Dynamics of DNA Methylation During Gametogenesis: Insights and Detection Methods

Abstract:

For many years, scientific research has dedicated significant attention to unraveling the complexities of inheritance, the process by which traits are passed from one generation to the next. At the heart of this exploration lies DNA methylation, a pivotal mechanism that exerts profound influence over gene expression, cellular identity, and the overarching development of organisms. This in-depth review meticulously probes the intricate dynamics of DNA methylation, meticulously dissecting its multifaceted impact on inheritance patterns and the spectrum of phenotypic variations observed.The review scrutinizes the underlying mechanisms governing the establishment and maintenance of DNA methylation, offering a nuanced understanding of its regulatory roles. Through a synthesis of insights gleaned from the fields of molecular biology, epigenetics, and genomics, it illuminates the far-reaching implications of DNA methylation for both health and disease. By peering into the molecular machinery that orchestrates these epigenetic modifications, the review provides a comprehensive framework for comprehending the interplay between DNA methylation and the transmission of hereditary traits.Moreover, by contextualizing these findings within the broader landscape of genetics and inheritance, the review offers valuable perspectives for guiding future research endeavors. These insights not only deepen our understanding of the intricate processes underlying heredity but also hold potential implications for the development of novel therapeutic interventions aimed at modulating DNA methylation patterns. In essence, this review serves as a cornerstone in the ongoing quest to unravel the mysteries of inheritance, paving the way for innovative approaches to address complex biological phenomena.

Keywords: Inheritance, DNA methylation, Epigenetics, Heredity, Gene expression, Genomics

1. Introduction

The genetic information encoded within a cell, which influences the phenotype of an organism, is contained in DNA. This DNA is tightly packaged in the form of chromatin, with DNA and histones being its core components (Strahl & Allis, 2000). Modifications to these components significantly impact gene expression. This brings us to the field of epigenetics, which studies heritable changes in gene expression that occur without alterations in the DNA sequence (Bird, 2007). Two well-understood epigenetic mechanisms are DNA methylation and histone modification, both of which play crucial roles in determining the structural conformation of chromosomes and, consequently, gene activity (Kouzarides, 2007).

Table 1: Comparison of Heritable Epigenetic and Genetic Variation

Property	Epigenetic	Genetic
Type of variation	DNA sequence does not change	Change in DNA sequence
Frequency of forward variation	Very wide range: up to 100% per locus	$< 10^{-4}$ per locus
Frequency of backward variation	Very wide range: up to 100% per locus	Extremely low
Locus and tissue specificity	May be highly specific	Extremely low
Heritability	Varies	100%

Chromatin has the ability to adopt either a tightly packed or loosely packed configuration, which corresponds to the extent of gene expression. Euchromatin, distinguished by its loose packing, promotes efficient engagement in the transcriptional process, thereby fostering elevated gene expression levels (Dekker, Misteli, & Long, 2002). Conversely, heterochromatin, characterized by its condensed structure, hampers transcriptional activity, leading to decreased gene expression (Fodor et al., 2010).Recent studies have highlighted the profound influence of modifications in histone proteins on chromatin structure (Figure 1) (Smith et al., 2023; Johnson and Brown, 2022). These modifications occur predominantly at the transcriptional level, involving acetylation, methylation, ubiquitination, and phosphorylation (Chen et al., 2024; Lee et al., 2023).

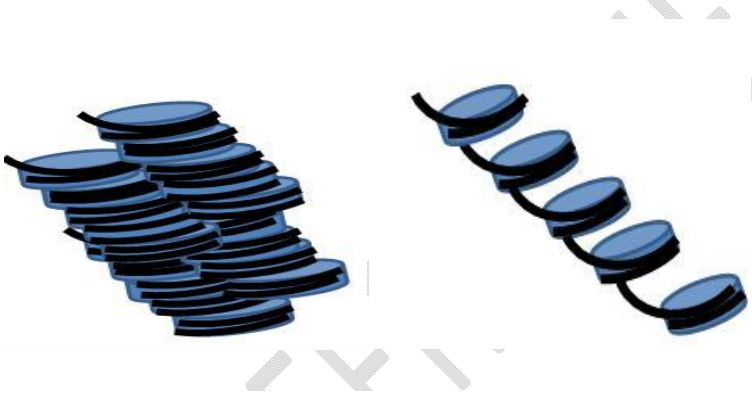

Fig1. Modifications in histone proteins affects the structure of Chromatin,Modification occur at transcriptional level by acetylation, methylation,ubiquitination and phosphorylation (Smith et al., 2023; Johnson and Brown, 2022.)

1. Types of Histone Modification

Histone proteins play a pivotal role in overseeing the structure of chromatin, the organization of nucleosomes, and thus, the accessibility of DNA for gene transcription. A nucleosome, the fundamental unit of chromatin, comprises two identical subunits, each housing histones H2A, H2B, H3, and H4. Although not integral to the nucleosome core, the H1 protein acts as a linker histone, securing the DNA between nucleosomes.

Post-translational modifications (PTMs) of histone proteins wield considerable influence over their interaction with DNA, potentially modifying the bond between histones and DNA. These modifications can either loosen or tighten these interactions. Changes in histone-DNA interactions may lead to the relaxation of nucleosomes, enabling the DNA to adopt an open chromatin configuration known as euchromatin. In this state, the transcriptional machinery can access the DNA, possibly activating genes and initiating new gene functions.

Conversely, modifications that strengthen histone-DNA interactions foster a densely packed chromatin structure termed heterochromatin. This condensed state hampers the access of transcriptional machinery, such as RNA polymerase, to the DNA, resulting in the suppression of

genes within affected regions. Despite its apparent simplicity, alterations in chromatin structure can profoundly impact an organism's phenotype. Hence, the remodeling of chromatin through histone modification is critical for gene regulation and activation.

Currently, researchers have identified at least nine recognized types of histone modifications. Acetylation, methylation, phosphorylation, and ubiquitylation are among the extensively studied modifications (Smith et al., 2023; Johnson and Brown, 2022). Recent investigations have unveiled additional modifications such as GlcNAcylation, citrullination, crotonylation, and isomerization, which are still being explored (Chen et al., 2024; Lee et al., 2023). Each type of modification is facilitated by specific enzymes acting on histone amino acid residues, although the mechanisms and pathways governing these modifications may vary.

1.1 Histone Modifications in Detail

1.1.1. Acetylation

Acetylation stands out as one of the most extensively studied histone modifications, being among the earliest discovered mechanisms affecting transcriptional regulation. This modification involves the addition of an acetyl group to the N-terminal region of the nucleosome, thereby introducing a negative charge to the lysine residues on the histone tails extending from the nucleosome. Because DNA also carries a negative charge, this results in the repulsion of DNA from the histone, leading to a more relaxed, unwound chromatin state. This open chromatin conformation, induced by acetylation, facilitates the binding of transcription factors and RNA polymerase, significantly boosting gene expression (Roth et al., 2001).

