The C. elegans MEX-1 protein is present in germline blastomeres and is a P granule component

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SUMMARY

In the nematode Caenorhabditis elegans, germ cells arise from early embryonic cells called germline blastomeres. Cytoplasmic structures called P granules are present in the fertilized egg and are segregated into each of the germline blastomeres during the first few cleavages of the embryo. Mutations in the maternally expressed gene mex-1 disrupt the segregation of P granules, prevent the formation of germ cells, and cause inappropriate patterns of somatic cell differentiation. We have cloned the mex-1 gene and determined the distribution pattern of the mex-1 gene products. The MEX-1 protein contains two copies of an unusual ‘finger’ domain also found in the PIE-1 protein of C. elegans. PIE-1 has been shown to be expressed in germline blastomeres, and is a component of P granules. We show here that MEX-1 also is present in germline blastomeres and is a P granule component, although MEX-1 is a cytoplasmic protein while PIE-1 is present in both the nucleus and cytoplasm. We further show that MEX-1 is required to restrict PIE-1 expression and activity to the germline blastomeres during the early embryonic cleavages.

Key words: Caenorhabditis elegans, mex-1, pie-1, P granules, germ cells

INTRODUCTION

Analysis of the pattern of cell division and differentiation, or lineage, of the Caenorhabditis elegans embryo has shown that germ cells have an invariant origin (Sulston et al., 1983). Germ cells arise from only one branch of the embryonic lineage; cells in this branch are called germline blastomeres (see Fig. 1). Cells in all other branches produce only somatic cell types, and are called somatic blastomeres.

How do germline blastomeres differ from somatic blastomeres? Some somatic cell fates in the early embryo appear to be specified in part by two maternally provided transcription factors, SKN-1 and PAL-1 (Bowerman et al., 1992; Hunter and Kenyon, 1996). Germline blastomeres also contain SKN-1 and PAL-1, but do not appear to respond to these factors (Bowerman et al., 1993; Hunter and Kenyon, 1996). Recent studies have shown that embryonic transcription is repressed in germline blastomeres (Seydoux et al., 1996), presumably making these blastomeres refractory to factors that might otherwise direct somatic differentiation. Transcriptional repression in germline blastomeres requires the maternal PIE-1 protein, which is a predominantly nuclear protein that is present in each of the germline blastomeres (Mello et al., 1996; Seydoux et al., 1996). The molecular function of PIE-1 has not yet been determined, nor has the basis for the germline-restricted distribution of the PIE-1 protein.

A second difference between germline and somatic blastomeres is that cytoplasmic structures called P granules are found only in germline blastomeres (Strome and Wood, 1982). P granules are associated with germ cells, or germ cell precursors, at all stages of the C. elegans life cycle in embryos, larvae and adults (Strome and Wood, 1982). Thus P granules may function in germ cell development, though this function has not been determined. P granules contain uncharacterized RNA molecules that are capped and polyadenylated (Seydoux and Fire, 1994). Recently, the proteins MEX-3 and PIE-1 have been shown to associate with P granules in the germline blastomeres of early embryos, but not with the P granules found in germ cell precursors in later embryos, larvae or adults (Draper et al., 1996; Mello et al., 1996). MEX-3 is a presumptive RNA-binding protein that is present in the cytoplasm of both somatic and germline blastomeres; the function of MEX-3 in germline blastomeres has not been determined (Draper et al., 1996). PIE-1 is a novel, predominantly nuclear protein with two copies of an unusual ‘finger’ domain characteristic of the vertebrate TIS11/Nup475/TTP protein (Mello et al., 1996). As described above, PIE-1 is required to repress embryonic transcription in germline blastomeres (Seydoux et al., 1996).

