A conserved RNA-binding protein controls germline stem cells in *Caenorhabditis elegans*

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Germline stem cells are defined by their unique ability to generate more of themselves as well as differentiated gametes[1]. The molecular mechanisms controlling the decision between self-renewal and differentiation are central unsolved problems in developmental biology with potentially broad medical implications. In *Caenorhabditis elegans*, germline stem cells are controlled by the somatic distal tip cell[2,3]. FBF-1 and FBF-2, two nearly identical proteins, which together are called FBF ('*fem-3* mRNA binding factor'), were originally discovered as regulators of germline sex determination[4]. Here we report that FBF also controls germline stem cells: in an *fbf-1 fbf-2* double mutant, germline proliferation is initially normal, but stem cells are not maintained. We suggest that FBF controls germline stem cells, at least in part, by repressing *gld-1*, which itself promotes commitment to the meiotic cell cycle[5,6]. FBF belongs to the PUF family ('Pumilio and FBF') of RNA-binding proteins[7]. Pumilio controls germline stem cells in *Drosophila* females[8,9], and, in lower eukaryotes, PUF proteins promote continued mitoses[10,11]. We suggest that regulation by PUF proteins may be an ancient and widespread mechanism for control of stem cells.

In the *C. elegans* germ line, mitotic cells reside distally and differentiating gametes are proximal (Fig. 1a). The mitotic region includes germline stem cells. By analogy with other tissues, the mitotic region may also contain cells with a more limited capacity to proliferate, sometimes called transit amplifying cells[1]. Germline mitotic divisions are controlled by the somatic distal tip cell (DTC), which uses Notch signalling to promote mitosis and prevent meiosis[2,3]. Therefore, the DTC provides a niche for maintaining germline stem cells. Once germ cells reach the transition zone, they enter meiosis and then progress through the pachytene stage of meiotic prophase and gametogenesis as they move proximally[3,12]. Downstream of Notch signalling, the *gld-1* and *gld-2* genes promote commitment to the meiotic cell cycle[5,6,13].

The *fbf-1* and *fbf-2* genes encode nearly identical (>91%) proteins that bind specifically to an RNA regulatory element in the *fem-3* 3′ untranslated region (3′UTR), and thereby promote the hermaphroditic switch from spermatogenesis to oogenesis[4]. To investigate FBF function in more depth, we generated an *fbf-1* single mutant and then induced an *fbf-2* mutation on the *fbf-1* chromosome to generate an *fbf-1 fbf-2* double mutant (Fig. 1b). The *fbf-1* deletion removes the RNA-binding region and alters the reading frame (Fig. 1b, top); *fbf-2(q704)* is a nonsense mutation (Fig. 1b, bottom). Both mutations are likely to abolish FBF activity. Consistent with this idea, similar results have been obtained using another single mutant, *fbf-1(ok224)* and another double mutant, *fbf-1(q662) fbf-2(q655)*. Furthermore, RNA-mediated interference (RNAi) of either *fbf-1* or *fbf-2* targets both *fbf* messenger RNAs and has the same effect as the double mutant (not shown).

The phenotypes of the *fbf-1* single mutant and the *fbf-1 fbf-2* double mutant suggest that FBF-1 and FBF-2 are largely redundant. Thus, *fbf-1* mutant hermaphrodites were fertile and virtually wild type, although they made more sperm than normal (Fig. 1c). By contrast, *fbf-1 fbf-2* double mutants were sterile with severe germline defects (Fig. 1c, e, g, h). The *fbf-1 fbf-2* double mutant had two prominent germline defects. First, no switch from spermatogenesis to oogenesis occurred, as predicted from RNAi experiments using single-stranded RNA[4]. Second, all cells in the mitotic region entered meiosis in late stage 4 larvae (L4). Early germline development appeared normal: newly hatched animals possessed two germline precursor cells in wild type[3] and in the double mutant ($n = 19$). Early germline divisions generated an average of 31 descendants by L3 in wild type ($n = 10$, range 26–38), and 32 descendants by the

