

Identification of Grandchildless Loci Whose Products Are Required for Normal Germ-Line Development in the Nematode *Caenorhabditis elegans*

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ABSTRACT

To identify genes that encode maternal components required for development of the germ line in the nematode *Caenorhabditis elegans*, we have screened for mutations that confer a maternal-effect sterile or "grandchildless" phenotype: homozygous mutant hermaphrodites produced by heterozygous mothers are themselves fertile, but produce sterile progeny. Our screens have identified six loci, defined by 21 mutations. This paper presents genetic and phenotypic characterization of four of the loci. The majority of mutations, those in *mes-2*, *mes-3* and *mes-4*, affect postembryonic germ-line development; the progeny of mutant mothers undergo apparently normal embryogenesis but develop into agametic adults with 10–1000-fold reductions in number of germ cells. In contrast, mutations in *mes-1* cause defects in cytoplasmic partitioning during embryogenesis, and the resulting larvae lack germ-line progenitor cells. Mutations in all of the *mes* loci primarily affect the germ line, and none disrupt the structural integrity of germ granules. This is in contrast to grandchildless mutations in *Drosophila melanogaster*, all of which disrupt germ granules and affect abdominal as well as germ-line development.

THE question of how cell fates are specified and expressed in developing organisms remains a key unresolved issue in developmental biology. One of the best studied embryonic lineages is the germ lineage. In a wide variety of organisms, the primordial germ cells are set aside very early in development. A long history of experimentation suggests that in these organisms, cytoplasmic localization plays a major role in determining which cells follow a germ-cell fate [see DAVIDSON (1986) for review]. As early as 1910, BOVERI showed that in *Ascaris*, a localized region of polar ooplasm, the germ plasm, determines which cells become germ-line precursors. More recently, cytoplasmic transplantation experiments in *Drosophila melanogaster* have demonstrated that polar plasm from oocytes or early embryos is sufficient to induce the formation of primordial germ cells or "pole cells" at ectopic locations in embryos (ILLMENSEE and MAHOWALD 1974, 1976; ILLMENSEE, MAHOWALD and LOOMIS 1976). This indicates that a primary determinant of germ cells is provided maternally and localized in the egg cytoplasm. Further evidence in support of the importance of maternal factors comes from genetic analysis of the *grandchildless-knirps* class of genes in *D. melanogaster*. These genes were identified by maternal-effect lethal mutations that cause two developmental defects. Embryos from homozygous mutant mothers fail to form pole cells and display defects in abdominal segmentation (BOSWELL and MAHOWALD 1985; SCHÜPBACH and WIESCHAUS 1986; LEHMANN and NÜSSLEIN-VOLHARD 1986). Both defects are

thought to result from a failure in formation of polar granules, cytoplasmic organelles that normally are localized at the posterior end of oocytes and early embryos and in the pole cells and germ cells of later stages. Consistent with this, at least one of the *grandchildless-knirps* genes, *vasa*, is known to encode a polar-granule component (HAY *et al.* 1988). These results, along with the observation that germ-line-specific granules are nearly ubiquitous among species, have led to the model that germ granules carry maternally provided determinants of germ-cell fate.

In this paper, we describe a genetic approach to identifying maternal factors that control germ-line determination and development in the nematode *Caenorhabditis elegans*. In *C. elegans*, the germ lineage arises from the P4 founder cell, which is generated early in development via a series of unequal, stem-cell-like divisions (DEPPE *et al.* 1978). Like the pole cells in *Drosophila*, P4 and its descendants contain germ-line-specific granules, termed P granules. However, instead of being prelocalized in the oocyte, P granules are progressively partitioned to the germ lineage during the series of unequal divisions (STROME and WOOD 1982; WOLF, PRIESS and HIRSH 1983). Although P granules are excellent candidates for germ-cell determinants, at present it is not known what role, if any, they play in specification or expression of germ-cell fate.

To identify maternal components that contribute to development of a normal germ line, we have screened for and characterized grandchildless muta-

tions in *C. elegans*. We describe four loci that appear to encode maternal factors required primarily, if not exclusively, for germ-line development. One locus is required for generation of the germ-line progenitor cells, and the other three loci are required for normal post-embryonic proliferation of the germ line. In contrast to mutations in the *grandchildless-knirps* genes in *Drosophila*, none of the *C. elegans* grandchildless mutations analyzed to date appear to affect the assembly of germ granules.

MATERIALS AND METHODS

General techniques and strains: Maintenance and genetic manipulation of *C. elegans* were carried out described by BRENNER (1974). Strains were provided by the *Caenorhabditis* Genetics Center. In addition, *sqt-3(sc63)* was a gift from KAYE EDWARDS; *lin-7(n1413)unc-52(e444)* was a gift from CARL JOHNSON; *hDp20* was a gift from KIM MCKIM and ANN ROSE; *mes-1(q222)* and *mes-1(q367)* were gifts from ERIC LAMBIE and JUDITH KIMBLE; *lin-25(n545ts)*, *arDf1* and *rol-4(sc8)unc76(e911)* were gifts from SIMON TUCK and IVA GREENWALD; *itDf2* was a gift from KEN KEMPHUES. *C. elegans* variety Bristol, strain N2 was the wild-type parent of all nematode strains used. Markers, duplications and deficiencies used are listed by chromosome:

I: *unc-11(e47)*, *unc-38(e264)*, *dpy-5(e61)*, *lev-10(x17)*, *sDf4*, *sDp2*, *hDp20*.

II: *unc-4(e120)*, *rol-1(e91)*, *lin-7(n1413)*, *unc-52(e444)*.

III: *unc-32(e189)*, *dpy-17(e164)*.

IV: *unc-5(e53)*, *unc-24(e138)*, *fem-3(q22ts)*, *dpy-20(e1282)*, *him-3(e1147)*, *egl-23(n601)*, *eDf18*, *eDf19*.

V: *dpy-11(e224)*, *rol-4(sc8)*, *sqt-3(sc63ts)*, *lin-25(n545ts)*, *him-5(e1490)*, *unc-76(e911)*, *arDf1*, *itDf2*.

X: *lon-2(e678)*, *lin-2(e1309)*, *unc-9(e101)*, *unc-84(e1410)*, *unc-3(e151)*.

Standardized *C. elegans* genetic nomenclature, as described by HORVITZ *et al.* (1979), has been used. All grandchildless mutations isolated have been given gene designations *mes* for maternal-effect sterile mutation.

Nonconditional *mes* mutations were maintained as balanced stocks. *mes-2* stocks were balanced with *mnC1*, which suppresses recombination over 80% of chromosome II (HERMAN 1978). *mes-4* and *mes-6* stocks were balanced with the reciprocal translocation chromosomes *nT1[unc(n754)let](IV, V)* (FERGUSON and HORVITZ 1985). This balancer (abbreviated *D-nT1*) has a dominant Unc and recessive lethal phenotype. *mes-3* stocks were maintained as heterozygotes over a marked chromosome I or balanced with either the free duplication *sDp2* (I; f) (ROSE, BAILLIE and CURRAN 1984) or *hDp20* (I, V; f) (MCKIM and ROSE 1990).

