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GENETIC ASPECTS OF THE NORMAL AND PATHOLOGICAL TRAITS IN HUMANS

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Genetic aspects of the normal and pathological traits in humans

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1. Organization of storage and usage of genetic information

1.1 Principles of genetic information storage

Genetic information is stored in the primary structure of DNA molecules - in the order of nitrogen bases of nucleotides. Strict rules for DNA storage, use, protection and transfer ensure that:

• the risk of DNA damage is as small as possible. Information from genes is not used directly but only through copies made by transcription – RNA molecules;

• only currently needed information (gene) is always used and no mistakes happen in its usage. This function is provided by proteins of different types and function that are either associated with DNA or are involved in the execution of its functions;

• its replication and transmission to daughter cells takes place only after inspection and repairing of defects.

Prokaryotic genome is relatively small and simply designed. It is formed by a circular double strand DNA molecule, which is in one place (ORI = start of replication) attached to the inner face of cell's cytoplasmic membrane. Information is localized in the cytoplasm and readily available.

Eukaryotic genome is composed of nuclear and mitochondrial (and chloroplast in plants) genomes. For simplicity, we will only consider the genome, which is located in the nucleus.

Nuclear genome of eukaryotes is large and segmented. It is divided into an even number of double strand linear DNA sequences that closely communicate with different types of proteins and are localized in the cell nucleus. Transcription takes place in the nucleus. Translation begins in the cytoplasm and is carried out in cell's ribosomes (mostly attached to endoplasmic reticulum).

1.2 Spatial arrangement of the nuclear (genomic) DNA in prokaryotes

Prokaryotic genome forms in space seemingly chaotically arranged loops, called nucleoid. They are result of DNA coiling and supecoiling, in order to achieve its minimal energy level (Fig. 1).

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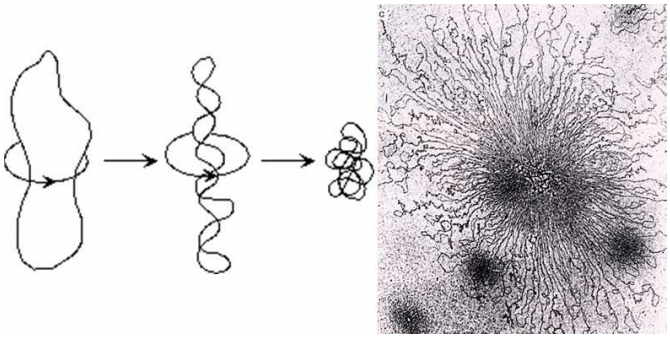


• **Figure 1.** Coiling and supercoiling of bacterial chromosome

It was assumed for a long time that coiling of prokaryotic DNA in nucleoid is random, even if it was known that supercoiling of DNA involves at least two enzymes - **gyrase** (helps coiling) and **topoisomerase** (facilitates unwinding of DNA and protects it from damage). It was assumed that there are no assistance (regulatory) proteins, there. Only recently, it was found that structural and regulatory proteins (DNA binding proteins – DNAbp) are associated even with bacterial DNA. These are histone-like proteins (HLPs) that have a fundamental role in regulation of the nucleoid construction and in management of genetic information usage in bacteria. Main HLPs are proteins HU, FIS, IHF and H-NS.

HU dimers, where there are a few, associate to each other and to DNA. Linked HU dimers form small local clusters, that contributes to DNA coiling.

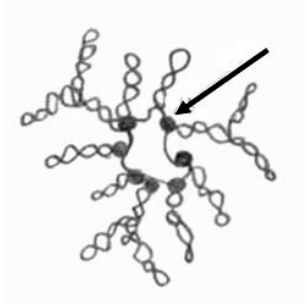
In high concentration of HU opposite effect occurs – DNA become linear and HU bind to helical structure of DNA.

H-NS form bridges between distal segments of DNA and "enwrap" double-stranded DNA.

IHF evokes a formation of "sharp loops" of DNA, if it binds to sequence-specific sites or smaller folds, in non-specific sites.

FIS dimers bind to DNA and form loops of it.

Dodecamers DPs induce close formations of DNA, which protect it against damage. It is assumed that bacterial DNA in endospores also has this configuration.



It was found that although the bacteria do not have cytoskeleton; there is probably a system of connected fibers (arrow) in the nucleoid, which are visible in the nucleoid cross-section, and which, together with DNA, form a "rosette" composed of arc-shaped domains (Fig. 2).

These fibers pass along the whole bacteria cell and ORI of the prokaryotic chromosome is attached to them in one “pole” and TER (end of replication) is found at the other one.

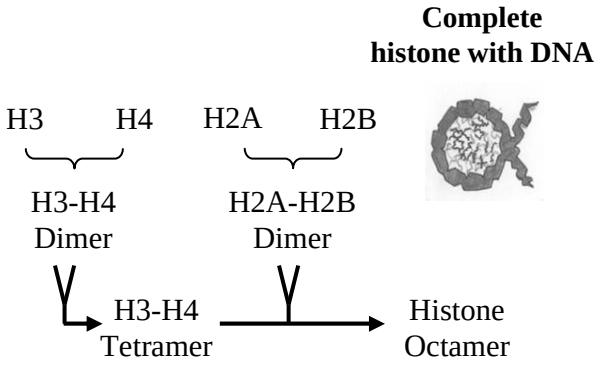
• **Figure 2.** DNA rosettes around longitudinal fibers

1.3 Spatial arrangement of the nuclear (genomic) DNA in eukaryotes

Eukaryotic genome is divided into linear DNA molecules of variable length, which closely communicate with structural and functional proteins. Together they form the internal content of nucleus, called **chromatin.**

Proteins, associated with DNA and involved in its protection and use of genetic information, are divided into histones and nonhistones ones.

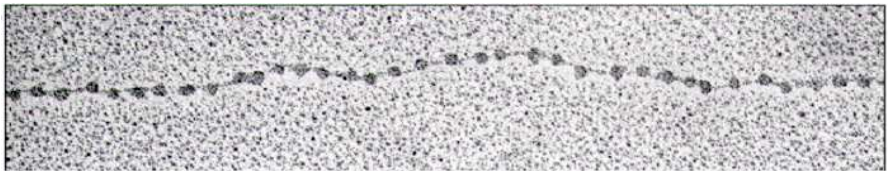
Histone proteins have structural and also regulatory functions. They are basic proteins with a positive charge. There are several types of histones, but five of them: H1, H2A, H2B, H3 and H4, play a role in the development of chromatin. They are very old (conserved) and are able to dimerise and oligomerise. Dimers H2A-H2B and H3-H4 form tetramers and together they form octamers. They contain “helix - turn - helix“ motifs, which allow them to fix the DNA, which (because of opposite charge) wraps around them 1.65 times, in the length of 146 base pairs. The values given in different literary sources may vary, which does not change the fact that the association of histones, together with each other and also with DNA, is a process that takes place spontaneously and so histones fulfill its structural function. DNA wrapping on the histone octamer creates basic functional and regulatory structure of chromatin – the **nucleosome** (Fig. 3).



• **Figure 3.** Scheme of nucleosome formation

Known image (Fig. 4) of nucleosomes connected together by a short section of DNA, called “beads-on-a-string” and forming of so-called 10 nm fiber, is an artifact, because it cannot be found in this form in nature, as the "linker" DNA would be destroyed. If the octamer breaks apart and DNA is linear – it is a short-term event (during transcription or replication), which takes place in a limited range and is strictly controlled and managed.

Dissociation (disintegration) of histone octamers and linearization of DNA occurs after targeted aminoacylation of histones. Its reorganization is the result of deacylation. This process can be blocked by histone methylation. So is performed histone regulatory function - gene expression can occur only after DNA section with located gene is accessible (linearized).

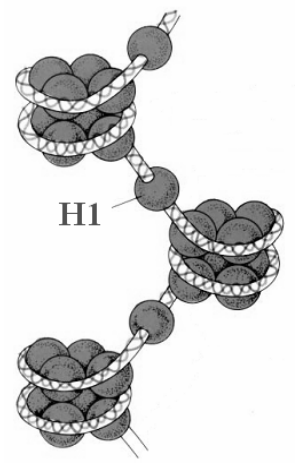


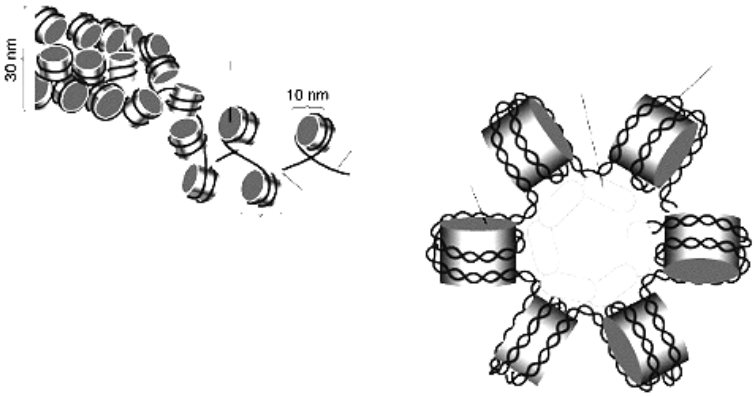
• **Figure 4.** 10 nm strand “beads-on-a-string”

Histone H1 binds to DNA between nukleosomes in 20 bp distance (Fig. 5). It has fixing and oligomerisation function. It connects to nucleosome and so fixes DNA. Complex composed of H1 and nucleosome is called **chromatosome** and it is considered to be the (second level) basic unit of chromatin.

H1 molecules have also the ability to merge with each other, and so 6-8 chromatosomes form circular **solenoid** structure. Solenoid (Fig. 6) is single "floor" of helical structure called **30 nm chromatin fiber** (Fig. 7).

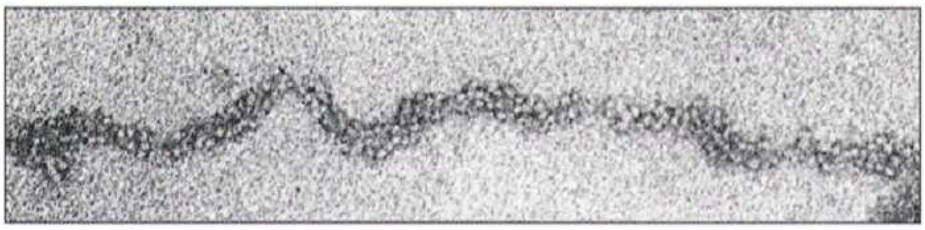
Chromatin fiber forms loops that are connected to nonhistone (s.c. scaffold) proteins of chromosome and nuclear matrix, which makes the base for ultrastructure and spatial configuration of eukaryotic chromosomes in nucleus. In euchromatine, loops are loose and transcription of genes can take place. In heterochromatine, loops are condensed, which prevents the use of genetic information they contain.





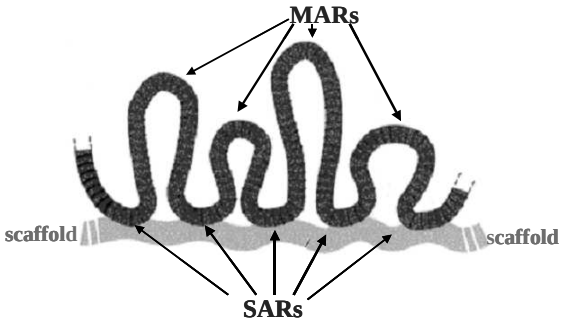
• **Figure 5.** Linkage of H1 to DNA

• **Figure 6.** Formation of solenoids and 30 nm chromatin fiber



• **Figure 7.** 30 nm chromatin fiber

Bases and tops of chromatin fibers loops contain non-coding DNA in the length from 10 to 90 kb. SARs (scaffold attachment regions) are attached to scaffold proteins forming the longitudinal axis of chromosomes and MARs (matrix attachment regions) to the lamin fibres of nuclear matrix (Fig. 8). They are developmentally conserved and rich in adenine-thymine pairs.



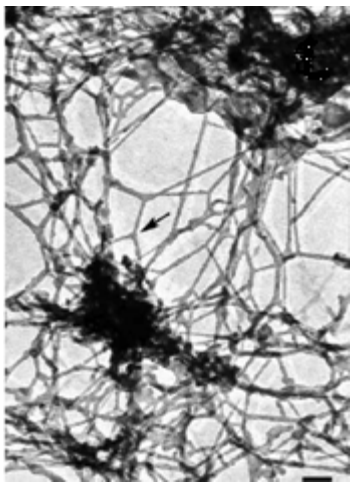
• **Figure 8.** Loops of 30 nm chromatin fiber and their attachment area

Regulation of gene expression takes place after unwinding of chromatin fiber, when corresponding regulatory protein (main transcription factor) binds to reporter octamer, on DNA associated H2A. Z protein, which indicates the area where target gene is located. Other regulatory proteins cause disconnection of H1 (by aminoacylation) and dissociation of nucleosomes that are closest to the gene regulatory region. This will enable DNA linearization and subsequent binding of general transcription factors. Transcription takes place so that the smallest possible number of chromatosomes is dissociated; therefore they are reconstructed immediately after the use of genetic iánformation.

Nonhistone proteins have mainly regulatory function and manage the internal organization of interphase nucleus. They are scaffold (structure and associated) proteins forming ultrastructure of chromosomes and proteins, which ensure the protection of chromosome territories and chromatin dynamics inside them.

Scaffold proteins can be divided into two main groups - structural and associated. Structural proteins form the longitudinal axis of the chromosome and are lamins of nuclear matrix.

Proteins forming the longitudinal axis of chromosome, have properties of linear proteins and are able to overlap each other and form more complex fibrous formations. Example is tropomyosin, which is composed of two chains arranged in α-helix. Tropomyosin ends can overlap each other and by this way form fibrous figures. It allows maintaining of linear architecture of chromosome axis, to which bottoms off loops of chromatin fibers are connected. Apart from this, in prophase and prometaphase of cell division, they ensure condensation of chromosome by their lengthwise gliding down, which makes the chromosome shorter and thicker, without endangering of chromatin loops. In anaphase and telophase they slid opposite direction.



Nuclear matrix proteins form a continuous layer covering the inner face of inner membrane of the nuclear envelope (the lamina) and spatial network inside the nucleus (matrix). These proteins are called lamins and divided into three types - a, b, c.

Lamins a and lamins c, compose intranuclear matrix, net consisting of 10 nm long fibrous Y-shaped subunits (Fig. 9). In some areas (“crossings”) of this net, are located enzyme clusters ("factories") in which transcription or replication takes place – s.c. **transcriptosomes** and **replicosomes**. They are not distributed symetrically – their number increases towards the centre of nucleus.

• **Figure 9.** 10 nm lamin subunits (arrow) forming the nuclear matrix

Lamin b has three functions – (1) it anchores lamin protein layer (lamina) to the inner face of the inner membrane of the nucleus envelope, (2) telomere loops of chromosomes are connected to it, and (3) it communicates with the intermediate cytoskeleton filaments, which pass through the nuclear pores. It is attached to nuclear envelope throughout the whole cell cycle and has a crucial role in the reorganization of the nucleus in telophase. At the beginning of prophase lamins are phosphorylated, what cause disassociation of matrix and release of chromosomes, lamins a and c. Nuclear envelope disintegrates into vesicles, which remain connected to the cytoskeleton filaments, by lamins b. Lamins are again dephosphorylated in the telophase. Chromosomes rejoin by telomers to lamin b, lamina and matrix are restored; vesicles of nuclear envelope are rejoined and new nucleus is form

The fundamental importance of lamins for inner nuclear organization of chromatin indicates mutation in 22B splicing site of intron 10 in HGPS gene (Hutchinson-Gilford progeria syndrome), coding both lamin a and c. The consequence is progeria, fatal disease characterized by premature aging and children death, caused by defects of regeneration and dying of cells, which cannot generate correct nuclear matrix.

The **non-histone proteins associated with the scaffold and nuclear matrix** are divided into four groups:

• Enzymes with a specific function – e.g. topoisomerases. Their task is to eliminate torsional tension in the DNA resulting from repeated coiling and uncoiling (winding and unwinding) of chromatin fibers. Topoisomerase I cleaves a single strand of DNA, allows it to unwind and adds or removes one base pare. Topoisomerase II, after cleaving of both DNA strands and its relaxing, adds or

removes two pairs of bases. There is no danger of information losses, because MARs and SARs contain lot of not-coding DNA, there.

• Proteins that carry out transcription and replication. They are assembled in the "crossings" of nuclear matrix net. They are not arranged a symmetrically in the nucleus – as they are closer to the nucleus center so more of them are present. The role of assistance proteins is to transport those parts of chromatin loops to them, which contain genes that need to be transcribed. The same way it works with the order in which different types of chromatin are replicated.

• HMG (high mobility group) proteins are ubiquitous nuclear proteins that regulate and facilitate all the processes associated with DNA - transcription, replication, recombination and repair. They bind to DNA and to chromatin and act as "structural elements" of short-term and long-term changes in their "architecture". They also influence the activity of various regulatory molecules, e.g. receptors for hormones, p53, HOX-D9, and many transcription factors. Disorders of their structure and function are linked to some cancers, so they are target of some cytostatic drugs. The antibodies against HMG proteins are produced in some autoimmune diseases.

• Proteins ensuring chromosome territories and their internal organization:

a) Insulator proteins that are involved in regulation of genes expression, so, that in the territory of chromosome (or in its domain), they manage the internal organization of DNA. They provide "transfer" of chromatin loops containing particular genes closer to the nucleus center, to active transcription sites - transcriptosomes.

b) DBF (the domain boundary factors) proteins that define territories (“domains”) of chromosomes in the nucleus. They protect against random and unwanted interactions between regulatory sequences of genes. They also allow or support the use of genes inside the chromosome territory (domain). Some of them impede the penetration of the signal, which promotes heterochromatin formation to active chromosomal domains.

c) CMM (cellular memory modules) proteins are as yet little studied proteins, known in Drosophila melanogaster. They provide "memory patterns" of chromatin reorganization (the so-called "histone reset"), e.g. restoring of structural and functional arrangement of chromatin in the daughter cell nuclei, after the cell division. It is assumed that they have a crucial role in maintaining of cell´ differentiation rate and degree. Experts expect that, after studying them, it will be possible to remove (suppress) their activity and thus obtain a tool for converting differentiated cells to totipotent ones, which for example shall open the way to prepare “less preprogrammed” stem cells, to treat patients.

Each interphase chromosome has inside the nucleus its own territory, and contains clearly distinguished chromatin domains, sized from 1 Mb. Parts of the chromosome take up different positions in its territory – according to gene content and chromatin type. Gene-rich and early replicating chromatin is located closer to the center of nucleus. On the contrary – gene-poor and middle-to-late replicating chromatin is collected on the periphery of the nucleus. Territories of chromosomes are defined by interchromatide compartments, which contain different types of non-chromatide domains with enzymes and other proteins, for example DBF, transcriptosomes and replicosomes.

To regulate the expression of particular genes, interactions between chromatin and interchromatide compartments are necessary. Transcription status of genes is related to their position in the chromosome territory. Dynamic change of chromatin fibers position and thus the genes (except for pericentromeric chromatin) plays a fundamental role in the activation and silencing of transcription.

2. Methods of human genetics

Human genetics has its noticeable particularities arising from indisputable fact that in human is not applicable experimental mating (hybridization), as it is common in genetic research of plants and animals.

However, it is needed to recognize participation of inheritance on formation both normal and pathological traits and diseases, that are aim of study for human and clinical geneticists. So, researchers and geneticists had to discover special methods for studying of genetic background of human traits, diseases and probability of their appearance in current or future generation.

Clinical genetics field of interest are diagnostics and prognosis of inherited diseases and pathological traits. Because they can be caused by wide scale of changes in genetic material (e.g. single-gene inherited point mutations, polygene, multifactorially inherited and chromosomal aberrations) has human genetics many specific questions dealing with research and utilization of obtained information in medicine.

Practical application of genetics in medicine allow to recognize genuine reason of rapidly growing number of disease origin, appoint recurrence risk of disease in particular family and relatives, as well as prenatally determine genetic impair of fetus.

Essential methods of human genetics are:

- Gemelology – survey of twins
- Genealogy – analysis of family trees
- Genetics of populations
- Cytogenetics
- DNA and proteins analysis (molecular genetics and protenomics)

Molecular genetics require special textbook and cytogenetics will be described in separate chapter.

2.1 Gemelology (survey of twins)

This method is used to determine participation of inheritance an environmental (epigenetical) factors on certain trait origin. It arises from knowledge that twins are of two kinds – identical and not-identical (fraternal), with different probability of having common alelles.

Identical (**MZ**, **monozygous**) twins were originally single zygote. In early embryonal development morula divides into two clusters of cells and each of them develops separately. MZ twins have identical genotype. All possible differences in their phenotypes are mainly due to external factors.

Fraternal (**DZ, dizygous**) twins appear after coincident fertilization of two ova, each with other sperm. They are sibs as other ones, just developing simultaneously. Probability for having equal alleles is 50 %, as it is between other siblings.

Principle of twins survey method is comparison of congruity (**concordance**) and discongruity (**discordance**) in form of studied trait in each pair of twins. Interesting is equality in trait form between twins in pair, not form itself. For instance, in blood groups system AB0 we are not interesting, what kind of blood group have twins in each pair, but if they do have the some one.

Proportion of inheritance and environment on particular trait formation is calculated by H quantity (heritability). It is calculated by comparison of trait form concordance (in per cents) both, in identical ($\mathbf{C_{MZ}}$) and in fraternal twins ($\mathbf{C_{DZ}}$) with formula:

$$H = \frac{C_{MZ} - C_{DZ}}{100 - C_{DZ}}$$

H quantity varies in interval from 0 to 1. So like H tends to 1, trait is more determined by genetic background. If H tends to 0, trait was formed more by randomly acting epigenetical factors (genetical predisposition).

If C_{MZ} is 100 (%), than H=1 and trait is completely determined by single major gene. Conversely, in traits significantly affected by environment, the value of H tends to zero. If C_{MZ} is equal to C_{DZ} variable H is equal to zero – a trait it is totally affected by epigenetical factors.

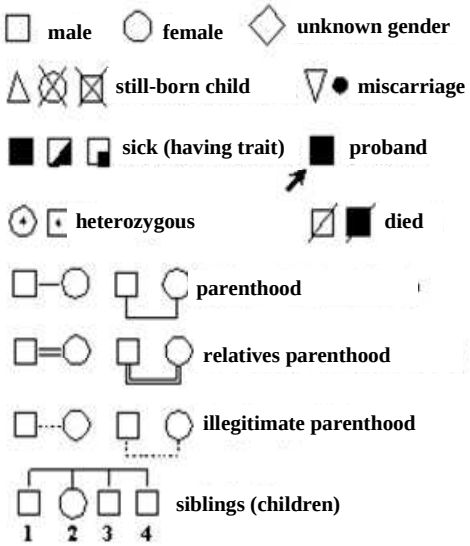
2.2 Genealogy method

Consists in the construction and analysis of the standard family trees (pedigrees) in families where studied trait (e.g. disease) appears. Data are obtained from a larger number of families, usually in the form of pre-prepared questionnaire. The processing of results allows:

- To determine the type of inheritance of analyzed trait, that have been demonstrated (by gemelology), that have strong hereditary conditionality. It is done by processing a large number of pedigrees of families where the trait appears.
- Record of occurrence of appreciable traits in a particular family, what is used in the activities of clinical geneticists.
- In recessive hereditary disease, identify carriers of pathological alleles (heterozygous) or express this assumption.
- Determine the risk of (another) disease appearance in the family, given the genotypes of parents.

The family member, from which begins genealogical analysis of the family is called the proband. In practice it is usually the first affected, or it's relative. In addition to survey the incidence of pathological statuses and anomalies, it is also observed the incidence of spontaneous abortions, still births, impaired fertility, consanguine marriage, etc.. Data must be, as much as possible, obtained from several sources and objective (health records, birth and parish registers, etc.). Obtained data are used to compile standard family tree using internationally recognized symbols (Fig. 10), according to the following rules:

- pedigree must be sufficiently developed in breadth and in depth;
- pedigree has captured at least three generations in a row (labeled from oldest to youngest in Roman numbers),
- if it is possible, in each generation capture all the members (including illegitimate), marked with Arab numbers from left to right, from oldest to youngest;
- legend is inevitable component part of the of family tree, which should include significant data, variations in the use of symbols and so on. Each family member can be identified by coordinates (number of generation / number in the order of generation).



• **Figure 10.** Basic symbols in standard family tree

3. Single gene inherited normal and pathological traits

Single-gene autosomaly inherited are traits, whose phenotypes are determined by the single gene (locus). In regard to locus position in the chromosome we distinguish inheritance autosomal or gonosomal, which may be according to the nature of the responsible allele dominant (codominant, intermediate) and recessive.

The essence of single-gene conditioned pathological traits is mutation on the level of gene (point mutation), which causes a deviation in the synthesis of the polypeptide chain. According to a function of gene product (protein) appear defects of different nature**.**

3.1 Basic terms used in single-gene inheritance

Gene – elementary unit of inheritance. It is region of DNA that is transcribed as a single unit and carries information for a discrete hereditary characteristic, usually corresponding to a single protein or single RNA.

Locus – the position of gene on the chromosome, where single allele is placed. Chromosomes having the same loci are homologous.

Allele – an alternative form of a gene (version of genetic information), that is located at a specific position (locus) in certain chromosome. In a diploid cell each gene will have two alleles, occupying the corresponding position (locus) in homologous chromosomes.

Trait is a genetically determined structural or functional feature of the organism. It may be qualitative or quantitative one, and it is the result of the impact of genotype and environment.

Incidence is a frequency of trait appearance in particular population.

Single-gene inheritance – trait is determined by one gene (locus). This way inherited traits are usually qualitative ones and environmental factors interfere to their form only occasionally.

Genome – term used both for sum of all genetic information in a cell or in an organism, in particular, the DNA that carries this information**.** Genome of eukaryotic cell is composed of nuclear and mitochondrial ones.

Genotype – genetic constitution of an individual (organism). Simultaneously is the term used for description of the particular combination of alleles determining certain trait.

Phenotype is term used both for sum of all trait of the individual, and for description of particular trait form.

Dominant – opposite of recessive. The member of a pair of alleles that is expressed in the phenotype of an organism while the other allele is not, even though both alleles are present.

Recessive - The member of a pair of alleles that fails to be expressed in the phenotype of the organism when the dominant allele is present.

Complete dominance is a condition in which the dominant allele of heterozygous completely suppresses the phenotypic expression of recessive alleles. To create a dominant form of trait a single dominant allele is needed. In this type of inheritance is not possible, form of trait (phenotype), distinguish if genotype is the homozygous dominant or heterozygous.

Expressivity is variability in forms of phenotype in individuals carrying a particular genotype. Commonly it is used as a description of pathology in inherited pathological traits and diseases.

Penetrancy is the frequency of trait expression in owners of certain genotype in the population. Fully penetrating gene occurs in all of its carriers. Incompletely penetrate gene occurs only in part (in per cents) of persons having the genotype (pair of alleles).

Homozygous is individual having for particular gene (locus) two identical alleles (pair). If alleles in the pair are dominant, genotype for the locus is dominant homozygous. If they both are recessive, it is recessive homozygous.

Heterozygous is individual having two different alleles for particular locus (dominant and recessive). In X-linked inheritance is for this genotype in females used term “carrier”.

Compound heterozygous is individual having for particular gene two different alleles, but each of them has different mutation and encodes distinct isoprotein.

Hemizygote – an individual having only a single copy of a particular gene. Commonly the term is used for alleles in X chromosome of male.

Intermediarity means that in the formation of single trait are in heterozygous one involved both alleles equally, so that trait form is between that of both homozygous parents (e.g. pink flowers).

Codominancy is a type of inheritance, when alleles are different and each of them separately determines its own form of trait (e.g. blood group AB).

3.2 Autosomal dominant inheritance

In this type of single-gene inheritance locus responsible for trait formation is located on particular autosomal chromosome. This inheritance is often named as "Mendelian" one. The trait occurs equally in men and women. Single dominant allele is enough to form a trait – it is present both, in dominant homozygous and in heterozygous genotypes. If parents are heterozygous, the probability that child will acquire dominant form of the trait is 75%. Dominant form of normal trait appears in family tree very often. The gender of the parents has no influence on the trait transmission. The transfer of trait from father to son is possible. If the individual has only pair of recessive alleles it is recessive homozygous one. Recessive allele is able to form recessive phenotype only in this genotype. If both parents carry recessive form of the trait then occurrence of this trait in their child is caused by *de novo* mutation.

3.2.1 Autosomal dominant (Mendelian) inheritance of the normal traits

Typical example of autosomal dominant inheritance of normal traits in man (s.c. Mendelian) are antigens (agglutinogens) on the surface of erythrocytes, that allow to sort out people into "blood groups". Their name originates from agglutination – name of reaction, how they are proven. 25 various types of agglutinogens are known and each of them has its own type of inheritance. The blood group systems AB0 and Rh are the best known.

Rhesus factor has no natural antibodies (agglutinins). Rh factor positive is characterized by presence of the antigen on erythrocytes and Rh negative persons do not have the mentioned antigen. It is inherited with autosomal dominancy. In Slovakia 85% of the population are Rh^+. The gene for Rh factor is located on the 1^{st} chromosome and single allele Rh^+ is enough (e.g. in heterozygous one) to create Rh agglutinogen (table 3). Rh negative is a person who is a recessive homozygous (rh^-rh^-).

• **Table 1.** The inheritance of Rh factor

Phenotype	**Genotype**
Rh positive	Rh^+Rh^+ or Rh^+rh^-
Rh negative	rh^-rh^-

The Fisher theory assumes that Rh factor is determined by three alleles (C, D, E), where the dominant and most important for Rh^+ formation is D allele (table 4). We can serologically prove antigens that are coded by alleles D, C, c, E, e on the correspondent locus. No antigen product of the allele d has been proved (recessive allele).

• **Table 2.** Inheritance Rh - Fisher theory

Phenotype	**Genotype**
Rh positivity	DD or Dd
Rh negativity	Dd

AB0 blood groups are determined by occurrence of antigens localized on the surface of the erythrocytes. There is an oligosaccharide base as an antigen determinant. The AB0 system is the oldest known blood system. In the blood plasma (serum) of people having blood group A, B or O group are present natural antibodies – agglutinins anti-A or anti-B (table 1).

Blood serum of the A and B group persons than can be used for investigation of the blood group of the examined persons. It is done by the hemaglutination – specific reaction ("crowding") of erythrocytes in examined drop of the blood by serum that contains one of the agglutinins.

• **Table 3.** Antigens and agglutinins in system AB0

Blood-group	**Antigen** (agglutinogen)	**Agglutinin**
A	A	anti B
B	B	anti A
AB	A and B	none
0	Any more	anti A and anti B

In the ABO system there are 3 alleles (A, B and 0). The alleles A and B are codominant and both are dominant to 0 allele. Therefore here are six possible pair of alleles combinations. Four possible phenotypes are formed by these combinations (table 2).

• **Table 4.** Phenotypes and genotypes in system AB0

Phenotype of blood-group	Genotype of blood-group
A	AA or A0
B	BB or B0
AB	AB
0	00

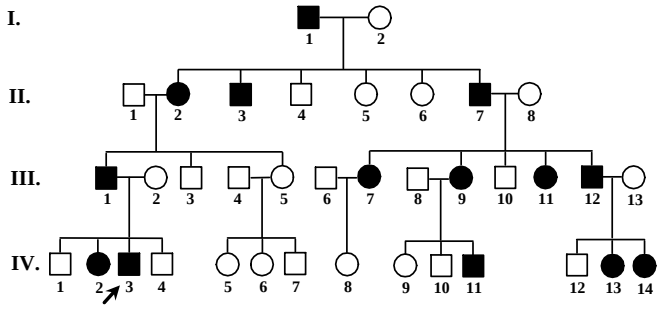
Due to the possibility of the heterozygous status in genotype of the blood group A and B (A0, B0) we cannot determine their genotype. Therefore we consider both possibilities (homozygous and heterozygous), when counting the probability of children´ blood groups.

3.2.2 Autosomal dominant inheritance of the pathological traits

Database OMIM (Online Mendelian Inheritance in Man; www.ncbi.nlm.nih.gov/sites/entrez) 16th April 2009 keeps records of 1101 diseases with autosomal dominant type of inheritance.

