Redundant control of the *Caenorhabditis elegans* sperm/oocyte switch by PUF-8 and FBF-1, two distinct PUF RNA-binding proteins

Jennifer L. Bachorik* and Judith Kimble*†‡§

*Program in Cellular and Molecular Biology, †Department of Biochemistry, and ‡Howard Hughes Medical Institute, University of Wisconsin, Madison, WI 53706

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PUF proteins control both growth and differentiation in the *C. elegans* germ line. These conserved RNA-binding proteins inhibit expression of target mRNAs, either by repressing translation or promoting degradation. Previous studies showed that PUF-8, a PUF protein with striking similarity to human Pumilio, prevents return of primary spermatocytes to the mitotic cell cycle [Subramaniam, K. & Seydoux, G. (2003) *Curr. Biol.* 13, 134–139]. We now report that PUF-8 is also critical for the hermaphrodite sperm/oocyte switch. Most *puf-8* mutant hermaphrodites make both sperm and oocytes and are self-fertile, but some make a vast excess of sperm and fail to switch into oogenesis. This *puf-8* defect is dramatically enhanced by removal of another *puf* gene called *fbf-1*: all *fbf-1 puf-8* double mutants fail in the hermaphrodite sperm/oocyte switch. Therefore, *puf-8* and *fbf-1* act redundantly to control this decision. Epistasis analyses place *puf-8* and *fbf-1* upstream of *fog-2*, a gene near the top of the germ-line sex determination pathway. Furthermore, the abundance of FOG-2 increases dramatically in the distal region of *fbf-1 puf-8* double mutants. We suggest that PUF-8 and FBF-1 may control *fog-2* expression, and that the sperm/oocyte decision occurs in the distal germ line.

germ line | PUF proteins | RNA regulation | sex determination | *fog-2*

PUF proteins (for Pumilio and FBF) are key regulators of gene expression. These broadly conserved RNA-binding proteins bind specifically to regulatory elements in target mRNAs and repress expression, by either translational repression or mRNA degradation (1). A typical PUF protein possesses eight Puf repeats, which are required collectively for RNA binding and mRNA repression (2–4). One ancient role of PUF proteins appears to be the control of stem cells, but PUF proteins also control other aspects of development (1, 5).

We have focused on how PUF proteins control the *Caenorhabditis elegans* sperm/oocyte switch. *C. elegans* can exist as either a self-fertile hermaphrodite or a male. Hermaphrodites produce sperm during the fourth larval stage (L4) and produce oocytes as adults. Previous work showed that two PUF proteins, called FBF-1 and FBF-2, are required for the hermaphrodite sperm/oocyte decision (2). FBF-1 and FBF-2 are >90% identical to each other throughout their full lengths (2). Therefore, RNA interference (RNAi) could not be used to deplete individual FBF proteins, but deletion mutants in each *fbf* gene have demonstrated that they are largely redundant (6, 7). Thus, most *fbf-1* and *fbf-2* single mutants are self-fertile, although rare *fbf-1* mutants make only sperm and rare *fbf-2* mutants make only oocytes. More importantly, all *fbf-1 fbf-2* double mutants fail to switch from spermatogenesis to oogenesis (2). Therefore, *fbf-1* and *fbf-2* function redundantly to promote the sperm/oocyte switch.

The distal adult germ line is composed of a population of mitotically dividing cells (see ref. 8 for review). As cells divide and move proximally, they exit the mitotic cell cycle and enter meiosis, ultimately differentiating as either sperm or oocytes. In addition to their role in the sperm/oocyte switch, FBF-1 and FBF-2 also control the mitosis/meiosis decision: all *fbf-1 fbf-2* double mutants fail to maintain germ cells in the mitotic cell cycle (6). Intriguingly, the *fbf* single mutants have gene-specific effects on number of cells in the mitotic region: the *fbf-1* mitotic region is smaller than normal, whereas the *fbf-2* mitotic region is larger than normal (6, 7). FBF-1 and FBF-2 have overlapping but distinct distributions in the distal germ line, which may explain the subtle differences in their effects on germ-line fates (6, 7). Therefore, although FBF-1 and FBF-2 are redundant in their capacities to promote germ-line mitotic divisions and oogenesis, they have acquired distinct patterning functions in the distal germ line.

