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Regulation of Cell Fate in *Caenorhabditis elegans* by a Novel Cytoplasmic Polyadenylation Element Binding Protein

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The *fog-1* gene of *Caenorhabditis elegans* specifies that germ cells differentiate as sperm rather than as oocytes. We cloned *fog-1* through a combination of transformation rescue experiments, RNA-mediated inactivation, and mutant analyses. Our results show that *fog-1* produces two transcripts, both of which are found in germ cells but not in the soma. Furthermore, two deletion mutants alter these transcripts and are likely to eliminate *fog-1* activity. The larger transcript is expressed under the control of sex-determination genes, is necessary for *fog-1* activity, and is sufficient to rescue a *fog-1* mutant. This transcript encodes a novel member of the CPEB family of RNA-binding proteins. Because CPEB proteins in *Xenopus* and *Drosophila* regulate gene expression at the level of translation, we propose that FOG-1 controls germ cell fates by regulating the translation of specific messenger RNAs.

Key Words: **germ line; spermatogenesis; translational regulation; CPEB protein; *fog-1; C. elegans*.**

INTRODUCTION

The dominant paradigm for how cell fates are specified relies on master transcriptional regulators. This paradigm was established, in large part, by study of the genes that regulate mating type in yeast (Nasmyth, 1982), but also applies to cell fate decisions in animals. For example, the MyoD/myogenin family of transcription factors regulates muscle cell fate in vertebrates (Olson, 1990; Sabourin and Rudnicki, 2000). In a similar manner, the genes of the Achaete-scute complex help specify the neuroblast fate in fruit flies (Skeath and Carroll, 1994).

The *Caenorhabditis elegans* germ line provides one of the leading systems for studying cell fate decisions in a model animal (Schedl, 1997; Ellis, 1998). This species has two sexes—*XO* animals, which develop as males, and *XX* animals, which develop as hermaphrodites. These hermaphrodites are essentially females that produce some sperm, which they store for later use in self-fertilization. This ability to self-fertilize simplifies the analysis of sterile mutations. Furthermore, although the germ line contains more cells than any other in the nematode, there are only a small number of cell fates to consider, and each is readily distinguished from the others (Hirsh *et al.*, 1976; Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; Sulston *et al.*, 1983). Germ cells at the distal end of the gonad undergo repeated mitotic divisions, and those that are forced away from this region by the mass of proliferating cells enter meiosis (Fig. 1A). In males, these meiotic cells all become sperm, whereas in hermaphrodites the first cells to enter meiosis become sperm, but later ones form oocytes.

Great strides have been made in learning how germ cell fates are controlled in nematodes. As one might expect, genes that control the animal's sexual identity play a critical role in the germ line (Meyer, 1997; Ellis, 1998). As with all tissues, germ cells respond to the levels of HER-1, a small, secreted protein that promotes both spermatogenesis and male somatic fates (Hodgkin, 1980; Perry *et al.*, 1993). In *XO* animals, HER-1 appears to bind and inactivate the TRA-2A receptor, leading to both spermatogenesis and male development (Hodgkin, 1980; Kuwabara *et al.*, 1992; Hodgkin and Albertson, 1995; Kuwabara, 1996b). Since hermaphrodites don't make HER-1, TRA-2A is active throughout the soma in *XX* worms, where it represses the activities of three proteins needed for male development—FEM-1, FEM-2, and FEM-3 (Doniach and Hodgkin, 1984; Kimble *et al.*, 1984; Hodgkin, 1986). In males, these FEM

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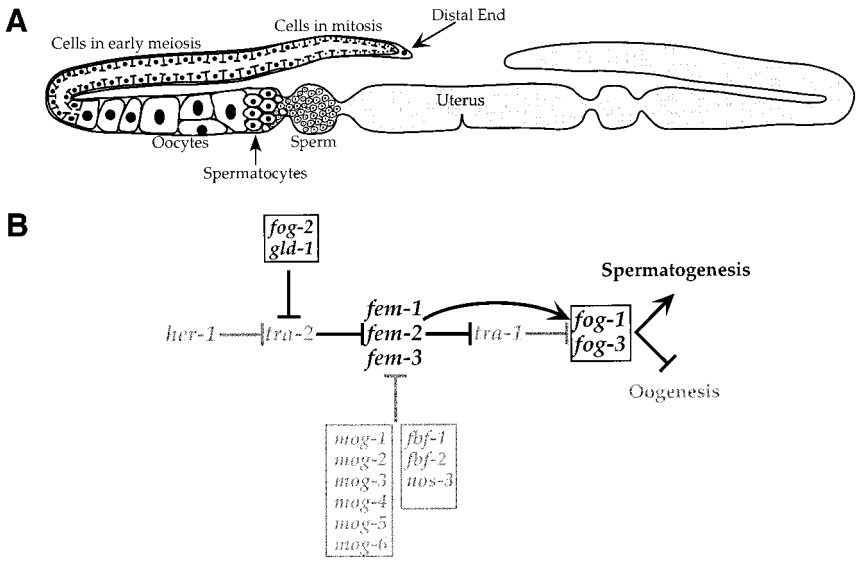

FIG. 1. *fog-1* acts at the end of the sex-determination pathway for germ cells. (A) Hermaphrodite gonad and germ line. For simplicity, only cells in the anterior arm are shown. (B) Sex determination in the germ line of an L4 hermaphrodite. Only genes that act downstream of the HER-1 signal are shown (for a review, see Ellis, 1998). Genes that are likely to be active are shown in black, those likely to be inactive in gray. Positive regulation is indicated with an arrow, and negative regulation with a line ending in a bar. Genes that act only in germ cells are shown in boxes.

proteins act within cells to repress the activity of *tra-1*, which encodes a zinc-finger protein that directly controls some, and perhaps all, somatic cell fates (Zarkower and Hodgkin, 1992; Conradt and Horvitz, 1999). To allow *XX* animals to develop as hermaphrodites, *fog-2*, *gld-1*, *fbf-1*, *fbf-2*, *nos-3*, and the *mog* genes modulate the activities of *tra-2* and *fem-3* in the germ line, so as to allow spermatogenesis in L4 larvae and oogenesis in adults (Fig. 1B).

Several results suggest that, in the germ line, *tra-1* might act through *fog-1* and *fog-3* to control whether germ cells become sperm or oocytes. First, mutations that inactivate either *fog-1* or *fog-3* cause all germ cells to differentiate as oocytes, even in males (Barton and Kimble, 1990; Ellis and Kimble, 1995). Thus, these genes differ from upstream regulators like *fog-2*, which function only in hermaphrodites. Second, mutations in *fog-1* and *fog-3* are epistatic to mutations in all other sex-determination genes, which indicates that *fog-1* and *fog-3* act at the end of the sex-determination pathway (Barton and Kimble, 1990; Ellis and Kimble, 1995). Genetic studies suggest that the *fem* genes also act at this position in the pathway, leaving unresolved the question of whether they act upstream of *fog-1* and *fog-3*. Third, however, molecular studies show that both *tra-1* and the *fem* genes regulate the expression of *fog-3*, but that *fog-1* does not (Chen and Ellis, 2000). Furthermore, these studies suggest that TRA-1A acts directly on the *fog-3* promoter (Fig. 1B).

Since FOG-3 is a member of the BTF family of proteins, one possibility is that it acts indirectly to regulate transcription in germ cells (Chen *et al.*, 2000). Indeed, many transcripts required for spermatogenesis are found only in males and L4 hermaphrodites (reviewed by L'Hernault, 1997). Mutations in these genes differ in one important way—*fog-1* is very sensitive to changes in gene dosage (Barton and Kimble, 1990), whereas *fog-3* is not (Ellis and Kimble, 1995). Thus, it is possible that changes in *fog-1* activity determine germ cell fate in living animals.

To learn how *fog-1* controls cell fate, we cloned it. We found that *fog-1* produces two major transcripts, but that only the larger one is essential for activity. This transcript contains a single long open reading frame, which encodes a novel cytoplasmic polyadenylation element binding protein. Members of this family of proteins bind to the 3′-UTRs of specific target messages and either promote polyadenylation and translation (Hake and Richter, 1994) or block translation (de Moor and Richter, 1999). These results suggest that translational regulation is critical for the direct specification of male germ cell fates.

MATERIALS AND METHODS

Genetic Nomenclature

The genetic nomenclature for *C. elegans* was described by Horvitz *et al.* (1979), with two exceptions. First, we use "female" to designate a hermaphrodite that makes oocytes but no sperm; by definition, female worms cannot self-fertilize. Second, we use capital letters and plain font to indicate the protein encoded by a gene. Thus, the protein produced by the *fog-1* gene is FOG-1.

TABLE 1
Genetic Mapping of *fog-1*

	Parental genotype	Recombinants picked	Segregation pattern
1	*ace-2 dpy-5/fog-1 (q187) unc-11*	Unc non-Fog	12/12 ⇒ *ace-2*
2	*ace-2 dpy-5/fog-1 (q507); ace-1*	Ace non-Dpy Dpy non-Ace	0/24 ⇒ *fog-1* 20/20 ⇒ *fog-1*
3	*fog-1(q507)/mek-2 ace-2 I; ace-1 X*	Ace non-Mek	4/19 ⇒ *fog-1*
4	*fog-1(q243) qP3/ sup-11 unc-11*	Unc non-Sup	4/15 ⇒ + + 7/15 ⇒ *fog-1* + 4/15 ⇒ *fog-1 qP3*

Note. After identifying recombinant F1 progeny, we isolated and characterized homozygous F2 animals. Ace-2 animals appear uncoordinated when tested in an *ace-1* background (Culotti *et al.*, 1981), Mek animals die (Church *et al.*, 1995), and Sup animals are sickly (Greenwald and Horvitz, 1981).

