Parental epigenetic control of embryogenesis: a balance between inheritance and reprogramming?

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Abstract

At fertilization, fusion of two differentiated gametes forms the zygote that is capable of forming all of the varied cell lineages of an organism. It is widely thought that the acquisition of totipotency involves extensive epigenetic reprogramming of the germline state into an embryonic state. However, recent data argue that this reprogramming is incomplete and that substantial epigenetic information passes from one generation to the next. In this review we summarize the changes in chromatin states that take place during mammalian gametogenesis and examine the evidence that early mammalian embryogenesis may be affected by inheritance of epigenetic information from the parental generation.

Introduction

Embryos develop from the fusion of two highly specialized haploid germ cells, oocytes and sperm, yet possess during early development the property of totipotency, the ability to give rise to every cell type in an organism. The acquisition of developmental potency is thought to arise from reprogramming of the parental germline epigenetic state to a new epigenetic state in the early embryo. The full extent of this reprogramming is, however, unknown, leaving open the question of how much epigenetic information present in mature gametes is retained in the embryo.

Epigenetic information is generally considered to represent heritable information in genome function that is not encoded by the DNA sequence. Based on studies of embryonic development and cellular differentiation, several mechanisms have been proposed for the transmission of epigenetic information. These include chemical alteration of DNA itself (most often by methylation or related chemical groups), post-translational modifications of histones, and transmission of RNA. Recent genome-wide chromatin analyses in a variety of cell types revealed that regulatory sequences of genes as well as of repetitive sequences are generally associated with DNA and/or distinct histone modifications and chromatin associated proteins [1]. The presence of a defined “epigenome” in embryonic stem (ES) cells raises the question about the ontogeny of such a pluripotency-related chromatin program: whether it is newly defined during pre-implantation development or inherited from gametes.

To establish a conceptual framework for future experiments, we propose two opposite models for epigenetic control of mammalian pre-implantation development (Figure 1): The *reprogramming model* proposes that chromatin states in gametes become reset upon fertilization to enable the acquisition of totipotency. This classical model is in part based on global changes in DNA methylation along the paternal and maternal genomes of pre-implantation embryos, as visualized by immuno-fluorescence microscopy [2] and the ability of the cytoplasm of oocytes and zygotes to partially reprogram the epigenome of differentiated nuclei [3]. Likewise, during primordial germ cell specification, somatic epigenetic programs

acquired during early embryogenesis are erased and subsequently replaced by female and male specific germ cell programs. In the case of DNA methylation in the context of genomic imprinting, however, part of the germline program escapes reprogramming in early embryos, safe guarding parental specific expression during somatic development [4].

In contrast, the *inheritance model* proposes that chromatin states in mature germ cells are inherited by embryos to direct transcriptional activation or silencing upon zygotic genome activation and subsequent development. Chromatin programs in mature germ cells may either be specified during gametogenesis, or originate from pre-implantation embryos or even from parental gametes, thereby enabling intergenerational or transgenerational inheritance of epigenetic information. Transmission of such pre-patterned chromatin states, constituting a default "*intrinsic inter-/transgenerational inheritance program*", could contribute to the high level of developmental potential of early embryos. We further postulate that "*acquired inter-/transgenerational inheritance*" results from a temporal exposure to environmental cues altering the cellular state of the germline in one generation and changing transitorily, for one or multiple generations, the execution of the default intrinsic inheritance program, and thereby affecting the phenotype of offspring [5]. Acquired inheritance would depend on the interaction between germ cells and somatic cells in the organism, either directly between gonadal somatic cells and germ cells, or indirectly by hormonal signaling. In general, acquired inheritance (and not the intrinsic inheritance program) is referred to as transgenerational inheritance [6].

Studies in non-mammalian model organisms have provided evidence for various mechanisms playing a role in inter-/transgenerational epigenetic inheritance. For instance, studies in plants demonstrated that altered patterns of DNA methylation (so-called epialleles) can be inherited over successive generations [7], [8]. Transmission of the chromatin state of a chromosomal regulatory element through both mitosis and meiosis has been shown in *Drosophila* [9], [10]. In *zebrafish* embryos, homeostatic and developmentally regulated genes are marked by permissive and repressive histone modifications prior to zygotic genome activation. This pre-patterning reflects in part chromatin marking observed in *zebrafish* sperm, providing means for intergenerational inheritance [11], [12]. In the context of RNA-based inheritance, transmission of silencing induced by siRNAs (for instance in *C. elegans*) [13] or maternal piRNAs (in *Drosophila*) [14]) has also been established. Below, we review the current knowledge on chromatin dynamics during germ cell development and intergenerational epigenetic inheritance in mouse.

