Course: **Biology and Genetics**

Field of Study: Cosmetology

Form of classes and number of hours: lectures 30 h, laboratory classes 30 h

Number of ECTS credits: 4

Learning outcomes:
Knowledge of the biological basis of the structure and functioning of organisms. Knowledge of the basic relationships between organisms in nature. Basic knowledge of genetics.

Evaluation methods of learning outcomes:
examination

Subject matter of the classes:

1. Structural levels of organism structure.
2. The functioning of the organism at various levels (elements of cytophysiology, physiology).
3. Selected issues of ecology.
4. Genetics.

Selected educational content includes genetics only.

References

Books

Błaszczyk M., *Podstawy genetyki*. Oficyna Wydawnicza PWSZ, Nysa 2017.

Brown K., *Introduction to Genetics: A Molecular Approach*. Taylor & Francis Inc., 2011.

Mann R., *Handbook of Genetics and Genomics*. Callisto Media Inc., 2019.

Shukla A. N., *Human Genetics*. Discovery Publishing House Pvt Ltd., 2016.

The following text covers chosen Genetics topics. The course ‘Biology and genetics’ consists of physiology-related topics as an addition thereto.

1. Cell cycles

Haploid organisms have a single set of DNA molecules, so there is only one DNA of a given type in each cell. **Diploid organisms**, on the other hand, have a double set of DNA, so DNA molecules come in pairs: one from the father and one from the mother.

Cells undergo cyclical divisions, which are divided into a period of growth and preparation for the next division. These successive phases of a cell's life are called repeating cell cycles.

Within one cell cycle, four basic phases can be distinguished: G_1, S, G_2 and M. The first three together constitute the interphase, i.e. the period between divisions, while M is the actual cell division (mitosis or meiosis). Cells may stop dividing temporarily or permanently by entering the resting phase of G_0.

The G1 phase (G = gap) is the period of cell growth and the accumulation of necessary substrate reserves until the G_1 phase control mechanisms confirm the cell's readiness for DNA replication.

The S phase is associated with the synthesis of histones and DNA (replication). The amount of DNA in a cell is doubled, so there is a quadruple set of DNA molecules.

The G2 phase involves the continued growth of the cell until the G_2 control mechanisms confirm that it is ready for mitosis. Then, among others, the elements of the cytoskeleton involved in mitosis are synthesized (building division spindles).

M is the phase of cell division, most commonly mitosis, after which the daughter cells enter the G_1 phase.

G_0 is a phase beyond the actual cell cycle and is referred to as 'resting', and this does not mean 'resting' from full metabolic activity, only from cell division. Mature cells in some tissues enter the G_0 phase after their growth period. They no longer undergo further mitotic divisions, but undergo differentiation, i.e. specialization to perform specific functions.

1.2. Cell divisions

In the course of cell division, the following occurs: karyokinesis, i.e. the division of the cell nucleus, and cytokinesis, the division of the cytoplasm and organelles. Before cells start their division, they usually double the amount of DNA.

Mitosis is the process of cell division by which two daughter cells are produced that are genetically identical to the parent cell.

There are several phases in the course of mitotic division. Chromatin condensation takes place in the prophase, as a result of the cytoskeleton reorganization, a division spindle is formed, e.g. from microtubules and titin. The nucleoli and the nuclear envelope disappear. On each chromatid, a protein complex – kinetochore – appears opposite the centromere, to which spindle elements are attached. In metaphase, each chromosome is made up of two chromatids linked by cohesin. Chromosomes are positioned in the equatorial plane of the cell. In anaphase, chromatids are attracted towards the poles of the cell by dynein as it moves along the microtubules of the spindle. In telophase, chromosomal decapitalization and the reconstruction of the nuclear envelope around both of the resulting nuclei take place.

Karyokinesis is followed by cytokinesis, i.e. the separation of the cytoplasm by tightening the contractile ring built in the anaphase of actin and myosin filaments arranged around the periphery (at the equator) of the cell.

Meiosis is the division of a cell to produce daughter cells that have twice as much DNA (and chromosomes) as the parent. It takes place during the synthesis of reproductive cells during spermatogenesis and oogenesis. Its aim is to reduce the amount of genetic material so that the zygote formed after fertilization has the same amount that was reproduced in the cells before meiosis. In the course of meiosis, two divisions follow one another. The first division is a **reductional division**.

Profhase I begins with leptotene, during which the chromosomes begin to condense and the sister chromatids unite. In the zygote, pairing of homologous chromosomes occurs: sister chromatids adhere to each other. The chromosomes are bound by the synaptonemal complex. During pachytene, the crossing-over process takes place, which ends in diplotene, which also involves disassembly of the synaptonemal complex and the disconnection of homologous chromosomes. At the end of prophase I, diakinesis takes place, the condensation of chromosomes ends, and the nuclear envelope and nucleoli disappear.

