A sisRNA/miRNA Axis Prevents Loss of Germline Stem Cells during Starvation in Drosophila

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SUMMARY

Animal reproduction responds to nutritional status. During starvation, Drosophila and Caenorhabditis elegans enter a period of reproductive diapause with increased apoptosis, while maintaining a stable pool of germline stem cells (GSCs). How GSCs are protected is not understood. Here, we show that a sisRNA/miRNA axis maintains ovarian GSCs during starvation in Drosophila. Starvation induces the expression of an ovary-enriched sisRNA sisR-2, which negatively regulates GSC maintenance via a fatty acid metabolism gene dFAR1. sisR-2 promotes the expression of bantam, which in turn inhibits the activity of sisR-2, forming a negative feedback loop. Therefore, bantam acts as a buffer to counteract sisR-2 activity to prevent GSC loss during starvation. We propose that the sisR-2/bantam axis confers robustness to GSCs in Drosophila.

INTRODUCTION

Noncoding RNAs (ncRNAs) have emerged as important players in gene regulation (Cech and Steitz, 2014; Kung et al., 2013; Rinn and Chang, 2012; Ghildiyal and Zamore, 2009; Matera et al., 2007). Recently, stable intronic sequence RNA (sisRNA) has been discovered in several organisms such as viruses, yeast, Drosophila, Xenopus, and mammals (Osman et al., 2016; Pek and Okamura, 2015). We are only beginning to appreciate the biological significance of these intronic transcripts, which are proposed to function as an added layer of gene regulation. sisRNAs have been suggested to regulate expression of their parental genes (host genes where the sisRNAs originate from), act as molecular sponges for proteins and microRNAs (miRNAs), and play a role in translational regulation (Osman et al., 2016). In Drosophila, two sisRNAs, sisR-1 and sisR-4, have been reported to regulate the expression of their parental genes (Pek, 2018; Tay and Pek, 2017; Pek et al., 2015). It remains unclear whether sisRNAs also engage in other forms of feedback loops to modulate gene expression. On the other hand, miRNAs are small ncRNAs that play important roles in many biological processes by regulating the expression of their target genes post-transcriptionally (Bushati and Cohen, 2007). They are often engaged in regulatory feedback/feedforward loops to fine-tune gene expression, and confer robustness in response to stress (Posadas and Carthew, 2014; Ebert and Sharp, 2012; Herranz and Cohen, 2010). Regulatory crosstalks between sisRNAs and miRNAs have not been reported.

Reproduction is highly sensitive to changes in nutritional status. Some organisms have evolved to reduce their reproductive capacity as a way to conserve resources (Tatar et al., 2001). Studies in the nematode worm Caenorhabditis elegans and Drosophila had revealed that, during starvation, animals enter a period of reproductive diapause that halts germline activity (Angelo and Van Gilst, 2009; Drummond-Barbosa and Spradling, 2001). In both cases, although much of the germline undergoes apoptosis, there is little or no loss of germline stem cells (GSCs). The molecular pathways ensuring the protection of GSCs under starvation are poorly understood. Currently, ncRNAs such as bantam, miR-184, sisR-1, and Piwi-interacting RNAs are known to play intrinsic roles in the regulation of GSC maintenance (Rojas-Ríos et al., 2017; Wong et al., 2017; Lovino et al., 2009; Yang et al., 2009; Shcherbata et al., 2007). Whether ncRNA regulatory axes confer robustness in GSCs is not explored.

RESULTS

sisR-2 Regulates the Number of GSCs

We focused on sisR-2, an ovary-enriched sisRNA from the mushroom bodies tiny (mbt) gene locus (Ng et al., 2018; Pek et al., 2015). To examine the function of sisR-2, we cloned the full-length sequence of sisR-2 by performing 5′ and 3′ rapid amplification of cDNA ends (Figures 1A and S1A) and generated two independent transgenic shRNA flies (Figure 1A). Using a germline-specific driver, knockdown was specific to sisR-2 but not the parental gene mbt mRNA or the Mbt protein (Figures S1B and S1C). Furthermore, RNAi against the mbt exon reduced mbt mRNA level but had no effect on the abundance of sisR-2 (Figures 1A and S1B). These results demonstrate that sisR-2 RNAi was specific to sisR-2 and did not target the mbt pre-mRNA.

Knockdown of sisR-2 using both RNAi lines resulted in a significant increase in the number of eggs laid per female
compared with sibling controls (Figure 1B), suggesting a role in oogenesis. The *Drosophila* ovary consists of several ovarioles, which are strings of progressively developing egg chambers. These egg chambers originate from the germarium, located at the anterior end of the ovariole. The anterior tip of the germarium contains two to three GSCs that can be identified by their location next to the cap cells and the presence of spherical spectrosomes (visualized using HTS or α-Spectrin staining) (Figure 1C) (Kai and Spradling, 2003). To understand how *sisR-2* regulates egg production, we first counted the number of ovarioles in the *sisR-2* RNAi flies, and did not observe any significant differences (Figure S1D). By staining with antibodies against α-Spectrin and Vasa (a germline marker), we observed an...
Figure 2. *sisR-2* Regulates GSC Maintenance via *dFAR1*

(A) Predicted secondary structure of *sisR-2*. Regions that base pair with *dFAR1* and *bantam* are indicated.

(B) Sequence of the exposed 3' end tail of *sisR-2*, indicating potential base pairing with *dFAR1*.

(C) The *dFAR1* locus showing regions of predicted *sisR-2* base pairing, primers, transposon insertion (mutant), and targeted by RNAi construct.

(D and L) Confocal images showing the germaria of the indicated genotypes with α-Spectrin (green) and Vasa (red).

(E and M) Chart showing the percentage of germaria with the indicated number of GSCs in different genotypes shown in (D and L), respectively. Fisher's exact test was performed. N = 21–30 germaria.

(F) Heatmap showing the relative levels of *sisR-2* and *dFAR1*. Red, high expression; white, low or undetectable expression.

(G) qPCR showing the relative levels of *dFAR1* in ovaries of the indicated genotypes.

(H) Representative RT-PCR showing the levels of *dFAR1* in ovaries of *sisR-2* RNAi flies.

(I) Graph showing the relative levels of *dFAR1* normalized to *act5C*, as shown in (H). Student's t test was performed. Error bars depict SD. N = 3 biological replicates.

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increase in the number of germaria with more GSCs in \textit{sisR-2} RNAi ovaries (Figures 1D and 1E). We only counted GSCs that were in close contact with the cap cells, to avoid including the cystoblasts that also contain spectrosomes (Figure 1C). In \textit{nos > sisR-2 RNAI-1} ovaries, 77% of the germaria counted had >2 GSCs, compared with only 55% in \textit{nos/+} (TM3) control ovaries. Similarly, in \textit{nos > sisR-2 RNAI-2} ovaries, 58% of the germaria counted had >2 GSCs, as compared with only 47% in \textit{nos/+} (CyO) control ovaries (Figures 1D and 1E). Consistently, by using pMad, a marker for active bone morphogenetic protein signaling in GSCs, we observed more germaria with increased pMad-positive GSCs in \textit{sisR-2 RNAI-2} ovaries (Figures S1E and S1F). Furthermore, the expression of \textit{sisR-2} was upregulated in cystoblasts (bam mutants) when compared with GSCs (\textit{dpp} overexpression), consistent with a role in promoting GSC differentiation (Figures S1G and S1H). Taken together, we conclude that \textit{sisR-2} negatively regulates GSC maintenance.

As \textit{sisR-2} is maternally deposited into \textit{Drosophila} oocytes, we asked if maternal \textit{sisR-2} also functions to regulate the number of GSCs in the progeny (Pek et al., 2015). We first generated a stable stock of \textit{sisR-2 RNAI} flies, whereby females from this stock would deposit reduced levels of \textit{sisR-2} into their oocytes. We crossed these females with wild-type males and counted the number of GSCs in their female progeny (Figure S1I). Flies with reduced levels of maternally deposited \textit{sisR-2} (maternal RNAI only) had an increase in GSCs (Figures 1F, 1G, and S1I). Interestingly, zygotic knockdown of \textit{sisR-2} in these flies (maternal and zygotic RNAI) had an additive increase in the number of GSCs (Figures 1F and 1G), suggesting that both maternal and zygotic \textit{sisR-2} functions to repress GSC number.

\textit{sisR-2} Regulates GSC Maintenance via dFAR1

To characterize the molecular function of \textit{sisR-2}, we predicted its secondary structure using the Vienna RNAfold software. \textit{sisR-2} was predicted to form a secondary structure with a protected 3' end consisting of several stable hairpins and an exposed 3' end tail (Figure 2A). The secondary structure is consistent with a distinct 3' end and heterogeneous 3' ends (Figure S1A). The predicted structure of \textit{sisR-2} resembles that of \textit{sisR-1}, which represses the ncrNA \textit{ASTR}, possibly via base pairing of its 3' tail with the target (Wong et al., 2017; Pek et al., 2015). We hypothesized that \textit{sisR-2} may also regulate its target gene(s) in a similar manner. By performing a BLAST search, we found that the 20-nucleotide 3' end of \textit{sisR-2} can form an 18-nucleotide base pairing with the internal untranslated region of the bi-cistronic transcript encoding the protein coding genes \textit{CG10096} and \textit{CG10097} (Figures 2A–2C and S2A). Using the RNAhybrid program, we confirmed the stability of this base pairing with a minimum free energy of \(-24.8\) kcal/mol (Figure 2B) (Rehmsmeier et al., 2004). \textit{CG10096} and \textit{CG10097} are highly similar genes with \(~50\%) identity. For our analysis, we focused on \textit{CG10096} because of the availability of mutants for genetic analysis. \textit{CG10096} is one of several \textit{Drosophila} homologs identified for the two human \textit{fatty-acyl-CoA reductase} (FAR) genes, \textit{FAR1} and \textit{FAR2}, which are involved in ether lipid synthesis in the peroxisomes (Faust et al., 2012). We therefore named \textit{CG10096} as \textit{dFAR1}. We first asked if \textit{dFAR1} plays a role in the regulation of GSCs. \textit{dFAR1} homozygous mutants displayed a GSC loss phenotype suggesting that \textit{dFAR1} regulates GSC maintenance (Figures 2D, 2E, and S2B). Since \textit{sisR-2} exhibit spatial and temporal expression patterns (Pek et al., 2015), we next asked if its predicted target, \textit{dFAR1} display reciprocal expression patterns. We made use of the modENCODE temporal and tissue expression data in FlyBase, and observed that \textit{dFAR1} exhibited a mutually exclusive temporal and spatial expression patterns to \textit{sisR-2}, suggesting that \textit{sisR-2} negatively regulates \textit{dFAR1} (Figure 2F). We then verified that \textit{dFAR1} is indeed present in GSC-like cells by examining the levels of \textit{dFAR1} in the ovaries overexpressing \textit{dpp} (Figure S1H). Expression of \textit{dFAR1} is higher in the ovaries overexpressing \textit{dpp} compared with control whole ovaries, indicating that \textit{sisR-2} and \textit{dFAR1} are co-expressed in the same cells (Figure 2G). To investigate whether \textit{sisR-2} regulates \textit{dFAR1} in vivo, we examined the levels of \textit{dFAR1} in the ovaries of \textit{sisR-2} RNAI flies. Knockdown of \textit{sisR-2} resulted in an upregulation of \textit{dFAR1} mRNA in the ovaries (Figures 2H and 2I). Since the \textit{FAR1} proteins in humans and \textit{Drosophila} show substantial similarity (Figure S2C), we used antibodies raised against a peptide containing residues 7–149 of human \textit{FAR1} to investigate the levels of \textit{dFAR1} in \textit{sisR-2 RNAI} flies (Figure 2C). A reduced amount of \textit{dFAR1} protein was detected in \textit{dFAR1} homozygous mutants, indicating that the human \textit{FAR1} antibody is able to detect \textit{dFAR1} (Figure S2D). Using this human \textit{FAR1} antibody, we observed an increase in the number of \textit{dFAR1} foci in the germaria of \textit{sisR-2} RNAI flies compared with controls (Figures 2J and 2K). In addition, the level of \textit{dFAR1} protein was also higher in \textit{sisR-2}}
Figure 3. *bantam* Regulates GSC Maintenance by Repressing *sisR-2* Activity

(A) Sequence of *bantam* indicating potential base pairing with *sisR-2*.

(B and D) Confocal images showing the germaria of the indicated genotypes with α-Spectrin (green) and Vasa (red).

(C and E) Chart showing the percentage of germaria with the indicated number of GSCs in different genotypes shown in (B and D), respectively. Fisher’s exact test was performed in (E). N = 28–44 germaria.

(F and G) qPCR showing the relative levels of *sisR-2* in ovaries of the indicated genotypes. Student’s t test was performed. Error bars depict SD. N = 3 biological replicates.

(H) qPCR showing the relative levels of dFAR1 in ovaries of the indicated genotypes. Student’s t test was performed. Error bars depict SD. N = 3 biological replicates.

(I) Confocal images showing the germaria of the indicated genotypes stained with GFP (green) and Vasa (red). Asterisks, GSCs. Arrowheads, somatic cells.

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RNAi ovaries (Figure S2D). Together, these results indicate that sisR-2 downregulates dFAR1 in the ovaries.

We next examined if sisR-2 regulates GSC maintenance by repressing dFAR1. Using an RNAi line (GD17564) designed to target two exons of dFAR1 (Figures 2C and S2E), we found that knockdown of dFAR1 could rescue the GSC phenotype in sisR-2 RNAi flies (Figures 2L and 2M). In contrast, no significant difference in GSC number was observed in the ovaries of dFAR1 knockdown flies in otherwise wild-type background compared with control (Figures S2F and S2G), confirming a specific genetic interaction between sisR-2 and dFAR1. Taken together, our data suggest that sisR-2 controls the number of GSCs in Drosophila by modulating dFAR1 (Figure 2N).

**bantam** Regulates GSC Maintenance by Repressing sisR-2 Activity

Since both sisRNAs and miRNAs are engaged in self-regulatory feedback loops, we considered the possibility of a sisR-2/miRNA axis (Tay and Pek, 2017; Pek et al., 2015; Posadas and Carthew, 2014; Ebert and Sharp, 2012; Herranz and Cohen, 2010). bantam has been reported to play a role in the maintenance of GSCs in the ovaries (Yang et al., 2009; Shcherbata et al., 2007). We identified a potential 17-nucleotide stable base pairing between bantam and sisR-2 (Figures 2A and 3A). Unlike canonical seed base pairing, the predicted interaction between bantam and sisR-2 is non-canonical, containing two G:U wobble base pairs. We first verified the cell-autonomous role of bantam in GSCs using transgenic flies expressing bantam sponge. Germline expression of bantam sponge led to the occurrence of germaria with no GSCs (Figures 3B and 3C). Conversely, germ-line overexpression of bantam resulted in an increase in the number of GSCs (Figures 3D and 3E). Thus, bantam plays a cell-autonomous role in maintaining GSCs.

We hypothesized that bantam may promote GSC maintenance by repressing the activity of sisR-2. Knockdown of sisR-2 could rescue the GSC loss phenotype observed in the bantam sponge flies, confirming that sisR-2 acts downstream of bantam in the regulation of GSCs (Figures 3B and 3C). Consistent with recent reports that non-canonical base pairing does not lead to target degradation (Gilot et al., 2017; Agarwal et al., 2015), we did not observe any up- or downregulation of sisR-2 in ovaries expressing bantam sponge or bantam, respectively (Figures 3F and 3G). These results suggest that bantam inhibits the activity, but not the abundance, of sisR-2. Furthermore, we observed an increase in dFAR1 levels in the ovaries of flies overexpressing bantam (Figure 3H). Our results are consistent with a model that bantam promotes GSCs by repressing the activity of sisR-2 (Figure 3N).

Next, we wondered if sisR-2 reciprocally regulates bantam, forming a feedback loop. By using a bantam sensor transgene to monitor the activity of bantam, we observed an increase in GFP signal in sisR-2 RNAi ovaries, indicating a reduction in bantam activity (Figures 3I, 3J, and S3). Similarly, knockdown of sisR-2 resulted in a downregulation of the primary bantam transcript in the ovaries, indicating that sisR-2 promotes the transcription of bantam (Figure 3K). We then asked if bantam acts downstream of sisR-2 in the regulation of GSCs. Overexpression of bantam was not able to enhance the GSC phenotype in sisR-2 RNAi flies (Figures 3L and 3M), thus consistent with our data that bantam acts upstream of sisR-2 in regulating GSCs (Figure 3N). Altogether, our data support a model for a sisR-2/bantam axis in the control of GSC maintenance (Figure 3N).

**sisR-2/bantam Axis Protects GSCs from Starvation**

During nutrient deprivation, Drosophila females exhibit a marked reduction in the rate of egg production. This is largely due to a drop in the rate of germline cell proliferation coupled with an increase in apoptosis at two checkpoints during oogenesis (Drummond-Barbosa and Spradling, 2001). The number of GSCs, however, remains unchanged, suggesting the presence of a mechanism that prevents loss of GSCs during starvation. We investigated the possibility that the sisR-2/bantam axis functions to protect GSCs during starvation. We monitored the expression of sisR-2 and bantam in ovaries of y w flies that were starved or fed with yeast. Interestingly, expressions of sisR-2 and bantam were both elevated in the ovaries of starved flies (Figures 4A, 4B, and S4A), while that of another germline-specific gene nanos was unchanged (Figure S4B), indicating that the activity of the sisR-2/bantam axis was upregulated during starvation. Interestingly, the expression of dFAR1 remained relatively unchanged (Figure S4C). We hypothesized that

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(J) Chart quantifying the fluorescence intensity in GSCs of the indicated genotypes shown in (I). Student’s t test was performed. Error bars depict SD. N = 3 germaria.

(K) qPCR showing the relative levels of pri-bantam in ovaries of sisR-2 RNAi flies. Student’s t test was performed. Error bars depict SD. N = 3 biological replicates.

(L) Confocal images showing the germaria of the indicated genotypes with α-Spectrin (green) and Vasa (red).

(M) Chart showing the percentage of germaria with the indicated number of GSCs in different genotypes shown in (L). Fisher’s exact test was performed. N = 25–27 germaria.

(N) Working model.

GSCs are marked by asterisks (*). Scale bars, 10 μm.
bantam acts as a buffer to counteract sisR-2 activity, thus preventing the repression of dFAR1 and the loss of GSCs during starvation. Consistent with previous reports, the number of GSCs in starved and fed y w flies had no significant differences (Figures 4C and 4D). Instead, bantam sponge flies were more sensitive to starvation by exhibiting a significant decrease in the number of GSCs (Figures 4C and 4D). As sisR-2 is upregulated during starvation, we wondered if flies overexpressing sisR-2 also display increased sensitivity to starvation. Indeed, we observed a decrease in the number of GSCs in flies overexpressing sisR-2 during starvation (Figures S4D–S4G). However, this decrease was not statistically significant, possibly due to the still intact negative feedback by bantam acting on sisR-2 in these flies (Figure 4E).

**DISCUSSION**

In summary, we have shown that the sisR-2/bantam feedback axis plays an important role in maintaining GSCs during starvation (Figure 4E). We show that sisR-2 regulates GSCs by modulating dFAR1. The functions of FAR genes in *Drosophila* have not been extensively characterized (Faust et al., 2012). dFAR1 is predicted to encode a peroxisomal protein that modulates fatty acid metabolism. This is particularly interesting in the light that fatty acid metabolism protects GSC loss during starvation in *C. elegans* (Angelo and Van Gilst, 2009), suggesting that lipid metabolism is a conserved downstream pathway.

In many animals, the highly conserved insulin-signaling pathway is responsible for coordinating changes in nutrition to the metabolic status and growth of the organism (Shim et al., 2013). In *Drosophila*, *Drosophila* insulin-like peptides (DILPs) are secreted by the brain in response to food. The *Drosophila* female germline is highly sensitive to changes in diet, and this is regulated partly via insulin signaling, which has been shown to promote both the maintenance and proliferation of GSCs (Hsu and Drummond-Barbosa, 2009). Since sisR-2 is elevated during starvation, whereas DILPs expression is reduced, it is tempting to speculate that the expression of sisR-2 is repressed by the insulin-signaling pathway.

One possible mode of sisR-2 action is to regulate dFAR1 mRNA decay and/or translation. Moreover, bantam may bind and regulate the activity of sisR-2 by remodeling sisR-2’s secondary structure. Finally, our study provides a paradigm for a negative feedback mechanism involving a miRNA and a sisRNA in preventing the loss of GSCs during nutritional stress. Analogous RNA/RNA regulatory cross-talks may be conserved in other species.

**Figure 4. sisR-2/bantam Axis Protects GSCs from Starvation**

(A and B) qPCR showing the relative levels of (A) sisR-2 and (B) pri-bantam in ovaries of y w flies raised in fed or starved conditions. Student’s t test was performed. Error bars depict SD. N = 3 biological replicates.

(C) Confocal images showing the germaria of the indicated genotypes, raised in fed or starved conditions, with α-Spectrin (green) and Vasa (red).

(D) Chart showing the percentage of germaria with the indicated number of GSCs in different genotypes shown in (C). Fisher’s exact test was performed. N = 40–43 germaria.

(E) Proposed model.

GSCs are marked by asterisks (*). Scale bars, 10 μm.
EXPERIMENTAL PROCEDURES

A detailed description of all methods is included in the Supplemental Information.

Fly Strains

Flies were maintained in standard cornmeal medium at 25°C. The following Gal4 drivers were used to drive UAS-transgene expression in the germline: MTD-Gal4 (Petrella et al., 2007), nanos-Gal4-VP16 (Van Doren et al., 1998), and vasa-Gal4 (gift from Y. Yamashita). Mbt RNAi (JF3311), and dFAR1[00276] were obtained from the Bloomington Stock Center. dFAR1 RNAi (GD17564) was obtained from Vienna Drosophila Resource Center. Bam[Δ86], c587-Gal4, and UAS-dpp were gifts from Y. Cai. UAS-bantam sponge, UAS-bantam.D, and bantam sensor were gifts from S. Cohen (Herranz et al., 2012; Brennecke et al., 2003). For collection of eggs, virgin females were fed with wet yeast for several days. For starvation experiments, newly eclosed flies were kept in vials containing 1% agarose, either fed with wet yeast or starved, for 3 days. Generation of dsRed-intron-myc overexpression flies was done as described previously (Pek et al., 2015). For generation of sisR-2 shRNA transgenic flies, shRNAs targeting sisR-2 were designed and cloned into Valium22 plasmid, performed as described previously (Ni et al., 2011). Sequences were chosen to avoid potentially off-target effects. Transgenic flies were generated by Genetic Services using phiC31 integrase-mediated insertion into 25C7 and 68A4 landing site (Bischof et al., 2007). Oligonucleotide sequences are available in Table S1. To compare the expression of sisR-2 between GSCs and the differentiated CBs, we used ovaries with tumorous germaria from c587>UAS-dpp and bam[Δ86]/bam[Δ86] flies. In c587>UAS-dpp flies, the tumorous germarium is filled with GSC-like cells (due to an expansion in niche signaling throughout the germarium) (Kai and Spradling, 2003; Xie and Spradling, 1998). bam[Δ86]/bam[Δ86] mutant flies also contain a tumorous germarium; however, their germarium is filled with CB-like cells instead. These cells are more like CBs as they are no longer receiving niche signaling (due to an expansion in niche signaling throughout the germarium) (Kai and Spradling, 2003; Xie and Spradling, 1998). bam[Δ86]/bam[Δ86] mutant flies also contain a tumorous germarium; however, their germarium is filled with CB-like cells instead. These cells are more like CBs as they are no longer receiving niche signals as they have left the niche, but they remain as single spermatocyte cells as the differentiation program in these cells cannot be turned on without bam expression (Chen and McKearin, 2003).

Immunostaining

Immunostaining was performed as described previously (Wong et al., 2017; Pek and Kai, 2011). Ovaries were fixed in a solution of 16% paraformaldehyde and Grace’s medium at a ratio of 2:1 for 20 min, rinsed, and washed with PBX solution (PBS containing 0.2% Triton X-100) three times for 10 min each, and pre-absorbed for 30 min in PBX containing 5% normal goat serum. Ovaries were incubated overnight with primary antibodies at room temperature, washed three times for 20 min each with PBX before a 4 hr incubation with secondary antibodies at room temperature. Ovaries were again washed three times for 20 min each with PBX. Primary antibodies used in this study are as follows: mouse monoclonal anti-α-Spectrin (3A9, 1:1; Developmental Studies Hybridoma Bank), guinea pig anti-Vasa (1:1,000) (Patil and Kai, 2010), rabbit anti-pMad (1:50; Cell Signaling Technology, cat. no. 9516), mouse anti-GFP (1:500, Invitrogen, monoclonal 3 × 10⁵, cat. no. A-11120), and rabbit anti-human FAR1 (1:100; Sigma-Aldrich, cat. no. HPA017322). Images were taken with a Carl Zeiss LSM 5 Exciter Upright microscope and processed using Adobe Photoshop.

Identification of GSCs

GSCs were identified based on the following criteria: presence of Vasa staining, position at the anterior tip of the germarium, next to the cap cells, and the presence of a single spherical spermatocyte stained using anti-α-Spectrin antibodies.

SUPPLEMENTAL INFORMATION

Supplemental Experimental Procedures, four figures, and one table and can be found with this article online at https://doi.org/10.7554/eLife.05005.

AUTHOR CONTRIBUTIONS

I.O. and J.W.P. conceived the project, performed the experiments, and wrote the paper.

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