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Review

# Mechanisms of human DNA methylation, alteration of methylation patterns in physiological processes and oncology

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ABSTRACT

DNA methylation is one of the epigenetic modifications of the genome, the essence of which is the attachment of a methyl group to nitrogenous bases. In the eukaryote genome, cytosine is methylated in the vast majority of cases. About 98% of cytosines are methylated as part of CpG dinucleotides. They, in turn, form CpG islands, which are clusters of these dinucleotides. Islands located in the regulatory elements of genes are in particular interest. They are assumed to play an important role in the regulation of gene expression in humans. Besides that, cytosine methylation serves the functions of genomic imprinting, transposon suppression, epigenetic memory maintenance, X- chromosome inactivation, and embryonic development. Of particular interest are the enzymatic processes of methylation and demethylation. The methylation process always depends on the work of enzymatic complexes and is very precisely regulated. The methylation process largely depends on the functioning of three groups of enzymes: writers, readers and erasers. Writers include proteins of the DNMT family, readers are proteins containing the MBD, BTB/POZ or SET- and RING-associated domains and erasers are proteins of the TET family. Whereas demethylation can be performed not only by enzymatic complexes, but also passively during DNA replication. Hence, the maintenance of DNA methylation is important. Changes in methylation patterns are observed during embryonic development, aging, and cancers. In both aging and cancer, massive hypomethylation of the genome with local hypermethylation is observed. In this review, we will review the current understanding of the mechanisms of DNA methylation and demethylation in humans, the structure and distribution of CpG islands, the role of methylation in the regulation of gene expression, embryogenesis, aging, and cancer development.

*Abbreviations:* 5caC, 5-carboxylcytosine; 5fC, 5-formylcytosine; 5hmC, 5-hydroxymethylcytosine; ACHY, acetylcholinesterase; AID/APOBEC, activation-induced deaminase/apolipoprotein B mRNA editing enzyme; APC, adenomatous polyposis coli; ATM, ataxia telangiectasia mutated gene; BER, base excision repair; BRCA, breast cancer antigen; BTB/POZ, broad-complex, tramtrack and bric-a-brac/poxvirus and zinc finger; CDKN2A, cyclin-dependent kinase inhibitor2A; CDKN2B, cyclin-dependent kinase inhibitor 2b; CHK2, checkpoint kinase 2; DMAP, DNA methyltransferase associated protein; DNMT, DNA methyltransferase; ESR1, estrogen receptor 1; FANCF, Fanconi anemia group F protein; HCC, hepatocellular carcinoma; HDAC, histone deacetylase; hoxa9, homeobox protein Hox-A9; HPSE, heparanase; HRAS, harvey Rat sarcoma virus; ICR/DMR, differentially methylated regions of imprinting control regions; IGF2, insulin-like growth factor 2; LIG4, DNA ligase 4; LINE, long interspersed nuclear elements; MAPK/ERK, mitogen-activated protein kinase/extracellular signal-regulated kinase signaling pathway; MAT, methionine adenosyltransferase; MBP, methyl-binding protein; MeCP, methyl-CpG-binding protein; METTL14, methyltransferase 14, N6-adenosine-methyltransferase subunit; METTL3, N6-adenosine-methyltransferase 70 kDa subunit; MLH, DNA mismatch repair protein; NCoR, nuclear receptor co-repressor; NRF, nuclear respiratory factor; $p16^{INK4a}$, cyclin-dependent kinase inhibitor 2A; PAF15, proliferating cell nuclear antigen (PCNA)-associated factor; PCNA, proliferating cell nuclear antigen; RASSF1, Ras association domain-containing protein; RBBP8, retinoblastoma-binding protein 8; SAH, S-Adenosyl-L-homocysteine; SAM, S-adenosylmethionine; SFRP1, secreted frizzled-related protein 1; SLE, systemic lupus erythematosus; SLIT2, slit guidance ligand 2; SOCS3, suppressor of cytokine signaling 3; SOX17, SRY-box transcription factor 17; SRA, stationary-phase-induced ribosome-associated protein; TDG, thymine-DNA glycosylase; TET, ten-eleven translocation protein family; TP53, tumor protein 53; TP73, tumor protein 73; TSG, tumor suppressor gene; UHRF, ubiquitin-like, containing PHD and RING finger domains; VIRMA, vir like m6A methyltransferase associated; WIF1, Wnt inhibitory factor 1; WTAP, Wilms' tumor 1-associating protein; XPG, xeroderma pigmentosum group G complementing factor; ZNF382, zinc Finger Protein 382.

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## 1. Introduction

Methylation is a crucial DNA modification that has significant effects on gene expression. Among the various forms of DNA methylation, cytosine methylation is the most frequent in eukaryotic cells. Cytosine methylation is an epigenetic mechanism in which the methyl group is transferred to the fifth carbon of cytosine, resulting in the formation of 5-methylcytosine (Fig. 1) (Moore et al., 2013). Although cytosines can be methylated in any context, more than 98% of DNA methylation occurs in cytosines located in the CpG dinucleotide in somatic cells. The main functions of this modification are genomic imprinting, X chromosome inactivation, suppression of transposons, regulation of gene expression, maintenance of epigenetic memory, and embryonic development (Field et al., 2018; Xiao et al., 2018).

The mammalian genome contains approximately 28 million CpG dinucleotides, which are unevenly distributed, and each can exist in a methylated or unmethylated state (Jin et al., 2011). It is assumed that in most cases, methylation of individual sites has no specific biological function (Edwards et al., 2013).

