In the hermaphrodite gonad an interaction between two equipotential somatic cells, Z1.ppp and Z4.aaa, is required for the specification of their fates. These two cells have variable fates: either one can become an anchor cell (AC), with the other becoming a ventral uterine precursor cell (VU) (Kimble and Hirsh, 1979). Laser ablation experiments have shown that the VU fate of Z1.ppp or Z4.aaa depends on an "AC-to-VU" signal emanating from the presumptive AC (Kimble, 1981; Seydoux and Greenwald, 1989). Specification of the AC and VU fates of Z1.ppp and Z4.aaa depends on lin-12 activity: in lin-12(0) hermaphrodites, which lack lin-12 activity, both Z1.ppp and Z4.aaa adopt the AC fate, while in lin-12(d) hermaphrodites, which by genetic criteria have elevated lin-12 activity, both Z1.ppp and Z4.aaa adopt the VU fate (Greenwald et al., 1983). Although lin-12 function is VU autonomous, the level of lin-12 activity seems to be assessed in both Z1.ppp and Z4.aaa before either cell commits to a restricted fate (Seydoux and Greenwald, 1989). The genetic analysis combined with the finding that lin-12 is predicted to encode a transmembrane protein with large extracellular and intracellular regions (Yochem et al., 1988) has led us to propose that the lin-12 protein functions as a receptor for the AC-to-VU signal (Seydoux and Greenwald, 1989).

An inductive soma-germline interaction is required for germline development. In this paper, we use the term inductive simply to refer to interactions that occur between different tissues or cell types. The hermaphrodite gonad has two arms; each arm has a distal-proximal axis with respect to the vulval position (Figure 1). The germline tissue is syncytial. Nuclei in the distal region of the germline are mitotic, while more proximal nuclei are meiotic. Laser ablation experiments have shown that the mitotic "fate" of distal germline nuclei in each arm depends on a somatic gonadal cell, the distal tip cell (DTC), which is thought to produce a DTC-to-germline signal that induces mitosis (or inhibits meiosis) in the germline (Kimble and White, 1981). The mitotic division of germline nuclei depends on the continued presence of gfp-1 activity within the germline (Austin and Kimble, 1983; Prish et al., 1987). The predicted product of gfp-1 is a transmembrane protein with extensive similarity to lin-12 (Yochem and Greenwald, 1989; Austin and Kimble, 1989). Thus, genetic and molecular evidence suggests that gfp-1 encodes a receptor for the DTC-to-germline signal (Austin and Kimble, 1987, 1989; Yochem and Greenwald, 1989).

The DTC-germline interaction occurs distally, while the AC-VU interaction takes place in the proximal part of the gonad in the vicinity of the most proximal region of the germline. We present evidence that, under certain conditions, the AC can inappropriately induce mitosis in the proximal germline.

Results

Development of the Wild-Type Hermaphrodite Gonad

C. elegans postembryonic development comprises four...
Gametogenesis are ordered from distal to proximal (Figure 3C; Hirsh et al., 1976). The most proximal end in the L4 stage and continues tally (Kimble and White, 1981). Gametogenesis begins at the DTC (distal germline nuclei) continue to divide mitotically (Hirsh et al., 1976). The germline progenitors between the anterior and posterior arms of the gonad. During the L3 stage, germ-line nuclei farthest from the DTC (proximal germline nuclei) have been apportioned between the anterior and posterior arms of the gonad. During this process, germline nuclei are excluded from the central region where the AC resides. It is not clear whether the fully differentiated AC initially maintains some direct contact with proximal germline nuclei. By the L3 stage, however, the AC is surrounded by somatic tissue and is no longer in close proximity to the germline (Figure 1). The fate of the AC in the adult is not known (Kimble and Hirsh, 1979).

**lin-12(0) Hermaphrodites Display Proximal Mitosis**

Examination of *lin-12(0)* young adults by Nomarski microcopy reveals that proximal germline nuclei exhibit the same morphology as distal nuclei. Gametogenesis is limited to the region between the distal and proximal mitotic nuclei. In this region, the normal proximal to distal order of spermatogenesis followed by oogenesis is observed (Figure 2B; also see Experimental Procedures).

To demonstrate that proximal germline nuclei in *lin-12(0)* hermaphrodites are in the mitotic cycle and are not arrested in an early meiotic stage, germline chromosome morphology was visualized by DAPI staining (Figure 3) (see Experimental Procedures). Proximal nuclei in *lin-12(0)* L4 and young adult hermaphrodites are in mitosis (Figure 3H; also see Experimental Procedures), demonstrating proximal germline proliferation. The most distal nuclei are also mitotic, as in wild type (Figures 3D and 3I). Two regions of meiotic prophase (separated by spermatocytes) are observed; the meiotic nuclei are farthest away from the proximal and distal mitotic regions (Figure 3G; also see Experimental Procedures).

Thus, in *lin-12(0)* hermaphrodites a second region of germline proliferation is generated in the most proximal portion of each gonadal arm. We term this phenotype "proximal mitosis." Based on this *Lin-12(0)* phenotype, we conclude that in wild-type hermaphrodites, *lin-12(+)* activity acts, either directly or indirectly, to prevent proximal mitosis.

We note that in the experiments preseented below proximal mitosis was scored in young adult hermaphrodites simply using Nomarski optics. Proximal germinal nuclei with a morphology typical of distal mitotic nuclei and absence of gametogenesis proximally (Figure 2B) were taken as evidence for proximal mitosis.

