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 hermaphrodite 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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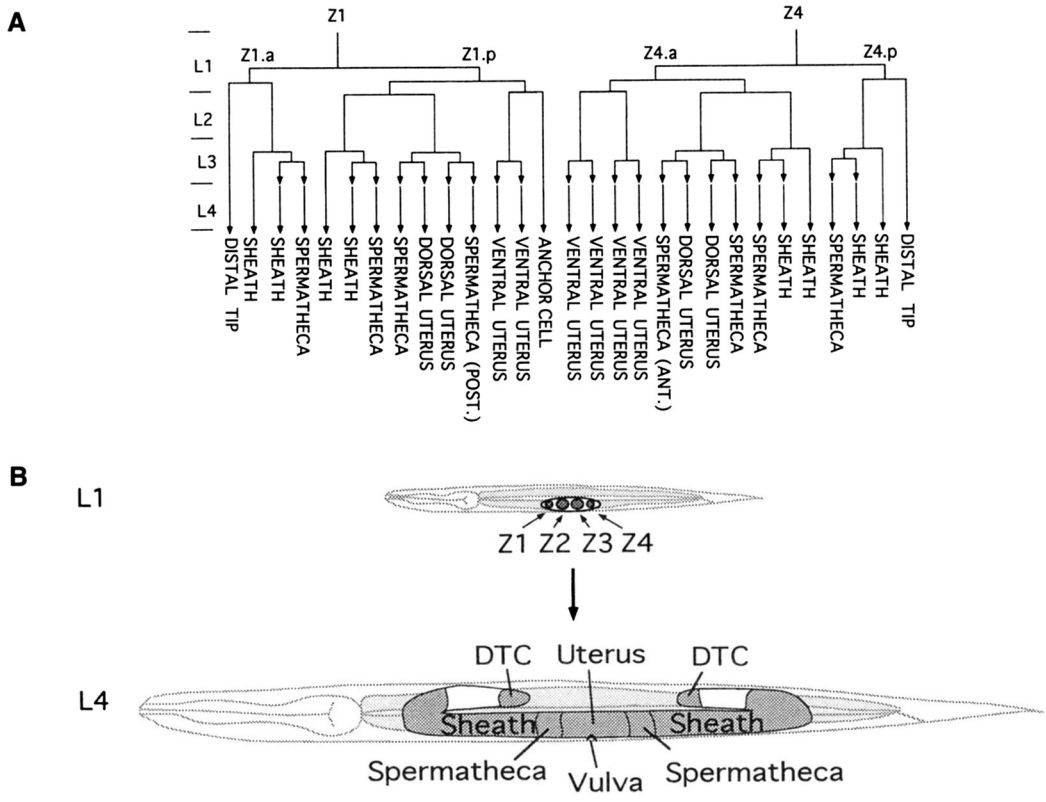

FIGURE 1.—Overview of hermaphrodite gonad development. (A) Z1 and Z4 each produce six descendants by the beginning of L2. Z1.ppp and Z4.aaa are members of an equivalence group; one of the two cells will become an anchor cell (AC) and the other will contribute 10 descendants to the ventral uterus. The primary derivatives of each cell present at the beginning of L2 are indicated. All cells except the AC and the distal tip cells undergo additional rounds of division, beginning in the early L3.

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 post-embryonic divisions in response to food. However, the stage-specific patterns of division of the somatic gonadal cells are unaffected by mutations in heterochronic genes such as *lin-14* and *lin-28* (AMBROS and HORVITZ 1984), which regulate developmental timing within non-gonadal tissues. This suggests that a separate regulatory program operates within gonadal tissues to control stage-specific division patterns.

