The Phenotype of *mes-2*, *mes-3*, *mes-4* and *mes-6*, Maternal-Effect Genes Required for Survival of the Germline in *Caenorhabditis elegans*, Is Sensitive to Chromosome Dosage

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Manuscript received July 22, 1997
Accepted for publication September 15, 1997

ABSTRACT

Mutations in *mes-2*, *mes-3*, *mes-4*, and *mes-6* result in maternal-effect sterility: hermaphrodite offspring of *mes*/*mes* mothers are sterile because of underproliferation and death of the germ cells, as well as an absence of gametes. Mutant germ cells do not undergo programmed cell death, but instead undergo a necrotic-type death, and their general poor health apparently prevents surviving germ cells from forming gametes. Male offspring of *mes* mothers display a significantly less severe germline phenotype than their hermaphrodite siblings, and males are often fertile. This differential response of hermaphrodite and male offspring to the absence of mes^+ product is a result of their different *X* chromosome compositions; regardless of their sexual phenotype, *XX* worms display a more severe germline phenotype than *X*O worms, and *XXX* worms display the most severe phenotype. The sensitivity of the mutant phenotype to chromosome dosage, along with the similarity of two MES proteins to chromatin-associated regulators of gene expression in Drosophila, suggest that the essential role of the *mes* genes is in control of gene expression in the germline. An additional, nonessential role of the *mes* genes in the soma is suggested by the surprising finding that mutations in the *mes* genes, like mutations in dosage compensation genes, feminize animals whose male sexual identity is somewhat ambiguous. We hypothesize that the *mes* genes encode maternally supplied regulators of chromatin structure and gene expression in the germline and perhaps in somatic cells of the early embryo, and that at least some of their targets are on the *X* chromosomes.

THE germline is crucial for the perpetuation of species. In metazoans, it is set apart from the soma relatively early in development, and must be specified to follow its unique developmental program of extensive proliferation, meiosis, and the production of gametes (for review see Marsh and Goode 1994). The task of identifying factors that participate in specifying the germline and initiating its developmental program can be approached genetically by screening for mutants defective in early aspects of germline development. In organisms that rely heavily on a maternal contribution of RNA and proteins, the crucial factors are likely to be encoded by maternal-effect genes. This logic prompted us to carry out extensive screens for maternal-effect sterile mutants in the nematode, *Caenorhabditis elegans* (Capowski *et al.* 1991). This paper describes analysis of the major class of mutants that came from those screens.

In *C. elegans*, the germline (P4 cell) is set apart from the soma by the 16–24-cell stage of embryogenesis. The primordial germ cell divides only once during embryogenesis. Extensive proliferation of the germline occurs during larval development, resulting in expansion of the germline from two cells in first stage larvae (L1s) to ~1500 cells in adult hermaphrodites and ~500 cells in adult males. Meiosis commences in the L3 stage, sperm are formed in the L4 stage, and in hermaphrodites oocyte production begins in the adult stage; males continue to make sperm throughout adulthood (see Kemphues and Strome 1997 and Schedl 1997 for reviews of germline establishment and development).

To identify genes encoding early-acting factors required uniquely for germline development, we screened for maternal-effect sterile (*mes*) mutants (Capowski *et al.* 1991). The phenotype of homozygous *mes* worms is dependent on the genotype of their mother: *mes* hermaphrodites produced by *mes*/+ mothers are fertile, whereas *mes* hermaphrodites produced by *mes* mothers are sterile. Even *mes*/+ hermaphrodites produced by mating *mes* mothers to wild-type males are sterile, revealing that maternal mes^+ product is both necessary and sufficient for normal germline development. Mutations in four of the five genes identified in this screen (*mes-2*, *mes-3*, *mes-4*, and *mes-6*) result in a common mutant phenotype: the hermaphrodite offspring of *mes* mothers undergo apparently normal germline development during embryogenesis but develop into sterile adults with a 10–100-fold reduction in the number of undifferentiated germ cells and no gametes (Capowski *et al.* 1991; Paulsen *et al.* 1995).

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As described in this paper, we see a dramatic difference in the severity of the Mes phenotype when comparing male and hermaphrodite siblings produced by *mes* mothers. In contrast to the severely affected germline seen in mutant hermaphrodites, mutant male siblings generally possess a well-proliferated and healthy-appearing germline, produce hundreds of sperm, and are fertile. We present evidence that the difference in expressivity of the Mes phenotype in hermaphrodites and males depends on *X* chromosome dosage and not on the sexual phenotype of the worms. Molecular analysis has revealed that two of the *mes* genes encode homologs of Polycomb group (Pc-G) proteins in Drosophila (R. Holdeman, S. Nehrt and S. Strome, unpublished results; I. Korf, R. Holdeman, Y. Fan and S. Strome, unpublished results). Pc-G proteins repress gene expression and are thought to act by controlling chromatin structure. A role for MES proteins in modulation of chromatin and repression of gene expression in the germline is consistent with the sensitivity of the mutant phenotypes to chromosome dosage. In addition to their essential role in the germline, the *mes* genes may serve a nonessential role in somatic cells of the early embryo. This is revealed by our unexpected finding that *mes* mutations can affect the sexual fate of worms, probably through an effect on dosage compensation in the early embryo.

MATERIALS AND METHODS

General techniques and strains: Maintenance and genetic manipulation of *C. elegans* were carried out as described by Brenner (1974). Unless stated otherwise, experiments were conducted at room temperature (20–22°). The permissive and restrictive temperatures for all temperature-sensitive mutations used in our experiments were 16° and 25°, respectively. Most strains were provided by the *Caenorhabditis* Genetics Center, including the tetraploid (4n) stock (strain SP346). *fog-1(q253ts)* was from Judith Kimble. *tra-1(e1099)* was from Barbara Robertson and William Wood. *tra-1(e1575gf)* was from Tim Schedl. *sdc-3(y52)* was from Barbara Meyer. *C. elegans* strain N2 variety Bristol was the wild-type parent of all nematode strains used. Mutations, deficiencies, and balancers used are listed by linkage group (LG):

LG I: *mes-3(bn35), fog-1(q253ts), unc-11(e47), dpy-5(e61), dpy-14(e188), glp-4(bn2ts), hDp20 (I;V,f)*
LG II: *mes-2(bn11, bn27, bn76), tra-2(e1095), dpy-10(e128), unc-4(e120), mnC1, rol-1(e91)*
LG III: *tra-1(e1099, e1575gf), plg-1(e2001), unc-32(e189), unc-79(e1068), ced-4(n1162), glp-1(q175), eT1 (III,V)*
LG IV: *mes-6(bn64, bn66), him-8(e1489), fem-3(q20ts,gf), unc-26(e205), DnT1[unc(n754)let] (IV;V)*
LG V: *mes-4(bn58, bn67), dpy-11(e224), sdc-3(y52, y128), unc-76(e911), DnT1[unc(n754)let] (IV;V)*

mes mutations were maintained as balanced stocks or *in trans* to appropriately marked chromosomes. *mes-2* mutations were balanced with *mnC1* (Herman 1978). *mes-3* mutations were maintained as homozygotes in combination with *hDp20* to provide *mes-3*$^+$ function, or in heterozygotes *in trans* to one of the following recessively marked chromosomes: *unc-11 dpy-5* or *dpy-5 dpy-14*. *mes-4* and *mes-6* mutations were balanced with the reciprocal translocation *DnT1*, which has a dominant Unc and a recessive lethal phenotype (E. Ferguson, personal communication).

Nomenclature: Standardized *C. elegans* genetic nomenclature (Horvitz *et al.* 1979) has been used. Genes and transcripts are given three-letter, lowercase, italicized designations, *e.g., mes-2*. Protein products are given nonitalicized, all uppercase designations *e.g.*, MES-2. The mutant phenotypes caused by mutating a particular gene are given the three-letter designation with only the first letter being uppercase, *e.g.*, Mes. In this paper, the term *bal* will be used to designate any of the balancer chromosomes or marked chromosomes described in the previous section, and Bal will be used to describe the phenotype of each.

Staining nuclei: To visualize nuclei in intact animals, single worms were placed in 1 μl of water on a polylysine-coated slide (Miller and Shakes 1995), and the slide was briefly waved over the flame of an alcohol burner until all the water had evaporated and the worms were dry. Eight μl of an equal volume mixture of self-hardening mounting medium (Gelutol; Monsanto, St. Louis, MO; Miller and Shakes 1995) and Hoechst Stain (40 mm NaC1, 10 mm Tris-Cl, pH 7.4, 1 mm EDTA, pH 8.0, 20% glycerin, 10 μg/ml Hoechst 33342) were put on an 18 × 18-mm coverslip and placed on the specimen.

To visualize nuclei in extruded gonads, worms were placed in a 10-μl drop of Solution A [100 μg/ml Hoechst 33342, 0.5× egg salts (Edgar 1995), and 0.25 μM levamisole to promote hypercontraction] on a polylysine-coated slide. After allowing the levamisole to take effect, worms were cut open with a scalpel just posterior to the pharynx or anterior to the anus. The released gonads (and other tissues) were incubated in Solution A for 2–3 min to allow the Hoechst dye to penetrate. Then 10 μl of Solution B (5% glutaraldehyde, 0.5× egg salts, 2 μg/ml Hoechst 33342) were added. Samples were covered with a coverslip and viewed immediately.

All samples were viewed on an Axioskop (Carl Zeiss, Thornwood, NY) equipped with Nomarski differential interference contrast and epifluorescence optics. Photographs were taken with Tri-X film and developed with D-76 developer (Eastman Kodak, Rochester, NY).

Synchronizing worms: Gravid hermaphrodites were cleaned of *Escherichia coli* by allowing them to crawl on agar plates lacking bacteria. Worms were incubated in 20 μl of distilled water at room temperature for 6–10 hr, and then cut in half with a scalpel to release their embryos. The embryos were put in a humid chamber overnight. By the next day, all the embryos had hatched and arrested as L1s. L1s were transferred to plates spread with bacteria and allowed to grow to the desired age.

Laser ablation of distal tip cells: A general description of laser microsurgery in *C. elegans* is described elsewhere (Bargmann and Avery 1995). Worms were mounted on 0.4% agarose pads containing 10 mm sodium azide and overlaid with coverslips. Ablations were performed in hermaphrodites at the late L2/early L3 stage of development using a VSL-337 laser (Laser Science Inc., Newton, MA) and coumarin dye (Avery and Horvitz 1987). Only one distal tip cell (DTC) was ablated per worm, leaving the other gonad arm to serve as a control for the behavior of the germline in the presence of the DTC. We monitored the effectiveness of our technique by ablating the DTC of wild-type hermaphrodites and demonstrating that all germ cells became sperm. After operating, worms were placed on individual plates, allowed to develop for 48 hr, Hoechst stained as described above, and assayed for the number of sperm, meiotic germ nuclei, and/or undifferentiated germ nuclei present. Results were scored blindly, without knowledge of the genotype of the specimen or which DTC had been destroyed.

Construction and analysis of *mes; glp-4* mutants: *mes/bal; glp-4* strains were created with the following *mes* alleles: *mes-2(bn11), mes-4(bn58), mes-4(bn67),* and *mes-6(bn66).* Crosses were done at 16° unless otherwise noted. (P0) *glp-4* males (raised at permissive temperature) were mated to *mes/bal* hermaphrodites. (F1) Progeny were allowed to mate with their siblings. The desired matings were between *mes/+; glp-4/+,* and *bal/+; glp-4/+* F1s to produce *mes/bal; glp-4* F2s. (F2) L4 hermaphrodites from these matings were picked to individual plates and allowed to produce self progeny for 24 hr. For the *mes-4* and *mes-6* constructions, Unc L4s (containing *DnT1*) were picked. After 30–50 F3s had been laid, the F2s were transferred to new plates. The first set of plates was moved to 25°, and the second set was kept at 16°. (F3) Worms raised at 25° were scored for the presence of 100% Glp sterile worms. For the *mes-2* construction, the plates were also scored for the presence of *mnC1* worms. (F4) 16° plates corresponding to broods with Glp steriles at 25° were scored for the presence of ~33% Mes sterile worms. This identified new strains that were *mes/bal; glp-4.*

To perform experiments with the *mes-2/mnC1; glp-4* strain, individual non-Dpy non-Unc hermaphrodites were allowed to lay eggs for 24 hr at 16° and were then transferred to 25°. *mes-2/mnC1; glp-4* hermaphrodites produced fertile progeny at 16°, including Dpy Uncs. Their germlines were non-Mes non-Glp-4. The siblings raised at 25° were non-Mes Glp-4. *mes-2; glp-4* hermaphrodites produced all sterile progeny at 16°, with no Dpy Uncs. Their germlines were Mes non-Glp-4. The siblings raised at 25° were Mes Glp-4.

To perform experiments with the *mes-4/DnT1; glp-4* and *mes-6/DnT1; glp-4* strains, individual Unc (containing *DnT1*) and non-Unc (lacking *DnT1*) hermaphrodites were allowed to lay eggs for 24 hr at 16° and were then transferred to 25°. The *mes/DnT1; glp-4* hermaphrodites produced fertile progeny at 16°. Their germlines were non-Mes non-Glp-4. The siblings raised at 25° were non-Mes Glp-4. *mes; glp-4* hermaphrodites produced all sterile progeny at 16°. Their germlines were Mes non-Glp-4. The siblings raised at 25° were Mes Glp-4.

