Published in final edited form as:

Curr Opin Genet Dev. 2015 April ; 31: 20–27. doi:10.1016/j.gde.2015.04.003.

Epigenetic Regulation of Germ Cells— Remember or Forget?

Lijuan Feng¹ and Xin Chen^{1,2}

¹Department of Biology, The Johns Hopkins University, Baltimore, MD 21218

Abstract

Unlike somatic cells, germ cells retain the potential to reproduce an entire new organism upon fertilization. In order to accomplish the process of fertilization, germ cells undergo an extreme cellular differentiation process known as gametogenesis in order to produce morphologically and functionally distinct oocyte and sperm. In addition to changes in genetic content changes from diploid to haploid, epigenetic mechanisms that modify chromatin state without altering primary DNA sequences have profound influence on germ cell differentiation and moreover, the transgenerational effect. In this review, we will go over the most recent discoveries on epigenetic regulations in germline differentiation and transgenerational inheritance across different metazoan species.

Keywords

Germ cells; somatic cells; germline stem cell; epigenetics; transcription; transgenerational inheritance

Introduction

The dichotomy of germline and soma represents the earliest lineage specification among many metazoan organisms. In a living organism, germ cells reside in microenvironments contributed by somatic cell types. Communications between germline lineage and their microenvironments are critical for their proper activity throughout the lifetime.

Epigenetic mechanisms play crucial roles in defining germ cell identity and regulating germ cell activity, which is required for precise differentiation into functional gametes and transmission of proper information to the next generation. Among all epigenetic modifications, the mechanisms of DNA methylation and histone modifications are the best understood. However, DNA methyltransferase activity is almost negligible in adult flies [1–4] and worms [5], simplifying epigenetic regulation in these organisms to occur mainly through changes in the modification or positioning of canonical histones (i.e. H3, H4, H2A

© 2015 Published by Elsevier Ltd.

²Correspondence to: Xin Chen, Ph.D. Department of Biology, 3400 North Charles Street, The Johns Hopkins University, Baltimore, MD 21218-2685, Tel: 410-516-4576; Fax: 410-516-5213; xchen32@jhu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

and H2B) and histone variants (e.g. H3.3, H2A.Z). Another important epigenetic regulator is contributed by small noncoding RNAs, such as microRNAs [6] and PIWI-interacting RNAs (piRNAs) [7], that regulate gene and transposon/retrotransposon expression or function, respectively.

Epigenetic regulation as intrinsic mechanisms to regulate germline stem cells (GSCs)

In several model organisms, gametogenesis depends on constant GSC functionality [8, 9]. Like many other types of adult stem cells, GSCs undergo asymmetric cell divisions to give rise to two daughter cells with distinct cell fates: one that self-renews to retain stem cell identity and another one that commits to differentiation. These two daughter cells arise from the same mitotic event and therefore inherit an identical genetic code. Although it is conceivable that the epigenetic information is different in these two cells, the nature of the distinct epigenetic code had not been identified. Using a dual-color labeling strategy to label pre-existing vs. newly synthesized canonical histone H3 precisely, our lab has shown that during the asymmetric division of the *Drosophila* male GSC, the preexisting H3 is selectively segregated to the self-renewed GSC daughter cell whereas newly synthesized H3 is enriched in the differentiating daughter cell [10] (Fig. 1). Since post-translational modifications of histones are key epigenetic information, our studies provide the first direct evidence suggesting that GSC may selectively retain preexisting histones that define their stem cell identity whereas the other daughter cell may preferentially reset their chromatin to prepare for differentiation. Another study using CO-FISH (chromosome orientation fluorescence *in situ* hybridization) technique combined with chromosome- and strand-specific probes has shown that sex chromosomes (X and Y) have biased segregation of sister chromatids in the same system (Fig. 1). Although autosomes do not display such a bias, they show another interesting co-segregation pattern [11]. An earlier paper using nucleoside analog 5-bromo-2-deoxyuridine (BrdU) incorporation assay showed lack of “immortal strand” in this system [12]. Together, these studies suggest that biased segregation of sister chromatids of sex chromosomes might be due to different epigenetic information they carry. However, the connection between asymmetric histone inheritance and biased sister chromatid segregation of sex chromosomes remains unclear. It would be interesting to find out whether mutations disrupting one segregation pattern will affect the other pattern.

