gon-2, a Gene Required for Gonadogenesis in Caenorhabditis elegans

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ABSTRACT

The gonad of the Caenorhabditis elegans hermaphrodite is generated by the postembryonic divisions of two somatic precursors, Z1 and Z4, and two germline precursors, Z2 and Z3. These cells begin division midway through the first larval stage. By the end of the fourth larval stage, Z1 and Z4 produce 143 descendants, while Z2 and Z3 give rise to ~1000 descendants. The divisions of Z2 and Z3 are dependent on signals produced by Z1 and Z4, but not vice versa. We have characterized the properties of five loss-of-function alleles of a newly described gene, which we call gon-2. In gon-2 mutants, gonadogenesis is severely impaired; in some animals, none of the gonad progenitors undergo any postembryonic divisions. Mutations in gon-2 have a partial maternal effect: either maternal or zygotic expression is sufficient to prevent the severe gonadogenesis defects. By cell lineage analysis, we found that the primary defect in gon-2 mutants is a delay (sometimes a complete block) in the onset and continuation of gonadal divisions. The results of upshift experiments using a temperature-sensitive allele suggest that zygotic expression of gon-2 begins early in embryogenesis, before the birth of Z1 and Z4. The results of downshift experiments suggest that Z1 and Z4 can generate the full complement of gonadal tissues even when gon-2 function is inhibited until the end of the second larval stage. Thus, gon-2 activity is probably not required for the specification of gonadal cell fates, but appears to be generally required for gonadal cell divisions.

In the nematode Caenorhabditis elegans, as in other metazoans, the division and differentiation of cells within separate tissues is coordinated throughout development (Horvitz and Sulston 1977; Sulston et al. 1983). However, experimental and mutational studies have clearly demonstrated that the development of individual tissues and organ systems is subject to separate regulation (for reviews, see Riddle et al. 1997). We have begun to investigate the mechanisms that govern cell division and differentiation within the C. elegans gonad, with the aim of determining how the development of this specific organ is regulated and how the timing of gonad development is controlled in relation to the other tissues in the animal.

The somatic cells of the C. elegans gonad are derived from two postembryonic blast cells, Z1 and Z4, which begin dividing midway through the first larval stage (L1). The timing and orientation of the divisions executed by Z1 and Z4 are predictable and sex-specific (Kimble and Hirsh 1979; Figure 1). In this article, we consider only the development of the hermaphroditic gonad. At the molt between the second (L2) and third (L3) larval stages, the 12 descendants of Z1 and Z4 assume a characteristic "somatic primordium" arrangement, which prefigures the organization of the adult gonad. Between early L3 and early L4, the somatic cells execute a series of well defined sublineages, coincident with the onset of differentiation into specific tissue types. Six major types of tissues are generated (Hirsh et al. 1976; Kimble and Hirsh 1979; see Figure 1): (1) distal tip cells (DTCs), which lead the extension and reflexion of the gonadal tube during the larval stages. The DTCs are required for distal germline proliferation throughout development (Kimble and White 1981). (2) Sheath cells, which form an epithelial casing that surrounds the proximal germline, and part of the distal germline. Sheath cells at different positions along the proximo-distal axis appear to have different functions, including regulation of ovulation, meiotic progression and mitotic proliferation of the germline (Greenstein et al. 1994; McCarter et al. 1997). (3) Spermathecal cells, which form a contorted tube that harbors spermatozoa. (4) Spermathecal/uterine junction cells, which create a valve-like connection between the spermatheca and the uterus. (5) Uterine cells, which form an epithelium that surrounds the uterine cavity, a temporary storage organ for fertilized eggs. A subset of the ventral uterine cells form connections with the vulval epithelial cells, creating an opening through which eggs are laid (Newman et al. 1996). (6) Anchor cell (AC), which provides an inductive signal that triggers underlying epidermal cells to execute vulval lineages during L3 (Kimble 1981). In late L4, the AC differentiates into a ventral uterine cell (Newman et al. 1996).

The germline is derived from another pair of postembryonic blast cells, Z2 and Z3, that are situated between Z1 and Z4 (Sulston et al. 1983). Z2 and Z3 begin to divide slightly before Z1 and Z4; their divisions continue exponentially throughout the larval stages and do.

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not follow a predictable spatio-temporal pattern (Kimble and White 1981). Differentiation of the germ cells begins in L4, when the most proximal germ cells undergo spermatogenesis. Subsequently, germ cells differentiate into oocytes.

Little is known about how the onset of division of the somatic gonadal cells is regulated. Like all other post-embryonic blast cells, Z1 and Z4 fail to initiate divisions when newly hatched larvae are starved. Therefore, both gonadal and non-gonadal tissues may respond to the same signal(s) that trigger postembryonic divisions in embryonic blast cells, 21 and 24 fail to initiate divisions as somatic gonadal cells is regulated. Like all other post-

In this article, we report the identification and characterization of gon-2, a gene that is specifically required for gonadogenesis in C. elegans. Mutations in gon-2 have no apparent direct effects on the development of non-gonadal tissues. The primary effect of mutations in gon-2 is a retardation or blockage of gonadal cell divisions. This effect can be explained most simply by a defect in somatic (rather than germline) tissues of the gonad, since mitotic proliferation of the germline is completely dependent on the presence of somatic gonadal cells (Kimble and White 1981; McCarter et al. 1997), whereas the divisions of Z1 and Z4 proceed normally even if Z2 and Z3 are killed early in the first larval stage (L1) (Kimble and White 1981). Our data indicate that gon-2 function is required for both the onset and continuation of postembryonic divisions of Z1 and Z4.

MATERIALS AND METHODS

General methods: Nematodes were cultured in Petri dishes with Escherichia coli as a food source, essentially as described by Brenner (1974). For initial work we used MYOB medium (Church et al. 1995), but most experiments were done using NGM-Lite, a modification of Brenner's NGM (Brenner 1974): 1.5 g/liter NaCl, 4 g/liter Bactotryptone (Difco), 3.0 g/liter KH₂PO₄, 0.5 g/liter K₂HPO₄, 8.0 mg/liter cholesterol, 20 g/liter agar. Variations in media did not result in any obvious differences in the growth or phenotypes of the strains used in this study. Stocks were maintained at 15°, 20° or 25°, depending on the experiment. Nutrient-free plates were made with MYOB mix lacking tryptone and cholesterol. All chemicals were obtained from Sigma, unless otherwise noted.

