Pelota controls self-renewal of germline stem cells by repressing a Bam-independent differentiation pathway

Rongwen Xi[1], Choung Doan[1], Dazhi Liu[1,2] and Ting Xie[1,2,*]

[1]Stowers Institute for Medical Research, 1000 E. 50th Street, Kansas City, MO 64110, USA
[2]Department of Anatomy and Cell Biology, University of Kansas School of Medicine, 3901 Rainbow Boulevard, Kansas City, KS 66160, USA
*Author for correspondence (e-mail: tgx@stowers-institute.org)

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Summary

In the *Drosophila* ovary, germline stem cell (GSC) self-renewal is controlled by both extrinsic and intrinsic factors. The Bmp signal from niche cells controls GSC self-renewal by directly repressing a Bam-dependent differentiation pathway in GSCs. *pelota* (*pelo*), which has been previously shown to be required for *Drosophila* male meiosis, was identified in our genetic screen as a dominant suppressor of the *dpp* overexpression-induced GSC tumor phenotype. In this study, we reveal the unexpected new role of Pelo in controlling GSC self-renewal by repressing a Bam-independent differentiation pathway. In *pelo* mutant ovaries, GSCs are lost rapidly owing to differentiation. Results from genetic mosaic analysis and germ cell-specific rescue show that it functions as an intrinsic factor to control GSC self-renewal. In *pelo* mutant GSCs, Bmp signaling activity detected by *Dad-lacZ* expression is downregulated, but *bam* expression is still repressed. Furthermore, *bam* mutant germ cells are still able to differentiate into cystocytes without *pelo* function, indicating that Pelo is involved in repressing a Bam-independent differentiation pathway. Consistent with its homology to the eukaryotic translation release factor 1α, we show that Pelo is localized to the cytoplasm of the GSC. Therefore, Pelo controls GSC self-renewal by repressing a Bam-independent differentiation pathway possibly through regulating translation. As Pelo is highly conserved from *Drosophila* to mammals, it may also be involved in the regulation of adult stem cell self-renewal in mammals, including humans.

Key words: Germline stem cells, Self-renewal, Pelota, Bmp, Differentiation, *Drosophila*

Introduction

A stem cell is characterized by its ability to self-renew and generate differentiated cells throughout the lifetime of an organism. Understanding how stem cell self-renewal is controlled is an important issue in stem cell biology and will help realize potentials of stem cell-based therapies. Studies from diverse systems indicate that stem cell self-renewal is controlled by both extrinsic factors (niche signals) and intrinsic factors (Fuchs et al., 2004; Spradling et al., 2001). Niche signals have been shown to control GSC self-renewal by directly repressing expression of differentiation-promoting genes in the *Drosophila* ovary (Chen and McKearin, 2003a; Song et al., 2004). Therefore, the identification of pathways and genes that repress stem cell differentiation is crucial for understanding how stem cell self-renewal is controlled.

In the *Drosophila* ovary, GSCs reside in a structure called the germarium, which is at the anterior end of an ovariole (Lin, 2002; Xie and Spradling, 2001). At the anterior tip of the germarium, three types of somatic cells (terminal filament cells, cap cells and inner sheath cells) constitute a niche that supports two or three GSCs (Lin, 2002; Xie and Spradling, 2001; Xie and Spradling, 2000) (Fig. 1A). One GSC divides to generate two daughter cells: the daughter cell maintaining contact with the cap cells through DE-cadherin-mediated cell adhesion renews itself as a stem cell, while the daughter cell moving away from the cap cells differentiates into a cystoblast (Song et al., 2002). The cystoblast divides four times with incomplete cytokinesis to form a 16-cell cyst, in which one cell becomes an oocyte and the rest become nurse cells (Spradling, 1993). Bmp signals produced by the niche, Dpp and Gbb, have essential roles in controlling GSC self-renewal, as reduction of Bmp signaling activity results in the loss of GSCs by differentiation and overexpression of *dpp* in the germarium produces GSC-like tumors (Song et al., 2004; Xie and Spradling, 1998). Bmps from the cap cells function as short-range signals that directly repress the transcription of *bam* in GSCs to maintain their self-renewal, and also allow cystoblasts lying one cell diameter away to differentiate (Chen and McKearin, 2003a; Song et al., 2004). *bam* is necessary and sufficient for germ cell differentiating in the *Drosophila* ovary (Ohlstein and McKearin, 1997). In addition, two other genes, *Yb* and *piwi*, function in the somatic niche cells to control GSC (Cox et al., 2000; King et al., 2001). *Yb* encodes a novel protein and directly regulates expression of *piwi* and *hh* in TFs; *hh* signaling also modulates GSC self-renewal though it is not essential (King et al., 2001). *piwi* encodes a family of conserved RNA-binding proteins and is required in the niche cells for controlling GSC self-renewal and inside GSCs for

their division (Cox et al., 1998; Cox et al., 2000). Two recent studies have shown that *piwi* also maintains GSC self-renewal by repressing *bam* expression through regulation of either the Bmp signaling pathway or a Bmp-independent signaling pathway (Chen and McKearin, 2005; Szakmary et al., 2005). However, it remains unclear how *piwi* controls GSC division intrinsically.

Two translational repressors, Nanos (Nos) and Pumilio (Pum), have been shown to be required for the maintenance of ovarian GSCs by preventing differentiation (Forbes and Lehmann, 1998; Wang and Lin, 2004). Pum/Nos repress differentiation of PGCs and GSCs through a Bmp-independent pathway, as their expression is not regulated by Bmp signaling and their mutations cannot suppress hyperactive Bmp signaling-induced PGC proliferation (Gilboa and Lehmann, 2004). It is likely that Nos and Pum are involved in repressing translation gene products that are important for germ cell differentiation and thereby for controlling GSC self-renewal. To identify further intrinsic factors that are required for Bmp-mediated GSC self-renewal, we performed a genetic screen to identify dominant suppressors of the *dpp*-induced GSC-like tumor phenotype (C.D. and T.X., unpublished). One of the suppressors is *pelota* (*pelo*), which has been studied for its role in the regulation of *Drosophila* male meiosis. Cellular and molecular analysis showed that *pelo* is required for the progression through meiosis in spermatogenesis and encodes an evolutionarily conserved protein that contains an eukaryotic release factor 1 α (eRF1α)-like domain at its C terminus (Eberhart and Wasserman, 1995). The studies on the yeast *pelo* homolog *dom34* suggest that Pelo is involved in translational regulation. Deletion of Dom34 causes growth retardation, defective sporulation and reduced polyribosomes (Davis and Engebrecht, 1998). *dom34* has a strong genetic interaction with *RPS30A*, which encodes ribosomal protein S30A; overexpression of RPS30A rescues the growth defects and reduced polyribosomes of *dom34* mutants (Davis and Engebrecht, 1998). Moreover, Dom34 specifically interacts with Hbs1, a small GTPase that is also implicated in translational regulation (Carr-Schmid et al., 2002). It has been recently shown that *pelo* knockout mice exhibit early embryonic lethality with defects in cell division and proliferation (Adham et al., 2003). Taken together, *pelo* may be involved in the regulation of meiosis and mitosis possibly through regulating translation. In this study, we have revealed an unexpected new role of Pelo in the control of GSC self-renewal and division in the *Drosophila* ovary, possibly through regulating translation.

Materials and methods

Constructs

To make a *UASp-pelo* construct, the *pelo* cDNA insert from an EST clone (AT07625) was subcloned to pBlueScript with restriction enzymes *Eco*RI and *Pst*I, and was then subcloned to a UASp vector with *Kpn*I and *Xba*I. The Gateway cloning technology (Invitrogen) was used to generate UASp-tagged *pelo* constructs. The destination vectors used in this study were kindly provided by Terence Murphy (for more information, see http://www.ciwemb.edu/labs/murphy/Gateway%20vectors.html). Briefly, the *pelo*-coding region was amplified from the EST using the *pfu* DNA polymerase (Stratagene). The PCR product was cloned into an ENTR vector using a TOPO gateway cloning kit (Invitrogen). The ENTR clone was subsequently used in combination with different destination vectors to generate constructs of the *pelo* gene with different tags under the control of UASp promoter using the LR reaction kit (Invitrogen). Inverse PCR mutagenesis was used to make mutations on the putative nuclear localization sequence. All constructs were confirmed by sequencing before they were introduced into flies.