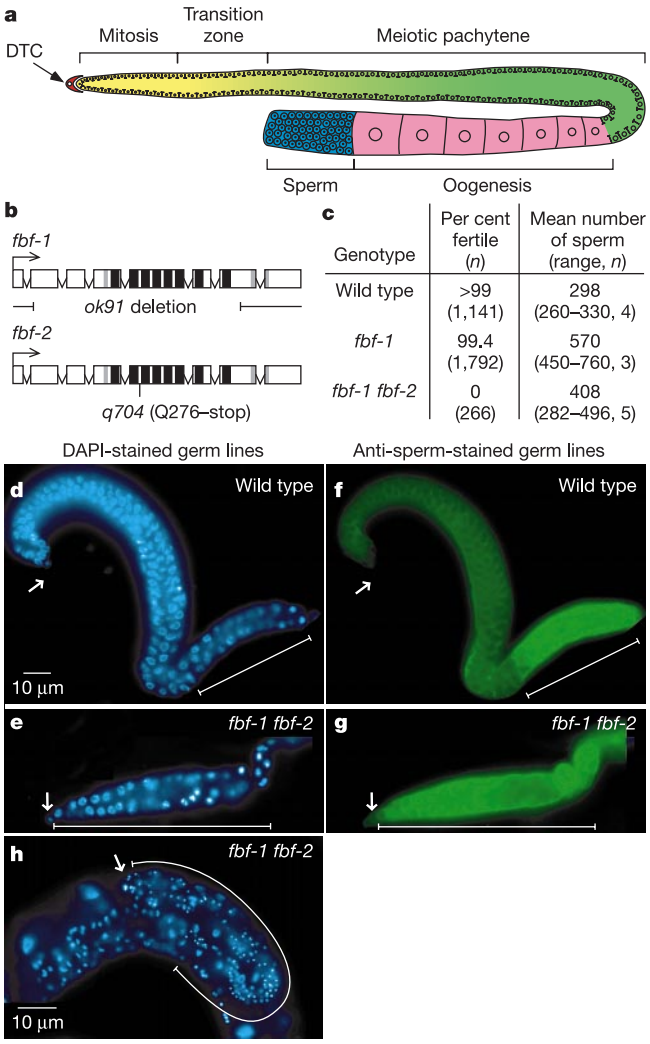

Figure 1 FBF is required for germline stem cells. **a**, Germline organization is the same in both sexes, except hermaphrodites make sperm and oocytes, whereas males make only sperm. **b**, Molecular lesions in *fbf* mutations. Black boxes, Puf repeats, which govern RNA binding; grey, Csp region[4]. **c**, Fertility in *fbf* mutants. *n*, number animals scored. **d**–**g**, L4 hermaphrodite germ lines stained with 4,6-diamidino-2-phenylindole (DAPI) and anti-sperm as described[23]. **d** and **f** are same germ line, as are **e** and **g**, and all are at the same magnification. **h**, Adult stained with DAPI as described[6]. Arrow, distal end; bar, spermatogenic region.

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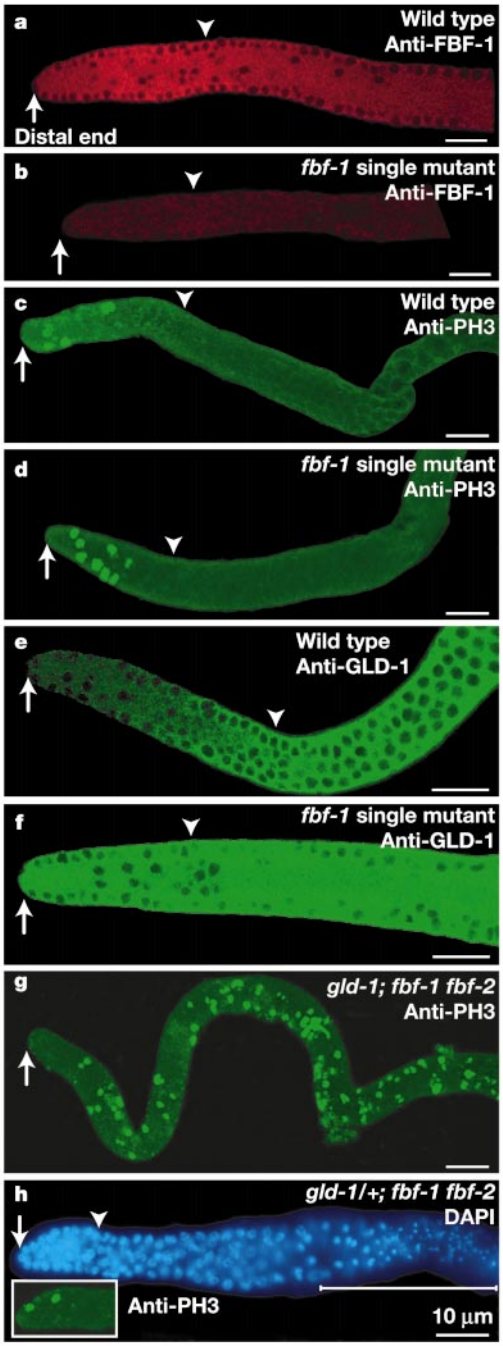


