2006

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Xiang Yu
Syracuse University

Valarie E. Vought
Syracuse University

Barbara Conradt
Dartmouth Medical School

Eleanor M. Maine
Syracuse University

Published 2006 Wiley-Liss, Inc.

genesis 44:412–418 (2006)

LETTER

Eukaryotic Translation Initiation Factor 5B Activity Regulates Larval Growth Rate and Germline Development in *Caenorhabditis elegans*

Xiang Yu,[1] Valarie E. Vought,[1] Barbara Conradt,[2] and Eleanor M. Maine[1]*

[1]Department of Biology, Syracuse University, Syracuse, New York
[2]Department of Genetics, Dartmouth Medical School, Hanover, New Hampshire

Received 17 April 2006; Revised 29 June 2006; Accepted 2 July 2006

Summary: In *C. elegans*, a population of proliferating germ cells is maintained via GLP-1/Notch signaling; in the absence of GLP-1 signaling, germ cells prematurely enter meiosis and differentiate. We previously identified *ego* (enhancer of *glp-1*) genes that promote germline proliferation and interact genetically with the GLP-1 signaling pathway. Here, we report that *iffb-1* (initiation factor five B) is an *ego* gene. *iffb-1* encodes the sole *C. elegans* isoform of eukaryotic translation initiation factor 5B, a protein essential for translation. We have used RNA interference and a deletion mutation to determine the developmental consequences of reduced *iffb-1* activity. Our data indicate that maternal *iffb-1* gene expression is sufficient for embryogenesis, and zygotic *iffb-1* expression is required for development beyond late L1/early L2 stage. Partial reduction in *iffb-1* expression delays larval development and can severely disrupt proliferation and differentiation of germ cells. We hypothesize that germline development is particularly sensitive to *iffb-1* expression level. genesis 44:412–418, 2006. **Published 2006 Wiley-Liss, Inc.**†**

Key words: eIF5B; *C. elegans*; germ line; GLP-1/Notch; translation initiation factor

Robust germline proliferation in *Caenorhabditis elegans* depends on inductive signals from the somatic gonad (Killian and Hubbard, 2004; Pepper *et al.*, 2003; Seydoux and Schedl, 2001) and on the expression of proteins that are critical for basic cellular processes, such as nuclear lamin, Aurora A kinase, and ribosomal proteins (e.g., Furata *et al.*, 2002; Liu *et al.*, 2000; Maciejowski *et al.*, 2005). In wildtype development, the first stage (L1) larva hatches with two germ cell precursors, which begin to divide during L1 stage; a population of proliferating germ cells is maintained throughout larval development and in adults (Seydoux and Schedl, 2001). Meiotic germ cells are not present until L3 and do not begin to differentiate as sperm until L4 stage. Without inductive signaling, mediated by the GLP-1/Notch pathway, all germ cell precursors prematurely enter meiosis during L2 and differentiate (Austin and Kimble, 1987).

One approach to studying the control of germline proliferation has been to identify genetic enhancers of a weak *glp-1* (loss-of-function) (*lf*) mutation. This approach has identified genes whose products function in the GLP-1 signaling pathway (e.g., LAG-1), regulate trafficking of a GLP-1 signaling pathway component (e.g., SEC-23, epsin), or act in parallel with GLP-1 to promote germline proliferation (e.g., ATX-2, EGO-1) (Maine *et al.*, 2004; Qiao *et al.*, 1995; Roberts *et al.*, 2003; Tian *et al.*, 2004; Vought *et al.*, 2005). Thus, analysis of *glp-1* enhancers has not only provided information about the mechanism and regulation of GLP-1/Notch signaling, but also identified other factors that promote germline proliferation.

In the course of other work, we observed that RNA interference (RNAi) of the annotated gene, Y54F10BM.2, enhanced a weak *glp-1(lf)* phenotype. Y54F10BM.2 was predicted to encode a protein closely related to eukaryotic translation initiation factor 5B (eIF5B) (http://www.wormbase.org). We determined the full-length Y54F10BM.2 cDNA sequence (see Methods) and confirmed that its predicted translation product has the same structure as eIF5B proteins characterized in other systems. The 1074 amino acid protein has a positively charged region of low sequence complexity at the N-terminus, followed by the conserved eIF5B function region (domains I-IV) (see Fig. 1). We named the gene *iffb-1* (initiation factor five B). Blast searches revealed no other known or predicted

*Correspondence to: E. M. Maine, Department of Biology, Syracuse University, 108 College Place, Syracuse, NY 13244. E-mail: emmaine@syr.edu

Contract grant sponsors: National Science Foundation; Syracuse University; Max Planck Society; Howard Hughes Medical Institute Award, Biomedical Research Support Program for Medical Schools; National Institutes of Health.