P granules are partitioned to germline blastomeres during each of the early embryonic cleavages (Strome and Wood, 1983). After fertilization there is a general flow of cytoplasm, including P granules, toward the posterior pole of the egg (Hird and White, 1993; Hird et al., 1996). While other cytoplasmic components cycle back anteriorly along the cortical margins of the egg, P granules appear to remain associated with the posterior cortex, and thus are partitioned by the first cleavage
into the posterior cell, a germline blastomere (Hird and White, 1993; Hird et al., 1996). This general pattern of asymmetric localization of P granules happens prior to each of the subsequent cleavages, although additional mechanisms appear to contribute to P granule localization at later cleavages (Hird et al., 1996).

Previous studies have shown that the maternal gene mex-1 is essential for the development of germ cells, and for the proper segregation of P granules during the first embryonic cleavages (Mello et al., 1992; Schnabel et al., 1996). P granules accumulate posteriorly in newly fertilized mex-1 mutant eggs, but they do not associate properly with the cortex and are thus spread throughout the posterior half of the egg. In the next and subsequent cleavages, this incomplete localization leads to the mis-partitioning of P granules into somatic blastomeres, and a progressive loss of P granules in cells that would normally be germline blastomeres (Mello et al., 1992; Schnabel et al., 1996).

mex-1 mutants also have complex defects in somatic differentiation (Mello et al., 1992; Schnabel et al., 1996). The SKN-1 transcription factor is required for the proper development of the ventral blastomeres MS and E, and is expressed at high levels in these blastomeres in wild-type embryos. In mex-1 mutants, SKN-1 is expressed at high levels in anterior blastomeres that inappropriately adopt MS-like (Mello et al., 1992) or E-like (Schnabel et al., 1996) fates. mex-1 mutants also have a temperature-sensitive defect in the E blastomere, such that at low temperature E has a pattern of development similar to a posterior blastomere called C (Mello et al., 1992). Thus mex-1 mutations alter the fates of certain somatic blastomeres such that they develop with characteristics of more posterior blastomeres (Mello et al., 1992; Schnabel et al., 1996).

We report here the cloning of mex-1 and the localization of the mex-1 gene products. mex-1 encodes a protein, MEX-1, with two ‘finger’ domains similar to those found in PIE-1. We show that MEX-1 is a cytoplasmic protein that is distributed unequally between the early blastomeres and that MEX-1, like PIE-1, is a component of P granules. Finally, we demonstrate that MEX-1 is required to restrict PIE-1 localization and activity to germline blastomeres.

**Materials and Methods**

**Strains and alleles**

Bristol strain N2 was used as the standard wild-type strain. The mex-1 alleles used were: *zu120, zu121*, and *zu140* (Mello et al., 1992), *ii9* (Kemphues et al., 1988), *zu221* (provided by R. Lin), *or4* and *or14* (provided by B. Bowerman), e2569 (provided by J. Ahringer), pie-1 (*zu154*) was used in in situ hybridization experiments. Nematode strains were cultured as described by Brenner (1974).

**Cloning and molecular analysis of mex-1**

A strain carrying the yeast artificial chromosome Y17G7 (provided by A. Newman and P. Sternberg) was shown by genetic crosses to complement mex-1(*zu120*) (B. Draper, unpublished results). The mex-1 locus was identified through a series of germline transformation experiments with cosmids and phage spanning Y17G7 (provided by A. Newman and P. Sternberg). Cosmids and phage were injected individually or in groups with the marker rol-6 (Mello et al., 1991) into the syncytial gonad of *mex-1(zu120)unc-4(e120)/mmC1II* animals. DNA was injected at a final concentration between 100 and 200 ng/µl.

Four transgenic lines segregating Rol Unc hermaphrodites were isolated after injection of the phage clone VT#Y1L7 (Papp et al., 1991) containing an approximately 20 kb insert of genomic DNA. The Rol Unc adults were unable to roll, but had the right-handed twist characteristic of Roller animals. Rol Unc hermaphrodites produced either inviable Mex embryos, or viable Unc, or Rol Unc, larvae. Unc larvae became adult hermaphrodites that produced all inviable Mex embryos. We were unable to obtain phenotypic rescue with subclones of VT#Y1L7.

