THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 287, NO. 11, pp. 8371–8382, March 9, 2012

Caenorhabditis elegans RNA-processing Protein TDP-1 Regulates Protein Homeostasis and Life Span*[S]◆

Received for publication, October 12, 2011, and in revised form, December 29, 2011 Published, JBC Papers in Press, January 9, 2012, DOI 10.1074/jbc.M111.311977

Tao Zhang[‡], Ho-Yon Hwang[‡], Haiping Hao[§], Conover Talbot, Jr.[§], and Jiou Wang[‡1]

From the [‡]Department of Biochemistry and Molecular Biology, Bloomberg School of Public Health, The Solomon H. Snyder Department of Neuroscience, and [§]Institute for Basic Biomedical Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 212105

Background: TDP-1 is the worm ortholog of TARDBP, which is a key protein in human neurodegeneration, including ALS and FTLD.
Results: Loss of TDP-1 alleviates proteotoxicity in worm models and extends life span.
Conclusion: TDP-1 has a function in regulating protein homeostasis and aging.
Significance: Learning the novel functions of TDP-1 may advance understanding of the role of RNA processing in protein homeostasis and aging.

Transactive response DNA-binding protein (TARDBP/TDP-43), a heterogeneous nuclear ribonucleoprotein (hnRNP) with diverse activities, is a common denominator in several neurodegenerative disorders, including amyotrophic lateral sclerosis and frontotemporal lobar degeneration. Orthologs of TDP-43 exist in animals ranging from mammals to invertebrates. Here, we systematically studied mutant *Caenorhabditis elegans* lacking the nematode TDP-43 ortholog, TDP-1. Heterologous expression of human TDP-43 rescued the defects in *C. elegans* lacking TDP-1, suggesting their functions are conserved. Although the *tdp-1* mutants exhibited deficits in fertility, growth, and locomotion, loss of *tdp-1* attenuated defects in several *C. elegans* models of proteotoxicity. Loss of *tdp-1* suppressed defects in transgenic *C. elegans* expressing TDP-43 or CuZn superoxide dismutase, both of which are associated with proteotoxicity in neurodegenerative diseases. Loss of *tdp-1* also reduced defects in mutant animals lacking the heat shock factor HSF-1. Transcriptional profiling demonstrated that the loss of TDP-1 altered expression of genes functioning in RNA processing and protein folding. Furthermore, the absence of *tdp-1* extended the life span in *C. elegans*. The life span extension required a FOXO transcriptional factor DAF-16 but not HSF-1. These results suggest that the *C. elegans* TDP-1 has a role in the regulation of protein homeostasis and aging.

Adult-onset neurodegenerative diseases are characterized by age-dependent and progressive loss of neurons. Proteinaceous inclusions are a common pathological hallmark in many neurodegenerative diseases. From amyloid-β peptides in Alzheimer disease (1) to many aggregation-prone proteins in different neurodegenerative diseases, there is much evidence suggesting that the maintenance of proteostasis is critical for these diseases (2). As a risk factor for neurodegenerative diseases, aging is also associated with regulation of protein homeostasis. In *Caenorhabditis elegans*, aging is dependent on heat shock factor 1 that controls the expression of molecular chaperones important for protein folding (3, 4). In addition, insulin/IGF-1 signaling, a conserved pathway known to influence life span, has been shown to influence protein aggregation and neurodegeneration in animal models (3, 5, 6). Therefore, alterations in protein quality control appear to be a mechanism that links aging to neurodegeneration.

ALS[2] is an age-dependent neurodegenerative disease characterized by progressive degeneration of motor neurons, with ~10% of all cases considered familial. To date, several genes have been linked to familial ALS, including CuZn superoxide dismutase (*SOD1*) (7), alsin (8, 9), dynactin (10), senataxin (11), VAMP-associated protein B (12), transactive response DNA-binding protein (*TARDBP*/TDP-43) (13, 14), fused in sarcoma/translocated in sarcoma (*FUS*/TLS) (15, 16), optineurin (17), valosin-containing protein (*VCP*) (18), ubiquilin 2 (19), and a hexanucleotide repeat expansion in the gene *C9orf72* (20, 21). Two themes have emerged among these ALS genes. First, perturbation of RNA processing might be implicated in ALS cases linked to *TARDBP*/TDP-43, *FUS*, and the hexanucleotide repeat expansion in *C9orf72* (20–23). Second, proteinaceous inclusions have been reported as a common pathology in ALS cases linked to *SOD1* (24, 25), *TARDBP*/TDP-43 (13, 14), *FUS* (15, 16), optineurin (17), *VCP* (18), and ubiquilin 2 (19).

TDP-43-positive proteinaceous inclusions are also observed in other neurodegenerative disorders, including FTLD, Alzheimer, Parkinson, and Pick disease (22, 23). In ALS and FTLD, the TDP-43-positive inclusions and concurrent deple-

* This work was supported, in whole or in part, by National Institutes of Health Grants NS062089 and NS07432 (to J. W.). This work was also supported by the Robert Packard Center for ALS Research at Johns Hopkins, the Muscular Dystrophy Association, and The Johns Hopkins Claude D. Pepper Older Americans Independence Center.
◆ This article was selected as a Paper of the Week.
[S] This article contains supplemental Tables 1–6, Figs. S1–S9, and additional references.
[1] To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology, The Johns Hopkins University, 615 N. Wolfe St., E8410, Baltimore, MD 21205. Tel.: 410-502-0927; Fax: 410-955-2926; E-mail: jiouw@jhmi.edu.

[2] The abbreviations used are: ALS, amyotrophic lateral sclerosis; FTLD, frontotemporal lobar degeneration; RRM, RNA recognition motif; lf, loss of function; FC, fold change; Q-PCR, quantitative PCR; hnRNP, heterogeneous nuclear ribonucleoprotein.

tion of nuclear TDP-43 represent a major pathology of the degenerating tissues (13). Linkage of over 30 TDP-43 mutations to ALS has confirmed the pathogenic role of TDP-43 in neurodegeneration (22). However, it remains unclear whether TDP-43 contributes to neurodegeneration through a gain of toxicity or through loss of its normal function (22, 23).

TDP-43 has protein domains that are characteristic of hnRNP proteins, which were originally defined as a set of proteins that bind to nascent transcripts to form protein-RNA complexes (26). TDP-43 is reported to regulate the transcription of the HIV-1 genome and the SP-10 mouse promoter (27, 28). TDP-43 also plays a role in RNA splicing, including promoting exon skipping during the alternative splicing of the cystic fibrosis transmembrane regulator and apolipoprotein A-II transcripts, as well as promoting exon inclusion during the splicing of the spinal muscular atrophy protein transcript (29, 30). Primarily a nuclear protein, TDP-43 has also been localized to cytoplasmic RNA granules under stress (31–33). TDP-43 has been shown to stabilize the transcripts of low molecular weight neurofilament, histone deacetylase 6, and Atg7 (34–36). TDP-43 also binds to its own transcript and autoregulates its protein levels (37–39). Despite recent studies uncovering roles of TDP-43 in pre-mRNA processing and gene expression regulation (40), the full range of multifaceted TDP-43 functions remains to be elucidated, and their relevance in the neurodegenerative diseases is unclear (22, 23).

Overexpression of TDP-43 causes severe toxicity in many experimental model organisms, including yeast, worm, fly, zebrafish, mouse, and rat (41–53). TDP-43 is highly conserved from mammals to lower metazoans. Knock-out studies suggested that TDP-43 and its orthologs were required in early embryonic development of animals from mouse to *Drosophila* (35, 54–56). However, the *C. elegans* ortholog of TDP-43, TDP-1, is dispensable for survival. The physiological function of TDP-1 in *C. elegans* is currently unknown.

Here, we have systematically characterized loss-of-function *tdp-1* mutants to explore the function of TDP-1. We demonstrate that human TDP-43 and *C. elegans* TDP-1 are functionally conserved. Combining genetic analysis and expression profiling of the loss-of-function *tdp-1* mutants, we describe the role of *C. elegans* TDP-1 in regulating protein homeostasis and aging. In several models of proteotoxicity, loss of TDP-1 alleviated lethality, protein aggregation, and neuronal dysfunction. Loss of TDP-1 also extended life span. These findings may contribute to understanding of the function of this class of hnRNP proteins in protein homeostasis and aging.

EXPERIMENTAL PROCEDURES

C. elegans Strains—N2 Bristol and mutant *C. elegans* strains were cultured using standard conditions at 20 °C unless indicated otherwise. The mutant strains obtained from the *Caenorhabditis* Genetics Center were as follows: RB929 (*tdp-1*(*ok803*)), VC549 (*tdp-1*(*ok781*)), PS3551 (*hsf-1*(*sy441*)), CX51 (*dyn-1*(*ky51*)), and CF1038 (*daf-16*(*mu86*)). The strain FX4439 (*fust-1*(*tm4439*)) was received from the National Bioresource Project of Japan. The *tdp-1*(*ok803*) and *tdp-1*(*ok781*) animals were backcrossed with N2 at least four times. Integrated lines expressing human TDP-C25-YFP (*iwIs22*), human SOD1-G85R-YFP (*iwIs8*), or YFP only (*iwIs25*) driven by the *snb-1* promoter have been described previously (6).

The human TDP-43 transgenic *iwEx21*gf strain was made by injecting 20 μg/ml plasmid *Ptdp-1*::TDP-43 with 76 μg/ml of a 1-kb ladder DNA and 4 μg/ml *Pmyo-2*::RFP. The expression of human TDP-43 by *Ptdp-1*::TDP-43 was driven by the worm *tdp-1* promoter, which is defined as a 291-bp genomic DNA fragment between the start codon and the neighboring 5′ gene F44G4.3. This 291-base fragment followed by the human TDP-43 complementary DNA was cloned into the pPD30.38 vector (Fire Lab Vector, Addgene) using HindIII and XhoI sites to generate *Ptdp-1*::TDP-43. *Pmyo-2*::RFP was cloned by inserting the red fluorescent protein coding sequence into the pPD132.102 vector (Fire Lab Vector, Addgene) with a *myo-2* promoter to drive pharyngeal expression of red fluorescent protein. One line containing the *iwEx21*gf extrachromosomal array was further treated with 30 μg/ml trimethylpsoralen (Sigma) and two doses of 300 μJ of 365-nm UV light, and the resulting integrant *iwIs21*gf that stably expressed the transgene was isolated. The *iwIs21* integrated line was backcrossed with the N2 strain four times. The *iwEx21*gf and *iwIs21*gf strains showed similar phenotypes. Additionally, a *Ptdp-1*::YFP extrachromosomal array was generated using the same methods and conditions.

Prediction of Protein Domains—Predictions of nuclear localization signal and nuclear export signal for *C. elegans* TDP-1 were made based upon the criteria of bipartite nuclear localization signal with two clusters of basic amino acids separated by a linker of greater than 10 amino acids and the class 2 nuclear export signal consensus pattern $\phi X\phi X_2\phi X\phi$, in which the hydrophobic residue (ϕ) may be Leu, Ile, Val, Met, or Phe and preferably at least two of the four hydrophobic residues are Leu or Ile, respectively (see detailed information in the supplemental material). Prediction of RRM domains was based on their homology to human TDP-43 RRM domains (22).