Figure 2 FBF-1 controls GLD-1 expression. Adult hermaphroditic gonads were stained[23] with anti-FBF-1 (**a**, **b**), anti-phosphohistone H3 (anti-PH3, Upstate Biotechnology), which stains dividing cells[24] (**c**, **d**, **g**, **h**), anti-GLD-1 (ref. 14) (**e**, **f**) or DAPI (**h**). Arrow, distal end; arrowhead, approximate end of mitotic region. **a**, FBF-1 in wild-type germ line. **b**, FBF-1 in *fbf-1* mutant; no staining seen. **c**, Mitotic region in wild type. **d**, Mitotic region in *fbf-1* mutant. Nuclei positive for anti-PH3 (green) can extend 16–20 germ-cell diameters from the distal end in wild-type ($n = 21$) and 12–14 cell diameters in the mutant ($n = 21$). **e**, Wild type. GLD-1 is low in distalmost cells ($n = 17$; also see ref. 14). **f**, *fbf-1* mutant. High GLD-1 extends to the distal end ($n = 16$ germ lines). **g**, The *gld-1(q485); fbf-1(ok91) fbf-2(q704)* germ line has many nuclei positive for anti-PH3 ($n = 16$ of 16). **h**, The *gld-1(q485)/ +* ; *fbf-1(ok91) fbf-2(q704)* germ line has a restored distal mitotic region with anti-PH3-positive nuclei (inset) and makes mature sperm. White bar, spermatogenic region. All scale bars, 10 μm.

same stage in the double mutant ($n = 10$, range 26–39). However, by L4, wild-type germ lines continued to proliferate (Fig. 1d), but all *fbf-1 fbf-2* germ cells had entered meiotic pachytene (Fig. 1e). Furthermore, whereas the wild-type germ line had a restricted region of spermatogenesis (Fig. 1f, green), all *fbf-1 fbf-2* germ cells entered spermatogenesis (Fig. 1g, green). No morphologically aberrant or dying germ cells were seen at any stage ($n > 10$, each stage). By adulthood, the *fbf-1 fbf-2* germ line consisted entirely of mature sperm (Fig. 1h). On average, each of the two gonadal arms made 50 germ cells (range 35–60; $n = 10$ arms), or a total of 100 germ cells per animal, making about 400 mature sperm (Fig. 1c). By contrast, wild-type adult hermaphrodites have about 2,000 total germ cells and about 300 mature sperm[2,3]. Therefore, the double mutant fails to switch into oogenesis and fails to maintain germline stem cells. A similar defect was observed in *fbf-1 fbf-2* mutant males (not shown). We conclude that FBF controls the maintenance of germline stem cells, but is not required for early larval germline proliferation.

Previous work showed FBF-1 in the L3 germline cytoplasm[4]. We now report that FBF-1 is present uniformly in germline cytoplasm during early larval stages, and becomes enriched in the mitotic region of L4 larval (not shown) and adult germ lines (Fig. 2a). There is no detectable staining in an *fbf-1* single mutant (Fig. 2b). Within the mitotic region of wild-type germ lines, the distalmost germ cells possess faint but easily detectable FBF-1 staining, which becomes intense in more proximal mitotic cells (Fig. 2a). As germ cells leave the mitotic region and enter early meiotic prophase, FBF-1 is reduced to a low level. This pattern is similar in males (not shown). Thus FBF-1 is found in mitotic germline cells in L4 larvae and adults, when FBF is required to maintain germline stem cells.

One mechanism by which FBF could maintain germline stem cells is to inhibit activity of a gene controlling commitment to meiosis. One such gene is *gld-1* (refs 5, 6). Using an affinity-purified antibody to GLD-1 (ref. 14), we first examined GLD-1 in an *fbf-1* single mutant. We chose the single mutant because the *fbf-1 fbf-2* double mutant dramatically changes germline cell fates, making the analysis of any changes in GLD-1 expression difficult to interpret. By contrast, *fbf-1* single mutants are nearly wild type with respect to germline fates and pattern, including the mitotic region (Fig. 2c, d). In wild-type germ lines, GLD-1 was either expressed at a low level or was undetectable in the distalmost germ cells (Fig. 2e)[14]. However, in *fbf-1* single-mutant germ lines, GLD-1 was detectable in all distal germ cells (16 of 18 germ lines), and abundant in most of them (12 of 18 germ lines) (Fig. 2f). This distal expansion of GLD-1 in the *fbf-1* mutant is consistent with the idea that FBF negatively regulates GLD-1 expression. The fact that the distal expansion of GLD-1 is not sufficient to drive germline stem cells into meiosis may reflect the fact that *fbf-2* remains or that other regulators are required in addition to GLD-1 (see below).

