Transformation of the germ line into muscle in *mes-1* mutant embryos of *C. elegans*

Susan Strome¹, Paula Martin¹*, Einhard Schierenberg² and Janet Paulsen¹†

¹Department of Biology and Institute for Molecular and Cellular Biology, Indiana University, Bloomington, IN 47405, USA
²Zoologisches Institut der Universität Köln, 50923 Köln, Germany

*Present address: Institute of Molecular Biology and Department of Biology, University of Oregon, Eugene, OR 97403, USA
†Present address: Genetics Institute, 87 CambridgePark Drive, Cambridge, MA 02140, USA

**SUMMARY**

Mutations in the maternal-effect sterile gene *mes-1* cause the offspring of homozygous mutant mothers to develop into sterile adults. Lineage analysis revealed that mutant offspring are sterile because they fail to form primordial germ cells during embryogenesis. In wild-type embryos, the primordial germ cell P₄ is generated via a series of four unequal stem-cell divisions of the zygote. *mes-1* embryos display a premature and progressive loss of polarity in these divisions: P₀ and P₁ undergo apparently normal unequal divisions and cytoplasmic partitioning, but P₂ (in some embryos) and P₃ (in most embryos) display defects in cleavage asymmetry and fail to partition lineage-specific components to only one daughter cell. As an apparent consequence of these defects, P₄ is transformed into a muscle precursor, like its somatic sister cell D, and generates up to 20 body muscle cells instead of germ cells. Our results show that the wild-type *mes-1* gene participates in promoting unequal germ-line divisions and asymmetric partitioning events and thus the determination of cell fate in early *C. elegans* embryos.

Key words: *C. elegans*, *mes-1*, germ line, cell fate, sterility

**INTRODUCTION**

Our understanding of how cell fates are specified in early embryos has grown considerably in the last few years, in large part due to the wealth of information and insights coming from analysis of maternal-effect mutations that affect early development. Such analysis has revealed that early development of *C. elegans* embryos is guided both by differentially partitioned maternal factors (Bowerman et al., 1992, 1993) and by cell-cell signalling (Priess and Thomson, 1987; Priess et al., 1987; Mello et al., 1994; Hutter and Schnabel, 1994; Schnabel, 1994). One graphic example of asymmetrically partitioned maternal factors is P granules, cytoplasmic structures that are delivered via a series of localization events to the primordial germ cell P₄ (Strome and Wood, 1982; Wolf et al., 1983). The best studied molecule involved in cell-cell signalling is the putative receptor GLP-1 (Yochem and Greenwald, 1989). This protein also displays differential localization, via translational control, which restricts GLP-1-mediated signalling events to certain blastomeres (Evans et al., 1994).

Generating cells of the correct sizes, in the correct positions, and with the correct contents requires the precise control of spindle placement and the proper localization of cytoplasmic components relative to the spindle (reviewed by Strome, 1993). In *C. elegans* embryos the machinery responsible for controlling spindle position and localizing certain components includes the microfilament cytoskeleton (Strome and Wood, 1983; Hill and Strome, 1988, 1990) and the products of the *par* (for partitioning defective) genes (Kemphues et al., 1988). Maternal-effect mutations in the *par* genes abolish the asymmetry of the early embryonic divisions and of at least some partitioning events. These defects result in the absence of some tissues, most notably intestine and germ line, and generally result in embryonic lethality (Kemphues et al., 1988; Morton et al., 1992).

Our interest is in how the germ line is specified in early *C. elegans* embryos. The primordial germ cell P₄ is generated by a series of four unequal stem-cell divisions of the zygote (Fig. 1) (Deppe et al., 1978; Sulston et al., 1983). Maternally contributed P granules are partitioned to the smaller daughter at each of the unequal divisions, come to reside in P₄, and remain associated with the germ nuclei throughout development (Strome and Wood, 1982). Although P granules serve as useful markers of the germ lineage, their composition and role(s) in germ-line development are not known. The analogous granules in *Drosophila*, termed polar granules, are much better understood and are clearly involved in formation of the germ line in early embryos (reviewed by Lehmann, 1992; Wilson and MacDonald, 1993). Some of the components of *Drosophila* polar granules are encoded by members of the ‘posterior group’ genes (Hay et al., 1988; Bardsley et al., 1993). Mutations in these genes result in failure to form polar granules and to form germ-line progenitor cells (Boswell and Mahowald, 1985; Lehmann and Nusslein-Volhard, 1986; Schupbach and Wieschaus, 1986). The current model of *Drosophila* polar granules is that they serve as scaffolds for...
delivered as yet unidentified germ-line determinants to the posterior pole, where primordial germ cells normally form. The granules also carry the abdominal determinant encoded by \textit{nanos}, and thus mutations in polar-granule genes result in maternal-effect embryonic lethality (Ephrussi et al., 1991; Lehmann and Nusslein-Volhard, 1991; Wang and Lehmann, 1991).

To understand better how formation of the germ line is controlled in \textit{C. elegans}, we screened for maternal-effect mutations that result in defects in specification or development of the germ line. By screening for maternal-effect sterile (\textit{mes}) mutants, we identified five genes whose products are maternally supplied and appear to be required solely for normal development of the germ lineage (Capowski et al., 1991). One of these genes, \textit{mes-1}, participates in generation of the primordial germ cell in the early embryo. The other four \textit{mes} genes do not appear to be required to establish the germ lineage during embryogenesis, but instead are required for normal post-embryonic development of the germ line. This paper presents a detailed analysis of the \textit{mes-1} mutant phenotype and evidence that in \textit{mes-1} embryos the primordial germ cell \textit{P4} is transformed into a muscle precursor, like its somatic sister cell \textit{D}. This cell-fate transformation apparently results from loss of polarity and defective cytoplasmic partitioning during the division that generates \textit{P4} and \textit{D}.