Published online in Wiley InterScience (www.interscience.wiley.com).

DOI: 10.1002/dvg.20232

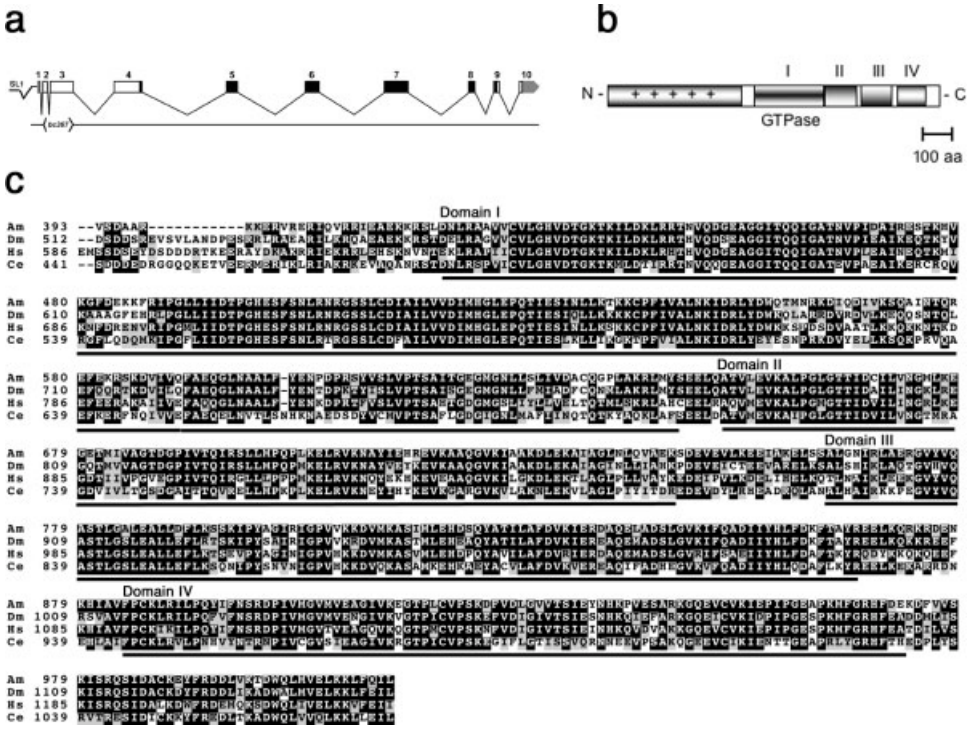

FIG. 1. *iffb-1* is predicted to encode the *C. elegans* ortholog of eukaryotic translation initiation factor 5B (eIF5B). (**a**) Diagrammatic representation of *iffb-1* gene structure. Exons are numbered. 5′ and 3′ untranslated regions are shaded gray; the region encoding domains I–IV is shaded black. The *bc367* deletion removes 720 nucleotides, shifts the predicted open reading frame at codon 32, and inserts a stop at codon 126. (**b**) Diagrammatic representation of the *C. elegans* IFFB-1/eIF5B protein. The N-terminal domain is positively charged and of low sequence complexity. The four conserved eIF5B structural domains (I–IV) are indicated (see Roll-Mecak *et al.*, 2000). (**c**) Alignment of the functional region (domains I–IV) of eIF5B from *C. elegans* and several other species. Each domain is underlined. Am, *Apis melifora*; Dm, *Drosophila melanogaster*; Hs, *Homo sapiens*; Ce, *Caenorhabditis elegans*.

Table 1
The Loss of eIF5B Activity Has Severe Developmental Consequences

Maternal genotype	*N*	% Larval arrest	% *sC1/sC1* Dpy[a]	% wt	*n*
Larval arrest phenotype is balanced by sC1 and strictly recessive					
iffb-1(bc367)/sC1	438	22.4	24.2	53.4	2
iffb-1(bc367)/+	1060	24.6	NA	75.4	6

Treatment	*N*	% Larval arrest	% Slow growth[b]	% Fertile adults	% Sterile adults	*n*
iffb-1 RNAi causes growth defects						
iffb-1 RNAi[c]	182	37	63	20	43	4

Animals were raised at 25°C. *N*, number of germ lines scored. *n*, number of broods scored.
[a]*sC1/sC1* animals were distinguished by their Dumpy (Dpy) body morphology.
[b]Wildtype controls produced adult progeny by 72 h. In contrast, adult *iffb-1(RNAi)* animals took 96–120 h to reach adulthood.
[c]Individual late L4 or young adult hermaphrodites were placed onto feeding plates at 25°C and transferred daily; their progeny were raised continuously in the presence of *iffb-1* dsRNA. See Materials and Methods.