Egg Laying at 25 °C and Hatching—*C. elegans* strains were cultured at 20 °C until they grew to the L4 larval stage. L4 larvae were individually transferred to new plates and then cultured further at 25 °C. These adults were allowed to lay eggs at 25 °C and were then transferred to new plates every 24 h thereafter until they stopped producing eggs (3 days). The number of hatched larvae and dead eggs was counted after 24 h of incubation at 20 °C following the transfer of adults.

Growth Speed at 25 °C—Twenty five synchronized *C. elegans* eggs laid within a 3-h period at 20 °C were transferred to 25 °C. The number of animals that had reached adulthood was counted after 48, 72, and 96 h.

Locomotor Assay—*C. elegans* at the L4 larval stage were transferred to M9 buffer (3 mg/ml KH_2PO_4, 6 mg/ml Na_2HPO_4, 5 mg/ml NaCl, and 1 mM $MgSO_4$) to observe their thrashing movements. To quantitate their relative motility, the number of the thrashes was counted for 1 min after 1 min of adaptation. A thrash was counted when both the head and the tail bent more than 45° away from the anterior-posterior axis.

Microscopy—For *C. elegans* low magnification imaging, Leica M165 FC stereo microscope, Leica DFC310 FX camera, and Leica Application Suite were used. For high magnification imaging, animals were immobilized with 100 mM muscimol and

examined with a Zeiss AxioObserver Z1 with Apotome imaging system. For nuclear staining, *C. elegans* were fixed in 500 μl of methanol on dry ice for 5 min. Following three washes of PBS with 0.1% Tween 20 (PBST), the fixed worms were mounted onto microscope slides in a solution of 2.5% 1,4-diazobicyclo[2,2,2]-octane in 100 mM Tris, pH 8.8, with 50% glycerol and 0.2 μg/ml 4′,6′-diamidino-2-phenylindole dihydrochloride (DAPI). For the quantitation of the fluorescent protein aggregates, the overall fluorescence of the inclusion bodies was measured by ImageJ software (National Institutes of Health).

Protein Aggregate Extraction Assay—*C. elegans* were collected by washing them off the NGM plates using M9 buffer. After five further washes with M9 buffer, worm pellets were resuspended in 200 μl of extraction buffer (10 mM Tris-HCl, pH 8.0, with 1 mM EDTA, 100 mM NaCl, and 0.5% Nonidet P-40) supplemented with mini-EDTA protease inhibitor mixture (Roche Applied Science) and 50 mM iodoacetamide (Sigma) and then homogenized by sonication on ice. The lysates were then transferred to an Airfuge (Beckman Coulter) and centrifuged at 25 p.s.i. (~130,000 × *g*) for 5 min. The supernatant was saved as the "S1" fraction. The remaining pellets were sonicated again in the extraction buffer and ultracentrifuged (~130,000 × *g*) for 5 min. The final pellet "P2" samples were resuspended in 100 μl of buffer containing 10 mM Tris-HCl, pH 8.0, with 1 mM EDTA, 100 mM NaCl, 0.5% Nonidet P-40, 0.5% deoxycholic acid, and 2% SDS. The S1 and P2 fractions were subjected to SDS-PAGE. The immunoblotting analyses were performed using 1:2,000 anti-YFP (BD Biosciences) and 1:1,000 anti-TDP-43 (Proteintech Group, Chicago) antibodies. Proteins were visualized using enhanced chemiluminescence.

Microarray—Total RNAs were extracted with TRIzol (Invitrogen) from triplicates of N2 and outcrossed *tdp-1*(*ok803*lf) *C. elegans* and purified with the RNeasy kit (Qiagen). The RNAs were labeled using the 3′ IVT Express labeling protocol described by Affymetrix. Briefly, 100 ng of total RNA was used to synthesize first strand cDNA using T7 oligo(dT) oligonucleotides and first strand enzyme mix (Affymetrix). The resulting single strand cDNA was subsequently converted into double strand cDNA using DNA polymerase and RNase H. The double strand cDNA was used to generate and label linearly amplified RNA through *in vitro* transcription followed by purification using magnetic beads. 15 μg of the labeled amplified RNA was fragmented and hybridized to the Affymetrix *C. elegans* genome array. Affymetrix Fluidics Station 450 was used to wash and stain the chips, removing the nonhybridized target and incubating with a streptavidin-phycoerythrin conjugate to stain the biotinylated amplified RNA. The staining was further amplified using a biotinylated anti-streptavidin antibody, followed by a second staining step with a streptavidin-phycoerythrin conjugate. Fluorescence was detected using the Affymetrix G3000 GeneArray Scanner, and image analysis of each GeneChip was performed through the Affymetrix GeneChip Command Console version 3.4 software.

The management and statistical analysis of the microarray data were performed using the Partek Genomic Suite (Partek Inc., St. Louis) and Spotfire DecisionSite software (TIBCO Software Inc., Palo Alto, CA). For the Gene Ontology analysis, the annotation file for the *C. elegans* genome was downloaded from the website of the Gene Ontology Consortium August, 2011.

The microarray data were also analyzed through the use of Ingenuity Pathways Analysis (Ingenuity Systems). For the network analysis, the microarray data set containing *C. elegans* gene identifiers and expression values was uploaded into the application. Each identifier was mapped to its corresponding gene product in the Ingenuity Knowledge Base. The molecules that met the selection criteria (*e.g.* expression fold changes above a threshold) were overlaid onto a global molecular network developed from information contained in the Ingenuity Knowledge Base. The relevant networks of selected molecules were then algorithmically generated based on their connectivity. Similarly, for the functional analysis, selected molecules that were associated with biological functions in the Ingenuity Knowledge Base were analyzed to identify the biological functions that were most significant to the data set. The raw data of the present microarray analysis can be found at the NCBI gene expression and hybridization array data repository (GEO, www.ncbi.nlm.nih.gov, accession number GSE34113).

Quantitative Reverse Transcription and Q-PCR—*C. elegans* was harvested, and RNA was isolated using a phenol/chloroform extraction with TRIzol reagent (Invitrogen), followed by purification using RNeasy mini kit (Qiagen). A two-step RT-PCR was employed to assess relative changes in transgenic transcripts using iScript cDNA synthesis kit and SYBR Green Supermix (Bio-Rad). Standard curves were generated for all primers used, and the worm *gdh-1* gene was used as the control.

Life Span Assay—Synchronized *C. elegans* eggs were isolated within a 3-h period of egg laying for life span assay. Animals were considered dead if they showed no response when probed with a platinum pick. At least 90 animals were used for each experiment. The animals were censored if they crawled out of the plate, had a ruptured vulva, or died as "bags of worms" with larvae hatching inside the adults. For *hsf-1*(*sy441*lf) mutants, which die at a high frequency as bags of worms, 100 μg/ml 5-fluorodeoxyuridine (Sigma) was included in the medium to prevent reproduction. Synchronized late L4 larvae were transferred from normal NGM plates to NGM plates containing 5-fluorodeoxyuridine. The life span data were analyzed with Prism 3 software.

Statistical Analysis—*p* values for all phenotypic analyses were obtained using Student's *t* test, with the exception of the life span data, for which the log rank test was used. For the microarray data, Student's *t* test was used to analyze the gene expressions. For the analysis of the transcriptome profiles using Gene Ontology and Ingenuity Pathways Analysis, Fisher's exact test was used.

RESULTS

C. elegans tdp-1 Is Required for Optimal Fertility, Growth, and Locomotion—Our bioinformatic analyses and searches in gene homology databases suggested that *tdp-1* (sequence name F44G4.4) is the sole ortholog of human TDP-43 in the *C. elegans* genome (Fig. 1*A* and supplemental Fig. S1). *C. elegans tdp-1* encodes a protein that has the same length, 414 amino acids, as its human ortholog. Human TDP-43 and *C. elegans* TDP-1 share the same alignment of protein domains character-