**MATERIALS AND METHODS**

**Strains and alleles**

The general maintenance and genetic analysis of \textit{C. elegans} strains were performed as described in Brenner (1974). The wild-type parent of all strains used was \textit{N2}, variety Bristol. The following \textit{X} chromosome markers were used: \textit{lon-2(e678)}, \textit{egl-15(n484)}, \textit{sma-5(n678)}, and the chromosome \textit{X} deficiency \textit{nDf19}, which is balanced by the reciprocal translocation \textit{scT1(LX)} (Fodor and Deak, 1985; McKim et al., 1988). Some strains were provided by the Caenorhabditis Genetics Center. The strain MT3353 \textit{egl-15(n484) sma-5(n678)} was a gift from Roger Hoskins. The transgenic strain PD56 containing the \textit{unc-54::lacZ} construct (Okkema et al., 1993) was a gift from Andy Fire.

**Genetic analysis**

The isolation of the EMS-induced alleles \textit{bn7}, \textit{bn24}, \textit{bn52}, and \textit{bn56} was previously described by Capowski et al. (1991). The EMS-induced alleles \textit{q222} and \textit{q367} were isolated by Eric Lambie and Judith Kimble. The alleles \textit{bn74}, \textit{bn84}, \textit{bn89}, and \textit{bn90} were induced by gamma radiation using the screen previously described by Capowski et al. (1991) with the following modifications: 137Cs was used as the mutagen at doses of 8-11 krad, and \textit{N2} was used as the parental strain. Complementation analysis was performed as previously described by Capowski et al. (1991).

The mapping of the \textit{mes-1} alleles \textit{bn7}, \textit{bn24}, \textit{bn52}, and \textit{bn56} was described by Capowski et al. (1991). The \textit{mes-1} alleles \textit{bn74}, \textit{bn84}, \textit{bn89}, and \textit{bn90} were placed between \textit{egl-15} and \textit{sma-5} by 3-factor mapping. The 3-factor analysis was carried out by placing onto individual plates \textit{Egl} non-\textit{Sma} recombinant progeny from \textit{egl-15 + sma-5} / + \textit{mes-1} + hermaphrodites and scoring for the presence of sterile progeny in the \textit{F3} generation, indicating the presence of the \textit{mes-1} allele. Twenty-eight/32 \textit{Egl} non-\textit{Sma} recombinants picked up the \textit{bn74} allele. The number of \textit{Egl} non-\textit{Sma} recombinants that picked up \textit{bn84}, \textit{bn89}, and \textit{bn90} were 59/69, 33/36, and 33/36, respectively.

\textit{mes-1/Df19} hermaphrodites were constructed by mating \textit{lon-2 mes-1/bn7 or bn52} males to hermaphrodites of the following genotype: / + / scT1 I; \textit{Df19} / + x \textit{scT1 [lon-2]X}. Non-Lon \textit{F1} \textit{L4} hermaphrodites were placed on individual plates at either 16°C or 25°C, and their broods were scored for dead embryos and for sterile vs. fertile progeny.

\textit{mes-1} hermaphrodites containing an \textit{unc-54::lacZ} fusion construction were generated by mating \textit{mes-1}(/\textit{bn7} or \textit{bn84}) males with hermaphrodites from the transgenic strain PD56. PD56 worms are \textit{tra-3(e1107am)}, but the \textit{tra-3} mutation is suppressed by an integrated copy of the construct pPD18.42, which contains the amber suppressor \textit{sup-7} and the \textit{E. coli lacZ} gene with a nuclear localization signal and under the control of the muscle-specific \textit{unc-54} promoter (Okkema et al., 1993). \textit{F1} hermaphrodites from the cross were placed on individual plates at 16°C. \textit{mes-1; sup-7 unc-54::lacZ} \textit{F2} hermaphrodites were identified by their production of approximately 70% sterile progeny at 25°C and of 100% progeny containing \textit{β}-galactosidase activity in their muscle cells. The strain was maintained at 20°C.

Embryonic lethality and expressivity of the maternal-effect sterile phenotype were determined as previously described by Capowski et al. (1991).

**Determination of the temperature-sensitive period**

For upshift experiments, embryos were dissected out of gravid \textit{mes-1} hermaphrodites that had been raised at the permissive temperature, 16°C. Embryos were transferred to the restrictive temperature, 25°C, at the one-, two-, and four-cell stages. To shift later stage embryos, two-cell embryos were maintained at 16°C for an additional 1 or 2 hours and then shifted to 25°C. The number of cells in these embryos at the time of the upshift was determined by fixing and staining some of each set of embryos with diaminobenzidine (DABP; see below for fixation) and counting DABP-stained nuclei. To shift newly hatched larvae, unstaged embryos were maintained at 16°C overnight, and hatched \textit{L1s} were shifted to 25°C. For downshift experiments, embryos were dissected out of \textit{mes-1} hermaphrodites that had been raised at 25°C. The stages at which embryos were shifted down to 16°C were one-cell, two-cell, four-cell, four-cell + 30 minutes, and four-cell + 60 minutes. Shifted embryos and larvae were grown to adulthood and scored for fertility vs. sterility.

**Microscopy**

Light microscopy was performed with a Zeiss Axioscope equipped with Nomarski differential interference contrast and epifluorescence optics. Photographs were taken on Kodak Tri-X or Plus-X film and developed in Diafine Two-Bath Developer (Acufine, Inc.). Confocal microscopy was performed with a Biorad MRC600 system attached to a Nikon Optiphot. Photographs were taken using a video graphic printer (Sony).

