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THE ROLE OF MAMMALIAN DNA METHYLTRANSFERASES IN THE REGULATION OF GENE EXPRESSION.

JUSTYNA TUREK-PLEWA and PAWEŁ P. JAGODZIŃSKI*

Department of Biochemistry and Molecular Biology,
Karol Marcinkowski University of Medical Sciences, ul. Święcickiego 6,
60-781 Poznań, Poland

Abstract: The term epigenetic modification denotes reversible traits of gene expression that do not include alterations to the DNA sequence. These epigenetic alterations are responsible for chromatin structure stability, genome integrity, modulation of tissue-specific gene expression, embryonic development, genomic imprinting and X-chromosome inactivation in females. Epigenetic changes include reversible DNA methylation and histone acetylation or methylation. The modification of mammalian genomic DNA includes the methylation at the 5-position of the cytosine (C) residue within cytosine-guanine dinucleotides (CpG), resulting in the formation of 5-methylcytosine (m⁵C). Regulatory DNA sequences in vertebrates often have little or no methylation. The methylation of mammalian genomic DNA is catalyzed by DNA methyltransferases (DNMTs), which play a special role in the initiation of chromatin remodeling and gene expression regulation. The mammalian DNMTs are DNMT1, DNMT3A and DNMT3B, which together with accessory proteins, like DNMT3L, are responsible for methylation pattern acquisition during gametogenesis, embryogenesis and somatic tissue development. Reversible epigenetic alterations lead to selective utilization of genome information through the activation or inactivation of transcription of functional genes during gametogenesis, embryogenesis and cell differentiation. Recently, several disparate isoforms of DNMT1 were identified in human somatic and

* Corresponding author: fax: (48 61) 865 95 86, e-mail: pjagodzi@am.poznan.pl

Abbreviations used: DNMT – DNA methyltransferase; MeCP – m⁵CpG-binding protein; MBD – m⁵CpG-binding domain; HDAC – histone deacetylase; HAT – histone acetyltransferase; HP1 – heterochromatin protein 1; DAPI – 4'-6-diamidino-2-phenylindole; AdoMet – S-adenosyl-L-methionine; AdoHcy – S-adenosyl-L-homocysteine; PCNA – proliferating cell nuclear antigen; PBD – (PCNA) binding domain; NLS – nuclear localization signal; ATRX – cysteine-rich zinc finger DNA-binding motif; PHD – polybromo homology domain; ICF – immunodeficiency disorder, centromere instability and facial anomalies syndrome.

female and male germ cells. Recent advances in the investigation of DNMT function in epigenetic DNA changes have formed the basis of the understanding of various disorder etiopathogeneses, and as a result, have facilitated and enabled new therapies with respect to these diseases.

Key Words: Chromatin Remodeling, Methylation, DNMT, DNA Methyltransferase, Gene Expression

INTRODUCTION

The term epigenetic modification refers to heritable traits of gene expression that do not include alterations of DNA sequences [1]. Epigenetic alterations are reversible and are transmitted during cell mitosis or meiosis. This process contributes to chromatin structure stability, genome integrity, modulation of tissue-specific gene expression, embryonic development, replication timing, genomic imprinting and X-chromosome inactivation in females [2-6].

Epigenetic changes include reversible DNA methylation and histone acetylation or methylation. Histone modifications seem to be a universal and evolutionally conserved epigenetic mechanism of transcription regulation in eukaryotic cells, whereas DNA methylation occurs in prokaryotic and eukaryotic cells. The methyl group can be attached at the N6 position of adenine, or the N4 or C5 positions of the cytosine residues of prokaryotic or eukaryotic genomic DNA.

The only modification of mammalian genomic DNA is the methylation at the 5-position of the cytosine (C) residue within the cytosine-guanine dinucleotides (CpG) resulting in the formation of 5-methylcytosine (m^5C), which is designated as the fifth base of DNA (Fig. 1) [7, 8].

