fog-1, a Regulatory Gene Required for Specification of Spermatogenesis in the Germ Line of *Caenorhabditis elegans*

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ABSTRACT

In wild-type *Caenorhabditis elegans*, the *XO* male germ line makes only sperm and the *XX* hermaphrodite germ line makes sperm and then oocytes. In contrast, the germ line of either a male or a hermaphrodite carrying a mutation of the *fog-1* (*f*eminization *o*f the *g*erm line) locus is sexually transformed: cells that would normally make sperm differentiate as oocytes. However, the somatic tissues of *fog-1* mutants remain unaffected. All *fog-1* alleles identified confer the same phenotype. The *fog-1* mutations appear to reduce *fog-1* function, indicating that the wild-type *fog-1* product is required for specification of a germ cell as a spermatocyte. Two lines of evidence indicate that a germ cell is determined for sex at about the same time that it enters meiosis. These include the *fog-1* temperature sensitive period, which coincides in each sex with first entry into meiosis, and the phenotype of a *fog-1*; *glp-1* double mutant. Experiments with double mutants show that *fog-1* is epistatic to mutations in all other sex-determining genes tested. These results lead to the conclusion that *fog-1* acts at the same level as the *fem* genes at the end of the sex determination pathway to specify germ cells as sperm.

IN *Caenorhabditis elegans*, *XX* diploid animals are hermaphrodite (essentially a somatic female that produces first sperm and then oocytes); *XO* animals are male. The genetic mechanism by which these two sexes are determined is reasonably well understood. The ratio of *X* chromosomes to sets of autosomes initially determines the sexual phenotype (MADL and HERMAN, 1979). This ratio is then interpreted by a small number of regulatory genes to specify the sex of the animal [see VILLENEUVE and MEYER (1989) for review]. For example, three *fem* genes are required to direct the male fate throughout the animal; loss-of-function mutations in any one of these *fem* genes lead to the transformation of both *XX* and *XO* animals to females (spermless hermaphrodites). Because all the major tissues (hypodermis, nerve, muscle, gut, somatic gonad and germ line) are sexually differentiated [see HODGKIN (1987a) for a review], these genes affecting the sex of the entire animal are thought to act globally.

It is not understood how genes regulating the sex of the entire animal act to direct one type of sexual differentiation in one tissue (*e.g.*, sperm or oocyte in the germ line) and a second type of sexual differentiation in another tissue (*e.g.*, vas deferens or uterus in the somatic gonad). To identify genes that control the differentiation of a single tissue, we have isolated mutants that alter the decision between spermatogenesis and oogenesis. Here, we describe our characterization of the gene, *fog-1* (for *f*eminization *o*f the *g*erm line). A preliminary report of *fog-1* can be found in DONIACH (1986). In *fog-1* mutants of either sex, germ cells that would normally differentiate as sperm become oocytes instead. Significantly, we see no somatic feminization in *fog-1* mutants. We propose that the wild-type *fog-1* gene encodes a tissue-specific regulatory component that directs spermatogenesis in the germ line.

MATERIALS AND METHODS

Culture and strains: Worms were cultured and mutagenized with ethyl methanesulfonate (EMS) as described (BRENNER 1974), except that EMS was used at a concentration of 0.01 M unless stated otherwise. All experiments were done at 20° unless stated otherwise. Nomenclature follows the guidelines of HORVITZ *et al.* (1979).

Mutations and rearrangements used in this study were: linkage group *I* (LG*I*): *sup-11(n403)*, *ace-2(g202)*, *unc-11(e47)*, *dpy-5(e61)*, *unc-13(e51)*, *sDp2*; LG*II*: *dpy-10(e128)*, *unc-85(e1414)*, *tra-2(e1095)*; LG*III*: *glp-1(q224)*, *tra-1(e1099)*; LG*IV*: *fem-3(q20gf)*, *fem-3(q60gf)*, *fem-3(q66gf)*, *fem-3(q95gf)*, *fem-3(q96gf)*, *dpy-20(e1282)*, *tra-3(e1107)*; LG*V*: *dpy-21(e428)*, *her-1(e1518)*, *him-5(e1490)*; LG*X*: *ace-1(p1000)*, *sup-7(st5)*. A description of these mutations and rearrangements can be found in HODGKIN *et al.* (1988). Note that *ace-1* and *ace-2* as single mutants are non-Unc, but that the *ace-1*; *ace-2* double mutant is Unc.

Isolation of *fog-1* alleles: All *fog-1* mutations isolated were backcrossed at least twice against the wild-type N2 before further analysis to eliminate extraneous mutations. Two *fog-1* alleles, *q155* and *q229*, were isolated after EMS mutagenesis in a general screen for self-sterile mutants (see Table 1) (S. MAPLES and J. KIMBLE, unpublished results). In this screen, referred to as the "brute force screen" below and in Table 1, *fog-1* mutations arose at a frequency of 1/5000 haploid genomes. For comparison, loss-of-function mutations in other genes arose at similar frequencies in this

screen: 1/2500 (*fog-2*), 1/5000 (*fem-1*), 1/10,000 (*fem-3*), 1/5000 (*tra-2*), and 1/5000 (*tra-1*), respectively.

Thirty-two *fog-1* alleles were isolated as dominant suppressors of *fem-3(q95gf* or *q96gf)* (see Table 1). At 15°, homozygous *fem-3(gf)* *XX* hermaphrodites are self-fertile, making both oocytes and sperm; however at 25°, they are self-sterile, making only sperm in an otherwise normal hermaphrodite body (BARTON, SCHEDL and KIMBLE 1987). For most experiments, L4 *fem-3(gf)* hermaphrodites were mutagenized with either EMS or γ-rays (4000 R) and picked 8–10 per Petri dish. Mutagenized animals were grown at 15° for 2 days to ensure fertility and then shifted to 25°. All F_1 progeny were sterile unless a dominant suppressor had been induced. After EMS mutagenesis of *fem-3(q96gf)*, *fog-1* mutations are isolated at a frequency of 1/13,000 haploid genomes. Given that only about half of the animals of genotype *fog-1/+*; *fem-3(gf)* are self-fertile, a lower frequency than that obtained in the brute force screen is expected.

Four alleles were isolated in a noncomplementation screen (Table 1). To do this, *fog-1(q253ts); fem-3(q96gf) dpy-20; him-5*; *ace-1* males were crossed to EMS mutagenized (0.025 M) *ace-2 dpy-5*; *ace-1* hermaphrodites at 15°. Crosses were shifted to 25° after 24–48 hr. F_1 cross progeny L4 *XX* animals were picked away from their brothers to prevent mating and screened the following day for females. Candidate females were crossed to wild-type males. Cross-progeny (F_2) were picked to individual plates (25°). Dpy-5 animals in their self-progeny broods (F_3) were examined by Nomarski optics to determine their phenotype. In no case was there a lack of Dpy animals in these broods, which would have indicated a lethal *fog-1* allele. *fem-3(q96gf)* was included in this screen because it greatly reduces the background of spurious F_1 females. To show that *fem-3(gf)* does not interfere with the isolation of new mutants, *fog-1(q253ts); fem-3(q96gf) dpy-20*; *him-5*; *ace-1* males were crossed to *qDf3/ unc-11 dpy-5* hermaphrodites. Wild-type cross progeny *XX* animals were picked away from their brothers and scored one day later: 10/31 developed as females.