eIF5B isoform in *C. elegans*, and we conclude that *iffb-1* encodes the sole *C. elegans* eIF5B protein.

eIF5B (called IF2 in prokaryotes) is a universally conserved translation initiation factor. It is required for subunit joining and may also function in selection/binding of the initiator methionine-tRNA (Guillon *et al.*, 2005; Marintchev and Wagner, 2004). To characterize the *iffb-1* strong *lf* phenotype, we isolated and sequenced a deletion in the gene (see Materials and Methods). The deletion allele, *iffb-1(bc367)*, lacks most of exons 2–3 and predicted to encode a 125 amino acid polypeptide that is likely to be null for function (Fig. 1a). We also used *iffb-1* RNAi to deplete the gene product, and compared the developmental defects associated with *iffb-1(bc367)* and *iffb-1(RNAi)*.

iffb-1(bc367) has a recessive larval arrest phenotype (Table 1; Fig. 2b; see Methods). The same phenotype is seen in a high percentage of *iffb-1(RNAi)* animals (see later). To determine the stage at which the *iffb-1(bc367)* larval arrest occurs, we counted seam cell and gonadal precursors. Wildtype L1 larvae have 10 seam cell precursors on each side of the body, four of which divide dur-

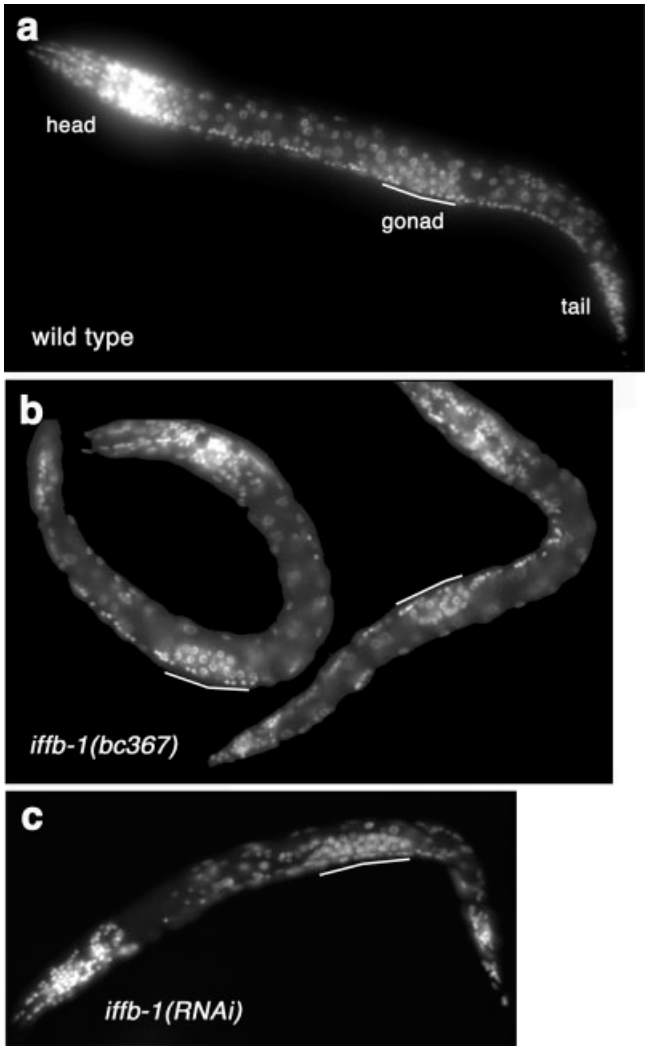

FIG. 2. Larval arrest is associated with the loss of *iffb-1* function. Each panel shows larvae stained with DAPI. The gonad primordia (somatic and germline precursors) are indicated with a bar. (**a**) A wildtype (N2) larva at early L2 stage. Head, tail, and gonad are labeled. (**b**) Two *iffb-1(bc367)* arrested larvae. (**c**) An arrested *iffb-1(RNAi)* larva. We note that only a subset of cells is visible in the focal plane of each image. See also the *iffb-1(bc367)* gonad shown in Fig. 4f.