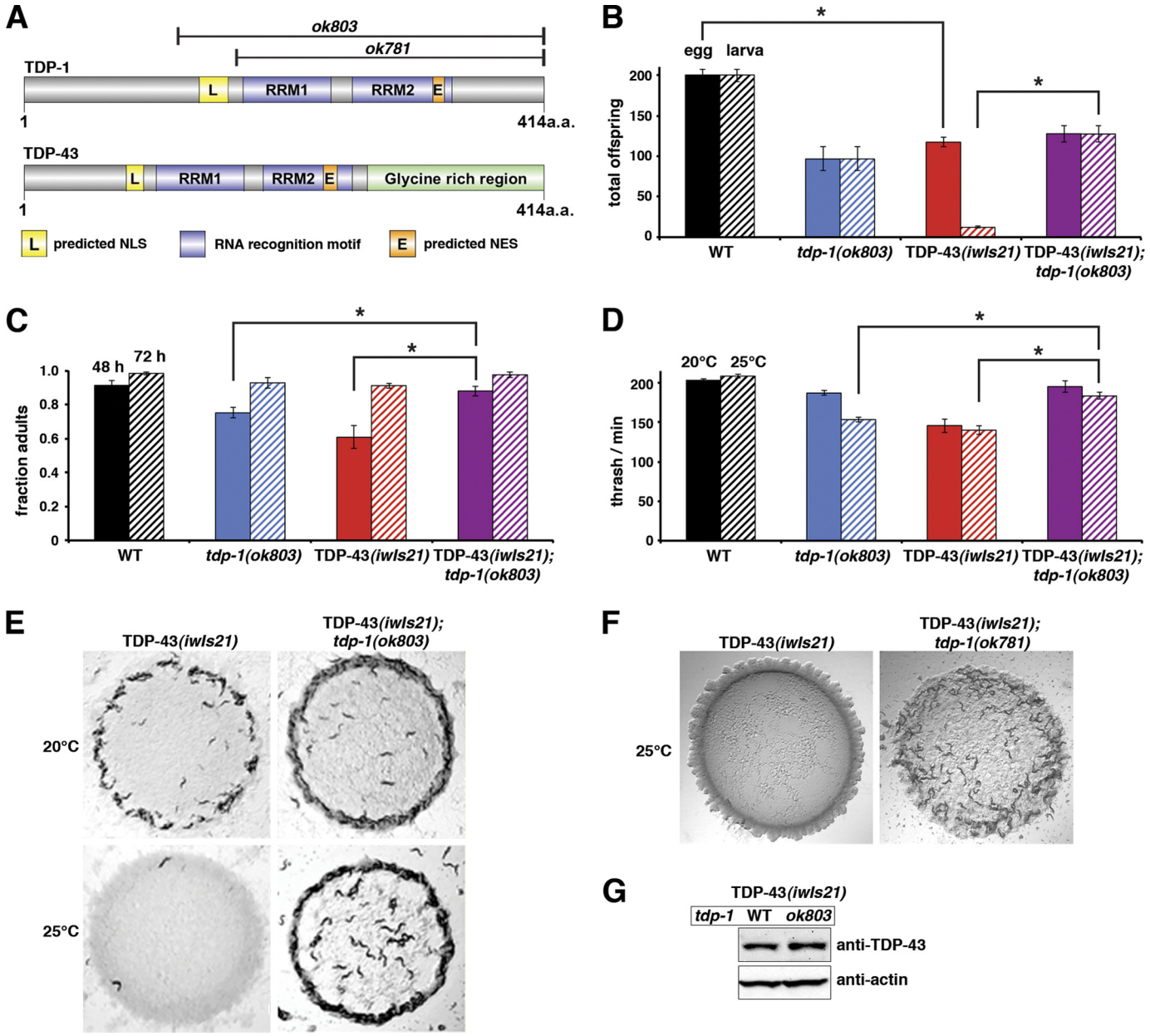

FIGURE 1. ***C. elegans* TDP-1 and human TDP-43 are functionally conserved.** *A,* schematic diagram of *C. elegans* TDP-1 and human TDP-43 proteins. Predictions of domains are described under "Experimental Procedures." Loss of TDP-1 protein regions caused by *tdp-1* mutant alleles, *ok803* and *ok781*, are indicated. NLS, nuclear localization signal; NES, nuclear export signal. *B–F,* loss of *C. elegans* TDP-1 (*ok803* or *ok781*) and expression of human TDP-43 (*iwIs21*) rescue each other's phenotypic defects in egg-laying, growth, and locomotion. *B,* mean number of eggs (*solid bars*) and corresponding larvae (*hatched bars*) produced when parental hermaphrodites at the L4 stage were transferred from 20 to 25 °C (± S.E.; $n = 10$). *, $p < 0.0001$. *C,* mean fraction of eggs laid at 20 °C reaching adulthood after 48 (*solid bars*) or 72 h (*hatched bars*) of growth at 25 °C (± S.E.; $n = 5$). *, $p < 0.02$. *D,* mean locomotor activity of L4 larvae, as indicated by the number of thrashes per min in liquid (± S.E.; $n = 25$) following culture at 20 °C (*solid bars*) and 25 °C (*hatched bars*). *, $p < 0.0001$. *E* and *F,* representative images of animals grown at 20 and 25 °C for 3 days. *G,* total TDP-43 protein level in TDP-43(*iwIs21*gf) animals is not affected by *tdp-1*(*ok803*lf).

istic of an hnRNP protein, including two RRM domains, a nuclear localization signal proximate to the N terminus, and a nuclear export signal embedded in the second RRM domain.

We obtained two mutant alleles of *C. elegans tdp-1*, *ok803* and *ok781*, each of which remove ~1.2 kb from *tdp-1*, including the two RRM domains and the nuclear export signal (Fig. 1*A* and supplemental Fig. S1). Both deletions are probably null mutations. These *C. elegans tdp-1* loss-of-function (lf) mutants exhibited discernible defects in fertility, growth, and locomotion. Most of the *tdp-1*(lf) data shown here were obtained using *ok803*, and the results were verified using the other allele *ok781*.

First, loss of *tdp-1* led to lower fertility in *C. elegans*. The *tdp-1*(*ok803*lf) hermaphrodites generated only half as many eggs as N2 wild-type (WT) animals, likely as a result of an early depletion of sperm (Fig. 1*B* and supplemental Fig. S2). Second, loss of function of *tdp-1* led to slower growth. At 48 h after egg laying, ~75% of *tdp-1*(*ok803*lf) animals had reached adulthood, as compared with ~91% of N2 WT animals (Fig. 1*C*). Finally, loss of *tdp-1* led to a locomotor deficit, which was exacerbated when the animals were cultured at 25 °C. Specifically the rate of thrashing by *tdp-1*(*ok803*lf) animals was ~74% that of N2 WT animals (Fig. 1*D*). Thus, intact *tdp-1* is required for optimal

developmental and physiological processes in *C. elegans*, including fertility, growth, and locomotion.

Human TDP-43 and C. elegans TDP-1 Are Functionally Conserved—To examine whether human TDP-43 could functionally replace TDP-1 in *C. elegans*, we expressed human TDP-43 under the control of the endogenous *tdp-1* promoter. Following generation of extrachromosomal transgenic arrays expressing human TDP-43, we further developed a stable integrated transgene *iwIs21.* Next, we constructed strains containing both the gain-of-function TDP-43(*iwIs21*gf) transgene and *tdp-1*(lf) mutations. The TDP-43(*iwIs21*gf) transgene moderately improved the fertility defects in the *tdp-1*(*ok803*lf) mutant (Fig. 1*B*, $p = 0.10$). Moreover, the human TDP-43(*iwIs21*gf) transgene rescued the slow growth phenotype in loss-of-function mutant *tdp-1*(*ok803*) (Fig. 1*C*). In addition, the TDP-43(*iwIs21*gf) transgene rescued the locomotor deficit caused by the *tdp-1*(*ok803*lf) mutation at 25 °C (Fig. 1*D*). These data demonstrated that human TDP-43 could substitute for *C. elegans* TDP-1, indicating that they are functional orthologs.

Transgenic expression of human TDP-43 in *C. elegans* can induce locomotor and developmental defects. The TDP-43(*iwIs21*gf) transgenic *C. elegans* exhibited locomotor defects as compared with WT animals. The rate of thrashing by the TDP-43(*iwIs21*) animals was ~70% that of WT animals at 25 °C (Fig. 1*D*). Notably, the TDP-43(*iwIs21*gf) transgene caused profound temperature-dependent developmental defects. When parental hermaphrodites harboring the TDP-43(*iwIs21*gf) transgene were transferred from 20 to 25 °C, most offspring died during embryogenesis (Fig. 1*B*), and many surviving larvae failed to grow to adulthood (Fig. 1*E*). By comparison, if the temperature elevation occurred after the parental hermaphrodites laid eggs at 20 °C, most of the offspring could grow to adulthood, albeit at a slower rate than the N2 WT animals (Fig. 1*C*). For example, when eggs laid at 20 °C were hatched subsequently at 25 °C, ~60% of TDP-43(*iwIs21*) animals reached adulthood at 48 h after egg laying, as compared with ~91% of WT animals (Fig. 1*C*). By a series of 25 °C incubations of varying duration administered at several time points during development, elevation of temperature for 2–3 h near the time of fertilization was found to be sufficient and necessary to induce embryonic arrest (supplemental Fig. S3). This temperature-dependent phenotype was solely dependent on the TDP-43 transgene but not its chromosomal integration site, because examination of extrachromosomal array transgenic animals demonstrated similar defects. The profound temperature-sensitive developmental defect in TDP-43(*iwIs21*gf) animals is consistent with the notion that the toxicity of TDP-43 protein is dependent on its misfolding (6), which can be exacerbated by rapid temperature elevation.

Next, we examined the expression pattern of the *tdp-1* gene. The promoter region of the *tdp-1* gene was cloned to drive expression of a YFP reporter. With the engineered *tdp-1* promoter, the YFP reporter was expressed in embryos before hatching. In both larvae and adults, the reporter expression was found in multiple *C. elegans* tissues, including body wall muscles, pharynx, and neurons (supplemental Fig. S4). It should be noted that the endogenous expression of *tdp-1* that is not recapitulated by the engineered promoter is still possible.

A diverse set of model organisms was shown to be sensitive to elevated levels of TDP-43 expression (41–53). If human TDP-43 is functionally redundant with TDP-1, lowering the level of endogenous TDP-1 would attenuate the toxicity of the TDP-43 transgene in *C. elegans*. Indeed, the *tdp-1*(*ok803*lf) mutation suppressed the observed defects in the TDP-43(*iwIs21*gf) animals. First, loss of *tdp-1* attenuated the embryonic lethality and slow growth phenotype observed in transgenic TDP-43(*iwIs21*gf) animals at 25 °C (Fig. 1, *B, C,* and *E*). Second, loss of *tdp-1* also suppressed the locomotor deficit observed in TDP-43(*iwIs21*gf) animals (Fig. 1*D*). Finally, another independent loss-of-function mutation *tdp-1*(*ok781*lf) suppressed the developmental defects caused by TDP-43(*iwIs21*gf) at 25 °C (Fig. 1*F*). The protein expression level of TDP-43 was not changed by the presence of the *tdp-1*(*ok803*lf) mutation (Fig. 1*G*). The insoluble protein aggregates in TDP-43(*iwIs21*) were not detected. These results showed that human TDP-43 can substitute for TDP-1 and that the removal of TDP-1 alleviates the toxicity of elevated levels of TDP-43, suggesting that human TDP-43 and *C. elegans* TDP-1 are functionally conserved.

Loss of tdp-1 Attenuates Neurotoxicity of Aggregation-prone Proteins—Next, we investigated whether loss of *tdp-1* confers any protection against the toxicity of TDP-C25, which is a 25-kDa carboxyl fragment of TDP-43 and a signature component of the TDP-43 positive inclusions in brain tissues from ALS and FTLD patients (13). TDP-C25 exhibits an unusually high propensity to form protein aggregates (6). In transgenic TDP-C25(*iwIs22*gf) *C. elegans* stably expressing the TDP-C25-YFP fusion protein under the pan-neuronal promoter of the *snb-1* synaptobrevin gene, TDP-C25-YFP forms discrete fluorescent protein aggregates in neuronal somas and axons (Fig. 2*B*) (6). TDP-C25(*iwIs22*gf) transgenic *C. elegans* exhibited severe locomotor deficits, which resulted from neurotoxicity of the misfolded proteins (6). Interestingly, loss of *tdp-1* significantly ameliorated the locomotor defects caused by TDP-C25(*iwIs22*gf) (Fig. 2*A*), suggesting a strong suppression of the neurotoxicity. In addition, the amount of fluorescent TDP-C25-YFP aggregates in neurons was reduced in TDP-C25(*iwIs22*gf);*tdp-1*(*ok803*lf) animals, as compared with animals carrying the TDP-C25(*iwIs22*gf) transgene alone (Fig. 2, *C* and *D*). Similar amelioration of TDP-C25 protein aggregation was also observed in animals carrying the independent loss-of-function mutation *tdp-1*(*ok781*) and the TDP-C25(*iwIs22*gf) transgene (supplemental Fig. S5). To examine the effect of loss of *tdp-1* on TDP-C25 aggregation, an aggregate extraction assay was used as described previously (6). When compared with the protein aggregates in animals carrying only the TDP-C25(*iwIs22*gf) transgene, insoluble TDP-C25 aggregates were significantly reduced in animals carrying both the TDP-C25(*iwIs22*gf) transgene and the loss-of-function mutation *tdp-1*(*ok803*) (Fig. 2*E*), but no change in soluble TDP-C25 protein levels was detected. These data suggest that loss of *tdp-1* may alleviate TDP-C25-associated neurotoxicity by suppressing protein aggregation.