Isolation of a *fog-1* deficiency: A deficiency of *fog-1* was isolated as follows. *unc-11 dpy-5/++* males were crossed into wild-type hermaphrodites that had been mutagenized with gamma-rays (7000 R). After allowing these animals to mate for 24 hr they were transferred to Petri dishes containing 1 mM aldicarb (Chem Service, Inc., Westchester, PA) in agar. Mutations in *unc-11* confer resistance to aldicarb (J. Rand and C. Johnson, personal communication); therefore, only those animals that acquire a lesion in *unc-11* will survive. (*dpy-5* mutants are hypersensitive to aldicarb, even if they are also *unc-11* mutants Therefore, the right end of deletions generated in this way will not extend into or past *dpy-5*.) Four *unc-11* mutations were recovered from approximately 6,000 chromosomes screened. Two were viable *unc-11* mutations with no other phenotype. Two others were lethal. One of the lethal *unc-11* mutations complements *fog-1(q187)* while the other *qDf3*, fails to complement *fog-1(q187)* (Table 2). Other complementation tests indicate that *qDf3* includes *ace-2* but does not include *sup-11* or *dpy-5* (Figure 1). Unc non-Dpy males were observed among the progeny of the cross *qDf3/+* male × *ace-2 dpy-5*; *ace-1*. However, only nonSup females were observed among progeny of cross *qDf3/+* male × *sup-11 fog-1*.

Complementation tests and mapping: All *fog-1* mutations fail to complement the reference allele, *q187*. *ql87* fails to complement *e1959*, an allele of *fog-1* obtained in a screen by DONIACH (1986). Also, all *fog-1* mutations map to LG*I* to the left of *unc-11*. One allele, *q187*, was more closely mapped. From a parent of genotype + *dpy-5 unc-13/fog-1* ++, 9/9 Unc recombinant carried *q187* and 3/3 Dpy recombinants did not carry *q187*. This places *fog-1* to the left of *unc-13*. In addition, from a parent of genotype (*sup-11* + *unc-11/+ fog-1* +, 5/13 Unc recombinants carried the *fog-1* mutation. Additional 3-factor data accumulated from all alleles is consistent with this map position: from parents of genotype *sup-11* + *unc-11/+ fog-1(x)* +, 38/65 Unc recombinants carried *fog-1(x)*. These data place *fog-1* on the map about 60% of the way between *sup-11* and *unc-11* (see Figure 1). From four complete broods of *fog-1(q187) unc-11/++* hermaphrodites raised at 20°, 15 Fog non-Uncs, 17 Unc non-Fogs, and 246 Unc Fogs were counted. This gives a distance of 3.1% between *fog-1* and *unc-11*. *fog-1* was also mapped relative to *ace-2*. 2/18 Unc recombinants from a mother of genotype *ace-2* + *dpy-5/+* fog-1(q187) +; *ace-1* carried the *fog-1* mutations, placing *ace-2* to the left of *fog-1*.

Scoring the *fog-1* phenotype: For epistasis experiments and for determining the phenotype of the reference allele, *q187*, hermaphrodites were scored by Nomarski optics: the Fog phenotype was scored as production of oocytes instead of sperm. For mapping and determining the phenotype of alleles other than *q187*, hermaphrodites were scored by dissecting microscope: the Fog phenotype was scored as production of unfertilized oocytes that stack up in the gonads of females, giving these animals a "striped" appearance in the dissecting microscope.

Males were scored for the Fog phenotype by examining them with Nomarski optics for the presence of oocytes. For males heterozygous for *fog-1*, 10 animals were scored for each allele except *q187*($n > 200$). For males homozygous for *fog-1*, 5–12 males were scored for each allele except *q187*($n = 100$). Fifty *XX* animals were scored for each *fog-1* allele.

Finally, all alleles were examined to make sure they did not cause either sex-specific lethality or a complete sexual transformation of either *XX* animals to male or *XO* animals to female. To do this, *fog-1/+* males were crossed to *fog-1* females; parents were removed from the Petri dish after 12–24 hr and numbers of male and female cross-progeny were counted. In no case did the sex ratio differ significantly from one, indicating that *fog-1* does not cause any sex-specific lethality nor does it transform males into perfect females. The number of sperm made by individual *fog-1/+* males was determined by picking *fog-1(q187) unc-11/++* L4 males to plates without hermaphrodites to prevent mating. By the next day, they had stopped making sperm and started making oocytes. They were then fixed and stained by DAPI (AUSTIN and KIMBLE, 1987). Sperm were visualized by fluorescence optics, and each animal's sperm number was counted twice.

Assaying for the presence of yolk proteins in *fog-1* males: Adult worms were fed *Escherichia coli* grown on ^{35}S-sulfate according to SHARROCK (1983, 1984). Proteins were analyzed by polyacrylamide gel electrophoresis (LAEMMLI 1970) followed by autoradiography of the gel.

Tests for suppression by *sup-7*: Unc *XX* animals segregating from hermaphrodites of genotype *fog-1 unc-11/++*; *sup-7* were examined using Nomarski optics for production of sperm. (For alleles *q372, q379, q380* and *q382, dpy-5* was used as a marker instead of *unc-11*.) *sup-7(st5)* suppresses amber mutations in many genes (WATERSTON 1981), including genes active in the germ line (HODGKIN 1985). Strains were homozygous for *sup-7* if they showed the cold sensitive lethal phenotype of *sup-7* (WATERSTON 1981). In no case was the number of animals that made sperm different from that expected from recombination of *fog-1* away from *unc-11* (*n* ranged from 7 to 53 in these experiments; average $n = 20$). Alleles tested at both 20° and 25° were *q182, q188, q191, q198, q203, q204, q205, q207, q229, q242, q250, q255,*

TABLE 1

fog-1 alleles

Allele	Method of Isolation	Mutagen
q155, q229	Screen for sterile mutants	EMS
q180, q181, q182, q187, q188, q190, q191, q192, q193, q198, q199, q200, q201, q203, q204, q205, q206, q207, q253ts, q254, q255, q256, q258, q259, q261, q262	Dominant suppressors of *fem-3(q96gf)*	EMS
q241, q242, q325	Dominant suppressors of *fem-3(q96gf)*	γ-Ray
q248, q250, q311ts	Dominant suppressors of *fem-3(q95gf)/+*	EMS
q372, q379, q380, q382	Noncomplementation screen	EMS

q256, q258, q262 and *q271*. Alleles tested only at 20° were: *q155, q181, q193, q200, q325, q372, q379, q380* and *q382*.

Temperature shift experiments: *Shift up*: Homozygous *fog-1(q253ts)* or *fog-1(q253ts); him-5(e1490)* adult hermaphrodites raised at 15° were picked to lightly seeded plates that had been preincubated at 15°. L1 worms were picked within 2 hr of hatching ($t = 0$) and shifted from permissive (15°) to restrictive (25°) temperature at specified intervals after hatching.

Shift down: Homozygous *fog-1(q53ts)* or *fog-1(q253ts): him-5(e1490)* adult hermaphrodites grown at 15° were shifted to 25° for 12 hr and then moved to lightly seeded plates that had been preincubated at 25°. Therefore, all of embryogenesis must have occurred at 25° for embryos laid on this new plate. L1 worms were picked within 1 hr of hatching ($t = 0$) and shifted from 25° to 15° at specified intervals after hatching.

Scoring shifted animals: Each hermaphrodite was scored as either self-fertile or female (Fog), by picking worms to separate plates and scoring them as adults. For several of the fertile animals the self brood size was counted. Each male was scored by Nomarski for presence of sperm and oocytes.

***fog-1; glp-1* double mutant:** Animals of genotype *fog-1(q253ts); glp-1(q231ts); him-5* were grown at 15°. Animals were shifted to 25° as L2 larvae (stage assessed using Nomarski optics) and scored as adults to determine germ-line sex ($n = 12$).