To investigate whether FBF acts genetically upstream of *gld-1*, we examined a *gld-1; fbf-1 fbf-2* triple mutant. In wild-type germ lines, mitotic cells are restricted to the distal region (Fig. 1a, 2c)[2], whereas in *gld-1* null mutants, proximal germ cells also divide mitotically[5]. The *gld-1; fbf-1 fbf-2* triple mutants displayed mitotically dividing cells throughout the germ line (Fig. 2g); in particular, distal germ cells were mitotic at all stages examined (early L4, late L4 and adult). Therefore, in the absence of *gld-1*, FBF is no longer necessary to promote germline proliferation. This epistasis is consistent with FBF acting as a negative regulator of *gld-1*. We also examined *fbf-1 fbf-2* double mutants that were heterozygous at the *gld-1* locus (*gld-1/ +*), and were surprised to find that the germ line regained a distal mitotic region (Fig. 2h). Indeed, these germ lines were considerably larger than in the *fbf-1 fbf-2* double mutant (270 germ cells per arm compared with 50 germ cells per arm) and made about 900 mature sperm per animal ($n = 4$), more than twice as many sperm as an *fbf-1 fbf-2* double mutant. Therefore, the distal

mitotic cells were both proliferating and generating gametes, two defining features of stem cells. Thus reduction of *gld-1* by one copy seems to restore germline stem cells to the *fbf-1 fbf-2* double mutant, which strongly supports the idea that *gld-1* repression is critical for the maintenance of germline stem cells.

We hypothesized that FBF-1 and FBF-2 repress *gld-1* expression by binding 3′UTR elements, and therefore examined the *gld-1* 3′UTR sequence for possible FBF-binding sites. FBF binding requires a UGUR tetranucleotide and adjacent sequences (D.B. and M.W., manuscript in preparation). Two potential FBF-binding elements (FBEs) were identified: FBE-a and FBE-b (Fig. 3a). To test FBF binding, we first used the yeast three-hybrid system[15] (Fig. 3b). FBF-1 and FBF-2 bound both sites, activating *lacZ* (Fig. 3c) and *HIS3* reporter genes (Fig. 3d, and not shown). Indeed, FBF-1 binding to FBE-a is one of the strongest interactions yet detected in the system (Fig. 3d)[16]. FBF–FBE interactions were specific by three criteria. Mutations in the UGU of FBE-a* and FBE-b* (Fig. 3a) disrupted binding (Fig. 3c–e); FBF did not bind other RNAs, including several that bind other PUF proteins (Fig. 3c, d, and not shown); and a distinct *C. elegans* protein, PUF-5, failed to bind either site (Fig. 3d). The FBF–FBE interactions were direct, as judged by gel retardation assays (Fig. 3e). Purified, recombinant FBF-1 labelled with glutathione *S*-transferase (GST) bound ^{32}P-radiolabelled FBE-a and FBE-b RNAs carrying wild-type, but not mutant, FBEs (Fig. 3e). We conclude that FBF binds specifically to two sites in the *gld-1* 3′UTR.

We have provided cytological, genetic and molecular evidence that FBF represses *gld-1* mRNA activity. A fourth line of evidence relies on *gld-1(oz10)*, which deletes 500 nucleotides from the *gld-1* 3′UTR, including FBE-a and FBE-b (Fig. 3a)[17]. Interpretation is complicated, because the mutant also carries a missense mutation[17]. Nonetheless, GLD-1 expression expands to the distal end in *gld-1(oz10)* mutants[14], supporting the hypothesis that the FBE sites mediate *gld-1* repression. The *gld-1* mRNA encodes a translational repressor of the STAR/Quaking/GSG family[17,18]. It is possible that PUF proteins repress such mRNAs more generally.

