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pop-1 Encodes an HMG Box Protein Required for the Specification of a Mesoderm Precursor in Early C. elegans Embryos

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Summary

In C. elegans embryogenesis, the MS blastomere produces predominantly mesodermal cell types, while its sister E generates only endodermal tissue. We show that a maternal gene, *pop-1*, is essential for the specification of MS fate and that a mutation in *pop-1* results in MS adopting an E fate. Previous studies have shown that the maternal gene *skn-1* is required for both MS and E development and that *skn-1* encodes a transcription factor. We show here that the *pop-1* gene encodes a protein with an HMG box similar to the HMG boxes in the vertebrate lymphoid-specific transcriptional regulators TCF-1 and LEF-1. We propose that POP-1 and SKN-1 function together in the early embryo to allow MS-specific differentiation.

Introduction

Blastomeres become committed to distinct patterns of differentiation during the very early cleavage stages of Caenorhabditis elegans embryogenesis. For example, by the 8-cell stage only one blastomere, a mesodermal precursor called MS, can produce both pharyngeal cells and body wall muscles when all other blastomeres are removed or destroyed (Mello et al., 1992). Because the cell types that MS produces can be identified easily in embryos, several experimental and genetic studies have focused on how the fate of MS is determined (reviewed by Wood and Edgar, 1994). In wild-type embryogenesis, the MS blastomere is born after an invariant sequence of three anterior–posterior cell divisions (Figure 1; Sulston et al., 1983). The posterior blastomere in a 2-cell-stage embryo invariably is the MS precursor; when it divides, its anterior daughter always becomes the MS precursor. Thus, the specification of the MS blastomere can be described as a sequence of decisions the embryo makes at the 2-cell, 4-cell, and 8-cell stages about which of two sister blastomeres becomes an MS precursor.

skn-1 is a maternally expressed gene that encodes a putative transcription factor required for MS development (Bowerman et al., 1992; Blackwell et al., 1994). In a 2-cell embryo, the sister blastomeres AB and P1 have unequal levels of SKN-1 protein (Figure 1; Bowerman et al., 1993). AB contains a low level of SKN-1, while its sister P1, the MS precursor, contains a high level of SKN-1. Maternal effect lethal mutations in the genes *par-1* and *mex-1* result in an equal distribution of SKN-1 protein in both sisters, and in these mutant embryos both sisters produce cell types characteristic of a wild-type MS blastomere (Kemphues et al., 1988; Mello et al., 1992; Bowerman et al., 1993). The *par-1* gene appears to encode a protein kinase that, in a 2-cell-stage embryo, is present on the membrane of the MS precursor, but is not present on its sister (Guo and Kemphues, 1995). Thus, establishing unequal distributions of the SKN-1 and PAR-1 proteins appears to be one of the initial steps in determining which blastomere ultimately adopts the MS fate.

When the P1 blastomere divides, its daughters EMS and P2 both inherit equal levels of SKN-1 protein (Bowerman et al., 1993). Therefore, factors other than SKN-1 must determine that in a 4-cell embryo EMS becomes the MS precursor while P2 does not. One such factor is the product of the maternal gene *pie-1*. Genetic studies suggest that in wild-type development *pie-1(+)* activity prevents the P2 blastomere from becoming an MS precursor by inhibiting *skn-1(+)* activity; in mutant embryos lacking *pie-1(+)* function, both P2 and EMS become MS precursors (Mello et al., 1992). *pie-1* recently has been shown to encode a protein that is present in P2, but not in EMS (C. Mello and J. R. P., unpublished data). Therefore the molecular events that create the asymmetric distribution of PIE-1 appear to restrict further which embryonic blastomere becomes MS.

In the 4-cell-stage embryo, the MS precursor is called EMS, because it later divides into sister blastomeres called E and MS. E produces only intestinal cells, in contrast with MS, which produces predominantly pharyngeal cells and body wall muscles (Sulston et al., 1983). MS and E have equal levels of SKN-1 protein, and neither sister has detectable PIE-1 protein (Bowerman et al., 1993; C. Mello and J. R. P., unpublished data), so factors other than SKN-1 and PIE-1 must specify the different fates of MS and E. No genes have been described previously that are candidates for determining which of these sisters becomes MS or E, but experimental studies have suggested that cell–cell interactions play a role in this decision (Schierenberg, 1987; Goldstein, 1992, 1993, 1995). In a 4-cell-stage embryo, EMS contacts a blastomere called P2. If the P2 blastomere is removed from the embryo at the beginning of the 4-cell stage, neither daughter of EMS develops like an E blastomere, and both daughters have some characteristics of a normal MS blastomere (Goldstein, 1992, 1993).

In this paper, we show that a maternal effect lethal mutation in the gene *pop-1* (for posterior pharynx defective) results in the MS blastomere adopting the fate of a wild-type E blastomere, suggesting that *pop-1(+)* activity plays a role in the specification of the MS blastomere in normal development. The *pop-1* gene encodes a protein with a previously described DNA-binding motif called an HMG box; the HMG box in *pop-1* is very similar to the HMG boxes in the vertebrate lymphoid-specific transcriptional regulators human T cell factor 1 (TCF-1) and mouse

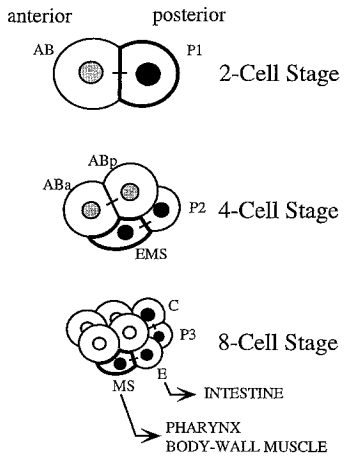

Figure 1. Origin of the MS Blastomere

Schematic diagrams of early embryos with pairs of sister blastomeres connected by short lines. The level of nuclear SKN-1 protein in each blastomere is indicated by the appearance of the smaller circle within: closed circle, high; stippled, low; open, very low or none (Bowerman et al., 1993). The MS blastomere and each of its precursors are outlined in bold (P1 at the 2-cell stage; EMS at the 4-cell stage). For further description of embryonic anatomy and blastomere fates, see Sulston et al. (1983).

lymphoid enhancer-binding factor 1 (LEF-1). We propose that POP-1 and SKN-1 regulate gene expression in the MS blastomere that allows MS-specific differentiation, represses E-specific differentiation, or both.