RESULTS

The *fog-1* mutant phenotype: Thirty-eight alleles of *fog-1* have been isolated, by several methods: (1) a simple screen for sterile mutants (2 alleles), (2) a genetic selection for dominant suppressors of gain-of-function mutations (gf) in *fem-3*, which by themselves masculinize the germ line (32 alleles), and (3) a noncomplementation screen (4 alleles) (Table 1). The *fog-1* mutations were isolated at a frequency typical of loss-of-function mutations (see MATERIALS AND METHODS), but none of the 25 alleles tested was suppressed by the amber suppressor *sup-7*. The *fog-1* locus maps on linkage group I between *ace-2* and *unc-11* (Figure 1).

In *fog-1* homozygotes, the germ line is sexually

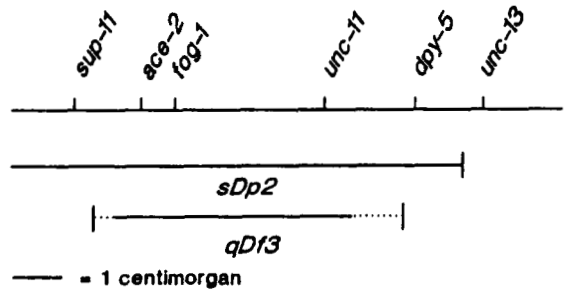

FIGURE 1.—Map position of *fog-1* and *qDf3* relative to other genes on the left end of linkage group *I*.

transformed to the female fate. In both sexes, germ cells that would normally have differentiated as sperm become oocytes instead. Whereas wild-type males make sperm continuously and never make oocytes (Figure 2, *A* and *B*), homozygous *fog-1* males make oocytes continuously and never make sperm (Figure 2, *C* and *D*). Similarly, whereas wild-type hermaphrodites make some sperm (about 160 per ovotestis) and then make oocytes continuously (Figure 3, *A* and *B*), homozygous *fog-1* hermaphrodite make oocytes continuously and never make sperm (Figure 3, *C* and *D*). For each sex, oogenesis begins at the time at which spermatogenesis would normally have begun. All 38 alleles are fully penetrant and cause an absence of sperm in both sexes.

In animals heterozygous for any of the *fog-1* alleles listed in Table 1, the germ line is partially feminized. [See below for the paradoxical result that *fog-1(deficiency)/+* heterozygotes do not show this feminization.] For *fog-1/+* males, some sperm are made and then oocytes are produced (Figure 4). This semidominant feminization is not due to a maternal effect since heterozygous males were feminized even when derived from a wild-type mother (10/13 male progeny feminized from the cross *fog-1(q187) unc-11/++* males × *dpy-10 unc-85* hermaphrodites). The average number of sperm made by a heterozygous male before oogenesis is 374 (range 280–522). All alleles are fully penetrant for this semi-dominant feminization of males. For *fog-1/+* hermaphrodites, we tested the canonical allele *q187* for changes in number of sperm produced. In *q187/+* hermaphrodites, fewer sperm are made than in wild type (Table 2).

Unlike the germ line, somatic tissues are not affected in *fog-1* mutants, whether heterozygous or homozygous (Figures 2–5). The somatic tissues of *fog-1(q187)* have been examined in most detail; in addition, no sign of somatic feminization has been observed in *q187/+* males or males carrying any other *fog-1* allele. We have examined *fog-1* and *fog-1/+* males by Nomarski microscopy and find that they possess a normal male somatic gonad and copulatory apparatus (male tail) and no sign of vulva formation (Figure 2, *C* and *D*, and Figure 4, and data not shown). Furthermore, they exhibit normal male mating behavior and

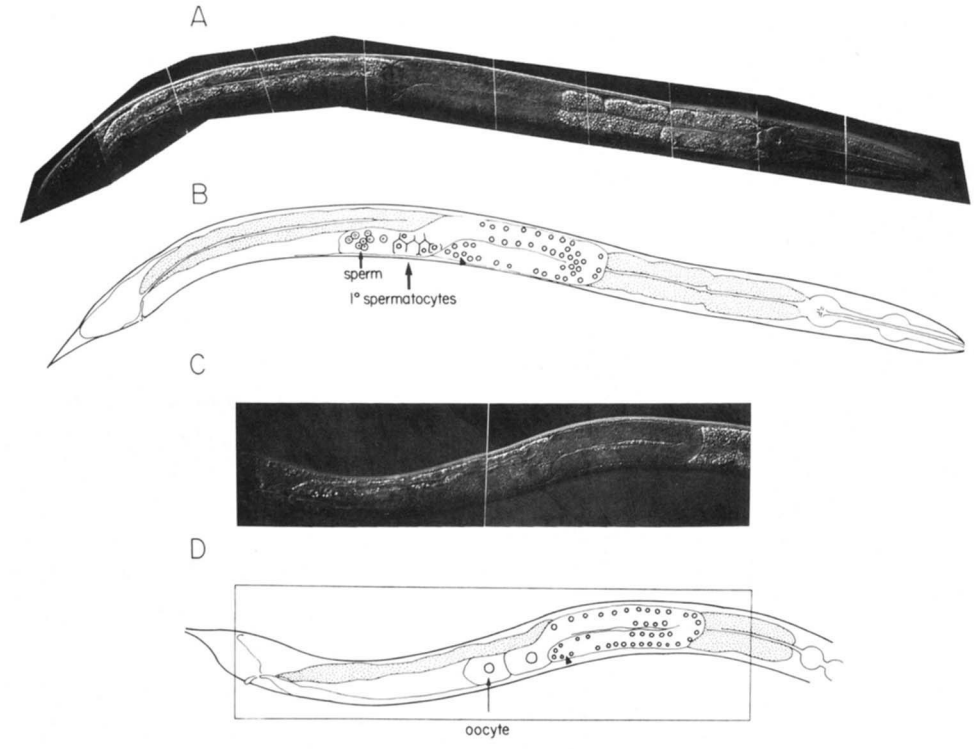

FIGURE 2.—The male *fog-1* phenotype. A and B, A wild-type L4 male, lateral view. Sperm are made continuously; the soma is male. C and D, A *fog-1* homozygous L4 male, lateral view. Oocytes are made continuously. In particular, the first germ cells to differentiate are shown. The somatic structures are all male despite the production of oocytes in the *fog-1* germ line. In both (B) and (D), an arrowhead indicates the mitotically proliferating pool of undifferentiated germ cells that remains throughout the lifetime of the animals and is the source of germ cells from which new gametes are made. Magnification bar equals 50 μm.

