REVIEW

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The mammalian genome experiences profound setting and resetting of epigenetic patterns during the life-course. This is understood best for DNA methylation: the specification of germ cells, gametogenesis, and early embryo development are characterised by phases of widespread erasure and rewriting of methylation. While mitigating against intergenerational transmission of epigenetic information, these processes must also ensure correct genomic imprinting that depends on faithful and long-term memory of gamete-derived methylation states in the next generation. This underscores the importance of understanding the mechanisms of methylation programming in the germline. *De novo* methylation in the oocyte is of particular interest because of its intimate association with transcription, which results in a bimodal methylome unique amongst mammalian cells. Moreover, this methylation landscape is entirely set up in a non-dividing cell, making the oocyte a fascinating model system in which to explore mechanistic determinants of methylation. Here, we summarise current knowledge on the oocyte DNA methylome and how it is established, focussing on recent insights from knockout models in the mouse that explore the interplay between methylation and chromatin states. We also highlight some remaining paradoxes and enigmas, in particular the involvement of non-nuclear factors for correct *de novo* methylation.

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DNA methylation, oocyte, chromatin

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Gavin Kelsey ( )

Mammalian oocytes have a distinctive epigenome

DNA methylation is considered a repressive epigenetic modification. In most mammalian somatic cells, it occurs across

is a process in which parent-of-origin allele-specific DNA methylation results in monoallelic expression of imprinted genes in offspring tissues[12]. Most imprinted genes in the mouse and human have their imprinting control regions (ICRs or germline differentially methylated regions [DMRs]) methylated in oocytes. These ICRs are CpG islands that are intragenic in oocytes and become methylated during oocyte growth[4]. Failure to establish methylation at ICRs in the oocyte results in severe developmental abnormalities[13], showing the importance of correct epigenetic programming in the oocyte for the next generation.

It might seem extravagant to methylate a large fraction of the genome to ensure methylation of a couple of dozen imprinted loci. However, *Dnmt3L*- and *Dnmt3a*-null oocytes, which lack most DNA methylation, are ostensibly normal, can be fertilised, and will support development of the embryo until beyond implantation, when imprinting defects cause developmental arrest[13,14]). But it is possible that methylation in the oocyte has significance beyond classical imprinted genes. Embryos developing from DNA methylation-deficient oocytes succumb to problems in trophoblast development, hence placental defects, for which a contribution of genes outside known imprinted genes has been implicated[15]. Thus, it seems likely that there are more extensive effects of oocyte methylation than classical imprinted genes.

D methylation requires active transcription

Consistent with the predominant gene-body pattern of methylation, *de novo* DNA methylation in the oocyte has been

