**glp-1** Is Required in the Germ Line for Regulation of the Decision between Mitosis and Meiosis in C. elegans

Judith Austin* and Judith Kimble**

* Laboratory of Cell and Molecular Biology
The Graduate School
University of Wisconsin
Madison, Wisconsin 53706

** Department of Genetics
College of Agriculture and Life Sciences
University of Wisconsin
Madison, Wisconsin 53706

Summary

In the wild-type C. elegans germ line there are both mitotic and meiotic germ cells. Mutations in **glp-1** cause germ cells that would normally divide mitotically to enter meiosis. This mutant phenotype mimics that for distal tip cell regulation. Mosaic analyses suggest that **glp-1** is produced in the germ line. We propose that **glp-1** acts as part of the receiving mechanism in the interaction between the distal tip cell and germ line.

Introduction

Interactions between cells are critical to the control of growth and differentiation of multicellular organisms. Such interactions were first observed in classic experiments with sea urchin and frog embryos (e.g., Driesch, 1891; Spemann and Mangold, 1924). More recently, cell interactions that regulate development have been described in the nematode Caenorhabditis elegans. In particular, signals from one cell to another can influence cell lineage (Sulston and White, 1980; Kimble, 1981; Sulston et al., 1983), proliferation of the germ line (Kimble and White, 1981), formation of the vulva (Kimble, 1981; Sternberg and Horvitz, 1986), and development of the pharynx (Priess and Thomson, 1987).

As a model for analysis of regulatory cell interactions during development, we have chosen to focus on the interaction in C. elegans that controls germ-line proliferation. A somatic cell, the distal tip cell, controls the decision between mitosis and meiosis in the germ line (Kimble and White, 1981). The distal tip cell influences germ cells that are not in direct cellular contact with it and therefore acts over a distance (Kimble and White, 1981). In both hermaphrodites and males, only the germ cells closest to the distal tip cells continue to divide mitotically throughout development. If the distal tip cell is removed by laser ablation at any time during development, all germ cells in the mitotic cell cycle enter meiosis and form gametes. Thus, the interaction between the distal tip cell and the germ line is necessary for proliferation of the germ line during larval development and maintenance of a germ-line stem cell population during adulthood.

Molecular models of signal transmission have emerged from biochemical studies of neurotransmitters, hormones, and growth factors in higher animals. Essentially, one cell produces the signal; another cell produces the receptor and transduction machinery (e.g., G proteins and effector proteins such as adenylate cyclase) necessary to respond to that signal. However, the identification and in vivo manipulation of molecules that mediate specific regulatory interactions between cells during development are still technically difficult in higher animals. To identify the signaling and receiving components of a cell interaction that controls development, we have sought mutants defective in the distal tip cell-germ line interaction.

In this paper, we describe a genetic and developmental characterization of one gene, **glp-1** (germ-line proliferation defective). We suggest that **glp-1** functions as part of the receiving mechanism in the interaction between the distal tip cell and germ line. Although mutations of **glp-1** were first detected by their effect on germ-line growth, further analysis revealed a requirement for maternally produced **glp-1** during early embryogenesis. Our observations, together with those reported in the accompanying paper, Priess et al. (1987), indicate that **glp-1** is also involved in induction of the anterior pharynx. Thus, **glp-1** is essential to at least two cell interactions that are distinct both in time and in the cells affected.

Results

Development of the C. elegans Germ Line

Development of the wild-type C. elegans gonad is diagrammed in Figure 1. In both hermaphrodites and males, a gonadal primordium is present at hatching. Two cells in this primordium, Z1 and Z4, give rise to the somatic structures of the gonad (e.g., uterus and vas deferens); the two other cells, Z2 and Z3, generate the germ line. As the gonad develops, it forms a double reflexed tube in hermaphrodites and a single reflexed tube in males. In L3, some germ cells enter meiosis, while others continue to divide mitotically. Germ cells farthest from the distal tip cells enter meiosis first and become gametes, while germ cells near the distal tip cells remain mitotically active even in the adult. The term germ cells is used to refer to descendants of Z2 and Z3. Although the germ-line tissue is syncytial, each germ-line nucleus occupies its own membrane-bound alcove of cytoplasm located at the edge of a common anuclear cytoplasm (Hirsh et al., 1976). Each germ-line nucleus and its cytoplasm is called a germ cell in this paper.
glp-1 Is Required for Germ-Line Proliferation

We have identified eight recessive mutations that define the gene glp-1. These mutations fail to complement each other, and they map to the same location on LGIII. None is suppressible by the amber suppressor sup-7 (Waterston, 1981; see Experimental Procedures).

The glp-1 mutations each affect proliferation of the germ line in essentially the same way: germ cells that would normally continue to divide mitotically instead enter meiosis and differentiate into gametes (Table 1; Figure 2). Animals homozygous for any of four nonconditional glp-1 alleles (q46, q158, q172, or q175) or a temperature sensitive (ts) glp-1 allele (q275) at the restrictive temperature have the same, fully penetrant germ-line phenotype. In both hermaphrodites and males, only 4-8 descendants of Z2 and Z3 are produced (Table 1). These germ cells all enter meiosis and differentiate into sperm (Figure 2). Another glp-l(ts) allele, q31, has a nearly identical phenotype but produces a few more germ cells (Table 1). In animals homozygous for any of these six alleles, the somatic gonad is morphologically normal. In particular, the distal tip cells are present and appear normal. The glp-1 hermaphrodite ovoids is U-shaped as normal; in hermaphrodites, this shape is regulated by the distal tip cell (Kimble and White, 1981), indicating that at least one function of the distal tip cell is unaffected in glp-1 hermaphrodites.