**Lineage analysis**

The complete embryonic lineage of \textit{C. elegans} is described by Sulston et al. (1983). To examine the early lineage of \textit{mes-1} embryos, typically three embryos were dissected from \textit{mes-1} mothers and mounted on an agar pad for analysis at 23°C, as described by Bossinger and Schierenberg (1992). Most to all of the blastomere divisions could be followed up to the 28-cell stage, after which \textit{P4} and \textit{D} and their descendants were preferentially monitored. The embryos were retrieved from the agar pads and transferred to individual plates. After hatchng, the \textit{L1s} were mounted for viewing of the gonad primordium and scoring for the presence or absence of the primordial germ cells \textit{Z2} and \textit{Z3}. The \textit{L1s} were transferred to individual plates, grown to adulthood, and scored for sterility versus fertility.

**Blastomere nomenclature**

In wild-type embryos, \textit{P4} is adjacent to the Ep blastomere, is smaller than its sister cell \textit{D}, and has a longer cell cycle than \textit{D}. In \textit{mes-1} embryos, we refer to the blastomere closest to \textit{Ep} as \textit{P4}, and indeed this \textit{P4} cell is usually somewhat smaller than and has a longer cell cycle than its sister cell. However, in some \textit{mes-1} embryos in which
the P₃ division has a pronounced left-right orientation, P₄ and D cannot be unambiguously identified. These less certain embryos were included in the lineage analysis (Fig. 3); a potential mis-naming of P₄ and D does not alter interpretation of the lineage results. Such embryos were not included in the laser ablation experiment (Table 3) in which confidence in blastomere identity was required.

Immunofluorescent staining

Embryos and larvae were fixed and processed for indirect immunofluorescence microscopy as described by Hill and Strome (1990). For single label experiments, the mouse monoclonal antibody OIC1D4 (IgG) (Strome, 1986) was used to stain P granules, and the mouse monoclonal antibody 5-6 (IgG; kindly provided by David Miller) (Miller et al., 1983) was used to stain myosin heavy chain. For co-staining of P granules and myosin heavy chain, the mouse monoclonal anti-P-granule antibody K76 (IgM) (Strome and Wood, 1983) was used in combination with the 5-6 anti-myosin antibody. K76 is selectively recognized by rhodamine-conjugated anti-mouse IgM (mu-chain-specific) secondary antibody (U.S. Biochemicals). Both K76 and 5-6 are recognized by fluorescein-conjugated anti-mouse IgG (heavy and light chain-specific) secondary antibody (U.S. Biochemicals).

Staining of β-galactosidase activity in transgenic worms

Transgenic adults containing the unc-54::lacZ construct (Okkema et al., 1993) were processed for staining of β-galactosidase activity as follows. Worms were picked into a small drop of water on a gelatin-coated slide and allowed to dry. The slides were placed in acetone at room temperature for 5 minutes and allowed to dry. Worms were stained with 30-100 μl of 0.2 M sodium phosphate pH 7.5, 1 mM MgCl₂, 5 mM K₂Fe(CN)₆, 5 mM K₃Fe(CN)₆, 4 μg/ml SDS, 24 μg/ml X-gal (Fire et al., 1990) for 1-2 hours at room temperature. After the staining solution was wipped off the samples, a coverslip with a drop of mounting fluid containing Gelvatol (Monsanto) was placed over the stained worms. Counting of muscle nuclei was done only in ‘optimally stained’ worms, in which the small body muscle nuclei in the head were well stained and ‘countable’, and in which there were no ambiguities in muscle counts. The only exception to the last criterion is that the anal depressor muscle, which is small and usually stains very weakly, may have been included in some of the body muscle counts.

Laser ablation of embryonic blastomeres

The nuclei of embryonic cells were destroyed by the laser microbeam method of Sulston and White (1980), using a VSL-337 laser (Laser Science, Inc.) and coumarin dye (Avery and Horvitz, 1987). The laser-to-microscope adapter (Laser Science, Inc.) was attached to a dual lamp house (Zeiss) on a Zeiss Axioscope. The intensity of the laser beam was controlled by varying the opening of the attenuator slot in the laser module. Embryos were obtained from gravid hermaphrodites, and typically 5-7 one- to two-cell embryos were mounted on an agar pad, as described in the lineage section. The laser beam was focused on the nucleus of selected blastomeres, and nuclei were hit with approximately 30-60 pulses of laser light until substantial debris accumulated (Bowerman et al., 1992). The last blastomere to be ablated in each operated embryo was either P₄ or D. In mes-1 embryos, this last ablation was performed only when the orientation of the P₃ division was such that P₄ and D could be unambiguously identified. The embryos were allowed to develop overnight and examined by Nomarski optics to verify that blastomeres had not lysed and to look for contracting muscle cells. Any embryos that had not been completely operated on or in which cell lysis caused embryo death were destroyed by using the laser to burn a hole in the eggshell. Embryos were retrieved and fixed and stained with anti-myosin antibody, as described in the immunofluorescent staining section.

Alleles analyzed

As indicated in Table 1, no single mutant allele of mes-1 appears to be significantly more severe than the others. We report the results of analysis of the bn7 allele. In addition, most experiments were performed with at least one additional mes-1 allele, and similar results were obtained for that allele and bn7.

