

The Metabolism of 5-methylcytosine Residues in DNA

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Summary

The fundamental biochemical processes of 5-methylcytosine (5-mC) synthesis, maintenance, conversion and removal determine the time and spatial pattern of DNA methylation. This has a strong effect on a plethora of physiological aspects of cellular metabolism. While the presence of 5-mC within the promoter region can silence gene expression, its derivative – 5-hydroxymethylcytosine exerts an opposite effect. Dysregulations in the metabolism of 5-mC lead to an altered DNA methylation pattern which is linked with a disrupted epigenome, and are considered to play a significant part in the etiology of several human diseases. A summary of recent knowledge about the molecular processes participating in DNA methylation pattern shaping is provided here.

Key words

Epigenetics • DNA methylation • DNA demethylation • 5-methylcytosine

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Introduction

Emerging evidence indicates that in the etiology of numerous human diseases, their pathological phenotype is a consequence of disturbances in epigenetic regulatory mechanisms with an impact on gene

expression (Aslani *et al.* 2016, Kwak and Park 2016, Ludwig *et al.* 2016). Contrary to genetic mutations, which affect the DNA sequence, disorders in epigenetic regulation may alter gene expression *via* silencing or overexpression (Weinberg and Morris 2016, Willis *et al.* 2016). Aberrant expression of genes may underlie the changes leading to switching of the phenotype from normal to pathological (Gupta *et al.* 2009). Recently, the expanding group of “epigenetic disorders” has come to include diverse diseases such as neurological (Carrick *et al.* 2016), psychological (Klengel and Binder 2015), immunological (Ciechomska and O'Reilly 2016), cardiovascular (Ahuja *et al.* 2017, Kim and Stansfield 2017), neoplastic (Feinberg *et al.* 2016, Upchurch *et al.* 2016) and metabolic diseases (Kamat *et al.* 2016, Hur *et al.* 2017). Increasing knowledge about the etiology of epigenetic disorders is aligned with the progress of therapies focusing on prevention or reversal of the pathological phenotype through modulation of the activity of several molecular members of the epigenetic regulatory machinery (Bär *et al.* 2016, Kgatele *et al.* 2016, Mund and Lyko 2010, Yoo and Jones 2006).

DNA methylation

The term epigenetics defines the heritable changes in gene function that do not involve changes in their DNA sequence and the processes through which genes produce their effect on the phenotype of the organism (Ho and Burggren 2010). Epigenetic regulation plays an essential physiological role in the activation or

silencing of appropriate genes during development, differentiation, and environmentally stimulated phenotypic plasticity. Within the cells, DNA and histones are chemically modified by covalent modification of several distinct groups that lead to the changes in chromatid structure and chromosomal architecture. The covalent modifications of DNA and histones are reversible and dynamic depending on the developmental state, the tissue specificity and the influence of environmental factors. At the molecular level, covalent modifications of DNA participate in the regulation of chromosomal level of condensation, which can exist in a transcriptionally active or inactive states named as euchromatin or heterochromatin, respectively. In addition to changes in chromosomal structures that exert an effect on transcriptional activity the level of already synthesized gene-specific mRNA molecules may be downregulated in the presence of specific miRNA molecules (Rassoulzadegan *et al.* 2006, Saetrom *et al.* 2007).

In human cells, the predominant covalent modification of DNA is the enzymatically catalyzed methylation of cytosine residues to 5-methylcytosine (5-mC; D'Alessio and Szyf 2006, Gupta *et al.* 2010, Zilberman and Henikoff 2007). The methylation of DNA in differentiated cells occurs primarily but not solely at CG residues present in CG islands. The CG islands comprise approximately 60 % of the nucleotides at the 5'-end region of the gene promoters, wherein approximately 60-80 % of the CG residues remain methylated after differentiation. The methylation of CG residues at promoter regions represses transcription, whereas the active promoters remain un-methylated. DNA methylation either directly prevents binding of transcription factors to their target binding sites (Prendergast and Ziff 1991) or provides binding sites for methyl-binding domain containing proteins that are able to suppress gene repression (Nan *et al.* 1998) or control alternative promoter usage (Rauch *et al.* 2009). In addition, DNA methylation prevents chromosomal instability by silencing non-coding DNA and transposable DNA elements. Contrary to methylation in promoter and enhancer regions, the presence of 5-methylcytosine residues within the gene sequences is positively correlated with its transcription (Lister *et al.* 2009, Rauch *et al.* 2009).