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 KH_2PO_4, 0.5 g/liter K_2HPO_4, 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. (1988), unless indicated otherwise]: *smg-2(r863)* (HODGKIN *et al.* 1989), *fog-1(q253)* (BARTON and KIMBLE 1990), *dpy-5(e61); unc-13(e51), lin-10(e1439), ceh-6(mg60)* (T. BURGLIN and G. RUVKUN, personal communication), *fer-1(hc1), unc-29(e1072); nDf23, nDf24, nDf25, nDp4.*

Isolation of *gon-2* alleles: *q362* and *q388* were identified during the course of F_1 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 F_3 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° (to render them self-sterile) and then mutagenized with 75 mM EMS for 4 hr (at room temperature). Groups of approximately five mutagenized animals were then mated (at 15°) with ~20 males of genotype *gon-2(q388),* which had been raised at 15°. Adults were transferred to new plates every 12 hr to promote developmental synchrony among the eggs laid on each plate. Soon after the F_1 progeny reached adulthood (after 7 days at 15°), 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°, each plate was shifted to 25° for 48 hr, then returned to 15°. This temperature regimen rescues the sterility of *q388/** 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° 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 ~3000 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/m^2/sec 310 nm UV light for 10 sec, then mated with hermaphrodites of genotype *gon-2(q388) unc-29(e1072)* at 15°. Approximately 1000 non-Unc F_1 hermaphrodites were cloned at 15°, allowed to lay eggs for 48 hr, then transferred to new plates at 25°. *dxDf1* was recovered from the 15° replica of a clone that segregated Gon F_2s at 25°.

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° 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° 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; ISNENGHI *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° or 20° until L4, then shifted either to 15° (for subsequent upshifts) or 25°(for subsequent downshifts) until reaching adulthood. For embryos of genotype *fog-1(q253) gon-2(q362) unc-75(e950)gon-2(q388),* females of genotype *fog-1(q253) gon-2(q362) unc-75(e950)* were mated with *gon-2(q388)* males at 15° (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° or 25°, 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. Mothers 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 SULSTON (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°, 20° or 25°. Starved L1 larvae were then transferred to seeded plates and incubated for ~12 hr at 20° or 25° before being mounted for cell lineage analysis. All animals were maintained at 25° during the experiment. At the L2/L3 molt, animals whose cell lineages had been followed were returned to plates, incubated at 25°, 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 F_1 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 F_3 progeny. In this article, we use the term "Gon" to refer to vulvaless 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-*

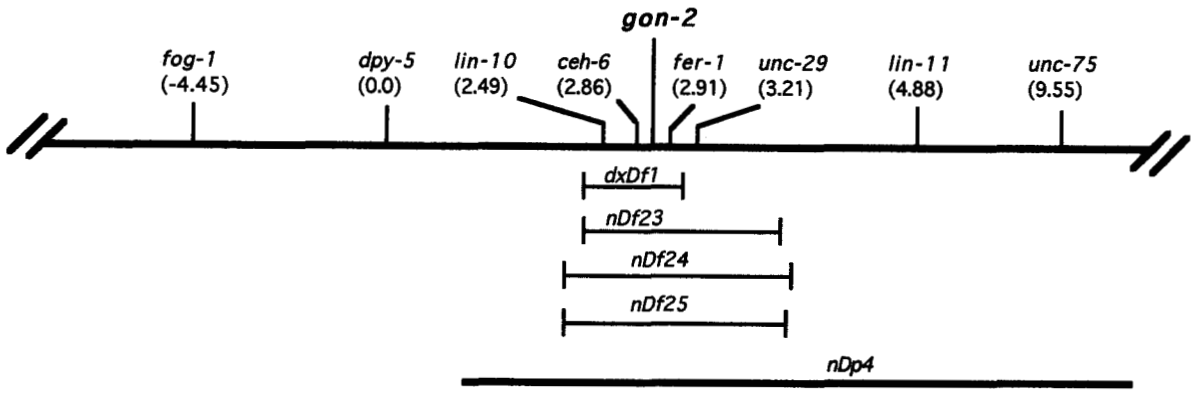

FIGURE 2.—Position of *gon-2* on the genetic map of chromosome I. Locations of genetic markers and rearrangement endpoints in the vicinity of *gon-2* are based on the genetic map assembled by J. HODGKIN, R. DURBIN and S. MARTINELLI (personal communication). Map units, in cM, are indicated within parentheses.