In **metaphase I**, pairs of chromosomes are arranged in the equatorial plane of the cell.

In anaphase I, chromosomes spread randomly to the poles of the cell, without separation of sister chromatids. Thus, in the daughter nuclei, the number of chromosomes will be reduced by half (first meiotic division is a reductional division, daughter cells have a haploid number of chromosomes, 1n).

There is no typical **telophase** here, a pair of haploid cells immediately enter the second division.

The second meiotic division (**equational division**) is essentially identical to mitosis. The enzyme separase cuts the junctions (cohesive complexes) between sister chromatids. This allows them to diverge in anaphase II to opposite poles of the cell.

The result of both divisions is the formation of 4 haploid cells with the number of 1n chromosomes, each of which has one chromatid. The daughter cells are not identical – their genetic material is different.

2. Classical genetics

2.1. The first law of inheritance and basic concepts of classical genetics

The first scientific theory of inheritance was developed by J. Mendel in the middle of the 19th century. Mendel noticed that crossing plants with specific traits resulted in specimens having this trait in the next generation in predictable proportions. The clearest example is the pea plant with white or purple flowers. Mendel separated plants with different flower colours from each other, then over several generations he selected specimens with only purple flowers or – separately – only white flowers for further reproduction. Currently, it can be described as obtaining two pure lines, i.e. homozygous lines (homozygotes, i.e. individuals having both alleles of this gene the same, designated e.g. VV or vv – heterozygotes i.e. individuals having different alleles of one gene, Vv).

Mendel assumed that the ‘inheritance factors’ of a specific trait pass from both parents to offspring, occurring in pairs in each individual. These ‘factors’ are now known as **genes**. Genes can take various forms (**alleles**, the word allele is short for ‘allelomorph’), determining the different form of a given trait (e.g. different colour of flower petals, different blood groups, etc.). These alleles can be **dominant** or **recessive**. In genetics, the dominant alleles are conventionally marked with capital letters, in the described case V (violet), recessive alleles with lower case letters, here: v. A dominant allele is the one that only needs one in a gene pair for the trait form determined by that allele to occur (e.g. if there is a Vv heterozygote, the V trait form is present).

A **genotype** is a set of alleles of a given organism (all alleles of all genes, or for the sake of simplicity, only a record of alleles of a given gene or genes). **A phenotype** is a set of observed characteristics of an individual, i.e. a physical manifestation of the genotype, taking into account the fact that some recessive alleles may not be reflected in the phenotype.

Mendel, after crossing the VV and vv plants, noticed that in their offspring (in the F1 generation) all plants had purple flowers (having a Vv set of alleles). This is how he defined domination and recessivity. In the further part of the experiment, Mendel subjected the obtained heterozygous F1 plants to self-pollination. In the F2 generation, he obtained 75% purple-flowered plants and 25% white-flowered plants.

Thanks to this experiment, Mendel showed that from each parent individual to the offspring, one allele of the pair (in the germ cells – gametes) passes, which is the basic mechanism (and law) of inheritance.

2.2. The second law of inheritance

Mendel's second law ('the law of independent assortment') states that different traits inherit from each other independently. However, it applies only to features whose genes lie on different chromosomes or whose locations cross-over occurs.

On the other hand, if genes lie on the same chromosome, they will be inherited in a conjugated manner: whole DNA molecules (with all their alleles on them) pass randomly to gametes. Only the crossing-over process can disturb that.

2.3. Multi-gene inheritance

One gene usually describes the structure of one protein, so its form (allele) is directly responsible for the form of this protein and the function it performs. However, a large proportion of the phenotypically observed traits, not in biochemical studies, but in simple observation, are directly or indirectly influenced by at least a few genes. In the example of iris colour already given, at least sixteen genes are involved, although the OCA2 gene is of particular importance, which determines the production of the melanin pigment (and thus also affects the colour of the skin and hair (called *oculocutaneous albinism type 2*, a disease caused by one form of alleles), forms of albinism), the HERC2 gene regulating its expression, and the EYCL1, 2, 3 genes.

2.4 Epigenetics

Epigenetics deals with the influence of environmental factors on gene expression. Under the influence of such external factors, changing the activity of genes, cells can change their functions and undergo remodeling to a very large extent. There are many examples of epigenetic mechanisms, including: **epistasis**, i.e. the influence of some genes and their expression products on the expression of other genes. A given allele may be present, but the expected trait will not manifest itself phenotypically due to the presence of specific alleles of other genes. Another example is **pleiotropy**. It is based on the influence of one gene on more than one trait. Still other ones are genomic, imprinting, reprogramming, etc.

