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Reflections on the Significance of DNA Methylation in Living Organisms: from Bacteria to Humans

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Short Commentary

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ABSTRACT

Epigenetics refers to the study of heritable changes in gene expression which occur without a change in DNA sequence and DNA methylation constitutes one of the most important causative components of this regulation. Unlike DNA mutations, changes in DNA methylation can be influenced by the environmental factors, they are stable at the time scale of an individual and present different levels of heritability. Efficient resistance systems against phage infections in bacteria take advantage of methylation mechanisms and these defence systems became invaluable tools in biotechnological applications. An evolutionary pattern of genome methylation guides us for a better understanding of specific functions in diverse organisms. In human diseases, in contrast to mutations which typically occur at a wide range of sites, aberrant methylation of specific promoter regions is a consistent feature of cancer. Thus, the determination of these circulating methylation epigenotypes is quite advantageous in terms of reflecting the early stages of carcinogenesis and predetermining the future cancer type. In this commentary, we aim to provide an overview of the concepts and molecular mechanisms related with the methylation in living organisms. Understanding how methylation dynamically contributes to both prokaryotic and eukaryotic life forms holds much promise for perceiving the value of epigenetic processes in evolutionary and clinical practice.

INTRODUCTION

While genetics refers to the research of information inherited on the basis of gene sequence, epigenetics is the research of reversible changes that occurs without any change in DNA sequence [1-2]. Epigenetic modification types include DNA methylation, histone modification, microRNA (miRNA)- and long non-coding RNA (lncRNA)- mediated regulation [3]. Dynamic chromatin states are controlled by reversible epigenetic patterns of DNA methylation and histone modifications and thus these two patterns orchestrate DNA organization and gene expression [4]. Epigenetic players vigorously interact with each other for final determination of gene expression; the effect of DNA methyltransferases (DNMTs) on DNA methylation is influenced by their interaction with histones and nucleosomes and DNA methylation can also mediate histone and nucleosome modifications [5]. DNA methylation is essential for normal embryonic development and has many important functions such as gene regulation, cell differentiation control, chromatin modification, mutation accumulation, silencing of endogenous retroviruses, chromosomal integrity, genomic imprinting control and X chromosome inactivation [6].

The establishment and maintenance of DNA methylation is generated by specific enzymes known as DNA methyltransferases. The methyl group may be incorporated on the N6 position of the adenine or at different molecular positions of the cytosine (N4

or C5) by distict DNA methyltransferases dependent upon the organism. Adenine methylation is found in Eubacteria and Archea but restricted to some unicellular organisms such as *Tetrahymena* in Eukaryotes and the chloroplastic genome of land plants. Cytosine methylation at the C5 position is common throughout all life domains and is the only DNA modification reported in multicellular eukaryotes [7].