Even though the histone variant H3.3 is inherited symmetrically at a global level during GSC asymmetric division [10], another histone variant H2Av, the *Drosophila* homolog of mammalian H2A.Z/H2A.X, is required for male GSC maintenance. Accordingly, the ATP-dependent chromatin-remodeling factor Domino required for proper H2Av association with DNA also help maintain GSC [13]. However, H2Av is not required for germ cell differentiation, as several differentiation markers show proper expression in *H2Av* mutant germ cells. Lack of H2Av does not result in global changes in H3K4me3 or H3K27me3 immunostaining pattern; neither does it causes altered Janus kinase signal transducer and activator of transcription (JAK-STAT) response in GSCs, a critical pathway for GSC maintenance (reviewed by [14]). However, it is possible that *H2Av* mutation disrupts H3K9me2/3-enriched heterochromatin structure in GSCs, as shown in somatic cells

previously ([15]). Phosphorylation of H2Av (γ H2Av) serves as a specific marker for double-strand DNA break which often indicates early response to DNA damage. Consistently, it was reported that mutations in H2Av enhance *Drosophila* male germline defects caused by DNA damage, resulting from mutations in a Polycomb group (PcG) gene *multi sex combs* (*mxc*) [16]. Suppression of *mxc* phenotype by reduction in H3 and enhancement by *H2Av* mutation are not restricted to germline but also apply to somatic cells, suggesting a broader connection between histones and PcG function that may not be mediated through a particular histone modification.

Our understanding of the biological functions of particular histone modifications is primarily derived from studies of mutations that inactivate the particular histone modifying enzyme that ‘writes’, ‘reads’, or ‘erases’ such a modification. For example, *eggless/dSETDB1* encodes a H3K9 methyltransferase and *egg* mutants show GSC self-renewal defects in *Drosophila* ovary. However, the GSC loss defects are not caused by loss-of-function in the well-known bone morphogenetic protein (BMP) signaling pathway in female GSCs. Therefore these findings suggest a potential H3K9me3-dependent but BMP-independent GSC maintenance mechanism [17]. Another study on *Eggless* revealed its function in transcriptional regulation of piRNA clusters [18]. Activated piRNAs act with the PIWI protein to protect germline genome by preventing transposable element activity, which may be a conserved mechanism for germline genome integrity across species [19–23]. A few microRNAs have also been found to regulate *Drosophila* female GSC maintenance or differentiation. For example, *miR-184* [24] and *bantam* [25] are both required for the balance of GSC maintenance vs. differentiation in ovaries. Together, these results strongly suggest GSCs have a unique epigenetic state to help maintain stem cell fate probably by activating and maintaining ‘stemness’ gene expression while repressing differentiation gene expression.

Epigenetic mechanisms cooperate with extrinsic signaling to regulate germline function

Extrinsic cues from the somatic cells in the ‘microenvironment’ or ‘niche’ must interplay with intrinsic factors in germ cells to determine their functionality. The somatic cells in gonads could play a passive role for germ cell differentiation. For example, two PcG chromatin regulators Psc and Su(z)2 act in somatic gonadal cells to maintain their identity and restrict them from overproliferation. Loss of their function leads to somatic cell tumor and affect germline function by physically displacing GSCs from their niche [26]. Recent studies demonstrate that somatic gonadal cells also play active roles in determining germ cell identity and activity. For example, inactivation of the H3K27me3-specific ‘writer’ E(z) methyltransferase in somatic gonadal cells causes the neighboring germ cells to adopt somatic cell fate [27]. This germline cell fate change is restricted to early-stage germ cells including GSCs, suggesting that epigenetic plasticity declines during cellular differentiation. It is conceivable that this non-cell-autonomous regulation of germline through somatic gonadal cells must act through some signaling pathway(s). Indeed, multiple signaling pathways have been identified for their critical roles for germ cell function, such as JAK-STAT, BMP, epidermal growth factor (EGF) [28], Notch [29], ecdysone [30], insulin [31]

and Slit-Roundabout [32] pathways. Interestingly, in many cases signaling pathways act through chromatin regulators. For example, the steroid hormone ecdysone signaling promotes female GSC maintenance through chromatin remodeling factors such as ISWI and Nurf301 acting in GSCs [33]. A functional interaction between ecdysone hormone signaling and Nurf301 was also seen in *Drosophila* male GSCs, even though in this context the ecdysone signaling acts in somatic cells to regulate germline [34], suggesting a sex-specific response. Recently, technological advancements have allowed identification of direct target genes in a cell-type-specific manner for both chromatin factors and transcription factors downstream of signaling pathways, which has helped facilitate our understandings of such a crosstalk. For example, the Wnt and EGF pathway genes are identified as direct targets of H3K27me3-methyltransferase E(z) in somatic cells and *egfr* mutants show strong genetic interaction with *E(z)* mutant in fly testes [27]. The H3K9me3-methyltransferase Eggless is required in escort cells to regulate *Drosophila* female germ cell differentiation partially through controlling BMP signaling [17] (Fig. 2). Epigenetic ‘erasers’ such as histone demethylases also regulate germ cells non-cell-autonomously. For example, the H3K27me3-specific demethylase dUTX removes the repressive H3K27me3 mark at the upstream region of the *Socs36E* gene in somatic cells of fly testes. Because *Socs36E* is an inhibitor of the JAK-STAT pathway, this function of dUTX prevents hyperactivation of JAK-STAT signaling and maintains proper gene expression and architecture of the testis niche and male GSC function [35]. In *Drosophila* ovary, H3K4me1/2-demethylase Lsd1 function in escort cells on a diverse group genes, including both BMP-related and BMP-unrelated genes [36]. Therefore, Lsd1 regulates germline differentiation by preventing ectopic BMP signaling outside of niche [36, 37] as well as using BMP-independent mechanisms. In addition to histone modifying enzymes, nuclear lamina regulates specific nucleoporin distribution which in turn promotes nuclear localization of phosphorylated ERK, the downstream effector of the EGF pathway [38] (Fig. 2). This activity of Lamin occurs in somatic gonadal cells but affects the neighboring germline function, which shows how nuclear structure acts as another layer of epigenetic regulation to modulate readout of signaling pathway and regulate germ cell activity.