3. Molecular genetics

3.1. Structure of nucleic acids

DNA, or **deoxyribonucleic acid**, is a polymer of nucleotides. A single nucleotide is composed of a deoxyribose molecule linked by an N-glycosidic bond with a purine nitrogen base (adenine (A) and

guanine (G)) or a pyrimidine base (cytosine (C) and thymine (T)) and phosphorylated (most often at the position of the fifth carbon atom in deoxyribose).

Nucleotides can chain together to form phosphodiester bonds between one nucleotide phosphate residue (attached to the fifth carbon atom of deoxyribose) and the third carbon atom of another deoxyribose molecule. In such a chain, one side of the molecule always ends with a ‘free’ third carbon atom in the deoxyribose molecule, and the other with a fifth (‘free’ or unphosphorylated). This is referred to as the 3’ and 5’ ends.

In a DNA molecule, most often there are two chains, lying in parallel. The nitrogen bases go towards the other chain, so they lie opposite to each other, which allows the formation of hydrogen bonds between them. Adenine and thymine make two such bonds, cytosine and guanine – three, so the bases always pair this way. This is the **principle of complementarity**.

These chains lie opposite to each other with 3’ and 5’ ends: the 3’ end of one chain lies opposite the 5’ end of the other one; this is called anti-parallel arrangement. A DNA molecule can be linear, but it can also close to a circular shape. The strands are twisted around each other to form a double, right-handed helix.

In the cell nucleus of every almost living cell in the body there is a complete double set of DNA molecules. Each of them is several to several dozen centimeters long. Human DNA is almost 2 meters long. The cell nucleus is only a few µm in diameter. DNA must be arranged in the nucleus in such a way as to ensure rapid access of enzymes in the event that a gene needs to be used for transcription, while at the same time the DNA must never become entangled. The layout must therefore be very precise. This is largely due to histone proteins.

More or less fused DNA together with histones form **nuclear chromatin**. The more chromatin is condensed, the lower its transcriptional activity: active DNA must be de-spiralized in order for enzymatic transcription complexes to attach to and move along its molecule. Strongly fused chromatin (**heterochromatin**) is inactive.

In prokaryotes and in mitochondria, DNA is not linear, but circular: the 3’ ends are linked by a phosphodiester bond to the 5’ ends.

Ribonucleic acid (**RNA**) has a structure similar to that of DNA, it is also a chain of nucleotides. The difference is that the core of the molecule together with phosphoric acid builds ribose instead of deoxyribose, and instead of thymine, uracil is one of the nitrogen bases with similar properties. Another difference is that DNA is usually double-stranded in cells while RNA exists as a single-stranded molecule. A characteristic type of RNA is tRNA.

3.2. Genetic code

The genetic information located in DNA describes the structure of proteins.
DNA encodes twenty of the naturally occurring amino acids. There are more than twenty proteins synthesized in cells, because they undergo post-translational modifications, such as adding functional groups (methyl, acetyl, hydroxyl, phosphoryl, etc.), carbohydrate molecules, lipids, changing the chemical structure, creating disulfide bridges, removing fragments of the polypeptide chain, etc.

DNA, as described earlier, is a polymer of four types of nucleotides containing nitrogen bases: adenine, thymine, cytosine, and guanine. Thus, information in such a molecule must be written with four ‘characters’, A, T, C, G, respectively.

Since we have four different letters, three-letter ‘words’ (**codons**) are required to write the 20 amino acids.

Several codons can code for one amino acid, these are called synonyms.

Successive nucleic acid codons designate successive amino acids of the polypeptide chain being formed; writing is continuous (except for introns), usually not overlapping.

The AUG codon describes methionine. At the same time, it is the codon that marks the start of translation: the translation complex moves along the mRNA until it hits the first AUG sequence and starts protein synthesis from there. Thus, any newly synthesized protein starts with methionine (which is often removed later). At the same time, the AUG codon sets the so-called **reading frame**. This means that, starting from the AUG sequence, successive triples of bases are treated as successive codons that define subsequent amino acids.

The UAA, UAG, and UGA codons are called nonsense codons, they do not code for any amino acid, but mark the end of polypeptide synthesis at this point.

3.3. Organization of genetic information

Genetic information, i.e. information about the sequence of amino acids in proteins, is contained in DNA in the form of genes, each of which includes information about the amino acid sequence of one protein or peptide. Depending on the size of the encoded molecule, genes have length. The number of genes depends on the species.