Results

pop-1 Mutants Lack MS-Derived Pharynx

The *pop-1(zu189)* mutation was identified in a genetic screen for maternal effect lethal mutants with defects in pharyngeal development (see Experimental Procedures). We show below that the *pop-1* gene is expressed zygotically as well as maternally and that the *pop-1(zu189)* mutation appears to affect only the maternal expression. We refer in this paper to the embryos produced from *pop-1(zu189)* homozygous mothers as *pop-1* mutant embryos.

pop-1 mutant embryos have a small pharynx, about half the size of a wild-type pharynx, and a larger than normal intestine (Figure 2). In wild-type embryogenesis, the pharynx is comprised of cells that originate from two early blastomeres called AB and MS (Figure 1; Sulston et al., 1983). AB-derived pharynx forms as the result of cell–cell interactions that involve the GLP-1 receptor, a homolog of the Drosophila NOTCH protein (Priess et al., 1987; Yochem and Greenwald, 1989; Artavanis-Tsakonas et al., 1995). The formation of MS-derived pharynx does not require cell–cell interactions or *glp-1(+)* function; *glp-1* mutants lack AB-derived pharynx, but have a partial pharynx that is derived entirely from MS (Priess et al., 1987). If the partial pharynx in *pop-1* mutants is derived from MS, it should still be present in *pop-1;glp-1* double mutants, whereas if it is derived entirely from AB it should not be present in the double mutant. We find that most *pop-1; glp-1* double mutant embryos lack pharyngeal cells altogether (Table 1; Figure 3), suggesting that the partial pharynx in *pop-1* mutants is derived from AB.

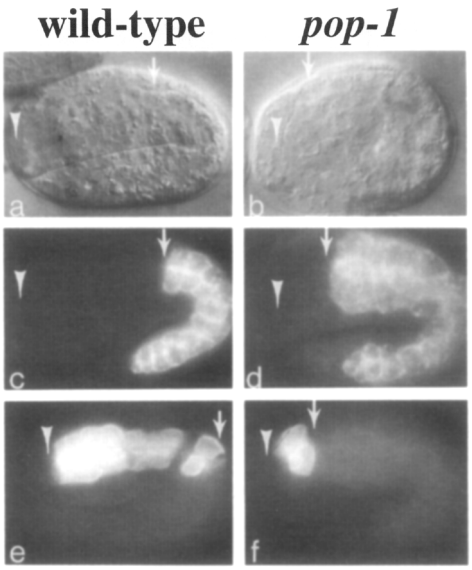

Figure 2. Differentiation in Wild-Type and *pop-1* Mutant Embryos

Tissue differentiation in wild-type embryos (left) and *pop-1* mutant embryos (right). (a) and (b) are light micrographs. (c)–(f) are immunofluorescence micrographs of intestinal cells stained with MAbICB4 (c and d) and pharyngeal muscles stained with MAb3NB12 (e and f). Pharyngeal cells are enclosed by a prominent basement membrane; the anterior tip of the pharynx is indicated by an arrowhead, and the posterior tip is indicated by a white arrow at the junction between the pharynx and the intestine. Note that the pharynx in the *pop-1* mutant is much smaller than the wild-type pharynx. Terminal stage *pop-1* mutant embryos do not hatch, but produce tissues and organs that appear fully differentiated in the light microscope. The *pop-1* embryo shown is undergoing morphogenesis; proper body morphogenesis requires that dorsal epithelial cells, called hypodermal cells, migrate across the ventral surface to enclose the embryo. About 70% of the *pop-1* mutant embryos do not complete enclosure, presumably because of abnormal MS descendants present on the ventral surface.

We next asked whether the partial pharynx in *pop-1* mutant embryos develops like normal AB-derived pharynx. The cell–cell interactions that result in AB-derived pharynx require that the MS blastomere functions as a signaling cell during the 12-cell stage of embryogenesis. If the MS blastomere is killed in wild-type embryos at the 8-cell stage, AB-derived pharynx does not develop (Hutter and

Table 1. Pharynx in *pop-1* and *pop-1;glp-1* Mutant Embryos

	Embryos Lacking Pharyngeal Cells (%)	
Genotype	15°C	25°C
pop-1	8 (n = 178)	6 (n = 272)
pop-1;glp-1(e2142ts)	9 (n = 132)	87 (n = 106)

glp-1(e2142ts) is a temperature-sensitive, maternal effect lethal mutation. At permissive temperature (15°C), *glp-1(e2142ts)* mutant embryos contain both AB- and MS-derived pharynx. At restrictive temperature (25°C), these embryos all produce MS-derived pharynx, but about 91% (n = 220) lack AB-derived pharynx. Pharynx was assayed by MAb9.2.1. antibody staining.

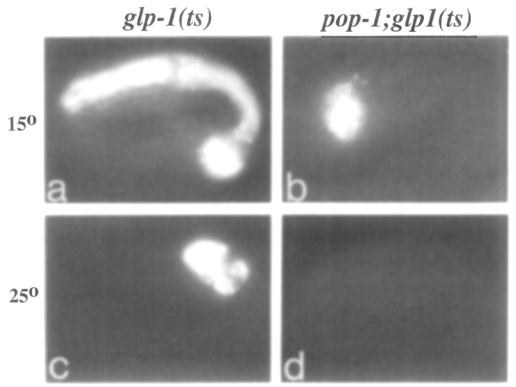

Figure 3. Pharyngeal Development in *pop-1* Embryos Requires *glp-1(+)*

Immunofluorescence micrographs of terminal stage fixed embryos stained with MAb9.2.1 to show pharyngeal development. To the left are embryos from mothers homozygous for *glp-1(e2142ts)*, a temperature-sensitive mutation, at the permissive (a) and nonpermissive (c) temperatures. To the right are embryos from mothers homozygous for *pop-1(zu189);glp-1(e2142ts)* mutations at permissive (b) and nonpermissive (d) temperatures.

Schnabel, 1994; Mango et al., 1994a). However, if the MS descendants are killed at the 28-cell stage, after cell signaling is completed, the AB descendants already are committed to producing pharyngeal cells and form a partial pharynx (Priess and Thomson, 1987). We found that *pop-1* mutant embryos did not produce any pharyngeal tissue after the MS blastomere was killed at the 8-cell stage, but did form a partial pharynx when the MS descendants were killed later at the 28-cell stage (Table 2), suggesting that MS functions as a signaling cell. In a 28-cell-stage embryo there are 16 AB descendants; in normal development, only three of these descendants are signaled to produce pharyngeal cells. If these three AB descendants are killed in wild-type embryos, no AB-derived pharynx is formed (Priess and Thomson, 1987). To test whether the same three AB descendants are the sole source of pharyngeal cells in *pop-1* mutant embryos, we killed these descendants at the 28-cell stage and found that the resulting embryos did not contain pharyngeal cells (Table 2). Taken together, these results demonstrate that *pop-1* mutant embryos appear to form normal AB-derived pharynx, with MS functioning as a signaling cell, but that MS itself does not produce pharyngeal cells.

MS Adopts the Fate of Its Sister E in *pop-1* Mutant Embryos

The fates of individual 8-cell-stage blastomeres can be assayed by killing all other blastomeres and allowing the partial embryos to develop. In such experiments, a wild-type MS blastomere produces pharyngeal cells and body wall muscles, while its sister, the E blastomere, produces only intestinal cells, corresponding to the fates of MS and E, respectively, in normal development (Mello et al., 1992). We find that the MS blastomere from a *pop-1* mutant embryo does not produce any pharyngeal cells or body wall muscles, but instead produces only intestinal cells (Table 2). The E blastomere in a *pop-1* mutant embryo also produces only intestinal cells in these experiments (Table 2). Indeed, the development of both the MS and E blastomeres in *pop-1* mutant embryos appears identical to the development of a wild-type E blastomere in terms of the number of intestinal cells produced and the morphological differentiation of these intestinal cells.