Germ cell development in mammals

In several well-studied model organisms germ cells arise via asymmetric distribution of germline inducing factors, known as germplasm, providing an obvious mechanism for intergenerational inheritance [15]. This is not the case in mammals, where primordial germ cells

(PGCs) are specified from a small population of posterior proximal epiblast cells (Figure 2) [16], [17]. Prior to undergoing overt cellular differentiation, PGCs undergo changes in gene expression state and DNA and histone methylation levels that are believed to represent re-programming of these cells [18], [19]. The balance between epigenetic reprogramming versus inheritance in PGCs is, however, not understood, in part due to the technical challenges of working with these rare and relatively inaccessible cells. Following the early events of germline reprogramming, germ cells in male and female embryos embark on drastically different differentiation programs, which may suggest different capacities for intergenerational inheritance between the sexes.

DNA Methylation

Methylation of the fifth position of cytosine on DNA (5mC) represents one well-studied mechanism of epigenetic transmission in the mouse. This mechanism provides evidence for both germline reprogramming and germline inheritance. During PGC migration to the developing gonad, germ cell chromatin undergoes substantial changes [19]. During the same time period levels of 5mC in germ cells also begin to decrease [18]. PGC DNA demethylation continues after their arrival in the gonad and effects around 80-90% of the genome [20]. The mechanism behind this demethylation has been the subject of many years of research. It was hypothesized that, following a paradigm established in plants [21], deamination and subsequent DNA repair might be involved in DNA demethylation. Deletion of the cytidine deaminase *Aid* in the mouse provided some support for this model as PGCs in these animals display modest DNA hypermethylation [20]. Further support was provided by the observation that PGC demethylation is also associated with the presence of DNA single stranded breaks [22]. A similar mechanism for demethylation of the paternal genome may exist in the early embryo as well [23]. Another recent advance was the discovery that the hydroxylated form of methylated DNA (5hmC) is present in substantial quantities in mammalian genomes and that this modified base could be formed from 5mC by proteins of the Tet family [24], [25]. Three different Tet proteins exist in mice. While deletion of *Tet1* leads to no obvious mutant phenotypes [26], *Tet2* seems to function as a tumor suppressor during hematopoiesis [27], [28]. Maternal deletion of *Tet3* leads to defects in early development with 50% of maternally *Tet3*-deficient embryos failing to develop to term [29]. What differences exist between these two classes of *Tet3*-deficient embryos remains unknown. Likewise, it is unclear whether this observed embryonic lethality is due to impaired 5mC to 5hmC conversion of the paternal genome in the zygote versus changes to the maternal epigenome, inherited from the mutant oocyte.

While removal of DNA methylation across the majority of the genome in PGCs would seem to preclude the inheritance of this mark, certain DNA sequences, such as the Intracisternal A Particle (IAP), appear rather resistant DNA demethylation in PGCs [30]. A well known example of maternal epigenetic inheritance in the mouse is the agouti viable yellow (A^{vy})

allele of the agouti locus which contains an IAP retrotransposon sequence inserted near the promoter of the gene [31]. Gene expression from this allele correlates positively with the degree of DNA methylation of the IAP sequence in soma of the individual itself as well as of the mother. Furthermore, the methylation state is susceptible to environmental cues such as maternal diet [32]. Nonetheless, DNA methylation at the A^{vy} allele inherited from the oocyte is lost in pre-implantation embryos arguing that DNA methylation is not the primary mediator of intergenerational epigenetic inheritance [33]. Comparable observations were made for the *Axin-fused* allele [34], [35].

Unlike IAP retrotransposons, DNA methylation at imprinted control regions (ICRs) is erased in PGCs [30]. ICRs become subsequently methylated in either the male or female germline and maintain their methylation state following fertilization to direct parental specific expression during embryonic and postnatal development. Thus, developmental changes in DNA methylation at ICRs represent an one generational cycle of epigenetic reprogramming (in PGCs) and inheritance (in early embryos). Intriguingly, while DNA methylation is clearly involved in epigenetic inheritance of imprinted DNA methylation, recent work indicates that feedback to DNA sequence via the Krüppel-associated box-containing zinger finger protein Zfp57 is required for efficient maintenance of imprinted DNA methylation in early embryos [36] and in ES cells [37], [38].

