fog-2, a Germ-Line-Specific Sex Determination Gene Required for Hermaphrodite Spermatogenesis in Caenorhabditis elegans

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ABSTRACT

This paper describes the isolation and characterization of 16 mutations in the germ-line sex determination gene fog-2 (fog for feminization of the germ line). In the nematode Caenorhabditis elegans there are normally two sexes, self-fertilizing hermaphrodites (XX) and males (XO). Wild-type XX animals are hermaphrodite in the germ line (spermatogenesis followed by oogenesis), and female in the soma. fog-2 loss-of-function mutations transform XX animals into females while XO animals are unaffected. Thus, wild-type fog-2 is necessary for spermatogenesis in hermaphrodites but not males. The fem genes and fog-1 are each essential for specification of spermatogenesis in both XX and XO animals. fog-2 acts as a positive regulator of the fem genes and fog-1. The tra-2 and tra-3 genes act as negative regulators of the fem genes and fog-1 to allow oogenesis. Two models are discussed for how fog-2 might positively regulate the fem genes and fog-1 to permit spermatogenesis: fog-2 may act as a negative regulator of tra-2 and tra-3, or fog-2 may act positively on the fem genes and fog-1 rendering them insensitive to the negative action of tra-2 and tra-3.

In the nematode Caenorhabditis elegans, there are normally two sexes. The initial signal for sex determination is the ratio of the number of X chromosomes to sets of autosomes (MADL and HERMAN 1979). Diploid XX animals are self-fertilizing hermaphrodites; diploid XO animals are males. Most tissues of these two sexes differ morphologically and/or biochemically. The term “hermaphrodite,” when applied to C. elegans, describes a self-fertile animal with a female soma and a germ line that is first male, producing sperm, and then female, producing oocytes. Hermaphrodites of C. elegans reproduce either by self-fertilization or by cross-fertilization after mating with males.

The C. elegans hermaphrodite soma is essentially female. It is morphologically indistinguishable from the female soma of a closely related male/female nematode species Caenorhabditis remanei (SUDHAUS 1974), and is extremely similar to the female soma of Panagrellus redivivus (STERNBERG and HORVITZ 1981, 1982). The hermaphrodite soma is also indistinguishable from the soma of C. elegans females that arise as a consequence of mutations in sex determining loci (e.g., fem mutations). In the hermaphrodite germ line, spermatogenesis occurs first, beginning during the last larval stage of development (L4) and ending soon after the molt into adulthood. From this brief period of spermatogenesis, about 320 sperm per hermaphrodite are produced from about 40 primary spermatocytes in each of two gonads. Then, each gonad switches to oogenesis and oocytes are produced continuously throughout adulthood. The sexual duality of the XX germ line in a female soma suggests that hermaphroditism in C. elegans is a property of the germ-line tissue. This is to be distinguished from hermaphroditism in the annelid Lumbricus terrestris (earthworm) in which each animal has a separate ovary and testis (sexual duality in both the germ line and soma) and reproduction occurs by mating (HICKMAN, HICKMAN and HICKMAN 1973).

In C. elegans, the X/A ratio is transduced by a set of genes that direct both sex determination and dosage compensation (VILLENUEVE and MEYER 1987; NUSSEBAUM, L. MILLER, J. PLENESFISCH and B. MEYER, personal communication). These “transducer” genes, in turn, regulate both genes that direct dosage compensation plus genes that specify the sexual phenotype. Here, we focus on the later group of sex-determination genes; the transducer genes and dosage compensation genes are beyond the scope of this paper.

Seven sex-determining genes have been identified that specify sexual fate in all tissues of the animal—both somatic and germ line (HODGKIN and BRENNER 1977; HODGKIN 1980; DONIACH and HODGKIN 1984; HODGKIN 1986). In addition, one sex-determining gene has been identified that affects the sexual fate of a single tissue, the germ line (DONIACH 1986b; M. K. BARTON, personal communication). The germ-line and somatic mutant phenotypes of these sex-determining genes are summarized in Table 1. These genes must act downstream from the X/A ratio,
TABLE 1

Summary of sex determination genes used in this study

<table>
<thead>
<tr>
<th>Genotype</th>
<th>XX phenotype</th>
<th>XO phenotype</th>
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<tr>
<td></td>
<td>Germ Line</td>
<td>Soma</td>
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<tr>
<td>Wild type</td>
<td>Male then female (self-fertile)</td>
<td>Female</td>
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<tr>
<td>her-l(lf)*</td>
<td>Male then female (self-fertile)</td>
<td>Female</td>
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<tr>
<td>fem-1(lf)−</td>
<td>Female</td>
<td>Female</td>
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<tr>
<td>fem-2(lf)−</td>
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<tr>
<td>fem-3(lf)−</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>tra-1(lf)−</td>
<td>Male then female</td>
<td>Male</td>
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<tr>
<td>tra-2(lf)−</td>
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<tr>
<td>tra-3(lf)−</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>fog-lk−</td>
<td>Female</td>
<td>Female</td>
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</tbody>
</table>

*lf, loss-of-function; for these genes this is the probable null phenotype. gf, gain-of-function. Phenotypes are of homozygotes. For details of mutant phenotypes, consult references and text.

*Hodgkin (1980).

† Homozygous mutant derived from a homozygous mutant mother m(−/−), z(−/−).


§ Hodgkin (1986).


* Hodgkin (1987); T. Schedl, unpublished observations. tra-1(lf) is included in this table for comparison with tra-2(lf) and tra-3(lf). The interaction of tra-1 and fog-2 mutations will be discussed elsewhere.

* Gonad abnormal, Hodgkin (1987); T. Schedl, unpublished observations.

† Hodgkin and Brenner (1977); T. Schedl, unpublished observations.

‡ Doniach (1986a), this paper.

<table>
<thead>
<tr>
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<th>Soma</th>
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<tbody>
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<td>Male</td>
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<tr>
<td>her-l(lf)*</td>
<td>Male then female (self-fertile)</td>
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<td>fem-1(lf)−</td>
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<td>fem-3(lf)−</td>
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<td>tra-1(lf)−</td>
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<td>tra-2(lf)−</td>
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Because mutations in them override this initial signal. Based on the results of a series of experiments in which the epistasis of mutations in these genes was analyzed, Hodgkin (1980, 1986) proposes that the sex-determining genes act in a cascade of negative regulation to control the state of tra-1, which in turn specifies somatic sexual phenotype.

The wild-type function of each of the sex-determining genes has been deduced from the phenotype of animals homozygous for a loss-of-function (lf) mutation in that gene. Thus, fem-1(lf), fem-2(lf), and fem-3(lf) XX and XO mutant animals are female instead of hermaphrodite and male, respectively (Table 1); therefore the wild-type fem-1, fem-2, and fem-3 genes are required for male development in both the XX germ line and all XO tissues (Nelson, Lew and Ward 1978; Doniach and Hodgkin 1984; Kimble, Edgar, and Hirsh 1984; Hodgkin 1986). Similarly, her-l(lf) XO animals are hermaphrodite instead of male; therefore, the wild-type her-l gene is required for the development of male somatic tissues and for continuous spermatogenesis in XO animals (Hodgkin 1980; C. Trent, P. Schedin and W. Wood, personal communication). Because her-l(lf) mutants are self-fertile (Table 1), wild-type her-l is not needed for hermaphrodite spermatogenesis. Finally, tra-1(lf), tra-2(lf), and tra-3(lf) XX animals are masculinized. The details of masculinization by mutations in each of the tra genes varies (Table 1). Both loss-of-function and gain-of-function mutant phenotypes of tra-2 make this gene stand out as necessary for the switch from spermatogenesis to oogenesis in the hermaphrodite germ line. In contrast, the role of tra-1 in the hermaphrodite germ line is unclear (Hodgkin 1987a; T. Schedl, unpublished observations). Therefore, the wild-type tra-1, tra-2, and tra-3 genes are necessary for female development, but their roles in specification of that development differ (Hodgkin and Brenner 1977).

The production of first sperm and then oocytes by the C. elegans hermaphrodite raises two major questions about the regulation of sex determination in the XX germ line: (1) how is male germ-line development initiated within a female soma? and, (2) how is the switch from male to female germ-line development affected? Since all uncommitted germ cells of the XX hermaphrodite are XX, whether a precursor of sperm or oocyte, the X/A ratio is not the primary signal for sexual choice in the hermaphrodite germ line. Instead, control of tra-2 and fem-3 activities appear to be important to germ-line sex determination in hermaphrodites. In particular, the phenotypes of gain-of-function (gf) mutations of tra-2 and fem-3 provide some insight into the genetic mechanisms of control over hermaphrodite germ-line development. Both tra-2(gf) and fem-3(gf) mutations affect the
sexual fate of the XX germ line, but have little or no effect on the XO germ line or the soma of either XX or XO animals. The XX germ line of tra-2(gf) mutants is feminized: germ cells that would normally differentiate as sperms become oocytes instead, and oogenesis continues throughout adulthood (Donaich 1986a). Conversely, the XX germ line of fem-3(gf) mutants is masculinized: sperms are produced continuously, generating a vast excess of sperms, with no sign of oogenesis. Thus, germ cells that would normally differentiate as oocytes become sperm instead (Barton, Schedl and Kimble 1987). Feminization of the XX germ line by tra-2(gf) mutations suggests that, in wild-type XX animals, tra-2 activity might be modulated to permit spermatogenesis. Donaich (1986a) suggests that tra-2 is no longer sensitive to this modulation in tra-2(gf) mutants. Similarly, masculinization of the XX germ line by fem-3(gf) suggests that, in wild-type XX animals, fem-3 activity is negatively regulated to permit the switch to oogenesis. Barton, Schedl and Kimble (1987) suggest that fem-3(gf) mutants are no longer sensitive to this negative regulation.

This paper describes loss-of-function mutations in a germ-line specific sex-determination gene, fog-2 (fog for feminization of the germ line). XX animals homozygous for fog-2 mutations are female, while XO animals are unaffected. Therefore, a homozygous fog-2 strain can reproduce as a male/female strain, but not as a self-fertilizing hermaphroditic strain. This mutant phenotype indicates that fog-2 is a regulator of hermaphroditic spermatogenesis. Analysis of the interaction of fog-2 mutations with mutations in other sex determining genes provides a framework for placing fog-2 within the regulatory hierarchy of sex determination as it applies to the XX germ line.

MATERIALS AND METHODS

General methods for culturing nematodes have been described by Brenner (1974). Experiments were performed at 20° unless specified in the text. For all experiments worms were under continuous growth conditions and were not starved or recovering from the dauer state.

Nomenclature: For certain genes in the C. elegans sex determination pathway, some loss-of-function mutations exhibit dominance and some gain-of-function mutations are recessive. We therefore designate alleles as gain-of-function with the suffix gf and loss-of-function with the suffix gf instead of abbreviations for dominant and recessive. Numerically designated alleles without a suffix are assumed to be loss-of-function unless indicated to the contrary: this avoids confusion between "1" and "1" (see Barton, Schedl and Kimble 1987 for a further description). For experiments where maternal and zygotic genotypes are important they are indicated by m( ) and z( ), respectively. For example, m(−/−), z(−/−) indicates a heterozygous mutant derived from a heterozygous mutant mother. All other nomenclature follows Horvitz et al. (1979).

Strains: C. elegans var. Bristol isolate N2 is defined as wild type, and is the strain from which all other stocks are derived. Most of the mutations used in this study are listed in Hodgkin and Riddle (1988) and Swanson, Edgar and Riddle (1984). The phenotypes of sex determination mutants are shown in Table 1 and are described and referenced explicitly in the text. The following mutations and chromosome rearrangements were used [daf (abnormal dauer larva formation), dpy (dumpy), emb (embryonic lethal), fem (feminization of germ line and soma), fog (feminization of germ line), her (hermaphroditization), lon (long), sup (suppressor), tra (transformer), unc (uncordinated)].

Linkage group (LG): I − fog-1(q812), LG II: dpy-10(e128), tru-2(e1095), e1425, e1941gf, e2046gf, e2020gf, unc-4(e120), unc-50gf. LG III: fem-2(e2115), dpy-19(e1289), dpy-18(e364). LG IV: fem-1(e1991), unc-24(e138), fem-3(e1966), q20gf, q97gf, q99gf, dpy-15(m081), dpy-20(e1282), unc-30(e191), tra-3(e1107). LG V: her-1(e1520), r1561, tru-3(e1490), dpy-21(e428), emb-4(hre60), unc-51(e369), e1189. LG X: sup-7(st5), unc-2(e678).