Two other glp-1 alleles, q50 and q35, have phenotypes with distinctive features. Most animals homozygous for glp-1(q50) make only a small number of germ cells that differentiate into sperm, but 16% of glp-1(q50) hermaphrodites and males produce a normal germ line (Table 1). In glp-1(q50) hermaphrodites, the germ-line phenotype is determined separately for each ovotestis: in some animals one ovotestis has only a few germ cells, while the other has a normal germ line. glp-1(q35), in addition to its recessive effect on germ-line proliferation, has a semidominant Muv (multivulva, Horvitz and Sulston, 1980) phenotype: in both hermaphrodites and males, small protrusions of the ventral hypodermis are observed. This somatic phenotype, which is not seen in any of the other glp-1 alleles, is typical of dominant mutations in lin-12 (Greenwald et al., 1983), a gene that maps 0.02% from glp-1 (see Figure 7). However, the glp-1(q35) and lin-12(d) Muv phenotypes are distinct, and glp-1/lin-12(d) animals have a normal germ line and are self-fertile (see Experimental Procedures).

The eight glp-1 mutations described here are all likely to be the result of a lowered amount or activity of the glp-1 gene. The effect of glp-1 mutations on the germ line is recessive in all cases, and glp-1 mutants arise at a frequency of $3 \times 10^{-4}$ after ethyl methane sulfonate mutagenesis. This frequency is similar to that of loss-of-function mutations in other C. elegans genes (Brenner, 1974). The null, or complete loss-of-function, phenotype of glp-1 is probably that of the most severe glp-1 alleles (q46, q158, q172, q175, q224ts25).

### Entry into Meiosis Occurs Prematurely in glp-1 Homozygotes

Direct observation of gonadogenesis in hermaphrodites homozygous for a strong allele of glp-1 shows that the only detectable defect is early entry of all germ cells into meiosis. In glp-1(q46) hermaphrodites, the gonadal primordium and cell lineage of Z1 and Z4 are normal during L1 and L2, but Z2 and Z3 divide only once during L1. By mid-L2, germ-line mitoses have ceased; by mid-L3, all germ cells have become primary spermatocytes. No cell death has been observed.

The unusually early appearance of primary spermatocytes in glp-1(q46) hermaphrodites indicates that germ

---

**Table 1. Total Number of Germ Cells Produced by glp-1 Homozygotes**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of Germ Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>q46&lt;sup&gt;+&lt;/sup&gt;</td>
<td>~1500 (~600)</td>
</tr>
<tr>
<td>q46&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5-7 (4-8)</td>
</tr>
<tr>
<td>q158&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5-7 (4-8)</td>
</tr>
<tr>
<td>q172&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5-8 (5-8)</td>
</tr>
<tr>
<td>q175&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4-8 (4-7)</td>
</tr>
<tr>
<td>224F(25°C)</td>
<td>6-8 (6-8)</td>
</tr>
<tr>
<td>233F(25°C)</td>
<td>7-25 (7-16)</td>
</tr>
<tr>
<td>n&lt;sup&gt;50&lt;/sup&gt;c</td>
<td>47-198 (15-22)</td>
</tr>
<tr>
<td>n&lt;sup&gt;50&lt;/sup&gt;c</td>
<td>6-21 (6-17)</td>
</tr>
<tr>
<td>n&lt;sup&gt;50&lt;/sup&gt;c</td>
<td>~800 (~600)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Both control and glp-1 animals (except for q50) are homozygous for unc-22(e189). glp-1(q46) is linked to the translocation chromosome eT1 (see Experimental Procedures).

<sup>b</sup> Number of germ cells is total number of 22 and 23 descendants prior to meiotic divisions at a time that is 24 hr after L4. Since mitotic cells are still present, this number will increase as the animal ages. (n = 2)

<sup>c</sup> Number of germ cells is total number of Z2 and Z3 descendants prior to meiotic divisions. No more germ cells are ever made. (n = 10)

<sup>d</sup> Two alternative phenotypes are seen in glp-1(q50) homozygotes (see text). For this table, only glp-1(q50) hermaphrodites in which both ovotestes had the same phenotype were scored.

---

Figure 1. Gonadogenesis in Wild-Type Hermaphrodites and Males

Each diagram depicts a developing gonad during consecutive stages of postembryonic development. Anterior is to the left, dorsal is up. L1, L2, L3, and L4 are first, second, third, and fourth larval stages, respectively; A indicates adult. Lethargus (left) is the period just prior to molting at the end of each larval stage. Somatic gonadal tissue is striped; distal tip cells are marked by an asterisk; germ nuclei in the mitotic cell cycle, by open circles; germ nuclei in meiotic prophase, by filled circles; sperm, by stippling; oocyte nuclei, by squares. For more detail see Hirsch et al. (1976), Kliss et al. (1976), Kimble and Hirsh (1979), and Kimble and White (1981).
Cells enter meiosis earlier in glp-1 mutants than in normal wild-type animals. Therefore, we compared the first sign of entry into meiosis in glp-1(+) and glp-1(q46) animals. Larvae at different stages of development were stained with diamidino-phenylindole (DAPI), and the first appearance of chromosomes typical of the pachytene stage of meiotic prophase was used to detect entry into meiosis (Figure 3). As had been previously observed (Kimble and White, 1981), we found that in glp-1(+) hermaphrodites and males, pachytene nuclei are not seen until late L3 lethargus and mid-L3, respectively (Table 2; Figure 3). However, in glp-1(q46) hermaphrodites and males, pachytene nuclei were seen in late L2 (Table 2; Figure 3). The first mature sperm were observed about one larval stage after the first appearance of pachytene nuclei in both glp-1(+) and glp-1(q46) animals (Table 2). Thus, both entry into meiosis and spermatogenesis occur earlier in glp-1 than in wild-type animals.