Locus of the pathological gene is on certain autosome. The disease occurs in the following generations (vertical type of heredity). Affected person is rarely homozygous ones. The course of the disease is worse in homozygous ones, because of severity of clinical signs. Every affected individual has got affected (at least) one of the parent, but the parents is usually not relatives. Transmission and appearance of disease is not sexually influenced. The transfer from father to son is possible. If one parent is affected dominant homozygous and second parent is healthy (recessive homozygous), all children will be affected (sick). In case, that affected parent is heterozygous and second one is healthy (recessive homozygous), the theoretical rate of affected and healthy children is 50 % : 50 %. If both parents are heterozygous, the theoretical rate of affected and healthy children is 75 % : 25 %. Healthy persons do not transfer this way inherited diseases to their children – if it appears between them – it is *de novo* mutation.

The gene products here are mainly affected basic structural proteins (e.g. transport proteins or cellular receptors). Affections of enzymes are very rare, here. Figure 11 shows typical family tree of the pathological trait with the autosomal inheritance.



• **Figure 11.** Typical the family tree of pathological trait with the autosomal inheritance

3.2.2.1. An examples of diseases with the autosomal dominant inheritance

3.2.2.1.1 Huntington disease

Degenerative disorder of the neural cells of basal ganglia with progressive advancement of disease was first time described by George Huntington in 1872. It is typical disease with late-onset – the majority of the patients do not have problems before age of 30. The average age of the first symptoms is 38 years. Development of disease takes 5 - 10 years and leads to total loss of motor control and intellectual skills. The disease is characterized by uncontrolled choreatic movements and gradual progressive dementia. It is programmed death of the brain. The disease leads to mental and physical ability decline.

The Huntington disease (chorea) has autosomal dominant inheritance with the full penetrancy, incidence is 1: 15 000 to 1: 20 000 deliveries. Late-onset of disease is the reason, why is this disease heritable. Clinical signs are equal in dominant homozygous and heterozygous persons.

Gene of Huntington chorea belongs to the first ones that where mapped exclusively on a base of family studies and gene-linkage analyses by using polymorphisms of the DNA. Nearly ten years of research of many scientific teams were successfully ended up in 1993 - when wide international consortium announced identification of the IT 15 gene, mutation of which is the causal reason of the Huntington´ disease. The gene with the size of 200kb has 67 exons and it is localized on the 4th chromosome (4p16.3). Protein huntingtine is the gene product and it is composed of 3144 amino acids and it has 348kDa.

The huntingtine was identified nearly in all tissues, mainly in the brain (the biggest concentration is in the cerebellum, hippocampus and cortex). The increased level has not been found in the striatum area which is affected the most. The last results outline that huntingtine is essential for the normal development and for microtubules dependent intracellular transport.

The molecular base of the disease is enormous increase of CAG triplet repetitions in first exon of gene. A normal number of repetition is 9 – 35. If it is 35 – 39 times, s.c. permutation appear and the number of repetition has achieved certain critical limit. In patients there is triplet expansion 39 – 100 times. The most greatest described expansion so far was 250 copies. So like is expansion larger, so earlier become signs of the disease.

Indirect methods of DNA analysis (RFLP, VNTR) were used in diagnostics – based on identification of the mutation co-segregation of the certain allele polymorphism in families. It was used only until the gene was detected. Currently it is used only in some cases, e.g. prenatal diagnostics.

Currently is possible to use the direct DNA diagnostics (detection of CAG triplet expansion by using PCR method).

Some families with the symptoms similar to Huntington disease (HD) did not show mutations in locus 4p16.3. They are called Huntington Disease Like and are called HDL 1, 2 and 3, whereby HDL3 has autosomal recessive inheritance.

3.2.2.1.2 Neurofibromatosis type 1

This disease affects nerve system in the skin, visceral organs and brain. It is characterized by multiple fibromas (benign tumors of the soft tissue) that are dispersed in the whole body and angiomas (benign tumors of the blood vessels) manifesting as red spots. Special traits are blots with the color of white coffee (café-au-lait). There is high probability of the malignant disease in fibromatosis patients such as neuroblastoma, meningioma and astrocytoma (neural tissue tumors). Skeleton alterations, bones deformations and scoliosis are here typical. Mental retardation and muscular disability is also frequent. Inheritance is autosomal dominant and incidence is 1 : 2 500 – 1 : 3 000 deliveries.

A responsible is tumor-suppressor gene NF1–GRP, with size of 350kb, contains 60 exons and is localized on 17th chromosome (17q11.2). Protein neurofibromin 1 is the gene product and it is composed of 2818 amino acids and its size is 327kDa. .

3.2.2.1.3 Neurofibromatosis type 2

Neurofibromatosis type 2 is limited on the auditory nerve area (commonly bilateral). Tumors of brain can be also present. The incidence of neurofibromatosis 2 is 1 in 40 000.

A responsible tumor-suppressive gene NF2–Merlin, with size of 219kb, contains 24 exons and is localized on 22nd chromosome (22q12.2). Protein neurofibromin 2 is the gene product and it is composed of 595 amino acids and its size is 696kDa.

Disease is caused by deletions in tumor suppressor gene NF2, most commonly in exons 2 and 3.

3.2.2.1.4 Achondroplasia (chondrodystrophy, dwarfism)

It belongs to dysplasias - developmental abnormalities of a certain tissue, particulary to unidysplasias – where are affected tissues derived from single embryonic germ layer. It is the failure of growth (epiphyseal) plate function of long bones, which cartilage (“chondra”) does not growth and thus is cancelled its growth in length. It results to short stature in adulthood – males about 130 and

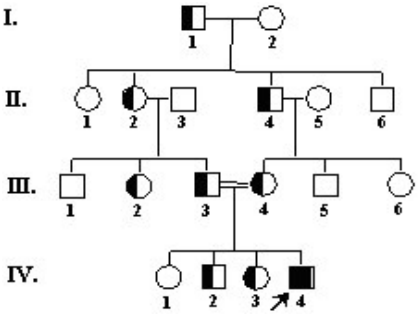
females about 120 cm. Characteristic here is the combination of markedly short limbs, larger head and normal size of trunk. Deformities in the femur neck and hip joint lead to the swinging walk. Otherwise, the development is appropriate, including adequate intelligence.

This disease has different types of inheritance, but in terms of clinical relevance is of the utmost important autosomal dominant type. Dominant homozygous individuals for achondroplasia are unable to live (s.c. tanatoform or death bringing genotype). Many phenotypes of this group are incompletely dominant, this means that heterozygous individuals have less severe symptoms. Incidence of disease is 5 – 15 : 100 000 deliveries.

Responsible gene is the gene for the fibroblast growth factor receptor 3 (FGFR 3), which has a size of 16.5 kb, contains 19 exons and is located on the 4 chromosome (4p16.3). A gene product is a receptor with tyrosine kinase activity, which is composed of 806 amino acids and has a molecular weight of 115 kDa.

3.3 Autosomal recessive inheritance of pathological traits

Autosomal recessive traits are in majority of single gene inherited cases the diseases. OMIM database 16th April 2009 keeps records of 1132 diseases with autosomal dominant type of inheritance. The major gene causing pathological trait has the locus at autosomal chromosome and affected person has recessive homozygous genotype. Disease need not to appear in every generation of family, but when once appear, is more frequent between the siblings (horizontal type of inheritance). So like parents are more closely related, so is probability of affected child birth higher. Healthy parents, if both of them are dominant homozygous or one of them is heterozygous, will have all children healthy. Parents of affected child are commonly healthy but heterozygous, so they can have recessive homozygous and affected children with a probability of 25 %. In case, that one parent is healthy (dominant homozygous) and another parent is affected (autosomal recessive homozygous), all children will be healthy, but heterozygous ones. If one parent is healthy (but heterozygous) and second parent is affected (autosomal recessive homozygous), the probability of having affected and healthy children will be 50 % : 50 %.



Pathological gene product is mostly enzyme defect - enzymopathy. Figure 12 represent family tree of pathological trait with autosomal recessive type of inheritance.

• **Figure 12.** A typical family tree of a pathological trait with autosomal recessive type of inheritance

3.3.1 Examples of diseases with autosomal recessive type of inheritance

Product of recessive mutated allele is modified protein. In recessive homozygous individual then occur mostly defect of particular enzyme (s.c. enzymopathy) and pathological status has the nature of inherited metabolic disorder. **Enzymopathy** is mostly disease (disorder), caused by the lack of a specific enzyme (or production of unworkable enzyme), with subsequent accumulation of the substrate, the body can not metabolize. They have a traditional name (eg, Tay-Sachs disease, Wilson's disease) or they are called with name of the indecomposable substrate + emia (if it accumulates in the blood – e.g. galactosemia) or + uria (if it is excreted in urine – e.g. alcaptonuria, phenylketonuria). Some of them have typical symptoms, but usually in the forefront of the clinical picture is retardation of physical and mental development of the organism caused by intoxication with stored substrate.

3.3.1.1 Alcaptonuria

It is an inherited disorder of connective tissue. The defect of the enzyme homogentisate 1,2-dioxygenase (HGD) causes the accumulation of homogentisic acid (or alkapton), normal intermediate product of tyrosine metabolism, in blood. Secondarily is alkapton excreted in urine. That causes main symtoms of disease – the dark color of urine and storing of ochronotic pigment (oxidation polymer of homogentisis acid) into various connective tissues – sclera, cartilages, ligaments, intima of larger vessels, endocardium and epidermis. It is commonly associated with prostatic and renal stones, even in

childhood. Greatest symptom is afflicting of large joints and spine (ochronotic arthropathies), which occurs between 20 - 40 year of life and gradually fully disable a patient.

Inheritance is autosomal recessive and world-wide incidence is 1 : 100 000 - 250 000. In Slovakia it is 1:19 000 deliveries, the highest in the world.

Responsible gene is HGD, which has a size of 55 kb, contains 14 exons and is located on the 3rd chromosome (3q21-q23). Gene products is homogentisate 1,2-dioxygenase, which consists of 445 amino acids and has a molecular weight of 50 kDa.

Currently are known more than 40 different mutations in this gene (CCC trinucleotide is preferentially mutated motif). In the population of Slovakia were found 10 different types of mutations (changing sense, frameshift and splicing mutations).

In the diagnosis is used PCR amplification with subsequent detection of the substitution mutation by restrictive enzyme and/or heteroduplex analysis. The results of these examinations always have to be confirmed by DNA sequencing.

3.3.1.2 Phenylketonuria

Phenylketonuria (PKU) is a relatively common metabolic disorder, characterized by „a mousy odor" of the urine, eczemas, epileptic seizures, albinism, microcephaly and, later, severe mental disability. Disease is caused by a deficiency of in liver produced enzyme phenylalanine hydroxylase. This enzyme converts phenylalanine to another amino acid, tyrosine. Impaired enzyme can not catalyze phenylalanine and the amino acid accumulates in the blood and body tissues of patient. There is no cure, but PKU is one of the few genetic diseases that can be controlled by diet. A diet low in phenylalanine and high in tyrosine can be a very effective treatment.

Inheritance is autosomal recessive and incidence of PKU is 1 : 10 00 – 12 000 (between gypsies in Slovakia it is 1: 1 000).

A responsible gene is PAH, with size of 90kb, it has 13 exons and is localized on 12th chromosome (12q22-q24). Hepatic enzyme phenylalanine hydroxylase is the gene product and it is composed of 452 amino acids and its molecular weight is 55kDa.

Prenatal diagnostics is possible by DNA analysis – PCR.

3.3.1.3 Cystic fibrosis

Cystic fibrosis is characterized by generalized dysfunction exocrine glands. This disease is caused by failure of pumping of chloride ions in slime (mucus) produced by exocrine glands. Consequence is that these glands in cystic fibrosis produce very viscous mucus containing proteins, leading to plugging of gland opening. Viscose mucus also narrows bronchi, what embarrass ventilation of lungs and besides that, support development of inflammatory (both viral and bacterial) diseases, e.g. bronchitis and pneumonia.

Cystic fibrosis is most common hereditary defect of Caucasoid race. Inheritance is autosomal recessive and incidence is 1:2 500 deliveries. In Slovakia it is 1 : 1 800).

A responsible gene is CFTR, with size of 250 kb, contains 27 exons and is localized on 7th chromosome (7q31.2). Protein CTRF (Cystic Fibrosis Transmembrane Regulator) is the gene product and it is composed of 1480 amino acids and its molecular weight is 168kDa.

Mutation stops synthesis of CTRF protein what leads to disorder in chloride anions transport and water in cells of glands and production of viscous mucus. In CFTR gene were identified more than 1300 mutations and most common one is F508del (lack of triplet 508). In Slovakia F508del appears in 59.4 % of cystic fibrosis patients, but frequent are also mutations like G542X (5.6 %), R553X (3.5 %) or N1303K (3.0 %).

Prenatal diagnosis is done by direct DNA diagnostics – PCR – amplified and sequenced is area with most common mutations.

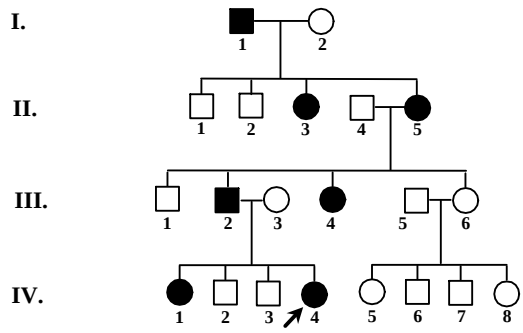
4. Normal and pathological single-gene X-linked traits

Gonosomal inheritance is linked to sex chromosomes X and Y. There are several specific differences in comparison to autosomal inheritance. Male has single X chromosome, (is so-called hemizygous) and therefore for each locus in the X chromosome male has just one allele.

X chromosome is the sixth biggest human chromosome and it has more than 800 genes. Y chromosome belongs among the smallest human chromosomes. It contains more than 40 genes, but just one of them is known to play a role in determining male sex specification (TDF-testes determining factor). Its mutation evokes gonadal dysgenesis. Therefore Y-linked kind of inheritance (holandric heredity) has no practical importance for clinical genetics.

4.1 Pathological traits with X-linked dominant type of inheritance

For X-linked dominant type of inheritance it is common that the locus of such pathological gene is on X chromosome. There are more female than male patients and transmission from father to son is not possible. If the mother is affected and heterozygote), there is a 50 % probability for both - daughters and sons to gain pathological allele. If the mother is dominant homozygous, she shall transmit disease (mutation) to all her children. The male with a disease has no son afflicted by the disease (if the mother is healthy) but all his daughters shall be afflicted. Male patients go through more complicated or even a lethal disease development. Heterozygous women (X_DX) have also normal allele, which reduces the effect of the pathological allele. Clinical-genetic units (diseases) with X–linked dominant type of inheritance are rare. In figure 13 is an image of a family tree with pathological trait of X – linked dominant type of inheritance.



• **Figure 13.** Family-tree model of a pathological trait by X-linked dominant heredity type

4.1.1 An example with the gonosomal dominant type of inheritance

4.1.1.1 Hypophosphathemic rachitis, resistant to vitamin D

This disease is caused by phosphorus deficiency and it is the reason of bone mineralization, failure bone growth and development failure and malfunction. Genetic mutation causes a decrease of re-absorption of phosphorus in kidney proximal tubulus to 42 – 72 % (normal is 85 – 95 %), which causes a low level of phosphorus in serum. Activity of alkalic phosphatases is multiple increased. Compared to rachitis as a result of avitaminosis D, hypocalcaemia is here missing (low level of calcium in the blood). In this type of rachitis the physiological amount of vitamin D has no therapeutic effect. Frequency of occurrence is 1 – 10 : 1 000 000 births. Women with this disease are twice as frequent as men.

Responsible gene is PHEX (phosphate regulating endopeptidase homolog), with the size of 220 kb, containing 22 exons and it is localized on short arm of X chromosome (Xp22.1). Protein PHEX is the gene product and it is composed of 749 amino acids and has molecular weight of 86 kDa.

The reason for this disease are different mutations of PHEX gene, e.g. various point mutations with substitution character, frame shift mutations, deletions of one or more exons, mutations of the splicing sites between introns and exons.

4.1.1.2 Rett syndrome

It is a complex genetic disorder of neural development, found only in female patients. There are known cases of Rett syndrome found in boys, too, but it is usually a lethal form of syndrome, causing miscarriage, stillbirth or an early death of infant. Initial development of a child (6 to 18 months of age) usually seems to be normal. Then a period of temporary stagnation or regression begins when the child loses speech ability and wilful hands use. The main feature of Rett syndrome is dyspraxis, i.e. disability of body control or motoric movements. There are stereotypic repetitive movements of hands, clapping and characteristic picture of washing hands, micro-cephalic and walking defection. There are additional epileptic hits, increased muscular tonus, deformation of rachis (back-bone) and joints, and muscular atrophy.

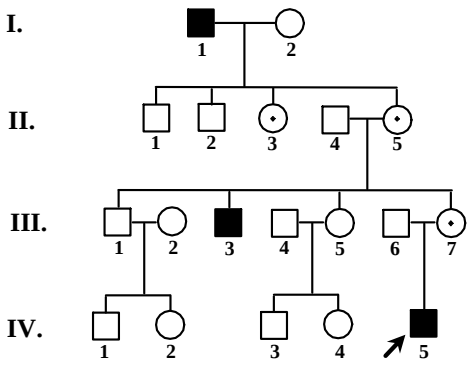
Inheritance is X-linked dominant, with an incomplete penetrancy and variable expressivity. Occurrence ratio is of 1:10 000 – 1:23 000 of females births.

The responsible gene is MECP2 (methyl CpG binding protein 2), with the size of 76kb, containing 4 exons and is localized on X chromosome (Xq28). The gene product is on methylated CpG binding protein composed of 486 amino acids with molecular weight of 52.4 kDa. This protein is important for normal development of the brain.

4.2 Pathological traits with X-linked recessive type of inheritance

In this type of inheritance it is known that the pathological gene is located on the X-chromosome. Among affected, male sex is predominated, however, the transmission from father to son is not possible. In case of a healthy father and the pathological allele being transmitted by a healthy mother (carrier), the possibility of defection of their sons is 50 %. Their daughters are healthy, but there is a 50 % possibility that they shall be carriers. Affected father transmit the pathological allele to his daughters and they become carriers, if the allele coming from their mother is "healthy", i.e. normal. Defection of a female can occur just in case if they have received a mutated allele from each parent. Different level of clinical manifestation according to Lyon´s theory can occur in the carriers (if the carrier has more than 50 % cells with the inactivated chromosome with normal allele).

Figure 14 shows the family tree with X- linked recessive inheritance of pathological trait.



• **Figure 14.** Family tree model of a pathological trait with X-linked recessive inheritance

4.2.1 Examples of X-linked recessive type of inheritance

4.2.1.1 Haemophilia A

It is the best known, the most serious and the most common genetic defection of blood clotting (coagulation). It occurs in 80 % cases of haemophilias. Symptoms include abnormal bleeding even from minor wounds. It is caused by an absence, decrease or loss of coagulation factor VIII activity. It leads to its defection in the function as a co-factor for X-factor activation, which is important in the process of blood clotting (change of prothrombin to thrombin and fibrinogen to fibrin). Fibrin clot, which is very weak in people with haemophilia, is not able to form a stable fibrin clot in the bleeding wound. Inheritance of haemophilia A is X-linked recessive. It is more common in boys with the incidence frequency of 1:5 000 – 1:10 000 of male births.

HEMA (hemA gene - F8) is the responsible gene, with the size of 186 kb; it contains 26 exons and it is located in X chromosome (Xp28). Genetic product is factor VIII; which consists of 2332 amino acids and has a molecular weight of 330 kDa.

The causes of disease are different kinds of mutations – inversion, point and frame shift mutations, deletions, duplications and insertions. Diagnosis is based on their identification.

4.2.1.2 Haemophilia B

It is a less common defection of coagulation. The cause is deficiency or mutation of a gene for IX factor.

The responsible gene is HEMB (hemB gene - F9), with the size of 35kb, which contains 8 exons and it is localized on X chromosome (Xq27.1-q27.2). Genetic product is the IX factor, which consists of 418 amino acids and it has the molecular weight of 56kDa.

4.2.1.3 Duchenne muscular dystrophy

It is a severe and progressive muscular disease that affects males and is lethal. Patients have just a small chance to live up to the age of twenty. Typical clinical symptoms include easy fatigability, weakness, muscular atrophy and wasted facial muscles. Pulmonary complications are also frequent. Inheritance is X-liked recessive. Disease has the incidence of 1: 3 500 births.

The responsible gene is DMD, with the size of 2.4Mb, containing 79 exons and it is localized on the X chromosome (Xp21.2). Dystrophin protein is the gene product, it is composed of 3685 amino acids and its molecular weight is 427kDa.

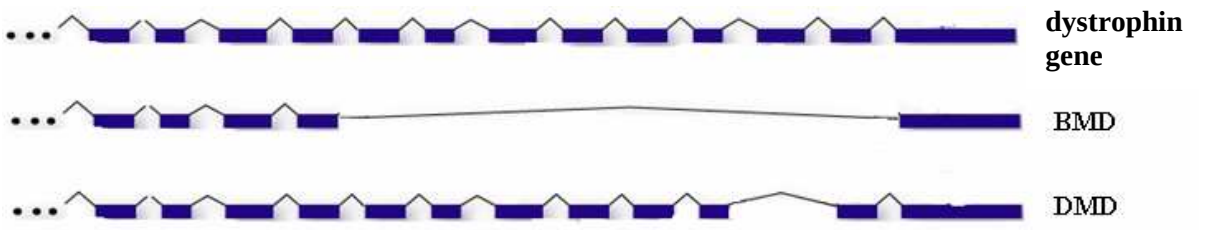
DMD gene is huge one; it forms 0.1 % of the whole human genome. Its size is caused by extremely long introns. 32 % of complete length of introns is formed by repetitive sequences. Complete mRNA is 14kb long. Transcription of this gene is controlled by 7 promoters, which regulate development and tissue specific expression.

There are 65 known allelic variants caused mostly by deletions (45 %), point mutation (35 %), duplications and insertions (6 %).

4.2.1.4 Becker muscular dystrophy

It is a less severe and serious form of dystrophy with later onset and a slower progress of muscular weakness. It may be connected with mental retardation. Inheritance is of X-linked recessive and incidence is 1: 35 000 of male births.

The responsible gene is DMD, the same gene as for Duchenne muscular dystrophy (Fig. 15). Both diseases are caused by different allele mutations of that same gene (intra-allelic heterogenity).



• **Figure 15.** Huge deletions within Becker's muscular dystrophy (BMD) do not change reading frame and the consequences for patients are less serious than in the case of small deletions within Duchene muscular dystrophy (DMD), which change the reading frame

4.2.1.5 Daltonism

Majority of daltonistic persons cannot distinguish red and/or green colours. Literary sources say that there are as many as 8 % colour-blind males in the Western Europe and 75 % of them are green – blind and 25 % are red-blind.

The responsible gene is IKBKG (inhibitor of kappa light polypeptide gene), with the size of 23kb, containing 10 exons and it is localized on the X chromosome (Xq28). Gene product is a protein, which consists of 412 amino acids and has a molecular weight of 48 kDa.

4.2.1.6 Adrenoleucodystrophy

It belongs to the most common inherited disorders of peroxisomes. The disease is mostly manifested in male population and its incidence is 1: 21 000 (USA) – 1: 15 000 (France). In spite of X-linked recessive course of inheritance, there are less severe forms of the disease in female population. The main pathobiochemical feature of this disorder is the accumulation of carboxylic acids with very long chains in tissues of central nervous system, peripheral nerves, adrenal glands and in body fluids. Histological and biochemical examination of adrenal gland tissue is characteristic for this disease. The disease leads to a progressive degradation of behaviour, recognition and neurological functions. First symptoms are emotional unstableness, speech difficulty, dyspraxis, bad orientation in space and visual function decline. Clinical symptoms in school age are manifested by degradation of achievement, behavioural changes and mind deterioration. Signs of dementia and epileptic convulsions can occur in advanced stages of disease. 90 % of patients suffer from adrenal gland insufficiency.

The responsible gene is ABCD1 (ATP-binding cassette, sub-family D), with the size of 21kb, containing 10 exons and it is localized on X-chromosome (Xq28). Gene product is the membrane protein of peroxisomes, it is composed of 745 amino acids and its molecular weight is 83kDa.

RFLP and sequencing are used in diagnostics. Up to now there are as many as 866 of registered mutations causing adrenoleucodystrophy, where 453 mutations single nucleotide ones, 61 % are mutations that change the sence, 23 % are deletions and insertions.

4.2.1.7 Fabry Disease

It is a hereditary metabolic disorder, which is characterised by a failure of glycosphingolipids degradation. These are then accumulated in different tissues in the form of thick deposits. This leads to defection of the structure and function of more organs, mainly the cardiovascular system and kidney. It belongs amongst rare diseases. Its incidence is 1: 40 000 births of males.

The responsible gene is GLA (galactosidase alpha), with the size of 10.15 kb, containing 7 exons and it is localized on X chromosome (Xq22.11). Alpha-galactosidase is the gene product and it is composed of 429 amino acids and its molecular weight is 48.8 kDa.

PCR and sequencing are used in diagnostics. 370 mutations of this gene have been identified so far.

5. Multifactorial inheritance

Multifactorial (complex) trait is the result of a combination of genetic and non-genetic factors. Genetic factors (precondition) are referred to as genetic (hereditary) predisposition. Non-genetic factors include ones from external environment (components of food, age, sex, geographical conditions, stressful socio-economic situation, daily routine, etc.), act in a randomly fluctuating quantity and intensity. As these traits have complex etiology, the rules of their occurrence are called multifactorial inheritance.

In this context, it is necessary to clarify the differences between polygene inheritance and genetic predisposition. **Polygene inheritance** means that one qualitative trait is conditioned by more than one major gene. For example, in the formation of iris of eye color are involves 2 genes and skin color three major genes. As many dominant alleles they are there, as darker is the color.

Hereditary predisposition is provided by a large number of minor genes, which (in terms of the pathological trait formation) have additive effect. So-called familial occurrence of pathological trait (it occurs more frequently in certain families than in the general population) usually warns about its hereditary predisposition. Regarding the minor genes, a single gene does not have a significant impact on formation of the trait, its mutation does not change the form of character and alleles of these genes are expressed in various combinations and time. The complexity of this arrangement provides a large impact for external (environmental) factors.

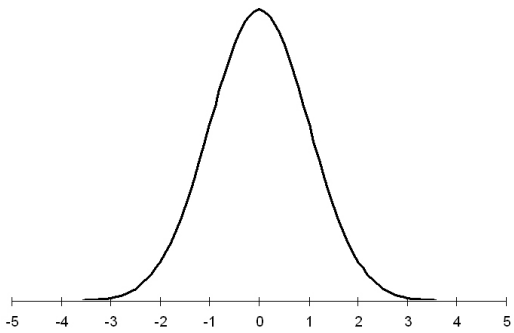
In particular (individual) cases of pathological statuses (e.g. developmental defect or disease), it can be often very difficult to decide whether in its etiology plays real rule one of the environmental factors, or a predisposition of an individual – as an increased sensitivity to this epigenetical factor. This not only causes serious problems in explaining of the cause of pathological form of trait in a particular person, and determining prognosis of the disease evolution, but also in estimating of the likelihood of its recurrence risk in offspring.

In prognostics and prenatal diagnosis of these traits it is essential to search and study the so-called candidate genes. They are (by their phenotypic expression) major genes that are usually discovered indirectly, for example, in study of s.c. micro-deletions syndromes (MDS). If symptoms typical for a certain multifactorially conditioned pathological trait are also included into a clinical picture of the MDS, a search for the candidate gene starts in the deleted segment. Mutation of the gene or a specific relationship of its product (coded protein) to a particular molecule can induce a situation for creating a similar pathological phenotype. If it is confirmed that the pathological phenotype may be caused also by that gene, possibilities for its diagnosis, prevention and therapy extremely expand.

5.1 Normal variability

The majority of normal quantitative traits (expressed in metric units, e.g. body height) have a continuous (normal) distribution (variability) in the population. Individual forms of traits follow continually in a row (without interruption) and the differences between their forms are provided by intra-species variability. Bell (binary, Gaussian, Pearson´) curve (Fig. 16) shows their distribution best way.

The occurrence of a quantitative trait is characterized by arithmetic average (or median) and curve steepness, which describes the dispersal and variability of the trait. The interval between the two standard deviations above and below the average contains more than 94 % of all observations. Measured trait´ values, in population, which exceed out of two standard deviations in both directions (± 2 SD), are regarded as extremes. Trait values that are beyond extreme (e.g. dwarfism and gigantism) are considered as pathological ones and have different etiologies. Dwarfish growth is usually single-gene inherited disorder and gigantism is mostly caused by overproduction of growth hormone in the pituitary gland.



• **Figure 16.** Gaussian curve

To the traits with unimodal population distribution (with one peak of the curve) and multifactorial inheritance belong not only body height and weight, but also total number of papillary lines (TRC) and intelligence as measured by intelligence quotient (IQ).

5.2 Tendency to the average values

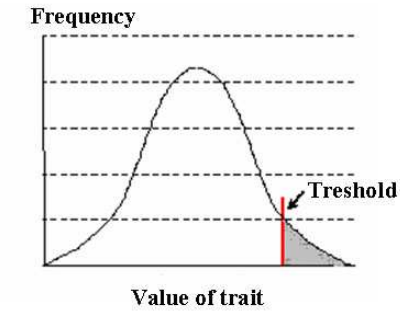
In the familial studies of metric (quantitative – expressed in metric units, for example, height in centimeters) traits is often observed tendency to the average values, that can be also seen in common observations. For instance, parents who reach extreme levels of trait (in terms of positioning the value of their trait in the normal distribution curve) have children who reach values closer to the population average.

5.3 Heritability

The expression "heritability" is a result of efforts to distinguish the proportion of predisposition (genes) and environment factors in given multifactorial trait formation. **Heritability** is defined as the proportion of additive genetic variance to total phenotypic variance of a trait. Heritability is a measure of fact, if the proportion of genetic factors (predisposition) for the emergence of a phenotype (or predisposition to the phenotype) is large or small. So like higher is heritability, so higher is proportion of genetic factors. Information are obtained by twin survey (gemelology method).

5.4 Traits with threshold effect

Qualitative multifactorial traits are characterized by description or comparison with standards. They have different distribution in populations (Fig. 17). Curve looks similar, but the distribution is discontinuous. This means that the two forms of trait are recognized – normal and pathological ones. Each of them has its own variability. The form of the trait that is considered as a pathological one (a disease or developmental disorder) is called the trait´ **threshold**. Here is also a distinct distribution – more severe pathological features occur less frequently.



• **Figure 17.** Threshold scheme

5.5 Inborn defects with multifactorial way of inheritance

Inborn (congenital) defects (ID) and diseases with multifactorial way of inheritance in humans can be divided according to the frequency of occurrence into:
• Rare defects and rare diseases (population frequency <1 %), for example cleft lip and cleft palate, neural tube defects, heart ID, defective development of the hip joints;
• Defects and diseases with median frequency of occurrence in the population (<5 %), for example majority of severe mental illnesses such as schizophrenia, biopolar disease, oligophrenia*;*
• Diseases with high frequency – e.g. high blood pressure, diabetes (insulin-independent type), ulcerative digestive tract disease, atopy, etc..

In practice of genetic consultation of families with multifactorial defects (diseases) theoretically can be in calculation of probability of trait´ reappearance used Edwards's formula or s.c. empirical risks charts. Empirical risks of the first or repeated occurrence of pathological trait are based on the processing of many family trees of families with given defect or disease. In general, it is a rule that with increase of affected persons between close relatives, increase also the risk of disability for other members of family. On the other hand, so like more distant relatives are affected, the risk of disability is lower. If the disorder or disease affects both sexes in varying degree, than the disability of less affected sex increase the risk for relatives (children). For instance underdeveloped hip joints occur 5 times more frequently in girls. If a boy with such disorder is born, the presumption of disability risk is higher in the family.

Edwards's formula expresses the presumption of disability risk for relatives of the affected individual.

Disability risk, r = √ relative frequency of disease in the population

The formula is valid to 1st degree relatives (parents, children, siblings). Assuming that are affected more 1st degree relatives of the person for whom the prognosis is calculated, the result of the formula is multiplied by their number. For instance – father and son suffer from the same ID. What is the risk that another child is born with the same ID, when the frequency of this disorder in the population is 0.09 %?

$$r = \sqrt{0.0009} \times 2 = 0.03 \times 2 = 0.06 = 6\ \%$$

In multifactorially conditioned inborn defects and illnesses, their demonstration involves environmental factors in addition to genetic ones. For instance in diseases with threshold effect - adverse environmental factors may push the threshold to the left – the trait is demonstrated at lower predisposition values. Conversely, by reducing the negative impact of external factors, there is an increase of the threshold, and hence the probability of disease is lower. Diseases of cardio-vascular system are generally known examples of threshold "drift". If the predisposition is high (and it is not a single-gene conditioned pathology), healthy lifestyle of the family members can help them to delay onset or mitigate the symptoms of the disease.

