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MEDICAL BIOLOGY

TEXT BOOK

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CYTOLOGY

CHAPTER 1

Biology as a Science. Development of Biology. Characteristics of life. Life organization levels

Biology as a Science

Biology is a science about life. This life science studies the structure, growth and development, reproduction, heredity and variation, ontogeny and phylogeny of living organisms. Biology is considered as the basics of medicine, and is highly important for future doctors. It is a multidisciplinary science that involves subjects like anatomy, physiology, biochemistry, biophysics etc.

The term "BIOLOGY" was proposed by J. B. Lamarck in 1802. Development of Biology as a science started from ancient times by Aristotle, Hypocrite, who studied the morphology, reproduction and development of organisms. In medieval period the church ruled the scientific development and turned it to metaphysical concepts: everything is created by God and cannot undergo evolutionary changes.

The renaissance period was announced as progressive time of Biology as a science.

1665 Robert Hooke discovered cells by invention of simple microscope.

1839 Theodor Schwann and Mattias Schleiden proposed cell theory.

1859 Charles Darwin presented principles of evolution.

1865 Gregor Mendel formulated main laws of Genetics.

1953 Watson and Crick discovered the molecular structure of DNA that launched development of molecular genetics.

In 1970s discovery of methods of genetic engineering provided the development of DNA technology: cloning and gene therapy.

Living organisms. Characteristics

In regard to modern concepts, the life or living substance is a complex of two biopolymers (nucleic acids and proteins), which are bound to other chemicals and thus form a reproducing, regulated and an ordered open system, which realizes directed flow of materials and energy under certain environmental conditions. All the living organisms are characterized by following features:

1. Ordered structure. The living organism is a living whole, unity of discrete parts, which have a perfect order. They are organized in ***hierarchic pattern,*** which is the arrangement of organization levels in increasing order, and each level of biological structure is belt on the level below. For example, the cell is a higher level for organelles, and it is a sublevel for a tissue. The hierarchic organization of an organism is as follows: atom, molecule, organelle, cell, tissue, organ, organ-system, organism. The living organisms are ***unite*** on one hand and ***discrete*** on the other.

2. Metabolism. It is the flow of energy and cycling of material between living organisms and environment.

Metabolism consists of two opposite pathways: ***anabolism*** and ***catabolism***. Anabolism is characterized as synthetic reaction when simple molecules make up a more complex one, and this process requires energy, e.g., synthesis of glycogen polymer from glucose monomers. Catabolism is

a breakdown reaction, in which complex substances are broken to simple ones, and energy is released, e.g., breakdown of glycogen into many glucose monomers in the liver and muscle cells to provide normal amount of glucose in blood.

All the metabolic reactions are performed by biologically active proteins – ***enzymes***. There are as many enzymes in the organism as many reactions, since every enzyme is specific to the substrate it binds to. Activity of an enzyme depends on the temperature, pH of the medium as well as concentration of the substrate.

The organism is an ***open system***. This means that energy and the matter can be transferred between the living organism and surroundings. For example, an animal obtains starch, proteins and other complex molecules through food. As catabolic pathways break these molecules down, the animal releases CO_2 and H_2O to environment, and the energy generated is used for the needs of the organism. Every energy transformation makes the universe more disordered. Scientists use a quantity called ***entropy*** as a measure of disorder, or randomness. The more random a matter is, the greater its entropy. In contrast to non-living matter, the living organisms counteract to the increase of entropy. The energy generated during catabolism is distributed through the body unevenly, so that the organ in work is more intensively supplied with energy. The cells create ordered structures from less organized starting materials. The entropy of an open system (organism) may decrease so long as the total entropy of universe increases, thus ***negative entropy*** is common for the living organisms.

3. Reproduction. It is the ability of organism to produce a copy of own kind to continue the species. On molecular level reproduction is common for DNA, which is the only molecule that can copy itself. This is known as DNA replication. Cell reproduction is performed in cell division (mitosis).

4. Repairing (regeneration). It is the ability to repair the damaged cells or tissues. The mutated molecule of DNA can also be repaired. Tissue repair is known as regeneration.

5. Regulation. The organism continuously adjusts to the always changing environment, otherwise it would undergo abnormal changes or even die. Relative stability (constant condition) of the internal environment in the body is called ***homeostasis***. Regulatory mechanisms maintain homeostasis within tolerable narrow range even though the environment can alter severely. The control is provided by humoral (*humor* – liquid, i.e. blood) and neural regulations. Hormones are biologically active substances than are produced in glands and provide humoral regulation through blood circulation. Neuromediators (messengers of neural signals) like acetylcholine and noradrenaline, provide neural regulation which spread through nerve endings to the target organ.

The regulation on molecular level is realized by ***feedback*** mechanism. This is of two types – negative and positive. In negative feedback the changes in regulated and regulating substances have opposite directions: excessive amount of regulated substance inhibits synthesis of the substance which regulates it, and the low level of regulated substance stimulates increase of regulating substance. For example, when the level of blood thyroxin (regulated substance) falls then the production of thyroxin stimulating hormone (TSH) in pituitary is elevated. In positive feedback (less common in living systems) the changes in the regulated and regulating substances have the same directions, e.g., increase of blood sugar leads to increase of insulin synthesis by pancreas cells, and low blood sugar leads to decrease of insulin synthesis.

6. Response and motion. Response is the ability of an organism to react the external stimuli. It is usually expressed in motion. Response of unicellular organisms is known as **taxis** (positive –

towards the stimulus; negative – opposite to stimulus). Response in multicellular organisms with neural system is known as **reflex.**

7. Heredity and variation. Heredity is the transmission of traits through generations. The carrier of genetic information is the DNA molecule, which is replicated and transmitted to daughter cells. Messenger RNA transfers the genetic message to realize expression of genetic information. Along with inherited similarity, there is also variation – ability of organisms to acquire new traits during life span. This helps them to increase adaptability.

8. Growth and development. Growth is characterized as increase of body weight and sizes. It is provided by cell division (mitosis). Development of an organism occurs while it grows up. The individual development of the organism, from fertilization till its death, is called ***ontogeny***. The growth of an organism can occur either through whole life span (undefined or unlimited), e.g. in fish, plants, or it can be limited (defined) during certain period of life (e.g., mammals).

9. Adaptation. It is the ability to get used to new environment conditions. Life evolves as a result of interaction between organisms and their environment. Organisms are open systems that interact continuously with environment and adjust to it. If not adapted, the homeostasis of the body is disrupted and the organism may die.

Life organization levels

Life is hierarchically organized into structural levels, with specific properties resulting from specific structure at each level. Three main levels of life organization are differentiated: biological microsystem, mesosystem and macrosystem. Biological microsystem includes molecular, subcellular and cellular sublevels. Mesosystem constitutes are tissues, organs, organ-systems and organism. Biological macrosystem consists of population, community, ecosystem, Biosphere. Each sublevel has its elementary unit and elementary phenomenon, which provide development of life in the given sublevel.

Biological microsystem. The elementary unit of *molecular-genetic level* is the gene. DNA is the chemical substance of gene, which is the unit of inheritance that transmits information through generations of offspring. DNA replication is the phenomenon of molecular-genetic level. At this level the living organisms are similar – proteins of living organisms are made up of amino acids, their DNA and RNA carry the same type of nucleotides (triplets), and the energy source is the ATP. The molecular level is studied by biochemical and X-ray analysis methods.

Subcellular level is presented by cell organelles. Structural organization of cells is different at this sublevel. Eukaryotes have nucleus, membranous and non-membranous organelles. Prokaryotes are missing these structures. Subcellular level is studied by electron microscope.

All organisms are composed of cells, which are the structural and functional units of life. *Cell* is the elementary unit, and *metabolism* is considered as a phenomenon of *cellular level.* On this level the plant and animal cells are distinguished. The plant cells are autotrophic: they convert the solar energy to chemical energy, synthesizing organic substances from non-organic ones (water and carbon dioxide). Animal and fungi cells are heterotrophic, they acquire energy through the plant and animal food. This level is studied by cytological methods.

Biological mesosystem. The constitutes of mesosystem are tissue, organ, organ system and organism. Cells with similar structure and function are grouped into tissues. Tissues arise due to increase of cell number and their differentiation. The animal tissues are: connective, epithelial (covering), muscle and neural. The tissue sublevel is studied by histological methods.

The organ sublevel is studied by morphological methods. The organs are formed by different tissues, they have certain location in the body, specific structure and function.

The elementary unit of organism sublevel is the individual organism. Individual development of an organism is known as *ontogeny*, which is considered as the phenomenon of this sublevel.

Biological macrosystem consists of population, community, ecosystem and biosphere sublevels. Population is a group of organisms belonging to the same species that occupies a given area. ***Population*** on its own is the elementary unit, and evolutionary *change of the population gene pool* (set of genes of a population of a particular species) is considered as a phenomenon of this sublevel.

Populations of different species living in the same area make up a biological ***community***. Interactions within community (living things or biotic factors) with non-living (abiotic) features of the environment, such as soil, radiation, humidity, concentration of oxygen and carbon dioxide, etc form an ***ecosystem***, e.g., a forest or lake.

Biosphere is the ultimate level of all the systems of living organisms and ecosystems. The biosphere is the entire sphere of the Earth including all the ecosystems (aquatic, terrestrial and air) that are inhabited by living organisms. The phenomenon of biosphere is the *evolution*.

Light microscope

Modern microscopes use two magnifying lenses. The microscope that magnifies the object by using several lenses is called **compound microscope**. The specimen is mounted on a slide glass and positioned on the flexible **specimen stage** of the microscope. The light source (sun light or lamp) is focused by a **condenser lens** onto the stage holding the specimen. Light from the specimen is passes through the **objective lens**. The image on the objective focal plane is magnified by the **ocular lens,** or **eyepiece**. The total magnification is a product of the magnification of all the lenses: if the lens magnifies 100x and the eyepiece magnifies 10x, the final magnification will be 1000x. Along with magnifying ability the microscope is characterized also by resolution power. The **resolving power** is the ability of microscope to produce a clear image (differentiate the distance between closest points). The light microscope cannot produce an image of an object that is smaller than the length of the light wave. The value for the resolution of a light microscope is 200 nm (for electron microscope it is 0.5 nm).

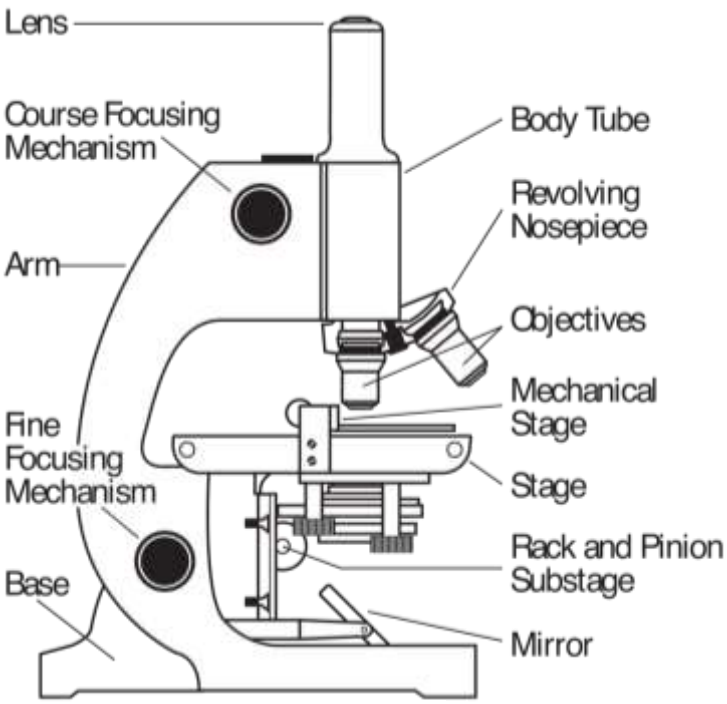


Fig. 1. Light microscope

The compound microscope consists of three parts: mechanical, optical and light parts.

Mechanical part consists of: base, stand, ocular tube, revolver (nosepiece).

The light part is presented by mirror or lamp illuminator, iris diaphragm, condenser. The stand is attached to the base and mechanical stage, and two focusing knobs (for coarse focus and fine

focus adjustment) are fitted to the stand. The amount of the light is controlled by opening or closing the diaphragm, changing the position of condenser as well as by the mirror (concave surface collects light, and convex surface disperses the light).

The optical part is presented by different lens systems involved in the eyepiece and objectives. Eyepiece is fitted into the ocular tube, it may have various magnifications that depend on the model of microscope. For example, some may have eyepieces magnifying 7x, 10x, 15x. There are few types of objectives of various magnifications, which are held and rotated in the revolver: low magnification objectives (4x, 8x, 10x, 20x), high magnification objective (40x), oil-immersion objective (90x) used with cedar wood oil.

IA

1. Elementary phenomenon of life organization on cellular level is:

A. Stable condition of cell structure
B. Cell organelle
C. Energy flow
D. Energy and material cycling

2. Elementary phenomenon of life organization on population level is change of:

A. Mutation
B. Gene pool
C. Genotype
D. Individual organism

3. Living organisms are represented as:

A. Closed system
B. Open system
C. Mixed system
D. System promoting entropy to increase

4. Ability of living organisms to react to the external or internal stimuli is called:

A. Adaptation
B. Homeostasis
C. Entropy
D. Response

IB

1. Molecular level is not studied by the:

A. Physical-chemical method
B. Biochemical method
C. Microscopic method
D. Roentgen-structural method

2. Self-reproduction as a property is not typical for:

A. DNA molecules
B. Cells
C. Proteins
D. Organisms

3. Response of single-cell organisms is not manifested by means of:

A. Taxis
B. Positive taxis
C. Reflex
D. Negative taxis

4. Subcellular level is not constitutes from:

A. Cell ingredients
B. Membranous organelles
C. Non-membranous organelles
D. Cells

5. The following is not characteristic for living substance:

A. Response
B. Self-regulation
C. Self-reproduction
D. Correct answer is absent

II

1. The following is typical for living substances:

1. Self-reproduction
2. Self-regulation
3. Increase of entropy
4. Self-repair
5. Constant entropy

A. 1,2,4 B. 1,3,5 C. 1,4 D. 2,5

2. Biological microsystem levels are:

1. molecule
2. subcellular
3. organ
4. cellular
5. tissue

A. 1,3,5 B. 1,2,4 C. 2,3 D. 1,4

3. Characteristics of life in organism level are:

1. heredity
2. strict diversity of discrete parts
3. tissue formation and differentiation
4. ontogeny
5. complex interactions between discrete parts

A. 1,2 B. 2,3 C. 2,4,5 D. 3,4,5

4. What processes take place during catabolism?

1. Protein synthesis
2. DNA replication
3. Organic substances breakdown
4. Energy release
5. Participation of enzymes

A. 1,2 B. 3,4,5 C. 1,3,5 D. 2,4

5. The metabolic reactions are:

1. Strictly determined
2. Regulated
3. Interrelated
4. Not mutually interrelated
5. Not determined

A. 1,2,3 B. 2,3,5 C. 1,4,5 D. 3,5

CHAPTER 2
Chemical composition of cell

Cell Organic Ingredients

Structural and chemical similarity of different cells proves the concept of their common origin. Most of the chemicals that make up living organisms are based on the element *carbon.* Compounds containing carbon are said to be organic.

Proteins. The importance of proteins is implied by their name, which comes from Greek "*proteios*", meaning "at first place". All proteins consist of C, H, O, N atoms. Some proteins can have also S, Fe, Zn, Cu atoms. Protein amount for more than 50% of the dry weight of most cells, and they have big molecular mass.

Proteins are made up from monomers called **amino acids**. Amino acids are organic molecules possessing both carboxyl (COOH) and amino groups (-NH_2). At the center of the amino acid is an asymmetric carbon atom. Its four different partners are an amino group, a carboxyl group, a hydrogen atom, and a variable group symbolized by ***R,*** also called **radical** or **side chain**. There are 20 types of amino acids that may build up millions of proteins. Amino acid subunits are linked covalently by *peptide bonds.* Such bonds are the result of a condensation reaction (one molecule of water is released) between the carboxyl group of one amino acid and the amino group of the other one. The unit of two joined amino acids is called a *dipeptide,* and the polymer of amino acids is called a *polypeptide*.

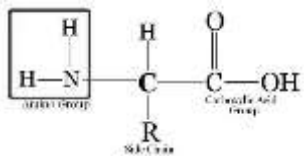


Fig. 2. Amino acid structure.

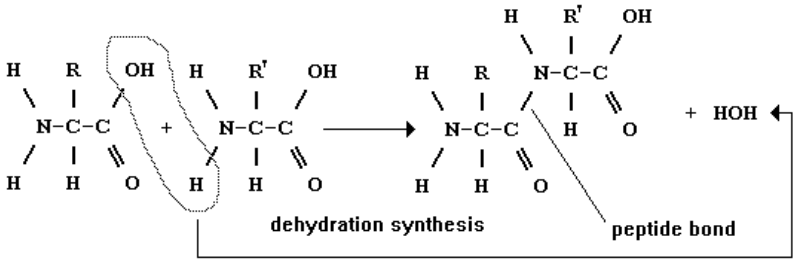


Fig. 3. Formation of peptide bond between two amino acids.

The proteins differ in the sequence, set and number of their amino acids. Millions of different proteins are possible to build up by various set, sequence and number of amino acids. So it is the proteins that provide the diversity of life (living organisms). Each species has its specific proteins, and a protein of one species serves as an antigen for another species organism.

The function of the protein closely deals with its amino acid structure and sequence in the polypeptide chain. The amino acid sequence in polypeptide chain is considered the **primary structure** of protein.

Secondary structure is provided by hydrogen bonds between different amino acids of the folding chain (between NH group of one and CO group of another). Hydrogen bonds are weak, but high amount of such bonds can support particular shape of the protein (***alfa-helix*** or ***beta-sheet***).

Tertiary structure of protein is constructed by various interactions between different chemical groups: hydrogen bonds, hydrophobic interactions, ionic and disulfide bonds. Amino acids with hydrophobic side chain usually congregate in clusters at the core of the protein, out of contact with water. The conformation of a protein may be reinforced further by strong, covalent

bonds called disulfide bridges (S-S) formed between sulfur-containing amino acids. Ionic bonds can be formed between charged radicals.

Some proteins have also a **quaternary structure** made by integration of several polypeptides and/or non-proteinous components (e.g., hemoglobin consists of 4 polypeptide chains each having a hem group with iron).

The three-dimensional conformation of a protein can be either fibrous (thread-like), e.g. keratin, collagen, or globular (spherical), e.g. hemoglobin.

Protein conformation depends on physical and chemical factors like pH, temperature. These agents may alter the native conformation of protein, and this is known as ***denaturation***. Denaturation can be reversible (primary structure of protein is not changed) and irreversible (peptide bonds are broken).

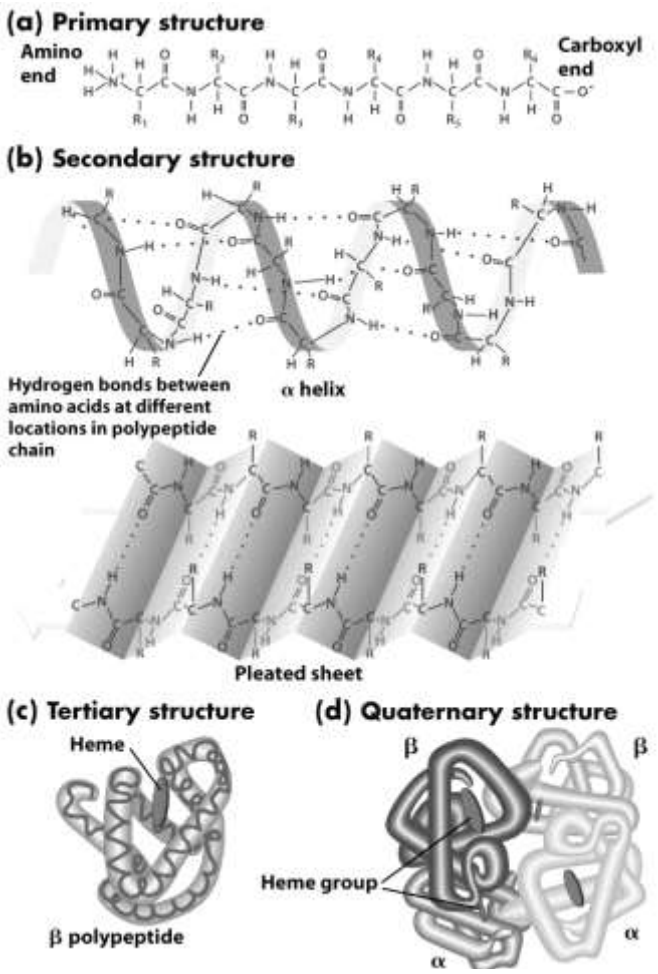


Fig. 4. Protein folding.

Functions of Proteins

1. **Structural function** concerns the proteins that are included in the membrane structures of the cell (plasma membrane, nuclear membrane and membranous organelles), tissues (collagen is a protein of connective tissue), chromosomes (histone proteins).
2. **Catalytic function.** Enzymes are catalytic proteins that speed up the rate of reaction without being consumed by the reaction. Catalytic activity of the enzyme is provided by its **active center**. Catalytic activity of many enzymes requires non-protein helpers called ***co-enzymes***. The helpers can be organic molecules (e.g., vitamins) or metal ions (Zn, Co, Fe). The active center may contain also. In enzymatic reactions, the substrate binds to the active site to form an enzyme-substrate complex (key-to-lock matching). Enzyme activity can be affected by environmental factors like temperature and pH. There are as many enzymes in the organism as many reactions, since every enzyme is specific to the substrate.
3. **Regulatory function.** Hormones can be biologically active proteins, which regulate different functions of the organism. For example, insulin regulates glucose metabolism, growth hormone provides growth of the body.
4. **Signaling.** This is performed by membrane proteins called receptors. Reversible denaturation of the receptors under chemical or physical influence stimulates signal reception and transduction into the cell.

5. **Contractile function.** The proteins of cilia and flagella (*tubulin*), as well as of muscle cells (*actin* and *myosin*), help in motility. Interaction between actin and myosin provide contraction of muscle cells. Cilia and flagella are made of tubulin protein, which forms microtubules by polymerization.
6. **Transport function**. Some proteins transfer chemicals. For example, hemoglobin transfers oxygen, carbon dioxide in blood. Transport proteins of plasma membrane provide selective transport of various chemicals.
7. **Immune protection.** In response to antigens (foreign proteins of viruses, bacteria) the cells of immune system (B-lymphocytes) produce specific proteins called antibodies. Antibodies specifically bind to antigens and neutralize them. This mechanism of immune protection is known as humoral immunity.
8. **Energy source.** In some conditions (when lipids and carbohydrates are expired as primary source of energy) proteins may generate energy (1g protein produces 17.6 kJ energy). The proteins break down completely first to amino acids, and then – to CO_2, water, nitrogen wastes.

Carbohydrates are organic substances that consist of carbon (C), hydrogen (H) and oxygen (O). Carbohydrates account for about 1% of the dry weight of animal cells, and about 5% in liver and muscle cells. The simplest carbohydrates are the ***monosaccharides***, single sugars also known as simple sugars. ***Disaccharides*** are double sugars, consisting of two monosaccharide monomers. ***Oligosaccharides*** (*oligo* - few) consist of about 20 monosaccharide monomers. The ***polysaccharides*** are polymers of many sugars.

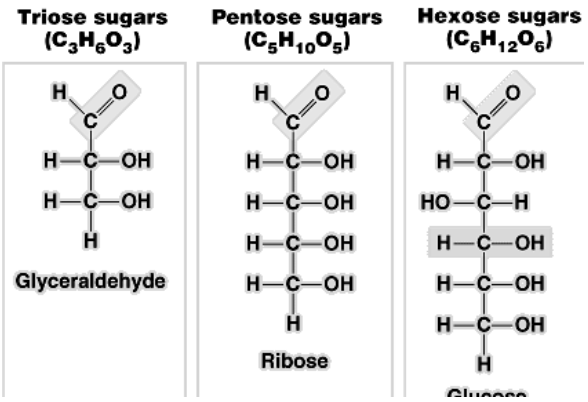


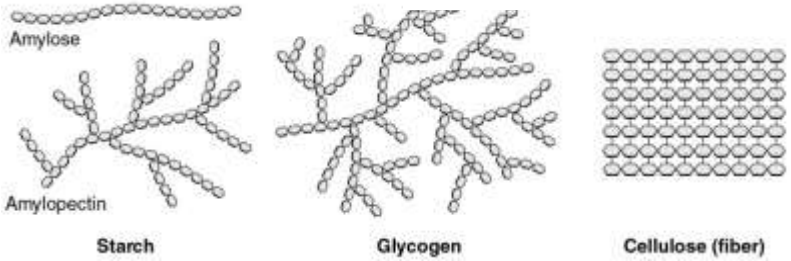
Fig. 6. Monosaccharides.

Monosaccharides are well soluble in water and have a sweet taste. General formula is $C_n(H_2O)_m$. Depending on number of carbon atoms the monosaccharides are: trioses (3 carbon sugars), tetroses (4 carbon sugars), pentoses (5 carbon sugars), hexoses (6 carbon sugars). Ribose and deoxyribose are pentose sugars with structural function (they are involved in RNA and DNA, respectively). Glucose, fructose and galactose are hexose sugars that serve as source of energy. One gram of sugar generates 17.6 kJ energy.

Disaccharides are sucrose (glucose+fructose), lactose (glucose+galactose) and maltose (glucose+glucose). These saccharides also have energetic function. They break up into monosaccharides and are utilized by cells.

Oligosaccharides (*oligo* - few) are found in the structure of glycocalix in animal cells. They are involved in glycoproteins and glycolipids.

Fig. 7. Polysaccharides.



Polysaccharides are starch, cellulose and glycogen. They arise via

polymerization (condensation) of monosaccharides. Solubility and sweet taste of polysaccharides decreases along with increased number of monomers. Glucose is the monomer of these three polysaccharides.

Starch is a storage polysaccharide of plants. Cellulose makes the structure of plant cell wall; it is not soluble in water and is not branched. Though cellulose is not digested in human intestine, it stimulates production of mucus in intestines and provides faster evacuation of the bowel. Glycogen structurally resembles the starch but is more branched. It is a storage polysaccharide in animal cells (liver, muscles, heart). Hydrolysis of glycogen releases glucose on demand.

Lipids are a group of organic substances which are not solved in water (hydrophobic) and are solved in organic solvents (lipophilic). Hydrophobic behavior of lipids is based on their molecular structure. The most important families of lipids are **fats and oils, phospholipids**, **steroids**.

Fats make about 5-10% of the dry weight of animal cell, however some cells (subcutaneous layer) can contain about 90% fat. The structure of fat is made by glycerol and 3 fatty acids (*triglycerid*). A fatty acid has a long carbon skeleton (16-18 atoms length). The fatty acids in animal fat molecule have no double bonds between carbons, and are called *saturated*. This makes the animal fats solid at room temperature. The fats of plants and fish are usually liquid at room temperature and referred to as oils. The fatty acids in oils are *unsaturated* (have double bonds).

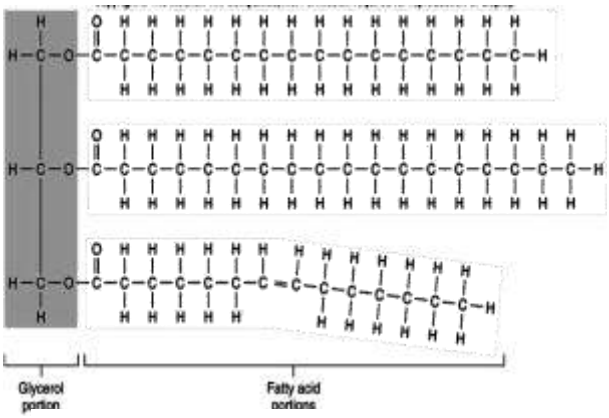


Fig. 8. Triglycerid.

Fats have important roles:

a) They are a rich source of energy and provide about 20-30% of energy in the cell. From 1g of fat 38.9 kJ energy is generated.

b) Subcutaneous lipid layer is an effective thermoregulatory barrier and prevents from cold.

c) Fats are considered as water generation source – 1kg fat oxidates into 1.1kg water. That is why many desert animals can survive for long time without water intake.

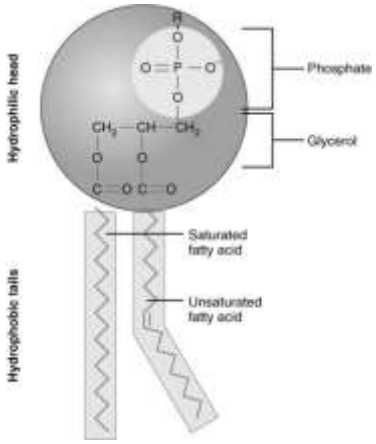


Fig. 9. Phospholipid.

Phospholipids consist of glycerol connected to phosphate group and 2 fatty acids. The glycerol along with phosphate group form the hydrophilic (lipophobic) head of the phospholipid, and fatty acids make the hydrophobic (lipophilic) tails. Significance of phospholipids is their structural role: they are the major component of all the biological membranes (cell membrane, nuclear membrane, membranous organelles).

Steroids. Steroids are characterized by carbon skeleton consisting of four fused rings. Such important substances as *cholesterol*, fat-soluble vitamins (A, D, E, K vitamins), sex hormones (testosteron, estrogen, progesteron), cortisol (hormone of

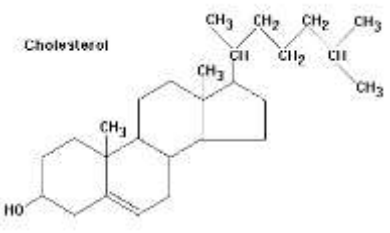


Fig. 10. Cholesterol.

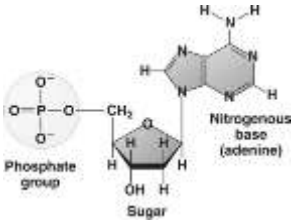
adrenal gland) have steroid structure.

Cholesterol is the precursor from which other steroids (hormones and vitamins) are synthesized. It is also a structural component of animal cell membrane and gives stability to it. Thus, cholesterol is an essential substance in animals, although a high level of it in blood may contribute to ***atherosclerosis*** (*atheros* – fat*, sclerosis* – stiffening).

Nucleic acids are informational polymers that store and transmit hereditary information. Firstly, Swiss scientist Frederic Miescher has isolated them from nucleus in 1869. There are two types of nucleic acids: DNA and RNA. DNA resides in the nucleus, mitochondria, and plastids. The amount of DNA in the cells is constant. Human somatic cells contain 6,6 x 10^{-12} g DNA, and germ cells – 3,3 x 10^{-12} g.

James Watson and *Francis Crick* first proposed the double helix model as three-dimensional structure of DNA in 1953 and were awarded a Nobel prize.

DNA molecule consists of two helically twisted strands (double helix). The width of the macromolecule is 2 nm. Each strand of deoxyribonucleic acid is a polymer of monomers called **nucleotides.** Each nucleotide is itself composed of three parts: nitrogenous base, a pentose – deoxyribose, and a phosphate group. There are two families of nitrogenous bases: double-ring **purines** and single-ring **pyrimidines.** The members of purine family are adenine (A) and guanine (G). The pyrimidines are cytosine (C), thymine (T), and uracil (U). Adenine, guanine, cytosine are found in both types of nucleic acids (DNA and RNA), thymine is found only in DNA, and uracil – only in RNA. A nitrogenous base is joined to number 1′ carbon of sugar, and the phosphate group is attached to number 5′ carbon of the pentose. In polynucleotide, nucleotides are joined by covalent bonds called phosphodiesther linkages between the sugar C3′ carbon of one nucleotide and the phosphate of next (sugar-phosphate backbone). The sequence of nucleotides joined by phosphodiester bonds makes the *primary structure* of DNA.



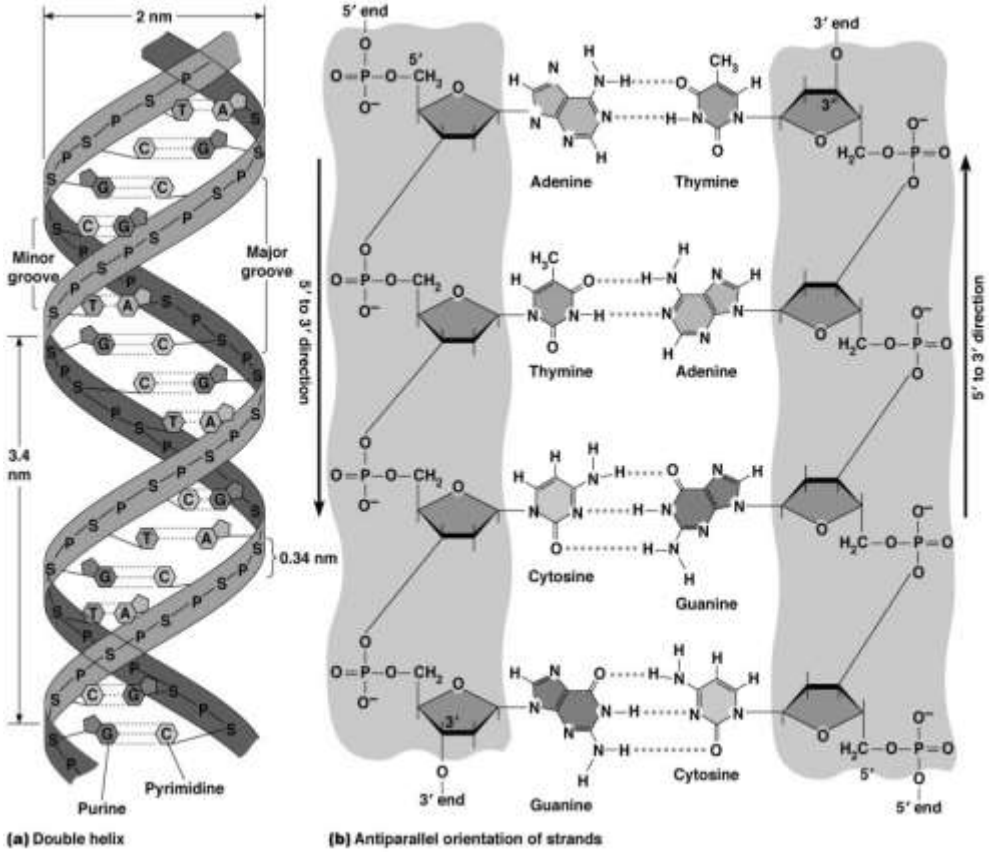


Fig. 11. Nucleotide and DNA.

The nitrogenous bases are paired by hydrogen bonds, by which the *secondary structure* of DNA is formed. Only certain bases in the double helix are compatible with each other, A always pairs with T (2 hydrogen bonds), and G – with C (3 hydrogen bonds). The two strands of the double helix are ***complementary,*** each the predictable counterpart of the other. If we were to read the sequence of bases along the one strand as we traveled the length of double helix, we would know the sequence of bases along the other strand. In addition, two strands of DNA are also ***antiparallel*** (C3′ end of one strand faces C5′ of second strand). Complementarity and antiparallelism of two strands are important prerequisites for DNA replication.

The *tertiary structure* of DNA is presented as a double helix molecule.

According to ***Chargaff's*** rules, in DNA:

1. A=T, G=C or A/T=1 and G/C=1
2. G+T=A+C or (G+T)/(A+C)=1
3. (A+T)/(G+C)=constant for each species (1.53 for human).

RNA is a single stranded polynucleotide. The pentose sugar is the ribose, it contains uracil (U) instead of thymine (T). Quantity of RNA in the cells is not constant. It increases during synthesis of proteins (translation). RNA synthesis on DNA template is known as transcription. There are three types of RNA:

Transport RNA (tRNA) transfers amino acids to the ribosomes during protein synthesis. The primary structure of tRNA is a sequence of about 80 nucleotides. The secondary structure is formed by hydrogen bonds between complementary nucleotides within this strand so that it resembles a clover leaf. The loop protruding from one end includes the ***anticodon***, the specialized triplet that is complementary to the codon of mRNA associated with ribosome. The 3′ end carries CCA triplet, which is the attachment site for an amino acid and is called ***acceptor***. There are about 40 types of different tRNAs.

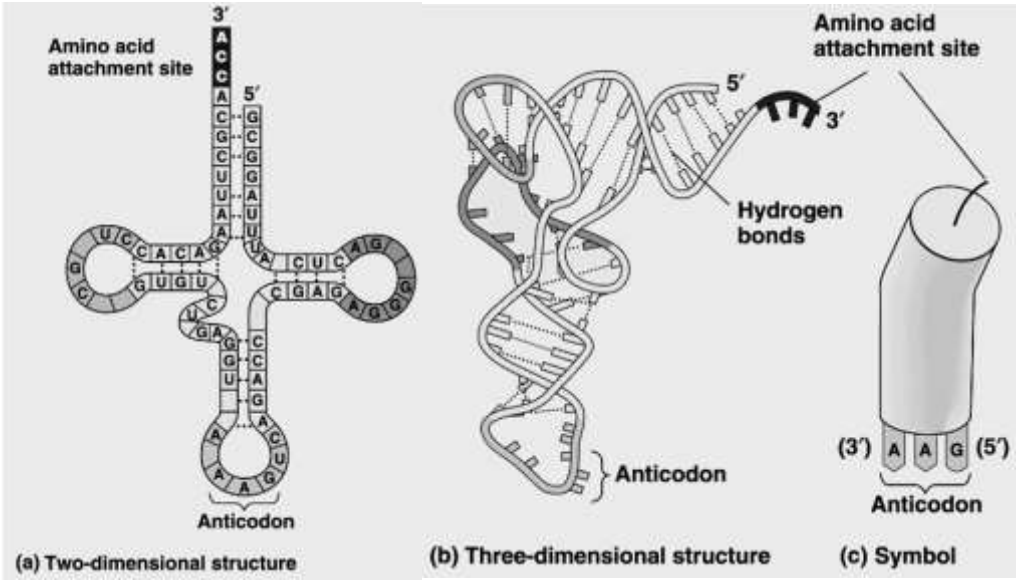


Fig. 12. Transport RNA.

Ribosomal RNA (rRNA) is a molecule about 3000-5000 nucleotides long, which is structural component of ribosome. Ribosome subunits containing rRNA are formed in nucleolus, and they leave to cytoplasm, where whole ribosomes are assembled during translation.

Messenger RNA (mRNA) carries the genetic message from nucleus (DNA) to cytoplasm for protein synthesis.

Thus, all three types of RNAs take part in translation process.

ATP (adenosine triphosphate) is the universal carrier of energy. The amount of ATP is especially high in muscles and nerve tissue. ATP is a nucleotide that has nitrogenous base adenine, pentose (ribose) and three phosphate groups. ATP, in contrast to nucleotides of nucleic acids, has a chain of three phosphate groups. The bonds between the phosphate groups (macroergic bonds) are not stable and can easily break. When the terminal phosphate bond is broken, a molecule of inorganic phosphate leaves the ATP, which becomes ADP (adenosine diphosphate). Breaking of second bond leads to AMP (adenosine monophosphate). These hydrolysis reactions release high amount of energy utilized by cells.

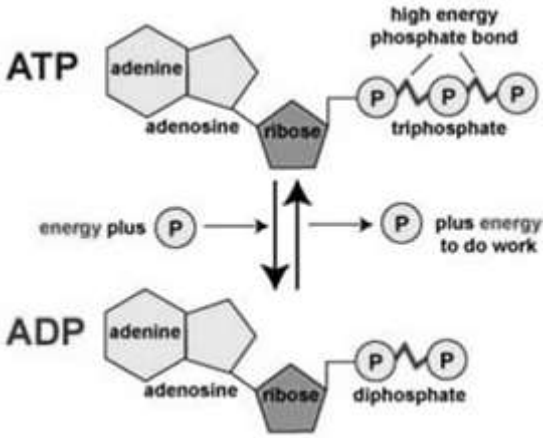


Fig. 13. ATP.

A

1. The polypeptide chain of protein is considered as:
A. primary structure
B. secondary structure
C. tertiary structure
D. quaternary structure

2. In reversible denaturation:
A. primary structure is changed
B. primary structure is not changed
C. peptide bonds are broken down
D. amino acids sequence is changed

3. In polynucleotide chain of DNA the nucleotides are joined together by:
A. hydrogen bonds
B. peptide bonds
C. phosphodiester bonds
D. hydrophobic interactions

4. Which of the followings is specific for a species?

A. A+G T+C	**B. A+T G+C**	C. T+G C+A	D. A+C

B

1. Which of the following is not included in DNA structure?
   A. thymine
   B. guanine
   C. uracil
   D. cytosine

2. Which of the following is not true about DNA?
A. the model of replication is semiconservative
B. the molecule is double helix
C. it is a polypeptide
D. two strands of DNA are antiparallel

3. What function is not common for protein?
A. structural
B. catalytic
C. storage
D. protective

4. Which of the following is not true about RNA?
A. the quantity in the cell is not constant
B. its amount increases during protein synthesis
C. it has the same nucleotides as DNA
D. it is a one polynucleotide chain

II

1. Which of the followings are monosaccharides?
1. ribose
2. starch
3. glycogen
4. cellulose
5. fructose
a. 1,3 b. 2,4,5 c. 1,5 d. 2,3

2. Lipids have the following functions:
1. structural
2. signaling
3. energetic
4. transport
5. contractile
a. 1,3,5 b. 3,4,5 c. 1,3 d. 4,5

3. The nucleotide may have:
1. purine base
2. amino acid
3. pyrimidine base
4. pentose
5. glycerol
a. 1,3,4 b. 2,3,4 c. 1,4,5 d. 2,5

4. There are following bonds in DNA molecule:
1. hydrogen
2. phosphodiester
3. peptide
4. disulfide
5. hydrophobic
a. 1,3,4 b. 1,2 c. 1,4,5 d. 4,5

CHAPTER 3
Genetic code. DNA replication. Transcription. Processing. Translation. Central Dogma of Biology

Genetic code

Information flow in the cells provides synthesis of important proteins for the organism and transfer of traits through generations of cells and organisms. Information is encoded basically in the nuclear, partly (10%) in mitochondrial and plastid DNA.

