure of chromosomes. As noted above, the familiar usually X shaped chromosomes appear only in dividing eukaryotic cell, and when the cell is not dividing, chromosomes exist as regions of nuclear chromatin the borders between which are not visually distinguishable.

• In the beginning of cell division, each chromosome consists of two *sister chromatids*.

- The sister chromatids contain per one copy of the same DNA (because cell doubles its DNA before division).

- The sister chromatids will be separated and delivered to different daughter cells during division. Hence, both daughter cells will have same DNA.

- The sister chromatids are linked together in the region which is called *centromere* (or *primary constriction*).

- The centromere is bound with a protein complex kinetochore. This complex connects with microtubules (microtubules pull the chromatids apart during division). Hence, the centromere is the region where microtubules link to the chromosome.

- The chromosome regions on the sides of centromere are called arms. Each sister chromatid has 2 arms, so a chromosome in the beginning of division must have 4 arms.

- The arms of a chromosome may have different length. Hence the arms are called *short* (or *p arm*) and *long* (or *q arm*). Based on the length, chromosomes can be of 3 basic types:

  – *Acrocentric chromosomes* are those in which p arm is very short compared to the q arm.

  – *Submetacentric chromosomes* are those in which the length of arms is slightly unequal.

  – *Metacentric chromosomes* have q and p arms of almost same length.

- Terminal regions of arms are called *telomeres*. They contain specific DNA nucleotide sequence that repeats many times (in vertebrates, including human it is TTAGGG).

- DNA of telomeres forms loops which hide the ends of DNA.

- This prevents misrecognition of DNA ends by enzymes as fragments of damaged DNA.

- Telomeres of DNA shorten after every copying of DNA. So, another function of telomeres might be protection of other DNA regions from shortening.

- Arms of some chromosomes may have narrow region which is called secondary constriction. Some parts of these constrictions indicate sites of nucleolus formation — nucleolar organizing regions.

Functions of chromosomes. The main function of chromosomes is storage of genetic information and its equal distribution to daughter cells when cell divides.

- All species have their unique genetic information (unique genes — instructions for creation of proteins) which is contained in molecules of DNA.

- For example, all genes of human are contained in 23 DNA (in other species it is different).

- Each of those DNA is unique, has own length and set of genes.

- Each of those DNA is contained in its own chromosome.

- Humans have double set of all genes, i. e. 23 chromosomes + 23 same chromosomes. Cells with such double chromosome set are called *diploid* and denoted as *2n*.
- Hence, every chromosome has a pair — another chromosome with same genes. Such chromosomes are *homologs* (or *homologous chromosomes*).
- Homologs have same genes, though even same genes in different chromosomes might be slightly different (i. e. the instructions for creation of the same sort of protein in different chromosomes may be slightly different).
- Every cell of the body has full set of chromosomes.
- All individuals of the same species have same chromosomes. The only exception is the last pair of chromosomes in many animals (including humans): women have two X chromosomes while men have one X and one Y chromosomes.

The Denver system of chromosome classification. The Denver system of chromosome classification, established in 1959, identified the human chromosomes by their length and the position of the centromeres (tabl. 1).

Table 1

The Denver system of chromosome classification

Chromosomes	Group
#1–3	A
#4–5	B
#6–12 and X	C
#13–15	D
#16–18	E
#19–20	F
#21–22 and Y	G

The Paris nomenclature of chromosomes. In 1968, it was demonstrated that stained chromosomes showed a distinct staining. Each chromosome exhibits unique banding pattern and could be identified this pattern.

- There are various methods of chromosomes staining (Q-banding, G-banding, R-banding).
- In 1971, at the Paris Conference, a new system for the classification of chromosomes, based on Q, G, and R banding patterns, was introduced to identify individual chromosomes and chromosome regions.
- This was followed in 1978 by a new document entitled «An International System for Human Cytogenetic Nomenclature».
- Chromosome banding also allows to indicate gene location. For example, the location of the gene of the enzyme phenylalanine hydroxylase (PAH) is 12q23.2. This means that the gene is situated in:
  - 12th chromosome;
  - q (long) arm;
  - region 2;
  - band 3;
  - sub-band 2.

3. CELL CYCLE. MITOSIS AND MEIOSIS

Cell cycle. Cell cycle (mitotic cell cycle) is the complete sequence of cellular events from one cell division to the next.

• Cell cycle consists of four successive phases: G_1 *phase* → *S phase* → G_2 *phase* → *M phase*. They are illustrated in the fig. 4.

Fig. 4. Cell cycle

• The G_1, S, and G_2 phases are together referred to as *interphase*. During interphase cell prepares itself for division.

• Division occurs at the M phase.

• The M phase in mother cell is followed by G_1 phase starting in two daughter cells.

• The «M» means *mitosis*. Mitosis is the division of the nucleus in eukaryotes. The division of cytoplasm also occurs during M phase and is called cytokinesis.

• Some cells go through repeated cell cycles. Other cells never or rarely divide once they are formed.

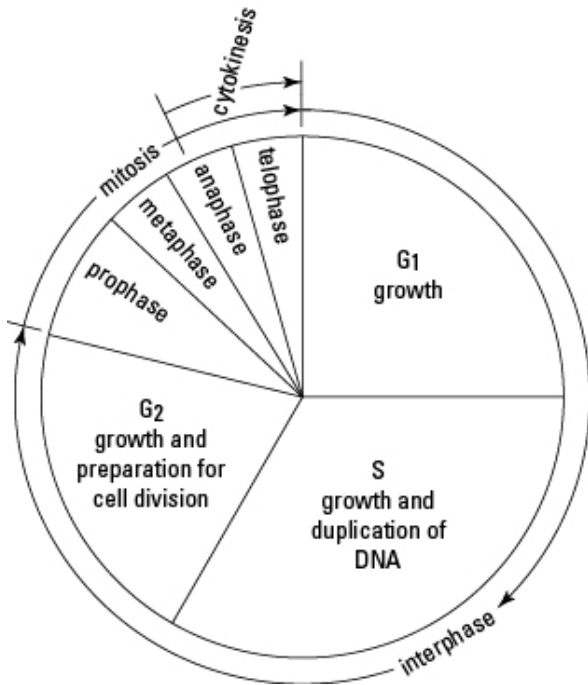• During all the cell cycle, diploid cells stay diploid (2n). Though chromosomes start to consist of two daughter chromatids (2chr) only after replication. After separation of sister chromatids, each chromosome has only one (1chr).

Interphase. Interphase stars from G_1 phase. The «G_1» means first gap or first growth phase.

• In this phase cell determines whether it must divide or exit the cell cycle.

• If the cell leaves the cell cycle, it inters the phase called the G_0 phase.

• Particular stimuli may make the cell return from G_0 to the G_1 phase.

• During G_1 phase, the cell grows and synthesizes mRNA and proteins histones that are required for DNA synthesis.

• The S phase (synthesis phase) follows the G_2 phase.

• It is the phase of the cell cycle in which DNA is replicated (i. e. doubled).

• DNA replication is required to successful cell division as each daughter cell must have same copies of DNA.

• The set of genetic material changes from 2n1chr to 2n2chr.

• Cell continues to grow.

• The S phase is followed by G_2 phase. The «G_2» means second gap or second growth phase.

• During this phase, cell grows more and completes preparations for cell division.

Mitosis. Mitosis of the nucleus is usually divided into five stages: prophase, prometaphase, metaphase, anaphase, and telophase. Cytokinesis usually occurs at approximately the same time with telophase.

Before mitosis starts, cell has nucleus with loose chromatin and nucleolus, and two centrosomes adjacent to the nucleus. The centrosomes were formed earlier by replication of a single centrosome.

1. *Prophase:*
– Nucleoli disappear;
– Loose fibers of chromatin condense into observable chromosomes, composed of two identical sister chromatids;
– Mitotic spindle forms. It is composed of microtubules which extend from two centrosomes;
– Centrosomes move apart, propelled by lengthening of the microtubule bundles between them.

2. *Prometaphase:*
– Nuclear envelope breaks into membrane vesicles.
– Spindle microtubules extend from each pole (because centrosomes are at opposite cell poles) toward the cell's equator.
– Some microtubules attach to the kinetochores and chromosomes start to move as microtubules change their length.

3. *Metaphase:*
– During metaphase, chromosomes move to the *metaphase plate*, the plane at the equator of the cell between the poles where centrosomes are situated.
– Centromeres of all chromosomes are aligned on the metaphase plate.

4. *Anaphase:*
– It begins when paired centromeres of each chromosome move apart.
– Microtubules attached to kinetochore shorten and pull sister chromatids of each chromosome to different cell poles.
– Sister chromatids split apart into separate chromosomes and move towards opposite poles of the cell.
– Simultaneously, the poles of the cell move farther apart, elongating the cell.
– At the end of anaphase, the two poles have identical collections of chromosomes.
– The set of genetic material of dividing cell (2n2chr) becomes 2n1chr at each cellular pole.

5. *Telophase:*
• Daughter nuclei begin to form at the two poles.
• Nuclear envelopes form around the chromosomes from fragments of the parent cell's nuclear envelope and portions of the ER.
• Nucleoli reappear.
• Chromosomes become less condensed and form loose chromatin mass of the nucleus. This occurs at every pole of the dividing cell.

• Cytokinesis occurs and the cytoplasm of the mother cell is divided into two. Two new cells are formed.

Checkpoints in the cell cycle. There are 3 checkpoints in the cell cycle.

1. The *G_1 checkpoint* controls G_1/S transition. It is the point when cell «decides» to divide or not to divide. At this step cell «insures» that:

• It has enough nutrients to divide.

• Molecular signals from the environment suggest that division is possible.

• DNA is not damaged.

2. The *G_2 checkpoint* controls G_2/M transition. Cell can pass through this checkpoint if:

• DNA is not damaged.

• All DNA is completely replicated.

3. The *M checkpoint* or *spindle checkpoint* controls transition from metaphase to anaphase. Cell can pass through this checkpoint if:

• Cell «examines» whether all the sister chromatids are correctly attached to the spindle microtubules.

Regulation of the cell cycle by cyclins and cdks. The proteins *cyclin-dependent kinases* (*Cdks*) synchronize the ordered sequence of cell cycle events. I. e. they regulate passing from one cell cycle phase to another by rhythmic changes in the activity of certain kinases.

• Kinases are the enzymes that transfer a phosphate group from ATP to a target protein. Phosphorylation, in turn, either activates or inactivates the target protein.

• The target proteins eventually cause activation of other proteins and expression of particular genes associated with another phase of the cell cycle.

• Cyclical changes in kinase activity are controlled by other regulatory proteins called *cyclins*. Their concentrations change cyclically during the cell cycle.

• Cdks that regulate cell cycles are active only when attached to a particular cyclin. Hence, *changes in cyclin concentration control passing to the next phase of the cell cycle*.

An example of a cyclin-Cdk complex is MPF (maturation promoting factor), which controls the cell's progress through the G_2 checkpoint to mitosis.

• Cyclin is produced at a uniform rate throughout the cell cycle, and it accumulates during interphase.

• Cyclin combines with Cdk to form active MPF, so as cyclin concentration rises and falls, the amount of active MPF changes in a similar way.

• MPF phosphorylates proteins that participate in mitosis and initiates the chromosome condensation, dispersion of the nuclear envelope.

• In the latter half of mitosis, MPF activates enzymes that destroy the cyclin. This causes the reduction of MPF activity (the Cdk portion of MPF is not degraded).

Meiosis. Unlike mitosis, meiosis is not used for common reproduction of cells.

• Meiosis is the first step in *sexual reproduction* of organisms.

• Unlike mitosis, producing two diploid cells (2n) form one diploid cell, meiosis produces 4 haploid (n) cells from one diploid cell.

• Unlike *diploid cells* (2n) having two same sets of chromosomes (23 + 23 = 46 for human), *haploid cells* (n) have only one set of unique chromosomes (23 for human). The second set of same chromosomes is absent in them.

• At the next step of sexual reproduction, two haploid cells (usually from different organisms) fuse together into a single diploid cell (n + n = 2n).

• Such diploid cell is called *zygote*. Haploid cells that fuse into zygote are called *gametes*.

• Every human (or almost all other animals), develops from a zygote.

• Zygote forms when father's sperm cell (n) fuses with mother's ovum (n).

• Sperms and ova are formed by meiosis.

• As same genes of homologous chromosomes might have some differences, sexual reproduction creates new cells with new combinations of gene variants. This is the main «aim» of sexual reproduction.

• Hence, mitosis is common division producing same cells. Meiosis is specialized division producing gametes for sexual reproduction.

The mechanism of meiosis. Meiosis consists of two divisions which are called meiosis I and meiosis II. Both divisions have four phases: prophase I, metaphase I, anaphase I, telophase I and prophase II, metaphase II, anaphase II, telophase II.

Meiosis I:

1. Prophase of meiosis I is complicated. There occur processes of *synapsis* and *crossing over* of homologous chromosomes.

• Synapsis is a connection of homologous chromosomes. Regions of one homologous chromosome connect to the same regions of the other homolog.

• Two connected homologs are called *bivalents* («bi» = 2) or *tetrads* of chromatids («tetra» = 4).

• Crossing over is exchange of the same segments of homologous chromosomes. The sites where it occurs are relatively random. This process increases the diversity of gene variants from one parent in a gamete.

• Centrosome movement, spindle formation, and nuclear envelope breakdown occur as in mitosis.

2. Metaphase I:

• Bivalents are located along the equator of the cell.

3. Anaphase I:

• Microtubules pull homologous chromosomes from every bivalent to different sides of the cell. Notice that chromosomes, but not sister chromatids, are delivered to cell's poles.

• Each chromosome still contains 2 sister chromatids.

• Each cell pole has the set of genetic material 1n2chr instead 2n2chr that was previously present in the mother cell.

4. Telophase I:

• New nuclei appear.

• Cytokinesis occurs.

• Two haploid daughter cell are formed.

5. Period between the two divisions of meiosis is interkinesis.

• Meiosis II is more similar to mitosis.

6. Prophase II:

• A spindle apparatus forms.

• Chromosomes, each still composed of two chromatids associated at the centromere, are moved by microtubules toward the metaphase II plate.

7. Metaphase II.

• Chromosomes are on the equator of the cell.

8. Anaphase II.

• Chromatids are pulled apart as in mitosis.

• Chromosome set at each cell pole becomes 1n1chr.

9. Telophase II is similar to that of mitosis.

• Nuclei form, the chromosomes begin decondensing, and cytokinesis occurs.

• Four daughter cells are formed.

4. THE STRUCTURE AND FUNCTIONS OF NUCLEIC ACIDS

The structure of DNA. Nucleic acids are DNA and RNA.

• They are chains of repeating units which are called nucleotides.

• A nucleotide of DNA consists of:

1. Sugar deoxyribose, which has 5 carbon atoms. Each carbon has own number from 1' to 5'.

2. Phosphate connected to the sugar's carbon «#5».

3. Nitrogenous base connected to the sugar's carbon «#1». Bases in DNA can be of 4 types:

– *Adenine* (A);

– *Guanine* (G);

– *Cytosine* (C);

– *Thymine* (T) — nucleotides of RNA have similar base *uracil* (U) instead of T.

• A and G are *purine* bases; C and T (or U in RNA) are pyrimidine bases (this is related to their chemical structure).

• DNA consists of two strands. Each strand is a nucleotide chain (RNA has just one).

• Nucleotides in a chain connect to one another with phosphodiester bonds: the phosphate of one nucleotide connects to the carbon «#3» of the next one.

• Hence, deoxyriboses and phosphates form the sugar-phosphate backbone of nucleotide strands.

• One end of the backbone has phosphate on the carbon «#5» of the first nucleotide's sugar and the other end has hydroxyl group on the «#3» of the last nucleotide's sugar. Hence, ends of a strand can be denoted as 3' and 5' ends.

• DNA strands are antiparallel: the 3' end of one strand is near the 5' end of the other one and vice versa.

• Two strands of DNA are connected to one another. Bases in one strand connect to the bases of the other one forming *base pairs*.

• Bases cannot connect randomly. The base A can bind only with T of the other strand (or vice versa), the base G can bind only with C (or vice versa). This rule of base pairing is called *complementarity*.

• There are hydrogen bonds between bases in a base pair. A and T are linked with 2 hydrogen bonds, G and C are linked with 3 bonds.

• The first consequence of complementarity is that the number of T in DNA is equal to the number of A and the number of G is equal to the number of C (if there is A in one strand, then there must be T in the other strand and so on).

• The seconds consequence of complementarity is that the number of purine bases is equal to that of pyrimidines.

• The rules that A=T, G=C, A+G=T+C were discovered earlier than the structure of DNA by Erwin Chargaff. Now they are known as Chargaff's rules.

The structure and function of RNA. RNA is another nucleic acid. It consists of nucleotides. Though there are difference s between DNA and RNA:

• RNA nucleotides have sugar ribose instead of deoxyribose (chemically they are very similar).

• RNA nucleotides may have bases A, G, C, but not T. They have uracil (U) instead of T (chemically U and T are very similar).

• DNA consists of two coiled strands. RNA consists of only one strand which can be twisted into 3-dimentional structure resembling that of proteins. This is due to connection of complementary nucleotides in the RNA strand.

• DNA only stores genetic information. Some RNA also can serve as temporary storage of genetic information, but some RNA in addition may catalyze chemical reactions (commonly proteins catalyze reactions). The RNAs which can catalyze reactions are called ribozymes.

• Common RNA is many times shorter than DNA.

• In eukaryotes, most of DNA is in the nucleus (in chromosomes), the RNA is present both in the nucleus and cytoplasm. This is because of their different functions.

Depending on functions, RNA can be of many types:

1. Messenger RNA (mRNA):

− It is temporary copy of a gene (or several genes in prokaryotes).

− Since a gene is an «instruction» for creation of particular protein, this gene copy (i. e. mRNA) must travel from the nucleus to the cytoplasm where ribosomes can «read» it and build a protein.

2. Ribosomal RNA (rRNA):

– All types of rRNA are the structural components of ribosomes responsible for their normal functioning. rRNA are not carriers of «instructions» for protein creation. They are working RNA of the cell.

3. Transfer RNA (tRNA):

– This is another «worker» RNA which carries amino acids (structural components of proteins) to the working ribosome.

4. Other types of RNA may work in a complex with proteins against mRNA and prevent the synthesis of particular proteins (miRNA, siRNA, piwiRNA), other RNAs can help to modify mRNA in the nucleus (small nuclear RNA). RNA of other types perform many other different functions.

The central dogma of molecular biology. In 1957 Francis Crick summarized the concepts explaining relation between DNA, RNA and proteins in a single concept. He named this concept *the central dogma of molecular biology*.

• The dogma can be formulated as «DNA makes RNA and RNA makes protein». Though further discoveries made this statement more complicated:

• DNA can be copied. This is called *replication*.

• RNA is copied from a DNA template. This is called *transcription*.

• Some RNA sequences can be copied in the form of DNA. This is *reverse transcription*.

• In some viruses, RNA can be copied from another RNA template. This is *RNA replication*.

• RNA template is used to build proteins. This is called translation.

Replication of DNA. DNA replication is doubling of DNA by cell's enzymes. In eukaryotes, this occurs at the S phase of the cell cycle.

• DNA replication begins at special sites called *origins of replication* (*ori*). Origins have a specific nucleotide sequence. DNA of bacteria and viruses have only one origin, the chromosomes of eukaryotic have hundreds or thousands of origins.

• Specific proteins required to initiate replication bind to each origin. They open the DNA double helix.

• The regions of separated strands at origins are called *replication bubbles*. The Y-shaped half of a replication bubble where one group of enzymes works and replicates DNA is called replication fork.

• In a replication fork, enzymes add new complementary nucleotides (according to base pairing rule) to each strand of the mother DNA. Hence, after replication each DNA copy has one old and one new strand. This is called *semiconservative synthesis*.

• Many different enzymes work in every replication fork.

• The strands of the DNA that must be replicated are continuously separated by *helicase*.

• New DNA strand are assembled by the enzymes called *DNA polymerases*. DNA polymerase links nucleotides to the growing strand.

● DNA polymerases can only add nucleotides to a fragment of ready-made strand, but cannot start synthesis of new strand from the beginning. For this reason, another enzyme called *primase* creates a short complementary RNA fragment which is called *primer* on the template strand.

● DNA polymerase adds new nucleotides to the primer. Later, primers are removed and replaced with DNA nucleotides.

● When DNA polymerase links the nucleotides to the growing strand, these strands grow in the 5' → 3' direction since new nucleotides are added only to the 3' end of the growing strand.

● As the strands of the mother DNA are antiparallel, one strand replicated continuously, the DNA polymerase follows the helicase separating the DNA strands and the DNA polymerase on the other strand must move in opposite direction.

● *Leading strand* is the DNA strand which is synthesized as a single polymer in the 5' → 3' direction towards the replication fork.

● *Lagging strand* is the DNA strand that is discontinuously synthesized against the overall direction of replication.

● *Lagging strand* is produced as a series of short segments called *Okazaki fragments*.

Transcription. Transcription is the first step in expression of genes. Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product (protein or RNA). It is illustrated in the fig. 5.
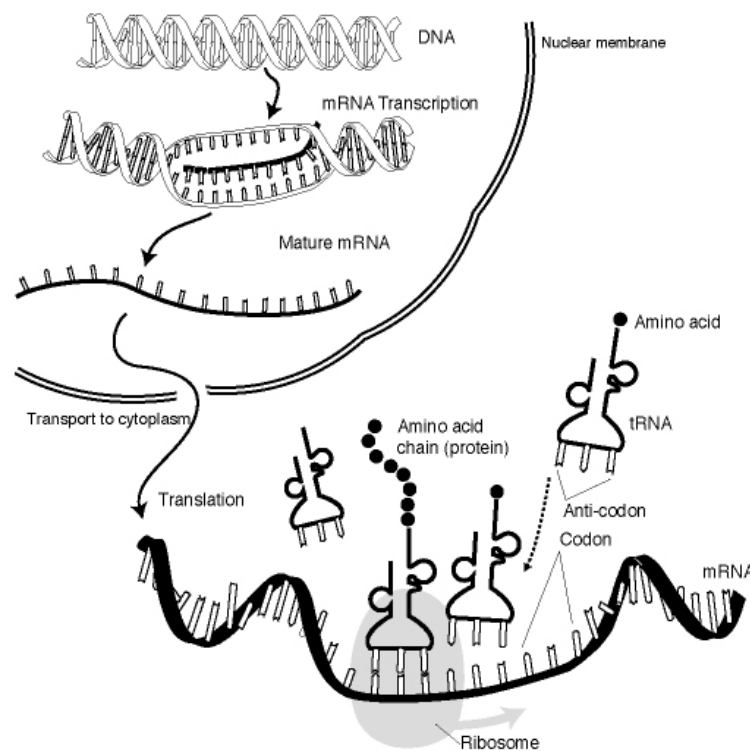

Fig. 5. Diagram of gene expression

23

Transcription is the synthesis of RNA using DNA as a template.

• The mRNA carrying information about the protein structure is called mRNA.

• Transcription is performed by enzymes RNA polymerases.

• RNA polymerase starts transcription from special DNA sequences called promoters.

• RNA polymerase untwists and opens a short segment of DNA exposing about ten nucleotide bases.

• One of the exposed DNA strands is the template. The RNA polymerase «reads» add complementary RNA nucleotides to the template.

• RNA grows one nucleotide at a time in the 5' → 3' direction.

• As the mRNA strand elongates it peels away from its DNA template.

• The nontemplate strand of DNA behind the RNA polymerase reforms hydrogen bonds with the template (i. e. DNA strands connect again).

• Transcription proceeds until RNA polymerase transcribes a DNA sequence called a *terminator*. At this step transcription is finished.

• The newly made RNA in eukaryotes is modified and transported to the cytoplasm where ribosomes use it for synthesis of proteins i. e. for translation.

Genetic code. To understand translation, we need to discuss how nucleotide sequences define the structure of proteins.

• Proteins are long chains of amino acids.

• The genetic information written in DNA and RNA is the instruction describing the amino acids which are required to build certain protein and order of these amino acid in the protein.

• One amino acid is encoded by 3 adjacent RNA nucleotides. Such nucleotide triplet is called codon. For example, the codon ACU codes for amino acid threonine, AAU codes for asparagine, AAA = lysine.

• The number of all the possible codons is $4^3 = 64$ (there are 4 possible nucleotides, one codon consists of 3 nucleotides).

• The number of amino acids which can be encoded by DNA is 20. As $64 > 20$, some different codons code for the same amino acid. For example, UCU, UCA, UCG, UCC — all code for the same amino acid serine.

Ribosomes and tRNA. Apart from mRNA, translation involves ribosomes and tRNA.

tRNA is a «worker» RNA which delivers amino acids to ribosome.

• The molecule of tRNA has several arms. One arm carries amino acids, the other one contains an *anticodon*.

• Anticodon is complementary to a codon which codes for the amino acid carried by tRNA.

• For example, the amino acid methionine is coded by the codon AUG. Hence, the anticodon of the tRNA carrying methionine must be UAC.

Ribosomes consist of large and small subunits which connect only during translation. The large subunit has several sites for tRNA.

● tRNA with new amino acid come to the *A site*.

● From the A site tRNA passes to the to the *P site* and then exits the ribosome leaving its amino acid.

Translation. Translation is the process in which ribosomes attach amino acids to one another in the order defined by an mRNA. The chain of amino acids is called polypeptide. It is not yet a mature protein ready to perform its function. Translation occurs in the cytoplasm.

