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DEPS-1 promotes P-granule assembly and RNA interference in *C. elegans* germ cells

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P granules are germ-cell-specific cytoplasmic structures containing RNA and protein, and required for proper germ cell development in *C. elegans*. PGL-1 and GLH-1 were previously identified as critical components of P granules. We have identified a new P-granule-associated protein, DEPS-1, the loss of which disrupts P-granule structure and function. DEPS-1 is required for the proper localization of PGL-1 to P granules, the accumulation of *glh-1* mRNA and protein, and germ cell proliferation and fertility at elevated temperatures. In addition, DEPS-1 is required for RNA interference (RNAi) of germline-expressed genes, possibly because DEPS-1 promotes the accumulation of RDE-4, a dsRNA-binding protein required for RNAi. A genome wide analysis of gene expression in *deps-1* mutant germ lines identified additional targets of DEPS-1 regulation, many of which are also regulated by the RNAi factor RDE-3. Our studies suggest that DEPS-1 is a key component of the P-granule assembly pathway and that its roles include promoting accumulation of some mRNAs, such as *glh-1* and *rde-4*, and reducing accumulation of other mRNAs, perhaps by collaborating with RDE-3 to generate endogenous short interfering RNAs (endo-siRNAs).

KEY WORDS: Germ granules, RNAi, Gene expression

INTRODUCTION

Germ granules are large non-membrane bound organelles composed of RNAs and proteins important for germ cell specification. They are found in the cytoplasm of developing germ cells in many organisms, and are commonly perinuclear (Eddy, 1975). Germ granules in different organisms are now known to share a number of components, including proteins related to the *Drosophila* DEAD-box helicase VASA, but many organism-specific germ granule components have also been described that appear to be important for germ granule formation or function (reviewed by Seydoux and Braun, 2006; Strome and Lehmann, 2007). The molecular functions of germ granules are likely to be complex but, based on their compositions and subcellular localization, it has been argued that germ granules may post-transcriptionally regulate mRNAs, sort newly transcribed mRNAs as they leave germ cell nuclei, and/or facilitate the localization of mRNAs and proteins to primordial germ cells of embryos with maternally inherited germ plasm (Seydoux and Braun, 2006). Furthermore, recent findings in *Drosophila* and mice suggest that germ granules might play a role in the biogenesis of small RNAs (Kotaja et al., 2006; Lim and Kai, 2007).

In the nematode *Caenorhabditis elegans*, germ granules are known as P granules. P granules are germ cell-specific at all stages of development (Strome and Wood, 1982) and are important for germ cell development, as removing some of the constitutive components of P granules both alters the structure of P granules in the adult germ line (Schisa et al., 2001), and causes sterility and germ cell underproliferation (Kawasaki et al., 2004; Kawasaki et al., 1998; Kuznicki et al., 2000). Specifically, the nematode-specific predicted RNA-binding protein PGL-1 and the VASA-like RNA helicase GLH-1 are both constitutively associated with P granules and required for proper germ cell proliferation in *C. elegans* at elevated temperatures. Immunofluorescence-based analyses of the relationships between PGL-1, GLH-1 and other P-granule proteins have suggested that *pgl-1* and *glh-1* define a pathway for P-granule assembly or stability (Amiri et al., 2001; Kawasaki et al., 2004; Kawasaki et al., 1998). For example, PGL-1 localizes poorly to P granules when GLH-1 function is compromised (Kawasaki et al., 1998), while GLH-1 appears to localize normally to P granules when PGL-1 is absent (Kawasaki et al., 2004).

We report here the identification of mutations in a new gene, called *deps-1* (*de*fective *P* granules and *s*terile), that is important for the assembly of PGL-1 onto P granules. Like PGL-1 and GLH-1, DEPS-1 protein associates with P granules and is required for proper germ cell proliferation at elevated temperatures. Consistent with these phenotypic similarities, *deps-1* mutant germ lines display reduced levels of *glh-1* mRNA and protein, and a diffuse distribution of PGL-1. However, DEPS-1 also appears to have novel functions that are not mediated by reduced GLH-1 or diffuse PGL-1. Specifically, DEPS-1 promotes the expression of *rde-4* (for *RNAi DEfective*) mRNA and protein, and efficient RNA interference in the *C. elegans* germ line. In addition, DEPS-1 represses the expression of a subset of genes the expression of which is also repressed by RDE-3. We propose that DEPS-1 plays a role in some of the RNA regulatory processes mediated by P granules in the *C. elegans* germ line, and that those processes may include the generation of small RNAs that repress the accumulation of endogenously expressed transcripts.

MATERIALS AND METHODS

Strains and culture

Nematodes were cultured as described by Brenner (Brenner, 1974). Alleles and transgenes were: *deps-1(bn113, bn121, bn124* or *bn128)* I, *rde-3(ne298)* I, *bnIs1[pie-1::gfp::pgl-1 unc-119(+)]* I (Cheeks et al., 2004), *glh-1(ok439)* I, *unc-11(e47)* I, *rde-4(ne301)* III, *unc-119(ed3)* III, *pgl-1(bn101)* IV and *pgl-3(bn104)* V. Unmarked *deps-1* alleles were balanced by *hT2[qIs48]* I;III. Transformation rescue experiments used a *deps-1(bn121) unc-11(e47)* I; *sDp2* (I,f) strain.

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Genetic screen

bnIs1[gfp::pgl-1] L4 stage hermaphrodites were mutagenized with 50 μM EMS, allowed to self-fertilize and their F1 progeny picked to individual plates. F2 hermaphrodites were examined for diffuse GFP::PGL-1 in young, unlaid F3 embryos on a dissecting microscope. GFP::PGL-1 was examined in the germ lines and oocytes of candidate mutants and sterile hermaphrodites on a compound microscope at higher power. Worms were raised at 24-25°C to promote GFP::PGL-1 expression from *bnIs1*. Approximately 8700 haploid genomes were screened, and five recessive mutations causing diffuse GFP::PGL-1 were recovered.

DEPS-1 antibodies

DEPS-1 coding sequences from cDNA yk605c11 were PCR-amplified and cloned into modified pET or pGEX vectors (J. Suh, personal communication) using Gateway cloning technology (Invitrogen) to generate four fusion proteins: 6XHIS::DEPS-1 (amino acids 1-619), GST::DEPS-1 (amino acids 1-619), GST::DEPS-1N (amino acids 1-316) and GST::DEPS-1C (amino acids 313-619). Fusion proteins were purified using Ni-NTA agarose (Qiagen) or as inclusion bodies. Antibodies were raised against the 6XHIS fusion protein in rabbits (Cocalico), immuno-affinity purified against a GST fusion protein coupled to CNBr-activated sepharose 4B (Amersham) and eluted in 0.2 M glycine and 0.15 M NaCl. Antibodies purified against GST::DEPS-1C had the least cross-reactivity with a ~120 kDa nuclear antigen and are shown. All antibody preparations recognized DEPS-1 in wild-type animals but not in *deps-1* mutants.

pie-1::deps-1::gfp transgene

A 3.3 kb region from cosmid W03C10 was subcloned into pBluescript SK+ to make pCS306. Site-directed mutagenesis of pCS306 replaced the *deps-1* stop codon with an *Age*I site, and a 0.9 kb *Age*I fragment from pID3.01 (Pellettieri et al., 2003) was inserted to make pCS320 (*deps-1::gfp*). *deps-1(coding)::gfp* sequences were PCR amplified and inserted into pID2.02 (D'Agostino et al., 2006) using Gateway cloning technology (Invitrogen) to make pCS336 (*pie-1::deps-1::gfp*). *deps-1*-specific primer sequences were: ATGTCAGAACGCCAATCCAA (5′) and TTTGGTTGGATAACGGGTAG (3′). pCS336 was introduced into worms by biolistic transformation of *unc-119* (Praitis et al., 2001), and an integrated line expressing DEPS-1::GFP was identified.

