All are equal, but some are more equal than others: Epigenetic regulation of germline stem cell fate in *Drosophila melanogaster*

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Adult stem cells are capable of both symmetric and asymmetric cell divisions. Asymmetric cell division allows self-renewal and gives rise to intermediate cells that ultimately differentiate into specific cell types. Consequently, adult stem cells play a key role in development and tissue homeostasis during the life span of an organism. Typically, adult stem cell divisions are regulated through coordination between non-autonomous signaling from the niche and cell-autonomous influences from stem cell-intrinsic factors. Although localized distribution of proteins, RNA and organelles during cell division contributes significantly to the differences between fates of daughter cells, recent studies have also implicated epigenetic factors in this process. A number of epigenetic modifications remain associated with the chromosomes during mitosis and serve as a template to reestablish fates after mitosis. Whether the distribution of epigenetic modifications is random on each chromatid or there is a bias in their distribution is therefore under extensive investigation. The nonrandom distribution of epigenetic modifications on mitotic chromosomes provides an attractive possible explanation of how bias is generated during chromatid segregation. In *Drosophila* male germline stem cells, the histone modifications present in the stem cells are distinct from those in the differentiating daughter cells. These modifications help to retain pre-existing histones in the mother cell while imparting newly synthesized histones to the daughter cell. Importantly, the retention of pre-existing histones in the stem cells is a prerequisite to maintain their ability to self-renew. Here we summarize recent studies that focus on the role of different epigenetic modifications in the regulation of asymmetric adult germline stem cell divisions in *Drosophila*. We further describe how epigenetic modifications potentially lead to variations in the otherwise equivalent chromatids, and discuss the role of biased chromatid segregation in asymmetric cell divisions.

Key words: adult stem cells, asymmetric cell divisions, epigenetic regulation, histone modifications, *Drosophila melanogaster*

INTRODUCTION

Adult stem cells constitute a special population of cells that support highly regenerative adult tissues. These cells demonstrate two unique capabilities: self-renewal and the capacity to generate cells that ultimately differentiate into diverse cell types. Adult stem cells undergo asymmetric cell division, where one daughter cell retains the ability to function as a stem cell via self-renewal while the other acquires competence to differentiate (Betschinger and Knoblich, 2004). Generation and maintenance of asymmetry is critical for creating a diverse population of cells in an adult organism as well as maintaining tissue homeostasis, because it plays a critical role in replenishing cells lost due to natural cell death or injury over the organism’s life span. Defects related...
to maintaining a balance between stem cell renewal and differentiation are associated with several disease conditions such as cancer, diabetes and various neurodegenerative disorders. Hence, there is a keen interest in understanding the mechanisms underlying stem cell specification, maintenance and asymmetric cell division.

Over the course of development, two types of adult stem cells are specified: germline stem cells (GSCs) and somatic stem cells (SSCs). Both the germline and somatic stem cells are required for the proper development of the gonad. GSCs give rise to the germline proper, whereas SSCs contribute to generation of the supporting somatic cell types. Unlike the embryonic stem cells, the regenerative potential of stem cell populations such as GSCs and SSCs is restricted as they can give rise to only a defined, tissue-specific population of cells belonging to either a single or multiple lineages. Interestingly, however, they retain the ability of limited self-renewal via mitotic division cycles (Weissman, 2000; Rossant and Tam, 2004).

In the context of a tissue, adult stem cells reside in a special microenvironment referred to as the niche (Morrison and Spradling, 2008). The niche allows interaction between the stem cells and different cell-extrinsic signals. In some instances, these signals are mediated via direct cell-to-cell communication or cell-to-matrix interaction. Another category of signals comprises diffusible signaling ligands, which regulate various transcriptional programs in the stem cells. These interactions are crucial, as they are capable of regulating the maintenance of stem cells as quiescent cells, as well as self-renewal or commitment to differentiation (Morrison and Spradling, 2008). The availability of genetic tools allowing lineage tracing and manipulation of gene expression in a clonal manner has yielded extensive characterization of a variety of stem cell populations and their niches in *Drosophila*. These include, but are not limited to, the intestinal stem cells, the neuronal stem cells, and the male and female GSCs and SSCs in the testis and ovary, respectively. By employing similar labeling techniques in the mouse, different lineages that arise from stem cells in the testis and small intestine have also been characterized (Barker et al., 2007; Oatley and Brinster, 2012). *Drosophila melanogaster* has thus served as an excellent model to study the germline and somatic stem cell microenvironment, as well as pathways that regulate adult stem cell identities.

