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Original Article

CBP-1 Acts in GABAergic Neurons to Double Life Span in Axenically Cultured Caenorhabditis *elegans*

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Abstract

When cultured in axenic medium, Caenorhabditis *elegans* shows the largest life-span extension compared with other dietary restriction regimens. However, the underlying molecular mechanism still remains elusive. The gene *cbp-1*, encoding the worm ortholog of p300/CBP (CREB-binding protein), is one of the very few key genes known to be essential for life span doubling under axenic dietary restriction (ADR). By using tissue-specific RNAi, we found that *cbp-1* expression in the germline is essential for fertility, whereas this gene functions specifically in the GABAergic neurons to support the full life span–doubling effect of ADR. Surprisingly, GABA itself is not required for ADR-induced longevity, suggesting a role of neuropeptide signaling. In addition, chemotaxis assays illustrate that neuronal inactivation of CBP-1 affects the animals' food sensing behavior. Together, our results show that the strong life-span extension in axenic medium is under strict control of GABAergic neurons and may be linked to food sensing.

Keywords: biology of aging, *Caenorhabditis elegans*, dietary restriction

Reducing food intake without malnutrition extends life span in a wide variety of species, ranging from single-celled organisms to mammals (1). How dietary restriction (DR) increases life span and decreases the onset of age-related diseases are questions of major interest in biomedical research. Research in model organisms has provided seminal information on the genetic pathways involved in the pro-longevity effect of DR (2). In Caenorhabditis *elegans*, a broad range of DR regimens have been used and they all extend life span to various degrees by separate or partially overlapping molecular pathways (3). Apart from bacteria-based methods, DR can also be obtained by culturing worms in sterile, chemically defined or undefined liquid media (4,5). Axenically cultured worms show DR-like phenotypes such as slowed development, reduced fecundity, a slender appearance, and prolonged life span. Hence, the term axenic dietary restriction (ADR) is often applied (6,7).

ADR-induced longevity may be distinct from life-span extension caused by most other forms of DR, as its effect is unusually large and independent of most key factors such as DAF-16, PHA-4, and SKN-1 (6,8,9). Up till now, the underlying molecular mechanisms supporting this robust longevity effect remain enigmatic. Our recent results confirmed an earlier study showing that CBP-1, the worm ortholog of p300/CBP (cAMP response element binding [CREB]–binding protein) homolog, is one of the very few genes of which RNAi knockdown in adults completely blocks ADR-induced life-span extension (8,10).

CBP-1 is a transcriptional coactivator with histone aceytltransferase activity, and it is predominantly localized in the nuclei of most if not all somatic cells (11–13). CBP-1 acts as an important regulator of longevity by different interventions, and it exhibits a neuron-protective effect during normal aging (10,14). For example, CBP-1 is required for neuronal protection by a few drugs from glucose toxicity (15) and for life-span extension of the *mir-80* mutant that shows a DR-like phenotype (16). Life-span extension by the DR mimetic D-beta-hydroxybutyrate also depends on CBP-1 (17). In addition, several proteins implicated in life-span extension are CBP-1 binding partners, like DAF-16, HSF-1, and SKN-1 (10,18), suggesting that CBP-1 constitutes a common factor integrating multiple longevity pathways and ultimately supports healthy aging. Therefore, it is of great importance to investigate how CBP-1 regulates aging and to study its potential function in health and life span.

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In this study, we focus on the tissues where CBP-1 acts to influence life span and we investigate how it may relate to sensing of bacterial food.

Methods

C. elegans Strains

The wild-type *C. elegans* used was Bristol N2 male stock. The tissue-specific RNAi strains used in this study are listed in Supplementary Table 1. The strain OD95 was used for reproductive system imaging (19). All strains were obtained from the Caenorhabditis Genetics Center (CGC), Minneapolis, MN. The RNAi clone targeting *cbp-1* was obtained from the Ahringer RNAi library (20). The bacterial strain containing the empty vector L4440 was used as a control.