Isolation of fog-2 alleles: Four methods were used to isolate fog-2 alleles: 1. Screen for germ-line feminizing mutants. L4 hermaphrodites (Po), either N2 or dpy-19 +/+ unc-32 (markers used were for reasons irrelevant to this work), were mutagenized with 0.05 M ethyl methanesulfonate (EMS) for 4 hr (Brenner 1974) and individual F1 self-progeny were picked to agar-filled Petri plates. A dissecting microscope, the F2 self-progeny were screened for the presence of females (spermless hermaphrodites) at 25°. Four fog-2 alleles were isolated from 12,458 mutagenized haploid genomes; q70 and q71 were from N2 Po while q154 and q226 were from dpy-19 +/+ unc-32 Po.

2. Screen for mutations that fail to complement fog-2(q71), unc-51 hermaphrodites were mutagenized with EMS as described above and crossed with fog-2(q71) males, either at 15° or 25°. Non-Unc F1 cross-progeny were picked away from XO males at the L4 stage (to ensure virginity) in groups of 25 to 50. Plates were screened for F1 females among self-fertile sibs when all animals were adults. Females arising from the failure of the putative fog-2 allele (unc-51 fog-2(new) to complement q71) in trans were crossed with N2 males and 8 to 12 F2 L4 XX progeny were picked. For a new fog-2 allele one expects the F2 to be self-fertile and about half to segregate Unc-females (unc-51 is tightly linked to fog-2, see Figure 1) while the remaining 2 F2 animals segregate non-Unc (q71) females. The possibility of recessive lethal events that were induced in cis to unc-51 and that failed to complement fog-2(q71) was tested by searching for F2 hermaphrodites that did not segregate either Unc or female animals; none of this class was found. New dominant Fem/Fog mutations, e.g., tra-2(gf), see below, caused up to 50% of the F2 cross progeny to be female. Seven fog-2 alleles were isolated from 23,407 mutagenized haploid genomes; q86, q123, q124, were obtained at 25°, while q166, q167, q170, and q177 were obtained at 15°. q86 was isolated in cis to unc-51(e369) while all others were in cis to unc-51(e1189). To ensure independence of fog-2 alleles one mutant from a given cross was retained.

3. Extragenic suppressors of fem-3(q20gf), fem-3(gf) mutants are self-fertile at 15° and sterile (Mog) at 25°. Mog animals are sterile because they produce a vast excess of sperm and no oocytes and thus have a masculinization of the germline phenotype (Table 1; Barton, Schedl and Kimble 1987). Mutations in fog-2 suppress fem-3(gf), so the fog-2: fem-
3(gf) double mutant is self-fertile (see RESULTS). fem-3(q20gf)
dpy-20 L4 XX animals were mutagenized with EMS and allowed to produce self-progeny at 15°C. Adult F1 animals were picked, shifted to 25°C and plates were screened for self-fertile F2 animals. One fog-2 allele, fem-j(q20gf)l+, was obtained by this procedure.

4. Complementation suppression of fem-3(q95gf)/+; fog-2(q71)/+. The strongest fem-3(gf) allele is q95, which is 100% Mog as a heterozygote at 25°C (Barton, Schedl, and Kimble 1987). It was found that fem-3(q95gf)/+; fog-2(q71)/+ is also Mog at 25°C. However, when fog-2(q71) is homozygous, fem-3(q95gf)/+ is no longer a dominant sterile (about 80–90% of animals are self-fertile). Thus a newly induced fog-2 allele that fails to complement fog-2(q71) in trans will suppress the dominant sterility of fem-3(q95gf)/+; fog-2(q71)/+. unc-51(1189) L4 XX animals were mutagenized with EMS and then crossed at 25°C with fem-3(q95gf) dpy-20; him-5 fog-2(q71) males (from a 15°C stock). Non-Unc F1 animals were screened for self-fertile hermaphrodites (or eggs on the plate) among a sea of Mog animals and males. Any F1 self-fertile animals were picked and new fog-2 alleles were sought as F2 Unc females. In a number of cases, the F1 self-fertile animal had been mated by a sibling male (as judged by male progeny in the F2), and as a result, more than one type of mutagenized unc-51 chromosome was present. If the hermaphrodite had been mated, 12 non-Unc F2 L4 XX animals were picked and F3 Unc females were sought. As in the "screen for mutations that fail to complement fog-2," candidates were examined for sterile or lethal non-complementing alleles, but none was found. Four fog-2 alleles (q247, q249, q251, and q263) were obtained from this procedure. In addition, a sterile mutation that does complement fog-2, and that does not suppress fem-3(q95gf)/+, Ste(q265), was fortuitously isolated in this mutagenesis. Ste(q265) is closely linked to fog-2 (Figure 1), and thus useful in balancing unc-51 fog-2 doubles. The phenotype of Ste(q265) XX animals is an arrest in gonadal development, lack of a vulva, and a reduced number of germ cells in which the only gametes to develop are sperm.

All putative fog-2 alleles were out-crossed at least four times to N2. Where applicable, fem-3(q20gf) or q95gf) dpy-20 were removed during out-crossing based on loss of the tightly linked dpy-20 marker and the absence of the Mog phenotype of fem-3(gf) at 25°C. All alleles were then tested (or retested) for failure to complement fog-2(q71). Mapping showed that all alleles are tightly linked to unc-51 on the right arm of chromosome V (see below). The fog-2 alleles isolated linked to unc-51 were maintained as heterozygotes balanced by emb-4(hc60) or by Ste(q265). For construction of male/female strains (see below), the linked unc-51 was removed by two factor crosses.

Analysis of fog-2 mutants: Females can be distinguished from hermaphrodites either using a dissecting microscope or by Nomarski differential interference microscopy. With a dissecting microscope, adult females are non-egg-bearing, and as such, the ventrally located uterus is empty and appears as a clear patch. Further, unfertilized eggs accumulate in females giving the proximal arm of the gonads a striped appearance. To identify females unequivocally, animals were examined by Nomarski optics with a Zeiss Planoapo 63X lens at ×630 magnification. By Nomarski, females in L1 lethargus or as young adults lack sperm and primary spermatocytes in both the gonad and the spermatheca. The gametes that develop most proximally are oocytes (see RESULTS and Figure 2 for further description).

To determine the penetrance of the Fog phenotype, XX L4 animals were picked to individual plates and examined by the dissecting microscope as adults [about 24 hr (20 or 25°C) or about 36 hr (15°C)] for a female morphology and the absence of eggs/larvae. Selected animals were further examined by Nomarski. In some situations (such as strain constructions), L4 XX animals were transferred in groups of 20 to 50 and scored as above.

Males (XO) were examined by the dissecting microscope for the presence of a male tail, the absence of a vulva and for mating behavior. The following sexually dimorphic structures were examined by Nomarski optics for morphology and size to learn if there was any feminization of males: the germ line, the male gonad consisting of a single reflexed arm and vas deferens (Klass, Wolf and Hirsh 1976; Kimble and Hirsh 1979), and the bursal fan, sensory rays (9 pairs), and copulatory spicules of the male tail (Sulston, Albertson and Thomson 1980). Further, the type and position of gametes and germ cells within the gonad (Hirsh, Oppenheim and Klass 1976) and the presence of yolk in the pseudocoelom (refractive droplets, Kimble and Sharrock 1983; Doniaich 1986a) were scored. For each of the fog-2 alleles, more than 40 XO animals

**Figure 1.**—Map of the right arm of chromosome V. The positions of fog-2, emb-4, and Ste(q265) (above the line) were determined by two- and three-factor crosses (Table 2; see MATERIALS AND METHODS). The positions of loci shown below the line are from Swanson, Erdley and Riddle (1984) and Hodgkin and Riddle (1988).

**Figure 2.**—Wild-type and fog-2(q71) XX germ-line phenotypes. (a and b), Composite photomicrographs using Nomarski optics. Focal plane was adjusted to show the two gonad arms. Scale bar = 40 μm. Line drawings are shown below. (c and d), Higher magnification photomicrographs of boxed gonad region from (a) and (b), respectively. Scale bar = 10 μm. Line drawings of gamete type and position in gonad are shown below. (a and c), Wild-type young adult hermaphrodite. (b and d), fog-2 young adult female. (c), Gametes in the proximal part of the anterior gonad arm of a hermaphrodite. The first, most proximal, germ cells have differentiated as sperm. Subsequent germ cells have differentiated as oocytes. (d), Gametes in the proximal part of the posterior gonad arm of a fog-2 female. The first, most proximal, germ cells have a transformed sexual fate and have differentiated as oocytes instead of sperm. There is no evidence of sperm or spermatogenesis. Note that in both the hermaphrodite (c) and the female (d) the spermatheca (striped in drawings) is empty. Thick arrows—oocytes. Oocytes are very large cells, with a large smooth nucleus, and have a granular cytoplasm. Immature oocytes are smaller, have a large nucleolus, and also have a granular cytoplasm. Thin arrow—sperm. Sperm are small, with a tiny elevated refractile nucleus. Arrow head—primary spermatocyte. Note that the pattern of gametogenesis in the anterior and posterior gonads are equivalent in the hermaphrodite, and similarly, they are equivalent in the fog-2 female. For further morphological details see Hirsh, Oppenheim and Klass (1976) and Kimble and Ward (1988).
were examined. Because feminization (the appearance of
vill and oocytes) can be observed only in older adult males
for certain mutations (DONIACH 1986a; and see below), fog-
2 alleles q71, q86, q123, and q247 were scored over
time; about 20 males for each were examined at about 24, 48,
72, 96, and 120 hr. post L4 at 20°.
For all of the fog-2 alleles (except q177), XO animals
were shown to be functional males by construction and
propagation of male/female strains. Adult fog-2 females
were crossed with fog-2/+ males from which ten single
male cross progeny (either fog-2 or fog-2+/+) were mated
with a single adult fog-2 female. Male/female strains
were established from matings in which both parents were homo-
zygous fog-2. In general, the fog-2 male/female strains
were the source of animals for strain constructions, scoring
phenotypes and were continuously propagated by mating
a single male and a single female. A sex ratio of one was
observed for these male/female strains (e.g., fog-2(q226)
mating produced 107 females and 108 males), in contrast
to what is observed for some other male/female nematode
species (TRIANTAPHYLLOU 1973).

The mating efficiency of fog-2 males was found to be
equivalent to wild-type males. This was determined by a
mating competition assay. Twenty-four young adult males,
12 for fog-2(q71) and 12 for N2, were placed with four fog-
2 females (2 hr at 20°), and then the males were removed.
The genotype of the cross progeny was determined by picking
XX L4 animals over 2 days and scoring their phenotype. Female progeny were generated by mating with
a fog-2 male; hermaphrodite progeny by an N2 male. From
two such mating competitions, 186 females and 171 self-
fertile hermaphrodites were found.

All fog-2 mutants were tested for heat sensitivity (alleles
isolated at 23–25°) or cold sensitivity (alleles isolated at
15°). Male/female strains or unc-51 fog-2(q177) self-fertile
animals were placed at the appropriate temperature for at
least two generations. Then, the proportion of L4 XX
animals that developed as female or self-fertile adults was
determined. None of the fog-2 alleles was temperature
sensitive.

The recessive nature of fog-2 alleles was shown by
crossing fog-2 males with unc-51 animals; non-UNC XX
progeny [m(+/+), x(+/+)] were scored for self-fertility.
The possibility of maternal effects was tested in strong fog-
2 mutants. A maternal rescue effect was tested by deter-
mining the proportion of unc-51 fog-2 [m(+/+), x(−/−)]
females segregating from an unc-51 fog2/+ mother. All
Unc self-fertile animals found were recombinants. A ma-
ternal absence effect was tested by determining the pro-
portion of fog-2+/+ [m(−/−), x(+/−)] females segregating
from a cross of fog-2 females by N2 males. No maternal
effects were found (see results).

Tests for amber alleles of fog-2: The amber suppressor
tRNA mutation sup-7(st5) (WATERSTON 1981; WILLS et al.
1983) was used to test whether any of the fog-2 alleles
were amber mutations. For fog-2 alleles linked to the unc-51
marker, Unc females were crossed with dpy-18(e3644) ++ ;
sup-7/0 males, and non-UNC XX cross progeny picked. In
the next generation, Unc L4 animals were removed and scored
as self-fertile or female. Since only Unc
fog-2 males would be homozygous for sup-7, greater
than 60 Unc animals were scored for each allele tested. No
suppression was observed in tests at either 20° or 25°
for fog-2(q86, q123, q124, q166, q167, q247, q249, q263).

