The *mog-1* Gene Is Required for the Switch From Spermatogenesis to Oogenesis in *Caenorhabditis elegans*

Patricia L. Graham*† and Judith Kimble*‡

*Laboratory of Molecular Biology, Graduate School, †Department of Genetics and ‡Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison, Wisconsin 53706

Manuscript received September 16, 1992
Accepted for publication December 12, 1992

ABSTRACT

*Caenorhabditis elegans* hermaphrodites make first sperm, then oocytes. By contrast, animals homozygous for any of six loss-of-function mutations in the gene *mog-1* (for masculinization of the germ line) make sperm continuously and do not switch into oogenesis. Therefore, in *mog-1* mutants, germ cells that normally would become oocytes are transformed into sperm. By contrast, somatic sexual fates are normal, suggesting that *mog-1* plays a germ line-specific role in sex determination. Analyses of double mutants suggest that *mog-1* negatively regulates the *fem* genes and/or *fog-1*: *mog-1; fem* and *mog-1; fog-1* double mutants all make oocytes rather than sperm. Therefore, we propose that wild-type *mog-1* is required in the hermaphrodite germ line for regulation of the switch from spermatogenesis to oogenesis rather than for specification of oogenesis per se. In addition to its role in germ-line sex determination, maternal *mog-1* is required for embryogenesis: most progeny of a *mog-1; fem* or *mog-1; fog-1* mother die as embryos. How might the roles of *mog-1* in the sperm/oocyte switch and embryogenesis be linked? Previous work showed that *fem-3* is regulated post-transcriptionally to achieve the sperm/oocyte switch. We speculate that *mog-1* may function in the post-transcriptional regulation of numerous germ-line RNAs, including *fem-3*. A loss of *mog-1* might inappropriately activate *fem-3* and thereby abolish the sperm/oocyte switch; its loss might also lead to misregulation of maternal RNAs and thus embryonic death.

The nematode *Caenorhabditis elegans* develops as one of two sexes: XX animals are hermaphrodite, while XO animals are male. Hermaphrodites are essentially somatic females that produce sperm and then oocytes; they can reproduce either by self-fertilization, using their own sperm, or by cross-fertilization. In most organisms, including *C. elegans*, sex determination requires the coordinated regulation of cells in a given individual to adopt one of two alternative fates. However, in *C. elegans* hermaphrodites, male gametes must be produced briefly in an otherwise female animal. The restriction of spermatogenesis to a specific time and place of hermaphrodite development suggests that temporal, spatial, and tissue-specific controls must influence the sex-determining machinery to achieve this short burst of male development. Sex determination in the hermaphrodite germ line may therefore serve more generally as a paradigm for understanding how cell fates are controlled in a pattern during development.

The choice of sexual fate in *C. elegans* is controlled by a set of genes that act both in somatic tissues and in the germ line. Of most importance to this paper are the genes that regulate sexual fate only: *her-1* (HODGKIN 1980; TREN'T, WOOD and HORVITZ 1988), *tra-1* (HODGKIN and BRENNER 1977; HODGKIN 1987; SCHEDL et al. 1989), *tra-2* (KLASS, WOLF and HIRSH 1976; HODGKIN and BRENNER 1977), *tra-3* (HODGKIN and BRENNER 1977), *fem-1* (NELSON, LEW and WARD, 1978; DONIACH and HODGKIN 1984), *fem-2* (KIMBLE, EDGAR and HIRSH 1984; HODGKIN 1986), and *fem-3* (HODGKIN 1986; BARTON, SCHEDL and KIMBLE 1987). In addition, three sex determination genes act early during embryogenesis to control both sex determination and dosage compensation (MILLER *et al.* 1988; NUSBAUM and MEYER 1989; VILLENEUVE and MEYER 1987; VILLENEUVE and MEYER 1990). The primary signal for sex determination is the ratio of X chromosomes to sets of autosomes, or the X/A ratio (MADL and HERMAN, 1979). The sex determination genes appear to function as a series of alternating on/off switches with the activity of each gene controlled by the state of one or more upstream genes (HODGKIN 1980; HODGKIN 1986). One important distinction between the functions of these genes in the germ line and soma is that the *fem* genes are the terminal regulators in the germ line, whereas *tra-1* plays this role in the soma (HODGKIN, 1986, 1987; SCHEDL et al. 1989).

The specification of sexual fate in the germ line depends on germine-specific sex determination genes in addition to the globally acting genes described above. The *fog-1* gene (for feminization of the germ line) is required for specification of germ cells as sperm and acts with the three *fem* genes at the end of the
sex-determination pathway to direct spermatogenesis in both XX and XO worms (BARTON and KIMBLE, 1991). Two other genes, *fog-2* (SCHEDL and KIMBLE 1988) and *mog-I* (for masculinization of the germ line; this paper), are required for the transient production of male gametes in the hermaphrodite germ line.

The pattern of sex determination in the hermaphrodite germ line requires two steps of regulation. First, the X/A ratio must be circumvented to initiate male development in XX animals. At least two genes mediate this control. The *tra-2* gene must be negatively regulated to achieve hermaphrodite spermatogenesis (DONIACH, 1986; SCHEDL and KIMBLE, 1988) and *fog-2* is required for the onset of hermaphrodite spermatogenesis. One attractive hypothesis is that *fog-2* functions by negatively regulating *tra-2* (SCHEDL and KIMBLE, 1988). Second, male development must be turned off and female development turned on to accomplish the switch from spermatogenesis to oogenesis. Here, the male-determining *fem-3* gene is negatively regulated to switch from spermatogenesis to oogenesis (BARTON, SCHEDL and KIMBLE 1987); this regulation is post-transcriptional and acts via a regulatory element in the *fem-3* 3′-untranslated region (AHRINGER and KIMBLE, 1991).

In this paper, we introduce the germ line-specific sex determination gene, *mog-I*, and demonstrate that it is required for regulation of the sperm/oocyte switch in the hermaphrodite germ line. In addition, we show that maternal *mog-I* is essential for embryogenesis and speculate that *mog-I* may play a role in the post-transcriptional regulation of several germ line RNAs, including *fem-3* and maternal RNAs.

MATERIALS AND METHODS

Maintenance: Worms were maintained as described by BRENNER (1974). Experiments were done at 20°C unless otherwise noted.

Nomenclature: The suffix "gf" indicates a loss-of-function mutation, the suffix "g" indicates a gain-of-function mutation, and the suffix "mx" indicates a mutation with mixed loss and gain of function character. All other nomenclature conforms to HORVITZ et al. (1979).