5.5.1 Cleft lip and cleft palate

Cleft lip (CL) and cleft palate (CP) are relatively common IDs, which make about 5 – 6 % of all congenital defects. Cleft lip and cleft palate do not form a homogeneous genetic unit. They can be divided into two genetically independent groups (Fig. 18 - 20).

The first group consists of CL with or without CP. The frequency of their occurrence is 1 : 1 000 live-born children. Lateral CL is formed around the 35th day of intrauterine development after a partial or faulty connection of the maxillar and medial nasal processi.

Left-sided cleft is twice as more frequent than the right one; and more often found in male (70:30). Medium cleft of upper lip is very rare; there is either a defective development of both medium nasal processi or a disorder in the development of the upper lip philtrum (0.2 – 0.7 % of all cases of cleft lip disorders). Medial CL is sometimes associated with ID of face and brain – e.g. holoprosencephaly in mutation of gene for SHH protein.

The second group of clefts includes isolated CP with a frequency of 1 : 2 500 live newborns. It is twice more frequent in female than in male. Secondary palate is formed by merging up primary palate with lateral palateal plates, which grow out of maxillary processi in horizontal direction, during the 8th week and merge from front to dorsal direction in the 10th week. In female embryos they merge about a week later, so cleft palate is more frequent in women.

Congenital (inborn) cleft lip and palate is a representative example of fact, that the resulting pathological phenotype, may have several possible causes of appearance. In other words – the number of ID types is smaller than the sum of etiological factors that can cause them. Cleft lip and palate (together or separately) may be:

• caused by mutations in (at least ten) major genes (though some of them are still candidate genes), located on chromosomes 1, 2, 3, 4, 6, 11 and 13;
• a part of the phenotype in more than 200 syndromes – e.g. Apert, Marfan and Larsen;
• a part of pathological phenotypes of syndromes associated with chromosomal aberrations, for example trisomy of 13, 18 or 21, but also in the syndrome 49, XXXXY;
• one of the consequences of specific action of harmful factors in the developing embryo and fetus, such as teratogens (e.g. isotretionin and phenytoin) or mechanical oppression (strangulation) violence (e.g. amniotic bands);
• a consequence of the interaction between genetic predisposition and external factors (multifactorial inheritance).

Recent studies based on DNA analysis revealed predisposition to the cleft defects formation depending on the presence of certain alleles in locus for TGF-alpha. In formation of orofacial clefts can be involved the following groups of genes:

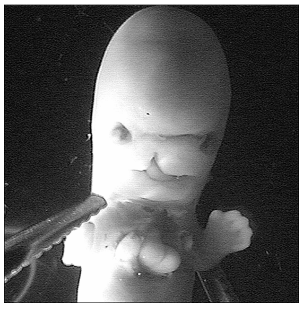
• genes that express in some parts of the embryo or in a certain period of palatine arcs development, for example genes for transforming growth factor alpha and beta (TGF-α, TGF-β2, TGF-β3);
• genes, which have their biological activity indirectly associated with development of orofacial structures (e.g. receptor for retinoic acid, receptor for methylenetetrahydrofolate-reductase, and folic acid receptor);
• loci or genes identified in experiments on animals as homeiotic genes MSX-1 and MSX-2;
• genes involved in interactions with the metabolism of xenobiotics, for example in cytochrome system P-450.

Appointing of clefts and other IDs ethiology is important for determining of the recurrence risk (in the family).

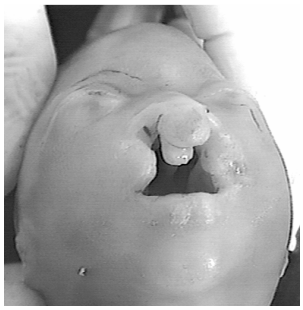
Multifactorially conditioned group forms a substantial part of all CL and CP. When estimating the risk of recurrence, it must also be taken into account the health status of parents, as well as the number of already-born disabled and healthy children in a particular family. With increasing numbers of disabled children in the family, the likelihood of disability increases; on the contrary, it decreases with increasing numbers of healthy children (table 5).

The risk level is also influenced by a degree of defect and the patient's gender. From multifactorial inheritance model with threshold effect it is apparent, that with increase of patient defect severity, the predisposition in the family is greater, which increases the likelihood of such pathology (defect) reappearance in next offspring. Patient's gender has a similar effect on risk level.

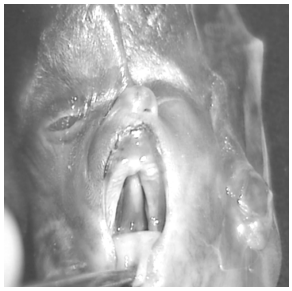
The empirical risk values in different populations are significantly different. Therefore, the genetic consultation activities should be always based on the data from particular population which the patient comes from, and the results of other populations should only be used with caution.



• **Figure 18.** Unilateral cleft lip (l.dx.)



• **Figure 19.** Bilateral cleft lip and cleft palate



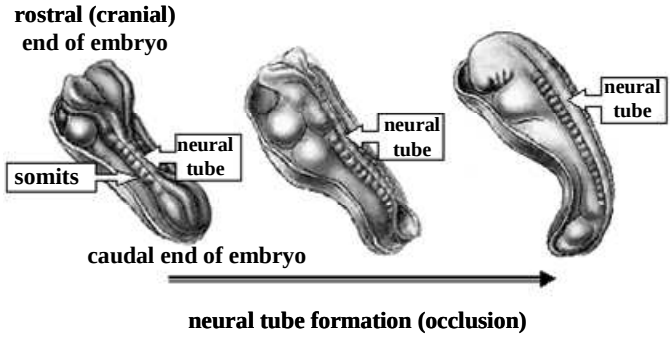
• **Figure 20.** Cleft of secondary palate

• **Table 5.** The risk of cleft lip and cleft palate (CL / CP) and isolated cleft lip (CP) for next child in %

Affected parents	Affected siblings	Affected relatives	CL/CP	CP
0	0	0	0.1	0.04
0	1	0	4	2
0	1	1	4	7
0	2	0	9	–
1	0	0	4	6
1	1	0	17	15

5.5.2 Neural tube defect

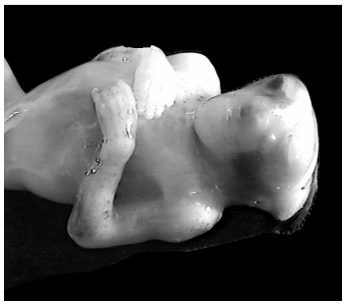
In normal conditions, neural tube closes completely around the 28th day of embryogenesis. First it closes approximately in the middle of embryonic body; later its closing continues in caudal and rostral (“cranial”) direction. Frontal neurophorus shall be closed during the 25th to 26th day of development and about two days later the dorsal neurophorus closes (Fig. 21), as well.



• **Figure 21.** Scheme of the neural tube occlusion

If the neural tube does not occlude, a cleft – neural tube defect (NTD) appear. NTD includes, in order of severity, anencephaly, encephalocele and spina bifida. They often occur in affected families simultaneously and are assumed to have a common origin. Cause of small proportion of NTD (less than 10 %) can have evident reason:

• amniotic bands;
• single-gene inheritance (e.g. Meckel syndrome - autosomal recessive inheritance, locus on 17q22-q23)
• syndromes conditioned by chromosome aberrations (in particular trisomy 13, 18, triploidy),
• the effect of certain teratogens (e.g. aminopterin, thalidomide, valproic acid).

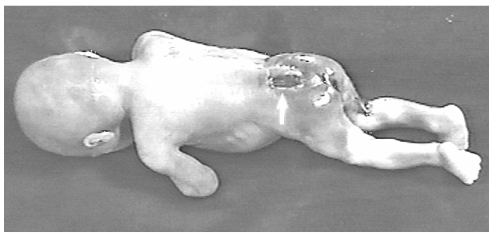


Anencephaly is a congenital disorder of development, in which did not develop majority of the brain and the calva of the skull is missing (Fig. 22). Defect is a consequence of neural tube unocclusion at its rostral end. Neural tube cavity remains open at the head. Affected child can only live for several hours after the birth at most – anencephaly incidence at misscarriages is 5 times higher than in newborns. It often occurs in association with spina bifida (up to 17 %), cleft lip and cleft palate (in 2 %), but also with other malformations. About 2/3 of those affected are girls.

• **Figure 22.** Anencephalus

Encephalocele (cranial - occipital meningocele) is a malformation in which the skin is intact, and the brain and its envelopes are "flowing out" through the defect in the cranial (occipital) bone. The incidence is 0.3 – 0.6 : 1 000 live newborns. Multifactorial type of inheritance may be one of the reasons for this disorder, but it can be a part of single-gene syndromes (Walker-Warburg syndrome, autosomal recessive inheritance, locus is at 9q31), as well as a result of teratogens action. This disorder occurs in combination with other disorders of development - hydrocephalus, spina bifida, microcephaly, etc.

Spina bifida (rhachischisis, meningocele, myelomeningocele) is a developmental defect in which the spinous processes and the vertebrae arcs do not develop, either at one or at more of the adjacent vertebrae. Bone cover of the spinal cord therefore remains open in the middle plane (Fig. 23). Defect is most commonly located in the lower thoracic, lumbal or sacral regions. The frequency of spina bifida occurrence in the human population is variable. Geographical (from 0.3 in Japan to 4.1 in South Wales per 1 000 newborns) and ethnical differences (it is more frequent in Caucasoid race) are here significant. It can also be part of certain single-gene inherited syndromes; in addition to multifactorial inheritance (e.g. Meckel syndrome); as well as chromosomal aberrations (trisomy 13 and 18, deletion in 22q11 and triploidy). Spina bifida could be also associated with other central nervous system (CNS) and lower limbs anomalies.



• **Figure 23.** Spina bifida

5.5.3 Pyloric stenosis

Pyloric stenosis is a pathological state caused by hypertrophy of sphincter muscle, which restricts opening of the pylorus and emptying of the stomach. Obstruction is treated surgically. It manifests itself in early childhood from approximately the 3^{rd} – 4^{th} week of newborn life. To explain the high incidence of pylorus stenosis in some families, multifactorial inheritance model with threshold effect was developed (table 6).

• **Table 6.** The incidence of pyloric stenosis in offspring of a patient compared to its incidence in normal population

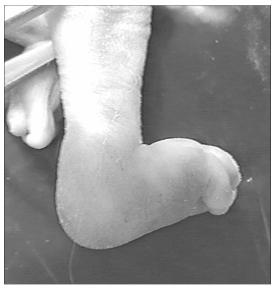
Relatives	Risk	Population risk multiple
Sons of affected male	1:18	x 11
Daughters of affected male	1:42	x 24
Sons of affected female	1:5	x 40
Daughters of affected female	1:14	x 70

Pyloric stenosis is about 5-times more frequent among boys than in girls (boys 5:1 000, girls 1:1 000 the same gender newborns).

Offspring of affected women have a higher average predisposition to pyloric stenosis than the offspring of affected male. The threshold for the trait appearance is lower for boys than for girls, and therefore, irrespective of whether the pathological state is inherited from the father or mother, sons have generally higher probability to develop this disease, than daughters. Sons of affected mothers are at the highest risk (approximately 20 %).

5.5.4 Pes equinovarus

Pes equinovarus is a flexion of foot (s.c. clubfoot) or hand (s.c. clubhand) bones connection in the joint capsule (Fig. 24). The incidence is 1.2 : 1 000 of live newborns. It is the most frequently occurring deformity of foot. It may be unilateral or bilateral. The ratio boys : girls is 2 : 1. Pes equinovarus may be caused by genetic and epigenetic factors, as well. Here may be a genetic predisposition in an inferior ligament apparatus of a joint. Other IDs, such as skeletal dysplasia (failure of proper development or growth), limb reducing defects or CNS anomalies (spina bifida) are often associated with pes equinovarus. Factors of external environment, as well as lack of space in the uterus (e.g. oligohydramnion - reduced amount of amniotic fluid, amnitic bands syndrome and uterine tumors), can also be the cause of this developmental anomaly. Pes equinovarus is also part of several genetically determined syndromes (e.g. Ecsobar sy., Germany-Gruber sy., trisomies of chromosomes 13 and 18).



• **Figure 24.** Pes equinovarus

5.5.5 Congenital heart defects

Congenital heart defects belong to most common IDs in children. These can include short cut faults, when oxygenated blood mix with deoxinated one, for example when stay open connections between pulmonary artery and aorta, as well as short cuts on the level of septal openings (in atria and/or ventricles). There are also faults, with constriction or extension of head vessels or heart defects with altered myocard muscle structure.

Tetralogy of Fallot is characterized by four defects - truncus pulmonale stenosis (narrowing of pulmonary artery), defects of intraventricular septum, transposition of aorta and hypertrophy of right ventricle. It can also be part (3 – 4 % of cases) of chromosome 21 trisomy (Down syndrome). Incidence varies between 2.5 to 3.5 : 10 000 live born; and represents 4 – 7.5 % of all heart defects.

It is manifested by clubbed fingers, or bluish skin, which suggests poor blood oxidization. Children have shallow breath, and are excessively tired. Hypertension and hypertrophy of right

ventricle is also present, which can lead to severe disturbances in heart rhythm. Due to the insufficient oxygenation of the brain; child's development is subsequently damaged.

The only solution is surgery, without it 10 % of affected live up to the age of 20, and only 3 % of the disabled live up to 40 years of age. The treatment is most effective, if the operation is carried out before the child age of 12.

Ductus arteriosus is the connection between pulmonary artery and descending aorta, by which blood of the fetus bypasses pulmonary circulation. Under normal circumstances it closes shortly after birth. Open (appertus) ductus arteriosus is one of the most common major vascular anomalies (incidence 1 : 3 000 live births) and may occur alone or in conjunction with other heart defects. Permanently open ductus arteriosus causes that the blood from aorta flow into pulmonary artery Consequence is increase of the pressure in the lungs, followed with hypertrophy of right heart ventricle muscles. Surgery treatment is necessary.

Defect of atrial septum – in 25 to 30 % of people remains a small opening in the foramen ovale (hole between the right and left atrium present in fetal period, closes in the first minutes after birth - under normal circumstances), but it does not cause a function failure. Rarely this opening is much bigger and then oxygenated blood from the left atrium flows through it into the right atrium. This return of blood causes an overload of the right side of the heart. This ID often occurs with other severe congenital birth defects, such as pulmonary valve stenosis or transposition of the aorta. Sometimes the whole atrium septum does not develop, at all.

Defect of ventricular septum occurs less frequently than atrial septal defects. The openings in the septa are usually oval or round, 1 – 2 cm wide in average. As the consequence of this defect, the blood flows from left to right ventricle, which causes dilatation (enlargement) of right ventricle. Absence of the entire interventricular septum is very rare.

6. Mutagenesis and reparation mechanisms

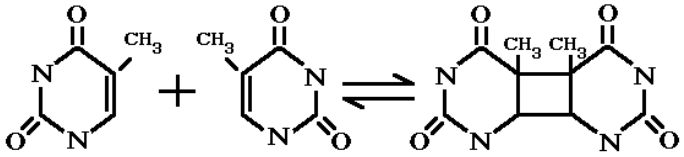
6.1 Mutagenesis

Mutation is a sudden change of quality and/or quantity, as well as configuration of genetic information. A consequence depends on position where it appears, its type and effect, if it will be replicated and if it will be expressed into phenotype. Terminology committee for genetics defines the term "mutation" as "a change in nucleotide sequence of a genome, which involves substitution, deletion or insertion".

From the formal point of view (as an agreement of geneticists), inter-individual differences of genome, independently from their phenotypic manifestation, are called either **polymorphism**s of DNA (if there are present in more than 1 % of population) or **mutation**s (if they are in less than 1 % of population members).

The term "mutant" (carrier of mutation) can have different meanings. Classically – in experiments on model organisms (e.g. Drosophila melanogaster), mutant is an individual, in which the mutation is manifested in its phenotype. In human genetics, the term "mutant individual" can be understood as an individual who has a certain allele for particular locus. Mutated allele changes phenotype and depends on, if it is dominant or recessive. The examples are described in the chapter dedicated to single-gene inheritance.

Mutagens are physical, chemical or biological factors, which can be pertinent to formation of a mutation. To **physical** ones belong different kinds of irradiation. Their effects depend on mechanism of activation, intensity, as well as the dose and duration of exposure. The effects of X-rays or radioactivity cause ionisation of bases or formation of hydroxyl radicals from water, which can react with DNA bases and change their structures. Gamma irradiation can evoke splitting of N–glycoside and phosphodiester bonds in DNA strands and, in cases of great doses, even disruptions and sequential reconstructions of chromosomes can occur. Ultraviolet light induces in DNA strands formation of pyrimidine – mainly thymine – dimmers, which can stem from cyclization of two nearby thymines (Fig. 25). Thymine dimmer inhibits the replication and transcription.



• **Figure 25.** Thymine dimmer

Chemical mutagens incorporate a large group of substances. There are various mechanisms of their influence, from alternative bases to DNA degradation. According to the mechanism of their action, three kinds of chemical mutagens are distinguished:

- substances, that directly deteriorate DNA – chemical substances, which can modify DNA structure. For instance cytosine deamination (removing of NH_2 group) results in its change to uracil pairing with adenine; instead of pairing with guanine what can change sense (coded amino acid). Or they can be directly integrated into DNA and cause a frameshift (shift of reading frame and consequent mistake in coded amino acids) in genetic code (e.g. nitrous acid, hydroxylamine, alkylators and acridine dyes);
- nitrogen bases analogues – are substances, which have a similar chemical structure as nitrogen bases found in the primary DNA structure. If they are present during DNA replication, they can be integrated into polynucleotic chain, but later they are not recognized in transcription and cause frameshift (e.g. 5-bromuracil, 2-aminopurine);
- substances, which decrease the activity of reparation enzymes and so (secondarily) increase amount of mutations (e.g. caffeine in bacteria).

Various mobile genetic elements (e.g. proviruses of retroviruses) can be considered as **biological** mutagens, if they are accidentally integrated into the coding part of a gene and then cause insertion mutations.

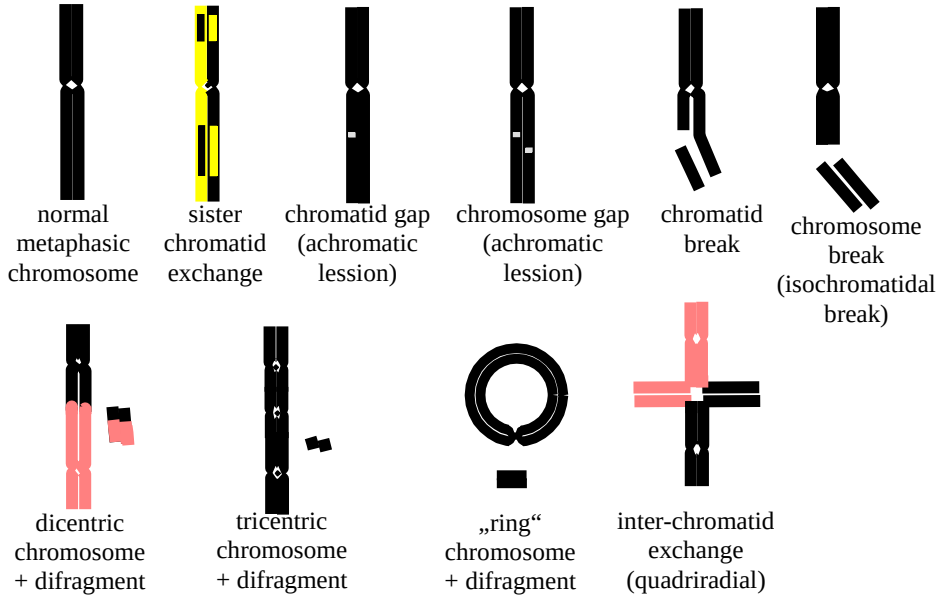
For the evaluation of mutation it is important to consider the following factors:

- Characteristic features of factors, which invoke mutations;

- Mechanisms, by which mutations are done. In case of **spontaneous mutations** formation are not known causal factors and mechanisms of their origin. The probability that each human gene will mutate within one generation is approximately from 1×10^{-5} to 1×10^{-10}. So like are new alleles identified, their population frequency – their appearance in genetic pool, are also calculated. **Dominant mutations**, notably those of them which can seriously affect individuals, generally cannot enter into the genetic pool (due to selection). If they reappear (mostly) as new mutations (s.c. mutations *de novo*). **Recessive mutations** are transmitted through heterozygous parents and appearance of affected recessive homozygous individuals is very rare (5 - 10 per 10.000 newborns).

Induced mutations are caused by known physical and chemical or biological mutagens, mostly in the experiment. In testing of potential mutagens, it is important to prove that applied factors in the survey did cause statistically significant increase of the spontaneous mutations frequency at the model organism (system);

- Factors, that influence the activity of mutagens, like the way of they enter in an organism, their detoxication and metabolism;
- The capacity and conditions for the activity of reparation mechanisms, which can repair defects (mutations);
- The location (place) of mutagen intervention and the type of mutation it causes. If **the mutation occurs in non-coding sequences** and the integrity of DNA molecules is not affected, the result need not be manifested in phenotype. It is just observed the change of DNA polymorphism length. If the **regulatory sequences** of the gene is affected, three situations can occur, in regard to where and what happen. First consequence could be "switch-off" – gene is disabled from transcription and no protein (coded by affected gene) will be synthesized. Second possibility is down-regulation of the gene followed by insufficient synthesis of coded protein. Third variant of regulatory sequence damage is the abnormal increase of transcription intensity – its over-expression. **Mutations in coding sequences** of gene (exons) manifest themselves, if they are out of "gene code degeneration" range. We recognize two kinds of them – point and frameshift mutations. **Point mutation** is the change of one or more bases, where the result is a triplet bases change. Consequently this results in a coding of distinct amino acid or the origin a stop–codon instead of normal codon. Phenotypic demonstration of point mutation in coding sequences depends on divergence of mutated product (protein) from the original one – from isoproteins to pathological ones (e.g. sickle cell anaemia). **Frameshift mutations** develop as a result of insertion or deletion of one or more bases. The result is then shift in reading of genetic code frame (transcription), as it is for instance in Duchenne and Becker´s muscular dystrophy. Here belongs also mutations of splicing sites for maturation of pre-mRNA, where appear abnormally long (short) mRNAs and consecutively proteins;
- Phenotypic manifestations (consequences) of mutations for cell. If mutation affects a gene essential for cell existence, the cell dies (by necrosis or apoptosis). In case mutations of genes participating in the cell cycle regulation, malignant transformation of the cell may occur. If a cell's less essential gene is mutated, an aberrant protein is synthesised, which may (or not) influence phenotypic manifestations in the cell and the organism. If the mutation does not manifest itself immediately after its origin, it is so-called "silent" mutation. For example, if mutation of one allele of tumour-suppressor gene RB1 occurs, coded protein rb1 does not change its capability in cell cycle regulation (fact, that the mutation happen is clinically not recognizable). Retinoblastoma (cancer of retina) develops only after subsequent mutation, when the cell loses latest normally functioning allele, what then results in the loss of heterozygousity (LOH);
- Types of cells in which the mutation has occurred. In case of mutations brought by germ cells – **gametic** mutations (both inherited and *de novo*) – are after fertilisation present in all cells of the new organism. In **somatic** mutations, an isolated body cell is affected, and the mutation is transmitted just to the cells formed by its division (clone). Phenotypic consequences of somatic mutation are diverse – from no expression until malignant transformation or cell death. Radiotherapy of tumours is an example of intentional evocation of massive mutations in tumours. These can activate immunity or mechanisms initiating apoptosis, e.i.the process of silent cell death;
- Level of genetic material organization, that is affected. According to this criterion, we distinguish mutations of genomes, chromosomes and genes. **Genome mutations** are called numerical aberrations and they change number of whole chromosomal sets or single chromosomes. Details of their origin mechanisms and consequences see in Chapter 9. **Mutations of chromosomes** (structural aberrations) are changes in organization and/or completeness of chromosomes (see chapter 9). In testing of mutagens we are faced to different types of damages of chromosomes (Fig. 26 - 28).



• **Figure 26.** Scheme of chromosome mutations, the action of mutagens

• **Figure 27.** Reunion complex

• **Figure 28.** Chromosomal disruption (break)

Gene (point) **mutations** are changes in DNA structure, concerned to one or more nucleotides (deletion, addition, substitution, inversion and frameshift mutations).

Deletion – loss of bases (part of chromosome) – in range from 1 base up to mega bases. They are frequent in non-coding regions and rare in coding ones. The simplest one is the loss of one base (e.g. G**A**TC → GTC), which causes frameshift mutation.

Addition (insertion) – is an entering of one or more bases (e.g. GATC → G**G**ATC). They are common in non-coding regions. They are rare in coding regions and cause frameshift mutations. Another, significantly longer DNA sequence can be inserted into DNA strand, e.g. a mobile genetic element. In this case this is called **duplication** (e.g. **GA**TC → **GAGA**TC) or **expansion** – i.e. multiplication of joined motive of bases. Example for expansion are s.c. CpG isles, an unstable repetition of trinucleotides, like CGG CGG CGG → CGG **CGG CGG** CGG CGG, and it could be the reason for serious diseases, e.g. Huntington disease.

Substitution – exchange of bases (e.g. **G**ATC → **C**ATC). If a purine replaces another purine, or pyrimidine another pyrimidine, it is **transition**. In case of substitution of purine with pyrimidine and vice versa, we call it **transversion**. According to the results of these changes, we distinguish the following types (and consequences) of substitutions:

- **silent** – because of genetic code degeneration, the substitution of bases does not evoke change in coded amino acid, because some amino acids can be coded by more than one type of triplets. The most common is the substitution of the third base in the triplet, because the first two bases are the most responsible for amino acid coding;

UAU → UAC
Tyr → Tyr

- **missense -** substitution of one base change the codon and it codes another amino acid;

UAU → UCU
Tyr → Ser

• **nonsense** – the most serious type of mutations are the substitutions, which change normal triplet to a stop codon. It results to a shorter protein product.

UAU → UAA
Tyr → STOP

Another case is, when the stop codon is changed to amino acid coding triplet. The result is an abnormally extended protein. Obtained mRNA then ends by the next coming stop codon (e.g. on the end of following gene);

• **splice site** mutations appear on a border of exon and intron, which lead to wrong splicing in maturation of pre-mRNA. Result is either retaining of intron or splicing out exon with previous and following exons (e.g. in laminA gene and progeria).

Inversion – 180° turn of the base sequence (e.g. G**AT**C → G**TA**C).

Frameshift – is the result of deletion, insertion or inversion of one or two nucleotides (not three or their multiple). It causes a shift in reading frame of genetic code and change of the amino acids order.

Contemporary international nomenclature defines individual rules for description of various types and positions of mutation. The original gene is defined as a **wild–type** one. Amino acids are marked by capital letters, e.g. K means lysine, L is leucine, G is glycine, R is arginine and X is a stop codon. We can also use three-letter abbreviations, e.g. Arg (arginine) and His (histidine). The order of amino acids is counted from the protein molecule N–terminus and also from the adenine nitrogen base at the beginning of start triplet.

Examples:
G52R or *Gly52Arg* – glycine, a 52nd amino acid in the line has changed to arginine
K320X – a 320th amino acid in the line, originally lysine, has changed to a stop codon
nt400(C→G) – a 400th cytosine in line has changed for guanine
nt5445(del4) – deletion of four nucleotides, from position 5445
Δ*F508* – deletion of fenylalanine codon, in 508th position
nt317(insA) – adenine has been inserted after a nucleotide in position 317

Mutations of genomes and chromosomes are analysed by cytogenetic methods. Mutations of genes are mostly analysed by methods of molecular biology.

6.2 Reparation mechanisms

During the evolution process the organisms evolved enzymatic mechanisms, which enable repair or at least toleration of DNA damages. Therefore they are called **reparation mechanisms**. After the signal informing about the presence of mutagene or damage of DNA strand, the cell starts producing most of these enzymes. Decrease in reparation enzymes' activity lead to serious consequences.

Reparation of thymine dimmer can be done in two ways. The first is called **photo reactivation** and it is carried out by **photolyase** enzyme. This enzyme links to dimmer and splits bonds between dimmer's thymine bases. The second reparation system **(nucleotide excision reparation)** contains a specific endonuclease, which cuts DNA strand in the place of dimmer. Due to the effect of DNA polymerase I a short oligomer containing the thymine dimmer is cleaved out; the same enzyme completes the gap by adding of missing nucleotides.

Another reparation mechanism is **base excision reparation**. Reparation enzyme **DNA–glycosidase** distinguishes the base that has been altered and breaks the N–glycoside bond, which binds the base to deoxyribose. This creates an AP location, which can be recognised by **AP– endonuclease**; which then cleaves the phosphodiester bond of DNA strand in the place of the missing base and cleaves out several nucleotides. DNA–polymerase I afterwards completes missing nucleotides and DNA–ligase joins both ends of the strands.

SOS reparation mechanism, in which can be activated as many as 20 SOS genes, does not usually repair damage without mistake. It runs in critical situations, when the reparation of DNA is vital act and its task is to master a large amount of DNA damages. In an undamaged cell, the product of gene *lexA*+ acts as a repressor for 20 SOS genes. In case of damage, SOS signal is produced, the *recA* gene *is* activated, and RecA protein is formed, which then starts cleaving LexA proteins. With a decrease of LexA proteins amount in the cell – other SOS genes are also activated. *RecA* was the first

recombination reparation gene that was discovered and it is the most important component in SOS reparation. There are usually about 1.000 – 10.000 of *RecA* monomers in a cell. With SOS induction after DNA damage its expression increases up to 50 times. These ways of reparation collaborate perfectly together.

6.2.1 Syndromes with defective DNA reparation mechanism (syndromes with spontaneous instability of chromosomes)

Five diseases (Xeroderma pigmentosum, Fanconi anemia, Bloom syndrome, Ataxia teleangiectasia and Cockayne syndrome) can be found under this common title. These can develop as consequence of mutations of genes for reparation enzymes. They are autosomal recessive inherited diseases with a very low frequency of occurrence. Affected individuals are secondarily extremely sensitive to influence of mutagens. Mutations accumulating in their DNA lead to high risk of cancer development.

6.2.1.1 Xeroderma pigmentosum

It appears with frequency 1: 250 000. It is caused by a mutation in one of the genes, which code proteins necessary for reparation of damage caused by UV radiation (photo reactivation and excision DNA reparation). People suffering from this disease are born with the mutation. Seven groups (XPA - XPG) are here distinguished according to damage type (table 7)

• **Table 7.** Xeroderma pigmentosum

Group	Locus	Gene size (kb)	Number of exons	Number of amino acids	Protein size (Da)
XPA	9q22.3	22.41	6	273	31 368
XPB	2q21	36.89	15	782	89 300
XPC	3p25	33.90	16	940	105 981
XPD	19q13.2	20.73	23	760	86 909
XPE	11p12	24.26	10	427	47 864
XPF	16p13.3	28.18	11	905	103 289
XPG	13q33	29.93	15	1186	133 329

Breaks, dicentric, acentric and ring chromosomes can be found in the patient's karyotype. Characteristic feature is an increased sensitivity to solar radiation and disorders of skin pigmentation. Patients suffering from this disease have dry, scaly skin (xeroderma). Very high risk of skin cancer is characteristic for the disease; the probability is 2000 times higher then in a healthy persons. Patients are affected with cancer at the age of eight (on average); which is 50 – 60 years earlier than it is in the rest of population.

6.2.1.2 Fanconi anemia

Its occurrence is 1: 350 000. It is caused by a genetic mutation of FANCA (Fanconi anemia complementation group A) gene (79.1 kb), located on 16q24.3. It has 43 exons, a coded protein has the molecular weight 162 775 Da and it consists of 1455 amino acids. This type of mutation results in DNA reparation failure and we distinguish 8 groups (FAA - FAH). There are breaks, gaps and quadriradials could be found in the karyotype. Insufficient function of haematopoesis, growth retardation, congenital defects of upper limbs, malfunction of skin pigmentation and even leukaemia are characteristic manifestations of this disease.