We then asked whether the protective effect of loss of *tdp-1* on neurotoxicity associated with protein misfolding and aggregation was specific to the TDP-43 polypeptides. Mutations in

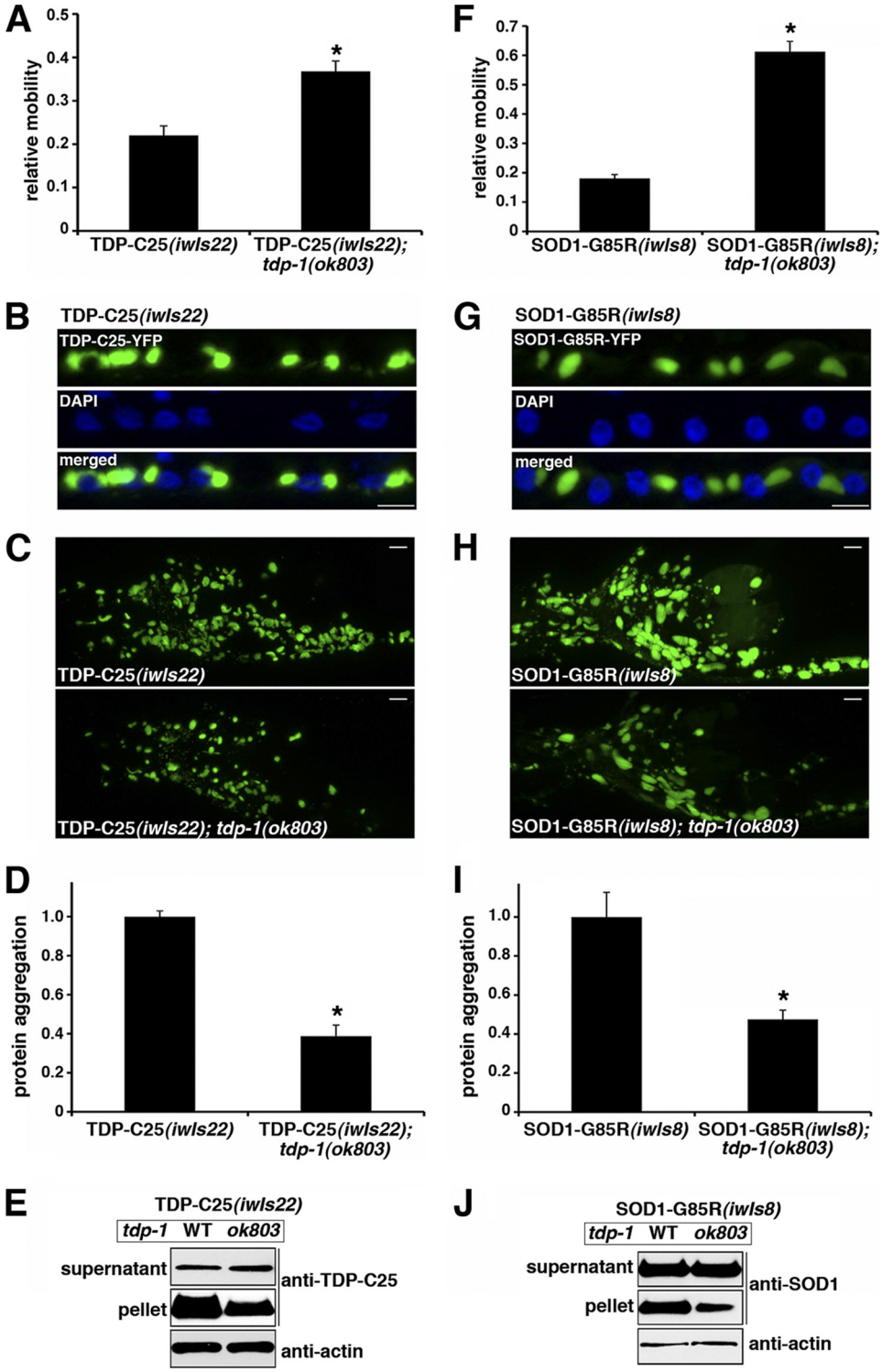

FIGURE 2. **Loss of *tdp-1* alleviates locomotor deficits and protein aggregation in *C. elegans* expressing neuronal TDP-C25(*iwIs22*gf) or SOD1-G85R(*iwIs8*gf).** *A* and *F,* relative locomotor activity of 1-day-old adults as indicated by thrashing rates in liquid normalized against control strains expressing YFP only (± S.E.; $n = 32$). *, $p < 0.0001$. *B* and *G,* cytoplasmic protein aggregates indicated by YFP fluorescence and nuclei by DAPI staining (*blue*) in neurons expressing TDP-C25-YFP (*iwIs22*gf) or SOD1-G85R-YFP (*iwIs8*gf). *Scale bar,* 5 μm. *C* and *H,* protein aggregates indicated by YFP fluorescence in head neurons expressing TDP-C25-YFP or SOD1-G85R-YFP from live *C. elegans* animals are compared between WT and loss-of-function *tdp-1*(*ok803*lf) backgrounds. *Scale bar,* 5 μm. *D* and *I,* protein aggregation was quantified by measuring the YFP fluorescence intensity of the inclusions (± S.E.; $n = 3$). *, $p < 0.05$. *E* and *J,* protein levels of TDP-C25-YFP and SOD1-G85R-YFP in soluble (*supernatant*) and insoluble (*pellet*) fractions of differentially extracted tissues from animals in the WT or *tdp-1*(*ok803*lf) mutant background.

CuZn superoxide dismutase (SOD1) have been implicated in ~20% of familial ALS cases, and many of these mutations lead to increased protein aggregation (57). A glycine-to-arginine substitution at amino acid 85 (G85R) renders SOD1 particularly aggregation-prone, and the *C. elegans*-stable transgene *iwIs8* expressing an SOD1-G85R-YFP fusion protein under the

snb-1 pan-neuronal promoter causes pronounced locomotor defects and formation of protein aggregates in neurons (Fig. 2*G*) (58). The loss-of-function *tdp-1*(*ok803*) mutation was found to significantly improve locomotor activity and reduce fluorescent aggregates in animals carrying the SOD1-G85R(*iwIs8*gf) transgene (Fig. 2, *F*, *H*, and *I*). Detergent extraction assay further indicated reduction in SOD1-G85R-YFP protein aggregate levels in *C. elegans* carrying both the transgene SOD1-G85R(*iwIs8*gf) and the loss-of-function mutation *tdp-1*(*ok803*), as compared with animals carrying the SOD1-G85R(*iwIs8*gf) transgene alone (Fig. 2*J*). Thus, loss of *tdp-1* may alleviate the neurotoxicity caused by TDP-43 and SOD1 through a shared mechanism.

We also tested whether the effects of TDP-1 on protein aggregation could be seen in another RNA-binding protein. Similar to TDP-43, FUS is also an hnRNP protein that has been implicated in the neurodegenerative diseases ALS and FTLD. Mutations in FUS were linked to familial ALS, and its protein products were found in ubiquitin-positive inclusions in the central nervous system of a subset of ALS and FTLD patients (15, 16). The *C. elegans* ortholog of *FUS* is *fust-1*(C27H5.3), and a large deletion allele, *tm4439*, is probably a null allele of *fust-1*. We generated *C. elegans* carrying both the loss-of-function mutation *fust-1*(*tm4439*) and the transgenic TDP-C25(*iwIs22*gf). Our examination indicated no alleviating effects of *fust-1*(*tm4439*) on the protein aggregation pathology. The amount of fluorescent TDP-C25-YFP aggregates in neurons was similar between animals carrying TDP-C25(*iwIs22*gf);*tdp-1*(*ok803*lf) and those carrying the TDP-C25(*iwIs22*gf) transgene alone (supplemental Fig. S6). Although *fust-1* may potentially differ from *tdp-1* in many aspects, including their temporal and spatial expressions, this result suggested that the role in suppressing protein aggregation may be specific for *tdp-1*.

Loss of tdp-1 Alleviates Defects in the Absence of Heat Shock Factor 1—The observation that loss of *tdp-1* suppressed proteotoxicity and aggregation of distinct proteins such as TDP-43 and SOD1 suggested that *tdp-1*(lf) might suppress defects caused by mutations that interfere with overall protein quality control. In response to heat-induced stress, heat shock factor 1 induces transcription of many genes, including molecular chaperones responsible for protection from protein misfolding and aggregation (59). A loss-of-function mutation of the *C. elegans* heat shock factor 1, *hsf-1*(*sy441*lf), causes temperature-dependent growth and egg-laying defects (60). Therefore, we used the mutant *hsf-1*(*sy441*lf) animals as a model for compromised protein quality control independent of ectopic transgene expressions. To test whether loss of *tdp-1* improves the compromised protein quality control in the absence of *hsf-1*, animals carrying both *hsf-1*(*sy441*lf) and *tdp-1*(*ok803*lf) were constructed and examined. The *hsf-1*(*sy441*lf);*tdp-1*(*ok803*lf) double mutant animals showed significant improvement in growth and egg laying as compared with the *hsf-1*(*sy441*lf) single mutant animals (Fig. 3, *A* and *B*). Next, to address whether the protective effect of *tdp-1*(*ok803*lf) was general to all temperature-sensitive mutants, we examined another temperature-sensitive mutant, the dynamin GTPase *dyn-1*(*ky51*lf), previously shown to be influenced by proteotoxicity (61). Unlike

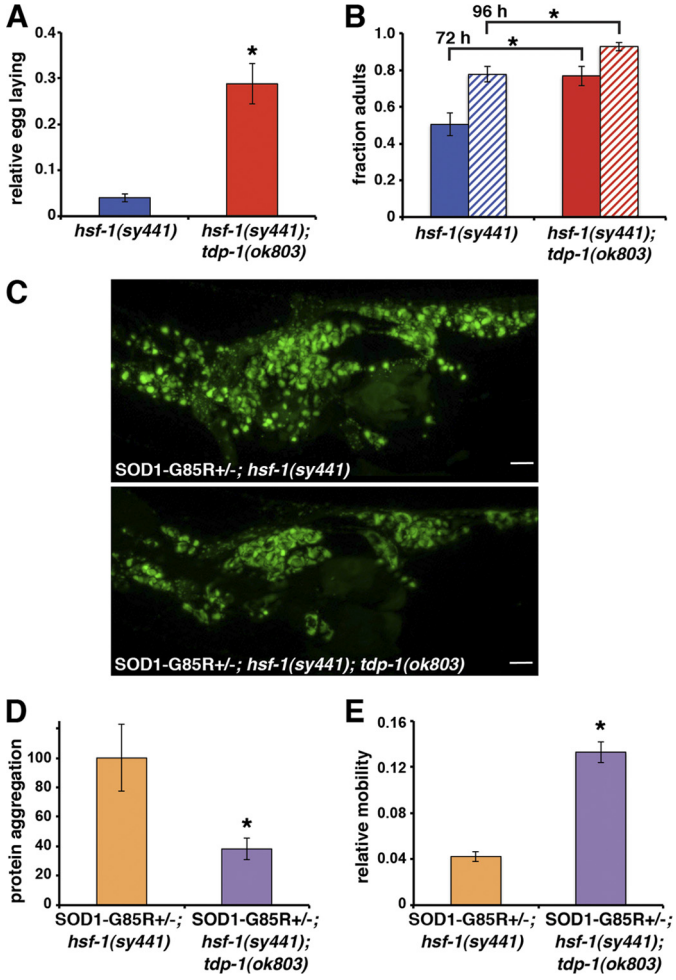

FIGURE 3. ***tdp-1*(*ok803*lf) mutation alleviates defects in loss-of-function mutant *hsf-1*(*sy441*lf).** *A*, relative number of eggs (± S.E.; $n = 20$) laid per animal as normalized against the WT N2 strain following culture at 25 °C. *, $p < 0.0001$. *B*, mean fraction of eggs laid at 20 °C reaching adulthood after 72 (*solid bars*) or 96 h (*hatched bars*) of growth at 25 °C (± S.E.; $n = 5$). *, $p < 0.05$. *C*, protein aggregates indicated by YFP fluorescence in head neurons expressing hemizygous SOD1-G85R-YFP (*iwIs8*gf) from live *C. elegans* L4 larvae are compared between the *hsf-1*(*sy441*lf) and double mutant *tdp-1*(*ok803*lf);*hsf-1*(*sy441*lf) backgrounds. *Scale bar*, 5 μm. *D*, protein aggregation was quantified by measuring the YFP fluorescence intensity of the inclusions (± S.E.; $n = 3$). *, $p < 0.05$. *E*, relative locomotor activity of L4 larvae as indicated by thrashing rates in liquid normalized against control strains expressing hemizygous SOD1G85R-YFP (*iwIs8*gf) only (± S.E.; $n = 25$). *, $p < 0.0001$.

hsf-1(*sy441*lf), the locomotor and egg-laying defects of *dyn-1*(*ky51*lf) were not alleviated but worsened by *tdp-1*(*ok803*lf) (supplemental Fig. S7). These data indicated that loss of *tdp-1* specifically protected against the defects caused by the absence of normal HSF-1 in *C. elegans*.