Strains: All strains are derivatives of *C. elegans* var. Bristol strain N2, the designated wild type. Most mutations used are described in HODGKIN et al. (1988). The sex determination genes are referenced explicitly in the text. The following mutations and chromosomal rearrangements were used (*dpy* (dumpy), *fem* (feminization of germ line and soma), *fog* (feminization of the germ line), *gfp* (germ line proliferation defective), *lin* (lineage defective), *her* (hermaphroditization), *him* (high incidence of males), *mog* (masculinization of the germ line), *sma* (small), *sup* (suppressor), *tra* (transformer), *unc* (uncoordinated): LG I: *fog-1(q180), unc-11(e47)*.

LG II: *tra-2(q122gf), q270, q276, e1941(mx, e1095), unc-4(e120), mmCl (HERMAN 1978).*

LG III: *fem-2(e2105), dpy-17(e164), dpy-19(e1259ts), sma-2(e502), unc-32(e189), lin-12(n941), gfp-1(q46), mog-1(q151, q161, q223, q370, q471, q473), unc-69(e587), tra-1(e1099, e1781lam), dpy-18(e364), eT1 (ROSENBLUTH and BAILLIE 1981), qCl (AUSTIN and KIMBLE 1989), dpD3 (AUSTIN and KIMBLE 1987), ndD40 (HENGARTNER, ELLIS and HORVITZ 1989).*

LG IV: *unc-129(el138), unc-3(e1996), dpy-20(e1282), tra-3(e1107am), DnT1.*

LG V: *her-1(e1518), him-5(e1490), dpy-21(e428), fog-2(q71). *

LG X: *sup-7(st5), unc-6(e78).* 

**Isolation of mog-1 alleles:** Nine mutants that produce excess sperm and no oocytes in XX animals were isolated in a screen for self-sterile mutations (S. MAPLES, P. BALANDYK and J. KIMBLE, unpublished). Specifically, L4 hermaphrodites (*Po*), either N2 or *dpy-19+/+ unc-32*, were mutagenized with either 1 μM/ml or 4 μM/ml ethyl methanesulfonate (EMS) for 4 hr, individual *F1* self-progeny picked to separate droplets, and *F2* were screened for sterile mutants. Sterile *F2* were examined by Nomarski microscopy and those with a Mog (for MASCULINIZATION of the germ line) phenotype were out-crossed at least twice to N2. Mapping and complementation analysis (see below) revealed that four Mog mutations, *q151, q161, q223 and q370*, were alleles of a single gene, which we call *mog-I*. *mog-I(q151, q161 and q223*) were isolated after screening 9,467 haploid genomes mutagenized with 1 μM/ml EMS (a mutation frequency of 3 × 10⁻⁵), whereas *mog-I(q370*) was isolated after mutagenesis with 4 μM/ml EMS. Five other mutations with a Mog phenotype were single alleles of other genes (P. L. GRAHAM, T. SCHEDL and J. KIMBLE, in preparation) and will not be discussed further.

Two other *mog-I* alleles, *q471* and *q473*, were isolated in a screen for mutations that fail to complement *mog-I(q223)*. For this non-complementation screen, *dpy-19, him-5* L4 males raised at 15°C were mutagenized with 2 μM/ml EMS for 4 hr at 20°C and mated with hermaphrodites of genotype *dpy-19 mog-I(q223) unc-69, dpy-3/dpy-19+ mog-l(+). Crosses were left at 20°C overnight and then shifted to 25°C. Non-Dpy non-Unc hermaphrodite cross progeny (1500 *F1*) were picked to individual plates as L4s or young adults (*dpy-19* males at 25°C and males carrying *dpD3* do not mate, so adults were not mated). *F2* progeny were dissected by microscopy for plates on which all *Dpy* *F2* were sterile. Such *Dpy* sterile progeny were examined by Nomarski microscopy to ask if they were Mog.

**Scoring the mog-I phenotype:** All *mog-I(x), mog-I(x)/mog-I(y), mog-I(x)/nDf40, mog-I(x)/qCl, nDf40/qCl, double mutant, and temperature shifted worms were scored by Nomarski microscopy. In addition to the germ line phenotype, each worm was scored for morphogenesis of its tail, vulva and somatic gonad and for production of yolk (refractile droplets in the pseudocoelom (KIMBLE and SHARROCK 1983)).

For mapping experiments, segregation analysis, and amber suppression tests, the Mog phenotype was scored by dissecting microscope. At this level of resolution, Mog worms can be detected because they have no embryos and exhibit a dark longitudinal stripe (the intestine) flanked by clear stripes (probably accumulated yolk).

To examine *XO* worms, *mog-I (q223); him-5* males were examined by Nomarski microscopy for an alteration in any of the following: male gonad (KLAAS, WOLF and HIRSH 1976; KIMBLE and HIRSH 1979), bursal fan and sensory rays of the tail (SULSTON, ALBERTSON and THOMSON 1980), position of spermatocytes within the gonad (HIRSH, OPPENHEIM and KLAAS 1976) and absence of yolk (refractile droplets) from the pseudocoelom (KIMBLE and SHARROCK 1983; DONIACH 1986).

**Penetrance:** For each allele, at least 100 *Unc* progeny
from mog-I(x) unc-69/+ mothers raised at either 15° or 25° were scored by dissecting microscope for sterility. Any self-fertile Unc progeny were picked to separate plates and progeny tested to ask whether they were recombinants. At 15°, mog-I(x) unc-69 worms became adults approximately one day later than dpy-19 unc-69 worms grown in parallel. All mog-I(x), except mog-I(q473), were fully penetrant.

We next examined the penetrance of mog-I(q473) more carefully. Individual mog-I(q473) unc-69/+ L4s were placed on preincubated plates (either 15° or 25°) and transferred to fresh plates daily so that their progeny were roughly synchronized. From each plate, at least 35 gonadal arms from Unc adult progeny were examined by Nomarski microscopy. Unc progeny with oocytes were progeny tested to ask if they were recombinants. At 25°, 4/120 mog-I(q473) unc-69 gonadal arms contained oocytes, a fraction that remained fairly constant regardless of parental age. By contrast, at 15°, the percent of gonadal arms containing oocytes was 6, 6, 4, 24, 33, 19.

Mapping: The mog-I locus maps to chromosome III between dpy-19 and unc-69 (Figure 1). Three-factor data from hermaphrodites of genotype mog-I(x) dpy-19 unc-69 were obtained for each mog-I allele. For example, with mog-I(q223), 11/22 Dpy non-Unc recombinants and 11/21 Unc non-Dpy recombinants carried mog-I. Similar results were obtained with the other five alleles. Two-factor data was obtained for mog-I(q223). From four complete broods of mog-I(q223) unc-69(e387)/+ raised at 20°, 1098 wild-type, 308 Unc, Mog, 7 Mog, and 4 Unc were counted. Map distance was calculated using the formula $P = 1 - \sqrt{0.5 - 2\pi}$ (Brennner, 1974) where $R$ = number of recombinants/total number of progeny. These data show that mog-I is located 0.8 map units to the left of unc-69 (Figure 1).