6.2.1.3 Bloom syndrome

Its occurrence is 1:100 000. It is caused by mutation of LIG4 gene for DNA ligase, (8.9 kb), with 2 exons, located on 13q33.3. Protein's molecular mass is 103.97 kDa and it consists of 911 amino acids. Disease does not have variants (groups). In the karyotype there are breaks, higher rate of sister chromatids exchanges, even chromosome fragility. Butterfly-shaped facial edema, growth retardation, hypersensitivity to infections and various kinds of malignity belong to typical manifestations of the syndrome.

6.2.1.4 Ataxia teleangiectasia

Its occurrence is 1: 40 000. It is caused by mutation of ATM gene, located on 11q22.3 (143 kb); it has 64 exons. Protein consists of 3056 amino acids and has the molecular weight of 350. 64 kDa. We distinguish 6 groups (ATA - ATF). Breaks and translocations of chromosomes are found in the karyotype. Its key symptoms are: disorders of walking, skin defects, respiratory infections, changes found in brain and lungs. There is also an increased probability of lung cancer, there.

6.2.1.5 Cockayne syndrome

Its occurrence is 1: 250 000. It is caused by mutation of CSA and CSB genes, resulting in excision reparation failure. CSA is located on 5q12.1, (71.23 kb), it has 12 exons, coded protein has molecular weight of 44055 Da and it consists of 396 amino acids. CSB gene, located on 10q11 (80.36 kb) has 21 exons, and codes a protein which consists of 1493 amino acids (with the molecular weight of 168416 Da). We distinguish three groups (CSA, CSB and CSIII). Breaks of chromosomes are found in the karyotype. Symptoms, which occur, include: mental retardation, precocious turning gray, geriatric face, long limbs and hearing losses. No particular type of cancer is linked to this syndrome.

6.2.2 Using mutagenesis in discovery the gene function

In the process of human genome sequencing increases number of genes with unknown function. Knowledge about gene' nucleotides sequence and three-dimensional structure of protein is not sufficient to explain the function of this protein. A lot of proteins (mainly those, which are part of multi-enzymatic complexes) do not have any independent activity; on the contrary, some proteins (e.g. kinases and molecular motors) participate in various processes in the cell.

In effort to explain the function of a certain gene, its mutants inactivating only particular protein are constructed, in order to specify the protein's function. It is most useful to follow up mutants that produce only one protein, which is active just in certain temperature. These proteins are easily inactivated by change of temperature and consequent lack of function is than studied.

Techniques of recombinant DNA molecules enable manufacturing of genetic alternations in in-vitro conditions, and consequently return such mutated gene to its original organism and watching change of its function. These techniques enable to change coding sequences in gene, forming partly active or inactive proteins, or change gene´ regulatory region; which subsequently change the amount of protein. Organisms, which contain genes modified by recombination techniques, are called transgenetic organisms. Haploid organisms (bacteria, yeast) are the most common ones, where the gene can be substituted, knocked out (knock out technique) or added. It is more complicated to substitute a gene in diploid organisms e.g. mice, therefore genes are inserted into embryonic cells, which ensures that at least a part of offsprings will have the mutated gene integrated permanently into their organisms. Similar techniques are being used in gene therapy.

Controlled mutagenesis can be used to change one or more amino acids in a protein. This technique can be used to recognize which part of polypeptide chain is important for creating its quaternary structure, protein interaction with a ligand or enzymatic catalysis.

6.2.3 Tests of potential mutagens

In experimental genetics and in mutagens testing, the organism bearing a mutation is called a mutant (mutant type). As a standard for comparison is used so-called "wild type", is a normal form of gene and trait, found in wild living organisms. This classification is relative. Mutation from the "wild type" to a mutant is called a **direct mutation** and the mutation happening in the opposite direction is called a **reverse mutation**. Reverse mutants (revertants) are organisms in which the wild-type of phenotype is restored by ("second") mutation.

Toxicology deals with testing of external factors, which can be harmful for cells and organisms. Experimental testing of potential mutagens is also a part of this investigation. It is necessary to prove that the tested factor (under defined circumstances) evokes a significant (statistically important) increase in the amount of mutations in a model system– in comparing to their known spontaneous occurrence.

There is no universal object or methodology for testing, which would enable detecting all kinds of mutations. Therefore a wide range of tests and various biological models, such as viruses, bacteria, mammalian or human cells, etc. are used. The result is determining the risk of the tested factor for a human, who is being exposed to it.

Internationally approved general **criteria for mutagenetic testing** demand:

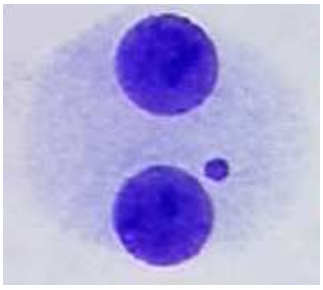
- high sensibility – they must not provide any false neither negative nor positive results. It is inevitable to prove the relation between the dose and effect, i.e. increase in the amount of mutations (or their importance) with increasing the dose of tested factor;
- survey and evaluation of all kinds of genetic damages;
- tests must be methodically precisely specified and repeatable. In case of using the same model, conditions of performance, dosage and interpretation, the results must be consistent (regardless where they were done);
- tests, after their final interpretation, must serve as a tool for determining the risk for men.

Three types of tests for potential mutagens detection are used – compulsory, for verification and supplementary.

Compulsory tests are prescribed e.g. for newly synthesized substances and in the process of registering of new drugs, etc. Here belong:

- tests for gene mutations done on bacteria (e.g. *Salmonella typhimurium, Escherichia coli*), yeast (e.g.. *Saccharomyces cerevisiae*) and flies (e.g. *Drosophila melanogaster*);

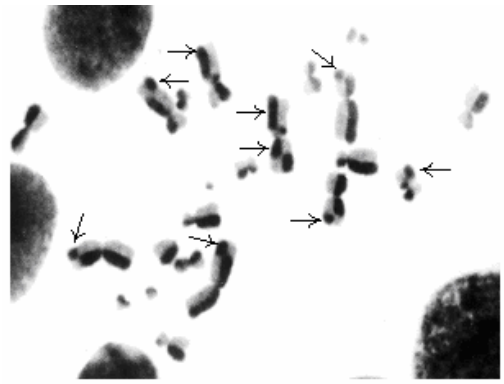
- tests for chromosomal aberrations – after application of tested factor to the model system, its effect is watched by cytogenetical analysis of cell division in bone marrow of laboratory animals (e.g. China hamsters or mice) and of cultivated lymphocytes of human venous blood. Micronucleus test done on cultivated animal and human cells is commonly used (Fig. 29).



• **Figure 29.** Micronucleus

- reparation tests for detecting of DNA damage and repair. Bacteria (*Salmonella typhimurium* and *Escherichia coli*) are most frequently used as model organisms.

Verification tests are used in cases when not the whole set of compulsory tests had been used or in case that at least one of them showed a positive result. Here belong tests for DNA damage detection and repair and gene mutation in mammalian cells, as well as tests of transmissible translocations in mice and hamsters.



Supplementary tests are used to determine details about special features and effects of the mutagen. Here belong for example dominant lethal test (on Drosophillas), test for sperms abnormalities (both, animal and human), sister chromatides exchange (Fig. 30) and a test of mitotic recombination at *in vitro* cultivated cells.

• **Figure 30.** Exchange of sister chromatides

In each experiment (test) are models (cells or organisms) divided into the following experimental groups:

- groups with different dosage of tested factor (usually three are used);
- negative control (treated with solvent, only) – it serves as recognition of potential interfering of any unknown factor;
- positive control (application of known mutagen) – to verify if the reaction of model system genetic material is, as it had been expected.

7. Chromosomal analysis in interphase and mitosis

Cytogenetics is a scientific discipline, which studies organization of genetic material on the chromosomal level (chromatin) – number and shape of chromosomes, or even their segments. It is used to diagnose numerical and structural chromosomal aberrations in various pathological statuses.

Cytogenetics can be divided, in regard to the different methodical approach, to:

- **classical –** analysis is based on microscopic evaluation of (by staining visualized) material of chromosomes in mitosis (chromosomes are examined) and in interphase (sexual heterochromatin is examined)**;**
- **molecular** – analysis is based on the identification of chromosomes or their parts after hybridization of DNA with labeled probes.

7.1 Classical cytogenetics

7.1.1 Cytogenetic analysis in mitosis

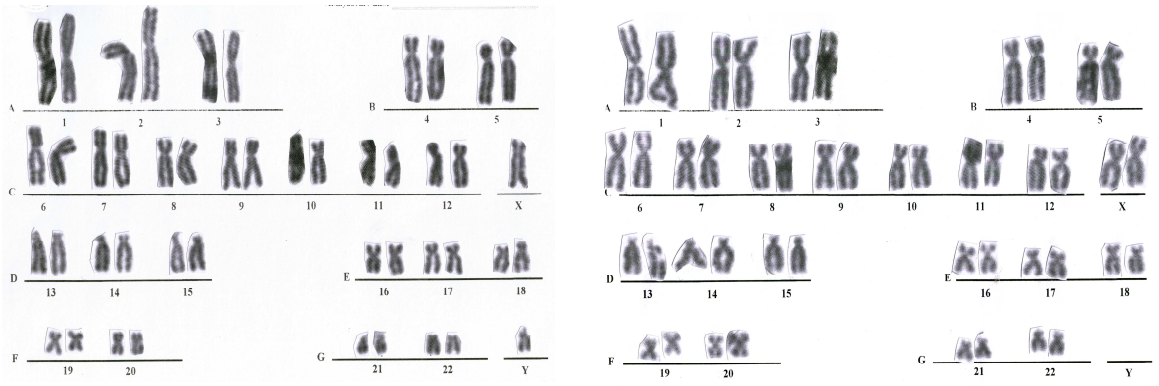
Viable cells, capable of proliferation, are needed for examination. It is necessary, via cell cultivation, to gain sufficient amount of dividing cells in the mitosis, mainly in metaphase, when are the chromosomes best observable.

Mitosis metaphase is suitable for numerical and structural cytogenetical analysis, because the chromosomes are maximally condensed and organized in metaphase plate (equatorial plane). When the mitosis course is stopped in metaphase (continuation to anaphase is halted) by adding mitotic poison (e.g. colchicine), we are speaking about c-metaphase.

The preparation of karyologic slides for metaphase chromosomal analysis consists of following steps:

- trypsinization – the cells cultivated in vitro release from cultivation substrate and they stay in suspension;
- hypotonization – osmotic lysis of cell spread chromosomes, so they are more suitable for analysis;
- fixation – stabilize examined material;
- staining – conventional (i.e. diffuse – usually by Giemsa dye) or identification (i.e. G, Q, C and R banding)

After processing the cells by the above mentioned procedure, metaphase chromosomes, arranged and analyzable in microscope, are obtained. Classical or digital methods are used to capture the picture of s.c. “metaphase figures” in microscope (microphotography). Chromosomes are then “cut out” from microphotography and arranging into pairs and groups, according to standard classification – a karyotype is obtained (Fig. 31).

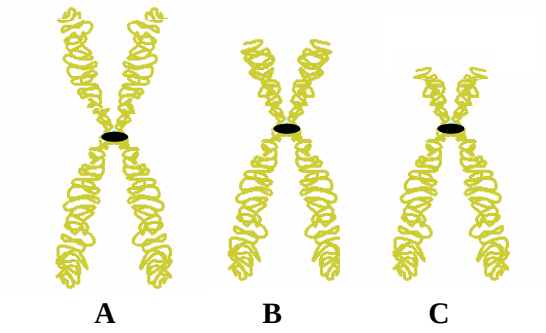


A **B**

• **Figure 31.** Normal male (A) and female (B) karyotype (conventional staining)

Used staining technique determines the resolution level of particular chromosomes and their subsequent classification.

Denver classification (1959) – chromosomes are arranged into groups (Fig. 32) according to their relative length (big, medium, and small) and the position of centromere. Human chromosomes are metacentric (with the centromere almost in the middle), submetacentric (centromere closer to one of the telomeres) and acrocentric (centromere close to telomere).



• **Figure 32.** Classification of chromosomes according to the centromere position (A – metacentric, B – submetacentric, C – acrocentric)

By using this classification only the total number of chromosomes and their number in particular group (A – G) can be evaluated:

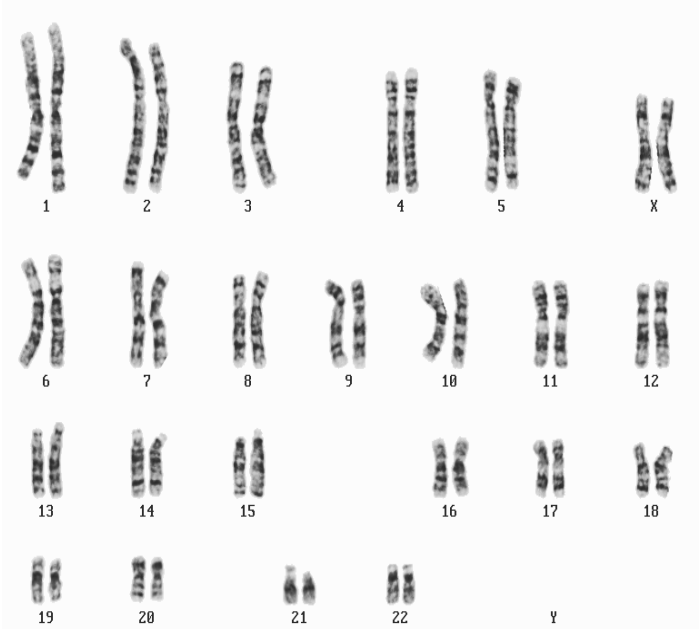
- group A – big metacentric chromosomes 1 – 3;
- group B – big submetacentric chromosomes 4 – 5;
- group C – medium submetacentric chromosomes 6 – 12 + chromosome X;
- group D – medium acrocentric chromosomes 13 – 15;
- group E – small metacentric to submetacentric chromosomes 16 – 18;
- group F – small metacentric chromosomes 19 – 20;
- group G – small acrocentric chromosomes 21 – 22 + Y chromosome

However, this method does not allow identification of individual chromosomes within the group and smaller chromosomal reconstructions, because the chromosomes are stained diffusely – conventional way.

Paris classification (named after the town where the first conference in 1979 was held) not only enables the exact determination (identification, description) of particular chromosomes (1 – 23), but also a detection of chromosomal structural aberrations. Detection of structural aberrations is possible by using various staining techniques, which provide **banding** of chromosomes. Number, size and location of these bands are unique and typical for each chromosome and results from its euchromatin and heterochromatin structures. The most common identification techniques are G-, Q-, R- and C- banding.

Q–band was the first method enabling banding. Its principle is that chromosomes are first treated by heat and then is applied the fluorescence dye (quinacrine mustard) which binds specifically to heterochromatin chromosomal sections, thus in ultraviolet light in fluorescent microscope both, more and less intensive fluorescent bands (Q-bands), are visible.

G–band is the most commonly used identification staining method; during which the chromosomes are partially digested by proteolytic enzymes (e.g. trypsin) and afterwards stained with Giemsa solution. Bands (G-bands, named after Giemsa), which are now visible, correspond with Q-bands and represent condensed heterochromatin (dark bands) and euchromatin (light bands) blocks in chromosome (Fig. 33).



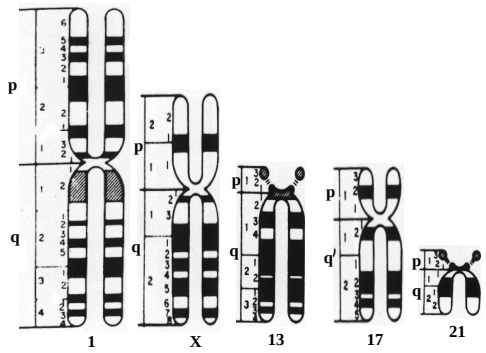
• **Figure 33.** Normal female karyotype – 46, XX (G–band)

R–band is a method, in which bands of reverse character (to Q- and G-bands) are obtained after incubation of chromosomal preparations at high temperature (90ºC). The reason of R-banding is better study of bands which are in Q and G banding light, so less investigable.

C–band is a method, which provides demonstration of constitutive heterochromatin blocks in centromeric region of all chromosomes; and in pericentromeric region of chromosomes 1, 9, 16 and Yq (C-bands). This method is often used in prenatal diagnostics.

High resolution technique – HR provides more detailed analysis of all chromosomes, prometaphasic to prophasic (little condensed), as well. The number of bands is from 800 – 1400, while in metaphase karyotype only 400 bands can be identified.

Paris classification evaluates the quantity, length and position of bands on short (p) and long (q) arm of each chromosome (Fig. 34). Each arm contains of 1 to 6 regions with a certain (standard) number of bands. The numbering begins from the centromere towards the telomere of chromosome. To identify a particular chromosomal band it is needed to determine the number of chromosome, the arm symbol, number of respective region, and number of individual band. All these data are written successively without any gaps or punctuation (e.g. 1q24 means chromosome number 1, long arm, region 2 and band 4). HR techniques allow partition of one band to several subbands, which are written after the dot, e.g. 12th subband in 1q24 is recorded as 1q24.12.



• **Figure 34.** Examples (scheme) of Paris classification

7.1.2 Cytogenetic analysis in interphase

The first method used for identification of sex determining chromosomes in non-dividing interphase cells was the analysis of sex chromatin of gonosomes X and Y. In the early seventies of previous century it was revolutionary method used for testing of sportswomen' chromosomal gender. It is also used in screening examination of patients with a suspicion for chromosomal aberration of X or Y chromosomes. It is based on detection of specific chromatin in interphase nuclei.

According to classic cytogenetics, the chromosomes consist of euchromatin and heterochromatin.

Heterochromatin is a strongly condensed chromosomal area, "poor" in genes (genetic deserts or genetically "mute"). Two types of heterochromatin are recognized according to their position and mechanism of origin:

- **constitutive heterochromatin,** found in all cells of an individual and during all the stages of development. It is placed in the centromere regions of all chromosomes. Besides it makes large heterochromatin blocks in pericentromeric regions of long arms of chromosomes 1, 9, 16 and a majority of long arms of chromosome Y;
- **facultative** – depends on the cell type and its stage of development. The example is female heterochromatin X resulting from one X chromosome inactivation in each female cell during the earliest embryogenesis stage (between 9^{th} and 12^{th} day of development). Inactivation is done by condensation and massive methylation of one of the X chromosome from pair (including its euchromatin regions). Thus the genetic information located on inactivated X chromosome becomes inaccessible. It is a mechanism providing (according to Lyon's theory) compensation of gene dose – in women only one X chromosome remains active; as it is in men.

Mary F. Lyon's theory (1961) – only one X chromosome is active in human somatic cells. The second X chromosome in women is condensed and inactived (in interphase somatic cells is visible as a sex X-chromatin). In each cell of a female embryo, in the second week of development, is randomly inactivated one X chromosome – of mother or father. The inactivation is for a cell population arising from each cell (clone) than stable and permanent – the inactivated X-chromosome is also replicated, but later is again inactivated. The only exceptions are oogonia, where is inactivation abolished in the interest of full validity of the genetic variability arising from development of gametes.

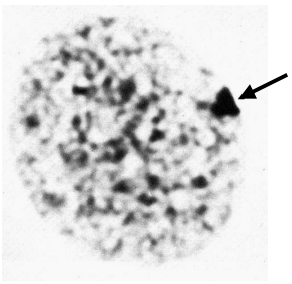
Therefore each woman is a **physiologic mosaic** for X chromosome – she has (statistically) in one half of all her somatic cells active father' X chromosome and in the second half it is the mother' X chromosome active. But in reality, local tissue mosaic can have different ratio of cells, due to random character of cell populations used in growing of embryonic germ layers formation and development of organs. It is extremely important in case of X-linked carrier females. One of best known examples is hypohydrotic ectodermal dysplasia, when carrier woman has skin with islands of normal skin and islands of "dry skin", where is sweat glands dysfunction. This explains the presence of phenotypical manifestations in carriers of X-bound recessive diseases – these occur, when (randomly) the cells with active X chromosome with pathological allele are superior in number in the target tissue.

X chromosome inactivation is **incomplete**. For example, the gene XIC - X chromosome inactivation center - its product – XIST (X inactive specific transcript) manages the X chromosome inactivation by methylation of GC dinucleotids.

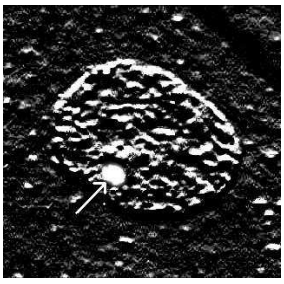
So-called **pseudoautosomal genes are located** on both X and Y chromosomes in s.c. pseudoautosomal regions (PARs), which are not inactivated. Under normal circumstances, they manifest themselves biallelically – in pairs, like it is in Mendelian inheritance. This explains phenotypic manifestations in women with polysomy and monosomy of X chromosome.

Inactivated X chromosome (sex heterochromatin X) is visible as a dark lens-shaped object, so-called Barr body, in about 20 % of interphase cells. It is situated on the inner face of inner membrane of nuclear envelope (Fig. 35). Number of X chromosomes is n + 1 (n = number of Barr bodies). This enables determination of the number of X chromosomes in examined cell.

Similarly, Y chromosome can be proved in interphase nucleus as a point, shining under UV light – a so-called F element (Fig. 36). This method is based on a strong affinity and intercalacy of fluorescent dye (chinacrine) to huge heterochromatin block on Y chromosome long arms (Yq).

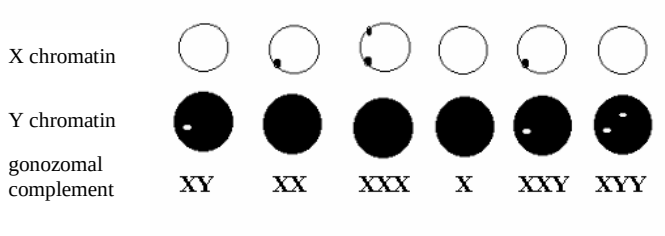


• **Figure 35.** Barr body (marked by an arrow)



• **Figure 36.** F element (marked by an arrow)

Simultaneous X and Y chromatin examination gives a general picture about s.c. gonozomal complement of examined cells (Fig. 37). However, later it is necessary to examine the metaphase chromosomes for exact diagnostics and for detection of structural aberrations of X and Y chromosomes (iXq, iYq, delYq).



• **Figure 37.** Scheme of X and Y chromatin examination

7.2 Molecular Cytogenetics

Molecular cytogenetics is based on the probe hybridization (labeled single-strand DNA molecule with known nucleotides sequence). Probe is complementary to a sequence in DNA, analyzed in situ (in chromosome) – directly in nucleus or in karyologic slides (metaphase figures on slides). By radioactive isotopes-labeled probes are used in ISH (in situ hybridization). Probes labeled by fluorescent dye (fluorochrome) are used in FISH (fluorescent in situ hybridization). Classical FISH method is in majority cases based on the extreme affinity between biotin (vitamin B7 or rather vitamin H) and streptavidin. Biotin is "connected" to probe. Fluorochrome – i.e. so-called "reporter group", is bound to streptavidin. After successful probe hybridization, it is possible to identify its presence and position (in fact of biotin) by adding streptavidin with fluorochrome. By using of various different probes and fluorochromes emitting different colors, it is possible to mark more target sequences in the same nucleus. For example, in prenatal genetic diagnostics, utilizing of probes identifying chromosomes 13, 18, 21, X and Y most common aneuploidies, can be detected in the amniotic fluid cells (amniocytes).

Great advantage of FISH and its modifications, in comparison to the classical cytogenetics, is that they can be used also in the interphase, when the cell cultivation process is unnecessary or impracticable. Another benefit is the possibility to analyze archived material, as well.

According to the site of hybridization, specificity and length of probe it is possible to analyze the following:

- specific chromosomal structures – e.g. satellite regions of chromosomes by satellite probes, centromere regions by centromere probes or telomere regions by telomere probes;
- specific DNA sequences, i.e. chromosomal regions responsible for the origin of certain pathological states (locus specific probes, enabling analysis of particular gene structures or sequences within a gene – in the diagnostics of microdeletion syndromes);
- whole chromosomes - using chromosome identifying probes.

Nowadays, there are several FISH modifications:

- **comparative genomic hybridization** (CGH) - has contributed to chromosomal changes' analysis in oncological diseases. It is a quantitative assessment of differently labeled DNA signals from normal and tumor tissues. On the basis of different fluorescence, analyzed by computer; minor changes in the amount of DNA can be revealed in the examined tissue, i.e. changes in chromosomal number. However, CGH does not allow to detect structural aberrations of chromosomes, which are not accompanied by a change in DNA amount;
- **multicolor FISH (mFISH**) – this method uses differently labeled probes for each chromosomal pair. It is not only used for distinguishing of each chromosome but for structural aberrations of chromosomes, as well. Digital displaying technology is necessary for evaluation, especially a high-sensitivity camera and high-performance computer with software for analysis of pictures obtained by this method;
- **spectral karyotyping (SKY)** - links colored chromosomal visualization with classical banding of chromosomes. It is a simultaneous hybridization by a mixture of special probes marked by five different fluorochromes enabling to distinguish up to 31 target sequences. Similar technical equipment as used in the mFISH method is needed for visualization. The result is colored banding, specific for each chromosome.

8. Gametogenesis

Gametogenesis, as the process of specialized germ cells origin and development from preliminary gamete cells (s.c. gametogonia), is the basis for sexual reproduction.

During gametogenesis the number of chromosomes is reduced to one half, in order to maintain (after fertilisation) the species specific amount of genetic information, in sexual reproduction. Gametogenesis also ensures creation of new genetic combinations – each individual, except for identical twins, has a unrepeatable and unique combination of alleles and forms of traits.

Genetic variability generated in gametogenesis increases chance of species survival even in changing conditions.

Variability is conditioned by two phenomena, which accompany sexual reproduction. First of these are processes that are associated with meiosis. It is the crossing-over (in prophase of the first meiotic division) and random segregation of chromosomes (in anaphase of the first and second meiotic divisions).

There may be as many as 2^{23} original combinations of originaly parental chromosomes, i.e. more than 8 million possibilities. Crossing-over allows further allele recombinations. The second phenomenon, creating new genetic combinations, is fertilization randomness. Fusion of gametes maintains a specific number of chromosomes for different species.

The process of gametogenesis takes place exclusively in the gonads (primary sexual organs).

It can be divided into spermatogenesis (formation of male gametes - sperms) and oogenesis (development of female gametes – ova). Sperms develop in the male gonad (testis) and ovum in the ovary – a female gonad. Mature gametes of either gonad are transferred to the genital tract (male and female) through which they are transported into the physiological point of their destination.

The development of gametes can be divided into three phases:

• **Phase of multiplication** – by repeated mitotic division of gametogonia (spermiogonia or oogonia) their number increases;
• **Phase of growth -** the name of this phase is derived from microscopically observable increase in the volume (diameter) of gametogonia. Diploid gametogonia synthesize cell organelles and prepare for division. At the end of this stage we are talking about primary gametocytes (primary spermatocytes and primary oocytes) or gametocytes of the 1st order, which are still diploid;
• **Maturation phase –** this matches with meiotic division. In the sperm development, this phase ends with spermiocytogenesis. The result of this phase is a formation of haploid gametes.

8.1 Meiosis

Meiosis consists of two consecutive cell´ divisions, but DNA replication happen only once – before the first meiotic division.
First meiotic division (also called maturing, reductive or heterotypic division):

The prophase of the first meiotic division is very long and much more complicated than it is in mitosis. It has the following stages:

• **proleptotene stage** - condensation of chromosomes begins. Cell nuclei are filled with diffuse chromatin and variable amounts of more condensed chromatin granules;
• **leptotene stage** - chromosomes condensation continues. Partly condensed, long and thin chromosomes are still attached to the nuclear membrane by their telomeres (Fig. 38);

• **Figure 38.** Leptotene stage

• **zygotene stage** - homologous chromosomes make pairs and longitudinally attach themselves to each other by their homologous non-sister chromatids. Chromosome synapsis appear and so-called bivalents are formed (Fig. 39). Synapsis is conditioned by a protein complex, which we call synaptonemal complex;
• **Figure 39**. Zygotene stage

• **pachytene stage –** condensation of bivalents continues further (Fig. 40). Two sister chromatids are visible on each of lined-up chromosomes; this formation is therefore called tetrade. Joined non-sister chromatids of tetrade, crosses and "wrap around" each other, as spirals. This phenomenon is known as crossing-over and crossover points are called chiasmata (Fig. 41). Recombination knots are found in the regions of synaptonemal complexes, containing high molecular weight proteins that are responsible for recombination of chromatids;

• **Figure 40.** Pachytene stage

• **Figure 41.** Crossing-over progress

• **diplotene stage** - synaptonemal complexes disappear and homologous chromosomes begin to separate from each other. Their separation is however, more difficult in crossovered regions. Here can happen breakages and subsequent exchanges of homologous sequences, between non-sister chromatids. Recent findings suggest that these activities are specifically managed by topoisomerase enzymes. So is, thus the mechanism of recombinant chromatids creation, which contains both originally paternal and maternal parts (alleles). This is the mechanism that allowed sexually reproducing organisms to reach almost unlimited variability and hence adaptability (Fig. 42);

• **Figure 42.** Diplotene stage

• **diakinesis** - condensation of chromatids continues and chiazmata move towards the chromosomes' ends – s.c. chiazmata termination (Fig. 43). At this stage there is a breakdown of nuclear envelope membranes and disappearance of nucleolus. Dividing apparatus is formed.

Meiosis than progresses in the same manner as mitotic division – to prometaphase, metaphase, anaphase and telophase of first meiotic division.

• **Figure 43.** Diakinesis

The end of prophase I of meiotic division is followed by metaphase, anaphase and telophase of the 1st meiotic division. Short interphase follows after the end of the 1st meiotic division, which however does not include the S-phase and therefore, there is no DNA replication present.

The result of the 1st meiotic division is the random separation of homologous chromosomes into daughter cells. These cells (secondary gametocytes) have only single set of chromosomes (n), but each of these chromosomes is still composed of two chromatids – in humans there are 23 chromosomes but 46 chromatids, there.

During **the 2nd meiotic division** (also called as equational or homeotypic), which is in fact a mitotic division, are sister chromatids separated and randomly distributed into daughter cells – haploid gametes. Each gamete thus contains only one piece of each type of chromosomes (in humans there are 22 autosomes and one gonosome.

The result of meiosis is a reduction of the number of chromosomes by half (2n → n) and creation of new genetic combinations by crossing-over and random segregation of parental chromosomes. Haploid (n) secondary spermatocytes and oocytes are the products of the 1st meiotic division. These then enter the 2nd meiotic division, the result of which is an oocyte (+ 3 polar bodies) or 4 spermatids, which later differentiate into mature sperms in the process of spermiohistogenesis.

8.1.1 Disorders of the normal course of meiosis

Normal course of gametogenesis and meiosis in it has for human's existential character as a mechanism needed to provide normal offspring, and thus the continuity of life. Like any other process in nature, also gametogenesis is a process with a certain error percentage. Consequences of defective gametogenesis mean formation of chromosome aberrations in the progeny, which are in most cases almost incompatible or completely incompatible with life. Causes of defective gametogenesis may include:

• **Failures in pairing of homologous chromosomes**:

a) formation of univalents or partially asynaptic bivalents, which are selected in the further course of meiosis and result in formation of aneuploid gametes

b) non-homologic pairing or lateral pairing of a univalent, which leads to the formation of structural chromosomal aberrations;

• **Unequal crossing-over** in prophase of 1st meiotic division - the result is deletion of a certain segment in one, and its duplication in the other one chromatid.

• **Nondisjuncion** – failure of sister chromatids separation (disjunction) before start of anaphase. The result is that one daughter cell has both chromosomes of the pair and another daughter cell will have no one:

a) If nondisjunction happens in anaphase of first meiotic division, it results to formation of aneuploid gametes; two of which are disomic (with an additional chromosome) and two nulisomic (without a chromosome) – no normal gametes appear;

b) Nondisjunction in anaphase of 2nd meiotic division leads to formation of a disomic and a nulisomic gamete. Two remaining gametes are normal.

• **Anaphase lag** is failure of single chromosome "journey" towards the centrosome (cell's pole) on time, due to kinetochore microtubules failure. Lagging chromosome will not be included into the newly formed nucleus. It is lost in cytoplasm and nucleus has one chromosome less. Anaphase lag can happen during meiosis, but it has great importance in post-zygotic mitotic division – in mosaics formation.