To further test the effects of loss of *tdp-1* on *hsf-1*-dependent proteotoxicity, we used the transgene SOD1-G85R(*iwIs8*gf) as a reporter. Loss-of-function *hsf-1*(*sy441*lf) mutant alone significantly worsened the protein aggregation and locomotor defects caused by the transgene SOD1-G85R(*iwIs8*gf). In the presence of homozygous *hsf-1*(*sy441*lf), most animals homozygous for the transgene SOD1-G85R(*iwIs8*gf) were too sick to grow to adulthood, so we used animals hemizygous for the transgene SOD1-G85R(*iwIs8*gf) for our analysis. Specifically, we constructed and compared a triple mutant that is hemizygous for SOD1-G85R(*iwIs8*gf) and homozygous for *hsf-1*(*sy441*lf);*tdp-*

1(*ok803*lf) with a double mutant that is hemizygous for SOD1-G85R(*iwIs8*gf) and homozygous for *hsf-1*(*sy441*lf). The presence of *tdp-1*(*ok803*lf) attenuated the protein aggregation and locomotor defects in the triple mutant animals, as compared with the double mutant animals (Fig. 3, *C–E*).Thus, *tdp-1* may act downstream of *hsf-1* or in a parallel pathway to influence proteotoxicity. Together, these results support a role for *C. elegans tdp-1* in regulating protein quality control.

Transcriptional Profiling of tdp-1 Loss-of-function Mutant—To examine the global gene expression changes induced by loss of *tdp-1*, *C. elegans* transcriptomes were compared between N2 WT animals and *tdp-1*(*ok803*lf) mutant animals. Messenger RNAs were isolated from triplicate samples of WT or *tdp-1*(*ok803*lf) *C. elegans*, and linearly amplified RNA probes were used to hybridize to the Affymetrix *C. elegans* genome array that probes over 22,500 gene transcripts. Q-PCR was used to verify the gene expression data from the microarrays. Correlation analysis indicated that the microarray data were highly reproducible with the Q-PCR assay. Overall, the microarray and Q-PCR values exhibited a good linear correlation for 29 representative genes tested ($R > 0.90$). In the absence of TDP-1, there were more genes down-regulated than those up-regulated. If the threshold was set at a fold change (FC) of 1.5 by the microarray measurement, 712 genes (3.15%) were differentially regulated (485 down and 227 up) in the *tdp-1*(lf) mutant. If the threshold was set at FC of 1.2, 4381 genes (19.3%) were differentially regulated (2600 down and 1781 up) in the *tdp-1*(lf) mutant (supplemental Table S1).

To examine biological processes that are significantly affected in the *tdp-1*(*ok803*lf) mutant, we conducted gene ontology analysis on the transcriptome profiling results using annotation from the Gene Ontology Consortium (62). We asked which biological processes as defined by Gene Ontology terms were most significantly represented by the top 712 differentially regulated genes in the *C. elegans* genome. This analysis indicated that the most significantly affected biological processes in *tdp-1*(*ok803*lf) were those involving molting cycle, growth, locomotion, determination of adult life span, and endoplasmic reticulum unfolded protein response (Fig. 4*B* and supplemental Table S2). These top-ranked biological processes matched well with the phenotypes observed in the *tdp-1*(lf) mutant *C. elegans*. For example, 98 out of 1466 genes related to locomotion were represented in the differentially expressed set ($p < 10^{-9}$) (supplemental Table S2). Consistently, locomotor defects were observed in the *tdp-1*(lf) mutant (Fig. 1). Also, 132 out of 2329 genes related to growth were represented in the differentially expressed set ($p < 10^{-7}$) (supplemental Table S2), and loss of *tdp-1* caused growth defects in the mutant *C. elegans* (Fig. 1). Finally, there was a trend of down-regulation for the genes that encode molecular chaperones (supplemental Table S2). Q-PCR assays confirmed the trend of down-regulation for a few molecular chaperones in the *tdp-1*(lf) mutant (supplemental Fig. S8*A*), suggesting that there is a reduced burden on protein quality control in these mutants.

To further understand the molecular consequences of loss of *tdp-1* at the molecular level, we analyzed the differentially regulated genes using the Ingenuity Pathways Analysis from Ingenuity Systems, which is supported by a repository of evidence-based biological interactions and functional annotations. The

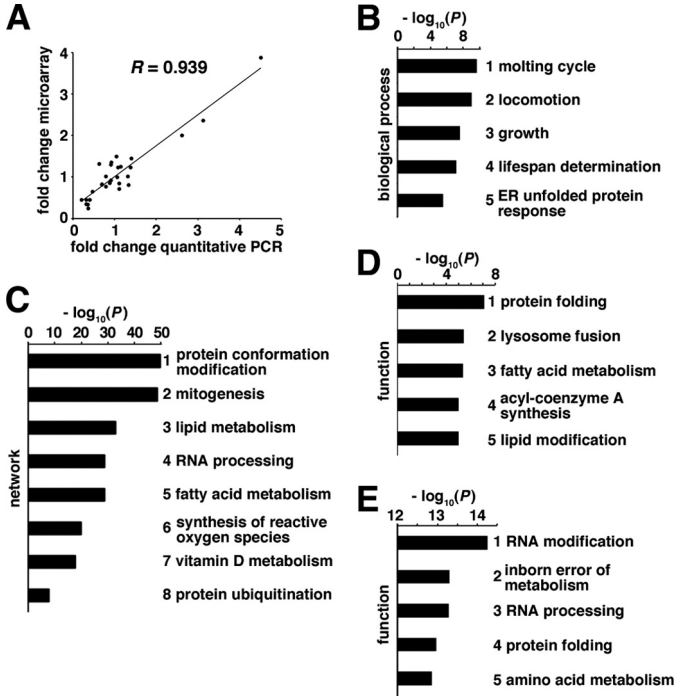

FIGURE 4. **Transcriptional profile analysis of mutant *C. elegans* lacking *tdp-1*.** *A*, results from *C. elegans* genome microarrays are validated with quantitative PCR. The relative fold changes in gene expression of 29 representative genes between *tdp-1*(*ok803*lf) and the WT N2 animals are shown. The values from the microarray and the quantitative PCR experiments show a linear relationship. *R* is the Pearson correlation coefficient. *B*, biological processes that are most affected in *tdp-1*(*ok803*lf) according to the Gene Ontology analysis. *ER*, endoplasmic reticulum. *C*, molecular networks that are most affected in *tdp-1*(*ok803*lf) according to the Ingenuity Pathways Analysis. *D* and *E*, molecular and cellular functions that are most affected in *tdp-1*(*ok803*lf) according to the Ingenuity Pathways Analysis, with the threshold at FC >1.5 (*D*) or FC >1.2 (*E*). *P* is the probability value from Fisher's exact test.

differentially regulated genes were highly enriched in several functional categories that involve protein post-translational and RNA post-transcriptional modifications (Fig. 4, *C–E*). For the top 712 differentially regulated genes (FC >1.5), the most relevant molecular network included proteins that function in protein conformational modification and mRNA processing (Fig. 4*C* and supplemental Table S3). Additionally, the Ingenuity Pathways Analysis of over-represented molecular functions pointed to a few cellular functions, including protein folding (Fig. 4*D* and supplemental Table S4). To gain a full picture of expression of genes involved in protein quality control, the genes involved in protein folding and ubiquitination were selected with a lower threshold of expression fold changes (FC >1.2) (Table S5). At this threshold (FC >1.2), RNA modification was the top-ranked molecular function represented by the differentially regulated genes (Fig. 4*E* and supplemental Table S6). The loss of *tdp-1* had pleiotrophic consequences on the expression of diverse RNA-processing proteins, including those functioning in the processing and splicing of messenger RNA, ribosomal RNA, transfer RNA, and small noncoding RNA (supplemental Table S6). For example, the loss of *tdp-1* altered the expression of hnRNP genes, including the *C. elegans* homolog of hnRNPA1, *hrp-2* (supplemental Fig. S8*B*). The expression of genes involved in RNA splicing, such as *snr-5*, which encodes a small nuclear ribonucleoprotein, was also

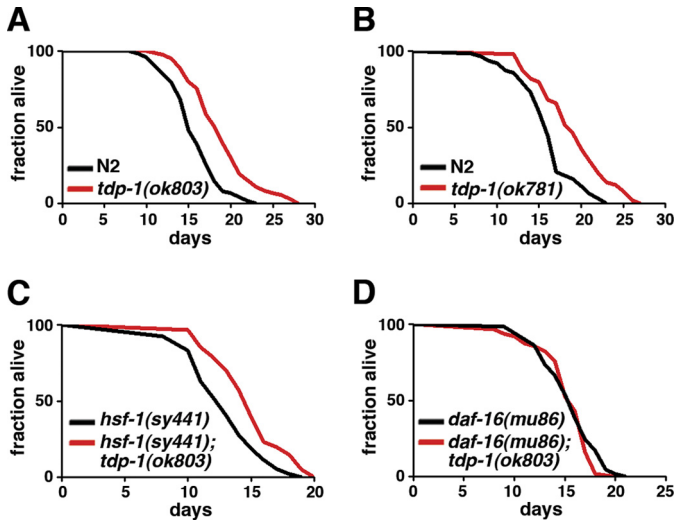

FIGURE 5. ***tdp-1*(lf) mutation leads to a life span extension that is dependent on *daf-16* but not on *hsf-1*.** *A,* survival of the WT N2 (*black*) and *tdp-1*(*ok803*lf) (*red*) animals. $p < 0.0001$. *B,* survival of N2 WT (*black*) and *tdp-1*(*ok781*lf) (*red*) animals. $p < 0.0001$. *C,* survival of *hsf-1*(*sy441*lf) animals (*black*) and *hsf-1*(*sy441*lf);*tdp-1*(*ok803*lf) double mutants (*red*) at 25 °C using 100 μg/ml of 5-fluorodeoxyuridine. *D,* survival of *daf-16*(*mu86*lf) animals (*black*) and *daf-16*(*mu86*); *tdp-1*(*ok803*lf) double mutants (*red*). $p < 0.0001$.

affected (supplemental Fig. S8*B*). Together, the transcriptome profiling of the *tdp-1* loss-of-function mutant showed a prominent change in the expression of genes involved in protein post-translational and RNA post-transcriptional modifications.