Whereas the two FBF proteins are distant cousins of *Drosophila* and human Pumilio, *C. elegans* PUF-8 is more related by amino acid sequence (1). Within the Puf repeats, the FBF amino acid sequence is only ≈32% identical to fly or human Pumilio, but PUF-8 is ≈45% identical within that same domain (1). Previous work identified a role for *puf-8* in spermatogenesis: in *puf-8* mutants raised at 25°C, primary spermatocytes dedifferentiate and enter the mitotic cell cycle (9). Here we identify a role for PUF-8 in the hermaphrodite sperm/oocyte switch. A *puf-8* deletion mutant has a low penetrance effect on the switch, but *fbf-1 puf-8* double mutants are fully defective. In addition, the *puf-8* gene affects the size of the adult mitotic region of the germ line. We find that *fbf-1* and *puf-8* act upstream of *fog-2*, a germ-line-specific sex determination gene that functions near the top of the sex determination pathway. We do not know whether PUF-8 controls *fog-2* directly, but FOG-2 protein abundance increases dramatically in *fbf-1 puf-8* double mutants. This FOG-2 increase is largely confined to the distal region of the germ line, which suggests that the sperm/oocyte decision may be made in this part of the germ line. We discuss these results in light of our current knowledge of regulators of the sperm/oocyte switch, and suggest a model that can explain, at least in part, how PUF-8 and FBF-1 may work together to regulate the switch.

Materials and Methods

C. elegans* Methods.** All strains were maintained at 20°C unless otherwise noted. The *puf-8(q725)* deletion was isolated by standard methods (10). Mutations and balancers used in this work include: ***LG II: *fbf-1(ok91)*, *fbf-2(q738)*, *puf-8(q725)*, *puf-8(ok302)*; *mIn1[mIs14 dpy-10(e128)]*; ***LG III***: *fem-2(e2105)*, *unc-45(r450)*, *dpy-1(e1)*; ***LG IV***: *fem-1(e1991)*, *dpy-20(e1282)*, *unc-5(e53)*; and ***LG V***: *fog-2(q71)*, *rol-9(sc148)*, *unc-51(e1189)*. To confirm that *fbf-1 puf-8; fog-2 rol-9* feminization was not due to the *rol-9* mutation, we examined *fbf-1 puf-8; rol-9* triple mutants and found that they were masculinized ($n = 90$). To assess oocyte function, five wild-type males were placed on a Petri dish with

Abbreviation: RNAi, RNA interference.

§To whom correspondence should be addressed. E-mail: jekimble@facstaff.wisc.edu.

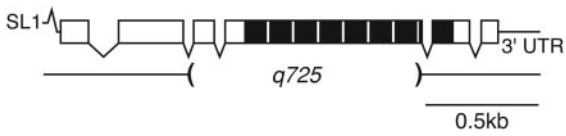


Fig. 1. The *puf-8(q725)* deletion mutant. A schematic of the *puf-8* gene is shown. Boxes, coding region; connecting lines, introns and 3′ UTR; black boxes, Puf repeats. Extent of *puf-8(q725)* deletion shown below the gene structure.

either one *fog-2*, *fog-2 rol-9* or *fbf-1 puf-8; fog-2 rol-9* female. For simplicity, we omit the *rol-9* marker in the text.

Molecular Methods. The *puf-8* SL1 *trans*-spliced leader and exon/intron boundaries were determined by sequencing RT-PCR-generated clones by standard methods. Total RNA prepared from N2 and *puf-8(q725)* young adults was reverse transcribed by using oligo(dT) primer. The resultant cDNA was amplified by seminested PCR, using an SL1-specific primer and either of two *puf-8*-specific primers (details available on request). The *puf-8* 3′ UTR was determined by PCR using the λAE.1 oligo(dT) primed *C. elegans* mixed stages phage cDNA library.