RESULTS

Summary of germ-line development in wild-type animals

The germ line is continuous throughout the life cycle (see stippling in Fig. 1) and is set apart from the somatic lineages during early embryogenesis (Deppe et al., 1978; Sulston et al., 1983). The 1-cell embryo P₀ divides unequally to generate a large somatic founder cell and a smaller germ-line cell P₁. P₁ reiterates this unequal cleavage pattern, as do the germ-line daughter cells P₂ and P₃. The final P-cell daughter P₄ is the primordial germ cell. P₄ divides equally into Z₂ and Z₃ at about the 100-cell stage. Z₂ and Z₃ undergo extensive postembryonic proliferation during the four larval stages (L1-L4), generating the approximately 2000 germ cells in the adult hermaphrodite. In hermaphrodites the first germ cells to enter meiosis (during the L₄ stage) differentiate into sperm, and later germ cells differentiate into oocytes.

Mutations in mes-1 result in a maternal-effect sterile phenotype

The mes-1 gene was identified in screens for mutations in maternal components required for germ-line development in C. elegans. Such mutations confer a maternal-effect sterile (mes) or ‘grandchildless’ phenotype: homozygous mutants produced

<table>
<thead>
<tr>
<th>Allele</th>
<th>% sterile progeny*</th>
<th>% embryonic lethality of progeny*</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild-type</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>mes-1(bn7)</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>mes-1(bn24)</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>mes-1(bn32)</td>
<td>47</td>
<td>12</td>
</tr>
<tr>
<td>mes-1(bn56)</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>mes-1(q222)</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>mes-1(q367)</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>mes-1(bn74)</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>mes-1(bn84)</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>mes-1(bn7) lon-2/nDf19</td>
<td>17</td>
<td>35</td>
</tr>
<tr>
<td>mes-1(bn2) lon-2/nDf19</td>
<td>29</td>
<td>33</td>
</tr>
<tr>
<td>n.d. n.d.</td>
<td>25</td>
<td>21</td>
</tr>
</tbody>
</table>

*The number of F₁ progeny scored ranged from 687 to 5006.

†Isolated by E. Lambie and J. Kimble.

‡These values are taken from a separate experiment (Capowski et al., 1991) in which embryonic lethality was generally lower.

§Two additional alleles induced by gamma radiation, bn89 and bn90, also display temperature-sensitive and incompletely expressed maternal-effect sterility, but % sterile progeny and % embryonic lethality were not quantitated for those alleles.

¶The deficiency nDf19 removes mes-1 and other loci. Homozygous nDf19 embryos are lethal and account for approximately 25% of the embryonic lethality seen in mes-1/nDf19 strains.

n.d. = not done.
by heterozygous mothers are themselves fertile, but produce sterile progeny (Capowski et al., 1991). Six of the \textit{mes-1} mutations were induced by EMS mutagenesis, and four were induced by gamma radiation.

An unusual feature of \textit{mes-1} is that all 10 mutant alleles are both temperature-sensitive and incompletely expressed: homozygous mutant mothers produce a low percentage of sterile progeny at the permissive temperature (16°C) and a high percentage of sterile progeny at the restrictive temperature (25°C) (Table 1). Mutant mothers also produce a low percentage of inviable embryos at the permissive temperature and a somewhat elevated percentage at the restrictive temperature (Table 1). The temperature-sensitive and partially expressed Mes phenotype appears to be the null phenotype for \textit{mes-1}, based on two lines of evidence. (1) \textit{mes-1}/deficiency hemizygous hermaphrodites do not display a more severe phenotype than homozygous \textit{mes-1} hermaphrodites (Table 1; the \textit{mes-1}/deficiency values for embryonic lethality include the approximately 25% embryos that die from being homozygous for the deficiency), indicating that \textit{mes-1} mutations behave similarly to a deficiency of the region. (2) Transformation rescue experiments have delimited the \textit{mes-1}-containing region to 17 kb, and the \textit{bn74} allele is a 20-25 kb deletion that lacks the entire 17 kb of rescuing genomic DNA (Laura Berkowitz and S.S., unpublished result). Thus, the temperature sensitivity of all ten alleles of \textit{mes-1} suggests that the gene participates in an inherently temperature-sensitive process.

The temperature-sensitive period for \textit{mes-1} is during early embryogenesis

Because we think that \textit{mes-1} participates in a temperature-sensitive process, we expected the TSP for \textit{mes-1} to indicate when that process is occurring. To determine the TSP for \textit{mes-1}, embryos obtained from mutant mothers raised at either 16°C or 25°C were shifted to the opposite temperature at different stages of early development. Based on the results of both the upshift and downshift experiments, the TSP for \textit{mes-1} extends from the 1-cell to the 24- to 28-cell stage of embryogenesis (Fig. 2). This is when the embryo is undergoing the series of unequal divisions that set the germ lineage apart from the somatic lineages. The TSP suggests that the \textit{mes-1} gene product participates in controlling the divisions that ultimately form the primordial germ cell.

\textit{mes-1} mutant embryos display defects in early cell divisions and fail to generate the primordial germ cell \textit{P}_4

To determine the embryonic defects that lead to sterility, we compared the early cell lineage of embryos produced by \textit{mes-1} mutant mothers at restrictive temperature to the lineage of wild-type embryos. Fig. 3 shows the relative order of cell divisions of three wild-type embryos (leftmost column) and eight \textit{mes-1} embryos (rightmost three columns) that were...
monitored from the 2- to 4-cell stage through the 100- to 120-cell stage (when the two D cells divide into four), scored for the presence of the germ-line cells Z2 and Z3 after the embryos hatched (Fig. 4), and scored for fertility vs. sterility as adults.

