The Levels of the RoRNP-Associated Y RNA Are Dependent Upon the Presence of ROP-1, the *Caenorhabditis elegans* Ro60 Protein

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**ABSTRACT**

The Ro ribonucleoproteins (RoRNP) consist of at least one major protein of 60 kD, Ro60, and one small associated RNA, designated Y RNA. Although RoRNP have been found in all vertebrate species examined so far, their function remains unknown. The *Caenorhabditis elegans* rop-1 gene previously has been identified as encoding a Ro60 homologue. We report here the phenotypic characterization of a *C. elegans* strain in which rop-1 has been disrupted. This is the first report regarding the inactivation of a major RoRNP constituent in any organism. The rop-1 mutant worms display no visible defects. However, at the molecular level, the disruption of rop-1 results in a dramatic decrease in the levels of the ROP-1-associated RNA (*CeY RNA)*. Moreover, transgenic expression of wild-type rop-1 partially rescues the levels of *CeY RNA*. Considering that transgenes are poorly expressed in the germline, the fact that the rescue is only partial is most likely related to the high abundance of the *CeY RNA* in the adult germline and in embryos. The developmental expression pattern and localization of *CeY RNA* suggest a role for this molecule during embryogenesis. We conclude that, under laboratory culture conditions, ROP-1 does not play a crucial role in *C. elegans*.

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ture in the 5S rRNA molecule (Shi et al. 1996). Ro60 has thus been proposed to lead these mutant 5S RNAs to degradation, suggesting a role in 5S rRNA biogenesis for the RoRNP. This putative role has been recently reinforced by electron microscopy observations showing that RoRNP components are present in nucleoli and ribosome-rich cytoplasmic areas (Farriss et al. 1997).

We and others have previously reported the identification of a Ro60 homologue in the nematode Caenorhabditis elegans (Labbé et al. 1995; Van Horn et al. 1995). This protein, designated ROP-1, binds to a single Y RNA (CeY RNA) of 105 nucleotides, which is predicted to fold in the same manner as its mammalian and frog counterparts, but that lacks a pyruridine tail that would permit the binding of the protein La (Van Horn et al. 1995). We wished to take advantage of the powerful genetics of C. elegans to investigate the biological function of ROP-1. To that effect, we studied a worm strain in which the gene encoding ROP-1, rop-1, has been inactivated by the insertion of the Tc1 transposon. We report here the phenotypic and molecular characterization of this C. elegans rop-1::Tc1 mutant strain.

MATERIALS AND METHODS

General methods and strains: C. elegans strains were cultured as described by Brenner (1974). All animals were grown at 20°C unless otherwise stated. The wild-type strain used was the Bristol N2 strain. The strain NL733 rop-1(pk93) was received from Ronald Plasterk of The Netherlands Cancer Institute, and was generated by PCR-screening a randomly inserted Tc1 transposon frozen nematode library (Zwaal et al. 1993). The strain MO470 rop-1(pk93) was generated by backcrossing NL733 hermaphrodites 10 times with MO259 unc-42(e270) males to eliminate the mutator activity as well as other Tc1 insertions present in the original strain.

Generation of transgenic nematodes: Animals were transformed using two previously described methods. In the first one, plasmid pCerO4146, containing the wild-type rop-1 gene, was co-injected with the transformation marker rprF4 [containing a dominant mutation in the collagen gene rol-6(su1006)] in rop-1(pk93) animals, both at a concentration of 50 μg/ml (Mello and Fire 1995). The extrachromosomal array of one of the three resulting strains, rop-1(pk93);qmEx156, was integrated in the genome using the γ-ray-induced integration technique (Mello and Fire 1995). Alternatively, to increase the transgenic expression level of rop-1, pCerO4146 was also co-injected with both rprF4 and wild-type genomic DNA prepared as previously described (Kelly et al. 1997). This technique generates more complex arrays that should allow better expression of a given transgene. All the strains were assayed for the rescue of CeY RNA expression.

For lacZ expression, the rop-1 promoter was cloned in vector pPD22.11 so that it would drive the expression of a nuclear localization signal (NLS)-containing β-galactosidase gene product, with the 3’ UTR of unc54 (Fire et al. 1990). The resulting plasmid pCerO4107 was co-injected with rprF4, at respective concentrations of 100 μg/ml and 50 μg/ml. The extrachromosomal array of one of the seven resulting strains was integrated in the genome using the γ-ray-induced integration technique (Mello and Fire 1995). All the strains were stained with X-β-Gal as described previously (Fire 1992), and the expression pattern of rop-1::lacZ was thus visualized.