● Ribosomes «read» the mRNA in the direction from their 5' end to the 3' end.

● Translation starts not from the end of mRNA, but from the *start codon* AUG. This codon codes for the amino acid methionine.

● Translation stops not in the end of mRNA, but when a ribosome reaches a stop codon. There are 3 possible stop codons: UAA, UGA or UAG.

● The mRNA segment between 5' end and the start codon is called *leader* (or *5' untranslated region*), the segment between the stop codon and 3' end is called *trailer* (or *3' untranslated region*).

Translation has 3 steps: initiation, elongation and termination.

1. In initiation the first tRNA (with methionine) binds to the start codon of mRNA, and ribosomal subunits also bind this region. The first tRNA does not pass through A site and is in the P from the beginning of initiation.

2. In elongation several steps repeat many times:

● Another tRNA comes to the A site.

● Next actions occur only if its anti-codon is complementary to the mRNA codon which is exposed in the A site.

● The amino acid of the tRNA from the P site binds to the amino acid of the tRNA which had just come to the A site. Hence the first tRNA no longer has amino acid while the new one has two.

● At this step ribosome moves one codon forward, and the new tRNA with all its amino acid passes from the A site to the P site. The tRNA which previously was in the P site is ready to leave the ribosome.

● The A site is ready to receive another tRNA with its amino acid (if its anti-codon matches to the mRNA codon exposed in the A site).

● When new tRNA brings an amino acid, these steps repeat and the amino acid chain elongates.

3. Termination occurs when a stop codon enters the A site. None of the stop codons codes for amino acids. In termination the protein called *release factor* binds to the codon and causes separation and release of polypeptide and tRNA from the ribosome, separation of the small and large ribosomal subunits.

Lecture 3. ARRANGEMENT OF GENETIC MATERIAL

Outline:
1. Basic principles in regulation of gene expression.
2. Regulation of gene expression in prokaryotes.
3. Regulation of gene expression in eukaryotes. Histones.
4. Regulation of transcription in eukaryotes.
5. Post-transcriptional RNA modifications.
6. Cytoplasmic inheritance.

1. BASIC PRINCIPLES IN REGULATION OF GENE EXPRESSION

Connection between genes and metabolism. Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product (RNA or protein). Gene expression includes transcription and translation (see the previous lecture).

• To understand why gene expression must be thoroughly regulated, you have to understand the following:

• The processes occurring in the cell may change (e. g. when cell needs to adjust itself to changing environment).

• All activities of the cell are performed by various types of proteins (and some types of RNA).

• Hence, any change in the cell's activities is caused by activation/inactivation or creation/destruction of certain proteins responsible for these activities.

• Consequently, some genes must be expressed only at particular moments in cell's life and stay silenced when they are not required.

• For example, the bacterium *E. coli* has gene LacZ coding for the enzyme breaking the sugar *lactose*. Production of this enzyme (i. e. expression of the LacZ gene) is useless (and even deleterious) when lactose is absent in the medium as it is waste of cell's resources.

• To control the production of certain proteins, cells can regulate: rate of transcription, mRNA's lifespan, protein's lifespan.

Feedbacks in regulation of gene expression.

Gene expression is regulated through *positive and negative feedbacks*.

• Example of positive feedback: protein A stimulates the creation of the protein B, the protein B stimulates the production of protein A. The more protein A is in the cell, the more protein B is created, this increases the amount of protein A produced, hence the amount of the protein A continues to increase and so on.

• Example of negative feedback: protein A stimulates the creation of the protein B, the protein B suppresses the production of protein A. The more protein A is in the cell, the more protein B is created, this decreases the amount of protein A produced, hence the production of protein B decreases, this, in turn increases the production of the protein A as it is not inhibited by the protein B and so on.

• Positive feedbacks help to cause rapid changes in the system. Negative feedbacks commonly help to maintain the balance is the system.

2. REGULATION OF GENE EXPRESSION IN BACTERIA

Operon. Metabolism of one substance may require several different proteins which catalyze different steps in a chain of chemical reactions. Proteins which work together must be created together i. e. their genes must be expressed together. For this reason, in prokaryotic DNA such genes are clustered together. Several genes then transcribed to the same mRNA because they are situated between the same promoter and terminator.

Promoter is the DNA region that includes the site where RNA polymerase binds and where transcription begins.

Terminator is the site where transcription is finished.

The group of genes which are clustered together and transcribed to the same mRNA is called *operon*. Operons are present in prokaryotes, but not found in eukaryotes.

Operons can be inducible or repressible.

• Inducible operons are not active, unless particular molecule called *inducer* is in the cell.

• Repressible operons are active unless particular molecule called *corepressor* is in the cell.

Lac operon. Lac operon is an example of *inducible operon*. The regulation of its work is presented in the fig. 6.

In the absence of lactose, the *lac* repressor binds the operator, and transcription is blocked.

	promoter	operator	*lacZ*	*lacY*	*lacA*	terminator

RNA polymerase ⟶✕✕▶ repressor
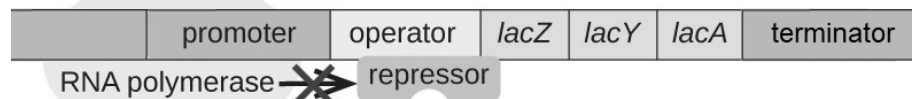
In the presence of lactose, the *lac* repressor is released from the operator, and transcription proceeds at a slow rate.

	promoter	operator	*lacZ*	*lacY*	*lacA*	terminator

RNA polymerase ⟶

repressor — lactose
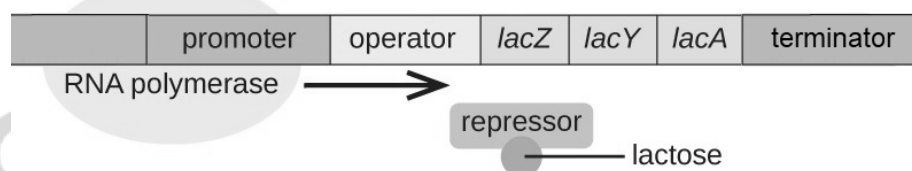
Fig. 6. The work of the lac operon

• The bacterium *E. coli* can feed on milk sugar *lactose*.

• 3 enzymes involved in metabolism of lactose are clustered together into the *Lac operon*.

• When lactose is absent, the enzymes metabolizing it are not required. Hence, when lactose is absent, the transcription in the lac operon does not occur.

• When lactose is present, the transcription in the lac operon occurs and enzymes are produced.

• As the lac operon is induced by lactose, it is an inducible operon.

Lac operon consists of promoter (where RNA polymerase binds) and terminator (where transcription is terminated) with 3 genes between them. Between the promoter and the first gene is the nucleotide sequence called *operator*.

• Operator is involved in activation/inactivation of gene expression.

• There is protein called *repressor* which can bind the Lac operator.

• The repressor for the lac operon is created by the expression of another gene (LacI) which is not in the lac operon and is always active.

• When LacI repressor binds to the operator, RNA polymerase cannot transcribe the genes in the lac operon.

• As transcription does not occur, the enzymes metabolizing lactose are not created.

• If molecules of lactose pass to the cell, they can bind with repressor. An isomer of lactose binding with repressor makes it not active.

• Inactivated repressor is unable to bind the operator, so RNA polymerase can transcribe the lac operon moving from promoter to terminator.

• mRNA created during transcription is used by ribosomes for creation of enzymes. One of the enzymes is β-galactosidase which eventually metabolizes the lactose.

• As lactose is absent, it cannot inactivate the new doses of LacI repressor. The repressor binds the operator and prevents lac operon from transcription.

Trp operon. Trp operon is an example of *repressible operon*.

Tryptophan is an amino acid. It is required by all living organisms. If a cell is low on tryptophan, it must synthesize it from other products. Creation of new tryptophan molecules requires several enzymes. The genes coding for 5 of them are clustered together between the same promoter and terminator. These DNA regions and their operator together comprise trp operon.

• When tryptophan is present, enzymes creating tryptophan are not required. Hence, when tryptophan is present, the transcription in the trp operon does not occur.

• When tryptophan is absent, the transcription in the trp operon occurs and enzymes are produced.

• As the transcription of the trp operon is suppressed by tryptophan, it is a repressible operon.

• The mechanism of transcriptional regulation is similar to that of the lac operon: repressor binds the operator and prevents the transcription.

• The major issue differentiating the repressible trp operon from the lac operon is that repressor cannot bind with the operator unless it contacts with trypto-

phan. Hence, tryptophan makes repressor active while lactose makes its repressor inactivated.

- Tryptophan is corepressor for the trp operon.

3. REGULATION OF GENE EXPRESSION IN EUKARYOTES. HISTONES

Difference between gene expression in bacteria and eukaryotes. The difference between gene expression in bacteria and eukaryotes is considered in the tabl. 2.

Table 2

Difference between gene expression in bacteria and eukaryotes

	Bacteria	Eukaryotes
Participation of proteins histones in gene expression	There are no histones. Condensation of DNA is limited and does not essentially regulate gene expression.	DNA of eukaryotes is bound with proteins histones which condense chromatin. Highly condensed DNA is not expressed.
Regulation of transcription	Regulation of transcription involves not many proteins. They act close to the promoter (e. g. repressors binding the operator)	Large number of proteins are involved in transcriptional control (e. g. general transcription factors required for transcription to occur). The regulatory proteins may act at sites close to or far from promoter (i. e. at proximal and distal control elements).
Post-transcriptional RNA modifications	Rare	Very common, RNA contains non-coding fragments introns which are removed from mature mRNA.

Histones and gene expression. Histones are small proteins that are rich in positively charged amino acids and bind to negatively charged DNA, forming chromatin.

Histone proteins associated with DNA are responsible for DNA packing in eukaryotes. The histones form nucleosomes around which DNA wraps.

- A nucleosome includes of a protein core which consists of eight protein molecules.
- These molecules are per two copies each of four types of histone (H2A, H2B, H3, H4).
- DNA wraps around such protein core making 1.7 turns.
- A fifth histone (H1) attaches near the bead when the chromatin undergoes the next level of packing.
- Nucleosomes may control gene expression by controlling access of transcription proteins to DNA.
- For example, acetylation of histones changes the chromatin so it becomes less condensed, and the DNA becomes accessible for transcription.
- Methylation of histones has opposite effect: DNA becomes more tightly packaged and inaccessible for transcription.

● Methylation may occur not only in histones, but in the DNA itself. For example, methylation of a promoter typically acts to repress gene transcription.

Condensation of DNA. Apart from gene expression, histones play major role in DNA condensation.

1. Nucleosomes are responsible for the first packing level of eukaryotic DNA.

● Binding with nucleosomes typically decreases its length by 5–6 times.

● Because of their appearance, nucleosomes on the DNA are often compared with «beads on a string». The diameter of such fiber is near 10 nm.

Higher levels of DNA packing include the following:

2. The 30-nm chromatin fiber.

● It consists of a tightly wound cylinder-like coil with six nucleosomes per turn.

● Formation of this structure requires histone H1.

● DNA becomes near 6 times shorter.

● However, some recent studies demonstrate that in the nuclei, DNA is packed in tightly associated 10-nm fibers that are not compacted into 30-nm fibers.

3. In the next level of higher-order packing, the 30-nm chromatin fiber forms looped domains.

● These looped domains are attached to a nonhistone protein and contain 20,000 to 100,000 base pairs.

● The diameter of such fiber may be near 300 nm.

● DNA becomes 20 times shorter.

● Interphase looped domains attach to a scaffolding inside the nuclear envelope (nuclear lamina); this helps organize areas of active transcription.

● Chromatin fibers of different chromosomes do not become entangled as they occupy restricted areas within the nucleus.

4. Coil and fold, further compacting the chromatin into a mitotic chromosome characteristic of metaphase.

● DNA becomes 10-20 times shorter.

● The result of condensation at all these levels is shortening the DNA near 10 000-fold.

4. REGULATION OF TRANSCRIPTION IN EUKARYOTES

There are 3 types of RNA polymerase in eukaryotes. The RNA polymerase II catalyzes mRNA synthesis, so it transcribes genes that will be translated into proteins.

● Other types of RNA polymerases catalyze synthesis of other RNA types.

● In eukaryotes, mRNA codes for synthesis of only one protein because a transcription unit (the DNA sequence which is transcribed from DNA to RNA) contains a single gene.

- RNA polymerases bind to DNA at promoters (i. e. the regions where transcription begins).

- The eukaryotic promoter is about 100 nucleotides long.

- Apart from the site, where transcription begins (it is called *initiation site*) promoter contains a few nucleotide sequences recognized by specific proteins called *transcription factors*.

- Transcription factors (TF) are the DNA-binding proteins which help initiate transcription.

- There are two types of transcription factors:
  – *General transcription factors* which act at the promoter of all genes.
  – *Specific transcription factors* that bind to *control elements* that may be close to or farther away from the promoter. Specific TF control not all, but specific, individual genes.

- In eukaryotes, RNA polymerases cannot recognize the promoter without the help of transcription factors. Eukaryotic RNA polymerase II, is bound with many transcription factors before transcription starts.

- A few general transcription factors bind to specific DNA sequences within the promoter, but many bind to proteins, including other transcription factors and RNA polymerase.

- The interaction of general transcription factors and RNA polymerase II with a promoter usually leads to a low rate of initiation and production of few RNA.

- High levels of transcription in eukaryotes at the appropriate time and place depend on specific transcription factors.

- Specific transcription factors, either activators or repressors, bind to the nucleotide sequences called the control elements.

- The control elements can be situated closely to the promoter (*proximal control elements*) or very far from it — thousands of nucleotides upstream or downstream of a gene or even within an intron (distal control elements).

- The specific transcription factors can be either *activators* or *repressors*.

- Activators stimulate transcription of particular gene e. g. making it easier for RNA polymerase to bind to the promoter of the gene. Repressors suppress transcription.

- Activators or repressors may bind to distal control elements which are called *enhancers* or *silencers* respectively.

- A given gene may have multiple control elements, each active at a different time or in a different cell type or location in the organism. Each element is generally associated with only one gene and no other.

Although enhancers and silencers are situated far from the promoter, the proteins which bind with them must contact with the complex of RNA polymerase and transcription factors. Such contact is provided by other proteins.

• As the expression of a gene is generally regulated by many specific transcription factors, *cell can control the expression the gene by production of specific transcription factors*.

• Transcription itself in eukaryotes is generally similar to those in all other cells including bacteria: the two DNA strands are separated at the initiation site, and transcription begins; RNA polymerase II starts moving along DNA exposing the template DNA strand for basepairing with RNA nucleotides; as mRNA strand elongates, it peels away from its DNA template and double DNA helix is re-formed. Transcription proceeds until the RNA polymerase II transcribes a terminator.

5. POST-TRANSCRIPTIONAL RNA MODIFICATIONS

Post-transcriptional RNA modifications. RNA in eukaryotes are modified after transcription. The sum of all the modifications is referred to as RNA processing. The processing includes:
  – Modification of the 3' and 5' ends.
  – Removal of certain RNA regions which do not code for proteins.
The mRNA before processing is called *primary transcript* or *pre-mRNA*.
The diagram of mature eukaryotic mRNA is shown in the fig 7.
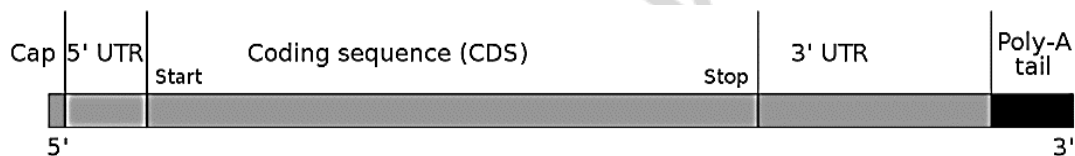
Fig. 7. Diagram of mature eukaryotic mRNA

Modification of the 3' and 5' ends. The 5' end of the primary transcript is modified with *5' cap*.

• *5' cap* is a modified guanine nucleotide.

• It is added to the 5' end of mRNA shortly after transcription begins (i. e. to the leader, but not to the start codon).

• It protects the growing mRNA from degradation by hydrolytic enzymes and helps small ribosomal subunits recognize the attachment site.

The 3' end, which is transcribed last, is modified by enzymatic addition of a poly-A tail, before the mRNA exits the nucleus.

Poly (A) tail added to the 3' end is a sequence of about 30 to 200 adenine nucleotides.

It is added to the trailer, but not to the stop codon. This occurs before the RNA exits the nucleus.

The poly (A) tail may inhibit degradation of mRNA in the cytoplasm, facilitate attachment to small ribosomal subunit, facilitate mRNA's export from the nucleus to the cytoplasm.

RNA splicing. Unlike genes of bacteria, eukaryotic genes commonly contain introns.

- *Introns* are noncoding regions in eukaryotic genes.
- Introns are between the protein-coding gene regions which are called *exons*.
- The pre-mRNA contains all the nucleotide sequence of all introns and exons.
- As introns do not carry any «instructions» for protein synthesis, they must be removed from the RNA. Only exons are transcribed and expressed.
- The process in which introns are removed and adjacent exons are connected to one another is called *RNA splicing*.
- RNA splicing also occurs during post-transcriptional processing of tRNA and rRNA.
- Splicing is performed by *spliceosomes* which are large molecular catalytic complexes containing molecules of proteins and RNA.

As introns do not code for protein, at first view their presence in genes may look a nonsense. Though introns actually might be one of the reasons of the evolutionary success of eukaryotes.

- Some intron DNA sequences may control gene activity.

In general, an exon may code for particular protein domain. The protein domains are relatively independent regions of protein which may be associated with different functions of the protein.

- Introns may allow a single gene to direct the synthesis of different proteins due to the process called *alternative splicing*.
- Alternative splicing means that different cells perform different splicing of the same gene: one cell can remove all introns and link exons, a cell of another tissue may remove one of the exons with introns, so the resulting protein would lack one of domains it could have. Hence, one gene may code for several variants of proteins.
- Presence of intron-exon structure may facilitate the evolution of genes: recombination of exons from different genes can produce new proteins with unique shape and set of functions.

6. CYTOPLASMIC INHERITANCE

Cytoplasmic inheritance. Cytoplasmic inheritance, also known as extranuclear inheritance, is the transmission of genes situated in the cytoplasm.

- Extranuclear DNA is present in cytoplasmic organelles such as mitochondria and chloroplasts.
- Presence of own DNA in these organelles is due to their origin: they evolved from bacteria engulfed by an ancient proto-eukaryotic cell.
- Other source of extranuclear DNA is presence of cellular parasites such as viruses or bacteria.

- Cytoplasmic genes are inherited from mother as zygote's cytoplasm is the cytoplasm of the mother's ovum, but not sperm.
- In animals, mitochondria of the zygote are received from the ovum, in plants, a zygote receives its plastids from the ovum, not from pollen.
- Some cases when paternal mitochondrial DNA was inherited were reported in 2018, though it is not enough to consider such inheritance pattern of mitochondrial genome regular and common.
- These cytoplasmic genes are not *inherited in Mendelian fashion*, because they are not distributed by segregating chromosomes during meiosis.

Cytoplasmic genes in plants were first described by Karl Corens in 1909. He noticed that plant coloration of an ornamental species was determined by the seed bearing plants and not by the pollen producing. It is now known that maternal plastid genes control variegation of leaves.

Inheritance of mitochondrial genes was described by Boris Ephrussi, 1949. He noticed that some colonies of yeast grow slowly and are smaller than others (petite colonies). This was caused by inheritance mutation in mitochondrial genome.

The mitochondrial DNA (mtDNA) of human contains near 16500 base pairs and contains 37 genes. 13 of them code for proteins, 22 code for tRNA, 2 code for rRNA. The proteins encoded by mtDNA participate in transfer of electrons during cellular respiration (cytochrome c oxidase, cytochrome b, NADH dehydrogenase) and ATP synthesis (ATP synthase).

There are human diseases caused by inheritance of dysfunctional mitochondria. Common symptoms often include poor growth, loss of muscle coordination, muscle weakness, learning disabilities, visual problems, hearing problems.

Examples of such diseases are: mitochondrial myopathy, Leber's hereditary optic neuropathy (LHON), Neuropathy, ataxia, retinitis pigmentosa, and ptosis (NARP) and others.

Many mitochondrial diseases are associated with inheritance of nuclear genes as many genes coding for mitochondrial proteins were transferred to the nucleus in course of evolution (e. g. myoneurogenic gastrointestinal encephalopathy — MNGIE).

Lecture 4. GENETIC ENGINEERING AND BIOTECHNOLOGY

Outline:

1. Introduction.
2. DNA sequencing.
3. DNA cloning.
4. Amplification of DNA.
5. Southern blot.
6. Gene therapy.

1. INTRODUCTION

DNA technology. The properties of an organism depend on its genes. Modern technologies allow to manipulate genome of an organism and change its characteristics. Such DNA manipulations are called *DNA technology*. The methods of DNA technology can be used for:

– Fundamental science — studying the functions of different genes, the role of some genes in the cell or developing embryo.

– Creating the organisms with new properties e. g. microorganisms producing proteins used in medicine.

– Gene therapy — treatment of hereditary disorders caused by gene mutations.

2. DNA SEQUENCING

DNA sequencing. DNA sequencing is the process of determining order of nucleotides in DNA.

Many techniques in genetic engineering and biotechnology are not possible without the knowledge of nucleotide sequence in a desired gene.

There are many methods of sequencing. Many of them were expensive and hard to perform. The program for sequencing of human genome (Human Genome Project) was started in 1990 and successfully finished in 2003. The project was international and very expensive.

Modern methods of sequencing are cheaper and faster. They made the sequence of human genome almost routine procedure.

In one new method, a DNA is placed in a chamber with electrolyte. The chamber is separated into two by a membrane. The membrane has a very small pore (a nanopore). Electric current is applied and ions start to flow through the nanopore. DNA strand also moves through the pore and interrupts the free flow of ions. Nucleotides of different types interrupt the flow of ions differently. The current strength changes with every nucleotide of the DNA strand, these changes are analyzed and interpreted as a nucleotide sequence.

So far researchers had completed the sequencing of thousands of genomes among which are the genomes of modern and ancient humans, other animals and numerous microorganisms.

Enzymes used in genetic engineering and biotechnology. The manipulations with DNA such as cutting, joining, copying of nucleotide sequences require some tools. Fortunately, such tools already exist in the nature — every cell has enzymes performing such functions.

1. *DNA restriction endonucleases* (*restriction enzymes*) are the enzymes which can cut DNA. Commonly, restriction endonucleases recognize particular DNA site and cut it.

• For example, the enzyme EcoR I recognizes the sequence $\frac{5'\ GAATTC\ 3'}{3'\ CTTAAG\ 5'}$ and cuts it between G and A in both strands. Notice that both strands contain the same sequence GAATTC, but one of them must be read in opposite direction.

• The DNA containing such region is cut into two forming fragments with ends $\frac{G}{CTTAA}$ and $\frac{AATTC}{G}$. Both fragments contain a single-strand region TTAA5' which can bind to the same region (due to complementarity) forming weak connection with to hydrogen bonds.

• As such complementary single-strand regions can «stick» to one another, they are called sticky ends.

• The nucleotide sequence recognized by particular restriction endonuclease (e. g. GAATTC in this example) is called *restriction site*.

• The DNA which do not contain the restriction sites for certain endonuclease cannot be cut by that endonuclease. This problem can be solved by use of another restriction enzyme recognizing another nucleotide sequence.

• Researches use restriction enzymes to cut DNA.

2. *Ligases* are the enzymes which can connect DNA fragments. For example, they can connect A and G cut by EcoR I in previous example.

• Researches use ligases to join DNA fragments together.

3. *DNA polymerases* are the enzymes which assemble a complementary DNA sequence using another DNA sequence as a template.

• As noted in the lecture 2, DNA polymerase cannot start the synthesis of new strand, but can only continue to elongate a short ready-made nucleotide sequence.

• Such sequence is called primer.

• Researches use DNA polymerases to make copies of DNA fragments in a laboratory.

4. *Reverse transcriptase* is an enzyme which uses RNA as a template for creation of a complementary DNA (cDNA).

• It also requires a primer.

• Researches use reverse transcriptase when need to copy nucleotide sequences from RNA to DNA.

Many other tools can be used in biotechnology. For example, CRISPR/CAS9 can be «programmed» to target any nucleotide sequence and cut it.

3. DNA CLONING

DNA cloning. To work directly with specific genes, these genes must be obtained in multiple identical copies and separated from the plenty of other DNA sequences of the cell. Certain genes or other nucleotide sequences can be multiplied by the methods of DNA cloning. This method is based on the fact that cells double their DNA for further division.

• The desired DNA is commonly cloned in bacteria such as *Escherichia coli*.

• The genome of bacteria has a bacterial chromosome (i. e. *nucleoid*) and multiple small circular DNA called *plasmids*.

• Each plasmid containing just a few genes.

• Before bacterial cell divides, it replicated its DNA. The number of plasmid's copies increases as well.

• To clone pieces of DNA using bacteria, researchers first obtain a plasmid and insert the desired DNA fragment into it.

• The plasmid carrying the foreign gene is called a *recombinant DNA*.

• The DNA, such as a plasmid, which is used for making recombinant DNA, is called a *vector*. The plasmid used for DNA cloning is called *cloning vector*.

• The plasmid is then inserted into a bacterial cell. The bacterium carrying a recombinant DNA is called a *recombinant bacterium*.