## 2. Enzymatic DNA methylation systems

The process of cytosine modification in animals is mediated by DNA methyltransferases (DNMTs), leading to the formation of 5-methylcytosine (5mC) (Bacalini et al., 2021). There are 5 DNMTs encoded in the human genome: DNMT1, DNMT2, DNMT3a, DNMT3b, and DNMT3l. DNMT1, DNMT3a, and DNMT3b are canonical methyltransferases that directly catalyze the addition of methyl groups to cytosine. DNMT1 usually localizes to replication foci in the S phase of the cell cycle and methylates a new daughter strand, earning its name as the "maintenance methyltransferase." The N-terminal domain of DNMT1 contains several subdomains that provide recognition of distinct targets. For example, the CXXC domain acts as a sensor for unmethylated CpG (Pradhan et al., 2008) while DMAP is required for the recruitment of the transcriptional repressors DMAP1 and HDAC (Rountree et al., 2000). DNMT1 also contains a proliferating cell nuclear antigen (PCNA) interaction region required to target the replication fork. Recent studies indicate that the active center of the C-terminal domain interacts specifically with a preference 30–40-fold higher for hemimethylated DNA (Loaeza-Loaeza et al., 2020). The multidomain protein UHRF1 plays a crucial role in directing methyltransferase to DNA replication sites by binding specifically to hemimethylated DNA via its SET and SRA domains (Miyashita et al., 2022). Furthermore, this protein is an E3 ubiquitin ligase that ubiquitinates histone H3 and PAF15 (PCNA-Associated Factor 15), resulting in DNMT1 recruitment (Veland et al., 2017; Petryk et al., 2020).

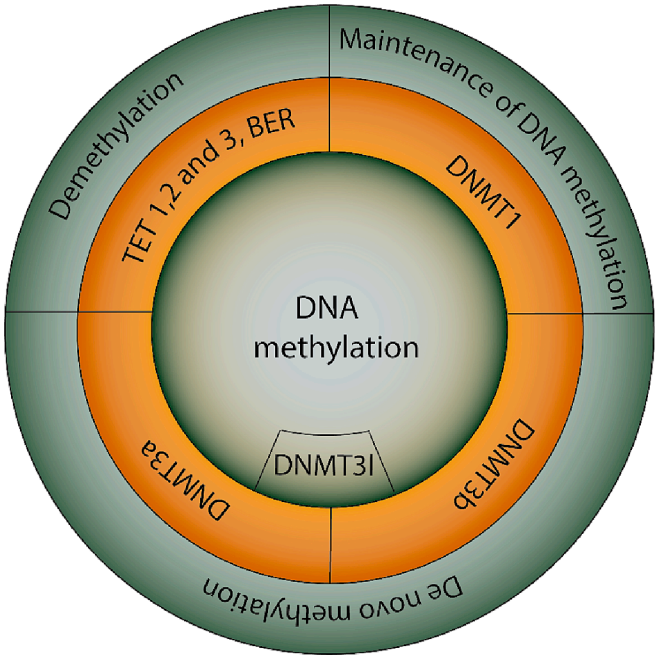


**Fig. 1.** Enzymatic system of regulation of methylation in mammals.

In contrast to DNMT1, DNMT3a and DNMT3b mediate *de novo* methylation (Fig. 1). DNMT3a specifically methylates imprinted genes in germ cells and the *XIST* gene on the X chromosome, while DNMT3b can methylate centromeric satellite repeats. Hence, both methyltransferases can methylate almost all DNA loci (Li et al., 2007, Jin et al., 2013).

The enzymatic system of DNA methylation includes the supporting DNA methyltransferase DNMT1 and the methyltransferases DNMT3a and DNMT3b, which are responsible for the *de novo* methylation of DNMT3l the supporting enzyme. RNA methylation is performed by the enzymes METTL3 and METTL14. Active demethylation is catalyzed by enzymes belonging to the TET family and those of the excision repair complex.

The N-terminal domain of DNMT3 contains the PWWP and ADD regulatory subdomains, and six CXXC repeats followed by the C-terminal moiety. Although the PWWP domain is positively charged and can interact with negatively charged DNA, it is not involved in the catalytic activity of the enzyme, unlike the CXXC motif (Loaeza-Loaeza et al., 2020).

DNMT3l has no catalytic activity but it can stimulate methylation by DNMT3a and DNMT3b methyltransferases, thereby increasing their ability to bind to S-adenosylmethionine (SAM) (Li et al., 2007, Jin et al., 2013, Gao et al., 2020). Mammalian cells also have the DNMT2 methyltransferase, also known as TRDMT1. Initially, it was assumed that it performs the function of DNA methylation since it is a homologue of other representatives of this family. However, it is now established that DNMT2 is involved in the methylation of cytosine-38 in the anticodon loop of the aspartic acid transfer RNA, which is important for maintaining tRNA stability, methylating RNA and playing a role in the immune response against many pathogens, including RNA viruses (Jin et al., 2011; Bhattacharya et al., 2021, Li et al, 2018).

The functionality of methyltransferases can be affected by various factors. For example, in systemic lupus erythematosus, DNA methylation aberrations are associated with DNMT1 expression, with significantly lower levels of DNMT1 and DNMT3A transcripts observed. In disorders causing decreased expression or functionality of DNMT1, there is no complete transfer of the methylation pattern to a newly generated cell after mitosis, which leads to passive demethylation. DNMT1 activity is regulated by the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling pathway. Environmental exposures stimulate reactive oxygen species production, which could lead to DNA methylation aberrations by disrupting the MAPK/ERK pathway (Lanata et al., 2018).

SAM molecules serve as a source of $CH_3$ groups for methylation. SAM is produced during the "methionine cycle" reactions from methionine by the enzyme methionine adenosyltransferase (MAT). Next, SAM is converted into S-Adenosyl-L-homocysteine (SAH) by methyltransferases, which is then hydrolyzed into homocysteine by acetylcholinesterase (ACHE) (Fig. 2) (Razumkina et al., 2018). S-adenosylmethionine is synthesized in the cytoplasm of every cell. Its synthesis is especially active in hepatocytes. The process of attaching a methyl group to cytosine is a multistep process. The conversion of methionine to homocysteine is catalyzed by a complex of enzymes such as MAT, DNMT, and ACHE. At the stage of transformation of S-adenosylmethionine into S-adenosylhomocysteine the transfer of methyl group to cytosine with its transformation into 5-methylcytosine occurs (Razumkina et al., 2018). SAM levels are influenced by dietary intake of folic acid, vitamins B6 and B12 (Lanata et al., 2018). Notably, low methionine levels can affect DNA methylation, thereby leading to the dysregulation of gene expression