For fog-2 alleles that were not marked, fog-2/unc-51(e369)
strains homozygous for sup-7 were constructed. The pheno-
type of unc-51(e369 or e1899) is paralyzed, dumpy and
egg laying defective (Egl). The unc-51 allele e369 is sup-
pressible by sup-7 and unc-51(e369); sup-7(st5) homozygotes
have a coiyl Unc, non-Dpy non-Egl phenotype at 20° and
25°. fog-2 males were crossed to sup-7; unc-51(e369) her-
maphrodites, non-coiyl cross progeny picked and allowed
to self. F2 animals were picked individually to obtain the
stock fog-2/unc-51(e369); sup-7 identified by producing coiyl
Unc, but no paralyzed Unc progeny. From this, greater
than 50 non-UNC L4 animals were picked to search for
suppressed fog-2 animals that do not segregate Unc. No
suppression was observed at either 20° or 25° for fog-2
(q70, q71, q123, and q226). The leaky alleles q177, q254,
and q251 were not tested.

Genetic mapping: fog-2 was initially mapped to the right
tip of LG V near unc-51. fog-2 was positioned by three-
and two-factor crosses (Table 2, A and B, respectively) in
relation to dpy-21 and unc-51. Two loci [Ste(q263) and emb-
4] used for balancing marked fog-2 mutants were also
positioned in the same factor crosses. Figure 1 summarizes
the map of the right arm of LG V and was derived from
Table 2 and the current C. elegans map (HOKKIN and RIDDLE
1988).

tra-2(gf) mutants: Two tra-2(gf) mutants (q122gf and
q179gf) were isolated as dominant XX F2 females in the
course of the fog-2 complementation screen. They were
characterized in a manner similar to that detailed above
for fog-2 mutants. These mutants are similar to the tra-
2(gf) alleles described by DONIACH (1986a), XX animals are
females while XO animals are essentially wild type males
(see Table 1).

The tra-2(q122gf) allele shows a strong dominant XX
feminizing phenotype: 100% of q122gf/q122gf, q122gf/+,
and q122gf/tra-2(gf) [for either tra-2(e1095 and e1425)]
were female in both the germ line and the soma (n > 200
for each). Males (XO) were found to be functionally normal
as judged by construction of male/female strains. When
examined by Nomarski, q122gf homozygotes were
morphologically normal except in two regards. First, 8% lacked
germ cells in the adult gonad. Second, older males (72 hr
or more post L4 at 20°) showed feminization in the germ
line [50% (n = 78) showed evidence of oogenesis] and in
the intestine (50% had yolk in the pseudocoelom). Three-
factor mapping of q122gf with respect to dpy-10 unc-4 on
LG II was consistent with the position of tra-2(gf): of 11
Dpy recombinants, one was dpy-10 tra-2(e1095 and e1425)
two were dpy-10 +, and of 11 Unc recombinants, nine were
tra-2(q122gf) unc-4 and two were unc-4. dpy-10 is about
0.1% to the left of tra-2 while unc-4 is 1.2% to the right
(HOKKIN and RIDDLE 1988). Demonstration that the
q122gf mutation is an allele of tra-2 was obtained by
isolating tightly linked revertants of the dominant XX germ
line feminizing phenotype. These revertants have a tra-
2(gf) phenotype and fail to complement tra-2(gf) (P.
OKKEMA, personal communication).

The tra-2(q179gf) allele, while exhibiting a dominant
gain-of-function phenotype in the germ line, also has
recessive loss-of-function (hypomorphic) characteristics
in the soma (see DONIACH (1986a) for a further discussion
of this phenotype). The germ-line feminization is incomplete:
73% of q179gf/q179gf animals were female (n = 108), 85%
of q179gf/+ animals were female (n = 66), and q179gf/
tra-2(e1095) animals were 100% self-fertile (n = 66). So-
matic masculinization was observed in q179gf XX homozy-
gotes. They had a truncated tail and some animals were
Egl. The penetrance of the tail masculinization and the Egl
phenotypes was increased in q179gf/tra-2(e1095) animals.
XO males were morphologically and functionally normal.
Mapping yielded four dpy-10 tra-2(q179gf) and nine dpy-10
+ recombinants and 15 tra-2(q179gf) unc-4 and three +
### Table 2

**Map data for region of Chromosome V around fog-2**

**A. Three-factor crosses**

<table>
<thead>
<tr>
<th>Gene (allele)</th>
<th>Parental genotype</th>
<th>Recombinant phenotype</th>
<th>Recombinant genotype</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>fog-2(q71)</td>
<td>fog-2/dpy-2 unc-51</td>
<td>Dpy non-Unc</td>
<td>dpy-21 + fog-2</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>dpy-21 unc-51 +</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
<td>+ unc-51 +</td>
<td>135</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unc non-Dpy</td>
<td>dpy-21 unc-51 +</td>
<td>33</td>
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<tr>
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<td></td>
<td></td>
<td>dpy-21 +</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dpy non-Fog</td>
<td>dpy-21 + fog-2</td>
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<td></td>
<td></td>
<td></td>
<td>dpy-21 +</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ste(q265) q265/fog-2</td>
<td>unc-51 fog-2</td>
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<td></td>
<td></td>
<td>unc-51 + q265</td>
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</tr>
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<td></td>
<td></td>
<td>emb-4(hc60)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>dpy-21 emb-4</td>
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<td></td>
<td>dpy-21 + unc-51</td>
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<td>Dpy non-Unc</td>
<td>dpy-21 + unc-51</td>
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<td></td>
<td></td>
<td>dpy-21 +</td>
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<td></td>
<td></td>
<td>Unc non-Dpy</td>
<td>+ emb-4 unc-51</td>
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<td></td>
<td></td>
<td>dpy-21 + unc-51</td>
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<td></td>
<td>unc-51 e369</td>
<td>dpy-21 unc-51</td>
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<td></td>
<td></td>
<td></td>
<td>dpy-21 unc-51</td>
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</tbody>
</table>

**B. Two-factor crosses**

<table>
<thead>
<tr>
<th>Gene (allele)</th>
<th>Heterozygous parent</th>
<th>Segregants</th>
<th>Map distance&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>fog-2(q71)</td>
<td>dpy-21 fog-2/ +</td>
<td>647 wild type</td>
<td>11.5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>192 Dpy Fog</td>
<td>52 Dpy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 Fog</td>
<td></td>
</tr>
<tr>
<td>unc-51 e369</td>
<td>dpy-21 unc-51/ +</td>
<td>690 wild type</td>
<td>11.4%</td>
</tr>
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<td>147 Dpy Unc</td>
<td>33 Dpy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48 Unc</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> The embryonic lethality of *emb-4(hc60)* (Miwa et al. 1980) was scored by cloning 12 L4 progeny from each of the initial recombinants and scoring for a brood of dead embryos at 25°.

<sup>b</sup> Map distance (or recombination frequency, p) was calculated from the formula $R = p - p^2/2$ (Brenner 1974). Two-factor crosses were performed at 20°.

unc-4 recombinants. A recessive roller mutation is tightly linked to *q179gf* and has not yet been separated.

Three other *tra-2(gf)* alleles have also been employed in this study (*q101gf, q103gf, and q244gf*; Z. Rosenquist, M. K. Barton and P. Okkema, personal communication). Each of these alleles is strong; *tra-2(gf)* and *tra-2(gf)/+* XX are 100% female (*n > 50*). Homozygous XX animals also have a normal female soma while XO males are unaffected.

**Double heterozygote constructions:** Double mutant heterozygotes were generated by crosses as detailed below and their phenotypes analysed by picking individual L4 XX cross progeny and examining them as adults about 24 hrs later (20°) for female morphology and the presence of eggs and larvae on plates. In some cases animals were further examined by Nomarski. The crosses to generate the desired maternal and zygotic genotypes were as follows. For *fog-1 m(−/−), z(−/+),* XX females homozygous for *fog-1(q187)* (M. K. Barton, personal communication) were crossed to
fog-2(q71) or control N2 males. For fem-1 m(+/+), z(+/+), XX females homozygous for fem-1(e1991) dpy-20, an amber, putative null allele (Doniach and Hodgkin 1984), were crossed to fog-2 or N2 males. For fem-1 m(+/+); z(+/+) females were crossed to dpy-20; fog-2 females or to control unc-24 dpy-20 hermaphrodites and Dpy cross progeny scored. For fem-2 m(+/+); z(+/+), XX females homozygous for the putative null allele (Hodgkin 1986) fem-2(e1205) were crossed with fog-2 or N2 males. For fem-3 m(+/+), z(+/+), XX females homozygous for the putative null allele (Hodgkin 1986) fem-3(e1996) dpy-20 were crossed with fog-2 or N2 males. For fem-3 m(+/+); z(+/+), XX females homozygous for the putative null allele were obtained by crossing Unc hermaphrodites unc-24 fem-3(e1996) + dpy-20 unc-24 + daf-15 + to homozygous dpy-20; fog-2 or control dpy-20; him-5 males and Dpy progeny scored. For fem-3 m(+/+), z(+/+), + fem-3(e1996) dpy-20/+ + males were crossed to unc-24 + dpy-20; fog-2 females or control unc-24 + dpy-20 hermaphrodites. The statistical significance of the difference between proportions [females/(females + self-fertiles)] for given genotypes was determined by the z-test (FLeUAND 1973) at $P < 0.05$.

**her-1(£f) fog-2 double mutant constructions:** XO animals homozygous for a putative null allele of her-1(e1520) (Hodgkin 1980) have a soma that is female and a germ line that first makes sperm and then oocytes; these XO animals are self-fertile (Table 1). him-5(e1490) was used to generate XO animals by nondisjunction of the X chromosome of progeny homozygotes (Hodgkin, Horvitz and Brenner 1979). dpy-21(e904) was used to distinguish XX and XO animals independent of sexual phenotype; XX animals are Dpy while XO animals are non-Dpy (Hodgkin 1980, 1985, 1986). dpy-21 fog-2(q71) non-Dpy (XO) males were mated with her-1 him-5 dpy-21 Dpy (XX) hermaphrodites. Dpy cross progeny + + dpy-21 fog-2 her-1 him-5 dpy-21 + were cloned; they segregated F2 Dpy females (dpy-21 fog-2 homozygotes), Dpy hermaphrodites (her-1 him-5 dpy-21 homozygotes), and Dpy parental hermaphrodites (+ + dpy-21 fog-2 her-1 him-5 dpy-21 +). Twenty-one Dpy F2 hermaphrodites were picked to individual plates in which a cross-over event had occurred between him-5 and fog-2 (distance of about 18%, see Figure 1). Three recombinant F2, her-1 him-5 dpy-21 fog-2 her-1 him-5 dpy-21 +, were found and they segregated the following progeny types: her-1 him-5 dpy-21 homozygotes that were self-fertile Dpy(XX) or non-Dpy(XO), parental her-1 him-5 dpy-21 fog-2 her-1 him-5 dpy-21 + self-fertile Dpy(XX) or non-Dpy(XO), and her-1 him-5 dpy-21 fog-2 homozygotes that were female Dpy(XX) or female non-Dpy(XO). The genotype of the non-Dpy (XO) females was confirmed by mating with fog-2 males. For one such cross, 31 cross-progeny were males, and 10 were females. In addition, a number of dead embryos were observed. These data suggest that the non-Dpy females were XO and homozygous for fog-2.

**tra-2(f£): fog-2 double mutant constructions:** XX animals homozygous for putative null alleles of tra-2(e1095 and e1423 amber) are transformed into incomplete males (Table 1, Hodgkin and Brenner 1977). Heterozygotes show a semi-dominant Egl phenotype (TRENT, TSUNG and HORVITZ 1983; DONIACH 1986a). The semi-dominant nature of tra-2; fog-2 double mutants was confirmed with either e1095 or e1425 and with one of three alleles of fog-2 (q71, q124, q247). + tra-2(e1425) unc-4 dpy-10 + unc-4 hermaphrodites were crossed with either fog-2(q71 or q124) males. For one such cross, 31 cross-progeny were males, and 10 were females. In addition, a number of dead embryos were observed. These data suggest that the non-Dpy females were XO and homozygous for fog-2. For one such cross, 31 cross-progeny were males, and 10 were females. In addition, a number of dead embryos were observed. These data suggest that the non-Dpy females were XO and homozygous for fog-2, more than 60 animals were examined at each time point for both q71 and q124. About 1% of the Unc animals were recombinants (female soma and self-fertile) of the genotype tra-2(e1425) unc-4 + based on progeny testing. The remaining (99%) Unc animals were incomplete males indistinguishable from Nomarski from tra-2(e1425) unc-4 alone.

