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Review Article

# Epigenetic Re-Programming during Mammalian Preimplantation Embryogenesis and PGC Development

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## Abstract

Epigenetic programming and reprogramming, by means of DNA methylation and histone modifications etc., control the mammalian development to a large extent. They are also artificially altered for cell fate conversion and regeneration. Though epigenetic modifications change with slow dynamics during somatic cell lineage differentiation, they undergo a genome-wide dramatic change with extensive DNA de-methylation and histone modification during two specific time windows, the early embryogenesis and the Primordial Germ Cell (PGC) development stage. Here we reviewed these global epigenetic reprogramming occurred during normal development, mainly focusing on DNA methylation, histone modification and X-chromosome inactivation. Epigenetic reprogramming participates in many key biological processes such as genomic imprinting, X-chromosome inactivation, gene expression regulation and tumorigenesis and genome stability. Understanding the mechanisms of epigenetic reprogramming during the early embryogenesis and PGC formation would facilitate our knowledge of the developmental process and disease progress.

**Keywords**: Epigenetic reprogramming; DNA methylation; De-methylation; Histone modifications; X chromosome inactivation; Embryogenesis; PGCs

**Abbreviations:** PGCs: Primordial Germ Cells; XCI: X Chromosome Inactivation; 5mC: 5-methylcytosine; 5hmC: 5-hydroxymethylcytosine; Xp: Paternal X Chromosome; 5fC: 5-formylcytosine; 5caC: 5-carboxylcytosine; ICM: Inner Cell Mass; PRC2: Polycomb Repressive Complex 2; IAPs: Intracisternal-A-Particles; CGIs: CpG Islands; gDMRs: Germline Differently Methylated Regions; Xi: Inactive X Chromosome; qRT-PCR: Quantitative Reverse Transcription Polymerase Chain Reaction

## Introduction

Epigenetic landscape, including both DNA methylation and histone modifications, is maintained in a relatively stable state in mammalian somatic issues. However, during the Preimplantation embryogenesis and the post-implantation germ cell development processes, two waves of genome-wide epigenetic programming and reprogramming occur. In these processes, DNA methylation erasure and reestablishment and histone remodeling change dynamically and cooperatively to construct the epigenome, which restores the genome to a pluripotent state and impose on the following differentiation.

In mammals, upon fertilization, the zygotic genome experiences a comprehensive reprogramming process, which contributes to the transition of the zygote into a totipotent state. And during this reprogramming process, epigenetic reprogramming plays an essential role in regulating the zygotic gene expression.

The second wave of epigenetic reprogramming starts to emerge together with the specification of PGCs at E7.25 [1]. During this process, DNA de-methylation is completed thoroughly, with the erasure of the methylation pattern in imprinted loci between E10.5 and E12.5 after they migrate into the genital ridge [2,3]. And this makes the genome ready to reset to a gender-specific state along with the mature of germ cells.

Coupling with epigenetic remodeling in females, a signature of the placental mammals-X chromosome inactivation occurs, which balances the expression dosage of genes on X chromosome between males (XY) and females (XX). Since one of the two X chromosomes in female somatic cells is transcription ally silenced, namely the X Chromosome Inactivation (XCI), the expression of X-linked genes is monoallelic. There are two forms of XCI in mice, imprinted and random ones, and their dynamics correlates with the two waves of DNA methylation variation during embryogenesis *in utero*.

## Epigenetic reprogramming during the Preimplantation embryogenesis

**DNA de methylation in the early embryogenesis:** During early embryogenesis, with a global DNA de-methylation observed in mouse embryos, the DNA methylation level drops to the lowest at the blastocyst stage [4,5], which then gradually reverts to the somatic level after implantation happens due to functions of the *de novo* DNA methyl transferase, namely Dnmt3a and Dnmt3b [6], and maintains stable thereafter resulting from the activity of Dnmt1, the maintenance methylase. But during embryo development processes between the two-cell and the blastocyst stage, there is no Dnmt1, which results in a replication-dependent manner of gradual reduction in DNA methylation. Thus the so-called passive de-methylation occurs in the maternal genome (Figure 1) [4,7]; Whereas the paternal genome DNA is actively demethylated at the one-cell stage in a quite different pattern, that is, through the replication-independent pathway (Figure 1) [8-10]. During the de-methylation process, most genes are demethylated throughout the whole genome, including housekeeping genes and repeat sequences like LINE1. However, the imprinted genes can surprisingly escape from this fate successfully and remain hypermethylated, similar to the ones in gametes.