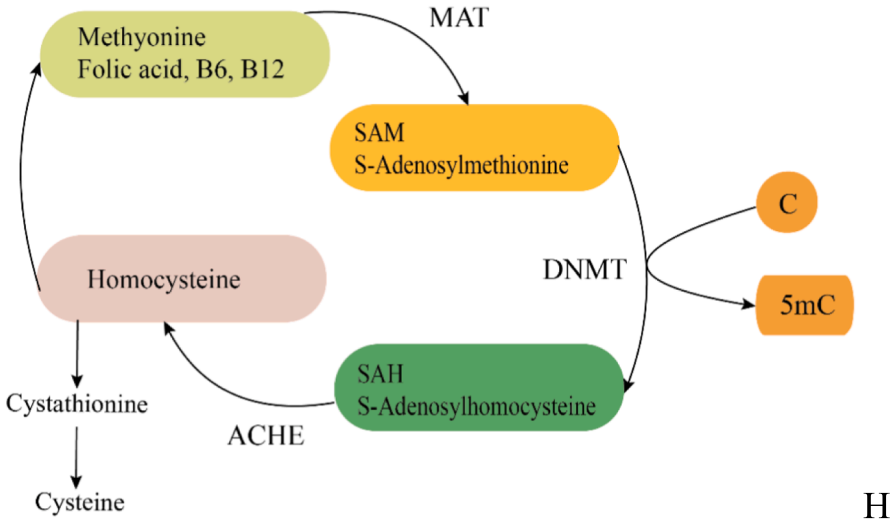


**Fig. 2.** Scheme of reactions of the methionine cycle leading to methylation of cytosine.

(Zhang et al., 2018).

Nutrigenomics is a modern scientific discipline that studies the influence of nutrition, one of the most important environmental factors, on epigenetic processes. It is known that several dietary molecules can act as epigenetic modulators. The Mediterranean diet, which is characterized by a high consumption of fruits, vegetables, seafood, and olive oil, is a preferred diet type. Some fruits and vegetables, which contain vitamin B12 and folic acid, are methyl group donor molecules. Insufficient intake of these foods can cause DNA hypomethylation. For example, a reduced LINE-1 methylation pattern can cause genomic instability and increase cancer risk (Caradonna et al, 2020).

DNA methylation is not a static modification. Patterns are altered by methylation and demethylation in both normal and various human pathologies. There is active and passive demethylation. The passive one occurs as a result of sequential processes of DNA replication when the newly synthesized strand is not yet methylated, and there is an absence or lack of a mechanism for maintaining methylation in the cell. Demethylation is the process of sequentially transforming 5-methylcytosine through deamination and/or oxidation. Deamination occurs more frequently spontaneously. The resulting product is recognized by the base excision repair (BER) system to replace the modified nucleotide with cytosine (Bhutani et al., 2011). 5mC can be chemically modified at two sites: an amino group and a methyl group. As a result of deamination to a carbonyl group using the AID/APOBEC complex, 5mC is converted to thymine. Thus, a G/T mismatch, which induces a BER pathway to correct the base, is created (Moore et al., 2013).

Active demethylation is a key enzymatic process in which a methyl group is modified or removed. This process involves proteins of the TET (ten-eleven translocation methylcytosine dioxygenase) family (TET1, 2, and 3); the main domain of these enzymes uses molecular oxygen to catalyze the oxidative decarboxylation of α-ketoglutarate. The cysteine-rich helper domain plays a regulatory role by stabilizing TET-DNA binding. Depending on the substrate, different representatives of TET catalyze the demethylation reaction. Thus, TET2 has lower activity against 5mCpC and 5mCpA than against 5mCpG (Wu and Zhang, 2017; Kohli and Zhang, 2013). In both active DNA demethylation mechanisms mentioned, thymine DNA glycosylase cleaves the modified residue during excision repair (Lio et al., 2020).

TET-mediated demethylation can be performed by either dependent ('passive') or independent ('active') mechanisms of DNA replication. The replication-dependent DNA demethylation is based on the fact that DNMT1/UHRF1 complex does not recognize 5hmC, 5fC or 5caC. As a result, the sites gradually lose their methylation tags. The second demethylation mechanism involves thymine-DNA-glycosylase (TDG), a repair enzyme that can remove 5fC and 5caC. The resulting products are then removed by the excision repair mechanism (Onodera et al., 2021).

The first intermediate in the demethylation of 5-methylcytosine is 5-hydroxymethylcytosine (5hmC), which is formed as a result of oxidation by TET enzymes. The degree of 5hmC methylation and its occurrence vary between different tissues, with its highest frequency observed in the brain, where ~ 1% of cytosines are represented in the form of 5-hydroxymethylcytosine. Among cytosine modifications, 5-hmC is primarily associated with demethylation and the active state of chromatin. In embryonic stem cells, it is associated with the regulatory elements of pluripotency-associated genes. The subsequent demethylation products include 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). 5caC can be formed either as an oxidation product directly from 5mC or during sequential oxidation with the formation of an intermediate 5fC. Studies show that 5-formylcytosine plays an important role in the regulation of gene expression in embryonic stem cells. Additionally, 5-carboxycytosine is a marker of active demethylation and is also found in the promoters of highly transcribed genes during the embryonic development (Wu and Zhang, 2017; Kohli and Zhang, 2013; Bochtler et al., 2017).

DNA methylation and demethylation processes in cells are strictly regulated by enzymatic systems and depend on various external and internal factors. Despite the high level of regulation, abnormalities can sometimes be observed that can lead to various diseases.

## 3. CpG islands

The gradual decrease in the number of CpG dinucleotides observed during evolution results in a frequency lower than the theoretically expected one. Specifically, in the human genome, the total content of guanine and cytosine is 42%, while the expected content of CpG dinucleotides is 4.4%. Spontaneous deamination of methylcytosine and its conversion into thymine contributes to a decrease in the number of CpG sites, resulting in a CpG frequency in the human genome of less than one-fifth of the expected frequency (Babenko et al., 2017).