**lin-12 Activity Is Required in the Somatic Gonad to Prevent Proximal Mitosis**

To determine the focus of *lin-12* activity necessary to prevent proximal mitosis, we examined the germline of genetic mosaics containing defined *lin-12(0)* cells among *lin-12(+)* cells. Mosaics were isolated among segregants of the strain *ncl-7 uric-36 lin-12; qDp3* as described in Beyerdoux and Greenwald (1989) and Experimental Procedures. Two mosaics, designated type I in Figure 4, lacked *lin-12* activity in the germine (P descendent) but retained *lin-12* activity in the somatic gonad (Z1 and Z4 descendent).

---

**Figure 1. Gonadogenesis in Wild-Type Hermaphrodites**

Each diagram represents the gonadal anatomy at defined points during successive stages of postembryonic development, based on observations by Hirsh et al. (1976), Kimble and Hirsh (1979), and Kimble and White (1981). Stippled region: somatic gonadal tissue. See text for a brief description of gonadogenesis. The AC precursor (Z1.ppp or Z4.aaa) is born in the L1-L2 molt and becomes morphologically recognizable as an AC by the end of the L2 stage. No AC seems to be present in the adult; its fate is not known (Kimble and Hirsh, 1979).

---

Ultrastructural studies have shown that both potential ACs, Z1.ppp and Z4.aaa, directly contact the germline in the mid-L2 stage (Kimble, 1978). A morphologically distinct AC does not appear until the late L2 stage, when a group of somatic cells move around the AC precursor to form the somatic primordium (Kimble and Hirsh, 1979).
Figure 2. Germline of Wild-Type and lin-12(q269) Hermaphrodites
Composite photomicrographs using Nomarski optics showing a single gonadal arm. Line drawings are shown below. Bar, 40 μm.
(A) Wild-type (N2) young adult hermaphrodite. Distal germline nuclei are mitotic, while oocytes and sperm are visible proximally.
(B) lin-12(q269) young adult hermaphrodite. There is no sign of gametogenesis in the most proximal region. Instead, proximal germline nuclei appear like the distal mitotic germline nuclei. Oocytes and sperm are visible only in the loop region. lin-12(q269) hermaphrodites seem to have strongly reduced but not null lin-12 activity. lin-12(0) hermaphrodites exhibit the same germline phenotype as lin-12(q269) hermaphrodites, except that the region of proximal mitosis is often more extensive in lin-12(0) hermaphrodites (see Experimental Procedures).

These animals did not display proximal mitosis; instead gametes were observed proximally as in wild type. This result suggests that lin-12 activity is not required in the germline to prevent proximal mitosis.

We also characterized the germline phenotype of 14 mosaics in which lin-12 activity was absent from either the Z1 lineage or the Z4 lineage but was present in the rest of the animal, including the germline (type II and III mosaics, Figure 4). We refer to these mosaics as Z1/Z4 mosaics. Most Z1 descendants contribute to the anterior arm of the gonad, while most Z4 descendants contribute to the posterior arm (Kimble and Hirsh, 1979). Hence, Z1/Z4 mosaics have essentially one somatic gonadal arm that is genotypically lin-12(+) and one somatic gonadal...
Figure 3. Morphology of Germline Nuclei in Wild-Type and lin-12(0) Hermaphrodites
Photomicrographs of young adult hermaphrodites stained with DAPI.
(A-D) Wild type. (E-I) lin-12(0) (unc-26(e251) lin-12(mdd1))
(A and E) Low magnification view of an anterior gonadal arm. The vulval region is to the right.
arm that is genotypically lin-12(0). In 13 of 14 Z1/Z4 mosaics, the lin-12(u) arm displayed the proximal mitosis phenotype observed in lin-12(0) mutants (type II and III mosaics, Figure 4; Figure 5). The lin-12(+) arm in each of these mosaics was phenotypically wild type. These results suggest that lin-12 activity is required in the somatic gonad to prevent proximal mitosis.

In 1 of 14 Z1/Z4 mosaics, both gonadal arms had a wild-type germline phenotype. A wild-type phenotype was also observed in 3 of 8 additional mosaics that lacked lin-12 activity in the Z1 lineage or the Z4 lineage as well as in other nongonadal lineages (data not shown). Thus, in a minority of mosaic animals (4 of 22), proximal mitosis is suppressed in both arms although lin-12 activity is present in only one arm. It is possible that in such animals the lin-12(+) somatic gonadal cells from the lin-12(+) arm are sufficient to prevent proximal mitosis in the lin-12(0) arm. However, as described above, in the majority of mosaic animals (18 of 22), lin-12(+) activity prevents mitosis locally, i.e., only in the lin-12(+) gonadal arm.

Mosaic animals in which lin-12 activity is absent from either the Z1 or the Z4 lineage have a lin-12(0) AC (Seydoux and Greenwald, 1989). All mosaics of this type observed in the present study (72 of 22) had at least one phenotypically wild-type gonadal arm. These data demonstrate that lin-12 activity is not required in the AC to prevent proximal mitosis if other somatic cells are lin-12(+). We do not know whether lin-12 activity in the AC would be sufficient to prevent proximal mitosis if all other somatic cells were lin-12(0).