For RNAi feeding experiments, we generated bacterial strains by standard methods (11, 12). *puf-8* cDNA nucleotides 427-1608, *fem-3* cDNA nucleotides 1–1167, and *fog-1* cDNA nucleotides 61–990 (13) were subcloned into vector L4440 and transformed into HT115 cells. L4s were fed HT115 carrying the dsRNA of interest, and self progeny were examined. For *fog-3* RNAi, we injected double-stranded RNA corresponding to nucleotides 2–740 at 1 mg/ml into N2 and *fbf-1(ok91) puf-8(q725)* balanced by *mnIn1[mIs14 dpy-10(e128)]* and examined self progeny for defects.

Immunocytochemistry. Gonad dissections, fixation, and antibody staining were performed as described (14, 15). Extruded germ lines (≈24 h past mid-L4) were costained with DAPI. The sperm-specific antibody, SP56, was a gift from S. Ward (University of Arizona, Tucson; ref. 16); α-RME-2 was a gift from B. Grant (Rutgers, The State University of New Jersey, Piscataway; ref. 17) ; α-FOG-2 was a gift from T. Schedl (Washington University School of Medicine, St. Louis; ref. 15). Images were obtained on either a Bio-Rad MRC1024 confocal microscope or, for DAPI images, a Hamamatsu Orca camera with OPENLAB software (Improvision, Lexington, MA), and processed by using PHOTOSHOP (Adobe Systems, San Jose, CA). All compared images were processed identically.

Results

The *puf-8* Single Mutant Phenotype. To investigate *puf-8* function, we isolated *puf-8(q725)*, a 1,026-bp deletion that removes seven of the eight Puf repeats (Fig. 1). Because all eight repeats are required for RNA binding (2–4), *puf-8(q725)* is likely to have little or no PUF-8 activity. Using RT-PCR, we examined mRNAs from both wild-type and *puf-8(q725)* animals. Wild-type *puf-8* is SL1 *trans*-spliced and contains 1,812 nucleotides, excluding its poly(A) tail (Fig. 1). In contrast, *puf-8(q725)* mRNA splices exon 2 to exon 5, and it is predicted to terminate prematurely. Like *puf-8(ok302)* (9), *puf-8(q725)* mutants developed proximal tumors at 25°C (83%, $n = 64$). Thus, both *q725* and *ok302* appear to be strong loss-of-function alleles. Our genetic analyses rely primarily on the *puf-8(q725)* deletion.

We examined *puf-8* single mutants at 20°C. Most *puf-8(q725)*, *puf-8(ok302)* and *puf-8(RNAi)* hermaphrodites were self-fertile and produced the normal number of sperm (*puf-8(q725)*: average 155 sperm per gonad arm, range 125–223, $n = 10$ arms). However, some animals made excess sperm and failed to switch into oogenesis, the Mog phenotype (for masculinization of the germ line) (Table 1). Therefore, *puf-8* affects germ-line sex determination.

***puf-8* and *fbf-1* Redundantly Promote the Sperm/Oocyte Switch.** Because both *fbf-1* and *puf-8* have low penetrance Mog phenotypes (*fbf-1:* <1%, (ref. 6); *puf-8:* 3%, Table 1), we asked whether *fbf-1* and *puf-8* might be redundant for the sperm/oocyte switch. Whereas most *fbf-1* and *puf-8* single mutants made both gametes (Fig. 2*A*) (6), *fbf-1 puf-8* double mutants and *fbf-1 puf-8(RNAi)* animals were Mog: all made excess sperm and failed to switch to oogenesis (Table 1 and Fig. 2 *A* and *C*). To confirm this effect, we used sperm- and oocyte-specific markers. *puf-8* single mutants that were self-fertile stained positively for both markers ($n = 16$ arms) (Fig. 2*B*), but the Mog *fbf-1 puf-8* double mutants stained positively only with the sperm-specific marker ($n = 51$ arms) (Fig. 2*D*). We conclude that *fbf-1* and *puf-8* are redundant for the sperm/oocyte switch.

We also examined *fbf-2 puf-8* double mutants, and found that most *fbf-2 puf-8* double mutants were fertile, indicating that these two *puf* genes are not redundant for the sperm/oocyte switch or for any other major germ-line function (Table 1). Therefore, *puf-8* is redundant with *fbf-1*, but not with *fbf-2*. This finding underscores the idea that *fbf-1* and *fbf-2* regulatory roles are not identical, as reported (7).