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

TABLE 1

Partial maternal effect of *gon-2(q388)*

	Genotype						
	Zygotic	Maternal	Paternal	% Gon[a]	% Wild type	% Other	*n*
A.	*gon-2(q388)*[b]	*q388*	*q388*	98.6	0.3	1.1[c]	739
Conclusion: The Gon phenotype is highly penetrant in the absence of maternal and paternal expression.							
B.	*gon-2(q388)*[d]	*q388/+*	*q388/+*	0.3	0	99.7[e]	347
Conclusions: Zygotic expression is not usually necessary to prevent the Gon phenotype. Zygotic expression is necessary for hermaphrodite fertility.							
C.	*q388* and *q388/+*[f]	*q388*	*q388/+*	50	48	2[c]	1304
Conclusions: Zygotic expression is usually (or always) sufficient to prevent the Gon phenotype. Paternal expression is not sufficient to prevent the Gon phenotype.							
D.	*q388/+*[g]	*q388*	+/+	0	100	0	437
Conclusions: Maternal expression is not necessary to prevent the Gon phenotype. Zygotic expression is sufficient to prevent the Gon phenotype and to prevent sterility.							
E.	*q388* and *q388/+*[h]	*q388/+*	*q388*	3	57	40[e]	130
Conclusions: Maternal expression is usually sufficient to prevent the Gon phenotype. Paternal expression is not necessary 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.

[a] Defined as vulvaless (Vul) animals with a gonad ≤50 μm in diameter, as scored by dissecting microscope.

[b] Obtained by selfing homozygous *q388 unc-29(e1072)* hermaphrodites.

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

[d] Obtained by selfing *q388 unc-29(e1072)/++* hermaphrodites.

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

[f] 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 F_1.

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

[h] Obtained by crossing purged *q388 unc-29(e1072)/++* hermaphrodites with *q388* males.

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 *q362* homozygotes that are derived from mothers 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].

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.

Fortuitously, three alleles of *gon-2*, *q388*, *dx12*, and *dx22*, are temperature sensitive and have relatively high penetrance. As a simple way of characterizing each allele, we shifted animals of genotype *q388*, *dx12* and *dx22* from permissive to restrictive temperature immediately after hatching. A mutation that is temperature sensitive

