Published in final edited form as:

Curr Opin Cell Biol. 2010 December ; 22(6): 737–743. doi:10.1016/j.ceb.2010.09.004.

Epigenetic regulation of germ cell differentiation

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Abstract

Germ cells and somatic cells have the identical genome. However, unlike the mortal fate of somatic cells, germ cells have the unique ability to differentiate into gametes that retain totipotency and produce an entire organism upon fertilization [1]. The processes by which germ cells differentiate into gametes, and those by which gametes become embryos, involve dramatic cellular differentiation accompanied by drastic changes in gene expression, which are tightly regulated by genetic circuitries as well as epigenetic mechanisms [2,3]. Epigenetic regulation refers to heritable changes in gene expression that are not due to changes in primary DNA sequence. The past decade has witnessed an ever-increasing understanding of epigenetic regulation in many different cell types/tissues during embryonic development and adult homeostasis. In this review, we focus on recent discoveries of epigenetic regulation of germ cell differentiation in various metazoan model organisms, including worms, flies, and mammals.

Keywords

Chromatin; epigenetics; germ cells; differentiation; development

Introduction

In eukaryotic nuclei, DNA is organized into nucleosomes by wrapping around histone octamers, which act as fundamental units to form higher order chromatin structure. Epigenetic mechanisms alter chromatin structure by changing the position, organization and composition of nucleosomes, while preserving primary DNA sequence. Epigenetic mechanisms contribute significantly to “cellular memory”, which maintains a particular cell fate through both mitotic and meiotic cell divisions [4–6]. Although the heritability of different epigenetic mechanisms is still under debate [7], for practical reasons, the epigenetic regulation discussed in this review comprises any change at the chromatin structural but not at the DNA sequence level. This includes DNA methylation, non-covalent chromatin remodeling, covalent post-translational modifications of histones (e.g. phosphorylation, acetylation, methylation, ubiquitination, etc. [8]), histone variants, and histone substitutes deposition (Note #1). The dynamics of epigenetic regulation is demonstrated by the variety of different mechanisms and the cross-talk among them, such as interactions between DNA

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methylation and histone modifications [9]. Recent research has identified a cohort of specific enzymes and factors that generate and recognize particular histone modifications, known as epigenetic “writers” and “readers”, respectively. For example, in mammals, methylation of H3K4 is “written” by the MLL(Mixed Lineage Leukemia) family histone methyl-transferase, which can be “read” by different proteins to execute a wide range of biological functions [10]. In addition, a third group of enzymes can remove (or “erase”) particular histone modifications, such as histone demethylases and deubiquitinating enzymes [11,12], allowing plasticity and reversibility of epigenetic regulation.

Among all known cell types, germ cells are unique due to their abilities to produce the next generation of an entire organism upon fertilization [1]. Because of their immortal nature, it is critical for germ cells to not only undergo proper cellular differentiation within one generation, but also retain accurate information to initiate the next generation. Specifically, epigenetic mechanisms may play roles in: (1) ensuring meiosis and terminal differentiation program during gametogenesis; (2) reliably retaining information in gametes for the next generation; (3) erasing improper features in zygotes before initiating a new life cycle, in order to prevent inheritable epimutations. Studies in many labs have contributed to a better understanding of how germ cells achieve these missions using various epigenetic mechanisms. In this review, we will discuss recent exciting findings following the journey of germ cells in both male and female model organisms. We will begin by discussing the epigenetic mechanisms that regulate gametogenesis, followed by those employed by gametes to retain proper information for embryogenesis while evading accumulation of deleterious epimutations.

Chromatin landscapes in undifferentiated germline stem cells (GSCs) and transit-amplifying germ cells

In many species, gametogenesis is initiated by GSCs, which undergo transit-amplification before committing to meiosis and terminal differentiation. The chromatin structure of GSCs acts as an intrinsic mechanism to maintain their unique self-renewal ability and block differentiation. Interestingly, the ATP-dependent chromatin remodeling factor, Imitation Switch (ISWI), is essential for both male and female GSC maintenance, at least partly by preventing premature differentiation, suggesting a common epigenetic mechanism employed by both sexes. In the *iswi* mutant flies, both female and male GSCs fail to self-renew and undergo premature differentiation due to precocious expression of the *bag of marbles* (*bam*) gene, which is necessary and sufficient to induce differentiation [13,14]. In male, it has been further characterized that such a requirement is specifically regulated by one particular ISWI-containing chromatin remodeling complex, the nucleosome-remodeling factor (NURF) complex [14].