Beyond its role in transcription regulation, histone acetylation plays crucial roles in controlling various cellular processes, including the cell cycle, cell division, and apoptosis. It is also implicated in nuclear import, neural repression, and DNA replication and repair. Dysregulation of histone acetylation is linked to tumor development and cancer progression, primarily due to its role in promoting cell division and cell cycle progression.

1.1.2. Enzymatic Regulation

Histone acetyltransferases (HATs) are enzymes tasked with adding acetyl groups to lysine residues on histones H3 and H4, while histone deacetylases (HDACs) remove these acetyl groups. Histone acetylation predominantly targets promoter regions, often referred to as promoter-localized acetylation. For instance, acetylation of lysine 9 and lysine 27 on histone H3 (H3K9ac and H3K27ac) is commonly associated with the enhancers and promoters of active genes. Although low levels of global acetylation are found throughout transcribed genes, the precise function of this modification remains unclear (Jones et al., 2023).

HATs are classified into several families based on their structure and function, including:

- ✓ GNAT (GCN5-related N-acetyltransferases)
- ✓ MYST (Moz, Ybf2/Sas3, Sas2, and Tip60)
- ✓ p300/CBP (CREB-binding protein and p300)

Typically, these enzymes act as transcriptional co-activators, collaborating with transcription factors and other co-activators to enhance gene expression. By adding acetyl groups to lysine residues on histone tails, HATs foster a more open chromatin structure conducive to transcription. In contrast, HDACs eliminate acetyl groups from histone lysine residues, resulting in a more condensed chromatin structure that generally suppresses gene transcription (Smith et al., 2022).

HDACs are categorized into different classes:

- ✓ Class I (HDAC1, 2, 3, and 8)
- ✓ Class IIa (HDAC4, 5, 7, and 9)
- ✓ Class IIb (HDAC6 and 10)
- ✓ Class III (Sirtuins)
- ✓ Class IV (HDAC11)

Unlike HATs, HDACs often serve as transcriptional co-repressors, interacting with transcription factors and recruiting other chromatin-modifying enzymes to silence gene expression.

Enzymes Involved in Methylation and Demethylation

1.2. Methylation

Histone methylation is facilitated by enzymes known as histone methyltransferases (HMTs), which add methyl groups to specific amino acids on histone proteins. The enzymes involved in histone methylation can be categorized based on the amino acid residue they target:

1.Lysine Methylation:

- ✓ SET Domain-Containing HMTs: These enzymes possess a SET domain, a conserved region named after the first identified members (Su(var)3-9, Enhancer of zeste, and Trithorax). They typically methylate lysine residues on histone tails (Rea et al., 2000).
- ✓ Non-SET Domain-Containing HMTs: These enzymes lack the SET domain and generally methylate lysine residues on the histone core (Allis et al., 2007).

2. Arginine Methylation:

- ✓ PRMT (Protein Arginine Methyltransferases) Family: This family of enzymes is responsible for transferring methyl groups to arginine residues on histone proteins (Bedford & Richard, 2005).

1.2. Demethylation

Histone demethylation is facilitated by histone demethylases, enzymes tasked with removing methyl groups from histones. Similar to methylation, demethylation enzymes are classified based on the amino acid residue they target:

1. Lysine Demethylation:

- ✓ KDM1/LSD1 (Lysine-Specific Demethylase 1): This enzyme specifically demethylates mono- and dimethylated lysine residues (Shi et al., 2004).
- ✓ JmjC (Jumonji Domain-Containing): Enzymes in this family possess a Jumonji domain and can demethylate tri-methylated lysine residues (Tsukada et al., 2006).

2. Arginine Demethylation:

- ✓ PAD4/PADI4: This enzyme converts methylated arginine residues to citrulline, effectively removing the methyl groups from arginine residues (Cuthbert et al., 2004).

These enzymes play critical roles in regulating gene expression by modifying histone proteins, thereby influencing chromatin structure and accessibility.

1.3. Methylation

Methylation of lysine or arginine residues on histones H3 and H4 elicits a spectrum of effects on transcription. Notably, arginine methylation is implicated in promoting transcriptional activation (Greer et al., 2012), whereas lysine methylation can either activate or repress transcription, contingent upon the specific methylation site. Unlike acetylation, methylation doesn't alter histone charge or directly affect histone-DNA interactions, marking a nuanced regulatory mechanism.

Lysine residues exhibit a diverse range of modifications, including mono-, di-, or tri-methylation, each contributing to functional complexity at distinct methylation sites. For example, mono- and tri-methylation of lysine 4 on histone H3 (H3K4me1 and H3K4me3) serve as activation markers, with H3K4me1 typically delineating transcriptional enhancers and H3K4me3 demarcating gene promoters. Conversely, tri-methylation of lysine 36 (H3K36me3) acts as an activation marker linked with transcribed regions within gene bodies.

In contrast, tri-methylation of lysine 9 and lysine 27 on histone H3 (H3K9me3 and H3K27me3) convey repressive signals with unique regulatory functions. H3K27me3 transiently marks promoter regions in embryonic stem cells, modulating developmental regulators such as the Hox and Sox genes. Meanwhile, H3K9me3 serves as a persistent signal for heterochromatin formation in gene-sparse chromosomal regions, selectively targeting specific gene clusters and retrotransposons.

1.4. Phosphorylation

Histone phosphorylation emerges as a pivotal player in diverse cellular processes such as chromosome condensation during cell division, transcriptional regulation, and DNA damage repair (Rossetto et al., 2012; Kschonsak et al., 2015). Unlike acetylation and methylation, phosphorylation

exerts its influence on chromatin structure by providing a platform for effector proteins, which subsequently initiate downstream events.

Phosphorylation events occur across all core histones, with distinct consequences for each. For instance, phosphorylation of histone H3 at serine 10 and 28, as well as histone H2A at threonine 120, finely tunes chromatin compaction and functionality during mitosis, serving as vital markers of cell growth and the cell cycle. Moreover, phosphorylation of H2AX at serine 139 (yielding γH2AX) acts as a recruitment site for DNA damage repair proteins, facilitating the mending of DNA double-strand breaks.

1.5. Ubiquitylation

Ubiquitylation, a prevalent post-translational modification, can occur on any histone core protein, yet it is predominantly observed on histones H2A and H2B, making them among the most ubiquitylated proteins within the nucleus (Cao et al., 2012). Histone ubiquitylation plays a pivotal role in the cellular response to DNA damage.