A 7.2 kb BamHI-HindIII genomic DNA fragment isolated from VT#Y1L7 was used to screen a mixed stage *C. elegans* cDNA library (provided by A. Fire). Positive cDNAs were used to construct Blue-script plasmids pJP5G1 (2.7 kb insert) and pJP5G4 (1.1 kb insert). Antisense RNAs corresponding to the full cDNA inserts were produced using an in vitro transcription kit (Promega). RNA injections were carried out as described by Guo and Kemphues (1995). RNAs from pJP5G1 and pJP5G4 were injected at concentrations of 0.5 and 1.8 µg/µl, respectively. Approximately 60% of the resulting embryos resembled *mex-1* mutant embryos. 4 different *mex-1* cDNAs were sequenced on both strands. A potential SL1 trans-spliced acceptor (Krause and Hirsh, 1987) was analyzed by reverse transcription of total mixed stage RNA (provided by M. Morrison and M. Roth) using a random hexamer primer mix (Boehringer Mannheim), followed by polymerase chain reaction (PCR) using a *mex-1*-specific primer and a primer specific for either the trans-spliced leader SL1 or SL2 as described by Spieh et al. (1993). A single product with the predicted size was obtained only when the SL1 primer was included; this product was shown by Southern analysis to hybridize to a *mex-1* cDNA, and coincided with the 5’ end of the *mex-1* cDNA by sequence analysis. *mex-1* alleles were examined for deletions in the *mex-1* gene by single-worm PCR (Barstead and Waterston, 1991) using combinations of *mex-1*-specific primers. The regions of genomic DNA surrounding deletions in the *mex-1(zu40)* and *mex-1(zu221)* alleles were isolated by PCR and sequenced.

**In situ hybridization**

In situ hybridization using either *pes-10* or *mex-1* probes was done according to the method of Seydoux and Fire (1994). *mex-1* sense and antisense RNA probes labeled with digoxigenin-11-dUTP (Boehringer Mannheim) were made from pJP5G9, a plasmid containing part of the *mex-1* cDNA that includes the entire coding region and 387 bp of the 3’ UTR. The detection reaction with alkaline phosphatase-conjugated anti-digoxigenin (Boehringer Mannheim) was allowed to develop for approximately 45 minutes. Photographs were taken with Ektachrome 160T professional color reversal film (Kodak). Images were digitized using a Nikon Coolscan slide scanner (Nikon). The digitized images were assembled using Adobe Photoshop 3.0 (Adobe).

**Production of antibodies and immunostaining**

Rabbit polyclonal antisera *8230I and 8325I* were generated against the predicted MEX-1 protein (see Fig. 2A). Peptides were synthesized using a prepared 4-branch MAPs core attached to a Wang resin (Novabiochem) on an Applied Biosystems model 430 peptide synthesizer. Peptide sequences were chosen to exclude the highly conserved finger domains of the MEX-1 protein. Immunizations (Lin et al., 1995) and affinity purification (Robinson et al., 1988) of antibodies were as described previously. Antibodies were eluted from matrix-bound antigen with 0.2 M glycine with 1.0% bovine serum albumin, pH 2.5. Embryos were fixed and stained with antibodies, and with DAPI to visualize DNA, as described previously (Lin et al., 1995). The antisera 8230I and 8325I gave indistinguishable patterns of staining on wild-type embryos. No staining was detected with the 8325I antisera in embryos from homozygous *mex-1(zu120)* mothers, nor with the 8230I antisera in embryos from *zu120, zu121, zu221*, or e2569 mothers; staining was detected in embryos homozygous...
gous for the weak mex-1 mutation i97. P granule/MEX-1 double staining experiments were as described by Draper et al. (1996). Embryos were stained for PIE-1 with a mouse monoclonal antibody, P4G5, that was raised against PIE-1-specific peptides and that will be described elsewhere (C. Schubert unpublished results); we obtained similar embryonic staining patterns with a rabbit polyclonal antibody described by Mello et al. (1996). Embryos were prepared and stained as described previously (Mello et al., 1996). All images were collected on a DeltaVision SA3.1 wide-field deconvolution optical sectioning microscope (Applied Precision, Inc.) and assembled using Adobe Photoshop 3.0 (Adobe). The MEX-1 protein and P granule co-localization images were deconvolved using the reiterative constrained method of Sedat and Agard (Hiraoka et al., 1991).

