Biochemical Society Transactions (2019) **47** 997–1003
https://doi.org/10.1042/BST20180218

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Review Article

A model for the aberrant DNA methylomes in aging cells and cancer cells

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DNA methylation at the fifth position of cytosine is a major epigenetic mark conserved in plants and mammals. Genome-wide DNA methylation patterns are dynamically controlled by integrated activities of establishment, maintenance, and removal. In both plants and mammals, a pattern of global DNA hypomethylation coupled with increased methylation levels at some specific genomic regions arises at specific developmental stages and in certain abnormal cells, such as mammalian aging cells and cancer cells as well as some plant epigenetic mutants. Here we provide an overview of this distinct DNA methylation pattern in mammals and plants, and propose that a methylstat, which is a *cis*-element responsive to both DNA methylation and active demethylation activities and controlling the transcriptional activity of a key DNA methylation regulator, can help to explain the enigmatic DNA methylation patterns in aging cells and cancer cells.

Introduction

DNA methylation at the fifth position of cytosine is a major epigenetic mark present in plants and mammals. Through regulation of transcription activities and genome stability, 5-mC DNA methylation is involved in many important biological processes, such as fruit ripening in plants and aging in humans [1,2]. Both plants and mammals use *S*-adenosyl-methionine as the methyl donor for DNA methylation that is catalyzed by DNA methyltransferases with conserved catalytic domains. The landscape of DNA methylome is dynamically shaped by integrated activities of establishment, maintenance, and removal [1,2]. In both plants and mammals, alterations in genomic DNA methylation patterns can be triggered by intrinsic developmental programs and by certain environmental factors. For instance, aging in mammals is accompanied by a general loss of DNA methylation particularly at repeat sequences and transposable elements on the autosomal chromosomes [3], while a recent study reported a trend of acceleration in Y-chromosomal DNA methylation with increasing age [4]. Besides, locus-specific DNA hypermethylation and the concomitant gene silencing are being increasingly recognized as potential biomarkers for diseases such as cancer [5,6].

DNA methylation in plants and mammals

In mammals, DNMT3 (DNA methyltransferase 3) family members catalyze *de novo* DNA methylation [7]. Mammalian piRNAs, which are small RNAs of 25–30 nucleotides, are thought to pair with nascent transcripts and recruit *de novo* DNA methyltransferases [8]. In another proposed model, DNMT3A is activated or recruited to its target loci via protein interaction with DNMT3L, a noncatalytic DNMT3 paralog that binds unmethylated histone 3 lysine 4 (H3K4) [9,10]. Once established, mammalian DNA methylation is maintained by DNMT1, which restores hemimethylated DNA to a fully methylated status during DNA replication [7].

Received: 30 April 2019
Revised: 28 June 2019
Accepted: 1 July 2019

Version of Record published:
18 July 2019

Biochemical Society Transactions (2019) **47** 997–1003
https://doi.org/10.1042/BST20180218

Plant DNA methylation occurs in all cytosine contexts and can be established through the RNA-directed DNA methylation (RdDM) pathway, in which complementary pairing between 24-nt siRNAs and nascent scaffold RNAs, together with protein–protein interactions, recruits the protein machinery for DNA methylation [1,11]. Once established, DNA methylation in the CG context is maintained by the DNMT1 ortholog MET1 (Methyltransferase 1), while CHG (H represents A, T, or C) methylation is maintained by CMT2 (Chromomethylase 2) and CMT3 through a reinforcing loop that involves KYP4-mediated histone H3K9 methylation [12]. The asymmetric CHH methylation is maintained through persistent *de novo* methylation catalyzed either by DRM2 (Domains Rearranged Methylase 2) through the RdDM pathway or by CMT2 that requires the chromatin remodeling protein DDM1 (Decreased DNA Methylation 1) [13].

Active DNA demethylation is accomplished through a base excision repair pathway. In contrast with mammals, which initiate active DNA demethylation with oxidation and/or deamination of 5-mC, plants directly excise the 5-mC base utilizing 5-mC DNA glycosylases [14–16].

DNA methylome in aging cells and cancer cells

In some mammals including humans, the DNA methylome undergoes global alterations at certain developmental stages. After fertilization, the zygote experiences genome-wide demethylation in the paternal pronuclei, followed by a replication-dependent passive DNA demethylation in the maternal genome [16–18]. At the stage of blastocyst formation, *de novo* DNA methyltransferases reestablish genomic DNA methylation in both parental origins [16,19]. Global demethylation also occurs in primordial germ cells and is followed by extensive chromatin rearrangement, presumably allowing for epigenetic reprogramming toward totipotency [20]. Incomplete and aberrant DNA methylation reprogramming has been postulated as a cause of the high frequency of cloning failure and of developmental abnormalities in cloned animals [14,21,22].

