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FOG-2, a novel F-box containing protein, associates with the GLD-1 RNA binding protein and directs male sex determination in the *C. elegans* hermaphrodite germline

Robert Clifford[1,2], Min-Ho Lee[1], Sudhir Nayak[1], Mitsue Ohmachi[1], Flav Giorgini[1,3] and Tim Schedl[1,*]

[1]Department of Genetics, Washington University School of Medicine, Campus Box 8232, 4566 Scott Ave, St Louis MO, 63110, USA
[2]Laboratory of Population Genetics, National Cancer Institute, Bethesda, MD 20892, USA
[3]Department of Genetics, University of Washington, Seattle WA, 98195-7360, USA
*Author for correspondence (e-mail: ts@genetics.wustl.edu)

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SUMMARY

Male sex determination in the *Caenorhabditis elegans* hermaphrodite germline requires translational repression of *tra-2* mRNA by the GLD-1 RNA binding protein. We cloned *fog-2* by finding that its gene product physically interacts with GLD-1, forming a FOG-2/GLD-1/*tra-2* 3′untranslated region ternary complex. FOG-2 has an N-terminal F-box and a novel C-terminal domain called FTH. Canonical F-box proteins act as bridging components of the SCF ubiquitin ligase complex; the N-terminal F-box binds a Skp1 homolog, recruiting ubiquination machinery, while a C-terminal protein-protein interaction domain binds a specific substrate for degradation. However, since both *fog-2* and *gld-1* are necessary for spermatogenesis, FOG-2 cannot target GLD-1 for ubiquitin-mediated degradation. We propose that FOG-2 also acts as a bridge, bringing GLD-1 bound to *tra-2* mRNA into a multiprotein translational repression complex, thus representing a novel function for an F-box protein. *fog-2* is a member of a large, apparently rapidly evolving, *C. elegans* gene family that has expanded, in part, by local duplications; *fog-2* related genes have not been found outside nematodes. *fog-2* may have arisen during evolution of self-fertile hermaphroditism from an ancestral female/male species.

Key words: Translational repression, GLD-1, FOG-2, F-box, germline sex determination, *tra-2* mRNA, *Caenorhabditis elegans*

INTRODUCTION

Translational regulation is an important mechanism for temporal and spatial restriction of gene expression. Biological processes utilizing translational repression/activation include anterior-posterior axis formation in *Drosophila*, sex determination in *C. elegans*, and iron homeostasis in mammalian cells (reviewed by Anderson and Kimble, 1997; Hentze and Kuhn, 1996; van Eeden and St Johnston, 1999). Translational repression, in many cases, is controlled by sequences in the 3′UTR of the mRNA. While sequences in 3′UTRs that are important for translational repression and proteins that bind these sequences have been identified, the mechanism(s) by which repression occurs is largely unknown (Gray and Wickens, 1998).

C. elegans GLD-1, a germline-specific cytoplasmic protein, is necessary for three aspects of germline development (Francis et al., 1995a,b; Jones et al., 1996). First, *gld-1* has an essential function in meiotic prophase progression and oocyte development. Second, it is necessary for spermatogenesis in the hermaphrodite. Third, it directs the initiation of meiotic development in a pathway that is redundant with the activity of the *gld-2* gene (Kadyk and Kimble, 1998). Recent data indicates that GLD-1 carries out at least some of these functions by acting as a translational repressor (see below: Jan et al., 1999; M.-H. Lee, B. Grant, D. Hirsh, and T. Schedl, unpublished data). GLD-1 is a member of a family of proteins, including mouse quaking and *Drosophila* How, that contain an approx. 200 amino acid region of similarity called the GSG or STAR domain (Di Fruscio et al., 1998; Jones and Schedl, 1995; Vernet and Artzt, 1997), which consists of a centrally located approx. 100 amino acid KH RNA binding region flanked by an approx. 75 amino acid conserved N-terminal region and an approx. 25 amino acid conserved C-terminal region. GSG domain proteins differ from other KH RNA binding proteins (e.g. fragile X protein FMR1) in three respects: (1) the KH domain contains an additional approx. 26 amino acids present in two conserved loops (Gibson et al., 1993; Musco et al., 1996; Siomi et al., 1993); (2) there is conservation outside the KH region; and (3) there is only a single KH domain.

To begin to understand how GLD-1 functions as a translational repressor for diverse aspects of germline development, we performed a yeast two-hybrid screen to identify proteins that associate with it. This screen identified the *fog-2* gene product that, like GLD-1, acts in the specification of the male sexual fate in the hermaphrodite

germline (Schedl and Kimble, 1988). Below we briefly review hermaphrodite germline sex determination.

In *C. elegans* there are two sexes, hermaphrodites and males. The hermaphrodite has a female soma but a germline that makes sperm then oocytes. The first approx. 40 germ cells that differentiate in each gonad arm develop as sperm in the fourth larval stage, then a cell fate switch occurs so that all remaining germ cells develop as oocytes in the adult. Sexual fate in the germline is determined by a negative regulatory cascade (Fig. 1; reviewed by Ellis, 1999; Hansen and Pilgrim, 1999; Kuwabara et al., 1998). The genes *fem-1*, *fem-2* and *fem-3* and *fog-1* and *fog-3* direct spermatogenesis (Barton and Kimble, 1990; Ellis and Kimble, 1995; Hodgkin, 1986). The activities of these genes are negatively regulated by *tra-2* and *tra-3* to allow oogenesis. *tra-2* encodes a transmembrane receptor protein (Kuwabara et al., 1992). The TRA-2 cytoplasmic domain binds FEM-3; this interaction appears to lead to FEM-3 sequestration or inactivation (Mehra et al., 1999). In males, secreted HER-1 product presumably binds to the extracellular domain of TRA-2, freeing FEM-3 from the TRA-2 intracellular domain, resulting in continuous spermatogenesis (Kuwabara et al., 1992; Perry et al., 1993). Control in the hermaphrodite germline is more complex as there is a brief period of spermatogenesis during larval development before the switch to oogenesis in the adult. Furthermore, *her-1* is not responsible for inhibiting *tra-2* to allow hermaphrodite spermatogenesis (Hodgkin, 1980; Perry et al., 1993; Trent et al., 1991). Instead, the gene *fog-2* acts at an equivalent position in the cascade, being necessary for spermatogenesis in the hermaphrodite and functioning upstream of *tra-2* (Schedl and Kimble, 1988). The function of *fog-2*, unlike the *fem* genes and *fog-1* and *fog-3*, is restricted to the hermaphrodite.

Posttranscriptional control of *tra-2* and *fem-3* is important for the hermaphrodite pattern of spermatogenesis then oogenesis. *tra-2* mRNA is translationally repressed to allow hermaphrodite spermatogenesis. Gain-of-function (gf) mutations that disrupt one or both of the 28 base **D**irect **R**epeat **E**lements (DRE) in the *tra-2* 3′UTR cause feminization of the hermaphrodite germline; a role of the DREs in translational repression is inferred from the shift of *tra-2* mRNA containing gf mutant 3′UTR into larger polysome fractions compared to mRNAs containing a wild-type 3′UTR and on derepression of a *lacZ* reporter when the *tra-2* 3′UTR contains gf mutations (Doniach, 1986; Goodwin et al., 1993; Schedl and Kimble, 1988). Goodwin and coworkers identified GLD-1 as a protein that binds the DREs and used reporter constructs and antibody staining to identify that its function is in the translational repression of *tra-2* mRNA (Jan et al., 1999). This is consistent with the loss-of-function (lf) phenotype of *gld-1* (feminization of the hermaphrodite, and not the male, germline; Francis et al., 1995a) where the absence of *tra-2* translational repression would result in high levels of TRA-2 protein that could bind and inactivate FEM-3.

fem-3 mRNA is posttranscriptionally repressed to allow oogenesis. Mutations that alter the *fem-3* 3′UTR result in a gf masculinization where the switch to oogenesis fails to occur (Ahringer and Kimble, 1991; Barton et al., 1987). The *fbf-1* and *fbf-2* Pumilio homologs and the genes *mog-1* to *mog-6* are important for this negative regulation (Gallegos et al., 1998; Zhang et al., 1997).