Three nucleotide units in DNA are transcribed into mRNA nucleotide triplets called **codons.** The genetic code is a triplet code, with codons of three bases coding for specific amino acids. Each triplet codon specifies only one amino acid, but an individual amino acid may be specified by more than one codon.

Properties of genetic code

1. Code is **triplet**: triplets of nucleotide bases are the units that can code for all the amino acids. If each arrangement of three consecutive bases specifies an amino acid, there can be 64 ($=4^3$) possible codes, except three codons (ATT, ATC, ACT in DNA, and UAA, UAG, UGA in RNA) that do not designate amino acids, they are "stop" codons (termination signals) or nonsense codons, marking the end of translation.

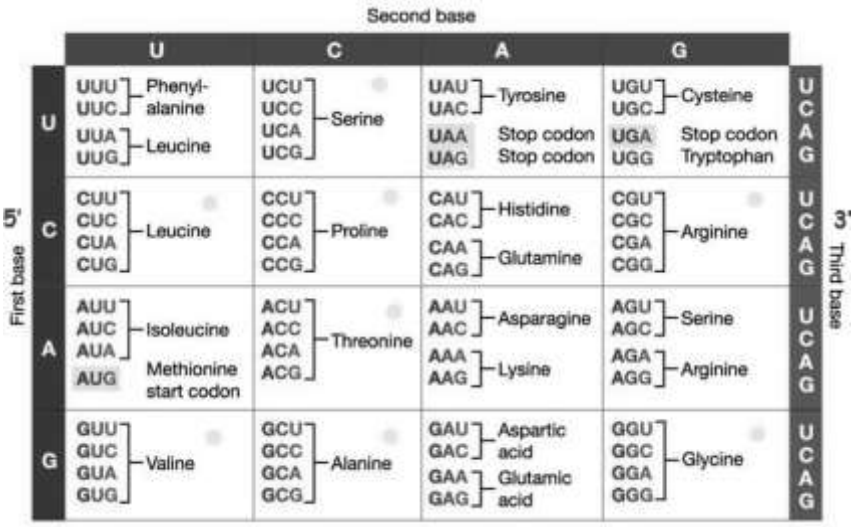


Fig. 14. Genetic code.

2. The code is **redundant**. There are 61 triplets coding for 20 amino acids: one to 6 triplets can code for each amino acid. This has antimutagenic significance, since in case of mutation of one of the triplets, there are still others that will code the amino acid, and the protein structure would not change.
3. The code is **specific**: each triplet codes for specific amino acid.
4. The genetic code is **universal** for all organisms, meaning that in every species the amino acids have the same codes.
5. The code is **non-overlapped**. Genetic information is encoded as a sequence of non-overlapping codons, each of which is translated into a specific amino acid during protein synthesis. Given nucleotide belongs only to given triplet.
6. The code is **continuous** or **not interrupted**: there are no spaces between codons.
7. The code has ***colinearity***, where the sequence of codons in mRNA corresponds to sequence of amino acids in polypeptide chain.

DNA sequences and repeats

According to frequency of nucleotide repeats in the genome (haploid set of chromosomes) there are following sequence of DNA:

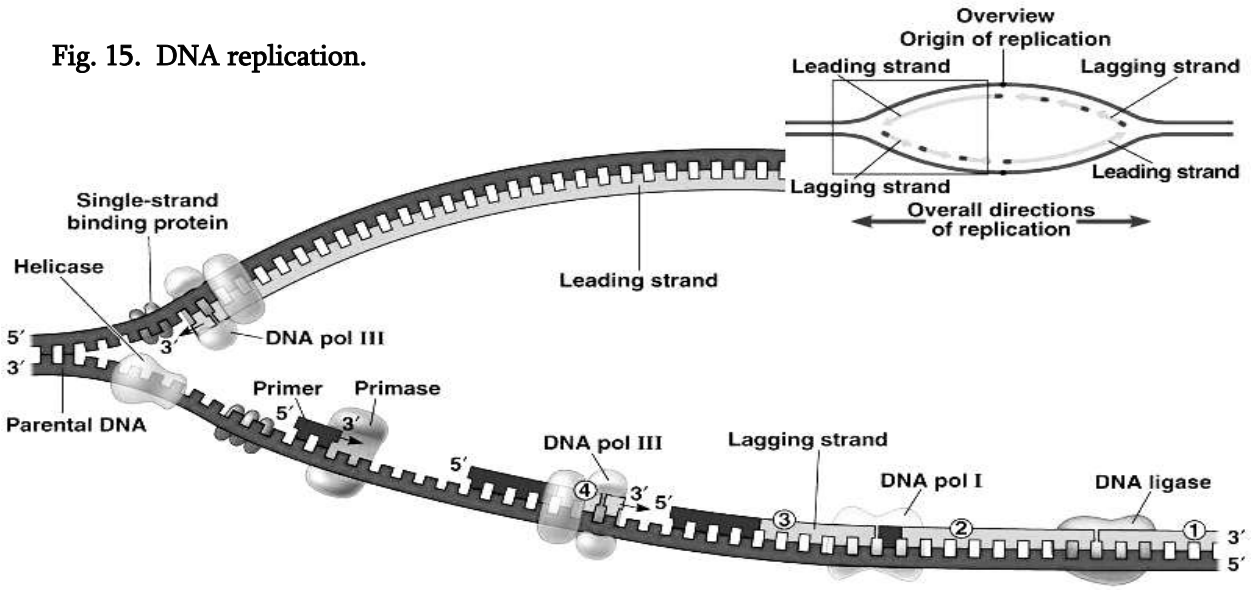
1. **Unique sequences** (structural genes). These sequences are presented only in a single copy and are known as unique sequences. They encode for various proteins (enzymes, hormones, etc) providing certain traits.
2. **Moderately repeating** sequences (constitutive or "house-keeping" genes). They repeat 100-10000 times and encode for histone proteins, rRNA and tRNA, enzymes of replication, transcription, translation. Several repeats prevent from expression of mutations of these genes (antimutagenic significance).
3. **Highly repetitive sequences**. Most of non-coding sequences (satellite DNA, and spacers – fragments between genes) consist of ***highly repetitive DNA***, nucleotide sequences that are present in many copies in a genome (10^4-10^6), usually not within genes. The repetitive DNA sequences are encountered at chromosomes telomeres and centromeres, suggesting that this DNA plays a structural role for chromosomes. The DNA at centromeres is essential for disjunction of chromatids during cell division and they may help organize the chromatin within the interphase nucleus. In addition, homologous chromosomes can be recognized by this region during meiosis. The repeated sequences of telomeres prevent the ends of chromosomes from sticking and, in addition, they prevent shortening of DNA after each round of replication.

Some of the repetitive DNA are not next to each other but are scattered about the genome. These sequences are mobile genetic elements – *transposons*.

DNA replication

The basic feature of DNA is **replication** that provides transfer of hereditary material from cell to cell, from one generation to the next. The fragment of DNA beginning from the origin of replication up to the end of replication is known as a **replicon** – the unit of replication. In prokaryotes there is just a single replicon (single DNA molecule), while in eukaryotes replicons are

Fig. 15. DNA replication.



thousands. This enables completing replication of huge eukaryotic genome in rather short period.

DNA replication takes place during S stage of interphase by the help of ***helicase***, ***topoisomerase***, ***DNA-polymerase***, ***RNA-primase***, ***DNA-ligase*** enzymes as well as single strand binding proteins (***SSBP***). The *helicase* that initiates the replication, recognizes and untwists the double helix by cutting the hydrogen bonds between complementary nucleotides. The Y-shaped region where the new strands of DNA are elongating is called a *replication fork.* To maintain the untwisted condition of the already untwisted single strands *SSBP* proteins*, or double helix destabilizing proteins* (they destabilize the double helix condition, and stabilize untwisted, single stranded condition), line up along the separated strands and prevent their rejoining. Along with separation of the double helix by helicase, supercoils appear ahead of the replication fork. These knots can hinder the further advancing of helicase. Therefore, a special enzyme known as *topoisomerase* cuts (binds reversibly to) the one strand ahead of helicase. The cut strand rotates around the second one, and thus the tension is released. The main enzyme of replication is *DNA-polymerase*, which adds a new nucleotide to 3-OH′-group of the previous nucleotide forming a phosphodiester covalent bond. Each added nucleotide is complementary to the one in the template strand. Peculiarity of the DNA-polymerase is that it cannot start the replication (attaching the first nucleotide) from the beginning, since it needs a free 3-OH′-group already bound to the template. This is provided by *primer,* which is an RNA-fragment (10 nucleotides). It is synthesized by *primase*.

DNA-polymerase is able to elongate the chain only in one direction – from 5′ to 3′ end of a new strand. It moves along the template as the replication fork progresses. The DNA strand made by this mechanism is called the **leading strand.**

To elongate the other new strand of DNA, polymerase must work along the template away from the replication fork and synthesize short segments of DNA called *Okazaki fragments* (1000-2000 nucleotides long in prokaryotes, and 100-200 nucleotides long in eukaryotes). Each of them is directed to the 5′ to 3′ end of the second new strand (analogous to the sewing method called back-stitching) and every time starts from a primer. Then all the primers are removed, and fragments are joined by *ligase*. This strand is referred to as a **lagging strand**. When the double helix replicates, each of the daughter molecules will have one old (parent) strand and one newly made strand. This is the ***semiconservative*** model of DNA replication.

Transcription

Transcription is the process of transfer of genetic information from DNA to RNA. Transcription is provided by RNA-polymerase enzyme and proceeds in three stages: *initiation*, *elongation* and *termination*. The stretch of DNA that is actually transcribed is called a *transcription unit* or *transcripton*.

1. Initiation. Transcription is initiated by binding of the RNA-polymerase to the *promoter* (start point located on 3′ end of template strand of DNA). This is enabled by transcription factor proteins first bound to the promoter. The template strand is known as antisense strand, and the second strand of DNA is known as sense or coding strand, since it carries the same “sense” of transcribing RNA.

2. Elongation. RNA-polymerase ***elongates*** an RNA molecule with a nucleotide sequence complementary to the template strand. Elongation of new nucleotides takes place in 5′ to 3′ direction.

3. Termination. Eventually the RNA polymerase transcribes a ***terminator*** that signals the end of transcription, i.e. termination. Thereafter the RNA is released, and polymerase dissociates from the DNA.

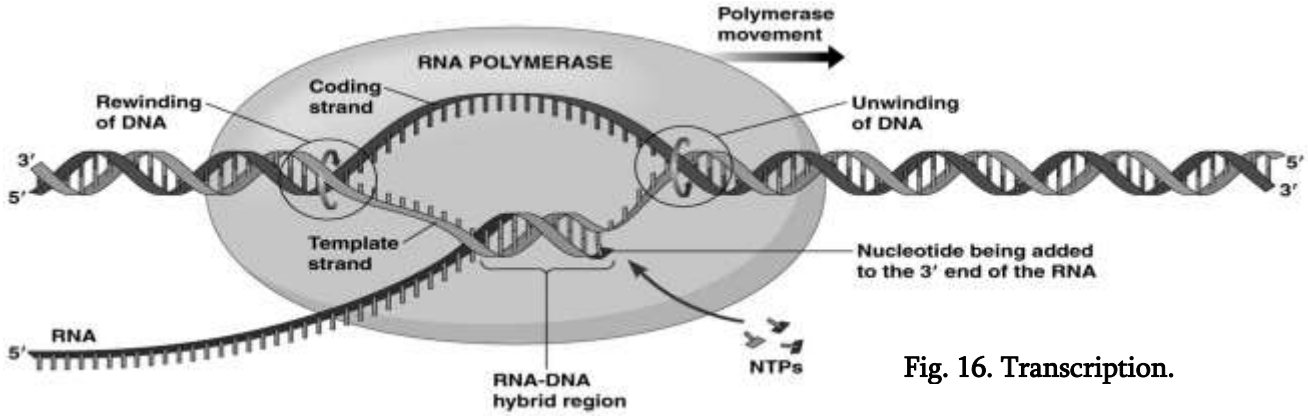


Fig. 16. Transcription.

The primary transcript RNA is called pre-mRNA, because it must undergo certain modifications to become mature for translation. Whole process of maturation is called ***processing***, which occurs before the RNA leaves the nucleus.

Processing of pre-mRNA includes modification of 5′ end, which becomes a *cap* (methylated GTP), and modification of 3′ end *(poly-A tail)* by adding up to 300 Adenines. The cap helps attachment of ribosome subunits, and the 3′-tail prevents degradation of mRNA and provides export of mRNA to cytoplasm.

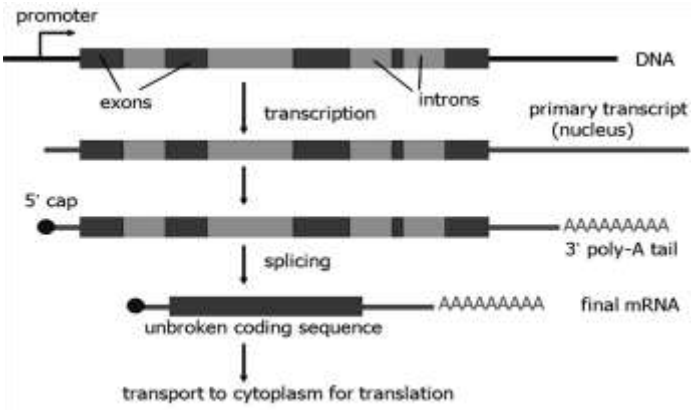


Fig. 17. Processing.

In eukaryotes the transcription unit of DNA contains regions called ***exons***, which carry information about protein structure, and non-coding segments called ***introns*** that lie between coding regions. Prokaryotes lack introns. During processing the introns are removed by ***restrictase*** enzyme, and the exons are spliced together (***splicing***) by ***ligase*** enzyme. In some cases the exons are spliced in alternative orders, and the same genetic information coded in DNA provides different proteins (e.g., antibodies). This is known as ***alternative splicing***.

Translation

Mature mRNA in the cytoplasm performs the *translation,* the process in which a cell interprets a genetic message and builds a protein accordingly. As a molecule of mRNA slides through a ribosome, codons are translated into amino acids, one by one. In process of translation tRNA, mRNA, amino acids, ATP and enzymes participate.

Translation is the process of transfer of nucleotide triplet sequence from mRNA to the sequence of amino acids in polypeptide. It consists of three stages:

1. ***Initiation.*** The small and big subunits of ribosomes attach to the mRNA. Each amino acid is activated by joining to the respective tRNA by a specific enzyme called an ***aminoacyl-tRNA synthetase***. This process is called ***recognition***. Aminoacyl-tRNA moves to the ribosome, two subunits of ribosome attach to 5' end of mRNA and a tRNA bearing the first amino acid (Methionine) of the polypeptide arrives to ribosome.

2.***Elongation***. Ribosome "rolls" along the mRNA from 5′ to 3′ direction. An incoming aminoacyl-tRNA binds to the codon of mRNA by hydrogen bonds. The ribosome catalyzes formation of a peptide bond between the previous and newly arrived amino acid. The previous tRNA is discharged and leaves the ribosome. This cycle is repeated until the polypeptide is elongated and completed.

3.***Termination***. The final stage of translation is *termination.* Elongation continues until a ribosome reaches a stop codon. Three special triplets UAA, UAG, UGA act as *stop codons.* Then the ribosome subunits and the polypeptide dissociate from mRNA.

After completion of the synthesis of the polypeptide (primary structure of protein) it undergoes modification (protein folding) in ER and Golgi for gaining the tertiary/quaternary structure and becoming functionally active protein.

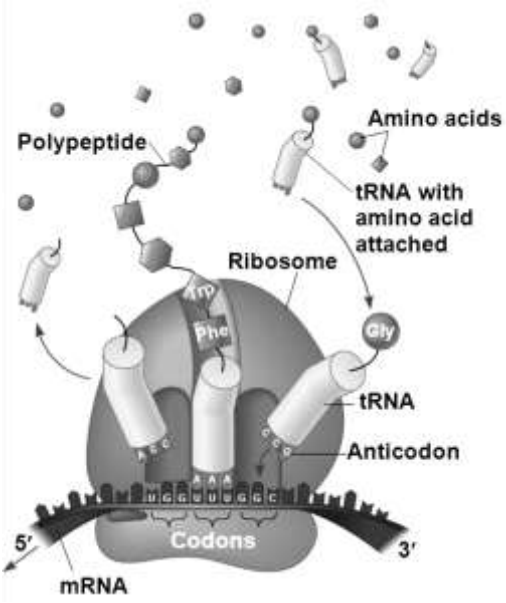


Fig. 18. Translation.

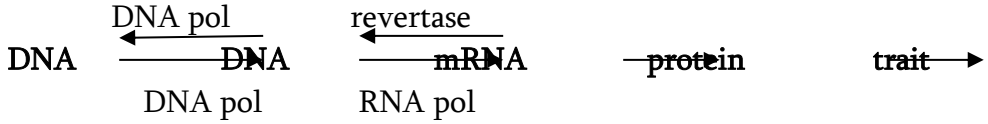
Central dogma of Biology

Information flow is realized from DNA to RNA via the process of transcription, and hence – to protein via translation:

DNA ↔ DNA → RNA → Protein

Although originally called dogma, this idea has been tested repeatedly until it was found that retroviruses possess reverse transcriptase enzyme (*revertase* or RNA-dependent DNA polymerase), which enables reverse transcription – synthesis of DNA on template of mRNA. Recently it was also found another direction of information flow: RNA synthesis on RNA template in viruses, which is realized by RNA dependent RNA polymerase enzyme.

Final expression of DNA genes is manifested as a certain trait and is mediated by proteins:



IA

1. DNA-topoisomerase enzyme:
   A. breaks down hydrogen bonds of DNA 2 strands
   B. releases tension of DNA strands
   C. joins Okazaki fragments
   D. provides synthesis of primer
2. Repetitive DNA is included in:
   A. structural genes
   B. rRNA genes
   C. tRNA genes
   D. spacers
3. DNA-topoisomerase enzyme:
   A. opens the helix
   B. prevents formation of knots
   C. cuts introns
   D. produces Okazaki fragments
4. The result of transcription is:
   A. exon and intron
   B. mRNA
   C. enzyme
   D. protein
5. Elongation is the process of:
   A. joining of amino acid with t-RNA
   B. mRNA synthesis
   C. extending of polypeptide chain
   D. beginning of polypeptide chain synthesis

IB

1. Which of the following is not true about genetic code?
   A. it has triplet structure
   B. it is redundant
   C. it is individual
   D. it is universal
2. Helicase cannot:
   A. break down hydrogen bonds of DNA strands
   B. untwist DNA strands
   C. join nucleotides
   D. form replication fork
3. What does not occur during translation?
   A. mRNA maturation
   B. polypeptide chain elongation
   C. initiation
   D. recognition
4. What is not true about alternative splicing?

A. exons join in different sequences
B. from one gene different mature mRNAs are formed
C. introns join in different sequences
D. one gene can have information about a few proteins

5. What does not occur during translation?
   A. moving of ribosome along mRNA
   B. joining of amino acids
   C. recognition
   D. elongation of mRNA

II

1. The phases of translation are:
   1. termination
   2. initiation
   3. splicing
   4. processing
   5. elongation

A. 2,3,4 B. 1,3 C. 1,2,5 D. 3,5

2. Highly repetitive DNA:
1. makes up satellite DNA
2. aids in recognition of homologous chromosomes in meiosis
3. is found in telomeres
4. repeats in genome 100-1000 times
5. encodes for structural genes

A. 1,3,4 B. 2,4 5 C. 4,5 D. 1,2,3

3. What takes place during transcription?
   1. all types of RNAs are synthesized
   2. transferring of genetic information onto ribonucleic acids
   3. recognition
   4. processing
   5. joining of tRNA with amino acids

A. 1,2,5 B. 3,4,5 C. 1,2 D. 1,4,5

4. The genetic code is:
   1. specific
   2. universal
   3. colinear
   4. overlapping
   5. individual

A. 1,2,3 B. 2,4,5 C. 4,5 D. 1,3,5

5. DNA-topoisomerase enzyme:
   1. breaks down phosphodiester bonds of one strand
   2. breaks down hydrogen bonds
   3. synthesizes SSBP proteins
   4. releases tension of DNA strands
   5. joins together new synthesized DNA fragments

A. 1,2,3 B. 3,4,5 C. 1,4 D. 2,4 5

CHAPTER 4
Non-cellular and cellular forms of life. Viruses, viroids, prions. Prokaryotes and eukaryotes. Cell structure.

The living organisms are divided into cellular and non-cellular types in accordance with their structural organization. The non-cellular forms of life do not have common cell structure (cell membrane, nucleus, cytoplasm). These are ***viruses***, ***viroids*** and ***prions***. The cellular organisms are prokaryotes and eukaryotes.

Non-cellular organisms

Viruses are discovered by *Ivanovski D.I.* in 1892. The viruses have too small sizes (tiniest viruses are 20 nm) and they are obligate intracellular parasites. The viruses crystallize out of cells and can reproduce only within a host cell. Growth is not common for them.

The structure of all viruses involves a nucleic acid and protein sheet called ***capsid***. Some viruses may have also additional layer of ***envelope*** made of glycoproteins. In regard to the type of genetic material contained in the viruses there are two types of these organisms: DNA viruses (e.g., Herpes virus) and RNA viruses (e.g., HIV, flu virus, hepatitis B viruses).

The viruses may affect all types of cellular organisms. In this sense the viruses are specific, meaning that they affect only particular, specific types of cells.

The viruses that affect bacteria are called ***bacteriophages***, or just ***phages***. They are composed of head containing the genetic material (DNA or RNA), tail and tail fibers made of protein. The reproduction of phages may proceed in ***lytic*** or ***lysogenic*** cycles.

During lytic cycle the phage attaches to the host cell of bacteria and lyses the cell wall by tail fibers. The phage injects its genetic material to the host cell. The phage uses the cell machinery to produce own particles, which assemble into whole phages. The reproduced phages lyse the host cell and leave to affect new cells.

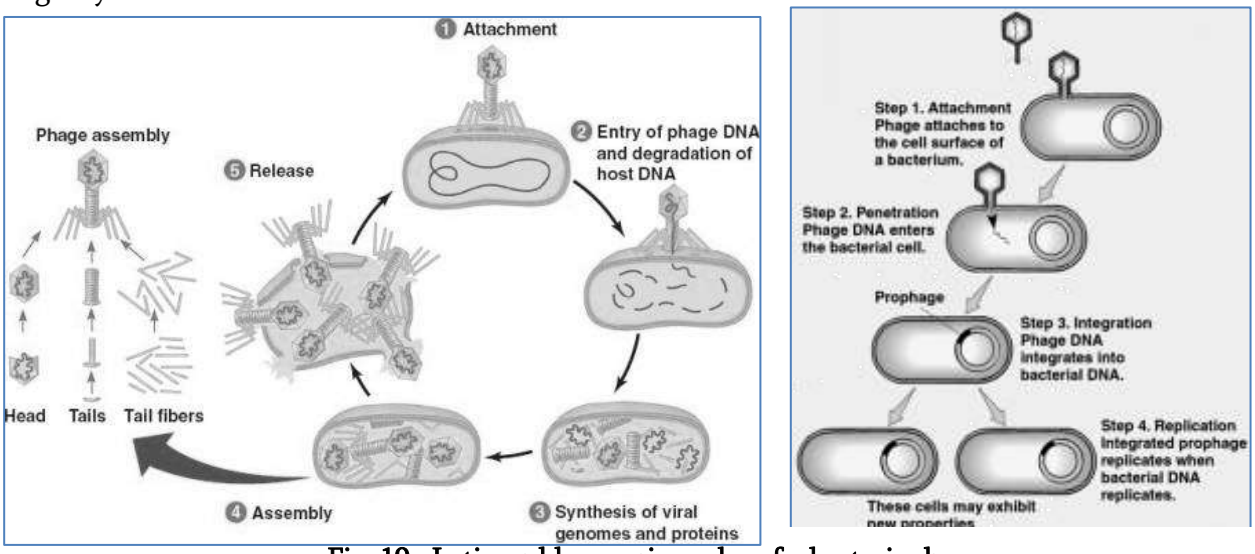


Fig. 19. Lytic and lysogenic cycles of a bacteriophage.

In contrast to lytic cycle, the lysogenic cycle of phages replicates the viral genome without destroying the host. Within the host cell phage DNA integrates with bacterial DNA and transmits it through generations. The bacteria then are called ***lysogenic***, and the phage is called as ***prophage*** (precursor of the phage) or ***temperate phages***. The environment factors like radiation or chemicals can activate the prophage and switch the lysogenic cycle to lytic.

Viroids present as a nucleic acid and lack protein capsid. The genetic material is the short RNA (200-300 nucleotides long), which can affect only damaged plant cells. The only viroid of humans described is the *delta agent* that causes hepatitis D. It is incorporated in the capsid of hepatitis B virus (HBV) and may affect human liver cells only along with HBV.

Prions are infective protein agents that do not have any genetic material. Prions affect brain tissue and cause neurodegenerative diseases (e.g. Kuru, *scrapie*, "mad cow's" disease). Prion diseases can be both acquired (through infected meat) and hereditary (gene mutation).

Cellular organisms

Prokaryotes are single-cell organisms measuring 0.5-5 mkm. They lack true nucleus (*pro* – before, *karyon* – nucleus). These are bacteria, mycoplasmas and blue-green algae.

The genetic material of prokaryotes is presented as a single circular DNA molecule called ***nucleoid*** (like a nucleus), which is not connected to histone proteins. It is located free in the cytoplasm, since there is no nuclear membrane.

Prokaryotes have no membranous organelles, instead they have ***mesosomes*** which are infoldings of plasma membrane. The non-membranous organelle in prokaryote is ribosome, which is smaller (70S) than in eukaryotes (80S). Prokaryotes have cell wall made of muramic acid, while in eukaryotes the cell wall is present in plant cells and is made of cellulose. Additionally, some prokaryotes may have also a capsule. Arrangement of microtubules in flagella and cilia of prokaryotes is "9+0", and the eukaryotic motility organelles are arranged as "9+2". Division of prokaryotes is amitosis.

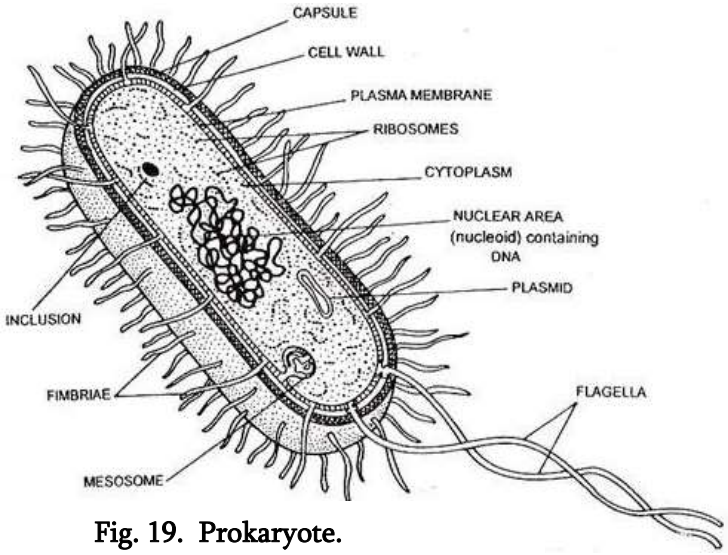


Fig. 19. Prokaryote.

Eukaryotes can be both unicellular and multicellular organisms. The eukaryotic cell is larger (5-100 mkm) than prokaryotes. Eukaryotes (*eu* – true, *karyon* – nucleus) have true nucleus: the genetic material is linear and is bound to histone proteins organizing chromosomes; nuclear membrane separates genetic material from cytoplasm. Membranous organelles (e.g., endoplasmic reticulum, Golgi apparatus, etc.) provide ***compartmentation*** of the cytoplasm (partitioning into membranous parts), which allows simultaneous processing of multiple metabolic reactions in various parts of the cell.

Theodor Schwann and *Mattias Schleiden* have summed up all the acquired knowledge about cells and proposed the cell theory (1839), which has the following major concepts:

1. Every living organism consists of one or many cells.
2. Cell is the structural and functional unit of life.
3. Every cell arises from preexisting cell.

The eukaryotic cell consists of cell membrane (plasma membrane), nucleus and the cytoplasm with organelles.

Plasma membrane consists of phospholipid bilayer with proteins embedded in it. The phospholipids face by their hydrophobic tails, which provide fluidity, and the proteins have

mosaic arrangement in the two layers of phospholipids. Such a structure of plasma membrane is known as ***fluid-mosaic model.*** Cholesterol is a component of animal cell membrane. It provides stability of the membrane.

In animal cells the plasma membrane is associated with **glycocalix**, which is a 20 nm layer made of glycolipids and glycoproteins formed by union of oligosaccharides (about 20 monomers) and lipids or proteins, respectively. It functions for recognition of adjacent cells of the same tissue as well as signaling.

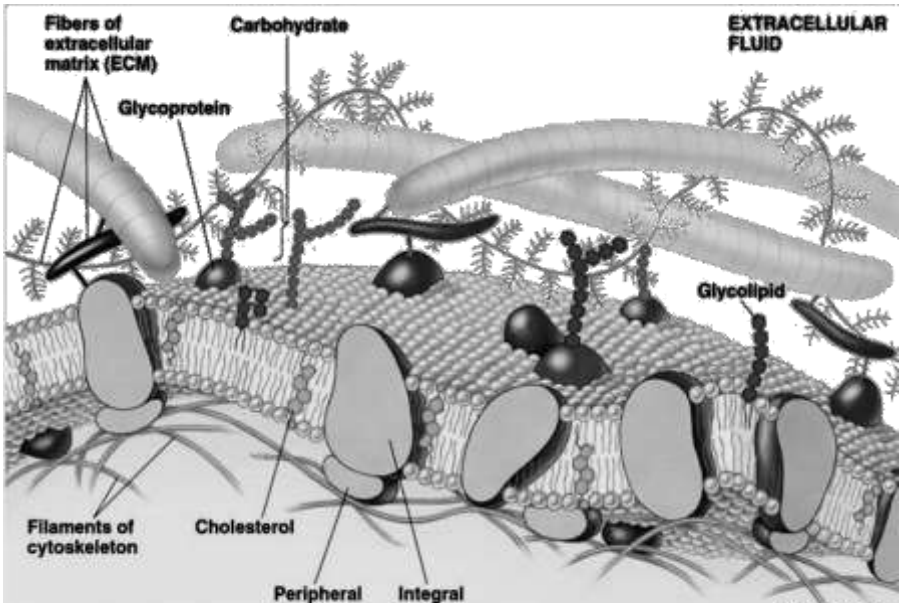


Fig. 20. Cell membrane.

Functions of plasma membrane are following:

1. **Protection and shaping**.
2. **Catalytic function**.

Some proteins in the plasma membrane are enzymes that catalyze specific reactions.

3. **Transport**.

The cell membrane has selective permeability for different substances. For example, the hydrophobic part of the phospholipid bilayer is permeable for lipids, steroid vitamins and hormones, water, CO_2, O_2. These substances cross the membrane easily by diffusion (*passive transport*). The membrane is relatively impermeable for charged atoms. Hydrophilic substances that cannot pass through hydrophobic layer of the membrane are transported by carrier-proteins that can act like gates (*facilitated diffusion*), or present channels (e.g., Na^+-K^+ pump). Large molecules are transported in membrane-bounded vesicles (*endo- and exocytosis*).

Following types of transport are differed:

a) Passive transport (diffusion, facilitated diffusion, osmosis).
b) Active transport.
c) Endocytosis and exocytosis.

Passive transport is the movement of molecules across a biological membrane from higher to lower concentration (down concentration gradient) without use of energy. When this refers to solute molecules, it is called *diffusion*. *Facilitated diffusion* is realized by membrane proteins, which take and release a molecule across membrane (e.g., sugar molecules).

Osmosis is the passive transport of water (solvent). Transport of water is balanced in both directions of the membrane of cells that are in isotonic medium (it is considered concentration

of NaCl=0.9%). In hypertonic medium (NaCl>0.9%) the water moves out from the cell and it shrinks (*plasmolysis*). In hypotonic medium (NaCl<0.9%) water moves to inside the cell, which swells and bursts (if the cell is an RBC, the process is known as hemolysis).

Active transport is against concentration gradient (from lower to higher concentration), so it uses energy. For example, the transport of Na^+ and K^+ ions is realized by channel-proteins that involve ATP-ase enzyme (Na^+ moves out of the cell, K^+ transfers into the cell).

Endocytosis is the process by which cell ingests larger materials. It is of two types: phagocytosis and pinocytosis. Phagocytosis (cell eating) is ingestion of solid particles like food, viruses, bacteria. It is common for some leukocytes (white blood cells), amoeba. In pinocytosis (cell drinking) cell engulfs liquid particles (e.g., fat droplets). *Exocytosis* is the process opposite to endocytosis. Several secretory proteins and waste products are eliminated from the cell through exocytosis.

4. **Signaling**. The ligands that are hydrophilic (e.g. protein hormones, neuromediators) cannot pass through the hydrophobic part of bilayer. These substances bind to specific proteins of the membrane – receptors, and transmit the signal to the cell. For example, adrenaline realizes its effect through adrenoreceptors. In case of receptor deficiency there is no response despite of presence of the signaling molecule. For example, in testosterone receptor deficiency (due to gene mutation) the male sex hormone cannot perform its action, and the organism with male karyotype (46,XY) expresses female secondary sex traits. This pathology is known as ***testicular feminization*** or ***Morris syndrome***, which is an example of *male pseudohermafroditism* (organism with male sex glands – testes, but female secondary sex traits).
5. **Intercellular interactions (contact function)**. Adjacent cells contact with each other by specific structures in cell membrane. The types of intercellular interactions are:

a) tight junctions (between intestinal cells),
b) desmosomes (between epithelial cells, provide toughness),
c) gap junctions (in heart muscle cells).

Cell organelles

Cytoplasm is a semifluid environment of the cell, which contains the organelles. The cell organelles are of two types – membranous and non-membranous. Membranous organelles in turn are single-membranous (endoplasmic reticulum, Golgi complex, lysosomes, microbodies) and double-membranous (mitochondria and plastids). Non-membranous organelles are centrioles, ribosomes, microtubules and microfilaments.

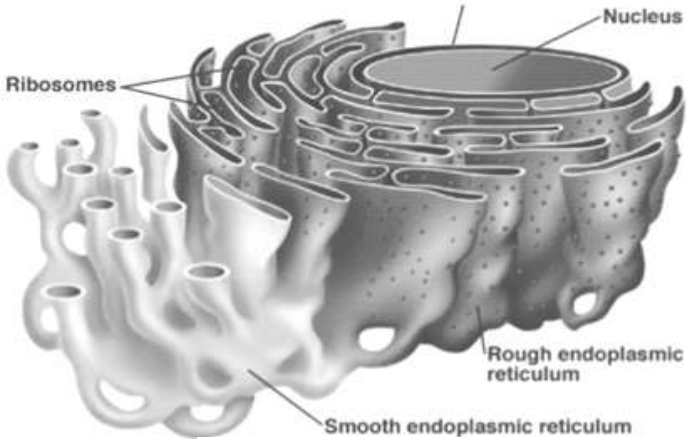


Fig. 21. Endoplasmic reticulum.

Endoplasmic reticulum (ER) is a membranous network inside the cytoplasm stretching from nuclear membrane to plasma membrane. It consists of membranous tubules and sacs. ER is of two types – smooth endoplasmic reticulum (SER) and rough endoplasmic reticulum (RER). RER contains ribosomes which make the ER

rough.

Functions of ER include:

1. Compartmentation of the cell.
2. Synthesis of proteins (membrane proteins, enzymes, hormones) on RER.
3. Synthesis of lipids (involved in membranes, steroid hormones). In case of gene mutation of enzyme producing cortisol hormone in adrenal gland, cells produce more testosterone, and the organism with female karyotype (46,XX) develops male sex traits. This is known as adrenogenital syndrome, which is a female pseudohermafroditism (organism with female sex glands ovaries but male secondary sex traits).
4. Regulation of Ca2+ ions transport in muscle cells ER (sarcoplasmic reticulum). When Ca^{2}+ transport is disrupted, cell metabolism is intensified and this leads to malignant hyperthermia (temperature is 42°C-43°C).
5. Detoxification of poisons in liver cells. The ER of hepatocytes neutralizes both endogenous toxins (bilirubin) and exogenous poisons (alcohol, drugs). That is why hepatic failure may lead to severe intoxication of organism.

Golgi apparatus consists of membranous tubules, sacs and vesicles. The structural unit of Golgi apparatus is a **dictyosome** which is made of 3-12 flattened sacs with vesicles on tips. Functions of Golgi apparatus are:

a) modification of substances produced in and transported from ER. For example, lipids are phosphorylated to form phospholipids, oligosaccharide branches are added to proteins and lipids to form glycoproteins and glycolipids, respectively. Thus, Golgi apparatus provides innovation of plasma membrane and formation of glycocalix;
b) packing and shipping of the modified products in the form of membranous vesicles to specific sites of the cell or storing them in Golgi;
c) synthesis of polysaccharides;
d) formation of primary lysosomes.

Lysosomes are single-membranous sacs with hydrolytic enzymes, which can hydrolyse proteins, polysaccharides, fat, nucleic acids. These enzymes are active in acidic medium (pH=5.0) and are inactive in the neutral cytoplasm (pH=7.0). Function of lysosomes is intracellular digestion.

Types of lysosomes:

a) ***primary lysosomes*** contain hydrolytic enzymes and are formed in Golgi apparatus.
b) ***prelysosomes*** are formed in a result of endocytosis (e.g., food or foreign organisms) or contain own particles of the cell (e.g., old cell organelles).

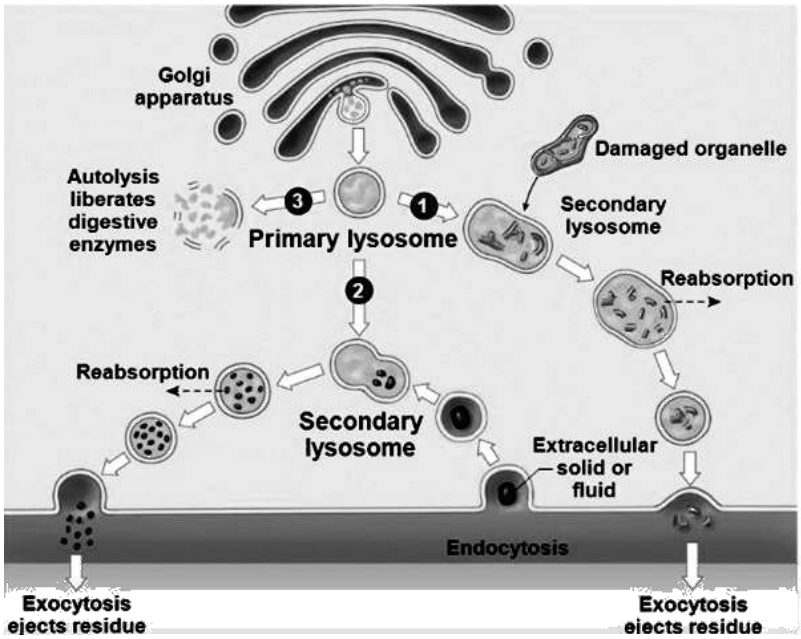


Fig. 22. Intracellular digestion by lysosome.

c) ***secondary lysosomes*** develop from fusion of primary and prelysosomes. They are of two types: *heterolysosome* and *autolysosome (phagosome)*. If the secondary lysosome contains a particle entered by endocytosis, then a heterolysosome is formed. If the digested material is a cell particle, then an autolysosome is formed.

d) ***postlysosome*** develops after digestion inside the secondary lysosome and it contains waste products, which eliminate from the cell by exocytosis.

Lysosomal storage diseases. Mutations of genes coding for lysosomal enzymes, lead to genetic diseases in which a substance that should be digested by the given enzyme, is accumulated and stored in the cell. The extra amount of this substance damages the cell causing storage diseases. Examples of storage diseases are glycogenosis (accumulation of glycogen in muscles or liver), Tay-Sachs' disease (accumulation of specific lipids in brain).

Lysosomes realize also autodigestion of the cell during the programmed cell death called ***apoptosis***.

Microbodies (peroxisomes). Peroxisomes contain enzyme *peroxidase* that breaks long-chain fatty acids. During this oxidation a by-product hydrogen peroxide (H_2O_2) is formed which is very toxic to the cell and is neutralized by another peroxisomal enzyme *catalase* (antioxidative enzyme).

Mitochondria are double-membranous organelles. The outer membrane is smooth, and the inner membrane is convoluted. The infoldings are called ***cristae***; they increase surface for cell respiration. Inside the mitochondrion, the inner membrane encloses a space called ***matrix***, where a mitochondrial DNA (circular molecule) and 70S ribosomes are located. Own DNA provides synthesis of about 40% proteins required for mitochondrial function (including enzymes of ATP synthesis). Synthesis of rest of the proteins is under control of the nuclear DNA. That is the reason why the mitochondria are called ***semiautonomous*** organelles. The number of mitochondria in the cell correlates with its activity. Cells with high metabolism (nerve, muscle and liver cells) are rich in mitochondria.

