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Epigenetic Is Promising Direction In Modern Science

T.V. Fartushok, O.A. Kovalyshyn, Yu.M. Fedevych, I.A. Tanchyn, V.S. Zhykovskiy[1]

[1]Danylo HalitskyLviv National Medical University

Summary:

Epigenetics studies the inherited changes in a phenotype or in expression of genes than change of sequence of DNA nucleotide.

The most distinguished epigenetic tools are: modifications of histones, enzymatic DNA methylation, and gene silencing mediated by small RNAs (miRNA, siRNA).

The resulting m5C residues in DNA substantially affect the cooperation of proteins with DNA.

It is organized by hormones and aging-related alterations, one of the mechanisms controlling sex and cellular differentiation. DNA methylation regulates all genetic functions: repair, recombination, DNA replication, as well as transcription.

Distortions in DNA methylation and other epigenetic signals lead to diabetes, premature aging, mental dysfunctions, and cancer.

Key words: epigenetics, 5-methylcytosine, DNA methylation, histones.

Introduction

Recent scientific studies have highlighted the important role of fine-tuning mechanisms in the processes of genome functioning and its interaction with the environment.

The obtained results proved that a considerable part of these data lies in those processes that are described by epigenetic approaches< namely studies of hereditary properties of the organism, which are not directly related to the change of the nucleotide sequence in a DNA molecule [1,2].

Despite the successes of molecular biology and genetics, it is still unclear how the usualgrowth of an organism occurs, as cells containing the same genetic data from the beginning, precisely and correctly implement the peculiarities of certain parts of the genome in a specific phenotype. Epigenetics helps to answer the following questions: carcinogenesis, plasticity of stem cells, cell identity (specificity), regeneration of cells and tissues in animals and plants, aging, programmed death [3, 4].

There are a number of factors that regulate the expression of genes: methyl CpG DNA-binding and other proteins, histone modifications, hormone-receptor complexes, DNA methylation, nucleosome remodeling and chromatin overall rearrangement, and interaction with short non-encoding RNAs. Definition of genes needscomplicated complexes and ensembles of above 100 proteins engaged in the elongation and initiation of transcription from one promoter and in the processing of rRNA. Epigenetic control can amplify the initial signal (the promoterstimulation) or carry out the silencing of the gene. Epigenetic memory is sometimesrelatedtoparticularalterations of histones in chromatin. [1, 3].

In particular, this can occur with gel seylensing; this prohibition (conformational "lock") in a specificarea of the chromosome may become substantial as a consequence of induced (allowed) methylation of DNA by this chromatin state [2].

The factors related to the environmentcan have a noticeable effect on the enzymes' activity, which carry out alterations of DNA and histones. These cofactors incorporate: acetylcoensyme A for acetylase, ATP for kinases,S-

adenosylmethionine (SAM) for methyltransferases. The level of those cofactors in the cell can differremarkably and relyupon the environment. Adequate epigenetic control is formed on the basis of many factors'balance. [3].

How data and information concerning the chromatin'sstructure is realized to the daughter from the mother cell? This can happen in this way. This is clear that the of "core" histone proteins'synthesis is clearly organized in the cycle of cell. In the process of chromatin formation, certain proteins, relyingupon the nature and degree of their enzyme alterations, can bind to the DNA to shape corresponding sites for transcription [5].

Changes in the character of histone and DNA methylation have been detected; structural alterations in the nucleosome are not resulted from a gene that has undergone mutations directly, therefore, it can be regarded as epigenetic aberration. That also is applicable to mutations in the synthesis and utilization of SAM genes: the absence of SAM results in a violation of trans-methylation responses in the cell and inactivation of a number of enzymes for which SAM is an allosteric effector. However, although epigenetic changes are transmitted by heredity, this is not infinitely [6]. The central biology dogma of DNA ↔ RNA → protein is currently supplemented with knowledge regardingprions proteins that are capable of replication, inherited without the participation of DNA and RNA matrices [1, 7, 8].

Chromatine proteins histonesare inhibitors of transcription: the DNA free of them is much better transcribed than chromatin bound to them. There was an impression that for effective transcription it seemsvital to "undress" the DNA, releasing it from the histones. Nonetheless, V. Allfrey and A. Mirsky demonstrated that histones can be proacetylated to activate transcription of inactive chromatin, which is accompanied by a significant weakening of the binding of these proteins

to DNA [9]. Opened and describedmodifications of histones – acetylation, phosphorylation, methylation, ubiquitination, etc [10].