In all eight mes-1 embryos shown in the figure, as well as in many other partially lineaged mes-1 embryos, the timing of divisions and sizes of blastomeres appeared normal up until the 7-cell stage and after that differed from wild-type embryos in the following ways. (1) The divisions of P2 and P3 did not display the pronounced asymmetry seen in wild-type embryos. This was especially apparent in the division of P3. Both daughter cells of P3 usually looked similar in size when the cleavage furrow began to form, but by the end of cytokinesis one cell usually appeared somewhat smaller. (2) The orientation of the P3 division was often skewed in mes-1 embryos, resulting in daughter cells positioned more along the left-right axis than in wild type. (3) P4 divided precociously in most mes-1 embryos. (4) P4 underwent at least one extra round of division in many mes-1 embryos. (See Materials and Methods for explanation of blastomere nomenclature in mes-1 embryos.)

The four mes-1 embryos shown in the second column in Fig. 3 illustrate several of the above points. P4 divided at nearly the same time as its sister cell D (at approximately the 50-cell stage), and then the two P4 descendants divided again approximately when the D descendants divided from two to four cells. The L1s that resulted from these four embryos lacked Z2 and Z3 (Fig. 4B) and developed into sterile adults. Thus, sterility of mes-1 animals appears to be due to defects in the divisions that normally generate the primordial germ cell P4.

The three mes-1 embryos shown in the third column of Fig. 3 displayed fairly normal P3 behavior. Although the division

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**Fig. 2.** The temperature-sensitive period for mes-1 is during early embryogenesis. Open circles indicate the percentage of fertile adults obtained from mes-1(bn7) embryos that were shifted from the restrictive (25°C) to permissive (16°C) temperature at the stages indicated (see Materials and Methods for details). Closed diamonds indicate the percentage of fertile adults obtained after shifting mes-1(bn7) embryos from permissive to restrictive temperature at the stages indicated. A minimum of 23 adults were scored for each time point. Embryos grown continuously at 16°C generated 87% fertile adults and embryos grown continuously at 25°C generated 29% fertile adults, as indicated by the arrows to the right of the figure.

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**Fig. 3.** Sequence of cell divisions in wild-type and mes-1 embryos. The scale on the left indicates time in minutes (at 23°C) after division of EMS; the earlier sequence of divisions is normal in mes-1 embryos (not shown). Rounds of divisions within a lineage are indicated by the lineage name preceded by the number of cells in the lineage that were dividing at the indicated time. The two rounds of AB division in parentheses were not observed, but presumably occurred. The leftmost column shows the sequence of cell divisions in wild-type embryos up until the division of the D descendants from two to four cells, and indicates the presence of Z2 and Z3 in L1s and the development of these animals into fertile adults. The rightmost three columns show three lineage patterns observed in mes-1(bn7) embryos (see Materials and Methods for explanation of blastomere nomenclature in mes-1 embryos). In four mes-1 embryos, P4 divided precociously (at about the same time as D) and underwent at least one extra round of division (0-18 minutes after the 2D division); the resulting larvae lacked Z2 and Z3 and developed into sterile adults. In three mes-1 embryos, P4 divided on a fairly normal schedule (after D and only once during the period of observation); the resulting L1s contained Z2 and Z3 and developed into fertile adults. In one mes-1 embryo, P4 divided somewhat precociously and underwent an extra round of division at 125 minutes; the resulting larva contained Z2 and Z3 and developed into a fertile adult. Lines between the columns highlight the similar timing of the division of P3 and the differences in timing of the P4 division in different embryos.
of P₄ into Z₂ and Z₃ occurred somewhat earlier than in wild-type embryos, Z₂ and Z₃ did not divide during the approximately 2 hours that they were observed. The L₁s that hatched from these three embryos contained normal appearing Z₂ and Z₃ cells (Fig. 4A) and developed into fertile adults. Thus, fertile mes-I adults arise from embryos that undergo an apparently normal lineage.

One mes-I embryo (fourth column of Fig. 3) displayed an unusual lineage. The division of P₄ was precocious, and the P₄ descendants divided from two to four cells approximately 20 minutes after the corresponding division of the D cells. However, the resulting L₁ contained apparently normal Z₂ and Z₃ cells and developed into a fertile adult. This embryo illustrates one type of hybrid behavior that P₄ can display: dividing more like D than P₄, but generating germ cells like P₄ normally does.

Our lineage analysis also revealed defects that lead to the low percentage embryonic lethality observed in the progeny of mes-I mothers. Several of the mes-I embryos whose early lineage we had observed died, apparently from defective development of the C lineage, which in wild-type embryos generates the hypodermal cells that cover the posterior of the embryo (Sulston et al., 1983). These mes-I embryos arrested as late stage embryos or early L₁s that lacked the normal hypodermal covering of the posterior end (Fig. 4C). The first two divisions of the C lineage were delayed relative to other lineages in two mes-I embryos and occurred at the normal time in two other embryos (data not shown). Because the percentage of inviable embryos produced by mes-I mothers is relatively low (7-16%; see Table 1), we have focused in this paper on analyzing the defects that lead to the predominant phenotype, maternal-effect sterility.