A Single AC Is Necessary and Sufficient for Proximal Mitosis in lin-12(0) Hermaphrodites

Mosaic analysis indicates that a defect in the somatic gonad rather than in the germline causes the proximal mito-

---

Figure 4. Germline Phenotype of lin-12 Genetic Mosaics

Each diagram shows an abbreviated C. elegans lineage (Sulston et al., 1983). A horizontal line represents one cell division; a dotted line represents six cell divisions. P1 is the zygote. The embryonic blastomeres AB, MS, E, C, and D are precursors for the somatic, while P1 gives rise to the germline. Z1 and Z4 give rise to the somatic structures of the gonad: most Z1 descendants contribute to the anterior gonadal arm, while most Z4 descendants contribute to the posterior arm. The Ncl phenotype (+ for Ncl+ and − for Ncl−) is indicated for each lineage that was scored in mosaic animals isolated among segregants of the strain ncl-1(e1855) unc-35(e251) lin-12(n947); qDp3 (see Experimental Procedures). qDp3 carries the wild-type alleles of ncC7, uric-36, and lin-12. Thus, the genotype of Ncl− cells is expected to be ncl-1(e1855) unc-35(e251) lin-12(n947); qDp3, and the genotype of Ncl+ cells, in which qDp3 has been lost, is expected to be ncl-1(e1855) unc-35(e251) lin-12(n947). Cells derived from the embryonic precursor E cannot be scored reliably for the Ncl− phenotype. The presence of qDp3 in the germline was assessed by scoring for phenotypically wild-type progeny. n = number of mosaic individuals of each type obtained. The phenotype of the germline is also indicated. (Note: the fates of the somatic cells of these mosaic animals were previously reported in Seydoux and Greenwald, 1989.)

---

Figure 5. Germline in a Z1/Z4 Mosaic Hermaphrodite

The diagram depicts the germline phenotype of 13 of 14 mosaic hermaphrodites in which lin-12 activity was absent either from the Z1 lineage or the Z4 lineage but present in the rest of the animal, including the germline (type II and III mosaics from Figure 4). In these mosaics, one somatic gonadal arm is genotypically lin-12(+) and the other is lin-12(0). The lin-12(+) arm is phenotypically wild-type and the lin-12(0) arm exhibits the proximal mitosis defect.
**Figure 6. Laser Ablation Experiments in lin-12(O) Hermaphrodites**

(A) Late L1 lin-12(O) somatic gonad. Somatic nuclei are schematically represented as they appear in the gonad or a late L1 lin-12(O) hermaphrodite. The name (i.e., Z1.ppa), and the fate of each cell is indicated. AC, anchor cell; VU, ventral uterine precursor cell; DTC, distal tip cell; SHISP, sheath cells and spermatheca precursor; SHISPIDU, sheath cells, spermatheca, and dorsal uterus precursor. Note that in lin-12(O) hermaphrodites, Z1.ppa and Z4.aaa always become AC, while Z1.ppa and Z4.aap rarely do so (Greenwald et al., 1983, Experimental Procedures). A horizontal line represents a cell division.

(B) Ablations in lin-12(O) hermaphrodites. Ablations were performed in unc-36(e251) lin-12(nq41) hermaphrodites as described in Experimental Procedures. All ablations were completed by the L2 stage except for type V ablaiions, which were performed in the L2 molt. Diagrams depict late L1 somatic gonads as shown in (A). In diagram V, an L2 molt gonad is schematically represented, and only the two ablated ACs are shown. The number of animals with proximal mitosis of the total number of operated hermaphrodites is indicated for each type of ablation. Proximal mitosis was not verified by DAPI staining, but was scored using Nomarski optics (see Experimental Procedures).

* In these animals, no gonadal arms were formed since the DTCs are required for their outgrowth from the gonadal primordium (Kimble and White, 1981). Thus, all germline nuclei remained proximal. These germline nuclei proliferated into adulthood. In contrast, in similarly ablated wild-type hermaphrodites, germline nuclei enter meiosis by the L3–L4 molt (Kimble and White, 1981). We have also ablated the DTCs in L3 hermaphrodites in which partial gonadal arms were formed (see Figure 1). In 7 of 7 thus operated hermaphrodites, proximal germline nuclei remained in mitosis while distal germline nuclei entered meiosis and gametogenesis.

** In these animals, no gonadal arms were formed (type I ablation, Figure 6B), a single AC is sufficient to promote proximal mitosis (type IVa and IVb ablations, Figure 7B). In all cases, however, ablation of the SHISPIDUs, VUs, and AC (type Vb ablation, Figure 7B) does not result in proximal mitosis, suggesting that the AC is necessary for proximal mitosis. Conversely, ablation of all somatic gonadal cells except for 1 DTC and 1 AC (type VI ablation, Figure 7B) results in one gonadal arm with proximal mitosis.
Ectopic Germline Mitosis in C. elegans

A. Wild-type somatic gonad

B. Wild-type ablations

proximal mitosis in operated wild-type hermaphrodites since ablation of the DTCs together with ablation of other somatic cells results in an abnormal gonad with variable germline growth (data not shown). A DTC is not, however, necessary for proximal mitosis in lin-12(0) hermaphrodites (type I ablation of Figure 6B).

We propose that the AC is both necessary and sufficient to promote proximal mitosis in wild-type hermaphrodites when neighboring somatic gonadal cells are ablated. Furthermore, we conclude that the neighboring somatic gonadal cells normally act to prevent the AC from promoting proximal germline mitosis.