Fly stocks and markers

Unless otherwise stated, flies were reared at 25°C on standard molasses-based food. The fly stocks used for this study include: $pelo^1$, a strong P-element-induced allele (Eberhart and Wasserman, 1995); bam^{86}, a null allele (McKearin and Ohlstein, 1995); Sec61α-GFP (ZCL0488) (Kelso et al., 2004); *Dad-lacZ* (Tsuneizumi et al., 1997); *bam-GFP* (Chen and McKearin, 2003b); *nosgal4VP16* (Van Doren et al., 1998); and *c587-gal4* (Song et al., 2004).

Generating marked clones

Marked clones were generated using the FLP-mediated FRT recombination technique according to the published procedures (Song et al., 2002; Xie and Spradling, 1998). These marked clones were analyzed and quantified 3 days, 10 days, 17 days and 24 days after clone induction (ACI). For examination of *Dad-lacZ* and *bam-GFP* expression in the marked $pelo^1$ mutant GSCs, the ovaries were analyzed 14 days ACI. The genotypes used for clonal analysis in this study are shown as follows:

(1) *hs-flp/+; FRT 40A* $pelo^1$*/ FRT40A arm-lacZ*
(2) *hs-flp/+; FRT 40A* $pelo^1$*/ FRT40A ubi-GFP; Dad-lacZ/ +*
(3) *hs-flp/+; FRT 40A* $pelo^1$*/ FRT40A arm-lacZ; bam-GFP/ +*.

Immunohistochemistry

Antibody staining of ovaries was performed using our published protocol (Song et al., 2002). The following antisera and dilutions were used: rabbit anti-β-galactosidase (1:100; Molecular Probes); mouse anti-β-galactosidase (1:50; Molecular Probes); rabbit anti-GFP (1:100; Molecular Probes); monoclonal anti-Hts 1B1 (1:4; DSHB); monoclonal anti-HtsRC (1:4; DSHB); monoclonal anti-Orb (1:30; DSHB); and rabbit anti-Vasa antibody (1:1000; a gift of Dr Paul Lasko). Secondary antibodies including goat anti-rabbit or anti-mouse IgG conjugated to Alexa 488 or Alexa 568 (Molecular Probes) were used at a dilution of 1:200.

ApopTag staining and BrdU labeling

Ovaries from 2-day-old females for ApopTag staining and BrdU labeling were dissected in Grace's medium. ApopTag in situ apoptosis detection kit (Serologicals, Clarkston, GA) was performed according to the manufacture's manual. For BrdU labeling, the ovaries were incubated in the medium at a final concentration at 75 μg/ml at room temperature for 1 hour. Fixation and BrdU detection were described previously (Zhu and Xie, 2003).

Results

pelo is required for controlling self-renewal of ovarian GSCs

As we reported previously, *dpp* overexpression in inner sheath cells driven by the *c587-gal4* driver completely blocks germ cell differentiation, resulting in the formation of GSC-like tumors and consequently female sterility (Song et al., 2004). Such female sterility is very sensitive to genetic changes of any *dpp* downstream components; for example, removal of one copy of any Bmp downstream gene such as *punt*, *mad* and *Med* can sufficiently reverse the sterility phenotype, leading to fertile females (Doan and Xie, data not shown). To identify the genes that are potentially involved in Bmp signaling in GSCs, we performed a dominant suppressor screen using the existing

Table 1. *pelo* is essential for the maintenance of GSCs in the *Drosophila* ovary

	pelo^{1}/CyO				*pelo*^{1}/*pelo*^{1}			
	No. GSC/GM*			Average number of cysts/germarium†	No. GSC/GM			Average number of cysts/germarium
	0	1	⩾2		0	1	⩾2	
2 days	0	2	38	8.95 (*n*=40)	7	47	42	3.00 (*n*=89)
7 days	0	0	40	8.98 (*n*=40)	51	34	4	0.50 (*n*=38)

*Number of GSCs per germarium.
†Average number of germline cysts per germarium, calculated as the total number of cysts in the germaria divided by the total number of germaria. Germaria containing at least one GSC were chosen for analysis, and the total number of germaria counted is shown in parentheses.

deficiency kit, which covers 65% of the *Drosophila* genome. In the screen, we have identified a small deficiency, Df(2L)s1402 (30C-30F), that can rescue *dpp* overexpression-induced female sterility. Among 19 existing mutations in the genomic region, *pelota* is identified as dominantly suppressing *dpp* overexpression-induced sterility (Doan and Xie, data not shown). Although it has been identified for its role in *Drosophila* male meiosis, its mutant females are also semi-fertile, and ovaries are overtly small (Eberhart and Wasserman, 1995), suggesting that it is also involved in the regulation of *Drosophila* oogenesis. However, it remains unclear whether the *pelo* mutation affects GSCs.

To determine if *pelo* is required for maintaining GSCs, we quantified GSCs in *pelo*^{1} homozygous mutant germaria. *pelo*^{1} is a P-element-induced strong or null allele, which is based on the evidence that it produces a truncated *pelo* transcript deleting most of the eRF1α domain (Eberhart and Wasserman, 1995). The ovaries from 2-day-old or 7-day-old *pelo*^{1} homozygous and heterozygous females were immunostained with anti-Vasa and anti-Hts antibodies. Vasa is expressed specifically in germ cells (Lasko and Ashburner, 1988), while Hts is preferentially rich in spectrosomes (GSCs and cystoblasts), fusomes (2-, 4-, 8- and 16-cell germline cysts) and membranes of somatic follicle cells in the germarium and egg chambers (Lin et al., 1994). GSCs can be reliably identified at the tip of the germarium by their anteriorly localized spectrosome and direct contact with cap cells (Lin, 2002; Xie and Spradling, 2001). In *pelo*^{1} heterozygous control females, 2-day-old and 7-day-old germaria had an average of 2.35 and 2.40 GSCs, respectively, and more than 97% of the germaria contained two or more GSCs (Table 1; Fig. 1B), which closely resembles wild-type. The 2-day-old *pelo*^{1} homozygous germaria contained an average of 1.4 GSCs, with some having two GSCs (Fig. 1C) and the other containing only one GSC (Fig. 1D), indicating that *pelo* is required for GSC establishment or GSC maintenance. Furthermore, the 7-day-old *pelo*^{1} germaria had an average of 0.47 GSCs with 57.3% (51/89) of them containing no GSC (Table 1; Fig. 1E). Consistent with the previous study showing that *pelo*^{1} is a strong or null allele (Eberhart and Wasserman, 1995), the *pelo*^{1}*/df(2L)s1402* mutant ovaries behaved just like the homozygous *pelo*^{1} mutant ovaries in terms of GSC numbers at different ages (Fig. 2E). This also suggested that the mutation in *pelo* is responsible for GSC loss phenotype in the *pelo*^{1} homozygous mutant ovaries. Together, these results demonstrate that Pelo is required for maintaining GSCs in the *Drosophila* ovary.