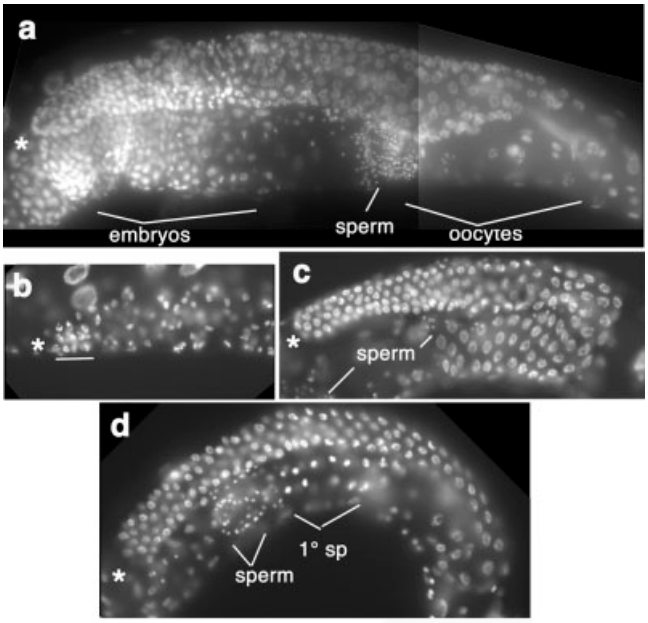

FIG. 3. Germline defects are associated with the partial loss of *iffb-1* function during larval development. Asterisk indicates the distal end of the germ line, where proliferating germ cells normally reside. (**a**) Wildtype (N2) hermaphrodite germ line. Sperm, oocytes, and embryos are indicated. (**b**) Severely defective germ line in an *iffb-1(RNAi)* adult escaper. Bar indicates the small cluster of undifferentiated germ cells. (**c**,**d**) Germ lines of *iffb-1(RNAi)* adult escapers containing sperm (c,d) and primary spermatocytes (1° sp) (d), but no oocytes.

ing L2 stage (Sulston and Horvitz, 1977). Using a transgenic marker, *scm::gfp*, to visualize seam cells (see Methods), we observed 10 seam cells/side in *iffb-1(bc367)*; *scm::gfp* larvae ($n = 15$ animals). Wildtype early L2 larvae have a total of ~24 gonad precursor cells: 12 somatic precursors and ~12 germ cell precursors (Kimble and Hirsh, 1979). (Germ cell divisions are somewhat variable.) Arrested *iffb-1(bc367)* mutants had a total of 20 (±6) cells in the developing gonad (including both germline and somatic precursors) (Fig. 2b) ($n = 14$). By these two measures, therefore, *iffb-1(bc367)* larvae appear to arrest at late L1/early L2 stage. *iffb-1(bc367)* germ cells did not differentiate (Fig. 2b). This is not surprising, since the developmental arrest occurs well before the normal onset of meiosis and differentiation (see earlier). When *iffb-1(bc367)* mutants were allowed to age in parallel with their heterozygous siblings, their germ cells became irregular in size and morphology and did not divide further (data not shown). Although we did not quantify these characteristics, we noted that mutant larvae survived for many days and became slightly longer (the latter presumably due to an increase in cell size as is seen also in wildtype adults).

We used *iffb-1* RNAi to investigate the effect of a partial loss of IFFB-1/eIF5B activity and to confirm the specificity of the *bc367* larval arrest phenotype. We put wildtype adult hermaphrodites onto *iffb-1* dsRNA feeding plates and characterized the development of their progeny, which were raised continuously in the presence of *iffb-1* dsRNA (see Methods). Importantly, 37% of the (hatched) progeny arrested as young larvae (Table 1), with characteristics similar to *iffb-1(bc367)* mutant arrested larvae (Fig. 2c). Given the specificity of RNAi (see Fire *et al.*, 1998), this result indicates that the larval arrest associated with *iffb-1(bc367)* is caused by the loss of eIF5B/IFFB-1 activity and not by a linked mutation in another gene. Sixty-three percent of the *iffb-1(RNAi)* animals developed successfully to adulthood, although they took 24–72 h longer than wildtype to reach adulthood, and most of them were sterile (Table 1; Fig. 3). *iffb-1* RNAi also produced dead embryos, especially in the latter part of the brood (data not shown). The simplest ex-

Table 2
A Reduction in eIF5B Activity Enhances the glp-1(ts) *Phenotype in the Germ Line*

Genotype	% Iffb-1[a] sterile (range)	% Glp-1[b] sterile (range)	% Glp-1[c] fertile (range)	% Fertile non-Glp-1 (range)	*N*
Continuous RNAi during development; assayed as adults					
iffb-1(RNAi)	35.5 (10–61)	0	0	53 (39–90)	576
unc-32 glp-1(ts)	0	1	7	92	149
iffb-1(RNAi) unc-32 glp-1(ts)	47 (33–61)	25 (22–29)	18 (6–30)	11 (7–11)	180
Genotype	% Glp-1[d]	% with MZ	% Other	*N*	
RNAi initiated at L4 stage; assayed after 72 h					
unc-32 glp-1(ts)	6	94	0	70	
unc-32 glp-1(bn18ts); gfp(RNAi)	5	95	0	42	
iffb-1(RNAi)[e]	0	100	0	>60	
iffb-1(RNAi) unc-32 glp-1(ts)	58.5	41.5	3.5	118	

