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Caenorhabditis elegans germline patterning requires coordinated development of the somatic gonadal sheath and the germ line

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Abstract

Interactions between the somatic gonad and the germ line influence the amplification, maintenance, and differentiation of germ cells. In *Caenorhabditis elegans*, the distal tip cell/germline interaction promotes a mitotic fate and/or inhibits meiosis through GLP-1/Notch signaling. However, GLP-1-mediated signaling alone is not sufficient for a wild-type level of germline proliferation. Here, we provide evidence that specific cells of the somatic gonadal sheath lineage influence amplification, differentiation, and the potential for tumorigenesis of the germ line. First, an interaction between the distal-most pair of sheath cells and the proliferation zone of the germ line is required for larval germline amplification. Second, we show that insufficient larval germline amplification retards gonad elongation and thus delays meiotic entry. Third, a more severe delay in meiotic entry, as is exhibited in certain mutant backgrounds, inappropriately juxtaposes undifferentiated germ cells with cells of the proximal sheath lineage, leading to the formation of a proximal germline tumor derived from undifferentiated germ cells. Tumors derived from dedifferentiated germ cells, however, respond to the proximal interaction differently depending on the mutant background. Our study underscores the importance of strict developmental coordination between neighboring tissues. We discuss these results in the context of mechanisms that may underlie tumorigenesis.

Keywords: Germline; Gonadal sheath cell; Soma/germline interaction; Tumor; Cell–cell interaction; Proliferation; Mitosis; Meiosis; Proximal proliferation

Introduction

The control of cell proliferation within a developing organ or tissue is a fundamental problem in developmental biology. Since proliferation is often influenced by signals coming from the surrounding cellular environment, this question becomes even more complex if cells experience changes in their environment as they proliferate. In addition to signals that dictate cell fate status such as competence to proliferate versus differentiate, an equally important and perhaps less well-understood aspect of development is the control of the rate or extent of proliferative growth. Many tissues and organ primordia undergo a period of proliferation during their development that later resolves into a slower rate of homeostatic stem cell-based maintenance (Fuchs et al., 2004). If cell–cell interactions guide the extent of proliferation during development, then correct temporal and spatial coordination of these interactions is critical to ensure sufficient growth and prevent inappropriate proliferation.

One example of cell proliferation that occurs in the context of a changing developmental environment is the early proliferation of the germ line (Saffman and Lasko, 1999). The germ line of many animals undergoes extensive proliferation prior to meiosis and gametogenesis. The extent of proliferation during development must be tightly controlled: insufficient proliferation could deplete reserves of germline stem cells and excessive proliferation could lead to formation of germline tumors. In mammals, the proliferation of undifferentiated germ cells occurs in the context of the still-developing somatic gonad (McLaren, 2003). In males, this proliferation resolves into stem cell proliferation followed by transit-amplifying divisions to maintain homeostasis. In female mammals, extensive proliferation early in development is countered by extensive cell death (McLaren, 2001, 2003).

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In *Caenorhabditis elegans*, a similar phase of germline amplification occurs within the developing somatic gonad and produces a stem cell population or proliferation zone (Fig. 1). In the wild-type hermaphrodite, two gonad arms elongate away from a medial (proximal) somatic gonad primordium. Germ cells proliferate throughout the gonad arm until mid-way through the third larval stage (L3), at which time the proximal-most germ cells enter meiosis (Fig. 1; Hansen et al., 2004a; Kimble and White, 1981). A distal proliferation zone is thereby established and is maintained by an interaction between the distal tip cell (DTC) and the germ line (Kimble and Hirsh, 1979; Kimble and White, 1981). The DTC/germline interaction is mediated by the GLP-1 receptor, a member of the LIN-12/Notch family (Austin and Kimble, 1987; Yochem and Greenwald, 1989).

An interaction between somatic cells of the gonadal sheath/spermatheca (SS) lineage and the germ line is also required to promote robust amplification of the germ line (McCarter et al., 1997). The SS lineage/germline interaction acts in parallel to the GLP-1-mediated interaction: in the

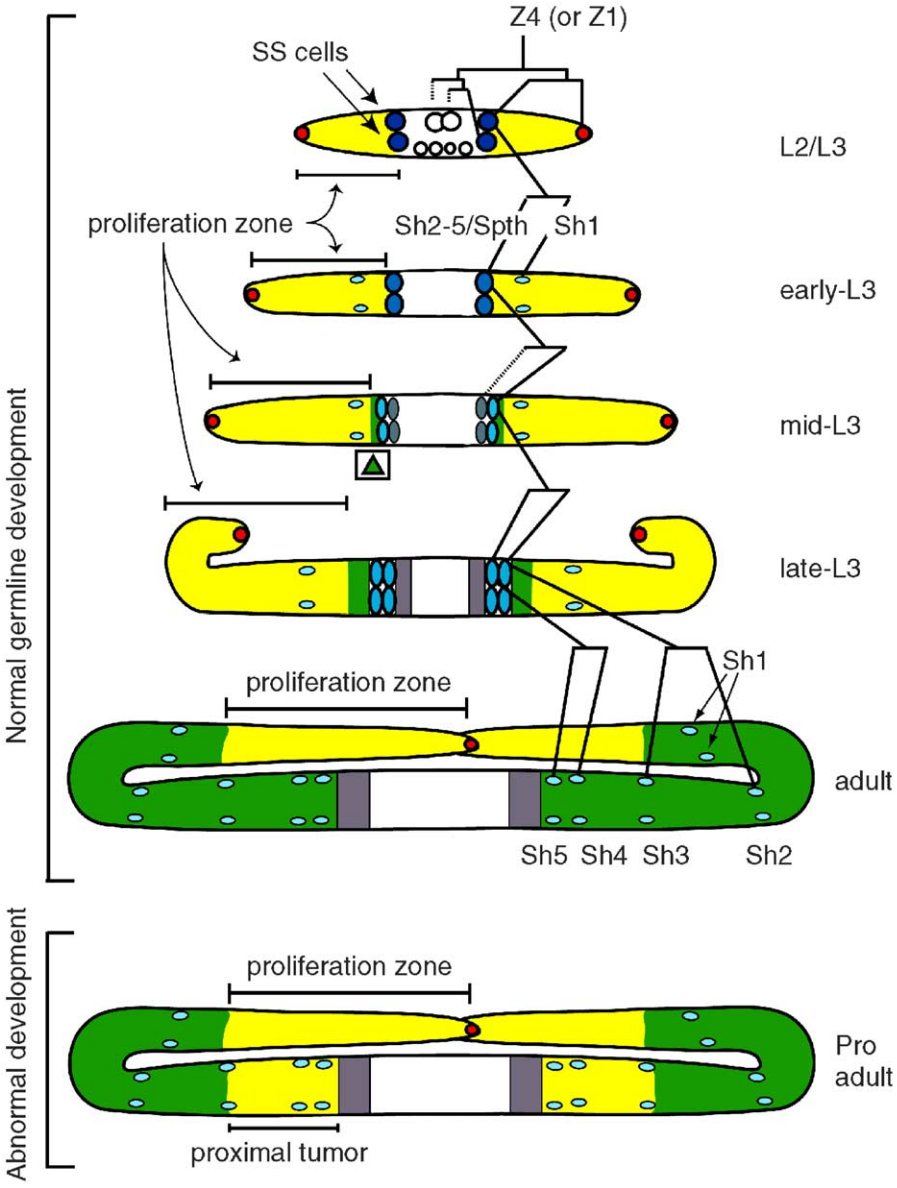

Fig. 1. Cartoon representation of hermaphrodite gonadogenesis. The positions of nuclei of SS cells and their descendents are shown with the sheath lineage connected by solid lines and additional lineages in dotted lines (the lineage is depicted in only one of the two SS cells after the L3). Vertical distance in the depicted lineage is not proportional to time between divisions. The sheath lineage nuclei are indicated in blue (increasingly light as the lineage progresses to differentiated sheath cells); the sheath cell bodies are not indicated (see Fig. 2). Yellow indicates germ cells in the mitotic cell cycle and green indicates germ cells in meiosis or gametogenesis or gametes. Grey represents the spermatheca and white represents the uterine and anchor cell lineages. The distal tip cell is indicated in red. Green boxed triangle indicates initial meiotic entry. Proximal germline tumors characteristic of the proximal proliferation (Pro) phenotype, are depicted in both gonad arms of the Pro adult. Lateral views are depicted for the L2/L3 and adult gonads. The proximal gonad of all other stages is depicted as a dorsal or ventral view, but the extent of gonad migration at each stage is represented laterally.

context of constitutive GLP-1 activity, the SS lineage is still needed for robust proliferation of the germ line (McCarter et al., 1997). The molecular basis for this interaction is unknown. The sheath cells are born in five lateral pairs adjacent to the proximal germ line of each gonad arm and grow out over the germ cells, eventually forming a thin single-cell layer between the germ line and the basement membrane surrounding the gonad (Hall et al., 1999; Hirsh et al., 1976; Fig. 1).

Here, we explore somatic gonad/germ cell interactions in *C. elegans* and establish that (1) the distal pair of sheath cells (Sh1) promotes larval germline amplification, (2) larval germline amplification is required for proper gonad arm elongation and hence influences the developmental stage at which germ cells escape the mitosis-promoting/meiosis-inhibiting activity of the DTC, and (3) an inappropriate interaction between the proximal sheath lineage and undifferentiated germ cells can drive the formation of a proximal germline tumor. Our studies illustrate the importance of strict temporal coordination of the development of juxtaposed tissues and a dramatic consequence of its disruption. Moreover, we demonstrate the existence of a counter-intuitive basis for tumor formation: early under-proliferation of the germ line causes a transient delay in differentiation, setting the stage for an inappropriate interaction that, in turn, causes hyperplasia of the same developmentally delayed germ line. We propose that similar developmental defects may underlie tumor formation in other systems.