Microscopy and imaging: Live nematodes were mounted on thin pads of 3% agarose, essentially as described by Horvitz and Sulston (1977). Microscopy was performed using a Nikon Microphot-SA fitted with standard epifluorescence filters and Nomarski differential interference contrast optics. Video imaging was done using a Sony AVC-D7 CCD camera. For image acquisition, the camera was connected to a Scion LGIII frame grabber installed in a Quadra 700 computer and frames were captured using NIH Image software (as modified by Scion). Images were manipulated and labeled using Adobe Photoshop 3.0.

Nematode strains: All nematode stocks were derived from the wild-type strain N2 of Caenorhabditis elegans (Brenner 1974). Strain constructions and genetic analyses were done according to standard methods (Sulston and Hodgkin 1988).

The following mutations and rearrangements of chromosome I were used [reviewed and summarized by Hodgkin et al. (1995)].
al. (1988), unless indicated otherwise: img-2(r683) (HODGRIN et al. 1988), fgl-1(q253) (BARTON and KIMBLE 1990), dpy-5(e61); unc-13(e51), lin-10(e1439), celh-6(mg60) (T. BURGILIN and G. RAVIKUN, personal communication), fer-1(hc1), unc-29(e1072); nD23, nDP24, nDP25, nDP4.

Isolation of gon-2 alleles: q362 and q388 were identified during the course of F1 clonal screens designed to identify sterile and/or lethal mutations (e.g., LAMBIE and KIMBLE 1991). In each case, animals with a Gon (gonadless) phenotype were observed among the F2 progeny of N2 hermaphrodites that had been mutagenized with 50 mM EMS (BRENNER 1974). Fertile siblings were then outcrossed to map and characterize these mutations.

The following non-complementation screen was done to isolate additional alleles of gon-2. Hermaphrodites of genotype unc-29(e1072) fer-1(hc1) were raised at 25°C (to render them self-sterile) and then mutagenized with 75 mM EMS for 4 hr (at room temperature). Groups of five mutagenized animals were then mated (at 15°C) with ~20 males of genotype gon-2(q388), which had been raised at 15°C. Adults were transferred to new plates every 12 hr to promote developmental synchrony among the eggs laid on each plate. Soon after the F1 progeny reached adulthood (after 7 days at 15°C), they were suspended in M9 buffer and plated on 12-well plates that were seeded with E. coli (at a density of approximately three worms per well). After 16–24 hr at 20°C, each plate was shifted to 25°C for 48 hr, then returned to 15°C. This temperature regimen rescues the sterility of gon-2/* animals (where * is a new allele of gon-2) produced early in the brood, but causes a Gon phenotype among q388/* siblings that undergo embryogenesis and early larval stages at restrictive temperature. The return to 15°C allows recovery of new alleles from the fertile animals of genotype q388/*; three new alleles, dx12, dx22 and dx23, were isolated as a result of screening ~5000 mutagenized genomes. Each new allele was backcrossed to N2 at least three times before being characterized.

Isolation of dxDF1: Adult N2 males were irradiated with ~25 J/m²/sec 310 nm UV light for 10 sec, then mated with hermaphrodites of genotype gon-2(q388) unc-29(e1072) at 15°C. Approximately 1000 non-Unc F1 hermaphrodites were cloned at 15°C, allowed to lay eggs for 48 hr, then transferred to new plates at 25°C. dxDF1 was recovered from the 15°C replica of a clone that segregated Gon F3s at 25°C.

Mapping of gon-2: Initial mapping experiments indicated that gon-2 is located on the right arm of chromosome I, in the vicinity of fer-1 (Figure 2; E. J. LAMBIE, unpublished results). To map gon-2 relative to fer-1, hermaphrodites of genotype lin-10(e1439) fer-1(hc1) unc-29(e1072)/gon-2(q388) were raised at 25°C and recombinant progeny were isolated. Among 14 Lin Non-Fer recombinants, three failed to segregate progeny of genotype lin-10(e1439) gon-2(q388). Therefore, gon-2 is to the left of fer-1, and closer to fer-1 than to lin-10. To position gon-2 relative to ceh-6, hermaphrodites of genotype dpy-5(e61) ceh-6(mg60) unc-29(e1072)/gon-2(q388) were selfed at 25°C and recombinant progeny were isolated. Among seven Unc Non-Ceh recombinants, three failed to segregate progeny of genotype gon-2(q388) unc-29(e1072). Therefore, gon-2 is located to the right of ceh-6. We have confirmed this map position by transformation rescue of gon-2(q388) using cosmids situated within the ceh-6–fer-1 interval (A. Y. SUN and E. J. LAMBIE, unpublished results).

Temperature-sensitive period: Temperature-sensitive periods (TSPs) were determined by performing reciprocal temperature shifts (HIRSH and VANDERSLICE 1976; ENSENGAH et al. 1983). For embryos of genotype gon-2(q388)/gon-2(q388) and gon-2(dx12) fer-1(hc1) unc-29(e1072)/gon-2(dx12) fer-1(hc1) unc-29(e1072), embryos were derived from homozygous mutant hermaphrodites that were raised at 15°C or 20°C until L4, then shifted either to 15°C (for subsequent upshifts) or 25°C (for subsequent downshifts) until reaching adulthood. For embryos of genotype fgl-1(q253) gon-2(q388) unc-75(e950) gon-2(q388), females of genotype fgl-1(q253) gon-2(q386) unc-75(e950) were mated with gon-2(q388) males at 15°C (only upshifts were done for this genotype).

In all cases, two-cell stage embryos were dissected from adults and then placed on plates that were pre-equilibrated to either 15°C or 25°C, as above. For time points before hatching, reciprocal shifts were done by transferring embryos to plates of the appropriate temperature. For larval timepoints, staging was done by picking newly hatched animals or by identifying individuals in lethargus (for molting stages). Gonadal and vulval phenotypes were scored by dissecting microscope once animals had reached adulthood.

Starvation experiments: For starvation of L1 larvae, eggs were collected from stocks growing on seeded plates and transferred to nutrient-free plates (see above). Forty-eight to 72 hr after hatching, L1 larvae were transferred to plates containing E. coli grown to adulthood and then scored. Males were starved by picking L4s to nutrient-free plates, dissecting eggs from them after they reached adulthood, then transferring eggs to plates containing E. coli. A combination of these two methods was used in experiments in which both the L1 larvae and their mothers were starved.

Cell lineage analysis: Cell lineages were followed using methods similar to those described by HORVITZ and SULLSTON (1977). We found it necessary to use relatively thick agarose pads (1 mm) to achieve long-term survival of continuously mounted animals; presumably, the extra pad thickness facilitates gas diffusion and thereby prevents drowning.