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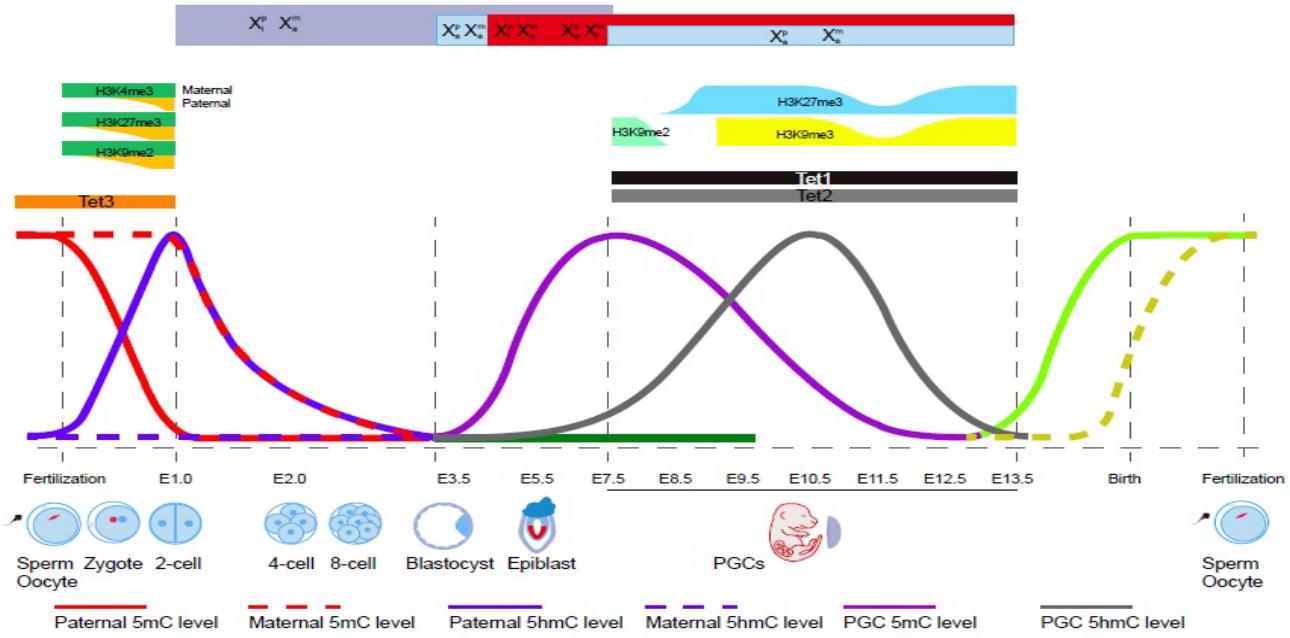


