MES-1, a protein required for unequal divisions of the germline in early *C. elegans* embryos, resembles receptor tyrosine kinases and is localized to the boundary between the germline and gut cells

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**SUMMARY**

During *Caenorhabditis elegans* embryogenesis the primordial germ cell, P₄, is generated via a series of unequal divisions. These divisions produce germline blastomeres (P₁, P₂, P₃, P₄) that differ from their somatic sisters in their size, fate and cytoplasmic content (e.g. germ granules). *mes-1* mutant embryos display the striking phenotype of transformation of P₄ into a muscle precursor, like its somatic sister. A loss of polarity in P₂ and P₃ cell-specific events underlies the Mes-1 phenotype. In *mes-1* embryos, P₂ and P₃ undergo symmetric divisions and partition germ granules to both daughters. This paper shows that *mes-1* encodes a receptor tyrosine kinase-like protein, though it lacks several residues conserved in all kinases and therefore is predicted not to have kinase activity. Immunolocalization analysis shows that MES-1 is present in four- to 24-cell embryos, where it is localized in a crescent at the junction between the germline cell and its neighboring gut cell. This is the region of P₂ and P₃ to which the spindle and P granules must move to ensure normal division asymmetry and cytoplasmic partitioning. Indeed, during early stages of mitosis in P₂ and P₃, one centrosome is positioned adjacent to the MES-1 crescent. Staining of isolated blastomeres demonstrated that MES-1 was present in the membrane of the germline blastomeres, consistent with a cell-autonomous function. Analysis of MES-1 distribution in various cell-fate and patterning mutants suggests that its localization is not dependent on the correct fate of either the germline or the gut blastomere but is dependent upon correct spatial organization of the embryo. Our results suggest that MES-1 directly positions the developing mitotic spindle and its associated P granules within P₂ and P₃, or provides an orientation signal for P₂- and P₃-specific events.

**Key words:** *C. elegans*, MES-1, Germline, Asymmetric division, Mitotic spindle, P granules

**INTRODUCTION**

Asymmetric localization of cytoplasmic factors and unequal cell division are fundamental to the development of all eukaryotes. When these mechanisms are used to generate distinct daughter cells during development, the two events must be coordinately regulated, to ensure the proper segregation of factors to each daughter. This is observed in such diverse systems as bud formation in the yeast *Saccharomyces cerevisiae* and neurogenesis in *Drosophila* (Madden and Snyder, 1998; Hawkins and Garriga, 1998; Jan and Jan, 2000; Schweisguth, 2000). As these events have become better understood, it has become apparent that many molecular components of cellular asymmetry are conserved (Drubin and Nelson, 1996; Shulman et al., 2000). Thus, elucidating new players and mechanisms for guiding asymmetric events can provide insights that extend beyond the system of study.

Early *C. elegans* embryos provide an ideal system in which to study asymmetry. These embryos undergo a series of stem-cell-like asymmetric divisions to establish the germline and somatic founder cells. The one-cell zygote, P₀, divides to form a large somatic cell, AB, and a smaller germ line cell, P₁ (Deppe et al., 1978; Sulston et al., 1983). P₁ and its daughter (P₂) and granddaughter (P₃) each divide asymmetrically to generate a somatic and a germline cell. The last of these divisions generates the primordial germ cell, P₄. Besides the difference in size, the germline cells (P₁, P₂, P₃, P₄) differ from their somatic sisters in their fate, in the timing of their subsequent divisions and in their cytoplasmic content. The latter is strikingly illustrated by the presence of P granules, cytoplasmic structures that are specifically segregated to the germline cell at each division and that are required for fertility (Strome and Wood, 1982; Kawasaki et al., 1998).

Many of the components required for early *C. elegans* asymmetry have been elucidated. In the newly fertilized embryo, the sperm entry point specifies the posterior end (Goldstein and Hird, 1996). The sperm component(s) that accomplishes this has not yet been identified, but likely candidates are the centrosomes and their associated microtubules, which may cause cytoplasmic and cortical
showed that in cells, Z2 and Z3, and as a result develop into sterile adults produced from homozygous mothers lack the primordial germ cell. This results in alignment of the spindle along the dorsal-ventral axis, asymmetric positioning of the spindle closer to the ventral pole and delivery of the majority of P granules to the ventral cytoplasm. These events ensure that P granules are delivered to the small, ventral (germline) daughter cell. These events in P2 and P3 are altered and uncoordinated in mes-I mutant animals. The nucleus-centrosome complex does not migrate and P granules become segregated to one side instead of one pole of the spindle, resulting in their distribution to both daughter cells (Fig. 1). It is postulated that, in addition to P granules, other factors, such as muscle determinants, are also mislocalized; the presence of muscle determinants in both P4 and D causes both daughters to produce muscle.

Here, we present molecular characterization of mes-I and show that it encodes a transmembrane protein with similarity to receptor tyrosine kinases, though it is unlikely to have kinase activity. We also show that MES-1 is localized to a restricted portion of the cell membrane in the P cells of four- to 24-cell embryos. This pattern, on the P-cell membrane adjacent to its gut-cell neighbor, correlates with the location at which the next P cell forms. Thus, MES-1 localization is consistent with its role in the asymmetric divisions that generate P3 and P4. Analysis of its distribution in various cell-fate and patterning mutants suggests that the localization of MES-1 is not dependent on the correct fate of either the P cells or the gut cells, but is dependent upon correct spatial organization of the embryo.