Epigenetic regulation during germ cell differentiation pathway

Before commitment for meiosis and terminal differentiation, progenitor germ cells normally undergo a transit-amplification stage as mitotic cells. Transit-amplifying (TA) germ cells differ from *bona fide* GSCs in several ways. The progression from GSC to TA germ cells in a loss of asymmetric histone inheritance [10] and the potential to turn on a somatic-specific transcription factor [27]. In spite of these differences at the morphological level, TA germ cells have the potential to become GSC-like cells through a dedifferentiation process (reviewed by [39, 40]). However, it remains unclear whether dedifferentiated GSC-like cells recover both molecular and cellular characteristics like *bona fide* GSCs. For example, the well-oriented centrosomes in GSCs are not maintained in dedifferentiated GSC-like cells, leading to a G2/M arrest and declined mitotic activity [41]. More studies are needed to compare molecular features, including chromatin structure and transcriptional profile, between GSCs and dedifferentiated germ cells, to fully understand this *in vivo* cellular reprogramming process.

Different from transit-amplifying cells, germ cells cannot dedifferentiate once turning on the meiotic program [42], suggesting fundamental differences of chromatin state between mitotic and meiotic germ cells. High-throughput approaches have been used to identify meiosis-specific histone modifications and interestingly many of them are conserved across species, suggesting potentially common epigenetic mechanisms during meiosis. For example, it is feasible to use yeast sporulation for a genetic screen to identify histone mutants that affect spermatogenesis in higher organisms. This method has led to discoveries of H4S1P and H3T11P as key meiotic modifications in flies and mice [43, 44]. Another proteomic approach identified a novel H3K23me3 as another conserved meiosis-specific histone modification from *Tetrahymena* to *C. elegans* [45].

Intriguingly, meiotic epigenetic regulation has sex-specificity. In the *Drosophila* ovary, only one germ cell per germline cyst differentiates into oocyte while the rest enter into a cell cycle variation known as an endocycle to become nurse cells. Conversely, in male germline, all mitotic germ cells enter meiosis and differentiate to sperm. Transdetermination from oocyte to nurse-like cells was observed when PcG components E(z) and Su(z)12 are knocked down in *Drosophila* female germline. This cell fate change results from derepression of Cyclin E and cyclin-dependent kinase inhibitor Dacapo upon loss of the repressive H3K27me3 mark [46]. However, such a cell fate switch does not occur in male germline, suggesting that differences between female and male germline differentiation pathways require distinct epigenetic regulators. Consistently, important post-transcriptional regulators microRNAs have distinct roles in regulating male versus female germline differentiation in *Drosophila*. Bag-of-marbles (Bam) has critical but distinct roles in regulating male versus female germ cell differentiation [47]. In meiotic male germ cells, microRNAs downregulate Bam protein but not *bam* mRNA through their interactions with the *bam* 3'UTR. Failure to achieve this downregulation leads to sperm differentiation defects [48]. However, this regulation does not happen in female germline. Another example is male germline-specific regulation of Wnt signaling pathway by microRNAs: both β -catenin and the downstream transcription factor TCF are downregulated by microRNAs in *Drosophila*. Loss of such an antagonization leads to male germline differentiation defects and decreased fertility [49]. In addition, piRNAs are the largest group of small non-coding RNAs and play important roles in germline. For example, a recent study using *Maelstrom* mutant mice lacking piRNAs shows increased retrotransposon LINE-1 activity and extensive elimination of oocytes, providing an interesting connection between the piRNA level and fetal oocyte quality control [50]. In *Maelstrom* mutant adult male mice, lack of piRNAs may indirectly lead to failure in translation of many mRNAs required for sperm differentiation, resulting in male sterility [51].