TDP-1 Regulates Life Span via a Pathway Requiring DAF-16 but Independent of HSF-1—The transcriptional profiling analysis also showed that genes functioning in the determination of adult life span were differentially regulated in the *tdp-1*(lf) mutant (Fig. 4*B* and supplemental Table S2). Like neurodegeneration, aging has been linked to deleterious effects of protein misfolding (3, 4). Reasoning that the improved protein homeostasis resulting from *tdp-1*(lf) might be associated with altered life span, we examined the life span of *tdp-1*(lf) mutants. Interestingly, the loss-of-function *tdp-1*(*ok803*) mutants lived ~20% longer than the N2 wild-type animals when cultured at 20 °C (Fig. 5*A*). An independent loss-of-function allele, *tdp-1*(*ok781*), also extended the life span of *C. elegans* to a similar extent (Fig. 5*B*). Our combined results indicate that *C. elegans* TDP-1 is a negative regulator of aging.

There are two characterized transcriptional factors, encoded by *hsf-1* and *daf-16*, that regulate both aging and protein homeostasis (3, 4). Because *hsf-1* and *daf-16* are two key genes that positively regulate life span, we asked whether the life span extension conferred by *tdp-1*(lf) requires the normal function of *hsf-1* or *daf-16*. We observed that *hsf-1*(*sy441*);*tdp-1*(*ok803*lf) double mutants lived longer than the single mutant *hsf-1*(*sy441*), suggesting that *tdp-1*(lf) influenced life span at least through some mechanisms independent of *hsf-1* (Fig. 5*C*). In contrast, a loss-of-function mutant *daf-16*(*mu86*lf) completely blocked the life span-extending effects of *tdp-1*(*ok803*lf) (Fig. 5*D*), indicating that the long lived phenotype conferred by loss of *tdp-1* required intact *daf-16*.

DISCUSSION

In this study, we systematically investigated the function of TDP-1 in *C. elegans*. TDP-1 shares significant homology with its mammalian ortholog TDP-43. The genetic interactions between TDP-1 and TDP-43 suggest that they are functionally conserved. Loss of TDP-1 appeared to increase the tolerance of *C. elegans* to proteotoxicity. Furthermore, loss of TDP-1 extended life span in *C. elegans*. Together, these results suggest a novel role for a conserved RNA-processing protein in the regulation of protein homeostasis and life span.

C. elegans TDP-1 is highly homologous to mammalian TDP-43. TDP-1 has the same protein domains as mammalian TDP-43 (Fig. 1). Although the C-terminal region of TDP-1 is not as glycine-rich as mammalian TDP-43, there is significant homology in this region, and the C-terminal part of TDP-1 can replace its mammalian counterpart and maintain the RNA splicing activity of the mammalian protein (46). The genetic interactions between *C. elegans* TDP-1 and human TDP-43 suggest that they are functionally conserved. For example, heterologous expression of human TDP-43 in *C. elegans* is able to suppress the defects caused by loss of *tdp-1* (Fig. 1). *C. elegans* TDP-1 is not required for survival, unlike other TDP-43 orthologs in more complex metazoans from *Drosophila* to mice (35, 54–56). Although it is likely that TDP-43 in higher animals may have acquired distinct properties compared with TDP-1, it is also possible that their conserved function is required for survival of complex organisms but not for survival of relatively simple *C. elegans*. Such low stringency of requirement for survival in *C. elegans* as compared with mammals has been observed in loss-of-function studies of many other genes. For example, loss of the type III RNase Dicer1 leads to lethality early in mouse development (63), but *C. elegans* lacking the ortholog DCR-1 are viable (64). Thus, the nonlethal phenotype of *C. elegans* lacking TDP-1 provides a simple system to study the conserved functions of this family of proteins.

RNA-processing proteins such as hnRNPs have multiple roles in RNA metabolism, including transcription, splicing, and nucleocytoplasmic transport of RNAs (26). The function of TDP-1, like its mammalian ortholog TDP-43 (40), is probably multifaceted. Loss of TDP-1 caused changes in expression levels of many RNA-processing genes, including orthologs of hnRNP proteins and splicing factors (supplemental Table S6 and Fig. S8). The effect of loss of TDP-1 on RNA processing could lead to the observed pleiotrophic phenotypes in *C. elegans*, including defects in fertility, growth, and locomotion (Fig. 1). Consistent with these observations, gene ontology analysis of the transcriptome profiles indicated altered expression of genes that have related functions such as growth and locomotion (Fig. 4*B*).

Furthermore, our characterization of *tdp-1* loss-of-function mutant suggested a role for TDP-1 in the regulation of protein homeostasis and life span in *C. elegans*. Loss of TDP-1 increased the solubility of an aggregation-prone C-terminal fragment of TDP-43 as well as that of a mutant SOD1. Loss of TDP-1 also conferred resistance to HSF-1 depletion in *C. elegans*. These data suggested that long term reduction in the function of TDP-1 led to a resistance to

stress on proteostasis. In accordance with our whole-animal observations from *C. elegans*, a recent study showed that knockdown of the *Drosophila* ortholog of TDP-43 protected against the neurotoxicity of overexpressed mutant VCP, an AAA^+ ATPase functioning in protein quality control, in a transgenic fly model (65). The molecular mechanisms through which TDP-1 regulates proteostasis in *C. elegans* remain to be elucidated. We propose three potential models that are not mutually exclusive. First, the removal of the TDP-1 protein itself may lessen the burden on the protein folding machinery in the cell. This model is supported by recent observations that the TDP-43 protein is highly prone to misfold and aggregate *in vitro* and *in vivo* (6, 66) and that a TDP-43 fragment can form amyloid fibrils (67). Second, diverse activities of TDP-1 in RNA processing may allow it to act as an orchestrator to adapt to proteotoxic stress through the regulation of RNA. RNA processing could affect all aspects of protein homeostasis, from protein synthesis to degradation. Loss of TDP-1 may alter global RNA levels and in turn protein homeostasis, resulting in adaptation of the cell to stress on protein quality control systems. Consistent with this model, the microarray analysis showed a trend of decreasing mRNA levels (supplemental Table S1). Finally, TDP-1 may regulate proteostasis through specific pathways. Our analysis of the life span extension in the *tdp-1* loss-of-function mutant suggested that the phenotype required intact DAF-16 but not HSF-1 (Fig. 5). Therefore, the transcriptional factor DAF-16, which is known to promote protein homeostasis (3, 6), may be an important player mediating the effect of TDP-1 on proteostasis.

Our finding that loss of TDP-1 reduces proteotoxicity is consistent with the observation that the loss-of-function mutant animals had extended life span. Although the molecular connections between aging and protein homeostasis are not fully understood, two pathways have been found to regulate both aging and protein homeostasis, *i.e.* the heat shock response and the insulin/IGF-1 signaling (3, 4). HSF-1 and DAF-16 are the master transcriptional factors that mediate the heat shock response and the insulin/IGF-1 signaling, respectively. Whereas reduced activity in TDP-1 reversed the defects in a loss-of-function mutant of HSF-1 (Fig. 3), the depletion of HSF-1 did not block the life span-extending effects of the TDP-1 reduction (Fig. 5). By contrast, the loss of DAF-16 completely blocked the life span-extending effects of the TDP-1 reduction. In simplest genetic interpretations, TDP-1 acts upstream of DAF-16 but independently of HSF-1 to influence life span (supplemental Fig. S9). However, complex networks rather than the linear pathways are possible. Nevertheless, the life span extension in the absence of TDP-1 requires intact DAF-16, an evolutionarily conserved FOXO family transcriptional factor at the intersection of regulations of aging, immunity, and stress responses (68).

In summary, we identified a new function of TDP-1 in regulating protein homeostasis and life span. Given the established functions of the TDP-1 homologs in RNA processing, this study suggests a layer of regulation of proteostasis and aging imparted by RNA-processing proteins. Further studies on these new functions of TDP-1 may help understand the conserved function of this class of RNA-processing proteins in metazoan evolution.

Acknowledgments—We thank D. Drummond-Barbossa, M. Matunis, P. Coulombe, and members of Wang laboratory for helpful discussions.

REFERENCES

1. Glenner, G. G., and Wong, C. W. (1984) Alzheimer disease. Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun.* **120,** 885–890
2. Balch, W. E., Morimoto, R. I., Dillin, A., and Kelly, J. W. (2008) Adapting proteostasis for disease intervention. *Science* **319,** 916–919
3. Hsu, A. L., Murphy, C. T., and Kenyon, C. (2003) Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science* **300,** 1142–1145
4. Morley, J. F., and Morimoto, R. I. (2004) Regulation of longevity in *Caenorhabditis elegans* by heat shock factor and molecular chaperones. *Mol. Biol. Cell* **15,** 657–664
5. Cohen, E., Bieschke, J., Perciavalle, R. M., Kelly, J. W., and Dillin, A. (2006) Opposing activities protect against age-onset proteotoxicity. *Science* **313,** 1604–1610
6. Zhang, T., Mullane, P. C., Periz, G., and Wang, J. (2011) TDP-43 neurotoxicity and protein aggregation modulated by heat shock factor and insulin/IGF-1 signaling. *Hum. Mol. Genet.* **20,** 1952–1965
7. Rosen, D. R., Siddique, T., Patterson, D., Figlewicz, D. A., Sapp, P., Hentati, A., Donaldson, D., Goto, J., O'Regan, J. P., and Deng, H. X.. (1993) Mutations in CuZn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* **362,** 59–62
8. Yang, Y., Hentati, A., Deng, H. X., Dabbagh, O., Sasaki, T., Hirano, M., Hung, W. Y., Ouahchi, K., Yan, J., Azim, A. C., Cole, N., Gascon, G., Yagmour, A., Ben-Hamida, M., Pericak-Vance, M., Hentati, F., and Siddique, T. (2001) The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. *Nat. Genet.* **29,** 160–165
9. Hadano, S., Hand, C. K., Osuga, H., Yanagisawa, Y., Otomo, A., Devon, R. S., Miyamoto, N., Showguchi-Miyata, J., Okada, Y., Singaraja, R., Figlewicz, D. A., Kwiatkowski, T., Hosler, B. A., Sagie, T., Skaug, J., Nasir, J., Brown, R. H., Jr., Scherer, S. W., Rouleau, G. A., Hayden, M. R., and Ikeda, J. E. (2001) A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. *Nat. Genet.* **29,** 166–173
10. Puls, I., Jonnakuty, C., LaMonte, B. H., Holzbaur, E. L., Tokito, M., Mann, E., Floeter, M. K., Bidus, K., Drayna, D., Oh, S. J., Brown, R. H., Jr., Ludlow, C. L., and Fischbeck, K. H. (2003) Mutant dynactin in motor neuron disease. *Nat. Genet.* **33,** 455–456
11. Chen, Y. Z., Bennett, C. L., Huynh, H. M., Blair, I. P., Puls, I., Irobi, J., Dierick, I., Abel, A., Kennerson, M. L., Rabin, B. A., Nicholson, G. A., Auer-Grumbach, M., Wagner, K., De Jonghe, P., Griffin, J. W., Fischbeck, K. H., Timmerman, V., Cornblath, D. R., and Chance, P. F. (2004) DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). *Am. J. Hum. Genet.* **74,** 1128–1135
12. Nishimura, A. L., Mitne-Neto, M., Silva, H. C., Richieri-Costa, A., Middleton, S., Cascio, D., Kok, F., Oliveira, J. R., Gillingwater, T., Webb, J., Skehel, P., and Zatz, M. (2004) A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. *Am. J. Hum. Genet.* **75,** 822–831
13. Neumann, M., Sampathu, D. M., Kwong, L. K., Truax, A. C., Micsenyi, M. C., Chou, T. T., Bruce, J., Schuck, T., Grossman, M., Clark, C. M., McCluskey, L. F., Miller, B. L., Masliah, E., Mackenzie, I. R., Feldman, H., Feiden, W., Kretzschmar, H. A., Trojanowski, J. Q., and Lee, V. M. (2006) Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* **314,** 130–133
14. Sreedharan, J., Blair, I. P., Tripathi, V. B., Hu, X., Vance, C., Rogelj, B., Ackerley, S., Durnall, J. C., Williams, K. L., Buratti, E., Baralle, F., de Belleroche, J., Mitchell, J. D., Leigh, P. N., Al-Chalabi, A., Miller, C. C., Nich-