We are not certain which somatic gonadal cells must necessarily be ablated to allow the AC to promote proximal mitosis. Ablation of all VU cells is neither necessary (type IV ablation, Figure 7B) nor sufficient (type II ablation, Figure 7B). Although it appears that there is a cumulative effect of ablation of somatic gonadal cells (i.e., the greater the number of ablated cells, the more likely it is that proximal mitosis will occur), a qualitative role for certain cells has not been ruled out since we have not systematically ablated all combinations of Z1.p and Z4.a descendants.

After the L2–L3 Molt, the AC Is No Longer Necessary to Promote Proximal Mitosis

In lin-12(0) hermaphrodites as well as in operated wild-type hermaphrodites (type III ablation, Figure 7B), ablation of the AC(s) after the L2–L3 molt does not prevent proximal mitosis. Indeed, ablation of the AC(s) in the L3 stage had no apparent effect on the germline of lin-12(0) hermaphrodites and did not prevent proximal mitosis in operated wild-type hermaphrodites (Table 1, a and c). In contrast, ablation of the DTC at all stages in development, in wild-type as well as in lin-12(0) hermaphrodites, results in distal germline nuclei entering meiosis (Kimble and White, 1981; Figure 6B legend). One possibility is that, by the L3 stage, another cell in addition to the AC can promote mitosis proximally. We have not been able to identify such a cell, although we have ruled out the involvement of P(3–8).p, the hypodermal cells induced by the AC to form the vulva in the L3 stage (Sulston and Horvitz, 1977; Kimble, 1981). Ablation of P(3–8).p in the L1 stage followed by ablation of the AC in the L3 stage in lin-12(0) as well as in operated wild-type hermaphrodites still results in proximal mitosis (Table 1, b and d).
**gpl-l Activity Is Required for Proximal Mitosis in Operated lin-12(+) Hermaphrodites**

*gpl-l* activity is required for continued mitosis of distal germline nuclei (Austin and Kimble, 1987). To test whether *gpl-l* activity is also required for proximal mitosis in operated *lin-12(+)* hermaphrodites, we repeated our ablations in *lin-12(+) gpl-l(q237)* hermaphrodites. At 25°C, all germline nuclei in *gpl-l(q237)* hermaphrodites enter meiosis prematurely in the L2 stage, resulting in a sterile animal with only a few sperm. In contrast, at 15°C *gpl-l(q237)* hermaphrodites have a wild-type germline, and proximal germline nuclei enter meiosis in the L3 stage as they do in wild type (Austin and Kimble, 1987; G. S., unpublished data). However, the level of *gpl-l* activity in *gpl-l(q237)* hermaphrodites grown at 15°C is not fully wild type since the *gpl-l* maternal effect lethal phenotype (Austin and Kimble, 1987; Priess et al., 1987) is observed at a low penetrance (G. S., unpublished data). Ablation of the SH/SP/DUs and the VUs (as in ablation type III. Figure 7) had no apparent effect on the germline phenotype of *gpl-l(q237)* hermaphrodites grown at 15°C. The same result was obtained with another *gpl-l(tts)* allele, *gpl-l(q224)*. In contrast, ablation of the same cells caused proximal mitosis in wild-type hermaphrodites grown at 15°C (Table 2). These results suggest that the proximal mitosis observed in operated *lin-12(+)* hermaphrodites requires *gpl-l(+) activity.

We did not do a similar experiment with *lin-12(0)* hermaphrodites since the *lin-12(q269) gpl-l(q237)* double mutant has a larval lethal phenotype (J. Kimble, personal communication).

**Discussion**

**Certain Somatic Gonadal Cells Prevent a Potential Inductive Interaction between the AC and the Germline**

In intact wild-type hermaphrodites, a signal from a somatic gonadal cell, the DTC, induces mitosis (or inhibits meiosis) of the distal germline nuclei in each gonadal arm (Kimble and White, 1981). In this study, we have defined two conditions under which a different somatic gonadal cell, the AC,
can induce germline mitosis. The AC, which is located proximally, can induce the proliferation of proximal germline nuclei (prosimal mitosis). In wild-type hermaphrodites, the AC can induce proximal mitosis if certain neighboring somatic gonadal cells (SH/SP/DUs and VUs) are ablated by a laser microbeam. The AC is both necessary and sufficient to induce proximal mitosis. Thus, cell–cell interactions (see below) involving somatic cells neighboring the AC must normally prevent the AC from inducing mitosis in proximal germline nuclei.

In lin-12(0) hermaphrodites, the AC can induce mitosis of proximal germline nuclei even in the presence of all other somatic cells. As in wild type, the AC is both necessary and sufficient to induce proximal mitosis. In addition, mosaic analysis demonstrates that lin-12 activity is required in the somatic gonad but not in the germline or in the AC to prevent proximal mitosis locally.

We believe that the proximal mitosis of lin-12(0) and of operated wild-type hermaphrodites is a consequence of the same underlying mechanism. Indeed, in both cases, an AC is necessary and sufficient for proximal mitosis. Thus, we conclude that prevention of proximal mitosis by somatic gonadal cells requires lin-12 activity.

Models for the AC–Germline Interaction and Its Prevention by Somatic Gonadal Cells

The AC has the potential to induce mitosis of proximal germline nuclei. This potential AC–germline interaction requires gpl-1 activity, but it is normally prevented by the presence of somatic gonadal cells neighboring the AC and by lin-12 activity. Several models can account for these observations.