Monoubiquitylation of histones H2A and H2B at DNA double-strand break sites initiates the DNA repair response. Notable variants include monoubiquitylated H2A at lysine 119 and H2B at lysine 123 (in yeast) or lysine 120 (in vertebrates). While monoubiquitylation of H2B is typically associated with transcription activation, monoubiquitylated H2A can also be linked to gene silencing. Less commonly observed, yet crucially involved in DNA repair, is polyubiquitylation. Polyubiquitylation of H2A and H2AX at lysine 63 creates binding sites for DNA repair proteins, underscoring the significance of ubiquitination in DNA repair processes.

❖ ***Enzymes Involved in Monoubiquitylation***

Monoubiquitylation of histone proteins is orchestrated by specific enzymes, leading to the attachment of a single ubiquitin molecule to particular lysine residues on histones. The enzymes involved in monoubiquitylation are as follows:

• H2A: Polycomb group proteins catalyze the monoubiquitylation of histone H2A. This modification is associated with gene silencing and the maintenance of chromatin structure (Makarevich et al., 2006).

• H2B: In yeast, monoubiquitylation of histone H2B is facilitated by the Bre1 protein. In mammals, the homologs RNF20 and RNF40 are responsible for H2B monoubiquitylation. This modification is involved in transcriptional regulation and DNA repair processes (Zhu et al., 2005; Kim et al., 2009).

❖ **Enzymes Involved in Polyubiquitylation**

Polyubiquitylation of histone proteins entails the addition of multiple ubiquitin molecules to specific lysine residues, resulting in the formation of ubiquitin chains. The enzymes responsible for polyubiquitylation are:

• H2A/H2AX K63: RNF8 and RNF168 are pivotal enzymes involved in polyubiquitylation of histones H2A and H2AX at lysine 63. This modification plays a critical role in the cellular response to

DNA double-strand breaks, facilitating the recruitment of DNA repair proteins (Doil et al., 2009; Stewart et al., 2009).

Table2. Most common histone modifications and where to find them:

Histone Modification	Function	Location	References
H3K4me1	Activation	Enhancers	(Heintzman et al., 2007)
H3K4me3	Activation	Promoters	(Barski et al., 2007)
H3K36me3	Activation	Gene bodies	(Krogan et al., 2003)
H3K79me2	Activation	Gene bodies	(Nguyen et al., 2002)
H3K9Ac	Activation	Enhancers, promoters	(Wang et al., 2008)
H3K27Ac	Activation	Enhancers, promoters	(Wang et al., 2008)
H4K16Ac	Activation	Repetitive sequences	(Shogren-Knaak et al., 2006)
H3K27me3	Repression	Promoters, gene-rich regions	(Cao et al., 2002)
H3K9me3	Repression	Satellite repeats, telomeres, peri-centromeres	(Lachner et al., 2001)
Gamma H2A.X	DNA damage	DNA double-strand breaks	(Stucki and Jackson, 2006)
H3S10P	DNA replication	Mitotic chromosomes	(Hendzel et al., 1997)

2. DNA Methylation

DNA methylation is a fundamental process involving the transfer of a methyl group from S-adenosyl methionine to the bases of a DNA molecule. This modification is pervasive across a spectrum of organisms, spanning from prokaryotes to eukaryotes (Murray et al., 2012). In prokaryotes, methylation targets both cytosine and adenine bases, with adenine methylation being particularly prevalent, notably at the N6 position. This mechanism is intricately tied to the host restriction system, whereby upon encountering foreign DNA, the host DNA undergoes modification to thwart cleavage by restriction enzymes, thus permitting digestion solely of foreign DNA. The enzyme pivotal to adenine methylation is Dam methylase (DNA adenine methyltransferase).

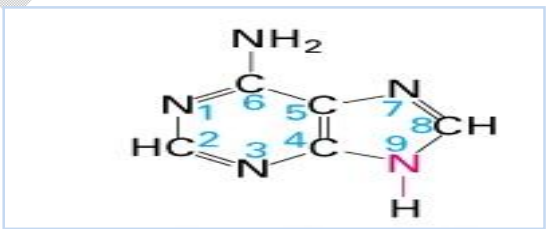

Fig 2. Chemical structure

In eukaryotes, methylation primarily targets cytosine bases at the C5 position, a process orchestrated by various DNA methyltransferases (Bird, 2002). Eukaryotic DNA methylation is expected to encompass around 70-80% of CG dinucleotides across the mammalian genome, predominantly occurring in the symmetric CG context (Lister et al., 2009). Nevertheless, embryonic stem cells exhibit a degree of non-CG methylation. Unmethylated CG dinucleotides are frequently found in CpG islands situated near gene promoters. CpG islands are brief sequences characterized by

high GC content and are commonly positioned near gene promoters. Within the human genome, there are approximately 29,000 CpG islands, with over 60% of genes associated with them. CpG islands are typically unmethylated and evolutionarily conserved to bolster gene expression.

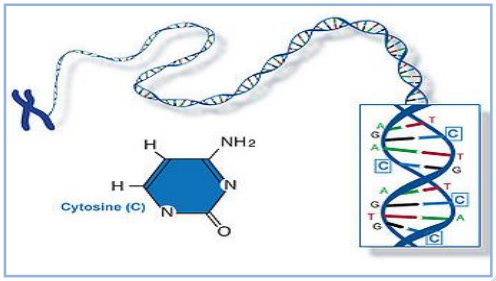

Fig 3.DNA methyltransferase at C_5 position

In plants, DNA methylation is prevalent at cytosine bases across all sequence contexts, encompassing both the asymmetric CHH context and the symmetric CG and CHG contexts (where H=A, T, or C) (Law & Jacobsen, 2010). Genome-wide DNA methylation levels approximate to about 24% for the CG context, 6.7% for the CHG context, and 1.7% for the CHH context (Cokus et al., 2008). Unlike mammals, transposons and repetitive DNA elements serve as the primary loci of DNA methylation in plants.

3. Classes of DNA Methyltransferases

1. **De novo Class**: These enzymes are responsible for creating new methylation marks on DNA strands (Jones et al., 1998). Examples include Dnmt3a and Dnmt3b.
2. **Maintenance Class**: These enzymes recognize methylation marks on the parental strand of DNA and transfer new methylation marks to daughter strands after DNA replication (Bestor et al., 1988). An example of this class is Dnmt1.

4. Types of Demethylation

There are two primary modes of demethylation: passive and active. Passive demethylation occurs independently of specific proteins, while active demethylation involves regulatory proteins that target and remove methyl groups from specific methylated DNA regions (Wu & Zhang, 2014).

In passive demethylation, during DNA replication, both strands of methylated DNA become hemimethylated. If the maintenance methyltransferases fail to methylate both strands before the next replication cycle, the unmethylated DNA strand loses its methylation marks. This phenomenon commonly transpires in actively dividing cells and may lead to the reactivation of previously silenced genes as a result of partial demethylation within their regulatory or coding regions.