The importance of DNA methylation in bacteria is protecting the bacterial genome from invasion of extracellular DNA. There are an estimated 10^{31} viruses on Earth and most of these are the phages that infect bacteria [8]. Thus, it is not surprising that huge number of phages oblige bacteria to develop counter attacks as defense mechanisms against these prolific invaders. The defense systems of prokaryotes can be classified into two broad groups according to their modes of action; the first group includes defense systems functioning on the principle of self-non-self-discrimination and the second group of defence systems is based on programmed cell death or dormancy induced by infection. Self-non-self-discrimination based defense systems contain at least three types; the best characterized one is restriction-modification (R-M) system that uses methylation to label the 'self' genomic DNA and recognizes and cleaves unmodified 'non-self' DNA. Another defense system called as DNA phosphorothioation labels DNA by phosphothiolation and destroys unmodified DNA. These two systems represent the innate immunity in prokaryotes. Unlike these mechanisms, the third one represents the prokaryotic adaptive immunity system and is called as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas (CRISPR-associated genes) which is the most known case of Lamarckian inheritance [9]. Bacteria can integrate short stretches of phage-derived sequences (spacers) within CRISPR loci to become phage resistant and both CRISPR locus and phage genomic regions are subject to rapid evolutionary changes. The key feature in CRISPR-mediated resistance is that the newly acquired spacer (between 29 and 31 nucleotides in size) must be identical to the phage genomic sequence to provide resistance [10]. CRISPR-CAS system found in roughly half of sequenced bacterial genomes and over 80% of archaeal ones has several analogies with the eukaryotic RNA interference (RNAi) systems, especially with the piwi-interacting RNAs (piRNA) [11,12]. The best characterized restriction-modification (R-M) system in which protection is conferred by usually methylation is present in over 90% of sequenced bacterial and archaeal genomes and the best known type II systems are widely used in molecular biology [12,13]. Though R-M systems have been exhaustively studied, particularly in genetic engineering, recent researches clarified that both systems seem compatible, cleave invading DNA and provide bacteria increased phage resistance. Moreover, CRISPR-Cas system can cleave phage DNA previously methylated by the R-M system [12]. Recently, a novel defense system denoted as BREX (Bacteriophage Exclusion) was discovered. This system methylates chromosomal DNA at a specific motif and methylation seems to be essential for the activity of the system. So, BREX is a phage-defense system providing protection against a wide array of phages including both virulent and temperate ones and the research of the distribution of BREX systems across microbial species clarifies the extensive horizontal transfer [14]. The absence of DNA methylation in some eukaryotes such as yeast, roundworm and fruit fly is related with the evolutionary loss of DNA methyltransferase homologs [15]. 'Genome-defense' hypothesis can also be suggested for mammals. Genomic parasites such as transposable elements can be controlled by DNA methylation which blocks mobile element transcription. Many methylated cytosines in mammalian genomes are found within mobile elements and when DNA methyltransferases are inactivated, some endogeneous retroviruses tend to become demethylated. Since there are some exceptions for this hypothesis such as *C. intestinalis*, this issue needs to be further investigated [16].

DNMTs performing DNA methylation share a conserved catalytic domain referring a common and ancient origin [16]. In most animal phyla, DNA methyltransferases consist of a family of three subdivisions called as DNMT1, DNMT2, and DNMT3. DNMT1 is a post replication maintenance DNA methyltransferase involved in the addition of methyl groups at the C5 position and thus ensures that DNA methylation status is faithfully copied on the newly synthesized DNA strand. DNMT3 methylates DNA during animal development and is called as de novo DNA methyltransferase. DNMT2's methylation role is minor and its enzymology largely directed to tRNA [17]. Besides with the common origin of DNMTs, methylation is conserved with clear preference for exons in most organisms [18].

DNA methylation in animal genomes occurs almost exclusively at cytosines followed by guanines, so-called 'CpG dinucleotides'. Chemically unstable methylated cytosines tend to undergo spontaneous deamination and mutate to thymines. As a result, DNA methylation leads to a high frequency of point mutations from CpGs to TpGs and methylated genomic regions gradually lose their CpGs. The depletion of CpG dinucleotides in genomic regions is measured by a metric termed 'CpG O/E: observed CpG frequency normalized by the expected CpG frequency' which is a straightforward and robust method to evaluate DNA methylation levels of different genomes [16].

One of the sources for evolutionary novelty is gene duplication events. However, gene duplications in genome dynamics can also lead to the stoichiometric imbalance of proteins which reduces the fitness of an organism. Evidences from comparative analysis of gene duplicates and selected gene families support the role of differential DNA methylation and epigenetic changes at protecting duplicate genes from pseudogenization [19]. When multiple human and chimpanzee tissues are investigated, depending on the tissue 12-18% inter-species differences in gene expression levels might be partly explained by changes in DNA methylation patterns [20]. However, there are some circumstances including plasminogen sequences where cytosine metylation patterns appear to be conserved [21]. Handel and Ramagopalan's debate focuses on the nature of epigenome. Since epigenome is dynamic and the environment exerts a key influence over this, epigenetic marks reflect an individual's environmental exposures and tend to change during the lifetime of a cell/tissue and thus are acquired all the time [22].

There are 3 important effects of DNA methylation on the genome in the development of disease mechanisms: 1) mutational burden of 5-methylcytosine, 2) epigenetic effects of promoter methylation on gene transcription, and 3) potential gene activation and induction of chromosomal instability by DNA hypomethylation [23]. Epigenetics was clearly linked to a variety of diseases in humans, such as developmental diseases, autoimmunity disorders, neuropsychiatric disorders, pediatric syndromes and cancer [4,24].

