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The *pie-1* and *mex-1* Genes and Maternal Control of Blastomere Identity in Early C. elegans Embryos

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Summary

During C. elegans embryogenesis an 8-cell stage blastomere, called MS, undergoes a reproducible cleavage pattern, producing pharyngeal cells, body wall muscles, and cell deaths. We show here that maternal-effect mutations in the *pie-1* and *mex-1* genes cause additional 8-cell stage blastomeres to adopt a fate very similar to that of the wild-type MS blastomere. In *pie-1* mutants one additional posterior blastomere adopts an MS-like fate, and in *mex-1* mutants four additional anterior blastomeres adopt an MS-like fate. We propose that maternally provided *pie-1(+)* and *mex-1(+)* gene products may function in the early embryo to localize or regulate factors that determine the fate of the MS blastomere.

Introduction

The early blastomeres in C. elegans embryos undergo very different patterns of cell differentiation (Sulston et al., 1983). For example, one of the 8-cell stage blastomeres produces only intestinal cells, a second produces pharyngeal cells and body wall muscles, and a third produces hypodermal cells and neurons. Although the fates of some blastomeres in C. elegans appear to be determined by early cell–cell interactions (Priess and Thomson, 1987; Schierenberg, 1987; Schnabel, 1991; Wood, 1991), the fates of other early blastomeres appear to be specified, at least in part, by factors that are unequally distributed in the early embryo (Laufer et al., 1980; Cowan and McIntosh, 1985; Edgar and McGhee, 1986; Priess and Thomson, 1987; Schierenberg, 1987). For example, if immediately after the first division of the embryo the anterior and posterior sister blastomeres are separated, both are able to produce hypodermal cells, but only the posterior blastomere is able to produce intestinal cells, pharyngeal cells, and body wall muscles (Laufer et al., 1980; Priess and Thomson, 1987). Furthermore, if cytokinesis is arrested at the 2-cell stage, only the posterior blastomere can undergo intestinal or muscle-specific differentiation (Laufer et al., 1980; Cowan and McIntosh, 1985). Thus, the first two blastomeres already appear to have intrinsic differences in their abilities to produce certain cell types.

Previous genetic studies in C. elegans have suggested that the initial specification of blastomere fates may be coupled to early events that establish the anterior–posterior polarity of the embryo. Mutations in any of four genes, called *par-1*, *par-2*, *par-3*, and *par-4*, result in changes in the number and types of differentiated cells produced during embryogenesis. For example, *par-1* mutant embryos fail to produce intestinal cells and produce extra pharyngeal cells (Kemphues et al., 1988). In addition, the *par* mutations reduce or eliminate several visible anterior–posterior differences in the early embryo (Kemphues et al., 1988). In a wild-type 2-cell stage embryo, the posterior blastomere is smaller than the anterior blastomere, has a slower cell cycle period, has a different cleavage pattern, and uniquely contains cytoplasmic structures called P granules (Strome and Wood, 1982, 1983; Yamaguchi et al., 1983; Wolf et al., 1983; Schierenberg, 1984). Mutations in the *par* genes can lead to defects in all of these early differences. For example, in *par-1* mutants the anterior and posterior blastomeres are nearly equal in size, they divide synchronously, and P granules are distributed equally between the first two blastomeres (Kemphues et al., 1988). Although it is not known how *par* mutations affect both blastomere fate and the polarity of the embryo, it has been proposed that they may do so by interfering with the proper localization of cytoplasmic factors necessary for both of these processes (Kemphues et al., 1988).

To understand what properties of, or factors in, the early blastomeres allow them to produce different cell types, we have begun a genetic analysis of how pharyngeal cell fates are specified. We have focused on pharyngeal development because the first two blastomeres differ in their ability to produce pharyngeal cells, and because pharyngeal cells can be identified easily with the light microscope even in embryos with highly abnormal morphogenesis (Priess and Thomson, 1987; Priess et al., 1987; Kemphues et al., 1988). Because it is likely that maternally expressed gene products function in establishing some of the initial differences between blastomeres in C. elegans, we have isolated and analyzed maternal-effect mutations that result in embryos with either no pharyngeal cells or too many pharyngeal cells. The only maternal-effect mutants identified thus far that do not produce pharyngeal cells, but produce other fully differentiated tissues, all have mutations in the gene *skn-1* (Bowerman et al., 1992). The predicted *skn-1* protein product contains a sequence similar to the DNA-binding domain of a family of transcription factors called "bZIP" proteins, suggesting that *skn-1* may regulate early transcriptional events leading to the production of pharyngeal cells (Bowerman et al., 1992).

In this study we describe maternal-effect mutations in the genes *pie-1* and *mex-1* that cause embryos to produce too many pharyngeal cells (as do some of the *par* mutations), but do not disrupt the visible aspects of anterior–posterior polarity in the early embryo. We show that embryos from both mutant strains overproduce pharyngeal cells as a consequence of transformations in cell fate in the early embryo. In both mutant strains multiple 8-cell stage blastomeres adopt a pattern of cell cleavage and

differentiation characteristic of a single 8-cell stage blastomere, called MS, that produces pharyngeal cells in addition to other cell types. We propose that *pie-1(+)* and *mex-1(+)* activity is required during wild-type development to differentially localize or regulate factors that specify the fate of the MS blastomere. We show that *skn-1(+)* activity is required for many, if not all, characteristics of the MS blastomere, suggesting that one function of the *pie-1* and *mex-1* gene products may be to localize or regulate *skn-1(+)* activity.

Results

Background

The pharynx in C. elegans is an organ used for feeding and consists of pharyngeal muscle cells, gland cells, neurons, and structural cells (Albertson and Thomson, 1976). Pharyngeal cells appear to secrete, and become enclosed by, a prominent basement membrane, and thus can be identified easily with the light microscope (Figure 1). Pharyngeal cells also can be identified with fluorescence microscopy after staining embryos with antibodies that recognize pharyngeal cell types (see Figure 2 and Experimental Procedures). The first two embryonic blastomeres are called AB and P1, and both produce pharyngeal cells in normal development. However, when these blastomeres are separated and cultured in isolation, only P1 is able to produce pharyngeal cells (Priess and Thomson, 1987). AB descendants appear to become committed to producing pharyngeal cells through interactions with descendants of P1; these interactions require maternal expression of the *glp-1* gene (Priess and Thomson, 1987; Priess et al., 1987). The *glp-1* gene encodes a possible transmembrane protein (Austin and Kimble, 1989; Yochem and Greenwald, 1989) that is very similar in structure to the products of the *lin-12* gene (Greenwald, 1985; Yochem et al., 1988) and the *Notch* gene (Wharton et al., 1985; Kidd et al., 1986), which are required in several different cell–cell interactions in C. elegans and Drosophila, respectively. In embryos from mothers homozygous for *glp-1* mutations, P1 produces its full complement of pharyngeal cells, but AB appears to produce neuronal cells instead of pharyngeal cells (Priess et al., 1987).

pie-1 and *mex-1* Mutant Embryos Produce Excess Numbers of Pharyngeal Cells

Maternal-effect lethal mutants were isolated in a screen of approximately 30,000 mutagenized haploid genomes using a previously described method (Priess et al., 1987; Kemphues et al., 1988; see Experimental Procedures). Five mutant strains produced embryos with extra pharyngeal cells and wild-type patterns of early cleavage. The early blastomeres in these mutant embryos appear identical to wild-type embryos in terms of size, cleavage rate, and division axes up to approximately the 28-cell stage (data not shown). Two of these mutations define the gene *pie-1*, and three define the gene *mex-1*.

The *pie-1* and *mex-1* embryos do not undergo normal body morphogenesis (Figure 1), although *pie-1* mutant embryos occasionally develop a fairly normal head containing too many pharyngeal cells (data not shown). The pharyngeal cells in both types of mutant embryos form structures with many of the morphological characteristics of the wild-type pharynx (Figure 1). For example, the pharyngeal muscles are usually associated with a specialized cuticular structure called the grinder, which is one of the final structures formed in wild-type embryogenesis. In addition to producing too many pharyngeal cells, *pie-1* mutant embryos also appear to produce too many intestinal cells (see Figures 1b, 5c; *pie* stands for pharyngeal and intestinal excess), and *mex-1* mutant embryos appear to produce

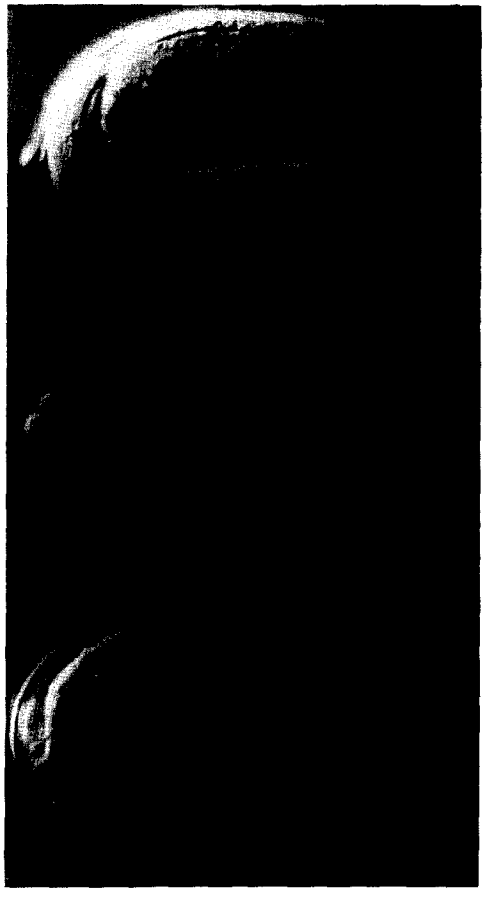

Figure 1. Wild-Type, *pie-1*, and *mex-1* Embryos

Light micrographs of wild-type (A), *pie-1* (B), and *mex-1* (C) embryos taken 14 hr after first cleavage at 22°C. The wild-type embryo has elongated into a worm and is near hatching. The *pie-1* and *mex-1* mutant embryos have not undergone normal body morphogenesis. The wild-type pharynx is enclosed by a basement membrane ([A], small arrows) and contains a specialized cuticular structure called the grinder ([A], large arrow). Each of the mutant embryos has large clusters of cells that appear to be surrounded by a basement membrane (small arrows), and some of these clusters contain cuticular structures resembling the wild-type grinder ([B], [C]; large arrows). Note that most of the left half of the *mex-1* mutant embryo appears to be comprised of pharyngeal tissue (see also Figure 2c). Nuclei of intestinal cells (arrowheads) are visible in all embryos. Note that most of the right half of the *pie-1* embryo appears to be comprised of intestinal cells (see also Figure 5c). In all figures embryos are oriented with anterior to the left; and all embryos shown are approximately 50 μm in length.