Animals were raised at 20°C. The full genotype of the *glp-1(ts)* strain was *unc-32(e189) glp-1(bn18ts)*. *N*, total number of germ lines scored; MZ, mitotic zone. At least two independent *iffb-1* RNAi experiments were done in each case. Wildtype and *glp-1(ts)* animals were treated in parallel with *iffb-1* dsRNA. RNAi experiments were done as follows. In continuous RNAi during development, adults were placed onto the bacterial feeding strain; their F1 progeny were raised continuously in the presence of dsRNA and scored as adults. In RNAi initiated at L4 stage, L4 larvae were placed onto the bacterial feeding strain and scored after 72 h (see Methods). We note that the penetrance of the *iffb-1(RNAi)* defects was highly variable at 20°C as compared with 25°C (reported in Table 1). For RNAi data, the % listed is the average of multiple experiments; because the efficacy of *iffb-1* RNAi is highly variable at 20°C, we also list the range of outcomes.

[a]The Iffb-1 sterile germ line was severely reduced in size compared with wild type and contained either no gametes or only sperm. A population of undifferentiated germ cells was present that, based on nuclear morphology, did not appear to be actively proliferating or undergoing meiosis (see Fig. 3).

[b]In Glp-1 steriles, all germ cells had entered meiosis and differentiated at an early point in development; only sperm were present in the germ line at time of assay.

[c]In Glp-1 fertiles, germ cells had proliferated extensively before prematurely entering meiosis; early meiotic germ cells, oocytes, and sperm were present at time of assay.

[d]In this P0 assay, Glp-1 indicates that all distal germ cells had exited mitosis and entered meiosis prior to the time of assay; typically, the distal most germ cells were either in leptotene–zygotene ("transition") or pachytene stage of first meiotic prophase.

[e]The relative proportion of germ cell nuclei in leptotene–zygotene stages was enlarged compared with wildtype controls (see text).

planation of this result is that the RNAi treatment depleted maternally-encoded IFFB-1/eIF5B, which is essential for embryogenesis.

Taken together, our data suggest that maternal IFFB-1/eIF5B is sufficient for development through embryogenesis, and zygotic eIF5B is necessary for development past late L1–early L2 stage. The *iffb-1* mRNA expression pattern has been characterized by the Nematode Expression Pattern Database consortium (NEXTDB) and is consistent with our phenotypic data. *iffb-1* mRNA was detected by in situ hybridization at all developmental stages and was particularly abundant in early embryos and in the germ line of L4 larvae and adults (see http://nematode.lab.nig.ac.jp/db2/ShowCloneInfo.php?clone=270b2).

We observed a range of germline defects in *iffb-1(RNAi)* sterile adults (Fig. 3b–d). In the most severe cases, only a few, undifferentiated germ cells were present (Fig. 3b). In milder cases, germ cells proliferated extensively and produced sperm, but no oocytes. In some of these germ lines, active gametogenesis was not visible (Fig. 3c). In others, spermatogenesis was ongoing, although the germ line should have begun producing oocytes by this time (Fig. 3d). We conclude that a partial reduction in eIF5B activity impairs germline proliferation and differentiation, and may prevent or delay the sperm-oocyte switch.

We further investigated the interaction between *iffb-1* and *glp-1*. In *glp-1(bn18ts)* mutants at 25°C, all germ cells enter meiosis and differentiate early in larval development, indicating little GLP-1 signaling activity (Kodoyianni *et al.*, 1992). At 20°C, a population of mitotic germ cells is maintained in >99% of *glp-1(bn18ts)* adult germ lines, indicating substantial GLP-1 signaling activity (Qiao *et al.*, 1995) (see Table 2). When *glp-1(bn18ts)* animals were raised in the presence of *iffb-1* dsRNA at 20°C and survivors were assayed at 24–48 h into adulthood, ~25% of the adult survivors had a Glp-1 phenotype: All the proliferating cells had prematurely entered meiosis during larval development (Table 2). In contrast, we observed a Glp-1 phenotype in 8% of *glp-1(bn18ts) iffb-1*(+) controls and in none of the *iffb-1(RNAi) glp-1*(+) controls (Table 2). In addition, many *iffb-1(RNAi) glp-1(bn18ts)* adults had a severe Iffb-1 phenotype, containing only a few, undifferentiated germ cells, similar to the phenotype shown in Fig. 3b (Table 2). Therefore, it appears that a moderate reduction in eIF5B function can enhance *glp-1* whereas a severe reduction in eIF5B is epistatic to *glp-1*. Consistent with this interpretation, at semi-permissive and restrictive temperatures, the phenotype of *iffb-1(bc367) glp-1(bn18ts)* double mutant arrested larvae resembled that of *iffb-1(bc367)* single mutant arrested larvae (Fig. 4e,f). At 20°C, *iffb-1(bc367) glp-1(ts)* double mutant arrested larvae had an average of 18 (±3) gonad cells ($n = 10$), in comparison with 20 (±6) gonad cells in *iffb-1(bc367)* single mutants (see earlier). At 25°C, *glp-1(bn18ts)* germ cells prematurely enter meiosis in L2 stage and differentiate. However, *iffb-1(bc367) glp-1(bn18ts)* mutant arrested larvae raised at 25°C resembled those raised at 20°C, and did not make sperm (data not shown).

