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Early Embryos Reprogram DNA Methylation in Two Steps

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While DNA cytosine methylation is relatively stable in somatic tissues, it is highly dynamic during preimplantation development. A recent study in *Nature* by Meissner and colleagues (Smith et al., 2012) now reveals dramatic shifts in DNA methylation during the earliest stages of mouse embryogenesis at genome scale and base resolution.

DNA methylation at the 5-position of cytosine (5mC) in mammals largely occurs at CpG dinucleotides and is required for normal embryogenesis. A global DNA methylation landscape is first established by de novo DNA methyltransferases Dnmt3a and Dnmt3b in the inner cell mass (ICM) of blastocysts and is stably inherited in somatic tissues through the action of maintenance methylation machineries. DNA methylation in somatic cells generally displays a bimodal distribution, in which the majority of CpG sites are methylated and unmethylated CpGs are primarily found in clusters termed CpG islands (CGIs) that are frequently associated with gene promoters (Deaton and Bird, 2011). It is broadly accepted that DNA methylation at gene promoters and other regulatory sequences such as enhancers inversely correlates with transcription and may facilitate lineage restriction during development.

While the methylation pattern is stably maintained in somatic tissues, mammalian preimplantation development is accompanied by a wave of genome-wide demethylation and remethylation. Early studies using immunofluorescence staining and locus-specific bisulphite sequencing have indicated that DNA methylation in the paternal genome is rapidly removed via a replication-independent process a few hours after fertilization, while the maternal DNA methylation level is gradually reduced in a replication-dependent manner with the lowest level occurring at the blastocyst stage (Mayer et al., 2000). Recent studies have revealed that loss of 5mC in the paternal genome in the zygote is primarily initiated by Tet3 (Gu et al., 2011; Wossidlo et al., 2011), a member of the Ten-eleven translocation (Tet) family of DNA dioxygenases capable of converting 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) through iterative oxidation (Wu and Zhang, 2011). High-resolution metaphase chromosome immunostaining of various stages of preimplantation embryos using antibodies specific for 5mC, 5hmC, 5fC, and 5caC suggested that the bulk 5mC in the paternal genome is first oxidized to 5hmC/5fC/5caC in zygotes, followed by replication-dependent dilution of 5hmC/5fC/5caC during preimplantation development (Inoue et al., 2011; Inoue and Zhang, 2011) (Figure 1A). Although these studies have revealed a global picture of how 5mC is lost during preimplantation development, it lacks the resolution for us to tell exactly which part of the genome is subject to or protected from this wave of DNA demethylation. Bisulfite sequencing has been the method of choice to generate single-nucleotide resolution maps of DNA methylation, and genome-wide bisulphite sequencing of somatic and cancer genomes continues to shed light on genomic distribution and regulatory function of DNA methylation in tissue-specific gene expression and tumorigenesis. However, similar analysis for preimplantation embryos has been difficult due to limitations on the number of embryos available.

In a recent issue of *Nature*, Meissner and colleagues have successfully overcome this technical hurdle by using the reduced representation bisulphite sequencing (RRBS) technique and have generated base-resolution and genome-scale maps

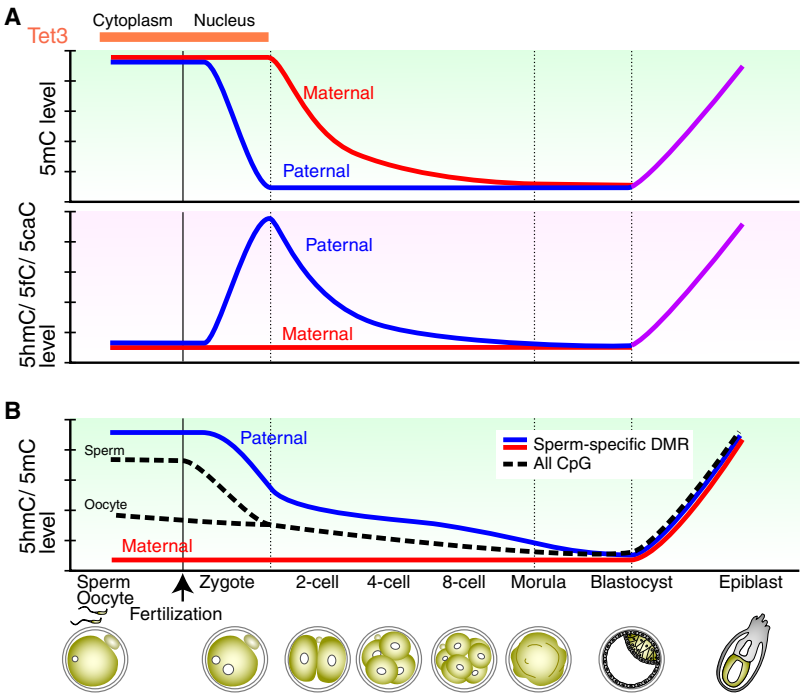

Figure 1. DNA Methylation Dynamics during Mouse Preimplantation Development
(A) Methylation dynamics revealed by immunostaining. Tet3 is expressed and localized to paternal pronucleus in zygote to mediate oxidation of 5mC to 5hmC/5fC/5caC. The 5mC oxidation derivatives undergo replication-dependent dilution during preimplantation development, while the 5mC in the maternal genome follows the same dilution process without going through the oxidation process.
(B) Methylation dynamics revealed by RRBS. Shown is the 5mC level of 74 CpG sites within sperm-specific DMRs (blue and red) as well as the average of all CpGs (broken lines). (Figure credit: Shinpei Yamaguchi.)