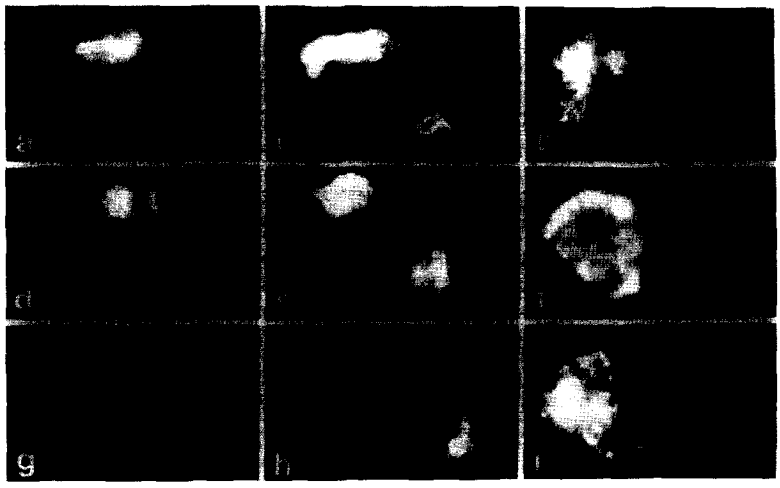

Figure 2. *pie-1* and *mex-1* Mutant Embryos Produce Extra Pharyngeal Cells

Immunofluorescence micrographs of embryos stained with either 3NB12 (a–c) or 9.2.1 (d–l), which are monoclonal antibodies that stain pharyngeal-specific muscle cells (Epstein et al., 1982; Priess and Thomson, 1987). Each column contrasts results from various genetic or physical experiments on embryos from mothers that were either wild type for both *pie-1* and *mex-1* (left column), homozygous for *pie-1(zu127)* (middle column), or homozygous for *mex-1(zu120)* (right column) mutations. (a–c) Pharyngeal cells in wild-type (a), *pie-1* (b), and *mex-1* (c) embryos at about 8 hr after first cleavage at 22°C. (d–f) Pharyngeal cell development at about 14 hr after first cleavage at 25°C in *glp-1* (d), *pie-1;glp-1* (e), and *mex-1;glp-1* (f) embryos. In *glp-1* single mutants (d), only the EMS blastomere produces pharyngeal cells (see also Priess et al., 1987). Note that both *pie-1;glp-1* and *mex-1;glp-1* double mutant embryos produce more pharyngeal tissue than *glp-1* single mutants. It is difficult to count accurately the number of pharyngeal cells present in any embryo; however, estimates were made after staining *glp-1* and *pie-1;glp-1* embryos with the 3NB12 antibody. *glp-1* mutant embryos appeared to contain an average of 9 positively staining cells (range of 7–12; n ≈ 16), and *pie-1;glp-1* embryos contained an average of 15 cells (range of 12–18; n = 26). (g-i) Pharyngeal cell development after the EMS blastomere was killed in *glp-1* (g), *pie-1;glp-1* (h), and *mex-1;glp-1* (i) embryos. Each staining pattern represents results from at least 10 experiments.

too many body wall muscle cells (Figure 5e; *mex* = **m**uscle **ex**cess). Body wall muscles are morphologically and immunologically distinct from pharyngeal muscles (Epstein et al., 1982; Miller et al., 1983; for simplicity we will refer in this paper to "body wall muscles" as "muscle cells"). The terminal phenotypes of both alleles of *pie-1* are indistinguishable from each other and do not vary at culture temperatures between 16°C and 25°C (data not shown). The terminal embryonic phenotypes of the three *mex-1* alleles appear indistinguishable under standard culturing conditions (22°C); however, each of the *mex-1* alleles shows a cold-sensitive (16°C) defect in producing intestinal cells (see Table 1).

The *pie-1* and *mex-1* mutations result in recessive, strict maternal-effect lethality; all embryos produced by heterozygous mutant mothers are viable, and all embryos produced by homozygous mutant mothers die regardless of their zygotic genotype (genetic tests are described in Experimental Procedures). The *pie-1* mutations map on chromosome III between *spe-6* and *unc-25*, and the *mex-1* mutations map on chromosome II between *rol-1* and *unc-52*. The *pie-1* and *mex-1* mutations were identified at a relatively low frequency but within the range expected for simple loss of function mutations (Brenner, 1974). Embryos produced by homozygous *pie-1* mutant mothers are identical in phenotype to those produced by mothers heterozygous for a *pie-1* mutation and a deficiency of the *pie-1* chromosomal region, indicating that a 50% reduction in dose of the mutant gene does not exacerbate the maternal *pie-1* defect nor reveal zygotic ones (see Experimental Procedures). No deficiencies exist at present for the *mex-1* region of chromosome II.

Table 1. Intestinal Development in *mex-1* Mutants

	Embryos with Intestinal Cells (%)	
Allele	25°C	16°C
zu120	99	63
zu121	98	28
zu140	99	65

From 200 to 1000 embryos were collected from homozygous *mex-1* hermaphrodites and allowed to develop for either 15 hr at 25°C or 20 hr at 16°C. Intestinal cell development was scored in the light microscope using polarizing optics to detect intestine-specific gut granules. In the cases where no intestinal cells were observed, the embryos contained larger numbers of hypodermal cells than were visible in the embryos that produced intestinal cells (see also Figure 3).

The above data suggest that the *pie-1* and *mex-1* genes are expressed maternally and that their gene products are required for normal embryonic development. However, these mutations have not been demonstrated to be null, so it remains possible that the *pie-1* and *mex-1* genes have additional functions in development. For example, the genes *glp-1* and *skn-1*, which mutate to maternal-effect lethality at a frequency similar to *pie-1* and *mex-1*, are required zygotically during germline and intestinal development, respectively (Austin and Kimble, 1987; Bowerman et al., 1992). The *pie-1(zu127)* and *mex-1(zu120)* alleles were used in all of the following experiments unless indicated otherwise.

pie-1 and *mex-1* Mutants Do Not Require *glp-1(+)* Activity to Produce Pharyngeal Cells

The *pie-1* and *mex-1* mutant embryos appear to contain approximately twice the wild-type amount of pharyngeal tissue (compare Figure 2a with Figures 2b and 2c). Because approximately half of the pharynx in a wild-type embryo develops as a result of cell–cell interactions in early embryogenesis (Priess and Thomson, 1987; Priess

et al., 1987), it was possible that the extra pharyngeal cells in the *pie-1* and *mex-1* mutants resulted from inappropriate cell–cell interactions. In a wild-type embryo these interactions involve descendants of the P1 blastomere and require *glp-1(+)* activity (see Background).

To determine whether *glp-1(+)* activity is required for the production of the extra pharyngeal cells in the mutant embryos, we constructed *pie-1;glp-1* and *mex-1;glp-1* double mutant strains and compared the size of the pharynx in each of the double mutant embryos with the pharynx in the respective single mutant embryos. The pharynx in *pie-1;glp-1* double mutants (Figure 2e) is reproducibly smaller than the pharynx in *pie-1* single mutants (Figure 2b and data not shown), indicating that the production of some pharyngeal cells in *pie-1* mutant embryos requires *glp-1(+)* activity. However, the pharynx in *pie-1;glp-1* double mutant embryos (Figure 2e) is approximately twice as large as the pharynx in *glp-1* single mutant embryos (Figures 2d), indicating that the production of many of the extra pharyngeal cells in *pie-1* mutant embryos does not require *glp-1(+)* activity. The *mex-1;glp-1* double mutants (Figure 2f) produce approximately the same amount of pharyngeal tissue as *mex-1* single mutant embryos (Figure 2c), and much more than in *glp-1* single mutants (Figure 2d). Thus the production of most, if not all, of the extra pharyngeal cells in *mex-1* mutant embryos does not require *glp-1(+)* activity.

In a *glp-1* mutant embryo the P1 daughter called EMS is the only blastomere that produces pharyngeal cells (Priess et al., 1987). We find that after the EMS blastomere in a *glp-1* mutant embryo is killed with a laser microbeam, no pharyngeal cells are produced (Figure 2g). In contrast, when the EMS blastomere is killed in either *pie-1;glp-1* or *mex-1;glp-1* double mutant embryos, numerous pharyngeal cells are produced (Figures 2h and 2i). Thus, in both *pie-1* and *mex-1* mutant embryos, blastomeres other than EMS appear to produce pharyngeal cells and do not require *glp-1(+)* activity to do so.

Multiple Early Blastomeres in *pie-1* and *mex-1* Mutant Embryos Develop Like a Wild-Type MS Blastomere

To determine the source of the extra pharyngeal cells in the *pie-1* and *mex-1* mutant embryos, we used a laser microbeam to kill all but one of the blastomeres at the 2-, 4-, or 8-cell stages of development. The cell types produced by the single remaining blastomere were analyzed in the light microscope or by fluorescence microscopy after staining with cell type–specific probes (see Experimental Procedures). When single blastomeres from 2- or 4-cell stage embryos are allowed to develop, they produce

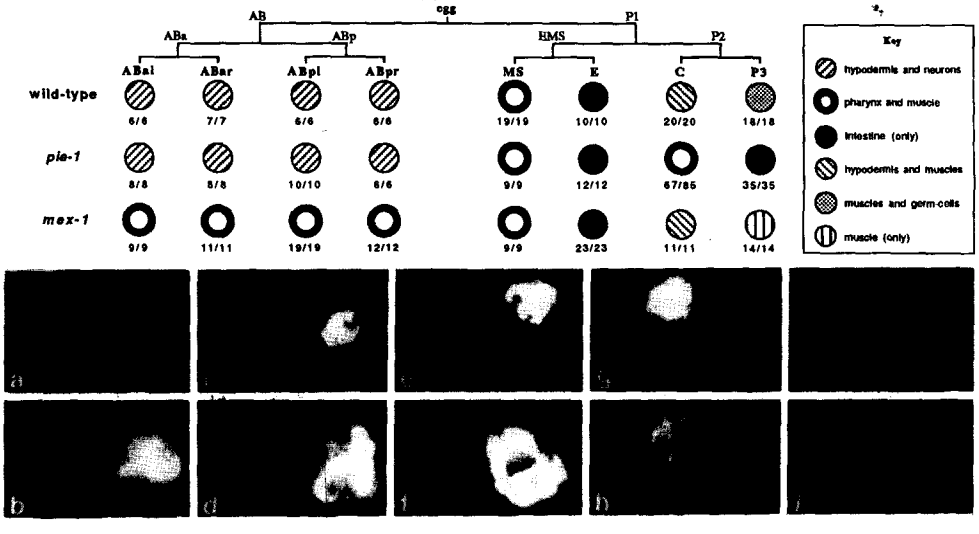

Figure 3. *pie-1* and *mex-1* Mutations Alter Early Blastomere Fates

In the top diagram, the lineal relationships and names of all embryonic blastomeres up to the 8-cell stage are shown. For example, the 8-cell blastomeres named ABal and ABar are both daughters of the 4-cell blastomere named ABa. The coded circle underneath each name of an 8-cell stage blastomere indicates the types of cells that blastomere produced after all other blastomeres were irradiated with a laser microbeam; the differentiated cell types represented by each coded circle are listed in the key (top right panel; see Experimental Procedures). The top, middle, and bottom rows of coded circles represent experiments on wild-type, *pie-1*, and *mex-1* embryos, respectively; the numbers below each coded circle represent the number of experiments performed. In *pie-1* mutants the C blastomere showed two patterns of differentiation; in 18/85 cases the C blastomere produced only intestinal cells. All experiments shown were performed at 22°C. Because intestinal development in *mex-1* mutants shows some temperature dependence (Table 1), the development of the E blastomere was also examined at 16°C: the E blastomere produced only intestinal cells in 12/24 experiments, but produced hypodermal cells and muscles (and no intestinal cells) in the remaining cases.