During spermatid maturation, the switch from histones to protamine is a conserved process to ensure highly compact paternal genome (reviewed by [52, 53]). The histone-to-protamine switch requires coordinated activity of histone variants and modified histones to destabilize nucleosome structure followed by histone degradation and replacement with small basic proteins such as protamine. Both H4 hyperacetylation [54] and H3K79 methylation [55, 56] are reported to be conserved modifications prior to this transition. The following replacement of histones with protamines is initiated by degrading core histones in an

acetylation-dependent but polyubiquitin-independent manner in mice [57]. Independent of histone removal, assembly of protamine-based chromatin in sperm requires function of the canonical histone chaperone CAF1 [58].

Transgenerational epigenetic inheritance

As discussed earlier in this review, histone modifying enzymes and modified histones are known for their important roles in regulating germline identity and activity. However, it is not well understood whether and how this information in germline from parents could pass onto their progenies; and if so, whether somatic cells and/or germ cells of the progenies inherit this information. Recent published work sheds some light on this process. A recent study in *C. elegans* utilized elegant genetics to generate embryos that lack H3K27me3-writing enzyme and can only inherit H3K27me3 from parents. Interestingly, under this condition H3K27me3 stays with the genome where the marker is generated for quite a few cell cycles in early embryos, suggesting that both sperm and oocyte can pass epigenetic information to early embryos. However, maintenance of histone modification requires H3K27me3-writing enzyme, suggesting that modified histones and histone modifying enzymes cooperate to pass the epigenetic information across generations (Fig. 3). Similar effect was also observed for another repressive modification H3K9me2 acting with H3K9me2-writing enzyme [59]. In addition to repressive marks, modifications associated with active transcription such as H3K4me2 can also be inherited through many generations. For example, in *C. elegans* the H3K4me2-demethylase is encoded by *spr-5*, whose mutants display decreased fertility across generations along with global accumulation of H3K4me2 [60]. This progressive sterility phenotype provides a sensitized background to screen for genetic modifiers, from which a cohort of histone modifying enzymes have been identified as either enhancers or suppressors, including H3K4me1/2-writing enzyme, H3K9me3-writing, reading and erasing enzymes [61] (Fig. 3). Because these enzymes have antagonizing functions toward either active (e.g. H3K4me1/2) or repressive (e.g. H3K9me3) mark, it suggests that a balance between euchromatin and heterochromatin is needed for transmitting epigenetic information properly to the offspring. Noticeably, a very recent study demonstrates a potential connection between transgenerational epigenetic inheritance and obesity in mice and human [62].

In vertebrates DNA methylation is another important epigenetic player in addition to histone modifications. Recent study in zebrafish (Fig. 3) show that paternal but not maternal DNA methylation profile retained during spermatogenesis has similar pattern with those in early stage embryos [63]. On the other hand, the maternal DNA methylome is reprogrammed and acts with paternal DNA methylome to establish a totipotent zygotic epigenome [64]. Because studies in mice and human show similar phenomenon [65, 66], it is of particular interest to understand how gametogenesis resets chromatin while retains certain epigenetic information; and whether abnormality in this process leads to transgenerationally inheritable diseases.

In addition to histone modifications and DNA methylation, piRNAs also contribute to transgenerational epigenetic inheritance. In *Drosophila*, maternally deposited piRNAs are important for transposon silencing in the next generation, without which progenies become

sterile [67]. Recent studies in multiple strains from two different *Drosophila* species demonstrate that transgenerationally transmitted piRNA can serve as a trigger to promote piRNA biogenesis by changing the chromatin state at piRNA precursor region in progenies [68, 69] (Fig. 3). It has been shown that piRNAs and their associated proteins contribute to transgenerational gene silencing in germline in *C.elegans* [70]. More studies are needed to understand how different epigenetic mechanisms cooperate to ensure proper transgenerational inheritance to maintain somatic fitness as well as germline integrity and functionality.

Conclusions and perspectives

Because germ cells carry a unique mission to pass information from generation to generation, it is critical for them to ‘remember’ proper but ‘forget’ wrong information. Therefore gametogenesis faces challenges to not only turn on gene expression for meiosis and terminal differentiation of gametes but also erase markers that would be harmful for next generations. Studies in model organisms reveal multiple intrinsic regulators and extrinsic signaling pathways acting to make these decisions. Together with the ever-improving genetic and genomic tools, we anticipate that the next a few years will witness a more comprehensive and detailed picture of epigenetic regulation in germ cells.

Acknowledgements

We apologize to people whose work cannot be discussed here due to space limitation. Supported by NIH R01HD065816 and R01GM112008, the David and Lucile Packard Foundation, Johns Hopkins University start-up (X.C.).