**MATERIALS AND METHODS**

**Strains and alleles**

Maintenance and genetic manipulation of *C. elegans* were carried out as described in Brenner (1974). *C. elegans* variety Bristol, strain N2 was used as the wild-type strain. The following mutations, polymorphisms, balancers and deficiencies were used in this study. LGI: glp-4(bn2ts), unc-13(e1091), mom-4(or39), hT1(I,V), pop-l(za189), dpy-5(e61), hT2[bi-4(e937) let-7(h661)](I,III), unc-101(m1), par-6(za222), hT1[unc-54(h1040)]. LGII: rol-1(e91), mes-1(za121), mmC1[dpy-10(e128) unc-52(e444)]. LGIII: hT2[bi-4(e937) let-7(h661)](I,III), pie-1(za154), unc-25(e156), dpy-19(e1259), glp-1(g339), par-2(bv32), unc-45(e286ts), scC1[dpy-1(e1) let-7(?), lon-1(e185), par-3(i771), qC1[dpy-19(e1259ts) glp-1(q339)]. LGIV: hT1(I,V), rol-4(s8), par-1(b274), nT1(I,V), DnT1[unc-754(let)](IV), unc-9(e152), unc-22(s7), let-99(s1201), unc-31(e169). LGV: dpy-11(e1180), mom-2(ow42), nT1(I,V), him-5(e1490), DnT1[unc-754(let)](IV), par-4(i75ts). LGX: mes-1(bn74rs), mes-1(bn7ts), mom-4, mom-2, let-99, pop-1 and pie-1 strains were from the Caenorhabditis Genetics Center. Strains containing par mutations were a gift from K. Kemphues; mes-1 was a gift from G. Seydoux; and pos-1 was a gift from J. Priess.

**Cloning mes-1**

*mes-1* was mapped to cosmid C38D5, as described in Browning et al. (1996). Restriction fragments of C38D5 were cloned into pBS-Bluescript (Stratagene) using standard methods. Cosmid DNA and the cytoplasm destined for the somatic daughter cell. It is unknown if this disappearance is due to degradation or disassembly of the granules. The nucleus-centrosome complex rotates and migrates to the ventral side of the P cell, and then forms the spindle. When the nuclear membrane breaks down, the associated P granules are released. Thus, in P2 and P3, rotation and migration of the nucleus-centrosome complex accomplishes three results: orientation of the spindle along the dorsal-ventral axis, asymmetric positioning of the spindle closer to the ventral pole and delivery of the majority of P granules to the ventral cytoplasm. These events ensure that P granules are delivered to the small, ventral (germline) daughter cell. These events in P2 and P3 are altered and uncoordinated in mes-I mutant animals. The nucleus-centrosome complex does not migrate and P granules become segregated to one side instead of one pole of the spindle, resulting in their distribution to both daughter cells (Fig. 1). It is postulated that, in addition to P granules, other factors, such as muscle determinants, are also mislocalized; the presence of muscle determinants in both P4 and D causes both daughters to produce muscle.

Here, we present molecular characterization of mes-I and show that it encodes a transmembrane protein with similarity to receptor tyrosine kinases, though it is unlikely to have kinase activity. We also show that MES-1 is localized to a restricted portion of the cell membrane in the P cells of four- to 24-cell embryos. This pattern, on the P-cell membrane adjacent to its gut-cell neighbor, correlates with the location at which the next P cell forms. Thus, MES-1 localization is consistent with its role in the asymmetric divisions that generate P3 and P4. Analysis of its distribution in various cell-fate and patterning mutants suggests that the localization of MES-1 is not dependent on the correct fate of either the P cells or the gut cells, but is dependent upon correct spatial organization of the embryo.
Sequence analysis and 5’ end isolation

cDNAs were initially sequenced using Sanger dideoxy-mediated chain termination (Sambrook et al, 1989). Additional sequencing was performed as described in Holdeman et al. (1998). The 5’ end was isolated by RT-PCR. First-strand cDNA synthesis used an oligonucleotide primer that corresponded to the 5’ end of the longest isolated partial cDNA. PCR on first-strand RT reaction products used a 5’ oligonucleotide that corresponded to the predicted 5’ end and an internal 3’ primer. The DNA fragment was cloned into pBS-Bluescript (Stratagene) and sequenced. The 5’ end was confirmed by repeating the PCR using an oligonucleotide that hybridized to spliced-leader (SL1) sequence for the 5’ primer (Spieth et al., 1993).

For sequencing the mes-1 alleles, genomic DNA was prepared from homozygous mutant worms carrying each of the eight mutant alleles. The mes-1 gene was amplified using PCR as five segments that covered the entire coding region, some introns and all intron-exon boundaries. Each segment was cycle sequenced using ABI Prism Big Dye Cycle Sequencing (PE Applied Biosystems).

Northern analysis

Northern hybridization analysis was performed as described in Holdeman et al. (1998), using the rpp-1 transcript, which encodes a ribosomal protein, as a loading control (Evans et al., 1997). The intensity of the transcript bands was determined either from a scanned autoradiograph using NIH Image software or using a Molecular Dynamics PhosphorImager. The relative levels were expressed as a ratio of the mes-1 signal intensity to the intensity of the rpp-1 signal.