FBF repression of *gld-1* maintains mitotically dividing germline cells, including stem cells. In *fbf-1 fbf-2* double mutants, all germline cells enter meiosis, whereas in *gld-1; fbf-1 fbf-2* triple mutants, mitoses are found throughout the germ line. Remarkably, removal of even one wild-type copy of the *gld-1* gene seems to restore stem cells to the *fbf-1 fbf-2* double mutant. Therefore, low GLD-1 activity is essential for germline stem cells. Yet we have not been able to show the converse: high GLD-1 may not be sufficient to drive germ cells into meiosis. One explanation is that the increased GLD-1 observed in *fbf-1* and *gld-1(oz10)* mutants does not represent fully unregulated wild-type protein. In *fbf-1* mutants, FBF-2 remains, and in

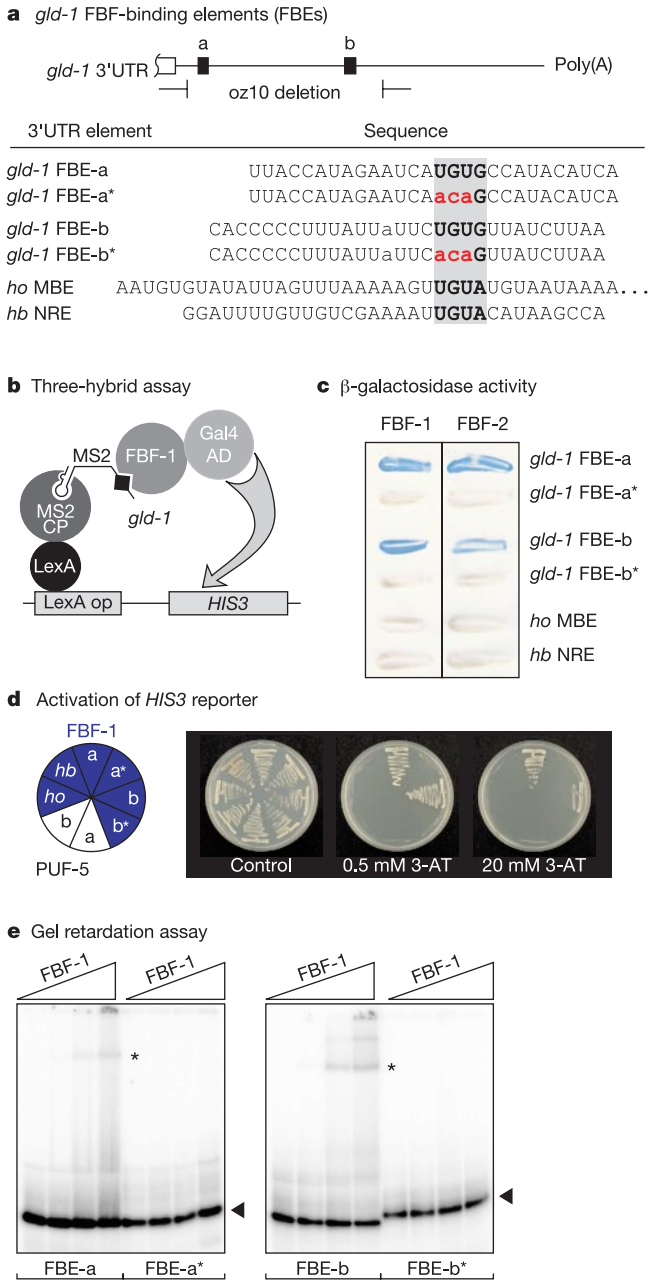
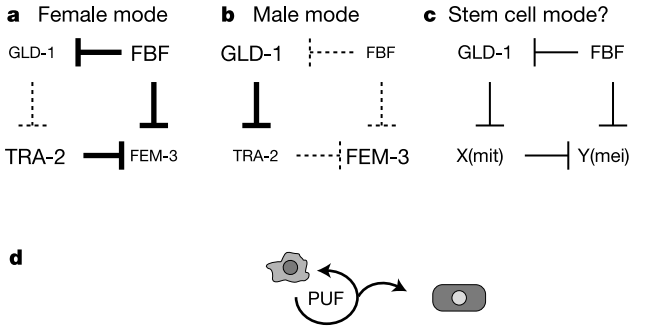


