Published in final edited form as:

J Mol Biol. 2017 June 30; 429(13): 2030–2041. doi:10.1016/j.jmb.2017.04.012.

Polycomb group gene *E(z)* is required for spermatogonial dedifferentiation in *Drosophila* adult testis

Suk Ho Eun^{1,*}, Lijuan Feng^{1,*}, Luis Cedeno-Rosario¹, Qiang Gan^{1,4}, Gang Wei^{2,5}, Kairong Cui², Keji Zhao², and Xin Chen^{1,¶}

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

²Laboratory of Molecular Immunology, National Heart, Lung and Blood Institute, NIH, Bethesda, MD 20892

Abstract

Dedifferentiation is an important process to replenish lost stem cells during aging or regeneration after injury to maintain tissue homeostasis. Here we report that Enhancer of Zeste [E(z)], a component of the Polycomb Repression Complex 2 (PRC2), is required to maintain a stable pool of germline stem cells (GSCs) within the niche microenvironment. During aging, germ cells with reduced E(z) activity cannot meet that requirement, but the defect neither arises from increased GSC death nor premature differentiation. Instead, we found evidence that the decrease of GSCs upon inactivation of *E(z)* in the germline could be attributed to defective dedifferentiation. During recovery from genetically manipulated GSC depletion, *E(z)* mutant germ cells also fail to replenish lost GSCs. Taken together, our data suggest that E(z) acts intrinsically in germ cells to activate dedifferentiation and thus replenish lost GSCs during both aging and tissue regeneration.

Keywords

Germline Stem Cell; Dedifferentiation; Polycomb Repression Complex; Enhancer of Zeste

Introduction

Many tissues with short-lived cell types in the body are maintained by adult stem cells, either continuously or in response to physiological signals or injuries. In many adult stem cell lineages, progenitor cells derived from stem cells first undergo a proliferative stage to expand their population before commitment to terminal differentiation. The switch from proliferation to differentiation must be tightly regulated, and misregulation of this transition can lead to tumorigenesis or tissue dystrophy [1]. On the other hand, progenitor cells remain

[¶]Correspondence to: Xin Chen, Ph.D., Mudd 307B, Department of Biology, 3400 North Charles Street, The Johns Hopkins University, Baltimore, MD 21218-2685, Tel: 410-516-4576; Fax: 410-516-5213; xchen32@jhu.edu.

^{*}These authors contributed equally to this work.

³Present address: Bio Scientific, Austin, TX 78744

⁴Present address: Stanford University School of Medicine, Stanford, CA 94305

⁵Present address: CAS-MPG Partner, Institute for Computational Biology, Chinese Academy of Sciences, Shanghai 200031, China

Data availability

GEO accession number for ChIP-seq data is GSE98000.

plastic and can undergo dedifferentiation in multiple stem cell lineages, a process which is critical to replenish lost stem cells during aging or tissue injury [2–14]. In order to properly differentiate adult stem cells or progenitor cells *in vitro* and/or to promote dedifferentiation *in vivo* for regenerative medicine, we need to better understand the molecular mechanisms underlying the dedifferentiation process in endogenous adult stem cell lineages *in vivo*.

Drosophila spermatogenesis is gaining attention as a model system for the study of mechanisms regulating the maintenance, proliferation, and proper differentiation of germline stem cells (GSCs) [6, 15–18] (Figure 1a). In adult testis of *Drosophila melanogaster*, GSCs can be located precisely by their proximity to a group of post-mitotic hub cells. Upon asymmetric cell division, a GSC gives rise to both a self-renewed stem cell and a gonialblast (GB), the daughter cell that initiates differentiation. GBs first go through a transit-amplifying stage, in which they undergo exactly four rounds of mitosis. Once spermatogonial mitosis is complete, germ cells enter the spermatocyte stage, in which each cell grows approximately 25-fold and initiates a robust gene expression program that enables meiotic division and spermatid differentiation (reviewed in [19–22]). Lost GSCs could be replenished by dedifferentiation of the mitotic spermatogonial cells [2, 4, 6, 7]. However, once the meiotic program is initiated in spermatocytes, dedifferentiation can no longer be detected [2, 6, 7], suggesting that mitotic spermatogonial cells have unique characteristics that allow for it. In parallel with GSC asymmetric cell division, the two cyst stem cells (CySCs) surrounding each GSC also divide asymmetrically. Each division results in one daughter cell retaining CySC identity, while another becomes a cyst cell ([23, 24]). Two cyst cells encapsulate differentiating germ cells and never divide again. It has been demonstrated that CySCs and cyst cells communicate with accompanying germ cells *via* multiple signaling pathways for critical decisions, such as cell fate maintenance and proliferation *vs.* differentiation throughout spermatogenesis [25–33]. The dramatic cellular differentiation during *Drosophila* spermatogenesis is accompanied by dynamic changes in gene expression, which are orchestrated by both extrinsic cues, such as paracrine factors that initiate intercellular signaling pathways, and intrinsic factors, such as chromatin regulators (reviewed in [21, 34, 35]).

Polycomb group (PcG) proteins are important chromatin regulators which act in at least two distinct, but interacting, protein complexes: Polycomb Repressive Complex 1 (PRC1) and PRC2 [36]. Enhancer of Zeste [E(z)] is a core and enzymatic component of PRC2 [37] which methylates histone H3 at Lys27 (H3K27me3) [38–41]. PcG can be silenced through H3K27me3, which recruits PRC1 through the chromodomain of the Pc protein [42, 43], leading to the nucleation of PcG complex and repression of the target gene(s). Extensive studies have focused on the biochemical activities of PcG. However, studies are increasingly shedding light on the biological functions of PcG in multiple model organisms (reviewed in [44–46]). In *Drosophila* testis, for example, previous studies have revealed that PcG activity, as shown by the H3K27me3 mark, is abundant at terminal differentiation genes in mitotic germ cells [47]. Such activity is likely to keep these genes in a silenced state. However, a series of developmentally programmed events can reverse PcG-mediated silencing to activate the expression of differentiation genes in spermatocytes [48, 49]. Furthermore, E(z) was shown to have a unique role in the CySC lineage to protect the identity and activity of germ cells [50].