Antibody production

A bacterial 6xHis fusion expression construct was made by cloning a 1418 base pair fragment, corresponding to intracellular amino acids 495-966, into the SacI and HindIII sites of the pET-28a vector (Novagen). This fragment was generated by PCR of the original cDNA isolate using oligonucleotides containing a SacI site (5’ primer) and a HindIII site (3’ primer). The fusion protein was expressed in E. coli strain BL21(DE3) and purified using Ni-NTA agarose columns (Qiagen). The purified protein, isolated in a polyacrylamide gel slice, was injected into rats with Freund’s adjuvant. Anti-MES-1 antibodies were purified from crude antiserum by blot affinity purification and elution by 0.2 M glycine, pH 2.8 (Olmswed, 1986). Following dialysis against PBS, the antibodies were passed over a column of 6xHis-MES-6 (Korf et al., 1998) attached to Affi-GEL active ester agarose 10 (Biorad) to remove nonspecific and denatured antibody. Anti-MES-1 antibodies were not able to detect MES-1 on a western blot from total embryo extract, but were able to detect MES-1 if immunoprecipitation was performed first.

Immunofluorescence analysis

To visualize MES-1 localization, animals were cut in a drop of M9 and fixed in cold methanol followed by cold acetone as described in Strome and Wood (1983). Samples were blocked in PBS, 1.5% bovine serum albumin, 1.5% non-fat dried milk prior to applying 1:3 dilution of affinity-purified anti-MES-1 antibodies. Crude rabbit anti-PGL-1 antisemur (Kawasaki et al., 1998) diluted 1:30,000, rabbit anti-penta-acetyl-histone H4 (kindly provided by D. Allis; Lin et al., 1989) diluted 1:6000, and rabbit anti-actin (against C-terminal peptide, Sigma) diluted 1:250, were also used. Serum from one animal used to produce anti-MES-1 antibodies contains anti-centrosome antibodies as well as anti-MES-1 antibodies. The centrosomal staining is unrelated to MES-1, since it is still detectable in bn74 worms (a complete deletion allele of mes-1). Secondary antibodies used were affinity-purified fluorescein-conjugated goat anti-rat, rhodamine-conjugated goat anti-rabbit, Cy3-conjugated goat anti-rabbit (Jackson) or Alexa 488 goat anti-rat (Molecular Probes), diluted 1:250. Samples were mounted in Vectashield anti-fade mounting media (Vector Laboratories). In one series of staining, ethidium bromide was included in the mounting medium to detect DNA.

Blastomere isolation

Blastomeres were isolated as described by Shelton et al. (1996), with modifications suggested by A. Skop (personal communication). Briefly, wild-type worms were cut in a drop of M9. The eggshell of young embryos (one-, two- and four-cell stages) was removed with hypochlorite (5-6% stock solution, diluted 1:6) treatment. Embryos were washed in egg salt buffer and the eggshell was further digested away in 5 U/ml chitinase, 20 mg/ml chymotrypsin in egg salt buffer for 7 minutes. Embryos were washed in three droplets of simplified growth medium (SGM) supplemented with 35% heat-treated calf serum. The vitelline membrane was removed by pipetting the embryos repeatedly through a narrow-bore drawn glass capillary. Cells were washed in serum-free SGM and fixed in 2% paraformaldehyde. The cells were then affixed to GCP-coated slides (0.2% gelatin, 0.002% chromakey alun, 1 mg/ml polylysine) and stained with antibodies as described in the above section.

RESULTS

MES-1 is partially similar to receptor tyrosine kinases

To investigate how MES-1 participates in the asymmetric divisions of P2 and P3, we analyzed the gene. The mes-1 gene is located in the egl-15-sma-5 interval on LGX. Because there were few useful genetic markers for recombination mapping in this interval, macrorestriction analysis was used to search for allele-associated restriction fragment length polymorphisms (RFLPs) (Browning et al., 1996). This analysis used infrequently cutting restriction enzymes and pulsed-field gel electrophoresis to examine large fragments of DNA. DNA from two alleles of mes-1 showed an RFLP that mapped to cosmid C38D5 (Browning et al., 1996; data not shown). In DNA transformation rescue tests, the Mes-1 phenotype was rescued by the C38D5 cosmid, as well as by a 17 kb fragment of C38D5. This fragment was used to screen a cDNA library (Barstead and Waterston, 1989), and four different CDNA that mapped to C38D5 were identified. It has been found that RNA prepared from cloned genes, when injected into wild-type hermaphrodites, produces a gene-specific phenocopy (Guo and...
Differences in the subcellular localization of MES-1 indicate that this protein functions in RNA interference (RNAi), a process that is essential for C. elegans development. MES-1 is present in the cytoplasm and is concentrated in the P granules, which are organelles involved in RNAi. The figure shows the distribution of MES-1 in wild-type and mes-1 mutant embryos, highlighting the role of MES-1 in the partitioning of P granules.

**Fig. 1.** Division and P-granule partitioning in P2 in wild-type and mes-1 four-cell embryos. Nuclei are shown in black, centrosomes in green and P granules in blue. Anterior is towards the left and ventral is downwards. In wild-type embryos, the majority of P granules associate perinuclearly and the nucleus-centrosome complex rotates and migrates to the ventral side of P2, where P granules are deposited and the spindle forms. In mes-1 embryos P granules associate perinuclearly, but the nucleus-centrosome complex does not rotate and migrate. This results in a symmetrically placed spindle, which generally comes to lie along the correct axis. P granules are usually segregated to one side of the spindle and distributed to both daughters.

Kemphues, 1995; Rocheleau et al., 1997; Fire et al., 1998). This phenomenon, termed RNA interference (RNAi), was used to determine which of the four cDNAs from C38D5 corresponded to mes-1. Injection of RNA from one cDNA phenocopied the Mes-1 defects. This cDNA was confirmed as mes-1 by failure to detect a transcript in northern analysis of mRNA from a deletion allele of mes-1 and by sequencing mutant alleles (see below).