**After the L1 Stage, glp-1 Function Is Required Continuously during Germ-Line Development**

Temperature-shift experiments were done with glp-1(ts) mutants to determine when glp-1 is required for normal germ-line development. When raised continuously at the permissive temperature, these mutants have a wild-type germ line; when grown at the restrictive temperature, they are Glp. For the purposes of this paper, Glp denotes the mutant phenotype of glp-1. In these experiments, staged animals were shifted from permissive to restrictive temperature, or vice versa, at different times of development...
Figure 3. Premature Entry into Meiosis of Germ Cells in glp-1 Homozygotes

Each picture includes a single ovotestis in which the distal end is to the left. A small arrow indicates the distal tip cell. Animals in (C) and (D) are stained with DAPI. (A and C) unc-32 glp-1(e177) L3 hermaphrodites; (B and D) unc-32 glp-1(q46) L3 hermaphrodites. In the glp-1(e177) ovotestes, there are numerous germ cells and mitotically dividing nuclei can be seen (metaphase plates are indicated by large arrows in (C). In each glp-1(q46) ovotestis, only three germ cells are present (arrowheads in [B] and [D]). All three germ cell nuclei in (D) are in the pachytene stage of meiotic prophase. Bar, 10 µm.

Table 2. Developmental Timing of Germ-Line Maturation

<table>
<thead>
<tr>
<th>Genotype&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Sex</th>
<th>Animals with Pachytene Nuclei/Total Animals&lt;sup&gt;c&lt;/sup&gt;</th>
<th>First Appearance of Pachytene Nuclei&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>eL2</td>
<td>mL2</td>
</tr>
<tr>
<td>+</td>
<td>♂</td>
<td>0/4</td>
<td>2/2</td>
</tr>
<tr>
<td>q46</td>
<td>♂</td>
<td>0/5</td>
<td>2/3</td>
</tr>
<tr>
<td>+</td>
<td>♀</td>
<td>0/5</td>
<td>2/3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Sex</th>
<th>Animals with Sperm/Total Animals&lt;sup&gt;c&lt;/sup&gt;</th>
<th>First Appearance of Sperm&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>eL3</td>
<td>mL3</td>
</tr>
<tr>
<td>+</td>
<td>♂</td>
<td>0/4</td>
<td>7/7</td>
</tr>
<tr>
<td>q46</td>
<td>♂</td>
<td>0/5</td>
<td>2/5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Presence of germ cells in the pachytene stage of meiotic prophase was scored in animals stained with DAPI.

<sup>b</sup> Both control and glp-1 animals are homozygous for unc-32(e1100).

<sup>c</sup> Each larval stage was divided into early (e), middle (m), late (l), and lethargus (leth).

<sup>d</sup> Presence of sperm was scored using Nomarski optics.

and later scored for the presence of continued germ-line proliferation. Data are shown for glp-1(q231); similar results were obtained with glp-1(q224).

Experiments in which animals were shifted up from permissive to restrictive temperature indicate that glp-1 function is required continuously for germ-line mitoses. When glp-1(q231) hermaphrodites were shifted from 15°C to 25°C at any time after hatching, adults possessed no germ cells in mitosis (Figure 4A). (L4s and adults shifted were scored 24 hr later.) All germ cells had entered meiosis and differentiated as sperm or oocytes, depending on when the temperature shift occurred (Figure 4B). The number of germ cells produced also depended on the stage of development at which the animals were shifted (Figure 4B). Hermaphrodites shifted up during L1 made the same number of germ cells as animals raised continu-
g/p-l Regulates the Mitotic/Meiotic Decision

593

![Figure 4](image1.png)

Figure 4. Effect of Temperature Shifts on Germ-Line Proliferation in g/p-l (ts) Hermaphrodites

The abscissa represents developmental time in hours at 25°C. The rate of development at 25°C is twice as fast as that at 15°C (data not shown; Hirsh and Vanderstichele, 1976). Incubation times at 15°C have been normalized to 25°C hours by halving the actual time. (A) Effect of temperature shifts on germ-line mitoses in g/p-l(q231) hermaphrodites. Staged animals were shifted from 15°C (permissive temperature) to 25°C (restrictive temperature), or from 25°C to 15°C. Shifts up, open circles, n = 10 for each time point; shifts down, filled circles, n > 20 for each time point. (B) Total number of germ cells produced by g/p-l(q231) hermaphrodites shifted from 15°C to 25°C at consecutively later times during development, n = 5 for all time points. Shifts in which germ cells differentiate as sperm, open squares; shifts in which germ cells produce both sperm and oocytes, open triangles.