mes-I embryos display defects in cytoplasmic partitioning

Germ-line-specific P granules are normally partitioned to the small P-cell daughter at each of the asymmetric divisions of the P lineage (Fig. 5B,D) (Strome and Wood, 1982, 1983). This requires the coordinate regulation of P-granule segregation and spindle placement - P granules must be segregated along the axis defined by the orientation of the spindle and to the pole of the spindle destined for the small P-cell daughter. The reduction or loss of asymmetry observed in the divisions of P₂ and P₃ in mes-I embryos prompted us to analyze the patterns of P-granule segregation in mutant embryos. We observed that P granules were correctly partitioned to P₁ and to P₂ in all 2- to 7-cell embryos examined (Fig. 5F and Table 2). However, many later stage embryos showed evidence of mis-partitioning (Fig. 5H,J,L and Table 2). Most 8- to 15-cell embryos contained P granules in only P₃ (as seen in wild type), although some embryos contained P granules in both P₃ and C (Fig. 5H) or in only C. The frequency of mis-partitioning was higher at the division of P₃ into P₄ and D: the majority of 16- to 46-cell embryos examined contained P granules in both P₄ and D (Fig. 5J and Table 2). Thus, as with the division defects, defects in P-granule partitioning appear to be confined to the last two of

Table 2. P-granule distributions in mes-I embryos and L₁s

<table>
<thead>
<tr>
<th>Stage*</th>
<th>n</th>
<th>Cell(s) with P granules†</th>
<th>% of animals with each P-granule distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryos</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2- to 3-cell</td>
<td>27</td>
<td>P₁</td>
<td>100</td>
</tr>
<tr>
<td>4- to 7-cell</td>
<td>44</td>
<td>P₂</td>
<td>100</td>
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<tr>
<td>8- to 15-cell</td>
<td>48</td>
<td>P₃</td>
<td>71</td>
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<td></td>
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<td>P₃ + C</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>8</td>
</tr>
<tr>
<td>16- to 46-cell</td>
<td>51</td>
<td>P₄</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P₄ + D</td>
<td>80</td>
</tr>
<tr>
<td></td>
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<td>D</td>
<td>2</td>
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<tr>
<td>L₁s</td>
<td>113‡</td>
<td>Z₂ + Z₃</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Z₂ + Z₃ + ectopic</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ectopic</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>none</td>
<td>4</td>
</tr>
</tbody>
</table>

*Embryos and larvae were obtained from mes-I(bn7) mothers raised at 25°C. Embryonic stages were assessed by counting DAPI-stained nuclei.
†The cell(s) containing P granules was identified by its position in a DAPI-stained embryo, and by assessing the cell-cycle stage of that cell and others in the embryo (see Fig. 5 for examples). All of the wild-type embryos examined contained P granules in only the P cell (n=32 for P₁, n=58 for P₂, n=78 for P₃, and n=117 for P₄).
‡The values for L₁s are from Capowski et al. (1991).

n = number of embryos and L₁s analyzed.
the four asymmetric divisions of the P lineage and are observed more frequently in the last asymmetric division.

A clue to how P granules become distributed to both P3 and C and to both P4 and D in mes-1 embryos came from examining P2 and P3 cells during metaphase and anaphase. We observed many examples of mitotic P2 and P3 cells with P granules partitioned to the side of the metaphase plate and therefore to the side of the spindle. This configuration of P granules relative to the spindle would probably result in their distribution to both daughter cells. (Fig. 5F) mes-1(bn7) embryo at the 15-cell stage. P granules are in both P3 (posterior-most cell) and C (cell in anaphase). (I,J) mes-1(bn7) embryo at the 24-cell stage. P granules are in both P4 and D. (K,L) mes-1(bn7) embryo with >100 cells. P granules are in Z2 and Z3 (two bright cells in center) and probably cells of the D lineage. Bar, 10 μm.

Thus, in mutant embryos P granules appear to be partitioned to a restricted region of P2 and P3, but there is loss of the coordinate regulation between spindle orientation and P-granule partitioning. As a result, both daughter cells (P3 and C, and P4 and D) in mes-1 embryos often inherit what is normally partitioned to P3 and then P4 in wild-type embryos.

During the partitioning of P granules to P3 and P4 in wild-type embryos, some P granules are occasionally distributed to C and D, but granules are not observed in the daughters of C or D (Strome and Wood, 1983). This suggests that P granules are stable in germ-cell cytoplasm but not in somatic-cell cytoplasm. To determine whether mis-partitioned P granules disappear in the C- and D-derived cells in mes-1 embryos, we fixed and immunostained mutant L1s produced at 25°C. In addition to larvae with the normal pattern of P-granule staining of Z2 and Z3 (see Fig. 6A), some larvae displayed staining of Z2/Z3 and additional cells along the anterior body wall (see Fig. 6C), and many larvae showed staining of cells along the anterior body wall but lacked staining in the region of Z2/Z3 (Table 2 and Capowski et al., 1991). We assume that the 27% of L1s with detectable P granules only in Z2 and Z3 correspond to the 18% of embryos with P granules only in P4. The 69% of L1s with ectopic P-granule staining probably correspond to the 82% of embryos with P granules in both P4 and D or in D alone. One explanation for these results is that in many mes-1 embryos the descendants of P3 (perhaps even the descendants of P2) are hybrid in character; they follow somatic fates but retain the germ-line trait of maintaining P granules.

In mes-1 embryos, the primordial germ cell is transformed into a muscle precursor

Two observations discussed above suggest that in a majority of mes-1 embryos P4 follows the fate of its sister D and generates body muscle instead of germ line. First, P4 displays a cell division cycle characteristic of D and then fails to generate the normal germ-line daughters Z2 and Z3 (Fig. 3). Second, the pattern of ectopic P-granule staining in mes-1 L1s coincides with the approximate position of body muscle cells (Fig. 6C and Capowski et al., 1991). To verify that ectopic P
granules are in muscle cells, mes-1 L1s were co-stained with anti-P-granule antibodies and antibodies to muscle-specific myosin heavy chain. In mes-1 L1s, ectopic P granules were usually observed in the same regions and focal planes as the body muscle thick filaments stained by the anti-myosin antibody (Fig. 6C,D). Furthermore, the pattern of thick filament staining was sometimes disorganized in regions containing ectopic P granules (Fig. 6D), further suggesting that the ectopic P granules are in muscle cells.