Only one of the two strands of DNA contains information – it is the **template strand**. It is the one that is used to synthesize the mRNA molecule, which will be involved in the synthesis of the protein. This strand is also referred to as non-coding and antisense strand. The second strand is a non-matrix, coding, meaningful strand.

Genes are often assembled into groups of genes: in bacteria, there are **operons**, i.e. groups of genes coding for proteins with related functions, in higher organisms – **multigene families**, including genes assembled not so much because of similar functions, but because of the need for a similar number of copies .

Within DNA there are coding (containing specific genetic information describing the structure of proteins) and non-coding sequences. Within the non-coding DNA there are so-called **pseudogens**.

Large areas of non-coding genes, accounting for 25% of the total genome, are sometimes referred to as *gene deserts*. Currently, their regulatory functions are suggested. For example, disorders within them are associated with several types of cancer.

3.4. Enzymes modifying nucleic acids

Nucleases are enzymes that cut the phosphodiester bond between nucleotides, which breaks the nucleic acid strand.

Ligases combine nucleic acid chains that have been cut to form phosphodiester bonds. This takes place both during DNA repair and during recombination, RNA splicing, and also during DNA replication.

Polymerases work similarly to ligases, building phosphodiester bonds between nucleotides, but in their case it is a ‘tape’ action that takes place during the synthesis of nucleic acid.

Helicases are enzymes that break hydrogen bonds between opposite nucleotides. Thanks to this, two strands of DNA can unravel, and only in this state DNA can be replicated or transcribed.

Topoisomerases are enzymes that regulate the degree of twist of the DNA double helix and enable the solution of some problems resulting from the closure of DNA into a circular form.

3.5. DNA replication

DNA replication is the process of duplication of a DNA molecule before a cell divides. Thus, just before division, the cell is tetraploid, and after division, both daughter cells are diploid (they have a double set of DNA molecules).

Replication occurs in the template of both molecules of the double-stranded DNA helix. It is described as a semi-conservative or 'semiconservative' process: a new, complementary molecule is added to each of the two single-stranded molecules. As a result, both daughter cells will have double-stranded DNA molecules, one strand of which is derived from the parent cell and the other is freshly synthesized.

Due to the specificity of the functioning of DNA polymerases, which move along the template DNA in the 3'-5' direction, DNA synthesis must always be in the 5'-3' direction.

Generally speaking, the replication process begins with the disentanglement of the double helix by the helicase enzyme at sites rich in A = T pairs. These sites are referred to as ori (origin). The double strand is separated, thus creating a **replication fork**. Single strand binding (SSB) proteins bind to the separated strands to prevent the strands from reattaching.

DNA polymerase is unable to initiate DNA synthesis. It can only lengthen the chain from the 3' end by pairing the nucleotides with the template. Therefore, first a short piece of an RNA primer is synthesized with the participation of **primase**, which is an RNA polymerase. Only then **DNA polymerase can** begin to work, attaching another nucleotide and creating further phosphodiester bonds. Due to the necessity to run the synthesis in the 5'-3' direction, the synthesis on one strand is continuous, on the other one, in sections, the so-called Okazaki fragments.

If the DNA is non-circular, there is no room for addition of an RNA primer to the 5' end of the DNA delayed strand. Therefore, with each successive division, the DNA should shorten, lose end sections, which therefore cannot be replicated (approximately 50-150 bp for each cell division). In order to avoid this, there are **telomeres** at the ends of the molecule. These are short, repeating sequences (in human: 5'TTAGGG3', 5-30 kbp in length). The 3' end of the leading strand extends beyond the 5' end of the lagging strand. The **telomerase** enzyme has an RNA fragment complementary to this sequence, extends the leading strand (on the template of its own RNA segment) and detaches. This process is repeated many times. Thus, losses from successive replication and cell division are related to the telomeres and not to the coding sequences.

3.6. Transcription

Transcription is the synthesis of mRNA (*messenger RNA*) on the basis of information from DNA.

There are three stages of transcription: initiation, elongation, and termination. RNA polymerase is responsible for the course of transcription.

Initiation occurs when RNA polymerase binds via the σ subunit, together with protein transcription factors, to the promoter of a gene. RNA synthesis must start exactly at the point where a specific gene begins. After RNA polymerase is attached to the promoter, a closed promoter complex is formed. The two DNA strands are separated in an area rich in adenine-thymine base pairs. At this stage, the σ subunit detaches from the polymerase (referred to as an 'open promoter complex'). Then the free ribonucleotides may already be 'matched' to the next codons of the template DNA and combined together.

This is the second stage of transcription, **elongation**. The RNA polymerase moves along the DNA molecule in the 3’ to 5’ direction, attaching subsequent ribonucleotides to the free 3’ end of the newly emerging RNA chain.