***puf-8* Promotes Germ-Line Proliferation.** The *fbf-1 puf-8* double mutants maintained a mitotic region throughout adulthood (Fig. 2*C*). Therefore, *puf-8* and *fbf-1* are not redundant with respect to the mitosis/meiosis decision. To explore the possibility of a more subtle effect of *puf-8* on germ-line proliferation, we examined the mitotic regions in *puf-8* single mutants as well as in *fbf-1 puf-8* and *fbf-2 puf-8* double mutants. We first measured mitotic region length by DAPI staining. Specifically, we counted

Table 1. Defects in *puf-8* single and *fbf puf-8* double mutants

Genotype	Mog, %	Other sterile*, %	$n^{\dagger}$	Mean brood size (range)	$n^{\ddagger}$
Wild type	0	0	1,279	320 (291–343)	4
puf-8(RNAi)	2	0	286	ND	NA
puf-8(q725)	3	0	1,952	198 (123–254)	5
puf-8(ok302)	17	4	642	77 (35–143)	4
fbf-1(ok91) puf-8(RNAi)	98	2	326	ND	NA
fbf-1(ok91) puf-8(q725)	100	0	500	ND	NA
fbf-2(q738) puf-8(q725)	6	9	1,420	146 (105–209)	5

All animals were maintained at 20°C. Mog, masculinization of the germ line; ND, not determined; NA, not applicable.

*Animals with a variety of other defects causing sterility: defective oocytes, vacuoles, and underproliferated germ lines.

†Number of gonad arms scored.

‡Number of broods scored.

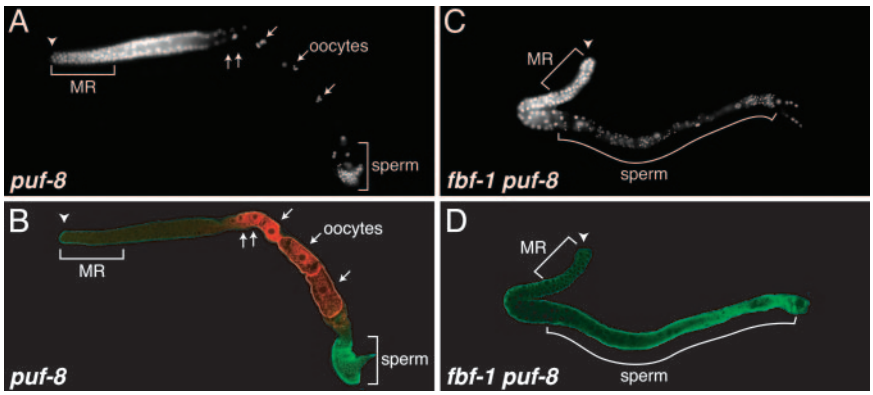


Fig. 2. *puf-8* and *fbf-1* redundantly promote the sperm/oocyte switch. (*A–D*) Dissected germ lines. Arrowhead, distal end; MR, mitotic region; arrows, oocytes; distal, left. (*A* and *C*) DAPI stained. (*B* and *D*) Costained with the sperm-specific antibody SP56 (green) and oocyte-specific antibody α-RME-2 (red). *A* and *B* and *C* and *D* are the same germ line of genotype *puf-8(q725)* or *fbf-1(ok91) puf-8(q725)*, respectively.

the number of germ cell diameters from the distal end to the transition zone, a region where germ cells have entered early stages of meiotic prophase. To confirm this length, we also assessed the proximal-most PH3-positive cell, which should coincide with the border between the mitotic region and the transition zone. Finally, we counted total number of germ cells occupying the mitotic regions. Wild-type hermaphrodite germ lines had a normal sized mitotic region (≈20 cell diameters in length) with an average of 262 total germ cells (Fig. 3*A* and Table 2). By contrast, *puf-8* had a shorter mitotic region (≈15 cell diameters in length) with an average of 116 germ cells (Fig. 3*B* and Table 2). Thus, the *puf-8* mitotic region is significantly smaller than wild type. The *fbf-1 puf-8* and *fbf-2 puf-8* mitotic regions were not dramatically different (Fig. 3 *C* and *D* and Table 2).

