The establishment of Caenorhabditis elegans germline pattern is controlled by overlapping proximal and distal somatic gonad signals

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Abstract
We investigated the control of proliferation and differentiation in the larval Caenorhabditis elegans hermaphrodite germ line through analysis of glp-1 and lag-2 mutants, cell ablations, and ultrastructural data. After the first several rounds of germ cell division, GLP-1, a receptor of the LIN-12/Notch family, governs germline proliferation. We analyzed the proximal proliferation (Pro) phenotype in glp-1(ar202) and found that initial meiosis was delayed and spatially mispositioned. This is due, at least in part, to a heightened response of the mutant GLP-1 receptor to multiple sources of the somatic ligand LAG-2, including the proximal somatic gonad. We investigated whether proximal LAG-2 affects germline proliferation in the wild type. Our results indicate that (1) LAG-2 is necessary for GLP-1-mediated germline proliferation and prevention of early meiosis, and (2) several distinct anatomical sources of LAG-2 in the larval somatic gonad functionally overlap to promote proliferation and prevent early meiosis. Ultrastructural studies suggest that mitosis is not restricted to areas of direct DTC-germ line contact and that the germ line shares a common cytoplasm in larval stages. We propose that downregulation of the GLP-1 signaling pathway in the proximal germ line at the time of meiotic onset is under tight temporal and spatial control.

Keywords: Germ line; Meiosis; Proliferation; GLP-1/Notch; LAG-2; Gonadogenesis; C. elegans

Introduction
In all animal systems studied, the germ line is amplified by mitosis prior to meiotic entry. In many systems, germline stem cells are established and provide a continuous supply of cells that enter meiosis and form gametes. Stem cells in this context are defined as cells that both self-renew and give rise to cells that differentiate (Spradling et al., 2001; Watt and Hogan, 2000). It is not clear what mechanisms govern the relative competence of individual early germ cells to become stem cells or to differentiate as the gonad develops. In male mammals, Drosophila and Caenorhabditis elegans, mitotic germ cells and somatic cells are intermingled in the early gonad, while mitotic stem cells are relegated to one section of the adult gonad. The proper adult arrangement of stem cells and differentiating germ cells is critical for fertility, yet the anatomical and molecular mechanisms responsible for the proper spatial and temporal establishment of this pattern are not well understood in any organism.

The C. elegans hermaphrodite gonad provides a relatively simple model system to explore the coordinate development of the germ line and somatic gonad. Germline proliferation occurs in the larval gonad prior to the onset of meiosis (Fig. 1), generating a set of mitotic nuclei that are all descendents of the primordial germ cells Z2 and Z3. During the third larval stage (L3), a subset of these germ cells enters meiosis in the proximal-most part of each developing arm of the germ line, an event we term initial meiotic entry or initial meiosis. Once initial meiosis occurs, cells distal to the point of initial meiosis remain mitotic and continue to contribute cells to the meiotic pathway. Thereafter, the distal tip cells (DTC) create a functional stem cell

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niche for the germ line (Kimble and White, 1981; Watt and Hogan, 2000) and maintain the distal-to-proximal pattern of mitosis, meiosis, and gametogenesis.

After the first several divisions of the primordial germ cells in the L1, germline proliferation in *C. elegans* is dependent on GLP-1-mediated signaling (Austin and Kimble, 1987). GLP-1 is a member of the conserved LIN-12/Notch family of receptors (Yochem and Greenwald, 1989). In response to ligand binding, Notch receptors undergo proteolytic cleavage, releasing the intracellular domain from the membrane and allowing it to enter the nucleus. There, the intracellular domain interacts with nuclear factors to effect changes in gene expression (see Baron et al., 2002 for a review). Loss of GLP-1 signaling in the *C. elegans* germ line results in a severe proliferation defect and early meiotic entry (Austin and Kimble, 1987), while constitutive activity of the receptor causes all germ cells to remain mitotic (Berry et al., 1997). GLP-1 in the germ line is activated by the membrane-bound ligand LAG-2, a DSL-family member (Henderson et al., 1994; Tax et al., 1994).

Fig. 1. Hermaphrodite gonadogenesis. Schematic diagram of hermaphrodite gonadogenesis (see Kimble and Hirsh, 1979 for details). The germ line is largely syncytial, but each nucleus and its surrounding cytoplasm is, by convention, referred to as a "germ cell." In newly hatched larvae, the germline founder cells Z2 and Z3 are flanked by the somatic gonad precursor cells, Z1 and Z4. Germline proliferation begins midway through the first larval stage (L1) and continues through adulthood. Somatic gonad precursors proliferate in the L1, giving rise to 12 somatic cells. At the end of the L2, the 10 proximal somatic gonad cells form the somatic gonad primordium, while the distal tip cells (DTCs) remain at the anterior and posterior ends of the gonad arms. Somatic gonad rearrangement separates proliferating germ cells into anterior and posterior populations and alters the position of germ cells relative to somatic gonad cells. Initial meiosis occurs in the mid-to-late L3 in the proximal-most part of the germ line, at the border of the gonad "arms" and the centrally located somatic gonad. In DAPI-stained preparations, initial meiosis is visible with the appearance of "transition" nuclei ("transition" corresponds to leptotene and zygotene stages of prophase of meiosis I). Pachytene nuclei appear at the proximal border of the germ line late in the L3 (Austin and Kimble, 1987; Kimble and White, 1981). In later stages, the proximal-most cells are the first to form gametes. Yellow denotes mitotic cells, green denotes meiotic cells, light blue indicates spermatocytes, dark blue indicates sperm, and pink indicates oocytes. The central block shown for the L3 and subsequent stages represents cells in the proximal somatic gonad. Gonad sheath cells are not depicted.
LAG-2 is expressed at high levels in the two DTCs, where it interacts with germ cells to promote mitosis and/or inhibit meiosis (Henderson et al., 1994; Lambie and Kimble, 1991). Reporter expression studies also indicate that lag-2 is expressed in two proximal somatic gonad cells, Z1.ppp and Z4.aaa (Wilkinson et al., 1994). The role of LAG-2 in these cells is best characterized in the context of the anchor cell/ventral uterine precursor cell (AC/VU) decision, where it acts as a ligand for the receptor LIN-12 (Wilkinson et al., 1994). A potential role for the proximal gonad in germline proliferation was first suggested by studies from Seydoux et al. (1990). They found that in the absence of lin-12 or in the absence of specific proximal somatic gonad cells, glp-1-dependent ectopic proliferation occurs in the proximal germ line (Seydoux et al., 1990). The appearance of mitotic germ cells proximal to mature gametes defines the proximal proliferation (Pro) phenotype (Qiao et al., 1995; Schedl, 1997). The lin-12(null) and ablation-induced Pro phenotypes are also dependent on the presence of an AC precursor in the proximal somatic gonad (Seydoux et al., 1990). A Pro phenotype was also observed and investigated by Henderson et al. (1997), where it occurred in response to expression of a non-membrane-bound form of the LAG-2 ligand. The Pro phenotype was dependent on the presence of both an AC precursor and the DTC in the corresponding arm of the gonad (Henderson et al., 1997).