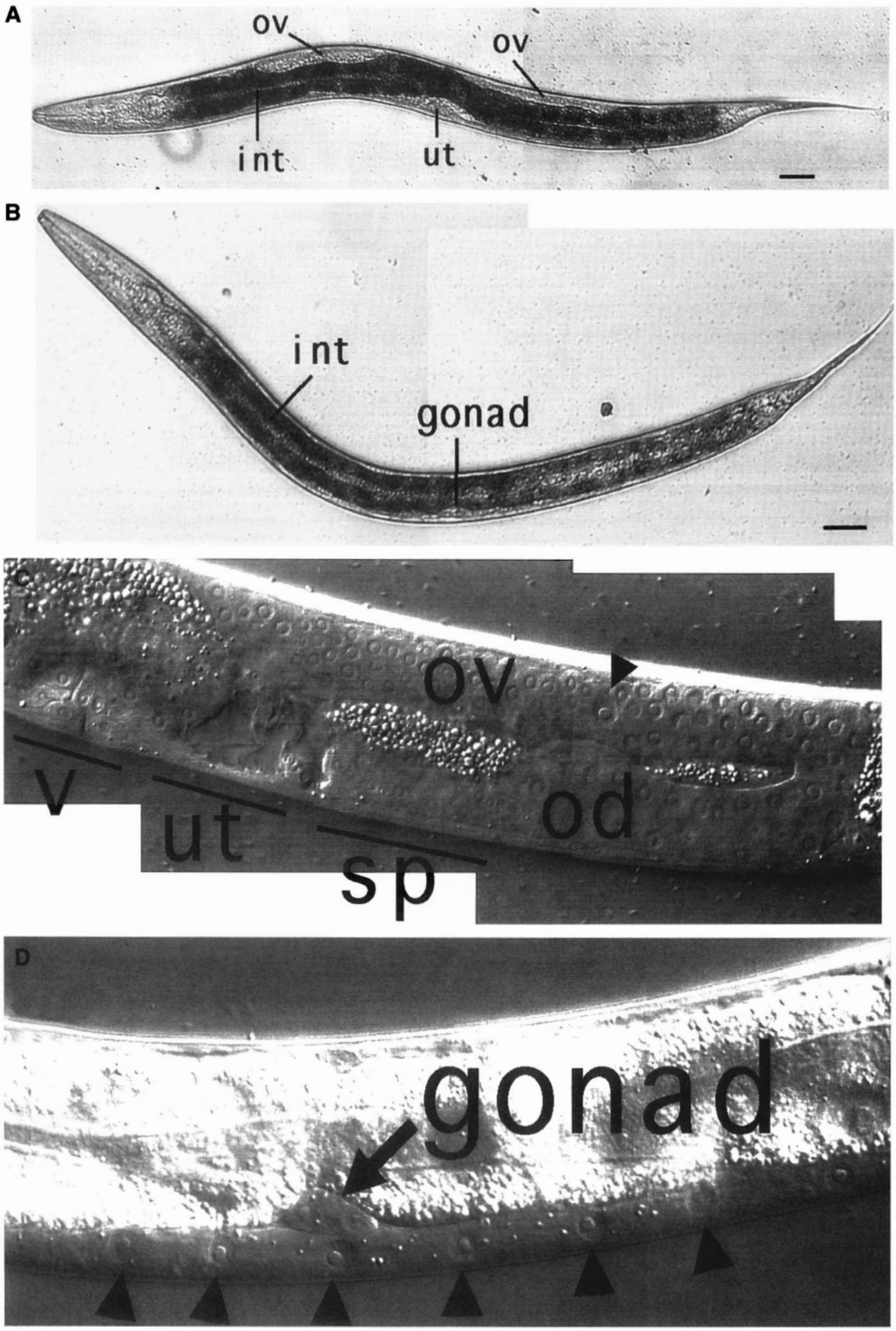

FIGURE 3.—Comparison of wild-type and *gon-2* L4 larvae. (A) Wild type, low magnification; the dark intestine is displaced by the developing gonad. Bar, 50 μm. (B) *gon-2(q388),* low magnification; in the absence of gonadal development, the intestine fills the body cavity. Bar, 50 μm. (C) Wild type, Nomarski DIC; same animal as in A. Arrowhead, distal sheath cell nucleus. (D) *gon-2(q388),* Nomarski DIC; same animal as in B. Arrowheads, nuclei of descendants of P5.p, P6.p and P7.p, which would give rise to the vulva in a wild-type hermaphrodite. only a rudimentary gonad is present. OV, ovary; OD, oviduct; SP, spermatheca; UT, uterus; V, vulva. In all panels, anterior is to the left, dorsal is up. See MATERIALS AND METHODS for imaging procedures.

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

Genotype	Temperature (°)	% Gon[a]	% Wild type	% Other[b]	*n*
q388	15	0	100	0	1009
q388	25	95	0	5	889
q388/Df[c]	25	97	0	3	106
q388/q388/+ and *q388/q388*[d]	25	0	72[e]	28[f]	367
q362	15	0.25	99.5	0.25	2718
q362	20	0.2	99.4	0.4	2124
q362	25	7	87	6	1704
q362/q388[g]	25	90	4	6	191
q362/Df and *q362/+*[h]	25	44	53	3	97
dx12[i]	15	4	90	6	380
dx12[i]	25	38	18[j]	44	270
dx12/q388[k]	25	81	3	16	143
dx12/Df[m]	25	82	2[j]	16	104
dx22[i]	15	0	100	0	430
dx22[i]	25	69	15[j]	16	404
dx22/q388[k]	25	81	1	18	217
dx22/Df[m]	25	85	2[j]	13	60
dx23[i]	15	0	100	0	419
dx23[i]	25	5	91[j]	4	351
dx23/q388[k]	25	40	46	14	168
dx23/Df[l]	25	34	40[j]	26	68

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; nDp4*[*gon-2(+)*]. 75% of the progeny are expected to carry the duplication.

[e] Presumed genotype *q388/q388;nDp4[gon-2(+)]*. Thirty-three of 266 were Egl, but non-Vul. *nDp4* 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 reflexed 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(e1029)*.

[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(e1029)*.