of DNA methylation in the sperm, oocyte, zygote, 2-, 4-, and 8-cell cleavage stage embryos, as well as in the early ICM and postimplantation embryos (Smith et al., 2012). The RRBS method is well-suited for analyzing DNA methylation patterns in preimplantation embryos from which only a limited amount of DNA can be extracted. Using the methylation-insensitive restriction enzyme MspI (C^CGG) to enrich for CpG-containing genomic fragments, the RRBS method can cover approximately 1 million CpG dinucleotides (~5% of all CpGs), with about half located within promoters/CGIs and the rest distributed in relatively CpG-poor sequences or various classes of repetitive elements (Meissner et al., 2008). The authors could thus quantitatively compare methylation profiles within these gene regulatory or repetitive sequences at each developmental stage. Notably, while the sperm methylation profile is more similar to somatic methylation patterns in postimplantation embryos and adult tissues, oocytes exhibit a relatively hypomethylated profile that more closely resembles that of early embryonic stages and preimplantation ICM. The strong inverse correlation between CpG density and methylation levels that are observed in the sperm and somatic cells is also not as apparent in the oocyte and preimplantation embryos.

The unique methylation profiles of gametes and preimplantation embryos prompted the authors to search for dynamic changes in DNA methylation between consecutive developmental stages. This analysis revealed two phases of dramatic changes in methylation levels: a marked decrease in methylation levels from sperm to zygote and a massive remethylation from the early ICM to postimplantation embryos. However, because bisulphite sequencing cannot distinguish between 5hmC and 5mC or between 5caC and unmethylated cytosine (Wu and Zhang, 2011), the demethylation events in the paternal genome detected by RRBS may represent the conversion of 5mC/5hmC into 5caC (Inoue et al., 2011; Inoue and Zhang, 2011). Moreover, demethylation in the bulk zygotic genome appears to be primarily dependent on replication-dependent passive dilution of 5mC/5hmC, which predicts an ~50% decrease in 5mC/5hmC signals during each cell division. The lack of dramatic changes in methylation levels between zygote and preimplantation cleavage stages (2-, 4-, and 8-cell stages) suggests that a substantial fraction of genomic regions covered by RRBS are targeted by Tet3-mediated oxidation of 5mC/5hmC to produce 5caC and/or by base-excision repair mechanisms to actively regenerate unmethylated cytosine (passive dilution/enzymatic conversion of 5caC to C is undetectable by RRBS) before the first cell division commences. Building on these results, the authors determined which genomic features exhibit the most dramatic methylation changes during the sperm to zygote transition. Interestingly, repetitive sequences including specific families of long interspersed elements (LINEs, e.g., L1 elements) and long terminal repeat (LTR) retroelements showed marked loss of methylation after fertilization, whereas other repeat elements such as intracisternal A-particles (IAPs) retain their high methylation levels during preimplantation development.

Finally, the authors identified differentially methylated regions (DMRs) contributed by each gamete and examined methylation dynamics of these parent-of-origin-specific DMRs during early embryogenesis. This analysis revealed 4,894 sperm-contributed and 376 oocyte-contributed DMRs. Notably, oocyte-contributed DMRs are primarily located in CpG-rich promoters, whereas sperm-contributed DMRs are predominantly found in CpG-poor intergenic regions. Classical imprinting control regions (ICRs) maintain their allele-specific methylation through adulthood. Interestingly, in agreement with a recent study (Smallwood et al., 2011), a large number of oocyte-contributed CGI-containing DMRs retain intermediate methylation levels in preimplantation embryos before gradually becoming unmethylated through ICM specification to acquire a somatic methylation pattern of CGIs. Using known single nucleotide polymorphisms (SNPs) in hybrid strains, the authors tracked 74 CpG sites within sperm-contributed DMRs

during early development and confirmed that paternal genome-derived DMRs undergo demethylation in the zygote and preimplantation embryos before they are remethylated in postimplantation embryos (Figure 1B).

The results by Meissner and colleagues provide important insights into how global mammalian DNA methylation patterns are dynamically regulated in the zygote and preimplantation embryos. Notably, all previous immunostaining-based studies of the one-cell zygote revealed comparable 5mC levels between maternal and paternal pronuclei before paternal DNA demethylation commences, but the RRBS-based method suggests that the maternal genome is broadly hypomethylated before fertilization (Figure 1; compare 1A and 1B). This discrepancy is probably due to the fact that RRBS analysis only covers a small fraction of all CpG dinucleotides (~5% of all CpGs) with a strong bias toward CpG-rich regions that are usually unmethylated (Meissner et al., 2008). It remains to be determined quantitatively whether bulk maternal genome that is not currently covered by RRBS also has little 5mC at fertilization. Moreover, the binary information encoded by bisulphite sequencing data cannot fully reveal the exact modification status of cytosine when 5mC is further oxidized by Tet proteins (5mC/5hmC as methylated, and 5caC/C as unmethylated). Thus, new technologies such as single-molecule real time (SMRT) sequencing are needed to generate high-resolution maps of 5mC and its oxidation derivatives in the zygote and preimplantation embryos, where 5hmC/5fC/5caC is highly enriched and dynamically regulated (Inoue et al., 2011; Inoue and Zhang, 2011). Elucidation of methylome dynamics of preimplantation embryos should provide new and exciting information about how epigenetic mechanisms contribute to the formation of pluripotency and perhaps suggest novel means to improve somatic cell reprogramming.

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