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

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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 *m*asculinization *o*f the *g*erm 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 germline 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; TRENT, 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 germline-specific sex determination genes in addition to the globally acting genes described above. The *fog-1* gene (for *f*eminization *o*f the *g*erm 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-1* (for *m*asculinization *o*f the *g*erm 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-1*, 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-1* is essential for embryogenesis and speculate that *mog-1* 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° unless otherwise noted.

Nomenclature: The suffix "*lf*" indicates a loss-of-function mutation, the suffix "*gf*" indicates a gain-of-function mutation, and the suffix "*mx*" indicates a mutation with *m*ixed 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), *glp* (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, e1941mx, e1095), unc-4(e120), mnCl* (HERMAN 1978).

LG III: *fem-2(e2105), dpy-17(e164), dpy-19(e1259ts), sma-2(e502), unc-32(e189), lin-12(n941), glp-1(q46), mog-1(q151, q161, q223, q370, q471, q473), unc-69(e587), tra-1(e1099, e1781am), dpy-18(e364), eT1* (ROSENBLUTH and BAILLIE 1981), *qC1* (AUSTIN and KIMBLE 1989), *qDp3* (AUSTIN and KIMBLE 1987), *nDf40* (HENGARTNER, ELLIS and HORVITZ 1992).

LG IV: *fem-1(e1991am), unc-24(e138), fem-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 (P_0), either N2 or *dpy-19* +/+ *unc-32*, were mutagenized with either 1 μl/ml or 4 μl/ml ethyl methanesulfonate (EMS) for 4 hr, individual F_1 self-progeny picked to separate plates, and F_2 were screened for sterile mutants. Sterile F_2 were examined by Nomarski microscopy and those with a Mog (for *M*asculinization *o*f the *g*erm 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-1*. *mog-1(q151*, q161 and *q223)* were isolated after screening 9,467 haploid genomes mutagenized with 1 μl/ml EMS (a mutation frequency of 3×10^{-4}), whereas *mog-1(q370)* was isolated after mutagenesis with 4 μl/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*-1 alleles, *q471* and *q473*, were isolated in a screen for mutations that fail to complement *mog-1(q223).* For this non-complementation screen, *dpy-19; him-5* L4 males raised at 15° were mutagenized with 2 μl/ml EMS for 4 hr at 20° and mated with hermaphrodites of genotype *dpy-19 mog-1(q223) unc-69; qDp3[dpy-19(+)mog-1(+)].* Crosses were left at 20° overnight and then shifted to 25°. Non-Dpy non-Unc hermaphrodite cross progeny (1500 F_1) were picked to individual plates as L4s or young adults (*dpy-19* males at 25° and males carrying *qDp3* do not mate, so adults were not mated). F_2 progeny were screened by dissecting microscope for plates on which all Dpy F_2 were sterile. Such Dpy sterile progeny were examined by Nomarski microscopy to ask if they were Mog.

Scoring the *mog-1* phenotype: All *mog-1(x), mog-1(x)/mog-1(y), mog-1(x)/nDf40, mog-1(x)/qCl, nDf40/qCl,* double mutant, and temperature shifted worms were scored by Nomarski microscopy. In addition to the germline 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, *dpy-19 mog-1(q223); him-5* males were examined by Nomarski microscopy for an alteration in any of the following: male gonad (KLASS, 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 KLASS 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

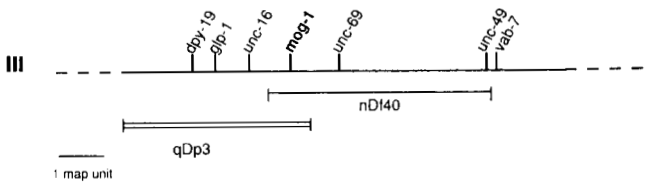

FIGURE 1.—Map position of *mog-1* relative to neighboring genes near the center of linkage group III.

from *mog-1(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-1(x) unc-69* worms became adults approximately one day later than *dpy-19 unc-69* worms grown in parallel. All *mog-1(x)*, except *mog-1(q473)*, were fully penetrant.

We next examined the penetrance of *mog-1(q473)* more carefully. Individual *mog-1(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-1(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, 41, 24, 33, 19 or 8%.