Previous work showed that PUF-8 functions in spermatogenesis to inhibit mitotic divisions and promote progression through meiosis (9). However, we find that *puf-8* functions in the distal germ line to maintain the normal number of germ cells in the mitotic cell cycle. Unlike FBF-1 and FBF-2, which are required for maintenance of stem cells (6), PUF-8 is required for extending the mitotic capacity of the distal germ line. Therefore, PUF-8 can function in opposite modes, inhibiting the mitotic cell cycle in spermatogenic cells (9) and promoting the mitotic cell cycle in undifferentiated germ cells. We do not understand how PUF-8 functions in these opposite modes, but suggest that PUF-8 acts in a combinatorial fashion with other regulators to control key components of the cell cycle machinery.

***fbf-1 puf-8* Acts at the Top of the Germ-Line Sex Determination Pathway.** Fig. 4*A* shows a simplified version of the germ-line sex determination pathway (reviewed in ref. 18). In this pathway, *fog-1* and *fog-3* are terminal regulators and essential for sperm specification. Other genes function as negative (i.e., *tra* and *fbf* genes) or positive (i.e., *fem*, *fog-2*, and *gld-1* genes) regulators of *fog-1* and *fog-3*. To determine where *fbf-1 puf-8* functions in this pathway, we conducted epistasis experiments. Specifically, genes required for sperm specification were depleted in *fbf-1 puf-8* double mutants, and the presence of sperm or oocytes was assayed. The result was the same for all of the experiments: depletion of any *fem* or *fog* gene from *fbf-1 puf-8* mutants led to production of oocytes only, which places *fbf-1* and *puf-8* upstream of all these genes (Fig. 4*B*).

The presence of oocytes in *fbf-1 puf-8; fog-2* triple mutants was surprising, in part because *fbf-1 fbf-2* acts downstream of *fog-2* (2, 13). Therefore, we examined *fbf-1 puf-8; fog-2* with gamete-specific markers, and found that the germ lines stained positively with an oocyte-specific marker, but failed to stain with a sperm-specific marker ($n = 26$ arms, Fig. 4 *C* and *D*). In contrast, *fbf-1 fbf-2; fog-2* mutants produced sperm, and their germ lines stained positively with the sperm-specific marker, but not with the oocyte-specific marker ($n = 18$ arms, Fig. 4 *E* and *F*). We conclude that *fbf-1* and *puf-8* act genetically upstream of *fog-2*, and that *fog-2* activity is required for the *fbf-1 puf-8* Mog phenotype.

FOG-2 Expression Increases in *fbf-1 puf-8* Mutants. PUF proteins are conserved mRNA repressors, and their removal often results in an increase in the protein encoded by its target mRNA (1). To ask whether FOG-2 protein increases in *fbf-1 puf-8* mutants, we stained germ lines with α-FOG-2 antibodies. FOG-2 is a cytoplasmic protein present in proliferating and meiotic prophase germ cells (15). We found FOG-2 abundance to be similar in

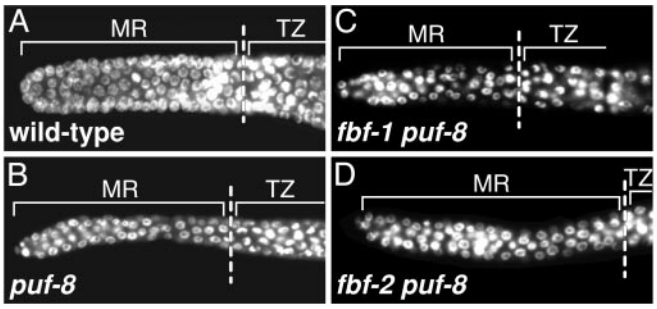
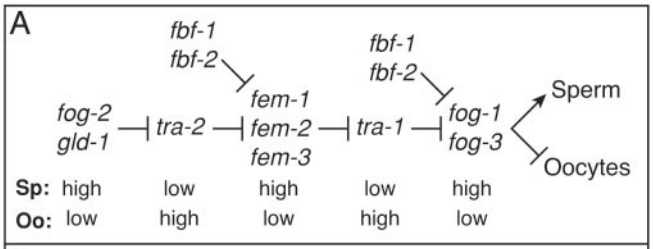


Fig. 3. *puf-8* affects the size of the mitotic region. DAPI-stained dissected germ lines with focus on distal end. Dashed line, boundary between mitotic region (MR) and transition zone (TZ).