Figure 3 FBF binds specifically to the *gld-1* 3′UTR. **a**, Predicted FBF-binding elements (FBEs) in *gld-1* 3′UTR (FBE-a and FBE-b, 896 and 498 nt upstream of polyadenylation site, respectively). The UGUR motif is common among PUF-binding sites (grey box). Red letters are point mutations. Controls, *hunchback* (*hb*) Nanos response element (NRE)[25] and *ho* MPT-5/PUF-5 element (MBE)[26]. **b**, Three-hybrid assay[15,16]. **c**, β-galactosidase activity. **d**, *HIS3* activation. Left, letters indicate hybrid RNAs ('a' is *gld-1* FBE-a, and so on). Cells expressing FBF-2 (blue sectors) or *C. elegans* PUF-5 (white sectors). Right, growth was monitored on media lacking histidine and containing 3-AT, a competitive inhibitor of *HIS3*. **e**, Gel retardation assays. ^{32}P-RNAs were incubated with 0, 50, 250 or 500 pmol GST–FBF-1. Asterisk, specific complex; arrowhead, free RNA.



d

Organism	PUF	Stem cell/mitosis	Differentiaton	Ref.
C. elegans	FBF	Germline stem cell	Meiosis	This work
Drosophila	Pumilio	Germline stem cell	Cystoblast	8, 9
Dictyostelium	PUFA	Vegetative mitoses	Morphogenesis	10
S. cerevisiae	Puf5p	More mitoses	Fewer mitoses	11

Figure 4 PUF protein regulation of cell fates. **a**, **b**, Regulation of sex determination. Large letters refer to active regulators; smaller letters refer to repressed regulators. Thick bars represent active repression; dashed bars represent lack of repression. **c**, Regulation of mitosis and meiosis. Letters and bars are given equal strength since this circuit is speculative. See text for further explanation. **d**, Role of PUF proteins in the nematode worm *C. elegans*, the fruitfly *Drosophila melanogaster*, the ameboid protozoan *Dictyostelium discoideum*, and the budding yeast *Saccharomyces cerevisiae*.

gld-1(oz10), GLD-1 protein carries a missense defect[17]. In addition, other regulators (such as GLD-2; ref. 6) may be required to drive germ cells into meiosis.

FBF provides a direct link between two major germline decisions: maintenance of stem cells (this work) and germline sex determination[4]. Intriguingly, GLD-1 provides a second and opposite link between these same decisions[5,6,13,18]. Thus, FBF promotes mitosis and oogenesis, whereas GLD-1 promotes meiosis and spermatogenesis. We suggest the existence of a multiply reinforced translational switch that controls germline fate decisions. The circuitry is best understood for sex determination, where a series of negative regulators have been identified[3] (Fig. 4a, b). A similar circuit may also control the mitosis/meiosis decision (Fig. 4c). Although some GLD-1 target mRNAs have been identified[19,20], their role in stem cell control remains unknown. Identification of FBF and GLD-1 target mRNAs (X(mit) and Y(mei) in Fig. 4c) is needed to elucidate how the circuit controls the decision between mitosis and meiosis.

Our results, together with those of others, suggest a conserved role for PUF proteins (Fig. 4d). FBF control of germline stem cells in *C. elegans* is reminiscent of the Pumilio control of germline stem cells in *Drosophila* ovaries[8,9]. Remarkably, PUF proteins in yeast and the protozoan *Dictyostelium* also promote mitosis at the expense of an alternate state (Fig. 4d). In *Dictyostelium*, PufA mutants cease vegetative mitotic divisions, aggregate and undergo morphogenesis prematurely[10]. In yeast, PUF5/MPT5/UTH4 mutants divide fewer times and age early, whereas cells overexpressing this protein divide more times and age late[11]. The common theme is that PUF proteins promote cell division at the expense of differentiation. This conservation suggests that additional components of the circuitry may also be conserved, an idea that awaits identification of PUF targets critical for stem cell maintenance in multiple organisms. □

Methods

Mutants

The *fbf-1(ok91)* deletion mutant was isolated in a screen based on polymerase chain reaction (PCR) as described[21]. Primers were: 201A, GTCAACGAGAGGAAATCTTCG; 202S, CCAGTGGCCATAATCGTGTG; 203A, GCGTAATGAATTATTTTAGTGTTG; 204S, CCTGAATAATGATTGTGATTCTC. To obtain the *fbf-1 fbf-2* double mutant, *fbf-1(ok91)* males were mutagenized with ethyl methane sulphonate and crossed into *mIn1[mIs14 dpy-10(e128)]* homozygotes, which carry a green fluorescent protein (GFP)-marked balancer for chromosome II obtained from M. Edgley. Non-green progeny from *fbf-1*/mIn1* heterozygotes were screened for sterility. Potential doubles were tested by complementation to a previously isolated double mutant and then sequenced to determine the *fbf-2* lesion. Each mutant was outcrossed against wild type at least six times. *fbf-1(ok91)* was maintained as a homozygous stock. *fbf-1(ok91) fbf-2(q704)* homozygotes were offspring of *fbf-1 fbf-2/mIn1[mIs14 dpy-10(e128)]* hermaphrodites. *gld-1; fbf-1 fbf-2* and *gld-1/+; fbf-1 fbf-2* mutants were progeny from *gld-1(q485)/ccIs4251 unc-15; fbf-1(ok91) fbf-2(q704)/mIn1[mIs14 dpy-10(e128)]* hermaphrodites.