In mammalian cells, aging is characterized by a general loss of DNA methylation and repressive histone marks in heterochromatic regions [23]. In addition to the global hypomethylation, locus-specific DNA hypermethylation also occurs during the process of aging. The age-dependent hypermethylation often exists in actively transcribed genomic regions, including regulatory genes for differentiation, apoptosis, and transcription [23,24]. Aging-associated genome hypomethylation may result from the gradual reduction in the levels of the DNA methyltransferase DNMT1 [25]. Aging-associated DNA methylome is also subject to the impacts of certain environmental factors such as dietary folate and oxidative stress. Aging with dietary deficiency of folate, which is a key source of the methyl group for DNA methylation, causes not only promoter hypomethylation of a couple of proto-oncogenes but also promoter hypermethylation of some tumor suppressor genes including p53 [26]. Oxidative stress due to mitochondria dysfunction occurs during aging and the stress can be enhanced by environmental toxins such as heavy metals and ozone [27,28]. During oxidative stress, accumulation of reactive oxygen species (ROS) can either directly cause DNA lesion or disrupt cellular metabolism and thereby affect TET-mediated DNA demethylation, leading to alterations in epigenomic landscapes [27,29,30]. The locus-specificity and the reproducibility of aging-associated DNA methylation changes indicate that DNA methylation may play a regulatory role in certain biological processes during aging, instead of simply being a stochastic consequence of cellular deterioration. The gradual changes in DNA methylation during aging have been proposed to be an 'epigenetic clock' [31,32]. Indeed, in certain cases such as human blood DNA, changes in DNA methylation levels are clearly associated with the aging process, and are so highly reproducible that they can be used for age prediction [33].

Besides aging, cancer cells also generally display not only global hypomethylation at heterochromatic regions, but also loci-specific DNA hypermethylation [34]. Increased methylation of tumor suppressor genes is an early event in many tumors, suggesting that altered DNA methylation patterns could be one of the first detectable neoplastic changes associated with tumorigenesis [35,36]. ctDNA bearing cancer-specific methylation patterns have been investigated as feasible biomarkers in cancers [37]. In both aging cells and cancer cells, DNA hypermethylation commonly occurs at loci that are targeted by Polycomb-group (PcG) proteins [38–40]. Because PcG-mediated transcriptional silencing can be antagonized by active histone modifications such as H3K4me3, additional DNA methylation at PcG-targeted loci seems to serve a double-locking role to secure transcriptional silencing. In the *min*$^{-}$ mouse model of cancer that displays tumor at ages of 3–5 months, it was shown that the appearance of cancer can be prevented by consecutive chemical inhibition of DNA methylation starting from birth, but not by treatments that started from 3 months of age [41]. These observations suggest that DNA methylation, possibly through developmental accumulation, contributes to tumorigenesis. In addition, DNA methylation abnormalities at gene promoters in elderly cells phenocopy cancer cells; particularly,

aging-associated DNA hypermethylation can occur at the promoter regions of some tumor suppressor genes [24,42–44]. Thus cancer formation appears to be tightly correlated with the changes in DNA methylation during the aging process.

Dynamic regulation of DNA methylome in plants

Similar to mammals, plants also go through massive alterations in their DNA methylome at specific developmental stages. In *Arabidopsis thaliana*, both the male and female gamete companion cells undergo DME (DEMETER)-mediated global demethylation, resulting in genome-wide DNA hypomethylation with reinforced CHH methylation at transposons in the endosperm [1]. During ripening, tomato fruits display DML2 (DEMETER Like 2)-mediated progressive DNA demethylation at hundreds of gene loci, many of which are known to regulate fruit ripening [45,46]. Chemical inhibition of DNA methylation causes premature ripening of tomato fruits, whereas a loss of DML2 function results in non-ripening fruits [45–47]. Thus the changes in DNA methylome during fruit ripening serve a crucial function.