RNA procedures: Staged and mixed-staged worms were grown and collected as described previously (Lewis and Fire 1995). Total RNA was extracted by treating the worm pellets with TRIzol reagent (BRL) according to the manufacturer’s instructions. Reverse transcription-PCR (RT-PCR) analysis was done as previously described (Ewbank et al. 1997). For Northern blot analysis, 5 μg of total RNA were resolved on a 1.5% formaldehyde-agarose gel electrophoresis in 1× 3-(N-morpholino)propanesulfonic acid (MOPS) buffer (200 mM MOPS; 50 mM Na acetate; 10 mM EDTA). Following migration, the gel was rinsed twice for 20 min in 20× SSC (3 mM NaCl; 0.3 mM Na citrate) and the RNA was transferred overnight by capillarity with 20× SSC onto Nytran filters (Schleicher & Schuell, Keene, NH). DNA probes were labeled by the random priming method using the PrimeAid kit (Stratagene, La Jolla, CA). The membrane was then UV irradiated (UV Crosslinker, FisherBiotech) and prehybridized in RNA hybridization solution (100 μM NaCl; 50 mM PIPES, pH 6.8; 50 mM phosphate buffer, pH 7.0; 1 mM EDTA; 5% SDS) at 60°C for 1 hr. Hybridization was performed in the same buffer at 60°C for 12–16 hr. Following hybridization, the membrane was washed twice for 15 min in 2× SSC; 0.1% SDS at 60°C. The membrane was subsequently exposed wet to autoradiography on Kodak BioMax film.

For ribosomal RNA extraction, worm pellets were ground in liquid nitrogen and resuspended in NET-2 buffer (40 mM TrisHCl, pH 7.4; 150 mM NaCl; 0.05% NP-40). The extracts were spun at 5,000 rpm for 15 min at 4°C to eliminate the collagen cuticles. The supernatants were then ultracentrifuged at 35,000 rpm for 2 hr at 4°C in a Beckman (Fullerton, CA) SW50.1 rotor. The resulting pellets were resuspended in 100 μl of RNase-free water and subjected to two rounds of phenol/ chloroform extractions, followed by a precipitation with Na acetate. RNA was collected by centrifugation for 15 min at 4°C.

For individual 5S rRNA cloning, 2.5 μg of total or ribosomal RNA were precipitated and resuspended in 1 μl of poly(A) polymerase buffer (50 mM TrisHCl, pH 7.9; 250 mM NaCl; 10 mM MgCl2; 2.5 mM MnCl2; 250 μM ATP; 20 units of RNase inhibitor (Stratagene); 0.5 μg/ml of bovine serum albumin). One unit of poly(A) polymerase (BRL) was then added to the mixture. The reaction was incubated 15 min at 37°C to allow polymerization, followed by 15 min at 65°C to inactivate the polymerase. RT was performed on 2 μl of polyadenylated RNA sample in a total reaction volume of 25 μl using primer X BpolY(T). PCR was then performed on 5 μl of RT product using primers X BpolY(T) and S1CCESS. Vent polymerase (New England Biolabs, Beverly, MA) was used to minimize the misincorporation events. The PCR products were ligated in the pBlueScript vector (Stratagene) and sequenced individually by the dyeoxy chain termination technique using a T7 sequencing methodology (Pharmacia, Piscataway, NJ). To rule out the possibility of selecting for a particular mutation, the whole procedure was repeated three times for each cellular 5S rRNA pool.

Immunoblotting: Western blots were carried out as previously described (Rokeach et al. 1991) and bound antibodies were detected using the DuPont (Wilmington, DE) Renaissance detection system, according to the manufacturer’s instructions.

