The conserved NAD(H)-dependent corepressor CTBP-1 regulates *Caenorhabditis elegans* life span

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Edited by Richard H. Goodman, Vollum Institute, Portland, OR, and approved December 5, 2008 (received for review March 17, 2008)

CtBP (C-terminal binding protein) is an evolutionarily conserved NAD(H)-dependent transcriptional corepressor, whose activity has been shown to be regulated by the NAD/NADH ratio. Although recent studies have provided significant new insights into mechanisms by which CtBP regulates transcription, the biological function of CtBP remains incompletely understood. Here, we report that genetic inactivation of the *Caenorhabditis elegans* homolog, *ctbp-1*, results in life span extension, which is suppressed by reintroduction of the *ctbp-1* genomic DNA encoding wild-type but not NAD(H)-binding defective CTBP-1 protein. We show that CTBP-1 possibly modulates aging through the insulin/IGF-1 signaling pathway, dependent on the forkhead transcription factor DAF-16, but independent of the NAD-dependent histone deacetylase SIR-2.1. Genome-wide microarray analysis identifies >200 potential CTBP-1 target genes. Importantly, RNAi inhibition of a putative triacylglycerol lipase gene *lips-7*(C09E8.2) but not another lipase suppresses the life span extension phenotype. Consistently, metabolic analysis shows that the triacylglycerol level is reduced in the *ctbp-1* deletion mutant, which is restored to the wild-type level by RNAi inhibition of *lips-7*. Taken together, our data suggest that CTBP-1 controls life span probably through the regulation of lipid metabolism.

aging | CtBP | transcription corepressor

CtBP is a transcriptional corepressor that is evolutionarily conserved from *Caenorhabditis elegans* to human (1). CtBP shares sequence homology with the NAD/NADH-dependent 2-hydroxy acid dehydrogenases (2-Hacid_DH) (2), and has been shown to exhibit dehydrogenase activity in vitro (3–5), although the physiological substrates and functional significance of this enzymatic activity remain unclear. CtBP binds NAD and NADH, and the NAD/NADH ratio appears to regulate the interactions of CtBP with DNA-binding transcription factors (6, 7), suggesting a potential role for CtBP as a sensor of cellular redox states. CtBP represses transcription by recruiting multiple histone modifying enzymes including the histone H3 lysine 9 (H3K9) methyltransferase G9a/HMTase1 and the histone H3 lysine 4 (H3K4) demethylase LSD1 (3, 8). Previous studies suggest a role for CtBP in mouse development, apoptosis, and hypoxia-induced tumor migration (9–12). However, by and large, the biology of CtBP is still incompletely understood.

Aging is a complex process regulated by an interacting network of factors. The insulin/insulin-like growth factor-1 (IGF-1) signaling pathway, the JNK anti-stress pathway and the mitochondria respiratory chain, have all been shown to regulate the aging process (13). Besides genetic factors, environmental conditions including stress and nutrient availability, have also been demonstrated to influence longevity (13–15). Transcription factors including DAF-16 and the NAD-dependent histone deacetylase SIR2 are at the converging points to integrate these different signals and regulate longevity through modulating gene transcription (13, 15). Similar to SIR2, CtBP is also an NAD(H) dependent transcriptional corepressor, prompting us to examine whether CtBP might play a role in regulating longevity.

We investigated CtBP function in *C. elegans* through the analysis of a worm CtBP (*ctbp-1*, F49E10.5) deletion mutant and by RNAi inhibition of *ctbp-1* expression. Significantly, we find that loss of *ctbp-1* expression leads to an extended adult life span and increased resistance to stress. Furthermore, genetic complementation experiments show that the NAD(H) binding motif is important for the ability of *ctbp-1* to regulate life span. Our epistasis analyses suggest that CTBP-1 functions in the insulin-like pathway, upstream of DAF-16 and likely downstream of the NAD dependent histone deacetylase SIR-2.1. Genome-wide expression profiling studies identify a wide range of CTBP-1 target genes. Strikingly, RNAi inhibition of a CTBP-1 target gene, a putative triacylglycerol (TAG) lipase gene *lips-7* suppresses the life span phenotype associated with the loss of CTBP-1, whereas inhibition of another TAG lipase K08B12.1 has no such effect. Depletion of the TAG lipase also results in the increase of TAG, which is down-regulated in the *ctbp-1* mutant. Taken together, our findings suggest that the evolutionarily conserved NAD(H)-dependent transcription corepressor CtBP controls life span by regulating transcription of gene(s) important for lipid metabolism.