8.1.2 Comparison of meiosis and mitosis

- Meiosis occurs only in the gonad tissues. Mitosis takes place in the cells of all tissues.
- Spermiogenesis and mitosis occur continuously, oogenesis is a periodical process.
- Spermiogenesis and mitosis involve a large number of cells. Only one functional ovum is the outcome of oogenesis.
- Haploid gametes are the result of meiosis. Diploid somatic cells are formed by mitosis.
- Gametogenesis itself is a special type of cell differentiation. In somatic cells differentiation takes place after the division, i.e. in G0 phase of cell cycle interphase.
- Replication of DNA happens in gametogenesis only once, prior to the 1st meiotic division. The DNA replication takes place before each mitotic division.
- In meiosis, homologous chromosomes pairing and crossing-over occurs. In mitosis, these processes are not normally present.
- Cells created by meiosis bear different genetic information (alleles). Daughter cells resulting from mitosis are genetically identical.
- Genetic significance of meiosis is in maintaining species-standard number of chromosomes and in creating hereditary variability. By mitosis is genetic information transferred from mother to daughter cells unrevised.
- Gametic mutations emerge as a consequence of any disturbances of meiosis and are subsequently (after fertilization) transmitted to all cells of new individual. Mutations in somatic cells may have various consequences.

8.2 Spermiogenesis

The spermiogenesis in human is a continuous process – from puberty until late old age (with individual variation). The process is continual and takes 64 to 72 days (about 10 weeks). Sperms are produced in the quantity of 100 – 200 million in a single ejaculate. In the Slovak Republic it is considered as normal to have at least 30 million sperms in 1 ml of one semen (ejaculate). World Health Organization (WHO) considered, that minimum in s.c. "developed countries" is 20 million sperms per ml of ejaculate.

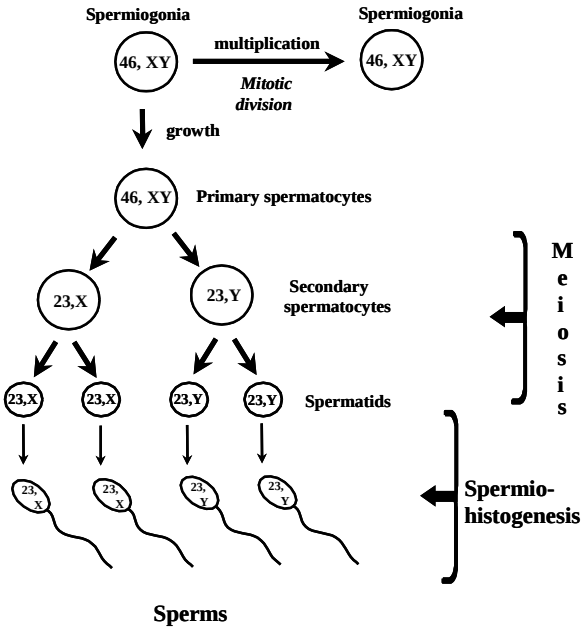
The problem is in decrease of spermiogenesis, mainly because of increased temperature of testes. Normally are testes of mammalian males located so, that they are 2 ºC bellow the body core temperature. Wearing of cotton-based underwear and jeans increases temperature and decreases amount of developed sperms.

In spermiogenesis develop from one spermatogonia 4 mature, functionally equivalent motile sperms. The cells originated by 2nd meiotic division are called spermatids. Their later development is spermiohistogenesis – by development of head, neck, connecting part and tail they change into matured sperms (Fig. 44).

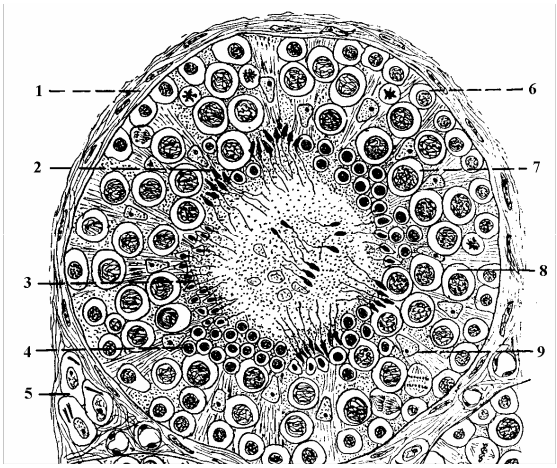
Spermiogenesis takes place in convoluted seminiferous tubules of the testes (tubuli seminiferi contorti, Fig. 45). The periphery of seminiferous tubules is the place for intense mitotic division of spermatogonia.

Bellow the spermatogonia layer (towards the lumen of seminiferous tubules) are primary spermatocytes, which have gathered supply matter and increased their volume (growth phase).

Secondary spermatocytes, derived from the 1st meiotic division, are located in another layer. Spermatids and flagella of sperm, visible in the lumen off seminiferous tubules, undergoing spermatohistogenesis (maturation phase) are found under them.



• **Figure 44.** Diagram of spermiogenesis

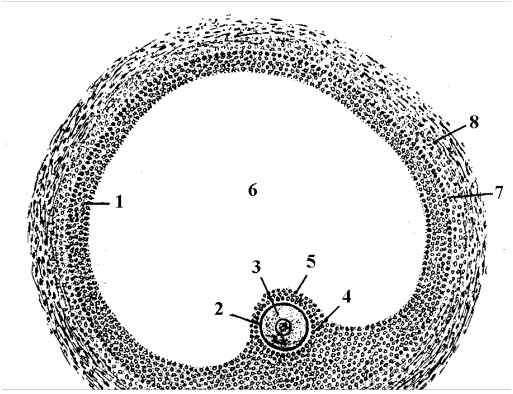


• **Figure 45.** Cross-section of seminiferous tubules 1 - membrane propria, 2 - sperm in contact with Sertoli cells, 3 - spermatohistogenesis, 4 - spermatids, 5 - insterstitial cells of Leydig, 6 – spermiogonial cells, 7 - secondary spermatocytes, 8 - primary spermatocytes, Sertoli cell with a nucleus.

8.3 Oogenesis

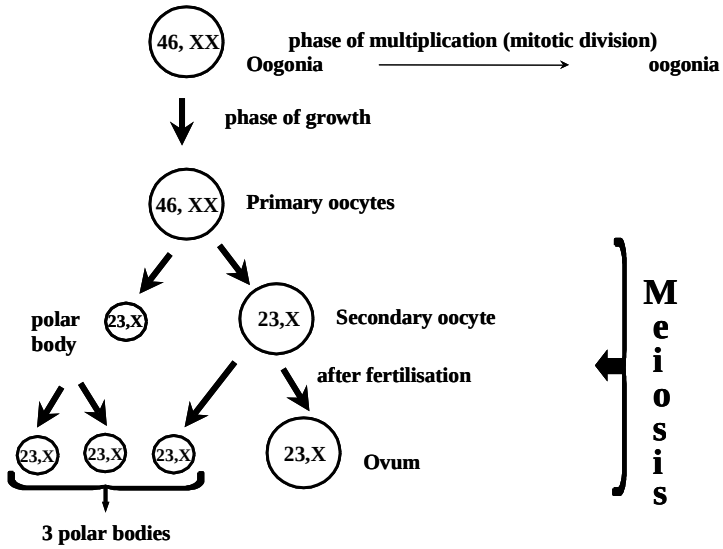
Oogensis begins in the early stages of embryogenesis in the cortex layer of ovaries. Primary oocyte is enveloped by follicular epithelial cells and thus a primordial follicle is formed. Until the delivery, prophase of the 1st meiotic division takes place in all oocytes - up to diplotene stage. Then, until puberty, all oocytes remain inactive.

The ova are further developed (since puberty) in monthly cycles. Follicles begin to grow – multi-layered epithelium and a secondary follicle are formed by follicular cell multiplication. Then, by drawing aside of follicle cells a follicle with a cavity is formed, called a tertiary follicle, in which an oocyte is found, located eccentrically in the hillock of cells - cumulus oophorus. Follicle with a large cavity is called a mature or Graaf´ follicle (Fig. 46). Here the oocyte completes 1st meiotic division and undergoes prophase and metaphase of the 2nd meiotic division. This is the time when ovulation occurs. 2nd meiotic division is completed only after fertilization.

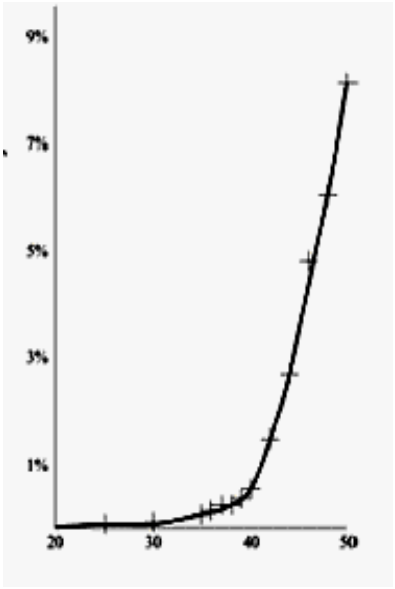


• **Figure 46.** Graaf follicle scheme - 1 - membrana granulosa, 2 - the zona pellucida, 3 - secondary oocyte, 4 - corona radiata, 5 - cumulus oophorus, 6 - cavum foliculi, 7 - theca interna foliculi, 8 - theca externa foliculi)

The first meiotic division gives rise to two non-equivalent cells: a secondary oocyte and polar body (which can later divide). The second meiotic division results in a mature ovum and the second polar body (Fig. 47).



• **Figure 47.** Scheme of oogenesis



About 2.6 million primary oocytes can be found in the ovaries of a newborn girl; approximately 400 of which mature during reproductive age, while the rest of them are eliminated by apoptosis. Oogenesis is a process, which starts at about the 4th month of intrauterine development and finish by menopause at the 45th – 50th year of women's age. Development of a specific oocyte can therefore take several decades, which increases the likelihood of nondisjuncion in the 1st meiotic division and thus the risk of a birth of a child with numerical aberration of chromosomes (Fig. 48).

• **Figure 48.** The age of the mother and the risk of giving birth to a child with numerical aberration of chromosomes

9. Chromosomal aberrations

In cytogenetic analysis can be in karyotype find different variations, called in clinical genetics as chromosomal aberrations. They relate to the changes in structure or number of chromosomes. These changes are very significant, because they can cause chromosomaly conditioned pathological statuses (syndromes).

Chromosomal aberrations, according to the nature of deviation, can be divided into **numerical** and **structural.** Depending on which chromosomes are affected by aberrations, it is distinguished between aberrations of autozomes and gonozomes.

All chromosome aberrations are large changes in the genetic material caused by **mutation**s. Depending on the type of cells in which they occur, mutations are divided to:

- **gametic,** found in the gametes (inherited or formed *de novo*) and transmitted to all child cells, which arise after fertilization of such gametes;
- **somatic,** arising in different stage of development of an individual after formation of zygote (**postzygotic mutation**s**)**. They are transmitted only to the cells that are formed by mitotic division of the affected cells (clone). Thus, the mutation occurs only in part of the cells that form the body of an individual. This situation, when the organism is composed of two (or more) populations of in genotype different cells is called **mozaicism**s.

9.1 Numerical chromosomal aberrations

These aberrations mean that the number of chromosomes in somatic cells differs from diploid (euploid) number (2n). Two types of numerical aberrations are recognized:

• **polyploidy**, which have supernumerary set (or sets) of chromosomes – for example **triploidy** (3n - 69 chromosomes) or **tetraploidy** (4n - 92 chromosomes);

• **aneuploidy**, in which the number of chromosomes differs from euploidy – particular chromosome is more or less. The resulting number of chromosomes is odd – in majority of cases is final number of chromosomes 46 ± 1. If the number of certain chromosome is increased, it is called **polysomy**, trizomy is the most common. If the number of chromosomes is reduced – particular chromosome is missing – it is **monosomy.**

9.1.1 Polyploidy

In humans, triploidy and tetraploidy are the most common. They are relatively frequent in early miscarriages (especially triploidy) as they are not compatible with life.

Triploidy (3n) is mostly the result of multiple fertilization (fertilization by two sperms). It can however, also be a consequence of pathological gametogenesis (formation of a diploid oocyte or sperm) or non-expelling the 2nd polar body from a fertilized oocyte. Triploidy represent 17 – 20 % of chromosomal aberrations detected in early miscarriages. Live birth, however, is very rare and the newborns survive a few days, at most.

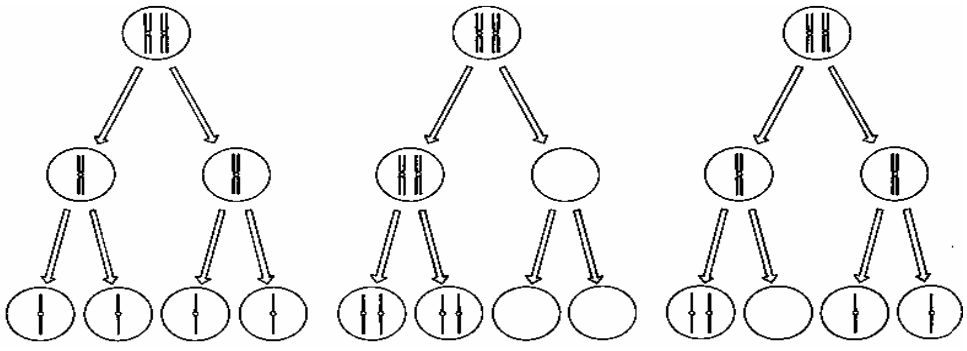
Tetraploidy (43n) is mostly caused by endoreduplication (S-phase is repeated) in the first mitotic division of a zygote, or it may also be a result of multiple fertilization. This state is however, incompatible with human´ life, even in the early stages of embryogenesis.

9.1.2 Aneuploidy

Aneuploidy can be caused by:

- **nondisjuncion** in anaphase of the 1st or 2nd meiotic division. Because the sister chromosomes (in a beginning of anaphase) do not part (disjunct), one daughter cell will possess 24 and the second one 22 chromosomes (Fig. 49). When each of them will be fertilized with normal gamete – aneuploidy in zygote will appear;
- **anaphase lag** in the 1st or 2nd meiotic division. In this disorder, one chromosome (from particular pair of homologous chromosomes) does not succeed, during anaphase, arrive to the centrosome and is lost in cytoplasm. It results into different number of chromosomes in daughter cells. If anaphase lag happens in meiosis – one cell will have 22 and another one 23 chromosomes. If it happens during mitotic division, the mosaic appear;

- **disorder of homologous chromosomes pairing** in prophase 1 of the meiotic division. If no pairing occurs, univalents or partially asynaptic bivalents are formed, which get lost in the further course of meiosis and give rise to aneuploid gamete formation.



normal meiosis | nondisjunction in the 1st meiotic division | nondisjunction in the 2nd meiotic division

• **Figure 49.** Nondisjunctions in first and second meiotic divisions

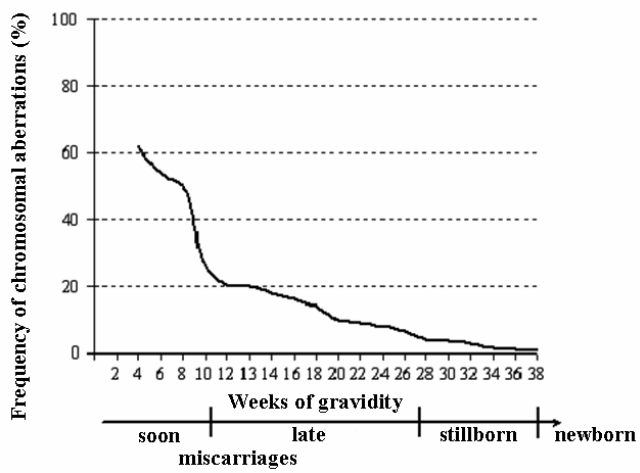
Aneuploidy of each chromosome can happen. Generally, in organisms is the additional genetic information (e.g. trisomy) tolerated better than its absence (e.g. monosomy), and aneuploidy of autosomes have more serious consequences than aneuploidy of gonosomes.

Monosomy of autosomes is incompatible with life even in the early stages of embryogenesis. The only monozomy compatible with life in humans is monozomy X – of which 99 % dies in prenatal age, but in 1 % of surviving girls (it is mostly the mosaic form), paradoxically, it causes a relatively mild postnatal disability.

Trizomies of large chromosomes (with large numbers of genes) have more severe consequences than trisomies of small chromosomes.

Chromosomal aberrations have an important role as a cause of miscarriages. The highest incidence of the most severe forms of aberrations is assumed in the first two weeks of intrauterine development, however these are miscarried before pregnancy is clinically diagnosed. After pregnancy diagnosis; chromosome aberrations in the first trimester appear in 50 % of miscarriages. Most common here are (except for polyploidy) trisomy of chromosome 16 and monosomy of X, but also any other trisomy may occur, except trisomy of chromosome 1 (which is assumed to be lethal already at gamete stage).

In further development, as the consequence of natural selection, the percentage of chromosomal aberrations appearance decreases in miscarriages and stillborn newborns (Fig. 50).



• **Figure 50.** Frequency of chromosomal aberrations in miscarriages, stillborn (6 %) and alive born fetuses (0.6 %)

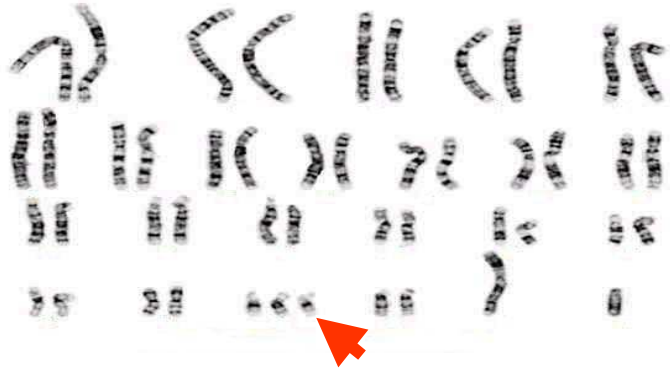
With progress of pregnancy, the recorded aberrations spectrum narrows, also as a result of selection – so like more disabled the embryo (fetus) is, so sooner it dies and is miscarried or born prematurely as a stillborn.

One of the factors that significantly increase the probability of numerical aberrations occurrence is the mother age higher 35 years (increased risk of nondisjunction in the 1st meiotic division), as described in the chapter “Gametogenesis”.

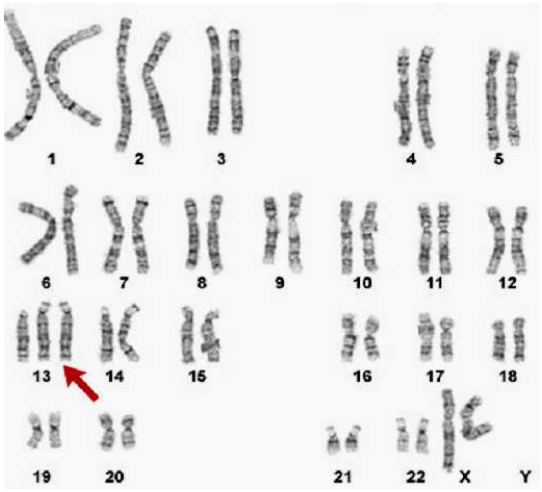
Phenotype manifestations of chromosomal aberrations are severe and usually also include a certain degree of psychomotor retardation. They form a complex of symptoms and abnormalities (syndrome) that are characteristic for specific chromosomal aberrations, although some of them also occur in other syndromes.

Numerical aberrations of autosomes:

Down syndrome (prevalence of 1 : 700 live newborns) is conditioned by trizomy of chromosome 21 (Fig. 51). Phenotypic manifestations include psychomotor retardation, musclar hypotony and hyperflexibility of joints, single transverse palmar groove, a broad flat face, large protruding tongue, epicantus of the eyelids, congenital heart defects, susceptibility to infections and cancer (leukemia). The form of Down syndrome caused by free supernumerary chromosome 21 in all cells of an individual is called a **trizomic (free) form.** In addition to this form, are recognized also **mosaic** and **translocation form** of Down syndrome (explained below).

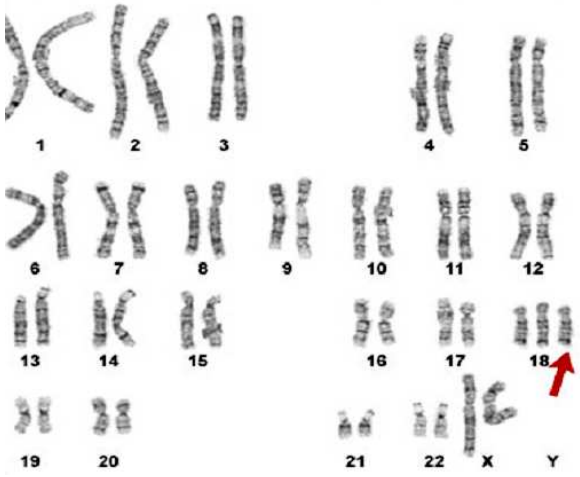


• **Figure 51.** Trizomy 21 karyotype



Patau syndrome (1 : 10 000) is conditioned by trizomy of chromosome 13 (Fig. 52). Phenotypic manifestations include psychomotor retardation, deafness, cleft lip and palate, polydactyly, congenital heart defects, abnormal kidneys and genital. Disabled individuals usually die till the end of the 1st month of life.

• **Figure 52.** Trizomy 13 karyotype



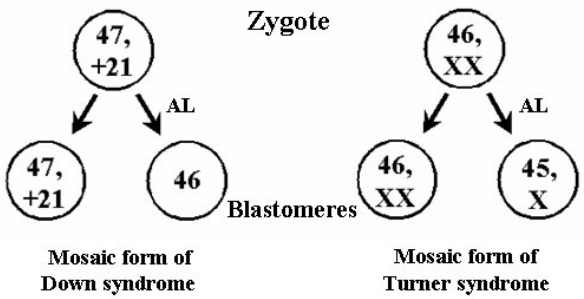
Edwards syndrome (1 : 7 500) is conditioned by trizomy of chromosome 18 (Fig. 53). Phenotypic manifestations include psychomotor retardation, malformations of many organs, mandibular hypoplasia, small mouth and nose, finger deformities and pes equinovarus. Disabled usually die within half a year after birth.

• **Figure 53.** Trizomy 18 karyotype

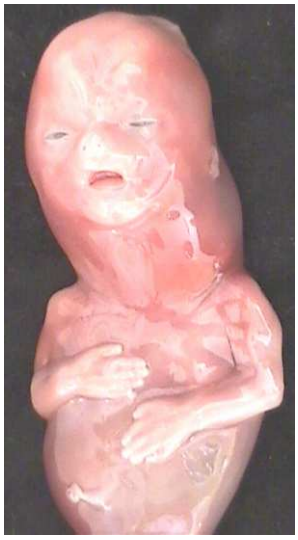
Numerical aberrations of other autosomes are rare. If they occur, they are found mostly in the mosaic form, because the presence of a normal cell line has a dilutive effect on the severity of phenotypic manifestations. The degree of disability is associated with tissue distribution and percentage of normal and pathological cell lines proportion.

Mosaic form of chromosomal aberration may arise during mitotic divisions in post-zygotic period as a result of nondisjunction or anaphase lag.

Consequences of anaphase lag and a mosaic formation may be different (Fig. 54). If it occurs in one of the blastomeres, originated by mitotic division of trisomic zygote, the result is a dilution effect and an individual with a mosaic form (e.g. Down syndrome). As soon as the anaphase lag of additional chromosome occurred, the less severe are the clinical manifestations, because "normalized" population of cells (now with normal number of chromosomes) is larger. On the contrary, after anaphase lag and the loss of chromosome X in a blastomere originating from a normal zygote with genome 46, XX, the outcome is mosaic form of monosomy X. Women with this form of the Turner syndrome have as less severe clinical symptoms, as later anaphase lag happened.



• **Figure 54.** Origin of mosaic forms of chromosomal aberrations



• **Figure 55.** Miscarried fetus of Turner syndrome

Numerical aberrations of gonosomes:

Turner syndrome (1 : 1 000) is conditioned by monosomy X. Women have small figure, are sterile, do not have developed primary and secondary sexual characteristics and typical skin fold on the neck (pterygium colli). In this syndrome there is a significant discrepancy between the severity of disability; prenatally and postnatally. Although 99 % of them are miscarried, postnatal disability is relatively mild (Fig. 55).

Syndrome with three X chromosomes (1 : 1 000) has a karyotype 47, XXX. These women are fertile, but with more frequent miscarriages. They are often moderately mentally retarded.

Klinefelter syndrome has a karyotype 47, XXY (1 : 1 000). Males with this syndrome do not have to have any problems until adolescence. They are sterile, because of primary defect of Sertoli cells in testes. Some individuals can have developmental disorders of genital, gynecomastia, female type of pubic hair, tall figure. They may also be mentally retarded.

Syndrome (Jacobs) with two Y chromosomes has karyotype 47, XYY (1 : 1 000). Men do not usually have severe phenotypic manifestations.

9.2 Structural chromosomal aberrations

Structural chromosomal aberrations are changes in the organization of the genome, altering the structure of one or more chromosomes.

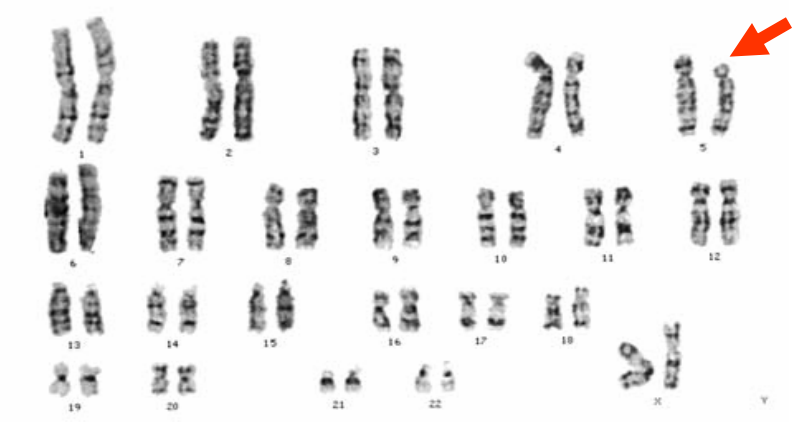
They are formed by **chromosomal breaks** (disruption of chromosome continuity) or as a consequence of **crossing-over disorder.**

The unequal crossing-over causes duplication of particular part in one chromosome and its lack in another chromosome. A failure may also occur in chromosomes pairing. A change in chromosome structure can also occur in pairing of non-homologous chromosomes and exchange of their sections, or as a consequence of lateral pairing of univalent.

Structural chromosomal aberrations include:

• **deletion** - A portion of the chromosome is missing or deleted. If the end segment is missing, it is called **terminal deletion**, if the aberration is located within the chromosome, it is called **interstitial deletion**.

In newborns, the most common structural aberration is deletion of the terminal segment of p arm of chromosome 5 (Fig. 56), which causes a cat cry syndrome (cri du chat). It is manifested by severe psychomotor retardation and anomaly of laryngeal development; which causes the typicalvoice;

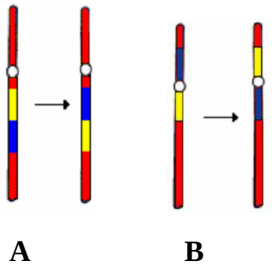


• **Figure 56.** Cat cry syndrome (karyotype).

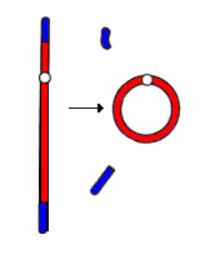
• **duplications** – a part of the chromosome is duplicated, resulting in extra genetic material;

• **inversion** – a portion of the chromosome has broken off, turned upside down and reattached, therefore the genetic material is inverted by 180º. If the concerned portion is without a centromere, it is called **paracentric**; if the inverted section includes a centromere, it is a **pericentric** inversion (Fig. 57);

• **ring chromosome** – telomeres of a chromosome has broken off and its ends form a ring. This can happen with or without loss of genetic material (Fig. 58);



• **Figure 57.** Schemes of paracentric (A) and pericentric inversion (B)



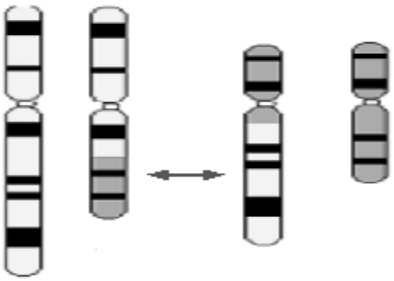
• **Figure 58.** Ring chromosome

• **Isochromosome** – is a consequence of mistake in sister chromatids separation – they do not part from each other longitudinally, but transversely. Two mediocentric chromosomes, one of which consists of two identical long (p) and the other one of two short (q) arms, are the result. In fact it is duplication of one, and deletion of the other chromosome arm (Fig. 59);

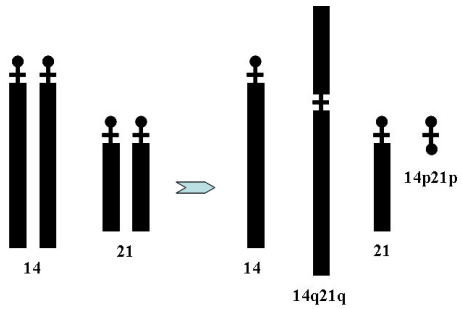
• **Figure 59.** Isochromosome

- **translocations** occur when (after break) a part of one chromosome is transferred from one locality, to another one. There are two main types of translocations, there. In a **simple translocation**, part of chromosome moves to another place of the same chromosome or to another chromosome. In a **reciprocal translocation**, segments from two different chromosomes are exchanged (Fig. 60). A special type of translocation is **Robertsonian translocation** (Fig. 61).

Two acrocentric chromosomes (from D and G groups of karyotype) join together in the centromere and form one submetacentric or metacentric chromosome.



• **Figure 60.** Reciprocal translocation



• **Figure 61.** Robertsonian translocation

Consequences of structural chromosome aberration depend on the type of aberration, its position on the chromosome and its extent. In case of multiplication or loss of genetic information (e.g in translocations and inversions), the consequence may not even occur. In this case it is a **balanced** chromosomal rearrangement, carriers of which may not have any phenotypic manifestations.

If there is a change in the quantity of genetic material, these are called **unbalanced chromosomal translocations**, and carriers have phenotypic manifestations. Unbalanced translocations in children are formed either as *de novo* mutations, or appear as a result of the **chromosomes segregation** during gametogenesis in **carriers of balanced translocations**.

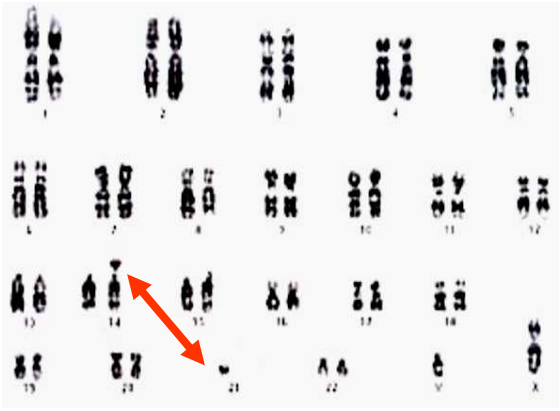
Carriers of balanced translocations form gametes without modifications, gametes with balanced translocations, but also gametes with unbalanced translocations. Fertilization of gametes with unbalanced translocations may cause a condition incompatible with life, or birth of a disabled child.

For instance a carrier of balanced Robertsonian translocation 14/21 (Fig. 62) forms gametes which are:

• normal, without rearrangement of chromosomes;

• without chromosome 14 (after fertilization, a zygote with monosomy 14 is formed; this is incompatible with life and causes misscarriage);

• without chromosome 21 (after fertilization, a zygote with monosomy 21 is formed; this is incompatible with life and causes misscarriage);



• **Figure 62.** Balanced translocation 14/21 karyotype

• with balanced rearrangement (after fertilization, a zygote with identical findings as in a parent – the carrier of balanced translocation – is formed);

• with additional chromosome 21 (after fertilization, a zygote with trisomy 21 is formed – creating a translocation form of Down syndrome, which is clinically equal to the so-called free form (i.e. trisomic) - 47, +21;

• with additional chromosome 14 (after fertilization the zygote has an extra 14th chromosome; trisomy 14; which is a state incompatible with life, and causes misscarriage).

The life-compatible combinations of chromosomes in a balanced translocation 14/21 gamete carrier, after fertilization with a normal gamete:

• combination without chromosomal translocation;

• balanced translocation without subsequent phenotypic changes after fertilization;

• unbalanced translocation containing two chromosomes 21, after fertilization, a translocation form of Down syndrome appear.