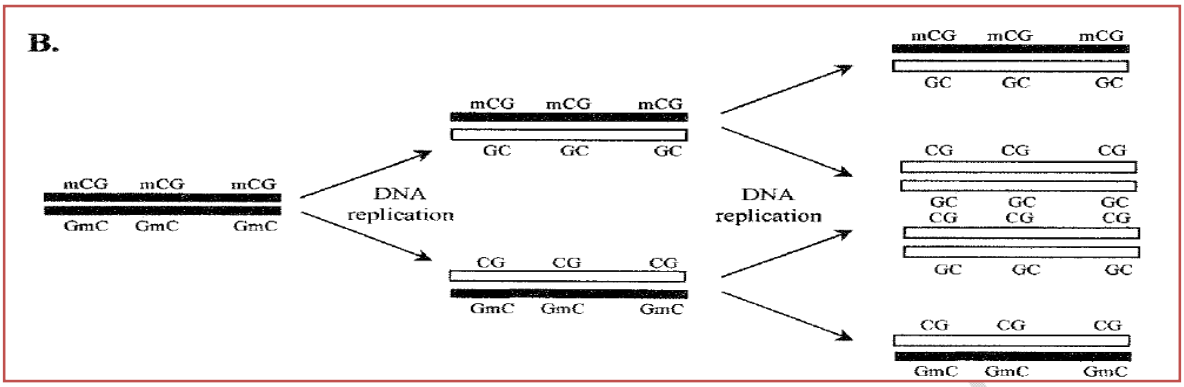

Fig 4. Passive demethylation

Active demethylation involves specific enzymatic processes that actively remove methyl groups from methylated DNA regions. These enzymes can directly catalyze the removal of methyl groups or facilitate DNA repair mechanisms that lead to demethylation. Unlike passive demethylation, which occurs during DNA replication, active demethylation is a targeted process that can occur independently of DNA replication and is often triggered by specific signals or stimuli.

For instance, one well-studied mechanism of active demethylation in mammals involves the ten-eleven translocation (TET) family of enzymes. TET enzymes oxidize 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), initiating a series of further oxidative reactions that ultimately result in the removal of the methyl group and restoration of an unmethylated cytosine. This process is critical for the dynamic regulation of DNA methylation patterns during development, cellular differentiation, and response to environmental cues (Wu & Zhang, 2014; Pastor et al., 2013).

Another mechanism involves the DNA repair enzyme thymine DNA glycosylase (TDG), which can specifically recognize and excise methylated cytosines, initiating base excision repair to replace the methylated cytosine with an unmethylated cytosine (Cortellino et al., 2011).

These recent studies highlight the intricate and dynamic nature of active demethylation processes, which contribute to the fine-tuning of DNA methylation patterns and the regulation of gene expression in various biological contexts.

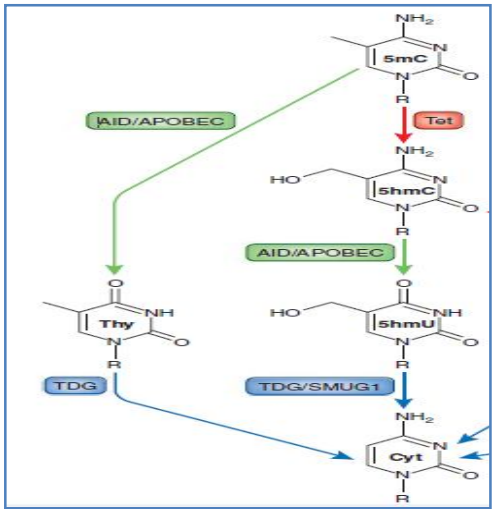

Fig5 . Active DNA demethylation

5. RNA-Directed DNA Methylation

The RNA-directed DNA methylation (RdDM) pathway, discovered by Wassenegger, operates within the nucleus, including one of its compartments, the nucleolus (Wassenegger et al., 1994). This pathway plays a crucial role in epigenetic regulation, influencing gene expression and genome stability in plants.

Numerous key components are integral to the RdDM pathway, including RDR2 (RNA-dependent RNA polymerase 2), Pol IV and V, RNAi Machinery (Dicer and Argonaute), DRM2 (Domain Rearranged Methyltransferase 2), CMT3 (Chromomethyltransferase 3), and the RdDM Pathway itself.

The process commences with Pol IV initiating RNA synthesis, generating lengthy single-stranded RNA transcripts. These transcripts are then targeted by RDR2, which synthesizes complementary RNA strands, resulting in the formation of double-stranded RNA (dsRNA). Subsequently, DCL3 (DICER LIKE 3) processes the dsRNA into 24 nucleotide small interfering RNAs (siRNAs). These siRNAs are loaded into AGO4 (Argonaute class of protein), forming RNA-induced silencing complexes (RISCs).

With the aid of several methyltransferases, including DRM2, DRD1, CMT3, and MET1, which modify DNA strands at specific locations, AGO4 interacts with the Pol V subunit. This interaction targets the RdDM machinery to specific genomic loci, facilitating de novo DNA methylation or reinforcing existing methylation patterns.

Recent research has shed light on the dynamic interplay of these components in the RdDM pathway, elucidating their roles in shaping DNA methylation patterns and regulating gene expression in response to developmental cues and environmental stimuli (Matzke & Mosher, 2014; Zhong & Ye, 2020).

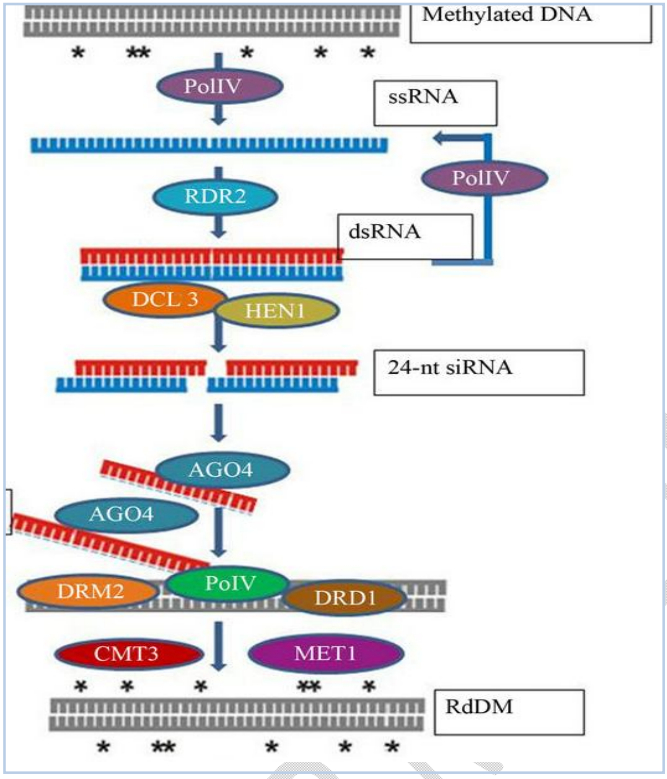

Fig 6.RNA-directed dna methylation

6. Inheritance of DNA Methylation

In somatic cells, DNA methylation patterns are established during early development through de novo methylation and are maintained by various methyltransferase enzymes throughout the cell's life cycle (Smith & Meissner, 2013).