### CELL-EXTRINSIC FACTORS REGULATING STEM CELL FATE

**Female GSC niche** *Drosophila melanogaster* females possess a pair of ovaries, each comprising arrays of developing oocytes known as the ovariola. GSCs and the associated niche are located at the anterior tip of an individual ovariola in a structure known as the germarium (Lin and Spradling, 1993; Roth and Lynch, 2009). The ovarian niche comprises at least three types of somatic cell populations: terminal filament cells (TFCs) at the tip of the germarium, cap cells situated at the base of the TFCs, and escort stem cells (Fig. 1A) (Xie & Spradling, 2000; Decotto & Spradling, 2005). Two to three ovarian GSCs tightly associate with about five to seven cap cells. The physical proximity and/or attachment between the cell types is crucial as the loss of adherens junctions between cap cells and GSCs and/or SSCs leads to defects in the maintenance of these stem cells and results in their precocious differentiation (Song and Xie, 2002; Song et al., 2002).

The asymmetric cell divisions of both the male and female GSCs are regulated via coordination between non-autonomous signaling from the niche and cell-autonomous influences in the form of stem cell-intrinsic factors. In females (also in males; see below) JAK/STAT signaling, in conjunction with bone morphogenetic protein (BMP) signaling, plays an essential role in maintenance of the GSCs. Therefore, tightly controlled levels of JAK-STAT signaling and repression of the master differentiation gene *bag-of-marbles* (*bam*) are required for stem cell maintenance and self-renewal (Fig. 1B). Hyperactivation of this pathway leads to stem cell over-proliferation. In contrast to the mammalian system, a much less complex version of the JAK/STAT pathway is present in *Drosophila* and all the component genes of the pathway are present in a single copy (Tulina and Matunis, 2001). The signaling pathway is triggered upon binding of the secreted Unpaired (Upd) ligand to its cognate cell surface receptor Domeless, which activates the downstream JAK Hopscotch kinase, ultimately resulting in phosphorylation-mediated dimerization of STAT92E and its translocation into the nucleus. STAT92E dimers bind to defined canonical binding sites and regulate target gene expression (Bausek, 2013; Zeidler and Bausek, 2013). In the ovary, Upd and related cytokines are produced by the TFCs, and their secretion activates JAK-STAT signaling in cap and escort stem cells. The cap cells in the ovary also produce BMP ligands, Dpp and Ghb, that activate BMP signaling in the GSCs, while the escort stem cells encapsulate the GSCs and contribute to their fate via regulation of both JAK-STAT and BMP signaling (Decotto and Spradling, 2005) (Fig. 1B). Binding of BMP ligands to their receptor in GSCs prevents transcription of the master differentiation gene *bam*, which is both necessary and sufficient for the differentiation of early GSC lineages (Song et al., 2002). Following an oriented GSC division, only the proximal daughter cell usually retains adhesion to the cap cells, while the distally positioned daughter cell does not. This leads to activation of *bam* transcription in the distal daughter cell and causes it to differentiate into a cystoblast, which will give rise to a new germline cyst (Song and Xie, 2002; Song...
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et al., 2002; Xia et al., 2010). Remarkably, transcription of *bam* is activated only one cell diameter away from the GSCs, which involves the establishment of a steep gradient of BMP signaling in the niche. Mechanisms that regulate the establishment of this BMP signaling gradient in the niche are not completely understood. In this regard, it is noteworthy that the components of the extracellular matrix also play a role in maintaining GSC identity. Extracellular matrix components such as type IV collagens bind to Dpp and limit its diffusion, while Dally, a heparan sulfate proteoglycan, stabilizes Dpp and facilitates its binding to its receptors on the GSCs. High levels of *dally* expressed in the niche, in the proximity of cap cells, prevent differentiation of GSCs, while low levels of *dally* outside the niche lead to downregulation of BMP signaling, resulting in activation of *bam* expression in cystoblasts (Hayashi et al., 2009). Other pathways that also contribute to regulation of BMP signaling in the niche include the targeted degradation of Dpp receptors in cystoblasts by the BMP-induced ubiquitin E3 ligase.
Smurf, which is essential for their differentiation (Xia et al., 2010). Taken together, these observations indicate that the binding of BMP signals by the GSCs outside the niche is controlled by multiple mechanisms.