Complementation tests: For mog-I(q161, q370, q471 and q473), mog-I(x)+ males were crossed to Unc hermaphrodites of genotype mog-I(q223) unc-69 dpy-19 unc-69. For mog-I(q131), mog-I(q151)+ males were crossed to mog-I(q223)/dpy-19 unc-69 hermaphrodites that had been allowed to self-fertilize until purged of all self-sperm. Tests for amber suppressible alleles: Each mog-I mutation was tested for suppression by the amber suppressor tRNA mutation sup-7(sts5) (Waterston 1981; Wills et al. 1983). The initial cross was done in one of two ways: (1) dpy-19 mog-I(q223) or q370++ hermaphrodites that had been purged of all self-sperm were mated with sup-7(sts5)/0 males. (2) dpy-19 mog-I(q151, q161, q471 or q473)/dpy-19 unc-69 Dpy hermaphrodites were mated with sup-7/0 males. From both types of crosses, F1 L4 hermaphrodites were picked to separate plates at 20° and 25° and allowed to self-fertilize. All Dpy F2s from two or three broods were scored for self-fertility at each temperature. Self-fertile Dpy F2s were progeny tested to distinguish between suppression and recombination.

Counting mog-I sperm by DAPI staining: For staining with diamidino-phenylindole (DAPI), adult mog-I(q223) homozygotes raised at 25° were washed in M9 salts, incubated 5-10 min in methanol or ethanol containing 200 ng/ml DAPI, washed once in distilled water, and mounted on agarose pads for observation and photography (E. Lambie, personal communication). Four mog-I gonadal arms had 313 ± 23, 364 ± 6, 391 ± 57 or 314 ± 13 sperm, where each arm was counted 3 times and the average taken.

Activation with pronase: Sperm from adult mog-I(q223) were released into sperm buffer (Ward, Hogan and Nelson 1983) or sperm buffer with 200 µg/ml pronase at room temperature. After a 5-10 min incubation, sperm were examined by Nomarski microscopy for extension of pseudopods (Ward and Carrel 1979). As a control, sperm from virgin N2 males were tested in parallel.

Antibody staining of mog-I sperm: Sperm distribution in mog-I and wild-type gonads was visualized by immunofluorescence. To extrude gonads, worms were cut on a polylime-treated slid into 8 µl of M9 + 0.25 M levamisole. The gonads were then fixed with 1% paraformaldehyde (10 min), treated with 0.1% Triton X100 (5 min), washed with 100 µl Tris-buffered saline containing 0.5% bovine serum albumin (BSA) (15 min to 1 hr), and incubated with TR II antibody diluted 1:500 in Tris-buffered saline (overnight, 4°). TR II, a mouse monoclonal antibody directed against C. elegans sperm-specific proteins, was a gift from Sam Ward (Ward et al. 1986). Worms were washed with 100 µl Tris-buffered saline containing 0.5% BSA 3-4 times (15-30 min/wash), then incubated with rhodamine labeled, donkey-anti-mouse secondary antibody diluted 1:100 (Jackson ImmunoResearch) and DAPI (1-2 hr). Next, worms were washed as above, then mounted in 8 µl mounting medium containing 1,4-diazabicyclo[2.2.2]octane (DABCO) and para-phenylenediamine. mog-I(q223) and mog-I(q223) ++/+ unc-69 adults grown at 25° were stained in parallel.

Scoring the mog-I maternal effect: To characterize the maternal-effect lethal phenotype of mog-I, eggs from each double mutant (e.g., mog-I; fem-3) were picked to a separate plate and scored 1 day later. Unhatched embryos were examined by Nomarski to assess the stage of arrest and the presence of specific tissues (e.g., pharynx). Fluorescence was used to score gut granules (Babu 1974; Lauffer, Bazzy- Camacho and Wood 1980). Embryos that hatched were scored 3 days later for viability. As a control, worms homozygous for each feminizing mutation were crossed with N2 males at 20°, and embryos scored as described.

Construction of strains: mog-I/nDf40: mog-I(x) unc-69/+ + males were crossed to nDf40 dpy-18/unc-32 dpy-18 Dpy hermaphrodites. Since nDf40 uncovers unc-69, Unc cross progeny were picked as L4s and scored one day later.

mog-I/qC1: qC1/unc-32 dpy-18; him-5 males were crossed to mog-I(x) unc-69/dpy-19 unc-69 Unc hermaphrodites. Since qC1 carries dpy-19, non-Dpy XX cross progeny were picked as L4s and scored as described above. Self-fertile worms were progeny tested and found to be of genotype unc-32 dpy-18/mog-I(x) unc-69 or unc-32 dpy-18/dpy-19 unc-69.

qC1/nDf40: qC1/unc-32 dpy-18; him-5 males were crossed with nDf40 dpy-18/unc-32 dpy-18 Dpy hermaphrodites. Non-Dpy, non-Unc XX L4 cross progeny were picked and scored by dissecting microscope for self-fertility 1 day later; any sterile progeny were scored by Nomarski microscopy. When tested, self-fertile non-Dpy non-Unc hermaphrodite cross progeny were of genotype qC1/unc-12 dpy-18.

mog-I(q223)/mog-I(q473) and nDf40/mog-I(q473); dpy-19 mog-I(q473); him-5 males were crossed with either mog-I(q223) unc-69/dpy-19 unc-69 hermaphrodites or nDf40 dpy-18/dpy-19 unc-69 hermaphrodites (nDf40 removes unc-69). To ensure the phenotypes of these two strains could be compared, the two crosses were carried out together on plates housed in the same box. From each cross, non-Dpy, non-Unc XX 1.4 F1, cross progeny were picked to individual plates and scored one day later. Any such F1 hermaphrodites
that were self-fertile were progeny tested to determine their genotype. Because ndf40 has a semidominant lethal phenotype at 15°C (data not shown), mog-1(q223) and ndf40 were compared at 20°C.

mog-1; her-1: To examine both XX and XO mog-1; her-1 double mutants, we incorporated dpy-21 into the strain, which marks XX and XO animals independently of sexual phenotype: XX dpy-21 animals are Dpy while XO dpy-21 animals are non-Dpy (Hodgkin 1980). To obtain this strain, her-1 him-5 dpy-21 XX Dpy hermaphrodites were mated with mog-1(q223 or q370) unc-69/+ males and mog-1(q223 or q370) unc-69/++; her-1 him-5 dpy-21 animals were identified by progeny testing.

mog-1; fog-2: Unc progeny of fog-2+/+; mog-1 unc-69/++ hermaphrodites were examined. One-quarter should be homozygous for fog-2, but all were Mog. To be sure the double mutants were not dying as embryos or larvae, 30–40 eggs were picked from each fog-2+/+; mog-1 unc-69/++ hermaphrodite and scored for viability.

mog-1; fem-1(fj): To obtain mog-1; fem-1 from a fem-1 homozygous mother, fem-1 unc-24; dpy-19 mog-1+/+ self-fertile hermaphrodites were picked to individual plates and their Dpy progeny were examined.