Identification of *mes* mutations: The general screen used to identify *mes* mutations is shown in Figure 1. Ethyl methanesulfonate (EMS) mutageneses were performed as described in BRENNER (1974) except that concentrations used ranged from 24 to 60 mM. Three variations of this screen were used. (1) Either N2 or *him-5* or *egl-23 him-3* L4 hermaphrodites were mutagenized and transferred to plates in groups of approximately 20. F₁ hermaphrodite progeny were picked to individual plates, allowed to lay 20–40 embryos and then removed. F₂ progeny were scored as adults, and broods displaying excessive lethality or any sterility were discarded. The F₂ adults were washed off the plates to restrict their brood size. F₃ embryos, which stuck to the plates, were allowed to mature, and the plates were scored

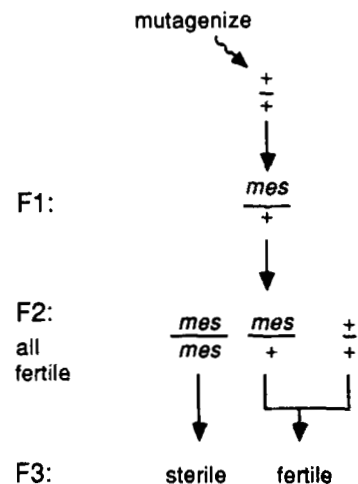


FIGURE 1.—Screen for maternal-effect sterile mutations. Hermaphrodites are mutagenized, and F₁ progeny are picked onto individual plates. Animals heterozygous for a newly induced *mes* allele produce all fertile F₂ progeny, including fertile homozygous *mes* animals. These homozygous mutant F₂ animals produce sterile progeny, resulting in an F₃ mixed population of sterile and fertile worms. (See MATERIALS AND METHODS for more detailed description.)

for the presence of any sterile animals. Candidate broods containing sterile animals were tested for maternal-effect sterility as follows. Ten to 20 fertile hermaphrodite siblings were picked to individual plates and allowed to lay a small clutch of embryos. If the candidate brood carried a *mes* allele then usually one or more of the individuals picked was a homozygous mutant animal and produced sterile progeny. The stock was then maintained from sister plates containing heterozygous siblings. Ten *mes* alleles were isolated from 5974 haploid genomes screened. (2) The mutagenized strain was either *dpy-5* or *dpy-5 lev-10*. Mutagenized animals were mated to wild-type or *him-5* males and outcross F₁ progeny were picked and screened as above. Eight *mes* alleles were isolated from 2953 haploid genomes screened. (3) *dpy-11/D-nT1* hermaphrodites were mutagenized. F₁ Unc progeny were picked and screened as above except that the F₂ animals had small enough broods that washing the adults off was unnecessary. Six *mes* alleles were isolated from 2292 haploid genomes screened. For all three types of screens, mutagenized animals were raised at either 16° or room temperature (22–24°), and all progeny were maintained at room temperature.

Newly isolated alleles were linkage mapped by mating *mes/+* males to *dpy-5* I; *rol-1* II; *unc-32* III or *unc-5* IV; *dpy-11* V; *lon-2* X hermaphrodites. F₁ outcross progeny were picked to individual plates and allowed to lay 20–30 embryos at 25°, then transferred to a fresh plate at 16° to lay the rest of their brood. The F₃ generations on the 25° plates were scored for the presence or absence of sterile animals to identify F₁ *mes* heterozygotes. F₂ Rol, Unc, Lon or Dpy progeny from these *mes* heterozygous F₁ animals were picked from the 16° plates. Their progeny were scored for fertility or sterility, and markers showing significant deviations from a 3:1 fertile:sterile ratio were judged to be linked to the *mes* mutation being tested.

Once several complementation groups had been identified, newly induced *mes* alleles were often not linkage mapped. Instead, they were assigned to loci according to the results of complementation tests. *mes/+* males were mated to appropriately marked *mes-1*, *mes-2*, *mes-3*, *mes-4*,

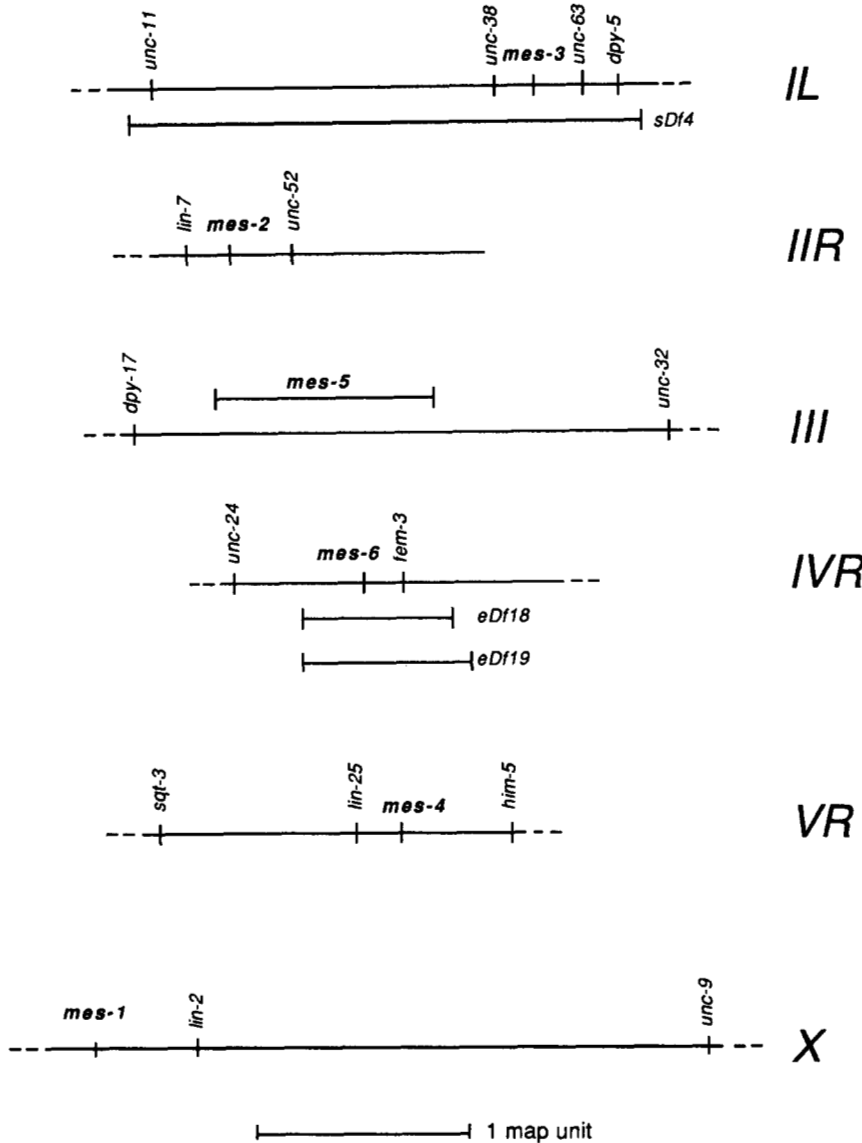


FIGURE 2.—Map positions of the six *mes* loci. Partial map of the *C. elegans* chromosomes showing the position of *mes* loci (in boldface) relative to markers. Positions were determined from mapping described in MATERIALS AND METHODS. Deficiencies that fail to complement *mes-3* or *mes-6* are shown.

mes-5 or *mes-6* strains, and F₁ outcross hermaphrodites were scored for production of fertile or sterile progeny. If a newly induced *mes* mutation failed to complement an assigned allele, it was two- or three-factor mapped to the same chromosomal location to insure that it was not a second-site noncomplementing mutation.

