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Research Article

Enzymatic DNA Methylation: What it is Needed for in the Cell

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Abstract

Enzymatic DNA methylation is an important constituent of the cell epigenetic regulatory system. It is tissue (cell) and age specific and involved in regulation of all genetic functions including transcription, DNA replication and repair, gene transposition, cell and sex differentiation. The methylation pattern of DNA is inherited, significant distortions in it result in defects of growth and development and they are responsible for induced epigenetic diseases especially such as cancer, diabetes, serious mental disorders, some of which lead to social misbehavior. Thus, the normal life is impossible without proper genome methylations. Unfortunately, many crucial aspects of DNA methylation, particularly the directed genome modification, are still poorly investigated. Therefore, further comprehensive study of various details of DNA methylation is especially needed. It is essential for both elucidations of mechanisms of gene functioning in the cell and of genome evolution. Besides, it is very important for the further progress in medicine, agriculture, biotechnology and social life.

ABBREVIATIONS

SAM: S-Adenosyl-*L*-Methionine; GMO: Gene Modified Organism; m^6A: N_6-Methyladenine; m^5C: 5-Methyl Cytosine; siRNA: Small Interfering RNA

INTRODUCTION

Almost 70 years ago it was already known that so-called "minor" bases 5-methylcytosine (m^5C) and N_6-methyladenine (m^6A) can occur in DNA [1,2]. The origin of these bases in DNA was unknown for a long period. Only in 1964 the specific enzymes DNA-methyl transferases were observed in bacteria [3] and then in eukaryotes; these enzymes site specifically transferred methyl groups from S-adenosyl-L-methionine (SAM) onto definite cytosine or adenine residues in DNA chains. It became clear that minor bases (m^5C and m^6A) do not incorporate into DNA in a ready-made form but originate there as a result of enzymatic modification (methylation) of common bases (C or A, respectively) in DNA chains that are forming or already formed. Nevertheless, the specificity and functional role of DNA methylations were still unknown for a long period. Moreover, the concept that these minor bases do not have any essential significance both in the structure of DNA itself and its functioning was quite widely disseminated. However, we were always sure that minor methylated bases in DNA as well as the enzymatic genome modification should not be traceless in the genome organization and they must obligatorily affect cell functions. First, methylation of cytosine residues stabilizes DNA double helix and, second, it strongly affects DNA binding with various proteins including regulatory ones. In many cases cytosine DNA methylation prohibits binding of specific nuclear proteins involved in transcription and other genetic processes. On the other hand, there are proteins that bind specifically to methylated DNA sequences and arrange on DNA an entire ensemble of proteins controlling gene expression. Thus, in fact, DNA methylation is a factor of negative or positive control of transcription.

DISCUSSION

The non-enzymatic DNA methylation

Interestingly, the non-enzymatic DNA methylation in the presence of SAM is conjugated with rapid spontaneous oxidative deamination of the m^5C residues formed with their transformation into thymine residues [4]. This was evidence that methylation of cytosine residues in DNA may often result in C → T transition, and 5-methylcytosine residues in DNA are "hot" mutation points. This phenomenon seems to be responsible for known evolutionary disappearance (suppression) of some CpG sequences from genes and genomes of various organisms. During enzymatic DNA methylation this C → T conversion is not expressed markedly. This means that some peculiar mechanisms protecting m^5C from deamination on the enzymatic DNA methylation should exist. On the other hand, if this methylation type still takes place in the cell, it can result in appearance of wrong codons and this could be a sort of aging program [5].

Methylome

A great success was achieved recently in development of the bisulphite DNA sequencing methods that allowed us to establish, as it was declared, complete methylome. But it is not complete yet

as far as the methylation prophile of the DNA adenine residues to it should be added to make the general methylome picture full. Today the determination of methylome reminds the momentary snapshot but full documentary film of the whole life situation with genome modification is still a dream.

In fact, the level of DNA methylation in the life cycle is very dynamic and flexible; it depends on many intra- and exocellular factors and environmental conditions. Anyway, at each moment of life or cell cycle it reflects the balance between methylation and demethylation processes. Therefore, inspection of two sides of the medal is more or less equally important. The different mechanisms of DNA demethylation are already described. They include the m^5C excision with subsequent DNA repair, direct m^5C demethylation (removal of CH_3-group), demethylation through 5-hydroxymethylcytosine formation and others). Some of these reactions are still needed in verification and most of them in description of respective enzymes involved.