The nucleotide sequence of the mes-1 cDNA (Accession number AF200199, listed in GenBank under reference cosmid F54F7, Accession number Z67755, which extensively overlaps with cosmid C38D5) predicts that mes-1 encodes a protein of 966 amino acids (Fig. 2A) that shows overall structural similarity to receptor tyrosine kinases (RTKs; for review, see Hanks and Quinn, 1991; Van Der Greer et al., 1994). RTKs are a very large, well conserved family of proteins that are involved in signaling pathways for cell growth and differentiation. Consistent with it belonging in this class of proteins, MES-1 contains a signal sequence, a single transmembrane domain, and an intracellular kinase-like domain. The extracellular region of MES-1 contains seven potential glycosylation sites (consensus NXS/T where X is any amino acid except P and D) but no recognizable domains or motifs. The kinase region of RTKs consists of 11 subdomains, called I-XI, that have the following functions: I-III bind the phosphate donor Mg^{2+}/ATP; IV is structural; V-VII are catalytic; VIII-IX provide substrate recognition; X and XI are undefined. The predicted MES-1 protein lacks any significant sequence similarity to subdomains I and II, and its subdomains III-XI show only 20-25% identity with other RTKs, compared with >35% identity for typical kinases. Furthermore, even within these subdomains, several amino acids required for catalytic activity are absent from MES-1 (Fig. 2A). Specifically, MES-1 has changes in 10 of the 21 invariant and nearly invariant amino acids that are found in almost all kinases, including serine/threonine kinases (Hanks and Quinn, 1991). The lack of a recognizable nucleotide binding site and the above described sequence differences within the kinase region suggest that MES-1 is not a functional kinase.

**mes-1 alleles**

Two alleles of mes-1 contain substantial alterations of the gene. bn74 has a 25 kb deletion that removes the entire coding region of mes-1 (Browning et al., 1996). bn89 is a more complex rearrangement consisting of both a deletion and a duplication: a 5.5 kb deletion removes the upstream region and 0.5 kb of coding sequence and a approx. 3 kb duplication contains much of the coding sequence. As expected, neither of these mes-1 alleles produces mRNA or protein (Figs 3A, 4D and data not shown).

The other eight alleles of mes-1 produce mRNA and were sequenced to determine their molecular lesions (Fig. 2B). The entire coding region and all intron-exon boundaries were sequenced. For one allele, bn7, no changes were found. Three alleles, bn24, bn90 and bn84, contain base pair changes that result in a premature stop codon. Allele bn56 contains a change in a 3’ splice site. Two alleles, q367 and q222, contain missense amino acid changes. Allele bn52 contains two mutations: a 50 bp deletion within intron 1 and a missense change of Gly890 to Glu in subdomain IX (Fig. 2B). RTKs have an invariant serine at that position. Only allele bn52 produces detectable embryo staining (data not shown). The distribution of MES-1 in bn52 is the same as in wild type, but the protein does not persist as long. Interestingly, all ten mes-1 alleles result in equally severe phenotypes.

**mes-1 transcript is enriched in adults and embryos**

The mes-1 gene produces a 3.7 kb transcript, which is first detected at the L4 stage, accumulates in adults, and is present in embryos (Fig. 3B). To investigate if the mes-1 transcript is germline specific, northern analysis was performed using RNA from glp-4(bn2) worms, which essentially lack germ cells (Beanan and Strome, 1992). The mes-1 transcript level in adult glp-4(bn2) worms is 20-30% of the wild-type adult level (data not shown). The enrichment of mes-1 in the germline of worms and its presence in embryos are consistent with the known function of mes-1 in early embryos. The presence of significant levels of mes-1 RNA in somatic cells (i.e. in glp-4 worms) was unexpected since mes-1 mutants display a strictly maternal-effect sterile phenotype. No specific pattern of MES-1 staining was detected in somatic tissues when wild-type animals were compared with bn74 animals.
MES-1 localization appears to correlate with the position to which the nucleus-centrosome complex migrates (Hird et al., 1996). To determine the relative positions of MES-1 and the spindle, embryos were stained with antibodies that detect MES-1 localization at the junction between the germline cell and the gut cell

To address when and where MES-1 protein functions, its subcellular localization was determined by immunofluorescence microscopy. Rat antisera were raised against a 6×His-tagged fusion protein containing the 475 amino acid intracellular domain of MES-1 and were then affinity purified. The specificity of the antibody was confirmed by the fact that MES-1 staining was undetectable in worms homozygous for a deletion allele of \textit{mes-1} (allele \textit{bn74}; Fig. 4D). MES-1 is detected as a thin crescent, initially in four-cell embryos between P2 and EMS and then in eight- to 12-cell embryos between P3 and E (Fig. 4A,B). The EMS blastomere gives rise to E, which produces gut (intestine), and MS, which produces pharynx and muscle (Deppe et al., 1978; Sulston et al., 1983). In this paper we refer to EMS as a gut cell, though it generates more than just gut.

MES-1 persists through the formation of P4 at the 24-cell stage (Fig. 4C). After P4 is generated, MES-1 begins to fade and is usually not detectable beyond the 30-40-cell stage (data not shown).

MES-1 is predicted to span the membrane. To confirm that MES-1 was located at the cell periphery, embryos were costained with an antibody to actin, which is concentrated in the cell cortex (Fig. 5A-E). Where MES-1 staining was present, it was adjacent to and sometimes overlapped with actin staining. The crescent shape of MES-1 staining outlined a portion of the contact area between the germline and the gut cell; the tips of the crescent point dorsally, towards the interior of the embryo.

MES-1 localization at the junction between the germline cell and the gut cell

**Fig. 2.** MES-1 protein sequence and allele mutations. (A) Predicted MES-1 amino acid sequence. Potential glycosylation sites are marked with asterisks (consensus NXS/T) and the transmembrane domain is underlined. Roman numerals show the approximate locations of kinase subdomains described in the text. Amino acids that are invariant or nearly invariant in RTKs are boxed. Blue boxes indicate the residue is conserved in MES-1 and red boxes indicate the residue is not conserved. (B) Schematic drawing of MES-1 showing the relative positions and amino acid changes for seven of the eight non-deletion alleles of \textit{mes-1}. The shaded boxes represent the transmembrane and the kinase-like domains.

**Fig. 3.** Northern analysis of \textit{mes-1} transcripts. (A) \textit{mes-1} mRNA is detectable in wild type (N2) and undetectable in the \textit{mes-1} deletion allele \textit{bn74}. (B) \textit{mes-1} mRNA accumulation during development. PolyA+ RNA was isolated from wild-type hermaphrodites, which were synchronized at each of the six developmental stages shown. A, adults; E, embryos; L1-L4, four larval stages. In (A) and (B) a 2.6 kb partial cDNA clone of \textit{mes-1} was used as a probe to detect the 3.7 kb transcript. The transcript of the ribosomal gene, \textit{rpp-1}, was used as a loading control. Relative levels of the \textit{mes-1} transcript are shown at the bottom.
centrosomes (Fig. 6A-B). From late prophase through anaphase, the more ventral centrosome was closely juxtaposed to the MES-1 crescent, with the two appearing to almost touch (Fig. 6A). By telophase, MES-1 and the centrosome had separated (Fig. 6B).

The pattern of MES-1 staining is consistent with its function in the asymmetric divisions of P2 and P3. The stages that exhibit MES-1 localization temporally correlate with the P2/P3-specific role for MES-1. The loss of MES-1 after the formation of P4 reinforces the idea that MES-1 is only needed until this cell is formed. Its localization between the P cell and the gut cell also spatially correlates with where the future P cell will form (Hird et al., 1996).

**MES-1 is localized in the germline cell starting with P2**

As a consequence of the localization of MES-1 to the area of contact between P2 and EMS and between P3 and E, it was not apparent from examining intact embryos whether the P cell or the gut cell or both contained protein. To address this issue, the P2 cell was dissociated from four-cell embryos and both it and the remaining embryo portion were fixed and stained for MES-1 (Fig. 7). MES-1 was detected on the surface of P2 and not on any other cells of the remaining embryo, most notably not on the EMS cell. Within the isolated P2 cell, the distribution of MES-1 was still in a crescent shape. Given the restriction of MES-1 to the P2 cell at the four-cell stage, it is reasonable to assume that in later-stage embryos MES-1 is present on the surface of P3 and P4, although this was not tested by staining isolated P3 and P4 cells. Consistent with this assumption, co-staining of MES-1 and actin (see Fig. 5) shows the cytoplasmic domain of MES-1 to be on the P-cell side of the zone of germline-gut contact. These immunostaining patterns suggest that MES-1 functions cell autonomously within the P cell.

**Effects of cell fate and polarity defects on MES-1 localization**

The distinct localization of MES-1, on P2 and P3 at the border with the gut cell, raised several questions, which were addressed by staining various early embryonic mutants for MES-1.

First, is the germline fate of the P cells required for MES-1 expression and localization? In pie-1 mutants P2 develops like its somatic sister, EMS (Mello et al., 1992). PIE-1 represses transcription of somatically expressed genes in the P cells, and the loss of this repression in pie-1 mutants causes the fate transformation of P2 (Seydoux et al., 1996). MES-1 localization appeared wild-type in pie-1 mutant embryos (data not shown). This suggests that P2 can lose at least one germline trait (transcriptional repression) and need not manifest a germline fate to properly express and localize MES-1.

Second, is the gut fate of EMS required for correct MES-1 localization? Fig. 4. MES-1 localization in wild-type and mes-1 mutant embryos. Projections of multiple confocal optical sections. Anterior is towards the left and ventral is downwards. Top row stained with anti-MES-1 antibodies. Middle row stained with anti-PGL-1 and anti-acetyl-histone H4, which detect P granules and DNA, respectively. Bottom row shows schematic drawings of the embryos, with the germline and the gut cells indicated. Cells containing P granules are shaded gray, and the MES-1 crescent is shown as a thick black line. Delineation of the cells is only approximate and is based on the positions of the nuclei. Wild-type (N2) embryos at the four-cell stage (A), 12-cell stage (B) and 26-cell stage (C). MES-1 is localized as a crescent between the germline and the gut cells, specifically between P2 and EMS (A), P3 and E (B), and P4 and Ep (C). (D) bn74, a complete deletion of the mes-1 gene, lacks detectable MES-1 staining; eight-cell stage embryo shown. Scale bar: 10 μm.
In pop-1 embryos, EMS produces two E cells instead of an E and an MS cell (Lin et al., 1995). In mom-2 and mom-4 embryos, EMS produces two MS cells (Thorpe et al., 1997; Rocheleau et al., 1997). MES-1 localization appeared to be the same as in wild type in these mutant embryos (data not shown), suggesting that proper MES-1 localization is not dependent on the correct specification or fate of the EMS lineage.

Last, do factors that regulate establishment of polarity and unequal divisions in one- and two-cell embryos affect the

Fig. 5. MES-1 and actin localization in a wild-type 15-cell embryo. Projections of multiple confocal optical sections. Anterior is leftwards and ventral is downwards. Left column is anti-MES-1 staining. Middle column is anti-actin staining. Right column contains merged images. Red shows actin, green shows MES-1. MES-1 is localized at the cell periphery. (A) Projection of upper sections showing half the crescent. (B) Projection of lower sections showing the other half of the crescent. (C) Projections of all confocal sections. These sections show that one half of the crescent is on the left side of the embryo, and the other on the right, and that the tips of the crescent are pointed dorsally. (D) DNA stained with ethidium bromide. (E) Enlargement of the merged MES-1 crescent and actin.

Fig. 6. MES-1 and centrosome localization in wild-type six- to seven-cell embryos. Left panels show staining with antibodies to MES-1 and centrosomes. Right panels show staining of DNA with ethidium bromide. Projections of multiple confocal optical sections. Anterior is leftwards and ventral is downwards. Arrows point to P₂ cell in (A) pro-metaphase and (B) telophase of mitosis. Arrowhead in (A) points to MES-1 crescent ‘sandwiched’ between posterior centrosome of EMS (left of MES-1) and ventral centrosome of P₂ (right of MES-1).
distribution of MES-1 in four-cell and later stages? Mutants used in this analysis exhibited MES-1 staining patterns that fell into two general categories.

The first category is a lack of detectable MES-1. This pattern was seen in mutant embryos from par-1, par-3 and par-4 animals. par-1 embryos undergo a symmetric first division and distribute P granules to both daughter cells (Kemphues et al., 1988). par-4 embryos show fairly normal first division asymmetry, but like par-1 distribute P granules to both daughter cells. par-3 embryos undergo a symmetric first division and sometimes distribute P granules to both daughters. MES-1 was not detectable in embryos from any of these par mutants (Fig. 8A and data not shown).

The second category displays a variety of MES-1 staining patterns which include ‘wild-type’, ‘ectopic’ and absent. Classification of the type of MES-1 staining was based on the following criteria: ‘wild-type’ – the MES-1 crescent was asymmetrically distributed on one side of a posteriorly located cell that contained P granules, but the embryo itself did not always appear to be the same as wild type; ‘ectopic’ – a MES-1 crescent was present on more than one face of a single cell or on more than one cell or on a cell that was not at the posterior end of the embryo. Mutants that produce a variable MES-1 pattern are pos-1, par-2, par-6, let-99 and mex-1 (Table 1 and Fig. 8B-E). pos-1 embryos display several defects, including symmetric division of P 2 and P 3 and distribution of P granules to both daughters of P 3 (Tabara et al., 1999), which we speculate may result from an absence or altered distribution of MES-1. par-2 embryos correctly segregate P granules in the first division, but show an altered P 1 division pattern and distribute P granules to both daughters of P 1 (Kemphues et al., 1988; Boyd et al., 1996). Most par-6 embryos correctly segregate P granules in the first division, but in the four-cell stage, P granules are detected in all four blastomeres (Watts et al., 1996). In let-99 embryos spindles are not properly oriented, but nevertheless P granules are segregated properly in the first two divisions (Rose and Kemphues, 1998). In mex-1 embryos all founder lineages, including the germline, display cell-fate defects, suggesting that MEX-1 plays a role in general polarity of the embryo (Schnabel et al., 1996). mex-1 embryos often correctly segregate P granules during the division of P 0 but then mis-segregate them in subsequent divisions (Mello et al., 1992). As stated above, all of these mutants display a variable, and often aberrant, distribution of MES-1 (Table 1). Together, these results indicate that MES-1 distribution depends, either directly or indirectly, on polarity cues established earlier in the embryo.

Table 1. Percentage of mutant embryos displaying aberrant MES-1 localization

<table>
<thead>
<tr>
<th>Mutant strain</th>
<th>Type of aberrant MES-1 staining</th>
<th>2-3</th>
<th>4</th>
<th>6-7</th>
<th>8</th>
<th>10-14</th>
<th>15-20</th>
<th>21-28</th>
</tr>
</thead>
<tbody>
<tr>
<td>pos-1 (zu148)</td>
<td>Ect</td>
<td>10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>79</td>
<td>87</td>
<td>75</td>
</tr>
<tr>
<td>mex-1 (zu121): 16°C</td>
<td>Ect</td>
<td>20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20</td>
<td>11</td>
<td>25</td>
<td>74</td>
<td>89</td>
<td>71</td>
</tr>
<tr>
<td>mex-1 (zu121): 25°C</td>
<td>Ect</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>53</td>
<td>41</td>
<td>48</td>
<td>36</td>
</tr>
<tr>
<td>par-2 (kw32)</td>
<td>Ect</td>
<td>0</td>
<td>12</td>
<td>17</td>
<td>32</td>
<td>31</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Abs</td>
<td>65</td>
<td>17</td>
<td>44</td>
<td>56</td>
<td>52</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>let-99 (s1201)</td>
<td>Ect</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>15</td>
<td>48</td>
<td>42</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Abs</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>11</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>par-6 (zu222)</td>
<td>Ect</td>
<td>0</td>
<td>50</td>
<td>50</td>
<td>78</td>
<td>71</td>
<td>74</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Abs</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>7</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>For each stage a minimum of twenty embryos were counted.

<sup>b</sup>Ect - ectopic MES-1 pattern.

<sup>c</sup>Abs - absent or undetectable MES-1.

<sup>d</sup>Since MES-1 is not normally detected prior to the four-cell stage, the precocious staining is considered aberrant.
DISCUSSION

MES-1 is a predicted transmembrane protein that shows overall structural similarity to receptor tyrosine kinases, although it is unlikely to have kinase activity. MES-1 is at the surface of P2 and P3, which correlates both spatially and temporally with its role in the asymmetric divisions of these cells. Early embryonic mutants that alter embryonic polarity cause MES-1 to be ectopically localized or absent.

Function of MES-1 in asymmetric germline divisions

Based on the defects in migration of the nucleus-centrosome complex in mes-1 mutants and on the membrane localization of MES-1, we propose the following two models for how it may function. MES-1 may directly cause the movement of the nucleus-centrosome complex and its associated P granules in P2 and P3. In this model, MES-1 probably interacts with microtubules emanating from the centrosomes; MES-1 may bind microtubules directly or via a microtubule-associated protein or microtubule motor (for examples, see Thaler and Haimo, 1996; Stearns, 1997). MES-1, together with any associated proteins, pulls the nucleus-centrosome complex towards itself, resulting in proper alignment and asymmetric positioning of the spindle. The close physical proximity between the MES-1 crescent and one centrosome of the spindle seems to support this model. Alternatively, MES-1 could provide an orientation signal for events in P2 and P3. Its location may mark an area of the cell and signal where the next P cell is to be generated. The nucleus-centrosome complex and P granules would then respond to this signal and migrate towards it. These two models are not necessarily mutually exclusive.

MES-1 also participates in orienting a gradient of activity that stabilizes or destabilizes P granules. As described earlier, P granules not only aggregate perinuclearly, but they also disappear from the portion of the cell destined for the somatic daughter. This behavior is probably due to a gradient of P-granule stabilizing and/or destabilizing activity. In mes-1 mutant embryos, P granules disappear from the wrong region...
of the cell (see Fig. 1). As a result, P granules come to lie along one side, instead of one pole, of the spindle and consequently are partitioned to both daughter cells. The role of MES-1 in positioning the P-granule gradient, but not in forming the gradient, is analogous to that of Insuteable in Drosophila neuroblasts. The determinant Numb is asymmetrically localized as a crescent and placement of the crescent, but not its formation, is dependent on Insuteable (Kraut et al., 1996).

In formulating models for MES-1 function, the temperature-sensitivity of the Mes-1 phenotype needs to be considered. All ten alleles of mes-1, including a deletion of the entire coding sequence, exhibit a large percentage (70-90%) of sterile progeny at high temperature and a low percentage (10-15%) at lower temperature (Capowski et al., 1991). Indeed, division asymmetry in P2 and P3 are usually normal in mes-1 embryos at low temperature and altered at high temperature (Strome et al., 1995; E. Schierenberg and S. S., unpublished). This reveals that the process that controls unequal division and partitioning at low temperature and altered at high temperature (Strome et al., 1995; E. Schierenberg and S. S., unpublished). This reveals that the process that controls unequal division and partitioning in P2 and P3 is inherently sensitive to temperature and does not absolutely require MES-1 at low temperature. Through an interaction or dimerization, MES-1 may stabilize a factor that is prone to inactivation at high temperature. Alternatively, there may be redundant pathways for achieving correct division of P2 and P3, one controlled by MES-1 and a separate one that is prone to inactivation at high temperature.

Possible mechanisms of MES-1 function
While MES-1 shows overall similarity to receptor tyrosine kinases, it lacks several subdomains and invariant amino acids that are required for kinase activity. Thus, it is probably not catalytically active. How then does MES-1 function?

MES-1 may acquire kinase activity through association with another kinase, either another RTK (e.g. Qian et al., 1992, 1994; Wada et al., 1990; Pinkas-Kramarski et al., 1996) or a non-receptor kinase. An associated kinase could either phosphorylate MES-1, creating docking sites (see below), or itself generate an intracellular signal to orient P2 and P3 events, as discussed above. An alternative scenario is that association of MES-1 with a kinase may instead have a dominant-negative effect and inhibit the activity of the kinase.

MES-1 may assemble into and function as part of a localized multi-component complex, similar to many kinases and phosphatases (Faux and Scott, 1996; Pawson and Scott, 1997). Indeed, MES-1 contains four potential binding sites for Src-homology-2 (SH2) domains. SH2 domains recognize phosphotyrosines in a short motif and mediate protein-protein interactions (Pawson, 1995). Budding in yeast provides a striking example of assembly and use of a localized complex to orient cell division. The bud site contains multiple proteins, including GTPases and Bni1, a scaffold protein that links the GTPases with the actin cytoskeleton (for review see Cabib et al., 1998; Lee et al., 1999). Localized reorganization of the actin cytoskeleton leads to reorientation of the mitotic spindle. A similar series of events may occur through MES-1 and an associated complex in P2 and P3.

If MES-1 is a receptor, what is the nature and source of its ligand? The observation that MES-1 is seen only on the surface of P2 and P3 where they contact the gut cell suggests that the gut cell supplies the ligand and that the ligand may be membrane-associated itself. MES-1 and its ligand may interact through direct cell-cell contacts, in a manner analogous to that of the Eph subfamily of RTKs and ephrin membrane-associated ligands (for review see Brüchner and Klein, 1998). Interaction with this hypothetical ligand may cause MES-1 to aggregate to only the portion of the P cell that contacts the gut cell, which may activate MES-1.