592

Experiments in which animals were shifted down from restrictive to permissive temperature indicate that the g/p-l function is not required during the first mitotic divisions of the hermaphrodite germ line. When g/p-l(q231) hermaphrodites were shifted from 25°C to 15°C during L1 and examined as adults, their germ lines were phenotypically normal: some germ cells had remained in mitosis, and proliferation of the germ line was normal (Figure 4A). However, animals shifted down at mid-L2 or later had no mitotic germ cells as adults: all germ cells had entered meiosis and formed gametes.

Maternal Wild-Type g/p-l Gene Product Is Required for Embryogenesis

Embryos produced by g/p-l hermaphrodites arrest during embryogenesis (Figure 5). The mutant embryos contain several hundred cells and show signs of differentiation: some cells have gut granules, other cells resemble neurons, and the presence of muscle cells is indicated by contractions. A compact group of cells with morphological signs of pharyngeal differentiation is seen within the embryo. In addition, morphogenesis is defective, and a recognizable worm is not made. This phenotype is seen with little variation in g/p-l(q35), g/p-l(q50), g/p-l(q224ts), and g/p-l(q237ts) embryos. In the case of g/p-l(q35ts) mutants, embryos develop normally at permissive temperature, but when hermaphrodites are shifted up to restrictive temperature late in larval development or as adults, embryos arrest as described. Since g/p-l(q224ts) at restrictive temperature appears to have a null phenotype for germ-line proliferation, it is likely that the embryonic phenotype we describe here is the null phenotype.

Maternal effect tests indicate that the embryonic lethality associated with mutations in g/p-l is due to a lack of maternally contributed g/p-l(+) gene product. When g/p-l(q237) hermaphrodites, shifted from 15°C to 25°C as L4 larvae, laid eggs, 0/1072 hatched. Similarly, when g/p-l(q231) hermaphrodites were shifted from 15°C to 25°C as adults and crossed to wild type males, 0/113 g/p-l(q237)/+...
eggs produced by the mating hatched. For this cross, the hermaphrodite's self-sperm had been previously exhausted so that all eggs produced had to be glp-1(+), from the mating (see Experimental Procedures). Therefore, glp-1(ts) contributed by the father is not sufficient for normal embryogenesis. In contrast, 404/414 eggs laid by self-fertilized glp-1(q231)/+ hermaphrodites hatched; 21% of the live progeny were glp-1(q231)/* glp-1(q231). Similar results have been seen for glp-1(q35), glp-1(q50), and glp-1(q224).

Temperature-shift experiments indicate that glp-1(+) activity is required early in embryogenesis (Figure 6). When embryos from self-fertilized glp-1(q231) hermaphrodites were shifted from restrictive (25°C) to permissive (15°C) temperature at the 2 cell or 4 cell stage, they hatched and developed to adulthood. However, embryos shifted down later did not hatch. When glp-1(q231) embryos were shifted from 15°C to 25°C at the 2 cell stage, they did not hatch, whereas most embryos shifted up at about the 28 cell stage developed normally. These experiments suggest that glp-1 has an early function, between the 4 cell and 28 cell stages of embryogenesis, that is required for normal embryogenesis.

An intriguing result was obtained when glp-1(q231) embryos were shifted from 15°C to 25°C at the 8 cell stage. Most of these embryos hatched, but they were deformed and arrested as L1 larvae. In 12/12 of these larvae, the anterior bulb of the pharynx was missing. An analogous effect (i.e., absence of cells that normally make up the anterior pharynx) has been seen in blastomere-removal experiments that suggest cell interactions during early embryogenesis (Priess and Thomson, 1987; see accompanying paper, Priess et al., 1987, for a more detailed discussion of this phenotype).

Meiosis and Gametogenesis Do Not Require glp-1

Although glp-1 is required for mitotic proliferation of the germ line, it is not required for either meiosis or gametogenesis. By morphological criteria (Nomarski image and DAPI-stained chromosomes), both meiosis and gametogenesis are essentially normal in glp-1 mutants. (The only abnormality observed is that, in animals homozygous for certain alleles of glp-1, some sperm have a vacuolated appearance.) Furthermore, glp-1 sperm are functional, as demonstrated in the following test. Individual glp-1(q235) males were mated to unc-32; fem-1 females, which contain only oocytes and cannot self-fertilize. Of 19 such matings, 4 resulted in some offspring (1–5 progeny per male). All eggs hatched and developed normally. glp-1(q231) males raised at 25°C can also mate and give rise to cross-progeny (data not shown).

The embryonic lethality associated with mutations in glp-1 is not due to a defect in oogenesis. As described above, the embryonic period of temperature sensitivity for glp-1 begins after fertilization. In glp-1(q231) L4 hermaphrodites shifted from 15°C to 25°C, all oogenesis occurs at the restrictive temperature. Yet, when embryos produced by such animals are shifted back to 15°C at the 2 cell stage, the embryos develop normally. Thus, glp-1(ts) hermaphrodites can produce functional oocytes at the restrictive temperature.

glp-1 Acts in the Germ Line for Continued Germ-Line Mitoses

The glp-1 germ-line phenotype, arrest in germ-line mitoses followed by entry into meiosis, is strikingly similar to the effect of killing the somatic distal tip cell. This suggests that glp-1 may function in the interaction between distal tip cell and germ line. If so, then glp-1 might encode part of the signaling mechanism that emanates from the distal tip cell or part of the receiving mechanism that transduces the signal to effect germ-line growth. To distinguish between these possibilities, we examined genetic mosaic animals. In C. elegans, genetic mosaic animals can be generated by use of a mitotically unstable free duplication (Herman, 1984). Briefly, loss of a free duplication from a precursor cell during development will uncover a homozygous mutation under that duplication. If wild-type gene product must be produced in the descendant cells of the precursor for normal development, then loss of the duplication will result in a mutant phenotype.