Results and Discussion

Worm CTBP-1 has been shown to be the homologue of human CtBP and it has the characteristic NAD(H)-dependent 2-Hacid_DH domain and represses transcription (16). The *C. elegans ctbp-1*(*ok498*) mutant lacks the NAD(H)-binding domain and most of the dehydrogenase catalytic domain [Wormbase, F49E10.5 (www.wormbase.org) and Fig. 1*A*]. *ctbp-1*(*ok498*) was backcrossed with N2 4 times and yielded 3 independent lines, namely A26, D22 and D51. These 3 lines behaved identically, i.e., they showed no overt phenotypes in morphology, developmental rate, fecundity or formation of a diapause state known as dauer (supporting information (SI) Fig. S1 and data not shown). However, compared with the wild-type worms, all 3 lines showed a significant increase in mean adult life span (≈20%) and maximal life span (≈10–20%) (Fig. 1*B* and Table S1). A similar life span extension by *ctbp-1*(*ok498*) was also observed at a lower temperature of 20 °C (Fig. 2*B* and D). This ≈20% change in life span is in line with the recent reports of a number of genes whose de-regulation also resulted in a similar increase of life spans (17, 18).

Several lines of evidence further support the notion that the life span extension phenotype is caused by the loss of *ctbp-1*. First, depletion of *ctbp-1* by RNAi (Fig. S2*A*) in worms resulted in a comparable life span extension (Fig. 1*C* and Table S1). The

Author contributions: S.C., J.R.W., and Y.S. designed research; S.C., J.R.W., S.G., and J.A.H. performed research; S.C., S.G., J.A.H., R.R.G., and P.G. analyzed data; and S.C., R.R.G., and Y.S. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0802674106/DCSupplemental.

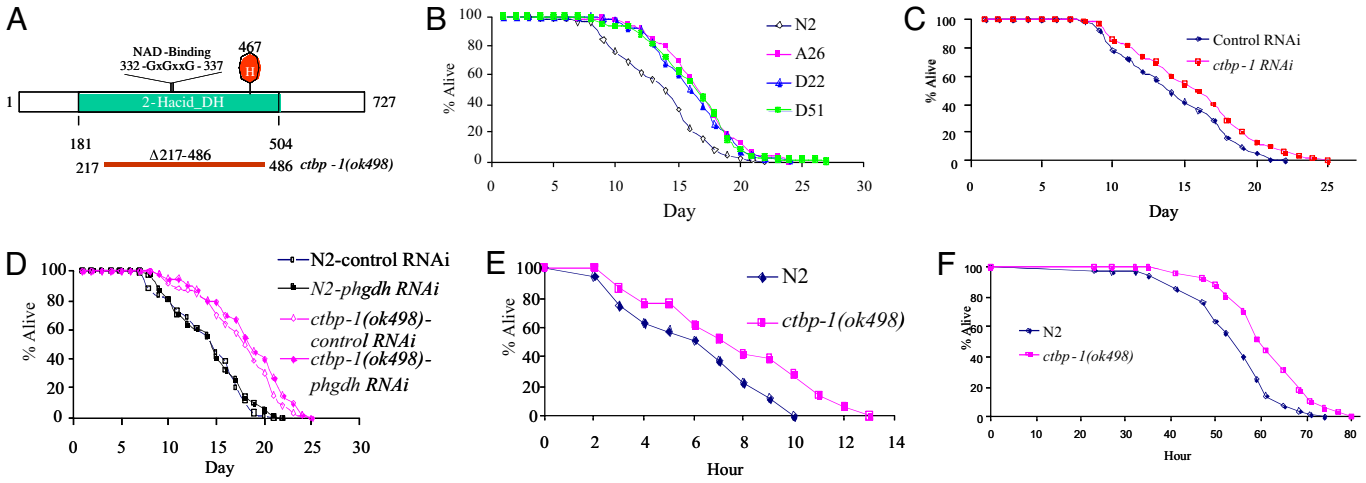

Fig. 1. CTBP-1 regulates life span in *C. elegans*. (*A*) A schematic presentation of the *ctbp-1*(*ok498*) mutant. (*B*) All 3 independent lines derived from *ctbp-1*(*ok498*) had similar increase in life span compared with N2. Mean adult life span of each strain is (mean ± SEM): N2, 13.8 ± 0.4 days; A26, 16.9 ± 0.4 days ($P < 0.0001$); D22,16.4 ± 0.4 days ($P < 0.0001$); D51, 16.5 ± 0.4 days ($P < 0.0001$). *P* values were calculated against N2. The maximal life span of each is N2, 22 days; A26, 24 days; D22, 25 days; D51, 27 days. Statistical data are summarized in Table S1. (*C*) *ctbp-1* RNAi increases worm life span. Control RNAi: 14.5 ± 0.3 days; *ctbp-1* RNAi: 16.0 ± 0.3 days ($P = 0.0002$). (*D*) RNAi depletion of a possible *ctbp-1* homologue *phgdh* failed to alter life span in N2 or *ctbp-1*(*ok498*). (*E*) *ctbp-1*(*ok498*) worms are more resistant to oxidative stress. N2 and *ctbp-1*(*ok498*) were exposed to 150 mM papraquat and the survival time for N2 and *ctbp-1*(*ok498*) are 6.1 ± 0.4 h and 7.8 ± 0.6 h, respectively ($P = 0.006$). (*F*) *ctbp-1*(*ok498*) is more resistant to heat stress. Worms were exposed to 32 °C on the first day of adult. Mean survival time for N2 was 54.0 ± 0.9 h and 61.6 ± 0.9 *for ctbp-1*(*ok498*) ($P < 0.0001$). Similar results were obtained in 3 independent experiments for paraquat and heat resistance assay.