Mapping: The *mog-1* locus maps to chromosome *III* between *dpy-19* and *unc-69* (Figure 1). Three-factor data from hermaphrodites of genotype *mog-1(x)/dpy-19 unc-69* were obtained for each *mog-1* allele. For example, with *mog-1(q223)*, 11/22 Dpy non-Unc recombinants and 11/21 Unc non-Dpy recombinants carried *mog-1*. Similar results were obtained with the other five alleles. Two-factor data was obtained for *mog-1(q223)*. From four complete broods of *mog-1(q223) unc-69(e587)/++* raised at 20°, 1028 wild-type, 308 Unc Mog, 7 Mog, and 4 Unc were counted. Map distance was calculated using the formula $P = 1 - \sqrt{10 - 2R}$ (BRENNER, 1974) where R = number of recombinants/total number of progeny. These data show that *mog-1* is located ≈0.8 map units to the left of *unc-69* (Figure 1).

Complementation tests: For *mog-1(q161, q370, q471* and *q473), mog-1(x)/+* males were crossed to Unc hermaphrodites of genotype *mog-1(q223) unc-69/dpy-19 unc-69*. For *mog-1(q151), mog-1(q151)/+* males were crossed to *mog-1(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-1* mutation was tested for suppression by the amber suppressor tRNA mutation *sup-7(st5)* (WATERSTON 1981; WILLS *et al.* 1983). The initial cross was done in one of two ways: (1) *dpy-19 mog-1(q223 or q370)/++* hermaphrodites that had been purged of all self-sperm were mated with *sup-7(st5)/0* males. (2) *dpy-19 mog-1(q151, q161, q471* or *q473)/dpy-19 unc-69* Dpy hermaphrodites were mated with *sup-7/0* males. From both types of crosses, F_1 L4 hermaphrodites were picked to separate plates at 20° and 22° and allowed to self-fertilize. All Dpy F_2 from two or three broods were scored for self-fertility at each temperature. Self-fertile Dpy F_2 were progeny tested to distinguish between suppression and recombination.

Counting *mog-1* sperm by DAPI staining: For staining with diamidinophenylindole (DAPI), adult *mog-1(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-1* gonadal arms had 313 ± 23, 364 ± 6, 391 ± 37 or 514 ± 13 sperm, where each arm was counted three times and the average taken.

Activation with pronase: Sperm from adult *mog-1(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-1* sperm: Sperm distribution in *mog-1* and wild-type gonads was visualized by immunofluorescence. To extrude gonads, worms were cut on a poly-lysine-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-diazidabicyclo[2.2.2]-octane (DABCO) and paraphenylenediamine. *mog-1(q223)* and *mog-1(q223) +/+ unc-69* adults grown at 25° were stained in parallel.

Scoring the *mog-1* maternal effect: To characterize the maternal-effect lethal phenotype of *mog-1*, eggs from each double mutant (*e.g.*, *mog-1; 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; LAUFER, BAZZICALUPO 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-1/nDf40: mog-1(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-1/qC1: qC1/unc-32 dpy-18; him-5 males were crossed to *mog-1(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-1(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-32 dpy-18*.

mog-1(q223)/mog-1(q473) and nDf40/mog-1(q473): dpy-19 mog-1(q473); him-5 males were crossed with either *mog-1(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* L4 F_1 cross progeny were picked to individual plates and scored one day later. Any such F_1 hermaphrodites

that were self-fertile were progeny tested to determine their genotype. Because *nDf40* has a semidominant lethal phenotype at 15° (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(lf): 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-2++/+ mog-1 unc-69* were picked to individual plates and progeny tested to identify a recombinant of genotype *fem-2 + +/fem-2 mog-1 unc-69*. From these *fem-2* homozygous mothers, *fem-2 mog-1 unc-69* Unc progeny were scored.

mog-1; fem-3: Dpy Unc progeny from F_1 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)*, the canonical null, and *tra-1(e1781)*, an amber allele. *XX tra-1(e1099)* homozygotes 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; SCHEDL *et al.* 1989); *XX tra-1(e1781)* homozygotes have a male soma, but make oocytes much more frequently than *tra-1(e1099)* mutants (HODGKIN 1987; SCHEDL *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* hermaphrodites and L4 Unc cross progeny were picked 5/plate. The next generation were screened by dissecting scope for Unc worms with male tails. The mog-1 *unc-69 tra1* 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(lf); 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)* homozygotes 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)* homozygotes are completely transformed males and are cross-fertile (T. SCHEDL, personal communication). Each *tra-2(x); mog-1(y)* double mutant was identified among the progeny of *tra-2(x)/+; mog-1(y) unc-69/++* hermaphrodites. F_2 Unc *XX* males of genotype *tra-2(y); mog-1(x) unc-69* were scored by Nomarski microscopy as above. For each strain, *XX tra-2; unc-69* males were scored in parallel to control for marker effects.