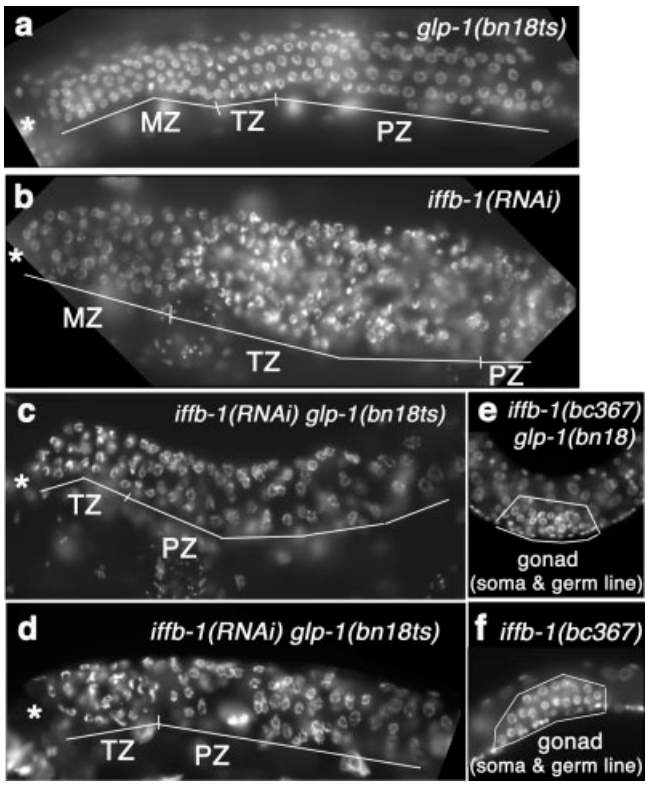

FIG. 4. A weak *glp-1(lf)* phenotype is enhanced by *iffb-1(RNAi)*. Panels a–d show relevant portions of the adult hermaphrodite germ line stained with DAPI. Asterisk indicates the distal tip of the germ line. Proliferating, leptotene–zygotene, and pachytene nuclei are indicated MZ (mitotic zone), TZ ("transition" zone), and PZ (pachytene zone), respectively. All animals were raised at 20°C, a semi-permissive temperature for *glp-1(bn18ts)*. (**a**) Distal germ line of a *glp-1(bn18ts)* mutant at 72 h post-L4 stage. A mitotic zone is clearly present. (**b**) Distal germ line of a wildtype (N2) animal treated with *iffb-1* RNAi for 72 h beginning at L4 stage. A mitotic zone is still present. (**c**, **d**) Distal germ lines of two *glp-1(bn18ts)* animals treated with *iffb-1* RNAi for 72 h beginning at L4 stage. Distal germ cells have prematurely entered meiosis, so that the mitotic zone is absent and meiotic germ cells in leptotene–zygotene stage extend to the distal end of the gonad. Pachytene nuclei are present more proximally. Nuclei in *iffb-1(RNAi)* germ lines (b–d) have a clumped distribution compared with nuclei in panel a. (**e**) The gonad of an *iffb-1(bc367) glp-1(bn18ts)* double mutant (arrested larva). Differentiated gametes are absent. (**f**) An *iffb-1(bc367)* gonad. Note the resemblance to (e).

To investigate *glp-1* enhancement without the complication of other *iffb-1(lf)* defects, we began *iffb-1* RNAi treatment at L4 stage and evaluated animals after 72 h of treatment at 20°C (see Methods). This approach guaranteed that germ cells had proliferated substantially and proximal germ cells had entered meiosis prior to RNAi treatment. At 72 h post-L4, all *iffb-1(RNAi) glp-1*(+) controls retained a mitotic population (Fig. 4b; Table 2) as did 94–95% of *glp-1(bn18ts)* and *gfp(RNAi) glp-1 (bn18ts)* controls (Table 2). In contrast, 58.5% of *iffb-1(RNAi) glp-1(bn18ts)* germ lines had a Glp-1 phenotype, with leptotene–zygotene or pachytene germ cells extending to the distal end of the gonad arm (Fig. 4c,d; Table 2). Meiotic progression also appeared to be impaired by this *iffb-1* RNAi treatment. The leptotene–zygotene region was expanded in the *iffb-1(RNAi)* controls, and some pachytene nuclei were clumped together, characteristic of a pachytene progression defect (Church *et al*., 1995) (Fig. 4b). Clumped pachytene nuclei were also visible in *iffb-1(RNAi) glp-1(bn18)* germ lines (Fig. 4c,d). Hence, both proliferation and differentiation of germ cells was disrupted by *iffb-1(RNAi)*.