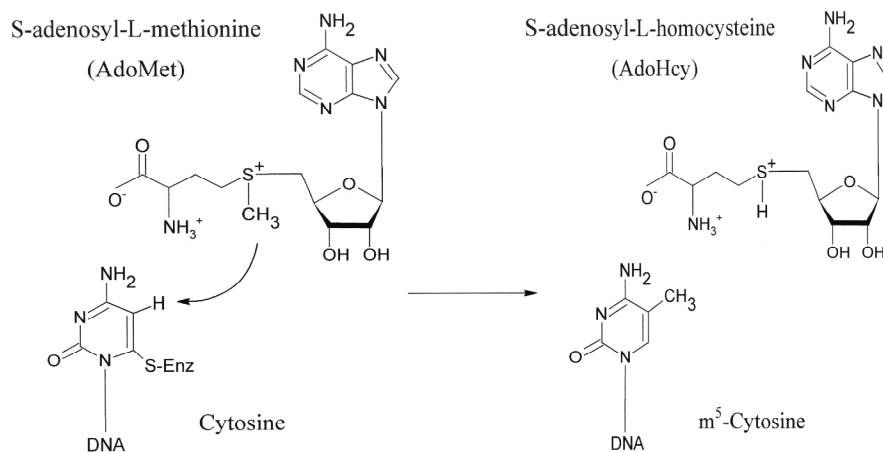


Fig. 1. The methylation mechanism of cytosine (C) to 5-methylcytosine (m^5C). Methylation of C within CpG dinucleotides is conducted by methyltransferases that use AdoMet as a donor of a methyl group.

In the genomic DNA of mammalian cells, 60-90% of the C residues located within CpG dinucleotides are methylated [9]. In higher eukaryotes, CpG pairs are major mutational hotspots because of the spontaneous deamination of m⁵C to thymine (T). The C→T transition results in the progressive elimination of methylated CpG sites from the coding sequence during evolution [10-12]. The 0.5-5kb DNA fragments with clusters of CpG dinucleotides in GC-rich (60-70%) DNA regions are designated as CpG islands, and are mainly located within the first exon and promoter of numerous genes [13]. Hypermethylation of gene promoters or hypomethylation of various parts of the genome may contribute to malignant or autoimmune disease development [14, 15].

Epigenetic alterations help lead to the selective utilization of genome information through the activation or inactivation of functional gene transcription during gametogenesis, embryogenesis and cell differentiation [16].

THE ROLE OF EPIGENETIC MODIFICATIONS IN GENE TRANSCRIPTION

The methylation status of regulatory DNA sequences correlates with the transcriptional activity of genes. Unmethylated CpG islands are sometimes present in first exon and promoter of “housekeeping” and tissue-specific genes. In higher eukaryotes, DNA demethylation and the methylation and acetylation of certain histones are associated with the formation of transcriptionally active chromatin (euchromatin) (Fig. 2).

Histone methylation and acetylation or deacetylation are also reversible epigenetic modifications, respectively responsible for the activation or stable silencing of transcription (Fig. 2, Tab. 1) [17]. The acetylation level of chromatin histones is maintained by histone acetylase (HAT) and histone deacetylase (HDAC) (Fig. 2, Tab. 1). Histones can be also methylated by histone methylases at position ε, and by the guanidine amine groups of Lys (K) and Arg (R) residues (Tab. 1).

Euchromatin structure often contains unmethylated first gene exons, acetylated histones and the methylated K4 amino acid residue of the H3 histone. By contrast, the presence of methylated DNA, and the deacetylated and methylated K9 amino acids of the H3 histone residue can be associated with transcriptionally inactive chromatin (heterochromatin) that is strongly stained with 4'-6-diamidino-2-phenylindole (DAPI) [18]. The formation of heterochromatin may start with the deacetylation of H3 histone K9 by HDAC, which enables the methylation of H3 histone K9. The methylated K9 amino acids of H3 histone are recognized by heterochromatin protein 1 (HP1), which can stabilize the condensed form of heterochromatin structure [19]. However, mice cells with HP1 knock-out still have DAPI-strong heterochromatin [18]. The N-terminal chromo domain of HP1 interacts with methylated H3 histone K9, whereas the C-terminal chromoshadow domain binds to non-histone proteins. However, the precise role of HP1 in heterochromatin stabilization and gene repression is still under investigation [20].