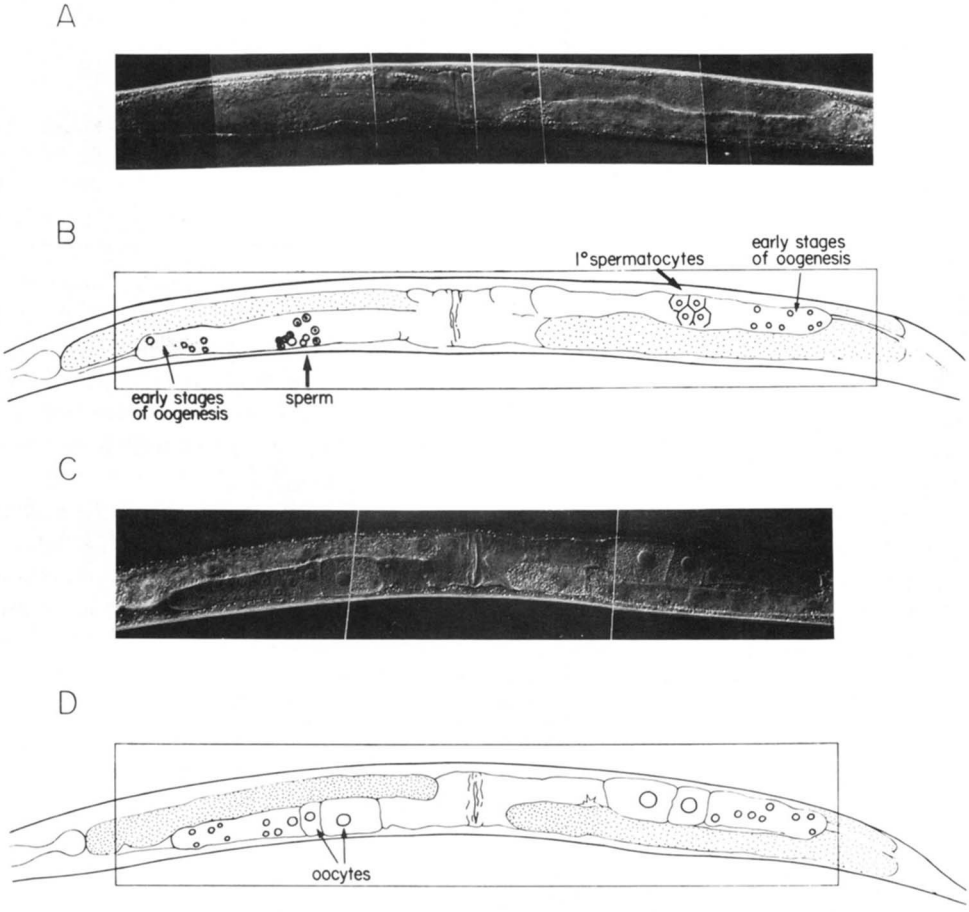

FIGURE 3.—The hermaphrodite *fog-1* phenotype. A and B: A wild-type young adult hermaphrodite, ventral view. Both sperm and oocytes are made. C and D, a *fog-1* homozygous young adult hermaphrodite, ventral view. Only oocytes are made. In particular, the first germ cells to differentiate are shown. The somatic structures are unchanged from the wild-type hermaphrodite. Magnification bar equals 50 μm.

there is no evidence of yolk synthesis in adult *fog-1* or *fog-1*/+ males (Figure 5). Yolk proteins are normally made by the hermaphrodite intestine (KIMBLE and SHARROCK 1983) (Figure 5, lanes 1 and 2) and are similarly made by *fog-1 XX* females (Figure 5, lane 3). However, yolk proteins are not made by wild-type males (Figure 5, lane 4), *fog-1*/+ males (Figure 5, lane 5), or *fog-1* homozygous males (Figure 5, lanes 6–8). Therefore, feminization associated with *fog-1* mutations appears to be limited to the germ line. Finally, we see no alteration in the hermaphrodite soma. The wild-type hermaphrodite soma normally shows only

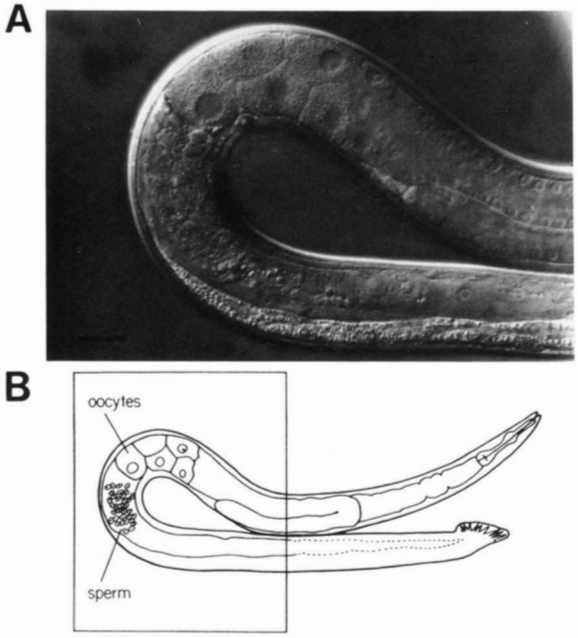

FIGURE 4.—A and B, The semi-dominant male phenotype of *fog-1*. An adult *fog-1*/+ male, lateral view. First sperm and then oocytes are produced. No change in somatic structures is observed. Magnification bar equals 50 μm.

female characteristics and it remains female in *fog-1* mutants.

Dosage studies of the *fog-1* locus: Table 2 shows the effect of changing the dosage of *fog-1* on germline feminization in both males and hermaphrodites. As described above, *fog-1/fog-1 XX* and *XO* animals make only oocytes whereas *fog-1*/+ *XX* and *XO* animals make both sperm and oocytes. We examined the effect of *fog-1* dosage by using the free duplication *sDp2*, which carries a wild-type copy of *fog-1*. Significantly, there is no feminization of the *XO* male germ line in the presence of two copies of wild-type *fog-1* and one mutant copy of *fog-1*. In this experiment, the sex of the germ line is dictated by the number of wild-type *fog-1* copies rather than the number of mutant *fog-1* copies. Therefore, the semidominance of the *fog-1* mutations must be explained either by haplo-insufficiency or by a weak gain-of-function effect of the *fog-1* mutations.

To test the possibility that *fog-1* may be haploinsufficient, we isolated a deficiency of the *fog-1* region (see MATERIALS AND METHODS). We found that animals carrying a *fog-1* mutation in *trans* to this deficiency make only oocytes (Table 2). Because this phenotype is identical to that of the *fog-1/fog-1* animal, those alleles obtained by screening for mutations that fail to complement a *fog-1* mutation (noncomplementing alleles) might have been equivalent to this deficiency. Paradoxically, the deficiency of the *fog-1* region, unlike all other *fog-1* alleles, is not semidominant.

TABLE 2

Effect of copy number of *fog-1*(+) on sperm/oocyte decision

Genotype	*XO* phenotype	*XX* brood size[a]
++/+*unc-11*	Sperm only[b] (n=61)	315 ± 28 (n=8)[b] (range 273–379)
++/*fog-1 unc-11*	Sperm, then oocytes[c] (n > 200)	257 ± 31 (n = 6)[c] (range 202–299)
fog-1 unc-11/fog-1 unc-11	Oocytes only[d] (n = 100)	0 (n = 100)
sDp2/+*unc-11*/+*unc-11*	Sperm only[e] (n = 9)	237 ± 25 (n = 10)[f] (range 179–309)
sDp2/+*unc-11*/*fog-1 unc-11*	Sperm only[g] (n = 29)	226 ± 15 (n = 18)[f] (range 169–280)
sDp2/*fog-1 unc-11*/*fog-1 unc-11*	Sperm, then oocytes[h] (n = 6)	133 ± 21 (n = 8)[f] (range 90–174)
qDf3/+*unc-11*	Sperm only[i] (n = 36)	Not determined
qDf3/*fog-1 unc-11*	Oocytes only[j] (n = 34)	0 (n = 27)[k]

[a] ± values indicate 95% confidence limits of the mean. The brood size of ++/+ *unc-11* animals is significantly different from that of ++/*fog-1 unc-11* animals ($P < 0.01$, t test). Brood size of sDp2/*fog-1 unc-11* is significantly different from both sDp2/+*unc-11* and sDp2/+*unc-11*/*fog-1 unc-11* ($P < 0.01$, t test).

[b] Cross progeny of N2 males × *unc-11* hermaphrodites.

[c] Cross progeny of N2 males × *fog-1*(*q187*) *unc-11* female.

[d] Unc males from cross: *fog-1*(*q187*) *unc-11*/++ males × *fog-1*(*q187*) *unc-11* female.

[e] Non-Unc male progeny from cross *unc-11* males × *sDp2*/*unc-11* hermaphrodite.