The ending of the RNA synthesis is called termination. In prokaryotes, this occurs on palindromic sequences. These are sequences where the second part repeats the first in reverse order and with base replacement to complement (e.g., ATTCG-CGAAT). In RNA then, the structure of the so-called hairpins (the molecule ‘folds’ to form a double-stranded, complementary section).

An mRNA is formed before translation in the cell nucleus as a pre-mRNA. It contains non-coding sequences (**introns**) between the coding sequences (**exons**). The introns are removed and the sections of the exons are spliced together.

It is possible to combine exons in different combinations, i.e. not in the order ‘1-2-3-4-5’, but e.g. ‘1-3-4-5-2’ etc. This results in greater protein diversity. It is the one of the mechanisms by which several different proteins can be made from one gene. This process is called **alternative splicing**.

In order to prevent digestion of mRNA by nucleases crossing 3’-5’ bonds, the so-called a **cap**, i.e. 7-methylguanosine connected with a 5’-5’ triphosphate bond, resistant to these enzymes. At the same time, the cap enables the ribosome to recognize the end of the mRNA molecule. In turn, a **poly A tail**, i.e. about 250 adenine nucleotides, is attached to the 3’ end. This polyadenylation is likely to protect against exonuclease.

3.7. Translation

Translation is the process of synthesizing peptide chains on an mRNA template. The order of the amino acids in the molecule is crucial for the properties of a protein. This information is transferred from DNA to mRNA and used in the translation process, in which subsequent amino acids are combined in the order determined by the mRNA. For this to be possible, the amino acids must be properly prepared, i.e. linked to a transport RNA (tRNA).

The tRNA molecule is composed of approx. 74-95 nucleotides. Its second row structure is shaped like a clover leaf; It has 4-5 arms, i.e. loops formed by RNA hairpins. The tRNA includes the following arms in the molecule: dihydrouridine, anticodon, additional, pseudouridine and acceptor. It is the tRNA molecules that carry specific amino acids. The attachment of an amino acid to the tRNA acceptor arm is called aminoacylation, the reaction product is **aminoacyl-tRNA**; it is catalysed by a specific aminoacyl-tRNA synthetase, which recognizes both the amino acid and the tRNA.

Thus, aminoacyl-tRNA molecules are the substrate in the process of translation, and energy from the hydrolysis of ATP and GTP is also needed. Ribosomes are also essential as coordinating structures for the mRNA and tRNA activity during protein synthesis, ensuring appropriate spatial arrangement of all the elements necessary in this process.

Translation begins with initiation. At the site adjacent to the AUG sequence (encodes START and methionine), a small ribosome subunit binds to the mRNA (in *Eucaryota*, the small ribosome subunit moves from the 5’ end cap until the AUG sequence is recognized). This codon corresponds to the anticodon of the methionine transfer tRNA, so such a molecule (specific, initiator: $tRNA_i^{Met}$) binds to the mRNA and together with a small subunit of the ribosome forms an initiation complex. At this stage, with the participation of protein initiators, the large ribosomal subunit binds and the elongation phase begins. There are two aminoacyl-tRNA binding sites on the large ribosomal subunit: the peptide site (P), currently occupied by $tRNAi^{Met}$, and the aminoacyl site (A). Another aminoacyl-tRNA molecule with an anticodon corresponding to the first mRNA codon after the start AUG goes to the site A. With the participation of the so-called polypepti-dyltransferase centre,

a peptide bond is created between the amino acid of this aminoacyl-tRNA and methionine. This is followed by a translocation: the newly attached aminoacyl-tRNA is shifted to the P site. The tRNA molecule is then cut off from the already attached amino acids, and another aminoacyl-tRNA molecule goes to the A site. For these processes to take place, further protein factor, elongation, are necessary. Elongation continues until one of the STOP codons is encountered. Instead of another aminoacyl-tRNA, the A site of the large subunit of the ribosome moves to a termination factor that releases the synthesized polypeptide molecule. The energy necessary for the individual stages of translation to take place comes from the hydrolysis of GTP and ATP.

4. DNA mutations, DNA repair

During replication and meiosis, sometimes errors occur, as well as mutations caused by mutagenic external factors. A mutation is an inherited change in genetic material.

Spontaneous mutations (without external factors) are for example: deamination, depurination, tautomerisation, slipped strand mispairing mutation. These mutations occur mainly due to replication errors and repair processes.

Mutations can also be induced by external factors: chemical, physical and biological.

Chemical **mutagenic factors** include: nitrogen bases, nitric acid, alkylating agents, methyl methanesulfonate, psoralens, nitrogen base analogs, transposons, reactive oxygen species, free radicals.