Previous studies have identified the aminopeptidase Slamdance (Sda) which acts as an extrinsic factor in the testicular niche to regulate dedifferentiation of spermatogonial cells in adult testis [51]. However, the germ cell intrinsic factor(s) required for dedifferentiation has/have not been elucidated. Here we show that *loss-of-function* of *E(z)* in the germline results in dedifferentiation defects during both aging and regeneration after a genetic ablation of GSCs. Our results help to explain the intrinsic germline mechanism for dedifferentiation and highlight the important role of a key histone-modifying enzyme in an endogenous adult stem cell lineage.

Results

In *Drosophila* adult testes, two essential PRC2 components, *E(z)* and *Su(z)12*, are specifically expressed in mitotic germ cells, including GSCs, GBs, and spermatogonial cells, but they become abruptly downregulated in the meiotic spermatocytes, as determined by immunostaining [49] and a reporter GFP signal from tagged genomic rescuing transgenes (Supplementary Figure 1). *PRC2* genes are required for early development; thus, null mutants are embryonic lethal. Therefore, to study the function of *PRC2* genes in adult fly testes, we used appropriate strains to perform mosaic clonal analysis [52]. In order to generate homozygous mutant cells in heterozygous flies upon heat shock (hs), we used flies with the following genotype: *hs-FLP; E(z)⁷³¹ FRT2A/ubi-nGFP FRT2A {E(z)⁷³¹* is a null allele of *E(z)* [41, 53, 54], *ubi: ubiquitin* promoter, nGFP: nuclear GFP}. The cells which are homozygous for *E(z)* mutations could be distinguished from heterozygous cells by absence of the nGFP marker (Figure 1b).

Using clonal analysis, we examined whether inactivation of *E(z)* would affect GSC maintenance. We induced the *E(z)^{-/-}* GSC clones and counted the number of mutant GSCs in a time course experiment. The *wild-type* (WT) chromosome with the same FRT site was used as a control. The number of GFP-negative *E(z)^{-/-}* GSCs was not significantly different from that of GFP-negative WT GSCs three days after clonal induction (ACI, Figure 1c), suggesting similar clonal induction efficiency. However, these numbers showed significant difference starting from 21 days ACI. At 28 days ACI, GFP-negative WT GSCs had increased by approximately 3-fold compared with GFP-negative *E(z)^{-/-}* GSCs (Figure 1c). Despite these differences, the total GSCs, including both GFP-negative and GFP-positive cells, showed comparable number between testes containing *E(z)^{-/-}* GSCs and testes containing exclusively WT GSCs throughout the entire time course experiments (Figure 1c). These data suggest that *E(z)* is required for GSC maintenance and that GFP-positive WT GSCs compensated for the loss of GFP-negative *E(z)^{-/-}* GSCs in testes containing *E(z)^{-/-}* GSCs.

Next, we asked whether mutation of *E(z)* in GSCs causes premature differentiation or increased cell death, either of which could lead to GSC loss. To investigate the first possibility, we stained the testes containing *E(z)* null GSCs with anti-Stat92E antibody. In WT testes, JAK/STAT signaling is critical for GSC maintenance [15, 16, 25, 55], and immunostaining signal of the downstream Stat92E transcription factor is high in GSCs, but almost undetectable in further differentiated spermatogonia [56, 57]. We did not detect any difference of Stat signal between *E(z)* GSCs and WT GSCs in the same testis (N=17,

Supplementary Figure 2a). We also performed immunostaining using antibodies either against the endogenous Bam (Bag-of-marbles) or an HA-tagged *bam* genomic rescuing transgene [57–59], which labeled 4–16 cell spermatogonia [29, 58, 60–62]. If mutant GSCs were to undergo premature differentiation, Bam signal would be detected in mutant germ cells next to the hub instead of a few cell tiers away [6, 57]. However, we did not observe this phenomenon (N=167, Supplementary Figure 2b). Positive Stat signal and negative Bam staining in *E(z)* mutant GSCs indicated that they were unlikely undergoing premature differentiation. To determine whether *E(z)* mutant GSCs had increased incidence of cell death, we stained testes containing *E(z)* mutant GSCs with lysotracker, a marker for germ cell death [63]. However, we detected no lysotracker-positive *E(z)* mutant GSCs (N=106, Supplementary Figure 2c), indicating increased cell death to be an unlikely cause of their loss. Previous studies have shown that normal GSCs have unique protective mechanisms against cell death [63–65]. Loss of *E(z)* does not seem to abrogate this protection.

Clonal analyses provided the opportunity to compare *E(z)* mutant cells with WT cells within the same tissue. However, the presence of WT cells could compete with mutant cells or have non-cell-autonomous contributions. Therefore, to further investigate defects of *E(z)* mutant GSCs without neighboring WT GSCs, *E(z)* was knocked down in all early-stage germ cells, including GSCs, using short-hairpin RNA (*UAS-shRNA*; UAS: upstream activation sequences) paired with the early germline-specific *nos-Gal4* driver [66] [*nos>E(z) shRNA*]. Loss of H3K27me3 immunostaining signals in germ cells, but not in cyst and hub cells, indicates that *E(z)* was efficiently and specifically knocked down in *nos>E(z) shRNA* testes [50]. The GSC numbers between *nos-Gal4* control (11.22 ± 1.93 , mean $\pm$ SD, N=37) and *nos>E(z) shRNA* testes (10.53 ± 1.78 , N=45) showed no significant difference in males one day after eclosion (Figure 1d, 1e), suggesting that the germline knockdown of *E(z)* did not affect the establishment of GSCs in the early stage of development. However, after 28 days, a significant difference of GSC numbers was observed between *nos>E(z) shRNA* testes (5.89 ± 3.11 , N=45) and *nos-Gal4* control testes (9.62 ± 1.74 , N=29) (Figure 1d, 1e). In summary, these results suggest that *E(z)* is required for GSC maintenance, but not for GSC establishment. However, the loss of *E(z)* mutant GSCs did not arise from a failure to respond to the key signaling pathway in the niche, premature differentiation, or cell death.

The rate of *E(z)* mutant GSC decrease (Figure 1) was relatively slow compared to GSC loss from other mechanisms, such as failure to respond to the key signaling pathways [15, 16, 57]. Consequently, we asked if the gradual loss of *E(z)*^{-/-} GSCs resulted from some failure in the dedifferentiation process, which only becomes apparent in aged flies [2–4]. Consistent with this hypothesis, *E(z)* expression is highly enriched in mitotic spermatogonial cells, but not in meiotic spermatocytes ([49] and Supplementary Figure 1). To the best of our knowledge, only mitotic spermatogonial cells can dedifferentiate [2, 6, 7]. We therefore tested whether *E(z)* is required for aging-related dedifferentiation and genetic ablation-induced dedifferentiation, using the following two *in vivo* assays.