DNA methylation in mitochondria

DNA methylation in mitochondria and plastids is yet poorly investigated and certainly it is needed to be studied more comprehensively. We know only for sure that DNA in these sub cellular organelles in animals and plants is methylated but the nature and character of DNA methylation there is different compared with that in the nuclei [4,6]. Unfortunately, the mechanisms of DNA modification in these particles are practically unknown, nothing is known on the nature, origin, specificity action and other properties of DNA-methyltransferases and functional role of DNA methylation there. It might be suggested only that it all may to some extent be similar to methylation of nuclear DNA and it may be involved in a control for genetic processes in mitochondria and plastids. Anyway, some so-called mitochondrial diseases seem to be due to distortions of mitochondrial DNA methylation.

Adenine DNA methylation

An especial attention now is paid to adenine DNA methylation in higher eukaryotes in connection with recent detection of N_6-methyladenine in DNA of Drosophila [7], nematode and in mouse embryonic stem cells [8]. Thus, m^6A is an important element of the epigenetic gene regulatory system in mammals. This adenine DNA modification is involved in the gene silencing in mammalian cells. m^6A presents also in DNA of algae, fungi, protists and plants. Unlike in animals, the mitochondria in plants (wheat) possess adenine DNA-methyltransferases but not cytosine DNA-methyltransferases. The enzyme (wadmtase) isolated from wheat mitochondria methylates in DNA an internal adenine residue in the TG**A**TCA sequence [9] and it seems to take part in regulation of mitochondrial DNA replication.

DNA methylation in plants

DNA methylation in plants is much richer and more complex than that in animals [10]. Plants possess a larger set of DNA-methyltransferases, some of which are unique for these organisms and have the ubiquitin-binding domain. Unlike in animals, knockout of many DNA-methyl transferase genes in plants is not lethal. One and the same plant gene can be methylated on both adenine and cytosine residues. Adenine DNA methylation can influence the cytosine DNA methylation and vice versa. Thus, like cytosine methylation, it may control DNA replication and gene expression in eukaryotes.

DNA methylation is a key mechanism of regulation of gene activity and cellular differentiation

It is established that cytosine DNA methylation is tissue (cellular) [11], sub cellular (organelle), and age [12] specific. Detection of tissue specificity of DNA methylation [11] allowed us first to declare that DNA methylation should be a mechanism of regulation of gene expression and cell differentiation. These observations have drawn attention of many investigators and pushed ahead the intense study of DNA methylation throughout the world.

The DNA methylation pattern in neurons but not other brain cells was changed during rat training [13]. Changes in the neuronal DNA methylation pattern induced by training were the first evidence that genome takes part in memory formation. Dynamic changes in DNA methylation occur in post mitotic neurons, the methylation-mediated chromatin remodeling may play critical roles in the gene expression modulation involved in long-lasting neuronal responses. DNA methylation represents one of the most permanent mechanisms of cellular memory. Interestingly, high levels of DNA methylation and demethylation activities persist in the brain cells throughout the whole life.

Interestingly, DNA methylation is involved in the social life. It was observed in social insects (bee) and mammals. Adverse events in early life can leave persistent epigenetic marks on specific genes that may prime susceptibility to neuroendocrine and behavioral dysfunctions. In general, the post mitotic epigenetic modifications in neuronal function can serve to facilitate or disfavor physiological and behavioral adaptations. Transcription and functioning of the mother care genes depend on their methylation status. Disfunctions of these genes due to aberrant methylation in the early life can subsequently lead to misbehavior, crime and suicides.

DNA methylation and cancer

"As early as 1977 a Russian team looked at normal cells from cows, then compared those with cells from cows who had a type of cancer known as lympholeukemia. In general, the overall DNA methylation was lower in cells from animals with the cancer. This was one of the early clues that methylation, DNA methylation, at least, might be involved in cancer, either as cause or as a result" [14]. Now it is no doubt that some changes in DNA methylation induce cancer, premature aging, apoptosis and death. For example, liver hepatoma was obligatory developed in rats fed with methionine-deficient diet. The DNA methylation patterns in normal and malignant cells are different. Therefore, DNA methylation profile of genome or separate genes serves already as a true marker of different cancer varieties even at the early stages of carcinogenesis. Inhibitors of DNA methylation (5-azacytidine and others) are efficiently used for treatment of some cancers (skin cancer). Many hopes on successful cancer treatment are associated with development of methods of siRNA silencing of metastatic genes, in particular. This type of the gene silencing may be essentially due to siRNA-directed DNA methylation.