Pelo could maintain GSCs by controlling either self-renewal or survival. Thus, the GSC loss in *pelo* mutant ovaries could be due to either differentiation or cell death. To differentiate these two possibilities, we examined cell death of GSCs in the *pelo* mutant ovaries using the ApoTag cell death labeling system, which has been successfully used in our previous studies (Zhu and Xie, 2003). In this experiment, the spectrosomes and fusomes were also labeled for facilitating identification of GSCs, cystoblasts and germline cysts. Among

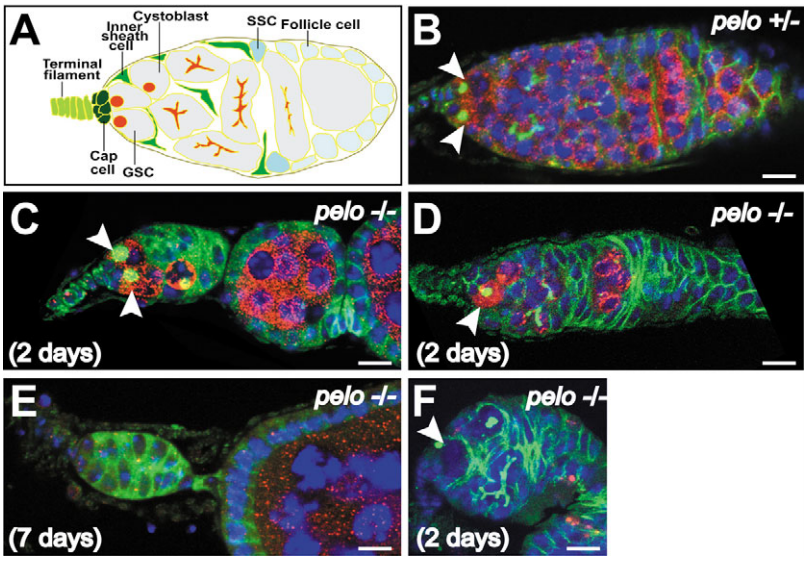
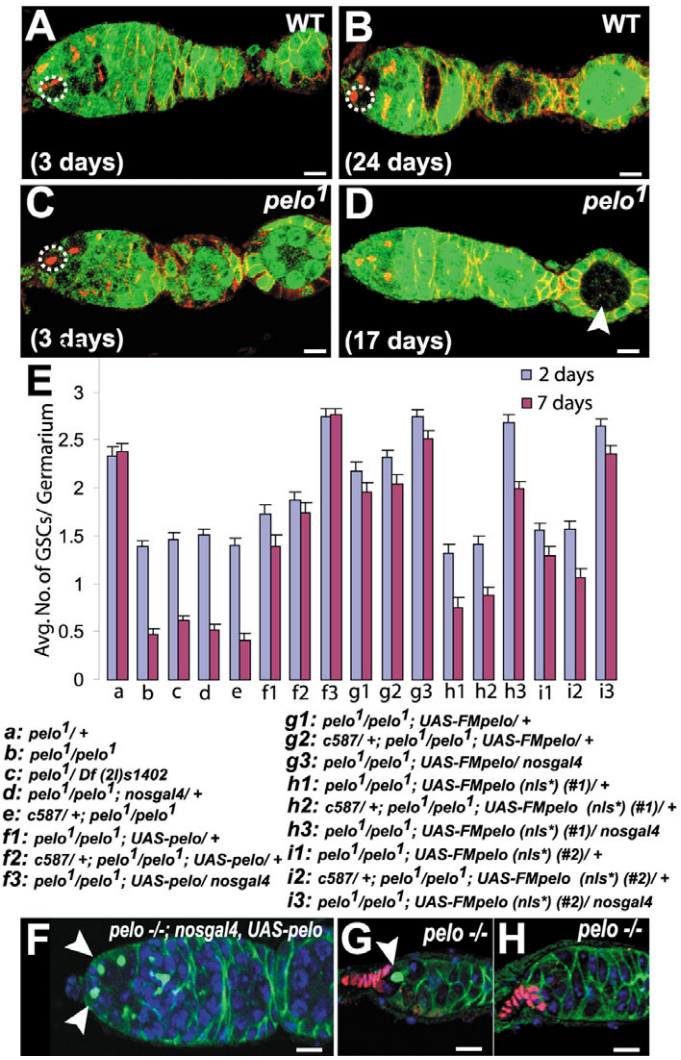


Fig. 1. *pelo* is required for controlling ovarian GSC self-renewal. All germaria or ovarioles in this and subsequent figures represent one confocal section and are shown with anterior towards the left. (A) A schematic diagram of the germarium. Red circles indicate spectrosomes (i.e. GSCs and cystoblasts) and branched red structures indicate fusomes (i.e. germ cell cysts). Ovarioles from *pelo*^{1} homozygous mutant (C-E) and heterozygous control (B) flies labeled for Vasa (red, germ cells) and for Hts (green, spectrosomes and fusomes). (B-E) Germaria contain two GSCs (B) (indicated by arrowheads), two GSCs (C), one GSC (D) and no GSCs (E). (F) A *pelo*^{1} mutant germarium labeled by TUNEL (red), showing that the GSC (arrowhead) was not apoptotic but there are some dying follicle cells (red) in the posterior. Scale bars: 10 μm.

156 *pelo*^{1} heterozygous control germaria, no dying GSCs (>250 GSCs examined) were observed, and there were eight dying cysts. This is consistent with the previous result that some germline cysts die naturally (Drummond-Barbosa and Spradling, 2001). Among 264 *pelo*^{1} homozygous germaria, there were also no dying GSCs detected (>300 GSCs examined) but 49 dying cysts were observed (Fig. 1F). Clearly, the *pelo* mutation does not affect GSC survival but appears to increase dying cysts [from 5% of the 2-day-old wild-type germaria carrying dying cysts (n=40) to 18% of the 2-day-old *pelo*^{1} mutant garmaria carrying dying cysts (n=89)]. These observations suggest that Pelo is required for GSC self-renewal and also for cyst survival.

We also noticed that the *pelo* mutant germaria that still harbored two GSCs were extremely small and contained very few germline cysts (Fig. 1C). As the *pelo* mutation resulted in only 18% of the germaria carrying one or more dying cysts, the *pelo* mutant GSC division rate probably also decreased. Then, we used BrdU incorporation to label S-phase cells to determine whether *pelo* is required for controlling GSC division. In the 2-day-old females, 10.7% of *pelo* heterozygous control GSCs (n=525) were BrdU positive, whereas only 2.5% of *pelo* homozygous GSCs incorporated BrdU (n=202). This result shows that *pelo* is also required for controlling GSC division.



pelo is required intrinsically for controlling GSC self-renewal

Pelo could control GSC self-renewal by acting either inside the GSC, in the niche or both. Our mRNA in situ hybridization and gene expression profiles of agametic ovaries show that *pelo* mRNAs were ubiquitously expressed at lower levels throughout the germarium, suggesting that *pelo* could function in GSCs or the somatic niche cells, or both (data not shown). We used FLP-FRT-mediated mitotic recombination to determine whether Pelo functions inside GSCs for controlling their self-renewal (Xu and Rubin, 1993). The FLP-mediated FRT recombination has been used to generate marked mutant GSCs and determine their loss rates for deducing the role of a particular gene in GSC maintenance (Xie and Spradling, 1998). According to published experimental procedures (Xie and Spradling, 1998; Song et al., 2002), the ovaries of the females of appropriate genotypes were dissected at 3, 10, 17 and 24 days after clone induction (ACI) mediated by heat-shock treatments, and marked wild-type and *pelo* mutant GSCs were identified by the lack of *arm-lacZ* expression and the presence of an anteriorly anchored spectrosome. In the wild-type control, 55.0% of the marked GSCs (from 52.8% of the germaria carrying one or more marked control GSCs at 3 days ACI to 28.9% of the germaria carrying one or more marked control GSCs at 24 days ACI) were still maintained for 3 more weeks (Xie and Spradling, 1998) (Fig. 2A,B). By contrast, 97% of the marked *pelo* mutant GSCs (from 48.6% of the germaria carrying one or more marked mutant GSCs at 3 days ACI to 1.7% of the germaria carrying one or more marked mutant GSCs at 24 days ACI) were lost during the same 3-week period (Table 2; Fig. 2C,D). These results demonstrate that *pelo* is required in GSCs for controlling their self-renewal. It appears that these marked *pelo* mutant GSCs are lost slower than the GSCs in the homozygous *pelo* mutant ovaries. This could be explained by the possibility that Pelo is very stable or has functions in both GSCs and soma. If Pelo is very stable, it