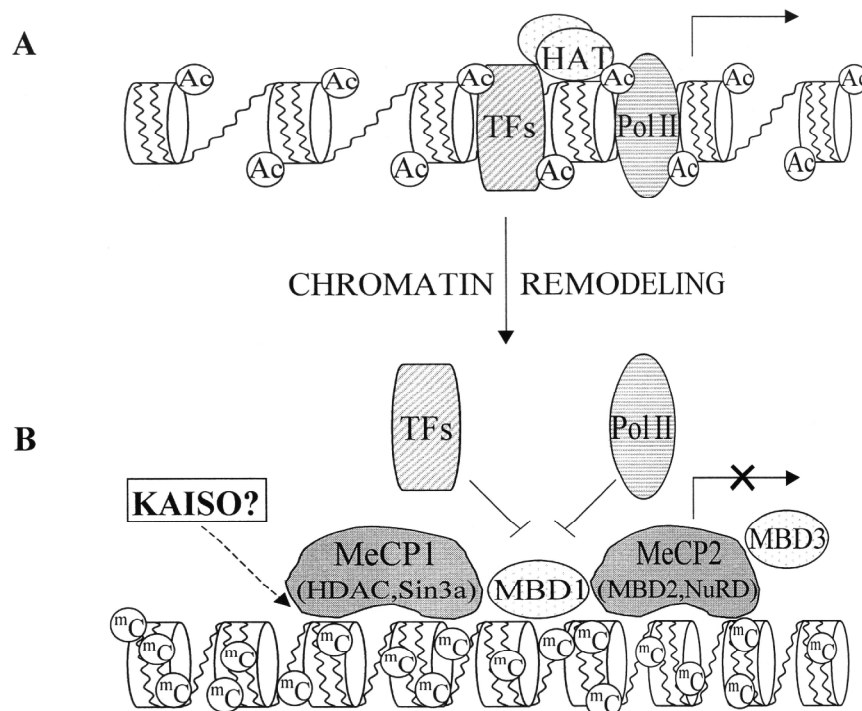


Fig. 2. The effect of DNA methylation on the repression of transcription. The transcriptionally active state of chromatin can be associated with a hypomethylated promoter and hyperacetylated (Ac) histones that can bind transcription factors (TFs) and RNA polymerase II (Pol II) to the promoter sequence (A). The transcriptionally inactive state of chromatin contains hypermethylated DNA and hypoacetylated histones that recruit m^5 CpG-binding proteins (MeCP1 and 2), corepressor protein (Sin3a), m^5 CpG-binding domain 2 (MBD2) protein, ATP-dependent remodelling protein (NuRD) and histone deacetylase (HDAC), and other protein repressors interfering with TFs and RNA Pol II binding to the promoter sequence (Tab. 1) (B). HAT represents histone acetyltransferase.

The methylation of mammalian genomic DNA is catalyzed by DNA methyltransferases (DNMTs) that can be divided into *de novo* and maintenance methyltransferases (Fig. 3) [21, 22]. *De novo* DNMTs can effectively methylate C to m^5 C post-replicatively in unmethylated DNA, whereas maintenance DNMT preferentially attaches a methyl group to hemimethylated DNA during replication (Fig. 3).

The methylation level of DNA can also be controlled by demethylation, the details of which remain unclear. During or after replication, DNA regions may bind sequence-specific proteins which block the attachment of the methyl group to CpG dinucleotide and the formation of methylation patterns unique for each tissue [23]. Demethylation of DNA regulatory sequences can be conducted in a

passive or active manner [10]. It has been suggested that an active mechanism is probably catalyzed by enzymes with demethylation activity. Although m⁵CpG-binding domain 2 (MBD2) protein and 5-methylcytosine DNA glycosylase have been postulated as candidates for demethylation of DNA, the regulation of this process requires additional detailed studies [10].

Tab. 1. Proteins participating in chromatin remodelling. MBD: m⁵CpG-binding domains, MeCP1: m⁵CpG-binding proteins, HDAC: histone deacetylase, HAT: histone acetyltransferase.

Proteins modifying chromatin structure	Function	
DNA methyl-transferases	DNMT1	maintains DNA-methylation pattern during replication [10, 19, 35]
	DNMT2	has very weak methylating activity [19, 35]
	DNMT3A	involved in <i>de novo</i> acquisition of DNA-methylation pattern [10, 19, 35]
	DNMT3B	
	DNMT3L	involved in maternal genomic imprinting affects activity of DNMT3A and 3B [35]
m ⁵ CpG-binding proteins	MBD1	m ⁵ CpG-binding domains that act as transcription repressors [10, 19]
	MBD2	
	MBD3	component of the chromatin remodelling protein complex Mi-2/NuRD [10, 19]
	MBD4	DNA glycosylase involved in DNA mismatch repair [19]
	MeCP1	MBD2-NuRD complex binding to methylated promoters that represses transcription [10]
	MeCP2	forms a complex with HDAC, co-repressor protein (Sin3a) and functions as a transcription uncharacterized protein lacking MBD domain but that binds to methylated DNA [10, 19]
	Kaiso complex	
	HDAC1, HDAC2	deacetylate histones [19]
Histone-modification enzymes	H3K4 MTases	methylate H3 histone K4 [19]
	Suv39h1, Suv39h2	methylate H3 histone K9 and K27 [19]
	G9a, Eu-HMTase1, ESET/SETDB1	methylate H3 histone K9 [19, 63-65]
	P300/CBP, PCAF, AF250, Gcn5	acetylate histones [19]
	p160 family	nuclear receptor that has histone acetyltransferase activity [19]
ATP-dependent remodelling proteins	Mi-2/NuRD	protein complexes that have ATPase activity introducing conformational changes in nucleosomal DNA [19]
	SWI/SNF/Brm	
	ISWI	