RESULTS

MEX-1 contains a repeated ‘finger’ motif

Transformation rescue of the mutant strain mex-1(zu120) was obtained with a single phage (see Materials and Methods) and probes from this phage were used to isolate candidate mex-1 cDNAs. Antisense RNA transcribed from the cDNAs SG1 and SG4 resulted in mex-1-like embryonic phenotypes when injected into the gonads of wild-type adult hermaphrodites (data not shown), suggesting that these cDNAs correspond to the mex-1 gene. The sequence derived from SG1, SG4, and overlapping cDNA clones is shown in Fig. 2A, with the sequence of the predicted protein. We found that two mex-1 mutants have deletions in the gene defined by these cDNAs. mex-1(zu221) has a 437 bp deletion in the 5’ end of this gene that would remove the first 36 amino acids of the predicted protein, mex-1(zu140) has an approximately 600 bp deletion toward the 3’ end that would remove the C-terminal 80 amino acids of the predicted protein. These several lines of evidence together indicate that this gene is mex-1.

The mex-1 cDNA can encode a 494 amino acid protein that contains two copies of a predicted Cys/His ‘finger’ motif with the unusual spacing CX5CX2CX3H, initially described in the mouse TIS11/Nup475/TPP protein (Fig. 2B; Varnum et al., 1989; Dubois et al., 1990). This class of ‘finger’ domain has been identified in proteins from several organisms including Saccharomyces cerevisiae, Drosophila and C. elegans (Ma et al., 1994; Ma and Herschman, 1995; Mello et al., 1996), however the biochemical function of this domain has not been determined. The PIE-1 protein in C. elegans has two finger domains similar to the predicted MEX-1 protein (Mello et al., 1996). PIE-1 is a predominantly nuclear protein that is localized to germ line blastomeres, and that is required to repress embryonic transcription in these blastomeres (Mello et al., 1992; Seydoux et al., 1996; see Introduction). The vertebrate TIS11/ Nup475/TPP protein can be found in the nucleus (Dubois et al., 1990) and is induced in 3T3 cells in their immediate response to growth factor or serum stimulation, suggesting it may also play a role in regulating gene expression (for review, see Herschman et al., 1994). The predicted MEX-1 protein does not have significant similarity to PIE-1 or TIS11/Nup475/TPP outside of the finger domains, and does not contain any known nuclear localization sequence or other motifs that might suggest its function or distribution.

The mex-1 gene products localize predominantly to germ line blastomeres

We analyzed the distribution of the mex-1 mRNA by in situ hybridization (see Materials and Methods). mex-1 mRNA is detected in the syncytial core of the gonad and in oocytes at all stages of maturation (Fig. 3A). In embryos, mex-1 mRNA has a distribution that is similar to a pattern described previously in C. elegans for several unrelated maternally expressed messages, collectively called class II mRNAs (Seydoux and Fire, 1994). There is no genetic evidence for embryonic expression of mex-1 (Mello et al., 1992), therefore we consider it likely that the expression pattern we describe here represents maternally expressed mex-1 mRNA, though this has not been tested directly. Like class II maternal messages, mex-1 mRNA is distributed uniformly in 1-cell and 2-cell stage embryos (Fig. 3B,C) but then appears to be degraded rapidly in somatic blastomeres (Fig. 3D-H). After each of the subsequent cleavages, mex-1 mRNA persists at high levels in the germline blastomere, but gradually decreases to undetectable levels in the somatic sister of the germline blastomere (compare Fig. 3H with 3G). After the division of the final germline blastomere, P4, mex-1 mRNA can no longer be detected in the embryo (data not shown).