Immunocytochemistry

Embryos and germ lines were fixed using methanol/acetone (Strome and Wood, 1983). Antibody dilutions were 1:500-1:1000 anti-DEPS-1, 1:4000 anti-PGL-3 (Kawasaki et al., 2004), 1:10,000 anti-GLH-1 (Kawasaki et al., 2004), 1:1000 PA3 [a gift from M. Monestier (Monestier et al., 1994)] and 1:500 Alexa Fluor 488 goat anti-rabbit IgG, Alexa Fluor 594 goat anti-mouse IgG and Alexa Fluor 594 goat anti-rat IgG (Molecular Probes). Images were acquired with a Nikon Eclipse TE200 microscope and UltraVIEW LCI spinning-disk confocal laser using UltraVIEW software (Perkin Elmer).

Western blots

Experimental conditional were as follows: (1) for DEPS-1 western, 30 *deps-1* M–Z– or wild-type gravid adults (20°C) were loaded per lane; (2) for GLH-1 western, 30 *deps-1* M+Z–, M–Z– or wild-type gravid adults (15°, 24.5°C) were loaded per lane; (3) for RDE-4 western, 50 μg acetone-precipitated protein isolated from 1-2 ml *deps-1* M–Z– or wild-type worms enriched for gravid adults (20°C) was loaded per lane. Protein was isolated by dounce homogenization of worms in 50 mM Tris pH 7.5, 10 mM KOAc, 100 mM KCl, 1 mM EDTA, 1 mM DTT, 1% Triton X-100, 2.5× protease inhibitors (Roche) followed by centrifugation at 41 ***g*** in a Beckman JA20 rotor at 4°C to remove insoluble material. Primary antibodies were 1:2000 anti-DEPS-1, 1:5000 anti-GLH-1, 1:1000 anti-RDE-4 (Tabara et al., 2002) and anti-α-tubulin (DM 1a, Sigma) at 1:250 for GLH-1 western, 1:1000 for DEPS-1 western or 1:10,000 for RDE-4 western. Secondary antibodies were 1:5000-1:10,000 horse radish peroxidase (HRP)-conjugated goat anti-rabbit IgG, 1:5000 HRP-conjugated goat anti-mouse IgG, alkaline phosphatase (AP)-conjugated goat anti-rabbit IgG and AP-conjugated goat anti-mouse IgG (Jackson Labs). Antibody signals were detected using SuperSignal West Pico Chemiluminescent Substrate (Pierce) and autoradiography film (DEPS-1, RDE-4 westerns) or the Enhanced Chemifluorescence detection system and Typhoon 9200 (Amersham) (GLH-1 western).

RNA interference

A *deps-1(RNAi)* clone was generated by subcloning a 1.4 kb genomic *Bam*HI fragment from the *deps-1* gene into L4440 (Timmons and Fire, 1998) to generate pCS302. Other RNAi clones (Kamath et al., 2003) were purchased (Geneservice). RNAi was essentially as described (Kamath et al., 2001) but used plates containing 0.2% lactose to induce dsRNA expression (E. Lambie, personal communication).

Real-time quantitative RT-PCR and microarray analysis

RNA was isolated from 50 worms or 50-100 dissected gonads (50 for PCR, 100 for microarrays) as described (Chi and Reinke, 2006). Worms were subjected to three rapid freeze-thaw cycles in Trizol (Invitrogen) prior to RNA isolation. RT-PCR was performed in triplicate either as described (Bender et al., 2006) or using an Mx3000p QPCR system (Stratagene) and iQ SYBR Green Supermix (BioRad) containing 30 nM reference dye (Stratagene). All data were normalized to *ama-1* and the Pfaffl method (Pfaffl, 2001) used to calculate relative fold changes. Gonad dissection and microarray analysis of linearly amplified polyadenylated RNA was as described by Bender et al. (Bender et al., 2006). Confidence levels were determined using a *z* test performed in Excel followed by a correction for multiple testing in which the *P*-value was multiplied by 17,539 (the number of genes on the microarrays). After this correction, all genes with a fold change of >1.8-fold, $P<0.05$ were selected for analysis. The GEO accession number for microarray data is GSE 9993.

RESULTS

deps-1 is required for the proper localization of PGL-1 to P granules

Previous studies suggested that mutations in *pgl-1* and *glh-1* define a genetic pathway for normal P-granule formation in *C. elegans* (Kawasaki et al., 2004; Kawasaki et al., 1998). For example, the P-granule-associated protein PGL-1 is required for proper P-granule morphology (Schisa et al., 2001) and the localization of PGL-1 to P granules is disrupted in *glh-1(lf)* mutants (Kawasaki et al., 1998) (C.A.S. et al., unpublished). To identify other participants in P-granule formation, we screened for mutations that phenocopy the PGL-1 localization defect of *glh-1(lf)* mutants. We identified five new mutations that partially disrupt the localization of GFP-tagged PGL-1 (GFP::PGL-1) to P granules (Fig. 1 and data not shown). These mutations include *bn125,* a new allele of *glh-1* that will be described elsewhere, and four independent mutations in the *deps-1* (*de*fective *P* granules and *s*terile) gene (*bn113*, *bn121*, *bn124* and *bn128*). Like *glh-1(lf)* mutations, *deps-1* mutations disrupt the localization of endogenous PGL-1 and the related protein PGL-3 to P granules (data not shown). This suggests that *deps-1* is a new member of the P-granule formation pathway, and that it functions upstream of the PGL family of proteins.

DEPS-1 is a novel P-granule-associated protein

deps-1 was mapped to the left arm of LGI using snip-SNP bulk segregant analysis (Wicks et al., 2001) and other standard genetic techniques (data not shown). The PGL-1 localization defect of *deps-1(bn121)* animals was rescued by germline transformation with genomic sequences containing the gene Y65B4BL.2, which by in situ hybridization appears to be strongly expressed in germ cells at all stages of development (Y. Kohara, personal communication). Furthermore, RNA interference (RNAi) of the Y65B4BL.2 gene disrupted the localization of GFP::PGL-1 to P granules and caused sterility at 24.5ºC, two distinctive phenotypes that are characteristic of *deps-1* mutants (Figs 1, 3; data not

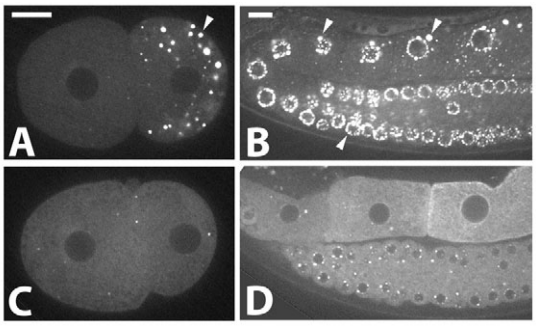

Fig. 1. GFP::PGL-1 is mis-localized in *deps-1* mutants.
(**A**,**B**) GFP::PGL-1 localizes to P granules in a wild-type two-cell embryo (A) and in an adult hermaphrodite germ line (B). P granules (arrowheads) are cytoplasmic in the posterior cell of two-cell embryos (A), perinuclear in pachytene germ cells (lower half of B), and both cytoplasmic and perinuclear in oocytes (upper half of B). (**C**,**D**) GFP::PGL-1 localizes poorly to P granules in a *deps-1(bn121)* two-cell embryo (C) and hermaphrodite germ line (D). Single-section confocal images of the mid-regions of embryos and germ lines are shown. Equivalent exposure times and settings were used to image A and C, and B and D. Scale bars: 10 μm.

shown). Finally, sequence analysis of Y65B4BL.2 identified allele-specific lesions in all four *deps-1* mutants (Fig. 2A), indicating that Y65B4BL.2 is *deps-1*.

deps-1 encodes a novel protein with a serine-rich C-terminal domain of low amino acid complexity (Fig. 2A). DEPS-1 is 45-51% identical to predicted proteins in the closely related nematodes *C. briggsae* and *C. remanei*, but does not resemble identified proteins in other organisms. Affinity-purified polyclonal antibodies raised against DEPS-1 stain both P granules and nuclei in germ lines, and developing embryos of all stages (Fig. 2C-F, 2I-N and data not shown). Several findings suggest that the P-granule stain is due to DEPS-1, but that the nuclear stain is due to cross-reactive material. First, in *deps-1* mutants, the P-granule stain and a DEPS-1-sized band (~69 kDa) on western blots are absent, while nuclear stain and a band of ~120 kDa persist (Fig. 2B,E). Second, a DEPS-1::GFP fusion protein expressed in the *C. elegans* germ line is cytoplasmic and concentrated on P granules in adult germ lines and late stage embryos; nuclear GFP is not observed (Fig. 2G,H). These imaging results reveal that DEPS-1 is a new constitutive component of P granules.