TABLE 1

***mog-1 XX* germ-line phenotype**

Genotype[a]	*XX* Germ-line phenotype[b]	*n*[c]
+/+	Sperm and oocytes	>100
mog-1(x)/+	Sperm and oocytes	>100
mog-1(x)/mog-1(q223)	Excess sperm, no oocytes[d]	>10
mog-1(x)/nDf40	Excess sperm, no oocytes[e]	>10
mog-1(x)/qC1	Excess sperm, no oocytes	>10
nDf40/qC1	Excess sperm, no oocytes	13
mog-1(q223)/q223)/+[f]	Sperm and oocytes	>100

[a] *mog-1(x)*: *q151, q161, q223, q370, q471* or *q473*.
[b] 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.
[c] *n* = number of worms scored for each allele. Each worm possesses two ovotestes.
[d] For most *mog-1(x)*, all ovotestes made excess sperm and no oocytes; the exception was *mog-1(q473)*: *62/63 mog-1(q473)* ovotestes produced excess sperm and 1/63 had an oogenic core.
[e] For most *mog-1(x)*, all ovotestes made excess sperm and no oocytes; the exception was *mog-1(q473)*: *41/42 mog-1(q473)* ovotestes produced excess sperm and no oocytes and 1/42 produced oocytes.
[f] *mog-1(+)* was carried on *qDp3*.

mog-1; tra-3(lf): Hermaphrodites of genotype *mog-1 unc-69/+; tra-3* were first identified. From these parents, Unc progeny of genotype *mog-1 unc-69; tra-3* were scored and compared to +/+; *tra-3* pseudomales segregating from a +\+; *tra-3* hermaphrodite.

tra-2(gf); mog-1: This double mutant was constructed in two ways. (1) *unc-4 tra-2(q122gf)* Unc females were crossed with *dpy-19 mog-1(q223); him-5* males. F_1 females and males of genotype *unc-4 tra-2(q122gf)/++; dpy-19 mog-1(q223)/++* were crossed with each other, F_2 Dpy Unc L4s hermaphrodites of genotype *unc-4 tra-2(q122gf); dpy-19 mog-1(q223)* were separated from their siblings, and scored the following day. (2) *mog-1(q223) unc-69/dpy-19 unc-69* Unc hermaphrodites were crossed with *tra-2(q122gf)* males. From this cross, single F_1 females were mated with single F_1 males and the cross in which both parents were *tra-2(q122gf)/+; mog-1(q223) unc-69/++* identified by the presence of F_2 Unc Mog progeny. From this cross, Unc *XX* progeny were isolated as L4s and examined when adult.

tra-2(mx); mog-1: The *tra-2(e1941mx); mog-1(q223)* double mutant was constructed in a manner analogous to that described in (2) for the *tra-2(q122gf); mog-1* double mutant.

