

pos-1* encodes a cytoplasmic zinc-finger protein essential for germline specification in *C. elegans

Hiroaki Tabara^{1,*}, Russell J. Hill^{2,*}, Craig C. Mello³, James R. Priess^{2,4,‡} and Yuji Kohara¹

¹Department of Genetics, Graduate University of Advanced Studies and Gene Network Lab, National Institute of Genetics, Core Research for Evolutional Science and Technology, Japan Science and Technology Corporation, Mishima 411, Japan

²Fred Hutchinson Cancer Research Center, Howard Hughes Medical Institute, Seattle, WA 98109, USA

³University of Massachusetts Cancer Center, Worcester, MA 01605, USA

⁴Zoology Department, University of Washington, Seattle WA 98195, USA

*These authors contributed equally to this work

‡Author for correspondence (e-mail: jpriess@fred.fhrc.org)

Accepted 14 October; published on WWW 3 December 1998

SUMMARY

Germ cells arise during early *C. elegans* embryogenesis from an invariant sequence of asymmetric divisions that separate germ cell precursors from somatic precursors. We show that maternal-effect lethal mutations in the gene *pos-1* cause germ cell precursors to inappropriately adopt somatic cell fates. During early embryogenesis, *pos-1* mRNA and POS-1 protein are present predominantly in the germ precursors. POS-1 is a novel protein with two copies of a CCCH finger motif previously described in the germline proteins PIE-1 and MEX-1 in *C. elegans*, and in the mammalian TIS11/Nup475/TTP protein. However,

mutations in *pos-1* cause several defects in the development of the germline blastomeres that are distinct from those caused by mutations in *pie-1* or *mex-1*. The earliest defect detected in *pos-1* mutants is the failure to express APX-1 protein from maternally provided *apx-1* mRNA, suggesting that POS-1 may have an important role in regulating the expression of maternal mRNAs in germline blastomeres.

Key words: Germ cells, CCCH finger, Embryogenesis, *Caenorhabditis elegans*, POS-1

INTRODUCTION

In higher animals, sperm and oocytes differentiate from special cells called germ cells. Since the germ cells must reproduce the entire organism with each new generation, they are considered to be totipotent. Thus mechanisms must exist that maintain totipotency in the embryonic cells destined to produce germ cells, although very little is understood about this process for any animal embryo.

In *C. elegans* embryogenesis, the germ cells arise from a stem cell-like lineage pattern (see Fig. 1; Sulston et al., 1983). Blastomeres in the branch of the lineage that leads to germ cells are called germline blastomeres; the sisters of the germline blastomeres produce only somatic tissues and are called somatic blastomeres (for general reviews of *C. elegans* embryogenesis, see Kempthues and Strome, 1997; Schnabel and Priess, 1997). The final germline blastomere is called P₄ and it produces only germ cell descendants.

Several differences have been described between the germline blastomeres and somatic blastomeres of the *C. elegans* embryo. For example, many maternally provided mRNAs persist longer in the germline blastomeres than in the somatic blastomeres (Seydoux and Fire, 1994). Cytoplasmic granules of unknown function called P granules are partitioned asymmetrically into the germline blastomeres during each of

the early divisions, ultimately becoming localized into the P₄ blastomere (Strome and Wood, 1983). The relationship between the persistence of maternal mRNAs, or P granule localization, and the totipotency of the germline blastomeres has not been determined.

Another difference between the germline and somatic blastomeres is in the initiation of embryonic gene transcription; after embryonic transcription initiates at the 4-cell stage of development, new mRNA transcripts are detected only in the somatic blastomeres (Seydoux and Fire, 1994; Seydoux et al., 1996). This result suggests that transcription is repressed or blocked in the germline blastomeres. The apparent lack of transcription provides an explanation for why germline blastomeres do not respond to transcription factors such as the SKN-1 and PAL-1 proteins. SKN-1 and PAL-1 are translated from maternally provided mRNAs in both germline and somatic blastomeres (Bowerman et al., 1993; Hunter and Kenyon, 1996). Although these transcription factors promote specific patterns of somatic differentiation in the somatic blastomeres, they appear to have no effect on the development of the germline blastomeres (Bowerman et al., 1992; Hunter and Kenyon, 1996). Thus, transcriptional repression appears to be at least part of the mechanism that maintains the totipotency of the early germline blastomeres.

Mutations in the genes *pie-1* and *mex-1* cause the germline blastomeres to adopt somatic fates, suggesting that they function in maintaining the totipotency of the germline blastomeres (Mello et al., 1992). In *pie-1* mutants, embryonic transcription appears to initiate simultaneously in both somatic and germline blastomeres (Seydoux et al., 1996), such that the germline blastomeres become competent to respond to SKN-1 and PAL-1. These results suggest that *pie-1(+)* functions in repressing transcription in wild-type germline blastomeres. *mex-1* appears to play a distinct role from *pie-1*, because at least some transcription is properly repressed in the germline blastomeres of *mex-1* mutants (Guedes and Priess, 1997). PIE-1 is a novel, predominantly nuclear, protein of unknown function that is localized to a germline blastomere at each of the early embryonic cell divisions (Mello et al., 1996). MEX-1 is a novel, cytoplasmic protein that is localized predominantly to a germline blastomere at each early division (Guedes and Priess 1997). PIE-1 and MEX-1 are both components of P granules, and both proteins contain two copies of a CCCH-type 'finger' domain originally described in a vertebrate protein of unknown function called TIS11/Nup475/TTP (Varnum et al., 1989; DuBois et al., 1990; Lai et al., 1990).

We have taken two complementary approaches to identify additional genes that establish germline/soma differences in *C. elegans*. As part of the *C. elegans* cDNA project, we have used molecular screens to look for mRNAs that are localized asymmetrically in the early embryo (Y. Kohara, unpublished data). In a second approach, we have used genetic screens to identify maternal-effect lethal mutations that prevent the development of germ cells. In this report we describe the *pos-1* gene, which was identified independently in both screens. We show that the *pos-1* mRNA is distributed asymmetrically after the first cleavage of the embryo, and is present predominantly in germline blastomeres. Like MEX-1, POS-1 is a cytoplasmic protein localized predominantly to germline blastomeres. Like both MEX-1 and PIE-1, the POS-1 protein is a component of P granules and has two copies of the CCCH finger motif. We show that *pos-1* mutants have developmental defects that are distinct from both *mex-1* and *pie-1* mutants, suggesting that the POS-1, MEX-1 and PIE-1 proteins have at least some distinct roles in the specification of germline blastomeres.

MATERIALS AND METHODS

Molecular analysis of *pos-1*

Sequencing of the *pos-1* cDNA yk61h1 (accession number AB006208) showed that the *pos-1* mRNA is 1138 bases in length excluding the poly(A) tail. The presence of the splice leader SL1 in *pos-1* mRNA was confirmed by reverse transcription-PCR from embryonic RNA using primers specific for SL1 or SL2 (Spieth et al., 1993) and sequencing of the products. In order to physically map *pos-1*, digoxigenin (DIG)-labeled probe was prepared from the insert of yk61h1 (Hodgson and Fisk, 1987) and hybridized to an ordered grid of YAC clones covering most of the *C. elegans* genome (Coulson et al., 1988). Hybridization was seen to Y75G4 and Y5C20, located between *rol-3* and *her-1* on chromosome V. The cosmids C37F1 and W03E11 were shown to contain the *pos-1* gene by PCR. An 8 kb genomic region containing *pos-1* was amplified by extended PCR (Barnes, 1994) and sequenced by the shotgun sequencing strategy

(Anderson, 1981). This sequence matches sequence within F52E1 (accession number U41109) obtained subsequently by the *C. elegans* sequencing consortium. Nucleotide sequencing of *pos-1* from mutant strains was performed by extracting DNA (Williams et al., 1992) from animals homozygous for a single *pos-1* allele. The 5' and 3' regions of *pos-1* were sequenced after amplification using primer pairs GCAGCATTCTTGCTGGTGA/ATGGTTGACAACGATTTCCTG and TCCACATAACGAGGGCTATG/TGCTTACAAACGCAGTCA-GG, respectively.

Nematode strains

The general methods for handling *C. elegans* strains are described by Brenner (1974). The *pos-1* alleles *ne51*, *zu125*, *zu146*, *zu148*, *zu175* and *zu363* were isolated using procedures described previously (Priess et al., 1987). For experiments described in this paper, *zu148*, *zu175* and *zu363* were in *cis* to *unc-42(e270)*, and *zu125* was in *cis* to *dpy-11(e224)*. *zu363* was isolated by K. Mickey, and *ne51* by C. Rocheleau. In addition, the following genetic reagents were used: LG II: *mex-1(zu120)*, *mex-1(zu121)*, *rol-1(e91)*, *mnCI*; LG III: *pie-1(zu154)*, *unc-25(e156)*, *qC1*; LGV: *sDf35*, *nT1*.