Strains

We cultured *C. elegans* as described by Brenner (1974) and raised strains at 20°C unless indicated otherwise. All strains were derived from the Bristol strain N2 (Brenner, 1974), with the exception of TR403 (Collins *et al.*, 1989). We used these mutations: *LGI, sup-11(n403)* (Greenwald and Horvitz, 1982), *mek-2(n2678)* (Kornfeld *et al.*, 1995), *fog-1(q187), fog-1(q241), fog-1(q242), fog-1(q253ts), fog-1(q329)* (Barton and Kimble, 1990), *fog-1(q491), fog-1(q492), fog-1(q493)* (Ellis and Kimble, 1995), *ace-2(g72)* (Culotti *et al.*, 1981), *unc-11(e47), dpy-5(e61)* (Brenner, 1974), *glp-4(bn2ts)* (Beanan and Strome, 1992); *LGII, tra-2*(*b202*ts) (Klass *et al.*, 1976); *fem-1*(*hc17*ts) (Nelson *et al.*, 1978), *fem-3*(*q96*gf,ts) (Barton *et al.*, 1987), *dpy-20(e1282ts)* (Hosono *et al.*, 1982); *LGV, him-5(e1490)* (Hodgkin *et al.*, 1979); *LGX, ace-1(p1000)* (Culotti *et al.*, 1981). In addition, we used the following chromosomal rearrangements: *qDf3 I* (Barton and Kimble, 1990) and *szT1(I;X)* (Fodor and Deak, 1985; McKim *et al.*, 1988).

The *fog-1(q507)* mutation was isolated as a dominant suppressor of *fem-3*(*q96*gf,ts) from a screen of 800 F_1 animals, following mutagenesis with UV light (Hartman, 1984).

Genetic Mapping

Our results suggested that the published location of *fog-1* might be incorrect (Barton and Kimble, 1990). To map *fog-1* with respect to *ace-2*, we carried out crosses 1 to 3, described in Table 1. These data show that *fog-1* maps close to *ace-2*, but to its left.

Physical Mapping

Using the cosmid C01G7 as a probe for Southern analysis (Southern, 1975), we identified an *Eco*RI fragment that is approximately 5 kb in N2 DNA and 5.2 kb in TR403 DNA. We named this polymorphism *qP3*. We present data showing that *fog-1* maps to the left of *qP3* in cross 4 of Table 1. Probes for Southern analysis were labeled with [^{32}P]dCTP using either random priming or *Taq* polymerase (Promega).

We used the PCR to determine if the DNA amplified by pairs of primers from the region was present in homozygous deficiency embryos or in *fog-1* adults (Ellis and Kimble, 1995). The primers we used are listed in Table 2; their positions on the physical map are diagrammed in Figs. 2A and 3B, and their precise location can be determined by a BLAST search (http://www.sanger.ac.uk/Projects/C_elegans/blast_server.shtml) of the *C. elegans* genome sequence (*C. elegans* Genome Sequencing Consortium, 1998).

Transgenic Nematodes

We used transformation rescue to determine the precise location of the *fog-1* gene. To produce stable lines of transgenic animals, we injected *fog-1*(*q253*ts) animals with both the plasmid pRF4 [*rol-6(su1006*dm)] at 100 ng/μl (Mello *et al.*, 1991) and a test YAC or genomic fragment. After identifying stable, transformed lines of worms that showed the Rol phenotype of our marker gene, we tested animals at the restrictive temperature of 25°C to see if the extrachromosomal array allowed them to produce sperm.

Analysis of fog-1 cDNAs

The central portion of the *fog-1L* cDNA was isolated by reverse-transcriptase PCR (RT-PCR) using primers RE85 and RE86. The 5′ end was isolated by rapid amplification of cDNA ends (RACE; Frohman *et al.*, 1988), using primers Q_0 and RE87 for the primary amplification and Q_1 and RE88 for the secondary amplification. The 3′ ends were also isolated by RACE, using primers Q_0 and RE89 first and then Q_1 and RE84. These PCR products were each sequenced on one strand using the dideoxy nucleotide method (Sanger *et al.*, 1977) with fluorescently labeled terminators (Halloran *et al.*, 1993).

We also amplified the 5′ ends of *fog-1L* and *fog-1S* using a primer corresponding to the SL1 *trans*-spliced leader sequence (Krause and Hirsh, 1987). We used RE88 with SL1 to amplify *fog-1L* and RE90 with SL1 for *fog-1S*.

Northern Analysis

We used acid guanidinium thiocyanate–phenol–chloroform extraction (Chomczynski and Sacchi, 1987; Chomczynski, 1993) to isolate total RNA from nematodes grown in liquid culture (Sulston and Brenner, 1974). From these total RNA samples, we prepared poly(A) RNA by selecting for transcripts that bind oligo(dT) cellulose. To analyze these RNAs by Northern analysis (Alwine *et al.*, 1977), we separated them on an agarose gel containing formaldehyde, transferred them to a positively charged nylon membrane, and used an antisense RNA probe from the region between primers RE101 and RE107. As a control for RNA purity and loading, we probed the same blots with a DNA probe to the *actin-3* message (Krause *et al.*, 1989).

RNA-Mediated Interference

To prepare double-stranded RNA, we used primers RE91 and RE92 to make the template for dsRNA#2 by RT-PCR and primers RE93 and RE94 to make the template for dsRNA#1. Each template was flanked by T7 promoters. We prepared RNAs by *in vitro* transcription, precipitated them, resuspended each in 1× injection buffer (Fire, 1986), and allowed each to anneal at 37°C after brief denaturation. We estimated the final concentrations to be 1 mg/ml, by ethidium bromide staining. Procedures for injection are described by Guo and Kemphues (1995) and Fire *et al.* (1998).

TABLE 2
Primers Used in These Experiments

RE61	AAGCCAACGTACGTGGTGTT
RE62	AAGGTCACGTTGGCCAAACA
RE63	ATTATTGCGAAATGGAGACCA
RE64	TCAATCGAGCAGAATAACGAA
RE65	CAACAAAGTTCTATCGGTGGA
RE66	TGCTCGATCCATAAATGTTAGA
RE67	ACATTATTCGCAACAAACGGA
RE68	TGGATGGAACGAGTAAAGGA
RE69	ACAAATGTGGAGAATCAGGAT
RE70	TTCCGATTGGCAGTTGAAGT
RE71	AGCCGGTTATGCAGTATTCAA
RE72	TTGCAAGTTTTCTTGAGCCTT
RE73	TAACAGTTCGAAAGCTCGGT
RE74	TTTTGAGCTGCTGCGTTGAC
RE75	CGGGGTCCGAGGAGCTCA
RE76	GGCTTAGGATTTTGGCGGA
RE77	TCGCTCCACCAGACGCCT
RE78	CGTTCTACGTCCATTACGTTCC
RE79	CCTCAAACTCCATCAAATCC
RE80	CTGATGATCTTCTCGCGG
RE81	CGTTATTTCGGTGTTTTTGGC
RE82	TCCCACAGTTGCGAAACGG
RE83	GGAGTGTGGGGACTCTTGTG
RE84	TCCATCCACCCAACTATCACC
RE85	CATCACGACGACGAGTTCAG
RE86	GTCCTTTCTCGGACGACG
RE87	GACGAGAAGAACAACTCCG
RE88	GCCAAGAGCTCGAGTTGGAG
RE89	ATAGAATTCCCGGTCCGAATGAATCCAGAAGCC
RE90	GGCCAATCGACGAAAACCG
RE91	GGATCCTAATACGACTCACTATAGGGAGACATCAC GACGACGAGTTCA
RE92	GGGATCCTAATACGACTCACTATAGGGAGAGCCAA GAGCTCGAGTTGGAG
RE93	GGATCCTAATACGACTCACTATAGGGAGACGTTGG TGGAATCTCGC
RE94	GGATCCTAATACGACTCACTATAGGGAGAG ACGAGAAGAACAACTCCG
RE95	AGCTTAAAACCAATGTTTCCCAGTGGT
RE96	CGTTGGTGGAATCTCGC
RE97	CACATCATATTTCGGAGCAGG
RE98	TTCAAATCCGTCCCTGG
RE99	TGATGGAAGATGTTGGATGTG
RE100	CCGGCGGTGGGAATTGTG
RE101	GTCCTTTCTCGGACGACG
RE102	GCCGGATTCCACGAAGC
RE103	TTGAATCCGGCATCCATTTTCG
RE104	GGCGTGCACCTACTCAAACCGGGA
RE108	ACAGTCCTGCAGATGTTTCCCAGTGGTCACAA TG
RE109	ACAGTCGTCGACCTACTTTCCCATATTAAC AAGGTACATAT
RE110	GCATTTACCTCTAGAAACGTGTGC
RE111	GCACACGTTTCTAGAGGTAAATGC
RE112	GCACACGTTTCTAGAGATGGAAAG
RE113	TATATAGGTACCGCCCATCTCGGAGGT
RE114	TATATAGGGCCCGTGCTCTAGTGACAAGTG
RE115	CGCCATTGGATCCTACTCGT
RE116	ACGAGTAGGATCCAATGGCG
RE117	GCCCGTCTTGCAGCTGCC
RE118	ACGGGAAATTGTGGCCGCAC
RE119	GAACTCCATCCGGAGCACTG
RE120	GCCTGGAACATCATCCTCATC
RE121	GGGATCCTAATACGACTCACTATAGGGAG ATCCATCCACCCAACTATCACC
RE122	CACAAGAGTCCCCACACTCCTCGTTCTGT AAGCTTAATGAATAC
RE123	TGACCACTGGGAAACATTGG
RE124	GCGGTCTGTGCATTTTGACG
RE125	GAGCCAACTGGGCGAGCA
RE126	CTGTCGGCGGCAATACATCA

Note. The sequence of each primer is listed, with pairs grouped together whenever possible.

