Essays in Biochemistry (2019) **63** 691–705
https://doi.org/10.1042/EBC20190043

PORTLAND PRESS

Review Article

The role and mechanisms of DNA methylation in the oocyte

Gintarė Sendžikaitė[1] and **Gavin Kelsey**[1,2]

[1]Epigenetics Programme, Babraham Institute, Cambridge CB22 3AT, U.K.; [2]Centre for Trophoblast Research, University of Cambridge, Cambridge CB2 3EG, U.K.

Correspondence: Gintarė Sendžikaitė (gintare.sendzikaite@babraham.ac.uk) or Gavin Kelsey (gavin.kelsey@babraham.ac.uk)

Epigenetic information in the mammalian oocyte has the potential to be transmitted to the next generation and influence gene expression; this occurs naturally in the case of imprinted genes. Therefore, it is important to understand how epigenetic information is patterned during oocyte development and growth. Here, we review the current state of knowledge of *de novo* DNA methylation mechanisms in the oocyte: how a distinctive gene-body methylation pattern is created, and the extent to which the DNA methylation machinery reads chromatin states. Recent epigenomic studies building on advances in ultra-low input chromatin profiling methods, coupled with genetic studies, have started to allow a detailed interrogation of the interplay between DNA methylation establishment and chromatin states; however, a full mechanistic description awaits.

Introduction

All cells within an organism have the same genome but acquire different appearance and function. The identity of a cell is defined by selective activation of transcriptional programmes and subsequent maintenance during cell division. Epigenetic mechanisms, such as DNA methylation and histone tail post-translational modifications (PTMs), play a crucial role in cell lineage specification during development and faithful maintenance during cell division by regulating chromatin function [1].

DNA methylation

DNA methylation is a covalent modification in which a methyl group from the donor S-adenosyl methionine (SAM) is attached to the carbon-5 atom of cytosine residues by DNA methyltransferases (DNMTs) [2,3]. In vertebrate genomes, it is found mostly, but not exclusively, within a CpG dinucleotide context. Since CG dyads are methylated symmetrically, i.e. on both DNA strands, their methylation can be heritable during cell division, thus providing the means for 'epigenetic memory' [4]. Although CpG dinucleotides are under-represented in vertebrate genomes, they are mostly methylated in somatic tissues. A notable exception is CpG islands (CGIs), where CpGs are clustered together [5,6]. Many CGIs found within gene promoters and transcription start sites (TSSs) are constitutively unmethylated, but some exhibit a lineage-specific DNA methylation status that helps shape the transcriptional landscape. Overall DNA methylation is considered to be a repressive mark, especially at heterochromatin, pericentromeric regions, gene promoters, repetitive and transposable elements [7]. In contrast, methylation over gene bodies is associated with active transcription. Functionally, DNA methylation alters binding of transcription factors and other chromatin interacting proteins, chromatin structure and accessibility, thus fine-tuning gene expression [7].

Received: 17 September 2019
Revised: 29 October 2019
Accepted: 29 October 2019

Version of Record published:
29 November 2019

Histones

In order to be packaged into the cell nucleus, DNA is wrapped around nucleosomes, octamers containing two each of histones H2A, H2B, H3 and H4. Histones have protruding terminal tails that can acquire a plethora of PTMs, such as methylation or acetylation [8]. In addition to

Essays in Biochemistry (2019) **63** 691–705
https://doi.org/10.1042/EBC20190043

PTMs, histones have non-canonical variants that are often incorporated outside DNA replication and which can add another layer of chromatin control. Many histone PTMs are associated with specific genomic regions, activity states and functions. For example, histone 3 lysine 4 trimethylation (H3K4me3) is found at active promoters and TSSs and is associated with gene activation [9], while H3K36me3 marks actively transcribed gene bodies and prevents spurious initiation at cryptic intragenic TSSs [10,11]. Similarly, developmental regulator genes can be marked by both activating H3K4me3 and repressive H3K27me3 marks, and are referred to as being 'bivalent' or 'poised' for transient activation [12]. Together, DNA methylation and histone PTMs control chromatin accessibility and packaging, allowing gene activation or repression.

Epigenetic transitions in development

The ability of chromatin to undergo dynamic transitions is especially important during gamete and early embryo development, when two major epigenetic reprogramming waves are observed in mammals. Reprogramming is required to abolish established patterns determining cell lineage and to restore pluripotent potential [13–15]. Both sperm and egg are terminally differentiated gametes. After fertilisation, embryonic cells undergo epigenetic reprogramming to erase the gametic epigenome and regain totipotency [16]. During pre-implantation development, paternal DNA is rapidly demethylated, by a mechanism that is still not fully understood, partly involving activity of Ten-eleven translocation (TET) enzymes, while the maternal DNA methylation is lost in a passive manner during cellular proliferation. By the time the inner cell mass of the blastocyst is formed, the DNA methylation and histone PTM patterns that characterised the gametes are almost completely lost and only a small subset of gamete differentially methylated regions (gDMRs) and histone PTMs are retained [16,17]. After implantation, from the epiblast stage to gastrula (in the mouse between embryonic days E4.5 and E6.5), DNA methylation is regained and established in a lineage-specific pattern [18].

In mammals, the germline arises from somatic cells of the early post-implantation embryo. In mice, primordial germ cells (PGCs) are specified at E6.5–E7.25 in the yolk sac endoderm, and a second epigenetic reprogramming event is observed in these cells at E10.5–E11.5 [13,19]. During this time, parent-of-origin epigenetic marks are erased. As PGCs proliferate, they migrate to the genital ridge and differentiate into prospermatogonia or oogonia, depending on gonadal sex. Gamete-specific epigenomes are established in the germline soon after birth in male and in adulthood in female mice. Oogonia in the fetal ovary arrest at prophase I of the first meiotic division, and after puberty oocytes develop in readiness for ovulation and resumption of meiosis prior to fertilisation [20]. During this prolonged non-replicative period the oocyte develops from the primary non-growing oocyte (NGO) to the fully grown oocyte (FGO) germinal vesicle (GV) stage (Figure 1A), ultimately experiencing transcriptional arrest. With ovulation, the GV breaks down and the oocyte attains the meiosis II (MII) stage, where it remains until fertilisation. The process of oocyte development is accompanied by global transcriptional and epigenetic changes that are crucial for successful fertilisation and later development (Figure 1A) [21].

Aim of this review

Although epigenetic reprogramming during early embryogenesis erases much of the gametic epigenomes, there are epigenetic features inherited from the oocyte and subsequently maintained in offspring. This is exemplified by imprinted genes, which are monoallelically expressed in offspring on account of DNA methylation differences acquired on these genes in oocytes compared with sperm [22]. This legacy of the gametic epigenome could provide the potential by which genetic and environmental factors that affect the oocyte epigenetic landscape give rise to intergenerational epigenetic inheritance [23], although the extent to which this occurs is still unclear. Nevertheless, it underlines the importance of understanding the normal processes of epigenetic programming that occur in the oocyte. In this respect, CpG methylation is of particular interest not only because the methylation pattern is remarkably different between male and female gametes, but also because of its potential for maintenance after cell division. Complete or partial loss of oocyte methylation is known to cause embryonic lethality and congenital diseases, highlighting the importance of faithful methylome establishment. This review focusses on established and recent knowledge of chromatin dynamics and key mechanisms responsible for faithful DNA methylation establishment in the oocyte. We discuss findings in mouse oocytes as a paradigm for mammalian systems.

Oocyte DNA methylation and chromatin dynamics

Oocyte methylation landscape

The oocyte is a terminally differentiated cell with a unique DNA methylation pattern, distinct from sperm or soma. In sperm DNA methylation is evenly dispersed and covers approximately 90% of the genome, with the notable exception