While imprinted loci have been extensively studied, the role of inherited DNA methylation throughout the genome is less well understood. Recently, two genome-wide studies of DNA methylation on developing mouse oocytes revealed that DNA methylation established in the female germline by the *de novo* DNA methyltransferase Dnmt3a and its non-catalytic paralog Dnmt3l correlates with DNA methylation profiles measured in blastocyst embryos [39],[40] . Surprisingly, the DNA methylation level in blastocysts was several-fold higher than expected on the basis of the widely cited model of active and passive demethylation that are thought to reprogram the paternal and maternal genomes during pre-implantation development. Kobayashi and colleagues also demonstrated that ICRs versus retrotransposons have a differential requirement for *Dnmt3l* for DNA methylation establishment [40]. Nonetheless, embryos derived from oocytes deficient for *Dnmt3a* or *Dnmt3l* are capable of developing to midgestation in the mouse [41], [42] suggesting that maternally inherited DNA methylation does not play a critical role in pre-implantation development.

Oogenesis and chromatin

Female germ cells initiate meiosis during fetal life, arresting their cell cycle at the diplotene stage of meiotic prophase (Figure 2). Beginning a few days after birth (and continuing periodically throughout the reproductive lifespan of the organism) oocytes are recruited into a growing phase, which associates with increased transcriptional activity. At the end of this

growing phase, oocytes are induced to resume meiosis by a surge of luteinizing hormone (LH), re-arresting at metaphase of meiosis II (M-II). It has long been known that morphological changes in chromatin are associated with growing oocyte development [43]. During the early growing phase, chromatin of mouse oocytes exists in a de-condensed configuration known as the Non-Surrounded Nucleolus (NSN) state [44], [45], [46]. As oocytes reach the final stage of oocyte growth they undergo a change in chromatin state, forming condensed rings of chromatin (containing pericentric heterochromatin [47] around the prenucleolar body), forming the Surrounded Nucleolus (SN) state [44], [45], [46]. The transcriptional activity of oocytes correlates with their chromatin configuration: NSN oocytes display transcriptional activity while SN oocytes are transcriptionally repressed. However, the transcriptional repression found in mature oocytes is not dependent on their SN chromatin state. Despite failing to achieve the SN chromatin configuration [47], oocytes deficient for the histone chaperone Nucleoplasmin 2 *(Npm2*) undergo transcriptional repression [48]. Importantly, oocyte chromatin configuration does, however, correlate strongly with embryonic competence [49], [50].

The linker histone H1 variant *H1foo* is specifically expressed in growing oocytes [51], [52]. Knockdown of this variant in growing oocytes by morpholino antisense oligonucleotides leads to a reduced capacity of these oocytes to resume meiosis [53]. Oocyte chromatin also contains the H2A variant macroH2A throughout the growing phase, as well as during meiotic resumption [54]. This variant also remains associated with maternal chromatin following fertilization [54], providing a possible mediator for inheritance. As expected for non-replicating cells, growing oocytes do not incorporate the replication-dependent H3 variants, H3.1 and H3.2 [55]. They do, however, robustly incorporate the replication-independent variant H3.3, suggesting that ongoing changes in chromatin composition occur during oocyte growth [55]. H3.3 incorporation was observed in the nuclei of oocytes where transcription had ceased, indicating the existence of continuous, transcription-independent nucleosome turnover in oocytes [55].

Changes in histone modifications during oogenesis have been extensively cataloged in many publications (reviewed in [56]), however, only a limited number of studies have identified functional roles for these modifications. Growing oocyte development is associated with increasing levels of histone acetylation, followed by abrupt de-acetylation during meiotic resumption [57]. Modulation of HDAC (histone de-acetylase) activity in oocytes can alter the condensation of chromatin in these cells, with increased HDAC activity leading to premature chromatin condensation [58], and HDAC inhibition leading to chromatin de-condensation [48]. Genetic ablation of *Hdac1* and *Hdac2* severely impairs oocyte growth and transcription leading to female sterility [59]. Histone de-acetylation is also important for the development of oocytes following meiotic resumption, with failure to de-acetylate histones impairing chromosome elongation and alignment during M-II. These defects are thought to be caused in part by failure centromeric heterochromatin binding by the chromatin remodeler Atrx in these cells [60].