(HIRSH and VANDERSLICE 1976; ISNENGHI *et al.* 1983). For each timepoint, we determined the fraction of animals expressing a weak mutant phenotype (partial mutant; see below, under *Anatomical defects*), a strong mutant phenotype (Gon), and no mutant phenotype (WT). We use the term "non-Gon" to refer to animals that exhibit either a WT phenotype *or* a partial-mutant phenotype. This category is useful, because the percentage of non-Gon animals is expected to be the best indicator of low levels of *gon-2* gene activity, and therefore it should provide the most accurate measurement of the beginning of the TSP in upshift experiments and the end of the TSP in downshift experiments. The production of WT animals should require a relatively high level of *gon-2* activity, and so should provide the most sensitive measure of the beginning of the TSP in downshift experiments.

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).

In the reciprocal experiment, we found that *q388* animals downshifted as late as the L2/L3 molt exhibit significant levels of gonad development. This suggests that gonadogenesis may be able to proceed at least partially even if it is initiated during a later larval stage than usual (see below for further consideration of this issue).

TABLE 3

Effects of starvation on penetrance of *gon-2* alleles

Genotype	Temperature (°)	Starvation	% Gon	% Wild type	% Other	*n*
q362	15	—	0.25	99.5	0.25	2718
q362	15	L1s	26	68	6	117
q362	20	—	0.2	99.4	0.4	2124
q362	20	L1s	66	8.4	25.6	674
q362	20	Mothers	76.8	14.8	8.4	122
q362	20	Mothers and L1s	60	20	20	83
q362 unc-75(e950)	20	—	4.6	93	2.4	565
q362 unc-75(e950)	20	L1s	63	24	13	209
smg-2(r863) q362 unc-75(e950)	20	L1s	4.4	91	4.6	204
q388	20	—	0	100	0	1372[a]
q388	20	L1s	0	100	0	>200[a]
q388	25	—	95	0	5	1151
smg-2(r863) q388	25	—	90	0	10	623
dx12	15	—	4.2	90	5.8	380
dx12	15	L1s	5	95	0	142
dx22	15	—	0	100	0	430
dx22	15	L1s	1	95	4	230
dx23	15	—	0	100	0	419
dx23	15	L1s	0	100	0	106

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.

[a] 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 adless) 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