Mapping of *mes* loci: The map positions of the six *mes* loci identified are shown in Figure 2. Positions were assigned on the basis of two- and three-factor mapping as described below. In general, *trans* three-factor mapping was carried out by constructing *mes/m1 m2* strains (where *m1* and *m2* are appropriate markers) and cloning F₁ M1 non-M2 and M2 non-M1 recombinant progeny. Two generations later, the F₃ progeny were scored for the presence or absence of sterile animals to determine whether the *mes* allele was present in the F₁ recombinant. *cis* two-factor mapping was performed by constructing *m mes/++* strains, cloning F₁ M progeny and scoring their progeny for sterile or fertile offspring.

mes-1 (X): *mes-1* was most precisely mapped using the allele *bn7*. Two-factor crosses relative to *lon-2*, *unc-84* and *unc-9* place it approximately 13 map units to the right of *lon-2* and 3 map units to the left of *unc-9*. It was also three-factor

mapped relative to *lin-2 unc-9*. None of the 20 Lin non-Unc and all of the 21 Unc non-Lin recombinants had acquired the *bn7* allele, suggesting that *mes-1* is to the left of *lin-2*. This was confirmed by the isolation of a *bn7 lin-2 unc-9/+ lin-2 unc-9* recombinant from a *bn7 +/+ lin-2 unc-9* heterozygote. The other alleles of *mes-1* were mapped to the same place as follows: *bn24* was *cis* three-factor mapped relative to *lon-2 unc-9*; *bn52* was two-factor mapped relative to *lon-2*; *bn56*, *q222* and *q367* were three-factor mapped relative to *lon-2 unc-3*.

mes-2 (II): The allele *bn11* was used to position *mes-2*. Two-factor data relative to *unc-4* and *rol-1* place it on the right end of the chromosome near *unc-52*. Three-factor crosses with *lin-7 unc-52* place *bn11* between the two markers (2/13 Lin non-Unc recombinants picked up the *mes-2* allele). The other two alleles, *bn27* and *bn48*, were two-factor mapped relative to *unc-4* and *rol-1* respectively to place them at the same locus as *bn11*.

mes-3 (I): The map position of *mes-3* was determined by three-factor mapping the allele *bn35* relative to *unc-38 dpy-5*. Thirteen/42 Unc non-Dpy and 40/55 Dpy non-Unc recombinants picked up *bn35*, placing it between the two markers. It was also mapped to the left of *unc-63* by two

criteria: first, three-factor mapping relative to *unc-63 dpy-5* resulted in 0/32 Unc non-Dpy and 26/26 Dpy non-Unc recombinants that contained *bn35*; second, a *bn35 unc-63 dpy5/+ unc-63 dpy-5* recombinant was isolated from the heterozygous strain *bn35 +/+ unc-63 dpy-5*. The other two *mes-3* alleles, *bn21* and *bn53*, mapped to the same position when three-factor mapped relative to *unc-11 dpy-5*.

mes-4 (V): *bn23* was the most carefully mapped *mes-4* allele. A three-factor cross between *bn23* and *dpy-11 unc-76* produced 66/83 Dpy non-Unc and 27/81 Unc non-Dpy recombinants that contained *bn23*, placing it two map units to the left of *unc-76*. It was mapped to the right of *lin-25* by two criteria: first, a *lin-25 bn23 +/lin-25 + unc76* recombinant was isolated from a *lin-25 unc-76/bn23* heterozygote; second, the deficiency *arDf1*, which extends leftward from *lin-25* (S. TUCK, personal communication), complements *bn23*: 20 *arDf1/sqt-3 bn23* hermaphrodites, which were Sqt, produced fertile progeny. It was placed to the left of *him-5* as follows: first, a *rol-4 unc-76/bn23 him-5* heterozygote produced Rol non-Unc recombinant progeny that were subsequently shown to have recombined between *bn23* and *him-5* (i.e., *rol-4 + him-5 +/rol-4 + + unc-76*); second, the deficiency *itDf2*, which extends rightward from *him-5* (K. KEMPHUES, personal communication), complements *bn23*: 83 *itDf2/bn23 him-5* hermaphrodites, which were Him, produced fertile progeny. The other three *mes-4* alleles, *bn50*, *bn58*, and *bn67*, were three-factor mapped to the same position relative to *dpy-11 unc-76*.

mes-5 (III): The *mes-5* allele, *bn37*, was three-factor mapped relative to *dpy-17 unc-32*. 6/9 Unc non-Dpy and 3/9 Dpy non-Unc recombinants picked up the *mes-5* allele, placing it between the two markers.

mes-6 (IV): The *mes-6* locus was mapped most precisely using the allele *bn38*. A three-factor cross relative to *unc-24 dpy-20* produced 9/40 Unc non-Dpy and 18/25 Dpy non-Unc recombinants that contained *bn38*, placing it between the two markers. In addition, three-factor mapping relative to *unc-5 fem-3* placed it approximately 0.3 map unit to the left of *fem-3* (33/38 Unc non-Fem recombinants picked up *bn38*). The other three alleles, *bn64*, *bn66*, and *bn69*, were three-factor mapped to the same position relative to *unc-24 dpy-20*.

Complementation tests with chromosomal deficiencies:

The chromosome I deficiency *sDf4* fails to complement all *mes-3* alleles. *mes-3 dpy-5/++* males were mated to hermaphrodites of the genotype *sDf4/bli-4(e937) dpy-14(e188)*. Because the deficiency removes the *dpy-5* locus, Dpy-5 outcross hermaphrodites were cloned; these animals are *mes-3 dpy-5/sDf4*. Eleven/16 *mes-3(bn21) dpy-5/sDf4* mothers, 12/13 *mes-3(bn35) dpy-5/sDf4* mothers and 8/9 *mes-3(bn53) dpy-5/sDf4* mothers produced sterile progeny. The remaining animals did not survive to adulthood or produced no progeny at all. These last two phenotypes do not appear to be caused by the *mes-3* alleles since the parental hermaphrodite strain behaves in a similar manner.

The chromosome IV deficiencies *eDf18* and *eDf19* fail to complement all *mes-6* alleles. Individual *Df/+* males were mated with *mes-6/D-nT1(IV)*; *dpy-11/D-nT1(V)* or *mes-6 dpy-20/D-nT1(IV)*; *+/D-nT1(V)* hermaphrodites, and 20 non-Dpy, non-Unc F₁ progeny were cloned. Half the progeny should have been *Df/mes-6* and approximately half the animals produced sterile progeny.

Maternal effect tests: The strictness of the maternal effect was tested by mating wild-type males to appropriately marked *mes* hermaphrodites and scoring outcross progeny (*m mes/++*, phenotypically wild type) and self-cross marked progeny (*m mes/m mes*) for sterility or fertility. Greater than 500 outcross progeny or the total broods of ten *mes* mothers

were scored. For the temperature-sensitive alleles, crosses were carried out at 25°.

Embryonic lethality and expressivity: Homozygous mutant *mes* hermaphrodites were picked to individual plates as L4s at 16° or 25° and transferred to fresh plates every 24 or 12 hr, respectively. Plates were scored for unhatched embryos a sufficient amount of time after the mother had been removed (>24 hr for 16° experiments and >12 hr for 25° experiments). Hatched progeny were allowed to grow to adulthood, counted and scored for fertility or sterility.

mes-3(bn21)/sDf4 hermaphrodites were constructed by crossing *bn21(I)*; *him-5(V)* males to *dpy-5/sDf4* hermaphrodites (hermaphrodites are phenotypically Dpy) and shifting individual L4 outcross hermaphrodite progeny to 25°. *bn21/sDf4*; *him-5/+* animals were distinguished from *bn21/dpy-5*; *him-5/+* siblings by the absence of Dpy progeny. Embryonic lethality and expressivity experiments were carried out as described above.

Immunofluorescence and nuclear staining: Embryos and larvae were fixed and stained for P granules as described in STROME and WOOD (1983). Monoclonal antibodies used included K76 (STROME and WOOD 1983), OIC1D4, PIF4B11, PIG4, N123.A3 and PIIaH4A6 (STROME 1986). Adult animals were fixed and stained with diamidinophenolindole (DAPI) as previously described (STROME and WOOD 1983). All staining was visualized on a Zeiss Axioskop equipped with Nomarski differential interference contrast and epifluorescence optics.