Genetic studies of *tra-2* gf and *fem-3* gf mutants suggested that a balancing of TRA-2 feminizing and FEM-3 masculinizing activities controls the hermaphrodite pattern of spermatogenesis, then oogenesis (Barton et al., 1987; Schedl and Kimble, 1988). This can now be understood in terms of posttranscriptional regulation of *tra-2* and *fem-3* mRNAs as well as inactivation of FEM-3 by binding to the intracellular domain of TRA-2. In larvae, translational repression of *tra-2* mRNA would lead to a lower TRA-2/FEM-3 ratio resulting in spermatogenesis, while in adults, posttranscriptional repression of *fem-3* mRNA would lead to a higher TRA-2/FEM-3 ratio resulting in oogenesis. However, it is not known how the switch from spermatogenesis to oogenesis is achieved (Gallegos et al., 1998). Is translational repression of *tra-2* mRNA constant over time or is it relieved in the adult? Similarly, is the posttranscriptional repression of *fem-3* implemented only in the adult? One route to understanding this switch is to characterize the mechanism of *tra-2* mRNA translational repression. We have identified FOG-2 as a cofactor that physically associates with GLD-1 in the translational repression of *tra-2* mRNA. FOG-2 contains an F-box motif, which is usually associated with proteins involved in ubiquitin mediated degradation of substrates. We propose that in *C. elegans* sex determination a FOG-2/GLD-1/*tra-2* 3′UTR complex functions in translational control, representing a novel role for an F-box protein.

MATERIALS AND METHODS

Strains

Standard methods were used for culturing and handling *C. elegans* (Brenner, 1974) at 20°C. *fog-2 oz* alleles were isolated in a screen for failure to complement *fog-2(q71)* feminization of the hermaphrodite germline phenotype following EMS mutagenesis (*oz123*, *oz168*, *oz169*, *oz170* and *oz184*), psoralen mutagenesis (*oz40*) or gamma-ray mutagenesis (*ozDf1* and *ozDf2*).

Analysis of *fog-2* gene structure

fog-2 corresponds to the predicted gene Y113G7B.5. We determined the intron and exon boundaries of *fog-2* by sequencing cDNA clones *cm16g5* (ACeDb) and CD13.1 (this study). *fog-2* contains four exons that correspond to nucleotides 11,630-11,702, 11,834-11,911, 11,959-12,598 and 12,649-13,008 of Y113G7B (gi:5824688; EMBL:AL110477.1). The start codon is at nucleotides 11662-11664; the termination codon is at nucleotides 12871-12873.

Our mRNA sequence includes nucleotides 12,806-12,850 and thus differs from EST *y335g6* and the predicted Y113G7B.5 sequence. We believe *y335g6* represents a mis-spliced or rare form of the *fog-2* message because the putative intron is present in *cm16g5*, CD13.1 and each of the four 3′ cDNA clones we isolated. In addition, we cannot detect the *y335g6* mRNA species by RT-PCR using a primer specific to the isoform or primers flanking it.

Sequence analysis

To identify *fog-2* lesions, we isolated genomic DNA from two mutant worms to serve as template for a PCR reaction (Williams et al., 1992). PCR products were purified and then used for sequencing. Sequence alignments were performed with the Lasergene (DNAStar) and ClustalW 1.7 (Thompson et al., 1994). Phylogenetic analysis was performed using PHYLIP (Lim and Zhang, 1999; version 3.57C) and homology searches were performed using the NCBI BLAST (Version 2.0) server (http://www.ncbi.nlm.nih.gov:80/BLAST/).

Plasmids used for the two-hybrid screen

GLD-1 constructs were cloned into the pAS1 vector (Fields and

Sternglanz, 1994) to generate in-frame fusions to the DNA binding domain of Gal4p at the C terminus. The amino acids included in each 'bait' construct are indicated in parentheses; pAS1-GLD-1(84-457), pAS1-GLD-1(84-227), pAS1-GLD-1(84-341), pAS1-GLD-1(245-457), pAS1-GLD-1(245-341), pAS1-GLD-1(273-457), and pAS1-GLD-1(1-270). A set of mutant *gld-1* cDNAs kindly provided by A. Jones was used to construct two-hybrid vectors containing the missense mutations *oz10*, *oz17*, *oz47*, *q126*, *q266* and *q361* in pAS1-GLD-1(84-341).

The complete *fog-2* coding region was PCR amplified from the cDNA *cm16g5* using Vent polymerase (NEB). The following pAS-1 FOG-2 constructs were used for two-hybrid analysis; pAS1-FOG-2(1-327) and pAS1-FOG-2(79-327). *ftr-1* was obtained by RT-PCR and used to construct pAS1-FTR-1(72-314).

Two-hybrid screen and assay

Yeast strain Y190 was transformed as described by Yamada et al. (1998) using R. Barstead's oligo(dT)-primed and random-primed *C. elegans* cDNA two-hybrid libraries (Kraemer et al., 1999). Two-hybrid screens were performed with the bait constructs pAS1-GLD-1(1-270), pAS1-GLD-1(84-227), pAS1-GLD-1(84-341) and pAS1-GLD-1(373-457). Transformants were selected on $His^-/Leu^-/Trp^-$ medium with 25 mM 3-amino-triazole (Sigma). $His^+Leu^+Trp^+$ yeast colonies were tested for expression of the β-galactosidase reporter gene by a nitrocellulose filter lift assay (Durfee et al., 1993). The library plasmid was isolated from β-galactosidase-positive yeast grown in $His^-/Leu^-/Trp^-$ liquid medium using the glass bead miniprep and then rescued in *E. coli* by electroporation. Minipreps were prepared from two bacterial colonies from each transformation and re-tested for specificity (Bartel et al., 1993) using GLD-1, Tat and p53. Clones showing specific interaction with GLD-1 were sequenced for further analysis.

Antibodies

The C-terminal FOG-2 peptide (Fig. 7A, CFDSALKIEKVSITE-DDLALL) was coupled to KLH using the Imject Activated Immunogen Conjugation kit (Pierce) and used to immunize a rabbit. A peptide affinity column was prepared using the Sulfolink kit (Pierce) and antibodies specific to the peptide were purified essentially as described by Harlow and Lane (1988). Gonad dissections (Francis et al., 1995b), whole-mount staining of L1 to L3 larvae, antibody incubations and washes, and western blots (Jones et al., 1996) were performed as described. Affinity purified anti-FOG-2 antibody was used at a 1:100 dilution. Epifluorescent images were captured with a Zeiss Axioskop equipped with a Hamamatsu digital CCD camera (Hamamatsu Photonics), and processed with Photoshop 5.5 (Adobe).

UV crosslinking and immunoprecipitation

^{32}P-labeled RNAs were synthesized from PCR products that have a T3 primer site with [^{32}P]UTP (ICN) and T3 RNA polymerase at 37°C for 2 hours. Template DNAs were then removed by incubating with 10 Units RNase-free DNase (Roche) at 37°C for 15 minutes and ^{32}P-labeled RNAs were purified through Sephadex G-50 quick spin column (Roche). A volume of RNA giving 10^5 cts/minute was incubated with cytosol extracts in 5 mM Hepes (pH 7.6), 1 mM $MgCl_2$, 75 mM KCl, 1 mM DTT, 1% glycerol, 300 μg/ml tRNA, 3 mg/ml Heparin in a final volume of 30 μl for 20 minutes at room temperature and UV-crosslinked in a Stratalinker UV Crosslinker 2400 (Strategene) at maximum output for 10 minutes on ice. 80 μg RNase A was then added and incubated for 20 minutes at room temperature to digest any uncrosslinked RNA. After digestion, 30 μl of homogenization buffer (HB, 15 mM Hepes pH 7.6, 10 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM EGTA, 44 mM sucrose; Lichtsteiner and Tjian, 1995) containing 100 mM NaCl was added and immunoprecipitation was performed with anti-GLD-1 antibody, anti-FOG-2 antibody, or control IgG (SC94; Santa Cruz Biotech.) bound to protein G-Sepharose (Sigma) for 1 hour at 4°C. The beads were then isolated, washed 4 times with HB containing 100 mM NaCl, and boiled in SDS sample buffer. The supernatant was resolved in 10% SDS-PAGE and exposed to X-OMAT film (Kodak).

Primers used for sequencing and RT-PCR, details of the various pAS1-GLD-1 and pAS1-FOG-2 constructs, results and methods for the GST-pull downs, and details of the *fog-2/ftr-1* gene family can be accessed from our web site (http://www.genetics.wustl.edu/tslab/SN_FOG-2_Page.html).