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

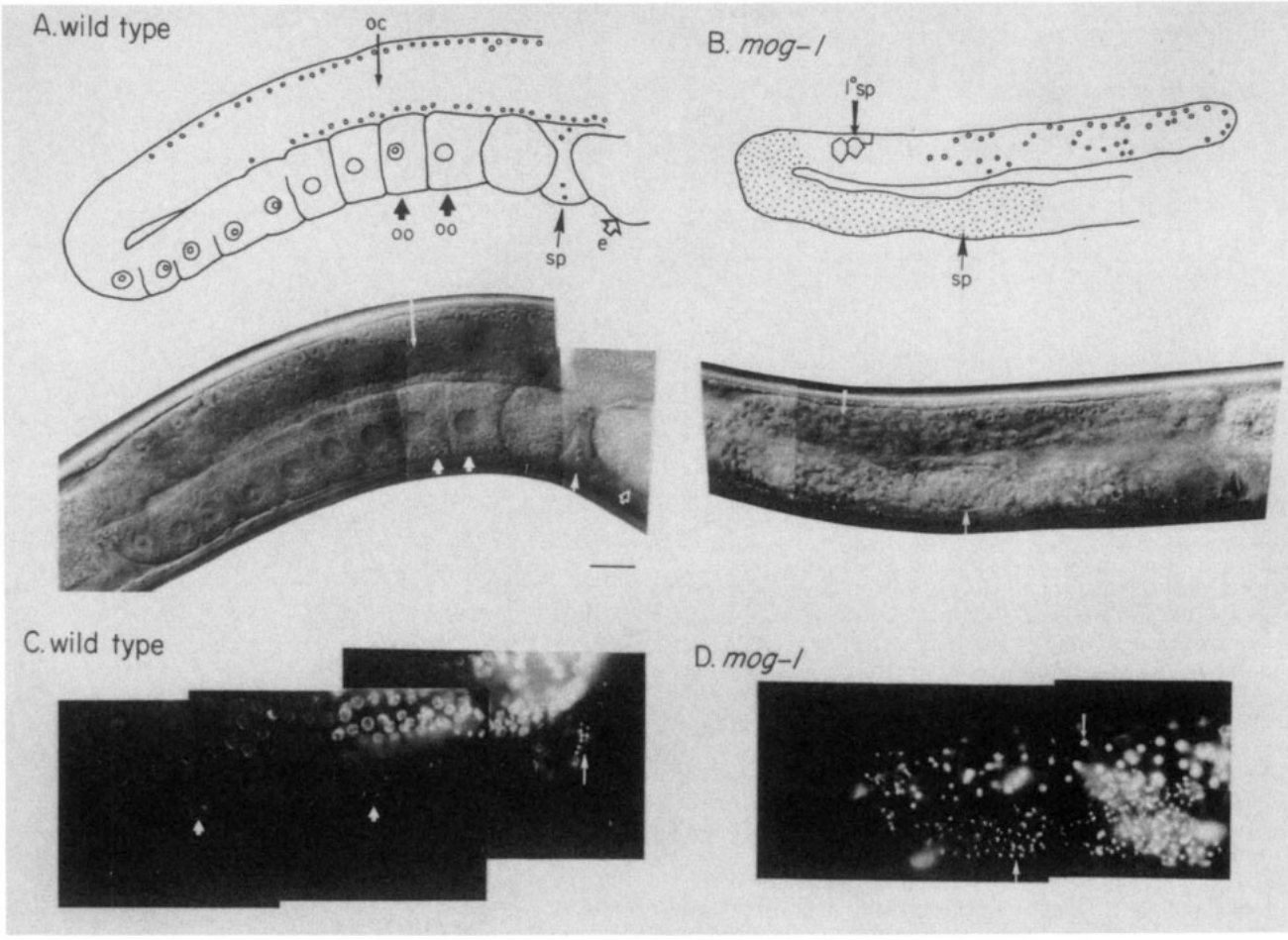

FIGURE 2.—The *mog-1* mutant phenotype: masculinization of the germline. (A) and (B) Nomarski photomicrographs with drawings above. (C) and (D) DAPI-stained gonadal arms. The magnification bar in (A) represents ≈50 μm and is appropriate for A–D. (A and C) wild-type *(mog-1(q223) +/+ unc-69)* adult hermaphrodites, 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-1(q223)/mog-1(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-1(q473)* is incompletely penetrant at both temperatures (MATERIALS AND METHODS). The phenotype of *mog-1*, described below, is the same for all five non-conditional *mog-1* mutants, and for most (97%) *mog-1(q473)* animals at restrictive temperature (25°).

The *mog-1* 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-1 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-1*. The morphology of *mog-1* sperm is normal (Figures 2 and 3). Furthermore, *mog-1* sperm, like wild type (WARD, HOGAN, and NELSON 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-1* mutants, *XX mog-1* 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-1 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-1* 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-1* mutants is consistent with the idea that *mog-1* does not regulate the somatic sexual phenotype.

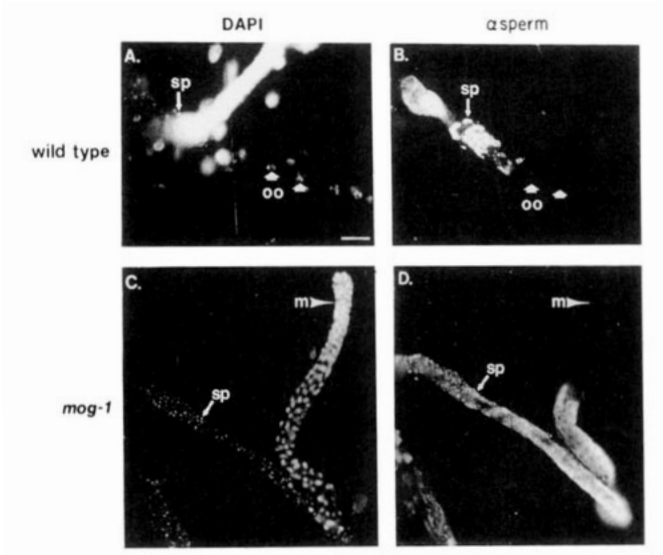

FIGURE 3.—*mog-1* sperm react with anti-sperm antibody. (A and C) DAPI-stained gonadal arms. (B and D) same gonad treated with TRII, an antibody that detects *C. elegans* sperm (WARD *et al.* 1986) (see MATERIALS AND METHODS). Magnification bar in (A) represents ≈50 μm and is appropriate for A–D. (A 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.