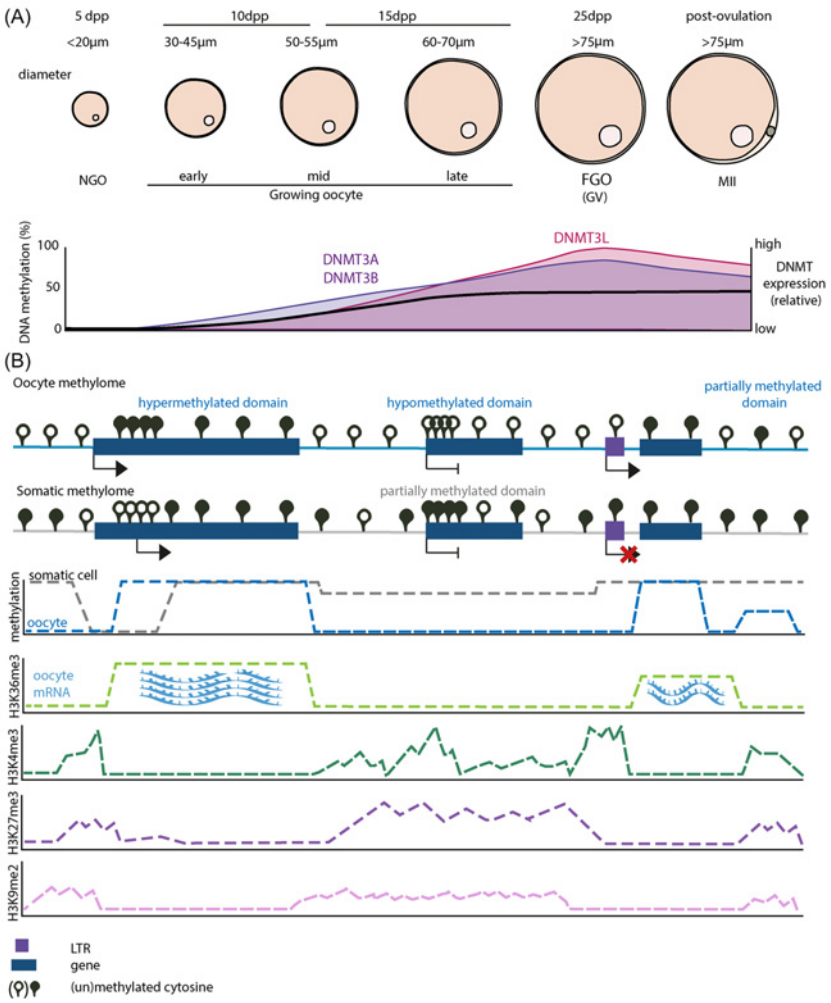

Figure 1. DNA methylation and histone mark patterns in oocyte

(**A**) At day 5 post-partum (5dpp), the majority of oocytes are non-growing (NGOs). Oocytes gradually grow postnatally in mice, which is manifested by an increase in diameter. Representative sizes of oocyte at different dpps are shown. DNA metyltransferases DNMT3A, DNMT3B and DNMT3L are increasingly expressed from early growing oocytes, which coincides with the start of DNA methylation establishment. DNMT expression peaks in FGOs, when DNA methylation is completed at approximately 40% genome coverage. Ovulation initiates maturation of the FGO and transition to MII stage, when a polar body is formed (depicted in grey) and the MII oocyte remains arrested until fertilisation. Oocyte transcription is globally repressed at the MII stage, corresponding with decrease in DNMT3 transcript levels. DNA methylation is depicted by the black line, relative expression levels for DNMTA/DNMT3B and DNMT3L are in violet and magenta, respectively. Abbreviation: dpp, days post partum. (**B**) Schematic representation of DNA methylation and histone marks in the FGO. Unlike the methylome of somatic cells, which shows relatively high and even levels of DNA methylation across the genome, the oocyte methylome has distinct bimodal and clustered hyper- and hypo-methylation domains. In somatic cells, promoter CpG methylation status is linked to expression, where actively transcribed genes tend to have an unmethylated promoter, but in oocytes this relationship is more ambiguous. Hypermethylated domains in the oocyte are associated with actively transcribed genes and the H3K36me3 mark. Levels of H3K36me3 positively correlate with levels of gene expression at the locus. Notably, a subset of oocyte transcripts arise from oocyte-specific or LTR-driven promoters. Hypomethylated domains span transcriptionally inactive parts of the genome and only a small fraction of the oocyte methylome falls into a partially methylated domain category; these domains tend to overlap intergenic areas. H3K4me3 in the FGO has a non-canonical distribution, where it spreads from promoters, forming broad low to medium enrichment domains over hypo- and partially methylated domains, but not hypermethylated domains. Similarly, H3K27me3 has a non-canonical distribution over hypo- and partially methylated domains, some of which overlap H3K4me3, forming bivalent chromatin. Bivalent chromatin in the oocyte does not fully recapitulate bivalency found in embryonic tissues, as the enrichment of H3K27me3 is lower. Meanwhile, H3K9me2 covers approximately a quarter of oocyte genome, again, with exclusion of hypermethylated domains. Height of the curves indicates relative enrichment of histone marks. Abbreviation: LTR, long terminal repeat.

Essays in Biochemistry (2019) **63** 691–705
https://doi.org/10.1042/EBC20190043

of most CGIs that escape methylation. Meanwhile, mature oocytes show 40% global DNA methylation. Consequently, there are thousands of germline differentially methylated domains (gDMRs) [24–26].

The acquisition of DNA methylation in the oocyte is gradual throughout its growth and development. NGOs present in the primordial follicle before folliculogenesis is initiated are practically unmethylated, and methylation is acquired as the oocyte increases in size, primarily at the later stages of follicle development [24,25] (Figure 1A). What initiates *de novo* methylation is unclear, but might simply be the availability of appropriate DNMT activity coupled with permissive underlying chromatin state (see below). There does not seem to be much selectivity in the timing at which different genomic features become methylated, although there is a pronounced asynchrony amongst them, including CGIs and imprinted gDMRs [27–29]. Unlike somatic cells, where most CpGs are methylated, with the exception of active gene promoters, CpGs in FGOs exhibit a distinctly bimodal and clustered methylation distribution. Large genomic domains that are either hyper- (>75%) or hypo- (<25%) methylated form a signature oocyte methylome (Figure 1B). Only a small fraction of CpGs fall into a partially methylated category, and these domains are usually found at intergenic areas. This bimodal pattern is conserved in all mammalian oocytes so far studied [30,31].

Additionally, the oocyte has relatively high levels of non-CpG (CpH) methylation. It occurs mostly, but not exclusively, in the context of CpA dinucleotides [32,33]. CpG and CpH DNA methylation show very strong positive correlation and similar dynamics during oocyte maturation. CpH methylation is absent from NGOs and increases until and possibly beyond the FGO stage, where it is mostly found over active genes [25,29,33]. The significance of CpH methylation is unclear, but a new hypothesis has recently been advanced of its possible role in transcriptional regulation in human oocytes (https://www.biorxiv.org/content/10.1101/651141v1.full).

The functional role of DNA methylation in the oocyte

DNA methylation appears to be dispensable for oocyte development and competence, as genetic ablation of oocyte methylation allows for successful fertilisation and ensuing embryonic development until the mid-gestation stage [34–38]. But it is essential for genomic imprinting: a subset of the gDMRs that evade embryonic reprogramming during early development result in parent-of-origin specific gene expression of the associated genes – imprinted genes. Failure to establish imprints leads to lethality or congenital diseases in both mice and humans, the pathologies observed are linked to placental and foetal growth, brain and metabolic function. Interestingly, only three imprinted loci are conferred by methylation in male gametes, while there are at least 26 DNA methylation-dependent imprinted regions conferred in the oocyte [22,24,25]. Imprinted loci contain a single or a cluster of genes, whose expression patterns are determined by imprinting control region (ICR) DNA methylation status. DNA methylation at ICRs is set up during oocyte growth in a transcription-dependent manner. For example, at the imprinted *Gnas* locus, transcriptional activity from an oocyte-specific promoter upstream of the *Nesp* gene is required for methylation of that locus. Disruption of transcription leads to failure of DNA methylation establishment over the ICR disrupting monoallelic expression of *Gnas* locus imprinted genes and viability in mice [39]. Oocyte ICRs are composed of CGIs enriched for a specific CG-rich hexameric motif recognised by the KRAB zinc-finger protein 57 (ZFP57). ZFP57 protects imprinted sites from demethylation during embryonic reprogramming by recruiting KAP1, SETDB1, HP1 and NP95 to form a robustly silenced locus [40,41]. Imprinted genes exhibit stable and heritable monoallelic parent-of-origin specific gene expression that can be maintained throughout the lifecourse, and which is only overwritten during PGC reprogramming. The complex mechanisms of ICR establishment and maintenance have been extensively studied and recently reviewed [22,42].

Apart from ICRs, some gDMRs show transient or tissue-specific inheritance post fertilisation [43,44]. Maternal non-imprinted gDMRs were shown to play a role in regulation of placental development in mice and humans [38,45]. The majority of such loci lose parent-of-origin methylation upon implantation, but it is currently not well understood how they affect pre-implantation development; whether gDMRs have any impact on zygotic genome activation, and if unaccounted demethylation escapees serve a more general purpose.

Complete loss of DNA methylation in oocytes manifests in embryonic lethality at E10.5, which was originally attributed to defects arising from imprint loss [34,36]. However, more recent evidence from various mouse knockouts (discussed in relevant contexts below) suggest that disruption of the oocyte methylome could lead to developmental defects unrelated to effects of disrupted imprinting (Table 1). For example, oocyte-ablation of *Kdm1a* or *Mll2*, which impair few or no imprints, respectively, show that minor global loss of gene-body methylation results in maternal-to-zygotic transcriptional transition or ovulation failure [46–49]. However, the effects of *Kdm1a* and *Mll2* knockouts could also be mediated by chromatin alterations. Meanwhile, *Stella* and *Uhrf1* knockout oocytes, which also show limited alteration to imprints but a strong global change in DNA methylation, arrest at the blastocyst stage

Table 1 Summary of known genetic oocyte-specific knockout models and their impact on DNA methylation

Factor	Function	Impact on imprinted gDMRs in oocyte	Impact on global oocyte DNA methylation	Impact on post-fertilisation development	Reference
Dnmt3a	DNA methylation	Severe loss of DNA methylation	Global loss of DNA methylation	Incorrect imprint establishment, E9.5-E10.5 lethality	[24,33,34,35]
Dnmt3b	DNA methylation	Not affected	Not affected	Normal germ cell and post-fertilisation development	[33,34,35]
Dnmt3l	*De novo* methylation targeting	Severe loss of DNA methylation	Global loss of DNA methylation	Incorrect imprint establishment, E9.5-E10.5 lethality	[24,33,35,36]
Dnmt1	Maintenance of DNA methylation	Not affected	Slight loss of global DNA methylation, mostly at hemimethylated sites	Partial failure to maintain imprinted gDMRs, prenatal lethality	[79,80]
Kdm1a	H3K4me1/2 and H3K9me2 demethylase	Loss of methylation at *Gnas1A*, *Cdh15*	Minor loss of genic DNA methylation	Arrest at two-cell stage	[46,48,61]
Kdm1b	H3K4me1/2 demethylase	DNA methylation loss mostly at late methylating gDMRs	Loss of genic DNA methylation	E10.5 lethality	[46,60]
Mll2	H3K4me2/3 methyltransferase	Not affected	Minor loss of gene body methylation due to decreased transcription	Oocytes fail to ovulate and die prior to fertilisation	[47,49]
Setd2	H3K36me3 methyltrasnferase	Loss of methylation at all imprints	Global inverse pattern, hypermethylated domains lose methylation, hypomethylated domains gain methylation	Preimplantation lethality; post-implantation lethality in cytosolic rescue	[102,104]
Uhrf1	Recruitment of DNMT1 to hemimethylated DNA	Significant loss only at *Gnas1A, Peg10, Mest*	Minor loss of global DNA methylation, mostly over intermediately methylated and inactive domains	Lethality around blastocyst stage	[109]
Stella	Protection of genome from methylation	Not affected	Two-fold global hypermethylation	Lethality around blastocyst stage	[51,52]
G9a	H3K9me2 transferase, DNA methylation recruitment	Minor loss of methylation at *Gnas1A*, *Mest*	Minor loss of DNA methylation	Blastocyst or peri-implantation stage lethality (not fully penetrant)	[119,128]
Sall4	Transcription factor	Loss of methylation at all imprints	Major whole genome DNA methylation loss	Oocytes fail to mature	[120]
Hdac1/Hdac2	Histone deacetylases	Loss of methylation at all imprints	Global loss of DNA methylation	Oocytes fail to mature	[62,63]
Sin3a	Member of HDAC repressor complex	Loss of methylation at selected imprints	n/a	Lethality at two-cell stage (knockdown experiment)	[62,126,127]
Cfp1	SETD1 H3K4 methyltransferase DNA binding subunit	n/a	Global loss of DNA methylation	Lethality at two-cell stage	[89]
Hira	H3.3 deposition chaperone	Reduction in DNA methylation at imprints	Global loss of DNA methylation	Lethality immediately after fertilisation	[58]

[50–52]. Together, these findings suggest that although DNA methylation is not required for development or maturation of the oocyte and fertilisation *per se*, it is indispensable for embryonic development beyond imprinting in ways that are not yet fully understood.