If only the E blastomere in a wild-type embryo is killed, all other blastomeres appear to differentiate normally, and a partial embryo forms that does not contain an intestine (Table 2; Junkersdorf and Schierenberg, 1992). We find that when either the E blastomere or the MS blastomere in a *pop-1* mutant embryo is killed, the resulting embryo always contains intestinal cells (Table 2). However, if both MS and E are killed in the same embryo, no intestinal cells are formed (Table 2). Thus, in *pop-1* mutants both MS and E produce intestinal cells, and no additional blastomeres appear to produce intestinal cells. These results demonstrate that the lack of MS-derived pharynx and the presence of an abnormally large intestine in *pop-1* mutant embryos are both a consequence of a change in the fate of the MS blastomere.

Table 2. Pharynx and Intestine in Wild-type and *pop-1* Partial Embryos

	Embryos with Pharyngeal Cells		Embryos with Intestinal Cells	
Blastomeres Ablated	Wild Type	*pop-1*	Wild Type	*pop-1*
MS at 8-cell stage	0 of 25	0 of 11	25 of 25	11 of 11
$(MS)^2$ at 28-cell stage	—	8 of 8	—	—
$(AB)^3$ at 28-cell stage	—	0 of 8	—	—
All except MS	25 of 25	0 of 24	0 of 25	23 of 24[a]
All except E	0 of 9	0 of 19	9 of 9	19 of 19
E at 8-cell stage	10 of 10	24 of 24	0 of 10	24 of 24
MS and E	0 of 8	2 of 27[b]	0 of 8	0 of 27

Pharyngeal and intestinal differentiation was scored in embryos after killing selected early blastomeres with a laser microbeam (see Experimental Procedures). The blastomeres $(MS)^2$ and $(AB)^3$ are, respectively, the two MS-derived and the three AB-derived pharyngeal precursors in 28-cell-stage embryos (MSaa and MSpa and ABalpa, ABaraa, and ABarap; see Sulston et al., 1983). The differentiation of pharynx and intestine was scored by MAb3NB12 and MAbICB4, respectively.

[a] The single MS blastomere that did not produce intestinal cells instead produced hypodermal cells and muscle cells.

[b] MS normally induces AB to produce pharyngeal cells. It is likely that the MS blastomere was not ablated sufficiently to prevent signaling in these embryos and that the observed pharyngeal cells were derived from AB.

Because the MS blastomere in *pop-1* mutants produces intestinal cells like its sister the E blastomere, we asked whether MS had additional E-like properties. In wild-type embryogenesis, E descendants never express the *hlh-1* gene, which is a homolog of the vertebrate *MyoD* gene, but all MS descendants express *hlh-1* in 28-cell-stage embryos (Krause et al., 1990; Chen et al., 1992). We stained 28-cell-stage *pop-1* mutant embryos with an antibody that recognizes the protein product of *hlh-1* and did not detect *hlh-1* expression in any MS descendants (none of 20 embryos examined). A second difference between E and MS in wild-type embryogenesis is the cell cycle periods of their daughters (Sulston et al., 1983). The cell cycle period of the E daughters is about 45 min, while that for the MS daughters it is about 20 min. In *pop-1* mutants, the MS daughters have a cell cycle period nearly as long as wild-type E daughters, varying between 30 and 42 min.

In wild-type embryogenesis, E does not appear to be required for any AB-specific differentiation. However, as described above, MS functions at the 12-cell stage as a signaling cell required for the development of AB-derived pharynx. We have shown in this paper that in *pop-1* mutants the MS blastomere also functions as a signaling cell for AB-derived pharynx. This result suggests either that the MS blastomere in *pop-1* mutants retains some wild-type MS characteristics or that a wild-type E blastomere might also have the ability to signal. The latter may not have been detected in previous studies because in wild-type embryos the AB descendants that contact E are not competent to respond to signaling. To test this possibility, we took advantage of the recent finding that mutations in the maternal gene *apx-1* cause all AB descendants to become responsive to signaling at the 12-cell stage (Mango et al., 1994a; Mello et al., 1994). In wild-type embryos, we find that killing the MS blastomere prevents AB descendants from producing pharyngeal cells (Table 3), as reported previously by others (Hutter and Schnabel, 1994; Mango et al., 1994a). However, most *apx-1* mutant embryos still produce pharyngeal cells even after the MS blastomere is killed (Table 3), indicating that an additional blastomere(s) is functioning as a signaling cell. If both MS and E are killed, no pharyngeal cells are produced in most *apx-1* mutant embryos, suggesting that E can function as a signaling cell. Thus, although MS is the only signaling cell for AB-derived pharynx in wild-type embryogenesis, the E blastomere may also have the potential for signaling. Taken together, our analysis of *pop-1* mutants shows that each of the properties of the MS blastomere is consistent with the properties of a wild-type E blastomere, suggesting that the MS blastomere is adopting the fate of its sister E.

Table 3. Pharynx Induction in Wild-type and *apx-1* Mutant Embryos

Blastomeres Ablated	Embryos with Induced Pharyngeal Cells	
	Wild Type	*apx-1*
MS	0 of 25	25 of 29
MS and E	0 of 8	1 of 24

The differentiation of pharynx was assayed by the antibody MAb9.2.1.

pop-1(+) Activity Is Required for MS Development

We have shown that a maternal effect lethal mutation in the *pop-1* gene prevents proper MS development but appears to have no effect on the development of other embryonic blastomeres. Previous studies have identified mutants in which multiple embryonic blastomeres have some characteristics of a wild-type MS blastomere (Mello et al., 1992; Kemphues et al., 1988). These ectopic MS-like blastomeres are located anterior to the normal position of the MS blastomere in *mex-1* mutants or posterior to MS in *pie-1* mutants (Mello et al., 1992). In *par-1* mutants, the MS-like blastomeres are both anterior and posterior to MS (Bowerman et al., 1993). If *pop-1(+)* activity is essential for the specification of the MS fate, the development of each of these ectopic MS-like blastomeres should be effected by *pop-1* mutations regardless of the positions of these blastomeres in embryos. We constructed *pop-1; mex-1*, *pop-1;pie-1*, and *pop-1;par-1* double mutants to test this possibility. We find that blastomeres that adopt MS-like fates in the single mutant strains can adopt E-like fates in the double mutant strains (Figure 4; Table 4). For example, in *pie-1* single mutants the EMS and P2 blastomeres both produce MS-like and E-like daughters, resulting in pharyngeal cells and intestinal cells, respectively (Mello et al., 1992). In contrast, in *pop-1;pie-1* double mutants EMS and P2 produce only intestinal cells (Table 4). In *mex-1* single mutants, the daughters of ABa and ABp can produce pharyngeal cells like a wild-type MS blastomere; in most *pop-1;mex-1* double mutant embryos, ABa and ABp produce only intestinal cells or produce both intestinal and pharyngeal cells (Figure 4; Table 4). Thus, *pop-1(+)* activity appears to play a role in allowing blastomeres that would otherwise produce intestinal cells to produce pharyngeal cells instead, as an MS blastomere does in wild-type embryogenesis.