To examine MEX-1 protein localization, we used two rabbit polyclonal antisera raised against different regions of the MEX-
1 protein and found identical results. No staining was detected with these antisera in several mex-1 mutant strains (see Material and Methods). MEX-1 is first detected at low levels in the cytoplasm of mature oocytes (data not shown). The level of MEX-1 increases markedly after fertilization; at the late 1-cell stage, MEX-1 appears most abundant in the posterior half of the embryo (Fig. 4A). During the early cleavage stages, MEX-1 is present in the egg cytoplasm of each of the germline blastomeres and is not detected in nuclei (Fig. 4C–K). After each division of a germline blastomere, MEX-1 is detected initially in both daughters, but then disappears from the somatic daughter later in that cell cycle (compare the early 4-cell embryo in Fig. 4E with the late 6-cell embryo in Fig. 4G).

**MEX-1 is present in the germline blastomere P4, but cannot be detected in the daughters of P4 (see Fig. 5F and data not shown).**

**MEX-1 is a component of P granules**

In addition to the cytoplasmic staining described above, distinct granules are stained prominently by the MEX-1 antisera in the cytoplasm of each germline blastomere (Fig. 4). These granules closely resemble P granules in size, number and distribution (Strome and Wood, 1982). When embryos were stained simultaneously with a MEX-1 antisera and an anti-P granule antibody, we found that the staining patterns of the granules coincided for each of the germline blastomeres P0, P1, P2, P3, and P4 (the P2 blastomere is shown in Fig. 5A–C). P granules

**Fig. 2. Cloning and molecular analysis of mex-1. (A)** Sequence of a mex-1 cDNA and corresponding protein. The mex-1 mRNA is trans-spliced with these antisera in several mex-1 mutant strains (see Material and Methods). MEX-1 is first detected at low levels in the cytoplasm of mature oocytes (data not shown). The level of MEX-1 increases markedly after fertilization; at the late 1-cell stage, MEX-1 appears most abundant in the posterior half of the embryo (Fig. 4A). During the early cleavage stages, MEX-1 is present in the egg cytoplasm of each of the germline blastomeres and is not detected in nuclei (Fig. 4C–K). After each division of a germline blastomere, MEX-1 is detected initially in both daughters, but then disappears from the somatic daughter later in that cell cycle (compare the early 4-cell embryo in Fig. 4E with the late 6-cell embryo in Fig. 4G).

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are present in both daughters of the P₄ blastomere (Fig. 5D), however MEX-1 is not detectable in either daughter (Fig. 5F). Similarly, P granules are present in the germ cells of larvae and adults, but MEX-1 is not detectable at these later stages (data not shown). Thus MEX-1 may either associate with P granules only transiently during the early embryonic cleavages, or MEX-1 may be masked in P granules at all other stages.

Ectopic repression of pes-10 in mex-1 mutants

We have shown that the MEX-1 and PIE-1 proteins share a common ‘finger’ motif, and that both are present in germline blastomeres (Mello et al., 1996; this study). Because PIE-1 has
have been shown to be required for the proper regulation of embryonic transcription in germline blastomeres (Seydoux et al., 1996), we asked whether MEX-1 might have a similar role. Transcription in the early embryo can be monitored conveniently by analyzing expression of the pes-10 gene (Seydoux and Fire, 1994). In wild-type embryos, pes-10 mRNA is present at high levels in all somatic blastomeres from the 8-cell stage until the 15-cell stage, but is not detected in germline blastomeres at any stage (Seydoux and Fire, 1994). For example, in a wild-type 15-cell stage embryo, only one blastomere does not contain detectable levels of pes-10 mRNA; this blastomere is the germline blastomere P3 (Fig. 6A; see also Seydoux and Fire, 1994). In this paper we refer to the absence of pes-10 mRNA in germline blastomeres as pes-10 repression, though the biochemical mechanism that establishes the soma/germline difference in pes-10 mRNA levels has not been determined. In pie-1 mutant embryos, pes-10 mRNA accumulates in all blastomeres, indicating that pie-1(+) is required for the germline-specific repression of pes-10 (Seydoux et al., 1996).

