ABSTRACT

Thioredoxins are a class of small proteins that play a key role in regulating many cellular redox processes. We report here the characterization of the first member of the thioredoxin family in metazoans that is mainly associated with neurons. The Caenorhabditis elegans gene B0228.5 encodes a thioredoxin (TRX-1) that is expressed in ASJ ciliated sensory neurons, and to some extent also in the posterior-most intestinal cells. TRX-1 is active at reducing protein disulfides in the presence of a heterologous thioredoxin reductase. A mutant worm strain carrying a null allele of the trx-1 gene displays a reproducible decrease in both mean and maximum lifespan when compared to wild type. The identification and characterization of TRX-1 paves the way to use C. elegans as an in vivo model to study the role of thioredoxins in lifespan and nervous system physiology and pathology.
1. INTRODUCTION

Thioredoxins (TRX) are small redox proteins present in all organisms. They function as general protein-disulfide reductases. TRX are maintained in the reduced active form by electrons donated by NADPH and transferred via the flavoenzyme thioredoxin reductase (TRXR), which altogether constitute the thioredoxin system [1]. While there is only one thioredoxin system composed of two TRX and one TRXR in bacteria, eukaryotic organisms have at least two different general thioredoxin systems, one located in the cytoplasm and one in mitochondria [2]. Tissue specificity in metazoan thioredoxins is restricted to axonemes of Chlamydomonas flagella and sea urchin sperm [3,4], to testis and ovary in fruit flies [5], and to spermatozoa in mammals (reviewed in [6]).

The functions of the different thioredoxin systems are broad, mostly relying on their redox properties, such as antioxidant defense, regulation of transcription factor DNA binding activity, cell growth and differentiation or immune system signaling, among others [7]. Moreover, thioredoxin systems have also been implicated in many important pathologies such as neurodegenerative and cardiovascular diseases, cancer, male infertility or diabetes [6,7]. However, the molecular basis of the implication of thioredoxin systems in the processes described above is still far from known, as knockout mice for any of the components of either the cytoplasmic or the mitochondrial thioredoxin systems result in embryonic lethality [8-11].

For this reason we have turned to the nematode Caenorhabditis elegans, an excellent animal model system, to dissect in vivo and at the molecular level the otherwise more complicated physiological and pathological scenarios in higher organisms. We analyzed the completely sequenced C. elegans genome and found two thioredoxin reductases (TRXR) and at least eight different thioredoxins (TRX). Here we describe the identification and characterization of one of the C. elegans thioredoxins, TRX-1. TRX-1 is expressed in ASJ ciliated sensory neurons involved in chemosensation, and to some extent also in the posterior-most intestinal cells. ASJ neurons have previously been implicated in the regulation of entry into and exit from the dauer larval stage, a specialized developmental stage designed for enduring and surviving harsh environmental conditions [12]. Mutations in several genes of the dauer pathway have
also been reported to affect worm lifespan [13]. We show here that the novel thioredoxin TRX-1, expressed in ASJ neurons, also affects the lifespan of *C. elegans*, thus providing an amenable model to study the role of thioredoxins in nervous system biology and aging.
2. MATERIALS AND METHODS

C. elegans strains and assays. The techniques used for culturing C. elegans were essentially as described [14]. The following strains were used: wild type N2 Bristol, RB1332 *trx-1(ok1449)* II, VZ1 *trx-1(ok1449)* II (which is derived from RB1332 after six rounds of outcrossing with N2), MT1642 *lin-15* (n765ts) X, CB1370 *daf-2* (e1370) III, CF1038 *daf-16* (mu86) I, NL2099 *rrf-3* (pk1426) II, GR1373 *eri-1* (mg366) IV, OE3010 *lin-15* (n765ts) X; ofEx4 [pBHL98 (lin-15ab+); *trx-1::gfp*]. Possible visible phenotypes and simple phenotypical assays evaluated in this work are essentially the same as described [15] and were carried out at 20°C (except for dauer formation assays which were performed at 25°C).