Worms were harvested as L3s or young adults. The age of the worms was confirmed by examination of ventral hypodermal and vulval cells under Nomarski optics (Sulston and Horvitz 1977). Worms were stained with Hoechst to assess the appearance of mutant germ nuclei.

Construction and analysis of *mes; glp-1* mutants: *mes/bal; unc-32 glp-1/++* stocks were created using *mes-2(bn11), mes-2(bn27), mes-4(bn58), mes-4(bn67),* and *mes-6(bn69).* (P0) *unc-32 glp-1/eT1* males were crossed to *mes/bal* hermaphrodites. (F1) Outcross male progeny (*unc-32 glp-1/++* or *eT1/+,* in combination with *mes/+* or *bal/+*) were mated *en masse* to new *mes/bal* hermaphrodites. (F2) L4 hermaphrodites were picked to individual plates, allowed to produce self progeny, and scored for the presence of the desired phenotypic classes: F3 Unc Glp sterile worms, F3 Bal worms, and F4 Mes sterile worms. (If *DnT1* was the balancer chromosome used, then the dominant phenotype allowed its presence to be identified and balanced worms to be selected from among the F2). The resulting *mes/bal; unc-32 glp-1/++* stocks were maintained by cloning fertile *mes/bal* (or *mes/mes*) worms from broods that also contained Unc Glp worms. Experiments were performed by comparing the germlines of non-Mes Unc Glp worms from *mes/bal; unc-32 glp-1/++* mothers to the germlines of Mes Unc Glp worms from *mes; unc-32 glp-1/++* mothers. Germlines were analyzed in intact young adults that were stained with Hoechst.

Construction and analysis of *mes; ced-4* mutants: We constructed *mes/+; unc-79 ced-4/++* strains using *mes-2(bn11), mes-4(bn67),* and *mes-6(bn66).* (P0) N2 males were mated to *unc-79 ced-4* hermaphrodites. (F1) Heterozygous male cross progeny were mated to *mes/bal* hermaphrodites. (F2) Non-Bal L4 hermaphrodite offspring were placed on individual plates and allowed to produce self progeny. (F3) Broods were scored for the presence of Unc worms and the absence of Bal worms, indicating that the F2 parent was the desired heterozygote: *mes/+; unc-79 ced-4/++.* F3 Unc Ced and non-Unc non-Ced hermaphrodites were placed on individual plates. Approximately one fourth of each class was expected to be *mes/mes.* (F4) Broods were scored for fertility or sterility. We compared the extent of germline proliferation between Mes Unc Ced F4s (produced by *mes; unc-79 ced-4* F3s) and Mes non-Unc non-Ced F4s (produced by *mes; unc-79 ced-4/++* or *mes; ++* F3s). Germlines were analyzed in intact young adults stained with Hoechst.

Construction and analysis of *mes; him-8* mutants: *mes-2(bn11)* and *mes-3(bn35)* strains, in the configuration *mes/bal; him-8,* were made and analyzed as follows: (P0) *him-8* males were crossed to *mes/bal* hermaphrodites. (F1) Male progeny (*mes/+; him-8/+* or *bal/+; him-8/+*) were mated to outcross hermaphrodite siblings (*mes/+; him-8/+,* genotype was confirmed by allowing the worms to lay eggs before mating them and then analyzing the self progeny). (F2) L4 hermaphrodites were placed on individual plates and allowed to produce two generations of progeny. (F3) Plates containing Bal worms and males were retained. (F4) The desired strain was identified by the presence of Mes sterile hermaphrodites. To compare the germlines of *mes; him-8* males and hermaphrodites, non-Bal L4 hermaphrodites were isolated and allowed to produce self progeny. Approximately one third of the non-Bal hermaphrodites were *mes; him-8,* and they produced all sterile Mes hermaphrodite progeny and semifertile Mes male progeny.

mes-4(bn67)/DnT1; him-8/DnT1 was made by crossing *him-8* males to *mes/DnT1* hermaphrodites (P0). (F1) *mes/+;him-8/+* male progeny were crossed to *mes/DnT1* hermaphrodites. (F2) Individual L4 Unc (containing *DnT1*) hermaphrodites were allowed to produce two generations of progeny. (F4) The desired strain contained Mes sterile hermaphrodites and males. We also constructed a *mes-6 him-8* recombinant chromosome and balanced it over *DnT1* to create *mes-6(bn66) him-8/DnT1.* For analysis, Mes males and hermaphrodites were obtained from unmated, fertile, non-Unc (*mes*; *him-8*) hermaphrodites.

Mes siblings of each sex were raised at 20°, harvested between 68 and 74 hr after hatching, stained with Hoechst to visualize DNA, and then examined.

Construction and analysis of *mes-4; fog-1* mutants: We constructed *mes-4/DnT1; fog-1* strains using *mes-4(bn58)* and *mes-4(bn67),* and a *fog-1* male stock that was established from a rare spontaneous male. (P0) *fog-1* males (raised at permissive temperature) were crossed to *mes-4/DnT1* hermaphrodites. (F1) Outcross males were mated to their hermaphrodite siblings, also at 16°. (F2) Unc (containing *DnT1*) L4 hermaphrodite offspring were placed on individual plates at 16° and allowed to lay ~30 embryos. Then the F2s were transferred to new plates at 25°. (F3) The 25° F3 progeny were scored. Broods consisting of 100% Fog steriles were noted, and the corresponding 16° plates were retained at 16° for one more generation. (F4) The 16° plates were examined for the presence of Mes sterile worms. Strains were maintained at 16° by cloning fertile Unc hermaphrodites.

Fertile non-Unc (*mes-4; fog-1*) hermaphrodites were transferred from 16° to 25° to examine the feminized germline of their offspring. Fertile non-Unc hermaphrodites were also maintained at 16° to generate Mes offspring without a feminized germline. Fertile Unc (*mes-4/DnT1; fog-1*) hermaphrodites were transferred from 16° to 25° to verify that non-Mes germlines were feminized by *fog-1.* Worms were harvested as young adults and stained with Hoechst.

To examine Mes males with a feminized germline, fertile

non-Unc (*mes-4; fog-1*) hermaphrodites were transferred individually from 16° to 25° and mated to *fog-1* males (raised at permissive temperature). Outcross *mes-4*/+; *fog-1* males were recovered for analysis. These were compared to *mes-4*/+; *fog-1*/+ males, which were obtained by mating *mes-4; fog-1* hermaphrodites and N2 males. Worms were harvested as young adults and stained with Hoechst.

Construction and analysis of *mes-4; fem-3(gf)* mutants: We created *mes-4*/*DnT1; fem-3(gf)*/*DnT1* strains using *mes-4(bn58)*, *mes-4(bn67)*, and a *plg-1; fem-3(gf); him-5* strain. The *plg-1* mutation causes males to deposit a gelatinous plug at the vulva of their mating partners, and the *him-5* mutation causes hermaphrodites to produce ~33% *X*O male offspring. The *plg-1* and *him-5* mutations were not retained in the *mes-4*/*DnT1; fem-3(gf)*/*DnT1* strains. (P0) *plg-1; fem-3(gf); him-5* males (raised at permissive temperature) were crossed to *mes-4*/*DnT1* hermaphrodites at 16°. (F1) Non-Unc (lacking *DnT1*) F1 males were mated to new *mes-4*/*DnT1* hermaphrodites at 16°. (F2) L4 Unc (containing *DnT1*) hermaphrodites were placed on individual plates at 16° and left there for two generations. (F4) From plates containing Mes non-Unc sterile worms, fertile Unc hermaphrodites were transferred individually to 25°. (F5) Those that produced Fem-3(gf) non-Unc sterile and non-Fem-3(gf) Unc fertile worms identified the desired *mes-4*/*DnT1; fem-3(gf)*/*DnT1* construct.

Fertile non-Unc [*mes-4; fem-3(gf)*] hermaphrodites were transferred from 16° to 25° to examine the masculinized germline of their offspring. Fertile non-Unc hermaphrodites were also maintained at 16° to generate Mes offspring without a masculinized germline. Fertile Unc [*mes-4*/*DnT1; fem-3(gf)*] hermaphrodites were transferred from 16° to 25° to verify that non-Mes germlines were masculinized by *fem-3(gf)*. Worms were harvested as young adults and stained with Hoechst.

Construction and analysis of *mes-4; tra-1* mutants: We created a *mes-4(bn67)*/*DnT1; tra-1(e1099)*/+ strain using the following procedure: (P0) On one plate, *tra-1 XX* males were crossed to *mes-4*/*DnT1* hermaphrodites. On a second plate, wild-type *X*O males were crossed to *mes-4*/*DnT1* hermaphrodites. (F1) From the first mating, Unc hermaphrodite progeny were placed individually onto new plates and allowed to lay ~30 F2 embryos. [The purpose of this step was to identify F1 outcross hermaphrodites (*DnT1*/+; *tra-1*/+) that would produce one fourth *tra-1* male F2s.] Each F1 was then transferred to a new plate and mated to multiple non-Unc (*mes*/+) F1 males from the second P0 mating plate. (F2) Self progeny of F1 hermaphrodites were scored for the presence of Tra males. From corresponding F1 mating plates, L4 F2 Unc hermaphrodites were picked onto new plates and allowed to produce two generations of progeny. (F3) Broods that segregated Tra males were retained. (F4) The presence of Mes sterile hermaphrodites in this generation identified the desired strain: *mes-4*/*DnT1; tra-1*/+. To maintain the stock, Unc hermaphrodites were picked individually from plates that segregated males.

Experiments were performed as follows. Non-Unc L4 hermaphrodites (either *mes-4; tra-1*/+ or *mes-4;* +) were separated from males to prevent mating. Hermaphrodites that were fertile at maturity (*i.e.*, offspring of *mes-4*/*DnT1* mothers) were placed onto individual plates. *mes-4; tra-1*/+ hermaphrodites produced all sterile progeny, one quarter of which were Tra males. We compared the germlines of these Tra Mes males to the germlines of their non-Tra Mes hermaphrodite siblings. We also examined Tra non-Mes control males produced by *mes-4*/*DnT1; tra*/+ hermaphrodites. All worms were harvested as young adults and stained with Hoechst.

Construction and analysis of *mes-4; tra-2* mutants: We made a *mes-4(bn67)*/*DnT1; tra-2*/+ strain using the following procedure. (P0) Fertile *mes* males (from a *mes-4*/*DnT1* male stock) were crossed to *tra-2*/*dpy-10 unc-4* hermaphrodites. (F1) Outcross males (*mes-4*/+; *tra-2*/+ or *mes-4*/+; *dpy-10 unc-4*/++) were mated *en masse* to *mes-4*/*DnT1* hermaphrodites. (F2) From successful F1 matings, L4 Unc F2s were placed onto individual plates and allowed to produce two generations of progeny. (F3) Plates with Tra males were retained. (F4) The presence of Mes sterile hermaphrodites identified the desired strain: *mes-4*/*DnT1; tra-2*/+. Experiments were performed by placing fertile non-Unc hermaphrodites (either *mes-4; tra-2*/+ or *mes-4;* +) onto individual plates. The former produced ~25% Mes Tra males. We harvested the Mes Tra males and their Mes non-Tra hermaphrodite siblings as young adults, stained them with Hoechst, and compared their germlines. We also examined Tra non-Mes males produced by *mes-4*/*DnT1; tra-2*/+ mothers.

Construction and analysis of *mes-4; tra-1(gf)* mutants: A strain containing the dominant *tra-1(e1575gf)* allele (Hodgkin 1987) was maintained by mating *tra-1(gf)*/+ *XX* females to wild-type *X*O males. Offspring included *tra-1(gf)*/+ *XX* females (self-sterile, but fertile when mated), *tra-1(gf)*/+ *X*O females (self-sterile, but slightly fertile when mated), +/+ *XX* hermaphrodites (self-fertile), and +/+ *X*O males (fertile). Potential *tra-1(gf)*/+ *XX* and *X*O females, along with +/+ *XX* hermaphrodites, were separated from male siblings as L4s. This step was performed to prevent worms from mating with males. In particular, it was necessary to prevent *tra-1(gf)*/+ *XX* females from mating because the production of embryos would make the females indistinguishable from +/+ *XX* hermaphrodites. Upon reaching maturity, *tra-1(gf)*/+ sterile females were classified as being *XX* or *X*O based on size and tail morphology: *tra-1(gf)*/+ *XX* females are the size of wild-type hermaphrodites and have a normal spike tail; *tra-1(gf)*/+ *X*O females are smaller and have a truncated, stumpy tail.

We generated *mes-4(bn67); tra-1(gf)*/+ *X*O hermaphrodites using the following procedure: (P0) *tra-1(gf)*/+ *XX* females were mated to *mes-4 X*O males (derived from a *mes-4*/*DnT1* male stock). (F1) Outcross *mes-4*/+; *tra-1(gf)*/+ *XX* females (identified by examining the tail morphology of sterile worms that had been separated from males at the L4 stage) were mated to new *mes-4 X*O males. (F2) Outcross *XX* females [*mes-4; tra-1(gf)*/+ or *mes-4*/+; *tra-1(gf)*/+] were identified and then mated individually to new *mes-4 X*O males. (F3) Broods that were all sterile identified mothers that were *mes-4; tra-1(gf)*/+. Stumpy-tailed individuals were presumed to be *mes-4; tra-1(gf)*/+ *X*O females, and spike-tailed worms were presumed to be either *mes-4; tra-1(gf)*/+ *XX* females or *mes-4;* + *XX* hermaphrodites. Young adults were harvested and stained with Hoechst. We compared the germlines of *mes-4; tra-1(gf)*/+ *X*O females, *mes-4; tra-1(gf)*/+ *XX* females, *mes-4*; + *XX* hermaphrodites, and *mes-4;* + *X*O males.