slightly lower degree of life span extension of the RNAi treated animals is probably due to an incomplete knockdown of the endogenous *ctbp-1*. As an important specificity control, we also RNAi depleted C31C9.2 (Fig. S2*B*), a phosphoglycerate dehydrogenase (PHGDH), which is the only other *C. elegans* ORF that is potentially related to CtBP (Wormbase), and found no effects on the life span of either N2 or *ctbp-1*(*ok498*) (Fig. 1*D*). Second, reintroduction of the genomic DNA encompassing the *ctbp-1* gene rescued the life span phenotype (Table 1), indicating that the expended life span is caused by the loss of CTBP-1 function. Taken together, these results show that CTBP-1 regulates life span in *C. elegans* without affecting development or fertility.

To further establish the role of CTBP-1 in life span regulation, we investigated the ability of *ctbp-1*(*ok498*) to respond to stress, given that increased stress resistance is a common feature of many long-lived worms (19). As shown in Fig. 1 *E* and *F*, we found that, compared with the N2, *ctbp-1*(*ok498*) displayed

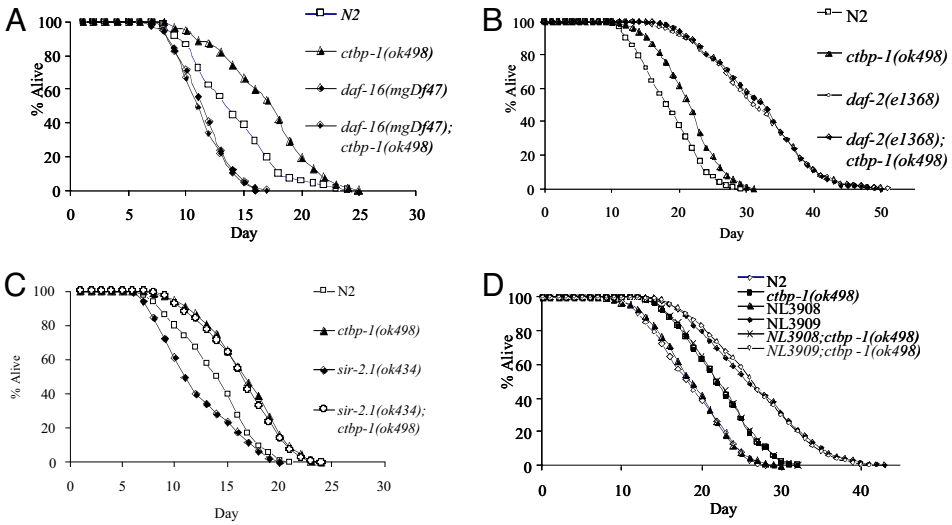

Fig. 2. *ctbp-1* interaction with the insulin-like and the *sir-2.1* pathways. (*A*) Extension of life span in *ctbp-1*(*ok498*) depends on *daf-16*. Mean life span of each strain is: N2, 14.3 ± 0.4 days; *ctbp-1*(*ok498*), 17.2 ± 0.5 days; *daf-16*(*mgDf47*), 11.9 ± 0.3 days; *daf-16*(*mgDf47*);*ctbp-1*(*ok498*), 11.6 ± 0.3 days [$P = 0.63$ compared with *daf-16*(*mgDf47*)]. The experiment was repeated 3 times with similar results. (*B*) *ctbp-1*(*ok498*) was crossed into the *daf-2*(*e1368*) mutant. N2, 17.7 ± 0.3 days; *ctbp-1*(*ok498*), 21.6 ± 0.3; *daf-2*(*e1368*), 31.6 ± 0.4; *daf-2*(*e1368*);*ctbp-1*(*ok498*), 31.9 ± 0.4 [$P = 0.96$ compared with *daf-2*(*e1368*)]. (*C*) *ctbp-1*(*ok498*) was crossed into *sir-2.1*(*ok434*). N2, 14.1 ± 0.2 days; *ctbp-1*(*ok498*), 16.8 ± 0.2 days; *sir-2.1*(*ok434*), 12.2 ± 0.3 days ($P < 0.0001$ compared with N2); *sir-2.1*(*ok434*);*ctbp-1*(*ok498*): 16.6 ± 0.3 days [$P = 0.4$ compared with *ctbp-1*(*ok498*)]. (*D*) *ctbp-1*(*ok498*) was crossed into the *sir-2.1* overexpression line NL3909 and its control line NL3908. N2, 18.8 ± 0.3 days; *ctbp-1*(*ok498*), 22.3 ± 0.3 days; NL3908, 19.1 ± 0.3 days; NL3909, 26.7 ± 0.4 days; *NL3908*;*ctbp-1*(*ok498*), 22.5 ± 0.3 days ($P < 0.0001$ compared with NL3908); *NL3909*;*ctbp-1*(*ok498*), 26.9 ± 0.4 days ($P = 0.71$ compared with NL3909). The data were pooled from 3 independent experiments. The life span assays in *B* and *D* were carried out at 20 °C. The full statistical analyses of the data are listed in Table S1.