In Situ Hybridization

In situ hybridization was performed as described (Seydoux and Fire, 1995; Jones *et al.,* 1996). Briefly, over 100 extruded gonads of N2 young adult males were treated with fixing solution (3% paraformaldehyde, 0.25% glutaraldehyde, 90 mM K_2HPO_4) for 1.5 h. After fixation, the gonads were washed with 1× PBS with 0.1% Tween 20 three times, treated with ice-cold methanol for at least 2 h, and then digested with 25 μg/ml proteinase K for 30 min. Finally, the extruded gonads were preincubated for 1 h at 37°C in a hybridization solution that contained 16% formamide, and then DIG-labeled oligonucleotides were added to a final concentration of 0.5 μg/ml each. These probes were specific to *fog-1L* and had the sequences IS1 (GAGTCCATTTTTCATTGT-GACCACTGGGAAAC), IS2 (GTTGTGGGCAGTCTGTGC-GACGCTGGAACG), and IS3 (CTCCGAGAGCTTGACACT-GTTGCCGGCGGGCTC). After overnight incubation, the samples were washed four times with hybridization buffer and visualized using α-DIG antibody conjugated with alkaline phosphatase (Roche).

Plasmid Constructs

To make the small genomic plasmid, we used *Pwo* polymerase to amplify from genomic DNA an *Apa*I/*Xba*I fragment (primers RE110 and RE114), an *Xba*I/*Bam*HI fragment (primers RE111 and RE116), and a *Bam*HI/*Kpn*I fragment (primers RE115 and RE113). We digested each fragment with the indicated enzymes and ligated them into the pCR2.1 vector (Invitrogen). Finally, the genomic/cDNA fusion was made by replacing the *Xba*I/*Bam*HI fragment with an *Xba*I/*Bam*HI fragment amplified from cDNA, using primers RE112 and RE116. The structure of each plasmid was verified by sequencing.

RESULTS

Identification of fog-1

To clone *fog-1,* we used molecular and genetic strategies to find its position on chromosome *I.* Our results showed that *fog-1* maps to the right of the *mek-2* gene and to the left of both the restriction fragment length polymorphism *qP3* and the *ace-2* gene (Fig. 2A, Table 1). We used PCR to test homozygous deficiency embryos for the presence of DNA sequences from this region. We found that the deletion *qDf3* breaks within Y54E10; since *fog-1* mutations fail to complement this deletion (Barton and Kimble, 1990), *fog-1* should lie under *qDf3* or near its endpoint. These data suggested that *fog-1* mapped to the right side of the YAC Y54E10. With the exception of W01B11 (which does not contain *fog-1*), this region is not represented in any cosmid or phage libraries.

To precisely locate *fog-1,* we designed probes from this region of Y54E10, based on sequence data from the *C. elegans* Genome Sequencing Consortium (1998). Each probe was used to test for rearrangements in DNA prepared from *fog-1* mutants that had been induced with γ-irradiation, UV light, or trimethyl psoralen. Eventually, we identified a region of about 6 kb that was altered by two different *fog-1* mutations, *q241* and *q492* (Figs. 2B and 2C). This region was predicted to contain a single gene, Y54E10A_156.D.

To see if Y54E10 could rescue a *fog-1* mutant, we isolated five transgenic lines following injection of Y54E10. At the restrictive temperature of 25°C, one of these extrachromosomal arrays restored self-fertility to 85% of *fog-1*(*q253*ts) mutants (n = 20). This result confirmed that *fog-1* was located on Y54E10 and established transformation rescue as an assay for the precise location of the gene. We then tested fragments from the putative *fog-1* region for their ability to rescue *fog-1*(*q253*ts) (Fig. 2C). Our results indicate that the region encoding the predicted protein Y54E10A_156.D is sufficient to rescue *fog-1* mutants.

The Mutation fog-1(q241) Is a Molecular Null Allele

To learn the null phenotype of *fog-1,* we investigated the mutations *fog-1(q241)* and *fog-1(q492),* which each showed a rearrangement in this region. By sequence analysis, we found that *q492* is a small deletion of 155 nucleotides. Since it spans two exons (see below), it eliminates only 90 nucleotides from the mature transcript, leaving the final message in frame. The *q241* mutation is a more complex rearrangement, which appears to contain two linked deletions. We tested the primer pairs shown in Fig. 3B by the PCR, to learn which target sequences were present in homozygous *q241* females. Our results show that *q241* deletes a large region upstream of *fog-1* and also much of the coding region (Figs. 3A and 3B). Furthermore, although *q241* retains some *fog-1* DNA, RT-PCR assays show that the levels of the truncated transcript are reduced (Fig. 3C), as would be expected for messages that were degraded by the *smg* surveillance system (Hodgkin *et al.,* 1989; Pulak and Anderson, 1993). These results suggest that *q241* is a molecular null allele.

What is the null phenotype of *fog-1*? Most *fog-1* mutations show some haploinsufficiency (Barton and Kimble, 1990). However, although two large deletions uncover *fog-1,* one is haploinsufficient in males and the other is not (Ellis and Kimble, 1995). These results left the null phenotype in doubt. Fortunately, our current studies show that the small deletion *q241* is likely to be a molecular null allele. This allele shows haploinsufficiency in males, just like the large deficiency *qDf4* (Barton and Kimble, 1990; Ellis and Kimble, 1995). Furthermore, this allele shows a typical Fog phenotype when homozygous—germ cells differentiate as oocytes rather than as sperm, but somatic cell fates are not affected. These traits appear to define the null phenotype for *fog-1.*

Although *q241* also deletes the predicted gene Y54E10A_156.C, *fog-1(q241)* animals resemble other *fog-1* mutants. Thus, either Y54E10A_156.C is not a true gene or it has no obvious function that can be detected in a *fog-1* mutant background.

fog-1 Produces Two Major Classes of Transcripts

To identify *fog-1* messages, we used Northern analysis and found that the *fog-1* region produces two major transcripts, which we call *fog-1L* (2.3 kb) and *fog-1S* (1.8 kb, Fig. 4A). The small transcript predominates in poly(A) purified RNA (Fig. 4A), but the large transcript is more common in total RNA (Fig. 5). We amplified internal portions of the Y54E10A_156.D message by RT-PCR and then used RACE (Frohman *et al.,* 1988) to isolate the 5′ and 3′ ends (see Materials and Methods). Using 5′ RACE, we identified two different start sites for the gene. Based on their sizes, one start site should produce a message of the right size to be the large transcript and the other a message of the right size to be the small one. Both transcripts are *trans*-spliced to the SL1 leader sequence. We also observed two different 3′ ends for *fog-1,* located near one another 241 and 343 nucleotides downstream of the stop codon. We used RT-PCR and primers specific for each start site and each termination site to show that all four possible *fog-1* messages are present in the worm (Materials and Methods). Throughout this paper, we use *fog- 1L* to describe the transcripts that start at exon 1, but have different 3′ ends, and *fog-1S* to describe the transcripts that start with exon 5, but have different 3′ ends. Although all four transcripts have been deposited in the EMBL database (Accession Nos. AJ297846–9), none corresponds exactly to the structure of Y54E10A_156.D predicted by the Genefinder program (Favello *et al.,* 1995).