Epigenetic signals in the organism and cell are many, they are different. Among these signals are currently known [3, 11].

- Methylation of DNA;
- Severalhistones'enzymatic modifications;
- Chromosomal and genomic rearrangements;
- Small non-coding RNA (siRNA, or so-called small interfering RNA).

The main subject of the study of epigenetics is the study of epigenetic signals, their nature, their interaction and the physico-chemical impacts that they cause, their biological action in the cell at several functional levels of the organism and in different conditions of the external and internal environment.

Methylation of DNA - an epigenetic mechanism for monitoring the genetic functions of the body.

Scientists have discovered that the features of all higher plants'DNA issomewhat high content of their additional base - 5-methylcytosine (m^5C). Later in plant DNA, as in bacteria, was found N^6-methyladenin (m^6A) (Table. 1) [11].

Table 1 Minor methylated bases in DNA (Vanyushin B., 2013)

Organisms	Minor bases, %	
	m^5C	m^6A
Bacteria	0,01-1,53	0,02-0,70
Algae	0,20-3,50	0,10-0,60
Mushrooms	+	0-0,5

The simplest		0,3-1,0
Plants	2,0-10,0	0,5-1,0
Invertebrates	0,1-2,5	?
Vertebrates	0,7-3,5	+

For several years, these bases' origin in the DNA stayedunrevealed. Just in 1963 were first detected in bacteria, and then in eukaryotes enzymes, which in the existence of a donor of methyl groups of S-adenosylmethionine selectively methylated individual residues of cytosine and adenine in the DNA chains. Scientists have investigated that the "minor" bases (m^5C and m^6A) found in the DNA molecule are not embedded in them in the finished form.They are formed as a consequence of enzymatic alteration (methylation) of the corresponding bases (Fig. 1) in the shaped chains of DNA [12, 13].

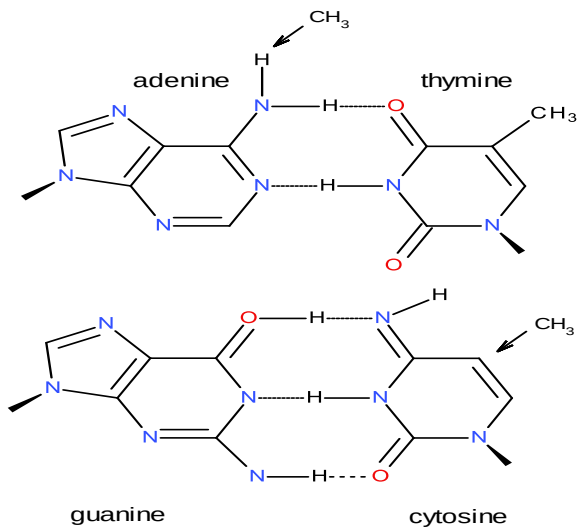

Fig. 1. Modifications (methylation) of the corresponding bases in the DNA chains. (Arrows show the locations of the methylated points).

In this case, the enzyme DNA-methyltransferase forms a covalently linked complex with DNA extracted from the double-stranded DNA of the modified base DNA and methylates it (Fig. 2).

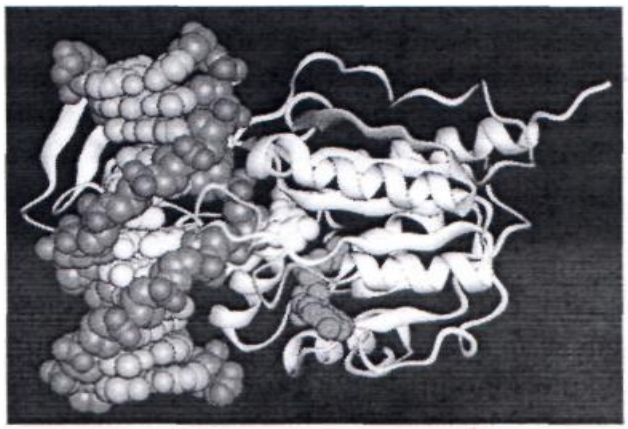

Fig. 2. Complex of cytosine DNA-methyltransferase from DNA.