The lower two panels are immunofluorescence micrographs showing partial embryos resulting from some of the experiments described above. These partial embryos were stained with antibodies that recognize pharyngeal cells (top row) or muscle cells (bottom row). (a, b) Development of the C blastomere in wild-type embryos after all other blastomeres are destroyed. (c, d) Development of the C blastomere in *pie-1* mutant embryos. (e, f) Development of the MS blastomere in wild-type embryos. (g, h) Development of the ABpl blastomere in *mex-1* mutant embryos. (i, j) Development of the ABpl blastomere in wild-type embryos. Pharyngeal cells were stained with 9.2.1 (see Figure 2) and muscle cells with 5–6, a monoclonal antibody that recognizes body wall–specific myosin (Miller et al., 1983).

a spectrum of differentiated cell types consistent with the development of their respective 8-cell stage descendants (data not shown); we describe in this article results from experiments on 8-cell stage blastomeres (Figure 3).

In experiments on wild-type 8-cell stage embryos, only the MS blastomere is able to produce pharyngeal cells (Figure 3). The MS blastomere in *pie-1* and *mex-1* mutant embryos also produces pharyngeal cells in similar experiments. However, additional 8-cell stage blastomeres can produce pharyngeal cells in both mutant strains: the C blastomere produces pharyngeal cells in most *pie-1* mutant embryos (67/85 experiments; in the remaining cases the C blastomere produced intestinal cells), and the four granddaughters of AB produce pharyngeal cells in *mex-1* mutant embryos (Figure 3). We noticed that these mutant blastomeres each produce approximately the same amount of pharyngeal tissue as is produced by an MS blastomere (compare Figures 3c, 3e, and 3g) and also produce muscle cells, as does the MS blastomere (Figures 3d, 3f, 3h). These observations suggested that the mutant blastomeres might be adopting an MS-like pattern of development and led us to ask whether these blastomeres had other MS-like characteristics.

In wild-type embryogenesis the MS blastomere produces the first cells to express CeMyoD, which is a C. elegans homolog of the vertebrate MyoD family of myogenic regulatory genes (Krause et al., 1990). To compare the pattern of CeMyoD expression in wild-type embryos with *pie-1* and *mex-1* mutant embryos, we introduced a CeMyoD–β-galactosidase fusion construct into each strain and stained the transgenic embryos for β-galactosidase activity (see Experimental Procedures). Wild-type embryos show variable staining patterns, but in 28-cell stage embryos we never find more than four positively staining cells. In all cases in which the positively staining cells can be identified unambiguously, they are the first 4 descendants of the MS blastomere (Figure 4A; see also Krause et al., 1990). In *pie-1* and *mex-1* mutant embryos we find that additional 28-cell stage blastomeres can express the CeMyoD–β-galactosidase reporter (Figures 4B and 4C). In *pie-1* mutant embryos the additional positively staining blastomeres are always in the posterior of the embryo where the 4 descendants of the C blastomere are located, and in *mex-1* the additional positively staining cells are in the anterior and middle of the embryo where the 16 descendants of the AB granddaughters are located. These results suggest that the C blastomere in *pie-1* mutants, and the AB granddaughters in *mex-1* mutants, exhibit an early pattern of CeMyoD expression similar to that of a wild-type MS blastomere.

Another characteristic of the MS blastomere is that one of its descendants undergoes the first programmed cell death in C. elegans embryogenesis (see Horvitz et al., 1982 for review of cell death in C. elegans). The cell that dies is generated from an invariant sequence of anterior–posterior cleavages and is called MSpaapp (cells are named in C. elegans according to their position at birth; for example MSp is the posterior daughter of the MS blastomere). To test whether the MSpaapp cell death could be used as a marker for an MS pattern of development, we asked whether this cell death occurred when all blastomeres other than MS were destroyed with a laser microbeam. We find that the MS descendant born from the same series of anterior–posterior cleavages that normally generates MSpaapp undergoes cell death, even though this cell is no longer in the position that MSpaapp occupies in an intact embryo (Figure 4D).

To determine whether the MS-like blastomeres in *pie-1* and *mex-1* mutants produce a cell death equivalent to MSpaapp, we followed the development of each of these blastomeres in intact embryos. In both *pie-1* and *mex-1* mutant embryos MSpaapp undergoes programmed cell death (Tables 2 and 3). As described above, the C blastomere in *pie-1* mutant embryos resembles an MS blastomere in its ability to produce pharyngeal cells and CeMyoD–β-galactosidase–positive descendants. The descendant of the C blastomere that would correspond in origin to MSpaapp is called Cpaapp, and in wild-type embryos it does not undergo cell death (Sulston et al., 1983). We found that the C blastomere in *pie-1* mutant embryos divides with a pattern and timing of cleavage similar to the MS blastomere and that the Cpaapp descendant undergoes programmed cell death in most embryos (Figure 4E and Table 2). Similarly, in *mex-1* mutants each of the four MS-like blastomeres (the AB granddaughters) divide with an MS-like pattern and timing of division and produce a descendant corresponding in origin to MSpaapp that undergoes programmed cell death (Figure 4F and Table 3).

We examined several other descendants of the MS-like mutant blastomeres and found that their development was different from wild type, but identical, or very similar, to the development of the corresponding descendants of the MS blastomere (Figures 4G–4I; Tables 2 and 3). For example, the C blastomere in *pie-1*, and the ABpl blastomere in *mex-1*, both generate a descendant corresponding in origin to MSpaap that, like a wild-type MSpaap, produces pharyngeal cells (Figure 4G).

The Parents of the MS and C Blastomeres Appear to Be Identical in *pie-1* Mutant Embryos

The sister of the MS blastomere, called E, produces all of the intestinal cells found in a wild-type embryo, and no other cell type (Sulston et al., 1983). Moreover, only the E blastomere has the ability to produce intestinal cells after all other blastomeres are destroyed in wild-type embryos (Bowerman et al., 1992; see Figure 3). In contrast, a second blastomere in addition to E always produces intestinal cells after similar experiments on *pie-1* mutant embryos; this blastomere is called P3 (Figures 3, 5a, 5b).

A wild-type P3 blastomere divides unequally, and the smaller daughter becomes the germ cell precursor, which undergoes only one additional division during embryogenesis (Sulston et al., 1983). We followed the development of the P3 blastomere in *pie-1* mutant embryos and found that its pattern of cleavage is different from a wild-type P3 blastomere, but very similar to a wild-type E blastomere. Like a wild-type E blastomere, the P3 blastomere in *pie-1* mutant embryos divides equally, and both daughters undergo several additional rounds of cleavage to produce only intestinal cells (Figure 5b and data not shown).

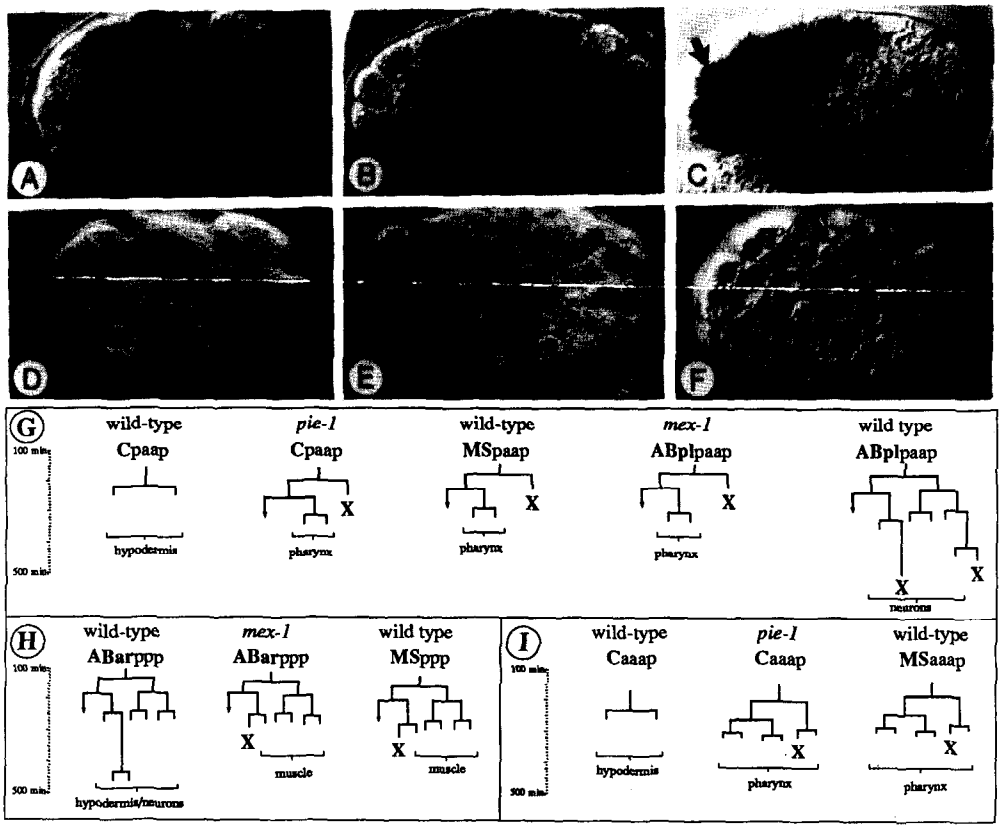

Figure 4. *pie-1* and *mex-1* Mutant Embryos Contain Multiple MS-like Blastomeres

(A–C) Light micrographs of wild-type (A), *pie-1* (B), and *mex-1* (C) embryos that are transgenic for an CeMyoD–β-galactosidase fusion construct. Each embryo was fixed at about the 28-cell stage of development and stained for β-galactosidase activity. The wild-type embryo (A) contains four positively staining cells, which were identified as the first 4 descendants of the MS blastomere (see Experimental Procedures; see also Krause et al. [1990]). The *pie-1* mutant embryo (B) contains eight positively staining cells; four of these are MS descendants (these appear in about the same positions as the MS descendants in (A) but are just below the focal plane), and the posterior group of four (arrow) are the first 4 descendants of the C blastomere. All of the anterior blastomeres in the *mex-1* mutant embryo (C) stain positively (arrow), consistent with the locations of the descendants of the AB granddaughters.