**Male GSCs and their niche** *Drosophila* male GSCs reside in a stem cell niche, which comprises hub cells and cyst stem cells (CySCs) (Fig. 1C) (Fuller and Spradling, 2007). Hub cells consist of 8–16 somatically derived post-mitotic cells that remain attached to the apical wall of the testis (Hardy et al., 1979). Between seven and twelve GSCs surround the hub cells such that each GSC is physically attached to a hub. Typically, a male GSC undergoes an asymmetric division that produces one GSC and one gonialblast (GB). The GBs then develop into spermatogonia and spermatocytes, which eventually undergo meiosis and generate spermatids (Yamashita and Fuller, 2005; Yamashita et al., 2005). The somatic stem cells or CySCs also divide asymmetrically, producing one CySC and a cyst cell. Like the escort cells in the female GSC niche, a pair of CySCs further encapsulates a GSC and the hub cells via its cellular processes, while a pair of cyst cells ensheaths developing GBs, spermatogonia and spermatocytes. The GB divides synchronously to form a 16-cell cyst that eventually gives rise to 64 sperm cells. Unlike the escort cells in females, the CySCs not only divide to produce cyst cells but under certain circumstances can also differentiate into hub cells. Replenishment of the hub is required for maintenance of the niche. A similar phenomenon is unknown in the female GSC niche (Voog et al., 2008; Cheng et al., 2011). Although the SSCs are not required for the self-renewal of GSCs, both GSCs and SSCs are required for the completion of gametogenesis and their loss results in aberrant development of the structures, resulting in rudimentary gonads (Murray et al., 2010).

The male GSC niche in the testis utilizes similar signaling pathways to that of the ovary (Fig. 1C). Germ cells directly contacting the hub cells via adherens junctions self-renew, whereas the daughter cells further from the hub cells undergo differentiation and initiate cyst formation. The hub cells secrete Upd, which initiates JAK-STAT signaling in the GSCs and CySCs. However, the signal transduction downstream of JAK-STAT signaling results in distinct consequences: it strengthens the physical association between the hub cells and GSCs, whereas in CySCs it represses the transcription of differentiation factors. The CySCs also secrete Gbb and Dpp, which activate BMP signals and repress the transcription of *bam* in GSCs (Leatherman and Dinardo, 2008). A steep gradient of BMP signaling is established in the male GSC niche, which represses the expression of *bam* within one cell diameter from the hub cells. Thus, the differentiation of both male and female GSCs is repressed by a cascade of JAK/STAT and BMP signaling and is contingent upon at least two distinct cell types in the niche (Fig. 1B and C, Table 1).

**CELL-INTRINSIC DETERMINATION OF STEM CELL FATE**

In addition to non-autonomous signaling, cell-autonomous intrinsic factors significantly contribute to the establishment and/or maintenance of stem cell identity and the cells’ subsequent development. Interestingly, work from Fuller and Yamashita has documented the unique participation of centrosomes in the determination of stem cell fate. These data suggest that male GSCs retain the mother centrosome, which remains closely anchored to the GSC-hub interface. By contrast, the daughter cell inherits the newly synthesized centrosome and undergoes differentiation. This asymmetric segregation of centrosomes is critical for proper positioning of the spindle and for subsequent spindle orientation with reference to the hub. Importantly, it precedes the asymmetric division of the GSCs, implying their critical role for this asymmetric segregation. Consistent with this proposition, mispositioning of the centrosomes results in a failure of mitotic spindle formation, and mitotic arrest of the GSC (Yamashita et al., 2003; Yamashita et al., 2007). It is unclear, however, whether the non-autonomous signaling and cell-intrinsic transcriptional regulators coordinately control the duplication and uneven separation of the centrosomes among the daughter cells. Furthermore, it remains to be determined whether centrosome assembly and the emanating microtubule network can also modulate the signaling machinery via a feedback mechanism. In this regard, it is interesting to note that although most stem cells need the niche environment for self-renewal and to initiate asymmetric cell division, it is not essential in all contexts. For instance, adult neuroblasts can maintain their identity and are capable of dividing asymmetrically in the absence of a conventional niche (Prehoda, 2009). Asymmetric divisions of larval neuroblasts are not oriented with respect to an external axis, and these cells retain the capability to divide asymmetrically even in culture (Ceron et al., 2006). During embryonic development, when neuroblasts delaminate, they inherit their apical-basal polarity from the epithelial cells in the neuroectoderm. As these neuroblasts undergo multiple rounds of asymmetric divisions along this apical basal axis, their axis aligns relative to the axis of the previous cell division. The centrosome plays a crucial role in maintaining the neuroblast’s apical-basal axis of polarity by serving as a reference point and allowing apical accumulation of the Par complex (Rebollo et al.). This further regulates proper orientation of the mitotic spindle and asymmetric localization of cell fate determinants in the dividing neuroblast (Knoblich, 2008).
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Despite their identical genomic sequence, adult stem cells and differentiated cells possess distinct chromatin structure and gene expression profiles, which are imparted, in part, by epigenetic modifications. Typically, epigenetic changes operate at the level of chromatin structure while leaving the primary DNA sequence unperturbed. Epigenetic modifications include alterations at the DNA level, such as DNA methylation, or post-translational modifications of DNA/nucleosome-associated proteins such as histones. For instance, H3 and H4 hyperacetylation is enriched at constitutively active genomic regions, whereas H3K27 trimethylation and H3K79 dimethylation mark repressive regions. Thus, in addition to specific transcription factors, the cell-intrinsic components required for stem cell identity include chromatin modulators involved in epigenetic regulation (Jaenisch and Young, 2008).