Muscle cells containing P granules could have arisen either from P4 following a muscle fate or from a P-granule-containing D cell following the normal D fate, which is to generate 20 anterior body muscle cells (Sulston et al., 1983). The former possibility predicts that sterile mes-1 adults should contain extra body muscle cells and that fertile mes-1 adults should not. We tested this prediction by introducing a transgenic muscle reporter into mes-1 mutant strains. This construct contains the E. coli lacZ gene with a nuclear localization signal and under the control of the muscle-specific unc-54 promoter (O'kema et al., 1993). Staining of the muscle nuclei in sterile mes-1 animals revealed that the worms indeed contain extra body muscle cells. Wild-type adults contain 95 body muscle cells (Sulston and Horvitz, 1977), and with the transgenic muscle reporter show staining of 89-96 (94.7±1.5) muscle nuclei (Fig. 7A). (The ‘extra’ muscle nucleus observed in some worms may be the anal depressor muscle; see Materials and Methods.) In contrast, the majority of sterile mes-1 adults contain greater than 96 and up to 115 (103±7.8) β-galactosidase-positive muscle nuclei (Fig. 7C). The presence of up to 20 extra body muscle cells (D normally generates 20) and the anterior location of the extra body muscle cells (data not shown) give additional support for a transformation of P4 into a D-like muscle precursor in many mes-1 embryos. Fertile mes-1 animals, in which P4 apparently followed its normal germ-line
DISCUSSION

In mes-1 embryos P4 follows a muscle fate

Mutations in the mes-1 gene cause a majority of embryos produced by homozygous mutant mothers at 25°C to develop into sterile adults with extra body muscle cells. The basis for this phenotype is a transformation of the P4 blastomere’s fate from germ line to muscle. The strongest evidence for this is that in laser-operated mes-1 embryos, an isolated P4 cell can generate differentiated muscle cells. In the majority of mes-1 embryos, P4 and its sister D are similar in size, show a D-like division pattern and differentiation program, but show the germ-line trait of maintaining P granules. Thus, we speculate that both cells may actually have a hybrid (P4/D) identity.

P4 having a P4/D hybrid identity may explain an unexpected result. Isolated P4 cells displayed the potential to generate muscle in a larger proportion (18/19) of mes-1 embryos than predicted by the percentage (70%) of sterile mes-1 offspring. This difference, which by χ² analysis is statistically significant (P < 0.05), suggests that P4 often inherits the potential to generate both germ line and muscle, and that in intact embryos there are signals or cell-cell interactions that suppress the muscle potential and/or promote the germ-line potential of P4, leading to fertile mes-1 adults. Both inductive and repressive interactions are known to occur between other blastomeres in C. elegans embryos (Priess and Thomson, 1987; Schnabel, 1994).

Mis-partitioned P granules do not specify germ-line fate

The hypothesis that germ granules instruct the cells that receive them to follow a germ-line fate has received strong support from investigations done in Drosophila. The most convincing evidence is that when germ granules are induced to form in an ectopic position in the embryo, primordial germ cells form at that same ectopic position (Ephrussi and Lehmann, 1992; Bardsley et al., 1993). Based on these and other results from Drosophila, we expected that mis-partition of P granules in C. elegans might cause otherwise somatic cells to follow a germ-line fate. However, this is not observed in mes-1 embryos. Although P granules are mis-segregated to both P4 and D in a majority of mes-1 embryos, D never appears to follow a germ-line fate. Instead, P4 usually follows a muscle fate.

We offer three possible explanations. (1) When P granules are partitioned to both P4 and D, neither cell inherits enough germ-line-determining factors to promote germ-line development. (2) In addition to mis-partitioning P granules, mes-1 embryos mis-partition factors involved in specifying muscle-cell fate, and muscle determinants in P4 and D are dominant over germ-line determinants. (3) P granules are not sufficient for or not involved in specification of germ-line fate in C. elegans.

Whatever signals the germ line to develop (P granules or other factors) may be more permissive than instructive: the germ-line signal may not instruct cells how to develop, but instead protect them from somatic differentiation signals. Elimination or mis-partitioning of this protection would allow an underlying somatic fate to be expressed. In mes-1 embryos, the germ-line cell P4 expresses the fate of its somatic sister D, perhaps as a result of mis-partitioning of protective factors. In pie-1 embryos, the germ-line cell P4 may express the fate of its somatic sister EMS because it lacks protection from the somatic determinant SKN-1 (Mello et al., 1992; Bowerman et al., 1993).