For gon-2(q362), newly hatched L1 larvae were incubated on nutrient-free plates (see above) for 24–48 hr at 15°C, 20°C or 25°C. Starved L1 larvae were then transferred to seeded plates and incubated for ~12 hr at 20°C or 25°C before being mounted for cell lineage analysis. All animals were maintained at 25°C during the experiment. At the L2/L3 molt, animals whose cell lineages had been followed were returned to plates, incubated at 25°C, then scored as adults.

Non-gonadal lineages: To investigate the possibility that mutations in gon-2 affect non-gonadal lineages, we examined the Gon progeny of gon-2(q388) gon-2(q388) hermaphrodites. No apparent defects were observed in coordination, general body morphology, stage-specific expression of alae, dauer formation, or male tail morphology. Therefore, at this level of analysis, the effects of gon-2 appear to be confined to the gonad.

RESULTS

Identification and mapping of gon-2: Two alleles of gon-2, q362 and q388 were isolated incidentally during the course of clonal F1 screens for sterile and/or lethal mutations (e.g., LAMBIE and KIMBLE 1991; E. J. LAMBIE, unpublished results). Both mutations were identified based on the presence of Gon (gonadless) animals among the F2 progeny. In this article, we use the term "Gon" to refer to vulvalless animals in which the gonad has a diameter of ≤50 μm (see Figure 3); this usage is different than that of HIRSH and VANDERSLICE (1976), who used Gon to describe animals that fail to produce eggs or progeny. Some gon-2 mutant animals exhibit less severe defects in gonadogenesis (see below, under Anatomical defects); we refer to this as the "partial-mutant" phenotype.

Since q362 and q388 were obtained from screens that were not specifically conducted to identify alleles of gon-
2, the frequency of allele isolation (~1/15,000 mutagenized genomes) is expected to be an underestimate of the actual frequency of occurrence. Three additional alleles of gon-2, dx12, dx22 and dx23 were isolated by screening for mutations that fail to complement q388 (MATERIALS AND METHODS). These alleles were found at a frequency of 1/1000 mutagenized genomes, consistent with a loss-of-function character. gon-2 is not likely to be a complex locus, because all pairwise combinations of mutant alleles exhibit failure to complement (A. Y. SUN and E. J. LAMBIE, unpublished results).

Standard three-factor mapping methods were used to determine that gon-2 is located between ceh-6 and fer-1 on the right arm of chromosome I (MATERIALS AND METHODS; Figure 2). All of the analyses reported in this article are based on results obtained with hermaphrodites; however, the Gon phenotype is expressed in both males and hermaphrodites (A. Y. SUN and E. J. LAMBIE, unpublished results).

Partial maternal effect of gon-2: Mutations in gon-2 confer a partial maternal effect Gon phenotype; i.e., either maternal or zygotic expression of a wild-type allele is sufficient to prevent expression of the Gon phenotype (Figure 3; Table 1, A–C). Paternal expression has no effect on the penetrance of the Gon phenotype (Table 1D). Therefore, gon-2 is expressed maternally, and its gene product is probably provided to the oocyte in the form of protein and/or mRNA.

Although zygotic expression of gon-2 is not required for early stages of gonadogenesis, homozygous q388 hermaphrodites derived from a heterozygous mother are sterile (Table 1B). It is not clear why these animals are sterile; most of them appear to have an approximately normal number of uterine, spermathecal and sheath cells and eventually make both sperm and oocytes (A. Y. SUN and E. J. LAMBIE, unpublished results). A more detailed characterization of the zygotic sterile phenotype of gon-2 will be presented elsewhere.

Dosage analysis: By several criteria, all of the alleles of gon-2 that we have characterized appear to be loss-of-function mutations. Each is recessive, temperature sensitive, and has a similar (usually stronger) mutant phenotype when placed in trans to a deficiency (Tables 1, 2). Furthermore, increasing the dosage of gon-2(+) (by means of the duplication, nDp4) eliminates the mutant phenotype of gon-2(q388), as expected for a loss-of-function mutation (Table 2).

Three lines of evidence suggest that q388 is the strongest allele, approximating a complete loss of gene function at restrictive temperature. First, q388 exhibits the highest penetrance (Tables 1–3). Second, only q388 causes zygotic sterility (see above; Table 1). Third, the penetrance of the other alleles of gon-2 is comparably enhanced when placed in trans to either q388 or a deficiency that removes gon-2 (Table 2). However, q388 is not likely to be a null allele, because it does not cause a Gon phenotype at 15°, unlike two other putative hypomorphic alleles, q362 and dx12.

Starvation sensitivity of q362: The penetrance of the Gon phenotype among gon-2(q362) animals can be enhanced more than 100-fold by starving newly hatched L1s for 24 hr before transferring them to plates with a bacterial food supply (Table 3). This suggests that the mRNA and/or protein product derived from the q362 allele is probably unstable under conditions of nutritional stress. Starvation does not substantially increase the penetrance of other alleles of gon-2 (Table 3).

To examine the possibility that q362 affects mRNA stability, we tested the effects of smg-2(r863) on starvation sensitivity. The smg-2(r863) mutation is expected to stabilize classes of mRNA that would otherwise be rapidly degraded, e.g., a mutant mRNA with a premature stop codon (HODGKIN et al. 1989; PULAK and ANDERSON 1993). We found that smg-2(r863) is an efficient suppressor of the starvation sensitivity of q362. This suppression is not due to a nonspecific effect on gonadogenesis, because smg-2(r863) does not suppress q388 (Table 3).

Interestingly, starvation of q362 hermaphrodites is sufficient to increase the penetrance of the Gon phenotype, even if their progeny are fed immediately after hatching (Table 3). Possibly, starvation of q362 hermaphrodites during oogenesis results in a decrease in

![Figure 2. Position of gon-2 on the genetic map of chromosome I. Locations of genetic markers and rearrangement endpoints in the vicinity of gon-2 are based on the genetic map assembled by J. HODGKIN, R. DURBIN and S. MARTINELLI (personal communication). Map units, in cM, are indicated within parentheses.](image-url)
the quantity or quality of maternally contributed gon-2 mRNA, and zygotic activity from the q362 allele during larval development is not sufficient to compensate for this deficit. Consistent with this possibility, the penetrance of the Gon phenotype is very high among homozygotes after hatching increases the quantity or quality of maternally contributed gon-2 mRNA, and zygotic activity from the q362 allele during larval development is not sufficient to compensate for this deficit. Consistent with this possibility, the penetrance of the Gon phenotype is very high among homozygotes after hatching.