Histone methylation appears to be inherited by embryos through the female germline. In early embryos, heterochromatic sequences are differentially marked in pronuclei of maternal and paternal origins [61]. Maternal heterochromatin carries *Suv39h2*-dependent H3K9 trimethylation, which is inherited from the oocyte and is required for maintenance of the canonical heterochromatic state in embryos. In paternal heterochromatin, which lacks this mark, proteins of the Polycomb Repressive Complex 1 (a major repressive complex implicated in epigenetic repression of e.g. developmental regulatory genes during development [62]) mediate transcriptional repression of heterochromatin associated satellites sequences [61]. In addition to roles for histone methylation associated with repressive chromatin, trimethylation of H3K4, an active mark, has also been shown to be important in the female germline. Oocyte-specific deletion of *Mll2*, an H3K4 methyltransferase, results in decreased levels of H3K4 methylation in peri-ovulatory oocytes and impairs either ovulation or pre-implantation development, depending on the timing of conditional deletion during oogenesis [63].

Global chromatin remodeling during spermiogenesis

In contrast to female germline development, meiosis begins in male germ cells only after birth (Figure 2). With respect to chromatin, changes are clearly detectable from the onset of meiosis. Chromatin changes may be responsible for localization of meiotic recombination (Box 1) and the specialized meiotic behavior of the sex chromosomes (Box 2). Following meiosis, haploid round spermatids undergo dramatic and extensive chromatin remodeling. This process results in the genome-wide exchange of histones by spermatogenesis-specific basic DNA packaging proteins (initially transition proteins and ultimately protamines) [64], [65]. Nevertheless, in human and mouse approximately 10 and 1% of histones are retained in spermatozoa respectively [66]. Since these retained histones harbor post-translational modifications, as in somatic cells, they may function as mediators of epigenetic inheritance between generations.

Some histone variants such as testis specific histone H2B variant, TH2B and testis specific histone H1, H1t are present from early spermatogenic cells to the round spermatid stage [67], [68], [69]. However, variants of H2A such as H2AL1/2, H2A.Bbd, or of H1, such as H1t2 and Hils1, are specifically incorporated during the histone-to-protamine exchange in round/elongating spermatids [70], [71], [72], [73]. While H2AL1/2 mark pericentric heterochromatin during spermatogenesis, they are quickly displaced from paternal heterochromatin after fertilization [74], potentially restricting a functional role in paternal inheritance. H2A.Bbd was found to destabilize nucleosomes, thus its presence in nucleosomes was suggested to facilitate replacement of histones by protamines [71]. Recently, another H2A variant, H2A.Lap1 has been shown to be loaded onto the X chromosome and autosomes in round spermatids and suggested to have a role in transcription of repressed genes [75]. Among testis-specific H1 variants, it was shown that Hils1 localizes to the same sites as Tnp2 and

Prm1 [73], while deficiency for H1t2 was found to result in reduced sperm mobility and condensation defects [72].

In addition to incorporation of histone variants, post-translational modifications of histones and their read-out play a critical role for genome-wide chromatin remodeling during late spermatogenesis. Histones are hyperacetylated beginning at the round spermatid stage with increasing levels detectable in the elongating spermatid stage [76], [77]. This hyperacetylation is thought to facilitate global chromatin remodeling through creation of a more accessible chromatin environment. Hyperacetylated histones are recognized by the bromodomain containing protein Brdt [78], [79]. Deletion of the first bromodomain of *Brdt* in mice results in abnormal chromatin remodeling and male infertility [80]. *Brdt*-mutant sperm is, however, capable of supporting normal embryonic development when used for intracytoplasmic sperm injection (ICSI) [80]. Histones are also ubiquitinated in elongating spermatids [81]. In particular, the E3 ubiquitin ligase Rnf8 is required for normal histone eviction during spermatogenesis [82]. *Rnf8*-deficient spermatids are also deficient for spermatid histone acetylation, suggesting interplay between these two pathways [82]. Recently, a global survey of histone modifications identified crotonylation as a new histone modification that was found at high levels in elongating spermatids. Histone crotonylation correlates with gene expression with a specific enrichment on sex chromosomes in spermatogenic cells. The exact role that histone crotonylation play in spermatogenesis (and elsewhere) remains to be determined [83].