The free duplication, qDp3, carries wild-type alleles of glp-1, unc-36 and ncl-1 (Figure 7). The strain we used for analysis of glp-1 mosaic was ncl-1 unc-36 glp-1(q40); qDp3. With this strain, absence of qDp3 from a given cell could be detected by scoring for the unc-36 and/or ncl-1 mutant phenotypes. The ancestors of embryonic precursor cells, somatic gonad, and germ line are shown in Figure 9A. The unc-36(+) product is required for normal movement in cells descended from AB (Kenyon, 1986). The ncl-1(+) product is required for maintenance of a normal-sized nucleolus in most somatic cells. If a cell...
g/p-l Regulates the Mitotic/Meiotic Decision

Figure 7. Map Position of g/p-l

Figure 8. Nuclei Scored for the Ncl Phenotype in Genetic Mosaic Animals

Table 3. Genetic Mosaics with Loss of qDp3 in the Somatic Gonad

<table>
<thead>
<tr>
<th>Presence (+) or Absence (−) of qDp3</th>
<th>Germ-Line Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal</td>
<td>Z1</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>−</td>
</tr>
</tbody>
</table>

of qDp3 in the germ line can be assessed by progeny testing. All 3 animals produced progeny that carried qDp3, confirming the presence of qDp3 in the germ line of these mosaics. These results show that when gip-1f (+) is absent from the distal tip cell, but present in the germ line, germ-line development is normal. We conclude that gip-1 is not required in the distal tip cells for normal germ-line development.

The second type of genetic mosaic had a duplication loss that resulted in a Glp germ-line phenotype. We screened for non-Unc Glp animals. Such animals must have qDp3 in AB, because they move normally, but they must have lost qDp3 elsewhere to cause the Glp phenotype. Once such mosaics were identified, individual cells (Figure 8B) were scored for the Ncl phenotype to learn where qDp3 had been lost. The 20 non-Unc Glp mosaics identified fall into six classes (Figure 9B). In class I (3 animals), all descendants of MS, C, and D were Ncl, suggesting that qDp3 had been lost from Pn. In class II (7 animals), only descendants of D were Ncl, indicating a loss of qDp3 from either D or Pn. In class III (5 animals), no sign of somatic duplication loss was detected. Since gip-1f (+) was present in the distal tip cells of animals in classes II and III, gip-1f (+) in this signaling cell is not sufficient for normal germ-line proliferation. In classes IV (3 animals) and V (1 animal), at least two duplication losses had occurred. In class IV, losses were from MS or EMS and from D or Pn, losses were from MS or EMS and C. In class VI (1 animal), only a single somatic duplication loss was observed, at MS or EMS.

From the genetic mosaics examined, it is clear that gip-1f (+) in the distal tip cell is neither necessary nor sufficient for normal germ-line development. Where then is gip-1f (+) produced? The pattern of duplication losses observed in the non-Unc Glp mosaic animals strongly suggests that gip-1f (+) is required in the germ line. Although the presence of qDp3 cannot be assayed in a Glp germ line, there is evidence in most of these mosaics for a duplication loss in the embryonic precursor cells leading to the germ line.
**g/p-l Functions in Regulation of the Mitotic/Meiotic Decision**

For another free duplication (E. Hedgecock, M. Salvato, fore, propose that the g/p-l gene product required for nor-

Glp animals. Presence (+) or absence (-) of 9Dp3 in the embryonic

germline; AB, MS, E, C, and D are embryonic precursors of the soma

PO, P1, Pp, Pa, and P4 are embryonic precursor cells leading to the

C. elegans. (A) Embryonic origin of the somatic gonad and germ line.

(S&ton et al., 1983). (B) Interpretation of mosaics found as non-Uric

precursors MS, C, and D is indicated. The embryonic precursor E gives

rise only to intestinal cells in which the Ncl phenotype cannot be reli-

ably scored. All animals had a Glp germ line: therefore the presence or

absence of 9Dp3 in the germ line could not be assessed in P4.

Normally, in C. elegans, some germ cells divide mitoti-

cally throughout development, while others enter meiosis and form gametes. The phenotype of putative null alleles of gip-1 is a switch of all germ cells from mitosis to meiosis. This effect is seen in both sexes and effectively stops germ line proliferation. Since the process of mitosis, meiosis, and gametogenesis can all occur normally in gip-

1 mutants, it is likely that the wild-type gip-1 gene product acts to regulate the decision between mitosis and meiosis.

**glp-1 May Be Involved in Regulation of the Germ Line by the Distal Tip Cell**

In C. elegans, the germ-line decision between mitosis and meiosis is controlled by a somatic regulatory cell, the dis-

tal tip cell (Kimble and White, 1981). The effect of glp-1 muta-

tions on the germ line is essentially the same as that of ablating the distal tip cell early in development. In both cases, a small number of germ-line divisions occur, fol-

lowed by entry of all germ cells into meiosis. A possible expanation of the gip-1 mutant phenotype might therefore have been that development of the distal tip cells is defec-

tive. However, no alteration of the distal tip cell is seen in glp-1 mutants.