Materials and methods

Strains

Strains were derived from the Bristol strain N2, grown at 20°C unless otherwise noted, and constructed using standard procedures (Brenner, 1974). The following mutations were used: LGI: *gld-1(q485)* (Francis et al., 1995a); LGII: *pro-1(na48)* (Killian and Hubbard, 2004); LGIII: *glp-1(ar202)* (Pepper et al., 2003a), *mIn1[dpy-10(e128) mIs14]* (Edgley and Riddle, 2001) was used as a balancer for LGII and *hT2[qIs48]* (Mathies et al., 2003; McKim and Rose, 1990) as a balancer for LG I and III. *mIs14* and *qIs48* are independent insertions of *ccEx9747* with markers: *myo-2*::*GFP* expressed in the pharynx, *pes-10*::*GFP* expressed in embryos, and a gut promoter driving GFP in the intestine.

Markers

The following transgenes were used as markers for cells of the somatic gonad in all strains in all experiments described in this study: LGIV: *tnIs6[lim-7*::*GFP rol-6(su1006)]* marks gonadal sheath cell pairs 1-4 (Hall et al., 1999); LGV: *qIs19[lag-2*::*GFP rol-6(su1006)]* marks the anchor cell, the DTC, and its precursors (Blelloch et al., 1999). *lag-2*::*GFP* persists in non-DTC lineages of the somatic gonad for several divisions (Siegfried et al., 2004) and was also used here as a marker for the SS cells and their immediate daughters. Here, we refer to strains carrying both *tnIs6* and *qIs19* transgenes as *Somatic Gonad hermaphrodite*::*GFP* (*SGh*::*GFP*).

Time course analysis and cell counts

Animals were synchronized essentially as described (Pepper et al., 2003a). Synchronized L1s were allowed to develop to the stages indicated and then were dissected and fixed (see below). Germ cells were identified as mitotic or meiotic based on nuclear morphology as revealed by DAPI staining. The crescent-shaped morphology of germ cells undergoing transition into meiosis is a suitable indicator of initial meiosis based on their coincident expression of an early meiosis marker (Hansen et al., 2004a). In all cases where cell counts or cell-diameter counts were used to determine the size of the proliferation zone, the proliferation zone was defined as the region between the distal tip and the first transition nucleus (consistent with counts reported by Hansen et al., 2004a).

Laser microsurgery

Cell ablations were carried out essentially as described in McCarter et al. (1997). Animals were immobilized on 5% agar pads containing 3 mM sodium azide and specific cells of the somatic gonad were identified based on GFP expression (see above) and position. Cells were ablated using a nitrogen pulse laser (Laser Science Inc, VSL337) as described (Bargmann and Avery, 1995). Only one arm was targeted per animal, though anterior and posterior arms were targeted in different individuals. Unoperated gonad arms in operated individuals served as controls, as did animals reared with and mounted on the same slides as operated animals. Successful ablation of the distal pair of sheath cells (Sh1) was verified in live adults: only 6 *lim-7*::*GFP*-positive sheath cells were present in these animals and no distal sheath was observed. Successful ablation of Sh2-5/Spth resulted in an adult gonad arm with only 2 *lim-7*::*GFP*-positive sheath cells (Sh1) and a greatly reduced spermatheca. Sh1 cells often remained in a more proximal position in Sh2-5-ablated animals than in unablated animals. Successful 2SS ablations resulted in an adult gonad arm with no *lim-7*::*GFP*-positive sheath cells. Animals were dissected, except where indicated, and DAPI stained (see below).

Dissections, fixation, and microscopy

Following live DIC and GFP scoring at indicated intervals post-ablation, animals were placed into depression slides in a drop of M9 buffer containing 400 μM levamisole and cut with syringe needles (25 gauge) just posterior to the pharynx or at the tail. The extruded gonads were fixed with 3% paraformaldehyde for 10 min. Fixed gonads were

moved on an eyebrow hair to 5% agar pads covered with 2.5 μl Vectashield mounting medium containing 4′,6-diamido-2-phenylindole (DAPI) (Vector Laboratories H-1200). Where indicated, whole animals were fixed in 95% ethanol for 10 min and mounted as described above. Imaging was performed with a Zeiss Axioplan II microscope and Openlab software (Improvision).

Results

We wished to determine the functional relationship between the developing gonadal sheath and the establishment of germline proliferation/differentiation pattern (Fig. 1). Therefore, we conducted a simultaneous anatomical analysis of the sheath lineage and germ line over time and assessed the contribution of specific cells of the sheath lineage to proliferation and differentiation under both normal and abnormal germline patterning conditions.

Coordinate development of the gonadal sheath and the germ line

Previous data suggest that the founder cells of the sheath/spermatheca lineage (SS cells) first divide around the time that germ cells first enter meiosis (Hansen et al., 2004a; Kimble and Hirsh, 1979; Kimble and White, 1981). These two events, however, have not been subject to simultaneous analysis and the subsequent anatomical relationship between the developing sheath and the larval germline has not been previously established. Therefore, to determine the anatomical relationship between cells of the developing somatic gonadal sheath and germline proliferation and differentiation, we examined individual gonad arms using simultaneously detectable markers for the somatic gonad cells (*SGh::GFP)* and germline nuclear morphology at several time points (see Materials and methods; Fig. 2).

At the L2/L3 molt (prior to initial meiotic entry), we found that the two SS cells in each gonad arm are in direct contact with undifferentiated (proliferative) germ cells (Fig. 2A). In the early L3, the SS cells divide (two SS cells per gonad arm). The distal pair of SS daughter cells do not divide further, and differentiate (“sheath pair one” (Sh1); Figs. 1 and 2B). In contrast, the proximal daughters of each SS cell undergo further divisions and ultimately give rise to the proximal sheath (sheath pairs 2–5) and most of the spermatheca (Sh2-5/Spth; Figs. 1 and 2; Kimble and Hirsh, 1979). The Sh2-5/Spth cells remain adjacent to the proximal-most germ nuclei in the early L3 (Fig. 2B).

In the mid-L3, meiotic development is initiated in the germ cells that lie between Sh1 and the daughters of the Sh2-5/Spth cells (Figs. 1 and 2C). Sh1 associates with germ cells of the proliferation zone (Fig. 2C). The Sh2-5/Spth cells divide before (temporally) initial meiosis and both pairs of resultant daughter cells are proximal to meiotic germline nuclei (Figs. 1 and 2C). Thus, the only somatic cells in direct contact with the proliferation zone after the initial onset of meiosis are Sh1 and the DTC (Figs. 1 and 2).

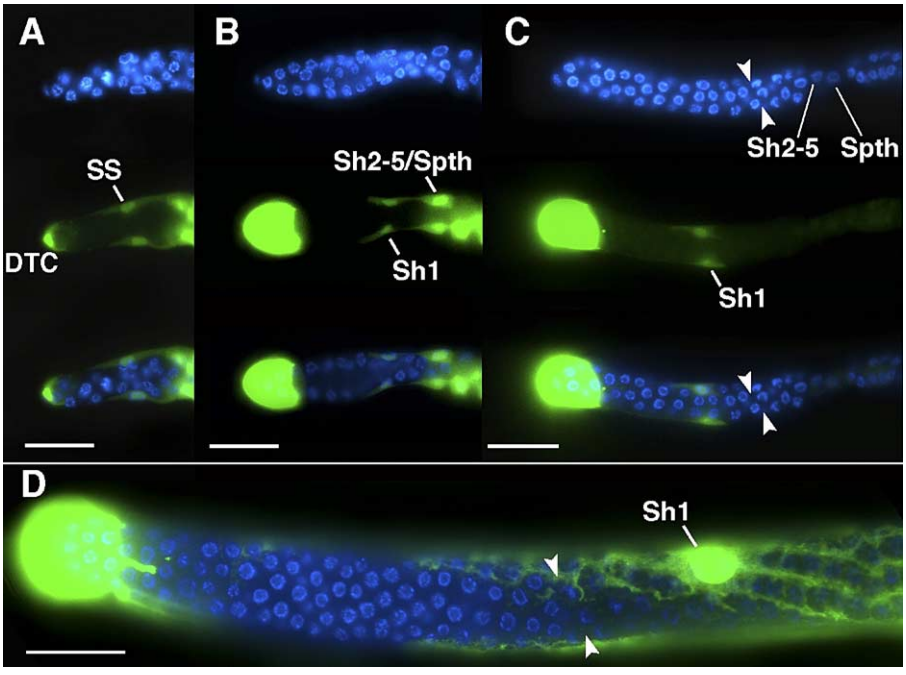

Fig. 2. Coordinate development of the somatic gonad and germ line. Dissected gonads from (A) L2/L3, (B) early L3, (C) mid-L3, and (D) early adult individuals carrying the *SGh::GFP* markers (see Materials and methods). From top to bottom, images show DAPI, GFP, and the overlay (A–C), and DAPI/GFP overlay only (D). Arrowheads indicate the proliferation zone/transition zone border. Scale bars = 25 μm. (A and B) The SS cells and their distal daughters (Sh1) extend over a large area of the pre-meiotic germ line (Pepper et al., 2003b). (C) Only one of each pair of the Sh2-5 and Spth cells is shown; the other cells are out of the plane of focus.

Throughout the L3 and L4, Sh1 maintains close association with the proliferation zone, but this association gradually shifts such that Sh1 contacts fewer cells in the proliferation zone and a greater number of meiotic cells. The centrally positioned Sh1 nuclei and the proliferation/transition border are aligned at the mid-L4, and the Sh1 cell bodies still extend well into the proliferation zone at the L4/adult molt (Fig. 2D). We observed that in embryo-producing adults, the distal-most edge of Sh1 only reaches the transition zone, consistent with the findings of Hall et al. (1999). Therefore, our observations indicate that all or part of the Sh1 cells contact proliferative germ cells in the L3 and L4.