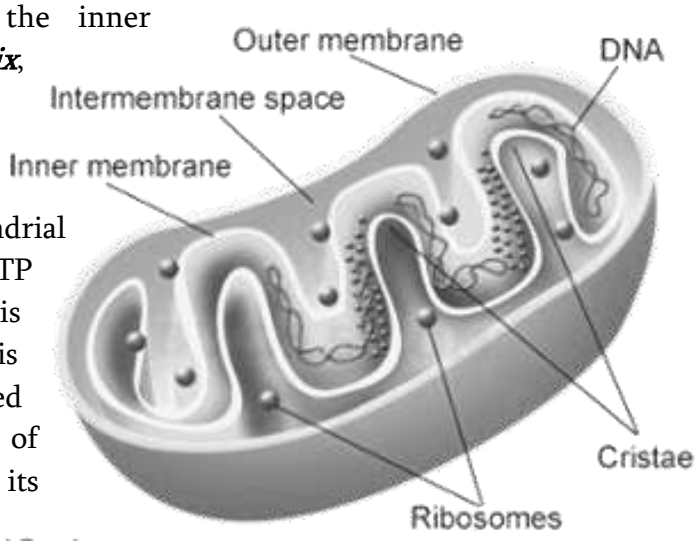


Fig. 23. Mitochondrion.

Main function of mitochondria is ATP synthesis (cell respiration) which occurs on inner membrane of cristae. Hereafter, the mitochondria are known as power stations of the cell. Mitochondria can replicate their DNA and double.

Mutations of mitochondrial DNA may lead to diseases that affects nervous system and muscles, which are highly active tissues requiring energy (ATP). Known diseases are ***spina bifida*** (split spinal cord) and ***Leber's optic nerve atrophy.***

Plastids are double-membranous organelles common only for plant cells. They have much in common with mitochondria. The outer layer is smooth, and inner layer is convoluted (***thylakoids***), inner membrane encloses a space called ***stroma***. Plastids are semiautonomous organelles as well (have own DNA, 70S ribosomes). There are three types of plastids:

chloroplasts (green plastids), chromoplasts (colored plastids) and leuko- or amyloplasts (white plastids).

Chloroplasts have green pigment chlorophyll and can transform sun light energy to the chemical energy of organic substances (e.g., glucose), the process known as ***photosynthesis***.

Ribosomes are 15-30 nm in size and consist of small and big subunits formed in nucleolus (nucleolar organizer regions). Each of the subunits contains rRNA and protein. Ribosome subunits transfer through nuclear pores to cytoplasm where they assemble onto mRNA to start protein synthesis (translation). A group of ribosomes on mRNA that produce the same type of polypeptide make up a ***polysome***, or a ***polyribosome***. Big ribosomes (80S) are found in cytoplasm of eukaryotes (free ribosomes) and on RER (bound ribosomes). Small ribosomes (70S) are located in prokaryotes as well as in plastids and mitochondria.

Microtubules and microfilaments. These non-membranous organelles are both involved in cell skeleton (cytoskeleton) which supports cell shape.

Microtubules are hollow rods 25 nm in diameter. They are made by polymerization of ***tubulin*** protein. Cortical layer of cytoplasm (layer under cell membrane) is rich in microtubules and provide cell shaping. They also make the structure of centrioles, spindle fibers, cilia and flagella.

Microfilaments are solid rods about 7 nm in diameter. They are made from ***actin*** filaments. In muscle cells actin filaments interact with myosin microfilaments to provide muscle contraction, which requires also ATP and Ca^{2+} ions. In plant cells actin filaments provide cytoplasmic streaming, and in some protozoa (e.g. amoeba) – also amoeboid movement by false feet (pseudopods).

Centrosome (cell centre) locates close to nucleus and consists of two centrioles which are perpendicular to each other. Each centriole is formed by nine triplets (9x3) of microtubules. They are found in animal cells and function in formation of mitotic spindle fibers during cell division. In case the spindle fibers are not formed properly, the chromosomes or chromatids are not disjuncted normally, and daughter cells acquire unequal number of chromosomes. This leads to genome mutations (change of chromosome number) and development of chromosomal diseases.

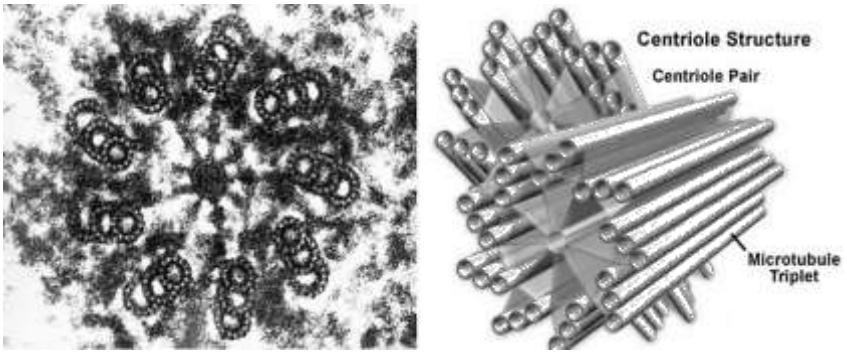


Fig. 24. Centrioles.

Cell inclusions are temporary components of the cell. They have no specific shape and present different materials (for example, pigments, fat droplets, glycogen granules) involved in vesicles.

IA

1. The cortical layer of cytoplasm is rich in:
   A. ER
   B. microtubules and microfilaments
   C. Golgii complex
   D. ribosomes
2. Which of the following is synthesized in RER of gland cells?
   A. any protein
   B. excretory proteins
   C. steroid sex hormones
   D. polysaccharides
3. Which of the following is formed in Golgi comlex?
   A. primary lysosomes
   B. autolysosomes
   C. heterolysosomes
   D. secondary lysosomes
4. Peroxisomes contain:
   A. peroxidase and ligase
   B. catalase and restrictase
   C. catalase and peroxidase
   D. peroxidase and helicase

IB

1. Prokaryotes do not have:
   A. DNA
   B. thylakoids
   C. muramic acid
   D. plastids
2. Which of the followings is not a function of mitochondria?
   A. synthesis of enzymes
   B. cellular respiration
   C. synthesis of rRNA
   D. synthesis of polysaccharides
3. Which of the followings is not a cellular form of life?
   A. mycoplasma
   B. fungi
   C. phages
   D. protozoa
4. Which of the followings is not a compartment of the cell?
   A. mitochondria
   B. microtubules
   C. lysosomes
   D. dictyosome
5. What is not true for centrioles?
   A. take part in chromosome disjunction
   B. Form spindle fibers
   C. consist of tubulin
   D. contain actin and myosin

II

1. Viruses:
   1. have protein coat
   2. have both DNA and RNA
   3. are intracellular parasites
   4. can reproduce
   5. can grow

   a. 1,2,3 b. 2,4 c. 1,3,4 d. 4,5
2. Glycocalyx consists of:
   1. polysaccharides
   2. glycoproteins
   3. glycolipids
   4. nucleoproteins
   5. phospholipids

   a. 1,2,3 b. 2,3 c. 2,3,5 d. 1,4,5
3. The functions of plasma membrane are:
   1. protein synthesis
   2. synthesis of ATP
   3. transport
   4. signaling
   5. detoxification of poisons and drugs

   a. 3,4 b. 2,3 c. 3,4,5 d. 1,4,5
4. Ribosomes:
   1. consist of two subunits
   2. are made up of proteins, DNA
   3. are made up of proteins, RNA
   4. are formed in nucleolus
   5. have cytoskeleton function

   a. 1,3,5 b. 2,4,5 c. 1,3,4 d. 4,5
5. Centrioles:
   1. have cylindrical form
   2. are composed of 9 triplets of microtubules
   3. consist of tubulin protein
   4. contain DNA or RNA
   5. are found in plant and animal cells

   a. 1,2,4 b. 2,3,5 c. 3,4,5 d. 1,2,3

CHAPTER 5

Nucleus. Chromosomes. Cell cycle. Extensions to mitosis. Apoptosis.

Nucleus

Nucleus is the main compartment of non-dividing eukaryotic cells. Nucleus consists of nuclear membrane (envelope), nuclear matrix (karyoplasm), chromatin and nucleolus.

Nuclear envelope has double-membranous structure. Two membranes are separated by ***perinuclear space*** and are fused by pores through which different substances are transported in both directions from nucleus to cytoplasm, and vice versa. For example, mRNA, tRNA, rRNA (ribosome subunits) are transported from nucleus, and proteins (histones, enzymes of replication and transcription), nucleotides, ATP enter the nucleus. The internal surface of nuclear membrane is covered by ***nuclear lamina*** to which the chromatin is attached during interphase.

Nuclear matrix (karyoplasm) is the inner content of the nucleus where the nucleoprotein material of chromatin is organized.

Nucleolus is present in the non-dividing stage and consists of DNA (genes of rRNA), rRNA and protein. In prophase of mitosis nucleolus is dissolved, and is reformed again in telophase. In humans the nucleolus is made up by 5 pairs of satellite chromosomes (number 13, 14, 15, 21, 22) which have ***nucleolar organizer regions (NORs)***. The large and small subunits of ribosomes are formed in nucleolus.

Chromatin. Within the nucleus, the DNA is organized along with proteins into material called chromatin, which is the decondensed condition of genetic material present during interphase. Chromatin as a nucleoprotein is composed of DNA (40%) and protein (40% histone and 20% non-histone). During cell division chromatin coils up (condenses), becoming thick structure called ***chromosomes***, which are stained well and can be visible by light microscope. There are two types of chromatin:

1. Euchromatin presents coding sequences (genes) located on chromosome arms and appears in uncoiled condition, which is poorly stained and transcriptionally active.

2. Heterochromatin presents condensed condition of genetic material in interphase, it is well stained and transcriptionally inactive. There are two types of heterochromatin: constitutive and facultative. ***Constitutive*** or ***structural heterochromatin*** locates on the tips of chromosomes called ***telomeres*** and primary constriction or ***centromere*** of chromosomes (recall highly repetitive non-coding DNA).

Facultative heterochromatin is presented by sex chromatin in females (***Barr body***), which is the one of two X chromosomes. It is inactivated since the third week of embryonal development and condenses into heterochromatin state. It is studied in buccal epithelium and leukocytes found under the nuclear membrane.

Chromosomes

Chromosomes are formed in prophase of mitosis by coiling and condensation of chromatin. In metaphase they reach the maximal condensation and consist of two sister chromatids, which are joined in a primary constriction called centromere. Centromere divides the chromosome into two arms. According to the position of the centromere there are several types of chromosomes:

a) **metacentric** – the centromere is located in the center of the chromosome and divides it into equal arms.

b) **submetacentric –** the centromere is shifted from the center, and one arm is longer (indicated as *q*) than the other (short arm is indicated as *p*).

c) **acrocentric –** the centromere is almost on the tip of the chromosome, and arms are quite unequal. Some acrocentric chromosomes have a secondary constriction, which is followed by the "satellite" region of chromosome. These are known as **satellite** chromosomes. There are 5 pairs of such satellite chromosomes in humans: 13, 14, 15, 21, and 22, where the nucleolus is formed. The region of secondary constriction is called *nucleolar organizer region (NOR)* and presents rRNA genes.

a) **telocentric –** the centromere is on the tip of chromosome, and this is not a normal chromosome, since it misses one arm due to mutation (deletion). Telomeres are the parts of chromosomes that prevent them from sticking, thus providing their individual structure.

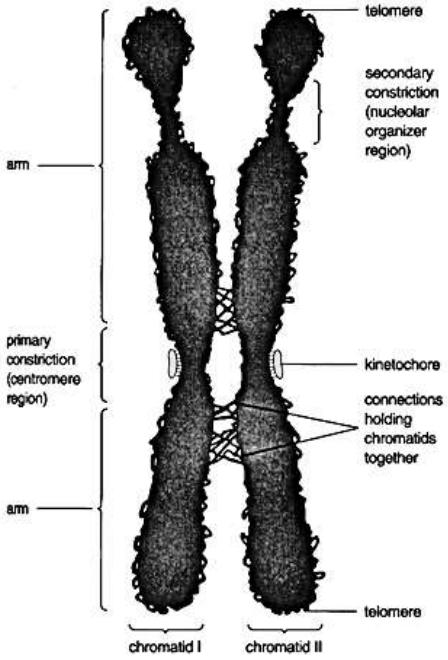


Fig. 25. Chromosome structure.

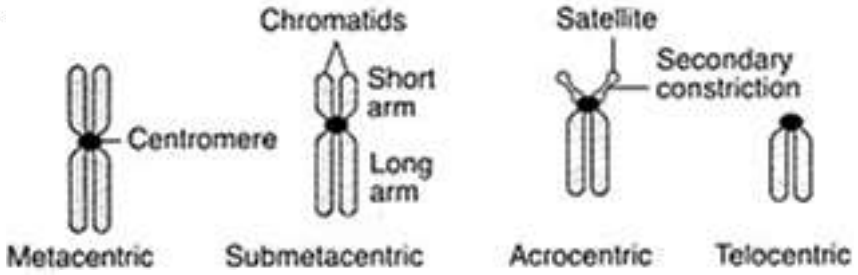


Fig. 26. Types of chromosomes.

DNA packing. Chromosome organization

DNA molecule that is 2 nm in diameter and few centimeters long in eukaryotes, cannot fit into microscopic cell nucleus. That is why it undergoes folding and forms chromosomes, which are much shorter (few micrometers) and thicker.

The chromatin of eukaryotic cells presents a nucleoprotein structure composed of DNA and proteins (histone and non-histone). These proteins help to pack DNA and organize chromosomes. In addition, histone proteins provide also gene activity regulation. Histones are basic proteins rich in basic amino acids; they are of 5 types (H1, H2A, H2B, H3, H4).

The levels of DNA packing are:

1. **Nucleosome** is composed of DNA wound around a protein core made of pairs of four histones (H2A, H2B, H3, H4). The diameter of DNA thickens up to **11 nm**. Nucleosomes are like beads on a string.
2. **30 nm chromatin fiber** is formed by H1 histone, which is attached to *linker DNA* (DNA between two nucleosomes) and brings together adjacent nucleosomes making them closer. H1 histone can regulate gene activity: when it attaches to DNA, the transcription is stopped (genes are inactivated), and when it leaves the DNA then genes become are transcriptionally active.

3. **Looped domains (300 nm thick)** are formed by attachment of far apart nucleosomes to non-histone protein scaffold.
4. **Interphase chromatin (700 nm thick).** The loops that are identical by chemical structure form blocks and condense the previous level forming an interphase chromatin.
5. **Metaphase chromosome** has **1400 nm** of diameter, which is the ultimate level of condensation present during metaphase stage of mitosis. The length of chromosome is few micrometers (depending on length of different DNA molecules), and it consists of two sister chromatids joined by centromere. This structure is well observed by light microscope (during karyotype studies).

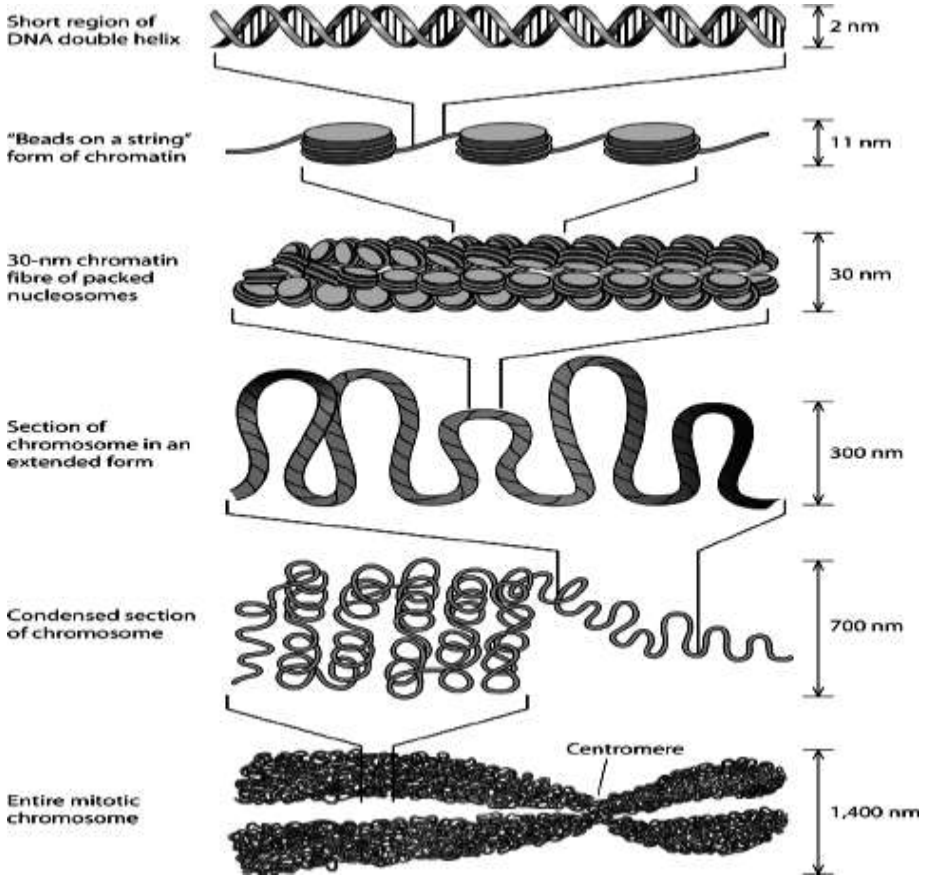


Fig. 27. DNA packing.

Chromosome rules

1. The number of chromosomes is **constant**: before the cell divides DNA within chromosomes doubles (2 chromatids) and is distributed to daughter cells during mitosis. The number of chromosomes in species is preserved constant due to mitosis, meiosis and fertilization. The number of chromosomes is constant for each species and does not depend on its evolutionary development level.
2. Chromosomes are **continuous**: it is provided by DNA replication and transmission to daughter cells. The chromosomes maintain their structure and are "copied" through cell generations.
3. The number of chromosomes is **even**: somatic cells have diploid set of chromosomes, which means there are two copies (a homologous pair) from each chromosome type. One from each

chromosome pair is maternal, and the second is paternal. Homologous chromosomes have same structure, shape and gene set, but combination of alleles in homologous loci may vary.

4. Non-homologous chromosomes are **individual**: they have an individual structure with specific set of genes, position of centromere.

Karyotype

Karyotype is the diploid set of chromosomes of a species (also organism or cell) and is characterized by specific number, structure and shape of chromosomes. Study of karyotype can reveal various genetic disorders related to the structure or number of chromosomes (chromosome diseases).

To examine the human karyotype, bone marrow or skin fibroblast cells or lymphocytes from peripheral blood are usually cultured, since they are either easy to obtain and/or can intensively divide. Sometimes there is a need to study fetal cells (during pregnancy) for pre-natal diagnosis of some genetic diseases.

The cells then are treated with drug (phytohemagglutinin) to stimulate mitosis. Colchicin (mitotic poison) is then added to arrest mitosis at metaphase, when the chromosomes, each consisted of two joined sister chromatids, are ultimately condensed. The cells are then treated with hypotonic solution due to which they swell and their chromosomes spread out. A drop of the cell suspension in fixative is spread on a microscope slide, dried and stained (homogeneously or in bands). After, the chromosomes are photographed to make karyograms and are studied.

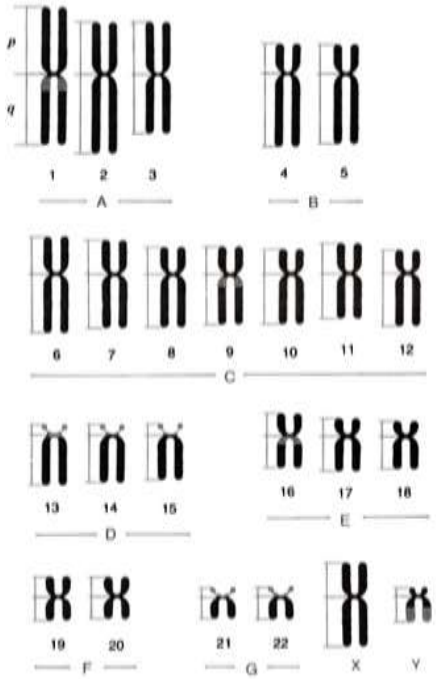


Fig. 28. Karyotype. Denver classification.

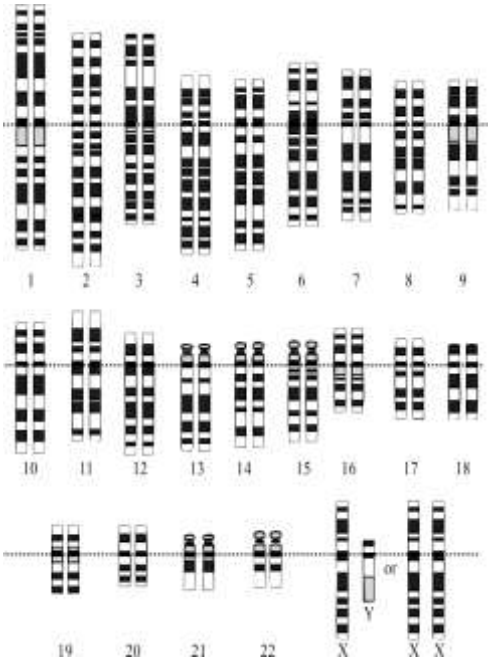


Fig. 29. Karyotype. Paris classification.

The first classification of human chromosomes was presented in Denver congress, in 1960. The complex set of chromosomes is studied in ***karyograms*** (or idiograms). In the karyogram the homologous pairs of identified chromosomes (numbered from 1 to 23) are arranged in alphabetical groups by centromere position and decreasing sizes (from largest - number 1, to smallest – number 22). This presents the ***Denver classification*** of chromosomes (metacentric, submetacentric, acrocentric chromosomes in A, B, C, D, E, F, G groups). Big metacentric X and small acrocentric Y chromosomes are known as sex chromosomes or allosomes (23rd pair). Female somatic cells contain

22 pairs of autosomes and two X chromosomes, while male karyotype is presented in 22 pairs of autosomes and X and Y chromosomes.

Since several chromosomes have almost the same size and centromere position, some difficulties may arise regarding their identification. Hence, methods of *differential staining of chromosomes* were developed with the help of fluorescence dyes. Such method stains the chromosomes in light and dark bands and enables precise identification of all the chromosomes because each chromosome has individual pattern of banding (sequence of light and dark bands). This classification is known as ***Paris classification*** (was proposed in Paris, 1971).

And nowadays, more modern techniques enable staining of each chromosome into specific colour.

Karyotyping helps in diagnosis of numeric and structural mutations of chromosomes (***genome mutations*** and ***chromosome aberrations***, respectively), leading to chromosomal diseases. Moreover, Paris classification allows also diagnosis of some ***gene mutations***.

Cell cycle

The cell cycle is a cycle of repeating events lied between two cell divisions or a division and cell death. Cell cycle includes:

a) mitotic cycle that involves **interphase** (a preparing stage) and cell division or **mitosis**.
b) stage of performing specific functions.

There are cells that undergo only mitotic cycle (cells of embryo). These cells perform specific functions following their differentiation.

Interphase. In this stage the cell prepares for division. Interphase consists of three stages:

G1 – in presynthetic or postmitotic stage the cell prepares for synthesis (S) stage, in which DNA is replicated. So, in G1 there is synthesis of DNA replication enzymes, histones, ATP.

S (synthesis) stage – is the longest stage during interphase (and whole mitotic cycle). Here DNA is replicated, and two sister chromatids are formed each presenting a DNA molecule. The genetic material is presented as 2n4c (2n – diploid number of chromosomes, 4c – 4 chromatids in one homologous pair of chromosomes).

G2 – postsynthetic or premitotic stage, in which the cell is preparing for division to form two daughter cells. There is increase of cytoplasm volume, replication of mitochondrial DNA, doubling of organelles (mitochondria, centrioles), synthesis of tubulin (involved in centrioles, spindle fibers) and ATP.

Mitosis

There are two main types of cell division into two daughter cells: **mitosis** or indirect division, and **amitosis** or a direct division.

Mitosis is an indirect division, since it is not a simple division into two, but occurs with the help of mitotic apparatus (particularly spindle fibers), which equally distributes the genetic material into daughter cells. The word "***mitos***" in Greek means thread or fiber.

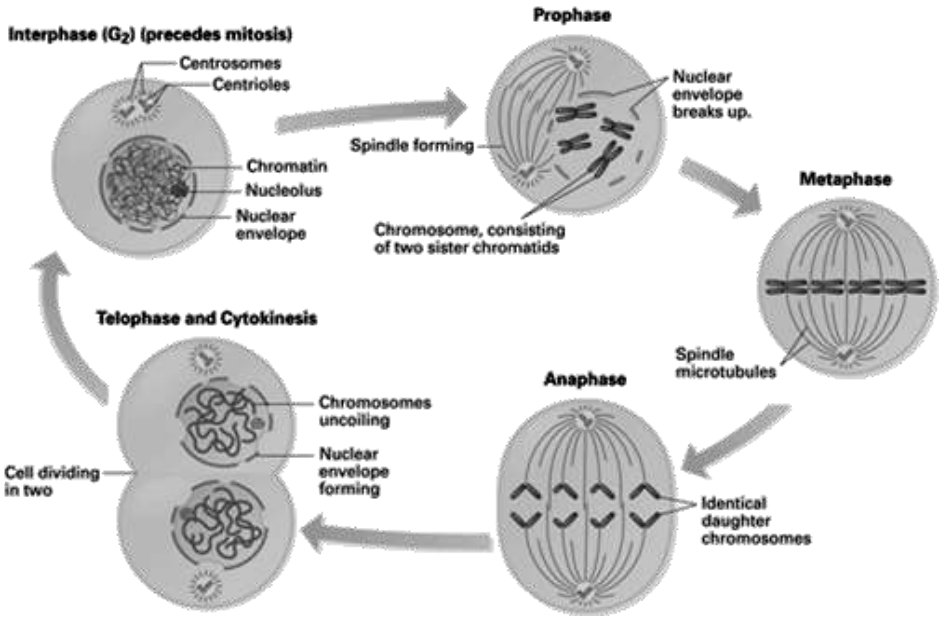


Fig. 30. Mitotic cycle.

In multicellular organisms mitosis occurs in diploid somatic cells. Mitotic division consists of four stages: prophase, metaphase, anaphase, telophase. These stages divide the nucleus and all together are referred to as ***karyokinesis***. It is followed by division of cytoplasm known as ***cytokinesis***.

Prophase: chromatin undergoes condensation and the chromosomes become visible by microscope, nuclear membrane and nucleolus disperse, doubled centrioles move towards the opposite poles of the cell, mitotic spindle starts to form. *Metaphase:* chromosomes, each consisting of 2 chromatids, line up the metaphase plate (central plate of the cell), spindle fibers attach to the centromeres (kinetochores) of chromosomes from both sides. Thus, a ***mitotic apparatus*** is formed made of chromosomes on metaphase plate, centrioles and spindle fibers attached to chromosomes. In metaphase stage the chromosomes are observed to be made of two sister chromatids joined at centromere. They are the most condensed in this stage. *Anaphase: s*ister chromatids are ***disjuncted*** (separated) and move to the poles of the cell by shortening (contractions) of spindle microtubules. *Telophase:* chromosomes made of single chromatid uncoil into chromatin, nuclear membrane and nucleolus reform, and at each pole of the cell there is a diploid nucleus with 2n2c genetic material.

Cytokinesis *divides the mother cell into two diploid daughter cells (2n2c each).*

Biological significance of mitosis

a. Maintaining constant number of chromosomes through generations of diploid cells, since before each division there is replication of DNA. The daughter cells get equal genetic material identical with that of the mother cell, hence they are alike in morphology and functions.
b. Providing growth and development of organisms by increasing the number of cells.
c. In unicellular eukaryotes mitosis is a pattern of reproduction (e.g. amoeba and Paramecium).

Amitosis is direct division of the cells without formation of spindle fibers. It divides the genetic material into relatively equal parts and is faster than mitosis. Amitosis is common in prokaryotes and cancer cells.

Extensions to mitosis

1. **G_0 cells** are cells that undergo no more mitosis after several rounds of divisions. Following the last division they enter a stage called G_0 stage, and the cells are called G_0 cells. For example, these are nerve and muscle cells. In some conditions G_0 cells can restart mitosis, e.g., if leukocytes are treated with phytohemagglutinin, the cell starts to divide. In Gurdon's experiments the nerve cell nucleus of a frog is transferred to the enucleated egg cytoplasm, and this cell also restarts division, even a tadpole develops.
2. **Endomitosis** is the division (disjunction) of replicated chromatids inside the nucleus, which is not followed by karyokinesis and cytokinesis. It results in polyploid cell with multiplied set of chromosomes (e.g. 2n2c→4n4c→8n8c etc.).

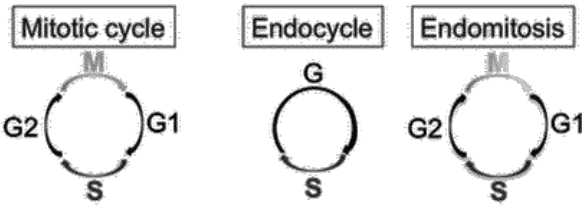


Fig. 31. Mitotic cycle and extensions.

3. **Polyteny.** This is a condition in which the DNA repeatedly replicates (endocycling) without subsequent disjunction of chromatids, and the number of chromosomes remains diploid. This results in a ***giant*** or ***polytene chromosome***, consisting of about 1000 parallel chromatids and often showing conspicuous transverse banding. Polyteny is observed in the cells of certain insects, notably in the salivary glands of *Drosophila* fruit fly larva. Uncoiled parts called ***puffs*** are observed on giant chromosomes at specific stages in the fast development of insect (egg-larva-pupa-adult) and present active sites of transcription. The coiled inactive parts are known as ***disks***. Giant chromosomes are well stained and easily observed, thus are used in genetic studies of chromosomes (genetic mapping).

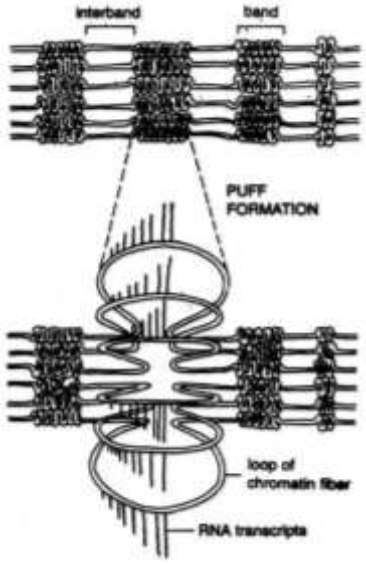


Fig. 32. Polytene chromosome structure.

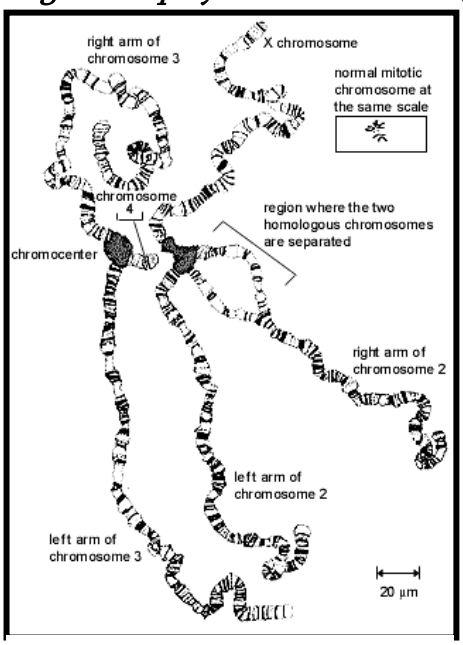


Fig. 33. Polytene vs. normal chromosomes in Drosopha.

4. **Aneuploidy (or heteroploidy)**. The cells with normal set of chromosomes are known to be ***euploid*** (somatic cells are diploid and germ cells are haploid). During cell division the spindle fiber formation can be disrupted, and this leads to non-disjunction of chromosomes

(chromatids) with subsequent unequal distribution of chromosomes to daughter cells. If the number of chromosomes between daughter cells differs in one, two, etc., then the genetic disorder is known as ***aneuploidy*** (2n±k), for example Down syndrome (47XX,21^{+}). The cells with this genome mutation have three copies of chromosome 21, instead of two homologues (Down syndrome is also known as trisomy of 21st chromosome). If the number of chromosomes is multiple of haploid set (e.g., 3n, 4n, etc.) this is known as ***polyploidy***. It is normally common in some plants and is abnormal for animals, humans.

Mitotic activity of cells

Mitotic activity of cells can be affected by various factors. The ***mitotic activity*** is defined as number of mitotically dividing cells among 1000 cells studied. **Factors regulating mitosis** are of two types: ***mitogenic*** and ***amitogenic***.

Mitogenic factors stimulate mitosis. There are exogenous factors (light, temperature, radiation) and endogenous factors (hormones of pituitary, thyroid and adrenal glands). ***Phytohemagglutinin*** (plant hormone) that stimulates leukocyte division is a mitogenic factor.

Amitogenic factors suppress or stop mitosis. For example, ***colchicin*** prevents polymerization of spindle fibers, ***keylons*** are used in treatment of benign tumor.

According to mitotic activity there are several types of tissues:

1. ***stable tissues*** – no mitosis is seen in the tissue cells (e.g. neurons, muscle cells);
2. ***growing tissues*** – mitotic division can be observed in some cells of the tissue (liver, kidney cells);
3. ***innovating tissues*** – mitotic divisions are observed in all the cells of the tissue, and newly formed cells replace the periodically dying out old cells (epithelial cells of intestines, epidermis, stem cells in bone marrow).

Apoptosis

The cells can die from various reasons including both exogenous and endogenous factors. Among exogenous factors there are infective pathogens (viruses, bacteria), high radiation and temperature, different chemicals; endogenous chemical example is bilirubin.

All multicellular organisms including humans carry genetic information about the **programmed death** of the cell, which is called **apoptosis**. Apoptosis is a biological phenomenon, which eliminates old and mutated cells (e.g., cancer cells) from the organism. In mature organism the apoptosis maintains relatively constant number of cells in the body. The program starts when the cell receives a signal from receptors of plasma membrane and transfers it to the nucleus. There is activation of genes that determine apoptosis, synthesis of specific enzymes, which destruct cell. In humans, apoptosis occurs by the help of lysosomal proteolytic enzymes of **caspase** family.

In some cases the apoptosis can be triggered prematurely, and in other pathological conditions it can be stopped. For example, HIV virus which causes acquired immunodeficiency syndrome (AIDS), binds to receptors of plasma membrane of T-lymphocytes and stimulates their apoptosis that cause immunocompromised condition.

A specific **protein p53** is one of the main anticancerogenic factors (tumor suppressor protein) in human organism, which stops the mitotic cycle of mutated cells in G1 and G2 stages, and

prevents onset of mitosis to allow the cell to repair mutations or enter apoptosis. Oncogenic viruses can impede the normal function of p53 protein, as a result the apoptosis fails. The cells start to divide continuously to form a tumor – a mass of abnormal cells.

IA

1. In S phase of interphase:
   A. mitotic spindle is formed
   B. DNA is replicated
   C. nucleolus dissolves
   D. nuclear membrane disappears
2. Nucleolar organizer is a:
   A. primary constriction of chromosome
   B. secondary constriction of chromosome
   C. telomere of chromosome
   D. satellite part of chromosome
3. Mitosis is disrupted if:
   A. mitotic spindle is damaged
   B. nuclear membrane is dissolved
   C. nucleolus disappears
   D. centrioles are replicated
4. Constitutive heterochromatin:
   A. carries information about proteins
   B. contains unique sequences of DNA
   C. is located next to centromeres region
   D. carries information about rRNA and tRNA
5. What process occurs in nucleolus?
   A. synthesis of tRNA
   B. synthesis of histones
   C. formation of ribosomal subunits
   D. synthesis of mRNA

IB

1. In G_2 phase dose not occur:
   A. syntheses of tubulin protein
   B. replication of mitochondrial DNA
   C. syntheses of histone proteins
   D. ATP syntheses

2. Which of the followings does not participate in mitosis?
   A. centrioles
   B. ribosomes
   C. microtubules
   D. chromosomes

3. There is a lack of euchromatin in:
   A. Transcriptionally active DNA
   B. less stained parts of chromosome
   C. centromere region
   D. arms of chromosome

4. Nuclear envelope does not participate in:
   A. connection of nucleus and cytoplasm
   B. exchange of substances
   C. separation of transcription from translation
   D. formation of ribosomal subunits

5. Denver classification of chromosomes does not allow to study:
   A. shape of chromosomes
   B. number of chromosomes
   C. individual chromosomes
   D. chromosome structure

II

1. The nucleolus:
1. has globular structure with its own membrane
2. consists of proteins and tRNA
3. is formed in nucleolar organizer
4. takes part in formation of ribosomal subunits
5. is not a permanent structure of nucleus

a. 1,2,3 b. 3,4,5 c. 1,3,4 d. 1,5

2. Euchromatin regions of chromosome are:
1. less stained
2. intensively stained
3. highly condensed
4. less condensed
5. transcriptionally active

a. 1,4,5 b. 2,3,4 c. 3,5 d. 2,5

3. Facultative heterochromation:
1. is less condensed part of chromosome
2. can not convert to euchromatin
3. can convert to euchromatin
4. is presented as one X chromosome in female
5. makes Barr body

a. 1,2,3 b.3,4,5 c. 1,4,5 d. 2,5

4. Binding of histones to DNA in chromosomes:
1. enables the transcription
2. disable the transcription
3. suppress the activity of the genes
4. stimulate the activity of the genes
5. increases the length of DNA molecule

a. 2,3 b. 1,2,3 c. 3,4,5 d. 4,5

5. The stable tissues:
1. contain G_0 cells
2. are composed by neural cells
3. have the higher mitotic activity
4. have the lower mitotic activity
5. usually are not dividing

a. 1,2 b. 3,4 c. 1,4 d. 1,2,5

CHAPTER 6
Reproduction. Meiosis. Gametogenesis. Gametes. Fertilization. Parthenogenesis

Reproduction is the process by which an organism produces an individual of its kind and continues the species. There are two main types of reproduction: asexual and sexual. They are common for both unicellular organisms **(monocytogenic reproduction)** and multicellular organisms **(polycytogenic reproduction)**.

Asexual reproduction is realized by one parental organism, without gametes (germ cells), and the offspring is numerous and identical to mother organism.

Types of monocytogenic asexual reproduction:

a. Binary fission – division of cell into two daughter cells by mitosis (protists – amoeba, paramecium) or amitosis (bacteria).
b. Schizogony or multiple fission – first the nucleus is divided into multiple pieces; then each of them is surrounded by cytoplasm and gives rise to multiple offspring (e.g., malarial Plasmodium).
c. Budding – the parent cell gives off a small outgrowth (bud), which then pinches off as a new cell (e.g., yeasts).
d. Sporogony – reproduction of haploid cells of malarial agent (Plasmodium) producing sporozoites in mosquito.

Types of polycytogenic asexual reproduction:

a. Fragmentation – division of the body into fragments, which create a new organism by regeneration of missing parts (ringworms).
b. Strobilation – division of the body into equal fragments (coelenterates).
c. Budding – overgrowth of cells' group from mother organism that detaches and develops as an individual (e.g., hydra).
d. Polyembryony – in early stages of development (stages of 8-16 blastomeres) the embryo (developed in a result of sexual reproduction) divides into several parts, which then develop identical organisms (common in armadillo; also monozygotic or identical twins in humans).
e. Vegetative reproduction – it is peculiar for plants that reproduce via vegetative organs (e.g., root, stem, leaf).

For **sexual reproduction,** usually two parents and germ cells (gametes) are required. The offspring expresses variation, since gametes develop by meiosis.

Monocytogenic sexual reproduction is of two types – ***conjugation*** and ***copulation.***

Conjugation occurs in bacteria and Paramecium. In bacteria conjugation is one-way process, whereby two bacteria (donor and recipient) come in contact and the donor transfers genetic material to recipient.

In *Paramecium* conjugation is a bidirectional process. It is the temporary union of two cells with exchange of their genetic material through conjugation tube, which results in recombination between two organisms.

Copulation is a sexual process in unicellulars. Two individuals acquire sexual differences, i.e. transform into gametes, and fuse into a zygote. At initial stages of evolution the gametes were similar and were referred to as isogametes (the process – *isogamy,* e.g., in unicellular algae). Later

they differed in sizes (*anisogamy* – in Volvox). In some species the difference is too expressed; female gamete is much larger and immotile compared to the small and motile male gamete. This particular case of anisogamy is called *oogamy* (in Plasmodium).

The types of ***polycytogenic sexual*** reproduction are: ***fertilization*** and ***parthenogenesis (without fertilization).***

Gametes that fuse during fertilization are formed by gametogenesis, which occurs by meiosis.

Meiosis

Meiosis is the division of germ cell precursors (*2n4c*), which results in haploid germ cells or gametes (*nc*). During **meiosis** the number of chromosomes is reduced twice (*meios* - reduction), from diploid (2n) to haploid (n).

Meiosis consists of two subsequent divisions (meiosis I, II). A rest stage in between them is called ***interkinesis***, where no DNA replication occurs. Meiosis I and II consist of 4 stages (prophase, metaphase, anaphase, telophase).