Postembryonic germ-line proliferation: Wild-type and *mes* larvae were staged according to the number and position of vulval precursor cells (SULSTON and HORVITZ 1977) and fixed as previously described (STROME and WOOD 1983). L4 and adult animals were stained with DAPI while earlier stages were stained with anti-P-granule antibodies as well as DAPI (see above). Each data point plotted in Figure 5 represents the average of at least nine larvae. The full genotype of the *mes-2* larvae was *unc-4(e120)mes-2(bn11)*. *unc-4(e120)* larvae exhibit wild-type germ-line proliferation (P. MARTIN, unpublished results) so any reduction seen is a result of the *mes-2* allele.

RESULTS

Screen for grandchildless mutants: We have used the screen diagrammed in Figure 1 to identify grandchildless genes in *C. elegans*. This screen takes advantage of the fact that worms lacking embryos are easily distinguished from fertile animals at the resolution of the dissecting microscope (Figure 3). In sterile animals, the space normally taken up by embryos is empty, resulting in an easily-scored clear phenotype. Animals carrying newly induced grandchildless or maternal-effect sterile (*mes*) mutations were identified as F₁ heterozygotes that produced fertile progeny in the F₂ generation and fertile plus sterile worms in the F₃ generation. This screen allows isolation of recessive alleles in genes whose products are maternally supplied and required for normal germ-line development. It will not identify dominant alleles, mutations in non-maternal-effect genes required for germ-line development, or mutations in genes required for additional developmental processes unless rare alleles result in a *Mes* phenotype. We expected to isolate *mes* mutations that result from defects in cytoplasmic partitioning



FIGURE 3.—The sterile phenotype is easily scored at the resolution of the dissecting microscope. (a) Wild-type fertile hermaphrodite. (b) *mes* sterile hermaphrodite. The worms are oriented anterior-left and ventral-down.

TABLE 1
Maternal-effect sterile alleles

| | Locus | | | | | |
|------------|---|---|---|--|---------------|--|
| | <i>mes-1</i> | <i>mes-2</i> | <i>mes-3</i> | <i>mes-4</i> | <i>mes-5</i> | <i>mes-6</i> |
| Chromosome | X | II | I | V | III | IV |
| Alleles | <i>bn7ts</i> <i>bn24ts</i> <i>bn52ts</i> <i>bn56ts</i> | <i>bn11</i> <i>bn27</i> <i>bn48</i> | <i>bn21ts</i> <i>bn35</i> <i>bn53</i> | <i>bn23</i> <i>bn50</i> <i>bn58</i> <i>bn67</i> | <i>bn37ts</i> | <i>bn38</i> <i>bn64</i> <i>bn66</i> <i>bn69</i> |

because weak alleles of *par* genes, a class of maternal-effect lethal genes, exhibit an incompletely expressed Mes phenotype. Strong *par* mutations disrupt cytoplasmic partitioning during the first few embryonic cleavages causing embryonic lethality (KEMPHUES *et al.* 1988). The Mes phenotype of these mutations suggests that germ-line development is sensitive to partitioning defects. In addition to partitioning mutations, we expected to isolate mutations in genes encoding maternally provided products required for generation, determination, proliferation, or differentiation of the germ line or establishment of the appropriate germ-cell environment, all of which might also cause a Mes phenotype.

We have screened 11,219 EMS-mutagenized haploid genomes and isolated and characterized 19 *mes* alleles. These have been assigned to six complementation groups (Table 1). The *mes-5* locus is the only one for which multiple alleles have not been obtained; it may represent a rare maternal-effect allele of a gene whose null phenotype is not Mes (for example, see PERRIMON *et al.* 1986). For the other five loci, alleles have been isolated at a frequency characteristic of loss-of-function mutations in *C. elegans* (BRENNER 1974). All mutations are fully recessive: heterozygous mothers produce one quarter homozygous *mes* hermaphrodites, which are themselves fertile, but produce sterile progeny.

Figure 2 shows the map position of each of the *mes*

TABLE 2
Strictness of the maternal effect of *mes* alleles

| Genotype of mother | Number of broods scored | Percent sterile self-cross | Percent sterile outcross |
|----------------------------|-------------------------|----------------------------|--------------------------|
| <i>lon-2(e678) mes-1</i> | | | |
| <i>bn7ts</i> ^a | 11 | 45 ^b | 54 ^b |
| <i>unc-4(e120) mes-2</i> | | | |
| <i>bn11</i> | 8 | 100 | 100 |
| <i>bn27</i> | 5 | 100 | 100 |
| <i>rol-1(e91) mes-2</i> | | | |
| <i>bn48</i> | 10 | 100 | 100 |
| <i>mes-3 dpy-5(e61)</i> | | | |
| <i>bn21ts</i> ^a | 14 | 100 | 100 |
| <i>bn35</i> | 9 | 100 ^c | 100 ^c |
| <i>bn53</i> | 11 | 100 | 100 |
| <i>dpy-11(e224) mes-4</i> | | | |
| <i>bn23</i> | 31 | 100 | 100 |
| <i>bn50</i> | 12 | 100 | 100 |
| <i>bn67</i> | 7 | 100 | 100 |

^a Crosses were done at the restrictive temperature. For other alleles, the data are from crosses done at 16° or both 16° and 25°.

^b These values are not significantly different according to a Student *t*-test with *t* = 1.3509, d.f. = 20, 0.1 < *P* < 0.2.

^c Fewer than 1% fertile progeny.

loci. Two of the six map to regions for which deficiencies exist. The chromosome I deficiency, *sDf4*, fails to complement *mes-3* alleles and the chromosome IV deficiencies, *eDf18* and *eDf19*, fail to complement *mes-6* alleles. In both cases, *mes/Df* hermaphrodites are themselves fertile, but produce sterile progeny, suggesting that the Mes phenotype is the loss-of-function and perhaps the null phenotype.

Four of the complementation groups, *mes-1*, *mes-2*, *mes-3* and *mes-4* have been well characterized, and a description of how these grandchildless mutations affect germ-line development is the subject of this paper. The Mes sterile phenotype is expressed in the progeny of homozygous mutant mothers. For the purposes of this paper, the terms “*mes* progeny,” “*mes* embryo” and “*mes* larva” refer to the offspring of a homozygous *mes* mother.

***mes* mutations primarily affect the germ line:** In order to more fully define the Mes phenotype and to determine whether *mes* mutations affect processes in addition to germ-line development, we characterized the strictness of the maternal effect, the expressivity, and the maternal-effect embryonic lethality associated with mutations in the four complementation groups. All *mes-2* and *mes-3* alleles and the three *mes-4* alleles tested are strict maternal-effect mutations: all of the outcross progeny of homozygous *mes* hermaphrodites mated with wild-type males were sterile (Table 2). Thus, maternal expression of these genes is required for normal germ-line development to occur. All *mes-1* alleles are incompletely expressed (see below); therefore, evaluating paternal or zygotic rescue is not a straightforward experiment for this locus. However,