We next quantitated the extent of proliferation in the mitotic zone with respect to SS cell and Sh1 contact after the formation of the somatic gonad primordium at the L2/L3 molt (Fig. 3). We counted germ cells in the proliferation zone at 10 time points from the L2/L3 molt through adulthood and simultaneously monitored sheath development (see Materials and methods). The number of germ cells in the proliferation zone increased dramatically during periods of SS cell and Sh1 contact, approximating exponential growth (Fig. 3). Specifically, in the 21 h between Sh1 birth to the L4/Adult molt, the proliferation zone increased by ~164 germ cells. The rate of amplification declined coincident with loss of the Sh1/proliferation zone contact (an increase of only 50 cells over the first 13 h of adulthood). As adulthood progressed, the number of cells in the proliferation zone slowly decreased, losing nearly 100 germ cells (38% loss) over the next 43 h (Fig. 3). Taken together, the results are consistent with the possibility that the size of the proliferation zone in the adult is largely determined by the earlier soma/germline interaction between the SS and Sh1 cells and the germ cells in the proliferation zone.

Sheath pair 1 is required for germline amplification

Previous cell ablation studies indicate that the DTC is essential to maintain the distal proliferation zone (Kimble and White, 1981), and that cells of the SS lineage are required for robust germline proliferation (McCarter et al., 1997). Given the results of our anatomical analysis, we asked if the diminished amplification of the germ line following ablation of both SS cells in a gonad arm (a "2SS ablation"; McCarter et al., 1997) could be solely due to the interaction between the larval proliferation zone and Sh1. To test this hypothesis, we ablated Sh1 in the early L3, shortly after the division of the SS cells, and evaluated the size of the mitotic zone in the adult relative to 2SS-ablated gonads

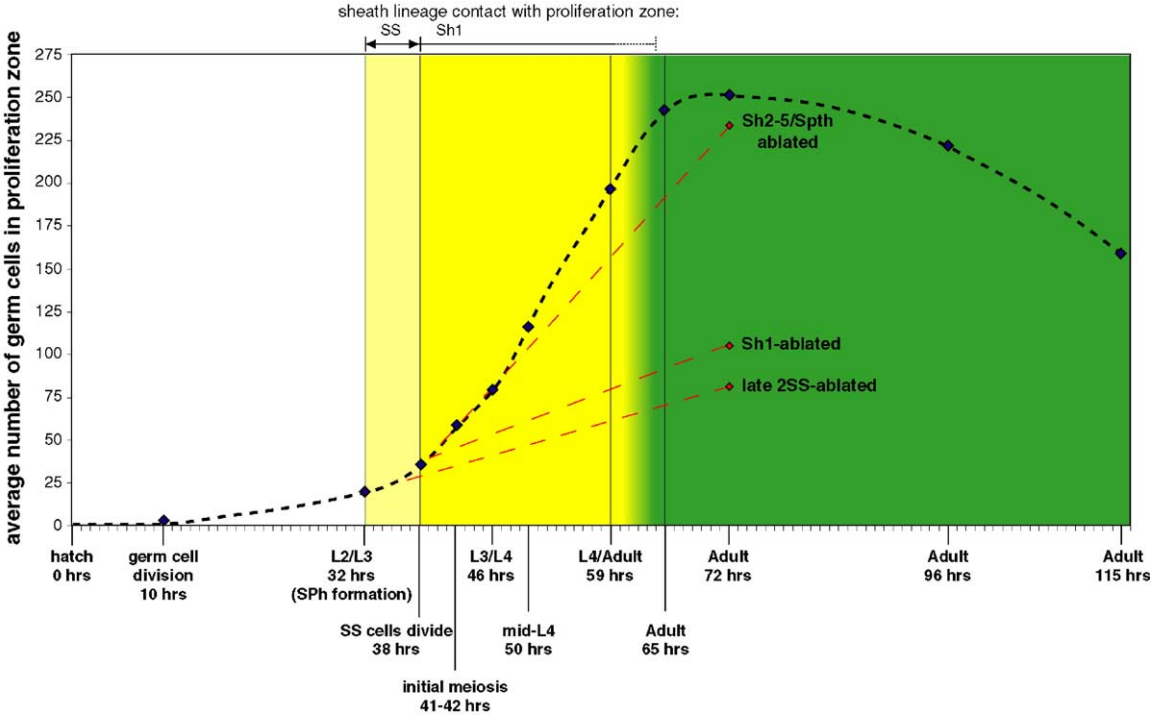

Fig. 3. Time course analysis of the amplification of the proliferation zone and its dependence on cells of the sheath lineage. The *x*-axis is time in hours and *y*-axis is numbers of germ cells in the proliferation zone. Lines between data points were drawn to indicate the approximate population dynamics of the proliferation zone. Average cell counts (± one standard deviation (*n*)) are as follows for time points at 32, 38, 41, 46, 50, 59, 65, 72, 96, and 115 h post-hatch: 19.6 ± 1 (13), 37.2 ± 3 (12), 60.6 ± 12 (13), 81.1 ± 8 (10), 121.5 ± 15 (16), 201.1 ± 17 (12), 243.8 ± 13 (12), 251.1 ± 22 (10), 219.6 ± 26 (11), and 156.5 ± 19 (11). Contact between proliferative germ cells and SS and Sh1 cells is indicated in pale and bright yellow, respectively, at times after formation of the somatic gonad primordium. Green indicates time during which Sh1 is no longer in contact with proliferative germ cells. Results of cell ablation studies (Table 1) are indicated at the 72-h time point, a point comparable to ~76 h in Table 1, given differences in experimental conditions. Red dotted lines originate just before and just after division of the SS cell for the 2SS-ablated and the other two ablations, respectively (Table 1). The time at which the SS cells were ablated is somewhat later than in experiments reported by McCarter et al. (1997).

Table 1
Sh1 is required for robust germline amplification

Ablated cells	Mitotic	Transition	Pachytene	*n*
(none)	271 ± 36	108 ± 21	194 ± 43	10
2SS	82 ± 6	12 ± 7	22 ± 8	5
Sh1	115 ± 25	112 ± 29	134 ± 42	9
Sh2-5/Spth	235 ± 12	32 ± 8	100 ± 31	6

Average numbers of germ cells in the adult gonad (~76 h after hatching) are indicated ± one standard deviation. *n* = the number of gonad arms scored. Cell ablations were performed in the *SGh::GFP* strain (see Materials and methods) in the early L3. For Sh1 and Sh2-5/Spth, both lateral cells were ablated in one gonad arm. Control (unablated) gonad arms include the unablated gonad arm in each operated individual as well as additional individuals (see Materials and methods). All control gonad arms contained mature sperm and oocytes. For the 2SS-ablated gonad arms, four contained spermatocytes and sperm, and one displayed neither signs of spermatogenesis nor gametes. For Sh1-ablated gonad arms, nine contained sperm and oocytes and one contained oocytes but no sperm. All Sh2-5/Spth-ablated gonad arms contained sperm but had not yet formed oocytes.

and unoperated controls (see Materials and methods; Table 1, Figs. 3 and 4). The adult proliferation zone in Sh1-ablated gonad arms contained many fewer cells than unoperated control arms, and only a slightly greater number of cells than in 2SS-ablated arms (Table 1; Figs. 3 and 4).

To determine if SS lineage cells other than Sh1 influence germline amplification, we ablated the Sh2-5/Spth precursors just after the SS cells divided, isolating the Sh1 pair as the only remaining sheath cells. Sh2-5/Spth-ablated gonad arms contained nearly wild-type numbers of cells in the adult proliferation zone (Table 1; Fig. 3). In the absence of the proximal sheath cells, Sh1 does not maintain as distal a position in late larvae, which may account for slightly less robust amplification than in the wild type. We conclude that the bulk of SS lineage-mediated germline amplification is due to the Sh1/proliferation zone interaction, but that the SS cells themselves also contribute to early germline amplification prior to the birth of Sh1. Our anatomical studies and cell-killing experiments suggest that, in addition to the DTC, the Sh1/germline interaction is required for sufficient germline amplification to generate a normal adult proliferation zone.