olson, G., and Shaw, C. E. (2008) TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* **319,** 1668–1672
15. Kwiatkowski, T. J., Jr., Bosco, D. A., Leclerc, A. L., Tamrazian, E., Vanderburg, C. R., Russ, C., Davis, A., Gilchrist, J., Kasarskis, E. J., Munsat, T., Valdmanis, P., Rouleau, G. A., Hosler, B. A., Cortelli, P., de Jong, P. J., Yoshinaga, Y., Haines, J. L., Pericak-Vance, M. A., Yan, J., Ticozzi, N., Siddique, T., McKenna-Yasek, D., Sapp, P. C., Horvitz, H. R., Landers, J. E., and Brown, R. H., Jr. (2009) Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* **323,** 1205–1208
16. Vance, C., Rogelj, B., Hortobágyi, T., De Vos, K. J., Nishimura, A. L., Sreedharan, J., Hu, X., Smith, B., Ruddy, D., Wright, P., Ganesalingam, J., Williams, K. L., Tripathi, V., Al-Saraj, S., Al-Chalabi, A., Leigh, P. N., Blair, I. P., Nicholson, G., de Belleroche, J., Gallo, J. M., Miller, C. C., and Shaw, C. E. (2009) Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* **323,** 1208–1211
17. Maruyama, H., Morino, H., Ito, H., Izumi, Y., Kato, H., Watanabe, Y., Kinoshita, Y., Kamada, M., Nodera, H., Suzuki, H., Komure, O., Matsuura, S., Kobatake, K., Morimoto, N., Abe, K., Suzuki, N., Aoki, M., Kawata, A., Hirai, T., Kato, T., Ogasawara, K., Hirano, A., Takumi, T., Kusaka, H., Hagiwara, K., Kaji, R., and Kawakami, H. (2010) Mutations of optineurin in amyotrophic lateral sclerosis. *Nature* **465,** 223–226
18. Johnson, J. O., Mandrioli, J., Benatar, M., Abramzon, Y., Van Deerlin, V. M., Trojanowski, J. Q., Gibbs, J. R., Brunetti, M., Gronka, S., Wuu, J., Ding, J., McCluskey, L., Martinez-Lage, M., Falcone, D., Hernandez, D. G., Arepalli, S., Chong, S., Schymick, J. C., Rothstein, J., Landi, F., Wang, Y. D., Calvo, A., Mora, G., Sabatelli, M., Monsurrò, M. R., Battistini, S., Salvi, F., Spataro, R., Sola, P., Borghero, G., ITALSGEN Consortium, Galassi, G., Scholz, S. W., Taylor, J. P., Restagno, G., Chiò, A., and Traynor, B. J. (2010) Exome sequencing reveals VCP mutations as a cause of familial ALS. *Neuron* **68,** 857–864
19. Deng, H. X., Chen, W., Hong, S. T., Boycott, K. M., Gorrie, G. H., Siddique, N., Yang, Y., Fecto, F., Shi, Y., Zhai, H., Jiang, H., Hirano, M., Rampersaud, E., Jansen, G. H., Donkervoort, S., Bigio, E. H., Brooks, B. R., Ajroud, K., Sufit, R. L., Haines, J. L., Mugnaini, E., Pericak-Vance, M. A., and Siddique, T. (2011) Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. *Nature* **477,** 211–215
20. DeJesus-Hernandez, M., Mackenzie, I. R., Boeve, B. F., Boxer, A. L., Baker, M., Rutherford, N. J., Nicholson, A. M., Finch, N. A., Flynn, H., Adamson, J., Kouri, N., Wojtas, A., Sengdy, P., Hsiung, G. Y., Karydas, A., Seeley, W. W., Josephs, K. A., Coppola, G., Geschwind, D. H., Wszolek, Z. K., Feldman, H., Knopman, D. S., Petersen, R. C., Miller, B. L., Dickson, D. W., Boylan, K. B., Graff-Radford, N. R., and Rademakers, R. (2011) Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* **72,** 245–256
21. Renton, A. E., Majounie, E., Waite, A., Simón-Sánchez, J., Rollinson, S., Gibbs, J. R., Schymick, J. C., Laaksovirta, H., van Swieten, J. C., Myllykangas, L., Kalimo, H., Paetau, A., Abramzon, Y., Remes, A. M., Kaganovich, A., Scholz, S. W., Duckworth, J., Ding, J., Harmer, D. W., Hernandez, D. G., Johnson, J. O., Mok, K., Ryten, M., Trabzuni, D., Guerreiro, R. J., Orrell, R. W., Neal, J., Murray, A., Pearson, J., Jansen, I. E., Sondervan, D., Seelaar, H., Blake, D., Young, K., Halliwell, N., Callister, J. B., Toulson, G., Richardson, A., Gerhard, A., Snowden, J., Mann, D., Neary, D., Nalls, M. A., Peuralinna, T., Jansson, L., Isoviita, V. M., Kaivorinne, A. L., Hölttä-Vuori, M., Ikonen, E., Sulkava, R., Benatar, M., Wuu, J., Chiò, A., Restagno, G., Borghero, G., Sabatelli, M., Heckerman, D., Rogaeva, E., Zinman, L., Rothstein, J. D., Sendtner, M., Drepper, C., Eichler, E. E., Alkan, C., Abdullaev, Z., Pack, S. D., Dutra, A., Pak, E., Hardy, J., Singleton, A., Williams, N. M., Heutink, P., Pickering-Brown, S., Morris, H. R., Tienari, P. J., Traynor, B. J., *et al.* (2011) A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* **72,** 257–268
22. Lagier-Tourenne, C., Polymenidou, M., and Cleveland, D. W. (2010) TDP-43 and FUS/TLS. Emerging roles in RNA processing and neurodegeneration. *Hum. Mol. Genet.* **19,** R46–64
23. Chen-Plotkin, A. S., Lee, V. M., and Trojanowski, J. Q. (2010) TAR DNA-binding protein 43 in neurodegenerative disease. *Nat. Rev. Neurol.* **6,** 211–220
24. Wang, J., Xu, G., and Borchelt, D. R. (2002) High molecular weight complexes of mutant superoxide dismutase 1. Age-dependent and tissue-specific accumulation. *Neurobiol. Dis.* **9,** 139–148
25. Bosco, D. A., Morfini, G., Karabacak, N. M., Song, Y., Gros-Louis, F., Pasinelli, P., Goolsby, H., Fontaine, B. A., Lemay, N., McKenna-Yasek, D., Frosch, M. P., Agar, J. N., Julien, J. P., Brady, S. T., and Brown, R. H., Jr. (2010) Wild-type and mutant SOD1 share an aberrant conformation and a common pathogenic pathway in ALS. *Nat. Neurosci.* **13,** 1396–1403
26. Dreyfuss, G., Matunis, M. J., Piñol-Roma, S., and Burd, C. G. (1993) hnRNP proteins and the biogenesis of mRNA. *Annu. Rev. Biochem.* **62,** 289–321
27. Abhyankar, M. M., Urekar, C., and Reddi, P. P. (2007) A novel CpG-free vertebrate insulator silences the testis-specific SP-10 gene in somatic tissues. Role for TDP-43 in insulator function. *J. Biol. Chem.* **282,** 36143–36154
28. Ou, S. H., Wu, F., Harrich, D., García-Martínez, L. F., and Gaynor, R. B. (1995) Cloning and characterization of a novel cellular protein, TDP-43, that binds to human immunodeficiency virus type 1 TAR DNA sequence motifs. *J. Virol.* **69,** 3584–3596
29. Bose, J. K., Wang, I. F., Hung, L., Tarn, W. Y., and Shen, C. K. (2008) TDP-43 overexpression enhances exon 7 inclusion during the survival of motor neuron pre-mRNA splicing. *J. Biol. Chem.* **283,** 28852–28859
30. Mercado, P. A., Ayala, Y. M., Romano, M., Buratti, E., and Baralle, F. E. (2005) Depletion of TDP 43 overrides the need for exonic and intronic splicing enhancers in the human apoA-II gene. *Nucleic Acids Res.* **33,** 6000–6010
31. Dewey, C. M., Cenik, B., Sephton, C. F., Dries, D. R., Mayer, P., 3rd, Good, S. K., Johnson, B. A., Herz, J., and Yu, G. (2011) TDP-43 is directed to stress granules by sorbitol, a novel physiological osmotic and oxidative stressor. *Mol. Cell. Biol.* **31,** 1098–1108
32. McDonald, K. K., Aulas, A., Destroismaisons, L., Pickles, S., Beleac, E., Camu, W., Rouleau, G. A., and Vande Velde, C. (2011) TAR DNA-binding protein 43 (TDP-43) regulates stress granule dynamics via differential regulation of G3BP and TIA-1. *Hum. Mol. Genet.* **20,** 1400–1410
33. Liu-Yesucevitz, L., Bilgutay, A., Zhang, Y. J., Vanderweyde, T., Vanderwyde, T., Citro, A., Mehta, T., Zaarur, N., McKee, A., Bowser, R., Sherman, M., Petrucelli, L., and Wolozin, B. (2010) Tar DNA binding protein-43 (TDP-43) associates with stress granules. Analysis of cultured cells and pathological brain tissue. *PLoS One* **5,** e13250
34. Strong, M. J., Volkening, K., Hammond, R., Yang, W., Strong, W., Leystra-Lantz, C., and Shoesmith, C. (2007) TDP43 is a human low molecular weight neurofilament (hNFL) mRNA-binding protein. *Mol. Cell. Neurosci.* **35,** 320–327
35. Fiesel, F. C., Voigt, A., Weber, S. S., Van den Haute, C., Waldenmaier, A., Görner, K., Walter, M., Anderson, M. L., Kern, J. V., Rasse, T. M., Schmidt, T., Springer, W., Kirchner, R., Bonin, M., Neumann, M., Baekelandt, V., Alunni-Fabbroni, M., Schulz, J. B., and Kahle, P. J. (2010) Knockdown of transactive response DNA-binding protein (TDP-43) down-regulates histone deacetylase 6. *EMBO J.* **29,** 209–221
36. Bose, J. K., Huang, C. C., and Shen, C. K. (2011) Regulation of autophagy by neuropathological protein TDP-43. *J. Biol. Chem.* **286,** 44441–44448
37. Polymenidou, M., Lagier-Tourenne, C., Hutt, K. R., Huelga, S. C., Moran, J., Liang, T. Y., Ling, S. C., Sun, E., Wancewicz, E., Mazur, C., Kordasiewicz, H., Sedaghat, Y., Donohue, J. P., Shiue, L., Bennett, C. F., Yeo, G. W., and Cleveland, D. W. (2011) Long pre-mRNA depletion and RNA missplicing contribute to neuronal vulnerability from loss of TDP-43. *Nat. Neurosci.* **14,** 459–468
38. Ayala, Y. M., De Conti, L., Avendaño-Vázquez, S. E., Dhir, A., Romano, M., D'Ambrogio, A., Tollervey, J., Ule, J., Baralle, M., Buratti, E., and Baralle, F. E. (2011) TDP-43 regulates its mRNA levels through a negative feedback loop. *EMBO J.* **30,** 277–288
39. Igaz, L. M., Kwong, L. K., Lee, E. B., Chen-Plotkin, A., Swanson, E., Unger, T., Malunda, J., Xu, Y., Winton, M. J., Trojanowski, J. Q., and Lee, V. M. (2011) Dysregulation of the ALS-associated gene TDP-43 leads to neuronal death and degeneration in mice. *J. Clin. Invest.* **121,** 726–738
40. Buratti, E., and Baralle, F. E. (2010) The multiple roles of TDP-43 in pre-mRNA processing and gene expression regulation. *RNA Biol.* **7,** 420–429
41. Johnson, B. S., McCaffery, J. M., Lindquist, S., and Gitler, A. D. (2008) A