Transcription and transposable elements

With the recruitment of primordial follicles into growth (from NGO to early growing oocyte), a definitive oocyte transcriptome is established. Once development has progressed to about the antral follicle stage (early to mid growing oocyte), expressed genes in oocytes start to acquire DNA methylation across their gene bodies. Methylation increases during oocyte growth and is completed by the FGO GV stage. High transcriptional levels, DNA methylation and H3K36me3 abundance show high correlation, and approximately 90% of methylome establishment can be attributed to transcription events (Figure 1B) [24–26,29,46,53]. In agreement with this, loss of transcription upstream of imprinted genes results in failure to set up methylation at ICRs and imprinting of these genes [26,39,54]. In the oocyte, subsets of transposable elements, especially Long Terminal Repeats (LTRs), are very active and highly expressed: they can act as promoters, TSSs or splice donors, thereby generating approximately 10% of oocyte-specific

Essays in Biochemistry (2019) **63** 691–705
https://doi.org/10.1042/EBC20190043

transcript species [26,55,56]. Transcriptional activity of these LTRs contributes to the generation of hypermethylated domains found downstream (Figure 1B) [26]. A recent study in mouse, rat and human oocytes identified that approximately one-sixth of all DNA methylation is linked to transcription initiated at LTRs [31]. LTR-dependent DNA methylation shows strong species specificity, and can be inherited to blastocyst or extraembryonic tissues [31]. Moreover, LTRs are suggested to be drivers of species-specific imprint establishment in humans and mice (https://www.biorxiv.org/content/biorxiv/early/2019/08/07/723254.full.pdf).

Local chromatin environment

As noted above, not all sites gain methylation simultaneously during oocyte growth [24,27,29]. The timing of DNA methylation of specific genes and genomic features is not linked to the underlying sequence but could rather be assigned to local chromatin environment, histone PTMs and nucleosome density. Although the oocyte is in a non-replicative state, nucleosome turnover, an inherent process during transcription, is required to aid oocyte maturation. Deletion of HIRA, a histone chaperone responsible for non-canonical histone deposition in quiescent cells [57], in the oocyte results in increased accessibility and loss of landmark histone modifications, which in turn leads to genome-wide hypomethylation [58]. At the same time, nucleosome depletion at certain sites increases accessibility and could allow easier access for DNMTs. Genes showing high accessibility at TSSs or across the gene body during oocyte development are associated with higher transcription and DNA methylation levels [53]. Genes that remain highly compacted throughout oocyte growth tend to remain silent and are not subjected to *de novo* methylation. Similarly, precocious expression of the *de novo* methyltransferases DNMT3A and DNMT3L accelerates imprint establishment at only a selection of loci and others appear to be protected by a restrictive chromatin environment [59].

Loci that acquire methylation late in oocyte growth are often CGI-rich, and require removal of H3K4me2 or H3K4me3, active chromatin marks that inhibit binding and activity of the DNMT3A/L complex [29,46]. The H3K4 demethylases KDM1A and KDM1B are expressed throughout oocyte growth or from mid-growth phase, respectively. Ablation of KDM1B, and to some extent KDM1A, in the oocyte resulted in failure to establish full DNA methylation over most imprinted genes and led to focal hypomethylation [46,48,60,61]. Similarly, histone deacetylase 1 and 2 (HDAC1/2) are expressed in early oocytes, with the former subsequently decreasing as growth progresses. Loss of HDAC1/2 results in altered chromatin environment and perturbed transcription, leading to both global and imprint-specific DNA methylation loss in the oocyte [62,63]. As noted above, 10% of DNA methylation in the oocyte is transcription-independent and these loci tend to be methylated quite late in oocyte growth. DNMT targeting to those sites is likely to involve histone modifications, remodellers or other chromatin-interacting proteins that would make local chromatin accessible and appropriately marked, but the precise mechanism(s) are unknown.

DNA methylation machinery

Although the oocyte has a unique DNA methylation pattern, it relies on an otherwise conventional DNA methylation machinery. The DNMT family in mammals consists of five members: one maintenance, three *de novo* methyltransferases and a cofactor. DNMT1, the maintenance DNMT, recognises and methylates the unmethylated strand on hemimethylated DNA [64]. During S phase, DNMT1 associates with replication foci through Ubiquitin-like, plant-homoeodomain (PHD) and ring finger-containing 1 (UHRF1) and ensures faithful methylation maintenance on the nascent DNA strand [65]. Homozygous deletion of *Dnmt1* results in embryonic lethality [66]. In addition to DNMT1, there are three *de novo* DNMTs, which use unmethylated DNA as a substrate. DNMT3A and DNMT3B show partial redundancy and are both required for epigenetic reprogramming during embryogenesis [67]. *Dnmt3a*$^{-/-}$ mice fail to survive longer than 3 weeks postnatally, while *Dnmt3b*$^{-/-}$ and *Dnmt3a*$^{-/-}$ *Dnmt3b*$^{-/-}$ mice die before E11.5. DNMT3C, a third, murine-specific *de novo* methyltransferase, has recently been discovered; it silences evolutionary young retrotransposons in prospermatogonia by methylating their promoters [68]. DNMT3L is the odd member of DNMT family, since it does not have an active catalytic domain [69]. DNMT3L is also less conserved between the species and is only found in mammals with genomic imprinting [70]. The C-terminal domain of DNMT3L can bind DNMT3A and DNMT3B C-terminal domains and significantly enhances their chromatin binding and/or catalytic activity by formation of tetramers [71,72].

DNMTs in the oocyte

Both DNMT3A and DNMT3B are detectable and localise to the nucleus of the GV oocyte [73]. However, DNMT3A and DNMT3L are the key players, both necessary for faithful DNA methylation establishment. Expression of DNMT3A, DNMT3B and DNMT3L in growing oocytes is coordinated, their expression levels increase as oocyte development proceeds, peaking towards the GV stage when *de novo* methylation is complete, and decrease once oocyte attains the MII stage (Figure 1A) [74]. DNMT3A is essential for catalysing the methylation, but relies heavily on

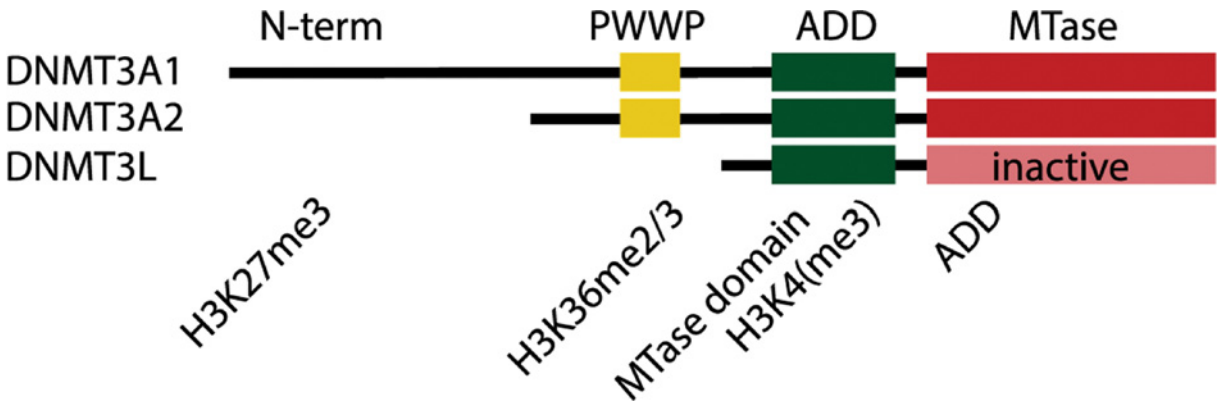

Figure 2. Schematic structures of DNMT3A and DNMT3L, and their predicted interactors

N-terminal domain of DNMT3A1 is required for localisation at bivalent chromatin shores in ES cells. Notably, this isoform is not expressed in the oocyte. The PWWP domain is poised to recognise H3K36me2/3, but this interaction has not been interrogated in the oocyte. The ADD domain forms a fold with the catalytic MTase domain to create an inactive allosteric conformation of DNMT3A. Recognition of unmethylated H3K4 tail alters this conformation and stimulates catalytic activity, while H3K4me3 repels the protein.

interaction with DNMT3L for genomic targeting [24,25,34–36]. Both *Dnmt3a*$^{-/-}$ or *Dnmt3l*$^{-/-}$ mice fail to establish germline methylation, but *Dnmt3l*$^{-/-}$ show a more severe phenotype [24,34–36,72,75]. In mice, the conditional deletion of DNMT3A or DNMT3L results in failure to establish DNA methylation in the oocyte and consequently a loss of maternal imprints in offspring [34–36]. DNMT3B, although expressed, does not appear to play a role in DNA methylation in the oocyte, and *Dnmt3b*$^{-/-}$ oocytes mature without failure and have unaffected phenotype [35]. However, in other contexts [76], DNMT3B is able to bind DNMT3L. It is possible that in the *Dnmt3a*$^{-/-}$ background, DNMT3B interacts with DNMT3L to rescue some of the methylation targets. Unlikely to be required as a maternal transcript prior to zygotic genome activation, the role of *Dnmt3b* expression in the oocyte remains elusive. Regarding DNMT1, the oocyte expresses an oocyte-specific isoform *Dnmt1o*, which arises from an alternative 5′ exon and is 118 amino acids shorter than the somatic isoform. DNMT1O is found in high abundance in growing oocytes and, although some nuclear localisation is retained, it is mostly cytoplasmic [73,77]. DNMT1O in the oocyte has a minor role in fully methylating hemimethylated sites, but the main purpose for accumulation of this protein in oocytes is likely to be after fertilisation [33,78,79].

Recruitment of DNMTs in the genomic context

Many studies have sought to understand how the oocyte-specific DNA methylation pattern is set. Overall, DNMT3s show rather limited target sequence specificity, which is also true for methylated CGIs in the oocyte [24,80]. However, N-terminal regulatory domains of these proteins – the ADD (ATRX-DNMT3-DNMT3L) and PWWP (Pro-Trp-Trp-Pro motif) domains – can interact with various histone PTMs and guide DNMT localisation and enzymatic activity (Figure 2). Recent advances in low-input chromatin immunoprecipitation methods, requiring as little as a few hundred cells [16], have allowed the interrogation of the localisation of histone marks with respect to specific genomic features and DNA methylation status. In combination with knockouts for specific histone modifier enzymes, such studies are shedding light on instructive and consequential interactions between different mechanisms. Since DNMT3A is responsible for the majority of DNA methylation in the oocyte, we focus on known and predicted mechanisms of its targeting to the genome.