The *pop-1* Gene Can Encode an HMG Box-Containing Protein

The *pop-1* mutation was mapped to the interval between the genes *lin-17* and *fog-1* (Figure 5). We obtained transformation rescue of *pop-1* mutant embryos with the cosmid BF8 from this interval and simultaneously identified a transposon insertion that comapped with the *pop-1(zu189)* mutation. DNA flanking the Tc1 insertion site was cloned and mapped with respect to the rescuing cosmid and used as a probe to screen C. elegans genomic and cDNA libraries. Figure 5 shows a 20 kb genomic interval containing the genomic and cDNA clones isolated with this probe.

To confirm that the cDNA we identified corresponds to the *pop-1* transcript, we asked whether anti-sense RNA generated from this cDNA could cause wild-type embryos to develop like *pop-1* mutant embryos. When anti-sense RNA derived from various portions of this cDNA was injected into the syncytial gonad of wild-type adults, the animals laid inviable embryos that appeared identical in morphology to *pop-1* mutant embryos (data not shown). Sequence analysis of the genomic and cDNA clones indicated that the *zu189* mutation is a Tc1 insertion in the last intron, downstream of the translation termination codon

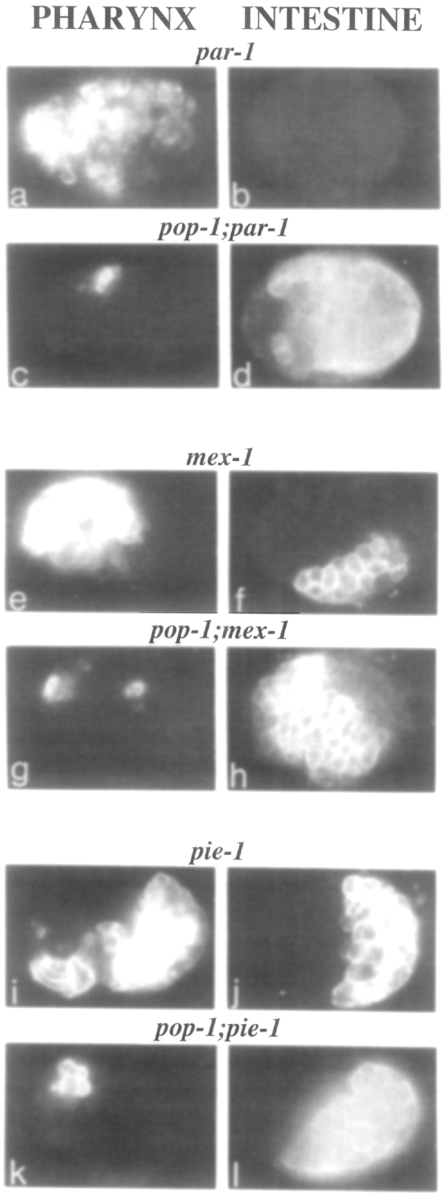

Figure 4. MS-like Fates Require *pop-1(+)* Activity

Immunofluorescence micrographs of terminal stage embryos stained with MAb3NB12 to show pharyngeal development (left column) or MAbICB4 to show intestinal development (right column). Each pair of two rows compares differentiation between a single mutant and the corresponding *pop-1* double mutant. *par-1*, *mex-1*, and *pie-1* single mutants have abnormally large amounts of pharyngeal tissue (a, e, and i, respectively; compare with Figure 2e) and zero, one, or two times the wild-type number of intestinal cells (b, f, and j, respectively; compare with Figure 2c). The double mutants with *pop-1* have much less pharyngeal tissue (c, g, and k) and many more intestinal cells (d, h, and l) than each of the corresponding single mutants. *pie-1* single mutants produce both AB-derived and MS-derived pharynx (i), and the AB-derived pharynx remains in *pop-1;pie-1* double mutants (k). The alleles used were *par-1(e2012)*, *mex-1(zu120)*, and *pie-1(zu154)*.

(Figure 6). We therefore conclude that this cDNA indeed corresponds to the *pop-1* transcript.

The *pop-1* cDNA can encode a 487 amino acid protein with a previously described DNA-binding domain called an HMG box (Figure 6A), which is found in a diverse group

Table 4. Pharynx and Intestine in *pop-1;mex-1* and *pop-1;pie-1* Mutant Embryos

Genotype	Blastomere	Embryos with Intestinal Cells	Embryos with Pharyngeal Cells
pie-1	EMS and P2	19 of 19	19 of 19
pop-1;pie-1	EMS and P2	11 of 11	0 of 11
mex-1	ABa	0 of 15	15 of 15
	ABp	0 of 15	15 of 15
	EMS	9 of 10	10 of 10
pop-1;mex-1	ABa	18 of 22	16 of 22
	ABp	13 of 20	7 of 20
	EMS	14 of 14	0 of 14

Individual blastomeres were allowed to develop after all other blastomeres were killed. Alleles used are *pie-1(zu154)* and *mex-1(zu120)*. Gut formation in the *mex-1(zu120)* mutant embryos is cold sensitive; therefore, *mex-1* and *pop-1;mex-1* mutant embryos were cultured at 22°C. In wild-type embryos, intestine is derived from the E blastomere and pharynx is derived from the MS blastomere when all other blastomeres are ablated.

of nuclear proteins (Jantzen et al., 1990; Laudet et al., 1993). HMG box proteins are divided into two groups: the HMG/upstream binding factor (UBF) subfamily consists of proteins with two or more HMG boxes that bind DNA with relatively low specificity (Laudet et al., 1993) and the TCF/SOX subfamily consists of proteins with a single HMG box that exhibit sequence-specific DNA binding. POP-1 contains a single HMG box that, by sequence similarity, belongs to the TCF/SOX subfamily of which many members have been shown to be transcriptional regulators. Within this family, POP-1 is most closely related to TCF-1 (van de Wetering et al., 1991), LEF-1 (Travis et al., 1991; Waterman et al., 1991), sex-determining region Y (SRY; Gubbay et al., 1990; Sinclair et al., 1990), and SOX-5, an SRY HMG box–related protein (Denny et al., 1992). The HMG box comprises a very loose consensus sequence with an average sequence identity among different HMG domains of about 25% (Laudet et al., 1993). In this region, POP-1 shares 50% identity with TCF-1 and LEF-1 and 26%–31% identity with SRY and SOX-5, but only 19% identity with HMG-1, a member in the HMG/UBF subfamily (Figure 6C). POP-1, TCF-1, and LEF-1 also have in common a proline-rich region N-terminal to their HMG boxes.