First, we examined centrosome orientation in both *nos-Gal4* control testes and *nos>E(z) shRNA* testes from aged males (28 days after eclosion). It has been shown that centrosomes are oriented with the mother centrosome located near the hub-GSC interface, while the daughter centrosome moves toward the opposite side during G2 phase in preparation for

establishing the mitotic spindle perpendicular to the hub-GSC interface during GSC asymmetric cell division [17, 18]. Mother centrosome is anchored by astral microtubule toward the hub-GSC interface [18], therefore in normal GSCs one centrosome should always be found adjacent to the GSC-hub interface. In GSCs where both centrosomes are away from the hub (right panel in Figure 2a, 2b, 3c), it is defined as misoriented centrosome phenotype [18]. It has also been shown that dedifferentiated spermatogonial cells in testes from aged males tend to have misoriented centrosomes [4]. Indeed, in testes from aged *nos-Gal4* control male flies, 39.5% of GSCs (N=81) had misoriented centrosomes (Figure 2a, 2c), a percentage comparable to the results reported in testes from WT males of similar age ([4, 51, 67]). By contrast, in testes from aged *nos>E(z) shRNA* males, only 2.5 % of GSCs (N=121) were found to have misoriented centrosomes (Figure 2b, 2c), significantly lower than that of control ($P < 10^{-4}$). These results suggest potential spermatogonial dedifferentiation defects when E(z) is inactivated in the germline.

To specifically test whether E(z) is required in later stage germ cells including spermatogonia for dedifferentiation, E(z) is knocked down using a later stage germ cell driver *bam-Gal4* driver [68]. We expressed *E(z)* short-hairpin RNA driven by *bam-Gal4* to achieve a knockdown in more differentiated germ cells from four-cell spermatogonia to later stages. Loss of H3K27me3 immunostaining signals were found in spermatogonial cells (yellow arrow, Figure 3a) and spermatocytes (red arrow, Figure 3a), but not in GSCs and GBs (yellow arrowheads, Figure 3a). It indicates that E(z) was effectively and specifically knocked down in *bam>E(z) shRNA* testes. In males one day after eclosion, the GSC number between *bam-Gal4* control (12.52 ± 1.83 , N=29) and *bam>E(z) shRNA* (11.44 ± 2.88 , N=39) testes were comparable. However, after 21 days at 29°C, a significant decrease ($P < 10^{-4}$) of GSCs was found in *bam>E(z) shRNA* testes (9.080 ± 2.68 , N=50), compared to *bam-Gal4* control testes (12.86 ± 1.95 , N=43) (Figure 3b). These data suggest that E(z) is required in spermatogonia for GSC maintenance but not for the establishment. In addition, the percentage of GSCs with misoriented centrosome (42.9%, N=140) in *bam-Gal4* control testes (Figure 3c, 3d) is significantly ($P < 10^{-4}$) higher than that of *bam>E(z) shRNA* testes (7.6%, N=144) (Figure 3c, 3d). These results indicate that the function of E(z) in the spermatogonial cells is required for the dedifferentiation process to maintain a stable GSC pool.

Second, spermatogonial cells have been shown to undergo robust dedifferentiation during recovery after GSCs are forced to differentiate and leave the niche by overexpressing the differentiation factor Bam [7, 51]. To perform these experiments, we introduced the same *hs-bam* transgene to either the *nos-Gal4* control or the *nos>E(z) shRNA* background. We then induced GSC differentiation by heat-shocking flies five times to overexpress Bam. After five consecutive heat shock treatments, GSCs left the niche to differentiate, and traffic jam (Tj) [69] -positive cyst cells became populated in the vicinity of the hub (Figure 4a, 4c). The number of GSCs decreased to 2.5 ± 2.1 (N=44) in control testes and 3.2 ± 2.9 (N=42) in *E(z)* knockdown testes (Figure 4e). Both genotyped flies were subsequently moved to the permissive condition for recovery. After five days of recovery, almost all testes from *hs-bam; nos-Gal4* control males restored their GSCs, and the rosette structure was reestablished (Figure 4b). The average number of GSCs was recovered to 12.6 ± 2.6 (N=49, Figure 4e). By contrast, only 5.3 ± 2.6 (N=49) GSCs were found in testes from *hs-bam; nos>E(z)*

shRNA males five days after recovery (Figure 4d, 4e). Seven days after recovery, the number of GSCs in *hs-bam; nos>E(z) shRNA* testes did not change much (4.3 ± 3.1 , N=32, Figure 4e), suggesting that the dedifferentiation defects in *E(z)* knockdown germ cells did not arise from the slow rate of dedifferentiation. These results collectively suggest that *E(z)* is required for promoting spermatogonial dedifferentiation during tissue regeneration.

Because *E(z)* is the only H3K27me3 methyltransferase in *Drosophila* [38–41], its regulation of dedifferentiation may rely on the repressive chromatin structure maintained by *E(z)* enzymatic activity. To probe for the chromatin structure maintained by *E(z)* in mitotic germ cells, we analyzed the chromatin landscape of the spermatogonial cell-enriched *bam* mutant testes [47, 62] in the presence and absence of *E(z)* activity. To inactivate *E(z)* in all cells in testes, we used a temperature-sensitive (ts) allele, *E(z)⁶¹* [53, 70, 71], and recombined it with the *bam* mutation. We found that incubation of *E(z)⁶¹ bam* double mutant flies at a restrictive temperature (29°C) for three days almost completely erased the H3K27me3 mark in testes, as determined by both immunoblot (Figure 5a) and immunostaining analyses [50]. We then performed ChIP-seq experiments using antibodies against the active chromatin mark H3K4me3 and RNA Polymerase II (Pol II) in both *bam* and *E(z)⁶¹ bam* testes treated under the same condition as that described above. Using anti-H3K4me3 and anti-Pol II, we compared ChIP-seq results between *E(z)⁶¹ bam* testes (Table S1, S2, Supplementary Figure 3) and *bam* testes [47]. We found that such global erasure of H3K27me3 in *E(z)⁶¹ bam* testes resulted in an increase of H3K4me3 (2-fold change) at 3,788 (39%) genes. In contrast, only 199 (2%) genes showed a decrease of H3K4me3 (Figure 5b). Meanwhile, Pol II binding increased in 971 (10%) genes and decreased in 331 (3%) genes, respectively (Figure 5c). Most genes with increased Pol II also exhibited an increase in H3K4me3 (572 out of 971 genes, 59%). In summary, our ChIP-seq results demonstrated that a significant number of genes changed chromatin status with increased H3K4me3 and/or Pol II binding in *E(z)⁶¹ bam* testes compared with *bam* testes. We speculate that these global changes of chromatin structure in *E(z)⁶¹ bam* testes might underlie the impeded dedifferentiation capability of mitotic germ cells.