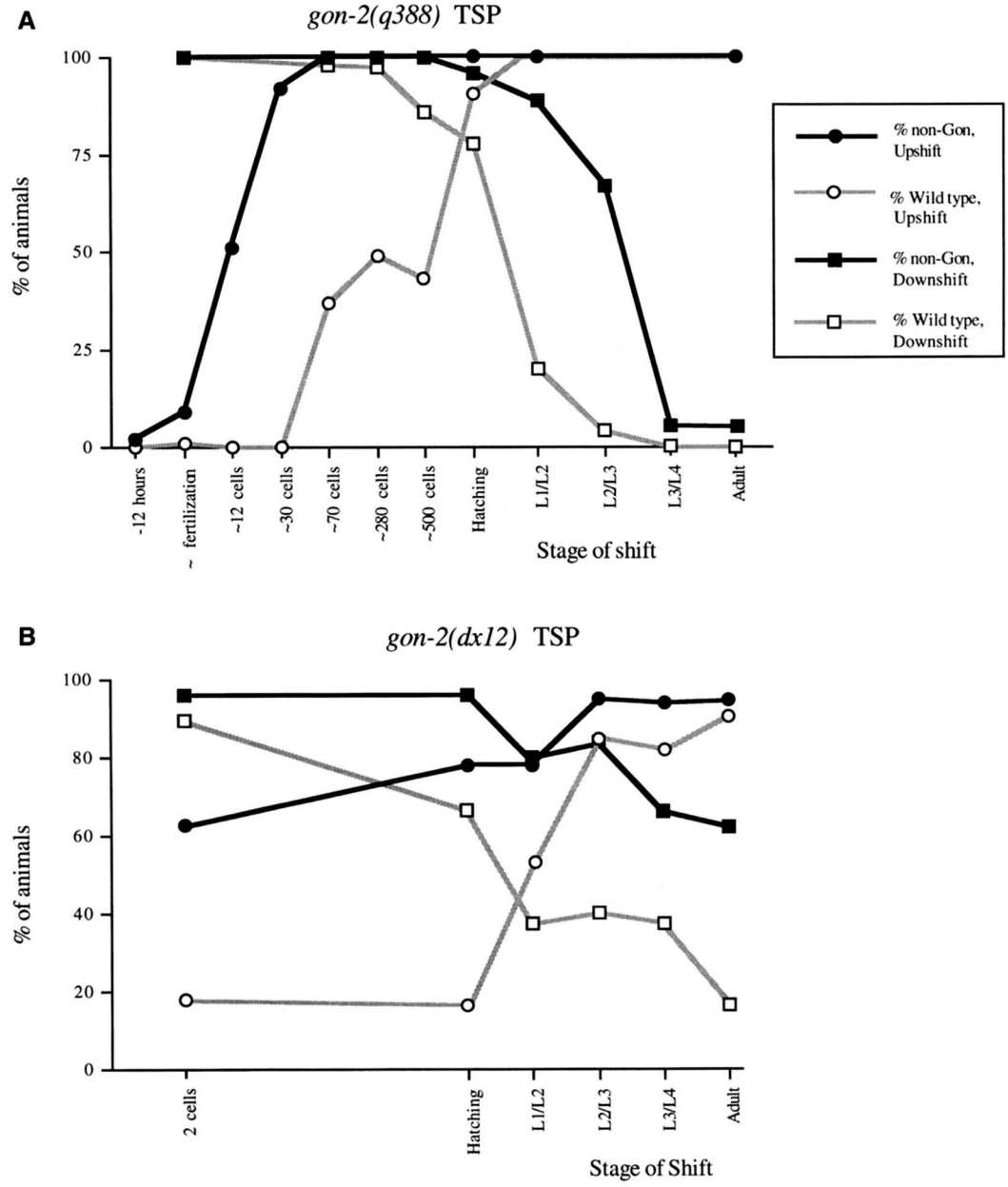

FIGURE 4.—Temperature-sensitive periods of *gon-2* alleles. Reciprocal temperature shifts were performed as described in MATERIALS AND METHODS. Distances on the *X*-axis are not directly proportional to elapsed time. Embryonic stages were estimated by examination of representative embryos and by comparison 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-Gon. 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-Gon. 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(hc1) unc-29(e1072)*.

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

TABLE 4

Maternal rescuing activity of *q388*

Maternal genotype	Oogenesis temp (°)	% Gon among Unc progeny	*n*
q362 unc-29/q362	15	23.8	109
q362 unc-29/q362	25	20.2	193
q362 unc-29/q388	15	1.0	174
q362 unc-29/q388	25	42.0	300

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 gametogenesis was evident and proximal somatic tissues could not be identified. In the other case, both gonad arms were