The ADD domain, H3 and H3K4me3

The ADD domain, present in all DNMT3s, is homologous to a conserved PHD zinc finger motif. ADD domains of DNMT3A and DNMT3L have a high affinity to the N-terminal region of histone 3, especially when unmethylated at lysine 4 (H3K4). This interaction promotes DNA methylation catalysis [81–84]. When DNMT3A is in complex with DNMT3L, which is presumably the case in the oocyte, recruitment of the DNMT3L ADD domain is sufficient to engage the whole complex [72,81]. Methylated lysine H3K4me3 in somatic cells is found at active gene promoters and TSSs, and inhibits DNMT3A activity [81–84]. Structural studies have found that DNMT3A is intrinsically in an autoinhibitory allosteric conformation, driven by the ADD domain: the ADD domain masks the DNA binding site of the catalytic domain. Recognition of unmethylated H3K4 specifically allows a structural shift and uncouples the

Essays in Biochemistry (2019) **63** 691–705
https://doi.org/10.1042/EBC20190043

ADD-catalytic domain interaction, allowing activation of DNMT3A enzymatic function [85]. Thus, DNA methylation and H3K4me3 are mutually exclusive in the genome. Engineering of the ADD domain to lose sensitivity to H3K4me3 results in aberrant gain of methylation over these domains [86]. The function of the ADD domain in somatic cells is relatively well studied with many mechanistic insights supported by structural work. Current evidence of methylation patterns in the oocyte suggests that the ADD domain plays a similar role in the oocyte, although no oocyte-specific studies have been conducted.

Curiously, H3K4me3 has an atypical broad domain pattern in the mouse FGO, where it covers approximately one-fifth of the genome. Broad domains are found not only over TSSs but over distal regions as well, and these domains tend to show low or intermediate levels of DNA methylation (Figure 1B) [47,87,88]. H3K4me3 appears as canonical sharp peaks in NGOs, and these loci are protected from *de novo* DNA methylation through ADD domain inhibitory mechanism [81,82]. During oocyte development, H3K4me3 spreads, simultaneously but at mutually exclusive locations with DNA methylation, and invades intermediately methylated domains from the mid to late oocyte growth stage, reaching the final distribution in FGO [47,87,88]. In the oocyte, the H3K4me3 methyltransferase MLL2 (KMT2B), expressed at mid- to MII stages, is responsible for non-canonical H3K4me3 establishment [47,49]. In MLL2 knockout oocytes DNA methylation spreads to some but a limited number of domains that should normally contain H3K4me3, whereas in DNMT3A knockout oocytes there is a more pervasive spread of H3K4me3 into normally DNA methylated domains [47]. This observation suggests that DNMT3A recruitment depends not only on absence of antagonistic H3K4me3, but also presence of an attractive histone PTM. Another H3K4 methyltransferase, SETD1, is assumed to be responsible for canonical promoter-associated H3K4me3 establishment via its DNA binding subunit CFP1, although this has not yet been validated by ChIP-seq analysis. Deletion of CFP1 in the oocyte resulted in loss of global DNA methylation, however, this could be an indirect effect of DNMT3A down-regulation following loss of promoter H3K4me3 [89]. These findings suggest a complex interaction, where localised H3K4me3 at TSSs prevents DNA methylation establishment, while hypermethylation at transcription-independent domains protects these loci from broad non-canonical H3K4me3.

The PWWP domain, H3K36me3 and bivalent chromatin

The PWWP domain is a member of the Tudor domain royal superfamily and is mostly found in chromatin-interacting proteins. It has an intrinsic and somewhat unspecific affinity to chromatin and modified histones [90]. In the DNMT family, the PWWP domain is only found in DNMT3A and DNMT3B, and is known to be required for methylation of major satellite repeats [90–92]. The PWWP domain contains a conserved aromatic cage that enables binding of methylated lysines, especially H3K36me3 [93,94]. Extensive biochemical work suggests that the PWWP domain of DNMT3A interacts specifically and exclusively with H3K36me2/3 [95–98]. Dhayalan et al. [95] show that a mutation (D329A) within the aromatic cage of the DNMT3A PWWP domain disrupts binding of H3K36me3 *in vitro*. H3K36me3 is universally found over expressed gene bodies, and studies suggest that in mouse embryonic stem cells DNMT3B and not DNMT3A is responsible for DNA methylation over H3K36me3 domains [11,99], a conclusion supported by a study expressing DNMT3B in yeast [100]. We used a mouse model to investigate the effect of the DNMT3A^{D329A} mutation but did not find any evidence of methylation defects over gene bodies and H3K36me3 domains in embryos or adult mouse brain [101], or in oocytes (unpublished). This raised a question whether DNMT3A in the oocyte indeed was recruited by the transcription-dependent H3K36me3 mark over gene bodies [46].

Xu et al. [102] tested the DNA methylation and H3K36me3 interaction from a different perspective and generated a mouse with an oocyte-specific SETD2 knockout: SETD2 is the histone lysine methyltransferase uniquely responsible for H3K36me3 deposition in mammalian cells [103,104]. H3K36me3 is already present in early oocyte growth stages and increases together with transcription and DNA methylation, persisting until the MII stage (Figure 1B). Depletion of H3K36me3 in NGO resulted in dramatic loss and redistribution of DNA methylation and affected all maternal imprints in FGO. It also led to altered transcriptome, reorganisation of H3K4me3 and H3K27me3 mark landscapes, and caused embryonic lethality [102]. This work suggests that H3K36me3 is a master regulator of the oocyte methylome, and is required to prevent H3K4me3 and H3K27me3 from the spreading into actively transcribed regions. Yet it remains unclear whether the recognition of this histone tail modification by the DNMT3A PWWP domain is the main driver of DNA methylation establishment.

The N-terminal domain and H3K27me3

DNMT3A has two major isoforms. DNMT3A1, the longer isoform that predominates in adult somatic tissues, and DNMT3A2, a shorter isoform found in the oocyte and embryonic tissues. DNMT3A1 has been shown to localise preferentially to the shores of Polycomb-regulated bivalent chromatin and follows the dynamics of the H3K27me3 mark during neuronal differentiation [105]. Bivalent chromatin comprises an active chromatin mark H3K4me3 and

repressive H3K27me3 and is protected from DNA methylation in somatic cells [12,106]. Bivalent domains are also present in the oocyte [47], although they appear to be less pronounced than in embryonic tissues (Figure 1B), while H3K27me3 shows a somewhat non-canonical distribution: it is anti-correlated with transcribed genes and mostly overlaps hypo- and partially methylated domains (Figure 1B) [47,107]. In soma, the DNMT3A and bivalent chromatin interaction depends on the N-terminal disordered domain that is specific to isoform 1. However, only DNMT3A2, lacking the N-terminal domain, is expressed in the oocyte [108]. Currently the mechanism of mutual exclusivity between DNA methylation and H3K27me3 at CGIs is not known. It is possible that lack of DNMT recruitment to bivalent chromatin shores simply results in those domains remaining unmethylated. This theory has not been put to test and H3K27me3 link to DNA methylation in the oocyte is unclear.

UHRF1, STELLA and DNMT1

While the loss of imprinted gDMRs in the oocyte causes mid-gestational embryonic lethality, excessive gain of methylation, even when gDMRs remain relatively unaffected, also results in failure of embryonic development [51,52], highlighting the importance of DNA methylation and lack thereof outside of the gene body context.

The UHRF1 ubiquitin ligase recognises hemimethylated DNA and recruits the maintenance DNMT1 to these sites [50,65]. UHRF1 is mostly cytoplasmic in the oocyte, however, when deleted, it results in loss of DNMT1 localisation to the nucleus [109]. UHRF1 knockout oocytes exhibit a decrease in global DNA methylation of approximately 8%, which is greater than what could be attributed to loss of symmetric 5mC at hemimethylated sites that is dependent on DNMT1, suggesting additional pathways of UHRF1-dependent methylation in oocytes. Interestingly the loss was observed in non-CpG methylation too, suggesting that UHRF1 is involved in *de novo* methylation targeting [109].

Work in NIH3T3 cells showed that UHRF1 is regulated by a maternal effect protein STELLA (also known as PGC7 and DPP3A). Overexpression of STELLA prevented localisation of UHRF1 and DNMT1 to the DNA replication fork and resulted in global hypomethylation [110]. STELLA is expressed in the oocyte and is known to be responsible for protection of 5mC during epigenetic reprogramming in early embryogenesis [111,112]. However, earlier work did not find any effect of *Stella* knockout in the oocyte [112]. Recently, two studies showed that knocking-out STELLA in the oocyte resulted in aberrant gain of DNA methylation of more than 28% [51,52]. In the context of the oocyte, STELLA is responsible for nuclear export of UHRF1 to the cytoplasm, thereby preventing methylation of regions normally unmethylated in the oocyte.

UHRF1 or STELLA knockouts in the oocyte cause hypo- and hyper-methylation, respectively, but the effect was localised to intermediately methylated regions harbouring low- or non-expressed genes [51,109]. Both proteins are required for proper embryonic development, and these knockouts result in early lethality. This work sheds some light on cellular regulation of DNMT1 in the oocyte, and highlights our lack of understanding of the roles of the UHRF1–DNMT1 interaction in *de novo* methylation. Notably, *Uhrf1* or *Stella* knockout oocytes exhibit severe global methylation changes that do not alter imprinting regions, yet embryos fail early in development [51,52,109], suggesting that oocyte methylation at non-imprinted domains has important consequences post-fertilisation.

G9A/GLP and H3K9me2

G9A/GLP (EHMT2/EHMT1) is a histone methyltransferase complex responsible for H3K9me2 primarily in euchromatic regions of the genome [113]. In somatic cells H3K9me2 is highly abundant and is involved in heterochromatin formation [114]. G9A initiates heterochromatinisation of the genome by establishing H3K9me2 during embryogenesis and, independent of its catalytic activity, recruits *de novo* DNMT3s for DNA methylation [115–117]. G9A is expressed from early oocyte stages, and levels of both G9A expression and H3K9me2 abundance increase as the oocyte progresses through the growth phase [118]. Broad domains of H3K9me2 cover more than a quarter of the mature oocyte genome but mostly where CpG methylation is low (Figure 1b) [119]. Oocyte-specific G9A knockout shows no effect on NGOs and only a slight loss of DNA methylation is observed in FGOs. Currently there are no studies conducted in the oocyte that explore GLP function, but the outcome is expected to be similar as in most situations G9A function is completely dependent on GLP. Thus, although H3K9me2 is abundant in the oocyte, it does not direct DNA methylation to specific genomic loci.