TABLE 3

Expressivity, embryonic lethality and brood size associated with *mes* alleles

| Genotype of mother | Percent sterile progeny ^a | | Percent embryonic lethality of progeny ^a | | Average brood size of mother ^b | |
|----------------------------|--------------------------------------|------------------|---|-----|---|-----|
| | 16° | 25° | 16° | 25° | 16° | 25° |
| Wild-type | 0 | 0 | 1 | 3 | 340 | 249 |
| <i>mes-1</i> | | | | | | |
| <i>bn7ts</i> | 11 | 67 | 3 | 8 | 289 | 118 |
| <i>bn24ts</i> ^c | 8 | 34 | 1 | 4 | 338 | 202 |
| <i>q222ts</i> ^d | 6 | 77 | 1 | 7 | 285 | 130 |
| <i>q367ts</i> ^d | 12 | 79 | 4 | 9 | 274 | 156 |
| <i>mes-2</i> | | | | | | |
| <i>bn11</i> | 100 | 100 | 1 | 6 | 220 | 139 |
| <i>bn27</i> | 100 | 100 | 2 | 5 | 241 | 135 |
| <i>mes-3</i> | | | | | | |
| <i>bn21ts</i> | 2 | 100 | 0.5 | 4 | 313 | 111 |
| <i>bn35</i> | 100 ^e | 100 ^e | 1 | 3 | 186 | 128 |
| <i>bn53</i> | 100 | 100 | 2 | 2 | 200 | 130 |
| <i>mes-4</i> | | | | | | |
| <i>bn23</i> | 100 | 100 | 2 | 4 | 245 | 166 |
| <i>bn50</i> | 100 | 100 | 3 | 1 | 259 | 151 |
| <i>bn67</i> | 100 | 100 | 2 | 3 | 229 | 133 |
| <i>bn58</i> | 100 | 100 | ND ^f | 3 | ND | 195 |
| <i>mes-3(bn21)/sDf4</i> | ND | 98 | ND | 36 | ND | ND |
| <i>+/sDf4</i> | ND | 0 | ND | 29 | ND | ND |

^a >1500 F₁ progeny were scored for each genotype except for *mes-3(bn21)/sDf4* where nine broods were scored and *+/sDf4* where 13 broods were scored.

^b Average brood size of >10 mothers.

^c Full genotype of mother was *lon-2(e678) mes-1(bn24ts)*.

^d Isolated by E. LAMBIE and J. KIMBLE.

^e Fewer than 1% fertile progeny.

^f Not done.

crosses between *lon-2 mes-1(bn7)* hermaphrodites and wild-type males resulted in no significant difference between the percent sterile outcross progeny and the percent sterile self-cross progeny (Table 2), suggesting that neither paternal nor zygotic expression of the wild-type *mes-1* gene rescues the sterility.

All alleles of *mes-2* and *mes-4* are nonconditional and the grandchildless phenotype is fully expressed: homozygous mothers produce 100% sterile progeny (Table 3). One of the *mes-3* alleles, *bn21*, is a conditional mutation that is fully expressed at the restrictive temperature. The other two are non-conditional; *mes-3(bn53)* is fully expressed, while *mes-3(bn35)* mothers occasionally produce a low number of fertile progeny (fewer than 1%). In contrast, all *mes-1* alleles are temperature-sensitive and incompletely expressed.

The germ lineage is not necessary for embryonic viability (SULSTON *et al.* 1983). In order to determine whether the *mes* loci are required for essential embryonic processes, lethality of *mes* embryos was compared to that of wild-type embryos (Table 3). None of the *mes* alleles tested showed embryonic lethality that is significantly different from wild type, indicating that the *mes* gene products do not affect developmental

processes that are essential for the production of viable embryos. In addition, neither *mes-3(bn21)/sDf4* mothers (Table 3) nor *mes-6(bn66)/eDf18* mothers (C. GARVIN and D. BREAZEALE, unpublished results) produced significantly more inviable embryos than *+/Df* controls, suggesting that the null phenotypes for *mes-3* and *mes-6* do not include high levels of maternal-effect embryonic lethality. However, *mes-3(bn21)/sDf4* mothers produced more fertile progeny than *mes-3(bn21)* mothers (Table 3). The significance of this result is unclear, although it has been observed that the genetic background of a *mes-3* strain can affect the expressivity of the Mes phenotype (E. CAPOWSKI, unpublished results).

Although *mes* mutations do not affect embryonic viability, they are associated with some adult phenotypes in addition to the maternal-effect sterility. For all four loci, fertile homozygous *mes* mothers have a reduced average brood size relative to wild-type controls (Table 3). This suggests that the *mes* mutations may affect the germ line of the fertile *mes* mothers as well as the sterile *mes* progeny. In addition, a small percentage of *mes* sterile progeny display secondary phenotypes. *mes-1* and *mes-3* sterile hermaphrodites occasionally show vulval defects: fewer than 4% of *mes-1* sterile offspring have multiple vulvae and fewer than 1% of *mes-3* sterile offspring have protruding vulvae. Also, a small number of *mes-4* sterile progeny exhibit a "sickly" phenotype: the animals appear sluggish and stiff. It is not clear what the relationship is between these minor maternal-effect phenotypes and the Mes phenotype. Overall, based on the analyses described above, it appears that mutations in *mes-1*, *mes-2*, *mes-3* and *mes-4* primarily affect development of the germ line.

Embryonic germ-line development in *mes* progeny: We have assessed embryonic germ-line development mainly by scoring for the presence of the two germ-line progenitor cells, Z2 and Z3, in newly hatched L1 larvae. These two cells are the daughters of the germ-line founder cell, P4, which is generated at the 16- to 24-cell stage of embryogenesis. P4 and its daughters can be visualized by Nomarski or by staining with antibodies to P granules, cytoplasmic organelles that are maternally contributed to the zygote, partitioned to the P4 cell and associated with the germ-line descendants of P4 throughout development (STROME and WOOD 1982). In addition to serving as markers for the germ lineage, P granules serve as an indicator of cytoplasmic partitioning during the early embryonic cleavages.

mes-1 larvae display abnormal P granule-staining patterns indicative of partitioning defects during embryogenesis. Figure 4, c-f, and Table 4 show the range of P granule-staining patterns observed in L1 larvae from *mes-1(bn7ts)* mothers raised at the restrictive