The methylation of cytosine is enzymatically catalyzed by a group of enzymes of the DNA methyltransferase family [E. C. number 2.1.1.37, recommended name: DNA (cytosine-5-)-methyltransferase] consisting

of three isoenzymes. DNA methyltransferase (Dnmt) 1 initiates *de novo* methylation (Bestor 1992), whereas Dnmt3a and Dnmt3b are involved in maintaining the methylation pattern (Okano *et al.* 1999). The donor of methyl groups is S-adenosylmethionine (SAM) and its availability is dependent on the capacity of the SAM cycle to generate SAM and regenerate methionine from homocysteine. The sufficient resynthesis of methionine from homocysteine may be limited by availability of vitamins B₁₂ and folic acid as well as enzymatic activity of methionine synthase. A deficiency of cells to resynthesize S-adenosylmethionine, due to insufficient levels of both vitamins may negatively affect the DNA methylation status (Niculescu and Zeisel 2002, James *et al.* 2003, Ulrey *et al.* 2005, Chang *et al.* 2011, Fernández-Roig *et al.* 2012). In addition, the increased level of unprocessed homocysteine may disturb the methylation reactions (Škovierová *et al.* 2015) by inhibiting the enzymatic activity of methyltransferases including Dnmts (Lin *et al.* 2014).

In mammalian cells, *de novo* methylation of cytosine by Dnmt3a is stimulated by non-catalytic paralogue Dnmt31 (Liao *et al.* 2012). In addition, similarly to Dnmt3a and Dnmt3b, Dnmt31 may interact with histone deacetylase (Aapola *et al.* 2002, Deplus *et al.* 2002) and block the transcription process. The space specific methylation by Dnmt1 is also regulated through its interaction with the protein UHRF1, which is able to sense the presence of methylated histone 3 and subsequently can recruit Dnmt1 to its proximity (Bronner *et al.* 2013). Both proteins, Dnmt31 and UHRF1, possess the capability to assign DNA methylation patterns with histone epigenetic marks, covalent modifications that may influence nucleosome positioning (Harikrishnan *et al.* 2005, Wysocka *et al.* 2006) and chromatin remodeling (Portela and Esteller 2010).

Removal of the methyl moiety

The methylation pattern of DNA is dynamic and varies in cell specific and time dependent manners. In contrast to methylation, the reverse process of 5-mC conversion to cytosine is not straightforward and requires several consecutive steps. Based on the enzymatic mechanisms underlying the demethylation, passive and active pathways can be recognized (Chen *et al.* 2013, Franchini *et al.* 2012, Piccolo and Fisher 2014, Wu and Zhang 2014).

Methyl moieties can be lost from the genome by

blocking Dnmt activity during cell replication, which is considered to be a passive DNA-demethylation pathway. Suppressed expression, enhanced degradation, nuclear exclusion or enzymatic inhibition of DNA methyltransferase activity will definitely lead to a progressive decline in the presence of 5-mC within the genome upon DNA replication. In mammals, the process of passive demethylation is supposed to occur following fertilization and during gametogenesis (Chen and Riggs 2011, Franchini *et al.* 2012, Piccolo and Fisher 2014).