We examined pes-10 mRNA levels in mex-1 mutants by in situ hybridization of 8-cell to 16-cell stage embryos. We found that pes-10 mRNA was not detectable in multiple blastomeres in all mex-1 mutant embryos examined (n>100), in contrast to the single blastomere lacking pes-10 mRNA in wild-type embryos. We identified these blastomeres in the mex-1 embryos by comparison with the positions of blastomeres in the wild-type as mex-1 progression, though the mRNA was not detected in all blastomeres in mex-1. In about 46% of the 8-cell stage mex-1 embryos, pes-10 mRNA was detectable in E and MS (Fig. 7C), while only 46% of the embryos contained relatively high levels of PIE-1 in E and MS. These results suggest that mex-1 mutations affect both the embryonic localization and cytoplasmic distribution of the PIE-1 protein, and that the mis-localization of PIE-1 in mex-1 mutants becomes more severe at low temperature.

DISCUSSION

MEX-1 and PIE-1 have different functions in germ cell development

In this study we have shown that the C. elegans MEX-1 protein contains two repeats of a ‘finger’ domain that has been found in several proteins of unknown function from both vertebrates and invertebrates. The only C. elegans protein described to date that contains similar domains is the PIE-1 protein (Mello et al., 1996). Both MEX-1 and PIE-1 are required for germ cell development, both are present in the germline blastomeres of early embryos, and both are components of P granules (Mello et al., 1992, 1996, this study). These similarities suggest the possibility that MEX-1 and PIE-1 might have common functions.
However, PIE-1 is a predominantly nuclear protein (Mello et al., 1996), while MEX-1 lacks a recognizable nuclear localization signal and appears to be predominantly, if not exclusively, cytoplasmic.

PIE-1 is required for the repression of embryonic transcription of genes such as pes-10 in the germline blastomeres (Seydoux et al., 1996). We have shown that MEX-1 is not required to repress pes-10 transcription. However, MEX-1 is required for the proper spatial pattern of pes-10 repression, apparently through its role in localizing PIE-1. Ectopic expression of pie-1 driven by a heat-shock promoter has been shown to reduce the level of mRNA from an embryonically transcribed reporter gene by about 75% in all blastomeres (Seydoux et al., 1996). This result has suggested that PIE-1 may be sufficient to repress embryonic transcription, and is consistent with our finding that ectopic PIE-1 in mex-1 mutants results in ectopic repression of pes-10.

**P granule localization to germline blastomeres**

Previous studies have shown that mex-1(+) activity is required for the proper cortical association of P granules in germline blastomeres during the early, asymmetrical cleavages (Mello et al., 1992; Schnabel et al., 1996). Our observation that MEX-1 protein is a component of P granules only during these cleavages suggests that P granule-associated MEX-1 might directly bind to proteins at the cortex, such as PAR-1 or PAR-2 (Guo and Kemphues, 1995; Boyd et al., 1996). Alternatively, MEX-1 could be required for the proper structure or assembly of P granules, and thus affect cortical localization indirectly. For example, analysis of *Drosophila* mutants defective in polar granules indicates that the proper assembly of these structures requires the sequential addition of specific components (for review see Lehmann and Ephrussi, 1994).