fem-2 mog-1: To construct a fem-2 mog-1 chromosome, progeny from fem-24+/+; meg-1 unc-69 were picked to individual plates and progeny tested to identify a recombinant of genotype fem-2-++/+; fem-2 meg-1 unc-69. From these two homozygous mothers, fem-2 meg-1 unc-69 Unc progeny were scored.

mog-1; fem-3: Dpy Unc progeny from F1, hermaphrodites of genotype mog-1(q223 or q370) unc-69/++; fem-3 dpy-20/++ were examined.

mog-1; fog-1: Unc progeny mog-1 unc-69/++; fog-1+/+ hermaphrodites were scored. One-quarter should be homozygous for fog-1. Because fog-1 was not marked, 30–40 eggs were picked from each fog-1+/+; mog-1 unc-69/++ mother and checked for viability.

mog-1 tra-1: We constructed mog-1 tra-1 double mutants with tra-1(e1099), an amber allele. XX tra-1(e1099) homzygotes have a male soma, rarely produce oocytes, and often have an expanded distal core containing granular material, which is indicative of early oogenesis (Hodgkin 1987; Schedel et al. 1989). XX tra-1(e1781) homzygotes have a male soma, but make oocytes much more frequently than tra-1(e1099) mutants (Hodgkin 1987; Schedel et al. 1989).

To make a mog-1 unc-69 tra-1 recombinant chromosome, unc-69/++ males were crossed into mog-1 unc-69/++; tra-1 homzygotes and L4 Unc cross progeny were picked 5/plate. The next generation were scored by dissecting for Unc worms with male tails. The mog-1 unc-69 tra-1 chromosome was retrieved and balanced with et1. The presence of mog-1 on the recombinant chromosome was validated by complementation. To control for marker effects, strains of genotype unc-69 tra-1(e1099 or e1781) et1 were grown and scored in parallel with the corresponding experimental strains. Two to three complete broods and several partial broods were scored for each strain.

tra-2(fj); mog-1: We constructed mog-1 tra-2 double mutants with three tra-2 loss-of-function alleles. Two alleles, tra-2(e1095) and tra-2(q270), are classical strong loss-of-function mutations: XX tra-2(e1095 or q270) homzygotes are transformed from hermaphrodite to male, though they have a slightly defective male tail and do not mate (Hodgkin and Brenner 1977). By contrast, XX tra-2(q276) homzygotes are completely transformed males and are cross-fertile (T. Schedel, personal communication). Each tra-2(e); mog-1(fy) double mutant was identified among the progeny of tra-

### TABLE 1

<table>
<thead>
<tr>
<th>Genotype&lt;sup&gt;a&lt;/sup&gt;</th>
<th>XX Germ-line phenotype</th>
<th>n&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>++</td>
<td>Sperm and oocytes</td>
<td>&gt;100</td>
</tr>
<tr>
<td>mog-1/x/++</td>
<td>Sperm and oocytes</td>
<td>&gt;100</td>
</tr>
<tr>
<td>mog-1/x/mog-1(q223)</td>
<td>Excess sperm, no oocytes&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&gt;10</td>
</tr>
<tr>
<td>mog-1/x/ndf40</td>
<td>Excess sperm, no oocytes&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&gt;10</td>
</tr>
<tr>
<td>mog-1/x/q370</td>
<td>Excess sperm, no oocytes&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&gt;10</td>
</tr>
<tr>
<td>ndf40/q370</td>
<td>Sperm and oocytes</td>
<td>15</td>
</tr>
<tr>
<td>mog-1(q223)/q223&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Sperm and oocytes</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

<sup>a</sup> mog-1(x): q151, q161, q223, q370, q473 or q473. <br><sup>b</sup> If worms were self-fertile, they were scored as making sperm and oocytes; if worms were sterile, their germ lines were examined by Nomarski optics to determine which type of gametes were produced. <br><sup>c</sup> n = number of worms scored for each allele. Each worm possesses two oocytes. <br><sup>d</sup> For most mog-1(x), all oocytes made excess sperm and no oocytes, the exception was mog-1(q473); 62/63 mog-1(q473) oocytes produced excess sperm and 1/63 had an oogenic core. <br><sup>e</sup> For most mog-1(x), all oocytes made excess sperm and no oocytes; the exception was mog-1(q473); 41/42 mog-1(q473) oocytes produced excess sperm and no oocytes and 1/42 produced oocytes. mog-1(+) was carried on qdp3.

**RESULTS**

**Identification of the mog-1 locus:** We isolated four mog-1 alleles in a general screen for sterile mutants and two others in a noncomplementation screen (see MATERIALS AND METHODS). These six mutations were assigned to the mog-1 locus by two criteria. First, all six map between dpy-19 and unc-69 on linkage group III (Figure 1) (see MATERIALS AND METHODS). Second, all six fail to complement the reference allele mog-1(q223) (Table 1, line 3). None of the six mog-1
FIGURE 2.—The mog-l mutant phenotype: masculinization of the germline. (A) and (B) Nomarski photomicrographs with drawings above. (C) and (D) DAPI-stained gonadal arms. The magnification bar in (A) represents ≈50 µm and is appropriate for A–D. (A and C) wild-type (mog-I(q223) +/+ unc-69) adult hermaphrodites, lateral view. From proximal to distal, the gonad contains: an embryo (e), sperm (sp), oocytes (oo), and nuclei arrested in meiotic pachytene that surround an oogenic core (oc). (B and D) mog-l(q223)/mog-l(q223) adults, lateral view. Sperm occupy the proximal gonad and primary spermatocytes (1*sp) are found in the distal arm. There is no oogenic core and no oocytes are made.

mutations is amber suppressible (MATERIALS AND METHODS). Five alleles, q151, q161, q223, q370 and q471, are fully penetrant at both 15° and 25°; whereas mog-l(q473) is incompletely penetrant at both temperatures (MATERIALS AND METHODS). The phenotype of mog-l, described below, is the same for all five non-conditional mog-l mutants, and for most (97%) mog-l(q473) animals at restrictive temperature (25°).

The mog-l mutant phenotype: The wild-type hermaphrodites ovotestis first makes sperm and then switches to oogenesis (Figure 2). When grown at 25°, a wild-type ovotestis produces 90–125 sperm (HIRSH, OPPENHEIM and KLASS 1976). In mog-l XX homozygotes, spermatogenesis begins at the normal stage of development, but it continues unabated; oogenesis is not observed (Figure 2). The number of sperm per mutant ovotestis was estimated to be 300–500 (see MATERIALS AND METHODS). Therefore, germ cells that would have become oocytes in wild type are transformed into sperm in mog-l. The morphology of mog-l sperm is normal (Figures 2 and 3). Furthermore, mog-l sperm, like wild type (WARD, HOGAN, and NEL-SON 1983), extend pseudopods when treated with pronase (data not shown) and stain with a monoclonal antibody that recognizes many sperm specific proteins (Figure 3).