Construction and analysis of 2*X*:3A *mes* mutants: Tetraploid stocks fall into two classes (Madl and Herman 1979): (1) hermaphrodites having four sets of autosomes and four *X* chromosomes (4*X*:4A) produce ~99% hermaphrodite self progeny, and (2) hermaphrodites having four sets of autosomes but only *3X* chromosomes (3*X*:4A) produce 25-40% male progeny. Male offspring of 3*X*:4A hermaphrodites are usually 2*X*:4A animals (*X*:A = 0.5). Triploid *mes* mutants were generated by mating 2*X*:4A tetraploid males to 2*X*:2A diploid *mes* mothers. The resulting outcross progeny were 2*X*:3A *mes*/+/+ mutants. All *mes* alleles used in this analysis were marked with a visible mutation. As a control, we also mated tetraploid males to diploid hermaphrodites that carried the marker mutation but were mes^{+}. The following *mes* mutations and markers were used: *mes-2(bn27)* marked with *unc-4*, *mes-3(bn35)* marked with *dpy-5*, and *mes-4(bn67)* and *mes-6(bn64)*, both marked with *dpy-11*. 2*X*:3A outcross progeny were scored for sexual phenotype by light microscopy and by staining with

Hoechst. Some of the 2*X*:3A outcross progeny were able to produce gametes, perhaps as a result of the extra copies of the *mes*$^+$ alleles from the tetraploid parent. The presence of oocytes allowed us to confirm that the worms were indeed triploids: oocytes contained six bivalents and six monovalents instead of the wild-type number of six bivalents (Madl and Herman 1979).

Construction and analysis of *mes; sdc-3(y52)* mutants: *sdc-3(y52)* was derived from the strain *sdc-3(y128)/sdc-3(y52) unc-76*. *sdc-3(y128)* disrupts dosage compensation, causing homozygous *XX* mutants to be Dpy. *sdc-3(y52)* disrupts sex determination, resulting in the transformation of homozygous *XX* mutants into males. Because these two *sdc-3* mutations affect different processes, they can be maintained as a *trans*-heterozygous stock (Klein and Meyer 1993). The *mes* mutations combined with *sdc-3(y52)* were *mes-2(bn76)* marked with *rol-1*, *mes-3(bn35)* marked with *dpy-5*, and *mes-4(bn67)* and *mes-6(bn66)* marked with *dpy-11*.

(P0) Wild-type males were crossed to *sdc-3(y128)/sdc-3(y52) unc-76* hermaphrodites. (F1) Individual male outcross were mated to single *mes marker/bal* hermaphrodites. (F2) L4 hermaphrodite progeny from successful mating plates were cloned and allowed to produce self progeny. (F3) Broods were scored for the presence of homozygous *mes marker* worms (Rol or Dpy) and homozygous *sdc-3(y52) unc-76* worms (Unc). *mes marker; sdc-3(y52) unc-76/++* hermaphrodites were placed on separate plates and allowed to produce progeny. (F4) All of the F4s were Mes steriles, and ~25% were also Unc males [*sdc-3(y52)*]. (The *unc* marker was scorable in a *rol* or *dpy* background.) We counted the number of Mes Sdc-3(Tra) worms present and determined their sexual phenotype using both light microscopy and Hoechst staining.

mes-6 and *dpy-11*, which are unlinked, were separated after the F1 cross. At subsequent steps, homozygous *mes-6* mutants were identified by the presence of all sterile progeny. Since *sdc-3* and *mes-4* are ~1 map unit apart on LGV, a recombination event was required to create the double mutant. Of 60 F2 hermaphrodites cloned, three recombinants were identified.

To control for the potential effects of the *marker*-bearing chromosomes, we also analyzed *marker; sdc-3(y52) unc-76* animals produced by *marker; sdc-3(y52) unc-76/++* hermaphrodites. The parent hermaphrodites were obtained from the *mes marker; sdc-3(y52) unc-76/++* strains described above, resulting either from recombination between the *mes* and *marker* genes (*mes-2*, *mes-3*, and *mes-4*) or from independent assortment of the *mes* gene from the *marker* gene (*mes-6*). Homozygous *marker* mutants (*mes*$^+$) were identified by the production of all fertile progeny.

Statistical analysis: Descriptions of all statistical analyses can be found in Ambrose and Ambrose (1987). The rank sum test, a nonparametric test for significant differences between two samples of equal or unequal size, was used to compare the average number of germ nuclei in different populations of worms. To select between two methods of calculating *t*, the *F* test was applied to establish whether or not the variances of the samples were significantly different. These tests were performed on the data presented in Figures 2, 6, 7, and 8. Results are significant at the 0.05 α level.

RESULTS

Germ cells in *mes* mutant hermaphrodites are unhealthy and prone to degeneration: In wild-type hermaphrodites, germ nuclei in the mitotic region are similar in size and uniformly arranged around the periphery of each syncytial gonad arm. In *mes* mutant hermaphrodites, germ nuclei are reduced in number compared to wild type, appear swollen, and show an abnormal arrangement in the gonad; the cytoplasm also appears "coagulated" (Capowski *et al.* 1991; Paulsen *et al.* 1995). Figure 1A shows wild-type and *mes-4* gonad arms that were released from adult hermaphrodites and stained with the DNA dye Hoechst 33342. The reduction in number and the altered appearance and ar-

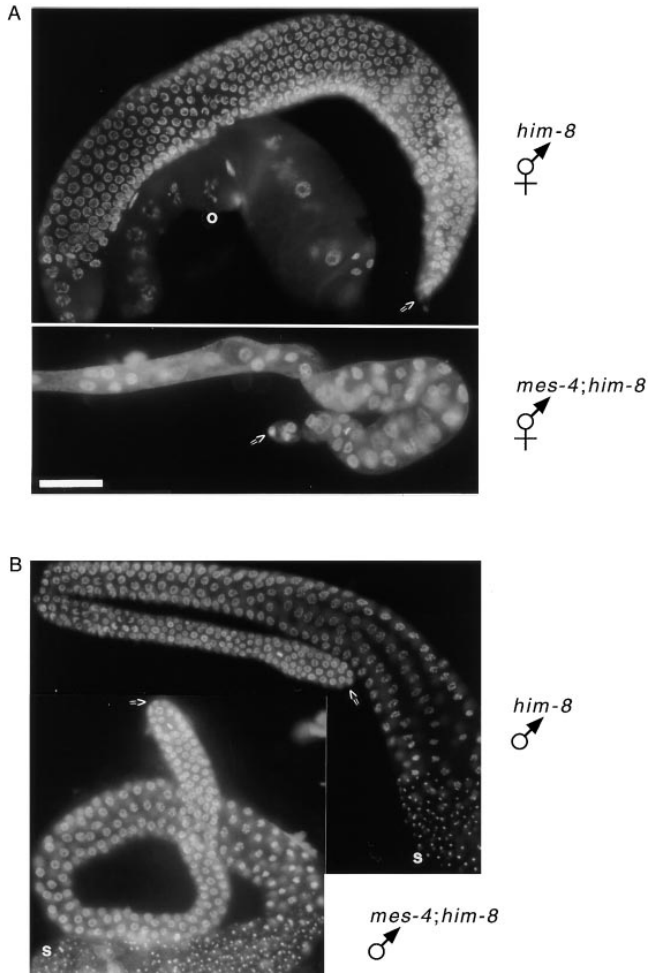

Figure 1.—Germlines from *mes-4*$^+$ and *mes-4* mutant hermaphrodites and males. The panels show fluorescence micrographs of *him-8* and *mes-4(bn67); him-8* mutant gonads that were dissected from adult worms and stained with Hoechst 33342 to visualize DNA (see materials and methods). The mutant gonads shown are representative of the more proliferated gonads observed among *mes-4* hermaphrodites and males. (A) Hermaphrodite gonad arms. The *him-8* gonad contains many evenly spaced germ nuclei that are progressing through various stages of mitosis (near the distal tip, indicated by an arrow), meiosis, and oogenesis (indicated by an o); the region of the gonad containing sperm is not shown. The *mes-4; him-8* gonad contains many fewer germ nuclei that are enlarged and unevenly spaced. (B) Male gonads. Both gonads contain many evenly spaced nuclei at various stages of mitosis (near the distal tip, indicated by an arrow), meiosis, and spermatogenesis (sperm are indicated by an s). Bar, 10 μm.

rangement of germ nuclei in the *mes-4* gonad arms are apparent.

For most *mes* alleles, late larval and adult hermaphrodites show a decrease in the number of germ cells relative to earlier larval stages (Capowski *et al.* 1991; Paulsen *et al.* 1995), indicating that at least some germ cells die. Programmed cell death is a normal fate choice for many somatic cells and some germ cells in wild-type hermaphrodites (Hengartner 1997). Paulsen *et al.* (1995) previously tested whether programmed cell death is responsible for the decline in the number of germ cells in *mes-3* mutant hermaphrodites. Mutations in *mes-3* were combined with a mutation in *ced-3* or *ced-4*. Mutations in either of these two *ced* genes prevent the programmed cell deaths that occur during normal development (Ellis and Horvitz 1986). Mutations in the *ced* genes did not suppress the germline death observed in *mes-3* mutants, indicating that germline death in *mes-3* worms is not caused by inappropriate activation of the programmed cell death pathway. To extend this analysis to the other *mes* genes, we constructed *mes; unc-79 ced-4*/++ hermaphrodites and examined the germlines of their homozygous *mes; unc-79 ced-4* offspring. *mes; unc-79 ced-4* hermaphrodites contained the same reduced numbers of germ cells as *mes* single mutants (Figure 2). Thus, as observed with *mes-3*, the Mes phenotype of *mes-2*, *mes-4*, and *mes-6* is not suppressed by a *ced-4* mutation. We conclude that *mes* mutations do not lead to germ cell death via the genetically regulated programmed cell death pathway. Rather, *mes* germ cells probably undergo a more general, necrotic type death, perhaps as a result of physiological stress or injury.

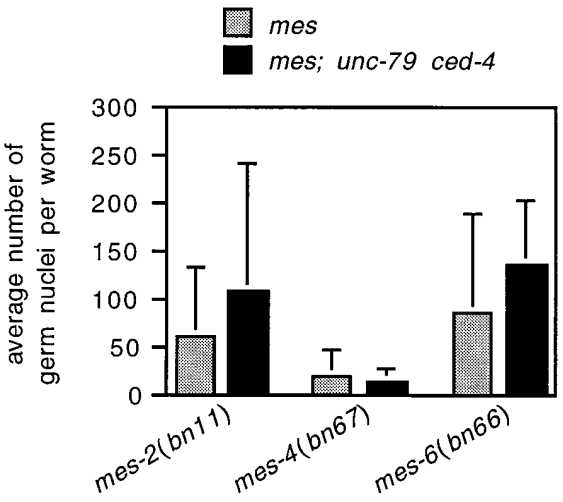

Figure 2.—Mutations in *ced-4* do not suppress the germline proliferation defects associated with mutations in *mes-2*, *mes-4*, and *mes-6*. Adult worms were stained with Hoechst to visualize DNA, and their germ nuclei were counted (see materials and methods). The histograms compare the average number of germ nuclei in Unc (black bars) and non-Unc (gray bars) offspring of *mes; unc-79 ced-4*/++ mothers; standard deviations are shown. Between 13 and 29 worms were scored for each genotype. For each *mes* allele, the average number of germ nuclei in *mes* hermaphrodites does not differ significantly from the average number in *mes; unc-79 ced-4* hermaphrodites (see materials and methods for statistical analysis).

In support of this conclusion, *mes* germ cells do not resemble wild-type cells dying by programmed cell death. Programmed cell death leads to nuclear condensation and formation of a button-like, refractile body (Sulston and Horvitz 1977; Sulston *et al.* 1983). In contrast, degenerative cell deaths seen in ion channel mutants of *C. elegans* are characterized by enhanced membrane cycling, cytoplasmic vacuoles, and swelling of the nucleus and cell (Chalfie and Wolinsky 1990; Hall *et al.* 1997). *mes* germ cells display cytoplasmic vacuoles and nuclear swelling (Paulsen *et al.* 1995; Figure 1A). Although the syncytial nature of the germline makes it difficult to directly compare the pathology of *mes* mutant germlines with degenerating somatic cells, we think that the *mes* mutant phenotype is most consistent with degeneration.

We postulated that the degeneration of *mes* germ nuclei might be a consequence of defects in the execution of mitosis, leading to aneuploidy or other chromosomal abnormalities. To test this idea, we constructed *mes* strains carrying a *glp-4* mutation, which arrests germline mitosis, and investigated whether mitotic arrest would prevent the degeneration of *mes* nuclei. In *glp-4* hermaphrodites raised at the restrictive temperature, germ nuclei are few in number (average of 12 in young adults) and appear to be arrested in mitotic prophase; they resemble some of the germ nuclei located in the distal mitotic region of a wild-type germline (Beanan and Strome 1992). In *mes; glp-4* hermaphrodites raised at restrictive temperature, the number of germ nuclei was the small number typical of *glp-4* mutants, but the nuclei had the diffuse appearance typical of degenerating *mes* nuclei (Figure 3). Such defective nuclei were seen both in *mes* and in *mes; glp-4* hermaphrodites as early as the L3 stage. We conclude that abnormalities seen in *mes* germ cells are probably not the result of accumulated damage from multiple rounds of faulty mitosis. Rather, mutant germ nuclei appear abnormal by L3, after they have undergone as few as two rounds of division (from two nuclei in L1s to eight nuclei in *glp-4* L3s).