Combinations of chromosomes incompatible with life in gametes of a carrier of balanced translocation 14/21, after fertilization with a normal gamete:

• a gamete without chromosome 14, after fertilization a zygote with monosomy 14 is formed;

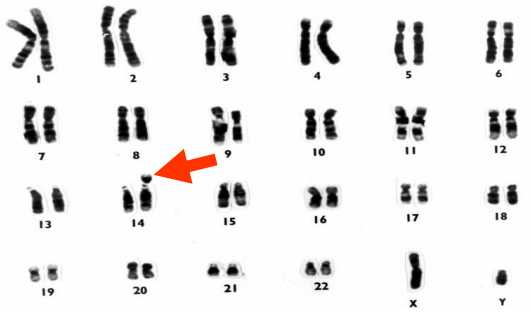
• a gamete without chromosome 21, after fertilization a zygote with monosomy 21 is formed;

• unbalanced translocation contains two chromosomes 14, after fertilization a zygote with trisomy 14 is formed.

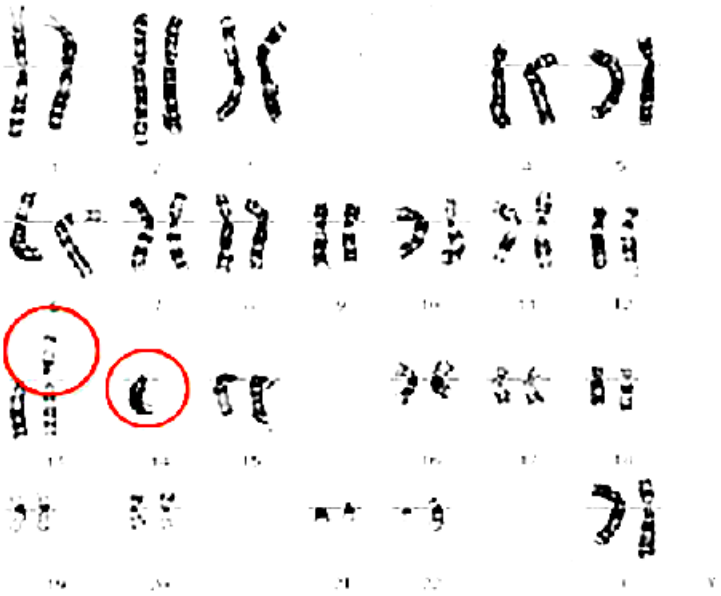
Carrier of a balanced translocation 14/21 (Fig. 63) therefore have a theoretical risk of 33 % that will have a child with Down syndrome (it transfers its balanced translocation to its offspring in a balanced form). Empiric risk is, however, smaller than the theoretical risk (5 – 10 %).

Because Robertsonian translocations can cover all acrocentric chromosomes (e.g. 14/13, Fig. 64), a child with Patau syndrome can be born also as translocated form of the 13th chromosome trisomy.

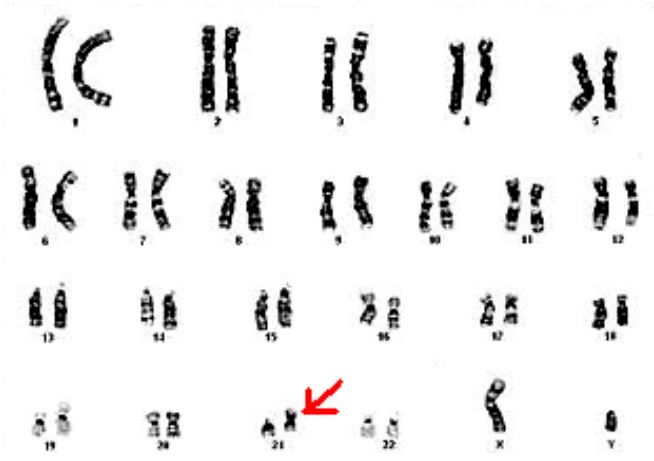
In case of balanced translocation of homologous chromosomes (e.g. 21/21) (Fig. 65), the carrier cannot produce normal gametes – the gametes can be either with both homologous chromosomes in the case of 21/21 translocation. The Down syndrome translocation form is found after fertilization, with 100 % risk or the gametes can be nullisomic, which causes life-incompatible monosomy of autosomes after fertilization. Carrier of a balanced reconstruction of the homologous chromosomes thus cannot have healthy offspring.



• **Figure 63.** Unbalanced translocation 14/21



• **Figure 64.** Balanced translocation 14/13



• **Figure 65.** Unbalanced translocation 21/21

10. Regulation and deregulation of a cell cycle

10.1 Principles of cell cycle regulation

Cell cycle starts with formation of a new daughter cell, and ends with its division into two daughter cells. If all external and internal conditions are accomplished, the cycle repeats. It produces clone (subpopulation) of cells.

In prokaryotes, due to their simplicity, the cell cycle is shortened. The cells are divided immediately after completing of the DNA replication. This direct cell division is called amitosis. The following DNA replication may start earlier than cytokinesis had finished.

If at the beginning of the cycle of a prokaryotic cell differentiation occurred, anabiotic stage – endospore, appear. The cycle continues only after the spores get into optimal environment. After that, the endospores change into a vegetative cell form, capable of division**.**

Eukaryotic cells are much more complicated. They have an extensive and complexly organized nuclear genome. Their DNA is linear, divided into sections and is associated with histone and non-histone proteins (see Chapter 1).

Daughter cells in multicellular organisms have to be, after division, genetically identical (somatic – after mitosis) or with haploid number of chromosomes (gametic – after meiosis).

The somatic (body) cell´ cycle must simultaneously guarantee checking and reparation of all errors in DNA – before replication, during it and also after its completion. If it does not succeed to be done within a time limit, the risk cell is eliminated by apoptosis, so that its malignant transformation does not endanger the entire organism.

For these reasons, the cell cycle of eukaryotic cells of multicellular organisms is governed by a complex system of control and leading (regulatory) mechanisms, which must be in balance.

Cell cycle is divided into a four phases and each of them has a check (control) point – a time-limited period in which, by interaction of regulatory proteins, is checked what previously had happen and have to be done. The cycle is in checkpoint arrested (in order to do corrections) and then it may continue further. If amount of mutations is too big, cell terminates by apoptosis.

Basic principles of management (regulation) of the somatic cell cycle are:

• normal somatic cell will divide only after receiving an adequate stimulus from its surroundings. This stimuli are so-called growth factors, coming either by endocrine or paracrine way. After its connection to the receptor for growth factor emerges a signal, which spreads by cascade of molecular interactions inside the cell. Only independent, i.e. malignantly transformed cells divide independently – without stimulus;

• **signal** (stimulus) for continuation in cell cycle is carried out by implementing of the so-called **cell signal pathways**. The result is usually a targeted gene (genes) expression or influence of the activity (activation or inhibition) of target protein (complex). The signal is gradually spreading (amplifying), which ensures the coordination of various processes that take place simultaneously. Many of them are controlled by a negative feedback or by individual signaling pathways meeting and influencing their course ("cross-talk"). Providing of their presence, activation and inhibition of proteins and their complexes in the cell (nucleus) has a specified time succession, which ensures correct order of the regulation processes.

Activity and quantity of interacting regulatory proteins, is of the utmost importance for proper regulation of particular cell cycle phases. Correct regulation of the cell cycle can only be achieved by a balance of regulatory proteins interactions. If this balance is disturbted, deregulation of cell cycle occurs and the risk of malignant transformation of cells increases.

The main mechanisms of management activities (effectiveness) of proteins involved in the cell cycle regulation are:

• **Control of gene** (genes) **expression**, which ensures proper timing of the protein presence in the nucleus or in the cytoplasm. There are two ways to achieve this in the cell:

a) signal pathway ends with expression of particular gene, which usually encodes the regulatory protein – transcription factor (e.g. c-myc). This protein then provides targeted transcription of certain other genes (in c-myc case, for example, genes for cyclin D and a subunit for ubiquitin ligase SCF). This procedure is used especially in cases of proteins with a major regulatory role;

b) signal pathway ends by expression of genes family, allowing consecutive or mass transcription of required genes. An example is the E2F family of genes for transcription factors – it allows, in a

short time after licensing of S-phase initiation, the expression of genes for all enzymes and regulatory proteins required for DNA replication;

• **activation and inactivation** of proteins which regulate cell cycle:

a) **enzymatic modification** – e.g. aminoacylation and deacylation of histones (regulating access to genes), as well as methylation, which has a blocking effect. These enzyme interventions belong to the s.c. post-translation modifications of proteins,

b) **enzyme subunits assembling** – e.g. in ubiquitin-ligase SCF

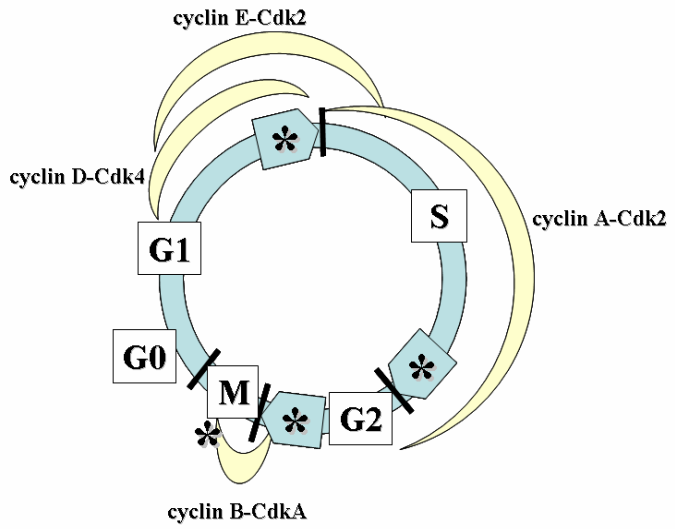
c) **phosphorylation and dephosphorylation** of target proteins, at the site of specific amino acids, changes their conformation (spatial structure), so that they are either activated or inactivated. The result depends on which amino acids are phosphorylated and on which (and on how many) points of the target protein. An example is cdc2 kinase in complex with cyclin B. Phosphorylation of Cdc2 on treonine in position 14 and tyrosine in position 15 are inhibitory. They outweigh the activation effect of treonin phosphorylation in position 160 (or 162, or 164 depending on the cell type).

Enzymes, which acquire phosphate (PO_4^-) from the donor (adenosinetriphosphate or guanosinetriphosphate) and add it to an amino acid with - OH group (serine, threonine or tyrosine) are called kinases. In cell cycle regulation utmost important are:

• receptors for growth factors with kinase activity of their intracellular domain;

• cytoplasmic transmitters – MEK and MAP kinases (mitosis activating proteinkinases), which transmit the signal to the nucleus;

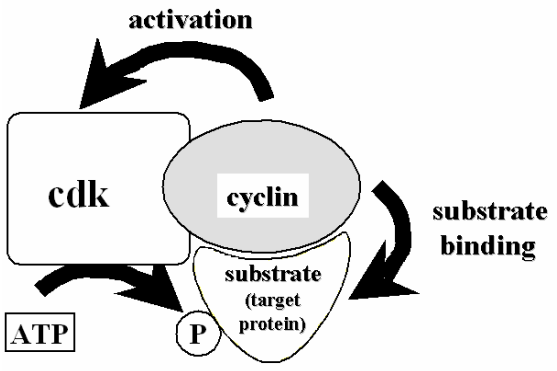
• cyclin-dependent kinases (Cdk or Cdc), which are active only when linked with cyclin and provide transitions to the particular stages of cell cycle (Fig. 66). They are permanently present in cells. More than 10 Cdk are known in humans; of which only Cdk 1, 2, 4, 6 and 7 play an important role in the regulation of cell cycle (others may have additional or regulatory function). In yeast, on which the function of most of them has been studied, they are referred to with the initials Cdc. To activate the Cdk, its link with cyclin is needed; as one Cdk phosphorylates different target proteins, according to which cyclin it is associated with, and vice versa, cyclin may alternate different Cdk - influencing different proteins (processes). In some cases (cyclin A-cdk2 or cyclin E-cdk2) for the activation of Cdk, only the cyclin binding itself is sufficient for Cdk activation, in other cases activating phosphorylation is necessary (cyclinB-cdk2) or adding of another regulatory component (cdk4-cdc37-cyclin D) may be also needed.



• **Figure 66**. Cyclin and CDK in cell cycle (*- check point)

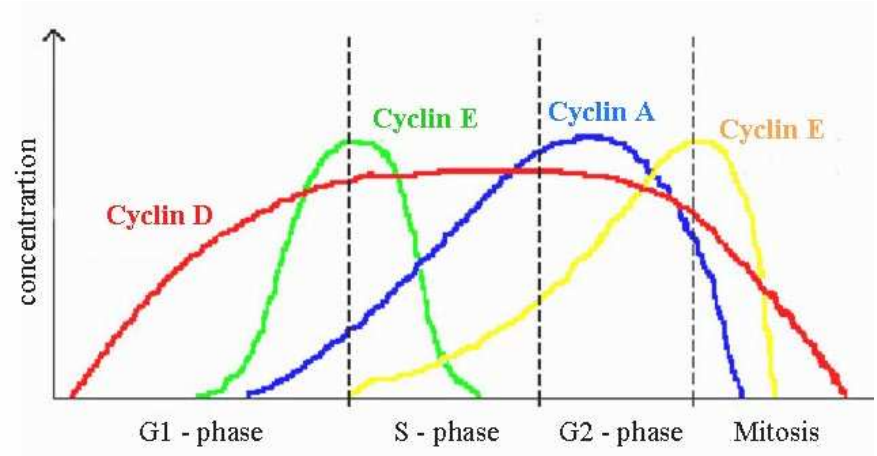
Enzymes, which take away phosphate from positions where it was attached by kinase, are called phosphatases (dephosphorylases). An example is the cdc25 - phosphatase, which also ensures the transition from G2 to M-phase by withdrawing of inhibiting phosphates from treonine 14 and tyrosine 15 on cdc2, which means that activating phosphorylation on treonine 160 * prevails, and this kinase complex with cyclin B triggers mitosis.

d) **activator binding** – e.g. cyclin to Cdk (Fig. 67). Cyclins are proteins that have a role to activate the Cdk.



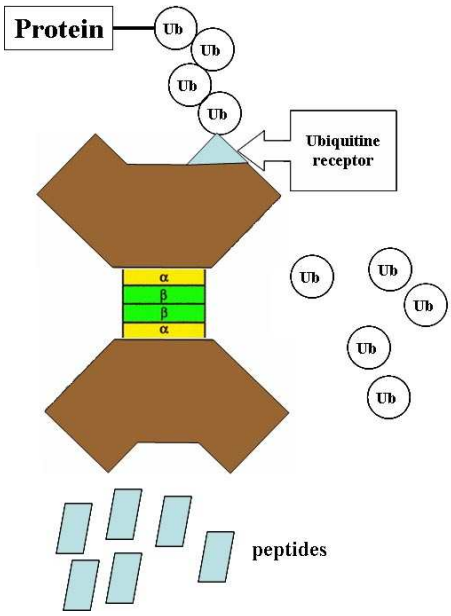
• **Figure 67.** Activation of Cdk by cyclin binding

More than 15 types of cyclins are known in eukaryotic cells, but only some of them are involved in the regulation of cell cycle - cyclin A, B, C, D, E and H. Individual cyclins are synthesized, present in the nucleus and (in the complex with Cdk) active in certain phases of cell cycle (Fig. 68). When completing the task (after ubiquitination) they are degraded in proteasomes. Cyclins A and B contain a destructive domain and cyclins D and E contain a PEST sequence (rich in proline, glutamic acid, serine and threonine), which is typical for proteins with a very short life-span. Dynamics of cyclin has thus a defined course and their elimination is the primary prevention of cell cycle processes repetition, which they regulate.



• **Figure 68.** Dynamics of cyclin during a cell cycle

e) **targeted elimination** – e.g. ubiquitination. The name of mechanism comes from the Latin ubique (everywhere present). This process is a universal method of targeted disposal of unwanted proteins. They are labeled with an ubiquitin chain and are destroyed in protease complex in the cytoplasm, which is called the 28S proteasome. Using of the so-called "E enzymes" - ubiquitin-activation enzyme (E1) and ubiquitin-conjugation enzyme (E2); ubiquitin chain is formed. This is then attached by ubiquitin ligase (E3) to the lysine of protein, which is intended for destruction. Subsequently E3 is disconnected and labeled protein is attached to the ubiquitin-detecting proteasome domain (Fig. 69) by ubiquitin chain. Ubiquitin chain is disconnected and broken-up – so the molecules can be used again. Protein taken by proteasome is broken into peptides, which are then split by other enzymes to recyclable amino acids.



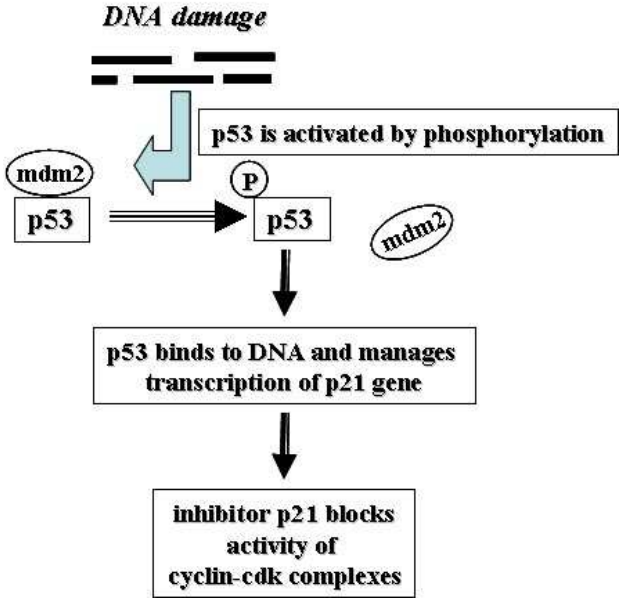
• **Figure 69.** Proteasome

Key players in this type of regulation of proteins activity are E3 enzymes - ubiquitin ligases. Two of them - the SCF and APC/C - are of particular importance in the regulation of cell cycle. SCF is responsible for destruction of inhibitor molecules p27 and p21, which slows down (p27) or blocks (p21) the transition from G1 phase into S-phase. APC/C is part of anaphase-triggering complex in mitotic check point in cell cycle – it has to ubiqiunate for example cyclin B and securing proteins.

f) **binding** of **inhibitor** – e.g. p27 and p21 proteins. Inhibitors of cyclin-cdk complexes activity play a major role in prevention of mutations occurrence and accumulation in the genome of the cells. Those inhibitors block the activity of the complexes, especially cyclin D-cdk4 and cyclin E-cdk2, to prevent them from strong phosphorylation of the protein rb1 and so – the transition from G1 to S-phase.

The protein with a molecular weight of 27 kDa (p27) has a particular role in blocking the activity of cyclin E-cdk2 complex and thereby slows down its contribution to rb1 phosphorylation. This provides, in common conditions, enough time for checking and repairing mutations that were formed in the DNA. p27 molecules are gradually disposed by using SCF, and their inhibitory effect ceases.

While p27 is a regular participant in the control of G1 / S transition; p21 is synthesized only if there is a critical DNA damage. The p21 role is to block (arrest) cell cycle progress and get more time to repair of mutations or to cell termination by apoptosis, if mutations amount is irreparable. Once the reporting system detects DNA damage, by the ATM-ATR system, check kinases Chk1 and Chk2 are activated. This leads to phosphorylation and subsequently activation of p53 protein, which is released from its bond to mdm2 protein and so p53 can bind to DNA. p53 ensures, by its transcriptional domain, transcription of gene for protein p21 (Fig. 70). Predilection site for the linkage and inhibitory action of p21 at the end of G1 phase is cyclin D-cdk4 complex, and in G2 phase (for correction of errors occuring in DNA replication) it is cyclin B-cdc2.



• **Figure 70.** Establishment and function of inhibitory protein P21

10.2 Course and check points of cell cycle

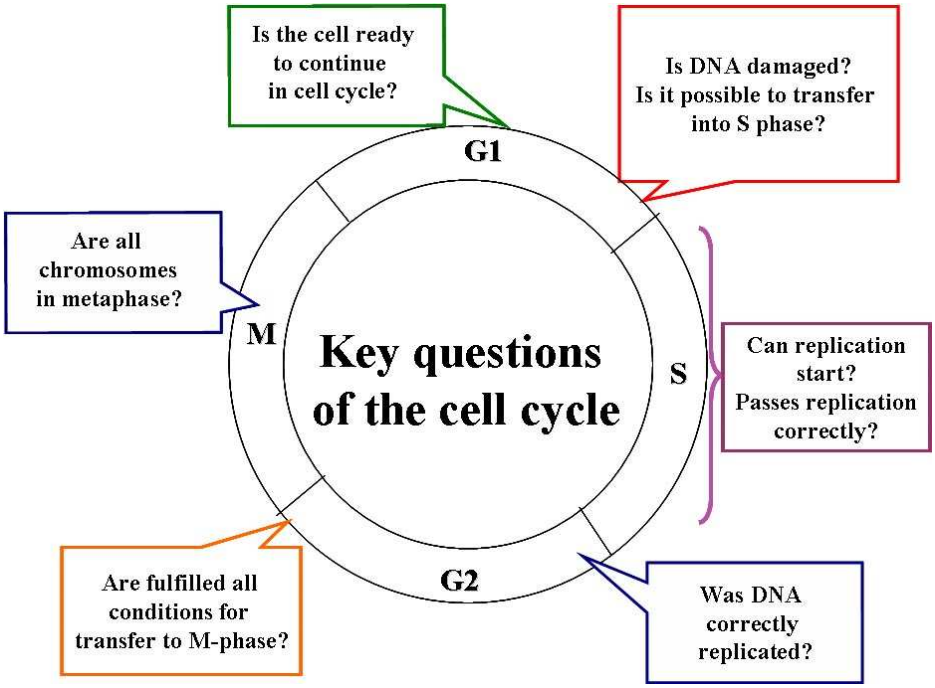
Particular cell cycle phases are characterized by a series of processes that take place in them. Main role of interphase is the protection of DNA quality and the same quantity (volume) of genetic information for both daughter cells must be ensured in mitosis.

The culmination of each series (cascade) of processes in each of the phases of cell cycle is a check point, which has one or several key molecules (players). The interaction of regulatory proteins either activates this key protein – which allows continuation of the cycle or blocks it (them) and stops the cycle; in order to correct mutations. If DNA damage is too big – cell destruction by apoptosis is instructed (see Chapter 11).

The principle is that death of one cell will not endanger the whole multicellular organism, but a clone arising from single malignantly transformed cell can kill this organism. To prevent this, in each phase of cell cycle, there are “questions”, which need to be “answered” (Fig. 71).

The degree of cell differentiation, functional status of cell, environmental conditions (e.g. temperature) etc., determine whether the cell cycle will continue or not. If it continues, the cell installs receptor(s) for growth signals on its surface.

For enabling of the transition from G1 to S phase, important is amount of mutations that happend in DNA. The key player licencing cycle continuation is rb1 protein, which allows synthesis of all proteins necessary for initiation of DNA replication, including cyclin A, which transfers the cell to S-phase. p53 protein plays a key role in arresting of the cycle progression (and possible induction of apoptosis).



• **Figure 71.** Key questions in cell cycle

During S-phase of cell cycle has key (control) role DNA polymerase III, which not only replicates the majority of DNA (synthesizes a complementary copy of the leading strand and Okazaki fragments), but also searches for and repairs errors (in bases' complementarity), which it may do. In case of a severe disruption of S-phase, a p21 inhibitor protein may interfere with it, and block its progress by binding to the cyclin A-cdk2 complex.

After the completion of S-phase (in the G2 phase), it is necessary to check the result of replication and repair errors, which were done in the process. A key player is cdc25, which removes inhibiting phosphorylations and thus activates the cyclin B-cdc2 complex, to induce cell mitosis.

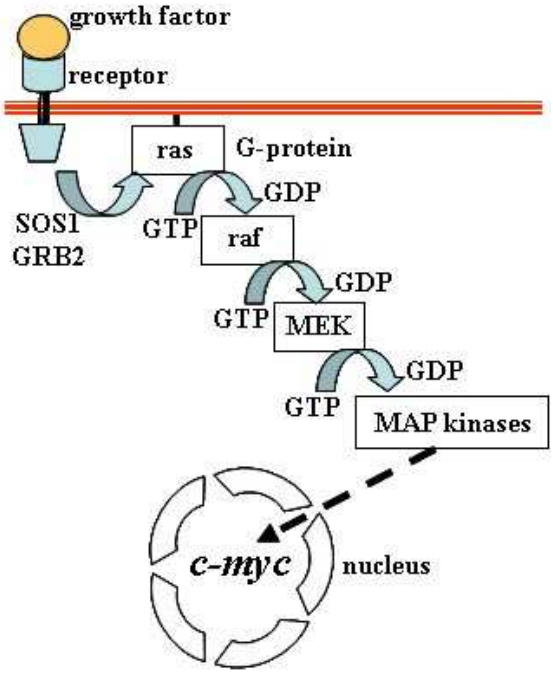
Aligning all duplicate chromosomes in the metaphase plate is essential for equal division of the same volume of genetic information (number and type of chromosomes) into daughter cells. Mad2 and APC/C are particular key proteins.

10.2.1 Check points of cell cycle phases

1st check point – in the end of G1 phase; it is traditionally called the main check point of the cell cycle. It provides licencing for transfer from G1 to S-phase of a cell cycle.

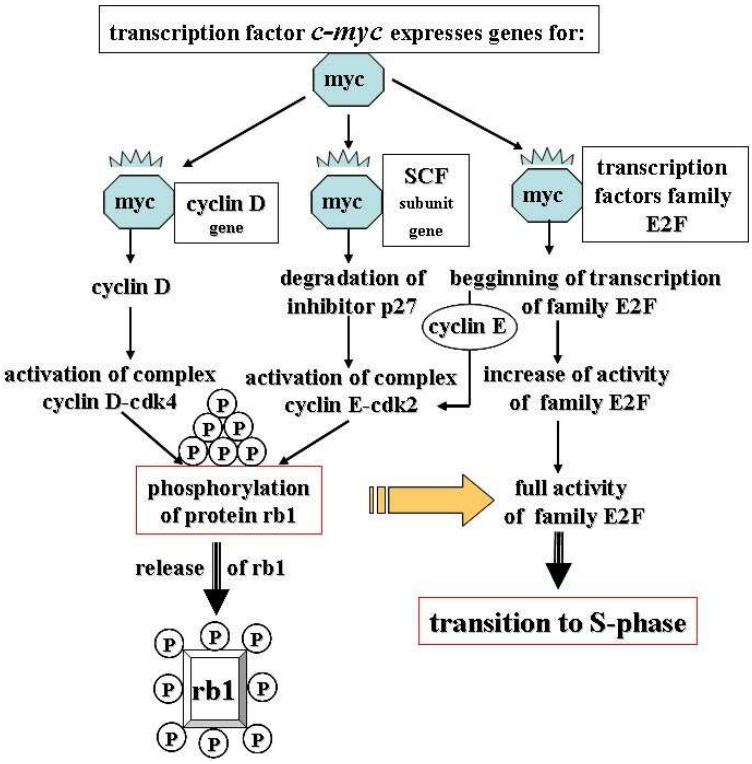
DNA mutations are detected and repaired here. After the repair it is either allowed for the cell to move to S-phase, or the cell is led to termination (due to irreparable mutations) - by apoptosis. Protein rb1 is the key player; it blocks the transcription proteins (factors) E2F gene family. After rb1 release (by strong phosphorylation) from its repressor action, the E2F family of transcription factors not only ensures the synthesis of S-phase cyclin A, but also of all other proteins necessary for replication of nuclear DNA (DNA polymerase I and III, primase, ssDNAbp etc.).

At the beginning of this period of cell life (Fig. 72) growth factor links to appropriate receptor on the surface of cytoplasm membrane. As a result, the receptor dimerizes and its intracellular part (domain) is activated – its kinase activity arises. The result is activation and clustering of assistant proteins (e.g. GRB2 and SOS1), which ends to activation of G-protein K-ras. Activation is preformed in such matter that from the K-ras is removed GDP and GTP (guanosinetriphosphate) which is added to it. K-ras splits-out one phosphate and by it phosphorylate another protein in the cascade – Raf. This subsequently phosphorylases (and so activates) cytoplasmic transmitters – MEK and MAP (mitosis activating protein) kinases, which finally enter signal the cell nucleus, through the pores in its envelope. Here is induced expression of the target gene for key transcription factor – e.g. c-myc protooncogene.



• **Figure 72.** The principle of extra-nuclear part of the signal transmission in cell cycle regulation

Transcription factor c-myc has (during the signal amplification) the task to provide the transcription of at least two genes and one family of genes (Fig. 73).



• **Figure 73.** Intra-nuclear part of the signal path for licensing of G1-S transition

C-myc first provides the synthesis of (particular) cyclin D. Following the formation of cyclin D-cdk4 complex, it begins to phosphorylate the repressor protein rb1. This is, however, not enough to release rb1 from the regulatory domain of transcription factors gene family E2F, which blocks its expression. Complex cyclin E-cdk2 comes to help (to hyperphosphorylation of rb1), but it is blocked by an inhibitor p27. However, c-myc provides expression of the gene for the last SCF subunit, which activates this ubiquitin ligase. This leads to consecutive elimination of inhibitor p27 (if there are not too many mutations and inhibitor p21 does not start action). The result is that the phosphorylation

effect of cyclin E-cdk2 can also be fully expressed, which leads to the detachment of rb1 from E2F regulatory region and thus to the entry into S-phase of cell cycle.

2nd check point – is in the S phase. Precondition for S-phase (DNA replication) is the presence of cyclin A-cdk2 complex, which manages the course of this phase of the cell cycle and blocking of cyclin B-cdk1 complex, which triggers mitosis.

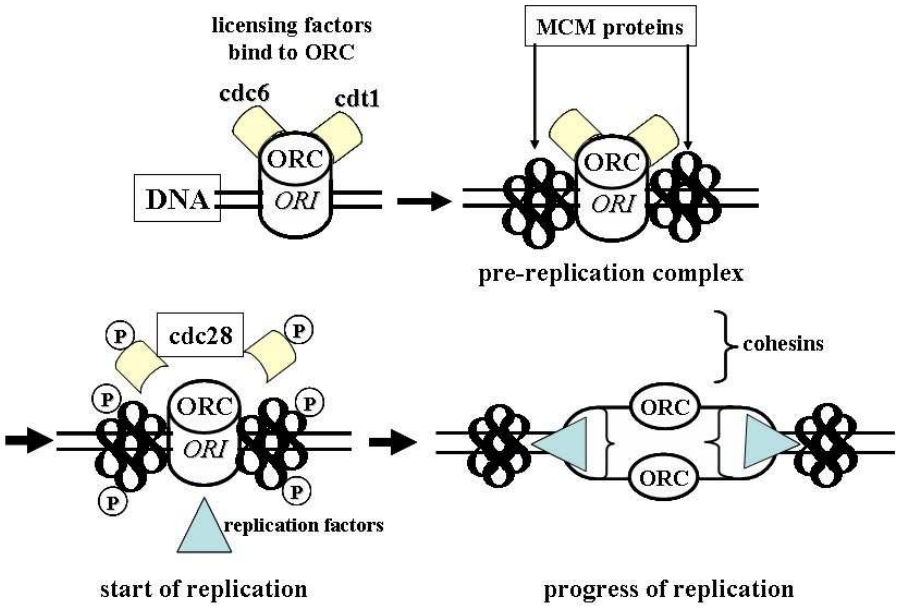
S-phase check point is implemented gradually. There are three locks, with only the last of them is being gradually unlocked - DNA polymerase III repairs mistakes, which it made in the course of replication.

The first lock, which "decides" on whether the replication of DNA in the cell can even begin (even though the move to S-phase has been allowed and all proteins, necessary for replication are present in the nucleus), is the presence of at least one free motif for connection of primer to telomere of chromosome(s). In the overhanging (leading) DNA strand (with 3'→5' orientation), a defined number (usually within 20) hexanucleotide motifs repeats are found – in human cells it is TTAGGG, but they have in different eukaryote organisms large variability.

In initiation of replication, primer binds to this hexanucleotide, so DNA polymerase III can anchor to it, in order to start continuous replication of the leading DNA strand. Primer binding site is not replicated (instead of situation in the synthesis of Okazaki fragments complementary with lagging DNA strand), so overhanging strand is therefore getting shorter – with each following DNA replication. When all hexanucleotides are used up, the cell dies via apoptosis. This is the primary prevention against cells, which have exceeded their lifetime. In embryonic (and in some malignantly transformed) cells, gene for telomerase enzyme is active. This ribonucleoprotein is able to supplement the lacking motifs, so the cell becomes immortal.