Table 1. CTBP-1 and CTBP-1(H467A) but not CTBP-1(G332/334V) rescue the extended life span phenotype in *ctbp-1*(*ok498*)

Strain	Adult life span, mean ± SEM	*N*	Percentage *v.* N2	*P v.* N2	*P v.* *ctbp1*(*ok498*);*pRF4*	Maximal adult life span
N2	13.6 ± 0.2	212				21
ctbp-1(*ok498*)	16.3 ± 0.3	196	20.0	<0.0001		23
ctbp-1(*ok498*); *pRF4*	16.7 ± 0.2	198	22.9	<0.0001		25
ctbp-1 transgenic lines						
ctbp-1(*ok498*); *Ex122*	13.8 ± 0.3	126	1.6	0.75	<0.0001	21
ctbp-1(*ok498*); *Ex256*	14.4 ± 0.2	213	6.1	0.005	<0.0001	21
ctbp-1(*G332/334V*) transgenic lines						
ctbp-1(*ok498*); *ExN337*	16.2 ± 0.3	177	18.9	<0.0001	0.29	23
ctbp-1(*ok498*); *ExN373*	16.4 ± 0.2	207	20.4	<0.0001	0.40	24
ctbp-1(*ok498*); *ExN3118*	16.8 ± 0.2	206	23.0	<0.0001	0.82	25
ctbp-1(*H467A*) transgenic lines						
ctbp-1(*ok498*); *ExH23*	14.3 ± 0.3	181	6.3	0.02	<0.0001	23
ctbp-1(*ok498*); *ExH235*	12.7 ± 0.3	106	-7.0	0.05	<0.0001	24

Life span of *ctbp-1*(*ok498*) rescued with wild-type and mutated *ctbp-1* genomic DNA. The life span of transgenic animals was compared to that of N2 and *ctbp-1*(*ok498*); *pRF4*. Life span data are pooled from 3 independent trials. The life span graphs can be found in Fig. S4*A*.

increased resistance to oxidative (induced by paraquat) and heat stress, but not to DNA (UV) damage, starvation and pathogen stress (*Pseudomonas aeruginosa* PA14) (Fig. S3). This increased resistance to oxidative and heat stress is consistent with the life span extension phenotype associated with abrogation of the *ctbp-1* gene in worms.

CtBP has been shown to bind NAD(H) and possess dehydrogenase activity in vitro (3–5), although the physiological substrate and the biological significance of this activity have remained elusive. Previous studies further suggested that NAD(H) binding regulates CtBP function by influencing the interactions between CtBP and its partners (6, 7). To investigate the functional relevance of NAD(H) binding and the putative dehydrogenase activity of CTBP-1, we carried out genetic complementation experiments in *ctbp-1*(*ok498*). Point mutations predicted to compromise the dehydrogenase activity (H467A) or NAD(H) binding (G332/334V) were introduced into CTBP-1 (4). As shown in Table 1 and Fig. S4*A*, whereas the genomic fragment encoding wild type or the dehydrogenase-defective CTBP-1 fully suppressed the life span phenotype associated with *ctbp-1*(*ok498*), the NAD(H) binding mutant CTBP-1(G332/334V) failed to do so. This observation is consistent with the earlier report demonstrating that the putative dehydrogenase activity was not required for mammalian CtBP proteins to mediate transcriptional repression (12). The failure of *ctbp-1*(*G332/334V*) to rescue the mutant was not due to lower protein level as CTBP-1(G332/334) was expressed at a level comparable to those of the wild type and CTBP-1(H467A) in the transgenic worms (Fig. S4*B*). However, when they were fused to G4DBD and tested in transcription assay, CTBP-1(G332/334V) had a much weaker transcription repression activity than wild-type and CTBP-1(H467A) (Fig. S4*C*), although all 3 G4 fusion proteins were again comparably expressed (data not shown). Taken together, these findings suggest that CTBP-1 regulates life span in *C. elegans*, and, whereas the dehydrogenase activity appears dispensable, its ability to bind NAD(H) seems important for this function.