cDNA cloning of the C. elegans *trx-1* gene. Information available at [www.wormbase.org](http://www.wormbase.org) identifies the C. elegans gene B0228.5 as encoding a putative thioredoxin, with two different splice variants named *a* and *b*, respectively. B0228.5 has been assigned the name *trx-1*. *trx-1* genomic sequence was used to design the following specific primers, B0228.5a forward 5´-ATGTTGAACGATGCAACTTC-3´, B0228.5b forward 5´-ATGTTCTCTCACAAGGAGCC-3´ and B0228.5 reverse 5´-TCAATTGAGCAGATACGTCGCTC-3, to amplify by PCR the coding regions of both variants from a C. elegans cDNA library (gift from C. Haycraft, University of Alabama at Birmingham, AL, USA; library details are available on request). Since we only managed to amplify the *trx-1b* variant from this cDNA library, the *trx-1a* variant was obtained from the corresponding C. elegans ORFeome clone (http://worfdb.dfci.harvard.edu/). Both *trx-1a* and *b* ORF’s were cloned into pGEM-Teasy vectors and sequenced. The 3´ UTR common to both isoforms was amplified from the same C. elegans cDNA library, whereas the respective 5´ UTRs of the *trx-1a* and *trx-1b* variants were determined by RT-PCR and 5´ RACE experiments with L1 stage mRNA, using both the ThermoScript™ RT-PCR System (Invitrogen) and the BD SMART™ RACE kit (BD Biosciences).

GFP expression analysis. A translational GFP fusion construct was obtained by PCR amplification of the *trx-1* promoter and gene from C. elegans genomic DNA. The forward primer B0228.5-fGFP 5´-
CATTCTGCAGAGAATGGATACCTGATCATT-3 and the reverse primer B0228.5-rGFP 5’-TCGAGGATCCCTTGAGCAGATATCGTGCTCCA-3’ were used and the PCR product was cloned into the PstI/BamHI sites of the pPD95.77 vector. MT1642 lin-15(n765ts) X animals were microinjected with the trx-1::gfp fusion construct together with the marker plasmid pBHL98 that rescues the multi-vulva phenotype of the MT1642 strain. For precise imaging of GFP fluorescence in the bilaterally symmetrical ASJ-left and -right neurons (ASJL, ASJR), a Zeiss LSM 510 META confocal microscope setup (Carl Zeiss, Germany) was used. Worms were put into 0.5 μl M9 buffer on a very thin 2% agarose pad containing an anesthetic (10 mM NaN₃), and were observed with an inverted Zeiss Axiovert 200 M microscope at a 63x objective magnification (NA = 1.4). Serial images through the entire worm were acquired along the z-axis in the 488-nm channel, with a stack increment of 0.8 μm. 3D images were constructed from the stacks of image files using Zeiss LSM software. The resulting images were then processed with Adobe Photoshop® Software.

Lifespan assays. Lifespan determinations followed essentially published procedures [16]. In brief, gravid hermaphrodites were allowed to lay eggs for 5 hours to synchronize the populations. Adult progeny was transferred to fresh plates every day or every other day until they laid no more eggs. Adult worms were split into 10 animals per plate to a total of 200 animals per assay. Animals were scored as dead when not responding to nose and tail prodding. Animals that crawled off the plate, exploded or bagged were censored at the time of the event, which allowed them to be incorporated into the data set until the censor date and thus avoid loss of information.

Bacterial feeding RNA interference assay. The primers B0228.5f-RNAi 5’-ATGCTCTAGAATGTGAAACGATG-3’ and B0228.5r-RNAi 5’-GCATCTCGAGTCATTGAGCAGATAC-3’ were used to amplify the C. elegans trx-1a ORF from genomic DNA. The resulting PCR product was cloned into the XbaI/XhoI sites of the pL4440 plasmid. The pL4440/trx-1a construct was transformed into the HT-115 E. coli strain and worms were then fed on standard agar plates supplemented with 1mM IPTG to induce dsRNA production. HT-115 transformed with the empty pL4440 and pL4440/unc-22 constructs were used as controls.
**Recombinant protein production and enzymatic activity assays.** The TRX-1b coding region was amplified by PCR from the pGEM-Teasy/trx-1b construct using the primers B0228.5f-GST 5′-CATGGGATCCATGTCTCTACCAAGGAGCC-3′ and B0228.5r-GST 5′-CATGGGATTCATCATATTGAGCGATACGTGCTC-3′ and cloned into the BamHI/EcoRI sites of the pGEX-4T-1 vector. The resulting pGEX-4T-1 trx-1b construct was transformed into *E. coli* BL21(DE3) and GST-TRX-1b production was induced with 0.5 mM IPTG for 3.5 hours. The resulting GST-TRX-1b protein was bound onto a GSH-Sepharose 4B column (Pharmacia-Biotech, Sweden) and TRX-1b was released from GST by incubation with 20 U thrombin. TRX-1b concentration was determined from the absorbance at 280 nm using a molar extinction coefficient of 10130 M⁻¹ cm⁻¹.