**Temperature-sensitive periods:** Identification of the developmental period during which gon-2 activity is required for gonadogenesis should provide clues to the possible function of the gon-2 gene product. Since starvation of q362 homozygotes after hatching increases the penetrance of the Gon phenotype, gon-2 activity is likely to be required after hatching. However, this does not exclude the possibility that gon-2 also has essential functions during embryogenesis. Furthermore, the results obtained with q362 do not indicate which stages of gonadogenesis require gon-2 activity. These issues are better addressed using temperature-sensitive alleles.

The ideal type of temperature-sensitive allele for determining the time of function of a given gene product is one that causes a reversible inactivation of the protein product. In this case, reciprocal temperature shifts can be used to determine the temperature-sensitive period (TSP), which provides an accurate estimate of the developmental period during which the gene product is required. However, temperature sensitivity can also result from thermal-dependent defects in gene product synthesis or stability. In such cases, the TSP is heavily influenced by both the activity and the perdurance of gene product synthesized at permissive temperature. For the ideal type of temperature-sensitive allele, we shifted animals of genotype q362/Df or q362/q388, even if the progeny are not starved (Table 2). Alternatively, it could be that larvae that develop from eggs produced by starved mothers are themselves nutritionally stressed before hatching [e.g., if the level of maternal products (yolk?) is lower than usual].

**TABLE 1**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Zygotic</th>
<th>Maternal</th>
<th>Paternal</th>
<th>% Gon</th>
<th>% Wild type</th>
<th>% Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>gon-2(q388)$^a$</td>
<td>q388</td>
<td>q388</td>
<td>98.6</td>
<td>0.3</td>
<td>1.1</td>
</tr>
<tr>
<td>B.</td>
<td>gon-2(q388)$^b$</td>
<td>q388/+</td>
<td>q388/+</td>
<td>0.3</td>
<td>0</td>
<td>99.7</td>
</tr>
<tr>
<td>C.</td>
<td>q388 and q388/+</td>
<td>q388</td>
<td>q388/+</td>
<td>50</td>
<td>48</td>
<td>2</td>
</tr>
<tr>
<td>D.</td>
<td>q388/+</td>
<td>q388</td>
<td>++/+</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>E.</td>
<td>q388 and q388/+</td>
<td>q388/+</td>
<td>q388</td>
<td>3</td>
<td>57</td>
<td>40</td>
</tr>
</tbody>
</table>

Conclusions: Maternal expression is usually (or always) sufficient to prevent the Gon phenotype. Zygotic expression is necessary for hermaphrodite fertility.

All animals were raised at 25°, the restrictive temperature for q388. unc-29(e1072) was used as a cis marker to identify q388 homozygotes. Although only q388 was tested in all different combinations, the Gon phenotype is also absent among the progeny of hermaphrodites heterozygous for the other mutant alleles of gon-2, suggesting that maternal expression is probably sufficient to rescue the Gon phenotype caused by these mutations also.

*Defined as vulvaless (Vul) animals with a gonad &ge; 50 μm in diameter, as scored by dissecting microscope.

*Obtained by crossing q388 unc-29(e1072) hermaphrodites.

*Animals with incomplete or abnormal development of gonad and/or vulva, as visible by dissecting microscope.

*Obtained by crossing q388 unc-29(e1072)/++ hermaphrodites.

*Non-Vul, but sterile. ~90% of these had essentially normal vulva morphology. In the others, the vulva was everted.

*Obtained by crossing q388 unc-29(e1072) hermaphrodites with q388/+ males. Animals of genotype q388 unc-29(e1072)/++ and q388 unc-29(e1072)/q388 cannot be unambiguously distinguished from each other in the F1.

*Obtained by crossing q388 unc-29(e1072) hermaphrodites with wild-type males.

*Obtained by crossing purged q388 unc-29(e1072)/++ hermaphrodites with q388 males.
for function and/or gene product stability should exhibit relatively high penetrance under these conditions. However, a mutation that is primarily temperature sensitive for synthesis might exhibit relatively low penetrance, due to the persistence of gene product made at the permissive temperature. Our results (see Figure 4) suggest that *q388* is primarily temperature sensitive for synthesis and *dx12* is primarily temperature sensitive for function and/or stability. *dx22* may be temperature sensitive for some combination of synthesis, function and stability, because Gon animals are observed after upshift at hatching, but the penetrance (10/132; 7.5%) is relatively low (compare with Table 2).

Since *q388* and *dx12* appear to represent the two extremes, we characterized each in greater depth by performing reciprocal temperature shift experiments.
TABLE 2

Effects of temperature and dosage on penetrance of gon-2 alleles

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Temperature (°)</th>
<th>% Gon&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% Wild type</th>
<th>% Other&lt;sup&gt;b&lt;/sup&gt;</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>q388</td>
<td>15</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>1009</td>
</tr>
<tr>
<td>q388</td>
<td>25</td>
<td>95</td>
<td>0</td>
<td>5</td>
<td>889</td>
</tr>
<tr>
<td>q388/Df&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25</td>
<td>97</td>
<td>0</td>
<td>3</td>
<td>106</td>
</tr>
<tr>
<td>q388/q388/ + and q388/q388&lt;sup&gt;d&lt;/sup&gt;</td>
<td>25</td>
<td>0</td>
<td>72&lt;sup&gt;e&lt;/sup&gt;</td>
<td>28&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>99.5</td>
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<tr>
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<td>0.2</td>
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<td>0.4</td>
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</tr>
<tr>
<td>q362</td>
<td>25</td>
<td>7</td>
<td>87</td>
<td>6</td>
<td>1704</td>
</tr>
<tr>
<td>q362/q388&lt;sup&gt;f&lt;/sup&gt;</td>
<td>25</td>
<td>90</td>
<td>4</td>
<td>6</td>
<td>191</td>
</tr>
<tr>
<td>q362/Df and q362/+&lt;sup&gt;g&lt;/sup&gt;</td>
<td>25</td>
<td>44</td>
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<td>97</td>
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<td>38</td>
<td>18&lt;sup&gt;h&lt;/sup&gt;</td>
<td>44</td>
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<tr>
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<td>81</td>
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<td>2&lt;sup&gt;l&lt;/sup&gt;</td>
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<tr>
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<td>18</td>
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<tr>
<td>dx22/Df&lt;sup&gt;k&lt;/sup&gt;</td>
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<td>85</td>
<td>2&lt;sup&gt;m&lt;/sup&gt;</td>
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<td>0</td>
<td>419</td>
</tr>
<tr>
<td>dx23</td>
<td>25</td>
<td>5</td>
<td>91&lt;sup&gt;n&lt;/sup&gt;</td>
<td>4</td>
<td>351</td>
</tr>
<tr>
<td>dx23/q388&lt;sup&gt;j&lt;/sup&gt;</td>
<td>25</td>
<td>40</td>
<td>46</td>
<td>14</td>
<td>168</td>
</tr>
<tr>
<td>dx23/Df&lt;sup&gt;t&lt;/sup&gt;</td>
<td>25</td>
<td>34</td>
<td>40&lt;sup&gt;o&lt;/sup&gt;</td>
<td>26</td>
<td>68</td>
</tr>
</tbody>
</table>