PCR reaction primers: The following primers were used in PCR and RT-PCR reactions SL1, TTTAATTACCAAGGTTT; SL2, TTATACCCAGTTACTCAAG; XBpolY(T), GCCTCTAGA GGATCC(T); S1CCESS, AATGTGGACGTTCATGACATC; CL1, GAACTAATGCTAGTACGAGT; CL2, CTCTGCTTATGGGAAAGTACCGGAG; CL3, TGTCGACATACACAGCATCATGACTTAC; CL4, TTGAGAAGGCAACATCTGTTCTG; RI, TCA CACGCTGATGCTGACTCCACGTGCG; RI, TTCTGAAACAC TTGTTGTAAG.
RESULTS

The allele *pk93* is null for the expression of *rop-1*: The strain NL733 rop-1(pk93), carrying an insertion of the transposon *Tc1* in the *rop-1* gene, was obtained from Ronald Plasterk (The Netherlands Cancer Institute, Amsterdam). The original strain was outcrossed 10 times with the wild-type strain N2 to ensure a wild-type genetic background as far as possible. The presence of the transposon within the *rop-1* gene was confirmed by PCR after each outcross, using primer pairs CL1/R1 and CL2/R1I. The exact position of the *Tc1* insertion was determined by sequencing the PCR product obtained following the second round of amplification. *Tc1* disrupted *rop-1* in the third exon, 23 nucleotides after the 3′ splice site of the second intron (Figure 1). Northern blot analysis on total RNA, with the *rop-1* coding sequence used as a probe, shows that the allele *pk93* has a greatly reduced expression of *rop-1* mRNA when compared to wild-type levels (Figure 2A). Moreover, the residual signal is most likely nonspecific hybridization since RT-PCR analysis failed to amplify any specific band from the *rop-1* mutant strain (Figure 2B). Furthermore, Western blot analysis on total protein extracts, using ROP-1 specific antibodies, did not detect the presence of ROP-1 in the *rop-1* mutant when compared to wild type (Figure 2C). No ROP-1 protein was detected, even when the primary antibody concentration and exposure times were greatly increased (data not shown). These results clearly demonstrate that the allele *pk93* is null for the expression of *rop-1*.

*rop-1* mutants do not show any visible phenotype: *rop-1* mutant worms do not show any obvious phenotype. The morphology and behavior of the mutants are wild type. Since Ro60 is present in virtually every vertebrate cell, it is likely to play a ubiquitous role in cellular physiology. Its absence is expected to affect basic cellular processes that could result in phenotypes that generally affect the whole organism. Thus, we scored the brood size and the life span of *rop-1* mutants, but observed no difference between wild-type and *rop-1* mutant worms at either 15°C, 20°C, or 25°C (data not shown). Therefore, under the growth conditions tested, ROP-1 does not seem to play a crucial role in *C. elegans* physiology.

5S rRNA processing in the absence of ROP-1: Ro60 has been shown to bind defective 5S rRNA copies in *Xenopus* oocytes (O’Brien and Wolin 1994). It has been proposed that this binding might lead mutant 5S molecules to a degradation pathway. Consequently, the absence of Ro60 should significantly affect 5S rRNA processing. To this end, we verified the fate of 5S rRNA.
TABLE 1
Sequencing of individual 5S rRNA copies

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Cellular 5S rRNA pool</th>
<th>Percent of mutant 5S rRNA molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2</td>
<td>rop-1 (+)</td>
<td>Ribosomal</td>
<td>1.6 (63)</td>
</tr>
<tr>
<td>N2</td>
<td>rop-1 (+)</td>
<td>Total</td>
<td>7.5 (67)</td>
</tr>
<tr>
<td>MQ470</td>
<td>rop-1(pk93)</td>
<td>Ribosomal</td>
<td>7.9 (89)</td>
</tr>
<tr>
<td>MQ470</td>
<td>rop-1(pk93)</td>
<td>Total</td>
<td>7.6 (79)</td>
</tr>
<tr>
<td>MQ684</td>
<td>rop-1(pk93); qmEx156</td>
<td>Ribosomal</td>
<td>10.4 (77)</td>
</tr>
</tbody>
</table>

*a* Cellular pool from which 5S rRNA molecules were isolated. See materials and methods for a complete description of the extraction procedure.

*b* Percentage of 5S rRNA molecules containing one mutation in their 120-nucleotide sequence, followed in parentheses by the total number of 5S rRNA molecules sequenced.

*c* The difference in mutational frequency observed between the N2 and MQ470 ribosomal pools is statistically significant (*P* < 0.05).

molecules in the rop-1 mutant worms using the following approaches.