(D–F) Early programmed cell deaths in wild-type (D), *pie-1* (E), and *mex-1* (F) embryos. Each of the strains carries an additional mutation in the gene *ced-2(e1752)* to facilitate identification of cell deaths (Hedgecock et al., 1983). (D) A wild-type embryo in which the AB, P2, and E blastomeres were irradiated to prevent division. The MS blastomere was allowed to develop, and its division pattern followed visually until a descendant that was equivalent in lineal origin to MSpaapp was born. This descendant was born in a markedly unequal division and quickly underwent programmed cell death (arrow). The MSpaapp cell death in an intact embryo normally occurs in the center of the embryo where it is surrounded by descendants of the AB, E, and P2 blastomeres; in this embryo the MSpaapp cell death occurred on the top surface adjacent to undivided AB (top left) and P2 (lower right) blastomeres. (E) The development of the C blastomere was followed in an intact *pie-1* mutant embryo until the birth of Cpaapp. Cpaapp was born in an unequal division and underwent programmed cell death (arrow). (F) The development of the ABal and ABpl blastomeres were followed in an intact *mex-1* mutant embryo until the birth of ABalpaapp and ABplpaapp. Both of these descendants were born in unequal divisions, and both underwent cell death (arrows). Note that wild-type embryos at the same stage of development as the mutant embryos depicted in (E) and (F) would contain only the MSpaapp cell death (Sulston et al., 1983). The *pie-1* mutant in (E) contained an additional cell death on a lower focal plane, consistent with the location of MSpaapp, and there were three additional cell deaths on lower focal planes of the *mex-1* mutant embryo in (F).

(G–I) Lineage diagrams comparing the development of a single descendant of an 8-cell stage, MS-like blastomere in a *pie-1* or *mex-1* mutant embryo with both the development of the same descendant in a wild-type embryo, and with the development of the lineally equivalent descendant of a wild-type MS blastomere. To facilitate comparison, the part of each cell name that corresponds to an 8-cell stage blastomere is lettered in bold. For example, ABpl is an 8-cell stage, MS-like blastomere in a *mex-1* mutant embryo; the development of its descendant **ABpl**paap is compared with a wild-type **ABpl**paap and with a wild-type **MS**paap, which is the lineally-equivalent descendant of the MS blastomere. In the lineage diagrams a vertical line represents a single cell, and a branch represents a cell division with the anteriormost daughter indicated on the left and the posteriormost daughter indicated on the right. Vertical lines ending in arrowheads indicate that the cell continued to divide but was not followed further, and "X" indicates a programmed cell death. Because the individual 8-cell stage blastomeres are born at slightly different times the lineages begin at different positions relative to the time axis on the far left of each diagram; this axis represents the total elapsed time since first cleavage. The cell deaths diagrammed in the wild-type **MS**paap lineage, the *pie-1* **C**paap lineage, and the *mex-1* **ABpl**paap lineage (G) are the same cell deaths photographed in Figures 3D, 3E, and 3F, respectively. The total number of embryos examined for each lineage is shown in Tables 2 and 3. All lineages were analyzed at 22°C, and cell fates were assigned by criteria described in Experimental Procedures.

Table 2. Cell Differentiation in *pie-1* Mutant Embryos

Cell Name	Fate in Wild-Type	Fate in *pie-1*	Wild-Type MS Fate
Cpaapp	1 hyp	1 death (5/6)	1 death
aaap	2 hyp	5 phar, 1 death (1)	5 phar, 1 death
aaaapa	(Caaaap = 1 hyp)[a]	2 phar (1)	2 phar
paap	2 hyp	4 phar, 1 death (1)	3 phar, 2 deaths
papapp	(Cpapap = 1 death)[a]	2 phar (1)	2 phar
MSpaapp	1 death	1 death (5)	1 death

The name of each cell analyzed is listed in the first column, its fate in wild-type embryos is shown in the second column, and its fate in *pie-1* mutant embryos is shown in the third column. The fourth column shows the fate of the lineally equivalent descendant of a wild-type MS blastomere. Criteria for assigning cell fates are described in Experimental Procedures; data for wild-type fates are from Sulston et al. (1983). hyp, hypodermal cells; phar, pharyngeal cells; mus, body wall muscles.

[a] Caaaap and Cpapap do not divide again in wild-type development.

The results described above indicate that in *pie-1* mutant embryos the blastomeres C and P3 develop very much like the wild-type blastomeres MS and E, respectively. Therefore, in *pie-1* mutant embryos the P2 blastomere (the parent of C and P3) appears to develop like its sister EMS (the parent of MS and E; see lineage diagram in Figure 3). This suggests that the wild-type activity of the *pie-1* product may be required for the P2 blastomere to become different from its sister, EMS.

Previously described mutations in the gene *skn-1* cause a maternal-effect lethal phenotype nearly reciprocal to that caused by *pie-1* mutations. In *skn-1* mutant embryos, the EMS and P2 blastomeres develop similarly to wild-type P2 blastomeres (with the exception that only P2 produces the germ cell precursors; Bowerman et al., 1992). In *pie-1* mutant embryos, as described above, both EMS and P2 develop like wild-type EMS blastomeres. These observations raise the possibility that in wild-type development either the *skn-1(+)* or the *pie-1(+)* gene product may negatively regulate the activity of the other. If so, then the *pie-1; skn-1* double mutant phenotype would be expected to resemble one of the two single mutant phenotypes. For example, if the only function of the *pie-1(+)* product was to negatively regulate *skn-1(+)* activity in the P2 blastomere, then the *pie-1;skn-1* double mutant should appear identical to the *skn-1* single mutant. We find that *pie-1;skn-1* double mutant embryos appear very similar to *skn-1* single mutant embryos (Table 4). In both strains EMS and P2 develop similarly to a wild-type P2 blastomere; EMS and P2 both produce hypodermal cells and muscles (data not shown), but almost never produce pharyngeal cells (Table 4) or intestinal cells (Figure 5d; Table 4). However, in contrast to the P2 blastomere in *skn-1* single mutant embryos, the P2 blastomere in *pie-1;skn-1* double mutants does not produce any cells resembling the germ cell precursors (data not shown). These results suggest that in wild-type embryogenesis *pie-1(+)* activity may be required to prevent *skn-1(+)* activity in the P2 blastomere and may also be required for the development of the germ cell precursors (see Discussion).

skn-1 Activity Is Required for Ectopic MS-like Fates in *mex-1* Mutant Embryos

In *mex-1* embryos all four granddaughters of the AB blasto-

Table 3. Cell Differentiation in *mex-1* Mutant Embryos

Cell Name	Fate in Wild-Type	Fate in *mex-1*	Wild-Type MS Fate
ABalpaapp	4 hyp	1 death (5/6)	1 death
pppap	3 neur, 2 deaths	1 mus, 1 death (2)	1 mus, 1 death
pppp	7 neur, 4 deaths	4 mus (1)/6 mus (1)	4 mus
ABarpaapp	2 hyp	1 death (4)	1 death
papp	4 hyp	6 mus (1)	6 mus
pppap	1 hyp, 2 neur	1 mus, 1 death (2)	1 mus, 1 death
pppp	4 hyp	4 mus (2)	4 mus
apaapa	1 phar, 1 death	1 death (1)	1 death
ABplpaapp	4 neur, 1 death	1 death (5)	1 death
paa	16 neur, 1 death	12 phar (1)/7 phar, 1 death, 1 ND (1)	10 phar, 3 deaths
ppppa	3 hyp, 1 neur	2 mus (1)	2 mus
aaap	4 hyp	6 phar (2)	5 phar, 1 death
ABprpaapp	4 neur, 1 death	1 death (2)	1 death
aaaa	6 neur, 3 deaths	8 phar (1)	5 phar, 4 neur, 1 death
MSpaapp	1 death	1 death (3)	1 death

The names of each cell analyzed is listed in the first column, its fate in wild-type embryos is shown in the second column, and its fate in *mex-1* mutant embryos is shown in the third column. The fourth column shows the fate of the lineally equivalent descendant of a wild-type MS blastomere. In the single case in which ABalpaapp did not die in a *mex-1* mutant, its sister (ABalpaapa) underwent programmed cell death. hyp, hypodermal cells; phar, pharyngeal cells; neur, neurons; mus, body wall muscles; ND, not determined. Criteria for assigning cell fates are described in Experimental Procedures; data for wild-type cell fates are from Sulston et al. (1983).

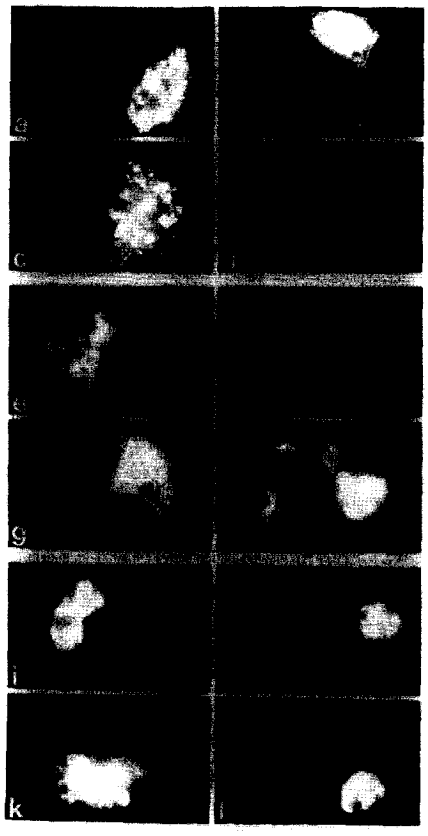

Figure 5. Mutations in the *skn-1* Gene Prevent the Development of MS-like and E-like Blastomeres

(a–d) Embryos viewed with polarization optics showing intestinal-specific granules. (a) A terminal stage *pie-1* mutant embryo in which all of the early blastomeres except E were destroyed. As in wild-type embryos, the E blastomere produces only intestinal cells (see also Figure 3). (b) A terminal stage *pie-1* mutant embryo in which all of the early blastomeres except P3 were destroyed. The mutant P3 blastomere also produces only intestinal cells. (c) A terminal stage, unoperated *pie-1* mutant embryo showing the large numbers of intestinal cells made by both the E and P3 blastomeres. The posterior half of this embryo consists almost entirely of intestinal cells. (d) A terminal stage, unoperated *pie-1;skn-1* double mutant embryo containing no intestinal cells. (e–h) Immunofluorescence micrographs of terminal stage mutant embryos showing muscle cells produced by single early blastomeres after all other blastomeres are destroyed. (e) The AB blastomere in a *mex-1* mutant produces numerous muscle cells. (f) The AB blastomere in a *mex-1;skn-1* double mutant does not produce muscle cells. However, muscle cells are produced by both the EMS blastomere (g) and the P2 blastomere (h) in *mex-1;skn-1* double mutants embryos. (i-l) Immunofluorescence micrographs of terminal stage *mex-1;pie-1* double mutant embryos showing pharyngeal cells produced by single 4-cell stage blastomeres after all other blastomeres are destroyed. The ABp, ABa, and EMS blastomeres each produced pharyngeal cells in all experiments as shown in (i), (k), and (l), respectively. Each of the experiments illustrated represents results from at least 10 embryos analyzed. The P2 blastomere (j) in *mex-1;pie-1* double mutants produced pharyngeal cells in 35/39 experiments. In three experiments P2 produced only intestinal cells, and in one experiment it produced hypodermal cells and muscles. Although in almost every case where P2 produced pharyngeal cells it also produced intestinal cells, we noticed that in five cases P2 produced numerous pharyngeal cells but no intestinal cells. *pie-1(zu154)* was used in the construction of the *mex-1;pie-1* double mutant strain. Muscle cells were stained with antibody 5–6 (see Figure 2) and pharyngeal cells with antibody 9.2.1.