Meiosis I

Prophase I. In addition to condensation of chromatin, dissolving of nuclear membrane and nucleolus, and centrioles movement to the poles, there is ***conjugation*** of homologues (the homologues make pairs), and an important event called ***crossing over*** (exchange of homologous loci between non-sister chromatids of homologous chromosomes). Crossing over results in recombination between maternal and paternal genes and contributes to ***recombination variation***.

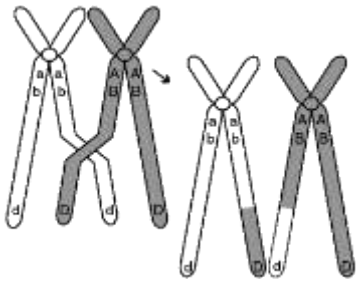


Fig. 34. Crossing over

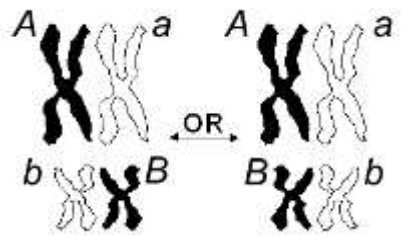


Fig. 35. Independent assortment of two pairs of chromosomes.

Metaphase I. Pairs of homologous chromosomes line up randomly the metaphase plate, and spindle fibers attach to the centromeres. ***Independent assortment*** of non-homologous chromosomes takes place, which is also a source of ***recombination variation***. The number of possible recombinations of non-homologous chromosomes in the gametes depends on haploid set, and is equal to 2^n (in humans it is 2^{23}, which is over 8 millions).

Anaphase I. The homologous chromosomes are disjuncted rather than chromatids.

Telophase I. Each pole of the cell has a haploid nucleus with chromosomes consisted of sister chromatids (**n2c**).

Meiosis II is resembling mitosis with a difference that the dividing cells are haploid (**n2c**), and from one such a cell two haploid cells are formed (**nc**), with chromosomes consisted of a single chromatid.

Biological Significance of meiosis

1. Meiosis reduces twice the diploid set of chromosomes and forms haploid gametes. This is important since the fertilization restores the diploid set in the zygote, which then cleaves by mitosis. Meiosis, fertilization and mitosis together provide constant number of chromosomes through generations of organisms and species.

2. Genetic variation (recombinations) due to crossing over and independent assortment of non-homologous chromosomes provides higher adaptability of offspring.

Gametogenesis

Gametogenesis is the process of producing haploid gametes (ovum and sperm).

Spermatogenesis is the process of sperm production, which takes place in testes – male gonads (sex glands). *Oogenesis* is the process of egg production that occurs in ovaries – female gonads.

Spermatogenesis: Occurs in the seminiferous tubules of the testes. It proceeds through 4 zones of development:

a) at the outer edge (the wall) of the tubule – in ***multiplication zone***, there are diploid cells called *spermatogonia* – the primordial cells that undergo rounds of mitotic divisions to maintain their number.

b) the spermatogonia differentiate into *primary spermatocytes* (2n), which grow in sizes in the ***growth zone*** of tubular wall.

c) in ***maturation zone*** the primary spermatocyte (2n4c) undergoes first meiotic division and converts into two *secondary spermatocytes* (1n2c) with haploid set of chromosomes but each consisting of 2 identical chromatids. Here the second meiotic division produces 4 *spermatids*, with ***nc*** genetic information each.

d) in ***transformation zone*** spermatids then differentiate into mature *sperm*. The process is known as ***spermiogenesis***. During spermiogenesis the nucleus of spermatids condenses, Golgi

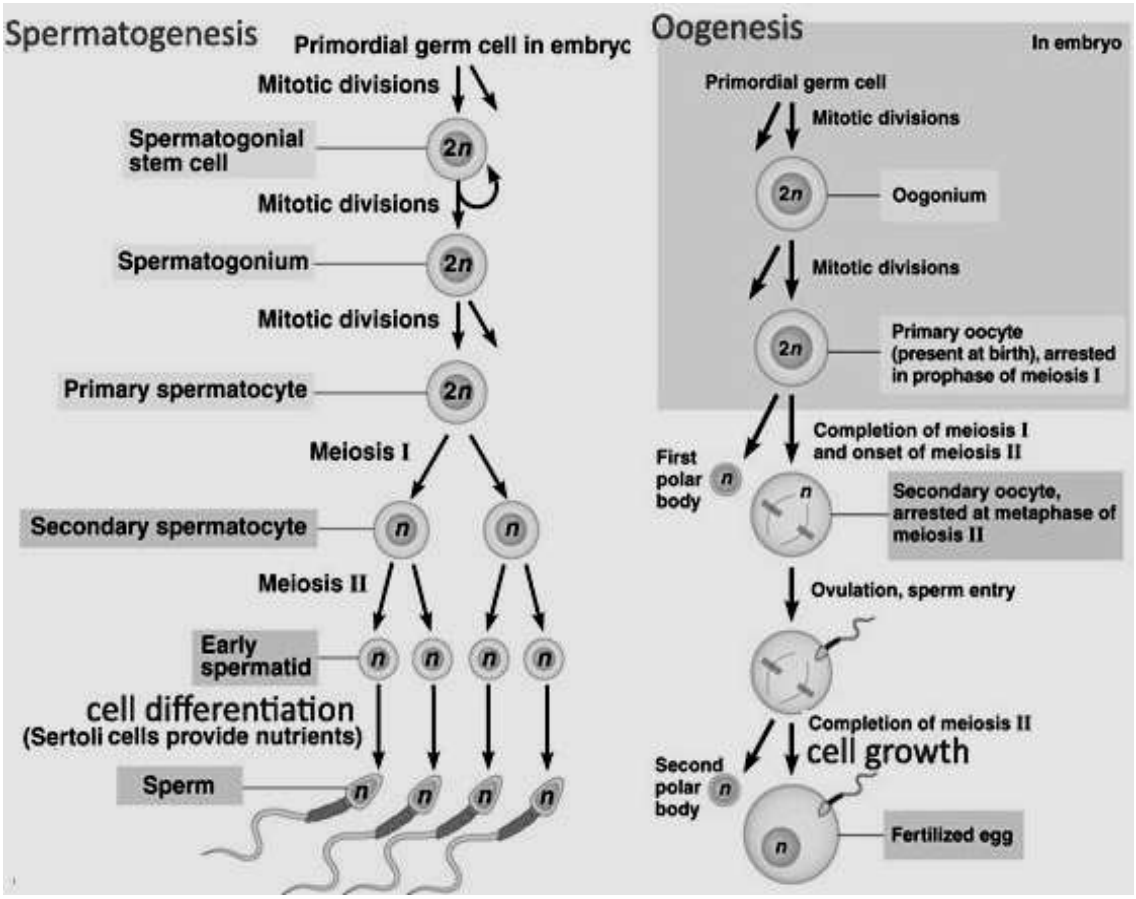


Fig. 36. Gametogenesis.

apparatus is modified to acrosome containing proteolytic enzymes (hyaluronidase), a flagellum is formed, and the mature sperms move to the lumen of the tubule.

Oogenesis: In human being*, oogonia* are formed from primordial germ cells by the second month of embryogenesis and increase in numbers by mitosis (***multiplication zone***). The sizes of oogonia grow and form *primary oocytes* (2n4c) in the ***growth zone***. Here they are arrested as primary oocytes in prophase I (**dictyotene stage**). At puberty there are about 400.000 primary oocytes that remain suspended in this arrest stage until meiosis I is completed at the time of first ovulation (puberty period by about 12 years of age). It results in a single *secondary oocyte* and first *polar body* (n2c each) and is completed once per month (***menstrual cycle***) until menopause. Second meiotic division is completed only if fertilization occurs. At fertilization, from a secondary oocyte the meiosis II produces a fertilized *ovum* (zygote) and a small second polar body (nc). The first polar body may also divide to produce two more polar bodies. Totally, an ovum and three polar bodies are formed from a primary oocyte.

Comparison of oogenesis and spermatogenesis

1. In oogenesis, during **dictyotene**, the rRNA genes of chromosomes are replicated for multiple times (this is called **gene amplification),** undergo decondensation (transcriptional activation) and transform into **lampbrush chromosomes.** Enormous number of rRNA is produced on the uncoiled fragments of DNA, which will provide synthesis of embryonic proteins in the oocyte.

   The longer the dictyotene stage lasts for, the higher is the rate of influence of mutagenic environmental factors on the prophaseI-suspended oocyte. This increases the frequency of non-disjunctions and chromosomal diseases (most common is Down syndrome). The frequency of ***Down syndrome*** occurrence increases especially in females over 35 years of age.
2. The spermatogenesis starts since puberty and continues almost during whole life. While in female, oogenesis (division of oogonia in multiplication zone) starts in a 2-3 month fetus and stops at prophase I of meiotic division (dictyotene) until ovulation. Second meiotic division occurs just after fertilization.
3. The transformation stage is absent in oogenesis.
4. Each primary spermatocyte gives rise to four sperms. And each primary oocyte gives rise to 1 ovum and 3 polar bodies. During female life span about 450-500 ova can be formed, and the male produces billions of sperm cells.

Characteristics of gametes

a. Gametes are haploid cells that have slow metabolism, which is then increased in fertilized egg. Gametes enter no mitotic cycle, only fertilized egg undergoes division (cleavage).

Ovum is characterised by ***ovocytoplasmic segregation***; the selective distribution of oval cytoplasm before fertilization into different parts, which gives rise to different tissues and organs during differentiation.

The human ovum is about 170 mkm in size. It is covered by ***follicle cells*** that were released along with the egg during ovulation (release of the ovum from follicle of ovary to fallopian tube). A capacitated sperm cell must migrate through this layer of follicle cells before it reaches the ***zona pellucida***, the layer of extracellular matrix of the egg over its plasma membrane. Under the plasma

membrane is the cortical layer of cytoplasm that is rich in microtubules and microfilaments, as well as cortical granules containing biologically active substances.

The ova are of following types according to the distribution of yolk (*lecithine*):

a) *isolecithal* – equal distribution in cytoplasm (lanceolate),
b) *centrolecithal* – yolk is concentrated in the centre of egg (insects),
c) *telolecithal* – peripheral distribution on the vegetal pole (birds, reptiles),
d) *alecithal* – almost no yolk is present (mammals).

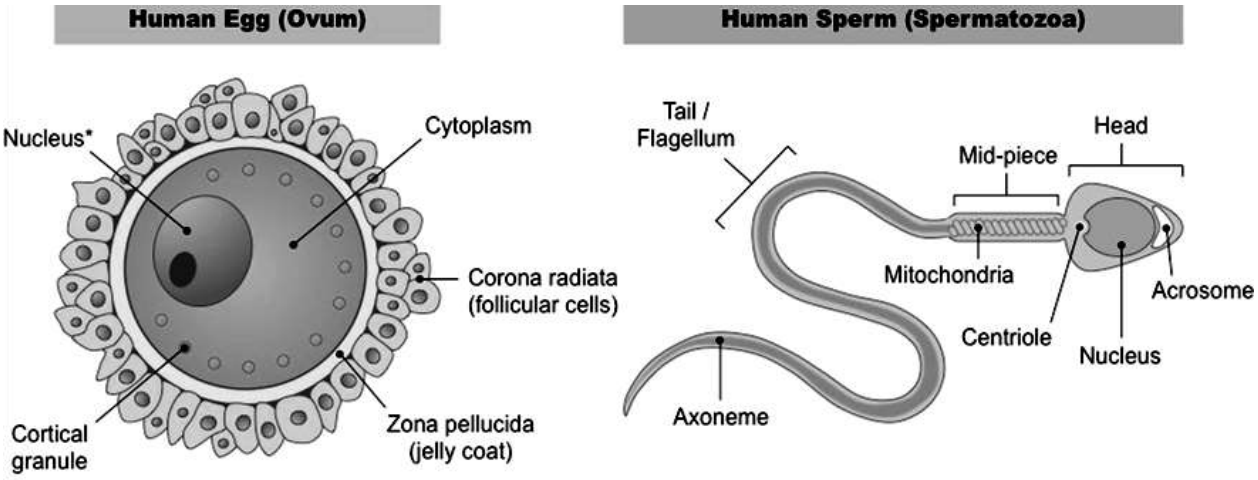


Fig. 37. Structure of ovum and sperm.

A human **sperm** cell is about 70 mkm in size. It has a head, neck and tail. The **head** containing the haploid nucleus is tipped with a special body, the **acrosome** (a modified Golgi complex), which contains enzymes that help the sperm penetrate into the egg. Behind the head, is the neck part with a single large mitochondrion that provides ATP for movement of the tail, and two centrioles, one of which enters the egg along with neck during fertilization, and the second is attached to tail. Tail is a flagellum consisted of microtubules.

Fertilization

Fertilization is a process of fusion of male and female haploid gametes resulting in diploid zygote, which gives rise to a new organism. The process consists of 3 phases:

1. attraction of gametes by copulation and capacitation;
2. egg activation (acrosomal and cortical reactions);
3. fusion of haploid nuclei of gametes, or *syngamy.*

Sperm transfer to fallopian tube is accomplished by copulation. Secretions (*gamons*) in mammalian female reproductive tract alter certain molecules on the surface of sperm cells and increase its motility. This enhancement of the sperm function in the female reproductive system is called a ***capacitation***, which requires about 6 hours in humans. Capacitation helps to attract gametes.

The binding of the sperm head to egg receptors induces the acrosome to release its contents by exocytosis in an **acrosomal reaction**: the proteolytic enzymes spilled from the acrosome enable the sperm cell to penetrate through the layer of follicle cells to the *zona pellucida,* then reach and

fuse with the plasma membrane of the egg. The binding of the sperm cell to the egg triggers depolarization of the egg membrane, which functions as a ***fast block to polyspermy***, because it prevents more than one sperm from fusing with the egg plasma membrane.

Another major effect of the fusion of egg and sperm plasma membranes is the **cortical reaction**, a series of changes in the cortical zone of the egg cytoplasm (just below the cell membrane) rich in cortical granules. The fusion of egg and sperm triggers a signal-transduction pathway that causes the egg endoplasmic reticulum to release Ca^{2+} into cytoplasm. The high concentration of Ca^{2+} brings about a change in cortical granules, which lie just under the egg's plasma membrane. In cortical reaction the granules of the cortex of the egg release their contents to the outside of the cell. Enzymes released from the granules, catalyze alterations of the zona pellucida, which then functions as a ***slow block to polyspermy***.

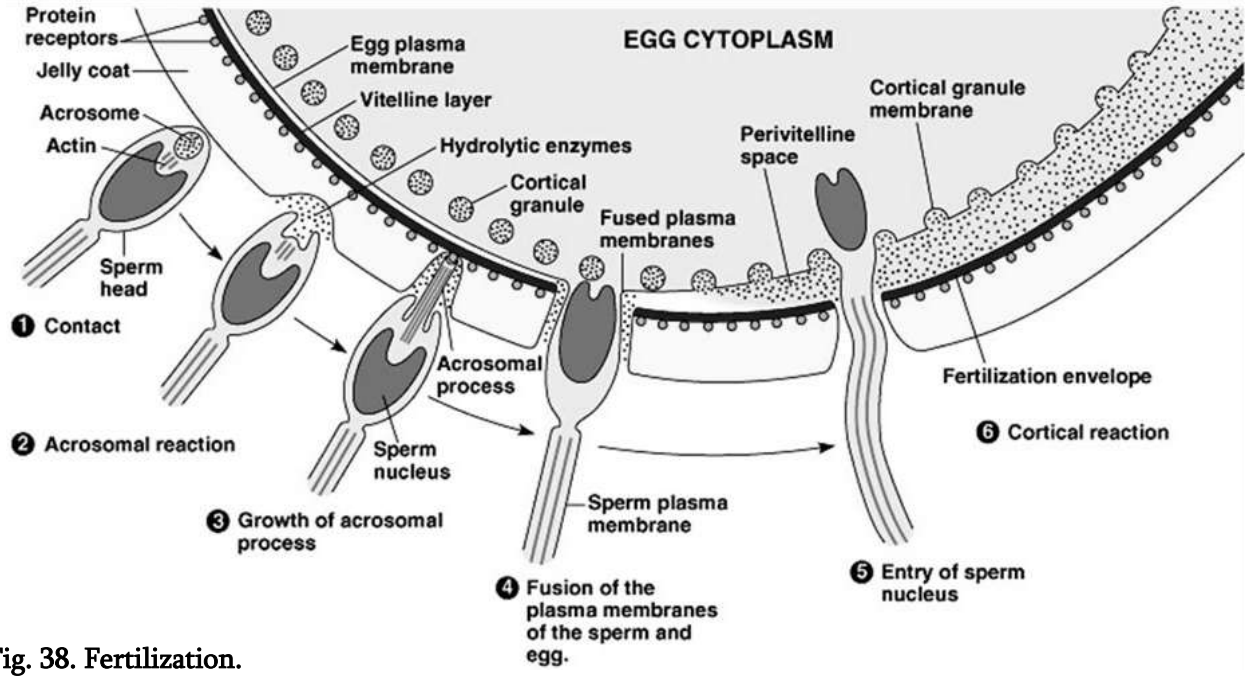


Fig. 38. Fertilization.

After penetration of sperm nucleus, which is known as male pronucleus, it fuses with female pronucleus thus forming a *synkaryon*: syngamy or synkaryogamy occurs. Just before fusion the meiosis II is completed in the ovum and the diploid set of chromosomes is restored in a zygote. The fertilization is said to be random, for example, in humans the given sperm, which is a one out of 2^{23} fertilizes an ovum, which is also 1 out of 2^{23} possible. So there are $1/2^{23} \times 1/2^{23}$ possible chromosome combinations in different zygotes. In addition to crossing over and independent assortment of chromosomes in meiosis, the random fertilization is also a source of recombination variation.

The zygote which undergoes fast, subsequent equal mitotic divisions (not followed by growth of daughter cells) called ***cleavage***. The fertilization of ovum occurs in fallopian tube, after which the zygote cleaves and moves to the uterus. This is where the ***implantation*** takes place in about 5-6 days following the fertilization.

In human, only one sperm out of thousands surrounding the egg fertilizes it. The ovum is viable for fertilization for about 24 hours following the ovulation, while the sperm cells – till 48 hours after they enter the female reproductive tract.

The fertilized egg is a **totipotent** cell, which means it has a potential to develop all types of tissues and a whole organism by further cleavage and differentiation.

Parthenogenesis

Development of an organism from a non-fertilized egg is known as parthenogenesis (*parthenos* – virgin, *genesis* – origin). Biological significance of parthenogenesis is the maintenance of the species, which during evolution have had obstacles (natural barriers such as mountains, lakes, etc.) in encountering partners.

Types of parthenogenesis are:

1. Natural and artificial
2. Obligate and facultative
3. Haploid and diploid
4. Gynogenesis and androgenesis.

Parthenogenetic development of an egg under natural conditions is called ***natural parthenogenesis***. Parthenogenesis produced experimentally in the laboratory is called ***artificial parthenogenesis***. For example, it can be caused by mechanical irritation of an egg, treating with chemicals (Ca^{2+}, acids, bases), exposing to high temperature, etc. Natural parthenogenesis can be ***obligatory*** and ***facultative***. In few species of animals the parthenogenesis is the only way of reproduction. This type of parthenogenesis is called obligatory. Populations having obligatory parthenogenesis consist entirely of females, for example, Caucasian lizard. Facultative parthenogenesis is peculiar for daphnia (microscopic aquatic arthropod) – the parthenogenetic reproduction of female individuals in summer switches into reproduction by fertilization in autumn (cyclic mode).

Haploid parthenogenesis is found in many insects (drones, male ants etc.). Haploid egg develops into haploid offspring that always develop into males. The fertilized eggs, being diploid, develop into females.

In ***diploid*** *parthenogenesis* the eggs either do not undergo any reduction division or undergo only incomplete meiosis without reduction of chromosome number. It occurs in some arthropods, e.g. daphnia, cyclops.

Gynogenesis refers to the species, which develop from unfertilized ovum. The ovum is just activated by sperm, which triggers oval cytoplasm to cleave, but it does not fuse with female pronucleus, for example in turkeys.

Androgenesis is found only in artificial parthenogenesis pattern when the organism develops from only sperm pronucleus. The egg is enucleated and is exposed to sperm cells. Haploid pronuclei of two sperms fuse in egg cytoplasm, and male individual develops (e.g., silk worm).

1. Multiple sperm penetration into egg is prevented by:

A. substances secreted by the sperm
B. cortical reaction
C. autodigestion in lysosomes
D. degeneration of fertilization membrane

2. Homologous chromosomes move to opposite poles of a dividing cell during:

A. mitosis
B. meiosis I
C. meiosis II
D. binary fission

3. What is common for asexual reproduction?

A. single individual is a parent
B. no gametes take part
C. the offspring is identical
D. all the answers are correct

4. Gametogenesis is the:

A. mitotic division of somatic cells
B. zygote formation
C. meiotic division of somatic cells
D. development of germ cells

5. Significance of meiosis is following:

A. keeping the diploid set in somatic cells
B. twice the reduction of chromosome set
C. source of genome mutations
D. twice the increase of chromosome set

IB

1. Gametes are not characterized by:

A. diploidy
B. slow metabolism
C. ability to fuse
D. haploidy

2. Meiosis does not result in:

A. germ cells
B.haploid cells
C. reduction of chromosome number
D. somatic cells

3. What is not common for zygote?

A. high mitotic activity
B. cleavage
C. low mitotic activity
D. diploidy

4. Which of the following is not a monocytogenic asexual type of reproduction?

A. copulation
B. schizogony
C. budding
D. binary fission

5. What is not referred to ovum?

A. slow metabolism
B. its formation by meiosis
C. diploid set of chromosomes
D. ability to fuse with sperm

II

1. Fertilization is:

1. fusion of male and female germ cells
2. process of attraction of gametes, egg activation, syngamy
3. a type of parthenogenesis
4. leads to zygote formation
5. a type of sexual reproduction

A. 3,4 B. 1,2,4,5 C. 1,2,5 D. 2,3,5

2. During spermiogenesis:

1. spermatids are formed
2. sperms are formed
3. sperm nucleus condenses
4. sperm tail is formed
5. Golgi complex transforms to acrosome

A. 2,5 B. 1,4,5 C. 2,3,4 D. 2,3,4,5

3. Zygote is formed due to:

1. spermatogenesis
2. fusion of gametes
3. mitosis of somatic cells
4. fertilization
5. ovogenesis

A. 2,4 B. 1,5 C. 3,4 D. 2,3

4. The types of parthenogenesis are:

1. constitutive
2. artificial
3. androgenesis
4. facultative
5. schizogony

A. 2,3,4 B. 1,2,4 C. 3,5 D. 1,3,5

5. In contrast to sexual reproduction the asexual reproduction provides:

1. uniform generation
2. high adaptation of generation
3. no variation
4. numerous generation
5. participation of 2 parents

A. 1,3,4 B. 1,5 C. 2,4,5 D. 1,4,5

GENETICS

CHAPTER 7

History of Development of Genetics. Language (terminology) of Genetics. Monohybrid Cross. Mendelian Laws of Uniformity and Segregation. Extensions to Segregation Law. Blood Groups and Rhesus Factor

Genetics is a science that studies different patterns of inheritance, the ways and principles of transmission of inherited traits. The history of development of Genetics is divided into three main periods:

The first period begins with the discovery of main laws of Genetics by Gregor Mendel (in 1865). He demonstrated that parents pass onto their offspring discrete heritable factors, which retain their individuality through generations.

In 1909, Danish scientist W. Johanssen came up with the term "gene" to denote the basic unit of heredity. By this period all genetic studies were conducted on organism level.

At the second period the genetic studies were done on cellular level (cytogenetics). Thomas Morgan and his colleagues discovered linked inheritance (in 1911) and proposed the chromosomal theory of inheritance.

The third period is characterized by the development of molecular Genetics. In 1941, G. Beadle and E. Tatum concluded that each gene provides synthesis of an enzyme: "one gene – one enzyme". In 1953, J. Watson and F. Crick proposed the structure of DNA and formed the basis for molecular genetics. In 1970s, a new direction of genetics – *gene engineering,* was developed. During 1990s nearly 6000 human genes have been mapped. By 2003, 30.000 human genes' sequencing was completed in Human Genome Project.

Along with Genetics progress various disciplines evolved.

- *Human Genetics* studies the genes during ontogeny and evolution process as well as the heredity and variation on molecular, cellular, organism and population levels of organization.
- *Medical Genetics* is interrelated with medicine. The knowledge about the mechanisms of disease can lead to prevention and treatment of the disorder.
- *Molecular Genetics* studies the role of the nucleic acids in maintenance and realization of genetic information, the mechanisms of regulation of genes activity, etc.
- *Cytogenetics* studies chromosomes in cells. In particular, the chromosome structure and alterations in number can be studies by karyotyping.
- *Population Genetics* studies the frequencies of occurrence of the alleles, genotypes and phenotypes in a given population. It helps to determine the carrier (heterozygotes) frequencies and mutation rate when the disease incidence is known.
- *Pharmacogenetics* is an important branch of genetics that studies genetically determined individual differences in response to drugs in humans.

Terminology of Genetics

Heredity is the ability to inherit traits and provide with similarity between parents and offspring generations. In fact, heredity makes possible the continuity of living organisms during alternation of generations. It tightly correlates with process of reproduction. The sexual reproduction leads to formation of individuals from male and female germ cells – gametes. However, the offspring does not completely resemble the parents' appearance and somehow differs from them showing a **variation**. It is a result of gene recombination and serves as a biological basis for the evolution of new species, as well as for development of adaptation. During asexual reproduction a new organism is formed from a single or group of somatic cells and is as same as the mother cell(s). Thus, heredity and variation are the two essential factors which maintain **similarity** and **diversity** of the living organisms.

Inheritance is the process of transmission of the hereditary traits.

Genotype is the genetic constitution of an individual, which is formed during fertilization when the maternal and paternal alleles join in the zygote. That is why all the organisms (except identical twins) differ in their genotypes.

Phenotype is the set of traits of an organism resulting from the combined action of genotype and environment.

Character and trait. Character is determined as a heritable feature (such as eye color) that varies among individuals. Each variant for a character (such as blue eye, brown eye) is called a **trait**.

Genome is a full set of genes in the haploid set of chromosomes of an individual. Genome is also a characteristic of a species.

Alleles are the alternative variants of genes, which detect alternate traits of same character (left and right handedness). **Allelic genes** are located on homologous loci of the homologous chromosomes in somatic cells. Allelic genes develop due to mutation. The mutant allele usually develops from wild one in a result of a direct mutation (A→a). Due to reverse mutation rarely the mutant allele can transform into wild type (a→A).

If the two parental alleles are identical for a particular character, the organism is said to be **homozygous (AA or aa)**. When the paired genes denote the contrasting (alternative) features of the same character, the organism is called **heterozygous (Aa)** for that character.

A character is said to be **dominant** if the allele expresses the same effect in both heterozygous and homozygous state (e.g., brown eyes are provided by AA and Aa). A character is said to be **recessive** if the allele expresses an effect only in the homozygous (blue eye).

Mendel's Laws of Inheritance. Monohybrid Cross

Mendel work can be considered as the discovery of genes and the pattern of their inheritance. In acknowledgement of his enormous contribution the term **Mendelian** is now applied both to different patterns of inheritance (Mendelian inheritance) shown by single gene characteristics and to disorders resulted from defects in a single gene (***monogenic*** trait **or *Mendelian trait***).

On the basis of Mendel's experiments three main principles were established. They are known as the Law of Uniformity (Law of Dominance), the Law of Segregation and the Law of Independent Assortment.

Law of Uniformity (Law of Dominance)

The law of uniformity states that all the first generation (F_1, F – *filia*, children) of monohybrid cross expresses identical dominant phenotype.

A ***monohybrid cross*** is a cross between individuals with one pair of alternate traits, or a cross between hybrids of single *(mono)* trait.

For example, brown eye color is dominant (A) over blue eye color (a) which is a recessive trait. If one of the parents is homozygous for brown eyes (AA), and second parent has blue eyes (aa), then all of their children (F_1) will have brown eyes and will be heterozygous for the trait (Aa).

P AA x aa
G A a
F_1 Aa 100%

Law of Segregation

In the second generation of monohybrid cross (a cross between F_1 hybrids), there is a segregation of traits in F_2 generation by 3:1 ratio. For example, in the generation of parents who are both heterozygous for right-handedness Aa (dominant trait), the 75% (3/4) of the offspring present with that trait, and in 25% (1/4) left-handedness is expressed – a recessive trait (aa) that was absent in parents. Genotypic segregation is as 1:2:1 or 1AA:2Aa:1aa.

P AA x aa
F_1 Aa 100%
P_{F1} Aa x Aa
G A a A a
F_2 1AA : 2Aa : 1aa

During meiosis each of the two alleles is transmitted (segregated) into different gametes, and the maternal and paternal alleles combine randomly in fertilization when the zygote is formed.

Test Cross

Since the dominant phenotype can be determined by both homozygous (AA) and heterozygous (Aa) genotypes (AA=Aa), often it is important to find out the genotype of that dominant trait. To test this, that organism is crossed with an individual of recessive trait (recessive homozygote). For example, black rabbit can be homozygote (AA) and heterozygote (Aa). To clarify the genotype, it may be crossed with a recessive white rabbit (aa).

P AA x aa
G A a
F_1 Aa 100%

P Aa x aa
G A a a
F_1 Aa : aa
50% 50%

If the offspring does not show off any segregation (i.e. it is uniform), then the tested organism was homozygote. If the offspring shows segregation as 1:1, then the organism with dominant trait was heterozygote.

Test cross is not applicable in humans.

Extensions to Mendel's Law of Segregation

Sometimes the expected ratio 3:1 is not expressed in a monohybrid cross, and in these cases the law of segregation is said to be extended. The cases of extensions to the Mendel law of segregation are: incomplete dominance, lethal genes, multiple alleles and codominance

Incomplete Dominance

In addition to complete dominance described by Mendel (dominant allele completely suppresses the expression of recessive one in heterozygote condition), some genes can show also incomplete dominance, in which the phenotype of the heterozygous is intermediate between that of either homozygote.

The heterozygote expression is usually measured as a range, such as a level of an enzyme that lies between the normal amount and complete absence. A more obvious incomplete dominant trait in humans is the wavy hair. The homozygote dominant condition is the curly hair, and the homozygote recessive phenotype is straight hair. The heterozygote has wavy hair.

A classic example of incomplete dominance occurs in the snapdragon plant. A red-flowered plant of genotype *RR* crossed to a white-flowered *rr* plant can give rise to a *Rr* plant, which has pink flowers (RR>Rr>rr).

Familial hypercholesterolemia. A person with disease-causing two dominant alleles (AA) lacks the receptors on liver cells that take up cholesterol from blood. A heterozygous person (Aa) has half the number of receptors, and only homozygote recessives (aa) are normal. The dominant homozygotes die still children from heart attack (hypercholesterolemia increases the risk of cardiovascular mortality due to coronary vessel obstruction), while the heart attack in heterozygotes can occur in adult age.

Cystinuria (increased concentration of cystine) also has a pattern of intermediate inheritance between cystine stone disease AA (recurring formation of cystine stones in urinary tract) and the normal state (aa). Since cystine is the most insoluble amino acid (derivate of cystein amino acid), the elevated urinary cystine predisposes to the formation of renal calculi (kidney stones). Complications of kidney stones include infection, hypertension, renal failure. Cystinuria is caused by mutation of genes coding for amino acid transporter enzymes present on plasma membrane of epithelial cells of proximal tubules of the nephron that results in disruption of amino acid reabsorbtion.

Lethal genes

Most of the mutations are usually harmful, and some of them are not compatible with life and cause death of an organism in homozygote condition (*AA* or *aa*). They are known as lethal genes, and expected Mendelian ratio of 3:1 is altered into 2:1. There are two types of lethal genes: dominant and recessive. The change in gene structure leads to an abnormal enzyme synthesis. The effect of these mutant genes can interrupt the embryonal development leading to death (abortion), and the genes are known as *lethal*. If the mutant genes affect the vital functions later after birth, such genes are called *sublethal*. An example of a dominant lethal gene is ***brachydactylia*** (short fingers). Homozygote dominants (AA) die still embryos in a result of skeletal abnormalities incompatible with life. Heterozygotes (Aa) present only with short fingers, and the recessive homozygotes (aa) are normal.

Sickle-cell anemia is inherited by a dominant sublethal gene, since dominant homozygote genotype (HbSHbS) may lead to death in few years because of severe anemia and cardiovascular, cerebral complications. People with sickle-cell anemia have sickle hemoglobin (HbS) due to substitution of glutamic amino acid (Glu) by valin amino acid (Val).This turns the normal (double-concave) RBC into sickle-shaped and hinders circulation of these RBCs through capillaries.

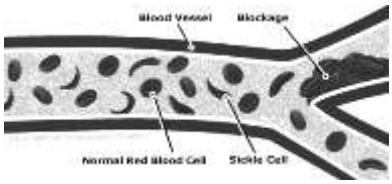


Fig. 39. Sickled RBCs in a capillary.

The mutation is present in African tropics, where a severe type of malaria (*falciparum* malaria) is also common. However, the population is resistant to the latter since *Plasmodium falciparum* cannot parasitize in sickled RBCs.

Cystic fibrosis and ***Tay-Sachs' disease*** are recessive mutations with sublethal effects. The children affected with Tay-Sachs' disease (*aa*) can die until 3-4 years of life because of accumulation of lipids in brain (lysosomal storage disease).

People suffering from cystic fibrosis sublethal mutation have a combination of symptoms including stiflingly sticky mucus in the lungs and bronchi, severe respiratory infections (pneumonia, bronchitis), and poor weight gain due to insufficient pancreatic function.

Multiple Alleles and ABO Blood Groups

Multiple alleles develop when three or more alleles of the same gene in the same locus occur in a result of mutations. A common example of multiple alleles are ABO blood groups, which are expressed due to three different alleles: I^A, I^B and I^O, the first two are dominant to I^O. There are four types of blood groups in ABO system are: O, A, B, and AB. They are differentiated depending on various combinations of antigens and antibodies in human blood. The two antigens – A and B are found on the cell membrane of the RBCs and are provided by I^A and I^B alleles, respectively. The natural antibodies – anti-A (α-agglutinin) and anti-B (β-agglutinin) are found in blood serum. Below are presented the 4 blood groups with respective genotypes, antigens and antibodies:

Table 1. ABO Blood Groups.

Phenotype (blood group)	Genotype	Antigen (on RBCs)	Antibody (in blood serum)
O	I^OI^O	None	anti-A and anti-B
A	I^AI^A, I^AI^O	A antigen	anti-B
B	I^BI^B, I^BI^O	B antigen	anti-A
AB	I^AI^B	both A and B antigens	none

A phenomenon of simultaneous expression of different dominant alleles located in homologous loci is known as a **codominance**. The AB blood group is based on expression of codominant alleles (I^AI^B). The AB blood group individuals show off both antigens equally.

During incompatible blood transfusion immunological reaction between the same antigen and antibody (e.g. A and anti-A) will cause agglutination of RBCs (cells clump together). Compatibility means that the recipient and donor in a transfusion belong to the same group.

Blood typing was once used in forensic medicine to identify paternity (it can be rejected but not confirmed) when the identity of the father is suspected, but not known for certain. Today, paternity is more likely to be proven by DNA fingerprinting – a procedure that is more precise but is much more expensive.

Rhesus (Rh) Factor

There are many other blood grouping systems known in addition to the ABO series. Another important blood group system is the Rhesus. Rh factor was discovered in 1940 in blood from rhesus monkey. The Rh factor is a protein substance in RBC membrane present in many people (about 85%). Such blood was typed Rh positive (Rh+). The blood of the remaining 15% that lacked the factor was typed as Rh negative (Rh-).

Rh factor is detected by three pairs of alleles (C, D, E) completely linked on number 1 chromosome (no crossing over between them), hence it is inherited as a monogenic (Mendelian) trait and is determined by presence of main antigen D. Rh+ is dominant over Rh-.

Rh-incompatibility develops in case of blood transfusion from Rh+ to Rh-, as well as between Rh-negative mother (dd) and Rh+ fetus (the father is Rh positive (DD or Dd)). The child of first pregnancy is usually not harmed but during delivery, some of the fetal Rh-positive RBCs (from umbilical cord) may get into mother's bloodstream through ruptured capillaries of placenta. Since the child's RBCs containing the Rh factor are foreign to the mother's immune system, she produces antibodies against them. Trigger of mother's immune response is referred to as sensitization. The anti-rhesus antibodies (immunoglobulins) penetrate easily to fetal bloodstream during next pregnancy. And when mother's antibodies pass into the circulation of subsequent fetuses, they may destruct the fetal RBCs (hemolysis), causing a severe ***hemolytic disease of newborns (HDN)*** known also as ***erythroblastosis fetalis (immature or blast forms of RBCs develop in blood)***. If the hemolysis is massive, it leads to anemia and high blood count of bilirubin (metabolite of hemoglobin). Bilirubin is a yellow pigment, and high bilirubin causes jaundice. Moreover, high amount of bilirubin damages also the brain. Sometimes there can be dead births.

HDN can be prevented for many Rh^- women, if they are not already sensitized. Anti-Rh immunoglobulin (RhoGam) is given to mother via injection within 72 hours of delivery to suppress her ability to react to the fetal Rh-positive RBCs. Anti-Rh-antibodies also should be given to an Rh-negative woman after a miscarriage, an induced abortion, or a blood transfusion with Rh-positive blood. Injection must be repeated with each pregnancy (starting from the first one). Treatment of HDN is realized by exchange blood transfusion (transfusion of Rh negative blood of the same group following the removal of fetal blood portions).

IA

1. An organism with contrasting alleles at one or more loci is:
   A. homozygote
   B. hemizygote
   C. heterozygote
   D. monozygote
2. Cross that tracks the inheritance of a single character is:
   A. monohybrid cross
   B. dihybrid cross
   C. test cross
   D. none of the above
3. Lethal genes cause death in:
   A. embryonal development
   B. childhood
   C. adulthood
   D. birth
4. A human with type O blood group has:
A. both A and B antigens
B. only A antigens
C. neither A nor B antigens
D. only B antigens
5. Prevention of Rh conflict is possible by injection of:
   A. antibiotics
   B. anti-Rh-antibodies
   C. Rh antigen
   D. vaccine

IB

1. Genotype is not:
   A. genetic make-up of organism
   B. set of genes of organism
   C. detected during fertilization
   D. set of traits of organism
2. Which traits are not inherited by incomplete dominance pattern?
A. cystinuria
B. sickle-cell anaemia
C. brown eyes
D. familial hypercholesterolemia
3. Which antigen is absent in Rh+ A blood group?
   A. B
   B. A
   C. D
   D. all
4. Which of the followings is not a test cross?
   A. Aa x aa
   B. AAx aa
   C. Aa x Aa
   D. AaBb x aabb
5. Inheritance of which of the traits is not extended from Mendel laws?
   A. Rh factor
   B. ABO blood groups
   C. hypercholesterolemia
   D. brachydactylia

II

1. Law of segregation is defined by segregation of:
   1. alternative alleles into gametes
   2. alternative gametes into alleles
   3. alternative alleles into somatic cells
   4. offspring phenotypes by 3:1 ratio
   5. offspring genotypes by 3:1 ratio

A. 1,3,4 B. 1,4 C. 3,4 D. 4,5

2. Recessive homozygote organism expresses:
   1. dominant trait
   2. recessive trait
   3. both dominant and recessive traits
   4. usually the mutant allele
   5. only maternal traits

A. 1,5 B. 3 C. 4,5 D. 2,4

3. Sublethal genes:
   1. cause immediate intrauterine death
   2. cause death after few years of life
   3. do not cause death
   4. develop Tay-Sachs' disease
   5. develop brachydactylia

A. 3,5 B. 2,5 C. 2,4 D. 1,4

4. Blood group of human is detected by antigens:
   1. A
   2. B
   3. C
   4. O
   5. D

A. 1,2,4 B. 3,5 C. 1,2,5 D. 1,2

5. Rh factor is coded by:
   1. single gene
   2. multiple alleles
   3. CDE genes
   4. three genes
   5. genes of A and B antigens

A. 3,4 B. 1,2 C. 2,5 D. 2,3,4

CHAPTER 8

Law of Independent Assortment. Law of Independent Assortment. Dihybrid Cross.

Law of Independent Assortment. Dihybrid Cross.

The law of independent assortment states that in the second generation of dihybrid cross there is independent segregation of traits by 9:3:3:1 ratio. The reason is that during formation of gametes (meiosis) members of segregating gene pairs assort into different gametes (and then offspring), independently of one another, since they locate on different pairs of chromosomes. When gametes are formed in diploid organism, the segregation of each gene pair does not affect the segregation of other gene pairs as long as the gene pairs are on separate chromosomes (unlinked). For example, suppose a cross between individuals differing in two characters: AaBb x AaBb, or a dihybrid cross. *Aa* represents a gene pair for handedness (A – right-handed, a – left-handed), and *Bb* represents the second gene pair for eye colour (B – brown eyes, b – blue eyes).

