DNA DAMAGE AND REPAIR. MOLECULAR MUTATIONS

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Abstract. *The process that allows living organisms to repair damage that occurs in DNA is called repair. All repair mechanisms are based on the fact that DNA is a double-stranded molecule, i.e. there are 2 copies of genetic information in the cell. If the nucleotide sequence of one of the two chains turns out to be damaged (altered), the information can be restored, since the second (complementary) chain is preserved. DNA is the main reserve of information and a source of transmission for generations.*

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The repair process takes place in several stages. At the first stage, a violation of the complementarity of DNA chains is detected. During the second stage, the non-complementary nucleotide or only the base is eliminated, at the third and fourth stages, the chain integrity is restored according to the principle of complementarity. However, depending on the type of damage, the number of steps and enzymes involved in its elimination may be different. Very rarely there are damages affecting both DNA chains, i.e. violations of the structure of the nucleotides of the complementary pair. Such lesions in germ cells are not repaired, since complex repair involving homologous recombination requires the presence of a diploid set of chromosomes.

Spontaneous damage. Violations of the complementarity of DNA chains can occur spontaneously, i.e. without the participation of any damaging factors, for example, as a result of replication errors, deamination of nucleotides, depurinization. Replication errors: The accuracy of DNA replication is very high, but about once per 105-106 nucleotide residues, pairing errors occur, and then, instead of a pair of A-T, G-C nucleotides, nucleotides that are not complementary to the nucleotides of the matrix chain are included in the DNA daughter chain. However, DNA polymerases δ, ε are capable of taking a step back (in the direction from the 3' to the 5' end) after attaching the next nucleotide to the growing DNA chain and cutting out the last nucleotide if it is not complementary to the nucleotide in the DNA matrix chain. This process of correcting mating errors (or correction) sometimes does not work, and then non-complementary pairs remain in the DNA at the end of replication, especially since DNA polymerase a lacks a corrective mechanism and "mistakes" more often than other polymerases.

In case of improper pairing, unusual bases do not appear in the primary structure of the DNA daughter chain, only the complementarity is violated. The system of repair of non-complementary pairs should occur only on the daughter chain and replace non-complementary bases only in it. Enzymes involved in the removal of the wrong pair of nucleotides recognize the matrix chain by the presence of methylated adenine residues in the -GATC- sequences. While the bases of the nucleotide residues in the daughter chain are unmethylated, the enzymes must have time to identify the replication error and eliminate it. Recognition and removal (the first stage) of

a non-complementary nucleotide occur with the participation of special proteins mut S, mut L, mut H. Each of the proteins performs its own specific function. Mut S finds the wrong pair and binds to this fragment. Mut H attaches to the methylated (by adenine) site -GATC- located near the non-complementary pair. The binding between mut S and mut H is the protein mut L, its addition completes the formation of an active enzyme. The formation of the mut S, mut L, mut H complex at the site containing the error contributes to the manifestation of endonuclease activity in the mut H protein. The enzymatic complex hydrolyzes the phosphoester bond in the unmethylated chain.

Exonuclease (the second stage) is attached to the free ends of the chain. By splitting off one nucleotide in the direction from the 3'- to the 5'-end of the daughter chain, it eliminates the section containing the non-complementary pair. The gap is built up by DNA polymerase β (the third stage), the connection of the main and newly synthesized sections of the chain catalyzes the enzyme DNA ligase (the fourth stage). For the successful functioning of exonuclease, DNA polymerase p and DNA ligase, participation in the repair of helicase and SSB proteins is necessary.

Depurinization (apurinization). The DNA of each human cell loses about 5,000 purine residues per day due to the rupture of the N-glycoside bond between purine and deoxyribose. Then, in the DNA molecule, a site devoid of nitrogenous bases is formed in place of these bases, called the AP-site (AP-site, or apurine site). The term "AP-site" is also used in cases when pyrimidine bases fall out of DNA and apyrimidine sites are formed. This type of damage eliminates the enzyme DNA insertase (from the English, insert - insert), which can attach a base to deoxyribose in accordance with the complementarity rule. In this case, there is no need to cut the DNA chain, cut out the wrong nucleotide and repair the gap. Deamination. The reactions of cytosine deamination and its transformation into uracil, adenine into hypoxanthine, guanine into xanthine occur much less frequently than depurinization, and amount to 10 reactions per genome per day.