Table 2. Summary of effects of *puf-8* mutants on size of the mitotic region

Genotype	Average length MR	Proximal-most PH3+ cell	Average no. of cells in MR
Wild type	20 ± 0.5	20	262 ± 15
	$n = 34$	$n = 23$	$n = 9$
puf-8	15 ± 1	15	116 ± 15
	$n = 29$	$n = 29$	$n = 10$
fbf-1 puf-8	14 ± 2	15	123 ± 21
	$n = 27$	$n = 19$	$n = 10$
fbf-1 puf-8	20 ± 1.5	16	158 ± 25
	$n = 28$	$n = 20$	$n = 13$

PH3+, phosphohistone H3-positive cell.



B

Genotype	Gametes Produced: Sp+Oo	Sp Only	Oo Only	n
Wild-type	100	0	0	1279
fbf-1 puf-8	0	100	0	500
fbf-1 puf-8; fem-1(e1991)	0	0	100	72
fbf-1 puf-8; fem-2(e2105)	0	0	100	34
fbf-1 puf-8; fem-3(RNAi)	0	0	100	158
fbf-1 puf-8; fog-1(RNAi)	0	0	100	94
fbf-1 puf-8; fog-2(q71)	0	0	100	105
fbf-1 puf-8; fog-3(RNAi)	0	0	100	138

Sp = sperm, Oo = oocytes, n = number of gonad arms scored

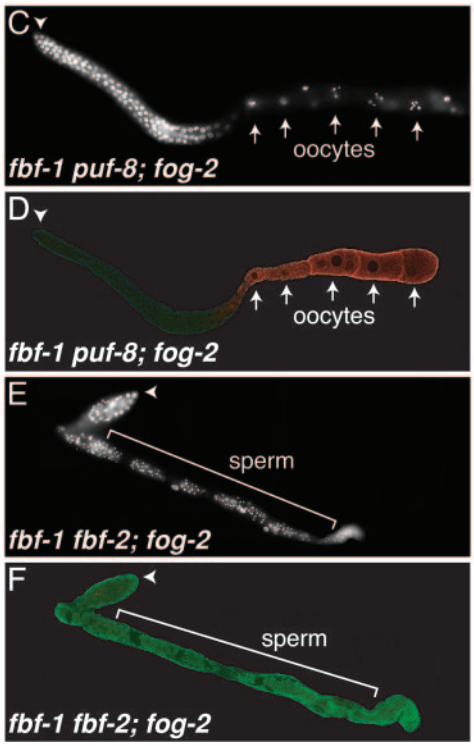


Fig. 4. *puf-8* and *fbf-1* function upstream of *fog-2* to promote the sperm/oocyte switch. (*A*) Simplified version of the hermaphrodite germ-line sex determination pathway. Sp, sperm; Oo, oocytes. Low and high refer to levels of above gene activities. (*B*) Epistasis analysis. For simplicity, genes are not listed according to their linkage group. (*C–F*) Dissected germ lines. Arrowhead, distal end. (*C* and *E*) DAPI-stained. (*D* and *F*) Costained with sperm-specific antibody SP56 (green) and oocyte-specific antibody α-RME-2 (red). *C* and *D* are the same germ line of genotype *fbf-1(ok91) puf-8(q725); fog-2(q71)*. *E* and *F* are the same germ line of genotype *fbf-1(ok91) fbf-2(q704); fog-2(q71)*.