***mes* germ cells apparently adopt a germline fate, but are limited in their ability to execute that fate:** The DTC of the somatic gonad, located at the distal end of each hermaphrodite gonad arm, promotes proliferation of nearby germ cells (Kimble and White 1981). When wild-type germ cells are displaced proximally by continued proliferation, they are released from the influence of the DTC, enter meiosis, and differentiate into sperm or oocytes. In *mes* hermaphrodites, germ cells rarely differentiate into gametes. This aspect of the phenotype could result from (1) prolonged proximity of prolifera-

tion-defective germ cells to the DTC, (2) failure in germline specification, or (3) an inability of correctly specified germ cells to execute meiosis and gametogenesis. To address which of the above possibilities is most likely, both genetic and microsurgical approaches were used to eliminate the DTC signaling pathway in different *mes* mutant backgrounds. This analysis was done previously with *mes-3* (Paulsen *et al.* 1995) and extended in this study to *mes-2*, *mes-4*, and *mes-6*.

The *glp-1* gene encodes the germline receptor for the DTC signal, and failure to express functional GLP-1 causes normal germ cells to prematurely enter meiosis and differentiate into sperm (Austin and Kimble 1987; Crittenden *et al.* 1994). The resulting hermaphrodites have an underproliferated germline containing ~20 sperm and are sterile. To determine if *mes* germ cells respond normally to the absence of GLP-1, we introduced a *glp-1* null allele into different *mes* backgrounds. Control worms showed the expected phenotypes: *mes*$^{+}$*; glp-1* worms lacked undifferentiated germ cells and contained ~20 sperm (Austin and Kimble 1987), and *mes; glp-1*$^{+}$ contained variable numbers of undifferentiated germ cells, with occasional gamete-like cells in worms bearing weaker alleles, such as *mes-2(bn27)* and *mes-4(bn58)*. The experimental *mes; glp-1* worms had a greatly reduced average number (at most five) of undifferentiated germ cells compared to *mes; glp-1*$^{+}$ controls, indicating that *mes* germ cells require the DTC signal to proliferate (Figure 4A and Paulsen *et al.* 1995). Thus, *mes* germ cells are normal in this aspect of response to lack of DTC signaling. However, only rare *mes; glp-1* worms contained sperm (Figure 4A and Paulsen *et al.* 1995), which were few in number and appeared defective. For example, none of the *mes-2(bn27); glp-1* worms (n = 19) contained sperm, and only 5% of *mes-4(bn58); glp-1* worms (n = 21) had sperm-like structures. These data indicate that when *mes* germ cells are deprived of the signal to undergo mitosis, they generally do not undergo meiosis and become sperm. Thus, *mes* germ cells are abnormal in this aspect of response to lack of DTC signaling.

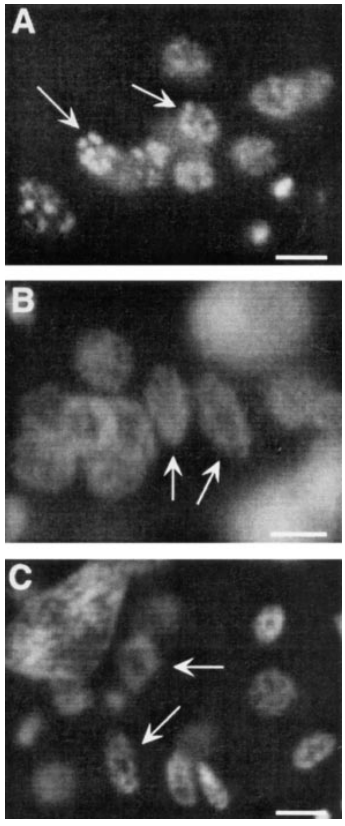

Figure 3.—Germ nuclei in young adult *mes-2; glp-4* hermaphrodites have a Mes-2, not a Glp-4, morphology. Sterile worms were stained with Hoechst to visualize DNA. (A) *glp-4* worms raised at a restrictive temperature (25°). (B) Offspring of *mes-2(bn11); glp-4* mothers raised at a permissive temperature (16°). (C) Offspring of *mes-2(bn11); glp-4* mothers raised at a restrictive temperature. Arrows point to undifferentiated germ nuclei. The germ nuclei in *glp-4* single mutants raised at a restrictive temperature appear to be arrested in prophase I of mitosis, and they contain distinct condensed chromosomes. Germ nuclei in *mes-2; glp-4* mutants are large and diffuse, resembling those in *mes-2* single mutants. Bars, 5 μm.

Another way to assess the behavior of *mes* germ cells that are free from the influence of the DTC is to destroy the DTC with a laser microbeam. Kimble and White (1981) demonstrated that laser ablation of the DTC during the mid to late L3 stage causes all germ cells in the operated gonad arm to enter meiosis and differentiate into sperm. Thus, DTC ablation phenocopies a *glp-1* mutation. To confirm the above *mes; glp-1* results using a different method of eliminating DTC signaling, we ablated the DTC in *mes-4* and *mes-6* mutant larvae. The DTC in one gonad arm of L3-stage hermaphrodites was ablated, and the other gonad arm was left unoperated to serve as a control for the behavior of the germline in the presence of the DTC. After surgery, worms were allowed to recover and mature for 48 hr, before being analyzed for the number and type of germ cells present. In the operated gonad arm of wild-type worms, results were as predicted: all germ cells became sperm. In the unoperated gonad arm of the same worm, germline development was normal. Ablations performed on *mes* worms resulted in the cessation of germ cell proliferation, but did not result in spermatogenesis (Figure 4B). Unoperated *mes-4(bn67)* gonad arms (n = 11) contained an average of 34 undifferentiated germ nuclei and no sperm. The operated gonad arms of the same worms contained an average of only three undifferentiated germ cells and no sperm. Thus, the behavior of *mes-4(bn67)* germ cells in a DTC-less gonad arm is like that of *mes-4(bn67)* germ cells in a *glp-1*–null background. Similar outcomes were obtained when the DTC was ablated in other *mes-4* and *mes-6* mutants (Figure 4B and data not shown) and in *mes-3* mutants (Paulsen *et al.* 1995): ablated arms contained fewer germ cells than unablated arms, and there was no

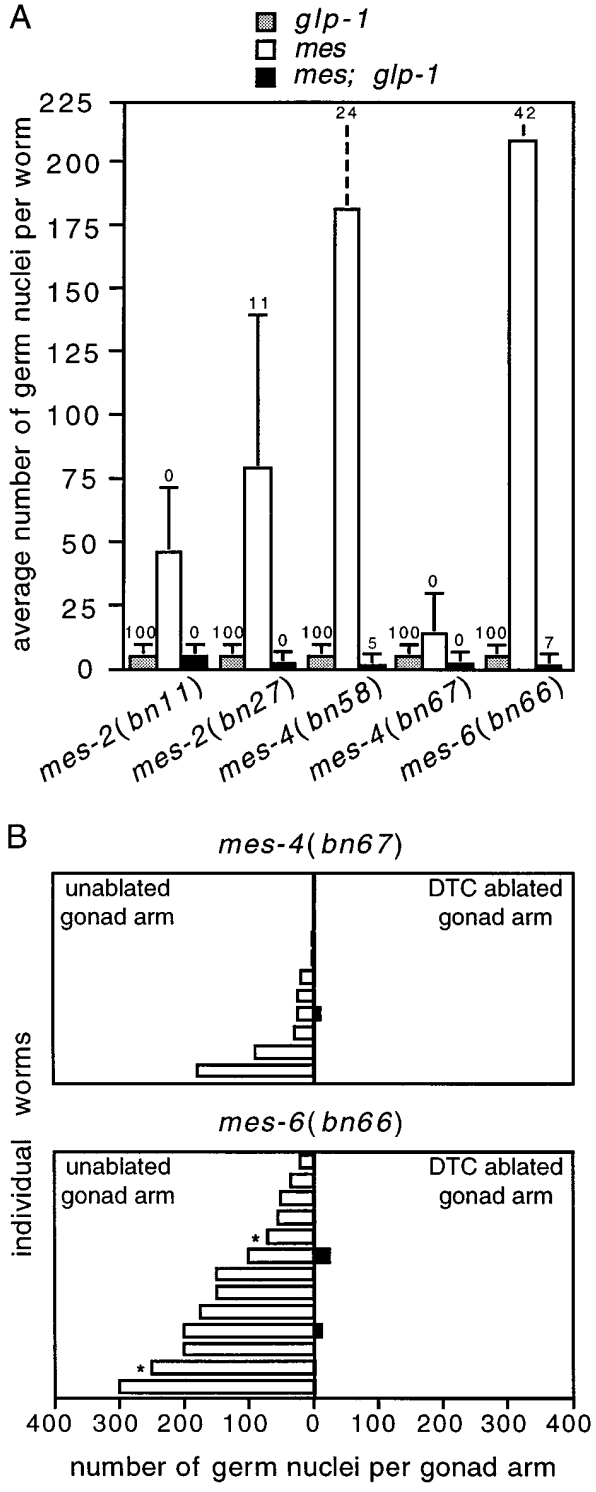

Figure 4.—Tests of *mes-2*, *mes-4*, and *mes-6* germ cell behavior in the absence of DTC signaling. (A) In the absence of *glp-1*$^+$ function, *mes* mutant germ cells fail to proliferate but do not differentiate. Bars show the average number of undifferentiated germ cells in adult hermaphrodites; standard deviations are shown. Numbers above each bar indicate the percentage of worms with gametes or gamete-like structures. (Gray bars) *unc-32 glp-1* offspring of *mes*/+; *unc-32 glp-1*/++ mothers. All germ cells differentiated into sperm. (White bars) *mes;* ++ or *mes; unc-32 glp-1*/++ offspring of *mes; unc-32 glp-1*/++ mothers. Most germ cells remained undifferentiated or died. A few oocyte-like and/or sperm-like structures were observed in some worms bearing weak *mes* alleles. (Black bars) *mes; unc-32 glp-1* offspring of *mes; unc-32 glp-1*/++ mothers. Most germ cells remained undifferentiated or died, but there were a few sperm-like structures in 5% of *mes-4(bn58)* worms and 7% of *mes-6(bn66)* worms. Worms represented by the open and black bars were siblings. Between 13 and 25 worms were scored for each genotype. Standard deviations for *mes-4(bn58)* and *mes-6(bn66)* germlines were ±231 and ±248, respectively. (B) Ablation of the DTC in *mes-4* and *mes-6* mutant worms reduces germ cell proliferation and does not induce gamete formation. The DTC of one gonad arm was ablated at the L3 stage. The unablated gonad arm served as a control for the level of germline proliferation and gamete production in mutant worms. Each horizontal pair of bars shows the two gonad arms of an individual worm scored 48 hr after microsurgery: white bars show the unoperated gonad arm, and black bars show the arm in which the DTC was ablated. Asterisks indicate that a few sperm were present.

apparent increase in meiosis or gametogenesis. These results provide further evidence that the ability of *mes* germ cells to proliferate is dependent on the presence of the DTC, but their general inability to differentiate is not caused by proximity to the DTC. Instead, it is likely that the inability of mutant germ cells to undergo meiosis and gametogenesis is a consequence of some intrinsic physiological defect.

The germlines of *mes* males are healthier than those in sibling *mes* hermaphrodites: In *C. elegans*, the choice of sexual fate is determined by the ratio of *X* chromosomes to sets of autosomes (Madl and Herman 1979). Diploid embryos containing two *X* chromosomes (*X*:A ratio = 1.0) develop into hermaphrodites. Those with only a single *X* chromosome (*X*:A ratio = 0.5) develop into males. Self progeny of wild-type 2*X*:2A hermaphrodites consist primarily of 2*X*:2A hermaphrodites; 1*X*:2A males are generated at much lower frequency (~0.3%) as a result of meiotic nondisjunction of the *X* chromosome (Hodgkin *et al.* 1979). We examined the germlines of rare *mes* males found in our stocks and were surprised to find that they exhibit much milder germline defects than *mes* hermaphrodites. To fully characterize the germline phenotype of *mes* males, we used a mutation in *him-8* (high incidence of males), which increases *X* chromosome nondisjunction during meiosis and results in ~37% males among the self progeny of homozygous mutant hermaphrodites (Hodgkin *et al.* 1979). We constructed *mes; him-8* hermaphrodites and examined the germlines of their male and hermaphrodite offspring.