While specific histone modifications serve as indicators of gene activity, the precise mechanisms underlying their mode of action during the acquisition of unique functional traits of stem cells and their differentiating progeny remain to be determined (Motamedi et al., 2004; Fischle et al., 2005).

In this regard, it is noteworthy that the protein components thought to be involved in chromatin remodeling appear to act in conjunction with the proteins/enzymes involved in epigenetic modifications (Table 2). For instance, proteins involved in chromatin remodeling such as members of the NURF complex and Scrawny (H2B ubiquitin protease) are required in the GSC niche in both males and females. ISWI, an ATP-dependent chromatin remodeler and component of the NURF complex, is specifically required for the repression of *bam* in GSCs (Cherry and Matunis, 2010; Yan et al., 2014). By contrast, the chromatin regulator No child left behind seems to function only in males (Casper et al., 2011; Matunis et al., 2012). Work from Ting Xie’s laboratory showed that in *iswi* mutants, GSCs are unable to interpret the BMP-mediated signaling from the niche correctly and repress cell differentiation. This results in defective self-renewal of GSCs and ultimately leads to their loss. On the other hand, mutations in another ATP-dependent chromatin remodeler, *domino*, lead to a significant reduction of SSCs while causing no change in the numbers of GSCs, suggesting that the self-renewal abilities of different stem cell populations even in close proximity are regulated by different chromatin remodeling factors (Xi and Xie, 2005). As in the case of *iswi* mutants, mutations in the *Drosophila* homolog of the histone lysine methyltransferase SETDB1, known as eggless (*egg*), result in GSC loss and/or premature germ cell differentiation. SETDB1 generates repressive histone H3K9 methylation, which seems to be required for maintaining GSCs. These authors showed that marked GSC clones of *egg* mutants exhibit diminished levels of H3K9 trimethylation (H3K9me3) and were rapidly lost from the niche. Although BMP-mediated repression of *bam*...
remained unperturbed in egg mutants, the egg mutant progeny cells continued to divide and differentiate to form a 16-cell cyst, suggesting that Egg activity is required for the self-renewal of GSCs but is dispensable for their differentiation. Furthermore, RNAi-mediated knockdown of egg specifically in the escort stem cells leads to a gradual loss of escort cells (ECs) and germ cell differentiation defects. These results indicate that Egg is an important H3K9 trimethylase in the Drosophila ovary that regulates the self-renewal of GSCs, perhaps via repressing a bam-independent differentiation pathway, and prevents the spread of BMP signaling in the differentiating ECs (Wang et al., 2011). These data underscore the importance of epigenetic mechanisms in influencing cell fates in the context of stem cells and their differentiated progeny.

