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glh-1, a germ-line putative RNA helicase from *Caenorhabditis*, has four zinc fingers

(DEAD-box protein/glycine-rich repeats/adenylylation control elements/*nos* response elements/*Ascaris*)

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ABSTRACT **We have cloned a family of putative RNA helicases from the free-living nematode *Caenorhabditis elegans*. One of these, a cDNA that we call *glh-1*, most closely matches in sequence and expression the previously described germ-line helicases PL10 from mouse and vasa from *Drosophila*. The amino terminus of the predicted protein of *glh-1* contains a set of glycine-rich repeats similar in location and sequence to those in the predicted vasa protein. However, unlike all other putative RNA helicases, glh-1 also contains four retroviral-type zinc fingers. The RNA expression pattern of this *Caenorhabditis* helicase correlates with the presence of germ-line tissue in the parasitic nematode *Ascaris lumbricoides* var. *suum* and with the presence of germ cells in wild type and several germ-line mutants of *Caenorhabditis*. In the germ-line mutants *glp-4* and *glp-1*, additional larger species of *glh-1* RNA exist, which correspond to different adenylylated forms of the *glh-1* transcript; these may be specified by motifs in the 3′ untranslated region of *glh-1* that are similar to adenylylation control elements and *nos* response elements.**

RNA helicases are proteins that unwind helical secondary structures in RNA molecules. An *in vitro* ATP-dependent RNA helicase activity has been demonstrated for the cellular proteins eIF-4A, a eukaryotic translation initiation factor (1) and P68, a nuclear protein (2). Other proteins have been assigned to the same family of RNA helicases on the basis of conserved sequences, primarily within seven different "helicase" motifs spanning ≈300 aa (3, 4). This family of RNA helicases shares a "DEAD box" (Asp-Glu-Ala-Asp) in helicase motif II, the B motif of the ATP-binding domain (5). More recently, proteins with a related "DEAH" motif have been included as a distinct subset (6).

In yeast and *Drosophila*, several proteins belong to the DEAD/H-box family of RNA helicases (7–11). One family member present in probably all eukaryotes is eIF-4A. The eIF-4A gene is highly conserved in mouse (1), yeast (12), tobacco (13), and nematodes (14). Other RNA helicases are much larger than eIF-4A, primarily because of additional amino-terminal sequences which may provide additional specificity to the RNA helicase activity or even additional protein function. Thus, the increasing number of proteins in the DEAD/H family may reflect different functions, RNA substrates, or tissue specificities of RNA helicases (6, 8).

Most putative RNA helicases have a role in one of two cellular processes, protein synthesis or RNA splicing; this includes the assembly of ribosomes and initiation factor complexes of the protein synthesis machinery and the assembly/disassembly of spliceosomes and the actual splicing steps in the pre-RNA splicing machinery (6, 8). Several putative RNA helicases have a role in cell growth and development, including the murine PL10 protein, which is expressed in the male germ line (15), the *Xenopus* An3 protein, which is localized to the animal pole of the *Xenopus* oocyte and embryo (16), and the *Drosophila* proteins vasa (9, 10) and ME31B (11), which are expressed primarily in the female germ line. These may impart a developmental or tissue-specific level of regulation to RNA translation, RNA splicing, or yet uncharacterized RNA helicase functions.

To examine the role of RNA helicases in the development of *Caenorhabditis elegans*, a free-living soil nematode for which the entire cell lineage has been established, we have cloned several *C. elegans* RNA helicases. We have focused on those DEAD/H-box proteins that display tissue-specific or developmentally regulated expression. Here, we describe the germ-line pattern of expression for a gene that we call *glh-1*. The presence and level of *glh-1* RNA in four different germ-line mutants correlate with the presence and relative number of germ cells and/or germ cell nuclei. The predicted glh-1 protein† is most similar to a subset of RNA helicases that includes vasa (9, 10), PL10 (15), An3 (16), and the splicing-machinery helicase Ded1 (17). However, glh-1 is distinguished by features not observed for other RNA helicases. There appear to be different polyadenylylated forms of the *glh-1* RNA. The polyadenylylation differences may be specified by motifs in the 3′ untranslated region (UTR) of *glh-1* that are similar to adenylylation control elements (ACEs) (18) and *nos* response elements (NREs) (19) found in maternal RNAs of other organisms. The predicted glh-1 protein is also the only putative DEAD-box RNA helicase that has retroviral-type zinc finger motifs.

MATERIALS AND METHODS

Polymerase Chain Reaction (PCR). Degenerate oligodeoxynucleotide primers were designed from the amino acid sequences PTRELA and DEADRM: 5′-CCAAC(C/T)(A/C)GNGA(A/G)(C/T)TNGC-3′ and 5′-CATNC(T/G)(A/G)TCNGC(C/T)TC(A/G)TC-3′, respectively. Reaction conditions were as described (20). A standard 50-μl reaction mixture contained 50 ng of *C. elegans* genomic DNA and 25 pmol of primer. Twenty-five cycles of amplification were performed in a temperature cycler (Coy, Ann Arbor, MI) with denaturation (94°C) for 1 min, primer annealing (49°C) for 1.5 min, and primer extension for 0.5 min, followed by a 10-min incubation at 72°C. Reaction products were purified and cloned.

Sequence Analysis. Both strands of all clones and subclones were sequenced by a combination of dideoxy chain termination, with Sequenase II (United States Biochemical) and [α-[^{35}S]thio]dATP, and *Taq* "Dye Deoxy Terminator Cycle"

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Abbreviations: DEAD, Asp-Glu-Ala-Asp; ACE, adenylylation control element; NRE, *nos* response element; UTR, untranslated region; NBP, nucleic acid-binding protein; CNBP, cellular NBP.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. L19948).

reactions, with an automated sequencer (Applied Biosystems), using T3, T7, and *glh-1*-specific primers (automated sequencing and primer synthesis were done at the DNA Core Facility of the University of Missouri-Columbia).

cDNA and Genomic Library Screens and Genomic Southern Blot Analysis. The insert of a PCR clone, rhel-1, was ^{32}P-labeled to screen a cDNA library made from mixed-stage RNA of *C. elegans* (provided by S. Kim, University of California, San Francisco) and a genomic library constructed from high molecular weight DNA from the N2 strain of *C. elegans* (K.L.B., unpublished data). Hybridization was at 68°C 5× SSPE, (1× is 0.18 M NaCl/10 mM sodium phosphate, pH 7.4/1 mM EDTA) containing 5× Denhardt's solution, 0.1% SDS, and denatured salmon sperm DNA at 100 μg/ml; the highest stringency washes were in 0.2× SSPE/0.5% SDS at 65°C for 1 hr. Genomic Southern blots and yeast artificial chromosome (YAC) grid filters were hybridized under the same conditions to ^{32}P-labeled cDNA fragments.

Growth and Maintenance of Worms. Wild-type *C. elegans* N2 var. Bristol (21) and temperature-sensitive mutant strains were cultivated in liquid culture (22). The germ-line proliferation mutants *glp-4* (*bn-2*) (23) and *glp-1* (*q231*, a weak allele) (24) were kindly provided by S. Strome and J. Kimble, respectively. The feminizing mutant *fem-1(lf)* (*hc17*) (25) and the masculinizing mutant *fem-3(gf)* (*q24*) (26) were provided by the *Caenorhabditis* Genetics Center, University of Missouri-Columbia. Populations of wild-type and mutant worms used as permissive temperature controls were grown at 15°C and were mostly adults with some third- and fourth-stage larvae (L3 and L4) when harvested for RNA. Synchronous cultures of mutant worms grown from embryos to adults at the restrictive temperature of 25°C were obtained as described (22). In brief, eggs were isolated from worms grown at 15°C and then hatched at 25°C for 24 hr in M9 medium. After addition of food, worms were grown to adults (60–72 hr) at 25°C and then harvested for RNA.

RNA Isolation and Northern Analysis. Poly(A)$^+$ RNA was isolated from nematodes as described (27). The developmental Northern filter was a generous gift from B. Dalley and M. Golomb (University of Missouri-Columbia) (28). Northern blots were hybridized at 42° for 36 hr to ^{32}P-labeled DNA fragments in the hybridization solution described above, with 50% (vol/vol) formamide.

RNase H/Oligo(dT) Analyses. These analyses were performed as described (29) with slight modifications. In brief, 3 μg of poly(A)$^+$ RNA and 750 ng of $(dT)_{12-18}$ in 10 μl of water were denatured at 100°C for 3 min, cooled for 5 min, combined with 4 μl of 5× RNase H buffer (100 mM Tris·HCl, pH 7.5/100 mM KCl/5 mM $MgCl_2$/0.5 mM Na_2EDTA), and annealed for 10 min at 22°C. For subsequent digestion at 37°C for 40 min, 25 μl of 1× RNase H buffer and 4 units of RNase H were added to the RNA/primer mixture. The RNase H reaction products were phenol/chloroform-extracted and ethanol-precipitated prior to Northern blot analysis.

RESULTS AND DISCUSSION

Generation of Hybridization Probes by PCR. We used a PCR strategy to amplify DNA fragments of the DEAD family of RNA helicase genes in the *C. elegans* genome. Sequence analysis of random PCR clones identified several potential nematode RNA helicases. One 220-bp PCR clone, which we call rhel-1, was most similar to a subfamily of putative RNA helicases (6) that includes vasa from *Drosophila*, PL10 from *Mus*, An3 from *Xenopus*, and Ded1 from *Saccharomyces*. The rhel-1 PCR clone was further used to screen a mixed-stage *C. elegans* cDNA library; at least two different genes were represented in the multiple cDNAs selected.

Cloning and Primary Sequence Analysis. The predicted amino acid sequence of one cDNA, *glh-1* (Fig. 1*A*), shares 94% identity in DNA and predicted protein sequence with the PCR clone rhel-1. Another related cDNA, *glh-2*, corresponds to the PCR clone rhel-1 (unpublished data). The *glh-1* cDNA is 2332 bp and has an open reading frame of 707 aa. All seven helicase motifs—I, Ia, and II–VI (3, 4)—are present. The DEAD motif is located at aa 443 in glh-1. The amino terminal portion of the predicted glh-1 protein is glycine-rich (51 of 100 aa); many are present in a motif of 10 aa, FGGG(N/K)-(N/T)GG(T/S)G, that is tandemly repeated five times from position 24 to 73 (Fig. 1 *A* and *B*). Adjacent to these repeats is the first of four imperfect repeats of a 20-aa sequence, which include a zinc finger motif of the $CX_2CX_4HX_4C$ type found in the retroviral NBPs (30) and the CNBP that binds a sterol receptor (31) (Fig. 1 *A* and *C*).

The initiating methionine was tentatively assigned to the first methionine of the open reading frame, leaving only 4 nt

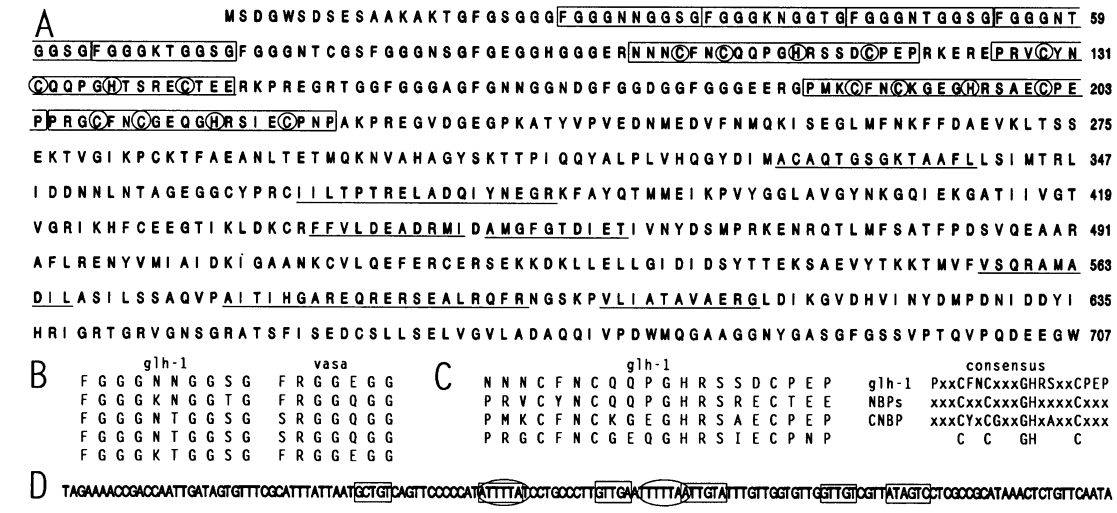

FIG. 1. Sequence analysis of *glh-1*. (*A*) Predicted amino acid sequence of the *glh-1* cDNA. The seven conserved helicase motifs are underlined. The glycine-rich repeated motifs are boxed. Four retroviral zinc finger motifs (with the characteristic CC/HC amino acids circled) are also boxed. (*B*) The five tandem repeats of a glycine-rich sequence in glh-1 are aligned vertically for comparison with those in the predicted protein of *Drosophila* vasa (9, 10). (*C*) Alignment of a 20-aa repeat in glh-1. The consensus sequence for these four repeats is compared with zinc finger motifs in retroviral nucleic acid-binding proteins (NBPs) (30) and the cellular nucleic acid-binding protein (CNBP) (31). (*D*) Potential 3′ UTR translational regulatory elements, ACE and NRE, are circled or boxed, respectively.

of 5′ noncoding sequence; therefore, this may not be a full-length cDNA. There are 157 nt of 3′ noncoding sequence including a consensus polyadenylylation site and a 14-nt poly(A) tail. Within the 3′ UTR are two motifs, (A/T)-TTTTA(T/A) (Fig. 1*D*), similar to the ACEs present in the 3′ UTRs of several maternal transcripts in mouse and *Xenopus* (18, 32). In addition, the 3′ UTR of *glh-1* has three motifs (Fig. 1*D*) that are similar to the bipartite NREs, GTTGT-N_5ATTGTA, present in the 3′ UTRs of the *Drosophila* hunchback and bicoid mRNAs (19).

Comparison of glh-1 with the DEAD Family of RNA Helicases. Within the region of conserved sequence among RNA helicases, glh-1 is most like four other helicases that have been previously grouped as a distinct subfamily of DEAD-box RNA helicases (6). Exact identities/similarities over the 399-aa conserved region of glh-1 (aa 286–684) with these predicted proteins are as follows: *Drosophila* vasa, 44%/56%; mouse PL10, 42%/58%; *Xenopus* An3, 44%/57%; yeast Ded1, 41%/55%; glh-1 therefore appears to fit into this subgroup. However, a 10-aa gap must be introduced prior to motif IV in other members of this group for maximal alignment with glh-1. And, the spacing between motifs I and Ia is intermediate in glh-1 compared with the other four. Identity of this same region of glh-1 with other RNA helicases—for example, eIF-4A (24% identity) and P68 (32% identity)—is much less.

Four zinc finger domains, present in the amino-terminal half of the predicted protein (Fig. 1 *A* and *C*), distinguish glh-1 from other known RNA helicases. The zinc fingers are of the CC/HC type found in the retroviral NBPs and the CNBP, the former of which has been shown to bind Zn^{2+}, viral genomic RNA, and coding-strand DNA (30, 33, 34). The presence of this type of zinc finger motif further predicts that the glh-1 protein interacts with a single-stranded nucleic acid, possibly an RNA or population of RNAs. The RNA-binding motif associated with helicase activity has not yet been determined for the DEAD-box family of proteins. However, because no other DEAD-box protein has zinc finger motifs, it is unlikely that the zinc fingers of glh-1 are required for helicase activity. They may instead confer additional RNA specificity to the putative helicase activity or represent an additional function unrelated to helicase activity.

The variable amino- and carboxyl-terminal portions of RNA helicases may further define their function. The similarities between the *Caenorhabditis* glh-1 and *Drosophila* vasa predicted proteins extend into these regions. The putative amino termini are MSDGWSD and MSDDWDD for glh-1 and vasa, respectively; the carboxyl termini are DEEGW and EEEGWQ for glh-1 and vasa, respectively. In addition, the amino termini of both predicted proteins contain five tandemly repeated motifs rich in glycine, although the charge and length of the repeat sequence are different (10 in glh-1 compared with 7 in vasa) (Fig. 1*B*). Other RNA helicases have glycine-rich regions (16), but none have glycine-rich tandem repeats. And, although neither a structural nor functional role has been determined for these repeats, it is unlikely that they represent random sequence arrangements. They may even distinguish these two putative RNA helicases in terms of function. RNA-binding proteins, the structural protein of plant cell walls (grp-1), and some keratins—for example, loricin—have glycine-rich domains, some punctuated with aromatic amino acids (35). The uncharged and nonhelical glycine-rich domain of some of the structural proteins is proposed to function in protein aggregation (36). Perhaps the glycine-rich repeats of vasa and glh-1 have a structural role in protein aggregation and/or localization. This possibility is especially intriguing because the vasa protein, in addition to being required for oogenesis, is also a component of the germ-line polar granules that are localized to the pole cells (germ-line lineage) of the *Drosophila* embryo. Alternatively, the glycine-rich repeats may function to bind RNA, as previously proposed (10).

Developmental and Tissue-Specific Expression of *glh-1*. To determine whether the expression of this *Caenorhabditis* gene is developmentally regulated, we conducted Northern analyses of RNAs from various nematode tissues and developmental stages. The *glh-1* cDNA hybridized to a 2.5-kb message in *C. elegans* poly(A)$^+$ RNA isolated from a mixed-stage population of nematodes (Fig. 2*A*, lane 4); upon longer exposures, cross-hybridization to the larger, 3.2-kb *glh-2* RNA was detected with the full-length *glh-1* cDNA but not with a *glh-1*-specific, 300-bp restriction fragment containing sequence encoding the glycine-rich repeats (data not shown). Because isolation of *Caenorhabditis* tissues for RNA preparation is not feasible, poly(A)$^+$ RNA was isolated from dissected tissues of *Ascaris lumbricoides* var. *suum*, a much larger, parasitic nematode closely related to *C. elegans* (37). In ovarian poly(A)$^+$ RNA, a 2.1-kb transcript hybridized to *glh-1* at high stringency and not to *glh-2* (Fig. 2*A*, lane 3, and data not shown). Longer exposures revealed a less abundant, similar-sized transcript in *Ascaris* male gonadal tissue (Fig. 2*A*, lane 2, and data not shown) but no *glh-1*-like transcript in poly(A)$^+$ RNA from either gut tissue (Fig. 2*A*, lane 1, and data not shown) or first- and second-stage larvae of *Ascaris* (data not shown). Thus the *Ascaris glh-1*-like RNA is present in germ-line tissue but not in the *Ascaris* somatic tissues examined. Further analysis of total RNA from developmental stages of *C. elegans* demonstrated that expression of the *glh-1* transcript is developmentally regulated, first detectable in the third to fourth larval stage and most abundant in the adult (Fig. 2*B*). Upon longer exposures, a weak hybridization signal was observed in RNA from the third larval stage (lane 5), but no *glh-1* transcript was detected in the embryo through second larval stages (lanes 1–3). Therefore, the timing of *glh-1* RNA expression is coincident with that of germ-line proliferation.

Expression of *glh-1* in Germ-Line Defective Mutants. To address whether the developmentally regulated expression of *glh-1* in fourth-stage larvae and adults of *C. elegans* is primarily due to germ-line expression, as also suggested by the tissue distribution in *Ascaris*, we analyzed RNAs from several germ-line mutants of *Caenorhabditis*. In wild-type *C. elegans* hermaphrodites, germ-line proliferation begins with mitotic proliferation of the germ nuclei within the syncytium of the developing gonad. In the third to fourth larval stage, the first few hundred germ nuclei enter meiosis and become male germ cells, the sperm, which are stored for fertilization. As the germ line continues to proliferate, those germ nuclei that enter meiosis become oocytes. Mature oocytes package cytoplasm from the syncytial core, which is believed to contain RNA and protein products from the germ nuclei. We

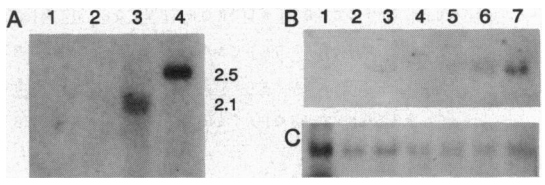

FIG. 2. Developmental Northern analysis of *glh-1*. RNAs from nematode tissues and different stages of the life cycle were hybridized to ^{32}P-labeled *glh-1* cDNA. (*A*) Each lane contained 3 μg of poly(A)$^+$ RNA from *A. lumbricoides* gut tissue (lane 1), male gonadal tissue (lane 2), and ovarian tissue (lane 3) and *C. elegans* mixed-stage worms (lane 4). RNA sizes were estimated from the mobility of an RNA ladder (BRL). (*B*) Each lane contained 10 μg of total RNA from *C. elegans* embryos (lane 1), first-, second-, dauer-, third-, or fourth-stage larvae (lanes 2–6, respectively), or adults (lane 7). (*C*) As a control, the Northern blot in *B* was also hybridized to *Caenorhabditis* heat shock protein (HSP70) cloned DNA.

examined the pattern of *glh-1* expression in germ-line mutants defective either in germ-line proliferation or in the switch from producing male to female germ cells. Poly(A)$^+$ RNA was prepared from the following temperature-sensitive mutants grown from embryos to adults at permissive (15°C) or restrictive (25°C) temperatures: *glp-4* (*bn2*), a germ-line-proliferation mutant that produces manyfold fewer germ nuclei and no oocytes or sperm (23); *glp-1* (*q231*, a weak allele), a germ-line-proliferation mutant that produces some sperm but which contains no germ nuclei due to a premature entry into meiosis (24); *fem-3(gf)* (*q24*, a weak allele), a gain-of-function masculinizing mutant that produces manyfold more sperm and no oocytes (26); and *fem-1(lf)* (*hc17*), a loss-of-function feminizing mutant that produces normal numbers of unfertilized oocytes and no sperm (25). As adult hermaphrodites, these mutants only display germ-line defects; their somatic gonads appear otherwise normal.

Both germ-line-proliferation mutants showed reduced levels of *glh-1* RNA compared with wild type. In the *glp-4*/25° mutant (no oocytes or sperm) very little *glh-1* transcript was present. Although adult *glp-4*/25° worms produce no germ cells, with age they do continue germ-line proliferation, albeit at a much slower rate, to progressively produce more germ nuclei. Because the *glp-4*/25° worms used for this study were adults for 36–48 hr, moderate germ-line proliferation had most likely occurred. Thus, if the *glh-1* transcript is produced by germ nuclei, then the low level of *glh-1* RNA in the *glp-4*/25° mutant probably reflects the reduced number of germ nuclei present. Similarly, in the *glp-1*/25° mutant (only a few sperm) the level of *glh-1* RNA was much less than that in wild type (Fig. 3). It is not known whether glh-1 RNA is synthesized before or after germ nuclei prematurely enter meiosis. Because somatic transcript levels appear elevated in this mutant, it is difficult to evaluate the relative level of *glh-1* in the *glp-1*/25° RNA. However, both germ-line proliferation mutants examined displayed a reduction in *glh-1* RNA level that paralleled their reduced numbers of germ nuclei or germ cells.

Analysis of both a feminizing and a masculinizing mutant, which have germ nuclei and either oocytes or sperm, respectively, showed no reduction in *glh-1* RNA compared with wild type. The level of *glh-1* RNA in the *fem-1(lf)*/25° mutant (oocytes only) was similar to that in wild type (Fig. 3). This could reflect *glh-1* RNA synthesis primarily in germ nuclei, oocytes, or both. However, in the *fem-3(gf)*/25° mutant (additional sperm in place of oocytes), *glh-1* RNA levels were greater than wild type (Fig. 3). Because this mutant is reported to produce and retain many more sperm than wild type, the increase in *glh-1* RNA may reflect low levels of *glh-1* RNA produced by a large number of male germ cells. Alternatively, if *glh-1* RNA is made in uncommitted germ nuclei, then increased numbers of these nuclei in *fem-3(gf)*/25° could account for the higher relative level of *glh-1* RNA. As a control for somatic transcript levels, pharyngeal myosin (38) and vitellogenin (39) gene probes were also hybridized to each Northern filter (Fig. 3). Neither control RNA was reduced in the germ-line defective mutants and their levels were parallel, even in the *glp-1*/25° mutant, which had consistently higher levels of somatic transcripts. At permissive temperatures, *glh-1* and somatic control transcript patterns were essentially wild type (Fig. 3 and data not shown).

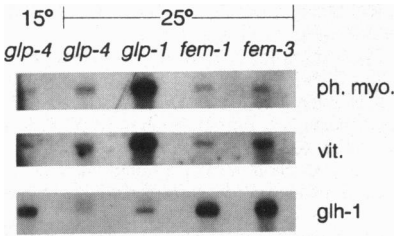

FIG. 3. Expression of *glh-1* in germ-line mutants. Each lane contained 3 μg of poly(A)$^+$ RNA from a different temperature-sensitive germ-line mutant grown at permissive (15°C) or restrictive (25°C) temperature. The Northern blot was hybridized with three cDNAs: pharyngeal myosin [ph. myo., a somatic transcript control (38)], vitellogenin [vit., a gut-specific, adult-somatic control (39)], and *glh-1*. Sizes of these RNAs are 6.1, 5.1, and 2.5 kb, respectively.

In each mutant studied, the presence of the *glh-1* transcript correlates with that of the germ-cell precursors, oocytes, or sperm in the adult hermaphrodite. Therefore, the simplest explanation is that *glh-1* is primarily a germ-line transcript. However, the data are also consistent with a germ-line-dependent but somatically expressed *glh-1* transcript. In either event, we cannot exclude additional low levels of somatic (germ-line-independent) expression of *glh-1*.

Although nematode germ-cell development differs significantly from that of *Drosophila*, *Xenopus*, or *Mus*, when compared with the transcription pattern of other RNA helicases, *glh-1* is most similar to *Drosophila* vasa. The vasa RNA is primarily detected in the female germ-line tissue but is also present in the male germ-line stem cells, cyst progenitor cells, and primary spermatocytes (40).

Polyadenylylation State of *glh-1* RNAs in the Germ-Line Mutants *glp-4* and *glp-1*. In addition to quantitative differences in *glh-1* expression in the germ-line mutants, there was also a qualitative difference. A larger, 2.8-kb *glh-1* transcript was also present in RNA from the *glp-4* and *glp-1* mutants grown at restrictive temperature (Figs. 3 and 4); larger *glh-1* transcripts were not detected in wild type or the other mutants even upon very long exposures (Figs. 2 and 3; data not shown). Multiple *glh-1* RNAs ranging in size from 2.8 to 2.5 kb, which appeared as a smear of hybridization, were also detected in *glp-4*/25° RNA. Although these RNAs could result from different promoters, alternative splicing, or different adenylylation states, the presence of potential polyadenylylation and translational regulatory sequences in the 3′ UTR suggested that *glh-1* RNAs of different polyadenylylation states may exist (Fig. 1*D*). Removal of the polyadenylate tract by first binding oligo(dT) to the RNA and then digesting with RNase H eliminated the *glh-1* transcript size variation present in both the *glp-4*/25° and *glp-1*/25° mutant RNA samples. After digestion, there was a single *glh-1* RNA species the same size as *glh-1* RNA in the treated controls, *glp-4*/15° RNA, and *glp-1*/15° RNA (Fig. 4). These results are observed with either the entire *glh-1* cDNA or a gene-specific *glh-1* cDNA subfragment (5′-most 300 bp) as a hybridization probe (Fig. 4, and data not shown); of course, hybridization signal strength was less with the smaller probe. Therefore the polyadenylylation differences are within the *glh-1* transcript and are not additional forms of the related, but distinct, *glh-2* RNA helicase. The somatic (gut-specific) esterase transcript

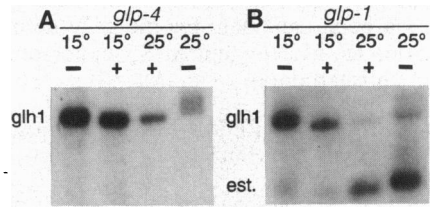

FIG. 4. Different polyadenylylated forms of *glh-1* RNA in the *glp-4* and *glp-1* mutants. RNAs from *glp-4* and *glp-1* mutant worms grown at permissive (15°C) or restrictive (25°C) temperature were hybridized to excess oligo(dT) and digested with RNase H. Treated (+) RNA samples were compared with untreated (−) control RNAs by Northern blot analysis. Each lane contained 3 μg of poly(A)$^+$ RNA from either *glp-4* (*A*) or *glp-1* (*B*) mutant worms. The hybridization probes were the 2.3-kb *glh-1* cDNA (*A*) and the 5′-most 300-bp fragment of the *glh-1* cDNA (a *glh-1* specific probe) and the esterase (est.) cDNA (control) (41) (*B*).

(41) does not exist in multiple polyadenylylated forms in these mutants (Fig. 4*B* and data not shown).

In mouse and *Xenopus* oocytes the ACE motifs present in some maternal RNAs are required for both the cytoplasmic deadenylylation of the primary transcript for storage, as well as later cytoplasmic readenylylation, presumably for translation (32). A similar process may occur in *C. elegans*. A primary, adenylylated *glh-1* transcript may persist in the *glp-1* and *glp-4* mutants because there are no (or very few) germ cells where the deadenylylation process normally takes place. Alternatively, if *glh-1* RNA is synthesized by the mitotic germ nuclei, then deadenylylation may occur in the cytoplasm of the syncytium. The slow progression of germ-line proliferation in *glp-4*/25° mutants could include slower deadenylylation, resulting in intermediate forms of deadenylylated *glh-1* RNA. By this same model, the small amount of a discrete-sized, more adenylylated *glh-1* RNA in the *glp-1*/25° mutants may remain due to premature entry into meiosis. However, we cannot rule out that these *glp-1* and *glp-4* mutations affect hyperadenylylation of the *glh-1* transcript. Further analysis of *glh-1* expression may determine whether these mutants provide a freeze-frame of the dynamic regulation of polyadenylylation in normal development or only in the defective germ-line development specific to *glp-4* and *glp-1*, and whether the conserved elements are functional.

We expected that other *C. elegans* genes also have ACE and NRE sequences in their 3′ UTRs. The related *glh-2* cDNA (unpublished data) and two B-type cyclin cDNAs (K.L.B., J. Richards, and M. Kreutzer, unpublished data) have potential ACE- and NRE-like elements. In *Drosophila*, the NREs present in the 3′ UTR of hunchback RNA are required for the translational repression of hunchback protein levels by a posterior protein, the nanos (*nos*) gene product (19, 42). Regulatory proteins that interact with the ACEs have yet to be identified. If either the ACE- or the NRE-like sequences control the polyadenylylation state of *glh-1*, then this may represent a different form of regulation by polyadenylylation than that described in sex determination of *Caenorhabditis* with another putative 3′ control element (29).

Genomic Organization of the Nematode RNA Helicase. Genomic Southern analysis suggests that *glh-1* is a single-copy gene (data not shown). The genomic clone Ce-*glh-1* maps between *dpy-14* and *unc-15* on LGI of the physical map of the *C. elegans* genome. The cosmid to which *glh-1* maps was previously shown to encode a 2.5-kb RNA transcript (43). Recent mutant-rescue experiments in this region of the genome, in which many essential genes have been identified (44), have localized four lethal mutants to the same cosmid that contains *glh-1* (S. McKay and A. Rose, personal communication). Three of these mutants also map to an adjacent, partially overlapping cosmid that does not contain *glh-1*. The other lethal mutant, *let-545*, maps exclusively to the cosmid containing the *glh-1* gene and has an adult sterile phenotype, making *glh-1* a good candidate for the gene defined by the *let-545* mutation (S. McKay and A. Rose, personal communication; unpublished data).

Note Added in Proof. An NRE-like element is functional in the *Drosophila* cyclin B 3′ UTR (45).

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