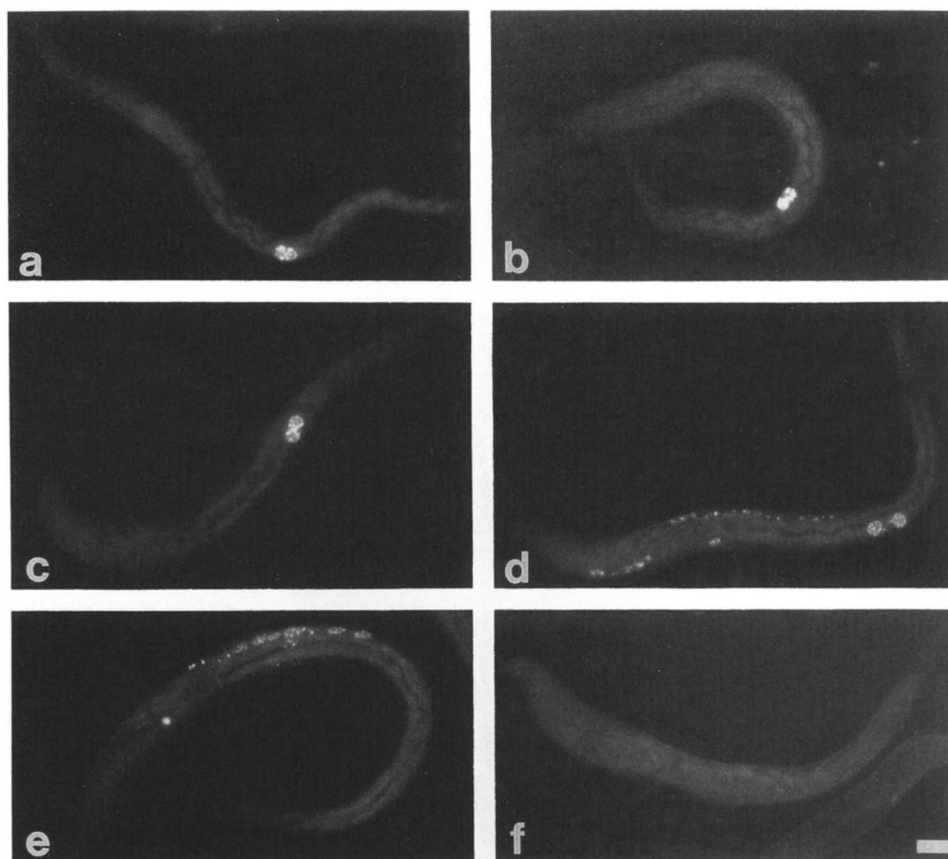


FIGURE 4.—P granule staining of L1 larvae. For all larvae, anterior is left. (a) Wild-type larva showing staining in the germ-line progenitor cells, Z2 and Z3. (b) *mes-2(bn11)* larva with staining in Z2 and Z3. (c) *mes-1(bn7)* larva with staining in Z2 and Z3. (d) *mes-1(bn7)* larva showing staining in Z2 and Z3 and also cells along the anterior body wall. (e) *mes-1(bn7)* larva with staining in cells along the anterior body wall and not in the region of Z2 and Z3. (f) *mes-1(bn7)* larva with no detectable P granule staining. Bar = 10 μ m.

TABLE 4
P granule staining in *mes-1(bn7)* L1 larvae

| Percent fixed and stained larvae with P granule staining in: ^a | | | | |
|---|------------------------|----------------|---------------|---|
| Germ cells only | Germ cells + body wall | Body wall only | No P granules | Percent sterile adult siblings ^b |
| 27 | 19 | 50 | 4 | 62 |

^a 113 larvae were scored.

^b 307 adult hermaphrodites were scored.

temperature. Forty-six percent of the larvae had normal staining in Z2 and Z3 (Figure 4, c and d, Table 4), with a subset of these animals showing additional staining along the anterior body wall (Figure 4d). In half of the animals, no staining was detectable in the region of the germ-line progenitor cells, but other cells along the anterior body wall stained positively (Figure 4e, Table 4). In a small number of animals, no P granule staining was detectable (Figure 4f); these animals costained with an antibody to a pharyngeal antigen (data not shown), indicating that they were permeable to antibody and suggesting that P granules were not present. The *mes-1* larval staining experiment was carried out on populations of L1 larvae, half of which were stained while the other half were allowed to grow to adulthood at the restrictive temperature and scored for fertility or sterility. Sixty-two

percent of the *mes-1* adult hermaphrodite progeny were sterile (Table 4). This is slightly greater than the number of L1s that lacked P granule staining in the region of Z2 and Z3, suggesting that in a small percentage of *mes-1* animals, sterility can occur when P granules are present in Z2 and Z3. Detailed lineage analysis has recently revealed that those *mes-1* L1s that lack P granule staining in the region of Z2 and Z3 lack Z2 and Z3 altogether, and that this is probably due to defects in division and cytoplasmic segregation during embryogenesis (P. MARTIN, J. PAULSEN and S. STROME, manuscript in preparation). Thus, the *mes-1* locus appears to participate in the generation of the germ line during embryogenesis; defects in this process lead to the production of sterile adults.

In contrast, the progeny of *mes-2*, *mes-3* and *mes-4* mutant mothers appear to undergo normal embryonic germ-line development. Mutant L1 larvae contain Z2 and Z3, and those cells stain positively for P granules. Figure 4b shows P-granule staining in a *mes-2(bn11)* L1 larva; *mes-3* and *mes-4* L1 larvae show the identical staining pattern. Staining was carried out with at least three different anti-P granule monoclonal antibodies to test for multiple P granule epitopes; all antibodies tested resulted in P granule-staining patterns identical to wild type. To verify that the normal pattern of P granule staining in L1s reflects normal P granule partitioning during embryogenesis, mutant embryos

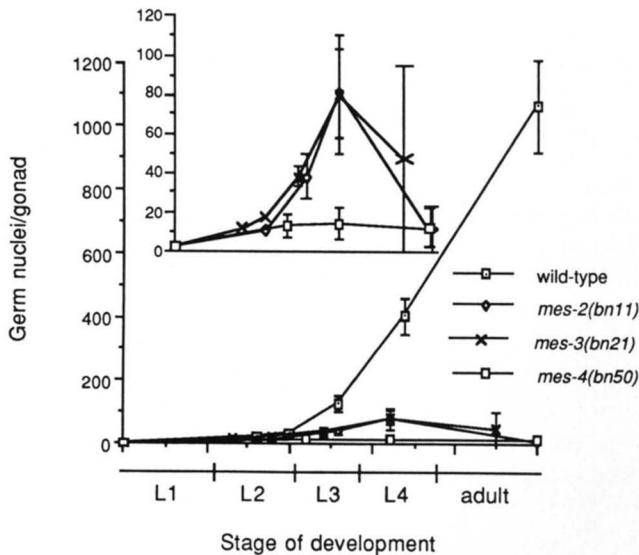


FIGURE 5.—Postembryonic germ-line proliferation in wild-type, *mes-2(bn11)*, *mes-3(bn21)* and *mes-4(bn50)* animals. L1, L2, L3 and L4 are the four larval stages. Inset is an expansion of the bottom tenth of the plot to show the *mes* mutant proliferation patterns.

were examined. They also show normal patterns of P granule staining (data not shown). These results suggest that mutations at the *mes-2*, *mes-3* or *mes-4* loci neither disrupt the structural integrity of P granules nor affect their partitioning to the germ lineage. Furthermore, these maternal-effect mutations do not appear to affect the generation or first division of the germ lineage.

Postembryonic germ-line development in *mes-2*, *mes-3* and *mes-4* progeny: As described above, wild-type larvae hatch with two germ-line progenitor cells, Z2 and Z3. These begin to divide late in the L1 stage and continue to proliferate throughout the three subsequent larval stages, reaching a level of approximately 1000 germ nuclei per gonad in the adult hermaphrodite (for a more detailed description, see KIMBLE and WHITE 1981). *mes-2*, *mes-3* and *mes-4* larvae also hatch with two apparently normal germ-line progenitor cells, which begin to divide late in the L1 stage, but the numbers of germ nuclei do not accumulate to wild-type levels. To assay germ-line proliferation, animals at different stages of development were stained with DAPI or DAPI plus anti-P granule antibodies, and germ nuclei were counted. The data collected for the most severe alleles of *mes-2*, *mes-3* and *mes-4*, along with data from wild-type animals, are plotted in Figure 5. For *mes-4(bn50)* larvae, it appears that only a few rounds of division occur before proliferation ceases; adults have essentially the same number of germ nuclei (12) as mutant L3 larvae. *mes-2(bn11)* larvae exhibit slightly more proliferation than *mes-4(bn50)* animals, accumulating up to 80 germ nuclei per gonad by the L4 larval stage. The average number of germ nuclei then decreases, with adults having on average 9 per gonad. *mes-3(bn21ts)* progeny