Most of the *deps-1* mutations identified in our screen are predicted to generate truncated DEPS-1 proteins (Fig. 2A). However, truncated proteins were not detected on western blots of any of the *deps-1* mutants (Fig. 2B and data not shown), suggesting that all four *deps-1* mutations are strong loss-of-function or null. Consistent with this observation, all four *deps-1* mutants and *deps-1/Df* animals display similar defects at high temperature (24.5°C, data not shown). We consider *deps-1(bn124* and *bn121)*, the two alleles with the earliest premature stop codons (Fig. 2A), to represent likely *deps-1(null)* alleles and describe their phenotype in the following sections.

deps-1 is important for fertility and germ cell proliferation

Lack of the two constitutive P-granule components PGL-1 and GLH-1 causes a germ cell proliferation defect that is sensitive to temperature and maternal genotype (Kawasaki et al., 2004; Kawasaki et al., 1998) (C.A.S. et al., unpublished). We examined the fertility of *deps-1* mutants at multiple temperatures (15, 20 and 24.5°C) and found that lack of DEPS-1 causes similar defects.

deps-1 mutant embryos from heterozygous (*deps-1*/+) mothers inherit maternally provided DEPS-1 but are unable to synthesize zygotic DEPS-1. These animals, referred to as *deps-1* M+Z– mutants, are typically >90% fertile at 15-24.5°C. The self-progeny of *deps-1* M+Z– mutant hermaphrodites lack maternal DEPS-1 and are unable to synthesize DEPS-1. These *deps-1* M–Z– mutants tend to be sterile at high temperature (24.5°C) and fertile at lower temperatures (15 and 20°C) (Fig. 3A and data not shown). For example, 93% of *deps-1(bn121)* M–Z– mutants raised at 24.5°C are sterile, while only 22-31% of *deps-1(bn121)* M–Z– mutants raised at 15-20°C are sterile (Fig. 3A). These results indicate that *deps-1* sterility is both temperature-sensitive and maternal effect, similar to null mutations in *pgl-1*, which cause 75-85% sterility at 25°C and 7-19% sterility at lower temperatures in the M–Z– generation (Kawasaki et al., 1998).

Most *deps-1* M–Z– sterile hermaphrodites raised at 24.5-26°C fail to make embryos or oocytes and have an empty uterus (Fig. 3A and data not shown). We examined the germ lines of hermaphrodites raised at 24.5°C and found that they frequently lack gametes and have reduced numbers of germ cells compared with wild type (Table 1). For example, roughly half (56%, *n*=48) of the *deps-1(bn121)* germline arms examined had fewer than 200 germ cell nuclei and 63% lacked both sperm and oocytes. On average, *deps-1(bn121)* adult hermaphrodites raised at 24.5°C have 254 germ cells per gonad arm (s.d.=236, *n*=16, range 10-762), while wild-type adults have an average of 586 germ cells (s.d.=45, *n*=6, range 526-651). Furthermore, decreased numbers of germ cells are often observed in *deps-1(bn121)* M–Z– adult males and L2-L4 stage hermaphrodite larvae raised at 24.5°C when compared with wild-type animals (data not shown). Taken together, these observations suggest that *deps-1* M–Z– mutant larvae raised at 24.5°C have partially penetrant defects in germ cell proliferation similar to those described for *pgl-1* M–Z– mutants at 26°C (Kawasaki et al., 2004; Kawasaki et al., 1998).

deps-1 is important for embryonic viability

Fertile M–Z– *deps-1* mutants raised at 15-20°C often have very few progeny, and sterile hermaphrodites at 15°C typically lay eggs that fail to hatch (Fig. 3A and data not shown). To investigate whether DEPS-1 promotes embryonic viability, we counted the number of eggs laid by *deps-1* M+Z– and M–Z– mutants, and determined whether they were able to complete embryonic development and hatch into larvae (Fig. 3B). *deps-1* mutations cause variably penetrant embryonic lethality with no uniform stage of developmental arrest (data not shown). This lethality appears to be cold sensitive and influenced by maternal genotype: it is most penetrant in eggs laid by *deps-1* M–Z– mutants at 15°C (Fig. 3B and data not shown). In addition, the total number of eggs laid by *deps-1* mutants is also influenced by maternal genotype (Fig. 3B and data not shown). Notably, the amount of *deps-1* embryonic lethality observed in our experiments (44-74% in the M–Z– generation at 20°C) is considerably higher than that seen in *pgl-1* M–Z– animals at the same temperature (Kawasaki et al., 2004), suggesting that DEPS-1 has functions in the *C. elegans* germ line and embryo in addition to promoting the localization of PGL-1 to P granules.

DEPS-1 promotes the accumulation of *glh-1* mRNA and protein

Loss-of-function mutations in *glh-1*, which encodes a P-granule-associated VASA-like RNA helicase (Gruidl et al., 1996), disrupt the localization of PGL-1 to P granules and cause germ cell proliferation