SALL4

In addition to chromatin modifications and modifiers, other DNA-interacting proteins, such as transcription factors, could be involved in DNA methylation regulation. The transcription factor SALL4 is expressed from the primary follicle stage throughout the growth of the oocyte, and is found in the nucleus. After the GV to MII transition, it relocates to the cytoplasm. Oocyte-specific ablation of *Sall4* shows that oocytes failed to reach the mature stage or undergo GV breakdown, required to proceed to the MII stage. Interestingly, *Sall4* knockout oocytes show loss of DNMT3A

Essays in Biochemistry (2019) **63** 691–705
https://doi.org/10.1042/EBC20190043

nuclear localisation and dramatically reduced levels of 5-mC. Imprinted regions were almost completely unmethylated, while repetitive elements were fairly hypomethylated. SALL4 is a transcriptional regulator of several histone lysine demethylases: *Sall4* knockout oocytes show higher expression of *Kdm5b*, and lower expression of *Kdm6a* and *Kdm6b*, consistent with lower H3Kme3 and higher H3K27me3 levels, respectively, as assessed by immunofluorescence [120]. This finding again links to the importance of dynamic chromatin changes in oocyte growth, and exemplifies the upstream involvement of transcription factors in DNA methylation.

Summary

- The oocyte has a unique methylome of hyper- and hypo-methylated domains that are gradually established by DNMT3A and DNMT3L during oocyte growth.
- The majority of methylated domains are associated with active transcription units, the remainder require local chromatin reorganisation.
- DNMT3A and DNMT3L are recruited to chromatin through their regulatory domain interactions with modified histone tails.
- Access of the DNA methylation machinery to the genome is regulated by cellular localisation of DNMTs and local chromatin environment.
- Although much of the oocyte methylome may be dispensable, faithful methylation establishment at both imprinted and non-imprinted loci is essential for embryonic development. Further work is needed to elucidate the role of the oocyte methylome in early embryogenesis and beyond.

Competing Interests

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

Abbreviations

ADD, ATRX-DNMT3-DNMT3L; CFP1, CxxC finger protein 1; CGI, CpG island; ChIP-seq, chromatin immunoprecipitation sequencing; DNMT, DNA methyltransferase; dpp, days post-partum; EHMT1/2, euchromatic histone lysine methyltransferase 1 and 2, same as GLP and G9A; FGO, fully grown oocyte; gDMR, gamete differentially methylated region; GLP, G9A-like protein, same as EHMT1; GV, germinal vesicle; G9A, same as EHMT2; HDAC1/2, histone deacetylase 1 and 2; HIRA, histone cell cycle regulator; HP1, heterochromatin protein 1; H2/3a/3b/4, histone 2 or 3a or 3b or 4; H3K4me1/2/3, histone 3 lysine 4 mono- or di- or trimethylation; H3K9me2, histone 3 lysine 9 dimethylation; H3K27me3, histone3 lysine 27 trimethylation; H3K36me2/3, histone 3 lysine 36 di- or trimethylation; ICR, imprinting control region; KAP1, KRAB-associated protein 1; KDM1A/B, lysine demethylase 1a or 1b; KRAB, Krüppel associated box; LTR, long terminal repeat; MLL2, myeloid/lymphoid or mixed-lineage leukaemia; MTase, methyltransferase domain; MII, meiosis II; NGO, non-growing oocyte; NP95, nuclear protein 95; PGC, primordial germ cell; PHD, plant-homoeodomain; PTM, post-translational modification; PWWP, Pro-Trp-Trp-Pro motif; SALL4, Sal-like protein 4; SAM, S-Adenosyl methionine; SETD1/2, histone-lysine N-methyltransferase; SETDB1, SET domain bifurcated histone lysine methyltransferase 1; SIN3A, SIN3 transcription regulator family member a; STELLA, also known as primordial germ cell protein 7 or developmental pluripotency-associated 3; TET, ten-eleven translocation; TSS, transcription start site; UHRF1, Ubiquitin-like, PHD and ring finger-containing 1; ZFP57, KRAB zinc-finger protein 57; 5mC, 5-methylcytosine.

References

1 Bernstein, B.E., Meissner, A. and Lander, E.S. (2007) The Mammalian Epigenome. *Cell* **128**, 669–681, https://doi.org/10.1016/j.cell.2007.01.033
2 Ehrlich, M. and Wang, R.Y. (1981) 5-Methylcytosine in eukaryotic DNA. *Science* **212**, 1350–1357, https://doi.org/10.1126/science.6262918
3 Okano, M., Xie, S. and Li, E. (1998) Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. *Nat. Genet.* **19**, 219–220, https://doi.org/10.1038/890
4 Bird, A. (2002) DNA methylation patterns and epigenetic memory. *Genes Dev.* **16**, 6–21, https://doi.org/10.1101/gad.947102
5 Bird, A., Taggart, M., Frommer, M., Miller, O.J. and Macleod, D. (1985) A fraction of the mouse genome that is derived from islands of nonmethylated, CpG-rich DNA. *Cell* **40**, 91–99, https://doi.org/10.1016/0092-8674(85)90312-5

6 Illingworth, R.S., Gruenewald-Schneider, U., Webb, S., Kerr, A.R.W., James, K.D., Turner, D.J. et al. (2010) Orphan CpG Islands Identify Numerous Conserved Promoters in the Mammalian Genome. *PLos Genet.* **6**, e1001134, https://doi.org/10.1371/journal.pgen.1001134
7 Schübeler, D. (2015) Function and information content of DNA methylation. *Nature* **517**, 321–326, https://doi.org/10.1038/nature14192
8 Kouzarides, T. (2007) Chromatin Modifications and Their Function. *Cell* **128**, 693–705, https://doi.org/10.1016/j.cell.2007.02.005
9 Santos-Rosa, H., Schneider, R., Bannister, A.J., Sherriff, J., Bernstein, B.E., Emre, N.C.T. et al. (2002) Active genes are tri-methylated at K4 of histone H3. *Nature* **419**, 407–411, https://doi.org/10.1038/nature01080
10 Bannister, A.J., Schneider, R., Myers, F.A., Thorne, A.W., Crane-Robinson, C. and Kouzarides, T. (2005) Spatial Distribution of Di- and Tri-methyl Lysine 36 of Histone H3 at Active Genes. *J. Biol. Chem.* **280**, 17732–17736, https://doi.org/10.1074/jbc.M500796200
11 Neri, F., Rapelli, S., Krepelova, A., Incarnato, D., Parlato, C., Basile, G. et al. (2017) Intragenic DNA methylation prevents spurious transcription initiation. *Nature* **543**, 72–77, https://doi.org/10.1038/nature21373
12 Bernstein, B.E., Mikkelsen, T.S., Xie, X., Kamal, M., Huebert, D.J., Cuff, J. et al. (2006) A Bivalent Chromatin Structure Marks Key Developmental Genes in Embryonic Stem Cells. *Cell* **125**, 315–326, https://doi.org/10.1016/j.cell.2006.02.041
13 Seisenberger, S., Andrews, S., Krueger, F., Arand, J., Walter, J., Santos, F. et al. (2012) The Dynamics of Genome-wide DNA Methylation Reprogramming in Mouse Primordial Germ Cells. *Mol. Cell* **48**, 849–862, https://doi.org/10.1016/j.molcel.2012.11.001
14 Cantone, I. and Fisher, A.G. (2013) Epigenetic programming and reprogramming during development. *Nat. Struct. Mol. Biol.* **20**, 282–289, https://doi.org/10.1038/nsmb.2489
15 Lee, H.J., Hore, T.A. and Reik, W. (2014) Reprogramming the Methylome: Erasing Memory and Creating Diversity. *Cell Stem Cell* **14**, 710–719, https://doi.org/10.1016/j.stem.2014.05.008
16 Xu, Q. and Xie, W. (2018) Epigenome in Early Mammalian Development: Inheritance, Reprogramming and Establishment. *Trends Cell Biol.* **28**, 237–253, https://doi.org/10.1016/j.tcb.2017.10.008
17 Smith, Z.D., Chan, M.M., Mikkelsen, T.S., Gu, H., Gnirke, A., Regev, A. et al. (2012) A unique regulatory phase of DNA methylation in the early mammalian embryo. *Nature* **484**, 339–344, https://doi.org/10.1038/nature10960
18 Borgel, J., Guibert, S., Li, Y., Chiba, H., Schübeler, D., Sasaki, H. et al. (2010) Targets and dynamics of promoter DNA methylation during early mouse development. *Nat. Genet.* **42**, 1093–1100, https://doi.org/10.1038/ng.708
19 Guibert, S., Forné, T. and Weber, M. (2012) Global profiling of DNA methylation erasure in mouse primordial germ cells. *Genome Res.* **22**, 633–641, https://doi.org/10.1101/gr.130997.111
20 Tang, W.W.C., Kobayashi, T., Irie, N., Dietmann, S. and Surani, M.A. (2016) Specification and epigenetic programming of the human germ line. *Nat. Rev. Genet.* **17**, 585–600, https://doi.org/10.1038/nrg.2016.88
21 Bonnet-Garnier, A., Feuerstein, P., Chebrout, M., Fleurot, R., Jan, H.-U., Debey, P. et al. (2013) Genome organization and epigenetic marks in mouse germinal vesicle oocytes. *Int. J. Dev. Biol.* **56**, 877–887, https://doi.org/10.1387/ijdb.120149ab
22 Tucci, V., Isles, A.R., Kelsey, G., Ferguson-Smith, A.C., Tucci, V., Bartolomei, M.S. et al. (2019) Genomic Imprinting and Physiological Processes in Mammals. *Cell* **176**, 952–965, https://doi.org/10.1016/j.cell.2019.01.043
23 Xavier, M.J., Roman, S.D., Aitken, R.J. and Nixon, B. (2019) Transgenerational inheritance: how impacts to the epigenetic and genetic information of parents affect offspring health. *Hum. Reprod. Update* **25**, 518–540, https://doi.org/10.1093/humupd/dmz017
24 Smallwood, S.A., Tomizawa, S., Krueger, F., Ruf, N., Carli, N., Segonds-Pichon, A. et al. (2011) Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. *Nat. Genet.* **43**, 811–814, https://doi.org/10.1038/ng.864
25 Kobayashi, H., Sakurai, T., Imai, M., Takahashi, N., Fukuda, A., Yayoi, O. et al. (2012) Contribution of Intragenic DNA Methylation in Mouse Gametic DNA Methylomes to Establish Oocyte-Specific Heritable Marks. *PLos Genet.* **8**, e1002440, https://doi.org/10.1371/journal.pgen.1002440
26 Veselovska, L., Smallwood, S.A., Saadeh, H., Stewart, K.R., Krueger, F., Maupetit-Méhouas, S. et al. (2015) Deep sequencing and de novo assembly of the mouse oocyte transcriptome define the contribution of transcription to the DNA methylation landscape. *Genome Biol.* **16**, 209, https://doi.org/10.1186/s13059-015-0769-z
27 Lucifero, D., Mann, M.R.W., Bartolomei, M.S. and Trasler, J.M. (2004) Gene-specific timing and epigenetic memory in oocyte imprinting. *Hum. Mol. Genet.* **13**, 839–849, https://doi.org/10.1093/hmg/ddh104
28 Hiura, H., Obata, Y., Komiyama, J., Shirai, M. and Kono, T. (2006) Oocyte growth-dependent progression of maternal imprinting in mice. *Genes Cells* **11**, 353–361, https://doi.org/10.1111/j.1365-2443.2006.00943.x
29 Gahurova, L., Tomizawa, S., Smallwood, S.A., Stewart-Morgan, K.R., Saadeh, H., Kim, J. et al. (2017) Transcription and chromatin determinants of de novo DNA methylation timing in oocytes. *Epigenetics Chromatin* **10**, 25, https://doi.org/10.1186/s13072-017-0133-5
30 Okae, H., Chiba, H., Hiura, H., Hamada, H., Sato, A., Utsunomiya, T. et al. (2014) Genome-Wide Analysis of DNA Methylation Dynamics during Early Human Development. *PLos Genet.* **10**, e1004868, https://doi.org/10.1371/journal.pgen.1004868
31 Brind'Amour, J., Kobayashi, H., Albert, J.R., Shirane, K., Sakashita, A., Kamio, A. et al. (2018) LTR retrotransposons transcribed in oocytes drive species-specific and heritable changes in DNA methylation. *Nat. Commun.* **9**, 3331, https://doi.org/10.1038/s41467-018-05841-x
32 Tomizawa, S., Kobayashi, H., Watanabe, T., Andrews, S., Hata, K., Kelsey, G. et al. (2011) Dynamic stage-specific changes in imprinted differentially methylated regions during early mammalian development and prevalence of non-CpG methylation in oocytes. *Development* **138**, 811–820, https://doi.org/10.1242/dev.061416
33 Shirane, K., Toh, H., Kobayashi, H., Miura, F., Chiba, H., Ito, T. et al. (2013) Mouse Oocyte Methylomes at Base Resolution Reveal Genome-Wide Accumulation of Non-CpG Methylation and Role of DNA Methyltransferases. *PLos Genet.* **9**, e1003439, https://doi.org/10.1371/journal.pgen.1003439
34 Kaneda, M., Okano, M., Hata, K., Sado, T., Tsujimoto, N., Li, E. et al. (2004) Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. *Nature* **429**, 900–903, https://doi.org/10.1038/nature02633