Table 4. Effect of *skn-1* Mutations on Pharyngeal and Intestinal Development in *pie-1* and *mex-1* Strains

Strain	Embryos with Pharyngeal Cells (%)	Embryos with Intestinal Cells (%)
Wild-type	100	100
pie-1	100	100
pie-1;skn-1	2	15
mex-1	100	99
mex-1;skn-1	3	1
skn-1	2	23

Embryos (200 to 1200) from wild-type hermaphrodites and from each of the homozygous mutant hermaphrodites were collected and allowed to develop for 15 hr at 22°C. Intestinal cells were scored as in Table 1. Pharyngeal cells were scored by staining embryos with the antibody 9.2.1, which recognizes pharyngeal muscle cells (see Figure 2). Almost all of the *pie-1;skn-1* and *mex-1;skn-1* embryos that were scored as positive contained only a few pharyngeal or intestinal cells, compared with the numerous pharyngeal and intestinal cells present in *pie-1* single mutants or pharyngeal cells present in *mex-1* single mutants (see Figure 2). The alleles used were *pie-1(zu127)*, *mex-1(zu120)*, and *skn-1(zu67)*.

mere produce pharyngeal cells, muscle cells, and an early cell death with a pattern of development nearly identical to a wild-type MS blastomere. *skn-1(+)* activity is required for the MS blastomere to produce pharyngeal cells in wild-type embryos (Bowerman et al., 1992) and for both the MS and C blastomeres to produce pharyngeal cells in *pie-1* mutant embryos. To determine whether *skn-1(+)* activity is required in *mex-1* embryos for the AB granddaughters to produce pharyngeal cells, we constructed and analyzed a *mex-1;skn-1* double mutant strain. We find that in almost all of the double mutant embryos the AB granddaughters do not produce pharyngeal cells (Table 4).

As described above, the AB granddaughters in *mex-1* single mutant embryos produce numerous muscle cells (Figure 5e; see also Figure 3). Because *skn-1(+)* activity was not thought to be required for muscle cell development (Bowerman et al., 1992), we were surprised to find that the AB granddaughters do not produce muscle cells in *mex-1;skn-1* double mutant embryos (Figure 5f). Moreover, the AB granddaughters in *mex-1;skn-1* double mutants do not produce the early cell deaths corresponding to MSpaapp described above (data not shown). Instead, the AB granddaughters produce numerous hypodermal cells, as they do in wild-type embryogenesis (data not shown). Therefore, *skn-1(+)* activity appears to be required for each of the MS-like characteristics of the AB granddaughters in *mex-1* mutant embryos.

Although *skn-1(+)* activity is required in *mex-1* mutants for the MS-like blastomeres to produce muscle cells, *skn-1(+)* activity is not required for other blastomeres to make muscles in wild-type or in *mex-1* embryos. Both EMS and P2 produce muscles in *mex-1;skn-1* double mutants (Figures 5g, 5h), as they do in *skn-1* single mutants and in wild-type embryos (Bowerman et al., 1992).

Both *mex-1* and *pie-1* mutant embryos produce multiple MS-like blastomeres, and *skn-1* activity is required for the expression of these MS-like fates in each mutant strain.

We have shown that in 4-cell stage *mex-1* mutant embryos three of the four blastomeres produce MS-like descendants. The only one that does not produce MS-like descendants is the P2 blastomere. We therefore asked whether P2 would produce MS-like descendants in a *mex-1;pie-1* double mutant, as it does in a *pie-1* single mutant embryo. We used a laser microbeam to kill all but one of the blastomeres in 4-cell stage *mex-1;pie-1* double mutants and stained the resulting embryos with an antibody that recognizes pharyngeal cells. We find that all 4 blastomeres are able to produce pharyngeal cells (Figures 5i–5l), suggesting that in the *mex-1;pie-1* double mutant embryos each of the 4-cell stage blastomeres produces an MS-like descendant. In these experiments both the EMS and P2 blastomeres produce intestinal cells (data not shown) in addition to pharyngeal cells, as they do in *pie-1* single mutant embryos. The AB blastomeres in the *mex-1;pie-1* double mutant embryos develop very much like the AB blastomeres of *mex-1* single mutant embryos, producing both pharyngeal cells (Figures 5i, 5k) and muscle cells (data not shown). The observation that the *mex-1;pie-1* mutant phenotype appears to be a combination of both single mutant phenotypes suggests that the *mex-1* and *pie-1* mutations may affect separate developmental processes.

pie-1 and *mex-1* Mutant Embryos Have Early Defects in the Germ Cell Lineage

The germ cell lineage in C. elegans embryogenesis consists of four unequal cleavages, beginning with the first division of the egg and ending with the division of the P3 blastomere (see Figure 3). The smaller daughter of the P3 blastomere (called P4) divides only once more in embryogenesis, producing the two germ cell precursors. The first visible defect in the cleavage pattern of *pie-1* and *mex-1* mutant embryos is the division of the P3 blastomere; in both mutants the daughters of P3 are equal in size. These daughters continue to divide equally at least three more times and differentiate as intestinal cells in *pie-1* mutant embryos and as muscle cells in *mex-1* mutant embryos (Figure 3).

At each cleavage in the wild-type germ cell lineage, cytoplasmic structures called P granules are localized into the smaller daughter blastomeres (Strome and Wood, 1982, 1983; Wolf et al., 1983). P granules have no known function, but are useful markers for blastomere polarity in these cleavages. We examined P-granule localization in the *pie-1* and *mex-1* mutants by staining embryos at various cleavage stages with an antibody specific for P granules (Figure 6). In *pie-1* mutants the distribution of P granules appears identical to that in wild-type embryos until the division of P3 (Figure 6g). When P3 divides in wild-type embryos, all the P granules are localized into the smaller daughter (P4), which produces only germ cells. In *pie-1* mutants P granules are distributed equally between both daughters of P3, and they continue to be distributed equally at each subsequent division until all descendants of P3 stop dividing and differentiate as intestinal cells (Figure 6i). In *mex-1* mutants P granules are localized correctly to the P1 blastomere at the first division (Figure 6b). However, the P granules remain dispersed throughout the P1 blastomere instead of becoming associated with the posteriormost cortex as in wild-type and *pie-1* embryos (compare Figures 6a and 6b). At each of the subsequent divisions in *mex-1* mutant embryos, most of the P granules are localized correctly to the smaller daughters, but a substantial fraction remains in the larger daughters (Figures 6d, 6f, 6h). Therefore, *mex-1* mutants have early defects in the localization of at least one component of the embryonic cytoplasm.

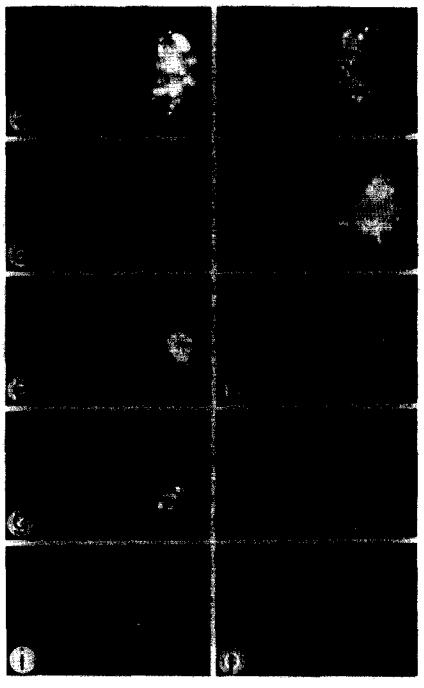

Figure 6. P-Granule Distribution in *pie-1* and *mex-1* Mutant Embryos

Immunofluorescence micrographs showing early cleavage stage *pie-1* (left column) and *mex-1* (right column) mutant embryos after staining with an antibody that recognizes P granules. (a, b) 2-cell stage. (c, d) 4-cell stage. (e, f) 8-cell stage. (g, h) 28-cell stage. (i, j) Terminal stage. The P-granule distribution in *pie-1* mutant embryos is identical to wild-type embryos until the 28-cell stage (g). In *pie-1* mutant embryos P granules are distributed equally between P4 and D ([g], arrow). In *pie-1* mutants P granules appear to be distributed equally to all of the daughters of both P4 and D, which continue to divide several times (i). In *mex-1* mutants P granules are localized correctly to the posterior blastomere at the first division (b). (d, f, h) After each of the subsequent embryonic cleavages the majority of the P granules are located in the correct blastomeres; however, numerous P granules are found in their sister blastomeres (arrows). The distribution of P granules in the *mex-1; pie-1* double mutants was indistinguishable from the distribution pattern in *mex-1* single mutants (data not shown).

Discussion

Maternal Control of Blastomere Identity

We have shown that maternal-effect mutations in the *pie-1* and *mex-1* genes lead to changes in blastomere fate. In *pie-1* and *mex-1* mutant embryos, multiple 8-cell stage blastomeres produce pharyngeal cells as well as other cell types through a pattern of development that by several

criteria is very similar to that of the MS blastomere: first, they do not require *glp-1(+)* activity to produce pharyngeal cells; second, they continue to produce pharyngeal cells when all other blastomeres are killed with the laser microbeam; and third, they produce pharyngeal cells as well as muscle cells and cell deaths with a pattern of cell division and differentiation very similar to that of the wild-type MS blastomere.

The observation that single, recessive maternal-effect mutations can result in multiple MS-like blastomeres suggests two general possibilities for how the MS fate normally is restricted to only one of the 8-cell stage blastomeres. In wild-type embryos blastomeres other than MS might contain the factors necessary for the MS fate; however, these blastomeres could contain other factors that specify alternative fates. Mutations in *pie-1* and *mex-1* might disrupt the specification of these alternative fates and thus result in multiple MS-like blastomeres. A second possibility is that dominant-acting factors that specify the MS fate are localized in wild-type embryos. Mutations in *pie-1* and *mex-1* might then prevent the proper localization of these factors. Although we cannot distinguish between these possibilities at present, the observation that *mex-1* mutants are defective in localizing the P granules (Figure 6) suggests *mex-1* mutants might also be defective in localizing other cytoplasmic components.

The pattern of blastomeres affected by the *pie-1* and *mex-1* mutations can be explained by a simple model for wild-type embryogenesis in which *mex-1(+)* and *pie-1(+)* activities are required during the first two divisions to restrict spatially the MS fate (Figure 7). This restriction could occur by preventing MS development as described above, or by localizing factors necessary for MS development. In this model *mex-1(+)* activity is required at the first division to restrict the potential for an MS fate to the posterior blastomere (P1). In *mex-1* mutants this restriction does not occur, so both blastomeres (AB and P1) produce MS-like descendants. In the second division *pie-1(+)* activity is required to restrict the potential for an MS fate to the anterior daughter of P1 (the EMS blastomere). In *pie-1* mutants this restriction does not occur, so both daughters of P1 (EMS and P2) produce MS-like descendants. In the *mex-1;pie-1* double mutants neither restriction occurs, so all of the 4-cell stage blastomeres produce MS-like descendants.