After that, the covalent bond between the DNA and the enzyme is broken, the complex breaks down, and the methylated base (m^5C) gets back to its position in the DNA structure. [14].Methylation of DNA has a substantial effect on its relation (binding) with different proteins. Particularly, proteins that specially bind to the regulatory elements of rRNA genes have been detected in plant nuclei and indicated that some of those nuclear proteins binding is modulated by methylation of in vitro cytosine DNA residues [1, 3].

In several cases, the methylation of DNA by cytosine residues stop its binding to specifically reacting with it nuclear proteins that carry out several genetic procedures, such as replication, transcription, as well as DNA repair [2, 3]. On the other hand, so-called m5SrG DNA-binding proteins are known, which are specifically arranged on the DNA of an entire ensemble of complex protein complexes, control and express the genes [15].

If protein-free DNA is incubated with S-adenosylmethionine labeled in the methyl group, then after a while this label is already present in the DNA in the form of the formed 5-methylcytosine and thymine. Thus, non-enzyme methylation of DNA was discovered (Fig. 3) [3].

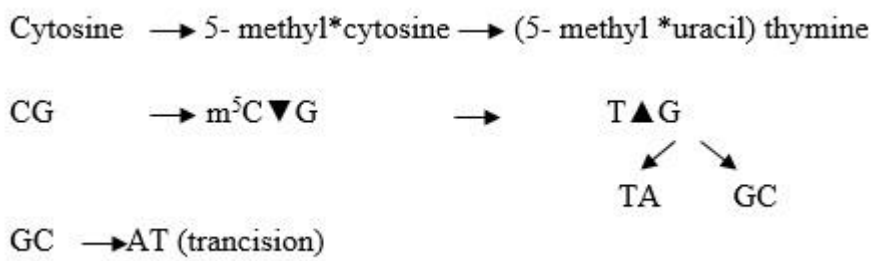

Fig. 3. Non-enzyme methylation of DNA.

Regarding that case, the labeled thymine in DNA was more than m5C. Therefore, it was discovered that the non-enzyme methylation of DNA in the solution is accompanied by rapid oxidative deamination of the formed residues m^5C with conversion to the remainders of thymine. This proved that the cytosine residues methylation in DNA can result in C → T transitions (GC-pair of bases is substitutedfor AT-pair), and the residues of 5-methylcytosine act as mutational points [16].

Specificity of enzyme methylation of DNA

The existence in the nature of the potential of methylation of DNA seems to have been used by special protein-enzymes DNA-methyltransferases, which, unlike chaotic, non-enzyme methylation, DNA modify cytosolic or adenine residues in certain nucleotide sequences [11].

Methylation of DNA is also carried out in animal cells, which has a significant biological significance. The cytosine residues,methylation in those and asymmetric

sequences is basicallyseen in the methylation of DNA induced by small RNA [1, 12, 16, 17].

Methylation of DNA in animals and plants, carried out by site-specific enzymes with cytosine DNA-methyltransferases, - leads to the occurrence of 5-methylcytosine residues (m^5C) in the sequences of CG, CNG and SNN. The plants also have adenine methylation of DNA. The appearance of m5C residues in DNA significantly affects the interaction of DNA with proteins. DNA methylation often blocks the binding of DNA to such proteins and prevents gene transcription, and on the other hand, it is a prerequisite for binding proteins [5]. There are special m^5CrG DNA binding proteins. Linking proteins with DNA arranges the entire ensemble of proteins of the transcription apparatus and is required for its activity [3, 15].

In animals and plants, along with the species, there is also tissue (cellular) subcellular (organoid) and age-specific DNA methylation. Methylation of DNA in animals decreases with age. Some researchers tend to believe that the degree of methylation of DNA can be judged by age and predicted life expectancy [2, 3, 18].

It has been proven that in the nucleus and the mitochondria of the same DNA, the DNA is methylated in different ways. In the mitochondrial DNA of the heart of the bull, 5-methylcytosine is detected. Together with these scientists, cytosolic DNA-methyltransferase has been isolated from animal mitochondria and it has been shown that this enzyme has another site specificity compared to nuclear DNA-methyl transferase. Unlike animals, 5-methylcytosine was not found in plants in DNA of mitochondria, but N^6-methyleneden was detected in them [12].