Mutations are often caused by reactive oxygen species (ROS), which can be partially regarded as spontaneous mutations, because ROS are formed as a by-product of basic aerobic cellular metabolism. Hydroxyl ($OH^{\cdot}$), Superoxide (O_2^-) and hydrogen peroxide (H_2O2) radicals cause 3000 to 5000 mutations per generation in *E. coli*.

Mutagenic physical factors are e.g. UV radiation: A (with a wavelength of 320-400 nm), B (280-320 nm), C (200-280 nm – absorbed by ozone). UV B and C radiation causes the formation of cyclobutane pyrimidine dimers and 6-4 photoproducts. Other physical factors are ionizing radiation: X and γ, α, β particles, neutrons.

Mutations in one nucleotide (a pair of nucleotides) within a single gene are called **small scale mutations**. Among these the following can be distinguished:

- substitution, which consists in replacing one nucleotide with another,
- insertion, i.e. insertion of one nucleotide,
- deletion, i.e. removal of one nucleotide.

Mutations can lead to different effects: e.g. replacing one nucleotide with another (substitution) can change the amino acid described by a particular codon. As a consequence, one other amino acid may be inserted into the protein molecule. This type of mutation is referred to as a missense mutation. In practice, such a protein may perform its functions unchanged, but it may also turn out that the protein is unable to perform its function. If the mutation occurs at the third position of the codon, there may be no change at all, it can result in a synonym codon. This is called silent mutation.

Another type of mutation in which one nucleotide is replaced by another is a nonsense mutation in which one of the STOP codons is produced in the wrong place.

Insertion or deletion of one nucleotide changes the reading frame. The entire codon is read at once: three nucleotides representing one amino acid. If a deletion causes removal of one of them, or an insertion adds one, the correct codons will not be read, but triples shifted by one position. This will result in the insertion of completely different amino acids during translation.

In addition to point mutations, there are also larger mutations, including large fragments of chromosomes: DNA molecules, tens, thousands or more pairs of nucleotides, definitely going beyond one gene. **Large scale mutations** take the form of insertions, deletions, rearrangements (translocations, shifts of genome fragments in relation to each other), inversion (turning a fragment of the DNA chain by 180˚), amplification (gene duplication). It is also possible to close the arms of the chromosome, forming a ring chromosome, and the formation of the so-called an isochromosome in which one of the arms is absent and the other, duplicated, takes its place. There is also a loss of heterozygosity, consisting in the loss of one of the pairs of alleles (due to deletion or recombination).

Changes in DNA can also affect entire chromosomes or large parts of them. These can include aberrations in the structure or number of chromosomes. In the case of a number, it can be referred to as aneuploidy (when the set of chromosomes is enlarged or reduced by one or more of them: nullisome – no chromosomes of a given pair, monosome – presence of a single chromosome instead of a pair, trisome – presence of three chromosomes instead of a pair) and polyploidy (increase of whole sets of chromosomes compared to the standard number 1n or 2n, e.g. 3n triploidy, 8n octaploidy, etc.).

DNA repair. It is estimated that about 104-106 DNA damage is generated in the human body per day. In order not to lead to cell dysfunction or cancer formation, it is necessary to repair the damage. This repair is energy-intensive, so if there is too much damage, the cell is directed to the apoptotic pathway. There are at least five basic models for repairing damaged DNA. A distinction is made between direct defect removal, nucleotide excision repair, base excision repair, base mismatch repair, and repair of double-stranded cracks. DNA repair is a complex process and makes up a significant part of cell metabolism. At least 130 genes (proteins) are involved in repair.

5. Human genetics

The normal human genotype includes 46 chromosomes: 23 pairs. 22 of them are called autosomes, one pair (XX or XY) are sex chromosomes. Each chromosome contains one DNA molecule. Thus, in every human cell (except the reproductive cell) containing a cell nucleus, there are 46 DNA molecules (including 23 from the mother's egg and 23 from the father's sperm).

The sequencing of the human genome was announced in 2003, when the Human Genome Project described 99% of the genome with an accuracy of 99.9%.

Of the 3 billion base pairs of human DNA, the encoding DNA comprises less than 2% of the human genome. Coding DNA sequence contains approximately 20,400 protein-coding genes. In contrast, 98% of the human genome is non-coding DNA sequence, including 14,600 pseudogens.

Compared to other catarrhine monkeys, humans have one fewer chromosomes. Human chromosome II was created from two other chromosomes linked by telomeres. The human genome differs from the chimpanzee genome in changes in 35 million nucleotides, 5 million insertions/deletions, rearrangements. The differences represent only 1.23% of the genome. However, as much as 6% active genes different in humans and in chimpanzees, which the latter species does not have must be added to this.