The distribution of CpG dinucleotides throughout the genome is uneven. Their accumulations form CpG islands (CGIs), which have a length of more than 200 bp, the content of the available number of CpGs to the expected number of more than 60% (O/E), the total content of guanine and cytosine over 50% (Gardiner-Garden et al., 1987). Varios methods, such as CpGProD, CpGIS and CpGplot, have been developed to predict CpG islands. They rely mainly on such properties of CpG islands as sequence size, the relative amounts of guanine and cytosine, and the O/E ratio (Yang et al., 2016).

CGIs are surrounded by "shores", "shelves", "open sea" regions

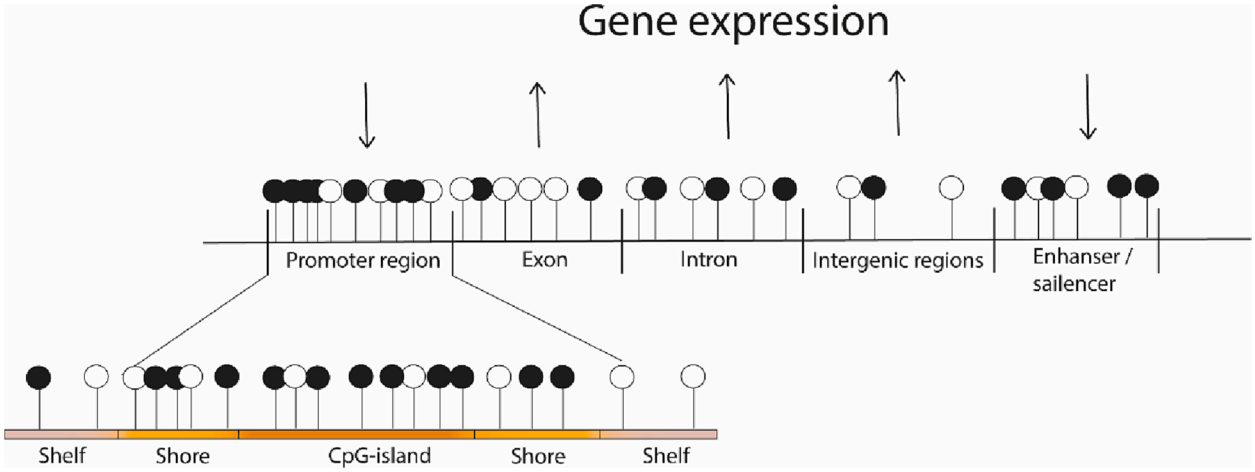


**Fig. 3.** Scheme of the structure of the CpG island.

(Fig. 3). Methylation levels vary from region to region. The number of CpG dinucleotides fluctuates in different regions, with the highest density observed in the body of the island, which gradually decreases towards the "open sea" region, where it reaches the lowest density. The "shores" are regions located at a distance of 0–2 kb from the island on both sides, where methylation levels are most susceptible to tissue differentiation, reprogramming, aging, and diseases, including cancer and Alzheimer's disease (Rao et al., 2013; Mitsumori et al.,2020). Notably, in the aggressive form of breast cancer, the "shores" of the island are characterized by a low level of methylation (Chae et al., 2016). The "shelves" are the regions at a distance of 2–4 kb and are also characterized by a decrease in methylation levels, as are the "shores" (Visone et al., 2019). The remaining regions are considered "open sea" (Rechache et al., 2012). It was noted that in oncological diseases, the average level of methylation of the island increases, while in the "open sea" region, it decreases (Lietz et al., 2020). Loss of methylation in the "open sea" regions of cancer has been linked to loss of imprinting, transcriptome alteration and chromosomal instability (Ibrahim et al., 2022).

Methylated cytosines are marked in black and unmethylated cytosines in white. The CpG island is surrounded on both sides by "shores," "shelves," and "open sea" regions. The separation depends on the density of CpG dinucleotides and their distance from the "island".

CpG islands are unevenly distributed throughout the genome. They are localized in promoter regions, enhancers, exons, introns, repeated sequences, subtelomeric regions, and intergenic regions. Fig. 4 presents the distribution of CpG cites throughout the human genome (Mahmood, Rabbani, 2019). Figure 5shows the distribution of CpG islands in the human genome.

The approximate relative distribution of methylation in different compartments of the genome is shown. Unmethylated fractions are marked in red. Introns and enhancers are classified as "Other compartments".

CpG islands are areas where CpG sites are densely arranged. There are certain criteria for identifying CpGIs and automated ways to identify them as well. The localisation of CpGIs is uneven along the human genome, which may be due to the different needs of genomic loci to be regulated through DNA methylation.

## 4. Regulation of gene transcription

Epigenetic regulation of transcription through DNA methylation can occur via two mechanisms: direct and indirect. The direct mechanism is based on the steric hindrance of methyl groups to the interaction of transcription factors with DNA. The indirect mechanism requires the involvement of proteins with high affinity to 5mC, which inhibit DNA

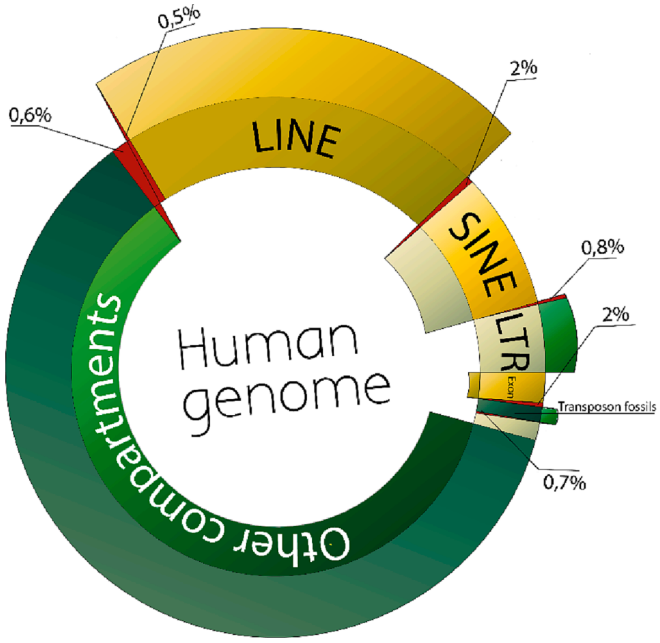


**Fig. 4.** Distribution of DNA methylation in the human genome.

binding to transcription factors (Loaeza-Loaeza et al., 2020).