The Long fog-1 Transcript Is Necessary and Sufficient for Activity

To learn if the long *fog-1* transcript was necessary for germ cells to become sperm rather than oocytes, we used RNA-mediated interference (RNAi; Fire *et al.,* 1998) to

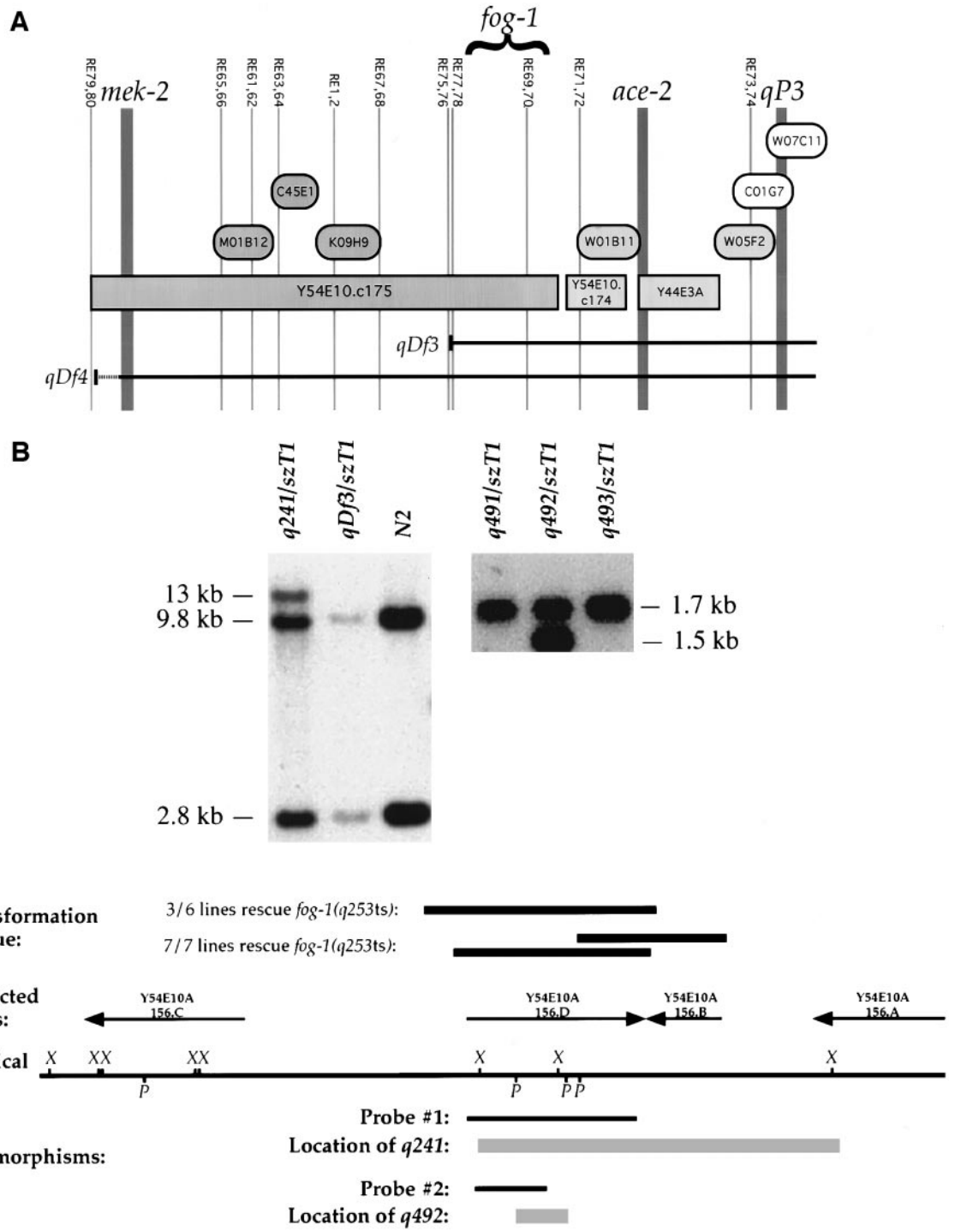

FIG. 2. Identification of the *fog-1* gene. (A) Alignment of the physical and genetic maps in the *fog-1* region. YACs are depicted as rectangles, cosmids as ovals. Clones that have been sequenced are colored gray. The *ace-2* and *mek-2* genes have been cloned (Church *et al.*, 1995; Kornfeld *et al.*, 1995; Wu *et al.*, 1995; Grauso *et al.*, 1998), and *qP3* is detected by both C01G7 and W07C11 (Materials and Methods). Primer pairs and deletion mapping are described under Materials and Methods. (B) Southern analysis of *fog-1* mutant DNA. DNA was prepared from strains in which a *fog-1* mutation was maintained *in trans* to the balancer chromosome *szT1*. Alleles are described under Materials and Methods. We used probe 1 for the *Xba*I digests shown on the left blot and probe 2 for the *Pvu*II digests shown on the right (see C). Each probe was prepared from cDNA from the indicated region. (C) Physical map of the *fog-1* region. The *C. elegans* Genome Sequencing Consortium (1998) determined the DNA sequence and predicted likely genes in this region. The probes used in B are shown below the line, along with the locations of the polymorphisms they detect. Transformation rescue experiments were carried out as described under Material and Methods, using two long-template PCR fragments that were co-injected (below) or a single genomic clone (above).

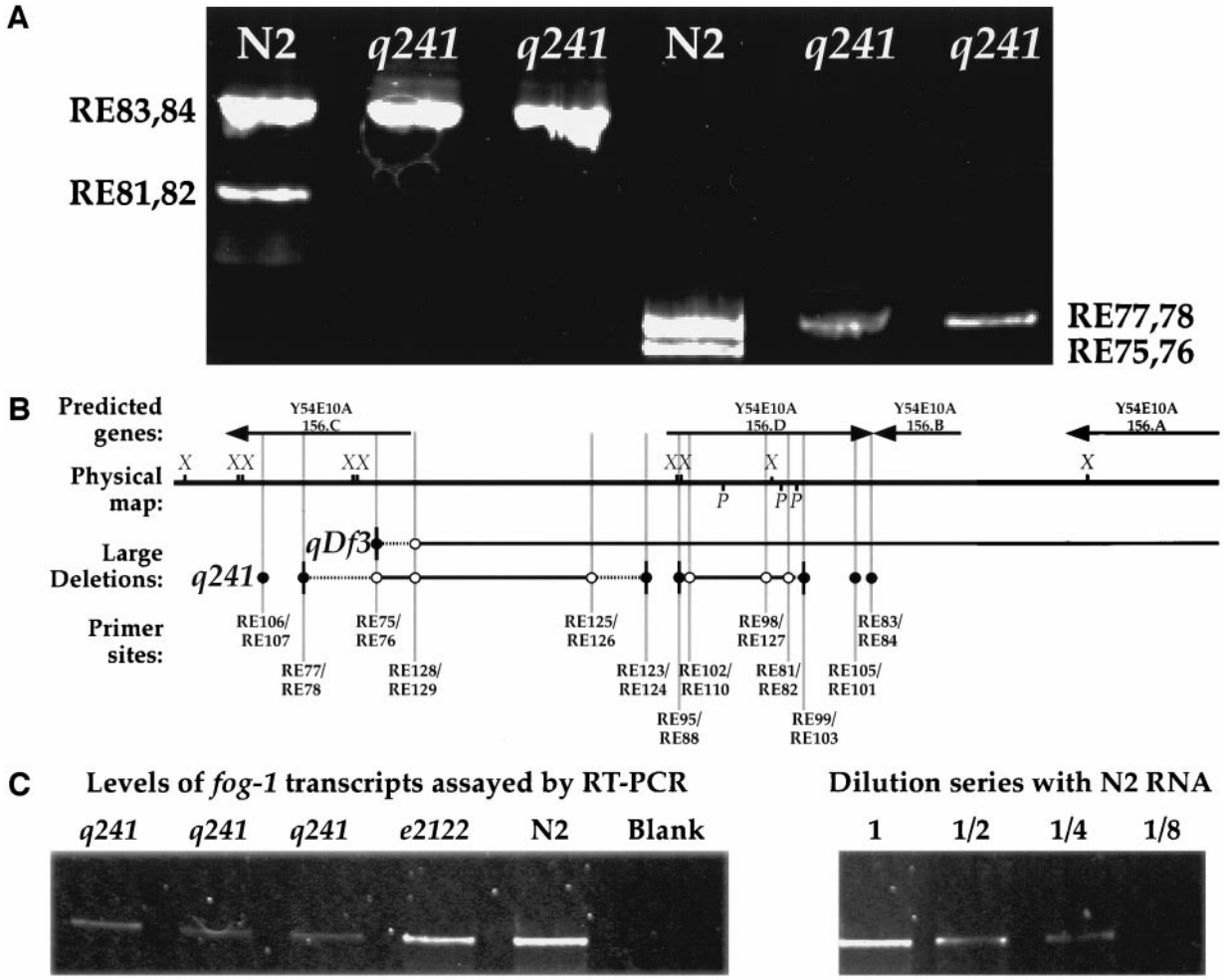

FIG. 3. The *fog-1(q241)* allele is a pair of linked deletions. (A) Acrylamide gel analysis of PCR products from *fog-1(q241)* females. Individual *fog-1(q241)* or wild-type females were prepared and analyzed by the PCR, as described under Materials and Methods. (B) Physical map of the *fog-1* region. The *C. elegans* Genome Sequencing Consortium (1998) determined the DNA sequence and predicted likely genes in this region. The lengths of the *q241* deficiencies were determined by examining homozygous deletion embryos by PCR, as described under Materials and Methods. Open circles and solid lines represent deleted DNA, dashed lines represent DNA that might be deleted, and black circles represent DNA present in *q241* animals. (C) Quantitative RT-PCR analysis of *fog-1* expression. The *fog-1* transcripts were amplified from RNA prepared from batches that each contained five L4 male nematodes, using primers RE83 and RE84, which are not deleted by *q241* (B). The *e2122* mutation, which is a missense mutation (unpublished results), is included as a control. The dilution series on the right shows that *fog-1* transcript levels were amplified in a linear range in this experiment.

eliminate either the long transcript alone or both transcripts together. This approach relies on the great specificity that RNAi shows for messages that contain the target sequence (Montgomery *et al.*, 1998) and on the assumption that the *fog-1S* transcript never contains sequences from the first four exons, even prior to *trans*-splicing. Because the two transcripts are coextensive, we could not selectively eliminate the smaller one. We found that males or hermaphrodites in which the long transcript alone had been targeted by RNAi appeared identical to *fog-1* mutants in all respects (Figs. 4B, 4C, and 4D). We obtained similar results when both transcripts were targeted by RNAi (Figs. 4C and 4D). These results confirm our identification of the *fog-1* gene and show that the long transcript is necessary for *fog-1* activity.