Genetic disruption of key methylation regulators leads to abnormal DNA methylome in plants. Notably, dysfunction of *Arabidopsis* MET1 or one of the RdDM core factors results in not only global hypomethylation but also hypermethylation at specific loci across the genome [48,49]. The locus-specific increase in DNA methylation is attributed to the activity of the remaining DNA methyltransferases combined with the reduced gene expression of the DNA demethylase ROS1 (Repressor of Silencing 1), whose promoter region harbors a methylation monitoring sequence (MEMS) [50]. DNA methylation at MEMS positively regulates ROS1 gene transcription through a transcription activation complex that contains the SUVH (Suppressor of Variegation 3–9 Homolog) DNA methylation readers [51,52]. Similar to the observations that changes in DNA methylation levels are highly correlated with age changes [33], DNA methylation level of the MEMS is quantitatively associated with the extent of binding of the SUVHs [51]. ROS1 antagonizes MET1 and RdDM activities at several thousands of genomic regions [49], thereby providing a coordination between DNA methylation and demethylation. Similar to the observations that DNA hypermethylation in aging cells and cancer cells preferentially occurs at regions with H3K27me3, ROS1-targeted regions are enriched with this repressive histone modification [49]. Because the methylation level of MEMS is co-regulated by MET1, the RdDM pathway, and ROS1, this unique element is considered as a methylstat that maintains homeostasis of ROS1-dependent DNA methylation across the genome of *Arabidopsis thaliana* [1,50]. Methylation-sensitive regulation of demethylase gene expression has also been observed in rice, maize, and *A. lyrata* [53–55]. Thus, the methylstat may be a conserved mechanism for regulating DNA methylation dynamics in plants.

A model for the altered DNA methylome in aging cells and cancer cells

Methylstats may also exist in mammals and contribute to shaping the DNA methylome. In such a scenario, the increased DNA methylation at specific loci in aging cells and cancer cells could result from DNA hypomethylation of a methylstat, which controls the expression of a key regulator of DNA methylation or demethylation reactions. In the methylstat model (Figure 1), DNA hypomethylation in aging cells and cancer cells leads to transcriptional activation of the methylstat-controlled DNA establishment or maintenance enzyme or its regulator; alternatively, DNA hypomethylation in aging cells and cancer cells may lead to transcriptional repression of the methylstat-controlled DNA demethylation enzyme or its regulator.

Unlike the autosomal chromosomes that show a general hypomethylation pattern during aging, the Y chromosome shows age-dependent increases in CG methylation [4]. It is possible that the Y chromosome is intensively targeted either by methylstat-mediated demethylation that is anti-correlated with aging, or by methylstat-mediated methylation that is positively correlated with aging. While the methylstat does not necessarily locate in the promoter region of the methylation/demethylation factor that it controls, its methylation levels should be quantitatively correlated with the transcription levels of the methylation/demethyaltion factor. This expectation may help with the searching for the methylstat, whose methylation levels decline in an age-dependent manner, as well as for the methylation/demethylation factor that it controls. In addition, abnormal gene expression levels of the methylation/demethylation factor would be common in the aging-related diseases that are associated with aberrant DNA methylation patterns, as well as in the individuals that show abnormal

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Biochemical Society Transactions (2019) **47** 997–1003
https://doi.org/10.1042/BST20180218