RESULTS

FOG-2 binds GLD-1 in the yeast two-hybrid system

We used the yeast Gal4p two-hybrid system (Fields and Sternglanz, 1994) to identify proteins that physically interact with GLD-1. We recovered two identical cDNAs in two-hybrid screens (Fig. 2B, row 7). One (OG2.3) using GLD-1 residues 84-341 and the other (CD13.1) using residues 273-457, both fused to the Gal4p DNA binding domain (Fig. 2A, rows 1 and 2). The two cDNAs are products of the gene Y113G7B.5 (Fig. 3A; Consortium, 1998), located on chromosome V in the vicinity of the genetically mapped germline sex determination gene *fog-2* (Schedl and Kimble, 1988). Since the lf phenotype of *fog-2* is essentially identical to the lf germline sex determination phenotype of *gld-1* (Francis et al., 1995a,b), the *fog-2* product is a candidate GLD-1 binding protein.

Three lines of evidence indicate that Y113G7B.5 is the *fog-2* gene. First, RNA mediated interference (RNAi; Fire et al., 1998) reveals that the lf phenotype of Y113G7B.5 is identical to that of *fog-2*. Introduction of dsRNA synthesized from cDNA clone CD13.1 produces a hermaphrodite-specific feminization of the germline: 91% of gonad arms (n=292) in somatically female cross-progeny have feminized germlines (contain oocytes where sperm would normally be produced) while none of their male siblings (n=100) show any germline or somatic sex determination defect. Second, 18 *fog-2* mutations are in the Y113G7B.5 transcription unit. These *fog-2* alleles include six different nonsense mutations, three unique missense mutations, three distinct splice site mutations and one complex lesion consisting of a trans-splice acceptor mutation and a missense mutation (Table 1). Finally, the Y113G7B.5 protein is not detected in animals homozygous for the *fog-2* nonsense alleles *oz40* or *q71* (Figs 4 and 6).

Characterization of the interaction between GLD-1 and FOG-2

We further characterized the GLD-1-FOG-2 interaction using the yeast two-hybrid system (Fig. 2A). GLD-1 amino acids 84-341, 273-457 or 273-341 fused to the Gal4p DNA binding domain interact with FOG-2 residues 79-327, while GLD-1 amino acids 1-270 failed to interact. Fusion proteins containing the Gal4p DNA binding domain and either residues 1-327 or residues 79-327 of FOG-2 interact with amino acids 283-463 of GLD-1. These results indicate that amino acids 283-341 of GLD-1 bind FOG-2 and demonstrate that the N-terminal part of FOG-2 (F-box) is not required for this interaction. The 58 amino acids of GLD-1 (283-341) that interact with FOG-2 contain the third alpha helical region of the KH domain (Lewis et al., 2000; Musco et al., 1996) as well as the C-terminal part of the redefined GSG domain (Di Fruscio et al., 1998).

A number of *gld-1* mutations alter residues in the portion of the protein that interacts with FOG-2 (Jones and Schedl, 1995).

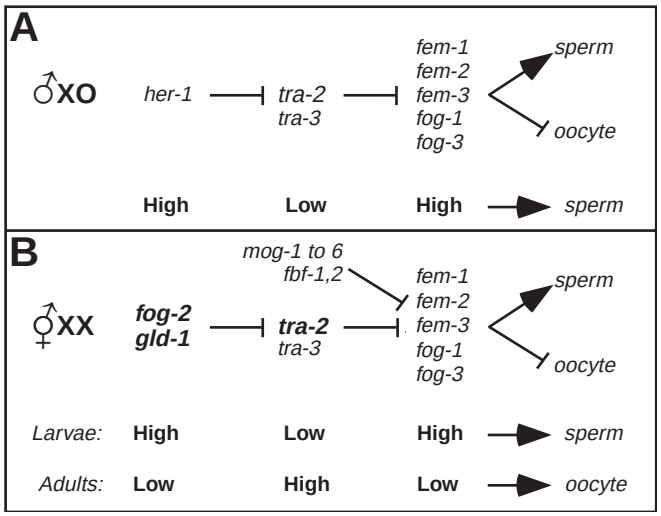

Fig. 1. Genetic pathway for germline sex determination in the male (A) and hermaphrodite (B). Arrows indicate positive regulation, barred lines indicate negative regulation. Relative activities are indicated as either High or Low. Upstream regulation by the primary sex determining signal, the ratio of X chromosomes to autosomes where hermaphrodites are XX and males are XO (Meyer, 1997), has been omitted. In males (A), *her-1*-mediated repression of *tra-2* activity allows the downstream *fem-1, 2, 3* and *fog-1, 3* activities to direct spermatogenesis and/or inhibit oogenesis. In hermaphrodite larvae (B), *tra-2* activity is maintained at low levels by *fog-2* and *gld-1* allowing a transient period of spermatogenesis. In the adult, presumably high levels of *tra-2* activity, as well as negative regulation by *mog-1* to *mog-6* and *fbf-1*, *fbf-2* result in oogenesis (see text).

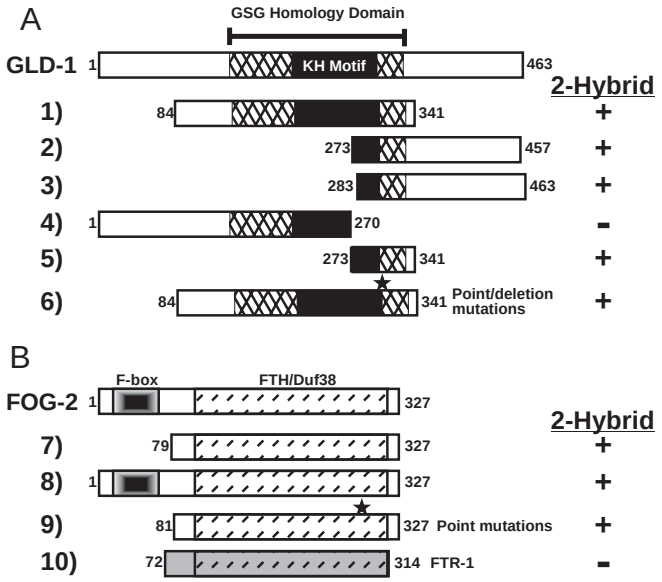

Fig. 2. Two-hybrid analysis of GLD-1 and FOG-2. Various GLD-1 and FOG-2 constructs were tested for interaction in a two-hybrid system and scored as + or – based on growth on selective media ($Leu^-/Trp^-/His^-$) after 72 hours. (A) GLD-1 bait constructs tested for binding to FOG-2 (aa79-327). The KH domain is shaded black, the GSG homology domain is crosshatched. Position of the point mutations and deletion tested are noted by the star. All GLD-1 constructs with the exception of GLD-1 (aa1-270) were positive for interaction with FOG-2. (B) FOG-2 and FTR-1 bait constructs tested for binding to GLD-1 (aa84-457). The F-box motif is shaded black, the FTH (Duf38) domain is stippled, and FTR-1 (aa72-314) is shaded gray. Position of the point mutations tested is indicated by the star. All FOG-2 constructs tested were positive for GLD-1 interaction. Row 7 corresponds to clones OG2.3 and CD13.1. FTR-1 was unable to interact with GLD-1 (row 10). Strains were verified to produce GLD-1, FOG-2 or FTR-1 fusion proteins by western blot.

These include the missense mutations *q126* G308E, *oz47* D310N, and *oz10* P335S and the *q266* mutation, an in-frame deletion of residues 322-331. Both the *q126* and *q266* mutants show strong feminization of the germline phenotypes (Francis et al., 1995a; Francis et al., 1995b), which might be due to a failure of GLD-1 to bind FOG-2. To test if any of these mutations, as well as *q361* G227D and *oz17* G248R, disrupt the interaction between GLD-1 and FOG-2, each was separately introduced into the Gal4 DNA binding domain construct containing residues 84-341 of GLD-1. Surprisingly, all the mutant fusion proteins retain the ability to interact with FOG-2 (Fig. 2, row 6), although we cannot rule out a small quantitative decrease in binding. The feminization phenotype of *gld-1 q126* and *q266* mutants thus appears not to be caused by a defect in the GLD-1-FOG-2 interaction.

To corroborate the GLD-1-FOG-2 interaction, we used an in vitro system. GLD-1 protein synthesized in the reticulocyte lysate system was tested for binding to GST-FOG-2 fusion protein linked to beads. Consistent with the two-hybrid assay results, we found that amino acids 84-457 of GLD-1, which do not bind GST, do bind residues 79-327 of FOG-2 fused to GST (data not shown).