[f] Self-broods of animals segregating from mother of genotype *sDp2*/*fog-1*(*q187*) *unc-11*/+ *unc-11*. Note that *sDp2*/+ animals make smaller broods than wild-type (ROSE, BAILLIE and CURRAN 1984). In these experiments only live progeny were counted. Thus brood sizes of animals carrying sDp2 cannot be compared with those of animals not carrying sDp2.

[g] Non-Unc male progeny from cross *unc-11* males × *sDp2*/*fog-1*(*q187*) *unc-11* hermaphrodite.

[h] Non-Unc male progeny from cross *sDp2*/*fog-1*(*q187*) *unc-11* males × *fog-1*(*q187*) *unc-11* female.

[i] Unc cross progeny males from cross *unc-11*/+ male × *qDf3*/+ hermaphrodite.

[j] Unc males either from cross *qDf3*/+ male × *fog-1 unc-11* hermaphrodites (n = 20) or from cross *fog-1 unc-11*/++ males × *qDf3*/+ hermaphrodite (n = 14). These males showed no female characteristics other than the production of oocytes.

[k] Unc females either from cross *qDf3*/+ male × *fog-1 unc-11* (n = 17) or from cross *fog-1 unc-11*/++ × *qDf3*/+ (n = 10).

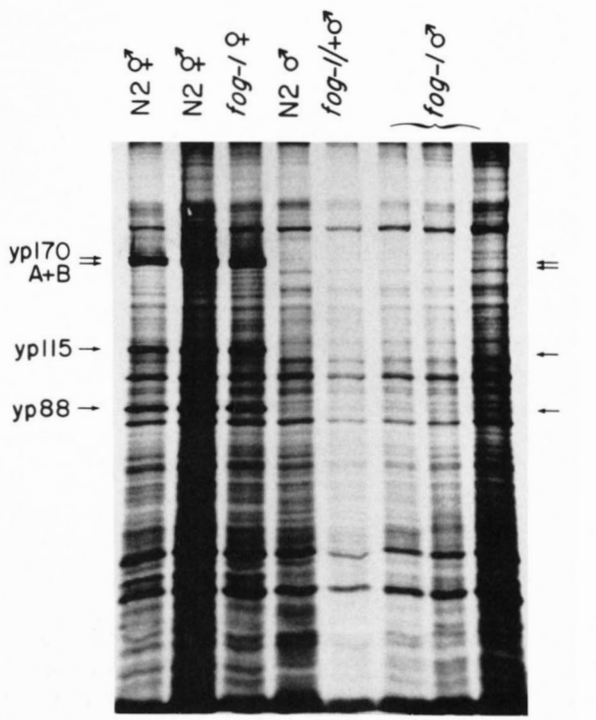

FIGURE 5.—Absence of yolk in *fog-1* mutant males. Although oocytes are made by the *fog-1* male germ line, yolk, which is normally made in the hermaphrodite intestine, is not made in the *fog-1* male. Therefore, there is no feminization of the intestine by *fog-1*. Four yolk proteins are made by wild-type adult hermaphrodites: yp88, yp115, yp170A and yp170B (SHARROCK 1983). These proteins are observed in lanes 1 (3 wild-type adult hermaphrodites), 2 (6 wild-type adult hermaphrodites), and 3 (6 *fog-1* adult females). The yolk proteins are not observed in lanes 4 (6 wild-type adult males), 5 (6 *fog-1*/+ adult males), 6 (6 *fog-1* adult males), 7 (12 *fog-1* adult males), or 8 (24 *fog-1* adult males). In all cases, the allele of *fog-1* was *q187*.

Whereas *fog-1* /+ males begin making oocytes one day after L4, hemizygous males continued to make sperm and had not switched over to oogenesis three days after L4 (Table 2). The simplest interpretation of the lack of germ-line feminization in *fog-1*(*deficiency*)/+ *XO* males is that *fog-1* is not haplo-insufficient.

Temperature shift experiments with a *fog-1*(*ts*) mutant: Figure 6A shows the effect of shifting *fog-1*(*q253ts*) hermaphrodites from permissive temperature (15°) to restrictive temperature (25°), and vice versa, at various times during development. All hours are normalized to 25° developmental time. When shifted up at or prior to 24 hr, hermaphrodites made only oocytes, but when shifted up at or after 32 hr, all made some sperm. Conversely, when shifted down at or prior to 22 hr, most made some sperm, but when shifted down at or after 26 hr, they made only oocytes. These shifts define a window of development, from about 22 hr to 32 hr, when *fog-1* activity is required to direct spermatogenesis in hermaphrodites. The beginning of this period, 22 hr, just precedes the time at which pachytene figures are first seen in hermaphrodite germ cells (26 hr) (KIMBLE and WHITE 1981).

The number of sperm made by a hermaphrodite is correlated with the time of its temperature shift. For shifts up, more sperm were made the later a shift to restrictive temperature was done. For shifts down, fewer sperm were made the later a shift to permissive temperature was done (Table 3). Germ cells do not mature synchronously but rather in a spatial gradient. The dependence of sperm number on time of shift is expected if *fog-1* is needed as individual germ cells reach the point in their maturity at which the sperm/oocyte decision is made. From the data provided in the previous paragraph, this point appears to coincide with entry into meiosis.

TABLE 3

Number of hermaphrodite sperm after temperature shifts

Shift up[a] (hr)	Brood size[b]	Shift down[a] (hr)	Brood size[b]
25	27 ± 16 (n = 21)	22	137 ± 41 (n = 15)
32	116 ± 41 (n = 11)	24	62 ± 18 (n = 15)
35	161 ± 24 (n = 10)	26	6 ± 13 (n = 15)
38	128 ± 22 (n = 12)		
45	229 ± 25 (n = 7)		

[a] Hours normalized to 25°.

[b] The number of hermaphrodite sperm can be determined by counting the number of self-progeny, because each sperm is efficiently used for fertilization of an oocyte. ± values indicate 95% confidence limits of the mean. Vertically adjacent values are significantly different from one another ($P < 0.05$, t test).

Figure 6B shows the effect of temperature shift experiments with *fog-1*(*q253ts*) males. Again, all times are normalized to developmental time at 25°. When shifted up at or prior to 21 hr, all *fog-1*(*ts*) males made only oocytes. When shifted up later, they made sperm, but their germ line subsequently switched to oogenesis. Because *fog-1*(*q253ts*) males grown at permissive temperature never switched to oogenesis (n=20 followed for 72 hr), there must be an ongoing requirement for *fog-1* in males. Shift down experiments gave results complementary to the shift up experiments. All *fog-1*(*ts*) males shifted from restrictive to permissive temperature at or prior to 19 hr made sperm continuously and did not switch to oogenesis. However, those shifted down at or after 28 hr never made sperm, instead they made only oocytes. Thus, *fog-1* activity is needed during a window of development, 19 to 36 hr, for the initiation of spermatogenesis in males. The beginning of this period, 19 hr, just precedes the time at which pachytene figures are first observed in male germ cells (23 hr) (KIMBLE and WHITE 1981).