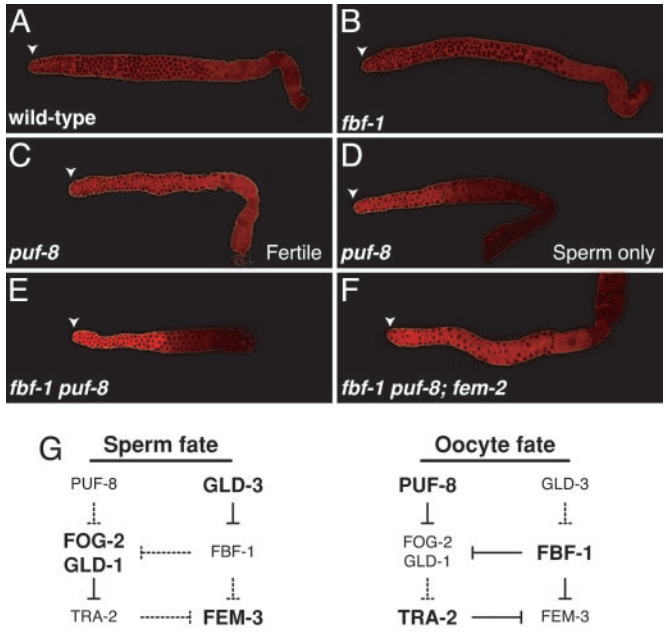


Fig. 5. FOG-2 regulation by *fbf-1* and *puf-8*. (*A–F*) Dissected germ lines stained with α-FOG-2 antibodies (red). Arrowhead, distal end. (*A–E*) Germ lines were treated identically, and confocal images were taken with the same settings at the same magnification for comparison. (*F*) Representative image. (*G*) Model for control of sperm/oocyte switch by PUF-8 and FBF-1. See text for explanation.

wild-type (26 of 26 arms; Fig. 5*A*) and *fbf-1* mutants (19 of 19 arms; Fig. 5*B*), but elevated in both fertile *puf-8* mutants (24 of 24 arms; Fig. 5*C*) and Mog *puf-8* mutants (2 of 2 arms; Fig. 5*D*). A dramatic effect was seen in *fbf-1 puf-8* double mutants: the level of FOG-2 increased compared to both wild-type and *puf-8* Mog mutants and was largely confined to the distal region of the germ line, including both the mitotic region and transition zone (58 of 58 arms; Fig. 5*E*). This FOG-2 increase was also seen in *fbf-1 puf-8; fem-2* females (13 of 13 arms; Fig. 5*F*), and is therefore independent of germ-line sex. We conclude that *fbf-1* and *puf-8* act upstream of *fog-2* and affect FOG-2 abundance. We suggest that the FOG-2 increase in *fbf-1 puf-8* double mutants may, at least in part, explain the failure of this mutant to switch to oogenesis. If true, the sperm/oocyte decision may take place in the distal germ line.

To ask whether *fbf-1 puf-8; fog-2* oocytes are functional, we assayed their ability to be fertilized and support embryogenesis. To this end, we mated either *fog-2* or *fbf-1 puf-8; fog-2* females with wild-type males. Most *fbf-1 puf-8; fog-2* females (22 of 29) could produce cross progeny and, thus, had functional oocytes, but they made many fewer embryos than *fog-2* females (*fbf-1 puf-8; fog-2* laid a total of 293 embryos from 12 crosses; *fog-2* laid a total of 2,008 embryos from 10 crosses), and many embryos did not survive (35% dead, $n = 293$ embryos). Therefore, *fbf-1 puf-8; fog-2* can produce functional oocytes, but some appear to be defective.

Discussion

This work presents two major findings. First, *puf-8* acts redundantly with *fbf-1* to promote the sperm/oocyte switch. Second, *puf-8* and *fbf-1* function upstream of *fog-2* to promote the sperm/oocyte switch. We discuss the implications of our findings for the control of germ-line sex determination.

PUF-8 and FBF-1 proteins are redundant for the sperm/oocyte switch: in most animals, either regulator is sufficient to promote the switch from spermatogenesis to oogenesis. We do not yet understand the molecular basis of this redundancy. Although PUF-8 and FBF-1 are both PUF proteins, they are distant relatives and appear to have distinct RNA-binding specificities (L. Opperman and M. Wickens, personal communica-

tion). One possible explanation of their redundancy is that PUF-8 and FBF-1 bind distinct regulatory elements within the same mRNA. Alternatively, PUF-8 and FBF-1 may be able to bind the same element under certain conditions that have not yet been defined (e.g., posttranslational modification or binding partners). A third explanation is that PUF-8 and FBF-1 may function in parallel to influence the same process. For example, PUF-8 and FBF-1 may regulate repressor-specific mRNAs that encode interdependent proteins, such as components of a complex. By this scenario, either FBF-1 or PUF-8 could effectively control activity of the complex by repressing one of its components. We favor this explanation because of the emerging regulatory circuitry (see below). However, the other ideas remain plausible, and should be tested once PUF-8 target mRNAs are known.