FOG-2 is a cytoplasmic germline protein

If FOG-2 and GLD-1 interact in vivo they must be co-expressed at the appropriate time and reside in the same subcellular compartment. We used an affinity purified polyclonal antibody raised against a unique 20 amino acid sequence at the C terminus of FOG-2 to examine its accumulation during nematode development. The anti-C-terminal antibodies are highly specific because they detect a single polypeptide with a mobility similar to the predicted molecular mass of FOG-2 (37 kDa) in wild-type adult hermaphrodites and males but not in the *fog-2* nonsense mutants *oz40* and *q71* (Fig. 4), and they fail to stain *oz40* or *q71* larvae or dissected adult gonads (Fig. 5, data not shown).

FOG-2 is detected uniformly throughout the germline of L1, L2 and L3 larvae (Fig. 5). In dissected gonads from L4 (not shown) and young adult hermaphrodites and males, FOG-2 accumulates in proliferating and meiotic prophase germ cells with the amount of protein decreasing in late gametogenesis such that it is not observed in sperm or in full grown oocytes.

FOG-2 accumulates exclusively in the cytoplasm, as is the case for GLD-1 (Jones et al., 1996). FOG-2 was not detected in somatic cells of larvae or somatic tissue liberated in gonad dissections (Fig. 5) and was not observed in western blots of *glp-1* mutants that lack a germline (Fig. 4). Therefore, FOG-2 appears to be limited to the germline. The presence of FOG-2 in proliferating and early meiotic prophase germ cells is consistent with its genetically defined role in germline sex determination. Importantly, this pattern overlaps, although is not identical to, that of GLD-1 (Jones et al., 1996). The

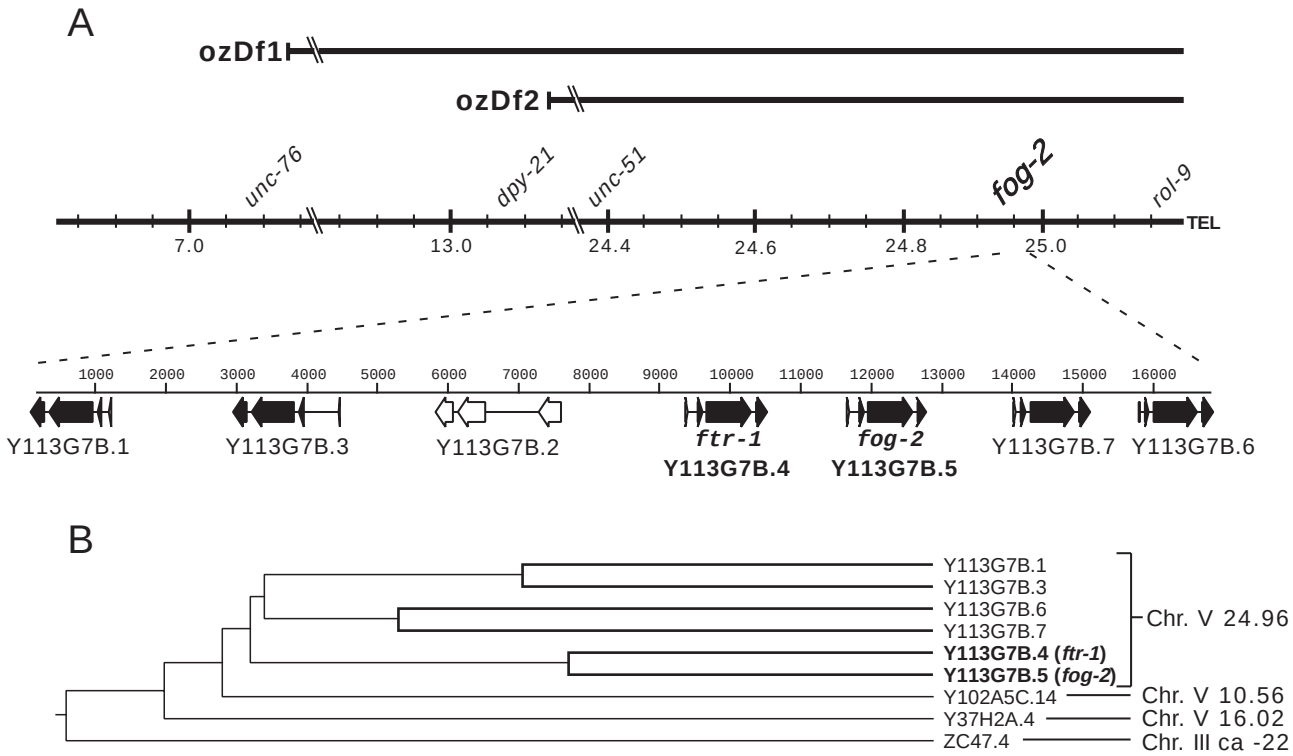

Fig. 3. Analysis of the fog-2 cluster. (A) Genetic/physical map of the region surrounding *fog-2* on chromosome V. Genetic position indicated below the linear map. Deletions (*ozDf1* and *ozDf2*) covering the *fog-2* region are above with breaks denoted by // marks. The physical map of the *fog-2* region from YAC Y113G7B is shown below. Arrows indicate predicted exons (actual for *fog-2* and *ftr-1*) and intervening lines represent introns oriented to illustrate direction of transcription; filled-in arrows denote *fog-2*-related genes. (B) Partial phylogenetic tree relating members of the *fog-2* family. Phylogenetic analysis, performed using MegAlign (DNASTAR), of six genes in the local *fog-2* cluster as well as three unlinked family members. Double-thick lines indicate inferred gene duplications. A more complete analysis can be found at (http://www.genetics.wustl.edu/tslab/SN_FOG-2_Page.html).

significance of FOG-2 accumulation in the male germline or during gametogenesis, both of which are unaffected in *fog-2* mutants, is unknown.

FOG-2 and GLD-1 form a ternary complex with the *tra-2* 3′UTR

Since GLD-1 binds to the *tra-2* 3′UTR to mediate translational repression (Jan et al., 1999) and FOG-2 binds to GLD-1, perhaps FOG-2, GLD-1 and the *tra-2* 3′UTR are present in a complex. To examine whether FOG-2 might bind directly to the *tra-2* 3′UTR, or associate indirectly via GLD-1, we used UV-induced RNA/protein cross-linking followed by immunoprecipitation (IP) with either anti-GLD-1 or anti-FOG-2 antibodies (Materials and Methods). ^{32}P-labeled wild-type or *e2020* mutant *tra-2* 3′UTRs were incubated with adult hermaphrodite cytoplasmic extracts from either wild-type, *gld-1(q361)* or *fog-2(oz40)* mutants, the RNA cross-linked to protein by UV treatment, and the unprotected RNA removed by RNaseA treatment. The ^{32}P-labeled RNA-protein complexes were either untreated (no IP), IP with anti-GLD-1 antibodies, or IP with anti-FOG-2 antibodies and the products resolved by SDS-PAGE (Fig. 6). The *tra-2 e2020* gain-of-function mutant 3′UTR is a negative control as it fails to bind GLD-1 (Jan et al., 1999) and is not translationally repressed. Both the anti-GLD-1 IP and the anti-FOG-2 IP identify a doublet protein band labeled with the wild-type *tra-2* 3′UTR but not *e2020* mutant 3′UTR. The bands co-migrate with the two isoforms of GLD-1 on western blots (data not shown; Jones et al., 1996). The *gld-1(q361)* extract (Fig. 6B) demonstrates that the presence of labeled GLD-1 in both IPs depends on GLD-1 and its ability to bind the *tra-2* 3′UTR; the *q361* missense mutation is in a GLY residue that is absolutely conserved in all KH-domain containing proteins and GST-GLD-1(*q361*) does not bind the *tra-2* 3′UTR in vitro (Jones and Schedl, 1995; Jan et al., 1999). We did not detect FOG-2 labeled with wild-type or *e2020* mutant *tra-2* 3′UTR in the anti-FOG-2 IP (Fig. 6; data not shown) indicating that FOG-2 does not directly bind the *tra-2* 3′UTR. The IP of GLD-1 by anti-FOG-2 from worm cytoplasmic extracts therefore provides further evidence of a physical interaction between the two molecules. GLD-1 was found to bind to the *tra-2* 3′UTR even in the absence of FOG-2 (Fig. 6B, *fog-2(oz40)*). These results suggest that translational repression of *tra-2* mRNA is mediated by a FOG-2/GLD-1/*tra-2* 3′UTR ternary complex, where FOG-2 associates with the *tra-2* 3′UTR indirectly via GLD-1.