P AABB x aabb

F_1 AaBb 100%

P_{F1} AaBb x AaBb

G AB Ab aB ab AB Ab aB ab

G	***AB***	***Ab***	***aB***	***ab***
AB	AABB	AABb	AaBB	AaBb
Ab	AABb	AAbb	AaBb	Aabb
aB	AaBB	AaBb	aaBB	aaBb
Ab	AaBb	Aabb	aaBb	aabb

F_2 9AB : 3aB : 3Ab : 1ab

1AABB 1aaBB 1AAbb 1aabb

2AaBB 2aaBb 2Aabb

4AaBb

2AABb

Each of the male gamete AB, Ab, aB, ab can fuse with any of the female gametes (AB, Ab, aB, ab), and 16 possible combinations of gametes can develop, out of which 9 have both dominant traits (right-handed with brown eyes, AB), 3 have first trait dominant and the second as recessive (right-handed with blue eyes, Ab), 3 with first trait recessive and the second dominant (left-handed with brown eyes, aB), and lastly 1 will have both traits recessive (left-handed with blue eyes, ab). That is, there are 4 possible phenotypes segregated by 9:3:3:1 ratio, and presented by 9 possible genotypes.

The gene pairs are behaving independently from one another, since they are on different pairs of chromosomes, that is unlinked. Then we can use the multiplication rule for independent probabilities to figure out the expected proportion of gametes. ***Multiplication rule*** states that the probability of two or more independent events occurring together is the product of their individual probabilities. If more complicated crosses are done, say trihybrid cross or a cross involving more number of traits, then *Punett* squares become too complex. Here the application of multiplication rule can be more useful.

Number of developed gametes depends on the rate of hybridization, and equals 2^n, where *n* is the rate of hybridization. For example, in monohybrid cross n=1, so number of gametes is 2 (2^1). The segregation in F_2 undergoes the Newton binom pattern.

Table 2. Segregation in F_2 in various crosses.

Hybridization	gametes	phenotypes	genotypes	segregation
Aa x Aa	2^1=2	2	3	3:1 $(3+1)^1$
AaBb x AaBb	2^2=4	4	9	9:3:3:1 $(3+1)^2$
AaBbCc x AaBbCc	2^3=8	8	27	27:9:9:9:3:3:3:1 $(3+1)^3$
polyhybrid cross	2^n	2^n	3^n	$(3+1)^n$

Linkage

Mendel's law of independent assortment is not always true as genes which are close together on one chromosome tend to be inherited together, i.e. they are "linked". The number of genes in a cell is far greater than the number of chromosomes (in humans it is 30.000 genes versus 23 pairs of chromosomes); in fact, each chromosome has hundreds of genes.

Thomas Hunt Morgan was the first to associate a specific gene with a specific chromosome (1910). For his work he selected a species of fruit fly, *Drosophila melanogaster*, a common, generally innocuous insect that feeds on the fungi growing on fruit. Fruit flies are prolific breeders: a single mating will produce hundreds of offspring, and a new generation can be bred fast – every two weeks. Another advantage of the fruit fly is that it has only four pairs of chromosomes easily distinguishable with light microscope, and has multiple alternate characters and mutations. These characteristics make the fruit fly a convenient organism for genetic studies.

Morgan experiments show how the linkage between genes affects the inheritance of two different characters – body colour and wing size. Wild type (dominant) flies have gray body and normal wings (BBVV). Mutant phenotypes (recessive) for these characters are black body and vestigial wings (*bbvv*), which are much smaller than normal wings.

P *BBVV* x *bbvv*

G *BV* *bv*

F_1 *BbVv* *(100% uniform)*

Morgan crossed male dihybrids (*BbVv*) with females that had both of recessive (mutant) phenotypes, black body and vestigial wings (*bbvv*). According to Mendel's law of independent assortment, Morgan's *Drosophila* testcross would result in four phenotypic classes of offspring in equal ratio (1:1:1:1) – 1 gray colour-normal wings **:** 1 gray-vestigial : 1 black-normal : 1 black-vestigial. But actual results were very different. There was equal ratio (1:1) of only two phenotypes of wild type (gray-normal) and double-mutant (black-vestigial) flies among offspring, which corresponded to two parental phenotypes.

P ♂*BbVv* x *bbvv*♀

G *BV, bv* *bv*

F_1 *1 BbVv* : 1 *bbvv*

Morgan reasoned that body colour and wing shape are usually inherited together because genes of these two characters are located on one chromosome pair, that is – are linked. The result resembles the monohybrid testcross when single pair of chromosomes is considered.

However, the ratio among offspring produced from a testcross between female dihybrids (BbVv) and males that had both mutant phenotypes (black bodies and vestigial wings, *bbvv*)

was not as in previous case, and recombinant phenotypes presenting with one wild and one mutant trait from each character (gray-vestigial and black-normal) also were shown off. These recombinant phenotypes numbered fewer (per 8.5% and 8.5% each) than expected based on independent assortment, and the frequencies of parental phenotypes offspring made for 41.5% each.

P ♀ *BbVv* x *bbvv* ♂

G *BV, Bv, bV, bv* *bv*

41.5% 8.5% 8.5% 41.5%

F1 *BbVv : Bbvv : bbVv : bb vv*

41.5% 8.5% 8.5% 41.5%

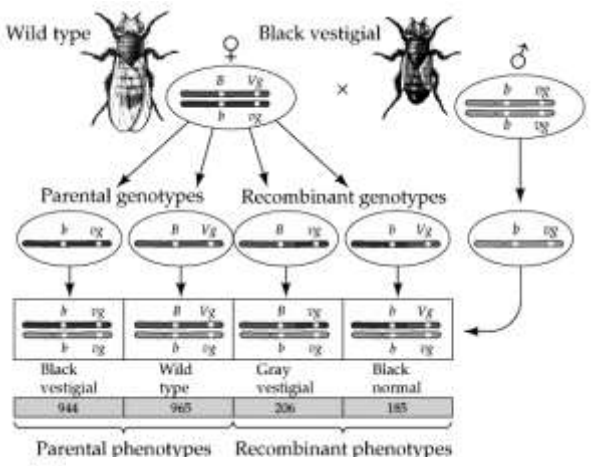


Fig. 40. Morgan experiment.

The actual results do not conform neither to 1:1:1:1 ratio if there was independent assortment, and nor to the ratio of 1:1 (only parental phenotypes) when complete linkage of these two genes could be expected. Most of the offspring had parental phenotypes suggesting linkage between two genes, but 17% of the flies were recombinants. So, although there was ***linkage***, it appeared ***incomplete***. Morgan proposed that some mechanism that exchanges segments between homologous chromosomes must occasionally ***break the linkage*** between two genes. Subsequent experiments have demonstrated that such an exchange – **crossing over** – accounts for the recombination of linked genes. Recombinant chromosomes bring together alleles in new combinations introduced to gametes. Such gametes are known as ***crossing over gametes***, since they arise if only crossing over occurs. It was shown also that there is gene excluding crossing over in male fruit fly, and only two phenotypes (1:1) occur in offspring of testcross between male dihybrids (*BbVv*) and double-mutant females (*bbvv*).

Study of inheritance of another pair of characters showed that the crossing over frequency was kept the same for that pair but different from other trait pairs. This observation evidences that genes on the same chromosome have linear arrangement. In fact, crossing over breaks the linkage between genes and leads to incomplete linkage.

Chromosome Theory of Heredity

In 1902 with evidence of the behaviour of chromosomes, *Walter Sutton* (an American graduate student) and *Theodor Boveri* (German biologist) recognised independently that the inherited factors described in Mendel's paper could be explained by consideration of the behaviour of chromosomes during meiosis. It is known as the *Sutton-Boveri* ***chromosome theory of heredity.*** According to this theory Mendelian genes are located on chromosomes, and it is the chromosomes that undergo segregation and independent assortment.

1. The genes on the chromosome locate linearly and make a group of linkage.

Human species genome has 23 linkage groups in females and 24 groups of linkage in males, who have one extra type of chromosome – Y.

2. The farther apart are two genes, the higher the probability that a crossover will occur between them, and therefore a higher recombination frequency and the less the linkage is.

3. The unit of genetic distance is called a ***morganid (m)***. One percent recombination equals 1/100 of a morganid, or a ***centimorganid (cm)***.

Recombination is rare if the linked genes are close. When the recombination frequency between linked genes that are far apart on chromosome is 50%, they behave like unlinked genes, and a result is indistinguishable from that for genes on different chromosomes. Contrarily, when two loci are so close together (3M) that crossing over is not observed, the genes are said to be completely linked like the three genes (C, D, E) of Rh factor inherited as a monogenic trait. The proof for the chromosomal theory of heredity came from experiments done by T.H. Morgan and Calvin Bridges on *Drosophila melanogaster*.

Genetic Maps

Using measures of recombination between genes geneticists created maps of entire genomes. The process of recombination between genes is random, but in large populations the number or recombinants are very predictable. That's why the maps are very accurate and very detailed. The methods of mapping among eukaryotes and prokaryotes vary based on different organization patterns of their genetic material.

There are three ways of mapping in eukaryotes: crossover method, cytogenetic method and somatic cell hybridization method.

1. Crossover method. A Genetic Linkage Map shows the order of genes on a chromosome. And the sequence is based on the recombination frequency data between the genes.

Alfred H. Sturtevant, a student in T. H. Morgan's laboratory, was the first to use recombination frequencies between genes to create chromosome maps in 1913. The map included genes on the X-chromosome of the fruit fly. He used recombination frequency data to assign relative distances between the genes in map units, or ***morganids***, and construct genetic linkage maps.

When constructing a genetic map, one simultaneously monitors the recombination among three or more linked genes far apart enough for crossing over to occur. Thus, *A. Sturtevant* first studied 3 genes for body colour (*b*), wing shape (*vg*) and cinnabar eye colour (*cn*): *b* and *vg* genes on a chromosome show a recombination frequency of 17%. Sturtevant knew that the recombination frequency between the (*vg)* gene and (*cn*) gene was 9%. Now he realised that if chromosomes where linear entities then there were two possibilities: ***b cn vg*** where the recombination frequency between *vg* and *cn* is expected to be 8%, or: ***cn b vg*** where the recombination frequency between *b* and *cn* is expected to be 26%.

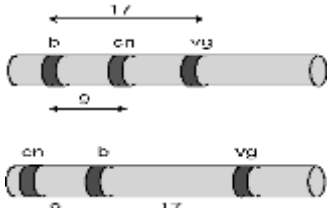


Fig. 41. Possible arrangement of *b, cn* and *vg* genes on a chromosome.

Sturtevant found that the recombination frequency between *b* and the *c* genes was 26% therefore assumed map number 2 was correct.

A **linkage map** is a genetic map based on recombination frequencies – is not really a picture of a chromosome size. The frequency of crossing over is not actually the uniform over the length of chromosome, and therefore the map units do not have absolute size (in nanometres, for instance).

2. Cytogenetical method (karyotyping) uses selective staining of chromosomes into specific sequences of bands (genetically active euchromatin and

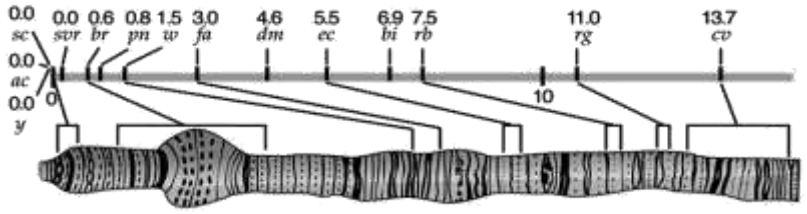
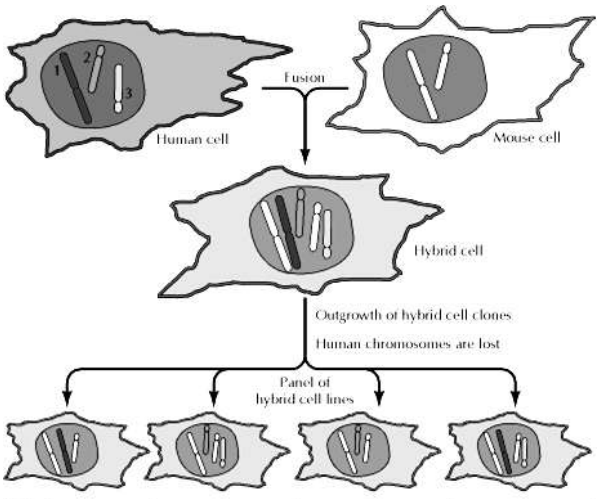


Fig. 42. Colinearity of Drosophila polytene X-chromosome banding and its genetic map for

inactive heterochromatin). This mapping is known as a *cytological map*. Cytological maps are convenient to study on large chromosomes (e.g. polytene chromosomes of Drosophila).

3. Somatic cell hybridization method. Somatic hybrids are formed by fusion of somatic cells of the same or different species. Cultured cells from two different species can be induced to fuse by the help of *uv*-radiation-treated *Sendai virus* to form hybrid cells. The nuclei also fuse forming a single nucleus (a *heterokaryon*). The heterokaryon of mouse-human somatic cell hybrids contain 86 chromosomes (40+46). They are useful for mapping of human genes. They are usually made using established mouse cell culture lines and human fibrocytes or leukocytes.



Hybrid cell line	A	B	C	D
Human chromosomes	1	2 + 3	2	1 + 3

Fig. 43. Somatic cell hybridazion.

In interspecific fusions, as the hybrid cell goes through subsequent mitotic divisions, chromosomes are randomly lost. When mouse-human hybrid cells are cultured, they selectively loose many of the human chromosomes, and along with this several genes quit expression with no further synthesis of proteins encoded by them. This allows the specialists to construct genetic maps for the lost human chromosomes, concluding that the eliminated genes are linked on the eliminated chromosome.

The somatic hybridization of human and mouse cells allowed finding all the linkage groups of human genome. The maximal number (over 300) of genes was established on X-chromosome. ABO blood group gene is linked with the gene of nail-patella syndrome (defects of nails and patella) on number 9 chromosome, and the Rh factor genes are linked with elliptocytosis (elliptic RBCs) gene on chromosome 1.

Genetic maps of human chromosomes are of great importance. Knowledge of where particular genes are located on human chromosomes can often be used to tell if a fetus carries a genetic disorder for which it is at risk.

The genetic engineering techniques have recently permitted the geneticists to isolate specific genes and determine their nucleotide sequence (DNA sequencing). The knowledge of the normal sequence and its changes during gene mutations may suggest a successful therapy for particular gene diseases and substitute the dysfunctional (mutated) genes with normal ones – that is ***gene therapy.***

Human Genome Project

Begun in 1990, the Human Genome Project is a 13-year effort coordinated by different research laboratories in different countries (Europe and the USA), which was completed by 2003. Project goals were to:

1. *identify* all the approximately 30.000 genes in human DNA,
2. *determine* the sequences of the 3 billion chemical base pairs that make up human DNA,
3. *store* this information in databases,
4. *improve* tools for data analysis.

Genetic Maps of Prokaryotes

1. The first method of making genetic maps in prokaryotes is based on interruption of conjugation process at various time points and observing expression of new trait expression in the recipient bacterium. Whole conjugation lasts for about 2 hours (120 min). Artificial interruption of the conjugation process will stop the passage of certain genes, and only some of donor bacterial genes will manage to move to recipient bacterium. In this case the measure of distance between genes is considered as a unit of time, e.g. a minute.

2. The second method in prokaryotic gene mapping uses data of crossover between homologous loci of partially diploid DNA fragments which arise following the conjugation. This is as same as the crossover method applied for chromosome mapping in eukaryotes.

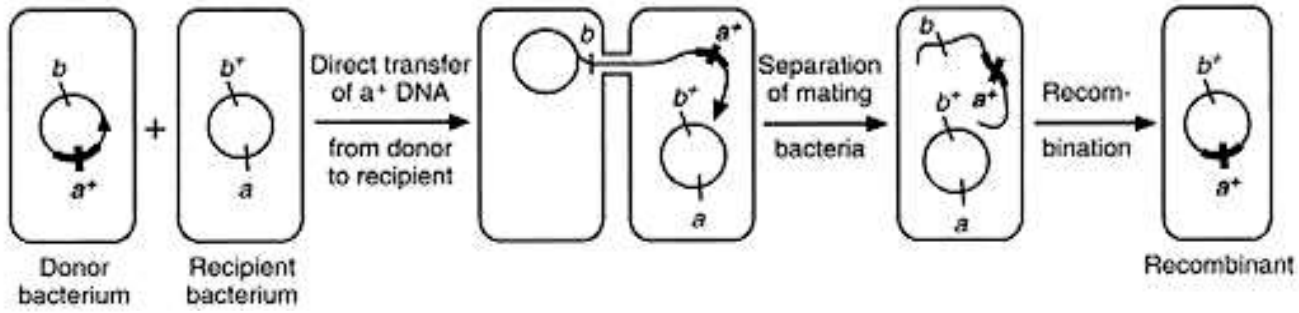


Fig. 44. Conjugation of bacteria and formation of recombinants.

1A

1. The genes are completely linked, if the distance between them is:

A. less than 3 m
B. more than 3 m
C. less than 50 m
D. more than 50 m

2. The number of linkage groups of an organism is equal to:

A. diploid set of chromosomes
B. haploid set of chromosomes
C. number of genes in the genome
D. number of autosomes

3. Dihybrid cross of drosophila in F2 showed linkage of the traits, because the genes were located on:

A. same pair of chromosomes
B. different pair of chromosomes
C. only sex chromosomes
D. only autosomes

4. *Morganid* is a gene distance unit that is equal to:

A. 1 nm
B. 1% of crossing over between genes
C. 1% of all the genome genes
D. 1 triplet

5. The genetic mapping of prokaryotes is based on:

A. ability to divide fast
B. losing the parts of their genes
C. conjugation
D. encapsulation

1B

1. Which of the followings is not a genotype of right-handed brown-eyed man?

A. AABB
B. AaBb
C. AaBB
D. aaBb

2. Which linkage is not complete? If the distance between genes is:

A. 2 morganids
B. 1 morganids
C. 3 morganids
D. 4 morganids

3. What is not detected by somatic cell hybridisation?

A. gene expression
B. linkage groups
C. gene sequence on chromosome
D. gene set of chromosomes

4. What is not the intention of Human genome project?

A. treatment of gene diseases
B. treatment of genome mutations
C. detection of gene nucleotide sequences
D. detection of gene sequences on chromosomes

5. Crossing over frequency is not:

A. directly proportional to gene distance
B. reversely proportional to gene distance
C. reversely proportional to linkage force
D. dependent on sex

II

1. Law of independent assortment is based on independent assortment of:

1. non-homologous chromosomes during meiosis I
2. non-homologous chromosomes during meiosis II
3. non-homologous chromosomes during mitosis
4. genes during meiosis I
5. genome during mitosis

A. 1,5 B. 3,4 C. 2,4 D. 1

2. The genes are completely linked if they:

1. locate on distance of less than 3M on a chromosome
2. locate on distance of more than 3M on a chromosome
3. locate on distance of more than 50M on a chromosome
4. sometimes undergo a crossing over
5. always are inherited together

A. 1,4 B. 1,5 C. 2,5 D. 3,4

3. *Drosophila melanogaster* is used in genetic studies because it:

1. has many mutatuins
2. is easy to breed
3. has few pairs of chromosomes
4. develops few offspring
5. has multiple linkage groups

A. 2,3 B. 1,2,3 C. 4,5 D. 1,3,5

4. Which of the statements refer to chromosomal theory of inheritance?

1. genes locate on chromosomes
2. chromosomes locate in nucleus
3. chromosome number is even in diploid cell
4. number of linkage groups is equal to haploid set of chromosomes
5. number of linkage groups is equal to diploid set of chromosomes

A. 1,4 B. 1,5 C. 2,3,4 D. 3,4,5

5. Genetic maps in eukaryotes are constructed on the basis of:

1. recombination frequencies
2. somatic cell hybridisation
3. crossing over frequencies
4. duration of conjugation
5. duration of copulation

A. 3,4 B. 1,2 C. 1,2,3 D. 3,5

CHAPTER 9
Inheritance of Sex. Sex-linked Inheritance. Cytoplasmic inheritance

Inheritance of Sex

The sex of an individual is the set of morphological, physiological, biochemical and behavioural characters that provide reproduction. Sexual traits are divided into primary and secondary types. The *primary sexual traits* are referred to the sex glands and sex hormones, which are formed already in a newborn. The *secondary sexual traits* develop in puberty on the basis of primary ones and include modifications in skeleton, muscles, larynx (voice), hair coverings, behaviour etc.

The sex in humans and other mammals is determined by the sex chromosome pair. In humans, a person who inherits two X chromosomes (XX), one from each parent, develops as a female. A male develops from a zygote containing X and Y chromosomes. The human females are said to be ***homogamete*** *sex*, since each of the ova contains the same X chromosome. In contrast, the male is known as ***heterogamete*** *sex* because sperms are categorised into X-carrying and Y-carrying types. Moreover, the human males are called also ***hemizygotes*** (*hemi* – half) as the genes on X and Y chromosomes are presented in single copy, while females have two copies of genes linked to X chromosomes and can either homo- or heterozygote for those genes.

Sex of the organism is inherited as a Mendelian trait and is detected during fertilization. The probability for having a male or female offspring is 50/50 (1:1).

The sex ratio shows the relation between males and females in a population. The ***primary sex ratio*** shows the relations of sexes among zygotes (at conception), it is equal to 100 females per 150 males. At birth (among newborns) the relation is called ***secondary sex ratio*** and equals approximately 100/105. In adult population the relation of sexes is called ***tertiary sex ratio***: among 20 year old population it is as 100/100, among 50 years old – 100/85, and among 85 year old ones – 100/50.

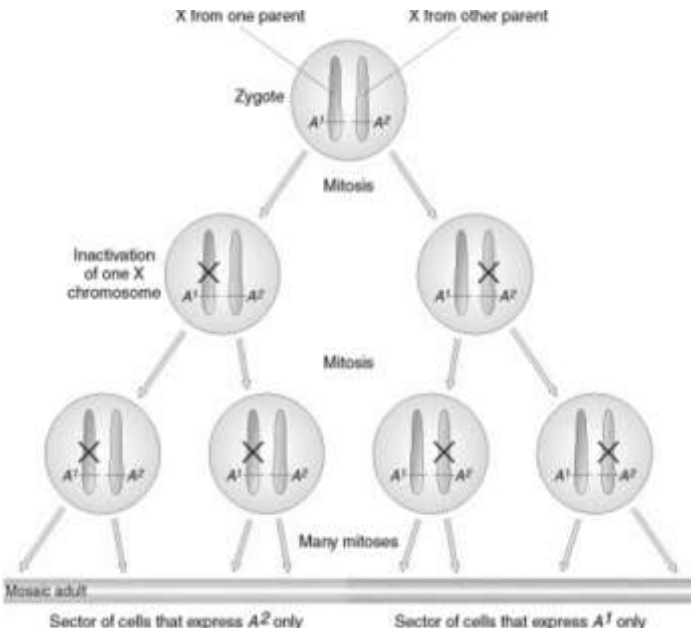


Fig. 45. Random inactivation of X chromosome.

As seen from sex ratio in different age groups of human population, it is obvious that females have higher survival rate. Higher survival of females is somehow explained by their two X chromosomes versus a single X in males. These two X chromosomes are active for 16 days of embryogenesis, after which one of them is inactivated (becomes a Barr body – sex chromatin). Since then the **dosage of X chromosome genes** is almost equal in females and males (females and males have one active X chromosome). This would result in **dosage compensation.** In half of the female cells there is *random* inactivation of paternal X chromosome, whereas in other half of the cells the maternal X chromosome is inactivated. As a consequence of *random* inactivation, females become **mosaics for the X chromosome** with the half of their cells having active

maternal X and the other half containing the paternal X active. Mosaicism of X chromosome alleles in females provides their higher adaptability versus males.

Once X-chromosome is inactivated in a cell, it will remain inactive in all the generations of that cell. Therefore, the X inactivation is said to be *fixed*. Moreover, the inactivation of X chromosome is *incomplete*: only 80% of genes are inactivated, while 20% remain active. That is the reason why Klinefelter syndrome (47,XXY) is not a normal male, and Turner syndrome (45,X) is not a normal female.

Different species have different systems to determine sex. In mammals (including humans) and Drosophila, females are homogamete sex (XX) and males are heterogamete (XY), but in fruit fly the situation is more complicated than in mammals; it is really the ratio of X chromosomes to the number of autosome chromosomes which determines the sex. *Sex index* (ratio of X chromosome number to the number of autosomes) in females is equal to 1, i.e. 2X/2A, in males it is equal to 0.5, i.e. XY/2A.

Some insects (e.g., bed bugs) present with heterogamete male individuals having only one X-chromosome (XO). In birds individuals with the same sex chromosome are males (ZZ); those with different sex chromosomes are females (ZW). In bees and ants unfertilized eggs become males (haploid), while fertilized eggs become females.

Disorders in sex inheritance: hermaphroditism. Hermaphroditism (*Hermes+Aphrodite, Greek mythology gods*) is a disorder of sexual differentiation characterised by sexual ambiguity (ambiguity of sex gonads and genitalia). Sexual differentiation process is a complex cascade of events which take place between 6-14 weeks of embryonic development. Some errors that may occur during that differentiation, can lead to sexual ambiguity or to discordance between chromosomal sex and the appearance of external genitalia. There are two types of hermaphroditism – true and pseudohermaphroditism.

True hermaphroditism is extremely rare condition when an individual has both testicular and ovarian tissues, often in association with ambiguous genitalia. In these patients an ovary can be found on one side and a testis on the other. Alternatively, there can be a mixture of ovarian and testicular tissue in the gonad which is known as ovotestis. Most patients with true hermaphroditism have a 46,XX karyotype, and in many of these individuals the paternally derived X chromosome carries Y chromosome-specific genes (SRY) as a result of incorrect crossing over between homologous pseudoautosomal regions (PAR) of X and Y chromosomes during meiosis I in spermatogenesis.

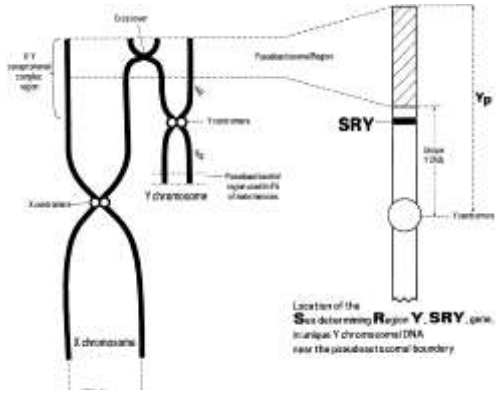


Fig. 46. Crossover between PARs of X and Y chromosomes.

In **pseudohermaphroditism** there is gonadal tissue of only one sex, but genitalia of the opposite sex. Thus in male pseudohermaphroditism there is a 46,XY karyotype with usually female genitalia. The most widely recognised cause of *male pseudohermaphroditism* is androgen insensitivity, which is also known as testicular feminisation syndrome (Morris syndrome). In *female pseudohermaphroditism* the karyotype is female, and the external genitalia are virilised (masculine). It occurs mainly in congenital adrenal hyperplasia because of mutation of cortisol-producing enzyme gene.

Sex Linkage

Because some of the chromosomes have a special role in determining gender, the genes on those chromosomes have a special kind of linkage called **sex-linkage.** The classic example of sex-linkage inheritance pattern as discovered by Morgan involved the *White-eye mutation* (termed *w*) of Drosophila melanogaster. When males carrying this mutation were mated to wild-type females with red eyes X^WX^W, the F_1 flies all had red eyes, suggesting that the red-type allele (*W*) was dominant over the white allele.

P X^WX^W x X^wY

G X^W X^w, Y

F_1 X^WX^w, X^WY – 100% red eyes

When the white-eyed female (X^wX^w) was mated to a red-eyed male (X^WY) there was 1:1 segregation but with surprising result: the white eyes were present only in males.

P X^wX^w x X^WY

G X^w X^W, Y

F_1 red X^WX^w : X^wY white

The explanation to this phenomenon is that eye colour was linked to female sex chromosome (X) and is absent on Y chromosome.

Sex-linked genes are divided into three groups:

1. genes linked only to X-chromosome,
2. genes linked to both X- and Y-chromosomes (PARs),
3. genes linked only to Y-chromosome (*holandric traits*).

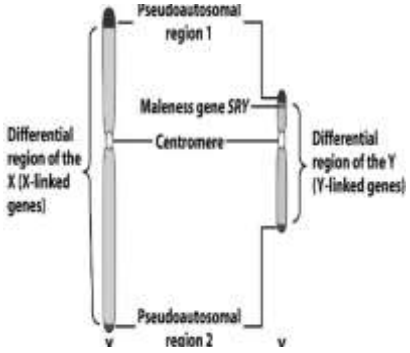


Fig. 47. Sex linkage.

X-linked Traits

X-linked genes account for more than 300, among them also the genes that provide development of female pattern organism. For X-linked genes the males are hemizygous and any allele present on it is expressed, while females can be either homozygous or heterozygous for X-linked genes.

X-linked recessive mutations

For X-linked recessive traits the heterozygote females are considered as "carriers" of the mutation. The effect cannot be seen in them, yet half of their sons are affected by the mutation. Affected father transmits the mutant allele to half of his daughters. Mother-to-son and father-to-daughter transmission is known as *criss-cross* pattern.

1. Haemophilia – a high frequency of sex-linked haemophilia has been common in the royal families of Europe (the British queen Victoria). It is defined by absence of certain protein (VIII factor) required for blood clotting. Haemophiliacs bleed excessively when injured; the most seriously affected individuals may bleed to death even after relatively minor skin abrasions or cuts.

2. Red-Green colour blindness (Daltonism) – the genes for the two protein receptors which recognise red and green light are located on the X chromosome. The two genes are located close to each other and mutations often occur which eliminate one or the other of the pair.

3. Duchenne muscular dystrophy (DMD) – This is the most severe and the most common among all muscular dystrophies. It affects about 1/3.500 males of all ethnic groups. The

symptoms of DMD are usually seen before 5 years: clumsiness, muscle weakness. Pseudohypertrophy of the calves is often seen early in the course of the disease. Most DMD patients are confined to wheelchairs by age 11. The heart and respiratory musculature become impaired, and death occurs usually from cardiac or respiratory failure.

4. *IgA deficiency (agammaglobulinemia)* leading to inborn immunodeficiency
5. *Morris syndrome*
6. *Glucose-6-phosphate dehydrogenase deficiency*

X-linked dominant mutations. An individual need inherit only a single copy of an X-linked dominant disease gene to manifest the disorder. Because females have two X-chromosomes, either of which can potentially carry the gene, they are twice as commonly affected as males (unless the disorder is lethal in males). Affected fathers cannot transmit the trait to sons. All of their daughters must inherit the disease gene, so all are affected. Affected females are usually heterozygotes, so they have 50% chance of passing the mutation to their daughters and sons.

1. **Hypophosphataemic rickets**, a disorder in which kidneys are impaired in their ability to reabsorb phosphates. This results in abnormal ossification with bending and distortion of bones, which cannot be treated by vitamin D (vitamin D non-dependent rickets).

2. **Fragile-X syndrome,** is characterized by mental retardation, also by distinctive facial appearance, with large ears and long face, hypermobile joints, and enlarged testes in postpubertal males. The term "fragile X" is derived from the fact that the X chromosome of affected individuals sometimes exhibits breaks and gaps near the tip of long arm. The gap region possesses more than 200 copies of tandemly repeated CGG triplets, which make the DNA unstable. Fragile X syndrome has 80% penetrance in males and 30% penetrance in females. The lower degree of penetrance in females is thought to be related to X-inactivation.

Linkage on Both Sex Chromosomes

The tips of short arms of X and Y chromosomes contain fragments homologous to each other, hence two different sex chromosomes have highly similar DNA sequences. This means that during meiosis (spermatogenesis) X and Y chromosomes can behave themselves as autosomes, and crossing over can occur between their homologous parts. This makes the linkage of these genes incomplete. These parts are known as pseudoautosomal regions (PAR). Example of a disease developed due to mutation of genes located on PARs is ***retinitis pigmentosum*** (progressive visual field loss and night blindness).

Y-linked traits

The small acrocentric Y chromosome contains relatively few genes. They are known also as ***holandric*** genes, since they are situated on male chromosome and can transfer only from father to son. These include:

1. gene that initiates differentiation of the embryo into a male (sex-determining region on the Y, or ***SRY gene***), several genes encoding for spermatogenesis factors.
2. webbed toes,
3. hypertrichosis (hairy ear lobs),
4. ichthyosis (fish scale-like skin),

Sex-limited and Sex-influenced Inheritance

Confusion sometimes exists regarding traits that are ***sex-linked*** and those that are ***sex-limited*** or ***sex-influenced***. Sex-limited and sex-influenced traits are usually autosomal traits but

often the expression rate (penetrance) or expression intensity (expressivity) of autosomal genes may be sex-dependent.

A *sex-limited* trait occurs in only one of the sexes, due to anatomical differences, e.g., heritable defects of uterine or testis express only in females or males, respectively. In contrast to sex-limited traits, the *sex-influenced* traits can be expressed in both of the sexes but differently. This is provided by different hormonal conditions of the male and female organisms that dictate how the sex-influenced traits will express. An example is the male-pattern baldness – the form of baldness in which hair is lost first from the crown of the head. It can occur both in males and females, but much more commonly in males. It is not an X-linked trait and is thought to be inherited as an autosomal dominant trait in males, whereas in females it is inherited as an autosomal recessive. Females display the baldness only when they are homozygote. Even then, they are more likely to show marked thinning of the hair rather than complete baldness.

Cytoplasmic or Extranuclear Inheritance

Although there is evidence that eukaryotic genes have specific loci on chromosomes and that their behaviour is explained by Mendel's laws, there are exceptions to the chromosome theory of inheritance. Not all of the genes in the cell are located on chromosomes, or even in the nucleus. Most of these extranuclear genes are found in cytoplasm – within semiautonomous organelles, such as mitochondria in animal cells and plastids in plants. These cytoplasmic genes are not inherited in Mendelian fashion because they are not distributed by segregating chromosomes during meiosis.

The extranuclear inheritance is also referred to as **cytoplasmic inheritance**. Genes located outside of the nucleus are: mitochondrial DNA (mtDNA), chloroplast DNA (cpDNA), and in prokaryotes these are **plasmids**, **episomes.** All of the genetic factors in whole cytoplasm are totally called as **plasmon,** and the separate elements as plasmogens. mtDNA and cpDNA are almost always **uniparentially inherited**, with only one sex (the female) transmitting the genomes to their offspring.

The mitochondria are located in cytoplasm, and the egg cell always contributes much more cytoplasm to the zygote than does the sperm. The cytoplasm provided by the female gamete contains several components – mtDNA, mRNA, proteins, and other factors for early development. Thus, any trait associated with a mitochondrial gene must be transmitted from mother to all her children, both male and female. Males inherit their mtDNA from mothers but cannot transmit to their offspring because sperm cells contain only few mitochondria that do not usually enter the egg.

The human mtDNA contains 37 genes coding 22 tRNAs, 2 rRNAs, and 13 proteins involved in ATP synthesis (oxidative phosphorylation). Mitochondrial DNA is free of introns. The mutation rate of mtDNA is about 10 times higher than that of the nuclear DNA. This is caused by lack of DNA repair mechanisms in mtDNA and possibly by damage of free oxygen radicals released during oxidative phosphorylation process. This is how explained one of the aging mechanisms.

Since each cell contains a population of mtDNA molecules, a single cell can have some molecules that present with mtDNA mutation and other molecules that do not. This heterogenety in mtDNA composition, termed **heteroplasmy**, is an important cause of variable expression in mitochondrial diseases. The larger the proportion of mutant mtDNA molecules, the more severe the expression of the disease is. Mitochondrial diseases are often associated

with defects in mitochondrial oxidative phosphorylation and affection of the tissues that have high requirements of energy (brain and spinal cord, skeletal and heart muscles, kidney, liver).

Mitochondrial Diseases

1. Spina bifida (split spinal cord) – occurs due to lack of fusion of vertebral arches and extrusion of the spinal cord (spinal hernia) during embryogenesis.
2. *Leber's* hereditary optic neuropathy. This is characterised by rapid, irreversible loss of vision in the central visual field since third decade of life as a result of optic nerve death.
3. Maternally inherited myopathy and cardiomyopathy – MMC.

1A

1. Chromosomes which determine individual's sex are called:

A. autosomes
B. somatic chromosomes
C. sex chromosomes
D. all of the above

2. Which trait is X and Y linked?

A. Duchenne muscular dystrophy
B. xeroderma pigmentosum
C. shortsightedness
D. Daltonism

3. Which of the diseases is transmitted by X-linked recessive pattern?

A. hemophilia
B. fragile-X syndrome
C. rickets
D. albinism

4. Pseudoautosomal regions are located on:

A. autosomes
B. pseudoautosomes
C. both X chromosomoes
D. X and Y chromosomes

5. What is common for both X and Y linkage?

A. retinitis pigmentosum
B. hemophilia
C. hypertrichosis
D. colour-blindness

1B

1. Homogamete sex is not:

A. having one type of gametes
B. having different type of gametes
C. the male in human
D. female in birds

2. What is not common for holandric traits?

A. transmission from father to son
B. Y-linkage
C. X-linkage
D. appearing in hemizygote condition

3. Which of the followings is not a Y-linked trait?

A. ichthyiosis
B. webbed neck
C. webbed toes
D. male sex development

4. Female phenotype is not common for:

A. male pseudohermaphrodite
B. female pseudohermaphrodite
C. Turner syndrome
D. Morris syndrome

5. What is not common for X-and-Y linked genes?

A. incomplete linkage
B. positioning in homologous loci
C. complete linkage
D. autosomal-like transmission

II

1. Hemizygote human organism:

1. is the male
2. is the female
3. has X and Y sex chromosomes
4. has haploid genome
5. is heterogamete

A. 2,4 B. 3,5 C. 2,3,5 D. 1,3,5

2. True hermaphroditism develops in the organism that has:

1. testes
2. ovaries
3. ambiguous genitalia
4. only male genitalia
5. only female genitalia

A. 1,2,3 B. 2,5 C. 1,4 D. 3

3. What is the pattern of inheritance for hemophilia?

1. dominant
2. recessive
3. Y-linked
4. X-linked
5. autosomal

A. 2,5 B. 2,4 C. 3 D. 1,4

4. Sex of a human individual depends on:

1. temperature influence
2. which gametes are fertilized
3. sex chromosomes of the individual
4. synthesis of sex hormones
5. influence of chemicals

A. 1,3 B. 2,3,4 C. 2,4,5 D. 3,5

5. Human somatic cells may have following chromosomes:

1. 44+XX
2. 44+XY
3. 22·2+XX
4. 23+X
5. 23+Y

A. 4,5 B. 3,4,5 C. 1,2,3 D. 1,2

CHAPTER 10
Gene and Its Properties. Interaction Between Genes. Molecular Genetics.

Gene and Its Properties

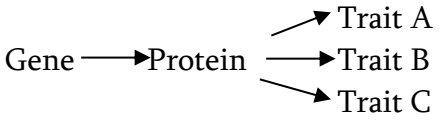
Gene is the functional unit of heredity. It is composed of thousands of nucleotide sequences.

Recombinations and mutations can occur in the genes. *Recon* is a smallest fragment of the gene which is able to recombine. *Muton* is a small fragment of gene that can undergo mutation.

There are two categories of genes: *structural* and *functional*. The genes that code for enzymes, proteinous hormones, histones and rRNA, tRNA, are known as *structural genes*. Functional genes modulate or regulate the function of structural genes. The gene has an ability to repair or restore the structural changes. **Properties of genes** are following:

1. Gene is ***discrete;*** a single gene is responsible for one trait (gene – protein – trait).
2. Gene is ***stable***; it can be transmitted through generations unchanged if no mutation occurs.
3. Gene is ***specific***. Each single gene is responsible for development of a certain trait.
4. Genes have ***dosage*** effect: normal gene dosage in somatic cells is two copies of genes on homologous chromosomes. If dosage is not balanced, genetic disease is a result. For example, in Klinefelter syndrome there is extra dosage of X chromosome genes in males (47,XXY or 48,XXXY). In Turner syndrome the gene dosage is less than normal (45,X).
5. Genes may have **pleiotropic** action. ***Pleiotropy*** is the effect of a single gene on more than one characteristic (*pleios* – many). There are two types of pleiotropic effects: primary and secondary.

Primary pleiotropy is expressed when single gene provides several traits simultaneously. The primary pleiotropy can be expressed as following:



An example of primary pleiotropy is the ***Marfan syndrome***. Marfan syndrome is an autosomal (N15 chromosome) dominant defect of fibrillin – a connective tissue protein. The protein is abundant in the eye lens, aorta (the largest artery in the body, leading from the heart), and the bones of limbs, fingers, and ribs. The Marfan syndrome symptoms are: ***lens dislocation***, ***long limbs***, spider fingers (*arachnodactyly*), and a *caved-in chest*. The most serious symptom is a life-threatening weakening and dilation of the aortic wall (*aortal aneurysm*), sometimes causing the vessel to suddenly burst.

In secondary pleiotropy the primary effect of the gene is followed by a consequent effect, the expression of which is mediated by previous one.

Scheme for secondary pleiotropy is: Gene → Protein → Trait A → Trait B → Trait C. For example, the sickle-cell anemia. The primary effect of the sickle-cell allele is the sickled RBCs due to abnormal hemoglobin (HbS). Secondary results develop due to anemia – weakness, cardiac failure, splenomegaly (spleen enlargement) etc.

Interaction Between Genes

It is known that every gene encodes for a certain trait. However, the action of different genes in the organism is interrelated. Two types of gene interactions are defined: allelic and non-allelic.

Interactions between allelic genes are: complete dominance, incomplete dominance superdominance, codominance, allelic exclusion. Interactions between non-allelic genes are: epistasis polymery,complementation (complementarity) position effect.