An apparent paradox arises from the results of our late shift up and shift down experiments. If *fog-1*(*ts*) is shifted late from permissive to restrictive temperature, oocytes are produced; however if *fog-1*(*ts*) is shifted late from restrictive to permissive temperature oocytes continue to be produced. We interpret the inability of the animals shifted down to produce sperm as a requirement during larval development for initiation of the spermatogenic pathway. This may occur

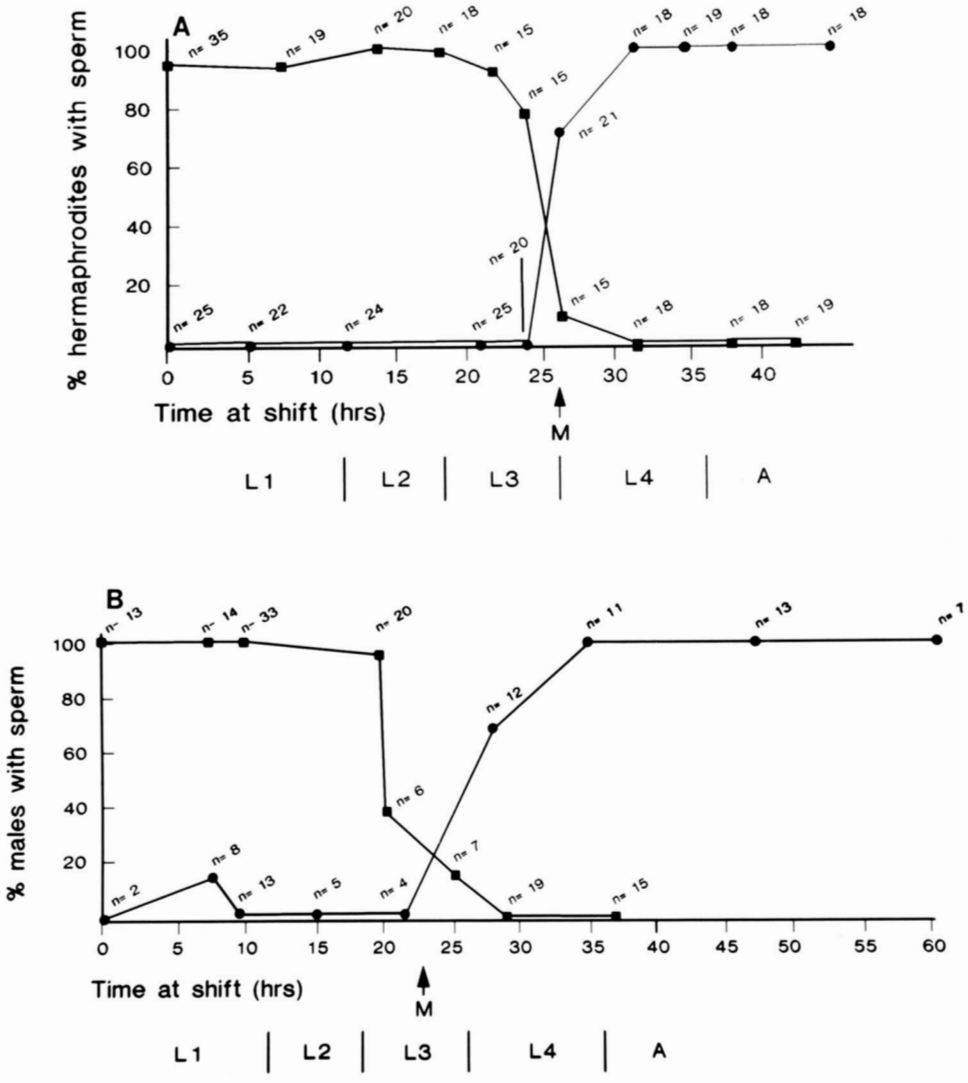

FIGURE 6.—Temperature sensitive periods of *fog-1*. In both (A) and (B), the ordinate shows the percent animals that possess any sperm and the abscissa shows the time in 25° hours of development. Circles denote shifts from permissive (15°) to restrictive (25°) temperature. Squares denote shifts from restrictive to permissive temperature. 0 hr = hatching L1, L2, L3, L4 and A indicate the durations of larval and adult stages. M (meiosis) indicates the time at which the first pachytene figures are observed. A, Temperature sensitive period of *fog-1* hermaphrodites. The stage at which *fog-1(q253ts)* is either synthesized or active during hermaphrodite development occurs in a window between 22 and 32 hr. This period is close to the time of entry into meiosis (M). B, Temperature sensitive period of *fog-1* males. The stage at which *fog-1(q253)* is either synthesized or active during male development occurs in a window between 19 and 36 hr. This period is close to the time of entry into meiosis (M).

because the *fog-1* product made during larval development differs from that made later (either in quality or quantity) or because regulators of *fog-1* differ at the two different stages of development.

Phenotype of a *fog-1*; *glp-1* double mutant: To further define the time during which *fog-1* is needed to specify that a germ cell be a spermatocyte, we used a *glp-1(ts)* mutant to alter the time at which germ cells enter meiosis. If *glp-1(ts)* animals are shifted to restrictive temperature during L1, the few germ cells that have been produced enter meiosis prematurely and differentiate as sperm (AUSTIN and KIMBLE 1987). At most, one mitotic cell division occurs before entry into meiosis after a shift of *glp-1(ts)* to restrictive temperature (J. AUSTIN, personal communication). We therefore shifted *fog-1(ts)*; *glp-1(ts)* double mutant animals to restrictive temperature as L2 larvae. We found that the germ cells forced to enter meiosis early in the *fog-1*; *glp-1* double mutant never differentiate as sperm but instead develop as oocytes or oocyte-like cells (Figure 7). Because the germ cells in *glp-1* single mutants make sperm when they enter meiosis during L2, active *fog-1* product must be available at that time. However, because no sperm were made in the *fog-1*; *glp-1* double mutant, *fog-1* must have been inactivated soon after the shift. Therefore, we conclude that the *fog-1* gene product specifies cell fate sometime during

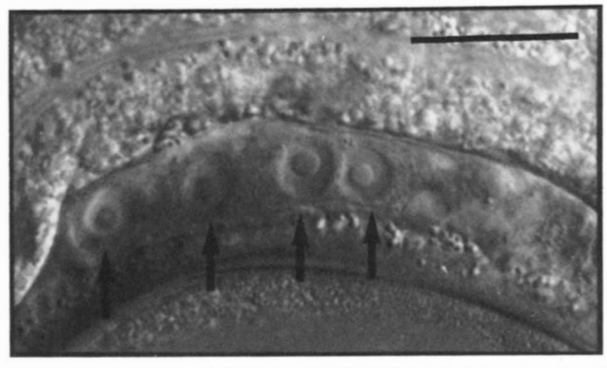

FIGURE 7.—A *fog-1(q253ts)*; *glp-1(q231ts)* adult hermaphrodite that was shifted to restrictive temperature as an L2 larva. Germ cells have cytoplasm and nuclei characteristic of oocytes. In *glp-1* single mutants, germ cells would have differentiated as sperm during L3. Larger, more typical looking oocytes can also be seen in these animals. Magnification bar equals 50 μm.

TABLE 4

Double mutant phenotypes

Genotype	Germ line phenotype	Somatic phenotype
tra-1[a]	Sperm only or sperm and oocytes	Pseudomale
fog-1; *tra-1*	Oocytes only[b] (n = 27)	Pseudomale
tra-2[c]	Continuous sperm	Pseudomale
fog-1; *tra-2*	Oocytes only[d] (n = 18)	Pseudomale
tra-3[e]	Continuous sperm (n = 11)	Pseudomale
fog-1; *tra-3*	Oocytes only[e] (n = 27)	Pseudomale
fem-3(gf) 25°[f]	Continuous sperm (Mog)	Female
fog-1; *fem-3(gf)* 25°	Oocytes only[g] (n = 17)	Female
her-1[h]	Sperm and oocytes	Female
fog-1; *her-1 him-5*	Oocytes only[i] (n = 19)	Female

[a] HODGKIN and BRENNER (1977), HODGKIN (1987b), SCHEDL *et al.* (1989).

[b] Unc pseudomales from self-progeny of *fog-1(q187) unc-11*/++; *tra-1(e1099)*/+ mothers. One Unc pseudomale made both sperm and oocytes; this was most likely a recombinant.