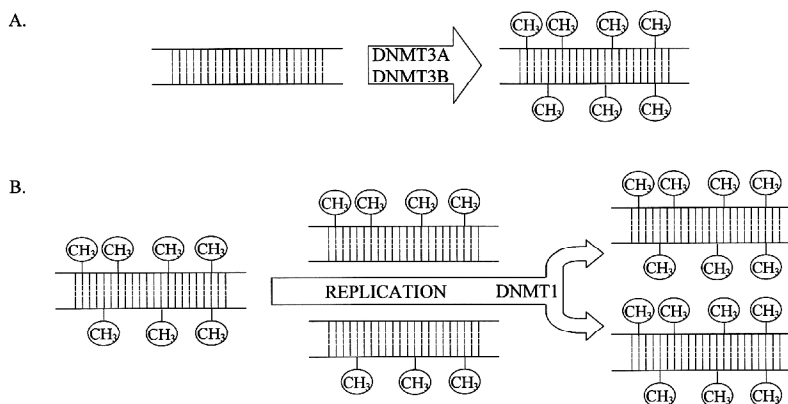


Fig. 3. The family of methyltransferases (DNMTs) is divided into *de novo* (A) and maintenance (B) DNMT. *De novo* DNMTs can create $m^5\text{CpG}$ dinucleotides in unmethylated DNA, whereas maintenance DNMT preferentially attaches methyl groups to hemimethylated DNA during replication.

The presence of $m^5\text{CpG}$ dinucleotides in the first gene exon or promoter may have an effect on gene transcription in a direct or indirect manner (Tab. 1). The direct mechanism involves the interference of $m^5\text{CpG}$ dinucleotides with transcription factors binding to a promoter. However, the indirect mechanism of gene regulation is preceded by DNA binding $m^5\text{CpG}$ dinucleotide-specific proteins which block the interaction of transcription factors with certain DNA sequences (Fig. 2, Tab. 1) [24]. These protein suppressors of promoters mainly include MBDs and $m^5\text{CpG}$ -binding proteins (MeCPs). These proteins are able to form complexes with HDAC, co-repressor (Sin3a) and ATP-dependent chromatin remodeling proteins, which are involved in the stabilization of heterochromatin structure (Fig. 2, Tab. 1).

DNA methyltransferase 1 (DNMT1) activity is crucial for the maintenance of DNA methylation and the appropriate histone H3 modification, which is important for the organization of chromatin domains. DNMT1 recruits chromatin-modifying enzymes including HDAC1, HDAC2 and histone methyltransferase (Suv39h1) [25, 26].

This indicates that DNMT activity is important for the initiation of chromatin remodeling and gene expression regulation. Mammalian DNMTs include DNMT1, DNMT3A, DNMT3B and DNMT3L, which are responsible for methylation pattern acquisition during gametogenesis, embryogenesis and somatic tissue development.

THE STRUCTURE AND FUNCTION OF EUKARYOTIC DNA METHYLTRANSFERASES

The DNMTs use S-adenosyl-L-methionine (AdoMet) as a donor of methyl groups (Fig. 1) [27]. The first DNMT was isolated and cloned from the

bacterium *Haemophilus haemolyticus*. This bacterial enzyme is composed of 327 amino acid residues and is three times smaller than eukaryotic DNMTs [28]. The eukaryotic DNMT family has five members: DNMT2, DNMT3A, DNMT3B, DNMT3L and DNMT1. DNMT3A and DNMT3B are *de novo* methyltransferases, whereas DNMT1 is involved in the maintenance of DNA methyltransferase (Fig. 3) [21, 22].