Sh1-mediated germline amplification influences gonad elongation and the developmental time at which germ cells enter meiosis

In addition to the germline amplification defect we observed after ablation of Sh1, we also noted that adult Sh1-ablated gonad arms contained fewer pachytene cells (Table 1) and exhibited a delay in reproductive maturity (data not shown). We considered two possibilities to account for this defect. One possibility is that Sh1 is required for meiotic progression and germ cells were unable to efficiently exit the pachytene stage of meiotic prophase

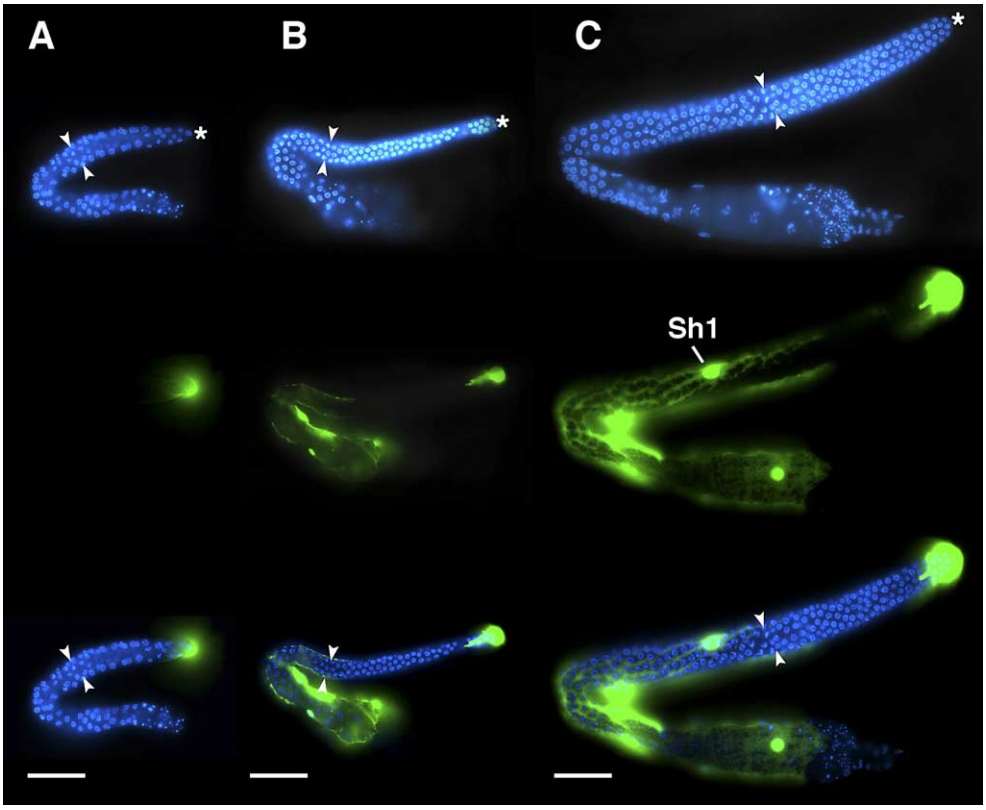

Fig. 4. The distal sheath cells (Sh1) are required for robust germline amplification. Dissected gonads following (A) 2SS, (B) Sh1, and (C) control (mock) cell ablations. All images are shown at the same magnification. Asterisks indicate the distal end of each arm. Scale bars = 25 μm. All other details are as given in Fig. 2.

(Pex phenotype), as was observed following a 2SS ablation (McCarter et al., 1997). However, we did not observe a Pex phenotype following Sh1 ablation (n = 9; Fig. 4), suggesting that the progeny of Sh2-5/Spth are sufficient for pachytene exit.

Another possibility to account for our observations is that initial meiotic entry is delayed in Sh1-ablated gonad arms and, as a consequence, gamete production is subsequently delayed. To determine if Sh1 is required for the proper timing of initial meiosis we ablated either Sh1 or Sh2-5/Spth in the early L3 and determined the furthest stage of meiotic progression at two subsequent time points (Table 2). Whereas unoperated mid-L4 gonads contained many pachytene germ cells and several spermatocytes, Sh1-ablated gonad arms (at the same larval stage) contained only a few transition nuclei, consistent with a delay of initial meiotic entry (Table 2; Materials and methods). The delay is significant: in the late L4, most Sh1-ablated animals had not begun spermatogenesis. Ablation of Sh2-5/Spth did not cause an observable delay of initial meiosis (Table 2). Furthermore, we did not observe a Pex phenotype following Sh2-5/Spth ablations (n = 6), suggesting that Sh1 is sufficient for pachytene exit. Thus, together with the absence of a Pex phenotype after Sh1 ablation, these data suggest that either part of the lineage (either Sh1 or Sh2-5/Spth) is sufficient to promote pachytene exit.

We further considered two possibilities for how Sh1 influences the timing of initial meiosis. One possibility is that Sh1 itself promotes meiosis. This is unlikely since our anatomical investigation indicated that Sh1 has little contact with meiotic germ cells at the time of initial meiosis. The second possibility is that robust germline amplification facilitates gonad elongation and is therefore required to properly position the DTC far enough from proximal germ cells to permit their timely meiotic entry. Cells within a critical distance from the DTC are prevented from entering meiosis due to GLP-1-mediated signaling (Austin and Kimble, 1987; Hansen et al., 2004b; Kimble and White, 1981).

If the extent of distal gonad elongation is dependent on both endogenous DTC leader function (Kimble and White, 1981) and robust germ cell proliferation, then under conditions of insufficient early germline amplification, the DTC may remain too close to proximal germ cells and thereby delay their entry into meiosis via GLP-1-mediated signaling. This scenario is consistent with our results that Sh1 is required for germline amplification and for timely meiotic entry.

This model would further predict that once a critical distance from the DTC is reached, initial meiosis should occur in germ cells furthest from the DTC. To test this prediction, we examined the size of the distal proliferation zone at the time of initial meiosis under normal, 2SS-, and Sh1-ablated conditions. Normally, initial meiosis occurs in the mid-L3 at a reproducible distance of 13 germ cell diameters from the DTC (Hansen et al., 2004a) and at an average of 61 ± 12 germ cells in the proliferation zone (n = 13; Fig. 3). Initial meiosis in 2SS- and Sh1-ablated gonad arms was delayed and occurred in the mid-L4. The proliferation zone at this stage averaged 59 ± 10 and 53 ± 3 germ cells, respectively (Table 2), also 13 cell diameters from the DTC (n = 2 and 3, respectively). Thus, delayed meiotic entry occurred when the proximal germ cells in the sheath-ablated arms reached the normal critical distance from the DTC. We conclude that SS- and Sh1-mediated germline amplification facilitates gonad elongation and thus influences the developmental stage at which meiosis first occurs. We also note that after Sh1 ablation, although initial meiosis is delayed, it occurs prior to the formation of the proximal sheath.

Table 2
Sh1-mediated germline amplification influences the timing of initial meiosis

Ablated cells	Stage	Mitotic	Transition	Pachytene	n
(none)	mid-L4	102 ± 21	20 ± 6	36 ± 16	11
2SS	mid-L4	59 ± 10	6 ± 0	0	2
Sh1	mid-L4	53 ± 3	3 ± 1	0	3
Sh2-5/Spth	mid-L4	108 ± 30	24 ± 11	25 ± 13	6
(none)	late-L4	123 ± 12	34 ± 9	48 ± 15	6
Sh1	late-L4	91 ± 12	19 ± 3	22 ± 4	4
Sh2-5/Spth	late-L4	107 ± 11	35 ± 8	46 ± 11	6

Average (± one standard deviation) germ cell counts at indicated stages of meiotic progression (mid-L4 = 20–22 h post-ablation; late L4 = 26–28 h post-ablation). Other details are as in Table 1. At the mid-L4, none of the ablated gonad arms had reached spermatogenesis, while 6/11 control arms contained spermatocytes. The furthest extent of gametogenesis for the late-L4 is as follows: for control arms, 3/6 contained spermatocytes and 3/6 contained sperm; for Sh1-ablated gonads, 1/4 contained spermatocytes; for Sh2-5/Spth-ablated arm, 3/6 contained spermatocytes and 2/6 contained sperm.

Cells of the proximal sheath lineage contribute to germline tumor formation

Delayed initial meiosis can correlate with proximal germline tumor formation. Several mutants that form a proximal germline tumor (Pro phenotype; Fig. 1) also display a severe delay of initial meiosis, including reduction-of-function and loss-of-function mutations in *pro-1* (Killian and Hubbard, 2004) and *lin-12* (Killian thesis, 2004; Seydoux et al., 1990), respectively, and gain-of-function alleles of *glp-1* (Pepper et al., 2003a,b). Therefore, we considered the possibility that the Pro phenotype is a secondary consequence of delayed initial meiosis. In this model, a transient delay in meiotic entry could disrupt the critical temporal coordination of soma/germline interactions resulting in inappropriate cell–cell contact that promotes tumor formation. Specifically, we hypothesize that a delay in initial meiosis causes the inappropriate juxtaposition of undifferentiated germ cells and proximal somatic gonadal sheath cells, and that this inappropriate contact promotes tumor formation. In the wild type, proximal somatic gonadal sheath cell pairs 2–5 are

born after initial meiosis has occurred (Fig. 1). Therefore, these cells and their progeny normally contact only meiotic germ cells. However, if initial meiosis is delayed beyond the mid-L4, proximal sheath cells will contact undifferentiated germ cells.

To test if inappropriate interaction between cells of the proximal sheath lineage and undifferentiated germ cells could influence proximal germline tumor formation, we ablated the Sh2-5/Spth cells in several different Pro mutant backgrounds (see Materials and methods). We found that tumor formation was completely abrogated in *pro-1(na48)* gonad arms following ablation of Sh2-5/Spth (Table 3). Suppression of proximal tumor formation was not limited to *pro-1(na48)* gonads: 22% of Sh2-5/Spth-ablated *glp-1(ar202)* gonad arms contained tumors compared to 97% of unoperated gonad arms (Table 3, Fig. 5). Both *pro-1* and *glp-1* proximal germline tumors derive from undifferentiated germ cells (Killian and Hubbard, 2004; Pepper et al., 2003b). Therefore, cells of the Sh2-5/Spth lineage possess a latent activity that can promote germline tumors derived from undifferentiated germ cells. These ablations eliminate the proximal four pairs of sheath cells and most of the spermatheca. Because the sheath cells are normally in extensive direct contact with the germ line, it is likely that these results are due to the proximal sheath (here, referring to sheath pairs 2–5)/germline interaction. It is, however, formally possible that the spermathecal cells of this lineage possess the latent tumor-promoting activity.