35 Kaneda, M., Hirasawa, R., Chiba, H., Okano, M., Li, E. and Sasaki, H. (2010) Genetic evidence for Dnmt3a-dependent imprinting during oocyte growth obtained by conditional knockout with Zp3-Cre and complete exclusion of Dnmt3b by chimera formation. *Genes Cells* **15**, 169–179, https://doi.org/10.1111/j.1365-2443.2009.01374.x

36 Bourc'his, D., Xu, G.-L., Lin, C.-S., Bollman, B. and Bestor, T.H. (2001) Dnmt3L and the Establishment of Maternal Genomic Imprints. *Science* **294**, 2536–2539, https://doi.org/10.1126/science.1065848

37 Hata, K., Okano, M., Lei, H. and Li, E. (2002) Dnmt3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice. *Development* **129**, 1983–1993

38 Branco, M.R., King, M., Perez-Garcia, V., Bogutz, A.B., Caley, M., Fineberg, E. et al. (2016) Maternal DNA Methylation Regulates Early Trophoblast Development. *Dev. Cell* **36**, 152–163, https://doi.org/10.1016/j.devcel.2015.12.027

39 Chotalia, M., Smallwood, S.A., Ruf, N., Dawson, C., Lucifero, D., Frontera, M. et al. (2009) Transcription is required for establishment of germline methylation marks at imprinted genes. *Genes Dev.* **23**, 105–117, https://doi.org/10.1101/gad.495809

40 Li, X., Ito, M., Zhou, F., Youngson, N., Zuo, X., Leder, P. et al. (2008) A Maternal-Zygotic Effect Gene, Zfp57, Maintains Both Maternal and Paternal Imprints. *Dev Cell.* **15**, 547–557, https://doi.org/10.1016/j.devcel.2008.08.014

41 Quenneville, S., Verde, G., Corsinotti, A., Kapopoulou, A., Jakobsson, J., Offner, S. et al. (2011) In Embryonic Stem Cells, ZFP57/KAP1 Recognize a Methylated Hexanucleotide to Affect Chromatin and DNA Methylation of Imprinting Control Regions. *Mol Cell.* **44**, 361–372, https://doi.org/10.1016/j.molcel.2011.08.032

42 Li, Y. and Li, J. (2019) Technical advances contribute to the study of genomic imprinting. *PLos Genet.* **15**, e1008151, https://doi.org/10.1371/journal.pgen.1008151

43 Proudhon, C., Duffié, R., Ajjan, S., Cowley, M., Iranzo, J., Carbajosa, G. et al. (2012) Protection against De Novo Methylation Is Instrumental in Maintaining Parent-of-Origin Methylation Inherited from the Gametes. *Mol. Cell* **47**, 909–920, https://doi.org/10.1016/j.molcel.2012.07.010

44 Rutledge, C.E., Thakur, A., O'Neill, K.M., Irwin, R.E., Sato, S., Hata, K. et al. (2014) Ontogeny, conservation and functional significance of maternally inherited DNA methylation at two classes of non-imprinted genes. *Development* **141**, 1313–1323, https://doi.org/10.1242/dev.104646

45 Sanchez-Delgado, M., Court, F., Vidal, E., Medrano, J., Monteagudo-Sánchez, A., Martin-Trujillo, A. et al. (2016) Human Oocyte-Derived Methylation Differences Persist in the Placenta Revealing Widespread Transient Imprinting. *PLos Genet.* **12**, e1006427, https://doi.org/10.1371/journal.pgen.1006427

46 Stewart, K.R., Veselovska, L., Kim, J., Huang, J., Saadeh, H., Tomizawa, S. et al. (2015) Dynamic changes in histone modifications precede de novo DNA methylation in oocytes. *Genes Dev.* **29**, 2449–2462, https://doi.org/10.1101/gad.271353.115

47 Hanna, C.W., Taudt, A., Huang, J., Gahurova, L., Kranz, A., Andrews, S. et al. (2018) MLL2 conveys transcription-independent H3K4 trimethylation in oocytes. *Nat. Struct. Mol. Biol.* **25**, 73, https://doi.org/10.1038/s41594-017-0013-5

48 Ancelin, K., Syx, L., Borensztein, M., Ranisavljevic, N., Vassilev, I., Briseño-Roa, L. et al. (2016) Maternal LSD1/KDM1A is an essential regulator of chromatin and transcription landscapes during zygotic genome activation. *eLife.* **5**, e08851, https://doi.org/10.7554/eLife.08851

49 Andreu-Vieyra, C.V., Chen, R., Agno, J.E., Glaser, S., Anastassiadis, K., Stewart, A.F. et al. (2010) MLL2 Is Required in Oocytes for Bulk Histone 3 Lysine 4 Trimethylation and Transcriptional Silencing. *PLoS Biol.* **8**, e1000453, https://doi.org/10.1371/journal.pbio.1000453

50 Bostick, M., Kim, J.K., Estève, P.-O., Clark, A., Pradhan, S. and Jacobsen, S.E. (2007) UHRF1 Plays a Role in Maintaining DNA Methylation in Mammalian Cells. *Science* **317**, 1760–1764, https://doi.org/10.1126/science.1147939

51 Li, Y., Zhang, Z., Chen, J., Liu, W., Lai, W., Liu, B. et al. (2018) Stella safeguards the oocyte methylome by preventing de novo methylation mediated by DNMT1. *Nature* **564**, 136, https://doi.org/10.1038/s41586-018-0751-5

52 Han, L., Ren, C., Zhang, J., Shu, W. and Wang, Q. (2019) Differential roles of Stella in the modulation of DNA methylation during oocyte and zygotic development. *Cell Discov.* **5**, 1–4, https://doi.org/10.1038/s41421-019-0081-2

53 Gu, C., Liu, S., Wu, Q., Zhang, L. and Guo, F. (2019) Integrative single-cell analysis of transcriptome, DNA methylome and chromatin accessibility in mouse oocytes. *Cell Res.* **29**, 110, https://doi.org/10.1038/s41422-018-0125-4

54 Smith, E.Y., Futtner, C.R., Chamberlain, S.J., Johnstone, K.A. and Resnick, J.L. (2011) Transcription Is Required to Establish Maternal Imprinting at the Prader-Willi Syndrome and Angelman Syndrome Locus. *PLos Genet.* **7**, e1002422, https://doi.org/10.1371/journal.pgen.1002422

55 Peaston, A.E., Evsikov, A.V., Graber, J.H., de Vries, W.N., Holbrook, A.E., Solter, D. et al. (2004) Retrotransposons Regulate Host Genes in Mouse Oocytes and Preimplantation Embryos. *Dev. Cell* **7**, 597–606, https://doi.org/10.1016/j.devcel.2004.09.004

56 Franke, V., Ganesh, S., Karlic, R., Malik, R., Pasulka, J., Horvat, F. et al. (2017) Long terminal repeats power evolution of genes and gene expression programs in mammalian oocytes and zygotes. *Genome Res.* **27**, 1384–1394, https://doi.org/10.1101/gr.216150.116

57 Ray-Gallet, D., Quivy, J.-P., Scamps, C., Martini, E.M.-D., Lipinski, M. and Almouzni, G. (2002) HIRA Is Critical for a Nucleosome Assembly Pathway Independent of DNA Synthesis. *Mol. Cell* **9**, 1091–1100, https://doi.org/10.1016/S1097-2765(02)00526-9

58 Nashun, B., Hill, P.W.S., Smallwood, S.A., Dharmalingam, G., Amouroux, R., Clark, S.J. et al. (2015) Continuous Histone Replacement by Hira Is Essential for Normal Transcriptional Regulation and De Novo DNA Methylation during Mouse Oogenesis. *Mol. Cell* **60**, 611–625, https://doi.org/10.1016/j.molcel.2015.10.010

59 Hara, S., Takano, T., Fujikawa, T., Yamada, M., Wakai, T., Kono, T. et al. (2014) Forced expression of DNA methyltransferases during oocyte growth accelerates the establishment of methylation imprints but not functional genomic imprinting. *Hum. Mol. Genet.* **23**, 3853–3864, https://doi.org/10.1093/hmg/ddu100

60 Ciccone, D.N., Su, H., Hevi, S., Gay, F., Lei, H., Bajko, J. et al. (2009) KDM1B is a histone H3K4 demethylase required to establish maternal genomic imprints. *Nature* **461**, 415–418, https://doi.org/10.1038/nature08315