We have analyzed three P granule components in mex-1 mutants; an unknown antigen recognized by the K76 antibody (Strome and Wood, 1982), the MEX-3 protein (Draper et al., 1996), and the PIE-1 protein (Mello et al., 1996). In mex-1 mutants, both the K76 antigen and the MEX-3 protein appear, by immunofluorescence, to be present in P granules at wild-type levels (S. Guedes and J. Priess, unpublished observations). However, we consistently detect abnormally low levels of PIE-1 staining in the P granules of mex-1 mutants. Because the level of nuclear PIE-1 in the P3 blastomere of mex-1 mutants appears only slightly lower than in a wild-type P3, we do not know whether the diminished PIE-1 staining of P granules can be explained entirely by the reduced level of PIE-1. Alternatively, MEX-1 may have a specific function in the association of PIE-1 with P granules; in future experiments it will be interesting to determine whether there are direct interactions between the MEX-1 and PIE-1 proteins. For example, P granule-associated MEX-1 might recruit PIE-1 into the granules through the common ‘finger’ domains of MEX-1 and PIE-1.

It is possible that PIE-1 becomes mislocalized in mex-1 mutants; an unknown antigen recognized by the K76 antibody (Strome and Wood, 1982), the MEX-3 protein (Draper et al., 1996), and the PIE-1 protein (Mello et al., 1996). In mex-1 mutants, both the K76 antigen and the MEX-3 protein appear, by immunofluorescence, to be present in P granules at wild-type levels (S. Guedes and J. Priess, unpublished observations). However, we consistently detect abnormally low levels of PIE-1 staining in the P granules of mex-1 mutants. Because the level of nuclear PIE-1 in the P3 blastomere of mex-1 mutants appears only slightly lower than in a wild-type P3, we do not know whether the diminished PIE-1 staining of P granules can be explained entirely by the reduced level of PIE-1. Alternatively, MEX-1 may have a specific function in the association of PIE-1 with P granules; in future experiments it will be interesting to determine whether there are direct interactions between the MEX-1 and PIE-1 proteins. For example, P granule-associated MEX-1 might recruit PIE-1 into the granules through the common ‘finger’ domains of MEX-1 and PIE-1.
mutants simply because PIE-1 is a P granule component, and P granules are mislocalized in mex-1 mutants. However the basis for the wild-type pattern of PIE-1 localization has not been determined, and may not involve the association between PIE-1 and P granules (Mello et al., 1996). For example, PIE-1 appears to be concentrated at the centrosomes of cells prior to division, and is not detected in P granules when cells are dividing (Mello et al., 1996). In dividing cells, PIE-1 is detected at high levels in the centrosome destined for the germline daughter, but is present at much lower levels, or not detected, in the centrosome destined for the somatic daughter. In mex-1 mutants, we have observed some mitotic spindles with PIE-1 at similar levels in both centrosomes (S. Guedes, unpublished results). Thus mex-1 mutations may cause defects in the polarity of germline blastomeres that are independent of P granule structure or function.

Germline/soma defects in mex-1 mutants

If P granules contain germ cell determinants, the lack of germ cells in mex-1 mutants could result from either the mislocalization of P granules, or from changes in the composition of P granules. We have shown that PIE-1, a component of P granules, is mislocalized in mex-1 mutants. We do not yet know whether this defect is sufficient to explain the lack of germ cells, or whether other factors that contribute to, or that can interfere with, germ cell development also are mislocalized. In mex-1 mutants, P3 and P4 contain slightly less than wild-type levels of PIE-1. Nevertheless, this level of PIE-1 is sufficient to repress pes-10 transcription in P3 and P4, as PIE-1 does in wild-type embryos. However, the lower levels of PIE-1 could be insufficient to prevent the action of transcription factors, such as SKN-1 and PAL-1, that might promote somatic differentiation in P3 and P4 (Bowerman et al., 1992, 1993; Hunter and Kenyon, 1996).