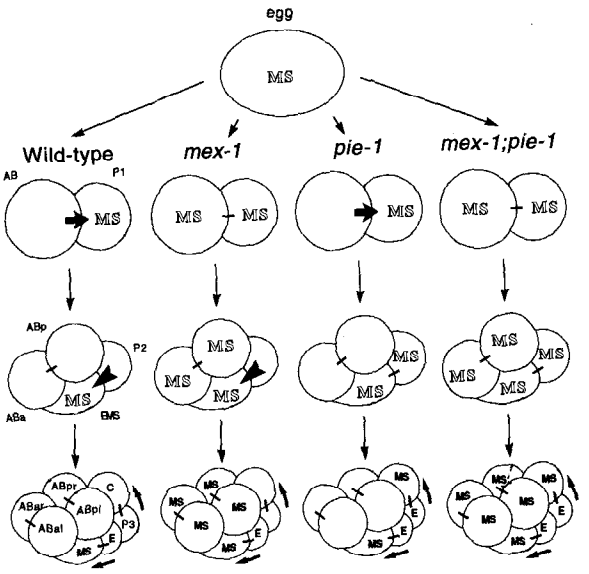

Figure 7. Specification of the MS Blastomere

In this model a factor(s) that can specify an MS pattern of development is imagined to exist in an inactive form in the fertilized egg. (This factor is represented as a hollow-lined "MS.") At the 2-cell stage in wild-type embryogenesis the MS factor is localized, or its activity restricted, to the posterior blastomere (P1) in a process that requires the *mex-1* gene product (bold arrow). At the 4-cell stage the MS factor is localized, or its activity restricted, to the anterior daughter of P1 (the EMS blastomere) in a process that requires the *pie-1* gene product (bold arrowhead). At the 8-cell stage the MS factor becomes active only in the anterior daughter of EMS (the MS blastomere, indicated by the solid-lined "MS"), as the result of unknown, posteriorly restricted, factors (thin arrows). The *mex-1*, *pie-1*, and *mex-1;pie-1* mutant phenotypes at the 8-cell stage are shown as arising from earlier errors in these two steps of localizing, or restricting the activity of, the MS factor.

The *skn-1* Product May Function to Activate the MS Pattern of Development

Maternal expression of the *skn-1* gene has been shown to be required for the production of at least three pharyngeal-specific cell types made by the MS blastomere (and for the E blastomere to produce intestinal cells; Bowerman et al., 1992). The *skn-1* gene appears to encode a protein product with some sequence similarities to the bZIP family of transcription factors and thus may function early in embryogenesis to regulate the activity of zygotic genes required for pharyngeal development (Bowerman et al., 1992). In extensive mutant screens, only four maternal effect mutations were identified that result in embryos completely lacking pharyngeal cells (but with apparently normal differentiation of most other cell types). All of these mutations are alleles of the *skn-1* gene, suggesting that there may not be many maternally expressed genes other than *skn-1* required for the MS blastomere to produce pharyngeal cell types (Bowerman et al., 1992).

In our analysis of the ectopic MS-like blastomeres in *pie-1* and *mex-1* mutants, we found that *skn-1* activity was required not only for the production of pharyngeal cells, but for all aspects of MS development that we examined. These results suggest that the *skn-1* product may activate part or all of the MS pattern of development. If so, then the *pie-1* and *mex-1* gene products could be required in wild-type development to localize or regulate the activity of the *skn-1(+)* product.

Intestinal Cell Fates Are Restricted to Posterior Blastomeres in the Early Embryo

In wild-type development the EMS blastomere divides into two daughters, MS and E, that have very different patterns of development. MS produces primarily pharyngeal cells and muscle cells, while its sister E produces only intestinal cells. Mutations in the gene *skn-1* alter the fates of both the MS and E blastomeres such that they both produce hypodermal cells and muscle cells (Bowerman et al., 1992). This indicates that there is at least one common step to the specification of the daughters of EMS and is

consistent with a simple model in which *skn-1* acts as an EMS "determinant." However, three results from the present study indicate that the specification of the EMS blastomere is more complex. Although the P2 blastomere in a *pie-1* mutant usually develops like an EMS blastomere, and requires *skn-1* activity to do so, in 20% of the embryos P2 does not produce an MS-like daughter and instead produces two E-like daughters. Second, in *mex-1* embryos the two anterior blastomeres (ABa and ABp) each produce two MS-like daughters, but never produce E-like daughters. Finally, in *mex-1* embryos cultured at low temperature the E blastomere occasionally produces hypodermal cells and muscles (as it does in *skn-1* mutant embryos), but the development of the MS blastomere is not affected. These observations suggest that some additional mechanism or factor present in the posterior sister blastomeres EMS and P2 (but not present in ABa and ABp) may be required for the posterior blastomeres to produce both MS-like and E-like daughters.

Studies on the development of the E blastomere had suggested that there might be an intestinal "determinant" localized to the E blastomere during the early cleavages (Laufer et al., 1980; Cowan and McIntosh, 1985; Edgar and McGhee, 1986; Priess and Thomson, 1987). However, more recent work has suggested that specification of the E blastomere may be more complicated, possibly involving cell–cell interactions between the P2 and EMS blastomeres (Schierenberg, 1987; Goldstein, 1992). If cell–cell interactions are required for EMS to produce "E" in addition to "MS" daughters, the anterior blastomeres in *mex-1* mutants may not be in the necessary positions to participate in these interactions. Similarly, the ability of the P2 blastomere in some *pie-1* mutant embryos to produce two E-like daughters could result from inappropriate cell–cell interactions in the posterior of the embryo.

Multiple Maternal Factors Appear to Specify Muscle Development in C. elegans Embryos

Our analysis of the development of *mex-1* mutants demonstrates that *skn-1(+)* activity is required for the MS-like descendants of the anterior blastomeres to produce muscle cells. This result suggests that *skn-1(+)* activity may be required to initiate a developmental program that includes the production of muscle cells. However, *skn-1* had not previously been thought to be involved in muscle specification, because the MS blastomere in *skn-1* mutant embryos produces muscle cells (Bowerman et al., 1992). A possible explanation for these apparently conflicting results is that in the absence of *skn-1(+)* activity the MS blastomere produces muscle cells through a default program it does not use in normal development.

In an 8-cell stage wild-type embryo there are three blastomeres that produce almost all of the muscle cells present at hatching; these blastomeres are MS, C, and P3 (Sulston et al., 1983). Each of these blastomeres produces apparently identical muscle cells through very different lineage patterns. For example, in addition to producing muscle cells the MS blastomere produces pharyngeal cells, the C blastomere produces hypodermal cells, and P3 produces germ cells. In *skn-1* mutants all aspects of the development of the P3 and C blastomeres appear normal; however, the development of the MS blastomere (and its sister the E blastomere) is altered dramatically. Instead of producing pharyngeal cells and muscle cells, the MS and E blastomeres in *skn-1* mutant embryos both produce hypodermal cells and muscle cells (Bowerman et al., 1992), which are cell types normally produced by the C blastomere in wild-type embryos (Sulston et al., 1983). These observations raise the possibility that in the absence of *skn-1(+)* activity, the MS and E blastomeres may produce muscle cells through a mechanism similar to that of a wild-type C blastomere.

The hypothesis that there are different genetic pathways (*skn-1* dependent and *skn-1* independent) for the maternal specification of muscle cell precursors during C. elegans development is consistent with recent analysis of CeMyoD. CeMyoD is the C. elegans homolog of the vertebrate MyoD family of myogenic regulatory genes and is expressed by the MS, P3, and C descendants that produce muscle cells. Deletion analysis of the CeMyoD promoter indicates that there may be distinct sequences that control expression of CeMyoD in these different early muscle cell precursors (A. Fire, personal communication).

The *pie-1* and *mex-1* Mutations Affect Both Somatic Cell and Germ Cell Development

In addition to their somatic defects, *pie-1* and *mex-1* mutant embryos also do not produce any cells that resemble the normal germ cell precursors in terms of morphology or cleavage pattern. In *pie-1* mutants the fates of all descendants of the P2 blastomere, including the germ cell precursors, are transformed to fates characteristic of the EMS blastomere. In *pie-1;skn-1* double mutant embryos, the development of the P2 blastomere is more nearly wild type (it produces hypodermal cells and muscles); however, the P2 blastomere still does not produce cells resembling the germ cell precursors. This result suggests that *pie-1(+)* activity may play some role in the development or specification of the germ cell precursors irrespective of its role in preventing the P2 blastomere from adopting an EMS-like fate.

Mutations in the *mex-1* gene result in a transformation in fate of all of the anterior embryonic blastomeres. The only posterior defect in *mex-1* mutant embryos is that the germ cell precursors continue to divide and differentiate as muscle cells. These observations suggest that the *mex-1* gene product may be required for both somatic cell and germ cell specification during wild-type development. As described above, *mex-1* mutants are defective in the localization of P granules during the early cleavages. Therefore, it is possible that *mex-1* mutations affect both somatic and germ cell fates by failing to localize cytoplasmic factors required for these fates. In this respect *mex-1* mutations may be analogous to maternal-effect mutations in certain of the Drosophila "posterior group" genes. During oogenesis in Drosophila structures called polar granules are assembled and localized at the posterior pole, and mutations that disrupt the assembly of these structures interfere with the specification of both germ cell and abdominal cell fates (reviewed by St Johnston and Nusslein-

Volhard, 1992). Future analysis of the *mex-1* gene may reveal whether there are mechanistic similarities between the way C. elegans and Drosophila embryos define posterior cell fates.

Anterior–Posterior Polarity in *pie-1* and *mex-1* Mutant Embryos

The MS-like blastomeres in *pie-1* and *mex-1* mutant embryos are located either anterior or posterior to the normal position of the MS blastomere and have different sets of neighbors. Nevertheless, the MS-like blastomeres produce a lineage pattern nearly identical to that of the wild-type MS blastomere, including the many anterior–posterior differences that occur within the MS lineage (see Figures 4G–4I; see also Sulston et al., 1983). For example, after the MS blastomere divides, or after the MS-like mutant blastomeres divide, it is always their posterior daughters that produce the first programmed cell death (equivalent to MSpaapp). If the anterior–posterior polarity of the wild-type MS cell lineage was controlled by local interactions with neighboring blastomeres, the ectopic MS lineages might be expected to be oriented randomly in the *pie-1* and *mex-1* mutant embryos. The finding that these ectopic MS cell lineages maintain the wild-type polarity suggests that there may be general cues throughout the embryo for determining anterior–posterior polarity.

Are the Fates of Some Early Blastomeres Determined by Intrinsic Factors?