[c] HODGKIN and BRENNER (1977).

[d] Unc pseudomales from self-progeny of *fog-1(q187) unc-11*/++; *tra-2(e1095)*/+ mothers.

[e] Unc pseudomales from self-progeny of *tra-3(e1107)*; *fog-1* (*q187*) *unc-11*/+ mothers.

[f] BARTON, SCHEDL and KIMBLE (1987).

[g] Unc progeny from self-progeny of *fem-3(q96)*; *fog-1(q187) unc-11*/++ mothers. In addition, we have looked at the self-progeny of *fem-3(gf)*; *fog-1(q187)*/+ for three other *fem-3* alleles. For each, about 1/4 of the progeny made only oocytes: for *fem-3(q20)*, 6/27 were female, for *fem-3(q60)* 22/89 were female, and for *fem-3(q66)*, 20/77 were female.

[h] HODGKIN (1980).

[i] Unc progeny from self-progeny of *fog-1(q187) unc-11*/+; *dpy-21 her-1 him-5*. In *dpy-21* strains, *XX* animals are Dumpy and *XO* animals are nonDumpy. Both Dumpy (n = 10) and nonDumpy (n = 9) Unc progeny were female.

or after the last mitotic cell division that occurs before entry into meiosis.

Epistasis experiments with *fog-1* and mutations in other sex-determining genes: To gain insight into the function of *fog-1* within the hierarchy of sex determination genes, we examined the phenotypes of double mutants homozygous for both *fog-1* and mutations in other sex-determining genes. All experiments, except that with *her-1*, were done solely in *XX* animals. In *fog-1(q187)*; *x* double mutant animals (where *x* is either *tra-1*, *tra-2*, *tra-3*, *fem-3(gf)* or *her-1*), only oocytes were made. For *tra-1*, *tra-2*, *tra-3*, and *fem-3(gf)*, *XX* animals were examined; for *her-1*, both *XX* and *XO* animals were examined. Table 4 presents the phenotypes of both single mutants and *fog-1*; *x* double mutants. The soma of *XX* animals masculinized by a *tra* mutation was not affected by the state of *fog-1* consistent with the idea that *fog-1* is a tissue-specific regulatory gene. These results place *fog-1* together with the *fem* genes at the end of the regulatory network in the germ line (Figure 8).

We have also examined the phenotype of *XX* animals that are heterozygous for *fog-1* but homozygous for either *tra-1*, *tra-2*, or *tra-3*. These *fog-1*/+; *tra* double mutants have the same germ-line phenotype seen in *fog-1*/+ heterozygotes (data not shown). Likewise, *XX* animals homozygous for *fem-3(gf)* and heterozygous for *fog-1* often make both sperm and oocytes at 25° and are therefore self-fertile hermaphrodite. This self-fertility explains the isolation of *fog-1* mutations as dominant suppressors of *fem-3(gf)*. Animals that are heterozygous for *fog-1* and homozygous for *fem-3(gf)* can also be Mog at 25°. Thus, when *fem-3* is misregulated as in *fem-3(gf)* mutants, *XX* animals

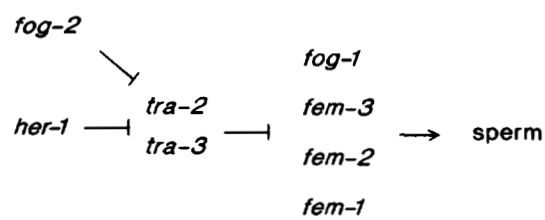

FIGURE 8.—A model for the role of *fog-1* in germline sex determination. ⊣, negative regulation; →, positive regulation. For a detailed review of the genetic pathway of *C. elegans* sex determination see VILLENEUVE and MEYER (1989). Briefly, the *X:A* ratio, with the *xol* and *sdc* genes, sets the state of *her-1* to be ON or OFF. In males, *her-1* is ON, *tra-2* is OFF, and the *fem* genes are ON; therefore spermatogenesis occurs. In hermaphrodites, *her-1* is OFF, *tra-2* is ON, and the *fem* genes are OFF; therefore oogenesis occurs. To allow for the burst of spermatogenesis in hermaphrodites, *fog-2* is thought to allow the *fem* genes to be ON by transiently repressing *tra-2*. Epistasis experiments place *fog-1* at the same level of epistasis as the *fem* genes. This position is consistent with the idea that *fog-1* specifies the "sperm" cell type. [Since it is not understood what role *tra-1* plays in the germ line (HODGKIN 1987b; SCHEDL *et al.* 1989), we have omitted *tra-1* from this germline regulatory pathway.]

can continue to make sperm even in the presence of only *one* functional copy of *fog-1*.

The self-fertility observed in *fog-1*/+; *fem-3*(*gf*) double is not allele specific. Mutations of *fog-1* isolated by brute force (*e.g.*, *q155*) and those isolated by suppression (*e.g.*, *q187*) are similar in their ability to limit the number of sperm made by a *fem-3*(*gf*) animal. Therefore, the *fog-1* defects associated with mutations that were isolated by suppression are not significantly different from those isolated by brute force. Consistent with its lack of semidominance in males, *qDf3*, is not a dominant suppressor of *fem-3*(*gf*) (data not shown). Finally, *fog-1*(*q187*) suppresses all four *fem-3*(*gf*) alleles tested: *fem-3*(*q20gf*), *fem-3*(*q60gf*), *fem-3*(*q66gf*) and *fem-3*(*q96gf*).

DISCUSSION

***fog-1* is a tissue-specific sex-determining gene:** The majority of known sex-determining genes influence the sex of most, or all, tissues of the organism [*e.g.*, see reviews by BAKER and BELOTE (1983), HODGKIN (1987a), and EICHER and WASHBURN (1986)]. In *C. elegans*, these global sex-determining genes affect both somatic and germ-line tissues. For example, loss-of-function mutations in each of the *fem* genes feminizes somatic and germ-line tissues in *XO* animals and each feminizes the germ line of *XX* animals. The *fog-1* gene, in contrast, is tissue specific. Mutations in *fog-1* lead to a sexual transformation of the germ line but not of the soma in both *XX* and *XO* animals: germ cells that normally would differentiate as sperm become oocytes instead [DONIACH (1986); this paper].

Other known tissue-specific sex determining genes include *fog-2* (SCHEDL and KIMBLE 1988), *fog-3* (T. SCHEDL, M. K. BARTON, and J. KIMBLE, unpublished results), and *mog-1* (for *m*asculinization *o*f the *g*erm line; P. GRAHAM and J. KIMBLE, unpublished results). Mutations in the *mab-3* gene affect several somatic tissues in the male; this gene has been proposed to act downstream of the sex determination pathway to effect male development in those tissues (SHEN and HODGKIN 1988). The role of the *fog-2* gene in sex determination differs significantly from that of *fog-1*. The *fog-2* gene is required for the onset of spermatogenesis only in hermaphrodites, not in males; therefore *fog-2* normally functions to permit a female animal to make sperm (SCHEDL and KIMBLE 1988). The genetic characterization of *fog-3* and *mog-1* has not progressed far enough to date to know their roles in germ-line sex determination.

The *fog-1* mutations are probably loss-of-function alleles: What is the nature of the *fog-1* mutations? Several lines of evidence argue that they reduce activity at the *fog-1* locus. These include the frequency with they were isolated, the identical phenotypes and full penetrance of all alleles, and the results of dosage studies. However, the most compelling evidence comes from the result of our unbiased noncomplementation screen for *fog-1* alleles. This screen was designed to detect *fog-1* alleles that behave like a deficiency and it generated "typical" semidominant *fog-1* alleles at a frequency expected for loss-of-function mutations (BRENNER 1974). Therefore, these alleles are likely to eliminate the function of *fog-1*.