In the course of constructing tra-2(e1095); fog-2(q71 or q247) double mutants, it was found that tra-2(f£) apparently suppresses the Fog phenotype. unc-51 fog-2(q71 or q247) females were crossed with tra-2(e1095);tra-2(q247) males. XX L4 cross progeny removed en masse and self-fertile animals picked to individual plates [tra-2(q122gf)/+; XX animals are female]. In the F2 far more Unc animals were self-fertile than expected by recombination (see Table 2 and Figure 1). Unc self-fertile animals were picked and segregated Unc Tra (29%), Unc females (54%), and Unc self-fertile (17%; n = 141 total animals for two broods of tra-2(e1095);tra-2(q247) hermaphrodites). Crossing of Unc self-fertiles with N2 males and analysis of F2 progeny confirmed that the original genotype was tra-2(e1095)+; unc-51 fog-2(q247). Analysis of >30 adult Unc incomplete males segregating from a tra-2(e1095)+; unc-51 fog-2 parent by Nomarski showed no difference from tra-2(f£) alone. In this case the fog-2 genotype is m(–/–), z(–/–). Similar results were obtained when fog-2(q71) was employed (data not shown).

Suppression of fog-2 was also observed with animals heterozygous for a deficiency (mdf30, SIGURDSON, SPANNER and HERMAN 1984) of the region around tra-2. A strain mdf30 unc-4 + unc-4; fog-2(q71) was constructed. Analysis of two broods showed segregation of dead embryos (27%, presumably mdf30 homozygotes), Unc females (57%) and Unc self-fertile hermaphrodites many of which were Egl (16%, n = 181 total embryos and animals for two broods). The genotype of self-fertile animals was verified by crossing with dpy-10 + males (dpy-10 is deleted by mdf30). From this cross, about one-fourth of the F1 cross progeny were Dpy and fog-2 segregated among progeny of all individually picked F1 males.

**tra-3: fog-2 double mutant constructions:** XX animals homozygous for a putative null allele of tra-3(e1107) amber when derived from a homozygous mutant mother [m(–/–), z(–/–)] are transformed into incomplete males (Table 1, Hodgkin and Brenner 1977). This phenotype is somewhat temperature sensitive; at 25° no animals are self-fertile, while at 15° some are self-fertile (Hodgkin 1986). At 25°, most animals have a male shaped somatic gonad (see below), while at 15° most animals have an intersexual somatic gonad.

tra-3(e1107) hermaphrodites were purged (allowed to exhaust all their sperm) crossed with unc-51 fog-2(+)+ (either q71 or q247) males and L4 cross progeny were individually picked. Non-Unc F2 animals were then individually picked, one-sixth of which have the genotype tra-3(e1107);tra-3(e1107); unc-51 fog-2(+)+. Such animals segregate a brood of all XX incomplete males with Unc animals homozygous for fog-2. Unc L4 animals were removed en masse and scored by Nomarski about 24 hr later (all at 25°). The same protocol employing unc-51+ males provided a control population for comparison. The phenotype of tra-3(e1107) m(–/–), z(–/–); unc-51 fog-2 was essentially identical to tra-3 m(–/–), z(–/–); unc-51+ (+). For both, tra-3 with either fog-2 or fog-2(+), all animals had a partially masculinized tail and all had yolk in the pseudocoelom. With fog-2(q71), 17% had an abnormal/intersexual somatic gonad, 40% had a male somatic gonad with a male germ line and 41% had a male somatic gonad with a germ line.
of first sperm and then oocytes (n = 59). Similar results were obtained with fog-2(q724). With fog-2(+), 21% had abnormal/intersex gonadal gonad, 42% had a male somatic gonad with a male germ line and 37% had a male somatic gonad and a germ line of first sperm and oocytes (n = 51). Note that an additional approximately 30% of Unc Tra animals for both fog-2 and fog-2(+) were dead at the time of scoring due to the inability to defecate as a result of a defective anus. Essentially identical germ line and somatic phenotypes were also observed when comparing a marked tra-3, unc-50 tra-3 m(=/-), z(=/-) with or without fog-2(q71) [data not shown].

fem-3(3f); fog-2 double mutant construction: Gain-of-function fem-3 mutants are self-fertile at 15° and Mog at 25°. The markers unc-24 and dpy-20 map about 1% to the left and right of fem-3 respectively. Thus Unc Dpy animals segregating from unc-24 fem-3(3f) dpy-20/+ + will be (>99%) homozygous for fem-3(3f). Homozygous fog-2 males were mated with unc-24 fem-3(3f) dpy-20 hermaphrodites (raised at 15°) and XX F1 cross progeny were picked and shifted to 25° as adults. The F2 includes unsuppressed Mog Unc Dpy animals and suppressed self-fertile Unc Dpy animals. Self-fertile unc-24 fem-3(3f) dpy-20; fog-2 stocks were established, and verified to be homozygous for both fem-3(3f) and fog-2 by crossing Unc Dpy animals with N2 males (15°), picking and shifting 12 F1 XX adults to 25°. All 12 F1 hermaphrodites segregated Unc Dpy Mogs and non-Unc non-Dpy females. Animals heterozygous for fog-2(q54 or q71) and homozygous fem-3(q20g) were obtained by crossing unc-24 fem-3(q20g) dpy-20; fog-2(q54 or q71) hermaphrodites with fem-3(q20g) dpy-20; him-5 males (at 15° or 25°) and scoring XX Dpy cross progeny.

Scoring the interaction of fem-3(3f) with fog-2 was determined as follows. L4 Unc Dpy XX animals were picked to individual plates and examined as adults either: (a) after 24 hrs later (25°) or 48 hr later (15°) for self-fertile hermaphrodite, male, and Mog phenotypes. The Unc Dpy animals were from homozygous fem-3(3f); fog-2 stocks. All worms that did not have eggs or larvae on the plate were scored by Nomarski. Animals found with clearing of fertilized eggs were scored as self-fertile hermaphrodites. Females were scored as defined above. Animals were scored as Mog if they did not have any self-progeny by 24 (25°) or 48 (15°) hr after the L4 stage and if their lack of progeny was due to a vast overproduction of sperm. Such animals include those that never make oocytes, and those that make oocytes days later than normal after producing a great excess of sperm. This procedure was also used to score the interaction between tra-2(3g) and fem-3(3g) and between tra-2(3g) and fog-2 in a fem-3(3g) background (see below).

An unmarked fem-3(q20g); fog-2(q71) stock was constructed by crossing dpy-20; fog-2(q71) males with fem-3(q20g); unc-51 hermaphrodites (15°) and obtaining a non-Dpy, non-Unc F2 XX suppressed self-fertile animal (25°) that fails to segregate Dyps or Uncs in the next generation. The stock was verified by crossing with N2 males as described above.

tra-2(3g); fem-3(3g) double mutant construction: By a strategy analogous to the fog-2; fem-3(3g) constructions described above, tra-2(3g) males were mated with unc-24 fem-3(3g) dpy-20 hermaphrodites and F2 Unc Dpy self-fertile animals (25°) were picked to obtain a suppressed self-fertile stock. As above, the stock was verified to be homozygous unc-24 fem-3(3g) dpy-20; tra-2(3g) by crossing with N2 males and analyzing the progeny segregating from 12 F1 animals (at 25°). Scoring of the interaction between

**tra-2(3g) and fem-3(3g) was also analogous to that described above for fog-2; fem-3(3g).**

**tra-2(3g); fog-2 double mutant constructions:** Male/female strains homozygous for both tra-2(3g) and fog-2 were constructed. tra-2(q122g) homozygous males were crossed to dpy-10 unc-4; fog-2 homozygous females. F1 males and females from the cross were mated to each other en massee followed by 32 matings of non-Unc non-Dpy single F2 males with single F2 females. Two single matings were identified in which both parents were tra-2(3g)/dpy-10 unc-4; fog-2 homozygous based on all >30 Dpy Uncs being females. This was followed by three generations of single male/female matings to obtain lines that fail to segregate Dpy Uncs. A male/female line was verified to be homozygous tra-2(q122g); fog-2(q71) by: (1) crossing males with dpy-10 unc-4 hermaphrodites and obtaining only non-Unc non-Dpy cross progeny; (2) intercrossing the F1, males and females from (1) and showing that about one-fourth the Dpy Uncs animals were female (because the original line is homozygous for fog-2); and (3) crossing males with unc-24, intercrossing the F1 males and females to show that about two-thirds of the Uncs females were females (because the original line is homozygous tra-2(q71)). Males and females of tra-2(q122g); fog-2(q71) were examined by Nomarski and found to be indistinguishable from tra-2(q122g) alone (see above). Similarly, a tra-2(q179g); fog-2(q71) male/female strain was constructed and verified to be homozygous for both q179g and q71. It has the recessive XX truncated tail and roller phenotypes expected of q179g homozygotes (see above).

**Triple mutants:** To construct fog-2(3f); tra-2(3g); fem-3(3g) triple mutants, homozygous XX unc-24 fem-3(3g) dpy-20; tra-2(3g) hermaphrodites were crossed with fog-2(q71) males and F1 L4 cross progeny picked (all at 25°). Unc Dpy F2 animals were picked and scored for Mog, self-fertile, and Fog phenotypes as described above for the fem-3(3g); fog-2 double. All Unc Dpy animals are homozygous for fem-3(3g), while one-sixteenth are homozygous for both fog-2 and tra-2(3g). In some cases, Unc Dpy triple mutant females were crossed with N2 males. Males cross progeny were examined for feminized phenotypes. Additionally, the Unc Dpy triple mutant females were shown to be homozygous for fem-3(3g) by picking 12 F1 progeny and showing that all segregate Unc Dpy Mogs at 25°.

A homozygous strain tra-2(e1941g); unc-24 fem-3(q20g) dpy-20; fog-2(q71) was obtained by repeated rounds of picking self-fertile Unc Dpy animals and testing for the presence of e1941g and q71. The genotype was verified by crossing with N2 males. All the male cross progeny tested (20) were shown to carry e1941g, q20g and q71 by mating single males with dpy-10 unc-4 or unc-51 hermaphrodites and examining the phenotype of F2 animals at 25°. F2 dpy-10 unc-4 females were homozygous for q71, unc-51 females were homozygous or heterozygous for e1941g and unc-24 dpy-20 Mogs were homozygous for q20g.

**RESULTS**

Isolation and characterization of fog-2 alleles: Sixteen recessive fog-2 alleles have been isolated by four different protocols (see MATERIALS AND METHODS for details). The phenotype of homozygous fog-2 mutants is the same for all alleles: XX animals are transformed from self-fertile hermaphrodites to females while XO males are unaffected (Figure 2; Table 3). In wild-type young adult hermaphrodites, sperm and pri-
primary spermatocytes are the first and most proximal gametes to differentiate within the gonad, followed by maturing oocytes (Figure 2, a and c). In fog-2 XX young adults, the first and most proximal gametes to differentiate are oocytes (Figure 2, b and d). There is no evidence of sperm, spermatogenesis, or germ cell death in fog-2 females. Further, oogenesis begins in fog-2 mutants at about the time spermatogenesis begins in wild type. In late L4, signs of oogenesis are observed in the proximal arm of each gonad and not that of primary spermatocytes. This is in contrast to the maternal effects observed for the fem genes. A maternal absence effect is observed for fem-3(+/f) such that fem-3/+ m(−/−), z(−/−) XX and XO animals are partially feminized (see Hodgkin 1986; Barton, Schedl and Kimble 1987 for details; Table 4). Thus, the absence of maternally contributed fem-3(+) impairs male germline and male somatic development in heterozygous progeny. A maternal rescue effect is observed for fem-1 and fem-2, XX and XO, and for fem-3 XO animals such that fem/fem m(−/−), z(−/−) animals are only incompletely feminized (Doniach and Hodgkin 1984; Hodgkin 1986). Here, maternally contributed fem(+) partially rescues homoygous fem(−) progeny such that some male development occurs in both the germline and soma.