To determine TRX-1b enzymatic activity we used the insulin assay which was performed as previously described [17]. Briefly, different amounts of TRX-1b were added to a 40 μl reaction mix and the reaction was initiated by the addition of 5 μl thioredoxin reductase from calf thymus (50 A₄₁₂ unit). Incubation was continued for 20 min at 37°C, then the reaction was stopped by the addition of 0.5 ml of 6M guanidine–HCl and 1mM DTNB, and the absorbance at 412 nm was measured. Human TRX-1 was used as a control.

**Antibody production and western blot analysis.** The peptides (NH₂–) NFKNQVKYFQC (–COOH) and (NH₂–) MSLTKEPILELADMC (–COOH) specific for the TRX-1a and b variants, respectively, were used to immunize rabbits and the immune serum was affinity-purified against the same peptides (Agrisera, Sweden). For full-length protein polyclonal antibodies, recombinant GST-TRX-1b (see above) was used to immunize rabbits (Agrisera, Sweden) and the immune serum was affinity-purified against GST-cleaved TRX-1b. Whole *C. elegans* extracts were separated by SDS-PAGE and transferred to a nitrocellulose membrane. Membranes where first incubated with affinity-purified anti-TRX-1 antibodies or peptide antibodies, subsequently conjugated with anti-rabbit IgG horseradish-peroxidase and developed using the Immun-Star HRP Substrate Kit (Bio-Rad, USA).
3. RESULTS AND DISCUSSION

During recent years the different thioredoxin systems have been attracting increasing attention as they are being implicated in a growing number of physiological and pathological processes [7]. Despite those facts, very little is known about the molecular pathways and partners of thioredoxins in eukaryotic cells due to the lack of an appropriate animal model for such studies. So far, knocking out any gene of either the cytoplasmic or the mitochondrial thioredoxin systems resulted in embryonic lethality in mice [8-11] or in flies [18,19]. Thus, we set out to evaluate the nematode C. elegans as an amenable model to study the thioredoxin systems in a whole organism.

A homology search through the C. elegans genome identified clear orthologues of mammalian thioredoxins TRX-1, TRX-2, TXL-1 and ERDJ5, while sperm-specific thioredoxins SPTRX-1, SPTRX-2, SPTRX-3 and TXL-2, all present in mammalian spermatozoa, were absent [6]. Similarly, putative orthologues of cytoplasmic and mitochondrial thioredoxin reductases, TRXR-1 and TRXR-2 were also present in the C. elegans genome [6,20], while TGR, a fusion protein of a glutaredoxin and a thioredoxin reductase domain, highly expressed in mammalian testis, is not present in worms [21]. Crawling C. elegans sperm lacks a flagellar propulsive system characteristic for mammalian sperm [22], thus explaining the absence in the nematode genome of putative orthologues of sperm-specific SPTRX-1, SPTRX-2, SPTRX-3, TXL-2 and TGR. These conservation patterns described above thus provide strong evidence for the essential role of the ubiquitous/general thioredoxin systems in eukaryotic cells.

As a first approach we aimed to characterize the C. elegans gene trx-1 (B0228.5), which gave the highest homology score when compared to human TRX-1. Although no ESTs have been isolated for this gene, two splice variants (named a and b) have been reported (http://www.wormbase.org). The two variants differ in only the first 14 amino acid residues (Figure 1A). The C. elegans trx-1 messages consist of 24 nt (a variant) and 68 nt (b variant) of 5´ UTR, 348 nt (a variant) and 345 nt (b variant) of ORF and 180 nt of 3´ UTR (common between both the a and b variants) including a non-canonical polyadenylation signal [23] (Supplemental Figure 1). We analyzed both the trx-1a and trx-1b 5´ UTRs...
by RT-PCR and 5’ RACE and could not detect any evidence for SL1 or SL2 trans-splicing. *trx-1a* is organized into 3 exons in the *C. elegans* genome, while the *trx-1b* variant consists of 4 exons (Figure 1B).