Mammalian DNMTs contain at least three structural regions, namely: the N-terminal regulatory domain, which is responsible for the localization of DNMTs in the nucleus; the C-terminal catalytic domain, which resembles that of the prokaryotic enzyme; and the central linker, which consists of repeated GK dipeptides (Fig. 4) [29]. The N-terminal domain plays a regulatory role, and contains a proliferating cell nuclear antigen-binding domain (PBD), a nuclear localization signal (NLS), a cysteine-rich zinc finger DNA-binding motif (ATRX), a polybromo homology domain (PHD), and a PWWP tetrapeptide chromatin-binding domain (Fig. 4) [22, 30]. By contrast, the primary sequence of the C-terminal catalytic domain of eukaryotic DNMTs contains ten different characteristic sequence motifs (Fig. 4). Six of them are evolutionally conserved: motifs I, IV, VI, VIII, IX and X (Fig. 4).

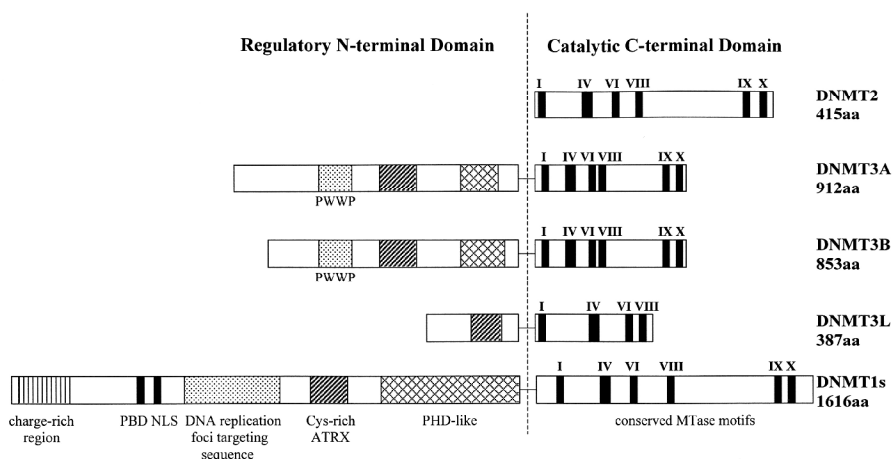


Fig. 4. The general structure of the members of the mammalian DNMT family [21, 22, 46, 48]. The N-terminal domain contains a proliferating cell nuclear antigen-binding domain (PBD), a nuclear localization signal (NLS), an ATRX cysteine-rich zinc finger DNA-binding motif, a polybromo homology domain (PHD) targeting DNMT to the replication foci, and a tetrapeptide PWWP, essential for DNMT binding to chromatin. The C-terminal domain includes six conserved motifs: I is involved in the formation of the AdoMet binding site; IV binds the substrate cytosine at the active site; VI contains the glutamyl residue serving as a proton donor; VIII's function is unclear; IX maintains the structure of the substrate-binding site; and X participates in the formation of the AdoMet binding site. aa – number of amino acids.

DNMT2 is the smallest mammalian DNA methyltransferase. It is composed solely of the C-terminal domain, and does not possess the regulatory N-terminal region. The DNMT2 catalytic domain does not exhibit particular *de novo* or maintenance methyltransferase activity in embryonic stem cells (ES) or adult somatic tissue. The structure of DNMT2 suggests that this enzyme can be involved in the recognition of DNA damage, DNA recombination and mutation repair [31, 32].

DNMT3A AND DNMT3B

DNMT3A and DNMT3B exhibit a high degree of primary structure homology, but these enzymes are encoded by different genes mapped to chromosomes 2p23 and 20q11.2, respectively [33]. These methyltransferases methylate CpG dinucleotides without preference for hemimethylated DNA, and are responsible for the *de novo* methylation of DNA, particularly during embryogenesis. DNMT3A and DNMT3B activity is reduced upon differentiation of ES cells and remains low in adult somatic tissues. The expression of *DNMT3A* is ubiquitous, while *DNMT3B* is expressed at very low levels in most tissues except the testis, thyroid and bone marrow [33]. DNMT3B level is profoundly increased in various tumor cell lines, indicating that it plays an important role in tumorigenesis [34, 35].