Two striking similarities between the distal tip cell and glp-1 controls suggest that glp-1 functions in the interaction between distal tip cell and germ line. First, both distal tip cell–germ line interaction and glp-1 activity are re-

quired continuously for germ-line mitoses. Ablation of the distal tip cells at any time during larval development or adulthood results in the entry of all mitotic germ cells into meiosis (Kimble and White, 1981). Similarly, a shift from permissive to restrictive temperature of glp-1(tts) animals at any time during larval development or adulthood results in the entry of all mitotic germ cells into meiosis. Second, both the effect of distal tip cell ablation and the phenotype of glp-1 mutants can be limited to one ovotestis. In her-

maphrodites, one distal tip cell is present at the end of each ovotestis; ablation of one distal tip cell affects germ-

line proliferation only in the corresponding ovotestis. Animals homozygous for the variable allele, glp-1(aq50), can be Glp in either one or both ovotestes. Therefore, the action of glp-1, like control by the distal tip cell, can be ovotestis-specific.

One difference between the glp-1 phenotype and the ef-

fect of distal tip cell ablation has been observed. In glp-1 mutants, meiosis starts prematurely (at the end of L2), while after early ablation of the distal tip cell, it starts at the normal time (at the end of L3). The earlier entry into meiosis in glp-1 mutants may simply reflect a difference between the effects of physical and genetic manipulations. For example, a glp-1 homozygous animal produces only delective glp-1 product, whereas in an ablation experiment, the animal's germ line has been exposed to a wild-

type distal tip cell or its ancestor. Furthermore, even after laser ablation, debris of the distal tip cell persists for some hours and may retain some function. Alternatively, glp-1 may influence the mitotic/meiotic decision independently of the distal tip cell during L1 and L2. Subsequently, glp-1 may act with the distal tip cell signal to define a set of germ cells that continue to proliferate throughout development.

**Discussion**

**glp-1 Functions in Regulation of the Mitotic/Meiotic Decision**

Normally, in C. elegans, some germ cells divide mitoti-

...
A Maternal Contribution of Wild-Type gpl-1 Is Necessary for Embryogenesis

In addition to its function in germ-line proliferation, a maternal contribution of gpl-1 is necessary for normal embryogenesis. The embryonic temperature-sensitive period is after fertilization, between the 4 cell and 28 cell stages. This suggests that the gpl-1 gene product is packaged into oocytes and utilized early in embryogenesis. The postfertilization temperature-sensitive period implies either that the effect of temperature on the gpl-1(ts) product is reversible or that the gpl-1 product is present in a form that is not temperature-sensitive prior to fertilization.

The apparent null phenotype of embryos derived from gpl-1(gpl-1) mutant mothers is complex. The embryo contains several differentiated tissue types, but little morphogenesis occurs. The embryonic phenotype of gpl-1 differs from its germ-line phenotype in that proliferation is not severely affected and differentiation is apparently not premature. This difference in phenotype implies that the function of the gpl-1 product is not specifically, or solely, to control proliferation and differentiation.

The embryonic phenotype of gpl-1 has been independently analyzed by Priess et al. (1987). Among the alleles used in their study, a novel embryonic defect was found. Mutant embryos accomplished some morphogenesis, but the resulting worm lacked the anterior half of the pharynx. With the alleles used in our study, this embryonic defect was observed only when gpl-1(ts) embryos were shifted from the permissive to the restrictive temperature in the middle of the embryonic temperature-sensitive period. It is possible that this defect was not detected in the other gpl-1 embryos that we examined because of their severely defective morphogenesis.

Blastomere-removal experiments have shown that interaction between blastomeres during early embryogenesis is necessary for formation of the muscle cells that make up the anterior pharynx (Priess and Thomson, 1987). The similarity between the effect of blastomere removal and gpl-1 mutations on development of the anterior pharynx suggests that gpl-1 is involved in the cell interaction required for formation of the anterior pharynx.

The gpl-1 Product Functions as Part of the Receiving Mechanism in the Distal Tip Cell Germ Line Interaction

The gpl-1 gene product appears to be required for at least two distinct cell interactions. What might its role be in these interactions? Does gpl-1 encode a component of the signaling mechanism or the receiving mechanism? Our analysis of genetic mosaics indicates that the gpl-1 activity necessary for germ-line mitoses is produced by the germ line. This implies that gpl-1 encodes a component of the receiving machinery for the distal tip cell--germ line interaction. The mosaic analysis also shows that gpl-1 activity in the distal tip cell is neither necessary nor sufficient for germ-line development. Thus, gpl-1 does not encode the distal tip cell signal.

The function of gpl-1 in mediating cell interaction is not known. Two simple possibilities are consistent with its role at the receiving end of the interaction. One is that gpl-1 encodes the receptor for the distal tip cell signal; another is that it encodes an element that transduces the signal from the receptor to proteins that act downstream to control the decision between mitosis and meiosis. The gpl-1 product that functions in the distal tip cell--germ line interaction might be identical to that in the embryo, or it might be an alternative product of the same gene. In either case, it seems likely that the function of gpl-1 will prove to be intrinsic to the receiving mechanism of two or more interactions that regulate specific developmental events.