Methylation changes are common characteristic of different cancer types and occur early in cancer development. Circulating tumor DNA (ctDNA) isolated from bodily fluids such as plasma, serum or urine provides a tremendous potential for non-invasively monitoring changes in tumor burden in cancer patients and thus is so helpful for tailoring treatment after surgery or during treatment. Cancer-specific circulating DNA methylation offers a range of promising targets such as improving patient management, reducing unnecessary drug toxicity and accelerating data acquisition from clinical trials [23,25]. Unlike mutations, methylation always occurs in defined regions (CpG islands) and can be detected with high-sensitivity and high-resolution techniques. Moreover, each type of tumor apparently seems to have its own signature of methylated gene patterns [2]. Pathogen infections also contribute to human cancers and these infections can cause aberrant methylation profiles. *Helicobacter pylori* infection induces DNA methylation accumulation in the gastric mucosa, leading to an epigenetic field defect and thereby increasing the risk of gastric cancer development. Epstein-Barr virus (EBV) infection induces extensive DNA methylation in gastric epithelial cells with a unique methylation epigenotype. Human papillomaviruses (HPVs) infect cutaneous or mucosal epithelia in which cervical neoplasia can develop. HPV long control region (LCR) contains viral promoter sequences of early genes including viral oncogenes E6 and E7, viral transcriptional enhancer and viral origin of DNA replication. DNA methylation studies displayed the association between the degree of CpG methylation at HPV16 LCR sequences and the severity of the disease [26,27].

The main methylation mechanism and a brief recapitulation of the models in bacteria and humans are depicted in **(Figure 1).**