Unlike XX animals, XO animals homozygous for any of the fog-2 alleles are unaffected in either germ line or soma. Detailed examination of the morphology of XO males by Nomarski (Table 3, see MATERIALS AND METHODS) showed no evidence of feminization for any of the alleles. Animals homozygous for four of the strong alleles (q71, q86, q123, and q247) were examined over time, with no evidence of feminization, even in old adults. For 15 alleles, males were shown to be fertile based on ability to mate and thus propagate biparentally as homozygous fog-2 male/female strains. In a mating competition experiment, fog-2(q71) males were indistinguishable from N2 males in ability to sire cross progeny (see MATERIALS AND METHODS).

No maternal effects have been observed for fog-2—neither a maternal absence effect [m(−−), z(−−)] nor a maternal rescue effect [m(−+), z(−−)] (Table 4, second and third rows). Thus, zygotic fog-2 activity is necessary and sufficient for hermaphrodite spermatogenesis. This is in contrast to the maternal effects observed for the fem genes. A maternal absence effect is observed for fem-3(+/f) such that fem-3/+ m(−/−), z(−/−) XX and XO animals are partially feminized (see Hodgkin 1986; Barton, Schedl and Kimble 1987 for details; Table 4). Thus, the absence of maternally contributed fem-3(+) impairs male germline and male somatic development in heterozygous progeny. A maternal rescue effect is observed for fem-1 and fem-2, XX and XO, and for fem-3 XO animals such that fem/fem m(−/−), z(−−) animals are only incompletely feminized (Doniach and Hodgkin 1984; Hodgkin 1986). Here, maternally contributed fem(+) partially rescues homoygous fem(−) progeny such that some male development occurs in both the germline and soma.

The recessive nature of fog-2 alleles was demonstrated by crossing fog-2 males with unc-51 hermaphrodites and showing that all heterozygous XX cross progeny were self-ferrile (Table 4, top row). Further, the brood size, which is limited by the number of functional sperm made (Ward and Carrel 1979), of fog-2(q71)/+ is not significantly different from that of wild type (306 ± 36 vs 328 ± 45, respectively, n = 10 in both cases; Student t-test, >95% confidence level). The penetrance of the XX fog-2 phenotype has been ranked for the 16 alleles and is shown in Table 3. There are two classes of fog-2 alleles. Four alleles are leaky (q177, q154, q251, and q124) and 12 are strong with complete penetrance of the Fog pheno-

---

**TABLE 3**

**Phenotype of fog-2 alleles**

<table>
<thead>
<tr>
<th>fog-2 allele</th>
<th>% Female</th>
<th>% Self-ferrile (hermaphrodite)</th>
<th>XO Morphology</th>
<th>Mating*</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>0</td>
<td>00 (n = 117)</td>
<td>Male</td>
<td>+</td>
</tr>
<tr>
<td>q177</td>
<td>73</td>
<td>27 (n = 309)</td>
<td>Male</td>
<td>ND</td>
</tr>
<tr>
<td>q154</td>
<td>93</td>
<td>7 (n = 442)</td>
<td>Male</td>
<td>+</td>
</tr>
<tr>
<td>q251</td>
<td>98</td>
<td>2 (n = 420)</td>
<td>Male</td>
<td>+</td>
</tr>
<tr>
<td>q124</td>
<td>99.6</td>
<td>0.4 (n = 117)</td>
<td>Male</td>
<td>+</td>
</tr>
<tr>
<td>q70, q71, q86</td>
<td></td>
<td>100%</td>
<td>Male</td>
<td>+</td>
</tr>
<tr>
<td>q113, q123, q166</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>q167, q170, q226</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>q247, q249, q263</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* See MATERIALS AND METHODS for scoring of phenotypes.
† Male/female strains.
‡ ND = not determined.
type (q70, q71, q86, q113, q123, q166, q167, q170, q226, q247, q249, q263). None of the alleles is temperature sensitive, although all alleles were isolated at one of the standard growth extremes (15°C, 4 alleles and 25°C, 12 alleles) for C. elegans.

The fog-2 alleles that have been isolated are likely to be loss-of-function. Eleven of the fog-2 mutants were isolated at a frequency of 3.2 × 10⁻⁴ and 3 × 10⁻⁵ per haploid genome after EMS mutagenesis (by the "screen for feminizing mutations" and the "screen for mutations that fail to complement fog-2" respectively, see MATERIALS AND METHODS). This is within the bounds (10⁻³ to 10⁻⁴) for the frequency of EMS induced loss-of-function mutations observed for other C. elegans genes (BRENNER 1974; GREENWALD and HORVITZ 1980; HODGKIN 1986). Additionally, all fog-2 mutants are recessive. The four leaky alleles probably represent partial loss-of-function (hypo-

morphic) mutations that retain some fog-2(+) activity. The 12 strong alleles are indistinguishable and completely penetrant; they are likely to be complete loss-of-function or null alleles. The argument for nullity is weakened somewhat by the fact that none of the fog-2 alleles is amber (see MATERIALS AND METHODS) and no deficiencies in the region have been isolated. However, since eleven fog-2 mutants were obtained by either complementation screens or complementation suppression, alleles that have a sterile or lethal phenotype should have been recovered. Since no sterile or lethal alleles of fog-2 have been isolated, the fully penetrant Fog phenotype of the 12 strong alleles probably represents the effect of the complete absence of the fog-2(+) activity. The strong allele q71 was used for all double mutant constructions and is designated as the canonical putative fog-2 null allele. In a number of cases, other strong alleles (q86, q123, or q247) were also employed and equivalent results were obtained (see below and see MATERIALS AND METHODS).

The apparent null phenotype of fog-2, in which XX animals are females while XO animals are normal males, indicates that the fog-2 product is necessary for specification of the male germ cell fate in an otherwise normal XX female soma. Also, fog-2(+) masculinizing activity is restricted to a single tissue, the germ line. The fact that fog-2 mutants have no effect on XO males indicates that the fog-2 product is not necessary per se for specification of the male germ cell fate. This contrasts with fem-1, 2, 3, and fog-1 which are each necessary for spermatogenesis in both XX and XO animals.

Interaction of fog-2 with other feminizing mutations: To test whether fog-2 mutants could enhance germ-line feminization in XX animals with other feminizing mutations, double heterozygotes were constructed and analyzed. Homozygotes of fog-1, fem-

1, fem-2, and fem-3 feminize the germ line of both XX and XO animals while fem-1, 2, and 3 also feminize the XO soma (Table 1). (Interactions with gain-of-function feminizing mutations in tra-2 are discussed below; interactions with gain-of-function mutations in tra-1 are beyond the scope of this paper.)

No fog-1/+ , fem-1/+ , or fem-2/+ heterozygotes are female (Table 4). In combination with fog-2/+ , no females were observed for either fog-1/+ or fem-2/+ (Table 4). For fem-1/+ ; fog-2/+ , a very low frequency of females was found, but it is unclear if the single events are significant. Of the three fem gene products, fem-2 is thought to be required in the smallest amount for normal male development, while fem-3 is required in the largest amount (HODGKIN 1986). The failure to observe a feminizing effect in the fog-2/+ ; fem-1/+ or fem-2/+ double heterozygotes may be a consequence of the small amount of fem-1 and 2 products necessary for normal spermatogenesis. Heterozygotes for fem-3(fly) show XX germ-line feminization that is dependent on the maternal genotype of fem-3 (HODGKIN 1986; BARTON, SCHELD and KIMBLE 1987). fem-3/+ animals were 4%, 10%, or 15% female depending on whether the mother was fem-3(+/-), fem-3+ , or fem-3/fem-3, respectively (Table 4). This is a consequence of both a haplo-

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Interaction of fog-2 with fog-1, fem-1(fly), fem-2(fly) or fem-3(fly) alleles in XX animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maternal (m) and zygotic (z) genotype of other feminizing loci</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>fog-1</td>
<td>m(-/-), z(+/-)</td>
</tr>
<tr>
<td></td>
<td>m(-/-), z(-/-)</td>
</tr>
<tr>
<td>fem-1</td>
<td>m(-/-), z(+/-)</td>
</tr>
<tr>
<td></td>
<td>m(-/-), z(-/-)</td>
</tr>
<tr>
<td></td>
<td>m(+/-), z(+/-)</td>
</tr>
<tr>
<td></td>
<td>m(-/-), z(-/-)</td>
</tr>
<tr>
<td>fem-2</td>
<td>m(-/-), z(+/-)</td>
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<tr>
<td></td>
<td>m(-/-), z(-/-)</td>
</tr>
<tr>
<td>fem-3</td>
<td>m(-/-), z(+/-)</td>
</tr>
<tr>
<td></td>
<td>m(-/-), z(-/-)</td>
</tr>
<tr>
<td></td>
<td>m(+/-), z(+/-)</td>
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<tr>
<td></td>
<td>m(-/-), z(-/-)</td>
</tr>
<tr>
<td></td>
<td>m(+/-), z(+/-)</td>
</tr>
<tr>
<td></td>
<td>m(-/-), z(-/-)</td>
</tr>
</tbody>
</table>

a Alleles used are: fog-1(q187), fem-1(es1991), fem-2(es2105) and fem-3(es1996).

b fog-2(q71).

c Results indicated with the same letter are significantly different from each other (P < 0.05; z-test [FREUND 1973]).
insufficiency for fem-3 in fem-3/+ XX animals and a maternal absence effect in fem-3/+ progeny from fem-3 mutant mothers. An enhancement of the XX germ-line feminization phenotype occurred when fem-3/+ was in combination with fog-2/+ . For a given maternal genotype of fem-3, the percentage of females was significantly increased when animals were also fog-2/+ (Table 4). Further, the difference in frequency of fem-3/+; fog-2/+ females was significantly different when mothers were either m(−/−) or m(−/+ ) for fem-3. These results indicate that reduced amount of zygotic fog-2 activity (as fog-2/+ ) in combination with a reduced amount of zygotic and/or maternal fem-3 activity caused a significant decrease in the number of XX animals that were able to initiate spermatogenesis and become self-fertile. This is consistent with fem-3 being the fem gene required in the largest amount for normal male development.

Interactions between fog-2 and her-1(lf): Loss-of-function alleles of her-1 transform XO males into self-fertile hermaphrodites—the soma is female and the germ line produces sperm and then oocytes (Hodgkin 1980; Table 1). The her-1(lf) fog-2 double mutant was constructed to ask if the spermatogenesis that occurs in a her-1 XO mutant hermaphrodite is dependent on fog-2 activity.

Self-fertile strains were constructed that were heterozygous for fog-2(q71) and homozygous for the putative her-1 null allele el520, her-1 him-5 dpy-21 fog-2/her-1 him-5 dpy-21 + (see MATERIALS AND METHODS). Such heterozygous strains segregated her-1 him-5 dpy-21 fog-2 homozygotes that were either XX or XO as a consequence of the him-5 mutation (Hodgkin, Horvitz and Brenner 1979). The dpy-21 mutation permits XX (phenotypically Dpy) and XO (phenotypically non-Dpy) animals to be distinguished independently of sexual phenotype (Hodgkin 1980, 1983, 1986). The heterozygous strains segregated both Dpy(XX) females and non-Dpy(XO) females. Thus her-1 transforms XO males into XO hermaphrodites and, in combination with fog-2, the XO hermaphrodites are transformed into females. This indicates that the spermatogenesis observed in a her-1 XO hermaphrodite is dependent on fog-2 activity. Further, although fog-2 mutants exhibit no phenotypic effect on XO animals that have a male soma, they do have an effect on XO animals that lack her-1 activity and have a female soma and hermaphrodite germ line.

The additive effect of phenotypes in XO animals for the her-1 fog-2 double mutant was also demonstrated with the temperature sensitive her-1 allele, el561. At the permissive temperature (15°) her-1(el561) XO animals are male, while at the restrictive temperature (25°) they are self-fertile hermaphrodites (Hodgkin 1984). A her-1(el561) fog-2(q71) male/female strain, propagating biparentally as XX females and XO males was obtained at 15°. When mated adults were shifted to 25° and progeny examined, both XX and XO females were obtained. A proportion of the females were shown to be XO by crosses using males marked with the X-linked gene lon-2 and observing Lon male cross progeny.

Interactions between fog-2 and tra-2(lf): Loss-of-function mutations in tra-2 transform XX animals into incomplete males. Homozygotes for putative tra-2 null alleles (el095 or el425 amber) are similar to XO males, except that tail structures are incompletely masculinized and there is no mating behavior (Hodgkin and Brenner 1977; Table 1). However, the germ line is identical to that of a wild-type male.