The architecture of the DNMT3A and DNMT3B enzymes is consistent with the general structure of the DNMTs (Fig. 4). The DNMT3A and DNMT3B PWWP domains may interact with chromatin, and thus the regulatory regions of these enzymes are able to bind to various transcriptional repressors (Fig. 4) [36]. DNMT3A can bind co-repressor RP58, oncogenic factor PML-RAR or HP1 β protein, whereas DNMT3B can be associated with Sin3a, condensin, KIF4A, SUMO-1/Ubc9 and ATP-dependent chromatin remodeling enzyme (hSNF2H) (Tab. 1) [32, 37]. DNMT3A and DNMT3B may also interact with DNMT1 and activate HDAC1, which deacetylates histones and represses gene transcription. This indicates that DNMT3A and DNMT3B may be involved in chromatin remodeling associated with the modulation of gene transcription.

Kelly and Trasler observed that DNMT3A is responsible for the *de novo* acquisition of methylation patterns during prenatal male germ cell development, while DNMT3B is involved in the maintenance of *de novo* methylation in the early stages of male germ cell mitosis [38]. The different roles of DNMT3A and DNMT3B in male gametogenesis suggest that both enzymes are essential in this process and cannot be substituted for each other [22, 38].

DNMT3A exhibits a lower level of enzymatic methyltransferase activity as compared to DNMT1. This may indicate that DNMT3A requires a small protein or co-factors for optimal activity. Furthermore, DNMT3A exhibits a preference for methylation sites that are flanked by pyrimidines [35]. Although DNMT3A is highly specific for CpG methylation, this enzyme is also able to methylate

cytosine at the CpA and CpT dinucleotides; however, the function of this DNA modification is still unknown [39].

DNMT3B is specialized for the methylation of CpG dinucleotides within repeated sequences of the pericentric satellite regions of chromosomes. Mutations within the DNMT3B gene can be associated with human genetic immunodeficiency disorder, centromere instability and facial anomalies syndrome (ICF syndrome) [40]. Individuals with ICF syndrome usually carry alleles with a mutation in the C-terminal DNA methyltransferase domain of DNMT3B. The chromatin abnormalities of ICF syndrome-affected patients include completely unmethylated DNA within the pericentric regions of chromosomes 1, 9 and 16 [22, 37, 38].

DNMT3B mRNA may form numerous alternative spliced variants which have a range of DNA binding affinities from very low to high. The existence of DNMT3B isoforms suggests that other factors can be involved in the binding of DNMT3B to a particular DNA region [34].

The DNA cytosine-like 5-methyltransferase (DNMT3L) protein lacks methyltransferase active site motifs and must cooperate with other *de novo* DNMTs [21, 41]. The *DNMT3L* gene is located on chromosome 21q22.3 and is mainly expressed in the postnatal female germ line during the acquisition of DNA methylation patterns. DNMT3L contains an active nuclear localization signal sequence (NLS) and the ATRX zing finger motif, which respectively enable this protein to translocate to the nucleus and bind to DNA. The same NLS and ATRX sequences were also found in the DNMT3A and DNMT3B enzymes (Fig. 4) [42-44].

The conserved PHD-like motif of DNMT3L interacts with and activates HDAC1. The role of DNMT3L in the activation of HDAC1 indicates that this protein is also involved in histone deacetylation, chromatin remodeling and transcription repression. This data also suggests that DNMTs are multi-functionally epigenetic proteins that also activate histone modification and chromatin structure rebuilding.

DNMT3L binds to the carboxyl-terminal part of DNMT3A and DNMT3B and increases the level of activity of these enzymes [45]. The interaction of mouse DNMT3L with DNMT3A induces conformational changes which increase the binding affinity of AdoMet to the substrate binding site. Human DNMT3L enhances the activity of DNMT3A and DNMT3B 1.5- to 3-fold, but does not affect the activity of DNMT1. Additionally, the DNMT3A/DNMT3L complex exhibits a higher binding affinity to DNA than DNMT3A does.

This suggests that DNMT3L is translocated to the nuclei, binds to DNA and may serve as a substrate exchange factor for functional DNMT3A and DNMT3B methyltransferases.

DNMT1 IS A MAJOR MAINTENANCE DNA METHYLTRANSFERASE

DNMT1 is the major enzyme responsible during replication for maintenance of the DNA methylation pattern (Fig. 3). During the replication of eukaryotic genomic DNA, approximately 40 million m⁵CpG dinucleotides are converted into the hemimethylated state in the newly synthesized DNA strand. These hemimethylated CpG sites must be methylated precisely to maintain the original DNA methylation pattern. DNMT1 is located at the replication fork and methylates newly biosynthesized DNA strands directly after the replication round (Fig. 3) [46]. DNMT1 displays a 5- to 40-fold higher activity *in vitro* for hemimethylated DNA than for unmethylated DNA [22, 46]. However, this enzyme also exhibits very weak *de novo* methylation activity which is stimulated by DNMT3A [47].