• When bacteria multiply, they replicate the recombinant plasmid, so the number of the desired DNA fragment increases together with it.

• The cloned DNA can be isolated from bacteria and used for any other purposes.

Cloning a eukaryotic gene in a bacterial plasmid. This process is demonstrated in the fig. 8 where it is simplified for clarity. A typical gene-cloning procedure includes the following steps.
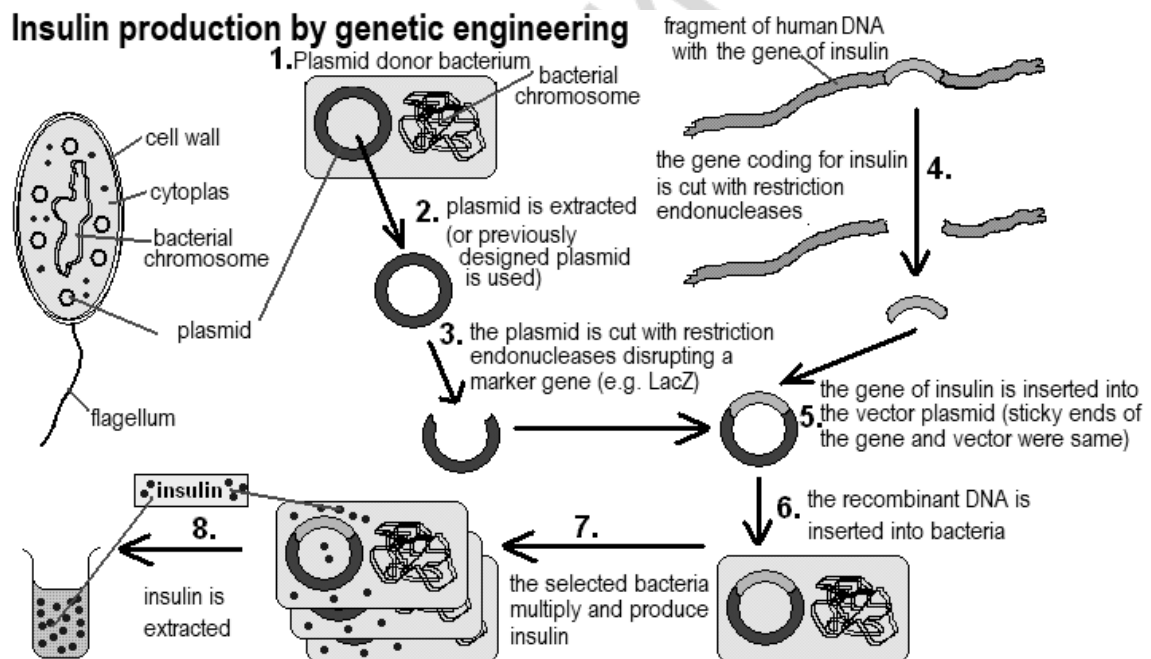

Fig. 8. Simplified process of insulin production by expression of the eukaryotic gene in a bacterial cell

Step 1: Isolation of the gene-source DNA and the vector.

• Vectors (e. g. bacterial plasmids) and the DNA (e. g. human DNA) containing the desired gene are isolated.

• The plasmid vector must contain the genes which allow to identify the cell containing it, for example genes *amp^R* (for antibiotic resistance to ampicillin) and *lacZ* (coding for b-galactosidase that metabolizes lactose).

• The restriction site for the endonuclease used in this example *must be situated within the lacZ gene*.

Step 2: Insertion of desired DNA fragment into the plasmid vector.

• The same restriction enzyme is used to cut the plasmid at the restriction site (and, hence, disrupt the lacZ gene) and the human DNA.

• As the same restriction enzyme was used for the plasmid and the DNA, both the plasmid and the DNA fragment containing the gene have same type of sticky ends.

• The fragments of DNA are then mixed with the cleaved plasmid. The sticky ends of the DNA fragments and plasmids connect with hydrogen bonds forming recombinant DNA molecules.

• Ligases are added. These enzymes link the two DNA molecules at the restriction sited with covalent bonds. The formation of recombinant is done.

• After ligation, mixture contains randomly joined DNA. The desired products are present among them; though other random combinations of DNA fragments are also usually present.

Step 3: Introduction of recombinant DNA into bacterial cells.

The recombinant DNA is added to a bacterial culture. Some bacteria of the culture will intake the DNA by transformation.

Step 4: Cloning of cells containing the foreign DNA.

• The bacteria are allowed to reproduce at a medium containing ampicillin. As the vector contained the gene providing ampicillin resistance, only the bacteria having the recombinant DNA can survive and multiply.

Step 5: Identification of cell clones carrying the gene of interest.

• A modified sugar called X-gal is added to the bacterial culture. This sugar is used as it turns into a blue product when hydrolyzed by b-galactosidase. Bacterial colonies metabolizing the X-gal appear blue.

• Only the cells containing functional LacZ gene can produce b-galactosidase. Remember that the restriction site where the desired DNA fragment inserted was within the LacZ gene. Hence, the blue colonies of bacteria are not those containing the desired recombinant DNA.

2. Cloning and expressing eukaryotic genes.

To allow the cloned gene being expressed, another type of vector must be used. It is called *expression vector*. They must contain promoter upstream the cloned gene. Even so, the expression of eukaryotic genes in bacteria can be associated with a number of problems:

1. Bacteria cannot process RNA to remove introns. The genes for expression in bacteria must lack introns. Such genes can be created:

• By *synthesis of artificial genes* without introns instead of *isolation of genes* from cell's genome.

• By synthesis of genes by *reverse transcription*. This process allows to create DNA copies of RNA that was formerly processed and lacks introns.

2. Bacteria are not always able to modify the protein product.

This problem, alongside with the previous one, can be solved by using eukaryotes (e. g. yeasts or cell cultures) as hosts for the recombinant DNA.

Yeasts are as easy to grow as bacteria as they are unicellular fungi, in addition, they have plasmids, which is rarity among eukaryotes.

Cell cultures are cells grown in artificial media in laboratories. As eukaryotes commonly do not have plasmids, the desired DNA is delivered to their genome by special virus (common property of viruses is the ability to insert their genome to the genome of the cell). It this case the *genome of a virus is a vector* which carries the desired gene.

3. Not all cells actively intake the foreign DNA from the medium.

• The basic method for insertion of DNA into bacteria is their ability for *transformation*. In this case some bacteria intake foreign DNA by themselves. The state when cell is capable of transduction is called competence.

• As noted above, some recombinant DNA can be delivered to particular cell by *infection with modified viruses*.

• In *electroporation*, a brief electric pulse applied to a cell solution. This causes temporary holes in the plasma membrane, through which DNA can enter.

• DNA can be *injected directly into a eukaryotic cell* with thin needles.

• Gene gun, a device for delivering exogenous DNA to cells, can fire the DNA attached to microscopic metal particles into plant cells.

• Plant cells can be also modified by means of Agrobacterium. These bacteria are natural gene engineers which can insert specific DNA into plant cell's genome.

4. AMPLIFICATION OF DNA

Amplification of DNA. Polymerase chain reaction. DNA amplification is the production of multiple copies of a desired DNA sequence. The technique used for this is called polymerase chain reaction (PCR).

PCR is based on duplication of a DNA fragment in a sample by DNA polymerases. As the reaction mixture must be heated to near 90°, the DNA polymerases of most animals cannot be used in PCR (because most protein denature at high temperature). For this reason, researches use the *Taq polymerase* — the DNA polymerase of a microorganism living in hot springs. It is resistant to high temperature.

PCR consists of 3 stages which repeat many times.

1. Denaturing. The reaction is heat to ~95 °C for 15 sec. At this temperature hydrogen bonds holding the complementary strands of DNA break and they separate. This allows to use each DNA strand as a template.

2. Annealing. The temperature is lowered to ~50 °C for 30 sec. At this temperature, primers can join the complementary regions in the sample DNA.

• Two types of primers are necessary (one primer for each DNA strand).

• The primers flank the DNA region which must be amplified as DNA polymerase can extend their 3' end, but not the 5' end.

• If primers cannot bind the DNA in a sample (this happens when complementary regions are not present is the sample DNA), PCR does not proceed. This fact can be used to detect certain DNA in a sample. This allows to use PCR for diagnosis of many diseases.

3. Extension. The temperature is increases to $72\,°C$ for 90 sec. The Taq polymerase extends primers by adding nucleotides complementary to the template.

After these 3 steps the number of DNA copies in a reaction mixture is doubled. This can be repeated until the number of target DNA reaches millions.

Despite its speed and specificity, PCR cannot substitute for gene cloning in cells because of occasional errors occurring in PCR. They limit the number of good copies and the length of DNA fragments that can be copied.

5. SOUTHERN BLOT

Southern blot. Southern blot is a method of molecular biology used for detection of a specific DNA sequence in DNA samples.

1. Restriction endonucleases are used to cut DNA from the sample into smaller fragments.

2. The DNA fragments are then separated by size. This is done by the technique called *gel electrophoresis*.

• DNA fragments are placed into wells situated on one side of a square-shaped block of *agarose gel*.

• Electric current is applied to the gel.

• As DNA has charge, DNA fragments start to move from the wells through the agarose gel in the direction from the negative electrode to the positive electrode.

• Shorter DNA fragments move through the gel faster, so all the DNA fractions ultimately separate into fractions of different length.

3. The DNA gel (with DNA in it) is placed into an alkaline solution to denature the double-stranded DNA.

• A sheet of nitrocellulose is placed on top of the gel and a stack of paper towels and a weight on are placed on the top. A buffer solution moves by capillary action from through the gel, membranes and paper towels. This transfer of buffer takes the DNA to the membrane due to the negative charge of the DNA and positive charge of the membrane.

• The membrane is processed to permanently attach the transferred DNA to the membrane.

• The membrane is then exposed to a hybridization probe. The probe is a single-strand DNA fragment with a specific nucleotide sequence complementary to the target which must be detected. The probe DNA is labelled by radioactive tags. The probes attach to the complementary DAN regions.

• After hybridization, excess probe is washed from the membrane

• The pattern of hybridization is visualized on X-ray film by autoradiography. Only the bands corresponding to the DNA fractions containing the target DNA are visualized.

6. GENE THERAPY

Gene therapy. The methods of DNA delivery into the cell can be used for the treatment of genetic disorders.

One type of severe combined immunodeficiency (SCID) caused by defect in a single gene. This was tried to be cured by gene therapy inserting the normal version of the gene. The treatment includes:

• Insertion the RNA version of normal allele into a viral vector.

• Taking patient's bone marrow cells and their culturing.

• Infection of the cells with the viral vector.

• The virus inserts the normal version of the gene into the genomes of the cells.

• Cells are injected back to the patient.

The trials of such therapy were done in France in 2000. Ten young children with SCID were treated. Nine of them showed significant, definitive improvement after two years. However, three of the patients subsequently developed leukemia and one of them died.

Recently the first successful clinical gene transfer in hemophilia B was reported. The hemophilia B is caused by coagulation factor IX deficiency.

The methods of biotechnology promise gene therapy of many diseases. Different techniques are used in tries to cure AIDS, various types of cancer and other genetic disorders.

Lecture 5. GENETIC AND PHENOTYPIC VARIATION

Outline:
1. The variability of living matter.
2. Phenotypic plasticity.
3. Genetic variation and sexual reproduction.
4. Mutations.
5. Mutations and cancer.

1. THE VARIABILITY OF LIVING MATTER

Basic concepts and types of variation. In biology *variation* means any difference between cells, individual organisms, or groups of organisms of any species caused either by genetic differences or by the effect of environmental factors on the expression of the genetic potentials.

• The variation primarily means the difference in phenotypes.

• *Phenotype* is the sum of all the characteristics of an organism as determined by the interaction of its genetic constitution and the environment.

• Phenotype of any organism or cell is determined by:

1. Its genotype. Genotype is the set of all cell's or organism's genes and gene variants (alleles).

2. The environment. Changes in the environment cause organisms to adapt to the new environmental factors.

3. Stochastic processes (i. e. randomness). This is based on unpredictable motion of individual molecules in cell's media, inconsiderable difference in enzyme concentrations and etc.

The variation caused by genetic differences is called *genotypic variation*.

• This variation is based on *recombination of parental alleles* and occurrence of new alleles via *mutations in DNA*.

The variation caused by effect of environmental factors is called *phenotypic variation* or *phenotypic plasticity*.

2. PHENOTYPIC PLASTICITY

Phenotypic plasticity is based on changes in an organism's behavior, morphology and physiology in response to a unique environment.

• Commonly it includes *modifications* of the phenotype which *adapt the organism to new environmental factors*.

• Examples:

– suntan occurs as protection from the excess of ultraviolet by accumulation of melanin in skin cells;

– the length of the hair coat of animals increases by winter;

– the leaf shape of a water plant *Sagittaria* depends on the location of the leaves (in water, on the water surface, above water).

– Plants growing in dryer soil grow longer roots than the same plants in moist soil.

– The concentration of hemoglobin and red blood cells in blood increases at altitude (as saturation of blood with oxygen at altitude decreases).

• The changes of the phenotype occur without the changes of the genotype.

• As the genotype is not affected, the modifications of the phenotype are not inherited by descendants.

• The phenotypic modifications are reversible.

• The reaction to particular environment is normally same in all individuals of the same species (or even in closely related individuals of different species). Hence, the changes in the phenotype can be predicted as they are not random.

The possible range of phenotypical changes caused by the environment is called the *reaction norm*. For example, leafs of a tree may have different size, though there are minimal and maximal sizes. The range within which the leaf size can vary is the reaction norm for this trait.

3. GENETIC VARIATION AND SEXUAL REPRODUCTION

Genetic variation and fitness. Although all individuals of the same species have same genes, they are different. Mostly this occurs because the same gene may have several variants.

- Different variants of the same gene are called *alleles*.

- One gene may have many different alleles, but every person may have two of them (because we have 2 copies of all genes due to diploid (2n) set of chromosomes).

- As alleles of the same gene are different, they may code for slightly different variants of the same type of protein.

- These differences may have effect on *fitness* of an individual.

- Fitness defines how good an animal is adopted to its environment.

- Organisms with higher fitness are those who adapted to the environment better than other organisms of the same species.

- For example, a sand-colored fish might be adapted for the life on the bottom better than a dark fish of the same species because it is less visible for predators. The sand-colored fish are eaten less frequently. Though if the same species leaves in a lake where the bottom is covered by dark stones, the sand-colored fish would have lower fitness as it is more visible for predators than the darker fish.

- Those who have higher fitness must produce more progeny. The progeny has the alleles from parents. The number of individuals carrying «better» alleles increases because they multiply better. This process is an example of natural selection.

- The difference in such phenotypes is often determined by different alleles of a gene and by various combinations of the alleles of different genes.

- The existence of different variants of the same gene can be useful for species as at least part of individuals may have the alleles increasing the fitness in a new environment.

Genetic variation due to recombination of parental alleles. The new combinations of parental alleles can be created by sexual reproduction (the asexual reproduction only copies all the parental genes). Gene recombination in sexual reproduction includes 3 main mechanisms:

1. Recombination by crossing over.

Crossing over is the exchange of the same regions of homologous chromosomes.

For example, there is a chromosome containing the genes *A, B, C, D* in a cell.

The other homologous chromosome must have same genes situated in the same loci (regions). Though the alleles of these genes might be not same, e. g. *a, b, c, D* (in this example we use capital and small letter to denote different alleles of the same gene, e. g. *A* and *a*, *B* and *b* and etc.).

In prophase of meiosis I homologous chromosomes connect (this process is synapsis) and form a bivalent. The bivalent must have the genes of both the chromosomes: $\frac{ABCD}{abcD}$.

When crossing over occurs, the alleles are exchanged, and the regions where crossing over occurs are relatively random. That is why different cells may produce different allele combinations in chromosomes of the same bivalent: $\frac{aBCD}{AbcD}$,

$\frac{abCD}{ABcD}$, $\frac{abcD}{ABCD}$, $\frac{aBcD}{AbCD}$.

Hence, the chromosomes in all the gametes produced after meiosis will be different.

2. Random combination of chromosomes in meiosis.

• Cells dividing by meiosis are diploid (2n). They have double set of chromosomes.

• Let's consider a hypothetic cell which has 2 pairs of chromosomes (2n=4). Such cell must have 2 chromosomes #1 (let's denote them as 1_a and 1_b) and 2 chromosomes #2 (2_a and 2_b).

• After meiosis, daughter cells are haploid (1n). They have one set of chromosomes. Every cell inherits random chromosome from each pair of chromosomes.

• In our example, daughter cells may have four different combinations of chromosomes: 1_a+2_a, 1_b+2_b, 1_a+2_b, 1_b+2_a.

• The cell with 3 pairs of chromosomes must have 8 different chromosome combinations, that with 4 pairs — 16 combinations. Humans have 23 pairs of chromosomes, so we can produce $2^{23} = 8\ 388\ 608$ different types of gametes with different combinations of chromosomes.

• This does not take crossing over into consideration, the actual number of different allele combinations is more by several orders.

3. Random combinations of different gametes in fertilization.

• As shown above, every individual may produce very large number of allele combinations in its gametes.

• The number of zygotes with different allele combination is equal to the number of all types of ova produced by a mother multiplied by the number of all types of sperms produced by the father.

• Even without considering the crossing over the number of all combinations of parental chromosomes in a human's zygote is near 7×10^{13} ($8\ 388\ 608^2$).

All these 3 mechanisms provide creation of individuals with the new combinations of existing alleles. The new alleles can appear or the number of genes can change when *mutations* occur.

4. MUTATIONS

General characteristics of mutations. Mutation is a relatively permanent heritable change in genetic material of the cell.

• The creation of mutations is called *mutagenesis*.

• Mutations can occur as errors in DNA replication, repair, or recombination that result in base-pair substitutions, insertions, or deletions.

44

• The agents that interact with genetic material to cause mutations are called *mutagens*.

• Mutations may occur *spontaneously* or they can be *induced* by researchers in a laboratory.

• Mutations may occur *in somatic cells*, then they may change the phenotype of the individual; such mutations are inherited via asexual reproduction. Other mutations may occur *in gametes* so they have effect on the phenotype of descendants transmitted by sexual reproduction. If mutations occur in embryo's cells, they can have effect on its phenotype, and also can be present in gametes.

• The effect of mutations can be beneficial (seldom), deleterious (more commonly) or neutral (most of point mutations in human).

Based on the level where mutation occur, they can be divided into:

• Gene mutations — changes in the nucleotide sequences.

• Chromosome mutations — alternations of chromosome structure.

• Genome mutations — alternations of the chromosome number.

Gene, chromosomes and genome mutations. The mutations affecting nucleotide sequences include point mutations which are changes of a single base pair.

Types of point mutations:

− *Transition* is a mutation that substitutes a nucleotide with another nucleotide of the same type (purine with another purine or pyrimidine with another pyrimidine: A ↔ G, C ↔ T).

− *Transversion* is a mutation that substitutes a purine nucleotide with pyrimidine one or vice versa: pyrimidine nucleotide with purine (A ↔ C; G ↔ T).

− *Insertions* and *deletions* are the mutations is which base pairs are added or deleted from DNA.

The effect of such mutation on genes can be different (fig. 9):

• *Silent mutations* occur when the encoded amino acid is not changed as several codons may code for the same amino acid.

• *Missense mutations* occur when the encoded amino acid changes.

• *Nonsense mutations* occur when a codon is altered into a stop codon (UAA, UAG, UGA). The synthesis of a protein is terminated at a stop codon.

• *Reading frame shift* is deletion of a base pair (or several base pairs) or insertion of one or several base pairs which changes the reading of all further codons in a gene. This occurs when the number of deleted or inserted base pairs in indivisible by 3.

Chromosome mutations may involve one or several chromosomes.

• *Deletion* is loss of a chromosome region. This may occur due to unequal crossing over. *Deficiency* is a deletion of a terminal chromosome region.

• *Duplication* is doubling of a chromosome region. This also may occur due to unequal crossing over.

• *Inversion* is alternation of genes' arrangement in a chromosome.

Type of Change	Mutation in the DNA	Example
None	None	5′–A–T–G–A–C–C–G–A–C–C–C–G–A–A–A–G–G–G–A–C–C–3′* Met – Thr – Asp – Pro – Lys – Gly – Thr –
Silent	Base substitution	5′–A–T–G–A–C–C–G–A–C–C–C–**C**–A–A–A–G–G–G–A–C–C–3′ Met – Thr – Asp – Pro – Lys – Gly – Thr –
Missense	Base substitution	5′–A–T–G–**C**–C–C–G–A–C–C–C–G–A–A–A–G–G–G–A–C–C–3′ Met – **Pro** – Asp – Pro – Lys – Gly – Thr –
Nonsense	Base substitution	5′–A–T–G–A–C–C–G–A–C–C–C–G–**T**–A–A–G–G–G–A–C–C–3′ Met – Thr – Asp – Pro – **STOP!**
Frameshift	Addition/deletion	5′–A–T–G–A–C–C–G–A–C–**G**–C–C–G–A–A–A–G–G–G–A–C–C–3′ Met – Thr – Asp – **Ala** – **Glu** – **Arg** – **Asp** –

Fig. 9. Consequences of point mutations within the coding sequence

• If telomere regions of both chromosome's arms are deleted, connection of the remaining ends into a ring may occur and form *ring chromosomes.*

• Translocations occur when a chromosome region is transferred to another chromosome.

• *Robertsonian translocation* is connection of two acrocentric chromosomes at the region of their centromeres.

Genome mutations include events alternating the number of chromosomes.

• *Haploidy* is a single chromosome set.

• *Polyploidy* is multiple chromosome set (3n, 4n, 5n)

• *Heteroploidy* (aneuploidy) is an abnormal number of chromosomes which is indivisible by *n* (2n ± 1, 2n ± 2 and etc.).

– *Trisomy* is presence of 3 homologous chromosomes instead of two.

– *Monosomy* is absence of one chromosome in a homologous pair.

Mutagens. Mutagens, the agents interacting with genetic material to cause mutations, can be of 3 types.

1. *Physical mutagens* are ionizing radiations such as X-rays, gamma rays and alpha particles.

• Ionizing radiations damage DNA and other molecules (proteins, carbohydrates and etc.).

• The damage can be caused directly by ionization or excitation.

• The damage can be caused indirectly through highly reactive *free radicals* produced by radiolysis of cellular water.

• Ultraviolet rays are absorbed by macromolecules and cause their excitation. This often leads to cross-linking of adjacent pyrimidine bases in nucleotide chains (TT, TC, CC, UU).

2. *Chemical mutagens* are the chemical agents causing mutations.

• They can modify bases of DNA, so bases can be changed into their analogs. The analogs may basepair differently from original bases, so base substitution may occur after replication.

• Some chemical mutagens (e. g. HNO_2) cause deamination (i. e. removal of amino groups) of bases. For example, deamination of cytosine results in formation of uracil by replacing $-NH_2$ group with $-OH$ group. Therefore, after replication base pair C-G is replaced by U-A pair.

• Alkylating agents may cause addition of an alkyl groups. Such base modifications may cause loss of the alkylated base by breaking the between the base and deoxyribose.

• Intercalating agents such as certain dyes (e. g. acridine orange) can insert themselves in DNA (i. e. intercalate the DNA) between the bases in adjacent pairs. They distort the DNA and cause deletion or insertion after replication of DNA molecule.

3. *Biological mutagens* include:

– Viruses which may insert their genetic material into the cell's genome and disrupt genes or DNA sequences controlling their expression.

– Transposons — the nucleotide sequences which may «travel» within the genome. Despite they are natural in genomes of cells, their behavior is similar to that of viruses. Transposons contain genes providing their movement within the genome. When insert themselves into new DNA location, they also may disrupt functional regions in DNA.

Not all mutations cause deleterious effect on cells. This is due to several factors.

• Cells have mechanisms for DNA repair.

• As DNA has 2 strands, it stores genetic information in 2 copies.

• Diploid chromosome set provides the second copy of every gene, so if one of them is damaged, the other one can continue to work.

• Some genes may have more than one copy in the genome.

• Different codons may specify the same amino acid. Hence, some point mutations do not change the sense of the codon.

• Most of eukaryotic DNA does not code for proteins and may even have no distinct functions. Mutagens can damage such DNA regions not reaching functional genes.

• Antimutagens may reduce frequency of mutations. Such substances may activate mechanisms of DNA repair or interact with mutagens to preven mutations.

Repair of DNA. Cell may use several different mechanisms to repair their DNA.

• Pyrimidine dimers can be repaired by the enzyme *photolyase*. The work of this enzyme requires absorption of blue or ultraviolet light, so the DNA repair by photolyase is called *photoreactivation*. This process is very common unicellular organisms, but does not occur in humans.

• Nucleotide excision repair is used by human cell to repair such damage as pyrimidine dimers. The damaged nucleotides are removed from the DNA strand together with a number of nucleotides upstream and downstream the damaged re-

gion. The gap in the DNA strand is repaired by DNA polymerase using the other strand as a template in a process similar to replication.

• Some physical mutagens, such as high-energy radiation, can cause double-stranded breaks in DNA (i. e. DNA is cut into two). In this case the two broken ends of the chromosome can be simply joined back together (this is called *non-homologous end joining*). The disadvantage of this mechanism is that it typically causes the loss or addition of a few nucleotides at the cut site. Alternative repair mechanism is called *homologous recombination*. In this case the information from the homologous chromosome or from a sister chromatid is used to repair the break.