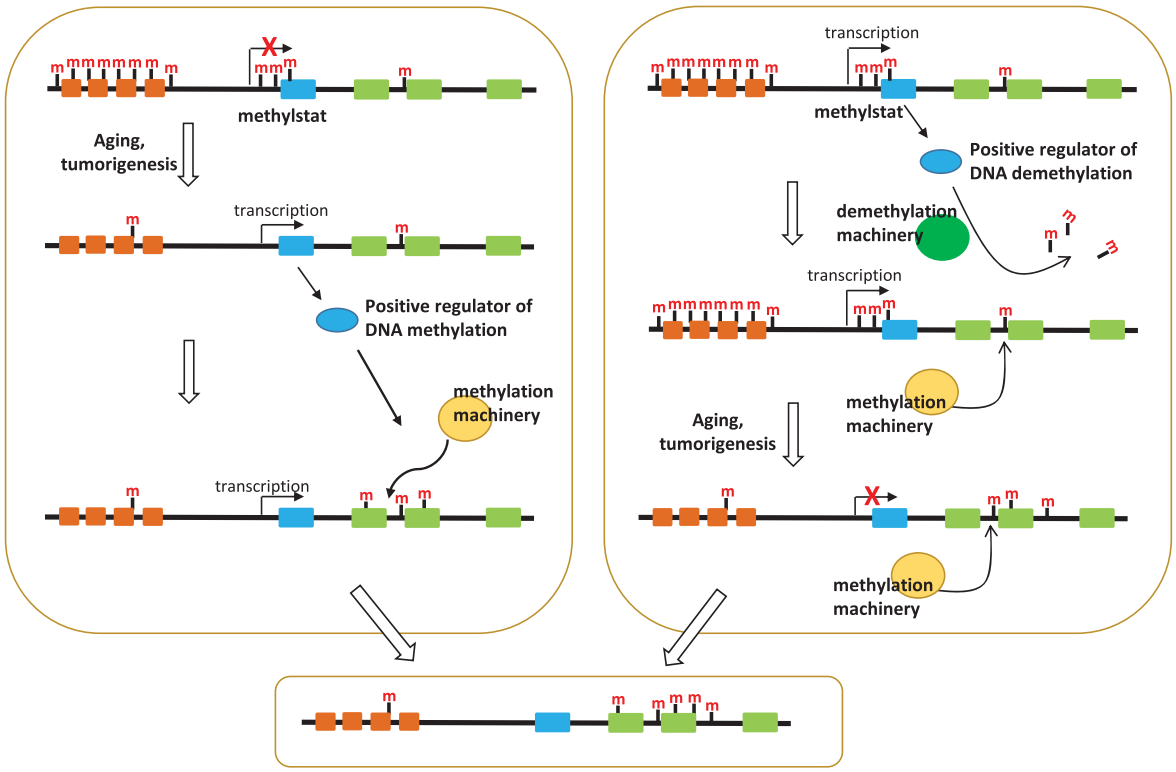

Figure 1. A model for the altered DNA methylome in aging cells and cancer cells.

Regulation of the DNA methylome in mammalian cells may involve a methylstat, which responds to DNA methylation levels and regulates gene expression in *cis*. The methylstat may contribute to the formation of DNA methylation patterns in aging cells and cancer cells in two possible ways. Left upper panel, aging or tumorigenesis causes DNA hypomethylation and consequently transcription activation of the methylstat. The methylstat controls a positive regulator of DNA methylation, the expression of which results in DNA hypermethylation. Right upper panel, DNA methylation in *cis* is required for transcriptional activation of the methylstat. The methylstat controls a positive regulator of DNA demethylation, whose expression results in the active removal of DNA methylation. Aging or tumorigenesis causes DNA hypomethylation and consequently transcriptional repression of the methylstat-controlled demethylation regulator. As a result, DNA methylation accumulates in regions that are normally pruned by the demethylation machinery. Methylation at the fifth position of cytosine is denoted by a red letter m.

rates of aging. DNA hypermethylation in both aging cells and cancer cells preferentially occurs at PcG-targeted loci [38–40]. It would be interesting to determine whether DNA methylation at these loci is pruned by active demethylation, and if so, whether the corresponding demethylation factors themselves are subject to methylation-dependent transcription regulation. An EZH2 (Enhancer of Zeste Homolog 2)-containing PcG complex was reported to recruit the methyltransferases for DNA methylation in cancer cells [56]. Thus it would be also interesting to examine whether DNA methylation negatively regulates the PcG-recruited DNA methylation machinery including the methyltransferases.

Summary

- Importance of the field: DNA methylation confers epigenetic regulation on many important biological processes. The genome-wide hypomethylation coupled with locus-specific hypermethylation is a hallmark of aging and tumorigenesis. Understanding the dynamic regulation of DNA methylome may help us understand and eventually manage aging and tumorigenesis.

- Summary of current thinking: Because the regulation of DNA methylation shares conserved features in plants and mammals, we propose that, similar to plants, mammalian cells also dynamically control genome-wide DNA methylation through a methylstat-dependent mechanism, and that this dynamic regulation contributes to the seemingly contradictory changes in DNA methylome in aging cells and cancer cells. This methylstat-based model implies that the locus-specific methylation increases observed in aging cells are not due to random epigenetic drift. Importantly, targeted intervention of the DNA methylation of the methylstat may slow down or prevent the DNA methylation increases in genes important for senescence and other old-age disorders and thus avert aging.

- Comments on future directions: While the methylstat model is enticing, the putative methylstats in mammalian cells have yet to be identified. Future investigations on this methylstat model may be aided by clues from the specific features of euchromatic regions where abnormal DNA methylation occurs.

Abbreviations

MEMS, methylation monitoring sequence; ROS, reactive oxygen species; ROS1, Repressor of Silencing 1; RdDM, RNA-directed DNA methylation; SUVH, Suppressor of Variegation 3–9 Homologue.

Acknowledgements

The authors apologize to those colleagues whose work is not cited owing to space constraints. H.Z. and J.K.Z. are supported by the Chinese Academy of Sciences.

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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