In addition to chromatin remodeling, dynamic regulation of histone modifications is also required to maintain GSC identity. In *Drosophila*, a deubiquitinating enzyme encoded by the *scrawny* (*scny*) gene regulates the mono-ubiquitination status of the canonical histone H2B. Like Iswi, Scny is also required for both male and female GSC maintenance. In female GSCs, mutations in *scny* result in elevated levels of the acetylated H3 (H3Ac) in addition to ubH2B, which leads to more open chromatin and active transcription possibly at differentiation gene loci [15]. Therefore, distinct histone modifications may cross-talk and act in coordination to maintain the undifferentiated status of GSCs.

In mammalian embryonic and adult stem/progenitor cells, bivalent histone modifications (both active H3K4me3 and repressive H3K27me3) and paused RNA polymerase II (Pol II) for differentiation genes were identified as a unique chromatin landscape [16–19], which is thought to poise genes for robust expression upon stimuli [20]. In *Drosophila* testes, the

function of *bam* is also required for the transition from transit-amplifying spermatogonial cells to differentiating spermatocytes [21]. Mutations in *bam* result in accumulation of undifferentiated cells [22,23]. We found that in *bam* testes, in contrast to mammalian stem/progenitor cells, differentiation genes are labeled by either the repressive H3K27me3 alone or by no mark (active H3K4me3 or repressive H3K27me3). Furthermore, these genes are not associated with paused RNA polymerase II (Pol II). These apparent differences might result from the unipotent feature of *Drosophila* male germline, which may not require an intermediate status before turning on differentiation genes for spermatogenesis. Alternatively, the bivalent and poised status of genes may prevent DNA methylation, which represses gene expression in a more stable manner in mammals. Lack of DNA methylase activity in adult flies [24] may render these two chromatin signatures dispensable in *Drosophila* adult testes [20]. It will be interesting to explore whether a similar chromatin structure exists in the germ cells of other organisms.

Epigenetic regulation during meiosis and cellular differentiation of germ cells

Meiosis is a process unique to germ cells. At the genome-wide level, initiation of meiosis in *Drosophila* male gonads is accompanied by drastic down-regulation of many chromatin regulators and histone modifying enzymes with opposite enzymatic activities, for example, Pcaf histone acetyl-transferase (HAT) and Rpd3 histone deacetylase (HDAC) [25]. Therefore it is possible that the chromatin structure in meiotic cells is less labile compared to GSCs and transit-amplifying cells [26]. Down-regulation of these factors coincides with up-regulation of a group of cell-type and stage-specific homologs of general transcription machinery, the tTAFs (testis-specific TBP-associated factors), in *Drosophila* spermatocytes at meiotic prophase [27,28]. Function of the tTAFs counteracts Polycomb group (PcG) repression established in GSCs and transit-amplifying cells, by displacing Polycomb protein away from differentiation genes. At the same time, the tTAFs recruit Trithorax group (TrxG) activities to these genes and induce their robust expression [29]. Since PcG and TrxG oppose each other through establishing the repressive H3K27me3 and the active H3K4me3 histone modifications [4], the tTAFs appear to activate differentiation genes by controlling the switch from H3K27me3-enriched to H3K4me3-enriched pattern. It is also conceivable that histone modifying enzymes that remove and generate these histone modifications must act cooperatively to ensure these changes.

Indeed, histone modifying enzymes with similar or distinct activities act in an orchestrated manner in regulating germ cell differentiation. For example, both dSETDB1/Eggless and Su(var)3-9 are histone methyltransferases that generate the H3K9me3 mark, which recruits heterochromatin protein1 (HP1) and propagates heterochromatin formation. Interestingly, these two enzymes act in a temporal-specific manner in *Drosophila* ovaries: dSETDB1/Eggless is essential for early germ cells including GSCs, while Su(var)3-9 is required for maintaining the H3K9me3 mark in later staged germ cells [30,31]. It is possible that this sequential mode of action of histone modifying enzymes is mediated by co-factors, such as Windei, a dSETDB1/Eggless binding partner [32]. Through distinct co-factors in different stages of germ cell differentiation, the resulting H3K9me3 may become enriched at different targets to silence their transcription. In addition, histone modifying enzymes with opposing activities are both found to be required for proper meiosis. For example, mutations in enzymes that generate the repressive H3K9 methylation (e.g., Suv39 for H3K9me3 and G9a for H3K9me1/me2 [33,34]) and the active H3K4 methylation (e.g., Meisetz for H3K4me3 [35]) both exhibit defects in meiotic prophase during mouse spermatogenesis. The observation that the functionally opposite methylation of H3K9 and H3K4 are both involved in the same process of germ cell differentiation further highlights the dynamic nature of epigenetic regulation during meiosis.