Before describing hermaphrodite/male differences in the Mes phenotype, we want to note that, as observed by Capowski *et al.* (1991), the expressivity of the Mes phenotype is sensitive to genetic background. For example, we observed that the hermaphrodite offspring of *mes-2(bn11); him-8* and *mes-6(bn66); him-8* mothers displayed a somewhat higher average number

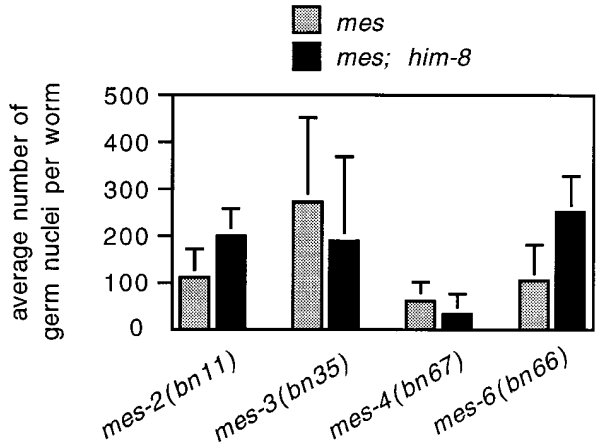

Figure 5.—Effects of the *him-8* genetic background on germline proliferation in *mes* mutant hermaphrodites. Histogram bars show the average number of germ nuclei in synchronized populations of hermaphrodite offspring produced by homozygous *mes* (gray bars) and *mes; him-8* (black bars) mothers; standard deviations are shown. Between 13 and 30 worms were scored for each genotype.

of germ nuclei than the hermaphrodite offspring of mothers bearing the same *mes* allele without *him-8* (Figure 5). However, the genetic background effects are much smaller than the differences observed between the *XX* and *X*O animals described below.

Several aspects of the germline phenotype are improved in *mes; him-8* males compared to *mes; him-8* hermaphrodites (Figures 1B and 6): (1) Males have a significantly higher average number of undifferentiated germ cells, (2) germ nuclei in males are usually normal in appearance and arrangement within the gonad tube, (3) germ cells in males often undergo meiosis and differentiate into sperm, and (4) males are often fertile (data not shown).

The difference in the average number of germ nuclei between males and hermaphrodites (Figure 6) is significant for each of the *mes* alleles tested (see materials and methods for statistical analysis). For instance, *mes-2(bn27)* males contained 480 ± 52 germ nuclei, while their hermaphrodite siblings contained 196 ± 129. In wild-type worms, the average steady-state number of germ cells in males and hermaphrodites is

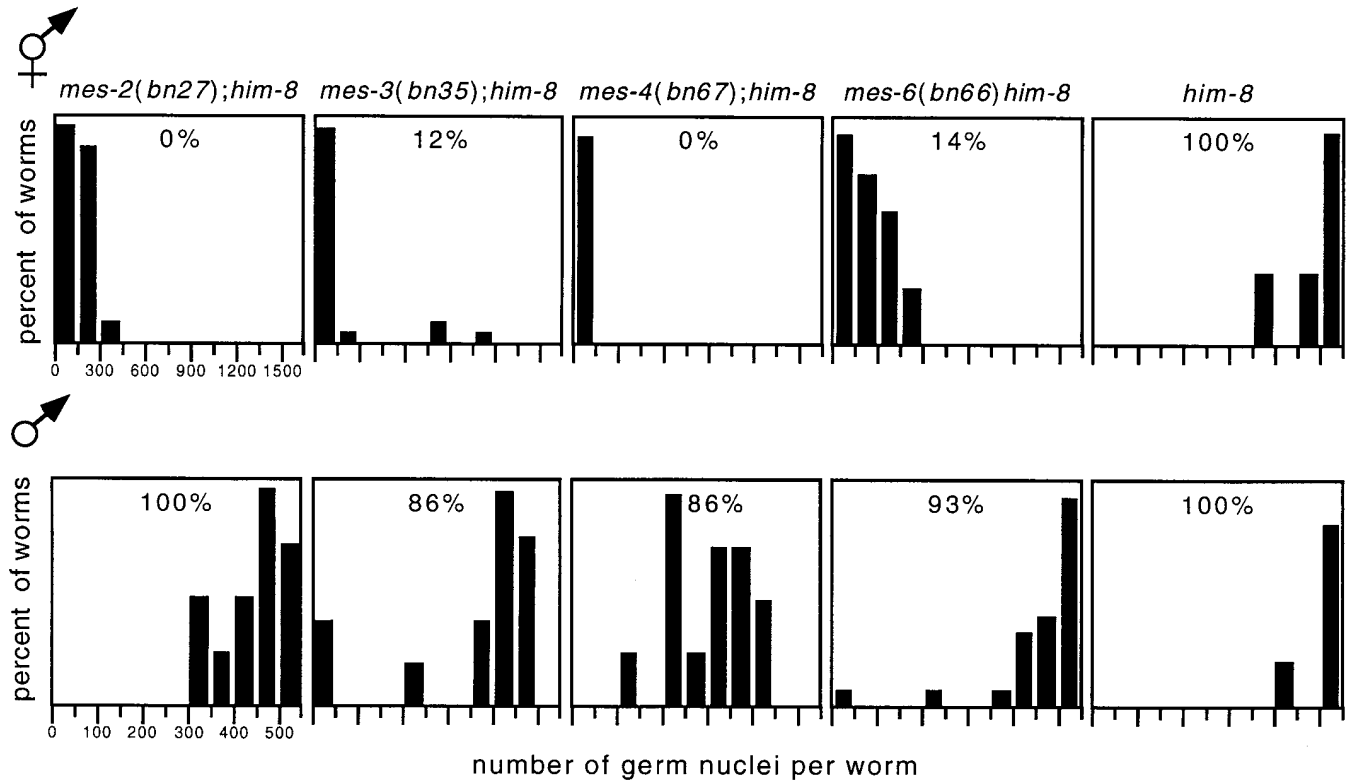

Figure 6.—Mutations in all the *mes* genes cause more severe germline defects in *XX* hermaphrodites than in *X*O males. Sibling hermaphrodites and males produced by homozygous *mes; him-8* mothers were stained with Hoechst to visualize and count germ nuclei. Between 12 and 30 worms were scored for each genotype. Each bar represents the percentage of worms containing a similar number of undifferentiated germ nuclei, within a range of 150 germ nuclei for hermaphrodites and 50 germ nuclei for males. Thus, each panel shows the frequency distribution of worms containing different numbers of germ nuclei. The percentage of worms that contained gametes or gamete-like structures is indicated at the top of each panel. For each *mes* mutant, the average number of germ nuclei in *mes; him-8 XX* hermaphrodites is significantly different from the average number in *mes; him-8 X*O males.

~500 and ~1500, respectively (Figure 6 legend). Thus, *mes-2(bn27)* males typically contain 96% of the normal number of germ cells, while *mes-2(bn27)* hermaphrodites contain only 13% of the normal number.

Meiotic germ cells and gametes are observed very infrequently in *mes* hermaphrodites, and when present, they are few in number and abnormal in appearance. In contrast, *mes* males frequently contain meiotic germ cells and sperm, and the number of sperm often approaches the wild-type level (Figure 1B). The percentage of males with sperm ranged from 86% for *mes-4(bn67)* and *mes-3(bn35)* to 100% for *mes-2(bn27)*. In the majority of these worms, sperm were too numerous to count. Because of their well-developed germlines, we routinely use *mes* males in matings; the success of the matings attests to the fertility of mutant males.

In addition to showing that the Mes phenotype is more highly expressed and penetrant in hermaphrodites than in males, the above analyses also reveal that not all mutant males have a normal germline; in fact, some display a severe germline phenotype. For instance, 14% of *mes-3(bn35); him-8* and 4% of *mes-6(bn66); him-8* males had <10 germ cells and no sperm (Figure 6). We conclude that the *mes* genes are essential for development of the hermaphrodite germline, but that their role is not limited to hermaphrodites.

The Mes phenotype is more severe in *XX* than in *X*O worms, regardless of sexual phenotype: Hermaphrodites and males differ in the following: (1) sex of the germline, (2) sex of the soma, and (3) *X*-chromosome constitution (see White 1988; Schedl 1997; Meyer 1997 for reviews). To test which, if any, of these differences is responsible for the differential response of the two sexes to *mes* mutations, we genetically altered the phenotypic sex of *XX* and *X*O *mes* worms. We created *mes* strains that carry a second mutation in one of several genes that function in sex determination (Table 1). Depending on the sex determination allele used, mutant animals were sexually transformed in the germline only or in both the germline and soma. We focused our analysis on *mes-4(bn67)* because, of the *mes* mutations characterized, this allele causes the most severe reduction in hermaphrodite germline proliferation and gametogenesis and the most dramatic hermaphrodite/male difference in Mes phenotype. We also did some experiments with a weaker allele, *mes-4(bn58)*.

TABLE 1

Genetic transformations of germline and somatic sex do not alter the differential sensitivity of *XX* and *X*O worms to mutations in *mes-4*

mes-4 allele	Zygotic genotype[a]		Temp.	Sex of soma[b]	Sex of germline[b]	Avg. number of germ nuclei	Presence of gametes[c]
bn67	*mes-4; fem-3(q20 ts)gf*	*XX*	16	H	H	1 ± 2	None
	mes-4; fem-3(q20 ts)gf	*XX*	25	H	M	5 ± 7	None
bn58	*mes-4; fem-3(q20 ts)gf*	*XX*	16	H	H	41 ± 67	4% had sperm
	mes-4; fem-3(q20 ts)gf	*XX*	25	H	M	35 ± 28	None
bn67	*mes-4; fog-1 (q253 ts)*	*XX*	16	H	H	3 ± 4	None
	mes-4; fog-1 (q253 ts)	*XX*	25	H	F	7 ± 10	None
	mes-4/+	*XX*	22	H	H	32 ± 26	None
	mes-4/+	*X*O	22	M	M	227 ± 93	38% had sperm
	mes-4/+; fog-1 (q253 ts)	*X*O	16	M	M	169 ± 88	86% had sperm
	mes-4/+; fog-1 (q253 ts)	*X*O	25	M	F	181 ± 69	35% had oocytes
bn58	*mes-4; fog-1 (q253 ts)*	*XX*	16	H	H	23 ± 21	None
	mes-4; fog-1 (q253 ts)	*XX*	25	H	F	32 ± 25	None
	mes-4/+; fog-1 (q253 ts)	*X*O	16	M	M	200 ± 44	83% had sperm
	mes-4/+; fog-1 (q253 ts)	*X*O	25	M	F	n.d.[d]	80% had oocytes
bn67	*mes-4; tra-1(e1099)/+* or *mes-4; +/+*	*XX*	22	H	H	9 ± 6	None
	mes-4; tra-1(e1099)	*XX*	22	M	M	11 ± 6	None
	mes-4; tra-2(e1095)/+ or *mes-4; +/+*	*XX*	22	H	H	14 ± 13	None
	mes-4; tra-2(e1095)	*XX*	22	M	M	15 ± 14	None
	mes-4; tra-1(e1575)gf/+ or *mes-4; +/+*	*XX*	22	H	H or F[e]	16 ± 15	None
	mes-4; +/+	*X*O	22	M	M	193 ± 127	42% had sperm
	mes-4; tra-1(e1575)gf/+	*X*O	22	H	F	335 ± 186	50% had oocytes 4% had sperm

[a] All worms were offspring of homozygous mutant *mes* mothers. Between 15 and 60 worms were examined for each genotype.

[b] H, hermaphrodite; M, male; F, female.

[c] In general, worms with a large number of undifferentiated germ nuclei contained gametes, and worms with few undifferentiated germ nuclei lacked gametes.

[d] *mes-4(bn58)/+; fog-1* worms raised at 25° appeared to contain at least as many germ nuclei as those raised at 16°. The exact number of germ nuclei was not determined.

[e] These two genotypic classes of worms were phenotypically indistinguishable because both were sterile offspring of *mes-4* mothers.

To determine whether the relative health of *mes-4* male germlines is caused by some distinctly masculine quality of the germline itself, we combined mutations in *mes-4* with a gain-of-function (gf) mutation *fem-3*. This *fem-3* mutation masculinizes the germline of *XX* worms: instead of making oocytes, *fem-3(gf) XX* worms raised at a restrictive temperature make sperm throughout adulthood and are sterile (Barton *et al.* 1987). We observed that *mes-4(bn67 or bn58); fem-3(gf) XX* worms raised at a restrictive temperature did not display an increase in the average number of germ nuclei (Table 1) or an improvement in germline health relative to *mes-4; fem-3(gf) XX* worms raised at a permissive temperature. Thus, masculinization of the germline in *mes-4* hermaphrodites does not affect the Mes mutant phenotype; *mes-4* germ cells exhibit a proliferation and differentiation defect that is independent of their sexual identity.

We used a mutation in *fog-1* to create a reciprocal sexual transformation of the germline. This mutation causes feminization of male and hermaphrodite germlines at a restrictive temperature, causing both to make only oocytes (Barton and Kimble 1990). When *fog-1* was combined with either *mes-4(bn67)* or *mes-4(bn58)*, the phenotype of *mes-4* male and hermaphrodite germlines was unchanged. In particular, the germlines of *mes-4; fog-1* males raised at a restrictive temperature were similar to the germlines of *mes-4; fog-1* males raised at the permissive temperature in terms of both the number of undifferentiated germ cells and the presence of gametes (Table 1). The results with *fog-1* confirm the results with *fem-3(gf)*, namely that the degree of proliferation and differentiation seen in *mes-4* germ cells, is independent of the sex of the germ cells.