The *pos-1* reference allele *zu148* behaves as a strict maternal-effect lethal mutation in the following tests. All progeny produced by self-fertilization of homozygous *zu148 e270* hermaphrodites arrested during embryogenesis ($n=4984$). 99% of progeny produced by self-fertilization of *zu148 e270/+* + hermaphrodites successfully completed embryogenesis ($n=2668$) and none of the 19 dead embryos had a Pos-1 mutant phenotype. All F₁ embryos produced by mating wild-type males to homozygous *zu148 e270* hermaphrodites purged of sperm arrested during embryogenesis with a Pos-1 mutant phenotype ($n=473$).

Whole-mount in situ hybridization

To detect *pos-1* mRNA, in situ hybridization was performed as described by Tabara et al. (1996). To detect *pes-2* and *apx-1* mRNAs, embryos were isolated from gravid adults by alkaline bleach, washed three times in M9 buffer, and transferred onto a poly L-lysine-coated slide using a siliconized pipette tip. To digest the eggshells, an equal volume of 3 mg/ml chitinase (Sigma C-6137), 2 mM DTT in basal EH buffer (Tabara et al., 1996) was added for 3 minutes at 22°C, followed immediately with 1/4 volume of 4% gelatin, 2% bovine serum albumin (BSA; Sigma A-7638) in M9 buffer at 37°C. Excess liquid was removed and the slide overlaid with a cover-slip and placed on dry ice for 10 minutes. The coverslip was snapped off and the slide immersed in methanol at -20°C. The embryos were then fixed and hybridized with DIG-labeled probe as described by Tabara et al. (1996). Hybridized probe was detected with an alkaline phosphatase mediated color reaction described previously (Seydoux and Fire, 1994).

The *pes-2* cDNA was identified by differential screening for genes with early embryonic expression (to be published elsewhere); a *pes-2* sequence has been previously submitted by Arnold and Hope (accession no. X94701).

Production of antibodies and immunostaining

Rabbit antisera were generated against either of two branched-chain peptides SEQLANRDPCTVPDDLREE or RSMDLNQSLPIRQSDLVRAFARA as described by Guedes and Priess (1997). Rat antiserum was raised against a POS-1 fusion protein as follows. A portion of *pos-1* encoding the first 96 amino acids was amplified by PCR from yk61h1 using primers tailed with *AseI* and *XhoI* sites (GCACATTAATATGGTTGACAACGATTTC and TGAACCTCGAGTTCCTTTCTCCTTTGAC). The product was cloned into pET23b (Novagen) using *AseI* and *XhoI* sites, and the clone was confirmed by sequencing. The POS-1 fusion protein was expressed in BL21(DE3)pLysS and purified by metal-chelated chromatography (Invitrogen) and gel filtration on Sephadex G-100 (Pharmacia Biotech).

Rat POS-1 antiserum was preabsorbed as follows. Protein was extracted from a mixed-stage embryo population, run on SDS-PAGE, and proteins over 50 kDa were blotted onto PVDF membrane (Pall Biosupport). The membrane was blocked in storage buffer (1% BSA [Sigma A-7638], 0.1% NaN₃, 0.01% Tween 20, PBS). Antiserum was diluted 1:100 in storage buffer and preabsorbed onto the membrane overnight at 4°C.

The preabsorbed antiserum stained a single band of about 30 kDa on western blots as follows. Protein was extracted from pregastrulation embryos, run on SDS-PAGE and blotted onto PVDF membrane. The membrane was incubated overnight at 4°C with blocking buffer (5% skim-milk, 0.01% NaN₃, 0.1% Triton X-100 in PBS) and then incubated overnight at 4°C with preabsorbed antiserum diluted at 1:500 in blocking buffer. Alkaline-phosphatase-conjugated anti-Rat IgG (Jackson Immunoresearch) was used as secondary antibody and phosphatase activity was detected as above.

To detect POS-1, embryos were immunostained by one of two protocols. Embryos treated by either protocol have detectable POS-1 in the cytoplasm and in the P granules of P₁ through P₄. However, P granule-associated POS-1 is detected at higher levels by protocol I than by protocol II. In protocol I, embryos were treated as described by Lin et al. (1995), except that the second methanol bath and the rehydration series were omitted. In protocol II, embryos were prepared and stored as for in situ hybridization (Tabara et al. 1996). Then, slides at 22°C were immersed in acetone for 7 minutes, in acetone:PBS (1:1) for 5 minutes, in PBT (1× PBS, 0.1% BSA (fraction V; Sigma A-7906), 0.1% Triton X-100) three times for 5 minutes each, and blocked for 30 minutes in 10% normal goat serum (Wako, Japan) in PBT. Embryos were incubated at 4°C overnight with preabsorbed antiserum diluted at 1:250 in PBT with 0.01% NaN₃; then with Cy3-conjugated anti-Rat IgG antibodies (Jackson Immunoresearch) for 2 hours (22°C). After each antibody incubation, slides were washed 3-4 times with PBT; the final wash contained 20 ng/ml DAPI. The POS-1 staining patterns described in this paper can be competed away with excess POS-1 fusion protein (data not shown).

Staining with mAbK76 or APX-1 antiserum were done as described by Strome and Wood (1983); Mickey et al. (1996).

RNAi

RNAi experiments were done as described by Powell-Coffman et al. (1996); Rocheleau et al. (1997). To prepare *pos-1* RNA, the insert of the clone yk117h11 (accession number D73358) was amplified using M13 and M13 reverse primers. To prepare *ama-1* RNA, a region of the *ama-1* locus (corresponding to nucleotides 9540 to 11381 of cosmid F36A4) was amplified from genomic DNA using oligos tailed with T3 and T7 polymerase sites. The amplified DNA was purified with Wizard PCR preps (Promega) and RNA was transcribed from the DNA using the Ribomax RNA production system (Promega). Microinjection of RNA into wild-type or homozygous *zul48* adult hermaphrodites induced a penetrant RNAi phenotype in F₁ embryos fertilized more than 24 hours after microinjection. All *ama-1*(RNAi) embryos arrested with 100-150 cells and without tissue differentiation as previously described by (Powell-Coffman et al., 1996; n=480).

Blastomere ablations

Laser ablation experiments were performed as previously described (Mello et al., 1992). P₄ was analyzed after ablation of ABa, ABp, EMS, C, and D. E (or MS) was analyzed after ablation of ABa, ABp, P₂ and MS (or E). In order to determine whether the descendants of ABp produced pharynx in *pos-1* mutants, ABa was ablated at the 4-cell stage and the descendants of EMS were ablated at the 12-14 cell stage. In control experiments to determine whether the descendants of ABa produce pharynx, ABp was ablated at the 4-cell stage and MS (or its daughters) at the 12 (or 14) cell stage. In these control experiments, 7 of 12 *pos-1* mutant embryos and 12 of 12 wild-type embryos produced pharynx.

Tissue type scoring and videomicroscopy

The presence of different tissues was scored by immunostaining with mAb3NB12 (pharynx; Priess and Thomson, 1987), mAb5.6 (body wall muscle; Miller et al., 1983), or mAbICB4 (intestine and valve cells; Okamoto and Thomson, 1985); by expression of a *hlh-1::GFP* transgene (muscle); by the birefringence of rhabditiin granules under polarized light (intestine); or by morphological criteria with Nomarski optics for hypodermis and germ cells (Mello et al., 1992).

The fate of ABp descendants was determined using a 4D microscope system described by Thomas et al. (1996). To calculate the volume of blastomeres, duplicate area measurements were made from videomicrographs. The volume was then calculated using the assumption that the blastomeres were spherical.

RESULTS

***pos-1* mRNA and POS-1 protein are distributed asymmetrically**

We used in situ hybridization to examine the distribution patterns of approximately one thousand mRNAs in early embryos as part of the *C. elegans* cDNA project (Y. Kohara, unpublished data). Probes from the cDNA clone yk61h1 reproducibly showed stronger hybridization to the posterior blastomere of a 2-cell stage embryo than to the anterior blastomere; this posterior blastomere is called P₁, and is a