Human TRX-1 is ubiquitously expressed in the cytoplasm of all human cells. It can translocate into the nucleus upon stimulation [24]. To determine the precise tissue and cellular expression pattern of *C. elegans* *trx-1* we used a translational GFP fusion construct to generate transgenic worms. Stable transgenic lines expressing the *trx-1::gfp* translational fusion always resulted in GFP expression in a single pair of amphid neurons (Figure 2A and 2B). A detailed anatomical analysis identified the ciliated chemosensory neuron pair ASJ-left and -right (ASJL, ASJR) as the cells expressing GFP. Fluorescence was readily detected in cilia, dendrites, cell bodies (cytoplasm and nucleus) and axons of the ASJ neurons (Figure 2A and 2B). GFP fluorescence co-localizing with the fluorescent dye DiI in only one neuron pair, the ventral-most of the fluorescent dye-filling neurons, further confirmed ASJ as the neuron type expressing *trx-1* in *C. elegans* (data not shown). A detailed sex- and stage-specific analysis of GFP expression showed that GFP fluorescence is first detectable in both male and hermaphrodite in late three-fold stage embryos, immediately prior to hatching. *trx-1::gfp* remains expressed in ASJ neurons through all stages, including dauer, until the animal dies. ASJ is a ciliated neuron type that extends its dendrite to the very tip of the worm head where cilia are exposed directly to environment [25]. As worms hatch they start receiving (chemical) cues from their surroundings. The fact that *trx-1* expression is triggered at this point supports a potential role for this gene in the pathways that control the integration and transduction of environmental signals.

To some extent (in most transgenic animals at all larval stages and in adults, but not in dauers) we have also detected GFP fluorescence in the intestine, readily distinguishable from gut autofluorescence, typically in the posterior-most intestinal cells. However, since the number of intestinal cells expressing GFP varied greatly between individuals, we believe that the intestinal GFP expression pattern could be due to genetic mosaicism, given that the transgenic animals analyzed in this study carry only extrachromosomal arrays and not integrated transgenes.

The fact that human TRX-1 is ubiquitously expressed in all cell types, while *C. elegans* TRX-1 is found in ASJ neurons and to some extent in the posterior-most part of the intestine, could mean that *C.
elegans TRX-1 is not a functional orthologue of human TRX-1, despite the high degree of sequence conservation. We speculate that C. elegans TRX-1 could have arisen from a common TRX-1 ancestor and then have acquired a specific function in neurons. Further studies of the remaining thioredoxins encoded in the C. elegans genome will clarify this point.

To determine whether both trx-1a and trx-1b gene variants are expressed from the trx-1 promoter we used peptide antibodies targeting the specific N-termini of the TRX-1a and TRX-1b protein variants (Figure 1A) on western blots of extracts of the transgenic worm strain OE3010 expressing the trx-1::gfp translational fusion. As shown in Figure 2C, we were able to detect only the TRX-1b::GFP fusion protein, but not TRX-1a::GFP. This result, together with the fact that in the genome of C. briggsae, a related nematode species, only homologous regions are found that match the 5’ portions of the C. elegans trx-1b gene variant but not that of the trx-1a gene variant, strongly suggests that it is mainly TRX-1b protein which is expressed from the C. elegans trx-1 promoter.

Thioredoxins are redox proteins that function as general protein-disulfide reductases by using the reducing power of NADPH via thioredoxin reductase [1]. To demonstrate that C. elegans trx-1 encodes a functional thioredoxin, we expressed TRX-1b in bacteria and evaluated its capability of reducing the disulfide bonds of insulin in the presence of a mammalian thioredoxin reductase and NADPH. As shown in Figure 3, C. elegans TRX-1b is able to reduce insulin, albeit at a lower ratio as compared to mammalian TRX-1. As most of the conserved residues for maintenance of three-dimensional structure and enzymatic activity are conserved in C. elegans TRX-1b (Figure 1A), the slower kinetics could be explained by the use of the heterologous mammalian thioredoxin reductase in our assay (C. elegans thioredoxin reductases are not yet properly characterized). We conclude that C. elegans trx-1 encodes a functional thioredoxin.