To learn if the long transcript was sufficient for *fog-1* activity, we designed a transgene in which the regions likely to contain the promoter and transcriptional start site for *fog-1S* had been eliminated (Fig. 4D). The segments that we excised from the original genomic construct were intron 4, which should contain the transcriptional start site for *fog-1S*, and intron 5, which lies nearby. This transgene restores production of sperm to *fog-1*(*q253*ts) mutants, which suggests that the large transcript might be sufficient

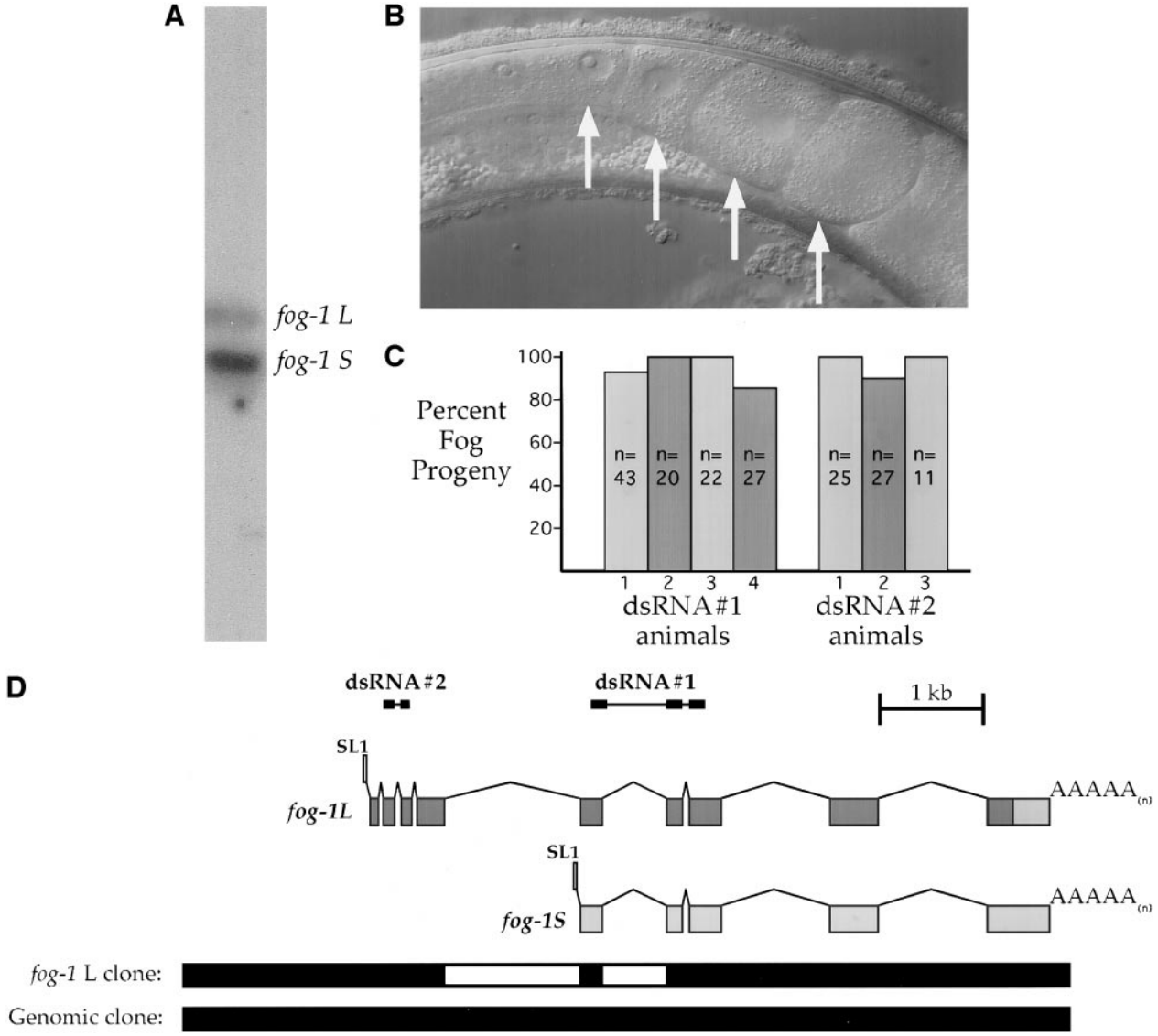

FIG. 4. *fog-1* produces two major classes of transcripts. (A) Northern blot of poly(A) purified RNA prepared from a mixed population of males and hermaphrodites that carried the *him-5(e1490)* mutation. The probe corresponded in sequence to dsRNA#1 (Fig. 4D). (B) Nomarski photomicrograph of the gonad and germ line in a *fog-1(RNAi)* male. The double-stranded RNA used was dsRNA#2 (Fig. 4D). Developing oocytes are indicated with white arrows. Anterior is to the left and ventral is down. (C) Bar graph showing the frequency of the Fog phenotype among the progeny of hermaphrodites injected with dsRNA#1 or #2. Animals were scored as Fog if they produced no self-progeny, but did make oocytes. (D) Structure of the *fog-1* transcripts. The sequence of the transcripts was determined by sequencing clones prepared by RT-PCR and RACE. The intron/exon boundaries were located by comparison with the published genome sequence. In the two clones used for transformation rescue, black indicates sequence included in the final construct.

for spermatogenesis (Table 3). However, these transgenic worms do not show the robust rescue, lasting from generation to generation at 25°C, that we observed for the full-length genomic clone. Furthermore, we were unable to test this construct in *fog-1* null mutants, since even full-length genomic clones do not rescue such mutants (unpublished data). Two models could explain why the *fog-1L* construct does not show the same long-lasting rescue as the genomic clone—first, the small transcript might contribute to *fog-1* activity or second, a site in the fourth or fifth introns might promote strong expression of *fog-1L*.

fog-1 Is Expressed in the Germ Line

In many animals, germ cell fates are determined by signals from nearby somatic tissues. We used two different