Three cytosine DNA-methyltransferases types and above ten genes encoding DNA-methyltransferases have been identified in plants. This is much more than in the eukaryotes. These enzymes carry out the methylation of the GrG sites for DNA replication, generally ensuring the conservation and transfer of the inherent

inheritance of the overall picture of the methylation of these sites in the genome [1, 3].In plants, methylation of DNA is controlled by phytohormones and specific regulators of plant growth and development. Due to the influence of various phytohormones in plants, methylation of DNA in the cell cycle significantly decreases [9, 19, 20].

In humans, three enzymes, the so-called DNA-methyltransferases 1, 3a and 3b (DNMT1, DNMT3a and DNMT3b), correspond to the DNA methylation process. It is anticipated that DNMT3a and DNMT3b are de novo synthesized methyltransferases that form the formation of DNA methylation in the early stages of development, while DNMT1 carries out DNA methylation on subsequent stages of the body's life. The enzyme DNMT1 has a high affinity for 5-methylcytosine. When DNMT1 finds a "semi-methylated site" (a site where methylated cytosine is only in one DNA strand), it methylates cytosine of the second thread on the same site. The function of methylation is to activate / inactivate the gene. In most cases, methylation of the promoter regions of the gene leads to inhibition of gene activity. It is shown that even minor changes in the degree of methylation of DNA can significantly alter the genetic expression [16, 20].

DNA-methyltransferase - redox-sensitive enzymes. The DNMT1 enzyme in vivo is responsible for the proper development of the embryo, imprinting, inactivation of the X chromosome and restoring the DNA structure after replication. It is present in most cases in somatic cells and is localized in the tricks of DNA replication, interacting with the nuclear antigen of proliferating cells (PCNA). Enzymes DNMT3a and DNMT3b are involved in de novo methylation. Expressedin embryonic and somatic cells of an adult. DNMT 3L is present in the germ cells and interacts with DNMT3a and DNMT3b in imprinting of parent genes. DNMT2 is

detected in all cell types, but does not exhibit enzyme activity, so its function is not yet established [6, 19].

A number of diseases, in particular cancer, are accompanied by abnormal hypomethylation of DNA and hypermethylation of CpG-islets in the promoter portions of proapoptic genes (suppressors of oncogenes), which leads to sustained repression of transcription [4, 9, 12, 18, 21]. The transcriptional repression in this case is mediated by proteins that can bind to methylated CpG dinucleotides. Methylcytosine-binding proteins activate histone deacetylase (HDAC) and other factors involved in chromatin remodeling [10].

The formed complex may modify histones, forming a condensed transcriptionally inactive structure of heterochromatin. The effect of methylation of DNA on chromatin structure is of great importance for the development and functioning of a living organism. In particular, the absence of methylcytosine-binding protein 2 (MeCP2) due to, mutations in the corresponding gene, leads to the development of Rett syndrome in humans [12, 21].

The inactivation of methylcytosine-binding protein domain 2 (Methyl-CpG binding domain protein 2 - MBD2), which is involved in the repression of transcription of hypermethylated genes, is observed in oncological diseases [22].

Methylation of DNA in animals and plants has a lot in common. Nevertheless, in plants it has several specific characteristics. The methylated CNG proportion and asymmetric DNA sequences in plant genomes is more substantial compared to that of in animals. Plants own a significantly more complex system of methylation of genomes than animals [1, 3].

Contrary to animals, plants ownparticular organelles – plastids (amyloplasty, leukoplates,chloroplasts, chromoplasts), which have their own, different from nuclear, system of modification (methylation) of DNA. Those systems can have asubstantial role in the functioning and differentiation of plastids. DNA

Methylation (mtDNA) in plant mitochondria is different from that in the nucleus. N^6-methyladenine was found in plant mtDNA, but not 5-methylcytosine, which is inherent in animal mtDNA [12, 13].

In plant there is a system of restriction – modification of the genome. The plants have S-adenosylmethionine-dependent endonuclease, which are sensitive to the status of methylation of DNA. Plant endonuclease is to some extent similar to a typical bacterial restriction endonuclease [3].

In the modification of the genome involved, at least, three components of this complex system; Substrate - DNA, enzyme (DNA-methyltransferase) and donor of methyl groups S-adenosylmethionine (Fig. 4). Control over the modification of DNA and the effectiveness of this process is carried out at the level of all these components, and with the participation of other diverse components of cellular metabolism. In addition, the methylation of the genome in a differentiated cell at a certain stage of ontogeny also depends on the activity of the enzymes that demethyate the DNA.