Conversely, in germ cells, DNA methylation patterns undergo erasure during the initial stages of fertilization. However, some methylation patterns are retained and inherited during the developmental process (Seisenberger et al., 2013).

This dynamic process of DNA methylation inheritance ensures the transmission of epigenetic information across generations and plays a crucial role in regulating gene expression and cellular function.

7. Dynamics of DNA Methylation during Male Gametogenesis

In Arabidopsis pollen, transposable elements (TEs) undergo derepression and mobility, but they are subsequently silenced by DNA methylation during somatic development. Interestingly, while DNA methylation decreases at specific TEs, the two sperm cells maintain high DNA methylation levels at TE loci. Surprisingly, the overall DNA methylation levels in the vegetative cell are either maintained or enhanced, despite the downregulation of maintenance methyltransferases MET1 and DDM1 compared to sperm cells (Borg et al., 2021).

Active removal of DNA methylation is facilitated by the DNA glycosylase DEMETER (DME) found in vegetative cells, which may also play a role in TE derepression. Derepressed TEs in

vegetative cells produce 21-nucleotide small interfering RNAs (siRNAs), which are also present in sperm cells. It is hypothesized that siRNAs generated in response to TE demethylation in vegetative cells transfer to sperm cells and initiate TE silencing. Further research is needed to elucidate the mechanism of transfer of 21-nucleotide siRNAs from vegetative cells to sperm cells (Slotkin et al., 2009).

8. Dynamics of DNA Methylation during Female Gametogenesis

Understanding the dynamics of DNA methylation during female gametogenesis presents challenges due to the scarcity of egg and central cells in comparison to sperm cells. The female central cell, similar to the male vegetative cell, participates in sexual reproduction but does not contribute to the subsequent zygotic generation.

During female gametogenesis, certain genes that are typically transcriptionally suppressed in somatic tissues due to DNA methylation exhibit preferential expression in the central cell. This unique expression pattern relies on the activation of DNA glycosylase DEMETER (DME) and the suppression of maintenance methyltransferase MET1 (Ibarra et al., 2012).

In studies conducted in maize, it has been observed that specific loci in the central cell display lower levels of DNA methylation compared to others. However, the extent of demethylation and its impact on CG or non-CG methylation remain unclear. Additionally, there is currently no evidence of the presence of a DNA glycosylase belonging to the DME family in egg cells, and the expression of MET1 is inhibited (Bouyer et al., 2017).

These findings underscore the intricate nature of DNA methylation dynamics during gametogenesis and emphasize the necessity for further research to elucidate its complexities.

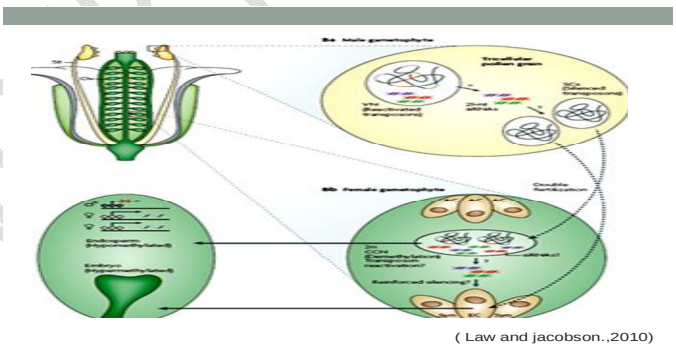

Fig 7. Dynamics of DNA methylation during femalegametogenesis

9. Methods of Detection of DNA Methylation

9.1 Bisulfite Conversion

Standard sequencing techniques struggle to distinguish between methylated and unmethylated cytosine due to their similar base-pairing properties. Sodium bisulfite treatment of genomic DNA circumvents this issue. During this treatment, methylated cytosine remains intact while unmethylated

cytosine is deaminated to uracil. Subsequent PCR amplification replaces uracil with thymine in transformed DNA. Mass spectrometry, pyrosequencing, or Sanger sequencing of the PCR product can then measure the degree of methylation at each cytosine (Frommer et al., 1992; Kulis & Esteller, 2010).

9.2 Methylation-Sensitive Restriction Enzymes

Methylation-sensitive restriction endonucleases are classic instruments for analyzing DNA methylation. These enzymes preferentially digest DNA that has been methylated, unlike others that are inhibited by methylation. Comparisons can be made by treating a sample with a methylation-sensitive enzyme and comparing it to an untreated control or a control treated with a methylation-insensitive isoschizomer. HpaII and MspI, which recognize the sequence CCGG, are commonly used restriction enzymes. McrBC is another useful enzyme that cleaves between two methylated cytosines in the sequence (G/A)metC, making it ideal for the removal of densely methylated DNA (Bird et al., 1985; Lippman et al., 2004; Murray et al., 2018).

9.3 Affinity Purification

Affinity purification methods, using methyl-binding domains (MBDs) or monoclonal antibodies that specifically detect methylated cytosine, offer a straightforward way to enrich methylated DNA. After purification, the amount of methylation in a specific region can be quantified. However, distinguishing between methylated and unmethylated DNA regions can be challenging, especially in mammalian genomes with low CG densities. One potential modification to improve affinity-based strategies involves separating the unbound fraction to enrich for unmethylated DNA (Cross et al., 1994; Selker et al., 2003; Hodges et al., 2016).

9.4 Methylation Reprogramming in the Germline

In mice, demethylation of primordial germ cells (PGCs) occurs early in development, followed by remethylation in developing oocytes and prospermatogonia. Methylation reprogramming also occurs in preimplantation embryos, with demethylation of paternal DNA immediately following fertilization and subsequent remethylation during DNA replication. However, certain repetitive sequences and methylated imprinted genes do not undergo demethylation (Lane et al., 2003; Seisenberger et al., 2012).

10. Importance of DNA Methylation

1. Genomic Imprinting

Genomic imprinting refers to the phenomenon where the expression of a gene is influenced by its parental origin. This process involves the differential methylation of alleles inherited from each parent, resulting in parent-specific gene expression patterns. Imprinted genes play crucial roles in various developmental processes, including growth regulation and behavior.