Table 3
Inappropriate interaction between the proximal sheath lineage and germ cells contributes to tumor formation

Relevant genotype	Ablated cells	Proximal tumors/total
pro-1(na48)	(none)	9/10
pro-1(na48)	Sh2-5/Spth	0/7
glp-1(ar202)	(none)	34/35
glp-1(ar202)	Sh2-5/Spth	4/18
gld-1(q485)	(none)	10/10
gld-1(q485)	Sh2-5/Spth	7/7
puf-8(RNAi)	(none)	16/29[a]
puf-8(RNAi)	Sh2-5/Spth	0/11[a]

Data are given as ratios of gonad arms that contained mitotic germ cells proximal to meiotic germ cells or gametes over the total number of gonad arms scored. Control arms include unoperated arms in operated individuals. All experiments were scored first by DIC microscopy and then after fixation and DAPI staining with the exception of *pro-1* controls and 3/7 ablated arms that were scored by DIC alone (the presence of proximal tumors versus proximal gametes was evident by DIC microscopy for this strain). In addition, 4/7 *pro-1(na48)* ablated gonad arms and all *glp-1(ar202)* gonads were dissected prior to fixation and staining. All strains also contained the *SGh::GFP* markers (see Materials and methods). All animals were raised at 25°C prior to and after ablation with the exception of *gld-1(q485)* that was raised at 20°C. All ablations were performed at the early L3 stage, and animals were scored as adults at the following times post-ablation (roughly equal developmental stages): 48 h for *pro-1*, 18 h for *glp-1*, 48 h for *gld-1*, and 24 h for *puf-8*. *puf-8(RNAi)* animals were generated by feeding as described (Timmons et al., 2001) using reagents from the MRC gene service (Kamath et al., 2003).

[a] These results are consistent with the incomplete penetrance of tumor formation previously reported for *puf-8(RNAi)* conditions (Subramaniam and Seydoux, 2003). These data include nine individual animals in which one gonad arm was ablated while the other served as the control unablated arm. In these individuals, 9/9 of the control arms formed tumors while none of the ablated arms formed tumors.

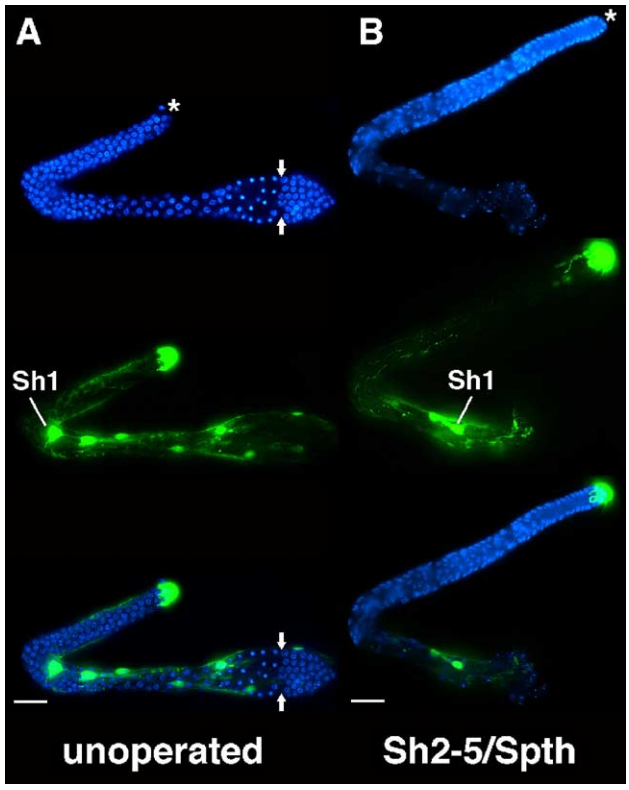

Fig. 5. The proximal sheath/spermatheca lineage promotes tumor formation. Dissected gonads from *glp-1(ar202)* individuals after (A) mock or (B) Sh2-5/Spth cell ablations (see Table 3). Arrows indicate the spermatocyte/proximal tumor border. Location of Sh1 is indicated; ablation of the proximal sheath precursor cells interferes with normal Sh1 positioning. Scale bars = 25 μm.

We next asked whether proximal tumors derived from dedifferentiated (as opposed to undifferentiated) germ cells are also dependent upon interaction with the proximal sheath lineage. Loss of *gld-1* (Francis et al., 1995a) or *puf-8* (Subramaniam and Seydoux, 2003) activity in the germ line results in dedifferentiated germline tumors. In these mutants, germ cells enter and progress through meiosis (to pachytene in *gld-1* and to spermatogenesis in *puf-8*) and then exit meiosis and return to mitosis, generating a proximal tumor (Francis et al., 1995a; Subramaniam and Seydoux, 2003). To test if the proximal sheath lineage contributes to the formation of these tumors, we ablated Sh2-5/Spth cells in *gld-1(q485)* and *puf-8(RNAi)* animals. We found that germline tumors always form in *gld-1* in the absence of the proximal sheath (Table 3; Figs. 6A–D). While *gld-1* tumors always formed, the tumors were consistently smaller than controls, suggesting that the growth of these tumors is sensitive to the presence of the proximal sheath lineage (Fig. 6E). In contrast, *puf-8(RNAi)*-induced proximal tumors, though also derived from dedifferentiated germ cells, are dependent upon the presence of the proximal sheath (Figs.

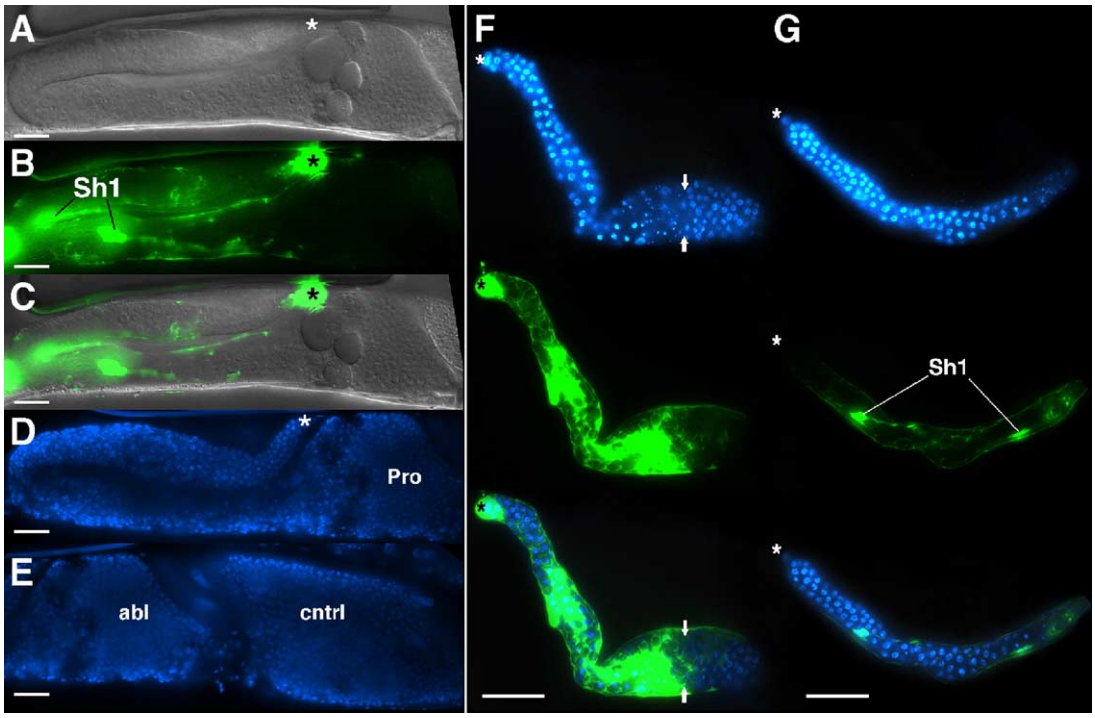

Fig. 6. The proximal sheath/spermatheca lineage is not required for *gld-1* germ cell tumor formation but is required for *puf-8* tumor formation. (A–D) One gonad arm of a *gld-1(q485)* animal after ablation of Sh2-5/Spth in live (DIC and GFP) and fixed (DAPI) preparations: (A) DIC, (B) GFP, (C) DIC/GFP overlay, and (D) DAPI stained. (E) Both gonad arms of the same individual shown in A–D with ablated (abl) and control (cntrl) arms indicated. (F and G) Dissected gonad arms from an individual *puf-8(RNAi)* animal: (F) unablated arm and (G) ablated (Sh2-5/Spth ablation) arm. (F and G) The images are DAPI, GFP, and the overlay from top to bottom. Arrows mark the border between sperm and the proximal germline tumor in (F). (G) The proximally shifted Sh1 nuclei are indicated; the DTC in this particular arm expressed GFP in vivo but was damaged during dissection and did not express GFP after fixation. Asterisks indicate distal end of each arm. Scale bars = 25 μm.

6F and G). Thus, the formation of proximal germ cell tumors that derive from undifferentiated germ cells largely depends on an interaction with the proximal sheath/spermathecal lineage whereas the formation of dedifferentiated germ cell-derived tumors cannot be predicted on this basis alone (see Discussion).

Two distinct mechanisms for delay of meiotic entry and for proximal tumor formation

Taken together, our data suggest a model in which a transient delay in meiotic entry can ultimately lead to proximal germline tumor formation by permitting the juxtaposition of undifferentiated germ cells with proximal sheath cells (Fig. 7). In both *glp-1(ar202)* and *pro-1(na48)*, initial meiosis is severely delayed, but for different reasons. The delay in *glp-1(ar202)* is due to germline-intrinsic hyperactivity of the GLP-1 receptor (Pepper et al., 2003a) that prevents timely meiotic entry. Our previous results indicate that wild-type *pro-1* activity is required in the SS lineage (not the germ line) to prevent tumor formation (Killian and Hubbard, 2004). Furthermore, the distal germline in *pro-1(na48)* mutant animals is reduced (Killian and Hubbard, 2004). Therefore, the delay in initial meiotic entry in *pro-1(na48)* mutants may be a secondary consequence of insufficient early distal germline proliferation that, in turn, prevents proper extension of the gonad arm such that the critical distance from the DTC is reached later than in the wild type.