Worm Culture and RNAi Treatment

Eggs, prepared by hypochlorite treatment of gravid adults, were allowed to hatch overnight in S basal (21), and the resulting first stage larvae (L1) were inoculated onto nutrient agar plates seeded with *Escherichia coli* K12 bacteria. Worms were grown synchronously, and the day of L4-to-adult transition was designated as day 0 of adulthood (D0). Subsequently, RNAi treatment was carried out following standard bacterial feeding protocols (22) for 5 days during early adulthood after which the worms were switched to their final dietary regimens (Supplementary Figure S3).

UV-Killing of Bacteria

Bacteria were killed by irradiation (1 J/cm^2) using the UV Stratalinker (Stratagene, USA). To check the efficiency of this treatment, a sample of killed bacteria was added to fresh LB medium and grown overnight at 37°C.

Life-Span Assays

After RNAi treatment, animals were collected for life-span assays. Previously, our group showed that axenic medium supplemented with growth-arrested *E coli* bacteria can be used as an appropriate control for ADR (23). However, for the fully fed (FF) setup, we chose to use nematode growth medium (NGM) seeded with *E coli* OP50 as this setup is more practical and considered the golden standard in *C. elegans* life-span analysis. In the FF setup, approximately hundred worms per strain were placed on small NGM plates (10 to 12 worms per plate) seeded with *E coli*. Worms were transferred to fresh NGM plates every week and survival was scored at regular time intervals. Individuals were considered dead if they did not respond to gentle prodding with a platinum wire. For ADR, about hundred worms were transferred to small polystyrene snap-cap tubes (3 to 5 worms per tube) containing 0.3 mL of liquid axenic medium consisting of 3% soy peptone (Sigma-Aldrich, St. Louis, Missouri) and 3% yeast extract (Becton-Dickinson, Franklin Lake, New Jersey), final concentrations (f.c.), supplemented with 0.05% hemoglobin f.c. (bovine; Serve, Heidelberg, Germany) diluted from a 100× stock in 0.1M KOH (autoclaved for 10 minutes). To prevent bacterial contamination, 0.1 g/L (f.c.) ampicillin and 0.05 g/L (f.c.) kanamycin were added. Worms in axenic medium were observed every other day using a stereomicroscope and were scored dead if no movement could be detected, even after gently tapping the tubes.

Progeny production was avoided by the addition of 5-fluoro-2′-deoxyuridine at 100 µM and 50 µM f.c. for FF and ADR, respectively. Worms that died of protruding vulva or crawling off the plates were censored. All life-span assays were conducted at 20°C and were repeated at least twice independently (pooled data are shown).

Data were analyzed using the online application for survival analysis (OASIS) described before (24). In all cases, life-span data are indicated as mean ± s.e.m. and *p*-values were calculated using the log-rank (Mantel–Cox) method. To evaluate the impact of *cbp-1* RNAi knockdown on ADR life-span extension, the mean life span ($\bar{x}$) was calculated from each set of four life-span curves covering two dietary regimens, FF and ADR, for worms submitted to *cbp-1* RNAi and empty vector control. The relative life-span extension (RLE) due to ADR for any given pair of FF and ADR survival tests was calculated as the ratio of the mean life span in both culture conditions minus one:

$$\mathrm{RLE} = \frac{\bar{x}_{\mathrm{ADR}}}{\bar{x}_{\mathrm{FF}}} - 1$$

Confocal Imaging

The transgenic reporter strain OD95 was used to observe germline morphology and early embryonic development. Animals were grown synchronously to late L4 stage on nutrient agar plates seeded with *E coli* K12 bacteria. Subsequently, a two-day *cbp-1* RNAi treatment was carried out on plates after which worms were randomly picked for confocal imaging. A subsample of worms was collected for hypochlorite treatment and eggs were imaged at regular time intervals over a 12-hour period. Images were taken with a Nikon TiE-C2 confocal microscope using 488-nm excitation wavelength with a 520/30-nm band pass filter for green fluorescence and 561-nm excitation wavelength with a 605/55-nm band pass filter for red fluorescence.