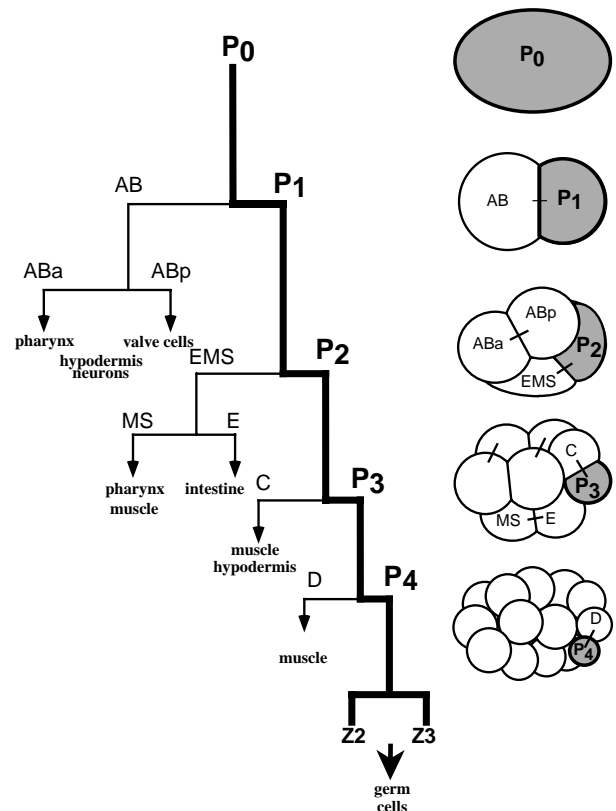
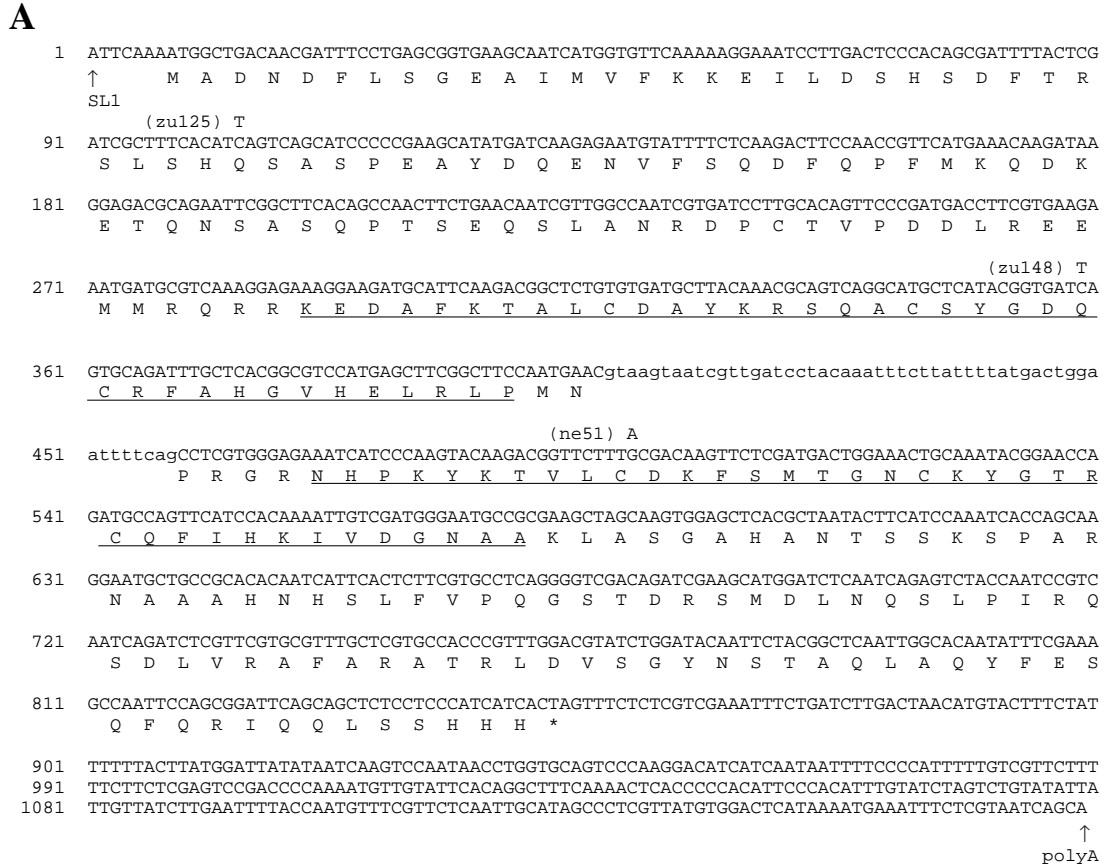
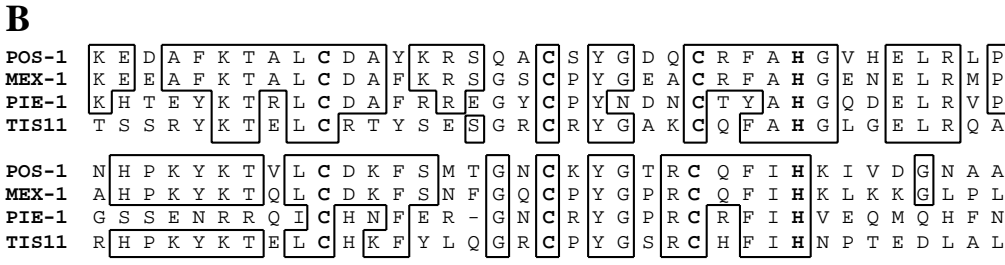


Fig. 1. Embryonic development of *C. elegans*. An abbreviated lineage diagram is shown on the left. Diagrams of embryos at corresponding stages are shown on the right (anterior is to the left, dorsal is up). Germline blastomeres are indicated by bold in the lineage diagram, and by shading in the schematic diagrams. Some of the tissue types produced by somatic blastomeres are listed.

Fig. 2. The *pos-1* gene. (A) Genomic sequence of the *pos-1* gene. The first base is the addition site of the *trans*-spliced leader SL1 and corresponds to base 31788 of cosmid F52E1. The final base corresponds to the start of a poly(A) tract. The single intron is shown in lowercase. Northern analysis shows that the *pos-1* mRNA is 1.3 kb in length in early embryos and adults (data not shown). Point mutations in *pos-1* alleles are indicated above the DNA sequence; the alleles *zu363* and *zu175* are deletions of bases 148-417 and 206-347, respectively. The predicted POS-1 protein is shown below the DNA sequence with the two CCCH finger domains underlined.



(B) Alignment of CCCH fingers. The top and bottom panels compare the first and second CCCH fingers of POS-1 with the corresponding fingers in MEX-1 and PIE-1 (*C. elegans*) and TIS11 (Mouse). Amino acid residues shared by POS-1 and MEX-1 are boxed.



germline blastomere (Fig. 1). We named the corresponding gene *pos-1* (posterior localization/posterior lineage defective). Sequence analysis of *pos-1* cDNAs and genomic DNA showed that the *pos-1* gene can encode a protein, POS-1, of 264 amino acids (Fig. 2A). Database searches with the predicted protein sequence showed that POS-1 is most similar to the *C. elegans* protein MEX-1 (Guedes and Priess, 1997) and that the greatest similarity between the two proteins is in two regions that each contain a copy of a CCCH-type of 'finger' motif (Fig. 2B).

The CCCH finger motif was first identified in a mammalian protein of unknown function called TIS11/Nup475/TTP (Varnum et al., 1989; DuBois et al., 1990; Lai et al., 1990). This motif has a different primary sequence from the 'zinc finger' domains found in transcription factors related to TFIIIA or GATA factors, and the biochemical function of the motif has not been determined (see Discussion). This motif also is found in the PIE-1 protein of *C. elegans* (Fig. 2B; Mello et al., 1996) and in other proteins predicted from the Genome Sequencing Project and the cDNA project of *C. elegans* (data not shown).

To define the expression pattern of the *pos-1* mRNA, we

performed in situ hybridization experiments on animals at different stages of development. During postembryonic development, *pos-1* mRNA is detected in the immature gonads of fourth stage larvae and in the mature gonad of adults (data not shown). *pos-1* mRNA is distributed uniformly in oocytes and in newly fertilized embryos (Fig. 3A). During the first embryonic cell division, *pos-1* mRNA appears more abundant in the posterior half of the embryo, such that at the 2-cell stage, *pos-1* mRNA is present at higher levels in the germline blastomere P₁ than in the somatic blastomere (Fig. 3C). *pos-1* mRNA initially is present at similar levels in both daughters of P₁ (Fig. 3E), but persists at high levels only in the germline daughter, P₂ (Fig. 3G). This asymmetrical distribution pattern is seen at each of the subsequent divisions of germline blastomeres (Fig. 3I,K). After the division of the final germline blastomere P₄, *pos-1* mRNA is not detectable in either daughter (Fig. 3M).

To determine the distribution of the POS-1 protein, we raised antisera against a fusion protein containing the N-terminal region of POS-1, and against synthetic peptides corresponding

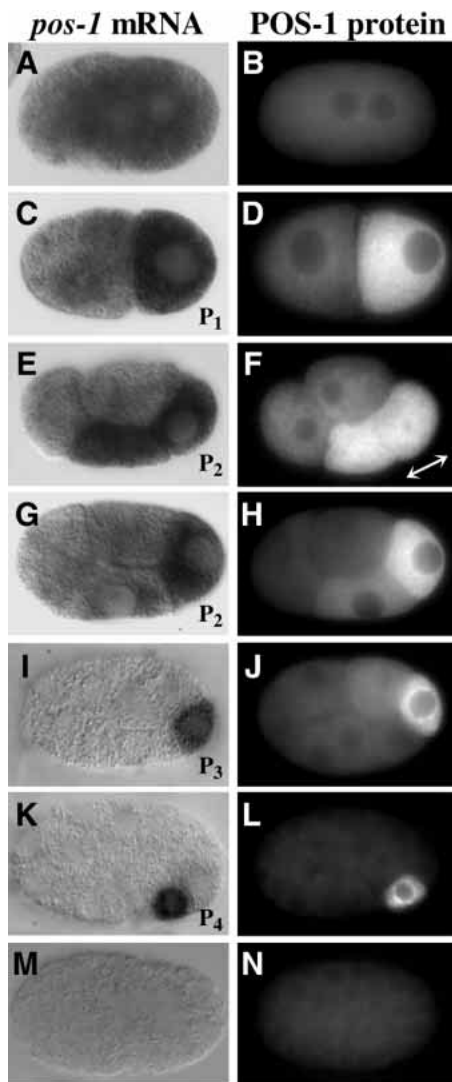


Fig. 3. Distribution of *pos-1* gene products. Each row compares two embryos at a comparable stage. The left column shows brightfield images of embryos after in situ hybridization for *pos-1* mRNA (darkly staining regions), and the right column shows immunofluorescence micrographs of embryos after staining with POS-1 antiserum (white regions). Embryos are oriented as in Fig. 1. (A,B) Newly fertilized, precleavage egg; (C,D) 2-cell stage; (E,F) early 4-cell stage, double-headed arrow in F indicates the sister blastomeres EMS and P₂; (G,H) late 4-cell stage; (I,J) 15-cell stage; (K,L) approximately 28-cell stage. (M,N) Embryos midway through embryonic development after the division of P₄.