If our model is correct, we might expect that tumor formation would occur in Sh1-ablated gonad arms since meiotic entry is delayed. We did not observe proximal tumors in these animals (Table 2, Fig. 4). Because Sh1 ablation delayed meiotic entry but did not cause proximal tumor formation, we reasoned that meiotic entry must have occurred just prior to the formation of the proximal sheath in these gonad arms. We also reasoned that a further retardation of meiotic entry should uncover the tumor-promoting potential of the proximal sheath/undifferentiated germ cell interaction. To test this idea, we turned to the *glp-1(ar202)* mutant background at 15°C, a temperature at which *glp-1(ar202)* does not form proximal tumors but is nonetheless sensitized (Hansen et al., 2004a; Killian and Hubbard, 2004; Pepper et al., 2003a). Ablations were performed in 10 *glp-1(ar202)* individuals. All Sh1-ablated gonad arms (10/10) displayed proximal tumors while none of the control arms formed tumors (n = 18 total control arms, including the unablated arms in the 10 individuals; Fig. 8). These results are consistent with our model.

Our model also predicts that inefficient germline elongation caused by a failure of the DTU to undergo

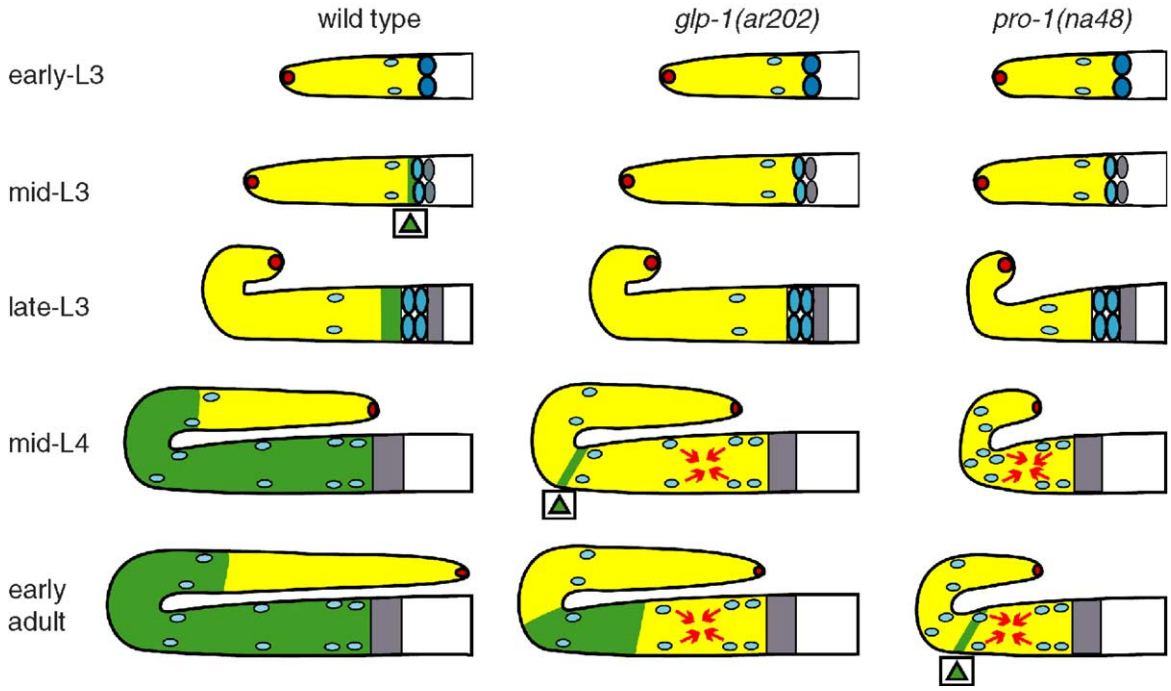

Fig. 7. Model of proximal tumor formation/Pro phenotype in *glp-1(ar202)* and *pro-1(na48)*. Color scheme is the same as Fig. 1. Red arrows represent germline proliferation-promoting activity of proximal sheath cells. See text for details.

sufficient centrifugal migration in early larval stages could cause proximal tumor formation. This prediction is borne out in *hlh-12(RNAi)* animals (R. Voutev and E. J. A. H., unpublished observations) and *mig-24/hlh-12* mutant animals, for example, where early DTC migration is impaired (K. K. Tamai and K. Nishiwaki, personal communication).

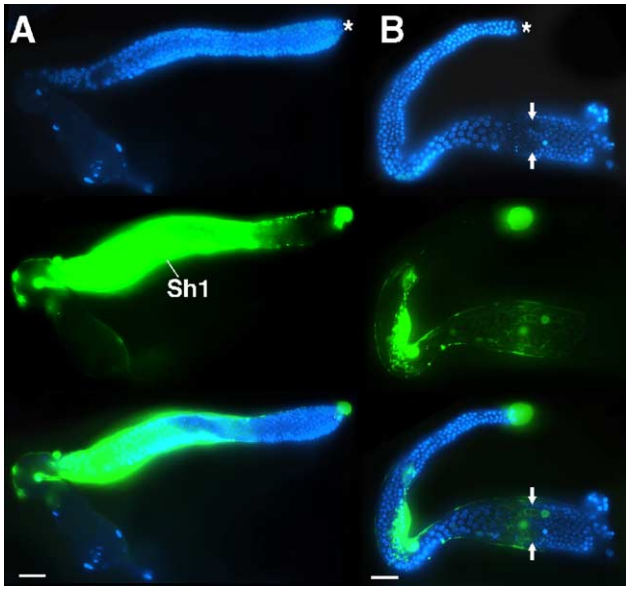

Fig. 8. Sh1 ablation in a sensitized genetic background causes proximal tumor formation. Dissected gonad arms from the same *glp-1(ar202)* individual after (A) mock or (B) Sh1 ablations. Arrows indicate the sperm/proximal tumor border. Asterisks indicate distal end of each arm. Scale bars = 25 μm.

Discussion

This study provides a clear example of temporal coupling between the development of the somatic gonad and the germ line and the tumorigenic consequences of its disruption. We have identified two anatomically distinct cell non-autonomous proliferation-promoting properties of the gonadal sheath lineage that impact germline development: a normal distal activity and a latent proximal activity. First, our study demonstrates that the SS cells and distal pair of sheath cells, Sh1, are required for amplification of the larval germ line and determine the size of the proliferation zone. Second, we show that Sh1-mediated proliferation is important for proper gonad elongation and to prevent a secondary delay in initial meiotic entry. Third, our analysis reveals a latent cell non-autonomous proliferation-promoting activity of the proximal sheath/spermatheca lineage that acts on undifferentiated germ cells and on certain dedifferentiated germ cells.

Anatomical studies place sheath pairs 1 and 2 together as distinct from pairs 3–5 (e.g., actin filaments are present in both, but the latter are contractile and contain both thick (myosin) and thin filaments; Hall et al., 1999; Strome, 1986). In our studies, the distal sheath pair (Sh1) is treated separately because it is born earlier and differentiates earlier than the other sheath cells. It is possible that if Sh2 were in the same anatomical position as Sh1, it would contribute to germline amplification, but this possibility cannot be evaluated at present.

The somatic gonadal sheath is required for several different aspects of germline development. Previously,

McCarter et al. (1997) demonstrated that the SS lineage is required for robust germline proliferation. The reduction in proliferation that occurs in the absence of the entire SS lineage occurs nonetheless in a germ line with constitutive GLP-1 activity, suggesting that the SS lineage proliferation-promoting activity is not mediated by *glp-1* signaling (McCarter et al., 1997). Our results extend this observation and pinpoint the SS cells themselves and their distal-most daughters (Sh1) as the normal proliferation-promoting cells of the SS lineage. Moreover, we show that contact between these cells and germ cells correlates with an exponential amplification phase of germline development.

McCarter et al. (1997) also reported defects in pachytene exit (Pex phenotype), sex determination, and ovulation (Emo phenotype) following ablation of the SS cells. Our results indicate that ablation of Sh1 or Sh2-5/Spth alone does not confer a Pex phenotype, suggesting that a pachytene exit-promoting activity is redundant in these two parts of the sheath. The proximal sheath also contributes to ovulation (Greenstein et al., 1994; Iwasaki et al., 1996; McCarter et al., 1997, 1999; Rose et al., 1997) and engulfment of germ cells that have undergone programmed cell death (Gumienny et al., 1999). Our data suggest another activity of the proximal sheath, albeit an activity only evident in abnormal conditions, in promoting proximal germline tumor formation.

The distal sheath and germline amplification

How does the presence of the SS and Sh1 cells promote a high rate of germline proliferation during larval development? One possibility is that in the absence of distal sheath cells, the germ line contacts basement membrane proteins that inhibit germline proliferation. We do not favor this explanation since sheath cells are present in *pro-1(na48)* mutants but robust distal proliferation does not occur. Another possibility is that the distal sheath cells provide "nutrition" or structural support that is necessary for robust proliferation. Nutritional studies suggest that the germ line is sensitive to sterol deprivation, but a more pronounced effect is observed on oogenesis than on early germline amplification (Merris et al., 2003; Shim et al., 2002). A temporal delay in gametogenesis has been reported as a result of defects in ubiquinone biosynthesis (*clk-1* mutation), presumably through its reduction of the oxidation of LDLs. This phenotype can be suppressed by reducing cholesterol intake or by reducing the level of superoxide dismutase (Shibata et al., 2003). Unlike the proximal sheath cells that appear to facilitate the entry of yolk through pores and into oocytes, the distal sheath does not contain pores but rather is rich in vesicles, Golgi, and rough ER (Hall et al., 1999; Hirsh et al., 1976). Therefore, a secretory mechanism may be involved in the distal proliferation-promoting activity. Other possibilities for non-autonomous mechanisms to promote proliferation are hormone-mediated or growth factor-mediated signaling pathways. The latter would also be consistent with a high secretory activity of the distal sheath. Further genetic studies will be required to determine the molecular mechanism(s) that underlie this interaction.