Chemotaxis Assay

After a 5-day *cbp-1* RNAi treatment (D1 to D5), animals were rinsed from the plates and washed three times with S basal. Worms were transferred to freshly prepared NGM solid plates for chemotaxis assays. Three types of behavioral assays (25) were performed as outlined in Figure 5. Briefly, a drop of freshly cultured *E coli* OP50 (10 µL) served as an attractant, whereas distilled water was used as blank. To paralyze the animals reaching the spot of preference, 1 µL of 1 M sodium azide was added to the mixtures prior to application to the plate. Around thirty to fifty worms were placed in a drop on the plate as indicated in the diagram (Figure 5A–C) and the liquid was allowed to evaporate shortly in the laminar flow bench. Plates were sealed and kept for 1 hour in the incubator at 20°C and later stored at 4°C till scoring.

The chemotactic index (CI) was calculated to precisely evaluate the worms' response to the food (25). In the type 1 assay, CI equals to the percentage of worms counted within the inner circle compared with the total number of worms present on the plate. For the type 2 assay, the number of worms in both control quadrants was subtracted from the number of worms in the test quadrants and this number was divided by the total number of worms that have left the central spot. In the third assay type, CI was calculated as the number of worms on the bacterial spot minus those on the blank spot, divided by the total number of scored worms on the plate. A score of +1.0 indicates maximal attraction toward the target and represents 100% of the worms arriving in the quadrants containing the food. An index of −1.0 is evidence of maximal repulsion.

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Thrashing and Touch Assays

RNAi-treated animals were rinsed off the plates and washed three times with S basal. Thrashing was measured by transferring individual adults (D6) into a drop of S basal on an NGM plate at room temperature. After equilibrating for 30 seconds, the number of body bends was counted over a period of 30 seconds. A single thrash was defined as a complete change in the direction of bending at the mid body (26). Rates were measured for at least 10 worms for each RNAi-treatment. Response to nose touch was measured as described (27). In this assay, at least 20 animals were tested for each condition.

Fertility Assay

Animals were grown synchronously to L4 stage on NA plates seeded with *E coli* K12. Subsequently, individual L4 hermaphrodites were transferred to NGM plates with a 25-μL spot of bacteria expressing *cbp-1* dsRNA. Animals were transferred to fresh plates daily during the reproductive period. After 24 hour incubation at 20°C, hatched larvae and unhatched eggs on each plate were counted. Fertility was calculated as total hatched larvae per worm, whereas the fecundity was calculated as the total number of hatched and unhatched eggs produced. At least five worms from per replicate (at least 3) were analyzed for each strain and for each condition.

Results

Contribution of Specific Tissues to the *cbp-1* Phenotypes Under FF Conditions

Prior to studying the role of *cbp-1* in ADR longevity, we analyzed the pleiotropic phenotypes of *cbp-1* knockdown under normal feeding conditions. Maternally provided CBP-1 regulates non-neuronal cell differentiation in early embryos, and therefore, null alleles result in sterility due to developmental arrest (12). We confirmed that this phenotype also occurs upon *cbp-1* RNAi for 2 days starting at L4 stage (Supplementary Figure S1A and C). RNAi knockdown of *cbp-1* is also known to decrease life span by approximately 30%–40% due to unknown reasons (8,10). As CBP-1 is expressed in many (if not all) tissues (12), it is not clear whether the sterility and life-span phenotypes are linked to the lack of CBP-1 activity in specific overlapping or nonoverlapping tissues.

Therefore, we knocked down *cbp-1* specifically in the neurons, intestine, germline, hypodermis, muscle, uterus, and the mesoderm and somatic gonad precursor cells using tissue-specific RNAi strains (Supplementary Table S1). *cbp-1* knockdown in neurons and germline phenocopied the life span–shortening effect (38% and 31%, respectively) of systemic RNAi (40%), indicating that CBP-1 activity in these tissues is crucial for normal life span. To a lesser extent, CBP-1 also acts in the intestine to influence life span, as indicated by the 24% life span reduction upon intestinal knockdown. In other tissues, *cbp-1* RNAi had no significant effect on life span (Figure 1A). Survival data are summarized in Supplementary Table S2.