to regions that are divergent in sequence from the MEX-1 protein. Both antisera gave identical immunostaining patterns; results shown here were obtained with the antiserum to the fusion protein except where otherwise stated. POS-1 protein is first detectable at low levels in some 1-cell embryos (data not shown). POS-1 is detected at high levels at the 2-cell stage, when it appears predominantly in the germline blastomere P₁ (Fig. 3D). The distribution of POS-1 protein after the 2-cell stage is similar to the distribution of the *pos-1* mRNA. When P₁ divides, for example, the POS-1 protein initially is present at equal levels in both P₁ daughters (double-headed arrow, Fig.

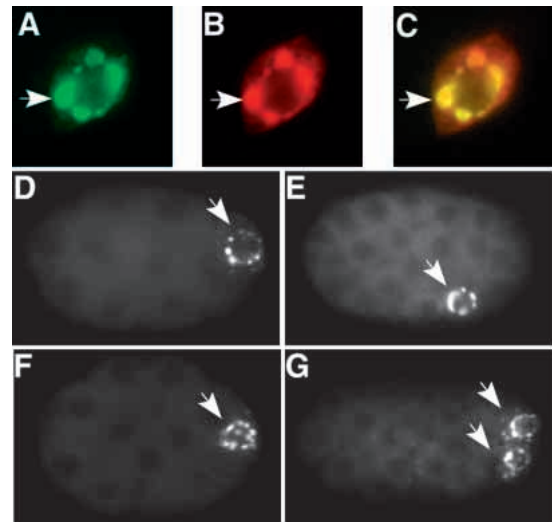


Fig. 4. P-granules in wild-type and in *pos-1* mutant embryos. The top row shows a high magnification view of a single, wild-type P₄ nucleus surrounded by granules (arrow) that stain positively with (A) mAbK76, which recognizes P granules, and with (B) a rabbit POS-1 antiserum. The combined image is shown in C. (D-G) Embryos stained with mAbK76 to visualize P granules. (D,E) Wild-type embryos showing P granules localized to P₃ (D) and P₄ (E). (F,G) *pos-1* mutant embryos showing P granules localized to P₃ (F) and to both P₄ and D (G).

3F), but persists at high levels only in the germline daughter P₂ (Fig. 3H). After the division of the final germline blastomere P₄, POS-1 is not detectable by immunostaining in any cell (Fig. 3N).

All antisera against POS-1 show strong, specific staining of granules within the cytoplasm of germline blastomeres. In double labeling experiments with an antibody specific for P granules (Strome and Wood, 1983), we determined that POS-1 is associated with cytoplasmic and perinuclear P granules (Fig. 4A-C). Although P granules are present in the germline at all stages of embryonic and postembryonic development, the POS-1 antisera only show staining of P granules in the germline blastomeres P₁, P₂, P₃ and P₄. Thus the *pos-1* gene is expressed maternally, and POS-1 is a cytoplasmic, P granule-associated protein that is present predominantly in germline blastomeres during early embryogenesis.

Identification of *pos-1* mutants

In genetic screens, we identified six independent mutant strains that had maternal-effect lethality and that produced inviable embryos with very similar phenotypes (see below). Genetic tests showed that these mutants are defective in a single locus. Since the genetic map position of this locus was similar to the molecular map position of *pos-1*, we tested whether the *pos-1* gene was defective in the mutant strains. We sequenced the *pos-1* gene from 5 strains and in each case found mutations that would be predicted to alter the POS-1 protein (Fig. 2A). In addition, we found that the POS-1 antisera failed to detect POS-1 protein in embryos from three of the mutant strains (*zu125*, *zu148*, and *zu363*; data not shown). Finally, using the technique of RNA mediated interference (Guo and Kempthues, 1995), we found that wild-type adults injected with *pos-1* RNA produced

Table 1. Intestinal differentiation in *pos-1* embryos

Embryo type	% embryos with intestine	<i>n</i>
<i>ne51/ne51</i>	38	585
<i>zu175/zu175</i>	8	1094
<i>zu125/zu125</i>	6	490
<i>zu363/zu363</i>	2	793
<i>zu148/zu148</i>	1	991
<i>zu148/sDf35</i>	2	507
<i>pos-1(RNAi)</i>	2	477
<i>zu148/zu148;pos-1(RNAi)</i>	0.3	610

embryos (hereafter referred to as *pos-1(RNAi)* embryos) that were phenocopies of embryos from the mutant strains (Table 1 and data not shown). From these several lines of evidence, we conclude that the 6 maternal-effect lethal mutations are alleles of *pos-1*. We consider *zu148* to be a null allele of *pos-1* since the phenotype it causes is not altered when *zu148* is placed in *trans* to a genetic deficiency of the *pos-1* locus (Table 1), and *zu148* mutants do not stain with the POS-1 antisera. Our analysis of the *pos-1* phenotype uses the *zu148* allele, except where it is noted otherwise. For purposes of convenience, the term ‘*pos-1* mutant embryo’ indicates an embryo produced from a mother homozygous for a mutation in *pos-1*.

pos-1 mutant embryos undergo approximately the normal number of cell divisions and produce well differentiated tissues (data not shown). However, terminal stage *pos-1* mutant embryos lack several cell types including intestinal cells (Table 1) and germ cell progenitors. In addition, *pos-1* mutants have an abnormally large amount of pharyngeal tissue, and they fail to hatch due to defects in body morphogenesis (data not shown). The germline defects and the somatic defects of *pos-1* mutants are described separately below.

***pos-1* mutants lack germ cell progenitors**

In wild-type embryogenesis, the germline blastomere P₄ divides once to produce the two germ cell progenitors, called Z₂ and Z₃ (see lineage diagram in Fig. 1). In late stage embryos, Z₂ and Z₃ are large cells with a distinctive morphology. Terminal stage *pos-1* mutant embryos lack any cells that resemble Z₂ or Z₃ (data not shown). To examine the development of P₄ in *pos-1* mutants, we used a laser microbeam to kill all other blastomeres; we refer to such experimental embryos as ‘laser-operated embryos’. In laser operated wild-type embryos, P₄ produces the two germ cell progenitors (Mello et al., 1992). In laser-operated *pos-1* mutant embryos, we found that P₄ does not produce germ cell progenitors (0 of 21 embryos made germ cell progenitors). P₄ instead undergoes multiple rounds of division and produces descendants that become muscles: these P₄ descendants are contractile, stain positively with the muscle-specific antibody mAb5.6 (14 of 19 embryos), and express the muscle-specific marker *hllh-1::GFP* (2 of 2 embryos). Since the sister of P₄, the D blastomere, produces muscle cells in wild type (see Fig. 1), we propose that P₄ adopts the fate of D in *pos-1* mutant embryos.

Defects in germline blastomeres in *pos-1* mutants

Since the germline blastomere P₄ has an altered fate in *pos-1* mutants, we asked whether defects could be detected in earlier germline blastomeres. Wild-type germline blastomeres have several characteristics that distinguish them from somatic

Table 2. Cleavage asymmetry of P₀ through P₃

Embryo type	Percentage volume segregated to P daughter				
	P ₀	P ₁	P ₂	P ₃	<i>n</i>
Wild type	38	40	25	32	6
<i>pos-1(zu148)</i>	38	40	39	51	8
<i>ama-1(RNAi)</i>	41	38	26	28	2
<i>pos-1(zu148);ama-1(RNAi)</i>	37	38	33	48	4

Each column indicates the volume of the blastomere that is inherited by its P daughter. For example, when a wild-type P₀ divides, 38% of its volume goes into its daughter P₁ (see Materials and Methods).

blastomeres: they have asymmetrically positioned mitotic spindles that result in asymmetric cell cleavage, they have relatively long cell cycle periods, they segregate P granules exclusively to daughter germline blastomeres, and they contain high levels of the proteins PIE-1 and MEX-1. In *pos-1* mutants, the first two germline blastomeres P₀ and P₁ appear normal for each of these characters, whereas the later germline blastomeres P₂, P₃ and P₄ have prominent defects in at least one of these characters. In all *pos-1* mutants examined, P₂ has less cleavage asymmetry than in wild type and P₃ has little to no cleavage asymmetry (Table 2). P₂, P₃, and P₄ have abnormally short cell cycle periods (Table 3). For example at the fifth embryonic cell cycle, P₄ has a period of 92 minutes in wild type and 31 minutes in *pos-1* mutants. In *pos-1* mutants the P granules, PIE-1 and MEX-1 are partitioned correctly into P₃ (95 of 102 embryos, see Fig 4F; 102 of 102 embryos, 41 of 41 embryos; respectively). However after the division of P₃, P granules (Fig. 4G) and PIE-1 are often present at equal levels in both P₃ daughters (55 of 58, and 20 of 67 embryos; respectively). Finally, the levels of cytoplasmic and P granule-associated PIE-1 and MEX-1 protein are diminished in the daughters of P₃ (data not shown).

