BIOLOGY

for international students studying «Pharmacy»

Lecture course

Minsk BSMU 2016

МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ РЕСПУБЛИКИ БЕЛАРУСЬ
БЕЛОРУССКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИЙ УНИВЕРСИТЕТ
КАФЕДРА БИОЛОГИИ

БИОЛОГИЯ
ДЛЯ ИНОСТРАННЫХ СТУДЕНТОВ ПО СПЕЦИАЛЬНОСТИ «ФАРМАЦИЯ»

BIOLOGY
FOR INTERNATIONAL STUDENTS STUDYING «PHARMACY»

Курс лекций

Минск БГМУ 2016

УДК 57(811.111) – 054.6(076.5)
ББК 28.0 Англ –923
Б 63

Рекомендовано Научно-методическим советом университета
в качестве курса лекций 15.06.2016 г., протокол № 10

А в т о р ы**:** доц. В. Э. Бутвиловский; ассист. В. В. Григорович; ассист. Е. А. Романовский; доц. А. В. Бутвиловский; ассист. Е. А. Черноус

Р е ц е н з е н т ы**:** канд. биол. наук, доц. А. В. Колб; канд. мед. наук, доц. О. Н. Ринейская

Биология для иностранных студентов по специальности «Фармация» = Б 63 Biology for international students studying «Pharmacy» : курс лекций / В. Э. Бутвиловский [и др.]. – Минск : БГМУ, 2016. – 72 с.

ISBN 978-985-567-545-8.

Содержит теоретический материал 9-ти тем лекций по биологии.
Предназначен для студентов 1-го курса медицинского факультета иностранных учащихся, обучающихся на английском языке по специальности «Фармация».

УДК 57(811.111) – 054.6(076.5)
ББК 28.0 Англ –923

Учебное издание

Бутвиловский Валерий Эдуардович
Григорович Виктор Васильевич
Романовский Евгений Александрович и др.

БИОЛОГИЯ
ДЛЯ ИНОСТРАННЫХ СТУДЕНТОВ ПО СПЕЦИАЛЬНОСТИ «ФАРМАЦИЯ»

BIOLOGY
FOR INTERNATIONAL STUDENTS STUDYING «PHARMACY»

Курс лекций
На английском языке

Ответственный за выпуск В. Э. Бутвиловский
Переводчики: В. В. Григорович, Е. А. Романовский
Компьютерная верстка А. В. Янушкевич

Подписано в печать 15.06.16. Формат 60×84/16. Бумага писчая «Снегурочка».
Ризография. Гарнитура «Times».
Усл. печ. л. 4,18. Уч.-изд. л. 3,97. Тираж 30 экз. Заказ 537.

Издатель и полиграфическое исполнение: учреждение образования
«Белорусский государственный медицинский университет».
Свидетельство о государственной регистрации издателя, изготовителя,
распространителя печатных изданий № 1/187 от 18.02.2014.
Ул. Ленинградская, 6, 220006, Минск.

ISBN 978-985-567-545-8

LECTURE 1
Topic: CELL. CELL THEORY. ORGANIZATION OF SUBSTANCES AND ENERGY OFLWS IN THE CELL

Plan

1. Cell theory; its modern state.
2. Basic forms of cellular organization.
3. Structure, properties and functions of an plasma membrane.
4. Organization of a substances flow in the cell.
5. Organization of an energy flow in the cell.

In 1665 an English physicist R. Hooke studying a slice of cork tree's bark noticed little boxes which he called *«cellula»*, a cell.

Studying the cell ran together with the development of microscopic techniques.

A Dutchman A. van Leeuwenhoek revealed unicellular organisms in water. In 1825 a Chech scientist Ya. Purkyne described a semi-fluid, jelly-like content of the cell and called it «protoplasm» (greek *protos* — the first, *plasma* — formation).

In 1831 an English Botanist R. Brown revealed a *nucleus* in the cell.

In 1838–1839 German researchers Teodor Schwann (a zoologist) and Matthias Schleiden (a botanist) joined the received findings and formulated a cell theory. Its basic concepts are:

1) the cell is a basic structural unit of animals and plants;

2) the process of cell formation results in growth, development and differentiation of tissues of plants and animals.

In 1858 the research «Cellular pathology» by a German pathologist R. Virchov was published. It had two important concepts:

1) every cell originates from another cell due to division;

2) all diseases of the organism are due to some changes of the cellular structure and function.

The basic concepts of a modern cell theory:

1. The cell is a structural, functional and genetic unit of all living things; it is an open self-regulating system constantly passing flows of substances, energy and information.
2. Cells of all organisms have a similar structure, chemical composition and processes of vital activity.
3. Cells of a multicellular organism perform various functions and form tissues.
4. New cells form as result of the division of a mother cell.

Cytology (latin *cytos* — a cell, *logos* — a science) studies the structure, chemical composition, multiplication, development of cells and their interaction in a multicellular organism.

Prokaryotes do not have a formed nucleus. Their genetic apparatus is a circular DNA molecule not bound with proteins-histones that is called a *nucleoid* (fig.1).

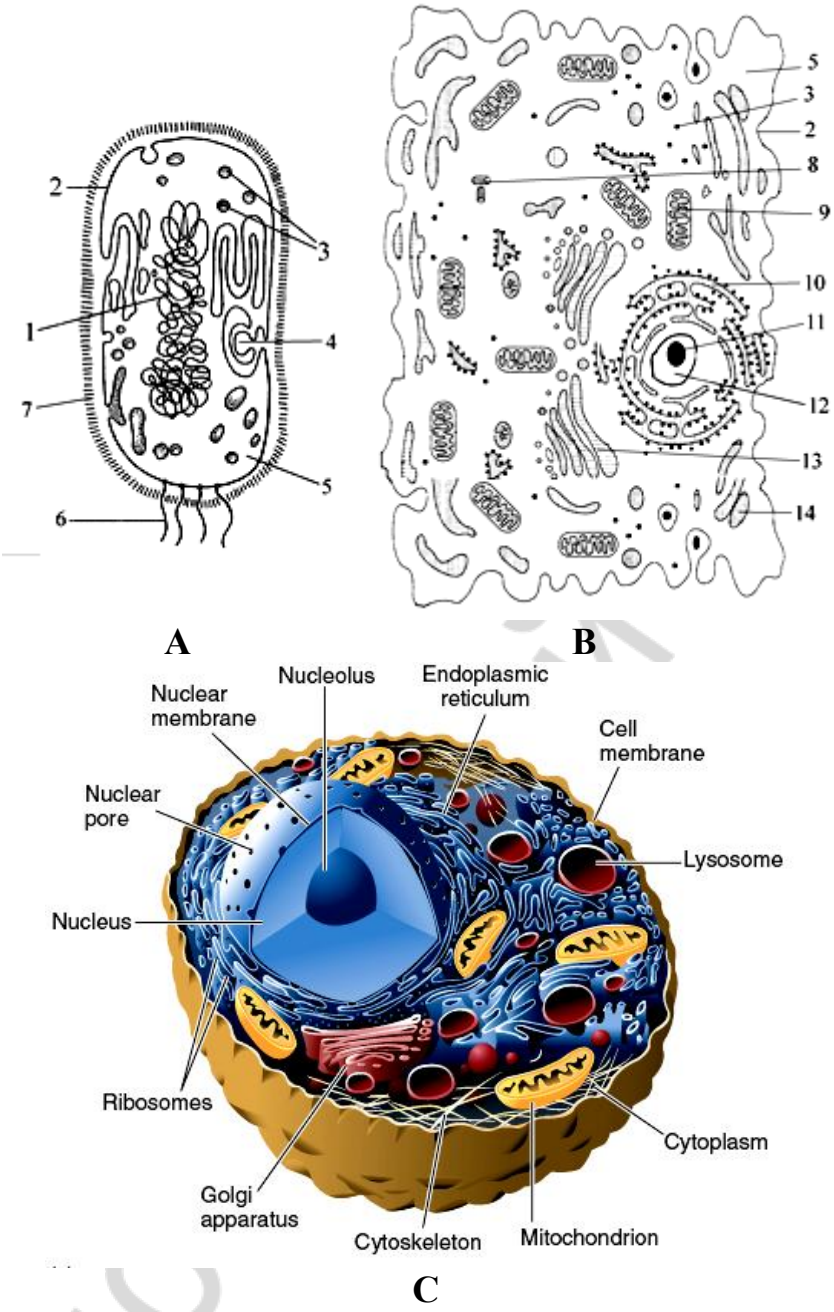


Fig. 1. Structure of prokaryotic (A) and eukaryotic (B, C) cells:
1 — a nucleoid; 2 — plasma membrane; 3 — ribosomes; 4 — mesosome; 5 — cytoplasm; 6 — filaments; 7 — cell wall; 8 — cell center; 9 — mitochondria; 10 — granular ER (endoplasmic reticulum); 11 — nucleolus; 12 — nucleus; 13 — Golgi complex; 14 — smooth ER

The most primitive prokaryotes are *mycoplasms*. Their age is 3 billion years; the diameter is 0.1–0.25 μm.

The majority of them are symbionts and facultative parasites of mammals, insects and plants. Unlike viruses, they are capable of self-reproduction, and unlike bacteria, they do not have a cell wall.

Affection of human fetuses with mycoplasms causes *mycoplasmoses*. They affect the lungs and the central nervous system. (table 1).

Table 1

Basic types of cellular organization

Prokaryotes (prenuclear)			**Eukaryotes (nuclear)**	
Mycoplasms	Bacteria	Cyanobacteria	Unicellular organisms (protists)	Cells of multicellular organisms

They can produce a teratogenic action, causing chromosomal defects. In adults mycoplasmoses occur in the lungs, respiratory and urogenital organs (table 2).

Table 2

Differences between prokaryotes and eukaryotes

Prokaryotes	**Eukaryotes**
No nucleus but nucleoid	There is a nucleus
Mycoplasms, bacteria, cyanobacteria	Protists, plant and animal cells
Sizes: 1–10 μm	10–100 μm
DNA is not bound with proteins-histones	DNA is linked with proteins-histones
There is no mitosis, no membrane organelles*, their functions are performed by mesosomes — ingrowths of the cellular membrane	There is mitosis, membrane organelles (fig. 1)

* Mesosomes perform their function — drawing in the cellular membrane.

Eukaryotes have a formed nucleus surrounded by a membrane. The genetic apparatus is a complex structure of DNA bound with proteins-histones.

The internal content of the cell is presented by a *cytoplasm* and *karyoplasm* (nucleus). In the cytoplasm, we can see a *gialoplasm* (cytoplasmatic matrix), *organelles* and *inclusions*. From outside the cell is covered with a membrane, the basic component of which is an elementary (biologic) membrane.

PLASMA MEMBRANE

N. Davson and R. Danielli proposed the first model of the plasma membrane in 1943. It was a **sandwich model** (fig. 2 part A).

Two layers of lipid molecules are located between two layers of protein molecules. Each lipid molecule has two ends: a *hydrophilic one* (soluble in water) and *hydrophobic one* (insoluble in water). Hydrophobic parts of the molecule are directed to each other, while hydrophilic parts are directed to protein molecules.

In 1972 S. Singer and G. Nicolson proposed a better **fluid mosaic** model which corresponds to the properties and functions of the plasma membrane (fig. 2 parts B and C).

Basic membrane components, *lipids*, comprise 20–80 % of their mass. They are phospholipids, lecithin and cholesterol. Protein molecules are in a double layer of lipid molecules that form a «lipid sea». Protein molecules passing through the

two layers of lipid molecules are *integral*. Those molecules, some of which are in a bilipid layer, are called *semi-integral*. *Peripheral proteins* are on the surface of lipids. The third component of the plasma membrane is glycoproteins forming a receptor apparatus (*glycocalix*) on its surface.

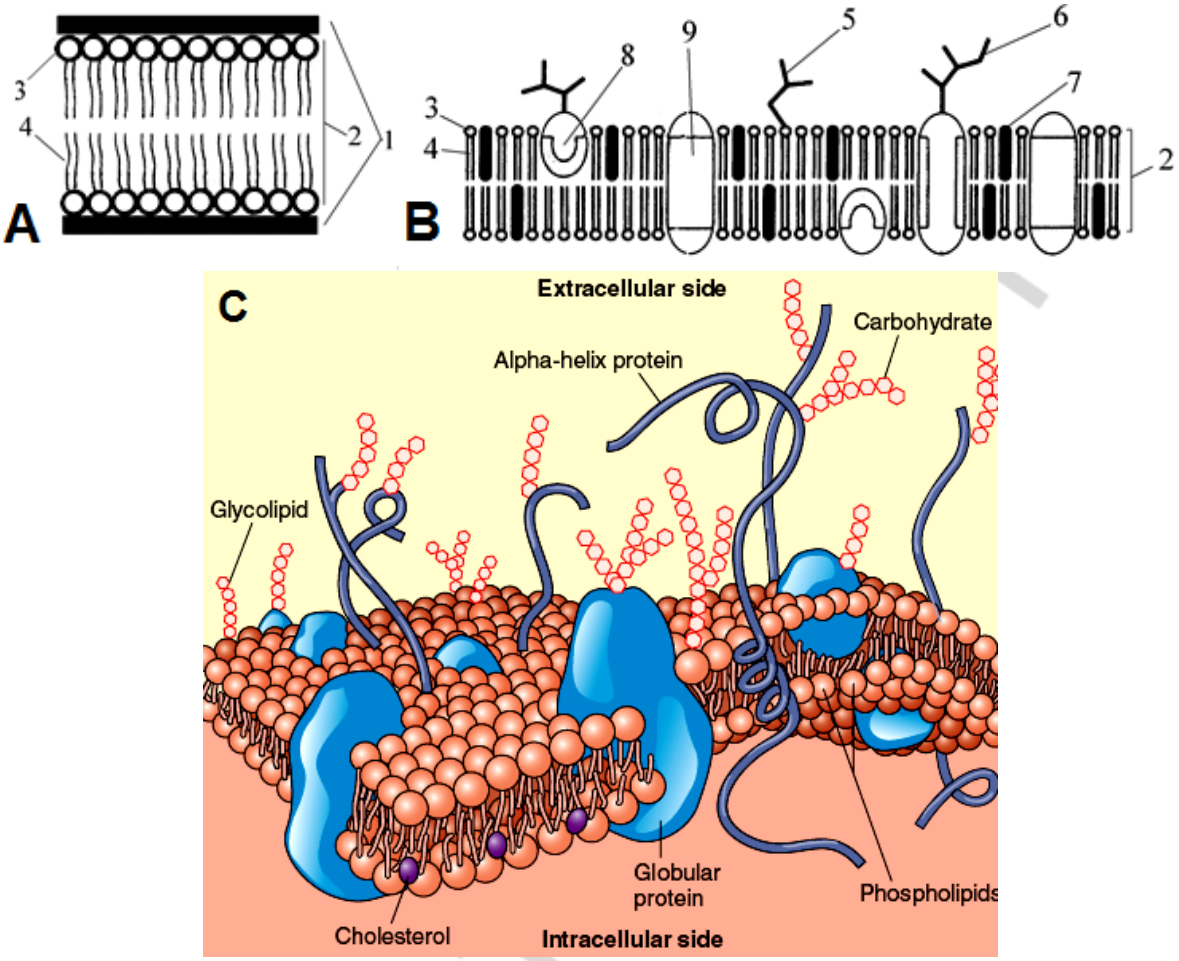


Fig. 2. Diagram of plasma membrane models:

A — sandwich model; B, C — fluid-mosaic model: 1 — solid protein layers; 2 — bilipid layer; 3 — hydrophilic heads of phospholipids; 4 — hydrophobic tails of phospholipids; 5 — oligosacharide chain of glycolipids; 6 — oligosacharid chain of glycoprotein; 7 — cholesterole molecule; 8 — semi-integral protein; 9 — integral protein; 10 — peripheral proteins

Basic membrane components, *lipids*, comprise 20–80 % of its mass. They are phospholipids, lecithin and cholesterol. Protein molecules are situated in a double layer of lipids that form a «lipid sea». Protein molecules embedded into two layers of lipid molecules are *integral*. Proteine molecules that are partially embedded into the bilipid layer are called *semi-integral*. *Peripheral proteins* are on the surface of lipids. The third component of the plasma membrane is glycoproteins forming a receptor apparatus (*glycocalix*) on its surface.

Properties of the plasma membrane:

– plasticity (restores quickly after injury; also stretches and compresses in cell movements);

– semi-permeability (selectively passes specific molecules);

– ability for self-locking (formation of phagosomes and vacuoles in feeding an amoeba).

Functions of the plasma membrane:

– structural (the membrane principe of the organelle structure: membranes are components of all cellular organelles except ribosomes and centrosomes);

– barrier (defends the cell from external effect and sustains its composition);

– participates in metabolic processes (many biochemical reactions take place on membranes);

– receptor (receiving and differentiating signals, substances).

ORGANIZATION OF A SUBSTANCE FLOW IN THE CELL

The substance flow in the cell undergoes three phases: passing of substances into the cell (membrane transport), transformation and distribution of substances in the cell, excretion of metabolites from the cell.

There are various mechanisms of membrane transport (fig. 3).

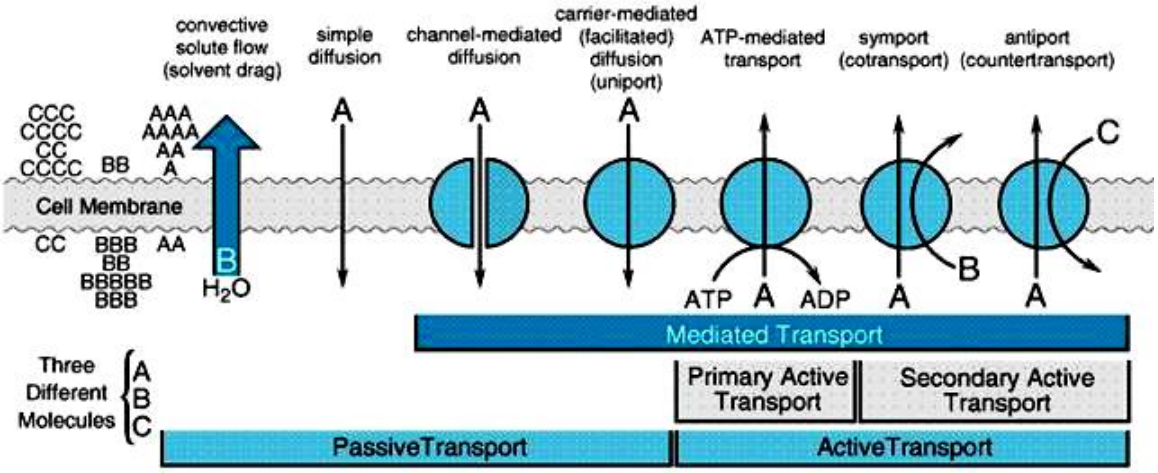


Fig. 3. Mechanisms of membrane transport

Passive transport runs in conformity with a concentration gradient without any waste of energy. Water and small molecules can pass into the cell by *osmosis* and *diffusion*, through pores or during dissolution in lipids. *Facilitated diffusion* is associated with participation of carrier proteins that are called *permeases*. In this way amino acids, sugars, fatty acids get into the cell.

Cytosis is participation of the membrane itself in capturing particles or molecules and transferring them into the cell (*endocytosis*) or excreting them from the cell (*exocytosis*). Cytosis means reversible changes of membrane architectonics (outlines). Transport of macromolecules or solid particles is *phagocytosis*, while transport of fluid drops is called *pinocytosis* (fig 4).

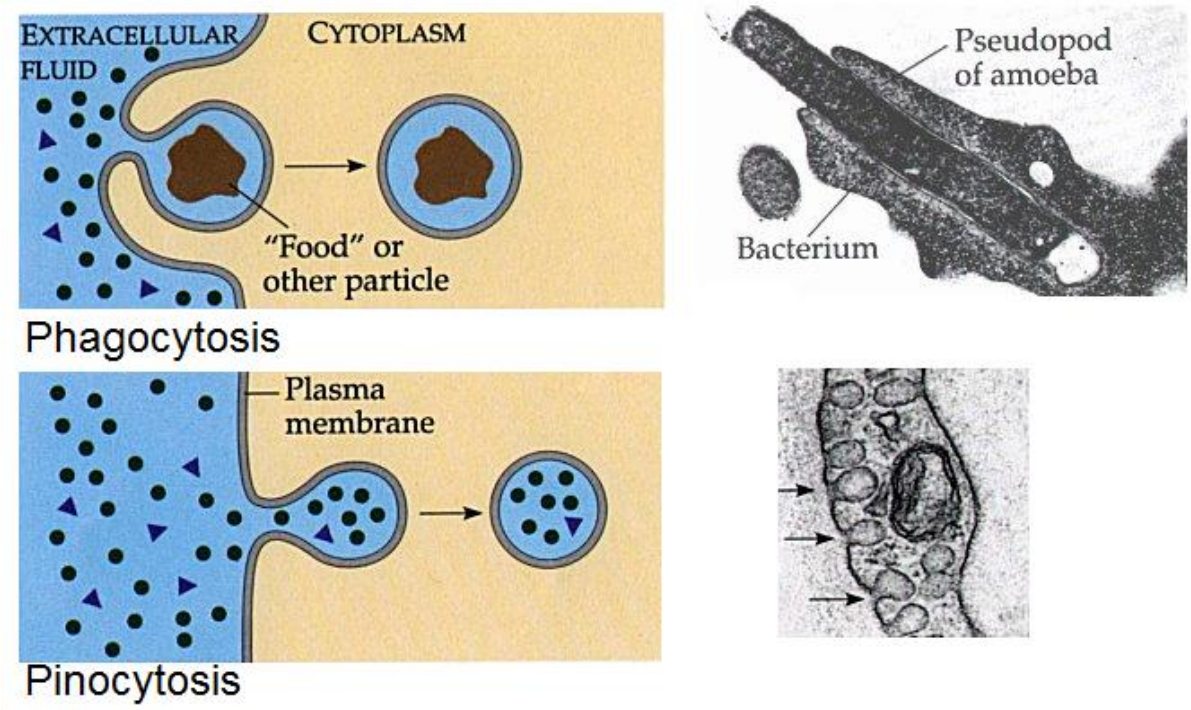


Fig. 4. Endocytosis

Substances and molecules that have passed the plasma membrane are distributed throughout the cell (fig 5).

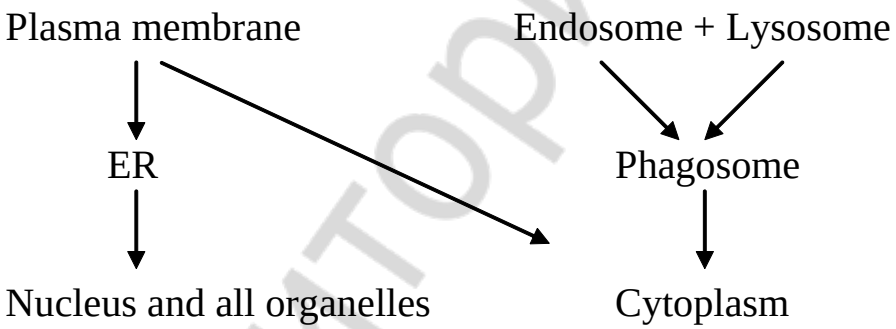


Fig. 5. Transport of substances in the cell

Assimilation reactions take place in the anabolic system of the cell. It includes organelles: ribosomes, endoplasmic reticulum (ER), Golgi complex.

Organelles are isolated areas of the cytoplasm that have a constant structure and perform specific functions (fig. 6).