In *C. elegans*, *Drosophila* and mammals, one of the well studied pathways that control life span is the insulin/IGF-1 pathway, which involves the insulin/IGF-1 receptor DAF-2 and the forkhead transcriptional regulator DAF-16 in worms (20). Mutations that reduce the activity of the insulin-like pathway extend life span, whereas deletion of *daf-16* inhibits this phenotype. To determine the relationship, if any, between *ctbp-1* and the insulin/IGF-1 signaling pathway, we first investigated the genetic relationship between *ctbp-1* and *daf-16* in life span regulation. When crossed into the *daf-16*(*mgDf47*) null mutant (21), *ctbp-1*(*ok498*) failed to extend its life span (Fig. 2*A* and Table S1), demonstrating that *daf-16* is required for *ctbp-1* regulation of life span. To further determine whether CTBP-1 operates in the insulin-like signaling pathway, *ctbp-1*(*ok498*) was crossed into the long-lived *daf-2* hypomorphic mutant *daf-2*(*e1368*) (22). We found no increase of life span of the double mutant versus the single *daf-2*(*e1368*) mutant (Fig. 2*B* and Table S1). Because the *daf-2* allele is not null, the epistasis between *daf-2* and *ctbp-1* cannot be determined unequivocally. However, taken together, our data suggest that *ctbp-1* functions in the insulin-like pathway to regulate life span.

Over-expression of Sir2 has been shown to increase life span in yeast, *C. elegans*, *Drosophila* and zebra fish (23). However, loss of *sir-2.1* reduces life span in *C. elegans* (24). Previous studies suggest that SIR-2.1 functions in the insulin-like pathway (25), possibly upstream of DAF-2 (26). We therefore examined whether there is any genetic interaction between *sir-2.1* and *ctbp-1* in life span regulation. When crossed into the *sir-2.1* null mutant *sir-2.1*(*ok434*) (24), *ctbp-1*(*ok498*) had no reduction in life span in the absence of *sir-2.1* (Fig. 2*C* and Table S1), suggesting that CTBP-1 acts either downstream of SIR-2.1 or in a parallel pathway. Next we tested whether loss of *ctbp-1* and over-expression of *sir-2.1* has any additive or synergistic effects on extending life span. We crossed *ctbp-1*(*ok498*) into the *sir-2.1* over-expression line NL3909 *pkls1642 [unc-119 sir-2.1]* and found that *NL3909*;*ctbp-1*(*ok498*) had similar life span as that of NL3909, whereas, as expected, loss of *ctbp-1* extended the life span of the control strain NL3908 *pkls1641 [unc-119]* (Fig. 2*D* and Table S1). Taken together, these data suggest that *ctbp-1* may function in the same pathway as *sir-2.1*, likely downstream of *sir-2.1*.

Interestingly, previous studies show that a reduction in CtBP binding to the transcription repressor HIC-1 [hypermethylated in cancer (27)] results in an up-regulation of the human Sir2 homologue SIRT1 expression in primary fibroblasts (28), suggesting that SIRT1 may be a downstream target of CtBP. However, the expression of *C. elegans sir-2.1* and other possible Sir2 homologues (*sir-2.2*, *sir-2.3* and *sir-2.4*) was unaltered in the absence of a functional CTBP-1 in our study (Fig. S5 and data not shown). The reason for this lack of regulation remains unclear, and could be because there is no close *C. elegans* homologues of HIC-1, which was shown to be required for the transcription regulation of SIRT1 by CtBP (28). Together with the epistatic analysis, our results suggest that CTBP-1 does not regulate life span through up-regulation of SIR-2.1.

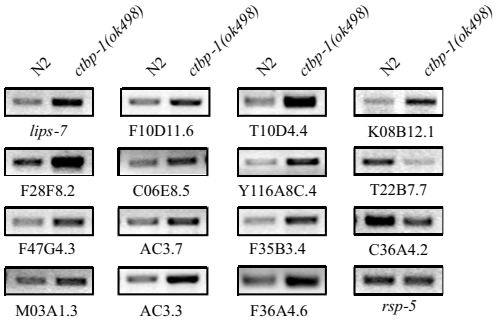

Fig. 3. RT-PCR verification of both up-regulated and down-regulated genes from the microarray analysis. *rsp-5* was used as a loading control. The complete gene list is in Table S2.