In contrast to the germline masculinization observed in mog-l mutants, XX mog-l homozygotes show no somatic masculinization. Specifically, the tail, which is a particularly sensitive indicator for perturbations of sex determination, is a simple spike in both wild-type and mog-l XX worms (Figure 4). Masculinization of the tail might have been observed either as a short, blunt tail or by the presence of a fan and rays (SULSTON et al. 1980). In addition, mog-l animals have a normal vulva and produce yolk [refractile droplets seen by Nomarski microscopy (KIMBLE and SHARROCK 1983)]. This lack of somatic masculinization in mog-l mutants is consistent with the idea that mog-l does not regulate the somatic sexual phenotype.
FIGURE 3.—mog-1 sperm react with anti-sperm antibody. (A and C) DAPI-stained gonadal arms. (B and D) same gonad treated with TRII, an antibody that detects C. elegans sperm (WARD et al. 1986) (see MATERIALS AND METHODS). Magnification bar in (A) represents =50 μm and is appropriate for A–D. (A and B) Gonad dissected from a wild-type (mog-1(q223) +/+ unc-69) adult hermaphrodite. Proceeding from proximal to distal, the gonad contains sperm (sp), then oocytes (oo). The distal mitotic region is not shown. Part B shows clearly that anti-sperm antibody stains mature sperm, which are found only in the proximal gonad. (C and D) Gonad dissected from a mog-1(q223)/mog-1(q223) adult. Sperm are found in the entire proximal gonad. A second gonad is seen at lower left. Part D shows that the anti-sperm antibody stains mature sperm in the proximal gonad, and also stains maturing spermatocytes in the distal gonad. Mitotically dividing nuclei (m) do not stain.

Finally, XO mog-1 homozygotes are typically male. Morphologically, the somatic gonad, germ line and tail are all normal, and no yolk is observed in the pseudocoelom (data not shown). Furthermore, mog-1 males exhibit normal mating behavior and sire cross progeny, indicating that mog-1 XO sperm are functional.

In sum, the XX mog-1 germ line is sexually transformed from hermaphrodite to male, and no defect is seen in the somatic tissue of either XX or XO mog-1 homozygotes. We therefore call this locus mog-1 (for masculinization of the germ line).

Germ-line masculinization is probably the null phenotype of mog-1: Three lines of evidence indicate that the mog-1 mutant phenotype is due to a reduction of mog-1 activity. First, all six mog-1 mutations are recessive. XX animals of genotype mog-1(x)/+ or mog-1(q223)/mog-1(q223)/+ are typically hermaphrodite, making sperm and then oocytes (Table 1). Furthermore, mog-1(x)/+ worms do not make excess sperm before switching into oogenesis (Table 2). Since hermaphrodite sperm are used efficiently for self-fertilization (WARD and CARREL 1979), the number of self-progeny provides an excellent estimate of the number of sperm made. The brood sizes of mog-1(x)/+ hermaphrodites are similar to those of wild-type herma-

FIGURE 4.—mog-1 does not masculinize the hermaphrodite soma. Nomarski photomicrographs. Magnification bar =50 μm. (A) The wild-type (mog-1(q223) +/+ unc-69) adult hermaphrodite tail ends in a spike. (B) The mog-1(q223)/mog-1(q223) adult tail also ends in a hermaphrodite-like spike. If the tail were masculinized, it would be blunter and might possess a fan and rays (SULESTON, ALBERTSON and THOMSON 1980).
Animals were examined. The results are summarized in Tables 1 and 2. For all double mutants, except mog-1; her-1, only XX and XO animals were examined. The results are summarized in Tables 3 and 4.

Double mutant experiments: Double mutants were examined to learn about the functional relationships between mog-1 and other sex-determining genes. In these experiments, we also explored the possibility that a somatic effect of mog-1 might be observed in partially masculinized XX or feminized XO animals. In constructing these double mutants, the best candidates for strong loss-of-function or null alleles were used for each gene. Most double mutants were constructed with each of two mog-1 alleles: q223 and q270. For all double mutants, except mog-1; her-1, only XX animals were examined. The results are summarized in Tables 3 and 4.

Double mutants of mog-1 with feminizing mutations: The her-1 gene is required in XO animals for specifying male development. XO her-1(1f) homozygotes are transformed from males into self-fertile hermaphrodites (Hodgkin 1980). mog-1; her-1 XX and XO worms were examined to determine whether the switch into oogenesis observed in her-1 mutants depends upon mog-1 activity. We found that both the XX and XO mog-1; her-1 double mutants have a female soma and a male germline (Table 3). Therefore, the oogenesis seen in her-1(1f) XX and XO hermaphrodites is dependent on mog-1 gene activity. Further, no somatic masculinization was seen in either the XX or XO double mutant, consistent with the idea that mog-1 regulates sexual fate in the germ line and not in the soma.

The fog-2 gene is required for the onset of hermaphrodite spermatogenesis (Schedl and Kimble 1988). XX fog-2 homozygotes are transformed from hermaphrodites into females that make only oocytes, whereas XO fog-2 homozygotes are male. We found that mog-1; fog-2 double mutants have a typically hermaphrodite soma but produce only sperm (Table 3). Therefore, the oogenesis seen in fog-2 females depends on mog-1 activity. Moreover, in the absence of mog-1, fog-2 activity is not required for the onset of spermatogenesis.

The three fem genes are required for male development in both somatic and germ-line tissue (Donia and Hodgkin 1984; Kimble, Edgar and Hirsh 1984; Hodgkin 1986; Barton, Schedl and Kimble 1987). In fem-1, fem-2 or fem-3 loss-of-function mutants, both XX and XO worms are transformed into females (spermless hermaphrodites). Double mutants were examined to determine whether the oogenesis seen in the fem(1f) mutants depends on mog-1. Because both fem-1 and fem-2 show maternal rescue, these double mutants were derived from mog-1(+/); fem/fem mothers. By contrast, fem-3 causes complete feminization of the XX germ line, irrespective of the maternal genotype, so we examined these double mutants from fem-3(+/)+ mothers. We found that all three mog-1; fem double mutants make only oocytes (Table 3). The mog-1; fem-3 and mog-1; fem-1 double mutants are female in both soma and germline. Therefore, mog-1 is not absolutely required for the specification of germ cells as oocytes. Furthermore, spermatogenesis in mog-1 mutants depends on wild-type fem-1 and fem-3 products. The fem-2 mog-1 double mutant has a female soma, but shows a range of germ-line phenotypes—when derived from a fem-2 homozygous mother (Table 3). One explanation of the variability in the fem-2 mog-1 worms is that the fem-2 allele used might not be null, and that the presence of some fem-2 product results in this variability. In support of this idea, we note that the XO phenotype of fem-2(e2105) is temperature sensitive; in particular fem-2(e2105) causes incomplete feminization of XO worms at temperatures below 25° (Hodgkin 1980). The mog-1