We first analyzed the levels of 5S rRNA by Northern blotting on total RNA extracts from mixed-stage worms. Using this method, we could not observe any difference between the levels of 5S rRNA in wild-type and rop-1 mutant strains (data not shown). As a control, we also analyzed the levels of five different tRNAs, which are transcribed by RNA polymerase III via a different set of transcription factors from those used for 5S rRNA. Yet the levels of tRNA in wild type and the rop-1 mutant strain appeared the same (data not shown). Thus, the amounts of 5S rRNA seem unaffected by the absence of ROP-1.

Next we verified the quality of individual 5S rRNA molecules transcribed in wild-type and rop-1 mutant strains. To this end, both total and ribosomal RNA was extracted from each strain and 5S rRNA molecules were selectively amplified by RT-PCR using primers XB-poly(T) and S1CE5S. Since the primer S1CE5S anneals to the first 18 nucleotides of the 5S molecule, no mutation could be detected from this region. In order to minimize nucleotide misincorporation events during the PCR reactions, a thermostable DNA polymerase containing a proofreading activity was used for DNA amplification (Vent DNA polymerase, New England Biolabs). As shown in Table 1, sequencing of the individual 5S rRNA copies from total RNA extracts revealed no substantial difference between wild-type and rop-1 mutant strains, with ~8% of the 5S RNA molecules containing a single mutation. However, sequencing of 5S rRNAs extracted from ribosomes of wild type displayed only 1.6% of mutation frequency, whereas those extracted from ribosomes of rop-1 mutants showed 8% of mutation frequency. The mutations observed maintained in general the ratio of purine:pyrimidine in the 5S molecule and were principally located in the regions defined as loop B-stem III-loop C and stem V-loop E (data not shown; O'Brien and Wolin 1994). Some mutations were found in more than one cellular pool and in both strains, demonstrating that they indeed occur in vivo and are not the result of the PCR amplification. These results suggest that ROP-1 is directly involved in the quality control of 5S rRNA. Transgenic animals expressing wild-type ROP-1 from a transgene were generated to rescue this phenotype. Nevertheless, the 5S rRNA molecules extracted from the ribosomes of these transgenic worms display 10% of mutation frequency. This lack of rescue by a rop-1 transgene could be explained either by the presence of another mutation affecting 5S rRNA processing in the MQ470 strain or by a general problem in rescuing events occurring in the germline (see below).

The levels of ROP-1-associated Y RNA are severely decreased in rop-1 mutants: It has previously been shown that the C. elegans ROP-1 protein associates with the CeY RNA (Van Horn et al. 1995). We studied the levels of CeY RNA by Northern blot analysis on C. elegans total RNA extracts. While CeY RNA is an abundant molecule in wild-type cells, its levels decrease dramatically in a rop-1 mutant background (Figure 3B; compare lanes 1 and 2). Because ROP-1 normally associates with CeY RNA, the simplest explanation would be that the absence of ROP-1 alters the stability of the small RNA. Alternatively, ROP-1 could be directly involved in the transcription of CeY RNA.

To show that the decrease in CeY RNA is due to the absence of ROP-1, we set out to rescue its expression by reintroducing the wild-type rop-1 gene in the rop-1 mutant. We generated three strains in which each transgenic array expresses ROP-1 at a different level. In C. elegans, transgenic arrays are maintained extrachromosomally and display some mitotic instability (Mello and Fire 1995). Therefore, not all the cells of a given animal contain the transgene. To circumvent this instability, the extrachromosomal array of one of these three strains was integrated in the genome of C. elegans by γ-ray treatment. Western blot analysis showed that the transgenic worms were indeed producing ROP-1 in all
Disruption of rop-1 in C. elegans

CeY RNA is present at higher levels in the adult germ-line and in embryos: Since the rescue of CeY RNA is partial, we further investigated this phenomenon. Even though transgenic arrays are usually well expressed in most somatic lineages, they are poorly expressed in the germ-line of C. elegans (Kelly et al. 1997). Therefore, it is difficult, at best, to rescue an event that occurs during oogenesis or early embryogenesis. To investigate this point, we analyzed the expression of RoRNP components throughout the developmental stages of the nematode by Northern blot analysis on staged-worm total RNA extracts. As shown in Figure 4A, rop-1 mRNA levels are stable during C. elegans development. Western blot