We present an analysis of the proximal proliferation (Pro) phenotype in a gain-of-function glp-1 mutant, glp-1(ar202) (Pepper et al., 2003). glp-1(ar202) is one of three temperature-sensitive gain-of-function mutants that we previously identified and characterized genetically. As a class, glp-1(Pro) mutants (1) encode mis-sense mutations in the extracellular domain of the receptor, (2) share unusual genetic properties, and (3) exhibit the Pro phenotype. Our genetic analysis indicated that, although glp-1(ar202) can, at a low penetrance, bypass the larval lethality of a lag-2(null) allele, the receptor is still sensitive to the presence of the ligand (Pepper et al., 2003). Here, we present evidence that the cellular basis for the glp-1(ar202) Pro phenotype is a delay and mispositioning of initial meiotic entry. Cell ablations in this mutant indicate that the Pro phenotype is sensitive to LAG-2 produced by the proximal somatic gonad, suggesting that the glp-1(Pro) phenotype is due to enhanced or prolonged response to early proliferation signals from the proximal gonad.

Our analysis of the glp-1(Pro) phenotype prompted us to further investigate how development of the somatic gonad and germ line are normally coordinated to set the stage for meiotic entry. Previous work (Austin and Kimble, 1987; Kimble and White, 1981) established that early ablation of the DTC or loss of glp-1 causes all germ cells to cease mitosis and enter meiosis. However, the germ cells of the DTC-ablated animals enter meiosis at the correct time, whereas those in the glp-1(null) enter meiosis early. These results led to the proposal that either (1) DTC-independent activation of GLP-1 occurs in the early germ line and that this activation is important to prevent early meiosis or (2) residual signaling occurs after the ablation (Austin and Kimble, 1987). We present results supporting the first hypothesis: we find that GLP-1-dependent early germline proliferation is dependent on the LAG-2 ligand but that the LAG-2 signal impinges on the germ cell population from multiple cells within the early larval somatic gonad, includ-

Fig. 2. The adult glp-1(ar202) Pro phenotype. Photomicrographs of live wild-type (A) and glp-1(ar202) (D) hermaphrodites under Nomarski optics and corresponding schematic diagrams (B), (E). (C) and (F) are in situ DAPI-stained preparations of individual (different individuals from those in A and D) wild-type and glp-1(ar202) animals, respectively. The color scheme in (B) and (E) is as described in Fig. 1. The asterisk in (C) and (F) indicates the distal tip. Bar, 50 μm.
ing proximal gonad cells. An ultrastructural analysis at the L2/L3 stage further indicates that germ cells are already syncytial and continue to proliferate at a distance from direct DTC contact. Thus, LAG-2 produced by the proximal somatic gonad, in addition to LAG-2 produced by the DTC, promotes germline proliferation during normal development. Based on these results, we propose a model whereby the entire early somatic gonad establishes a niche that is subsequently remodeled during gonadogenesis to allow the formation of a germline developmental axis with stem cells at one end and gametes at the other.

Materials and methods

Strains, genetic manipulations, and general analysis methods

Strains were constructed and maintained by using standard techniques. The wild type in all cases was the C. elegans var. Bristol N2 strain (Brenner, 1974), and mutants used in this study were derived from N2: glp-1(ar202) (Pepper et al., 2003), unc-32(e189) (Brenner, 1974), lag-2(q420) (Lambie and Kimble, 1991), and fem-1(hc17) (Nelson et al., 1978). Unless otherwise noted, strains were raised at 15°C, synchronized as described (Pepper et al., 2003), and shifted to 25°C for 48 h prior to analysis, and (n) represents the number of gonad arms scored.

Synchronization, time course analysis, and DAPI staining

Animals were tightly synchronized by a hatch-off protocol, fixed, and stained with DAPI as described (Francis et al., 1995; McCarter et al., 1997; Pepper et al., 2003). For time course analyses, glp-1(ar202), lag-2(q420), and N2 animals were collected at time points covering the late L1 to adult (13-48 h postshift, 20 time points in all with 2-20 animals scored per time point). At each time point, the developmental age of the animals was assessed and germline nuclei were staged and counted. Nuclear morphology was used to distinguish mitosis, “transition” (leptotene and zygotene), pachytene, spermatocytes, sperm, and oocytes. Since the growth rate of the mutants is more variable than that of the wild type, comparisons were made on animals stage-matched by molts and by the extent of vulval development.

Immunofluorescence analysis

Gonad dissections, fixation, and immunohistochemistry were carried out as described (Francis et al., 1995), except that 3% paraformaldehyde was used instead of formaldehyde. Dilutions used for primary antisera were as follows: αPGL-1-1, 1:5000, and SP56, undiluted (kindly provided by S. Strome), α-phospho-histone H3, 1:300 (Upstate Biotechnology, 06-570), and α-GLP-1(LNG), 1:10 (kindly provided by S. Crittenden and J. Kimble). Secondary antibodies (IgG; Jackson Immunoresearch Laboratories, Inc.) were as follows: Rhodamine-Red-X-conjugated AffiniPure Goat Anti-Rabbit to visualize αPGL-1, αGLP-1(LNG), and α-phospho-histone H3, and Fluorescein-conjugated AffiniPure Goat Anti-Mouse to visualize SP56. Samples were mounted in 5 μl Vectashield with DAPI (Vector Laboratories H-1200) on 5% agar pads for observation. Analysis was performed on a Zeiss Axioplan II microscope with Openlab software (Improvision) and on a Leica TCS SP II Confocal system (Leica Microsystems Inc.) with Leica Confocal Software. Worms of the genotype gld-2(q237) gld-1(q175); unc-32(e189) glp-1(q175) (kindly provided by D. Hansen and T. Schedl) were used as a negative control for α-GLP-1(LNG) staining (data not shown).