FOG-2 possesses two conserved motifs

The *fog-2* gene encodes a predicted protein of 327 amino acids (see Materials and Methods). FOG-2 is acidic and lacks an obvious signal sequence or nuclear localization sequence. However, it possesses two conserved motifs as determined by

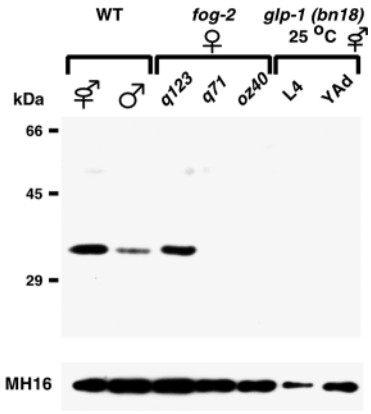

Fig. 4. Western blot analysis of FOG-2. FOG-2 accumulation was examined in wild-type (WT) adult hermaphrodites and males, *fog-2* adult females (*q123, q71, oz40),* and L4 and adult *glp-1(bn18)* hermaphrodites that essentially lack a germline (Kodoyianni et al., 1992). Each lane contains total extract from 100 worms of a given genotype. Samples were run on an SDS-polyacrylamide gel, blotted and probed with the anti-FOG-2 peptide antibody. Paramyosin, detected with monclonal antibody MH16 (Francis and Waterston 1985), was used as a loading control.

sequence alignment and by searching against the Pfam protein pattern database (Bateman et al., 2000).

Amino acids 16-65 of FOG-2 constitute an F-box motif (Fig. 7B; Bateman et al., 2000), which is a protein-protein interaction domain conserved in plants, fungi and animals (Bai et al., 1996). Residues 102 to 317 form the second conserved motif in FOG-2 (Fig. 7C), which we term the **F**OG-**2** **H**omology domain (FTH). This motif was previously described as Pfam entry Duf38 (**D**omain of **U**nknown **F**unction **38**) by Bateman et al. (2000): http://pfam.wustl.edu/hmmsearch.shtml. Unlike the F-box, which has been conserved throughout eukaryotic evolution, we have not detected the FTH domain outside of *C. elegans* and *C. briggsae*. The FTH region is critical for FOG-2 function, as two residues within this domain are altered by three missense mutations: G219R (*oz170*, *q113* and *q170*), G271R (*q123*) and G271E (*q70*) (Fig. 3a; Table 1).

Table 1. Molecular lesions in *fog-2* alleles

Allele*	Severity	Nucleotide‡	Amino acid§
q124	partial *lf*	C –3 A; G 562 A	trans-splice acceptor, Asp 118 Asn
q154, q251	partial *lf*	G –1 A	trans-splice acceptor
q177	partial *lf*	G 204 A	splice acceptor 1
oz40	strong *lf*	T 250 A	Tyr 29 STOP
oz169	strong *lf*	G 361 A	Arg 51 STOP
oz184	strong *lf*	C 515 T	Gln 103 STOP
q247	strong *lf*	C 595 T	Arg 129 STOP
q71, q226	strong *lf*	G 654 A	Trp 148 STOP
oz170, q113, q170	strong *lf*	G 865 A	Gly 219 Arg
oz123	strong *lf*	A 964 T	Arg 252 STOP
oz168, q167	strong *lf*	G 1019 A	splice acceptor 3
q123	strong *lf*	G 1071 A	Gly 271 Arg
q70	strong *lf*	G 1072 A	Gly 271 Glu

*All *q* alleles are described in Schedl and Kimble (1988), the *oz* alleles are described in the text. *fog-2(q71* and *oz40)* are possible null alleles as the homozygous phenotype is identical to the single mutant in *trans* to *ozDf1* for both XX and XO animals.

‡1 in nucleotides corresponds to the first nucleotide of the start ATG.

§1 in amino acids corresponds to the AUG start codon.

The best characterized F-box-containing proteins are components of the SCF complex, which directs ubiquitin-mediated degradation of specific substrates (Deshaies, 1999). However, since both *gld-1* and *fog-2* promote spermatogenesis, FOG-2 cannot function simply by targeting GLD-1 for ubiquitin-mediated degradation (see Discussion). F-box containing proteins (e.g. Cdc4) act as bridges; the N-terminal F-box recruits the ubiquitin ligase machinery while a C-terminal protein-protein interaction domain (e.g. WD40 for Cdc4) binds specific substrates. FOG-2 containing the FTH domain, but lacking the F-box, can physically associate with GLD-1 (Fig. 2, row 7). We thus propose that the C-terminal FTH motif represents a novel protein-protein interaction domain, analogous to the WD40 domain of Cdc4. The three *fog-2* missense mutations in the FTH domain may be defective in GLD-1 binding as they produce stable protein at essentially wild-type levels (Fig. 4, data not shown), yet have a strong lf mutant phenotype. The, *oz170*, *q123* and *q70* mutations were separately introduced into Gal4 DNA binding domain fusion constructs containing residues 81-317 of FOG-2 and tested for interaction with GLD-1. The three mutant constructs were able to interact with GLD-1 equivalent to wild-type FOG-2 (Fig. 2, row 9). Thus, the two FOG-2 residues affected by these mutations are not required for interaction with GLD-1 in yeast, but may instead be necessary for interaction with another factor.

fog-2 is part of an extensive nematode gene family

There are approx. 102 predicted *C. elegans* proteins related to FOG-2, each containing an N-terminal F-box and a C-terminal FTH (Duf38) domain. We have called these *ftr* genes (for ***f*****og-2** **r**elated). The most similar gene, *ftr-1*, lies just 5′ to *fog-2* and is 63% identical at the amino acid level (Figs 3A and 7A). Eight of the *ftr* genes (including *ftr-1*) may be functional, as ESTs have been identified (Y. Kohara, personal communication). There is a further set of approx. 75 predicted genes that contain only the FTH domain, some of which have EST hits. From the small amount of *C. briggsae* sequenced to date (approx. 10%) two predicted genes apparently containing only the FTH domain have been identified. (For additional information see http://www.genetics.wustl.edu/tslab/SN_FOG-2_Page.html).

Many of the *ftr* genes are found in clusters. *fog-2* itself lies within a group of six tandemly arranged *ftr* genes (Fig. 3A). Based on phylogenetic analysis, the *fog-2* cluster has expanded from 3 to 6 members by local gene duplication such that *fog-2* is most similar to *ftr-1*, Y113G7B.7 to Y113G7B.6 and Y113G7B.1 to Y113G7B.3 (Fig. 3B). For the most part, *ftr* genes from different clusters are more distantly related while genes within a cluster are more closely related. *ftr* genes, whether in clusters or not, tend to be found in the chromosomal arms, which in *C. elegans* are thought to be somewhat heterochromatic.