The development of individual early blastomeres in laser-operated wild-type embryos suggests that at each of the embryonic cleavages leading to the 8-cell stage, the ability to produce pharyngeal cells (independent of *glp-1(+)* activity) is restricted to only one blastomere. At the 2-cell stage this restriction appears to reflect intrinsic differences between the sister blastomeres, because only the posterior blastomere (P1) can produce pharyngeal cells when cultured in isolation (Priess and Thomson, 1987). Because it has not been possible to isolate and successfully culture blastomeres from later stages, we do not know whether the descendants of P1 also differ intrinsically from their neighbors. The ability of these descendants to produce pharyngeal cells after all neighboring blastomeres are killed could result from intrinsic factors, although it remains possible that the laser-killed cells influence pharyngeal development in these experiments.

Maternal-effect mutations in the *pie-1* and *mex-1* genes lead to changes in the fates of the early blastomeres but do not disrupt the normal anterior–posterior differences in blastomere size, cleavage rate, and cleavage pattern. This suggests that maternally-expressed genes in C. elegans may be involved in determining the fates of early blastomeres beyond simply establishing the initial anterior–posterior polarity of the embryo. In this study we have analyzed maternal-effect mutations that result in embryos with too many pharyngeal cells. The observation that each of the mutants identified overproduces pharyngeal cells through a reproducible lineage pattern that is nearly identical to that of the MS blastomere suggests that maternal factors may specify the fate of the MS blastomere, rather than the fates of individual cell types such as pharyngeal cells. Differential localization of factors that can activate or repress the MS pattern of development might then account for the different abilities of the early blastomeres to produce pharyngeal cells.

Experimental Procedures

Strains and Alleles

The Bristol strain N2 was used as the standard wild-type strain. The marker mutations, duplications, and deficiencies used are listed by chromosome as follows: LG*I*: *dpy-5(e61)*. LG*II*: *bli-2(e768)*, *mel-14(it24)*, *mel-16(b298)*, *mel-17(b299)*, *mel-20(b317)*, *mnC1*, *rol-1(e91)*, *unc-4(e120)*, *unc-52(e444)*. LG*III*: *dpy-17(e164)*, *dpy-18(e364)*, *eDf2*, *egl-35(n694)*, *eDp6*, *eT1(III;V)*, *glp-1(e2142)*, *nob-1(ct223)*, *spe-6(hc49)*, *unc-25(e156)*, *zyg-8(b235)*. LG*IV*: *ced-2(e1752)*, *nT1(IV;V)*, *skn-1(zu67)*, *unc-5(e53)*. LG*V*: *dpy-11(e224)*, *him-5(e1490)*. LG*X*: *lin-2(e1309)*, *lon-2(e678)*. *skn-1(zu67)* was obtained from Bruce Bowerman, *nob-1(ct223)* from Lois Edgar, and *spe-6(hc49)* from Sam Ward. The strain *PD280* was obtained from Andy Fire. This strain carries a CeMyoD–β-galactosidase fusion gene integrated on LG*I* (Andy Fire, unpublished data). All other mutant alleles listed above were obtained, or are available, from the C. elegans genetic stock center. The basic methods of C. elegans culture, mutagenesis, and genetics were as described in Brenner (1974).

Genetic Analysis

The *pie-1* and *mex-1* mutations were isolated in screens for recessive, nonconditional, maternal-effect, embryonic-lethal mutants. The basis of the screening procedure has been described previously (Priess et al., 1987; Kemphues et al., 1988). Ethylmethane sulfonate (50 mM) was used to mutagenize animals as described in Brenner (1974). Four separate populations were mutagenized, and a total of 15,000 F1 progeny (30,000 mutagenized haploid genomes) were cloned to individual culture plates. Strains producing embryos with too many pharyngeal cells were identified by examination under a compound microscope equipped with Nomarski optics. Mutant strains producing embryos with too many pharyngeal cells were kept for an additional generation, and the early cleavage patterns were examined. Mutations with early defects in blastomere size and cleavage pattern were either tested for complementation with existing *par* mutations or were frozen.

The *pie-1(zu127)* and *mex-1(zu120)* mutant alleles were mapped in standard two-factor linkage tests using the strains MT465 (*dpy-5*; *bli-2*; *unc-32* [*I;II;III*]) and MT464 (*unc-5*; *dpy-11*; *lon-2* [*IV;V;X*]) (provided by H. R. Horvitz). Three-factor crosses using visible mutations on LG*III* and LG*II* were used to map *pie-1* and *mex-1* mutations, respectively. The *pie-1* alleles *zu127* and *zu154* each were mapped to the interval between *spe-6* and *unc-25*, approximately 1.3 map units to the left of *unc-25*. The *zu127* and *zu154* mutations fail to complement each other and also fail to complement the deficiency *eDf2*. The *pie-1* alleles complement *zyg-8*, *nob-1*, and *egl-35*, which map near *pie-1*. The *mex-1* alleles *zu120*, *zu121*, and *zu140* each were mapped to the interval between *rol-1* and *unc-52* on chromosome II, approximately 1 map unit to the right of *rol-1*. The *mex-1* alleles fail to complement each other, but complement *mel-14*, *mel-16*, *mel-17*, and *mel-20*, which map near *mex-1* and also are maternal-effect lethals.

The *pie-1* and *mex-1* mutant strains were backcrossed at least eight times against the wild-type C. elegans strain N2, and the left and right chromosome arms were crossed off to insure that secondary mutations were removed from the respective strains. Genetic tests for the necessity/sufficiency of maternal expression for both the *pie-1* and *mex-1* genes were performed as follows.

Maternal necessity

No viable self-progeny were produced by hermaphrodites homozygous for *dpy-18pie-1(zu127)* (0%, n > 10,000) or *rol-1mex-1(zu120)* (0%, n > 10,000), and no viable cross progeny were produced after mating hermaphrodites purged of sperm from either mutant strain with wild-type males (0%, n = 1013 for *pie-1*; 0%, n = 582 for *mex-1*).

Maternal sufficiency

Purged wild-type hermaphrodites produced similar numbers of viable cross progeny after mating with either homozygous *dpy-18pie-1(zu127)* males (97%, n = 2267), *rol-1mex-1(zu120)/unc-4mex-1(zu120)* males

(99%, n = 553), or wild-type males (96%, n = 1284), suggesting that paternal expression of *pie-1(+)* and *mex-1(+)* is not required for embryogenesis. The *pie-1* and *mex-1* heterozygous mutant hermaphrodites produced viable self-progeny at a frequency similar to wild-type hermaphrodites, indicating that the *pie-1* and *mex-1* mutations do not cause zygotic lethality: 99% viable self-progeny (n = 1369) for *dpy-18pie-1(zu127)/++* hermaphrodites, 99% (n = 1588) for *rol-1mex-1(zu120)/++* hermaphrodites, and 99% (n = 726) for wild-type hermaphrodites.

dpy-18pie-1(zu127)/eDf2 heterozygous hermaphrodites were constructed by mating *dpy-18pie-1(zu127)* homozygous males to *eDf2/eDf2; eDp6* L4 stage hermaphrodites. A total of 99% (n = 540) of the eggs produced by the mated *eDf2/eDf2; eDp6* hermaphrodites hatched. The resulting *dpy-18pie-1(zu127)/eDf2* deficiency heterozygous males and hermaphrodites had no morphological defects visible in the dissecting microscope. The adult *dpy-18pie-1(zu127)/eDf2* hermaphrodites produced approximately wild-type numbers of eggs (all embryos were inviable; n > 1000). The early cleavages and terminal phenotypes of embryos from the *pie-1* deficiency heterozygotes were examined with Nomarski optics. The early cleavages appeared wild type up to approximately the 28-cell stage, as they do in embryos from *pie-1* homozygous mothers. A total of 73% (n = 137) of the terminal stage embryos examined were indistinguishable from those produced by *pie-1* homozygous mothers. All other embryos resembled *pie-1* embryos in terms of producing too many pharyngeal and intestinal cells, but tissues in these embryos were only poorly differentiated. These latter embryos were probably *eDf2* homozygotes, which have an early zygotic lethal defect with similar poorly differentiated tissue morphology (C. C. M., unpublished observations). A *pie-1(zu154)unc-25/eDf2* deficiency heterozygote was constructed as above for *dpy-18pie-1(zu127)/eDf2*, and the development of embryos from both strains examined after all 4-cell blastomeres except P2 were killed with a laser microbeam. Results from these experiments were similar to those from experiments on embryos from *pie-1* homozygous mothers: The P2 blastomere made intestinal cells in 24/24, 67/67, 13/13, and 22/22 experiments on embryos from *zu127/eDf2*, *zu127/zu127*, *zu154/eDf2*, and *zu154/zu154* mothers, respectively. The P2 blastomere made pharyngeal cells in 21/24, 57/67, 12/13, and 16/22 experiment on embryos from *zu127/eDf2*, *zu127/zu127*, *zu154/eDf2*, and *zu154/zu154* mothers, respectively.

Microscopy

Light microscopy was performed with a Zeiss Axioplan microscope equipped for epifluorescence, polarizing, and differential interference contrast (DIC) optics. Photographs were taken on Kodak Technical Pan film and developed in HC110 developer. Embryos were processed for light microscopy following the procedures of Sulston et al. (1983). Embryos were processed for immunofluorescence microscopy as in Albertson (1984) and Bowerman et al., (1992). P granules were visualized by staining with monoclonal antibody K-76 (Strome and Wood, 1983). Laser ablation experiments were performed using a VSL-337 laser (Laser Science, Inc.) attached to a Zeiss Axioscope microscope as described by Avery and Horvitz (1989). The laser beam was focused on the nucleus and centrosomes of selected blastomeres, and blastomeres were hit with approximately 30–60 pulses of light. This dose was sufficient to cause large quantities of refractile cytoplasmic debris to form in nuclei and to destroy the yolk-free zone that normally surrounds centrosomes. In most experiments the irradiated blastomere underwent some form of cytokinesis at least once. The development of the unirradiated blastomeres appeared identical irrespective of whether or not the irradiated blastomeres divided. The experiments in Figure 3 were performed as described here in detail for the MS blastomere: At the 4-cell stage ABa, ABp, and P2 were irradiated with the laser microbeam, and the E blastomere was irradiated immediately following the division of EMS. The resulting embryo was allowed to develop for about 12 hr, examined in the light microscope, then processed for immunofluorescence microscopy as above. In some experiments on the MSpaapp cell death in *ced-2* mutants (Figure 4D), a 297 nm, high intensity, ultraviolet monochromator (Bausch and Lomb) was used as a light source instead of the laser beam for killing cells. Both techniques for killing cells gave identical results.