POP-1 Is Localized to the Nuclei of Early Blastomeres

The polyclonal antiserum 94I was generated against a POP-1 fusion protein to analyze the distribution of POP-1 in embryos (Figure 7; see Experimental Procedures). Affinity-purified 94I stains early wild-type embryos, but not *pop-1* mutant embryos. We therefore conclude that the 94I antiserum recognizes the POP-1 protein. In immunostaining experiments, POP-1 protein is detected first in the nuclei of maturing oocytes in the gonad (Figure 7b). After fertilization, POP-1 is detected in the nuclei of most early embryonic cells (Figures 7d, 7f, and 7h). 94I does not stain cells during mitosis, and after the 360-cell-stage POP-1 is detected only in a subset of tissues (data not shown). Analysis of embryos homozygous for a deficiency of the *pop-1* locus indicates that the POP-1 staining de-

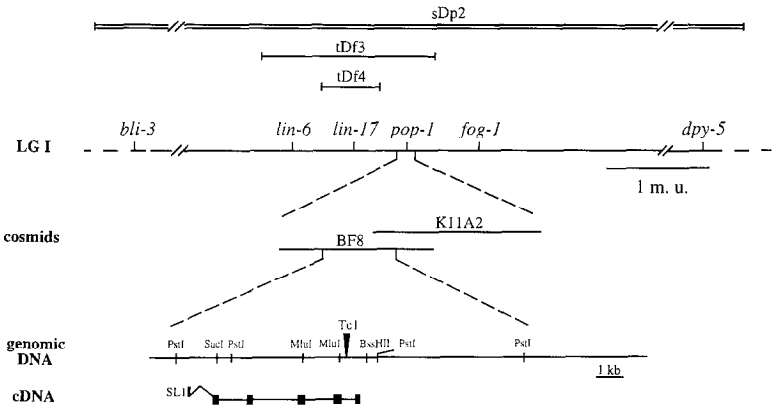

Figure 5. Molecular Cloning of *pop-1*

The *pop-1* mutation is complemented by the duplication *sDp2* and the deficiency *tDf4*, but not by the deficiency *tDf3*; these deficiencies and this duplication are shown above the genetic map of the *pop-1* locus on linkage group I (LGI). The inviability of *pop-1* mutants is rescued by the cosmid BF8, but not by the overlapping cosmid K11A2. A *pop-1* cDNA clone is shown beneath the genomic restriction map; the *pop-1(zu189)* mutation is a Tc1 insertion (closed arrowhead). Exons are shown as closed boxes and introns as lines. The *trans*-spliced leader SL1 (Krause and Hirsh, 1987) is present on the 5′ end of the *pop-1* message.

tected before the 28-cell stage represents maternal expression of the *pop-1* gene (see Experimental Procedures). Zygotic expression of *pop-1* is required for POP-1 staining in later stages and will not be described further here. *pop-1(zu189)* mutants do not have detectable staining in oocytes or embryos before the 28-cell stage, although they appear to have wild-type levels of staining after the 28-cell stage (data not shown). Similarly, we found that embryos from wild-type mothers injected with *pop-1* anti-sense RNA lacked POP-1 staining before the 28-cell stage (data not shown). Thus, the *pop-1* mutant phenotype appears to result from a low level, or absence, of POP-1 protein in the early embryo.

Although POP-1 protein is present in all early blastomeres, the 94I antiserum often shows different staining intensities in two pairs of sister blastomeres in 8-cell embryos. In about 70% of the embryos (n = 100), E stains less intensely than its sister MS, and P3 stains less intensely than its sister C (Figure 7h). However, in all other embryos at the same stage, these blastomeres appear to have equivalent staining intensities, suggesting they have equal levels of POP-1 protein.

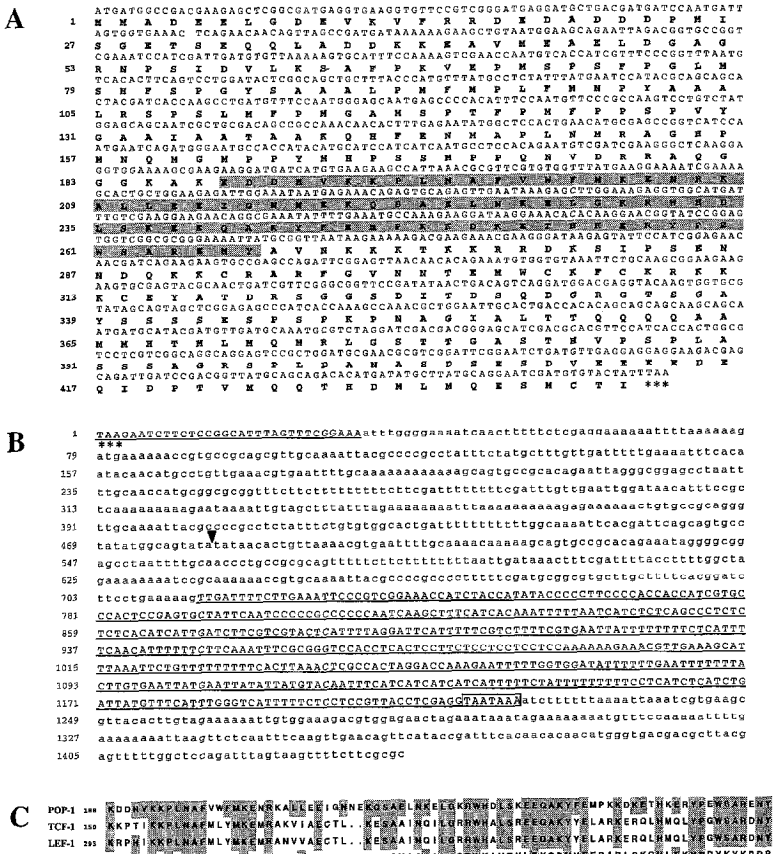

Figure 6. Sequence of the *pop-1* Gene

(A) The *pop-1*-coding sequence and deduced amino acid sequence. The HMG box stippled. The translation termination codon is indicated by three asterisks.

(B) Genomic sequence of the *pop-1* gene 3′ to the termination codon (three asterisks) shown in (A). The underlined sequences are part of the second to the last exon (1–32) and the last exon (716–1124) of the predicted *pop-1* transcript. A potential polyadenylation signal is boxed, and proceeds the poly(A) addition site at positions 1133–1136. The arrowhead indicates the Tc1 insertion site in *pop-1(zu189)*.

(C) Comparison of the HMG box from the predicted POP-1 protein with the HMG boxes from TCF-1 and HMG-1A in humans and LEF-1, SOX-5, and SRY in mice. Residues that POP-1 shares with at least one of these proteins are shown stippled.

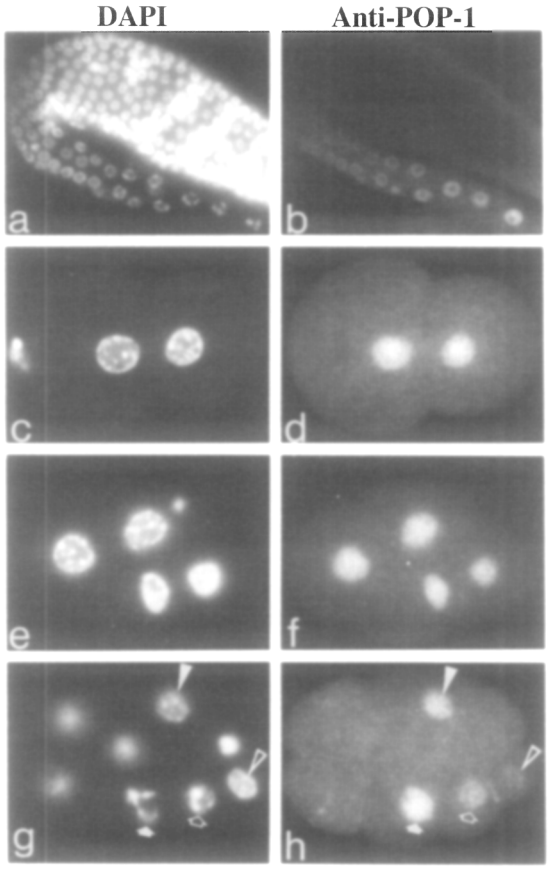

Figure 7. Localization of POP-1 Protein

Fluorescence micrographs of a wild-type adult gonad (a and b) and early embryos (c–h) stained with either diamidophenylindole (DAPI) to visualize nuclei (left column) or POP-1 antisera (right column). Embryos are oriented as shown in Figure 1.