*Unless otherwise noted, animals were generated by selfing homozygous hermaphrodites at the indicated temperature.
+a Defined as vulvaless (Vul) animals with a gonad ≤50 μm in diameter, as scored by dissecting microscope.
+b Non-Vul, but sterile. ~90% of these had essentially normal vulva morphology. In the others, the vulva was everted.
+c Full genotype: q388 unc-29(e1072)/dxDf1. Generated by crossing dxDf1/unc-29(e1072) males with q388 unc-29(e1072) hermaphrodites. Essentially the same results were obtained when q388 was tested over three other deficiencies, ndf23, ndf24 and ndf25.
+d Generated by selfing hermaphrodites of genotype q388/q388; ndf4[gon-2(+)]. 75% of the progeny are expected to carry the duplication.
+e Presumed genotype q388/q388; ndf4[gon-2(+)]. Thirty-three of 266 were Egl, but non-Vul. ndf4 causes an incompletely penetrant Egl phenotype (ElliS and Horovitz 1991).
+f Sterile, non-Vul; presumed genotype: q388.
+g Full genotype: fog-1(q253) q362 unc-75(e950)/q388. Generated by crossing q388 males with fog-1(q253) q362 unc-75(e950) females.
+h Generated by crossing dxDf1/unc-29(e1072) males with q362 unc-75(e950) hermaphrodites.
+i Full genotype: gon-2(dx12,22 or 23) fer-1(hc1) unc-29(e1072).
+j Wild type indicates the presence of a normal vulva, a reflected gonad, and the production of sperm and oocytes. Fertility was not directly scorable in animals homozygous for fer-1(hc1) that were raised at 25°, because they produce defective sperm.
+k Full genotype: gon-2(dx12,22 or 23) fer-1(hc1) unc-29(e1072)/q388. Generated by crossing q388 males with hermaphrodites of genotype gon-2(dx12,22 or 23) fer-1(hc1) unc-29(e1072).
+l Full genotype: gon-2(dx12,22 or 23) fer-1(hc1) unc-29(e1072)/dxDf1. Generated by crossing dxDf1/unc-29(e1072) males with hermaphrodites of genotype gon-2(dx12,22 or 23) fer-1(hc1) unc-29(e1072). For q388, we found that the fraction of non-Gon animals begins to increase before fertilization, reaches half maximum by the 12-cell stage and peaks by the 70-cell stage (Figure 4A). Since q388 is likely to be temperature sensitive for synthesis, this suggests that q388 affects some aspect of expression that begins before fertilization and extends into embryogenesis. In the absence of molecular data, it is not possible to determine which step(s) in gene product synthesis are affected by q388; however, these results do indicate that certain scenarios are unlikely (see DISCUSSION).

For q388, we found that the fraction of non-Gon animals begins to increase before fertilization, reaches half maximum by the 12-cell stage and peaks by the 70-cell stage (Figure 4A). Since q388 is likely to be temperature sensitive for synthesis, this suggests that q388 affects some aspect of expression that begins before fertilization and extends into embryogenesis. In the absence of molecular data, it is not possible to determine which step(s) in gene product synthesis are affected by q388; however, these results do indicate that certain scenarios are unlikely (see DISCUSSION).
TABLE 3

Effects of starvation on penetrance of gon-2 alleles

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Temperature (°C)</th>
<th>Starvation</th>
<th>% Gon</th>
<th>% Wild type</th>
<th>% Other</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>q362</td>
<td>15</td>
<td>—</td>
<td>0.25</td>
<td>99.5</td>
<td>0.25</td>
<td>2718</td>
</tr>
<tr>
<td>q362</td>
<td>15</td>
<td>L1s</td>
<td>26</td>
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<td>6</td>
<td>117</td>
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<tr>
<td>q362</td>
<td>20</td>
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<td>20</td>
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<td>66</td>
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<td>25.6</td>
<td>674</td>
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<tr>
<td>q362</td>
<td>20</td>
<td>Mothers</td>
<td>76.8</td>
<td>14.8</td>
<td>8.4</td>
<td>122</td>
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<tr>
<td>q362</td>
<td>20</td>
<td>Mothers and L1s</td>
<td>60</td>
<td>20</td>
<td>20</td>
<td>83</td>
</tr>
<tr>
<td>q362 unc-75(e950)</td>
<td>20</td>
<td>—</td>
<td>4.6</td>
<td>93</td>
<td>2.4</td>
<td>565</td>
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<tr>
<td>q362 unc-75(e950)</td>
<td>20</td>
<td>L1s</td>
<td>63</td>
<td>24</td>
<td>13</td>
<td>209</td>
</tr>
<tr>
<td>smg-2(r863) q362 unc-75(e950)</td>
<td>20</td>
<td>L1s</td>
<td>4.4</td>
<td>91</td>
<td>4.6</td>
<td>204</td>
</tr>
<tr>
<td>q388</td>
<td>20</td>
<td>—</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>&gt;200*</td>
</tr>
<tr>
<td>q388</td>
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<td>L1s</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>1372*</td>
</tr>
<tr>
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<td>25</td>
<td>—</td>
<td>95</td>
<td>0</td>
<td>5</td>
<td>1151</td>
</tr>
<tr>
<td>smg-2(r863) q388</td>
<td>25</td>
<td>—</td>
<td>90</td>
<td>1</td>
<td>0</td>
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<td>15</td>
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<td>L1s</td>
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<td>100</td>
<td>0</td>
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<td>419</td>
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<tr>
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<td>L1s</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>106</td>
</tr>
</tbody>
</table>

All experiments were done by selfing homozygous stocks at the indicated temperature. The dx12, dx22 and dx23 stocks were also homozygous for fer-1(hc1) and unc-29(e1072). Starvation was performed as described in METHODS.

*In the course of stock maintenance, we have observed that q388 homozygous stocks sometimes contain Gon animals at low frequency when raised at 20°.