Figure 3.—The levels of CeY RNA are decreased in rop-1 mutants and are partially rescued by transgenic expression of rop-1. (A) Western blot analysis of ROP-1 in transgenic strains. A total of 100 μg of total protein extracts was resolved on SDS-PAGE, transferred, and probed with an antibody specific for ROP-1. Each transgenic strain expresses ROP-1 at a different level when compared with each other. (B) Northern blot analysis of CeY RNA in transgenic strains. A total of 5 μg RNA was resolved by formaldehyde-agarose gel electrophoresis, transferred, and probed with the complete yrn-1 gene (Van Horn et al. 1995). This result shows that the levels of CeY RNA are greatly decreased in rop-1 mutant worms. These levels are also different in each transgenic strain and are proportionally related to the increase of ROP-1 in the same strain (compare corresponding lanes in A and B). (C) For quantification in Northern blot analysis, the membrane was stripped and reprobed with a cDNA encoding a portion of a gene encoding actin (plasmid pCeA103; Krause et al. 1989), thereby demonstrating that all lanes were loaded with comparable amounts of RNA. Lane 1, N2 extracts; lane 2, rop-1(pk93) extracts; lane 3, rop-1(pk93); qmEx158 extracts; lane 4, rop-1(pk93); qmEx156 extracts; lane 5, rop-1(pk93); qmEx160 extracts; lane 6, rop-1(pk93); qmIs11 extracts.

Figure 4.—CeY RNA is transcribed at higher levels in the adult germ-line and in embryos. Total RNA was prepared from staged worms and 5 μg of total RNA was resolved by formaldehyde-agarose gel electrophoresis, transferred, and probed with (A) a rop-1 cDNA, (B) the yrn-1 gene, or (C) a portion of the gene encoding actin. The levels of rop-1 mRNA and CeY RNA are both high in the embryos, but decrease at the subsequent larval stages. However, the levels of CeY RNA increase a little at the L4 stage and a lot at the adult stage. Lane 1, wild-type embryos; lane 2, wild-type L1 larvae; lane 3, wild-type L2 larvae; lane 4, wild-type L3 larvae; lane 5, wild-type L4 larvae; lane 6, wild-type nongravid adults; lane 7, glp-1(q231) mutant adults. (D) The intensity of each band obtained in (B) was determined by scanning the X-ray film with a LKB ultroscan XL enhanced laser densitometer (Pharmacia, Piscataway, NJ). Each individual band in (B) was corrected with the densitometric value obtained by scanning the band in the corresponding lane obtained in (C) with an actin probe. The corrected data were plotted on a graphical chart.
analysis on staged-worm protein extracts revealed that ROP-1 levels are also stable (data not shown). However, a differential expression pattern was observed for CeY RNA. CeY RNA levels are high in the embryos, as compared to the other stages but decrease at the L1 stage (Figure 4B; compare lanes 1 and 2). These levels are maintained through all the larval stages, but increase slightly at the L4 stage and considerably at the adult stage, a time when oogenesis starts (Bartron et al. 1987). The fact that the CeY RNA levels start to increase at the L4 stage and peak at the adult stage suggests that the CeY RNA is highly expressed in the germline. As a control, we checked the levels of CeY RNA in glp-1(q231) mutant adults grown at the nonpermissive temperature. This temperature-sensitive mutation results in worms whose germ cell precursors fail to undergo mitosis, thereby producing animals with no germline (Austin and Kimble 1987). We found that CeY RNA levels in glp-1 mutants are similar to those observed in the wild-type larval stages (Figure 4B). Taken together, these results clearly demonstrate that while rop-1 mRNA appears to be expressed at stable levels during development, CeY RNA is transcribed at higher levels in the adult germline and in embryos. Because transgenes are generally silenced in the germline, the levels of CeY RNA would not have been readily rescued by a transgene expressing wild-type rop-1.

The rop-1 promoter is not active in the germline when expressed from a transgene: To characterize the expression pattern of rop-1 from a transgene, we fused the rop-1 promoter to lacZ, with the 3' UTR of unc-54. The resulting strains express the enzyme β-galactosidase in the cellular types where the rop-1 transgenic promoter is active. As shown in Figure 5, the expression of rop-1::lacZ is mosaic and differs from animal to animal. This irregular pattern of expression cannot be explained only by the instability of transgenic arrays, because this pattern was still observed after one of the transgenic arrays was integrated in the genome of C. elegans. Nevertheless, by comparing the expression pattern of numerous worms and strains, we observed some β-galactosidase staining in every cell type of the nematode, except in the germline. The same results were obtained with a rop-1::glp reporter construct (data not shown). This ubiquitous expression further supports the notion of a basic cellular role for ROP-1, compatible with its involvement in 5S rRNA quality control. Furthermore, because CeY RNA is transcribed at higher levels in the germline and in embryos, the fact that the transgenic rop-1 is not expressed in the C. elegans germline provides an explanation for the partial rescue of CeY RNA levels we observed.