Examination of tra-2(lf); fog-2 homozygous double mutants showed they were phenotypically indistinguishable from tra-2(lf) alone for both the germ line and soma. This was observed for all the different combinations of alleles tested: tra-2(el425) with fog-2(q71, q124 or q247) and tra-2(el095) with fog-2(q71 or q247) (see MATERIALS AND METHODS for details). Self-fertile strains, tra-2(el095)/+ ; fog-2 (see below), were used to test the possibility that spermatogenesis in the tra-2(lf); fog-2 double had been a consequence of rescue by fog-2(+) present in the mother. It was found that tra-2(el095); fog-2(q71 or q247) (m(−/−), z(−/−)) animals were also indistinguishable from tra-2(lf) alone. Therefore, tra-2(lf) is epistatic to fog-2(lf). This indicates that the spermatogenesis observed in the tra-2(lf) XX mutants is not dependent on fog-2 activity.

Animals heterozygous for tra-2(lf) and homozygous for fog-2 are partially suppressed. When tra-2(el095)/+ ; fog-2 animals are examined, 26% (q247) or 28% (q71) were self-fertile (see MATERIALS AND METHODS). The same result was obtained if a deficiency for the region around tra-2, mnDf30 (Sigurdsom, Spanier and Herman 1984) was used; 33% of mnDf30/+ ; fog-2(q71) homozygotes were self-fertile. This suppression of the Fog phenotype suggests that fog-2 mutants fail to undergo spermatogenesis as a consequence of tra-2(+) feminizing activity. When tra-2(+) activity is reduced in the tra-2(lf)/+ ; fog-2 double mutant some spermatogenesis can occur in the absence of fog-2 activity. Note that in contrast to tra-2(lf); fog-2 homozygotes which have a masculinized soma (see above), in tra-2(lf)/+ ; fog-2 mutants, spermatogenesis occurs in animals with an essentially female soma. The suppression of fog-2 by tra-2(lf)/+ shown here, and the enhancement of fem-3(gf) by tra-2(lf)/+ described by Barton, Schedl and Kimble (1987) reveal a haplo-insufficiency for tra-2 in the germ line. A haplo-insufficiency for some somatic characteristics has also been demonstrated for tra-2 (Trent, Tsung and Horvitz 1983; Doniach 1986a).

Interaction of fog-2 with tra-3: An incomplete male phenotype is observed for XX tra-3 homozygotes
(using the putative null allele *e1107*) when they are derived from a homozygous mutant mother (Hodgkin and Brenner 1977; Table 1). *tra-3(e1107) m(−/−), z(−/−) XX pseudomales have an incompletely masculinized tail, continue to synthesize yolk, and, in general (79%), have a male somatic gonad. The germ line is also incompletely masculinized; 97% of animals first make sperm and then oocytes (n = 51, at 25°C). Essentially the same germ-line and somatic phenotypes were obtained when *tra-3(e1107) m(−/−), z(−/−)* was homozygous for either *fog-2(q71)* or *q247* (see MATERIALS AND METHODS). No additional feminization of either the soma or germ line was observed, (41%, n = 59 make sperm and then oocytes). Thus, *tra-3* is epistatic to *fog-2.*

Both *tra-2* and *tra-3* are epistatic to *fog-2.* In contrast, *fem-1, 2,* and 3 are epistatic to *tra-2* and *tra-3* in both germ line and soma (Doniaich and Hodgkin 1984; Hodgkin 1986), and *fog-1* is epistatic to *tra-2* and *tra-3* in the germ line (Doniaich 1986b; M. K. Barton, personal communication). These results suggest that the role of *fog-2* in promoting the male germ cell fate is fundamentally different from that of *fem-1, 2, 3,* and *fog-1.*

**fog-2 suppresses fem-3(gf):** Gain-of-function mutations in *fem-3* result in complete masculinization of the germ line of *XX* animals while the female soma is unaffected (Barton, Schedl and Kiemle 1987). Thus germ cells that would have normally undergone oogenesis instead undergo spermatogenesis. The three *fem-3(gf)* alleles used in this study have the following ranking of mutant strength: *q20gf* < *q96gf* < *q95gf.* Note that the *fem-3(gf)* allele *q20gf* (and probably *q96gf* and *q95gf*) does not simply increase the amount of *fem-3* but rather causes unregulated or inappropriate activity (Barton, Schedl and Kimble 1987).

When *fem-3(gf); fog-2* XX double mutants were constructed, the Mog and Fog phenotypes were both suppressed resulting in self-fertility at 25°C (Table 5). The penetrance of the suppression of *fem-3(q20gf)* by *fog-2* at restrictive temperature (25°C) was complete (100% self-fertile) for the seven *fog-2* alleles tested. Four of the alleles, *fog-2(q154, q124, q226,* and *q71,* were isolated independent of their suppression of *fem-3(gf)* while three, *fog-2(q113, q247,* and *q249,* were isolated in selections for *fem-3(gf)* suppressors. At 15°C, the permissive temperature for *fem-3(q20gf)*, the double mutants remain fully self-fertile. A similar result was obtained with doubles of *fem-3(q20gf)* and *fem-1(hc171)* or *fem-2(b2451s)* (Barton, Schedl and Kimble 1987). Two alleles, *fog-2(q71* and *q154,* were further tested for suppression in one copy and found to be weak dominant suppressors of *fem-3(q20gf).* Interestingly, the leaky allele, *q154,* suppresses as well in one or two copies as the putative null allele *q71* (Table 5). The stronger *fem-3(gf)* allele, (*q96gf*) was also completely suppressed by *fog-2(q71).* For the strongest *fem-3(gf)* allele, *q95, fog-2(q71)* did not suppress at 25°C, but at 15°C *q71* partially suppressed *q95,* significantly increasing its self-fertility (Table 5).

An unmarked *fem-3(q20gf); fog-2(q71)* strain was constructed and found to be essentially identical to wild type except for an increased brood size. Brood sizes for *fem-3(q20gf); fog-2(q71)* were 415(±24) at 15°C, 487(±39) at 20°C, and 296(±41) at 24°C compared to N2 which were 317(±32) at 15°C, 355(±37) at 20°C, and 247(±55) at 24°C. Males were functionally and morphologically normal. The normal male phenotype of *fem-3(gf); fog-2* contrasts to that of *fem-3(q20gf)* with *fem-1(hc171)* or *fem-2(b2451)* in which XO animals are still mutant due to lack of suppression of the XO somatic feminizing effects of *fem-1* and *fem-2* alleles (Barton, Schedl and Kimble 1987).

Thus, an essentially normal self-fertile strain is regenerated in the *fem-3(q20gf)* or *q96gf; fog-2* doubles by mutual suppression of the Fog and Mog phenotypes. The continued spermatogenesis observed in adult *fem-3(gf)* mutants must depend on *fog-2* germ-line masculinizing activity. In the absence of *fog-2* activity, adult spermatogenesis stops and oogenesis ensues. However, spermatogenesis (in late L4 and as a young adult) and the switch to oogenesis occurs in the absence of *fog-2* activity in the *fem-3(q20gf)* and *q96gf* mutants. Therefore, *fem-3(gf)* obviates the requirement for *fog-2* activity to initiate hermaphrodite spermatogenesis and switch to oogenesis.

**tra-2(gf) mutants:** Most gain-of-function alleles of *tra-2* have a dominant feminizing effect on the XX germ line, have essentially no effect on the XX soma, and have essentially no effect on the germ line or

---

**TABLE 5**

 Interaction of *fog-2* with *fem-3(gf)* in XX animals

<table>
<thead>
<tr>
<th>Allele</th>
<th>15°C % Self-fertile</th>
<th>25°C % Self-fertile</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>fem-3(gf)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>q20gf</em></td>
<td>+</td>
<td>100 (n &gt; 200)</td>
</tr>
<tr>
<td><em>q20gf</em></td>
<td>*q154/+</td>
<td>100 (n = 60)</td>
</tr>
<tr>
<td><em>q20gf</em></td>
<td>*q154</td>
<td>100 (n = 75)</td>
</tr>
<tr>
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<td>*q124</td>
<td>100 (n = 66)</td>
</tr>
<tr>
<td><em>q20gf</em></td>
<td>*q226</td>
<td>100 (n = 72)</td>
</tr>
<tr>
<td><em>q20gf</em></td>
<td>*q113</td>
<td>100 (n = 72)</td>
</tr>
<tr>
<td><em>q20gf</em></td>
<td>*q247</td>
<td>100 (n = 72)</td>
</tr>
<tr>
<td><em>q20gf</em></td>
<td>*q249</td>
<td>100 (n = 72)</td>
</tr>
<tr>
<td><em>q20gf</em></td>
<td>*q71/+</td>
<td>100 (n = 50)</td>
</tr>
<tr>
<td><em>q20gf</em></td>
<td>*q71</td>
<td>100 (n &gt; 200)</td>
</tr>
<tr>
<td><em>q96gf</em></td>
<td>+</td>
<td>100 (n &gt; 200)</td>
</tr>
<tr>
<td><em>q96gf</em></td>
<td>*q71</td>
<td>100 (n = 98)</td>
</tr>
<tr>
<td><em>q93gf</em></td>
<td>+</td>
<td>28 (n = 72)</td>
</tr>
<tr>
<td><em>q93gf</em></td>
<td>*q71</td>
<td>85 (n = 72)</td>
</tr>
</tbody>
</table>

* From stocks homozygous for both *fem-3(gf)* and *fog-2.* See MATERIALS AND METHODS for details.

* The remaining animals that were not self-fertile were *Mog.*

* Data from Barton, Schedl and Kiemle (1987).
soma of XO males (Table 1; Doniach 1986a; this paper). This phenotype contrasts with that of tra-2 (gf) alleles—masculinization of both germ line and soma of XX animals with no effect on XO males (Hodgkin and Brenner 1977). The gain-of-function phenotype of tra-2 (gf) alleles in the germ line of XX animals may be the result of increased, unregulated, or inappropriate tra-2 or fog-2 activity (Doniach 1986a).

Two tra-2 (gf) mutants were isolated in the “screen for mutations that fail to complement fog-2” (see Materials and Methods). The phenotype of tra-2 (q122gf) is strong: XX animals are female in both the germ line and the soma as homozygotes, as heterozygotes, and in trans to a putative tra-2 null allele. q122gf XO animals are generally unaffected, although old males show some oogenesis and oocyte synthesis. The phenotype of tra-2 (q179gf) is weaker: XX animals show semidominant germ-line feminization and recessive somatic masculinization (truncated tail and Egl). The somatic masculinization increases in trans to a putative tra-2 null allele (see Materials and Methods). tra-2 (q179gf) XO animals are unaffected. Weak tra-2 (gf) alleles like q179gf, which are gain-of-function in the germ line but partial loss-of-function in the soma, are discussed in more detail by Doniach (1986a).

No maternal absence effect, such as that of fem-3 (gf) (Hodgkin 1986; Barton, Schedl and Kimble 1987), was observed for tra-2 (gf) alleles. Neither heterozygous tra-2 (gf)/+ XO males derived from homozygous tra-2 (gf) mothers nor +/+ XX hermaphrodites derived from tra-2 (gf)/+ mothers were feminized (data not shown; Doniach 1986a).

Interaction of tra-2 (gf) with fem-3 (gf): Examination of tra-2 (gf)/fem-3 (gf) XX homozygous double mutants reveals mutual suppression of germline feminizing and masculinizing phenotypes resulting in self-fertility at both 15° and 25° (Table 6; Barton, Schedl and Kimble 1987). When fem-3 (q20gf) was used, the degree of suppression depended on the tra-2 (gf) allele. One group of alleles, q179gf and e1941gf, almost completely suppressed fem-3 (q20gf) such that while most animals were self-fertile, some remained Mog (Table 6). Another group of tra-2 (gf) alleles, q103gf, q122gf, q244gf, and e2046gf, completely suppressed fem-3 (q20gf) such that all animals were self-fertile. A final group of tra-2 (gf) alleles, q101gf and e2020gf, not only suppressed fem-3 (q20gf) but were partially epistatic to it since some animals were females (Table 6).

When the strongest fem-3 (gf) allele, q95gf, was used, it was fully epistatic to tra-2 (q122gf) at 25° (Table 6). However, q122gf does increase the self-fertility of q95gf at 15° (data not shown). By contrast, tra-2 (e2020gf) was able to partially suppress q95gf at 25° (Table 6). e2020gf was the strongest of the tra-2 (gf) alleles with respect to suppression of fem-3 (q20gf and q95gf). This allele also shows the strongest feminizing effect on XO males (Doniach 1986a).