Fig. 2. *pelo* functions in the cytoplasm of GSCs to control their self-renewal. The tips of the ovarioles (A-D) are labeled for *lacZ* (green) and Hts (red); the marked GSCs (broken lines) are identified by loss of *lacZ* expression and presence of a spectrosome on their anterior side. (A,B) Germaria showing the presence of 3-day-old (A) and 24-day-old (B) marked wild-type GSCs. (C) A germarium carrying a marked 3-day-old *pelo*^{1} homozygous GSC. (D) A germarium showing loss of a marked *pelo*^{1} mutant GSC evidenced by the presence of a marked germline cyst (arrowhead) in an egg chamber 17 days ACI. (E) Quantitative analyses of *pelo*^{1} GSC loss and phenotypic rescues by different *pelo* transgenes, which carry wild-type or mutant (nls*) epitope-tagged *pelo*. The *x*-axis shows different genotypes, while the *y*-axis indicates the average GSC number with a standard error. The blue bars indicate GSC numbers of the 2-day-old germaria of different genotypes, while the red bars represent GSC numbers of 7-day-old germaria. For each genotype at either time point, 40-130 germaria were examined. (F) A germarium with two GSCs (arrowheads) labeled for Hts (green) and DAPI (blue, nuclei) showing that germline expression of *pelo* is sufficient to rescue GSC loss in *pelo*^{1} mutants. (G,H) Mutant germaria with one (G) or no (H) GSC labeled for *hh-lacZ* (red) and Hts (green), showing the normal number of cap cells. Scale bars: 10 μm.

Table 2. *pelo* is required intrinsically for controlling the maintenance of GSCs but not SSCs in the *Drosophila* ovary

	Genotypes	3 days	10 days	17 days	24 days
Marked GSCs	Wild type	52.8%* (216)	45.3% (265)	31.0% (229)	28.9% (214)
	pelo^{1}	48.6% (212)	33.5% (197)	19.3% (202)	1.7% (181)
Marked SSCs	Wild type	68.1%† (216)	55.9% (265)	46.7% (229)	46.7% (214)
	pelo^{1}	74.1% (212)	63.0% (197)	52.5% (202)	46.0% (181)

*Percentage of germaria containing marked GSCs, total number of germaria counted is shown in parentheses.
†Percentage of germaria containing marked SSCs.

takes longer for residual wild-type Pelo that is made before clone induction to be degraded in the marked *pelo* mutant GSCs. Later, we will try to address whether *pelo* has a function in the soma to control GSC maintenance.

To confirm further that Pelo is indeed required intrinsically for controlling GSC self-renewal and that the *pelo* mutation is responsible for the GSC loss phenotype, we expressed a *UASp-pelo* transgene specifically in the germ cells using a germ cell-specific GAL4 driver, *nos-gal4VP16*, in *pelo* mutant ovaries. A *UASp-pelo* construct was made to be expressed in germline or soma using different GAL4 drivers (Rorth, 1998); *nosgal4-VP16* can drive a UAS transgene to be expressed in both GSCs and later germ cell cysts (Van Doren et al., 1998). Interestingly, introduction of one copy of *UASp-pelo* transgene alone into the *pelo* homozygous females was able to partially rescue the GSC loss phenotype, suggesting that there is a leaky expression of the transgene even without a GAL4 driver (Fig. 2E). The stem cell loss phenotype in *pelo* mutant ovaries was fully rescued by *nosgal4VP16*-driven *pelo* expression in the germline, including GSCs, restoring the normal GSC number (Fig. 2F). This result shows that the mutation in *pelo* is responsible for the GSC loss phenotype, and further confirms that *pelo* is required intrinsically for controlling GSC self-renewal.

Pelo has no obvious role in the somatic cells of the germarium including SSCs

Although *pelo* is required intrinsically for controlling GSC self-renewal, it does not rule out the possibility that a somatic function of *pelo* is also involved in regulating GSC self-renewal as it is also expressed in the somatic cells of the germarium. First, we examined whether the *pelo* mutation affects the survival of cap cells. A *hh-lacZ* enhancer trap line used in this study has been used to label terminal filament cells and cap cells in the *Drosophila* ovary (Forbes et al., 1996). In the *pelo* homozygous germaria carrying one or no GSCs, the cap cell number appeared to be normal, ranging from five to seven (Fig. 2G,H), indicating that Pelo function is at least not required for the formation or survival of cap cells. However, *pelo* could still be required in the surrounding somatic cells for controlling GSC function through regulating production of signals. To further test whether *pelo* has a somatic function in GSC regulation, we used the *c587-gal4* driver, which is expressed in IGS cells and follicle progenitor cells, and the same *UASp-pelo* transgene to test whether somatic expression *pelo* can rescue the *pelo* mutant GSC loss phenotype. However, c587-driven *pelo* expression in the somatic cells could only confer very limited rescue of the *pelo* mutant GSC loss phenotype, in addition to the partial rescue conferred by the *UAS-pelo* transgene alone, suggesting that *pelo* has little role in IGS cells and follicle cell progenitors for GSC self-renewal (Fig. 2E). As we have not tested whether *pelo* expression in terminal filaments/cap cells can mitigate the GSC loss phenotype of the *pelo* mutant ovaries, our results could not completely rule out the possibility that Pelo has a function in somatic cells for controlling GSC self-renewal.

As *pelo* is expressed in all the somatic cells, including SSCs, we then determined whether *pelo* has a role in SSC maintenance using FLP-mediated FRT recombination to generate marked *pelo* mutant SSC clones. The marked SSCs were identified as the *arm-lacZ*-negative somatic cells residing at the 2a/2b boundary and generating *arm-lacZ*-negative (marked) follicle cells in the germarium and egg chambers, according to our previous studies (Song and Xie, 2002; Song and Xie, 2003). In the control, 68% of marked wild-type SSCs were maintained for 3 weeks, supporting the fact that SSCs have a slow natural turnover (Table 2). Similarly, 62% of the marked *pelo* SSCs were maintained for 3 weeks, indicating that *pelo* plays little or no role in SSC maintenance (Table 2). The marked *pelo*^{1} mutant follicle cell clones exhibited a very minor phenotype: they appeared slightly thinner compared with wild-type follicle cells (data not shown). Although *pelo* is ubiquitously expressed throughout the germarium, the main function of *pelo* is primarily restricted to GSCs and their progeny in the ovary.

Pelo protein is localized to the cytoplasm for controlling GSC self-renewal

Drosophila Pelo has a putative nuclear localization signal sequence (PRKRK) at its N terminus (Eberhart and Wasserman, 1995; Nair et al., 2003); this sequence is perfectly conserved from *Drosophila* to human, raising an interesting possibility that Pelo is a nuclear protein. If Pelo indeed functions in the nucleus, we would expect that the disruption of the putative nuclear localization sequence leads to loss of its function. To directly test the idea, we generated a mutant version of Pelo with the replacement of PRKRK by RSRS as ablation of helix-breaking residue proline and reduction of basic residues can abolish the function of the nuclear localization signal (Conti et al., 1998). In S2 cells, the mutant Pelo protein tagged with 3xFlag and 6xMyc at its N terminus [F-M-Pelo(nls*)] was localized in the cytoplasm in the same way as the wild-type version tagged with the same tags at its N terminus (F-M-Pelo) (data not shown). To further determine whether the NLS of Pelo is important for Pelo function in controlling GSC self-renewal in vivo, we generated transgenic flies carrying either UASp-F-M-Pelo or UASp-F-M-Pelo(nls*). Two independent insertion lines for UAS-F-M-Pelo could fully rescue the *pelo* mutant GSC loss phenotype when they were driven to be expressed specifically in the germ cells by *nos-gal4VP16*, indicating that FLAG and MYC tags do not

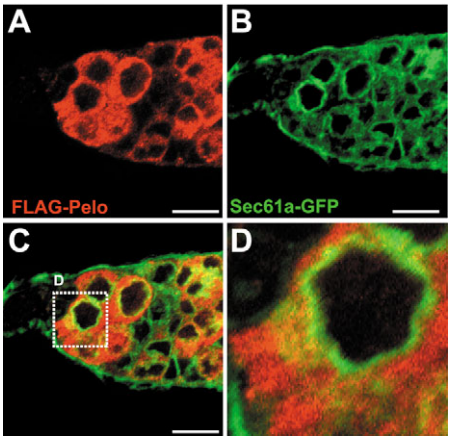


Fig. 3. Pelo is primarily localized in the cytoplasm of germ cells. (A-D) A *nos-gal4VP16*; *UAS-FLAG-Pelo*; *Sec61a-GFP* germarium labeled for FLAG (red, A) and GFP (green, B). The merged image (C) shows FLAG-tagged Pelo is localized primarily in the cytoplasm but is not particularly rich in ERs (green). (D) The enlarged area of a GSC marked in C. Scale bars: 10 μm.