It is noteworthy that approximately 40% of the genes encoding human proteins contain CpG islands in the promoter regions. Generally, housekeeping genes (60%) are characterized by the presence of CGIs in the promoter region (Mancini et al., 2021; Aoto et al., 2020). CGIs located in the promoter zone of actively expressed genes are almost always unmethylated. As a result, these genes are actively transcribed. CpG promoter islands may not have common promoter regions, such as TATA boxes. CpG dinucleotides can be part of motifs that serve as binding sites for transcription factors (Moore et al., 2013). The overall methylation pattern of the CpG island is now considered more important than the methylation of individual positions. CpG dinucleotides can facilitate the binding of transcription factors to DNA, thereby supporting gene expression. Earlier, it was assumed that high CpG dinucleotide densities would suppress gene function, and conversely, sequences with low CpG densities are associated with methyl-dependent transcription factors, such as NRF (nuclear respiratory factor) (Hartl et al., 2019).

In mammals, MeCP1 and MeCP2 proteins were initially implicated in the regulation of methyl-dependent gene expression, but later, it was

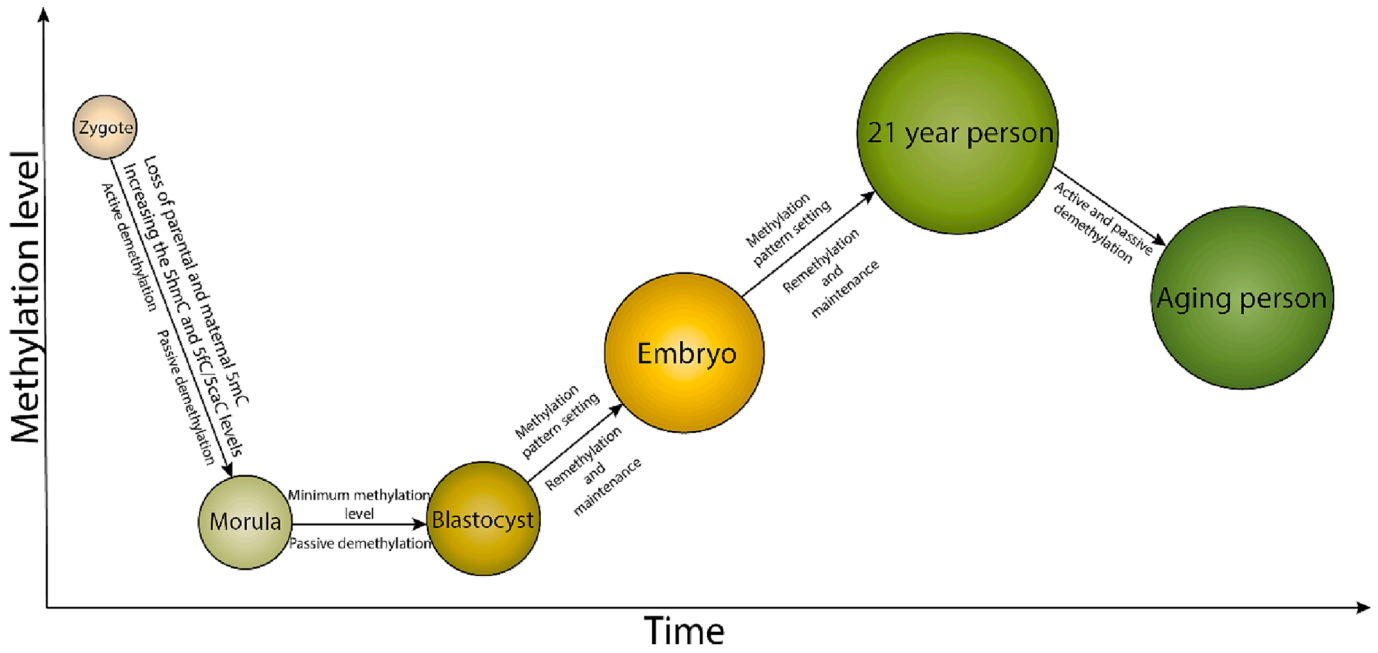


**Fig. 5.** Scheme of genome demethylation during embryogenesis.

discovered that MeCP1 is involved in chromatin remodelling. Consequently, MeCP2 is now considered to play a predominant role (Mahmood, Rabbani, 2019). The MeCP2 structure contains a methyl-CpG-binding domain (MBD) whose functional role is to recognize and bind methylated sites. Other proteins of the family possess unique domains that confer specific properties. For instance, the transcriptional repression domain (TRD) involved in gene silencing is present in MBD1, MBD2 and MeCP2 proteins. However, MBD5 and MBD6 proteins, despite having a methyl-binding domain, are unable to bind to methylated DNA (Shimbo et al., 2016). Both MBD5 and MBD6 proteins interact with polycomb proteins, and MBD5 contains a PWWP motif that allows it to bind to methylated histones (Moore et al., 2013).

In addition to proteins of the MeCP2 family, another family of 8 members has been found, one of which is the Kaiso protein (Hudson et al., 2018). Proteins of this family recognize methylated DNA using zinc fingers (Prokhortchouk et al., 2001). The BTB/POZ domain within Kaiso recruits repression complexes to methylated DNA (Kaplun et al., 2019). The SRA family, which contains SET- and RING-associated domains, was identified as the third family capable of binding methylated DNA. UHRF proteins belong to this family.