The role of several other chromatin regulators in spermatogenesis has been studied by knock-out mouse models. Deficiency for *Jmjd1a*, a demethylase for H3K9me1/2, was found to block spermatid elongation and cause infertility [84]. In contrast, the absence of *Kdm4d*, a demethylase for H3K9me3, did not influence the progression of spermatogenesis, perhaps because of functional redundancy with other histone demethylases [85]. Other recent studies showed that the histone methyltransferase Mll5 is required for proper spermatid maturation and fertility [86], [87].

Although there have been many studies investigating the role of histone variants and chromatin regulators in reorganization of the paternal genome, in general the mechanisms of their actions remain unknown. Several recent studies have shown that histones (and their modifications) retained in human sperm are not randomly distributed, but are instead enriched at regulatory elements of genes [88], [89], [66]. Intriguingly, differential histone modifications associate with functionally distinct set of genes suggesting that transmission of retained histones might guide transcription during early embryonic development [88], [66].

Conclusions

Embryonic development requires epigenetic reprogramming to regenerate totipotency from a germline state in each generation. This reprogramming is likely counterbalanced by the

inheritance of epigenetic information present in mature gametes. Many questions remain as to the relative contributions of these two forces in early embryognesis and what functional roles inherited epigenetic information plays in mammalian embryogenesis.

Establishing that a system actually utilizes e.g. chromatin based mechanisms for epigenetic inheritance is, however, difficult. For example, the recent finding that the maintenance of imprinted DNA methylation in embryos and ES cells requires feedback to the underlying genome via the Zfp57 protein, binding to specific methylated DNA sequences within ICRs, sheds a new light on "epigenetic" inheritance of DNA methylation during early development [36], [37], [38]. Furthermore, a number of chromatin modifying enzymes have been shown to possess catalytic activity towards non-histone proteins, potentially complicating the interpretation of genetic deficiency studies [90].

Nonetheless, to provide evidence for the "intrinsic inter-/transgenerational inheritance program" model, more in depth molecular genetic studies are needed to elucidate the germline function of major epigenetic regulators shown to be involved in inheritance during somatic development. To understand the function of paternally transmitted (modified) histones for embryogenesis, it is necessary to dissect the mechanisms of histone eviction versus retention during spermiogenesis. Ultimately, it will be required to study the effect of germline expression of histones with residue specific mutations on parent-of-origin transcription during embryogenesis.

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Boxes:

Box 1: Prdm9 and chromatin in meiotic recombination

Chromatin plays a role in one of the fundamental processes of gametogenesis: meiosis. The products of meiosis contain a genetic complement that differs from that of their parental cells because of the process of meiotic recombination. Recombination does not occur at equal rates throughout the genome, but instead preferentially occurs at specific sites, known as "hotspots," where the incidence of DNA double strand breaks (DSBs) is increased [91]. Little interspecific conservation of hotspot usage has been observed, and in mice various genetic strain

backgrounds also display different recombination patterns [92]. In species from yeast to mammals, recombination hotspots correlate with enrichment for H3K4me3 [93], [94]. In mammals, these sites are bound by Prdm9 (also known as Meisetz), a protein capable of methylating H3K4 [95] and which is required for the completion of both male and female meiosis in mice [96]. It was shown that variations in hotspot usage correlate with variation in the DNA binding domain of Prdm9 [95], [97]. It is currently unknown whether Prdm9's role in promoting DSB formation and completion of meiosis depends on its methyltransferase activity towards H3K4. Whether additional chromatin factors (beyond DNA sequence) serve to recruit Prdm9 to hotspots also remains unknown. Interestingly, hotspots strongly affect the inheritance of specific sequences, as their tendency to undergo gene conversion drives their removal from the genome [98]. This suggests that a chromatin-based system utilized during spermatogenesis contributes to paternal inheritance of DNA sequences.