Interaction of fog-2 with tra-2 (gf) (XX): There are two simple mechanisms by which fog-2 might promote hermaphrodite spermatogenesis: fog-2 could act as a negative regulator of tra-2 and tra-3, or alternatively, fog-2 could act independently and positively on the fem genes and fog-1, rendering them insensitive to the negative regulatory action of tra-2 and 3 (see Discussion; Figure 3, models 1 and 2). The loss-of-function phenotype of fog-2 is essentially identical to the gain-of-function phenotype of tra-2. This suggests that the nature of the gain-of-function lesions of tra-2 (gf) alleles can be used to distinguish between the two possible mechanisms of fog-2 action: negative regulation of tra-2, or positive regulation of the fem genes and fog-1. The nature of tra-2 (gf) alleles can be tested by removing fog-2 activity. If fog-2 negatively regulates tra-2, one might expect that the defect in some tra-2 (gf) alleles is insensitivity to this regulation. The phenotype of such fog-2 insensitive tra-2 (gf) alleles should not be affected (enhanced) by removal of fog-2 activity. Additionally, the phenotype of other tra-2 (gf) alleles may be affected (enhanced) by removal of fog-2 activity even though fog-2 negatively regulates tra-2. The defect in such tra-2 (gf) alleles may be hyperactivity or an inappropriate interaction with the downstream fem genes and fog-1 and yet they remain sensitive to fog-2 activity. However, if fog-2 and tra-2 act independently, then the phenotype of all tra-2 (gf) alleles should be affected (enhanced) by removal of fog-2 activity. In this case, the defect in all the tra-2 (gf) alleles may be hyperactivity or inappropriate.

### Table 6

<table>
<thead>
<tr>
<th>Allele</th>
<th>Phenotype at 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>fem-3(gf)</td>
<td>tra-2(gf)</td>
</tr>
<tr>
<td>q20gf</td>
<td>q179gf</td>
</tr>
<tr>
<td>q20gf</td>
<td>e1941gf</td>
</tr>
<tr>
<td>q20gf</td>
<td>q103gf</td>
</tr>
<tr>
<td>q20gf</td>
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<td>q244gf</td>
</tr>
<tr>
<td>q20gf</td>
<td>e2046gf</td>
</tr>
<tr>
<td>q20gf</td>
<td>q101gf</td>
</tr>
<tr>
<td>q20gf</td>
<td>e2020gf</td>
</tr>
<tr>
<td>q95gf</td>
<td>q122gf</td>
</tr>
<tr>
<td>q95gf</td>
<td>q20gf</td>
</tr>
<tr>
<td>q95gf</td>
<td>e2020gf</td>
</tr>
</tbody>
</table>

* From stocks homozygous for both fem-3(gf) and tra-2(gf) grown at 25°.
* Mog—masculinization of the germ line, see MATERIALS AND METHODS for a description of the phenotype and its scoring.

See Doniach (1986a) for a description of these tra-2(gf) alleles.

Data from Barton, Schedl and Kimble (1987).

An additional 2% of animals produced no self-progeny. However, they were not Mog and contained sperm and oocytes of normal morphology in the normal positions.
interaction with the downstream *fem* genes and *fog-1*. As described in detail below, the seven *tra-2*(gf) alleles tested are all sensitive to *fog-2* activity: all showed an enhanced *XX* germ-line feminization phenotype when *fog-2* was removed. This does not allow one to distinguish between either model (see above, and see discussion), since both models predict a class of *tra-2*(gf) alleles that are sensitive to *fog-2* activity.

The interaction of *tra-2*(q179gf) and *fog-2*(q71) was examined by constructing a homozygous male/female strain. The *tra-2*(q179gf) single mutant is incompletely penetrant; 73% of *XX* homozygotes are female while the remaining 27% are self-fertile (see materials and methods). Enhanced germ-line feminization was observed when *tra-2*(q179gf); *fog-2*(q71) was examined as 100% of *XX* animals were female (*n* > 250). This indicates that the incomplete penetrance (residual spermatogenesis) of *q179*gf) is dependent on *fog-2* activity.

For the remaining *tra-2*(gf) alleles which have the same fully penetrant phenotype as *fog-2*, the interaction was tested in a *fem-3*(gf) background. In this background, all *fem-3*(q20gf); *fog-2* double mutants are self-fertile (Table 5), and most *tra-2*(gf); *fem-3*(q20gf) double mutants are self-fertile (Table 6). One then tests whether *tra-2*(gf); *fem-3*(q20gf) becomes further feminized (female) when *fog-2* activity is removed in the triple mutant. Since the *tra-2*(gf); *fem-3*(gf); *fog-2* triple mutants are, in most cases, female (see below), the phenotype could not be assessed in animals from triple homozygous mutant strains. Instead, the germ-line phenotypes of *fem-3*(gf) Unc Dpy homozygotes segregating from a *unc-24 fem-3*(gf) *dpy-20/+; *tra-2*(gf)/+; *fog-2*/+ hermaphrodite were scored (see materials and methods). Among the segregants, one-sixteenth (6.25%) are the triple mutant homozygotes. The phenotypes of these *fem-3*(gf) Unc Dpy homozygous segregants are shown in Table 7. Note that since *tra-2* (+) and *fog-2* (+) are present in the mother, progeny with a Mog phenotype will be observed.

The five *tra-2*(gf) alleles (*q103gf, q122gf, q244gf, e2046gf, and q101gf) showed enhanced feminization when *fog-2* activity was removed as females were observed among the segregating triple mutants (Table 7). For the *tra-2*(gf) alleles (*q122gf, e2046gf, q103gf and q244gf), doubles with *fem-3*(q20gf) were 100% self-fertile (Table 6). In the segregating triple mutants for these four *tra-2*gf alleles, more than one-sixteenth of the animals were female (Table 7). Since about three-sixteenths of the *fem-3*(gf) segregants were female, not only were the *tra-2*(gf); *fog-2* homozygotes female, but some animals homozygous for either *fog-2* or *tra-2gf* and heterozygous for the other must also have been female. The *tra-2*(q101gf); *fem-3*(q20gf) double has a low percentage of females (4%, Table 6). Yet, among the segregants of *fem-3*(q20gf)/+; *tra-2*(q101gf)/+; *fog-2*/+, 22% of the *fem-3*(q20gf) segregates were female. Enhanced feminization was even observed in triple mutant segregants when *fem-3*(q95gf) was used. *fem-3*(q95gf) is epistatic to both *tra-2*(q122gf) (Table 6) and *fog-2*(q71) (Table 5). The enhanced feminization phenotype indicates that these five *tra-2*(gf) alleles are sensitive to *fog-2* and that the spermatogenesis observed in *tra-2*(gf); *fem-3*(q20gf) double mutants (Table 6) is a consequence of *fog-2* (+) activity. In the absence of *fog-2* activity in the triple mutant, spermatogenesis was not observed. When triple mutant female segregants were crossed with N2 males, all XO male progeny were wild type. This is in contrast to the maternal absence effect observed in XO progeny of *fem-3*(lf) females. Thus, even though *tra-2*(gf); *fog-2* mutations abolish *fem-3*(gf) activity (animals are female not Mog), this is not equivalent to the absence of *fem-3* activity in a *fem-3*(lf) female.

Females were not observed when the *tra-2*(gf); *fem-3*(q20gf); *fog-2*(q71) triple mutants were assessed using *tra-2*(q179gf or e1941gf) (Table 7). The phenotype of *tra-2*(e1941gf) was shown to be sensitive to *fog-2* activity by examining a strain homozygous for *tra-2*(e1941gf); *fem-3*(q20gf); *fog-2*(q71) (see materials and methods). The triple mutant strain was 100% self-fertile (*n* > 212). This represents an enhanced feminization compared to the *tra-2*(e1941gf); *fem-3*(q20gf) double mutant which was 91% self-fertile and 9% Mog (Table 6).

### Table 7

<table>
<thead>
<tr>
<th>Allele</th>
<th>Phenotype of segregants*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>fog-2</em></td>
<td><em>fem-3</em>(gf)</td>
</tr>
<tr>
<td>q71 q20gf</td>
<td>q179gf</td>
</tr>
<tr>
<td>q71 q20gf</td>
<td>e1941gf</td>
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<td>q103gf</td>
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</tr>
<tr>
<td>q71 q20gf</td>
<td>q244gf</td>
</tr>
<tr>
<td>q71 q20gf</td>
<td>e2046gf</td>
</tr>
<tr>
<td>q71 q20gf</td>
<td>q101gf</td>
</tr>
<tr>
<td>q71 q95gf</td>
<td>q122gf</td>
</tr>
</tbody>
</table>

* All segregants are *XX* animals homozygous for *unc-24 fem-3*(gf) *dpy-20* and are homozygous, heterozygous or wild-type for *tra-2*(gf) and for *fog-2*. All at 25°, see text for details.

### Interaction of *fog-2* with *tra-2*(gf) (XO): In an attempt to reveal an effect of *fog-2* on the XO male germline (or somatic) phenotype, male/female strains were constructed that were homozygous for both *fog-2*(q71) and *tra-2*(q122gf) or *tra-2*(q179gf). XO males were examined in the course of the construction and in the final strains. Double mutant males were found to be indistinguishable from q122gf or q179gf males alone. Thus, in contrast to the additive effect observed in *XX* *fog-2*(q71); *tra-2*(q122gf) animals in a
fem-3(gf) background, or fog-2(q71); tra-2(q179gf) alone (see above), there is not an additive effect for tra-2(gf) and fog-2 in the germ line of XO males.

DISCUSSION

Sex determination in the hermaphrodite germ line: The C. elegans hermaphrodite gonad makes sperm first and then oocytes. This raises two major questions about the control of sex determination in the hermaphrodite germ line. How is male germ-line development (spermatogenesis) initiated within a female soma? and how is the switch from male to female germ-line development (oogenesis) achieved? Sex determination in the hermaphrodite germ line is not specified during early development (BARTON, SCHEDL and KIMBLE 1987). Throughout larval and adult life, a mitotic stem cell population generates meiotic germ cells for the continued production of gametes (HIRSH, OPPENHEIM, and KLASI 1976; KIMBLE and WHITE 1981). By manipulating temperature and thereby the state of a temperature sensitive gain-of-function allele of fem-3, either spermatogenesis or oogenesis can be induced after the L4 stage, independent of the type of gametogenesis that occurred previously (BARTON, SCHEDL and KIMBLE 1987). Therefore, the choice between spermatogenesis and oogenesis appears to be made continuously in a population of uncommitted germ cells late in development.

fog-2 regulates the sex determining genes to initiate male development in a female soma: The results of this study indicate that the fog-2 locus is normally required for initiation of spermatogenesis in hermaphrodites. XX animals homozygous for a loss-of-function allele of fog-2 are transformed from self-fertile hermaphrodites to females, whereas XO fog-2(If) mutant males are indistinguishable from wild-type males. Therefore, fog-2 is required for spermatogenesis in XX hermaphrodites but not in XO males. Since the sexual fate of the germ line but not the soma is transformed in fog-2 mutants, fog-2 is a germ-line-specific sex determination gene. Further, XO mutant hermaphrodites, homozygous for her-1, are transformed into females in the absence of fog-2 (a her-1 fog-2 double mutant). Therefore, fog-2 has a hermaphrodite-specific mutant phenotype, rather than an XX specific one. Thus, fog-2 does not appear to be controlled by the ratio of X chromosomes to autosomes. The germline- and hermaphrodite-specific properties of the fog-2 mutant phenotype are expected for a gene that regulates the onset of spermatogenesis in hermaphrodites, but does not specify spermatogenesis per se.