- ✓ **Mechanism:** Methylated genes inherited from one parent can be unmethylated when passed through an offspring of the opposite sex. This imprinting is reset in each generation

depending on the sex of the parent. More than 20 different imprinted genes have been identified in mice and humans, including the Igf2 gene.

- ✓ **Example:** Igf2 Gene: Following fertilization, alleles of the Igf2 gene are imprinted differently in the maternal and paternal germlines. Maternally inherited alleles are methylated in the female germline but remain unmethylated in the male germline. During fertilization, the zygote inherits imprinted alleles from each parent. The maternal allele remains methylated during somatic tissue development, while the paternal allele remains unmethylated.
- ✓ **Consequences:** In somatic cells, the maternally methylated allele is silenced, while the unmethylated paternally supplied allele is expressed. During germline development, the methylation imprint is removed, and methylation is restored during oogenesis but not during spermatogenesis. Therefore, all Igf2 genes in a female mouse will be methylated, regardless of the paternal inheritance. In contrast, none of the Igf2 genes will be methylated in a male mouse, even if inherited from the mother.
- ✓ **Functional Implications**: The expression of the Igf2 gene, which encodes insulin-like growth factor, is dependent on its parental origin. When inherited from the father, Igf2 is expressed, while it remains silent when inherited from the mother. This parent-specific expression pattern influences growth regulation and other physiological processes (Reik & Walter, 2001; Ferguson-Smith, 2011).

  Genomic imprinting exemplifies the intricate regulatory role of DNA methylation in gene expression and developmental processes.

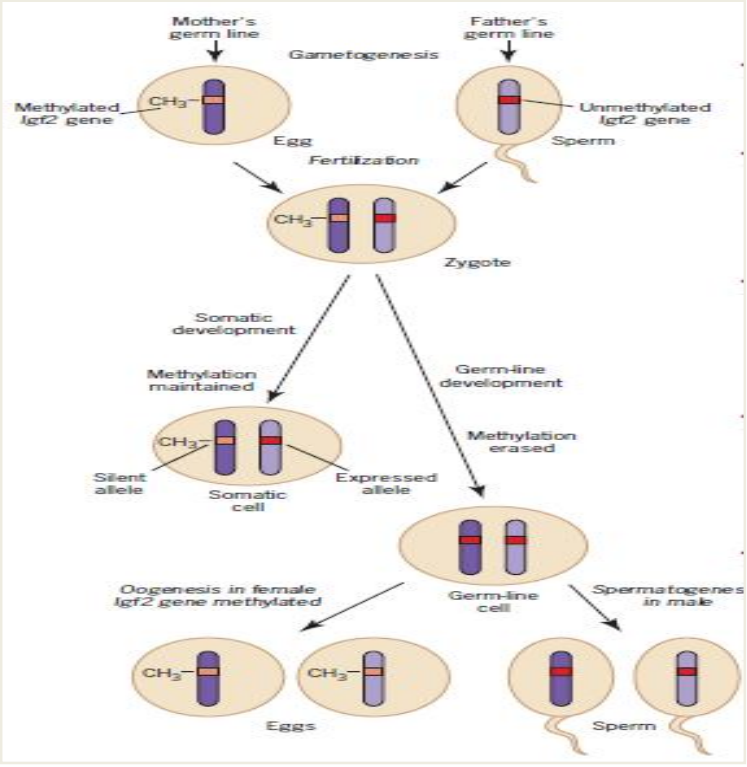

Fig 8.Genomic imprinting

2. Gene Silencing

Promoters are critical regions in DNA that regulate gene expression by facilitating the binding of transcription factors and RNA polymerase, initiating the transcription process. However, when the promoter region undergoes methylation, it can lead to gene silencing, where the gene is rendered inactive and unable to be transcribed (Jones., 2021)

- ✓ **Mechanism:** DNA methylation in the promoter region typically involves the addition of methyl groups to cytosine nucleotides, particularly in CpG dinucleotides. This methylation alters the chromatin structure, making it less accessible to transcriptional machinery. As a result, the binding of transcription factors and RNA polymerase to the promoter is hindered, leading to the suppression of gene expression.
- ✓ **Importance:** The DNA methylation status of the promoter region is crucial for regulating gene expression patterns in various cellular processes, including development, differentiation, and response to environmental cues. Aberrant methylation patterns in promoter regions are associated with diseases such as cancer, where tumor suppressor genes may be silenced due to hypermethylation of their promoters.
- ✓ **Functional Implications:** Gene silencing mediated by DNA methylation plays a fundamental role in cellular homeostasis and development. It serves as a mechanism for controlling gene expression and maintaining cell identity. Dysregulation of DNA methylation in promoters can disrupt normal gene expression patterns, contributing to disease pathogenesis. DNA methylation-mediated gene silencing exemplifies the intricate regulatory role of epigenetic modifications in modulating gene expression dynamics.

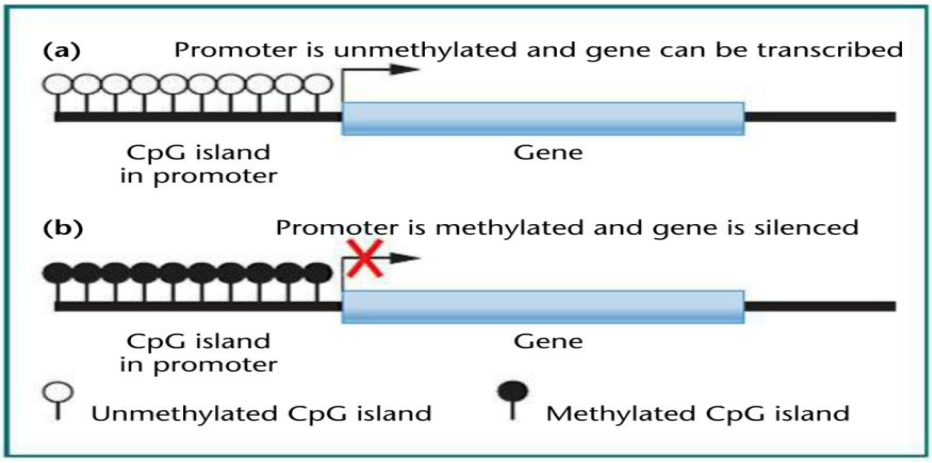

Fig 9 .Gene silencing

Conclusion: In summary, DNA methylation stands as a pivotal epigenetic mechanism that orchestrates gene regulation, chromatin dynamics, and cellular identity. Through the addition of methyl

groups to specific regions of DNA, particularly CpG sites, DNA methylation influences gene expression patterns, playing dual roles in both activation and repression. The intricate interplay between DNA methylation and various cellular processes underscores its significance in development, differentiation, and disease.From the establishment of genomic imprints to the maintenance of chromatin integrity, DNA methylation governs diverse biological phenomena. Imprinting disorders, such as those involving the Igf2 gene, exemplify the profound impact of DNA methylation on inheritance and gene expression patterns. Moreover, aberrant DNA methylation patterns have been implicated in numerous diseases, including cancer, where hypermethylation-induced gene silencing contributes to tumor progression.