ftr-1 does not have a major function in germline sex determination

The high level of sequence identity between FOG-2 and FTR-

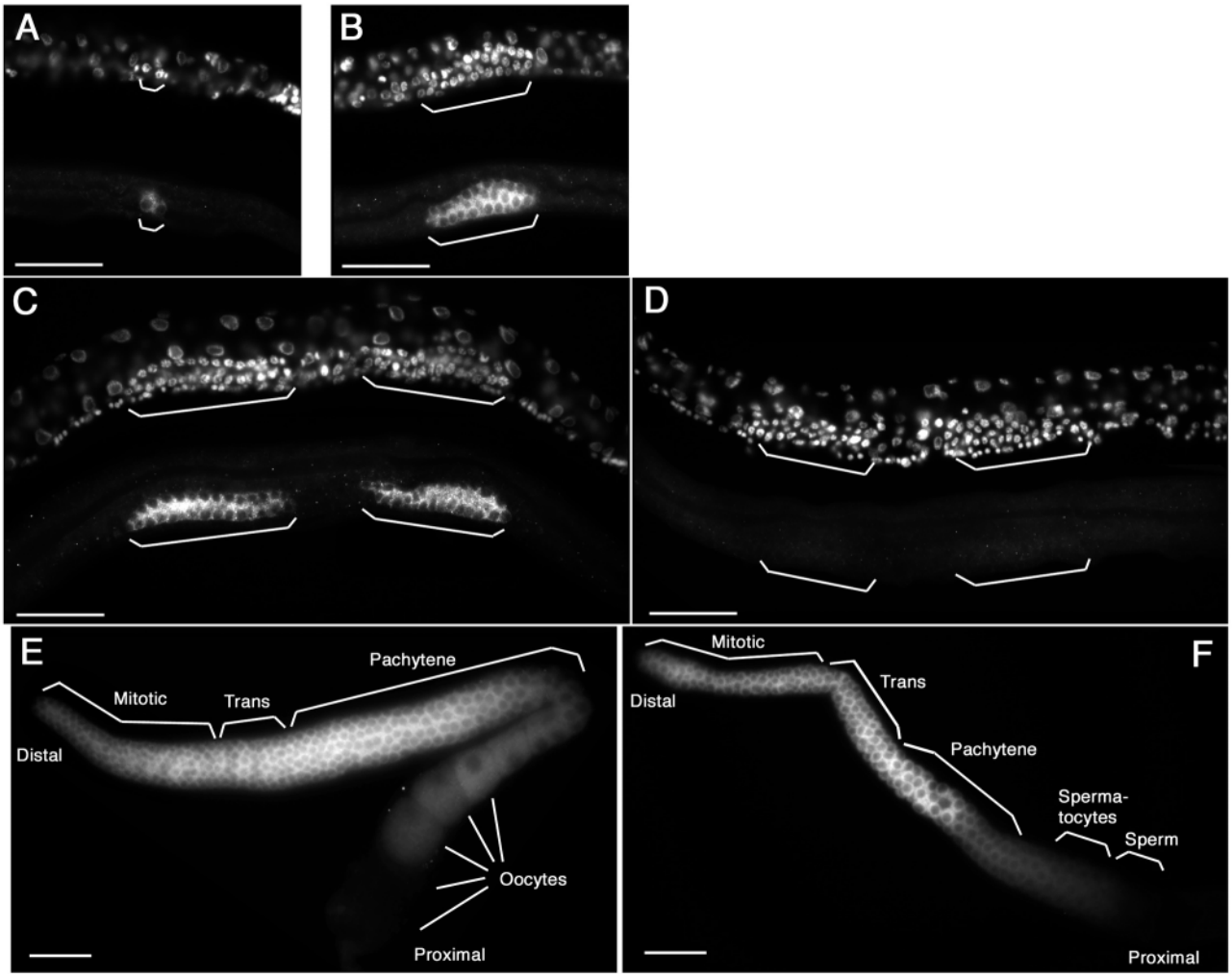

Fig. 5. Germline expression of FOG-2 during *C. elegans* development. (A-D) DAPI stained (top) and anti-FOG-2 stained (bottom) animals, where brackets demarcate the developing germline. (A-C) Wild-type hermaphrodites at the L1, L2 and L3 stages, respectively, with FOG-2 accumulating in the germline cytoplasm. (D) L3 *fog-2(oz40)* female, with staining similar to secondary antibody alone. (E-F) Dissected wild-type adult hermaphrodite and male gonads, respectively, distal to the left. Mitotic, transition, pachytene regions, spermatocytes, and sperm indicated with brackets. Scale bars, 20 μm.

1 suggests that they might function redundantly in sex determination or some other process. To investigate this possibility, we first used the yeast two-hybrid system to ask if FTR-1 could interact with GLD-1. We found that FTR-1 does not interact with GLD-1 using an FTR-1 fragment that was essentially identical to the FOG-2 fragment identified in the original screen (Fig. 2, line 10). We next examined the phenotypic consequences of loss of *ftr-1* function using RNAi. Using a 3′ region of *ftr-1* that has reduced identity with *fog-2*, we found that *ftr-1(RNAi)* essentially produces no phenotype in either wild-type hermaphrodites or males, in *fog-2* mutant hermaphrodites (females) or males or in a *fem-3(gf); fog-2* (Schedl and Kimble, 1988) background that is sensitized to additive feminization (data not shown). These results suggest that *ftr-1* does not work redundantly with *fog-2* in sex determination and that FTR-1 does not bind to GLD-1, analogous to FOG-2, to mediate translational repression in a separate context (e.g. RNAs involved in oogenesis).

DISCUSSION

The germline sex determination protein FOG-2 physically interacts with GLD-1

GLD-1, a cytoplasmic RNA binding protein, regulates multiple aspects of *C. elegans* germ cell development. Among these are meiotic prophase progression and oogenesis, male sex determination and initiation of meiotic development (Francis et al., 1995a,b; Kadyk and Kimble, 1998). To better understand how GLD-1 performs such diverse functions we used the yeast two-hybrid system to identify proteins that physically interact with GLD-1. One protein recovered in our two-hybrid screen was the product of the *fog-2* germline sex determination gene. Like *gld-1*, *fog-2* plays an important role in the adoption of the male sexual fate by germ cells developing in the hermaphrodite (Schedl and Kimble, 1988). GLD-1 appears to act as a translational repressor of *tra-2* mRNA by binding to the DRE sequences in its 3′UTR (Jan et al., 1999). A function in translational repression is supported by the observation that

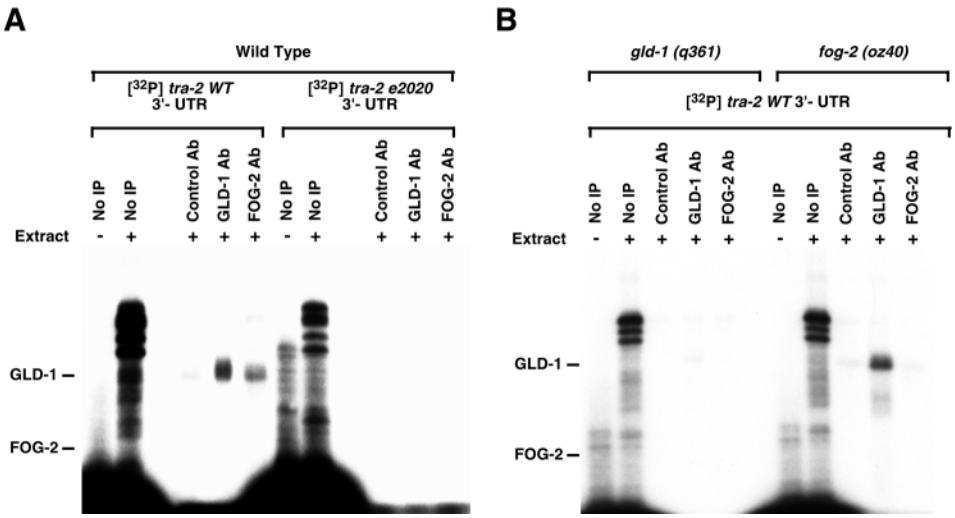

Fig. 6. FOG-2/GLD-1/*tra-2* mRNA ternary complex. ^{32}P-*tra-2* wild-type (WT) 3′UTR or ^{32}P-*tra-2(e2020)* 3′UTR were incubated with (+) or without (–) cytosolic extract from wild-type (A), *gld-1(q361)* or *fog-2(oz40)* (B) adult hermaphrodites, crosslinked with UV, digested with RNase, and immunoprecipitated (IP) with the indicated antibodies. Lanes labeled, 'No IP', were loaded with 20% of the material used in the experiment; 'GLD-1 Ab' were loaded with 40%; Control Ab and FOG-2 Ab' were loaded with 100%. Both *gld-1(q361)* and *fog-2(oz40)* extracts contain GLD-1 protein at wild-type levels (data not shown).

deletions affecting the DREs result in a gf feminization of the hermaphrodite germline, similar to the lf feminization phenotype of *gld-1* or *fog-2*, and causes missexpression of *tra-2* 3′UTR-containing reporter constructs (Doniach, 1986; Goodwin et al., 1993; Schedl and Kimble, 1988). Here we show that in worm cytoplasmic extracts FOG-2 and GLD-1 can form a ternary complex with the *tra-2* 3′UTR (Fig. 6). FOG-2 appears to associate indirectly with the *tra-2* 3′UTR by binding to GLD-1. Although GLD-1 does not require FOG-2 for binding to the *tra-2* 3′UTR (Fig. 6B), the completely penetrant *fog-2* lf feminization of the germline argues that FOG-2 function is important for *tra-2* translational repression in vivo and that GLD-1 is not sufficient. FOG-2 thus appears to act as a cofactor with GLD-1 in *tra-2* translational repression (Fig. 1).