Using loss-of-function mutations in *tra-1* (Hodgkin and Brenner 1977; Hodgkin 1987) and *tra-2* (Klass *et al.* 1976; Hodgkin and Brenner 1977), we addressed whether masculinization of somatic structures would affect the Mes-4 germline phenotype. The specific mutations used cause imperfect but complementary transformations of *XX* worms into males. *tra-1 XX* males display complete masculinization of the nongonadal soma, but only one third of these animals also have a fully male somatic gonad and germline, and are able to sire progeny (Schedl *et al.* 1989). *tra-2 XX* males have a fully transformed somatic gonad and germline, but some of the nongonadal structures are incompletely masculinized (Klass *et al.* 1976; Hodgkin and Brenner 1977). Using *mes-4(bn67)* and each of these *tra* mutations, we compared the germlines of *mes-4; tra XX* males with *mes-4; tra*/+ and *mes-4;* +/+ *XX* hermaphrodites (Table 1). The average number of germ nuclei (all undifferentiated) in *mes-4; tra-1 XX* males (n = 39 worms) was 11 ± 6, which is very similar to the 9 ± 6 germ nuclei observed in *mes-4; tra-1*/+ and *mes-4;* +/+ *XX* hermaphrodite controls (n = 50 worms; Table 1). Similarly, the average number of germ nuclei in *mes-4; tra-2 XX* males was 14 ± 13, compared to 15 ± 14 in *mes-4; tra-2*/+ and *mes-4;* +/+ *XX* hermaphrodites (Table 1 and Figure 7). Thus, the severe germline phenotype of *XX mes-4* worms is not rescued by masculinization of the germline or soma.

To perform the reciprocal test and determine whether feminization of the germline and soma causes *X*O worms to display a more severe Mes-4 phenotype, we used a gain-of-function mutation in *tra-1*, which acts dominantly to feminize both *XX* and *X*O worms (Hodgkin 1987). *XX tra-1(gf)*/+ worms are females, which produce normal oocytes and no sperm. These worms are self-sterile because of a lack of sperm, but they produce outcross progeny when mated. *X*O *tra-1(gf)*/+ worms display feminization of both the germline and the soma. These worms are self-sterile females, which produce variably abnormal oocytes and no sperm, and produce only a few progeny when mated. They also have stumpy tails, reflecting incomplete feminization of this somatic structure.

We wanted to compare the germlines of *mes-4; tra-1(gf)*/+ *X*O worms and *mes-4;* +/+ *X*O worms. To generate these worms, *mes-4; tra-1(gf)*/+ *XX* females were mated to *mes-4;* +/+ *X*O males. All F1s were homozygous for *mes-4*. The *XX* worms had a hermaphrodite-like spike tail, the *X*O *tra-1*+ worms had a male-like spade tail, and the *X*O *tra-1(gf)*/$^+$ worms had a stumpy tail. We analyzed 45 *mes-4;* +/+ *X*O control males (Table 1 and Figure 7), which contained an average of 193 ± 127 germ nuclei. Of these 45 worms, 26 lacked sperm, 9 contained 5–30 sperm, and 10 contained >200 sperm. The percentage of sperm-producing males (42%) was lower than the percentage reported above for *mes-4(bn67); him-8* males (86%), possibly because of an effect of genetic background. We analyzed 28 *mes-4; tra-1(gf)*/+ *X*O females with stumpy tails (Table 1 and Figure 7), which contained an average of 335 ± 186 germ cells. 50% of the worms contained what appeared to be endomitotic oocytes (Iwasaki *et al.* 1996); one worm contained 11 sperm. This frequency of gametogenesis is similar to the frequency observed in the *mes-4;* +/+ *X*O control males, although the type of gametogenesis (oogenesis *vs.* spermatogenesis) is altered by the *tra-1(gf)* mutation.

Two aspects of germline development were dramatically improved in *mes-4; tra-1(gf)*/+ *X*O females, compared to any *mes-4 XX* worms we have studied. From the standpoint of germline proliferation, the *mes-4; tra-1(gf)*/+ *X*O females contained ~23% the wild-type hermaphrodite level of germ cells, compared to a value of ~1% for *mes-4; tra-1*$^+$ *XX* hermaphrodites (see Figure 6). Moreover, the *mes-4; tra-1(gf)*/+ *X*Os attempted gametogenesis at a much higher rate (54% of worms) than *mes-4; tra-1*$^+$ *XX* worms (never observed). From these results, we conclude that *mes-4 X*O germ cells are able to undergo extensive proliferation and frequent differentiation within a hermaphrodite somatic envi-

ronment and when the sexual identity of the germline is female.

The *mes-4; tra-1* and *mes-4; tra-2* results demonstrate that a male body and germline is not sufficient for germline health, and the *mes-4; tra-1(gf)* results demonstrate that maleness is not necessary. Therefore, sexual phenotype probably does not play a role in making male germlines more tolerant of *mes-4* mutations than hermaphrodite germlines. The relevant factor is more likely to be the *X* chromosome constitution or the *X*:A ratio.

Our limited analysis of sexually transformed *mes-2* and *mes-6* worms suggests that the findings described above with *mes-4* extend to the other *mes* genes as well. *mes-2(bn11) XX* worms whose somatic tissues (and usually germlines) were masculinized by *tra-1* did not show an improvement in germline phenotype relative to their nontransformed siblings: *mes-2; tra-1 XX* males had 33 ± 32 germ cells, compared to 58 ± 55 in their sibling *mes-2; tra-1*/+ and +/+ *XX* hermaphrodites, and all worms lacked gametes. *mes-6(bn66) XX* worms whose somatic tissues (and usually germlines) were masculinized by *tra-1* had 47 ± 49 germ cells and no gametes. Sibling *mes-6; tra-1*/+ and +/+ *XX* hermaphrodites had 50 ± 60 germ cells. Three of the latter worms ($n = 43$) contained a few structures that looked like oocytes. Based on these results, we tentatively conclude that, as with *mes-4*, the severity of the germline phenotype caused by mutations in the other *mes* genes does not depend on sexual phenotype but is more likely to be sensitive to *X* chromosome dosage or the *X*:A ratio.

The Mes phenotype is more severe in *XXX* worms than in *XX* and *XO* worms: We described in the previous sections the less severe Mes phenotype displayed by *X*O *mes* worms compared to *XX* worms. To further test the sensitivity of the Mes phenotype to *X* chromosome dosage, we examined worms with an increased number of *X* chromosomes. Nondisjunction of the *X* chromosome results in diplo-*X* and nullo-*X* gametes and, as a result, can lead to the production of *XXX* offspring (dumpy hermaphrodites), as well as *X*O offspring (males; Hodgkin *et al.* 1979). From the broods of *mes; him-8* mothers, we compared the germlines of Dpy hermaphrodites (*XXX*) with the germlines of their non-Dpy hermaphrodite (*XX*) and male (*X*O) siblings. Using mutations in each of the four *mes* genes, we observed that the average number of germ nuclei in Dpy hermaphrodites was significantly reduced below the value in non-Dpy hermaphrodites, which was reduced below the value in males (Figure 8). This observation strength-

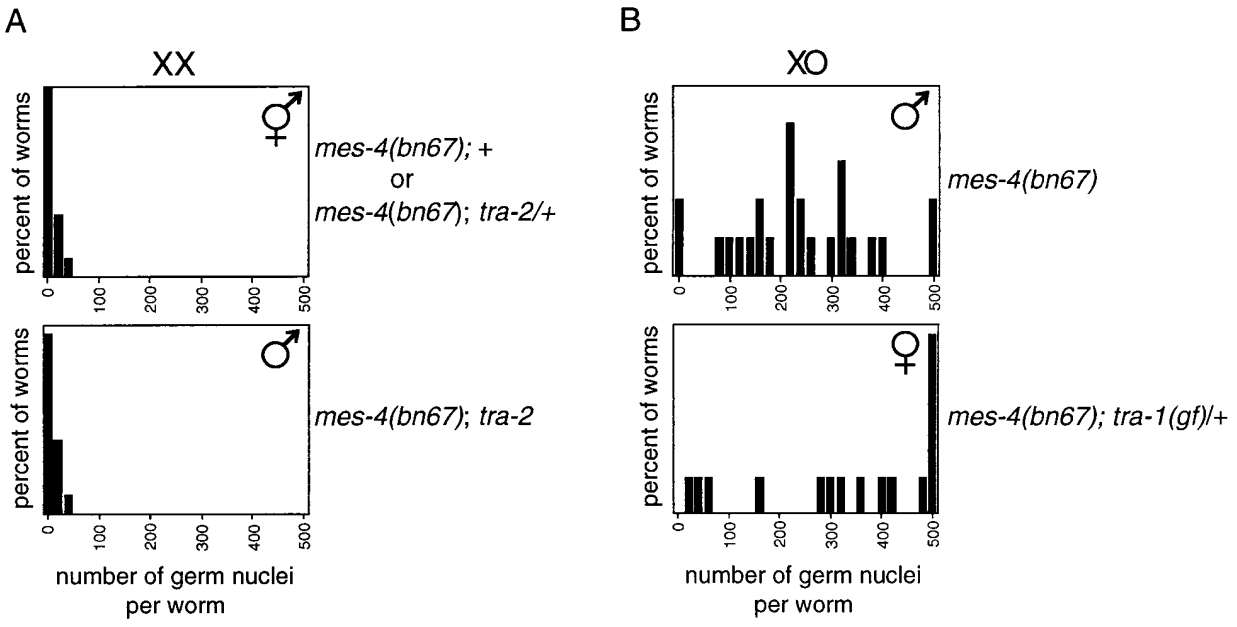

Figure 7.—The phenotype of *mes-4(bn67)* is more severe in *XX* than *X*O worms, regardless of the sex of the worms. Adult worms were stained with Hoechst to visualize and count germ nuclei. Between 28 and 48 worms were scored for each genotype. Each panel shows a frequency distribution of worms containing different numbers of germ nuclei (see legend to Figure 6). (A) Comparison of sibling *mes-4;* + or *mes-4; tra-2*/+ *XX* hermaphrodites and *mes-4; tra-2 XX* males produced by *mes-4; tra-2*/+ *XX* mothers. Both *XX* hermaphrodites and *XX* males had severely underproliferated germlines. (B) Comparison of sibling *mes-4;* + *X*O males and *mes-4; tra-1(gf)*/+ *X*O stumpy-tailed females produced by a mating between *mes-4; tra-1(gf)*/+ *XX* tapered-tail females and *mes-4;* + *X*O males. Both *X*O males and XO females had relatively well-proliferated germlines. The average number of germ nuclei does not differ significantly between different populations of *XX* worms (Mes hermaphrodites and Mes Tra-2 males) or between different populations of *X*O worms [Mes males and Mes Tra-1(gf) females]. However, the difference is significant between different populations of hermaphrodites/females [Mes *XX* hermaphrodites and Mes Tra-1(gf) *X*O females] and between different populations of males (Mes *X*O males and Mes Tra-2 *XX* males).

ens the correlation between the dosage of *X* chromosomes and the severity of the Mes phenotype.

***mes* mutations resemble dosage compensation *dpy* mutations in feminizing the germline and soma of male worms whose sexual identity is ambiguous:** The sensitivity of the Mes phenotype to *X* chromosome dosage or *X*:A ratio is reminiscent of the situation with mutations in dosage compensation, the process by which levels of *X* chromosome gene expression are equalized in animals bearing one *vs.* two *X* chromosomes. In *C. elegans*, dosage compensation in the soma is implemented in diploid animals bearing two *X*s: *XX* embryos reduce transcription from each *X* by half to achieve levels of *X* transcripts similar to those produced by the single *X* chromosome in *X*O males (Meyer and Casson 1986; Delong *et al.* 1987). Dosage compensation is an essential function in 2*X*:2A animals; 2*X*:2A embryos that fail to dosage compensate die from elevated *X*-linked gene expression (Hodgkin 1983; Meyer and Casson 1986; Plenefisch *et al.* 1989). Conversely, 1*X*:2A embryos that inappropriately implement dosage compensation die from underexpression of the *X* chromosome (Miller *et al.* 1988). Dosage compensation is controlled by the dosage compensation *dpy* genes (*dpy-21*, *dpy-26*, *dpy-27*, and *dpy-28*) and by upstream genes in the sex determination/dosage compensation pathway (Figure 9; reviewed by Meyer 1997). Mutations in the dosage compensation genes have a maternal effect and generally cause lethality of *XX* offspring, but do not affect *X*O worms. Rare *XX* survivors are abnormally short and fat, *i.e.*, dumpy, reflecting the deleterious effects of elevated *X*-linked gene expression. They are also fertile, indicating that the *dpy* dosage compensation activities are not required for normal germline proliferation and development. Molecular analysis of *dpy-26*, *dpy-27*, and the upstream genes *sdc-2* and *sdc-3* has led to the following model: a complex of dosage compensation proteins assembles specifically on the two *X* chromosomes in hermaphrodites and reduces gene expression by partially condensing interphase chromatin (Chuang *et al.* 1994, 1996; Lieb *et al.* 1996; Davis and Meyer 1997).