As far as DNA methylation takes part in regulation of all genetic functions, it proceeds through all our life starting from egg fertilization and early embryogenesis to terminal stages of ontogenesis and death. It participates in realization of development program. Sometime it is called even as a secondary genetic code. Can we change this code somehow? The answer is yes. But we have to do it very reasonably and carefully. Especially it is all important when we are trying to save the life or correct some crucial epigenetic errors. The mother nature does not like reckless games with DNA methylations, finely it is always inclined to bring the DNA methylation pattern back to initial one, but some changes in DNA methylation may be inherited and even evolved.

Investigation of DNA methylation in transgenic organisms is of a special interest. The DNA methylation and transcription patterns can be changed after incorporation of foreign DNA in genome of recipient cells [15]. These facts may to some extent increase the public suspicion to GMO and make desirable analysis of the DNA methylation profiles in such organisms. Changes in DNA methylation may be, at least, partially responsible for clonal changeability. This should be always kept in mind of those who deals with cell and organism cloning, extracorporal fertilization and artificial embryo cultivations. DNA methylation patterns are different even in homozygous twins and this may be responsible for their differences in development, behavior, sensitivity to infections and so on.

DNA methylation in animals and plants is under hormonal control [4], it is modulated also by antioxidants and short biologically active peptides [16]. Unfortunately, the molecular mechanisms of these modulations are unknown. Some short peptides can inhibit endonucleases action [16]. It is suggested that short peptides penetrating into nucleus might compete with various proteins including DNA-methyltransferases for respective DNA binding sites, and because of it these binding sites will be unmethylated [16] that is crucial for transcription of most genes.

RNA-directed DNA methylation and other epigenetic elements

Discovery of RNA-directed DNA methylation has a special significance for epigenetics and molecular biology. In particular, it helps to understand how specific DNA sequences to be de novo methylated. It looks like that these sites may be found by respective complementary siRNA and afterwards the DNA-RNA triplex formed induces de novo DNA-methyltransferases to act on addressed base in the DNA chain.

It is to be reminded that DNA methylation in the cell is a very complicated process, in which, at least, three partners (DNA itself, donor of methyl groups SAM and chromatin) are most essential. All of them are involved in regulation of DNA methylation. For instance, many epimutations responsible for deficiency of DNA methylation are associated with genes coding for enzymes of SAM synthesis and metabolism. DNA methylation depends also on the structural organization of chromatin and accessibility in it of respective DNA sites to be modified. This certainly means that a study of DNA methylation requires serious research of chromatin; further progress in this area is impossible without deciphering of fine fluctuating chromatin structures in the nucleus. In other words, someone who can selectively and purposefully direct the chromatin structure will rule the world.

It should be noticed that enzymatic DNA methylation is only one element of very complicated epigenetic cell system including also histone modifications, gene silencing by non-coding RNAs and others. These processes are well coordinated and interdependent. For example, histone methylations may induce DNA methylations and vice versa. Unfortunately, the mechanisms of these cooperative interactions between individual elements of cell epigenetic system are yet unclear. It seems that these controlling interactions between them proceed mainly at the chromatin level.

CONCLUSION

Cytosine and adenine DNA methylations are very important elements of the complex epigenetic regulatory system in the cell. These enzymatic DNA modifications play a crucial role in the life events such as cell differentiation, the organism development, aging, programmed cell death and evolution. DNA methylations take part in regulation of all genetic functions including gene transcription, DNA replication and repair, recombination. In bacteria these DNA modifications seem to initially serve as the tools protecting the cell against incorporation of foreign DNA into genome. In eukaryotes DNA methylations suppress the expression of already incorporated foreign DNA sequences making them silent. Nevertheless, the host restriction – modification phenomenon [17] or, at least, some its elements in higher eukaryotes should be still looked for. Our recent findings (plant and animal SAM-dependent endonucleases that are able to discriminate between methylated and unmethylated DNAs) may indicate that the DNA methylation functions defending genome similar to that in bacteria may exist also in animals and plants. Distortions in DNA methylations lead mostly to different diseases of epigenetic nature, premature aging and death. There is no life without proper DNA methylation.

There is no doubt that further investigation of mechanisms of DNA (de)methylations is very important for progress in modern biology, medicine, agriculture and biotechnologies.

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