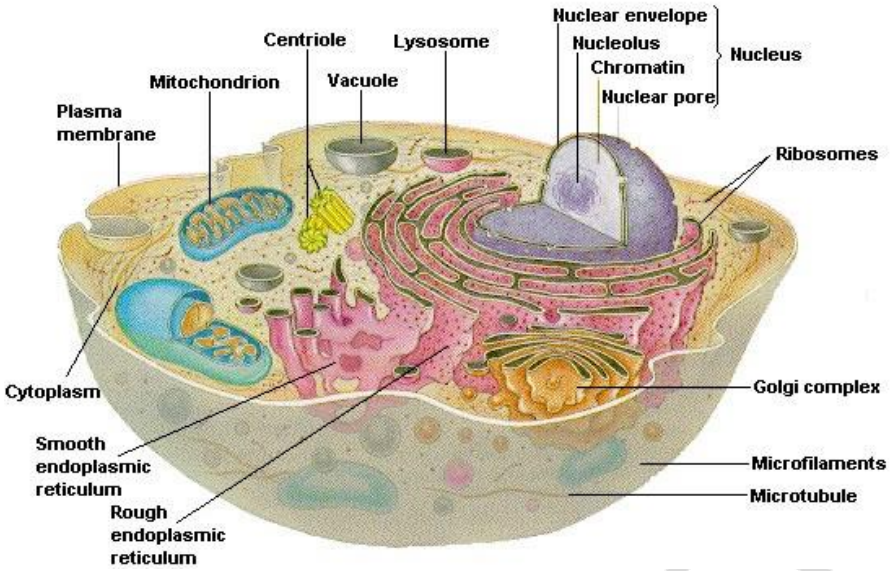


Fig. 6. Organelles of the cell

Ribosomes (fig. 7) are spherical bodies (15–35 nm in diameter) consisting of large and small subunits. They may stay freely in the cytoplasm, on the external nuclear membrane, on ER channels. A *large subunit* of a ribosome contains 3 various rRNA molecules and 40 protein molecules. A *small subunit* contains 1 rRNA molecule and 33 protein molecules. A ribosome assemble takes place in the pore areas of the nuclear membrane. The information about the rRNA structure and ribosome proteins is stored in nucleolar organizers (parts of a DNA molecule in the area of secondary constrictions of satellite chromosomes). Ribosomes take direct part in assembling protein molecules. Free ribosomes synthesize proteins for vital activity of the cell itself, the attached ones — proteins for excretion from the cell.

Endoplasmic reticulum (ER) is tubes and flattened membranous sacs located throughout the cell (fig. 8) and connected with a perinuclear space of the nucleus and the cavities of a Golgi complex. ER membranes perform division of cell cytoplasm into compartments where various biochemical reactions take place (the function of compartmentalization). *Granular (rough) ER* (ribosomes are located on its membranes) participate in biosynthesis of proteins that are later transported to a Golgi complex. Carbohydrates (glycogen) and lipids (cholesterol) are synthesized on membranes of a *smooth ER*. It participates in synthesis of steroid hormones, in excretion of chlorine ions (epithelial cells of the stomach), in detoxication by hepatic cells.

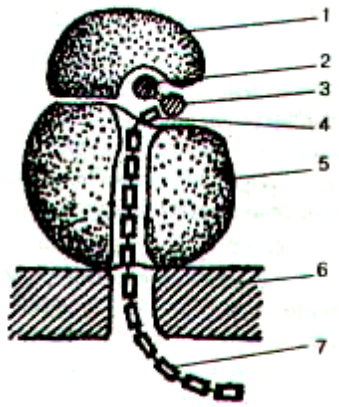


Fig. 7. Ribosome:
1 – small subunit, 2 – mRNA, 3 – tRNA, 4 – amimo acids, 5 – large subunit, 6 – membrane of ER, 7 – assembled protein.

Golgi complex (Golgi apparatus) consists of vesicles, tubes, sacs. Dictisomes (fig. 9) are basic elements of the complex.

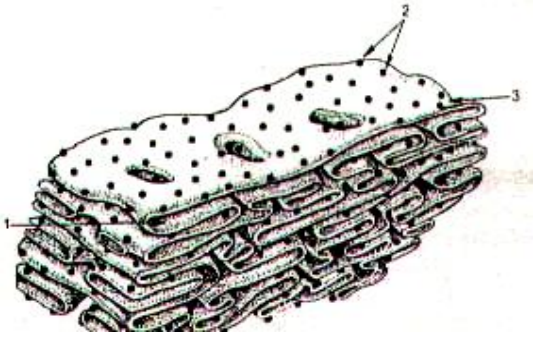


Fig. 8. Structure of granular ER:
1 – canal; 2 – ribosomes; 3 – membrane

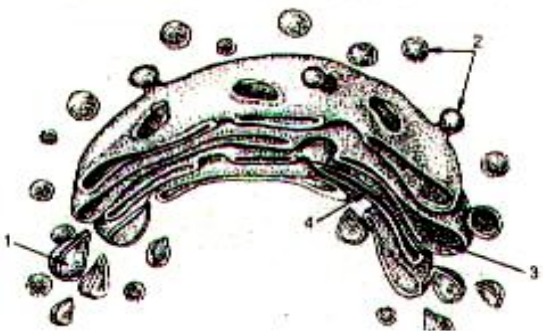


Fig. 9. Structure of Golgi complex:
1 – vacuole; 2 – vesicles; 3 membrane;
4 – canal

Dictisomes are piles of 10–15 plasma membranes that are dilated at the ends. These dilations form vesicles that separate and transform into lysosomes and vacuoles. Some of these vesicles excrete secrets and metabolites from the cell. Golgi complex has the following functions: 1) sorting out and packing substances, synthesized in ER, into vesicles; 2) formation of complex compounds (lipoproteins, glycoproteins); 3) formation of plasma membranes; 4) formation of lysosomes, glyoxysome and vacuoles; 5) secretion of substances.

Dissimilation reactions take place in the catabolic system of the cell. It includes mitochondria, lysosomes, microbodies (peroxisomes and glyoxysome).

Primary lysosomes are formed in Golgi complex. They look like spheric bodies (0.2–2 μm in diameter), covered with an plasma membrane. They carry approximately 50 various hydrolytic enzymes. Breaking down of substances takes place in *secondary lysosomes* that are formed when *primarylysosome* joins a *phagosome*. Lysosomes are capable of dissolving the structures of organelles.

Peroxisomes are formed in ER. Their enzymes (oxidizes) oxidize amino acids and form peroxide (H_2O_2).

Glyoxysome are formed in Golgi complex. Their enzymes transform fats into carbohydrates.

Mitochondria have a form of rods, filaments, granules when they are observed with a light microscope. The size of mitochondria is from 0.5 to 7 μm. Their number is different in cells with different activity. A wall of mitochondria consists of an *inner* and *outer* membrane. Ingrowths on the *inner* membrane form *cristae* enclosing a homogenic *internal matrix*. The interspace between membranes of mitochondria is filled with an *external matrix*. There are *3 enzyme systems* in mitochondria: enzymes of the *Krebs cycle* the (citric acid cycle) in the internal matrix; enzymes of tissue respiration on the *inner* membrane and in the external matrix; enzymes of *oxidative phosphorylation* in ATP-somes (on cristae). Mitochondria have an autonomous system of protein biosynthesis. There are ribosomes, various RNA and circular DNA molecules in its internal matrix.

Functions of mitochondria: ATP synthesis (transformation of energy of broken down compounds into that of phosphate bonds), synthesis of specific proteins and steroid hormones.

Energy exchange has three stages:

1. Preparatory stage takes place in the digestive system of organisms and in phagosomes of cells where complex organic compounds are broken down into simple ones: polysaccharides to monosaccharides, proteins to amino acids, fats to glycerol and fatty acids.

2. Anaerobic stage takes place in the cell cytoplasm. It involves 10 enzymes. Glucose is broken down into pyruvic acid (pyruvate) and 2 ATP molecules are formed. The pyruvate may pass into mitochondria (for further transformations). During muscular activity, the lactic acid is formed there.

3. Aerobic stage of energy exchange takes place in mitochondria (fig. 10).

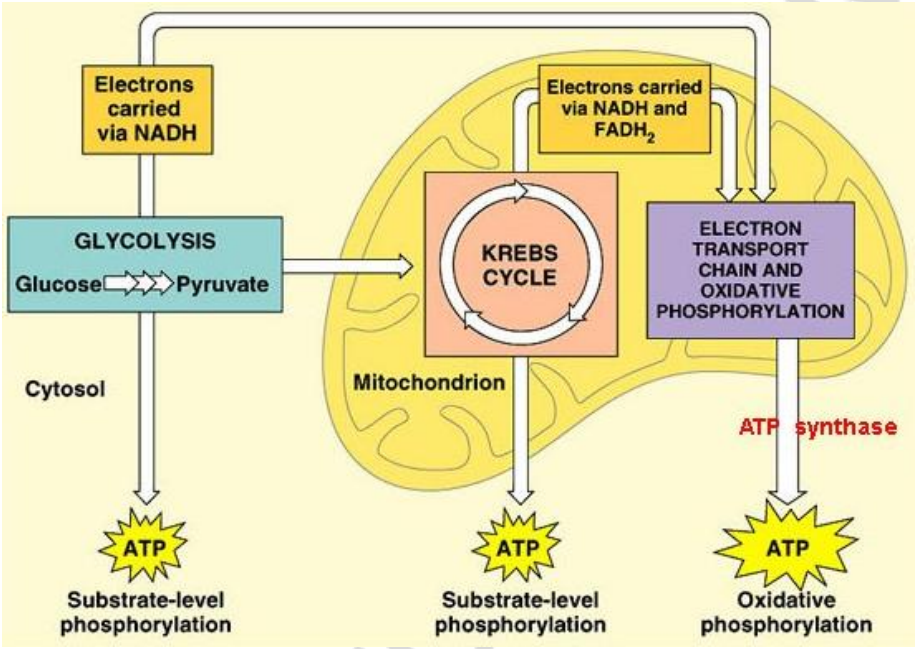


Fig. 10. Organization of an energy flow

Pyruvate in combination with coenzyme A (CoA) passes into the internal matrix of the mitochondrion. Hydrogen atoms are split out from an activated form of an acetic acid (Acetyl CoA).

The resulting CO_2 is released from a mitochondria while protons and electrons (from hydrogen atoms) pass to the enzyme system of tissue respiration Protons are accumulated on the outer surface of the internal membrane while electrons — on the inner one. On achieving a critical potential protons pass through the channels in ATP-somes.

Electrons give energy for attaching the residues of phosphoric acid to ADP and for forming ATP, and then they join the protons. Hydrogen atoms are formed and in association with oxygen form water molecules. During all transformations of 1 glucose molecule 36 molecules of ATP +2 molecules of the anaerobic stage are formed, in total 38 molecules of ATP.

LECTURE 2
Topic: ARRANGEMENT OF GENETIC MATERIAL (I)

Plan

1. Heredity and variation are fundamental properties of living things.
2. Gene: an evolution concept.
3. Structure and functions of nuclear acids.
4. Genetic code and its properties.
5. Properties of genes.
6. Classification of genes.

HEREDITY AND VARIATION ARE FUNDAMENTAL PROPERTIES OF LIVING THINGS

Heredity is the property of living things to preserve likeness of structural-functional organization in a number of generations.

Variation is the property of living things to acquire new characters under the influence of the environment.

Heredity is conservative. It consolidates and preserves characters of the organism and species. Variation, vice versa, permits organisms to acquire new characters and differ from parents.

The process of transmitting information from one generation to the other during sexual reproduction is called *inheritance* while the likeness degree of parents and children is called *inheritability*.

GENE: AN EVOLUTION OF THE CONCEPT

Ch. Darvin was the first to have written about heredity units. He called them heredity factors. In 1865 the work «Experiments with plant hybrids» by H. Mendel was published. He wrote there about *hereditary inclinations* that parental species pass to their offsprings in sexual reproduction. Mendel made experiments on peas. He wrote that hereditary inclinations are in gametes of parents; on fertilization they come together and form a zygote. Mendel's results were unusual for that time and scientists recognized them only in 1900 when H. de Freeze in the Netherlands, E. Chermak in Austria and K. Korrens in Germany got similar results and rediscovered Mendel's laws. 1900 is considered a year when the science of Genetics was born. In 1902 T. Bovery, E. W. Wilson and D. Setton proposed that hereditary factors were assocoated with chromosomes. In 1906 W. Betson introduced into Biology the term «genetics», and in 1909 W. Yohansen introduced the term «gene». In 1911 T. Morgan et al., conducting experiments on Drosophila, came to the conclusion, that genes were located in chromosomes in a linear order, and formulated a chromosomal theory of heredity. One question was unclear: what is the substance of heredity? In 1928 N. K. Koltsov presumed that the chromosome is a large protein molecule the radicals of which perform functions of genes.

STRUCTURE AND FUNCTIONS OF NUCLEIC ACIDS

In 1870 I. Misher described macromolecules in the nucleus and called them nucleic acids. They were DNA (deoxyribonecleic acid) and RNA (ribonucleic acid). The molecular structure of DNA was decoded in 1953 by J. Watson, F. Kreek and M. Wilkinson. They called it a «life thread».

Nucleic acids are polymers. Their monomers are *nucleotides*. A nucleotide contains a *nitrogenous base*, sugar *desoxyribose* or *ribose* and the *phosphoric acid residue*. There are 5 types of *nitrogenous bases*: guanine, adenine, cytosine, thymine, uracil. DNA nucleotides contain adenine, guanine, cytosine and thymine. RNA nucleotides contain adenine, guanine, cytosine and uracil. A, G are purine bases, T, C, U are pyrimidine bases.

The DNA molecule consists of two helixes. *Covalent phosphodiester bonds* between deoxyribase of one nucleotide and the phosphoric acid residue of the other form a sequence of nucleotides. Nitrogenous bases of two strands are linked according to a *complementarity* principle (self-supplementation) and are located inside the helix: A-T — 2 hydrogen bonds; G-C — 3 hydrogen bonds (fig. 11).

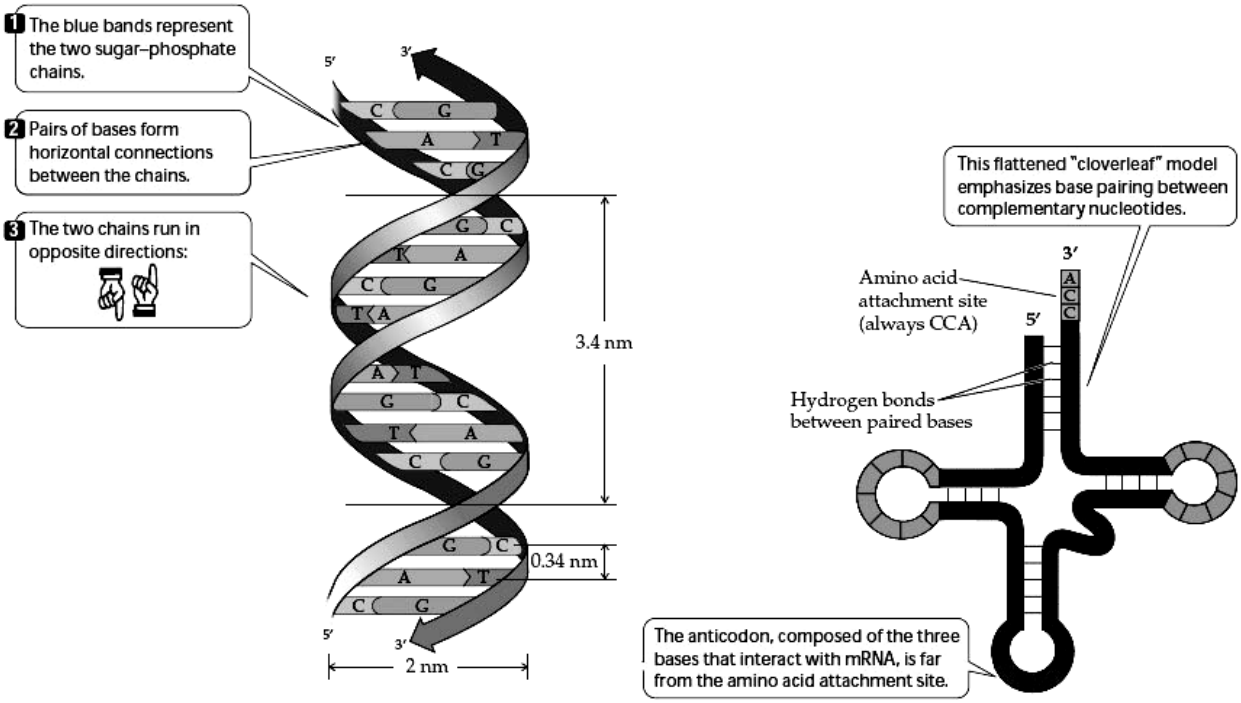


Fig. 11. Structure of a DNA molecule and tRNA

The character of complentarity of nitrogenous bases is expressed in the Chargaff's rules:

– the amount of purine bases in a double-strand DNA is equal to the amount of pyrimidine bases: A + G = C + T;

– the amount of adenine in a double-strand DNA is equal to that of thymine (A = T), the amount of guanine is equal to that of cytosine (G = C).

DNA is contained in a nucleus, mitochondria and plastids.

DNA possesses the *properties* of replication (self-reproduction) and the ability to repair (restoration of its structure after molecular impairment).

The DNA function is to store and transmit genetic information during multiplication of cells and organisms.

The RNA molecule is also a polynucleotide but it has one strand. It has uracil instead of thymine and has ribose instead of desoxyribose.

In some viruses, RNA is a store of hereditary information and has 2 strands in the molecule.

There are 3 types of RNA in the cell. 3–4 % of the whole cell's RNA is *messenger RNA* (mRNA): it «rewrites» genetic information from DNA and transmits it to ribosomes where protein molecules are being made. *Ribosomal RNA* (rRNA) comprises 80–85 % of the whole RNA of the cell. It is contained in ribosomes and provides spacial interlocation of mRNA and rRNA. *Transport RNA* (tRNA) transports amino acids from the cytoplasm to ribosomes. tRNA comprises 10–20 % of the whole RNA of the cell.

Ribonucleic acids are in the nucleus, cytoplasm, mitochondria and plastids.

Functions of RNA: participation in the synthesis of proteins (polypeptide molecules).

Genes perform two main functions in the cell. A *hetezrosynthetic function* is programming biosynthesis of protein in the cell. An *autosynthetic function* is DNA replication (self-duplication of DNA).

GENETIC CODE AND ITS PROPERTIES

– *tripletness:* one codon always corresponds to one amino acid in the molecule;

– *universality*: one and the same codon determines identical amino acids in all living organisms;

– *no overlapping*: one nucleotide is a part of only one triplet;

– *redundancy*: one amino acid can be coded by several different triplets (there are 20 amino acids, but 64 possible triplets;

– *continuity* (there are no separating characters between triplets);

– *unidirection* (reading of information from DNA is possible only in direction forma from end 3' to end 5' end; from an mRNA only in the direction from end 5' to end 3').

– presence of *codons-initiators* (they start protein biosynthesis) and *codons-terminators* (they terminate protein biosynthesis) among that 64 triplets.

– *co-linearity* is the correspondence of the nucleotide order in a DNA molecule to the amino acid order in a polypeptide molecule.

PROPERTIES OF GENES

1. *Specificity* — there is a unique sequence of nucleotides for any structural gene.

2. *Integrity* — the gene is indivisible as a functional unit.

3. *Discretion* — the gene can be divided into subunits responsible for various fuctions: a muton is responsible for mutation; a recon is responsible for recombination. A pair of nucleotides is their minimal size.

4. *Stability* — genes are relatively stable. The frequency of spontaneous mutation of a gene is about $1*10^{-5}$ per generation.

5. *Liability* — stability of genes is not absolute. They may change, mutate.

6. *Pleotropy* is a multiple action of a gene (one gene can be responsible for several characters.

7. *Expressivity* is a degree of phenotypical manifestation of the gene. It is associated with environmental factors and the influence of other genes.

8. *Penetrance* is the frequency of gene manifestation. It is calculated as the ratio (in percents) of the number of species having the character to the number of species having this gene.

CLASSIFICATION OF GENES

According to their function, genes are classified into structural and functional.

Structural genes contain information about enzymes, histones, sequences of nucleotides in various RNA. *Functional genes* regulate functioning of structural genes. *Genes-modulators, operators* and *regulators* belong to functional genes. Genes-modulators are *inhibitors, intensifiers, modifiers*. They enhance, attenuate or change the function of structural genes. *Genes-regulators* and *genes-operators* regulate structural genes.

The genotype of all somatic cells of the organism of one species is identical. However, cells of various tissues are very different. It is associated with functioning of various blocks of genes. The action area of this gene is called a *field of its action* (for example, the distribution of hair covering on the human body). As a rule, genes determining specific characters, do not work permanently (for example, genes determining the synthesis of sex hormones); their function considerably decreases with age. The functioning period of the gene is called the *time of its action*.

According to the place of action, genes are divided into 3 groups:

1. Genes functioning in all cells (for example, genes coding the enzymes of the energy exchange);

2. Genes functioning in cells of one tissue (determining the protein synthesis of myosin in muscular tissue);

3. Genes specific for one type of cells (genes coding for hemoglobin in immature erythrocytes).

Genes perform two main functions in the cell. As mentioned above, *hetezrosynthetic function* is programming biosynthesis of protein in the cell. An *autosynthetic function* is DNA replication (self-duplication of DNA).

DNA *replication* occurs during the synthetic period of the interphase of mitotic cycle. The synthesis of a DNA molecule is semi-conservative: in the duplicated DNA one strand is old (mother strand) and the other is new (daughter strand). This new strand is made according to the complementary concept. The

basic enzyme performing replication is a DNA polymerase (Arthur Korenberg, 1956).

The DNA helix is uncoiled and separated into two strands, each performs a role of the matrix (fig. 12) Replication starts simultaneously in several points of a DNA molecule. The DNA area from the beginning of one replication to the beginning of the other one is called a *replicon*. Chromosomes of eukaryote have many replicons, while a bacterial nucleoid has just one replicon. The DNA polymerase in the replicon may move along a mother strand only in the direction from 3' end to 5' end. That is why making new DNA strands is *anti-parallel*, it goes in opposite directions.

The replication area is called *a replication fork*. DNA strands are assembled simultaneously by several DNA-polymerases in each replication fork.

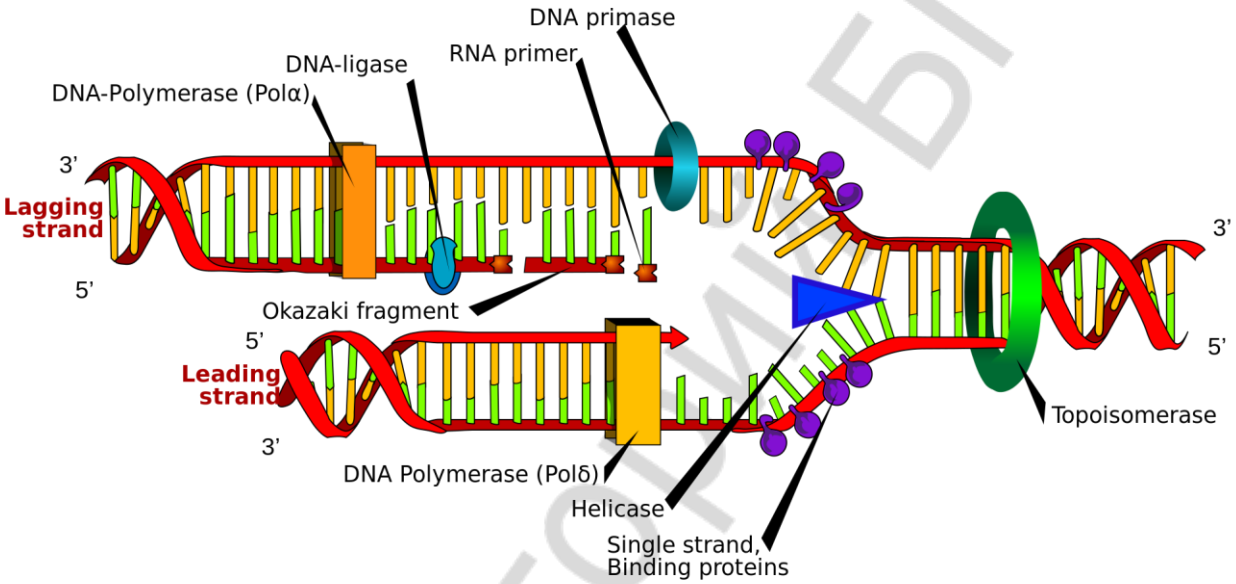


Fig. 12. Replication of a DNA molecule

In every replication fork, a DNA polymerase may continuously assemble one new DNA strand (as it is able to move in only one direction) on the leading strand. The second strand (lagging) is replicated by small segments containing 150-200 nucleotides. It is done by the DNA-polymerase that moves in the opposite direction. These areas are called Okazaki fragments. All synthesized fragments of the polynucleotide strand are linked by an enzyme ligase. All the cell's genome is replicated once during a mitotic cycle.