For dx12, we found that the fraction of non-Gon animals produced after upshift begins to increase before hatching, and reaches its maximum level by the L2/L3 molt (Figure 4B). This suggests that the major function of gon-2 may be completed by the end of L2. However, since dx12 is not fully penetrant, it may be that later stages of gonadogenesis are able to proceed relatively normally in this mutant background. As with q388, gonad development appears to be partially rescued among animals downshifted at the L2/L3 molt.

Maternal expression of q388: We investigated whether the q388 mutation prevents synthesis of maternally contributed gon-2 gene product (either mRNA or protein). The strategy employed hinges on the starvation sensitivity of q362. Since q388 is not starvation sensitive (Table 3), one would predict that animals of genotype q362 that are derived from hermaphrodites of genotype q362/q388 should be relatively resistant to starvation, due to maternal contribution from the q388 allele. However, if synthesis of maternal gon-2 activity derived from the q388 allele is temperature sensitive, then incubation of the mother at 25° should prevent maternal rescue of the Gon phenotype among the q362 unc-29 progeny. Our results indicate that q388 is able to provide nearly complete maternal rescuing activity when oogenesis takes place at 15°, but provides no activity when oogenesis takes place at 25° (Table 4).

Zygotic expression of q388: We performed the following temperature shift experiment to determine the time of onset of zygotic expression of gon-2. Embryos of genotype q362/q388 were generated at permissive temperature (by crossing males of genotype q388 with females of genotype fog-1(q253) gon-2(q362) unc-75(e950)) and then shifted to restrictive temperature at various timepoints. Starvation is not necessary in this experiment, because the penetrance of the Gon phenotype among q362/q388 animals is relatively high even in the presence of abundant food (Table 2). The percentage of non-Gon animals is not expected to begin increasing until after the q388 allele begins to be expressed. As can be seen in Figure 5, zygotic expression of q388 begins early in embryogenesis, before the 30-cell stage, and hence before the birth of Z1 and Z4 (which occurs at approximately the 200-cell stage; Sulston et al. 1983).

Anatomical defects: Mutations in gon-2 result in a range of defects in gonadogenesis. In the most extreme cases, none of the gonadal precursors divide. In slightly less severe cases, where one or two somatic divisions take place, the germline proliferates, but usually produces fewer than 50 germ cells; since no anchor cell is generated, these animals are vulvaless (Vul). By the dissecting microscope, representatives of both of these categories would be scored as having the Gon (gon-2) phenotype. In partial-mutant animals (listed under "Other" category in Tables 1–3), a reflexed gonad is formed that has representatives of most or all somatic gonad tissues; vulval induction usually takes place, but vulval morphogenesis is abnormal; some of these animals are fertile and some are not. The presence of gonadal reflex and vulval induction indicate that the distal tip cells and anchor cell, respectively, have been generated. Therefore, the initial set of gonadal divisions (which occurs during L1
in wild type) is likely to have occurred relatively normally. We did not directly follow gonadal sublineages in animals where partial gonads were formed. However, inspection of these animals as adults did not reveal the consistent absence of any particular gonadal tissue (A. Y. Sun and E. J. Lambie, unpublished results), so we consider it unlikely that gon-2 acts within a subset of the descendants of Z1 and Z4.

Cell lineage analysis: To directly determine the effects of gon-2 mutations on gonadal cell divisions, we followed the cell lineages of Z1 and Z4 in 10 hermaphrodites of genotype q362 (Figure 6). These animals were

FIGURE 4.—Temperature-sensitive periods of gon-2 alleles. Reciprocal temperature shifts were performed as described in MATERIALS AND METHODS. Distances on the X-axis are not directly proportional to elapsed time. Embryonic stages were estimated by examination of representative embryos and by comparison with the embryonic cell lineage (Sulston et al. 1983). Animals were scored and classified as described in Table 2. Values for 2-cell and adult are from Table 2 (constant temperature). (A) TSP of gon-2(q388). The data for the −12 hr timepoint are the pooled results obtained with two methods. In one method, L4 or young adult stage hermaphrodites were shifted from 15° to 25° and allowed to incubate for −12 hr before dissection of embryos; in this case, 2/152 progeny were non-Con. In the other method, the same temperature protocol was followed, but hermaphrodites were simply allowed to lay eggs at 25°; in this case, 14/737 progeny were non-Con. For the approximate fertilization timepoint, adult hermaphrodites were shifted from 15° to 25° and allowed to incubate for 1–2 hr before embryo dissection; this ensures that two-cell stage embryos collected for upshifting were fertilized at 25°. Sample sizes for upshifts/downshifts at each time point were as follows: two-cell (245/53); 12-cell (41/0); 30-cell (40/0); 70-cell (40/52); 280-cell (57/41); 500-cell (68/35); hatch (68/82); L1/L2 (55/102); L2/L3 (0/62); L3/L4 (0/67). B. TSP of gon-2(dx12). Sample sizes for upshifts/downshifts were as follows: hatch (112/93); L1/L2 (189/87); L2/L3 (85/62); L3/L4 (120/67). The full genotype of the strain used is gon-2(dx12) fer-1(hcl) unc-29(e1072).
TABLE 4
Maternal rescuing activity of q388

<table>
<thead>
<tr>
<th>Maternal genotype</th>
<th>Oogenesis temp (°C)</th>
<th>% Gon among Unc progeny</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>q362 unc-29/q362</td>
<td>15</td>
<td>23.8</td>
<td>109</td>
</tr>
<tr>
<td>q362 unc-29/q362</td>
<td>25</td>
<td>20.2</td>
<td>193</td>
</tr>
<tr>
<td>q362 unc-29/q362</td>
<td>15</td>
<td>1.0</td>
<td>174</td>
</tr>
<tr>
<td>q362 unc-29/q388</td>
<td>25</td>
<td>42.0</td>
<td>300</td>
</tr>
</tbody>
</table>

Hermaphrodites were incubated at either 15 or 25° from L4 to adult, then cut to release eggs. Eggs were incubated at 15° on nutrient-free plates, and hatchlings subsequently were starved for 72 hr prior to feeding. The Gon phenotype was scored after animals had reached adulthood. unc-29(e1072) was used as a cis marker to identify q362 homozygotes.

Observed continuously through the L1 and L2 stages, then returned to Petri plates and reexamined after reaching adulthood. In five animals, Z1 and Z4 did not divide through mid/late L2; in two animals, Z1 and Z4 each divided once at mid-L2; in one animal, Z1 divided once in mid-L2, but Z4 did not divide again during the observation period. All eight of these animals developed into adults with very small gonads (≤50 μm in diameter); however, it was often difficult to determine unambiguously whether the somatic cells had divided after the L2, due to variable, low-level proliferation of the germ cells.