5. MUTATIONS AND CANCER

Cancer is a group of diseases in which cells do not control their cell cycle and actively proliferate producing a *tumor*.

• Carcinogens are physical and chemical agents that cause cancer by mutating DNA.

• The mechanism causing the development of cancer is generally same — the activation of *oncogenes*.

• *Oncogene* is a gene which causes cancer when abnormally activated.

• Many oncogenes can be brought by the cell by some viruses.

• Most of oncogenes are essential for the cell as they regulate cell cycle and division, and they are called *proto-oncogenes* when work without errors.

Three types of mutations can convert proto-oncogenes to oncogenes:

1. Movement of DNA within the genome. Cancer cells often contain oncogenes separated from its normal control regions and transferred to new locations, even in another chromosome. In its new location, an oncogene may be near active promoters or other control sequences that enhance transcription.

2. Gene amplification. Sometimes more copies of oncogenes are present in a cell than is normal.

3. Point mutations. A slight change in a growth-stimulating protein can make it more active or more resistant to degradation than the normal protein.

Tumor suppressor genes code for proteins that normally inhibit growth. The mutations in such genes can make their proteins unable to suppress cell growth and division, so this promotes the development of cancer.

• The mutations occurring in different types of cancer commonly vary. Mutations of some genes are frequent, the mutations in others are not. For example, the mutation in the *ras* proto-oncogene or *p53* tumor suppressor gene are frequent (present in about 30 % and 50 % of human cancers respectively).

• The *ras* protein relays a growth signal from a growth factor receptor to other proteins ultimately stimulating promotion through the cell cycle. A mutated *ras* gene can produce a hyperactive version of the *ras* protein that stimulates the signal transduction cascade on its own, leading to excessive cell division.

• The tumor-suppressor protein p53 gene is a transcription factor of several genes that promotes the synthesis of growth-inhibiting proteins. p53 stops cell cycle, activates genes involved in DNA repair and when DNA damage is irreparable, activates the genes causing apoptosis (cell death). Mutations in the p53 gene can lead to excessive cell growth and cancer.

Transformation of normal cells into cancerous cells requires more than one somatic mutation. For example, in colorectal cancer develops gradually accumulating mutations in oncogenes and tumor-suppressor genes.

The development of several types of cancers (leukemia, liver cancer, cervical cancer) is associated with viruses. Viruses may add oncogenes to cells, disrupt tumor-suppressor genes DNA, or convert proto-oncogenes to oncogenes.

Lecture 6. GENETICS OF HUMAN

Outline:
1. The objectives of human genetics.
2. Methods used in human genetics
3. Methods of prenatal diagnosis.

1. THE OBJECTIVES OF HUMAN GENETICS

The Genetics of human explains many aspects of human nature. The understanding of genetic peculiarities of human being is of great importance for medicine.

The peculiarities of human genome:

• A genome is the sum of all nucleotide sequences of human contained in a haploid cell.

• The size of human genome is near 3×10^9 base pairs. This is near 1500 times larger than the genome of typical bacterium, but 5–10 times smaller than a genome of a plant (the genome of lilies is 60 times larger than human genome).

• The number of genes is near 20–25 thousand. This is comparable with other animals (mice have near 20 000 genes, drosophila — 14 000, corn plants — near 33 000, wheat — near 95 000).

• Despite human has the best intellect among animals, its genome is quite usual for the animal world. The genome of chimpanzee is 96 % genetically similar to humans, that of a cat is 90 % similar. This should be explained by the idea that not our genes make us unique, but how and when these genes work does.

• Approximately 5000 of genes in a human genome have never been the subject of a single dedicated paper and their functions are not clear.

• Many human diseases are associated with improper work of genes. Disorders can be caused by effect of a single defective gene (monogenic disorders), by the combinations of many genes. According to WHO, the global prevalence of all single gene diseases at birth is approximately 10/1000.

• Of recognized conceptions, 15 % result in spontaneous abortions, up to 60 % of which are due to chromosome abnormalities.

• Near 2 % of newborns may have chromosomal abnormalities.

2. METHODS USED IN HUMAN GENETICS

Various methods of human genetics can be used to study general genetic aspects of human or for diagnosis of genetic disorders.

Methods of molecular genetics. Methods of molecular genetics (e. g. PCR) can be used in researches and for diagnosis of genetic disorders. These methods work directly with DNA, RNA or proteins.
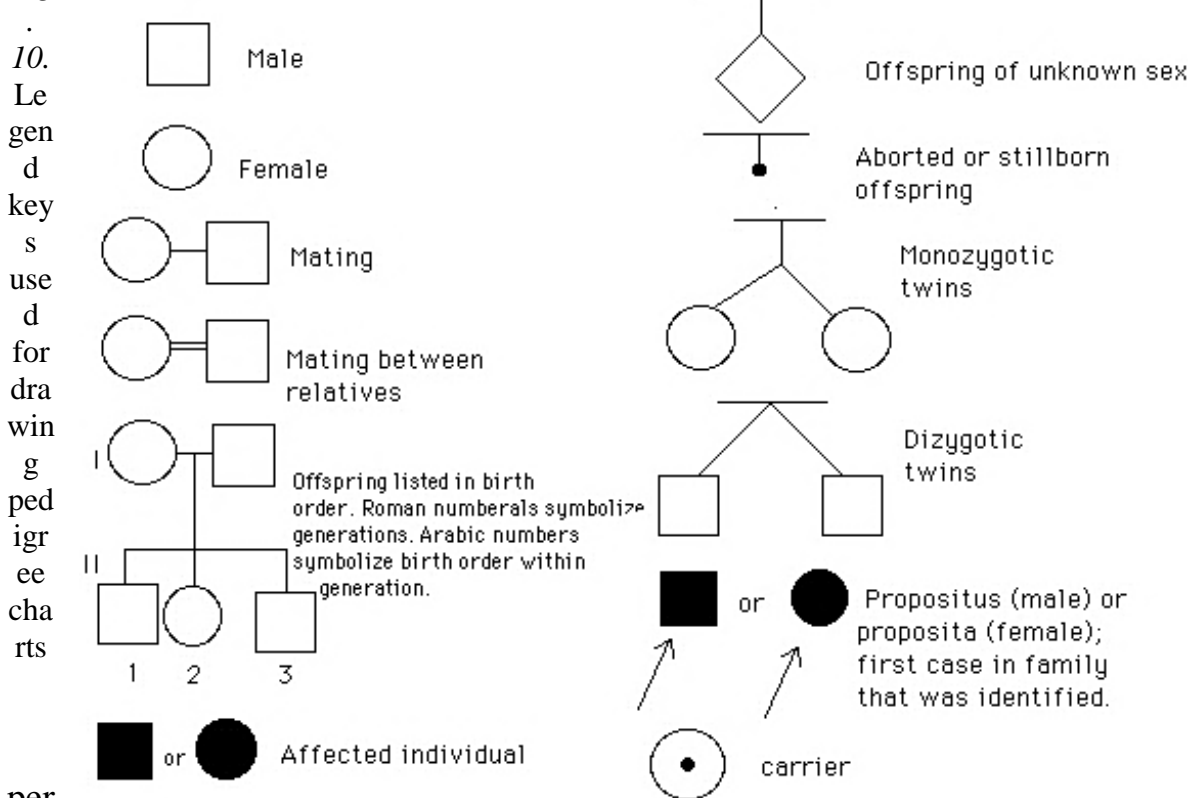
Genealogical method. Genealogical analysis was proposed by Francis Galton in 1883. It relies on drawing and analysis of *pedigree charts* that illustrate inheritance of a certain character (disorder).

The method allows to define:
– whether the character is hereditary or not;
– how it is inherited (type of inheritance);
– zygosity of family members (homo- or heterozygotes);
– gene penetrance (frequency of its manifestation);
– *genetic risk* — probability of giving birth to children with the character.

Legend keys used for drawing pedigree charts are shown in the fig. 10.

Fig. 10. Legend keys used for drawing pedigree charts

person who is studied and from whom the pedigree is composed is called *proband* or *propositus*. It is indicated with arrow.

Stages of genealogical analysis:
- collection of data about proband's relatives;
- drawing a pedigree chart;
- analysis of the chart and drawing conclusions.

As genes can be present in different chromosomes and have different alleles, five patterns of inheritance are possible in humans. This is illustrated in the tabl. 3.

Table 3

Five patterns of inheritance observed in pedigrees.

		Recessive gene	Dominant gene
Autosomal gene		Autosomal recessive inheritance (a)	Autosomal dominant inheritance (A)
Gonosomal gene	In the X chromosome	X-linked recessive inheritance (Xa)	X-linked dominant inheritance (XA)
	In the Y chromosome	Holandric inheritance (Y*)	

- *Autosomal dominant* pattern of inheritance:
- sick children are born by only sick parents;
- both men and women fall ill with equal probability;
- if one of the parents is dominant homozygote then the probability of the character for children is 100 %; if both parents are heterozygous — 75 %, if one parent is heterozygous and the other is recessive homozygote — 50 %.

- *Autosomal recessive* pattern of inheritance:
- sick children can be born by healthy parents;
- both men and women fall ill with equal probability;
- sick persons are not in every generation;
- if both parents are heterozygous then the probability of the character for their children is 25 %; if one parent is heterozygous and the other is a recessive homozygote — it is 50 %; if both parents are recessive homozygotes — 100 %.

- *X linked dominant* pattern of inheritance has the same signs as autosomal-dominant one. The key feature is the fact that a man having the X-linked dominant character can transmit the gene only to daughters (sons get his Y-chromosome).

- *X linked recessive* pattern of inheritance:
- mostly men fall ill;
- sick children can be born by healthy parents;
- sick persons are not in every generation;
- if parents are healthy then probability giving birth to a sick child is 50 % for boys, and 0 % for girls (i. e. 25 %).

- *Holandric* (*Y linked*) pattern of inheritance:
- only men can have the character;
- such men are in all generations;
- if a father is sick then all his sons are sick and vice versa.

51

Twin study. In 1876 Francis Galton proposed the method of twin study. The method is used to estimate roles of heredity and environment in development of a character.

- The frequency of giving birth to twins is near 1 %.

- Twins can be *monozygotic* (MT) if they develop from the same zygote and have identical genotype.

- *Dizygotictwins* (DT) develop from different ova that develop and are fertilized at the same period. Such twins have similar but not identical genotype (as siblings).

- Criteria of zygosity: MT always have same sex, blood group and fingerprints; in DT these factors can be different.

- The degree of twins' similarity on a character is called *concordance*, the degree of their difference is *discordance*. In other words concordance is percent of twin pairs who have the same trait among all twin pairs; discordance is the percentage of twin pairs who are different in the studied character.

- Roles of heredity and environment for development of a character can be calculated by the Holzinger's formula:

$$H = \frac{CMT\% - CDT\%}{100\% - CDT\%},$$

Key: *H* — role of heredity; *CMT* — concordance for monozygotic twins; *CDT* — concordance for dizygotic twins.

- The closer the *H* to 1.0, the more is the influence of heredity on the analyzed characteristic;

- If *H* tends to 0, then mostly environment affects the development of the characteristic and it is not heritable.

Karyotyping. Karyotyping is *studying all chromosomes* (*karyotype*) with microscope. The method reveals abnormalities in the number or structure of chromosomes.

Technique:

- Lymphocytes or cells of bone marrow are obtained from a patient and grown on culture medium.

- Mitosis is stimulated (as chromosomes become distinguishable only in a dividing cell).

- Mitosis is arrested (e. g. by colchicine destroying the mitotic spindle).

- The cells are treated with NaCl hypotonic solution. Cells rupture and release their chromosomes.

- The chromosomes are stained.

- Cells are studied under the microscope, photographs of cells where the chromosomes are clearly distinguishable are taken.

- These photographs are used to comply an ideogram (i. e. arrange all the chromosomes according to the Denver classification) and analyze it.

There are keys to write karyotypes and mutations:
- «*q*» — long arm of a chromosome,
- «*p*» — short arm of a chromosome,
- «+» — excess of genetic material,
- «–» — loss of genetic material.

For example, the karyotype writing for a man sick with Down syndrome caused by the presence of additional 21st chromosome is 47, XY, 21+ (i. e. there are 47 chromosomes, the sex chromosomes are XY, there is extra 21st chromosome), for a girl with Cri du chat syndrome — 46, XX, 5p- (i. e. there are 46 chromosomes, the sex chromosomes are XX, the p arm of the 5th chromosome has a deletion).

Biochemical tests. Biochemical genetic tests are used for revealing metabolic diseases by measuring activity of enzymes or the quantity of the reaction product that is catalyzed by an enzyme.

• Chromatography, fluorometry, radio immunological assay and other methods are used for revealing gene mutations causing metabolic diseases.

• For example, phenylketonuria (the impairment of phenylalanine (PhA) exchange) can be detected by measuring concentration of phenylalanine in blood: in healthy people it is 30–90 µmol/L, in sick ones it reaches 1200 µmol/L.

In different population 1 out of 30–40 people might be a heterozygous carrier of phenylketonuria. Carriage can be revealed by a *loading test*:

• Phenylalanine is injected to the examined person and its concentration in blood is recorded. If the concentration of PhA becomes normal slower than in other people, then the person is a carrier of phenylketonuria.

Hybridization of somatic cells. This method is based on another method called *cell culture*. It is growing somatic cells in nutritive media in a laboratory. This allows to produce multiple clones of a single cell. All of them have same genotype.

Cultivation of somatic cells offers opportunities for somatic cell hybridization — the technique which makes somatic cell fuse together (fig.11).

• Common cells in a medium unlikely fuse together. Though the addition of Sendai virus to a cell culture rapidly increases the incidence of cell hybridization.

• Heterokaryotes — cells containing two different nuclei from fused cells.

• After division of such cell, the daughter cells contain a single nucleus with the chromosomes of both previous nuclei. Such cells are called synkaryotes.

• The hybridization of cells allows to produce synkaryotes of cells taken from different species (human and mouse) and even of different phyla (human and mosquito).

• Synkaryotes are usually successfully obtained if cells are taken from species of the same class.

• Hybrid cells of human and mouse have 43 pairs of chromosomes (23 and 20 from human and mouse).

• Later on, chromosomes of the cell which has longer terms of division are eliminated: human chromosomes are eliminated from human-mouse synkaryote.
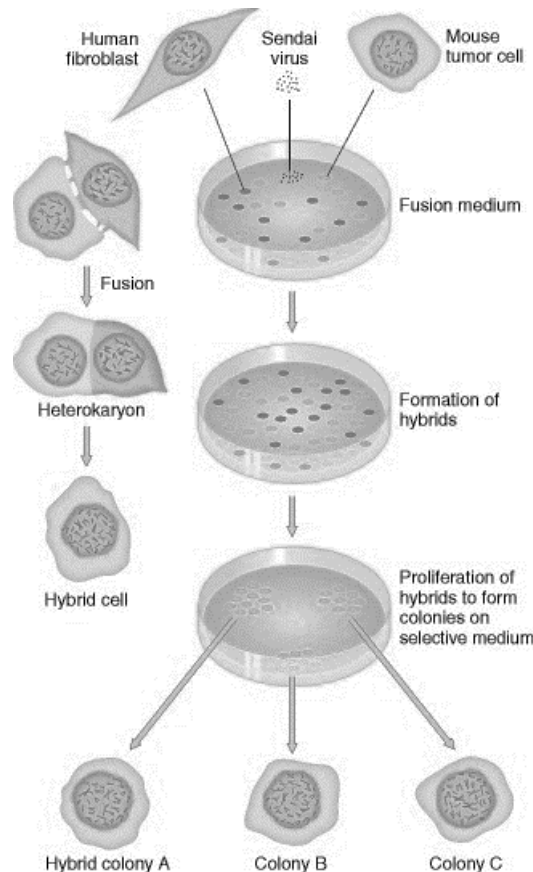

Fig. 11. Hybridization of somatic cells

Barr body test. Female mammals have two X chromosomes in somatic cells. One of them inactivates during embryogenesis. This happens early in embryonic development. The inactivation of X chromosomes is random — some cells inactivate the father's X chromosome while others inactivate the mother's one.

• In 1949 Barr and Bertram reported a difference of nerve cells from male and female cats. Cell nuclei from female cells showed a small darkly staining body near the nuclear envelope, while cell nuclei from males showed no such structure. The darkly staining structure found only in the nuclei of cells from females is known as the Barr body.

• The Barr body is the inactive X chromosome in a female somatic cell. As men have only one X chromosome, it stays active so men do not normally have Barr bodies in their cells. The number of Barr bodies visible at interphase is always one less than the total number of X chromosomes.

• Visualization of the Barr body allows to diagnose abnormalities in the number of X chromosomes (e. g. karyotypes 47, XXY or 45, X0).

Technique of Barr body staining:

1. A drop of water is placed in the center of the glass microscope slide.

2. Inside of person's cheek is gently scraped with the flat edge of a tooth-pick.

3. Cells are spread in the drop of water and the cover slip is placed on top of specimen.

4. One drop of methylene blue is applied along one side of the cover slip.

Dermatoglyphic analysis. Dermatoglyphic analysis studies skin patterns of the fingers, palms and feet. They often have some changes in case of malformations.

• Dermatoglyphic patterns are very individual and do not change during life. There are patterns of three types on finger tips: an arch (A), a loop (L) and a whorl (W).

• There are tri-radii in interfinger spaces: a, b, c and d. Near the bracelet crease is a palm tri-radius *t*. Connection of tri-radii a, d, t, with lines shows a main palm angle. Normally it is not more than 57°.

• The combination of radial loops on 4–5th fingers, palm angle of 60–86° and single transverse palmar crease (fusion of an oblique and transverse line) are typical for those who have congenital malformations.

Guthrie microbiological test (neonatal heel prick). It is used for diagnosis of phenylketonuria. This disorder is associated with increased concentration of phenylalanine in blood. Technique:

• A drop of newborn's blood is taken on a blotting paper.

• The paper is placed on agar medium where bacteria may grow.

• The medium also contains β-2-thienylalanine which inhibits bacterial growth. However, a high concentration of phenylalanine may cause bacteria to multiply and produce visible colonies.

• If the blood of a newborn contained a lot of phenylalanine bacterial colonies appear on the medium.

3. METHODS OF PRENATAL DIAGNOSIS

Prenatal diagnostic methods are the methods detecting a disease of a fetus before birth.

There are *direct* methods (examine the fetus itself) and *indirect* methods (examine the pregnant woman.

Alpha-fetoprotein test. Concentrations of α-fetoprotein (AFP) in the blood of a pregnant woman can be informative for doctors. α-fetoprotein is a protein produced by fetal cells and placenta.

• Low concentration of AFP at the 13–15th weeks of embryonic development might be caused by some trisomies, fetal growth retardation, threatened miscarriage or fetal death.

• High concentration of AFP might be associated with, plural pregnancy, nerve tube defects, congenital nephrosis and some other malformations.

Ultrasonography. Ultrasonography is a *direct non-invasive methods* (non-invasive = without tissues injury) of prenatal diagnosis. It is a diagnostic imaging

technique based on the application of ultrasound. It allows to obtain image of the fetus and embryonic membranes. All pregnant women are tested by the method because it is safe and can be repeated. Ultrasonography reveals vitality of the fetus, twin pregnancy and severe development defects of the skeleton, brain and spinal cord.

Direct invasive methods are the diagnostic procedures on the fetus with tissue injury. They are associated with some risk and performed only for *indications* such as:

– diagnosed hereditary disease in the family;

– mother's age over 37 years;

– carriage of X-linked recessive disorder by mother;

– cases of spontaneous abortions at early stages of pregnancy, stillbirths, children with multiple congenital anomalies and chromosome pathology;

– heterozygosity of both parents with an autosomal-recessive disorder.

Direct invasive methods are:

1. Chorion biopsy is taking chorion cilia through the uterine cervical canal for cytogenetic and biochemical investigations and DNA analysis. It is performed within 8th–13th weeks of gestation under control of ultrasonography. The method reveals gene, chromosome and genome mutations.

2. Amniocentesis is performed within 15–17th weeks. It is puncture of the amniotic sac through the abdominal wall under control of ultrasonography in order to take 15–20 ml of amniotic fluid with fetal cells. Complications in this method arise in 1 % of cases.

Lecture 7. GENETICS OF POPULATIONS

Outline:
1. The Hardy-Weinberg Equilibrium.
2. Natural selection.
3. Non-random mating.
4. Migration.
5. Genetic drift.

1. THE HARDY-WEINBERG EQUILIBRIUM

Genetics of populations. Population is a group of the organisms of the same species, which live in a particular geographical area, and have the capability of interbreeding.

All the genes carried by the members of a population form the *gene pool* of this population (remember that one gene may have different variants called alleles).

Hardy-Weinberg Equilibrium. The Hardy-Weinberg equilibrium is a principle stating that the frequencies of alleles and genotypes in a population will remain constant from one generation to the next in the absence of disturbing factors.

To see how this works, we consider the following example.

There is a gene which has two alleles: *A* and *a*. The gene is inherited in classic Mendel's pattern of complete dominance (the allele *A* is dominant, the allele *a* is recessive).

• There is a population of diploid organisms where the percentage of these alleles is equal (50 % each).

• Then the percentage of the genotype *AA* in this population must be 25 %, that of *Aa* — 50 %, *aa* — 25 %.

• In this conditions the percentage of couples *AA* x *AA* must be 6.25 % (0.25*0.25=0.0625) and all their children must have the genotypes AA (notice that their children must contribute 6.25 % of children in the population); the percentage of couples *AA* x *Aa* must be 12.5 % (0.25*0.5) half of their children have the genotype Aa, the other half — AA and etc.

• If we consider the children from all the families in the population awe will see that the percentages of their genotypes are *AA* = 25 %, *Aa* = 50 %, *aa* = 25 %.

• Hence, the percentage of alleles and genotypes in the new generation is same as in the previous one, as was predicted by Hardy-Weinberg Equilibrium.

The Hardy-Weinberg Equilibrium is a mathematical model which works only in ideal populations. The ideal population in this model has:

− no mutations (so new alleles do not appear);

− no migration (individuals carrying the alleles do not income from other populations);

− no natural selection (parents with all genotypes produce same number of children, none of the alleles increases the reproductive success).

− random mating (individual with any genotype choses a couple with random genotypes);

− infinitive number of individuals (this allows to avoid random statistical fluctuations).

2. NATURAL SELECTION

Natural selection. The idea of natural selection was developed by Charles Darwin.

Natural selection is the differential survival and reproduction of individuals due to differences in phenotype.

Those who have *«alleles providing better reproduction»* produce more progeny, so they produce more copies of these alleles. In the next generation carriers of these alleles again produce more progeny than the carriers of other alleles of the gene. So the number of copies of one allele increases from generation to generation while that of the other allele decreases.

Let's see how this proceeds in an example.

There is a population of haploid animals (for simplicity of the model).

• All the animals have same genes, one gene has two alleles: *B* and *b*.

• Those who have the allele *B* produce on average 2 % more progeny than those who have the allele *b*.

• 50 % of the animals have the allele *B* and 50 % have the allele *b* ($f_{(B)} = 50$ %, $f_{(b)} = 50$ %).

• After one cycle of reproduction, the ratio 50/50 changes as the carriers of *B* produce 2 % more progeny: $f_{(B)} \approx 50.5$ %, $f_{(b)} \approx 49.5$ %.

• After another cycle of reproduction $f_{(B)} \approx 51$ %, $f_{(b)} \approx 49$ %.

• The percentage of individuals carrying the «*allele of better reproduction*» *B* would increase until reaches 100 %.

Fitness is a value which measures the reproductive success of an organism or contribution of a genotype to the next generation compared to the contributions of alternative genotypes for the same locus.

The natural selection arises from several properties of living matter:

1. Reproduction. All species produce progeny.

2. Heredity. Progeny inherits the characteristics of parents. i. e. parents transfer their variants of genes to children.

3. Variation. Progeny differs from parents. Children have new combinations of parental alleles; genes can mutate and the mutations produce new alleles. Hence, descendants have new and different phenotypes.

With these different phenotypes, individuals in a population have different chance to produce fertile progeny (i. e. they have different fitness). This is because particular phenotypes can be more favorable for survival and reproduction in the environment where the population lives.

• For example, the Galapagos finches studied by Darwin had different size and shape of beaks.

• These shapes are evolutionary adaptations to different food sources. Average beak depth (an inherited trait) oscillates with rainfall.

• In wet years, birds preferentially feed on small seeds, and average beak depth decreases.

• In dry years, small seeds are less plentiful, so survival depends on the finches being able to crack the less preferred larger seeds, so the birds with can do it produce on average more progeny than the birds unable to crack the larger seeds. Average beak depth increases during dry years.

Types of natural selection. The heritable traits may vary among the population.

Natural selection can change the frequency distribution of heritable traits, depending on which phenotypes are favored in current environmental conditions. The natural selection can be of several types (see the fig. 12):

1. *Stabilizing selection* acts against both extreme phenotypes and favors intermediate variants of a heritable characteristic.

• For example, the number of eggs laid by certain bird species does not vary considerably. The number of the laid eggs is a heritable character.

• If genetic variants make the bird lay too many eggs, then the larger number of chicks hatching from them would require more food.

• The amount of food provided by two adults might be not enough, so the chicks have lower chance to survive.

• If genetic variants make the bird lay very small number of eggs, the chicks would receive much food, so their chance for survival is high. Though if the number of chicks raised by other bird families is more. So, the low number of descendants decreases the number of the gene variants providing laying small numbers of eggs.