LECTURE 3
Topic: ARRANGEMENT OF GENETIC MATERIAL (II)

Plan

1. levels of DNA packaging.
2. Protein biosynthesis in the cell.
3. Regulation of transcription in prokaryotes and eukaryotes.

Chromosomes of the interphase nucleus are represented by lumps of chromatin. *Chromatin* (DNP – deoxyribonucleoprotein), is DNA nucleoprotein fibers linked with proteins. Their common length in the nucleus of a somatic human cell is about 2 m, but the length of all chromosomes in the metaphase comprises approximately 150 μm. It is possible due to packaging of DNA occurring at 4 levels. They are based on spiralization of DNA (fig. 13).

The 1st one is the *nucleosome level*. A nucleosome is a globule consisting of 8 histone molecules (per 2 histone molecules of H_{2A}, H_{2B}, H_3 and H_4). The DNA makes about two turns around the proteins (about 200 pairs of nucleotides) and passes to the next protein globe. The diameter of a nucleosomal thread is about 10 nm. The length of DNA reduces by 6–7 times. The same occurs in the interphase.

The 2nd one is *supernucleosome level* (or solenoid). The nucleosomal thread condenses and a paralyze. Nucleosomes are connected by a histone H_1. One turn of the helix contains 6–10 nucleosomes. The helix diameter is about 25 nm. The thread length reduces 7 times. The supernucleosome packaging level can be seen under the electron microscope in the interphase and during mitosis.

The 3rd one is *chromatide level*. The spiralization is continued and a supernucleosomal thread forms bends and loops. It is the basis of the chromatid. The diameter of such loops is 50 nm. The DNA length reduces 10–20 times. Such packing level can be seen in the prophase of mitosis.

The 4th one is the *level of a metaphase chromosome*. During the metaphase chromatids are still being spiralized. The thread length reduces by 20 times. The length of metaphasal chromosomes ranges from 0.2 to 150 μm, the diameter is 0.2–5.0 μm. The total DNA condensation is 10 000 times (10^4).

Circular DNA molecules of prokaryotic cells («bacterial chromosomes») contain 5×10^6 pairs of nucleotides and form complexes with non-histone proteins.

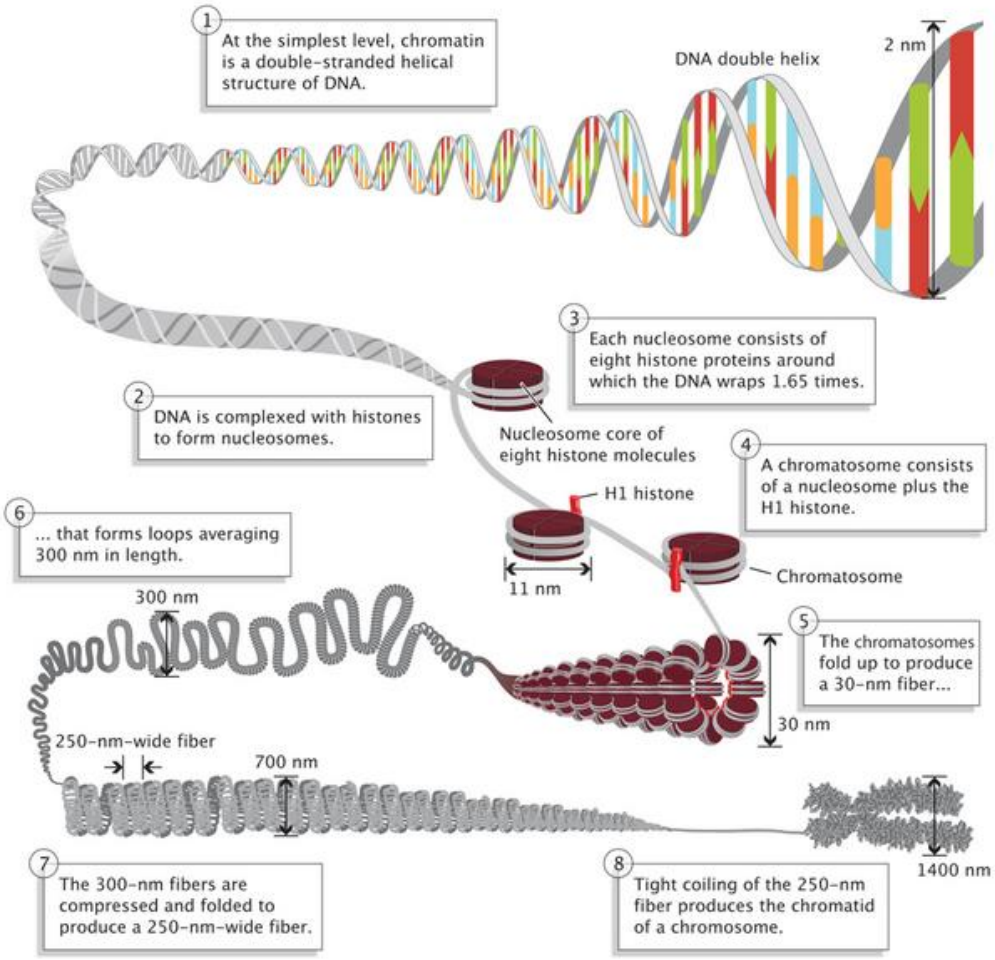


Fig. 13. Four package levels of DNP

PROTEIN BIOSYNTHESIS IN THE CELL

Protein biosynthesis is an enzymatic process where nucleic acids play the main role. An mRNA is synthesized in the nucleus on one of DNA strands: an RNA-polymerase transcribes the order of nucleotides of a segment of the DNA molecule (by complementarity rule). This process is called *transcription* (fig. 14). The mRNA enters the cytoplasm through nuclear pores and directs to ribosomes.

The process of *translation* occurs in ribosomes: the order of nucleotides in the mRNA defines the order of amino acids in the polypeptide molecule (protein). Small and large ribosome subunits attach to the mRNA (in the cytoplasm). One mRNA can be connected with many ribosomes. Such complex of ribosomes, united by one mRNA is a *polysome (polyribosome)*.

The beginning of translation is *initiation*, its end is *termination*. *Elongation* is the process when peptide bonds are formed between amino acids. The ribosome has two sites (fig. 16): the *aminoacyl (A-site)* and *peptidyl* (*P-site).* Therefore, there are two mRNA codons in the ribosome simultaneously, they are recognized by tRNA.

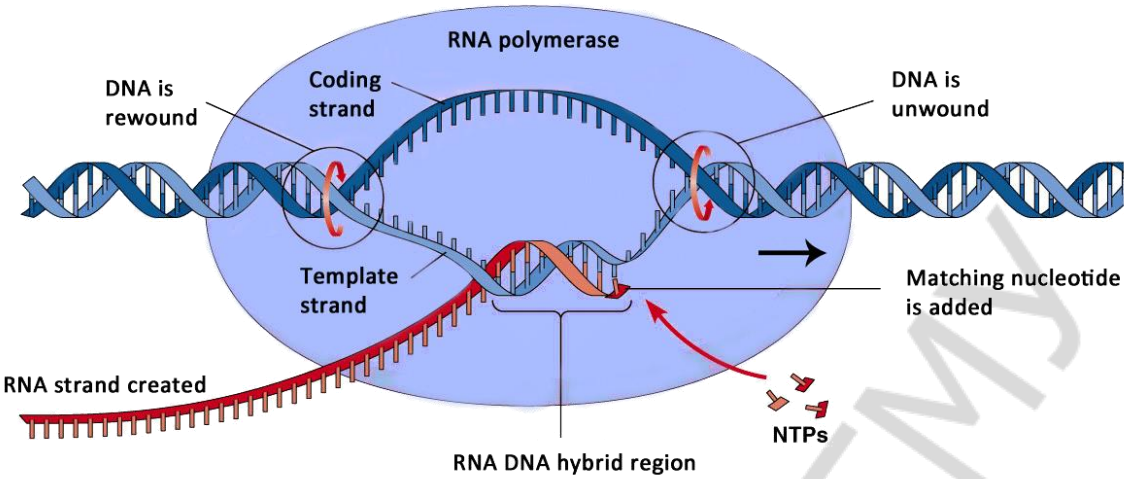


Fig. 14. Transcription

Recognition (recognizing of its own amino acid by tRNA) occurs in the cytoplasm. The transport RNA has a specific structure: one end of the molecule contains a nucleotide triplet called an *anticodon* and corresponds to only one definite amino acid, the other end is designed for carrying that amino acid (fig. 15). The amino acid joins the tRNA with the enzyme *amino-acyl-tRNA-synthetase* and ATP. The amino acid connected with its tRNA is a complex *called amino-acyl-tRNA.*The tRNA carries the amino acid into the A-site of the ribosome. If the tRNA anticodon and the mRNA codon are complementary then amino-acyl-t-RNA forms temporary bonds with mRNA. The ribosome shifts by one triplet, the amino-acyl-t-RNA passes into the P-site making the A-site free. When the second tRNA with its amino acid comes to the A-site, peptide bond sets between the first and second amino acids. The ribosome shift by one triplet again, the first tRNA leaves the ribosome and the second one passes into the P-site now. The process repeats many times. Termination of polypeptide synthesis is determined by stop-codons: UAA, UAG, UGA.

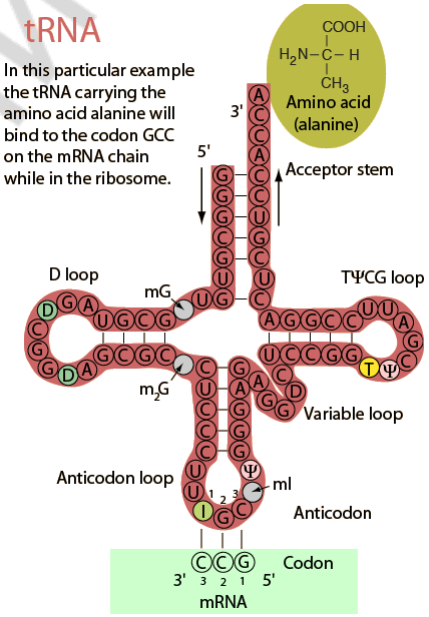


Fig. 15. Correspondence of a tRNA to a definite codon of mRNA.

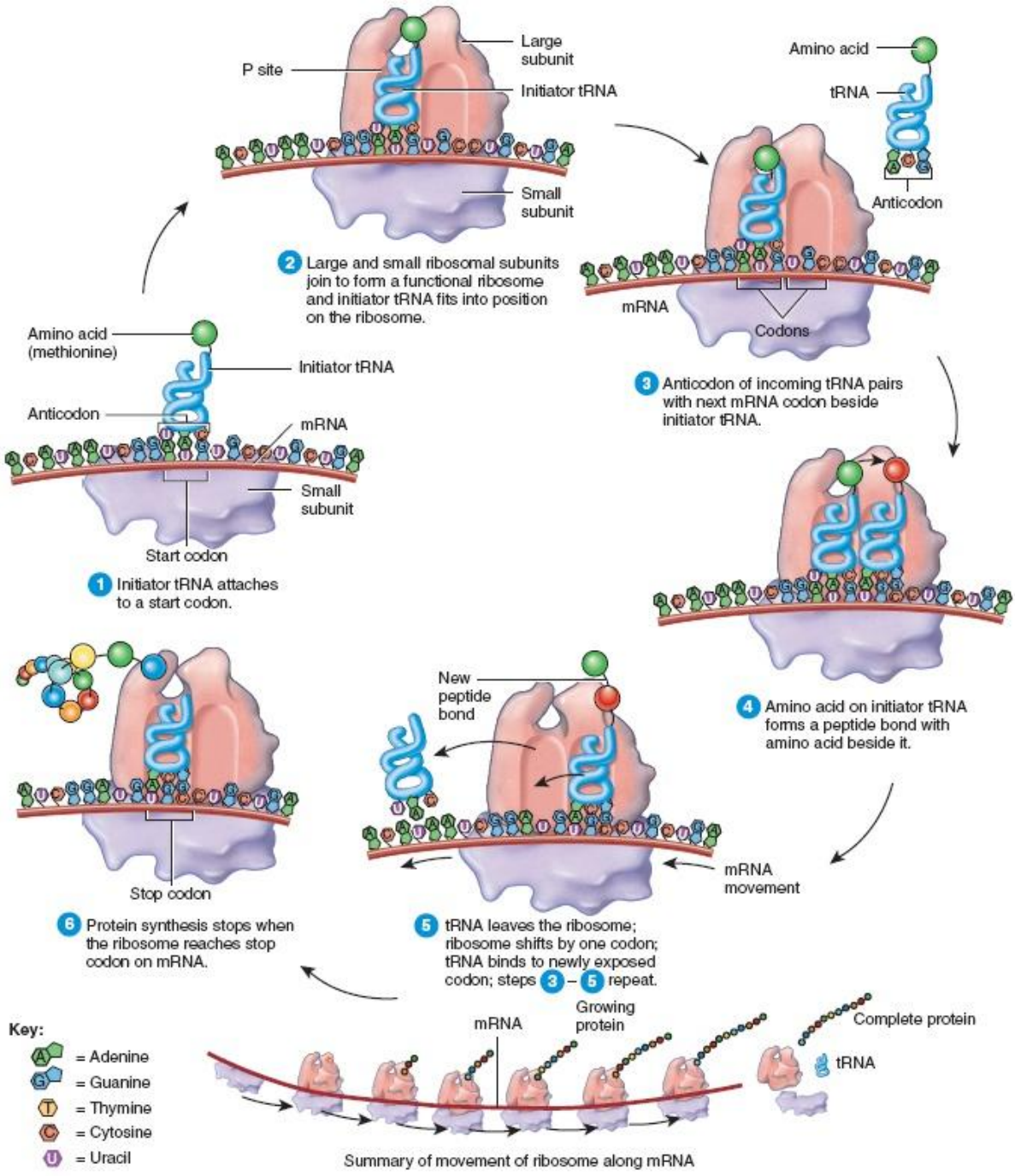


Fig. 16. Elongation

TRANSCRIPTION REGULATION IN PROKARYOTES AND EUKARYOTES

Francois Jacob and Jackques Monod described regulation action of genes in prokaryotes in 1961. The unit of prokaryotes transcription is called an operon (fig. 17). It includes structural-functional sections: a group of *structural genes* promoter, gene-operator and structural genes. The structural genes are not permanently active. The *gene-operator* starts and stops their functioning. Near the gene-operator is the site of RNA-polymerase attachment. It is called promoter. The operon also

includes an initiator (a sequence of nucleotides which starts the transcription) and a terminator of the transcription (it causes separation of the RNA polymerase and DNA).

Another necessary gene is the *gene-regulator* situated beyond the operon. It is permanently active. That gene carries information for the synthesis of the *protein-repressor*. The protein-repressor blocks the gene-operator to make the structural genes inactive. In this case operon doesn't act.

If the substance *inductor* passes into the cell, it binds the protein repressor. Inductor is the substance that can be split by enzymes encoded in the operon. In this case the gene-operator is released, complex of enzymes including RNA-polymerase breaks hydrogen bonds between DNA strands of structural genes. Then an mRNA is synthesized on one of the strands (according to complementarity principle. The mRNA is used for making enzymes that are responsible for breaking down the inductor (fig. 18).

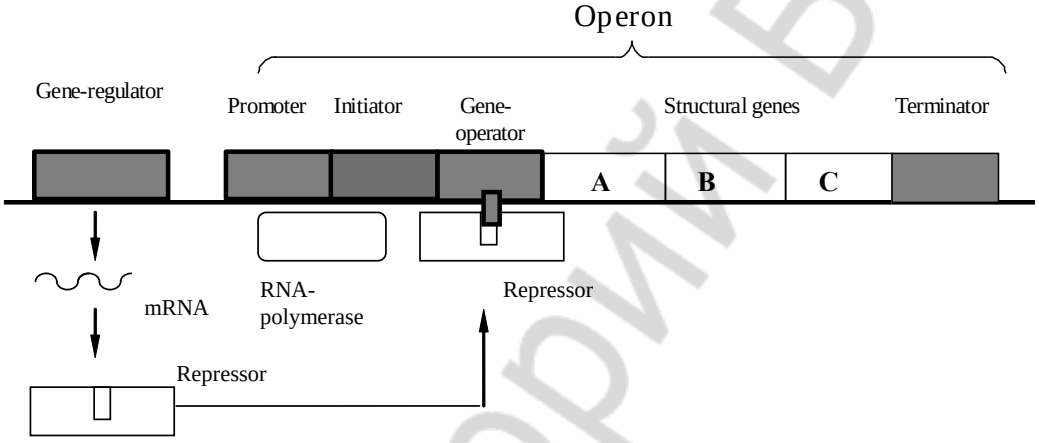


Fig. 17. Transcription regulation in prokaryotes. Operon doesn't act

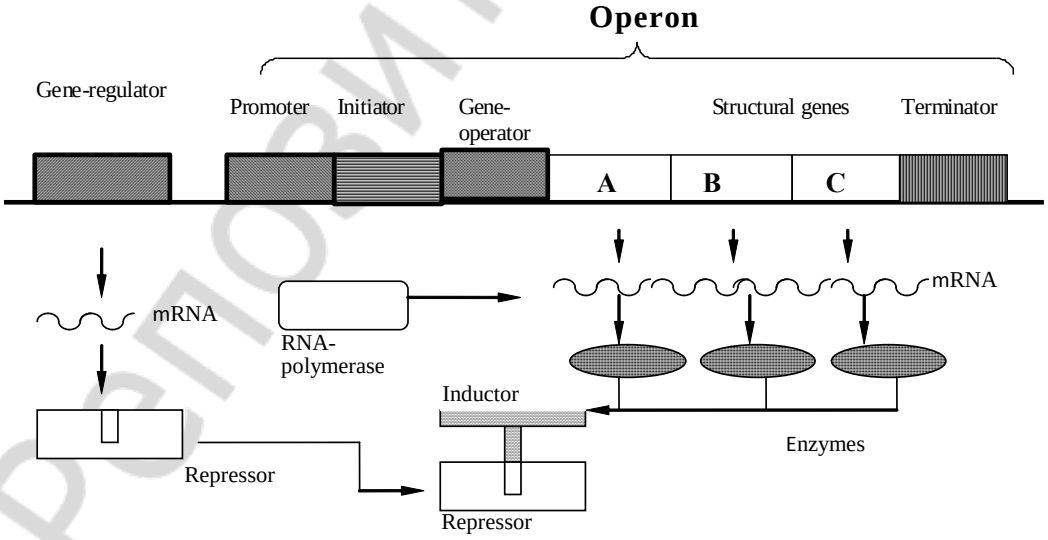


Fig. 18. Transcription regulation in prokaryotes. Operon acts

The operon acts unless the whole inductor is broken down. After that, the protein-repressor is released and able to blocks the gene-operator again. This switch off the structural genes are and proteins-enzymes are no longer synthesized. Each operon has its specific inductor (for example, lactose for the operon coding for enzyme lactase).

In 1972 G. P. Georgiev proposed a scheme explaining *regulation of genes' action in eukaryotes*. Conceptually it does not differ from that of prokaryotes. However, the structure of the scheme itself and the mechanism of its action are more complicated.

The transcription unit in eukaryotes is called a *transcripton*. It consists of a non-informative and informative zones. The *non-informative* or *acceptor* zone includes the promoter, initiator and a block of several genes-operators. The *informative* zone contains a structural gene with a terminator at the end. The structural gene has *introns* — non-informative DNA fragments, and *exons* — informative zones. A *block of several genes-regulators* rules the action of the transcripton. Several *proteins-repressors* are synthesized according to the information of that genes. The proteins block genes-operators.

Just as in case with the operon, reading of information from the structural gene occurs when *inductors* get into the cell. In the case with transcripton, the inductors are substances with complex composition (for example, hormones) requiring several enzymes for their splitting. The inductors release genes-operators from proteins-repressors. On one of DNA strands an mRNA is synthesized according to the complentarity principle, but it contains the information from the whole transcripton with all informative and non-informative zones. The synthesized RNA is a pre-mRNA.

The processing of the pre-mRNA takes place in the nucleus under the action of exo- and endonucleases. That enzymes break down the non-informative zones and split the pre-mRNA it into fragments. An mRNA is formed after splicing (connection) of informative sections (exons) by ligase. After such transformations, mRNA passes into the cytoplasm to ribosomes where enzymes breaking down the inductors are synthesized. As soon as the inductors have been broken down, genes-operators are blocked by proteins-repressors again and the transcripton is switched off (fig. 19).

The complexity of the regulation action of genes in eukaryotes and the structure of this scheme is as follows:

1. It participates in the action of genes-regulators and genes-operators.
2. The structural gene of exons has informative sections and introns, non-informative sections.
3. Initial formation of a pre-mRNA. Maturation of the mRNA is associated with processing and splicing.

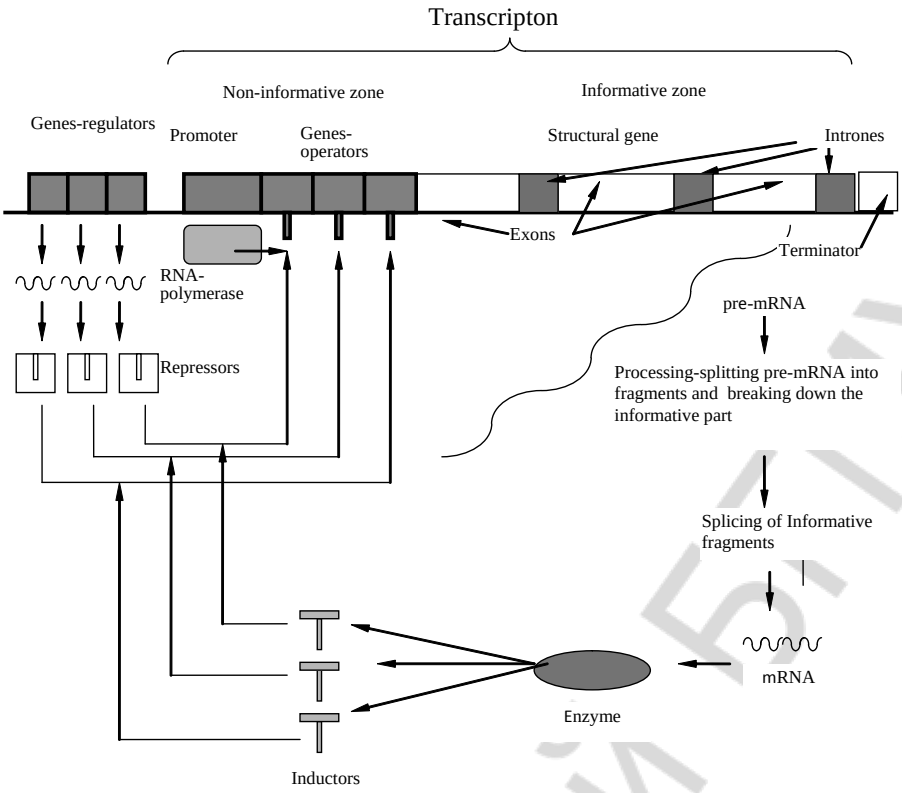


Fig. 19. Transcription regulation in eukaryotes

LECTURE 4
Topic: FUNDAMENTALS OF HUMAN GENETICS

Plan

1. Human genetics: the subject and tasks.
2. Specificity of human genetics.
3. Methods of studying human genetics.

HUMAN GENETICS: THE SUBJECT AND TASKS

Human genetics or **anthropogenetics** (Greek *antropos* — human) studies phenomena of inheritance and variation, laws of inheriting characters, their modifications under the influence of the environment. At present, there are a number of independent sections of human genetics: cytogenetics, biochemical and molecular genetics, radiation genetics, immune genetics, pharmacogenetics, population genetics.

One of the most important sections is *medical genetics* that has become an independent science. The subject of this science is studying hereditary pathology, development of diagnostic methods, treatment and prophylaxis of hereditary diseases.