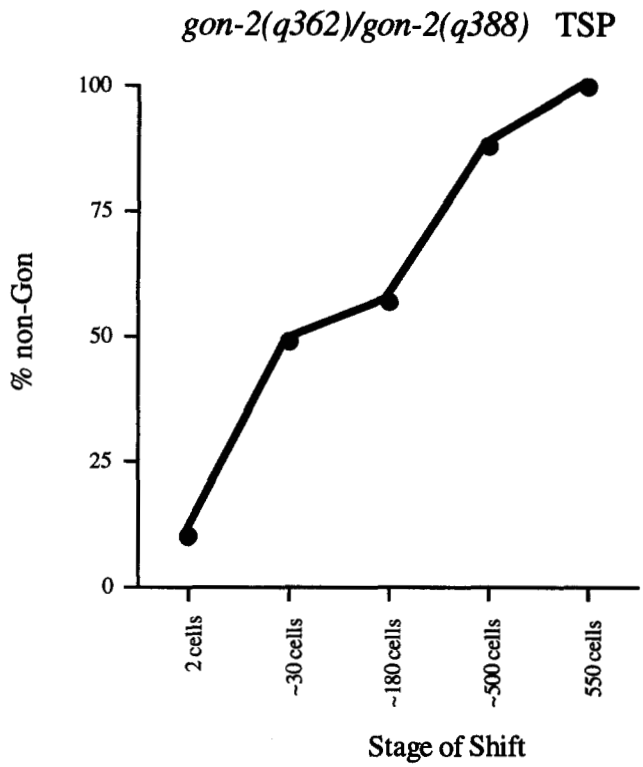

FIGURE 5.—TSP of *gon-2(q388)/gon-2(q362)*. Reciprocal temperature shifts were performed as described in MATERIALS AND METHODS. Distances on the *X*-axis are not directly proportional to elapsed time. Animals were scored and classified as described in Table 2. Sample sizes were as follows: two-cell (191; data from Table 2); 30-cell (47); 180-cell (14); 500-cell (26); 550 cell (31). The full genotype of the animals shifted is *gon-2(q388)/fog-1(q253) q362 unc-75(e950)*.

reflexed, ~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

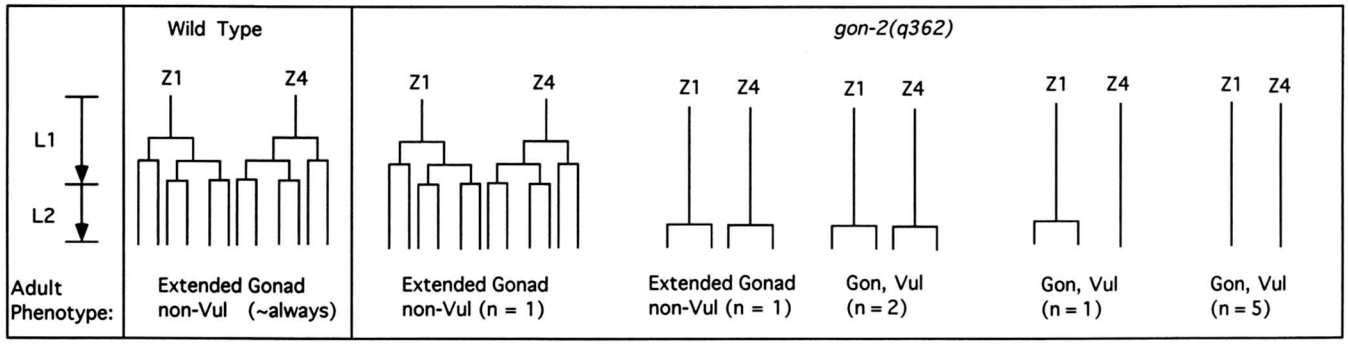

FIGURE 6.—Cell lineages of *gon-2(q362)* mutant animals. Lineaging was done as described in MATERIALS AND METHODS.

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 vulvaless 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 (HIRSH 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.

TABLE 5

Expression of *pes-1::lacZ*

Genotype	Stage scored	Fraction expressing *lacZ* in somatic gonad[a]
pes-1::lacZ	L1	93/106
pes-1::lacZ	Adult	0/40
q388; pes-1::lacZ	L1	14/22
q388; pes-1::lacZ	L2-L4	19/149
q388; pes-1::lacZ	Adult	16/236

The *pes-1::lacZ* reporter gene was present in the form of a chromosomal insertion, *leIs1*, 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).

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

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

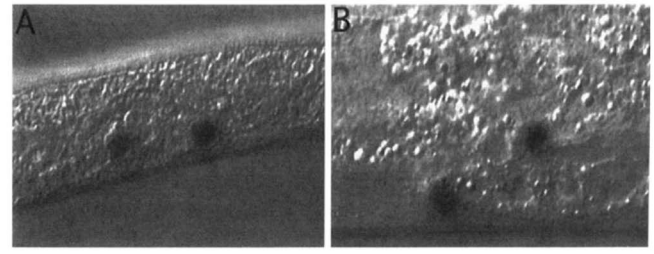

FIGURE 7.—Expression of *pes-1::lacZ*. (A) *leIs1* L1. (B) *gon-2(q388); leIs1*, adult. Animals were raised at 25°, then fixed and stained as described by HOPE (1994). In each case, Z1 and Z4 stain darkly with X-gal.

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(r863)* 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 *q388* 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 isoform 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 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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