In one animal, Z1 and Z4 each divided only once during the L2, but subsequent divisions (not directly observed) resulted in the execution of vulval induction and the formation of a normally reflexed gonad. In the remaining animal, Z1 and Z4 initiated divisions midway through the L1, resulting in a wild-type phenotype. In all cases, the only apparent effect of the gon-2 mutation was a delay in the timing of cell divisions. The orientation of cell division axes and the morphologies of daughter cells were similar to those seen in wild type. Therefore, we suspect that cell fate specifications per se are not altered by mutations in gon-2.

Delayed gonadogenesis in q388 and dx12: The results obtained with q362 suggest that a substantial delay in the onset of division of Z1 and Z4 does not preclude the formation of an approximately normal gonad. To determine whether other alleles of gon-2 reveal the same sort of temporal plasticity, we analyzed animals carrying q388 and dx12. In the case of q388, larvae that had been raised continuously at 25° were inspected by Nomarski at the time of the L2/L3 molt to identify animals in which Z1 and Z4 had failed to divide. These were then shifted down to 15° and allowed to grow to adulthood before scoring. Most of these animals developed into Gon adults, but in 2/19 cases sufficient gonadal divisions occurred to result in the formation of an extended gonad. Both animals were Vul. In one case, ≥200 germ cells were present, but no germogenesis was evident and proximal somatic tissues could not be identified. In the other case, both gonad arms were reflected, ≥500 germ cells were present and both spermatogenesis and oogenesis were successful; spermathecal cells were distinguishable among the proximal somatic tissues, but uterine cells could not be unambiguously identified. During the same experiment, an animal in the L2/L3 molt was identified in which Z1 and Z2 had not divided, but Z3 and Z4 had each divided once. After incubation at permissive temperature, this animal developed into a fertile adult; both gonad arms were fully reflexed, extensive spermathecal and uterine tissues were present and a normal vulva was formed. Sheath cells were also presumably present, but these were not scored. Thus, Z1 can generate the full complement of gonadal tissues, even if it does not begin to divide until after the L2/L3 molt.

In the case of dx12, animals grown at 15° were inspected at the L1/L2 molt, returned to 15°, and then scored after reaching adulthood. This experiment was done at low temperature for two reasons. First, even at 15°, dx12 produces a low percentage of Gon animals, indicating that the level of gon-2 activity is below the threshold required for normal development. Second, upshift experiments with dx12 (Figure 4B) indicated that the TSP for anchor cell formation is not complete until after the L1/L2 molt, suggesting that initial gonadal divisions are delayed relative to wild type. We...
found that the majority (10/12) of dx12 animals inspected at the L1/L2 molt had only four cells in gonad. Since only two of these developed into vulvalless adults, we conclude that dx12 causes a delay in the onset of gonadal divisions.

pes-1::lacZ expression: As an independent method of assessing the temporal specification of Z1 and Z4, we examined the expression of a pes-1::lacZ reporter gene (Hope 1991; Hope 1994) in gon-2 mutant animals. In a wild-type background, pes-1::lacZ is transcribed in Z1 and Z4 in early L1, then downregulated before the onset of gonadal divisions (Hope 1994). pes-1::lacZ is not expressed in any gonadal cells in wild-type adults. In gon-2(q388) mutant animals, we found that pes-1::lacZ continues to be expressed by Z1 and/or Z4 in the L2, L3, L4 and adult stages (Table 5; Figure 7). This is consistent with the idea that gon-2 function is required for Z1 and Z4 to proceed with postembryonic development.

**DISCUSSION**

Several classes of genes have been described that affect the development of the somatic cells of the C. elegans gonad (Hirsch and Vanderslice 1976; Greenwald et al. 1983; Hedgecock et al. 1987; Sternberg and Horvitz 1988; Seydoux et al. 1993). Most of these genes are also known to have important functions in the development of non-gonadal tissues. In this article, we present the characterization of five mutant alleles of gon-2, a gene required specifically for gonadogenesis. All of these alleles are recessive and are likely to be loss-of-function mutations (see RESULTS). In each case, homozygous mutant hermaphrodites produce progeny with severely reduced gonads (the gon phenotype). Our data suggest that gon-2 is required for the initiation and continuation of postembryonic gonadal cell divisions.

**Maternal and zygotic expression of gon-2:** Zygotic expression of a wild-type allele of gon-2 is sufficient to produce a wild-type phenotype among all of the progeny of a homozygous gon-2 mutant mother. Similarly, maternal expression of a wild-type allele of gon-2 (e.g., in a hermaphrodite of genotype gon-2(+)/gon-2(−)) is sufficient to prevent the Gon phenotype among nearly all of the homozygous mutant progeny. Thus, the initial gonadal divisions that generate the anchor cell and the distal tip cells can proceed if either the mother or the zygote carries a wild-type allele. Based on these data, we consider it likely that gon-2 is expressed in the maternal germline and that gon-2 gene product is contributed to the embryo in the form of mRNA and/or protein. Although maternally contributed gon-2 product is usually sufficient to prevent the Gon phenotype, zygotic expression is necessary to prevent hermaphrodite sterility. The role of gon-2 in hermaphrodite fertility is presently unresolved; there are no obvious somatic anatomical defects that correlate with this zygotic sterility. gon-2 could be required for the differentiation of somatic cells required for fertility, or gon-2 activity might be

**TABLE 5**  
Expression of pes-1::lacZ

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Stage scored</th>
<th>Fraction expressing lacZ in somatic gonad*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pes-1::lacZ</td>
<td>L1</td>
<td>93/106</td>
</tr>
<tr>
<td>pes-1::lacZ</td>
<td>Adult</td>
<td>0/40</td>
</tr>
<tr>
<td>q388; pes-1::lacZ</td>
<td>L1</td>
<td>14/22</td>
</tr>
<tr>
<td>q388; pes-1::lacZ</td>
<td>L2-L4</td>
<td>19/149</td>
</tr>
<tr>
<td>q388; pes-1::lacZ</td>
<td>Adult</td>
<td>16/236</td>
</tr>
</tbody>
</table>

The pes-1::lacZ reporter gene was present in the form of a chromosomal insertion, lds1, which was generously provided by Ian Hope. All animals were raised at 25° and stained for β-galactosidase as described by FIRE et al. (1993).

*Expression of pes-1::lacZ was restricted to one or two somatic cells in the gonad, presumably Z1 and/or Z4.