Staining

Anti-FBF-1 antibodies[4] are specific for FBF-1: they do not recognize FBF-2 on western blots of protein translated *in vitro* (not shown) or in stained gonads from *fbf-1* deletion mutants (Fig. 2b). Affinity-purified anti-GLD-1 (ref. 14) was a gift from T. Schedl. The sperm-specific monoclonal antibody, SP56 (ref. 22), was a gift from S. Ward. Images were obtained on a Bio-Rad MRC1024 confocal microscope or a Hamamatsu Orca camera with Openlabs software and processed using Adobe Photoshop. Images of FBF-1 in wild-type and *fbf-1* mutant backgrounds were processed identically, as were those of GLD-1 in the same backgrounds. To visualize dividing nuclei, we projected a z-series so that all nuclei positive for anti-phosphohistone H3 antibodies (anti-PH3) from a single germ line were visible.

Three-hybrid and gel retardation assays

Three-hybrid assays were performed as described[16]. The entire RNA sequences indicated in Fig. 3a were cloned into the *Xma*I and *Sph*I sites of the vector pIIIA/MS2-2, using annealed synthetic oligonucleotides. For the three-hybrid assay, a mutation (U to A, indicated by lower case in Fig. 3a) was introduced into *gld-1*–FBE-b to disrupt an oligo(U) tract that can cause RNA polymerase III termination in the three-hybrid assay. The RNA-binding domains of FBF-1 (amino acids 121–614), FBF-2 (amino acids 2–632) and PUF-5 (amino acids 1–553, Genbank accession number NM_063413) were cloned into pACT2. Plasmids encoding the hybrid RNA and chimaeric protein were co-transformed into strain YBZ-1 (ref. 16). Levels of 3-aminotriazole (3-AT) resistance were determined by assaying multiple transformants at ten different concentrations of 3-AT, up to 100 mM. β-galactosidase activities were determined using colony filter assays[16]. *In vitro* binding reactions (Fig. 3e) contained 50 pmol of ^{32}P-RNA and 0–500 pmol of protein, in 100 mM NaCl, 5 mM dithiothreitol, 5 mM $MgCl_2$, 2 mM HEPES buffer at pH 7.4, and 200 ng yeast RNA. RNA and protein were incubated at room temperature for 15 min, heparin was added to 10 mg ml^{-1}, and the mixture was run at 4 °C on a 6% native polyacrylamide gel that contained 0.5 × TBE with 5 mM $MgCl_2$.