(a and b) Low magnification pictures of an adult gonad. The gonad consists of a syncytium of nuclei joined by a common cytoplasm and of individual maturing oocytes (lower right in panels).

(c–h) High magnification pictures of 2-cell- (c and d), 4-cell- (e and f), and 8-cell-stage embryos (g and h).

(g and h) An example of an embryo with different staining intensities in sister blastomeres. Arrows point to the nuclei of the sister blastomeres MS (closed) and E (open). Arrowheads point to the nuclei of the sister blastomeres C (closed) and P3 (open). The gonad and all embryos shown were stained with the 94I antiserum.

Discussion

pop-1(+) Activity Is Required for the MS Blastomere Fate

The *pop-1* gene is expressed both maternally and zygotically during C. elegans development. In this study we have analyzed the role of *pop-1* in early embryogenesis using a mutation that appears to reduce or eliminate maternally supplied *pop-1(+)* activity and shown that *pop-1(zu189)* mutant embryos have little, if any, maternal POP-1 protein detected by POP-1-specific antisera. In addition, we have found that embryos from wild-type mothers injected with anti-sense *pop-1* RNA appear indistinguishable from *pop-1* mutant embryos.

Our genetic and molecular analysis of the *pop-1* gene leads to two major conclusions. First, the maternal *pop-1* gene product is essential for the correct specification of the MS blastomere. In wild-type embryogenesis, MS produces predominantly pharyngeal cells and muscle cells. Other early blastomeres, such as AB and P2, also produce pharyngeal cells and muscle cells, respectively, but do so through a pattern of cell cleavage and differentiation that is very different from the MS blastomere (Sulston et al., 1983). A maternal effect lethal mutation in *pop-1* prevents MS from producing either pharyngeal cells or muscle cells, but does not affect the ability of AB or P2 to produce these same cell types. The AB blastomere in *mex-1*, and the P2 blastomere in *pie-1*, mutant embryos produce both pharyngeal cells and muscle cells through an MS-like pattern of cell cleavages and differentiation (Mello et al., 1992); in *mex-1* and *pie-1* mutants, *pop-1(+)* activity appears to play a role in allowing AB and P2 to produce these cell types. These results indicate that *pop-1(+)* activity is not necessary for making pharyngeal or muscle cell types per se, but rather appears to be required for defining a set of properties of MS that make it different from all other 8-cell-stage blastomeres.

The second conclusion from our analysis is that the MS blastomere appears to contain all the factors required for developing like its sister, the E blastomere, but is prevented from doing so when *pop-1(+)* activity is present. In *pop-1* mutant embryos, the MS blastomere adopts an E-like fate in terms of its ability to produce intestinal cells, its cleavage rate, and its lack of *hlh-1* expression. We have shown that MS retains the ability to function as a signaling cell in *pop-1* mutants, but that a wild-type E may also have signaling properties. Finally, we have shown that blastomeres that inappropriately adopt MS-like fates in certain mutant strains can adopt E-like fates in the corresponding *pop-1* double mutant strains. The hypothesis that MS, or its descendants, contain factors that normally repress E-specific differentiation is supported by previous studies on the control of the *ges-1* gene (Aamodt et al., 1991). In wild-type embryos, *ges-1* is expressed in E, but not MS, descendants (Edgar and McGhee, 1986). However, a deletion in the 5′ promoter region of the *ges-1* gene results in *ges-1* expression in both MS and E descendants, suggesting that *ges-1* may be repressed in MS in normal development (Aamodt et al., 1991).

POP-1 and Transcriptional Regulation

The *pop-1* gene encodes a protein with a putative DNA-binding motif called an HMG box. Members of the TCF/SOX subfamily of HMG box proteins contain single HMG boxes that exhibit sequence-specific DNA binding (Laudet et al., 1993). POP-1 has a single HMG box that is most closely related to the lymphoid-specific transcription regulators TCF-1 and LEF-1 in the TCF/SOX subfamily (Travis et al., 1991; van de Wetering et al., 1991; Waterman et al., 1991). The mechanism by which TCF-1 and LEF-1 regulate gene expression is not fully understood, but it may involve alterations in local chromatin structure that allow enhancer-bound proteins to interact. The HMG domain of LEF-1 is capable of inducing a sharp bend in DNA

and can functionally substitute in vitro for bacterial integration host factor to bring together protein-binding sites that are widely separated on a DNA molecule (Giese et al., 1992). In vivo, LEF-1 appears to act in concert with other transcription factors to regulate lymphoid-specific expression of the T cell receptor α (TCRα) gene (Waterman and Jones, 1990; Travis et al., 1991; Waterman et al., 1991). A minimal enhancer for TCRα requires consensus binding sites for transcription factors such as CREB and ETS-1 in addition to binding sites for LEF-1, and the spacing between the CREB- and LEF-1-binding sites is critical for enhancer function (Ho and Leiden, 1990). These results suggest that in C. elegans POP-1 may interact with, or regulate interactions among, additional factors to activate or repress transcription.

POP-1 May Interact with SKN-1

The maternal gene *skn-1* encodes a putative transcription factor that, like POP-1, is required for the proper development of the MS blastomere and is present in the nucleus of MS (Bowerman et al., 1992, 1993). However, mutations in *pop-1* and *skn-1* cause different fate transformations in the MS blastomere. In wild-type development, MS produces primarily pharyngeal cells and body wall muscles. In *skn-1* mutant embryos, MS produces hypodermal cells and muscles, but in *pop-1* mutant embryos MS produces intestinal cells like a wild-type E blastomere. A simple explanation for the difference in MS development in *pop-1* and *skn-1* mutants is that *skn-1(+)* activity also is required for the proper development of the E blastomere. About 70% of the embryos produced by a *skn-1(zu67)* homozygous hermaphrodite lack intestinal cells, and in these mutant embryos the E and MS blastomeres both produce hypodermal cells and muscles (Bowerman et al., 1992). Thus, in wild-type embryos SKN-1 could function with POP-1 to specify MS development, and SKN-1 could function with other factors to specify E development. Indeed, *pop-1;skn-1* double mutants resemble *skn-1* single mutants in phenotype (R. L., unpublished data), suggesting that *pop-1(+)* cannot specify MS development independent of *skn-1(+)* activity.