Our data indicate that the *C. elegans* developmental rate depends on IFFB-1/eIF5B expression, and that a strong loss of IFFB-1/eIF5B function causes developmental arrest. Germ cells are more sensitive than somatic cells to a partial reduction in IFFB-1/eIF5B expression by RNAi, perhaps reflecting the fact that germ cells proliferate and differentiate in the adult, at a time when somatic development is complete. (Alternatively, the RNAi treatment may be substantially more effective in the germ line.) Our analysis of *iffb-1* brings this soma vs. germline difference into sharp focus, because the single eIF5B isoform is active in all tissues. (Other *C. elegans* eIFs for which there are developmental data, eIF5A and eIF4E, are present as tissue-specific isoforms (Amiri *et al*., 2001; Hanazawa *et al*., 2004; Keiper *et al*., 2000).)

The *iffb-1(lf)* developmental defects may reflect a general loss of protein production and a subsequent drop in protein levels. In the germ line, *iffb-1(RNAi)* presumably reduces translation of both mitotic and meiotic regulators, hence proliferation and differentiation are both impaired. By this logic, a mild *iffb-1(RNAi)* treatment may enhance a weak *glp-1(lf)* phenotype simply because the latter is especially sensitive to reduced translation of GLP-1 signaling pathway components. However, there may be additional, more specific, consequences of a reduction in eIF5B activity. Previous studies have suggested that a positive feedback mechanism may link proliferation and GLP-1 protein level in the distal germ line (see Hansen *et al*., 2004). *iffb-1(RNAi)* might interfere with such a mechanism by reducing proliferation, and thereby reducing expression of the already suboptimal GLP-1(bn18) protein. Another effect of reduced *iffb-1* expression may be to mimic the effect of specific translational repressors. For example, in (XX) hermaphrodites, the male germ cell fate requires translational repression of proteins that specify the female fate (e.g., TRA-2); the switch to oogenesis requires that this repression be relieved (Ellis and Schedl, 2006). *iffb-1(RNAi)* may prevent the sperm-oocyte switch by permanently preventing translation of TRA-2. In *Drosophila*, the developmental regulator, Vasa, is hypothesized to promote the translation of target mRNAs via its physical interaction with eIF5B (Johnstone and Lasko, 2004). Our analysis of IFFB-1/eIF5B has provided the background for future analysis of such translational regulation in *C. elegans*.

MATERIALS AND METHODS

Nematode Strains and Culture

Standard culture conditions were used (Epstein and Shakes, 1995). The wildtype strain was *C. elegans* variant Bristol

(N2). Mutant phenotypes were as described in Wormbase (http://www.wormbase.org) or as indicated. Mutations used were: *dpy-17(e164)*, *glp-1(bn18ts)*, *unc-32(e189)*, *let-706(s2480)*, *let-819(s2843)*, *let-831(2853)*, *iffb-1(bc367)* [this study]. *iffb-1(bc367)* was balanced with the chromosomal rearrangement, *sC1*. The *scm::gfp* transgenic array was used as a nuclear seam cell marker (J. Rothman, personal communication). Mutations in *let-706*, *let-819*, and *let-831* were reported to cause early larval arrest and map to an ~4 cM region on the left end of LGIII (Stewart *et al*., 1998). In our tests, an allele of each gene complemented *iffb-1(bc367)* for the larval arrest phenotype, indicating they are distinct from *iffb-1*. Although we did not characterize the phenotypes in detail, these mutants appeared to arrest shortly after hatching, at an earlier stage than *bc367* mutants.

Isolation of an *iffb-1* Deletion

The *iffb-1(bc367)* deletion was isolated by a standard PCR-based approach (Jansen *et al*., 1997). DNA was isolated from a library of *C. elegans* that had been mutagenized with EMS and used to do nested PCR using two sets of primers. One primer in each set was located upstream of *iffb-1* and one primer was located within exon 4. Primers for the initial PCR were: external left (el) 5′-ACTTTGTTGAACCTGGACCG-3′ and external right (er) 5′-CTTCCAGCAGCTTCATCTTCC-3′. Primers for the second round were: internal left (il) 5-CTCGAACACAGCATCATCGC-3′ and internal right (ir) 5′-GCAGCTGTCTGGTAGGTTCC-3′. An additional "poison" right primer (pp), 5′-TAGTTTTCCTCGGGCTCTGA-3′, was used in detection of the deletion. The wildtype amplification products were: el/er, 2,874 bp; il/ir, 2,616 bp; el/pp, 1,710 bp. An *iffb-1* deletion, *bc367*, was identified and then recovered (in heterozygous form) by sib selection. The region between the ir and il primers was sequenced on each strand, and a 720 bp deletion was identified that removes most of exons 2 and 3.