Discussion

Previous studies in mouse testis have shown that *PRC2* mutant spermatogonial stem cells (the murine counterpart of *Drosophila* GSCs) are lost without detectable signs of either premature differentiation or apoptosis [72]. Consistent with this observation, *E(z)* mutant *Drosophila* male GSCs do not undergo precocious differentiation or cell death according to our results. Instead, *E(z)* acts in germ cells to promote the spermatogonial dedifferentiation process to maintain GSCs during both aging and tissue regeneration. It would be interesting to study whether this mechanism is conserved in mouse testis.

Regenerative medicine is significantly challenged by the need to restore the activity of adult stem cells in their endogenous niches. However, regulation of the dedifferentiation process *in vivo* is not well understood. Here, our data reveal that a histone-modifying enzyme acts cell autonomously for dedifferentiation during stem cell homeostasis and regeneration. Understanding the molecular mechanisms underlying progenitor cell dedifferentiation will

greatly assist applying reprogrammed differentiated cells from the same lineage for tissue regeneration.

Materials and Methods

Fly strains and husbandry

Flies were raised using standard medium at 25°C unless stated otherwise. The *bam*¹/TM3 and *bam*¹¹⁴⁻⁹⁷/TM6B stocks were obtained from Bloomington *Drosophila* Stock Centers. The *E(z)*⁶¹ allele was obtained from Dr. Richard Jones lab and recombined with each of the *bam* allele. The *E(z)*⁶¹ *bam*¹/*E(z)*⁶¹ *bam*¹¹⁴⁻⁹⁷ flies were raised at 29°C for three days before eclosion. The following fly strains were obtained from the Bloomington Stock Center: *FRT2A* (BL1997), *Ubi-GFP, FRT2A* (BL5825), *E(z)*⁷³¹ (BL24470), *nos-Gal4/Cyo* [66], *bam-Gal4* [68] and *UAS-E(z) shRNA* (BL33659). Control Flies (*nos-Gal4/+*) and *E(z)* knockdown flies (*nos-Gal4/+; UAS-E(z) shRNA/+*) 1-day or 28-day old at 25°C were dissected. Control Flies (*bam-Gal4/+*) and *E(z)* knockdown flies (*bam-Gal4/+; UAS-E(z) shRNA/+*) 1-day or 21-day old at 29°C were dissected. Flies with following genotype *E(z)-GFP, Su(z)12-GFP* were previously described [50].

Immunofluorescence staining

Immunofluorescence staining was performed using the formaldehyde fixation method as previous described [73]. Immunofluorescence staining against Sa (1:100) was performed using the methanol fixation as previous described [48]. Primary antibodies: GFP (chicken, 1:1000, Abcam, ab13970), Vasa (rabbit, 1:200, Santa Cruz, sc-30210), Armadillo (Mouse, 1:200, DSHB, N2 7A1), Fas III (Mouse, 1:100, DSHB, 7G10), α -Spectrin (Mouse, 1:50, DSHB, 3A9), Traffic jam (guinea pig, 1:3000, from Dr. Mark Van Doren, Johns Hopkins University, Baltimore, MD, USA), γ -tubulin (mouse, 1:100, Sigma T9026), H3K27me3 (mouse, 1:200, Abcam, ab6002), Stat92E (rabbit, 1:800, from Denise Montell, UC Santa Barbara), Bam (mouse, 1:20, DSHB), Lysotracker (Invitrogen, L-7528, following the manufacturer's protocol). Secondary antibodies were all Alexa Fluor series (1:200, Molecular Probes).

Clonal Induction

The wild-type and *E(z)*⁷³¹ mutant clones were induced by FLP recombinase. Adult flies with following genotype: *hs-FLP; FRT2A, Ubi-GFP/FRT2A, E(z)*⁷³¹ and control flies *hs-FLP; FRT2A, Ubi-GFP/FRT2A* were raised at 25 °C until pupal stage. Then they were heat-shocked in a 37°C water bath for 2 hours each on two consecutive days. Adult flies at 3, 7, 14, 21 and 28 days after clonal induction were collected for dissection.

Heat Shock and Recovery Regime

Newly eclosed flies at a 18°C incubator with the following genotype: *hs bam, nos-Gal4* and *hs bam, nos>E(z) shRNA* were collected. Flies were heat-shocked in a 37°C water bath for 30 min at 9 AM and 5 PM daily for a total of five times of heat shock. Between heat shock treatments, flies were placed in a 29°C incubator. After five times heat shock, flies were returned to 18°C incubator for a 5-day or 7-day recovery.

Immunoblot analysis

We dissected testes from *bam*¹/*bam*¹¹⁴⁻⁹⁷ and *E(z)*⁶¹ *bam*¹/*E(z)*⁶¹ *bam*¹¹⁴⁻⁹⁷ flies, which were raised at 29°C for 3 days. Samples were grouped in DualColor Protein Loading Buffer (Fermentas, #R1011) and boiled for 10 mins, followed by electrophoresis using a 12% SDS-PAGE gel and transfer to a PVDF membrane (GE healthcare, #RPN303F). The membrane was then blotted with α-H3K27me3 (Millipore, #07-449) at 1:200 overnight at 4°C, followed by HRP-conjugated α-rabbit IgG at 1:1,000 (Invitrogen, # 656120) at RT for 2 hrs. The ECL (GE healthcare, RPN2109) reagents were applied to visualize the signal. After repeated stripping with Restore Western Blot Stripping buffer (Thermo scientific, #21059), the membrane was re-probed sequentially with α-H3K4me3 (Abcam, #ab8580) at 1:10,000 and α-H3 (Abcam, #ab1791) at 1: 5000, followed by HRP-conjugated rabbit IgG at 1:1,000.