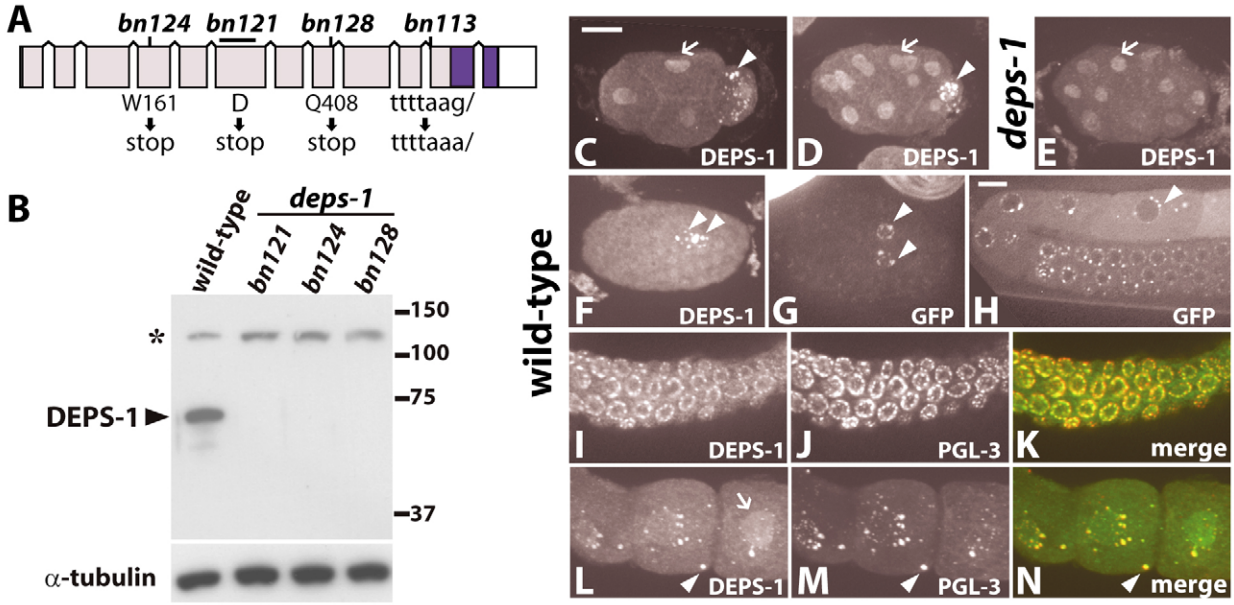

Fig. 2. DEPS-1 associates with P granules. (**A**) The predicted Y65B4BL.2/*deps-1* gene and locations of mutant alleles. Coding region is in gray and UTRs are in white. In blue is a 61 amino acid region containing 21 serines, 7 threonines, 8 arginines and 7 alanines. *bn121* removes nucleotides 988-1172 (horizontal line) and results in a predicted transcript with a stop immediately after amino acid M246. *bn113* mutates a conserved residue in the 3′ splice site. Amino acid and nucleotide sequence positions are with respect to the predicted translation start site. (**B**) Western blot analysis using affinity-purified anti-DEPS-1 and anti-α-tubulin as a loading control. The arrowhead indicates the position of DEPS-1. The asterisk indicates a ~120 kDa band present in wild type and in *deps-1* mutants. Molecular mass of protein standards in kDa is on the right. (**C-F**) Anti-DEPS-1 staining pattern of wild type (C,D,F) and *deps-1(bn121)* (E) embryos at advancing stages of development (C, four cell; D,E ~16 cell; F ~100 cell). (**G**,**H**) DEPS-1::GFP is concentrated on P granules in ~100-cell embryos (G) and in germ lines and oocytes (H). (**I-N**) DEPS-1 and PGL-3 co-localize on P granules in wild-type germ lines (I-K, surface of the pachytene region) and oocytes (L-N). Merged images show DEPS-1 in green and PGL-3 in red. Arrowheads and arrows indicate P granules and nuclei, respectively. H-K are single-section confocal images; all other images are confocal stacks. Scale bars: 10 μm. Images C-G and I-N use the scale bar in C.

defects similar to those described for *pgl-1* and *deps-1* (Kawasaki et al., 1998) (C.A.S. et al., unpublished). To determine whether GLH-1 localizes to P granules in *deps-1* mutants, we stained fertile *deps-1* hermaphrodites (M+Z– raised at 24.5°C) with an antibody that specifically recognizes GLH-1. GLH-1 localizes to P granules in *deps-1* mutants but protein levels appear to be significantly reduced (Fig. 4A-D). We used western blot analysis to quantify the relative amounts of GLH-1 present in wild-type and *deps-1* gravid adult hermaphrodites, and found that GLH-1 levels are reduced ~5- to 10-fold in M+Z– *deps-1* mutants raised at 24.5°C (Fig. 4E). The reduction in GLH-1 levels in *deps-1* mutants is sensitive to both temperature and maternal genotype (data not shown). In the M+Z– generation, the reduction in GLH-1 levels in *deps-1* relative to wild-type was ~2- to 3-fold or less at 15°C, compared with ~5- to 10-fold at 24.5°C. However, in the next generation (M–Z–), even 15°C *deps-1* hermaphrodites displayed a ~5- to 10-fold reduction in GLH-1.

We used quantitative RT-PCR to examine whether DEPS-1 is important for *glh-1* mRNA accumulation. The relative amount of *glh-1* mRNA was reduced ~5- to 10-fold in M+Z– *deps-1* gravid adult hermaphrodites raised at 24.5°C (Fig. 4E and data not shown). In contrast to GLH-1 protein levels, reduction in *glh-1* mRNA levels in *deps-1* mutants was not sensitive to temperature or maternal genotype: *glh-1* mRNA levels were reduced ~5- to 10-fold in *deps-1* mutants at all temperatures tested and in both the M+Z– and M–Z– generations (data not shown). This reduction is specific to *glh-1*, as mRNA levels of the germline-expressed genes *pgl-1* and *him-3* do not change significantly in *deps-1* mutants by quantitative RT-PCR (data not shown). These results suggest that DEPS-1 is required to produce or stabilize *glh-1* mRNAs.

Genome-wide microarray analysis identifies genes that are mis-regulated in *deps-1* germ lines

P granules contain mRNAs and may be involved in regulating mRNA transport as well as mRNA stability or translation (Pitt et al., 2000; Schisa et al., 2001; Seydoux and Braun, 2006). Because DEPS-1 localizes to P granules and promotes the accumulation of *glh-1* mRNA, DEPS-1 is a potential link between P granules and mRNA stability. Mutations in *deps-1* probably also alter the structure of P granules, as DEPS-1 promotes the localization and accumulation of PGL-1 and GLH-1, respectively, and both PGL-1 and GLH-1 appear to be important for proper P-granule morphology in the adult germ line (Schisa et al., 2001).

We performed a genome-wide analysis of mRNA levels to determine whether the P-granule defects described above generally alter mRNA levels in *deps-1* mutant germ lines. Total RNA was isolated from wild-type and *deps-1(bn124)* dissected gonads (M–Z– generation) raised at 20°C. Polyadenylated mRNAs were linearly amplified, labeled with Cy3 and Cy5 and hybridized to microarrays containing ~17,600 of the 20,000 predicted genes in the *C. elegans* genome (Chi and Reinke, 2006). These experiments identified only 13 genes, including *glh-1* and *deps-1*, that are downregulated and 32 genes that are upregulated at least 1.8-fold ($P<0.05$) in *deps-1* mutant germ lines compared with wild type (Table 2). Thus, despite

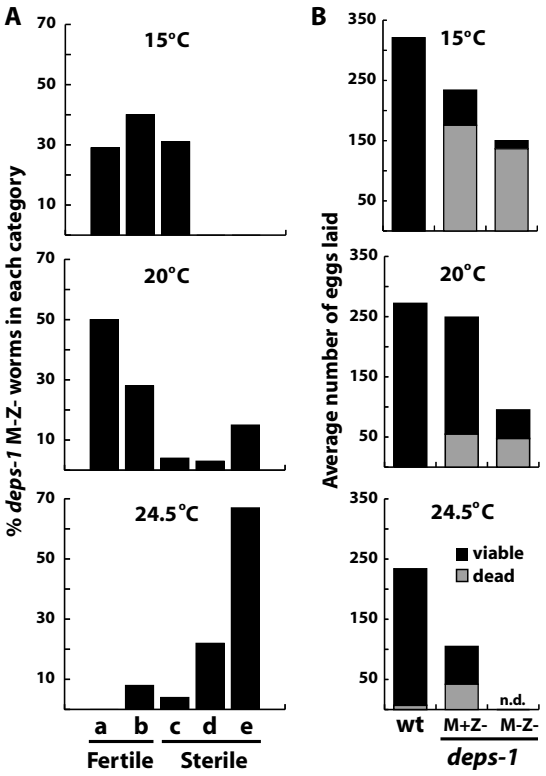

Fig. 3. *deps-1* mutants display temperature-sensitive sterility and embryonic lethality. (**A**) Fertility profiles of *deps-1(bn121)* M–Z– at 15, 20 and 24.5°C. Mutant hermaphrodites were picked to individual plates as L4s, and the fertility of each animal scored several days later. Animals that generated (a) >20 or (b) 1-20 larval progeny are defined as fertile. Animals that did not have larval progeny but (c) laid dead eggs, (d) produced oocytes or oocyte-like material, or (e) failed to make either embryos or oocytes are defined as sterile. Numbers of adults examined were 45 (15°C), 132 (20°C) and 105 (24.5°C). (**B**) Number and quality of eggs laid by *deps-1(bn121)* M+Z– and M–Z– adults at 15 and 20°C and *deps-1(bn121)* M+Z– adults at 24.5°C. n.d., not determined.