Temperature shift analysis

Synchronous populations of glp-1(ar202) animals were either shifted up from the permissive temperature (15°C) to the restrictive temperature (25°C) or shifted down (after an early L1 up-shift) from the restrictive temperature to the permissive temperature. Animals were staged at the designated time point by using Nomarski optics, and the Pro phenotype was similarly scored at the early adult stage. Animals shifted to the restrictive temperature as early adults were scored 24 h after the shift.

Germine feminization and RNA interference (RNAi) methods

RNAi feeding experiments were carried out by using HT115(DE3) Escherichia coli strain (Timmons et al., 2001) transformed with pLT63 (L4440 “double T7” vector with fem-1 cDNA insert) or the vector alone (Timmons and Fire, 1998) as described with minor modifications (Pepper et al., 2003). Animals from the second day of feeding were scored for both Fem and Pro phenotypes 48 h after the temperature shift. The criteria for the Fem phenotype were the absence of sperm and the characteristic columnar morphology of oocytes. Strains feminized by the fem-1(hc17) mutation were scored for Fem and Pro phenotypes 48 h post-hatch/temperature shift and after fixation and DAPI staining as described (Pepper et al., 2003).

Cell ablations

Wild-type animals were grown at 25°C, synchronized by a 1 hour hatch-off, and harvested just prior to the L1/L2 lethargus. Individual animals were mounted in M9 on 5% agar pads containing 10 mM sodium azide for anesthesia. Cells were identified by morphology and position in the hermaphrodite (Kimble and Hirsh, 1979) and were ablated in various combinations by using a nitrogen pulse laser (Laser Science Inc, VSL337) as described (Bargmann and Avery, 1995). For the meiotic entry experiments in the wild...
type, ablations were only performed on animals in which all four cells of interest (Z1.aa, Z1.pp, Z4.aa, and Z4.pp) were visible. This stage represents a short time window between the division of Z1.a and Z4.p to give rise to the DTCs and the time that Z1.pp and Z4.aa divide to give rise to Z1.ppa, Z1.ppp, Z4.aaa, and Z4.aap. Although Z1.ppp and Z4.aaa are the only proximal somatic gonad cells that normally express lag-2 (Wilkinson et al., 1994), their parents (Z1.pp and Z4.aa) were ablated since their sisters (Z1.ppa and Z4.aap) may produce lag-2 in their absence as evidenced by their ability to form an anchor cell (Seydoux et al., 1990). Animals were examined 18 h postablation by using Nomarski optics to verify the efficacy of the operation. Abnormal or sickly animals were discarded. The following additional criteria were used for scoring: after Z1.pp and Z4.aa ablations, only vulvaless animals were retained. Where one or both DTCs were ablated, only animals with no significant gonad arm migration were retained. Unoperated gonad arms were compared with operated arms as internal controls in animals for which one DTC was ablated. Animals that met these criteria were individually fixed in ethanol at 19 h postablation (L4 stage), stained with DAPI, and scored under 1000× magnification. Control animals were treated in parallel with ablated animals: control animals were picked from the same plates as operated animals, mounted on agar pads containing sodium azide, staged on the same microscope, and underwent the same recovery and subsequent growth conditions.

Cell ablations in glp-1(ar202) animals (and unoperated controls) were performed as above, except that the animals were grown at 20°C prior to the hatch-off, and all subsequent manipulations were performed at 23°C (rearing after the hatch-off, ablation, and growth after the ablation). In some cases, Z1.pp and Z4.aa were ablated prior to the completion of both cell divisions producing the DTCs. The same criteria noted above identified successful ablations; animals were fixed and scored for germline development as adults (36 h postablation).

**Electron microscopy methods**

Studies of gonad organization in wild-type larval animals utilized prints of six archival thin section series in the collection of the C. elegans Anatomy Center. These animals were fixed by immersion in chemical fixatives (either osmium tetroxide only, or buffered aldehydes followed by osmium tetroxide), and then dehydrated and embedded in plastic resin (Hall, 1995; Sulston et al., 1980). Where necessary, new TEM images were collected with a Philips CM10 electron microscope.

**Results**

Gonad development is well characterized in the C. elegans hermaphrodite (Kimble and Hirsh, 1979) and is summarized in Fig. 1. For simplicity, we refer to the first meiotic entry in the life of the worm as “initial meiotic entry.” This event constitutes the end of the all-mitotic phase of germline development, after which the distal-to-proximal germline pattern of proliferation and differentiation is established. Initial meiosis occurs in the L3 in the proximal-most part of the germ line. As a result of subsequent growth and morphogenesis of the gonad, later meiotic entry occurs in the distal germ line as cells exit the distal mitotic zone (Fig. 1).

**Mitotic germ cells occupy the proximal gonad in adult glp-1(ar202) animals**

glp-1(+) activity in the germ line maintains proliferation (Austin and Kimble, 1987). In glp-1(null) animals, only a few germ cell divisions occur and all germ cells enter meiosis (Austin and Kimble, 1987). Previously, we characterized three temperature-sensitive glp-1(Pro) mutants that define a novel class of glp-1(gain-of-function) alleles based on phenotypic and genetic characteristics (Pepper et al., 2003). The Pro phenotype of glp-1(Pro) mutants is striking: in the early adult hermaphrodite, a large mass of nuclei is present in the proximal gonad, between mature gametes and the proximal somatic gonad structures (Pepper et al., 2003; Fig. 2). This pattern contrasts with the wild-type germline pattern in which the distal germ line is mitotic and the proximal germ line contains gametes. The processes of mitosis, meiosis, and gametogenesis in these Pro mutants do not appear defective, rather their pattern is altered. To determine the cellular basis for the glp-1(Pro) phenotype we further analyzed a representative mutant, glp-1(ar202).