References and recommended reading

Special interest * Outstanding interest **

1. Richards EJ, Elgin SC. Epigenetic codes for heterochromatin formation and silencing: rounding up the usual suspects. *Cell*. 2002; 108(4):489–500. [PubMed: 11909520]
2. Lyko F, Ramsahoye BH, Jaenisch R. DNA methylation in *Drosophila melanogaster*. *Nature*. 2000; 408(6812):538–540. [PubMed: 11117732]
3. Hung MS, et al. *Drosophila* proteins related to vertebrate DNA (5-cytosine) methyltransferases. *Proc Natl Acad Sci U S A*. 1999; 96(21):11940–11945. [PubMed: 10518555]
4. Lyko F, et al. The putative *Drosophila* methyltransferase gene dDnmt2 is contained in a transposon-like element and is expressed specifically in ovaries. *Mech Dev*. 2000; 95(1–2):215–217. [PubMed: 10906465]
5. Simpson VJ, Johnson TE, Hammen RF. *Caenorhabditis elegans* DNA does not contain 5-methylcytosine at any time during development or aging. *Nucleic Acids Res*. 1986; 14(16):6711–6719. [PubMed: 3748820]
6. Chuang JC, Jones PA. Epigenetics and microRNAs. *Pediatr Res*. 2007; 61(5 Pt 2):24R–29R.
7. Castaneda J, Genzor P, Bortvin A. piRNAs, transposon silencing, and germline genome integrity. *Mutat Res*. 2011
8. Spradling A, et al. Germline stem cells. *Cold Spring Harb Perspect Biol*. 3(11):a002642. [PubMed: 21791699]
9. Kimble J, Seidel H. *C. elegans* germline stem cells and their niche. 2008
10. Tran V, et al. Asymmetric division of *Drosophila* male germline stem cell shows asymmetric histone distribution. *Science*. 2012; 338(6107):679–682. [PubMed: 23118191] ** This paper shows that during asymmetric division of *Drosophila* male GSCs, preexisting canonical histone

H3 are segregated to GSCs while newly synthesized H3 go to differentiating daughter cells. This selective inheritance does not apply to histone variants H3.3 or symmetrically dividing tumor-like GSCs.

11. Yadlapalli S, Yamashita YM. Chromosome-specific nonrandom sister chromatid segregation during stem-cell division. *Nature*. 2013 ** This paper provides direct evidence suggesting that sister chromatids of X and Y chromosome instead of autosomes undergo nonrandom segregation during asymmetric division of *Drosophila* male GSCs. This nonrandom segregation is disrupted in overproliferating as well as dedifferentiated GSCs.
12. Yadlapalli S, Cheng J, Yamashita YM. *Drosophila* male germline stem cells do not asymmetrically segregate chromosome strands. *J Cell Sci*. 2011; 124(Pt 6):933–939. [PubMed: 21325028]
13. Morillo Prado JR, Srinivasan S, Fuller MT. The histone variant His2Av is required for adult stem cell maintenance in the *Drosophila* testis. *PLoS Genet*. 2013; 9(11):e1003903. [PubMed: 24244183]
14. de Cuevas M, Matunis EL. The stem cell niche: lessons from the *Drosophila* testis. *Development*. 2011; 138(14):2861–2869. [PubMed: 21693509]
15. Swaminathan J, Baxter EM, Corces VG. The role of histone H2Av variant replacement and histone H4 acetylation in the establishment of *Drosophila* heterochromatin. *Genes Dev*. 2005; 19(1):65–76. [PubMed: 15630020]
16. Landais S, D'Alterio C, Jones DL. Persistent replicative stress alters polycomb phenotypes and tissue homeostasis in *Drosophila melanogaster*. *Cell Rep*. 2014; 7(3):859–870. [PubMed: 24746823]
17. Wang X, et al. Histone H3K9 trimethylase Eggless controls germline stem cell maintenance and differentiation. *PLoS Genet*. 2011; 7(12):e1002426. [PubMed: 22216012]
18. Rangan P, et al. piRNA production requires heterochromatin formation in *Drosophila*. *Curr Biol*. 2011; 21(16):1373–1379. [PubMed: 21820311]
19. Sienski G, Donertas D, Brennecke J. Transcriptional silencing of transposons by Piwi and maelstrom and its impact on chromatin state and gene expression. *Cell*. 2012; 151(5):964–980. [PubMed: 23159368]
20. Rozhkov NV, Hammell M, Hannon GJ. Multiple roles for Piwi in silencing *Drosophila* transposons. *Genes Dev*. 2013; 27(4):400–412. [PubMed: 23392609]
21. Le Thomas A, et al. Piwi induces piRNA-guided transcriptional silencing and establishment of a repressive chromatin state. *Genes Dev*. 2013; 27(4):390–399. [PubMed: 23392610]
22. Huang XA, et al. A major epigenetic programming mechanism guided by piRNAs. *Dev Cell*. 2013; 24(5):502–516. [PubMed: 23434410]
23. Malone CD, et al. Specialized piRNA pathways act in germline and somatic tissues of the *Drosophila* ovary. *Cell*. 2009; 137(3):522–535. [PubMed: 19395010]
24. Iovino N, Pane A, Gaul U. miR-184 has multiple roles in *Drosophila* female germline development. *Dev Cell*. 2009; 17(1):123–133. [PubMed: 19619497]
25. Yang Y, et al. The bantam microRNA is associated with *drosophila* fragile X mental retardation protein and regulates the fate of germline stem cells. *PLoS Genet*. 2009; 5(4):e1000444. [PubMed: 19343200]
26. Morillo Prado JR, Chen X, Fuller MT. Polycomb group genes Psc and Su(z)2 maintain somatic stem cell identity and activity in *Drosophila*. *PLoS One*. 2012; 7(12):e52892. [PubMed: 23285219]
27. Eun SH, et al. A non-cell autonomous role of E(z) to prevent germ cells from turning on a somatic cell marker. *Science*. 2014; 343(6178):1513–1516. [PubMed: 24675960] ** This paper demonstrates that E(z), the writer of repressive H3K27me3 marker, is required in somatic cells to antagonize germline-to-soma fate change non-cell-autonomously.
28. Matunis EL, Stine RR, de Cuevas M. Recent advances in *Drosophila* male germline stem cell biology. *Spermatogenesis*. 2(3):137–144. [PubMed: 23087833]
29. Kitadate Y, Kobayashi S. Notch and Egfr signaling act antagonistically to regulate germ-line stem cell niche formation in *Drosophila* male embryonic gonads. *Proc Natl Acad Sci U S A*. 2010; 107(32):14241–14246. [PubMed: 20660750]