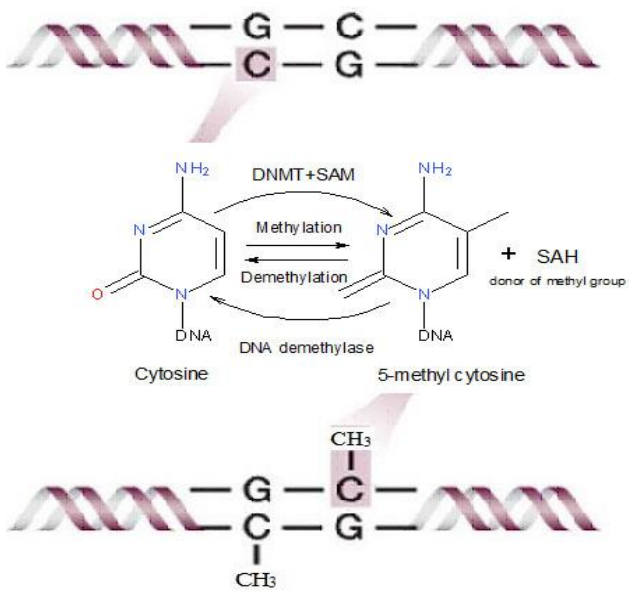

Fig. 4. Methylation and demethylation of DNA.

However, often in the presence of these active enzymes, a sufficient amount of S-adenosylmethionine (a donor of methyl groups and an enzyme activity modulator) and in the absence of appropriate inhibitors, these reactions in the nucleus are not possible due to the inaccessibility of the substrate-DNA in the chromatin for enzymes. Here it is important to organize the chromatin itself [9, 14].

In addition to the already mentioned multiple modifications of histones that modulate the chromatin organization and the availability of DNA for enzymes, many other proteins compete for the binding and interaction of DNA-methyltransferases with DNA. In particular, they can include proteins of hormone-receptor complexes.

The cytosine methylation of DNA controls plant growth and development (Vanyushin B.) and animals (Pugh), is involved in the regulation of all genetic processes, including transcription, replication, DNA repair, cell differentiation, genomic imprinting, and gene transcription [1, 7, 11].

The discovery of specific, so-called small RNAs (siRNAs, miRNAs) played an important role in representing the molecular mechanisms of gene expression regulation.These RNAs are encoded in the inverted sequences of the genome, which, as a result of reading and subsequent processing, appear in the form of short 12-14-member oligonucleotides.

Modification of histones

In the cell there are many other systems of epigenetic signals, they are diverse. In some of them the important role belongs not to DNA, but to proteins, including histones of chromatin. Due to one or another modification of histones the chromatin structure changes, which leads to inherited changes in the transcription of genes. Modification (methylation, phosphorylation, acetylation, ubiquitination

often determines whether the genes are active or not. To this also adjacent and a number of special non-gistone regulatory proteins that form complexes on DNA, which silencing genes, or, conversely, launch them into work. It is known that there is a correlation between the methylation of DNA and the modification of histones. The histonesmay act as a carrier of signals for the methylation of the genome [3, 8].

Although modifications of amino acids in histones occur throughout the protein molecule, the modification of N-tails is much more frequent. Modifications include: acetylation, phosphorylation, methylation.

Acetylationof histones

Acetylation is the most studied modification of histones (Fig. 5). Thus, acetylation with the acetyltransferase of the 14th and 9th lysines histone H3 (H3K14ac and H3K9ac, respectively) correlates with the transcriptional activity in this region of the chromosome. This is because the acetylation of lysine changes the positive charge to neutral, which makes it impossible to connect it with negatively charged phosphate groups in the DNA. As a result, the histones are disconnected from the DNA. Histones are able to maintain a modified state and act as a matrix for the synthesis of new histones that bind to DNA after replication [7].