First, to confirm that the ectopic mass in the proximal region of adult glp-1(ar202) consists of mitotic germ cells, we examined nuclear morphology and performed immunohistochemical analysis for germ cell and mitosis markers. Our analysis confirmed the presence of germline-specific P granules and the overlap of phosphohistone-H3 with mitotic figures evident in DAPI-stained preparations (Fig. 2; Materials and methods; and data not shown). We examined the expression of GLP-1 in the germ line of glp-1(ar202) animals in the early adult stage using antibodies that recognize the extracellular domain of the receptor (α-LNG) (Crittenden et al., 1994). Similar to previous results observed with lin-12(null) Pro animals (Crittenden et al., 1994), GLP-1 was localized to the membrane in the ectopic proximal mitotic zone of the glp-1(ar202) early adult Pro hermaphrodites (Fig. 3).

The adult Pro phenotype in glp-1(ar202) results from an earlier defect in initial meiotic entry

To determine the cellular basis for the Pro phenotype in glp-1(ar202) mutants, we performed a time-course analysis of germline development (see Materials and methods; Fig. 4; Table 1). The results indicate that the glp-1(ar202) Pro
phenotype is caused by spatial and temporal defects in initial meiotic entry. First, initial meiosis did not occur in the proximal-most germ cells, as it did in the wild type. Rather, the first cells to enter meiosis entered at a more distal position in the germ line. The cells closest to the proximal somatic gonad did not enter meiosis and instead remained mitotic. Second, relative to somatic development, the onset of meiosis was temporally delayed (Fig. 4; Table 1). The time course analysis ruled out several alternative possibilities for the Pro phenotype: proximal mitotic cells did not appear to be derived from distal stem cells that moved through differentiating cells to proliferate proximally, nor did they appear to derive from cells that entered meiosis normally and reverted to the mitotic cell cycle. In summary, the proximal-most cells in glp-1(ar202) Pro adults derive from cells that remained in the premeiotic state, while more distally located germ cells entered meiosis.

The Pro phenotype displays an early temperature dependence

If the Pro phenotype in glp-1(ar202) is caused by a defect in initial meiotic onset, the critical temperature-sensitive period for the Pro phenotype should coincide with or precede the time of initial meiosis. To test this prediction, we shifted worms from the permissive to the restrictive temperature at different time points and scored them for the Pro phenotype in the early adult stage (“up-shift”). glp-1(ar202) animals that were shifted to the restrictive temperature after the mid-L3 were non-Pro (wild-type proximal
germline pattern), while animals shifted to the restrictive temperature prior to the L3 displayed the Pro phenotype (Fig. 5). In a second experiment (“down-shift”), animals were shifted to the restrictive temperature as early L1 larvae and then shifted back to the permissive temperature at different stages. When animals were shifted back to the permissive temperature prior to the L3, they did not display the Pro phenotype (Fig. 5). To our surprise, in “down-shift” experiments, the proximal mitotic cells in L4 and adult animals did not enter meiosis even after the worms were shifted to the permissive temperature, suggesting that, if initial meiosis does not occur correctly in these animals, continued proliferation in the proximal germ line is irreversible. In summary, both temperature-shift experiments support the hypothesis that the Pro phenotype is caused by a defect in initial meiotic entry.

Fig. 4. Initial meiotic onset is delayed and displaced in glp-1(ar202). Representative individual L4 animals from the time course analysis, stage matched by vulva development. (A) and (B) are Nomarski images of one gonad arm and the vulva of the same individual animal shown in (C) after fixation and DAPI staining. (D), (E), and (F) are corresponding images of one individual glp-1(ar202) animal. A gonad arm of a later-stage glp-1(ar202) DAPI-stained individual is shown in (G). Arrowheads point to spermatocytes and arrows show the border of the germ line and proximal somatic gonad. Asterisks indicate the distal tip. The migration of the gonad arm in glp-1(ar202) was often slightly delayed compared with wild-type animals at a similar age. Bars, 10 µm.

Table 1
Initial meiotic entry is delayed in glp-1(ar202)

<table>
<thead>
<tr>
<th>Stage/Genotype</th>
<th>Mitotic</th>
<th>Transition</th>
<th>Pachytene</th>
<th>Spermatocytes</th>
<th>Sperm</th>
<th>Proximal Mitosis</th>
<th>Total</th>
</tr>
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<tr>
<td>Mid L2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2 (n = 20)</td>
<td>5 ± 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>glp-1(ar202) (n = 10)</td>
<td>8 ± 5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Mid L3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2 (n = 29)</td>
<td>48 ± 8</td>
<td>6 ± 7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>54</td>
</tr>
<tr>
<td>glp-1(ar202) (n = 10)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Mid L4</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2 (n = 20)</td>
<td>105 ± 15</td>
<td>27 ± 4</td>
<td>30 ± 6</td>
<td>4 ± 6</td>
<td>0</td>
<td>0</td>
<td>166</td>
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<tr>
<td>glp-1(ar202) (n = 8)</td>
<td>121 ± 11</td>
<td>53 ± 18</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>26 ± 17</td>
<td>200</td>
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<tr>
<td>Early Adult</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>N2 (n = 6)</td>
<td>167 ± 20</td>
<td>36 ± 12</td>
<td>61 ± 12</td>
<td>14 ± 4</td>
<td>29 ± 19</td>
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<td>glp-1(ar202) (n = 7)</td>
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<td>57 ± 5</td>
<td>13 ± 15</td>
<td>27 ± 47</td>
<td>107 ± 56</td>
<td>454</td>
</tr>
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</table>

Note. Average numbers of germ cells in mitosis and progressive meiotic stages per gonad arm (distal-to-proximal) in the wild-type N2 strain and glp-1(ar202) are given for various larval stages and the early adult (±1 standard deviation); see Materials and methods for details. n = the number of gonad arms scored for L3 and older. For the mid-L2, when germ cells are interspersed with and more difficult to distinguish from somatic nuclei, germ cell counts were obtained by counting the total number of nuclei within the gonad and subtracting 12, the number of somatic gonad nuclei present at this stage (Kimble and Hirsh, 1979). This value was then divided by two for comparison to germ cell counts per “gonad arm” given for later stages.
The glp-1(Pro) phenotype is independent of somatic or germ line sex-determination