Do EMS and E signal division asymmetry in P2 and P3?
It has been thought that the asymmetric divisions of the germline cells occur cell autonomously. Isolated P2 and P3 cells did not reorient their spindles in response to contact with a gut blastomere, suggesting that germline-gut contact does not control those P-cell divisions (Goldstein, 1995). Additionally, posterior fragments of P2 extruded from the eggshell early after formation of P2 were able to divide unequally (Schierenberg, 1987). However, posterior fragments of P3 extruded later in the cell cycle did not divide asymmetrically (Schierenberg, 1987). This suggests that the cue for asymmetric division is repositioned away from the posterior during the course of the cell cycle. This is consistent with a model in which EMS signals P2, possibly through MES-1, to attract the cortical site that controls asymmetry to the ventral-anterior side of P2. Our findings that MES-1 appears only where P2 contacts EMS revive the possibility that EMS indeed signals P2. With knowledge of the timing of appearance and spatial restriction of MES-1, it is important to re-examine the question of whether the asymmetric divisions of P2 and P3 occur cell autonomously or are influenced by their EMS/E neighbor.

MES-1 localization is affected by defects in embryonic polarity and correlates with P granules
The aberrant pattern of MES-1 seen in mutant embryos with polarity defects suggests that MES-1 localization requires correct embryonic organization, which is not surprising. However, some results were unexpected, in particular observing that certain par mutants show normal patterns of MES-1 and observing that most mutants that show altered patterns of MES-1 at later stages show a wild-type pattern early on. There is a general correlation between the severity and stage of MES-1 mislocalization and the severity and stage of P-granule mis-segregation in the various mutants. par-1, par-3 and par-4 embryos generally mis-segregate P granules during the first division and lack detectable MES-1 staining, whereas par-2, let-99, pos-1 and mex-1 generally segregate P granules correctly during the first division and generally show a normal MES-1 distribution early and then defective distributions later. Early P-granule mis-segregation is probably indicative of severe disruption of polarity, which in turn is likely to impair the cytoskeletal or cortical system that mediates correct localization of MES-1.

A further correlation between MES-1 and P granules was observed in those mutants that displayed wild-type or ectopic MES-1 distributions. In wild-type embryos, the cells that have MES-1 on their surface (P2, P3 and P4) always contain P granules. Similarly, in mutant embryos, cells that appeared to have MES-1 on their surface were almost always observed to contain P granules. These observations are consistent with P-granule segregation being directed by MES-1 toward the region of the cell where it is located. However, the converse was not necessarily true: cells containing P granules did not always display MES-1 on their surface. The presence of P granules in
cells that did not exhibit MES-1 may simply be the result of mis-segregation prior to the stages when MES-1 is present. The close physical association between MES-1 and one centrosome of the spindle observed in wild-type embryos was also observed in mutant embryos that exhibited ectopic MES-1. The correlation in these mutant embryos was not absolute, but this may be a consequence of our inability to assign which cell actually contains MES-1.

**MES-1 may be needed to maintain germline-gut contact**

Since P₀ and P₁ already have in place a mechanism for asymmetric divisions, why is a distinct P₂/P₃-specific mechanism needed? The answer probably relates to the phenomenon of polarity reversal and the hypothesis that germline cells need to be in contact with the gut for proper development. In wild-type embryos that have been released from the constraints of the eggshell, the P daughter of both P₀ and P₁ is formed to the posterior of its somatic sister. Then a switch occurs, termed polarity reversal, and the P daughter of both P₂ and P₃ is formed to the anterior of its somatic sister (Schierenberg, 1987). Had the divisions that produce the P cells to the posterior continued in P₂ and P₃, the germline cells P₃ and P₄ would have been separated from the gut. Thus, polarity reversal offers a mechanism to maintain contact between the germline and gut cells. Two observations suggest that MES-1 is involved in polarity reversal. First, it is localized in the proper place at the proper time to play a role. Second, mes-1 mutant embryos released from the constraints of the eggshell do not display polarity reversal (E. Schierenberg, personal communication).

Contact between the germline and gut is observed in many species. Embryos of the nematode *Acrobeloides nanus* (formally named *Cephalobus* sp.; Wiegner and Schierenberg, 1998) undergo the same P₀ and P₁ division patterns as *C. elegans* but then do not display a reversal of polarity during the divisions of P₂ and P₃ (Skiba and Schierenberg, 1992), resulting in all four P cells being formed to the posterior of their somatic sisters. This causes P₃ and P₄ to be separated from the gut cells. Intriguingly, just prior to gastrulation, cell migrations result in P₄ becoming positioned next to E, thereby re-establishing contact between the germline and gut. Since MES-1 is proposed to function in polarity reversal in *C. elegans*, it is predicted that *A. nanus*, which lacks reversal, would either lack MES-1 or use it for another purpose. In many other species, such as *Drosophila*, *Xenopus* and mice, primordial germ cells associate with the hindgut (Wylie, 1999). These examples suggest that germline-gut contact is a common feature and is likely to be required for normal germline development.

An association between the gut and germ cells may guide germ-cell ingestion during gastrulation. In *C. elegans*, the two gut cells (Ea and Ep) are the first cells to migrate inward during gastrulation. P₃ follows them into the interior of the embryo (Sulston et al., 1983). P₄ does not migrate inward in embryos in which migration of the two E cells has been inhibited (Schierenberg and Junkersdorf, 1992; Powell-Coffman et al., 1996). Similar events occur in *Drosophila*, where the primordial germ cells (PGCs) first travel passively to the hindgut during gastrulation and then actively migrate through the hindgut to the gonad (Jaglarz and Howard, 1994). Analysis of *Drosophila* PGC behavior in mutants that affect the gut suggests that the timing and direction of PGC migration out of the gut lumen depends upon developmental changes in the gut epithelium (Warrior, 1994; Jaglarz and Howard, 1995). Another role for the germline-gut contact in *C. elegans* is suggested by the observation of Sulston et al. (1983) that during late embryogenesis Z₂ and Z₃ project lobes into two cells of the gut, possibly receiving nourishment from this attachment.

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