To gain insights into the physiological processes regulated by CTBP-1 that may be potentially relevant to life span regulation in worms, we carried out a genome-wide expression microarray to identify downstream targets of CTBP-1, using young adult worms. Among ≈22,000 genes represented on the microarray, transcription of a total of 243 genes was changed by 2-fold or greater ($P < 0.05$) comparing *ctbp-1*(*ok498*) to N2 (Table S2). Approximately 90% (213) of these putative CTBP-1 target genes were up-regulated in *ctbp-1*(*ok498*) (Table S2), consistent with the idea that CTBP-1 functions primarily as a transcriptional corepressor. To confirm the microarray results, we compared the expression of these putative CTBP-1 target genes between N2 and the *ctbp-1*(*ok498*) by RT-PCR. We selected 20 genes that covered both up- and down-regulated ones (≈2- to 4.5-fold changes by microarray analysis), 19 displayed expression profiles consistent with the microarray data, which suggests that the microarray data are ≈95% reliable (Fig. 3 and data not shown).

We find that the up-regulated genes include those involved in metabolism, stress response and cell signaling, among others (Table S2). We used a gene ontology (GO) annotation tool DAVID (database for annotation, visualization, and integrated discovery) to analyze gene families and functional groups that are enriched in the microarray list (29). Genes with GO terms of ion transport, cuticle and collagen and lipases, among others, are significantly over-represented (Table S3). To determine which of these genes (and biological pathways) were important for CTBP-1 to regulate life span, we carried out a small scale genetic suppression experiments by using RNAi to inhibit a subset of CTBP-1 target genes in *ctbp-1*(*ok498*). Previous studies showed that genes associated with metabolism and stress response affect longevity (13, 30). Thus, in the suppression experiments, we focused on a small group of CTBP-1 target genes that may play a role in these processes. *lips-7* is predicted to encode a putative TAG lipase. It has the highly conserved GXSXG pentapeptide and the catalytic triad Ser:Asp:His, which are important for catalysis and are conserved among TAG lipases in different species (31, 32). The bacterial homolog of LIPS-7, LipA, has been demonstrated to have TAG lipase activity (33). Significantly, RNAi inhibition of *lips-7* (Fig. S2*C*) resulted in a complete suppression of life span extension associated with *ctbp-1*(*ok498*) (Table 2 and Fig. S6). This suppression was not due to sickness because inhibition of *lips-7* alone in N2 had no overt problems and worms had normal life span (Table 2 and Fig. S6). Knockdown of another TAG lipase K08B12.1 (Fig. S2*C*) that was also up-regulated in *ctbp-1*(*ok498*) (Fig. 3 and Table S2) had no significant effect on the life span of either N2 or *ctbp-1*(*ok498*) (Table 2), suggesting that the contribution of LIPS-7 to CTBP-1-mediated life span regulation is specific. Knockdown of F10D11.6 (Fig. S2*C*), a bactericidal/permeability-increasing protein, resulted in developmental defects and very early death (Table 2), precluding evaluation of its role in CTBP-1 life span regulation. RNAi of the remaining 4 CTBP-1 targets, which represent genes in metabolism, endoplasmic reticulum (ER) stress response and anti-bacteria response (Table 2) showed small or no suppression of the life span phenotype associated with *ctbp-1*(*ok498*). Although RNAi appeared to have significantly knocked down the expression of target genes (Fig. S2*C*), we could not exclude the possibility that the inhibition was still not sufficient enough to cause suppression. Alternatively, functional redundancy could also contribute to the apparent lack of suppression.

TAG lipases mediate triacylglycerol uptake, distribution and usage in mammals (34). Therefore, we investigated whether loss of CTBP-1 had any effects on the TAG level and whether *lips-7* depletion reversed these effects. Consistent with the increase in the level of the putative TAG lipase *lips-7*, *ctbp-1*(*ok498*) worms had a 16.8% decrease in the TAG level compared with N2 (Fig. 4*A*). When this gene was knocked down by RNAi, the level of TAG in *ctbp-1*(*ok498*) was increased to that of N2 (Fig. 4*A*). We obtained similar results by Nile Red staining, which visualizes the lipid contents (Fig. 4*B*). These data suggest that LIPS-7 is likely a functional TAG lipase, and that TAG levels play an important role in extending the life span of *ctbp-1*(*ok498*). However, RNAi inhibition of *lips-7* in N2, which led to a higher level of TAG, did not alter life span, suggesting that TAG increase alone is not sufficient to reduce life span in N2. Because TAG is the main form of fat storage, suppression of the life span phenotype seen upon inhibition of LIPS-7 suggests a potential link between fat metabolism and longevity that appears to be critical for CTBP-1-mediated life span extension.