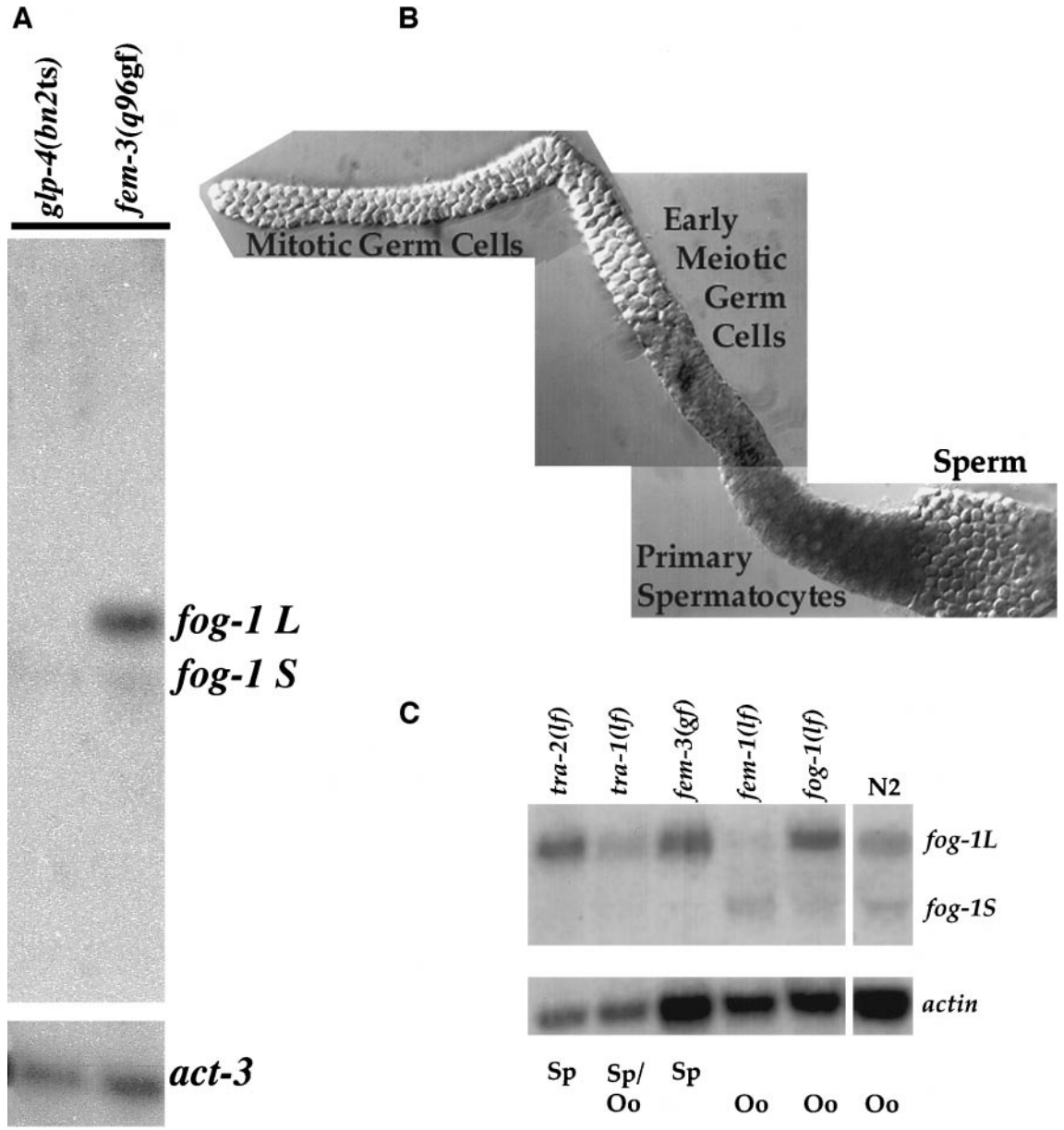

FIG. 5. *fog-1* functions in the germ line to control germ cell fates. (A) Northern blot. The *glp-4*(*bn2*ts) *XX* adults and *fem-3*(*q96*gf,ts) adults were raised at the restrictive temperature of 25°C. (B) Extruded gonad from adult male, stained with anti-*fog-1L* probes labeled with biotin and visualized with anti-biotin fluorescent antibodies. The distal tip of the gonad is at the left and the proximal end at the right. (C) Northern blot. The total RNAs were prepared from synchronous populations of *XX* adult animals. The wild-type and *tra-1* mutants were raised at 20°C and the others at the restrictive temperature of 25°C. The *tra-1(e1099)* animals have male bodies and produce sperm and oocytes, the *tra-2*(*b202*ts) animals develop male bodies and produce sperm, the *fem-1*(*hc17*ts) animals develop female bodies and produce oocytes, the *fem-3*(*q96*gf,ts) animals develop female bodies but produce only sperm, and the *fog-1*(*q253*ts) animals develop female bodies and produce oocytes.

approaches to determine if *fog-1* acts within the germ line itself to control germ cell fates or if instead it acts in the soma.

First, we used Northern analysis to see if *fog-1* transcripts were present only in animals with germ cells. To do this, we compared *fog-1* transcript levels between two strains: *fem-3*(*q96*gf,ts) adult hermaphrodites, which develop normally but produce sperm rather than oocytes (Barton *et al.*, 1987), and *glp-4*(*bn2*ts) adult hermaphrodites, which develop normally but produce neither sperm nor oocytes nor

TABLE 3
Transformation Rescue of *fog-1(q253*ts*)* Animals

Construct	Line	Percentage hermaphrodite	*n*
Overlapping genomic clones	A	63	8
	B	30	20
	C	62	13
	D	79	14
	E	71	7
Single genomic clone	A	90	10
	B	80	10
	C	40	10
	D	0	10
	E	0	10
	F	0	10
fog-1L clone	A	80*	20
	B	68*	9

Note. Individual L1 or L2 roller larvae were transferred to new plates and raised at 25°C. Hermaphrodites were self-fertile, which indicates that the *fog-1* phenotype had been complemented by the transgene. The asterisk indicates hermaphrodites from lines that could not be propagated indefinitely at 25°C.

even many immature germ cells (Beanan and Strome, 1992). We could not detect the *fog-1L* transcript in the Glp mutants (Fig. 5A). Thus, *fog-1L* is either produced in the germ line or produced in the soma following induction by germ cells.

To see if *fog-1* transcripts were physically present in germ cells, we used *in situ* hybridization (Crittenden *et al.*, 1994; Seydoux and Fire, 1995; Jones *et al.*, 1996). We focused on males, since they produce large numbers of sperm throughout adulthood, and observed that anti-*fog-1* probes specific to the large transcript stain the germ cells of extruded gonads. The most intense staining stretches from germ cells in early meiosis through primary spermatocytes, precisely where one might expect *fog-1* to act to promote spermatogenesis (Fig. 5B).

Expression of the Large Transcript Is Controlled by Sex-Determination Genes

How is the activity of *fog-1* regulated, so that males make sperm and adult hermaphrodites make oocytes? We used Northern analysis to see if *fog-1* is transcriptionally regulated by the sex-determination genes and found that young adults with mutations that promote spermatogenesis had high levels of the *fog-1L* transcript, whereas young adults with a *fem-1* mutation that promoted oogenesis did not express the *fog-1L* transcript (Fig. 5C). Furthermore, the levels of *fog-1S* seemed to be inversely correlated with those of *fog-1L*. By contrast, a mutation in *fog-1* itself did not prevent the expression of either transcript. These results suggest that genes of the sex-determination cascade regulate either the transcription or the stability of the large *fog-1* transcript, so that it is present at high levels during periods when sperm are made. Transcription of *fog-3* is controlled, at least in part, by the binding of TRA-1A to five sites in the *fog-3* promoter (Chen and Ellis, 2000). Since we observe four good TRA-1A binding sites in the promoter for *fog-1L* (Table 4), we suspect that the expression of *fog-1* is regulated in a similar way.

FOG-1 Is a Novel Member of the Family of CPEB Proteins

The large *fog-1* transcript contains a single long open reading frame, which encodes a protein of 619 amino acids. Because this transcript is necessary for *fog-1* activity, and perhaps also sufficient, we call its product FOG-1. Based on its sequence, FOG-1 is predicted to have a charge of 11 at pH 7.5 and a molecular weight of 71,018 daltons.

A BLAST search suggested that FOG-1 is a novel member of the family of cytoplasmic polyadenylation element binding (CPEB) proteins (Fig. 6). These proteins contain two RNA recognition motifs (RRM) and a novel zinc-binding domain at their carboxyl termini and regulate the translation of specific messenger RNAs (Hake and Richter, 1994; Hake *et al.*, 1998). This identification is supported by two observations. First, the RRMs of FOG-1 are much more like those of *Xenopus* CPEB than those of other RRM-containing proteins. For example, FOG-1 shares 28% identity with *Xenopus* CPEB in this region, but only 15% with *Drosophila* SXL-F. Second, FOG-1 appears to contain a zinc-binding C-H domain, which is characteristic of other known CPEB proteins, but not of most proteins that contain RRM domains. Although FOG-1 shows 27% identity and 46% similarity to *Xenopus* CPEB throughout the carboxyl half of the proteins, their amino termini are much more divergent.

These results suggested that FOG-1 might control cell fate by binding to and regulating specific messenger RNAs, an interaction that would presumably require the two RRMs. We found that *q492* is an in-frame deletion that removes amino acids 270 through 300 from the protein, which should inactivate RRM 1; the fact that this mutation causes a loss of function is consistent with the hypothesis that FOG-1 acts by binding target RNA molecules.

TABLE 4
Potential TRA-1A Binding Sites in the Promoter of *fog-1L*

Name	Orientation	Sequence
TRA-1A consensus		TTTTCnnnnTGGGTGGTC
−49 to −31	Reverse	TTTTCTTCGTGGGAGGTC
−217 to −199	Forward	TTTTCTTCATGGGCGGTC
−394 to −376	Forward	TTATCTTCGTGTGTGGTC
−566 to −548	Forward	TTTTCTTCGTGGGTGGTC

Note. The positions of each site are given relative to the first nucleotide of exon 1 (excluding the SL1 sequence). The TRA-1A consensus binding site was determined by Zarkower and Hodgkin (1993).