Another dynamic epigenetic regulation during mammalian gametogenesis is the establishment of differential DNA methylation at imprinted genes, which are differentially expressed according to their parental origin and important for embryonic development. In females, genomic imprinting occurs in oocytes arrested at meiosis I [36]. Recent studies have illuminated more molecular mechanisms regarding establishment and maintenance of methylation imprints in oocytes. As a novel mechanism, transcription across protein-coding sequence is required to establish DNA methylation pattern at several maternally imprinted genes in mouse [37], which is different from the involvement of noncoding RNAs in silencing imprinted genes in somatic cells [38]. Moreover, a histone modifying enzyme KDM1B is required for *de novo* DNA methylation at a subset of maternally imprinted genes during mouse oogenesis, which is likely dependent on its H3K4 demethylase activity [39]. In contrast to imprinting in oogenesis, the exact timing of paternal imprinting during mammalian spermatogenesis was not clear. A recent report has revealed that *de novo* DNA methylation in mouse male germline occurs mainly in spermatocytes at early meiotic prophase, in addition to spermatogonial cells [40]. Further studies are needed to understand more molecular mechanisms that underlie establishment and maintenance of paternally imprinted genes in mammals.

Epigenetic regulation in gametes: in preparation for the next generation

During spermiogenesis in post-meiotic germ cells from *Drosophila* [41] to mammals [3], histones are displaced by the transition nuclear proteins (Tnps) followed by protamines (Prms), allowing for extensive chromatin condensation and DNA packaging in sperm nuclei. This process is regulated by a variety of epigenetic mechanisms, including histone modifications, chromatin remodeling, and histone variants deposition.

Histone phosphorylation, acetylation, and methylation all act in a temporally distinct manner in the regulation of spermiogenesis. Enriched phosphorylation of H4S1 in post-meiotic gametes is evolutionarily conserved from yeast to flies and mice. An H4 S1A substitution or mutations in a member of the Ste20 kinase family abolish this phosphorylation and result in defective sporulation in yeast [42]. It will be interesting to examine whether similar mutations in flies and mice will lead to gametogenesis defects [43]. In addition to phosphorylation, the JmjC-domain-containing histone demethylase 1A/2A (JHDM1A/2A) is highly expressed in meiotic and post-meiotic male germ cells in mice. Demethylation of H3K9me2/1 by JHDM1A/2A is necessary to increase the expression of both *Tnps* and *Prms*. Interestingly hypomethylation of H3K9 also positively affects the global hyperacetylation of H3/H4, another known histone modification that ensures histone displacement and chromosome condensation. Therefore *Jmjd1A/2A* mutant male mice have smaller testes and are sterile [44,45]. Another chromatin regulator that facilitates H3 hyperacetylation in mice is a plant homeodomain (PHD) containing protein, called Pygopus 2 (Pygo2). Pygo2 is expressed in elongating spermatid, which may directly recruit a HAT enzyme to acetylate H3 and enhance expression of *Tnps* and *Prms* [46]. Before histones are displaced by Prms, a set of histone variants, including two H2A variants (H2AL1 and H2AL2) and a H2B variant (TH2B), reorganize the pericentric heterochromatin region for such a nucleoprotein transition in mice [47].