DNA methylation has long been thought to affect gene transcription alone. However, it has been found recently that attachment of the methyl group to cytosine may influence the alternative splicing process. Alternative splicing allows for increased diversity of mRNAs and their protein products. 95% of human multiexon genes undergo alternative splicing. The level of exon methylation was found to be higher than that of introns. In this way, these structural elements of genes can be differentiated. Methylation can affect alternative splicing in two ways. The first involves modulation of the rate of operation of RNA polymerase II using CTCF and MeCP2. CTCF and methylation play mutually exclusive roles by stimulating or inhibiting recognition of exons undergoing alternative splicing. In addition, CTCF attachment can support hypomethylation of a nearby region. The second method involves a heterochromatin protein (HP1). It recruits splicing factors to alternative splicing-exposed exons (Maunakea et al., 2013, Lev Maor et al., 2015, Shayevitch et al., 2018, Shayevitch et al., 2022).

Recent studies suggest that CGIs may play another regulatory role: they can enhance gene expression. Intragenic enhancers can influence host gene expression through RNA interference. In addition, it has been found that intragenic islands can affect pre-mRNA processing (Cain et al., 2022). Deaton and Bird make several suggestions about the functional significance of CpG island genes located in the body. These sites can initiate the transcription of regulatory noncoding RNAs, participate in the mechanisms of genomic imprinting, and influence alternative mRNA splicing (Kornblihtt, 2006).

One of the common features of CpG islands is that they contain fewer nucleosomes than other regions of DNA. Nucleosomes associated with CGIs are involved in enhancing gene expression (Moore et al., 2013). Thus, the question of whether DNA methylation represses or activates gene expression is highly locus- and stimulus-dependent (Dhar et al., 2021).

Methylation of CpG islands interferes with TF binding, thereby disrupting gene activity through the recruitment of methyl-dependent repressor proteins (Moore et al., 2013). However, for some genes, hypermethylation of promoter regions does not alter or even increases the level of expression. Some genes are characterized by the suppression of functional transcript synthesis when an island in the promoter is demethylated, as RNA polymerase II is then not recurrent. The methylation of individual CpGs is important for regulating expression, although the methylation status of these CpGs may be opposite to that of the island as a whole. For example, for the IL-6 gene, methylation levels at two CpG sites downstream of the transcription start site have been shown to influence gene expression. When CpG + 286 and CpG + 348 positions are hypermethylated, gene expression is suppressed. In addition, hypermethylation or deletion of CpG + 348 inhibits recruitment of CTCF (Lian et al., 2022).

Current scientific understanding suggests that methylation does not have a suppressive effect on gene expression, as the activity of some genes appears to be independent of methylation. Promoters with low CpG content can be transcriptionally active despite methylation. Sparse DNA methylation of promoters may repress transcription, but this effect could be overcome by an enhancer (Medvedeva et al., 2014).

Research has also shown both positive and negative correlations between tissue-specific methylation and gene expression. Regions with a positive correlation between methylation and expression are found to be more enriched in CpG dinucleotides in the promoter regions. This suggests that TFs bind to methylated regulatory elements and activate gene expression. In some regions, a positive correlation between methylation levels and gene expression is found. However, the presence of negative transcription regulators there suggests an indirect way of inhibiting gene

expression (Wan et al., 2015). In addition, blocking the binding of transcription factors to binding sites by methylation of individual CpGs is not expected to be a general regulatory mechanism of transcription (Medvedeva et al., 2014).

The regulation of gene expression by methylation mainly consists of suppression of expression in hypermethylated regions and its activation upon hypomethylation. In active genes, promoters are usually hypomethylated and internal CpG sites are hypermethylated. This prevents the formation of alternative forms of mRNA transcript. However, some transcription factors have an affinity for methylated sites. Thus, the expression of such loci is activated by the attachment of methyl groups.

## 5. The role of methylation in human embryonic development

Current understanding suggests that the epigenome plays a critical role in embryogenesis. The genome undergoes a two-stage reprogramming process during embryogenesis. The first wave of methylation tag loss occurs during the formation of primary germ cells. The significance of this process is to protect the cell lines of the future embryo from epimutations. This process is thought to be TET-dependent. Methyltransferases DNMT3a and DNMT3l then form new methylation patterns de novo, which leads to the formation of gender-specific gamete methylation patterns (Gopinathan, 2022).

Next wave of extensive genome demethylation occurs during early mammalian embryonic development (Li et al., 2018). The first stage occurs a few hours after the formation of the zygote. All methyl groups of the paternal pronucleus are removed within the first 6 h of forming the zygote, before any replication has occurred. At the morula stage, gamete methylation patterns are completely obliterated (Bernstein and Bernstein, 2020). Germline differentially methylated regions (gDMRs) and some retrotransposons, whose methylation is maintained by DNMT1, are not demethylated. Remethylation occurs at the blastocyst stage when DNMT3a and DNMT3b create de novo methylation patterns (Breton-Larrivée et al., 2019, Gopinathan, 2022).

The distribution of 5mC and 5hmC is asymmetric in the maternal and paternal pronuclei, whereas there is virtually no difference in 5fC and 5caC. During the postsynthetic period of zygotes, 5mC is lost and 5hmC is formed in the paternal pronucleus (Li et al., 2018). It is currently believed that a process catalyzed by TET3 is the primary mechanism for demethylation of the paternal genome. Presumably, both genomes can undergo active TET-dependent demethylation (Canovas et al., 2017). Notably, demethylation of TET persists beyond the zygotic stage and continues into later stages of embryogenesis (Li et al., 2018).

After fertilization, most of the methylated groups are removed through demethylation and hydroxylation. Before embryo implantation, the genome is methylated anew, forming a characteristic methylation pattern, which is then maintained in generations of cells.

After embryo implantation, remethylation leads to irreversible gene silencing, which persists steadily during cell division (Gopinathan, 2022). The primary remethylation pattern occurs through DNMT3a and DNMT3b activities in the outer layer of the discoblastula after embryo implantation. Subsequent changes occur during the cell differentiation phase. The resulting cell lineage-determined patterns are retained during further proliferation. Different methylation profiles are created in the gametes of embryos of different sexes (Guo et al., 2014; Veland et al., 2017).