The glp-1(Pro) phenotype is not dependent on hermaphrodite-specific somatic gonad development, since glp-1(ar202) males also display the Pro phenotype (data not shown). To determine whether the Pro phenotype was dependent on the sex of the germ line, we asked if female germ cells could form the ectopic proximal mass seen in glp-1(ar202) mutants. Hermaphrodites feminized by RNAi directed against fem-1 or feminized by the fem-1(hc17) mutation (see Materials and methods) displayed the Pro phenotype. Specifically, of 63 F1 self-progeny of glp-1(ar202) animals fed with bacteria producing fem-1 double-stranded RNA (Timmons and Fire, 1998), 51 were completely feminized. Of these 51 animals, 49 displayed a Pro phenotype. We also scored unc-32(e189) glp-1(ar202); fem-1(hc17) for Fem and Pro phenotypes: of 113 animals scored, 100% were Fem and 41% of these also displayed the Pro phenotype (this level of penetrance of Pro is comparable to 46% observed in the unc-32(e189) glp-1(ar202) strain; Pepper et al., 2003). Finally, the proximal germ cells in glp-1(ar202) animals do not appear to adopt a novel aberrant fate (in which they simultaneously express both a male fate and a proliferative capacity), since they are not recognized by SP56, a monoclonal antibody directed against an epitope expressed only in sperm and spermatocytes (Ward et al., 1986). SP56 staining was clearly visible in the spermatogenic cells distal to the ectopic mitotic germ cell mass (data not shown). In summary, the glp-1(ar202) Pro phenotype appears to be independent of sexual fate of the soma and germ line.

The glp-1(ar202) phenotype is sensitive to alteration of the proximal somatic gonad

Since lag-2 is expressed in the proximal somatic gonad cells Z1./pp and Z4.aaa (Wilkinson et al., 1994), one attractive explanation for the glp-1(Pro) phenotype is that it results from an elevated or persistent response to an early LAG-2/GLP-1 interaction in the proximal somatic gonad. Our previous analysis (Pepper et al., 2003) indicated that the glp-1(ar202) Pro phenotype can bypass zygotic lag-2, albeit inefficiently. That is, the few glp-1(ar202); lag-2(null) self progeny recovered from glp-1(ar202); lag-2(null)/+ mothers displayed a Pro phenotype. These results suggested that the receptor encoded by glp-1(ar202) can either (1) act constitutively, (2) respond to undetectable maternal levels of LAG-2, or (3) respond to another ligand. In combination with the reduction-of-function allele of lag-2, lag-2(q420), we observed a slight but significantly elevated penetrance of a wild-type germline pattern compared with glp-1(ar202) alone. Together, these results suggested that, in addition to a low level of ligand-independent activity, the receptor is still sensitive to the LAG-2 ligand (Pepper et al., 2003).

To test the dependence of the glp-1(Pro) phenotype on proximal somatic cells that express lag-2, we ablated Z1.pp and Z4.aa (the parents of Z1.bpp and Z4.aaa; see Materials and methods) in glp-1(ar202) L1 animals and compared their adult germline pattern phenotype with nonablated controls (Table 2). When the two proximal cells Z1.pp and Z4.aa were ablated, the frequency of the wild-type germline pattern was significantly elevated compared with the non-ablated control (from 5 to 32%). In one animal, germline proliferation could not be maintained at all despite the presence of the DTCs (Table 2). These results strongly suggest that the glp-1(ar202) phenotype is, at least in part, due to an elevated response to a signal from the proximal somatic gonad (see Discussion).

Early GLP-1-dependent germline proliferation in the wild type is governed by LAG-2/GLP-1 interactions

Our analysis of the glp-1(ar202) Pro phenotype led us to examine interactions between the soma and germ line that occur prior to and during initial meiosis in the wild type. glp-1(+) activity in the early germ line maintains proliferation and thus prevents premature meiosis (Austin and Kimble, 1987). The few germ cells that form in glp-1(null) animals reach the pachytene stage of meiosis I prophase in the L2 rather than the L3 (Austin and Kimble, 1987).

To determine whether the activation of GLP-1 by LAG-2 can account for the prevention of premature meiosis in the early larval germ line, we determined the time of meiotic entry in a lag-2 mutant. Strong loss-of-function alleles of lag-2 cause lethality prior to the time of initial meiosis, but a reduction-of-function allele, lag-2(q420), causes an incompletely penetrant temperature-sensitive glp-1-like germline proliferation defect (Glp) (Lambie and Kimble, 1991). We assessed the timing of initial meiosis in lag-2(q420) Glp animals and found that it was similar to that of the glp-1(null) (Table 3). We also determined the timing of initial meiotic entry in the portion of lag-2(q420) mutant animals that have relatively normal germline proliferation, and...
Ablation experiments were performed (see Materials and methods). Penetrance in glp-1(ar202) mitotic cells (Pepper et al., 2003) and is observed at a 22% (n wild-type in one arm, and the remaining three animals displayed the Class phenotype in one arm, one animal displayed Class A phenotype in both arms, one animal displayed Pro in one arm and wild-type in one arm, and two animals displayed Class A phenotype in both arms. If, as our genetic data suggest, LAG-2 is necessary for lag-2-expressing cells in the somatic gonad and asked if they affected early germline proliferation. In addition to lag-2 expression in the DTCs Z1.aa and Z4.pp (Fitzgerald and Greenwald, 1995; Henderson et al., 1994), lag-2 is expressed at a low level in two proximal somatic cells, Z1.pp and Z4.aa (Fitzgerald and Greenwald, 1995; Wilkinson et al., 1994). From the time Z1.pp and Z4.aa are born in the late L1 until the somatic gonad fully rearranges in the early L3, these cells are in contact with germ cells and are expressing lag-2 throughout the L2 (Wilkinson et al., 1994; X. Karp and I. Greenwald, unpublished observations). Although lin-12 and lag-2 are initially expressed at low levels in both Z1.pp and Z4.aa, LIN-12/LAG-2 interaction during the anchor cell/ventral uterine precursor cell (AC/VU) decision results in decreased expression of lin-12 and increased expression of lag-2 in one of the two cells, the cell that eventually adopts the AC fate (Seydoux and Greenwald, 1989; Wilkinson et al., 1994). After rearrangement of the somatic gonad in the late L2 and subsequent proliferation of the somatic gonad in the L3, the AC is separated from the germ line (Kimble and Hirsh, 1979).