ASJ neurons have been implicated in the decision-making process of entering into and exiting from the dauer larval stage [12,26]. The dauer larva is an alternative larval stage that is triggered by adverse environmental conditions or by hormonal cues [27]. ASJ neurons have also been shown to regulate longevity in worms. Ablation of ASJ neurons completely suppresses the lifespan extension produced by ablation of ASI neurons [16]. To elucidate the function of C. elegans TRX-1, we evaluated transient loss-of-function phenotypes arising from inactivating the trx-1 gene by using the bacterial
RNAi feeding technique [28] in N2 wild type, as well as in RNAi hypersensitive *eri-1* and *rrf-3* mutant backgrounds [29,30]. Among many potentially visible phenotypes looked for in those three strains we only observed a reproducible decrease in lifespan (data not shown). To fully address the function of *C. elegans* TRX-1, we used the mutant worm strain VZ1 *trx-1(ok1449)*, which carries a genomic deletion removing part of the proximal *trx-1* promoter region and all but the last exon of the *trx-1* gene (Figure 1B). This deletion results in a complete absence of TRX-1 protein as shown by western blot (Figure 4A). *trx-1(ok1449)* is therefore a null mutant allele. *trx-1* mutant worms develop and behave apparently normally under standard laboratory conditions. For example, they do not form dauer larvae in favorable environmental conditions, like in abundance of food. We then subjected *trx-1* mutants to conditions that induce dauer formation, such as starvation and exposure to dauer pheromone. Under both conditions, *trx-1* mutants produced dauers in similar numbers as compared to wild-type controls. Furthermore, upon placing animals back on food or upon removal of dauer pheromone, *trx-1* mutant dauers resumed growth normally (data not shown). We conclude that *trx-1* mutants are neither Daf-d (dauer-defective) nor Daf-c (dauer-constitutive). In addition to the dauer larval stage, *C. elegans* is also able to arrest at the L1 larval stage for several weeks when eggs hatch in the absence of food [31]. We also studied whether *trx-1* mutants had any defect in L1 larval arrest upon starvation, but observed no difference when compared to wild-type controls (data not shown). Next, based on our previous RNAi data we studied the longevity of *trx-1* mutants. As shown in Figure 4B and in Supplemental Table 1, *trx-1* mutants display a statistically significant decrease in both mean and maximum lifespan when compared to N2 wild type, which was not as pronounced as in well characterized short-lived *daf-16* mutants. Conversely, worms transgenically producing TRX-1b::GFP fusion protein in ASJ neurons have a marked increase in mean lifespan while their maximum lifespan is similar to that of N2 wild type. These results directly implicate *trx-1* in the ASJ-dependent mechanisms that regulate and control longevity.

Thioredoxins have already been proposed to play a role in longevity as transgenic mice expressing human TRX-1 live longer [32]. Although the mechanisms by which mammalian TRX-1 can affect longevity are not known, it has been proposed that this effect might be due to the modulation of the transcriptional activity of redox-sensitive transcription factors such as NF-kB, AP-1 and HIF-1, which
are activated during aging [33]. Furthermore, calorie restriction, the only condition that promotes longevity in all animal kingdoms, has been shown to inhibit mammalian TRX-1 translocation into the nucleus, thereby supporting the idea that TRX-1 redox-dependent activation of specific transcription factors plays an important role in lifespan modulation [33]. Another interesting possibility relies on the antioxidant capabilities of thioredoxins, as a causal connection between oxidative damage and organismal aging has clearly been demonstrated to exist [34]. The fact that *C. elegans trx-1* is expressed in ASJ chemosensory neurons, cells involved in lifespan extension [16], supports a regulatory or signaling role for the TRX-1 protein in the pathway(s) that control aging rather than a protective function as antioxidant, as such a protective function would be expected to be widely distributed in all worm cells.

With regard to dauer formation (ASJ neurons are dauer-promoting neurons) one might expect that a null mutation in a gene specifically expressed in those neurons could result in a dauer phenotype. Surprisingly, a *trx-1* null mutant is neither Daf-d nor Daf-c. One possible explanation could be that *trx-1* is involved in ASJ functions that specifically influence the aging, but not the dauer formation process. Another possibility is that the dauer formation assays we employed so far were not sensitive enough and that *trx-1* dauer phenotypes might only be revealed under sensitized (e.g. double mutant) backgrounds. Current efforts in our labs are directed toward combining the *trx-1* mutation with other mutations in genes known to regulate the dauer formation and/or the aging process, such as *daf-2* and *daf-16*, or *daf-11* and *daf-21* [13]. Particularly mutations in the last two genes are especially interesting for searching for potentially synthetic phenotypes with the *trx-1* mutation, as the genes *daf-11* and *daf-21* have been proposed to exert their effects in ASJ neurons [35].