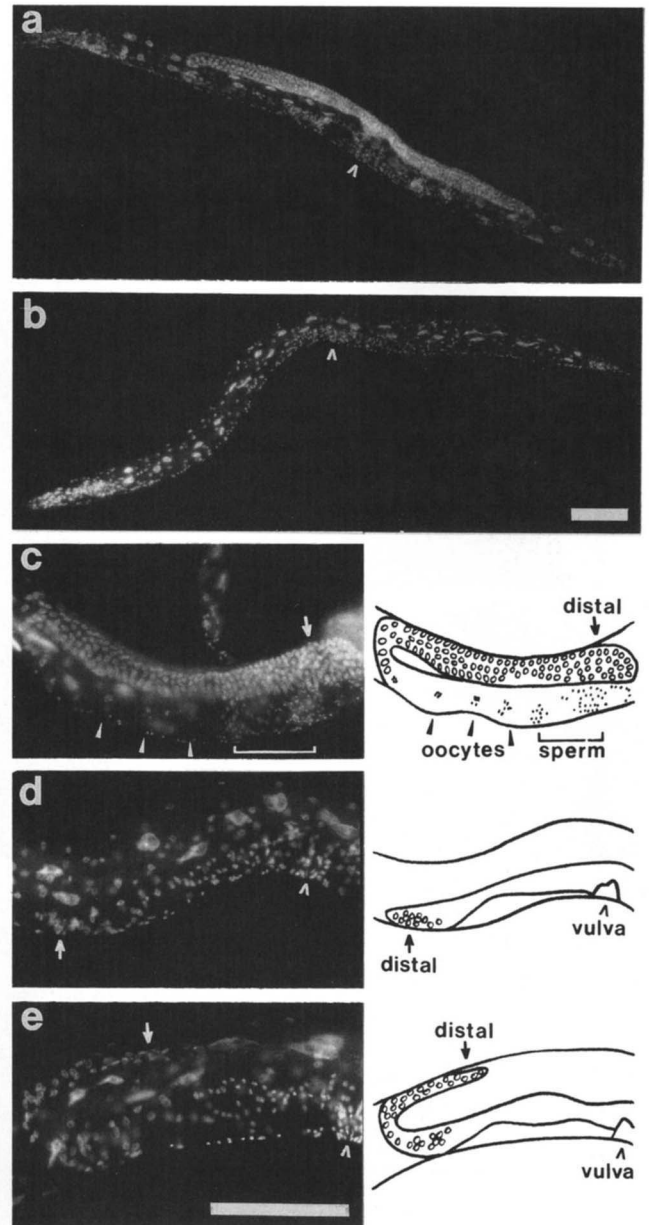


FIGURE 6.—DAPI-stained nuclei in wild-type and *mes* adult hermaphrodites. Anterior is left and ventral is down. The vulva is indicated with a carat (Λ). (a) Wild-type hermaphrodite. (b) Sterile *mes-3(bn21)* hermaphrodite. (c) One gonad arm of a wild-type hermaphrodite. Sperm (bracket), oocytes (arrowheads) and mitotic germ nuclei in the distal tip of the gonad (arrow) are indicated. (d) One gonad arm of a *mes-4(bn50)* hermaphrodite containing a small cluster of germ nuclei at the distal tip of the gonad (arrow). The gonad arm is unreflexed in this animal. (e) One gonad arm of a *mes-3(bn35)* hermaphrodite containing approximately 70 germ nuclei (arrow) and no sperm or oocytes. Cartoons of the germ nuclei within each gonad arm are drawn to the right of panels c–e. Bar = 100 μ m

raised at the restrictive temperature exhibit a very similar phenotype, with the number of germ nuclei also reaching a maximum of 80 germ nuclei per gonad in L4 larvae and dropping to an average of 14 germ nuclei in adults.

Figure 6 shows the adult phenotypes of the three

TABLE 5
Number of germ nuclei in sterile adult hermaphrodites

| Genotype of mother | Germ nuclei per gonad ^a | n |
|----------------------------|------------------------------------|----|
| Wild-type | 1069 ± 150 | 20 |
| <i>mes-2</i> ^b | | |
| <i>bn11</i> | 9 ± 14 | 28 |
| <i>bn27</i> | 4 ± 8 | 31 |
| <i>mes-3</i> | | |
| <i>bn21ts</i> ^c | 14 ± 28 | 12 |
| <i>bn53</i> | 25 ± 37 | 17 |
| <i>bn35</i> | 182 ± 97 | 26 |
| <i>mes-4</i> | | |
| <i>bn23</i> | 15 ± 11 | 22 |
| <i>bn50</i> | 12 ± 10 | 35 |
| <i>bn67</i> | 27 ± 15 | 10 |
| <i>bn58</i> | 162 ± 156 | 22 |

^a Mean ± standard deviation.

^b Full genotype of mother was *unc-4(e120) mes-2*.

^c *bn21* mothers and progeny were raised at the restrictive temperature.

mes loci. Hermaphrodites were fixed and stained with DAPI. In wild-type hermaphrodites, the germ nuclei are organized in two U-shaped gonad arms (Figure 6, a and c). Mitotic nuclei are located at the distal tip of each arm and meiotic nuclei and differentiating gametes are located more proximally (HIRSH, OPPENHEIM and KLASS 1976). The reduction in number of germ nuclei found in *mes* adults is strikingly apparent (Figure 6, b, d and e). None of the sterile hermaphrodites produced gametes, although meiotic figures resembling nuclei in diakinesis have occasionally been seen in sterile progeny of *mes-4(bn58)*, the weakest *mes-4* allele. Table 5 lists numbers of germ nuclei per gonad found in *mes-2*, *mes-3* and *mes-4* sterile adult hermaphrodites. The mutations show differing degrees of severity: *mes* animals bearing more severe alleles produce few or no germ nuclei. Animals carrying weaker alleles produce more germ nuclei, but still show approximately tenfold reductions in number relative to wild-type controls. In all cases, variability among individual animals was observed, as reflected in the large standard deviations. However, this apparently wide variability may reflect small variations in the number of divisions undergone by the germ nuclei.

In conclusion, *mes-2*, *mes-3* and *mes-4* progeny exhibit abnormal germ-line proliferation during larval development and fail to form mature gametes, resulting in sterile adults.

DISCUSSION

By screening for maternal-effect sterile mutations, we have identified six loci whose products are provided maternally and required for normal germ-line development in *C. elegans*. Mutations in these genes appear to affect two different phases of germ-line

development: generation of the germ-line founder cell during embryogenesis and proliferation of the germ line during postembryonic development. All of the *C. elegans* grandchildless mutations appear to primarily affect the germ line and none affects the structural integrity of germ granules. This is in contrast to grandchildless mutations identified in *D. melanogaster*, all of which disrupt germ-granule structure and affect abdominal development as well as germ-line development (see below).

The majority of *mes* mutations, those in *mes-2*, *mes-3* and *mes-4*, affect post-embryonic germ-line proliferation. (Preliminary analysis of *mes-6* indicates that it also belongs in this class of genes; C. GARVIN and D. BREAZEALE, unpublished results.) Early germ-line development in mutant progeny is normal: the germ-line founder cell is generated, divides once during embryogenesis, and begins to proliferate late in the L1 stage. However, accumulation of germ nuclei in *mes* progeny lags behind that found in wild-type animals. *mes-4* mutant animals exhibit the most severe proliferation defect. In *mes-4(bn50)* progeny, the number of germ nuclei does not increase past the number found in mutant L3 larvae. It is not known whether this defect is caused by cessation of nuclear divisions after the L3 stage, or if germ nuclei continue to divide but undergo concomitant degeneration. It appears that nuclear degeneration does occur in the germ line of *mes-2(bn11)* and *mes-3(bn21)* sterile animals, where germ nuclei accumulate until the L4 larval stage, after which the average number decreases. As with *mes-4*, the number of germ nuclei in *mes-2* and *mes-3* sterile adult hermaphrodites may reflect continued division and concomitant degeneration. In all three cases, mitotic figures have not been observed in DAPI-stained later stage animals such as those shown in Figure 6. However, this does not rule out the possibility that a small number of germ nuclei divide infrequently.