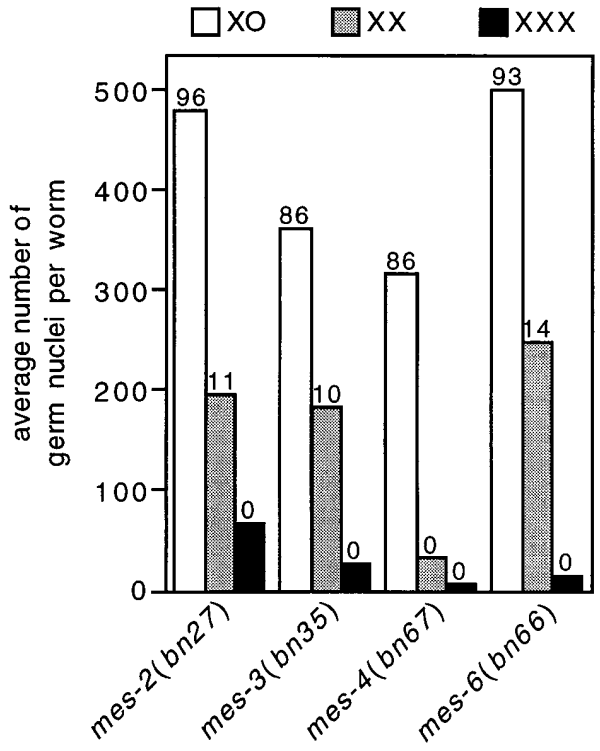

Figure 8.—Increasing the dose of *X* chromosomes enhances the germline defects caused by *mes* mutations. Comparison of the germlines in adult *X*O males (white bars), *XX* hermaphrodites (gray bars), and *XXX* Dpy hermaphrodites (black bars) produced by *mes; him-8 XX* mothers. Worms were stained with Hoechst to visualize and count germ nuclei. Between 11 and 44 worms were scored for each genotype. Each bar represents the average number of germ nuclei per worm. For each *mes* allele, the average number of germ nuclei is significantly different between *mes; him-8 X*O, *XX*, and *XXX* worms. Numbers above the bars indicate the percent of worms that contained gametes or gamete-like structures. Gametes in *mes-2; him-8*, *mes-3; him-8*, and *mes-6; him-8* males consisted of sperm only. Although sperm were not counted, there were hundreds of sperm in most males. Gametes in *mes-4; him-8* males included what appeared to be endomitotic oocytes in addition to, or instead of, sperm. This putative feminization of some *mes-4 X*O germlines has not been analyzed further. Gametes in *XX* hermaphrodites were few in number (usually less than 5) and included endomitotic oocytes, normal-appearing oocytes (with six condensed bivalents), and/or sperm (which sometimes looked normal and sometimes looked abnormal).

As described above for the *mes* genes, the mutant phenotype of the *dpy* genes is sensitive to *X* chromosome dosage: 3*X*:2A animals are more severely affected than 2*X*:2A animals, which are more severely affected than 1*X*:2A animals (DeLong *et al.* 1987). This similarity in mutant phenotype may indicate that the *mes* genes, like the dosage compensation genes, are involved in regulating the structure of chromatin and the expression of genes on the *X* chromosome. To explore this possibility, we tested for a further genetic similarity between the *mes* genes and the *dpy* genes. Disruption of dosage compensation has been observed to feed back on sex determination. Specifically, 2*X*:3A animals and *XX* animals carrying a mutation in the sex determination function of *sdc-3*, *i.e.*, *sdc-3(Tra)* mutation, develop as males, whereas 2*X*:3A and *sdc-3(Tra) XX* animals in which dosage compensation has been disrupted develop as hermaphrodites (Hodgkin 1987; Plenefisch *et al.* 1989; DeLong *et al.* 1993; see discussion). To determine whether mutations in the *mes* genes affect sex determination, we generated and analyzed 2*X*:3A *mes* mutants and *sdc-3(Tra); mes* mutants.

To generate 2*X*:3A animals, we mated tetraploid males (2*X*:4A) with *dpy* or *unc* diploid hermaphrodites (2*X*:2A; Madl and Herman 1979). Outcross progeny, *i.e.*, non-Dpy or non-Unc, are 2*X*:3A, resulting from the fusion of 1*X*:2A sperm with 1*X*:1A oocytes. In control

crosses of tetraploid males to *dpy* or *unc* hermaphrodites that were *mes*$^+$, the majority (88–99%) of the outcross progeny were male (Figure 10A). In experimental crosses of tetraploid males with *mes; dpy* or *mes; unc* hermaphrodites, the majority of outcross progeny were hermaphrodite (Figure 10A). The percentage of hermaphrodite progeny ranged from 63% for *mes-6(bn64)* to 93% for *mes-4(bn67).* These values can be compared to 55, 71, and 73% hermaphrodite progeny when the mothers carry mutations in *dpy-21*, *dpy-27*, or *dpy-28*, respectively (Hodgkin 1987; Plenefisch *et al.* 1989).

sdc-3 regulates sex determination and dosage compensation by separately mutable, independently functioning domains (DeLong *et al.* 1993; Klein and Meyer 1993; Davis and Meyer 1997). *sdc-3(Tra)* mutations, such as *sdc-3(y52),* affect sex determination but not dosage compensation: the majority of *sdc-3(Tra) XX* worms are transformed into nearly normal males (DeLong *et al.* 1993). Mutations in *dpy-26*, *dpy-27*, and *dpy-28* completely suppress the masculinization caused by *sdc-3(Tra)* mutations (DeLong *et al.* 1993). To determine whether *mes* mutations cause similar suppression, we created *mes; sdc-3(Tra) unc-76*/++ *XX* hermaphrodites and compared the sex of their Unc progeny, which are homozygous for *sdc-3(Tra),* to the sex of the Unc progeny from control *mes* $^+$*; sdc-3(Tra) unc-76*/++ mothers. Among the Unc progeny from control *mes*$^+$ mothers, 76% were males. In contrast, among the Unc progeny from *mes* mothers, 95–100% were hermaphrodites (Figure 10B).

Thus, in two tests of the effect of *mes* mutations on sex determination, *mes* mutations resemble mutations in the dosage compensation genes in their ability to feminize worms whose sexual identity is ambiguous.

DISCUSSION

Mutations in the four maternal-effect genes, *mes-2*, *mes-3*, *mes-4*, and *mes-6*, result in a common mutant phenotype: the hermaphrodite (*XX*) progeny of *mes*/*mes* mothers are sterile because of underproliferation and degeneration of the germline and an absence of gametes; in contrast, the male (*X*O) progeny have a substantially healthier germline and are often fertile. Interestingly, the severity of the germline phenotype depends on the *X* chromosome constitution and not the sexual phenotype of the worms. Thus, *XX* (and *XXX*) worms absolutely depend on maternally supplied *mes*$^+$ gene products for the survival of their germline; *X*O worms are less dependent on the *mes*$^+$ product. This differential requirement of *XX* and *X*O worms for *mes*$^+$ gene function may reflect involvement of *mes* gene products in control of gene expression from the *X* chromosomes. Consistent with a role in transcriptional regulation, two of the MES proteins are homologs of transcriptional regulators in Drosophila. Although the maternal-effect sterile phenotype suggests an essential role for the *mes* genes only in the germline, our observations that *mes* mutations can cause somatic sexual transformations suggest participation of the *mes* genes in somatic functions as well. We propose the following: (1) the *mes* genes encode maternally supplied regulators of gene expression, (2) their essential role is in the early germline, (3) they may serve a nonessential, modulatory role in early somatic cells, and (4) at least some of their targets are on the *X* chromosome.

***mes* mutant germ cells are proliferation defective and prone to degenerate, but they appear to be correctly specified:** The sterile hermaphrodite offspring of *mes* mothers contain reduced numbers of undifferentiated

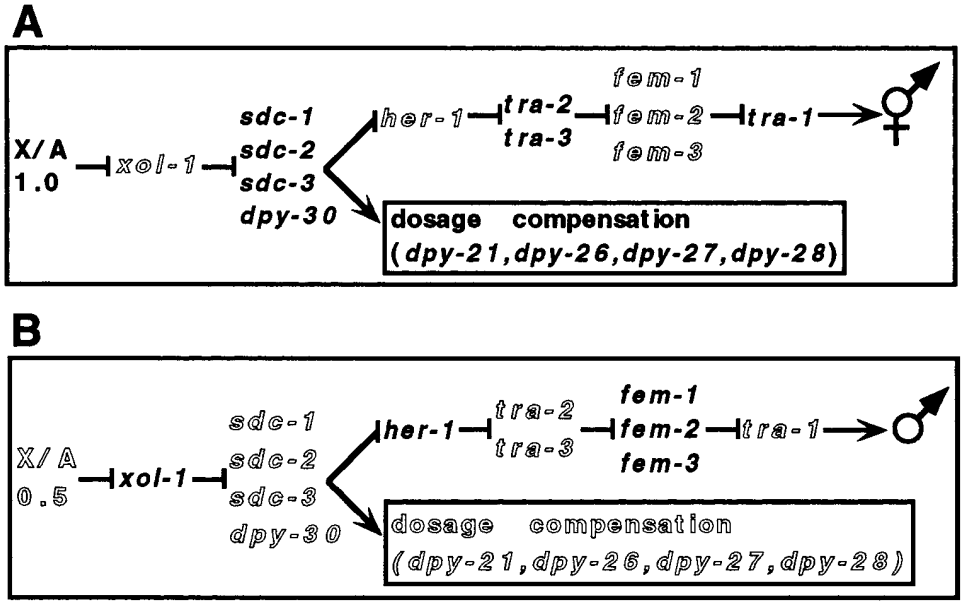

Figure 9.—The regulatory hierarchy controlling somatic sex determination and dosage compensation in *C. elegans.* The diagram shows the activity states of genes that act in a regulatory cascade to control both processes in hermaphrodites (A) and in males (B). Solid letters represent a high activity state and outlined letters represent a low activity state (modified from Hsu and Meyer 1994).

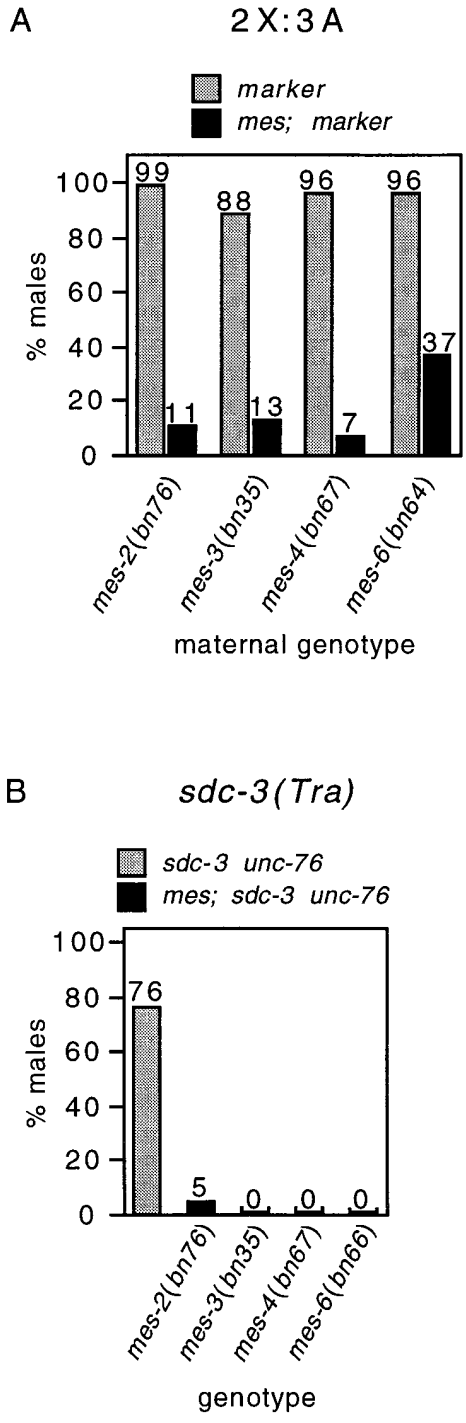

Figure 10.—Mutations in the *mes* genes can have strong feminizing effects on the soma. (A) Most 2*X*:3A offspring of *mes*$^+$ mothers are males, but most 2*X*:3A offspring of *mes*$^-$ mothers are hermaphrodites. Diploid hermaphrodites (2*X*:2A), homozygous for a mutation that causes a visible phenotype, were mated to unmarked tetraploid males (2*X*:4A), and the sexual phenotype of their 2*X*:3A cross progeny was determined. (Gray bars) Offspring of *mes*$^+$ mothers. (Black bars) offspring of *mes* mothers. At least 80 2*X*:3A worms were scored for each cross. (B) *mes* mutations suppress the masculinization of *XX* worms caused by a mutation that disrupts the sex determination activity of *sdc-3*. The mothers of the worms analyzed were *sdc-3(Tra) unc-76*/++, which were either wild-type or homozyous mutant for each *mes* gene. We examined the sexual phenotype of their Unc progeny. (Gray bar) Unc progeny of *sdc-3(Tra) unc-76*/++ mothers. (Black bars) Unc progeny of *mes; sdc-3(Tra) unc-76*/++ mothers. Between 35 and 418 worms were scored for each genotype. In both panels, the numbers above the bars indicate the percentage of males.

germ cells and no gametes. The proliferation defect appears to result from both a failure of germ cells to undergo the normal number of mitotic divisions and death of germ cells. We think that the main mode of *mes* germ cell death is degeneration, not programmed cell death, for two reasons. First, mutations that prevent programmed cell death do not suppress the Mes phenotype. Second, *mes* germ cells more closely resemble degenerating cells than cells undergoing programmed cell death. Thus, death is probably the result of physiological defects that arise as a consequence of lack of functional *mes*$^+$ product.