Understanding the biochemical roles of POP-1 and SKN-1 in the specification of the MS blastomere will require the identification of downstream target genes. At present only a few zygotically expressed genes have been identified that appear to function in pharyngeal and body wall muscle development, such as *pha-1* (Schnabel and Schnabel, 1990; Granato et al., 1994), *ceh-22* (Okkema and Fire, 1994), *pha-4* (Mango et al., 1994b), and *hlh-1* (Krause et al., 1990). *pha-4* and *hlh-1* are the best candidates for early zygotic genes that might be regulated by POP-1 and SKN-1. However, mutations in *pha-4* or *hlh-1* affect only a subset of MS descendants. Therefore, POP-1 and SKN-1 either regulate additional zygotic genes or regulate *pha-4* and *hlh-1* indirectly through an unknown gene or genes.

Does POP-1 Function in a Wild-Type E Blastomere?

Although each of the blastomeres in an 8-cell-stage embryo contains POP-1 protein, only the development of the MS blastomere appears to be effected by the *pop-1* mutation and by injections of *pop-1* anti-sense RNA. If POP-1 requires transcription factors such as SKN-1 to regulate gene activity, POP-1 might not be expected to function in any of the AB descendants, because these blastomeres lack SKN-1 protein. Similarly, POP-1 might not be expected to function in the P2 blastomere, because SKN-1 activity normally is prevented in P2 by the *pie-1* gene (Mello et al., 1992). Consistent with this hypothesis, we have shown here that *pop-1* can function in AB and P2 when these blastomeres acquire *skn-1(+)* activity, as in *mex-1* and *pie-1* mutant embryos. However, *pop-1* mutations do not appear to affect the development of the E blastomere, even though the phenotype of *skn-1* mutants suggests that *skn-1(+)* activity is present in E. What, then, is the difference between MS and E?

Cell–cell interactions appear to play an important role in determining the different fates of MS and E in wild-type embryogenesis. When the EMS blastomere divides, its anterior daughter always becomes MS and the posterior daughter always becomes E (see Figure 1). The P2 blastomere normally contacts the posterior surface of EMS where the E blastomere is born. However, if P2 is placed in contact with the anterior surface of EMS, the anterior daughter of EMS appears to become E-like and the posterior daughter becomes MS-like (Goldstein, 1995). If the P2 blastomere is prevented from contacting EMS altogether, EMS divides into two daughters that both have MS-like characteristics (Goldstein, 1992, 1993). These results suggest that contact with P2 somehow establishes anterior–posterior polarity in the EMS blastomere and that this polarity is required for EMS to produce a posterior daughter, E, with a different fate than the anterior daughter, MS. For example, the P2–EMS interaction could lead to an asymmetrical distribution within EMS of a dominant-acting transcription factor or result in the asymmetrical activation of a nonlocalized transcription factor.

Because a lack of *pop-1(+)* activity appears to convert an MS blastomere into an E-like blastomere, it is possible that the different fates of MS and E are specified in wild-type embryogenesis by lowering or inhibiting *pop-1(+)* activity in the E blastomere. Alternatively, the activity of POP-1 protein could be identical in both MS and E, but the E blastomere could lack an additional factor that normally functions with POP-1 to specify the MS fate. Intriguingly, the E blastomere often shows less staining with the 94I antiserum than does the MS blastomere. Many embryos also showed slightly less staining in P3 than in C: in *pie-1* mutants, where *skn-1(+)* activity is not repressed, P3 adopts an E-like fate and C adopts an MS-like fate. Thus, there is a correlation between low levels of POP-1 staining with the 94I antiserum and which of two sister blastomeres has the potential to adopt an E-like fate. In the future it will be important to determine whether the diminished staining in E results from lower levels of POP-1 protein, posttranslational modifications in POP-1, or associations between POP-1 and other factors that interfere with antibody binding.

Experimental Procedures

Strains and Alleles

The Bristol strain N2 was used as the standard wild-type strain. The

genetic markers, duplications, and deficiencies used in this paper are listed by chromosome as follows: linkage group I (LGI): *dpy-5(e61)*, *lin-17(n671)*, *fog-1(e2121)*, *sDp2*, *tDf3*, *tDf4*, *qDf3*, *qDf4*, *hDf10*, *hT1*, *hT2(I)*, *szT1*. LGII: *mex-1(zu120)*, *unc-4(e120)*, *mnC1*. LGIII: *glp-1(e2142ts)*, *pie-1(zu154)*, *unc-25(e156)*, *hT2(I)*. LGV: *him-5(e1490)*, *par-1(e2012)*, *rol-4(sc8)*, *hT1*. LGX: *lin-2(e1309)*, *lon-2(e678)*, *dpy-8(e130)*, *szT1*. The strains GE1549 and GE1386 were obtained from R. Schnabel and contain the deficiencies *tDf4* and *tDf3*, respectively. Strains JK323 and RE249 contain the deficiencies *qDf3* and *qDf4*, respectively, and were provided by R. Ellis. All other mutant alleles listed were obtained or are available from the C. elegans Genetic Stock Center. The basic methods for worm culture and genetics were performed as described by Brenner (1974).

Genetic Analysis

The *pop-1(zu189)* mutation was isolated in a previously described screen for recessive, nonconditional maternal effect embryonic lethal mutations using a transposon-mobilized strain RW7096 (*mut-6[st702]unc-22[st192::Tc1]*) (Mello et al., 1994). *pop-1(zu189)* was outcrossed ten times and positioned with respect to *lin-17*, *fog-1*, and *dpy-5* on LGI by standard procedures. Data for these crosses are available from the C. elegans Genetic Stock Center. The deficiency *tDf3* fails to complement the *pop-1* mutation, whereas the following deficiency strains complement *pop-1*: *tDf4*, *hDf10*, *qDf3*, and *qDf4*. One example of the complementation test is described as follows. A heterozygous deficiency was generated by mating *pop-1(zu189) dpy-5(e61)/hT1* males to *tDf3 dpy-5(e61)/szT1*; *dpy-8(e130)/szT1(lon-2[e687])* hermaphrodites. The Dpy progeny (*pop-1[zu189] dpy-5[e61]/tDf3 dpy-5[e61]*) were able to grow to adults that produced embryos. All embryos from these Dpy adults were inviable and appeared identical in phenotype to embryos from *pop-1(zu189)* homozygous hermaphrodites.

Maternal expression of *pop-1* is required for embryogenesis, as demonstrated by the following experiments. Hermaphrodites heterozygous for the *pop-1(zu189)* mutation produced all viable self-progeny, including homozygous *pop-1(zu189)* embryos (100% hatch, n > 1000). No viable progeny were produced from hermaphrodites homozygous for the *pop-1(zu189)* mutation (0% hatch, n = 2258). All cross-progeny of homozygous mutant hermaphrodites mated to wild-type males also were inviable (0% hatch, n = 366).