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1. Watt, F. M. & Hogan, B. L. Out of Eden: stem cells and their niches. *Science* **287,** 1427–1430 (2000).
2. Kimble, J. E. & White, J. G. On the control of germ cell development in *Caenorhabditis elegans*. *Dev. Biol.* **81,** 208–219 (1981).
3. Schedl, T. in *C. elegans II* (ed. Priess, J. R.) 241–269 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1997).
4. Zhang, B. *et al.* A conserved RNA-binding protein that regulates sexual fates in the *C. elegans* hermaphrodite germ line. *Nature* **390,** 477–484 (1997).
5. Francis, R., Barton, M. K., Kimble, J. & Schedl, T. *gld-1*, a tumor suppressor gene required for oocyte development in *Caenorhabditis elegans*. *Genetics* **139,** 579–606 (1995).
6. Kadyk, L. C. & Kimble, J. Genetic regulation of entry into meiosis in *Caenorhabditis elegans*. *Development* **125,** 1803–1813 (1998).
7. Wickens, M., Bernstein, D. S., Kimble, J. & Parker, R. A PUF family portrait: 3′UTR regulation as a way of life. *Trends Genet.* **18,** 150–157 (2002).
8. Lin, H. & Spradling, A. C. A novel group of *pumilio* mutations affects the asymmetric division of germline stem cells in the *Drosophila* ovary. *Development* **124,** 2463–2476 (1997).
9. Forbes, A. & Lehmann, R. Nanos and Pumilio have critical roles in the development and function of *Drosophila* germline stem cells. *Development* **125,** 679–690 (1998).
10. Souza, G. M., da Silva, A. M. & Kuspa, A. Starvation promotes *Dictyostelium* development by relieving PufA inhibition of PKA translation through the YakA kinase pathway. *Development* **126,** 3263–3274 (1999).
11. Kennedy, B. K. *et al.* Redistribution of silencing proteins from telomeres to the nucleolus is associated with extension of life span in *S. cerevisiae*. *Cell* **89,** 381–391 (1997).
12. Dernburg, A. F. *et al.* Meiotic recombination in *C. elegans* initiates by a conserved mechanism and is dispensable for homologous chromosome synapsis. *Cell* **94,** 387–398 (1998).
13. Francis, R., Maine, E. & Schedl, T. Analysis of the multiple roles of *gld-1* in germline development: interactions with the sex determination cascade and the *glp-1* signalling pathway. *Genetics* **139,** 607–630 (1995).
14. Jones, A. R., Francis, R. & Schedl, T. GLD-1, a cytoplasmic protein essential for oocyte differentiation, shows stage- and sex-specific expression during *Caenorhabditis elegans* germline development. *Dev. Biol.* **180,** 165–183 (1996).
15. SenGupta, D. J. *et al.* A three-hybrid system to detect RNA-protein interactions *in vivo*. *Proc. Natl Acad. Sci. USA* **93,** 8496–8501 (1996).
16. Bernstein, D. S., Buter, N., Stumpf, C. & Wickens, M. Analyzing mRNA–protein complexes using a yeast three-hybrid system: methods and applications. *Methods* **26(3),** 123–141 (2002).
17. Jones, A. R. & Schedl, T. Mutations in *gld-1*, a female germ cell-specific tumor suppressor gene in *Caenorhabditis elegans*, affect a conserved domain also found in Src-associated protein Sam68. *Genes Dev.* **9,** 1491–1504 (1995).
18. Jan, E., Motzny, C. K., Graves, L. E. & Goodwin, E. B. The STAR protein, GLD-1, is a translational regulator of sexual identity in *Caenorhabditis elegans*. *EMBO J.* **18,** 258–269 (1999).
19. Lee, M. H. & Schedl, T. Identification of in vivo mRNA targets of GLD-1, a maxi-KH motif containing protein required for *C. elegans* germ cell development. *Genes Dev.* **15,** 2408–2420 (2001).
20. Xu, L., Paulsen, J., Yoo, Y., Goodwin, E. B. & Strome, S. *Caenorhabditis elegans* MES-3 is a target of GLD-1 and functions epigenetically in germline development. *Genetics* **159,** 1007–1017 (2001).
21. Kraemer, B. *et al.* NANOS-3 and FBF proteins physically interact to control the sperm–oocyte switch in *Caenorhabditis elegans*. *Curr. Biol.* **9,** 1009–1018 (1999).
22. Ward, S., Roberts, T. M., Strome, S., Pavalko, F. M. & Hogan, E. Monoclonal antibodies that recognize a polypeptide antigenic determinant shared by multiple *Caenorhabditis elegans* sperm-specific proteins. *J. Cell Biol.* **102,** 1778–1786 (1986).
23. Crittenden, S. L., Troemel, E. R., Evans, T. C. & Kimble, J. GLP-1 is localized to the mitotic region of the *C. elegans* germ line. *Development* **120,** 2901–2911 (1994).
24. Hendzel, M. J. *et al.* Mitosis-specific phosphorylation of histone H3 initiates primarily within pericentromeric heterochromatin during G2 and spreads in an ordered fashion coincident with mitotic chromosome condensation. *Chromosoma* **106,** 348–360 (1997).
25. Wharton, R. P. & Struhl, G. RNA regulatory elements mediate control of *Drosophila* body pattern by the posterior morphogen *nanos*. *Cell* **67,** 955–967 (1991).
26. Tadauchi, T., Matsumoto, K., Herskowitz, I. & Irie, K. Post-transcriptional regulation through the HO 3′-UTR by Mpt5, a yeast homolog of Pumilio and FBF. *EMBO J.* **20,** 552–561 (2001).

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Competing interests statement

The authors declare that they have no competing financial interests.

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