30. Morris LX, Spradling AC. Steroid signaling within *Drosophila* ovarian epithelial cells sex-specifically modulates early germ cell development and meiotic entry. *PLoS One*. 7(10):e46109. [PubMed: 23056242]
31. Hsu HJ, Drummond-Barbosa D. Insulin signals control the competence of the *Drosophila* female germline stem cell niche to respond to Notch ligands. *Dev Biol*. 350(2):290–300. [PubMed: 21145317]
32. Stine RR, et al. Coordinate regulation of stem cell competition by Slit-Robo and JAKSTAT signaling in the *Drosophila* testis. *PLoS Genet*. 2014; 10(11):e1004713. [PubMed: 25375180]
33. Ables ET, Drummond-Barbosa D. The steroid hormone ecdysone functions with intrinsic chromatin remodeling factors to control female germline stem cells in *Drosophila*. *Cell Stem Cell*. 2010; 7(5):581–592. [PubMed: 21040900]
34. Li Y, et al. Steroid signaling promotes stem cell maintenance in the *Drosophila* testis. *Dev Biol*. 2014; 394(1):129–141. [PubMed: 25093968]
35. Tarayrah L, et al. Histone demethylase dUTX antagonizes JAK-STAT signaling to maintain proper gene expression and architecture of the *Drosophila* testis niche. *Development*. 2013; 140(5):1014–1023. [PubMed: 23364332]
36. Eliazar S, et al. Lsd1 restricts the number of germline stem cells by regulating multiple targets in escort cells. *PLoS Genet*. 2014; 10(3):e1004200. [PubMed: 24625679]
37. Eliazar S, Shalaby NA, Buszczak M. Loss of lysine-specific demethylase 1 nonautonomously causes stem cell tumors in the *Drosophila* ovary. *Proc Natl Acad Sci U S A*. 2011; 108(17):7064–7069. [PubMed: 21482791]
38. Chen H, Chen X, Zheng Y. The Nuclear Lamina Regulates Germline Stem Cell Niche Organization via Modulation of EGFR Signaling. *Cell Stem Cell*. 2013; 13(1):73–86. [PubMed: 23827710]
39. Yuan H, Yamashita YM. Germline stem cells: stems of the next generation. *Curr Opin Cell Biol*. 2010; 22(6):730–736. [PubMed: 20817500]
40. Sheng XR, Matunis EL. Make room for dedifferentiation. *Fly (Austin)*. 2009; 3(4):283–285. [PubMed: 19923919]
41. Cheng J, et al. Centrosome misorientation reduces stem cell division during ageing. *Nature*. 2008; 456(7222):599–604. [PubMed: 18923395]
42. Sheng XR, Matunis E. Live imaging of the *Drosophila* spermatogonial stem cell niche reveals novel mechanisms regulating germline stem cell output. *Development*. 2011; 138(16):3367–3376. [PubMed: 21752931]
43. Govin J, et al. Systematic screen reveals new functional dynamics of histones H3 and H4 during gametogenesis. *Genes Dev*. 2010; 24(16):1772–1786. [PubMed: 20713519]
44. Krishnamoorthy T, et al. Phosphorylation of histone H4 Ser1 regulates sporulation in yeast and is conserved in fly and mouse spermatogenesis. *Genes Dev*. 2006; 20(18):2580–2592. [PubMed: 16980586]
45. Papazyran R, et al. Methylation of histone H3K23 blocks DNA damage in pericentric heterochromatin during meiosis. *Elife*. 2014; 3:e02996. [PubMed: 25161194]
46. Iovino N, Ciabrelli F, Cavalli G. PRC2 controls *Drosophila* oocyte cell fate by repressing cell cycle genes. *Dev Cell*. 2013; 26(4):431–439. [PubMed: 23932903] * This paper shows that the key PRC2 component E(z) prevents oocyte from switching to nurse-like cell through repressing the expression of Cyclin E and Dacapo, whose functions are to drive endocycle in nurse cells.
47. Fuller MT, Spradling AC. Male and female *Drosophila* germline stem cells: two versions of immortality. *Science*. 2007; 316(5823):402–404. [PubMed: 17446390]
48. Eun SH, et al. MicroRNAs downregulate Bag of marbles to ensure proper terminal differentiation in the *Drosophila* male germline. *Development*. 2013; 140(1):23–30. [PubMed: 23154414]
49. Pancratov R, et al. The miR-310/13 cluster antagonizes beta-catenin function in the regulation of germ and somatic cell differentiation in the *Drosophila* testis. *Development*. 2013; 140(14):2904–2916. [PubMed: 23821034]
50. Malki S, et al. A role for retrotransposon LINE-1 in fetal oocyte attrition in mice. *Dev Cell*. 2014; 29(5):521–533. [PubMed: 24882376]