61 Wasson, J.A., Simon, A.K., Myrick, D.A., Wolf, G., Driscoll, S., Pfaff, S.L. et al. (2016) Maternally provided LSD1/KDM1A enables the maternal-to-zygotic transition and prevents defects that manifest postnatally. *eLife.* **5**, e08848, https://doi.org/10.7554/eLife.08848

62 Ma, P., Pan, H., Montgomery, R.L., Olson, E.N. and Schultz, R.M. (2012) Compensatory functions of histone deacetylase 1 (HDAC1) and HDAC2 regulate transcription and apoptosis during mouse oocyte development. *Proc. Natl Acad. Sci.* **109**, E481–9, https://doi.org/10.1073/pnas.1118403109
63 Ma, P., de Waal, E., Weaver, J.R., Bartolomei, M.S. and Schultz, R.M. (2015) A DNMT3A2-HDAC2 Complex Is Essential for Genomic Imprinting and Genome Integrity in Mouse Oocytes. *Cell Rep.* **13**, 1552–1560, https://doi.org/10.1016/j.celrep.2015.10.031
64 Hermann, A., Goyal, R. and Jeltsch, A. (2004) The Dnmt1 DNA-(cytosine-C5)-methyltransferase Methylates DNA Processively with High Preference for Hemimethylated Target Sites. *J. Biol. Chem.* **279**, 48350–48359, https://doi.org/10.1074/jbc.M403427200
65 Sharif, J., Muto, M., Takebayashi, S., Suetake, I., Iwamatsu, A., Endo, T.A. et al. (2007) The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* **450**, 908–912, https://doi.org/10.1038/nature06397
66 Li, E., Bestor, T.H. and Jaenisch, R. (1992) Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* **69**, 915–926, https://doi.org/10.1016/0092-8674(92)90611-F
67 Okano, M., Bell, D.W., Haber, D.A. and Li, E. (1999) DNA Methyltransferases Dnmt3a and Dnmt3b Are Essential for De Novo Methylation and Mammalian Development. *Cell* **99**, 247–257, https://doi.org/10.1016/S0092-8674(00)81656-6
68 Barau, J., Teissandier, A., Zamudio, N., Roy, S., Nalesso, V., Hérault, Y. et al. (2016) The DNA methyltransferase DNMT3C protects male germ cells from transposon activity. *Science* **354**, 909–912, https://doi.org/10.1126/science.aah5143
69 Aapola, U., Kawasaki, K., Scott, H.S., Ollila, J., Vihinen, M., Heino, M. et al. (2000) Isolation and initial characterization of a novel zinc finger gene, DNMT3L, on 21q22.3, related to the cytosine-5-methyltransferase 3 gene family. *Genomics* **65**, 293–298, https://doi.org/10.1006/geno.2000.6168
70 Yokomine, T., Hata, K., Tsudzuki, M. and Sasaki, H. (2006) Evolution of the vertebrate DNMT3 gene family: a possible link between existence of DNMT3L and genomic imprinting. *Cytogenet. Genome Res.* **113**, 75–80, https://doi.org/10.1159/000090817
71 Chen, Z.-X., Mann, J.R., Hsieh, C.-L., Riggs, A.D. and Chédin, F. (2005) Physical and functional interactions between the human DNMT3L protein and members of the de novo methyltransferase family. *J. Cell. Biochem.* **95**, 902–917, https://doi.org/10.1002/jcb.20447
72 Jia, D., Jurkowska, R.Z., Zhang, X., Jeltsch, A. and Cheng, X. (2007) Structure of Dnmt3a bound to Dnmt3L suggests a model for de novo DNA methylation. *Nature* **449**, 248–251, https://doi.org/10.1038/nature06146
73 Uysal, F., Ozturk, S. and Akkoyunlu, G. (2017) DNMT1, DNMT3A and DNMT3B proteins are differently expressed in mouse oocytes and early embryos. *J. Mol. Histol.* **48**, 417–426, https://doi.org/10.1007/s10735-017-9739-y
74 Lucifero, D., La Salle, S., Bourc'his, D., Martel, J., Bestor, T.H. and Trasler, J.M. (2007) Coordinate regulation of DNA methyltransferase expression during oogenesis. *BMC Dev. Biol.* **7**, 36, https://doi.org/10.1186/1471-213X-7-36
75 Bourc'his, D. and Bestor, T.H. (2004) Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L. *Nature* **431**, 96–99, https://doi.org/10.1038/nature02886
76 Suetake, I., Shinozaki, F., Miyagawa, J., Takeshima, H. and Tajima, S. (2004) DNMT3L Stimulates the DNA Methylation Activity of Dnmt3a and Dnmt3b through a Direct Interaction. *J. Biol. Chem.* **279**, 27816–27823, https://doi.org/10.1074/jbc.M400181200
77 Mertineit, C., Yoder, J.A., Taketo, T., Laird, D.W., Trasler, J.M. and Bestor, T.H. (1998) Sex-specific exons control DNA methyltransferase in mammalian germ cells. *Development* **125**, 889–897
78 Howell, C.Y., Bestor, T.H., Ding, F., Latham, K.E., Mertineit, C., Trasler, J.M. et al. (2001) Genomic Imprinting Disrupted by a Maternal Effect Mutation in the Dnmt1 Gene. *Cell* **104**, 829–838, https://doi.org/10.1016/S0092-8674(01)00280-X
79 Hirasawa, R., Chiba, H., Kaneda, M., Tajima, S., Li, E., Jaenisch, R. et al. (2008) Maternal and zygotic Dnmt1 are necessary and sufficient for the maintenance of DNA methylation imprints during preimplantation development. *Genes Dev.* **22**, 1607–1616, https://doi.org/10.1101/gad.1667008
80 Wienholz, B.L., Kareta, M.S., Moarefi, A.H., Gordon, C.A., Ginno, P.A. and Chédin, F. (2010) DNMT3L Modulates Significant and Distinct Flanking Sequence Preference for DNA Methylation by DNMT3A and DNMT3B In Vivo. *PLos Genet.* **6**, e1001106, https://doi.org/10.1371/journal.pgen.1001106
81 Ooi, S.K.T., Qiu, C., Bernstein, E., Li, K., Jia, D., Yang, Z. et al. (2007) DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature* **448**, 714–717, https://doi.org/10.1038/nature05987
82 Otani, J., Nankumo, T., Arita, K., Inamoto, S., Ariyoshi, M. and Shirakawa, M. (2009) Structural basis for recognition of H3K4 methylation status by the DNA methyltransferase 3A ATRX-DNMT3-DNMT3L domain. *EMBO Rep.* **10**, 1235–1241, https://doi.org/10.1038/embor.2009.218
83 Zhang, Y., Jurkowska, R., Soeroes, S., Rajavelu, A., Dhayalan, A., Bock, I. et al. (2010) Chromatin methylation activity of Dnmt3a and Dnmt3a/3L is guided by interaction of the ADD domain with the histone H3 tail. *Nucleic. Acids. Res.* **38**, 4246–4253, https://doi.org/10.1093/nar/gkq147
84 Li, B.-Z., Huang, Z., Cui, Q.-Y., Song, X.-H., Du, L., Jeltsch, A. et al. (2011) Histone tails regulate DNA methylation by allosterically activating de novo methyltransferase. *Cell Res.* **21**, 1172–1181, https://doi.org/10.1038/cr.2011.92
85 Guo, X., Wang, L., Li, J., Ding, Z., Xiao, J., Yin, X. et al. (2015) Structural insight into autoinhibition and histone H3-induced activation of DNMT3A. *Nature* **517**, 640–644, https://doi.org/10.1038/nature13899
86 Noh, K.-M., Wang, H., Kim, H.R., Wenderski, W., Fang, F., Li, C.H. et al. (2015) Engineering of a Histone-Recognition Domain in Dnmt3a Alters the Epigenetic Landscape and Phenotypic Features of Mouse ESCs. *Mol. Cell* **59**, 89–103, https://doi.org/10.1016/j.molcel.2015.05.017
87 Dahl, J.A., Jung, I., Aanes, H., Greggains, G.D., Manaf, A., Lerdrup, M. et al. (2016) Broad histone H3K4me3 domains in mouse oocytes modulate maternal-to-zygotic transition. *Nature* **537**, 548–552, https://doi.org/10.1038/nature19360
88 Zhang, B., Zheng, H., Huang, B., Li, W., Xiang, Y., Peng, X. et al. (2016) Allelic reprogramming of the histone modification H3K4me3 in early mammalian development. *Nature* **537**, 553–557, https://doi.org/10.1038/nature19361
89 Yu, C., Fan, X., Sha, Q.-Q., Wang, H.-H., Li, B.-T., Dai, X.-X. et al. (2017) CFP1 Regulates Histone H3K4 Trimethylation and Developmental Potential in Mouse Oocytes. *Cell Rep.* **20**, 1161–1172, https://doi.org/10.1016/j.celrep.2017.07.011
90 Qin, S. and Min, J. (2014) Structure and function of the nucleosome-binding PWWP domain. *Trends Biochem. Sci.* **39**, 536–547, https://doi.org/10.1016/j.tibs.2014.09.001

91 Ge, Y.-Z., Pu, M.-T., Gowher, H., Wu, H.-P., Ding, J.-P., Jeltsch, A. et al. (2004) Chromatin Targeting of de Novo DNA Methyltransferases by the PWWP Domain. *J. Biol. Chem.* **279**, 25447–25454, https://doi.org/10.1074/jbc.M312296200

92 Chen, T., Tsujimoto, N. and Li, E. (2004) The PWWP Domain of Dnmt3a and Dnmt3b Is Required for Directing DNA Methylation to the Major Satellite Repeats at Pericentric Heterochromatin. *Mol. Cell. Biol.* **24**, 9048–9058, https://doi.org/10.1128/MCB.24.20.9048-9058.2004

93 Wu, H., Zeng, H., Lam, R., Tempel, W., Amaya, M.F., Xu, C. et al. (2011) Structural and Histone Binding Ability Characterizations of Human PWWP Domains. *PLoS One* **6**, e18919, https://doi.org/10.1371/journal.pone.0018919

94 Rondelet, G., Dal Maso, T., Willems, L. and Wouters, J. (2016) Structural basis for recognition of histone H3K36me3 nucleosome by human de novo DNA methyltransferases 3A and 3B. *J. Struct. Biol.* **194**, 357–367, https://doi.org/10.1016/j.jsb.2016.03.013

95 Dhayalan, A., Rajavelu, A., Rathert, P., Tamas, R., Jurkowska, R.Z., Ragozin, S. et al. (2010) The Dnmt3a PWWP Domain Reads Histone 3 Lysine 36 Trimethylation and Guides DNA Methylation. *J. Biol. Chem.* **285**, 26114–26120, https://doi.org/10.1074/jbc.M109.089433

