The *Caenorhabditis elegans* Homologue of Thioredoxin Reductase Contains a Selenocysteyl-tRNA Insertion Sequence (SECIS) Element That Differs from Mammalian SECIS Elements but Directs Selenocysteine Incorporation*

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Thioredoxin reductases (TRR) serve critical roles in maintaining cellular redox states. Two isoforms of TRR have been identified in mammals: both contain a penultimate selenocysteine residue that is essential for catalytic activity. A search of the genome of the invertebrate, *Caenorhabditis elegans*, reveals a gene highly homologous to mammalian TRR, with a TGA selenocysteine codon at the corresponding position. A selenocysteyl-tRNA was identified in this organism several years ago, but no selenoproteins have been identified experimentally. Herein we report the first identification of a *C. elegans* selenoprotein. By radioisotope labeling of *C. elegans*, one major band was identified, which migrated with the predicted mobility of the *C. elegans* TRR homologue. Western analysis with an antibody against human TRR is the only known reductant of TR (7). The ability to synthesize selenoproteins is of fundamental importance in mammals, as deletion of the gene for selenocysteyl-tRNA leads to an embryonic lethal phenotype in a mouse model (5). The selenoprotein or proteins responsible for this lethality have not been determined. Cytoplasmic glutathione peroxidase, a selenoprotein functioning in breakdown of toxic hydroperoxides, appears not to be critical for normal development of mammals, probably due to redundancy in the glutathione peroxidase gene family (6). Among the 13 eukaryotic selenoproteins identified to date, thioredoxin reductase (TRR) is a strong candidate for an essential selenoprotein. TRR is a disulfide oxidoreductase with a broad substrate specificity; it reduces among many other substrates the active site disulfide in oxidized thioredoxin. Thioredoxin (TR) is an important cofactor in a large number of biological processes. While a knockout model for TRR has not been reported to date, targeted disruption of the TR gene causes early embryonic lethality, and TRR is the only known reductant of TR (7).

Two TRR isoforms have been described in several mammalian species, both of which contain a UGA codon encoding selenocysteine as the penultimate C-terminal amino acid (8–12). Putative SECIS elements are present in the 3'-UTRs of both sequences. In contrast to other selenoenzymes, it has been proposed that the selenocysteine residues in the TRRs are not in the predicted catalytic site, but function to carry reducing equivalents from the active site to substrates (13). Nonetheless, selenocysteine is critical for reducing thioredoxin, as demonstrated by the loss of activity upon chemical alteration (14) or proteolytic removal of this amino acid (13).

The *C. elegans* gene for selenocysteyl-tRNA was identified by Lee et al. in 1990 (15), indicating that selenoprotein genes would likely be present. To date, no selenoproteins have been experimentally identified in *C. elegans*. Since the complete genome of *C. elegans* has been sequenced, and this organism is amenable to genetic studies, it promises to be a valuable model system for the study of eukaryotic selenoprotein synthesis. In...
**SECIS Element of C. elegans TRR**

**Experimental Procedures**

75Se Labeling of C. elegans—C. elegans strain bristol (N2) was used throughout these studies (16). For 75Se labeling of worms, their food source, *Escherichia coli* strain OP50, was grown overnight in 1 ml of Luria broth containing 20 μCi of 75Se. 75Se-labeled bacteria were harvested by pelleting, resuspended in M9 buffer (22 mM KH2PO4, 42 mM Na2HPO4, 85 mM NaCl, 1 mM MgSO4), and dispensed on 60-mm plates containing 3 ml of nematode growth medium agar. Worms were grown at 22 °C on lawns of 75Se-labeled *E. coli* and harvested by washing them off the plates in M9 buffer, followed by sedimentation at 800 × g for 5 min and three washes in M9 buffer. The pellets were resuspended in phosphate-buffered saline and sonicated. As a protease inhibitor 1 mM phenylmethylsulfonyl fluoride was added. Lysates were stored at −20 °C until further use.

**Results**

**Identification of Selenoproteins in C. elegans**—To identify selenoproteins in *C. elegans*, we developed a 75Se labeling technique based on their food source, *E. coli*. *E. coli* were cultured in Luria broth media containing 75Se. This led to incorporation of 75Se predominantly into two *E. coli* selenoproteins. Worms were allowed to feed on a lawn of 75Se-labeled bacterial for 24 h, until bacteria were depleted, then harvested for analysis of 75Se incorporation. Since there are always trace amounts of bacteria in the gut of *C. elegans*, we also prepared a lysate of the 75Se-labeled bacteria. SDS-PAGE analysis of *C. elegans* homogenate reveals three major selenoprotein bands, two in the 80–95 kDa size range and one of 58 kDa. The two upper bands are also present in the bacterial lysate, suggesting that none of the bands visible by autoradiography are of RNA origin (data not shown).

**TRR Is the Only Eukaryotic Selenoprotein Homologue in C. elegans**—With RNase A. No difference in labeling pattern was seen compared with the untreated lysate, suggesting that none of the bands visible by autoradiography are of RNA origin (data not shown).