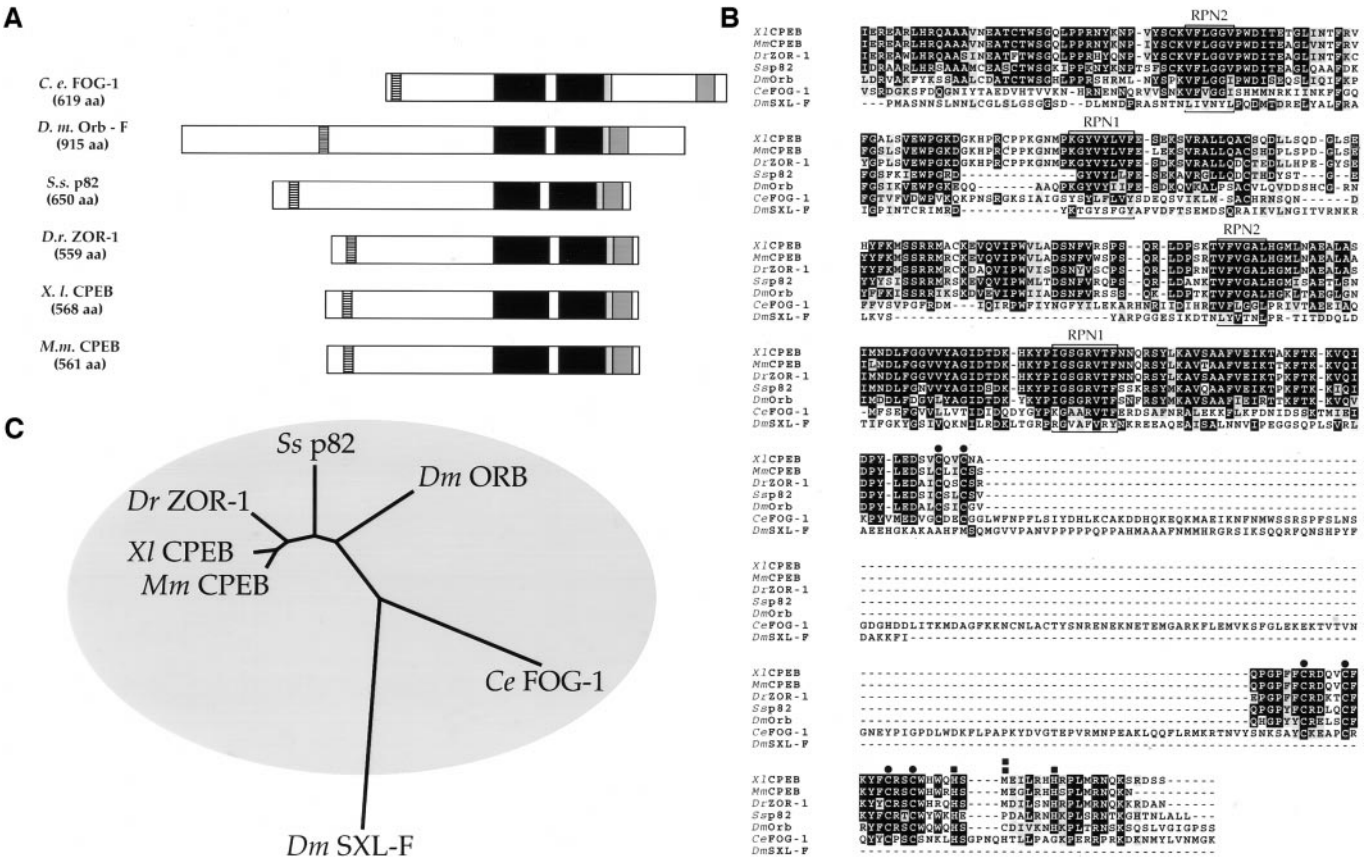

FIG. 6. FOG-1 is a novel member of the family of CPEB proteins. (A) Box diagrams comparing the structure of FOG-1 with that of other CPEB proteins. The RRM domains are shaded black, and the C-H domain is shaded gray. *Drosophila melanogaster* Orb (Lantz *et al.*, 1992), *Spisula solidissima p82* (Walker *et al.*, 1999), *Danio rerio* ZOR-1 (Bally-Cuif *et al.*, 1998), *Xenopus laevis* CPEB (Hake and Richter, 1994), *Mus musculus* CPEB (Gebauer and Richter, 1996) are also shown. (B) Box-shade alignment of FOG-1 sequence with those of other CPEB proteins. Conserved cysteines are marked with a circle and conserved histidines with a square, and the position of the final FOG-1 histidine is marked with two squares. The RPN1 and RPN2 structures within each RRM are defined by Burd and Dreyfuss (1994). The *X. laevis* CPEB sequence begins at residue 277, *M. musculus* CPEB at 274, *D. rerio* ZOR-1 at 268, *S. solidissima p82* at 373, *D. melanogaster* Orb at 540, *C. elegans* FOG-1 at 169, and *D. melanogaster* SXL-F at 93. (C) Unrooted tree showing the phylogenetic relationships between FOG-1, other described CPEB proteins, and SXL-F for the region shown in Fig. 5B. The tree was prepared using a neighbor-joining bootstrap method with 10,000 trials (Felsenstein, 1996); the ClustalX program was used for all calculations.

One aspect of FOG-1′s structure is unusual. Although it contains a domain rich in cysteines and histidines, which is common to all known CPEB proteins, and has been shown to bind zinc in *Xenopus* (Hake *et al.*, 1998), two features of this C-H domain are unique. First, this region of FOG-1 contains a large insertion not found in other CPEB proteins (Figs. 6A and 6B). Second, the spacing of the final histidine in the FOG-1 C-H domain differs from that found in other CPEB proteins (Fig. 6B). Although these changes might, in principle, alter the ability of FOG-1 to chelate zinc and bind RNA, the rest of the domain is highly conserved. We speculate that the large insertion might alter the specificity with which FOG-1 binds RNA or perhaps allow FOG-1 activity to be regulated in a manner not possible with other CPEB proteins.

DISCUSSION

fog-1 Encodes a CPEB Protein That Controls a Cell Fate Decision

In *C. elegans*, *fog-1* regulates a single cell fate decision—whether germ cells differentiate as sperm or as oocytes. In fact, analyses of the null mutants described in this report show that FOG-1 plays no detectable role in the development of other tissues (Barton and Kimble, 1990; Ellis and Kimble, 1995). We cloned *fog-1* and show here that it encodes a novel CPEB protein. This result suggests that FOG-1 regulates cell fates posttranscriptionally. In this respect, FOG-1 differs from other classical regulators of cell fate, like the yeast mating type genes *MATa* and *MATα* or the myoD gene of vertebrates.

This assertion is based on the assumption that FOG-1 directly controls germ cell fates. Is this so? Genetic tests reveal that five genes act at the end of the sex-determination pathway in the germ line—*fem-1, fem-2, fem-3, fog-1,* and *fog-3* (Hodgkin, 1986; Barton and Kimble, 1990; Ellis and Kimble, 1995). However, molecular data show that the levels of *fog-1* and *fog-3* transcripts are regulated by the *fem* genes (this paper; Chen and Ellis, 2000). Furthermore, additional mutations with similar phenotypes were not isolated from the screens that identified *fog-1* and *fog-3*. Thus, the simplest model is that these two genes act alone to specify germ cell fates and that their activities are regulated by the other sex-determination genes. However, we have been unable to determine if FOG-1 acts downstream of FOG-3, or upstream of it, or if the two act together, like MATa1 and MATα2 do to repress haploid mating functions in diploid yeast (Nasmyth, 1982). It is even possible that FOG-1 and FOG-3 play independent but essential roles in specifying germ cell fates, much as MATα1 and MATα2 play in specifying the α mating type in yeast.

Does the Drosophila Gene Sex lethal Function Like FOG-1 in Germ Cells?

In *Drosophila,* the sexual fate of all somatic cells is controlled by the master switch gene *Sex lethal* (reviewed by Cline and Meyer, 1996). The *Sex lethal* transcript can be spliced in two alternative ways—in *XX* flies, the female Sex lethal protein causes its own transcript to be spliced in the female manner and also regulates the splicing of transcripts that control downstream transcription factors; in males, the *Sex lethal* transcript is spliced into an inactive form. Thus, Sex lethal acts posttranscriptionally to control sexual identity, but it does so by regulating transcription factors that directly control cell fates.

However, in the germ line of *Drosophila,* Sex lethal acts downstream of other genes known to promote female germ cell fates (Oliver *et al.,* 1993). This result raises the possibility that Sex lethal might directly specify germ cell fates in a posttranscriptional manner, much as FOG-1 does in *C. elegans.* Despite this intriguing possibility, changes in the expression of Sex lethal do not appear to affect early steps in the sexual differentiation of germ cells (Steinmann-Zwicky, 1994), so Sex lethal might instead act during the growth, differentiation, or development of oocytes, rather than at the point when germ cells decide which fate to adopt.

The Role of FOG-1 in Males Indicates That CPEB Proteins Have Diverse Functions

Most of the characterized CPEB proteins play important roles in oocyte maturation or early development. These include *Xenopus* CPEB (Hake and Richter, 1994), mouse CPEB (Gebauer and Richter, 1996), zebrafish Zorba (Bally-Cuif *et al.,* 1998), *Drosophila* Orb (Christerson and McKearin, 1994; Lantz *et al.,* 1994), and clam p82 (Walker *et al.,* 1999). By contrast, analysis of mutants reveals that FOG-1 is not required for oogenesis, but instead promotes germ cells to differentiate as sperm (Barton and Kimble, 1990). Although *Drosophila* Orb also produces a transcript in the male germ line, this product has no known activity, so FOG-1 is the first example of a CPEB protein necessary for the development of male germ cells. Furthermore, FOG-1 is the first CPEB protein known to control a simple cell fate decision, in this case, whether germ cells differentiate as sperm or as oocytes.

Recent studies show that the CPB-1 protein of *C. elegans* is needed for primary spermatocytes to continue differentiation (Luitjens *et al.,* 2000) and that rat CPEB functions in specific neurons of the adult brain (Wu *et al.,* 1998). Taken together, these findings suggest that translational regulation by CPEB proteins is likely to play a broader role in development and behavior than initially appeared to be the case.

FOG-1 Is Part of a New Subfamily of CPEB Proteins

FOG-1 and CPB-1 resemble each other more than they do any other characterized CPEB proteins (our unpublished results; Luitjens *et al.,* 2000). However, BLAST searches reveal that the KIAA0940 protein, which is encoded by a cDNA found in human brain, also falls into this group (Nagase *et al.,* 1999). In addition, the *Drosophila* genome contains a potential gene that is very similar to KIAA0940. Since members of this subgroup are found in nematodes, insects, and vertebrates, the divergence of CPEB proteins into different subgroups probably predated the Cambrian explosion.