The second lock is a licensing of approach to ORI sequences, where replication starts and replication bubbles are formed. Immediately after replication of ORI, proteins of ORC (ORI recognising complex) bind to it. At the same time, security or so-called licensing factors - proteins cdc6 and cdt1, are also attached. Replication cannot start again unless they are removed. So-called MCM hexamers (minichromosome maintenance) proteins are added to both sides of this complex (at the beginning of the G1 phase of the new cycle). This entire conglomerate is then called pre-replication complex (fig. 74).

Replication can begin only when the licensing factors cdc6 and cdt1 are disconnected from ORC (and later ubiquitined), what is done by cdc28 and cdc7 kinases (Fig. 75). ORC is disconnected and replication factors (helicase, primase, DNA polymerase, etc.) form a replication bubble and replication starts. After ORI is replicated, ORC proteins associate with it. At the same time, new molecules of DNA are linked by cohesins. As this replication bubble moves forward, it pushes MCM proteins in front of itself. When two bubbles meet, they link together and MCM proteins dissociate.



• **Figure 74.** Start and course of replication

3rd check point of cell cycle – is in G2 phase, due to permit mitosis initiation. The precondition is post-replication control of DNA and reparation of defects. Phosphatase cdc25 is the key player here.

Similarly, as in the end of G1 phase, also here in G2 phase the system of reporter proteins reveals errors in DNA and transmits the signal to ATR / ATM system, which activates Chk1 and Chk2 kinases, with participation of BRCA1 protein. The result is phosphorylation of cdc25 (on serine in position 216) and binding of inhibitor protein 14-3-3 to this molecule. This blocks cdc25. Just like as in the G1 phase, it is also possible to activate p53 (in case of severe DNA damage) in the G2 phase, and so the cell can be eliminated by apoptosis.

After repair of mistakes done in replication of DNA, inhibitory phosphorylation cdc25 is abolished and inhibitor 14-3-3 is detached. Phosphatase cdc25 becomes active and removes inhibitory phosphates on treonine 14 and tyrosine 15 from the cyclin B-cdc2 complex. Active cyclin B-cdc2 complex can now trigger the prophase of mitosis.

4th check point - in the metaphase of mitosis it is necessary to provide the same volume of genetic information for both daughter cells. It is therefore a process which (except for semi-conservative replication of DNA) ensures identity of daughter cells' genomes. It consists of two parts, which are connected together – i.e. a control of sufficient linkage of microtubules to kinetochores (called SAC - Spindle Assembly Checkpoint) and a permission of the initiation (so-called promotion) of anaphase.

E3 ubiquitin ligase APC/C (anaphase promoting complex - cyclosome) is the key player. Emi1 protein binds to cdc20 and so blocks the APC/C from S-phase until prophase. In prometaphase, it is substituted by RASSF1A protein, in the inhibitory effect. It is released at the end of prometaphase, causing a partial activity of the cdc20-APC/C complex and leads to ubiquitination of, now unnecessary, cyclin A.

At the end of mitotic prometaphase, first phase of the mitotic check point starts - kinetochore microtubules (KM) gradually join kinetochores of sister chromatids. All duplicated chromosomes are gradually moved to the equatorial plane of the cell, by prolongation and shortenings of KMs.

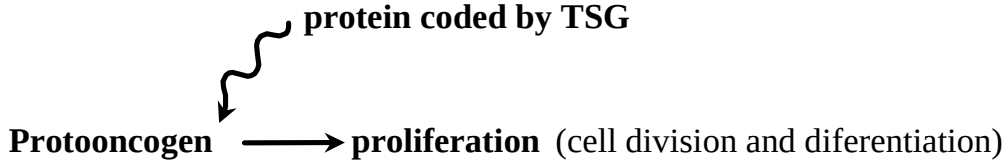
From the functional point of view, it is most important, that Mad2 (mitotic arrest deficient) proteins circulate around kinetochore, where they become phosphorylated. They strongly phosphorylate (in this state) and so block the activity of cdc20-APC/C complex. SAC is over, after every kinetochore is completely occupied by KMs. The consequence of SAC failure is non-disjunction and abnormal number of chromosomes, as described in the chapter on chromosomal aberrations.

As kinetochores are occupated by KMs, mad2 molecules remain unphosphorylated, cdc20-APC/C complex becomes active and removes anaphase inhibitors - cyclin B and securins by ubiquitination. Anaphase can be initiated.

Removing securins allows separase to cut Scc1 cohesin subunits even in the area between the sister chromatids (from the inner side), which leads to the ultimate separation of the daughter chromosomes. Motor proteins (kinesins and dyneins) are also activated and together with depolymerisation of kinetochore microtubules, lead to attracting chromosomes to centrosomes (poles) of cell.

10.3 Protooncogenes and tumor suppressor genes - their role in regulation and deregulation of the cell cycle

Cell cycle **regulation** involves two types of proteins, encoded by these genes – **tumor suppressor genes** (TSGs) with a control function and **protooncogenes** (PO), which ensure progression of the cycle. They are in close interaction and if the cell cycle is well-regulated, there is a balance between them. Their relationship is relatively antagonistic, but proteins, which are encoded by them (the products), interact at each step of the cell cycle (Fig. 75).



• **Figure 75.** The principle of tumor suppressor gene and protooncogene interaction

Many of these genes, as well as their function, have been already described in this chapter (e.g. cyclin, and some inhibitors). The aim of the following text is to give more data and deeper describe the relationship between TSG and the PO and their potential contribution to deregulation of cell cycle with a consequent risk of malignant transformation of cells.

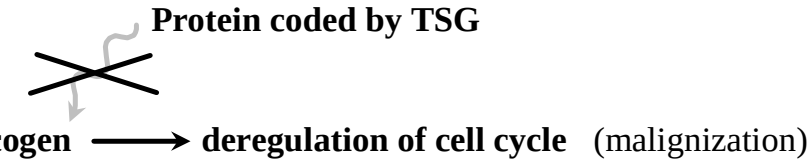
10.3.1 Tumor suppressor genes

These genes code proteins (Fig. 76), which control certain phase of cell cycle, influence signal transfer, allow DNA repair, and manage proper time for expression of certain genes, or direct cells to apoptosis. Number of known TSG is expanding, as cancer research is developing.

TSG	locus	product localization	process
RB1	13q	nucleus	regulation of transcription
TP53	17q	nucleus	regulation of transcription
p73	1p	nucleus	regulation of transcription
WT-1	11p	nucleus	regulation of transcription
NF-1	17q	cytoplasm	regulation of RAS
APC	5q21	cytoplasm	segregation of chromosomes
DCC	18q21	cytoplasm	cell adhesion
MLH	3p21	nucleus	DNA reparatiom
p16	9p21	nucleus	CDK inhibitor
BRCA1	17q21	nucleus	DNA reparation
PTEN	10q23	cytoplasm	lipid phosphatase
TGFbR11	3p22	cytoplasm	receptor kinase

• **Figure 76.** Examples of tumor suppressor genes

Due to the **loss of TSG function** – by mutation or by the effect of viral oncoproteins, which block the activity of proteins encoded by them – **the probability of malignant transformation of cells increases** (Fig. 77), as the control over proteins coded by PO fails.



• **Figure 77.** Scheme of the loss of tumor suppressor gene or the effect of protein encoded by this gene

Gene RB1 (localized on 13q14) has a length of 180 kb and contains 27 exons, with the length from 31 to 1889 bps. RB1 gene encodes protein rb1 (with a molecular weight from 105 kDa to 130 kDa). p105 consists of 928 amino acids. There are currently more than 930 known mutations of this gene. Most of them (80-85 %) lead to an preliminary stop-triplet origin, what gives rise to many different proteins (isoproteins). Known RB1 mutations are scattered from 1^{st} to 25^{th} exon, even affecting promoter region. About 80 % of de novo gametic RB1 mutations are of paternal origin.

Main function of rb1 protein (as has already been mentioned in the description of the check point in G1-S transition) is the repression of regulatory region of E2F gene family of transcription factors. After the repair of mutations in the DNA, rb1 is removed by strong phosphorylation, performed by cyclin D-cdk4 and cyclin E-cdk2 complexes.

Loss of function by **mutation** of RB1 describes Knudson´ theory of the two steps (hits). The first step is deletion (mutation) of one RB1 allele. An individual (in the gametic mutation) and cell (somatic mutation) become heterozygous - having one normal allele (dominant) and the second one mutated (recessive). rb1 protein is a dimer (each allele encodes a monomer) and the heterozygous state of the RB1 gene maintains (as most TSGs) rb1 function. Only after further mutation (step or hit), i.e. the loss of the other allele in the cell; resulting in recessive homozygous state – protein rb1 is not more functional. For this loss of (the last functional) allele, the abbreviation LOH (loss of heterozygosity) is used, and its consequences depend on whether the first loss was a gametic or somatic mutation.

Retinoblastoma – retinal tumor – is an example of tumors, formed in one of the tissues that are most exposed to mutagenic effects of UV light. In healthy humans, this malignancy is extremely

rare and is caused by LOH after previous somatic mutation in the same cell (clon). Therefore, it occurs only later in life, and as a unique tumor in one eye only (unilateral). Conversely, if the individual has the RB1 gene in heterozygous form in all cells of its organism (as a result of gametic mutation), there is a relatively high probability that LOH and tumor formation will occur in one of the retinoblasts in the retina. Therefore, this hereditary (familial) retinoblastoma occurs frequently in children age, in both eyes (bilaterally) and multiple.

Second type of loss of TSG action is **blocking** of the proteins encoded by tumor suppressor genes **activity** by the activity of DNA viruses oncoproteins. These include for example adenoviral oncoprotein E1A, T antigen (in SV40, Ebstein-Barr virus, etc.) and E6 and E7 proteins of serotypes 16 and 18) of human papilloma (wart) virus (HPV). These proteins belong to the so-called early ones and help the virus to completely occupy the cell on molecular level. To force the cell to synthesize all proteins that are necessary for viral DNA replication is a part of this process. To achieve this, the oncoprotein links to rb1 and releases it from repressor bond to regulatory area of E2F family.

The problem is that if the cell survives the virus attack and is at the end of G1 phase, nuclear genome replication may also occur – but without mutation repairs. This leads to accumulation of mutations, which is at the beginning of malignant transformation of cells. For HPV, the situation is more dangerous, because not only E7 releases rb1, but in addition - E6 blocks p53, which directly prevents both the repairs of mutations and apoptosis induction.

TP53 gene is located on chromosome 17 (17p13.1); coding region of gene is 2629 bps long. A large number of mutations of this gene are known. TP53 mutations were found in at least half of the tumors. **p53** protein consists of 393 amino acids. It forms a ternary complex, and each of the monomers has several functional domains, with three of them being the most important. The first is oligomerisation domain, that combines two dimers - each allele encodes one dimer (two monomers) into the tetramer (ternary complex). The second is domain which binds to DNA (DNAbd), with the nature of zinc finger – it binds the p53 into DNA large groove. The third is the transcriptional domain, by the means of which the p53 provides for example, the transcription of the gene for inhibitory protein p21.

The task of protein p53 is, as has been already described, binding to DNA – especially at the end of G1 phase. This consequently suspends the beginning of DNA replication and allows the specific enzyme systems (s.c. **reparation mechanisms**) to become active, as they detect and repair mutations in DNA. If there are too many mutations, it is p53, which induces processes leading to **apoptosis**.

Unlike most other TSGs, mutation of even one TP53 allele disrupts activity of the encoded p53 - aberrant heterotetramer appears. Its ability to bind to the DNA is reduced and its lifespan is extended (from the usual 1hour to 20 hours). Thus mutated p53 loses the ability to determine the time limit for repair of mutations in DNA. It also loses the ability to induce apoptosis.

Individuals, who have the gametic mutation of p53, suffer from the so-called Li-Fraumeni syndrome. DNA repair does not work properly, so even in case of a severe DNA damage apoptosis of risk cells is not induced. This leads to accumulation of mutations of other important TSGs and PO in their cells. First, there is a wide range of benign (non-malignant) tumors. However, it is only a matter of time for LOH of TP53 occurrence in one of their cells, or there is such a combination of mutations of other regulatory genes that the cell is transformed to the malignant one.

Here are few examples of the other TSG.

WT1 gene has the locus at 1p13; consists of 10 exons and has at least four different transcription variants. It encodes a tumor suppressor protein that has the character of DNA binding regulatory protein (DNAbd), which contains four zinc fingers. It is the transcription activator (or repressor) of certain genes and belongs to the key regulatory genes, for e.g. kidney and genitals development. WT1 mutations, in particular in its two areas of alternative splicing, occur for example in Wilm´ tumor of kidney.

NF-1 gene causes neurofibromatosis of the first type. It is located in 17q11.2, consists of 50 exons and has more than 300 kb of genomic DNA. Its function is to inactivate G-protein ras, which is essential for activation of the transmission of signals for cell division into the cell.

APC gene is localized in 5q21-q22, in locus belonging to DP2.5 gene. It has 15 exons and two alternative 5' UTR. Its transcript (mRNA) is 10386 bp long. There are currently more than 120 known mutations of this gene. They were found at FAP (Familial adenomatous polyposis), in Gardner syndrome and other tumors. FAP protein is composed of 2,843 amino acids. This TSG is working as one of the determining factors in the regulation of intestinal epithelium proliferation. It also

participates in the creation of extracellular protein connections (extracellular matrix). Therefore, typical FAP mutations are considered as the basic factor of hereditary (familial) predisposition for colon cancer.

DCC is one of the TSGs, mutation of which was found in the colon cancer. Its locus is on 18q21.3, near the telomere. Protein encoded by DCC has structures that are common to proteins that are involved in cell adhesive connections, and together with them participate in the interaction of cell-cell or cell-extracellular matrix. LOH of DCC gene (or deletion of the terminal part of chromosome 18) belongs to the final ones and rapidly aggravate patient prognosis at development of colon cancer (tumorigenesis). This is due to the disruption of extracellular contacts and facilitates the scattering (metastasis) of tumor cells into the body of the patient.

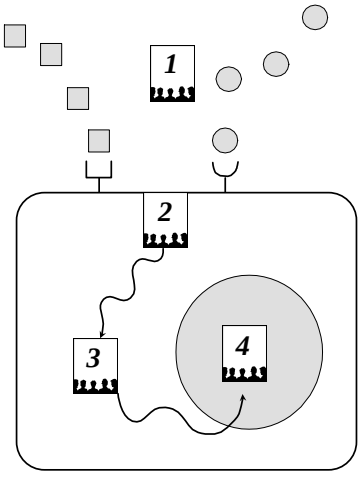
BRCA1 (breast cancer 1) has locus at 17q21, and is composed of 24 exons. It has many different transcription variants; its mRNA length is from 3559b to 7224b. It is the reason of wide range of isoproteins; containing from 680 to 1863 amino acids. BRCA1 mutations (over 300) are of different types and are found in various places in the gene. They are considered to be the main etiological factor in approximately 45 % of genetically determined tumors of breast (mammary gland) and in more than 80 % of genetically determined simultaneous forms of breast and ovarian cancer. As has already been mentioned, the BRCA1 protein plays a key role in stopping the cell cycle in G2 phase, and at inspection and reparation of errors happened in DNA replication. At the same time, the BRCA1 protein has a specific function - slowing down proliferation (as a negative growth regulator) of epithelia in the mammary gland and ovary. Mutations of this TSG lead to a reduction and even failure of both main functions of BRCA1encoded protein, thereby contributing to carcinogenesis, especially in the mentioned organs.

BRCA2 gene has the locus at 13q12.3. The gene contains 27 exons and its coding region has 10987 bps. BRCA2 protein contains 3418 amino acids. It cooperates with BRCA1, especially in repairing of breaks at both DNA strands, and at homologous recombinations. Failure of its function contributes to the emergence of the genetic component of most mammary gland tumors and/or ovarian cancer.

Gene **MLH-1** is located at 3p21.3 and consists of 19 exons, in the length about 100 kb. MLH-1 gene is a typical example of the so-called “mutator” genes. These encode proteins involved in the reparation of DNA, so mutations of these genes indirectly contribute to the increase of (uncorrected) mutations in the cells. MLH encoded protein belongs to the signal path of BRCA1. Mutations of this gene are directly related to some forms of colon cancer, as well as cancers of other organs.

10.3.2 Protooncogenes

They are the type of regulatory genes and code proteins, which direct the progress in the cell cycle. They are also known as the **proliferation genes**, that is, those that manage cell division. They are called as **oncogenes**, if they become the cause of malignant transformation of cells. Protooncogenes (POs) change to oncogenes either by mutation (normal amount of hyperactive protein is produced) or by over-expression (too big amount of normal protein is produced). Mutated POs usually transmit false signals into the cell, indicating continuation of the cycle – with no previous steps in the signal path. Over-expression of particular PO causes abnormal signal intensity in the path, that prevail action of tumor suppressor proteins. The result is deregulation of cell cycle.



Proteins encoded by protooncogenes occur at all levels of the cell cycle (Fig. 78).

• **Figure 78.** Localization of proteins encoded by protooncogenes
1 – mitotic activators, 2 - membrane proteins (receptors and G proteins), 3 - plasmatic transmitters (transmit a signal to nucleus), 4 - nuclear protooncogenes (transcription factors, cyclins etc.)

Growth factors (GF) are mitotic activators – e.g. PDGF, EGF and IGF. They are ligands for (mostly) receptors with kinase activity of their intracellular domain. They are either noticeably specific (acting only on one type of cell), or recognized by receptors of multiple cell types. To the cells are

transferred by the endocrine, but more often by paracrine regulation (e.g hormones in blood or as GF in tissue liquid).

PDGF (platelet derived growth factor) has its origin not only in the mentioned blood elements, but it is also produced by endothelial cells and placenta. It promotes proliferation of connective tissues, smooth muscle cells and also neuroglia.

EGF (epithelial growth factor) is produced in the cells of salivary and other glands. It supports proliferation of mesenchyma-derived cells, epithelium and neuroglia.

IGF-I (insuline-like growth factor) is synthesized in the liver. As a universal growth factor – it assists proliferation of many cell types.

Membrane proteins are divided into two groups - receptors and second messengers producers. It should be mentioned that while the growth factors are still present around the cells, the receptors for growth factors are transported to the surface of cell, only at the end of G1 phase, as the cell is ready to continue in the cycle.

Receptors for growth factors belong to those, which intracellular part (domain) is basically a kinase, mostly tyrosin, such as EGFR (EGF receptor). Activation of these receptors occurs by binding of the relevant growth factor, which usually leads to dimerisation (connection) of receptor subunits, triggering its kinase activity and autophosphorylation.

Receptor for epidermal growth factor (EGFR, ErbB-1 in humans known as HER1) is cell surface protein receptor for protein ligands from the EGF family. It is a member of the receptor tyrosine kinase family and Erb1 subfamily, which also includes HER1 and very similar HER2/c-neu, Her 3 and Her 4. They are activated by binding of specific ligands – EGF and TGFα (transforming growth factor alpha). After the ligand is attached, they dimerise – are transferred from the inactive monomer to active dimer form. This stimulates protein-tyrosine kinase activity of their intracellular part (domain). The result is autophosphorylation of its own tyrosines at positions 845, 992, 1045, 1068, 1148, and 1173. This means that the signal has been transmitted into the cell. To phosphorylation-activated receptor domain bind other proteins and the signal path, which ends in the nucleus, may be initiated.

Mutation that causes either excessive activity or excessive synthesis (overexpression) causes hyperactivity of this receptor and its conversion to oncogene, as has been described in many types of cancer, for example in lung cancer and in glioblastoma multiforme.

Producers of second messengers are proteins that convert the signal transmitted by a receptor into a form that can spread inside the cell and reaches to the target molecules. G-proteins have the greatest significance in regulation of the cell cycle. In particular, it is the Ras family - N-ras, H-ras and the most important is K-ras (locus at 12p12.1, protein has a molecular weight of 21 kDa), which belong to the proteins associated with receptors. Assistance proteins (e.g. SOS and GBR2) activate K-ras by withdrawing of the GDP and adding GTP to it. Activated K-ras is able to activate a serine-treonine kinase Raf, which starts transmitting of the signal (via MAP-kinases) through cytoplasm into the nucleus. K-ras mutations lead to elimination of its GTPase activity; thus GDP (from GTP) is not formed; K-ras remains permanently active and sends false signals to the cell, without being previously activated by the receptor. It is relatively common factor of deregulation, for example at pancreas cancer – where it occurs in up to 90 % of cases. K-ras mutations have particularly substitutional character and lead to a substitution of glycine for another amino acid at position 12 – e.g. for cysteine and arginine (in certain types of lung cancer) or valine (in some types of colon cancer).

Cytoplasmatic transmitters – transmit the signal to the nucleus. In the cell cycle regulation it is a kinase cascade, where they are activated by phosphorylation and activate another protein in the cascade by phosphate. The first is serine-treonine kinase Raf. Kinase Mek (MAP/Erk kinase-1) is next and then follow the other so-called MAP-kinases (mitosis activating protein-kinases). Each of them is activated in the control site (the T-loop) by phosphorylation, what changes its formation and triggers activity of its active center, capable of removing phosphate from a donor (ATP) and phosphorylate another kinase in the signal path. Other enzymes also cooperate with kinases. Kinases may form complexes and bind to scaffold protein MP1. In simplified terms, the task of kinases is to provide (regulate) the expression of certain genes, especially for the transcription factor. Some kinases, however, have the opposite effect – acting as a feedback – and inhibit other kinases.

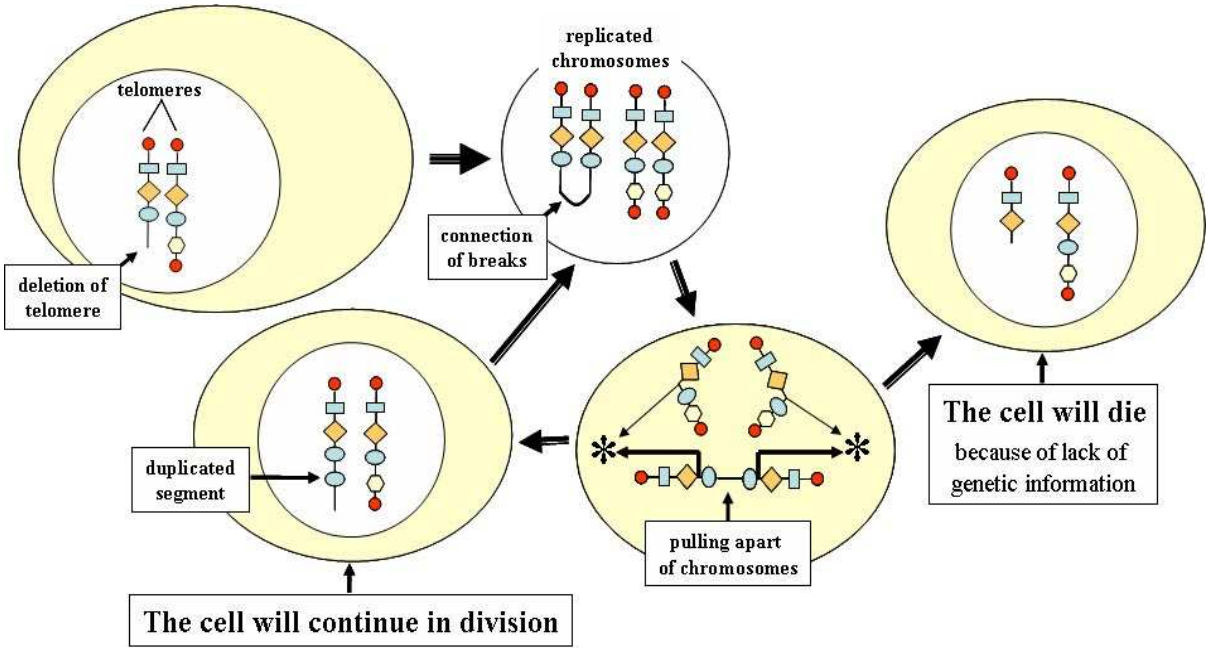
The classic case of converting cytoplasmic transmitter (MAP kinase) into an oncogene is Abelson´ kinase (abl). This happens after translocation (exchange) of q arms of chromosomes 9 and 22, resulting in formation of a typical marker – i.e. Philadelphia chromosome. After chromosome breakage, which is the prerequisite for translocation, only part that remains from bcr gene is the

activation domain and abl gene loses its regulatory region. The result of junction of these two genes (in locus 22q11.21) is the “fusion” gene bcr-abl. It encodes the permanently active bcr-abl kinase, which (in case of chronic myeloid leukemia – CML) sends false signals to the nucleus, to stimulate cell division.

Nuclear protooncogenes – e.g. cyclins and transcription factors. **Cyclins** are essential in regulation of various processes taking place during each of the cell cycle stages, as has already been indicated**.** These are regulatory molecules, which – after binding to the relevant cyclin-dependent kinase **(cdk)** – activate this kinase. It is important to emphasize, that at each stage of the cell cycle another cyclin is present (synthetized), and corresponding kinase is used. Cyclin is degraded (broken up) at proper time. On the other hand, kinases have a longer lifetime, but it is always only one type that is used, which has corresponding cyclins present in the nucleus.

C-myc (locus at 8q24.12-q24.13, protein has a molecular weight of 65 kDa) is an example of transcription factors, which (as already mentioned) has a fundamental role in regulation of expression of genes associated with G1-S transition. After translocation of this gene to chromosome 22 (into the area 22q11.2), in the place of the gene responsible for synthesis of heavy (lambda) chains of antibodies (immunoglobulins), which are permanently and very intensely transcribed, enhancer effect is extends to transferred c-myc and makes its abnormal transcription (overexpression), causing Burkitt lymphoma. But there are also known cases of this lymphoma, where a similar effect occurs as a consequence of hyperactivating c-myc mutations. However, serious consequences are due to amplification (multiplication of allele number) of this protooncogene, known for example in some colon tumors. Amplification may occur as the result of unequal crossing-over, but here is only one extra allele. It is assumed that multiple amplification, resulting in homogeneously stained area (HSR) on chromosomes, which are the result of repeated replication, done by "pulled apart" of chromosome, which had lost its telomere (Fig. 79).

Replicated parts of an incomplete chromosome (without telomere) stick together and chromosome with two centromeres is pulled apart in anaphase, usually in a position of original break. The cell, which has received the short part, dies due to lack of information. Cell, which has received the longer chromosome, continues in other cycles. Lengthening of the part before the break continues until the chromosome obtains a telomere (by a mutation of another chromosome).



• **Figure 79.** Gene amplification following deletion of telomere

Table 8 shows examples of protooncogenes amplification and tumor diseases, at which it has been detected.

• **Table 8.** Overview of protooncogenes amplification and its consequences

protooncogene	**amplification**	**tumor**
c-myc	20-times	leukemia and lung carcinoma
n-myc	5-1 000- times	neuroblastoma, retinoblastoma
l-myc	10-20- times	small cell lung cancer
c-abl	5- times	chronic myeloid leukemia
c-myb	5-10- times	acute myeloid leukemia, colon carcinoma
c-erbB	30- times	epidermoid carcinoma
K-ras	4-20- times	colon carcinoma

In addition to mentioned possibilities of overexpression origin, there are here also a viral, more exactly – retroviral etiologies of overexpression (e.g. c-myc and many other protooncogenes).

Regulatory overexpression may occur if a provirus, formed by reverse transcription of retroviral genome, is inserted in front of PO. LTR (long terminal repeat) sequence on the end of provirus is (within the distance of 3 kb) very potent enhancer of genes that follow. The result is increasing expression of PO, although it may not be dramatic - that's why such retroviruses are called non-acutely transforming ones.

Significantly different situation occurs when the retrovirus genome acquires viral oncogene (v-onc). This can happen in the host cell cytoplasm (when viral genome "travels" to packing place near cytoplasmic membrane of the cell). Because structure of retroviral genome is similar to mRNA – recombination of retrovirus genome with mRNA may occur. Problem appears, when the mRNA was done by transcription of (proto)oncogene (c-onc) of the cell. If the recombination happened in such place of its genome, that the retrovirus shall be capable of reverse transcription and insertion into nuclear genome in a new host cell – amplification happens – except for two own c-onc alleles the cell will have a third allele – v-onc. Serious problem occurs when the cell needs the protein encoded by this PO gene and all of its alleles present in the cell are expressed. LTR sequence on the beginning of the provirus is an extremely strong promoter, which causes extraordinary high over-expression of v-onc. A huge amount of this protein can be suddenly found in the cell, which leads to acute deregulation of the cell cycle. For this effect those retroviruses, which contain v-onc, are called acutely transforming retroviruses. In addition, there are also known cases of multiple amplification of v-onc (e.g. of v-myc in prostate cancer)

Finally, we will sum up the mechanisms by which the POs may be involved in deregulation of cell cycle and subsequent malignant transformation of cells (Fig. 80).

• **Figure 80.** The scheme of conversion of protooncogene to oncogene

Conversion of protooncogene to oncogene is due to the failure of proper function of protooncogene, as a result of mutations or its expression failure.

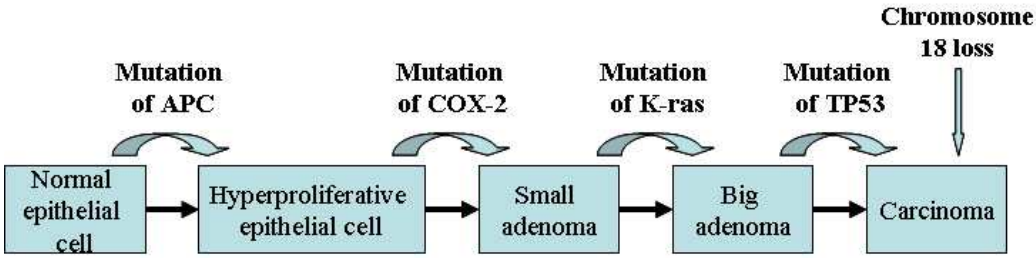
The first type is mutation of protooncogene. An example is so-called "fusion" gene (consisting of two damaged ones), as connection of the abl and bcr genes, in chronic myeloid leukemia.

The second type of a protooncogene conversion to oncogene **is excessive expression** (i.e. overexpression) of protooncogene. The result is abnormally big quantity of particular PO protein with the consequent deregulation of cell cycle. It arises either by regulatory overexpression (increased transcription of the normal number of alleles for a given protooncogene) or by amplification (multiplying of alleles number).

Regulatory over-expression may be caused by transfer (translocation) of protooncogene in the area behind the strong promoter (e.g. Burkitt lymphoma), or by pathological increase in promoter activity (its own regulatory region) of protooncogene (e.g. by the influence of final LTR activity of the provirus inserted in front of the gene).

Amplification may be due to a number of mechanisms of non-viral and retroviral etiology.

Process of cancerogenesis (carcinogenesis) – origin of cancer – is rarely so simply hereditary conditioned, as was indicated in previous cases. Usually it is a consecutive series of steps, starting by TSG loss, and then continuing with PO hyperactivity, followed by loss of TSG etc. Finally, certain gene for proteins of extracellular matrix usually mutates and metastases occur. An example is Kinzler and Volgestein scheme of developing cancer of the colon (Fig. 81)



• **Figure 81.** The scheme of colon cancer development

11. Apoptosis

Apoptosis means death of cells, which does not trigger a response in its surroundings. It was discovered at the beginning of the seventies of the last century, with the development of electron microscopy methods. It is such a discreet process that it is called a "silent cell death". This is reflected in its name - αποπτωσισ, which in the ancient Greek means falling of the tree leaves in autumn.

Termination of cells is event taking place continuously. It is connected to permanent rebuilding and regeneration of tissues, organs and organism. It also has a fundamental importance in remodeling processes during embryogenesis. Therefore, apoptosis is also called programmed cell death.

Apoptosis is also the way how the body protects itself against the risk arising from cells that have already exceeded their life-span or those that are potentially dangerous, for example, because of their risk to be malignantly transformed.

The oldest known mechanism of cell termination is a pathological process - **necrosis,** in which the disintegration of cell (cells) is accompanied by an inflammatory reaction of the surrounding area. One of the main processes that accompany necrosis is breaking of lysosomes, releasing of their enzymes and ingestion of the cell and its surroundings (i.e. autolysis).