Intriguingly, not all histones are substituted by Prms in mature human sperm. In fact, approximately 4% of the human haploid genome in sperm retains nucleosomes. More strikingly, genes required for early embryonic development, such as the *Homeobox* (*Hox*) genes and imprinted genes, retain nucleosomes. In addition, the bivalent chromatin signature for developmental regulators in embryonic stem cells (ESCs) is already detectable in sperm. The promoter regions of the developmental regulators are also hypomethylated in sperm [48]. It is possible that the bivalent chromatin signature of these developmental regulators

prevents them from being highly methylated in human sperm. In mice, the overall DNA methylation pattern in sperm is similar to that of ESCs and embryonic germ cells [49]. These exciting discoveries in mature mammalian sperm, together with a recent report that the establishment of the unique chromatin status in ESC occurs at the initiation of zygotic transcription in zebrafish [50], suggest that sperm epigenome has been substantially reset for pluripotency and such information could be “memorized” by embryonic cells even after zygotic transcription is initiated. However, exceptions are genes required for ESC pluripotency, such as *Nanog*, which is hypermethylated and silenced in mouse sperm but highly expressed in ESCs [49]. The difference in gametes and cells derived from early staged embryos (e.g. ESCs) suggests that the zygotic epigenome still needs to be reprogrammed, at least at particular genes, in order to return to pluripotency.

From gametes to embryos: remember and only remember what should be memorized

After fertilization, Prms are replaced by maternal histones in the male pronucleus. This process is initiated by a histone chaperon, HIRA, and facilitated by an ATP-dependent chromatin remodeler, CHD1, by assembling an intermediate nucleosome with the histone variant H3.3 in *Drosophila* [51–53]. HIRA is essential for H3.3-containing nucleosome formation only in male pronucleus [53]. Despite their essential functions in forming nucleosomes, removal of Prms was unaffected in both *hira* and *chd1* maternal mutants, suggesting that Prm removal is independent of, but may be prerequisite for, nucleosome formation in male pronucleus [52,53].

The formation of a zygote is a milestone in the onset of a new life. Somatic cells start a new cycle by erasing and re-establishing their epigenomes. Because of the immortal nature of germ cells, there are mechanisms that ensure the proper epigenetic memory is transmitted to the next generation's germ cells. Up to date, the best understood mechanism for epigenetic inheritance is DNA methylation. In post-fertilized embryos, primordial germ cells (PGCs) are specified from the soma and lead to the formation of GSCs in the new generation. In mice, such a process commences with genome-wide erasure of DNA methylation, followed by removal of several histone modifications and exchange of histone variants [54]. The AID-mediated deamination contributes to DNA demethylation in mouse PGCs [55]. Global erasure of DNA methylation in PGCs provides a potential molecular mechanism to prevent transgenerational epigenetic inheritance, in order to evade epimutations.

An intriguing study in *C. elegans* suggests that tight control of histone modifications is also required to restrict epimutations transgenerationally: An H3K4me2 demethylase SPR-5 has been shown to accumulate in adult gonads and early staged embryos, indicating its potential roles in resetting epigenomes between generations. Because of the demethylase activity of SPR-5, mutation in the *spr-5* leads to significant H3K4me2 retention at genes required for spermatogenesis, resulting in their mis-expression and eventual sterility. Interestingly, the *spr-5* mutant shows progressive sterility over 20 generations, suggesting that the H3K4me2 mark can be inherited transgenerationally in germ cells and the enzyme erasing this histone modification serves as the guardian to prevent this from occurring [56]. Not only does improper epigenetic inheritance of germ cells affect germline differentiation and fertility [56], it also reduces fitness of somatic cells and longevity. A recent study reported multiple histone modifying enzymes, including histone methyl-transferase and demethylase, act together to ensure proper H3K4me3 level in germline to maintain normal lifespan of *C.elegans* [57].

Conclusions and perspectives

Research in recent years has greatly improved our understanding of epigenetic regulation of germ cell differentiation and function in various model organisms (see Figure 1 for summary). Along the journey of germ cells from one generation to the next, they need to keep the appropriate epigenetic memory, which is ensured by dynamic establishment, erasure and re-establishment of their epigenome. However, it is still challenging to study epigenetic regulation at individual genes in particular staged germ cells, such as PGCs [55] and GSCs. A major technical hurdle is to obtain sufficient homogeneous cells to investigate their chromatin structure. New technology that can significantly reduce the required cell number for such studies will revolutionize the analysis of germ cell differentiation, which will be invaluable not only for treatment of diseases due to defects in germ cell differentiation, such as infertility and germ cell tumors, but also for applying germ cells in regenerative medicine because of the unique features of this cell type.