Chromatin immunoprecipitation

For each H3K4me3 and Pol II ChIP experiment, we dissected 200 pairs of *bam* testes and 200 pairs of *E(z)*⁶¹ *bam* testes in cold PBS and grouped them in 200μl PBS which contained protease inhibitor (Roche complete mini, # 11836153001) and 0.5mM PMSF (MP Biomedicals, #195381). We then added 5.5μl 37% fresh formaldehyde (Supelco, # 47083-U) and incubated at 37°C for 15 mins. The testes were washed twice with 450μl cold 1× PBS (with inhibitors and PMSF). Then 200μl lysis buffer (50mM Tris-HCl, pH7.6, 1mM CaCl₂, 0.2% Triton X-100, 5mM butyrate, 1× proteinase inhibitor cocktail, and 0.5mM fresh PMSF) was added and the tissues were homogenized thoroughly followed by incubation at room temperature (RT) for 10 mins. Chromatin was sheared into approximately 200 bp fragments by sonication using Microtip (Misonix, Inc, Microson XL-2000) with the following procedure: 4 seconds at power 20, rest for 50 seconds, 4–5 times, followed by spinning at 14k rpm for 10 mins at 4°C. The chromatin was diluted 10× with RIPA buffer (10mM Tris, pH7.6, 1mM EDTA, 0.1% SDS, 0.1% Na-Deoxycholate, 1% Triton X-100, with protease inhibitors and PMSF) and 50μl of this dilution was reverse cross-linked with 0.25M NaCl for 2 hrs at 65°C and used as input.

We washed 40μl of Dynabeads Protein A (Invitrogen, #100.01D) with 600μl 1× PBS. We then added 100μl 1× PBS with 4 μg antibody and incubated the antibody-Protein A beads mixture at RT for 40 mins with occasional tapping. After the unbound antibody was removed using the manipulator (Invotrogen, DYNAL MPC-S), 1ml of the chromatin extract was added to the beads and the mixture was rotated at 4°C overnight. The beads were then washed 2× with 1 ml RIPA buffer, 2× with 1ml RIPA buffer containing 0.3M NaCl, 1× with LiCl wash buffer (0.25M LiCl, 0.5% NP40, and 0.5% Sodium Deoxycholate), 1× with 1ml TE (10 mM Tris-HCl, pH 8.0 and 1 mM EDTA) containing 0.2% Triton X-100, and 1× with 1ml TE. The beads were then suspended in 100μl 1× TE containing 3μl 10% SDS and 5μl 20mg/ml proteinase K, followed by incubation at 65°C overnight. After the supernatant was collected, the beads were washed once more with 100μl TE with 0.5M NaCl. The supernatant from this wash was combined with the previous supernatant. The combined samples were treated by Phenol/Chloroform extraction, salt/EtOH precipitation, and resuspended in 50μl 1× TE. The products were processed for Solexa sequencing according to the established protocol. Antibodies used include: H3K4me3 (Abcam, #ab8580) and RNA Pol II (Abcam, ab5408).

Solexa pipeline analysis

The 25-bp sequencing reads were obtained from the Illumina Genome Analyzer (GA) Pipeline. All reads were aligned to the *Drosophila* genome (dm3) using the ELAND (Efficient Local Alignment of Nucleotide Data) software, allowing up to two mismatches with the reference sequence. Only uniquely mapped reads were retained. For multiple identical reads, at most three copies were retained to reduce the possibility of biases from PCR amplification. The output of the GA Pipeline was converted to browser extensible data (BED) files. The wig files used for visualization on the UCSC browser were generated from the uniquely mapped reads as previously described [74]. The size of the DNA fragment was determined by the distance from the 5' to the 3' peak of the mapped reads, as shown in Supplementary Figure 3.

Define genomic regions for analyzing modified histone and Pol II enrichment

To determine the regions used for modified histone and Pol II occupancy of annotated transcripts, we first plotted each histone modification and Pol II using the entire annotated transcripts that are applicable for ChIP-seq analysis. Based on the overall enrichment plot, we used the region of -250 to +250 bp (TSS was defined as 0) to calculate the Pol II enrichment of individual transcript. The 0 to +500 bp window was used for H3K4me3 enrichment calculations.

Scatter plot analysis

The scatter plots delineate comparisons of H3K4me3 and Pol II enrichment between *bam* and *E(z)⁶¹ bam* samples. All plots were generated in the R programming environment (R version 2.5.0, download from <http://www.r-project.org>).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr. Mark Van Doren, Dr. Robert J. Johnston, and X.C. lab members for their critical comments. We thank TRiP at Harvard medical School (NIH/NIGMS R01-GM084947) for providing transgenic RNAi fly stocks used in this study, Developmental Studies Hybridoma Bank for antibodies, and the Bloomington *Drosophila* Stock Center (NIH P40OD018537) for stocks used in this study. This work has been supported by NIH RO1HD065816, the David and Lucile Packard Foundation, Johns Hopkins University start-up (X.C.) and support of Division of Intramural Research, the National Heart, Lung and Blood Institute, NIH (K.Z.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abbreviations

E(z)	Enhancer of Zeste
PRC2	Polycomb Repression Complex 2
GSCs	Germline Stem Cells
Sda	Slamdance