Experimental Procedures

General Procedures and Strains

Routine maintenance was as described by Brenner (1974). All strains were raised at 20°C unless otherwise indicated. Observation of living animals was done using Nomarski differential interference contrast microscopy. Cell lineage analysis relied on the method of Sulston and Horvitz (1977); for the lineage reported here, two animals were examined continuously and many animals were examined at various times during development. DAPI staining of whole worms was performed as described by Ellis and Horvitz (1986), except that phenoxypropanol was not used. DAPI-stained worms were viewed using fluorescence microscopy. Presence of gut granules in embryos was scored using fluorescence optics (Lauber et al., 1980). Males analyzed were generated by use of him-5 mutant strains; him-5 causes a high incidence of males (Hoekgin et al., 1979). For many experiments, double mutants with gpl-1 and unc-32(e108) were constructed. The tight linkage between gpl-1 and unc-32 allowed gpl-1 homoyzgotes to be picked at a time when the Glp phenotype was not evident. Germ-line development is essentially wild-type in unc-32(e108), although the total number of germ cells produced is somewhat less than in N2. The phenotype of gpl-1 animals is the same in the presence and absence of unc-32(e108).

The wild-type parent of all strains used was C. elegans var. Bristol, strain N2 (Brenner, 1974). The following mutations were used in this study: LGII, dpy-1(e1), dpy-17(e164), ncl-1(e1865), unc-36(e2511), dpy-1(e2029), unc-32(e108), lin-12(dv170), lin-12(dv709), lin-12(dv709a909), lin-12(dv1377), lin-12(dv1377d20), lin-12(dv1377d32), unc-66(e587), and dpy-1(e1836i); LGIV, fem-1(hc71); LGV, him-5(e1490); LGX, sup-7(t25). Most of these mutations are described by Swanson et al. (1984); is-12 mutations are described by Greenwald et al. (1983) and Greenwald (1985). In addition, we used the translocation ots3(hc1), which suppresses recombinability in the right half of LGIII, including the region around gpl-1 (Rosenbluth and Baillie, 1981), and nm2037, a free duplication that includes most of LGIII (R. Herman and C. Kari, personal communication).

Isolation of gpl-1 Alleles

All gpl-1 alleles were obtained after mutagenesis with ethyl methanesulphonate (Brenner, 1974). L4 hermaphrodites (N2, dpy-1907, or dpy-1907 unc-32) were mutagenized, and F1 progeny were placed individually onto agar-filied petri dishes. If one-fourth of the F2 progeny were sterile, the sterile worms were examined with Nomarski optics. All mutations that had a Glp phenotype were isolated. All mutations that had a Glp phenotype (limited germ-line proliferation followed by entry of all germ cells into meiosis and formation of gametes) were found to be alleles of gpl-1.

Two additional gpl-1 alleles were isolated on the basis of their failure to complement gpl-1(q46). In the first noncomplementation screen, mutagenized dpy-19 hermaphrodites and males were crossed at 15°C. In individual F1 dpy-19 males were mated with unc-32 gpl-1(q46), nm2037 hermaphrodites. If sterile cross-progeny were observed, fertile siblings were used to isolate the new dpy-19-linked allele of gpl-1. From 113 matings, one allele, q528, was found. In the second noncomplementation screen, dpy-10 hermaphrodites were mutagenized and allowed to self-fertilize. Individual F1 hermaphrodites were then mated with gpl-1(q46) unc-32; him-5 males. When sterile cross-progeny were ob-
serviced, fertile siblings were used to recover the new dpy-19-linked allele of glp-1. From 524 matings, one allele, q172, was found.

Mapping, Complementation, and Amber Suppression Tests

The first allele of glp-1, q50, was tested to establish linkage with markers on all linkage groups and was found to be strongly linked to unc-22 on LGIII. All subsequent alleles have also been mapped to this region. One allele, q50, could not be mapped, because it arose on the translocation chromosome e17. Nonetheless, its location on e17 is consistent with its identification as an allele of glp-1.

A 4-factor cross was performed to establish the position of glp-1(q246) relative to the closest known genes in the region: dpy-19, unc-32, and lin-12. The strain dpy-19 glp-1(q46) unc-32 lin-12(n758) was grown at 25°C and scored for Dpy Non-Glio recombinants. The progeny of the recombinant animals were tested for the presence of unc-32 and lin-12 mutations. Altogether, 78/9287 Dyps were recombinant; 43/78 had recombined between dpy-19 and unc-32, 97/78 has recombined between unc-32 and lin-12(n758), and 6/78 had recombined between lin-12(n758) and glp-1(q246). Using these data and a 2-factor recombination frequency of 0.1%, the coding order of the unc-32 to lin-12 interval was established (C. Ferguson, personal communication), the distance between lin-12 and glp-1 was calculated as 0.02%. The candidate q50 allele was used for complementation with glp-1(q46). Heterozygotes of genotype glp-1(q46)glp-1(lx) were tested and scored using Nomarski optics for a Glp germ-line phenotype. In addition, glp-1(q50), glp-1(q231), and glp-1(q246) were tested for complementation of their maternal effect lethal phenotype with glp-1(q235). Heterozygotes were constructed and scored using Nomarski optics. These data were used to score the viability of cross-progeny from dpy-19 glp-1(q231) dpy-19 hermaphrodites placed on freshly seeded plates at 25°C. Larvae were killed at 1 hr of hatching (t = 0) and shifted from 25°C to 15°C at 6 hr intervals during development. The germ-line phenotype of the unc-32 glp-1(q231) homozygotes was scored at the adult stage using Nomarski optics.