In summary, the localization of *C. elegans* TRX-1 in ASJ neurons, the first predominantly neuron-expressed thioredoxin ever reported in any metazoan, together with the decreased lifespans of *trx-1* mutants provide an excellent model to dissect at the genetic level the role of thioredoxins in the aging process.
4. ACKNOWLEDGEMENTS

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5. REFERENCES


6. FIGURE LEGENDS

FIG. 1 A) Comparison of C. elegans TRX-1 with other eukaryotic thioredoxins. Identical residues are shaded in grey. The typical WCGPC active site of thioredoxins is boxed. Residues important for three-dimensional structure or function are highlighted with an asterisk. Peptide fragments used to generate a and b variant-specific antibodies are indicated by arrows. See also Supplemental Figure 1. B) Genomic structure of the C. elegans trx-1 locus on chromosome II and identification of the trx-1 (ok1449) deletion allele. Exons of the two gene variants (a and b) are shown as numbered shaded boxes connected by lines representing the introns. 5’ and 3’ UTRs are shown as white boxes. The trx-1 (ok1449) deletion allele is indicated by an interrupted line. The inset shows a PCR amplification of N2 wild type and VZ1 trx-1 (ok1449) genomic DNA using primers flanking the deletion (arrows in the schematic). A schematic of the translational trx-1::gfp fusion construct, used for the production of transgenic animals, which mainly express TRX-1b::GFP fusion protein, is shown at the bottom.

FIG. 2 A and B) Expression of trx-1::gfp fusion genes. Nomarski (top panels) and fluorescence (bottom panels) images of trx-1::gfp transgenic animals at the (A) dauer larval stage and (B) L4 larval stage. The transgenic strain OE3010 is shown. Only ASJ neurons show GFP expression in the entire cell: cilia, dendrites, cell bodies and axons. Scale bars represent 10 µm. C) Detection of the TRX-1b::GFP fusion protein variant from trx-1::gfp transgenic animals by immunoblotting. C. elegans total cell extracts were separated by SDS-PAGE, blotted and probed with peptide-specific antibodies against the TRX-1a and TRX-1b protein variants. For recombinant GST-TRX-1a and GST-TRX-1b, 10 ng were used. For C. elegans N2 wild type cell extracts 10 µg were loaded, for the strain OE3010 20 µg where used. Note that the peptide-specific antibodies are not sensitive enough to detect endogenous protein levels, present exclusively in one pair of sensory neurons (ASJL, ASJR) (data not shown).
FIG. 3 Disulfide-reducing enzymatic activity of *C. elegans* TRX-1b protein. Different concentrations of recombinant human TRX-1 (used as a control) and *C. elegans* TRX-1b were assayed in the presence of NADPH and calf thymus TRXR-1 as electron donors. Enzymatic activity is determined by measuring the production of reduced insulin [17].

FIG. 4 A) Demonstration by immunoblotting that the *trx-1 (ok1449)* deletion is a null allele mutation. *C. elegans* total cell extracts were separated by SDS-PAGE, blotted and probed with specific antibodies against recombinant full-length TRX-1b protein. For recombinant TRX-1b, 10 ng were used, while for *C. elegans* strains cell extracts 50 µg were loaded. The *C. elegans* strains RB1332 and VZ1 both carry the *trx-1 (ok1449)* deletion allele. B) Effect of the *trx-1 (ok1449)* deletion allele on worm lifespan. A null allele mutation, *trx-1 (ok1449)*, shortens *C. elegans* mean and maximum lifespan when compared to N2 wild type. This effect is not as pronounced as in *daf-16* mutants, used as control for short lifespans. Conversely, a transgenic strain overexpressing TRX-1b::GFP has a marked increase in mean lifespan, while maximum lifespan does not differ from that of N2 wild type. Several longevity assays were performed and a representative experiment is shown. Strains used were: N2 wild type (wt), CF1038 (*daf-16*), VZ1 (*trx-1*), OE3010 (carrying a translational *trx-1::gfp* fusion transgene, which overexpresses TRX-1b::GFP protein), CB1370 (*daf-2*). See also Supplemental Table 1.
Figure 1

**A**

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*Decoration #1*: Shade (with solid light gray) residues that match the Consensus exactly.

**B**

- 5'-UTR
- 3'-UTR
- 860 bp deletion

**trx-1 (ok1449)**

**N2 (wild type)**

**trx-1 (ok1449)**

** trx-1a**

**trx-1b**

**trx-1 (ok1449)**

**trx-1::gfp**
Figure 4

A

Recombinant TRX-1b  
N2 (wild type)  
RB1332 (trx-1)  
VZ1 (trx-1)  
0x outcrossed  
6x outcrossed

kDa

12 -

B

Fraction alive

Days of adulthood

wt  
daf-16  
trx-1  
trx-1::gfp  
daf-2

Miranda-Vizutte et al., Figure 4