Table 1. *deps-1* M–Z– sterile hermaphrodites have few germ cells and often lack gametes

		% Germline arms			
deps-1 allele	% Fertile adults* (n)	With few germ cells†	Lacking sperm	Lacking oocytes	Lacking gametes
+	100 (24)	0	2	10	0
bn113	13 (24)	31	44	77	33
bn121	0 (24)	56	79	79	63
bn124	13 (23)	65	59	83	59
bn128	8 (25)	56	58	72	52

All animals were raised at 24.5°C and stained with the DNA dye Hoechst as adults, 45-46 hours after feeding synchronized L1-stage larvae.
*Worms containing eggs in their uterus.
†Estimated to have fewer than 200 germ nuclei. These germ lines typically lack gametes.

the multiple P granule-related defects identified in *deps-1* mutants, DEPS-1 regulates the expression of relatively few genes, suggesting that GLH-1, localized PGL-1, and possibly P granules themselves do not play a global role in controlling mRNA levels in germ cells.

Although relatively few genes are mis-regulated in *deps-1* germ lines, we noted that many of the genes are members of gene families (Tables 2, 3). *glh-1* and *pgl-1* are both members of gene families and are partially redundant with other gene family members (Kawasaki et al., 2004; Kuznicki et al., 2000), suggesting that many genes related to P-granule function may have multiple genomic copies with redundant functions. Some cases of apparent mis-regulation in *deps-1* mutants of multiple members of a gene family may be due to cross-hybridization (see Table 3). However, analyses of the mRNA levels of specific gene family members by quantitative RT-PCR so far confirm the microarray results [Table 4; genes C38D9.2, F15D4.5, Y55H10B.1 and T21G5.3 (*glh-1*)]. In addition to gene families, several DEPS-1 regulated genes are clustered and either co-transcribed or possibly co-regulated (T20G5.2 and T20G5.11; F54H12.1 and F54H12.6; Y19D10A.4, Y19D10A.12 and Y19D10A.16; C01B4.6 and C01B4.7; K02B7.1 and K02B7.2; W09B7.1 and W09B7.2). Most of the clustered genes with decreased expression in *deps-1* germ lines (8/13 downregulated genes) appear to have functions related to carbohydrate metabolism (C01B4.6 and C01B4.7; Y19D10A.4, Y19D10A.12 and Y19D10A.16), the tricarboxylic acid (TCA) cycle (T20G5.2; F54H12.1) or RNA interference [T20G5.11 (*rde-4*); F54H12.1 (RNAi depletion causes RNAi resistance according to Kim et al. (Kim et al., 2005))], suggesting that *deps-1* germ lines may have defects in these processes. The idea that *deps-1* germ lines have RNAi-related defects is strengthened by the observation that many of the genes upregulated in *deps-1* germ lines are also upregulated in *rde-3* mutant worms, which are resistant to RNAi (Table 2). Connections between *deps-1* and RNAi are examined in the following sections.

DEPS-1 promotes the accumulation of *rde-4* mRNA and protein

As described, our microarray experiments identified *rde-4* as a gene that is downregulated nearly as much as *glh-1* in *deps-1* germ lines (Table 2). *rde-4* mRNA appears to be abundant in adult germ lines and may be maternally provided to early embryos (Baugh et al., 2003; Reinke et al., 2004; Y. Kohara, personal communication). We used quantitative RT-PCR to determine that *rde-4* mRNA levels are reduced 7- to 10-fold in *deps-1* gravid adult hermaphrodites (M–Z– generation) relative to wild-type animals (Fig. 4F, Table 4B and data not shown). Additional experiments indicated that *rde-4* mRNA levels are not significantly altered in *pgl-1* or *glh-1* mutants (data not shown), suggesting that this change in *rde-4* mRNA levels is specific to *deps-1*.

To investigate whether reduced *rde-4* mRNA levels in *deps-1* mutants result in reduced RDE-4 protein levels, we performed western blot analysis with an affinity purified anti-RDE-4 antibody (Tabara et al., 2002) on extracts enriched for gravid adults. RDE-4 protein levels are decreased ~10-fold in *deps-1* M–Z– mutants relative to wild-type adults (Fig. 4F). RDE-4 is a dsRNA-binding protein that is essential for RNAi in *C. elegans* (Tabara et al., 2002). At the molecular level, RDE-4 probably functions in the target tissue to recognize the long dsRNA molecules that initiate RNAi (Parker et al., 2006; Parrish and Fire, 2001; Tabara et al., 1999; Tabara et al., 2002), leading us to predict that a reduction in RDE-4 protein levels would cause measurable defects in RNAi in *deps-1* germ lines.

deps-1 mutants are resistant to germline RNAi

To determine whether *deps-1* mutants have defects in germline RNAi, we tested the effectiveness of RNAi against target genes known to be expressed in the maternal germ line and target genes

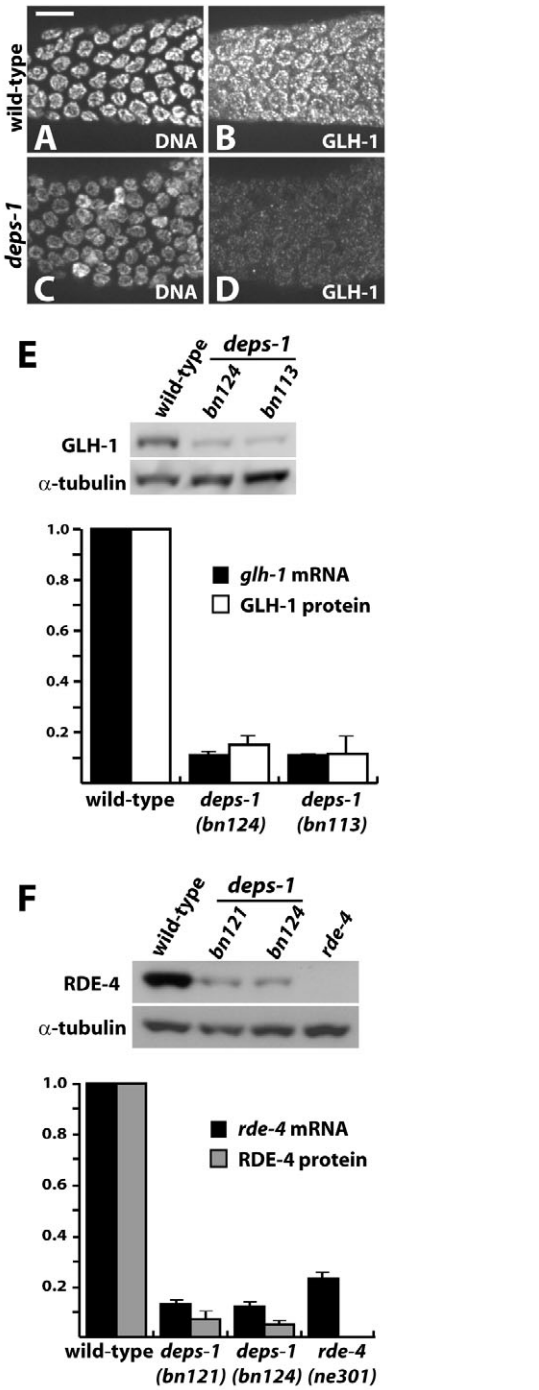

Fig. 4. DEPS-1 promotes the accumulation of *glh-1* and *rde-4* mRNA and protein. (**A-D**) Wild-type and *deps-1(bn124)* germ lines stained with PA3, a marker for chromatin (A,C), and anti-GLH-1 (B,D). A-D are stacks of three confocal sections taken at 1 μm intervals showing germ nuclei present on the surface of the pachytene region of the hermaphrodite germ line. Scale bar: 10 μm. (**E**) Western blot analysis (top panel) of GLH-1 in whole worm extracts, with α-tubulin as a loading control, and histogram (lower panel) of *glh-1* mRNA (measured by quantitative RT-PCR) and GLH-1 protein levels in *deps-1* mutants compared with wild type. (**F**) Similar analysis of *rde-4* mRNA and RDE-4 protein levels, but using partially purified worm extracts, and comparing with *rde-4* mutants and with wild type. The histograms show average ratios obtained in two independent experiments. The wild-type ratio was set at 1.0 in each experiment. For mutants, error bars indicate the s.e.m.

known to be zygotically expressed. RNAi was triggered by feeding L4 stage worms and their progeny *E. coli* expressing gene-specific dsRNAs (Timmons et al., 2001). RNAi against the maternally provided genes *pos-1*, *skn-1* and *pie-1* caused essentially all of the progeny of wild-type animals to arrest and die during embryogenesis, but did not cause a similar phenotype in *deps-1* mutants (Fig. 5A and data not shown). By contrast, RNAi against zygotically expressed genes required for proper locomotion (*unc-52*, *unc-22*, *pat-4*) or viability (*lin-26*) was not obviously different between wild type and *deps-1* mutants (Fig. 5B and data not shown).