• Hence, the birds laying moderate number of eggs are favored: they produce the number of descendants which can be fed and is enough to transfer genes to the next generation.
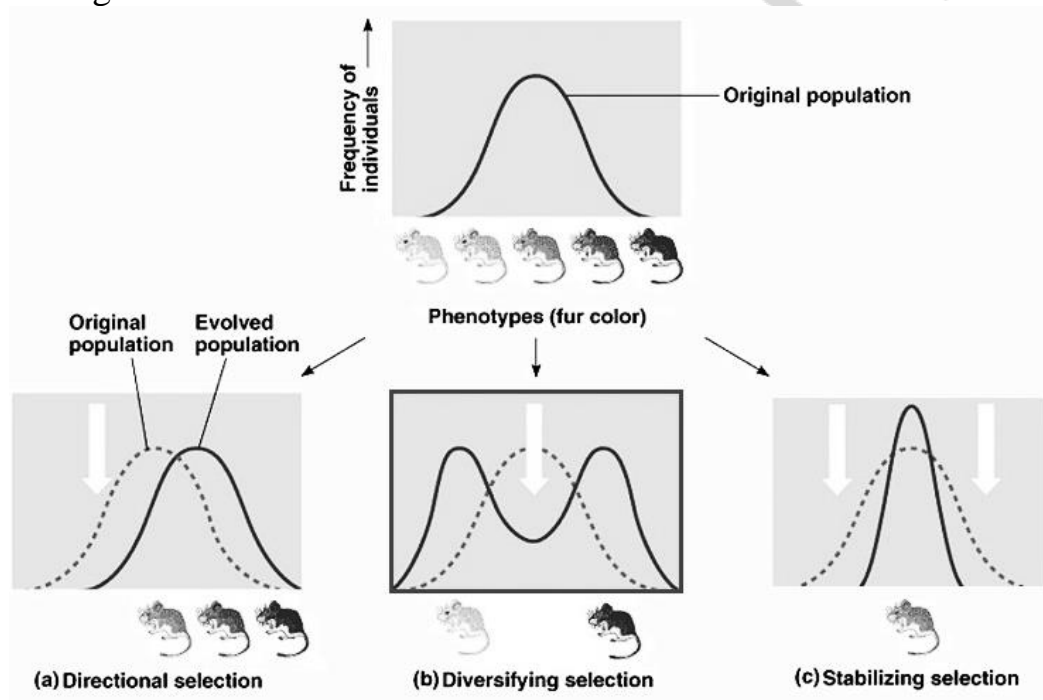

Fig. 12. Types of natural selection. The horizontal axis in the graphs is the darkness of coat of mice (from lighter to darker), the vertical axis is the number of mice of each color. In the original population, most mice of have moderate color and very light/very dark colors are in the smallest number of mice. If the population migrates to the dark terrain, the darker color is favored (*a*). In the new terrain consisting of black and light stones, the median color becomes the less favorable (*b*). If the new terrain has no dark and light elements, the median value of color is favored (c).

2. *Directional selection* acts against individuals exhibiting one extreme of a phenotypic range and favors individuals exhibiting other extreme of a phenotypic range.

• For example, many adult humans can drink milk and successfully metabolize lactose. This is not common in other adult mammals as they are intolerant to lactose.

• Milk of domestic animals could be additional source of food for ancient humans.

- Those who got mutations in particular DNA sequences and became more tolerant to lactose could use that food source, this could increase their chance to survive and produce progeny.
- By this mechanism the new genetic variants providing lactose tolearnce spread in many populations pf ancient humans.

3. *Disruptive selection* also known as *diversifying selection* occurs when conditions favor individuals at both extremes of a phenotypic range over individuals with intermediate phenotypes.

- For example, a population of black-bellied seedcracker finches may have two distinctly different beak sizes.
- Large-billed birds crack hard seeds while small-billed birds feed mainly on soft seeds.
- The birds with intermediate-sized bills are relatively inefficient at cracking both types of seeds and thus have lower relative fitness.

3. NON-RANDOM MATING

The Hardy-Weinberg Equilibrium works only if mating in the population occurs randomly. In real populations mating occurs *not* randomly.

The phenomenon occurring when members of one biological sex choose mates of the other sex to mate with, and compete with members of the same sex for access to members of the opposite sex is called *sexual selection*.

The sexual selection enhances the effect of natural selection. Preferring particular phenotypes is also at least partially heritable. Hence, those who choose the partner with higher fitness, provides both the genes of high fitness and genes for preferring such phenotypes.

4. MIGRATION

Migration. Migration is travel of individuals from the population (emigration) or into the population (immigration). These individuals carry their genes and genetic variants to/from the population and disturb the distribution of genetic variants in the population. The transfer of alleles between populations is called *gene flow*.

5. GENETIC DRIFT

Genetic drift. In small populations, allele frequencies fluctuate by chance over time. This process is called genetic drift. Large populations also exhibit genetic drift, but its effect is much weaker and often inconsiderable. Random fluctuation in gene frequencies are completely absent only in the populations with the infinitive number of individuals which is not possible in the nature.

Genetic drift in small populations can even eliminate one of the alleles after another fluctuation. This is shown in the fig. 13. By this mechanism, favorable mutations can become eliminated even if they increase fitness and deleterious mutations can reach large frequencies despite they decrease fitness. This is one of the

reasons why small populations often have unfavorable genetic characteristics, e. g. higher incidence of genetic disorders and malformations (another important factor is mating between related individuals which increases the chance of giving birth to recessive homozygotes).
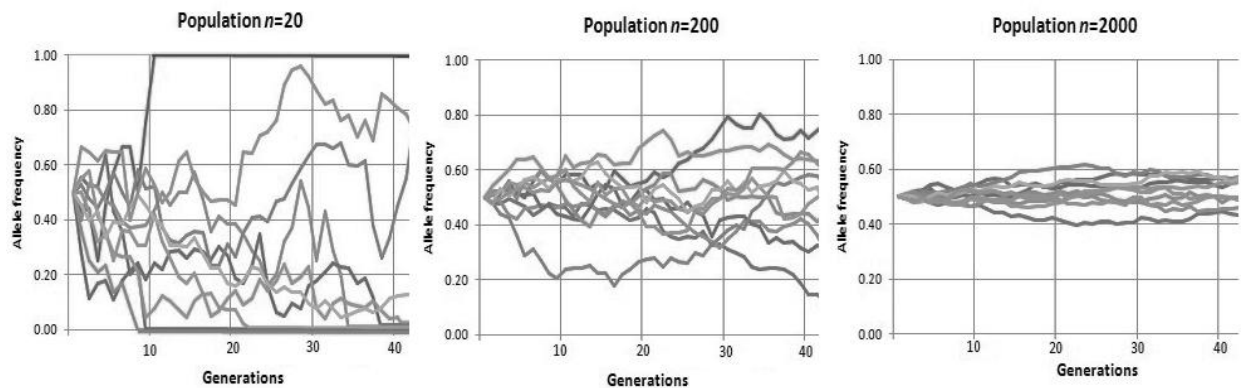

Fig. 13. Genetic drift. The frequency of ten different alleles is monitored.
The frequencies change stronger in smaller population and easily reach 100 % or 0 %

If the number of members in a population becomes small, genetic drift may increase. This is observed in founder effect and bottleneck effect.

• *Founder effect* is the loss of genetic variation that occurs when a new population is established by a very small number of individuals from a larger population. If one of the few founders has a rare genetic variant (by chance), the frequency of this variant in a growing population might be high.

• *Bottleneck effect* occurs when a population's size is reduced for at least one generation. Because genetic drift acts more quickly to reduce genetic variation in small populations, undergoing a bottleneck can reduce a population's genetic variation by a lot.

Lecture 8. ONTOGENY

Outline:
1. Fertilization.
2. Cleavage. Formation of blastula.
3. Gastrulation. Germ layers.
4. Organogenesis.
5. Mechanisms underlying cell differentiation.
6. Teratogenesis
7. Postnatal ontogenesis.

1. FERTILIZATION

Fertilization. The development of animal, including human development, begins from divisions of zygote.

• Zygote is a diploid cell formed when a sperm and egg merge in the process called *fertilization*.

• Fertilization not only unites the genetic materials from two parents, but activates the egg, so it starts to develop.

• Fertilization includes acrosomal reaction, cortical reaction, and activation of the egg.

Fertilization is well-studied on *sea urchins*:

1. The acrosomal reaction is the release of hydrolytic enzymes from the acrosome of a sperm. Acrosome is a cap-shaped vesicle in the head of the sperm which is a storage for these enzymes.

• The acrosome releases hydrolytic enzymes via exocytosis when contacts the jelly coat (the outer covering) of the egg.

• The enzymes cause the elongation of an acrosomal process and it penetrates the jelly coat.

• The tip of the process with its proteins contacts with the specific receptors on the egg.

• Due to its enzymes, acrosomal process penetrates through vitelline layer (one of the covering of the egg outside the plasma membrane) and contacts with the plasma membrane of the egg. The membranes of the sperm and egg fuse. The nucleus of the sperm enters the egg.

• This causes depolarization of the plasma membrane i. e. membrane loses its polarity. This is one of the two mechanisms necessary to prevent polyspermy (entrance of other sperms to the ovum). The depolarization of the egg is the *fast block to polyspermy*.

2. The cortical reaction is a process in which intracellular signals cause cortical granules to release their content from the egg cell. *Cortical granules* are the membrane vesicles situated in the outer layer of egg's cytoplasm adjacent to the plasma membrane.

• The fusion of egg and sperm causes the cell to release calcium (Ca^{2+}) from the egg cell's endoplasmic reticulum to the cytosol.

• The increasing concentration of Ca^{2+} causes the cortical granules to fuse with the plasma membrane and release their contents into the *perivitelline space* (the space between vitelline layer and the plasma membrane).

• The contents of the cortical granules separate the vitelline layer from the plasma membrane.

• Water is attracted to the perivitelline space by osmosis so it elevates the vitelline layer. The modified covering of the egg is called *fertilization membrane*.

• The fertilization membrane and other changes of the egg's surface prevent polyspermy (slow block of polyspermy) consisting of the fertilization membrane and other

3. Activation of the egg occurs when sperm enters the egg, but can be artificially induced by injection of Ca^{2+}. Hence, apart from stimulation of cortical reaction, the high concentration of Ca^{2+} also causes metabolic changes in the egg cell.

- Protein synthesis and ATP production rates increase.

- The nucleus of the sperm (which is now in the egg) swells and fuses with the nucleus of the egg. Zygote starts to replicate its DNA and begins first division in near 1.5 hours.

Fertilization in mammals. In general, the process of fertilization is sea urchin and mammals is similar, though some differences are present.

- Terrestrial animals, including humans, generally have internal fertilization.

- The secretion in the female's reproductive tract enhanced sperm function. This process is called capacitation.

- The secondary oocyte (egg) released at ovulation is surrounded by the layer of follicle cells (it is called *corona radiata*) and the sperm must migrate through this layer.

- The egg cell itself is covered with the network of cross-linked glycoprotein filaments. This covering is called *zona pellucida*.

- One of the glycoproteins in the zona pellucida can be recognized by proteins on the sperm's surface. Their binding stimulates an acrosomal reaction.

- Hydrolytic enzymes released from the acrosome help the sperm cell to penetrate the zona pellucida and reach the plasma membrane of the egg cell.

- The membranes of the sperm and egg cell fuse, the depolarization of egg's plasma membrane occurs.

- A cortical reaction occurs. Instead of raising a fertilization membrane, the contents of the cortical granules stimulate a hardening of the zona pellucida.

- The whole sperm is pulled into the egg cell.

- The basal body from the sperm's flagellum divides and forms the centrosomes of the zygote.

- Nuclei do not merge, but the chromosomes from both nuclei share a common spindle apparatus for the first mitotic division of the zygote.

2. CLEAVAGE. FORMATION OF BLASTULA

Cleavage is the process un which zygote undergoes many mitotic divisions into many smaller cells. These dividing cells are called *blastomeres*.

- The G_1 and G_2 phases in dividing blastomeres are very short.

- Very little gene transcription occurs during cleavage. In vertebrates, first generations of blastomeres use mainly the maternal mRNA accumulated in the ovum before.

- The zygote's cytoplasm is heterogenous. The blastomeres which inherited different pieces of the zygote's cytoplasm contain different cytoplasmic components.

- The polarity is observed in the eggs of most animals. It results from concentration gradients in the egg of such cellular components as mRNA, proteins, and yolk.

- The division of the zygote follows a specific pattern based on the location of poles.

- The yolk gradient is an important factor determining polarity of the zygote and influencing the cleavage pattern in frogs and other animals.

- The highest concentration of yolk is on the *vegetal pole* of the egg while the lowest one is on the opposite side — on the *animal pole*. The cleavage in the animal hemisphere is more rapid than in the vegetal hemisphere.

- A zygote of a frog has apparent yolk gradient. This causes different rate of cleavage on the poles, so the cells in the different hemispheres of the embryo have different size (smaller on the animal hemisphere as cells there divide more rapidly).

- Sea urchins and many other animals have small amounts of yolk in their eggs. Their blastomeres are about equal in size. Though animal and vegetal poles are present but determined by the concentration gradients of other cytoplasmic components.

- The complete division in eggs with little yolk or moderate amounts of yolk (e. g. sea urchins, frogs), is called *holoblastic cleavage*.

- In eggs which contain large amounts of yolk (e. g. birds, other reptiles), cleavage occurs only in a small disc of yolk-free cytoplasm at the animal pole of the egg. Such incomplete division of the zygote is called *meroblastic cleavage*.

- Cleavage produces a solid ball of cells called a *morula*.

- Then a fluid-filled cavity called *blastocoel* develops within the embryo. With this cavity the embryo is called a *blastula*.

- In sea urchins, the blastocoel is in the center of the blastula due to equal cell divisions. The wall of its blastula has one layer of cells.

- In frogs, the blastocoel is in the animal hemisphere because of unequal cell divisions (caused by yolk gradient).

3. GASTRULATION. GERM LAYERS

Gastrulation is the process in animal's embryonic development when blastula is rearranged to form an embryo with a *primitive gut* and *germinal layers*. Such embryo is called gastrula.

- Specific details of gastrulation may vary in different animal groups.

- The three layers produced by gastrulation are embryonic tissues called *embryonic germ layers*. The germ layers eventually develop into all organs and tissues of the adult animal.

- In sea urchin, gastrulation begins at the vegetal pole. This process is illustrated in the fig. 14.

- The wall of blastula there buckles inward forming a deep, narrow pouch which is the primitive gut. Such process is called *invagination*.

- Cells near the region undergoing invagination detach and migrate into the blastocoel.

• The opening of the primitive gut where invagination started is called blastopore. In adult sea urchins it forms the anus. The second opening forms at the other end of the archenteron, forming the mouth end of the rudimentary digestive tube.

• The cells of the archenteron in such embryo are mesoderm, the cells which stay on the surface are ectoderm, the cells which migrate into the blastocoel are mesoderm.

• Gastrulation during frog development also results in an embryo with the three embryonic germ layers and an archenteron that opens through a blastopore. Though its mechanism is more complicated as the wall of frog's blastula contains many layers of cells and the size of cells is different.
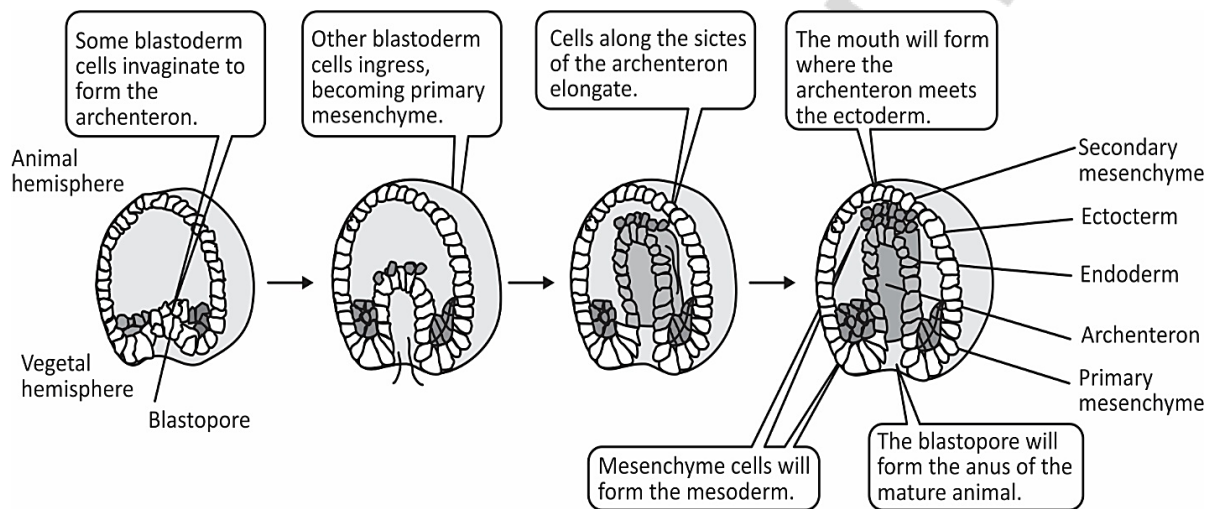

Fig. 14. Gastrulation in sea urchin

4. ORGANOGENESIS

Organogenesis. Organogenesis is the process in which the germ layers that developed during gastrulation give rise to rudimentary organs.

• The first organs developing in chordates (including frogs) are the notochord and neural tube.

• The notochord is formed when dorsal mesoderm above the archenteron condenses.

• The ectoderm above the notochord forms a *neural plate*. It thickens and sinks below the embryo's surface rolling itself into a *neural tube*. The neural tube will develop into the brain and spinal cord.

• Mesoderm cells that form the vertebrae gather around the elongating notochord.

• The vertebrae and muscles associated with the axial skeleton develop from *somites*. Somites are the mesodermal blocks formed from condensing mesodermal cells on the sides of the notochord.

• Further organogenesis, leads to development of other organs and tissues from the tissues of the germ layers (tabl. 4).

Table 4

The fate of embryonic germ layers

Germ layer	Location of the germ layer	Tissues derived from the germ layer
Ectoderm	Outermost layer of the gastrula	Nervous system and epidermis with its derivatives
Endoderm	Lines the archenteron	Lining of the digestive tract and associated organs such as liver, pancreas, lungs.
Mesoderm	Partly fills the space between the ectoderm and endoderm	Notochord, coelom lining, muscles, skeleton, gonads, kidneys and most of the circulatory system

Extraembryonic organs. Embryos of all vertebrates must develop in an aqueous environment. Eggs of fish and amphibians are laid in water. Terrestrial animals live in dry environments and protect their embryos in shelled egg (reptiles, birds) or the uterus (placental mammals).

• The aqueous environment for the developing embryos of reptiles, birds, and mammals is provided by a fluid-filled sac called the *amnion*. This adaptation to terrestrial life gave the name *amniotes* to these groups of animals.

• Another membrane forms a sac around all the embryo and other extraembryonic organs of amniotes is called chorion. It contributes to the placenta in mammals.

• The membranous sac that is attached to the ventral surface of the embryos of birds, reptiles, and some fishes and contains yolk is called *yolk sac*.

• There is another membranous sac called *allantois* growing out of the ventral surface of the hind gut of embryonic amniotes. It collects metabolic wastes produced by the embryo developing in the egg. In mammals it combines with the chorion to form the mammalian placenta.

Mammalian development. Fertilization occurs in the oviducts of most mammals and the early development occurs while the embryo travels down the oviduct to the uterus.

• Eggs of placental mammals contain small number of nutrients. The cleavage is holoblastic.

• At 7th day after fertilization the embryo forms a bubble-like structure consisting of about 100 cells surrounding a cavity. Such embryo is called *blastocyst*.

• The layer of cells surrounding the cavity is called *trophoblast*.

• The cells in the blastocyst's cavity are called *inner cell mass*. They are concentrated at one end of the cavity.

• The trophoblast will develop into fetal part of the placenta.

• The inner cell mass will develop into the embryo and some extraembryonic organs.

• The blastocyst reaches the uterus and implants into the uterine wall.

• The trophoblast secretes enzymes for the implantation. Contacting with the uterus, trophoblast extends fingerlike projections into the uterine tissues starting to form placenta.

• The inner cell mass separates into epiblast and hypoblast.

• The region where they contact will eventually contain the embryonic germ layers which will develop into the embryo.

5. MECHANISMS UNDERLYING CELL DIFFERENTIATION

Mechanisms of cell differentiation. The *differentiation* of cells is their specialization into cells of particular type. Cells become different as they start to express different genes and, hence, produce different proteins. The differentiation of cells in embryogenesis is caused by heterogeneity of egg's cytoplasm and induction.

• The heterogeneous distribution of cytoplasmic components (mRNA, proteins) in the unfertilized egg leads to the presence of different cytoplasmic components in the blastomeres inherited different regions of egg's cytoplasm.

• This results in expression of different genes in these blastomeres and cells eventually differentiate.

• Induction is the interactions between the embryonic cells. Such interactions are due to chemical signals or membrane components.

• In induction cells cause other cell to express particular genes.

The experiment done by Hans Spemann in 1924 shows that one group of cells can induce other cells to differentiate in a specific way.

• The experiment was done with embryos of amphibians.

• A small piece of gastrula was taken from the region of its blastopore (dorsal blastopore lip).

• It was transplanted to the ventral side of another gastrula.

• The recipient embryo formed a second notochord and neural tube in the region of the transplant, and eventually most of a second embryo developed.

• The transplanted dorsal lip *induced cells* in a different region of the recipient to form structures different from their normal fate.

• Finally, the transplanted dorsal lip caused the development of an entire extra embryo.

Cellular potency. Cellular potency is a cell's ability to differentiate into other cell types. The more cell types a cell can differentiate into, the greater its potency.

• The potency of most embryonic cells decreases as the embryo develop. The progressive restriction of a cell's developmental potential is called determination.

• *Totipotent cell* retains the zygote's potential to form new organism.

• For example, if the first two blastomeres after frog's zygote division are separated, each will develop into a normal tadpole.

• In mammals, cells of the embryo remain totipotent until they form blastocyst.

• *Determined cell* is one whose developmental fate cannot be changed by moving it to a different location in the embryo.

6. TERATOGENESIS

Teratogenesis. Teratogenesis is the creation of an abnormal organism with malformations due to a disruption in the structural formation of the organism during one of the developmental stages.

• Any agent that can disturb the development of an embryo or fetus is called *teratogen.*

• In general, each organ of the embryo passes through a period of development during which it is particularly susceptible to teratogenic factors. This developmental phase is commonly referred to as the *critical period* for that organ.

• In most cases the critical period corresponds to the time at which the organ is developing most rapidly.

Common malformations may include:

– congenital absence or abnormal development of an organ or part is *aplasia*;

– incomplete development of an organ or part is called *hypoplasia*;

– enlargement of an organ or part resulting from an increase in the size of the cells is *hypertrophy;*

– degeneration of an organ or tissue caused by loss of cells is *hypotrophy*;

– misplace of normal tissue is called *heterotopy*;

– the absence or closure of a normal body orifice or tubular passage is called *atresia*;

– constriction or narrowing of a duct or passage is called *stenosis*.

7. POSTNATAL ONTOGENESIS

Growth. Growth is the process which provides enlargement of sizes and mass of the body. The growth can be *unlimited* and *limited*. Unlimited growth lasts all the life (crawfishes, fish and reptiles) while limited one stops at the certain age (insects, birds, mammals).

Periods of the most intensive growth of human are:

• The first year of life when body length increases by 25 cm (+50 %) and the body bass by 7 kilograms (+200 %). The speed of growth gradually decreases and after the second year of life the height increases by approximately 12 cm.

• Puberty (the period of sexual maturation) begins in girls around ages 10–11 and ends around 15–17; boys begin puberty around ages 11–12 and end around 16–17. During this period the growth speed increases to 7–8 cm a year and then slows down.

The intensity of growth is not uniform for all different tissues:

• The whole body, muscles, skeleton, respiratory organs, liver follow the described pattern of growth and grow rapidly at the 1st year of life and puberty;

- The thymus, lymph nodes and the lymphoid tissue of the intestine, spleen, tonsils reach their maximal size by 11–12 years and then their volume decreases.
- The head, brain, eyes and spinal cord reach the size typical for adults earlier than other parts of the body — by 10–12 years;
- Organs of the reproductive system actively grow during puberty.

The body grows under the action of hormones.

- *Somatotropic hormone (growth hormone)* is produced by hypophysis. Its intensive production occurs since birth till 13–16 years. Hypofunction of hypophysis causes, *pituitary dwarfism,* hyperfunction causes *gigantism* and human height can surpass 2m. Production of hormone in adult age causes *acromegalia* — the enlargement of bones of palms, feet and face.

- *Thyroxin* enhances energy exchange in the body. Hypofunction of thyroid gland causes delayed growth, delayed puberty, impairment of body proportions, mental disturbance.

- *Sex hormones* have effect on metabolic processes as well. *Environmental factors* also have considerable effect on growth. Normal growth of a child requires balanced meal with vitamins and microelements. Synthesis of vitamin D is influenced by sunlight.

In recent decades, acceleration of physical and mental development of children and adolescents is observed. It is marked even at the stage of intrauterine development — the average body length of newborns increased 0.5–1.0 cm, body mass — 50–100 g, the terms of teeth cutting out changed. The human height has increased on an average by 8 cm over the recent 100 years. The numerous factors were supposed to cause acceleration e. g. increase the heterozygosity (mixed marriages), better food.

Constitution of human is the sum of all genetically conditioned peculiarities of human morphology, physiology and behavior. In 1927 M. V. Chernorutsky proposed the classification including three types of constitution:

- *Ectomorphic type (asthenics)*: a narrow chest, low position of the diaphragm, elongated lungs, short intestine provides lower absorption, thin bones and long extremities, thin layer of subcutaneous fat. Statistically, asthenics are characterized by high excitability. They more often have neuroses, hypotonia, ulcers, tuberculosis.

- *Mesomorphic type (sthenics)*: balanced constitution, moderate development of the subcutaneous fat tissue. They are usually people of action; more often have neuralgias, atherosclerosis and diseases of the upper airways.

- *Endomorphic type (hypersthenics)*: a broad chest, voluminous stomach and long intestine, considerable fat tissue. The amounts of cholesterol, uric acid, erythrocytes and hemoglobin in the blood are higher than in other constitution types. Assimilation processes predominate. Hyperstenics have tendencies to obesity, diabetes mellitus, hyper-tension, diseases of kidneys and gallbladder.

Age and ageing. *Biological age* is the correspondence of body functional capacities to certain age. *Chronological age* is the actual amount of time a person has been alive.

Aging is broadly defined as the time-dependent functional decline that affects most living organisms. It is characterized by a progressive loss of physiological integrity, leading to impaired function and increased vulnerability to death.

The science about ageing and old age is called Gerontology. It studies regularities of ageing of various organ systems and tissues. Geriatrics is the science about ageing-related diseases. It studies peculiarities of their development, course, treatment and prophylaxis.

Nine hallmarks of ageing were described in 2013:

• *Genomic instability*. There are evidences that accumulation of damage in genetic material throughout life is associated with ageing.

• *Telomere attrition*. Telomeres (terminal region of chromosomes) shorten in dividing cells due to inability of DNA polymerase to replicate the terminal DNA regions. Telomere exhaustion explains the limited division numbers of some in-vitro-cultured cells (this is called *Hayflick limit* after the scientist who described it). Telomere shortening is also observed during normal aging. Normal physiological aging can be delayed without increasing the incidence of cancer in adult wild-type mice by systemic viral transduction of telomerase — the enzyme which repairs telomeres.

• *Epigenetic alterations* involve alterations in DNA methylation patterns, post-translational modification of histones, and chromatin remodeling. There are multiple evidences suggesting that aging is accompanied by epigenetic changes and that epigenetic perturbations can provoke aging-like syndromes in model organisms.

• *Loss of proteostasis*. Aging and some aging-related diseases are linked to impaired protein homeostasis.

• *Deregulated nutrient sensing*. Current available evidence strongly supports the idea that anabolic signaling accelerates aging and decreased nutrient signaling extends longevity. A pharmacological manipulation that mimics a state of limited nutrient availability, such as rapamycin, can extend longevity in mice.

• *Mitochondrial dysfunction*. Mitochondrial dysfunction can accelerate aging in mammals, but it is less clear whether improving mitochondrial function can extend lifespan.

• *Cellular senescence*. Cellular senescence is a stable arrest of the cell cycle coupled to particular phenotypic changes. The senescence observed by Hayflick is caused by telomere shortening, but there are other aging-associated stimuli that trigger senescence independently of this telomeric process.

• *Stem cell exhaustion*. The decline in the regenerative potential of tissues is one of the characteristics of aging.

• *Altered intercellular communication*. Aging also involves changes at the level of intercellular communication, be it endocrine, neuroendocrine, or neuronal. Thus, neurohormonal signaling tends to be deregulated in aging as inflammatory

reactions increase, and the composition of the peri- and extracellular environment changes.

Clinical and biological death. Ageing of the organism is terminated by death. Death provides alternation of generations. Causes of death can be different. *Physiological death*, or natural death, occurs due to ageing. *Pathological death*, or untimely death, is the result of a disease or an accident.

• *Clinical death* occurs as a result of termination of vital functions (heart or respiration failure), but processes of substances exchange in the cells and organs are retained.

• *Biological death* is termination of processes of self-renewal in cells and tissues, impairment of chemical processes, autolysis and decay of cells. In the most sensitive cells of the cerebral cortex, necrotic changes are revealed already in 5–6 minutes after clinical death. Prolongation of the period of clinical death is possible by using general hypothermia of the organism that slows down metabolic processes and increases the resistance to anoxia.

• *Reanimation* is complex of actions performed to return a person to life from the state of a clinical death (when vital organs are not impaired) within 5–6 minutes while cells of the brain are still alive. Reanimation methods are used in medicine in any threatening conditions.

• *Euthanasia* is a medical assistance to pass from life for a terminally ill patient at his will or request.

Lecture 9. INTRODUCTION TO PARASITOLOGY

Outline:
1. Origin and age of parasitism. Criteria of parasitism.
2. Classification of parasites and hosts.
3. Transmission of parasites.
4. Other characteristics of parasites.
5. Examples of parasites.

1. PARASITISM

Parasitism. According to the study of Yevgeny Pavlovsky, «parasites are animals that live at the expense of individuals of other species; they are closely associated with these species biologically and ecologically during long or short period of their life cycle».

• Criteria of parasitism: are spacial interaction with the host, feeding at the expense of the host and pathogenic action on the host.

• The *host* of a parasite is an organism that provides the parasite with inhabitation and food and is harmed by it.

• A specific habitation is characteristic of the parasite.

• Primary habitation is the host's organism. It actively reacts to the presence of a parasite.

● The secondary habitation is external environment. The host is a link between the parasite and the environment.

● Parasitism is a most common form of symbiosis: all viruses, many bacteria, some kinds of fungi and higher plants are parasites. Parasites are 10 000 species of protozoans, 7000 species of arthropods, 20 000 species of helminthes. Some classes includes only parasites — Sporozoa, Flukes and Tapeworms.

Diseases caused by various parasites have different names: those caused by viruses and bacteria are called infections (flue, hepatitis, tuberculosis, etc.); by protozoans and helminthes are invasions (ascariasis, taeniasis, enterobiasis, etc.); diseases caused by arthropods (ticks, insects) are infestations (pediculosis, myiasis, scabies, etc.).

There are *various biological interactions* between species in the nature:

● *Competition* is interaction of organisms that require same existence conditions or resources.

● *Predation* is the interaction of organisms of different species in which one predator organism kills the other one — prey — and uses it for feeding.

● *Antibiosis* (Greek anti — against, bios — life) — interactions of organisms of different species in which metabolites of one of them suppress the development of other one. These substances have a chemical nature. An example is production of *antibiotics* by mildew fungi, secretion of *phytoncides* (Greek phyton — plant, caedo — kill) by some higher plants (pine, cedar, onion, garlic). Antibiotics and phytoncides are used in medicine for treating various diseases.

● *Symbiosis* is any form of interactions between different species. The term was introduced into biology by de Barry in 1879 (Greek sym — near, bios — life). The following forms of symbiosis are distinguished:

– *synoikia* or hosting (Greek syn — together, oikos — house) — one species uses the other one as habitation without causing any harm or benefit (cancroid sea acorns on a mollusks' shells);

– *commensalism* (French commensal — co-eater) — permanent or temporary co-habitation of individuals of different species in which one of them eats food remains or excretion products of the other one without any harm (shark and sticking fish);

– *mutualism* (French mutuus — mutually beneficial) — mutually beneficial co-habitation of organisms of different species;

– *parasitism* (para — near; sitos — feeding) is antagonistic symbiosis. The most common form of symbiosis is one variety of interspecies relations.

Age of parasitism. Theoretically, parasites presumably could appear together with protists as parasitic bacteria were found in the amoebae. Multicellular parasites existed in the paleozoic era: ichnolites of the stems of sea lilies (Echinodermata) had gall-like growths caused by nematodes.

Origin of parasitism:

1. Predator → ectoparasite. Medicinal leeches are temporary ectoparasites for the human; the leech can be predators for small animals as it sucks out a great amount of blood and the animal dies.

2. Free-living organism → attached mode of life → ectoparasitism. Free-living cirripedia may pass to an attached mode of life. They attach to underwater parts of wooden buildings or bottoms of ships. They pass to ectoparasitism if they attach to living objects — shells of mollusks or fish bodies.

3. Commensalism → ectoparasitism. Commensalism → endoparasitism. If a commensal settles on body coverings of the animal, it may become an ectoparasite. It becomes an endoparasite when gets inside the organism (in body cavities connected with the environment). Entamoeba coli is an endocommensal in the human organism.

4. Transit through the digestive tract → endoparasitism (larvae of a fly).

2. CLASSIFICATION OF PARASITES AND HOSTS

Classification of parasites.

1. According to interaction with the host:

– *obligate parasites* — parasitism is the only possible way of living for such species (Ascaris, lice);

– *facultative parasites* are free living organisms that can get into a living organism and behave as parasites (larvae of the domestic fly);

– *hyperparasites* or *superparasites* are parasites of parasites (bacteria in parasitizing protozoans).

2. According to location in the host:

– ectoparasites inhabit body coverings of the host (lice, fleas);

– endoparasites live inside the host's organism:

  – intracellular parasites (toxoplasma);
  – cavity parasites (Ascaris);
  – tissue parasites (liver fluke);
  – intradermal parasites (itch mite).

3. According to duration of the relation with the host:

– *permanent parasite* — all life cycle proceeds in the host (Ascaris);

– *temporary parasite* — some stages of their life cycle require development in the host: larval parasitism (larvae of a botfly); imago parasitism — parasitism of sexually mature individuals (mosquitoes, fleas).

Classification of hosts.

1. According the parasite's life stage:

– *definitive (principal) host* — a host where a parasite maturates and reproduces sexually (human for *Taenia solium*);

– *intermediate host* — a host where a parasite lives for period and reproduces asexually (human for malaria parasite);

– *supplementary or accessory host* — additional intermediate host (fish for a cat liver fluke).

– *reservoir host* — in this host invasive stage of the parasite accumulates (predatory fish for larvae of *Diphyllobothrium latum*).

2. According to conditions of parasite's development:

– *obligate (or natural) host* provides optimal conditions for parasite's development and there is biocenotic contact (natural ways of invasion) — the human for the *Ascaris lumbricoides*;

– *optional (or permissive, accidental) host* there are biocenotic contact, but no normal biochemical conditions for the parasite's development (the human for the *Ascaris suum* — affects pigs);

– *potential host* can provide normal biochemical conditions for the development of the parasite, but there are no biocenotic contact — no ways for invasion (Guiney pig for trichinella).

Parasite-host system. Parasitism is an ecological phenomenon. *Ecological Parasitology* studies interrelations of parasites and their populations with each other, with the host's organism and the environment.

The *parasite-host system* includes one host individual and a parasite (or an entire group of parasites) of the same species.

Conditions necessary for the formation of this system:

– Contact between the parasite and the host;

– The host must provide proper conditions for the development of the parasite;

– The parasite must resist host's protective reactions.

Evolution of the system tends to improve its stability, reach equilibrium, diminish antagonism between the parasite and the host.

Lessen of the antagonism is achieved due to co-adaptation:

– in the parasite — morphologic and biologic adaptations;

– in the host — complication of defense mechanisms.

Directions of evolution are also different (co-evolution):

– in the parasite — complication of adaptation mechanisms to the host;

– in the host — improving all defensive reactions (to destroy the parasite).

3. TRANSMISSION OF PARASITES

Parasite's transmission routes. Pathogens of diseases have four basic ways of entering to the body.

• They can be engulfed:

1. *Alimentary (fecal-oral) route* — orally with food and water (eggs of helminthes, cysts of protists);

• Infecting forms of parasites can be inhaled:

2. *Respiratory (droplet)* — through the respiratory tract (cysts of some Amoebae, some viruses and bacteria);

• Infecting forms of parasites can enter the body across the skin or mucous membranes during contacts:

3. *Indirect and direct contact* — contact with a sick one through household goods (itch mite) or with his body surface;

4. *Sexual* — in sexual contacts (*Trichomonas vaginalis*).

• Other routes are more diverse. As a rule, they are associated with direct or indirect contact of bloods or with presence of a transmitting factor which is uncommon for the nature.

5. *Vertical (transplacental)* — from mother to fetus (toxoplasma, malaria parasite);

6. *Iatrogenic* — due to medical procedures, for example transfusion of infected blood or usage of unsterile surgical instrument (trypanosomes, malaria parasite);

7. *Vector-borne* — carriage of a pathogen by an arthropod (trypanosomes, malaria parasite);

Vector transmitting the disease is an insect or another arthropod which carries pathogens of a disease and causing infection of a host.

• *Biological vector* is an arthropod in which a parasite multiplies or develops to become infective (malaria pathogen developing in mosquito).

• *Mechanical vector* is an organism where pathogen of a disease does not multiply or develop, but only carried on the body surface or appendages.

4. OTHER CHARACTERISTICS OF PARASITES

Adaptations to parasitism. Parasites are highly specialized organisms, maximally adapted to their inhabitation and way of living.

Morphological and physiological adaptations of parasites:

• Progressive adaptations:

– Optimization of the body size (up to 20 m in tapeworms);

– High development of the reproductive system;

– Hermaphroditism;

– Fixation organs (adhesive discs of Giardia lamblia, suckers of flukes, bothria or hooks of tape worms, claws of lice, etc.);

– Integument that protects the parasite from host's defense;

– Molecular mimicry — similarity of proteins of the parasite and the host;

– Excretion of anti-enzymes.

• Regressive adaptations:

– Simplification of sense organs — endoparasites have only tactile and chemical sense organ;

– Simplification of the organ systems — absence of alimentary tract in tape worms.

Biological adaptations are associated with structural peculiarities of the reproductive system, reproduction and life cycles of parasites:

• High fertility (*Taenia solium* excretes 100 thousand eggs with every mature segment, an ascaris — 250 thousand eggs per day);

- Diversity of asexual reproduction (schizogony in malaria parasite, polyembryony in flukes);
- Migrations within the host's organism (larvae of *Taenia solium* and *Ascaris lumbricoides*);
- Complex life cycles with alternation of hosts.

The results of interactions of the parasite and the host on the level of organism may be different: death of the parasite, death of the host and carriage of the parasite.

Pathogenicity is the ability to cause a disease. It may depend on:
– genotype of the parasite, its species;
– host's age (children and old people are more susceptible to invasion);
– diet regimen (improper diet weakens the organism and contributes to increasing the number of parasites in the organism and their sizes, reduces the terms of their development);
– dose and degree of invasion (the more eggs or larva get to the host's organism, the severer is the course of the disease);
– resistance of the host;
– presence of other parasites and diseases.

Specificity of the parasite is degree of a historically formed adaptation to certain hosts. Its types are:
– hostal specificity: monohostal parasites have one species of the host (Ascaris lumbricoides), polyhostal parasites have hosts of several species (trichinella);
– topical specificity (a site of parasitizing): Ascaris lumbricoides lives in intestine, head louse — on the hairy region of the head and etc.;
– age specificity: enterobiasis in more common for children;
– seasonal specificity: outbreaks of amebic dysentery are more typical for the end of spring and summer).

Pathogenic action of parasites:
– Mechanic: parasites harm tissues by their body mass (ball of Ascaris lumbricoides in the intestine, a cyst of echinococcus in the brain), by fixation organs (injury of the intestinal mucous membrane by suckers), impairment of skin, etc. This action is revealed due to a pain syndrome.
– Toxicoallergic action is produced by metabolites of parasites that are antigens; histolysins and decay products of dead parasites.
– Manifestations of this action: skin eruptions, dermatitis, eosinophilia, allergic reactions.
– Absorption of nutrients and vitamins results in avitaminosis (mainly A and C), loss of weight, exhaustion.
– Impairment of the metabolic process reduces host's resistance and increases sensitivity to pathogens of other diseases.
– Biologically active substances of some parasites have immune-depressive effect on the host.

– Some parasites stimulate oncogenesis: schistosomes may cause cancer of the bladder and rectum.

– Parasites produce an unfavorable effect on the course of pregnancy and development of a fetus (malaria parasite, toxoplasma, cat liver fluke, etc.).

Response of the host to parasitic invasion. The basis of all reactions is the host's immune response. Allergy is a kind of immune reactivity. The first reaction to a parasite is an attempt to kill it with enzymes, then — to neutralize factors of its «aggression» by proteases, inhibitors of enzymes.

Reactions at cellular level show as hypertrophy and modification of the shape of affected cells (erythrocytes in malaria).

At tissue level: isolation of the parasite from a healthy tissue (formation of a capsule in trichinellosis, formation of pseudocysts in toxoplasmas).

At organism level: humoral reactions (production of anti-bodies) and various forms of immunity: complete — relative, active — passive, inborn — acquired.

Biological basis of prophylaxis of parasitic diseases. K. I. Skriabin elaborated biological basis of prophylaxis to control parasitic diseases. It is a complex of prophylactic measures based on detailed studying of the pathogen's biology, migration ways, life cycle, biology of intermediate hosts. It is possible to interrupt any link of the parasite life cycle. The final practical aim of Parasitology is protection of the human, animals and plants from parasitic action and elimination of parasitic diseases.

Diagnosis of parasitic diseases. Accurate diagnosis of parasitic diseases is can be made due to the following methods:

Microscopy — detection of different forms of parasites in biological specimens of a patient.

Immunological methods — identification of diseases using laboratory techniques involving interaction of antigens with specific antibodies.

PCR — presence or absence of particular DNA can be confirmed by polymerase chain reaction.

5. EXAMPLES OF PARASITES

Entamoeba histolytica. *Entamoeba histolytica* (also known as dysenteric amoeba) is a pathogen of amoebiasis or amoebic dysentery (notice that the disease dysentery is caused by another pathogen!). The cases of the disease are reported everywhere, more commonly in countries with warm climate.

Entamoeba histolytica exists in two forms: trophozoite and cyst. Cysts (8–16 μm) contain 4 nuclei.

There are 3 forms of trophozoites of *Entamoeba histolytica*: minor vegetative form (lat. forma minuta), major vegetative form (lat. forma magna) and tissue form. Forma minuta is capable of movement and feeds on bacteria. It is not pathogenic. Forma magna and tissue form are pathogenic. The forma magna engulfs erythrocytes. Tissue trophozoites are very motile (tabl. 5).

Table 5

Forms of E. histolytica

	Forma minuta	Forma magna	Tissue form
Pathogenic	No	Yes	Yes
Size	12–20 µm	30–40 µm	20–25 µm
Location	Intestinal lumen	Intestinal lumen	Intestinal wall, affected organs
Confirms that person is sick	No, can be found in stool samples of carriers and after recovery	Yes, can be found in stool samples at acute stage of the disease	Seldom found in stool specimens

• Amoebiasis is transmitted via the fecal-oral route. Infection occurs by ingestion of cysts. Transmitting factors are contaminated vegetables, fruit and water. Mechanical vectors of cysts are flies and cockroaches. Four trophozoites (forma minuta) come out from each cyst in the intestine. As forma minuta is not pathogenic, the trophozoites can live in the host for a long time (feed, multiply) and transform into cysts. Such host person is called *cyst carrier*.

• When the host's organism is weakened, forma minuta can transform into forma magna and then invades the mucous membrane of the large intestine. In the intestinal wall it transforms into tissue form. Entamoeba causes lysis of epithelial cells causing formation of ulcers. When reach blood vessels, trophozoites can be carried into the liver, brain and other organs. In remission, the pathogenic trophozoites in the intestinal lumen transform into forma minuta and cysts.

• Symptoms of amoebiasis can vary from asymptomatic form or mild diarrhea to severe form with bloody diarrhea up to 10 times a day and more, intoxication, ache on the lower right side of the abdomen.

• Complications that may occur are amoebic abscess in the liver, lungs, brain, skin, perforation of the intestine and purulent peritonitis.

• Laboratory diagnosis. Diagnosis is made by microscopy of stool in order to find trophozoites (or cysts in case of asymptomatic form of the disease), immunodiagnosis (finding antibodies).

• Personal prophylaxis: observing hygiene rules (washing hands, washing vegetables and fruits with hot water, protection of food from flies and cockroaches).

• Social prophylaxis: finding and treatment of sick individuals and carriers; control over sanitary condition of water ponds, food manufacturers, shops and markets; prophylactic examination of workers of food manufacturers; elimination of flies and cockroaches; hygiene education.

Pathogen of malaria. Pathogens of human malaria refer to the order *Haemosporidia*, genus *Plasmodium*.

• There are of 5 species of plasmodia causing malaria in human: *Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, Plasmodium falciparum, Plasmodium knowlesi*.

• Malaria is common mostly in countries with a subtropic and tropic climate.

- Life cycle. The human is the intermediate host for the plasmodium while female mosquitoes are principal hosts and biological vectors.

- The disease is transmitted by female mosquitoes of the genus *Anopheles*. The mosquito injects *sporozoites* into the host while taking a blood meal. These parasites are taken by the bloodstream to the liver, where they invade liver cells (hepatocytes) and transform into *schizonts*.

- The hepatic schizonts grow and in 5–16 days reproduce asexually by schizogony (multiple fission) producing numerous *merozoites*. The invaded cells rupture and the merozoites are released to the bloodstream.

- This development of the parasite in liver at the first stages of the disease is *exoerythrocytic cycle* (exoerythrocytic schizogony, liver schizogony). There are no clinical signs and symptoms at this stage.

- The merozoites invade erythrocytes to continue their development and start *erythrocytic cycle*. Within the erythrocyte, the merozoite develops into a *ring* or *early trophozoite form*, which then develops into a *mature trophozoite.*

- The mature trophozoite undergoes schizogony to form numerous merozoites. The form of the parasite containing multiple merozoites is sometimes referred to as *morula*.

- The invaded erythrocyte containing merozoites ruptures, releasing them into the bloodstream (this process is *merulation,* at this moment attack of malaria starts).

- These merozoites invade erythrocytes again to repeat the cycle.

- Though, some of the merozoites develop within the erythrocytes into *micro- and macrogametocytes* (male and female gametocytes).

- To continue their development, the gametocytes must be ingested by female Anopheles mosquitoes during a blood meal.

- The multiplication of plasmodium in the mosquito is *sporogonic cycle* (sporogony). In stomach of the mosquito, the microgametes fertilize macrogametes to form *zygotes*.

- The zygotes turn into motile and elongated *ookinetes*. The ookinetes invade the midgut wall and develop into oocysts. The oocysts grow, rupture, and release sporozoites to the body cavity of the mosquito. The sporozoites accumulate in the salivary glands. Such mosquito becomes able to infect human.

- As noted above, infection of human occurs through a bite of a female Anopheles mosquito that injects sporozoites with saliva (vector-bone route of transmission).

- Infection is also possible in blood transfusion (transfusion-transmitted malaria) and transplacentally (congenital malaria). In this case the infecting stage for human is not sporozoite, but erythrocytic schizont.

- Clinical presentation. Incubation period depends on species of the parasite and may vary from 9 to 40 days. In case of *P. vivax* and *P. ovale* infection, exoerythrocytic cycle of some parasites can be delayed (due to *hypnozoites* —

dormant forms of the parasite) and this may prolong the incubation or cause relapses of the disease many months after recovery.

• Classically, malaria causes attacks that repeat every 48 hours (*P. falciparum, P. vivax, P. ovale — tertian malaria*), every 72 hours (*P. malariae — quartan malaria*) or every 24 hours (*P. knowlesi — quotidian malaria*).

• These periods are durations of their erythrocytic cycles. When parasites develop in the erythrocytes, numerous toxins accumulate there, and when the erythrocytes rupture these toxins are released to the bloodstream. These toxins cause the symptoms of malaria attack. The attack usually lasts 6–12 hours and consists of 3 stages:

1. *cold stage* lasts 1–2 hours, temperature elevates. Symptoms are shivering and sensation of cold;

2. *hot stage* lasts 5–8 hours; symptoms — fever 40–41 °C, aches of the head and lumbar area, sometimes vomiting.

3. *sweating stage* is the end of the attack when patient returns to normal temperature. This stage is characterized by profuse sweating.

• Malaria may complicate with anemia, jaundice, renal failure. One of the seveares complications is cerebral malaria (commonly caused by *P. falciparum*) characterized by impaired consciousness, delirium and focal and generalized convulsions. As the falciparum malaria causes severest complications, it is referred to as malignant tertian malaria.

• Laboratory diagnosis is usually based on finding parasites in blood (thick blood smear). It is necessary to take blood during the attack or immediately after. Species of plasmodia causing malaria in human have morphological differences that may help to identify them:

1. *Plasmodium vivax* has amoeboid-shaped trophozoite.

2. *Plasmodium ovale* similar to *P. vivax*, but affected erythrocytes have distorted elongated shape.

3. *Plasmodium falciparum* has gametocytes of crescent or semi-lunar shape.

4. Schizont of *Plasmodium malariae* band-shaped trophozoites.

Other methods used for diagnosis are based on antigen detection or PCR.

• Personal prophylaxis: prevention of mosquitoes bites (using repellents) and chemical prophylaxis.

• Social prophylaxis: revealing and treating sick people and carriers, hygiene education, elimination of mosquitoes of g. Anopheles.

• Fighting mosquitoes includes the following directions:

1. *Protection from bites* — wearing covering-up clothes, usage of repellents, nets on windows; zooprophylaxis — making biologic barriers (cattle-breeding farms) between places of mosquitoes' reproduction and dwelling houses, etc.).

2. *Elimination of adult mosquitoes* — dispersion of insecticides in places of wintering of mosquitoes (basements, garrets, cattle yards).

3. *Elimination of larvae.*

Opisthorchis felineus is a biohelminth, pathogen of *opisthorchiasis*. The disease is common in Siberia along the large rivers, though cases of opisthorchiasis were reported in other countries. Similar species cause infections in other regions of the world.

• Morphology. Adult worms are approximately 10 mm long. There are 2 testes lying one behind the other in the posterior portion of the body, the S-shaped excretory canal is between them.

• The ovary is anterior to the testes and a uterus is between the ovary and ventral sucker. The yolk glands (vitellaria) are situated on the lateral sides in the middle of the body. The two canals of the gut do not form lateral branches and lay between the vitellaria and other reproductive organs. Eggs are 26–30 × 10–15 μm size, have lid that opens to release miracidium on the narrow pole.

• Life cycle. The definitive hosts are human, cats, dogs and other fish-eating animals. The 1st intermediate host is freshwater snail (*Bithynia leachi*), the 2nd intermediate host is freshwater fish.

• Life cycle: *marita — egg — miracidium — sporocyst — redia — cercaria — metacercaria.*

• The adult flukes live in bile ducts and lay eggs that are released to the environment with feces. After ingestion by a suitable snail, the eggs release miracidia. Its development is similar to that of other flukes (sporocysts → rediae → cercariae). The cercariae escape from the snail to penetrate a freshwater fish which is the second intermediate host. In the fish, they encyst and transform into metacercariae.

• Infection of a human occurs when ingests undercooked fish containing metacercariae. Metacercariae excyst in the duodenum and ascend into the biliary ducts, where they mature into adults and start laying eggs in several weeks.

• Clinical presentation. Symptoms depend on the number of flukes invaded the person. Most of cases are asymptomatic. When symptoms develop, there can be diarrhea, abdominal pain, enlargement of liver and spleen. Severe cases can lead to fever, acute pain, significant enlargement of liver, jaundice.

• There is insufficient evidence of causal link between opisthorchiasis and liver cancer.

• Laboratory diagnosis is based on detection of eggs in stool specimens Immunological methods can be used if available.

• Prophylaxis. The disease can be prevented by proper cooking and salting fish; freezing the fish also kills the parasites. Other measures are treatment of sick people; prevention of water contamination with feces; hygiene education.

Ascaris lumbricoides is a geohelminth causing of *ascariasis* in humans. About billion people in the world are infected. According to WHO, ascariasis is found worldwide and occurs with greatest frequency in tropical and subtropical regions, and in any areas with inadequate sanitation. Ascaris infections cause approximately 60 000 deaths per year, mainly in children.

• Morphology. The body is cylindrical, sharpened at the ends. The length of an adult female is 15–40 cm, the lenght of a maleis 15–25 cm. Tail of males is curved, that of females is straight. There are cuticular lips on the anterior end of the body.

• Life cycle. Adult *A. lumbricoides* live and feed in the small intestine. Each female lays up to 240 000 eggs per day, the eggs are passed to the environment with feces. The eggs are unembryonated (larva is not developed yet) and thus non infective.

• Eggs develop in soil. This development requires favorable conditions (temperature 20–25 °C, humidity and oxygen) and lasts 21–24 days.

• Human become infected by ingestion of embryonated eggs with contaminated food or water.

• In the small intestine, larvae hatch out of the eggs, penetrate the intestinal wall and are carried to liver through portal circulation. With blood the larvae migrate through the heart and reach alveoli of lungs. From alveoli, the larvae then pass through bronchi and trachea to be swallowed. Larval migration lasts about 2 weeks.

• Larvae of other ascaris species (ascaris of pigs, dogs, etc.) may migrate in the human organism but do not reach maturity. The syndrome they cause is called Larva migrans.

• Clinical presentation. Most of people infected with ascariasis have no symptoms or mild symptoms. Infections with a large number of worms may cause severer symptoms.

• Clinical presentation of *pulmonary ascariasis* (larval migration): general weakness, transient eosinophilic pneumonitis, persistent spastic cough, skin rash, fever, perspiration.

• Clinical presentation of *intestinal ascariasis*: abdominal pain, nausea, vomiting, diarrhea, weight loss and growth retardation in children.

• *Complications of intestinal ascariasis*: intestinal obstruction, perforation of the appendix, migration of the parasite to the biliary tree or to the peritoneal cavity. There were cases when the parasites were found in frontal sinuses, cranial cavity, middle ear and ovaries.

• Laboratory diagnosis is based on microscopy of feces in order to find eggs of the parasite. Ascaris egg is round or oval, 60 × 45 µm size, have thick brown shell with rough surface. Sometimes larvae can be detected in sputum.

• Prophylaxis: to avoid contact with soil that may be contaminated with human faeces, to wash hands with soap and water before handling food, wash, peel or cook all raw vegetables and fruits, protect food from soil and wash or reheat any food that falls on the floor. Social prophylaxis requires finding and treating sick people, adequate sanitation, hygiene education.

Lice. There are two genera in order *Anoplura*: genus *Pediculus* and genus *Phthirus*. The genus *Pediculus* has only one species — *Pediculus humanus,* which, in turn, includes 2 subspecies — the *Pediculus humanus capitis* and the

Pediculus humanus humanus. Both of them can cross and produce fertile off-spring, though they have some morphological and biological differences.

Head louse (Pediculus humanus capitis).

• Morphology. The length of a male is about 2–3 mm, female — 3–4 mm. The posterior end of male's body is rounded, that of a female is slightly forked. Mouthparts are piercing-sucking.

• Life cycle. Lice live in the hairy area of the head. They feed on human blood 2–3 times a day, may starve for several days. The life cycle of the body louse includes stages of egg (**nit**), nymphs, and adult.

• Nits are attached to hair with a sticky secretion and develop about a week. During the whole life (up to 38 days) a female lays about 300 eggs. A larva comes from an egg and in several days transforms into imago (mature form).

Body louse (Pediculus humanus humanus).

• Morphology. Body lice are slightly larger than head lice (up to 4.7 mm), the visible fissures between segments are not as deep those of head lice, and the pigmentation of the body lice is less.

• Life cycle. Body lice live in clothing and travel to skin several times a day to feed on blood. Nits are stuck with a secretion to fabric (usually in the seams). The life span is up to 48 days, the development lasts not less than 16 days. By the end of its life female can have about 4000 offspring.

• Medical significance. Lice of the genus *Pediculus* cause *pediculosis*. Bites of lice cause itching. Intense itching leads to scratching which can cause sores and secondary bacterial infection of the skin. There may be post-inflammatory pigmentation of skin. Lice are biological vectors of *epidemic typhus* (caused by bacterium *Rickettsia prowazekii*) and a *louse-borne relapsing fever* (caused by bacterium *Borrelia recurrentis*), tularemia.

• Infection of epidemic typhus occurs by a specific contamination — rubbing louse feces or hemolymph into damaged skin during scratching.

• Pubic louse (*Phthirus pubis*).

• Morphology. Bodies of pubic lice are look short and wide compared to head or body lice. The length is to 1.5 mm.

• Life cycle is similar to that of other lice. The parasite affects areas with thick hair: pubic area, armpits, eyelashes, beard. The female lays about 50 eggs during its life. The life cycle from an egg to an adult insect form lasts 22–27 days.

• Medical significance. Pubic lice cause *phthiriasis* (severe itching usually in the pubic-hair area). Human can get phthiriasis by sexual contacts, rarely — through underwear and clothes.

• Diagnosis of pediculosis and phthiriasis. Lice and nits can be visible with the naked eye. Thus the diagnosis is performed by visual examination of scalp, hair, seams of clothing.

• Prophylaxis of pediculosis and phthiriasis. Avoid hair-to-hair contact, do not share clothing or towels, do not lie on beds, couches, pillows, carpets, or stuffed animals that have recently been in contact with an infected person.

Lecture 10. EVOLUTION OF ORGAN SYSTEMS IN CHORDATES

Outline:

1. Connection of the ontogenesis and phylogenesis, Biogenetic law, A. N. Sewertzoff's theory about phylembryogeneses.
2. Evolution of the nervous system.
3. Evolution of skull.
4. Evolution of the digestive system.

1. CONNECTION OF THE ONTOGENESIS AND PHYLOGENESIS, BIOGENETIC LAW, A. N. SEWERTZOFF'S THEORY ABOUT PHYLEMBRYOGENESES

Connection between the ontogenesis and phylogenesis. *Ontogenesis* is the individual development of an organism from the moment of zygote formation to death. This development is based on expression of certain genes by different cells and the effect of the environmental factors.

Phylogenesis is the evolutional history species development.

• Ontogenesis and phylogenesis are closely connected. Knowing the evolutional history of the human species, we can understand how and why some malformations develop.

• In 1866 Ernst Haeckel formulated the *biogenetic law: ontogenesis is a short and fast repeat of phylogenesis — «ontogeny recapitulates phylogeny»*.

• Ch. Darvin confirmed the correlation between onto- and phylogenesis and developed the theory of recapitulations. *Recapitulation* is a repeat of ancestral characters in embryos.

• In 1828 Karl Ernst von Baer made the theory more accurate. The ontogenesis does not repeat the entire ancestral forms, but the structures which eventually develop into different organs in ancestors and modern animal forms. This was expressed in the following laws:

− The more general characters of a large group of animals appear earlier in their embryos than the more special character;

− From the most general forms the less general are developed, and so on, until finally the most special arise;

− Every embryo of a given animal form instead of passing through the other forms, becomes separate from them;

− Fundamentally, therefore, the embryo of a higher form never resembles any other form, but only its embryo;

• The *hourglass model of embryonic evolution* predicts an hourglass-like divergence during animal embryogenesis. Embryos are more divergent at the earliest and latest stages but conserved during a mid-embryonic period that serves as a source of the basic body plan for animals within a phylum. That mid-embryonic period is called phylotypic stage.

• N. Severtsov elaborated the theory of phylembryogeneses. This theory explains relations between ontogenesis and phylogenesis. Phylembryogenesis is an

embryonic reconstruction that is preserved in adults and has adaptive nature. There are 3 types of phylembryogeneses:

1. Archallaxis is an early deviation from ancestral developmental pattern that occurs simultaneously with formation of the organ anlage (an example is development of a hair coat in mammals). Mutated genes get involved in morphogenesis at its initial stages and make the new course for development of the organ (recapitulations are absent);

2. Deviation development that begins in accordance with ancestral pattern and deviate in the middle of the course (an example is development of scales in reptiles). Initially morphogenesis proceeds according to ancestral patterns (partial recapitulation) but later on mutated genes activate and make new course for the organ's development.

3. Anaboly development that follows ancestral pattern up to its last stage and then new stages are added (a two-chambered heart into a four-chamber heart). At first all stages of the organ development recapitulate, and then mutated genes activate to form new character.

In cases of some malformations the body acquires characteristics of another orders or classes of chordates. They appear due to ontophylogenetic mechanisms such as recapitulations and parallelisms. Recapitulations occur as a result of incomplete anaboly or its absence. Examples of such disorders are three-chambered heart, preservation of embryonic vessels, two aortal arches, arrested development of kidneys, duplication of ureters. Parallelism is independent development of similar characters in closely related species during their evolution (human and animals that have similar origin). An example of parallelism in human is polymastia (abnormal number of nipples).

2. EVOLUTION OF THE NERVOUS SYSTEM IN CHORDATES

The nervous system originates from ectoderm and forms as a nerve tube.

1. Differentiation of the nerve tube into the brain and the spinal cord.

2. Evolution of the brain:

– transformation of 3 brain vesicles into 5 brain vesicles and therefore 5 brain regions;

– appearance of the cerebral cortex and enlargement of its surface due to its sulci (grooves) and gyri (folds);

– transformation of the ichthyopsidian brain into sauropsidian one and ultimately into mammalian brain.

3. Differentiation of the peripheral nervous system.

Lancelet. The CNS of lancelet is nerve tube. Its anterior part is dilated and has olfactory pit. Photosensitive cells (Hesse organs) are located throughout the whole length of the tube. The brain of mammals consists of 5 regions. It undergoes same stages during its formation. At first the nerve tube is formed and 3 brain vesicles appear in its anterior end: forebrain (prosencephalon), midbrain (mesencephalon) and hindbrain (rhombencephalon). Then the forebrain and hindbrain di-

vide to form 5 brain vesicles which transform into a certain brain region: cerebrum (*telencephalon*), interbrain (*diencephalon*), midbrain (*mesencephalon*), pons and cerebellum (*metencephalon*) and medulla oblongata (*myelencephalon*). There are cavities in the bran (cerebral ventricles) that are followed by the spinal canal in the spinal cord. The part of the brain located above the ventricles is called *roof* and the part below is the *floor* of the brain.

Fish. The brain of fishes is small. The cerebrum is not divided into hemispheres. The roof is epithelial; the floor of the brain consists of corpora striata. Olfactory lobes are small. The interbrain consists of thalamus and hypothalamus. The midbrain is large as it is the integrating center of the CNS (*ichthyopsidian* type of the brain). A flexure appears in the area of the midbrain. The cerebellum is developed well. There are 10 pairs of cranial nerves.

Amphibians. In amphibians: volume of the forebrain increases; cerebrum divides into 2 hemispheres; nervous tissue appears in the brain roof; corpora striata are well developed. Olfactory lobes are separated from the hemispheres. The interbrain consists of thalamus and hypothalamus. The midbrain is large and still serves as the integrating center. The cerebellum is poorly developed. The medulla oblongata is developed same as in fish. There are 10 pairs of cranial nerves.

Reptiles. In reptiles cerebrum is the largest brain region. Large olfactory lobes are differentiated, parietal lobes are separated. Hemispheres of the brain have primordial cortex on their lateral surfaces. The structure of the cortex is primitive (3 layers of cells) — *archipallium*. The corpora striata of the forebrain serve as the integrating center. Such type of the brain is called *sauropsidian (striatal)*. The size of the midbrain is lower than in amphibians (it is no longer the integrating center of the brain). The cerebellum is considerably larger than that of amphibians. The medulla oblongata forms a sharp flexure in the vertical plane. There are 12 pairs of cranial nerves.

Mammals. In mammals the forebrain reaches maximal development due to the secondary cortex (*neopallium*). In lower mammals the surface of the cortex is smooth, in higher mammals it has sulci and gyri. The secondary cortex i an integrating center (mammalian type of the brain). The forebrain covers the interbrain. The size of the midbrain decrease. This region consists of quadrigemina (2 superior colliculi are subcortical centers of vision, 2 inferior colliculi are subcortical centers of hearing). The cerebellum is considerably larger. It is differentiated into two hemispheres with the vermis in the middle. The brain has 12 cranial nerves.

There are 3 flexures of the brain: cephalic flexure at the level of the midbrain, cervical flexure in the region where the medulla oblongata passes into the spinal cord, pontine flexure in the area of the hindbrain.

3. EVOLUTION OF SKULL IN CHORDATES

Basic directions of evolution:

1. Accretion of the visceral cranium with the cerebral cranium; enlargement of the cerebral cranium.

2. The number of bones decreases by means of their accretion.

3. Replacement of cartilage with bone.

4. Movable connection of the skull and spine.

The cerebral cranium of vertebrates represents continuation of the axial skeleton. The visceral cranium supports the respiratory system and the first regions of the digestive system. The skull originates from 2 basic components: *parachordals* on the sides of the notochord (notochordal component) and *trabeculae* (perinotochordal component) in front of the notochord.

Trabeculae and parachordals overgrow and fuse together forming the brain case from beneath and the sides. It is complemented with nasal and otic capsules that accrete with it. Orbital cartilages form on lateral sides. The visceral cranium undergoes stages of connective, cartilaginous and bone tissues.

Fish. The cerebral cranium is cartilaginous; an occipital region appears. The visceral cranium consists of 5–6 cartilaginous arches that envelope an anterior region of the alimentary tube. The first one is mandibular arch. It consists of the palatoquadrate cartilage at the top and forms a primary upper jaw. Beneath, there is a Meckel's cartilage. It forms a primary lower jaw. The second one is hyoid arch consisting of 2 upper hyomandibular cartilages and 2 lower cartilages called hyoids. Each hyomandibular cartilage accretes to the base of the cerebral cranium; each hyoid connects with the Meckel's cartilage (*hyostylic type of skull*). The roof of the cerebral cranium includes paired frontal, nasal and parietal bones. The skull is immovably connected to the spine.

The skull of terrestrial vertebrates is connected with the spine movably.

Amphibians. Amphibians have secondary jaws. The palatoquadrate cartilage of the 1st arch accretes to the base of the cerebral cranium (*autostylic type of the skull*). The hyomandibular cartilage of the hyoid arch is no longer holder for the mandibular arch and transforms into an auditory ossicle (columella). The Meckel's cartilage is reduced, the hyoid transforms into processes of the hyoid bone. Other visceral arches (there were 6) are preserved as a hyoid bone and cartilages of the larynx.

Reptiles. In reptiles the skull ossified, many covering bones appeared. The connection of the visceral and cerebral crania occurs through ossified part of the reduced palatoquadrate cartilage. The skull is *autostylic*. The jaws are secondary. The secondary hard palate and zygomatic arches are formed.

Mammals. In mammals the roof of the skull consists of frontal and parietal bones. The mandible is one bone; its process forms a joint that connects it with the cerebral cranium. The palatoquadrate and Meckel's cartilages are transformed into an incus and a malleus. The upper part of the hyoid arch forms a stapes. Parts of 2nd and 3rd branchial arches form a thyroid cartilage of the larynx, 4th and 5th arches transform into other laryngeal cartilages. In higher mammals the volume of the cerebral cranium considerably increased. In human sizes of the visceral cranium are decreased in comparison with the cerebral cranium, the brain case is round and smooth. Zygomatic arches are formed (*synapsid type of the skull*).

4. EVOLUTION OF DIGESTIVE SYSTEM IN CHORDATES

The digestive system originates from the endoderm, its beginning and ending regions develop from the ectoderm.

Basic directions of evolution:

1. Differentiation of the alimentary tube into regions.
2. Appearance of digestive glands.
3. Appearance of teeth and their differentiation.
4. Enlargement of the absorption surface due to the elongation of the intestine and appearance of villi.

Lancelet. Lancelet's digestive system is presented by a straight tube that is differentiated into a pharynx and intestine. The pharynx has gill slits. The alimentary tube forms a hepatic cecum.

Fish. Fishes have jaws with homogenous teeth (homodontous animals). There are an esophagus, stomach, small and large intestines. The liver is well developed; there is a gallbladder. The pancreas is differentiated poorly.

Amphibians. Amphibians have an oropharyngeal cavity with homogenous teeth, esophagus, small and large intestine, liver, pancreas. A muscular tongue and salivary glands appear. There are no enzymes in saliva. Amphibians have a duodenum and rectum. The intestine ends with a cloaca.

Reptiles. Reptiles have an oral cavity that is separated from the pharynx, differentiation of teeth begins (fangs), walls of the stomach are thick. There is a primordial cecum, the intestine becomes longer and ends with a cloaca.

Mammals. Mammals are heterodonts (have incisors, canines and molars); lips appeared. The saliva contains enzymes. The intestine is differentiated into a small and large intestine, the caecum is well developed and has an appendix. The rectum ends with an anal opening. The mucous membrane of the intestine has a great number of folds, the small intestine has villi.

Ontophylogenetic etiology of congenital defects of skull and digestive system in human

Ontogenetic etiology of skull malformations: additional bone elements, nonunion of the hard palate (cleft palate), frontal suture; only one hearing bone; absence of the mental prominence.

Ontophyloigenetic etiology of malformations of digestive system: cervical fistulae (rupture gill pouch), homodontous teeth, additional lobes of the liver and pancreas, shortening of the intestine.

Lecture 11. POISONOUS AND VENOMOUS ORGANISMS

Outline:

1. Poisonous fungi.
2. Poisonous plants.
3. Poisonous and venomous animals.

1. POISONOUS FUNGI

Poisonous fungi. There are two morphological groups of fungi: micro- and macromycetes.

• The micromycetes are microscopic fungi. They are the most frequent agents of severe food intoxication caused by fungi (aspergillus, penicillium, fusarium, ergot). Macroscopic fungi are macromycetes. They usually cause intoxication when eaten by mistake (amanita, gyromitra).

• According to edibility, macromycetes (commonly known as mushrooms) are: edible, edible after proper cooking (blewit, sharp agaric), unpalatable, and poisonous (death cap amanita, fly amanita, gyromitra).

• Characteristics of the poisons. The poison of death cup amanita contains bicyclic polypeptides, amanitins and phalloidins. Fly amanita contains muscarine (para-sympathotropic agent) and hallucinogens (bufotenin, muscasone and others). Gyromitra contains gyromitrin which is similar to toxins of death cup amanita.

• Symptoms of poisoning: vomit, abdominal pains, hypersalivation and hyperhidrosis, lachrymation, breathlessness, miosis. In cases of severe poisonings — diarrhea, low arterial pressure, heart beat disorder, convulsions, collapse, coma. Death may occur as result of toxic hepatitis and acute heart failure.

• First aid: gastric lavage with suspending of activated carbon, 1 % potassium permanent, usage of saline laxatives.

2. POISONOUS PLANTS

• There are plants which produce and accumulate poisons which can cause toxication and even death of animals or human in various types of contacts. More than 10 000 species of poisonous plants are described. Such plants are lily of the valley, blister buttercup, marsh tea and others. There are extremely poisonous *plants such as devil's trumpets, black henbane, belladonna, Zwerg-Holunder* and others. Some plants can be poisonous only when grow in certain conditions.

• Characteristics of the poisons: plant poisons contain different groups of chemical compounds such as alkaloids, saponins, essential oils, glycosides, flavonoids, tannins, resinous substances, carboxylic acids, cyanic compounds and others.

• Toxicity of plant poisons is associated with a number of factors. Cardiac glycosides are not destroyed for a long time, are excreted through kidneys and affect them. Alkaloids have toxic effect on liver. Many glycosides are hydrolyzed and then broken into hydrocyanic acid which causes adverse effects.

• Symptoms: *The most clinically important syndromes in case of acute poisonings are: psychoneurological, respiratory, cardiovascular, gastro-intestinal, hepatic, renal.*

First aid in case of poisonings with phytotoxins.

1. *Removal of toxins from the organism*: gastric lavage, vomiting, removal of the intestinal content, usage of adsorbents such as activated carbon.

2. *Antidotes*: substance with opposite effect on the organism can be used for some toxins.

3. *Detoxication*: artificial diuresis, replacement transfusion, dialysis, hemosorption.

4. Relief of symptoms: *antishock therapy, normalization of work of respiratory, cardiovascular, central and peripheral nervous systems*.

3. POISONOUS AND VENOMOUS ANIMALS

Classification. *Venom* is a toxic secretoion of animals which is injected into the prey via bite ore sting.

• *Poison* is a toxic secretion or metabolite which is contained in a poisonous animal or exposed in its body surface.

• Animals can produce own toxins or accumulate them from the environment, there can be toxic metabolites or toxins produced by specialized glands, some animals can bite the victim while others cannot. This factors are the basis for the classification of animal toxicity.

• *Primarily-toxic* animals can produce own toxins. As a rule, toxicity of these animals is a character of the species (jellyfish, scorpions, snakes, fishes).

• *Secondarily-toxic* animals accumulate exogenous toxins from the environment. Such animals are *poisonous* if they are eaten by other organisms (fish adsorb industrial toxins from water).

• The primarily-toxic are divided into actively-venomous, actively-poisonous, passively-poisonous.

• *Actively-venomous* animals have glands producing venom and specialized biting apparatus (such as thread cells on tentacles of jellyfishes, a stinger in hymenoptera and fangs in snakes). Venom is injected into the body of the victim parenterally (avoiding the digestive tract).

• *Actively-poisonous* animals have glands producing poison, but no biting apparatus. Secretions of their glands are poisonous in case of direct contact with integuments of the victim (skin glands of amphibians, anal glands of insects).

• *Passively-poisonous* animals (fishes, caudate amphibians, mollusks) can have toxic metabolites that are accumulated in various organs and tissues. They are dangerous only when eaten by a victim.

Characteristic of animal toxins. Animal toxins (zootoxins) are biologically active substances that actively interact with biological structures of the body. Zootoxins are diverse by their chemical structure (alkaloids, histamine, various enzymes and their inhibitors).

There are various types of zootoxins such as:

1. *Neurotoxins* affecting predominantly the nervous system;

2. *Cytotoxins* damaging cells and tissues;

3. *Hemorrhagins* impairing normal permeability of blood vessels;

4. *Hemolysins* destroying erythrocytes.

The clinical presentation of toxication of human depends on composition of a poison or venom, the site of bite, sting or contact with a poison, the season of the year and the time of the day (animals can vary their toxicity) as well as a overall condition of the person.

Poisonous and venomous invertebrates. Coelenterates (orange-striped jellyfish and physalia) refers to *actively-venomous* animals. Thread cells release toxins with neurotoxic effects.

• *Clinical presentation*. In sites of stingby tentacles of the orange-striped jellyfish appears sharp pain, erythema, rash. Symptoms temperature rise, rapid decrease of muscle tone, pains in extremities and lumber area, impairment of consciousness, hallucinations, delirium, respiratory and cardiac affection, in severe cases — death.

• *First aid.* It is required to remove parts of tentacles and striking threads from the skin, treat the affected sites with alcohol or solution of soda.

• *Prophylaxis.* Not to bathe in the thicket of water plants and in places of jellyfish gatherings.

Phylum Arthropoda, class Arachnida, order Scorpions (yellow, Italian, black). They are actively-venomous, have venomous glands located in the last segment of the abdomen. They excrete neurotropic venom that blocks neuromuscular synapses.

• *Clinical presentation*. At the site of a bite appears a severe pain, edema, erythema, vesicles. Symptoms: headache, weakness, impairment of consciousness, and respiration, tachycardia in children. Lethal outcomes are possible.

• *First aid*. Sucking off the venom, applying cold to the site of a bite, taking pain-killers. Injection of specific antiserum.

• *Prophylaxis.* Protection from bites: examination of dwellings, bedding, clothes, shoes.

Order *Arachnida*. Spiders are actively-venomous. Ducts of their venomous glands open on chelicerae.

Karakurt has neurotropic venom that blocks neuromuscular synapses.

• *Clinical presentation*. At a bite site appears pain, numbness of extremities. Symptoms: pain quickly spreading throughout the body, headaches, breathlessness, heartbeat, bronchial spasms, vomiting and impairment of consciousness. Lethal outcomes are possible.

• *First aid.* Sucking off the venom, slight of the bite site, injection of an antikarakurt serum can be used. *Prophylaxis.* Prevention from getting karacurts to the places of human lodging for the night.

• *Tarantulas's* venom contains cytotoxins and hemorrhagins and impair permeability of capillary walls.

• *Clinical presentation*. At a bite site appear pain, reddening, edema, skin necrosis. Symptoms: malaise, sleepiness, chills, pulse acceleration, perspiration.

• *First aid*. To treat the site with disinfectants, ensure rest, abundant drinking, pain-killers for the patient. *Prophylaxis:* protection from bites.

Class Insecta, order Hymenoptera (bees, wasps). These insects are actively-venomous, have toxic glands and a sting at the end of the abdomen. The venom has a neurotropic and cytotoxic action and is a strong allergen.

• *Clinical presentation*. After a bite — pain, edema, erythema. Possible symptoms: allergic reactions.

Poisonous and venomous vertebrates. Toxic fishes are divided into 2 groups:

1. Venomous species having toxic glands; the secretion of these glands is injected into the wound made by fin rays, teeth or thorns of branchial covers. Representatives: sting ray, sea dragons, ruffs and perches, moray eels, devilfish, firefish. They are spread predominantly in tropic latitudes of the Pacific and Atlantic Oceans.

• *Pathogenic action and clinical presentation*. Toxins pass into the organism through a wound on the skin. At the moment of a prick victim feels pain that quickly spreads to the whole extremity. Then appear fear, breathlessness, heart pain, vomiting and sometimes loss of consciousness. Inflammation, sometimes ulcers and tissue necrosis develop at the bite site. A severe poisoning ends with death within a day.

• *Treatment*: sucking off the venom from the wound, applying a rope, symptomatic treatment. Prophylaxis includes putting on special clothes if deal with the fishes.

2. Fishes that are poisonous when eaten (moray eels, thons, perciformes, pufferfish). When these fishes are used as food, poisoning develops in 20–30 minutes. There appears numbness of the tongue and fingers, nausea, vomiting, breathlessness, respiratory and speech affection. The treatment is symptomatic. As prophylaxis, the mentioned fishes should be excluded from the diet.

Amphibians. There are some toxic substances in the skin of some amphibians. The most virulent poison is produced by African tree-frogs and tree-toads. Toxin of the Columbian cocoa frog (the length of 2–3 cm, the weight is a bit more than 1 g) is 50 times stronger than a tetanus toxin. Other toxic amphibians are not dangerous for the human (they have no mechanism for injecting the toxin into tissues). When their poison gets on the skin or mucous membranes, erythema and inflammation are observed. These symptoms are relieved by washing with water. It is necessary to take care lest amphibians' poison gets to the eyes.

Class *Reptila*. Families elapids and sea serpents (king cobra and Indian cobra, long-glanded coral snakes, sea kraits). These are primarily-toxic actively-venomous animals. They have toxic immobile fangs with canals for the venom on the anterior part of the maxilla.

• *Pathogenic action and clinical presentation*. The venom contains neurotoxins, cytotoxins, hemolysins. At a bite site develops pain, edema, inflammation. Symptoms: excitation and then depression of CNS; swallowing, speech and breathing are impaired. Lethal outcomes are possible.

Family *Viperidae* (blunt-nosed viper, phoorsa, Orsini's viper, copperhead snake, rattlesnakes). They are primarily-toxic actively-venomous animals. They have toxic glands and fangs with canals.

- *Pathogenic action and clinical presentation*. The venom contains neurotoxins, cytotoxins, hemolysins, they stimulate blood coagulation. At a bite site develops pain, edema, tissue necrosis. Symptoms: weakness, nausea, dizziness, impairment of blood coagulation. Lethal outcomes are possible.
- *First aid*. The bite site should be treated with an antiseptic and a compressing bandage should be applied. The patient should be transported in a lying position. Injection of snakes' antitoxins should be done.
- *Prophylaxis*: in places of snakes' inhabitance one should not touch them and wear high boots.

Lecture 12. HOMEOSTASIS AND CHRONOBIOLOGY

Outline:
1. Homeostasis and its maintenance.
2. Chronobiology and its medical aspects

1. HOMEOSTASIS AND ITS MAINTENANCE.

Homeostasis. Living organisms constantly contact with their environment. Environmental factors of their habitation are changing all the time. Any organism adapts to them and strives to maintain the constancy of its own morphology and physiology, physical and chemical properties of cells, tissues, interstitial fluid and blood.

Homeostasis is the property of living systems to maintain stability and relative constancy of their internal environment in changing environmental conditions. The term «homeostasis» (Greek *homois* — identical; *stasis* — immobility) was introduced into biology by an American physiologist W. Cannon in 1932.

Mechanisms of homeostasis. Mechanisms of homeostasis provide thermoregulation, regulation of the blood pressure and concentration of ions various media of the organism.

Homeostasis depends on:

1. *Substances* that perform various functions in cells (proteins, fats, carbohydrates, oxygen, inorganic compounds, etc.).

2. *Environmental factors* that have effect on cells (osmotic pressure, temperature, concentration of ions).

3. *Mechanisms* providing integrity of the body (immunobiological reactivity, regeneration and repair and others).

In the context of Cybernetics (science of control and communication) a living organism is a system in which input variables (a stimulus, irritant, cause) and output variables (an effect, reaction, response) interact. A system is a sum of all elements obeying definite behavioral law. The basis of the system functioning is

registration of deviations in output variables depending on the information received at the input. For all that, the system behavior changes according to the information coming to the control block through feedback channels.

Positive feedback enhances the action of input variables. This connection change the system to extreme states and ultimately causes its instability. Negative feedback weakens the action of input variables. Negative feedback is the most spread type of feedback in living organisms as it increases its stability.

Levels of homeostasis:

− *molecular-genetic*: DNA repair, regulation transcription;

− *cellular, tissue, organ*: regeneration (cristae of mitochondria, myofibrils, cisternae of Golgi complex, increase of the number of organelles, cell division, modifications in cells and intercellular substance);

− *organism*: neurohumoral regulation and the organizing role of the NS;

− *population-specious*: Hardy–Weinberg principle;

− *biocenotic*: self-regulation of the population number;

− *biospheric*: provides dynamic balance of living systems with the environment by trophic connections (food chains) and circulation of substances in the nature.

Adaptation of a biological system to changing conditions of internal or external environment is based on «metabolic adaptation» — quantitative changes of metabolic process in cells.

Maintaining the constancy of organism's internal environment and continuous adaptations to constantly changing external environment occurs by means of nervous and endocrine systems. *The nervous system* provides quick changes in the organism. The effect of *hormones* is slower bur lasts longer.

Immune mechanisms of homeostasis provide the organism defense from goreign genetic information (viruses, bacteria, protists, helminthes, proteins and modified cells of the organism itself). Homeostasis is regulated by the immune system consisting of the thymus, spleen, lymphatic nodes and red bone marrow.

The organism reacts to unusual and strong effects of external environment with *stress reaction*, which changes work of the majority organ systems. The stress reaction involves cerebral cortex, hypothalamus, hypophysis, adrenal glands (secrete adrenalin).

Stages of stress reaction:

− activation of defensive mechanisms;

− increasing the resistibility of the organism;

− attenuation of defensive mechanisms.

The 1st and 2nd links of this chain preserve mechanisms of homeostasis; the 3rd one causes failure of homeostatic mechanisms and development of pathologic changes in the organism.

Mechanisms of homeostasis are maximally reliable in mature age. In childhood and in course of aging, their efficiency decreases and general resistance of the organism during these periods of ontogenesis is low.

2. CHRONOBIOLOGY AND ITS MEDICAL ASPECTS.

Living organisms are surrounded by inanimate nature characterized by rhythmic processes. Rhythms are alternating deviations and restorations of an initial state of a system that occur at equal time intervals. For example, alternation of day and night, alternation of seasons. Living organisms have adapted to them by means of rhythmicity of their vital activity or biological rhythms (biorhythms). Rhythmic processes are observed at all levels of life from the molecular-genetic level to the biospheric one. C. Barr was the first who formulated the problem of biological time in 1861. The time associated with live phenomena is biological time. Chronobiology studies biological time and biorhythms (Greek *chronos* — time).

When rhythms are regulated by external factors, they are *exogenous* rhythms. *Endogenous rhythms* are regulated by internal factors. In fact, rhythms depend on both endo- and exogenous factors.

In many cases the main external factor regulating functional rhythmic activity of living organisms duration of light day (*photoperiod*). For example, flowers of the majority of plants open in the morning and close at night; Drosophilae come out of a chrysalis at dawn; 59 % of deliveries happens at night.

There are 5 types of biorhythms:

The 1st type: *rhythms of high frequency* last from fractions of a second to 30 minutes. Examples: heart contractions, respiration movements, peristalsis;

The 2nd type: *rhythms of moderate frequency* or *circadian rhythms* that last from 30 minutes to 28 hours. Examples: changes of respiration and growth in plants; changes of activity in animals (day-time and night-time animals). About 69 physiological processes in the human body are associated with circadian rhythms. They can change 3–5 and more times during the day. Examples of such changes in human:

contractile function of myocardium is higher at day-time;

maximal temperature of the body is reached at 18 o'clock;

arterial pressure in the human is higher at day-time and lower at night;

blood coagulation is higher at day-time;

speed of cell division is more in the morning than at night.

People can be divided according to their workability into:

	larks	doves	owls
♀	25 %	50 %	25 %
♂	50 %		50 %

Such difference in men and women can be explained by the fact that the gene regulating circadian rhythm is located in an X-chromosome.

The regulation of circadian rhythms is accomplished at a hypothalamus level.

The 3rd type is *month rhythms* (example: periods in women);

The 4th type is *annual or seasonal* (from some months to 1 year): depend on light day is its synchronizer. Examples: transmigration of birds; winter and summer hibernation in animals; maximal activity of adrenal glands in summer; arterial

pressure is higher in an autumn-winter period; incidence of bronchial asthma attacks is higher in January and April and less in summer months.

The 5th type — *rhythms of low frequency*: 3, 7, 11, 80–90-year changes of solar activity. With them are associated:

– 3-year rhythms of tuberculosis recurrence incidences in humans;

– epidemics of some infectious diseases; cardio-vascular and psychic diseases (their number increases at maximum solar activity). The dependence of physiological processes on solar activity cycles is studied by *heliobiology*.

There is many data about the influence of the Moon and its phases on living organisms in literature. The Moon phases repeat every 29.53 days. The Earth surface ascends 35.6 cm maximum and descends 17.8 cm under the influence of the Moon. Under the influence of the Sun, ascending of the surface is 16.4 cm and descending — 8.2 cm. Such «breathing» of the Earth's is caused by gravitation.

There are data that human workability, irritability of his nervous system, irritation increase at a full moon; at new moon there are weakness, lowering of activity, creative energy and abilities. Association of psychic diseases with the Moon phases was authentically proved. The least frequency of childbirth is marked at new moon, the highest — at full moon.

From the moment of birth, three activity cycles are observed in every human:

1 — *physiological* activity (23-day periodicity);

2 — *emotional* activity (28-day periodicity);

3 — *intellectual* activity (33-day periodicity).

There is a critical (zero) day in the middle of every period.

The first half of the cycle is a positive period, the 2nd half of the cycle is a negative period. All the critical days coincide once a year.

Applied sections of Chronobiology are Chronomedicine and more common sections — Chronopathology, Chronopharmacology, Chronotoxicology and Chronotherapy.

A *chronobiological* approach allows predicting exacerbations of chronic diseases and acceleration of patients' recovery.

Chronomedicine studies biological rhythms of a healthy and sick organism.

Chronopathology studies changes in the organism in the impairment of biorhythms. Discordance of biorhythms — *desynchronosis* — may be a sign of pathology in the organism or may lead to some pathology. It contributes to gastritis, ulcers, tumors, nervous disturbances. Desynchronosis produces a considerable effect on person's workability.

Chronopharmacology studies various efficiency of medicines at different time of the day — sensitivity may fluctuate from 0 to 100 %. Studying the organism sensitivity to medicines at different time of the day is the subject of *chronotoxicology*. For example, application of cyclophosphan at 18 o'clock increased the frequency of curing mice from leucosis 5 times as compared to its effect at 9 o'clock. Taking into consideration chronobiology, chronotherapy should revise medicines' doses, their administration at time when they are most effective. For

example, to prevent cardiac asthma and lung edema, which occur more often at night, the increasing of glycosides and prednisolone doses should be done not in the morning but in the evening.

Achievements of chronobiology and chronomedicine are used for elaborating *chronoprophylactic measures*:

– compilation of day chronograms of a norm;

– taking into account biorhythms for making up a rational regimen of work and rest, rational nutrition of people of various occupations — workers of night shifts, pilots and cosmonauts;

– prognosis of exacerbations of various diseases; settling the problems of acclimatization and adaptation.

Lecture 13. STEM CELLS, REGENERATION AND TRANSPLANTATION

Outline:
1. Stem cells.
2. Regeneration.
3. Transplantation.

1. STEM CELLS

Human body consists of more than 200 different types cells. Cells of these types are different to perform particular work, i. e. they are specialized for performing certain functions.

• The process in which cell acquires specialty is called differentiation.

• All the cells in the human body arise from a single cell (zygote) which is not specialized. As cell divide, they become differentiated.

• Stem cell is an unspecialized cell that can differentiate into specialized cells.

• It is known that differentiation of cells does not change their genes. This was confirmed by transfer of the nucleus from an epithelial cell of frog to a denucleated egg. The egg containing the nucleus of a differentiated cell developed into normal tadpole (John Gurdon, 1962).

• Though, a differentiated cell cannot become undifferentiated again or change their cell type. Once the differentiation pathway of a cell has been chosen, it can no longer become another type of cell.

• However, differentiated cells were turned back into the stem cells by methods of biotechnology. Several genes expressed in stem cells were inserted into mice fibroblasts by retroviruses (Shinya Yamanaka, 2006). Later on, human stem cells were successfully produced by this method. Such stem cells are called *induced pluripotent stem cells* (iPSC).

Stem cells can be obtained from embryonic or fetal tissues, cord blood or tissues of adults. Though these cells have different potencies (i. e. the «choice» for

differentiation is different). The potency of stem cells restricts with development of the embryo (see the lecture 8).

• Totipotent stem cells retain the zygote's potential to form new organism. For example, if the first two blastomeres after frog's zygote division are separated, each will develop into a normal tadpole.

• Pluripotent stem cells can differentiate into the cells of any germ layer. The example of such cells is the inner cell mass of the blastocyst. These stem cells can be obtained from in vitro fertilization clinics (as the number of produced zygotes exceeds the required number). This is associated with some moral and ethical questions, but 5-day old embryo has no even tissues developing into the nervous system. It is a mass of cells which cannot have personhood.

• Multipotent stem cells are cells that have the capacity to self-renew by dividing and to develop into multiple specialized cell types present in a specific tissue or organ. Such cells are present in adults.

Some important properties of stem cells:

• Undifferentiated state.

• Capability to divide and renew themselves. This is not possible for many specialized cell types (e. g. nervous system).

• Asymmetric division means that one daughter cell stays a stem cell, the other one differentiates. The fate of cells becomes programmed not after division, but before it is done. The state of stem cell is inherited epigenetically i. e. the properties of the cell are defined not by the specific genes it has, but by specific pattern of the gene expression.

• Capability to migrate in tissues.

The usages of stem cells include:

• Fundamental scientific researches (understanding of the mechanisms of cell differentiation, embryonic development, signaling cascades and gene expression).

• Test of drugs on cultures of stem cells programmed into particular cell types.

• Regenerative medicine — stem cell therapy for the repair of damaged tissues or organs (myocardial infarction, ischemic disease, stroke, diabetes, Alzheimer's disease, wounds and burns, and etc.)

2. REGENERATION

Regeneration renewal or restoration of body parts (or tissues) after injury or as a normal process.

Physiological regeneration is inherent in all organisms. In the process of vital activity, disruption of some anatomical structures may occur and they should be

Regeneration occurs at different levels:

• Cellular regeneration (e. g. nerve cells can grow new processes when they are lost);

• Tissue regeneration (e. g. wound healing);

- Organ regeneration (liver can grow bigger if its fragment was removed);
- Body part regeneration (axolotls can regenerate limbs);
- Whole organism regeneration (both fragments of a planarian cut into two refenerate and become independent organisms).

Regeneration can be physiological and reparative.

- *Physiological regeneration* is the replacement of cells which are lost during day-to-day activities. Categories of cells:
- Labile (or renewing) — regenerate regularly (epidermis — 10–12 days, Epithelium of gastrointestinal tract — 7 days, RBC — 120 days).
- Stabile (or expanding) — regenerate slowly (liver, kidneys, exo- and endocrine glands — 300-400 days).
- Static (or permanent) — no effective regeneration (neurons, myocardiocytes are unable to divide).
- *Reparative regeneration* is the replacement of lost body parts.

Types:
– Morphollaxis.
– Epimorphosis.
– Compensatory growth.

Morphollaxis is the regeneration of a part or organism from a fragment by its reorganization. In other words, a new smaller organism develops from each remaining part. Majority of regenerated tissue comes from already-present cells of the organism.

- An example of morphollaxis of the regeneration of planarian.
- Planarian is a flatworm of the class Turbellaria.
- When it is cut into pieces, then each piece can regenerate into a complete organism.
- An 1/279th of a planarian can regenerate.
- After a planarian has been transected, the wounded area is rapidly covered by a thin layer of epidermal cells.
- Undifferentiated cells then accumulate beneath the wound epithelium giving rise to an unpigmented structure referred to as the regeneration *blastema*.
- As regeneration proceeds, more of these undifferentiated cells continue to accumulate within the blastema, causing it to grow exponentially.
- Within one week of the transection, differentiation of the missing structures occurs.
- Regeneration occurs due to pluripotent stem cells *neoblasts* which contribute near 20 % of all worm's cells.

Epimorphosis (epimorphic regeneration) or — regeneration of a part or organism involving extensive cell proliferation followed by differentiation. e. g. a new limb of axolotl grows from the wound surface to replace the lost one).

- Axolotl is the larval stage of the salamander *Ambystoma* which reaches sexual maturity without undergoing metamorphosis.

• Within 1 day after limb amputation, the amputated surface is rapidly covered with epithelial cells. (dermis-free epithelial structure *wound epidermis*).

• After that, tissues beneath the wound epidermis undergo histolysis generating a population of undifferentiated cells (including proliferative and multipotent mesenchymal cells). At the end of the dedifferentiation stage, the mesenchymal cells build up a blastema — a cone-shaped mass of cells that is a structure comparable to the limb bud in limb development.

• Until the process of limb regeneration is completed, the blastema continues to grow distally by active proliferation of blastemal mesenchymal cells.

• Simultaneously with blastema elongation, redifferentiation and repatterning begins, and a complete limb structure is finally re-established.

• Regeneration does not occur if the limb is denervated (Schwann cells of the nerve produce a signaling molecule necessary to induce regeneration).

• Inactivation of macrophages also disturbs regeneration.

• Regeneration by epimorphosis is similar to the embryonic development of limbs. Many signaling pathways participate both in the embryonic development and regeneration. Activation of some of those causes some regenerative processes in the animals which are unable to regenerate, e. g. β-catenin activation caused partial wing regeneration in chicken embryo (Yasuhiko Kawakami et al., 2006).

Compensatory growth or endomorphosis — the part of an organ left after amputation enlarges as a result of cell enlargement and division. For example, if one kidney is missing, the other one becomes bigger to perform the functions of two kidneys.

Such enlargement of organs is based on two processes:

Hypertrophy — increase in the volume of an organ or tissue due to the enlargement of its cells.

Hyperplasia — increase in the volume of an organ or tissue due to the cell proliferation.

2. TRANSPLANTATION

Transplantation. Organ transplantation is medical procedure in which an organ or tissue (it is called *graft* or *transplant*) is removed from one body (from *donor*) and placed in the other body (to *recipient*), in order to replace a damaged or missing organ.

Important points in the history of transplantation were:

• Development of the technique of vascular suture and the transplantation of blood vessels (Alexis Carrel, The Nobel Prize 1912), Vladimir Demikhov's work with animals that included: first artificial heart, heart transplant, lung transplant, heart-lung transplant, liver transplant, mammary-coronary anastomosis, head transplant.

• The discovery of ABO blood group system by Karl Landsteiner, the discovery rhesus factor discovered. This lead to the discovery of HLA (human leuko-

cyte antigens) and the role of immune system for transplant rejection is revealed (Peter Medawar, The Nobel Prize 1960).

• The first successful heart transplant in human was done in South Africa by Christiaan Barnard in 1967.

Types of transplantation:

Based on the genetic relation of donor and recipient:

• *Autotransplantation* is the transplantation within the same organism.

• *Isotransplantation* is the transplantation from a genetically identical donor (i. e. from a twin).

• *Allotransplantation* is the transplantation from a genetically non-identical donor of the same species.

• *Xenotransplantation* is the transplantation in which donor and recipient belong to different genera, families.

Based on the location of the graft in the recipient:

• *Orthotopic transplantation* is the transplantation of the donor's organ to its native place;

• *Heterotopic transplantation* is the transplantation of the donor's organ to the place which is not the normal organ location;

Obtaining the organs for transplantation. Organs for transplantation can be taken from:

• Donation from a living donor. Living donors may donate a kidney, part of the liver, part of the lungs, stem cells, bone marrow.

• Donation from a dead donor. The organs and tissues which can be used as grafts are kidneys, heart, lungs, liver, pancreas, small intestine, corneas, skin, bone, tendons, cartilage, heart valves.

– Donation from a dead donor occurs after brain death.

– *Brain death* is the irreversible brain damage and loss of brain function, but other organs can still work and are kept functioning by life support.

– It should be distinguished from *biological death* (permanent cellular damage, resulting from lack of oxygen, that is not reversible) and *clinical death* (reversible cessation of blood circulation and breathing).

• Growing organs in laboratory is still under research. So far only organoids were obtained from stem cells.

– Organoids are tiny, self-organized three-dimensional tissue cultures that are derived from stem cells. They do not have the architecture of the original organ.

– Organoids can be used for fundamental medical researches, but are not yet applicable in practical medicine. However, these studies may lead to growing artificial organs in the future.

• 3D-bioprinting is the utilization of 3D printing–like techniques to combine cells, growth factors, and biomaterials to fabricate biomedical parts that maximally imitate natural tissue characteristics.

– This practice is under investigations.

– In 2017, 3D-bioprinted ears were transplanted to the children with congenital ear defects in China.

Tissue incompatibility. Tissue incompatibility is the complex immune response to foreign cells, tissues or organs. The immune system of recipient strives to damages the foreign cells of the transplant. This is the cause of transplant (graft) rejection.

– Major histocompatibility complex (MHC) is a group of protein markers that aid in the ability of the immune system to recognize own cells and distinguish them from foreign pathogens.

– Human MHC genes are situated in the p arm of the 6th chromosome.

– Human MHC are highly polymorphic due to numerous alleles of these genes.

Measures to overcome tissue incompatibility:

1. Selection of donor. The choose of donor having matching antigens (primarily for the locus D) allows to increase successful graft retention. Complete retention is possible only in monozygotic twins.

2. Suppression of the immune response. Therapy with immunosuppressive drugs, corticosteroids anti-lymphocyte serum, X- and gamma-rays.

REFERENCES

1. *Bekish, O.-Y. L.* Medical biology : textbook for student of higher educational establishments / O.-Y. L. Bekish. Vitebsk : VSMU Press, 2003. 346 p.

2. *Медицинская* биология для иностранных студентов 1-го года обучения = Medical biology for international students 1st year : курс лекций / В. Э. Бутвиловский [и др.] Минск : БГМУ, 2015. 141 с.

3. *Медицинская* биология для иностранных студентов, обучающихся по специальности «Лечебное дело» = Medical biology for international students studing General medicine : учеб.-метод. пособие / В. Э. Бутвиловский [и др.]. Минск : БГМУ, 2017. 200 с.

4. *Медицинская* биология и общая генетика : учеб. / Р. Г. Заяц [и др.]. 2-е изд., испр. : Минск : Выш. школа, 2012. 496 с.

5. *Частная* паразитология : учеб.-метод. пособие. / В. Э. Бутвиловский [и др.]. Минск : БГМУ, 2007. 107 с.

CONTENTS