Transcriptional regulation in *pos-1* mutants

Somatic blastomeres initiate transcription early in wild-type development while the germline blastomeres generally lack embryonically expressed mRNAs (Seydoux and Fire, 1994). For convenience, the absence of embryonic mRNAs in the germline blastomeres is called transcriptional repression, although the biochemical basis for the lack of mRNAs has not been established. PIE-1 is required for transcriptional repression in the germline blastomeres (Seydoux et al., 1996) and normally is present at high levels only in the germline blastomeres (Mello et al., 1996). Because POS-1 shares a sequence motif with PIE-1, we examined whether POS-1, like PIE-1, is required to repress transcription in the germline blastomeres.

We analyzed expression of the *pes-2* gene to determine whether germline blastomeres in *pos-1* mutants have inappropriate transcription. In our screen for asymmetrically localized mRNAs, we found that *pes-2* is expressed by the embryo at high levels starting at the 8-cell stage. Like other embryonically expressed genes described previously (see for example, Seydoux and Fire, 1994), expression of *pes-2* is repressed in the germline blastomeres by *pie-1(+)* activity. *pes-2* mRNA is not detected in the P₄ blastomere of wild-type embryos, which has high levels of PIE-1 (arrow, Fig. 5A), but is detected in P₄ in *pie-1* mutants lacking PIE-1 (arrow, Fig. 5B). *mex-1* mutants express PIE-1 ectopically in multiple

Table 3. Cell cycle periods of early blastomeres

Blastomere	Embryo type	Embryonic cell cycle					
		2	3	4	5	6	7
P lineage		(P ₁)	(P ₂)	(P ₃)	(P ₄)	(Z ₂ /Z ₃)	
	Wild type	19	25	34	92	no division	
	<i>pos-1(zu148)</i>	19	22	26	31	47	
	<i>ama-1(RNAi)</i>	23	28	40	103	no division	
	<i>pos-1(zu148); ama-1(RNAi)</i>	21	26	33	48	89	
AB	Wild type	18	18	19	23	33	38
	<i>pos-1(zu148)</i>	18	18	19	23	38	44
	<i>ama-1(RNAi)</i>	20	21	22	26	46	≥70*
	<i>pos-1(zu148); ama-1(RNAi)</i>	19	21	22	28	46	82
EMS	Wild type	–	21				
	<i>pos-1(zu148)</i>	–	20				
MS	Wild type			22	27	33	
	<i>pos-1(zu148)</i>			21	25	36	
E	Wild type	–	–	24	52		
	<i>pos-1(zu148)</i>	–	–	22	26		
C	Wild type	–	–	24	31	41	
	<i>pos-1(zu148)</i>	–	–	22	27	40	
D	Wild type	–	–	–	49		
	<i>pos-1(zu148)</i>	–	–	–	31		

The time (measured in minutes) of the cell cycle period of individual blastomeres is shown beginning with the second embryonic cell cycle (2-cell stage embryo). Each time is an average of measurements made on 4 embryos. Embryos were grown at 20±0.6°C.

*Division occurred in only two of four embryos examined.

blastomeres (Guedes and Priess, 1997), and appear to lack *pes-2* expression in these blastomeres (Fig. 5C). We examined *pes-2* mRNA in *pos-1* mutant embryos by in situ hybridization, and found that *pes-2* mRNA could not be detected in either the P₃ (data not shown) or the P₄ blastomere (arrow, Fig. 5D). We obtained similar results with a second gene, *pes-10* (Seydoux and Fire, 1994). In these experiments, embryonic expression of *pes-10* was monitored by GFP fluorescence from a *pes-10::GFP* transgene. As in wild-type embryos, GFP was not found in P₃ and P₄ of *pos-1* mutants (data not shown).

An additional reagent for evaluating transcription in the *C. elegans* embryo is the monoclonal antibody mAbH14 (Seydoux and Dunn, 1997). This antibody detects a phosphoepitope on the large subunit of RNA polymerase II (Kim et al., 1997). In *C. elegans*, high levels of the mAbH14 epitope correlates with the presence of active transcription; the nuclei of somatic blastomeres have high levels of the mAbH14 epitope, while the nuclei of germline blastomeres have low levels of the mAbH14 epitope (Fig. 5E; Seydoux and Dunn, 1997). In *pie-1* mutant embryos, the germline blastomeres have high levels of the mAbH14 epitope similar to the levels in somatic blastomeres (Seydoux and Dunn, 1997). In *pos-1*

mutant embryos, we found that P₂ and P₃ showed normal, low levels of staining with mAbH14 (data not shown). However the

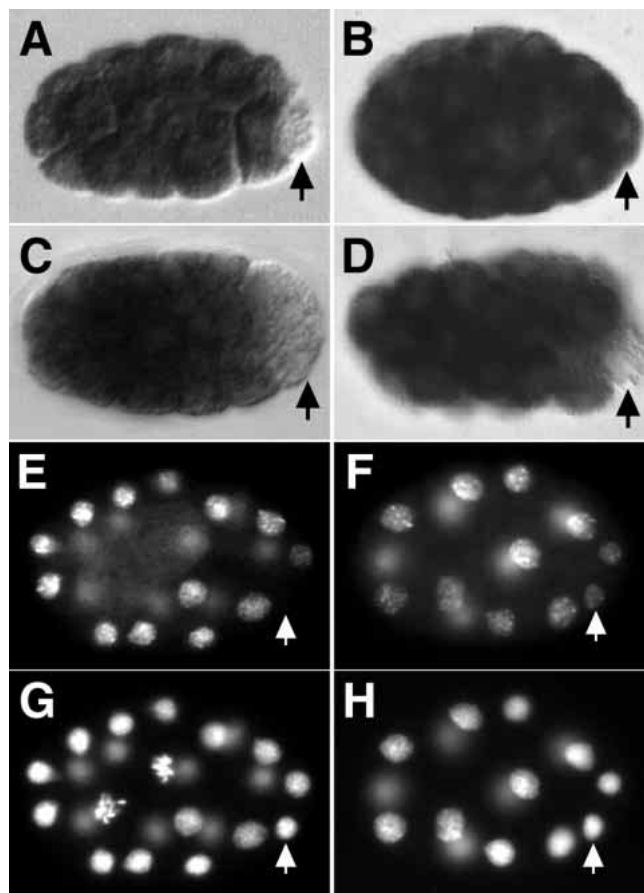


Fig. 5. Transcription in early embryos. In each panel the arrow points towards the position of the P₄ blastomere. (A-D) Analysis of transcription by in situ hybridization with a probe for *pes-2* mRNA (dark staining regions). (A) Wild-type embryo showing repression of *pes-2* only in P₄. (B) *pie-1(zu154)* mutant embryo showing no repression of *pes-2*. (C) *mex-1(zu121)* mutant embryo showing repression of *pes-2* in multiple blastomeres. (D) *pos-1* mutant embryo showing repression of *pes-2* only in P₄. (E-H) Analysis of transcription by immunostaining with mAbH14. (E) Wild-type embryo and (F) *pos-1* mutant embryo stained with mAbH14. (G,H) The same embryos as in E and F, respectively, stained with DAPI.

P₄ blastomere showed an abnormal, high level of staining (arrow, Fig. 5F). These results suggest that transcription is repressed properly in the early germline blastomeres in *pos-1* mutants, but that some transcription may initiate prematurely after the birth of P₄.

This analysis did not eliminate the possibility that low levels of transcription occur inappropriately in the early germline blastomeres of *pos-1* mutants. If such transcription occurred in *pos-1* mutants, it could be responsible for the defects in cleavage asymmetry and cell cycle that we have described. In wild-type development, the cleavage asymmetry and cell cycle of the germline blastomeres appears to be controlled by maternally, rather than embryonically, expressed genes because these processes are not affected by procedures that block embryonic transcription (Edgar et al., 1994; Powell-Coffman et al., 1996). Therefore, we reasoned that if inappropriate transcription was responsible for the germline defects of *pos-1* mutants, these defects would be suppressed by blocking embryonic transcription.