The active DNA-demethylation pathway involves several specific enzymes that are capable of converting 5-mC residues to products, which can subsequently be enzymatically substituted by cytosine (Fig. 1). The proteins from the ten-eleven translocation (TET) family are involved in the enzymatic conversion of 5-mC into its oxidized derivatives (Weber *et al.* 2016). TET proteins are capable of converting 5-mC in a series of reactions to 5-hydroxymethylcytosine, 5-formylcytosine and 5-carboxylcytosine (Piccolo and Fisher 2014).

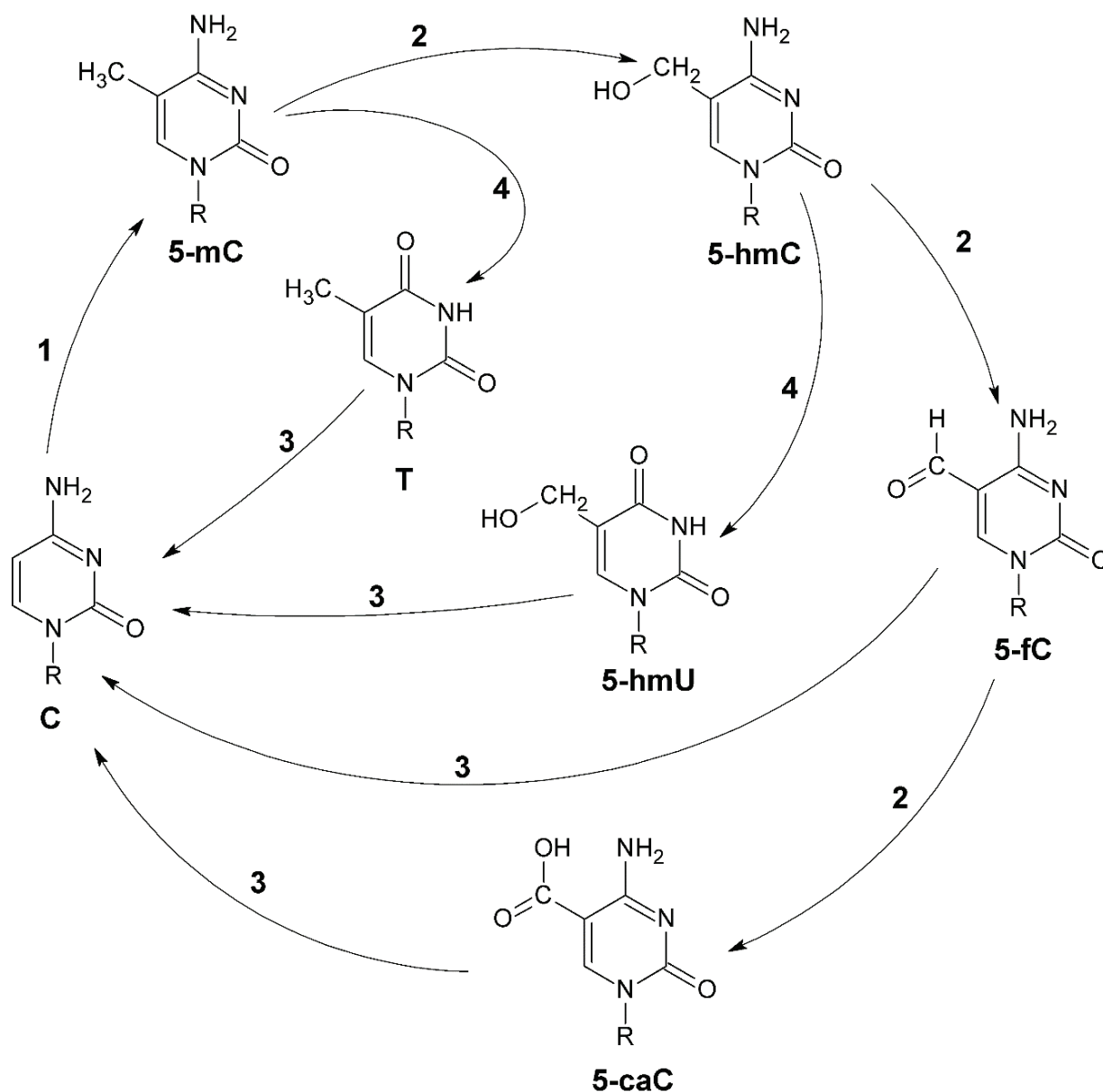


Fig. 1. Simplified scheme of active DNA-demethylation processes. A genomic cytosine nucleobase can be methylated by DNA methyltransferase (1) to generate a 5-methylcytosine residue (5-mC), which can be further enzymatically metabolized in two distinct pathways. TET enzymes can catalyze the oxidation of the methyl moiety of 5-mC in an ordered sequence of reactions (2) to 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC). Both, 5-fC and 5-caC, might be substituted by cytosine in a thymine glycosylase catalyzed base excision repair (BER) process (3). Deamination (4) of 5-mC and 5-hmC by the activation-induced cytidine deaminase/apolipoprotein B mRNA editing enzyme generates thymidine (T) and 5-hydroxymethyluracil (5-hmU) residues, respectively. They are both exchanged to cytosine by the BER process (3).

Oxidized derivatives of cytosine – 5-formylcytosine and 5-carboxylcytosine – can be removed from DNA molecules by thymine DNA glycosylase (TDG) and replaced by cytosine *via* the process of base excision repair (BER) (Maiti and Drohat 2011, Maiti *et al.* 2013, Ngo *et al.* 2016). The knowledge about other suggested mechanisms of demethylation is less comprehensive. They are supposed to include decarboxylation of 5-carboxylcytosine and enzymatic removal of a hydroxymethyl group from 5-hydroxymethylcytosine by the enzymes of the Dnmt family (Shen and Zhang 2013). Two additional pathways initiated by the class of cytidine deaminases – AID (activation-induced cytidine deaminase) and APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide), can facilitate