After fertilization, the paternal pronuclear is actively demethylated in one-cell stage (red line), inversely, the 5hmC level (blue line) increases in the paternal pronuclear. Whereas the maternal pronuclear maintains a hypermethylation state in one-cell stage and is passively demthylated in a replication-dependent manner (red dotted line). Immunofluorescence analysis in one-cell stage embryos identifies a consistent expression of several histone modifications, such as H3K4me3, H3K27me3 and H3K9me2 (green and orange box) in the maternal pronuclear, while the new incorporated histones in the paternal pronuclear acquire these modifications gradually during the developmental window. From two-cell to blastocyst stage, 5mC and 5hmC are diluted along with replication of cells and reach the lowest point at blastocyst stage (red dotted line and blue line, respectively). During this peroid, the paternal X chromosome is exclusively inactivated (cyan box). After implantation, genome in extraembryonic issues maintains a hypomethylation level (dark green line) and the paternal X chromosome is imprintedly inactivated (cyan box). However, ICM/epiblast cells restore a previous hypermethylation level (purple line). And these cells reactivate the imprinted inactivated X chromosome in 24 hours (E3.4-E4.5) (sky blue box). Then the two parental X chromosomes in epiblast cells are randomly inactivated (red box); While in PGCs, a second wave of genome-wide DNA demethylation is initiated, which is catalyzed by Tet1 and Tet2 (black and gray box), with a decline of 5mC (purple line) and an increase of 5hmC (gray line). During the process, H3K9me2 disappears gradually, and H3K27me3 and H3K9me3 accumulate soon afterwards (blue and yellow box). After entry into the gonads, PGCs have a concomitant loss of H3K27me3 and H3K9me3 (blue and yellow box). Accompanied with the loss of DNA methylation and repressed histone modifications, randomly inactivated X chromosome is reactivated in a replication-dependent way (sky blue box). Following gender-determination, *de novo* DNA methylation takes places in an asymmetrical pattern in male and female germ cell precursors. In male embryos, new DNA methylation is established before meiosis and is complished before birth (green line). While in female embryos, *de novo* DNA methylation is not initiate until birth and is established during the postnatal growth stage of oocytes (yellow dotted line).

**Figure 1:** Schedules of dymanic remodling of epigenetics in the early embryogenesis and the development of PGCs.

Though the phenomena of active and passive de-methylation are observed in the paternal and the maternal genomes respectively, the mechanisms of how active de-methylation happens and how imprinted genes are exempt from de-methylation are little known. Significant advancements have been made in deciphering the mechanisms behind active de-methylation, and the discovery of Tet proteins (Tet1, Tet2, Tet3), which catalyze the conversation of 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC) [11,12], was a hallmark among them. It is demonstrated that Tet3 mediates the active de-methylation of paternal pronuclear at the one-cell stage. Researchers uncovered an inverse correlation between 5mC and 5hmC, and this conversation was carried out by Tet3, since Tet3 knockout in mouse embryos eliminated this conversation and led to embryonic development failure [13]. Meanwhile, further studies identified that the converted 5hmC was not just an intermediate, for the reason that instead of disappearing soon, they were further converted into 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) [14,15]. Moreover, they were diluted in a replication-dependent way, a similar pattern like the DNA de-methylation in the maternal genome [14,16].

**Histone modifications in the preimplantation embryos:** Another crucial epigenetic factor influencing the development of preimplantation embryos remarkably is the modification of histones. They mark developmentally related genes and predetermine their expression before zygotic genome activation [17,18]. Increasingly more evidence suggests that a crosstalk between histone modifications and DNA methylation exists. Early studies demonstrated that there are changes of histone modification patterns happened along with the development of pre-implantation embryos [19-22]. Because of the protamine-histone transition that happens soon after fertilization, oocyte-derived unmodified histones embed into the paternal chromatin [23-26]. Thereafter newly synthesized modifications make the paternal and the maternal chromatin asymmetrically [24-26], which may contribute to the differences in de-methylation fates between the paternal and maternal genome. Immunofluorescence analysis demonstrate a consistent expression of several histone modifications, such as trimethylation on lysine 4 of histone 3 (H3K4me3), H3K27me3 and H3K9me2, in maternal pronuclear, while these modifications in paternal pronuclear gradually increase with the schedule of development in one-cell stage embryos (Figure 1) [20-22,27-29]. Although DNA de-methylation and several histone modifications present similar patterns, there is no direct evidence proving similarity in them. Previous studies implicated that stella/PGC7 protected the maternal genome against

from being demethylated and maintained the methylation level of several imprinted genes [30]. However, no bridge linking the histone modifications and DNA methylation was identified. Until 2012, based on their work published previously [30], Nakano's group demonstrated that stella/PGC7, through binding to H3K9me2, could block Tet3's binding to the maternal genome as well as several paternal imprinted genes, which protected them from active de-methylation [31]. This potential protection mechanism provides us with a better insight into this particular event, but more experiments are still required for a thorough understanding of it.