In contrast to fog-2, the fem genes, fem-1 (DONIACH and HODGKIN 1984), fem-2 and fem-3 (HODGKIN 1986) and fog-1 (DONIACH 1986b; M. K. BARTON, personal communication) promote spermatogenesis in both XX and XO animals, whether hermaphrodite or male. Also in contrast to fog-2, the fem genes and fog-1 are essential for specification of spermatogenesis. No sperm are made in animals that lack any of the fem genes or fog-1 in any genetic background tested to date. Thus, spermatogenesis does not occur in double mutants with fem-1(If), fem-2(If), fem-3(If), or fog-1 plus any of the tra genes (DONIACH and HODGKIN 1984; HODGKIN 1986; DONIACH 1986b; M. K. BARTON, personal communication) or plus any of several other germline masculinizing mutations (T. SCHEDL and M. K. BARTON, unpublished results). The essential nature of the fem genes and fog-1 in the germ line places their regulatory activity most proximate to the genes that direct male germ cell differentiation.

fog-2 appears to be the regulatory gene that activates the fem genes and fog-1 in the hermaphrodite germ line to achieve spermatogenesis. Positive regulation of fem-3 by fog-2 is indicated by the phenotypes of double mutants with fog-2 and fem-3. Thus, fog-2(If) enhances fem-3(If), but it suppresses fem-3(gf). How fem-1, fem-2, fem-3, and fog-1 interact to direct spermatogenesis is unknown, and therefore they will be considered here as a group. The regulatory action of fog-2, which may be indirect or direct (see below, Figure 3), needs only to affect the activity of one of these genes or gene products. Since fog-2 mutants show no maternal effect, regulation by fog-2 is likely to be zygotic.

The activity of fog-2 is opposite to that of tra-2 in regulating the onset of hermaphrodite spermatogenesis. Not only do loss-of-function alleles of fog-2 and tra-2 have opposite effects on the sexual phenotype of the germ line, but also fog-2(If) and tra-2(gf) have equivalent effects on that phenotype. The phenotypes of both fog-2(If) and tra-2(gf) are similarly restricted to hermaphrodites (either XX or XO), and both fog-2(If) and tra-2(gf) suppress fem-3(gf) to generate self-fertile hermaphrodites.

Figure 3 presents two models by which fog-2 may promote hermaphrodite spermatogenesis. In model 1, regulation is indirect: fog-2 promotes spermatogenesis by negatively regulating tra-2 and tra-3. The role of tra-2 and tra-3 as negative regulators of the fem genes and fog-1 is well documented (DONIACH and HODGKIN 1984; HODGKIN 1986; DONIACH 1986b; M. K. BARTON, personal communication). Further, based on the phenotype of gain-of-function alleles of tra-2, DONIACH (1986a) has suggested that the activity of tra-2 may be modulated in the hermaphrodite germ line to achieve spermatogenesis. In model 2, regulation is direct: fog-2 acts positively on the fem genes and fog-1, while independently, tra-2 and tra-3 act negatively on the fem genes and fog-1. In both models, oogenesis is represented as a default state that takes place in the absence of spermatogenesis,
Fern and Fog-2 Germ-line Sex Determination in C. elegans. For both models, the hierarchy of regulatory interactions exists in two sequential states which specify the hermaphrodite germ-line pattern of first sperm and then oocytes. In the first state (a), the three fem genes and fog-1 are active, and uncommitted germ cells develop as sperm. In the second state (b), the three fem genes and fog-1 are inactive, and uncommitted germ cells develop as oocytes. Models 1 and 2 differ primarily in the manner in which fog-2 acts as a positive regulator of spermatogenesis (part [a]). In model 1, positive regulation of spermatogenesis by fog-2 is indirect, by acting as a negative regulator of tra-2 and tra-3. In model 2, the regulation is direct, fog-2 acts positively on the fem genes and fog-1 rendering them insensitive to the independent negative action of tra-2 and tra-3. Maternally contributed fem (+) would, in both models, contribute to the net amount of masculinizing activity to be regulated. In part (b) of both models, oogenesis represents a default state that takes place in the absence of spermatogenesis; oogenesis occurs when the fem genes and fog-1 are inactivated as a result of tra-2 and tra-3 activity (Doniach and Hodgkin 1984; Hodgkin 1986; Doniach 1986b; M. K. Barton, personal communication). This is presumably a consequence of the switch from spermatogenesis to oogenesis being effaced is unknown (see text). It should be recognized that the interactions shown in models 1 and 2 are formalisms based on genetic experiments and do not imply molecular mechanisms of regulation. Further, since there is no evidence for physical interactions between these genes/products, it is possible that the regulation occurs through other unidentified elements. Finally, since gain-of-function mutations may change the mode of action of a gene/product in unpredictable ways, one must be cautious about conclusions reached using them.

Figure 3.—Two models for the genetic control of hermaphrodite germ-line sex determination in C. elegans. The interactions between genes in the two models are either negative (−) or positive (+) and determine whether a gene (or its product) is active or inactive. For both models, the hierarchy of regulatory interactions exists in two sequential states which specify the hermaphrodite germ-line pattern of first sperm and then oocytes. In the first state (a), the three fem genes and fog-1 are active, and uncommitted germ cells develop as sperm. In the second state (b), the three fem genes and fog-1 are inactive, and uncommitted germ cells develop as oocytes. Models 1 and 2 differ primarily in the manner in which fog-2 acts as a positive regulator of spermatogenesis (part [a]). In model 1, positive regulation of spermatogenesis by fog-2 is indirect, by acting as a negative regulator of tra-2 and tra-3. In model 2, the regulation is direct, fog-2 acts positively on the fem genes and fog-1 rendering them insensitive to the independent negative action of tra-2 and tra-3. Maternally contributed fem (+) would, in both models, contribute to the net amount of masculinizing activity to be regulated. In part (b) of both models, oogenesis represents a default state that takes place in the absence of spermatogenesis; oogenesis occurs when the fem genes and fog-1 are inactivated as a result of tra-2 and tra-3 activity (Doniach and Hodgkin 1984; Hodgkin 1986; Doniach 1986b; M. K. Barton, personal communication). This is presumably a consequence of the switch from spermatogenesis to oogenesis being effaced is unknown (see text). It should be recognized that the interactions shown in models 1 and 2 are formalisms based on genetic experiments and do not imply molecular mechanisms of regulation. Further, since there is no evidence for physical interactions between these genes/products, it is possible that the regulation occurs through other unidentified elements. Finally, since gain-of-function mutations may change the mode of action of a gene/product in unpredictable ways, one must be cautious about conclusions reached using them.

because no genes essential to specification of oogenesis (as the fem genes and fog-1 are essential to specification of spermatogenesis) have been identified to date.

The interactions between fog-2 and either tra-2 or tra-3 have been investigated in double mutants. The phenotype of tra-2(If); fog-2(If) is the same as that of tra-2(If) alone: sperm are made continuously in a masculinized soma. Similarly the phenotype of tra-3(If); fog-2(If) is essentially the same as that of tra-3(If) alone: some sperm are made in a masculinized soma. Thus, in the absence of tra-2 or tra-3 activity, fog-2 activity is not necessary to initiate spermatogenesis. One interpretation of the epistasis of tra-2(If) and tra-3(If) over fog-2 is that fog-2 acts to regulate tra-2 and tra-3 (model 1). In the absence of tra-2 or tra-3 activity, fog-2 is no longer required for negative regulation. An alternative interpretation is that fog-2 acts positively, rendering the fem's and fog-1 insensitive to the negative action of tra-2 and tra-3 (model 2). In the absence of tra-2 or tra-3, counteraction by fog-2 is no longer required.

Models 1 and 2 might have been distinguished by examination of interactions between fog-2 and tra-2(gf) mutations. If fog-2 negatively regulated tra-2 (model 1), a class of tra-2(gf) alleles would be predicted that is insensitive to fog-2 regulation. Such tra-2(gf) alleles would be unaffected by removal of fog-2 activity. tra-2(gf) alleles were tested in an attempt to find a class that is unaffected by removal of fog-2. However, all seven tra-2(gf) alleles tested were sensitive to the state of fog-2.

There is currently no evidence that favors one of the two models presented in Figure 3 over the other. It might be argued from the complete epistasis of the mutant phenotypes of tra-2 and tra-3 over that of fog-2 that model 1 is more likely. However, in support of model 2 is the apparent balancing of germ-line masculinizing and feminizing activities observed in double mutants. Thus, whereas fog-2(If) and tra-2(gf) mutants are feminized and fem-3(gf) mutants are masculinized, most fog-2; fem-3(gf) and tra-2(gf); fem-3(gf) double mutants are self-fertile hermaphrodites. Further, fog-2(If) is partially suppressed when tra-2(+) feminizing activity is reduced in tra-2(If)/+ double mutants.

It is likely that the activity rather than the synthesis of fem-3 is regulated by tra-2 and fog-2. The presence or absence of fem-3 in the hermaphrodite/female germ line can be deduced by examination of the sexual phenotype of her progeny. In particular, the XO progeny of mothers homzygous for fem-3(If) are feminized; therefore, fem-3 maternal product is required for normal sex determination of the embryo. However, no feminization has been observed among XO progeny of single mutant [fog-2 or tra-2(gf)], double mutant [tra-2(gf); fog-2], or triple mutant [tra-2(gf); fem-3(gf); fog-2] mothers. Instead, the sexual phenotype of these progeny is normal. Therefore, functional maternal product of fem-3 must be contributed to these embryos, even though it has not directed spermatogenesis in the mother’s germ line.

Fog-2 and the switch from spermatogenesis to oogenesis: The switch from spermatogenesis to oogenesis requires that the fem genes and fog-1 no longer direct spermatogenesis. Regulation of fem-3 appears to be key to the sperm/oocyte switch, because gain-of-function alleles of fem-3 no longer make the switch (Barton, Schedl and Kimble 1987). In addition, the
tra-2 gene appears to be required for the switch, because in the absence of tra-2, sperm are made continuously (Hodgkin and Brenner 1977). Given the proposed role of fog-2 in the onset of spermatogenesis, it is plausible that fog-2 must be negatively regulated to switch to oogenesis. In both models 1 and 2 (Figure 3), inactive fog-2 permits tra-2 and tra-3 activity to mediate the switch to oogenesis.

XX animals possessing fem-3(gf) make sperm continuously; no oocytes are seen. The double mutant, fem-3(gf); fog-2, makes sperm and then oocytes. Therefore, fem-3(gf) bypasses the need for fog-2 to initiate spermatogenesis. Yet, in a fem-3(gf) genetic background, fog-2(+) prevents the switch to oogenesis. However, an inactive fog-2 gene reinstates the switch. Perhaps fog-2 remains “on” (e.g., active, stable) in fem-3(gf) hermaphrodites, and it must become inactive to make oocytes. If so, then perhaps there is a regulatory feedback loop that normally inactivates fog-2 and it is defective in fem-3(gf) animals.

Conclusions and speculations: Sex determination in the germ line, production of sperm or oocytes, depends on the state of activity of the fem genes and fog-1. In hermaphrodites, these genes must first be active to direct male germ-line development, and then inactive to permit female germ-line development. The choice between spermatogenesis and oogenesis occurs in uncommitted germ cells that are present in the germ line even in the adult. Regulation of the fem genes and fog-1 depends on the activities of tra-2, tra-3, and fog-2. The fog-2 locus, a previously undescribed sex determination gene, is required for production of sperm in hermaphrodites, whereas tra-2 and tra-3 regulate sex determination in both somatic and germline tissues. We propose that fog-2, a germ-line- and hermaphrodite-specific sex determination gene, acts to regulate the sex determination hierarchy between two alternate states: active fog-2 leads to active fem genes and fog-1 and spermatogenesis, and inactive fog-2 leads to inactive fem genes and fog-1 and oogenesis. The mechanism by which the switch from spermatogenesis to oogenesis occurs, however, remains unknown.

It has been argued by Hodgkin (1987), based on the prevalence of male/female reproduction among nematodes, that hermaphroditism in C. elegans is likely to be a secondary specialization of an ancestral female sex. Given that the somatic tissues of C. elegans are extremely similar to those of females of C. remanei (Sudhaus 1974; T. Schedl, unpublished) and Panagrellus (Sternberg and Horvitz 1981; 1982), it is possible that the evolution of self-fertile hermaphrodites in nematodes involved changes in the germ line but not in the soma. The mechanism by which the sex determining genes could be regulated to achieve a hermaphrodite germ line would be simplified if the products of the masculinizing sex determination genes (e.g., the fem genes) were already present in the ancestral female germ line to direct male development in the embryo. In C. elegans, at least, all three fem genes do have maternal effects (Doniach and Hodgkin 1984; Hodgkin 1986). These maternal fem products in the female germ line must be negatively regulated to prevent them from directing spermatogenesis and thereby to permit oogenesis. Perhaps the fog-2 gene evolved to alleviate the negative regulation of the fem products briefly to permit some spermatogenesis in the otherwise female germline. One plausible ancestor of the fog-2 gene is her-1, a sex determination gene that negatively regulates tra-2 and tra-3 in response to the ratio of X chromosomes to autosomes.

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