Interactions of allelic genes

In ***complete dominance***, the dominant allele completely suppresses the recessive allele in the heterozygote, and the phenotypes of homo- and heterozygotes are identical (AA=Aa). Examples of traits inherited by complete dominance are polydactylia, achondroplasia (skeletal anomalies), Marfan syndrome, right-handedness, brown eyes, etc.

Alternatively, some genes show *incomplete dominance*, in which the heterozygous phenotype is intermediate between that of either homozygote. A more obvious incompletely dominant trait in humans is wavy hair. The homozygous dominant condition is straight hair, and the homozygous recessive phenotype is curly hair. The heterozygote has wavy hair. Other examples are cystinuria and familial hypercholesterolemia.

Different alleles that are both expressed in a heterozygote are ***codominant***. Example is the AB blood group in humans.

Superdominance is the phenomenon of over-expression of heterozygous condition compared to the homozygous dominant (Aa>AA). This is known as ***heterosis***. It results from hybridization of homozygotes (AAxaa), where the heterozygous hybrid offspring display greater vigor, size, resistance than the parents, i.e. is superior to parents. It is used in agriculture and animal breeding.

Allelic exclusion is introduced on the example of female X chromosome inactivation. Random inactivation of allele on one X chromosome excludes the expression of that allele and enables different phenotypic expression of the gene in cells mosaic for X chromosome (in half of them maternal X is active, in the rest half – paternal). Thus, females that are healthy carriers for hemophilia X^HX^h do not express clotting factor in half of the cells having active X^h, but the rest cells express that protein, and the heterozygote females have reduced count of that factor rather than normal amount as the homozygote dominant would have. The heterozygote female individual can be affected with hemophilia if the recessive allele of that mutation is not normally inactivated and has prevalence over dominant one among all the cells.

Interactions of non-allelic genes

Polymery. Most of the traits of the organism result from interaction of not a pair of alleles but several non-allelic genes. Hence, these traits are known as *polygenic* (rather than monogenic) and reflect the activities of more than one gene. This type of interaction for expression of a quantitative trait is known as *polymery.* In polymery there is an ***additive effect*** of genes, which means that the effects of the genes are cumulative, i.e. no one gene is dominant or recessive to another. Since several genes contribute to development of the same trait, they are designated by the same letter, however they are non-allelic, hence there are different indices for the alleles.

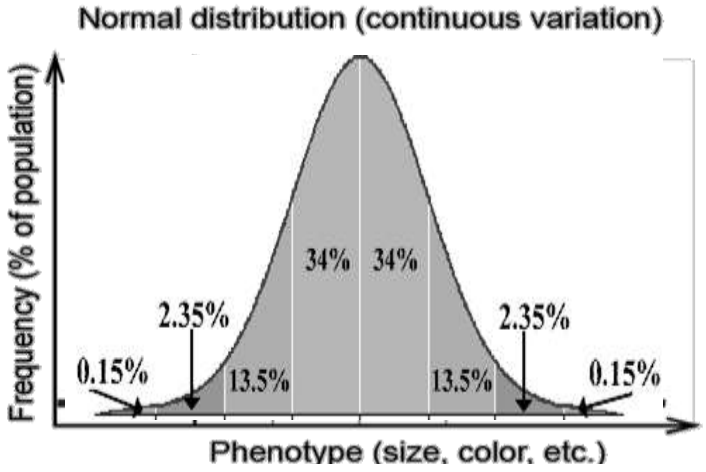


Fig. 48. Distribution of polygenic traits in population.

Distribution of possible phenotypes in polygenic trait in the population is said to be continuous and the graph has a "bell shape".

Human polygenic traits are: height, weight, eye color, skin color. The intensity of skin color is supported by melanin pigment concentration in skin cells. Skin color intensity is determined by 4 pairs of non-allelic genes $P_1P_2P_3P_4$. Four pairs of dominant alleles ($P_1P_1P_2P_2P_3P_3P_4P_4$) in genotype develop the maximum intensity of skin (African blacks). Total absence of dominant alleles (only recessive alleles – $p_1p_1p_2p_2p_3p_3p_4p_4$) is peculiar for Europoid race. Tetraheterozygotes present as mulattos ($P_1p_1P_2p_2P_3p_3P_4p_4$).

Different eye colors arise from two genes with two alleles each, that interact additively to produce eye colors from light blue to green and dark brown. The lightest color would have genotype aabb; the darkest – AABB.

Complementation (complementarity). It is a type of gene interaction in which one gene completes the action of another, and the trait is not shown off until all the genes for all metabolic pathways of the final product are expressed. In case of absence of any product coded by the genes, the complex trait fails to develop. For example, the ability to hear (hearing) depends on both normal structure of acoustic nerve – A, and the cochlea – B (bone in inner ear structure). If any of these units that is dominant character is not formed properly, then the individual develops an inborn deafness (becomes deaf-and-dumb) that may have different genotypes (aaBB, aaBb, AAbb, Aabb) lacking at least two different dominant alleles.

Complementation takes place also for the genes of testosterone synthesis enzyme and testosterone receptor, which complementarily provide development of male sex traits.

Epistasis. When one gene masks or suppresses the expression of another gene, a phenomenon is called *epistasis*. The gene that suppresses the expression of another non allelic gene is known as *epistatic*, and the suppressed gene is called *hypostatic*. Epistasis can be dominant and recessive.

When a dominant allele at one locus can mask the expression of both alleles (dominant and recessive) at another locus, it is known as dominant epistasis. In dominant epistasis the suppressing effect is expressed in both homozygote dominant and heterozygote conditions.

When recessive alleles at one locus mask the expression of both (dominant and recessive) alleles at another locus, it is known as recessive epistasis. The Bombay phenotype is an example of recessive epistasis and results from interaction of two genes: *I* and *H*. The relationship of these two genes affects the expression of the ABO blood groups. Dominant allele of epistatic gene provides synthesis of H antigen which is a precursor for both A and B antigens. The homozygotes for recessive allele *hh* cannot develop any antigen, and phenotypically these individuals are of O blood group (*Bombay phenotype*). The phenotype firstly was described in a family where O blood group mother and A blood group father gave rise to an AB blood group child. That unexpected phenotype then was considered from the mother who had I^B allele and homozygote condition for *h* allele (*hh*). So, the actual genotype of mother genotype hhI^BI^O, in which two recessive alleles suppressed expression of B antigen.

Position Effect. The functional activity of the gene depends on the neighboring alleles, which can effect expression of the certain gene. Different expressivities of genes depending on the position of closely located genes is discussed on example of Rh factor determining genes C-c, D-d and E-e linked on one pair of chromosomes. In the individuals with CDE/cDe genotype the amount of E antigens prevails over C antigens in blood RBCs, while in case of CDe/cDE genotype there are more C antigens than E. It means, when E and C are "neighbors", then E>C.

Position effect shows that in some cases a change of even genes' position and not only their structure or number, may lead to serious clinical consequences. For example, some cases of haemophilia A are caused by chromosome *inversion* that disrupts factor VIII gene on X chromosome. The "Philadelphia chromosome" is an aberrant chromosome formed due to *translocation* of genes between 9 and 22 chromosomes changing their positions and leading to blood cancer.

Molecular Genetics

Molecular genetics studies the molecular level of heredity and variation. Earlier in 20th century, identification of the molecules of inheritance appeared as a major challenge to biologists until the genetic role of DNA was proved. Successful genetic progress was much provided by the experimental objects selected for studies: viruses, bacteria, fungi. The advantages of these organisms over animals and plants present the following:

1. They have shorter life span, making easy the study of several generations during short period.
2. Numerous offspring in a single generation allowing statistically authentic data.
3. Their simple structure lacks histone-bound DNA and thus enables studies and regulation of any factor on reproduction, metabolism and other processes in the organisms.

Role of DNA as a carrier of genetic information was proved through the experiments of *transformation* and *transduction*.

Transformation

It is an uptake of genetic information (naked DNA) by a bacterium released from the dead bacteria in the environment. The discovery of the genetic role of DNA is traced back to 1928 with the work of an English bacteriologist, *Frederick Griffith*. The cells of *Streptococcus pneumoniae* are usually surrounded by a polysaccharide capsule. When grown on the surface of a solid culture medium, the capsule causes the colonies to have a glistening, smooth appearance. These cells are called **S-strain (*smooth)*** and are regarded as pathogenic. The bacteria that lack the capsule are non-pathogenic or harmless; their colonies do not have smooth but rough pattern of growth **(R-strain, *rough*)**.

Injection of S-strain will lead to death of a mouse from pneumonia. Injection of R cells is entirely harmless. When *Griffith* killed the pathogenic S strain bacteria by heating them and then mixed the cell remnants with living bacteria of non-virulent R strain, some of the living R cells were converted into pathogenic S-form and again killed the mouse. Furthermore, this new trait of pathogenicity was inherited in all the subsequent generations of the transformed bacteria. Thus, it was confirmed that some factor transformed the rough bacteria into smooth, although the identity of the transforming agent was not known. *Griffith* called the phenomenon **transformation,** defined as a change in a genotype and phenotype due to assimilation of external DNA by a cell. The recipient takes DNA up from the media, and there is no requirement for cell to cell contact.

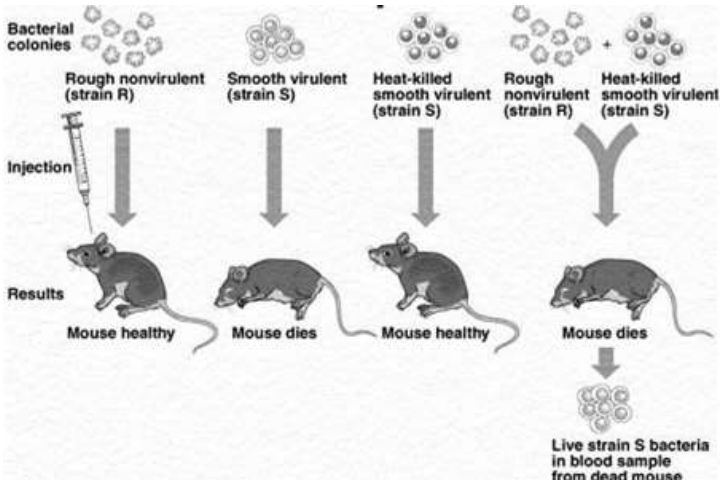


Fig. 49. Transformation of bacteria.

In 1944 *Oswald Avery* showed that the transforming substance was DNA. When he treated S strain bacteria with deoxyribonuclease (*DN-ase*) to destroy the DNA in them, the transforming ability was affected, while hydrolases of other organic substances could not cease

transformation. So DNA was the only material in the dead cells capable of transforming bacteria from one type to another. This fact evidences the genetic role of DNA.

Transduction

The phenomenon of transduction is another evidence for genetic role of DNA. Transduction is defined as the transfer of genetic information between bacterial cells through a virus (phage) particle. Transduction results from aberrations in phage reproductive cycle. When reproducing in a host cell (lytic or lysogenic cycles), in the process of assembling new virus particles, occasionally some host DNA may be incorporated into the phage capsid. When this phage infects another bacterium, it injects the fragment of donor bacterial DNA into the recipient where it can be exchanged for a homologous piece of the recipient's DNA (recombination) and provide synthesis of a new protein.

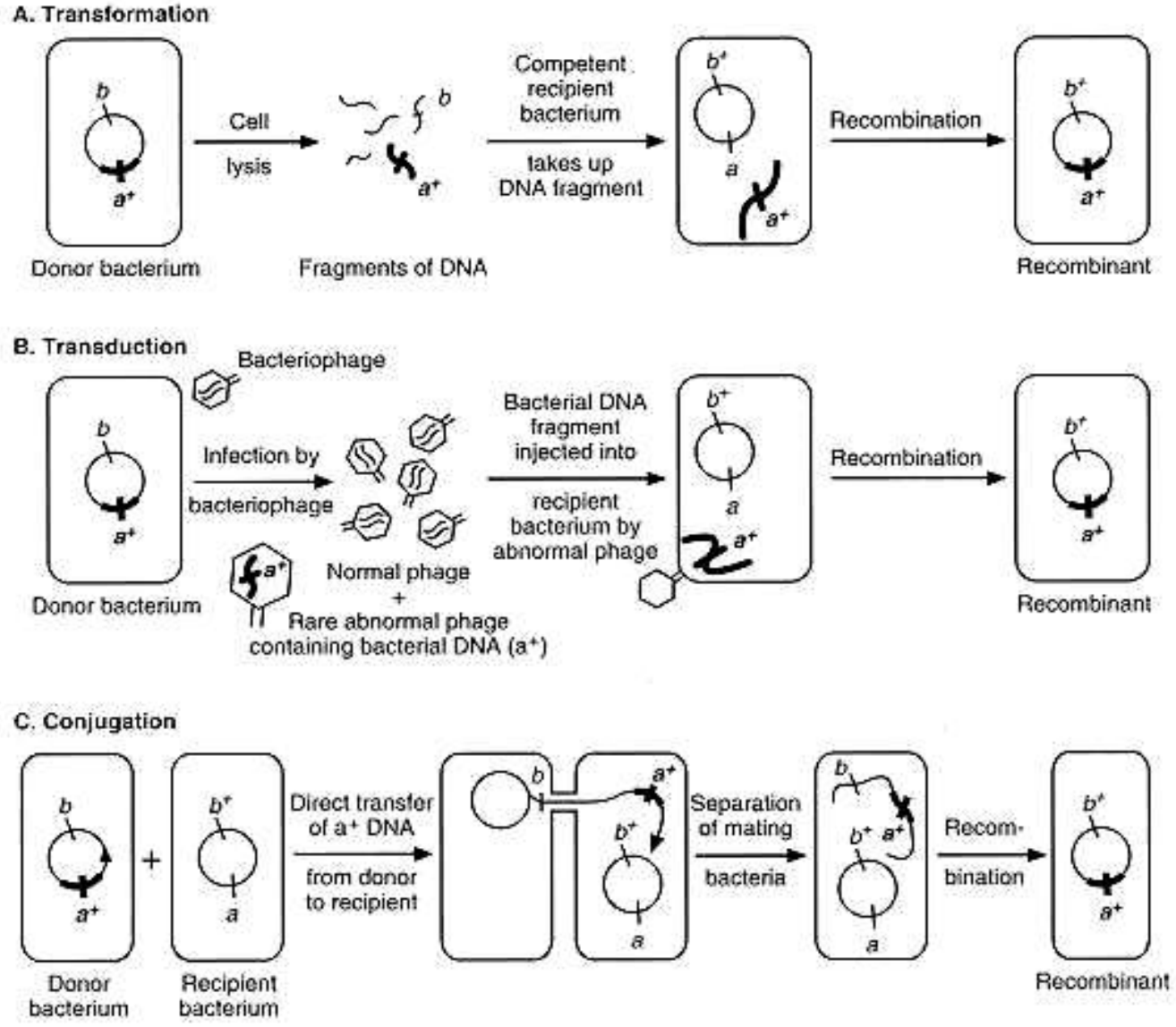


Fig. 50. Horizontal transfer of genes in bacteria: A) transformation, B) transduction, C) conjugation.

Conjugation. Plasmids. Episomes

Among alive bacteria the genetic material can be passed also directly, without bacteriophage intervention. This process is known as **conjugation** that is the transfer of bacterial

DNA from donor bacterium ("male F^+") to recipient bacterium ("female F^-") through a conjugation tube. This was first discovered in the bacterium, *E. coli*. The "male" bacterium has *sex pili* that are absent in "female" bacteria. The production of sex pili is enabled by F factor (F for fertility), which can exist either as a segment of DNA integrated in male bacterial chromosome, or as a separate unit of circular DNA called **plasmid**, which locates in cytoplasm separately from chromosome genome. A genetic element that can replicate either as a plasmid or as an integrated part of bacterial genome is known as an **episome**. In fact, episomes are plasmids that can integrate themselves into the chromosomal DNA of the host cell. In addition to some plasmids, temperate phages also qualify as episomes (recall lysogenic cycle). Plasmids and episomes are referred to as cytoplasmic inheritance in the prokaryotes.

During conjugation a sex pilus produced by the donor F+ bacterium binds to the female F- recipient. The copied F-plasmid DNA is then transferred from donor to the recipient. After conjugation the female F- cell gains F factor and transforms into F+ type.

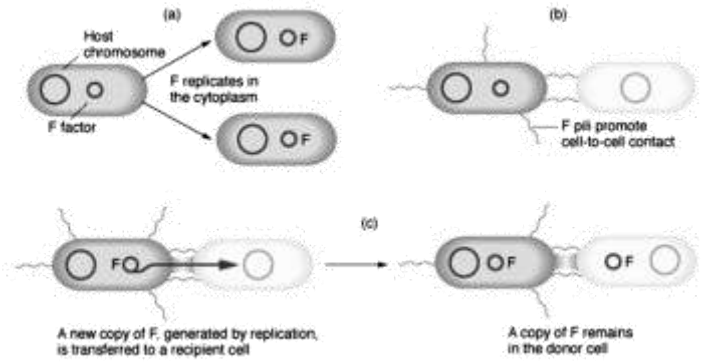


Fig. 51. Conjugation of bacteria.

There are three main classes of plasmids:

1. **Fertility** or F-plasmids which function to initiate conjugation process.

2. **Resistance** or R-plasmids contain genes that can provide resistance against antibiotic (drugs treating bacterial infections) or poisons. The bacterium containing R-plasmid is able to survive and grow in the host organism. Medical significance of bacteria carrying R-plasmid is that resistant strains of pathogens are hardly subject to antibiotic therapy. R plasmids also can transfer from one bacterial cell to another by conjugation, and even can pass in between different bacterial species (for example, *Shigella, E. coli*). Moreover, some R-plasmids may contain up to 10 genes for resistance to different antibiotics.

3. **Virulence** plasmids, which turn the bacterium into a pathogen.

Transposable Genetic Elements (Transposons)

Certain genetic elements have the capacity to move from one location to another in the genome (within the chromosome, from a plasmid to a chromosome or vice versa, or from one plasmid to another). Such mobile DNA parts are called **transposable elements (transposons)** or **jumping genes**.

There are **simple transposons** called **insertion sequences (IS)** and **composite transposons.** The **insertion sequences** contain just a *transposase gene* coding an enzyme transposase required for the movement and insertion of this element. Insertion sequences contain *inverted repeats* at their both ends (non-coding sequences about 20-40 nucleotides long, upside-down, backward versions of each other). Insertion sequences appear to cause mutations when they place within coding sequences and they are known to cause deletions in neighbouring genes. However, mutation of a given gene by transposon occurs only rarely – about once in every 10 million generations.

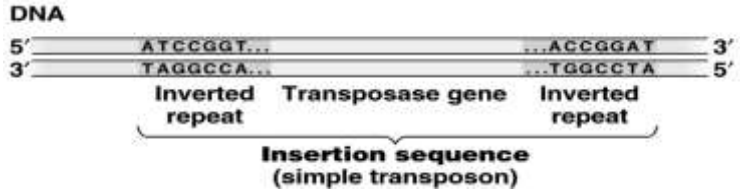


Fig. 52. Simple transposon.

for antibiotic resistance into a single R plasmid by moving the genes to that location from different plasmids. They have a significant role in evolution of R plasmids which can transfer within or between bacterial species and genera (*Shigella, Salmonella, Proteus*) all of which include pathogenic species.

Composite transposons are created when two IS elements insert near each other. Composite transposons contain also extra genes, for example genes for antibiotic resistance. Composite transposons are responsible for bringing multiple genes

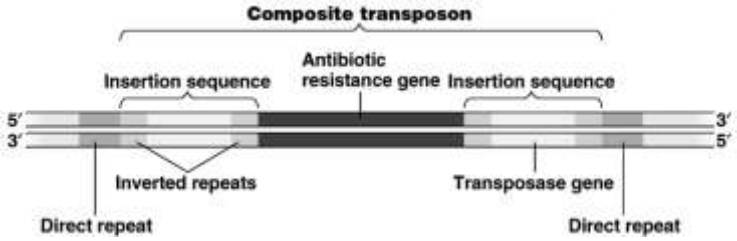


Fig. 53. Composite transposon.

CHAPTER 11
DNA repair. Genetic engineering. Cloning. Variation. Expressivity. Penetrance. IQ.

DNA Repair

The cells are permanently subject to damaging effect of the environment. To maintain the vital functions of a cell and the organism in whole, genetic material has to be resistant to spontaneous and induced DNA modifications. Certain mechanisms of DNA repair exist in all organisms including prokaryotes and highest eukaryotes (mammalians, humans). DNA repair minimizes cell killing, mutations, replication errors, which may lead to cancer and aging.

DNA repair types are:

I. Photoreactivation or Light Repair

DNA-damaging effect of ultraviolet radiation and the ability of light to correct it is observed in a variety of organisms involving both prokaryotes and eukaryotes. UV radiation (<300 nm) damages DNA by causing an extra covalent bond to form between adjacent pyrimidines (T-T, C-C or T-C) on the same strand. Particularly, the linked thymines are called thymine dimers. Their extra bonds kink the double helix sufficiently to disrupt replication. Photoreactivation repair system can reverse the UV-induced pyrimidine dimer formation. Dimers can be recognized by the enzyme **photolyase** activated under visible light (300-500 nm UV rays) and will directly split the dimer. This type of repair is called ***photoreactivation*** or ***light repair***.

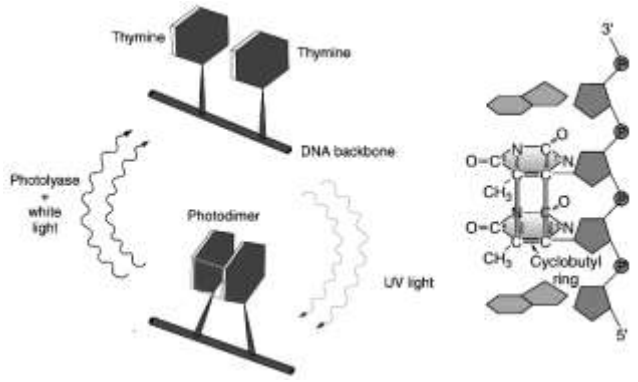


Fig. 54. DNA repair: photoreactivation.

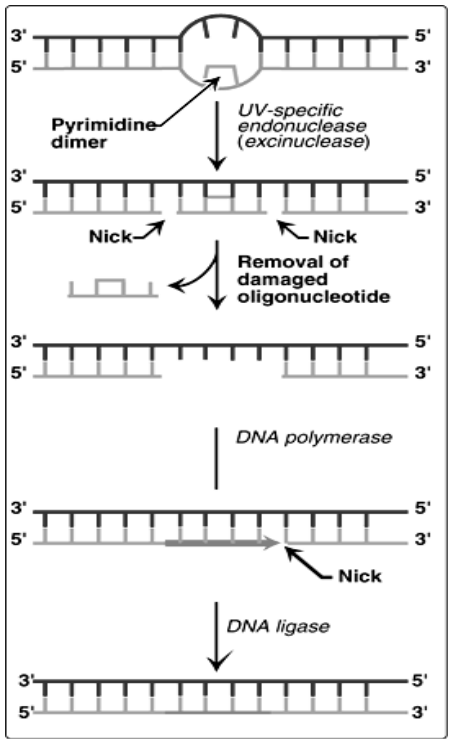


Fig. 55. DNA repair: excision.

II. Excision Repair or Dark repair is of four types:

1) UV damage repair or nucleotide excision repair. Thymine dimers that are not corrected by one-step photoreactivation under light, can be eliminated by excision repair in 5 steps:

a. ***endonuclease*** enzyme, detects T-dimer and nicks DNA strand on 5' end of the dimer.

b. ***exonuclease*** enzyme, nicks DNA 8 bases upstream and 4 or 5 bases downstream of dimer,

c. exonuclease eliminates the defective site.

d. ***DNA-polymerase*** now fills in the gap in 5' → 3' direction by complementarity with normal strand,

e. ***ligase*** seals the fragment.

2) Mismatch repair

Mismatch repair accounts for majority of all repairs. It follows behind replication fork. DNA-polymerase has also the activity of ***proofreading*** – that is proof matching of bases. This classic repair system is required to repair errors that escape detection by the proofreading systems during DNA replication. A specific DNA-pol

recognizes the replicative error and excises it. DNA-polymerase and ligase help to fill the gap with "matching" nucleotide.

3) Postreplicative (Recombinational) Repair

If T-dimer is not repaired through photoreactivation or excision repair that occur before DNA replication, then a ***postreplicative repair*** corrects the damage by recombination between two daughter DNA molecules. The complementary nucleotides opposite the T dimer cannot make hydrogen bonds with thymines during elongation of the new strand in front of defective template, and a gap arises when DNA-pol acts, while the normal mother strand serves a template for a normal new strand in second DNA molecule. The gap may be repaired by enzymes that excise a complementary segment from intact double helix, i. e. ***recombination*** occurs between two daughter molecules (*homologous recombination*). The gap that now arises in initially intact daughter helix, is filled by DNA-polymerase and ligase. The same happens to the gap originated after excision of the T dimer.

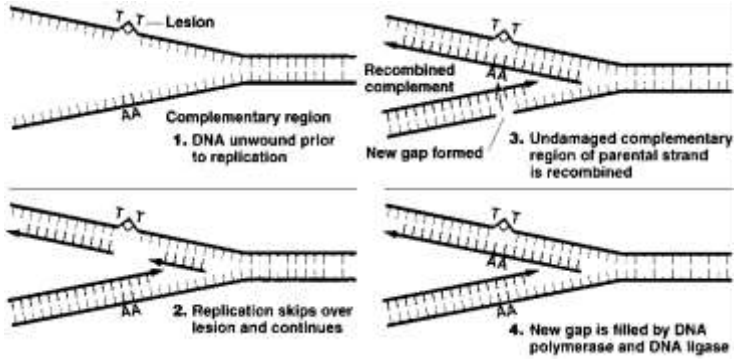


Fig. 56. DNA repair: postreplicative.

4) SOS Repair

When cells are overwhelmed by UV damage pre- and post-replicative DNA repair cannot manage all the corrections, and the ***SOS repair*** or ***inducible error-prone repair*** occurs. This allows the cell to survive but at the cost of mutagenesis.

Enzymes activated by SOS system, **inhibit cell division** in order to increase amount of time cell has to repair damage before replication. DNA replication processes are arrested if the level of DNA damage is critical, and the cell does not divide escaping transmission of mutations to offspring. This system repairs the DNA with errors and thus causes additional mutations.

Due to DNA repair mechanisms the rate of mutations decreases from 10^{-7} up to 10^{-9} (per base pair per cell division). So, DNA repair acts as an antimutagenic mechanism.

Human disease associated with DNA repair (nucleotide excision repair) disorders is ***xeroderma pigmentosum***. The patients have high sensitivity to sun light, extensive freckle-like lesions (hyperpigmentation) on sun-exposed skin and high risk of developing skin cancer.

Genetic Engineering

Genetic engineering is used for artificial modification of genetic information for creating new genotypes and providing desired phenotypes in cells or organisms. Genetic engineering realizes molecular genetics methods on gene, cellular and organism levels.

Gene (DNA) engineering is used to take genes and segments of DNA from one source and put them into another source. Gene engineering is carried out in following steps:

1. Obtaining the gene of interest through one of the following ways:

a) cutting the DNA at specific sites by *restrictase* enzyme,

b) synthesis of the gene of interest by *revertase* enzyme (producing a copy DNA from mRNA according to complementarity),

c) polymerase chain reaction (PCR).

2. Incorporation and transfer of the gene by a vector (phages, retroviruses, plasmids) to the specific site and getting a recombinant DNA.
3. Activation of the inserted gene and control of gene expression through further transcription and translation processes.

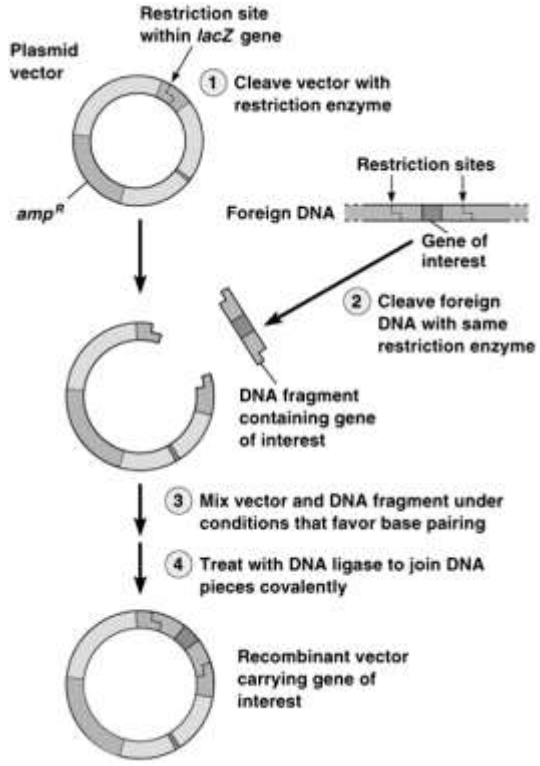


Fig. 57. Preparation of recombinant plasmid vector.

First chemically synthesised human gene was the gene of tRNA for *Alanine* amino acid (G. Khorana, 1970). After recombination with vector DNA, this gene was transferred and replicated in the host cell (bacteria, yeast or mammalian cell line).

Cloned genes can also be expressed in whole animal organism if introduced into the germ cells of an animal (e.g., mouse, fruit fly). Such an animal is called a **"transgenic organism"**. The transgenic mice can provide studies on mammalian gene expression and different models of genetic diseases.

Gene Therapy. Development of gene-transfer techniques is used for treating of inherited diseases in humans by **gene therapy**. The vectors that carry the genes of interest can be introduced both to somatic and germ cells. Several inherited diseases are likely to be treated by somatic-cell gene therapy. These include phenylketonuria (PKU), thalassaemias (haemoglobin deficiencies), sickle-cell anaemia, severe combined immunodeficiency disease (SCID).

Cloning

Reproductive cloning produces an exact copy of an existing organism. The first cloned mammal was a sheep named **Dolly** (Scotland, 1997). During cloning the nucleus from an ovum is removed and replaced with the DNA from a somatic cell of an adult animal. Then, the egg is implanted in a surrogate mother uterus, and the cloned embryo begins to divide as does a fertilized embryo. The human cloning is considered to be an immoral procedure, since there are major **ethical, religious** and **jurisdiction concerns** about human cloning.

While whole human organism is not cloned, it is possible to clone separate human organs. This procedure is called **therapeutic cloning**, which produces a healthy copy of a sick person's tissue or organ from transplant stem cells of the embryo that develops from an enucleated egg carrying a nucleus of the somatic cell of a donor organism (the patient). Stem cells are removed from the pre-embryo to produce a tissue or a whole organ for transplant back into the person who supplied the DNA. It prevents rejection of the transplanted organ, and cloned tissues can be transplanted back into donor's organism without being destroyed by own immune system.

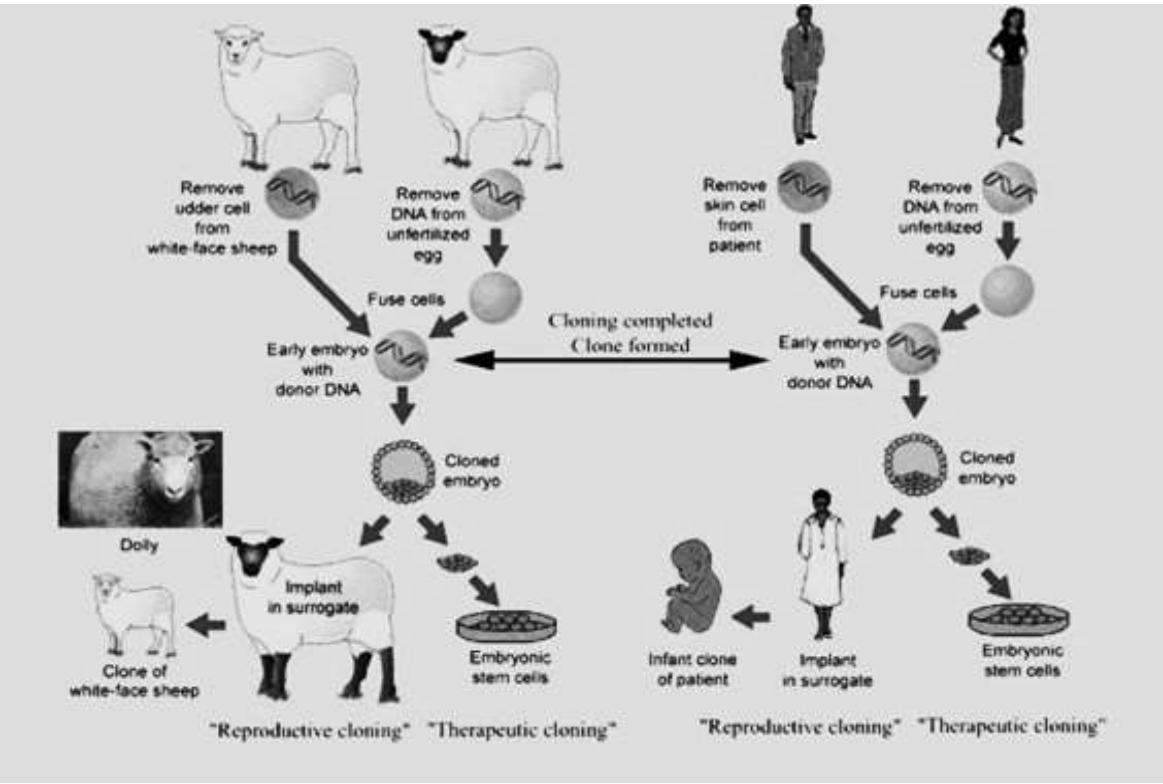


Fig. 58. Reproductive and therapeutic cloning.

Variation

Variation is the ability of an organism to gain new traits and is defined as any difference between cells, individual organisms, or groups of organisms within a species. If the difference is caused by variation in genotype, it is known as **genetic (or genotypic) variation** versus **phenotypic variation.** Variation is an important condition for phenotypic and genotypic polymorphism, biodiversity, adaptation, natural selection and speciation processes.

Genotypic variations are due to differences in number or structure of chromosomes or to changes in the genes. For example, differences in eye colour, nose form, and disease resistance are genotypic variations. Only genotypic changes can create a variation which will be subject to selection and relevant for evolution.

Phenotypic variation is caused by the effect of environmental factors that change phenotype without altering the genotype and is not transmitted to future generations. Phenotypic variation results from factors such as climate, food supply, and impact of other organisms. Phenotypic variations are often of adaptive character and not important in evolution. Natural selection occurs when there is phenotypic variation regardless of the source, but only variation with a genetic basis can be the source of evolutionary change.

Modification Variation. Various environmental factors can differently influence on the phenotypes of even genetically identical organisms in different nurturing conditions (e.g., modification variation causing differences between genetically identical monozygotic twins). Phenotypic changes that occur in individuals with the same genotypes under the known environmental impact are called **modifications**.

Modification of the trait is limited, and it cannot be modified randomly. The limit within which it can modify are genetically determined and is known as **norm of reaction**. Norm of reaction is the graphical representation of the **phenotypic value** of some trait of an individual genotype in a population. The norm of reaction thus depicts the phenotypic plasticity of a trait. Some traits have broader norm of reaction, usually these are quantitative characters, for example, skin colour intensity, body weight, etc. Narrow norm of reactions are characteristic for qualitative traits (like eye shape, blood group) which do not modify regardless of conditions. Norm of reaction for some traits is necessary to know for controlling of their phenotypic expression. The norm of reaction depends on both environment factors and individual genotypic differences (polymorphisms). Modifications do not exceed the norm of reaction. For example, change of human body weight along with different nutrition terms; change of RBC count in regard to partial pressure of air oxygen.

Phenocopy. Genocopy.

Phenocopy is a specific type of modification when an environmentally-caused (acquired) trait mimics the one caused by mutations. These cases should be precisely identified and differentiated by doctors. For example, ***thalidomide***, a pharmacological agent once used by pregnant women, caused limb defects in newborns. These abnormalities were mistaken for similar limb defects peculiar for gene or chromosomal inheritable mutations. Toxoplasmosis (protozoal infection) and rubella-virus infections during pregnancy have a high risk to develop embryonic malformations (cleft upper lip and palate, deafness, mental retardation) that resemble the ones of genetic origin (mutations).

Genocopy is introduced when aetiologically different genetic diseases exhibit the same clinical pattern of symptoms. For example, haemophilia A and B are caused by different gene

mutations, but clinically express in blood clotting defect. Mental retardation can be a symptom of fragile-X syndrome, Tay-Sachs disease or phenylketonuria (PKU).

Expressivity and Penetrance

The phenotypic expression of genotype is characterised by **expressivity** and **penetrance.**

Expressivity is a qualitative concept, which shows the variable expression of the trait. Not all the phenotypically expressed traits are manifested to the same degree. For example, the same symptom of the disease may vary in intensity among various individuals, e.g., there are three stages of severity of diabetes mellitus (mild, moderate and severe) which are dependent on external factors such as age, diet, exercise, drugs.

Expressivity is often affected by environmental factors, but sometimes specific gene combinations (e.g. gene dosage) may also alter this parameter. An example is skin pigmentation intensity that depends on both presence of number of dominant alleles in polygenic inheritance mode and *uv*-radiation.

Penetrance is a quantitative concept and shows the frequency (percentage) of expression of an allele among individuals who carry that allele. For example, if 8 of 10 people with the allele express symptoms, the disease is said to be 80% penetrant and shows incomplete penetrance. The trait is completely (100%) penetrant if everyone who inherits the allele shows it off. For epilepsy and diabetes mellitus the penetrance is incomplete and accounts for 67% and 57%, respectively. Fragile X syndrome has 80% penetrance in males and 30% penetrance in females. Incomplete penetrance is probably mediated by interaction of non-allelic genes in the genotype. One mechanism of 0% penetrance can be explained by phenomenon of epistasis.

Genetics of Human Behaviour. Intelligence Quotient (IQ).

The differences between individuals are not only of morphological, but also of behavioral and emotional nature. Some of these differences are associated with genetic factors, and the others are affected by environment. Human behaviour and emotional characteristics are tightly related to his intelligence. Whether nature or nurture influences intelligence sits at about 50/50. Intelligence is described as “the power of the mind to think in a logical manner and acquire knowledge”. It is considered that those who do well on one mental test also are likely to do well on other mental tests. Different subcategories of intelligence are detected by **Intelligence Quotient (IQ).**

In 1914 the measurement scale of IQ by dividing the subject’s mental age by his chronological age was suggested:

IQ= (Mental Age / Chronological Age) x 100

Mental age is detected by special tests. Intelligence Quotient indicates a person's mental abilities relative to others of approximately the same age. It has a bell-shape distribution with the greatest frequency (about 70%) in the centre. IQ scores reflect general capacity for performing intellectual tasks: arithmetic, logical, spelling skills, short term memory, algebraic, general knowledge, visual apprehension, geometric, vocabulary, intuition, computational speed.

Mentally inadequate people are considered as mentally retarded (e.g., ***oligophreny, idioty***). Low intelligence and mental retardation can be mediated by genetic factors (mutations), intrauterine (embryonal) development disorders caused by **teratogenic factors** (toxoplasmosis, viral and bacterial infections, drugs), hypoxia, intoxications (bilirubin, alcohol).

Table. 3. IQ score distribution among IQ test-takers.

IQ Score	Evaluation grade	Percent among test takers
40 – 55	Mentally inadequate (mentally disabled)	<1%
55 – 70	Low intelligence (learning difficulty)	2.3%
70 – 85	Below average	about 14%
85 – 100	Average	34%
100-115	Above average	34%
115 – 130	High intelligence (Gifted)	about 14%
130 – 145	Superior intelligence (Genius)	2.3%
145 – 160	Exceptionally gifted (extraordinary genius)	0.13%
over 160	"Unmeasurable" genius	–

1A

1. Genes which are responsible for synthesis of enzymes are called:

A. structural
B. functional
C. operator
D. modulator

2. Which one substance can join with repressor and detaches it from operator:

A. inducer
B. suppressor
C. regulator
D. co-repressor

3. An example of disorder in DNA repair is:

A. albinism
B. freckled skin
C. xeroderma pigmentosum
D. ichthyosis

4. Modification is:

A. phenotypic variation
B. genotypic variation
C. norm of reaction
D. a modified type of mutation

5. Phenocopy is a modification resembling a phenotype in:

A. mutation
B. recombination
C. genetic polymorphism
D. infection

1B

1. IQ does not depend on:

A. mental age
B. environment
C. heredity
D. academic progress

2. Gene engineering is not applied for:

A. obtaining of knock-out mice
B. obtaining of transgenic mice
C. gene therapy
D. somatic cell hybridisation

3. DNA repair is not:

A. antimutagenic mechanism
B. mutagenic mechanism
C. anti-aging mechanism
D. common for fungi

4. What is not common for phenotypic variation?

A. basis for evolution
B. adaptive character
C. change of phenotype
D. modifications

5. Phenocopy does not:

A. mimic gene mutation
B. develop by external factors
C. mimic infection
D. mimic chromosomal mutation

II

1. Which of the followings realise excision repair of DNA?

1. topoisomerase
2. ligase
3. DNA polymerase
4. photolyase
5. exonuclease

A. 1,2,3 B. 2,3,5 C. 4,5 D. 3.4

2. Which type of variation is heritable?

1. genotypic
2. phenotypic
3. recombinations
4. mutations
5. modifications

A. 1,2,4 B. 3,4,5 C. 1,3,4 D. 2,5

3. Gene engineering is targeted at:

1. synthesis of genes by reverse transcriptase
2. introduction of new genes to host genome
3. study of genome mutations
4. study of gene mutations
5. transfer of genes by vectors

A. 3,4 B. 1,4 C. 2,3,5 D. 2,4,5

4. Penetrance is:

1. intensity of expression of the trait
2. complete or incomplete
3. percent of expression of the trait
4. quantitative characteristic
5. qualitative characteristic

A. 1,2,5 B. 1,3,4 C. 2,3,5 D. 2,3,4

5. Which of the followings are phenocopies?

1. thalidomide-caused phocomelia
2. rubella-caused deafness
3. mental retardation in Down syndrome
4. mental retardation in toxoplasmosis
5. polydactyly caused by gene mutation

A. 3,4 B. 1,2,5 C. 1,2,4 D. 3,5

CHAPTER 12
Genotypic Variation. Mutations and Mutagenesis. Antimutagenic mechanisms.