To assess the effect of all somatic gonad lag-2-expressing cells on germline proliferation, we systematically ablated these cells singly and in combination. In these experiments, cells were ablated in the late L1 stage and individual animals were fixed and examined at a single informative time point in the L4 (see Materials and methods). We chose a time point late enough to allow the ablation to take effect but early enough to easily distinguish differences in stages of meiotic progression and gametogenesis (pachytene, spermatocytes, and sperm). Germ cells that had reached a later stage of meiotic progression at this single time point must have entered meiosis earlier than those that only reached an earlier stage of meiotic progression at the same time point. We assume that the rate of meiotic progression is not altered since there is no apparent change in the rate of meiotic progression once meiosis is initiated in glp-1(null) mutants (Austin and Kimble, 1987).

Our results indicate that all four potential lag-2-expressing cells influence early germline proliferation. First, consistent with previous results (Kimble and White, 1981), found that it was normal (Table 3). These results suggest that activation of GLP-1 by LAG-2 is necessary for promoting premature germline proliferation and hence preventing early meiotic entry.

Germline proliferation prior to somatic gonad rearrangement involves several sources of LAG-2

If, as our genetic data suggest, LAG-2 is necessary for GLP-1-mediated control of proliferation and prevention of early initial meiosis, then ablation of the relevant lag-2-expressing cell(s) in the somatic gonad should produce the same defects as those seen in glp-1(null) mutants. The DTC produces LAG-2, and ablation of the DTC causes all germ cells to enter meiosis. When a single DTC is ablated prior to the initial onset of meiosis, proliferation is reduced and all germ cells enter meiosis in the gonad arm adjacent to the ablation, but meiosis occurs at the correct time, rather than the early meiotic entry observed in glp-1(null) animals (Austin and Kimble, 1987; Kimble and White, 1981).

One possible explanation for the phenotypic discrepancy between DTC-ablation and the glp-1(null) is that DTC-independent activation of GLP-1 occurs in the early germ line and that this activation is important to prevent early meiosis. To test this possibility, we considered alternate lag-2-expressing cells in the somatic gonad and asked if they affected early germline proliferation. In addition to lag-2 expression in the DTCs Z1.aa and Z4.pp (Fitzgerald and Greenwald, 1995; Wilkinson et al., 1994), lag-2 is expressed at a low level in two proximal somatic cells, Z1.pp and Z4.aa (Fitzgerald and Greenwald, 1995; Wilkinson et al., 1994). From the time Z1.pp and Z4.aa are born in the late L1 until the somatic gonad fully rearranges in the early L3, these cells are in contact with germ cells and are expressing lag-2 throughout the L2 (Wilkinson et al., 1994; X. Karp and I. Greenwald, unpublished observations). Although lin-12 and lag-2 are initially expressed at low levels in both Z1.pp and Z4.aa, LIN-12/LAG-2 interaction during the anchor cell/ventral uterine precursor cell (AC/VU) decision results in decreased expression of lin-12 and increased expression of lag-2 in one of the two cells, the cell that eventually adopts the AC fate (Seydoux and Greenwald, 1989; Wilkinson et al., 1994). After rearrangement of the somatic gonad in the late L2 and subsequent proliferation of the somatic gonad in the L3, the AC is separated from the germ line (Kimble and Hirsh, 1979).

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Germline proliferation prior to somatic gonad rearrangement involves several sources of LAG-2

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A. Somatic gonad

B. Ablations

Fig. 6. Proximal and distal lag-2-expressing cells together prevent early meiotic onset. (A) The positions and identity of relevant somatic gonad cells are indicated at the time ablations were performed (see Materials and methods). (B) Ablated cells are marked by an “X.” In lines 2 and 4 (one DTC ablated), ablation of the anterior DTC is depicted, although roughly equal numbers of anterior and posterior DTC ablations were actually performed. Headings indicate the latest stage reached by germ cells in a given treatment in the operated gonad arm(s). Cells were ablated in late L1 larvae, and individual animals were fixed and examined at a single informative time point (see Materials and methods and Results). Assuming that the rate of meiotic progression is not altered (Austin and Kimble, 1987), the later the stage of the germ cells at the single time point, the earlier the germ cells must have entered meiosis. Data are reported as the number of worms with arm(s) containing cells at the indicated stage over the total number scored. In the last case (four cells ablated), four out of the six operated animals with sperm contained only mature sperm, indicating earliest meiotic entry, while the other two contained both spermatocytes and sperm. A previous report indicated that ablation of both Z1.a and Z4.p (like ablation of one DTC alone) does not influence the timing of meiotic entry (Kimble and White, 1981), while our results indicated a slightly earlier meiotic entry when their two distal daughters, Z1.aa and Z4.pp, were ablated. In the previous study, Z1.ap and Z4.pa were also eliminated as a consequence of ablating their parents (Kimble and White, 1981), and this may account for the slight difference between the results. More importantly, however, previous studies did not ablate the distal cells together with proximal cells. Our results demonstrate that ablation of the proximal lag-2-expressing cells together with the distal cells is necessary to observe a strong influence on the timing of meiotic entry.
The timing of meiotic entry was similar in mock-ablated animals versus animals in which one DTC was ablated. The timing of meiotic entry was also unaffected by ablation of Z1.pp and Z4.aa or Z1.pp and Z4.aa plus one DTC (Fig. 6). Finally, we compared the timing of meiotic entry in animals in which one DTC was ablated, both DTCs were ablated, and all four potential LAG-2-producing cells in the somatic gonad (Z1.pp, Z4.aa, and both DTCs) were ablated. Meiotic entry was slightly earlier when both DTCs were ablated compared with ablation of one DTC. Most striking, the ablation of all four cells (two proximal and two distal) resulted in early initial meiosis comparable with that observed in the lag-2(q420) and glp-1(null) mutant animals (Fig. 6). Together, our results indicate that both proximal and distal sources of LAG-2 contribute to proliferation across the germ line prior to initial meiosis and that their contributions are redundant and overlapping. In addition, our results are consistent with the possibility that other cells and other ligands do not contribute significantly to the activation of GLP-1 for its role in germline proliferation at this stage.