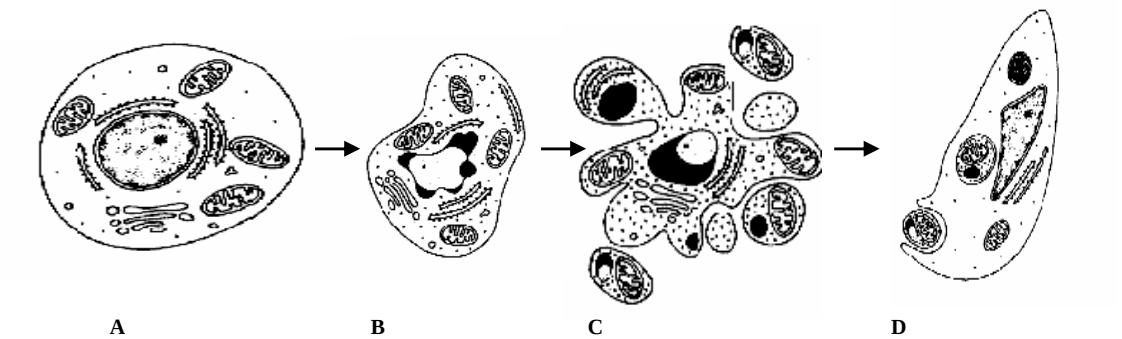
Apoptosis consists of a series of processes that are either induced externally or initiated by intracellular mechanisms. Apoptosis is provided by intracellular mechanisms, which must be specifically activated. They result is decomposition of cellular proteins and DNA, and the termination of cell in the form of membrane vesicles – so-called apoptotic bodies, which contain a different proportion of mentioned components (Fig. 82).

Apoptosis is the process during which the first activity is stopping of protein synthesis, including production of lysosomal enzymes. Subsequently DNAse degrades (splits up) nuclear DNA into short, 300bp-long fragments. Degraded chromatin stays, as a result of nuclear matrix disintegration, attached (aggregated) to the inner face of nuclear membrane. At the same time, proteins in the cytoplasm and organelles are also decomposed. The result is schrinkage (shriveling) of the cell.

Cleavage of proteins is executed by specific enzymes, called **caspases** because they cleave proteins at the site of two amino acids – cysteine and aspartic acid. They are present in the cells in an inactive form – as **procaspases**. There are several types of them and there is also hierarchy among them. The mechanism that triggers apoptosis, first activates one of the main caspases (8, 9, 10 or 12), by building it up from its subunits. So it becomes to be the active proteolytic enzyme, which activates one of the efficient caspases (3, 6 or 7) by its partial digestion.

Apoptotic process continues by forming small vesicles from the nuclear membrane, which contain chromatin residues. At the same time, the cytoplasmic membrane bulges (sc. blebbing) and small sacs, containing residues of cytoplasm, its contents and also the nucleus, are throttled off. This is the process of so-called **apoptotic bodies** formation.

Apoptotic bodies do not show any activity and are ingested by cells capable of phagocytosis, e.g. local phagocytes (macrophages). Material engulfed in the vesicles (phagosomes) is recycled in tissues.



• **Figure 82.** Apoptosis progression scheme (A - termination of synthetic processes in the cell; B - dense aggregation of chromatine on inner side of nuclear envelope and schrinkage of the cell; C - chromatin separates into vesiculesand the surface of cell is blebbing. The cell disintegrates into apoptotic bodies; D - apoptotic bodies are swallowed by surrounding cells (mainly by phagocytes) and decomposed in phagosomes)

Activation (induction) of apoptosis is a process, by which the cell triggers a series of mechanisms and reactions of molecules, ultimately leading to apoptosis. In general, there are two main types of apoptosis induction – exogenous and endogenous.

Exogenous activation starts after binding of signal molecule from extracellular environment to a specific receptor on the cell surface. This type is common for example in the development and action of specific immunity cells (T lymphocytes). FasL (Fas ligand) or TNF (tumor necrosis factor) are here the ligands. Their binding leads to trimerisation of three subunits of a so-called death receptor, and to activation of its intracellular domain. FADD (to Fas Associated Death Domain proteins) are added to it. These serve as an effector - attach themselves to procaspase 8 subunits and assemble a functional heterotrimer out of it. Caspase 8 then activates DNAse and efficient caspases, especially caspase 3.

Endogenous activation of apoptosis means, that signal inducing apoptosis arise inside the cell. The most important part of endogenous activation of apoptosis is related to DNA replication, thus mainly to accumulation of mutations in DNA and to "consumption" of primers binding motifs in telomeres. They are associated with p53 protein which was described in the cell cycle and its regulation.

Protein p53, through the system (cascade) of auxiliary proteins, releases **bcl2** protein from its association with the **bax** protein channel in the outer membrane of mitochondria. This then allows releasing of **cytochrome c** enzyme from the space between the outer and inner mitochondrial membranes, co cytoplasm. This enzyme binds to **Apaf1** protein (apoptosis activating factor 1), and together they create **apoptosome** complex, which activates caspase 9. This enzyme activates the subordinate caspases (3, 6, 7), which cleave cellular proteins. In addition, DNAses that cleave nuclear DNA are activated.

Another type of endogenous activation of apoptosis is the consequence of a toxic shock, when the detoxification mechanisms of endoplasmic reticulum are unable to master toxic compounds (e.g. radicals). There is Ca^{2+} ions and calpain excretion, leading to procaspase 12 activation; and subsequently it will manage already described processes.

The main functions of apoptosis are:

• programmed cell death:

a) the termination of cell which are over their life-span (depleted telomeres),
b) selection of cells (e.g. gametogonia and developing immune cells)
c) nidation and embryogenesis;

• controlled (silent) cell death in protection of multicellular organism against:

a) risky cells (e.g. malignantly transformed),
b) alien cells (e.g. parasites and transplants).

The main functions of apoptosis in embryogenesis are selections of defective (suspicious) or unnecessary cells, and remodeling of structures during embryogenesis. Physiological course of embryogenesis, in fact, depends on correct timing and spatial regulation of the ratio between cell proliferation and apoptosis.

Apoptosis is crucial in the development of heart, limbs (especially fingers), intestine, brain, excretory system and genital organs. Inadequately large or small activity of apoptosis in the development of these structures leads to congenital (inborn) defects.

Apoptosis is involved in many processes in the development of heart, but its failure has the most serious consequences in remodeling of ventricular septum and formation of primary and secondary septa between atria. This is a serious problem, since the failure of proper development of these compartments are responsible for nearly one-tenth of all inborn defects found in live newborns.

Apoptosis has essential remodeling importance in the development of extremities structures during the progression of apical ectodermal ridge. Examples are defects in the development of hands and fingers. The excessive (or prematurely started) apoptosis during the development of fingers causes polydactyly, and reversely, in its low intensity (or late entry), syndactyly is present.

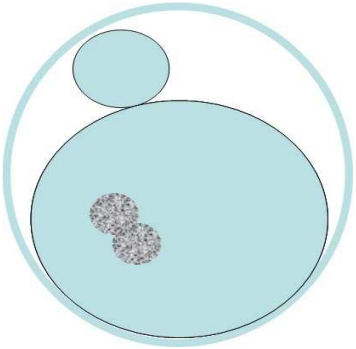
During development of intestine, especially in joining together of parts originating from mesoderm and endoderm (e.g. stomach to duodenum and sigma to rectum) apoptosis failures play role in the emergence of atresia (closure), duplication (doubling) and stenosis (narrowing) of bowels. Although inborn defects of the digestive system make up only 2 % of all reported IDs, most of them are life threatened atresias of oesophagus and rectum.

During brain development apoptosis stepwise terminates up to 50 % of originated neurocytes. First reason is adjusting to the number of target cells to which new-appeared cells may connect. Second reason is the permanent rebuilding of the brain, in the process of development, and in creation definitive connections of brain cells.

Apoptosis is also an important process in development of genitals. It is used in forming of a single cavity of the uterus and vagina and its disorders are responsible for the risk of duplication and forming of septa in these organs. In the vagina, insufficient apoptosis may cause its atresia. Another problem is development of masculine phenotype of the genitals, particularly the progression of urethra. Apoptosis is responsible for creating the “tunnel” between the corpora cavernosa, in which the urethra growths to the end of glans. In the case of excessive apoptosis, preliminary outfall (meatus) of urethra will happen. This is the origin of most common ID - hypospadia, which is recorded in more than 20 % of live-born boys.

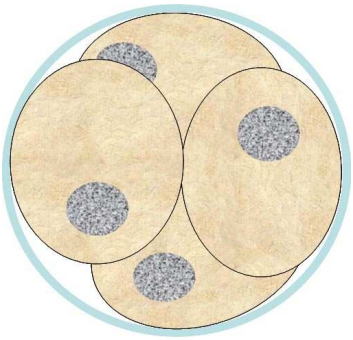
12. Introduction to genetics of development

From a functional point of view intrauterine development is divided into four stages - the pre-implantation stage, blastogenesis, embryogenesis and fetal period. They not only differ by the content of ongoing processes, but especially by the type of pre-programming of genetic information usage.



Fertilization occurs at the beginning of the **pre-implantation stage.** 2nd meiotic division of an oocyte is completed within 11 hours and the second polar body is formed (Fig. 83). Merging of paternal and maternal pronuclei and creation of a zygote takes place within 24 hours. Zygote is surrounded by a thin layer - zona pellucida, whose primary importance lies in preventing of repeated sperm penetration.

• **Figure 83.** Formation of a zygote



Zygote begins to divide and the whole complex travels in Fallopian tube to the uterine cavity. Daughter cells, as results of mitotic division of a zygote are called blastomeres (Fig. 84). After their division they cannot grow, their space is defined by zona pellucida; opposite - their dimensions are reduced. It is considered that this is one of the principal moments, which prevents differentiation of blastomeres. They are (the same as zygote) totipotent cells, and each one of them may give rise to a whole new organism (after separation) – as it is known in monozygous (identical) twins.

• **Figure 84.** Blastomeres

Cluster of equal cells (surrounded by zona pellucida) – **morula** – is formed by blastomeres division during their passing through the Fallopian tube. After it enters into the uterus cavity, zona pellucida ruptures and releases its content. This is the first signal for cell differentiation to begin. The result is formation of a structure with one cavity – a blastocyst. The following period is called **blastogenesis.**

On one side of a blastocyst, there is a cell cluster (called inner cell mass). By this side (pole) the blastocyst attaches to the endometrium (a mucosa covering cavity of uterus) and starts penetration into it. This process is called **nidation**. It is influenced by substances excreted from blastocyst (and later the young embryo), which induce apoptosis in endometrium, and also other its reactions, e.g. its proliferation and formation of new blood vessels. Another cavity is then formed in the inner cell mass (this object is then called gastrula). Embryoblast is formed from the cells between those two cavities and it first consists of ectoderm and endoderm. Later, mesoderm grows between them and a so-called embryonic disc is formed. This is a key moment in terms of further development. Primary pre-programming of differentiation happens here. Each cell receives the characteristics of the germ layer from which it has originated and can be further differentiated only to specifically defined types of cells - as showed experiences with stem cells.

Further development of all organs and organ systems continues in **embryogenesis.** Complex processes, described in details in embryology, and most of which are genetically programmed, is reached the state that from the embryonic disc with a diameter of 0.2 mm, finally 23 mm long embryo is formed. This stage ends with the development of external genitals and separation of fingers.

Fetal period is of varying duration – in different species of mammals and ends with birth. In this period the fetus further develops, grows and it prepares for (relatively) independent life after birth.

The most important development processes take place in embryogenesis. Also for understanding the management of cell differentiation, there is a very useful argument that the population (clone) of cells, created by a mitotic division of one pre-programmed cell, is a basic unit of embryonic development. This population must be (in sufficient number) in certain time and at the proper place in the embryo – and act (behavior) as it is expected.

Theory of elementary developmental processes describes four basic actions of mentioned populations that act in embryogenesis (proliferation, distribution, integration and reduction of cells).

Proliferation is division and differentiation of cell in embryo. The pre-programming of cells is fully exercised here, not only to create schemes for managing the genes expression and the variable use of genetic information, but also to prepare cells to be able to receive signals for differentiation from extra-cellular environment, thus inserting necessary (in types and number) receptors on their surface.

Distribution of cells, as change of population's position in space, is either active or passive. Only some cell types migrate actively, e.g. components of the future immune system or gametogonia. Usually the change of position is achieved by changing the proliferation activity of the population itself or of the surrounding populations. Often this is done with the cooperation with apoptosis, which provides necessary space.

Cells integration means physical, but mostly the information contact. Physical contact (direct or through the proteins of extra-cellular matrix) is an essential prerequisite for cell division. But, as shall be described on SHH as an example, it is not only the proper production of differentiation regulating protein that is essential, but also its undisturbed transport to the target (point of action).

Reduction of the unnecessary cell by apoptosis is a legal process and details of its role and defective effect on development are described in the respective chapter.

Differentiation is a condition where cells have the same genome, but they differ in phenotype. In multicellular organisms it is controlled by specific regulatory proteins (e.g. SHH) and by (pre)programming of gene expression. The basic mechanisms of differentiation are variable expressions of genes and alternative usage of genetic information.

Variable expression of genes means that different genes are used (expressed) in different cells. It is provided by regulatory proteins, which are under control of paracrine incoming signals. The cascades of consecutive expressions of genes are controlled by morphogens (such as SHH, HOX and PAX). Combinations of particular genes in morphogen' families are pre-programmed in the early periods of embryogenesis. In addition to the main pathways of expression (such as affiliation to the germ layer), different chemical signals can trigger the activity of other genes, but only in extent permitted in the cell. Use of other genes is blocked.

Alternative usage of genetic information is an explanation of the fact that although there are over 26 000 genes in the genome, there are more than 100 000 different proteins found in our bodies. Principle is that the properties of proteins depend only on their primary structure, i.e. the order of amino acids, they are composed of. Branch of molecular biology, which studies this field, is called "proteomics". With several types of interventions (alternative start of transcription, alternative splicing RNA editing and post-translation modification of proteins), various mRNAs can be obtained from the same gene and consequently different proteins are obtained, as well.

Alternative start of **transcription** means that there are more than one starts of transcription in the gene and regulatory proteins decide which of them is used. The results are different lengths of mRNA (and subsequently also proteins), which have different beginning and the equal end. Typical example is the longest human gene – for dystrophin. It is entirely transcribed only in the cells of cerebral cortex. About half of it is transcribed in the retina and only one-fifth in the neuroglia cells.

Alternative splicing allows pointed splicing-out (in maturation of pre-mRNA) copies of exons – together with introns. The result of this processing is mRNA (and proteins), which differ not only in the length but also in the number of exons, they contain. An example is calcitonin gene, which has 5 exons. While in the thyroid gland mRNA contains first four exons and the fifth one is spliced-out, in the nerve tissue the fourth exon is removed and the fifth one is present.

mRNA editing– by targeted enzymatic action one of the coding triplets (codons) is changed into a stop triplet, or to sequence activating poly(A)polymerase, which prematurely synthesizes poly-adenyl tail in mRNA. In both cases the results are shortened mRNA (encoded proteins), which has equal start, but a different end. An example is the gene for lipoprotein apoB. Entire gene is transcribed in the liver, and apoB100 protein (composed of 4,536 amino acids) is synthesized. In mucosal cells of intestine in the mRNA nucleotide 6666, cytosine is enzymatically replaced with uracil, so UAA stop triplet is prematurely formed and a protein apoB48 is synthesized, which contains only 2,152 amino acids.

Post-translation modification of proteins (mainly in the endoplasmatic reticulum and Golgi apparatus) leads to further variability of diverse proteins.

Proteins in the cell are associated by enormous amounts of interactions and primary increase of their variability by the above-described ways allows even more diverse relationships, and ultimately a high variability of differentiated cells.

For a very long time it has been known that the main stages of vertebrates embryonic development are almost identical. Later it was discovered, that the principles of genetic management of development and differentiation of cells are noticeably conserved, and that they are general, since insects. Knowledge, that some disorders of development have single-gene type of inheritance (e.g. autosomally dominant in brachydactyly), is more than 100 year old. But only discoveries in the eighties of last century allowed understanding of the principles of genetic management of development. Namely these were the discoveries of bithorax and antennapedia in Drosophila fruit flies.

In the case of bithorax it was the consequence of doubling of genes, governing the entire development of the thoracic segment of Drosophila´ body. In antennapedia it was a qualitative disorder – mutation of genes governing the development of head extremities (antennas) to thoracic ones.

These new discoveries led quickly to understanding, that there are genes and gene families that rule embryonic developments in Drosophila, particularly in the anterio-posterior axis. Later, their paralogs (analogous genes) were discovered in mammals and humans, as well.

At present it is supposed, that embryogenesis is controlled so, that certain cells of the embryonic disc (e.g. in the anterior lip of neuropore) produce and paracrine way excrete essential regulatory molecules, called inductors. They then act on other cells. Their effect is in the activation of both the cells that produce proteins as regulators of differentiation (e.g. SHH) and the activity of so-called gene families of morphogens (e.g. HOX) in other cells.

The presence and role of **differentiation proteins** of the HH (hedgehog) group was, as it often happens, found only after the discovery of their mutations in drosophila, which led to serious disturbances in the development of their embryo. Later, these proteins (and genes) were also described in humans. Among them the most important for development of the embryo is **SHH** (Sonic hedgehog) protein. It is produced by specific cell populations (centers), from which SHH molecules spread by diffusion into tissue liquid and by active movement of embryonal body fluids spread into it. This creates concentration gradient of molecules, i.e. in different parts of the tissue there is a different number of regulatory molecules acting on target cells. In order to exercise the differentiation and different effect, there must be specific receptors on the cytoplasmic membrane surface, able to evaluate not only the signal quality (ligand type), but also the quantity (number of acting molecules). These are specific receptors (called patch), which have a greater number of binding domains, and the type of signal transferred into the cell depends on the number of domains on which bind (are occupied by) signal molecules.

A typical example of the impact of differentiation regulator molecules on cells' behavior is the formation of neural tube – its occlusion and differentiation of motor neuron.

Center releasing SHH is found on the edges of neural crest. Here is the highest concentration of SHH, which leads to the proliferation of surrounding tissues and to neural tube occlusion. This also explains why the sensitive nerves enter the spinal cord by dorsal roots. Ventrally, the SHH concentration decreases, leading to differentiation of motor neurons on the opposite side (ventral) of the spinal cord.

SHH is also involved in the development of many other body structures. An example of its autosomally dominant mutation (that causes a reduction in its expression by 50 %) is holoprosencephaly. This is a disorder characterized by defects in the development of the face, brain and neurocranium. It has a variable expressivity – from cyclopia and anencephalia, cleft lip and palate, to relatively mild level of disability – a typical cone-shaped head, closely positioned eyes and single central incisor.

Morphogen families are genes families for transcription factors. They have a typical character of genes family – one common promoter (LCR – locus controlling region) governing the progression of gene expression successively - in a row. This is considered to be the main reason for the fact that the embryonic development progresses in one direction and cannot be "turned back" (to repair mistakes).

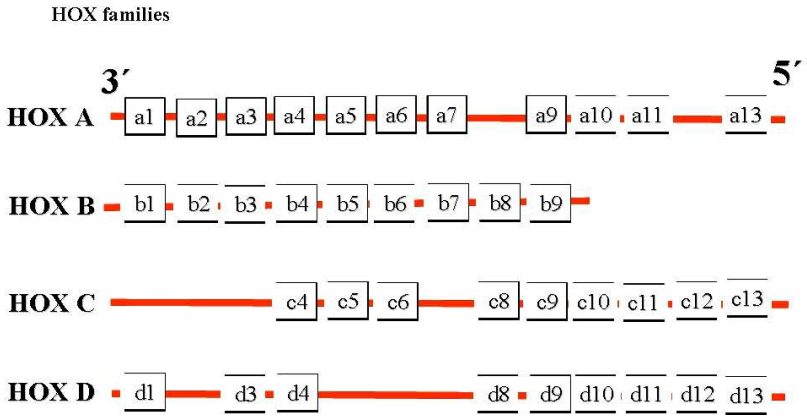
The most known is family of HOXes (boxes of homeotic genes). They include so-called homeiogens – highly conservative and the longest known regulatory genes. They have a length of 180 bp and code 60 amino acid homeodomains of DNA binding proteins that bind to the DNA large groove, before the gene. So they participate in both chromatin remodeling and regulation of selected genes expression, mainly from T2F family of transcription factors.

There are 38 different types of HOX genes associated into 4 families (table 9).

• **Table 10.** HOX families in human genome

HOX	Types	N. of genes	Locus
A	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12	11	7 p 14 - 15
B	1, 2, 3, 4, 5, 6, 7,8, 9	9	17 q 21 - 22
C	4, 5, 6, 8, 9, 10, 11, 12, 13	9	12q 13
D	1, 3, 4, 8, 9, 10, 11, 12, 13	9	2 q 31-32

Transcriptional factors coded by HOX, guide embryo development in the anterio-posterior direction. They are involved in the development of each structure in their complicated combinations and interactions (Fig. 85).



• **Figure 85.** HOX families

Examples of the consequences of their mutations are disorders of fingers development - polydactyly, syndactyly and synpolydactyly due to d13 gene mutation, which is one of the latest activated, during development of extremities.

In addition to HOX, other morphogens are involved in the development of different structures, for example from the PAX group, which also code on DNA binding regulatory proteins. Unlike HOX, these are spreaded across the genome (table 10).

• **Table 11.** Distribution of PAX in the genome.

PAX	Locus
1	20 p 11,2
2	10 q 24.3 – 25.1
3	2 q 35
4	7 q 32
5	9 p 13
6	11 p 13
7	1 p 36
8	2 q 12 - 14
9	14 q 12 - 13

PAXes are intensively studied and here is the list of roles of some of them:

- **PAX 1** plays a role in forming of the segmented structure of the embryo, especially in the rostral-caudal (later cranio-caudal) axis;

- **PAX 2** plays a role in the differentiation of kidney cells;
- **PAX 3** is exprimated during early neurogenesis. Its defect leads to a formation of a so-called Waardenburg syndrome (deafness and abnormal development of skin and eye);
- **PAX 5** is also known as BSAP and is included in the differentiation of B-lymphocytes, the development of central nervous system and spermiogenesis;
- **PAX 6** (oculorhombin) is a transcription factor important in the development of the eye and nose. Its defect causes aniridia (lack of iris), as well as autosomally dominant inherited partial absence of the iris.

13. Teratology

Teratology is an interdisciplinary scientific branch that deals with the study of inborn (developmental) defects, their causes and mechanisms of their origin. Its name comes from the 18th century and is derived from the Greek word teraton – monster.

Inborn defects (ID) are postnatally found deviation of intrauterine development, which is out of normal range of trait´ phenotypic variability - of a structure or function. Variability is determined by epigenetic factors, which act randomly, in fluctuating quantity and intensity.

This definition has been extended for experimental testing of potential harmful factors (teratogens) so, that it is needed to prove that, there has been a statistically significant increase in the frequency and/or severity of known ID at model organisms, in relation with the administration of the tested agent.

Sever disorders of intrauterine development are a topic of a separate chapter. These disorders lead to miscarriage of an embryo (fetus). We call them developmental defects, because pregnancy had not been finished by physiological delivery.

For each developing structure, the frequency of its pathological forms (IDs) is known. In European population, ID are diagnosed in approximately 2 – 3 % of live newborns. This value is affected by the method of recording and also by fact, what is considered to be ID. The incidence of ID shows significant regional differences not only in their number but also in the proportion of impair of various organ systems.

In IDs are distinguished so-called minus forms (absence of a part or the whole structure – e.g. anencephaly) and plus forms (more structures – e.g. polydactyly).

Various names are used for ID in clinical practice, such as malformations, anomalies, atypical growth, defects, developmental deviations, etc.

Two main groups of causes of intrauterine developmental disorders (origins of ID) are recognized:

- genetically caused diseases and syndromes, inherited or de novo mutations. It is estimated that they take part in etiology of ID not more than 25 %. Here belong – single gene inherited (e.g. holoprosencephaly), multifactorial (e.g. cleft lip an palate) and chromosomes aberrations syndromes (e.g. trisomy 21) etc.;
- influence on cells and their relations in an initially normal embryo (teratogensis). Developmental disorders can be induced by following groups of reasons:

a) mutagen action in some cells of embryo, that causes somatic mutations and harm their further role in development;

b) affection of the genes expression in embryo cells;

c) negative effects on intracellular communication of embryonic cells and regulation failure. This group is very large; it includes e.g.: lack of energy sources, inhibition of synthesis or enzymes' function, osmotic balance disorders, disturbing of membrane integrity and/or its function, cell communication (physical and paracrine), but also oppression (strangulation) damage (amniotic bands), etc.;

d) harmful factors or discomfort for embryo (fetus) from organism of the mother (e.g. endocrine disorders and malformations of the uterus);

e) infectious diseases during pregnancy (e.g. prime infections by rubella virus or by parasitic protozoon Toxoplasma gondii).

Above listed etiological causes are studied by teratology. Known factors (e.g. factors from the mother, infectious agents, chemicals, etc.) are involved in the etiology of ID in 10 % of cases. Reasons for the remaining 65 – 70 % of ID are not known (or till not identified).

Teratogen is a factor from the external (extra-embryonic) environment, which, after passage through transport channel, causes (or conditions) adverse affecting of embryogenesis in utero, with the consequent origin of ID.

Teratogen effect depends on:

- the period of embryonic development (so like the embryo is "younger", the teratogen induces more severe disorders)
- embryo sensitivity to harmful factors (within the intra- and inter-species variability, as described in chapter Multifactorial inheritance);
- teratogen dose and duration of exposure;

• mechanism of its action (including potential or inhibitory effect of different factors combined together and having effect at the same time);
• transport channel – the path, which teratogen have to pass, till acts to embryonic target structures. For chemical (mother´ metabolism) and biological (mother´ immune system) teratogens are crucial.

Types of teratogens:
• physical (e.g. penetrating and ionizing irradiation);
• chemical (e.g. drugs, xenobiotics, toxic agents, mutagens);
• biological (certain viruses, bacteria, protozoa).

Table 12 shows examples of the best-known drugs, whose teratogenic effects were discovered, and administration of these drugs to pregnant women is prohibited.

• **Table 12.** Examples of teratogenic drugs for humans

Isotretionin	craniofacial malformations, neural tube defects, cardiovascular defects
Lithium carbonate	various malformations of heart and arteries
Methotrexate	numerous malformations of face, skull, extremities and spine
Phenitoin	fetal hydantoint syndrome: intrauterine growth retardation, microcephalus, mental retardation, inner epicanthic folds, eyelid ptosis, deep nose bridge, phalanx hypoplasia
Thalidomide	abnormal development of limbs or limbs are missing completely, systemic abnormalities
Trimethadion	retarded growth, V-shaped eyebrows, low earlobes, lip and/or palate clefts
Valproic acid	craniofacial malformations, neural tube defects, frequent hydrocephalus, heart and skeleton defects

For the purpose of experimental teratology, i.e. study of the mechanisms of ID origin and testing of potential teratogens, a **theory of elementary morphological processes** (EMP) done by pre-programmed populations of cells was established (as described in chapter Genetics of development).

So like more active EMPs are - so more sensitive and responsive the embryo is - towards the teratogen activity. This explains the phenomenon, when sometimes one teratogen causes multiple ID and in other cases more teratogens together only cause single ID. Periods of development, in which the organ systems are most sensitive to teratogens action (because EMPs are here most active), are called "critical periods". In humans these are:

• for central nervous system (3^{rd} – 6^{th} week + declines until birth);
• cardiovascular system (mid-3^{rd} to 6^{th} week, declining by mid of 8^{th} week)
• lip and palate (6^{th} – 12^{th} week);
• extremities (half the 4^{th} to 7^{th} week).

These overview shows, that the most important processes of development happen in end of first and in second months of pregnancy, when many ladies do not know (or believe) that they are pregnant.

Summary of basic postulates of teratology:

• sensitivity to teratogenesis depends on the genotype of the embryo and the way of its interaction with factors of the external environment;

• sensitivity to teratogenesis vary in regard to the time of embryo development and the exposure duration (the critical periods) ;

• teratogens acts by certain mechanism to cells and tissues of the embryo and conditions abnormal embryogenesis (teratogenesis)

• the path of harmful exogenous factors to developing structures depends on the nature of the factors (agents);

• manifestation of damaged development increase with increasing doses of agent. As the ontogenetic age increases, the size of the dose relatively decreases (because of increase of body weight of mother and fetus);

• final manifestation of the abnormal development (in regard to extent of damage) could be – death, malformation, growth retardation or functional deficiency.

In regard to the medical and social importance of ID, it is necessary to study causes of their origin in humans. Basic methodological approaches are:

• **teratoepidemiology** - ID occurrence monitoring and search for potential teratogens by methods of epidemiology

• **experimental teratology** - verification of teratogen potential of observed agents in the experiments (from screening tests – to one generation reproduction studies – like is OECD 415).

The paradoxes of teratogens identification in humans are:

• the human embryo, the organism of a pregnant woman and feto-placental unit of humans are unique; therefore we do not have any adequate non-human model that would allow reliable transmission of results from experiments done on models to humans;

• order of human society – to test the teratogenic potential of newly introduced chemicals and pharmaceuticals, is very urgent;

• the result of situation is a compromise – finding out, if the tested compound passes through the placenta (this figure has priority) and examination of its teratogenic potential in experimental models. If in first, or both findings the result is positive, the compoundmust not be administered to pregnant women.

Models used for testing in experimental teratology:

In vivo:

• Subvertebrata (e.g. Hydra vulgaris);

• Vertebrata;

Amphibia (e.g .FETAX - a screening test with newt Xenopus laevis);
Aviary models (e.g. CHEST - a screening test in chicken embryos);
laboratory animals (mice, rats, rabbits, etc..) are considered the most appropriate, as this is the entire system of the mother - placenta - embryo (fetus).

In vitro:

• cultivated cells (to study the mechanism of teratogen action);

• cultivated parts of embryo (to study the mechanism of teratogen action)

• cultivated whole young animal embryos (screening tests).

Organization of embryotoxicity tests – effect on formation and survival of embryos; and teratogenicity (1-generation study OECD 415):

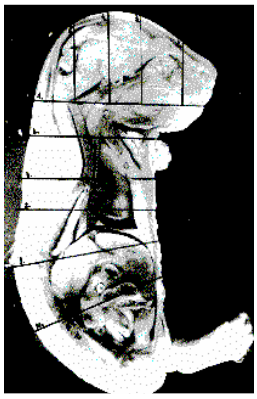
- selection of a suitable model organism, which adequately responds to potential teratogens;
- determining the of pregnancy beginning and embryogenesis stage;
- treatement – application of the tested substance (administration type should correspond with the expected exposure in humans);
- dosage (based on the estimated therapeutic dose or lethal dose). At least three experimental groups are used (with different doses) and a control group (solventis administered only). 10 to 20 animals should be in one group;
- treatment plan (applications and days schedule);
- the termination of the experimental animals (in laboratory animals with regard to maternal protein currency cannibalism) – the day before the end of physiological pregnancy.

In experiment are observed and recorded the following parameters:

- weight and behavior of females during the experiment (which may draw attention to non-specific effects of factors that may hide a specific effect);

- number of corpora lutea (how many oocytes were provided for fertilization), implants (the number of embryo that started to develop) resorbations (type of miscarriage in poly-embryonic animals), alive and dead fetuses;
- weight of fetuses, placentas and amniotic fluid;
- appearance of visceral and skeletal malformations.

Obtained values are statistically processed.



Evaluations of malformations in fetuses:

- **visceral malformations** are analysed (because of small size of fetuses), by s.c. Wilson´ method of razor sections. One third of dead fetuses are fixed in Bouin solution and than stored in spirit. In a semi-gross examination are 2 – 3 mm wide sections made, under prescribed protocol (Fig. 86). Each section is than analysed in microscope. Those of them, who are malformed or suspicious, are later examined by methods of histology.

• **Figure 86.** Sagital section through mouse fetus. Plains of Wilson´sections are indicated.

- **sceletal malformations** are analysed by differentiated staining of bones and cartilages of fetuses - by Petters. Randomly selected 2/3 of fetuses, after evisceration, are fixed in ethanol. Later they are brightened by KOH and stained with alizarin red and methylene-blue in steps. They are further kept in 20 % glycerol. In stereomocroscope is evaluated the status of bones (calcified structures are red) and cartilage (blue-green) development. The presence of anomalies and malformations, as well as the process of ossification, are monitored (Fig. 87).

• **Figure 87.** Differential staining by Petters

Contents

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Genetic aspects of the normal and pathological traits in humans

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