Table 2. Suppressor screens

Gene	Brief description	N2, mean ± SEM (*N*)	*ctbp-1*(*ok498*), mean ± SEM (*N*)	*P* value	No. of trials
Control		15.1 ± 0.1 (870)	18.2 ± 0.1 (832)	<0.0001*	11
AC3.3	Induced by blockage of the UPR in the ER	15.2 ± 0.3 (266)	18.0 ± 0.3 (261)	<0.0001*, 0.87†	3
C06E8.5	Bactericidal/permeability-increasing protein	15.3 ± 0.2 (295)	18.4 ± 0.2 (223)	<0.0001*, 0.63†	3
lips-7	Triacylglycerol lipase	15.5 ± 0.3 (154)	15.0 ± 0.3 (151)	0.28*, <0.0001†	3
F28F8.2	Long chain fatty acid acyl-CoA ligase	15.2 ± 0.3 (258)	18.6 ± 0.2 (258)	<0.0001*, 0.05†	3
F47G4.3	Glycerol-3-phosphate dehydrogenase	14.0 ± 0.2 (343)	16.1 ± 0.3 (331)	<0.0001*, 0.009†	4
K08B12.1	Triacylglycerol lipase	15.3 ± 0.2 (283)	18.5 ± 0.3 (263)	<0.0001*, 0.03†	3
F10D11.6‡	Bactericidal/permeability-increasing protein	—	—	—	—

Knock down of CTBP-1 target genes to examine their roles in life span regulation. Life span results represent pooled data from individual experiments. UPR, unfolded protein response.

*Comparing *ctbp-1*(*ok498*) with N2 treated with the same specific RNAi.

†Comparing *ctbp-1*(*ok498*) treated with the specific RNAi with that treated with control RNAi.

‡These worms were sick and died very early.

A

	N2 (μg/mg dry wt.)	*ctbp-1(ok498)* (? g/mg dry wt.)	Change compared to N2
Control RNAi	13.1 ± 0.4	10.9 ± 0.6	-16.8% *
lips-7 RNAi	25.0 ± 0.8	26.1 ± 0.7	4.4%
Change compared to control RNAi	90.8% *	139% *	

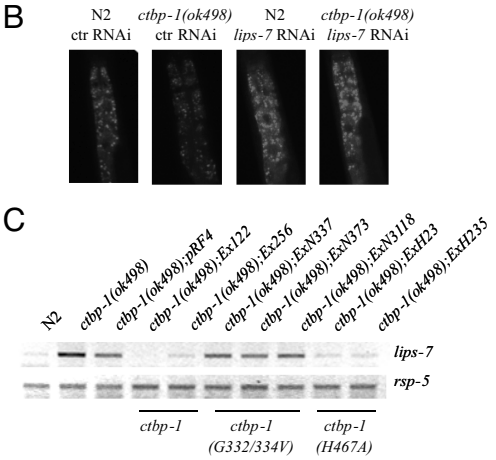

Fig. 4. TAG lipase is important for CTBP-1-mediated-life span regulation and its regulation by CTBP-1 depends on its NAD(H) binding motif. (*A*) Knockdown of *lips-7* increased TAG level which was decreased in *ctbp-1*(*ok498*). The TAG levels of N2 and *ctbp-1*(*ok498*) treated with *lips-7* RNAi or control RNAi were measured from 3 independent groups. *, $P < 0.05$, Student's paired 2 tailed *t* test. (*B*) Lipid storage was visualized with Nile Red. Shown are representative images from 8 worms examined in each group. (*C*) *lips-7* expression was reduced in *ctbp-1*(*ok498*) rescued with wild-type *ctbp-1* and *ctbp-1*(*H467A*) but not *ctbp-1*(*G332/334V*) DNA.

We next investigated whether the regulation of *lips-7* expression by CTBP-1 depends on its NAD(H) binding ability. Although up-regulation of *lips-7* transcription in *ctbp-1*(*ok498*) was suppressed by either CTBP-1 or CTBP-1(H467A), CTBP-1(G332/334V) failed to do so (Fig. 4*C*). These data demonstrate that CTBP-1 regulation of LIPS-7 depends on the NAD(H) binding motif, suggesting that CTBP-1 may regulate transcription and metabolism in response to the redox states of the organism.

We note that regulation of the TAG level through LIPS-7 is a significant contributor to life span regulation by CTBP-1 but this may not be the only factor. Our microarray analysis identified other genes downstream of CTBP-1 that may contribute to the regulation of longevity. In fact, some of the CTBP-1 target genes have already been shown to affect the aging process in other studies. For instance, we identified up-regulation of genes such as ABU (activated in blocked unfolded protein response) and PQN (prion-like Q/N proteins) involved in the ER stress response (35). The ER stress response is important to maintain protein homeostasis and has been implicated in different forms of stress response (36, 37). Interestingly, ABU and PQN proteins have been associated with resveratrol-induced life span extension (38). Thus, the increased expression of these proteins may contribute to the increased life span and stress resistance of *ctbp-1*(*ok498*). Our result that RNAi inhibition of AC3.3 (*abu-1/pqn-1*) did not suppress the extended life span in *ctbp-1*(*ok498*) may be due to insufficient knockdown or functional redundancy of these proteins because several other highly similar ABU/PQN proteins were also up-regulated (Table S2). In addition to the ABU/PQN proteins, the insulin-like peptide and peptidase genes are also up-regulated in *ctbp-1*(*ok498*) and could conceivably contribute to the extended life span by regulating the insulin-like signaling pathway (Table S2). The relationship of these proteins with the CTBP-1-mediated longevity, if any, is unclear and warrants further investigation.