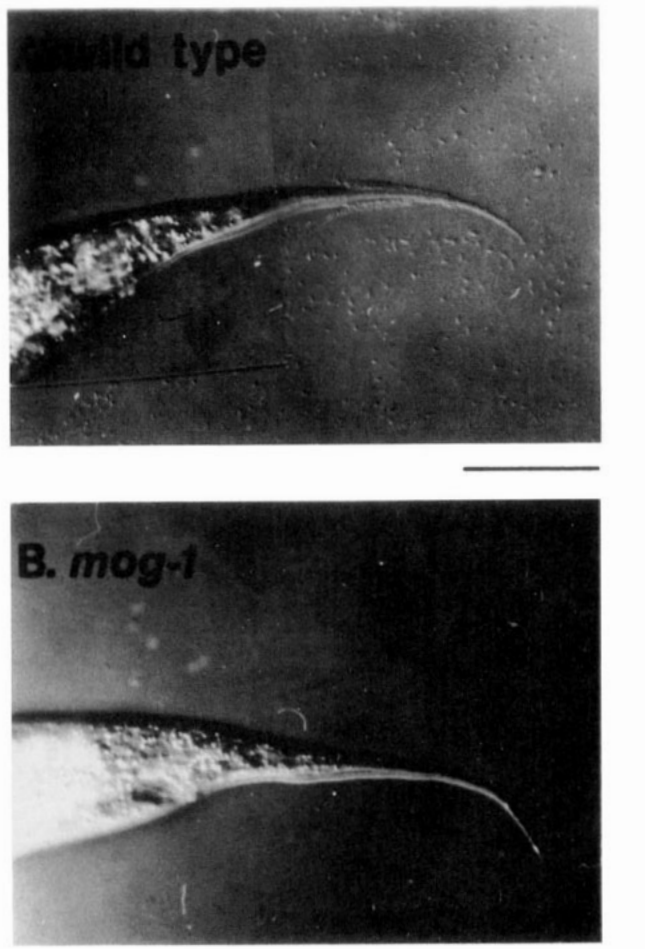

FIGURE 4.—*mog-1* does not masculinize the hermaphrodite soma. Nomarski photomicrographs. Magnification bar ≈50 μm. (A) The wild-type *(mog-1(q223) +/+ unc-69)* adult hermaphrodite tail ends in a spike. (B) The *mog-1(q223)/mog-1(q223)* adult tail also ends in a hermaphrodite-like spike. If the tail were masculinized, it would be blunter and might possess a fan and rays (SULSTON, ALBERTSON and THOMSON 1980).

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 *m*asculinization *o*f the *g*erm 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 hermaphrodites (Table 2). Second, *mog-1* alleles were isolated at a frequency typical of loss-of-function mutations (MATERIALS AND METHODS). Specifically, *mog-1* alleles were isolated at a frequency of 3×10^{-4}, which is similar to the frequency with which loss-of-function mutations in other genes were isolated in the same screen (BARTON and KIMBLE 1990). Third, the phenotype of *mog-1(x)* homozygotes is identical to that of *mog-1(x)/nDf40*, where *nDf40* removes at least part of the *mog-1* locus (Table 1, line 4). In addition, a rearrangement of chromosome *III*, called *qC1*, fails to complement each of the *mog-1* alleles (Table 1, line 5). The *qC1* chromosome, originally isolated as a γ-ray induced allele of *glp-1*, interferes with recombination over much of chromosome *III* and may therefore carry a chromosomal rearrangement.