The abnormal proliferation seen in *mes-2*, *mes-3* and *mes-4* animals could be due to defects in germ-cell identity or proliferation or differentiation. These could include defects in: (1) specification of germ-cell identity; (2) germ-line maintenance of germ-cell identity; (3) somatic maintenance of germ-cell identity; (4) delivery or functioning of maternal "proliferation factors" in the germ line; (5) somatic regulation of germ-line proliferation; (6) the ability of germ cells to differentiate; (7) somatic regulation of germ-line differentiation. Possibilities one, two, four and six imply that the focus of *mes* gene activity should be in the germ lineage, while possibilities three, five, and seven would suggest a somatic focus of activity. Unfortunately, mosaic analysis cannot be used with maternal-effect mutations to determine in which tissue the gene product is required; maternal-effect genes must be expressed in the mother for normal development of

tissues in the progeny. The availability of molecular probes for each of the *mes* genes might help resolve the issue of where the gene products are located and are likely to function.

No gametes are produced in *mes-2*, *mes-3* or *mes-4* sterile progeny. Possibilities one, two and three described above predict that germ cells cannot undergo gametogenesis because their germ-cell identity is lost. Similarly, possibilities six and seven predict a failure in the ability of the germ cells to initiate or execute gametogenesis. In contrast, according to possibilities four and five, the mutant germ nuclei present may be capable of forming gametes, but fail to do so because there is not sufficient proliferation to allow them to escape the influence of the somatic distal tip cells. The role of the distal tip cell is to maintain a mitotic stem cell population within the germ line, either by promoting mitosis or inhibiting meiosis in nearby germ nuclei (KIMBLE and WHITE 1981). One way to determine whether germ nuclei in sterile *mes* hermaphrodites are capable of gametogenesis is to eliminate the influence of the distal tip cells. This can be done surgically by laser-ablating the distal tip cells or genetically using mutations at the *glp-1* locus. Both approaches cause all of the mitotic germ nuclei to enter meiosis and differentiate into gametes (KIMBLE and WHITE 1981; AUSTIN and KIMBLE 1987). We are currently constructing *mes; glp-1* double mutants and plan to ablate the distal tip cells in *mes* animals to test the capacity of *mes* germ nuclei to differentiate into gametes.

In contrast to *mes-2*, *mes-3* and *mes-4*, progeny of *mes-1* mothers show defects in embryonic germ-line development and fail to generate germ-line progenitor cells. This absence of germ-line cells may be the result of abnormal cytoplasmic partitioning and cleavage asymmetry during the embryonic division that generates the germ-line founder cell (P. MARTIN, J. PAULSEN and S. STROME, manuscript in preparation). Such defects are reminiscent of the defects observed in embryos produced by *par* mutant mothers. Strong alleles of the *par* genes affect partitioning and cleavage asymmetry during the initial divisions of the zygote, those that normally generate essential somatic founder cells (KEMPHUES *et al.* 1988). As a result, strong *par* mutations are maternal-effect embryonic lethal. Weak *par* alleles display an incompletely expressed Mes phenotype, similar to the phenotype of *mes-1*. In fact, the abnormal patterns of P granule staining seen in L1 progeny of *mes-1* mothers are also observed in the progeny of *par-2* mothers carrying the weak allele, *e2030* (S. STROME, unpublished results). This raises the possibility that all six alleles of *mes-1* are weak alleles of a new partitioning-defective, maternal-effect lethal locus. Alternatively, *mes-1* may specifically affect partitioning and division pattern at a later stage of

embryogenesis than the *par* genes, leading to defects in the germ lineage but not in other lineages essential for embryo viability. The latter possibility is favored by the low embryonic lethality associated with *mes-1* mutations and may explain the abnormal P granule-staining patterns in mutant L1s. The extra staining cells in the anterior body wall, shown in Figure 4, d and e, could be descendants of the embryonic founder cell D into which P granules were incorrectly partitioned. Experiments are currently in progress to address these issues.

An intriguing aspect of *mes-1* mutations is that all six alleles exhibit a fully penetrant, incompletely expressed, temperature-sensitive Mes phenotype. If this is the null phenotype, then it suggests that in the absence of wild-type *mes-1* gene product, generation of the germ-line founder cell is a stochastic process that is sensitive to temperature. Maternal provision of wild-type *mes-1* product guarantees the success of the process at both low and high temperature. There are several other loci in *C. elegans* where all mutations, even putative null mutations, are temperature-sensitive. These include two genes involved in nuclear migrations during development, two dauer-formation genes and two sex-determination genes (SULSTON and HORVITZ 1981; GOLDEN and RIDDLE 1984; HODGKIN 1986). These mutations are thought to reveal either an inherently temperature-sensitive developmental process or one that becomes temperature-sensitive in the absence of wild-type gene product. An example of a locus in which all alleles are incompletely expressed is the *Drosophila* grandchildless gene, *tudor* (BOSWELL and MAHOWALD 1985). Mutant animals exhibit 30–50% maternal-effect embryonic lethality, suggesting that the *tudor* gene product, like the *mes-1* gene product, may be involved in a stochastic process.

The Mes phenotype appears to be the loss-of-function phenotype for at least two and perhaps five of the six loci identified in our grandchildless screens. For *mes-3* and *mes-6*, *mes* alleles hemizygous to deficiencies behave similarly to homozygous *mes* alleles, suggesting that maternal-effect sterility may be the null phenotype. For *mes-1*, *mes-2* and *mes-4*, both the frequency of isolation of mutant alleles and the similarity of phenotype displayed by different alleles of each locus suggest that maternal-effect sterility is the loss-of-function phenotype. Assuming that the *mes* alleles are loss-of-function mutations, we have used the frequency of isolation (24/11,000 haploid genomes) and the forward mutation rate for our EMS treatment ($3\text{--}4 \times 10^{-4}$ mutations/locus/haploid genome) to estimate that there are six to eight *mes* loci in the *C. elegans* genome. Although these calculations assume that maternal-effect genes involved in germ-line development are equally mutable and that there is little or no functional redundancy, they suggest that

we may have identified the majority of loci whose products are provided maternally and required primarily or solely for normal germ-line development.

Drosophila is the only other organism in which grandchildless mutations have been isolated. The grandchildless mutants in *Drosophila* produce embryos that lack detectable polar granules, and at least one of the grandchildless genes, *vasa*, encodes a polar-granule component (BOSWELL and MAHOWALD 1985; SCHÜPBACH and WIESCHAUS 1986; LEHMANN and NÜSSLEIN-VOLHARD 1986; HAY *et al.* 1988). In contrast, none of the *mes* mutations in *C. elegans* appears to disrupt the structural integrity of P granules. Thus, if any of the *mes* genes do encode P granule components, they are not required for granule assembly and stability. If none of the identified *mes* genes encode P granule components, and if the genome is close to saturation for this class of mutant, then our results suggest that either P granules do not participate in maternal control of germ-line development in *C. elegans* or they function in an additional process. When we screen for *mes* mutations, we screen for sterile animals in the F₃ generation. Thus, we might not recover mutations in P granule components as *mes* alleles if they affect the fertility of the F₂ homozygous mutant worms or the viability of their progeny. Most of the *Drosophila* grandchildless mutations were isolated in embryonic lethal screens and all affect embryonic viability as well as the structural integrity of polar granules and formation of the germ line. We have taken advantage of the hermaphrodite genetics of the nematode and the ease of visualizing sterile animals to isolate the pure grandchildless mutations described in this paper. Further analysis of the *mes* loci should shed light on how germ-cell fate is specified and expressed in *C. elegans*.

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