The timing of mes-1 function and its TSP

The earliest defect observed in mes-1 embryos at restrictive temperature is in the division of P2: P3 and C are more similar in size than they are in wild-type embryos, and a small percentage of mes-1 embryos display defects in partitioning of P granules (to both P3 and C) and in development of the C blastomere. In addition, mes-1 embryos that have been freed from the constraints of the eggshell display defects in the polarity of the P2 division (unpublished results). We think that these defects reflect a diminution of polarity. Polarity appears to be essentially lost by the P3 division in the majority of mes-1

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Table 3. Muscle development by individual blastomeres in wild-type and mes-1 embryos

<table>
<thead>
<tr>
<th>Genotype of mother</th>
<th>Unablated cell</th>
<th>Number of operated embryos that expressed muscle markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild-type</td>
<td>D</td>
<td>6/6</td>
</tr>
<tr>
<td>mes-1</td>
<td>D</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>P4</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>P4</td>
<td>18/19</td>
</tr>
</tbody>
</table>

Embryos were dissected from wild-type and from mes-1 (bu7 or bu84) mothers raised at 25°C and were operated on at 23°C. During the early embryonic divisions, the AB, EMS, and C blastomeres, and then either P3 or D were ablated with a laser microbeam, leaving a single unablated blastomere. The operated embryos were allowed to develop overnight, examined by Nomarski optics, and then assayed for the presence of differentiated body wall muscle cells by staining with the 5-6 anti-myosin antibody (see Materials and Methods). Many of the 5-6-positive embryos also had contracting masses of cells.
muscle cells, most likely derived from P4. In our model, wild-type muscle cells. These develop into sterile adults with extra body diminution or premature loss of polarity in the germ-line divisions. If this factor prematurely drops below the threshold level, resulting in division and cytoplasmic partitioning in the germ-line blastomeres. Genetic and molecular evidence suggest that the ten existing alleles of mes-1 display the null phenotype, and yet all ten alleles are temperature-sensitive and incompletely expressed. The temperature sensitivity of all alleles, especially a likely molecular null allele, suggests that the mes-1 gene participates in an inherently temperature-sensitive process. The partial expressivity of all alleles reveals that, even in the absence of wild-type mes-1 function, the process can be executed normally. Wild-type mes-1 function guarantees the successful execution of the process at both low and high temperature.

The TSP for mes-1 suggests that the wild-type gene product delivered by the mother is required from the 1-cell stage (well before the first visible defects) up to the 24-28-cell stage. Thus, the TSP encompasses all of the unequal P-cell divisions. Evidently, in the absence of wild-type mes-1 activity, the temperature at which the P0 and P1 divisions occur affects the fidelity of later divisions (measured as maternal-effect sterility). The TSP and the partial expressivity of mes-1 mutations support the model below.

**A model for mes-1**

At restrictive temperature mes-1 embryos display a premature and progressive loss of the ability to undergo polarized germ-line divisions and partitioning. We hypothesize that this is due to the progressive loss or inactivation of a cellular component that is required for the polarized divisions of the germ-line blastomeres and that is prone to inactivation, especially at high temperature. The role of wild-type mes-1 function is to stabilize this ‘polarity component’. In our model (Fig. 8), even in the absence of mes-1+ function, P0 and P1 contain sufficient concentrations of the active polarity component for normal polarized divisions. However, the level of active component falls below the required threshold in some P2 cells and in most P3 cells, leading to the progressively more severe defects in division and partitioning observed in mes-1 embryos. Consistent with this concentration model, when the separation of germ line from soma is delayed by experimental manipulation, the resulting germ-line blastomeres display premature loss of germ-line quality (Schierenberg, 1988; Schlicht and Schierenberg, 1991). Even in wild-type embryos, decay or inactivation of components required for polarized divisions may account for the cessation of unequal germ-line divisions after the generation of P4. Indeed, Boveri (1910) observed that Ascaris embryos occasionally undergo an extra germ-line division to generate a ‘P5’ cell.

What is the ‘polarity component’ with which mes-1 interacts? Known cellular components that participate in controlling the polarity of the germ-line divisions and cytoplasmic partitioning are the actin cytoskeleton (Strome and Wood, 1983; Hill and Strome, 1988, 1990) and the products of the par genes (Kemphues et al., 1988). Studies in yeast provide a clear parallel with the present study and illustrate how mes-1 could function to stabilize the actin cytoskeleton. In Saccharomyces cerevisiae the actin cytoskeleton is sensitive to high temperature; shifting wild-type yeast to high temperature results in a transient rearrangement of the actin cytoskeleton and affects actin-cytoskeleton-dependent processes, such as spindle orientation (Palmer et al., 1992; Lillie and Brown, 1994). Null embryos (see Fig. 8): P4 and D have similar sizes, both cells inherit P granules, and yet neither cell has sufficient germ-line quality to generate a functional germ line. Instead both cells generate body muscles. The range of defects observed in mes-1 embryos at restrictive temperature may all be explained by a premature and progressive loss of polarity in the germ-line blastomeres.

Fig. 8. Summary and model. The summary illustrates the phenotype displayed by the majority of mes-1 progeny at restrictive temperature. Loss of polarity in P3 results in approximately equal sized P4 and D cells, both of which inherit P granules (stippling) but follow a D-like muscle fate. The resulting larvae lack the primordial germ cells Z2 and Z3 and instead contain P granules in anterior muscle cells. These develop into sterile adults with extra body muscle cells, most likely derived from P4. In our model, wild-type mes-1 function stabilizes a factor that is required for polarized division and cytoplasmic partitioning in the germ-line blastomeres. In mutant embryos (dashed lines), the concentration or activity of this factor prematurely drops below the threshold level, resulting in diminution or premature loss of polarity in the germ-line divisions.
mutations in SAC6, which encodes the actin-associated protein fimbrin, have mild effects on cells at low temperature but cause arrest and lethality at high temperature (Adams et al., 1991). The actin cytoskeleton in C. elegans may also be prone to reorganization or disruption at high temperature, and mes-1 may encode an actin-associated protein that stabilizes it. Alternatively, mes-1 product may interact with and stabilize one of the par gene products. Molecular analysis of the mes-1 gene and localization of the protein product may reveal whether the gene product is associated with the actin cytoskeleton, interacts with one of the PAR proteins, or serves an unpredicted role in early embryos.

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**REFERENCES**


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