Three additional lines of evidence suggest that the *mog-1* mutant phenotype may be due to a complete loss of *mog-1* activity. First, two *mog-1* mutations with the typical Mog phenotype were isolated in a noncom-

TABLE 2

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

Parental genotype	T (°C)	Brood size[a]	Progeny phenotype (%)	
			Self-fertile	Mog
N2	25	225 ± 49 (n = 4)	100	0
mog-1(q151)/dpy-19 unc-69	25	250 ± 99 (n = 3)	75	25
mog-1(q161)/dpy-17 unc-32	25	189 ± 63 (n = 3)	72	28
mog-1(q223)/dpy-19 unc-69	25	247 ± 31 (n = 3)	73	27
mog-1(q473)/dpy-19 unc-69	25	288 ± 56 (n = 3)	72	28
N2	20	321 ± 46 (n = 5)	100	0
mog-1(q370)/sma-2 unc-69	20	342 ± 24 (n = 3)	73	27
mog-1(q471)/unc-69	20	340 ± 39 (n = 4)	74	26

[a] n = number of broods scored.

plementation screen. 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 *qC1/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.

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 *q370*. 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(lf)* 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(lf) 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 (DONIACH 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(lf)* 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*

TABLE 3

Phenotype of animals homozygous for *mog-1* and feminizing mutations

Genotype[a]	Germ-line phenotype (%)				n^b
	Sperm only	Sperm and oocytes	Oocytes only	Abnormal[c]	
mog-1	100	0	0	0	>100
her-1 XX	0	100	0	0	—
her-1; mog-1(q223)[d]	100	0	0	0	40
her-1; mog-1(q370)[d]	100	0	0	0	20
her-1 XO	0	100	0	0	—
her-1; mog-1(q223)[e]	100	0	0	0	28
her-1; mog-1(q370)[e]	100	0	0	0	34
fog-2 XX	0	0	100	0	—
fog-2; mog-1(q223)[f]	100	0	0	0	5[g]
fog-2; mog-1(q370)[f]	100	0	0	0	16[g]
fem-1 XX	0	0	100	0	—
fem-1; mog-1(q223)[h]	0	0	98	2	120
fem-1; mog-1(q370)[h]	0	0	100	0	18
fem-2 XX	0	0	100	0	—
fem-2; mog-1(q223)[i]	11	0	32	57	61
fem-2 mog-1(q370)[i]	3	6	47	44	66
fem-3 XX	0	0	100	0	—
fem-3; mog-1(q223)[j]	0	0	100	0	28
fem-3 mog-1(q370)[j]	0	0	100	0	34
fog-1 XX	0	0	100	0	—
fog-1; mog-1(q223)[k]	0	0	100	0	48[l]

[a] Alleles used were *mog-1(q223, q370)* (this paper), *her-1(e1518)* (HODGKIN 1980), *fog-2(q71)* (SCHEDL and KIMBLE 1988), *fem-1(e1991)* (DONIACH and HODGKIN 1984), *fem-2(e2105)* (HODGKIN 1986), *fem-3(e1996)* (HODGKIN 1986) and *fog-1(q180)* BARTON and KIMBLE 1990). See MATERIALS AND METHODS for construction of strains.

[b] *n* = number of ovotestes scored. For all of the single mutants, (—) means that no ovotestes were scored in this work, and that the phenotypes were obtained from references as listed in footnote *a*. For *fog-1* and *fog-2* strains, "*n*" is deduced, as described in footnotes *g* and *l*.

[c] Abnormal gametes were found in the proximal region of the ovotestes, they were approximately one-fourth the size of a typical oocyte, and they had a grainy cytoplasm and large nucleolus.

[d] Dpy Unc (*XX*) self-progeny of *mog-1 unc-69/++*; *her-1 him-5 dpy-21/her-1 him-5 dpy-21* mothers.

[e] Unc non-Dpy (*XO*) self-progeny of *mog-1 unc-69/++*; *her-1 him-5 dpy-21/her-1 him-5 dpy-21* mothers.

[f] Unc self progeny of *mog-1 unc-69/++*; *fog-2/+* mothers.

[g] The *fog-2* mutations was not linked to a morphological marker. All Unc worms hatching from *mog-1(q223* or *q370) unc-69/++*; *fog-2/+* mothers were scored by Nomarski microscopy; *fog-2* homozygotes should represent one-fourth of these Unc worms. Here, *n* = ¼ the number of total ovotestes scored. To be sure double mutants were not dying as embryos or young larvae, eggs were scored for viability (see MATERIALS AND METHODS for details); 90/90 eggs from *mog-1(q223) unc-69/++*; *fog-2/+* mothers, and 176/176 eggs from *mog-1(q370) unc-69/++*; *fog-2/+* mothers were viable.

[h] Dpy Unc self-progeny of *dpy-19 mog-1/++*; *unc-24 fem-1/unc-24 fem-1* mothers.