The distal tip cells do not extend long processes in the late L2

Our cell ablation analysis indicated that meiosis occurs slightly early in gonad arms in which both DTCs are ablated, and that this effect is enhanced with the additional removal of the two proximal lag-2-expressing cells. How can the DTC from one end of the gonad influence the proliferation of germ cells on the opposite end? Previous studies demonstrated the presence of long processes from
the adult DTC (an average of 8 cell diameters and a range of 2–20; Hall et al., 1999). If DTC morphology in the L2 were similar to that of the adult, the effective “reach” of each DTC could extend over a significant portion of the gonad, even to germ cells on the other side of the gonad. We examined the morphology of the DTC in transmission electron micrographs of serial sections of hermaphrodites in the late L2 (see Materials and methods; Fig. 7), and found that direct DTC-germ cell contact does not extend beyond two germ cell-lengths (each DTC touching a total of three germ cells). Given that the late L2 germ line contains ≈8–12 germ cells per arm, direct contact by the DTC processes is likely inadequate to explain continued proliferation prior to initial meiosis. Thus in the all-mitotic germ line of the L2/L3, the mitotic zone appears to extend beyond direct DTC/germ cell contact.

In the adult, it has been proposed that the DTC signal or intracellular products generated in response to it may be propagated within the germ line by transmission of signaling components via shared cytoplasm (Berry et al., 1997; Crittenden et al., 1994; Hall et al., 1999). Previous work established the syncytial nature of the late-larval and adult germ line (Hirsh et al., 1976), but connections between early germ cells have not been previously reported. Serial sections of L2/L3 animals show that germ cells are already connected by extensive cytoplasmic bridges (Fig. 7).

**Discussion**

*C. elegans* germline development has been conceptually separated into three phases: *glp-1*-independent onset of L1 proliferation, *glp-1*-dependent proliferation prior to initial meiosis, and continued DTC-dependent proliferation in the distal mitotic zone (Seydoux and Schedl, 2001). Results presented here provide new insight into the second of these proliferation phases, its termination at meiotic entry, and the transition to the last proliferation phase.

Germline proliferation prior to initial meiosis depends on LAG-2/GLP-1 interaction

After the first few germ cell divisions in the L1, all subsequent germline proliferation depends on GLP-1. Our studies indicate that GLP-1-dependent proliferation and delay of meiotic entry is LAG-2-dependent since reduction of *lag-2* [as in the *lag-2(q420)* mutant] results in early meiotic entry (Table 3). Our ablation analysis in the wild type further suggests that all potential *lag-2*-expressing cells in the somatic gonad, two DTCs plus the daughters of Z1.pp and Z4.aa in the proximal gonad, together influence the continuation of germline proliferation and prevent premature meiotic onset (Fig. 6). The DTCs appear to have an overlapping role since meiotic entry is slightly later when one DTC is ablated versus both DTCs. A proliferation-promoting role for the proximal *lag-2*-expressing cells was not previously appreciated since it is masked by the stronger influence of high levels of *lag-2* expression in the DTCs. Thus, the early germ line integrates signals from several anatomical sources that together promote proliferation throughout the germ line.

At the end of the L2, the somatic gonad undergoes an anatomical rearrangement as the 10 proximal cells come together in the center of the gonad and the germ cells are relegated to anterior and posterior populations with a DTC at each end (Kimble and Hirsh, 1979). The germ cells continue to proliferate in the early L3 prior to initial meiosis. Our ultrastructural data suggest that, at the L2/L3 molt, the DTCs have not yet formed long processes that are characteristic of the adult DTC morphology. Germ cells at this stage are already connected by extensive cytoplasmic bridges. The integration of the LAG-2/GLP-1-mediated mitogenic signal over the germ cell population could occur by cell–cell relay or through germine interconnections that permit transfer of the internalized ligand (Henderson et al., 1994), the intracellular domain of GLP-1, or its downstream effectors (Berry et al., 1997; Crittenden et al., 1994). The

<table>
<thead>
<tr>
<th>Stage/Genotype</th>
<th>Mitotic</th>
<th>Transition</th>
<th>Pachytene</th>
<th>Spermatocytes</th>
<th>Sperm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid L2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2 (n = 20)</td>
<td>5 ± 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>lag-2(q420)</em> non-Glp (n = 4)</td>
<td>4 ± 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>lag-2(q420)</em> Glp (n = 6)</td>
<td>0</td>
<td>0</td>
<td>4 ± 1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mid L3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2 (n = 29)</td>
<td>48 ± 8</td>
<td>6 ± 7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>lag-2(q420)</em> non-Glp (n = 20)</td>
<td>29 ± 8</td>
<td>2 ± 3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>lag-2(q420)</em> Glp (n = 12)</td>
<td>0</td>
<td>0</td>
<td>4 ± 1</td>
<td>3 ± 2</td>
<td>0</td>
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<tr>
<td>Early L4</td>
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<td></td>
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</tr>
<tr>
<td>N2 (n = 17)</td>
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<td>22 ± 5</td>
<td>14 ± 9</td>
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<td>0</td>
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<tr>
<td><em>lag-2(q420)</em> non-Glp (n = 11)</td>
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<td>13 ± 4</td>
<td>16 ± 6</td>
<td>1 ± 1</td>
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</tr>
<tr>
<td><em>lag-2(q420)</em> Glp (n = 6)</td>
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<td>0</td>
<td>0</td>
<td>2 ± 3</td>
<td>15 ± 10</td>
</tr>
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</table>

Note. Germ cell numbers were determined as described in Table 1 and Materials and methods. Two sets of data are given for *lag-2(q420)* for each stage; the top value is germ cell counts for fertile (non-Glp) animals and the bottom for sterile (Glp) animals (see text for details).
maintenance of GLP-1-mediated signaling by positive feedback (Berry et al., 1997; Kadyk and Kimble, 1998) may be particularly important after rearrangement of the somatic gonad to allow continued proliferation throughout the germ line in the early L3, prior to the onset of meiosis. Further ultrastructural analysis of the physical interactions between the germ line and the developing somatic gonad will be instructive.

An unexplored but formal possibility relevant to these observations is that the otherwise membrane-bound LAG-2 ligand could be soluble for a short time during development. While there is no direct evidence for a soluble form of LAG-2, this possibility has not been ruled out. There is evidence for cleavage of another member of the DSL family Delta (Qi et al., 1999), and potential proteolytic sites have been identified in LAG-2 (Tax et al., 1994). Engineered constitutively soluble forms of LAG-2 are active and can cause a Pro phenotype (Fitzgerald and Greenwald, 1995; Gao and Kimble, 1995; Henderson et al., 1997). Interestingly, the Pro phenotype induced by soluble LAG-2 cannot be suppressed by ablation of the DTC alone, but requires ablation of both proximal and distal LAG-2-producing cells (Henderson et al., 1997), likely reflecting the role for both proximal and distal LAG-2 in early germline proliferation. There is precedent in other systems for developmentally regulated soluble and membrane-bound stem cell factor (SCF) isoforms in mammalian spermatogenesis (Blanchard et al., 1998; Manova et al., 1993).