TABLE 2
Brood size and segregation analysis of mog-1(x)/+ worms

<table>
<thead>
<tr>
<th>Parental genotype</th>
<th>T (°C)</th>
<th>Brood size</th>
<th>Progeny phenotype (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>25</td>
<td>225 ± 49</td>
<td>100 0</td>
</tr>
<tr>
<td>mog-1(q151)/dpy-19 unc-69</td>
<td>25</td>
<td>250 ± 99</td>
<td>75 25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>189 ± 63</td>
<td>72 28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>247 ± 31</td>
<td>73 27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>288 ± 56</td>
<td>72 28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>20</td>
<td>321 ± 46</td>
<td>100 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>342 ± 24</td>
<td>73 27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>340 ± 39</td>
<td>74 26</td>
</tr>
</tbody>
</table>

a n = number of broods scored.

The original alleles of mog-1 were isolated on the basis of their germ-line phenotype. Therefore null alleles might have had a different phenotype. However, since mog-1(q223)/nDf40 is Mog (Table 1), we could have isolated mog-1 null mutations in a noncomplementation screen—even if the mog-1 null phenotype had been lethal. Second, the reference allele, mog-1(q223), behaves like the deficiency nDf40 when placed in trans to the weak mutation mog-1(q473) (Table 1, footnotes d and e). Third, if both qC1 and nDf40 eliminate mog-1, as might be predicted, the trans-heterozygote qCl/nDf40 would be a mog-1 null. We find that animals of genotype qC1/nDf40 are Mog (Table 1, line 6).

In sum, the accumulated evidence argues that the mog-1 mutations identified to date cause a reduction or complete loss of mog-1 activity. Therefore, the wild-type activity of mog-1 must be required either for oogenesis per se or for the switch from spermatogenesis to oogenesis.

The three fem genes are required for male development in both somatic and germ-line tissue (Donia and Hodgkin 1984; Kimble, Edgar and Hirsh 1984; Hodgkin 1986; Barton, Schedl and Kimble 1987). In fem-1, fem-2 or fem-3 loss-of-function mutants, both XX and XO worms are transformed into females (spermless hermaphrodites). Double mutants were examined to determine whether the oogenesis seen in the fem(1f) mutants depends on mog-1. Because both fem-1 and fem-2 show maternal rescue, these double mutants were derived from mog-1(+/); fem/fem mothers. By contrast, fem-3 causes complete feminization of the XX germ line, irrespective of the maternal genotype, so we examined these double mutants from fem-3(+/)+ mothers. We found that all three mog-1; fem double mutants make only oocytes (Table 3). The mog-1; fem-3 and mog-1; fem-1 double mutants are female in both soma and germline. Therefore, mog-1 is not absolutely required for the specification of germ cells as oocytes. Furthermore, spermatogenesis in mog-1 mutants depends on wild-type fem-1 and fem-3 products. The fem-2 mog-1 double mutant has a female soma, but shows a range of germ-line phenotypes—even when derived from a fem-2 homozygous mother (Table 3). One explanation of the variability in the fem-2 mog-1 worms is that the fem-2 allele used might not be null, and that the presence of some fem-2 product results in this variability. In support of this idea, we note that the XO phenotype of fem-2(e2105) is temperature sensitive; in particular fem-2(e2105) causes incomplete feminization of XO worms at temperatures below 25° (Hodgkin 1980). The mog-1
genetic background may reveal a similar temperaturesensitivity in fem-2(e2105) XX animals. Alternatively, a defect in the mog-1 gene may in fact be capable of bypassing the need for fem-2(+), in specifying sperm cell fate.

The fog-1 gene is required for spermatogenesis in both XX and XO worms (BARTON and KIMBLE 1990). The germ line of both XX and XO fog-1(f) mutants is feminized, but unlike the fem genes, fog-1 does not affect somatic sex. We find that fog-1; mog-1 double mutants make only oocytes (Table 3, Figure 5). Therefore, specification of sperm remains dependent on fog-1 activity, even in the absence of mog-1.

In sum, mog-1 is epistatic to her-1 and fog-2, but fem-1, fem-3 and fog-1 are epistatic to mog-1. These results place mog-1 in the middle of the regulatory hierarchy of germline sex determination (see DISCUSSION and Figure 6).

Double mutants of mog-1 with masculinizing mutations: Three tra genes are required for female development: XX animals homozygous for a loss-of-function mutation in tra-1, tra-2 or tra-3, are masculinized in both
TABLE 4
Phenotype of animals homozygous for mog-l and tra mutations

<table>
<thead>
<tr>
<th>Genotypea</th>
<th>Sperm only</th>
<th>Sperm and oocytes</th>
<th>Oocytes only</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>tra-1(e1099)</td>
<td>100d</td>
<td>0</td>
<td>0</td>
<td>97</td>
</tr>
<tr>
<td>tra-1(e1099) mog-l(q370)</td>
<td>100f</td>
<td>0</td>
<td>0</td>
<td>73</td>
</tr>
<tr>
<td>tra-1(e1781)</td>
<td>60</td>
<td>40</td>
<td>0</td>
<td>43</td>
</tr>
<tr>
<td>tra-1(e1781) mog-l(q223)</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>tra-2(e1095)</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>tra-2(e1095); mog-l(q223)</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>tra-2(e1095); mog-l(q370)</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>tra-3(e1107)</td>
<td>77</td>
<td>23</td>
<td>0</td>
<td>61</td>
</tr>
<tr>
<td>tra-3(e1107); mog-l(q223)m</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>tra-3(e1107); mog-l(q370)m</td>
<td>97</td>
<td>3</td>
<td>0</td>
<td>30</td>
</tr>
</tbody>
</table>

a Alleles used were mog-l(q223, q370) (this paper), tra-1(e1099, e1781) (HODGKIN, 1987; SCHEDL et al. 1989), tra-2(e1095, q270, q276) (HODGKIN and BRENNER 1977; OKKEMA and KIMBLE 1990; KUWABARA, OKKEMA and KIMBLE 1992) and tra-3(e1107) (HODGKIN and BRENNER 1977).

b Number of gonads scored.
c Unc self-progeny from unc-69 tra-1(e1099)++; mothers.
d Although no oocytes were observed, 22% of the gonads (21/97) had an oogenic core.
e Unc self-progeny from mog-l(q370) unc-69 tra-1(e1099);++ mothers.
f No oocytes or oogenic cores were observed.
g Unc self-progeny from unc-69 tra-1(e1781)++; mothers.
h Unc self-progeny from mog-l(q223) unc-69 tra-1(e1781);++ mothers.
i Unc self-progeny with male tails from tra-2(e1095)++; unc-69/+
mothers.
j Unc self-progeny with male tails from tra-2(e1095)++; mog-l(q223);unc-69/++; mother. Similar results were obtained with tra-2(q270);mog-l(q223) unc-69 (n = 17), and tra-2(q276);mog-l(q223) unc-69 (n = 18).
k Unc self-progeny with male tails from tra-2(e1095)++; mog-l(q370);unc-69 mothers.
l Self-progeny from tra-3(e1107);tra-3(e1107) mothers.
m Unc self-progeny from mog-l(q223) unc-69/++; tra-3(e1107)/tra-3(e1107) mothers.
n Unc self-progeny from mog-l(q370) unc-69/++; tra-3(e1107)/tra-3(e1107) mothers.