[i] Unc self-progeny of *fem-2/fem-2 mog-1 unc-69* mothers.

[j] Dpy Unc self-progeny of *mog-1 unc-69/++*; *fem-3 dpy-20/++* mothers.

[k] Unc self-progeny of *fog-1/+*; *mog-1 unc-69/++* mothers.

[l] Because *fog-1* was not linked to a morphological marker, all Unc worms hatching from *mog-1(q223 or q370) unc-69/++*; *fog-1/+* mothers were examined. About one-fourth of these Unc worms were females (36/136) and were scored as *fog-1* homozygotes. To be sure double mutants were not dying as embryos or larvae, eggs were scored for viability; 123/123 eggs were viable.

genetic background may reveal a similar temperature-sensitivity 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(lf)* 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-1* and *tra* mutations

Genotype[a]	Germline phenotype (%)			n^b
	Sperm only	Sperm and oocytes	Oocytes only	
tra-1(e1099)[c]	100[d]	0	0	97
tra-1(e1099) mog-1(q370)[e]	100[f]	0	0	73
tra-1(e1781)[g]	60	40	0	43
tra-1(e1781) mog-1(q223)[h]	100	0	0	50
tra-2(e1095)[i]	100	0	0	35
tra-2(e1095); mog-1(q223)[j]	100	0	0	33
tra-2(e1095); mog-1(q370)[k]	100	0	0	15
tra-3(e1107)[l]	77	23	0	61
tra-3(e1107); mog-1(q223)[m]	100	0	0	32
tra-3(e1107); mog-1(q370)[n]	97	3	0	30

[a] Alleles used were *mog-1(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] *n* = 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-1(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-1(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-1(q223)unc-69*/++ mothers. Similar results were obtained with *tra-2(q270);mog-1(q223)unc-69* (*n* = 17), and *tra-2(q276);mog-1(q223) unc-69* (*n* = 18).

[k] Unc self-progeny with male tails from *tra-2(e1095)*/+; mog-1(q370)/unc-69 mothers.

[l] Self-progeny from *tra-3(e1107)/tra-3(e1107)* mothers.

[m] Unc self-progeny from *mog-1(q223) unc-69*/++; *tra-3(e1107)/tra-3(e1107)* mothers.

[n] Unc self-progeny from *mog-1(q370)unc-69*/++; *tra-3(e1107)/tra-3(e1107)* mothers.

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-1* activity. Because *tra-3* mutants show maternal rescue, the *mog-1; tra-3* double mutant was derived from a *mog-1*/+; *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-1* 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-1 tra-1* and *mog-1; 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-1*. However, the somatic phenotype of the double mutant is typical of the particular *tra* mutation used, consistent with the idea that *mog-1* does not function in somatic sex determination.

For *tra-2; mog-1* 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(lf)* mutations, transforming *XX* animals into non-mating males, whereas *tra-2(q276)* is an unusual loss-of-function mutation that transforms *XX* worms into mating males (T. SCHEDL, personal communication). The germ lines of all three *tra-2; mog-1* double mutants, like those of