96 Bock, I., Kudithipudi, S., Tamas, R., Kungulovski, G., Dhayalan, A. and Jeltsch, A. (2011) Application of Celluspots peptide arrays for the analysis of the binding specificity of epigenetic reading domains to modified histone tails. *BMC Biochem.* **12**, 48, https://doi.org/10.1186/1471-2091-12-48

97 Mauser, R., Kungulovski, G., Keup, C., Reinhardt, R. and Jeltsch, A. (2017) Application of dual reading domains as novel reagents in chromatin biology reveals a new H3K9me3 and H3K36me2/3 bivalent chromatin state. *Epigenetics Chromatin* **10**, 45, https://doi.org/10.1186/s13072-017-0153-1

98 Weinberg, D.N., Papillon-Cavanagh, S., Chen, H., Yue, Y., Chen, X., Rajagopalan, K.N. et al. (2019) The histone mark H3K36me2 recruits DNMT3A and shapes the intergenic DNA methylation landscape. *Nature* **573**, 281–286, https://doi.org/10.1038/s41586-019-1534-3

99 Baubec, T., Colombo, D.F., Wirbelauer, C., Schmidt, J., Burger, L., Krebs, A.R. et al. (2015) Genomic profiling of DNA methyltransferases reveals a role for DNMT3B in genic methylation. *Nature* **520**, 243–247, https://doi.org/10.1038/nature14176

100 Morselli, M., Pastor, W.A., Montanini, B., Nee, K., Ferrari, R., Fu, K. et al. (2015) In vivo targeting of de novo DNA methylation by histone modifications in yeast and mouse. *eLife.* **4**, e06205, https://doi.org/10.7554/eLife.06205

101 Sendžikaitė, G., Hanna, C.W., Stewart-Morgan, K.R., Ivanova, E. and Kelsey, G. (2019) A DNMT3A PWWP mutation leads to methylation of bivalent chromatin and growth retardation in mice. *Nat. Commun.* **10**, 1884, https://doi.org/10.1038/s41467-019-09713-w

102 Xu, Q., Xiang, Y., Wang, Q., Wang, L., Brind'Amour, J., Bogutz, A.B. et al. (2019) SETD2 regulates the maternal epigenome, genomic imprinting and embryonic development. *Nat. Genet.* **51**, 844–856, https://doi.org/10.1038/s41588-019-0398-7

103 Sun, X.-J., Wei, J., Wu, X.-Y., Hu, M., Wang, L., Wang, H.-H. et al. (2005) Identification and Characterization of a Novel Human Histone H3 Lysine 36-specific Methyltransferase. *J. Biol. Chem.* **280**, 35261–35271, https://doi.org/10.1074/jbc.M504012200

104 Hu, M., Sun, X.-J., Zhang, Y.-L., Kuang, Y., Hu, C.-Q., Wu, W.-L. et al. (2010) Histone H3 lysine 36 methyltransferase Hypb/Setd2 is required for embryonic vascular remodeling. *Proc. Natl Acad. Sci.* **107**, 2956–2961, https://doi.org/10.1073/pnas.0915033107

105 Manzo, M., Wirz, J., Ambrosi, C., Villaseñor, R., Roschitzki, B. and Baubec, T. (2017) Isoform-specific localization of DNMT3A regulates DNA methylation fidelity at bivalent CpG islands. *EMBO J.* **36**, 3421–3434, https://doi.org/10.15252/embj.201797038

106 Brinkman, A.B., Gu, H., Bartels, S.J.J., Zhang, Y., Matarese, F., Simmer, F. et al. (2012) Sequential ChIP-bisulfite sequencing enables direct genome-scale investigation of chromatin and DNA methylation cross-talk. *Genome Res.* **22**, 1128–1138, https://doi.org/10.1101/gr.133728.111

107 Zheng, H., Huang, B., Zhang, B., Xiang, Y., Du, Z., Xu, Q. et al. (2016) Resetting Epigenetic Memory by Reprogramming of Histone Modifications in Mammals. *Mol. Cell* **63**, 1066–1079, https://doi.org/10.1016/j.molcel.2016.08.032

108 Chen, T., Ueda, Y., Xie, S. and Li, E. (2002) A Novel Dnmt3a Isoform Produced from an Alternative Promoter Localizes to Euchromatin and Its Expression Correlates with Activede Novo Methylation. *J. Biol. Chem.* **277**, 38746–38754, https://doi.org/10.1074/jbc.M205312200

109 Maenohara, S., Unoki, M., Toh, H., Ohishi, H., Sharif, J., Koseki, H. et al. (2017) Role of UHRF1 in de novo DNA methylation in oocytes and maintenance methylation in preimplantation embryos. *PLos Genet.* **13**, e1007042, https://doi.org/10.1371/journal.pgen.1007042

110 Funaki, S., Nakamura, T., Nakatani, T., Umehara, H., Nakashima, H. and Nakano, T. (2014) Inhibition of maintenance DNA methylation by Stella. *Biochem. Biophys. Res. Commun.* **453**, 455–460, https://doi.org/10.1016/j.bbrc.2014.09.101

111 Sato, M., Kimura, T., Kurokawa, K., Fujita, Y., Abe, K., Masuhara, M. et al. (2002) Identification of PGC7, a new gene expressed specifically in preimplantation embryos and germ cells. *Mech. Dev.* **113**, 91–94, https://doi.org/10.1016/S0925-4773(02)00002-3

112 Nakamura, T., Arai, Y., Umehara, H., Masuhara, M., Kimura, T., Taniguchi, H. et al. (2007) PGC7/Stella protects against DNA demethylation in early embryogenesis. *Nat. Cell Biol.* **9**, 64–71, https://doi.org/10.1038/ncb1519

113 Tachibana, M., Matsumura, Y., Fukuda, M., Kimura, H. and Shinkai, Y. (2008) G9a/GLP complexes independently mediate H3K9 and DNA methylation to silence transcription. *EMBO J.* **27**, 2681–2690, https://doi.org/10.1038/emboj.2008.192

114 Wen, B., Wu, H., Shinkai, Y., Irizarry, R.A. and Feinberg, A.P. (2009) Large histone H3 lysine 9 dimethylated chromatin blocks distinguish differentiated from embryonic stem cells. *Nat. Genet.* **41**, 246–250, https://doi.org/10.1038/ng.297

115 Epsztejn-Litman, S., Feldman, N., Abu-Remaileh, M., Shufaro, Y., Gerson, A., Ueda, J. et al. (2008) De novo DNA methylation promoted by G9a prevents reprogramming of embryonically silenced genes. *Nat. Struct. Mol. Biol.* **15**, 1176–1183, https://doi.org/10.1038/nsmb.1476

116 Dong, K.B., Maksakova, I.A., Mohn, F., Leung, D., Appanah, R., Lee, S. et al. (2008) DNA methylation in ES cells requires the lysine methyltransferase G9a but not its catalytic activity. *EMBO J.* **27**, 2691–2701, https://doi.org/10.1038/emboj.2008.193

117 Leung, D.C., Dong, K.B., Maksakova, I.A., Goyal, P., Appanah, R., Lee, S. et al. (2011) Lysine methyltransferase G9a is required for de novo DNA methylation and the establishment, but not the maintenance, of proviral silencing. *Proc. Natl Acad. Sci.* **108**, 5718–5723, https://doi.org/10.1073/pnas.1014660108

118 Kageyama, S., Liu, H., Kaneko, N., Ooga, M., Nagata, M. and Aoki, F. (2007) Alterations in epigenetic modifications during oocyte growth in mice. *Reproduction* **133**, 85–94, https://doi.org/10.1530/REP-06-0025

119 Au Yeung, W.K., Brind'Amour, J., Hatano, Y., Yamagata, K., Feil, R., Lorincz, M.C. et al. (2019) Histone H3K9 Methyltransferase G9a in Oocytes Is Essential for Preimplantation Development but Dispensable for CG Methylation Protection. *Cell Rep.* **27**, 282.e4–293.e4, https://doi.org/10.1016/j.celrep.2019.03.002

120 Xu, K., Chen, X., Yang, H., Xu, Y., He, Y., Wang, C. et al. (2017) Maternal Sall4 Is Indispensable for Epigenetic Maturation of Mouse Oocytes. *J. Biol. Chem.* **292**, 1798–1807, https://doi.org/10.1074/jbc.M116.767061
121 Hanna, C.W., Demond, H. and Kelsey, G. (2018) Epigenetic regulation in development: is the mouse a good model for the human? *Hum. Reprod. Update.* **24**, 556–576, https://doi.org/10.1093/humupd/dmy021
122 Guo, H., Zhu, P., Yan, L., Li, R., Hu, B., Lian, Y. et al. (2014) The DNA methylation landscape of human early embryos. *Nature* **511**, 606–610, https://doi.org/10.1038/nature13544
123 Smith, Z.D., Chan, M.M., Humm, K.C., Karnik, R., Mekhoubad, S., Regev, A. et al. (2014) DNA methylation dynamics of the human preimplantation embryo. *Nature* **511**, 611–615, https://doi.org/10.1038/nature13581
124 Xia, W., Xu, J., Yu, G., Yao, G., Xu, K., Ma, X. et al. (2019) Resetting histone modifications during human parental-to-zygotic transition. *Science* **365**, 353–360, https://doi.org/10.1126/science.aaw5118
125 Huntriss, J., Hinkins, M., Oliver, B., Harris, S.E., Beazley, J.C., Rutherford, A.J. et al. (2004) Expression of mRNAs for DNA methyltransferases and methyl-CpG-binding proteins in the human female germ line, preimplantation embryos, and embryonic stem cells. *Mol. Reprod. Dev.* **67**, 323–336, https://doi.org/10.1002/mrd.20030
126 Nabeshima, R., Nishimura, O., Maeda, T., Shimizu, N., Ide, T., Yashiro, K. et al. (2018) Loss of Fam60a, a Sin3a subunit, results in embryonic lethality and is associated with aberrant methylation at a subset of gene promoters. Buckingham M, Bronner M, editors. *eLife* **7**, e36435, https://doi.org/10.7554/eLife.36435
127 Jimenez, R., Melo, E.O., Davydenko, O., Ma, J., Mainigi, M., Franke, V. et al. (2015) Maternal SIN3A Regulates Reprogramming of Gene Expression During Mouse Preimplantation Development. *Biol. Reprod.* **93**, 89, https://doi.org/10.1095/biolreprod.115.133504
128 Zylicz, J.J., Borensztein, M., Wong, F.C., Huang, Y., Lee, C., Dietmann, S. et al. (2018) G9a regulates temporal preimplantation developmental program and lineage segregation in blastocyst. Akhtar A, editor. *eLife* **7**, e33361, https://doi.org/10.7554/eLife.33361