Embryonic transcription can be blocked with α -amanitin, or with RNA-mediated interference of the *ama-1* gene [*ama-1(RNAi)*], which encodes the large subunit of RNA polymerase II (Bird and Riddle, 1989). We found that *pos-1(zu148);ama-1(RNAi)* embryos appear similar to *pos-1(zu148)* embryos in terms of cleavage asymmetry (Table 2) and cell cycle periods (Table 3). For example, the cell cycle period of the P₄ blastomere is 48 and 31 minutes in the two types of embryos, respectively, compared to the much longer period of 92 minutes in wild-type embryos. In summary, *pos-1* mutants do not show the same types of defects in embryonic transcription that have been described previously for *pie-1* mutants, and it is unlikely that inappropriate transcription is the cause of the multiple defects observed in the early germline blastomeres of *pos-1* mutants.

Somatic defects in *pos-1* mutants

Defects in any of three distinct pathways have been shown to result in embryos with abnormally large amounts of pharyngeal tissue (excess pharynx; for review see Schnabel and Priess, 1997). We therefore addressed whether the excess pharynx prominent in *pos-1* mutant embryos resulted from similar defects. The first pathway localizes the activity of the SKN-1 transcription factor to the EMS blastomere. By immunostaining, we found that almost all *pos-1* mutant embryos had normal localization of SKN-1 (73 of 74 embryos). In wild-type development, SKN-1 activity causes the EMS blastomere to produce pharyngeal cells. Killing EMS with a laser prevents pharyngeal development in wild-type embryos, but not in mutant embryos such as *mex-1* that have ectopic SKN-1 activity (Mello et al., 1992). In *pos-1* mutants, we found that killing the EMS blastomere prevented pharyngeal development (0 of 20 embryos produced pharynx).

The second pathway is a Wnt-like (Wingless) pathway that polarizes EMS (Goldstein 1995; Rocheleau et al., 1997; Thorpe et al., 1997). This polarization results in the daughters of EMS, called MS and E, having different levels of the transcription factor POP-1 and different fates. We found that POP-1 was expressed correctly in *pos-1* mutant embryos (POP-1 was present at higher levels in MS than in E in 10 of 10 embryos, data not shown). Defects in the Wnt-like signaling pathway cause both MS and E to produce pharyngeal cells. In

contrast, we found in laser operated *pos-1* mutant embryos that neither MS nor E produced pharyngeal cells (0 of 20 and 0 of 16 embryos produced pharynx, respectively). Instead MS and E produced hypodermal cells (18 of 19, and 19 of 20 embryos, respectively; see Materials and Methods).

The third pathway is a Notch-like signaling pathway which regulates the development of the blastomeres ABa and ABp. These blastomeres are equivalent initially, but have different patterns of development because only ABp receives a signal from P₂. In the absence of this signal, ABp develops like ABa; for example ABp descendants fail to make valve cells and instead produce pharynx (Schnabel and Priess, 1997). We found that *pos-1* mutants lack valve cells (0 of 53 embryos showed valve cell staining with the antibody mAbICB4; see Materials and Methods). We followed the development of selected ABp descendants using videomicroscopy, and found that they adopted fates similar to the corresponding ABa descendants in wild-type embryos (Table 4). Finally, we found that ABp can produce pharynx in laser operated *pos-1* mutant embryos (20 of 29 embryos; see Materials and Methods). Thus ABp is the source of excess pharynx in *pos-1* mutants, and the altered development of ABp suggests a defect in P₂ to ABp signaling.

pos-1 mutants fail to express APX-1

In wild-type development, P₂ signals ABp with the ligand APX-1. *apx-1* mRNA is expressed maternally, and is present at equal levels in all blastomeres of 2-cell and 4-cell embryos (Mickey et al., 1996). However the APX-1 protein is expressed only in the germline blastomeres P₁ and P₂ at these stages

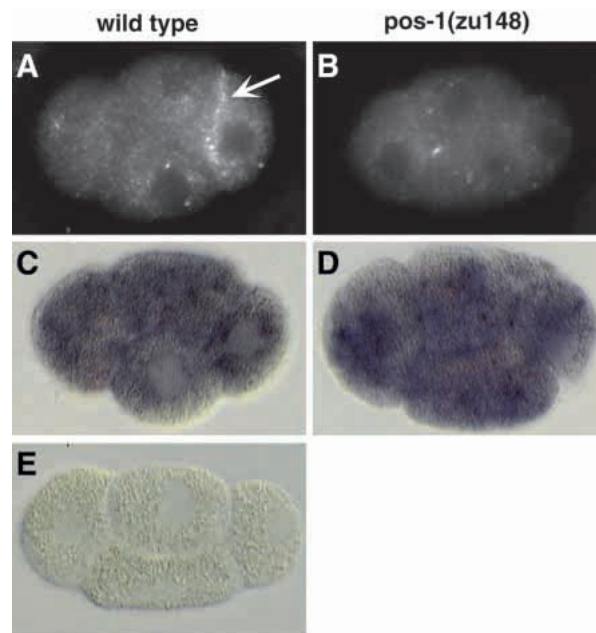


Fig. 6. Expression of APX-1 protein and *apx-1* mRNA in *pos-1* mutants. The genotypes of embryos are listed above the columns. (A,B) 4-cell stage embryos stained with an anti-APX-1 antiserum. In A, the arrow points to a band of APX-1 along the contact region between the germline blastomere P₂ and the somatic blastomere ABp. (C,D) Embryos after in situ hybridization with probes for *apx-1* mRNA (purple stain). (E) A control, wild-type 4-cell embryo hybridized with a sense probe for *apx-1*.

Table 4. ABp adopts an ABa-like fate in *pos-1* mutants

ABp descendant	Fate of ABp descendant in wild type	Fate of ABa descendant in wild type*	Fate of ABp descendant in <i>pos-1</i>
ABplpappap	death	hyp	hyp
ABpraapapa	division	death	death
ABprpaaapp	division	death	death
ABprppaaap	death	hyp	hyp
ABprpapapp	division	hyp	hyp
ABprpappap	division	hyp	hyp
ABprpapppa	division	hyp	hyp
ABprpapppp	vir(L/R)	hyp	hyp
ABprppppaa	division	hyp	hyp
ABprppppap	division	hyp	hyp‡

For each ABp descendant in column 1, the table lists: the fate of the descendant in wild-type development (column 2; Sulston et al., 1983); the fate of an analogous ABa descendant in wild-type development (column 3; see below); the fate of the ABp descendant in a *pos-1* mutant embryo (column 4). Fates in column 4 are from one of two *pos-1(zu125)* mutant embryos or a *pos-1(zu148)* mutant embryo. Fates are indicated as follows. Death: programmed cell death; hyp: hypodermis (epidermis); division: cell undergoes mitosis; vir(L/R): precursor to the left and right intestinal valve cells.

*ABp descendants can adopt one of two 'ABa-like' fates in the absence of P₂ signaling, depending on whether or not they contact a second signaling cell (for review see Schnabel and Priess, 1997). The fates listed here are those predicted in the absence of the second signal. The observation that ABp descendants often produce pharyngeal cells in *pos-1* mutants (described in text) suggests that they can respond to the second signal, though they did not do so in the experiments shown in this table.

‡15 other ABprp descendants showed the same transformation.

(Mickey et al., 1996; arrow, Fig. 6A). We used an antiserum that recognizes the APX-1 protein to stain *pos-1* mutant embryos, and failed to detect APX-1 in P₁ and P₂ (Fig. 6B; APX-1 was detected in 0 of 90 *pos-1* embryos and in 117 of 121 wild-type embryos, 3 to 7-cell stage). Because the APX-1 protein was not detectable, we asked whether the *apx-1* mRNA was present. We found that *apx-1* mRNA was readily detectable in 4-cell stage *pos-1* mutant embryos by reverse transcription PCR analysis (data not shown). By in situ hybridization, we found that *pos-1* mutant embryos had levels of *apx-1* mRNA equivalent to those found in wild-type embryos (Fig. 6). Thus, the germline blastomeres of *pos-1* mutants fail to express the APX-1 protein from the maternally supplied pool of *apx-1* mRNA.

DISCUSSION

We have shown that the maternal gene *pos-1* is required for the development of germ cells in *C. elegans*. Germ cells arise from lineally related embryonic blastomeres called germline blastomeres, and we have shown here that the POS-1 protein is expressed predominantly in the germline blastomeres. The POS-1 protein is first detected in the germline blastomere P₁, and the earliest defect we have observed in *pos-1* mutant embryos is the failure of P₁ to express the APX-1 protein. Subsequent germline blastomeres exhibit defects in cleavage asymmetry, cell cycle period, and partitioning of P granules. Because the cleavage asymmetry, cell cycle periods, and P granule partitioning of wild-type germline blastomeres do not appear to depend on interactions with somatic blastomeres (Schierenberg, 1988), our results indicate that

POS-1 has essential functions in the germline blastomeres themselves.

POS-1 also is required for the proper development of certain somatic tissues, raising the issue of whether POS-1 has essential functions within the somatic blastomeres. We have shown that the defects in the somatic blastomere ABp correspond to the types of defects that result from disruption of a Notch-like signaling pathway. In normal development, ABp uses the receptor GLP-1 to respond to the ligand APX-1 provided by the germline blastomere P₂. A wild-type ABp that is prevented from interacting with P₂ can use GLP-1 to respond to a second signal, and thereby produces pharyngeal cells. Our observation that ABp can produce pharyngeal cells in *pos-1* mutants therefore suggests that the GLP-1 response pathway is functional in ABp. Because P₂ in *pos-1* mutants fails to express APX-1, it is likely that the ABp defect in *pos-1* mutants results primarily from a defect in the germline blastomeres.