**TRR Is the Only Eukaryotic Selenoprotein Homologue in C. elegans with a UGA Selenocysteine Codon**—We performed a

**Color figure 1. 75Se labeling of worms and bacteria.** C. elegans were fed 75Se-labeled bacteria as food source, homogenized, and subjected to SDS-PAGE analysis, followed by autoradiography. Note that the upper two bands in the 80–95 kDa range seen in *C. elegans* are also present in the *E. coli* lane and are thus most likely of bacterial origin. The major band not found in *E. coli* migrates with a molecular mass of ~58 kDa.

**Fig. 1. 75Se labeling of worms and bacteria.** C. elegans were fed 75Se-labeled bacteria as food source, homogenized, and subjected to SDS-PAGE analysis, followed by autoradiography. Note that the upper two bands in the 80–95 kDa range seen in *C. elegans* are also present in the *E. coli* lane and are thus most likely of bacterial origin. The major band not found in *E. coli* migrates with a molecular mass of ~58 kDa.
search of the *C. elegans* genome data base with the sequences of all known mammalian selenoproteins. This search revealed a gene highly homologous to mammalian TRR, with a TGA selenocysteine codon at the corresponding position (Fig. 2, GenBank accession number 1397273). No homologues of any of the three isoforms of the iodothyronine deiodinases, selenoprotein P, or selenoprotein W were found. Homologues of glutathione peroxidases, selenophosphate synthetase (Sps2), and a 15-kDa selenoprotein (20) are present, but each contains a cysteine codon in place of the TGA selenocysteine codon found in the corresponding mammalian gene. Thus, the *C. elegans* thioredoxin reductase is the only homologue of a known mammalian selenoprotein containing a conserved selenocysteine codon.

Assignment of the 58-kDa Selenoprotein as a Member of the TRR Family—To obtain evidence for the identity of the 75Se-labeled 58-kDa protein as a thioredoxin reductase-like protein, we performed Western analysis using a polyclonal antibody against human TRR. The antibody was raised against an hTRR peptide sequence, which shares 17 of 21 amino acids (81% identity) with the corresponding *C. elegans* sequence (Fig. 2) and thus might be predicted to cross-react with the worm protein. Western analysis of the 75Se-labeled *C. elegans* lysate revealed an antibody-reactive protein of the predicted size (Fig. 3, hTRR antibody) and two additional cross-reacting bands. After extensive washing of the membrane and decay of the chemiluminescent signal, we used autoradiography to visualize the 75Se-labeled band. Superimposing the autoradiograph and chemiluminescence film revealed that the 75Se-labeled band bears homology to human TRR. The presence of additional cross-reacting bands prompted us to further examine the *C. elegans* data base for other sequences bearing homology to the human TRR peptide. After TRR, the next highest scoring matches were the glutathione reductase (GR) sequence and an uncharacterized pyridine nucleotide-disulfide oxidoreductase, C46F11, both exhibiting 57% identity (Fig. 2). GR and C46F11 do not contain UGA selenocysteine codons and do not correspond in their predicted sizes to the 58-kDa protein identified by 75Se labeling.

Identification of a Putative SECIS Element in *C. elegans* TRR—We next searched the sequence of the *C. elegans* TRR 3’-UTR for a potential SECIS element. Although there are several “AUGA” motifs in the 3’-UTR, none appeared in the context of a SECIS element. However, by extending the search to sequences which are similar but not identical to this motif, we identified a putative SECIS element that deviates from the vertebrate consensus by one nucleotide. The worm element,
beginning with “GUGA” instead of “AUGA”, is predicted to fold like a vertebrate form 2 SECIS element with a characteristic 10-base pair stem, an adenosine bulge, and an upper stem and small terminal loop (Fig. 4).

The C. elegans TRR SECIS Element Is Functional in Mammalian Cells—To investigate the ability of this putative SECIS element to direct selenocysteine incorporation in a mammalian cell line, we generated a construct containing the TRR SECIS element linked to the rat D1 coding region. This construct was transiently transfected into the human embryonic kidney-derived cell line HEK-293, and production of deiodinase activity was assessed. We have shown previously that a functional SECIS element is required for incorporation of selenocysteine into this protein, which in turn is required for maximal deiodinase activity (21) (18). The putative C. elegans SECIS element directed selenocysteine incorporation at an activity level slightly higher (134 ± 1.6%) than the wild type rat D1 SECIS element. Mutation of the invariant “G,” the third nucleotide in the “AUGA” motif, in either the rat glutathione peroxidase or rat D1 SECIS elements was shown previously to reduce activity by 6% (22) or to undetectable levels (3) (17), respectively. In the C. elegans SECIS element, this “G” to “A” mutation (Fig. 4) also resulted in near complete inactivation (3% of rat D1).