Environmental factors, dietary patterns and parental age can influence the levels of 5-methylcytosine in spermatozoa and oocytes. Nutrients from food serve as metabolites and co-substrates for TET enzymes, and deficiencies in these nutrients can lead to impaired demethylation and epimutations (Li et al., 2018). For example, according to nutrigenomics, poor supply of methyl donors may result in alterations in the DNA methylation patterns of newborns (Caradonna et al, 2020).

DNA methylation plays an important role in the regulation of human embryonic development. Methylation levels change several times during embryogenesis. Established patterns contribute to tissue-specific gene function during proliferation.

## 6. The role of methylation in aging and the development of oncology

In line with the dynamic nature of DNA methylation, age-related alterations in this modification, which are highly consistent among individuals, have been uncovered (Bacalini et al., 2021). More than 75% of CpG sites in tissue-specific genes are normally methylated in mammalian cells; however, the level of DNA methylation decreases with age, leading to a loss of transcriptional control. Conversely, DNA methylation often increases with age in CpG islands of promoter regions near transcription start sites for genes that regulate cell growth and development (Wilkinson et al., 2021). It is assumed that hypermethylation is an active process caused by aging, while associated hypomethylation is a passive process caused by stochastic or environmental reasons (Marttila et al., 2015). Notably, long-living individuals, who exhibit specific clinical, cellular, and molecular features associated with a healthy human phenotype, have lower levels of regulatory zone methylation than their peers who do not have longer lifespans (Li et al., 2013a; Pirazzini et al., 2021).

During aging, the following changes in the pattern of DNA methylation occur:

1. Hyper- and hypomethylation of individual CpG dinucleotides.
2. Hypomethylation of CpG dinucleotides in repeats.
3. Increased variability in specific CpG methylation levels in the general human population.
4. Increased number of random epimutations, i.e., changes in DNA methylation levels of a particular CpG site (Pirazzini et al., 2021).

In aging, the variation in methylation can affect different loci. At loci with high CpG dinucleotide frequencies, there is a relationship between individual methylation sites, which prevents the occurrence of variability at this site. Conversely, at low CpG site densities, differentially methylated sites are more likely to occur. In addition to the CpG site density, the nucleotide sequence itself may influence age-dependent changes in methylation patterns. Thus, certain SNPs can interfere with the relationship between CpG sites, which leads to variations and deterioration of the methylation pattern (Higham et al., 2022).

Recent research has investigated the relationship between age and changes in methylation. It was shown that in healthy individuals, only 8–18% of CpG sites change their methylation level over 6–10 years, while the majority of CpG sites remain unchanged. When comparing monozygotic twins aged 3 and 50 years, it was revealed that epigenetic differences between them increase throughout life (Lanata et al., 2018).

Tumorigenesis is characterized by a decrease in the overall level of DNA methylation with local hypermethylation, similar to the changes that occur during aging. Promoters and other regulatory elements of tumor suppressor genes and some proteins of the repair system are often hypermethylated. Hyperexpression of proto-oncogenes, growth factors, and proteins involved in the processes of tumor cell proliferation, invasion, and metastasis are also typical for cancers (Kabanov and Tishchenko, 2014; Luczak and Jagodzinski, 2006). Cancer cells are thought to exhibit altered methylation in approximately 600 CpG-rich regions compared to normal cells. Hypermethylation of tumor suppressor genes induces uncontrolled cell division, metastasis, apoptosis avoidance, and stimulation of angiogenesis, while the methylation of proteins of the repair system leads to genomic instability (Luczak and Jagodzinski, 2006).

In oncological diseases, alterations in methylation patterns result in a wide range of biological effects. Hypermethylation of tumor suppressor gene (TSG) promoters leads to the disruption of numerous biological functions: DNA repair (*MLH1*), cell cycle (*CDKN2A*), apoptosis (*WIF1*), and cell signaling (*RASSF1*) (Pan et al., 2021). Hypermethylation of tumor suppressor genes may be linked to a poor prognosis, such as in the

case of the *BRCA1* promoter hypermethylation, which has been associated with an increased risk of breast cancer and lymph node metastasis (Zhang and Long, 2016).

Hypomethylation of some genes in cancer leads to an increased expression of these genes. Specifically, low levels of DNA methylation enhance the expression of tumor genes such as heparanase, urokinase and calcium-binding protein, which facilitate the hematogenous and lymphogenous movement of metastatic cells (Luczak and Jagodzinski, 2006).

The insulin-like growth factor 2 (*IGF2*) gene serves as another example. In healthy cells, transcription of the gene occurs from one of the alleles. In tumor cells, there is a loss of imprinting resulting in *IGF2* being expressed from both alleles. This ultimately leads to the proliferation of tumor cells. Furthermore, genome destabilization resulting from decreased methylation promotes carcinogenesis. For example, hypomethylation of LINEs leads to tumor progression (Luczak and Jagodzinski, 2006).

*TP53 is* one of the most frequently mutated genes in cancer. Research indicates that in 25% of cases, mutations in the *P53* gene occur precisely at the CpG sites. This is due to the predisposition of 5mC to spontaneous deamination and conversion to thymine. Additionally, mutations can arise from the malfunctioning of methyltransferases or cytosine deamination by proteins of the APOBEC family (Kabanov and Tishchenko, 2014; Zhu et al., 2016). Tumor diseases are characterized by impaired functioning of methylation and demethylation proteins. It is noteworthy that various DNMT representatives can act both as tumor suppressor genes and as oncogenes, depending on tumor's localization. For instance, *DNMT1* acts as an oncogene in pituitary adenoma, thyroid cancer, pancreatic cancer, and stomach cancer. *DNMT3a* is an oncogene in pituitary adenoma, lung cancer, and melanomas but acts as a TSG in lung cancer and chronic lymphocytic leukemia. *DNMT3B* is an oncogene in breast cancer, hepatocellular carcinoma, colon and ovarian cancer, and a tumor suppressor gene in lymphomas (Zhang et al., 2020). The *DNMT3B* gene consists of 23 exons and 22 introns. The primary transcripts of the gene can produce 5 different isoforms: DNMT3b1, DNMT3b2, DNMT3b3, DNMT3b4 and DNMT3b5. Each of these isoforms is found in different cancers (Luczak and Jagodzinski, 2006). Hypermethylation of the *TET1* gene is common in cell lines and primary tumors of multiple carcinomas and lymphomas, tumors of the nasopharynx, esophagus, stomach, colorectal, kidney, breast, and cervical cancers, as well as non-Hodgkin's, Hodgkin's, and nasal natural killer/T-cell lymphomas. The TET1 catalytic domain has demethylase activity in cancer cells; it can inhibit CpG methylation of tumor suppressor gene (TSG) promoters and reactivate the expression of *SLIT2*, *ZNF382* and *HOXA9*. The reduced levels of 5hmC observed in cancer cells are likely due to impaired TET enzyme activity or reduced expression in TET proteins (Li et al., 2016).