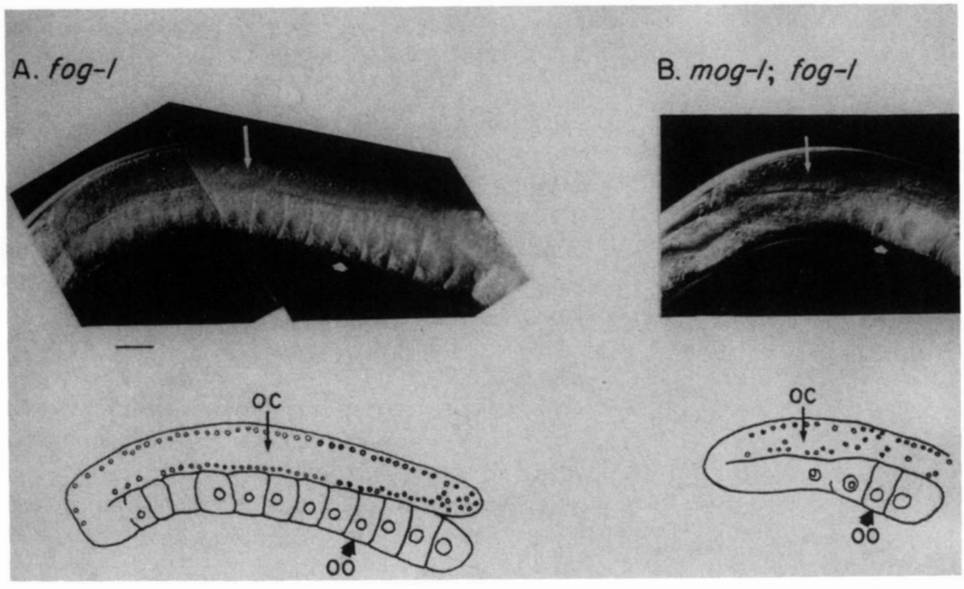

FIGURE 5.—*fog-1; mog-1* germ line is feminized. Nomarski photomicrographs with line drawings below. Magnification bar ≈50 μm. (A) *fog-1(q180)/fog-1(q180)* adult, lateral view. Only oocytes (oo) are made and an oogenic core (oc) is found. The right-most oocyte has entered the uterus, but is unfertilized. (B) *fog-1(q180)/fog-1(q180); mog-1(q223) unc-69/mog-1(q223) unc-69* adult, lateral view. Only oocytes (oo) are made and an oogenic core (oc) is observed. The right-most oocyte is in the spermatheca, but is unfertilized.

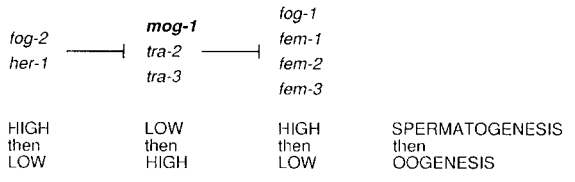

FIGURE 6.—A model for the role of *mog-1* in sex determination. In the hermaphrodite germ line, *fog-2* negatively regulates *tra-2*, thereby allowing the *fem* genes and *fog-1* to direct spermatogenesis. After approximately 300 sperm are made, *mog-1* becomes functional to direct the hermaphrodite switch from spermatogenesis to oogenesis. To achieve this switch, *mog-1* might positively regulate *tra-2* or negatively regulate the *fem* genes and *fog-1* (see DISCUSSION). Since the role *tra-1* in the hermaphrodite germ line is not understood (HODGKIN 1987; SCHEDL *et al.* 1989), we omit *tra-1* from this model.

TABLE 5

Phenotype of *mog-1* with *tra-2* regulatory mutants

Genotype	Germ-line phenotype (%) Sperm only	Sperm and oocytes	Oocytes only	n[a]
tra-2(q122gf)[b]	0	0	100	30
tra-2(q122gf);mog-1(q223)[c]	47	37	16	19
tra-2(e1941mx)[d]	0	0	100	14
tra-2(e1941mx);mog-1(q223)[e]	100	0	0	13

[a] n = number of ovotestes scored.
[b] SCHEDL and KIMBLE (1988).
[c] Unc Dpy cross progeny of genotype *tra-2(q122gf) unc-4; dpy-19 mog-1(q223).*
[d] DONIACH (1986).
[e] Unc Dpy cross progeny of genotype *tra-2 (e1941mx) unc-4; dpy-19 mog-1 (q223).*

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)*.

TABLE 6

Maternal effect lethality of *mog-1*

Maternal genotype[a]	Phenotype of progeny (%)[b] Embryonic lethal	Larval lethal	Viable adult	n[c]
fem-1	3	<1	96	242
fem-2	5	3	92	288
fem-3	2	<1	97	245
fog-1	1	0	99	262
tra-2(q122gf)	2	0	98	247
fem-1;mog-1(q223)	62	25	13	810
fem-1;mog-1(q370)	61	39	0	41
fem-2 mog-1(q223)	91	6	3	64
fem-2 mog-1(q370)[d]	95	2	3	214
fem-3; mog-1(q223)	68	21	11	252
fem-3; mog-1(q370)	61	18	21	316
fog-1;mog-1(q223)	98	1	1	206
tra-2(q122gf);mog-1(q223)	61	25	14	57

[a] Alleles used were *fem-1(e1991), fem-2(e2105), fem-3(e1996), fog-1(q180), fog-2(q71)* and *mog-1(q223, q370).*
[b] All progeny were obtaining by crossing females with N2 males at 20°. Embryos were scored for hatching and postembryonic growth by dissecting microscope.
[c] n = number of embryos scored.
[d] All Unc progeny from *fem-2* + +/*fem-2 mog-1 unc-69* mothers were crossed with N2 males as a group; 2/49 worms were hermaphrodites. Therefore, a fraction of the eggs scored may have been self-progeny.

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°, 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, SCHEDL 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.

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