FOG-2 is an F-box protein

FOG-2 contains an N-terminal F-box motif and a C-terminal FTH (Duf38) domain (Fig. 7). The F-box motif is distributed throughout eukaryotes, and within species multiple F-box proteins are found. The best characterized F-box proteins are components of the SCF complex that functions as an E3 ubiquitin protein ligase. F-box proteins, like Cdc4, act as bridges between the core ubiquitination complex and specific substrates (Fig. 8A). This occurs by the N-terminal F-box motif binding to Skp1, which recruits the core ubiquitination complex (CDC34, Rbx and CDC53; Deshaies, 1999). The C-terminal protein-protein interaction domain of the F-box protein (e.g. WD40 for Cdc4) binds specific substrates to be ubiquitinated and degraded (Winston et al., 1999). The C-terminal FTH domain of FOG-2 is likely a protein-protein interaction region as GLD-1 binds the C-terminal 246 amino acids of FOG-2, of which 215 belong to the FTH domain. Thus, FOG-2 has an overall arrangement that is typical of F-box proteins.

We propose that FOG-2 acts as a bridge or scaffold to recruit *tra-2* mRNA bound to GLD-1 and other proteins into a multiprotein complex (Fig. 8B). The FOG-2/GLD-1 multiprotein complex would then mediate translational repression of *tra-2* mRNA. This represents a novel function for an F-box containing protein. Typically, F-box proteins are part of the SCF complex that targets specific substrates for polyubiquitination and degradation by the 26S proteosome. However, both FOG-2 and GLD-1 promote male sex determination by acting to translationally repress *tra-2* mRNA; therefore, FOG-2 cannot target GLD-1 for ubiquitin mediated degradation.

How might FOG-2 function in the translational repression of *tra-2* mRNA? Here we consider three models for FOG-2 function. In the first, FOG-2 acts like a typical F-box protein of the SCF complex. In this case, the FOG-2 FTH domain binds an as yet unidentified positive regulator of *tra-2* translation (or a negative regulator of translational repression) while the FOG-2 F-box binds a Skp1 homolog to recruit the core ubitquitination machinery. Translational repression of *tra-2* mRNA occurs when the positive regulator is polyubiquitinated and degraded by the proteosome. Ubiquitin-mediated proteolysis appears to be important in the decay of cytokine mRNA that contain AU-rich elements in their 3′UTR (Laroia et al., 1999). For the second model, FOG-2 directs mono-ubiquitination of GLD-1 or an as yet unidentified co-factor that promotes translational repression of *tra-2* mRNA. Mono-ubiquitination might change the activity of the target protein or complex, but not lead to degradation. Examples of mono-ubiquitination include the alpha factor receptor, which results in ligand stimulated internalization (Terrell et al., 1998), and histones, which are associated with chromatin remodeling (Baarends et al., 1999). For the third model, FOG-2 assembles a novel multiprotein translational repression complex that does not involve covalent attachment of ubiquitin or ubiquitin related proteins. *S. cerevisiae* p58/Ctf13 is an example of an F-box protein that assembles a novel multiprotein complex, the Cbf3 centromere binding complex (Kaplan et al., 1997). Distinguishing between these and other models will require identification of other proteins that bind FOG-2.

GLD-1 appears to translationally repress a number of mRNAs. FOG-2 is likely specific for GLD-1-mediated translational repression of *tra-2* mRNA as *fog-2* mutants do not display other phenotypes observed in *gld-1* mutants and do not affect translational regulation of another GLD-1 mRNA target that encodes the RME-2 yolk receptor (M.-H. Lee, B. Grant, D. Hirsh, and T. Schedl, unpublished data). Specificity may be achieved by FOG-2 interacting, directly or indirectly, with

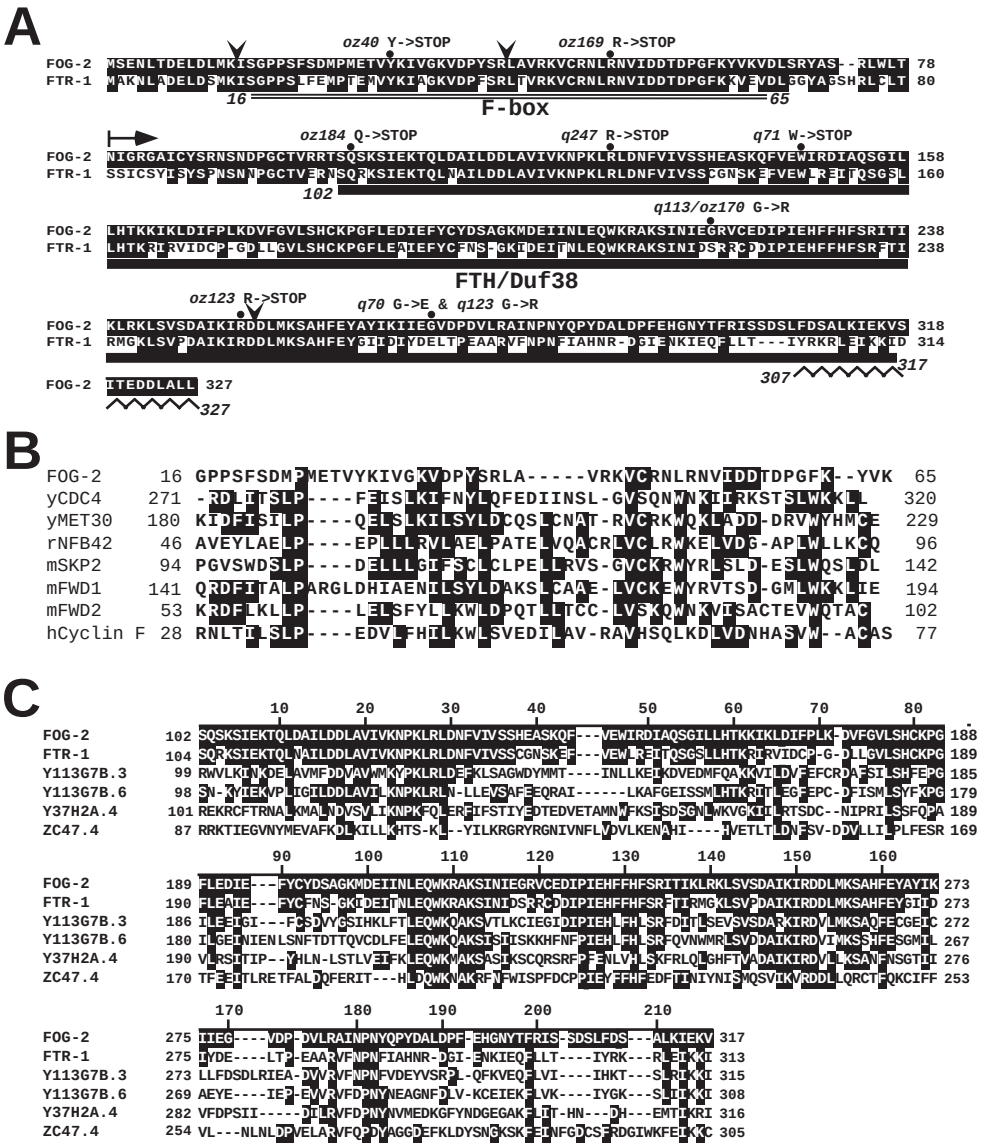

Fig. 7. Sequence analysis of FOG-2. (A) Alignment of FOG-2 and FTR-1 protein sequences. Black boxes indicate residues that are identical. The arrow indicates the N-terminal boundary of the FOG-2 clones (OG2.3, CD13.1) isolated in the two-hybrid screen. The F-box domain is indicated by the double line (aa 16-65), the FTH (Duf38) domain by a thick gray line (aa 102-317). The intron/exon junctions are indicated by the 'V'. The positions and identities of mutations are indicated above dots. The peptide used to generate the FOG-2-specific antibody is represented by the zigzag line (aa 307-327). (B) Alignment of the FOG-2 F-box region with F-box proteins from other species (y, yeast; r, rat; m, mouse; h, human). Identical residues are shaded black. (C) Alignment of the FOG-2 FTH (Duf38) region from close and distant *fog-2/ftr* family members. Residues identical to FOG-2 are shaded black. Alignments were performed using the CLUSTAL V (Higgins et al., 1992).

another RNA binding protein that specifically associates with *tra-2* mRNA.

Temporal control of *tra-2* translational repression

The hermaphrodite makes sperm in the L4 stage and then switches to oocytes in adulthood. Translational repression of *tra-2* is important to allow the *fem* genes and *fog-1* and *fog-3* to direct spermatogenesis while posttranscriptional repression of *fem-3* is important for oogenesis (Ahringer and Kimble, 1991; Barton et al., 1987; Doniach, 1986; Gallegos et al., 1998; Goodwin et al., 1993; Schedl and Kimble, 1988; Zhang et al., 1997). However, it is not known whether translational repression of *tra-2* (and/or control of *fem-3*) is modulated in larvae or the adult. *tra-2* translational repression occurs in males and the soma (Goodwin et al., 1993; Jan et al., 1999). An activity defined by RNA gel shift that binds the *tra-2* DRE (called DRF for **D**irect **R**epeat **F**actor) is found in wild-type hermaphrodites and in mutants that lack a germline (Goodwin et al., 1993). However, GLD-1 and FOG-2 are not detected in the soma and do not have essential functions in the specification of spermatogenesis in the male. Therefore, *tra-2* translational repression, and DRF components, differ between the hermaphrodite germline and the soma and males.