Another methylation protein associated with cancer is *UHFR1*. This gene itself is an oncogene leading to the development of hepatocellular carcinoma. *UHFR1* overexpression in cancer is still of uncertain etiology. Increased expression of the gene leads to suppression of DNMT1 activity (Petryk et al., 2020).

In conclusion, variations in gene methylation levels in different cancer types can result in multiple changes in cell functioning. In cancer, tumor suppressor genes tend to be hypermethylated and, as a consequence, their expression level is reduced. Whereas oncogenes change their methylation pattern, becoming hypomethylated and increasing their level of expression. Various genes that undergo changes in methylation levels in cancer are presented in Table 1.

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**Table 1**
Cancer-associated genes characterized by a change in methylation pattern in the promoters (genes characterized by hypermethylation are marked in red, and hypomethylation is marked in green; arrows indicate changes in expression level: increase or decrease).

| Gene | Type of cancer | Impact |
|---|---|---|
| *BRCA1* ↓ | Breast cancer, ovarian cancer (Zhang and Long, 2016) | Transcription, double-strand break repair, ubiquitinylation |
| *BRCA2* ↓ | Sporadic ovarian cancer (Bosviel et al., 2012) | Transcription, double-strand break repair, ubiquitinylation |
| *MLH1* ↓ | Colon tumor (Li et al., 2013) | Mismatch repair |
| *MSH2* ↓ | Endometrial cancer (Haraga et al., 2017) | Mismatch repair |
| *RBBP8* ↓ | Bladder cancer (Mijnes et al., 2018) | Homologous recombination, double-strand break repair |
| *CDKN2A* ↓ | Cancer of the esophagus, lung, head and neck, breast, hepatocellular, colon (Zhou et al., 2017;Shima et al., 2011) | Inhibition of cyclin-dependent kinases, cell cycle regulation |
| *CDKN2B* ↓ | Acute myeloid leukemia (Kamali et al., 2017) | Cell cycle regulation |
| *TP73* ↑ | Hepatocellular carcinoma, cancer of the gastrointestinal tract (Logotheti et al., 2021;Yao et al., 2019) | Apoptosis in response to DNA damage |
| *WIF1* ↓ | Colorectal cancer, osteosarcoma (Hu et al., 2018;Liu et al., 2017) | WNT/β-catenin signaling pathway |
| *SFRP1* ↓ | Colorectal, prostate cancer (Baharudin et al., 2020) | Cell proliferation, migration and invasion |
| *RASSF1* ↓ | Breast cancer, lung cancer, prostate cancer, kidney cancer, glioma, neuroblastoma (Malpeli et al., 2019) | Apoptosis, microtubule dynamics |
| *SOX17* ↓ | Lung cancer, breast cancer, colorectal cancer, hepatocellular carcinoma (Fu et al., 2015;Yin et al., 2012) | WNT/β-catenin signaling pathway, stem cell functions |
| *SOCS3* ↓ | Bladder cancer, breast cancer (Guan et al., 2017) | JAK-STAT signaling pathway |
| *HPSE* ↓ | Colorectal cancer (Zhang et al., 2021) | p53/P21 signaling pathway |
| *IGF22* ↓ | Breast cancer (Radhakrishnan et al., 2020) | Downregulation of the regulation of proliferation, growth, migration, differentiation of cells |
| *TP53* ↓ | Glioblastoma (Wei et al., 2019) | Cell cycle, apoptosis |
| *TET1* ↓ | Carcinomas, tumors of the nasopharynx, esophagus, stomach, colorectal cancer, cancer of the kidneys, breast and cervix, non-Hodgkin's, Hodgkin's lymphomas (Li et al., 2016) | Demethylation, suppression of expression of *SLIT2*, *ZNF382,* and *HOXA9* |
| *XPG* ↓ | Ovarian cancer (Sabatino et al., 2010) | Excision repair disorder |
| *LIG4* ↓ | Colorectal cancer (Kuhmann et al., 2014) | Disruption of non-homologous end joining |
| *FANCF* ↓ | Cervical cancer, squamous cell carcinoma of the lung and mouth (Narayan et al., 2004) | Disruption of DNA repair by disrupting the activation of signaling pathways associated with BRCA1, RAD51, ATM, NBS |
| *ATM* ↓ | Hepatocellular carcinoma (Yan et al., 2020) | Radiosensitivity of cells |
| *CHK2* ↓ | Non-small cell lung cancer (Zhang et al., 2004) | DNA damage signaling, cell cycle regulation, and DNA damage-induced apoptosis |
| *APC* ↓ | Cancer of the esophagus, colon, stomach, pancreas, and liver (Zhu et al., 2021) | β-catenin dependent Wnt signaling pathway |
| *HRAS* ↑ | Bladder cancer (Sun et al., 2012) | Stimulation of cells to carcinogenesis, disruption of intracellular signal transmission |
| $P16^{INK4A}$↓ | Squamous cell carcinoma of the larynx, salivary gland tumors, colorectal cancer (Agnese et al., 2006) | Cell cycle dysregulation |

**Data Availability Statement.**
Not applicable.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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