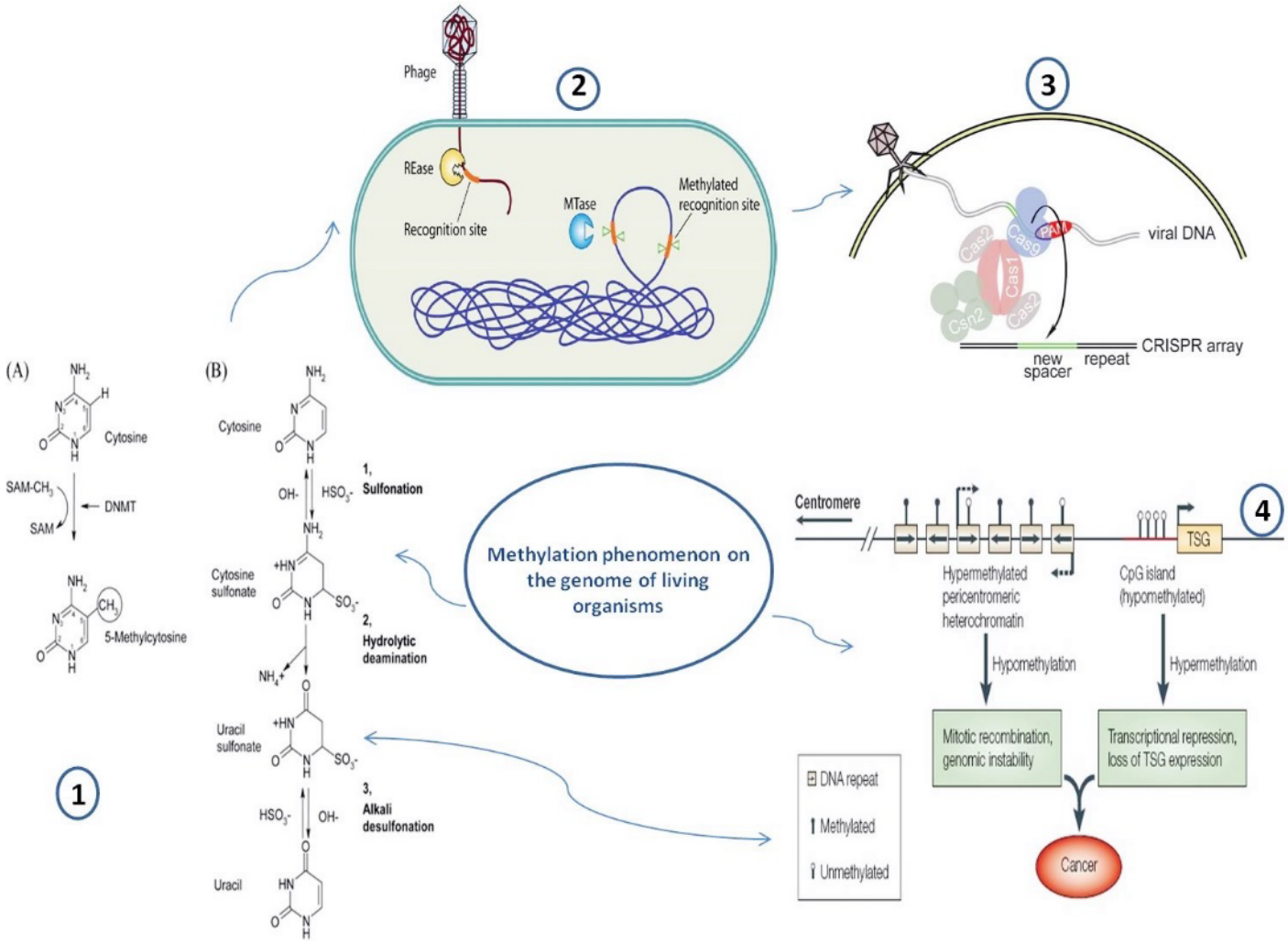

Figure 1. Methylation phenomenon on the genome of living organisms.

(1) Cytosine (CpG) methylation. (A) DNA methyltransferases 1, 3a, or 3b (DNMT) catalyzes the addition of a methyl group (the circled CH_3) at the fifth carbon of the pyrimidine ring of the cytosine nucleotide by using S-adenosyl methionine (SAM-CH_3) as a methyl donor. (B) C-to-T transition is initiated by the sulfonation of cytosine (cytosine to cytosine sulfonate), then hydrolytic deamination occurs (cytosine sulfonate to uracil sulfonate), process is concluded by alkali desulfonation (uracil sulfonate to uracil). Since methyl cytosine resists this chemical treatment, methylated versus unmethylated CpG can be distinguished by the subsequent molecular techniques. **(2)** Restriction-modification (R-M) systems as defense mechanisms. R-M systems recognize

the methylation status of incoming foreign DNA such as phage genomes. Methylated sequences are recognized as self, while recognition sequences on the incoming DNA lacking methylation are recognized as nonself and are cleaved by the restriction endonuclease (REase). The methylation status at the genomic recognition sites is maintained by the cognate methyltransferase (MTase). **(3)** CRISPR-Cas Model: After injection of the phage DNA an adaptation complex is formed by Cas9, Cas1, Cas2 and Csn2 uses the Cas9 PAM binding domain to specify functional protospacers. It is not known exactly how the protospacer sequence is extracted from the viral DNA to become a spacer. There are two models proposed: "cut and paste" and "copy and paste". In "cut and paste" model, a nuclease, possibly Cas1, cuts the viral DNA to generate the spacer. In the "copy and paste" model, the protospacer sequence is copied first. Once loaded with the selected protospacer sequence, this complex promotes the integration of this sequence into the CRISPR array, thus becoming a new spacer. **(4)** DNA methylation and cancer: A representative region of genomic DNA in a normal cell is shown. The region shown contains repeat-rich, hypermethylated pericentromeric heterochromatin and an actively transcribed tumour suppressor gene (TSG) associated with a hypomethylated CpG island. In tumour cells, hypomethylation of repeat-rich heterochromatin contributes to genomic instability through increased mitotic recombination events. De novo methylation of CpG islands also occurs in cancer cells and can result in the transcriptional silencing of growth-regulatory genes [28-31].

CONCLUSION

In conclusion, we can tell that methylation phenomenon is inevitably important in terms of both prokaryotic and eukaryotic life forms. It is valuable in archaea and bacteria in terms of genomic defense system modifications and responsible of gene regulation mechanisms in plants and animals. In humans, it is a very promising concept with the power of translating molecular genetics into novel clinical applications such as the determination of methylation epigenotypes of a variety of diseases and disease subtypes, predetermination of high-risk individuals, development of tailored molecular therapeutic regimes, monitoring changes in tumor burden following surgery and during treatment.

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