Box 2: Meiotic Sex Chromosome Inactivation

The heteromorphic nature of mammalian sex chromosomes means that these chromosomes cannot fully homologously pair during male meiosis. These chromosomes are held together during meiotic prophase by the formation of a single crossover at X-Y homologous sequences known as the pseudoautosomal region [99]. The unpaired regions of the chromosomes form a sub-domain on the nuclear periphery termed the sex body where they undergo transcriptional silencing in a process known as MSCI (meiotic sex chromosome inactivation) [100]. MSCI represents a specific instance of a common response to the existence of unpaired DNA regions during meiosis, first identified in the fungus *Neurospora*, known as MSUC (meiotic silencing of unpaired chromatin) [101]. The establishment of MSCI requires phosphorylation of the histone variant H2AX by the kinase ATR to form γH2AX [101]. While ATR is initially targeted to chromatin by the tumor suppressor BRCA1 [101], spreading of γH2AX along the sex chromosomes occurs in an MDC1-dependent manner [102]. Several additional histone variants and modifications have been proposed to regulate MSCI. For instance *Rnf8*, an E3 ubiquitin ligase is responsible for accumulation of ubiquitinated H2A on meiotic sex chromosomes. However, although deletion of *Rnf8* leads to a loss of this signal, MSCI is not impaired suggesting that H2A ubiquitination is not required for MSCI [82]. Additionally macro-H2A, a variant of H2A possessing a large non-histone domain, is strongly associated with the X and Y chromosomes during the early stages of MSCI, becomes lost (and, it is thought to be replaced with H2AZ) during late meiotic prophase [103], [104]. Intriguingly, canonical replication-loaded H3 (known in mammals as H3.1/H3.2) is depleted from sex chromosomes and replaced with the replication-independent variant H3.3 during the process of MSCI [105]. What functional role this global nucleosome replacement plays in MSCI and how this process is regulated by the known regulators of MSCI (such as ATR or MDC1) remain unknown. Subsequent to the initiation of MSCI and nucleosomal remodeling, meiotic sex chromosomes are dynamically methylated at various lysine residues of histone H3 and H4 [91],[105], as well as de-acetylated on the same histones.

Following meiosis, most single copy genes on the X and Y chromosomes remain in a transcriptionally silent state known as Post-meiotic Sex Chromatin (PMSC) [106], [101], [104], while genes present in multicopy arrays show expression in postmeiotic cells [107]. The role that PMSC plays in paternal inheritance in the early embryo is unknown. It has been proposed that imprinted inactivation of the paternal X chromosome in the pre-implantation embryo is established via a continuation of the PMSC state [108], [106]. However, this view remains controversial, as several studies indicated that imprinted X inactivation is initiated in the early embryo either in a *Xist*-dependent or *Xist*-independent manner [109], [110], [111]. In addition, the maternal X chromosome harbors a strong imprint that prevents its inactivation in early mouse embryos [112], [113].

Figure legends

Figure 1: **The *reprogramming* model (left) and *inheritance* model (right) of chromatin based epigenetic control of early embryonic development.**

See text for explanation.

Figure 2: **Life cycle of mammalian gametogenesis and embryogenesis**

Primordial germ cells (PGCs) arise from proximal epiblast cells and undergo extensive erasure of DNA methylation and chromatin changes during migration to and upon entry into the gonad. Directed by the somatic gonadal environment, germ cells enter the male or female fate. Male germ cells, initially called gonocytes, are cell cycle arrested and start to establish male specific DNA methylation patterns. During subsequent meiotic prophase, the X and Y chromosomes undergo meiotic sex chromosome inactivation (MSCI) characterized by major chromatin remodeling events. Following meiotic divisions, haploid spermatids undergo extensive nuclear and morphological changes including an almost genome-wide exchange of histones by protamines. Regulatory sequences, however, retain nucleosomes providing means for epigenetic inheritance. Female germ cells enter meiotic prophase in the embryo and complete the meiotic divisions upon hormonal induction in the adult ovary and fertilization by sperm. During the growing phase, oocytes establish DNA methylation at genes and imprinting control regions, undergo chromatin remodeling and acquire competence to direct embryogenesis. Upon fertilization, parental genomes form two pronuclei that are epigenetically distinct, reflecting the history of parental germline specific chromatin remodeling events. Paternal and maternal genomes undergo active and passive erasure of DNA methylation. The asymmetry in chromatin states at paternal and maternal chromosomes may potentially regulate activation and repression of *de novo* gene expression in pre-implantation embryos thereby directing embryogenesis.

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References of particular interest

*Johannes et al. 2009. **Assessing the impact of transgenerational epigenetic variation on complex traits.**

This study was one of the first studies showing that epialleles can be stably inherited over many successive generations in plants.

*Cavalli and Paro, 1998. **The Drosophila Fab-7 chromosomal element conveys epigenetic inheritance during mitosis and meiosis**

With this study, for the first time in *Drosophila* it was shown that the chromatin state of a chromosomal element, established by Polycomb and Trithorax group of proteins, could be transmitted via both mitosis and meiosis.