Control of mitotic/meiotic fate versus germline amplification

Our studies and those of McCarter et al. (1997) point to a distinction between germline proliferation that is promoted by the DTC–germline interaction (via the GLP-1/Notch signaling pathway) and that promoted by the distal sheath. The data are consistent with the notion that DTC–germline interaction imbues germ cells with a mitotic fate identity and/or inhibits their acquisition of the meiotic fate (Austin and Kimble, 1987) and promotes only a basal level of proliferation (Fig. 3; McCarter et al., 1997). Thus, the early amplification phase of the developing germ line requires the distal sheath/germline interaction to promote the proliferation of germ cells that are, due to GLP-1 signaling, undifferentiated. In the absence of signaling from the distal sheath, the germ line does not attain a sufficiently large proliferation zone and fertility and fecundity are compromised.

The larval amplification phase of germline development is anatomically distinct from the homeostatic phase of adult germline maintenance in which the distal sheath no longer contacts the proliferation zone. GLP-1-mediated proliferation is sufficient to keep the adult germ line in homeostasis during the early part of reproductive life. Our data indicate, however, that the number of cells in the proliferation zone drops during later reproductive life (Fig. 3), suggesting that the rate of proliferation is lower than the rate at which cells differentiate in older adults. Recently, the possibility that dividing germ cells exist in female mammals has been suggested (Johnson et al., 2004). These, too, apparently cannot keep pace with differentiation over time. The relationship between the reduction of proliferating germ cells and reproductive senescence in *C. elegans* has not yet been explored.

The proximal sheath lineage and tumor formation

How could the proximal sheath lineage promote tumor formation? The molecular mechanism for this interaction is not known, but several alternative possibilities can be envisaged. For example, the latent proliferation-promoting activity of the proximal sheath lineage could be due to the misinterpretation of a signal that normally promotes meiotic divisions during gametogenesis. Oocytes do not complete meiotic divisions until after fertilization in *C. elegans* hermaphrodites, but sperm undergo meiotic divisions in the proximal gonad, while in contact with proximal sheath cells (Hirsh et al., 1976). We do not favor this hypothesis since spermatogenesis (through meiotic divisions) appeared to progress normally in Sh2-5/Spth-ablated gonad arms (Table 1). Another possibility is that an as-yet unknown growth factor normally produced by the

proximal sheath is sufficient to drive proliferation of non-meiotic germ cells.

A third possibility is that the proximal sheath activates GLP-1 on the surface of non-meiotic germ cells with which it is in contact in certain mutants. DSL-family ligands activate Notch-family receptors, and there are at least 10 DSL-family ligands in *C. elegans* (Chen and Greenwald, 2004). If one or more of these ligands is produced by the sheath, it could inappropriately activate GLP-1 in adjacent undifferentiated germ cells. LIN-12 is the other Notch family member in *C. elegans* and is functionally interchangeable with GLP-1 (Fitzgerald et al., 1993). A *lin-12* reporter is expressed in the gonadal sheath (Wilkinson and Greenwald, 1995). Therefore, it is conceivable that a DSL ligand that normally binds the LIN-12 receptor is also expressed in the sheath. Our anatomical analysis indicates that under normal conditions, neither sheath pairs 2-5 nor the spermatheca contact germ cells that express a high level of GLP-1 on their surface (Crittenden et al., 1994). Therefore, the presence of a potential GLP-1-activating ligand in sheath pairs 2–5 would not adversely affect normal germline development. Because cleavage of the extracellular domain of Notch receptors is sufficient for activation (Struhl et al., 1993), a related possibility is that the proximal sheath produces a protease that promiscuously cleaves the extracellular domain of the GLP-1 receptor or otherwise results in unregulated (non-ligand-mediated) activation of GLP-1 in the germ line.

Models that invoke inappropriate activation of GLP-1 are consistent with our observation that the formation of undifferentiated germ cell tumors is dependent on the presence of the proximal sheath while germ cell tumors in *gld-1* mutants are not. In undifferentiated germ cell tumors, inappropriate activation of GLP-1 presumably initiates tumor formation. *gld-1* tumors are not dependent on GLP-1 (Francis et al., 1995b). That is, the initiation of tumor formation occurs within a germ cell-autonomous pathway downstream of *glp-1*. Our observation that *gld-1* tumors are smaller in the absence of the proximal sheath (Fig. 6E) is also consistent with a GLP-1 activation hypothesis since loss of *glp-1* activity in *gld-1* mutants also reduces *gld-1* tumor size (Francis et al., 1995b).

Our observation that *puf-8(RNAi)*-induced tumors are also dependent on the proximal sheath/spermatheca lineage is more difficult to reconcile with a GLP-1-activation model since these tumors form from cells that have already begun spermatogenesis (Subramaniam and Seydoux, 2003). The mechanism for spermatocyte dedifferentiation in these cells is unknown. A GLP-1-activation model could be reconciled if *puf-8*-mediated spermatocyte dedifferentiation is dependent on ectopic GLP-1 activation. Alternatively, these tumors could be proximal sheath-dependent by an alternate mechanism.

In addition to proximal sheath cell/germline interaction, other interactions may also be sufficient to drive inappropriate germline proliferation. Previous results indicate that the precursors to the AC can contribute to tumor formation in certain genetic backgrounds (Pepper et al., 2003b; Seydoux et al., 1990). This may account for our observation of incomplete suppression of *glp-1(ar202)* tumor formation in the absence of Sh2-5/Spth. Germ cells can also proliferate in the pseudocoelom outside the gonad, typically in close apposition to other tissues, as a result of a mutation in the laminin A gene *epi-1* (Huang et al., 2003). Thus, the basal lamina of the germ line likely acts to minimize inappropriate interactions between somatic cells and undifferentiated germ cells.

Tumorigenesis as a result of development gone awry

Together, our data suggest a model for the formation of the proximal germline tumor phenotype: the temporal coordination between the developing somatic gonad and the germ line is disrupted such that a transient delay in meiotic entry leads to the inappropriate contact of undifferentiated germ cells with the proximal sheath (see Results section; Fig. 7). This contact, in turn, supports unregulated germ cell proliferation in the proximal region of the germ line. Gametogenesis still occurs under these conditions, but it occurs late and distal to the tumor, precluding fertility. Thus, the genetic defect underlying tumor formation can be autonomous to the tumorous tissue (as in *glp-1(Pro)* mutants) or autonomous to the surrounding non-tumorigenic tissue (the sheath, as in *pro-1(na48)* mutants; Fig. 7).

One of the more counterintuitive aspects of our model for *pro-1(na48)* (Fig. 7) is that early under-proliferation of the germ line—via the subsequent delay in differentiation—causes later over-proliferation of the same germ line. Therefore, a delay in differentiation or failure to move away from a latent proliferation-promoting signal may cause inappropriate cell–cell interactions that allow tumor formation in the very same tissue that was initially slow to differentiate and/or proliferate. It is conceivable that similar developmental tumor-formation mechanisms exist in other organisms. If similar mechanisms exist in vertebrates, they would likely result in tissue-specific tumors. For example, zebrafish heterozygous for loss-of-function mutations in genes encoding certain ribosome components exhibit an increased incidence of peripheral nerve sheath tumors (Amsterdam et al., 2004). It seems more likely that reducing ribosomal function would lead to under-proliferation or growth retardation rather than to cell-autonomous over-proliferation. Therefore, we speculate that these mutations may induce tumors by uncoupling developmental coordination.

In male mammals, germ cells undergo several distinct phases of proliferation. In a situation analogous to the proliferation of germ cells during somatic gonad development in *C. elegans*, the testes forms while post-migratory primordial germ cells (gonocytes) proliferate. These gonocytes then become mitotically quiescent and either locate to the basement membrane of the seminiferous

tubule and become stem cells or they disintegrate (Lacham-Kaplan, 2004). Once stem cells are established, their daughters undergo proliferation as transit-amplifying cells prior to meiotic entry. Although changes in expression of germ cell markers have been documented as germ cells traverse different stages of proliferation (Lacham-Kaplan, 2004; Ohbo et al., 2003), this process is not understood in detail. Given that most (95%) human testicular cancers derive from germ cells (for a review, see Diez-Torre et al., 2004) and that many of these tumors are derived from undifferentiated germ cells, this regulation is important to understand. The progression of testicular germ cell tumors is thought to involve interaction between the somatic cells and germ cells (Diez-Torre et al., 2004). Our studies in *C. elegans* suggest that a transient delay in differentiation can cause inappropriate soma/germ cell interactions. It will be of interest to determine if inappropriate soma/germ cell interactions underlie human germ cell tumors.

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References

Amsterdam, A., Sadler, K.C., Lai, K., Farrington, S., Bronson, R.T., Lees, J.A., Hopkins, N., 2004. Many ribosomal protein genes are cancer genes in zebrafish. PLoS Biol. 2, E139.

Austin, J., Kimble, J., 1987. Glp-1 is required in the germ line for regulation of the decision between mitosis and meiosis in *C. elegans*. Cell 51, 589–599.

Bargmann, C.I., Avery, L., 1995. Laser killing of cells in *Caenorhabditis elegans*. Methods Cell Biol. 48, 225–250.