yeast TDP-43 proteinopathy model. Exploring the molecular determinants of TDP-43 aggregation and cellular toxicity. *Proc. Natl. Acad. Sci. U.S.A.* **105,** 6439–6444
42. Wegorzewska, I., Bell, S., Cairns, N. J., Miller, T. M., and Baloh, R. H. (2009) TDP-43 mutant transgenic mice develop features of ALS and frontotemporal lobar degeneration. *Proc. Natl. Acad. Sci. U.S.A.* **106,** 18809–18814
43. Kabashi, E., Lin, L., Tradewell, M. L., Dion, P. A., Bercier, V., Bourgouin, P., Rochefort, D., Bel Hadj, S., Durham, H. D., Vande Velde, C., Rouleau, G. A., and Drapeau, P. (2010) Gain and loss of function of ALS-related mutations of TARDBP (TDP-43) cause motor deficits *in vivo*. *Hum. Mol. Genet.* **19,** 671–683
44. Li, Y., Ray, P., Rao, E. J., Shi, C., Guo, W., Chen, X., Woodruff, E. A., 3rd, Fushimi, K., and Wu, J. Y. (2010) A *Drosophila* model for TDP-43 proteinopathy. *Proc. Natl. Acad. Sci. U.S.A.* **107,** 3169–3174
45. Hanson, K. A., Kim, S. H., Wassarman, D. A., and Tibbetts, R. S. (2010) Ubiquilin modifies TDP-43 toxicity in a *Drosophila* model of amyotrophic lateral sclerosis (ALS). *J. Biol. Chem.* **285,** 11068–11072
46. Ash, P. E., Zhang, Y. J., Roberts, C. M., Saldi, T., Hutter, H., Buratti, E., Petrucelli, L., and Link, C. D. (2010) Neurotoxic effects of TDP-43 overexpression in *C. elegans*. *Hum. Mol. Genet.* **19,** 3206–3218
47. Liachko, N. F., Guthrie, C. R., and Kraemer, B. C. (2010) Phosphorylation promotes neurotoxicity in a *Caenorhabditis elegans* model of TDP-43 proteinopathy. *J. Neurosci.* **30,** 16208–16219
48. Wils, H., Kleinberger, G., Janssens, J., Pereson, S., Joris, G., Cuijt, I., Smits, V., Ceuterick-de Groote, C., Van Broeckhoven, C., and Kumar-Singh, S. (2010) TDP-43 transgenic mice develop spastic paralysis and neuronal inclusions characteristic of ALS and frontotemporal lobar degeneration. *Proc. Natl. Acad. Sci. U.S.A.* **107,** 3858–3863
49. Stallings, N. R., Puttaparthi, K., Luther, C. M., Burns, D. K., and Elliott, J. L. (2010) Progressive motor weakness in transgenic mice expressing human TDP-43. *Neurobiol. Dis.* **40,** 404–414
50. Xu, Y. F., Gendron, T. F., Zhang, Y. J., Lin, W. L., D'Alton, S., Sheng, H., Casey, M. C., Tong, J., Knight, J., Yu, X., Rademakers, R., Boylan, K., Hutton, M., McGowan, E., Dickson, D. W., Lewis, J., and Petrucelli, L. (2010) Wild-type human TDP-43 expression causes TDP-43 phosphorylation, mitochondrial aggregation, motor deficits, and early mortality in transgenic mice. *J. Neurosci.* **30,** 10851–10859
51. Shan, X., Chiang, P. M., Price, D. L., and Wong, P. C. (2010) Altered distributions of Gemini of coiled bodies and mitochondria in motor neurons of TDP-43 transgenic mice. *Proc. Natl. Acad. Sci. U.S.A.* **107,** 16325–16330
52. Swarup, V., Phaneuf, D., Bareil, C., Robertson, J., Rouleau, G. A., Kriz, J., and Julien, J. P. (2011) Pathological hallmarks of amyotrophic lateral sclerosis/frontotemporal lobar degeneration in transgenic mice produced with TDP-43 genomic fragments. *Brain* **134,** 2610–2626
53. Zhou, H., Huang, C., Chen, H., Wang, D., Landel, C. P., Xia, P. Y., Bowser, R., Liu, Y. J., and Xia, X. G. (2010) Transgenic rat model of neurodegeneration caused by mutation in the TDP gene. *PLoS Genet.* **6,** e1000887
54. Wu, L. S., Cheng, W. C., Hou, S. C., Yan, Y. T., Jiang, S. T., and Shen, C. K. (2010) TDP-43, a neuro-pathosignature factor, is essential for early mouse embryogenesis. *Genesis* **48,** 56–62
55. Sephton, C. F., Good, S. K., Atkin, S., Dewey, C. M., Mayer, P., 3rd, Herz, J., and Yu, G. (2010) TDP-43 is a developmentally regulated protein essential for early embryonic development. *J. Biol. Chem.* **285,** 6826–6834
56. Chiang, P. M., Ling, J., Jeong, Y. H., Price, D. L., Aja, S. M., and Wong, P. C. (2010) Deletion of TDP-43 down-regulates *Tbc1d1*, a gene linked to obesity, and alters body fat metabolism. *Proc. Natl. Acad. Sci. U.S.A.* **107,** 16320–16324
57. Wang, J., Slunt, H., Gonzales, V., Fromholt, D., Coonfield, M., Copeland, N. G., Jenkins, N. A., and Borchelt, D. R. (2003) Copper-binding site-null SOD1 causes ALS in transgenic mice. Aggregates of non-native SOD1 delineate a common feature. *Hum. Mol. Genet.* **12,** 2753–2764
58. Wang, J., Farr, G. W., Hall, D. H., Li, F., Furtak, K., Dreier, L., and Horwich, A. L. (2009) An ALS-linked mutant SOD1 produces a locomotor defect associated with aggregation and synaptic dysfunction when expressed in neurons of *Caenorhabditis elegans*. *PLoS Genet.* **5,** e1000350
59. Sorger, P. K. (1991) Heat shock factor and the heat shock response. *Cell* **65,** 363–366
60. Hajdu-Cronin, Y. M., Chen, W. J., and Sternberg, P. W. (2004) The L-type cyclin CYL-1 and the heat-shock factor HSF-1 are required for heat-shock-induced protein expression in *Caenorhabditis elegans*. *Genetics* **168,** 1937–1949
61. Gidalevitz, T., Ben-Zvi, A., Ho, K. H., Brignull, H. R., and Morimoto, R. I. (2006) Progressive disruption of cellular protein folding in models of polyglutamine diseases. *Science* **311,** 1471–1474
62. Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., Harris, M. A., Hill, D. P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J. C., Richardson, J. E., Ringwald, M., Rubin, G. M., and Sherlock, G. (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat. Genet.* **25,** 25–29
63. Bernstein, E., Kim, S. Y., Carmell, M. A., Murchison, E. P., Alcorn, H., Li, M. Z., Mills, A. A., Elledge, S. J., Anderson, K. V., and Hannon, G. J. (2003) Dicer is essential for mouse development. *Nat. Genet.* **35,** 215–217
64. Knight, S. W., and Bass, B. L. (2001) A role for the RNase III enzyme DCR-1 in RNA interference and germ line development in *Caenorhabditis elegans*. *Science* **293,** 2269–2271
65. Ritson, G. P., Custer, S. K., Freibaum, B. D., Guinto, J. B., Geffel, D., Moore, J., Tang, W., Winton, M. J., Neumann, M., Trojanowski, J. Q., Lee, V. M., Forman, M. S., and Taylor, J. P. (2010) TDP-43 mediates degeneration in a novel *Drosophila* model of disease caused by mutations in VCP/p97. *J. Neurosci.* **30,** 7729–7739
66. Johnson, B. S., Snead, D., Lee, J. J., McCaffery, J. M., Shorter, J., and Gitler, A. D. (2009) TDP-43 is intrinsically aggregation-prone, and amyotrophic lateral sclerosis-linked mutations accelerate aggregation and increase toxicity. *J. Biol. Chem.* **284,** 20329–20339
67. Guo, W., Chen, Y., Zhou, X., Kar, A., Ray, P., Chen, X., Rao, E. J., Yang, M., Ye, H., Zhu, L., Liu, J., Xu, M., Yang, Y., Wang, C., Zhang, D., Bigio, E. H., Mesulam, M., Shen, Y., Xu, Q., Fushimi, K., and Wu, J. Y. (2011) An ALS-associated mutation affecting TDP-43 enhances protein aggregation, fibril formation and neurotoxicity. *Nat. Struct. Mol. Biol.* **18,** 822–830
68. Yen, K., Narasimhan, S. D., and Tissenbaum, H. A. (2011) DAF-16/Forkhead box O transcription factor. Many paths to a single Fork(head) in the road. *Antioxid. Redox. Signal.* **14,** 623–634