**Imprinted XCI and its reactivation:** When fertilization happens, the paternal X chromosome is inherited inactively, referred to as the imprinted XCI. This form of nonrandom XCI is initiated in embryos at the early cleavage stage and then maintained in extraembryonic tissues as well as their derivatives [32,33]. However, the imprintedly inactive paternal X chromosome is reactivated selectively in Inner Cell Mass (ICM) (Figure 1) [34]. Initiation of the imprinted XCI is dependent on the *cis* regulation of a long nocoding RNA – *Xist*, which coats the inactivated X chromosome [35]. Though the paternal X chromosome is inherited actively like the maternal X one [36], it soon becomes inactivated because of the expression of paternally originated *Xist*, which in turn wrap the paternal X chromosome up and leads to its silence; whereas the maternal *Xist* is repressed and not expressed until the morula stage [37]. During this period, the global genome is undergoing DNA demthylation, so DNA methylation may not likely participate in the imprinted XCI regulation. In addition, in *Dnmt1*-deficient embryos, imprinted XCI in extraembryonic tissues is not affected [38]. However, histone modifications alter dynamically. Immunofluorescence analysis demonstrated the accumulation of transcriptionally repressed markers, dimethylation of histone H3 lysine 9 (H3K9me2) and trimethylation of H3K27 (H3K27me3) starting from the 16-cell stage and continuing afterwards [39]. Additionally, hypoacetylation of H3K9 and hypomethylation of H3K4 were also detected from the 8-cell stage embryos [39], after the coating of *Xist* in Xp. These data indicates that *Xist* may cooperate with these modifications to regulate the activation of Xp. Indeed, recent work further reported an ncRNA (RepA) within *Xist* and identified it as a binding target for Polycomb Repressive Complex 2 (PRC2). RepA was demonstrated to be able to recruit PRC2 to the X chromosome, with Ezh2 serving as the binding subunit, ,then methylate lysine 27 on histone H3 [40] and subsequently silence the paternal X chromosome synergistically.

Albeit Xp is still inactive as determined by *Xist* coating, RNA Polymerase II (RNA Pol II) exclusion, H3K9 and H3K27 methylation as well as Eed/Enx1 accumulation at the early blastocyst stage [39], these depressed markers disappear late in the blastocyst stage [34,39]. This reactivation process resets cells in the ICM back to a pluripotent state and gets them ready for future lineage differentiation.

## Epigenetic reprogramming in germ cells

**DNA methylation reprogramming in germ cells:** At the blastocyst stage, embryos possess a globally low level of DNA methylation. However, the epiblast as well as the early PGCs, return to a hypermethylated state due to the function of *de novo* methyltransferases upon implantation [4,10,41,42]. After E8.5, with the migration and proliferation of PGCs, a genome-wide loss of DNA methylation is detected (Figure 1) [43]. Prior to E9.5, DNA de-methylation occurs on promoters, CpG islands, exons, introns and intergenic regions [44-46]. But until PGCs enter the genital ridges, imprinted genes are not erased [47,48]. The de-methylation happens later (E10.5 thereafter) is an active process mediated by Tet1 and Tet2 proteins (Figure 1) [49-51]. Since DNA methylation erasure is achieved in a thorough way, it is believed that epigenetic information cannot be passed on into the next generation, for epimutations were erased and corrected in this process [52-54]. However, there are still a handful of elements, Intracisternal-A-Particles (IAPs) for example, who escape from the systematic de-methylation. And that may provide an insight into the transgenerational epigenetic inheritance [2,46,47,55,56].

Following the gender-determination (~E12.5), *de novo* DNA methylation takes place and new methylation landscapes are established in an asymmetrical pattern in male and female germ cell precursors. In male germ cells, *de novo* methylation initiates before the onset of meiosis, and finishes prior to birth. While in female germ cells, *de novo* methylation occurs during the postnatal development of meiotic prophase I - arrested oocytes (Figure 1). Additionally, bisulfite sequencing analysis identifies ~900 oocyte-specific Methylated CpG Islands (CGIs) and ~60 sperm-specific methylated CGIs [57], among which Germline Differently Methylated Regions (gDMRs) of imprinted genes are included.