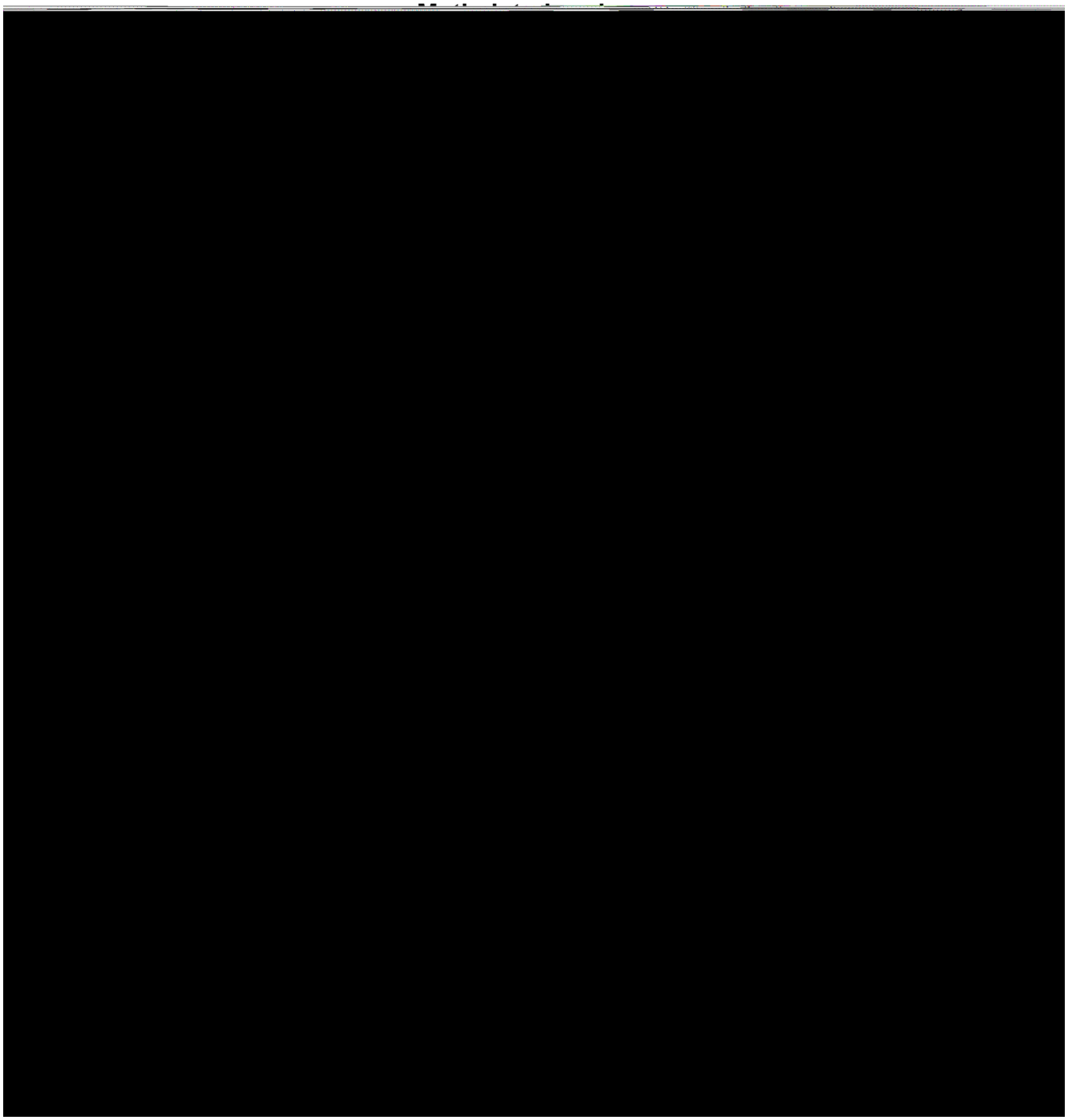

Figure 2. Models showing chromatin factors involved in DNA methyltransferase (DNMT) 3A/DNMT3L recruitment in methylated regions and factors inhibiting DNMT3A/DNMT3L binding at unmethylated regions. A) At actively transcribed gene bodies, SETD2-mediated H3K36me3 has been proposed to recruit DNMT3A/DNMT3L, whilst lysine demethylase 1B (KDM1B) seems to be required to prevent or remove histone 3 lysine 4 (H3K4) methylation. DNMT1 is needed for methylation of hemimethylated DNA. **B**) Ubiquitin-like, plant homeodomain and ring finger-containing 1 (UHRF1) is required for intermediate DNA methylation of some genic and intergenic regions, likely by recruiting one of the DNMT proteins, but the chromatin requirements are unknown. **C**) At active promoters, H3K4me3 is thought to prevent DNMT3A/DNMT3L binding. **D**) Transcriptionally inactive regions can be marked by mixed lineage leukaemia-2 protein (MLL2)-mediated H3K4me3 and/or polycomb repressive complex 2 (PRC2)-mediated H3K27me3, preventing recruitment of DNMT3A/DNMT3L to these regions. EED, embryonic ectoderm development; EZHIP, EZH inhibitory protein; SETD2, SET domain containing 2.

Single-locus analysis indicated that some imprinted gDMRs failed to become methylated in *Kdm1b*-null oocytes[37]. Subsequent genome-wide interrogation of methylation showed that most imprinted ICRs exhibit reduced methylation in *Kdm1b* knockout oocytes, as do many CpG islands that normally become methylated in oocytes[36] (Figure 2A). Why some imprinted ICRs are more sensitive to the loss of KDM1B is not fully known, but there is some evidence that ICRs and CpG islands acquiring methylation later in oocyte growth are more susceptible[11,

left by DNMT3A[5], i.e. to ensure symmetric methylation of CpG sites, thus demonstrating a role for DNMT1 outside of DNA replication (Figure 2A).

DNMT3 paradoxes

The model above indicates a role for H3K36me3 in recruiting DNMT3A to active gene bodies, which was predicted to be mediated via the PWWP domain of DNMT3A[27]. So far, this prediction has not been confirmed. Genomic studies in mouse embryonic stem cells (ESCs) have not identified

rationalised by a biochemical mechanism. The availability of quantitative and sensitive methylation profiling methods, including at the single-cell level

assembly of the mouse oocyte transcriptome define the contribution of transcription to the DNA methylation landscape. *Ge ▼me Bi●l.* 2015, **16**. 20 .
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