the deamination of 5-hydroxymethylcytosine. AID enzymes deaminate cytosine bases in DNA to yield uracil. AID and the larger family of APOBEC enzymes have been proposed as factors that affect DNA demethylation by deaminating 5-mC and 5-hmC in DNA to yield thymine and 5-hmU, respectively. As these are present in mismatched T:G and 5hmU:G base pairs, they are hypothesized to be excised by single-strand-selective monofunctional uracil DNA glycosylase 1, or thymine DNA glycosylase (Pastor *et al.* 2013).

Active and passive pathways of DNA demethylation may also act synergically in a replication-dependent manner *via* the formation of 5-mC. A symmetrically-methylated CpG sequence is converted during DNA replication into two asymmetrically methylated DNA strands. Hemimethylated CpG sites are recognized by UHRF1, the obligatory partner of the maintenance DNA methyltransferase DNMT1, which restores symmetrical methylation. TET proteins act at methylated CpG sites to generate symmetrically hydroxymethylated CpG sequences. 5-hmC and other oxidized methylcytosines may impair maintenance

methylation by inhibiting UHRF1 binding, DNMT1 activity, or both. As a result, the CpG sequence progressively loses DNA methylation through successive DNA replication cycles (Pastor *et al.* 2013).

Concluding remarks

The metabolism of 5-mC is considered to be one of the pillars in the process of epigenetic regulation of gene expression shaping the cellular phenotype. The processes leading to aberrant placement of epigenetic marks are important players in the etiology of several severe diseases (Portela and Esteller 2010) such as cancer (Cheray *et al.* 2013, Pacaud *et al.* 2014), autoimmune and metabolic diseases including diabetes mellitus (Nilsson *et al.* 2014, Rönn and Ling 2015). A better understanding of the fundamental biochemical pathways underlying the metabolism of DNA methylation may lead not only to better diagnostic methods (Vasanthakumar and Godley 2015) but also to improved prognosis and therapeutic approaches.

Conflict of Interest

There is no conflict of interest.

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