In this study, we report identification of the first selenoprotein and the first SECIS element in C. elegans. The protein migrates with the apparent molecular weight of a thioredoxin reductase homologue in the sequence data base and exhibits antibody cross-reactivity with members of this family of enzymes. Blast searches against the genome sequence of C. elegans (1998 number 85) using the protein sequences of all known vertebrate selenoproteins revealed the TRR sequence to be the only homologue of a vertebrate selenoprotein with a UGA codon at the corresponding position (Fig. 2). It has been speculated that this C. elegans homologue might be a selenoprotein based on the presence of the conserved UGA codon (23). Herein, we provide experimental evidence supporting the identity of the major C. elegans selenoprotein as TRR by Western analysis of 72-Se-labeled C. elegans homogenates and autoradiography of the same membrane, allowing superimposition of the two films. Our labeling studies indicate that this protein is by far the most prominent selenoprotein in C. elegans. In addition to the putative TRR homologue, a 72-Se-labeled band migrating at ~40 kDa was detected with varying intensity in different labeling experiments. The identity and source of this band are unknown; it may represent a degradation product derived from proteolysis of either the putative worm TRR or one of the bacterial selenoproteins.

All vertebrate selenoproteins contain at least one SECIS element, which is required for decoding UGA as a selenocysteine codon. An “AUGA” motif within the structural context of a SECIS element is invariant in all previously characterized eukaryotic selenoprotein sequences. Only after searching for SECIS-like structures, while allowing for deviations from the consensus, did we identify a putative stem loop structure meeting the requirements of a form 2 SECIS element. In this SECIS element, a “GUGA” motif is present instead of “AUGA”. This sequence still allows formation of the “GA quartet” shown recently to be critical for SECIS function in vertebrates (3). More importantly, the C. elegans TRR SECIS element directs selenocysteine incorporation in a mammalian cell line. Finally, a point mutation disrupting the ability to form the “GA quartet” resulted in near complete loss of activity, consistent with this element functioning analogously to mammalian SECIS elements. This suggests that the mechanism of selenocysteine incorporation may be evolutionarily conserved in eukaryotes.

It is not known whether the “GUGA” motif is specific for nematodes. The existence of an expressed sequence tag clone from the filarial nematode Onchocerca volvulus encoding a TRR homologue with a UGA codon at the corresponding position (GenBank™ accession number AA680606) suggests that in this subspecies TRR is also a selenoprotein. The sequence of the 3'-UTR, which would allow the search for a SECIS element in this gene, remains to be determined.

Since the genome of C. elegans has been completely sequenced, this sequence information could lead to the identification of components of the translational machinery required for selenoprotein synthesis. The genome of C. elegans contains selenocysteinyl-tRNA (Sel C) and selenophosphate synthetase (Sel D) genes, the only two components of the selenoprotein translational machinery identified in eukaryotes to date. Interestingly, whereas Drosophila and humans have two forms of selenophosphate synthetase (Sel D), one form being itself a selenoprotein, C. elegans has only one (Y45F10A4, GenBank™ accession number AF830888). This Sel D homologue exhibits similarity to both Sel D type 1 (50% identity) and type 2 (52% identity), but contains a cysteine codon at the position corresponding to Sec 63 in human Sel D type 2. A partial cDNA sequence from another filarial nematode, Brugia malayi, indi-
icates the presence of a cysteine-containing Sel D homologue (GenBank™ accession number AA585621).

Glutathione peroxidase (GPX) is a selenoenzyme in vertebrates, but in C. elegans, as well as in the filarial nematodes Dirofilaria immiti and Brugia phalangi, the homologues to GPX contain cysteine residues substituted for selenocysteine in the active site. The Dirofilaria immiti version of GPX exhibits a low level GPX activity compared with selenocysteine-containing GPX, when expressed in a bacterial expression system (24). This might indicate that in evolutionary terms it is more important to have a highly active, selenocysteine-containing TRR than a highly active GPX, providing the selective pressure to maintain the translational machinery required for selenoprotein synthesis.

Since the genome of Saccharomyces cerevisiae does not contain genes for selenocysteyl-tRNA or selenophosphate synthetase, and no selenoproteins have been identified experimentally in yeast, genetic studies of selenoprotein synthesis in eukaryotes have not been feasible to date. C. elegans could thus provide a long awaited model system for genetic dissection of this process in higher organisms. The technique of RNA-mediated interference in C. elegans, as well as in the filarial nematodes, but in C. elegans, the homologues to GPX contain cysteine residues substituted for selenocysteine in the active site. The Dirofilaria immiti version of GPX exhibits a low level GPX activity compared with selenocysteine-containing GPX, when expressed in a bacterial expression system (24). This might indicate that in evolutionary terms it is more important to have a highly active, selenocysteine-containing TRR than a highly active GPX, providing the selective pressure to maintain the translational machinery required for selenoprotein synthesis.

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Note Added in Proof—While this manuscript was in review, a publication describing 75Se labeling and partial purification of the C. elegans thioredoxin reductase appeared in press (25).