Any model for MEX-1 function must explain the defects in the development of somatic blastomeres that are observed in mex-1 mutants. Part of the specification of early blastomere fates in C. elegans involves the relative activities of PIE-1, that appears to repress embryonic transcription, and the maternally supplied transcription factors SKN-1 and PAL-1 (Bowerman et al., 1992, 1993; Draper et al., 1996; Hunter and Kenyon, 1996; Mello et al. 1996; Seydoux et al., 1996). SKN-1, but not PAL-1, plays a role in the development of the E blastomere in wild-type embryos, although both transcription factors are present in E. This is presumably because SKN-1 appears before PAL-1 in the parent of E, and PIE-1 is not present in that parent. In contrast, PAL-1, but not SKN-1, plays a role in the development of the C blastomere in wild-type embryos, although both factors also are present in C. This appears to be due to the fact that PIE-1 is present in the parent of C, blocking the activities of both PAL-1 and SKN-1. SKN-1 disappears immediately after C is born, allowing C to respond only to PAL-1. In about 50% of mex-1 mutant embryos cultured at low temperature, the E blastomere is transformed into a C-like blastomere (Mello et al., 1992). This is precisely the transformation in fate that would be predicted from our finding that PIE-1 is mislocalized to the E blastomere and the parent of E; PIE-1 would be expected to prevent SKN-1 from functioning in this lineage, allowing E to respond to PAL-1 once SKN-1 and PIE-1 were no longer present.

PIE-1 mislocalization, however, can not be the sole cause of somatic defects in mex-1 mutants. Anterior blastomeres in mex-1 mutants have abnormally high levels of SKN-1 protein and defects that are due, in part, to inappropriate skn-1(+) activity (Mello et al., 1992; Bowerman et al., 1993). We have never detected PIE-1 in these anterior blastomeres in mex-1 mutants, and previous studies have shown that these blastomeres do not return to a wild-type pattern of development in mex-1/pie-1 double mutants (Mello et al., 1992).

In a wild-type 2-cell stage embryo, SKN-1 is present at much higher levels in the posterior blastomere than in the anterior blastomere (Bowerman et al., 1993). It is not yet understood how this asymmetry is achieved; skn-1 mRNA is distributed equally between both blastomeres (Seydoux and Fire, 1994), suggesting that there is regulation at the level of skn-1 mRNA translation, or SKN-1 protein stability. In mex-1 mutants, SKN-1 is present at nearly equal levels in both anterior and posterior blastomeres. Thus MEX-1 might function as a regulator of skn-1 mRNA, or be required for the proper localization of such a regulator. For example, a regulator might be associated with P granules, and require MEX-1 for this association.

There are analogies between the somatic/germ cell defects associated with mutations in mex-1 and mutations in the ‘posterior group’ genes of Drosophila, many of which encode components of polar granules (see Mello et al., 1992). The abdominal determinant nanos is associated with polar granules; mutations in genes such as vasa that prevent polar granule formation prevent the proper posterior localization of nanos; and thus disrupt somatic differentiation indirectly (for review see Lehmann and Ephrussi, 1994). The germline cells, or germline precursors, in many animal embryos contain cytologically distinct structures similar to the polar granules of Drosophila or the P granules of C. elegans. It will be interesting to determine in future studies whether molecular components of these structures, such as MEX-1, have been conserved in animal evolution, and whether these structures in general serve to localize factors involved in both somatic and germ cell development.

We thank B. Draper, A. Newman, P. Sternberg for assistance in the cloning of mex-1; C. Sunkel, S. Parkhurst, M. Costa, R. Hill, B. Draper and W. Downs for comments on the manuscript, C. Schubert for communicating unpublished observations on pie-1 localization, and members of the Priess laboratory for advice and encouragement. S.G. is supported by fellowships from Programa Gulbenkian de Doutoramento em Biologia e Medicina (PGDBM) and the Fundação Luso-Americana para Desenvolvimento (FLAD). J. P. is supported by the Howard Hughes Medical Institute.

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MEX-1 is a P granule component


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(Accepted 17 November 1996)