Acknowledgments

We apologize to people whose work cannot be discussed in this review due to space limitation. We thank Chen lab members, Drs. Mark Van Doren and Keji Zhao for their critical comments on this review. The work in the Chen lab has been supported by Research Grant No. 05-FY09-88 from the March of Dimes Foundation, the R00HD055052 NIH Pathway to Independence Award and R21 HD065089 from NICHD, the 49th Mallinckrodt Scholar Award from the Edward Mallinckrodt, Jr. Foundation, the American Federation of Aging Research, the Lucile Packard Foundation, and the Johns Hopkins University start-up funding.

References and recommended reading

Note #1: The non-coding RNA regulation is discussed in another review in this series (refer to Bortvin's review).

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

** Buszczak 2009: This paper shows that a deubiquitinating enzyme for ubH2B, encoded by *scrawny* (*scny*), is essential for both male and female GSC maintenance. In addition to GSC, Scny is also required for *Drosophila* intestinal stem cell maintenance, implicating a common epigenetic mechanism used by multiple adult stem cell systems for their self-renewal abilities.

* Cherry 2010: This paper shows one of the three ISWI-containing chromatin remodeling complexes, the NURD complex, is required for *Drosophila* male GSCs. Together with a previous publication (Xi et al., 2005, Science 310:1487), ISWI chromatin remodeling complex has been shown to maintain both male and female GSC identities through interactions of distinct signaling pathways: JAK-STAT signaling in male and BMP (bone morphogenetic protein) signaling in female.

* Chotalia 2009: This paper shows a novel mechanism that transcription of protein-coding sequence is required to establish differential DNA methylation pattern at imprinted genes in mouse female germline, which is different from transcription of noncoding antisense RNAs in silencing monoallele of imprinted genes in somatic cells.

** Ciccone 2009: This paper shows that H3K4 demethylase KDM1B is required for *de novo* DNA methylation during mouse oogenesis, which makes a connection of erasing histone modification with establishing DNA methylation during germ cell differentiation.

** Farthing 2008: This paper shows that the global view of promoter methylation pattern in mouse sperms is very similar to that in embryonic stem cell or embryonic germ cell, suggesting that the sperm epigenome has been substantially reprogrammed for pluripotency and such information can be transmitted to cells in early staged embryos. However, DNA methylation pattern at pluripotent genes is different in sperm compared to embryonic stem cell, suggesting that such an epigenetic memory is gene-specific.

** Gan 2010 (Genome Biology): This paper demonstrates monovalent histone modification and unpoised status for most genes required for differentiation, using the *Drosophila bam* mutant testes that are enriched with undifferentiated cells. These data reveals that *Drosophila* male germ cells have a chromatin landscape different from embryonic stem cells or other mammalian stem/progenitor cells.

** Hammoud 2009: This paper demonstrates that developmental regulators for embryogenesis retain nucleosomal structure and are hypomethylated. These genes also carry bivalent histone modification similar to the pattern found in embryonic stem cell. These data suggest that some of the epigenomic information in sperm can be memorized in embryos.

** Katz 2009: This paper shows that mis-regulation of histone modification can be an epigenetic memory retained in germ cells and transmitted across many generations. Such an inappropriate memory leads to sterility.

* Konev 2007: This paper showed that the ATP-dependent nucleosome remodeling factor CHD1 is required for displacing Prm with canonical histones in male pronucleus in zygote, through an intermediate H3.3-containing nucleosomal structure. Interestingly, CHD1 is not needed to remove Prm as the first step for this transition.

* Popp 2010: This paper showed that AID acts as a positive regulator to erase DNA methylation in mouse PGC. In addition, mutations in *AID* have different effect on male and female PGC.

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Figure 1. Summary of different epigenetic mechanisms in regulating germ cell differentiation during metazoan life cycle

Male (blue circles) and female (pink circles) germ cells at equivalent differentiation stages are aligned vertically. Chromatin regulators, histone modifying enzymes and histone modifications regulated by them are placed according to the stages they are functional. Solid lines represent direct enzymatic reaction and dotted lines represent potential indirect regulation. Sperm and early embryonic cells in mammals share similar epigenomic signatures, indicated by the dotted circle. Different metazoan model organisms discussed in this review are labeled as *C. elegans* (C), *Drosophila* (D), and mouse (M). Refer to the text for detailed illustration and discussion of these data.