For tra-2; mog-l double mutants, we used three tra-2 alleles: one nonsense mutation, tra-2(e1095) (KUWABARA, OKKEMA and KIMBLE 1992) and two transposon insertions, tra-2(q270) and tra-2(q276) (OKKEMA and KIMBLE 1991). Two of these alleles, e1095 and q270, are typical tra-2(f) mutations, transforming X animals into non-mating males, whereas tra-2(q276) is an unusual loss-of-function mutation that transforms X worms into mating males (T. SCHEDL, personal communication). The germ lines of all three tra-2; mog-l double mutants, like those of soma and germ line (HODGKIN and BRENNER 1977; HODGKIN 1987; SCHEDL et al. 1989). Both tra-1 and tra-3 single mutants sometimes make oocytes (HODGKIN and BRENNER 1977; HODGKIN 1987; SCHEDL et al. 1989) (Table 4), but tra-2 XX single mutants make only sperm (HODGKIN and BRENNER 1977) (Table 4). Double mutants were examined to ask whether the oogenesis seen in tra-1 and tra-3 mutants is dependent on mog-l activity. Because tra-3 mutants show maternal rescue, the mog-l; tra-3 double mutant was derived from a mog-l/++; tra-3/tra-3 mother. Because tra-1 is genetically complex (HODGKIN 1987; SCHEDL et al. 1989), two tra-1 alleles were used. tra-1(e1099), the canonical null allele, makes few oocytes. Therefore, it is difficult to assess the relationship between mog-l and tra-1 using this allele. tra-1(e1781) is an amber suppressible allele and often makes oocytes. Similar results were obtained with both tra-1 mutations and tra-3. In mog-l; tra-1 and mog-l; tra-3 double mutants the germ line is completely transformed to the male fate (Table 4), indicating that the remaining oogenesis seen in the tra mutants is dependent on mog-l. However, the somatic phenotype of the double mutant is typical of the particular tra mutation used, consistent with the idea that mog-l does not function in somatic sex determination.

FIGURE 5.—fog-1; mog-l germ line is feminized. Nomarski photomicrographs with line drawings below. Magnification bar =50 μm. (A) fog-1(q180);fog-1(q180) adult, lateral view. Only oocytes (oc) are made and an oogenic core (oc) is found. The right-most oocyte has entered the uterus, but is unfertilized. (B) fog-1(q180);fog-1(q180); mog-l(q223) unc-69/mog-l(q223) unc-69 adult, lateral view. Only oocytes (oc) are made and an oogenic core (oc) is observed. The right-most oocyte is in the spermatheca, but is unfertilized.
each single mutant, are male. However, both tra-2(e1095); mog-1 and tra-2(q270); mog-1 worms had “clublike” tail structures that were visibly more severe than the corresponding tra-2 single mutant. These misshapen tails can be viewed either as a sign of feminization or as a nonspecific effect on development of the male tail. Such nonspecific effects are common (HODGKIN 1983; HODGKIN et al. 1989). In contrast, the tra-2(q276); mog-1 double mutant showed no phenotypic change in either soma or germ line when compared to the tra-2(q276) alone.

**Double mutants with tra-2 dominant regulatory mutations:** Two classes of dominant regulatory mutations exist for tra-2. XX heterozygotes of both classes, dubbed tra-2(gf) and tra-2(mx), are transformed from hermaphrodites into females (DONIACH 1986; SCHEDL and KIMBLE 1988). Molecular characterization of tra-2(mx) and tra-2(gf) mutations reveals that each class carries a diagnostic type of lesion (OKKEMA 1990).

Double mutants were made with a representative of each class of tra-2 regulatory mutation. The germ line of tra-2(mx); mog-1 double mutants is male, but that of tra-2(gf); mog-1 double mutants is variable (Table 5). Therefore, mog-1 is epistatic to tra-2(mx), but not to tra-2(gf).

**A maternal effect of mog-1 on embryonic viability:**
In mog-1 homozygotes, which make only sperm, maternal effects of mog-1 cannot be observed. However, various double mutants make oocytes (see above). Therefore, the influence of mog-1 mutations on embryogenesis can be examined with these double mutants.

Most (79–100%) progeny produced from mog-1; fem, mog-1; fog-1 or mog-1; tra-2(gf) double mutant mothers die (Table 6). The morphology of the dead progeny reveals no specific cause of death. Among the dead embryos, most (99%) arrest some time between gastrulation and hatching. This maternal effect lethality is observed with both alleles of mog-1 tested, which suggests that the lethality is not due to a separate linked mutation. By contrast with the mog-1 double mutants, most embryos from fem, fog-1 and tra-2(gf) single mutants are viable (DONIACH and HODGKIN 1984; HODGKIN 1986; BARTON, SCHEDL and KIMBLE 1987; BARTON and KIMBLE 1990) (Table 6). Therefore, death of the double mutant progeny is likely to result from absence of maternal mog-1.

**DISCUSSION**

**Zygotic mog-1 activity is required for the switch from spermatogenesis to oogenesis:** In this paper, we report the identification and genetic characterization of a new sex-determining gene mog-1. Normally, the hermaphrodite germ line produces first sperm, then oocytes; its soma is female. In hermaphrodites lacking...
mog-1 activity, spermatogenesis continues past the time when oogenesis would normally begin and no oogenesis occurs (Figures 2 and 3). Thus, germ cells that would normally become oocytes are transformed into sperm.

The transformation of germ cells from oocyte to sperm in mog-1 loss-of-function mutants suggests that mog-1 is required for oogenesis. What role might mog-1 play? One possibility is that mog-1 specifies germ cells as oocytes. This model predicts that oocytes cannot be made in the absence of mog-1. A second possibility is that mog-1 is critical for the hermaphrodite switch from spermatogenesis to oogenesis. This second model predicts that oocytes can be made in the absence of mog-1 if the sperm/oocyte switch is circumvented. To distinguish between these two models, we examined double mutants homozygous for both a mutation in mog-1 and a mutation in one of the three fem genes or fog-1. The fem genes and fog-1 are all required for the specification of the sperm fate; animals lacking any one of these genes make only oocytes (Doniach and Hodgkin 1984; Hodgkin 1986; Barton and Kimble 1990). In all double mutants (e.g., fog-1; mog-1), oocytes were produced. Therefore, mog-1 is not required for the specification of oocytes per se, but instead must be essential for the hermaphrodite switch from spermatogenesis to oogenesis.