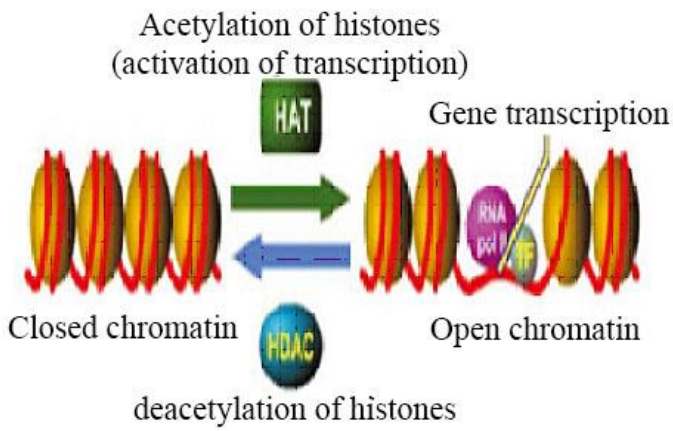

Fig. 5. Acetylation and deacetylation of histones.

Modifications of histones are indicators of active or suppressed chromatin. Degree of acetylation of histones is determined by activity of two types of enzymes - histone acetyltransferase (HAT) and deacetylase (HDAC) [14].

Phosphorylation of histones

Phosphorylation of histones occurs mainly on the remnants of serine and threonine, and affects chromatin changes, increasing the negative charge on the histone tail (Fig. 6).

This process is subject to dynamic regulation of the kinases (for example, phosphatase). Phosphorylation of histones plays a functional role in regulating the early response to expression, as well as in the dynamics of chromosomes during mitosis [8].

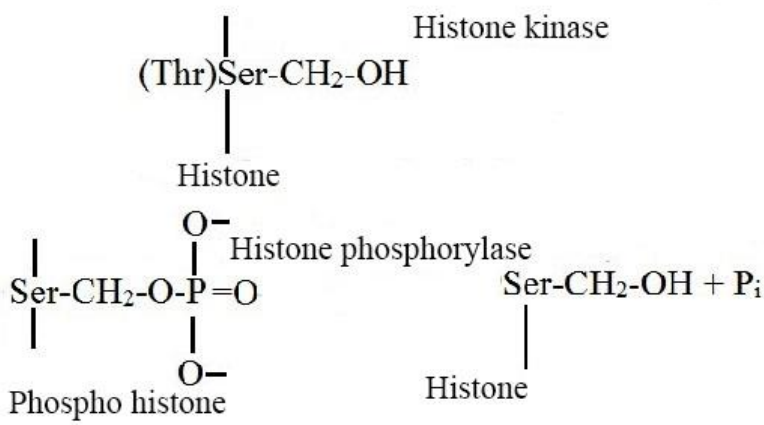

Fig. 6. Phosphorylation of histones.

Contrary tophosphorylation and acylation, histone methylation doesn't influence the nucleosome charge and can have a beneficial or repressive function in the expression of gene. Methyltransferases and dimethylases act on arginine and

lysine, and control the dynamic methylation process. [3, 23].Modification of histones leads to the induction or enhancement of methylation of CNG sites(Fig. 7).

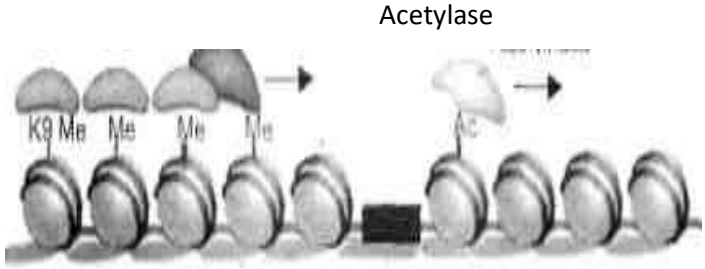

Fig. 7. Activation of chromatin as a result of demethylation of DNA and acetylation of histones.

In a number of these transformations, methylation of DNA can be a reason and a result of "silencing" genes [2].

Conclusions

The essential epigenetic signals in the cell is the methylation of DNA. n higher plants, DNA is methylated with cytosine residues; 5-methylcytosine is localized predominantly in the CG and CNG sequences. The ontogeny of plants and animals is generally impossible without the methylation of the genome.

In the nucleus, there are numerous DNA-methyltransferases, the methylation of DNA at different stages of replication may be carried out in different enzymes specificity. The state of methylation of the genome in a differentiated cell at a certain stage of ontogeny also depends on the activity of the enzymes that demethylated DNA. The degree and character of the methylation of the genome can be determined by the ratio of the activity of the enzymes that are methylating and demethyling the DNA.

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