Does the Small Transcript Play a Role in fog-1 Function?

Although *fog-1* produces two major groups of transcripts, three results suggest that the large transcript plays the dominant role in mediating *fog-1* activity. First, we showed that the *fog-1(null)* mutant phenotype is defined by the *q241* mutation, which causes a complex rearrangement that deletes much of the *fog-1* gene. When *fog-1L* is inactivated by RNA-mediated interference, the affected animals resemble *fog-1(null)* mutants in all respects, even though the small transcript should be unaltered by this treatment (Fire *et al.,* 1998; Montgomery *et al.,* 1998). Second, expression of *fog-1L* is correlated with spermatogenesis, whereas the small transcript is almost completely absent from animals that are making sperm. Since *fog-1* is needed for spermatogenesis to occur, this result implies that *fog-1L* carries out this function. Third, although the transcriptional start site for *fog-1S* should lie in the fourth intron of the *fog-1* gene, a transgene that lacks introns 4 and 5 can rescue *fog-1*(*q253*ts) mutants. This result suggests that the large transcript might be sufficient for *fog-1* activity. However, these transgenic animals do not show the robust rescue typical of a *fog-1* genomic DNA clone. Thus, it remains possible that the small transcript contributes

weakly to *fog-1* function. Alternatively, an enhancer for the large transcript might lie in one of the deleted introns.

The *fog-1S* transcript cannot encode a complete CPEB protein, since the first in-frame ATG is located in the middle of the region that encodes RRM 1. Furthermore, this ATG is located 151 nucleotides from the start of the transcript. Since the translation of most messages that have been *trans*-spliced to SL1 begins within 30 nucleotides of the *trans*-splice site (Blumenthal and Steward, 1997), it is possible that no FOG-1S protein is even produced. However, although *fog-1S* does not appear to be needed for spermatogenesis, it might play a regulatory role in development. For example, if a FOG-1S protein is made, it could bind to and inactivate other proteins needed for spermatogenesis. Alternatively, the *fog-1S* transcript might titrate out factors that regulate translation of both *fog-1L* and *fog-1S*. For example, consider *tra-2*, which produces three different transcripts (Okkema and Kimble, 1991). The larger transcript encodes TRA-2A, which is necessary and sufficient for somatic *tra-2* activity (Kuwabara *et al.*, 1992; Kuwabara and Kimble, 1995). Although the smaller *tra-2* transcripts are not found in *C. briggsae* (Kuwabara, 1996a), one of them appears to play a regulatory role in the control of germ cell fate in *C. elegans* (Kuwabara *et al.*, 1998).

Alternatively, *fog-1S* might have no function. It is not uncommon for genes in the sex-determination pathway to produce multiple transcripts, and usually only one of these messages is necessary and sufficient for activity. For example, *her-1* produces two transcripts (Perry *et al.*, 1993). The smaller one has its own promoter, but contains only the final two exons of the larger one and has no detectable function. Furthermore, the related nematode *C. briggsae* does not produce the smaller transcript (Streit *et al.*, 1999). In addition, *tra-1* produces two transcripts, which differ at their 3′ ends (Zarkower and Hodgkin, 1992). However, the protein encoded by the smaller transcript lacks the DNA binding activity of TRA-1A (Zarkower and Hodgkin, 1993). Furthermore, this smaller transcript is not found in *C. briggsae* (de Bono and Hodgkin, 1996). By analogy, we propose that *fog-1S* either has no function or plays a minor role in the regulation of *fog-1* activity.

Translational Regulation in the Germ Line

Our Northern analyses showed that the expression of *fog-1* depends on the germ line, and *in situ* hybridization demonstrated that *fog-1* transcripts are present in germ cells. The region of expression includes both cells in early meiosis and primary spermatocytes. If this pattern corresponds to the regions in which FOG-1 functions, then FOG-1 might be required not only to specify that germ cells become sperm, but also during early spermatogenesis.

Since FOG-1 is a member of the CPEB family of proteins, it is likely to regulate cell fate by binding to specific messenger RNAs and controlling their translation. *Xenopus* and mouse CPEB proteins promote the polyadenylation of *cyclin*, *Cdk2*, and *c-mos* mRNAs during oogenesis (Gebauer and Richter, 1996; Stebbins-Boaz *et al.*, 1996), and clam p82

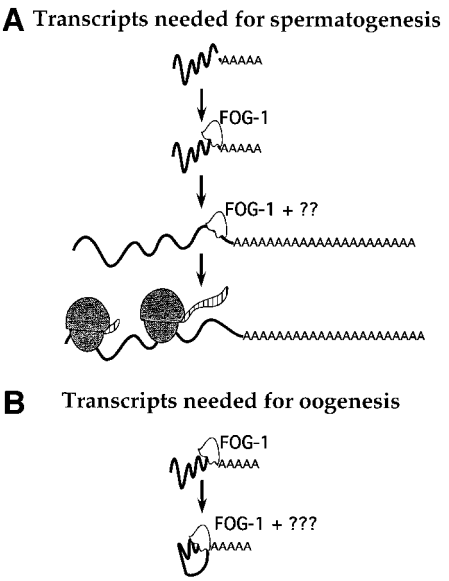

FIG. 7. FOG-1 regulates germ cell fates by controlling translation. (A) In this model, FOG-1 binds to transcripts needed for spermatogenesis and acts together with unknown factors to promote their polyadenylation. The longer poly(A) tails cause higher rates of translation for these messages, leading to spermatogenesis. (B) FOG-1 might also bind transcripts needed for oogenesis and act with unknown factors to block their translation.

promotes the polyadenylation of *cyclin* and *ribonucleotide reductase* mRNAs during oogenesis (Minshall *et al.*, 1999). Thus, one possibility is that FOG-1 promotes the translation of targets needed for spermatogenesis by promoting the extension of their poly(A) tails (Fig. 7A). However, the *Xenopus* and clam proteins have also been shown to block the translation of some messages (de Moor and Richter, 1999; Minshall *et al.*, 1999), so FOG-1 might instead mask specific mRNAs needed to initiate oogenesis (Fig. 7B).

Translational controls are also important at several earlier steps in the regulation of germ cell fate. For example, the sexual fate of germ cells depends on a signal from the soma that is received by the TRA-2A receptor (Kuwabara *et al.*, 1992; Kuwabara, 1996b). Translation of the *tra-2* message is regulated by sequences in its 3′-UTR (Goodwin *et al.*, 1993), which are recognized by GLD-1 (Jan *et al.*, 1999), an RNA-binding protein related to Sam68 (Jones and Schedl, 1995). Next, TRA-2A negatively regulates three FEM proteins, which act in the cytoplasm. One of these genes, *fem-3*, is translationally regulated (Ahringer and Kimble, 1991; Ahringer *et al.*, 1992) by the FBF proteins (Zhang *et al.*, 1997) and NOS-3 (Kraemer *et al.*, 1999). These FEM proteins appear to regulate germ cell fate in two ways, by directly promoting spermatogenesis and by negatively regulating TRA-1A (Doniach and Hodgkin, 1984; Hodgkin, 1986; Chen and Ellis, 2000). Surprisingly, although TRA-1A is a transcription factor related to the GLI proteins of mammals (Zarkower and Hodgkin, 1992), it also regulates

the subcellular distribution of *tra-2* messenger RNA (Graves *et al.*, 1999).

Why is translational control so important in the germ line? One possibility is that it allows transcripts needed for oocytes to be synthesized without being translated into unwanted proteins. If so, then translational control should not be required in male germ cells and might be present in *C. elegans* males only because some of the regulatory proteins are also used in self-fertile hermaphrodites. To explore this possibility, we are now studying sex determination in *C. remanei*, a related nematode that has male and female sexes. However, translational control of genes during spermatogenesis is also common in mammals (reviewed by Hecht, 1998), which suggests that it might be a general feature of male germ cell development. Since developing germ cells are part of a large syncytium in male mammals (Dym and Fawcett, 1971), just as in *C. elegans*, one possibility is that translational control helps restrict the production of proteins to specific germ cells or subcellular locations, despite the diffusion of messenger RNAs throughout the syncytium. We do not know if CPEB proteins regulate translation in the male germ line of species other than *C. elegans*, but the presence of male-specific *orb* transcripts in fruit flies suggests that they might (Lantz *et al.*, 1992).

CONCLUSION

Our results show that germ cell fates in *C. elegans* are controlled by a novel CPEB protein, FOG-1. This is the first CPEB protein shown to regulate a simple cell fate decision and perhaps the first translational regulator of any kind that directly controls cell fate. Furthermore, FOG-1 defines a new subgroup of CPEB proteins that appears to be conserved from nematodes to vertebrates. Since there are more than 60 *fog-1* mutations known, we have begun a molecular and genetic dissection of FOG-1 activity, to elucidate how CPEB proteins function. In addition, we are using suppressor analyses to identify proteins that might interact with FOG-1 to regulate translation. The combined power of these approaches should make FOG-1 a model for studying how translational regulators control cell fate decisions.

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