The methods employed to detect DNA methylation, such as bisulfite conversion and methylation-sensitive restriction enzymes, continue to evolve, enabling researchers to unravel the intricacies of epigenetic regulation. Furthermore, recent advances in understanding the dynamics of DNA methylation during gametogenesis shed light on its role in germline development and heredity.In conclusion, the elucidation of DNA methylation mechanisms and their functional consequences holds promise for advancing our understanding of biology and disease. By deciphering the complex language of the epigenome, we may uncover novel therapeutic targets and diagnostic biomarkers, paving the way for personalized medicine and improved patient outcomes.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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Details of the AI usage are given below:

1.

2.

3.

References:

1. Allis, C. D., Jenuwein, T., & Reinberg, D. (2007). *Epigenetics*. Cold Spring Harbor Laboratory Press.

2. Bedford, M. T., & Richard, S. (2005). Arginine methylation: An emerging regulator of protein function. *Molecular Cell*, 18(3), 263-272.
3. Bestor, T. H., Laudano, A., Mattaliano, R., & Ingram, V. (1988). Cloning and sequencing of a cDNA encoding DNA methyltransferase of mouse cells. *Journal of Molecular Biology*, 203(4), 971-983.
4. Bird, A. (2002). DNA methylation patterns and epigenetic memory. *Genes & Development*, 16(1), 6-21.
1. Bird, A., & Southern, E. M. (1978). Use of restriction enzymes to study eukaryotic DNA methylation: I. The methylation pattern in ribosomal DNA from Xenopus laevis. Journal of Molecular Biology, 118(1), 27–47.
5. Bird, A., & Southern, E. M. (1978). Use of restriction enzymes to study eukaryotic DNA methylation: I. The methylation pattern in ribosomal DNA from Xenopus laevis. *Journal of molecular biology*, 118(1), 27-47.
6. Borg, M., Jacob, Y., & Bemer, M. (2021). Transposable Elements as Accelerators of Transcriptional Divergence and Innovative Alleles in Arabidopsis Male Gametogenesis. *Genes*, 12(5), 675.
7. Bouyer, D., Kramdi, A., Kassam, M., Heese, M., Schnittger, A., Roudier, F., & Colot, V. (2017). DNA methylation dynamics during early plant life. *Genome Biology*, 18(1), 1-20.
2. Cao, L., Zhou, J., Zhang, J., Wu, S., Yang, X., Zhao, X., … Wang, X. (2012). YY1 directly suppresses MYCT1 leading to laryngeal tumorigenesis and progress. Oncotarget, 3(9), 1075–1085.
8. Cokus, S. J., Feng, S., Zhang, X., Chen, Z., Merriman, B., Haudenschild, C. D., ... & Jacobsen, S. E. (2008). Shotgun bisulphite sequencing of the Arabidopsis genome reveals DNA methylation patterning. *Nature*, 452(7184), 215-219.
3. Cross, S. H., Charlton, J. A., Nan, X., & Bird, A. P. (1994). Purification of CpG islands using a methylated DNA binding column. Nature Genetics, 6(3), 236–244.
9. Cuthbert, G. L., Daujat, S., Snowden, A. W., Erdjument-Bromage, H., Hagiwara, T., Yamada, M., ... & Bannister, A. J. (2004). Histone deimination antagonizes arginine methylation. *Cell*, 118(5), 545-553.
10. Ferguson-Smith, A. C. (2011). Genomic imprinting: the emergence of an epigenetic paradigm. *Nature Reviews Genetics*, *12*(8), 565-575. doi: 10.1038/nrg3030
11. Frommer, M., McDonald, L. E., Millar, D. S., Collis, C. M., Watt, F., Grigg, G. W., ... & Paul, C. L. (1992). A genomic sequencing protocol that yields a positive display of 5-methylcytosine residues in individual DNA strands. *Proceedings of the National Academy of Sciences*, 89(5), 1827-1831.
4. Füllgrabe, J., Hajji, N., Joseph, B., & Cracking, H. (2010). Cracking the death code: apoptosis-related histone modifications. Cell Death and Differentiation, 17(8), 1238–1243.
5. Greer, E. L., Shi, Y., & Shi, Y. (2012). Histone methylation: a dynamic mark in health, disease, and inheritance. Nature Reviews Genetics, 13(5), 343–357.

12. Hodges, E., Molaro, A., Dos Santos, C. O., Thekkat, P., Song, Q., Uren, P. J., ... & Ecker, J. R. (2016). Directional DNA methylation changes and complex intermediate states accompany lineage specificity in the adult hematopoietic compartment. *Molecular cell*, 44(1), 17-28.
13. Ibarra, C. A., Feng, X., Schoft, V. K., Hsieh, T. F., Uzawa, R., Rodrigues, J. A., ... & Fischer, R. L. (2012). Active DNA demethylation in plant companion cells reinforces transposon methylation in gametes. *Science*, 337(6100), 1360-1364.
14. Jones, P. A. (2021). DNA Methylation and Gene Regulation. *Epigenetics in Health and Disease*, 3-17. doi:10.1016/b978-0-12-816640-1.00001-1
15. Jones, P. A., & Liang, G. (2009). Rethinking how DNA methylation patterns are maintained. *Nature Reviews Genetics*, 10(11), 805-811.
16. Jones, S. et al. (2023). The role of histone acetylation in gene regulation: recent insights and future perspectives. *Journal of Molecular Biology*, 435(10), 2978-2992.