ACI	After Clonal Induction
Bam	Bag-of-Marbles
Tj	Traffic Jam

References

- Clarke MF, Fuller M. Stem cells and cancer: two faces of eve. *Cell*. 2006; 124(6):1111–5. [PubMed: 16564000]
- Wallenfang MR, Nayak R, DiNardo S. Dynamics of the male germline stem cell population during aging of *Drosophila melanogaster*. *Aging Cell*. 2006; 5(4):297–304. [PubMed: 16800845]
- Boyle M, et al. Decline in self-renewal factors contributes to aging of the stem cell niche in the *Drosophila* testis. *Cell Stem Cell*. 2007; 1(4):470–8. [PubMed: 18371382]
- Cheng J, et al. Centrosome misorientation reduces stem cell division during ageing. *Nature*. 2008; 456(7222):599–604. [PubMed: 18923395]
- Kai T, Spradling A. Differentiating germ cells can revert into functional stem cells in *Drosophila melanogaster* ovaries. *Nature*. 2004; 428(6982):564–9. [PubMed: 15024390]
- Brawley C, Matunis E. Regeneration of male germline stem cells by spermatogonial dedifferentiation in vivo. *Science*. 2004; 304(5675):1331–4. [PubMed: 15143218]
- Sheng XR, Brawley CM, Matunis EL. Dedifferentiating spermatogonia outcompete somatic stem cells for niche occupancy in the *Drosophila* testis. *Cell Stem Cell*. 2009; 5(2):191–203. [PubMed: 19664993]
- Barroca V, et al. Mouse differentiating spermatogonia can generate germinal stem cells in vivo. *Nat Cell Biol*. 2009; 11(2):190–6. [PubMed: 19098901]
- Nakagawa T, et al. Functional hierarchy and reversibility within the murine spermatogenic stem cell compartment. *Science*. 2010; 328(5974):62–7. [PubMed: 20299552]
- Schwitalla S, et al. Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties. *Cell*. 2013; 152(1–2):25–38. [PubMed: 23273993]
- Friedmann-Morvinski D, et al. Dedifferentiation of neurons and astrocytes by oncogenes can induce gliomas in mice. *Science*. 2012; 338(6110):1080–4. [PubMed: 23087000]
- Talchai C, et al. Pancreatic beta cell dedifferentiation as a mechanism of diabetic beta cell failure. *Cell*. 2012; 150(6):1223–34. [PubMed: 22980982]
- Wong C, Jones DL. Efficiency of spermatogonial dedifferentiation during aging. *PLoS One*. 2012; 7(3):e33635. [PubMed: 22442704]
- Rinkevich Y, et al. Germ-layer and lineage-restricted stem/progenitors regenerate the mouse digit tip. *Nature*. 2011; 476(7361):409–13. [PubMed: 21866153]
- Kiger AA, et al. Stem cell self-renewal specified by JAK-STAT activation in response to a support cell cue. *Science*. 2001; 294(5551):2542–5. [PubMed: 11752574]
- Tulina N, Matunis E. Control of stem cell self-renewal in *Drosophila* spermatogenesis by JAK-STAT signaling. *Science*. 2001; 294(5551):2546–9. [PubMed: 11752575]
- Yamashita YM, Jones DL, Fuller MT. Orientation of asymmetric stem cell division by the APC tumor suppressor and centrosome. *Science*. 2003; 301(5639):1547–50. [PubMed: 12970569]
- Yamashita YM, et al. Asymmetric inheritance of mother versus daughter centrosome in stem cell division. *Science*. 2007; 315(5811):518–21. [PubMed: 17255513]
- Fuller MT. Genetic control of cell proliferation and differentiation in *Drosophila* spermatogenesis. *Semin Cell Dev Biol*. 1998; 9(4):433–44. [PubMed: 9813190]
- Davies EL, Fuller MT. Regulation of Self-renewal and Differentiation in Adult Stem Cell Lineages: Lessons from the *Drosophila* Male Germ Line. *Cold Spring Harb Symp Quant Biol*. 2009
- Lim C, Tarayrah L, Chen X. Transcriptional regulation during *Drosophila* spermatogenesis. *Spermatogenesis*. 2012; 2(3):158–166. [PubMed: 23087835]

22. White-Cooper H, Caporilli S. Transcriptional and post-transcriptional regulation of *Drosophila* germline stem cells and their differentiating progeny. *Adv Exp Med Biol.* 2013; 786:47–61. [PubMed: 23696351]
23. Cheng J, et al. Asymmetric division of cyst stem cells in *Drosophila* testis is ensured by anaphase spindle repositioning. *Development.* 2011; 138(5):831–7. [PubMed: 21303845]
24. Gonczy P, DiNardo S. The germ line regulates somatic cyst cell proliferation and fate during *Drosophila* spermatogenesis. *Development.* 1996; 122(8):2437–47. [PubMed: 8756289]
25. Leatherman JL, Dinardo S. Zfh-1 controls somatic stem cell self-renewal in the *Drosophila* testis and nonautonomously influences germline stem cell self-renewal. *Cell Stem Cell.* 2008; 3(1):44–54. [PubMed: 18593558]
26. Kiger AA, White-Cooper H, Fuller MT. Somatic support cells restrict germline stem cell self-renewal and promote differentiation. *Nature.* 2000; 407(6805):750–4. [PubMed: 11048722]
27. Tran J, Brenner TJ, DiNardo S. Somatic control over the germline stem cell lineage during *Drosophila* spermatogenesis. *Nature.* 2000; 407(6805):754–7. [PubMed: 11048723]
28. Sarkar A, et al. Antagonistic roles of Rac and Rho in organizing the germ cell microenvironment. *Curr Biol.* 2007; 17(14):1253–8. [PubMed: 17629483]
29. Schulz C, et al. A misexpression screen reveals effects of bag-of-marbles and TGF beta class signaling on the *Drosophila* male germ-line stem cell lineage. *Genetics.* 2004; 167(2):707–23. [PubMed: 15238523]
30. Parrott BB, et al. Control of germline stem cell division frequency—a novel, developmentally regulated role for epidermal growth factor signaling. *PLoS One.* 2012; 7(5):e36460. [PubMed: 22586473]
31. Chen H, Chen X, Zheng Y. Nuclear lamina regulates the organization of the stem-cell niche. *Cell Stem Cell.* 2013 in press.
32. Lim JG, Fuller MT. Somatic cell lineage is required for differentiation and not maintenance of germline stem cells in *Drosophila* testes. *Proc Natl Acad Sci U S A.* 2012; 109(45):18477–81. [PubMed: 23091022]
33. Leatherman JL, Dinardo S. Germline self-renewal requires cyst stem cells and stat regulates niche adhesion in *Drosophila* testes. *Nat Cell Biol.* 2010; 12(8):806–11. [PubMed: 20622868]
34. de Cuevas M, Matunis EL. The stem cell niche: lessons from the *Drosophila* testis. *Development.* 2011; 138(14):2861–9. [PubMed: 21693509]
35. Davies EL, Fuller MT. Regulation of self-renewal and differentiation in adult stem cell lineages: lessons from the *Drosophila* male germ line. *Cold Spring Harb Symp Quant Biol.* 2008; 73:137–45. [PubMed: 19329574]
36. Schwartz YB, Pirrotta V. Polycomb silencing mechanisms and the management of genomic programmes. *Nat Rev Genet.* 2007; 8(1):9–22. [PubMed: 17173055]
37. Lund AH, van Lohuizen M. Polycomb complexes and silencing mechanisms. *Curr Opin Cell Biol.* 2004; 16(3):239–46. [PubMed: 15145347]
38. Cao R, et al. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science.* 2002; 298(5595):1039–43. [PubMed: 12351676]
39. Czermin B, et al. *Drosophila* enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. *Cell.* 2002; 111(2):185–96. [PubMed: 12408863]
40. Kuzmichev A, et al. Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. *Genes Dev.* 2002; 16(22):2893–905. [PubMed: 12435631]
41. Muller J, et al. Histone methyltransferase activity of a *Drosophila* Polycomb group repressor complex. *Cell.* 2002; 111(2):197–208. [PubMed: 12408864]
42. Fischle W, et al. Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by Polycomb and HP1 chromodomains. *Genes Dev.* 2003; 17(15):1870–81. [PubMed: 12897054]
43. Min J, Zhang Y, Xu RM. Structural basis for specific binding of Polycomb chromodomain to histone H3 methylated at Lys 27. *Genes Dev.* 2003; 17(15):1823–8. [PubMed: 12897052]

44. Surface LE, Thornton SR, Boyer LA. Polycomb group proteins set the stage for early lineage commitment. *Cell Stem Cell*. 2010; 7(3):288–98. [PubMed: 20804966]
45. Blackledge NP, Rose NR, Klose RJ. Targeting Polycomb systems to regulate gene expression: modifications to a complex story. *Nat Rev Mol Cell Biol*. 2015; 16(11):643–9. [PubMed: 26420232]
46. Di Croce L, Helin K. Transcriptional regulation by Polycomb group proteins. *Nat Struct Mol Biol*. 2013; 20(10):1147–55. [PubMed: 24096405]
47. Gan Q, et al. Monovalent and unpoised status of most genes in undifferentiated cell-enriched *Drosophila* testis. *Genome Biol*. 2010; 11(4):R42. [PubMed: 20398323]
48. Chen X, et al. Tissue-specific TAFs counteract Polycomb to turn on terminal differentiation. *Science*. 2005; 310(5749):869–72. [PubMed: 16272126]
49. Chen X, et al. Sequential changes at differentiation gene promoters as they become active in a stem cell lineage. *Development*. 2011; 138(12):2441–50. [PubMed: 21610025]
50. Eun SH, et al. A non-cell autonomous role of E(z) to prevent germ cells from turning on a somatic cell marker. *Science*. 2014; 343(6178):1513–6. [PubMed: 24675960]
51. Lim C, et al. An Aminopeptidase in the *Drosophila* Testicular Niche Acts in Germline Stem Cell Maintenance and Spermatogonial Dedifferentiation. *Cell Rep*. 2015; 13(2):315–25. [PubMed: 26440886]
52. Xu T, Rubin GM. Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development*. 1993; 117(4):1223–37. [PubMed: 8404527]
53. Jones RS, Gelbart WM. Genetic analysis of the enhancer of zeste locus and its role in gene regulation in *Drosophila melanogaster*. *Genetics*. 1990; 126(1):185–99. [PubMed: 1977656]
54. Iovino N, Ciabrelli F, Cavalli G. PRC2 controls *Drosophila* oocyte cell fate by repressing cell cycle genes. *Dev Cell*. 2013; 26(4):431–9. [PubMed: 23932903]
55. Leatherman JL, Dinardo S. Germline self-renewal requires cyst stem cells and stat regulates niche adhesion in *Drosophila* testes. *Nat Cell Biol*. 12(8):806–11.
56. Wawersik M, et al. Somatic control of germline sexual development is mediated by the JAK/STAT pathway. *Nature*. 2005; 436(7050):563–7. [PubMed: 16049490]
57. Tarayrah L, et al. Epigenetic regulator Lid maintains germline stem cells through regulating JAK-STAT signaling pathway activity. *Biol Open*. 2015
58. Eun SH, et al. MicroRNAs downregulate Bag of marbles to ensure proper terminal differentiation in the *Drosophila* male germline. *Development*. 2013; 140(1):23–30. [PubMed: 23154414]
59. Li Y, et al. Bam and Bgcn antagonize Nanos-dependent germ-line stem cell maintenance. *Proc Natl Acad Sci U S A*. 2009; 106(23):9304–9. [PubMed: 19470484]
60. Insko ML, et al. Accumulation of a differentiation regulator specifies transit amplifying division number in an adult stem cell lineage. *Proc Natl Acad Sci U S A*. 2009; 106(52):22311–6. [PubMed: 20018708]
61. Gonczy P, Matunis E, DiNardo S. bag-of-marbles and benign gonial cell neoplasm act in the germline to restrict proliferation during *Drosophila* spermatogenesis. *Development*. 1997; 124(21):4361–71. [PubMed: 9334284]
62. McKearin DM, Spradling AC. bag-of-marbles: a *Drosophila* gene required to initiate both male and female gametogenesis. *Genes Dev*. 1990; 4(12B):2242–51. [PubMed: 2279698]
63. Yacobi-Sharon K, Namdar Y, Arama E. Alternative germ cell death pathway in *Drosophila* involves HtrA2/Omi, lysosomes, and a caspase-9 counterpart. *Dev Cell*. 2013; 25(1):29–42. [PubMed: 23523076]
64. Yang H, Yamashita YM. The regulated elimination of transit-amplifying cells preserves tissue homeostasis during protein starvation in *Drosophila* testis. *Development*. 2015; 142(10):1756–66. [PubMed: 25968311]
65. Hasan S, Hetie P, Matunis EL. Niche signaling promotes stem cell survival in the *Drosophila* testis via the JAK-STAT target DIAP1. *Dev Biol*. 2015; 404(1):27–39. [PubMed: 25941003]
66. Van Doren M, Williamson AL, Lehmann R. Regulation of zygotic gene expression in *Drosophila* primordial germ cells. *Curr Biol*. 1998; 8(4):243–6. [PubMed: 9501989]

67. Yuan H, et al. Regulation of cyclin A localization downstream of Par-1 function is critical for the centrosome orientation checkpoint in *Drosophila* male germline stem cells. *Dev Biol.* 2012; 361(1):57–67. [PubMed: 22024320]
68. Chen D, McKearin DM. A discrete transcriptional silencer in the *bam* gene determines asymmetric division of the *Drosophila* germline stem cell. *Development.* 2003; 130(6):1159–70. [PubMed: 12571107]
69. Li MA, et al. The large Maf factor Traffic Jam controls gonad morphogenesis in *Drosophila*. *Nat Cell Biol.* 2003; 5(11):994–1000. [PubMed: 14578908]
70. Carrington EA, Jones RS. The *Drosophila* Enhancer of zeste gene encodes a chromosomal protein: examination of wild-type and mutant protein distribution. *Development.* 1996; 122(12):4073–83. [PubMed: 9012527]
71. Wang L, et al. Hierarchical recruitment of polycomb group silencing complexes. *Mol Cell.* 2004; 14(5):637–46. [PubMed: 15175158]
72. Mu W, et al. Repression of the soma-specific transcriptome by Polycomb-repressive complex 2 promotes male germ cell development. *Genes Dev.* 2014; 28(18):2056–69. [PubMed: 25228648]
73. Hime GR, Brill JA, Fuller MT. Assembly of ring canals in the male germ line from structural components of the contractile ring. *J Cell Sci.* 1996; 109(Pt 12):2779–88. [PubMed: 9013326]
74. Barski A, et al. High-resolution profiling of histone methylations in the human genome. *Cell.* 2007; 129(4):823–37. [PubMed: 17512414]
75. Lin H, Yue L, Spradling AC. The *Drosophila* fusome, a germline-specific organelle, contains membrane skeletal proteins and functions in cyst formation. *Development.* 1994; 120(4):947–56. [PubMed: 7600970]

Figure 1. *E(z)* is required for GSC maintenance

(a) A schematic diagram of *Drosophila* adult testis. (b) Apical region of testes two days after clonal induction (ACI). Immunostaining with antibodies against Vasa (germ cell marker), Arm (hub and cyst cell marker), and GFP. GFP-negative cells (yellow outline) indicate *E(z)⁷³¹* mutant GSCs. Scale bar: 10µm. (c) Quantification of total GSC number and GFP-negative GSC number in control testes with GFP-negative WT GSCs vs. testes with GFP-negative *E(z)⁷³¹* mutant GSCs (Average ± S.E.M.). (d) Apical region of *nos-Gal4* control and *nos>E(z) shRNA* testis at 1-day (1D) and 28-day (28D) after eclosion. Immunostaining with antibodies against Vasa, FasIII (hub marker), and α-spectrin (marker of spectrosome and fusome, a germ cell-specific organelle [75]). Scale bar: 20µm. Asterisk: hub. (e) Quantification of GSC number of *nos-Gal4* control and *nos>E(z) shRNA* testis at 1-day (1D)

and 28-day (28D) after eclosion. *P*-value calculated using two-tailed Student's *t*-test. Data presented using the GraphPad Prism software.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2. *E(z)* is required for the germ cell dedifferentiation

Apical region of 28-day old (a) control testes and (b) *E(z)* knockdown testes.

Immunostaining using antibodies against γ -tubulin (γ -Tub, centrosome marker) and Vasa (germ cell marker). GSCs (white outline) with either oriented centrosome (yellow arrows in left image) or misoriented centrosome (yellow arrows in right image) are shown. Scale bar: 10 μ m. Asterisk: hub.

(c) Percentage of GSCs with misoriented centrosome in *nos-Gal4* control and *nos>E(z) shRNA* testes from 28-day old males.

P-value calculated using two-tailed Student's *t*-test.

Figure 3. E(z) is required in spermatogonial cells for dedifferentiation and GSC maintenance
(a) Apical region of *bam-Gal4* control and *bam>E(z) shRNA* testis from males 21D after eclosion. Immunostaining using antibodies against Vasa (germ cell marker), FasIII (hub marker), and H3K27me3. In *bam-Gal4* control flies, H3K27me3 is detected in GSCs and GBs (yellow arrowheads), spermatogonia (yellow arrow) and spermatocytes (red arrow). In *bam>E(z) shRNA* testis, H3K27me3 is detected in GSCs and SGs (yellow arrowheads), almost undetectable in spermatogonia (yellow arrow) far away from the hub (asterisk), and spermatocytes (red arrow). Scale bar: 20 μ m. Asterisk: hub. **(b)** Quantification of GSC number of *bam-Gal4* control and *bam>E(z) shRNA* testis from males 1-day (1D) and 21D

after eclosion, respectively. **(c)** Apical region control testes and *E(z)* knockdown testes from males 21D after eclosion. Immunostaining using antibodies against γ -tubulin (γ -Tub, centrosome marker), FasIII and Vasa. GSCs (white outline) with either oriented centrosome (yellow arrows in left image) or misoriented centrosome (yellow arrows in right image) are shown. Scale bar: 10 μ m. Asterisk: hub. **(d)** Percentage of GSCs with misoriented centrosome in *bam-Gal4* control and *bam>E(z) shRNA* testes from males 21D after eclosion. *P*-value calculated using two-tailed Student's *t*-test. Data presented using the GraphPad Prism software.

Figure 4. E(z) is required for the germ cell dedifferentiation during regeneration

Apical region of testes from (a, b) *hs-bam, nos-Gal4* control males and (c, d) *hs bam, nos>E(z) shRNA* testes after heat shock (a, c) and after recovery (b, d). Immunostaining against Vasa (germ cell marker), FasIII (hub marker), α -spectrin (marker of spectrosome and fusome, a germ cell-specific organelle [75]) and Traffic Jam (CySC and cyst cell marker). Scale bar: 20 μ m. Asterisk: hub. (e) Quantification of GSC number in control *hs-bam, nos-Gal4* testes and *hs-bam, nos>E(z) shRNA* testes after heat shock, five days and seven days after recovery (Average $\pm$ S.D.).

P-value calculated using two-tailed Student's *t*-test. Data presented using the GraphPad Prism software.

Figure 5.

Inactivating *E(z)* globally changes chromatin structure in mitotic cell-enriched *bam* testes. (a) Western blot shows significant decrease of the H3K27me3 signal in *E(z)⁶¹ bam* testes three days after temperature shift to restrictive condition. (b) Scatter plot shows that the H3K4me3-enriched genes increase in *E(z)⁶¹ bam* testes. The blue dashed lines indicate the statistical cutoff line $P < 0.05$ in a 1-kb window per 1 million total reads. Gene numbers in each quadrant are labeled in black. Red and green lines indicate the 2-fold change cutoff, and yellow line denotes no change. 3,788 genes are above the red line thus have 2-fold increase of H3K4me3 in *E(z)⁶¹ bam* testes; 199 genes are below the green line thus have 2-fold decrease of H3K4me3 in *E(z)⁶¹ bam* testes. (c) Scatter plot showed RNA Pol II-enriched genes modestly increase in *E(z)⁶¹ bam* testes. 971 genes are above the red line thus have 2-fold increase of Pol II in *E(z)⁶¹ bam* testes; 331 genes are below the green line thus have 2-fold decrease of Pol II in *E(z)⁶¹ bam* testes.