In one model, we propose that in lin-12(0) and operated wild-type hermaphrodites, the AC is partially transformed to a DTC. Such a partially transformed AC would express the DTC-to-germline signal but would retain its normal proximal position, its typical nuclear morphology, and its ability to induce the vulva (Kimble, 1981). In its simplest form, this model does not account for the observation that in mosaic hermaphrodites, in which one somatic arm of the gonad is genotypically lin-12(0) and one arm is genotypically lin-12(+), only the lin-12(0) arm displays proximal mitosis. (In these mosaics, the AC is invariably lin-12(0) [Seydoux and Greenwald, 1989].) An additional mechanism by which the DTC-to-germline signal expressed from the AC is locally suppressed by lin-12(+) somatic cells must be invoked.

An alternate model is shown in Figure 9. This model relies on the strong similarity of the lin-12 and gpl-1 products, which are putative transmembrane proteins that are 50%–60% identical throughout most of their lengths and contain several different repeated peptide motifs in corresponding positions (Yochem and Greenwald, 1989). As described in the Introduction, the lin-12 and gpl-1 products may function as receptors for intercellular signals, the AC-to-VU signal and the DTC-to-germline signal, respectively (Seydoux and Greenwald, 1989; Yochem and Greenwald, 1989). Since we have observed that the AC can promote mitosis in the germline in a gpl-1-dependent manner, we postulate that the AC-to-VU signal, which normally binds to lin-12, is also able to activate the related gpl-1-encoded receptor, and does so in lin-12(0) and operated wild-type hermaphrodites. Although other models could account for our data, we find this model appealing because it depends on the known functional and structural similarities between lin-12 and gpl-1. There are precedents for the cross-activation of related receptors by related ligands. For example, the IGF-I receptor is similar to but distinct from the insulin receptor, yet IGF-I and insulin can interact with either receptor (for review see Czech, 1982). It should be noted that the molecular nature of the AC-to-VU and the DTC-to-germline signals are not known at this time.

How do somatic cells neighboring the AC normally prevent the AC from interacting with the germline? A number of types of cell–cell interactions can be considered. One possibility is that somatic gonadal cells interact directly with the proximal germline to render it resistant to the influence of the AC. This effect might be achieved by removing or inactivating gpl-1 in the proximal germline.

Another possibility is that somatic gonadal cells neighboring the AC isolate the interacting Z1,ppp and Z4,aaa cells from the germline. There are a number of ways in which this effect may be achieved. For example, somatic gonadal cells neighboring the AC could prevent the AC-to-VU signal from reaching the proximal germline by physically blocking AC–germline contact or by degrading the AC-to-VU signal. Neither of these mechanisms requires a direct role for lin-12 in preventing the AC–germline interaction; rather, altered morphology and/or cell types may be responsible for the inappropriate AC–germline interaction seen in lin-12(0) mutants. The arrangement of somatic gonadal cells in relation to the AC and to the proximal germline is not known at the ultrastructural level for the late L2 stage, which is the time when the AC–germline interaction could occur. Anatomical studies might rule out a physical blocking mechanism.

Figure 9 illustrates another mechanism in which, in contrast to the two mechanisms presented above, lin-12 plays a direct role in preventing the AC-to-VU signal from reaching the germline. In this model, the AC-to-VU signal is proposed to have a greater affinity for lin-12 than for gpl-1. Thus, when lin-12 is present, the AC-to-VU signal preferentially binds to the lin-12 product on the surface of somatic gonadal cells. When lin 12 activity is removed genetically (in lin-12(0) mutants) or physically (by laser ablation of somatic

### Table 2. gpl-1(+)) Activity Is Required for Proximal Mitosis in Operated in-12(+)) Hermaphrodites

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Ablated Cells</th>
<th>Ablation Stage</th>
<th>Proximal Mitosis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>SH/SP/DUs, VUs</td>
<td>L1 molt</td>
<td>1/3</td>
</tr>
<tr>
<td>gpl-1(q231)</td>
<td>SH/SP/DUs, VUs</td>
<td>L1 molt</td>
<td>0/3</td>
</tr>
<tr>
<td>gpl-1(q224)</td>
<td>SH/SP/DUs, VUs</td>
<td>L1 molt</td>
<td>0/3</td>
</tr>
</tbody>
</table>

* Number of hermaphrodites with proximal mitosis of the total number of operated hermaphrodites.

b Same ablation as type III ablation in Figure 7B, except that animals were grown at 15°C. gpl-1(q231) and gpl-1(q224) hermaphrodites grown at 15°C have phenotypically wild-type germlines and are fertile.
Differences between the AC-Germline and DTC-Germline Interactions