Sex determination occurs in human throught XX/XY chromosome system. Considering that the mother of a zygote can obtain only one of the two types of the X chromosome, and the father – with a probability of 1: 1, the X chromosome or the Y chromosome, this system enforces a 1: 1 female to male ratio.

The human sex chromosomes, X and Y, are very different: X contains about 800 protein coding genes, while Y contains only about 150 (of which as many as 73 code proteins).

During the development of the fetal female organism, one of the two X chromosomes is inactivated in almost all cells and transforms into a Barr body. The 'choice' of a chromosome to inactivate is random, thus different X chromosomes in a pair can be converted into a Barr body in different cells.

The Y chromosome recombines with X only in 5%. Recombination of the remaining 95% of the Y chromosome could be etrimental, so it is blocked (female organisms could develop with harmful genes derived from the Y chromosome, or male organisms, lacking some of the genes located on the Y chromosome).

6. Genetic aging of cells

Mammalian cells cannot divide indefinitely. L. Hayflick showed that for each species there is a maximum number of divisions. For human cells, it is approximately 40-60. This maximum number of divisions, the so-called The '**Hayflick limit**' was also found to correlate with telomere length. In subsequent cell divisions, the telomeres shorten as a result of replicative mechanisms. Active telomerase occurs in stem cells, and in adults, e.g. in the epidermis, sperm cell lines, lymphocytes, where it is necessary to proliferate rapidly throughout life.

Several conclusions can be drawn from Hayflick's theory, for example:

- attempts can be made to limit tumour growth with telomerase inhibitors,
- it is possible to try to restore tissues by activating telomerase (but risking the formation of cancer),
- 'cell renewal' (widely promoted in cosmetology) is not recommended – attempts to accelerate the cell replacement, e.g. in the epidermis through acceleration of cell multiplication. While telomerase is active in epidermal cells, frequent divisions (and frequent replications) increase the likelihood of tumour formation. In this case, 'cell renewal' is a necessary evil, not something to be intensified.

This is not the only genetic mechanism underlying cellular aging. The other is the gradual **accumulation of mutations**. Mutations occur continuously in every cell, and although the vast majority is repaired, some survive. Therefore, over the decades, the genotype gradually degenerates, and individual cells may lose their ability to function properly.

There are some **inherited genetic defects that accelerate aging**, caused by the presence of mutant alleles of genes responsible for DNA repair processes. Among the most obvious of them are:

- Cockayne syndrome, in which an allele coding for one of the proteins necessary for transcription-related repair is mutated, in this case has a life expectancy of 13 years on average,
- Huchinson-Gilford progeria, with a mutated allele encoding abnormal laminate A, a component of nuclear chromatin scaffolding, necessary for the repair of double-strand fractures (here, the survival rate is also on the order of 13 years),
- Werner syndrome with a mean survival rate of 47 years, associated with the presence of abnormal helicase and exonuclease repair with base excision.

The study of aging processes on individuals with exceptionally long lives revealed that they have above-average active DNA repair systems, for example PARP proteins: poly (ADP-ribose) polymerase, involved in the repair of single-stranded cracks.

7. Genetic engineering

Genetic engineering is carried out through a number of biochemical techniques such as DNA sequencing (i.e. the description of the nucleotide sequence in the nucleic acid chain), DNA cloning (i.e. obtaining multiple copies of DNA obtained bycutting out fragments by restriction enzymes, transfer of these fragments thanks to vectors such as plasmids, their recombination with bacterial DNA and multiplication of recombinant bacteria), polymerase chain reaction, nucleic acid hybridization, DNA microarray technique, targeted mutagenesis, horizontal genetic transfer.

7.1. Examples of genetic engineering applications

Genetic engineering techniques are gradually offering more and more possibilities. The practical application of these techniques is handled by white (industrial), green (agricultural) and red (medical) biotechnology. An example of the practical use of biotechnology are **gene therapies** aimed at combating diseases caused by abnormal alleles of specific genes. In addition to gene therapies, genetic engineering is used in medicine as a tool that allows the **production of drugs** by multiplying DNA-modified microorganisms (the first drug produced in this way was synthetic human insulin, in 1982, and nowadays there are hundreds of drugs produced by such methods (vaccines, antibodies, hormones, etc.). Of course, it is possible to produce proteins in this way for fields other than medicine, including cosmetology. In order to **identify carriers of recessive genes** that cause genetic diseases, tests can be carried out that can help prevent genetic diseases. **Personalized medicine** is gradually developing: genomes are studied in terms of existing links of groups of patients, groups of genes and diseases (*Genome-wide association study*, **GWAS**). The patient's genome is then analyzed, looking for specific alleles that may affect the health condition, susceptibility to diseases, drugs hypersensitivity, etc., more effective treatment in neoplastic diseases.