GLP-1 pathway signaling must be precisely downregulated in the proximal germ line at initial meiosis

Regardless of the exact mechanism by which GLP-1-pathway signaling is maintained in the premeiotic phase of germline development, subsequent meiotic entry must interfere with this activity. In the wild type, initial meiosis always occurs in the cells closest to the proximal somatic gonad. Therefore, GLP-1 activity must be downregulated proximally at the correct time for proper positioning and timing of initial meiosis and for the formation of the correct adult germline pattern.

The importance of tight spatial and temporal control of initial meiosis is highlighted by the radical pattern defects that can occur when initial meiosis is delayed and mispositioned, as in glp-1(Pro) mutants. The Pro phenotype was first described in lin-12(null) mutants (Seydoux et al., 1990), and likely also occurs in these mutants as a result of delayed and displaced meiotic entry due to inappropriate interaction between LAG-2 and GLP-1. In addition, Seydoux et al. (1990) demonstrated that ablation of selected somatic gonad cells in the L1 can also cause a Pro phenotype, and that the Pro phenotype in both the lin-12 mutant and in the ablated animals is dependent on glp-1(+) activity (Seydoux et al., 1990). Remarkably, the lin-12(null) Pro phenotype, once established, does not require the AC to be maintained (Seydoux et al., 1990). Therefore, even wild-type levels of GLP-1-mediated signaling, once inappropriately activated in proximal germ cells beyond the normal time of initial meiotic entry, can maintain proliferation in the absence of the AC. Thus, the proximal germ line exhibits a permissive to mitosis. This permissiveness may be a property of the proximal germ line throughout development since mutants of at least two loci exhibit reversion of meiotic cells to mitosis within in the proximal germ line: gld-1 (Francis et al., 1995) and puf-8 (Subramaniam and Seydoux, 2003).

The origin of the glp-1(Pro) phenotype

Given our results suggesting that LAG-2 in the proximal gonad contributes to early germline proliferation, the simplest hypothesis for the origin of the glp-1(Pro) phenotype is that the mutant receptor responds inappropriately to LAG-2 produced by the proximal somatic gonad. Although we have not formally ruled out the possibility that glp-1(ar202) is acting outside the germ line, our previous analysis indicates that glp-1(Pro) mutants display neither somatic gonad nor vulva phenotypes associated with glp-1 or lin-12 hypermorphic alleles (Greenwald et al., 1983; Berry et al., 1997; Pepper et al., 2003), consistent with glp-1(ar202) activity largely restricted to the germ line.

Here, using cell ablations, we extended our previous genetic analysis and found that the proximal somatic gonad influences the glp-1(ar202) phenotype. Most significantly, removal of Z1.pp and Z4.aa increased the frequency of the wild-type germline pattern or interfered with germline proliferation altogether (Table 2). We did not, however, observe a clear one-to-one correlation between the presence of the proximal somatic lag-2-expressing cells and the Pro phenotype, as would be expected in the simplest model for the glp-1(Pro) phenotype. The variability of our results likely reflects a combination of experimental limitations (e.g., inability to eliminate LAG-2 activity prior to the ablation and/or variable residual LAG-2 activity from membrane debris) and properties of the system itself. For example, our results may reflect the existence of both a ligand-dependent and ligand-independent activity of the glp-1(ar202)-encoded receptor, both of which are close to threshold levels for the mitosis/meiosis decision, and either of which may or may not trigger robust positive feedback necessary to produce the adult Pro phenotype in the absence of Z1.pp and Z4.aa. Additional observations support the idea that the glp-1(ar202) receptor responds to both proximal and distal sources of the ligand in a threshold manner. Ablation of one DTC in late L1 glp-1(ar202) hermaphrodites produces a range of phenotypes, including Glp, Pro, and a novel phenotype with mitosis in the proximal arm and gametogenesis in the distal region (n = 6; A.P. and E.J.A.H., unpublished observations). An alternative hypothesis is that additional mechanisms influence the Pro phenotype. For example, the receptor encoded by glp-1(ar202) could be preferentially resistant to mechanisms that actively downregulate GLP-1-pathway signaling in the proximal
germ line. Further clarification of these models will await the molecular characterization of other loci known to cause perturbations of initial meiosis that lead to a Pro phenotype, such as ego-3 (Qiao et al., 1995) and at least one additional locus (D.J.K. and E.J.A.H., unpublished observations).

**Early germline proliferation and the transition into germline stem cell proliferation**

Our results suggest that position rather than lineage within the germ line plays an important role in determining which germ cells will become stem cells and which will undergo early differentiation. Two distinct aspects of somatic gonad rearrangement effectively restructure a potential stem cell niche and determine where the onset of meiosis will occur. First, as the early larval gonadal cells divide and intermingle with germ cells, both distal and proximal cells together promote proliferation in the premeiotic germ line. Z1.a and Z4.p and/or their daughters promote proliferation early in the L1 (Kimble and White, 1981) and the DTCs together with Z1.ppp and Z4.aaa (and possibly their sisters) promote proliferation into the early L3. We propose that, until the somatic gonad rearranges and the DTCs migrate a sufficient distance from the proximal somatic gonad, germ cells are essentially equal in their stem cell potential since they appear to regulate both distal and proximal signals across the gonad. After rearrangement and further DTC migration, germ cells are presumably cut off from proximal LAG-2 (although the possibility of cellular processes extending from the AC has not been ruled out), and the only remaining source of LAG-2 for maintenance of the stem cells is the DTC. The regulation of early and late proliferation may not be identical. Several loci exhibit differential effects on early germline proliferation and later DTC-mediated proliferation. For example, loss of FBF activity specifically causes distal stem cell loss [a glp-1(loss-of-function)]-like phenotype, well after initial meiotic onset has occurred (Crittenden et al., 2002). What is clear from our studies is that anatomical and molecular mechanisms impose a tight restriction on the normal time and position for the initial onset of meiosis and that this restriction is essential for proper adult germline pattern formation. It will be of interest to see whether similar mechanisms occur during coordinated somatic gonad/germline development in other systems.

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**References**