Interestingly, recent data have also revealed a critical role played by histone H1 in the maintenance of GSCs. Conditional knockdown of H1 in GSCs using a germline FLIP-out system resulted in a drastic decrease in the total number of GSCs, suggesting that H1 is required for GSC maintenance. These observations thus provide evidence that H1 knockdown in GSCs led to a gradual loss of GSCs in testis and ovary. Previous studies had demonstrated that the loss of H1 in embryonic cells causes alterations of several histone modifications. These include methylation of H3K4, H3K9 and H3K27 at specific loci during embryonic stem cell differentiation in vitro (Zhang et al., 2012). Consistent with this, depletion of H1 in GSCs increased the level of H4K16 acetylation (H4K16Ac), which is a mark for hyperactive chromatin. Reciprocally, overexpression of the gene males absent on the first, which encodes an H4K16 acetyltransferase, increased the acetylation of H4K16 and resulted in dissociation of H1 from the chromosomes. This result further suggested an antagonism between H4K16Ac and H1’s association with specific genomic regions. Taken together, these data suggest that the regulation of H4K16Ac levels in GSCs is critical for their self-renewal and maintenance (Sun et al., 2015). Most critically, however, these studies documented the capability of histone H1, a constituent of nucleosomes, to contribute to stem cell fate.

Recent work also provides evidence for the role of multiple noncoding RNAs, including piRNAs, in the maintenance of stem cell identity in Drosophila. Genes such as Yb (female sterile (1) Yb) and piwi, which are implicated in piRNA production and the regulation of transposons, are widely expressed in both the germ cells and somatic cells in the Drosophila ovary, and have also been shown to be involved in somatic stem cell niches (Szakmary et al., 2009). A recent study demonstrated that knockdown of piwi in ECs causes a reduction in EC number and an accumulation of undifferentiated germ cells. These undifferentiated germ cells also exhibit activated BMP signaling, which suggests that Piwi is involved in the regulation of germ cell differentiation. By contrast, Piwi inactivation in the germline of the adult ovary leads to a gradual loss of GSCs and to germ cell differentiation defects, which indicates the role of Piwi in maintenance of adult GSCs (Ma et al., 2014).

It was subsequently demonstrated that in Drosophila ovaries, Piwi-piRNA complexes play roles in the repression of transposable elements (TEs) via H3K9 methylation, interaction with histone H1 and recruitment of heterochromatin protein 1a. Depletion of Piwi resulted in decreased accumulation of H1 at the TEs, leading to their derepression. Since Piwi has been implicated in the maintenance of adult GSCs in the Drosophila ovary,
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...it is possible that histone H1 is one of its targets and that its functions are mediated via regulation of H1 binding. The interaction between Piwi and H1 may in turn regulate transcription of various differentiation factors in the stem cell niche (Iwasaki et al., 2016).

Intriguingly, the functional involvement of histones and epigenetic mechanisms in cell fate determination poses an obvious challenge regarding the mechanisms employed to modulate the different epigenetic marks and their distribution during DNA replication. Many epigenetic marks are very dynamic and are likely erased during different phases of the cell cycle. For example, prior to mitosis the binding of heterochromatin protein 1 to trimethylated H3K9 is disrupted by the phosphorylation of serine 10 of histone H3 by Aurora B kinase (Fischle et al., 2005; Lansdorp et al., 2012). Additionally, transcription factors such as CTCF and polycomb proteins remain associated with chromosomes during DNA replication and mitosis (Petruk et al., 2012; Shen et al., 2015). This epigenetic memory also aids in resetting gene expression patterns in the daughter cells.

While some of the known chromatin marks are evenly distributed between both the sister chromatids, others may be distributed randomly and possibly unequally on both chromatids, thus making the two sister chromatids nonequivalent. Such a difference could lead to epigenetic differences between the two sister chromatids, ultimately resulting in the generation of variable gene expression patterns among different daughter cells. The regulation of epigenetic marks during DNA replication and later during mitosis can thus provide additional mechanisms for regulating differentiation and self-renewal of stem cells.

**Replication-coupled and replication-independent mechanisms of histone incorporation** During DNA replication, the majority of the canonical histones including H2A, H2B, H3 and H4 are synthesized and incorporated into the nucleosomes (Xu et al., 2011). This process is highly regulated and efficiently coordinated with the progression of the replication fork. It involves disruption and recycling of pre-existing nucleosome octamers, followed by the deposition of newly synthesized histones at the site of the replication fork (Corpet and Almouzni, 2009; Smith and Whitehouse, 2012). The incorporation of new histones at the replication fork requires various histone remodeling complexes and histone chaperones.