**DISCUSSION**

We report here the first phenotypic characterization of an organism devoid of Ro60 protein. The C. elegans rop-1 gene was disrupted by Tc1 transposon insertion at the 5' end of its third exon. The absence of ROP-1 synthesis was confirmed by Western blot analysis on total protein extracts from the rop-1 mutant strain. There was no visible phenotype observed in association with the disruption. However, in the rop-1 mutant strain, we observed a dramatic decrease in CeY RNA levels. The CeY RNA levels were partially rescued when the wild-type rop-1 gene was reintroduced in the rop-1 mutant worms. The partial rescue is probably related to the fact that the CeY RNA is transcribed at higher levels in the adult germline and in the embryo.

rop-1 mutant worms display no visible phenotype: The absence of a visible phenotype in rop-1 mutants is surprising considering the level of conservation of Ro60 proteins between human, mouse, frog, and nematode (Labbé et al. 1995; Van Horn et al. 1995; Wang et al. 1996). The fact that the rop-1 gene has been conserved during evolution strongly suggests that ROP-1 plays some role in the nematode's cellular physiology. The lack of observed phenotype might be due to the fact that the worms are cultured under ideal conditions, rather different from those they encounter in their natural habitat. However, growing the worms at different temperatures could not induce any effect in rop-1 mutants, but rop-1 could become essential in a variety of other natural stresses yet to be defined.

RoRNP components are present at higher levels during embryogenesis: RoRNP particles previously have been immunoprecipitated from C. elegans embryos (Van Horn et al. 1995). Our results showing the high abundance of CeY RNA in embryos corroborate this evidence and further demonstrate that CeY RNA is also transcribed in the adult germline. Because rop-1 expression appears to be stable throughout development and the
CeY RNA molecule is transcribed in the germline, it is not clear whether the CeY RNA is present naked, associated in RoRNP particles, or complexed to some other molecule. However, the fact that the CeY RNA is transcribed in the germline indicates that it is provided maternally to the developing oocyte. Similarly, it has been demonstrated that the multifunctional protein La also presents a developmentally regulated expression pattern in Drosophila melanogaster (Bai et al. 1994). Taken together, these observations suggest some function for the RoRNP complexes or individual components during early embryogenesis.

A link between ROP-1 and 5S RNA processing in C. elegans In Xenopus oocytes, Ro60 has been shown to interact with misfolded 5S RNA molecules that contain mutations, as well as a ~10-nucleotide extension at their 3' end (O’Brien and Wolin 1994; Shi et al. 1996). In this work, we have observed that in rop-1 mutants, the level of ribosome-associated 5S RNA mutant molecules is increased approximately fivefold. At this point, it is not clear whether this result is directly linked to the absence of ROP-1 or to another unidentified mutation present in the strain. However, because protein synthesis is performed at very high rates in the oocyte, the needs in 5S RNA are enormous, and a substantial amount of 5S RNA is deposited in the oocyte in a relatively short time (Wolffe and Brown 1988). Indeed, the amounts of 5S RNA in the oocyte are such that C. elegans embryos lacking the rs-1 locus (containing 110 tandem copies of the 5S rDNA) can develop normally and hatch only with the 5S RNA molecules supplied maternally (Ferguson et al. 1996). In this cellular context, the number of transcriptional errors in the maternal 5S rRNAs could be increased, justifying the need for an additional quality control mechanism. The fact that the fivefold increase in mutant 5S RNAs incorporated into ribosomes was not reverted in the rop-1 mutant carrying the transgenic wild-type rop-1 may be due to the failure of transgene expression in the germline. Supporting this argument, we have shown that the levels of CeY RNA are only partially restored in the transgenic worms. Likewise, the 5S RNA quality control function in early embryogenesis would not have been readily rescued. Taken together, these observations suggest that the Ro60-5S RNA association might specifically occur in oocytes and that Ro60 could be involved in the quality control of 5SrRNA. Because we only detect a difference in ribosome-associated 5S RNA mutant molecules between the wild-type and rop-1 mutant strain, this quality control would occur at the level of incorporation of 5S RNA into the ribosomes.

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