51. Castaneda J, et al. Reduced pachytene piRNAs and translation underlie spermiogenic arrest in Maelstrom mutant mice. *EMBO J.* 2014; 33(18):1999–2019. [PubMed: 25063675]
52. Rathke C, et al. Chromatin dynamics during spermiogenesis. *Biochim Biophys Acta.* 2014; 1839(3):155–168. [PubMed: 24091090]
53. Gaucher J, et al. From meiosis to postmeiotic events: the secrets of histone disappearance. *FEBS J.* 2010; 277(3):599–604. [PubMed: 20015078]
54. Awe S, Renkawitz-Pohl R. Histone H4 acetylation is essential to proceed from a histone- to a protamine-based chromatin structure in spermatid nuclei of *Drosophila melanogaster*. *Syst Biol Reprod Med.* 2010; 56(1):44–61. [PubMed: 20170286]
55. Dottermusch-Heidel C, et al. H3K79 methylation: a new conserved mark that accompanies H4 hyperacetylation prior to histone-to-protamine transition in *Drosophila* and rat. *Biol Open.* 2014; 3(6):444–452. [PubMed: 24795146]
56. Dottermusch-Heidel C, et al. H3K79 methylation directly precedes the histone-to-protamine transition in mammalian spermatids and is sensitive to bacterial infections. *Andrology.* 2014; 2(5): 655–665. [PubMed: 25079683]
57. Qian MX, et al. Acetylation-mediated proteasomal degradation of core histones during DNA repair and spermatogenesis. *Cell.* 2013; 153(5):1012–1024. [PubMed: 23706739]
58. Doyen CM, et al. Subunits of the histone chaperone CAF1 also mediate assembly of protamine-based chromatin. *Cell Rep.* 2013; 4(1):59–65. [PubMed: 23810557]
59. Gaydos LJ, Wang W, Strome S. Gene repression. H3K27me and PRC2 transmit a memory of repression across generations and during development. *Science.* 2014; 345(6203):1515–1518. [PubMed: 25237104] ** This paper shows that repression via PRC2-generated H3K27me3 can be transmitted to embryos from both sperm and oocyte in *C.elegans*. The H3K27me3 mark persists after several rounds of cell divisions in embryos without PRC2, but maintenance and propagation of H3K27me3 requires PRC2 activity during embryogenesis.
60. Katz DJ, et al. A *C. elegans* LSD1 demethylase contributes to germline immortality by reprogramming epigenetic memory. *Cell.* 2009; 137(2):308–320. [PubMed: 19379696] * This paper shows that mutants of the H3K4me2 demethylase-encoding *spr-5* gene leads to heritable accumulation of H3K4me2 and increased expression of spermatogenesis genes. Such changes cause progressive sterility over many generations in *C. elegans*.
61. Greer EL, et al. A histone methylation network regulates transgenerational epigenetic memory in *C. elegans*. *Cell Rep.* 2014; 7(1):113–126. [PubMed: 24685137]
62. Ost A, et al. Paternal diet defines offspring chromatin state and intergenerational obesity. *Cell.* 2014; 159(6):1352–1364. [PubMed: 25480298] * This paper demonstrates that *Drosophila* paternal sugar intake induces transgenerational metabolic reprogramming and causes adiposity in offsprings. Such a reprogramming is mediated through Polycomb and H3K9/K27 me3, and similar mechanism might exist in mice and human.
63. Jiang L, et al. Sperm, but not oocyte, DNA methylome is inherited by zebrafish early embryos. *Cell.* 2013; 153(4):773–784. [PubMed: 23663777] ** This paper shows that during early embryogenesis of zebrafish, paternal DNA methylation pattern is maintained but maternal methylome is reprogrammed to a paternal-like pattern.
64. Potok ME, et al. Reprogramming the maternal zebrafish genome after fertilization to match the paternal methylation pattern. *Cell.* 2013; 153(4):759–772. [PubMed: 23663776] ** This papers demonstrates that the totipotent chromatin state of zebrafish zygotes requires competent paternal DNA methylome and reprogrammed maternal DNA methylome.
65. Hammoud SS, et al. Distinctive chromatin in human sperm packages genes for embryo development. *Nature.* 2009; 460(7254):473–478. [PubMed: 19525931]
66. Hammoud SS, et al. Chromatin and transcription transitions of mammalian adult germline stem cells and spermatogenesis. *Cell Stem Cell.* 2014; 15(2):239–253. [PubMed: 24835570]
67. Brennecke J, et al. An epigenetic role for maternally inherited piRNAs in transposon silencing. *Science.* 2008; 322(5906):1387–1392. [PubMed: 19039138]
68. Le Thomas A, Marinov GK, Aravin AA. A transgenerational process defines piRNA biogenesis in *Drosophila virilis*. *Cell Rep.* 2014; 8(6):1617–1623. [PubMed: 25199836]