Prior to their incorporation into the nucleosomes the newly synthesized histones must undergo specific modifications such as acetylation of H3K56 and H4K5, which enable their binding to histone chaperones such as CAF-1 and ASF-1 (Verreault et al., 1996; Li et al., 2008). The incorporation of newly synthesized histones at the replication fork is mediated via a direct interaction between the histone chaperones and other factors such as prolifer...
ating cell nuclear antigen (PCNA), which is the processivity factor for DNA polymerase (Shibahara and Stillman, 1999). The acetylation marks on the newly incorporated histones are then removed by histone deacetylases. The histones are then further modified according to their location on the chromosomes. The polycomb group (PcG) proteins and TrxG proteins are crucial in maintaining the balance between repressive H3K27 trimethylation and active H3K4 trimethylation at different loci. These factors remain associated at the replication fork and regulate the modification of newly incorporated histones after the passage of the replication fork (Ringrose et al., 2004; Ringrose and Paro, 2004; Zhu and Reinberg, 2011). Studies examining histone methylation marks during S phase in cells from Drosophila embryos have shown that, unlike the modified histones, only the unmodified histones are in close proximity to PCNA. This suggests that the histone modifications are reestablished after the passage of the replication fork; however, the mechanisms underlying the recycling of the pre-existing histones and their reincorporation at specific locations are unclear (Petruk et al., 2013).

In contrast to canonical histones, the incorporation of histone variants occurs via a replication-independent and a transcription-coupled mechanism (Henikoff et al., 2004). Canonical H3 is replaced with the H3.3 variant at actively transcribed coding regions and origins of replication. The H3.3 variant has been shown to be important in maintenance of epigenetic memory during mitosis and meiosis (Szenker et al., 2011). The CENP-A variant of H3 is also associated with centromere regions, and is thought to play a critical role in distinguishing between the two sister chromatids during asymmetric cell divisions (Ahmad and Henikoff, 2002).

Non-random histone distribution and chromatid segregation in GSCs Studies from Xin Chen’s group have demonstrated that “old” and “new” canonical H3 histones are distinguished and differentially distributed during the asymmetric division of male GSCs; however, the histone variant H3.3, which is incorporated in a replication-independent manner, is not. In an ingenious set of experiments, they observed that pre-existing H3 histones are selectively segregated to the GSCs, whereas newly synthesized H3 molecules are enriched in the differentiating daughter cell (Tran et al., 2012). This non-random segregation of pre-existing H3 is specific to the asymmetric division of GSCs and is not observed in other symmetrically dividing progenitor cells. Additionally, if the GSCs are genetically manipulated to divide symmetrically, the asymmetric distribution of male GSCs, the pre-existing H3 histones are modified by phosphorylation of threonine 3 (H3T3P), which distinguishes them from the newly synthesized histone molecules (Fig. 2). Mutating the threonine residue to either alanine or aspartate, neither of which can be phosphorylated, prevented the asymmetric H3 inheritance and led to the loss of GSCs and formation of germline tumors (Xie et al., 2015). These observations thus demonstrated that H3T3P-like modifications have the potential to distinguish between the two sister chromatids. It will be worth exploring whether the pre-existing H3 histones are randomly incorporated among the sister chromatids or are selectively enriched on a preferred DNA strand (Xie et al., 2015).

A glass half full: Generation of asymmetry involving histone-based epigenetic modifications While these studies have shed light on unique modes of regulation underlying the establishment and/or maintenance of GSCs and their interaction with neighboring somatic cell types including the corresponding niche in male gonads of Drosophila, several unanswered questions remain. A recent study from Yukiko Yamashita’s laboratory has shown nonrandom asymmetric segregation of the X and Y chromosome during GSC divisions. The functional relationship between the two asymmetry-generating events, however, is uncertain at present (Yadlapalli and Yamashita, 2013).

Why only H3 and H4 histones are apparently involved in epigenetic inheritance mechanisms is equally elusive. It is possible that, unlike the H2A-H2B dimer, the parental H3-H4 tetramer does not dissociate during replication (Seale, 1975). PcG proteins have been shown to bind specifically to pre-existing histones carrying H3K27me3 marks during DNA replication, thus suggesting that dimerization of PcG aids in bridging the pre-existing H3–H4 tetramers before and after the replication fork to ensure their incorporation on a specific sister chromatid (Tran et al., 2013). In this context it is interesting to note that most of the histone modifications implicated in functional regulation are observed on histones H3 and H4, supporting an underappreciated regulatory role performed by H2A and H2B during epigenetic events. A challenge for the future will therefore be to assess if such mechanisms are indeed canonical and routinely deployed during asymmetric cell divisions of adult stem cells of various kinds. Moreover, since adult stem cells are also capable of dividing symmetrically, it is of considerable interest to understand how the individual epigenetic marks are inherited differentially during symmetric and asymmetric divisions of adult stem cells.

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