Blelloch, R., Anna-Arriola, S.S., Gao, D., Li, Y., Hodgkin, J., Kimble, J., 1999. The gon-1 gene is required for gonadal morphogenesis in *Caenorhabditis elegans*. Dev. Biol. 216, 382–393.

Brenner, S., 1974. The genetics of *Caenorhabditis elegans*. Genetics 77, 71–94.

Chen, N., Greenwald, I., 2004. The lateral signal for LIN-12/Notch in *C. elegans* vulval development comprises redundant secreted and transmembrane DSL proteins. Dev. Cell 6, 183–192.

Crittenden, S.L., Troemel, E.R., Evans, T.C., Kimble, J., 1994. GLP-1 is localized to the mitotic region of the *C. elegans* germ line. Development 120, 2901–2911.

Diez-Torre, A., Silvan, U., De-Wever, O., Bruyneel, E., Mareel, M., Arechaga, J., 2004. Germinal tumor invasion and the role of the testicular stroma. Int. J. Dev. Biol. 48, 545–557.

Edgley, M.L., Riddle, D.L., 2001. LG II balancer chromosomes in *Caenorhabditis elegans*: mT1(II;III) and the mIn1 set of dominantly and recessively marked inversions. Mol. Genet. Genomics 266, 385–395.

Fitzgerald, K., Wilkinson, H., Greenwald, I., 1993. Glp-1 can substitute for lin-12 in specifying cell fate decisions in *Caenorhabditis elegans*. Development 119, 1019–1027.

Francis, R., Barton, M., Kimble, J., Schedl, T., 1995a. Gld-1, a tumor suppressor gene required for oocyte development in *Caenorhabditis elegans*. Genetics 139, 579–606.

Francis, R., Maine, E., Schedl, T., 1995b. Analysis of the multiple roles of gld-1 in germline development: interactions with the sex determination cascade and the glp-1 signaling pathway. Genetics 139, 607–630.

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

Greenstein, D., Hird, S., Plasterk, R.H., Andachi, Y., Kohara, Y., Wang, B., Finney, M., Ruvkun, G., 1994. Targeted mutations in the *Caenorhabditis elegans* POU homeo box gene ceh-18 cause defects in oocyte cell cycle arrest, gonad migration, and epidermal differentiation. Genes Dev. 8, 1935–1948.

Gumienny, T.L., Lambie, E., Hartwieg, E., Horvitz, H.R., Hengartner, M.O., 1999. Genetic control of programmed cell death in the *Caenorhabditis elegans* hermaphrodite germline. Development 126, 1011–1022.

Hall, D.H., Winfrey, V.P., Blaeuer, G., Hoffman, L.H., Furuta, T., Rose, K., Hobert, O., Greenstein, D., 1999. Ultrastructural features of the adult hermaphrodite gonad of *Caenorhabditis elegans*: relations between the germ line and soma. Dev. Biol. 212, 101–123.

Hansen, D., Hubbard, E.J., Schedl, T., 2004a. Multi-pathway control of the proliferation versus meiotic development decision in the *Caenorhabditis elegans* germline. Dev. Biol. 268, 342–357.

Hansen, D., Wilson-Berry, L., Dang, T., Schedl, T., 2004b. Control of the proliferation versus meiotic development decision in the *C. elegans* germline through regulation of GLD-1 protein accumulation. Development 131, 93–104.

Hirsh, D., Oppenheim, D., Klass, M., 1976. Development of the reproductive system of *Caenorhabditis elegans*. Dev. Biol. 49, 200–219.

Huang, C.C., Hall, D.H., Hedgecock, E.M., Kao, G., Karantza, V., Vogel, B.E., Hutter, H., Chisholm, A.D., Yurchenco, P.D., Wadsworth, W.G., 2003. Laminin alpha subunits and their role in *C. elegans* development. Development 130, 3343–3358.

Iwasaki, K., McCarter, J., Francis, R., Schedl, T., 1996. Emo-1, a *Caenorhabditis elegans* Sec61p gamma homologue, is required for oocyte development and ovulation. J. Cell Biol. 134, 699–714.

Johnson, J., Canning, J., Kaneko, T., Pru, J.K., Tilly, J.L., 2004. Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature 428, 145–150.

Kamath, R.S., Fraser, A.G., Dong, Y., Poulin, G., Durbin, R., Gotta, M., Kanapin, A., Le Bot, N., Moreno, S., Sohrmann, M., Welchman, D.P., Zipperlen, P., Ahringer, J., 2003. Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. Nature 421, 231–237.

Killian, D.J., 2004. Proliferation, differentiation and tumor formation: roles of the developing gonadal sheath in patterning the *C. elegans* germ line. PhD thesis, New York University, New York, NY.

Killian, D.J., Hubbard, E.J., 2004. *C. elegans* pro-1 activity is required for soma/germline interactions that influence proliferation and differentiation in the germ line. Development 131, 1267–1278.

Kimble, J., Hirsh, D., 1979. The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. Dev. Biol. 70, 396–417.

Kimble, J., White, J., 1981. On the control of germ cell development in *Caenorhabditis elegans*. Dev. Biol. 81, 208–219.

Lacham-Kaplan, O., 2004. In vivo and in vitro differentiation of male germ cells in the mouse. Reproduction 128, 147–152.

Mathies, L.D., Henderson, S.T., Kimble, J., 2003. The *C. elegans* hand gene controls embryogenesis and early gonadogenesis. Development 130, 2881–2892.

McCarter, J., Bartlett, B., Dang, T., Schedl, T., 1997. Soma–germ cell interactions in *Caenorhabditis elegans*: multiple events of hermaphrodite germline development require the somatic sheath and spermathecal lineages. Dev. Biol. 181, 121–143.

McCarter, J., Bartlett, B., Dang, T., Schedl, T., 1999. On the control of oocyte meiotic maturation and ovulation in *Caenorhabditis elegans*. Dev. Biol. 205, 111–128.

McKim, K.S., Rose, A.M., 1990. Chromosome I duplications in *Caenorhabditis elegans*. Genetics 124, 115–132.

McLaren, A., 2001. Mammalian germ cells: birth, sex, and immortality. Cell Struct. Funct. 26, 119–122.

McLaren, A., 2003. Primordial germ cells in the mouse. Dev. Biol. 262, 1–15.

Merris, M., Wadsworth, W.G., Khamrai, U., Bittman, R., Chitwood, D.J., Lenard, J., 2003. Sterol effects and sites of sterol accumulation in *Caenorhabditis elegans*: developmental requirement for 4alpha-methyl sterols. J. Lipid Res. 44, 172–181.

Ohbo, K., Yoshida, S., Ohmura, M., Ohneda, O., Ogawa, T., Tsuchiya, H., Kuwana, T., Kehler, J., Abe, K., Scholer, H.R., Suda, T., 2003. Identification and characterization of stem cells in prepubertal spermatogenesis in mice small star, filled. Dev. Biol. 258, 209–225.

Pepper, A.S., Killian, D.J., Hubbard, E.J., 2003a. Genetic analysis of *Caenorhabditis elegans* glp-1 mutants suggests receptor interaction or competition. Genetics 163, 115–132.

Pepper, A.S., Lo, T.W., Killian, D.J., Hall, D.H., Hubbard, E.J., 2003b. The establishment of *Caenorhabditis elegans* germline pattern is controlled by overlapping proximal and distal somatic gonad signals. Dev. Biol. 259, 336–350.

Rose, K.L., Winfrey, V.P., Hoffman, L.H., Hall, D.H., Furuta, T., Greenstein, D., 1997. The POU gene ceh-18 promotes gonadal sheath cell differentiation and function required for meiotic maturation and ovulation in *Caenorhabditis elegans*. Dev. Biol. 192, 59–77.

Saffman, E.E., Lasko, P., 1999. Germline development in vertebrates and invertebrates. Cell. Mol. Life Sci. 55, 1141–1163.

Seydoux, G., Schedl, T., Greenwald, I., 1990. Cell–cell interactions prevent a potential inductive interaction between soma and germline in *C. elegans*. Cell 61, 939–951.

Shibata, Y., Branicky, R., Landaverde, I.O., Hekimi, S., 2003. Redox regulation of germline and vulval development in *Caenorhabditis elegans*. Science 302, 1779–1782.

Shim, Y.H., Chun, J.H., Lee, E.Y., Paik, Y.K., 2002. Role of cholesterol in germ-line development of *Caenorhabditis elegans*. Mol. Reprod. Dev. 61, 358–366.

Siegfried, K.R., Kidd III, A.R., Chesney, M.A., Kimble, J., 2004. The sys-1 and sys-3 genes cooperate with Wnt signaling to establish the proximal-distal axis of the *Caenorhabditis elegans* gonad. Genetics 166, 171–186.

Strome, S., 1986. Fluorescence visualization of the distribution of microfilaments in gonads and early embryos of the nematode *Caenorhabditis elegans*. J. Cell Biol. 103, 2241–2252.

Struhl, G., Fitzgerald, K., Greenwald, I., 1993. Intrinsic activity of the Lin-12 and Notch intracellular domains in vivo. Cell 74, 331–345.

Subramaniam, K., Seydoux, G., 2003. Dedifferentiation of primary spermatocytes into germ cell tumors in *C. elegans* lacking the pumilio-like protein PUF-8. Curr. Biol. 13, 134–139.

Timmons, L., Court, D.L., Fire, A., 2001. Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in *Caenorhabditis elegans*. Gene 263, 103–112.

Wilkinson, H., Greenwald, I., 1995. Spatial and temporal patterns of lin-12 expression during *C. elegans* hermaphrodite development. Genetics 141, 513–526.

Yochem, J., Greenwald, I., 1989. Glp-1 and lin-12, genes implicated in distinct cell–cell interactions in *C. elegans*, encode similar transmembrane proteins. Cell 58, 553–563.