There are about 5000 metabolic hereditary disorders in humans. Various congenital defects are revealed in every 30–40 per 1000 newborns.

The tasks of human genetics are:

1. Improving methods for early diagnosis of hereditary disorders.
2. Studying the pathogenesis, treatment and prophylaxis of human hereditary disorders.
3. Wide usage of genetic counseling.
4. Elaborating genetic aspects of immunity, transplantation, allergy and cancerogenesis.
5. Setting up a database, elaboration of genic therapeutic methods based on genic engineering.
6. Elaborating methods for protecting the human gene pool.

SPECIFICITY OF HUMAN GENETICS

The human as an object of genetic research has its peculiarities and faces a number of difficulties.

Difficulties of human genetics:

1. Impossibility to use the hybridological analysis and for humans.
2. There is a complex karyotype with many chromosomes and linkage groups.
3. Late puberty, a small number of children in the family, slow alternation of generations.
4. Diversity of ecological and social conditions; impossibility to establish identical conditions of life.

Advantages of the human as a genetic object:

1. High number of individuals in human populations.
2. International cooperation of geneticists and exchange of obtained information.
3. Humans are better clinically studied than other objects.
4. Elaboration of special methods to overcome difficulties while studying human genetics.

METHODS OF STUDYING HUMAN GENETICS

F. Galton suggested a **twin method of investigation** in 1875. It allows determining a correlative role of heredity and environment in manifestation of the character. The birth rate of twins is 1 %. Twins may be *monozygotic (uniovular*) (MZT). They develop from one zygote, have an identical genotype. If twins are *dizygotic* (DZT), they develop from different simultaneously fertilized zygotes. They have different genotypes, just as in siblings.

Criteria of zygosity in twins: MZT always have same sex, blood groups, skin patterns. In DZT these parameters can be different; in some cases they may coincide (except skin patterns).

The similarity of twins according to a studied character is called *concordance*, differences according to this character — *discordance* (table 3).

Table 3

Concordance according to some characters in MT and DT

Character	Concordance of MZT, %	Concordance of DZT, %
Tuberculosis	66.7	23
Oligophrenia	97	37
Schizophrenia	69	10
Blood group (AB0)	100	46
Eye color	99.5	25
Hypertension	20.2	10
Diabetes mellitus	65	18

The formula of Holzinger is used to determine a share of heredity and environment in the development of a specific character:

$$H = \frac{CMZ\% - CDZ\%}{100\% - CDZ\%}$$

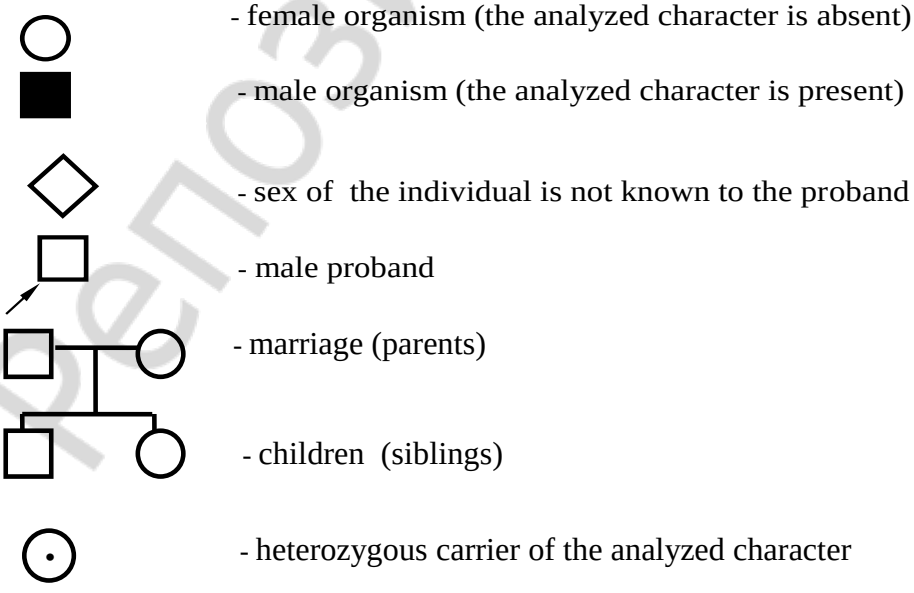
where H — a heredity share; CMZ — concordance in monozygotic twins; CDZ — concordance in dizygotic twins.

If H = 1.0, only heredity is responsible for the development of the character; if the amount of H closer to 0, then the environment is mostly responsible for the development of the character.

F. Halton suggested a genealogic analysis in 1876. It became a basis for a **clinical-genealogical method** — making up genealogies and analyzing inheritance of characters in a number of generations. This method allows establishing:

- degree of relationship;
- whether the character is hereditary or not (presence of a family disease);
- type of inheritance (dominant, recessive, autosomal, sex-linked, holandric);
- zygosity of the genealogy members (homozygotes or heterozygotes);
- genic penetrance ;
- probability of the character in offspring.

Designations used in making up a genealogy:



- female organism (the analyzed character is absent)
- male organism (the analyzed character is present)
- sex of the individual is not known to the proband
- male proband
- marriage (parents)
- children (siblings)
- heterozygous carrier of the analyzed character

A *proband* is the person from whom a genealogy starts, it is marked with an arrow.

Stages of the genealogical analysis:

– taking information about relatives of the proband;
– making up and analyzing the genealogy; making conclusions.

Autosomal-dominant type of inheritance (fig. 20):

– both males and females fall ill with equal probability;
– sick children only in families with sick parents;
– sick people are almost always in all generations;

Dominant homozygotes (AA) and heterozygotes (Aa) are sick, recessive homozygotes (aa) are healthy.

Probability of inheriting the character: 100 % if one of the parents is homozygous; 75 % if both parents are heterozygous, 50% of one parent is heterozygous and the second one is healthy (recessive homozygote).

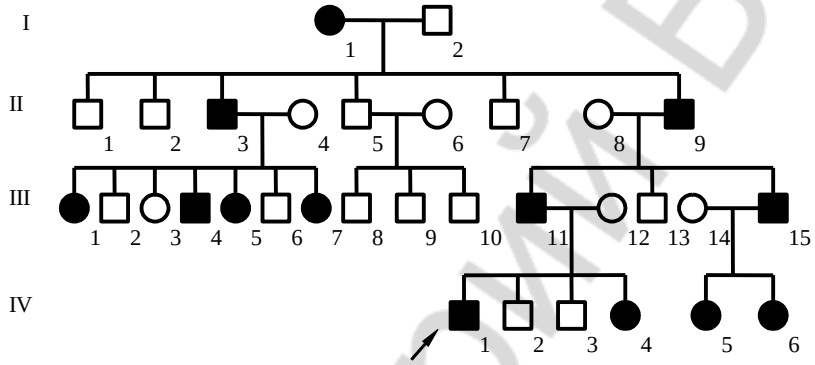


Fig. 20. Autosomal-dominant type of inheritance

Autosomal-recessive type of inheritance (fig. 21):

– both males and females fall ill with equal probability;
– sick people not in every generation;
– healthy parents can have a sick child;

Dominant homozygotes (AA) and heterozygotes (Aa) are healthy, recessive homozygotes (aa) are sick.

Probability of inheriting the character: 25 % if both parents are heterozygous; 50 % if one parent is heterozygous and the second one is sick (recessive homozygote); 100 %, if both parents are sick (recessive homozygote).

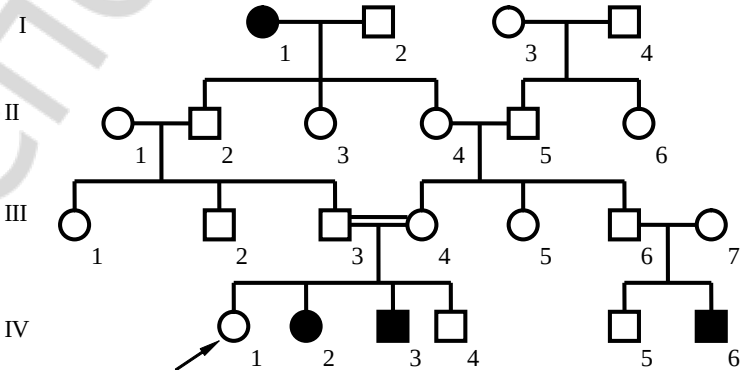


Fig. 21. Autosomal-recessive type of inheritance

X-linked dominant type of inheritance (fig. 22) is similar to an autosomal-dominant one, except the fact that *males can pass this character with an X-chromosome only to daughters.*

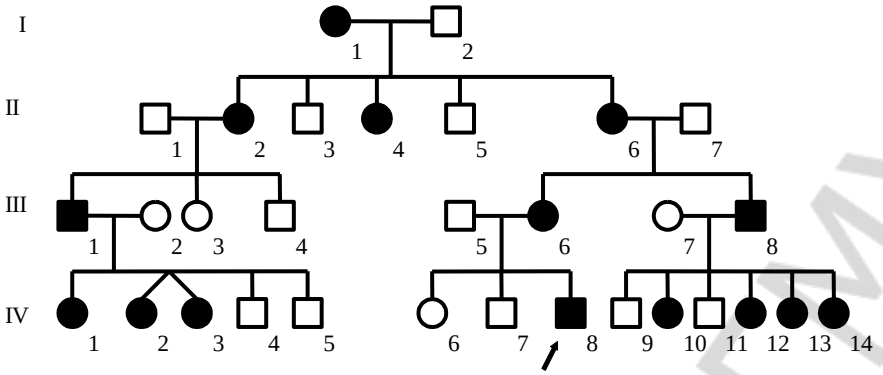


Fig. 22. X-linked dominant type of inheritance

X-linked recessive type of inheritance (fig. 23):

– predominantly males fall ill;
– patients are not in every generation;
– healthy parents have a sick child;
– probability of inheriting the character is 25 % in boys and 0 % in girls from all children, if both parents are healthy.

Over 200 X-linked recessive diseases are known, when males are affected and females are mostly carriers.

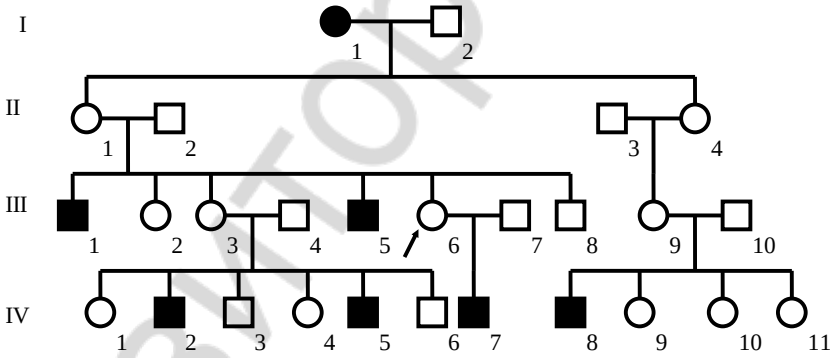


Fig. 23. X-linked recessive type of inheritance

Holandric type of inheritance (fig. 24):

– only males fall ill;

sick father always has only sick sons.

Population-statistical method of human genetics is based on the law of Hardy–Weinberg. The method was proposed by an English mathematician J. Hardy in 1908 and a German doctor-geneticist G. Weinberg.

The method allows studying the structure of human populations, determine the frequency rate of genes and genotypes in the population and the rate of heterozygous carriage of a gene. Using this method one can study genic geography of diseases. The incidence of hereditary diseases highly varies in different populations and geographical zones. The incidence of schizophrenia in common population is 1 %, in the population of relatives it is 7–16 %.

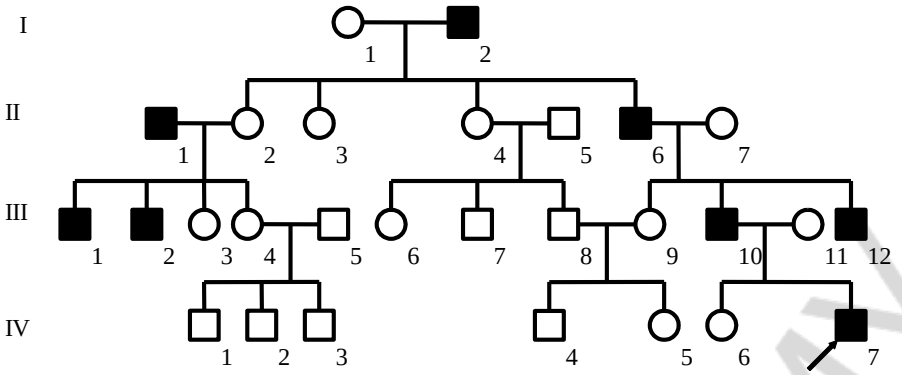


Fig. 24. Y-linked (holandric) type of inheritance

The stages of the method:
- selection of the population;
- taking material;
- statistical analysis.

Cytogenetic method is based on a microscopic study of the karyotype and determination of Barr bodies in nuclei of somatic cells. Cells of the bone marrow, lymphocytes and tumors are received and cultured. Mitotic division is stimulated, stopped at the stage of metaphase; then the cells are treated with a hypotonic solution of NaCl, and the chromosomes are stained. After making their photos, idiograms are composed and analyzed. An autoradiographic method is used for identification of chromosomes. A fluorescent analysis is used for making precise the karyotype and mapping chromosomes.

The method reveals genomic and chromosomal mutations. Special designations are used for transcribiong these mutations: *q* — a long arm of the chromosome, *p* — a short arm of the chromosome, «+» — redundancy of genetic material, «–» — deficiency of genetic material. A male with Down's syndrome — 47, XY+21. A female with monosomy of the 21st pair — 45, XX-21. Translocation (t) — 45, XXt (13q, 21q), the presence of a ring (*r*) X-chromosome — 46, X*r* (X).

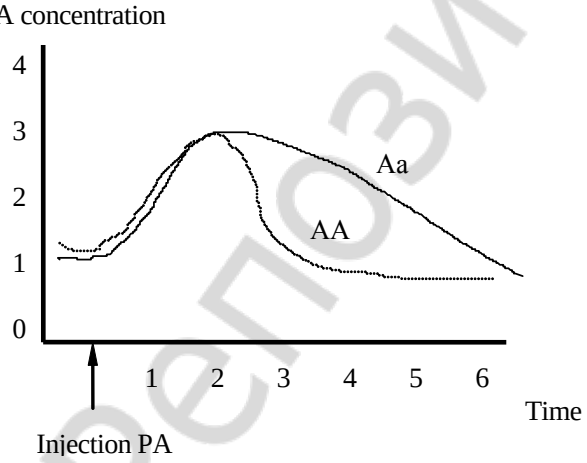


Fig. 25. Revealing heterozygotes by the biochemical method

Biochemical methods are used for revealing metabolic hereditary diseases by estimating enzyme activity or a quantitative final product of the reaction, which this enzyme catalyzes. Chromatographic, fluorometric, radioimmunological methods help revealing genic mutations (causes of metabolic diseases). For example, phenylketonuria is an impairment of phenylalanine exchange (PA). The incidence of this disease is 1:10000. Phenylketonuria can be revealed by the blood content of phenylalanine (fig. 25): in healthy people, it is 1–2 mg%, in the sick — 50–60 mg%. Every 30–40th person is a carrier of a phenylke-

tonuria gene. Heterozygosity can be revealed when phenylalanine is injected and its content in the blood is determined. If, after injecting PA, the curve of its content in the blood is slowly returning to its norm, then a person is heterozygous according to a phenylketonuria gene

Genetic methods are to reveal structure variations of the investigated DNA part and to decode the primary nucleotide sequence. In the majority of cases, it is sufficient to diagnose a disease or a heterozygous state.

To make the analysis it is necessary to get (amplify, multiply) a sufficient number of DNA fragments. It is done using a *polymerase* chain reaction (PCR) — in several hours one can obtain any number of fragments. The cycle of amplification includes 3 stages: temperature denaturation of DNA (separation of a 2-strand molecule into single-strand ones) → joining single-strand molecules to complementary parts → synthesizing polynucleotide strands on single-strand molecules using the polymerase. Restictases help obtain DNA fragments (per 4–6 pairs of bases). The fragments are separated using electrophoresis on the surface of polyacrylamide gel, then they are identified.

Modeling methods:

– Biological: studying hereditary human pathologies on animals. For example, hemophilia in dogs, diabetes in rats, cleft lip and palate in mice. The theoretical basis of applying the results, obtained on animals, for humans is a *law of homologous series* of N. I. Vavilov (genetically close species and genders have similar series of hereditary variation). The biological modeling is used for studying mutagenic and teratogenic effect of new medicines.

– Mathematical: is used in population genetics while determining the frequency rate of genes and genotypes in populations under various environmental conditions.

Express-methods are fast (screening-tests) preliminary methods (for example, examination of a newborn for phenylketonuria). These methods must be economic, reliable, diagnostically significant; the material for investigation should be taken in small amounts and be easily accessible (blood, urine).

Biochemical and chemical methods (colored reactions) are used for quick preliminary diagnosis of metabolic diseases. Determination of X- and Y-sex chromatin is shown in the table 4.

Table 4

Epithelial cells of the cheek mucus	
coloration	
acetorcein	acrychin-yperite
↓	↓
light microscope	luminescent microscope
↓	↓
determination of a number of X-chromosomes in the presence of Barr bodies (they exceed the number of lumps of X-chromatin by 1)	determination of a number of Y-chromosomes in the karyotype (an Y-chromosome gives bright green fluorescence)

Guthrie microbiological test. A drop of blood from the heel of a newborn is placed on the blotting paper and then on the agar bacterial culture containing a definite anti-metabolite of phenylalanine. The anti-metabolite inhibits growth of bacteria. However, if there is a lot of phenylalanine in the blood, anti-metabolite is broken down and microbes start growing. Changing metabolites, one can determine definite amino acids and carbohydrates in the blood.

A method of prenatal diagnosis of hereditary diseases is making diagnosis of a disease or development defects before birth. When it is incurable, birth prevention of such a child is discussed (interruption of pregnancy with the woman's consent). A modern level of development of prenatal diagnosis allows making a diagnosis of all chromosomal diseases, the majority of congenital development defects and diseases associated with the impairment of enzyme functions, if a biochemical defect is known.

Methods of prenatal diagnosis can be *indirect* (examination of a pregnant woman — obstetrical-gynecological, genealogical, bio-chemical) and *direct* (examination of the fetus).

One of indirect methods is determination of α-fetoprotein (AFP) in the blood serum of a pregnant woman. The AFP concentration reduces in chromosomal diseases. It may increase on the 13–15th week, when there is a menace of a miscarriage, intrauterine death of the fetus, multiple pregnancy, defects of the neural tube, congenital nephrosis.

Direct non-invasive methods (without tissue injury) include *ultrasonography* that is done for all pregnant women.

Ultrasonography is using ultrasound to get an image of the fetus and its membranes. The method is not dangerous for the fetus and can be re-used. Ultrasonographic diagnosis, made at different stages of pregnancy, reveals the fetus vitality, twin pregnancy, development defects of the brain (anencephaly, hydrocephaly, microcephaly), cranial and spinal hernias, hydronephrosis, polycyctosis, hypoplasia or anaplasia of kidneys, esophageal atresia, impairments of the bony system development. About 90 % of congenital defects are supposed to be revealed by ultrasound examination.

Direct invasive methods (with tissue injury): chorion biopsy, amniocentesis.

Chorionbiopsy is taking chorion cilia through the uterine cervix (transcervically) for cytogenetic and biochemical investigations and DNA analysis (fig. 26). It is done on the 8–13th week of gestation with an ultrasound apparatus. The method *allows revealing all mutations*: genic, chromosomal and genomic.

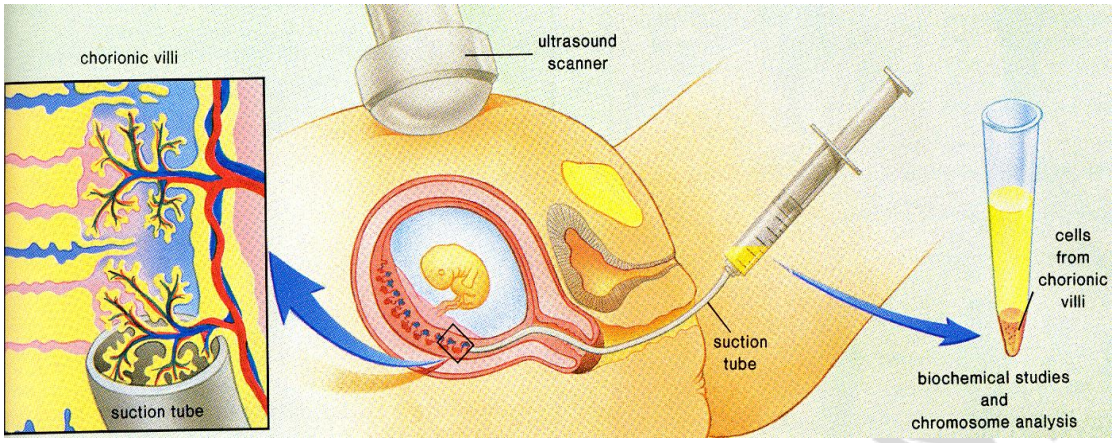


Fig. 26. Chorionbiopsy

Amniocentesis gives the best results. On the 15–17th week a puncture of the amniotic sac is made under the control of an US apparatus, and 15–20 ml of the amniotic fluid with fetal cells are taken by a syringe. The fluid is used for biochemical investigations. In fetal cells, one can determine genomic and chromosomal mutations, X- and Y-chromatin. DNA analysis determines genic mutations. While using the amniocentic method, complications do not exceed 1 %.

Indications for diagnosis by direct invasive methods are:

– the presence in the family of a hereditary disease;

– mother's age over 37 years (a mean risk value to have a fetus with a chromosomal anomaly for women at this age is 2–3 %, at the age of 45 years and over — 5–10 %);

– the presence of a gene X-linked recessive disease in the mother;

– spontaneous abortions at early terms of gestation, still birth, birth of children with multiple development defects and with chromosomal pathology;

– heterozygosity of both parents having one pair of genes with an autosomal recessive type of inheritance;

– pregnant women from the zone with an elevated adiation background.

LECTURE 5
GENETIC ENGINEERING

Plan

1. Stages of genetic engineering.
2. Obtaining the genetic material.
3. Polymerase chain reaction (PCR).
4. Incorporation of DNA fragments into the molecule-vector.
5. Introduction of recombinant DNA in the cell-recipient.
6. Using methods of genetic engineering in medicine.

STAGES OF GENETIC ENGINEERING METHODS:

The achievements of molecular biology, biochemistry and genetics put the beginning to a new branch of science — **genetic engineering**. Methods of genetic engineering help to create new genetic structures, organisms with a new genetic program according to a previously made plan. It became possible due to elaborating methods of transferring genetic information from one organism to the other. *Purpose of genetic engineering* — is designing of genetic structures according to a given plan (creation of organisms with a new genetic program by translocation of genetic information from one organism to the other).

Stages of genetic engineering:

1. Obtaining genetic material.
2. Translocation of DNA fragments into a molecule-vector.
3. Introduction of a recombinant DNA into a cell-recipient.
4. Selection of cellular clones containing molecules of a hybrid DNA (fig. 27).

OBTAINING GENETIC MATERIAL.

Chemical-enzymatic synthesis of genes. Short (8–16 nucleotides) single-strand DNA fragments are synthesized *in vitro*, then they are linked by lygases and treated with high temperature for the formation of double-strand DNA molecules. The gene should be sequenced for this method.

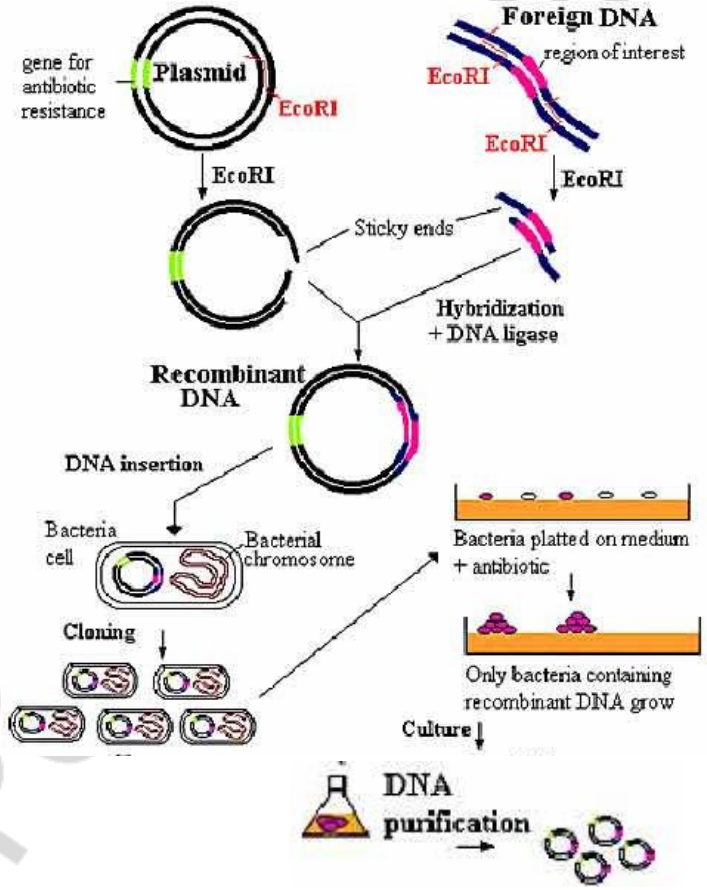


Fig. 27. Stages of genetic engineering methods

Enzymatic synthesis of complex genes. It is performed by recurrent transcription. An isolated mRNA is used as a matrix. Using an enzyme revertase, a coding DNA strand is synthesized, then it is replicated. The obtained genes do not function in cells as they have no promoter and regulation part. During transfer into a bacterium a promoter is added to structural genes, and the gene starts its work.

Isolating natural genes with restictases. Restrictases are enzymes causing DNA hydrolysis with formation of shorter fragments of the molecule. They affect DNA of any organisms if it has sites of recognition (usually they recognize very specific parts for every enzyme with 4–6 pairs of nucleotides in length). These parts are called *palindromes*.

At present there are over 500 restrictases in genetic engineering, they are able to cut the DNA in approximately 120 sites and form double-strand (*blunt*) ends or single-strand (*sticky*) ends in the DNA.

Gene isolation with restrictases has a number of disadvantages:

– it is not always possible to select restrictases, which allow to cut out a DNA part with a necessary gene;

– the cut out DNA fragment may contain introns, then recombinant DNA will not be able to work in prokaryotic cells due to disability for processing and splicing.

POLYMERASE CHAIN REACTION (PCR)

K. Mullis (1987) elaborated a method of polymerase chain reaction (PCR). PCR is performed in vitro using the enzyme of DNA-polymerase of a bacterium Thermus aquaticus, nucleotides (A, T, G and C) and short *primers*. The enzyme is persistent to high temperature.

Thanks to primers the DNA fragment is limited, it will be copied by DNA polymerase. The PCR has 3 stages (fig. 28):

1. *Denaturation* — a mixture, which contains a specimen of a needed DNA, is heated to 90 °C. Meanwhile, during 15 seconds there occurs breaking of hydrogen bonds between DNA strands, and two single-strand molecules are formed from one double-strand molecule.

2. *Hybridization of* ***primer****s* — the temperature is lowered to +50 °C and **primer**s are added. This stage lasts about 30 seconds.

3. *Polymerization* — the mixture is heated again to +70 °C. At this temperature the Taq-polymerase lengthens both **primer**s from their 3’ ends. The **primer**s grow up to the matrix sizes. This process takes 90 sec.

As a result, the number of DNA increases by many times. During 20 cycles the number of DNA copies reaches 10^6.

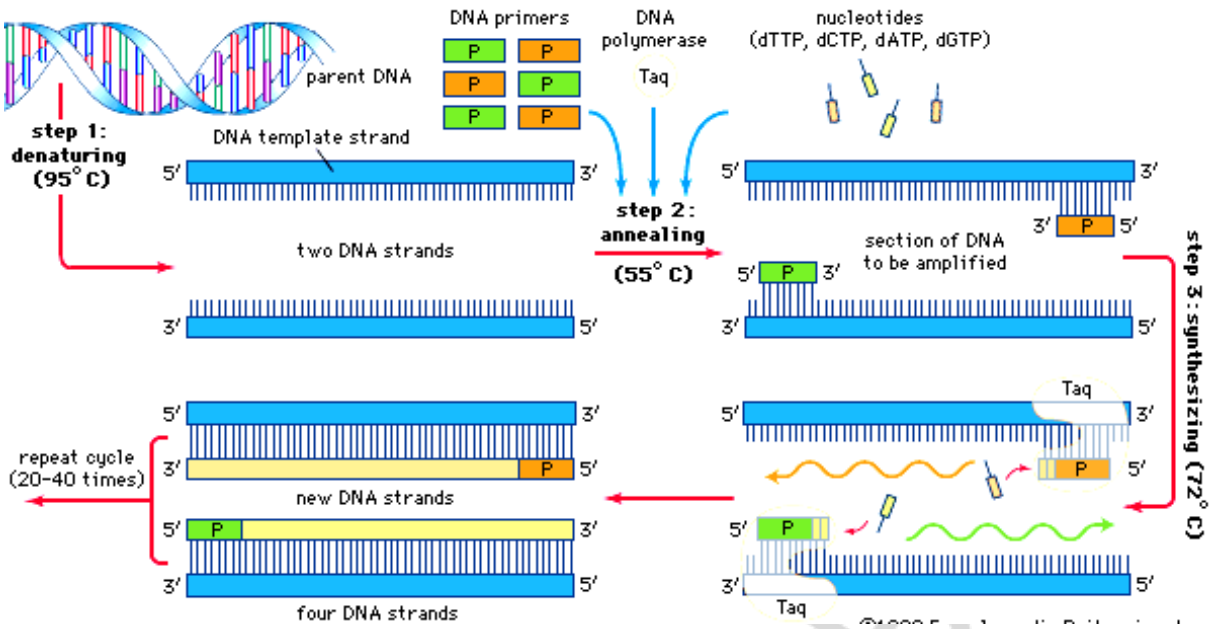


Fig. 28. Stages of PCR

INCORPORATION OF DNA FRAGMENTS INTO THE MOLECULE-VECTOR

Vector is a small autonomously replicated DNA molecule, which provides multiplication and work of the incorporated definite gene.

Vector molecules should:

– contain points of replication origin and replicate autonomously;

– permanently be inherited by a host cell;

– be contained in a great number of copies in the cell;

– possess a sufficient capacity, which allows cloning big genes in their composition;

– contain «convenient» sites of restriction;

– contain selective markers, which could be used for selecting cells that have received a cloned DNA segment and the marker itself.

The most useful of «vector-host» systems are those, in which the host role play *bacteria E. coli*, and the vector role — *plasmids.*

Plasmids — are ring autonomously replicated DNA molecules that are contained in bacterial cells (fig. 29).

Phage vectors are phage particles containing a recombinant DNA. Vectors for E. coli are constructed on the basis of phage λ and phage M 13.

Phage λ contains a double-strand DNA of 48 500 pairs of nucleotides in size. It is packed into the head as a linear molecule with sticky ends. After penetration into the cel, sticky ends are mutually paired, the molecule locks into a ring and is sewn by a DNA-lygase. It is possible to clone fragments of 15 000 pairs of nucleotides long in the content of vectors on the basis of phage λ.

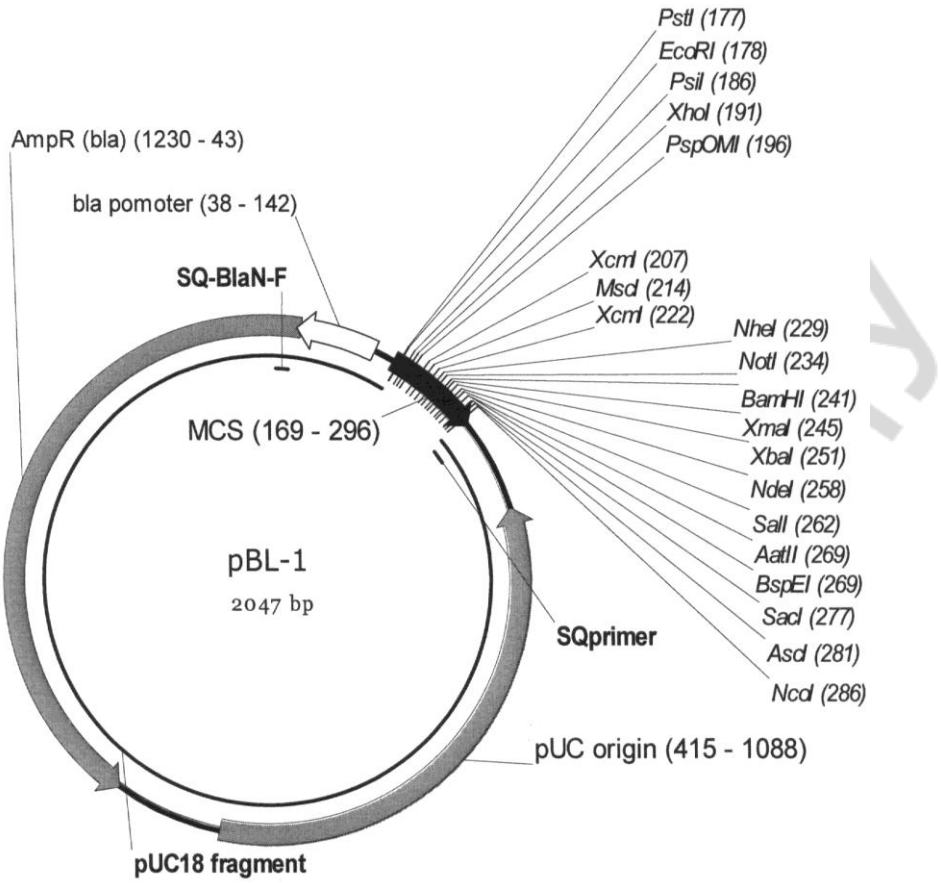


Fig. 29. The plasmid pBL-1

Cosmids are vectors made on the basis of plasmids and phage λ. Cosmids have cos-sites, which are located on both ends of a DNA molecule of phage λ. Complementary single-strand parts are 12 nucleotides long, due to which the phage has a linear shape, they join each other through cos-sites and form a long sequence of hundreds of phage DNA or concatameres.

Phasmids are hybrid vectors that can develop both as a phage and a plasmid. The capacity of plasmids is comparable to that of phage vectors.

INTRODUCTION OF RECOMBINANT DNA IN THE CELL-RECIPIENT

The following methods are used:

1. Conjugation — transmission of genetic material in bacteria may occur in direct intercellular contact. Genetic material is transmitted only in one direction.
2. Transformation — transmission of genes with a free soluble DNA (by plasmids), isolated from cells-donors;
3. Transduction — the transmission of DNA from a cell-donor to a cell-recipient may occur with participation of b vector acteriophages;
4. Transfection — infection with phages λ, ψ174 and T4;
5. Competence — ability of cells to absorb a DNA from the environment;
6. Microinjection of DNA molecules into animal cells;
7. Using liposomes for introducing DNA into animal cells. Liposomes are vesicles surrounded by one or several layers of lipids.

USING METHODS OF GENETIC ENGINEERING IN MEDICINE

Southern blot hybridization. The method developed in 1975 allows identifying restriction DNA fragments (fig. 30).

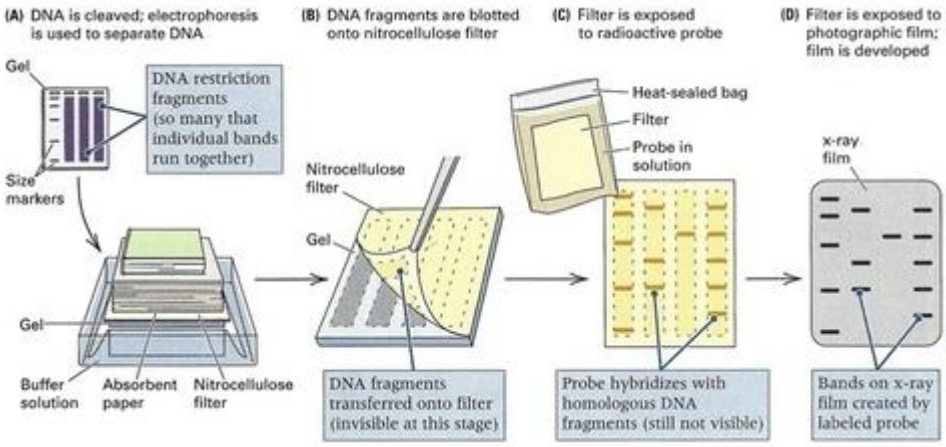


Fig. 30. Southern blot hybridization method

A DNA treated with restrictases is placed on agar jelly in a special chamber for electrophoresis, where an electric field is formed, and under its influence DNA fragments start moving. Short fragments move faster. After electrophoresis a mixture of DNA fragments forms some fractions located some distance from each other. Each such fraction corresponds to one DNA fragment. DNA fragments separated in the agar jelly are denaturated to single-strand molecules, and then the whole electrophoresic DNA specter is printed (blotting) on an applied to the jelly nitrocellulose film and is fixed by high temperature. Then the film is placed into the culture containing a radioactively marked DNA-probe. The probe can hybridize only with a specific complementary to it DNA fragment. After interaction with the DNA-probe the film is applied to the nitrocellulose membrane containing all obtained DNA fragments. After exposition there appear lighted spots corresponding to the arrangement of marked DNA fractions on the film (autoradiogram).

The method is used for revealing DNA sequences characteristic of mutated genes, it allows diagnosing gene mutations.

Gene dactyloscopy. There is a minisatellite DNA in the human genome, which presents short (9–64 nucleotide pairs), tandem repeats, variable DNA sequences. A tandem repeat consists of two or more identical DNA sequences located close to each other. The human has many different tandem DNA pepeats located in different chromosomes, which in total form a unique complement of minisatellite DNA for every human. The method of analyzing these fragments got the name of gene dactyloscopy (fingerprint of DNA).

The technology of gene dactyloscopy: a DNA is isolated from cells and cut into fragments of various length with the help of restrictases. Then the Southern-blot analysis is made. *Fractions containing a minisatellite DNA*, are revealed with a probe, which is complementary to a link from 13 recurrent nucleotides. The probe is radio-

active, it lights a roentgen film only in definite places, giving a picture of some tens of alternating dark fractions corresponding to separate minisatellites.

Genetic engineering helped to receive clones of cells of enteric bacterium that can produce insulin and somatotropin necessary for patients in great amounts. There were elaborated methods of producing anti-viral serum, VIII factor of blood coagulation, anti-genes of HIV, serum against hepatitis B. Under way are clinical trials of therapeutic methods for treatment of some malignant diseases, immunodeficient conditions and enzymopathies. The genetic engineering helped to create plants capable of assimilating nitrogen from the atmosphere, microorganisms synthesizing food proteins from carbohydrates of mineral oil.

The methods of genetic engineering are widely used for establishing genes banks of humans, animals and plants.

The future of genetic engineering is the development of genotherapy and genosurgery of hereditary diseases of man which is associated with transplantation of somatic cells of normal (instead of mutated) or lacking genes into the germ; the development of cloning embryonic cells for obtaining organs and tissues for transplantation.

The genetic therapy can also be used for humans to correct genetic defects in somatic cells or in embryonic cells and at early development stages of the zygote.

LECTURE 6
Topic: REPRODUCTION OF ORGANISMS

Plan

1. Reproduction as a universal feature of living things.
2. Forms of organisms' reproduction.
3. Evolution of the sex process.
4. Gametogenesis. The structure of sex cells (gametes).
5. Insemination. Fertilization.
6. Specificities of human reproduction.

Reproduction is one of the major universal features of living things, providing self-reproduction based on transmission of genetic information from generation to generation. Reproduction *on a molecular level* is replication of DNA (self-doubling), *on a subcellular level* — doubling of some organelles, on a *cellular* — amitosis, mitosis (cellular division). Cellular division is the basis of *organism's reproduction*.

FORMS OF ORGANISMS' REPRODUCTION

Asexual reproduction of organisms is the most ancient method. In parasites, asexual reproduction is a method of settling and increasing the number of species (table 5).

Table 5

<table>
<tr><td colspan="4">Asexual reproduction</td></tr>
<tr><td colspan="3">Vegetative (with body parts)</td><td>Sporulation(with special cells — spores)</td></tr>
<tr><td rowspan="2">in unicellular</td><td colspan="2">in multicellular</td><td rowspan="2"></td></tr>
<tr><td>in plants</td><td>in animals</td></tr>
</table>

Vegetative reproduction of unicellular organisms:

a) *half-and-half division* — the nucleus is divided mitotically, then the cytoplasm is divided in two by constriction; longitudinal division — in euglena, transverse — in infusoria.

b) *schizogony* — multiple division — at first the nucleus is divided into many parts, then the cytoplasm (in malaria plasmodium);

c) *budding* — a projection with a nucleus is formed on a mother cell (a bud); the bud is growing and separates from the maternal cell (in yeast and in sucking infusoria).

Vegetative reproduction in multicellular organisms:

A. *In plants* — by vegetative organs: root, stem, leaves.

B. *In animals*:

a) budding (in hydra);

b) fragmentation — division of the body by constrictions into several parts (celiac; and ring worms);

c) polyembryony — division of the fetus into several parts, each forming a whole organism (flukes).

Sporulation: specially formed cells — spores — give a start to a new organism (in water-plants, mushrooms, moss, lycopodium, horsetail and ferny). Spores in plants are formed in special organs — sporangia (table 6).

Table 6

<table>
<tr><td colspan="3">Sexual reproduction</td></tr>
<tr><td rowspan="2">with fertilization (gametic copulation)</td><td colspan="2">without fertilization (partenogenesis)</td></tr>
<tr><td>androgenesis</td><td>gynogenesis</td></tr>
</table>

EVOLUTION OF THE SEXUAL PROCESS

The sex process is the basis of sexual reproduction. It may take a form of conjugation (exchange of genetic information between two cells) or copulation — joining of genetic information of two cells. Conjugation is characteristic of infusoria and bacteria. During conjugation, infusoria join by a cytoplasmatic bridge and exchange parts of the micronucleus. Then they diverge and reproduce by asexual way.

At a definite period of the life cycle, individuals of protists perform the function of gametes. They fuse (copulation occurs) and then they reproduce by division. If there is fusion of cells, identical in size and mobility, the process is called *isogamy* (example: amoebas with shells). The process is called *anisogamy* if one cell is larger and immobile and the other — smaller and mobile (example: malaria plasmodia).

Copulation in sexual reproduction of multicellular organisms is *gametic*. Special cells, gametes, form in gonads (sex glands). Female gametes are formed in ovaries, male gametes — in testicles.

Ova have a round or a bit oval shape. They have 60 μm — some centimeters in diameter. They are immobile. Ova contain organelles and a store of nutrients (yoke). Their cytoplasm is specific for definite species. Ova are covered with various membranes, in mammals — also with cells of the follicular epithelium.

A **spermatozoon** consists of a head, neck and tail. It is mobile, has small sizes (40–500 μm). The sizes of a human spermatozoon are 52–70 μm. An *acrosome*, a modified Golgi complex, is at the end of the head. It provides passing of the spermatozoon into the ovum. The nucleus occupies the most part of the head; a thin layer of the cytoplasm surrounds it. There is a centersome in the neck and a helix, consisting of mitochondria. They produce energy for the tail to move (fig. 31).

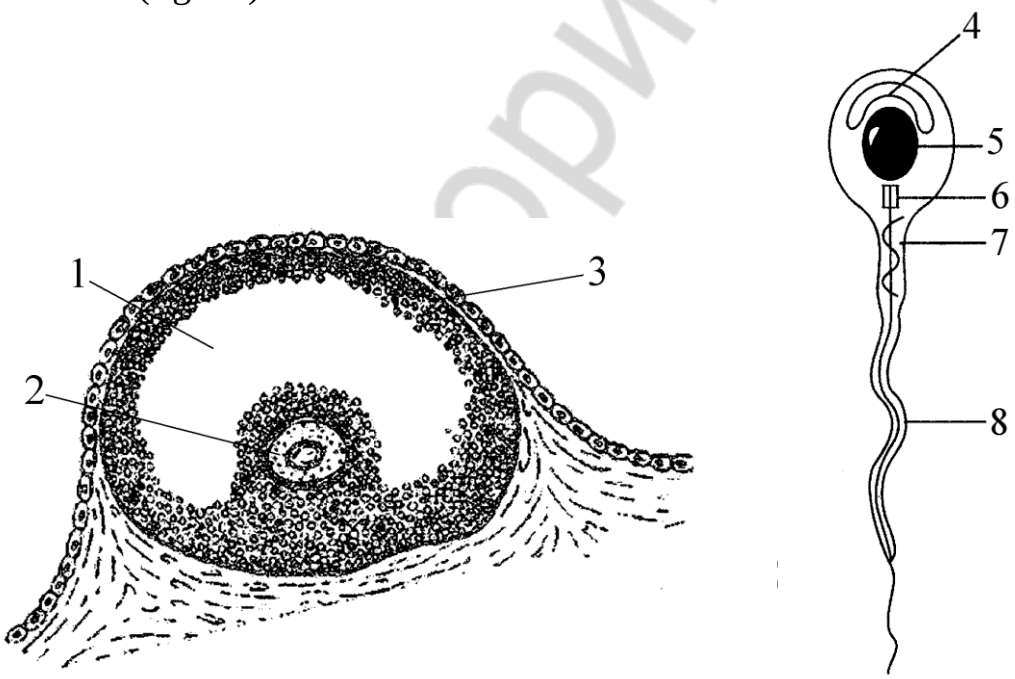


Fig. 31. The structure of an ovum and a spermatozoon:
1 — follicular cavity; 2 — ovum; 3 — follicular membrane; 4 — acrosome; 5 — nucleus; 6 — centriole; 7 — neck; 8 — tail

GAMETOGENESIS

Gametogenesis is a process of gamete formation: *ovogenesis* is formation of an ovum, *spermatogenesis* — formation of a spermatozoon. In gametogenesis, (fig. 32) haploid gametes are formed from diploid somatic cells of sex glands (gonads).

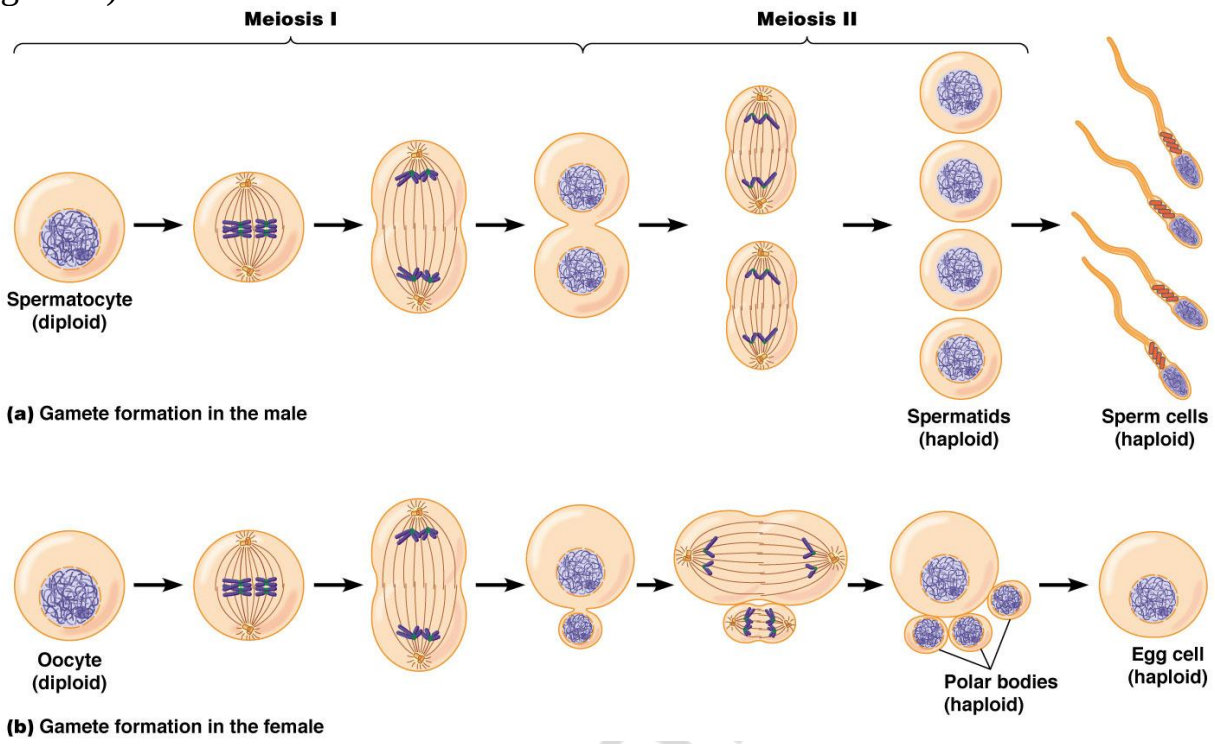


Fig. 32. Gametogenesis

Specificities of human gametogenesis

1. Mitotic division of ovogonia is completed before birth of the organism. Mitosis of spermatogonia starts from the maturation period.

2. In ovogenesis, a growth zone is particularly marked, in spermatogenesis a growth zone is practically unmarked.

3. In ovogenesis, the 1st meiotic division stops at diakinesis of the prophase till puberty. The 2nd meiotic division stops at the metaphase and is completed after fertilization.

4. In ovogenesis a formation zone is not marked, in spermatogenesis it is considerably marked.

A newborn girl has about 30 000 ovocytes in her ovaries, 300–400 (about 13 cells per year) of them reach their maturation. During the period of sex life a male organism produces up to 300 billion spermatozoa (several billion per one ovocyte of the II order).

At present, the last stages of ovogenesis are reproduced outside the organism and give the possibility of "conception" in vitro. At the stage of 8–16 blastomeres, the germ is transferred into the uterus of a woman-recipient.

In lower animals, sex cells are produced during all their life, in higher — during the period of their sexual activity.

The major advantage of sexual reproduction before asexual one is the enlargement of genetic variety of species and populations (table 7).

Table 7

Differences of reproduction forms

	Asexual	**Sexual**
Parental individual	1	2
Gamete production	–	+
Source of development of daughter individuals	Continuation of the development stage of a parental individual	Development starts with one cell — a zygote (fusion of haploid gametes → a diploid zygote
Genetic variety	–	+

Depending on the presence and functioning of sex glands (gonads) in the organism, we differentiate hermaphrodism and sexual dimorphism.

A hermaphrodite is an organism, which has male and female gonads producing sex cells in one individual. Such hermaphrodism occurs in flat and ring worms. It is a *true hermaphrodism*. Its variety may be hermaphrodism of mollusks, the sex gland of which periodically produces either male or female gametes. In case of a *false hermaphrodism,* one individual develops external sex organs and secondary characters of both sexes, but gonads of one sex (either male or female).

Organisms with separate sexes have either female or male gonads. Their sex organs are germinated in embryogenesis. Males and females are characterized by such signs of *sex dimorphism* as: differences in body dimensions, coloration, structure, voice characteristics, behavior and other features. *The signs of human sexual dimorphism are*: peculiarities of the bony-muscular system; distribution of subcutaneous adipose cellular tissue; the degree of hair covering development; voice tembre; specificities of the nervous system and behavior, etc.

INSEMINATION

A number of processes that provide a contact of female and male gametes is called *insemination*. In most of water animals insemination is external: gametes are excreted into the external environment, and their fusion occurs in water. In *internal insemination* (in ground animals) male gametes are introduced into sex ways of a female during intercourse.

The process of insemination is followed by a process of fertilization — fusion of gametes with formation of a zygote (fig. 33). The contact of zygotes is provided by:

– various charges of gametes;
– movement of spermatozoa and contraction of the walls of female sex ways;
– excretion of special chemical substances, gamones, by an ovum; spermatozoa react to them with a positive chemotaxis.

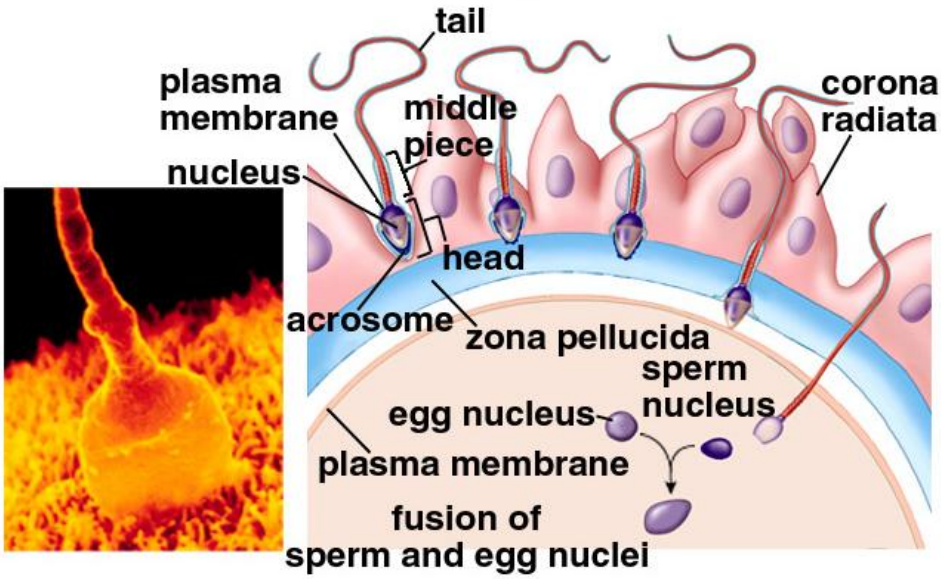


Fig. 33. Fertilization

The process of fertilization has an external and internal phases.

During the *external fertilization phase*, an ovum is activated and a spermatozoon is passing into it. There is an opening in the membrane of some ovum, a *micropile*, through which a spermatozoon enters an ovum. In the majority of cases, its passing into the ovum occurs due to an *acrosomal reaction*. During the contact with an ovum, the membrane of the acrosome breaks down and the enzyme of *hyaluronidase* is excreted. It dissolves the ovum membrane, the acrosome throws out an acrosomal thread and permeates through the egg membranes and fuses with the ovum membrane. A *receiving prominence* is formed on this part of the ovum; it grasps and brings the head, centriole and mitochondria of the spermatozoon into the cytoplasm of the ovum. If one spermatozoon enters the ovum (in mammals), the process is called *monospermy*. If several spermatozoa pass into the ovum (in insects, fish, birds), the process is called *polyspermy*. Activation of the ovum is associated with complex structural and physical-chemical changes: reconstruction of the cytoplasm, changed permeability of the membrane and metabolism. After the spermatozoon has passed into the ovum, a membrane of fertilization is formed on the surface of the ovum, and other spermatozoon cannot get inside. The external phase of fertilization is completed.

Synkaryogamy, the 2nd important process associated with *the internal phase of fertilization*, is fusion of haploid nuclei and formation of a diploid nucleus of the zygote. Colloidal features of the ovum cytoplasm change, its viscosity increases. *A male pronucleus* (spermatozoon nucleus) swells to the sizes of *a female pronucleus* (ovum nucleus), turns by 180^{o} and moves to a female pronucleus with its centersome.

Partenogenesis and its varieties represent a special form of sexual reproduction: gynogenesis and androgenesis — the development of organisms from unfertilized ovum.

Partenogenesis (Greek *partenos* — virgin, *genos* — birth) was described in the middle of the XVIII century by a Swiss naturalist Sh. Bonne. *Natural partenogenesis* occurs in lower cancroids, bees, butterflies, rock lizards. Nuclei of somatic cells of such individuals will be haploid. A diploid complement sometimes restores in fusion of ovum nuclei with the nucleus of a directive body. In 1886 A.A. Tikhomirov described *a natural partenogenesis*. He induced splitting of unfertilized eggs of a silkworm, affecting them with physical and chemical stimuli. B.L. Astaurov developed an industrial method of obtaining partenogenetic offspring in a silkworm.

Gynogenesis (Greek *gyne* — woman) is the development of the organism based on the information of a female pronucleus. A spermatozoon is an activator of the development. The spermatozoon nucleus does not take part in fertilization. If it gets into the ovum, it becomes broken down. Gynogenesis occurs in some fish species (e.g., silver crucian). Their offspring are only of females.

Androgenesis (Greek *andros* — man, *genesis* — birth) — the development of the germ occurs at cost of nuclei of one or two male gametes, which permeated into the cell with a broken nucleus. Such individuals are obtained in a silkworm and some wasps. All of them had only paternal characters.

SPECIFICITIES OF HUMAN REPRODUCTION

Specificities of human reproduction are associated with the fact that the human is not only a biological, but also a social being. The ability for reproduction in humans appears with puberty. Its signs are periods in girls (at an average age of 12–15 years) and pollution in boys (at 13–16 years). The duration of the reproductive period in women is to 40–45 years, in men — to old age. During one intercourse, about 200 million of spermatozoa are excreted with seminal fluid. Ovocytes in ovaries, precursors of future ovum, are germinated in embryogenesis. When puberty has come, once in a moon month an ovocyte of the 2^{nd} order is formed. Fertilization in humans occurs in the upper parts of uterine tubes, usually during the first 12 hours after ovulation. Spermatozoa preserve their ability for fertilization during 1–2 days after their entering the female sex ways.

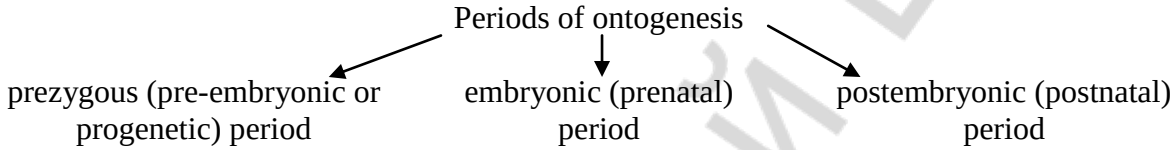
Human reproduction, unlike that of animals, has no seasonal prevalence. It depends on a number of social-economic factors. As a social being, the human can regulate the childbirth.

LECTURE 7
Topic: FUNDAMENTALS OF ONTOGENESIS

Plan

1. Periods of ontogenesis.
2. Embryogenesis.
3. Realization of genic actions in ontogenesis.
4. Critical periods of development. Teratogenesis.
5. Periods of postnatal ontogenesis.
6. Growth: laws and growth regulation. Constitution and habitus.
7. Aging and old age. Theories of aging.
8. Death, clinical and biological. Reanimation and euthanasia.

Ontogenesis is individual development from a zygote formation until death of the organism.



Prezygotic period or progenesis is the period of formation and maturation of parental gametes, which will form a zygote. The quality of gametes, the presence of mutated cells there will have a considerable influence on the health of future children.

Embryonic or prenatal period starts with the moment of a zygote formation and ends with birth of a new organism or its going out of ovum membranes.

Postembryonic or postnatal period starts with birth of an organism or going out of ovum membranes and to its death.

EMBRYOGENESIS

Human embryogenesis includes:

– Germinative or initial period is the 1^{st} week after fertilization, when splitting of a zygote takes place;

– Embryonic period — the 2^{nd}–3^{rd} weeks after fertilization, when a blastula and gastrula are formed; germination of germination sheets and axial organs takes place;

– Prefetal period — the 4^{th}–8^{th} weeks, when germination of organ systems and placenta takes place;

– Fetal period — from the 9^{th} week an embryo is called a fetus; growth of the fetus and formation of organs and organ systems take place.

A zygote is a unicellular stage of development (fig. 34) of multicellular organisms that is formed in zygosis (fusion of a male and a female gamete).

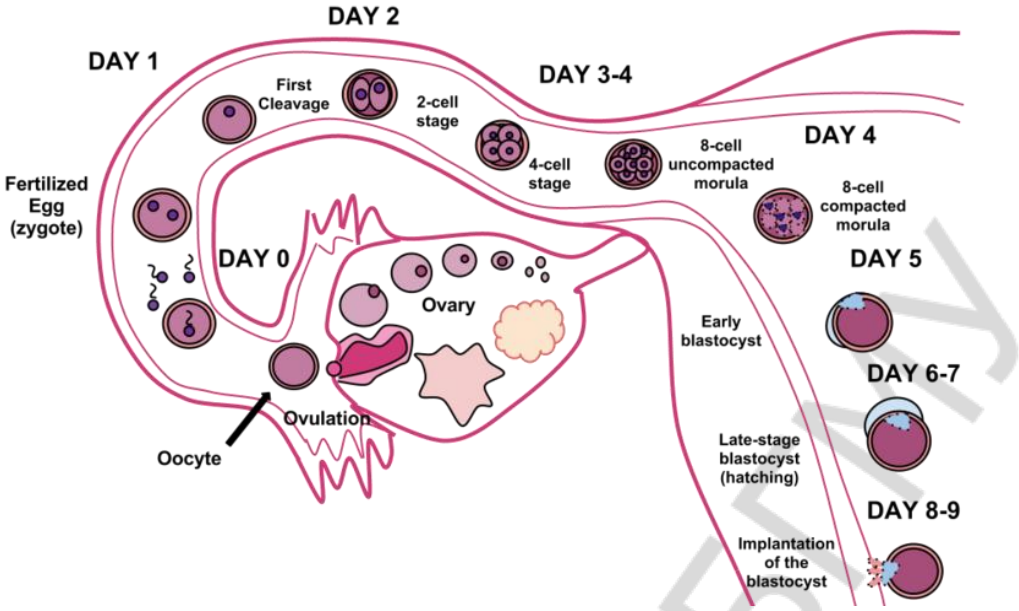


Fig. 34. Inichial stages of Human embryogenesis

The type of splitting a zygote is determined by the ovum type, which depends on the quantity of nutrients (yolk) and their distribution (fig. 35).

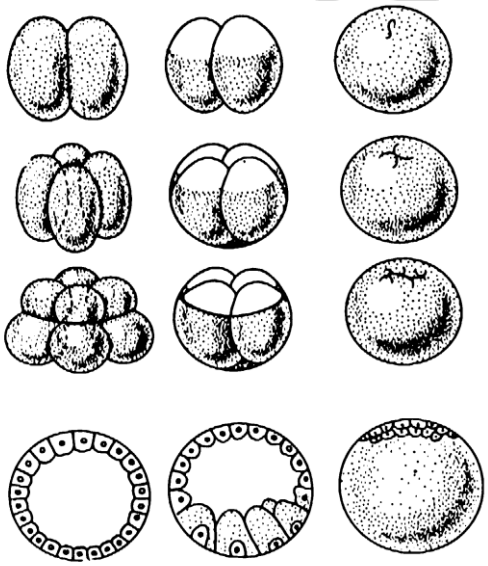


Fig. 35. Splitting types of various zygotes

Isolecytal ova: insufficient quantity of yolk, it is distributed regularly in the cell. Splitting of such eggs is complete, regular and synchronous (human).

Moderately telolecytal ova: moderate content of yolk at the pole, which is called vegetative. The pole, where the cytoplasm with the nucleus is placed, is called animal. Splitting is complete, irregular (amphibian).

Sharply telolecytal ova: a great amount of yolk is placed at the vegetative pole. A germinal disc is split; it is a small part of the cytoplasm with the nucleus. The splitting is called discoid (birds).

Centerlecytal ova: the center of the cell is occupied by the yolk; the cytoplasm forms a peripheral layer, where splitting takes place. It is called superficial (insects).

Cells that are formed during splitting of a zygote are called *blastomeres*. The germ of some animals reminds a raspberry or a mulberry in the process of splitting. It got the name of *morula*. Blastomeres of the morula are distributed on the periphery in one layer and form a *blastula* — a one-layer germ. A layer of cells is called a *blastoderm*. Cells of the blastoderm are called embryonic cells. The cavity of a blastula got the name of a primary cavity, *blastocele*. The germs of all types of animals undergo the blastula stage.

Gastrulation, formation of a gastrula, follows the blastula stage. Layers of gastrula cells got the name *germinal layers*. There are four types of gastrulation (fig. 36):

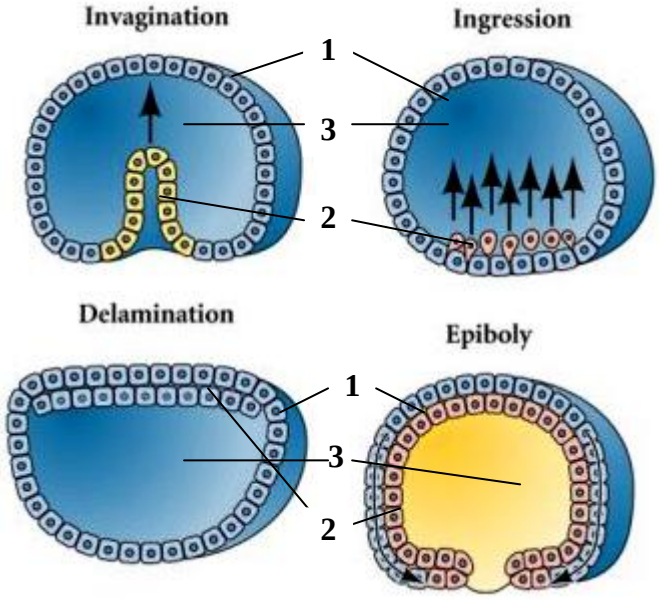


Fig. 36. Ways of gastrulation:
1 — ectoderm; 2 — entoderm; 3 — gastrocoel

1. *Invagination* — drawing in. The vegetative pole of the blastula is drawn inside, settling below the animal pole. A two-layer germ is formed. The external sheet gets the name of an *ectoderm*, the internal — an *entoderm*. The gastrula cavity is called a *gastrocele* or a primary intestine. The entrance to this intestine is a primary mouth or a blastopore. Its edges form *an upper and lower lip of the blastopore*. In the secondary-mouthed (echinodermata, chordates) it becomes an anal opening, while the mouth is formed at an opposite end of the germ.

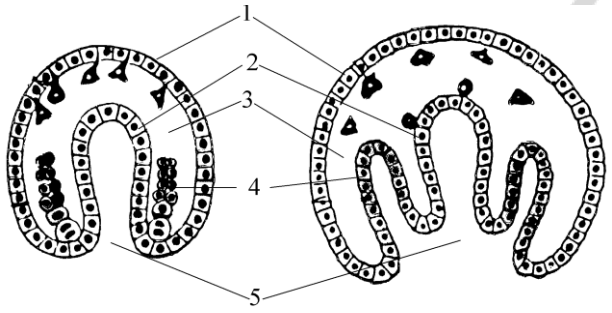
2. *Immigration* — «eviction» of some cells to a germinal cavity and formation of a second layer (endoderm) there. This way is characteristic of coelenterate.

3. *Epiboly* — becoming covered (in a telolecytal type of ova). Cells of the animal pole divide faster than cells of the vegetative pole, which form an endoderm.

4. *Dellamination* — splitting. All cells of one germinal layer divide parallelly to its surface and form two layers — an ectoderm and an entoderm.

In humans gastrulation goes according to a mixed type — several types are combined simultaneously.

At the stage of two germinal sheets, the development of sponges and coelenterate is completed. They refer to two-layer animals. All other animals, occupying higher evolution stages, are three-layer beings. Germination of the 3rd (middle) germination sheet, mesoderm, occurs in two ways — teloblastic and enterocelic (fig. 37).



Teloblastic Enterocelic

Fig. 37. Formation of 3rd germinal sheet:
1 — ectoderm; 2 — entoderm; 3 — gasrtocoel; 4 — mesoderm; 5 — blastopore

The teloblastic way is characteristic of many invertebrates. During gastrulation, one large cell, a teloblast, is formed at each of the two sides of the blastopore. They start dividing; small cells occupy the space between the ectoderm and entoderm and form the mesoderm.

The enterocelic way of germinating the mesoderm is characteristic of the chordates. On both side of the primary intestine, bulges (celomic sacs) are formed. They separate from the primary intestine, spread out between the ectoderm and entoderm and give a start to the mesoderm.

After the formation of germinal sheets, germination of axial organs occurs, *histogenesis* — the process of tissue formation and *organogenesis* — the process of organ formation.

Derivatives of germination sheets:

The *ectoderm* gives a start to external coverings, the central nervous system, initial and final parts of the gastrointestinal tube.

From the *entoderm* a chord, a middle part of the gastrointestinal tube and respiratory system are formed.

From the *mesoderm* the bony-muscular, cardio-vascular and urinary-genital systems are formed.

REALIZATION OF GENIC ACTIONS IN ONTOGENESIS

Genetic information (sequence of DNA nucleotides) provides the synthesis of mRNA, proteins-enzymes, which cause the development of characters. Manifestation of genic action depends on other genes. They may affect the given gene, proteins-enzymes that are coded by this gene, manifestation of the character. The given gene may affect the realization of action of other genes. The realization of genic action also depends on environmental factors that may change the structure of DNA, mRNA, proteins-enzymes and phenotypical manifestations of the gene (fig. 38).

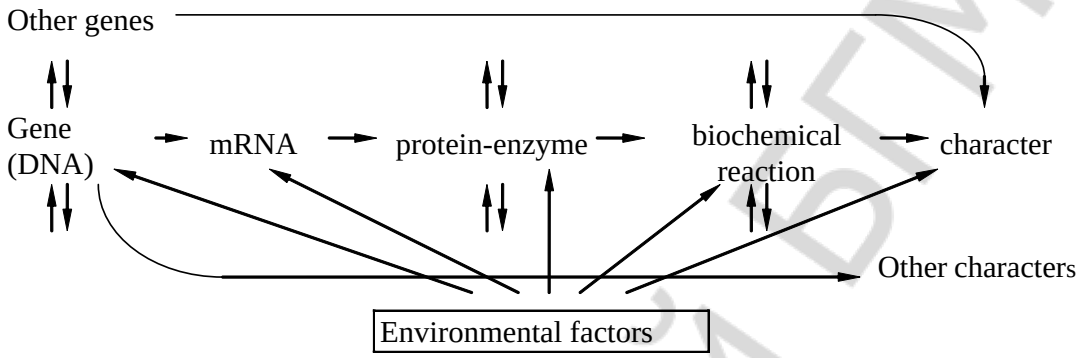


Fig. 38. Realization of genetic information

Mechanisms ensuring embryogenesis:

1. Differential genic activity — during the embryonic development, various blocks of genes have a definite order of repression and derepression.

2. Determination — the choice of a specific way of development, the acquisition by cells the ability to develop in a definite direction and simultaneous restriction of their future development possibilities. At the beginning of embryogenesis, blastomeres are totipotent (they may give a start to a whole organism) and their development depends on external inductors and adjacent cells. At later stages of embryogenesis, the cells become determinated (their development is predetermined), and they develop according to a definite plan.

3. Differentiation — biochemical, functional and morphological specialization of cells; modification of a developing structure, when relatively uniform formations become more and more different.

Differentiation phases:

– dependent (till the stage of an early gastrula);
– independent (at the stage of a late gastrula).

4. Morphogenesis — when new structures appear and their forms change in the process of ontogenesis.

Genetic basis of differentiation

Genetic differentiation is associated with uniqueness of the ovum. It shows in heterogeneity of the cytoplasm — different parts of the cytoplasm have different complements of chemical substances and have different potencies (fig. 39).

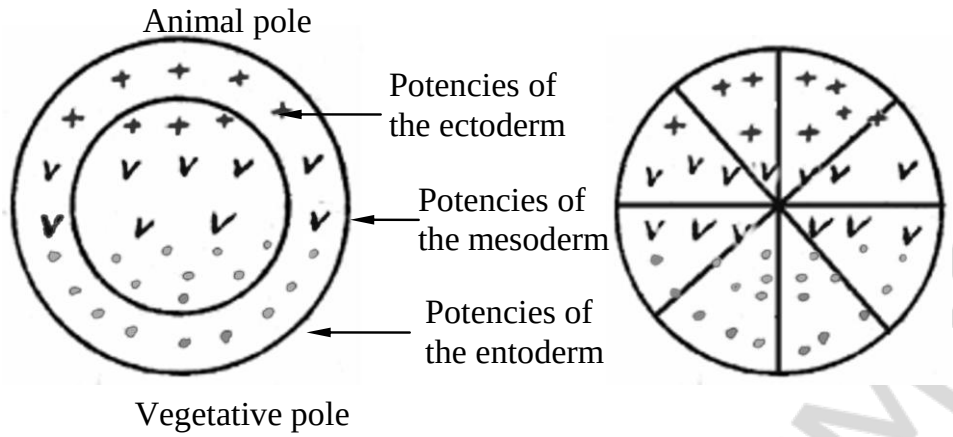


Fig. 39. Heterogeneity of the ovum cytoplasm and its subsequent splitting (various signs denote different chemical content of the cytoplasm)

Stages of differentiation

Chemical heterogeneity of the ovum cytoplasm (enhances after fertilization).

↓

Chemical heterogeneity of the blastomere cytoplasm.

↓

In different blastomeres are different inductors.

↓

Different inductors include different transcriptones.

↓

Different proteins-enzymes are synthesized; they catalyze different types of biochemical reactions.

↓

The synthesis of different typo- and tissue-specific proteins in different blastomeres.

↓

Different types of cells are formed, morphological heterogeneity is created.

↓

Different types of cells form different tissues.

↓

Different tissues form different organs.

Mechanisms of morphogenesis:

1. Embryonic induction — the impact of a group of embryonic cells on adjacent cells (G. Shpeman, G. Mangold). The primary inductor (upper lip of a blastopore) determines the formation of a nervous tube, then a chord is induced, and then — a gastrointestinal tube.

2. Morphogenetic fields (A. G. Gurvich) — distant interactions of cells having an electric or gravitational nature.

3. Gradient of physiological activity (Ch. Child) — intensity of metabolism is higher in a head part of the germ as compared to a tail part; it produces a regulating action on morphogenesis.

4. Positional information of the cell — using intercellular interactions every cell assesses its position in the germ of the organism, then it differentiates itself according to this position.

CRITICAL PERIODS OF DEVELOPMENT

The periods of the greatest sensitivity of the germ to environmental factors are called **critical periods**.

The human has three basic critical periods in embryogenesis:

1) *implantation* — instillation of the embryo into the mucus of the uterus (6^{th}–7^{th} day after fertilization);

2) *placentation* — the beginning of the placenta formation (14^{th}–15^{th} day after fertilization);

3) *delivery* — the outcome from the mother's organism, adaptation of the function of all organ systems, modification of feeding (39^{th}–40^{th} week).

Critical periods coincide with transitions from one period of development to the other and changes in living conditions of the germ.

The impairment of the natural course of embryogenesis under environmental conditions is called teratogenesis (Greek *teras* — monster). Factors causing teratogenesis are teratogenes. Teratogenes may be groups of medicines (anti-biotics, quinine, chloridine and anti-depressants), alcohol, nicotine, «toxins» of parasites, various types of radiation. The action of teratogenes causes development defects. Teratogenes affect gametes, causing *gametopathies*, and different stages of embryogenesis causing the development of *embryopathies*. Congenital defects may be primary due to a direct action of teratogenes (example: atresia of the Sylvius aqueduct) and secondary — because of complications of primary defects (example: hydrocephaly).

The science *teratology* studies causes, mechanisms of development and prophylaxis of development defects.

The occurrence frequency of development defects in human populations is 1–2 %.

The variety of congenital development defects includes:

– agenesia — absence of an organ (e. g., an extremity);
– hypogenesia — underdevelopment of an organ (e. g., gonads);
– hypergenesia — overdevelopment (e. g., polydactily);
– atresia — imperforation of natural openings and canals (e. g., esophagus, anus);
– ectopy — changing of the organ position (e. g., the heart on the right side).

The reasons of congenital defects:

1) genetic (various mutations);
2) exagenic (environmental factors);
3) multifactoral (joint action of factors of the 1^{st} and 2^{nd} groups);

4) interaction of the germ's parts (embryonic induction).

The postembryonic (postnatal) period is a period from the moment of birth or going out of ova membranes and to death. After morphogenesis is completed, puberty starts followed by reproduction and a final stage of ontogenesis — aging and death.

Types of ontogenesis

Direct development	Indirect development (with metamorphosis)
a) laying eggs with a great amount of yolk (birds)	a) incomplete metamorphosis-stages: egg – larva – mature individual (intestinal helminthes)
b) intrauterine (mammals)	b) complete metamorphosis-stages: egg – larva – chrysalis – mature individual (butterflies, 2-wing insects)

Periods of human postnatal ontogenesis

Neonatal period (1–10 days) is a complex period when reconfiguration of the whole organism occurs in order to adaptat to new existence conditions.

Infancy, or breastfeeding period (11 days – 12 months). A child is feed with mother's milk. The baby grows rapidly.

Early childhood (1–3 years). The child learns to walk and speak, gets acquainted with the world arround.

The 1st period of childhood (4–6 years). The child is interested in everything and tries to understand everything, get hang of basic game skills.

The 2nd *period of childhood* (7–11 years in girls, 7–12 years in boys). The growth slows, intensive development of the muscular system occurs. In this period children go to school.

Puberty, or adolescence (12–15 years in girls, 13–16 years in boys) Sexual maturation starts and growth speed intensity increases.

Juvenilty (16–20 years in girls, 17–21 years in young men) Sexual maturation, growth and physical development have completed.

1st period of middle age (21–35 years in women, 22–35 years in men) an optimal period for childbirth; mastering professional skills.

2nd *period of middle age* (36–55 years in women, 36–60 years in men) is a period of the most active professional activity. The first signs of ageing appear after 35 years).

Advanced age (56–75 years in women, 61–75 years in men). The processes of aging are going on; this is the age of retirement.

Senile age (76–90 years) Senile changes are marked; some people still can work creatively at this age.

Longevity (over 90 years).

There are critical periods in the postnatal human ontogenesis:

1. *Neonatal period* (the first days after birth) — reconfiguration of all organ systems for a new environment.

2. *Puberty period* (12–16 years) — a hormonal readjastment, formation of secondary *sex characters.*

3. *Period of sexual involution.* (about 50 years in women, 60–70 years in men). Reproductive function fades functional depression of gonads and endocrine glands occur.

There are three periods of postnatal ontogenesis in animals and men:

1) prereproductive (juvenile);
2) reproductive (mature);
3) postreproductive (aging).

GROWTH: LAWS AND GROWTH REGULATION

Growth is the enlargement of dimensions and body mass. Growth can be unrestricted (indefinite) — it lasts all life (crustacean, fish and amphibian) and restricted (definite) — it stops by a definite age (insects, birds, mammals). The process of growth in humans is irregular; the periods of fast growth alternate with the periods of slowed growth.

Laws of growth

The highest intensity of human growth is marked during the 1st year of life — the increment is 25 cm. On the 2nd year of life it is 10–11 cm, on the third — 8 cm. At the age of 4–7 years — it is 5–7 cm per year. At junior school age — 4–5 cm per year, during puberty the growth intensity increases up to 7–8 cm per year. Then the human growth slows down and up to 25 years it increases 1–2 cm per year.

Regulation of growth							
Hormones			**Environmental factors**				
Chondotropic hormone (hypophysiotropic hormone)	Thyroxin (thyroid-stimulating hormone)	Sex hormones	Light	Nutrition	Vitamins (A, B, D)	Micro-elements	Social-economic factors

The *somatotropic* (chondotropic) hormone is secreted from the moment of birth till 13–16 years. When the function of the gland is decreased, hypopituitary dwarfism develops. When it is increased, gigantism develops; the height of a person reaches 2 m and over. Secretion of this hormone in adulthood gives acromegaly — enlargement of the bones of the wrist, foot and face. *Thyroxin* increases energy exchange in the organism. The functional decrease of the gland results in growth retardation, impairment of body proportions, inhibition of sex development, mental impairment. *Sex hormones* affect all the processes of metabolism.

Environmental factors have a great effect on growth. For normal growth of a child, he should have a well-balanced nutrition including vitamins and microelements. A very important role for the synthesis of vitamin D (calciferol) plays the sun light.

Basic types of tissue and organs growth (fig. 40):

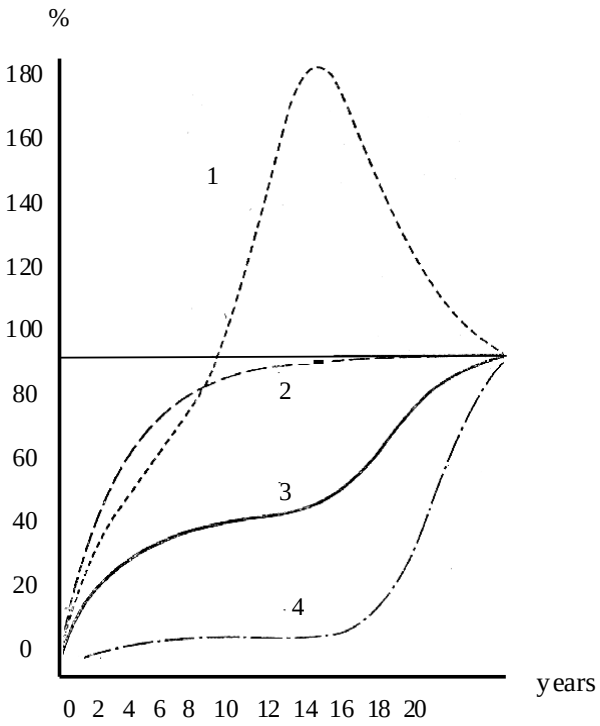


Fig. 40. Types of tissue and organs growth

1 — *lymphoid*; thymus, lymphatic nodules, lymphoid tissue of the intestines, spleen, tonsils; the maximum increment of their mass goes to 11–12 years.

2 — *cerebral*: the brain and spinal cord, eyes, the head develop earlier than other body parts — after birth and till 11–12 years.

3 — *general*: the whole body, muscles, the skeleton, respiratory organs, liver — a maximum growth during the first year of life and in the puberty period.

4 — *reproductive*: various parts of the genital system — a fast growth in the puberty period.

During the last decades there was marked *acceleration* of physical and physiological development of children. It shows already at the stage of intrauterine development — an increase of the body length in neonates by 0.5–1.0 cm, body mass — by 50–100 g, the terms of teeth eruption change. During the last 100 years the height of adult people increased on an average by 8 cm. Acceleration may be caused by the following factors: interracial marriages (heterozygosity increases), urbanization, enhancement of the radiation background, modification of the Earth magnetic field and a number of social factors.

Human age	
Biologic — the age he looks	Chronological — the number of years he/she has lived

The biological and chronological age do not always coincide.

Criteria for determination of the biological age:

– maturity of the skeleton: ossification of various parts of the skeleton occurs at a different age;

– teeth maturity: eruption of milk teeth and their substitution with permanent ones occur at a definite age.

– the time of appearance and the degree of development of secondary sex characters.

CONSTITUTION AND HABITUS

The constitution of a person presents genetically conditioned specificities of morphology, physiology and behavior.

In 1927 M. V. Chernorutsky advanced a classification which distinguished three main constitutional types.

An ectomorphic type (asthenics). They have a narrow chest, a low position of the diaphragm, lengthened lungs, relatively short intestines with low absorption, thin bones and long extremities, a thin layer of fat deposits. Asthenics are characterized by increased excitability, inclination to neuroses, hypotonia, ulcers, and tuberculosis.

Mesomorphic types (normosthenics) have a proportional constitution, moderately developed subcutaneous cellular tissue. The people of this type are energetic, mobile and prone to neuralgia, atherosclerosis and diseases of the upper respiratory tract.

An endomorphic type (hypersthenics) are characterized by a broad chest, a high position of the diaphragm, a voluminous stomach and long intestines with high absorption, a considerable layer of fat. The heart has relatively large dimensions; the blood contains an increased content of cholesterol, uric acid, erythrocytes and hemoglobin. In hypersthenics dominate the processes of assimilation; they are prone to atherosclerosis, obesity, diabetes, hypertension, the diseases of kidneys and gall bladder. The people of this type are well balanced, calm, and sociable.

As to the types of constitution, the majority of people occupy an intermediate position.

Peculiarities of morphology, physiology and behavior at a definite period constitute a habitus. Habitus reflects how a person feels, his health state at a given moment. It includes peculiarities of the constitution, carriage and gait, color of skin coverings, expression of the face, correspondence of biological and chronological age.

AGING AND OLD AGE

Aging is a general biological law, characteristic of all living organisms. Old age is a final stage of ontogenesis. The science about old age is called *gerontology*. Gerontology studies laws of aging of various organ systems and tissues. It includes the sections of gerontal hygiene and gerontal psychology.

Geriatrics is a science about diseases of old age; it studies peculiarities of their development, course, treatment and prophylaxis.

The process of aging includes all levels — a molecular, subcellular, cellular, tissue, organ and organism level. It results in decrease of vitality of the organism, weakening of homeostasis and adaptation mechanisms. The biological meaning of aging is inevitability of death.

Signs of aging in organs and organ systems

1. Cardio-vascular system. Vascular elasticity of blood vessels changes; the connective tissue in the heart and vascular walls grows out instead of the muscular tissue; blood circulation in tissues and organs becomes impaired. Functioning of blood-forming organs deteriorates.

2. Respiratory system. Interalveolar septa become disrupted, the respiratory surface of the lungs decreases; their vital capacity becomes less; the connective tissue grows out.

3. Gastrointestinal system. Loss of teeth, functional decrease of gastric glands, impairment of the motor function of the intestines.

4. Urinary system. Death of a part of nephrons, decrease of filtration intensity of kidneys.

5. Muscles and the skeleton. Atrophy of skeletal muscles, lessening of bone strength, domination of mineral substances in their composition.

6. Nervous system. Death of neurons, impairment of functional regulation of organs, slowing down the speed of impulse conduction, memory weakening. Functional decrease of all sensitive organs.

7. Weakening of the mechanisms of humoral and cellular immunity.

External manifestations of aging signs: carriage, body shape and gait change; grey hair appear, skin elasticity is being lost (wrinkles appear), sight and hearing deteriorate.

Gerontology proposes over 300 hypotheses of aging. The most common are:

1. *Energetic* (M. Rubner, 1908): the organism of every species has a definite energetic background. After it has been spent during life, the organism dies.

2. *Intoxicational* (I. Mechnikov, 1903): self-poisoning of the organism because of accumulation of nitrogenous waste and purification products in the colon of an individual.

3. *Associated with the connective tissue* (A. Bogomolets, 1922): as the connective tissue is a trophic regulator of cells and tissues, its changes impair interactions of tissues and cause aging.

4. *Overstraining of the central nervous system* (I. Pavlov, 1912; G. Selie, 1936): nervous shocks and prolonged nervous overstrains cause untimely aging.

5. *Changing of colloidal properties of the cellular cytoplasm* (V. Ruzhichka, M. Marinesku, 1922): the changed cytoplasm poorly retains water, that is why colloids from hydrophilic become hydrophobic, colloidal particles become larger and their biological properties change.

6. *The programmed number of cellular mitoses* (A. Khaflick, 1965): various species have an unequal number of cellular mitoses — the longer is life the greater number of them they have (fibroblasts of human embryos give about 50 generations, they are about 15 in mice and hens).

7. *Genetic:* accumulation of mutations; decrease of intensity and impairment of the processes of transcription, translation and repair; impairment of self-renewal of proteins.

Social factors produce a considerable effect on the process of human aging as well as conditions and style of life, various diseases. Human aging and life span also depend on the ecologic situation.

The science that studies a healthy style of life and conditions to prolong its duration is called *valeology*.

A theoretically possible human age is 150–200 years; a maximum registered is 115–120 years. An average life span in Republic of Belarus for men is 64–74 years, for women — 74–79 years. In some countries, an average life span is 35–40 years.

CLINICAL AND BIOLOGICAL DEATH

Aging of the organism is finished with *death*.

Death provides a change of generations. Causes of death may be different.

A *physiological* or natural death occurs due to aging.

A *pathological* or untimely death is a result of disease or an accident.

A clinical death occurs due to a failure of vital functions (heart and respiration failure), but metabolic processes in cells and organs are preserved.

A biological death occurs when the processes of self-renewal in cells and tissues stop, chemical processes are impaired, and autolysis and break down of cells take place.

Necrotic changes in the most sensitive cells of the brain cortex occur already in 5–6 minutes. It is possible to delay the period of a clinical death by general hypothermia, which slows down metabolic processes and enhances the persistence to oxygen deficiency.

Reanimation and euthanasia

Reanimation is a possibility to return a person to life after a clinical death (when vital organs are not impaired) in 5–6 minutes, while cells of the brain cortex are “alive”. Reanimation methods are used in medicine in any threatening circumstances.

In the middle of the XX century there appeared a trend in medicine, which was called euthanasia.

Euthanasia is a medical aid to pass from life for a seriously and terminally ill person according to his wish and request of his relatives. Euthanasia is legitimate in several countries. It requires solving many juridical and moral ethical problems.

LECTURE 8
Topic: ECOLOGICAL PARASITOLOGY

Plan

1. The subject of ecological parasitology.
2. Classification of parasites and their hosts.
3. The system «parasite – host».
4. Parasitic system.
5. About biological basics of prophylaxis of parasitic diseases.

Parasitism is an ecological phenomenon. Ecological parasitology became an independent science in 1930s thanks to works by Russian scientists B. A. Dogel, V. N. Beklemishev, E. N. Pavlovsky. It studies relations between parasites and their population, with the host organism and the environment. The work of E. N. Pavlovsky «The organism as an inhabitation medium» (1934) was very significant for the development of ecological parasitology. It determined the concept of «parasitocenosis» including all parasites (of various types) of only one host organism. For example, protists, flat- and ringworms, various bacteria may inhabit the digestive system.

There are definite interrelations between them:

– *synergism*: combination of helminthes and viruses, bacteria and protists; dysenteric amoeba, having left the cyst, consumes bacteria of the host's intestine, otherwise it won't become pathogennic;

– *antagonism*: for the most of helminthes, increase of one species number results in decrease of the other species number;

– *antibiosis*: several species cannot live in one environment due to their excretion of metabolites (cholera agent in hen does not go with tape worms, malaria plasmodia and ascaris).

THE SYSTEM «PARASITE – HOST»

The system «parasite – host» includes one host individual and one or a group of parasitic individuals of a definite species. To form this system one needs the following conditions:

a) contact between the parasite and the host;
b) host must provide conditions for the development of the parasite;
c) ability of the parasite to resist reactions from the host part.

The basic development of evolution is elaboration of a balanced system; antagonism between partners is smoothed down and reliability of the system increases. The dualism of the system «parasite – host» means that from one side partners relations are antagonistic, from the other — they are stabilized. Smoothing down of the antagonism occurs due to co-adaptation («co» means mutual):

– in the parasite — morphological and biological adaptations occur;
– in the host — complication of defense mechanisms takes place.

Evolutionary directions (co-evolution) are also different:

– in the parasite — it is complication of adaptation mechanisms to the host;

– in the host — mastering of defense reactions on all levels (for destruction of the parasite).

CLASSIFICATION OF PARASITES

1. According to their relations with the host:

– *obligate parasites* — this way of life is characteristic of all representatives of the given species (Ascaris, tapeworm, lice);

– *facultative or pseudoparasites* — as a rule, they are free-living but, having got into the human or animal organism, they may exist there producing harm (larvae of flies);

– *hyperparasites or superparasites* — they are parasites of parasites (bacteria in parasitic protists).

2. According to localization in the host:

– *ectoparasites* — they inhabit coverings of the host's organism (lice, fleas);

– *endoparasites* — they live inside the host's organism;

a) intercellular (malaria parasite);

b) interabdominal (helminthes in the intestine);

c) tissue (liver fluke);

d) intradermal (itch mite).

3. According duration of its relation with the host:

– *constant* — they live their whole life cycle in the host (ascaris, fish tapeworm);

– *temporal* — a part of their life cycle occurs in the host (larval parasitism fly's larvae; immaginal parasitism — only sexually mature individuals are parasites (mosquitoes, fleas).

CLASSIFICATION OF HOSTS

1. According a life stage of the parasite:

a) *definitive or final host* — the parasite reaches its sex maturation in his organism and undergoes its sexual reproduction (human for the beef tapeworm and liver fluke);

b) *intermediate host* — parasite's larvae inhabit the host's organism and undergo asexual reproduction (mollusks for flukes, humans for malaria parasite);

c) *additional host* (the 2nd intermediate) — predatory fish for larvae of the broad fish tapeworm;

d) *reservoir host* — the host where accumulation of parasite's invasion stages occurs (wild rodents for leishmania).

2. According to conditions of the parasite development:

a) obligate or natural hosts — provide optimal conditions for the parasite development in the presence of biocenotic relations (natural ways of infecting the human with ascaris and pinworm);

b) optional hosts — presence of biocenotic links, but the absence of biochemical conditions for the parasite development (a human for a pig ascaris);

c) potential hosts — presence of chemical conditions, but the absence of biocenotic links (a guinea pig for a trichinella).

Transmission rotes of parasites.

1) *alimentary* — orally with food and water (eggs of helminthes, cysts of protozoans);

2) *droplet (respiratory)* — through the respiratory tract (cysts of some Amoebae, some viruses and bacteria);

3) *vertical* — intrauterally from mother to fetus (toxoplasma, malaria parasite);

4) *iatrogenic* — due to medical procedures, for example transfusion of infected blood (trypanosomes, malaria parasite);

5) *sexual* — in sexual contacts (Trichomonas vaginalis);

6) *direct contact* — contact with a sick person or animal (itch mite);

7) *indirect contact* — contact with a sick person or animal through household goods (itch mite);

8) *vector-borne* — with participation of an arthropod (trypanosomes, malaria parasite):

– inoculation (through the proboscis of a vector during blood meal) — trypanosomes, malaria plasmodia;

– contamination (contamination of the skin by excrements of a vector, which contain the patogen and are rubbed into the skin during itching) — trypanosomes of Chagas disease, bacillus of plague;

Parasites are highly specialized organisms with maximal adaptation to their environment. On one side, there occurred simplification of some of their organs, on the other — complication of the other systems.

ADAPTATIONS OF PARASITES

Progressive morphological adaptations:

1. *enlargement of the body* (up to 20 m in tapeworms);

2. *the reproductive system it most developed* as compared to other systems;

3. *hermaphroditism*;

4. various fixation organs (adhesive discs of Giardia lamblia, suckers of flukes, botria or hooks of tape worms, claws of lice, etc);

5. *integument* — tegument or cuticle protect the patasite from host's enzymes;

6. *molecular mimicry* — similarity of proteins of the parasite and the host;

7. *excretion of anti-enzymes, histolysines.*

Regressive morphologicaladaptations:

1. *simplification of sense organs* — endoparasites have only tactile and chemical sense organ;

2. *simplification of the organ systems* — absence of the alimentary tract in tape worms.

Biological adaptations are associated with structural peculiarities of the reproductive system, reproduction and life cycles of parasites:

1. *high fertility* (Taenia solium excretes 100 thousand eggs with every mature segment, an ascaris — 250 thousand eggs per day);

2. *various forms of asexual reproduction* (schizogony in malaria parasite, polyembryony in flukes);

3. *migrations within the host's organism* (larvae of Taenia solium and Ascaris lumbricoides);

4. *complex life cycles* with alternation of hosts.

The results of interactions of the parasite and the host on the level organism may be different:

- *death of the parasite;*
- *death of the host and;*
- *carriage of the parasite*.

Every parasite has pathogenicity. Pathogenicity of the parasite is expression of extreme parasitism, its ability to cause the disease. Pathogenicity depends on a number of factors:

– *Parasite genotype*, its belonging to a species;

– *Host age* (children and elderly people are more sensitive to infection);

– *Diet* (parasitic protists are more numerous in the gastrointestinal tract of herbivorous animals; unwholesome diet of the host increases the number of parasites in the organism and their sizes, reduces the terms of parasites development;

– *Doses and invasion degrees* (the more eggs or parasite larvae are introduced into the host organism, the more severe will be the disease);

– *Resistance degrees of the host organism*;

– *Presence of other parasites and diseases*, which will weaken the host organism.

The following pathogenicity grades are marked out: carriage, subclinically and clinically marked parasitic diseases and lethality. Host pathogenicity is sharply marked in phylogenically young systems «parasite-host».