The development of the somatic blastomere EMS depends upon an interaction with P₂, which is mediated by a Wnt-signaling pathway. The defects we observe in EMS in *pos-1* mutants do not match the types of defects that result from disruption of the Wnt pathway, but instead are similar to the defects caused by mutations in the gene *skn-1*. These defects include the production of hypodermal cells by both daughters of EMS, and a failure of the daughters of E to initiate gastrulation of the embryo (our unpublished results). Since *pos-1* mutants have the wild-type pattern of SKN-1 expression, it is possible that *pos-1* mutants lack a factor(s) that functions in concert with SKN-1. POS-1 could be this factor, and function in the EMS blastomere with SKN-1. Alternatively, POS-1 function could be required within the germline blastomeres for the expression of a SKN-1 co-factor that is later inherited by EMS. SKN-1 itself provides an example of a protein that is expressed initially in a germline blastomeres before it is inherited by EMS (Bowerman et al., 1993).

POS-1 and transcriptional regulation

We have shown that the predicted POS-1 protein contains two copies of a CCCH 'finger' motif that also is present in the PIE-1 and MEX-1 proteins of *C. elegans*. A biochemical function for the CCCH motif has not been demonstrated, but several proteins with CCCH motifs have been implicated in different aspects of RNA metabolism. The U2AF³⁵ protein, which is involved in mRNA splicing, contains two CCCH fingers (Zhang et al., 1992). The 30 kDa subunit of Cleavage and Polyadenylation Specificity Factor (CPSF) contains 5 CCCH domains (Barabino et al., 1997), and the corresponding protein of *Drosophila*, Clipper, has been shown to act as an endoRNase in vitro (Bai and Tolia, 1996). Recent studies have shown that a murine CCCH protein called TTP can bind to the TNF- α mRNA (Carballo et al., 1998). Thus, it is possible that the finger domains of POS-1 mediate protein-nucleic acid interactions in *C. elegans*, for example in translational or transcriptional regulation.

The PIE-1 protein appears to function in transcriptional regulation by repressing embryonic transcription in the germline blastomeres (Seydoux et al., 1996). In contrast, we do not believe that POS-1 functions to repress transcription in the early germline blastomeres. First, our analysis of *pes-2* expression and mAbH14 staining in *pos-1* mutants suggests that transcription is repressed properly in the germline

blastomeres P₀, P₁, P₂ and P₃. Second, after blocking transcription in *pos-1* mutant embryos, we did not observe significant suppression of the defects in cleavage asymmetry and cell cycle period. Finally, other than the fact that *pos-1* and *pie-1* mutants fail to produce germ cells, they have almost no phenotypic similarity, suggesting that the POS-1 and PIE-1 proteins have different functions. Although both mutants have defects in APX-1, *pos-1* mutants lack APX-1 altogether, while *pie-1* mutants have variable abnormalities in APX-1 expression. APX-1 is a transmembrane protein in the germline blastomeres, and when APX-1 is segregated into a somatic blastomere such as EMS, it is internalized and degraded. In *pie-1* mutants, the P₂ blastomere adopts an EMS-like fate and often shows internalized APX-1. Thus we consider it likely that inappropriate transcription in the P₂ blastomere of *pie-1* mutants causes it to process APX-1 like a wild-type EMS.

Although inappropriate transcription does not appear to be the cause of the defects in the early germline blastomeres in *pos-1* mutants, transcription may initiate prematurely in the final germline blastomere, P₄. In *pos-1* mutants, the level of PIE-1 in the nucleus of P₄ is reduced, and previous studies on other mutants (Tenenhaus et al., 1998) have suggested that reduced levels of PIE-1 can cause a partial activation of transcription. Although *pes-2* does not appear to be transcribed in the P₄ blastomere of *pos-1* mutants, P₄ has a high level of mAbH14 staining that suggests active transcription. Thus the level of PIE-1 in P₄ might be sufficient to repress transcription of *pes-2*, but not other genes in *pos-1* mutants.

Premature transcription in P₄, or in the daughters of P₄, is a likely explanation for why P₄ produces muscles rather than germ cells in *pos-1* mutants. In wild type embryos, P₄ and its sister D, both contain the transcription factor PAL-1; *pal-1(+)* activity appears to promote muscle development in D descendants, but has no effect on the transcriptionally repressed P₄ blastomere (Hunter and Kenyon, 1996). If P₄ became transcriptionally competent in a *pos-1* mutant embryo, it might also be expected to produce muscles in response to *pal-1(+)* activity.

POS-1 and translational regulation

C. elegans appears to use transcriptional repression as a strategy for maintaining the totipotency of the early germline blastomeres. Although the germline blastomeres do not transcribe new mRNAs, each germline blastomere has a unique pattern of protein expression. For example, P₀ expresses PIE-1, P₁ expresses PIE-1 and APX-1, and P₂ expresses PIE-1, APX-1 and PAL-1 (Mickey et al., 1996; Hunter and Kenyon, 1996; Mello et al., 1996). These proteins are expressed from a pool of maternally provided mRNAs that is present in all of the germline blastomeres. Very little is known about how the temporal patterns of protein expression are achieved, but the diversity of the patterns suggests that the germline blastomeres have multiple mechanisms to control translation or protein stability.

We propose that POS-1 protein is part of the mechanism for regulating protein expression from maternal mRNAs in wild-type embryos. APX-1 protein is not detectable in *pos-1* mutants, though *apx-1* mRNA appears at wild-type levels. Since POS-1 expression closely precedes APX-1 expression in wild-type embryos, *pos-1(+)* activity may directly regulate *apx-1* mRNA translation. In a similar manner, the defects in cleavage

asymmetry, cell cycle period, and P granule partitioning observed in *pos-1* mutant embryos could result from a failure to translate unidentified mRNAs required for those processes.

POS-1 and MEX-1

POS-1 and MEX-1 are more similar in sequence to each other than to PIE-1, and neither appear to have the same function as PIE-1 in transcriptional repression in the germline blastomeres (our present study; Guedes and Priess 1997). This raises the issue of whether MEX-1 has a function that is similar to POS-1. We have found that *mex-1(zu120)* mutant embryos have lower than normal levels of POS-1 at all stages (our unpublished results), suggesting that MEX-1 could have a role in mRNA translation. *mex-1(zu120)* mutants also lack APX-1 expression, although we do not yet know if this is a consequence of the low level of POS-1 (K. Mickey, R. Hill, unpublished observations).

If POS-1 and MEX-1 have a similar biochemical function, then phenotypic differences between *pos-1* and *mex-1* mutants (Mello et al., 1992; Schnabel et al., 1996) could result from POS-1 and MEX-1 having different targets, or from differences in the time that the proteins act in the germline blastomeres. MEX-1 is expressed at high levels in 1-cell stage embryos (Guedes and Priess, 1997), while POS-1 is not expressed at high levels until the 2-cell stage. We do not yet know if forced expression of POS-1 at the 1-cell stage would allow it to substitute for MEX-1. However, since MEX-1 and POS-1 are expressed together after the 1-cell stage, the observation that *mex-1(+)* activity does not compensate for mutations in *pos-1* indicates that POS-1 must have some unique targets. The different requirements for POS-1 and MEX-1 in the development of the germline blastomeres suggests the possibility that diversity between germline blastomeres could be controlled by the sequential expression of different translational regulators. It will be interesting in future studies to determine how POS-1 and MEX-1 function biochemically, and whether the similar proteins predicted by the Genome Sequencing Project and the cDNA project of *C. elegans* have related roles in the development of the germline blastomeres of *C. elegans*.

We thank T. Motohashi for her excellent technical assistance, M. Okada for advice, A. Fire for the *hlh-1::GFP* line, G. Seydoux for the *pes-10::GFP* line, A. Coulson and the Sanger Centre (UK) for cosmid clones, and H. Chamberlin for comments on the manuscript. H. T. was a fellow of the COE program of the MESSC (Ministry of Education, Science and Sports and Culture of Japan). Y. K. is supported by a Grant-in-Aid for Scientific Research on Priority Areas on Genome Science from MESSC and by CREST (Core Research for Evolutional Science and Technology) of Japan Science and Technology Corporation. R. J. H. was a fellow of the Jane Coffin Childs Memorial Fund. C. C. M. is supported by a PEW scholarship, a Career Development Award from the American Cancer Society and a grant from the NIH. J. R. P. is supported by the HHMI and a grant from the NIH. Some of the strains used in this work are from the *Caenorhabditis Genetics Center* (Supported by NIH National Center for Research Resources).

REFERENCES

- Anderson, S. (1981). Shotgun DNA sequencing using cloned DNase I-generated fragments. *Nucl. Acids Res.* **9**, 3015-3027.