When the proliferation defect of *mes* germ cells is enhanced by a *glp-4* mutation, the germ cells still degenerate. This finding argues against a model in which the causes of germline degeneration are aberrant mitoses and aneuploidy. Instead, the "poor health" of *mes* germ cells is likely to precede and contribute to their inability to undergo the many rounds of divison that are typical of the cells in a wild-type germline.

The response of *mes* germ cells to elimination of DTC signaling provided two clues about their developmental potential. First, *mes* germ cells, like wild-type germ cells, depend on the DTC-signaling pathway to promote proliferation. This suggests that mutant germ cells are correctly specified and are expressing the GLP-1 receptor for the DTC signal. Second, *mes* germ cells rarely undergo meiosis and gametogenesis when the DTC signaling pathway is removed. Thus, the failure of mutant germ cells to form gametes in the presence of DTC signaling is not simply caused by DTC inhibition of meiosis. Rather, *mes* germ cells apparently lack the necessary cellular machinery to differentiate and/or are too unhealthy to execute meiosis and gametogenesis.

The severity of the *mes* germline phenotype is dependent on number of *X* chromosomes or *X*:A ratio, not on sexual phenotype: Among offspring of *mes* mothers, males generally have much healthier germlines than their hermaphrodite siblings. There are at least two components to the improvement: the average number of undifferentiated germ cells in males is significantly higher than in hermaphrodites, and gametogenesis is common in males but rare in hermaphrodites. Because of these differences, males are often fertile while their sibling hermaphrodites are sterile.

By examining *mes* worms carrying a mutation in one of several different genes in the sex determination pathway, we showed that the severity of the *mes* germline phenotype does not correlate with phenotypic sex

of the soma or germline: *XX* animals with a masculinized germline and soma display the severe Mes phenotype that is typical of *XX* hermaphrodites, and *X*O animals with a feminized germline and soma show the increased number and more normal appearance of germ cells typical of *X*O males. These findings suggest that, in terms of the consequences of eliminating maternal *mes*$^+$ product, the key difference between hermaphrodites and males is their *X* chromosome karyotype. This hypothesis is supported by the fact that 3*X*:2A *mes* hermaphrodites have an even more severe germline phenotype than 2*X*:2A *mes* hermaphrodites.

***mes* mutations and mutations in dosage compensation display striking similarities in phenotype:** As discussed in the last section of results, the *mes* genes show striking phenotypic similarities to the genes that control dosage compensation in the soma of *C. elegans*. Mutations in the dosage compensation *dpy* genes, *i.e.*, *dpy-26*, *dpy-27*, and *dpy-28*, result in maternal-effect death of *XX* animals and have no effect on *X*O animals (reviewed by Meyer 1997). Mutations in the *mes* genes result in maternal-effect death of the germline in *XX* animals, and have a milder effect on *X*O animals. Thus, by analogy to the *dpy* genes, whose function is to implement dosage compensation in the soma of *XX* animals, the *mes* genes may function to implement dosage compensation in the germline of *XX* animals. A further surprising parallel between the two classes of genes is their apparent participation in sex determination. Disruption of somatic dosage compensation has been observed to feed back on sex determination by transforming males whose sexual identity is ambiguous into hermaphrodites (Hodgkin 1987; Plenefisch *et al.* 1989; Delong *et al.* 1993). A similar feminizing effect is caused by mutations in the *mes* genes. The so-called "ambiguous" males can arise via an altered *X*:A ratio, *i.e.*, 2*X*:3A males, or by certain mutations in sex determination genes, *i.e.*, *XX* animals carrying a mutation in the sex determination domain of *sdc-3* (Figure 9). When 2*X*:3A or *sdc-3(Tra) XX* animals are the offspring of *dpy* mothers or *mes* mothers, the majority of them develop as hermaphrodites rather than males. What is the mechanism of dosage compensation, how does disruption of dosage compensation influence sex determination, and what clues can the parallels between *dpy* and *mes* mutations provide about *mes* gene function?

Based on the molecular analysis of *dpy-27*, *dpy-26*, *sdc-3*, and *sdc-2*, as well as a wealth of genetic information (Chuang *et al.* 1994, 1996; Lieb *et al.* 1996; Davis and Meyer 1997; reviewed by Meyer 1997), the current molecular view of dosage compensation in the soma is as follows: a complex of proteins, including DPY-27, DPY-26, SDC-3, SDC-2, and probably DPY-28 and other proteins, assembles on the two *X*s in all cells (with the notable exception of the primordial germ cell) of *XX* embryos at about the 30-cell stage. This protein complex reduces by half the level of transcripts made by each hermaphrodite *X* chromosome. DPY-27 is a member of the SMC family of proteins (Chuang *et al.* 1994), which are structural components of chromosomes known to be involved in the condensation of mitotic chromosomes in yeast and frogs (Strunnikov *et al.* 1993; Hirano and Mitchison 1994). Thus, the dosage compensation complex is envisioned as reducing transcription by partially condensing the *X* chromosomes.

Recent results suggest an attractive model for the mechanism by which disruption of dosage compensation feeds back on sex determination (Davis and Meyer 1997). The key player in this model is SDC-2, which participates directly in both dosage compensation and sex determination; it is a component of the dosage compensation protein complex, and it also negatively regulates the promoter of *her-1*, the first gene in the sex determination pathway (Nusbaum and Meyer 1989; D. Lapidus, H. Dawes, D. Berlin, and B. Meyer, unpublished results; Meyer 1997). In normal *X*O animals, high levels of XOL-1 repress synthesis of SDC-2; as a result, dosage compensation is not implemented, and expression of *her-1* leads to male development (Rhind *et al.* 1995; see Meyer 1997 for review). In normal *XX* animals, XOL-1 levels are low, and synthesis of SDC-2 leads to both assembly of the protein complex that mediates dosage compensation and repression of *her-1*, leading to hermaphrodite development. This dual role of SDC-2 predicts that the concentration of the protein in cells is crucial. Indeed, excess SDC-2 expressed from a transgenic array feminizes *sdc-3(Tra) XX* animals (Davis and Meyer 1997). This finding suggests that the mechanism by which disruption of dosage compensation feminizes *sdc-3(Tra) XX* and 2*X*:3A animals is by elevating the level of SDC-2 available to repress *her-1* expression, thereby leading to hermaphrodite development. SDC-2 levels may be elevated by release of SDC-2 from the dosage compensation complex or by increased expression of *sdc-2*, which is on the *X*. Potentially, any perturbation of the dosage compensation complex or of expression of *X*-linked genes in the early embryo may increase SDC-2 levels, resulting in feedback on sex determination. *mes* mutations may create such perturbations.

The MES proteins are predicted to regulate gene expression via modulation of chromatin structure: Cloning and molecular analysis has revealed that MES-2 and MES-6 are the *C. elegans* homologs of Enhancer of zeste [E(z)] and Extra sex combs (Esc) (R. Holdeman, S. Nehrt and S. Strome, unpublished results; I. Korf, R. Holdeman, Y. Fan and S. Strome, unpublished results), both members of the Polycomb group (Pc-G) in Drosophila (Jones and Gelbart 1993; Simon *et al.* 1995; Gutjahr *et al.* 1995). This group of proteins is best known for its role in long-term maintenance of repression of homeotic genes (see Orlando and Paro 1995; Simon 1995; Pirrotta 1997 for reviews). However, some members of the Pc-G group also show ge-

netic properties typical of the Drosophila Trithorax group (Trx-G), which antagonizes the Pc-G and maintains activation of homeotic genes, and some Pc-G members participate in the regulation of other genes in addition to the homeotic genes (Jones and Gelbart 1990; Phillips and Shearn 1990; Lajeunesse and Shearn 1996). The common theme among Pc-G and Trx-G proteins is the regulation of gene expression via modulation of chromatin structure (see Orlando and Paro 1995; Simon 1995; Pirrotta 1997 for reviews). The Pc-G proteins in particular are thought to assemble into multimeric protein complexes at specific sites on chromosomes; the complexes may repress expression of nearby genes by modifying nucleosomes or by altering higher-order chromatin structure. Evidence for formation of localized protein complexes comes from colocalization of Pc-G proteins on polytene chromosomes and from the ability to coimmunoprecipitate Pc-G proteins (see reviews above). Furthermore, Jones *et al.* recently demonstrated that E(z) and Esc interact directly (C. Jones, J. Ng, A. Peterson, K. Morgan, J. Simon, and R. Jones, unpublished results), raising the possibility that MES-2 and MES-6 interact. Indeed, MES-2 and MES-6 depend on each other to be correctly localized in nuclei. MES-3, which is a novel protein (Paulsen *et al.* 1995), is required for the correct localization of both MES-2 and MES-6 (R. Holdeman, S. Nehrt and S. Strome, unpublished results; I. Korf, R. Holdeman, Y. Fan and S. Strome, unpublished results).

In larvae and adults, MES-2, MES-6, and MES-3 are localized in the nuclei of the germline (R. Holdeman, S. Nehrt, and S. Strome, unpublished results; I. Korf, R. Holdeman, Y. Fan, and S. Strome, unpublished results). In early embryos, the three MES proteins are present in all nuclei. We hypothesize that the essential role of the maternal load of MES proteins in the primordial germ cells is to maintain or create a chromatin architecture that is compatible with the germline's program of gene expression. If the MES proteins also affect chromatin architecture in somatic cells of early embryos, then they may affect assembly of the dosage compensation complex on the *X* chromosomes or participate directly in regulating *X* chromosome gene expression. Even slight destabilization of the dosage compensation complex or elevation of *sdc-2* expression from the *X* in *mes* mutant embryos could sufficiently elevate the amount of SDC-2 available to feed back on sex determination via *her-1* regulation, as discussed above. An alternative scenario is that *mes* mutations affect expression of a dosage compensation gene or of *sdc-2* in the maternal germline, and that mutant mothers contribute an altered dosage of DPY or SDC-2 protein to their embryos.

A model for MES protein function: Our current model of MES protein function is based on the genetic results presented in this paper, our findings that two MES proteins are homologs of known regulators of gene expression in Drosophila, and studies of MES protein localization (R. Holdeman, S. Nehrt and S. Strome, unpublished results; I. Korf, R. Holdeman, Y. Fan and S. Strome, unpublished results). The MES proteins are maternally loaded and passed to the somatic and germline cells of the embryo. Their essential role is in the germline, where by analogy with Pc-G and Trx-G proteins, the MES proteins may function as protein complexes to organize chromatin into a conformation that promotes correct levels or patterns of expression of genes needed for normal germline development. Because a maternal load of wild-type protein is sufficient for fertility, we hypothesize that the MES proteins are involved in the establishment of chromatin architecture, but they are not required for its maintenance. Animals that lack a maternal load of wild-type protein are able to correctly specify the germline, but the germ cells are proliferation defective and prone to degenerate, perhaps as a result of inappropriate levels or patterns of gene expression.

What are the targets of MES function? The sensitivity of the Mes phenotype to *X* chromosome dosage suggests that at least some target genes are on the *X*. If all targets are on the *X*, then the role of the *mes* genes may be dosage compensation in the germline of *XX* animals. Two results argue against this model: first, some *mes* males show aspects of the mutant phenotype, and second, the MES proteins do not appear to bind exclusively to a single chromosome in the germline (R. Holdeman and S. Strome, unpublished results). Thus, we favor a model in which the *mes* target genes are on one or more autosomes, as well as on the *X*. Future tests of this model could include examining the sensitivity of the germline phenotype to the dosage of autosomes, *i.e.*, autosomal duplications and deficiences, and visualizing the distribution of the MES proteins on chromosomes in the germline, *i.e.*, using chromosome-specific hybridization probes (Chuang *et al.* 1994).

An unresolved issue is whether the MES proteins serve an important function in somatic cells as well as in the germline. In sexually normal *XX* and *X*O animals, the *mes* mutant phenotype appears to be restricted to the germline. A potential somatic function of the *mes* genes is revealed only under unusual conditions: 2*X*:3A or *sdc-3(Tra)* 2*X*:2A animals develop as males in a mes^+ background but as hermaphrodites in a maternally mes^- background. MES proteins are present in the nuclei of somatic cells during the period of embryogenesis when dosage compensation is initiated and the sexual fate of the soma is determined (Villeneuve and Meyer 1990; Rhind *et al.* 1995; Davis and Meyer 1997; R. Holdeman, S. Nehrt and S. Strome, unpublished results; I. Korf, R. Holdeman, Y. Fan and S. Strome, unpublished results). Thus, the MES proteins are present in the right place at the right time to participate in regulating chromosome structure and gene expression in the embryonic soma. Examining the effect

of *mes* mutations on the expressivity of certain types of mutations, *e.g.*, semidominant, that affect somatic tissues in the embryo would provide a further test of MES protein participation in modulating levels of gene expression in the soma. Since elimination of *mes*$^+$ function does not adversely affect development of somatic cells into healthy adult tissues, any modulatory role the MES proteins serve in the early embryonic soma must be considered nonessential.

We thank Barbara Meyer for strains, advice, and helpful suggestions on experiments and this paper; Tim Schedl for strains and stimulating discussions; and Phil Meneely for advice on working with polyploids. Some strains were supplied by the Caenorhabditis Genetics Center, which is funded by the National Institutes of Health (NIH) National Center for Research Resources. This research was supported by NIH grant GM34059 to S.S. S.S. gratefully acknowledges the support of American Cancer Society award FRA-399, and R.H. acknowledges the support of NIH Training Grant GM07227.

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Communicating editor: R. K. Herman