The structure of DNMT1 indicates that the DNMT1 gene could have been formed during the fusion of a prokaryotic DNMT gene with a mammalian DNA-binding protein gene [29, 48]. Mammalian DNMT1 is also composed of at least three major structural elements (Fig. 4). The first 621 amino acids of the N-terminus are not essential for DNMT1 activity [32]. However, the N-terminal DNMT1 domain is essential for discrimination between hemimethylated and unmethylated DNA strands and is responsible for a decrease in *de novo* methylation activity.

The charge-rich motif of the N-terminal domain of DNMT1 interacts with DNMT1 and represses transcription without the participation of HDAC (Fig. 4) [49]. The DNMT1 N-terminal domain can also interact with other proteins, including the proliferating cell nuclear antigen (PCNA), SNF2 family member ATP-dependent chromatin remodeling enzyme (hSNF2H), inhibitor of cyclin-dependent kinases (p21WAF1), E2F1 transcription factor, HDAC1 or HDAC2 (Fig. 4, Tab. 1) [6, 32, 35, 50]. The N-terminus of DNMT1 can also recognize the MBD1, MBD3, MeCP2 and HP1 proteins. The interaction of DNMT1 with numerous protein suppressors of promoters suggests that this DNA methyltransferase is also a crucial element of the transcription suppression complex.

The ATRX zinc finger motifs and the PCNA-binding domain (PBD) of DNMT1 also respectively interact with PCNA and DNA during replication. This causes a better presentation of targeting sequences (TS) and PBHD domains, which stabilizes the replication foci and induces an increase in the biosynthesis rate of new DNA strands (Fig. 4) [51]. DNMT1, which forms the core of the DNA replication machinery complex, is also involved in the mismatch repair system [52].

The primary structure of human DNMT1 suggests that the entire catalytic site of this enzyme is composed of 500 amino acids and is located at the C-terminal domain [32]. The C-terminal catalytic domain of DNMT1 is characterized by the presence of 10 conserved amino acids motifs. Five of these motifs, namely I, IV, VI, VIII, X, are involved in the binding of substrate to DNMT1 (Fig. 4) [29].

Recently, several disparate isoforms of DNMT1 were identified in human somatic and female and male germ cells [53-55].

DNMT1 ISOFORMS IN HUMAN GERM CELLS

Compared to the mouse model, little is known about the mechanisms responsible for the acquisition and maintenance of DNA methylation patterns in human germ cells and early embryos [38]. The human genomic DNA of the spermatozoon and oocyte exhibits different sex-specific methylation patterns that are essential for primary gene expression during early embryogenesis [53, 56]. The sex-specific DNA methylation patterns of germ cells are modified during somatic cell differentiation in embryogenesis. DNMTs with *de novo* activity are essential to establish new patterns of methylation in early embryos, and the level of this methyltransferase expression is reduced in differentiated somatic cells.

Although the DNA methylation level of primordial germ cells is low, sperm DNA is more methylated than oocyte genomic DNA [57, 58]. The function of sex-specific methylation patterns in the female and male germ line is under intensive investigation. The acquisition of a methylation pattern requires the involvement of a protein complex composed of DNMTs with *de novo* activity, DNA-binding proteins and chromatin structure modeling factors (Tab. 1).

The different expression patterns of DNMT3A, DNMT3B, DNMT3L and DNMT1 in the female and male germ lines are probably essential for the acquisition of a sex-specific methylation pattern [54]. The formation of the DNA methylation pattern is initiated in the female and male prenatal germ line. In female germ cells, methylation patterns are further acquired postnatally, during the oocyte growth phase, and are completed after the pachytene phase of meiosis. The male genomic DNA methylation pattern is partially formed in prospermatogonia before birth and is completed before the end of the spermatogenesis pachytene phase in the adult male [56].

The mRNA of DNMT1 is present at high levels in postmitotic female and male germ cells. However, the *DNMT1* gene is unique because of its possession of sex-specific promoters and a multi-potential first exon. The DNMT1 biosynthesis and localization during various stages of gametogenesis is controlled by unique sequences of first gene exons, which are formed during alternative splicing. The first exon of the DNMT1 sequence is different in transcript isoforms which were found in growing oocytes (DNMT1o), pachytene spermatocyte (DNMT1p) and somatic cells (DNMT1s) (Fig. 5) [59].