It is possible that *tra-2* is translationally regulated in two ways. In the hermaphrodite soma and in males, there may be only a basal or constitutive level of translational repression as there is no need for modulation. In the male, this regulation may help to adjust the concentration of TRA-2 to be in a range where regulation by HER-1 can occur. In the hermaphrodite germline, modulation of translational repression may be an important part of the sperm/oocyte switch: strong repression in larvae to allow for spermatogenesis and weak repression in the adult to assist in oogenesis. How might FOG-2 and GLD-1 participate in this modulation? FOG-2 and GLD-1 protein accumulation is unlikely to be a major factor as both are present in the germline throughout larval and adult life. Instead, protein modification mediated by FOG-2 is a possibility. For SCF-mediated degradation, often modification (phosphorylation) of specific substrates is the signal for destruction (Deshaies, 1999). If FOG-2 acts like a typical F-

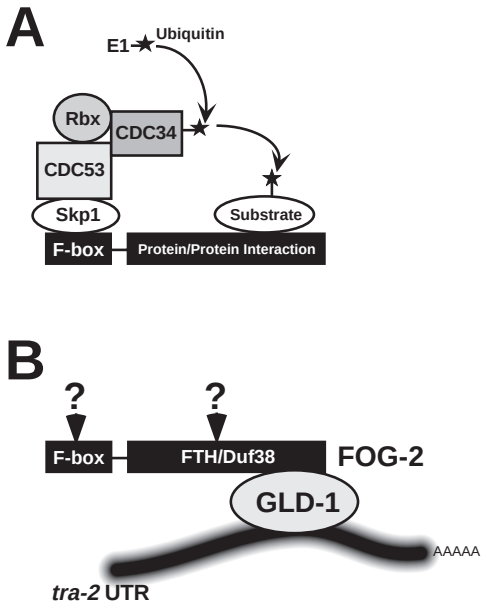

Fig. 8. The FOG-2 F-box protein may act as a bridge to assemble a *tra-2* mRNA multiprotein translational repression complex. (A) Diagram of a 'typical' SCF complex where the F-box containing protein (e.g. Cdc4) acts as a bridge between the ubiquitination machinery and the substrate (Deshaies, 1999). (B) The FOG-2 F-box protein may also act as a bridge to assemble a complex that represses the translation of *tra-2* mRNA. GLD-1, bound to the *tra-2* 3′UTR, associates with FOG-2 to form part of a translation repression complex. GLD-1 most likely interacts with the C-terminal portion of the FOG-2 FTH domain (the C-terminal 50 aa are necessary for FOG-2 to interact with GLD-1, S. N. and T. S. unpublished). We propose that the FOG-2 F-box binds a protein (?), possibly a worm Skp1 homolog, and that the FTH domain binds an additional protein (?). The idea that the FTH domain may bind an additional protein is supported by *fog-2* missense mutations in the FTH domain that have a strong lf phenotype yet produce stable protein that can bind GLD-1. See text for details. For simplicity, GLD-1 binding to the two DREs, as well as functioning as a multimer, are not shown.

box protein (first model above), then modification of a positive regulator of *tra-2* translation followed by degradation could lead to temporal modulation of repression. Alternatively, for the assembly of a multiprotein translational repression complex (third model above) modification of FOG-2 (like phosphorylation of the F-box protein p58/Ctf13; Kaplan et al., 1997) or another protein may control assembly and thus modulate translational repression.

Genetic studies suggest that *tra-2* translational repression may also occur in adults. *fem-3(gf)* mutants undergo spermatogenesis throughout adulthood (Barton et al., 1987). The adult spermatogenesis phenotype of *fem-3(gf)* is suppressed by *fog-2(lf)* and by *tra-2(gf)* suggesting that disruption of *tra-2* translational repression during adulthood will produce sufficient additional TRA-2 to counteract the excess FEM-3 produced by *fem-3(gf)*. If *tra-2* translational repression is temporally modulated, then modulation may be quantitative rather than qualitative.

fog-2 is a member of a rapidly evolving gene family that may be nematode specific

In *C. elegans* there are more than 100 genes related to *fog-2* (*ftr* genes) that contain an N-terminal F-box and a C-terminal FTH domain. There is an additional set of approx. 75 predicted genes that have only the FTH domain. The FTH domain (and thus FOG-2) does not appear to exist outside the nematodes. By contrast, the worm genome does have orthologs of conserved F-box-containing proteins (e.g. *sel-10* corresponds to Cdc4; Hubbard et al., 1997). The large number of predicted F-box proteins in *C. elegans* is therefore primarily due to the large number of *ftr* genes. Many of the *ftr* genes are found in clusters, with expansion within a cluster due to local gene duplications. The *ftr* genes thus appear to be rapidly evolving, and at least in *C. elegans* are greatly expanded. The expansion of worm-specific gene families such as *ftr* may account, in part, for the greater number of genes in *C. elegans* compared to flies (Consortium, 1998; Rubin et al., 2000).

fog-2 and *ftr-1* represent an example of a recent gene duplication followed by divergence: FOG-2 binds GLD-1 and is important for translational repression of *tra-2* mRNA while FTR-1 appears not to bind GLD-1 or have a role in germline sex determination. *fog-2* is likely under selection for function in sex determination while *ftr-1* is not. However, the *fog-2–ftr-1* divergence is non-random. First, exons 1, 2 and 3 are >80% identical at the nucleotide level while exon 4 is only 41% identical. Second, the introns are conserved, with intron 3 being 82% identical. The bulk of the amino acid changes are in exon 4 (Fig. 7A); surprisingly most of these are due to single base insertion/deletions that shift the *ftr-1* reading frame relative to *fog-2*. Thus, *ftr-1* remains under selection, although its function is not currently known.

fog-2 and the evolution of self-fertile hermaphroditism

Within the Caenorhabditis genus, there are species like *C. elegans* that have self-fertile hermaphrodites and males (androdioecious) and species like *C. remanei* that have females and males (gonochoristic). Fitch (1997) proposed that within the Rhabditidae family female/male reproduction is the ancestral state while self-fertile hermaphroditism has evolved independently multiple times. The important feature in the evolution of self-fertility is the ability of the germline to briefly undergo spermatogenesis, as *C. elegans* hermaphrodites and *C. remanei* females are otherwise essentially identical at the morphological level. *fog-2*, *gld-1* and *tra-2(gf)* mutants can be propagated as obligate XX female/XO male reproducing strains suggesting that implementation of germline translational control of *tra-2* mRNA may be significant in the evolution of self-fertile hermaphroditism (Doniach, 1986; Francis et al., 1995b; Haag and Kimble, 2000; Schedl and Kimble, 1988). *C. remanei* has a *tra-2* ortholog that functions in female sex determination, analogous to *C. elegans* (Haag and Kimble, 2000). *C. remanei* also has a homolog of *gld-1* (Jones and Schedl, 1995). A DRF-like activity is found in *C. remanei* extracts that can gel shift the 3′UTRs of both *C. remanei* and *C. elegans tra-2*, leading Haag & Kimble (2000) to propose that *tra-2* is also translationally regulated in *C. remanei*. However, the tissues or sex specificity of the *C. remanei* DRF-like activity is not known.

Since the *fog-2/ftr* gene family appears to be rapidly evolving, there is a distinct possibility that *C. remanei* may not have a *fog-2* ortholog, although it likely has *ftr* genes. In the absence of a *fog-2* ortholog, *C. remanei* would be unable to

translationally repress *tra-2* in the germline (or modulate translational repression), although it may retain a basal, *fog-2* independent translational repression mechanism in the female soma and/or the male, like *C. elegans*. With this scenario, *fog-2* would be a recently evolved gene that facilitates self-fertility in *C. elegans*. However, it is unlikely that the presence or absence of *fog-2* is the only difference between *C. elegans* and the ancestral female/male species given the temporal control of the sperm/oocyte switch, the regulation of *fem-3*, and the importance of the number of sperm produced for reproductive success (Hodgkin and Barnes, 1991).

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