The role of mog-1 in sex determination appears to be specific to the germ line. Mutations in mog-1 do not affect sexual differentiation in the XX soma, XO germ line or XO soma. Yet, two observations suggest that mog-1 mutations may have a somatic effect. First, mog-1 mutants grow more slowly than wild-type worms at 15°C, though no specific cellular defect was observed (see MATERIALS AND METHODS). Second, some tra-2; mog-1 double mutants have a slightly defective tail when compared to tra-2 single mutants. Therefore, mog-1 may play a minor or redundant role in some ubiquitous function, in addition to its major role in germ-line sex determination.

How might mog-1 achieve the switch into oogenesis? The most likely mechanism by which mog-1 achieves the sperm/oocyte switch is to regulate known members of the sex determination genes. The onset of hermaphrodite spermatogenesis relies on a germ line-specific sex determination gene called fog-2 (Schedl and Kimble 1988), and three fem genes and fog-1 are essential for specifying the sperm fate (Doniach and Hodgkin 1984; Hodgkin 1986; Barton and Kimble 1990). As diagrammed in Figure 6, fog-2 is thought to block tra-2 activity, which frees the fem genes and fog-1 to direct spermatogenesis. One mechanism by which mog-1 might mediate the switch from spermatogenesis to oogenesis is to negatively regulate one of the fem genes and/or fog-1. Since the three fem genes and fog-1 are all necessary for spermatogenesis, any one of them might be negatively regulated to achieve the switch. Alternatively, mog-1 might achieve the switch by positively regulating tra-2. mog-1 might either activate tra-2 directly or turn off fog-2.

To distinguish among the mechanisms outlined above, we examined double mutants carrying a mutation in mog-1 and a mutation in each of the other major genes in the germ-line sex determination pathway. We found that mog-1 is epistatic to her-1 and fog-2, but that fem-1, fem-3 and fog-1 are epistatic to mog-1. Based on the results of these double mutant experiments, we propose that mog-1 acts after her-1 and fog-2, but before the fem genes and fog-1 in the sex determination pathway (Figure 6). Therefore, mog-1 does not negatively regulate fog-2 to achieve the sperm/oocyte switch. The tra; mog-1 double mutants do not further define the position of mog-1 in the germline sex determination pathway; the increased masculinization of some tra; mog-1 double mutants could result from additive effects rather than epistasis. Therefore, we currently cannot distinguish between the remaining models. mog-1 might positively regulate tra-2 or negatively regulate the fem genes and/or fog-1.

Two major questions about the regulation of the hermaphrodite germ line remain unsolved: How is the temporal order of sexual fates regulated so that sperm are made first and then oocytes? And why does the switch from spermatogenesis to oogenesis occur after a certain number of sperm are produced? Now that regulators of sexual fate in the hermaphrodite germ line have been identified (i.e., tra-2, fem-3, fog-2 and mog-1), it will be possible to learn how these regulators are themselves regulated to establish the pattern seen in the hermaphrodite germ line.

Maternal mog-1 is required for embryogenesis: Whereas mog-1 single mutants make only sperm, certain double mutants (e.g., fog-1; mog-1) make oocytes despite the absence of mog-1 (see Table 4). Most progeny derived from females that lack mog-1 die as embryos or young larvae. Furthermore, a wild-type allele of mog-1 brought in from the father cannot rescue these progeny. This maternal effect lethality has been observed using two independently isolated alleles of mog-1. Since similar lethality is not observed among progeny of any of the single mutants used to feminize the mog-1 germ line, the embryonic death must be due to a lack of maternal mog-1.

The role of maternal mog-1 in embryogenesis and larval development is not understood. No specific developmental defect (e.g., cell fate transformation) is observed among the dying progeny. One explanation is that a maternal contribution of mog-1 may be required for normal development of the progeny. Alternatively, wild-type mog-1 may be required during
oogenesis for production of a fully competent oocyte. We currently cannot distinguish between these two models.

Speculations on the function of mog-1 in both germ-line sex determination and oogenesis/embryogenesis: The mog-1 loss-of-function phenotype is a failure in hermaphrodites to switch from spermatogenesis to oogenesis: mog-1(lf) mutants make excess sperm and no oocytes. A similar phenotype is observed in fem-3 gain-of-function mutants (BARTON, SCHERL and KIMBLE 1987). These fem-3(gf) mutations all map to a 5-base pair sequence within the 3′-untranslated region and may define a binding site for a negative regulator of fem-3 (AHRINGER and KIMBLE, 1991). Based on the results of double mutant phenotypes, we proposed above that mog-1 might negatively regulate one of the fem genes and/or fog-1. A more specific, but also a much more speculative model is that mog-1 regulates fem-3 through the regulatory element in its 3′-untranslated region.

The maternal requirement for mog-1 during embryogenesis seems at first to be entirely distinct from the zygotic requirement for mog-1 in germline sex determination. However, many maternal RNAs are regulated by elements in their 3′-untranslated regions [e.g., WICKENS (1990) for a review]. Moreover, fem-3 is a maternal RNA (AHRINGER et al. 1992). Therefore, a unifying hypothesis is that mog-1 may regulate numerous maternal RNAs, including fem-3. Oocytes lacking mog-1 may contain aberrantly activated maternal RNAs and therefore be unable to support subsequent development.

An intriguing extension of the idea that mog-1 may control both the sperm/oocyte switch and maternal RNAs is that this mechanism may have existed prior to the evolution of the C. elegans hermaphrodite. If true, we envision that the preexisting mog-1-mediated control of maternal RNAs was co-opted to achieve the switch from spermatogenesis to oogenesis, an essential step in the creation of a self-fertilizing hermaphrodite from a female.

We are grateful to SANDRA MAPLES and PHIL BALANDYK for the isolation of the first four mog-1 alleles. In addition, we express thanks to members of the Kimble laboratory for critical reading of the manuscript, with particular appreciation due to TOM EVANS and J. KIMBLE. We also thank KATHY BARTON, TIM SCHERL and SUZANNE SPRUNGER for comments on the manuscript. Technical illustrations were prepared with the superb assistance of LEANNE OLDS. This research was supported by National Institutes of Health (NIH) grant HD24663 to L.K. P.L.G. was supported for part of her graduate work by the NIH predoctoral training grant GM07133 awarded to the Department of Genetics. Some nematode strains used in this study were provided by the Caenorhabditis elegans Genetics Center, which is supported by contract number NO1-AG-9-2113 between the National Institutes of Health and the Curator of the University of Missouri.

LITERATURE CITED


C. elegans Sex Determination


Communicating editor: R. K. Herman