Genotypic Variation. Mutations and Mutagenesis

Genotypic variation occurs when the genotype of an organism changes, and this variation is heritable. Genotypic variation is of two types: recombination variation and mutation variation.

Recombination variation is associated with:

a. crossing over between homologous loci of homologous chromosomes during prophase of meiosis I,

b. independent assortment of non-homologous chromosomes on metaphase plate during meiosis I (2^n combinations of maternal and paternal chromosomes in gametes)

c. random fertilization ($1/2^n$ x $1/2^n$ possible chromosome combinations in zygotes).

Some organisms can develop also horizontal transmission of genetic material: i.e. transformation, transduction, conjugation that lead to new combinations of genes. Recombination increases genotypic and phenotypic polymorphism and contributes to natural selection and evolution process.

Drastic, permanent changes in the genetic material are called ***mutations***. Most of the mutations are harmful and recessive (wild type transformation into mutant), and except somatic mutations the rest are heritable.

Mutation theory developed in early 20th century by *Hugo de Vries* (1901-1903) according to which the mutations arise drastically, without transitions, they are constant, multidirectional (harmful and beneficial), and they can recur.

Here is the **mutation classification** offered by *S.G. Inge-Vechtomov* (1989) **in regard to:**

I. Origination:
1. spontaneous (natural, unknown factors)
2. induced (by known artificial mutagens)

II. Level of genotype involved:
1. gene (point) mutation
2. chromosomal aberrations (change in chromosome structure)
3. genome (change in chromosome number – aneuploidy)

III. Effects on phenotype:
1. harmful
2. neutral
3. beneficial

IV. Heterozygous expression:
1. dominant
2. recessive

V. Heritability:
1. somatic
2. germinal

VI. Location in cell:
1. nuclear
2. extranuclear

VII. Wild or mutant allele:
1. direct (wild (A) → mutant (a))
2. indirect (mutant (a) → wild (A))

The harmful heritable changes lead to natural selection of more viable organisms and species, they provide adaptation to environment and a material for evolution. On the other hand, in human populations *lethal and sublethal mutations* are the cause of many genetic diseases that may lead to death. *Neutral mutations* do not express phenotypically, they change only genotype and are called *silent mutations*.

Mutations are heritable when they involve germ cells – gametes. Changes of genetic material in somatic cells are not transmitted to progeny. For example, moles, cancer.

Since genetic information is located not only within nucleus but also in the cytoplasm (extranuclear inheritance), mutations can refer to the extranuclear genes as well, for example mutation in mitochondrial genes, plasmids.

According to the origination of the mutations they are of two types: ***spontaneous and induced. Induced mutation***s are caused by certain mutagens, different factors that damage DNA. ***Spontaneous mutations*** are those that occur without a known exposure to a mutagen. They may result from mistakes during or post DNA replication, or they may actually be caused by environmental mutagenic agents (e.g. natural radiation: sunlight, cosmic rays, the earth's crust). Another reason of spontaneous mutation is the shifting of transposons within the genome.

Mutation rate is the probability of particular kind of mutation per a period of time, e.g. number of mutations per gene per cell division (or per generation). Mutation rate at the nucleotide level is usually estimated as to be about 10^{-9} per base pair per cell division. At the level of a gene the mutation rate is quite variable, ranging from 10^{-4} to 10^{-7} per locus (gene) per cell division. **Mutation frequency** is the number of times a particular mutation occurs in proportion to a number of cells or individuals in a population.

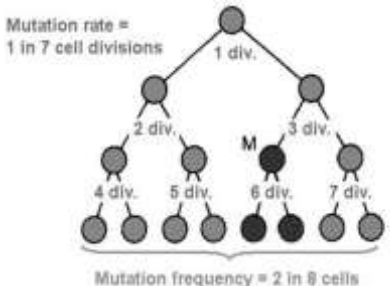


Fig. 59. Mutation rate vs. mutation frequency.

The rate of spontaneous mutations varies from gene to gene and from organism to organism. It can depend on gene size (the larger the more frequent), location (mitochondrial genes mutate at higher rate as well, since they lack DNA repair mechanisms), parent's age (e.g. Down syndrome correlates with mother's age, and Marfan syndrome – with father's age).

Inducible mutations are caused by mutagenic agents, which can be subdivided into physical, chemical, pharmacological and biological. The mutagens often may cause cancer, such mutagens are called ***cancerogens***.

1. Physical mutagens are the high temperature and radiation. The radiation can be ionizing and non-ionizing due the penetrating ability.

Non-ionizing radiation is the ultraviolet spectrum within 260-270 nm range where it is absorbed by nucleic acid and develops pyrimidine dimers. It can cause skin cancer. **Ionizing radiations** are **X-rays** and **gamma rays**, which have much more energy and penetrating power. They ionize mainly the cell water molecules to form radicals (molecular fragments with unpaired electrons) that can break different bonds in DNA (hydrogen, phosphodiester, bond between sugar and base), causing also deletions of chromosome fragments or loss of the bases. Radiation energy is able also to form abnormal bonds within DNA (e.g., pyrimidine dimers, cross-linking of non-complementary nucleotides, etc.). Gamma radiation is more powerful than X-rays; it is emitted from radioactive isotopes and is able to penetrate through all the body.

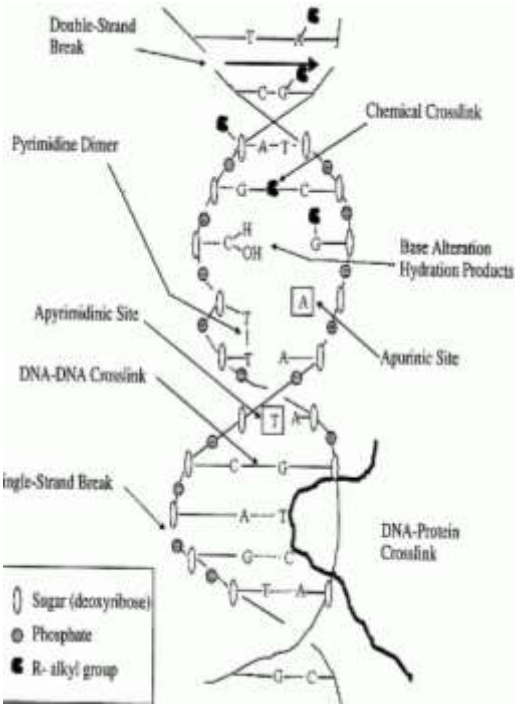


Fig. 60. Effects of physical mutagens.

2. Chemical mutagens. The chemical mutagens are the 5-bromuracil (base analogue), acridine dyes (3-ring molecules having the size of nucleotide pair), alkylating agents (have alkyl group, e.g. mustard gas and EMS (ethyl methyl sulfoxide) which cause change of nucleotide type and mispairing), colchicin.

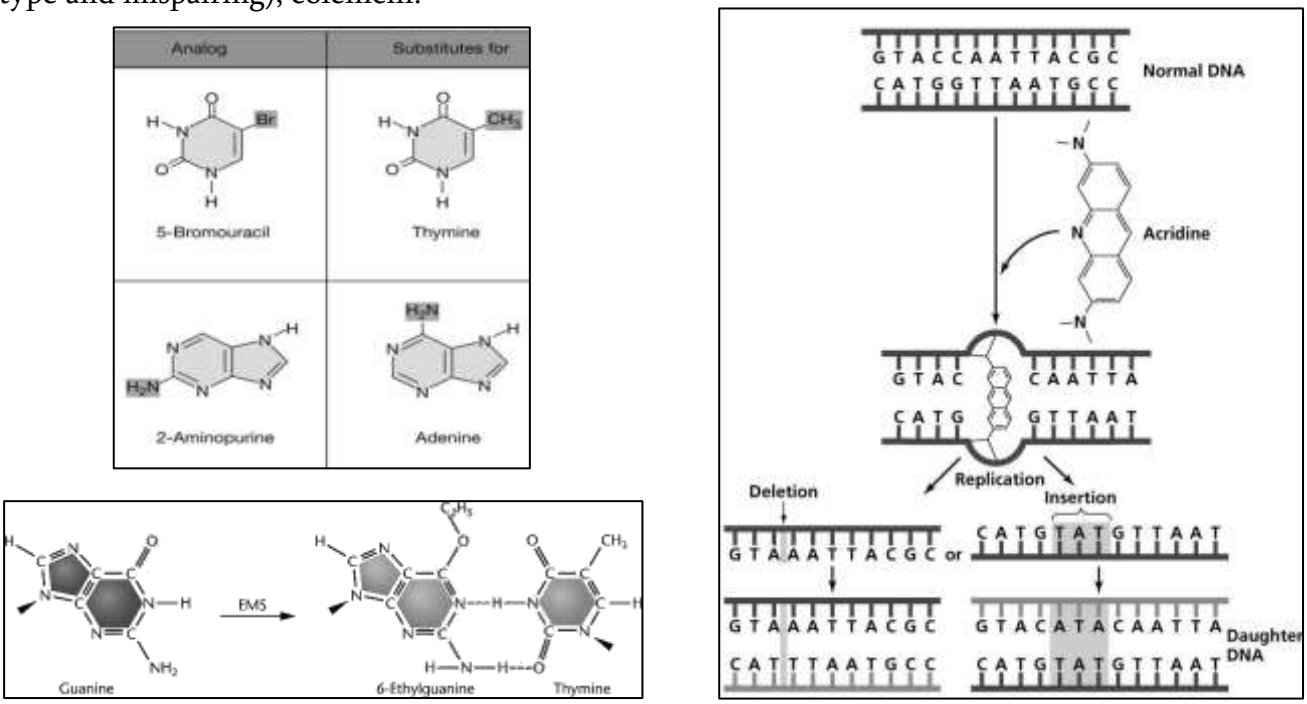


Fig. 61. Effects of chemical mutagens.

3. Pharmacological mutagens are certain drugs which can be mutagenic and are contraindicated especially during pregnancy (e.g. thalidomide), since they cause embryonic malformations and are known as ***teratogenic factors*** *(teratos - monster)*.

4. Biological mutagens are viruses, some bacterial toxins and transposable genetic elements. Viruses may lead to gene mutations and chromosomal aberrations, while transposons cause frameshift mutations.

Gene Mutations

Gene mutations are known also as point mutations, since genes are the structural and functional unit of genetic material, and physical changes in gene structure are so small that cannot be detected macroscopically. Gene mutations in relation to biochemical and clinical expression of the disease are also called ***genopathies*** or ***enzymopathies***, or ***molecular diseases***.

Types of gene mutations include ***dimer formation, substitution of a nucleotide, frameshifts (nucleotide addition or deletion), triplet repeat expansions.***

Nucleotide **substitution** is the replacement of base pairs in the nucleotide triplet during DNA replication. In regard to position of substitution it can result in different effects on polypeptide encoded by the gene. If a mutation does not alter the polypeptide product, this is termed as **silent mutation**. This happens if the substitution occurs in the third nucleotide of the triplet. Some gene mutations may lead to the change in the encoded polypeptide and can occur as missense or nonsense mutation. **Missense mutation** –a single base pair substitution that occurs on first or second position of the triplet and results in one wrong codon and one wrong amino acid. An example of missense substitution is ***sickle-cell anemia (***substitution of *valine* amino acid triplet with glutamin amino acid triplet). **Nonsense mutation** – change in the base

sequence results in transcription of a stop or nonsense codon. Therefore, translation of the mRNA will stop prematurely. **Frameshift mutations** are **additions** (insertions) and **deletions** of nucleotides. This causes a reading frame to shift, and all of the codons and all of the amino acids

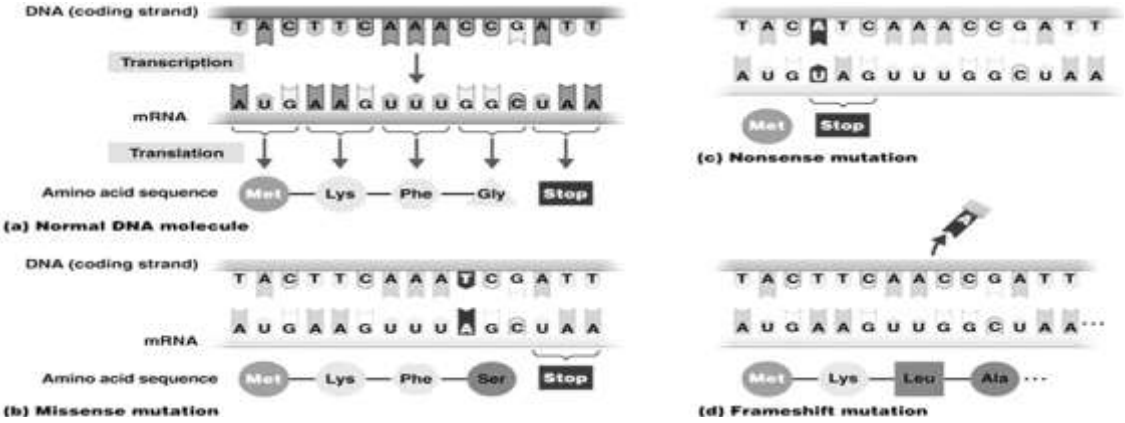


Fig. 62. Types of gene mutations.

after that mutation are usually wrong. Translation of the gene is "frameshifted" as well. Frameshifts often create new stop codons and thus generate nonsense mutations.

Various **triplets repeat expansions** present another type of gene mutation with expanded copies of certain repeated triplets. For example, **Huntington's disease** is a dominant autosomal mutation causing progressive mental retardation and uncontrollable movements of limbs, convulsions. Mutation is caused by multiple duplications (over 35) of CAG triplet. An inverse relationship exists between the repeat length and the age of onset. Repeats over 50 copies cause disease onset at an early age. **Fragile X syndrome** is characterised by more than 200 copies of repeated CGG triplets, which make the DNA unstable (fragile).

Chromosomal Aberrations

Changes in chromosome structure are known as chromosomal mutations or aberrations. Structural chromosome abnormalities may be **unbalanced** – when the rearrangement causes a gain or loss of chromosome material, and **balanced** – when rearrangement does not produce a gain or loss of chromosome material. Unlike aneuploidy (change in chromosome number), balanced structural abnormalities do not cause serious health consequences. Chromosomal abnormalities are of four types: **deletions, duplications, inversions, translocations.**

1. Deletion is a loss of any fragment of the chromosome. Deletions sometimes occur at both tips of a chromosome. The remaining chromosome ends can then fuse due to loss of telomeres, forming a **ring chromosome**. Ring chromosomes are often lost, resulting in monosomy for the chromosome. An **isochromosome** shows deletion of one arm with duplication of another. The most commonly found isochromosome is that which consists of two long arms of X chromosome. This accounts for about 15% of all cases of Turner syndrome.

"C*ri du chat*", or cat's cry syndrome, a condition where a deletion in the short arm of chromosome 5 leads to extreme mental retardation (IQ < 30), microcephaly, and a mewing-like cry due to abnormal larynx. Another case is a deletion of the short arm of chromosome 4 – **Wolf-Hirschhorn syndrome**.

2. Duplication is gain or addition of chromosome parts leading to partial trisomy. A reason for duplication can be unequal crossover during meiosis, which results in a loss of chromosome

material on one homologue and gain of material on the other (for example, some types of colour blindness).

3. Inversion is the result of two breaks on a chromosome followed by the reinsertion of the missing fragment at its original site but in inverted order. Thus, a chromosome symbolised as AB***CDE***FG might become AB***EDC***FG after an inversion. In some cases even a change of genes' position may lead to serious clinical consequences **(position effect)**. For example, some cases of

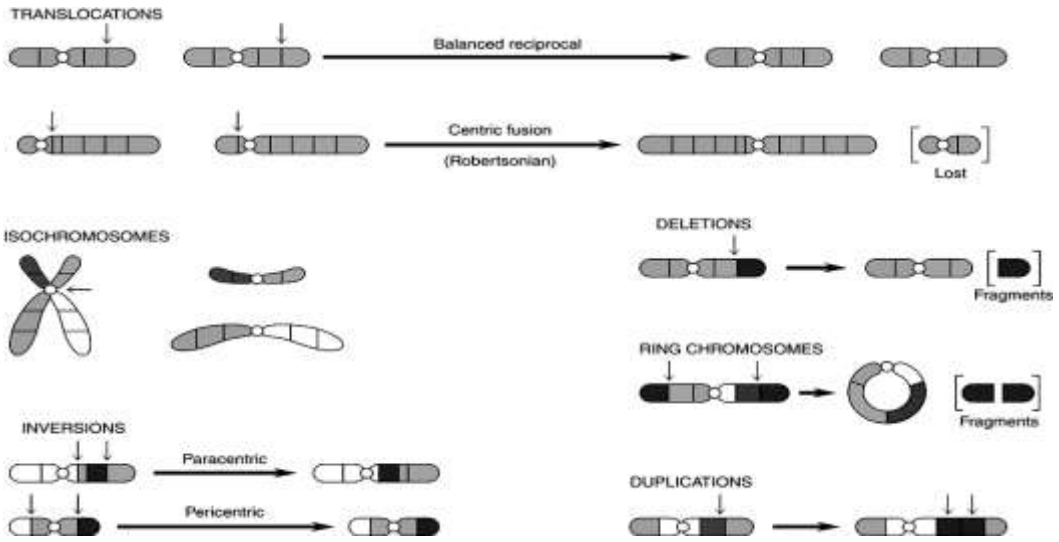


Fig. 63. Chromosomal aberrations.

haemophilia A are caused by chromosome inversion that disrupts factor VIII gene on X chromosome.

4. Translocations are the interchange of genetic material between non-homologous chromosomes. There are three basic types of translocations, termed **reciprocal, non-reciprocal** and **Robertsonian.**

Reciprocal translocations happen when breaks occur in two different (non-homologous) chromosomes, and the material is mutually exchanged (balanced translocation). For example, the Philadelphia chromosome is formed by reciprocal translocation between 9 and 22 chromosomes and forming of smaller chromosome 22. This causes a ***Chronic Myelogenous Leukemia (CML)*** – a type of a blood cancer (change of gene locations, i.e. position effect).

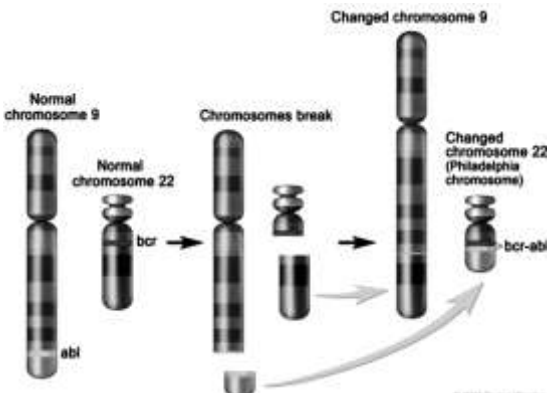


Fig. 64. Reciprocal translocation. Philadelphia chromosome.

Non-reciprocal translocations are termed those, in which a fragment from a chromosome is cut and translocated onto non-homologous one (balanced translocation).

In Robertsonian type of translocation the short arms of two non-homologous chromosomes (usually acrocentric – NN 13, 14, 15, 21 and 22) are lost and the long arms fuse, forming a single chromosome (unbalanced translocation). The carriers of Robertsonian translocation lose no essential genetic material, they have 45 chromosomes (one normal 14 chromosome, one normal 21 chromosome and one 14q21q) and are phenotypically normal. Some of their offspring (1/6) may develop 14q21q+21 gametes and result in ***translocation***

variant of Down syndrome after fertilization with normal gamete. Translocation Down syndrome individual presents with 46 chromosomes.

Genome Mutations

Genome mutation is the change in chromosome number which alters the genome. Genome mutation is caused when the "ploidy" of the genome (number of chromosome set) is changed. These are **aneuploidy** and **polyploidy**. The most common cause of aneuploidy is **non-disjunction**, the failure of chromosomes or chromatids to disjoin normally during meiosis. The resulting gamete either lacks a chromosome or has two copies of it, producing a monosomic or trisomic zygote, respectively.

Aneuploidy is the variation of chromosome number due to loss or addition of 1 or more of the chromosomes expressed as 2n±k. Variants of aneuploid cells are the monosomy, nullisomy, trisomy.

Monosomy (2n–1) results from a loss of a single chromosome. Example is Turner syndrome (45, X). ***Nullisomy*** (2n–2) results from a loss of homologous pair of chromosomes. ***Trisomy*** (2n+1) is presented as a set with one extra chromosome. For example, Down's syndrome – trisomy 21 (47,XX21^{+}), Patau syndrome – trisomy 13 (47,XY,13^{+} lethal), Edward's syndrome – trisomy 18 (47,XY,18^{+} lethal). Trisomies of sex chromosomes are: supermale syndrome – 47,XYY; superfemale syndrome – 47,XXX; Klinefelter syndrome – 47,XXY.

Usually few aneuploids, especially monosomics, can be viable in animals. Most of them, as well as the polyploidies terminate in spontaneous abortion. Sex chromosome (particularly X chromosome) aneuploidy occurs more often than autosome aneuploidy because in X-aneuploidy the individual (female) gains or loses functionally inactive genes on X chromosome, while majority of genes on extra or lost autosomes are active (**dosage imbalance**). It is estimated that approximately 50% of fetal deaths accounts for autosomal trisomies. All the autosomal monosomies are lethal.

Polyploid cells carry entire sets of chromosomes, for example 3n, 4n, etc. This is common in some plants and is not encountered in animal species.

Antimutagenic mechanisms

Along with many mechanisms that cause mutations there are also processes that prevent mutagenesis, otherwise irreversible changes could occur in the organism. Here are some of the antimutagenic mechanisms:

1. **DNA repair.** DNA repair process decreases the rate of mutations about 10 folds. The mechanisms of DNA repair are discussed above.
2. **Extra copies of genes.** Some genes exist in the genome in many copies, the activity of which can remain the same in case of mutation of either one. For example, there is more than one copy of genes of histone proteins, enzymes of DNA replication and proteins responsible for cell division ("house-keeping genes").
3. **Diploidy of the genetic material.** Somatic cells contain diploid set of chromosomes, and mutation on either of the homologues prevents immediate phenotypic expression of mutation, because if the genotype is homozygote dominant, recessive mutation will lead to heterozygote state and phenotype will not change.
4. **Triplet structure of genetic code and redundancy of triplets.** Degenerate form (redundancy) of triplets means that the same amino acid can be encoded by more than one

triplet, which differs only by a nucleotide on third position. The mutation of third nucleotide leads to a triplet that still encodes for the same amino acid, and therefore the mutation is called a *silent mutation*.

5. Antioxidative enzymes and vitamins. In some pathological conditions of the organism (oxidative stress, cancer, inflammation, etc.) lipid peroxidation process is activated and brings to free radical formation, which may damage biological membranes of the cells and also DNA, causing a mutation. The process of lipid peroxidation is prevented by several antioxidants (vitamins A, E, C, catalase, etc.) that neutralize damaging and mutagenic effects of free radicals.

6. T-killer lymphocytes. There are several populations of T-lymphocytes. T-killer lymphocytes are able to recognise mutant cells arising spontaneously and destroy ("kill") them. Otherwise a mutant cell line could develop in the organism.

7. Natural selection process. During natural selection many species that express fatal mutations, are eliminated from population (see lethal genes). In this concern, the natural selection itself can be an antimutagenic mechanism.

CHAPTER 13

Methods to Study Human Genetics. Genetic Counselling. Treatment of Genetic Disorders.

Methods to Study Human Genetics

Main goal of medical Genetics is to diagnose and prevent hereditary diseases. And recently, gene therapy enabled also the treatment of some genetic disorders.

To study Human Genetics one must know the specificities of the study methods. Here are listed some of them:

1. It is impossible to study the desired cross in human society.
2. Few offspring in the human generation, which makes difficult to conduct statistic analysis.
3. Long generation gap (about 25-30 years age) and relatively late puberty (13-15 years age).
4. Presence of many groups of linkage in human genome (23 linkage groups in female and 24 in male).
5. Huge genetic variation.
6. Because of presence of numerous genes in the human genome and interaction between most of the genes it becomes very complicated to study the effect of discrete genes, especially through several generations.

Genealogical Method. Pedigree analysis

"*Pied de grue*" means "crane's foot" after the appearance of the genealogical tree as a bird's foot. The classical cross fertilization breeding experiments as performed by Mendel are not allowed and impossible in humans, so the study of genetic traits in humans must rely on observations made while working with individual families usually in genetic counseling centers!

The person who interested the investigator when starting pedigree analysis is called ***proband***. First degree relatives that are related at the parent-offspring levelare called***siblings***. A pedigree is built of symbols (square, circle, dotted symbols for carriers, etc.) and lines showing different generations.

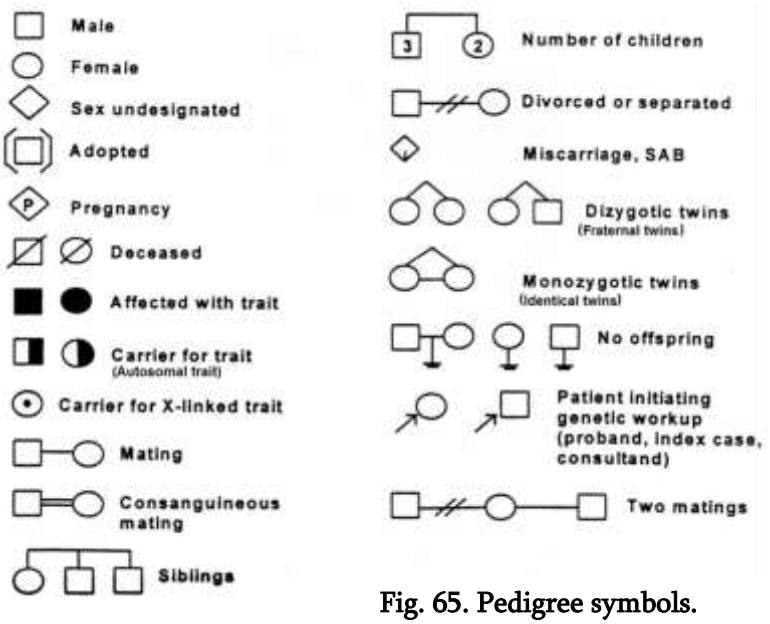


Fig. 65. Pedigree symbols.

The information presented in a pedigree can be analysed to determine:

a. whether a given physical trait is heritable or no;
b. the pattern of inheritance is (dominant or recessive);
c. information about linkage (linked or no; autosomal, sex-linked or mitochondrial);
d. penetrance and expressivity of the trait.

Autosomal traits can be inherited from both parents equally by daughters and sons.

Features of an autosomal dominant pattern of transmission are:

1) it is observed through consequent generations, and it is said to be a vertical transmission;

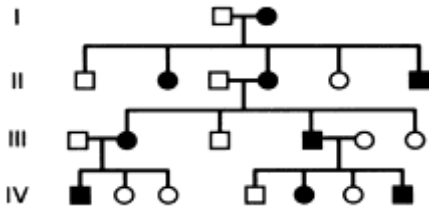


Fig. 66. Pedigree of autosomal dominant transmission.

2) all forms of transmissions between sexes are possible (i.e., female to male, female to female, male to male, male to female);

3) affected individuals who mate with unaffected individuals have a 50% chance of transmitting the trait to each child.

Features of autosomal recessive transmission are:

1) parents of the affected individual can be phenotypically healthy but are carriers of the trait;

2) pedigree involves rare traits, usually found in a single generation.

3) recurrence risk for a trait to appear in the generation of carrier parents is 25%;

4) the unaffected parents (carriers) of an affected individual may be related to each other (they are **consanguineous)**.

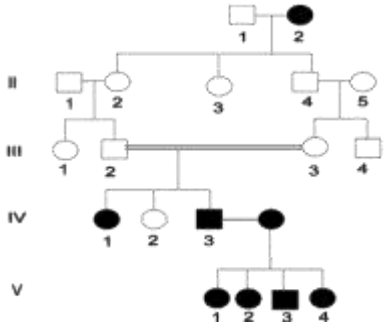


Fig. 67. Pedigree of autosomal recessive transmission.

5) transmission from both parents to sons and daughters.

Sex-linked inheritance patterns

X-linked dominant inheritance:

1) are seen in females twice as many as in males, because it is thought that hemizygous males are so severely affected that usually they do not survive to term (birth).

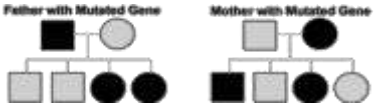


Fig. 68. Pedigree of X-linked dominant transmission.

2)heterozygous females, having one normal allele, tend generally to have milder expression of X-linked dominant traits (mosaicism).

3)affected males can transmit the disorder to their daughters but not sons (no father-son transmission).

X-linked recessive inheritance: transmission usually through unaffected carrier females to sons, from affected males to daughters (criss-cross), absence of father-son transmission.

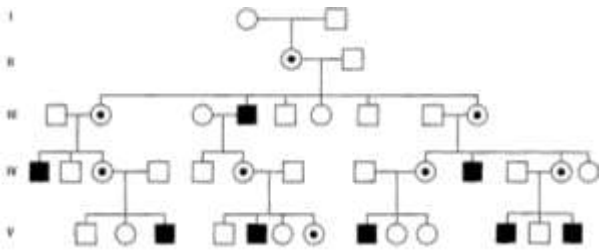


Fig. 69. Pedigree of X-linked recessive transmission.

Y-linked inheritance is peculiar only for males, with father-son transmission and affection of only males.

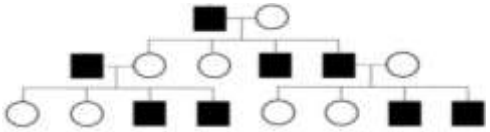


Fig. 70. Pedigree of Y-linked transmission.

Twins' Study

Twins can be identical – **monozygotic** (MZ) or non-identical – **dizygotic** (DZ). MZ twins develop from a single zygote due to its cleavage at the 8 or 16 cell stages and have identical genotypes. DZ twins originate from polyovulation (fertilization of two ova by two sperms) and are no more closely related genetically than brothers and sisters. Very late division of zygote occurring more than 2 weeks after fertilization can result in conjoined twins called ***Siamese*** twins.

Twins study can help in assessment of genetic and environmental influence on expression of traits because MZ twins have identical genotypes, and it is evident that any difference in their phenotypes would be the result of impact of different environments if the MZ twins are "raised apart".

For example, if among 100 MZ twin pairs mental retardation appears in 87 twin pairs, the twins are called 87% **concordant** for the given trait. And if among 100 DZ twin pairs mental retardation develops in 37 pairs and in the rest 63 pairs the trait expresses only in one of the twin members the other being normal, the twins are referred to as **discordant** for the trait. The discordance in this case is 63%. To detect the role of heredity and environment in certain traits expression, a formula is suggested:

H = (MZ concordance % – DZ concordance %) / (100 – DZ concordance %) x 100

When the heredity quotient (H) is equal to 1, then the trait completely depends on genotype, and the environment (E) has no role. For example, blood groups, gene mutations, etc. are completely the same in monozygotic twins. When H=0, no genetic factor influences the expression of the trait, and it is completely developed due to environmental impact (E=1). For example, this occurs in all infections, which are acquired from environment. Heredity quotient varies between0 and 1 for ***multifactorial diseases*** (e.g., schizophrenia, arterial hypertension, diabetes, peptic ulcer, cancer). Role of environment is determined by formula: **E=100% – H.**

If the concordance for schizophrenia in MZ twins is equal to 70%, and it is 13% in DZ twins, the heredity quotient H is calculated as following: (70-13)/(100-13)·100=0.65, or 65%. Then E=35%, meaning that the environment can partially also contribute to expression of schizophrenia.

Dermatoglyphics

The word **dermatoglyphics** comes from two Greek words (*derma, skin* and *glyphe, carve*) and refers to the friction ridge formations which appear on the fingers, palms and soles. Respectively, the methods are called **dactyloscopy,palmoscopy** and **plantoscopy.** The ridges are in fact the dermal papillae formed by dermal epithelium, on which the sweat glands open through pores.

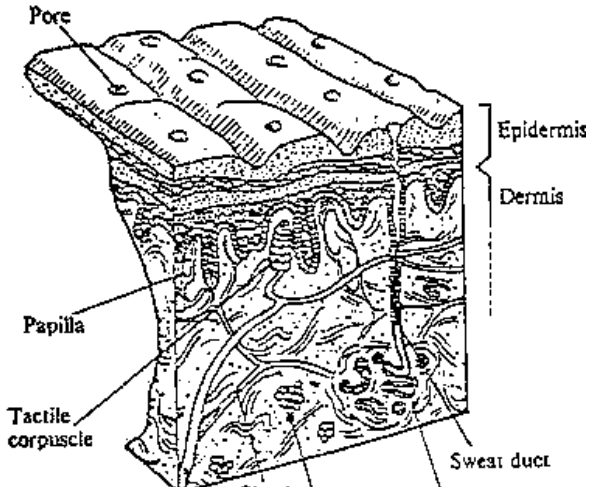


Fig. 71. Skin ridges formed by dermal papillae.

Ridge formations of the skin begin to appear during the third and fourth month of embryonal development and do not change through whole life span. There are not two individuals with totally same patterns of dermal ridges.

Dactyloscopy. Important dermatoglyphics on the fingers are situated only on the distal phalanges as fingerprints. A *triradius* is a point from which three ridge systems intercourse in three different directions, at angles of about 120°. There are three main types of finger ridge patterns depending on the number of ***triradia*** present by them: *arches*, *whorls* and *loops*. There are two triradia in whorl pattern, one in a loop and none in an arch. Fingerprint pattern contains a certain number of ridges crossing the straight line between the triradius and core of the pattern. Number of ridges varies in patterns and individuals.

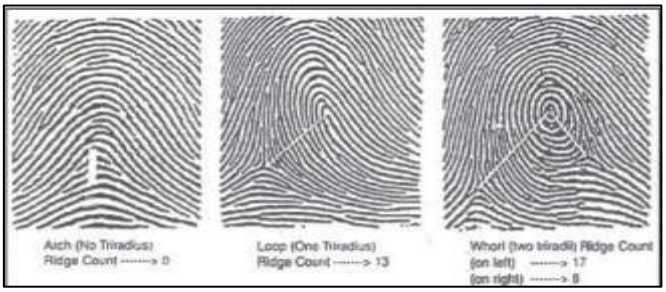


Fig. 72. Fingerprint patterns.

Fingerprints are important for **identification of zygosity** (MZ or DZ) and identification of the person in **criminology** practice (even though DNA analysis is the most precise method). If 7 or more fingers out of 10 have the same pattern in both of the twins pair, then they are MZ twins. When only 4-5 fingers coincide in finger skin patterns, the twins are DZ.

Palmoscopy. The palm patterns are defined mainly by five triradii: four digital triradia at the bases of the 2nd, 3rd, 4th and 5th fingers referred to as *a, b, c, d*. and an axial *triradiust* near the base of the palm and near palmar axis. The position of axial triradius is measured as the ***atd*** *angle*, which normally ranges between 48° and 57°. It changes in some genome mutations: Down syndrome – ∠atd = 81º, Turner syndrome – ∠atd = 66°, Patau syndrome – ∠atd = 108°, Klinefelter syndrome – ∠atd = 42°.

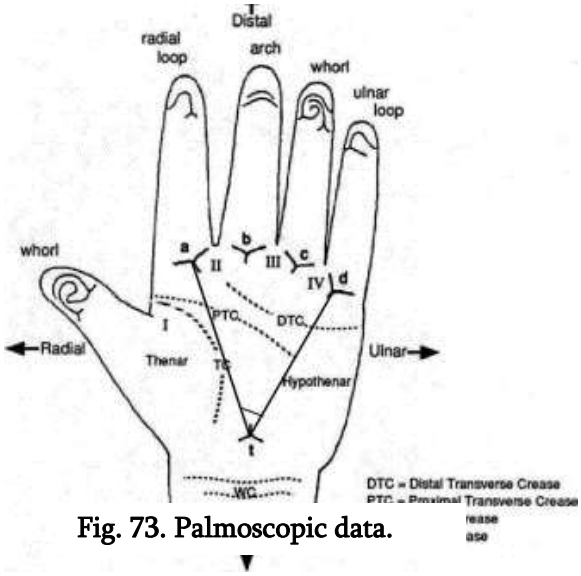


Fig. 73. Palmoscopic data.

The **flexion creases** – heart, head and life lines of palmistry are not dermal ridges, but they are formed at the same time. A **simian crease** (single transverse crease) in place of the usual two creases is common in Down syndrome, but it occurs approximately in 1% of normal individuals, at least on one hand.

Population Statistics Method. Population Genetics

Population genetics studies not individuals or families, but all of the alleles in a population, which constitute the ***gene pool***. Population genetics studies the frequencies of occurrence of the dominant and recessive alleles, genotypes (homozygotes, heterozygotes) and phenotypes in a given population. It helps to determine the carrier (heterozygotes) frequencies and mutation prevalence when the disease incidence is known.

Hardy-Weinberg genetic equilibrium formula predicts the expected genotype frequencies using the allele frequencies in a diploid, sexually reproducing population. Consider an ideal population in which there is a biallelic locus with the frequencies of alleles ***A*** and ***a*** equal to ***p*** and ***q***. These are the only alleles found on this locus, so that: (p + q) = 100% or 1.

This means: $(p+q)^2 = 1$. It is also correct that: $(p+q)^2 = p^2+2pq+q^2 = 1$.

In this formula, p^2 corresponds to the frequency of homozygous genotype AA, q^2 to aa, and 2pq to Aa. Since "AA, Aa, aa" are the three possible genotypes for a biallelic locus, the sum of their frequencies should be 1.

$$p^2 + 2pq + q^2 = 1$$
$$AA \quad Aa \quad aa$$

Frequency of recessive mutation in the population may allow detection of heterozygote carrier (2pq) in the population, so and the risk of having affected child from carrier parents in the population. Though the actual number of individuals with each genotype will change, as the population size increases or decreases, but their proportions (relative frequencies) remain constant.

Somatic Cell Hybridization Method

Hybridization of somatic cells is used to study heredity and variability of somatic cells. This method compensates otherwise impossible hybridological analysis of humans. It enables constructing genetic maps for the human chromosomes (*see Genetic maps*).

Cytogenetic Methods

1. Cytogenetic analysis enables microscopic study of chromosomes in human cells. Differential staining (banding) of chromosomes during ***karyotyping*** helps to identify chromosomes according to the pattern of band staining. After chromosome banding and preparing a karyogram, it is possible to **count total number of chromosomes (**diagnose genome mutations**)** and also **reveal some structural changes (chromosomal aberrations).**

2. Cytogenetic method includes also **sex chromatin (Barr body) detection**, which is applied as an express diagnostic tool for sex identification, as well as genome mutations of X-chromosome (Turner *45,X*; superfemale *47,XXX* and Klinefelter *47,XXY* syndromes). The cells of buccal (cheek) epithelium, and sometimes also the WBCs are the subject for study. The sex chromatin in the WBC nucleus is known as "*drum stick*". The number of Barr bodies is one as less as all the X chromosomes.

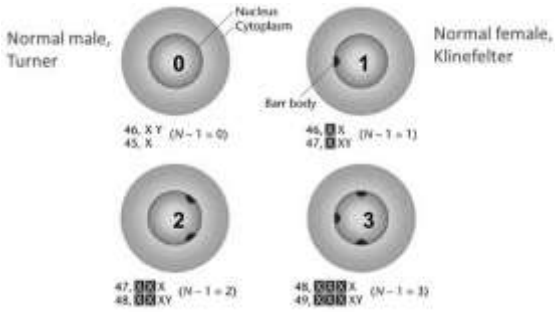


Fig. 74. Barr body detection.

Biochemical Method

While the genome mutations and chromosomal aberrations can be detected by cytogenetic methods (e.g., karyotyping), single gene disorders (molecular diseases or enzymopathies) can be diagnosed by biochemical tests, which reveal any product of the disrupted metabolism or the defective enzyme responsible for that metabolism in blood or urine. Pathologic effects are mainly due to toxic accumulations of substrates or intermediate metabolites.

For example, **phenylketonuria (PKU)** is an autosomal recessive inborn disorder of phenylalanin amino acid metabolism due to deficiency of a certain enzyme, which leads to accumulation of toxic metabolites of phenylalanin (phenylpyruvic acid and phenylketons) in blood and urine. Biochemical diagnosis of PKU is based on detection of phenylpyruvic acid in the urine. It is realized through a reaction between phenylpyruvate and ferric chloride ($FeCl_2$) which oxidation to $FeCl_3$ changes the colour of the kit into green. And nowadays the *Guthrie* test (a microbiological test) is used for PKU diagnosis.

Another excellent example of biochemical testing for inborn metabolic diseases is detection of blood glucose level in diabetes (it is high during insulin deficiency), or findings of high blood cholesterol in hypercholesterolemia.