The DNMT1o transcript contains the translation start codon at the methionine (ATG₄) in exon 1, whereas the DNMT1s initiation codon of translation (ATG₃) is located in exon 1s [48]. The DNMT1o transcript is used as a template for the biosynthesis of protein which lacks the first N-terminal 118 amino acid residues present in DNMT1s [7, 55]. The DNMT1o protein is stored in the cytoplasm of the mature metaphase II oocyte and in the cytoplasm of the pre-implantation cleavage stages of embryos. After implantation of the eight-cell-stage embryo,

DNMT1o is translocated to the nucleus [60, 61]. This may suggest that DNMT1o is not required for the acquisition of the maternal imprint but seems to be essential for maintaining imprints of the eight-cell blastomere [56].

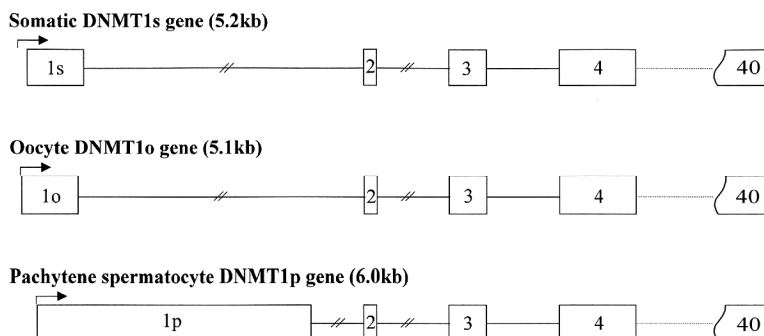


Fig. 5. A comparison of the first exon length of somatic (DNMT1s), oocyte (DNMT1o) and pachytene spermatocyte (DNMT1p) genes of maintenance DNA methyltransferase 1 (DNMT1). The first exon of the DNMT1 sequence is different in transcript isoforms which were found in somatic cells (DNMT1s), growing oocytes (DNMT1o) and pachytene spermatocytes (DNMT1p). The DNMT1o transcript contains a translation start codon at the methionine (ATG₄) in exon 4, whereas the DNMT1s initiation codon of translation (ATG₃) is located in exon 1s. DNMT1o transcript is used as the template for the biosynthesis of protein which lacks the first N-terminal 118 amino acid residues present in DNMT1s. By contrast, the DNMT1p transcript first exon sequence is designated as 1p and cannot be associated with polyribosomes. The spermatocyte-specific 1p exon sequence interferes with the translation machinery and prevents DNMT1 biosynthesis [53].

By contrast, the DNMT1p transcript first exon sequence is designated as 1p and cannot be associated with polyribosomes. The spermatocyte-specific 1p exon sequence interferes with the translation machinery and prevents DNMT1 biosynthesis (Fig. 5) [62]. Thus, DNMT1 activity is absent in spermatocytes during spermiogenesis.

The expression of DNMT1 is precisely regulated during female and male gametogenesis and the main differences are found in the pre- and perinatal expression of this enzyme. Transcripts of DNMT1o and DNMT1p are at a higher level, respectively in the testis and ovaries, relative to the levels in other tissues. Neither transcript is found in female and male mature germ cells. The transcriptional mechanism responsible for the suppression of DNMT1o and DNMT1p transcription in somatic cells and in other phases of gametogenesis is still unknown and requires further investigation.

The DNMT1p transcript is abundantly present in the pachytene spermatocytes, whereas the same cells lack DNMT1 protein. DNMT1 protein is present at significant levels in mature oocytes and pre-implantation embryos; however, the mRNA content of this enzyme is very low in the same cells [22]. The

significance of correlation loss between the DNMT1 transcript and the protein levels in germ and early embryonic cells is still unexplained [53]. This may suggest that other unidentified DNMTs may be responsible for the imprinting status in zygote and early embryos.

The presence of mRNAs in human ejaculated spermatozoa is well-determined, and approximately 3000 different mRNAs are present in fertile sperm [66-68]. We presume that the DNMT1p transcript may survive spermatid condensation and can be present in mature spermatozoa. After oocyte fertilization, DNMT1p mRNA may lose the 5'p fragment of the first exon and serve as a transcript for DNMT1 biosynthesis during zygotic and embryonic development. However, this hypothesis requires further detailed investigation. Recent advances in the investigation of DNMT function in the stabilization of chromatin structure formed the basis of the understanding of the abnormal regulation of gene expression associated with autoimmune and malignant diseases [11, 14].

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