*Brennecke et al. 2008. **An epigenetic role for maternally inherited piRNAs in transposon silencing.**

piRNAs play an important role in silencing of transposable elements in germ cells. This study showed that piRNAs transmitted by maternal germline are critical for embryonic silencing of transposable elements inherited from the male and female germlines.

*Lienert et al., 2011. **Identification of genetic elements that autonomously determine DNA methylation states.**

By using a system based on insertion of different DNA elements into the genome, the authors showed that underlying DNA sequence regulates DNA methylation status of a genomic locus, and this regulation is largely encoded within small methylation-determining regions (MDRs).

*Seki et al., 2005. **Extensive and orderly reprogramming of genome-wide chromatin modifications associated with specification and early development of germ cells in mice.**

This study is one of the initial examples describing the major changes in chromatin states occurring during development of primordial germ cells. During migration of germ cells to the gonad, H3K9 dimethylation and DNA methylation levels are greatly reduced, whereas H3K27 trimethylation levels are significantly increased.

*Popp et al. 2010. **Genome-wide erasure of DNA methylation in mouse primordial germ cells is affected by AID deficiency.**

This study measured DNA methylation levels in PGCs and showed that *Aid*-deficiency leads to globally higher levels of DNA methylation in primordial germ cells.

*Tahiliani et al. 2009. **Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1.**

5-hmC is quite widespread in the mouse genome, as determined by this study. It was also shown that Tet proteins can convert 5-mC to 5-hmC.

Smallwood et al. 2011. **Dynamic CpG island methylation landscape in oocytes and pre-implantation embryos.

By RRBS the authors determined genome-wide DNA methylation levels in mouse oocytes of various stages of development. The paper also shows that oocyte established methylation patterns are partially maintained in the embryo.

*Kobayashi et al., 2012. **Contribution of intragenic DNA methylation in mouse gametic DNA methylomes to establish oocyte-specific heritable marks**. This is the second study to examine global patterns of DNA methylation in mammalian oocytes and early embryos. The authors establish that methylation of different classes of sequences display differential requirements for Dnmt3l.

Akiyama et al. 2011. **Dynamic replacement of histone H3 variants reprograms epigenetic marks in early mouse embryos.

During early embryogenesis, paternal DNA becomes remodeled as the sperm chromatin is decondensed. This work reveals that maternal chromatin also undergoes remodeling during early development and reveals the timing of these changes.

*De La Fuente et al. 2004b. **ATRX, a member of the SNF2 family of helicase/ATPases, is required for chromosome alignment and meiotic spindle organization in metaphase II stage mouse oocytes.**

As oocytes differentiate, chromatin structure becomes more condensed. This study showed that HDAC activity is required for this condensation and that this activity is important for progression through meiosis.

Puschendorf et al. 2008. **PRC1 and Suv39h specify parental asymmetry at constitutive heterochromatin in early mouse embryos.

In early embryos male and female chromatin is marked by different repressive chromatin marks. This paper demonstrates that H3K9me3 established by the *Suv39h2* enzyme in oocytes serve as a default intergenerational signal for constitutive heterochromatin formation in the next generation. In its absence, Polycomb Repressive Complex 1 proteins form an alternative repressive state at major satellites underlying constitutive heterochromatin.

*Andreu-Vieyra et al. 2010. **MLL2 is required in oocytes for bulk histone 3 lysine 4 trimethylation and transcriptional silencing.**

MLL2 functions in oocytes to provide normal levels of H3K4me3 and deficiency for this gene during oogenesis results in developmental failure.

*Shang et al., 2007. **The first bromodomain of Brdt, a testis-specific member of the BET sub-family of double-bromodomain-containing proteins, is essential for male germ cell differentiation**

Brdt is a testis specific bromodomain-containing protein which could bind to acetylated lysines. Deletion of first bromodomain of Brdt in mice resulted in sterile males although intracytoplasmic sperm injection showed that mutant sperm could support embryonic development.

Tan et al., 2011. **Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification.

Using a proteomics approach, the authors identified many novel post-translational modification sites on histones. From those new modifications, histone lysine crotonylation was found to mark active genes and potentially regulate male germ cell differentiation.