Our suppression and metabolism analyses identified a potential link between fat metabolism and life span regulation mediated by CTBP-1. Increased fat storage has been associated with extended life span in worms (39–41); however, in other long-lived worm mutants, fat storage has been uncoupled from life span regulation (42). Furthermore, RNAi inhibition of an enzyme in the triacylglycerol synthesis pathway has been shown to extend life span in worms (43). In other organisms including mouse, the opposite correlation between fat storage and life span was also observed (44). The up-regulation of a TAG lipase and consequently down-regulation of TAG in *ctbp-1*(*ok498*) may reflect a possible change in energy utilization, which was implicated in caloric restriction animals and long-lived Snell dwarf mouse (45, 46). Thus, the increased expression of LIPS-7 in the *ctbp-1* mutant may contribute to life span extension by directly impacting energy metabolism and/or by changing lipid composition, membrane fluidity and signaling pathways.

Recent studies have identified roles for CtBP in mouse development, apoptosis, and hypoxia-induced tumor migration (9–12). Our study unveils a role for CtBP in regulating longevity in coordination with the insulin/IGF-1 pathway. We have also demonstrated that the life span regulation function of CtBP is likely to depended on its NAD(H) sensing ability, implicating that CTBP-1 may regulate transcription in response to nutrient availability and stress. This raises the possibility that CtBP may play an important role in sensing metabolic alterations and directly relaying this information to transcriptional regulation. Our data also show that lipid metabolism is important in CTBP-1 regulation of life span. Consistent with our demonstration of CTBP-1's involvement in lipid regulation, recent publications have documented a role for mammalian CtBP in regulating lipid storage and adipogenesis in mammalian cells and mice (47–49) while this manuscript was under review. Accumulation of lipid has been associated with aging-related diseases such as obesity, type II diabetes, insulin resistance and cancer (50, 51). Together, these findings suggest an important association between metabolism, aging-related diseases and longevity connected by the NAD(H) dependent transcription corepressor CtBP. Thus, CtBP, together with Sir2, represent an important group of energy-sensing transcriptional regulators that provide a direct link between transcription and metabolism, which impacts life span.

Materials and Methods

Strains and Routine Worm Culture. *ctbp-1*(*ok498*) was obtained from CGC. Routine maintenance of worms were performed as described in ref. 52.

Life Span Analysis. Life span analyses were carried out as prescribed (53) at 23.5 °C unless otherwise indicated. For *daf-2* mutant and *sir-2.1* overexpression line, 20 °C was used to prevent worms going into dauer and bagging. In that case, the whole assay were performed at 20 °C. Young adult worms (day 0 for life span) were plated on experimental plates, transferred to fresh plates approximately every 2 days during the reproductive period to eliminate progenies and transferred when necessary thereafter. Animals were scored every day and considered as dead when they did not respond to repeated tapping with the pick. All statistics were done with JMP software and *P* values were determined using log-rank statistics. For RNAi experiments, bacteria strains expressing gene specific dsRNA were grown on plates containing IPTG. Worms were left on the same plates for no more than 10 days to ensure the efficiency of RNAi.

Lipid Analysis. Age-matching young adult worms treated with RNAi were collected, washed multiple times with water and snap-frozen with liquid N2. The TAG levels were analyzed as described in ref. 54. Nile Red staining was performed on age-matching young adult worms as described in ref. 41.

Further details are in *SI Materials and Methods*.

ACKNOWLEDGMENTS. We thank David Sinclair, Keith Blackwell, Marcia Haigis, and the Y.S. laboratory members for helpful discussion and comments on

the manuscript. We thank Caenorhabditis Genetics Center for the *ctbp-1*(*ok498*) strain, Leonard Guarente (Massachusetts Institute of Technology, Cambridge, MA) for providing strain information on NL3908 and NL3909, Marc Vidal (Harvard Medical School, Boston) for the RNAi clones, Piali Sengupta (Brandeis University, Waltham, MA) for the dauer-inducing pheromone, and Deyu Fang (University of Missouri, Columbia, MO) and Frederick Ausubel (Harvard Medical School, Boston) for the *P. aeruginosa* PA14 strain and Christian Daly and Peter Boag for technical help. This work was supported by Ruth L. Kirschstein National Service Award GM 70095 (to J.R.W.), a grant from the Paul F. Glenn Labs for the Molecular Biology of Aging Pilot Project Grant Program (to Y.S.), and National Institutes of Health Grant GM 071004 (to Y.S.).

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