The second parasite characteristic is specificity, i. e. manifestation of historically established adaptation degree of the parasite to the host. Forms of specificity manifestation:

a) *hostal* (of the host): monohostal — the parasite has one type of the host (human Ascaris), polyhostal — the parasite has hosts of different types (leischmania, trichinella);

b) *topical* (place of parasitism): ascaris (intestines), fasciola (liver);

c) *relating to age* (the peak of parasitic diseases is marked in pre-school children);

d) *seasonal* (outbursts of amoebic dysentery — the end of spring – summer; of trichinosis — autumn – winter).

Pathogenic action of parasites on the host organism is due to their morpho-physiological peculiarities and it is in the range from an immunological reaction to clinical symptoms of the disease and fatal outcome.

1. Parasites produce *mechanical action* by their body mass (a ball of ascaris in the intestines, cyst of echinococcus in the brain), by their fixation organs (incarceration of the intestinal mucous membrane by suckers and tissue necrosis, integrity impairment of the mucus by hooks and irritation of nerve endings — spasm and obstruction of the intestines), integrity impairment of skin coverings by suckers larvae, proboscis of blood sucking arthropoda.

2. *Toxicoallergic action* is produced by parasites metabolites, being antigens for the host; excreted by parasites hemolysins, histolysins and waste products of ruined parasites; ectoparasites saliva action during blood sucking. Manifestations of this action in the host: skin eruption, dermatitis, eosinophilia, allergic reaction of various severities (up to anaphylactic shock).

3. *Absorption of nutrients and vitamins* in the host organism leads to avitaminosis (mainly A and C), loss of weight, emaciation.

4. *Impairment of metabolic processes* in the host organism causes weakening of resistivity and raise of sensitivity to causative agents of other diseases.

5. Biologically active substances of the parasite produce *immuno-supressive action*. They suppress phagocytic activity of leukocytes.

6. Some *parasites stimulate oncogenesis*, formation of malignant tumors: clonorchs and opisthorchs — cholangiocarcinoma, schistosomas — cancer of the gall bladder and rectum.

7. Parasites may produce *unfavorable effect on the course of pregnancy and fetus development* (malaria plasmodium, toxoplasma, opisthorch): cause gestational toxicosis, miscarriage, delivery complications (hemorrhages), impairment of the fetus development (congenital defects).

RESPONSES OF THE HOST ORGANISM

The basis of all reactions is immune defense of the host. Allergy is a kind of immunological reactivity.

The first reaction to a parasite — is an attempt to kill it with enzyme action, free radicals, and then — to neutralize factors of its «aggression» by proteases, enzyme inhibitors.

Defense reactions of the host manifest at a cellular, tissue and organism level. *Reactions on a cellular level*: hypertrophy and modification of affected cells (erythrocytes in malaria). *Tissue defense reactions* (toxoplasmosis, trichinellosis): isolation of the parasite from healthy tissue — capsule formation from the connective tissue, dilation of blood vessels and accumulation of leukocytes around the

parasite — incapsulation of a trichinella larvae, formation of the membrane of a toxoplasmatic pseudocyst. *On an organism level,* defense mechanisms manifest by humoral reactions (producing anti-bodies) and various forms of immunity: absolute — relative, active — passive, congenital — acquired. Absolute immunity is formed to leishmaniasis and trypanosomiasis, to malaria — relative. The most intense immunity arises to a larva stage. Immune reactions of the host decrease the speed of parasites reproduction and inhibit their development.

PARASITIC SYSTEM

Parasitic system (Beklemishev, 1956) forms on the population level. It includes a parasitic population of one species and one or several populations of the host or the host and the environment necessary for their existence. There are the following adaptations of parasites at the population level:

– *High fertility* is particularly important considering circulation of larva stages in the external environment and «search» of intermediate hosts;

– A movable larva or free living stages are for *active search of the host* in the cycle of parasites development;

– *The presence of dormant stages* (cysts, eggs) for waiting till unfavorable conditions are over;

– *Using reservoir hosts* for accumulation of invasive stages and transportation to their final hosts;

– *Synchronization of parasite development cycles and host's behavior.* For example, contamination of a human with schistosomes occurs during bathing, when suckers' cercaria get into blood vessels through the skin. A person comes into a water pond for bathing at the hottest time of the day, and just at this time one can observe a numerous excretion of cercaria from an intermediate host — a mollusk.

The result of the parasite impact on the host at the organism level, as a rule, is a disease, at the population level — morbidity (the number of sick individuals in a specific population). Mass diseases in human populations are epidemics, in animal populations — epizootics. Despite a clearly marked effect of the parasite on an individual host, its general influence on the host population may be insignificant.

Parasites are obligate components of biocenosis. V. N. Beklemishev proposed a conception "usefulness" of the parasite, i. e. the role of parasites in nature is stabilization of ecosystems. Parasitic diseases are a strong selective factor. The animal population without parasites will be doomed for death. Parasitism promotes the improvement of the host's immune defense.

Regulation of the number of host populations in parasitic systems follows a feedback principle (fig. 41).

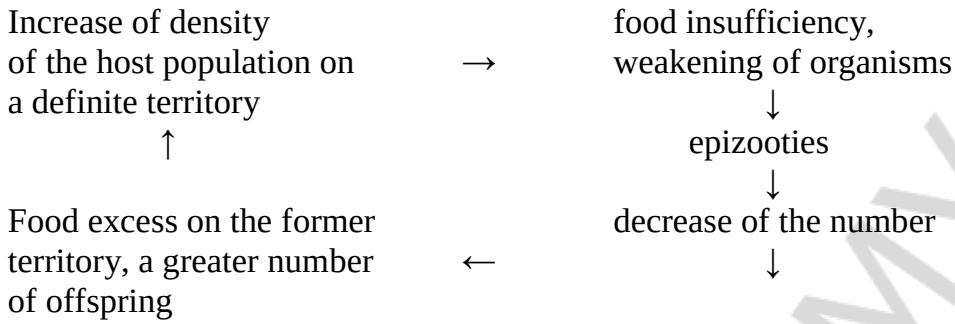


Fig. 41. Diagram of regulation of the host population quantity

Parasites bring considerable losses to plant-growing, cattle-breeding. All age groups of the population all over the world suffer from them. A number of objective causes hamper fighting against parasitic diseases: wide spreading of parasites, their great adaptive possibilities, the development of resistivity to antiparasitic preparations, difficulties of making vaccines.

Academician K. I. Skryabin developed *biological prophylactic basics* for fighting parasites.

It is «a complex of preventive measures based on detailed studying of causative agent's biology, migration ways of its development stages, biology of intermediate hosts that will make it possible to interrupt some link of a parasite development cycle».

The final practical aim of Parasitology is the defense of humans, animals and plants from parasites affect and stamping out parasitic diseases.

LECTURE 9
Topic: POISONOUS FUNGI AND PLANTS

Plan

1. Poisonous micro- and macromycetes. Classification of poisonous macromycetes.
2. Physiological characteristics of mycotoxins produced bymicro- and macromycetes.
3. Classification of poisonous plants.
4. Toxic agents produced by plants and mechanism of action.
5. Physiological characteristics of phytotoxin of thallophytes and embryophytes.

POISONOUS MICRO- AND MACROMYCETES. MACROMYCETES CLASSIFICATION

Toxicity – is the universal phenomenon in the wildlife and the most important mechanism of struggle for existence.

Substances that are used by the living organisms in the interspecific interactions are called allomones.

Classification of allomones according to the systematic belonging of the organisms that produce these substances.

- ***mycotoxins*** – fungi toxin;
- ***phytotoxins*** – plants toxin;
- ***zootoxins*** – animals toxin.

According to the morphological criterion within different classes of fungi usually mark out **macromycetas** – land plants and **micromycetas** – that have microscopic size.

There are 250 species of micromycetas that produce more than 100 items of toxic substances that poison food products of people and farming animals. Many micromycetas have grave consequences due to their cumulative effect, and also teratogenic, mutagenic and carcinogenic effect.

PHYSIOLOGICAL CHARACTERISTICS OF MICRO- AND MACROMYCETES MYCOTOXINS

Table 8

Toxic characteristic of mycotoxins of some micromycetas

Micromycetas	**Mycotoxins**	**Natural substrate**	**Toxic action**
Aspergillus flavus, A. parasiticus	Aflotoxins (10 compounds)	Peanuts, corn, other leguminous plants (seeds), nuts, vegetables, vegetable forage	Hepatotoxic, mutagenic, teratogenic, immunosuppressive
Penicillium viridicatum	Ochratoxins	Cereals, coffee, cheese, forage	Nephrotoxic, teratogenic
Genus Fisarium (Fisarium graminearum etc.)	Trihocens (40 compounds)	Cereals, forage, hay	Nephrotoxic, haemorrhagic, immunosuppressive, dermatoxic.
Ergot Claviceps purpurea	Ergotoxines	**More than 150 species of** wild and cultural cereals	Neurotoxic Declare itself in two forms: gangrenous («Saint Anthony's Fire») and convulsive

First Aid: gastric lavage by the suspension of the activated carbon in the 2% solution of baking soda, blended saline.

Preventive measures: control over the condition of the food and forage, removal of contaminated products.

Mycotoxins – thermotolerant compounds and in the process of usual culinary treatment of products are not destroyed.

Macromycetas classification:

– edible;

– conditionally edible: after proper culinary treatment they are suitable for eating: morel, gyromitra esculenta, russula foetens (stinking russula), coprinopsis atramentaria (common ink caporinky cap), boletus satanas (Devil's bolete or Satan's mushroom) etc;

– almost uneatable: many of the almost uneatable mushrooms (Tylopilus felleus, Chalciporus piperatus or Peppery bolete) do not produce mycotoxins, but have unremovable bitter taste, unpleasant odor, unpalatable pulp;

– poisonous: death cap, amanita, sulphur tuft, brown roll-rim (poison pax), yellow-staining mushroom.

The most poisonous macromycetas:

Death cap (*Amanita phalloides*) contains toxic polypeptides amanitin and phalloidin that mainly affect liver. In the result of disorder of protein, phospholipids and glycogen biosynthesis starts necrosis and fatty degeneration of the liver. Only 1/3 of a mushroom is enough for an intoxication. Most cases end with lethal outcome.

Fly agaric or fly amanita (*Amanita muscaria).* Its toxicity is caused by muscarine and muscaridine that stimulate M-cholinergic receptors autonomic nervous system. In case of a grave intoxication there can happen comatose state and collapse.

In case of intoxication by mycotoxins of macromycetes:

– gastric lavage by the suspension of the activated carbon;

– immediately ask for a skilled medical assistance;

– if possible keep the rest of the prepared food so that will be possible to identify mushroom species.

Prevention of intoxication by mycotoxins of macromycetes:

– do not collect unknown, old or overriped mushrooms;

– do not collect mushrooms near highways (mushrooms accumulate heavy metals and products of incomplete combustion of an automotive fuel);

– do not store mushrooms for a long time (it is a perishable product);

– forbid to sell salted and dried mushrooms.

POISONOUS PLANTS CLASSIFICATION

Poisonous plants are those that contain specific substances that in case of a certain exposition (dose and long-term exposure) can bring disease or death of a human or an animal.

Earth flora has more than 10 thousand species of poisonous plants, mainly in the tropics and subtropics, in the countries of moderate and frigid climate there are about 400 species.

Poisonous plants classification.

– unconditionally poisonous with the group of especially toxic;

– conditionally poisonous (poisonous in definite places of inhabitation, in case of improper storage vegetable feed or zymogenic influence of fungi and microorganisms).

Toxicity of different plants depends on:

– the place of a species in the geographic natural habitat ;

– the type of a soil;

– environmental conditions of a year;

– stage of an ontogeny and phenophase.

Veratrum that grows in Europe is a deadly poisonous plant, but in some areas of Armenia and Altai is a good fodder species.

Dull weather stimulates accumulation of alkaloids by plants.

During mild winter clover in the young sprouts accumulates cyanogenetic glycoside to protect itself from eating by snails that show early activity if January's isotherm higher than $+5^0$C.

Phytotoxins are accumulated in all parts of a plant or in certain organs.

For example, all part of the cursed buttercup (celery-leaved buttercup), stinking nightshade (black henbane), opium poppy are toxic.

Many of the Rosales (almonds, peach, apricot, cherry, plum) contains cyanide glycoside amygdalin in the cotyledons. When it dissociates hydrocyanic acid is generated in an organism. Consecration of cyanide in the cotyledons guarantees protection of juvenile sprouts.

It is usual for many plants that they contain phytotoxins during certain seasons of a year. In the storing subterranean organs there is a maximum of poison in the period of winter rest, and in the overground organs – in the period of blossoming.

The causes of poisoning by plant poison:

– eating (especially by children) attractive fruit, succulent roots, bulbs, stalks;

– an overdose by medicines made of plant material (lily-of-the-valley, foxglove, adonis, valerian, veratrum, magnolia vine, ginseng, etc.);

– respiratory poisoning caused by inhalation of poisonous secretion (ledum, fraxinella, conifers, rhododendron);

– contacts with plants (nettle, cow-parsnip, euphorbia, daphne mezereum, conium, etc.) causes allergic response;

– growing, preparation and processing of plant raw material (tobacco, belladonna, ranunculaceae, veratrum, red pepper, celandine, etc.);

– processing and chemical processing of wood (all of the conifers, oak, beech, alder, black locust, etc.);

– eating honey that contains pollen of poisonous plants (tobacco, atropa, ledum, paris, etc.), meat and milk of animals that ate poisonous plants (ranunculaceae, colchicum, veratrum, hemp nettle, yew, ephedra).

TOXIC AGENTS PRODUCED BY PLANTS AND MECHANISM OF ACTION

There are thousands of different kinds of toxic substances. According to their chemical character they are divided into several groups.

Alkaloids – nitrogen-containing organic compounds, mainly with heterocyclic structure. Science knows about 5000 alkaloids (many of them are toxic). They are contained in the form of organic acid (citric acid, malic acid, oxalic acid) in ~ 10% species of plants in number from 0,001% to 10-20% (cinchona bark). Especially many alkaloids are contained in the plants belonging to the families of Solanaceae, Papaveraceae, legumes, Rubiaceae, lily, Asteraceae, ranunculaceae). Most of the alkaloids are valuable medical products that are used as antihypertensive, raising arterial blood pressure, expectorant, stimulating central nervous system, analgetic, cholagogic, anticholinergic, antineoplastic, etc.

Organic acids – play an important role in metabolism of the plants, they are used in synthesis of amino acids, saponins, alkaloids, steroids and other compounds.

Groups of organic acids:

– **aliphatic:** formic, acetic, isovaleric acids (volatile, with pungent smell); citric, malic acids (non-volatile, all of the plants have them), oxalic acid, pyruvic acid;

– **aromatic:** benzoic (it is a part of volatile oils and balsams), gallic (is contained in tanning agents);

– **acyclic:** kinic (much is contained in bilberries, cranberries, coffee), shikimic.

Lipids – is a group of substances that are dissoluble in the low-polarity organic solvents (ether, benzol, carbon tetrachloride). They include fats (triglyceride of fatty acids), phospholipids, sterols and wax.

Groups of soft oil:

– **non-drying:** olive, castor, almond oils. They are used as a purge and for preparing solutions of sex hormones, camphor;

– **semidrying**: sunflower, maize, cottonseed oils. They are used for preparing ointment. Maize oil is used as an atherosclerosis disease prevention;

– **drying:** linseed and hempseed oil. They are used to prepare ointments to cure burns.

Terpenoids **–** oxygen-containing terpens derivative, hydrocarbon that consist of isoprene units (C_5H_8).

In the composition of essential oils they are used as spasmolytic, aseptic, expectorant remedy. The plans belonging to the families of Cucurbitaceae, Cruciferae (crucifers) and Scrophulariaceae (figwort family) contain terpenoids kukurbitatsins that have an antineoplastic action.

Steroid (cardiac) glycosides **–** sterane derivative. Have a cardiotonic action. At high doses – cardiac poison.

Cardiac glycosides are present in plants belonging to the families of the Ranunculaceae (the buttercup or crowfoot family), the Cruciferae (the crucifers), the Liliaceae (the lily family), the Scrophulariaceae (the figwort family), the Apocynaceae (the dogbane family) and the Asclepiadaceae.

Saponins are presented in plants in the form of steroids that contain 27 carbon atoms in molecule. They have bitter taste, cause irritation of mucous membranes, vomiting, increase secretion of bronchus. Saponins are almost not absorbed into digestive tract, but if they get into blood they cause paralysis of central nervous system and haemolysis of erythrocytes.

Flavonoids – crystalline phenol compounds that have a structure of C_6-C_3-C_6; they have white (catechins), yellow (flavones, flavonols), orange (chalcones), red, blue and violet (anthocyanins) colours. They have radioprotective, antioxidant, antineoplastic, estrogenic, spasmolytic, hypotensive and other kinds of action.

Tanning agents – high molecular polyphenols. They are contained in many plants, especially in dicotyledons (the Fabaceae (Leguminosae),the Myrtaceae (Myrtle family), the Rosales).Taning agents have astringent, bactericidal and tanning action.

Coumarins – oxygen-containing heterocyclic benzo-α-piron derivative. They are wide spread in plants (more than 200 compounds). They have spasmolytic, anticoagulant, bactericidal, photosensibilizing, dilating blood vessels and bactericidal actions. Dicumarin (bishydroxycoumarin) is an antagonist of the vitamin K.

Anthraquinones – anthracene derivative, mainly are glycosides. Many of them have cathartic action (leaf of senna, bark and fruit of Alder Buckthorn). Some of anthraquinones lower the level of haemoglobin, disturb the function of liver and kidneys.

PHYSIOLOGICAL CHARACTERISTICS OF PHYTOTOXIN OF THALLOPHYTES AND EMBRYOPHYTES

Algae. Toxic representatives (Microcystis, Anabaena variabilis, Nostoc pruniforme, Nostoc rivulare etc.) of blue-green algae inhabit the closed basins of the temperate zone. They cause «water bloom» in the period of mass propagation. «Water bloom» is accompanied by accumulation of toxic substances in the body of hydrobiontes and water environment. Anatoxin α causes depolarizing type blockade of neuromuscular junction, taking skeletal and respiratory muscular system.

Microcystine, cyclic polypeptide, that causes thrombocytopenia, haemorrhage of liver and lungs, vast thromboses, auxesis of liver up to 50%.

Intoxication of a human by blue-green algae happens when:

1) it gets into human body with water like toxic gastroenteritis: nausea, stomach pains, muscle and joint pains, vomiting, diarrhea;

2) swimming in basins: dermatitis, itching, conjunctivitis;

3) eating infected fish (pike, sander, burbot, etc.) causes Haff disease. During exercise stress rises sharp pain in the muscles that increase with the slightest movement, paralysis of respiratory muscular system is also possible.

Lycopodium (ground pines or creeping cedar). Overgroud part of plants belonging to all of the European species contains alkaloids (lycopodin, obscurin, clavatin, nicotine), spores are innocuous and are used as baby powder «lycopodium» (club moss). Lycopodium alkaloids have neurotropic action.

Equisetum (horsetail) contain large quantity of salt of silicic acid that causes mechanical injury and irritation of mucous membranes of digestive tract; палюстрин alkaloid effects cattle and causes indigestion, lowering of the yield of milk and its spoiling.

Fern. Is the most typical poisonous representative – dryopteris filix-mas (Common Male Fern). Rhizome is toxic, it contains folic acid, aspidinol, albaspidin. Main symptoms: sickness, vomiting, diarrhea , abdominal pains, headaches, giddiness, visual disturbance. Dried rhizome's extracts have anthelminthic effect, mainly paralyzing cestoda (tapeworms).

Gymnospermous that have toxic effect belong to the classes of gnetophytes and conifers. All organs of conifers contain resinous substance of a terpenoids nature. Damage to human's health is caused when chemical and mechanic treatment of wood is carried out.

Table 9

Typical representatives of the toxic angiosperms plants in the Republic of Belarus

Toxic organs	Phytotoxins	Intoxication symptoms
***Solonaceae family* Black Nightshade (*Hyoscyamus niger* L.)**		
Leafs and green fruit	Solanine (alkaloid) – irritates mucous membranes of the gastrointestinal tract, depresses activity of the central nervous system.	Abdominal pains, sickness, vomiting, cardiovascular collapse. In hard cases – comatose state.
***Solonaceae family* Jimson weed (*Datura stramonium* L.)**		
The whole plant and its seeds are toxic	Atropine, hyoscyamine, scopolamine (alkaloids) – irritate mucous membranes of the tract, depress activity of the central nervous system.	Dry mouth, aglutition, bloody diarrhea, dysfunction of the central nervous system (disorder of orientation and short-term memory, widened pupils).
Toxic organs	**Phytotoxins**	**Intoxication symptoms**
***The Poppy family (Papaveraceae)* Opium poppy (*Papaver somniferum* L.)**		
The whole plant is toxic, maximum - milky sap in the walls of the green bolls	More than 20 alkaloids. Morphine – analgesic, has a strong pain-relieving effect, but when repeatedly taken drug addiction is developing. papaverine has a spasmolytic and vasorelaxant action. Thebaine and protropin – convulsive poisons.	Sickness, vomiting, urinary difficulty, giddiness. Hallucinatory obscuration of consciousness is developing. Respiratory depression down to the complete cessation of breathing.

End of tabl. 9

Buttercup family (Ranunculaceae) **Cursed buttercup (*Ranunculus scleratus* L.)**		
Overground part	Lactones and flavonoids. Lactone protoanemonin has local irritating and narcotic action.	Sap from the leaves causes burns of skin and mucous membranes. If gets into the eyes – temporary blindness "night blindness". If gets into the body causes plentiful salivation, sickness, vomiting.
Thymelaeaceae family **February daphne (*Daphne mezereum* L.)**		
Bark, leaves, flowers and fruits	Terpenoid mezerin (strong irritating effect on the skin and mucous membranes) and cumarins (cause high bleeding tendency).	Contacts with skin cause dermatitis; if it gets into human body acts as hemorrhagic gastroenteritis
Ericaceae family ***Marsh Labrador tea* (*Ledum palustre*)**		
Overground part	Essential oil (cymene) causes inflammation of the gastrointestinal tract, depresses activity of the central nervous system.	Weakness, drowsiness , sickness, vomiting, lowering of the arterial blood pressure, tachycardia.
Cannabaceae family ***Cannabis sativa* L.**		
Tops of female plants, flowers and seeds are toxic	Dibenzopiran derivative (narcotic substance);	In case of long-term usage of hemp preparations (hashish, marijuana) – grave functional and mental disorder, dementia and disintegration of personality
Parsley family (Apiaceae) ***Sosnowsky's Hogweed* (*Heracleum sosnowskyi*)**		
The whole plant is toxic, maximum during generative phase	Alkaloids, triterpenoid saponin, flavonoids, furanocoumarins. Furanocoumarins possess the photodynamic activity, increasing sensitivity of skin to UV rays.	Hogweeds sap if contacts skin causes dermatitis that is similar to the sunburn. In grave cases second-degree burn is developing, there's also shivering, giddiness, fever.

First aid and preventive measures in case of phytotoxins intoxication:

- artificial vomiting;
- taking purgative;
- gastric lavage by the water suspension of absorbed carbon or 0,1% solution of potassium permanganate;
- taking neutralizers (soda, sour drinking) and enveloping substance (starch, egg-white, milk);
- through analyzing undigested remains detect the reason of intoxication;
- call for qualified medical aid.

In some cases milk, fats, acidic or soda solutions are contra-indicated.

Prevention of phytotoxins intoxication:

- do not eat unknown plants and use them for self-treatment;
- raise the level of ecological culture of people;
- mount preventive notices and barriers for livestock on the plantations of the poisonous plants;
- do not grow very toxic plants as decorative in the inhabited localities.

LITERATURE

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. *Medical* biology for international students 1st year : lecture course / V. E. Butvilovsky [et al.]. Minsk : BSMU, 2015. 141 p.

3. *Medical* biology for international students: учеб.-метод. пособие / В. Э. Бутвиловский [и др.]. Минск : БГМУ, 2016. 222 с.

4. *Медицинская* биология и общая генетика / Р. Г. Заяц [и др.]. 2-е изд. Минск : Выш. школа, 2012. 496 с.

CONTENTS