Robert et al. previously described that mutations in the *pgl-1* gene also cause a defect in germline RNAi (Robert et al., 2005). We confirmed that a *pgl-1(null)* mutant is resistant to *pos-1(RNAi)* in our assay (Fig. 5A). We also performed *pos-1(RNAi)* on *pgl-3(null)* and *glh-1(lf)* M–Z– mutants to determine whether the P-granule defects of *deps-1* mutants (reduced GLH-1, mis-localized PGL-1 and PGL-3) might promote resistance to germline RNAi. If this were the case, we would predict that (1) *pgl-3* mutants, which have no obvious P-granule or germline defects when *pgl-1* is wild type (Kawasaki et al., 2004), would not be resistant to RNAi, and (2) *glh-1(lf)* mutations, which disrupt the localization of both PGL-1 and PGL-3 to P granules (C.A.S. et al., unpublished), would be resistant to RNAi. We found that *glh-1* and *pgl-3* mutants both exhibit a strong RNAi response to *pos-1* dsRNA (Fig. 5A) and conclude that reduced GLH-1 levels and mis-localized PGL-1 and PGL-3 are not likely to be the cause of the *deps-1* germline RNAi defect. Furthermore, these findings support the emerging view that P-granule components serve diverse roles in the germ line.

deps-1 and *rde-3* repress the expression of overlapping sets of genes

The *rde-3* (formerly called *mut-2*) gene encodes a potential poly-A polymerase and, like *rde-4*, is essential for RNAi. RDE-3 probably functions downstream of RDE-4 in the RNAi pathway, and mutations in *rde-3* (but not *rde-4*) cause numerous germline-related phenotypes, including partially penetrant embryonic lethality, temperature-sensitive sterility and transposon activation (Chen et al., 2005; Tabara et al., 1999). Lee et al. recently identified 257 genes whose expression is at least twofold up-regulated in *rde-3(ne298)* worms (Lee et al., 2006). RDE-3 may repress the expression of some of these genes in normal worms by promoting the accumulation of gene-specific endogenous short interfering RNAs (endo-siRNAs) antisense to the coding strand of mRNAs (Ambros et al., 2003; Lee et al., 2006). Endo-siRNAs resemble the short interfering RNAs (siRNAs) generated during RNAi, and may regulate the levels of specific mRNAs in wild-type worms by RNAi-related mechanisms (Duchaine et al., 2006; Lee et al., 2006; Ruby et al., 2006). Interestingly, nearly 30% of the genes upregulated in *deps-1* germ lines (9/32) are also upregulated in mixed-stage mRNA preparations made from *rde-3(ne298)* worms (Table 2), and many of these are strongly upregulated in *rde-3* worms (5/9 increased >9-fold) (Lee et al., 2006). This degree of overlap is statistically significant ($P<2.2\times10^{-9}$) and striking considering that the *deps-1* and *rde-3* experiments used different stages (adult germ line versus mixed stage worms) and microarray platforms (amplicon versus oligo probes). *rde-3(ne298)* mutant worms do not display altered levels of *deps-1*, *glh-1* or *rde-4* mRNAs (Table 4B), and *deps-1* mutant germ lines and worms do not have altered levels of *rde-3* mRNA (0.91 *deps-1*/wild-type ratio by microarray analysis; 1.1 *deps-1*/wild-type ratio by quantitative RT-PCR on gravid adults),

Table 2. Microarray results for genes whose mRNA accumulation is affected at least 1.8-fold (*P*<0.05, corrected *z* test) in *deps-1* germ lines

Genes downregulated in *deps-1* relative to wild type

WormBase ID (gene name)	Gene family*	Primer pair	wt/*deps-1* ratio	Chr	*rde-3* regulation† (wt/*rde-3* ratio)
F54H12.6		F54H12.6	10.7	III	
Y65B4BL.2 (*deps-1*)		Y65B4B_13.B	6.5	I	
T21G5.3 (*glh-1*)	1	T21G5.3	3.3	I	
T20G5.11 (*rde-4*)		T20G5.11	3.2	III	
Y19D10A.12	2	Y19D10A.L	2.7	V	
F54H12.1 (*aco-2*)		F54H12.1	2.5	III	
Y19D10A.4	3	Y19D10A.D	2.4	V	
Y19D10A.16	4	Y19D10A.N	2.2	V	
C01B4.6	4	C01B4.6	2.0	V	
C01B4.7	3	C01B4.7	2.0	V	Down (2.5)‡
F55G1.8 (*plk-3*)		F55G1.8	1.9	IV	
T20G5.2 (*cts-1*)		T20G5.2	1.9	III	
Y116A8C.30		Y116A8C.30	1.9	IV	

Genes upregulated in *deps-1* relative to wild type

WormBase ID (gene name)	Gene family*	Primer pair	*deps-1*/wt ratio	Chr	*rde-3* regulation† (*rde-3*/wt ratio)
W09B7.2	5	W09B7.B	11.6	V	Up (39.1)‡
Y43F4A.3		Y43F4A.3	8.4	III	
F11A6.2		F11A6.2	6.5	I	
C38D9.2	6	C38D9.2	5.7	V	Up (45.6)
C07G3.9 (*ugt-64*)		C07G3.9	4.7	V	
W09B7.1	7	W09B7.C	4.4	V	
W03G1.3	8	W03G1.3	4.1	IV	
C40A11.8		C40A11.8	3.3	II	
K02B7.2	8	K02B7.2	3.2	II	Up (9.2)‡
K02B7.1§	9	K02B7.1	3.2	II	
C18D4.6		C18D4.6	3.1	V	
K02E7.2	8	K02E7.2	2.9	II	
F41G4.7	10	F41G4.6¶	2.6	X	Up (3.9)¶
T23G5.6		T23G5.6	2.6	III	Up (2.2)
F15D4.5	6	F15D4.5	2.4	II	Up (50.9)
K07E8.10	8	K07E8.10	2.3	II	
C04C3.5 (*dyf-3*)		C04C3.5	2.3	IV	
ZC15.3	10	ZC15.3	2.2	V	
K08D10.5		K08D10.5	2.1	IV	
C33H5.4 (*klp-10*)	11	C33H5.4	2.0	IV	
D2045.2	12	D2045.2	2.0	III	
T24B8.7		F37B12.4	2.0	II	
H04D03.3	12	H04D03.3	1.9	III	
R03G8.2**		R03G8.2	1.9	X	
Y39A3CL.6 (*pvf-1*)		Y39A3C_84.B	1.9	II	
C13B9.1		C13B9.1	1.8	III	
C04F12.9 (*rnh-1.3*)		C04F12.9	1.8	I	Up (17.2)
F30B5.4		F30B5.4	1.8	IV	
Y55H10B.1**	13	Y55H10B.D	1.8	IV	Up (5.2)‡
F57G4.3**	13	F57G4.3	1.8	V	Up (5.0)‡
T03D3.5		T03D3.5	1.8	V	
B0511.11		B0511.11	1.8	I	

*Numbers indicate different gene families. Members of each family are listed in Table 3.
†All *rde-3(ne298)* regulation data are from Table S1 in Lee et al. (Lee et al., 2006).
‡Oligos on *rde-3* arrays are not specific for this gene family member; some or all detect multiple gene family members.
§99% identical to R09E12.6, which is upregulated 20.88-fold in *rde-3(ne298)* mutants.
¶F41G4.6 is upregulated; F41G4.6 was recently merged into F41G4.7.
**Possible transposon (WormBase release WS172).

suggesting that RDE-3 and DEPS-1 do not regulate each other's expression. Instead, the two proteins might work together to regulate the expression of several genes in the *C. elegans* germ line.

Quantitative RT-PCR was used to verify that five of the genes upregulated in both *deps-1* and *rde-3* mutants by microarray analysis are indeed upregulated in *deps-1* mutant germ lines. Multiple cDNAs that may represent endogenous siRNAs were previously isolated for four of these genes (Table 4A), making them plausible targets of endo-siRNA-mediated repression (Ambros et al., 2003; Lee et al., 2006). All five genes were strongly upregulated (~4- to 326-fold) in *deps-1* germ lines compared with wild type (Table 4A). Strikingly, one of the genes examined (C38D9.2) is upregulated several hundred-fold in *deps-*

Table 3. Gene families mis-regulated in *deps-1* germ lines

Family*	Genes†
1	**T21G5.3**, *C55B7.1*
2	**Y19D10A.12**, *C01B4.9*
3	**Y19D10A.4**, **C01B4.7**
4	**Y19D10A.16**, **C01B4.6**
5	**W09B7.2**, F07B7.2‡
6	**C38D9.2**, **F15D4.5**
7	**W09B7.1**, F07B7.1‡
8	**W03G1.3**, **K02B7.2**, **K02E7.2**, Y55F3C.11‡, *R09E12.5*, C17B7.13‡, **K07E8.10**
9	W03G1.4, **K02B7.1**, K02E7.3, *Y55F3C.6*, *R09E12.6*, *C17B7.7*
10	**F41G4.7**, **ZC15.3**
11	**C33H5.4**, *C06G3.2*
12	**D2045.2**, **H04D03.3**
13	**Y55H10B.1**, **F57G4.3**, Y71A12B.7‡

*Gene families 2-5 and 7-9 have members with aligned sequences that are ⩾99% identical. The sole exception is K07E8.10, which is 94% identical to the other members of gene family 8. Other gene families are 86-96% (10-13) or 70-78% (1, 6) identical. Genes with less than 70% identity to other family members are not listed. Alignments were generated using genomic sequences and ClustalW (Chenna et al., 2003).
†Genes in bold were mis-regulated at least 1.8-fold (P<0.05, corrected z-test, Table 2). Genes in italics are co-regulated with other gene family members, but these changes are only statistically significant according to a less stringent statistical test (P<0.05, unpaired 2-tailed t-test).
‡Not independently represented on the microarrays.

1 germ lines (Table 4A) and in *rde-3(ne298)* and *deps-1* gravid adults (Table 4B). Because RDE-3 and RDE-4 may both be required to generate specific endo-siRNAs (Lee et al., 2006), and RDE-4 levels are decreased in *deps-1* mutants, we also examined the expression of all five genes in *rde-4* mutant germ lines; none of them was strongly upregulated (Table 4A). We conclude that the upregulation of these genes in *deps-1* germ lines is not due to the defect in *rde-4* expression, and that RDE-3 and RDE-4 may generally repress the expression of different genes in the *C. elegans* germ line. These conclusions are consistent with the idea that RDE-4 is essential for RNAi initiated by long dsRNA molecules, but not required for the initiation of other RNAi-related pathways that require RDE-3 (Grishok, 2005).

DISCUSSION

Loss of DEPS-1 and RNA accumulation defects

DEPS-1 is a P-granule-associated protein important for the localization of PGL-1 and the accumulation of *glh-1* and *rde-4* mRNA and protein. It is also required for fertility, embryonic viability, germline RNA interference and to repress the expression of genes also repressed by RDE-3 in the *C. elegans* germ line. It seems likely that many or all of these defects are the result of RNA accumulation defects in *deps-1* mutant germ lines: (1) reduced levels of *glh-1* mRNA and protein probably cause or contribute to the PGL-1 localization and fertility defects; (2) reduced levels of *rde*-4, and possibly F54H12.1 (*aco-2*), mRNA and protein probably cause or contribute to the germline RNAi defect; and (3) reduced levels of specific endo-siRNAs could cause the overexpression of genes repressed by RDE-3. Furthermore, localization of DEPS-1 to cytoplasmic P granules suggests that if DEPS-1 is directly involved in regulating mRNA or endo-siRNA accumulation, it does so post-transcriptionally. Although many RNAs are thought to pass through and be at least transiently concentrated in P granules in the adult germ line (Schisa et al., 2001), the loss of DEPS-1 does not dramatically affect the mRNA accumulation profile in germ lines. This suggests either that the compromised P granules present in *deps-1* mutants are largely functional or that P granules do not play a major role in stabilizing mRNAs in the *C. elegans* germ line.

Does DEPS-1 bind RNA?

More than 20 P-granule-associated proteins have been identified, and most are predicted to interact physically with RNA or are clearly implicated in RNA-related processes (Strome, 2005). These processes include translation (GLD-1, IFE-1), polyadenylation (GLD-2, GLD-3), splicing (Sm proteins), 5′ decapping and decay (CGH-1, DCAP-2), and P-granule assembly or stability (PGL-1, GLH-1) (reviewed by Strome, 2005; Seydoux and Braun, 2006). DEPS-1 does not have an obvious RNA-binding domain or motif but has a C-terminal serine-rich domain that also contains several arginines. This domain is distinct from the C-terminal Arg-Ser (RS) domains of splicing-related SR proteins, but shares at least one unusual characteristic with RS domains: both are predicted to lack defined structures (Haynes and Iakoucheva, 2006) (data not shown).

Table 4. Quantitative RT-PCR analysis of *deps-1*-regulated genes

A Genes upregulated in *deps-1* and *rde-3* mutants are not upregulated in *rde-4* germ lines (isolated gonads, 20°C)

		Amount of mRNA relative to wild-type gonads		
Wormbase ID	endo-siRNAs*	*deps-1(bn124)*†,‡	*rde-4(ne301)*‡	Wild type‡,§
C38D9.2	5	326.5	1.0	1.1
F15D4.5	10	28.9	1.1	1.4
T23G5.6	2	17.4	1.3	1.4
Y55H10B.1	0	7.0	2.0	0.9
C04F12.9	6	3.9	0.9	0.8

B *glh-1* and *deps-1* are not downregulated in *rde-3* or *rde-4* mutants (gravid adults, 20°C)

	Amount of mRNA in each mutant relative to wild type		
Wormbase ID (gene)	*deps-1(bn124)*†,‡	*rde-4(ne301)*‡	*rde-3(ne298)*‡
T21G5.3 (*glh-1*)	0.13	1.07	1.03
Y65B4BL.2 (*deps-1*)	0.04	0.92	0.86
T20G5.11 (*rde-4*)	0.12, 0.14	0.20, 0.26	1.14
C38D9.2	307.23	1.01	1330.44

*Number of small anti-sense cDNAs isolated from each gene according to Table S1 in Lee et al. (Lee et al., 2006).
†*deps-1* M-Z- mutants.
‡mRNA levels normalized relative to *ama-1* mRNA levels and compared with wild type.
§Two independent wild-type samples were compared.