The AC-germline interaction described in this paper is reminiscent of the DTC-germline interaction described by Kimble and White (1981), in that both interactions require glp-1 activity to induce mitosis (or inhibit meiosis) locally within the germline. However, these interactions differ in several respects. First, the DTC-germline interaction is required throughout the life of the animal for distal germline mitosis: ablation of the DTC at any time in development prevents distal mitosis. In contrast, the AC-germline interaction is apparently required only transiently for proximal mitosis: ablation of the AC after the L2 molt fails to restore proximal germline meiosis in lin-12(0) and operated wild-type hermaphrodites. One possible explanation is that in operated wild-type and lin-12(0) hermaphrodites in which the AC(s) has been ablated in the L3 stage, proximal nuclei cease mitosis in the L4 or adult stage but have also lost the ability to enter gametogenesis (no DAPI staining was performed in operated animals). Another possibility can be explained in the context of the model shown in Figure 9. For example, glp-1 and the DTC-to-germline signal could form an active complex that is rapidly degraded. Thus, a continuous source of signal would be required to maintain glp-1 activity in the germline. In contrast, if the oopec activation of glp-1 by the AC to VU signal resulted in the formation of a stable complex that is not degraded, an initial pulse of AC-to-VU signal would be sufficient to maintain glp-1 activity in the germline. Second, although both interactions require some glp-1 activity, the AC-germline interaction seems to be more sensitive to the level of glp-1 activity than the DTC-germline interaction. Indeed, the level of glp-1 activity in operated glp-1(q237) and glp-1(q224) hermaphrodites grown at 15°C is sufficient to allow distal germline mitosis but is not sufficient to allow proximal mitosis. This result might be due to different abilities of the AC-to-VU signal and of the DTC-to-germline signal to initially activate glp-1 and/or to some inherent differences between proximal and distal germline nuclei. Third, the AC-germline interaction is suppressed by lin-12 activity in the somatic gonad while the DTC-germline interaction is not. One possibility is that no somatic gonadal cells express lin-12 activity in the vicinity of the DTC. Alterna-
tively, the DTC-to-germline signal may preferentially bind to glp-1 even in the presence of lin-12.

Our data do not address the possibility that the AC-germline interaction has a wild-type function at some point in development. It remains possible, for example, that the AC (or its ancestors) is allowed to interact with the germline early in development (L1-L2 stages) to maintain germline nuclei in mitosis. We note that while in glp-1 mutants germline nuclei enter meiosis precociously in the L2 stage (Austin and Kimble, 1987), ablation of the DTC results does not advance the time of entry in meiosis. In such operated animals, meiosis starts in the L3 stage as in wild type (Kimble and White, 1981). Although this may reflect perdurance of the DTC-to-germline signal in cellular debris, it may also suggest that other somatic cells besides the DTCs promote germline mitosis in early development.

Prevention of Inappropriate Cell–Cell Interactions Appears to Be a Normal Process in Development

Previous studies have suggested that prevention of inappropriate interactions may be important in development. For example, studies in amphibians have suggested that lens formation may require the prevention of a potential interaction between neural crest cells and the presumptive lens ectoderm. In transplantation experiments, neural crest cells have been shown to inhibit lens formation (von Woelkenthal, 1961). In intact embryos, however, the optic vesicle directly contacts the presumptive lens ectoderm, and neural crest cells do not migrate in this region until after lens formation. Henry and Grainger (1987) have proposed that the optic vesicle defines the site of lens formation within a larger potential lens-forming field by preventing a potential interaction between neural crest cells and presumptive lens ectoderm (for review see Jacobson and Sater, 1988). Waring and Kenyon (1990) have proposed that in C. elegans prevention of cell–cell interactions may be required for the specification of the fate of the lateral hypodermal cell V6. In pal-1 loss-of-function mutant males, V6 adopts an inappropriate fate. Ablation of T, a neighbor of V6, in pal-1 mutants restores the wild-type fate of V6, whereas ablation of T in pal-1(+)* males does not affect the fate of V6 (Sulston and White, 1980). Based on these observations, Waring and Kenyon (1990) proposed a model whereby an undesirable signal from T to V6 is prevented by pal-1(+) activity.

Our results extend the proposals of Henry and Grainger (1987) and Waring and Kenyon (1990) by demonstrating directly that a potential interaction must normally be prevented in wild-type development. This potential interaction, between the AC and the germline, is understood at the cellular level and can be modeled at the molecular level. The cellular bases for both signaling and prevention of signaling have been defined: the AC is the source of the signal that induces proximal germline mitosis, and somatic gonadal cells neighboring the AC prevent that inappropriate signaling. Moreover, two genes have been directly implicated in these interactions: lin-12 and glp-1. Genetic studies of these two genes have established their roles in gonadal development (Greenwald et al., 1983; Austin and Kimble, 1987; Priess et al., 1987; Seydoux and Greenwald, 1989), and their predicted protein products have suggested plausible molecular roles as receptors for intercellular signals (Yochem et al., 1988; Seydoux and Groome, 1989; Yochem and Groome, 1989). In the case of the potential AC-germline interaction described in this paper, we propose that a cell signal destined for one receptor (lin-12) can be inappropriately received by another (glp-1). Structurally similar receptors and/or signals may be used to mediate different interactions to increase the developmental potential of an organism. These mediators may be derived by gene duplication/divergence, as is apparently the case for lin-12 and glp-1 (Yochem and Greenwald, 1989). However, the increase in diversity would be offset if the specificity of each interaction were lost. Thus, it becomes crucial to isolate different sets of interacting cells from each other, by temporal, spatial, or other mechanisms. Indeed, prevention of inappropriate cell–cell interactions may be as important to a developing organism as the occurrence of desired intercellular communication.