bc367 was balanced by crossing *sC1*/+ males into single hermaphrodites from the *bc367*/+ population, picking candidate cross-progeny to individual plates, identifying lines that carried *sC1*, and then testing for the presence of the deletion by PCR. Two *bc367/sC1* lines were chosen; in order to remove unlinked mutations, individuals from each line were out-crossed four additional times before phenotypic analysis. An identical larval arrest phenotype, balanced by *sC1*, was observed in both lines. To further confirm that *bc367* co-segregates with the larval arrest phenotype, PCR analysis was done with individual arrested larvae and non-arrested siblings. 12/12 arrested larvae were *bc367/bc367*, and 12/12 non-arrested siblings were *bc367*/+ or +/+.

Developmental Analysis

Germ cells were identified as mitotic or meiotic based on distinctive nuclear morphology, which was visualized by staining with the DNA dye, DAPI (see Qiao *et al*., 1995). We determined the *iffb-1(bc367)* larval arrest phenotype by scoring the full broods of *iffb-1(bc367)*/+ and *iffb-1(bc367)/sC1* animals. We placed individual L4 hermaphrodites onto plates, transferred them daily, and scored all of their progeny. Seam cells were counted by generating an *iffb-1(bc367)/sC1*; *scm::gfp* strain and counting the number of seam cells in *iffb-1(bc367); scm::gfp* arrested larvae. To examine the *iffb-1(bc367) glp-1(bn18ts)* phenotype, we constructed an *iffb-1(bc367) unc-32(e189) glp-1(bn18)/sC1 unc-32(e189) glp-1(bn18ts)* strain. *iffb-1 unc-32 glp-1* homozygotes were identified by their larval arrest phenotype.

cDNA Sequence

In other studies, we recovered a partial cDNA, pEL81, from a *C. elegans* cDNA library (kindly provided by R. Barstead). Partial *iffb-1* cDNA sequence was available from the *C. elegans* EST (Expressed Sequence Tag) Project. We determined the rest of the *iffb-1* cDNA sequence by sequencing pEL81 and by obtaining cDNAs yk270b2 and yk1408a07 from the EST Project and sequencing them. The 5′ end of yk1408a07 contains an SL1 trans-spliced leader sequence. The 3′ end of yk270b2 contains a consensus polyadenylation signal and a run of adenosines. Our cDNA sequence differs from the GeneFinder prediction in several respects; most notably, three exons that were predicted to lie between exon 4 and 5 are absent. There is extensive overlap between pEL81 and both yk1408a07 and yk270b2, and we have two-fold coverage for exons 2–6. The cDNA sequence has been deposited in GenBank (accession number DQ867020).

RNA Interference Assays

DsRNA was delivered by feeding the nematodes a bacterial strain that had been engineered to produce *iffb-1* or *gfp* dsRNA. Bacterial "feeding" strains were generated by cloning exons 2–6 of *iffb-1* or the entire *gfp* coding region into the L4440 vector, and transforming the plasmids into *E. coli* strain HT115, which is defective in the dsRNA-specific endonuclease, RNaseIII (Timmons *et al*., 2001). *iffb-1* RNAi was most effective at 25°C, as is the case for many genes. To investigate the developmental delay caused by *iffb-1* RNAi, L4 hermaphrodites were placed onto feeding plates and then transferred daily. Progeny were counted and removed to a new feeding plate once they reach L4 stage; they were scored for fertility once they reached adulthood. Animals were scored as fertile or sterile based on the presence/absence of embryos in the uterus, as observed in the dissecting microscope. A subset of sterile and fertile animals were DAPI-stained and evaluated in the compound microscope. We note that high throughput RNAi surveys previously reported that Y54F10BM.2 RNAi caused embryonic lethality (Sonnichsen *et al*., 2005) or adult sterility and larval arrest (Maeda *et al*., 2001).

ACKNOWLEDGMENTS

The authors thank Yuji Kohara and colleagues for the EST clones, Joel Rothman for the *scm::gfp* transgenic strain, Bob Barstead for the expression library from which we originally isolated pEL81, Xingyu She for assistance with the figures, and Scott Erdman, John Belote, Xingyu She, and Ying Liu for discussion. Some strains used in this study were provided by the *Caenorhabditis* Genetics Center, which is funded by the NIH Center for Research Resources.

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