CeMyoD Expression in Wild-Type and Mutant Embryos

Embryos were stained for β-galactosidase activity as in Fire et al. (1990). The CeMyoD–β-galactosidase reporter construct has been described previously (Krause et al., 1990). Although the *PD280* strain used in our experiments carries the CeMyoD–β-galactosidase construct stably integrated on LGI (Andy Fire, unpublished data), we observe the same variability in staining patterns in this strain as reported originally for transgenic lines carrying the same construct on an extrachromosomal array (see Krause et al., 1990). For example, 85% (n = 50) of 28-cell stage wild-type embryos examined showed staining of the first four MS descendants. Only the first four descendants of the MS blastomere were stained in any of these embryos. Approximately 60% (n = 26) of the *pie-1* embryos with early MS staining showed additional positively staining cells in the posterior of the embryo, and 89% (n = 35) of *mex-1* embryos with early MS staining showed additional positively staining cells in the anterior of the embryos. Embryos stained for β-galactosidase were stained subsequently with diamidinophenolindole (DAPI) as described by Krause et al. (1990). The identities of β-galactosidase-positive cells were determined by their positions relative to surrounding DAPI-stained nuclei as observed with fluorescence microscopy. These positions then were compared with diagrams of nuclear positions in 28-cell stage embryos (see Sulston et al., 1983).

Cell Fate Assignment and Lineage Analysis

Experimental embryos were examined in the light microscope to identify cell types on the basis of morphological criteria. In most instances embryos were examined further in the fluorescence microscope after staining with various antibodies. Criteria for scoring hypodermal cells, pharyngeal cells, body wall muscles, and intestinal cells were as described in Bowerman et al. (1992). In addition to morphological criteria for identifying hypodermal cells (Bowerman et al., 1992), we also used a fluorescent lectin (FITC–soybean agglutinin, Vector Laboratories Inc., Burlingame, CA) that stains an extracellular layer deposited by hypodermal cells. This lectin also stains an easily distinguishable extracellular layer associated with pharyngeal cells. In all respects the lectin staining pattern is identical to the staining pattern of the monoclonal "anti-cuticle" antibody originally isolated by S. Strome and B. Wood and described in Cowan and McIntosh (1985) (J. Priess, unpublished data). Because it is easy to identify hypodermal cells in the light microscope, lectin staining was used primarily to verify that embryos processed for immunofluorescence were, in fact, permeabilized sufficiently to permit staining with other cell type–specific antibodies. In such experiments FITC–soybean agglutinin at a final concentration of 0.01 mg/ml was mixed with rhodamine-conjugated secondary antibodies.

Lineage analysis was performed by visually following the cleavage pattern of individual cells viewed with Nomarski DIC optics. Because it is impossible to follow more than a few cells throughout embryogenesis, most of the results described in Table 2 represent a single lineage analysis on an individual embryo. However, in some embryos it was possible to follow two closely related lineages simultaneously until all cells stopped dividing and differentiated. These were: Caaap with Caaaapa, in *pie-1* mutants, and ABalpppap with ABalpppp, and ABarpppap with ABarpppp in *mex-1* mutants. Because MSpaapp and the lineally equivalent descendants from the MS-like blastomeres died relatively early in embryogenesis, it was sometimes possible to follow the cleavages leading to this early cell death in two different blastomeres simultaneously. These were Cpaapp with MSpaapp (two cases) in *pie-1* and ABalpaapp with ABplpaapp (three cases), ABarpaapp with ABprpaapp (two cases), and ABalpaapp with MSpaapp (two cases) in *mex-1* embryos.

Because individual, lineaged cells can not be followed with certainty as embryos are fixed and stained, it was necessary to use only morphological criteria observable in the light microscope for assigning cell fates. In normal development pharyngeal cells aggregate in distinctive compact clusters and become covered with a prominent basement membrane; in lineaged animals a cell was scored as "pharyngeal" only if it became part of a distinct cell cluster surrounded by a basement membrane. Once cells became part of such clusters, further morphological differentiation into pharyngeal structures usually was apparent; often the clusters would develop internal, cuticle-lined cavities similar to the cavity in a normal pharynx. We were not able to distinguish specific subclasses of pharyngeal cells by light microscopy. In normal development body-muscle cells become rounded, then develop small, motile, cytoplasmic processes resembling spikes (J. Priess, unpub-

lished observations; see also Gossett et al. [1982]). These several small spikes gradually coalesce into, or are replaced by, two prominent processes, and the presumptive body wall muscle cell begins twitching. We required lineaged cells to show both spikes and processes before identifying them as body wall muscles. Embryonic cells that migrate into the interior of the body are much harder to see than cells that stay at or near the surface; it was possible to assign pharyngeal cell fates to internal cells with certainty, but it was not possible to assign body wall muscle fates by our criteria. Therefore, in following body wall muscle lineages we scored only those cells that developed on the surface of the mutant embryos. To score muscle development in the ABalpppp, ABalpppap, and ABarpppp lineages in *mex-1* embryos, it was necessary to kill the ABp, EMS, and P2 blastomeres with the laser microbeam, because the ABa descendants invariably were covered over by other cells in unoperated *mex-1* embryos.

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References

Albertson, D. G. (1984). Formation of the first cleavage spindle in nematode embryos. Dev. Biol. *101*, 61–72.

Albertson, D. G., and Thomson, J. N. (1976). The pharynx of Caenorhabditis *elegans*. Phil. Trans. Roy. Soc. (Lond.) B *275*, 299–325.

Austin, J., and Kimble, J. (1987). *glp-1* is required in the germ line for regulation of the decision between mitosis and meiosis in C. elegans. Cell *51*, 589–599.

Austin, J., and Kimble, J. (1989). Transcript analysis of *glp-1* and *lin-12*, homologous genes required for cell interactions during development of C. elegans. Cell *58*, 565–571.

Avery, L., and Horvitz, H. R. (1989). Pharyngeal pumping continues after laser killing of the pharyngeal nervous system of C. elegans. Neuron *3*, 473–485.

Bowerman, B., Eaton, B. A., and Priess, J. R. (1992). *skn-1*, a maternally expressed gene required to specify the fate of ventral blastomeres in the early C. elegans embryo. Cell *68*, 1061–1075.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. Genetics *77*, 71–94.

Cowan, A. E., and McIntosh, J. R. (1985). Mapping the distribution of differentiation potential for intestine muscle and hypodermis during early development in Caenorhabditis elegans. Cell *41*, 923–932.

Edgar, L. G., and McGhee, J. D. (1986). Embryonic expression of a gut-specific esterase in *Caenorhabditis elegans*. Dev. Biol. *114*, 109–118.

Epstein, H. F., Miller, D. M., Gossett, L. A., and Hecht, R. M. (1982). Immunological studies of myosin isoforms in nematode embryos. In Muscle Development, M. Pearson and H. Epstein, eds. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 7–14.

Fire, A., Harrison, S. W., and Dixon, D. (1990). A modular *lacZ* fusion vector for studying gene expression in *Caenorhabditis elegans*. Gene *93*, 189–198.

Goldstein, B. (1992). Induction of gut in *Caenorhabditis elegans* embryos. Nature *357*, 255–257.

Gossett, L. A., Hecht, R. M., and Epstein, H. F. (1982). Muscle differentiation in normal and cleavage-arrested mutant embryos of Caenorhabditis elegans. Cell *30*, 193–204.

Greenwald, I. S. (1985). *lin-12*, a nematode homeotic gene, is homologous to a set of mammalian proteins that includes epidermal growth factor. Cell *45*, 583–590.

Hedgecock, E. M., Sulston, J. E., and Thomson, J. N. (1983). Mutations affecting programmed cell deaths in the nematode *Caenorhabditis elegans*. Science *220*, 1277–1279.

Horvitz, H. R., Ellis, H. M., and Sternberg, P. W. (1982). Programmed cell death in nematode development. Neurosci. Comment *1*, 56–65.

Kemphues, K. J., Priess, J. R., Morton, D. G., and Cheng, N. (1988). Identification of genes required for cytoplasmic localization in early C. elegans embryos. Cell *52*, 311–320.

Kidd, S., Kelley, M. R., and Young, M. W. (1986). Sequence of the *Notch* locus of Drosophila: relationship of the encoded protein to mammalian clotting and growth factors. Mol. Cell. Biol. *6*, 3094–3108.

Krause, M., Fire, A., Harrison, S. W., Priess, J., and Weintraub, H. (1990). CeMyoD accumulation defines the body wall muscle cell fate during C. elegans embryogenesis. Cell *63*, 907–919.

Laufer, J. S., Bazzicalupo, P., and Wood, W. B. (1980). Segregation of developmental potential in early embryos of Caenorhabditis elegans. Cell *19*, 569–577.

Miller, D. M., III, Ortiz, I., Berliner, G. C., and Epstein, H. F. (1983). Differential localization of two myosins within nematode thick filaments. Cell *34*, 477–490.

Priess, J. R., and Thomson, J. N. (1987). Cellular interactions in early C. elegans embryos. Cell *48*, 241–250.

Priess, J. R., Schnabel, H., and Schnabel, R. (1987). The *glp-1* locus and cellular interactions in the early C. elegans embryo. Cell *51*, 601–611.

Schnabel, R. (1991). Cellular interactions involved in the determination of the early C. elegans embryo. Mech. Dev. *34*, 85–100.

Schierenberg, E. (1984). Altered cell-division rates after laser-induced cell fusion in nematode embryos. Dev. Biol. *101*, 240–245.

Schierenberg, E. (1987). Reversal of cellular polarity and early cell-cell interaction in the embryo of *Caenorhabditis elegans*. Dev. Biol. *122*, 452–463.

St Johnston, D., and Nusslein-Volhard, C. (1992). The origin of pattern and polarity in the Drosophila embryo. Cell *68*, 201–219.

Strome, S., and Wood, W. B. (1982). Immunofluorescence visualization of germ-line-specific cytoplasmic granules in embryos, larvae, and adults of *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA *79*, 1558–1562.

Strome, S., and Wood, W. B. (1983). Generation of asymmetry and segregation of germ-line granules in early C. elegans embryos. Cell *35*, 15–25.

Sulston, J. E., Schierenberg, E., White, J. G., and Thomson, J. N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev. Biol. *100*, 64–119.

Wharton, K. A., Johansen, K. M., Xu, T., and Atravanis-Tsakonas, S. (1985). Nucleotide sequence from the neurogenic locus *Notch* implies a gene product that shares homology with proteins containing EGF-like repeats. Cell *43*, 567–581.

Wolf, N., Priess, J., and Hirsh, D. (1983). Segregation of germline granules in early embryos of *Caenorhabditis elegans*: an electron microscopic analysis. J. Embryol. Exp. Morphol. *73*, 297–306.

Wood, W. B. (1991). Evidence from reversal of handedness in C. elegans embryos for early cell interactions determining cell fates. Nature *349*, 536–538.

Yamaguchi, Y., Murakami, K., Furusawa, M., and Miwa, J. (1983). Germline-specific antigens identified by monoclonal antibodies in the nematode *Caenorhabditis elegans*. Dev. Growth Diff. *25*, 121–131.

Yochem, J., Weston, K., and Greenwald, I. (1988). The *Caenorhabditis elegans lin-12* gene encodes a transmembrane protein with overall similarity to *Drosophila Notch*. Nature *335*, 547–550.

Yochem, J., and Greenwald, I. (1989). *glp-1* and *lin-12*, genes implicated in distinct cell–cell interactions in C. elegans, encode similar transmembrane proteins. Cell *58*, 553–563.