interfere with Pelo function (Fig. 2E). Similarly, two independent transgenic lines of the NLS mutated version of Pelo could also fully rescue the *pelo* GSC loss phenotype (Fig. 2E), further supporting that the putative NLS is not important for Pelo function in GSCs and that Pelo functions in the cytoplasm to control GSC self-renewal (Fig. 2E). Interestingly, one of the transgenic lines [UASp-FM(nls*)#1] showed complete rescue for the *pelo* GSC loss phenotype with the *nos-gal4VP16* driver, whereas it exhibited little rescue for the GSC loss phenotype of the *pelo* mutant ovaries with the *c587* driver or without any *gal4* driver, further supporting our earlier conclusion that the mutation in the *pelo* gene is responsible for the GSC loss (Fig. 2E).

As the tagged Pelo is functional, we further determined the Pelo subcellular localization in GSCs using Flag or Myc antibodies. As in S2 cells, Pelo was mainly expressed in the cytoplasm in the germ cells (Fig. 3A). As Pelo protein has a domain showing homology to translation release factor 1a (RF1), we next examined whether Pelo is associated with the ER. Sec61α-GFP, a GFP fusion protein localized to ER membrane, was localized to ER membrane rich in the peri-nuclear area and spectrosome area of the GSC (Snapp et al., 2004) (Fig. 3B), while Pelo was evenly distributed throughout the cytoplasm of the GSC (Fig. 3C,D). There was only limited pattern overlap between Sec61α-GFP and F-M-Pelo, indicating that Pelo is primarily localized to the cytoplasm away from the ER membrane (Fig. 3D). Consistently, Pelo was also localized to the cytoplasm in S2 cells (data not shown). These findings show that Pelo functions in the cytoplasm of GSCs to control their self-renewal.

pelo modulates *dpp* pathway activity in GSCs

As discussed earlier, *pelo* was identified in a genetic screen looking for genes that can suppress *dpp* overexpression-induced GSC-like tumors, suggesting that *pelo* must somehow genetically interact with the *dpp/Bmp* pathway. To further reveal the relationship between *pelo* and Bmp signaling, we carefully examined the dose effect of *pelo* on *dpp*-induced GSC-like tumor formation. As reported previously (Xie and Spradling, 1998; Song et al., 2004), all the ovarioles overexpressing *dpp* by the *c587 gal4* driver (n=77) contained only single germ cells resembling GSCs (Fig. 4A). Among the *dpp*-overexpressing ovarioles also carrying one copy of the $pelo^1$ mutation, 36% of them (n=206) showed the same tumor phenotype, but the rest of the ovarioles contained differentiated germline cysts, developing egg chambers and even mature eggs (Fig. 4B), which could explain why *pelo* was identified in our suppressor screen. Among the *dpp*-overexpressing ovarioles also carrying two copies of the $pelo^1$ mutations, only 13.8% of them (n=261) contained only GSC-like single germ cells, 49.8% of them had a mixture of single germ cells and developing cysts (Fig. 4C). Interestingly, the rest (36.4%) were

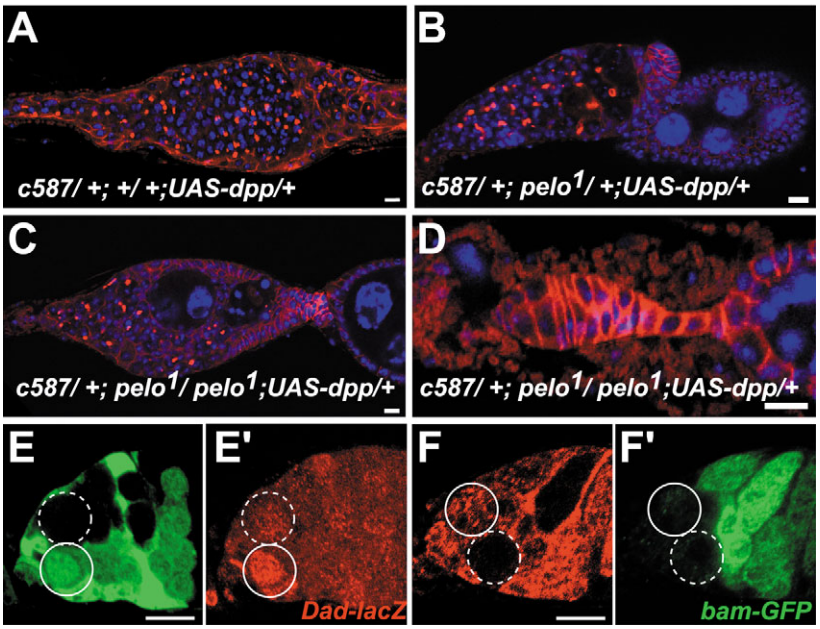


Fig. 4. *pelo* modulates *dpp* pathway activity, but is dispensable for *bam* suppression in GSCs. Germaria (A-D) are labeled for Hts (red) and DAPI (nuclei, blue). (A) A germarium showing that *dpp* overexpression causes accumulation of GSC-like cells. (B) A germarium showing that removal of one copy of functional *pelo* results in partial suppression of the GSC-like tumor phenotype induced by *dpp* overexpression, which is evidenced by the presence of egg chambers. (C,D) Germaria showing that removal of both copies of *pelo* partially (C) or completely (D) suppresses the tumor phenotype. (E,E′) A germarium labeled for GFP (green) and *lacZ* (red), showing that a marked *pelo* mutant GSC (broken lines, black in E) downregulates *dad-lacZ* expression (red, E′) in comparison with its neighboring wild-type GSC (solid circle, green). (F,F′) A germarium labeled for GFP (green) and *lacZ* (red), showing that a marked *pelo* mutant GSC (broken lines, black in F) does not upregulate *bam-GFP* expression (F′) in comparison with its neighboring wild-type GSC (solid circle, red). Scale bars: 10 μm.

reminiscent of the *pelo* GSC loss phenotype only (Fig. 4D). These results suggest that *pelo* functions as one of the Bmp downstream components or in a pathway parallel to the Bmp signaling pathway to control GSC self-renewal.

To further understand how *pelo* modulates Bmp signaling activity, we examined the expression of a Bmp direct target gene, *Dad*, in the *pelo* mutant GSCs. *Dad-lacZ* is a *lacZ* enhancer trap line for *Dad* (Tsuneizumi et al., 1997). Its expression is the strongest in the GSCs, and is quickly downregulated in the differentiating cystoblasts (Kai and Spradling, 2003; Song et al., 2004). The $pelo^{1}$ mutant GSCs marked by loss of *ubi-GFP* expression were generated by the FLP-mediated FRT recombination, and then were analyzed for *Dad-lacZ* expression 2 weeks after clone induction. Consistent with the idea that *pelo* is involved in modulating Bmp signaling, 69% (n=36) of the marked mutant *pelo* GSCs (GFP negative) showed the downregulation of *Dad-lacZ* expression in comparison with their neighboring wild-type GSCs (GFP-positive) (Fig. 4E,E′). We further determined whether *pelo* is also involved in Bmp-mediated *bam* repression in GSCs as Bmp signaling directly represses *bam* transcription in GSCs (Chen and McKearin, 2003a; Song et al., 2004). A *bam*-GFP transgene (a GFP reporter driven by a *bam* promoter) is repressed in GSCs, while its expression is upregulated in the differentiating cystoblasts (Chen and McKearin, 2003b). The marked *pelo* mutant GSCs (*lacZ* negative) were generated by the FLP-mediated FRT recombination and were examined for *bam* expression according to our previously published procedures (Song et al., 2004). Only about 5% (n=55) of the marked *pelo* mutant GSCs (*lacZ* negative) showed slight upregulation of *bam-GFP* in comparison with their neighboring unmarked wild-type GSCs (*lacZ* positive), while the rest of the marked $pelo^{1}$ mutant GSCs did not upregulate *bam-GFP* expression (Fig. 4F,F′). These findings indicate that Pelo is involved in modulating Bmp signaling in GSCs but plays little or no role in regulating Bmp-mediated *bam* repression, and further suggest that it functions in one branch of the responses of the Bmp signaling pathway to regulate GSC self-renewal.