Experimental Procedures

General Methods and Strains

Methods for handling and culturing C. elegans have been described by Brenner (1974). All experiments were performed at 20°C unless otherwise noted. The wild-type parent of all strains used was C. elegans strain Bristol N2 (Brenner, 1974). The following LG III duplication and mutations were used: gfp-3D (n175+), unc-36(e17), lin-12(+) (Austin and Kimble, 1987); glp-1(ex54), unc-32(e109), unc-36(e251) (Brenner, 1974); n175+ (E. Hedgpeth, personal communication). In addition, we used the glp-1 and lin-12 alleles described below. glp-1(t252) and glp-1(224) are two heat-sensitive alleles of glp-1 that cause all germline nuclei to enter meiosis prematurely at 25°C (Austin and Kimble, 1987). At 15°C glp-1(t252) and glp-1(224) hermaphrodites have a wild-type germline phenotype, but the glp-1 embryonic phenotype can be observed at a low frequency (G. S., unpublished data; J. Austin, personal communication).

lin-12(n41), lin-12(n38e1232::Tc1), lin-12(n70b920::Tc1), and lin-12(n137n20) are null alleles of lin-12 (Greenwald et al., 1983; Greenwald, 1985) that exhibit proximal mitosis and sterility. In lin-12(n137::Tc1-1::Tc1), the Tc1 is inserted into an exon encoding EGF-like repeats of the putative extracellular domain and hence would be expected to lead to premature chain termination (Greenwald, 1985 and unpublished data). At 20°C, unc-36(e251), lin-12(n176e202::Tc1) hermaphrodites have 2.10 ACs (n = 30); unc-36(e251) lin-12(n41) hermaphrodites have 2.12 ACs (n = 59); unc-32(e189) lin-12(n70b920::Tc1) hermaphrodites have 2.44 ACs (n = 9); and unc-32(e189) lin-12(n137n20) hermaphrodites have 2.55 ACs (n = 20). (In most cases where more than two ACs are present, the third and the fourth ACs have atypically enlarged nuclei and hence may not be truly ACs.) This statistically significant difference in average number of ACs may be due to the linked markers rather than to the specific lin-12 alleles. Other loss-of-function lin-12 alleles (lin-12(q269), lin-12(q341), and lin-12(q24)) also exhibit proximal mitosis (T. S., unpublished data). Unlike lin-12(q269) hermaphrodites, lin-12(q269) hermaphrodites are somewhat fertile and can be propagated as homozygotes, suggesting that they may retain some lin-12 activity (T. S., unpublished data).

Analysis of Germline Phenotype

The morphology of the germline of unc-36(e251) lin-12(n41) and lin-12(q269) hermaphrodites was examined in detail by Nomarski differential interference contrast microscopy throughout postembryonic development and compared with that of wild type (Hirsh et al., 1976; Kimble and Hirsh, 1979; Kimble and White, 1981). The germline of these hermaphrodites differs most from wild type in the adult stage. Young adult animals display proximal nuclei that have not begun gametogenesis and are morphologically similar to the mitotic nuclei observed in the distal region (Figure 2). In older unc-36(e251) lin-
Cells 950

12(n947) hermaphrodites, the proximal mitotic region has often expanded dorsally so that the entire proximal region of the hermaphrodite is filled with germcell nuclei (data not shown). Older adults often have disorganized germcell and burst at the ventral vulval protrusion. For this reason, we were not able to determine whether proximal mitosis continues in late adults. The disorganized germcell and bursting may be a result of proximal germcell nuclei and cytoplasm being forced into the spermathecae and uterus by contractions of the epithelial sheet cells, which usually push oocytes into the spermatheca for fertilization [Hirsh et al., 1976]. All other lin-12 mutants listed above were examined as young adults by Nomarski microscopy and found to exhibit proximal mitosis.

Germcell nuclear morphology in L4 and young adults unc-36(e257) lin-12(n947) and lin-12(q265) was examined by staining whole worms with DAPI (Elliis and Horvitz, 1986, as modified by E. Lambie, personal communication) and visualizing them by fluorescence microscopy. Proliferation was defined as the presence of mitotic (metaphase, anaphase, or telophase) nuclei and the absence of a mitotic prophase nucleus in the same region of the germcell. The nuclear morphologies observed in lin-12(e6) hermaphrodites were similar to those described in wild type (Hirsh et al., 1976; Klass et al., 1976; Herman et al., 1976; Austin and Kimble, 1986). We have not determined whether entry of germcell nuclei into meiosis is delayed in lin-12 hermaphrodites, as their growth is slow and variable.

The proximal mitotic region is less efficient at contributing nuclei to meiosis and gametogenesis than the distal mitotic region in unc-36(e257) lin-12(n947) and lin-12(q265) hermaphrodites. There are fewer nuclei in meiotic prophase in the proximal arm than in the distal arm (Figure 3). Although spermatogenesis occurs in both the proximal and distal regions, only the distal arm produces oocytes (Figure 2; unpublished data). Otherwise, germcell sex determination (specification of sperm then oocytes) and gametogenesis are apparently normal.

Proximal mitosis is more extensive in unc-36(e257) lin-12(n947) than in lin-12(q265) hermaphrodites. Both the size of the proximal mitotic region observed by Nomarski microscopy and the frequency of mitosis observed by DAPI staining is greater in lin-12(n947) than in lin-12(q265) as described above, lin-12(q265) is a null allele, while lin-12(q265) may retain some residual lin-12 activity. The proximal mitosis phenotype observed in wild-type ablated animals (see below) is similar to the phenotype of lin-12(q265) hermaphrodites (Figures 2 and 8).

Mosaic Analysis

The isolation of the mosaic animals presented in this paper has been described in detail by Seydoux and Greenwald (1989). In brief, segregants from the strain ncl-1 unc-36 lin-12; q0p3 were examined by Nomarski microscopy for NCIs cells derived from the C and D lineage (germcell mosaics) or for NC1 DTCs (Z4/aap mosaics) (Ncl- cells have enlarged nucleoli [Ed Hedgecock, personal communication]) and visualizing them by fluorescence microscopy. Proliferation was defined as the presence of mitotic (metaphase, anaphase, or telophase) nuclei and the absence of a mitotic prophase nucleus in the same region of the germcell. The nuclear morphologies observed in lin-12(e6) hermaphrodites were similar to those described in wild type (Hirsh et al., 1976; Klass et al., 1976; Herman et al., 1976; Austin and Kimble, 1986). We have not determined whether entry of germcell nuclei into meiosis is delayed in lin-12 hermaphrodites, as their growth is slow and variable.