Biochemical Screening. Most single gene diseases cannot subject to radical treatment but only supportive therapy is useful. The only aetiological (eliminating the cause) treatment for genopathies is gene therapy, which is not widely available for now. But there are few metabolic genetic disorders, early treatment of which can at least prevent the development of mental retardation. These early-preventable disorders are: ***PKU***, ***galactosaemia*** and ***congenital hypothyroidism (cretinism)***. For early diagnosis of these diseases and on-time prevention of mental retardation in these diseases there are established **newborn screening** programs conducted widely in newborn population to filter out (screen) the affected children.

DNA Analysis Methods. PCR. FISH

DNA technologies can be split into two main areas: DNA cloning (amplification) and methods of DNA analysis.

Polymerase chain reaction (PCR) is a method for making many copies (clones) of a specific segment of DNA to produce relatively large amounts of given DNA, which allows further analysis of its structure.

The PCR mimics DNA replication process, only it does it in a test tube (*in vitro*) and uses a specific ***DNA polymerase*** (from hot spring bacteria), which is heat-resistant – remains stable at high temperature applied during PCR. DNA nucleotides and a primer are also present in the test-tube. The three stages of PCR are carried out at different temperatures:

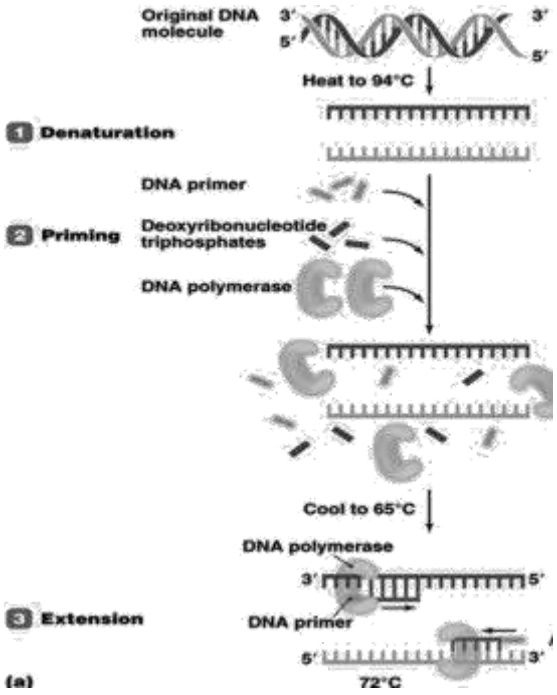


Fig. 75. PCR one cycle.

a. separation of two DNA strands (DNA denaturing or melting) by heating the vial to 90°-95° for 30 seconds.

b. attachment (annealing) of primers by cooling up to about 60°C for about 20 seconds.

c. elongation(extension) of new strands by heat-resistant DNA-polymerase at about 75°C.

One round of PCR takes less than 2 minutes and results in 2 copies of DNA fragment. But the cycle can be repeated 30 times, and amplification process has exponential nature (after 30 cycles 1 billion copies of a single piece of DNA can be produced).

Hence, if DNA amplification took place, it means the sample material did contain the DNA of interest, so it is a qualitative confirmation for gene mutations or diagnosis of infective diseases. If quantitative assessment of DNA amount before PCR is required, then amplified DNA undergoes agarose gel electrophoresis.

PCR allows analysis of DNA from any biological sample containing nuclei, and it is possible to start with a single molecule of DNA. It is applied in:

1. forensic medicine (person identification);
2. rapid prenatal diagnosis of gene diseases;
3. diagnosis of infectious diseases (viruses, bacteria, protists);
4. production of protein-medicine (insulin, clotting factor, growth hormone, etc.) for replacement therapy.

Fluorescent *In Situ* Hybridization (FISH)

In situ hybridization allows visualization and mapping of human chromosomes in a cell, including specific genes. This is important for diagnosis of chromosomal abnormalities and gene mutations. Unlike karyotyping, FISH does not have to be performed on actively dividing and metaphase-arrested cells. This technique uses single-stranded DNA fragments (**probes** produced by reverse transcriptase) that are complementary to the site of interest DNA. The probes are labelled by fluorescent dyes and have incorporated antigens. When the complementary probe binds to (is **hybridized** with) the analysed DNA, it is detected by antibodies corresponding to the antigens incorporated in the probes and is visualized by a fluorescent site by fluorescence microscope. This method of hybridization is known as **fluorescent in situ hybridization**, or **FISH**.

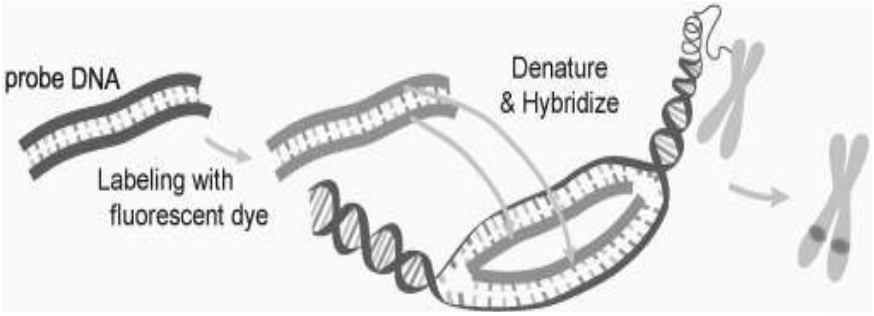


Fig. 76. FISH.

There are different types of FISH probes, which enable to detect gene mutations linked to specific chromosomes, genome mutations and chromosomal aberrations.

Prenatal Diagnosis

Prenatal (*pre* – before, *natus* – birth) diagnosis allows precise diagnosis of chromosomal diseases (genome and chromosomal mutations) and gene mutations before birth, early in pregnancy and allows termination of pregnancy (abortion) in early stages. The techniques include **amniocentesis, chorionic villi biopsy, non-invasive prenatal diagnosis, sonography** and **fetoscopy**.

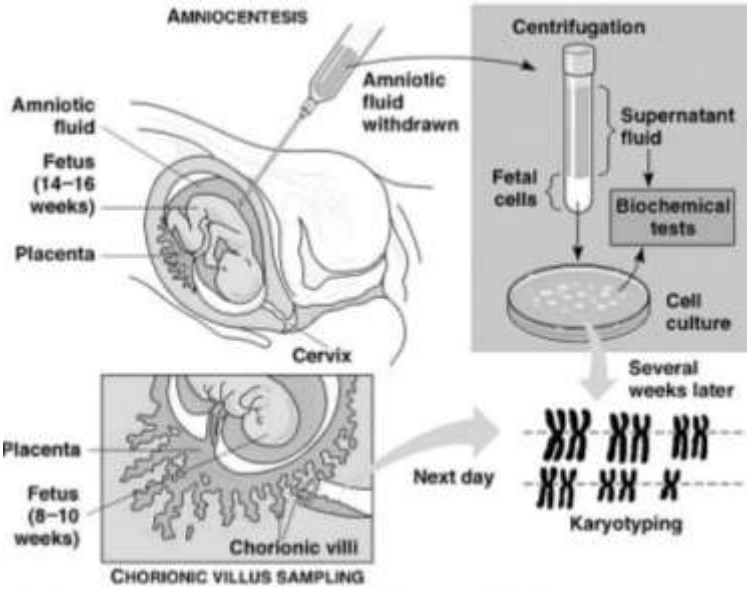


Fig. 77. Amniocentesis and CVB.

Amniocentesis. During intrauterine development, the fetus sheds cells of skin, intestines and urinary epithelium into the amniotic fluid, which can be analysed at 14-22 weeks of pregnancy. Approximately 10-30 ml of this fluid is removed, and the cells are recuperated by centrifugation of the specimen and cultured for a period of 5 to10 days for karyotyping. The amniotic fluid can be tested biochemically also for *alfa-fetoprotein*, which is decreased in Down syndrome. DNA in few cells obtained by amniocentesis can undergo also PCR technique and provide rapid prenatal diagnosis of gene diseases.

Chorionicvilli biopsy (CVB). The technique of CVB is realized by aspiration of a small amount of placental tissue (the ***chorion*** part that belongs to fetus) by a tube inserted through

the abdominal wall or through the vagina. It allows detection of chromosomal abnormalities in fetus by examination of cells at 8-12 weeks of pregnancy (versus 16 weeks in amniocentesis). And if an abortion is to be performed, it is a simpler process early in pregnancy. Another advantage of the method is that the fetal cells can be studied immediately without the need to culture them.

Non-invasive prenatal diagnosis (testing). Amniocentesis and chorionic villous sampling, which are invasive methods of prenatal diagnosis, carry a risk of spontaneous miscarriage. A new era in prenatal screening began with the clinical use of cell-free fetal DNA (cfDNA) analysis in maternal blood (non-invasive method) to screen for fetal aneuploidy starting from 10^{th} week of pregnancy. This technique involves sequencing fragments of cfDNA shed into the maternal circulation by the placenta, and quantifying the sequence reads of each chromosome. Sequence reads from any one specific autosomal chromosome are expected to have a 1:1 ratiowith reads from other autosomal chromosomes; cases of aneuploidy, in contrast, will deviate from this 1:1 ratio.

Sonography. It may reveal anatomical malformations (development defects) in fetus, e.g. heart and skeletal defects, other characteristic changes of organs or tissues. For example, characteristic ultrasound finding for Down syndrome fetus is absence of the nasal bone.

Fetoscopy. Fetoscopy is an endoscopic technique that allows to visualize the fetus around the end of the second trimester, by introducing a tube with optic fibres through the abdomen and the uterus allowing to biopsy fetal tissues or to perform surgical interventions.

Genetic Counselling

The process of identifying parents at risk for producing children with genetic defects and assessing the genetic state of early embryos is called **genetic counselling**. Genetic counselling is the process of evaluating family history and medical records (pedigree analysis), ordering genetic tests, evaluating the results of this investigation, and helping parents understand and reach decisions about what to do in future.

When any clinical disorder is diagnosed, the effected person and family members often need help to understand consequences of the disorder and possible ways to modify them. If the disorder is genetic, they need to know the genetic risk and the means available to prevent its transmission. Genetic counselling is the process of providing such information.

The most common situation in genetic counselling is that the consultants are the parents who have had a child with a disorder and want to know about the defect and its implication about their next children.

One should consider genetic counselling if any of the following risk factors is present:

1. Amniocentesis finds a chromosomal defect in the fetus.
2. Either parent or a close relative has an inherited disease or birth defect.
3. Either parent already has children with birth defects or genetic disorders.
4. The pregnant woman has had two or more miscarriages or babies that died in infancy.
5. The pregnant woman will be 35 or older when the baby is born (chances of having a child with Down syndrome increase with the mother's age: a 35-year-old woman has a one in 350 chance of conceiving a child with Down syndrome. This chance increases to one in 110 by age 40 and one in 30 by age 45).
6. Parents are concerned about genetic defects that occur frequently in their ethnic or racial group (African-American couples are most at risk for having a child with sickle cell

anaemia; Jewish couples of central or eastern European descent may be carriers of Tay-Sachs disease; couples of Italian, Greek or Middle Eastern descent may carry the gene for thalassemia; Armenian, Arabic and Jewish populations often present with familial Mediterranean fever - FMF).

If genetic counsellors have learned **prior to conception** that one of the couple is at high risk for having a child with a severe or fatal defect, the options might include:

a) pre-implantation diagnosis, which occurs when eggs that have been fertilized *in vitro* (***IVF***– in vitro fertilization, i.e. in a test-tube) are tested for defects at the 8-cell (blastocyst) stage, and only unaffected blastocysts are implanted in the uterus to establish a pregnancy,
b) using donor sperm or donor egg,
c) child adoption.

If the severe or fatal defect diagnosis is confirmed **during pregnancy** (prenatal diagnostics), the couple has a choice for:

a) preparing for the challenges after baby is born,
b) fetal surgery to repair the defect before birth (surgery can only be used to treat some defects, such as *spina bifida*)
c) interrupting the pregnancy (abortion).

1A

1. Pedigree analysis can detect:
A. personal identification
B. zygosity of twins
C. pattern of inheritance
D. chromosomal aberrations

2. What method is preferred to diagnose fetal Down syndrome if the pregnancy reached the 15th week?
A. amniocentesis
B. chorionic villi biopsy
C. blood cell karyotyping
D. fetoscopy

3. PCR can help in detection of:
A. viral infections
B. genome mutations
C. chromosomal aberrations
D. mRNA sequence

4. Which of the followings refers to dermatoglyphics?
A. fetoscopy
B. microscopy
C. palmoscopy
D. laparoscopy

5. The phenotypic similarity of twins is called:
A. penetrance
B. complementarity
C. concordance
D. expressivity

1B

1. Which are not the symbols used in pedigree construction?
A. circle
B. square
C. ellipsoid
D. dotted circle

2. What is not common for X-linked recessive traits?
A. expression in hemizygous males
B. transmission from carrier mother
C. transmission from father to sons only
D. expression in half of sons with carrier mother

3. The population statistics studies cannot detect frequency of:
A. alleles
B. genotypes
C. infections
D. phenotypes

4. Biochemical method cannot diagnose:
A. gene mutations
B. molecular diseases
C. enzymopathies
D. genome mutations

5. What diseases are not usually revealed by newborn screening?
A. Tay-Sachs
B. galactosaemia
C. PKU
D. congenital hypothyroidism

II

1. Genealogical method studies:
1. influence of environment on trait development
2. heritability of traits
3. inheritance pattern
4. type of linkage
5. concordance rate

A. 2,3,4 B. 1,2,4 C. 3,4,5 D. 3,5

2. Zygosity of twins can be detected by:
1. DNA analysis
2. dactyloscopy
3. concordance rate
4. biochemical tests
5. pedigree

A. 1,3,5 B. 2,4 C. 3,4,5 D. 1,2,3

3. Which data for atd palmar angle are correct?
1. Klinefelter syndrome - 42°
2. Down syndrome - 81°
3. Turner syndrome - 48°
4. Patau syndrome - 108°
5. normal angle - 66°

A. 3,5 B. 2,4 C. 1,5 D. 1,2,4

4. Somatic cell hybridisation is proceeded:
1. by Sendai virus
2. by delta viroid agents
3. fusing two somatic cells of different species
4. for hybridising two organism
5. for making chromosome maps

A. 2,4,5 B. 1,3,5 C. 1,3 D. 2,5

5. PCR is realised by:
1. RNA nucleotides
2. DNA nucleotides
3. helicase
4. special prokaryotic DNA-pol
5. eukaryotic DNA-pol

A. 1,3,4 B. 2,3,5 C. 1,3,5 D. 2,4

CHAPTER 14

Hereditary diseases: Chromosome disorders. Gene diseases. Mitochondrial Diseases. Multifactorial Diseases

Each human is estimated to have about 30.000 different genes. Alterations in these genes or in their combinations can produce genetic disorders. These disorders are classified into following major groups:

1. Chromosome disorders, in which the number or structure of chromosomes is altered. Numeric changes of entire chromosomes (lack or duplication) cause **genome mutations**, while structural changes lead to **chromosome aberrations.**

2. Single-gene disorders, in which single genes are altered (also are known as "Mendelian" conditions).

3. Mitochondrial disorders, relatively small number of diseases caused by alterations in the small mitochondrial DNA.

4. Multifactorial disorders, which are due to a combination of multiple genetic as well as environmental causes.

Chromosome disorders: Chromosome Number Abnormalities

Autosomal aneuploidies

Trisomy 21, Down syndrome. Trisomy 21 (47,XX+21 or 47,XY+21) produces Down syndrome, which is the most common aneuploid condition compatible with survival to term. There is a strong association between the incidence of trisomic Down syndrome and maternal age (but this correlation is absent in chromosome translocation-mediated Down syndrome). They present with group of clinical symptoms that help rapidly to suspect about diagnosis. The facial features include: upward sloping palpebral fissures, epicanthal folds (a skin fold at the inner angle of eye), nasal root, small and overfolded ears, usually large tongue that hangs out from mouth cavity, single fold (simian crease) on palm, wide palmar *atd* angle (∠atd=81º). Congenital cardiac abnormalities are present in about 40% of babies with Down syndrome, which are the most important single cause of decreased survival. In the absence of severe cardiac anomaly the average life span is 50-60 years. Moderate to severe mental retardation (IQ=20-70) is seen in most Down syndromes. Approximately 95% of Down syndrome cases are caused by non-disjunction, and only about 3% caused by Robertsonian translocation of chromosomes (e.g., 46,XX,t[21q,14q]). Approximately 1-3% of trisomy 21 present with mosaicism; they have some normal somatic cells and some cells with trisomy 21 (46,XX/47,XX+21).

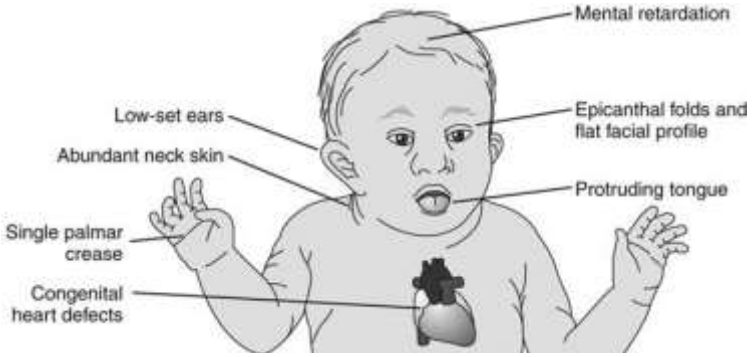


Fig. 78. Down syndrome.

Trisomy 13, Patau syndrome. The trisomy 13 (47,XX,+13) is also known as Patau's syndrome. The risk for bearing a child with this trisomy increases with advanced maternal age. Babies hardly

survive for very long if live born because of the multitude of anomalies. The individual is born with gross brain malformations, microphthalmia (sometimes cyclopy – single eye), hare lip and cleft palate, polydactyly and congenital heart disease. The syndrome is so severe that many babies die soon after birth.

Ultrasound can detect abnormalities (cardiac, gastrointestinal, urogenital) before birth in the fetus with aneuploid syndromes. Genetic testing by karyotyping or FISH through amniocentesis/CVB before birth or blood test (leukocytes) after birth can confirm the diagnosis. Additional tests include palmoscopy.

Trisomy 18, Edwards syndrome. This trisomy, also known as Edwards syndrome (47,XX,+18), is the second with a prevalence. There is a significant maternal age effect. Symptoms may include: mental retardation and delayed development, seizures, brain defects, small head (microcephaly), small eyes (microphthalmy), wide-set eyes (hypertelorism), epicanthal folds, congenital heart

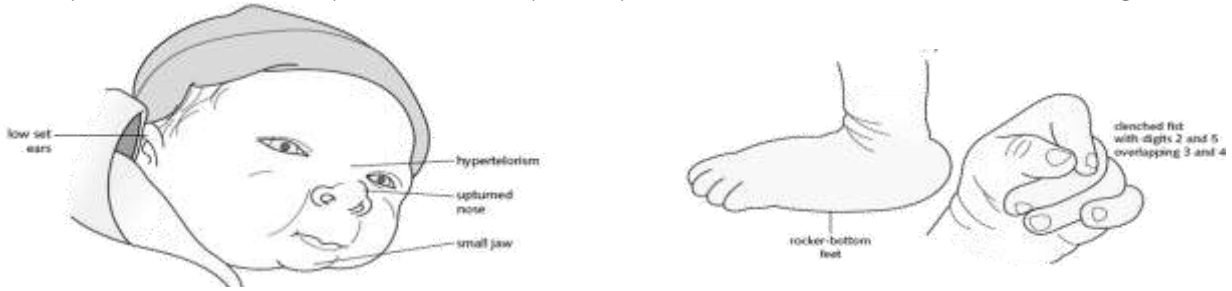


Fig. 79. Edward's syndrome.

defects, clenched hands with 2nd and 5th fingers on top of the others (the latter symptom allows clinicians to make initial diagnosis), malformations of the digestive, urinary and reproductive systems. Children with trisomy 18 hardly survive up to one year of age.

Sex Chromosome Aneuploidies

Primarily because of X inactivation, the consequences of this class of aneuploidy are less severe than those of autosomal aneuploidies. Monosomy for Y chromosome is always lethal.

Monosomy of X Chromosome. Turner syndrome (45,X). Individuals with Turner syndrome have characteristic female phenotype. The findings include: proportionate short stature (average adult height is 140-150cm), wide shield-like chest, broad ***webbed neck,*** congenital heart defects. They lack normal ovaries and do not usually develop secondary sexual characteristics, so most women are infertile. Turner syndrome females *are not mentally retarded*. Approximately 80% of X monosomy cases are caused by non-disjunction in paternal meiosis through a loss of any of sex chromosomes of father – X or Y (45,X sometimes is erroneously referred to as 45,X0). Most of live births with Turner syndrome are mosaics (45,X/46,XX). Very few cases of the

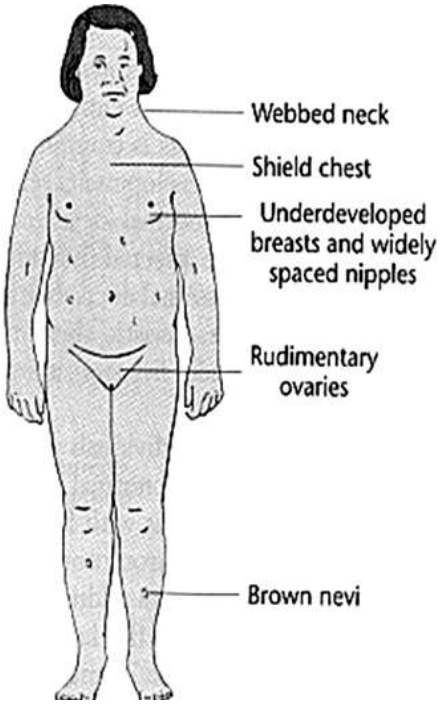


Fig. 80. Turner syndrome.

syndrome arise due to aberrations of X chromosome: isochromosome of long arm of one X chromosome (46,XXqi) or a ring X chromosome (46,XXr).

Diagnosis is confirmed by FISH, karyotyping and Barr body detection method (lack of Barr body).

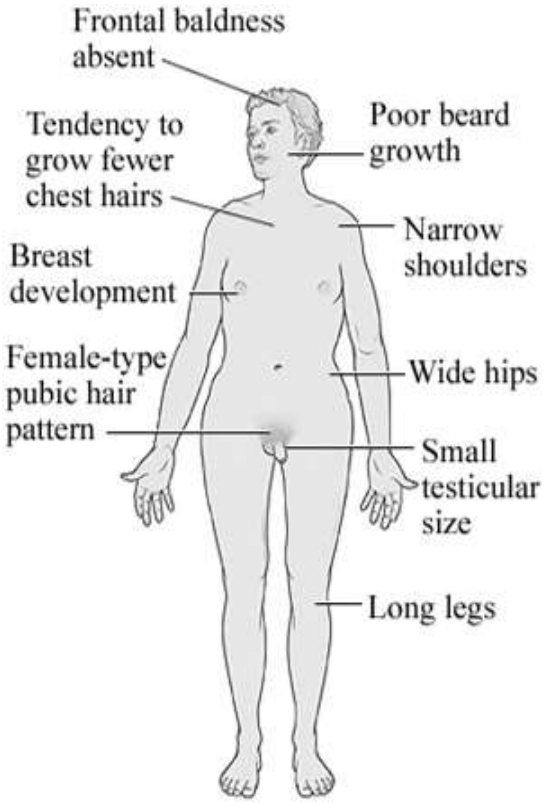


Fig. 81. Klinefelter syndrome.

Klinefelter syndrome. The person with 47,XXY karyotype is usually male because of the Y chromosome, but has lower levels of testosterone and may have some female-like features (because there are two X chromosomes). These pathological symptoms develop only after puberty. The individuals are taller than average, with disproportionately long arms and legs. Clinical examination of postpubertal patients reveals small testes (atrophy of seminiferous tubules), sterility is a possible complication. In some patients breast development (gynaecomastia) occurs and leads to increased risk of breast cancer. Although males with Klinefelter syndrome are usually not mentally retarded, but the IQ is below that of his siblings. The rarer subtypes 48,XXXY and 49,XXXXY of the syndrome may already lead to mental retardation. Treatment with testosterone beginning from puberty is beneficial for development of secondary sexual characteristics. Usually the karyotype and FISH of Klinefelter syndrome show additional X chromosome(s), and cytological analysis discovers one or more Barr bodies normally being absent in male cells.

47,XYY, Supermale syndrome. Individuals are often characterised by tallness and severe acne, and sometimes by skeletal malformations and insignificant mental deficiency. The affected male sometimes exhibits emotional immaturity and aggressive-impulsive behaviour. Non-disjunction arises in paternal gametogenesis. “Supermales” usually can give birth to normal individuals.

Chromosome disorders: Chromosome Structure Abnormalities (Aberrations)

In addition to the loss or gain of whole chromosomes, often parts of chromosomes can be lost or duplicated during gamete formation, and the arrangement of portions of chromosomes can be altered. Structural chromosome abnormalities may be **balanced** or **unbalanced**. In unbalanced aberrations the rearrangement causes a gain or loss of chromosomal material, while balanced aberrations are not associated by such type of rearrangement. Unlike aneuploidies, the balanced structural chromosomal abnormalities do not produce serious health problems. Chromosomal aberrations are of four types: deletion, duplication, translocation and inversion.

Deletion syndromes

The "cri-du-chat" or **"cat's cry syndrome"** is caused by deletion of the short arm of chromosome 5. The syndrome exhibits severe mental retardation and physical defects including structural defects of larynx giving the characteristic cat-like child's cry. **Wolf-Hirschhorn syndrome** is caused by a deletion of the short arm on chromosome 4. It is characterised by severe growth retardation and mental defect, microcephaly, "Greek helmet" face, wide spaced eyes and closure defects (cleft lip or palate and cardiac septal defects).

Duplication Syndromes

A specific case of chromosomal duplication (and deletion) is the isochromosome. Most isochromosomes observed in live births involve the X chromosome, and babies with isochromosome Xq present with clinical features of Turner syndrome.

Translocation syndromes

Translocation Down syndrome is another possible variant of Down's syndrome due to translocation between 21st and 13th, 14th or 15th (Robertsonian translocation). The baby inherits two normal chromosomes 21 (one from each parent), two normal chromosomes 14 (or 13 or 15), and a Robertsonian translocation chromosome from one of the parents that has 45 chromosomes. Translocation Down syndrome individual presents with 46 chromosomes (e.g. 46,XY,t[14q,21q]).

Chronic Myelogenous Leukosis (CML) arises due to reciprocal translocation between long arms of chromosome 22 and chromosome 9. It is designated as t(22q^{-};9q^{+}) and results in one chromosome 9 longer than normal and one chromosome 22 shorter than normal. The aberration chromosome 22 is called a **Philadelphia chromosome**. In CML, the haemopoetic cells that give rise to white blood cells in bone marrow grow uncontrollably leading to cancer.

Inversions are balanced aberrations, however in some cases the change of position of genes and their interrelation may also lead to pathological clinical symptoms, e.g., hemophilia.

Gene Mutation Diseases

There are over 10.000 single gene traits and disorders that have been identified to date. Single-gene diseases are also known as genopathies or sometimes enzymopathies, because there is often a mutation of enzymatic protein gene. Since these disorders develop because of deficiency or abnormal synthesis of only one type of molecule, they are also called molecular diseases. Types of gene mutations have been discussed in variability chapter.

Molecular diseases can be classified in following way, though different category disorders may in fact overlap.

1. Disorders of protein and amino acid metabolism
2. Disorders of lipid metabolism
3. Disorders of carbohydrate metabolism
4. Nucleic acid metabolism disorders
5. Disorders of mineral metabolism
6. Connective tissue diseases
7. Haemoglobinopathies

Amino Acid Metabolism Disorders

Phenylketonuria is an autosomal recessive mutation of enzyme converting the amino acid phenylalanine to tyrosine. Children with PKU, if untreated, are severely mentally retarded and often have convulsions due to affection of brain by toxic metabolites of phenylalanine (phenylpyruvic acid, phenylketones). Phenylketones are excreted in the urine (hence the name phenylketonuria). Deficiency of tyrosine consequently reduces melanin formation; affected children therefore often have blond hair and blue eyes.

PKU can be detected early in a newborn by screening. This is done by biochemical tests which detect the presence of phenylalanine or phenylpyruvic acid in the urine by its reaction with ferric chloride, or through increased level of phenylalanine in blood (Guthrie test). PKU can be treated by removal of phenylalanine from the diet. If PKU is detected early enough in infancy, the mental retardation can be prevented.

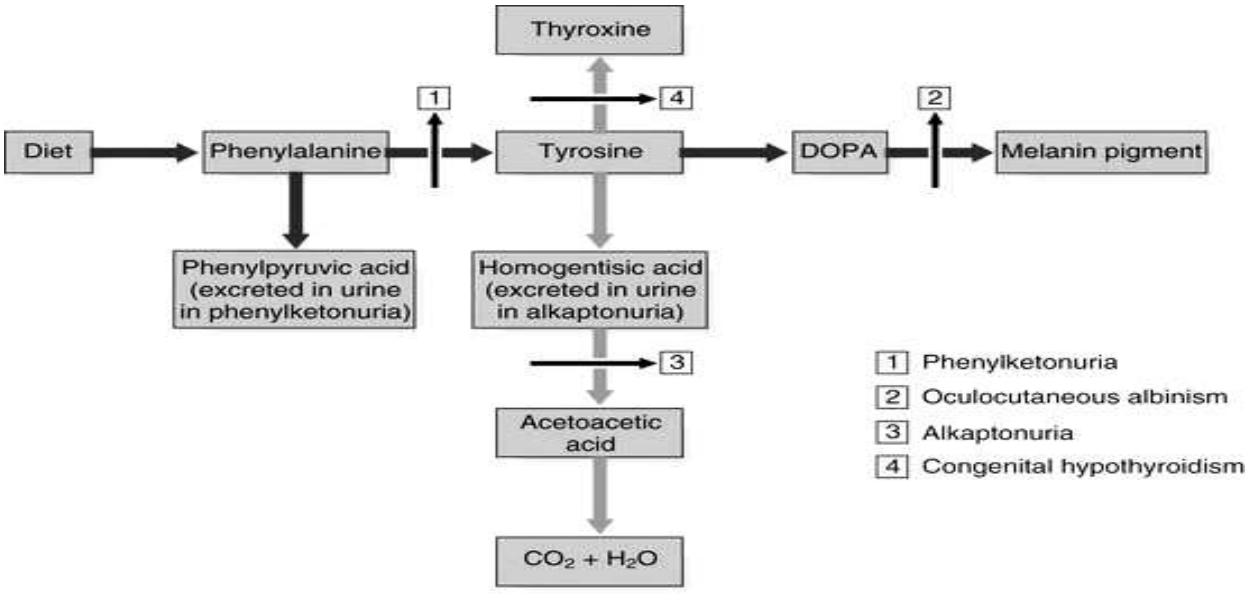


Fig. 82. Sites of biochemical block in phenylketonuria, alkaptonuria, congenital hypothyriodism and oculocutaneous albinism.

Alkaptonuria is an autosomal recessive inborn error of tyrosine metabolism. There is a block in breakdown of *homogentisic acid*, which accumulates and is excreted in urine, to which it imparts a dark colour on exposure to air. Dark pigment is deposited also in connective tissues such as cartilages of joints and ear, skin, and eye sclerae. Bluish discoloration of the nails is also characteristic.

Oculocutaneous Albinism is an autosomal recessive disorder due to deficiency of enzyme necessary for synthesis of melanin pigment. People with oculocutaneous albinism have little or no pigment in the eyes, skin, and hair (or in some cases in the eyes alone). The lack of the pigment in eye results in poor vision and typical uncontrolled pendular eye movement (*nystagmus*). *Strabismus,* which means that the eyes do not fixate and track together, is also common in albinism and is related to the altered development of the optic nerves.

Disorders of Carbohydrate Metabolism

Galactosemia. Galactosemia is an autosomal recessive disorder of galactose metabolism enzyme. Newborn infants with galactosemia cannot digest the galactose and lactose, and present with vomiting, failure to thrive and jaundice in second week of life. If untreated, complications like mental retardation, cataract and cirrhosis of liver may develop. These complications can be prevented by early diagnosis and feeding of affected infants with milk substitutes which do not contain galactose or lactose (the milk sugar which is broken down into galactose and glucose). Galactosaemia can be screened for in newborns by biochemical tests.

Glycogen storage diseases. Glycogen is the form in which the sugar glucose is stored in muscles and liver as a polymer, acting as a reserve energy source. There are several types of glycogen storage diseases, which are caused by accumulation of glycogen in excessive amounts within skeletal muscles, cardiac muscle and/or liver (e.g. von Gierke's disease, Pompe's disease).

Diabetes mellitus. It is of two types: type 1 or insulin dependent diabetes mellitus is related to genetic deficiency of insulin, and develops in childhood (juvenile type); and type 2 or insulin non-dependent diabetes mellitus, which usually develops in adults under influence of environment (multifactorial disease).

Disorders of Lipid Metabolism

Familial hypercholesterolaemia develops elevated cholesterol levels with a significant risk of developing early coronary disease due to deficiency of cholesterol receptors in liver cell membrane. The patients can present with subcutaneous deposition of lipids (*xanthoma*) in childhood or adolescence. The raised cholesterol level in blood is present from birth, and it leads to an early development of atherosclerosis and cardiovascular diseases. Heart attacks usually occur in 45-55 years old people.

Androgen insensitivity syndrome (Morris syndrome or **male pseudohermaphroditism**) is a result of X-linked recessive mutation of the gene that encodes for testosterone binding receptors. It develops normal male karyotype (46,XY) but female phenotype with external genitalia, and undergo breast development in puberty (testicular feminization). The patients lack uterus, fallopian tubes and ovaries and have misplaced underdeveloped testes tissue in abdomen. Affected individuals usually have a female sexual orientation but are obviously sterile (infertile). They require removal of their testes because of increased risk of testicular malignisation and development of testicular cancer.

Adrenogenital syndrome (**female pseudohermaphroditism)** is an enzymopathy leading to cortisol deficiency. The pituitary produces adrenocorticotrop stimulating hormone (ACTH), which cannot trigger cortisol synthesis because of genetic origin of its deficiency, still it triggers other adrenal hormones that have testosterone-like effects leading to so-called virilization (or masculinization). The karyotype is 46,XX with female gonads (ovaries), but these children have virilized external genitalia. Most of the affected females have also electrolyte metabolism disruption because of cortisol deficiency (cortisol regulates electrolyte balance, blood pressure).

Tay-Sachs' disease is an autosomal recessive sublethal mutation of a particular type of lipid (sphingolipids) metabolism in brain caused by deficiency of a lysosomal enzyme. As nerve cells become distended with fats, a deterioration of mental and physical abilities occurs. The child

becomes blind, deaf, and unable to swallow. Muscles begin to atrophy, and paralysis sets in. Children with Tay-Sachs disease usually die by age 4.

Disorders of Purine/Pyrimidine Metabolism

The disease associated with disrupted purine metabolism is the **gout**. The cause in most instances results from combination of genetic and environmental factors. Only a minority of persons who present with gout are found to have an inborn error of metabolism. Deposit of crystals of uric acid salts in small joints (mainly toes, fingers) causes joint inflammation - pain, swelling and tenderness. The diet of these patients should exclude meat and alcohol.

Disorders of Mineral Metabolism

Many of enzymes have co-factors which are commonly some vitamins or trace elements such as ions of heavy metals (zinc, copper, iron, etc.).

Disorder of Copper metabolism. Wilson Disease is caused by defective biliary excretion of copper which leads to affection of liver, kidneys, brain, eye lens.

Disorder of Iron metabolism (hemochromatosis). Lack of iron can cause anaemia but excessive iron is toxic. The body has few ways of disposing of unwanted iron, so the iron builds up in tissues causing damage and disease. Excess amounts of iron accumulate in liver, kidneys, heart, joints, pancreas. This may cause heart or liver failure, which can be fatal.

Cystic fibrosis is an autosomal recessive sublethal mutation related to disorder of chlorine transport in cell membranes. It expresses in salty tasting skin, dense mucus produced in bronchi, intestines, pancreas that predispose to frequent respiratory and gastrointestinal bacterial infections. These chronic infections often become the reason of life-shortening (lethality).

Hypophosphatemic rickets is an X-linked dominant mutation that disrupts the protein which reabsorbs phosphates from urine in kidney. The result is deformation of bones. It is not subject to vitamin D treatment.

Haemoglobinopathies

These are a group of inherited disorders **(sickle-cell anaemia, thalassaemia)** characterized by disorders of hemoglobin structure (mutations in globin chains' genes).

Sickle-cell anaemia presents with sickled and rigid RBCs due to HbS, while the normal RBCs that have normal HbA can bend and flex easily. Sickled RBCs result in blockage of capillaries which then stop the oxygen transfer. This in turn can lead to severe pain, hypoxia and damage to organs (liver, kidney, lungs, heart and spleen). Sickle-cell anaemia is sublethal dominant mutation (substitution gene mutation) when the dominant homozygotes may die in several years if not treated, while heterozygotes present with mild anaemia trait. The different kinds of sickle-cell disease and the different traits are found mainly in people whose families come from Africa, the Caribbean, the Eastern Mediterranean, Middle East and Asia.

Thalassemia (*thalassos*=sea, Greek) is a recessive autosomal mutation prevalent in Mediterranean region. It produces deficiency of either alfa or beta chains of hemoglobin (alfa- and beta-thalassemias, respectively), due to different deletions. Clinical symptom is anemia.

Connective tissue diseases. Marfan syndrome. It is an autosomal dominant mutation affecting fibrillin synthesis in connective tissues. Features of Marfan syndrome involve many structures (*primary pleiotropic effect*), including the skeleton, eyes, heart and blood vessels. The disease is characterized by unusually long and thin limbs, arachnodactylia, "hollow chest" or "pigeon chest",

ocular defects including lens ectopia. Most life-threatening defects are dilation of aorta (aneurysm), heart failure.

Mitochondrial Diseases

These diseases develop due to mutations of mtDNA (see cytoplasmic inheritance) and lead ATP deficiency, which influences nerve and muscle tissues primarily (Leber's optic nerve atrophy, spina bifida, maternally inherited myopathy and cardiomyopathy).

Multifactorial Diseases. Genetic susceptibility.

Medical genetics usually concentrates on the studies of unifactorial chromosomal and single gene disorders. But some diseases that are responsible for the majority of morbidity and mortality in developed countries are of even greater importance. These common diseases are **diabetes mellitus, some types of cancer, cerebrovascular** and **coronary artery disease, arterial hypertension, peptic ulcer, Alzheimer disease, schizophrenia** etc. They usually show a complex pattern of inheritance, when the genetic factors are usually multiple and interact with each other, and the environment matters as well. In these cases the hereditary quotient is not equal to 1 (H<1). It varies between 0 and 1 for multifactorial diseases.

Most of multifactorial disorders are considered as a result of an inherited predisposition or *genetic susceptibility.*

Genetic susceptibility for a particular disease can occur through single gene inheritance, for example early coronary artery disease arising from familial hypercholesterolemia. In an individual with a mutation in that gene the genetic susceptibility is the main determinant of the development of coronary artery disease, but this can be modified by environmental alteration, e.g. reduction in dietary cholesterol and avoidance of other risk factors such as obesity, smoking and lack of exercise.

Diabetes mellitus. Diabetes mellitus (DM) is a multifactorial disease with genetic susceptibility. This is a metabolic disorder characterised by inability to transport glucose from the bloodstream into cells due to insulin deficiency.

Alzheimer disease. Alzheimer's disease is characterised by dementia – an irreversible and progressive global impairment of intellect, memory, social skills and control of emotional reactions in the presence of normal consciousness. The onset of Alzheimer's disease starts with forgetfulness and difficulty in finding the right word. Alzheimer's disease is caused by affection of brain cells usually after age of 65, by deposition of abnormal protein (amyloid) in nerve cells.

Treatment of Genetic Disorders

The level at which the therapeutic intervention can be applied is influenced by the state of knowledge about the primary genetic defect, its effect, its interaction with environmental factors, and the way in which these may be modified.

1. Conventional Treatment. It is aimed at relieving the symptoms and preventing complications. For example, management of cystic fibrosis requires antibiotic treatment for prevention of respiratory and digestive infections, specific exercising for draining the air passages, etc.

2. Dietary control. Example is PKU which results in high concentrations of phenylalanin, causing mental retardation, seizures. The treatment consists of limiting dietary intake of phenylalanine to that essential for normal growth. In familial hypercholesterolemia fat consumption is limited and in diabetes – the carbohydrates are limited in the diet.

3. Life style modification. The effects of some genetic disorders may be minimized by changing the life style. For example, in diabetes and familial hypercholesterolemia high physical activity should be performed, and smoking is not recommended.

4. Surgical Management. Surgery plays an important role in various genetic disorders caused especially by genome mutations and chromosomal aberrations, which lead to congenital malformations (e.g. cleft upper lip (hare lip) and cleft palate, heart defects, aortal aneurism etc).

5. Replacement therapy substitutes either the protein that is a gene product or any substance, which is missing due to enzyme deficiency. For example, insulin – in diabetes mellitus, clotting factor – in haemophilia, growth hormone – in dwarfism replace the missing gene products. Congenital hypothyroidism can be treated with thyroxine replacement.

6. Gene therapy includes a treatment through introduction of normal gene into human body cells after removal of mutant one for treatment of gene diseases. There are reports on treatment of several gene diseases like ***severe combined immune deficiency, PKU, familial hypercholesterolemia, cystic fibrosis, hemophilia.***