Analysis of Embryos

Light microscopy was performed with a Zeiss Axioplan microscope equipped with epifluorescence, polarizing, and differential interference contrast (DIC) optics. Photographs were taken on Kodak Technical Pan film and developed in HC110 developer. Embryos were processed for light microscopy following the procedures of Sulston et al. (1983) and for immunofluorescence microscopy as described in Albertson (1984) and Bowerman et al. (1992). Intestinal cells were identified by their birefringent gut-specific granules and by staining with the monoclonal antibody MAbICB4 (Kemphues et al., 1988). Pharyngeal tissues were assayed by staining with either MAb3NB12, a monoclonal antibody that recognizes a subset of pharyngeal muscles (Priess and Thomson, 1987), or MAb9.2.1., a monoclonal antibody to pharyngeal-specific myosin (Epstein et al., 1982). Laser ablation was performed at 22°C using a VSL-337 laser (Laser Science) attached to a Zeiss Axioscope microscope as described in Avery and Horvitz (1989). After laser surgery, embryos were incubated at 16°C for 16–18 hr and examined in the light microscope and by immunofluorescence microscopy after antibody staining.

Cloning of *pop-1*

The *pop-1* mutation was mapped between the genes *lin-17* and *fog-1* to an interval of about 300 kb. Since the *pop-1* mutation was isolated from a mutator strain, we asked whether there was a novel transposon insertion associated with the *pop-1* mutation. A single Tc1 closely linked to the *pop-1(zu189)* mutation was identified by standard three-factor mapping. The DNA flanking the Tc1 was recovered by an inverse polymerase chain reaction (PCR) method as described in Hill and Sternberg (1992) and cloned into the BamHI site in the Bluescript vector KS(II) resulting in plasmid pRL155. The pRL155 insert was used to probe cosmids and yeast artificial chromosomes. Two yeast artificial chromosomes (Y71F9 and Y6A5) and one cosmid (BF8) hybridized with this probe; this result predicts a location for the *pop-1* gene consistent with results from genetic mapping experiments. BF8 and adjacent cosmids were injected separately into the gonad of *lin-17 pop-1; sDp2* hermaphrodites to assay for their ability to rescue the *pop-1* mutant phenotype using the procedures described by Mello et al. (1991). Phenotypic rescue was analyzed in the F2 progeny of injected worms.

The pRL155 insert also was used to screen a C. elegans genomic library and a mixed-staged cDNA library (provided by A. Fire). To verify that the cDNA clones correspond to the *pop-1* gene, we carried out injection of anti-sense RNA according to the procedure described by Guo and Kemphues (1995). When anti-sense RNAs derived from the putative *pop-1* cDNA were injected into the syncytial gonad of a wild-type adult, the animal produced dead embryos indistinguishable from *pop-1* mutant embryos. We also saw a similar effect using sense RNAs, analogous to the results reported by Guo and Kemphues (1995) in their analysis of the maternal gene *par-1*. It is not understood why both sense and anti-sense RNAs appear to reduce or eliminate endogenous gene function. However, using this technique it has been possible to phenocopy accurately each of the maternal mutants thus far tested, including *pie-1*, *mex-3*, and *apx-1*, with gene-specific sense or anti-sense RNAs (C. Mello, B. Draper, and J. R. P., unpublished data).

The sequence for the predicted *pop-1* transcript was obtained from several cDNA clones. The DNA sequence was determined by the dideoxy chain terminator method (Sanger et al., 1977). Reverse transcription coupled with PCR (RT–PCR) was used to determine the 5′ *trans*-spliced leader sequence and the 3′ untranslated region of the *pop-1* mRNA. RNA for RT–PCR analysis was isolated as described in Xie and Rothblum (1991). The 5′ RT step was performed with AMV reverse transcriptase at 37°C using a primer to the *pop-1* cDNA and either SL1 or SL2 primers. The SL1 and SL2 primers and reaction conditions are described in Spieth et al. (1993). The 3′ RT reaction was performed at 50°C using Superscript II (GIBCO BRL) to prevent potential secondary structure in the *pop-1* 3′ UTR. All reactions were carried out according to the suggestions of the manufacturer.

Generation and Characterization of POP-1 Antiserum

Two constructs with POP-1 fused to a His tag (Chen and Hai, 1994) were built. pRL166 was constructed by PCR amplification of the *pop-1* cDNA from a plasmid and cloning into the BamHI site of the expression vector pET-16B (Novagen). This construct generated a full-length POP-1 protein with 26 extra amino acids fused at the N-terminus. pRL174 is a fusion construct identical to pRL166 but with the HMG box deleted. This was generated by internally deleting the NsiI fragment (corresponding to a deletion of amino acids 167–366 in the POP-1 protein; see Figure 6) from pRL166.

The His–POP-1 fusion proteins were affinity purified on a nickel column according to the instructions of the manufacturer (Qiagen). Rabbits from the Jackson laboratory were immunized with a protein fraction that contained only POP-1 fusion protein. For each injection, 0.5 mg of affinity-purified fusion protein was injected subcutaneously. Animals were boosted every month and bled 2 weeks after each boost. Western blotting analyses were performed using bacterial extracts containing POP-1 fusion proteins to examine the immunoreactivity of each serum. To avoid cross-reactivity to other HMG box–containing proteins, we affinity purified the serum against the POP-1 fusion protein lacking the HMG domain. The affinity purification was performed with nitrocellulose-bound antigen as described in Robinson et al. (1988). This preparation is referred to as the 94I antiserum in the text.

To test whether POP-1 expression in late embryonic stages was due to zygotic expression of the *pop-1* gene, we stained embryos from hermaphrodites heterozygous for a deficiency of the *pop-1* locus (*tDf3*) with the 94I antiserum. All such embryos that were at the 28-cell stage or younger stained positively (n = 50), but about 25% of all later stage embryos did not stain (n = 100). We also examined embryos from hermaphrodites that are heterozygous for *pop-1(zu189)* and a deficiency of the *pop-1* locus; none of the embryos that were at the 28-cell stage or younger stained positively (n = 50), but about 75% of all later stage embryos stained positively (n = 100).

Immunofluorescence

Embryos were processed for staining with the anti-POP-1 antibody according to Bowerman et al. (1993) with the following modifications. Adults hermaphrodites were transferred to phosphate-buffered saline (PBS) on polylysine-coated slides. The hermaphrodites were cut open to release embryos and gonads, which immediately adhered to the

slides. After replacing the PBS with 15 μl of fixative (2% paraformaldehyde, 60 mM PIPES, 25 mM HEPES [pH 6.8], 10 mM EGTA, 2 mM $MgCl_2$), the embryos were flattened with a coverslip as described (Bowerman et al., 1993), incubated in a moist chamber for 30 min, and then frozen on dry ice. After 5 min, the coverslip was removed, and the slides were immersed in absolute methanol for 5 min at −20°, followed by 5 min in methanol at room temperature. The embryos were then rehydrated sequentially at room temperature in 90%, 70%, 50%, and 0% methanol in Tris–Tween (100 mM Tris–HCl [pH 7.5], 200 mM NaCl, 0.1% Tween). Embryos were treated with blocking solution (5% BSA and PBS) for 10 min and incubated with diluted affinity-purified POP-1 antibody overnight at 4°C and then with rhodamine-conjugated goat anti-rabbit secondary antibody for 2–3 hr at room temperature. Following each antibody incubation, the embryos were washed twice with Tris–Tween for 5 min. In the final wash, 20 ng/ml of DAPI was added to stain chromosomal DNA.

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