One result appears to complicate our interpretation of *fog-1* mutations as loss-of-function alleles. Whereas *fog-1*/+ males are partially feminized, *fog-1*(*deficiency*)/+ males make sperm continuously and show no feminization. Therefore, *fog-1* mutations do not behave like a deficiency in *trans* to the wild-type allele. There are two basic ways to explain this paradox. One possibility is that the deficiency may delete unidentified genes that, when removed, compensate for a haploinsufficiency of *fog-1*. Formally, the existence of regulatory portions of the *fog-1* gene is included among "unidentified genes." Another possibility is that the mutant *fog-1* gene displays "negative dominance," interfering with the activity of wild-type *fog-1* product if not physically removed. For a discussion of the many precedents for negative dominance, see HERSKOWITZ (1987). If the dominant negative effect were caused by mutant *fog-1* protein, this explanation predicts that the *fog-1* protein acts in a multimeric complex and/or that its activity is unusually sensitive to dose. Alternatively, the dominant negative effect might be caused by the presence of mutant *fog-1* DNA or RNA, both of which would be removed by the deficiency.

The collected evidence (noncomplementation alleles, mutation frequency, full penetrance, invariant expressivity, dosage studies) argues that the *fog-1* mutations are loss-of-function. It remains puzzling why *all* the *fog-1* mutations, except the deficiency, are semidominant. Perhaps a complete loss-of-function at the *fog-1* locus can only be obtained by removing *fog-1* DNA, RNA, and protein, and a "simple" loss-of-function, which eliminates *fog-1* protein but not DNA or RNA, is indeed semidominant. A similar but far less extreme difference between the heterozygote and hemizygote has also been observed for the *fem-3* locus (BARTON, SCHEDL and KIMBLE 1987).

The *fog-1* gene directs sperm differentiation in both sexes: From the phenotype of *fog-1* mutations that reduce activity of the *fog-1* locus, we deduce that the wild-type *fog-1* produce is required for specification of a germ cell as a spermatocyte. The results of temperature shift experiments indicate that *fog-1* is required both for the onset of spermatogenesis during larval development in both sexes and for the continuation of spermatogenesis in males. Given the fact that we have been unable to determine the complete loss-of-function phenotype unambiguously, it remains possible that the *fog-1* gene may have functions in addition to its role in germ-line sex determination.

The *fog-1* gene can be considered both a sex-deter-

mining gene (male *vs.* female fate) and a gene that regulates cell type (sperm *vs.* oocyte). Other genes that regulate cell type include the vertebrate *myoD* gene, which specifies muscle differentiation (DAVIS, WEINTRAUB and LASSAR 1987), and the yeast mating type genes (*e.g.*, HERSKOWITZ and OSHIMA 1981). Both *myoD* and the mating type genes have been implicated in transcriptional control of cell type-specific genes (JOHNSON and HERSKOWITZ 1985; LASSAR *et al.* 1989). Although functionally analogous, the molecular mechanism by which *fog-1* directs spermatogenesis is unknown.

Sex determination in the germ line probably occurs as a germ cell enters meiosis: The germ line consists of mitotically dividing nuclei that act as stem cells, nuclei arrested in the pachytene stage of meiosis, and differentiating gametes in both sexes (HIRSH, OPPENHEIM and KLASS 1976; KLASS, WOLF and HIRSH 1976). Some immature germ cells are not sexually determined, even in the adult gonad (KLASS, WOLF and HIRSH 1976; BARTON, SCHEDL and KIMBLE 1987). The *fog-1* temperature sensitive period for entry into spermatogenesis begins just before the time when germ cells are first seen to be in the pachytene stage of meiosis (KIMBLE and WHITE 1981). The temperature sensitive period continues for as long as sperm are made in the respective sex. Although the resolution of these experiments is limited, this timing is consistent with the idea that determination of a germ cell to be sperm or oocyte occurs as germ cells enter meiosis.

Using a temperature sensitive mutation in *glp-1*, which forces germ cells into meiosis within one division of a shift to restrictive temperature (AUSTIN and KIMBLE 1987), we found that a *fog-1(ts); glp-1(ts)* double mutant made no sperm when shifted to restrictive temperature during early larval development. Instead the few germ cells made appeared to differentiate as oocytes or oocyte-like cells. We interpret this result to mean that *fog-1* specifies the sperm fate during or after the mitotic cell division occuring just before entry into meiosis. This result supports the idea that germ-line sex determination occurs near the time that the cells enter meiosis.

***fog-1(ts)* males making oocytes do not make sperm when shifted to permissive temperature:** In *fem-3(gf)* mutants, animals making oocytes can be induced to produce sperm with the appropriate temperature shift (BARTON, SCHEDL and KIMBLE 1987). Why then is the same phenomenon not observed with *fog-1(ts)*? Sperm are not made when *fog-1(ts)* animals are shifted from restrictive to permissive temperature during or after L4? One plausible explanation is that there is a positive feedback of *fog-1* on *fog-1*, such that *fog-1* activity or synthesis during L4 and later requires prior *fog-1* activity. Such feedback might also explain the discrepancy between the dominance of all *fog-1* alleles and the lack of dominance of the *fog-1* deficiency. If, for instance, *fog-1* transcription requires *fog-1* product after it is initiated, then a nonfunctional copy of the gene might compete with the functional copy of the gene for activation of *fog-1*. In the deficiency hemizygote, there would be no genetic material to compete for binding *fog-1* and hence no dominance would be seen. This model predicts that, when *fog-1* levels fall below a certain threshold, *fog-1* is effectively turned off and cannot be reactivated.

***fog-1* acts with the *fem* genes at the end of the sex determination pathway to direct spermatogenesis in the germ line:** How does *fog-1* function to control the decision between spermatogenesis and oogenesis? In the germ line, the sex-determination genes act in a cascade of negative regulation to establish the activity of three *fem* genes that direct the sexual phenotype (KIMBLE 1988). By examining the phenotypes of double mutants, no combination has been found that overrides the requirement of *fog-1* for spermatogenesis. Similarly, no double mutant has been constructed that overrides the requirement for any of the *fem* genes in spermatogenesis (NELSON, LEW and WARD 1978; DONIACH and HODGKIN 1984; HODGKIN 1986). Thus, the *fem* genes and *fog-1* occupy the same epistasis level in the sex determination pathway of the germ line (Figure 8).

The functional relationships among the *fem* genes and *fog-1* are not known. One clue is that *fog-1* is epistatic to gain-of-function mutations of *fem-3*: whereas *fem-3(gf)* masculinizes the germ line, the *fog-1; fem-3(gf)* double mutant makes only oocytes. Therefore, even when *fem-3* is unregulated, the *fog-1* product is required for spermatogenesis. However, the products of these genes may act together (*e.g.*, in a multimeric complex) or in a dependent pathway of positive control. In either case, the *fem* genes and *fog-1* function at the end of the pathway to specify a germ cell as a spermatocyte.

Conclusions. In this paper we describe *fog-1*, a gene that acts with the *fem* genes at the end of the sex determination pathway to specify germ cells as sperm. The *fog-1* gene is needed for the onset of spermatogenesis during larval development of both sexes and for the continuation of spermatogenesis in males. Its temperature sensitive period coincides with the entry of germ cells into meiosis. The *fog-1* gene may serve as a link between the global sex-determination pathway and the tissue-specific response of spermatogenesis. As such, the further analysis of this gene may serve as a model to address the fundamental question of how a single pathway of control can act in multiple cell types to elicit a specific response.

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