pelo represses a *bam*-independent differentiation pathway in ovarian GSCs

bam is both essential and sufficient for cystoblast differentiation (McKearin and Ohlstein, 1995; Ohlstein and McKearin, 1997). Our observation that $pelo^{1}$ mutant GSCs are lost because of differentiation but do not upregulate *bam* expression suggests that *pelo* mutant GSCs differentiate using a *bam*-independent pathway. If so, we should expect *bam* mutant germ cells to be able to differentiate in the absence of *pelo* function. To test this idea, we investigated the genetic relationship between *bam* and *pelo*. The $pelo^{1}$ homozygous GSCs that were heterozygous for $bam^{\Delta 86}$, a deletion allele of *bam*, were still lost rapidly as in the $pelo^{1}$ mutant GSCs (data not shown); the $bam^{\Delta 86}$ homozygous germ cells that were also heterozygous for $pelo^{1}$ still failed to differentiate, as did the $bam^{\Delta 86}$ mutant one (Fig. 5A). Interestingly, in $pelo^{1}$; $bam^{\Delta 86}$ double homozygous germaria, most of the germ cells were cysts with branched fusomes, and some of them still retained a round spectrosome (Fig. 5B). The round spectrosomes in the remaining single germ cells were unusually larger than those in *bam* mutant single germ cells, suggesting the single germ cells could be growth-arrested cystoblasts but can continue to grow their spectrosome. The morphology of the branched fusomes of the double mutant cysts appeared abnormal. To further determine whether the oocytes form in these *pelo*; *bam* mutant cysts, we examined the expression of Orb protein in the double mutant germaria. Orb normally starts to accumulate in newly formed wild-type oocytes (Fig. 5C); however, no obvious Orb expression was detected in the *pelo*; *bam* mutant germaria, indicating that there is no oocyte formation in the double mutant cysts (Fig. 5D). As *pelo* mutant cysts can still form the oocyte, *bam* is probably required late for oocyte formation. These findings show that *pelo* mutant cystoblasts can differentiate without functional *bam*, and further suggest that *pelo* must repress a *bam*-independent differentiation pathway to maintain GSC self-renewal.

pelo is also required for survival and/or growth of germline cysts during mid-oogenesis

We also noticed that the *pelo* mutant females had some fertility after eclosion and quickly lost their fertility. Interestingly, in the 2-day-old *pelo* mutant ovaries, early egg chambers looked largely normal, and even a few mature eggs were present (Fig. 6A). By contrast, in the 7-day-old ovaries, egg chambers older than stage 9 were rarely observed, and even those early stage egg chambers exhibited condensed nurse cell DNA, which suggest that they are in the process of undergoing apoptosis (Fig. 6B,C). The egg chamber degeneration is not due to oocyte

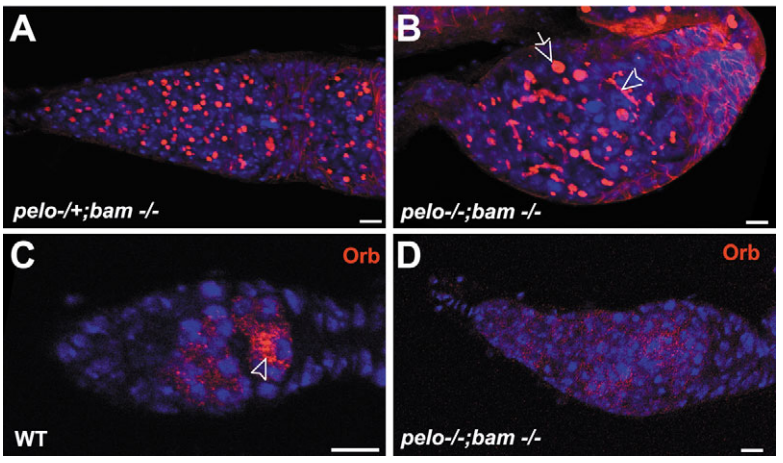


Fig. 5. A mutation in *pelo* can allow *bam* mutant cystoblast-like cells to differentiate into cystocytes. Germaria are labeled for Hts (red, A,B) or Orb (red, C,D) and DAPI (nuclei, blue). (A) A germaria homozygous for $bam^{\Delta 86}$ and heterozygous for $pelo^{1}$, showing undifferentiated single germ cells as evidenced by spectrosomes. (B) A germarium double homozygous for $pelo^{1}$ and $bam^{\Delta 86}$ showing the formation of cystocytes, as evidenced by branched fusomes (arrowhead). In addition, many persistent spectrosomes (arrow) are abnormally enlarged. (C) A wild-type germarium showing preferential accumulation of Orb in the newly formed oocyte (arrowhead). (D) A double *pelo* and *bam* homozygous germarium showing no oocyte formation, which is suggested by no preferential Orb accumulation in germ cells. Scale bars: 10 μm.

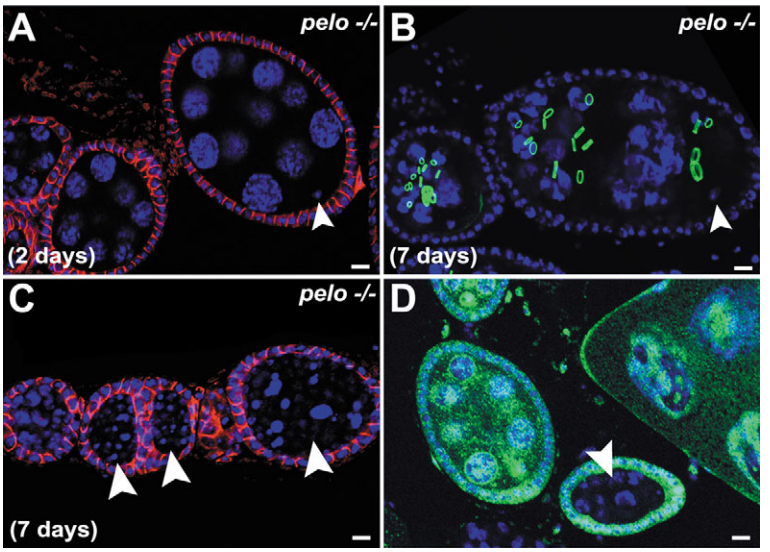


Fig. 6. *pelo* mutant ovaries exhibit age-dependent egg chamber degeneration. (A) Part of a 2-day-old *pelo*^{1} mutant ovariole labeled for Hts (red, follicle cells) and DAPI (nuclei, blue), showing normal nurse cell morphology and the oocyte (arrowhead). (B) Part of a 7-day-old *pelo*^{1} mutant ovariole labeled for HtsRC (green, ring canals) and DAPI (nuclei, blue), showing condensed nuclei of degenerating nurse cells in the egg chamber, in which the oocyte is indicated by an arrowhead. (C) Part of a 7-day-old *pelo*^{1} mutant ovariole labeled for Hts (red, follicle cells) and DAPI (nuclei, blue), showing several degenerating and growth-arrested egg chambers (arrowheads). (D) Part of an ovariole labeled for *lacZ* (green) and DAPI (nuclei, blue), showing a degenerating (unusually condensed nurse cell nuclei) and growth-arrested *pelo*^{1} mutant germline cyst (arrowhead, no *lacZ* expression). Scale bars: 10 μm.

formation defect because in these egg chambers the oocyte was present (Fig. 6B). In addition, the size of some egg chambers exhibiting condensed nurse cell DNA was also smaller than normal (Fig. 6C). The Pelo function also appeared to be germ-cell specific in egg chambers, as marked mutant *pelo* germ cells failed to grow to the normal size and became apoptotic (Fig. 6D). These results indicate that *pelo* is also required in the germ cells for their survival or normal differentiation during later oogenesis.

Discussion

In the *Drosophila* ovary, GSCs have been previously shown to be controlled by niche-derived Bmps and intrinsic factors such as Pumilio and Nanos. The Bmp signal controls GSC self-renewal by directly repressing Bam expression. In this study, we have revealed a new function of *pelo* in controlling GSC self-renewal in the *Drosophila* ovary. Our genetic clonal analysis and rescue experiment results show that Pelo is required intrinsically for controlling GSC self-renewal. It is involved in the regulation of some Bmp response in GSCs but is not required for repressing *bam* expression. Genetic analysis further indicates that it controls GSC self-renewal by repressing a *bam*-independent differentiation pathway (Fig. 7). Although translational control has been implicated in the regulation of GSC self-renewal, this is the first time it has been shown that a translational release factor-like protein controls GSC self-renewal. As Pelo is highly conserved from *Drosophila* to human, it is also possible that its mammalian homologs might also be involved in the control of stem cell self-renewal.

Pelo protein functions in the cytoplasm possibly as a translational regulator to control GSC self-renewal

Drosophila Pelo belongs to a family of evolutionarily conserved proteins with their primary function in the regulation of cell division. In the yeast, *dom34* (*pelo*) mutant cells grow slowly and have defects in the entry of meiosis, indicating that it is required for mitosis and meiosis. In mice, disruption of the *pelo* gene causes early embryonic lethality and defects in cell cycle progression (Adham et al., 2003). Although *pelo* is ubiquitously expressed throughout *Drosophila* development (Eberhart and Wasserman, 1995), the *pelo* mutants survive to adulthood without obvious defects in the body. In *Drosophila*, *pelo* has been shown to be required to control meiotic cell cycle progression in male germ cells. In this study, we show that *pelo* is required intrinsically for controlling self-renewal and division of GSCs but not SSCs in the ovary, which is supported by rescue and stem cell clonal analysis experiments. Even though Pelo members are required for regulating cell cycle progression from yeast to mammals, it remains unclear how they accomplish this function.

The only clue to the potential cellular function of Pelo comes from its high homology to the translation release factor 1. Its likely function as a translational regulator is further complicated by the presence of a highly conserved NLS sequence (Eberhart and Wasserman, 1995). Using an epitope-tagged Pelo that can rescue *pelo* mutants, we demonstrate that Pelo is mainly localized to the cytoplasm of both S2 cells and germ cells. Furthermore, the *pelo* gene with a mutated putative NLS is still fully functional. The yeast Pelo, Dom34, is also localized to the cytoplasm (Davis and Engebrecht, 1998; Huh

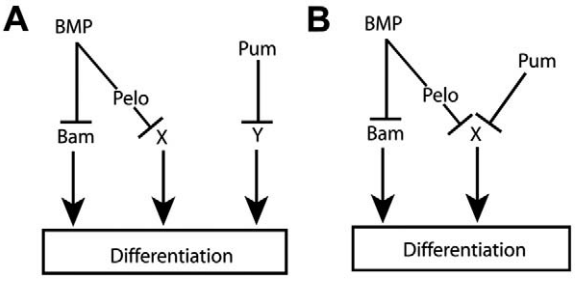


Fig. 7. Current working models to explain how Pelo is involved in controlling GSC self-renewal. Bmp signaling directly represses a Bam-dependent differentiation pathway and thereby controls GSC self-renewal. Pelo and Pum function separately to repress different Bam-independent pathways (X and Y in A) or function together to repress a Bam-independent pathway (X in B).

et al., 2003). These findings support the idea that Pelo proteins function in the cytoplasm as translational regulators in different organisms. If Pelo truly functions as a translational release factor, they must directly interact with ribosomes that are either associated with ER or in the cytoplasm. Consistent with this idea, some Pelo proteins are associated with ER membranes though the majority of Pelo proteins are not associated with ER membranes. In yeast, *dom34* mutants have dramatically reduced polyribosomes and can be rescued by a high-copy of the *ribosomal protein S30A* gene, indicating that Dom34 is involved in translation (Davis and Engebrecht, 1998). In addition, expression of *Drosophila pelo* in *dom34* mutants can rescue growth defects, indicating its conserved function during evolution. Therefore, Pelo is also likely a translational regulator in *Drosophila*, and is involved in regulating translation of a specific class of mRNAs that are important for germ cell function. In the future, it will be important to investigate whether Pelo is indeed involved in translational regulation and to identify its targets in germ cells.

Pelo participates in the Bmp pathway and a Bmp-independent pathway to control GSC self-renewal

Before this work, *pelo* has not been shown to be involved in regulating any signaling pathways in any organisms. The Bmp pathway is a major signaling pathway that is essential for controlling GSC self-renewal and division in the *Drosophila* ovary (Song et al., 2004; Xie and Spradling, 1998). The Bmp signaling activities can be reliably monitored by expression of *Dad* in GSCs (Kai and Spradling, 2003; Song et al., 2004). We anticipate that it must somehow interact with the Bmp pathway in controlling GSC self-renewal as *pelo* was also identified as a dominant suppressor of Dpp overexpression-induced GSC-like tumors. In this study, we show that the GSCs mutant for *pelo* downregulate *Dad*. These findings indicate that Pelo participates in Bmp signaling to control expression of *dpp* target genes in GSCs such as *Dad*.

In the *Drosophila* ovary, one of the ways in which Bmp signaling controls GSC self-renewal is to directly repress *bam* expression in GSCs. *bam* is necessary and sufficient for cystoblast differentiation in the *Drosophila* ovary (McKearin and Spradling, 1990; Ohlstein and McKearin, 1997). In this study, we show that Pelo is essential for controlling GSC self-renewal but is not involved in repressing *bam* expression. *pelo* mutant GSCs have normal *bam* repression but their progeny can still differentiate without *bam*, suggesting that *pelo* maintains GSCs by repressing a *bam*-independent pathway. During the preparation of this manuscript, two studies were published showing that *pum* controls GSC self-renewal by repressing a *bam*-independent pathway (Chen and McKearin, 2005; Szakmary et al., 2005). Pum is known to work together with Nos, which is also essential for *Drosophila* ovarian GSC self-renewal (Gilboa and Lehmann, 2004; Wang and Lin, 2004), to repress gene translation in the embryo (Barker et al., 1992; Forbes and Lehmann, 1998). As Pum/Nos does not participate in Bmp signaling (Gilboa and Lehmann, 2004) and Pelo is a translational release factor-like protein, we propose that Pelo works in a parallel genetic pathway with Pum in repressing the same or different Bam-independent differentiation pathways through regulating translation (Fig. 7). Although it is essential for repressing a Bam-independent pathway(s) in GSCs, Pelo is not so sufficient for doing so as Bmp signaling is for repressing *bam* as overexpression of *pelo* has no effect on the GSC maintenance and differentiation. In the future, it will be important to molecularly and genetically characterize the Bam-independent pathway repressed by Pelo and to further understand how Pelo represses it in relation to Pum.

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References

Adham, I. M., Sallam, M. A., Steding, G., Korabiowska, M., Brinck, U., Hoyer-Fender, S., Oh, C. and Engel, W. (2003). Disruption of the pelota gene causes early embryonic lethality and defects in cell cycle progression. *Mol. Cell. Biol.* **23**, 1470-1476.

Barker, D. D., Wang, C., Moore, J., Dickinson, L. K. and Lehmann, R. (1992). Pumilio is essential for function but not for distribution of the Drosophila abdominal determinant Nanos. *Genes Dev.* **6**, 2312-2326.

Carr-Schmid, A., Pfund, C., Craig, E. A. and Kinzy, T. G. (2002). Novel G-protein complex whose requirement is linked to the translational status of the cell. *Mol. Cell. Biol.* **22**, 2564-2574.

Chen, D. and McKearin, D. (2003a). Dpp signaling silences bam transcription directly to establish asymmetric divisions of germline stem cells. *Curr. Biol.* **13**, 1786-1791.

Chen, D. and McKearin, D. (2003b). A discrete transcriptional silencer in the bam gene determines asymmetric division of the Drosophila germline stem cell. *Development* **130**, 1159-1170.

Chen, D. and McKearin, D. (2005). Gene circuitry controlling a stem cell niche. *Curr. Biol.* **15**, 179-184.

Conti, E., Uy, M., Leighton, L., Blobel, G. and Kuriyan, J. (1998). Crystallographic analysis of the recognition of a nuclear localization signal by the nuclear import factor karyopherin alpha. *Cell* **94**, 193-204.

Cox, D. N., Chao, A., Baker, J., Chang, L., Qiao, D. and Lin, H. (1998). A novel class of evolutionarily conserved genes defined by piwi are essential for stem cell self-renewal. *Genes Dev.* **12**, 3715-3727.

Cox, D. N., Chao, A. and Lin, H. (2000). piwi encodes a nucleoplasmic factor whose activity modulates the number and division rate of germline stem cells. *Development* **127**, 503-514.

Davis, L. and Engebrecht, J. (1998). Yeast dom34 mutants are defective in multiple developmental pathways and exhibit decreased levels of polyribosomes. *Genetics* **149**, 45-56.

Drummond-Barbosa, D. and Spradling, A. C. (2001). Stem cells and their progeny respond to nutritional changes during Drosophila oogenesis. *Dev. Biol.* **231**, 265-278.

Eberhart, C. G. and Wasserman, S. A. (1995). The pelota locus encodes a protein required for meiotic cell division: an analysis of G2/M arrest in Drosophila spermatogenesis. *Development* **121**, 3477-3486.

Forbes, A. and Lehmann, R. (1998). Nanos and Pumilio have critical roles in the development and function of Drosophila germline stem cells. *Development* **125**, 679-690.

Forbes, A. J., Lin, H., Ingham, P. W. and Spradling, A. C. (1996). hedgehog is required for the proliferation and specification of ovarian somatic cells prior to egg chamber formation in Drosophila. *Development* **122**, 1125-1135.

Fuchs, E., Tumbar, T. and Guasch, G. (2004). Socializing with the neighbors: stem cells and their niche. *Cell* **116**, 769-778.

Gilboa, L. and Lehmann, R. (2004). Repression of primordial germ cell differentiation parallels germ line stem cell maintenance. *Curr. Biol.* **14**, 981-986.

Huh, W. K., Falvo, J. V., Gerke, L. C., Carroll, A. S., Howson, R. W., Weissman, J. S. and O'Shea, E. K. (2003). Global analysis of protein localization in budding yeast. *Nature* **425**, 686-691.

Kai, T. and Spradling, A. (2003). An empty Drosophila stem cell niche reactivates the proliferation of ectopic cells. *Proc. Natl. Acad. Sci. USA* **100**, 4633-4638.

Kelso, R. J., Buszczak, M., Quinones, A. T., Castiblanco, C., Mazzalupo, S. and Cooley, L. (2004). Flytrap, a database documenting a GFP protein-

trap insertion screen in Drosophila melanogaster. *Nucleic Acids Res.* **32**, D418-D420.

King, F. J., Szakmary, A., Cox, D. N. and Lin, H. (2001). Yb modulates the divisions of both germline and somatic stem cells through piwi- and hh-mediated mechanisms in the Drosophila ovary. *Mol. Cell* **7**, 497-508.

Lasko, P. F. and Ashburner, M. (1988). The product of the Drosophila gene vasa is very similar to eukaryotic initiation factor-4A. *Nature* **335**, 611-617.

Lin, H. (2002). The stem-cell niche theory: lessons from flies. *Nat. Rev. Genet.* **3**, 931-940.

Lin, H., Yue, L. and Spradling, A. C. (1994). The Drosophila fusome, a germline-specific organelle, contains membrane skeletal proteins and functions in cyst formation. *Development* **120**, 947-956.

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**, 2242-2251.

McKearin, D. and Ohlstein, B. (1995). A role for the Drosophila bag-of-marbles protein in the differentiation of cystoblasts from germline stem cells. *Development* **121**, 2937-2947.

Nair, R., Carter, P. and Rost, B. (2003). NLSdb: database of nuclear localization signals. *Nucleic Acids Res.* **31**, 397-399.

Ohlstein, B. and McKearin, D. (1997). Ectopic expression of the Drosophila Bam protein eliminates oogenic germline stem cells. *Development* **124**, 3651-3662.

Rorth, P. (1998). Gal4 in the Drosophila female germline. *Mech. Dev.* **78**, 113-118.

Snapp, E. L., Iida, T., Frescas, D., Lippincott-Schwartz, J. and Lilly, M. A. (2004). The fusome mediates intercellular endoplasmic reticulum connectivity in Drosophila ovarian cysts. *Mol. Biol. Cell* **15**, 4512-4521.

Song, X. and Xie, T. (2002). DE-cadherin-mediated cell adhesion is essential for maintaining somatic stem cells in the Drosophila ovary. *Proc. Natl. Acad. Sci. USA* **99**, 14813-14818.

Song, X. and Xie, T. (2003). Wingless signaling regulates the maintenance of ovarian somatic stem cells in Drosophila. *Development* **130**, 3259-3268.

Song, X., Zhu, C. H., Doan, C. and Xie, T. (2002). Germline stem cells anchored by adherens junctions in the Drosophila ovary niches. *Science* **296**, 1855-1857.

Song, X., Wong, M. D., Kawase, E., Xi, R., Ding, B. C., McCarthy, J. J. and Xie, T. (2004). Bmp signals from niche cells directly repress transcription of a differentiation-promoting gene, bag of marbles, in germline stem cells in the Drosophila ovary. *Development* **131**, 1353-1364.

Spradling, A. C. (1993). Developmental Genetics of Oogenesis. In *The Development of Drosophila Melanogaster*, Vol. 1 (ed. M. Bate and A. Martinez Arias), pp. 1-71. New York: Cold Spring Harbor Laboratory Press.

Spradling, A. C, Drummond-Barbosa, D. and Kai, T. (2001). Stem cells find their niche. *Nature* **414**, 98-104.

Szakmary, A., Cox, D. N., Wang, Z. and Lin, H. (2005). Regulatory relationship among piwi, pumilio, and bag-of-marbles in Drosophila germline stem cell self-renewal and differentiation. *Curr. Biol.* **15**, 171-178.

Tsuneizumi, K., Nakayama, T., Kamoshida, Y., Kornberg, T. B., Christian, J. L. and Tabata, T. (1997). Daughters against dpp modulates dpp organizing activity in Drosophila wing development. *Nature* **389**, 627-631.

Van Doren, M., Williamson, A. L. and Lehmann, R. (1998). Regulation of zygotic gene expression in Drosophila primordial germ cells. *Curr. Biol.* **8**, 243-246.

Wang, Z. and Lin, H. (2004). Nanos maintains germline stem cell self-renewal by preventing differentiation. *Science* **303**, 2016-2019.

Xie, T. and Spradling, A. (1998). Decapentaplegic is essential for the maintenance and division of germline stem cells in the Drosophila ovary. *Cell* **94**, 251-260.

Xie, T. and Spradling, A. (2000). A niche maintaining germ line stem cells in the Drosophila ovary. *Science* **290**, 328-330.

Xie, T. and Spradling, A. (2001). The Drosophila ovary: an in vivo stem cell system. In *Stem Cell Biology* (ed. D. R. Marshak R. L. Gardner and D. Gottlieb), pp. 129-148. New York: Cold Spring Harbor Laboratory Press.

Xu, T. and Rubin, G. M. (1993). Analysis of genetic mosaics in developing and adult Drosophila tissues. *Development* **117**, 1223-1237.

Zhu, C. H. and Xie, T. (2003). Clonal expansion of ovarian germline stem cells during niche formation in Drosophila. *Development* **130**, 2579-2588.