**Histone remodeling in germ cells:** During the formation of germ cells, histone modifications change in accord with the DNA methylation dynamics. A signature is the gradual loss of H3K9me2 which starts from E7.5 and afterwards (Figure 1) [43] in a G9a/EHMT2-independent way as G9a knockout does not affect the PGC specification [58]. However, it associates with the down regulation of GLP/EHMT1, which affects the G9a-GLP complex [59]. Another feature is the up regulation of H3K27me3 initiated at E8.5 that precede later on, concomitant with the accumulation of Ezh2 [43]. After PGCs enter the genital ridge, H3K4 methylation and H3K9 acetylation (H3K9ac), which are transcriptionally permissive, increase sharply [43], while transcriptionally repressive histone modifications, such as H3K9me3 and H3K27me3 are removed (Figure 1) [60]. These changes may make DNA demethylase accessible to the chromatin and achieve more extensive de-methylation, even on the imprinted gene loci [2,61].

**Random XCI and its activation:** Soon after implantation, the second wave of X chromosome inactivation and reactivation is initiated in parallel with to the genomic reprogramming occurring in PGCs [62]. At E4.5, the late blastocyst stage, the inactive X chromosome (Xi) is already reactivated (Figure 1). However, by E5.5, one of the two X chromosomes in most epiblast cells is chosen randomly to be inactivated by Xist, expressed either from the paternal or the maternal gene locus. The entire epiblast cells complete random X chromosome inactivation by E6.5, that is, the monoallelic expression of X-linked genes [63,64]. Since PGCs emerges after E6.25, marked by a PGC progenitors expressing protein, Blimp1 [65], it is indubitable that PGCs have undergone X chromosome inactivation before germ cell specification. Apart from the Xist coating, the nuclear accumulation of H3K27me3 participating in silencing X-linked gene expression was also detected by immunofluorescence staining [66].

With their migration and entry into the genital ridge, PGCs experience a Xi reactivation, which enable them ready for germ cell specification, for the X chromosomes in oocytes are active. FISH analysis revealed that decreased Xist expression beginning at ~E7.0, and reaching an undetectable level at ~E10.5 [67]. And there is a dramatic decline in H3K27me3 level from E7.5 to E9.5 [66]. However, even though X-linked genes initiate their biallelic expression from ~E7.75, they do not accomplish a thorough activation until E14.5 revealed by quantitative reverse transcription polymerase chain reaction (qRT-PCR) [66,67].

Compared with the reactivation in ICM, resetting the randomly inactivated Xi is a time-consuming event. These differences may account

for the involvement of different epigenetic remodeling that in PGCs it is a passive process requiring several cell divisions [66,67], while in ICM it is completed in one day [34,39]. The possibility is that in PGCs, *Xist* silences the X chromosome cooperating with DNA methylation as well as histone modifications [38,66,67], while in ICM no DNA methylation involved [38,43], making it possible for the erasure accomplished rapidly.

## Conclusion

Epigenetic reprogramming in the early embryogenesis and germ cells specification progress is complicated. Previous works have identified DNA methylase (Dnmt1, Dnmt3a and Dnmt3b) and DNA demethylases (Tet1, Tet2 and Tet3), and their function mechanisms have been revealed. However, when it comes to the complex biological processes, we know a little. Though it was demonstrated that H3K9me2 can protect the maternal genome and several imprinted genes from active de-methylation, this mechanism was not adapted to all the conditions. In addition, there indeed are regulatory elements escape de-methylation in PGCs [49,55] and epigenetic modification induced phenotype inheritable [68,69]. But no direct experimental evidence is given that epigenetic modifications/epimutations can be germline transmitted in mouse [52,54] as it is proven in zebrafish [70]. How DNA methylation and histone modifications interact with each other and function cooperatively is only a tip of the iceberg. To have a more comprehensive and thorough acknowledged of the epigenetic reprogramming, more works are still needed, which will deepen our understanding of the developmental regulation mechanisms.

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