Genetic engineering also offers hope of **recreating extinct species** of plants and animals. To do this, techniques such as those used to save currently endangered species are used.

Genetic engineering is also used, inter alia, in in historical research (genealogical lines, bone identification, etc., studies of the migration of population groups and their origin), biotechnology is also used to produce various substances in the chemical industry (citric acid, lactic acid – e.g. for polymers, glutamic acid, lysine, vitamin C, ethanol, oils), in paleontology, history and forensics (identification of biological samples).

GMO: genetically modified organisms.

Genetically modified organisms have a deliberately altered genotype compared to natural individuals in order to obtain traits that are profitable for e.g. a farmer. For example, artificially induced polyploidy has long been used in agriculture. Commonly used polyploid plants include:

- triploids: bananas, apples, citrus fruits, watermelon,
- tetraploids: apples, wheat (for pasta), potatoes, cotton, tobacco, pelargonium, leek, rapeseed,
- hexaploids: wheat (for cakes), triticale, kiwi, oats, chrysanthemum,
- octaploids: sugar cane, strawberry, dahlia, pansy, dahlia,
- dodecaploids: sugar cane.

Through genetic engineering, it is possible to make animal and plant resistant to diseases and climatic conditions, to accelerate growth, to increase or enable a given plant/animal to synthesize a given substance (e.g. a vitamin) by introducing alleles of genes for beneficial traits into the DNA. Genetically modified apples, potatoes (which do not darken when cut, their expression of polyphenol oxidase was suppressed), 'golden rice' with increased content of vitamin A is widely

used, and modified varieties of corn and bananas are enriched with vitamin A. Transgenic varieties of soybean, cotton, tobacco, sugar beet, sugarcane, maize, papaya, potatoes, cereals etc. resistant to insects, viruses, drought, frost etc. The USA are varieties modified in this way. Individual studies show different results, but in 2014 a meta-analysis of the available data showed that the use of GMOs reduced pesticide use by 37% while increasing yields by 22%.

Genes are introduced into cells by a variety of methods, including:

- viral vectors,
- the use of embryonic stem cells (DNA is inserted into the blastocyst and not into the zygote, so as the animal will be a chimera: only some of the cells will be changed),
- electroporation where an electrical impulse increases the permeability of a cell membrane by creating holes in it and transfers DNA between cells (electrotransfer of genes), electroporation can be reversible and irreversible, e.g. in cancer therapy,
- injection (microinjection),
- using a *gene gun* – metal particles coated with plasmid DNA are fired (this technique is used, for example, for DNA vaccines, in attempts to treat Alzheimer's disease, to introduce fluorescent protein genes.

As for animals, the following are some examples of the application of the genetic engineering.

Mosquitoes are modified by introducing a lethal gene into the males. In the Cayman Islands, this has reduced their population by 80%, which gives hope for a reduction of the malaria threat.

Modified fish with an overproduction of growth hormone are used in food production (AquAdvantage salmon introduced in 2015, trout, tilapia).

Numerous mammalian species are used in physiological/medical/genetic research. Mice, rats, rabbits, pigs and sheep have been model systems in these studies for several decades (e.g. models of physiological dysfunction, diseases, transplantation). This allows for better understanding of disease syndromes, the development of therapies and drugs, and the improvement of genetic engineering methods. For example, lines of *Knockout mouse* mice are created, having the selected gene replaced by an inactive gene. In this way, model animals are obtained that are used to study the function of the selected gene.

Genetic engineering in mammals is used to modify food-source animals. In 2006, pigs producing omega-3-unsaturated fatty acids were obtained. In 2011, the transgenic cows producing milk for humans were produced in China and Argentina. In Canada, in 2012, pigs were obtained containing the enzyme phytase, which allows phosphorus absorption to be more efficient (which, in turn, will reduce environmental pollution). In the same year, the cows with completely non-allergenic milk were produced in New Zealand.

Although this may sound odd, a patient undergoing gene therapy also meets the definition of a GMO.

Examples of questions:

1. Gene mutations.
2. Chromosomal mutations.
3. Frame shift mutations.
4. The translation processes.
5. The process and biological significance of meiosis.
6. Structure of RNA and DNA.
7. Sex chromosomes.
8. Gene therapies.
9. Mendel's laws. Telomeres.
10. Multi-gene inheritance.
11. Meaning of the terms: heterosis, haplotype, codomination, introns, genotype, homozygote, allele.
12. What is the splicing process?
13. The use of genetic engineering in food production.
14. The replication processes.
15. Aging by genetics.

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