To identify potential PUF-8 targets, we asked where PUF-8 acts in the hierarchy of germ-line sex determination regulators. Epistasis analyses place *puf-8* and *fbf-1* upstream of *fog-2*, a gene that regulates the onset of hermaphrodite spermatogenesis (19). Consistent with that placement, FOG-2 protein increases dramatically in *fbf-1 puf-8* double mutants. A simple hypothesis is that PUF-8 and FBF-1 bind elements in the *fog-2* mRNA and repress its expression; however, we have been unable to detect such binding (J.L.B., unpublished data).

Fig. 5*G* presents our working model for control of the sperm/oocyte switch by PUF-8 and FBF-1. This model incorporates our current findings with previous work on regulators of this switch. In brief, the model suggests that, when PUF-8 and FBF-1 are both absent (or inactive), FOG-2, GLD-1, and FEM-3 can specify the sperm fate, but when they are present (or active), FOG-2, GLD-1, and FEM-3 are all repressed and oogenesis ensues. [We highlight FEM-3 in this model for simplicity, but note that FBF also controls FOG-1 and probably FOG-3 (13).]

This model explains the PUF-8/FBF-1 redundancy by control of distinct mRNAs that encode proteins that work together. Thus, *gld-1* mRNA is a known FBF target (6), and *fog-2* expression is affected by PUF-8 and by both PUF-8 and FBF-1 (this work). The GLD-1 and FOG-2 proteins physically interact (15), and both are required for *tra-2* repression and hermaphrodite spermatogenesis (15, 19, 20). This model may also explain why PUF-8 is redundant with FBF-1 for the sperm/oocyte decision, but not for germ-line proliferation. Thus, FOG-2 appears specific to sex determination, whereas GLD-1 controls many aspects of germ-line development, including germ-line proliferation, sex determination and progression through meiosis. Although our model is clearly an oversimplification, it provides heuristic value for thinking about the increasingly complex regulation of the sperm/oocyte switch.

The mitosis/meiosis and sperm/oocyte decisions are controlled by many of the same regulatory proteins (refs. 8, 13, 21, and 22 and this work). Several lines of evidence suggest that the choice between spermatogenesis and oogenesis is made in the distal germ line. FOG-2 is present in the germ-line mitotic region and transition zone (15), and high levels of FOG-2 are largely confined to that region in *fbf-1 puf-8* double mutants (this work). In addition, FOG-1 is also localized to the distal germ line (13). The *fog-1* gene is essential for sperm specification (23), and FOG-1 protein is abundant in the proximal region of the mitotic germ line (13). Therefore, the choice between spermatogenesis and oogenesis is likely to be made in the distal germ line and may be further localized to the proximal region of the mitotic region. The idea that the sperm/oocyte switch is made in the distal germ line has implications for the question of why PUF-8 is redundant with FBF-1 and not with FBF-2. One simple possibility is that this difference reflects the distinct distributions of the two FBF proteins. Although FBF-1 and FBF-2 overlap within the mitotic region, FBF-1 is abundant more proximally and FBF-2 is more distal (6, 7). If the sperm/oocyte decision were made in the proximal part of the mitotic region, perhaps FBF-1 is in the right place to control this decision, but FBF-2 is located too distally.

We conclude that *puf-8* functions redundantly with *fbf-1*, and that these two genes act upstream of *fog-2* to promote the switch from spermatogenesis to oogenesis. By contrast, *fbf-1* also works redundantly with *fbf-2*, but the *fbf-1 fbf-2* control acts upstream of *fem-3*, *fog-1*, and *fog-3*, not upstream of *fog-2* (2, 13). Therefore, distinct combinations of PUF proteins act at multiple points in the germ-line sex determination pathway to control the sperm/oocyte switch. This web of redundancy provides a remarkably well buffered mechanism to ensure that hermaphrodite spermatogenesis occurs at the right time and in the right amount.

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