---

**Figure 6.**—Cell lineages of gon-2(q362) mutant animals. Lineaging was done as described in MATERIALS AND METHODS.

**Figure 7.**—Expression of pes-1::lacZ. (A) lds1 L1. (B) gon-2(q388); lds1, adult. Animals were raised at 25°, then fixed and stained as described by Hope (1994). In each case, Z1 and Z4 stain darkly with X-gal.
necessary within the germline to permit complete differentiation of sperm and/or oocytes.

Function of gon-2 after hatching: Results obtained with two alleles, q362 and dx12, suggest that gon-2 function is required after hatching. In the case of q362, starvation of L1s greatly increases the penetrance of the Gon phenotype. Similarly, shifting newly hatched dx12 animals from 15° to 25° results in an increase in the fraction of Gon animals. Both of these results suggest that gon-2 function is required after hatching. A possible caveat here is that these observations were made using mutant alleles. Therefore, it is formally possible that when gon-2 is expressed by a wild-type allele it can complete its function before hatching.

Nature of gon-2 alleles: All five of the gon-2 alleles that we have identified are conditional. However, the basis for conditionality and/or the degree of penetrance is different for each allele (Tables 2 and 3). Therefore, it is not likely that the absence of gon-2 function per se causes a conditional mutant phenotype. The phenotype of the most highly penetrant allele (q388) suggests that a null mutation would probably cause zygotic sterility (Tables 1, 2). Most of our characterization has involved three alleles, q362, dx12 and q388, that are discussed below.

q362: The penetrance of the Gon phenotype caused by q362 is greatly increased by starvation. Since this enhancement is suppressed by the smg-2(r363) mutation (which disrupts the degradation of defective mRNAs), the main effect of nutritional stress might be the destabilization of gon-2(q362) mRNA. In combination with the fact that starvation of a homozygous q362 hermaphrodite during oogenesis greatly increases the penetrance of the Gon phenotype among her progeny, this suggests that the maternally contributed form of gon-2 is probably mRNA. Effects of starvation on mRNA levels have not been widely investigated; however ANDERSSON et al. (1991) have reported that steady-state albumin mRNA levels are reduced in mice that have been deprived of food.

dx12: Analysis of the TSP of dx12 suggests that this allele could code for a protein whose activity or stability is diminished at restrictive temperature. However, even at permissive temperature, the level of gon-2 activity provided by dx12 is slightly below the threshold required for prevention of the Gon phenotype (see RESULTS). Therefore, dx12 could also conceivably act by perturbing the synthesis of gon-2 mRNA and/or protein.

q388: q388 homozygotes that have been shifted to restrictive temperature at the time of hatching do not express the Gon phenotype. This suggests that difference interferes with some synthetic step and does not render the gon-2 gene product thermolabile. Therefore, upshift experiments with q388 can be expected to provide a measure of when the temperature-sensitive stage of gon-2 synthesis has been completed. Our results indicate that thermal-resistant gon-2(q388) product (mRNA?) begins to be synthesized before fertilization, and by the 72-cell stage has reached sufficiently high levels to support at least the initial stages of gonadogenesis.

Synthetic steps that could possibly be affected by q388 include transcription, mRNA processing, mRNA degradation, translation and posttranslational modification. In the absence of molecular data, none of these can be unequivocally ruled out. However, certain scenarios can be excluded. For example, if maternal gon-2 is contributed exclusively in the form of mRNA (a likely possibility), then q388 cannot specifically affect translation, because the TSP for q388 begins before fertilization, and because q388 does not provide maternal rescuing activity when oogenesis takes place at 25°.

Early onset of zygotic gon-2 expression: Since our data indicate that gon-2(q388) is temperature sensitive for synthesis, the TSP measured for animals of genotype q388/q362 (where q388 is contributed paternally) should reflect the onset of zygotic transcription of gon-2. Through this assay, we found that expression of gon-2(q388) begins during the early embryonic cleavages, before the 30-cell stage of development.

Cellular basis for the Gon phenotype: The cell lineage data and pes-1::lacZ reporter results clearly indicate that gon-2 activity is required for at least the first two rounds of division of Z1 and Z4. Since mutations in gon-2 also cause heterogeneous defects in the organization and structure of the gonad (A. Y. SUN and E. J. LAMBIE, unpublished results), it is likely that gon-2 function is also required for subsequent division and or differentiation of the descendants of Z1 and Z4. We do not know whether gon-2 function is required within the germline; however, defects in the Z1 and Z4 lineages appear to be sufficient to explain all of the phenotypes seen in gon-2 mutant animals. Mosaic analysis will be necessary to resolve this issue unambiguously.

Tissue specificity of mutations in gon-2: Although mutations in gon-2 have profound effects on gonadal divisions, no other tissues are directly affected. While the simplest explanation is that gon-2 functions only in gonad development, it is also possible that gon-2 acts in multiple tissues, but its function is redundant in nongonadal tissues. Moreover, since we have not yet obtained a null allele of gon-2, it is possible that a complete loss of gene function will cause effects outside of the gonad.

Possible functions of gon-2: The dependence of gonadogenesis on gon-2 activity indicates that gon-2 acts in some way to positively regulate gonadal cell divisions. However, the genetic data provide no solid clues regarding the specific biochemical function of the gon-2 gene product. gon-2 could encode an organ-specific isofrom of a protein that is generally required for cell growth and division, or it could encode a component of a signal transduction pathway that specifically triggers the onset of postembryonic development in the gonad. In the latter case, gon-2 could act either within the gonadal precursors themselves or within the tissue(s) that trig-
ger postembryonic divisions in response to the availability of food.

One possibility is that gon-2 acts by negatively regulating the expression of the pes-1 transcription factor, which could itself act as a repressor of genes required for gonadal cell divisions. We should soon be able address these issues directly, since we have obtained transformation rescue of gon-2 using a cosmid clone that has been sequenced as part of the C. elegans genome project (COULSON et al. 1995; A.Y. SUN and E.J. LAMBIE, unpublished results).

Independence of gonadal and non-gonadal development: Analyses of the C. elegans heterochronic mutants have established that the events of gonadogenesis occur in a normal sequence even when the development of non-gonadal tissues is precocious or retarded (AMBROS and HORVITZ 1984). Our data complement these results, demonstrating that gonad development can proceed relatively normally, even if its onset is delayed as late as the L2/L3 molt. These results fit together well with Félix and STERNBERG’s (1996) observation that the onset of gonadal cell divisions is normally delayed until the second larval stage in certain relatives of C. elegans.

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LITERATURE CITED


HOPE, I. A., 1994 PES-1 is expressed during early embryogenesis in Caenorhabditis elegans and has homology to the fork head family of transcription factors. Development 120: 505–514.


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