- Bai, C. and Tolias, P. P. (1996). Cleavage of RNA hairpins mediated by a developmentally regulated CCCH zinc finger protein. *Mol. Cell Biol.* **16**, 6661-6667.
- Barabino, S. M. L., Hübner, W., Jenny, A., Minvielle-Sebastia, L. and Keller, W. (1997). The 30kD subunit of mammalian cleavage and polyadenylation specificity factor and its yeast homolog are RNA-binding zinc finger proteins. *Genes Dev.* **11**, 1703-1716.
- Barnes, W. M. (1994). PCR amplification of up to 35-kb DNA with high fidelity and high yield from lambda bacteriophage templates. *Proc. Natl. Acad. Sci. USA* **91**, 2216-2220.
- Bird, D. M. and Riddle, D. L. (1989). Molecular cloning and sequencing of *ama-1*, the gene encoding the largest subunit of *Caenorhabditis elegans* RNA polymerase II. *Mol. Cell Biol.* **9**, 4119-4130.
- Bowerman, B., Eaton, B. A. and Priess, J. R. (1992). *skn-1*, a maternally expressed gene required to specify the fates of ventral blastomeres in the early *C. elegans* embryo. *Cell* **68**, 1061-1075.
- Bowerman, B., Draper, B. W., Mello, C. C. and Priess, J. R. (1993). The maternal gene *skn-1* encodes a protein that is distributed unequally in early *C. elegans* embryos. *Cell* **74**, 443-452.
- Brenner, S. (1974) The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71-94.
- Carballo, E., Lai, W. S. and Blackshear, P. J. (1998). Feedback inhibition of macrophage tumor necrosis factor- α production by tristetraprolin. *Science* **281**, 1001-5.
- Coulson, A. R., Waterston, R. H., Kiff, J. E., Sulston, J. E. and Kohara, Y. (1988). Genome linking with yeast artificial chromosomes. *Nature* **335**, 184-186.
- DuBois, R. N., McLane, M. W., Ryder, K., Lau, L. F. and Nathans, D. (1990). A growth factor-inducible nuclear protein with a novel cysteine/histidine repetitive sequence. *J. Biol. Chem.* **265**, 19185-19191.
- Edgar, L. G., Wolf, N. and Wood, W. B. (1994). Early transcription in *Caenorhabditis elegans* embryos. *Development* **120**, 443-451.
- Goldstein, B. (1995). An analysis of the response to gut induction in the *C. elegans* embryo. *Development* **121**, 1227-1236.
- Guedes, S. and Priess, J. R. (1997). The *C. elegans* MEX-1 protein is present in germline blastomeres and is a P granule component. *Development* **124**, 731-739.
- Guo, S. and Kempthues, K. J. (1995). *par-1*, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. *Cell* **81**, 1-20.
- Hodgson, C. P. and Fisk, R. Z. (1987). Hybridization probe size control: optimized 'oligo labelling'. *Nucl. Acids Res.* **15**, 6295.
- Hunter, C. P. and Kenyon, C. (1996). Spatial and temporal controls target *pal-1* blastomere-specification activity to a single blastomere lineage in *C. elegans* embryos. *Cell* **87**, 217-226.
- Kempthues, K. J. and Strome, S. (1997). Fertilization and establishment of polarity in the embryo. In *C. elegans* II, (ed. Riddle, D. R., Blumenthal, T., Meyer, B. J. and Priess, J. R.), pp. 335-359. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Kim, E., Du, L., Bregman, D. B. and Warren, S. L. (1997). Splicing factors associate with hyperphosphorylated RNA Polymerase II in the absence of pre-mRNA. *J. Cell Biol.* **136**, 19-28.
- Lai, W. S., Stumpo, D. J. and Blackshear, P. J. (1990). Rapid insulin-stimulated accumulation of an mRNA encoding a proline-rich protein. *J. Biol. Chem.* **265**, 16556-16563.
- Lin, R., Thompson, S. and Priess, J. R. (1995). *pop-1* encodes an HMG box protein required for the specification of a mesoderm precursor in early *C. elegans* embryos. *Cell* **83**, 599-609.
- Mello, C. C., Draper, B. W., Krause, M., Weintraub, H. and Priess, J. R. (1992). The *pie-1* and *mex-1* genes and maternal control of blastomere identity in early *C. elegans* embryos. *Cell* **70**, 163-176.
- Mello, C. C., Schubert, C., Draper, B., Zhang, W., Lobel, R. and Priess, J. R. (1996). The PIE-1 protein and germline specification in *C. elegans* embryos. *Nature* **382**, 710-712.
- Mickey, K. M., Mello, C. C., Montgomery, M. K., Fire, A. and Priess, J. R. (1996). An inductive interaction in 4-cell stage *C. elegans* embryos involves APX-1 expression in the signalling cell. *Development* **122**, 1791-1798.
- Miller D. M. III, Ortiz, I., Berliner, G. C. and Epstein, H. F. (1983). Differential localization of two myosins with nematode thick filaments. *Cell* **34**, 477-490.
- Okamoto, H. and Thomson, J. N. (1985). Monoclonal antibodies which distinguish certain classes of neuronal and supporting cells in the nervous system of the nematode *Caenorhabditis elegans*. *J. Neurosci.* **5**, 643-653.
- Powell-Coffman, J.A., Knight, J. and Wood, W. B. (1996). Onset of *C. elegans* gastrulation is blocked by inhibition of embryonic transcription with an RNA polymerase antisense RNA. *Dev. Biol.* **178**, 472-483.
- 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 early *C. elegans* embryos. *Cell* **51**, 601-611.
- Rocheleau, C. E., Downs, W. D., Lin, R., Wittmann, C., Bei, Y., Cha, Y-H., Ali, M., Priess, J. R. and Mello, C. C. (1997). Wnt signaling and an APC-related gene specify endoderm in early *C. elegans* embryos. *Cell* **90**, 707-716.
- Schierenberg, E. (1988). Localization and segregation of lineage-specific cleavage potential in embryos of *Caenorhabditis elegans*. *Roux's Archives Dev. Biol.* **197**, 282-293.
- Schnabel, R., Weigner, C., Hutter, H., Feichtinger, R. and Schnabel, H. (1996). *mex-1* and the general partitioning of cell fate in the early *C. elegans* embryo. *Mech. Dev.* **54**, 133-147.
- Schnabel, R. and Priess, J. R. (1997). Specification of cell fates in the early embryo. *C. elegans* II (ed. Riddle, D. R., Blumenthal, T., Meyer, B. J. and Priess, J. R.), pp. 361-382. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Seydoux, G. and Fire, A. (1994). Soma-germline asymmetry in the distributions of embryonic RNAs in *Caenorhabditis elegans*. *Development* **120**, 2823-2834.
- Seydoux, G., Mello, C. C., Pettitt, J., Wood, W. B., Priess, J. R. and Fire, A. (1996). Repression of gene expression in the embryonic germ lineage of *C. elegans*. *Nature* **382**, 713-716.
- Seydoux, G. and Dunn, M. A. (1997). Transcriptionally repressed germ cells lack a subpopulation of phosphorylated RNA polymerase II in early embryos of *Caenorhabditis elegans* and *Drosophila melanogaster*. *Development* **124**, 2191-2201.
- Spiehl, J., Brooke, G., Kuersten, S., Lea, K. and Blumenthal, T. (1993). Operons in *C. elegans*: polycistronic mRNA precursors are processed by trans-splicing of SL2 to downstream coding regions. *Cell* **73**, 521-532.
- Strome, S. and Wood, W. B. (1983). Generation of asymmetry and segregation of germ-line granules in early *Caenorhabditis 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.
- Tabara, H., Motohashi, T. and Kohara, Y. (1996). A multi-well version of in situ hybridization on whole mount embryos of *C. elegans*. *Nucl. Acids Res.* **24**, 2119-2124.
- Tenenhaus, C., Schubert, C. and Seydoux, G. (1998). Genetic requirements for PIE-1 localization and inhibition of gene expression in the embryonic germ lineage of *Caenorhabditis elegans*. *Dev. Biol.* **200**, 212-224.
- Thomas, C., DeVries, P., Hardin, J. and White, J. (1996). Four-dimensional imaging: computer visualization of 3D movement in living specimens. *Science* **273**, 603-607.
- Thorpe, C. J., Schlesinger, A., Carter, J. C. and Bowerman, B. (1997). Wnt signaling polarizes an early *C. elegans* blastomere to distinguish endoderm from mesoderm. *Cell* **90**, 695-705.
- Varnum, B. C., Lim R. W., Sukhatme, V. P. and Herschman, H. R. (1989). Nucleotide sequence of a cDNA encoding TIS11, a message induced in Swiss 3T3 cells by the tumor promoter tetradecanoyl phorbol acetate. *Oncogene* **4**, 119-120.
- Williams, B. D., Schrank, B., Huynh, C., Shownkeen, R. and Waterston, R. H. (1992). A genetic mapping system in *Caenorhabditis elegans* based on polymorphic sequence-tagged sites. *Genetics* **131**, 609-624.
- Zhang, M., Zamore, P. D., Carmo-Fonesca, M., Lamond, A. I. and Green M. R. (1992). Cloning and intracellular localization of the U2 small nuclear ribonucleoprotein auxiliary factor small subunit. *Proc. Natl. Acad. Sci. USA* **89**, 8769-8773.