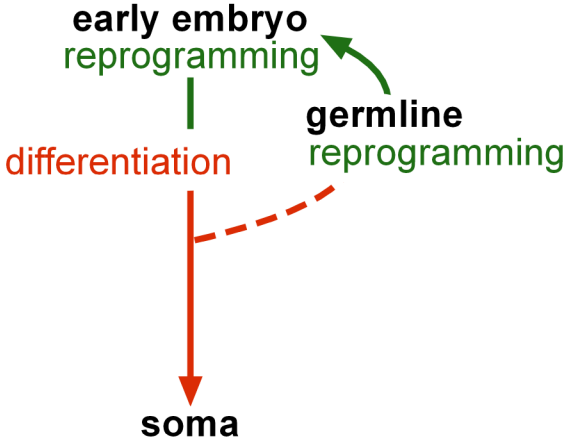
Hammoud et al., 2009. **Distinctive chromatin in human sperm packages genes for embryo development.

This study was the first to show genome-wide localization of histones and histone modifications in human sperm. The paper demonstrates that histones localize to specific sequences in human sperm and that the histone modifications H3K4me3 and H3K27me3 mainly localize to developmental promoters.

Brykczynska et al., 2010. **Repressive and active histone methylation mark distinct promoters in human and mouse spermatozoa.

This study shows that different histone modifications associate with distinct promoter sets in human sperm. H3K4me2 marks genes involved in spermatogenesis and cellular homeostasis,

while H3K27me3 marks developmental promoters. Importantly, the study also shows that histone modification states in human and mouse sperm are highly conserved.



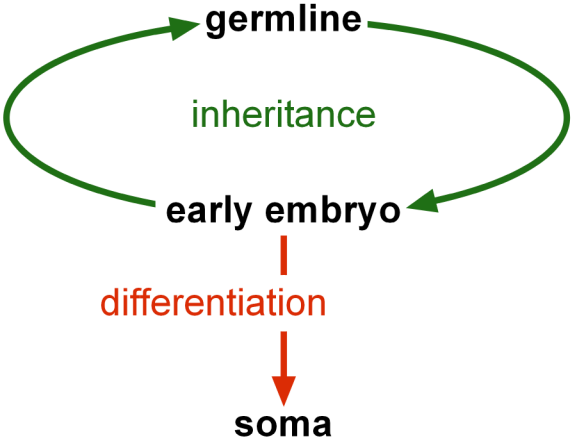


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Figure 1

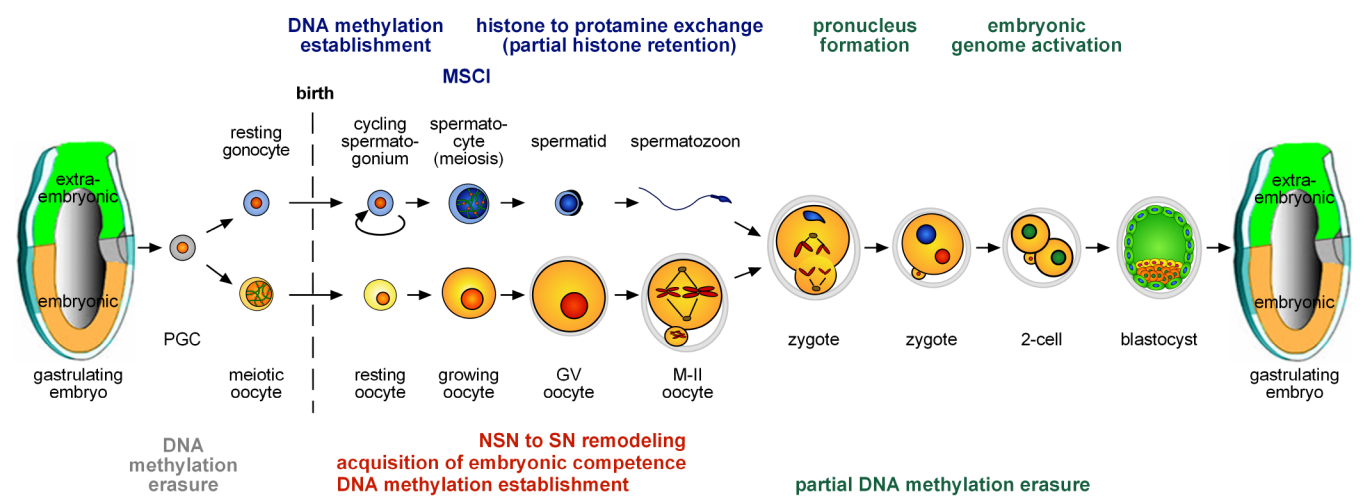

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Figure 2