The correction of this type of spontaneous damage occurs in 5 stages. DNA-N-glycosylase participates in the repair, hydrolyzing the bonds between the abnormal base and deoxyribose (the first stage), as a result, an AP site is formed that recognizes the enzyme AP-endonuclease (the second stage). As soon as a break occurs in the DNA chain, another enzyme, AP-exonuclease, enters into operation, which cleaves deoxyribose, deprived of a base, from the chain (the third stage). A gap in the size of one nucleotide appears in the DNA chain. The next enzyme, DNA polymerase p, attaches a nucleotide to the Z'-end of the broken chain according to the principle of complementarity (the fourth stage). To connect the two free ends (the 3' end of the embedded nucleotide and the 5' end of the main chain), another enzyme is required - DNA ligase (the fifth stage). The deamination of methylated cytosine is unrepaired and therefore dangerous. The product of its spontaneous deamination is thymine, a base normal for DNA that is not recognized by DNA-N-glycosylase.

Induced damage. Induced damage occurs in DNA as a result of exposure to a variety of mutagenic factors of both radiation and chemical nature. Formation of pyrimidine base dimers. Under the action of UFOs, the double bond between C5 and C6 carbon atoms in the composition of pyrimidine bases (thymine and cytosine) can be broken. The carbon atoms remain bound by a single bond. The distance between the parallel planes of the bases of the polynucleotide chain in which the break occurred is approximately 3.4. This distance allows the released valences between the C-C atoms of pyrimidine bases arranged sequentially in the DNA chain to form a cyclobutane ring. Depending on which bases are connected into a dimer, they are called thymine, cytosine dimers or thymine-cytosine dimers.

The removal of pyrimidine dimers occurs under the action of photoliase, the enzyme cleaves the newly formed bonds between neighboring pyrimidine bases and restores the native structure. There is a section in the photoliase that either absorbs photons itself (in the blue part of the spectrum), or binds to cofactors that adsorb light. Thus, light activates photolyase, which recognizes dimers in the irradiated DNA, attaches to them and breaks the bonds formed between the pyrimidine rings. After that, the enzyme is separated from the DNA. Nitrogenous bases in DNA can undergo various damages: alkylation, oxidation, reduction, or binding of the base to formamide groups. Repair begins with the attachment of DNA-N-glycosylase to the damaged base. There are many DNA-M-glycosylases specific to different modified bases. Enzymes hydrolytically cleave the N-glycoside bond between the altered base and deoxyribose, this leads to the formation of an AP site in the DNA chain (the first stage). The repair of the AP site can occur either only with the participation of DNA insertase, which attaches a base to deoxyribose in accordance with the complementarity rule, or with the participation of the entire complex of enzymes involved in the repair: AP-endonuclease, AP-exonuclease, DNA polymerase β and DNA ligase. Repair is necessary to preserve the native structure of genetic material throughout the life of the organism. A decrease in the activity of enzymes of repair systems leads to the accumulation of damage (mutations) in DNA. In patients, the activity of enzymes responsible for removing incorrect bases, "building" gaps and other functions is reduced in the repair system. The defect of the repair system manifests itself in hypersensitivity to UV light, which leads to the appearance of red spots on the skin, turning into non-healing scabs and often into skin cancer.

RNA polymerases. The biosynthesis of RNA is carried out by DNA-dependent RNA polymerases. 3 specialized RNA polymerases were found in eukaryotic nuclei: RNA polymerase I, synthesizing pre-rRNA; RNA polymerase II, responsible for the synthesis of pre-mRNA; RNA polymerase III, synthesizing pre-tRNA. RNA polymerases are oligomeric enzymes consisting of several subunits - 2α, β, β', σ. The o (sigma) subunit performs a regulatory function, it is one of the factors of transcription initiation, RNA polymerases I, II, III, recognizing different promoters, contain σ subunits of different structure.

Elongation. Elongation factors increase the activity of RNA polymerase and facilitate the divergence of DNA chains. The synthesis of the RNA molecule proceeds from the 5'- to the Z'-end of the complementary DNA matrix chain. At the stage of elongation, in the field of transcription.

Termination. Unwinding of the DNA double helix in the area of the termination site makes it accessible to the termination factor. RNA synthesis is completed in strictly defined areas of the matrix - terminators (termination sites). The termination factor facilitates the separation of the primary transcript (pre-mRNA) complementary to the matrix and RNA polymerase from the matrix. RNA polymerase can enter the next transcription cycle after the addition of the σ subunit.

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