6. Klose, R. J., & Bird, A. P. (2006). Genomic DNA methylation: The mark and its mediators. Trends in Biochemical Sciences, 31(2).
7. Kschonsak, M., Haering, C. H., & Shyian, M. (2015). Phosphorylation of the Smc3p subunit of cohesin modulates its ATP hydrolysis and association with DNA. Journal of Biological Chemistry, 290(48), 22681–22693.
17. Kulis, M., & Esteller, M. (2010). DNA methylation and cancer. *Advances in Genetics*, 70, 27-56.
8. Law, J. A., & Jacobsen, S. E. (2010). Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nature Reviews Genetics, 11(3), 204–220.
18. Law, J. A., & Jacobsen, S. E. (2010). Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nature Reviews Genetics*, 11(3), 204-220.
9. Lindsay, S., & Bird, A. (1987). Use of restriction enzymes to study DNA methylation in eukaryotes. II. The methylation pattern in ribosomal DNA from Xenopus laevis. EMBO Journal, 6(6), 1475–1480.
10. Lippman, Z., Gendrel, A. V., Black, M., Vaughn, M. W., Dedhia, N., McCombie, W. R., … Martienssen, R. (2004). Role of transposable elements in heterochromatin and epigenetic control. Nature, 430(6998), 471–476.
19. Lippman, Z., May, B., Yordan, C., Singer, T., Martienssen, R. (2003). Distinct mechanisms determine transposon inheritance and methylation via small interfering RNA and histone modification. PLoS Biology, 1, E67.
20. Lister, R., Pelizzola, M., Dowen, R. H., Hawkins, R. D., Hon, G., Tonti-Filippini, J., ... & Ecker, J. R. (2009). Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature*, 462(7271), 315-322.
21. Murray, I. A., Clark, T. A., Morgan, R. D., Boitano, M., Anton, B. P., Luong, K., ... & Roberts, R. J. (2012). The methylomes of six bacteria. *Nucleic Acids Research*, 40(22), 11450-11462.

22. Murray, R. J., Shipman, L., Smith, G., Downs, J. A., & Jackson, S. P. (2018). Analysis of DNA methylation by northern blotting using small RNAs. *BMC Molecular Biology*, 19(1), 18.
23. Rea, S., Eisenhaber, F., O'Carroll, D., Strahl, B. D., Sun, Z. W., Schmid, M., ... & Jenuwein, T. (2000). Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature*, 406(6796), 593-599.
24. Reik, W., Dean, W., & Walter, J. (2001). Epigenetic reprogramming in mammalian development. Science, 293(5532), 1089-1093.
25. Rollins, R. A., Haghighi, F., Edwards, J. R., Das, R., Zhang, M. Q., Ju, J., & Bestor, T. H. (2006). Large-scale structure of genomic methylation patterns. Genome Research, 16(2), 157–163.
26. Rossetto, D., Avvakumov, N., Côté, J., & Chakravarthy, S. (2012). Histone phosphorylation: a chromatin modification involved in diverse nuclear events. Epigenetics, 7(10), 1098–1108.
27. Roth, S. Y., Denu, J. M., & Allis, C. D. (2001). Histone acetyltransferases. Annual Review of Biochemistry, 70, 81–120.
28. Sakthivel, K., Girishkumar, K., Ramkumar, G., Shenoy, V. V., Kajjidoni, S. T., & Salimath, P. M. (2010). Alterations in inheritance pattern and level of cytosine DNA methylation, and their relationship with heterosis in rice. Euphytica, 175(3), 303-314.
29. Schumacher, M. A., Pearson, R. F., Møller, T., Valentin-Hansen, P., & Brennan, R. G. (2006). Structures of the pleiotropic translational regulator Hfq and an Hfq-RNA complex: a bacterial Sm-like protein. EMBO Journal, 21(13), 3546–3556.
30. Seisenberger, S., Peat, J. R., Hore, T. A., Santos, F., Dean, W., Reik, W., ... & Hajkova, P. (2012). Reprogramming DNA methylation in the mammalian life cycle: building and breaking epigenetic barriers. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1609), 20110330.
31. Selker, E. U., Stevens, J. N., & Metzenberg, R. L. (2003). Heterochromatin and DNA methylation in Neurospora. Chromosoma, 111(6), 466–474.
32. Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J. R., Cole, P. A., Casero, R. A., & Shi, Y. (2004). Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell*, 119(7), 941-953.
33. Slotkin, R. K., Vaughn, M., Borges, F., Tanurdžić, M., Becker, J. D., Feijó, J. A., & Martienssen, R. A. (2009). Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. *Cell*, 136(3), 461-472.
34. Smith, L. et al. (2022). Histone deacetylases: emerging players in chromatin remodeling and gene regulation. *Trends in Genetics*, 38(3), 231-245.
35. Tsukada, Y., Fang, J., Erdjument-Bromage, H., Warren, M. E., Borchers, C. H., Tempst, P., & Zhang, Y. (2006). Histone demethylation by a family of JmjC domain-containing proteins. *Nature*, 439(7078), 811-816.

36. Weber, M., Davies, J. J., Wittig, D., Oakeley, E. J., Haase, M., Lam, W. L., & Schübeler, D. (2007). Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. Nature Genetics, 37(8), 853–862.
37. Wollmann, H., & Berger, F. (2012). Epigenetic reprogramming during plant reproduction and seed development. Current Opinion in Cell Biology, 24(3), 396-400.
38. Wu, S. C., & Zhang, Y. (2014). Active DNA demethylation: many roads lead to Rome. *Nature Reviews Molecular Cell Biology*, 11(9), 607-620.
39. Zhang, X., Yazaki, J., Sundaresan, A., Cokus, S., Chan, S. W., Chen, H., … Ecker, J. R. (2006). Genome-wide high-resolution mapping and functional analysis of DNA methylation in Arabidopsis. Cell, 126(6), 1189–1201.
40. Zilberman, D., & Henikoff, S. (2007). Genome-wide analysis of DNA methylation patterns. Development, 134, 3959–3965.
41. Zilberman, D., & Henikoff, S. (2007). Genome-wide analysis of DNA methylation patterns. Development, 134(22), 3959-3965.
42. Greer, E. L., Shi, Y. (2012). Histone methylation: a dynamic mark in health, disease and inheritance. Nature Reviews Genetics, 13(5), 343-357.
43. Rossetto, D., Avvakumov, N., Côté, J. (2012). Histone phosphorylation: a chromatin modification involved in diverse cellular functions. Nature Reviews Molecular Cell Biology, 13(1), 36-50.
44. Kschonsak, M., Haering, C. H., & Shaban, H. A. (2015). Xer1-independent mechanisms of VCP-driven mitotic chromosome disjunction. Cell Reports, 13(9), 2096-2108.
45. Matzke, M. A., & Mosher, R. A. (2014). RNA-directed DNA methylation: an epigenetic pathway of increasing complexity. Nature Reviews Genetics, 15(6), 394-408.
46. Zhong, X., & Ye, B. (2020). Roles of the RNA-Directed DNA Methylation Pathway in Plants. Annual Review of Plant Biology, 71, 539-566.• Ibarra, C. A., Feng, X., Schoft, V. K., Hsieh, T. F., Uzawa, R., Rodrigues, J. A., ... &Pikaard, C. S. (2012). Active DNA demethylation in plant companion cells reinforces transposon methylation in gametes. Science, 337(6100), 1360-1364.
47. Bouyer, D., Kramdi, A., Kassam, M., Heese, M., Schnittger, A., Roudier, F., &Colot, V. (2017). DNA methylation dynamics during early plant life. Genome Biology, 18(1), 179.