The proximal mitotic region is less efficient at contributing nuclei to meiosis and gametogenesis than the distal mitotic region in unc-36(e257) lin-12(n947) and lin-12(q265) hermaphrodites. There are fewer nuclei in meiotic prophase in the proximal arm than in the distal arm (Figure 3). Although spermatogenesis occurs in both the proximal and distal regions, only the distal arm produces oocytes (Figure 2; unpublished data). Otherwise, germcell sex determination (specification of sperm then oocytes) and gametogenesis are apparently normal.

Proximal mitosis is more extensive in unc-36(e257) lin-12(n947) than in lin-12(q265) hermaphrodites. Both the size of the proximal mitotic region observed by Nomarski microscopy and the frequency of mitosis observed by DAPI staining is greater in lin-12(n947) than in lin-12(q265). As described above, lin-12(q265) is a null allele, while lin-12(q265) may retain some residual lin-12 activity. The proximal mitosis phenotype observed in wild-type ablated animals (see below) is similar to the phenotype of lin-12(q265) hermaphrodites (Figures 2 and 8).

Ablation Experiments

Ablations were performed by the method of Sulston and White (1980) using a laser microscope system essentially identical to that described by Sulston and Horvitz (1977) and Kimble and Hirsh (1979). Animals were scored for proximal mitosis as young adults using Nomarski microscopy and found to exhibit proximal mitosis.

AC Replacement Following Ablation of 24.aaa and Z4.aa

lin-12(0) hermaphrodites have at least two ACs. In 8 of 12 unc-36(e257) lin-12(n947) hermaphrodites in which two ACs were ablated early in the L2-L3 molt or the first half of the L2 stage, one AC was made by the L3 stage. However, in 0 of 9 unc-36(e257) lin-12(0) hermaphrodites, in which two ACs and Z4.aa were ablated in the L1-L2 molt or the first half of the L2 stage, one AC was made by the L3 stage. However, in 0 of 9 unc-36(e257) lin-12(0) hermaphrodites, in which two ACs and Z4.aa were ablated in the L1-L2 molt or the first half of the L2 stage just prior to primordium formation, no AC was made. These results indicate that Z1.ppa and Z4.aap have the potential to become ACs in the absence of Z1.ppp and Z4.aa, although this potential is lost earlier in wild-type than in lin-12(e6) hermaphrodites. Z1.ppp and Z4.aap normally become VUs that express a somewhat different lineage than the lineage expressed from the VU derived from Z1.ppp or Z4.aa. (In lin-12(e6) hermaphrodites Z1.ppp and Z4.aap can become ACs in the presence of Z1.ppp and Z4.aa, although this transformation is rarely observed [see above].)

Sterility Defect of lin-12(0) Hermaphrodites

lin-12(0) hermaphrodites are generally sterile. We wondered if this sterility was due to the low number and/or mispositioning of gametes caused by the proximal mitosis defect. If this were the case, ablation of Z1.ppp and Z4.aap, which prevents proximal mitosis, should restore wild-type fertility levels in lin-12(e6) hermaphrodites. Instead, we found that unc-36(e251) lin-12(n947) hermaphrodites in which Z1.pp and Z4.aa have been ablated gave an average of 0.6 progeny (n = 10) compared with an average of 29.6 progeny (n = 8) for similarly ablated unc-36(e251)lin-12(+) hermaphrodites. We conclude that the sterility defect observed in lin-12(e6) hermaphrodites is not simply due to proximal mitosis. In addition, we note that the two mosaic animals in which lin-12 activity was absent from the proximal germcell but present in the somatic gonad (type I mosaic, Figure 3) had an unusually low broad size (one mosaic had one progeny and the other had six progeny; only adult progeny were counted). This result may reflect a partial requirement for lin-12 activity in the germline necessary for wild-type fertility or may be due to the generally reduced vigor of lin-12(0) hermaphrodites.

Acknowledgments

We are indebted to Austin Kimble in whose laboratory T. S. first recognized the lin-12(0) proximal mitosis phenotype. We thank John Yochem for suggesting the model presented in Figure 9, Mal Steinberg and Eric Wiechauf for helpful discussions, and Judith Austin for sharing unpublished data on certain g/p-7 alleles. Finally, we thank Beth Bucher, Bob Horvitz, Simon Tuck, Hilary Wilkinson, and John Yochem for critical reading of the manuscript.

Research at Princeton University was supported by United States Public Health Service Grant GM37602, by the Searle Scholars Program/The Chicago Community Trust, and by a Dupont Young Faculty Grant to I. G., G. S. is a Howard Hughes Medical Institute Doctoral Fellow. Research at Washington University was supported by United States Public Health Service Grant HD25614 to T. S. Some nematode strains used in this study were provided by the Caenorhabditis genetics Center, which is supported by contract number no. 4-g-4-2113 from the National Institutes of Health and the Curator of the University of Missouri.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 8, 1990; revised April 4, 1990

References


Ectopic Germline Mitosis in *C. elegans*