Temperature-Shift Experiments (Germ-Line Development)

For shift-down experiments, egg-laying unc-32 glp-1(q231) dpy-19 hermaphrodites were used to eliminate any sperm from the matings. Animals were killed at 1 hr of hatching (t = 0) and shifted from 25°C to 15°C at 6 hr intervals during development. The germ-line phenotype of the unc-32 glp-1(q231) homozygotes was scored at the adult stage using Nomarski optics.

Characterization of the Maternal Effect Lethal Phenotype

In general, embryonic lethality was scored by counting the total number of eggs laid and then counting the number that hatched. To examine the viability of cross-progeny from dpy-19 glp-1(q231) and glp-1(q246) hermaphrodites, hermaphrodites were purged of their own sperm by allowing them to lay eggs at 16°C for several days and then mating with N2 males at 25°C.

For determination of the embryonic temperature-sensitive period of glp-1, 2 cell embryos were dissected from unc-32 glp-1(q231) dpy-19 hermaphrodites either grown at 15°C or raised to 25°C as L4s and maintained there for 20 hr. Dissections were done at the appropriate temperature. All shifts were done by transferring embryos to petri plates that had been preincubated at the appropriate temperature. Embryos were sacrificed for hatching; any embryos that hatched were scored for further development and, as adults, for production of a normal germ line.

Generation and Characterization of qDp3

qDp3 was generated by γ-ray mutagenesis of mndp37. mndp37 is a free duplication that covers most of chromosome III, including dpy-1 and dpy-18 (Figure 7, R. Herman and C. Kari, personal communication). To isolate the decrease of mndp37, L4 dpy-19 glp-1(q245) unc-69, mndp37 hermaphrodites were irradiated with 7000 roentgens at a dose rate of 1.28 × 10^4 roentgens/min. F1 progeny were screened for non-Dpy non-Glio Unc animals. One candidate contained qDp1, a duplication that covers dpy-19 and glp-1 but not unc-69 or dpy-18. L4 dpy-17 ncl-1 glp-1(q246); qDp1 hermaphrodites were irradiated as described above. F1 progeny were screened for Dpy non-Glio Unc animals. One candidate contained qDp3, a duplication that covers ncl-1 and glp-1 but not dpy-1 and dpy-17. When animals containing qDp3 or mndp37 were screened with DAPI, an extrachromosomal fragment was observed in both cases, but the qDp3-associated fragment was smaller than the mndp37-associated fragment.

Analysis of Genetic Mosaics

Animals with duplication losses in the somatic gonad were found by screening non-Unc L3 hermaphrodites from the strains ncl-1 unc-35 glp-1; qDp3 using Nomarski optics. Animals in which one or both distal tip cells had a Ncl phenotype were isolated and allowed to develop to adults and were then screened for the Ncl phenotype of other cells in the somatic gonad (Figure 8A) as well as their germ-line phenotype. The presence or absence of qDp3 in the germ line of these mosaic animals was determined by allowing them to lay eggs and looking for non-Unc, non-Glio progeny.

Mosaic animals with a Glp germ-line phenotype were scored for the Ncl phenotype of cells descended from MS, C, and D using Nomarski optics. The presence of the duplication in AB was confirmed by scoring the Ncl phenotype of neurons derived from AB A Ncl
g/p-7 Regulates the Mitotic/Meiotic Decision

phenotype in all the scored cells that were descended from a particular embryonic precursor was taken as evidence that the original precursor cell did not contain the duplication. A non Nol phenotype in some or all of the cells descended from a particular embryonic precursor was taken as evidence that the precursor cell had contained qDP3. Five non-Nol diploids found in our study were non-Nol in all cells scored (class III). These animals may result from an unscorable duplication loss (e.g., loss of qDP3 in P0) or loss of g/p-7(X) from qDP3 due to gene conversion, spontaneous mutation, or recombination with chromosome III. Because these animals have a Nol germ-line phenotype, it is not possible to test for the presence of qDP3 in their germ lines. Therefore these two possibilities cannot be distinguished.

Acknowledgments

We are grateful to Ed Hedgecock for providing ncl-1(e7865), to Bob Herman for providing mndp37, and to both for sharing unpublished data. We thank John Sulston for assistance in learning to identify particular cells, Iva Greenwald for consultations on lin-12, and Paul Sammack for advice on photographic techniques. We also thank Timothy Schedl for many helpful discussions and suggestions, as well as Phil Anderson, Julie Ahrringer, Bill Dove, Eleanor Maine, and Marv Wikkens for critical reading of the manuscript. Sandy Maples provided expert technical assistance; in particular, she isolated several alleles of g/p-7 described in this paper. Technical illustration was done by Leslie Ratas. Finally, we give special thanks to Jim Priess for communication of results prior to publication.

This research was supported by a U.S. Public Health Service grant GM31816, a Basil O'Connor Starter Research Grant no. 5-514 from the March of Dimes Birth Defects Foundation, and a Research Career Development Award 11D0000 to J.K.H. J. d. a. is a trainee of the NIH Predoctoral Training Program in Genetics, GM07133. Some nematode strains used in this study were provided by the Caenorhabditis elegans Genetics Center, which is supported by contract number N1-AI-7-2113 between 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 July 16, 1987; revised August 24, 1987.

References