69. Le Thomas A, et al. Transgenerationally inherited piRNAs trigger piRNA biogenesis by changing the chromatin of piRNA clusters and inducing precursor processing. *Genes Dev.* 2014; 28(15): 1667–1680. [PubMed: 25085419]
70. Ashe A, et al. piRNAs can trigger a multigenerational epigenetic memory in the germline of *C. elegans*. *Cell.* 2012; 150(1):88–99. [PubMed: 22738725]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1. Non-random segregation of sister chromatids and histone H3 during asymmetric division of GSCs

Asymmetric GSC division gives rise to two daughter cells: a self-renewed GSC (green) and a differentiating daughter cell (yellow). During this division, mother and daughter centrosomes with distinct microtubule nucleating capabilities are asymmetrically inherited; sister chromatids of X and Y chromosomes are segregated non-randomly; and preexisting H3 are preferentially segregated to GSC while newly synthesized H3 are mainly segregated to the other differentiating daughter cell. Potential crosstalk between non-random

segregation of sex chromosome sister chromatids and preexisting vs. newly synthesized H3 might exist.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2. Non-cell-autonomous regulation of germline through somatic gonadal cells

In somatic cells of *Drosophila* testis, H3K27me3-methyltransferase E(z) might maintain germ cell fate through downstream signaling, such as Wnt or Egfr pathway(s). Egfr signaling can also be regulated by nuclear lamina to promote germ cell differentiation. The H3K27me3 demethylase dUTX can modulate JAK-STAT signaling through activating JAK-STAT signaling inhibitor Socs36E. In addition, chromatin remodeling complex component, such as Nurf301, cooperates with ecdysone signaling to maintain GSCs. In *Drosophila* ovary, BMP signaling directly repress transcription of differentiation-promoting gene to maintain GSCs. In escort cells, H3K9me3-methyltransferase Eggless and H3K4me1/2-demethylase Lsd1 prevent ectopic BMP signaling outside the niche to regulate germline differentiation.

Figure 3. Transgenerational epigenetic inheritance in multiple organisms

In *Drosophila*, maternal piRNAs are transmitted to the next generation, whose function is important for transposon silencing in germline to maintain fertility. In addition, they also lead to H3K9me3 at piRNA clusters to promote piRNA biogenesis. In *C. elegans*, maternal and paternal PRC2-generated H3K27me3 can both be transmitted to embryos. Without the PRC2 enzyme, the H3K27me3 repressive mark only persists for several rounds of cell divisions. Different from H3K27me3, H3K4me2 needs to be erased by the Spr5 demethylase in germ cells at each generation. Failure of erasure leads to heritable

accumulation of H3K4me2 and misregulation of spermatogenesis gene expression in germ cells over 20 generations. Multiple chromatin factors, including H3K4me1/me2 and H3K9me3 methyltransferase, H3K9me3 demethylase, and H3K9me reader, can enhance or repress the sterility phenotype of *spr-5*. In *Zebrafish*, paternal DNA methylation pattern is maintained during embryo development, while the maternal DNA methylome undergoes reprogramming to resemble the paternal methylome.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript