ANALYSIS OF THE ROLES OF HISTONE DEMETHYLASES IN DROSOPHILA MELANOGASTER MALE GERM CELL MAINTENANCE AND DIFFERENTIATION

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ABSTRACT

Adult stem cells reside in microenvironments called niches, where they are regulated by both extrinsic cues such as signaling from neighboring cells and intrinsic factors such as chromatin structure. Here we report that in the *Drosophila* testis niche, an H3K27me3-specific histone demethylase encoded by *ubiquitously transcribed tetratricopeptiderepeat gene on the X chromosome* (*dUTX*) maintains active transcription of *Suppressor of cytokine signaling 36E* (*Socs36E*) gene by removing the repressive H3K27me3 modification near its transcription start site. *Socs36E* encodes an inhibitor of the Janus kinase signal transducer and activator of transcription (JAK-STAT) signaling pathway. While much is known about the niche-to-stem cell signaling, such as the JAK-STAT signaling critical for stem cell identity and activity, our results reveal that stem cells send feedback to niche cells to maintain the proper gene expression and architecture of the niche. We found that dUTX acts in cyst stem cells to maintain hub cell identity through activating *Socs36E* transcription and preventing hyperactivation of the JAK-STAT signaling. dUTX also acts in germline stem cells to maintain hub structure through regulating DE-Cadherin levels. In addition to the role of dUTX in the testis niche we report that in the *Drosophila* testis, an H3K4me3-specific histone demethylase encoded by *little imaginal discs* (*lid*) maintains germline stem cell self-renewal and prevents premature differentiation. Lid is required cell-autonomously in germ cells for proper expression of the Stat92E transcription factor, the downstream effector of JAK-STAT signaling pathway. Our findings support a germ cell autonomous role for the JAK-STAT pathway in maintaining germline stem cells and place Lid as an upstream regulator of this pathway. Our findings provide new insights into the biological functions of histone demethylases *in vivo* and shed light on the interaction between epigenetic mechanisms and signaling pathways in regulating stem cell activities.

In addition to our studies in the testis niche we report a role for dUTX in spermatocyte maturation. Our findings suggest that dUTX cooperates with the testis-specific homologs of TBP-associated factor (tTAFs) to sequester repressive factors to the nucleolar regions of spermatocytes and allow the transcription of terminal differentiation genes.

The readers of this thesis are: Dr. Allan Spradling, Dr. Xin Chen, Dr. Mark Van Doren, and Dr. Daniela Drummond-Barbosa.

TABLE OF CONTENTS

INDEX OF FIGURES

Chapter 1

Introduction to transcriptional regulation during *Drosophila* spermatogenesis and epigenetic regulation in adult stem cells and cancers

This chapter is based on previously published review articles (Lim et al., 2012; Tarayrah and Chen, 2013).

Overview of *Drosophila* spermatogenesis

Drosophila testis is a long tubular structure, which contains a stem cell niche at the apical tip and a linear distribution of germ cells with more differentiated ones toward the basal end of the tube. A cluster of post-meiotic cells, termed the hub, anchors both germline stem cells (GSCs) and cyst stem cells (CySCs) in the testis niche. The GSCs divide asymmetrically to self-renew and produce gonialblasts (GBs), which are displaced from the niche to start the cellular differentiation program. Each GB undergoes four rounds of mitosis as transit-amplifying spermatogonial cells, which remain interconnected by incomplete cytokinesis. These sixteen spermatogonia then undergo a meiotic S phase before switching to an elongated G2 phase as spermatocytes. A robust transcription program is turned on in spermatocytes to actively express genes required for meiotic division and terminal differentiation.

Along with GSC division, the CySCs, two of which encapsulate each GSC, also divide asymmetrically (Cheng et al., 2011). While the daughter cell of one CySC retains its stem cell identity, the other becomes a cyst cell, which never divides again. These two cyst cells continue to enclose the synchronously dividing and differentiating germ cells to form a distinct cyst until the individualization stage, when the spermatids are separated and released into the seminal vesicle as mature sperm.

Because of the physical association between cyst cells and germ cells, these two cell types act cooperatively throughout spermatogenesis. Here we will review transcriptional

regulation of *Drosophila* spermatogenesis in a step-wise manner and in the context of the intimate soma-germline interaction.

Transcriptional regulation in the stem cell niche

Adult stem cells normally reside in a microenvironment called niche. The *Drosophila* male GSC niche is one of the best characterized niches. In this niche, GSCs associate with two types of somatic cells: hub cells located at the tip of the testis and CySCs. The niche provides a polarized extrinsic environment where GSCs are maintained through cell-cell adhesion and niche-to-GSC signaling (Losick et al., 2011). Consequently, at the cellular level, GSCs undergo stereotypical asymmetric cell division (Yamashita, 2010; Yamashita et al., 2005) and at the molecular level, GSCs probably maintain a unique chromatin structure and gene expression profile (de Cuevas and Matunis, 2011; Eun et al., 2010). Because both signaling pathways and epigenetic mechanisms change the transcriptional profile of cells, we will discuss both of them here. Although highly anticipated, a direct link between these two mechanisms has not been reported in the male GSC niche.

Two major signaling pathways play important roles in the male GSC niche: Bone Morphogenetic Protein (BMP) pathway and JAnus Kinase-Signal Transducer and Activator of Transcription (JAK-STAT) pathway. In both pathways, ligands [Glass Bottom Boat (Gbb) and decapentaplegic (Dpp) for BMP; Unpaired (Upd) for JAK-STAT] emanating from the niche act upon their corresponding receptors at stem cells [Saxophone (Sax), Thick veins (Tkv) and Punt for BMP; Domeless for JAK-STAT] in order to promote phosphorylation and translocation of their downstream transcription factors [Mothers against Dpp (Mad) for BMP; Stat92E for JAK-STAT]. Activated transcription factors subsequently initiate a cascade of gene expression.

However, the direct target genes of both pMad and pStat92E are yet to be identified. It has been shown that GSCs unable to respond to the BMP pathway have ectopic transcription of a differentiation gene called *bag of marbles* (*bam*) and undergo premature differentiation (Kawase et al., 2004; Shivdasani and Ingham, 2003). Therefore, it is possible that normal BMP activity represses *bam* transcription in male GSCs, a phenomenon similar to what has been shown in female GSCs (Chen and McKearin, 2003a). Recent studies demonstrate that the major role of JAK-STAT in GSCs is to increase GSC-hub adhesion (Leatherman and Dinardo, 2010), suggesting that cell-cell adhesion molecules, such as *Drosophila* E-cadherin homolog (DE-cadherin, DE-cad), are potential downstream targets of Stat92E. In order to search for Stat targets at a genome-wide level, microarray analysis was performed to identify genes whose expression dramatically changes in response to hyperactivated Stat (Terry et al., 2006). Interestingly, validation of the Stat-responsive genes revealed that most of them are expressed in CySCs instead of GSCs, suggesting that active Stat signaling in somatic cells is predominant and required for maintaining GSCs. Consistent with this finding, ectopic expression of the Stat92E target gene Zinc-finger homeodomain protein 1 (Zfh-1) or Chronologically inappropriate morphogenesis (Chinmo) in cyst cells is sufficient for GSC self-renewal outside of the niche (Flaherty et al.; Leatherman and Dinardo, 2008). Zfh-1 is a transcription factor that guides GSC self-renewal probably by activating BMP signaling in CySCs (Leatherman and Dinardo; Leatherman and Dinardo, 2008), providing crosstalk between the BMP and JAK-STAT pathways. Another particularly interesting Stat target gene in somatic cells is *Suppressor of cytokine signaling 36E (Socs36E)*, which encodes an antagonist of the JAK-STAT pathway and acts to maintain the balance between CySCs and GSCs in the niche (Issigonis et al., 2009). Because the microarray analysis was performed using the entire tissue (i.e., testes), it does not

provide a cell type- specific transcriptional profile. Furthermore, because some of the identified target genes are transcription factors themselves, many of the genes with changed expression may not be their direct target genes. Further studies using purified cells in combination with chromatin immunoprecipitation (ChIP) using specific antibodies against pMad or pStat92E will reveal BMP and JAK-STAT direct target genes in the testis niche.

In addition to signaling pathways, epigenetic mechanisms also play important roles in regulating GSC activity (Eun et al., 2010). Epigenetic regulation changes chromatin structure and gene expression without changing DNA sequences. Two major classes of chromatin regulators have been identified for their functional roles in the male GSC niche: ATP-dependent chromatin remodelers and histone modifying enzymes. For example, the Nucleosome Remodeling Factor complex (NURF) has been shown to positively regulate JAK-STAT signaling required for both GSC and CySC maintenance (Cherry and Matunis, 2010). Because chromatin remodelers act in both transcriptional activation and repression, it is possible that NURF either promotes transcription of JAK-STAT activators or represses transcription of JAK-STAT inhibitors. A deubiquitinating enzyme of mono-ubiquitinated H2B encoded by *scrawny* (*scny*) serves as an example of the histone modifying enzyme. Since Scny normally functions in gene silencing, it was postulated to maintain GSCs by repressing the transcription of differentiation genes (Buszczak et al., 2009). Both NURF (Ables and Drummond-Barbosa, 2010; Xi and Xie, 2005) and Scny (Buszczak et al., 2009) also regulate female GSC function. By contrast, a novel chromatin factor encoded by *no child left behind* (*nclb*) specifically regulates male, but not female, GSC maintenance (Casper et al., 2011). Nclb is enriched at the chromatin region with active transcription, and *Stat92E* has decreased expression or accumulation in *nclb* mutant GSCs (Casper et al., 2011). Therefore, it seems that chromatin regulators may act with

signaling pathways to determine GSC fate in the niche. However, no direct connection between them has been reported, partly because of the difficulty in precisely mapping their direct target genes in different cell types from the niche. Finally, RNA-binding proteins such as Musashi (Msi) (Siddall et al., 2006), Held-out-wings (HOW) (Monk et al., 2010) and IGF-II messenger RNA binding protein (Imp) (Toledano et al., 2012) are all required for GSC maintenance, suggesting an important role of post-transcriptional regulation in the testis niche.

Transcriptional regulation in the transit-amplifying cells

When a GSC divides, two daughter cells take a distinct fate. One is retained as a self-renewed GSC, and another is displaced away from the niche to initiate a new gene expression program. Mitotically dividing GBs and spermatogonial cells contribute to a transit-amplifying stage between GSCs and meiotic germ cells.

The *bam* gene encodes a differentiation factor that is detected in 4- to 16-cell spermatogonia with a peak level in 8-cell spermatogonia (Gonczy et al., 1997) but not in GSCs (Insco et al., 2009; Schulz et al., 2004). Ectopic expression of Bam in GSCs causes premature differentiation or cell death of GSCs (Schulz et al., 2004; Sheng et al., 2009). The HOW RNA-binding protein (Monk et al., 2010) and a microRNA, *miR-7*, have both been implicated to bind to *bam* mRNA and downregulate *bam* expression (Pek et al., 2009) post-transcriptionally. Another RNA binding protein, Maelstrom (Mael), is required in spermatogonia to repress *miR-7* and upregulate *bam* expression so that GB can enter the normal transit-amplifying stage and divide as spermatogonia (Pek et al., 2009). Subsequently, the Bam protein accumulates to a

threshold level, which is required for spermatogonia to become spermatocytes (Insco et al., 2009). Therefore, in *bam* mutant males, this threshold is never reached and whole testes are filled with continuously dividing spermatogonial cells (Gonczy et al., 1997; McKearin and Spradling, 1990). Although expression of *bam* can be regulated at both transcriptional and post-transcriptional levels, the exact mechanism that Bam utilizes to regulate transit-amplifying cell differentiation is not clear. Studies in female germ cells suggest that Bam and another differentiation factor, Benign gonial cell neoplasm (Bgcn), form a protein complex to promote expression of differentiation genes in transit-amplifying cells (Li et al., 2009a). Because Bgcn is predicted to be a RNA-binding protein, further characterization of proteins and RNAs with which the Bam-Bgcn complex interacts will illuminate their functions in transit-amplifying cells.

In spermatogenesis, the switch from mitosis to meiosis is critical. Too early transition to meiosis may lead to decreased fertility, while failure in this transition may lead to germline tumor. The Epidermal growth factor (Egf) signaling pathway plays an important role in the regulation of this switch. The Egfr (Egf receptor) ligand Spitz is processed by Stet, a transmembrane protease, in germ cells (Schulz et al., 2002). Activated Spitz then acts on Egfr expressed in somatic cells (Kiger et al., 2000). Egf signaling acts through the guanine nucleotide exchange factor (GEF) Vav to activate Rac-type small GTPases, which are antagonized by the Rho-type small GTPases (Sarkar et al., 2007). Egfr signaling acts in cyst cells to restrict GSC self-renewal and spermatogonial proliferation, while promoting GSC-to-GB and spermatogonia-to-spermatocyte transitions (Kiger et al., 2000). Recent studies also demonstrate that Egfr signaling decreases GSC division frequency in adult but not larval testes, suggesting a dynamic mode of Egfr regulation (Parrott et al., 2012). In addition, mutations in a serine/threonine kinase signal transducer encoded by *raf* result in a phenotype similar to the *Egfr* mutant, suggesting that

the receptor tyrosine kinase (RTK) pathway is in general required in cyst cells for proper transit-amplification (Tran et al., 2000). The direct target genes for the Egfr/Raf pathway have not been identified; however, because compromised Egf signaling leads to defects of germline-soma interaction and overproliferation of spermatogonial cells, it is possible that the target genes regulate proper encapsulation of germ cells by cyst cells (Sarkar et al., 2007; Schulz et al., 2002). Furthermore, a recent study reported that a nuclear envelope component, Nucleoporin98-86, regulates proper GSC-to-GB and spermatogonia-to-spermatocyte transitions and that its function is upstream of the BMP, JAK-STAT and Egfr signaling pathways (Parrott et al., 2011). These results highlight the importance of nuclear structure in regulating cellular differentiation during spermatogenesis.

Both chromatin remodeling factors and histone modifying enzymes have abundant transcripts in *bam* mutant testes, which are enriched with transit-amplifying cells (Gan et al., 2010a). Studies using ChIP-seq (ChIP followed by high-throughput sequencing) revealed that terminal differentiation genes that are turned on in spermatocytes have either the repressive H3K27me3 alone or no mark (active H3K4me3 or repressive H3K27me3) in *bam* testes. Furthermore, these genes are not associated with paused RNA polymerase II (Pol II) (Gan et al., 2010b). This particular chromatin landscape is different from the bivalent chromatin structure (Bernstein et al., 2006) and paused Pol II at many differentiation gene promoters (Guenther et al., 2007) reported in embryonic stem cells (Buszczak and Spradling, 2006) and may help to prevent ectopic transcription of the terminal differentiation genes in transit-amplifying cells. On the other hand, this chromatin structure suggests that the terminal differentiation genes are subject to dramatic changes at their chromatin region to achieve robust transcription in spermatocytes. A recent study reported that *Plant Homeodomain Finger* 7 (*PHF7*) encodes an

epigenetic reader that is specifically expressed in GSCs and transit-amplifying cells. The PHF7 recognizes the active H3K4me2 histone modification and is required for GSC maintenance and proper spermatogonial differentiation (Yang et al., 2012). It will be interesting to investigate whether PHF7-target genes are H3K4me2/3-monovalent genes in GSCs and transit-amplifying cells.

It has recently been found that spermatogonia can dedifferentiate to reoccupy the niche and become GSC-like cells (Brawley and Matunis, 2004; Cheng et al., 2008; Sheng et al., 2009; Wallenfang et al., 2006; Wong and Jones, 2012). Although dedifferentiated spermatogonia have cellular features that are distinct from *bona fide* GSCs (Cheng et al., 2008), it is less clear whether they have different transcriptional profile and chromatin structure. Nevertheless, the dedifferentiated spermatogonia can undergo asymmetric cell division like GSCs (Cheng et al., 2008), suggesting that they may properly respond to niche-emanating signaling, possibly based on a permissive chromatin landscape and plastic transcriptional profile. By contrast, spermatocytes are unable to dedifferentiate to become GSCs (Sheng et al., 2009), suggesting that germ cells commit to an irreversible differentiation pathway at the spermatocyte stage.

Transcriptional regulation in meiotic spermatocytes

The transition from spermatogonia to spermatocytes is accompanied by a series of transcriptional, epigenetic and morphological changes. After transit-amplification, germ cells undergo the last S phase followed by an extended G2 phase as spermatocyte stage. Spermatocytes grow 25 times in volume and turn on a robust transcription program to activate genes required for spermatocyte maturation, as well as genes needed for meiotic divisions and

terminal differentiation (White-Cooper et al., 1998). Most genes required for meiotic divisions, as well as terminal differentiation, are under translational repression until a later time when their encoded proteins are functional.

The G2/M transition in meiosis I requires Cyclin B, Boule (a RNA-binding protein) and Twine (Cdc25 homolog), all transcribed in spermatocytes (Alphey et al., 1992; Courtot et al., 1992; White-Cooper et al., 1998). Boule translocates from the nucleus to the cytoplasm to trigger the G2/M transition in meiosis I by allowing translation of *twine* (Maines and Wasserman, 1999). At this time point, Cyclin B protein also escapes from translational repression and accumulates in the cytoplasm of spermatocytes (White-Cooper et al., 1998). In both *boule* and *twine* mutant testes, spermatid differentiation occurs independent of meiotic cell cycle progression, suggesting that these two processes can be uncoupled (Alphey et al., 1992; Eberhart et al., 1996). However, the discovery of two classes of genes expressed in early spermatocytes reveals a high degree of coordination between meiotic divisions and spermatid differentiation (Lin et al., 1996). Mutations in any of these genes arrest meiosis and block spermatid differentiation, leading to testes filled up with immature spermatocytes. These genes are named "meiotic arrest" genes, which are classified into "*aly*-class" and "*can*-class" based on morphological differences in the chromosomal structure of the mutant spermatocytes (Lin et al., 1996; White-Cooper et al., 1998) and distinct target genes they regulate (Ayyar et al., 2003; Beall et al., 2007; Chen et al., 2011a; Chen et al., 2011b; Hiller et al., 2004; Hiller et al., 2001; Jiang et al., 2007; Jiang and White-Cooper, 2003; Lin et al., 1996; Perezgasga et al., 2004; White-Cooper et al., 2000). For example, transcription of meiotic cell cycle genes such as *Cyclin B*, *boule* and *twine* rely on "*aly*-class" but not "*can*-class" genes (White-Cooper et al., 1998). However, Boule protein accumulation requires the "*can*-class" genes (Chen et al., 2005). Because "meiotic arrest" genes regulate

transcription or translation of meiotic cell cycle genes, they set up the crosstalk in which meiotic cell cycle progression awaits expression of terminal differentiation genes (Lin et al., 1996; White-Cooper et al., 1998).

The five known *aly*-class genes are *always early (aly), cookie monster (comr), matotopetli (topi), tombola (tomb)*, and *achintya+vismay (achi+vis)*. All of the *aly*-class genes, except for *achi/vis*, are expressed exclusively in primary spermatocytes (Ayyar et al., 2003; Jiang et al., 2007; Jiang and White-Cooper, 2003; Perezgasga et al., 2004; Wang and Mann, 2003; White-Cooper, 2009). Four of the five *aly*-class proteins have putative DNA binding domains: Comr contains a winged helix, Topi contains multiple Zn-finger motifs, Tomb has a CXC domain and Achi/Vis, products from gene duplication, has homeodomains. Thus, it is thought that these proteins regulate transcription of target genes by directly binding to DNA sequences, although their direct target genes have not been identified. Immuno-affinity purification studies have revealed that Aly and Tomb proteins are copurified with Mip40 (Myb interacting protein, 40kDa) to form the testis meiotic arrest complex tMAC, which also contains Topi, Comr and CAF1 (Beall et al., 2007). A second form of tMAC contains Aly, Comr and Achi/Vis (Wang and Mann, 2003). While the mode of action of tMAC is not fully elucidated, it is thought to have an active, rather than repressive, transcriptional role. Expression of Achi/Vis fused with a strong transactivation domain, VP16, rescued the *achi+vis* mutant phenotype, while the fusion of Achi/Vis with a repression domain, EnR, failed to rescue (Wang et al., 2008). Consistent with these findings, all tMAC subunits have been found to co-localize with euchromatin in primary spermatocytes (Jiang et al., 2007; Jiang and White-Cooper, 2003; Wang and Mann, 2003; White-Cooper et al., 2000).

The *can*-class genes encode testis-specific homologues of the ubiquitously expressed subunits of the general transcription factor II D ($TF_{II}D$). $TF_{II}D$ is one of the general transcription factors that constitute the RNA Pol II preinitiation complex composed of TATA-binding protein (TBP) and 13-14 TBP-associated factors (TAFs) (Cler et al., 2009; Matangkasombut et al., 2004; Tora, 2002). $TF_{II}D$ coordinates the interaction between RNA Pol II and gene promoter regions. The characterized *can*-class genes include: *cannonball (can, TAF5L), meiosis I arrest (mia, TAF6L), no hitter (nht, TAF4L), ryan express (rye, TAF12L)* and *spermatocyte arrest (sa, TAF8L)*. Among these five TAF homologs, four (Mia, Nht, Rye and Sa) share a similar structural domain called histone folding motifs for protein-protein interaction, and Can is a WD40-repeat-containing protein (Hiller et al., 2001). Indeed, Nht and Rye form a heterodimer *in vitro* (Hiller et al., 2004). These testis-specific TAFs (tTAFs) are thought to form a testis-specific complex required for the transcriptional activation of terminal differentiation genes (Hiller et al., 2004; Hiller et al., 2001). Such predicted functions of tTAFs suggest that they localize at euchromatin in spermatocyte nuclei. However, while some tTAFs associate with chromosomes in spermatocytes, most tTAF protein is localized to a subcompartment within the nucleolus (Chen et al., 2005; Metcalf and Wassarman, 2007). Interestingly, Polycomb (Pc) and other components of the Polycomb Repressive Complex 1 (PRC1) are localized to the same nucleolar subcompartment as tTAFs in spermatocytes. Furthermore, localization of PRC1 components to the spermatocyte nucleolus is coincident with tTAF expression and dependent on wild-type tTAF function (Chen et al., 2005). These results suggest that tTAFs act as derepressors by sequestering PRC1 to spermatocyte nucleolus to counteract Polycomb Group protein (PcG)-induced repression. However, removing PcG activity is not sufficient to turn on terminal differentiation genes in the absence of tTAFs (Chen et al., 2011b), suggesting that the chromatin-associated

tTAFs are required for activating terminal differentiation genes. ChIP and microarray analysis identified several tTAF direct target genes, such as *Mst87F, fuzzy onion* (*fzo*) and *don juan* (*dj*) (Chen et al., 2005; Chen et al., 2011b) Further ChIP analysis at *Mst87F, fzo* and *dj* promoter regions showed that levels of the repressive H3K27me3 mark and poised unphosphorylated Pol II are high, while levels of the active H3K4me3 mark are low in *can* and *aly* mutant testes (Chen et al., 2011a). These data suggest that tTAFs and tMAC might recruit the Trithorax group complex (TrxG) to methylate H3K4 at promoters of terminal differentiation genes (Chen et al., 2005).

Although the mode of interaction between tMAC components (*aly*-class) and tTAFs (*can*-class) is not fully understood, it was reported that the function of *aly* is required for both TAF8L's binding to target gene promoters and the proper nucleolar localization of several tTAFs as well as Pc in primary spermatocytes, suggesting that tMAC acts upstream of tTAFs (Chen et al., 2011a). This is consistent with transcriptional profiling assays using Northern blot, *in situ* hybridization, and microarray analysis (Chen et al., 2011b; Hiller et al., 2001; White-Cooper et al., 1998). In addition, while Mip40 is co-immunoprecipitated with tMAC components, loss of *mip40* results in spermatocytes with condensed chromosomes, a phenotype similar to mutants of *can*-class genes (Beall et al., 2007), suggesting that Mip40 might mediate the interaction between tMAC and tTAFs.

Transcriptional regulation in post-meiotic cells

After germ cells exit the extended G2 phase, they undergo two meiotic divisions followed by spermatid differentiation. One of the major epigenetic events in post-meiotic germ cells is the displacement of histones by the transition nuclear proteins (Tnps) followed by protamines (Prms)

(Rathke et al., 2007). The replacement of histones with protamines allows for DNA condensation and packaging in the sperm nuclei. To prepare for this replacement, histones undergo a series of post-translational modifications that open up the chromatin, including hyperacetylation of H3/H4 tails (Awe and Renkawitz-Pohl, 2010; Rathke et al., 2007), mono-ubiquitylation of H2A (Rathke et al., 2007), and phosphorylation of H4S1 (Krishnamoorthy et al., 2006).

The replacement of histones with protamines causes chromatin condensation, which is thought to block transcription in spermatids (Steger, 2001). In addition, autoradiography studies of nucleic acid synthesis demonstrated a lack of transcription in post-meiotic cells (Gould-Somero M., 1974; Oliveri G, 1965). Thus, it had been thought that spermatid differentiation genes are all transcribed in spermatocytes and under translational repression. For example, *dj* is transcribed in primary spermatocytes but remains translationally repressed until in elongated spermatids, where the Dj protein is required for sperm tail formation. However, 24 genes showed active transcription in *Drosophila* spermatids using a sensitive single-cyst quantitative RT-PCR method (Barreau et al., 2008). These results demonstrate that after global transcription is shut off in late spermatocytes, transcription can be reactivated at a set of genes during mid-elongation stage of spermatids, prior to the histone-to-protamine change. For example, *scotti* is one of the post-meiotically transcribed genes, which is required for normal actin cone progression during spermatid individualization. Consistent with its function, *scotti* mutant males are sterile (Barreau et al., 2008).

Active transcription in spermatids has been described in mammals (Reynard et al., 2007; Schultz et al., 2003). The new discovery of actively transcribed genes in *Drosophila* spermatids provides a striking similarity between fly and mammalian spermatogenesis. However, while genes transcribed in spermatids almost always encode components of the mature sperm in

mammals, most genes transcribed in *Drosophila* spermatids (22 of the 24) do not encode sperm proteins (Barreau et al., 2008). Further investigation of these genes is warranted to better understand their functions during spermatogenesis.

Conclusion and perspectives

In conclusion, *Drosophila* spermatogenesis has provided a great model system to study transcriptional regulation of the cellular differentiation pathway in an endogenous stem cell linage. Recently, new technologies, including high-throughput mRNA sequencing (RNA-seq) and ChIP-seq, have greatly improved our understanding of transcriptional regulation. In the future, combination of highly sensitive genomic techniques with purified cell types at distinct stages will reveal transcriptional profiles with much higher spatiotemporal resolution. Furthermore, ongoing efforts from several labs to identify direct target genes downstream of key signaling pathways will also illuminate regulatory networks that control transcriptional profile changes during spermatogenesis.

Epigenetic regulation in adult stem cells and cancer

Adult stem cells are defined as cells that have two central properties: self-renewal and differentiation. Many types of adult stem cells have the remarkable ability to undergo asymmetric mitotic divisions that produce two distinct daughter cells. Alternatively, they undergo symmetric divisions in a stochastic manner to produce more stem cells and differentiating cells. One daughter maintains the stem cell properties, while the other differentiates to replenish specialized cell types. The ability of adult stem cell derivatives to

divide and differentiate to replace damaged tissues provides the body with an internal repair system.

Previous studies on adult stem cells have focused on understanding how extrinsic signaling pathways regulate proper stem cell functions. In addition, recent evidence shows that intrinsic factors, such as chromatin structure of stem cells, play important roles in regulating stem cell identity and activity. Epigenetic mechanisms alter the chromatin state of genes without altering their primary DNA sequences. Three major epigenetic mechanisms are known to cooperate in stem cells: nucleosome repositioning driven by chromatin remodeling factors, DNA methylation, and post-translational modifications of histones, including methylation, phosphorylation, acetylation, ubiquitination, and sumoylation (Kouzarides, 2007). Together, these mechanisms may establish a distinct epigenetic state that leads to a unique gene expression pattern in stem cells (Cedar and Bergman, 2009). Perturbations of these epigenetic mechanisms may lead to premature differentiation or continuous self-renewal/proliferation of stem cells, a hallmark of cancer.

The relationship between carcinogenesis and changes in specific gene expression or genome stability has been well documented (Adjei, 2001; Boveri, 2008; Duesberg et al., 2004; Hanahan and Weinberg, 2000). Two major epigenetic mechanisms, DNA methylation and post-translational modifications of histones, have been shown to contribute to the initiation and progression of cancers (Blair et al., 2011; Chi et al., 2010; Esteller, 2008; Jones and Baylin, 2007; Rodriguez-Paredes and Esteller, 2011). Accumulation of aberrant genetic mutations or abnormal epigenetic profiles could lead to tumor initiation in adult stem cell lineages (Alison et al., 2011; Barker et al., 2009; Leedham et al., 2005). For example, using the lineage-tracing method, studies in mice have shown that aged intestinal stem cells (ISCs) accumulate cancer-

causing mutations (Barker et al., 2009; Zhu et al., 2009). However, while most studies characterize epigenetic alterations in cancers using cancer cell lines or the entire tumor, cells within a tumor display a wide degree of heterogeneity, and not all of them have the ability to initiate and sustain a tumor (Shipitsin and Polyak, 2008; Wang and Dick, 2005). Recently, it has been proposed that a small population of cancer cells, termed cancer stem cells (CSCs), is distinct from other tumor cells and has the capacity to drive tumor initiation and growth. By definition, CSCs are a subset of tumor cells that have the capacity to self-renew, the potential to develop into any other cells in the tumor, and the proliferative ability to drive continued tumor expansion (Jordan et al., 2006). In the past decade, CSCs were found to exist in a wide range of solid tumors (Al-Hajj et al., 2003; Bonnet and Dick, 1997; Eramo et al., 2008; O'Brien et al., 2007; Prince et al., 2007; Singh et al., 2004). CSCs are currently being targeted in cancer treatments; however, they are relatively resistant to a variety of chemo- and radiotherapy (Li et al., 2008). Therefore, a better understanding of the biology of CSCs, including epigenetic alterations that affect their function, is essential for developing effective cancer therapies. On the other hand, the existence of CSCs raises the concern that conclusions based on studies using entire tumors might not apply to CSCs.

In this review, we will start by discussing the most recent discoveries in epigenetic regulation of normal adult stem cell lineages in multiple stem cell systems and across several different model organisms. We will then take up the question of epigenetic regulation in cancers, focusing on recent data on CSCs and making comparisons with adult stem cells.

Epigenetic regulation in germline stem cells (GSCs)

Germ cells are a unique cell type because they are able to generate an entire organism upon fertilization (Cinalli et al., 2008). Because germ cells are responsible for initiating the next generations, it is crucial that they retain accurate genetic and epigenetic information and properly transmit such information across generations (Eun et al., 2010). In many organisms, GSCs initiate a tightly controlled cellular differentiation process called gametogenesis to produce gametes. Like other adult stem cells, GSCs are capable of both self-renewal and differentiation. In addition to extensive knowledge about the role of extrinsic signaling pathways in maintaining GSCs (Morrison and Spradling, 2008), recent studies have shown that epigenetic mechanisms control the decision of GSC self-renewal versus differentiation (Buszczak and Spradling, 2006; Li and Zhao, 2008).

Histone modifications play an essential role in intrinsically regulating GSC identity and activity. Recent studies have identified a cohort of enzymes called "epigenetic writers" and "epigenetic erasers" that generate or remove a particular histone modification (Sarmento et al., 2004; Seligson et al., 2005). These enzymes are shown to be important for stem cell activities. For example, members of the ASH-2 complex in *C. elegans* act as "epigenetic writers'" to generate the active trimethylation of histone H3 lysine 4 (H3K4me3). Deficiencies in members of the ASH-2 complex, such as WDR-5 and H3K4 methyltransferase (HMT) SET-2, lead to misregulation of a subset of genes required for worm longevity (Greer et al., 2010). Presence of an intact germline was necessary for lifespan regulation by members of the ASH-2 complex, suggesting that the "epigenetic landscape" of germ cells regulates somatic cell fitness. Additionally, mutations in *wdr-5*, whose function is required for ASH-2 complex stability and activity, lead to decreased GSCs and improper gametogenesis, suggesting another role for H3K4

methylation in maintaining GSC identity and proper differentiation (Li and Kelly, 2011) (**Fig. 1-3A**).

HMTs are also required for gametogenesis in *Drosophila melanogaster*. The *Drosophila* male and female GSC lineages are both paradigmatic systems to study adult stem cells in their physiological environment, or niche (Eliazer et al., 2011; Gan et al., 2010a; Lee et al., 2010; Tarayrah et al., 2013; Wang et al., 2011b; Yang et al., 2012). In females, 2-3 GSCs reside in the germarium located at the tip of each ovariole (Xie and Spradling, 2000), and each ovary contains about 16 ovarioles. Within the female GSC niche, GSCs directly associate with somatic cells (i.e., cap cells, terminal filaments, and escort cells, **Fig. 1-3B**). GSCs mutant for *eggless (egg),* a HMT that generates the repressive H3K9me3 modification, display both maintenance and differentiation defects (Wang et al., 2011b). Removal of *egg* function from germ cells using FLP-mediated FRT recombination leads to GSC maintenance defects in the niche, suggesting that Egg is required intrinsically for GSC self-renewal. Loss of *egg* in GSCs leads to decreased expression of bone morphogenetic protein (BMP) pathway components, which are necessary and sufficient for GSC self-renewal. Consistent with the results observed using loss-of-function alleles, knockdown of *egg* using an RNAi transgene leads to GSC loss (Wang et al., 2011b). However, using another RNAi transgene leads to enlarged germaria due to the accumulation of GSC-like cells, suggesting an intrinsic role for *egg* in regulating GSC differentiation (Rangan et al., 2011; Wang et al., 2011b). It is rare for a single gene to be required for both GSC maintenance and differentiation. The contradictory results could stem from one or both of the RNAi transgenes used having off targets. Interestingly, loss of *egg* in escort cells in the female GSC niche leads to germaria accumulating GSC-like cells, indicating that Egg is also required non-cell-autonomously for proper differentiation of GSCs. Most of the GSC-like cells away from

the niche still express high levels of BMP pathway components, suggesting that Egg acts in escort cells to prevent ectopic BMP signaling and allow proper GSC differentiation. It is remarkable that Egg regulates both GSC self-renewal and differentiation by having an opposite effect on the same signaling pathway in a cell type-specific manner (Wang et al., 2011b).

Another H3K9 methyltransferase in *Drosophila*, dG9a, is required for the formation of functional spectrosome, an organelle required for asymmetric divisions of female GSCs. As a result of spectrosomal dysfunction, germaria mutant for *dG9a* accumulate disorganized germline cysts that fail to specify the oocyte for oogenesis (Lee et al., 2010).

"Epigenetic erasers" reverse particular histone modifications, which have been shown to regulate adult stem cell maintenance (Eliazer et al., 2011; Tarayrah et al., 2013). For example, histone demethylases remove methyl groups from methylated lysine residues of histones (Klose et al., 2006). The lysine-specific demethylase 1 (Lsd1), which demethylates histone 3 on both lysine 4 and lysine 9 (H3K4/K9), was shown to function in the ovary to prevent GSC tumor formation and maintain proper egg chamber development (Eliazer et al., 2011).

In *Drosophila* testis, a group of 8-12 GSCs reside in a niche comprised of two types of somatic cells: hub cell and cyst stem cells (CySCs) (**Fig. 1-3C**). GSCs undergo asymmetric cell divisions to ensure the balance between self-renewal and differentiation (Inaba and Yamashita, 2012). Recent studies from our group reveal a very interesting phenomenon. Specifically, during GSC asymmetric divisions, preexisting histone 3 (H3) is preferentially retained in the GSC, while newly synthesized H3 is enriched in the other daughter cell called a gonialblast (GB) committed for differentiation. We further demonstrate that both asymmetric H3 segregation during GSC mitosis and post-mitotic rapid turnover of preexisting H3 in GB contribute to this asymmetric H3 distribution. Such asymmetric inheritance of H3 could be a mechanism for the

ability of GSC to maintain its unique gene expression profile, as well as allowing GB to reset its chromatin structure for differentiation (Tran et al., 2013; Tran et al., 2012). Interestingly, such an asymmetric H3 distribution pattern is abolished in testicular tumor in which GSCs are overproliferative (Tran et al., 2012), suggesting that this asymmetric H3 inheritance is related to different cell fates from asymmetric cell divisions. It will be interesting to investigate whether other stem cells use similar mechanisms for a reliable epigenetic inheritance.

Recently, several proteins that generate, recognize, or remove specific histone modifications have been reported to play essential roles in male GSC maintenance. For example, an "epigenetic reader" encoded by the *PHD finger protein 7* (*PHF7*) gene recognizes and associates with the active H3K4me2 mark. PHF7 is highly expressed in early germ cells and is required for GSC maintenance and spermatogonial differentiation (Yang et al., 2012). An "epigenetic eraser", *Drosophila* Ubiquitously transcribed tetratricopeptide repeat gene on the X chromosome (dUTX), is the sole enzyme that demethylates the repressive H3K27me3 mark(Smith et al., 2008). Our group found that dUTX regulates testis niche architecture by targeting the Janus kinase signal transducer and activator of transcription (JAK-STAT) signaling pathway, a major pathway required for GSC maintenance (Tarayrah et al., 2013). We further showed that dUTX maintains active transcription of an inhibitor of the JAK-STAT pathway encoded by *Suppressor of cytokine signaling at 36E (Socs36E)* gene. Specifically, dUTX removes the repressive H3K27me3 mark near the transcription start site (TSS) of *Socs36E* gene. In addition to its role in maintaining niche architecture, dUTX also functions intrinsically in male GSCs to maintain their adhesion to hub cells by regulating the transcription of *DE-Cadherin* (Tarayrah et al., 2013). Interestingly, mammalian UTX, also known as KDM6A, has been shown to regulate reprogramming: *Utx* mutant somatic cells cannot be induced to the ground state of

pluripotency (Mansour et al., 2012). In addition, mutations in the human homolog of UTX cause an increase in H3K27me3 levels and lead to human cancers (van Haaften et al., 2009). These observations suggest that UTX H3K27me3 demethylase maintains stem cell properties in multiple stem cell systems in different species.

Apart from histone modifying enzymes, dynamic regulation by chromatin remodeling factors is also required to maintain GSC activity and identity. Chromatin remodeling enzymes use ATP hydrolysis to alter histone-DNA contacts (Becker and Horz, 2002). In *Drosophila*, nine ATP-dependent remodelers have been classified into four families based on their structural similarities: (1) imitation switch (ISWI) family members which all have a SANT domain, (2) SWI2/SNF2-related proteins which share a bromodomain, (3) CHD family members which all have a chromodomain, and (4) Rad16 family members which possess a ring finger(Bouazoune and Brehm, 2006). Interestingly, ISWI maintains GSCs in both males and females, suggesting a common epigenetic mechanism in both sexes (Cherry and Matunis, 2010; Xi and Xie, 2005). ISWI and Nurf301 are two of the four subunits that form the nucleosome remodeling factor (NURF) complex. In male flies, mutations in either *iswi* or *nurf301* lead to decreased GSCs (Cherry and Matunis, 2010). In females mutant for either *iswi* or a second ATP-dependent remodeling factor known as *Domino (DOM)*, GSCs are lost as a result of premature differentiation (Xi and Xie, 2005). In both sexes, the premature differentiation of GSCs is caused by precocious expression of the *bag of marbles* (*bam*) gene, which is necessary and sufficient for GSC differentiation.

The role of chromatin remodeling factors in maintaining GSC activity is also evident in mammals. In mice, Sertoli cells maintain physical contact with germ cells throughout gametogenesis (**Fig. 1-3D**). They direct formation of the stem cell niche by coordinating the

functions of other support cell populations (Oatley et al., 2011). SIN3A, a nuclear corepressor that associates with histone deacetylase-1 (HDAC1), is highly expressed in Sertoli cells. HDACs remove acetyl groups from specific lysine residues on histone tails, and their activity is often associated with transcriptional repression. Testes from mice lacking *Sin3a* exhibit a wide range of defects from loss of GSCs and proliferative spermatogonia to failure of spermatid differentiation. GSC markers, such as *Oct4* and *Lin28*, are downregulated in *Sin3a* mutant testes (Gallagher et al., 2013; Payne et al., 2010) , suggesting that the chromatin structure of Sertoli cells is essential for maintaining active transcription of key regulators for GSC maintenance (Gallagher et al., 2013; Payne et al., 2010), probably through signaling pathways.

Epigenetic regulation in intestinal stem cells (ISCs)

The *Drosophila* midgut is the primary organ for food digestion and nutrient absorption. Therefore, its maintenance is essential for organismal growth and survival. The midgut in *Drosophila* comprises an epithelial monolayer that is surrounded by two layers of visceral muscle. Unlike GSCs, ISCs could not be easily identified based on their anatomic locations within the tissue. However, the lineage-tracing technique was utilized to successfully determine that ISCs reside at the basal side, adjacent to the basement membrane of midgut (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006). ISCs are multipotent in that they divide asymmetrically to self-renew and give rise to progenitor cells called enteroblasts (EBs). Activated Notch is sufficient for ISCs to differentiate to EBs, while activated Wnt signaling leads to ectopic ISC self-renewal (Lin et al., 2008; Ohlstein and Spradling, 2007). EBs further differentiate into two cell types: absorptive enterocytes (ECs) and enteroendocrine cells (ees) (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006) (**Fig. 1-4**). While many studies on

ISCs have focused on signaling pathways, such as Notch and Wnt signaling pathways (Wang and Hou, 2010), recent studies have uncovered important roles of epigenetic mechanisms in maintaining ISC identity and activity.

Several histone-modifying enzymes have been implicated in maintaining ISCs. One example is the Scrawny (Scny) enzyme that deubiquitinates mono-ubiquitinated H2B and functions in gene silencing. Adult flies mutant for *scny* rapidly lose ISCs due to inappropriate activation of the Notch pathway, which leads to ISC differentiation. Furthermore, *scny* mutant flies have decreased GSCs in testes and ovaries, as well as ISCs, suggesting that a single histone-modifying enzyme is required in multiple stem cell systems (Buszczak et al., 2009). Interestingly, cells mutant for *scny* have elevated ub-H2B and H3K4me3 signals, which probably leads to more open chromatin and active transcription of Notch target genes (Buszczak et al., 2009). Consistent with the requirement of ub-H2B for cellular differentiation, in female GSC lineage, ub-H2B signal is undetectable in GSCs, but detectable in the cystoblasts (CBs), the immediate daughter cells of GSCs committed for differentiation (Karpiuk et al., 2012). Recently, a histone acetyltransferase (HAT) encoded by the *Atac2* gene has been shown to regulate the activity of ISCs (Ma et al., 2013). HATs transfer acetyl groups to specific lysine residues on histone tails, a modification that is mostly associated with active transcription. Atac2 is a component of the Ada-Two-A-containing (ATAC) complex, which acetylates K16 on H4 (Suganuma et al., 2008; Suganuma et al., 2010). Loss of *Atac2* leads to increased ISCs, whereas overexpression of *Atac2* promotes ISC differentiation (Ma et al., 2013). The molecular mechanism by which Atac2 regulates ISC differentiation remains unknown, but one possibility is that Atac2 activates Notch target genes by generating the H4K16ac mark at their promoter regions.

In addition to histone-modifying enzymes, dynamic regulation of ISC activities is achieved by DNA modifications. DNA methylation at cytosines is usually associated with repressive gene expression (reviewed in (Cedar and Bergman, 2009)). Mammalian methyl-CpG-binding protein-2 (MeCP2) recognizes methylated DNA and associates with SIN3A and HDAC1 histone-modifying enzymes, acting as a bridging factor between DNA methylation and histone modifications (Nan et al., 1998). Unlike mammals, DNA methylation is only detectable in the early stages of *Drosophila* embryos (Lyko et al., 2000). Interestingly, expression of human MeCP2 (hMeCP2) in *Drosophila* ECs in midgut alters the cytological distribution of heterochromatin protein-1 (HP-1), as determined by immunofluorescence, and stimulates ISC proliferation. These observations suggest that hMeCP2 misregulates genes important for ISC maintenance (Lee et al., 2011).

Epigenetic regulation in hair follicle stem cells

In mammals, the stem cells within the hair follicle niche (HF-SCs) are required to sustain hair regeneration and pigmentation in a cyclical manner. HF-SCs refer to both epithelial hair follicle stem cells and melanocyte (i.e., pigment) stem cells, both of which reside at the base of the noncycling hair follicle in the bulge area (**Fig. 1-5**). Two hallmarks of HF-SCs are their extended state of dormancy and slow cycling, properties which predispose these cells to accumulate genetic mutations and epigenetic aberrations that lead to tumor formation (Morris, 2000). Remarkably, the proliferation and differentiation cycle of melanocytes is synchronized to the cycle of hair follicle cells in order to regenerate pigmented hair (Slominski and Paus, 1993). Hair follicles periodically undergo hair growth (anagen) followed by destruction (catagen) and rest (telogen), during which both stem cell populations remain quiescent for weeks in adulthood.

Several signaling pathways, including Wnt, BMP/TGF-β and mitogen-activated phosphokinase (MAPK) pathways, have been reported to play essential roles in activating both stem cell populations coordinately (Greco et al., 2009; Nishimura et al., 2010; Rabbani et al., 2011) in order to start a new cycle of hair follicle generation. Recent reports have uncovered key roles of specific histone-modifying enzymes in regulating the balance between quiescence and activation of HF-SCs. For example, Polycomb group (PcG) proteins, which are comprised of Polycomb repressive complex 1 (PRC1) and PRC2, have been shown to maintain the cyclical nature of hair follicle regeneration. Using chromatin immunoprecipitation, followed by ChIP-seq, a high-throughput sequencing technique, chromatin changes upon transition from HF-SCs to transit-amplifying progenies (HF-TA) have been characterized. In HF-SCs, PcG represses hair follicle differentiation by generating the repressive H3K27me3 mark at TSSs of key differentiation genes, which are repressed in HF-SCs, but expressed in HF-TAs. Reciprocally, genes required for HF-SC maintenance acquire high levels of H3K27me3 in HF-TA cells, which was found to be necessary for proper HF-TA differentiation (Lien et al., 2011). Because PRC2 components *Enhancer of Zeste homolog 1* (*Ezh1*) and *Ezh2* encode H3K27me3 methyltransferases in mice, *Ezh1/2* double knockout HF-SCs have reduced H3K27me3 levels and decreased proliferation. Real-time PCR (RT-PCR) and immunofluorescence analyses in mutant HF-SCs revealed increased transcription of the *Ink4b*/*Ink4a*/*Arf* gene locus, which encodes cell cycle inhibitors p16, p15 and p19 (Ezhkova et al., 2011). Increased expression of cell cycle inhibitors may lead to HF-SC proliferation defects.

Another recent study reported the role of Jarid2 in maintaining HF-SCs. Jarid2 is a member of the JumonjiC (JmjC) domain-containing family of proteins. Using ChIP, followed by quantitative PCR (qPCR) in *Jarid2* conditional knockout (cKO) neonatal keratinocytes,

H3K27me3 was demonstrated to have reduced levels at PRC2 target genes, suggesting that Jarid2 recruits PRC2 to their targets. These data are consistent with the function of Jarid2 in embryonic stem cells (ESCs) (Li et al., 2010a; Pasini et al., 2010). Although Jarid2 has been found to be dispensable for HF-SC establishment and maintenance, in *Jarid2* cKO mice, loss of *Jarid2* leads to increased expression of p16, which results in reduced proliferation and delayed hair follicle cycling of HF-SCs (Mejetta et al., 2011).

Abnormal epigenetic regulation in cancers

Self-renewal and proliferative abilities are essential for maintaining stem cell number and preventing tissue dystrophy. However, several mechanisms are required to tightly regulate stem cell self-renewal and proliferation in order to prevent uncontrolled cell expansion and tumor generation. The cancer stem cell (CSC) model proposes that a subpopulation of tumor cells self-renew and give rise to more differentiated cells that form the tumor (Al-Hajj et al., 2003; Bonnet and Dick, 1997; Eramo et al., 2008; Morrison et al., 2002; O'Brien et al., 2007; Prince et al., 2007; Singh et al., 2004). CSCs are highly proliferative and responsible for sustained tumor growth, as well as new tumor formation upon metastasis (Malanchi et al., 2012). Therefore, understanding the cellular and molecular characteristics of CSCs may have many implications for developing therapeutic strategies against cancers.

Several epigenetic mechanisms have been implicated in maintaining the identity and activity of CSCs. For example, global DNA hypomethylation has been shown to be a hallmark of many benign and invasive tumors (Feinberg and Vogelstein, 1983; Gama-Sosa et al., 1983; Goelz et al., 1985). *S100A4*, a metastasis-associated gene, has been found to be hypomethylated in colon cancer (Nakamura and Takenaga, 1998), and hypomethylation at the oncogene *R-RAS*

region is associated with gastric cancer (Nishigaki et al., 2005). DNA demethylation is a recently identified phenomenon with the discovery of the *ten-eleven-translocation* (*TET*) family genes. Members of the Tet family of proteins (Tet1/2/3) are dioxygenases that convert cytosine-5-methylation (5mC) to 5-hydroxymethyl-cytosine (5hmC) (Ito et al., 2010; Tahiliani et al., 2009), the removal of which contributes to the DNA demethylation process (Wu and Zhang, 2010). Interestingly, levels of 5hmC are substantially reduced in a number of human cancers, including breast, liver, lung and pancreatic cancers, which was found to be associated with dramatically reduced expression of all three *TET* genes (Yang et al., 2013). It is very likely that abnormal epigenetic regulation at *TET* genes' loci leads to their reduced expression. Significant loss of 5hmC is also a feature of human melanomas, and, interestingly, introduction of active *TET2* suppresses melanoma growth (Lian et al., 2012).

On the other hand, genetic mutations in *TET* genes have been found in other cancers, including leukemia and lymphoma (Huang et al., 2013b; Lian et al., 2012; Makishima et al., 2011; Pui et al., 2003), suggesting an essential role of DNA demethylation in carcinogenesis. Specifically, *TET2* has been shown to act as a critical tumor suppressor and is frequently mutated in leukemia and myeloid cancers (Ko et al., 2010; Mardis et al., 2009). *TET1* has also been shown to be a tumor suppressor in various cancers, including prostate and breast cancers (Hsu et al., 2012; Sun et al., 2013). Interestingly, while *TET* genes are frequently downregulated in tumors, a recent study reported that *TET1* is upregulated in *MLL*-rearranged leukemia which is accompanied by a global increase in 5hmC levels, suggesting a role for *TET1* as an oncogene instead of a tumor suppressor (Huang et al., 2013a). Such an observation highlights the importance of tissue context in understanding a gene's function since *TET1* can act as a tumor suppressor in solid tumors, but as an oncogene in leukemogenesis. Furthermore, while both Tet1

and Tet2 have similar catalytic activities, they play opposing pathological roles in leukemogenesis, probably due to different target genes.

On the other hand, increased DNA methylation has been detected at promoters of tumor suppressor genes, such as *p16* in melanoma (Gonzalez-Zulueta et al., 1995), *RB1* in retinoblastoma (Sakai et al., 1991), and *RUNX3* in human brain tumors (Avci et al., 2013). Hypermethylation was also detected at the promoter region of *Caspase 8 associated protein 2 (CASP8AP2)* gene in acute lymphoblastic leukemia (Li et al., 2013). DNA methylation is generated by DNA methyltransferase 1 (DNMT1) and maintained by DNMT3A and DNMT3B in humans (Bestor and Ingram, 1983; Chedin, 2011; Chen et al., 2007). DNA methylation has been shown to regulate CSC activity and tumor growth. For example, cKO of *Dnmt1* in mice with leukemia blocks further development of pre-existing leukemia. Furthermore, halving the level of Dnmt1 in wild-type mice leads to impaired leukemia stem cell self-renewal and survival, probably from hypomethylation and derepression of a number of tumor suppressor genes. Interestingly, using ChIP with H3K27me3 antibodies, the authors found that EZH2-controlled target genes are also derepressed in *Dnmt1* haploinsufficient mice. These data suggest that the PcG complexes might cooperate with DNA methylation to regulate leukemia stem cell activity and tumor growth (Trowbridge et al., 2012).

Consistent with the role of PcG in deterring tumor development, upregulation of EZH2 leads to aggressive progression of both breast and prostate cancers (Kleer et al., 2003; Varambally et al., 2002). A recent study reported that a high level of EZH2 expression leads to expansion of breast CSCs. Upregulation of EZH2 may lead to repression of the *RAD51* gene, which is known for DNA double-strand break repair. Failure in DNA repair results in increased genome instability and tumor progression (Chang et al., 2011). Furthermore, pharmacological

inhibition of PRC2 components, including EZH2, reduces expression of CSC markers and decreases tumor formation and growth in multiple types of cancers (Bao et al., 2012; Crea et al., 2011; Rizzo et al., 2011). Furthermore, knockdown of the oncogene BMI1 reduces expression of glioma stem cell genes and inhibits glioblastoma formation *in vivo* (Chatoo et al., 2009). BMI1 is a component of Polycomb repressive complex 1 (PRC1), which inhibits expression of tumor suppressor proteins *p16* and *p14*. Glioblastoma multiforme (GBM) is one the most common and lethal types of adult brain tumors (Stupp et al., 2009). Conditions such as hypoxia enhance the expression of glioma stem cell genes. Both hypoxia-inducible factor-1α (HIF1α) and HIF2α are preferentially expressed in glioma stem cells and are required for their maintenance (Heddleston et al., 2009; Li et al., 2009b; Seidel et al., 2010). Interestingly, knockdown of mixed-lineage leukemia 1 (MLL1), an H3K4me3 methyltransferase, inhibits expression of HIF2α and reduces glioma stem cell self-renewal and growth (Heddleston et al., 2012). These data suggest that epigenetic regulation of CSCs directly controls cancer initiation and growth. Histone demethylases have also been reported to regulate tumor formation and survival. For example, LSD1, which suppresses gene expression by converting dimethylated H3K4 to monomethylated and unmethylated H3K4, was shown to be highly expressed in pluripotent tumors. Pluripotent tumor cells express pluripotent stem cell markers, such as Oct4 and Sox2, and have the ability to differentiate into many cell types (Cheng et al., 2007; Jones et al., 2004a; Jones et al., 2004b; Strickland et al., 1980). Knockdown of *Lsd1* leads to growth inhibition of pluripotent tumor cells, such as in teratocarcinoma, embryonic carcinoma and seminoma (Wang et al., 2011a).

Conclusions and perspectives

In this review, we discussed recent advances in our understanding of epigenetic mechanisms in normal adult stem cell lineages and in tumorigenesis. Several epigenetic mechanisms have been shown to play important roles, including DNA methylation, covalent histone modifications, and chromatin remodeling. Further studies are needed to understand how different epigenetic mechanisms coordinate to ensure normal cellular differentiation in adult stem cell lineages and to prevent cancers. To better understand cancers, researchers are now focusing on the relationship between CSCs and normal stem cells. While both stem cell types have the ability to self-renew and differentiate, adult stem cells require niche cells to maintain their "stemness", whereas no niche has been identified for any type of CSCs. Additionally, while DNA methylation plays essential roles in tumorigenesis and CSC regulation, little is known about how DNA methylation regulates adult stem cells (Lyko et al., 2000). Multiple epigenetic factors are now considered targets for therapeutic strategies against cancer, and more studies are needed to elucidate the roles of epigenetic factors in tumor metastasis.

FIGURES

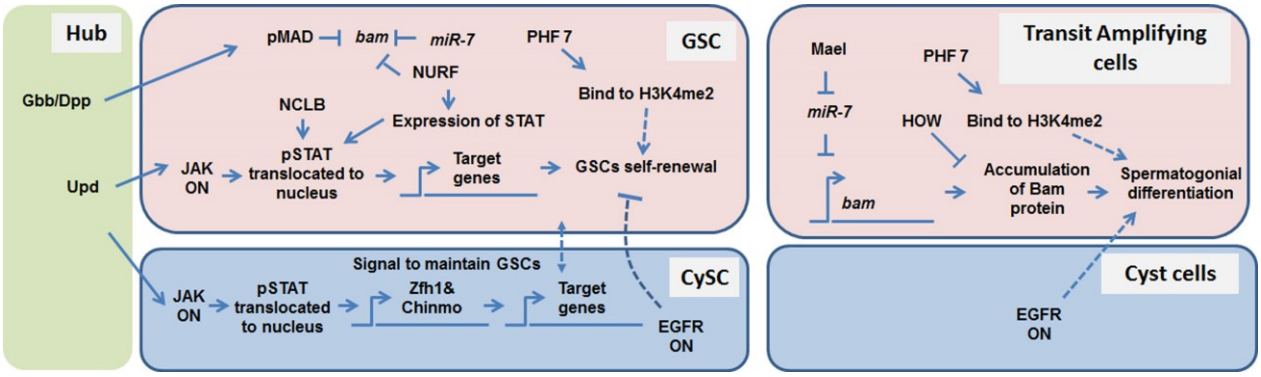


Figure 1-1. Summary of transcriptional regulation in stem cell niche and mitotic germ cells. Hub cells are in green, GSC and transit-amplifying cells are in pink, CySC and cyst cells are in blue. Solid lines denote direct regulation, dashed lines denote indirect regulation or lack of evidence for direct regulation. See text for detailed discussion.

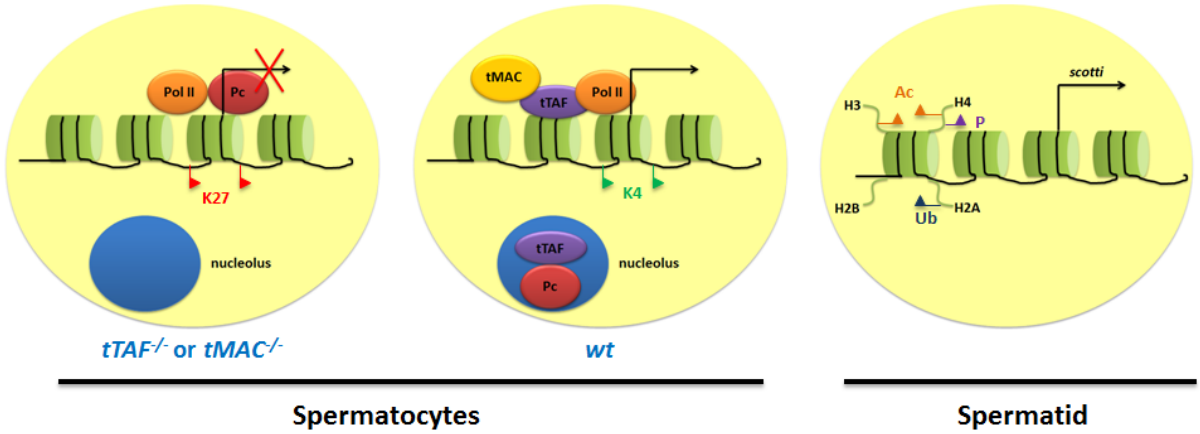
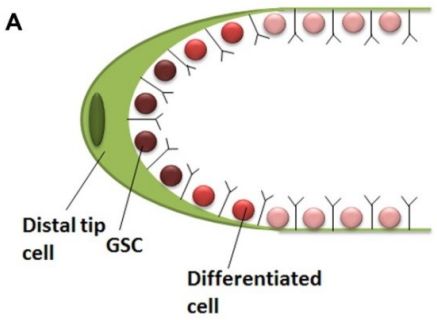


Figure 1-2. Summary of transcriptional regulation in meiotic and post-meiotic germ cells. A schematic diagram outlines potential chromatin state in spermatocytes mutant for tMAC or tTAF (left, analyzed with *can* or *aly* mutant testes) compared to mature wild-type (wt) spermatocytes (middle, analyzed with wt testes). K27: H3K27me3, K4:H3K4me3. Adapted from

Chen *et. al*.(Chen et al., 2011b) On the right a schematic diagram outlines potential chromatin state in spermatids prior to the histone-to-protamine transition. The *scotti* gene is transcribed in round spermatids. Ac: acetylation, P: phosphorylation, Ub: ubiquitylation.

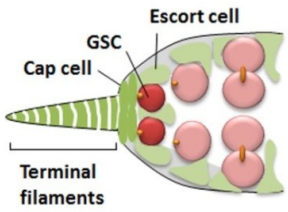
A



A'

Name of gene	Protein function	Cell type
wdr-5	H3K4 HMT	GSC

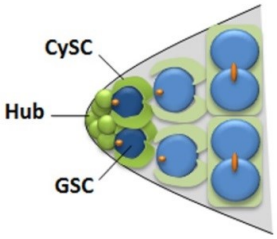
B



B'

Name of gene	Protein function	Cell type
egg	H3K9m3 HMT	GSC & Escort cells
dG9a	H3K9me3 HMT	GSC
lsd1	H3K4/K9 demethylase	GSC
iswi	Chromatin remodeling	GSC
dom	Chromatin remodeling	GSC
scny	Ub-H2B deubiquitinase	GSC

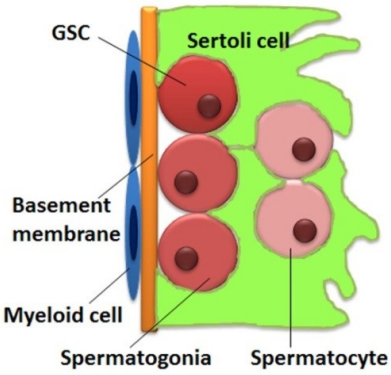
C



C'

Name of gene	Protein function	Cell type
PHF7	H3K4me2 reader	GSC & spermatogonia
dUTX	H3K27me3 demethylase	GSC & CySC
lsd1	H3K4/K9 demethylase	GSC
iswi	Chromatin remodeling	GSC
nurf301	Chromatin remodeling	GSC
scny	Ub-H2B deubiquitinase	GSC

D



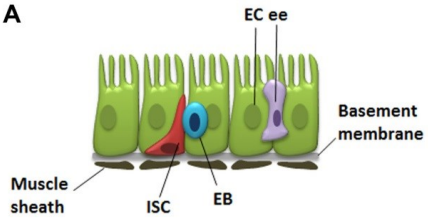
D'

Name of gene	Protein function	Cell type
Sin3a/HDAC1	Nuclear corepressor/ histone deacetylase	Sertoli cells

Figure 1-3. Illustrations of the GSC niches in *C. elegans*, male and female *Drosophila* and mouse. (**A**) *C. elegans* GSC niche. Illustration shows the distal tip cell which acts as a niche to

maintain GSCs. Dark red GSCs are within the influence of the niche and are maintained as GSCs. The lighter GSCs are outside the influence of the niche which causes them to differentiate. (**A'**) Summary of epigenetic factors that regulate the *C. elegans* GSC niche. (**B**) *Drosophila* female GSC niche. Illustration shows tip of the germarium with GSCs (dark pink, average 2-3) in the niche comprised of terminal filaments and cap cells (dark green). Escort cells are shown in light green. GSC progenies are shown in light pink. (**B'**) Summary of epigenetic factors that regulate the *Drosophila* female GSC niche. (**C**) *Drosophila* male GSC niche. Illustration shows tip of the testis with GSCs (dark blue, average 9-12; only 2 are shown here) in the niche comprised of hub cells and CySCs (dark green). Cyst cells are shown in light green. GSC progenies are shown in light blue. Round orange structures represent spectrosomes, and branched orange structures represent fusomes. (**C'**) Summary of epigenetic factors that regulate the *Drosophila* male GSC niche. (**D**) Mouse GSC niche. Illustration shows Sertoli cells which function as a niche to maintain GSCs. Myeloid cells and the basal membrane function as support cells to the niche. GSCs (dark red) differentiate to form spermatogonia (light red) which further differentiate to spermatocytes (pink). (**D'**) Summary of epigenetic factors that regulate the mouse GSC niche.

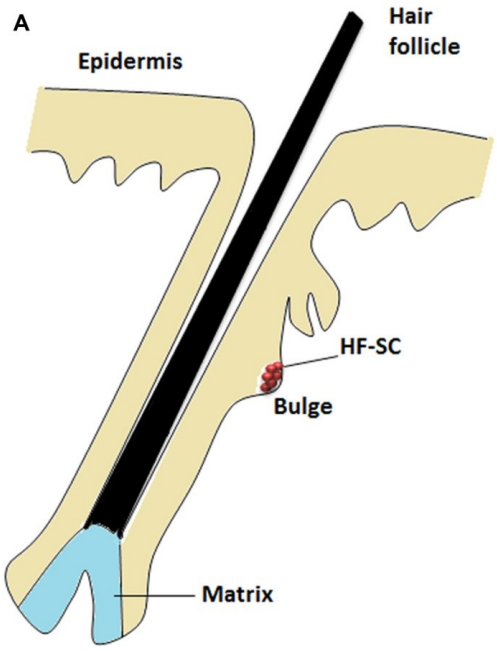
A



A’

Name of gene	Protein function	Cell type
scny	Ub-H2B deubiquitinase	ISC
Atac2	H4K16 acetyltransferase	ISC
MeCP2	Methyl-CpG-binding protein	ISC & EC

Figure 1-4. Illustration of the ISC niche in *Drosophila.* (**A**) *Drosophila* ISC lineage. Illustration shows an ISC (red) located at the basement membrane. Another daughter cell of ISC is an EB cell (blue), which further differentiates to EC (green) and ee (purple). (**A’**) Summary of epigenetic factors that regulate the *Drosophila* ISC niche.



A'

Name of gene	Protein function	Cell type
Ezh1 & *Ezh2*	H3K27me3 HMT	HF-SC & HF-TA
Jarid2	PRC2 recruiter	HF-SC

Figure 1-5. Illustration of the mammalian HF-SC niche. (**A**) Mammalian hair follicle and part of epidermis. Hair follicle stem cells or bulge stem cells reside in the bulge. (**A'**) Summary of epigenetic factors that regulate the HF-SC niche.

Chapter 2

Histone demethylase dUTX antagonizes JAK-STAT signaling to maintain proper gene expression and architecture of the *Drosophila* testis niche

This chapter is based on a previously published article (Tarayrah et al., 2013).

INTRODUCTION

Extrinsic signals in the stem cell niche are important to maintain appropriate interaction between stem cells and their niches (Morrison and Spradling, 2008). In addition, epigenetic regulation that changes chromatin structure without altering the associated DNA sequence acts intrinsically to regulate proper gene expression in stem cells (Clapier and Cairns, 2009). Both mechanisms are essential for regulating stem cell identity and activity (Cherry and Matunis, 2010; Eliazer et al., 2011). However, the crosstalk between them is not fully understood.

The *Drosophila* male germline stem cell (GSC) lineage is a paradigmatic system to investigate the molecular mechanisms that govern adult stem cell activity in their physiological environment (Kiger et al., 2001; Tulina and Matunis, 2001; Yamashita et al., 2003; Yamashita et al., 2007). *Drosophila* male GSCs reside in a microenvironment composed of two types of somatic cells: post-mitotic hub cells located at the tip of the testis and cyst stem cells (CySCs), two of which encapsulate each GSC (**Fig. 2-1A**). Hub cells and CySCs contribute to the niche of GSCs by providing critical signals to preserve GSC identity and activity (Kiger et al., 2001; Leatherman and Dinardo, 2008; Leatherman and Dinardo, 2010; Tulina and Matunis, 2001; Yamashita et al., 2003; Yamashita et al., 2007). Janus kinase signal transducer and activator of transcription (JAK-STAT) and bone morphogenetic protein (BMP) signaling pathways are the two major pathways that maintain the activity of both GSCs and CySCs. The JAK-STAT pathway is activated by the cytokine Unpaired (Upd) secreted from the hub cells, which initiates the downstream cascade to activate the Stat92E transcription factor in both GSCs and CySCs [reviewed in (de Cuevas and Matunis, 2011)]. Activation of Stat92E in CySCs initiates BMP

signaling required for GSC self-renewal, while activation of Stat92E in GSCs enhances their adhesion to the hub cells (Leatherman and Dinardo, 2008; Leatherman and Dinardo, 2010). On the other hand, *Suppressor of cytokine signaling 36E (Socs36E)* is expressed in both hub cells and CySCs, which attenuates JAK-STAT signaling (Terry et al., 2006) to maintain an appropriate balance between CySCs and GSCs in the testis niche (Issigonis et al., 2009).

In addition to signaling pathways, epigenetic mechanisms can profoundly influence critical decisions of stem cell maintenance versus differentiation. DNA wraps around four core histones (H3, H4, H2A and H2B) to form nucleosomes, the repeating basic units of chromatin. In *Drosophila,* there are two major epigenetic regulators: chromatin remodeling factors that use ATP hydrolysis to drive histone repositioning and histone modifying enzymes that covalently modify histones (Becker and Horz, 2002). Both mechanisms have been shown to act intrinsically to maintain GSCs in the testis niche (Buszczak et al., 2009; Cherry and Matunis, 2010).

Among the histone modifying enzymes, histone demethylases have been identified to act as "epigenetic erasers" that remove methyl-groups from methylated Lysine residues of histones (Klose et al., 2006). Among the 14 demethylases in *Drosophila* (Klose et al., 2006; Metzger et al., 2005; Shi et al., 2004), the *Drosophila ubiquitously transcribed tetratricopeptiderepeat gene on the X chromosome* (*dUTX*) encodes the sole demethylase that specifically removes the repressive trimethylation on lysine 27 of histone H3 (H3K27me3) (Smith et al., 2008). H3K27me3 is generated by a member of the Polycomb Group (PcG) family of proteins and has been shown to associate with silent regions of chromatin (Cao et al., 2002; Muller et al., 2002). Increased H3K27me3 levels have been reported to cause certain human cancers (Bracken et al., 2003; Kleer et al., 2003; Kondo et al., 2008; Varambally et al., 2002). Consistently, mutations that inactivate UTX, the mammalian homolog of dUTX, cause an increase of H3K27me3 and

lead to occurrence of human cancers (van Haaften et al., 2009). In *Drosophila*, dUTX has been reported to act as a suppressor of Notch- and Retinoblastoma-dependent tumors (Herz et al., 2010). Mammalian species have multiple H3K27me3-specific demethylases. Therefore, studying functions of dUTX in *Drosophila* greatly reduces the complications that might result from gene redundancy. The UTX protein is evolutionarily conserved and contains several tetratricopeptide repeats (TRP), as well as the catalytic Jumonji C (JmjC) domain (Klose et al., 2006). dUTX has been shown to physically associate with RNA Polymerase II (Pol II) *in vivo*, suggesting its involvement in transcriptional activation (Smith et al., 2008). To date, much of the knowledge about the epigenetic regulation of histone demethylases comes from biochemical studies *in vitro* or in cell culture, while their *in vivo* functions are not well understood. Therefore, to better understand the biological roles of dUTX, we examine its role in the *Drosophila* testis niche.

RESULTS

dUTX prevents overpopulation of Zfh-1-expressing cells around the hub

The *dUTX* gene encodes a histone demethylase that has been shown to remove H3K27me3 in somatic cells (Smith et al., 2008). To study the effect of *dUTX* loss on the level of H3K27me3 in testis, we used a strong *loss-of-function* allele of *dUTX* (*dUTX*^{1}) (Herz et al., 2010). The *dUTX*^{1}/Df hemizygous flies (referred to hereinafter as *dUTX*) are adult lethal, but survive up to the early pupal stage. Because of adult lethality of the *dUTX* flies, analysis of H3K27me3 levels in adult testes required the FLP/ FRT recombination system (Xu and Rubin, 1993). A dUTX-specific antibody raised against the N-terminal 153 residues (Smith et al., 2008) was absent in *dUTX*^{1} germline clones (**Fig. 2-2A,A"**), suggesting that *dUTX*^{1} is a strong loss-of-function allele. Consistent with the H3K27me3-specific demethylase activity (Herz et al., 2010),

dUTX homozygous germline clones showed an increase of the H3K27me3 signal using an H3K27me3-specific antibody (Chen et al., 2011b) when compared to the neighboring heterozygous germ cells (**Fig. 2-2B,B"**), demonstrating that dUTX acts as an H3K27me3 demethylase in germ cells. Using the mosaic analysis with a repressible cell marker (MARCM) system, we generated *dUTX* mutant cyst cell clones that are positively labeled by GFP (Lee and Luo, 1999), which showed increased H3K27me3 signal compared to a neighboring heterozygous cyst cell (**Fig. 2-2C-C"'**). These data demonstrate that dUTX also acts as an H3K27me3 demethylase in cyst cells in testis.

To determine the function(s) of *dUTX* in the male GSC niche, we analyzed testes isolated from the 3rd instar larvae of *dUTX* mutant males. Using antibodies against Armadillo (Arm) to label hub cells and the zinc finger homeodomain-1 protein (Zfh-1) to label CySCs and early cyst cells (Leatherman and Dinardo, 2008), we detected niche architectural defects in *dUTX* testes. In *wild-type* (wt) testes, Zfh-1-expressing CySCs surround GSCs and extend thin protrusions toward the hub, while their nuclei remain one-cell diameter away from the hub (**Fig. 2-1B,B'**). However, 48% of *dUTX* testes had 3 or more Zfh-1-expressing cells with their nuclei directly contacting the hub (**Fig.2-1C,C'**, arrows; **Fig. 2-1D**: compare the first and second columns). These Zfh-1-expressing cells whose nuclei contact hub cells directly stained positively for Traffic Jam (TJ), a transcription factor expressed in early cyst cell nuclei (Li et al., 2003), suggesting that they retain their identity as early cyst cells (data not shown). Overpopulation of Zfh-1-expressing cells was not, however, accompanied by an increase in the overall number of Zfh-1-expressing cells surrounding the hub [30.6±6.6 in wt testes (n=27) versus 31±9.5 in *dUTX* testes (n=30), P >0.05]. These results suggest that loss of *dUTX* does not affect Zfh-1-expressing

cell number but their behavior, which causes the Zfh-1-expressing cells to overpopulate around the hub area.

dUTX acts in CySCs and early cyst cells to prevent overpopulation of Zfh-1-expressing cells around the hub

To determine in which cell type dUTX is required to prevent overpopulation of Zfh-1-expressing cells around the hub, different cell type-specific Gal4 drivers were used in combination with a *UAS-dUTX shmiRNA* (small hairpin microRNAs) (Ni et al., 2011) to knockdown *dUTX* in a cell type-specific manner. Knockdown of *dUTX* exclusively in germ cells using *nanos* (*nos*)-*Gal4* (Van Doren et al., 1998) (**Fig. 2-3A,A'**), or in hub cells using *upd-Gal4* (Boyle et al., 2007) (**Fig. 2-3B,B'**), did not lead to overpopulation of Zfh-1-expressing cells around the hub. By contrast, knockdown of *dUTX* using cyst cell driver *c587-Gal4* (Manseau et al., 1997) led to a 45% increase of testes with overpopulated Zfh-1-expressing cells around the hub (**Fig. 2-4A,A'**, arrows; and **2-4D**: compare the third and fourth columns). Zfh-1-expressing cells also became overpopulated around the hub in 30% of *c587-Gal4* control males, which is probably due to Gal4 expression in cyst cells because a similar phenotype was observed in 35% testes carrying another cyst cell-specific *eya-Gal4* driver (Leatherman and Dinardo, 2008). To confirm that the *upd-Gal4* driving *dUTX shmiRNA* did reduce dUTX levels in hub cells, we stained testes from *upd>shmiRNA* males with the H3K27me3 antibody. As a control, the *c587>shmiRNA* testes were stained with the same anti-H3K27me3. The H3K27me3 signal in hub cells from *upd>shmiRNA* testes was higher than that in neighboring germ cells which had normal levels of dUTX (**Fig. 2-3C,C'**). By contrast, the H3K27me3 signal in hub cells from *c587>shmiRNA* testis was similar to that in the neighboring germ cells; because in this genotype both hub cells and germ cells have normal dUTX levels (**Fig. 2-3D,D'**). These results

demonstrate that normal function of dUTX is required in CySCs and/or early cyst cells, but not in hub cells, to prevent overpopulation of Zfh-1-expressing cells around the hub.

Function of dUTX in CySCs and early cyst cells depends on its demethylase activity

dUTX was reported to demethylate H3K27me3 *via* its catalytic JmjC domain (Smith et al., 2008). To determine whether the demethylase activity of dUTX is required for its function in CySCs and early cyst cells, $dUTX^{JmjC}$ was driven by the *c587-Gal4* driver at the *dUTX* mutant background. As a control, wild-type *dUTX* was expressed using the same driver. Overpopulation of Zfh-1-expressing cells in *dUTX* testes was rescued significantly by the wild-type *dUTX* transgene (**Fig. 2-4B,B'** and **2-4D**: compare the fifth and sixth columns), but not by the $dUTX^{JmjC}$ transgene (**Fig. 2-4C,C'** and **2-4D**: compare the fifth and seventh column). However, neither transgene completely rescued overpopulation of Zfh-1-expressing cells around the hub. This could be due to insufficient expression level or inappropriate expression timing using cDNA transgenes. In summary, these data demonstrate that the demethylase activity of dUTX is required for maintaining proper niche architecture.

dUTX demethylates H3K27me3 at *Socs36E* gene genomic locus for its active transcription

Because the overpopulation of Zfh-1-expressing cells around the hub in *dUTX* testes resembled the reported *loss-of-function* phenotype of the *Socs36E* gene (Issigonis et al., 2009), we used quantitative reverse-transcription PCR (qRT-PCR) to measure the *Socs36E* transcript level in *dUTX* testes. Since *Socs36E* is expressed specifically in hub cells and CySCs (Terry et al., 2006), we used a constitutively expressed somatic gene *Fringe* as an internal control. Indeed, we found that the *Socs36E* transcript level in *dUTX* testes decreased to ~65% of the level in the

wt control (**Fig. 2-5A**). However, using the entire testes may underestimate the change of *Socs36E* transcript level.

Previously, chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) data revealed that both the active histone modification H3K4me3 and RNA Polymerase II (Pol II) are enriched near the transcription start site (TSS) of the *Socs36E* gene (Gan et al., 2010b) (**Fig. 2-6A**). By contrast, the repressive histone modification H3K27me3 was depleted at the same region around the TSS of *Socs36E* gene (Gan et al., 2010b) (**Fig. 2-6A**). Because dUTX is an H3K27me3-specific demethylase (**Fig. 2-2B-C"'**), we next examined whether dUTX is required to remove H3K27me3 from the *Socs36E* TSS region, using anti-H3K27me3 ChIP followed by qPCR analysis. To generate high-resolution ChIP data, a 2kb genomic region around the *Socs36E* TSS was divided into 400-bp intervals and tested for H3K27me3 binding using a series of primer sets (p1-p5 in **Fig. 2-5B** and **Fig. 2-6A**). Control experiments were performed using two primer sets around the TSS of the control gene *Fringe* (p7-p8 in **Fig. 2-6B**), as well as a primer set within the *Socs36E* gene body (p6 in **Fig. 2-6A**). Consistent with a decreased transcription of *Socs36E* in *dUTX* testes (**Fig. 2-5A**), there was a ~4-fold enrichment of the repressive H3K27me3 at the p2 region in the *dUTX* testes compared to the wt control (**Fig. 2-5B**). The H3K27me3 binding profile at the *Socs36E* locus was consistent with the published ChIP-seq results (Gan et al., 2010b), which showed a peak enrichment of H3K27me3 at ~200-400bp downstream of the TSSs of target genes. By contrast, none of the control regions showed any difference of H3K27me3 binding between *dUTX* and wt testes (**Fig. 2-6A,B**: see numbers underneath control regions p6-p8). Furthermore, recently published ChIP-chip data using anti-dUTX antibody showed enrichment of dUTX around the *Socs36E* TSS region (**Fig. 2-6C**) (Tie et al., 2012). Taken together, these results demonstrate that dUTX

directly regulates *Socs36E* transcription by removing the repressive H3K27me3 histone modification near its TSS region.

We next examined whether overexpression of *Socs36E* independent of its genomic context is sufficient to rescue the niche architectural defects in *dUTX* testes. To achieve this, a *UAS-Socs36E-cDNA* transgene (Callus and Mathey-Prevot, 2002) was driven by either the *upd-Gal4* or the *c587-Gal4* driver. While *upd> Socs36E* failed to suppress the *dUTX* phenotype (**Fig. 2-5C**, arrows point to Zfh-1-positive cells around the hub), *c587> Socs36E* reduced overpopulation of Zfh-1-expressing cells around the hub in 93% of *dUTX* testes (**Fig. 2-5D**), further suggesting that *Socs36E* is the critical target gene of dUTX in CySCs. In summary, dUTX acts in CySCs and/or early cyst cells to directly regulate the chromatin state of *Socs36E* gene locus.

dUTX activates *Socs36E* transcription to control JAK-STAT signaling activity in the testis niche

Because Socs36E acts as a negative regulator of the JAK-STAT pathway (Terry et al., 2006), we next assessed the JAK-STAT signaling activity in the presence and absence of dUTX. In wt testes, Stat92E is enriched in GSCs and some of their immediate daughter cells called gonialblasts (GBs), but rapidly declines in further differentiated cells. Stat92E is also present in CySCs but is absent in hub cells (Leatherman and Dinardo, 2008) (**Fig. 2-7A,A'**). By contrast, Stat92E was ectopically turned on in *dUTX* testes (**Fig. 2-7B,B'**), including hub cells and further differentiated somatic cells. Using a *2×Stat-GFP* reporter, which reflects Stat92E activity in CySCs (Bach et al., 2007), we found that the GFP reporter was ectopically turned on in further differentiated cyst cells in *dUTX* mutant testes (**Fig. 2-8B,B'**), but not in the heterozygous

control (**Fig. 2-8A,A'**). Using qRT-PCR, we detected a ~1.5-fold increase of the *Stat92E* transcript in *dUTX* testes compared to that in the wt control (**Fig. 2-7C**). However, because we used whole testes for this analysis, the change in *Stat92E* transcript levels might be an underestimation. Furthermore, knockdown of *dUTX* using the cyst cell driver *c587-Gal4* (**Fig. 2-7E,E'**), but not the germ cell driver *nos-Gal4* (**Fig. 2-8C,C'** versus **2-8D,D'**) nor the hub cell driver *upd-Gal4* (**Fig. 2-8E,E'** versus **2-8F,F'**), led to ectopic Stat92E in further differentiated cells, similar to the phenotype observed in *dUTX* mutant testes. In addition, removing one copy of *Stat92E* using a strong loss-of-function *Stat92E* allele (Hou et al., 1996) suppressed overpopulation of Zfh-1-expressing cells around the hub in 90% of *dUTX* testes (**Fig. 2-7F,F'**), suggesting that hyperactivation of the JAK-STAT signaling is the causal reason for the niche architectural defects in *dUTX* testes. Together, dUTX acts in CySCs and early cyst cells to prevent ectopic JAK-STAT signaling activity.

dUTX acts in CySCs to maintain proper gene expression in hub cells

Our results also revealed dynamic communication among different cell types within the testis niche, where CySCs can send feedback to hub cells to maintain proper gene expression. We found that Zfh-1, a target gene of the Stat92E transcription factor (Leatherman and Dinardo, 2008; Terry et al., 2006), was ectopically expressed in hub cells in 92% of *dUTX* testes (**Fig. 2-1C,C'**; **Fig. 2-4E**: compare the first and second columns). However, the total number of hub cells did not change in *dUTX* testes [13±2.3 hub cells for *dUTX* 3rd instar testes (n=15) versus 13.6±2.0 hub cells for wt 3rd instar testes (n=18), P >0.05]. In addition, no hub cells undergo cell death in *dUTX* testes as determined by immunostaining with anti-Caspase 3, an apoptotic marker

(n=30) and none undergo mitosis as determined by immunostaining with anti-phospho-histone 3 (H3S10P, n=96), suggesting that hub cells maintain their number but turn on Zfh-1 expression ectopically. Because both hub cells and cyst cells in adult testes originate from the same group of somatic gonadal precursors (SGPs) in embryonic testes (Le Bras and Van Doren, 2006), one possibility for ectopic Zfh-1 expression in hub cells from adult testes is that Zfh-1 becomes misexpressed in hub precursor cells in *dUTX* embryonic testes. In order to test this possibility, we induced $dUTX^{1}$ mutant mitotic clones in adult testes and found it is sufficient to cause Zfh-1 misexpression in hub cells (**Fig. 2-9**). These results suggest that ectopic Zfh-1 expression in hub cells is due to loss of *dUTX* in CySCs and/or GSCs, the two cell types capable of forming mitotic clones next to the hub cells. Furthermore, we found that knockdown of *dUTX* in CySCs and/or early cyst cells using *c587-Gal4* (**Fig. 2-4A,A'** and **2-4E**: compare the third and fourth columns) is sufficient to turn on Zfh-1 expression ectopically in hub cells. By contrast, neither *nos-Gal4* (**Fig. 2-3A,A'**) nor *upd-Gal4* (**Fig. 2-3B,B'**) driving *dUTX shmiRNA* resulted in a similar phenotype. Together, these data demonstrate that loss of dUTX in CySCs leads to ectopic Zfh-1 expression in hub cells.

In addition, we found that the catalytic domain of dUTX is required to prevent ectopic Zfh-1 expression in hub cells because expression of the wild-type *dUTX* transgene rescued this phenotype (**Fig. 2-4B,B'** and **2-4E**: compare the fifth and sixth columns). We also observed partial rescue upon expression of the $dUTX^{JmjC}$ transgene (**Fig. 2-4C,C'** and **2-4E**: compare the fifth and seventh columns), suggesting a demethylase-independent role of dUTX in regulating proper gene expression in hub cells. Finally, restoring *Socs36E* expression in CySCs and early cyst cells (**Fig. 2-5D**) or removing one copy of *Stat92E* (**Fig. 2-7F,F'**) reduced ectopic Zfh-1 expression in hub cells of *dUTX* testes from 92% to 43-45%. Together, these data demonstrate

that dUTX acts as a histone demethylase in CySCs to prevent ectopic Zfh-1 expression in hub cells by maintaining proper JAK-STAT signaling activity.

dUTX maintains hub architecture by regulating DE-Cadherin levels in GSCs

We found that dUTX also acts in germ cells to maintain proper hub size. While dUTX directly regulates *Socs36E* transcription, unlike *Socs36E* testes (Issigonis et al., 2009), *dUTX* testes do not have decreased GSC number [12.8±3.0 for *dUTX* 3rd instar testes (*n*=98) versus 12.6±3.0 for wt 3rd instar testes (*n*=80), P >0.05]. This was due to a significant increase of hub area (**Fig. 2-10A-C**) in *dUTX* testes, which accommodated overpopulated Zfh-1-expressing cells around the hub without affecting GSC number. As mentioned previously, the increase in hub area in *dUTX* testes could not be attributed to an increase of hub cell number. However, we did observe an increase of individual hub cell size in *dUTX* testes compared to wt control (**Fig. 2-10D**). In addition, knockdown of *dUTX* using the germ cell driver *nos-Gal4*, but not the cyst cell driver *c587-Gal4* nor the hub cell driver *upd-Gal4*, led to increased hub area (**Fig. 2-10E**).

In wt testes, GSCs are attached to the hub *via* DE-Cadherin-mediated adherens junctions (Jenkins et al., 2003; Yamashita et al., 2003), resulting in a rosette-like structure (**Fig. 2-10A,A'**). The GSC-hub interface in wt testes averaged 4.3μm (**Fig. 2-11C**, first column). By contrast, the GSC-hub interface in *dUTX* testes was disrupted (**Fig. 2-10B,B'**, arrows). GSCs appeared to intrude into the hub area, leading to an increase of the GSC-hub interface to an average of 5.9μm (**Fig. 2-11C**, second column). We next examined whether this defect in *dUTX* mutant niche is due to mis-regulation of DE-Cadherin. Using qRT-PCR we detected a ~2-fold increase of the *DE-Cadherin* transcript level in *dUTX* testes compared to that in the wt control (**Fig. 2-11D**).

Additionally, we found that expression of a dominant-negative form of DE-Cadherin (*UAS-DE-Cad*^{dCR4h}) (Inaba et al., 2010) in germ cells suppressed the *dUTX* hub size defect (**Fig. 2-11A,A'**, and **2-11E**) and resulted in a decrease of the GSC-hub interface (**Fig. 2-11C**, third column). By contrast, overexpression of the wild-type DE-Cadherin (*UAS-DE-Cad*^{DEFL}) (Inaba et al., 2010) in germ cells enhanced the *dUTX* hub size defect (**Fig. 2-11B,B',** and **2-11E**) and led to an increase of the GSC-hub interface (**Fig 2-11B**, arrows and **2-11C**, fourth column). As a control, when both forms of DE-Cadherin were expressed in germ cells at the wt background, no obvious defect was detected (**Fig. 2-11C,E**). Although *DE-Cadherin* is one target gene of dUTX, it is unlikely the only target of dUTX in germ cells. Therefore, although mutations in dUTX lead to up-regulated *DE-Cadherin* transcript levels, overexpression of DE-Cadherin itself in germ cells is not sufficient to recapitulate the *dUTX* loss-of-function phenotype. In summary, our data demonstrate that dUTX acts in germ cells to maintain proper GSC-hub interface and hub size by regulating DE-Cadherin transcription.

DISCUSSION

In this study, we identify a new epigenetic mechanism that negatively regulates the JAK-STAT signaling pathway in the *Drosophila* testis niche (**Fig. 2-11F**): the H3K27me3-specific demethylase dUTX acts in CySCs to remove the repressive H3K27me3 histone modification near the TSS of *Socs36E* to allow its active transcription. Socs36E acts upstream to suppress Stat92E activity and to restrict CySCs from overpopulating the testis niche. In addition, dUTX acts in CySCs to prevent hyperactivation of Stat92E in hub cells, which otherwise ectopically turn on Zfh-1 expression. When we ectopically drove *Zfh-1* cDNA in hub cells using the *upd-Gal4* driver, no obvious defect could be identified. Therefore the biological consequence of

ectopic Zfh-1 expression in hub cells is currently unclear. However, ectopic Zfh-1 expression in hub cells and overpopulation of Zfh-1-expressing cells around the hub are two connected phenomena because both phenotypes are caused by loss of *dUTX* in CySCs.

dUTX also acts in GSCs to regulate DE-Cadherin levels to maintain proper GSC-hub interaction and normal morphology of the hub. It has been reported that differential expression of different cadherins causes cells with similar cadherin types and levels to aggregate (Friedlander et al., 1989; Steinberg and Takeichi, 1994). In wt testes, hub cells express higher levels of DE-Cadherin and therefore tightly associate with each other (Le Bras and Van Doren, 2006). Loss of *dUTX* in germ cells leads to higher levels of DE-Cadherin in GSCs, which probably allows them to intermingle with hub cells and causes disrupted hub architecture. It has been demonstrated that the major role of JAK-STAT in GSCs is for GSC-hub adhesion (Leatherman and Dinardo, 2010), suggesting that expression and/or activity of cell-cell adhesion molecules, such as DE-Cadherin, depend on JAK-STAT signaling. Therefore the abnormal DE-Cadherin activity in GSCs in *dUTX* testis could also result from mis-regulated JAK-STAT signaling in the testis niche.

dUTX is a new negative epigenetic regulator of the JAK-STAT signaling pathway

The JAK-STAT signaling pathway plays critical roles in stem cell maintenance in many different stem cell types across a wide range of species. Here our studies identify the histone demethylase dUTX as a new upstream regulator of the JAK-STAT pathway, which directly controls the transcription of *Socs36E*. In addition to acting as an antagonist of JAK-STAT signaling, *Socs36E* has been reported to be a direct target gene of the Stat92E transcription factor

(Terry et al., 2006). Therefore, increased Stat92E would upregulate *Socs36E* expression, but this was not observed in *dUTX* mutant testes. Instead, our data revealed that *Socs36E* expression decreased while *Stat92E* expression increased in *dUTX* testes, consistent with the hypothesis that *Socs36E* is a direct target gene of dUTX and acts upstream of *Stat92E*.

On the other hand, sustained activity of the JAK-STAT pathway in cyst cells has been reported to activate BMP signaling, which leads to GSC self-renewal outside the niche and gives rise to a tumor-like phenotype in testis (Leatherman and Dinardo, 2010). To examine the BMP pathway activity, we performed immunostaining experiments using antibodies against phosphoSMAD (pSMAD), a downstream target of BMP signaling. However, we did not detect any obvious difference of the pSMAD signal in the *dUTX* testes compared to the wt control (data not shown). Neither did we detect germline tumor in *dUTX* testes. We speculate that the germline tumor upon activation of the JAK-STAT pathway is secondary to the overproliferation of Zfh-1-expressing cells, which was not observed in *dUTX* testes.

dUTX coordinates crosstalk among different cell types within the testis niche

Our study also provides an example of the multidimensional cell-cell communication within a stem cell niche. Many studies of stem cell niche have focused on understanding niche-to-stem cell signaling in controlling stem cell identity and activity. For example, in *Drosophila* female GSC niche, Upd secreted from terminal filaments activates the JAK-STAT pathway in cap cells and escort cells, which subsequently produce BMP pathway ligand *decapentaplegic* (*DPP*) to activate BMP signaling and to prevent transcription of the differentiation factor *bag-of-marbles* (*bam*) in GSCs (Lopez-Onieva et al., 2008). In *Drosophila* intestinal stem cell (ISC)

niche, the visceral muscle cells underlying intestine secrete Wingless to activate the Wnt signaling and Upd to activate JAK-STAT signaling in ISCs, which are required for maintaining ISC identity and activity (Beebe et al., 2010; Jiang et al., 2009; Lin et al., 2008; Lin et al., 2010; Xu et al., 2011).

More studies now reveal the multi-directionality of signaling within stem cell niche. For example, in *Drosophila* female GSC niche, GSCs activate epidermal growth factor receptor (EGFR) signaling in the neighboring somatic cells, which subsequently represses expression of a glypican Dally required for stabilization and mobilization of the BMP pathway ligand DPP. Through this communication between GSCs and surrounding somatic cells, only GSCs maintain high BMP signaling (Liu et al., 2010). Here our studies establish another example for the multidimensional cell-cell communications within the testis stem cell niche where CySCs and GSCs have distinct roles in regulating hub cell identity and morphology.

Distinct biological functions of histone demethylases

Our data identified new roles of a histone demethylase in regulating an endogenous stem cell niche architecture and proper gene expression. Previous studies have reported *in vivo* functions of histone demethylases in several model organisms. For example, mammalian UTX has been shown to associate with the H3K4me3 histone methyltransferase MLL2 (Issaeva et al., 2007), suggesting its potential antagonistic role to the PcG proteins. The PcG proteins play a critical role in HOX gene silencing in both *Drosophila* and mammals (Beuchle et al., 2001; Ringrose and Paro, 2007; Schuettengruber et al., 2007; Schwartz and Pirrotta, 2007). Consistently, mammalian UTX has been reported to directly bind and activate the *HOXB1* gene locus (Agger et al., 2007). In addition to antagonizing PcG function, H3K27me3 demethylases

play critical roles during development. For example, in zebrafish, inactivating *UTX* homolog using morpholino oligonucleotides leads to defects in posterior development (Lan et al., 2007). And in *C. elegans*, the dUTX homolog is required for embryonic and postembryonic development (Vandamme et al., 2012), including gonad development (Agger et al., 2007). Furthermore, loss of UTX function in embryonic stem cells led to defects in mesoderm differentiation (Wang et al., 2012). And somatic cells derived from *UTX* loss-of-function human or mouse tissue fail to return to the ground state of pluripotency (Mansour et al., 2012). These reports demonstrate that UTX is not only required for proper cellular differentiation but also for successful reprogramming. However, despite multiple reports on the *in vivo* roles of H3K27me3-specific demethylases, little is known about their functions in any endogenous adult stem cell system.

While mammals have multiple H3K27me3-demethylases, *Drosophila* dUTX is the sole H3K27me3-specific demethylase. This unique feature, plus the well-characterized *Drosophila* adult stem cell systems, makes interpretations of the endogenous functions of histone demethylases in *Drosophila* unambiguous. Because mammalian UTX has been reported as a tumor suppressor (van Haaften et al., 2009), understanding the endogenous functions of dUTX in an adult stem cell system may facilitate using histone demethylases for cancer treatment.

In summary, our results demonstrate that stem cells send feedback to the niche cells to maintain their proper gene expression and morphology. Furthermore, this feedback is regulated through JAK-STAT signaling pathway whose activity is controlled by a chromatin factor, providing an example of crosstalk between these two regulatory pathways.

MATERIALS AND METHODS

Fly Stocks

Flies were raised on standard yeast/molasses medium at 25°C. The following stocks were used: *dUTX^{1} FRT40A (from A. Shilatifard), w^{1118}; Df(2L) BSC144 (Bloomington Stock Center, BL-9504), UAS-dUTX shmiRNA* (TRiP.HMS00575 from Bloomington Stock Center), *upd-Gal4* (from D. Harrison), *nanos-Gal4* (from M. Van Doren), *c587-Gal4* (from A. Spradling), *y,w; Ubi-GFP, Ubi-GFP, FRT40A (Bloomington Stock Center, BL-5189), hs-Flp122 (Bloomington Stock Center), Arm-LacZ, FRT40A (Bloomington Stock Center, BL-7371), UAS-dUTX and UAS-dUTX^{JmjC} were obtained from A. Shilatifard, refer to Materials and Methods in (Herz et al., 2010), UAS-Socs36E-45* (from B. Callus), *Stat92E^{06346}* (from N. Perrimon), *UAS-DE-Cad^{dCR4h}* and *UAS-DE-Cad^{DEFL}* (from Y. Yamashita), and *hs-FLP, UAS-GFP.nls, tub-Gal4/FM7; tub-Gal80 FRT40A/CyO (from E. Bach).*

Clonal Induction

dUTX^{1} clones that are negative with the GFP or LacZ marker were generated using the FLP/FRT recombination system. The flies used had the following genotypes: *hs-FLP122; Arm-LacZ, FRT40A/ dUTX^{1} FRT40A or hs-FLP122; Ubi-GFP, Ubi-GFP, FRT40A/ dUTX^{1}FRT40A*. The clones were induced by heat shocking pupae on days 8 and 9 for 2 hours at 37°C. After the second heat shock, flies were placed at 25°C and dissected and stained three days after clone induction. Mosaic Analysis with a Repressible Cell Marker (MARCM) clones were generated using flies with the following genotype*: hs-FLP, UAS-GFP.nls, tub-Gal4/+; tub-Gal80 FRT40A/ dUTX^{1} FRT40A*. The clones were induced by heat shocking pupae on days 8 and 9 for 2 hours at

37°C. After the second heat shock flies were placed at 25°C and dissected and stained one day after clone induction.

Immunofluorescence Staining

Immunofluorescence staining was performed following the procedure previously described in (Cheng et al., 2008). The primary antibodies used were: rabbit anti-Zfh1 (1:5000; from Ruth Lehmann, Skirball Institute of Biomolecular Medicine, NY, USA); mouse anti-Armadillo [1:100; developed by Eric Wieschaus and obtained from Developmental Studies Hybridoma Bank (DSHB)]; rat anti-Vasa (1:100; developed by Allan Spradling and Dianne Williams and obtained from DSHB); rabbit anti-dUTX (1:2000; from Ali Shilatifard, Stowers Institute for Medical Research, Kansas City, MO, USA); rabbit anti-trimethyl-Histon H3 (Lys27) (1:200; Millipore, Cat# 07-449); chicken anti-GFP (1:1000; Abcam, Cat# 13970); rabbit anti-Stat92E (1:800; from Denise Montell, Johns Hopkins School of Medicine, Baltimore, MD, USA); guinea pig anti-Traffic Jam (1:3000; from Mark Van Doren, Johns Hopkins University, Baltimore, MD); rabbit anti-phospho-Histone H3 (Ser10) (1:2000; Millipore, Cat# 06-570); and rabbit anti-Caspase 3 (1:100; BD Biosciences, Cat # 610322).

Isolation of Total RNA and Quantitative Reverse Transcription Polymerase Chain Reaction (qRT–PCR)

Total RNA was isolated from wt and *dUTX* 3rd instar larval testes using TRIzol reagent (Invitrogen, Cat# 15596-018) according to the manufacturer's instructions. Yield and quality of RNA were determined with a NanoDrop spectrometer (NanoDrop Technology, San Diego, CA). Reverse transcription was done using RevertAid First Strand cDNA Synthesis Kit (Fermentas,

Cat# K1621). Transcript levels were measured using SYBR Green PCR Master Mix (Fermentas, Cat# K0221) and normalized to *Fringe.* Sequences of primers used for qRT-PCR were as follows: CAGGATACGTTCCAGGACCAGG (*Fringe*-forward), TGGGCGGGCAGAAGCTGAAGTAG (*Fringe*-reverse), CTCACCTATTGGAATGCAGCT (*Socs36E*-forward), CGAAAAGCTCGATTGGCC (*Socs36E*-reverse), AAGGTGAGTGATTTGCTGTGCTGC (*Stat92E*-forward), CAACAAGCGAGCATGAGAATGCCA (*Stat92E*-reverse), GCGAATTGTCGCTGGCAATAACCT (*DE-Cadherin*-forward), TGGACGGCACCTCAGCCAATAATA (*DE-Cadherin*-reverse), CATGCTGCCCACCGGATTCAAGAAG (*RpL32*-forward), and CTCGTTCTCTTGAGAACGCAGGCGA (*RpL32*-reverse).

Chromatin Immunoprecipitation (ChIP)

ChIP was performed following the procedure previously described in (Gan et al., 2010b). For each biological replicate we dissected 200 pairs of *dUTX* testes and 200 pairs of wt testes. Sequences of primers used for qPCR were as follows: TGTGGAACTCATTGTGCCGTAG (p1-forward), GGAAACTTTCAAGAAGTTCTCATGGAGG (p1-reverse), TCGTTCCGTTTCTCCAGTGTGACT (p2-forward), TGTGCGACCACTTAGCCGAGTTAT (p2-reverse), TTATGCAAACAGCGAGCGGTTCAG (p3-forward), AGTAACGAGCACACCGAAACCA (p3-reverse), CATCATCACACAAGCGCACACACA (p4-forward), ACAGATACACAAGCGGACTCACAC (p4-reverse), GTGTGCTAATCCAATGATTCAAACTTCCA (p5-forward), CGCTCGAAAGTAACTTCGCGGTAA (p5-reverse), TATTCACGGGCTGTCGTTCGGTTT

(p6-forward), ATGGGCAGATTGTGAATGTGACGC (p6-reverse), GATATCCGTAGGCGCTCTGGTAGA (p7-forward), GCGAATCCTGCAAGCCATGATGTT (p7-reverse), AATAACTGTCAACACAGCGGCTCG (p8-forward), ACACTTTCTTATCCAACTCCCTATCA (p8-reverse).

Statistical analysis

Statistical significance was calculated using two-tailed Student's *t*-test or Fisher's test. *P*-values are denoted on figures or in figure legends. Error bars represent standard deviation (s.d.).

FIGURES

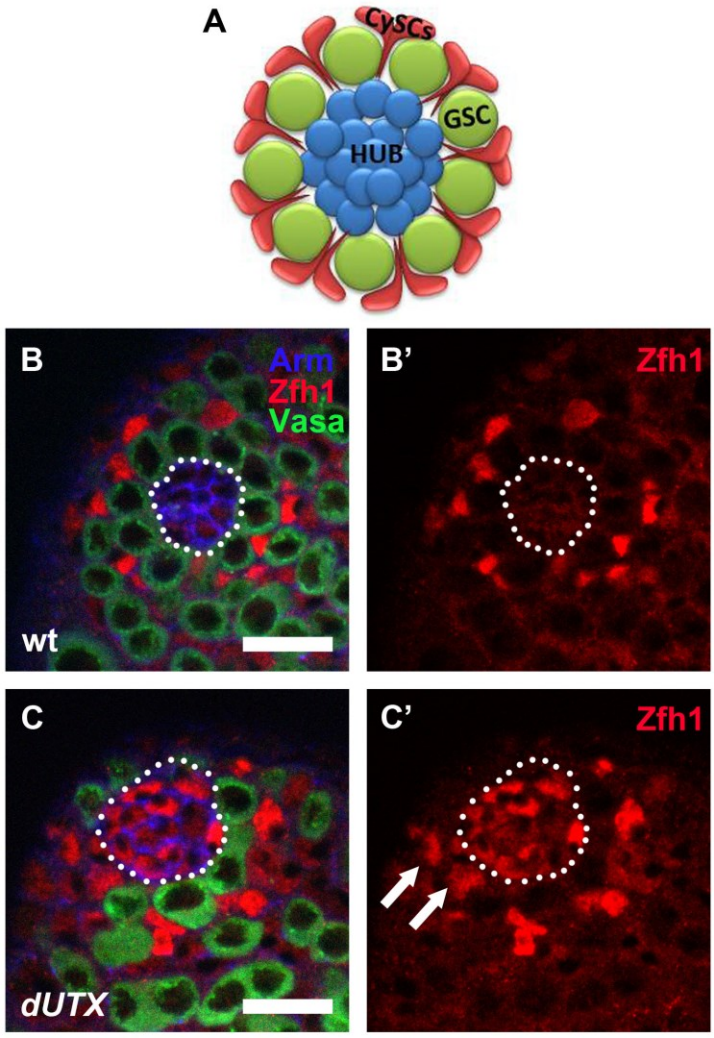


Figure 2-1. dUTX prevents Zfh-1-expressing cells from overpopulating the niche and represses Zfh-1 expression in the hub cells. (**A**) A schematic cartoon of the testis niche. (**B-C'**) Immunostaining using antibodies against Armadillo (blue), Vasa (green) and Zfh-1 (red) in (B-B') wt and (C-C') *dUTX* testes. Arrows point to overpopulated Zfh-1-expressing cells whose nuclei directly contact the hub (C'). Hub area outlined with white dotted line. Scale bar: 10μm.

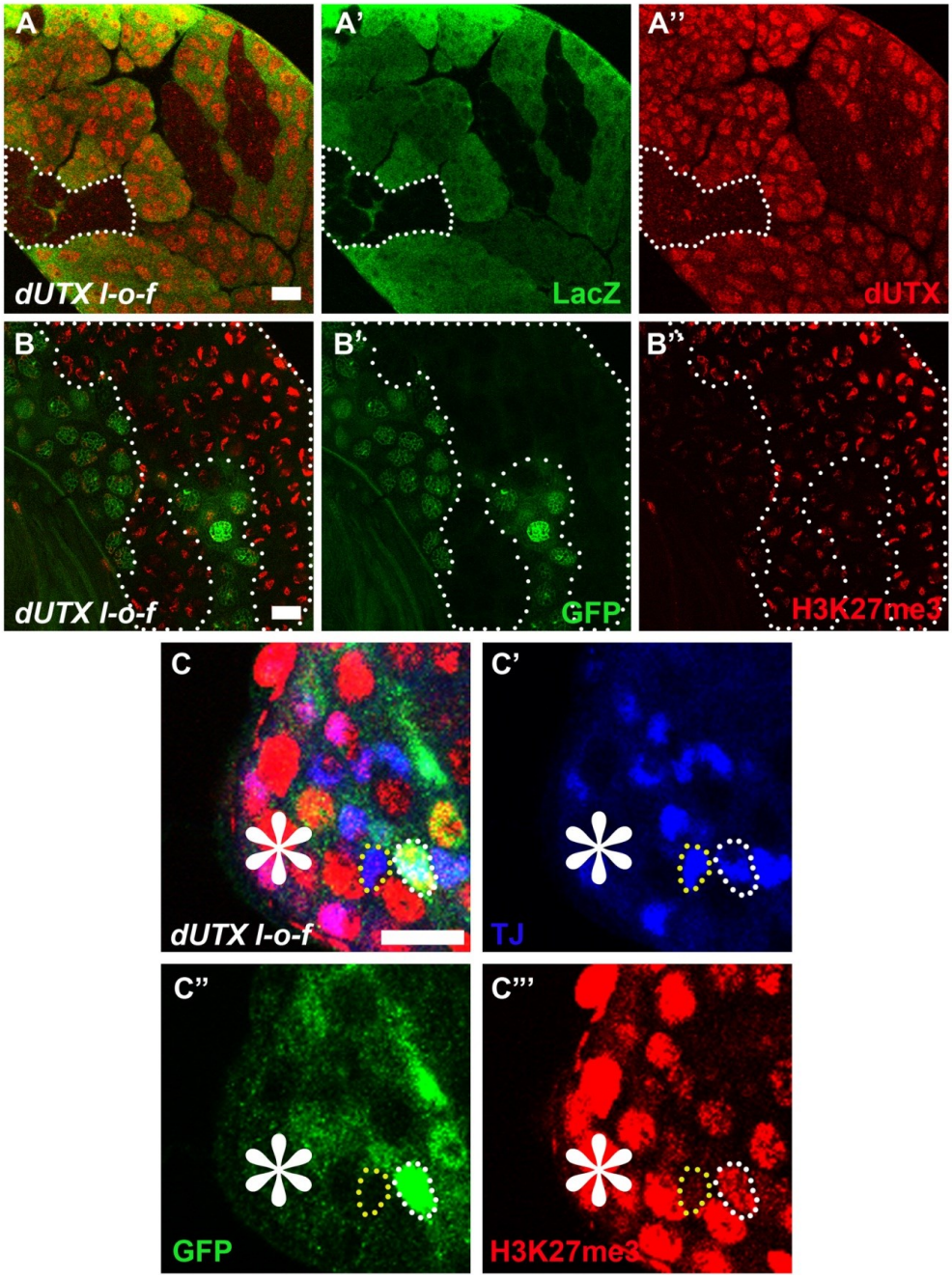


Figure 2-2. dUTX acts as an H3K27me3-specific demethylase in germ cells and cyst cells. (**A-A"**) wt testis with *dUTX* clones generated by conventional FLP/FRT system and immunostained with antibodies against LacZ (green) and dUTX (red). An outlined $dUTX^{-/-}$ clone negative for LacZ signal is absent for dUTX staining. (**B-B"**) wt ($dUTX^{+/-}$) testis with $dUTX^{-/-}$

clones generated by conventional FLP/FRT system and immunostained with antibodies against GFP (green) and H3K27me3 (red). *dUTX*$^{-/-}$ clone negative for GFP signal (outlined by white dotted line) has increased H3K27me3 signal. Scale bar: 20μm. (**C-C"'**) wt (*dUTX*$^{+/-}$*)* testis with *dUTX*$^{-/-}$ clones generated by MARCM system and immunostained with antibodies against TJ (blue), GFP (green), and H3K27me3 (red). *dUTX*$^{-/-}$ GFP-positive cyst cell (outlined by white dotted line) has increased H3K27me3 signal compared to neighboring heterozygous cyst cell (outlined by yellow dotted line). Scale bar: 10μm. Hub labeled by asterisks.

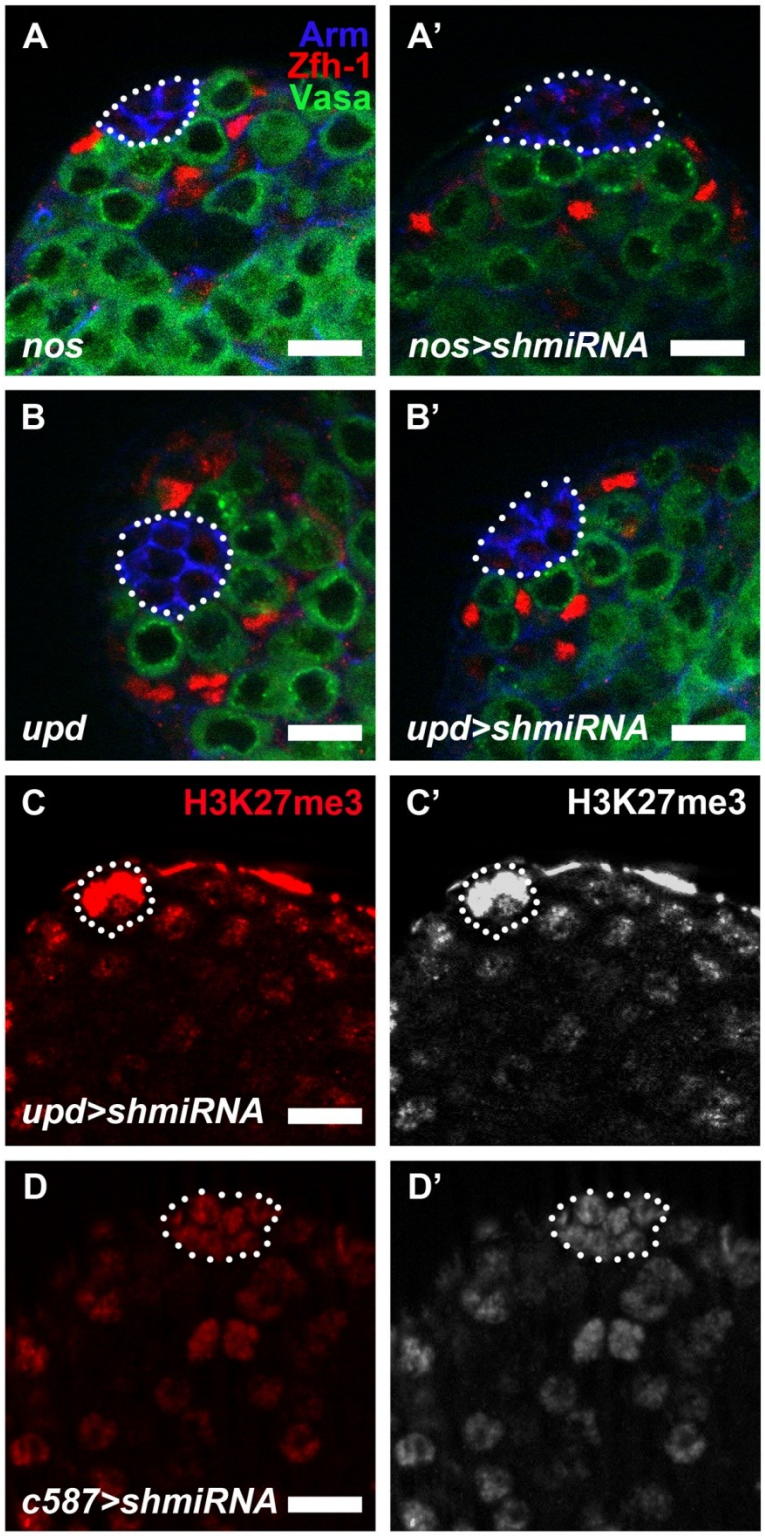

A
Arm
Zfh-1
Vasa
nos
A'
nos>shmiRNA
B
upd
B'
upd>shmiRNA
C
H3K27me3
upd>shmiRNA
C'
H3K27me3
D
c587>shmiRNA
D'

Figure 2-3. dUTX is not required in germ cells or hub cells to prevent overpopulation of Zfh-1-expressing cells around the hub area or ectopic Zfh-1 expression in hub cells. (**A-B'**) Immunostaining using antibodies against Armadillo (blue), Vasa (green) and Zfh-1 (red) in testes with the following genotype: (A) *nos-Gal4* control, (A') *nos-Gal4*; *UAS-dUTX shmiRNA*, (B) *upd-Gal4* control, (B') *upd-Gal4*; *UAS-dUTX shmiRNA*. (**C-D'**) Immunostaining using antibodies against H3K27me3 (red) on testes with the following genotype: (C-C') *upd-Gal4*; *UAS-dUTX shmiRNA*, (D-D') *c587-Gal4*; *UAS-dUTX shmiRNA*. Hub area outlined with white dotted line. Scale bar: 10μm.

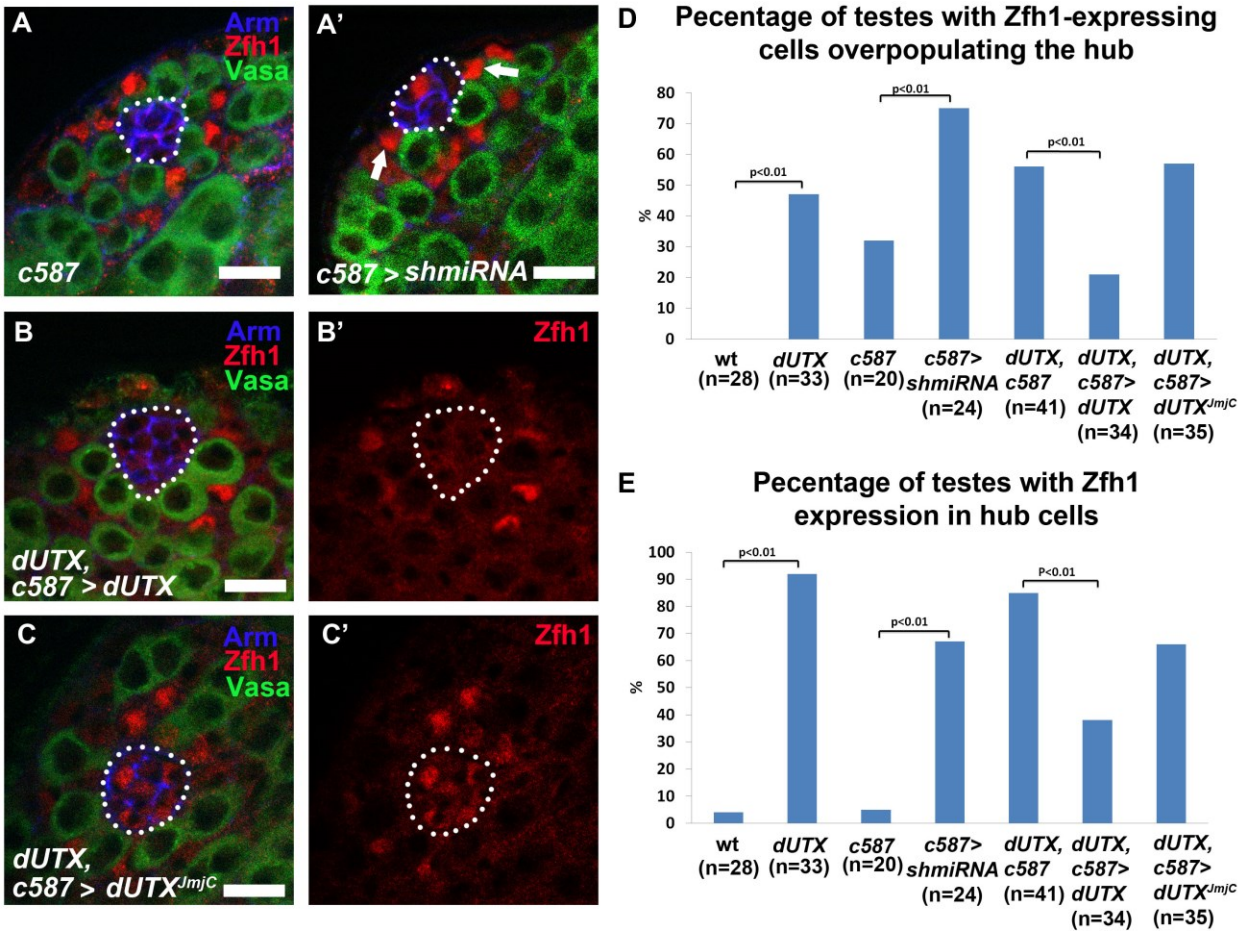


Figure 2-4. dUTX acts as a histone demethylase in CySCs and/or early cyst cells to repress overpopulated Zfh-1-expressing cells around the hub and ectopic Zfh-1 expression in hub cells. (**A-C'**) Immunostaining using antibodies against Armadillo (blue), Vasa (green) and Zfh-1 (red); hub area outlined with white dotted line. (A) *c587-Gal4*. (A') *c587-Gal4*; *UAS-dUTX shmiRNA*; arrows point to overpopulated Zfh-1-expressing cells whose nuclei directly contact the hub. (B-B') *c587-Gal4*; *UAS-dUTX* and (C-C') *c587-Gal4*; *UAS-dUTX*^{JmjC}, both at *dUTX* background. (**D**) Percentage of testes with overpopulated Zfh-1-expressing cells around the hub. (**E**) Percentage of testes with ectopic Zfh-1 expression in hub cells. Scale bar: 10µm. P-value calculated using Fisher's test.

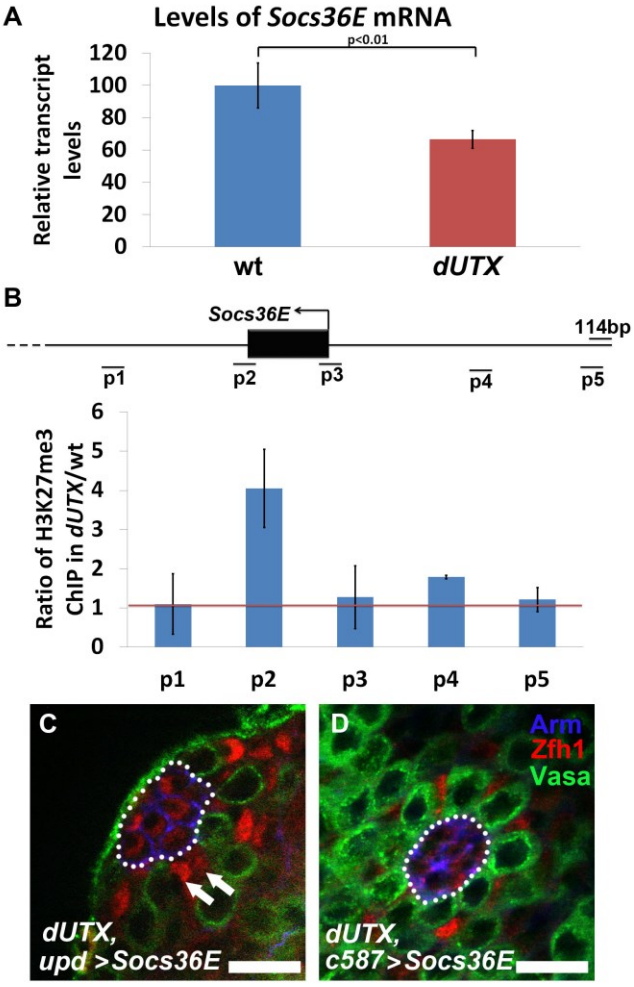


Figure 2-5. dUTX removes the repressive H3K27me3 histone modification at the *Socs36E* genomic locus and allows active transcription of *Socs36E*. (**A**) *Socs36E* mRNA measured by qRT-PCR in three independent biological replicates, normalized by *Fringe*. (**B**) Anti-H3K27me3 ChIPed DNA analyzed by qPCR, normalized to Input (Input%) and then compared between *dUTX* testes and wt controls, based on three independent biological replicates. (**C-D**) Immunostaining using antibodies against Armadillo (blue), Vasa (green) and Zfh-1 (red); hub area outlined with white dotted line. (C) *upd-Gal4*; *UAS-Socs36E-cDNA* transgene at *dUTX* background; arrows point to overpopulated Zfh-1-expressing cells whose nuclei directly contact

the hub. (D) *c587-Gal4*; *UAS-Socs36E-cDNA* transgene at *dUTX* background. Scale bar: 10μm. P-value calculated using Student's *t*-test. Error bars represent standard deviation (s.d.).

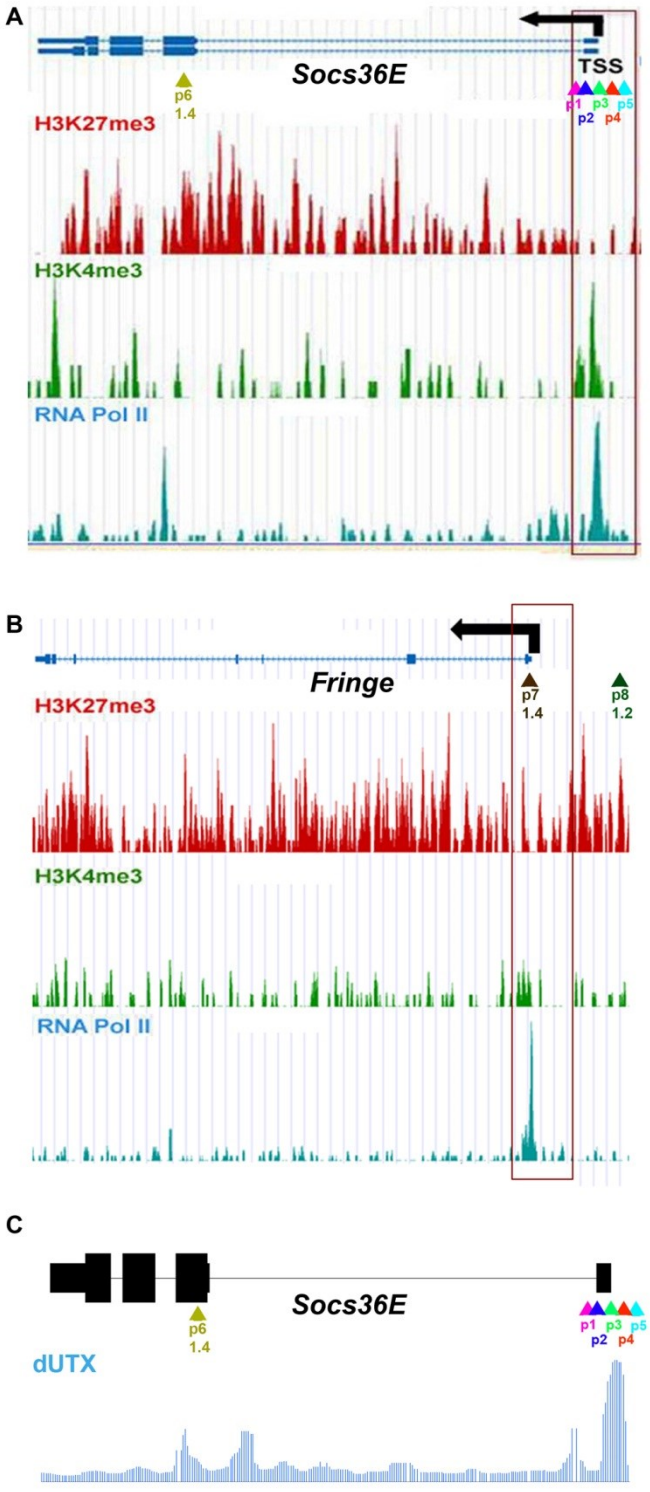

A
Socs36E
TSS
p6
1.4
p1 p3 p5
p2 p4
H3K27me3
H3K4me3
RNA Pol II
B
Fringe
p7
1.4
p8
1.2
H3K27me3
H3K4me3
RNA Pol II
C
Socs36E
p6
1.4
p1 p3 p5
p2 p4
dUTX

Figure 2-6. Genome browser screenshots show chromatin state of *Socs36E* and *Fringe* genomic loci. (**A-B**) ChIP-seq using antibodies against H3K27me3 (red), H3K4me3 (green) and RNA Pol II (blue) (Gan et al., 2010b) at (A) *Socs36E* and (B) *Fringe* genomic region. A 2kb-region around TSS for each gene is outlined by brown solid lined box. The five amplicons (p1-p5) used in ChIP-qPCR experiments were labeled in (A), which span across the TSS of *Socs36E*. The p6 amplicon is located inside the *Socs36E* gene body. The p7 and p8 amplicons are near the TSS of *Fringe*. Numbers underneath p6-p8 denoted anti-H3K27me3 ChIPed DNA in *dUTX* testes normalized by Input followed by comparison with *wt* testes, based on three independent biological replicates. (**C**) ChIP-chip using anti-dUTX showed enrichment of dUTX near the TSS of *Socs36E* gene (Tie et al., 2012), p1-p5 and p6 are labeled.

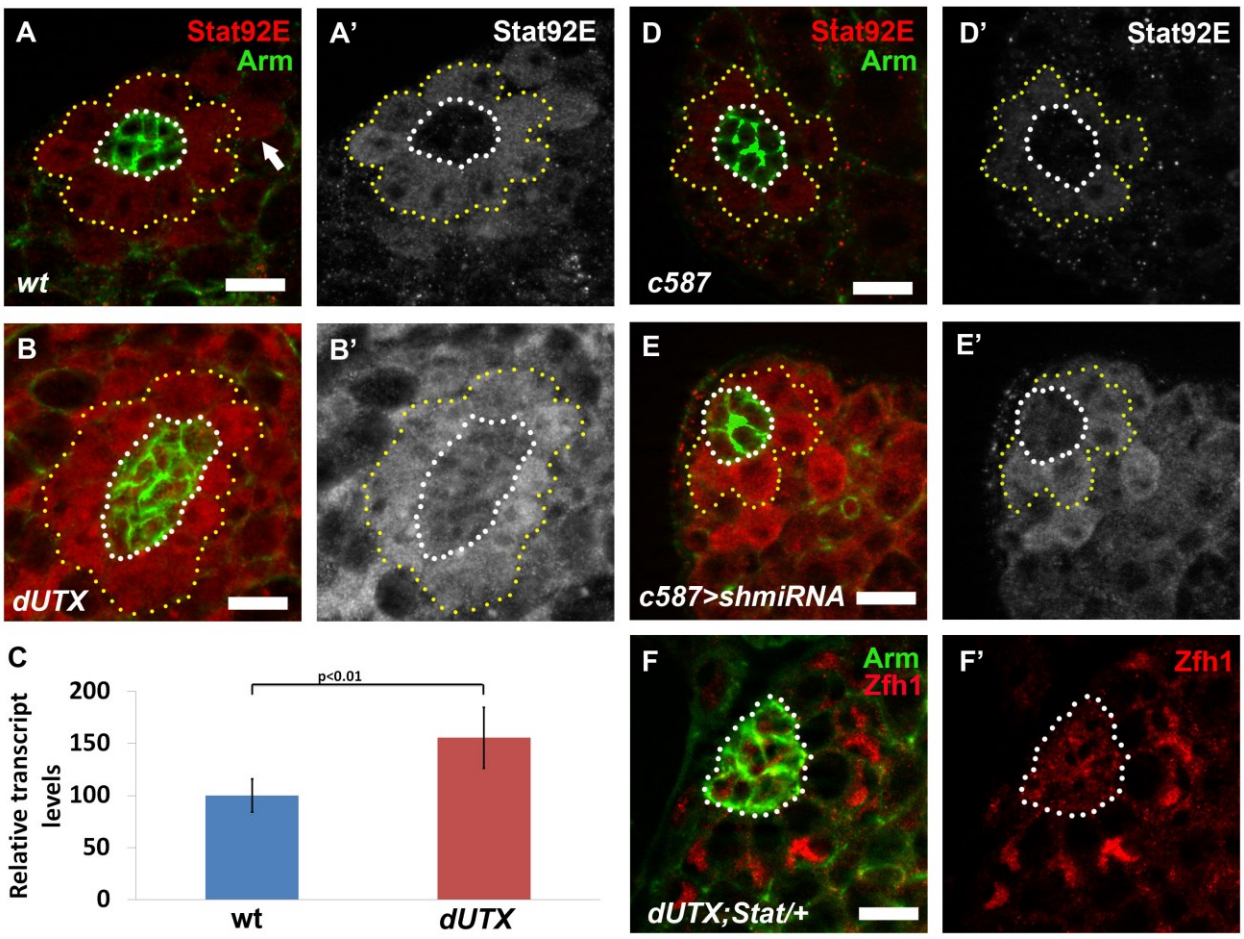


Figure 2-7. dUTX is required in CySCs and early cyst cells to prevent hyperactivation of the JAK-STAT signaling pathway. (**A-B'**) Immunostaining using antibodies against Armadillo (green) and Stat92E (red). Hub area outlined with white dotted line. Stem cell zone outlined with yellow dotted line. (A-A') wt testis. Arrow points to a GB positive with anti-Stat92E staining. (B-B') *dUTX* testis. (**C**) *Stat92E* mRNA measured by qRT-PCR in five independent biological replicates, normalized by *Fringe*. (**D-E'**) Immunostaining using anti-Arm (green) and anti-Stat92E (red) in (D-D') *c587-Gal4* control and (E-E') *c587-Gal4*; *UAS-dUTX shmiRNA* testes. (**F-F'**) Immunostaining using anti-Arm (green) and anti-Zfh-1 (red) in $dUTX^{-/-}$; $Stat92E^{+/-}$ testes.

Scale bar: 10μm. P-value calculated using Student's *t*-test. Error bars represent standard deviation (s.d.).

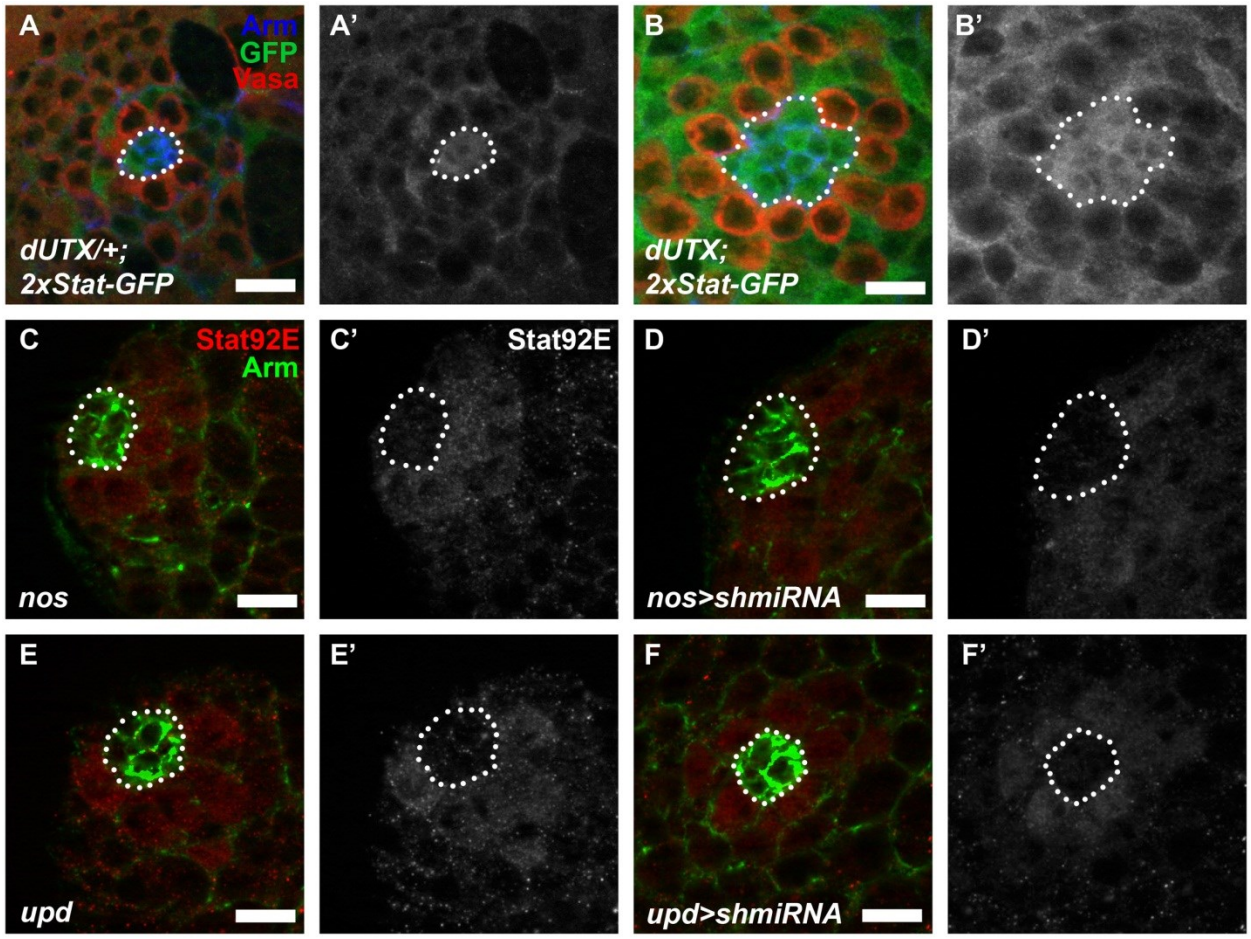


Figure 2-8. dUTX is required in cyst cells, but not in germ cells or hub cells, to maintain proper Stat92E expression in the niche. (**A-B'**) Immunostaining using antibodies against Armadillo (blue), Vasa (red) and GFP (green) in testes with the following genotype: (A-A') *dUTX/+; 2×Stat92E-GFP* control, (B-B') *dUTX*$^{-/-}$*; 2×Stat92E-GFP.* (**C-F'**) Immunostaining using antibodies against Armadillo (green) and Stat92E (red) in testes with the following genotype: (C-C') *nos-Gal4* control, (D-D') *nos-Gal4*; *UAS-dUTX shmiRNA*, (E-E') *upd-Gal4*

control, (F-F') *upd-Gal4*; *UAS-dUTX shmiRNA*. Hub area outlined with white dotted line. Scale bar: 10µm.

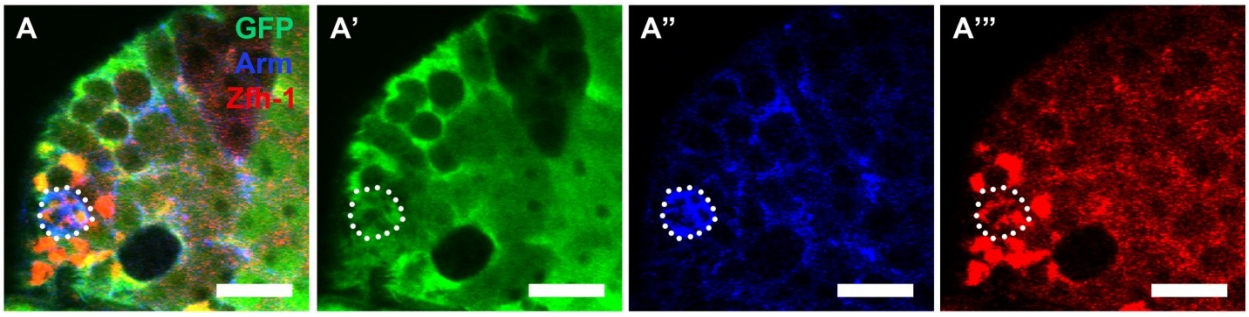


Figure 2-9. Zfh-1 is ectopically expressed in hub cells from testes with *dUTX*^{1} clones. (**A-A'"**) Immunostaining using antibodies against GFP (green), Armadillo (blue) and Zfh-1 (red) in a *dUTX*^{1} clone-containing testis.

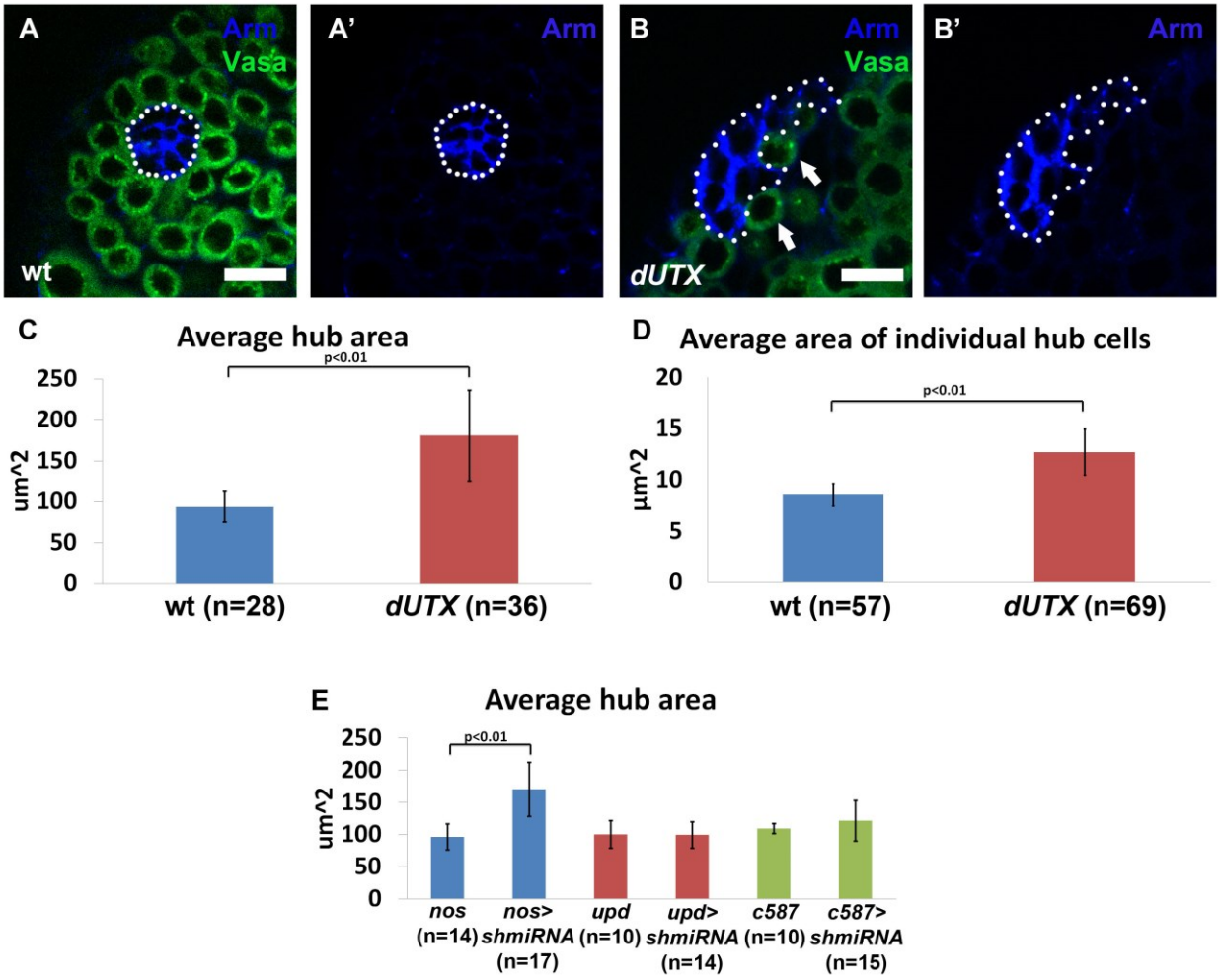


Figure 2-10. dUTX acts in germ cells to maintain proper hub size. (**A-B'**) Immunostaining using antibodies against Armadillo (blue) and Vasa (green); hub area outlined with white dotted line. (A-A') wt testis. (B-B') *dUTX* testis displays enlarged hub. (**C**) Quantification of average hub area: 94±18.65µm^2 in wt testes; 181±55.5µm^2 in *dUTX* testes. (**D**) Quantification of average area of individual hub cells: 8.5±1.1µm^2 in wt testes; 12.7±2.2µm^2 in *dUTX* testes. (**E**) Quantification of average hub area in testes from the following genotyped males: *nos-Gal4* control (96±20.35 µm^2), *nos-Gal4*; *UAS-dUTX shmiRNA* (170±41.7 µm^2, P <0.01), *upd-Gal4* control (100±21.4 µm^2), *upd-Gal4*; *UAS-dUTX shmiRNA* (99±20.3 µm^2, P >0.05), *c587-Gal4* control (109±7.7 µm^2), *c587-Gal4*; *UAS-dUTX shmiRNA* (121±31.4 µm^2, P >0.05).

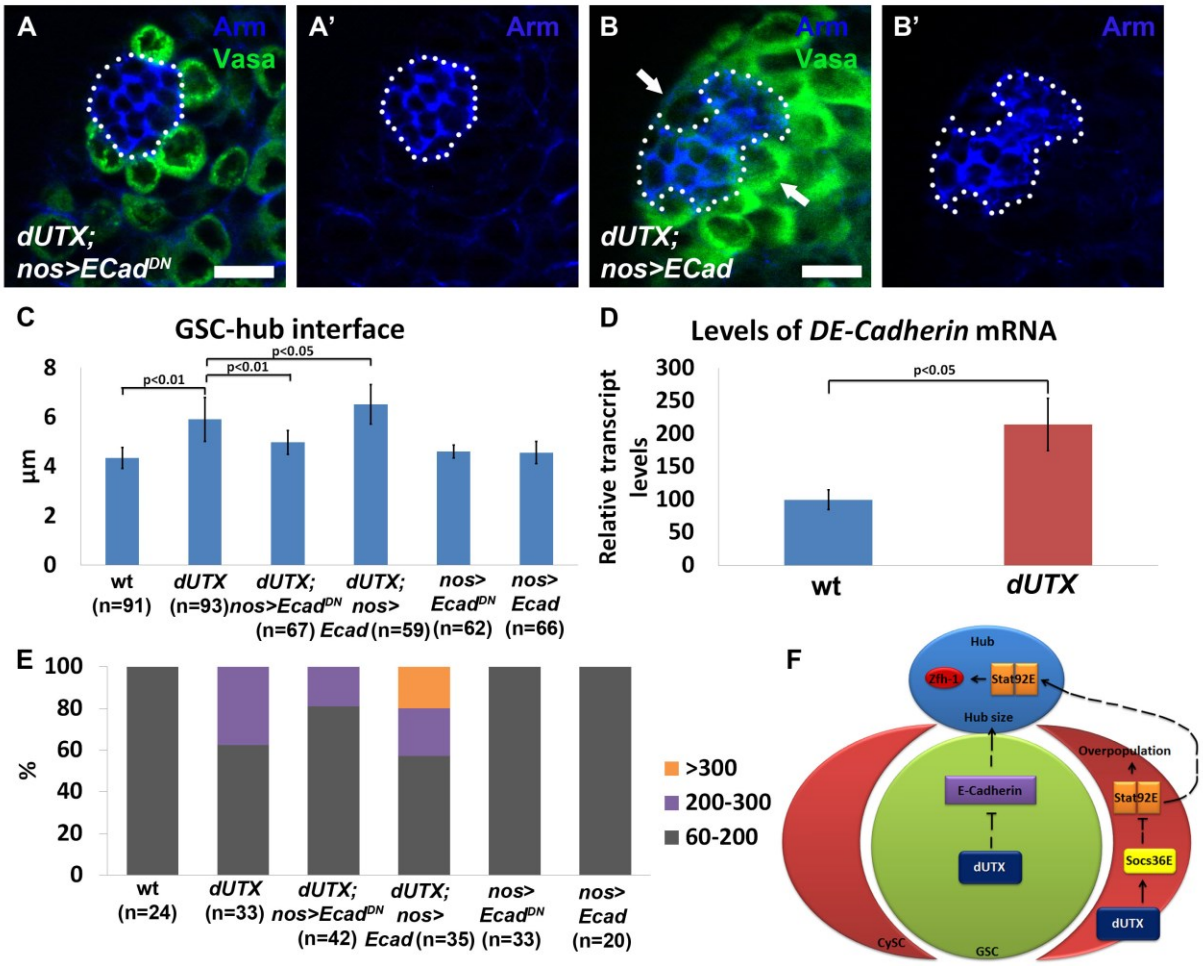


Figure 2-11. dUTX controls hub size through regulating DE-Cadherin levels in GSCs and a model. (**A-B'**) Immunostaining using antibodies against Armadillo (blue) and Vasa (green), hub area outlined with white dotted line. (A-A') *dUTX*; *nos>DE-Cad*^{dCR4h} testis. (B-B') *dUTX*; *nos>DE-Cad*^{DEFL} (**C**) Quantification of the average GSC-hub interface in testes from the following genotyped males: wt (4.3±0.4 µm), *dUTX* (5.9±0.9 µm), *dUTX*; *nos>DE-Cad*^{dCR4h} (4.9±0.5 µm), *dUTX*; *nos>DE-Cad*^{DEFL} (6.5±0.8 µm), *dUTX/+*; *nos>DE-Cad*^{dCR4h} control (4.6±0.3 µm), *dUTX/+*; *nos>DE-Cad*^{DEFL} control (4.5±0.4 µm). (**D**) *DE-Cadherin* mRNA

measured by qRT-PCR in three independent biological replicates, normalized by *RpL32*. (**E**) Quantification of percentage of testes with average hub area of 60-200 μm^2, 200-300 μm^2 or >300 μm^2, from the following males: 1- wt, 2- *dUTX* mutant, 3- *dUTX*; *nos>DE-Cad*^{dCR4h}, 4- *dUTX*; *nos>DE-Cad*^{DEFL}, 5- *dUTX/+*; *nos>DE-Cad*^{dCR4h} control, 6- *dUTX/+*; *nos>DE-Cad*^{DEFL} control. (**F**) A schematic diagram outlines functions of dUTX in testis niche, see details in Discussion.

Chapter 3

Histone demethylase Lid maintains germline stem cell through regulating JAK-STAT signaling pathway activity

This chapter is based on an article that has been recently submitted to Development.

INTRODUCTION

Extrinsic signals from cells comprising the stem cell niche are essential in maintaining stem cell activity (Morrison and Spradling, 2008). In addition, epigenetic regulation that changes stem cell chromatin structure without altering DNA sequences acts as an important intrinsic mechanism to maintain stem cells (Eun et al., 2010). Although both mechanisms are important to regulate stem cell activities, our understanding of the crosstalk between the two events is limited to a few examples (Cherry and Matunis, 2010; Eliazer et al., 2011; Tarayrah et al., 2013).

Drosophila spermatogenesis is a paradigmatic system to investigate the molecular mechanisms responsible for the maintenance of adult stem cell activities in their physiological environment (Kiger et al., 2001; Tulina and Matunis, 2001; Yamashita et al., 2003; Yamashita et al., 2007). The *Drosophila* male germline stem cell (GSC) niche is one of the best characterized niches in which GSCs associate with two types of somatic cells: hub cells located at the tip of the testis, and cyst stem cells (CySCs) two of which surround each GSC (**Fig. 3-1A**). Hub cells and CySCs contribute to a niche that provides the critical signaling necessary to preserve GSC identity and activity (Kiger et al., 2001; Leatherman and Dinardo, 2008; Leatherman and Dinardo, 2010; Tulina and Matunis, 2001). Janus kinase signal transducer and activator of transcription (JAK-STAT) and bone morphogenetic protein (BMP) signaling pathways are the two major pathways involved in the maintenance of the male GSC niche. Activation of the JAK-STAT pathway is initiated by the secretion of the cytokine Unpaired (Upd) from the hub cells. Upd binds the receptor Domeless activating Hopscotch (Hop), the JAK kinase homolog in *Drosophila*, and Stat92E, the STAT homolog, in both GSCs and CySCs [reviewed in (Hou et al., 2002)]. The intrinsic activation of Stat92E in CySCs activates BMP signaling and is thought to

be sufficient to cause continuous GSC self-renewal while Stat92E in the GSCs has been reported to only regulate their adhesion to the niche (Leatherman and Dinardo, 2008; Leatherman and Dinardo, 2010).

In addition to signaling pathways, the chromatin structure of GSCs can profoundly influence critical decisions of stem cell maintenance versus differentiation. In *Drosophila,* histone modifying enzymes and chromatin remodeling factors are important chromatin regulators (Becker and Horz, 2002). Both have been shown to act cell autonomously to maintain GSCs in the testis niche (Buszczak et al., 2009; Cherry and Matunis, 2010; Eliazer et al., 2014). Among the histone modifying enzymes, histone demethylases have been identified as "epigenetic erasers" that remove methyl-groups from methylated Lysine residues of histones (Klose et al., 2006). Specifically, the repressive trimethylation on lysine 27 of histone H3 (H3K27me3) and the active H3K4me3 histone modifications have been the focus of many studies. While H3K27me3 is laid down by a member of the Polycomb Group (PcG) proteins and associates with silent gene regions (Cao et al., 2002; Czermin et al., 2002; Kuzmichev et al., 2002; Muller et al., 2002), H3K4me3 is generated by the Trithorax Group (TrxG) family of proteins and has been shown to associate with active regions of chromatin (Byrd and Shearn, 2003). A recent study from our lab on the function of dUTX, a histone demethylase that targets the repressive H3K27me3 modification, revealed that dUTX regulates JAK-STAT pathway activity in the CySCs to maintain proper niche structure and gene expression (Tarayrah et al., 2013). Among the 14 demethylases in *Drosophila* (Klose et al., 2006)*, little imaginal discs* (*lid*) encodes a demethylase that specifically removes the active H3K4me3 modification (Eissenberg et al., 2007; Lee et al., 2007). Lid belongs to the JARID1 family of H3K4me3 demethylases (Secombe and Eisenman, 2007). While human cells encode four JARID1 family members (JARID1a,

JARID1b, JARID1c, and JARID1d), Lid is the sole *Drosophila* homolog (Eissenberg et al., 2007). The mammalian Lid homologue, JARID1a, was reported to play a critical role in breast cancer metastatic progression suggesting a role for H3K4me3 demethylases in inhibition of tumor progression and metastasis (Cao et al., 2014). Therefore, understanding the functions of Lid in an adult stem cell system might facilitate the targeting of histone demethylases for cancer treatment. Previous studies have shown that *lid* mutant adult flies have increased global H3K4me3 levels (Eissenberg et al., 2007; Lee et al., 2007) and demonstrated a role for Lid in dMyc-induced cell growth (Li et al., 2010b). However, the function of Lid in an endogenous stem cell system has not yet been elucidated. Here we report that Lid is required cell autonomously to prevent premature differentiation of male GSCs by maintaining Stat92E levels. Our findings support a cell autonomous role for the JAK-STAT pathway in maintaining GSCs and provide insight into the *in vivo* functions of a histone demethylase.

RESULTS AND DISCUSSION

Lid acts cell autonomously in the germline to maintain GSC number at the niche

lid encodes a histone demethylase that has been reported to specifically demethylate H3K4me3 *in vivo* (Eissenberg et al., 2007; Lee et al., 2007). To confirm the function of Lid as a specific H3K4me3 demethylase in the testis, we used a strong loss-of-function allele of *lid* (lid^{10424}) (Gildea et al., 2000; Li et al., 2010b). The lid^{10424}/Df hemizygous flies (referred to hereafter as *lid*) are pupal lethal with rare adult escapers. We analyzed H3K4me3 levels using immunoblot on testes isolated from third instar larvae of *lid* males and compared to wild-type (wt) third instar larval testes. Using antibodies against H3K4me3 and H3 as a control we found that loss of *lid* leads to an approximately 4-fold increase in H3K4me3 levels (**Fig. 3-2A-B**).

To determine whether overexpression of Lid in the germline is sufficient to reduce the H3K4me3 mark, we obtained a fly line carrying the *UAS-lid* transgene (Secombe et al., 2007). Germline clones overexpressing the *lid* transgene were marked by co-expression of the GFP marker, using the Gal4/UAS binary system as described in (Johnston et al., 1999). Indeed, we found that overexpression of Lid causes a marked reduction in H3K4me3 levels (**Fig. 3-2C-C"**). These data demonstrate that Lid can demethylate H3K4me3 in germ cells.

To determine the role of *lid* in the male GSC niche, we analyzed testes isolated from third instar larvae of *lid* males. We detected a marked decrease of GSC number in *lid* testes compared to wt testes. The decrease in GSC number was visible in immunostained images when comparing wt testes (**Fig. 3-1B**) with *lid* testes (**Fig. 3-1C**, dots). When quantified, we found that wt testes had an average of 9.7±1.6 GSCs compared to an average of 6.03±1.4 GSCs in *lid* testes ($p<0.01$, **Fig. 3-1D**). Furthermore, GSCs appeared to be detached from the hub cells in the majority of *lid* testes (**Fig. 3-1C**, arrow). These results suggest that Lid is required to maintain GSCs at the *Drosophila* testis niche.

Due to adult lethality we next analyzed the *lid* mutant phenotype in adult testes using the FLP-mediated FRT recombination system (Xu and Rubin, 1993). We generated mutant clones for *lid* using the strong loss-of-function allele lid^{k6801} (Gildea et al., 2000). We observed that the percentage of testes with at least one *lid* GSC clone declined over time compared to the wt control (**Fig. 3-1E**), suggesting that Lid is required cell autonomously to maintain GSCs at the *Drosophila* testis niche.

To confirm this conclusion, we used different cell-type specific Gal4 drivers in combination with a *UAS-lid* small hairpin microRNA (shmiRNA) (Ni et al., 2011) to knockdown *lid* in a cell type-specific manner. Knockdown of *lid* exclusively in early stage germ cells using

nanos (*nos*)-*Gal4* (Van Doren et al., 1998) led to a significant decrease in GSC number (**Fig. 3-1F-G**, dots; **3-1H**). By contrast, knockdown of *lid* using the early cyst cell driver *c587-Gal4* (Manseau et al., 1997) (**Fig. 3-1H** and **Fig. 3-3A-B**) or the hub driver *upd-Gal4* (Boyle et al., 2007) (**Fig. 3-1H** and **Fig. 3-3C-D**) did not lead to a change in GSC number. These results demonstrate that normal function of Lid is required in early stage germ cells including GSCs, but not in somatic gonadal cells, to prevent GSC loss at the testis niche.

We also found that Lid is required for proper hub architecture (compare hub region in **Fig. 3-1B** and **3-1C**). Loss of *lid* led to a dramatic increase in hub size compared to wt testes (**Fig. 3-4A**). The increase in hub size is a secondary effect due to germline loss because it was recapitulated by knocking down *lid* in germ cells but not in somatic gonadal cells (**Fig. 3-4B**), consistent with previous reports (Dinardo et al., 2011; Gonczy and DiNardo, 1996; Monk et al., 2010; Tazuke et al., 2002).

Lid is required to maintain GSC self-renewal and prevent premature differentiation

To determine the mechanism leading to GSC loss in the *lid* testes, we used phospho-Histone H3 (PH3) immunostaining to assess the ability of GSCs to divide. We observed a significant decrease in the mitotic activity of GSCs in *lid* testes compared to wt (**Fig. 3-5A**), suggesting a decreased self-renewal ability of *lid* mutant GSCs.

We next examined whether the loss of GSCs is accompanied by premature differentiation. Fusomes are branched cytoplasmic structures, comprised of membrane skeletal proteins such as α- and β-spectrin, that connect germ cells in a cyst (Deng and Lin, 1997). When we used antibody against α-spectrin, the fusome appears spherical (called spectrosome) in all GSCs (**Fig. 3-5B**, arrow) and becomes branched in further differentiated spermatogonia of wt

testes (n=44). By contrast, we observed spermatogonial cysts containing up to four interconnected germ cells in direct association with the hub region (**Fig. 3-5C**, yellow dotted outline) in 33% of the *lid* testes (n=52). This phenomenon could be due to premature differentiation of GSCs or dedifferentiation of spermatogonial cells. It has been reported that dedifferentiated spermatogonial cells tend to have a higher percentage of misoriented centrosomes when they home back to the niche (Cheng et al., 2008). We labeled centrosomes using antibody against γ-tubulin, a major component of the centrosome (Yamashita et al., 2003) and found that the percentage of GSCs with misoriented centrosomes was not significantly different in *lid* (6.3%, n=59) compared to wt testes (7.1%, n=104) ($p>0.05$). In addition to misoriented centrosomes, it has been reported that transiently disintegrating fusome remnants are detectable in dedifferentiated spermatogonial cells (Brawley and Matunis, 2004; Cheng et al., 2008). Using antibody against α-Spectrin, we did not observe any disintegrating fusome remnants in GSCs from *lid* testes (n=52). Taken together, these data suggest that GSCs in *lid* testes are lost due to decreased self-renewal ability and premature differentiation.

To further explore this, we stained for a germ cell differentiation marker, Bag of marbles (Bam) (Gonczy et al., 1997), in wt and *lid* testes. Bam is normally expressed in four-to-16-cell spermatogonial cells (Gonczy et al., 1997). Consistently, using a Bam-GFP reporter transgene (Chen and McKearin, 2003b), we found that the GFP signal is only detectable in differentiating spermatogonial cysts away from the hub in wt testes (n=20) (**Fig. 3-5D-D'**). By contrast, Bam-GFP-positive cells were detected in direct association with the hub cells in 15% of the *lid* testes (n=20) (**Fig. 3-5E-E'**, arrows in **3-5E'**), further indicating that Lid is required to prevent GSCs from undergoing premature differentiation.

Lid acts in germ cells to maintain the proper level of the Stat92E transcription factor

The JAK-STAT pathway is one of the major pathways that maintain stem cell activity and identity in the testis niche. Testes depleted of *Stat92E* display severe loss of both CySC and GSC populations due to premature differentiation (Kiger et al., 2001; Leatherman and Dinardo, 2008; Leatherman and Dinardo, 2010; Tulina and Matunis, 2001). In wt testes, Stat92E is highly enriched in GSCs and some of their immediate daughter cells, but rapidly declines in further differentiated cells (**Fig. 3-6A-A'**). By contrast, *lid* testes had no detectable Stat92E signal (**Fig. 3-6B-B'**), even though testes from both genotypes were immunostained together, mounted on the same slide and imaged using the same microscope settings. In addition, we used quantitative reverse transcription PCR (qRT-PCR) to measure the *Stat92E* mRNA levels. The constitutively expressed *RpL32* gene was used as an internal control. The *Stat92E* transcript level in *lid* testes decreased to ~50% of the level in the wt control (**Fig. 3-6C**). Finally, we compared *nos> lid shmiRNA* testes with the *nos-Gal4* control using the similar experimental strategy as described above. We also found a marked decrease of the Stat92E immunostaining signal at the niche in the *nos> lid shmiRNA* testes (**Fig. 3-6E-E'**) compared to the *nos-Gal4* control (**Fig. 3-6D-D'**). In summary, these results demonstrate that Lid acts in germ cells to maintain proper expression of the Stat92E transcription factor at both transcript and protein levels.

Lid regulates Stat92E in GSCs to maintain their self-renewal and prevent their premature differentiation

Because Lid is required for proper expression of Stat92E, we tested whether further reducing Stat92E levels could enhance *lid* mutant phenotype. By removing one copy of *Stat92E* using a strong loss-of-function allele ($Stat92E^{06346}$) (Hou et al., 1996) at *lid* mutant background,

we found a significant enhancement of the GSC loss and premature differentiation phenotypes. In wt testes, after GSC asymmetric cell division, the differentiating daughter cell called gonialblast (GB) undergoes four rounds of mitosis and then enters meiosis. During the elongated G2 phase of meiosis, male germ cells grow 25 times in volume as spermatocytes. Spermatocytes are distinguishable from spermatogonia based on their large size and distinct nuclear morphology (White-Cooper et al., 1998). In wt testes, spermatocytes are distant from the niche and never observed in close proximity with the hub cells (**Fig, 3-7A**, arrow). In *lid* mutant testes, although we found differentiated spermatogonial cysts in direct contact with hub cells, we have never observed spermatocytes in direct contact with the hub cells (**Fig. 3-7B**, arrow). However, in drastic contrast, we found that spermatocytes are in direct association with the hub cells in 100% of *lid; Stat92E/+* testes (**Fig. 3-7C**, arrows). In these testes, the earliest stage of germ cells we could detect is the eight-cell spermatogonial cells (**Fig. 3-7C**, yellow dotted outline). These results demonstrate that both the severity and the penetrance of the *lid* mutant phenotype are enhanced by loss of one copy of *Stat92E*, suggesting that Lid acts in synergy with Stat92E to prevent GSC premature differentiation. On the other hand, the enhanceable phenotype of *lid* mutant is consistent with the measurement of *Stat92E* transcript level in *lid* mutant testes (**Fig. 3-6C**), suggesting a partial loss of *Stat92E* phenotype in *lid* mutant testes. These results also explain the reason why GSCs mutant for Stat92E using a strong loss-of-function allele ($Stat92E^{06346}$) are lost at a more rapid rate (Tulina and Matunis, 2001) compared to *lid* mutant GSCs (**Fig. 3-1E**).

To explore whether Stat92E acts downstream of Lid, we next examined whether overexpression of a *Stat92E* cDNA is sufficient to rescue the *lid* mutant defects. We found that driving a *UAS-Stat92E* transgene (Bach et al., 2003) using *nos-Gal4* rescued both GSC loss (**Fig.**

3-7D, **3-7F** 3rd vs. 2nd column) and premature differentiation phenotype of *lid* mutant testes: the percentage of testes with spermatogonial cysts next to the hub decreased from 33% (n=52) in *lid* mutant to 5% (n=40) in *lid; nos>UAS-Stat* testes. In addition to *lid* mutant testes, we tested whether *nos>UAS-Stat* can rescue *nos>lid shmiRNA* phenotype in which Lid's function is only compromised in germ cells. We found that *nos>UAS-Stat* rescued GSC loss in *lid* knockdown testes (**Fig. 3-7E, 3-7F** 6th vs. 5th column). As a control for both experiments, we drove *UAS-Stat92E* expression using the same *nos-Gal4* at a wt background and did not observe an increase in GSC number. This result is consistent with the previous report that increasing Stat92E level in the germline does not lead to any ectopic phenotype (**Fig. 3-7F** last column) (Leatherman and Dinardo, 2008). In summary, these results demonstrate that Stat92E acts downstream of Lid in maintaining GSC self-renewal and preventing their premature differentiation.

Based on our data on Lid's function, we hypothesize that Lid is required for normal expression of Stat92E, which is required for GSC maintenance. Then loss of *Stat92E* in the germline should phenocopy the *lid* mutant phenotype. To test this hypothesis more directly, we compromised Stat92E expression by driving a *UAS-Stat92E shmiRNA* exclusively in early germ cells using the same *nos-Gal4* driver. We found that knockdown of *Stat92E* specifically in the germline led to similar phenotype of *lid* mutant testes in which differentiated spermatogonial cysts directly contact the hub cells in 48% of testes (n=33, **Fig. 3-7H**, arrow). By contrast, only spectrosome-containing GSCs are located next to the hub in all *nos-Gal4* control testes (n=30, **Fig. 3-7G**, arrow). The premature differentiation phenotype is more penetrant in *UAS-Stat92E shmiRNA* testes (48%) compared to *lid* mutant testes (33%), consistent with the partial loss of *Stat92E* phenotype in *lid* mutant testes as discussed previously. Taken together, these results

suggest that both Lid and Stat92E are required cell autonomously in GSCs to prevent them from undergoing premature differentiation.

A previous study has reported that Stat92E regulates adhesion of GSCs to the hub by maintaining DE-Cadherin levels (Leatherman and Dinardo, 2010). The increased detachment of GSCs in *lid* testes (**Fig. 3-1C**, arrow) could be due to decreased DE-Cadherin because of decreased Stat92E (**Fig. 3-6**). However, in addition to rescuing GSC number, the mitotic index of GSCs in *lid; nos>UAS-Stat* testes was also restored to the level in wt testes (**Fig. 3-7I**), suggesting a role for Stat92E in GSC self-renewal. Therefore our results support a cell-autonomous role for Stat92E in maintaining GSC self-renewal, consistent with two earlier publications (Kiger et al., 2001; Tulina and Matunis, 2001). The argument that germline Stat92E is only required for GSC adhesion to the hub cells (Leatherman and Dinardo, 2010) was based on an experimental design in which Stat92E is expressed solely in somatic gonadal cells of $Stat92E^{ts}$ mutants at a restrictive condition. Using this strategy, the authors observed that CySCs displaced GSCs at the hub but *Stat92E* mutant germ cells could be maintained in 89% of the testes eight days after inactivating *Stat92E* by temperature shift. However, there are two caveats in the interpretation of these results: first, *Stat92E* depletion was carried out using a $Stat^{ts}$ allele and it is very likely that some residual Stat92E activity in the germ cells support their maintenance. Another evidence that $Stat^{ts}$ is insufficient to deplete all Stat92E at restrictive temperature was that CySCs were retained but did not differentiate in *nos> Stat92E*; $Stat92E^{ts}$ testes. Second, in *Tj> Stat92E*; $Stat92E^{ts}$ testes, Stat92E activity could be up-regulated in CySCs which led them to outcompete GSCs from the niche, consistent with the phenotypes reported for the JAK-STAT repressor *Socs36E* mutant (Issigonis et al., 2009) and the Socs36E positive regulator the *dUTX* mutant (Tarayrah et al., 2013).

Since Lid is a demethylase that erases the H3K4me3 active histone modification, it is likely that Lid acts as a transcriptional repressor (Secombe and Eisenman, 2007). Then a JAK-STAT pathway negative regulator could be repressed by Lid which would lead to decreased Stat92E level when Lid function is compromised. To test this, we investigated genetic interactions between Lid and protein tyrosine phosphatase (Ptp61F), a negative regulator of the JAK-STAT pathway (Baeg et al., 2005) . Ptp61F is a tyrosine phosphatase that targets Stat92E and potentially Hop to inhibit their activity (Baeg et al., 2005). We found that removing one copy of *Ptp61F* using a loss-of function allele ($Ptp61F^{PBac}$) was sufficient to suppress the GSC loss phenotype in *lid* testes (**Fig. 3-8A-B**). Furthermore, when we drove *UAS-Ptp61F shmiRNA* using the *nos-Gal4* driver, we observed suppression of the GSC loss phenotype in *nos>lid shmiRNA* testes (**Fig. 3-8C-D**). As a control, the *UAS-Ptp61F shmiRNA* driven by the *nos-Gal4* did not lead to any significant change of GSC number (**Fig. 3-84D**), consistent with the previous observation that overexpression of Stat92E in germline does not lead to any ectopic phenotype (**Fig.3-7F**, last column). Therefore in both *lid* mutant and germ cell-specific *lid* knockdown testes, Ptp61F acts antagonistically with Lid in maintaining GSCs.

In this study, we report an important cell autonomous function of H3K4me3-specific histone demethylase Lid in male GSC maintenance in *Drosophila*, which is different from the H3K27me3-specific histone demethylase dUTX reported to have a non-cell autonomous role by our lab (Tarayrah et al., 2013). While both demethylases are required to regulate stem cell activities, Lid mainly regulates GSC while dUTX regulates CySC activities at the niche. Interestingly, both Lid and dUTX maintain the activities of their target stem cell population by regulating the JAK-STAT pathway. However, while dUTX controls the transcription of

Socs36E, an inhibitor of JAK-STAT in CySCs, Lid likely targets another JAK-STAT inhibitor Ptp61F in the germline. In summary, our results emphasize the importance of studying chromatin regulators' functions *in vivo* in the context of cell-cell communication, because their activities connect cell intrinsic mechanisms such as transcription with extrinsic signaling.

MATERIALS AND METHODS

Fly Stocks

Flies were raised on standard yeast/molasses medium at 25°C. The following stocks were used: *lid*^{10424} (Bloomington Stock Center, BL-12367), *lid*^{k06801}(Bloomington Stock Center, BL-10403), *w*^{1118}*; Df(2L) BSC184* (Bloomington Stock Center, BL-9612), *lid*^{k06801}*,FRT40A* (Bloomington Stock Center, BL-111088), *UAS-lid shmiRNA* (Valium 10, TRiP.HM05155 from Bloomington Stock Center, BL-28944), *UAS-lid shmiRNA* (Valium 22, TRiP.GL00612 from Bloomington Stock Center, BL-36652), *upd-Gal4* (from D. Harrison, University of Kentucky, Lexington, KY, USA), *nanos-Gal4* (from M. Van Doren, Johns Hopkins University, Baltimore, MD, USA), *c587- Gal4* (from A. Spradling, Carnegie Institution Department of Embryology, Baltimore, MD, USA), *y,w; Ubi-GFP, Ubi-GFP, FRT40A* (Bloomington Stock Center, BL-5189), *hs-FLP*^{122} (Bloomington Stock Center, BL- 33216), w; *FRT40A* (Bloomington Stock Center, BL-5756), w;; *Bam-GFP* (from D. McKearin, University of Texas Southwestern Medical Center, Dallas, TX, USA), *UAS-lid* (from J. Secombe, Albert Einstein College of Medicine, Bronx, NY, USA) [refer to Materials and Methods in (Li et al., 2010b; Secombe et al., 2007)], *Stat92E*^{06346} (from N. Perrimon, Harvard Medical School, Boston, MA, USA), *UAS-Stat92E* (from E. Bach, New York University School of Medicine, New York, NY, USA), *UAS-Stat shmiRNA* (Valium 20, TRiP.HMS00035 from Bloomington Stock Center, BL-3367), *UAS- Ptp61F shmiRNA*

(Valium 20, TRiP.HMS00421 from Bloomington Stock Center, BL-32426), *Ptp61F*^{PBac} (Bloomington Stock Center, BL-17698), and *Act5C<stop<Gal4, UAS-GFP* (from J. Secombe, Albert Einstein College of Medicine, Bronx, NY, USA).

Clonal Induction

lid^{k06801} clones were generated using the FLP/FRT recombination system. The flies used had the following genotypes: *hs-FLP*^{122}*; Ubi-GFP, Ubi-GFP, FRT40A/ lid*^{k06801} *FRT40A or hs-FLP122; Ubi-GFP, Ubi-GFP, FRT40A/ FRT40A.* The clones were induced by heat shocking pupae on days eight and nine for two hours at 37°C. After the second heat shock, flies were placed at 25°C and dissected and stained one, three and seven days after clone induction.

Overexpression clones were generated using the FLP/FRT recombination system. The flies used had the following genotypes*: hs-FLP122; Act5C<stop<Gal4, UAS-GFP; UAS-lid.* The clones were induced by heat shocking pupae on days eight and nine for two hours at 37°C. After the second heat shock, flies were placed at 25°C and dissected and stained three days after clone induction.

Immunofluorescence Staining

Testes were dissected in 1XPBS and fixed in 4% formaldehyde for 30 minutes. For immunostaining, testes were incubated with primary antibodies overnight at 4 °C, followed by washes in 1XPBST and incubation with secondary antibodies for two hours at RT. The following primary antibodies were used: mouse anti-Armadillo [1:100; developed by Eric Wieschaus, Princeton University, Princeton, NJ, USA, and obtained from Developmental Studies Hybridoma Bank (DSHB)]; rat anti-Vasa (1:100; developed by Allan Spradling and Dianne Williams and

obtained from DSHB); rabbit anti-Vasa (1:100; Santa Cruz, sc-30210); chicken anti-GFP (1:1000; Abcam, #13970); rabbit anti-Stat92E (1:800; from Denise Montell, Johns Hopkins School of Medicine, Baltimore, MD, USA); rabbit anti-phospho-Histone H3 (Thr3) (1:200; Millipore, #05-746R); mouse anti-α-spectrin (1:50; obtained from DSHB); mouse anti-γ-tubulin (1:100, Sigma, GTU-88); mouse anti-FasIII (1:50; obtained from DSHB, 7G10); rabbit H3K4me3 (1:200; Cell Signaling, #9751S). Alexa 488, 568 and 633-conjugated Goat anti-mouse, anti-rabbit, and anti-rat secondary antibodies were used (1:200; Molecular Probes/Invitrogen).

Isolation of Total RNA and Quantitative Reverse Transcription Polymerase Chain Reaction (qRT–PCR)

Total RNA was collected and isolated from wild-type (wt) and *lid* third instar larval testes using TRIzol reagent (Invitrogen, #15596-018) according to the manufacturer's instructions. The yield and quality of the RNA was determined using a NanoDrop spectrometer (NanoDrop Technology, San Diego, CA, USA). Reverse transcription was performed using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, #K1621). RNA Transcript levels were then measured using SYBR Green PCR Master Mix (Fermentas, #K0221) and normalized by *RpL32*. Sequences of primers used for qRT-PCR were as follows:

AAGGTGAGTGATTTGCTGTGCTGC (*Stat92E*-forward)

CAACAAGCGAGCATGAGAATGCCA (*Stat92E*-reverse)

CATGCTGCCCACCGGATTCAAGAAG (*RpL32*-forward)

CTCGTTCTCTTGAGAACGCAGGCGA (*RpL32*-reverse)

Western Blotting

Testes were dissected in 1XPBS. Samples were homogenized in 25 µl 1XPBS containing 4XDualColor Protein Loading Buffer (Fermentas, #R1011), boiled and loaded onto a 4-20% gradient SDS-PAGE gel (Novex, EC6065). Primary antibodies anti-H3K4me3 (1:1000; Cell Signaling, #9751S) and anti-H3 (1:5000; Abcam, ab1791) were used.

Statistical Analysis

Statistical significance was calculated using two-tailed Student's t-test or Fisher's test. P-values are indicated in figures or in figure legends. Error bars indicate s.d.

FIGURES

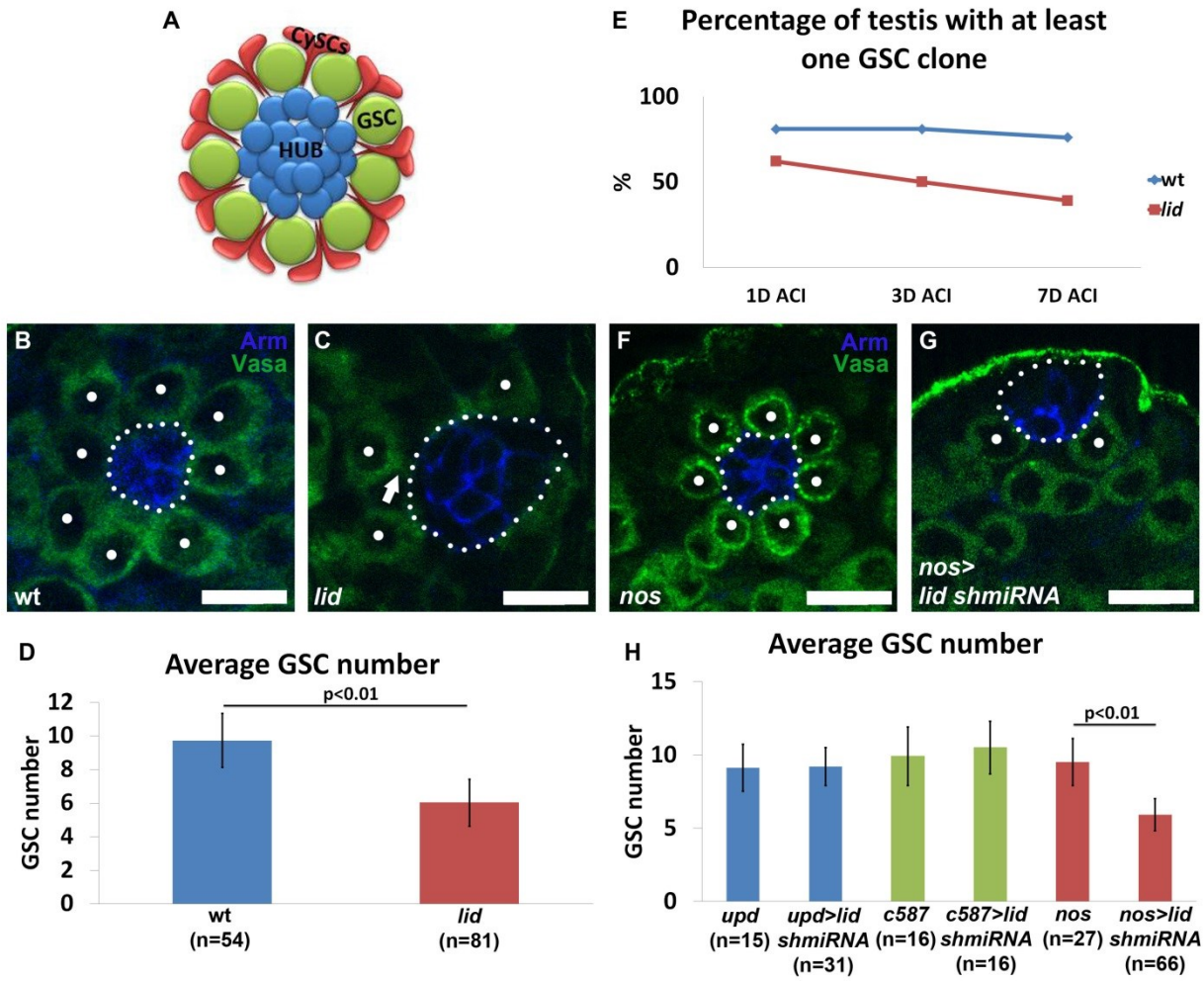


Figure 3-1. Lid acts cell autonomously in the germline to maintain GSC number at the niche. (**A**) Schematic of the *Drosophila* testis niche. CySCs, cyst stem cells; GSC, germline stem cell. (**B-C, F-G**) Immunostaining using antibodies against Armadillo (Arm) (blue) and Vasa (green) in wt (**B**), *lid* (**C**), *nos-Gal4* (**F**), and *nos-Gal4; UAS-lid shmiRNA* (**G**) testes. Arrow points to detached GSCs in *lid* (**C**) testes. Dots indicate GSCs which we identified as Vasa-labelled cells in direct contact with the hub. Hub area is outlined (white dotted line). (**D**)

Quantification of average GSC number: 9.7±1.6 in wt testes versus 6.03±1.4 in *lid* testes. (**E**) Quantification of the percentage of testes with at least one GSC clone, one, three, and seven days after clone induction by heat shock in wt and *lid* testes. (**H**) Quantification of average GSC number in testes from males of the following genotypes: *upd-Gal4* control (9.1±1.6); *upd-Gal4; UAS-lid shmiRNA* (9.2±1.3); *c587-Gal4* control (9.9±2); *c587-Gal4; UAS-lid shmiRNA* (10.5±1.8); *nos-Gal4* control (9.5±1.6); and *nos-Gal4; UAS-lid shmiRNA* (5.9±1.1). *P*-value calculated using Student's *t*-test. Error bars represent s.d. Scale bars: 10μm.

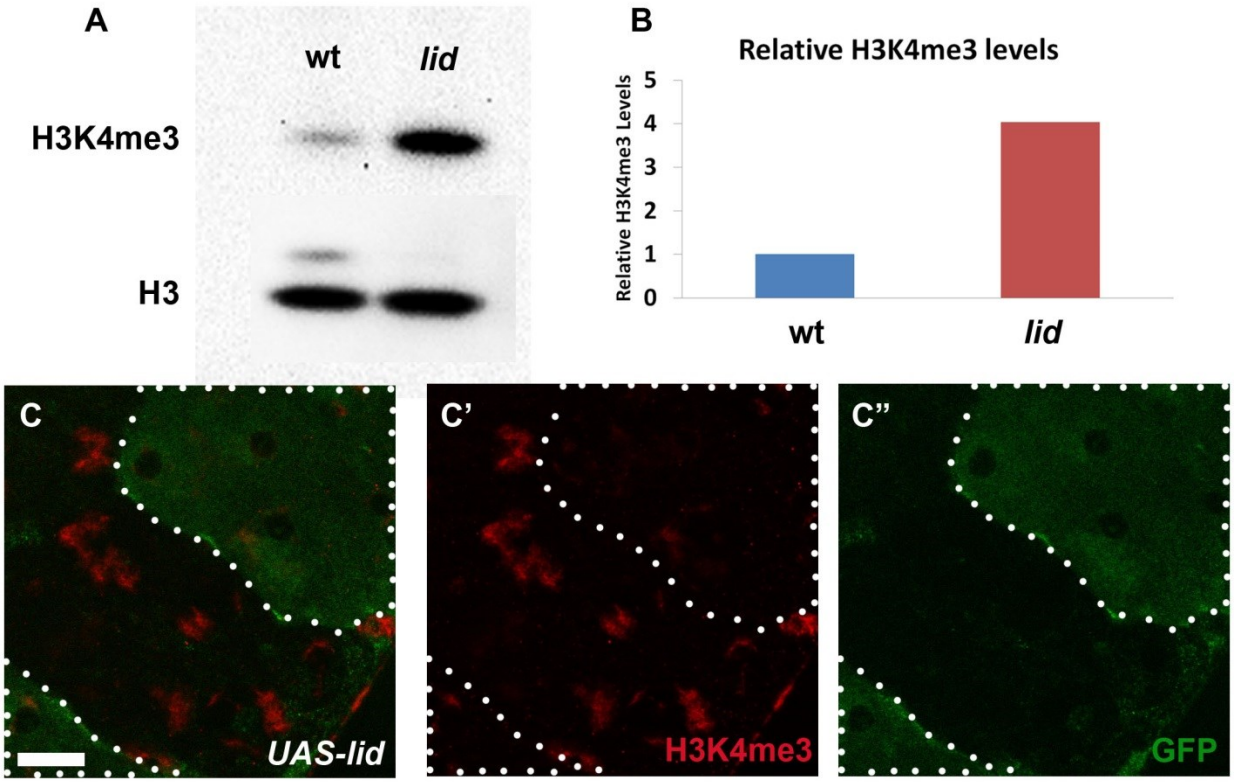


Figure 3-2. Lid acts as an H3K4me3 demethylase in the *Drosophila* testis. (**A**) Western analysis using antibodies against H3K4m3e and H3 in wt versus *lid* testes. (**B**) Quantification of relative H3K4me3 levels from the immunoblot in (**A**). (**C-C"**) Immunostaining using antibodies against H3K4me3 (red) and GFP (green) in testis overexpressing *lid* (*hs-Flp; Act5C-Gal4;UAS-lid*). Clones overexpressing the transgene are marked with GFP and outlined (white dotted line). Scale bar: 10µm.

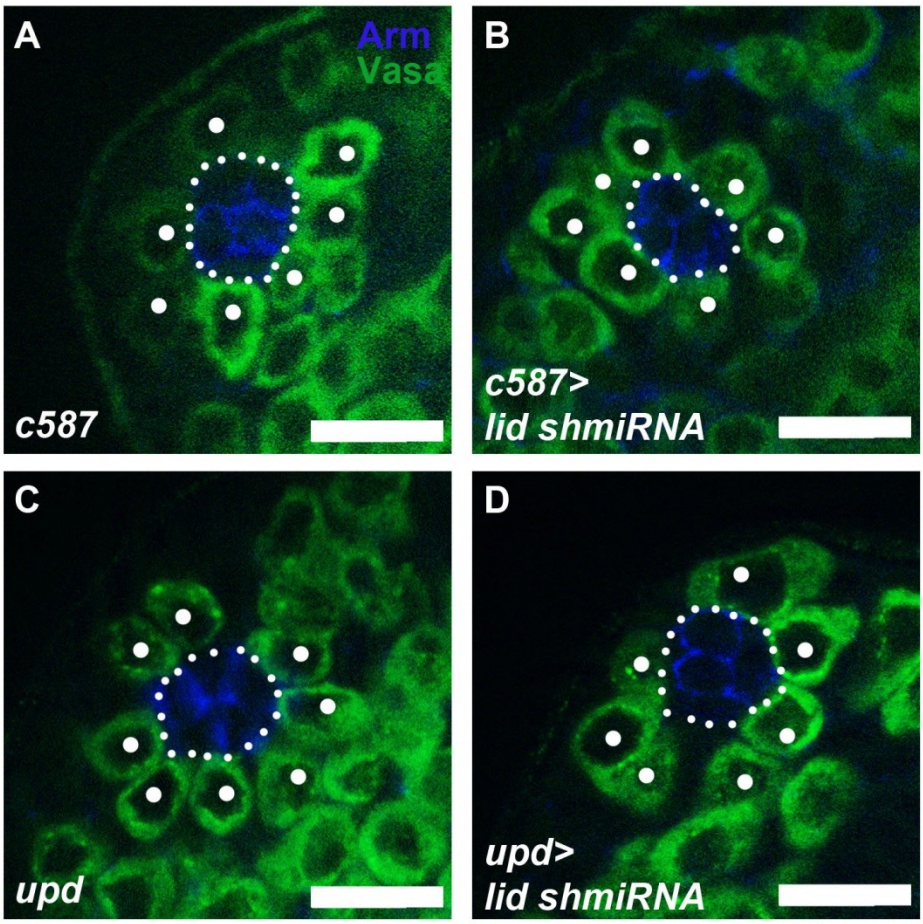


Figure 3-3. Loss of *lid* in cyst cells or hub cells does not affect GSC number. (**A-D**) Immunostaining using antibodies against Arm (blue) and Vasa (green) in *c587-Gal4* control (**A**), *c587-Gal4; UAS-lid shmiRNA* (**B**), *upd-Gal4* control (**C**), and *upd-Gal4; UAS-lid shmiRNA* (**D**). Dots indicate GSCs which we identified as Vasa-labelled cells in direct contact with the hub. Hub area is outlined (white dotted line). Scale bars: 10μm. dots label GSCs

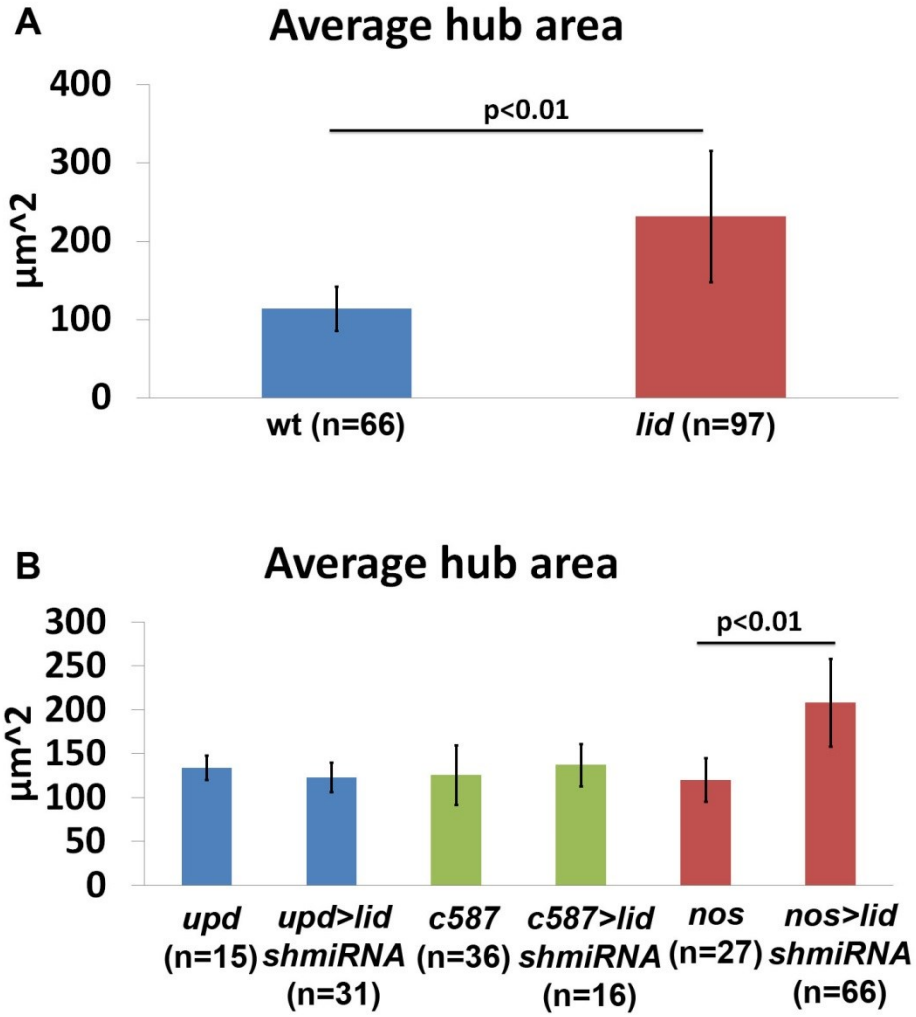


Figure 3-4. Lid acts in germ cells to maintain proper hub size. (**A**) Quantification of average hub area in wt (113.9±28.2 µm^2) versus *lid* (231.5±84.2 µm^2) testes. (**B**) Quantification of average hub area in testes from males of the following genotypes: *upd-Gal4* control (134±14 µm^2); *upd-Gal4; UAS-lid shmiRNA* (123±17 µm^2); *c587-Gal4* control (125±34 µm^2); *c587-Gal4; UAS-lid shmiRNA* (137±24 µm^2); *nos-Gal4* control (120±25 µm^2); *nos-Gal4; UAS-lid shmiRNA* (208±50 µm^2). *P*-values calculated using Student's *t*-test. Error bars represent s.d.

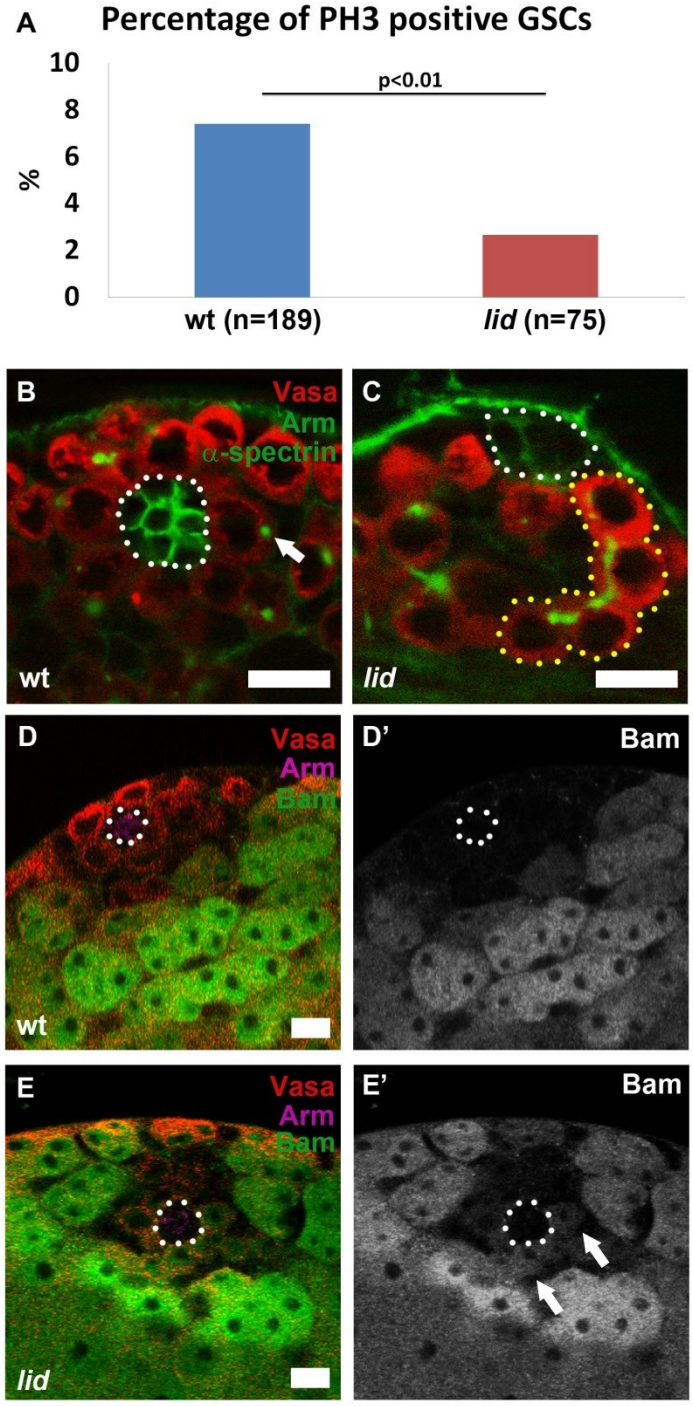


Figure 3-5. Lid is required to maintain GSC self-renewal and prevent premature differentiation. (**A**) Percentage of PH3 positive GSCs in wt (7.4%) versus *lid* (2.7%) testes. *P*-value calculated using Fisher's test. (**B-C**) Immunostaining using antibodies against Vasa (red),

Arm (green), and α-spectrin (green) in wt (**B**) and *lid* (**C**) testes. Arrow points to round spectrosome in (**B**). 4-cell spermatogonia cyst at the hub is outlined (yellow dotted line) in (**C**). Hub area is outlined (white dotted line). (**D-E'**) Immunostaining with antibodies against Vasa (red), Arm (magenta), and Bam-GFP (green) in *Bam-GFP* (**D-D'**) and *lid; Bam-GFP* (**E-E'**) testes. Arrows point to Bam-expressing GSCs in (**E'**). Hub area is outlined (white dotted line). Scale bars: 10μm.

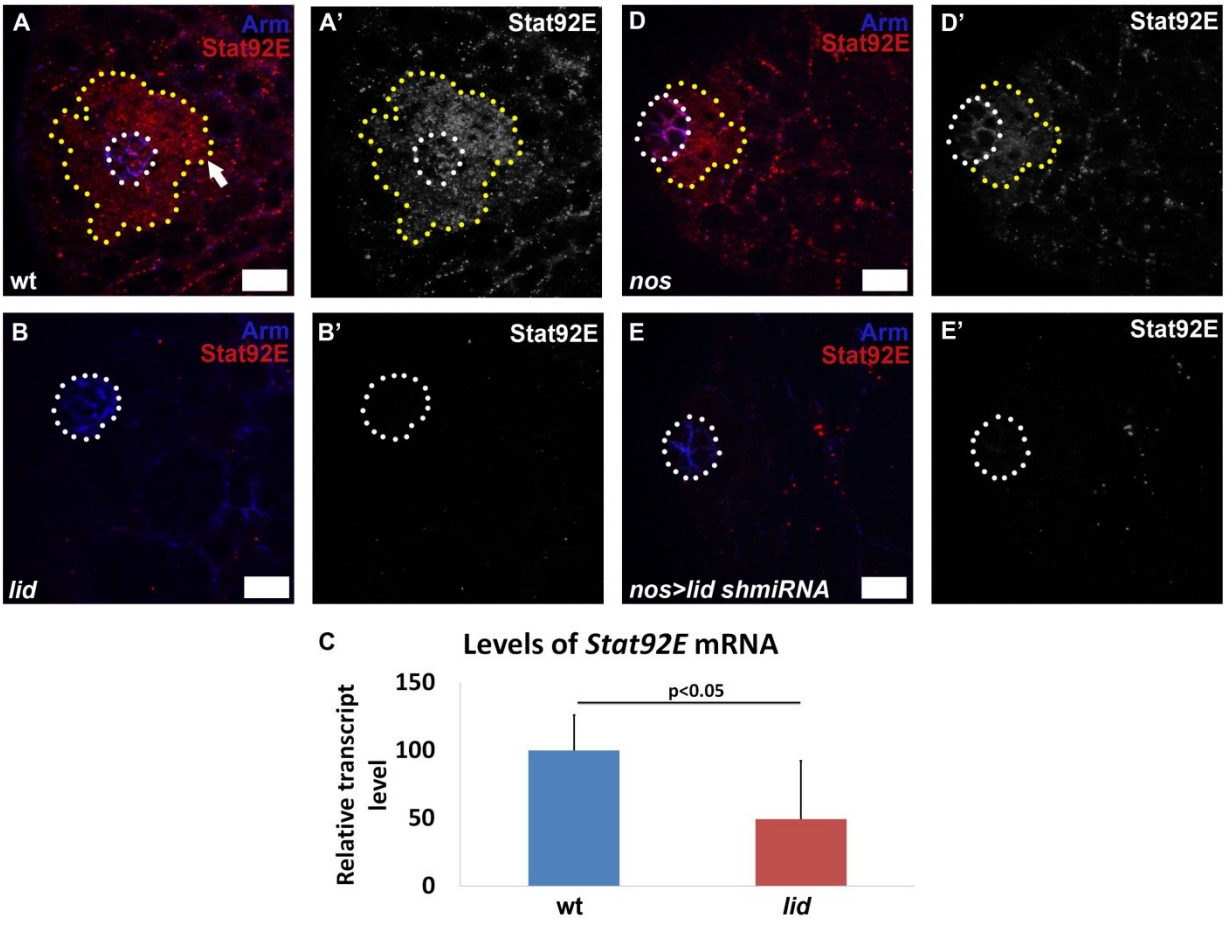


Figure 3-6. Lid acts in germ cells to maintain the proper level of the Stat92E transcription factor. (**A-B', D-E'**) Immunostaining with antibodies against Arm (blue) and Stat92E (red) in wt

(**A-A'**), *lid* (**B-B'**), *nos-Gal4* (**D-D'**), and *nos-Gal4; UAS-lid shmiRNA* (**E-E'**) testes. Stat92E-expressing cells are outlined (yellow dotted line) in (**A-A'**, **D-D'**). Arrow points to a Stat92E-expressing gonialblast in (**A**). Hub area is outlined (white dotted line). Scale bars: 10μm. (**C**) *Stat92E* mRNA levels measured by qRT-PCR in three independent biological replicates, normalized by *RpL32*. *P*-value calculated using Student's *t*-test. Error bars represent s.d. The Stat92E antibody may have a high detection threshold as it shows complete loss of Stat92E immunostaining signal in *lid* mutant testes.

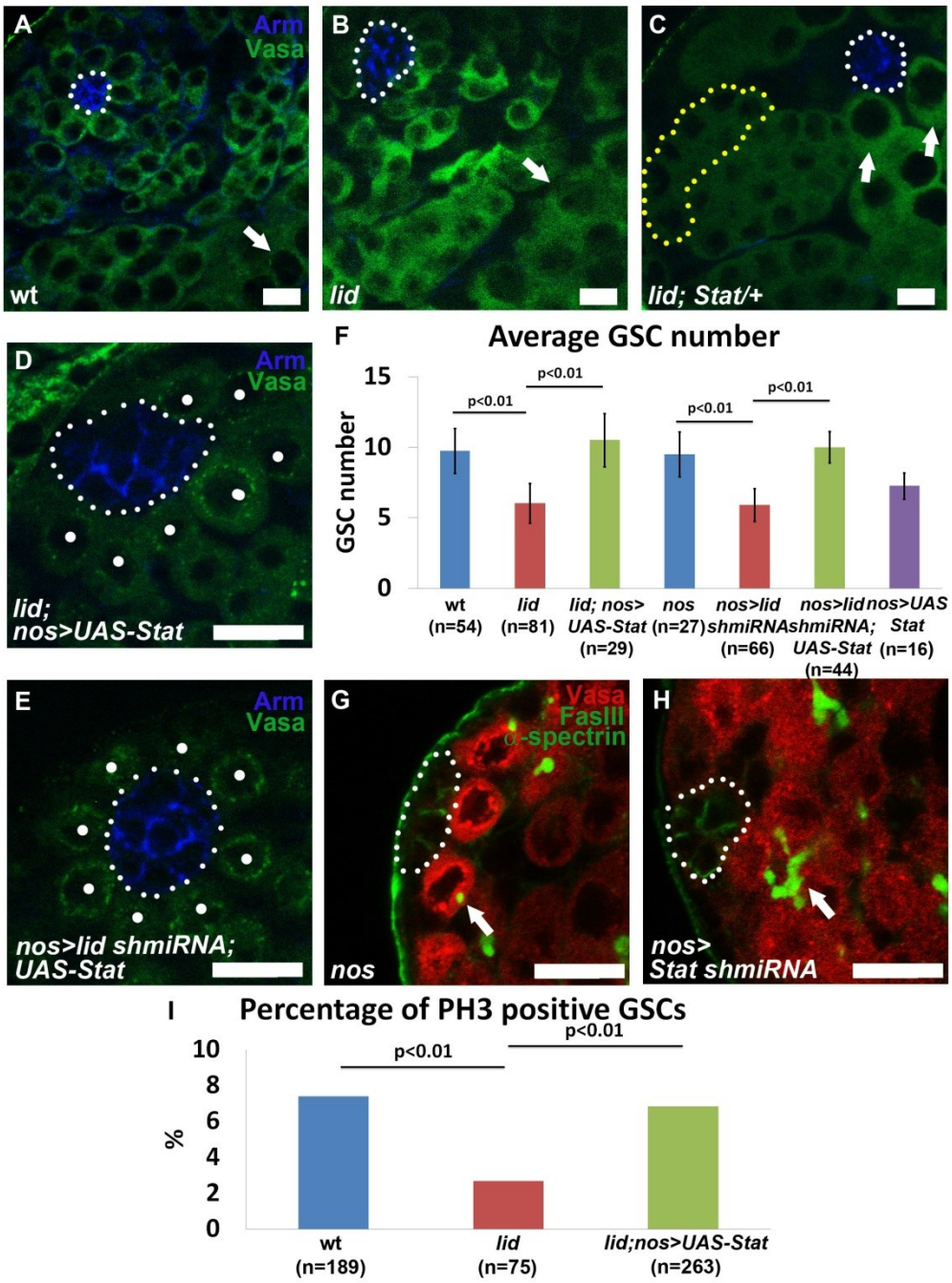


Figure 3-7. Lid regulates Stat92E in GSCs to maintain their self-renewal and prevent their premature differentiation. (**A-E**) Immunostaining using antibodies against Arm (blue) and Vasa (green) in wt (**A**), *lid* (**B**), and *lid; Stat92E/+* testes (**C**). Arrows point to spermatocytes. 8-cell spermatogonia cyst is outlined (yellow dotted line) in (**C**). (**D**) *lid, UAS-Stat92E; nos-Gal4*

with rescued GSC number. (**E**) *nos-Gal4; UAS-lid shmiRNA/UAS-Stat92E* testis with rescued GSC number. Dots indicate GSCs which we identified as Vasa-labelled cells in direct contact with the hub. Hub area is outlined (white dotted line). (**F**) Quantification of average GSC number in testes from males of the following genotypes: wt (9.7±1.6); *lid* (6.03±1.4); *lid, UAS-Stat92E; nos-Gal4* (10.5±1.9); *nos-Gal4* control (9.5±1.6); *nos-Gal4; UAS-lid shmiRNA* (5.9±1.1); *nos-Gal4; UAS-lid shmiRNA/UAS-Stat92E* (10±1.1); and *nos-Gal4; UAS-Stat92E* (7.3±0.9). *P*-values calculated using Student's *t*-test. Error bars represent s.d. (**G-H**) Immunostaining with antibodies against Vasa (red), FasIII (green), and α-spectrin (green) in *nos-Gal4* (**G**) and *nos-Gal4; UAS-Stat shmiRNA* (**H**) testes. Arrows point to round spectrosome in (**G**) and branched fusome in (**H**). Scale bars: 10μm. (**I**) Percentage of PH3 positive GSCs in wt (7.4%), *lid* (2.7%) and *lid, UAS-Stat92E; nos-Gal4* (6.8%) testes. *P*-value calculated using Fisher's test.

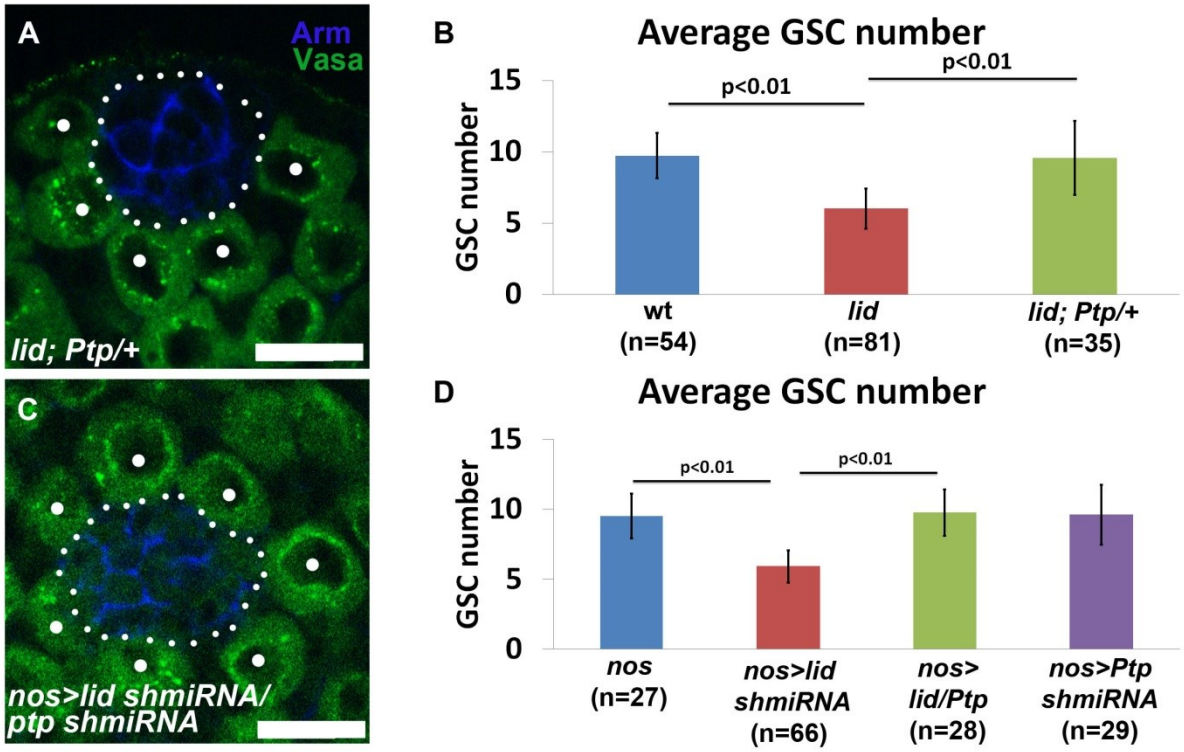


Figure 3-8. Ptp61F acts antagonistically with Lid to maintain GSC number. (**A, C**) Immunostaining using antibodies against Arm (blue) and Vasa (green). Dots indicate GSCs which we identified as Vasa-labelled cells in direct contact with the hub. Hub area is outlined (white dotted line). (**A**) *lid; Ptp61F/+* testes with rescued GSC number. (**B**) Quantification of average GSC number in testes from males of the following genotypes: wt (9.7±1.6); *lid* (6.03±1.4); *lid; Ptp61F/+* (9.3±2.4). (**C**) *nos-Gal4; UAS-lid shmiRNA/UAS-Ptp61F shmiRNA* testes with rescued GSC number. (**D**) Quantification of average GSC number in testes from males of the following genotypes: *nos-Gal4* control (9.5±1.6); *nos-Gal4; UAS-lid shmiRNA* (5.9±1.1); *nos-Gal4; UAS-lid shmiRNA/UAS-Ptp61F shmiRNA* (9.7±1.6); and *nos-Gal4; UAS-Ptp61F shmiRNA* (9.6±2.1). *P*-values calculated using Student's *t*-test. Error bars represent s.d. Scale bars: 10μm.

Chapter 4

Investigating the role of histone demethylase dUTX in *Drosophila* male germ cell differentiation

INTRODUCTION

Polycomb Group (PcG) proteins have been implicated in maintaining the proliferative state of stem cells by silencing terminal differentiation genes (Lessard and Sauvageau, 2003; Molofsky et al., 2003; Park et al., 2003). They consist of two distinct but interacting protein complexes, Polycomb Repressive Complex 1 (PRC1) and PRC2 (Schwartz and Pirrotta, 2007). Enhancer of Zeste [E(z)], a member of PRC2, is a methyltransferase that generates the H3K27me3 repressive mark in *Drosophila* (Cao et al., 2002; Czermin et al., 2002; Kuzmichev et al., 2002; Muller et al., 2002). [In mammalian systems, PcG proteins repress lineage-specific genes and removal of PcG is required for lineage commitment during differentiation in embryonic stem cells (ESCs) (Surface et al., 2010).] In addition, hematopoietic and neural stem cells have been reported to lose self-renewal capacity upon loss of mammalian PcG protein Bmi1 (Lessard and Sauvageau, 2003; Molofsky et al., 2003; Park et al., 2003).

Recent research has identified a cohort of specific enzymes named "epigenetic writers" or factors called "epigenetic readers" that generate or recognize particular histone modifications respectively, prompting studies of their biological functions during normal development and pathogenesis (Sarmento et al., 2004; Seligson et al., 2005). Among these newly identified enzymes, histone demethylases form a novel group with important roles in epigenetic regulation of gene expression (Klose et al., 2006; Schneider and Shilatifard, 2006; Shi, 2007). To date, much of the knowledge about the epigenetic regulation of histone demethylases comes from biochemical studies *in vitro* or in cell culture. In addition, little is known about the developmental program that reverses PcG repression in stem/progenitor cells, which is a prerequisite for normal differentiation and could potentially be used to treat cancers. Recent studies have identified the H3K27me3 demethylase whose enzymatic function directly

antagonizes PcG (Agger et al., 2007; De Santa et al., 2007; Klose et al., 2006; Lan et al., 2007; Xiang et al., 2007). In mammalian epidermal stem cell lineage, it has been demonstrated that the action of an H3K27me3 histone demethylase JMJD3 in reversing PcG-mediated epigenetic silencing is required for keratinocyte differentiation (Sen et al., 2008). Mammalian species have multiple genes encoding H3K27me3 demethylases. In contrast, the *dUTX* gene (*Drosophila* homolog of the mammalian *UTX* gene) encodes the sole fly H3K27me3 demethylase (Klose et al., 2006), thus greatly reduces complications that might result from gene redundancy. The dUTX protein has enzymatic activity that specifically demethylates H3K27me3 and H3K27me2, but not H3K27me1 *in vitro* (Smith et al., 2008). In addition, dUTX physically associates with RNA Polymerase II (Pol II) *in vivo*, suggesting its involvement in transcriptional activation (Smith et al., 2008).

Our previous work revealed another mechanism by which PcG-mediated repression is counteracted by the developmental program: in *Drosophila* testis, we found that several tTAFs allow robust transcription of differentiation genes by dissociating PRC1 components from their promoter region, followed by relocation of PRC1 components to the nucleolus (Chen et al., 2005). These studies highlight the importance of subnuclear localization of transcription regulators, providing another layer to the epigenetic regulation of gene expression in eukaryotic cells.

In this study we will investigate the cross-talk between these two mechanisms by studying the genetic interactions and biochemical associations between dUTX and tTAFs, which colocalize and potentially cooperate to antagonize PcG function in differentiating germ cells. In various stem cell lineages, reversal of PcG mediated repression may be a prerequisite to robustly turn on terminal differentiation genes (Buszczak and Spradling, 2006; Chen, 2008). Increased

PcG activity is associated with increased occurrences of cancers in humans (Bracken et al., 2003; Kleer et al., 2003; Varambally et al., 2002), while UTX has been reported to function as a tumour suppressor (van Haaften et al., 2009). Understanding the molecular mechanisms that antagonize PcG in an endogenous stem cell lineage may facilitate the design of therapeutic reagents to induce cancer cells (e.g. leukemic cells) to differentiate rather than unceasingly proliferate (Valk-Lingbeek et al., 2004).

In addition to the powerful *in vivo* adult stem cell system, we will employ state-of-the-art technologies to test these novel hypotheses. The strategies we propose here combine our expertise in fly molecular genetics with several innovative methods we have developed in our lab: (1) The ChIP-seq (chromatin immunoprecipitation followed by high-throughput sequencing) technique has been demonstrated to be a highly sensitive method for detecting binding sites of chromatin-associated proteins at a genome-wide coverage (reviewed in (Barski and Zhao, 2009; Schones and Zhao, 2008)). We have optimized the ChIP-seq assay using dissected testes (Gan et al., 2010b), in order to study the endogenous chromatin status; (2) a major concern in interpreting ChIP data is the impurity of the starting material, as found with intact tissue. We have therefore developed a protocol to isolate pure germ cells at specific stages, which will further enhance the resolution of our ChIP assays (Tran et. al., unpublished); (3) we have also developed a transcriptome assay using isolated single germ cell cysts, which allows us to directly compare transcriptional profiles of germ cells at distinct differentiation stages (Lim et. al., in preparation). These approaches will also be useful for investigators to study other systems, such as mammalian ESCs and adult stem cell lineages, to thoroughly understand the molecular features of stem cells and their derivatives by obtaining high-resolution molecular snapshots of their epigenomes and transcriptomes.

Stem cells have the remarkable ability to undergo asymmetric mitotic divisions that produce two distinct daughter cells. One daughter maintains the stem cell properties and regenerative potency while the other differentiates to replenish specialized cell types. The ability of adult stem cell derivatives to divide and differentiate to replace damaged tissues provides the body with an internal repair system. However, the molecular mechanisms that ensure a stem cell derivative properly differentiates are not well understood. Decoding the molecular mechanisms governing stem cell maintenance and differentiation holds great promise for developing stem cell-based therapies to treat diseases such as cancers and tissue dystrophy.

Drosophila male spermatogenesis has been shown to be a paradigmatic system to investigate the mechanisms responsible for the proliferation and maintenance of germline stem cells (GSCs) under their physiological condition (Brawley and Matunis, 2004; Kiger et al., 2001; Tulina and Matunis, 2001; Yamashita et al., 2003; Yamashita et al., 2007). Male GSCs attach to a cluster of somatic cells, called the hub, located at the tip of the testis. Hub cells contribute to a stem cell niche that provides a microenvironment that is necessary to preserve GSC identity. A male GSC divides asymmetrically to give rise to two daughter cells. One daughter cell retains stem cell identity while the other one gives rise to a gonialblast. Gonialblasts then enter a transit amplifying stage in which they undergo exactly four rounds of mitotic division as spermatogonial cells. Genes required for spermatid differentiation are silenced at the spermatogonial stage. After the fourth round of mitosis, the spermatogonia cells exit mitosis and enter early spermatocyte stage. Early spermatocytes remain at an elongated G2 phase of meiosis I, during which they grow 25 times in volume and mature into late spermatocytes. During this maturation spermatocytes initiate a robust transcription program. After exiting the spermatocyte stage germ cells undergo meiotic divisions and eventually differentiate into sperm. Defects in the

spermatogonia-to-spermatocyte transition or in the early-to-late spermatocyte maturation may lead to spermatogenesis defects and thus must be regulated precisely.

Epigenetic mechanisms play important roles in development by altering chromatin state of genes without altering their primary DNA sequences. Specifically, the active H3K4me3 and repressive H3K27me3 histone modifications have been suggested to mark developmental genes for either activation or repression during ESCs differentiation (Bernstein et al., 2006). [While H3K4me3 is laid down by the Trithorax group (TrxG) proteins and is associated with gene activation (Byrd and Shearn, 2003; Maines and Wasserman, 1999), H3K27me3 is generated by PcG proteins and has been shown to associate with gene silencing.]

Recent studies demonstrate that *dUTX* encodes the sole fly homolog of mammalian UTX (Smith et al., 2008). The UTX protein is conserved from worm to human with a highly homologous catalytic Jumonji C (JmjC) domain (Klose et al., 2006). The human UTX has been shown to specifically demethylate H3K27me3 (Smith et al., 2008). Mutations in the Caenorhabditis elegans homolog of *UTX* were shown to lead to abnormal gonad development (Agger et al., 2007). In addition, a high resolution transcriptome assay performed in our lab showed that the dUTX transcript is highly up-regulated in spermatocytes compared to spermatogonia, suggesting more requirement of dUTX in spermatocytes. Moreover, mammalian UTX associates with the H3K4me3 histone methyltransferase MLL2 (Issaeva et al., 2007), a homolog of *Drosophila* TrxG, suggesting that the connection between dUTX and TrxG, through tTAFs in my hypothesis, is conserved in mammals. I propose to study the hypothesis that tTAFs recruit dUTX to differentiation genes promoters to actively remove H3K27me3 to reverse PcG repression. In addition, through dUTX demethylase activity, it is required for differentiation gene expression and spermatocyte maturation.

In *Drosophila*, a PRC2 component, E(z), is a methyltransferase and is responsible for initiating PcG-mediated gene silencing (Cao et al., 2002). PRC1, also referred to as the maintenance complex, is involved in maintaining PcG target genes at a silent state. PRC1 component Polycomb (Pc) recognizes the H3K27me3 mark and interacts with PRC2 to repress transcription either by making the chromatin more compact (Francis et al., 2004), or by interfering with the transcription initiation (Dellino et al., 2004; Wang et al., 2004) or elongation (Stock et al., 2007).

We have found that the level of H3K27me3 at several differentiation gene promoters is significantly reduced upon the switch from spermatogonia to spermatocytes (Chen et al., 2011a). We have also shown that PRC2 expression is high in undifferentiated cells, including GSCs, gonialblasts and spermatogonia, but is abruptly downregulated in spermatocytes (**Fig. 4-1A**). At the same time, transition to spermatocytes is accompanied by turning on the tTAFs (**Fig. 4-1A'**). Five tTAF genes have been characterized in *Drosophila* - cannonball (can, tTAF5), meiosis I arrest (mia, tTAF6), spermatocyte arrest (sa, tTAF8), no hitter (nht, tTAF4) and ryan express (rye, tTAF12). In males mutant for any of these five tTAF genes, spermatid differentiation is blocked and the testes are filled up with immature early spermatocytes (Hiller et al., 2004; Hiller et al., 2001; Lin et al., 1996). In a previous study by Chen et. al. (Chen et al., 2005), tTAFs were shown to co-localize with several PRC1 components in the same sub-nucleolar compartment specifically in spermatocytes. The nucleolar localization of Pc coincides with tTAF expression and is dependent on wild-type function of tTAF. Furthermore, ChIP analysis revealed that Sa (tTAF8) is enriched at the promoters of several terminal differentiation genes, where it reduces Pc binding. Also, Sa enrichment on differentiation genes was concomitant with an increase of H3K4me3 at the promoters of these genes (Chen et al., 2005). These results suggest that tTAFs

might sequester PRC1 to the nucleolus and recruit or activate TrxG proteins to counteract PcG-regulated repression and turn on terminal differentiation genes.

In this study, I present evidence to support the hypothesis that histone demethylase dUTX cooperates with cell-type-specific transcriptional regulators to reverse PcG-mediated repression to allow for proper differentiation. I propose to study this mechanism both at the molecular level by identifying their target genes and at the cellular level by revealing how subnuclear localization contributes to such a regulation. Results from my study will be helpful in designing therapeutic strategies against cancers and in using stem cells for regenerative medicine.

RESULTS

dUTX acts as a PcG antagonist

To test whether dUTX antagonizes E(z) function via its demethylase activity, I performed fluorescence immunostaining using the specific H3K27me3 antibody (specificity was shown in (Chen et al., 2011a)) in *dUTX* loss- and gain-of-function conditions. To study whether *dUTX* loss-of-function mutation affects H3K27me3 levels in germ cells, I obtained a null allele of *dUTX* (*dUTX*^{1}) (Smith et al., 2008). The *dUTX*^{1}/Df hemizygous flies are adult lethal and survive up to the early pupal stage. Due to adult lethality of the *dUTX*^{1} mutant flies, comprehensive analysis of the mutant phenotype in adulthood requires the FLP/ FRT recombination system. In this system the FLP recombinase induces recombination between two FRT sites proximal to centromere. Distal to the FRT sites are either a lethal mutation or a cellular marker. Therefore recombination between the two FRT sites during mitosis can generate clones homozygous for the mutation in an otherwise heterozygous animal. Homozygous clones can be identified by the absence of the marker such as GFP or LacZ. Using this experimental design I have generated

dUTX mutant germ cell clones and found that a dUTX-specific antibody raised against the N-terminal 153 residues has greatly reduced immunostaining signal (**Fig. 4-2A-A''**). The reduction of the anti-dUTX staining in $dUTX^{1}$ clones is likely due to nonsense-mediated mRNA decay and therefore further supports that $dUTX^{1}$ is a null allele (Herz et al., 2010). I then stained the $dUTX^{1}$ mutant germline clones using anti- H3K27me3 and found a significant increase of H3K27me3 staining (in GFP-negative cells) compared to the wild-type neighboring cells (GFP-positive) (**Fig. 4-2B-B"**), consistent with the activity of dUTX as an H3K27me3 demethylase (Herz et al., 2010).

In addition to the loss-of-function studies, I tested whether overexpressing dUTX is sufficient to erase the H3K27me3 mark. I obtained a fly line carrying a *UAS-dUTX* construct. I used the Gal4/UAS binary system to overexpress dUTX in a subset of germ cells in testes. Cells overexpressing dUTX are positively labeled with a marker such as GFP, which have a reduction of H3K27me3 immunostaining signal (**Fig. 4-3A'-A"**). Furthermore, to investigate whether the JmjC domain is required for the demethylase activity of dUTX, I obtained another line with a UAS-$dUTX^{JmjC}$ transgene in which the JjmC domain is inactivated. Indeed, overexpression of $dUTX^{JmjC}$ had no effect on H3K27me3 staining (**Fig. 4-3B-B"**) demonstrating that the demethylase activity of dUTX in germ cells is dependent on the JmjC domain.

dUTX localizes to the nucleolus of spermatocytes in a manner dependent on tTAF function

To determine the pattern of dUTX localization we carried out an in situ hybridization experiment to detect dUTX RNA in the cells of the testis. Our results showed that the dUTX transcript is highly up-regulated in late spermatogonia/ early spermatocytes compared to the tip of the testis, suggesting a requirement for dUTX in spermatocytes (**Fig. 4-4A-B**).

To investigate the hypothesis that dUTX interacts with tTAFs to counteract PcG function, I studied the immunostaining profiles of Sa and dUTX in wild-type testes. I found that dUTX is localized to the nuclei in all stages of germ cells but becomes concentrated in the nucleolus only in spermatocytes (**Fig. 4-5A-A''**). In addition, dUTX is localized to the same sub-nucleolar compartment with Sa (**Fig. 4-5A-A"** insets). Recruitment of dUTX to the nucleolus coincides with the onset of Sa expression (**Fig. 4-5B**). The nucleolar localization of dUTX depends on wild-type tTAF activity because dUTX is found in nucleoplasm but not in nucleolus of *can* mutant spermatocytes (**Fig. 4-5C-C'**). Loss of nucleolar localization of dUTX in the *can* mutant spermatocytes is not caused by nucleolar loss. Fibrillarin, a marker of a fibrillar sub-compartment within the nucleolus, appears normal in *can* mutant spermatocytes (**Fig. 4-6A-A'**). Although the nucleolar localization of dUTX is dependent on tTAF function, the nucleolar localization of Sa is normal in the *dUTX* mutant spermatocytes (**Fig. 4-6B-B"**). These results suggest that tTAFs function upstream of dUTX for its proper sub-nuclear localization.

dUTX loss-of-function causes germ cell differentiation defects

To examine whether mutations in *dUTX* cause germ cell differentiation defects and whether *dUTX* mutant phenotype resembles *tTAF* mutant, I isolated testes from $dUTX^{1}/Df$ 3rd instar larval males and found that they are significantly smaller than the control testes at the same developmental stage (**Fig. 4-7A-B**). I also found that the size of the spermatogonial zone appears to be unchanged in $dUTX^{1}$ mutant testes; however, there is a significant decrease in the spermatocyte zone, leading to the overall smaller testis size (**Fig. 4-7A-B**). Using fluorescence immunostaining with anti-Caspase 3 antibody, I further determined that the smaller size of the mutant testes is not due to increased apoptosis. Therefore, the decreased spermatocyte zone in

dUTX mutant testes is likely due to failure of early spermatocytes to mature and differentiate into late spermatocytes. Indeed when I counted the number of spermatocytes in $dUTX^{1}$ mutant versus wild-type testes, the average number of spermatocytes in the $dUTX^{1}$ mutant testes (112±25) did not differ significantly from the wild-type control (119±22). Furthermore, *dUTX* mutant spermatocytes are much smaller than their wild-type counterparts (**Fig. 4-7C-D**), consistent with a critical role for dUTX in spermatocyte maturation. In addition to analyzing testes from the mutant males, I analyzed $dUTX^{1}$ mutant spermatocyte clones in mosaic testes. Since the FLP/FRT clonal induction depends on mitosis and spermatocytes are post-mitotic, all late spermatocyte clones must come from mitotic spermatogonial clones. Interestingly I found that while the number of early spermatocyte clones does not differ between *dUTX* mutant and *wild-type* control, the number of late spermatocyte clones significantly decreased in the mutant (**Fig. 4-7E-F**), supporting a role of dUTX in the maturation of early to late spermatocytes.

To probe for a potential genetic interaction between tTAFs and dUTX, I used the FLP/FRT system to generate dUTX mutant clones at a genetic background heterozygous for can (tTAF5). Our preliminary results indicate that two weeks after heat shock the number of late spermatocyte clones is further reduced compared to dUTX late spermatocyte clones at a wt background (data not shown), which suggests that tTAF mutation enhances dUTX mutant phenotype. To determine if dUTX and tTAFs regulate similar target genes we used qRT-PCR to measure the transcription level of known tTAF target genes *mst87F, don juan* (*dj*) and *fuzzy onion* (*fzo*), which are silent in spermatogonia and first transcribed in spermatocytes. We find that these differentiation genes were detectable in dUTX or tTAF single-mutant testis, although at a level lower than in wt (data not shown) further supporting a genetic interaction between dUTX and the tTAFs.

DISCUSSION

Based on the preliminary data, we propose a model how dUTX counteracts PcG activity in spermatocytes: (1) In the nucleolus, dUTX and tTAF sequester PRC1 to prevent it from repressing differentiation genes; (2) At the chromatin level, tTAF recruits dUTX to demethylate H3K27me3 and activate transcription of differentiation genes. We will further investigate this model using molecular, genetic, and biochemical approaches.

Investigate potential cooperation of dUTX with tTAF in counteracting PcG activity

Previous studies showed that the tTAF proteins counteract PcG activity by (1) converting the H3K27me3-enriched chromatin state of differentiation genes to H3K4me3-enriched, and (2) displacing PRC1 to the nucleoli of spermatocytes (Chen et al., 2005). I hypothesize that dUTX associates with the tTAFs to counteract PcG activity. In order to test the potential cooperation of dUTX with tTAFs in spermatocytes, we will first investigate genetic interactions between *dUTX* and *tTAF* genes in more detail. I will generate the *dUTX; tTAF* double-mutant strain and measure the transcript level of several differentiation genes, such as the known tTAF direct target genes, *mst87F, don juan (dj)* and *fuzzy onion (fzo)*, which are silent in spermatogonia and first transcribed in spermatocytes. We will compare the transcriptional level of these genes in *dUTX; tTAF* testis to that in the *dUTX* or *tTAF* single-mutant testis, using quantitative RT-PCR. These differentiation genes were detectable in *dUTX* or *tTAF* single-mutant testis, although at a level much lower than in wild-type, based on our preliminary data and previous studies (Gan et al.; Hiller et al., 2001; White-Cooper et al., 1998). If the *dUTX; tTAF* double mutant reduces differentiation gene expression to the same extent as the *tTAF* single mutant, it will support the

hypothesis that dUTX is recruited by tTAF to demethylate H3K27me3 at differentiation genes for their robust transcription.

The co-localization of dUTX and Sa in spermatocytes suggests that, in addition to the tTAFs, dUTX might be involved in sequestering PRC1 to the nucleolus to counteract PcG repression in spermatocytes. To test whether the nucleolar localization of Pc in spermatocytes depends on dUTX function, we are generating a strain that has the Pc-GFP transgene (Dietzel et al., 1999) at the $dUTX^{1}$/Df background, we will isolate larval testes from males of this strain to determine whether *dUTX* mutation affects Pc localization. Furthermore, the co-localization of dUTX, tTAFs and PRC1 to the same nucleolar compartment suggests that these proteins may physically interact with each other. To probe for the nature of the tTAF-associated protein complex(es) in spermatocytes, we have made transgenes encoding epitope-tagged versions of two different tTAFs: *3HA-sa* and *6myc-can* (Hiller et al., 2004). Both transgenes are expressed from their endogenous promoters and are fully functional *in vivo* (Chen et al., 2005). We will use commercially available antibodies against the epitope tags (HA or MYC) to immunoprecipitate tTAF-containing protein complexes from testes extracts, prepared using a published approach (Beall et al., 2007). We will then probe the precipitates with available anti-dUTX and anti-PRC1 (e.g. anti-Pc, anti-Ph and anti-dRing (Chen et al., 2005; Saurin et al., 2001)) antibodies to investigate whether the tTAFs, dUTX and PRC1 components associate with each other *in vivo*.

As an alternative approach to probe for physical interaction, we can utilize a highly specific GFP antibody (Heiman et al., 2008) to pull down Pc-GFP fusion protein from testes extracts and probe the immunoprecipitates with anti-dUTX and anti-Sa antibodies instead. We have had experience in performing protein immunoprecipitation experiments using *Drosophila* tissue (Chen et al., 2002).

Examine whether and how loss-of-function of *dUTX* causes germ cell differentiation defects

Results from the transcriptome assay developed in our lab and our immunostaining data demonstrate that both dUTX and PcG are ubiquitously expressed. However, demethylation of differentiation genes such as *mst87F, dj* and *fzo* occurs only upon transition from spermatogonia to spermatocytes, which suggests a dependence of this demethylation on tTAF function. If dUTX and tTAFs cooperatively antagonize PcG activity that sets up the chromatin landscape in undifferentiated cells, we hypothesize that the majority of dUTX and tTAF direct target genes will be genes that are H3K27me3-enriched in spermatogonia and H3K27me3-deprived in spermatocytes. As an initial effort, we have performed the following experiments and obtained very promising results: (1) we developed a sensitive technique to isolate wild-type and *tTAF* mutant spermatocyte cysts and profiled their transcriptomes using RNA-seq technology (Lim et al., in preparation). Comparison of these two transcriptomes identified all genes that depend on tTAF for robust transcription, including both tTAF-direct and indirect targets. (2) We analyzed H3K27me3 association with individual genes in purified wild-type spermatocytes using ChIP-seq assay (Tran V., unpublished). We then compared it with the published H3K27me3 ChIP-seq data using spermatogonia-enriched *bag-of-marbles (bam)* testes (Gan et al.). We found that approximately 400 genes that are enriched with H3K27me3 in *bam* testes become demethylated in spermatocytes. Interestingly, when we compared the H3K27me3 demethylated gene with the tTAF target genes, we found a significant overlap, including all known tTAF direct target genes, such as *mst87F, dj,* and *fzo* (Chen et al., 2005).. These data strongly suggest that the demethylase activity of dUTX cooperates with tTAF in turning on differentiation gene expression to ensure proper spermatocyte maturation.

The *tTAF* mutant spermatocytes have unique chromatin morphology, differentiation marker expression pattern, as well as PRC1 localization, distinguishable from wild-type spermatocytes (Chen et al., 2005; Hiller et al., 2004; Hiller et al., 2001; Lin et al., 1996). We will use the $dUTX^{1}$ *FRT40A* strain to generate marked mitotic clones in adult testis. Studying the effects of *dUTX* loss in this context provides me with an internal control. Using this setup We will perform immunostaining experiments using antibodies that recognize various differentiation markers, such as components of a testis-specific meiotic arrest complex (tMAC) (Beall et al., 2007; White-Cooper et al., 1998), as well as meiotic cell cycle regulator Boule (Maines and Wasserman, 1999). I will compare the data with the neighbouring wild-type spermatocytes, as well as with spermatocytes from *tTAF* mutant testes.

If indeed we find that dUTX germ cells have differentiation defects at the spermatocyte stage, as our preliminary data suggest, we will further test whether it is due to the enzymatic activity of dUTX. We will express the $dUTX^{JmjC}$ cDNA with a spermatocyte-specific *sa* promoter (Chen X., unpublished) and investigate whether it fails to rescue such defects. As a control, we will express wild-type *dUTX* with the same promoter to test whether it is sufficient to rescue. If the rescuing result is negative using $dUTX^{JmjC}$ but positive using dUTX, it will suggest that the requirement of dUTX in germ cell differentiation is dependent on its demethylase activity and is specific in spermatocytes.

In summary, the proposed experiments will uncover the molecular mechanisms that actively antagonize PcG-mediated repression, which have not been studied extensively in any other stem cell systems. Enhanced PcG activity has been associated with increased occurrence of cancer in human (Bracken et al., 2003; Kleer et al., 2003; Varambally et al., 2002), while mammalian UTX has been reported to function as a tumor suppressor (van Haaften et al., 2009).

A better understanding of the *in vivo* function of dUTX will be very useful in targeting PcG and using histone demethylase for cancer treatment.

MATERIALS AND METHODS

Fly Stocks

Flies were raised on standard yeast/molasses medium at 25°C. The following stocks were used: *dUTX^{1} FRT40A (from A. Shilatifard), w^{1118}; Df(2L) BSC144 (Bloomington Stock Center, BL-9504), y,w; Ubi-GFP, Ubi-GFP, FRT40A (Bloomington Stock Center, BL-5189), hs-Flp122 (Bloomington Stock Center), Arm-LacZ, FRT40A (Bloomington Stock Center, BL-7371), UAS-dUTX and UAS-dUTX^{JmjC} were obtained from A. Shilatifard, refer to Materials and Methods in (Herz et al., 2010), Act5C<stop<Gal4, UAS-GFP* (from J. Secombe, Albert Einstein College of Medicine, Bronx, NY, USA). The allelic combinations for *tTAF* mutants used were: *can^{2}/can^{12}* and *sa^{1}/sa^{2}* as well as *Sa-GFP* flies (refer to (Chen et al., 2005)).

Clonal Induction

dUTX^{1} clones were generated using the FLP/FRT recombination system. The flies used had the following genotypes: *hs-FLP122; Ubi-GFP, Ubi-GFP, FRT40A/ dUTX^{1} FRT40A or hs-FLP122; Ubi-GFP, Ubi-GFP, FRT40A/ FRT40A, hs-FLP122; Arm LacZ, FRT40A/ dUTX^{1} FRT40A or hs-FLP122; Arm LacZ, FRT40A/ FRT40A.* Overexpression clones were generated using the FLP/FRT recombination system. The flies used had the following genotypes*: hs-FLP122; Act5C<stop<Gal4, UAS-GFP; UAS-dUTX or hs-FLP122; Act5C<stop<Gal4, UAS-GFP; UAS-dUTX^{JmjC}*. The clones were induced by heat shocking pupae on days 8 and 9 for 2 hrs at 37°C.

After the second heat shock, flies were placed at 25°C and dissected and stained 3 days after clone induction.

Immunofluorescence Staining

Immunofluorescence staining was performed following the procedure previously described in (Cheng et al., 2008). The primary antibodies used were: mouse anti-βGal (1:5000, Promega, Cat# Z3783); rabbit anti-dUTX (1:2000; from Ali Shilatifard, Stowers Institute for Medical Research, Kansas City, MO, USA); rabbit anti-trimethyl-Histone H3 (Lys27) (1:200; Millipore, Cat# 07-449); chicken anti-GFP (1:1000; Abcam, Cat# 13970); rabbit anti-Vasa (1:100; Santa Cruz, sc-30210); mouse anti-Fibrillarin (straight, from P. Dimario and M. Pollard); guinea pig anti-Sa (1:100); anti-E(z) (1:100); and rabbit anti-Caspase 3 (1:100; BD Biosciences, Cat # 610322).

In situ hybridization

In situ hybridization was carried out following the protocol described previously in (Schulz, 2007). The *dUTX* sense and antisense probes were generated from a DNA fragment corresponding to bp 1628-2198 of the *dUTX* cDNA.

Isolation of Total RNA and Quantitative Reverse Transcription Polymerase Chain Reaction (qRT–PCR)

Total RNA was isolated from wt, $dUTX^{1}/Df$, and sa^{1}/sa^{2} 3rd instar larval testes using TRIzol reagent (Invitrogen, Cat# 15596-018) according to the manufacturer's instructions. Yield and quality of RNA were determined with a NanoDrop spectrometer (NanoDrop Technology, San

Diego, CA). Reverse transcription was done using RevertAid First Strand cDNA Synthesis Kit (Fermentas, Cat# K1621). Transcript levels were measured using SYBR Green PCR Master Mix (Fermentas, Cat# K0221) and normalized to *Rpl32.* Sequences of primers used for qRT-PCR were as follows:

CATGCTGCCCACCGGATTCAAGAAG (*RpL32*-forward)

CTCGTTCTCTTGAGAACGCAGGCGA (*RpL32*-reverse)

TCTTTATACTCAACAATCGATGG (*fzo*-forward)

TGTGACCATTTTGTAACTTTTGA (*fzo*-reverse)

ATGTCCTTGAGAACTTTAAGGAAG (*dj*-forward)

TTGCACTTTTTCTGCAACTTT (*dj*-reverse)

CACGAATATTAGATGAATACGAGG (*mst87F*-forward)

ATATACCTGTGCGTAACCAGATT (*mst87F*-reverse)

FIGURES

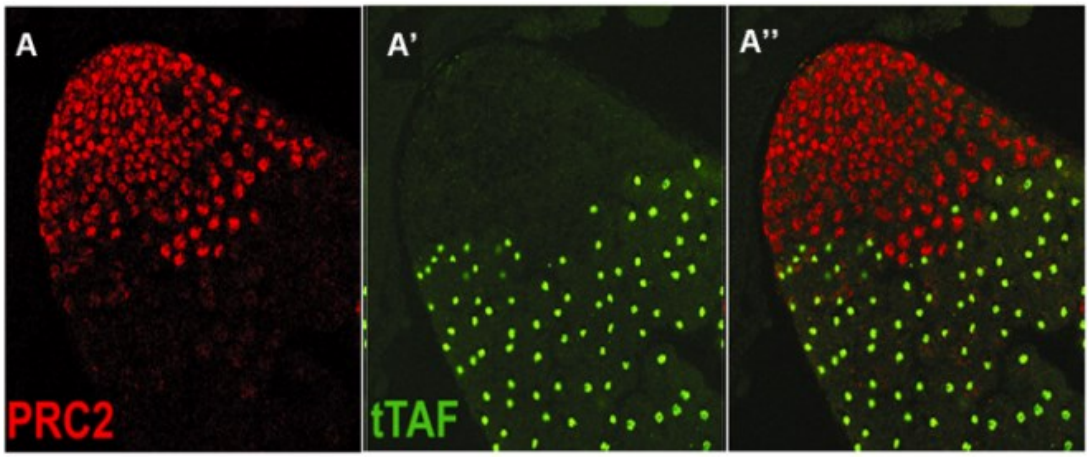


Figure 4-1. Down-regulation of PRC2 [E(z)] right before onset of tTAF (Sa) expression. (**A-A"**) Apical region of the wild-type testis: (A) anti-E(z) (red); (A') Sa-GFP (green); (A") merge.

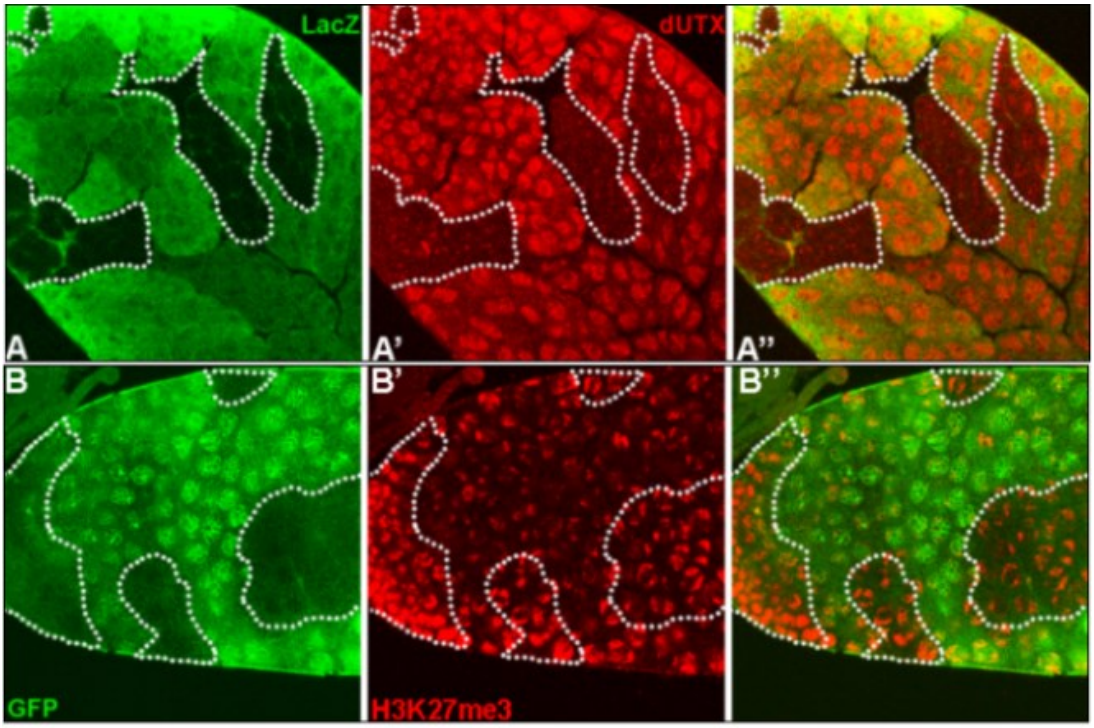


Figure 4-2. dUTX is a specific H3K27me3 demethylase. (**A-A"**) Testis with *dUTX* clones. Anti-LacZ (green), anti-dUTX (red): dUTX protein is highly reduced in *dUTX* mutant clones. (**B-B"**) Testis with *dUTX* clones. Anti-nGFP (green), anti-H3K27me3 (red): *dUTX* mutant clones have increased H3K27me3 staining. *dUTX* clones marked with white dotted line.

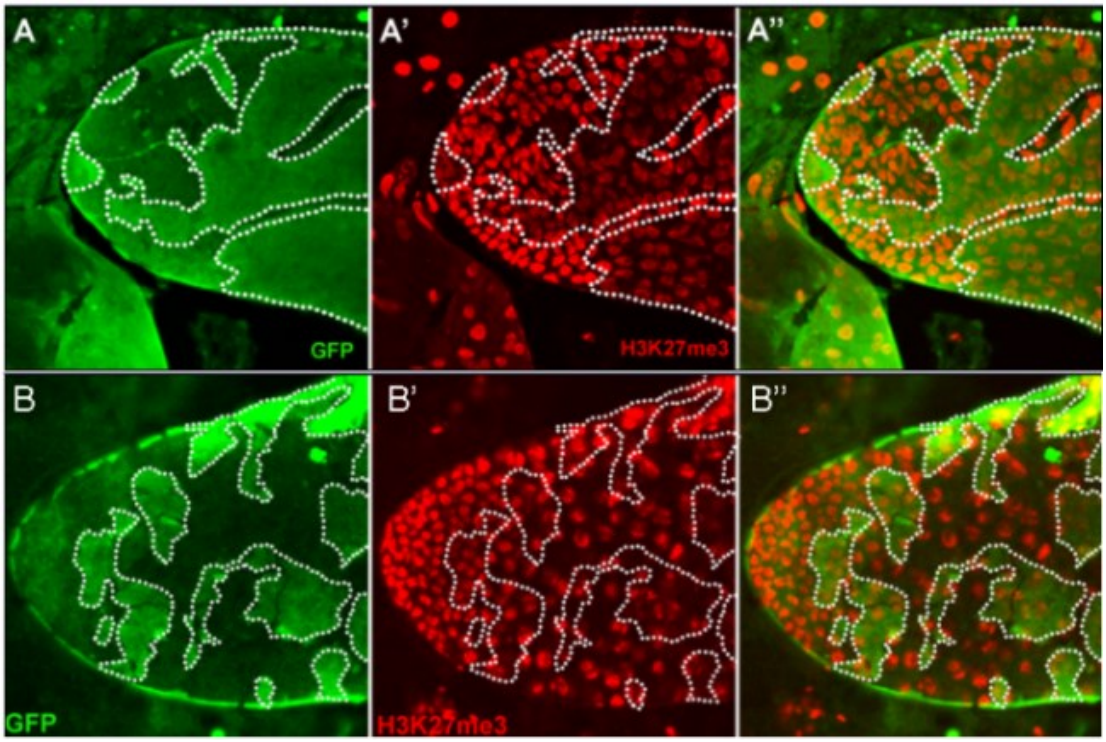


Figure 4-3. dUTX JmjC domain is required for H3K27me3 demethylation. Overexpression of *UAS-dUTX* but not *UAS-dUTX*^{JmjC} causes reduction of H3K27me3 staining. Anti-GFP (green), anti-H3K27me3 (red). (**A-A"**) Testis expressing *UAS-dUTX*. (**B-B"**) Testis expressing *UAS-dUTX*^{JmjC}. Overexpression clones marked with white dotted line.

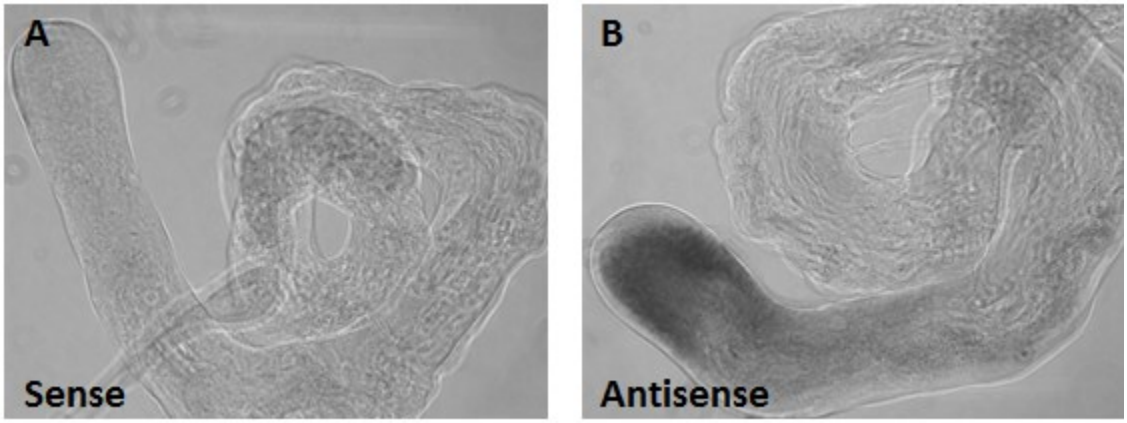


Figure 4-4. dUTX RNA is enriched in late spermatogonia and spermatocytes. (**A-B**) In situ hybridization with dUTX probe. Sense probe (A) and antisense probe (B) show enrichment of dUTX RNA in the late spermatogonia and spermatocyte regions.

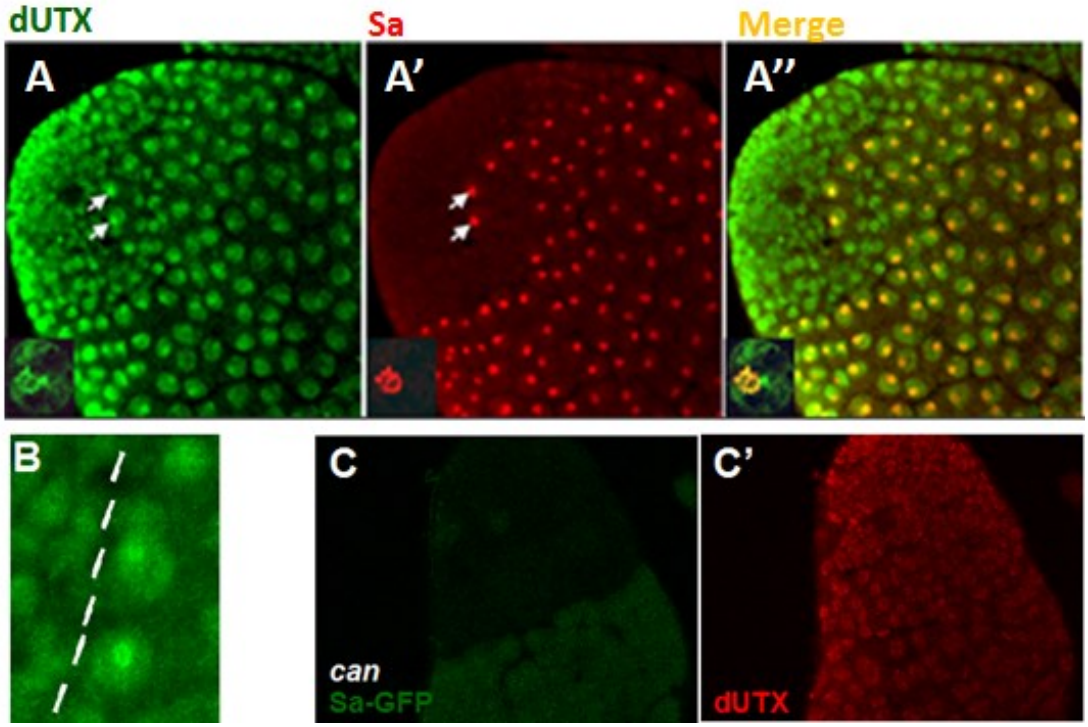


Figure 4-5. dUTX localizes to the nucleolus in spermatocytes, coincident with tTAF expression and dependent on tTAF function. (**A-A", insets**) dUTX co-localizes to the same

nucleolar compartment that contains Sa in wild-type testis. Anti-dUTX (green), anti-Sa (red). Arrows point to two early spermatocytes shown in (B). (**B**) To the right of dotted line: wild-type spermatocytes with tTAF expression, anti-dUTX (green). (**C-C'**) dUTX loses nucleolar localization in *can* mutant testis, Sa-GFP (green), anti-dUTX (red).

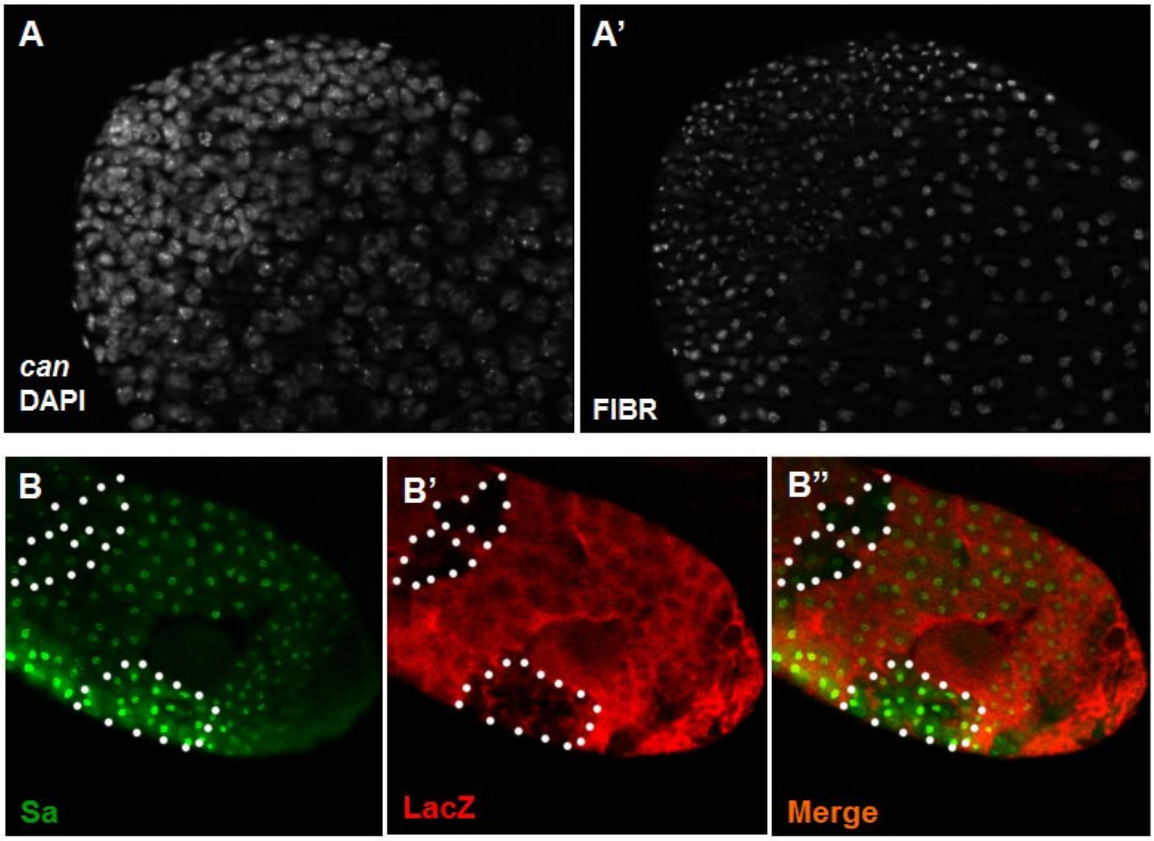


Figure 4-6. Loss of *can* does not affect nucleolar structure. Loss of *dUTX* does not affect Sa localization. (**A-A'**) *can* mutant testis stained with Dapi (A) and anti-Fibrillarin (A') has normal nucleolar structure. (**B-B"**) Testis containing *dUTX* clones stained with anti-Sa (green) and LacZ (red). *dUTX* clones marked with white dotted line and have normally localized Sa.

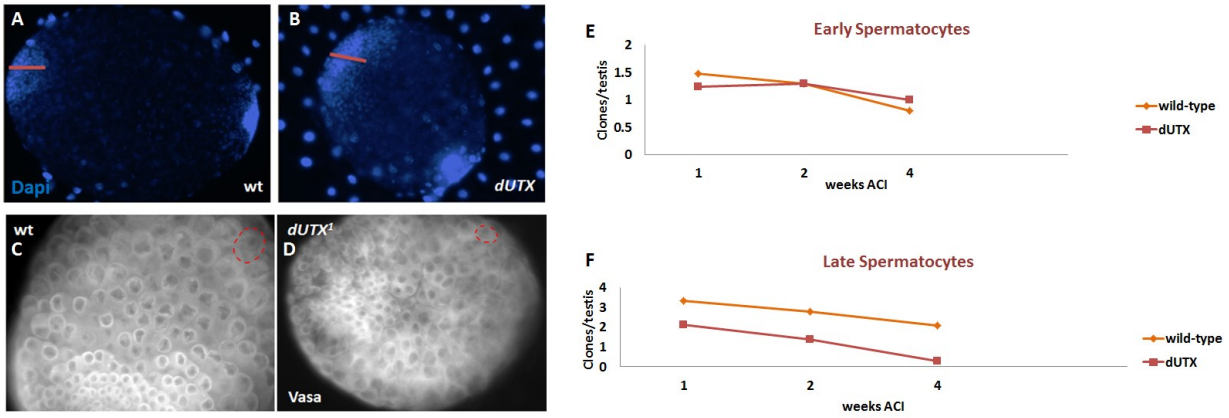


Figure 4-7. *dUTX* loss-of-function causes germ cell differentiation defects. (**A-B**) *dUTX* mutant testes (B) display a significant reduction in size compared to wild-type testes (A). Dapi (Blue), red line marks spermatogonial region recognized by a condensed Dapi signal. (**C-D**) dUTX is required for early-to-late spermatocyte maturation. Most mature spermatocyte outlined in red is smaller in *dUTX* testis (D) compared to wild-type (C). (**E-F**) Quantification of the number of wild-type and *dUTX* early spermatocyte clones (E) and late spermatocyte clones (F) per testis one, two and four weeks after clone induction.

REFERENCES

Ables, E. T. and Drummond-Barbosa, D. (2010). The steroid hormone ecdysone functions with intrinsic chromatin remodeling factors to control female germline stem cells in Drosophila. *Cell Stem Cell* **7**, 581-92.

Adjei, A. A. (2001). Blocking oncogenic Ras signaling for cancer therapy. *J Natl Cancer Inst* **93**, 1062-74.

Agger, K., Cloos, P. A., Christensen, J., Pasini, D., Rose, S., Rappsilber, J., Issaeva, I., Canaani, E., Salcini, A. E. and Helin, K. (2007). UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. *Nature* **449**, 731-4.

Al-Hajj, M., Wicha, M. S., Benito-Hernandez, A., Morrison, S. J. and Clarke, M. F. (2003). Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci U S A* **100**, 3983-8.

Alison, M. R., Lim, S. M. and Nicholson, L. J. (2011). Cancer stem cells: problems for therapy? *J Pathol* **223**, 147-61.

Alphey, L., Jimenez, J., White-Cooper, H., Dawson, I., Nurse, P. and Glover, D. M. (1992). twine, a cdc25 homolog that functions in the male and female germline of Drosophila. *Cell* **69**, 977-88.

Avci, C. B., Dodurga, Y., Susluer, S. Y., Sigva, Z. O., Yucebas, M., Caglar, H. O., Akalin, T., Dalbasti, T., Oktar, N. and Gunduz, C. (2013). Promoter hypermethylation-mediated down-regulation of RUNX3 gene in human brain tumors. *Ir J Med Sci*.

Awe, S. and Renkawitz-Pohl, R. (2010). 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* **56**, 44-61.

Ayyar, S., Jiang, J., Collu, A., White-Cooper, H. and White, R. A. (2003). Drosophila TGIF is essential for developmentally regulated transcription in spermatogenesis. *Development* **130**, 2841-52.

Bach, E. A., Ekas, L. A., Ayala-Camargo, A., Flaherty, M. S., Lee, H., Perrimon, N. and Baeg, G. H. (2007). GFP reporters detect the activation of the Drosophila JAK/STAT pathway in vivo. *Gene Expr Patterns* **7**, 323-31.

Bach, E. A., Vincent, S., Zeidler, M. P. and Perrimon, N. (2003). A sensitized genetic screen to identify novel regulators and components of the Drosophila janus kinase/signal transducer and activator of transcription pathway. *Genetics* **165**, 1149-66.

Baeg, G. H., Zhou, R. and Perrimon, N. (2005). Genome-wide RNAi analysis of JAK/STAT signaling components in Drosophila. *Genes Dev* **19**, 1861-70.

Bao, B., Ali, S., Banerjee, S., Wang, Z., Logna, F., Azmi, A. S., Kong, D., Ahmad, A., Li, Y., Padhye, S. et al. (2012). Curcumin analogue CDF inhibits pancreatic tumor growth by switching on suppressor microRNAs and attenuating EZH2 expression. *Cancer Res* **72**, 335-45.

Barker, N., Ridgway, R. A., van Es, J. H., van de Wetering, M., Begthel, H., van den Born, M., Danenberg, E., Clarke, A. R., Sansom, O. J. and Clevers, H. (2009). Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature* **457**, 608-11.

Barreau, C., Benson, E., Gudmannsdottir, E., Newton, F. and White-Cooper, H. (2008). Post-meiotic transcription in Drosophila testes. *Development* **135**, 1897-902.

Barski, A. and Zhao, K. (2009). Genomic location analysis by ChIP-Seq. *J Cell Biochem* **107**, 11-8.

Beall, E. L., Lewis, P. W., Bell, M., Rocha, M., Jones, D. L. and Botchan, M. R. (2007). Discovery of tMAC: a Drosophila testis-specific meiotic arrest complex paralogous to Myb-Muv B. *Genes Dev* **21**, 904-19.

Becker, P. B. and Horz, W. (2002). ATP-dependent nucleosome remodeling. *Annu Rev Biochem* **71**, 247-73.

Beebe, K., Lee, W. C. and Micchelli, C. A. (2010). JAK/STAT signaling coordinates stem cell proliferation and multilineage differentiation in the Drosophila intestinal stem cell lineage. *Dev Biol* **338**, 28-37.

Bernstein, B. E., Mikkelsen, T. S., Xie, X., Kamal, M., Huebert, D. J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K. et al. (2006). A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **125**, 315-26.

Bestor, T. H. and Ingram, V. M. (1983). Two DNA methyltransferases from murine erythroleukemia cells: purification, sequence specificity, and mode of interaction with DNA. *Proc Natl Acad Sci U S A* **80**, 5559-63.

Beuchle, D., Struhl, G. and Muller, J. (2001). Polycomb group proteins and heritable silencing of Drosophila Hox genes. *Development* **128**, 993-1004.

Blair, L. P., Cao, J., Zou, M. R., Sayegh, J. and Yan, Q. (2011). Epigenetic Regulation by Lysine Demethylase 5 (KDM5) Enzymes in Cancer. *Cancers (Basel)* **3**, 1383-1404.

Bonnet, D. and Dick, J. E. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* **3**, 730-7.

Bouazoune, K. and Brehm, A. (2006). ATP-dependent chromatin remodeling complexes in Drosophila. *Chromosome Res* **14**, 433-49.

Boveri, T. (2008). Concerning the origin of malignant tumours by Theodor Boveri. Translated and annotated by Henry Harris. *J Cell Sci* **121 Suppl 1**, 1-84.

Boyle, M., Wong, C., Rocha, M. and Jones, D. L. (2007). Decline in self-renewal factors contributes to aging of the stem cell niche in the Drosophila testis. *Cell Stem Cell* **1**, 470-8.

Bracken, A. P., Pasini, D., Capra, M., Prosperini, E., Colli, E. and Helin, K. (2003). EZH2 is downstream of the pRB-E2F pathway, essential for proliferation and amplified in cancer. *EMBO J* **22**, 5323-35.

Brawley, C. and Matunis, E. (2004). Regeneration of male germline stem cells by spermatogonial dedifferentiation in vivo. *Science* **304**, 1331-4.

Buszczak, M., Paterno, S. and Spradling, A. C. (2009). Drosophila stem cells share a common requirement for the histone H2B ubiquitin protease scrawny. *Science* **323**, 248-51.

Buszczak, M. and Spradling, A. C. (2006). Searching chromatin for stem cell identity. *Cell* **125**, 233-6.

Byrd, K. N. and Shearn, A. (2003). ASH1, a Drosophila trithorax group protein, is required for methylation of lysine 4 residues on histone H3. *Proc Natl Acad Sci U S A* **100**, 11535-40.

Callus, B. A. and Mathey-Prevot, B. (2002). SOCS36E, a novel Drosophila SOCS protein, suppresses JAK/STAT and EGF-R signalling in the imaginal wing disc. *Oncogene* **21**, 4812-21.

Cao, J., Liu, Z., Cheung, W. K., Zhao, M., Chen, S. Y., Chan, S. W., Booth, C. J., Nguyen, D. X. and Yan, Q. (2014). Histone Demethylase RBP2 Is Critical for Breast Cancer Progression and Metastasis. *Cell Rep*.

Cao, R., Wang, L., Wang, H., Xia, L., Erdjument-Bromage, H., Tempst, P., Jones, R. S. and Zhang, Y. (2002). Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science* **298**, 1039-43.

Casper, A. L., Baxter, K. and Van Doren, M. (2011). no child left behind encodes a novel chromatin factor required for germline stem cell maintenance in males but not females. *Development* **138**, 3357-66.

Cedar, H. and Bergman, Y. (2009). Linking DNA methylation and histone modification: patterns and paradigms. *Nat Rev Genet* **10**, 295-304.

Chang, C. J., Yang, J. Y., Xia, W., Chen, C. T., Xie, X., Chao, C. H., Woodward, W. A., Hsu, J. M., Hortobagyi, G. N. and Hung, M. C. (2011). EZH2 promotes expansion of breast tumor initiating cells through activation of RAF1-beta-catenin signaling. *Cancer Cell* **19**, 86-100.

Chatoo, W., Abdouh, M., David, J., Champagne, M. P., Ferreira, J., Rodier, F. and Bernier, G. (2009). The polycomb group gene Bmi1 regulates antioxidant defenses in neurons by repressing p53 pro-oxidant activity. *J Neurosci* **29**, 529-42.

Chedin, F. (2011). The DNMT3 family of mammalian de novo DNA methyltransferases. *Prog Mol Biol Transl Sci* **101**, 255-85.

Chen, D. and McKearin, D. (2003a). Dpp signaling silences bam transcription directly to establish asymmetric divisions of germline stem cells. *Curr Biol* **13**, 1786-91.
Chen, D. and McKearin, D. M. (2003b). A discrete transcriptional silencer in the bam gene determines asymmetric division of the Drosophila germline stem cell. *Development* **130**, 1159-70.
Chen, T., Hevi, S., Gay, F., Tsujimoto, N., He, T., Zhang, B., Ueda, Y. and Li, E. (2007). Complete inactivation of DNMT1 leads to mitotic catastrophe in human cancer cells. *Nat Genet* **39**, 391-6.
Chen, X. (2008). Stem cells: what can we learn from flies? *Fly (Austin)* **2**, 19-28.
Chen, X., Hiller, M., Sancak, Y. and Fuller, M. T. (2005). Tissue-specific TAFs counteract Polycomb to turn on terminal differentiation. *Science* **310**, 869-72.
Chen, X., Lu, C., Morillo Prado, J. R., Eun, S. H. and Fuller, M. T. (2011a). Sequential changes at differentiation gene promoters as they become active in a stem cell lineage. *Development* **138**, 2441-50.
Chen, X., Lu, C., Prado, J. R., Eun, S. H. and Fuller, M. T. (2011b). Sequential changes at differentiation gene promoters as they become active in a stem cell lineage. *Development* **138**, 2441-50.
Chen, X., Zhang, B. and Fischer, J. A. (2002). A specific protein substrate for a deubiquitinating enzyme: Liquid facets is the substrate of Fat facets. *Genes Dev* **16**, 289-94.
Cheng, J., Tiyaboonchai, A., Yamashita, Y. M. and Hunt, A. J. (2011). Asymmetric division of cyst stem cells in Drosophila testis is ensured by anaphase spindle repositioning. *Development* **138**, 831-7.
Cheng, J., Turkel, N., Hemati, N., Fuller, M. T., Hunt, A. J. and Yamashita, Y. M. (2008). Centrosome misorientation reduces stem cell division during ageing. *Nature* **456**, 599-604.
Cheng, L., Sung, M. T., Cossu-Rocca, P., Jones, T. D., MacLennan, G. T., De Jong, J., Lopez-Beltran, A., Montironi, R. and Looijenga, L. H. (2007). OCT4: biological functions and clinical applications as a marker of germ cell neoplasia. *J Pathol* **211**, 1-9.
Cherry, C. M. and Matunis, E. L. (2010). Epigenetic regulation of stem cell maintenance in the Drosophila testis via the nucleosome-remodeling factor NURF. *Cell Stem Cell* **6**, 557-67.
Chi, P., Allis, C. D. and Wang, G. G. (2010). Covalent histone modifications--miswritten, misinterpreted and mis-erased in human cancers. *Nat Rev Cancer* **10**, 457-69.
Cinalli, R. M., Rangan, P. and Lehmann, R. (2008). Germ cells are forever. *Cell* **132**, 559-62.
Clapier, C. R. and Cairns, B. R. (2009). The biology of chromatin remodeling complexes. *Annu Rev Biochem* **78**, 273-304.
Cler, E., Papai, G., Schultz, P. and Davidson, I. (2009). Recent advances in understanding the structure and function of general transcription factor TFIID. *Cell Mol Life Sci* **66**, 2123-34.
Courtot, C., Fankhauser, C., Simanis, V. and Lehner, C. F. (1992). The Drosophila cdc25 homolog twine is required for meiosis. *Development* **116**, 405-16.
Crea, F., Hurt, E. M., Mathews, L. A., Cabarcas, S. M., Sun, L., Marquez, V. E., Danesi, R. and Farrar, W. L. (2011). Pharmacologic disruption of Polycomb Repressive Complex 2 inhibits tumorigenicity and tumor progression in prostate cancer. *Mol Cancer* **10**, 40.
Czermin, B., Melfi, R., McCabe, D., Seitz, V., Imhof, A. and Pirrotta, V. (2002). Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. *Cell* **111**, 185-96.
de Cuevas, M. and Matunis, E. L. (2011). The stem cell niche: lessons from the Drosophila testis. *Development* **138**, 2861-9.
De Santa, F., Totaro, M. G., Prosperini, E., Notarbartolo, S., Testa, G. and Natoli, G. (2007). The histone H3 lysine-27 demethylase Jmjd3 links inflammation to inhibition of polycomb-mediated gene silencing. *Cell* **130**, 1083-94.
Dellino, G. I., Schwartz, Y. B., Farkas, G., McCabe, D., Elgin, S. C. and Pirrotta, V. (2004). Polycomb silencing blocks transcription initiation. *Mol Cell* **13**, 887-93.

Deng, W. and Lin, H. (1997). Spectrosomes and fusomes anchor mitotic spindles during asymmetric germ cell divisions and facilitate the formation of a polarized microtubule array for oocyte specification in Drosophila. *Dev Biol* **189**, 79-94.
Dietzel, S., Niemann, H., Bruckner, B., Maurange, C. and Paro, R. (1999). The nuclear distribution of Polycomb during Drosophila melanogaster development shown with a GFP fusion protein. *Chromosoma* **108**, 83-94.
Dinardo, S., Okegbe, T., Wingert, L., Freilich, S. and Terry, N. (2011). lines and bowl affect the specification of cyst stem cells and niche cells in the Drosophila testis. *Development* **138**, 1687-96.
Duesberg, P., Fabarius, A. and Hehlmann, R. (2004). Aneuploidy, the primary cause of the multilateral genomic instability of neoplastic and preneoplastic cells. *IUBMB Life* **56**, 65-81.
Eberhart, C. G., Maines, J. Z. and Wasserman, S. A. (1996). Meiotic cell cycle requirement for a fly homologue of human Deleted in Azoospermia. *Nature* **381**, 783-5.
Eissenberg, J. C., Lee, M. G., Schneider, J., Ilvarsonn, A., Shiekhattar, R. and Shilatifard, A. (2007). The trithorax-group gene in Drosophila little imaginal discs encodes a trimethylated histone H3 Lys4 demethylase. *Nat Struct Mol Biol* **14**, 344-6.
Eliazer, S., Palacios, V., Wang, Z., Kollipara, R. K., Kittler, R. and Buszczak, M. (2014). Lsd1 restricts the number of germline stem cells by regulating multiple targets in escort cells. *PLoS Genet* **10**, e1004200.
Eliazer, S., Shalaby, N. A. and Buszczak, M. (2011). Loss of lysine-specific demethylase 1 nonautonomously causes stem cell tumors in the Drosophila ovary. *Proc Natl Acad Sci U S A* **108**, 7064-9.
Eramo, A., Lotti, F., Sette, G., Pilozzi, E., Biffoni, M., Di Virgilio, A., Conticello, C., Ruco, L., Peschle, C. and De Maria, R. (2008). Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death Differ* **15**, 504-14.
Esteller, M. (2008). Epigenetics in cancer. *N Engl J Med* **358**, 1148-59.
Eun, S. H., Gan, Q. and Chen, X. (2010). Epigenetic regulation of germ cell differentiation. *Curr Opin Cell Biol* **22**, 737-43.
Ezhkova, E., Lien, W. H., Stokes, N., Pasolli, H. A., Silva, J. M. and Fuchs, E. (2011). EZH1 and EZH2 cogovern histone H3K27 trimethylation and are essential for hair follicle homeostasis and wound repair. *Genes Dev* **25**, 485-98.
Feinberg, A. P. and Vogelstein, B. (1983). Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature* **301**, 89-92.
Flaherty, M. S., Salis, P., Evans, C. J., Ekas, L. A., Marouf, A., Zavadil, J., Banerjee, U. and Bach, E. A. chinmo is a functional effector of the JAK/STAT pathway that regulates eye development, tumor formation, and stem cell self-renewal in Drosophila. *Dev Cell* **18**, 556-68.
Francis, N. J., Kingston, R. E. and Woodcock, C. L. (2004). Chromatin compaction by a polycomb group protein complex. *Science* **306**, 1574-7.
Friedlander, D. R., Mege, R. M., Cunningham, B. A. and Edelman, G. M. (1989). Cell sorting-out is modulated by both the specificity and amount of different cell adhesion molecules (CAMs) expressed on cell surfaces. *Proc Natl Acad Sci U S A* **86**, 7043-7.
Gallagher, S. J., Kofman, A. E., Huszar, J. M., Dannenberg, J. H., DePinho, R. A., Braun, R. E. and Payne, C. J. (2013). Distinct requirements for Sin3a in perinatal male gonocytes and differentiating spermatogonia. *Dev Biol* **373**, 83-94.
Gama-Sosa, M. A., Slagel, V. A., Trewyn, R. W., Oxenhandler, R., Kuo, K. C., Gehrke, C. W. and Ehrlich, M. (1983). The 5-methylcytosine content of DNA from human tumors. *Nucleic Acids Res* **11**, 6883-94.
Gan, Q., Chepelev, I., Wei, G., Tarayrah, L., Cui, K., Zhao, K. and Chen, X. Dynamic regulation of alternative splicing and chromatin structure in Drosophila gonads revealed by RNA-seq. *Cell Res* **20**, 763-83.

Gan, Q., Chepelev, I., Wei, G., Tarayrah, L., Cui, K., Zhao, K. and Chen, X. (2010a). Dynamic regulation of alternative splicing and chromatin structure in Drosophila gonads revealed by RNA-seq. *Cell Res* **20**, 763-83.
Gan, Q., Schones, D. E., Ho Eun, S., Wei, G., Cui, K., Zhao, K. and Chen, X. Monovalent and unpoised status of most genes in undifferentiated cell-enriched Drosophila testis. *Genome Biol* **11**, R42.
Gan, Q., Schones, D. E., Ho Eun, S., Wei, G., Cui, K., Zhao, K. and Chen, X. (2010b). Monovalent and unpoised status of most genes in undifferentiated cell-enriched Drosophila testis. *Genome Biol* **11**, R42.
Gildea, J. J., Lopez, R. and Shearn, A. (2000). A screen for new trithorax group genes identified little imaginal discs, the Drosophila melanogaster homologue of human retinoblastoma binding protein 2. *Genetics* **156**, 645-63.
Goelz, S. E., Vogelstein, B., Hamilton, S. R. and Feinberg, A. P. (1985). Hypomethylation of DNA from benign and malignant human colon neoplasms. *Science* **228**, 187-90.
Gonczy, P. and DiNardo, S. (1996). The germ line regulates somatic cyst cell proliferation and fate during Drosophila spermatogenesis. *Development* **122**, 2437-47.
Gonczy, P., Matunis, E. and DiNardo, S. (1997). bag-of-marbles and benign gonial cell neoplasm act in the germline to restrict proliferation during Drosophila spermatogenesis. *Development* **124**, 4361-71.
Gonzalez-Zulueta, M., Bender, C. M., Yang, A. S., Nguyen, T., Beart, R. W., Van Tornout, J. M. and Jones, P. A. (1995). Methylation of the 5' CpG island of the p16/CDKN2 tumor suppressor gene in normal and transformed human tissues correlates with gene silencing. *Cancer Res* **55**, 4531-5.
Gould-Somero M., H. L. (1974). The timing of RNA synthesis for spermiogenesis in organ cultures of Drosophila melanogaster testes. . *Wilhelm Roux's Arch Dev Biol* **174**, 133-148.
Greco, V., Chen, T., Rendl, M., Schober, M., Pasolli, H. A., Stokes, N., Dela Cruz-Racelis, J. and Fuchs, E. (2009). A two-step mechanism for stem cell activation during hair regeneration. *Cell Stem Cell* **4**, 155-69.
Greer, E. L., Maures, T. J., Hauswirth, A. G., Green, E. M., Leeman, D. S., Maro, G. S., Han, S., Banko, M. R., Gozani, O. and Brunet, A. (2010). Members of the H3K4 trimethylation complex regulate lifespan in a germline-dependent manner in C. elegans. *Nature* **466**, 383-7.
Guenther, M. G., Levine, S. S., Boyer, L. A., Jaenisch, R. and Young, R. A. (2007). A chromatin landmark and transcription initiation at most promoters in human cells. *Cell* **130**, 77-88.
Hanahan, D. and Weinberg, R. A. (2000). The hallmarks of cancer. *Cell* **100**, 57-70.
Heddleston, J. M., Li, Z., McLendon, R. E., Hjelmeland, A. B. and Rich, J. N. (2009). The hypoxic microenvironment maintains glioblastoma stem cells and promotes reprogramming towards a cancer stem cell phenotype. *Cell Cycle* **8**, 3274-84.
Heddleston, J. M., Wu, Q., Rivera, M., Minhas, S., Lathia, J. D., Sloan, A. E., Iliopoulos, O., Hjelmeland, A. B. and Rich, J. N. (2012). Hypoxia-induced mixed-lineage leukemia 1 regulates glioma stem cell tumorigenic potential. *Cell Death Differ* **19**, 428-39.
Heiman, M., Schaefer, A., Gong, S., Peterson, J. D., Day, M., Ramsey, K. E., Suarez-Farinas, M., Schwarz, C., Stephan, D. A., Surmeier, D. J. et al. (2008). A translational profiling approach for the molecular characterization of CNS cell types. *Cell* **135**, 738-48.
Herz, H. M., Madden, L. D., Chen, Z., Bolduc, C., Buff, E., Gupta, R., Davuluri, R., Shilatifard, A., Hariharan, I. K. and Bergmann, A. (2010). The H3K27me3 demethylase dUTX is a suppressor of Notch- and Rb-dependent tumors in Drosophila. *Mol Cell Biol* **30**, 2485-97.
Hiller, M., Chen, X., Pringle, M. J., Suchorolski, M., Sancak, Y., Viswanathan, S., Bolival, B., Lin, T. Y., Marino, S. and Fuller, M. T. (2004). Testis-specific TAF homologs collaborate to control a tissue-specific transcription program. *Development* **131**, 5297-308.
Hiller, M. A., Lin, T. Y., Wood, C. and Fuller, M. T. (2001). Developmental regulation of transcription by a tissue-specific TAF homolog. *Genes Dev* **15**, 1021-30.

Hou, S. X., Zheng, Z., Chen, X. and Perrimon, N. (2002). The Jak/STAT pathway in model organisms: emerging roles in cell movement. *Dev Cell* **3**, 765-78.
Hou, X. S., Melnick, M. B. and Perrimon, N. (1996). Marelle acts downstream of the Drosophila HOP/JAK kinase and encodes a protein similar to the mammalian STATs. *Cell* **84**, 411-9.
Hsu, C. H., Peng, K. L., Kang, M. L., Chen, Y. R., Yang, Y. C., Tsai, C. H., Chu, C. S., Jeng, Y. M., Chen, Y. T., Lin, F. M. et al. (2012). TET1 suppresses cancer invasion by activating the tissue inhibitors of metalloproteinases. *Cell Rep* **2**, 568-79.
Huang, H., Jiang, X., Li, Z., Li, Y., Song, C. X., He, C., Sun, M., Chen, P., Gurbuxani, S., Wang, J. et al. (2013a). TET1 plays an essential oncogenic role in MLL-rearranged leukemia. *Proc Natl Acad Sci U S A* **110**, 11994-9.
Huang, H., Jiang, X., Li, Z., Li, Y., Song, C. X., He, C., Sun, M., Chen, P., Gurbuxani, S., Wang, J. et al. (2013b). TET1 plays an essential oncogenic role in MLL-rearranged leukemia. *Proc Natl Acad Sci U S A*.
Inaba, M. and Yamashita, Y. M. (2012). Asymmetric stem cell division: precision for robustness. *Cell Stem Cell* **11**, 461-9.
Inaba, M., Yuan, H., Salzmann, V., Fuller, M. T. and Yamashita, Y. M. (2010). E-cadherin is required for centrosome and spindle orientation in Drosophila male germline stem cells. *PLoS One* **5**, e12473.
Insco, M. L., Leon, A., Tam, C. H., McKearin, D. M. and Fuller, M. T. (2009). Accumulation of a differentiation regulator specifies transit amplifying division number in an adult stem cell lineage. *Proc Natl Acad Sci U S A* **106**, 22311-6.
Issaeva, I., Zonis, Y., Rozovskaia, T., Orlovsky, K., Croce, C. M., Nakamura, T., Mazo, A., Eisenbach, L. and Canaani, E. (2007). Knockdown of ALR (MLL2) reveals ALR target genes and leads to alterations in cell adhesion and growth. *Mol Cell Biol* **27**, 1889-903.
Issigonis, M., Tulina, N., de Cuevas, M., Brawley, C., Sandler, L. and Matunis, E. (2009). JAK-STAT signal inhibition regulates competition in the Drosophila testis stem cell niche. *Science* **326**, 153-6.
Ito, S., D'Alessio, A. C., Taranova, O. V., Hong, K., Sowers, L. C. and Zhang, Y. (2010). Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. *Nature* **466**, 1129-33.
Jenkins, A. B., McCaffery, J. M. and Van Doren, M. (2003). Drosophila E-cadherin is essential for proper germ cell-soma interaction during gonad morphogenesis. *Development* **130**, 4417-26.
Jiang, H., Patel, P. H., Kohlmaier, A., Grenley, M. O., McEwen, D. G. and Edgar, B. A. (2009). Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the Drosophila midgut. *Cell* **137**, 1343-55.
Jiang, J., Benson, E., Bausek, N., Doggett, K. and White-Cooper, H. (2007). Tombola, a tesmin/TSO1-family protein, regulates transcriptional activation in the Drosophila male germline and physically interacts with always early. *Development* **134**, 1549-59.
Jiang, J. and White-Cooper, H. (2003). Transcriptional activation in Drosophila spermatogenesis involves the mutually dependent function of aly and a novel meiotic arrest gene cookie monster. *Development* **130**, 563-73.
Johnston, L. A., Prober, D. A., Edgar, B. A., Eisenman, R. N. and Gallant, P. (1999). Drosophila myc regulates cellular growth during development. *Cell* **98**, 779-90.
Jones, P. A. and Baylin, S. B. (2007). The epigenomics of cancer. *Cell* **128**, 683-92.
Jones, T. D., Ulbright, T. M., Eble, J. N., Baldridge, L. A. and Cheng, L. (2004a). OCT4 staining in testicular tumors: a sensitive and specific marker for seminoma and embryonal carcinoma. *Am J Surg Pathol* **28**, 935-40.
Jones, T. D., Ulbright, T. M., Eble, J. N. and Cheng, L. (2004b). OCT4: A sensitive and specific biomarker for intratubular germ cell neoplasia of the testis. *Clin Cancer Res* **10**, 8544-7.
Jordan, C. T., Guzman, M. L. and Noble, M. (2006). Cancer stem cells. *N Engl J Med* **355**, 1253-61.

Karpiuk, O., Najafova, Z., Kramer, F., Hennion, M., Galonska, C., Konig, A., Snaidero, N., Vogel, T., Shchebet, A., Begus-Nahrmann, Y. et al. (2012). The histone H2B monoubiquitination regulatory pathway is required for differentiation of multipotent stem cells. *Mol Cell* **46**, 705-13.
Kawase, E., Wong, M. D., Ding, B. C. and Xie, T. (2004). Gbb/Bmp signaling is essential for maintaining germline stem cells and for repressing bam transcription in the Drosophila testis. *Development* **131**, 1365-75.
Kiger, A. A., Jones, D. L., Schulz, C., Rogers, M. B. and Fuller, M. T. (2001). Stem cell self-renewal specified by JAK-STAT activation in response to a support cell cue. *Science* **294**, 2542-5.
Kiger, A. A., White-Cooper, H. and Fuller, M. T. (2000). Somatic support cells restrict germline stem cell self-renewal and promote differentiation. *Nature* **407**, 750-4.
Kleer, C. G., Cao, Q., Varambally, S., Shen, R., Ota, I., Tomlins, S. A., Ghosh, D., Sewalt, R. G., Otte, A. P., Hayes, D. F. et al. (2003). EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc Natl Acad Sci U S A* **100**, 11606-11.
Klose, R. J., Kallin, E. M. and Zhang, Y. (2006). JmjC-domain-containing proteins and histone demethylation. *Nat Rev Genet* **7**, 715-27.
Ko, M., Huang, Y., Jankowska, A. M., Pape, U. J., Tahiliani, M., Bandukwala, H. S., An, J., Lamperti, E. D., Koh, K. P., Ganetzky, R. et al. (2010). Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. *Nature* **468**, 839-43.
Kondo, Y., Shen, L., Cheng, A. S., Ahmed, S., Boumber, Y., Charo, C., Yamochi, T., Urano, T., Furukawa, K., Kwabi-Addo, B. et al. (2008). Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation. *Nat Genet* **40**, 741-50.
Kouzarides, T. (2007). Chromatin modifications and their function. *Cell* **128**, 693-705.
Krishnamoorthy, T., Chen, X., Govin, J., Cheung, W. L., Dorsey, J., Schindler, K., Winter, E., Allis, C. D., Guacci, V., Khochbin, S. et al. (2006). Phosphorylation of histone H4 Ser1 regulates sporulation in yeast and is conserved in fly and mouse spermatogenesis. *Genes Dev* **20**, 2580-92.
Kuzmichev, A., Nishioka, K., Erdjument-Bromage, H., Tempst, P. and Reinberg, D. (2002). Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. *Genes Dev* **16**, 2893-905.
Lan, F., Bayliss, P. E., Rinn, J. L., Whetstine, J. R., Wang, J. K., Chen, S., Iwase, S., Alpatov, R., Issaeva, I., Canaani, E. et al. (2007). A histone H3 lysine 27 demethylase regulates animal posterior development. *Nature* **449**, 689-94.
Le Bras, S. and Van Doren, M. (2006). Development of the male germline stem cell niche in Drosophila. *Dev Biol* **294**, 92-103.
Leatherman, J. L. and Dinardo, S. Germline self-renewal requires cyst stem cells and stat regulates niche adhesion in Drosophila testes. *Nat Cell Biol* **12**, 806-11.
Leatherman, J. L. and Dinardo, S. (2008). Zfh-1 controls somatic stem cell self-renewal in the Drosophila testis and nonautonomously influences germline stem cell self-renewal. *Cell Stem Cell* **3**, 44-54.
Leatherman, J. L. and Dinardo, S. (2010). Germline self-renewal requires cyst stem cells and stat regulates niche adhesion in Drosophila testes. *Nat Cell Biol* **12**, 806-11.
Lee, K. S., Yoon, J., Park, J. S. and Kang, Y. K. (2010). Drosophila G9a is implicated in germ cell development. *Insect Mol Biol* **19**, 131-9.
Lee, N., Zhang, J., Klose, R. J., Erdjument-Bromage, H., Tempst, P., Jones, R. S. and Zhang, Y. (2007). The trithorax-group protein Lid is a histone H3 trimethyl-Lys4 demethylase. *Nat Struct Mol Biol* **14**, 341-3.
Lee, S. H., Kim, I. J., Kim, J. G., Park, J. S., Kim, Y. S., Yamaguchi, M., Kim, C. M. and Yoo, M. A. (2011). Regulation of intestinal stem cell proliferation by human methyl-CpG-binding protein-2 in Drosophila. *Cell Struct Funct* **36**, 197-208.

Lee, T. and Luo, L. (1999). Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. *Neuron* **22**, 451-61.
Leedham, S. J., Thliveris, A. T., Halberg, R. B., Newton, M. A. and Wright, N. A. (2005). Gastrointestinal stem cells and cancer: bridging the molecular gap. *Stem Cell Rev* **1**, 233-41.
Lessard, J. and Sauvageau, G. (2003). Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. *Nature* **423**, 255-60.
Li, G., Margueron, R., Ku, M., Chambon, P., Bernstein, B. E. and Reinberg, D. (2010a). Jarid2 and PRC2, partners in regulating gene expression. *Genes Dev* **24**, 368-80.
Li, L., Greer, C., Eisenman, R. N. and Secombe, J. (2010b). Essential functions of the histone demethylase lid. *PLoS Genet* **6**, e1001221.
Li, M. A., Alls, J. D., Avancini, R. M., Koo, K. and Godt, D. (2003). The large Maf factor Traffic Jam controls gonad morphogenesis in Drosophila. *Nat Cell Biol* **5**, 994-1000.
Li, T. and Kelly, W. G. (2011). A role for Set1/MLL-related components in epigenetic regulation of the Caenorhabditis elegans germ line. *PLoS Genet* **7**, e1001349.
Li, X., Lewis, M. T., Huang, J., Gutierrez, C., Osborne, C. K., Wu, M. F., Hilsenbeck, S. G., Pavlick, A., Zhang, X., Chamness, G. C. et al. (2008). Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. *J Natl Cancer Inst* **100**, 672-9.
Li, X. and Zhao, X. (2008). Epigenetic regulation of mammalian stem cells. *Stem Cells Dev* **17**, 1043-52.
Li, Y., Minor, N. T., Park, J. K., McKearin, D. M. and Maines, J. Z. (2009a). Bam and Bgcn antagonize Nanos-dependent germ-line stem cell maintenance. *Proc Natl Acad Sci U S A* **106**, 9304-9.
Li, Z., Bao, S., Wu, Q., Wang, H., Eyler, C., Sathornsumetee, S., Shi, Q., Cao, Y., Lathia, J., McLendon, R. E. et al. (2009b). Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. *Cancer Cell* **15**, 501-13.
Li, Z. G., Jiao, Y., Li, W. J., Deng, G. R., Cui, L., Gao, C., Zhao, X. X., Wu, M. Y. and Jia, H. T. (2013). Hypermethylation of two CpG sites upstream of CASP8AP2 promoter influences gene expression and treatment outcome in childhood acute lymphoblastic leukemia. *Leuk Res*.
Lian, C. G., Xu, Y., Ceol, C., Wu, F., Larson, A., Dresser, K., Xu, W., Tan, L., Hu, Y., Zhan, Q. et al. (2012). Loss of 5-hydroxymethylcytosine is an epigenetic hallmark of melanoma. *Cell* **150**, 1135-46.
Lien, W. H., Guo, X., Polak, L., Lawton, L. N., Young, R. A., Zheng, D. and Fuchs, E. (2011). Genome-wide maps of histone modifications unwind in vivo chromatin states of the hair follicle lineage. *Cell Stem Cell* **9**, 219-32.
Lim, C., Tarayrah, L. and Chen, X. (2012). Transcriptional regulation during Drosophila spermatogenesis. *Spermatogenesis* **2**, 158-166.
Lin, G., Xu, N. and Xi, R. (2008). Paracrine Wingless signalling controls self-renewal of Drosophila intestinal stem cells. *Nature* **455**, 1119-23.
Lin, G., Xu, N. and Xi, R. (2010). Paracrine unpaired signaling through the JAK/STAT pathway controls self-renewal and lineage differentiation of drosophila intestinal stem cells. *J Mol Cell Biol* **2**, 37-49.
Lin, T. Y., Viswanathan, S., Wood, C., Wilson, P. G., Wolf, N. and Fuller, M. T. (1996). Coordinate developmental control of the meiotic cell cycle and spermatid differentiation in Drosophila males. *Development* **122**, 1331-41.
Liu, M., Lim, T. M. and Cai, Y. (2010). The Drosophila female germline stem cell lineage acts to spatially restrict DPP function within the niche. *Sci Signal* **3**, ra57.
Lopez-Onieva, L., Fernandez-Minan, A. and Gonzalez-Reyes, A. (2008). Jak/Stat signalling in niche support cells regulates dpp transcription to control germline stem cell maintenance in the Drosophila ovary. *Development* **135**, 533-40.
Losick, V. P., Morris, L. X., Fox, D. T. and Spradling, A. (2011). Drosophila stem cell niches: a decade of discovery suggests a unified view of stem cell regulation. *Dev Cell* **21**, 159-71.

Lyko, F., Ramsahoye, B. H. and Jaenisch, R. (2000). DNA methylation in Drosophila melanogaster. *Nature* **408**, 538-40.
Ma, Y., Chen, Z., Jin, Y. and Liu, W. (2013). Identification of a histone acetyltransferase as a novel regulator of Drosophila intestinal stem cells. *FEBS Lett* **587**, 1489-95.
Maines, J. Z. and Wasserman, S. A. (1999). Post-transcriptional regulation of the meiotic Cdc25 protein Twine by the Dazl orthologue Boule. *Nat Cell Biol* **1**, 171-4.
Makishima, H., Jankowska, A. M., McDevitt, M. A., O'Keefe, C., Dujardin, S., Cazzolli, H., Przychodzen, B., Prince, C., Nicoll, J., Siddaiah, H. et al. (2011). CBL, CBLB, TET2, ASXL1, and IDH1/2 mutations and additional chromosomal aberrations constitute molecular events in chronic myelogenous leukemia. *Blood* **117**, e198-206.
Malanchi, I., Santamaria-Martinez, A., Susanto, E., Peng, H., Lehr, H. A., Delaloye, J. F. and Huelsken, J. (2012). Interactions between cancer stem cells and their niche govern metastatic colonization. *Nature* **481**, 85-9.
Manseau, L., Baradaran, A., Brower, D., Budhu, A., Elefant, F., Phan, H., Philp, A. V., Yang, M., Glover, D., Kaiser, K. et al. (1997). GAL4 enhancer traps expressed in the embryo, larval brain, imaginal discs, and ovary of Drosophila. *Dev Dyn* **209**, 310-22.
Mansour, A. A., Gafni, O., Weinberger, L., Zviran, A., Ayyash, M., Rais, Y., Krupalnik, V., Zerbib, M., Amann-Zalcenstein, D., Maza, I. et al. (2012). The H3K27 demethylase Utx regulates somatic and germ cell epigenetic reprogramming. *Nature* **488**, 409-13.
Mardis, E. R., Ding, L., Dooling, D. J., Larson, D. E., McLellan, M. D., Chen, K., Koboldt, D. C., Fulton, R. S., Delehaunty, K. D., McGrath, S. D. et al. (2009). Recurring mutations found by sequencing an acute myeloid leukemia genome. *N Engl J Med* **361**, 1058-66.
Matangkasombut, O., Auty, R. and Buratowski, S. (2004). Structure and function of the TFIID complex. *Adv Protein Chem* **67**, 67-92.
McKearin, D. M. and Spradling, A. C. (1990). bag-of-marbles: a Drosophila gene required to initiate both male and female gametogenesis. *Genes Dev* **4**, 2242-51.
Mejetta, S., Morey, L., Pascual, G., Kuebler, B., Mysliwiec, M. R., Lee, Y., Shiekhattar, R., Di Croce, L. and Benitah, S. A. (2011). Jarid2 regulates mouse epidermal stem cell activation and differentiation. *EMBO J* **30**, 3635-46.
Metcalf, C. E. and Wassarman, D. A. (2007). Nucleolar colocalization of TAF1 and testis-specific TAFs during Drosophila spermatogenesis. *Dev Dyn* **236**, 2836-43.
Metzger, E., Wissmann, M., Yin, N., Muller, J. M., Schneider, R., Peters, A. H., Gunther, T., Buettner, R. and Schule, R. (2005). LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. *Nature* **437**, 436-9.
Micchelli, C. A. and Perrimon, N. (2006). Evidence that stem cells reside in the adult Drosophila midgut epithelium. *Nature* **439**, 475-9.
Molofsky, A. V., Pardal, R., Iwashita, T., Park, I. K., Clarke, M. F. and Morrison, S. J. (2003). Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. *Nature* **425**, 962-7.
Monk, A. C., Siddall, N. A., Volk, T., Fraser, B., Quinn, L. M., McLaughlin, E. A. and Hime, G. R. (2010). HOW is required for stem cell maintenance in the Drosophila testis and for the onset of transit-amplifying divisions. *Cell Stem Cell* **6**, 348-60.
Morris, R. J. (2000). Keratinocyte stem cells: targets for cutaneous carcinogens. *J Clin Invest* **106**, 3-8.
Morrison, S. J., Qian, D., Jerabek, L., Thiel, B. A., Park, I. K., Ford, P. S., Kiel, M. J., Schork, N. J., Weissman, I. L. and Clarke, M. F. (2002). A genetic determinant that specifically regulates the frequency of hematopoietic stem cells. *J Immunol* **168**, 635-42.

Morrison, S. J. and Spradling, A. C. (2008). Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. *Cell* **132**, 598-611.
Muller, J., Hart, C. M., Francis, N. J., Vargas, M. L., Sengupta, A., Wild, B., Miller, E. L., O'Connor, M. B., Kingston, R. E. and Simon, J. A. (2002). Histone methyltransferase activity of a Drosophila Polycomb group repressor complex. *Cell* **111**, 197-208.
Nakamura, N. and Takenaga, K. (1998). Hypomethylation of the metastasis-associated S100A4 gene correlates with gene activation in human colon adenocarcinoma cell lines. *Clin Exp Metastasis* **16**, 471-9.
Nan, X., Ng, H. H., Johnson, C. A., Laherty, C. D., Turner, B. M., Eisenman, R. N. and Bird, A. (1998). Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* **393**, 386-9.
Ni, J. Q., Zhou, R., Czech, B., Liu, L. P., Holderbaum, L., Yang-Zhou, D., Shim, H. S., Tao, R., Handler, D., Karpowicz, P. et al. (2011). A genome-scale shRNA resource for transgenic RNAi in Drosophila. *Nat Methods* **8**, 405-7.
Nishigaki, M., Aoyagi, K., Danjoh, I., Fukaya, M., Yanagihara, K., Sakamoto, H., Yoshida, T. and Sasaki, H. (2005). Discovery of aberrant expression of R-RAS by cancer-linked DNA hypomethylation in gastric cancer using microarrays. *Cancer Res* **65**, 2115-24.
Nishimura, E. K., Suzuki, M., Igras, V., Du, J., Lonning, S., Miyachi, Y., Roes, J., Beermann, F. and Fisher, D. E. (2010). Key roles for transforming growth factor beta in melanocyte stem cell maintenance. *Cell Stem Cell* **6**, 130-40.
O'Brien, C. A., Pollett, A., Gallinger, S. and Dick, J. E. (2007). A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* **445**, 106-10.
Oatley, M. J., Racicot, K. E. and Oatley, J. M. (2011). Sertoli cells dictate spermatogonial stem cell niches in the mouse testis. *Biol Reprod* **84**, 639-45.
Ohlstein, B. and Spradling, A. (2006). The adult Drosophila posterior midgut is maintained by pluripotent stem cells. *Nature* **439**, 470-4.
Ohlstein, B. and Spradling, A. (2007). Multipotent Drosophila intestinal stem cells specify daughter cell fates by differential notch signaling. *Science* **315**, 988-92.
Oliveri G, O. A. (1965). Autoradiographic study of nucleic acid synthesis during spermatogenesis in Drosophila melanogaster. . *Genetics* **125**, 535-550.
Park, I. K., Qian, D., Kiel, M., Becker, M. W., Pihalja, M., Weissman, I. L., Morrison, S. J. and Clarke, M. F. (2003). Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature* **423**, 302-5.
Parrott, B. B., Chiang, Y., Hudson, A., Sarkar, A., Guichet, A. and Schulz, C. (2011). Nucleoporin98-96 function is required for transit amplification divisions in the germ line of Drosophila melanogaster. *PLoS One* **6**, e25087.
Parrott, B. B., Hudson, A., Brady, R. and Schulz, C. (2012). Control of germline stem cell division frequency - a novel, developmentally regulated role for epidermal growth factor signaling. *PLoS One* **7**, e36460.
Pasini, D., Cloos, P. A., Walfridsson, J., Olsson, L., Bukowski, J. P., Johansen, J. V., Bak, M., Tommerup, N., Rappsilber, J. and Helin, K. (2010). JARID2 regulates binding of the Polycomb repressive complex 2 to target genes in ES cells. *Nature* **464**, 306-10.
Payne, C. J., Gallagher, S. J., Foreman, O., Dannenberg, J. H., Depinho, R. A. and Braun, R. E. (2010). Sin3a is required by sertoli cells to establish a niche for undifferentiated spermatogonia, germ cell tumors, and spermatid elongation. *Stem Cells* **28**, 1424-34.
Pek, J. W., Lim, A. K. and Kai, T. (2009). Drosophila maelstrom ensures proper germline stem cell lineage differentiation by repressing microRNA-7. *Dev Cell* **17**, 417-24.

Perezgasga, L., Jiang, J., Bolival, B., Jr., Hiller, M., Benson, E., Fuller, M. T. and White-Cooper, H. (2004). Regulation of transcription of meiotic cell cycle and terminal differentiation genes by the testis-specific Zn-finger protein matotopetli. *Development* **131**, 1691-702.

Prince, M. E., Sivanandan, R., Kaczorowski, A., Wolf, G. T., Kaplan, M. J., Dalerba, P., Weissman, I. L., Clarke, M. F. and Ailles, L. E. (2007). Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc Natl Acad Sci U S A* **104**, 973-8.

Pui, C. H., Chessells, J. M., Camitta, B., Baruchel, A., Biondi, A., Boyett, J. M., Carroll, A., Eden, O. B., Evans, W. E., Gadner, H. et al. (2003). Clinical heterogeneity in childhood acute lymphoblastic leukemia with 11q23 rearrangements. *Leukemia* **17**, 700-6.

Rabbani, P., Takeo, M., Chou, W., Myung, P., Bosenberg, M., Chin, L., Taketo, M. M. and Ito, M. (2011). Coordinated activation of Wnt in epithelial and melanocyte stem cells initiates pigmented hair regeneration. *Cell* **145**, 941-55.

Rangan, P., Malone, C. D., Navarro, C., Newbold, S. P., Hayes, P. S., Sachidanandam, R., Hannon, G. J. and Lehmann, R. (2011). piRNA production requires heterochromatin formation in Drosophila. *Curr Biol* **21**, 1373-9.

Rathke, C., Baarends, W. M., Jayaramaiah-Raja, S., Bartkuhn, M., Renkawitz, R. and Renkawitz-Pohl, R. (2007). Transition from a nucleosome-based to a protamine-based chromatin configuration during spermiogenesis in Drosophila. *J Cell Sci* **120**, 1689-700.

Reynard, L. N., Turner, J. M., Cocquet, J., Mahadevaiah, S. K., Toure, A., Hoog, C. and Burgoyne, P. S. (2007). Expression analysis of the mouse multi-copy X-linked gene Xlr-related, meiosis-regulated (Xmr), reveals that Xmr encodes a spermatid-expressed cytoplasmic protein, SLX/XMR. *Biol Reprod* **77**, 329-35.

Ringrose, L. and Paro, R. (2007). Polycomb/Trithorax response elements and epigenetic memory of cell identity. *Development* **134**, 223-32.

Rizzo, S., Hersey, J. M., Mellor, P., Dai, W., Santos-Silva, A., Liber, D., Luk, L., Titley, I., Carden, C. P., Box, G. et al. (2011). Ovarian cancer stem cell-like side populations are enriched following chemotherapy and overexpress EZH2. *Mol Cancer Ther* **10**, 325-35.

Rodriguez-Paredes, M. and Esteller, M. (2011). Cancer epigenetics reaches mainstream oncology. *Nat Med* **17**, 330-9.

Sakai, T., Toguchida, J., Ohtani, N., Yandell, D. W., Rapaport, J. M. and Dryja, T. P. (1991). Allele-specific hypermethylation of the retinoblastoma tumor-suppressor gene. *Am J Hum Genet* **48**, 880-8.

Sarkar, A., Parikh, N., Hearn, S. A., Fuller, M. T., Tazuke, S. I. and Schulz, C. (2007). Antagonistic roles of Rac and Rho in organizing the germ cell microenvironment. *Curr Biol* **17**, 1253-8.

Sarmento, O. F., Digilio, L. C., Wang, Y., Perlin, J., Herr, J. C., Allis, C. D. and Coonrod, S. A. (2004). Dynamic alterations of specific histone modifications during early murine development. *J Cell Sci* **117**, 4449-59.

Saurin, A. J., Shao, Z. H., Erdjument-Bromage, H., Tempst, P. and Kingston, R. E. (2001). A Drosophila Polycomb group complex includes Zeste and dTAFII proteins. *Nature* **412**, 655-660.

Schneider, J. and Shilatifard, A. (2006). Histone demethylation by hydroxylation: chemistry in action. *ACS Chem Biol* **1**, 75-81.

Schones, D. E. and Zhao, K. (2008). Genome-wide approaches to studying chromatin modifications. *Nat Rev Genet* **9**, 179-91.

Schuettengruber, B., Chourrout, D., Vervoort, M., Leblanc, B. and Cavalli, G. (2007). Genome regulation by polycomb and trithorax proteins. *Cell* **128**, 735-45.

Schultz, N., Hamra, F. K. and Garbers, D. L. (2003). A multitude of genes expressed solely in meiotic or postmeiotic spermatogenic cells offers a myriad of contraceptive targets. *Proc Natl Acad Sci U S A* **100**, 12201-6.

Schulz, C. (2007). In situ hybridization to Drosophila testes. *CSH Protoc* **2007**, pdb prot4764.

Schulz, C., Kiger, A. A., Tazuke, S. I., Yamashita, Y. M., Pantalena-Filho, L. C., Jones, D. L., Wood, C. G. and Fuller, M. T. (2004). A misexpression screen reveals effects of bag-of-marbles and TGF beta class signaling on the Drosophila male germ-line stem cell lineage. *Genetics* **167**, 707-23.
Schulz, C., Wood, C. G., Jones, D. L., Tazuke, S. I. and Fuller, M. T. (2002). Signaling from germ cells mediated by the rhomboid homolog stet organizes encapsulation by somatic support cells. *Development* **129**, 4523-34.
Schwartz, Y. B. and Pirrotta, V. (2007). Polycomb silencing mechanisms and the management of genomic programmes. *Nat Rev Genet* **8**, 9-22.
Secombe, J. and Eisenman, R. N. (2007). The function and regulation of the JARID1 family of histone H3 lysine 4 demethylases: the Myc connection. *Cell Cycle* **6**, 1324-8.
Secombe, J., Li, L., Carlos, L. and Eisenman, R. N. (2007). The Trithorax group protein Lid is a trimethyl histone H3K4 demethylase required for dMyc-induced cell growth. *Genes Dev* **21**, 537-51.
Seidel, S., Garvalov, B. K., Wirta, V., von Stechow, L., Schanzer, A., Meletis, K., Wolter, M., Sommerlad, D., Henze, A. T., Nister, M. et al. (2010). A hypoxic niche regulates glioblastoma stem cells through hypoxia inducible factor 2 alpha. *Brain* **133**, 983-95.
Seligson, D. B., Horvath, S., Shi, T., Yu, H., Tze, S., Grunstein, M. and Kurdistani, S. K. (2005). Global histone modification patterns predict risk of prostate cancer recurrence. *Nature* **435**, 1262-6.
Sen, G. L., Webster, D. E., Barragan, D. I., Chang, H. Y. and Khavari, P. A. (2008). Control of differentiation in a self-renewing mammalian tissue by the histone demethylase JMJD3. *Genes Dev* **22**, 1865-70.
Sheng, X. R., Brawley, C. M. and Matunis, E. L. (2009). Dedifferentiating spermatogonia outcompete somatic stem cells for niche occupancy in the Drosophila testis. *Cell Stem Cell* **5**, 191-203.
Shi, Y. (2007). Histone lysine demethylases: emerging roles in development, physiology and disease. *Nat Rev Genet* **8**, 829-33.
Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J. R., Cole, P. A. and Casero, R. A. (2004). Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* **119**, 941-53.
Shipitsin, M. and Polyak, K. (2008). The cancer stem cell hypothesis: in search of definitions, markers, and relevance. *Lab Invest* **88**, 459-63.
Shivdasani, A. A. and Ingham, P. W. (2003). Regulation of stem cell maintenance and transit amplifying cell proliferation by tgf-beta signaling in Drosophila spermatogenesis. *Curr Biol* **13**, 2065-72.
Siddall, N. A., McLaughlin, E. A., Marriner, N. L. and Hime, G. R. (2006). The RNA-binding protein Musashi is required intrinsically to maintain stem cell identity. *Proc Natl Acad Sci U S A* **103**, 8402-7.
Singh, S. K., Hawkins, C., Clarke, I. D., Squire, J. A., Bayani, J., Hide, T., Henkelman, R. M., Cusimano, M. D. and Dirks, P. B. (2004). Identification of human brain tumour initiating cells. *Nature* **432**, 396-401.
Slominski, A. and Paus, R. (1993). Melanogenesis is coupled to murine anagen: toward new concepts for the role of melanocytes and the regulation of melanogenesis in hair growth. *J Invest Dermatol* **101**, 90S-97S.
Smith, E. R., Lee, M. G., Winter, B., Droz, N. M., Eissenberg, J. C., Shiekhattar, R. and Shilatifard, A. (2008). Drosophila UTX is a histone H3 Lys27 demethylase that colocalizes with the elongating form of RNA polymerase II. *Mol Cell Biol* **28**, 1041-6.
Steger, K. (2001). Haploid spermatids exhibit translationally repressed mRNAs. *Anat Embryol (Berl)* **203**, 323-34.
Steinberg, M. S. and Takeichi, M. (1994). Experimental specification of cell sorting, tissue spreading, and specific spatial patterning by quantitative differences in cadherin expression. *Proc Natl Acad Sci U S A* **91**, 206-9.

Stock, J. K., Giadrossi, S., Casanova, M., Brookes, E., Vidal, M., Koseki, H., Brockdorff, N., Fisher, A. G. and Pombo, A. (2007). Ring1-mediated ubiquitination of H2A restrains poised RNA polymerase II at bivalent genes in mouse ES cells. *Nat Cell Biol* **9**, 1428-35.

Strickland, S., Smith, K. K. and Marotti, K. R. (1980). Hormonal induction of differentiation in teratocarcinoma stem cells: generation of parietal endoderm by retinoic acid and dibutyryl cAMP. *Cell* **21**, 347-55.

Stupp, R., Hegi, M. E., Mason, W. P., van den Bent, M. J., Taphoorn, M. J., Janzer, R. C., Ludwin, S. K., Allgeier, A., Fisher, B., Belanger, K. et al. (2009). Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol* **10**, 459-66.

Suganuma, T., Gutierrez, J. L., Li, B., Florens, L., Swanson, S. K., Washburn, M. P., Abmayr, S. M. and Workman, J. L. (2008). ATAC is a double histone acetyltransferase complex that stimulates nucleosome sliding. *Nat Struct Mol Biol* **15**, 364-72.

Suganuma, T., Mushegian, A., Swanson, S. K., Abmayr, S. M., Florens, L., Washburn, M. P. and Workman, J. L. (2010). The ATAC acetyltransferase complex coordinates MAP kinases to regulate JNK target genes. *Cell* **142**, 726-36.

Sun, M., Song, C. X., Huang, H., Frankenberger, C. A., Sankarasharma, D., Gomes, S., Chen, P., Chen, J., Chada, K. K., He, C. et al. (2013). HMGA2/TET1/HOXA9 signaling pathway regulates breast cancer growth and metastasis. *Proc Natl Acad Sci U S A* **110**, 9920-5.

Surface, L. E., Thornton, S. R. and Boyer, L. A. (2010). Polycomb group proteins set the stage for early lineage commitment. *Cell Stem Cell* **7**, 288-98.

Tahiliani, M., Koh, K. P., Shen, Y., Pastor, W. A., Bandukwala, H., Brudno, Y., Agarwal, S., Iyer, L. M., Liu, D. R., Aravind, L. et al. (2009). Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science* **324**, 930-5.

Tarayrah, L. and Chen, X. (2013). Epigenetic regulation in adult stem cells and cancers. *Cell Biosci* **3**, 41.

Tarayrah, L., Herz, H. M., Shilatifard, A. and Chen, X. (2013). Histone demethylase dUTX antagonizes JAK-STAT signaling to maintain proper gene expression and architecture of the Drosophila testis niche. *Development* **140**, 1014-23.

Tazuke, S. I., Schulz, C., Gilboa, L., Fogarty, M., Mahowald, A. P., Guichet, A., Ephrussi, A., Wood, C. G., Lehmann, R. and Fuller, M. T. (2002). A germline-specific gap junction protein required for survival of differentiating early germ cells. *Development* **129**, 2529-39.

Terry, N. A., Tulina, N., Matunis, E. and DiNardo, S. (2006). Novel regulators revealed by profiling Drosophila testis stem cells within their niche. *Dev Biol* **294**, 246-57.

Tie, F., Banerjee, R., Conrad, P. A., Scacheri, P. C. and Harte, P. J. (2012). The histone demethylase UTX and the chromatin remodeler BRM bind directly to Drosophila CBP and modulate its acetylation of histone H3 lysine 27. *Mol Cell Biol*.

Toledano, H., D'Alterio, C., Czech, B., Levine, E. and Jones, D. L. (2012). The let-7-Imp axis regulates ageing of the Drosophila testis stem-cell niche. *Nature* **485**, 605-10.

Tora, L. (2002). A unified nomenclature for TATA box binding protein (TBP)-associated factors (TAFs) involved in RNA polymerase II transcription. *Genes Dev* **16**, 673-5.

Tran, J., Brenner, T. J. and DiNardo, S. (2000). Somatic control over the germline stem cell lineage during Drosophila spermatogenesis. *Nature* **407**, 754-7.

Tran, V., Feng, L. and Chen, X. (2013). Asymmetric distribution of histones during Drosophila male germline stem cell asymmetric divisions. *Chromosome Res* **21**, 255-69.

Tran, V., Lim, C., Xie, J. and Chen, X. (2012). Asymmetric division of Drosophila male germline stem cell shows asymmetric histone distribution. *Science* **338**, 679-82.

Trowbridge, J. J., Sinha, A. U., Zhu, N., Li, M., Armstrong, S. A. and Orkin, S. H. (2012). Haploinsufficiency of Dnmt1 impairs leukemia stem cell function through derepression of bivalent chromatin domains. *Genes Dev* **26**, 344-9.
Tulina, N. and Matunis, E. (2001). Control of stem cell self-renewal in Drosophila spermatogenesis by JAK-STAT signaling. *Science* **294**, 2546-9.
Valk-Lingbeek, M. E., Bruggeman, S. W. and van Lohuizen, M. (2004). Stem cells and cancer; the polycomb connection. *Cell* **118**, 409-18.
Van Doren, M., Williamson, A. L. and Lehmann, R. (1998). Regulation of zygotic gene expression in Drosophila primordial germ cells. *Curr Biol* **8**, 243-6.
van Haaften, G., Dalgliesh, G. L., Davies, H., Chen, L., Bignell, G., Greenman, C., Edkins, S., Hardy, C., O'Meara, S., Teague, J. et al. (2009). Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer. *Nat Genet* **41**, 521-3.
Vandamme, J., Lettier, G., Sidoli, S., Di Schiavi, E., Norregaard Jensen, O. and Salcini, A. E. (2012). The C. elegans H3K27 demethylase UTX-1 is essential for normal development, independent of its enzymatic activity. *PLoS Genet* **8**, e1002647.
Varambally, S., Dhanasekaran, S. M., Zhou, M., Barrette, T. R., Kumar-Sinha, C., Sanda, M. G., Ghosh, D., Pienta, K. J., Sewalt, R. G., Otte, A. P. et al. (2002). The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* **419**, 624-9.
Wallenfang, M. R., Nayak, R. and DiNardo, S. (2006). Dynamics of the male germline stem cell population during aging of Drosophila melanogaster. *Aging Cell* **5**, 297-304.
Wang, C., Lee, J. E., Cho, Y. W., Xiao, Y., Jin, Q., Liu, C. and Ge, K. (2012). UTX regulates mesoderm differentiation of embryonic stem cells independent of H3K27 demethylase activity. *Proc Natl Acad Sci U S A*.
Wang, J., Lu, F., Ren, Q., Sun, H., Xu, Z., Lan, R., Liu, Y., Ward, D., Quan, J., Ye, T. et al. (2011a). Novel histone demethylase LSD1 inhibitors selectively target cancer cells with pluripotent stem cell properties. *Cancer Res* **71**, 7238-49.
Wang, J. C. and Dick, J. E. (2005). Cancer stem cells: lessons from leukemia. *Trends Cell Biol* **15**, 494-501.
Wang, L., Brown, J. L., Cao, R., Zhang, Y., Kassis, J. A. and Jones, R. S. (2004). Hierarchical recruitment of polycomb group silencing complexes. *Mol Cell* **14**, 637-46.
Wang, P. and Hou, S. X. (2010). Regulation of intestinal stem cells in mammals and Drosophila. *J Cell Physiol* **222**, 33-7.
Wang, S. L., Zheng, D. Z., Lan, F. H., Deng, X. J., Zeng, J., Li, C. J., Wang, R. and Zhu, Z. Y. (2008). Increased expression of hLRH-1 in human gastric cancer and its implication in tumorigenesis. *Mol Cell Biochem* **308**, 93-100.
Wang, X., Pan, L., Wang, S., Zhou, J., McDowell, W., Park, J., Haug, J., Staehling, K., Tang, H. and Xie, T. (2011b). Histone H3K9 trimethylase Eggless controls germline stem cell maintenance and differentiation. *PLoS Genet* **7**, e1002426.
Wang, Z. and Mann, R. S. (2003). Requirement for two nearly identical TGIF-related homeobox genes in Drosophila spermatogenesis. *Development* **130**, 2853-65.
White-Cooper, H. (2009). Molecular mechanisms of gene regulation during Drosophila spermatogenesis. . *Reproduction* **139**, 11-21.
White-Cooper, H., Leroy, D., MacQueen, A. and Fuller, M. T. (2000). Transcription of meiotic cell cycle and terminal differentiation genes depends on a conserved chromatin associated protein, whose nuclear localisation is regulated. *Development* **127**, 5463-73.
White-Cooper, H., Schafer, M. A., Alphey, L. S. and Fuller, M. T. (1998). Transcriptional and post-transcriptional control mechanisms coordinate the onset of spermatid differentiation with meiosis I in Drosophila. *Development* **125**, 125-34.

Wong, C. and Jones, D. L. (2012). Efficiency of spermatogonial dedifferentiation during aging. *PLoS One* **7**, e33635.
Wu, S. C. and Zhang, Y. (2010). Active DNA demethylation: many roads lead to Rome. *Nat Rev Mol Cell Biol* **11**, 607-20.
Xi, R. and Xie, T. (2005). Stem cell self-renewal controlled by chromatin remodeling factors. *Science* **310**, 1487-9.
Xiang, Y., Zhu, Z., Han, G., Lin, H., Xu, L. and Chen, C. D. (2007). JMJD3 is a histone H3K27 demethylase. *Cell Res* **17**, 850-7.
Xie, T. and Spradling, A. C. (2000). A niche maintaining germ line stem cells in the Drosophila ovary. *Science* **290**, 328-30.
Xu, N., Wang, S. Q., Tan, D., Gao, Y., Lin, G. and Xi, R. (2011). EGFR, Wingless and JAK/STAT signaling cooperatively maintain Drosophila intestinal stem cells. *Dev Biol* **354**, 31-43.
Xu, T. and Rubin, G. M. (1993). Analysis of genetic mosaics in developing and adult Drosophila tissues. *Development* **117**, 1223-37.
Yamashita, Y. M. (2010). Cell adhesion in regulation of asymmetric stem cell division. *Curr Opin Cell Biol* **22**, 605-10.
Yamashita, Y. M., Fuller, M. T. and Jones, D. L. (2005). Signaling in stem cell niches: lessons from the Drosophila germline. *J Cell Sci* **118**, 665-72.
Yamashita, Y. M., Jones, D. L. and Fuller, M. T. (2003). Orientation of asymmetric stem cell division by the APC tumor suppressor and centrosome. *Science* **301**, 1547-50.
Yamashita, Y. M., Mahowald, A. P., Perlin, J. R. and Fuller, M. T. (2007). Asymmetric inheritance of mother versus daughter centrosome in stem cell division. *Science* **315**, 518-21.
Yang, H., Liu, Y., Bai, F., Zhang, J. Y., Ma, S. H., Liu, J., Xu, Z. D., Zhu, H. G., Ling, Z. Q., Ye, D. et al. (2013). Tumor development is associated with decrease of TET gene expression and 5-methylcytosine hydroxylation. *Oncogene* **32**, 663-9.
Yang, S. Y., Baxter, E. M. and Van Doren, M. (2012). Phf7 controls male sex determination in the Drosophila germline. *Dev Cell* **22**, 1041-51.
Zhu, L., Gibson, P., Currle, D. S., Tong, Y., Richardson, R. J., Bayazitov, I. T., Poppleton, H., Zakharenko, S., Ellison, D. W. and Gilbertson, R. J. (2009). Prominin 1 marks intestinal stem cells that are susceptible to neoplastic transformation. *Nature* **457**, 603-7.

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Education

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Ph.D. Cell, Molecular, Developmental Biology and Biophysics (CMDB), December 2014
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BRIGHAM YOUNG UNIVERSITY (Provo, UT)
B.S. Biophysics, Minor Chemistry, August 2008
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Research Experience

CHEN LAB, JOHNS HOPKINS UNIVERSITY
Graduate student, August 2008-Present
My thesis work is focused on understanding the role of epigenetic mechanisms in regulating stem cell activities. I use *Drosophila melanogaster* as a model system and I am interested in dissecting the functions of histone demethylases in the male germline lineage.

WOODBURY LAB, BRIGHAM YOUNG UNIVERSITY
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Teaching Experience

PREPARING FUTURE FACULTY TEACHING ACADEMY, JOHNS HOPKINS UNIVERSITY
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THE SUGAR CODE, JOHNS HOPKINS UNIVERSITY
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Honors and Awards

2011-2014 Ruth L. Kirschstein National Research Service Award (NRSA) Predoctoral Fellowship from the National Institutes of Health (NIH)

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2010 CMDB Annual Retreat Poster Award

2009 Dupont Teaching Award

2004-2008 Undergraduate Full Academic Scholarship

2005 Die AnStifter Stuttgart Peace Prize

2004 Jerusalem's Youth Center Young Writer's Award

2002 DAAD Summer Exchange Program Stipendium

Publications

Tarayrah, L., and Chen, X. Epigenetic regulation in adult stem cells and cancers. Cell & Bioscience (2013) 3(1): 41.

Tarayrah, L., Herz, H.M., Shilatifard, A., Chen, X. Histone demethylase dUTX antagonizes JAK-STAT signaling to maintain proper gene expression and architecture of the *Drosophila* testis niche. Development (2013) 140: 1014-1023.

Lim, C.*, **Tarayrah, L.*,** Chen, X. (* Equal contribution). Transcriptional regulation during *Drosophila* spermatogenesis. Spermatogenesis (2012) 2(3): 158-166.

Gan, Q*, Chepelev, I*, Wei, G, **Tarayrah, L**, Cui, K, Zhao, K and Chen, X**.** (* Equal contribution). Dynamic regulation of alternative splicing and chromatin structure in Drosophila gonads revealed by RNA-seq. Cell Research (2010) 20(7): 763-783.