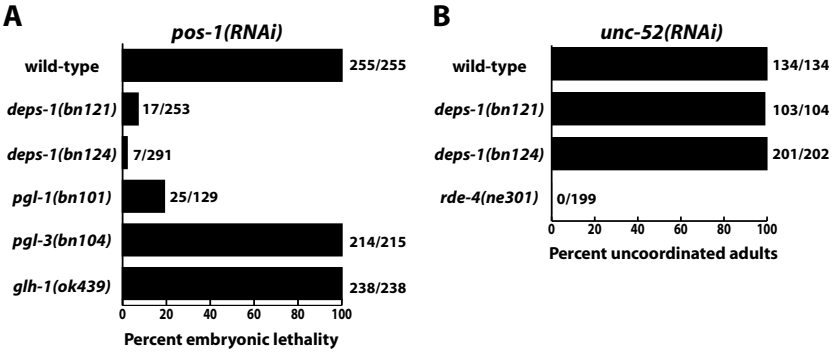

Fig. 5. *deps-1* mutants are resistant to RNAi of the germline-expressed gene *pos-1*. (**A**) *pos-1(RNAi)* causes highly penetrant embryonic lethality among the progeny of wild-type animals but not *deps-1* M+Z– or *pgl-1* M–Z– mutants raised at 20°C. The number of dead embryos/total embryos is indicated on the right. (**B**) *unc-52(RNAi)* causes the progeny of wild-type and *deps-1*, but not *rde-4*, mutants to become paralyzed as adults. The number of paralyzed/total worms is on the right.

Such 'intrinsically unstructured' regions are frequently important for protein function (Tompa, 2002). RS domains are important functional motifs that interact with other RS domains as well as RNA (Shen et al., 2004). We speculate that the serine-rich C-terminal domain of DEPS-1, also present in other *Caenorhabditis* DEPS-1-like proteins, is a protein or RNA interaction domain important for DEPS-1 function.

The conditional nature of the *deps-1* phenotype

One of the most intriguing observations about *deps-1*, *pgl-1* and *glh-1* is that animals with null or strong loss-of-function mutations in these genes have temperature-sensitive defects in germ cell proliferation (Kawasaki et al., 1998) (C.A.S. et al., unpublished). Studies of *pgl-1* and *glh-1* suggest that the conditional nature of these defects is due to functional redundancy with other members of their respective gene families. For example, *pgl-1; pgl-3* M–Z– and *glh-1 glh-4* M–Z– animals are sterile even at low temperatures (Kawasaki et al., 2004; Kuznicki et al., 2000) (C.A.S. et al., unpublished). Is a reduction in GLH-1 levels the cause of *deps-1* sterility? We think it is not the sole cause, because *deps-1* M–Z– animals display high sterility at 24.5°C, while *glh-1(lf)* M–Z– animals require higher temperatures (25-26°C) to display high sterility (C.A.S. et al., unpublished). DEPS-1 may therefore promote fertility and germ cell proliferation by regulating factors in addition to GLH-1. GLH-4 is an obvious candidate for such a factor; it appears to be well-expressed in *deps-1* mutants but localizes poorly to P granules (data not shown). It is possible that the function of GLH-4 is mildly impaired in *deps-1* mutants and this impairment enhances the sterility defect caused by reduced levels of GLH-1, resulting in highly penetrant sterility at 24.5°C but not at lower temperatures.

A second interesting aspect of the *deps-1* phenotype is the fact that, at 15°C, GLH-1 protein levels are reduced only ~2- to 3-fold in the M+Z– generation, even though *glh-1* mRNA levels are decreased 5- to 10-fold. This is different from 24.5°C, where *glh-1* mRNA and protein levels both are reduced 5- to 10-fold in the M+Z– generation. Several groups have noted that GLH-1 protein is more abundant at low temperatures than high temperatures (Orsborn et al., 2007; Walstrom et al., 2005) (data not shown), suggesting that GLH-1 is thermolabile. If GLH-1 is very stable at 15°C, perdurance of GLH-1 might obscure the effects of a reduction in *glh-1* mRNA levels in the M+Z– generation. In that case, we would expect GLH-1 levels to decrease 5- to 10-fold in the next generation, as we have observed for *deps-1* M–Z– animals at 15°C.

P granules, RNA interference and endo-siRNAs

Extensive genetic screens have been performed looking for mutants that are resistant to germline RNAi (Tabara et al., 1999) (C. Mello, personal communication). None of these screens identified mutations in *deps-1* as RNAi resistant. Two explanations seem likely. (1) *deps-1* mutations cause highly penetrant embryonic lethality in the M–Z– generation at low temperatures. This phenotype makes it difficult to maintain *deps-1* mutants as homozygotes, and also makes the RNAi resistance phenotype of *deps-1* mutants look less dramatic when a germline gene that causes embryonic lethality is targeted. (2) *deps-1* mutants may have a mild or hypomorphic defect in germline RNAi. In the course of our RNAi experiments with *deps-1*, we performed RNAi on the housekeeping gene *ama-1*, which is required maternally for embryonic viability. We found that *deps-1* M+Z– mutants produced viable eggs and were clearly resistant to *ama-1(RNAi)*, as *deps-1(+) ama-1(RNAi)*-treated control animals produced 100% dead eggs. We allowed the M–Z– progeny of these RNAi-treated M+Z– animals to grow up and found, to our surprise, that they produced increased proportions of dead eggs (data not shown). This observation suggests that *deps-1* germ lines may initiate RNAi after prolonged exposure to a dsRNA trigger and is consistent with the idea that RDE-4 levels are decreased, but not absent, in *deps-1* germ lines. Both *deps-1* phenotypes would make *deps-1* mutants unlikely to emerge in screens for homozygous viable mutants with strong defects in germline RNAi.

P granules are likely to be involved in multiple RNA-related processes in the *C. elegans* germ line (Pitt et al., 2000; Seydoux and Braun, 2006), so it is reasonable to think that P-granule components like DEPS-1 and PGL-1 could be directly involved in RNAi. Indeed, several connections between RNAi and germ granules have recently become apparent. Proteins involved in RNAi-related processes localize to germ granules (nuage or polar granules) in *Drosophila* (Lim and Kai, 2007; Megosh et al., 2006; Pane et al., 2007) and to P bodies, RNP particles in somatic cells that may be related to germ granules, in several organisms (Eulalio et al., 2007). Dicer, the endonuclease that processes precursor RNAs into siRNAs and miRNAs, localizes to germ granules in the male germ cells of mice and interacts with MVH, a mouse VASA homolog (Kotaja et al., 2006). Interestingly, the ectopic expression of at least some components of P granules in *C. elegans* somatic cells in retinoblastoma (Rb) pathway mutants is correlated with an enhanced response to RNAi (Wang et al., 2005). Despite these connections, we find that P-granule components are not generally required for RNAi (Fig. 5 and data not shown; D. Conte, personal communication). We think the simplest explanation for *deps-1* RNAi resistance is that DEPS-1 promotes the accumulation of RDE-4, a protein with a well-established role in RNAi (Tabara et al., 2002). The basis for *pgl-1* RNAi resistance is not currently known.

The observation that genes upregulated in a second mutant required for germline RNAi (*rde-3*) are frequently upregulated in *deps-1* germ lines suggests that *deps-1*, and possibly other P-granule

components, might be involved in a second RNAi-related process: the accumulation or function of specific endogenously expressed short interfering RNAs (endo-siRNAs). Endo-siRNAs appear to be a diverse group of small RNAs generated by multiple pathways. Their accumulation can depend on RDE-3, RDE-4 and other identified components of the RNAi machinery, suggesting that they repress gene expression by an RNAi-related mechanism (Duchaine et al., 2006; Lee et al., 2006; Ruby et al., 2006). Intriguingly, multiple components of *Drosophila* nuage promote the accumulation of a distinct group of short interfering RNAs, known as rasiRNAs, from repeated elements; rasiRNAs are thought to repress the expression of selfish genetic elements in the *Drosophila* germ line (Lim and Kai, 2007; Pane et al., 2007). DEPS-1 might function in an analogous manner and promote the accumulation of specific germline-expressed endo-siRNAs. Experiments are currently under way to determine whether endo-siRNAs associated with *rde-3/deps-1* upregulated genes accumulate normally in *deps-1* mutants (D. Conte, personal communication).

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