Systemic RNAi knockdown of *cbp-1* causes a >95% decrease in the number of hatched offspring (Figure 1B). Tissue-specific knockdown of *cbp-1* in the germline completely phenocopies this fertility defect, whereas *cbp-1* knockdown in the other tissues has no significant effect on fertility (Figure 1B). In accordance with the role of *cpb-1* in early development, worm fecundity (number of eggs layed per worm) was unaltered upon *cbp-1* knockdown (Supplementary Figure S1A and B). Despite its clear effect on embryonic development, *cbp-1* knockdown during adulthood has no obvious effect on

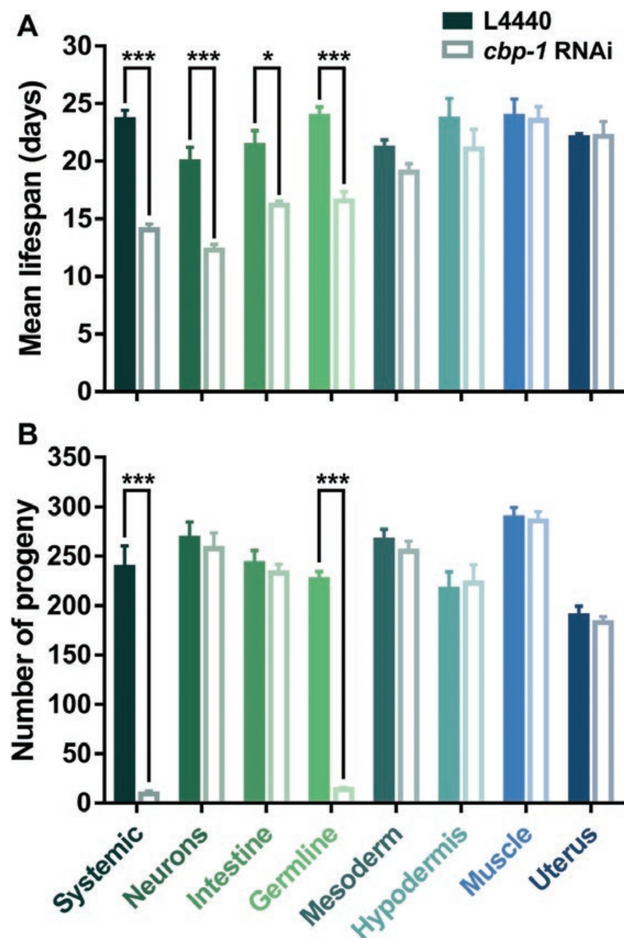

Figure 1. Systemic and tissue-specific knockdown of *cbp-1* differentially affect life span and fertility under FF conditions. (**A**) *cbp-1* knockdown in neurons, germline, and intestine shortens life span. Survival assays were performed using tissue-specific RNAi strains treated with *cbp-1* RNAi during the first 5 days of adulthood. Mean life-span data are derived from experiments shown in Figure 2 and Supplementary Table S2. (**B**) CBP-1 in germline only is required for reproduction. Error bars indicate standard error (standard error of the mean) of at least three independently grown replicates. *$p < .05$; **$p < .01$; ***$p < .001$.

gonad morphology (Supplementary Figure S1C). This observation contrasts with the decreased oocyte phenotype, observed earlier in *cbp-1(RNAi)* worms (19). In the latter study, late L4–larval stage worms were soaked in dsRNA for 24 hours, whereas in our experiment, L4-stage worms were fed bacteria expressing *cbp-1* dsRNA for 48 hours prior to imaging.

In summary, RNAi knockdown of *cbp-1* causes a decrease in life span mainly because of its vital functions in the neurons, germline, and the intestine. The developmental arrest phenotype of *cbp-1* is exclusively dependent on its function in the maternal germline. Thus, in bacteria-fed worms, the negative effect of *cbp-1* mutation on life span can be uncoupled from its sterility phenotype.

Life-Span Extension by ADR Requires Neuronal CBP-1

CBP-1 is one of the very few factors known to be involved in ADR-induced life-span extension (8,10), which is reconfirmed in our experiments. More specifically, *cbp-1* is responsible for the full life-span extension under ADR conditions (Figure 2A). We wondered whether the role of *cbp-1* in ADR longevity is linked to

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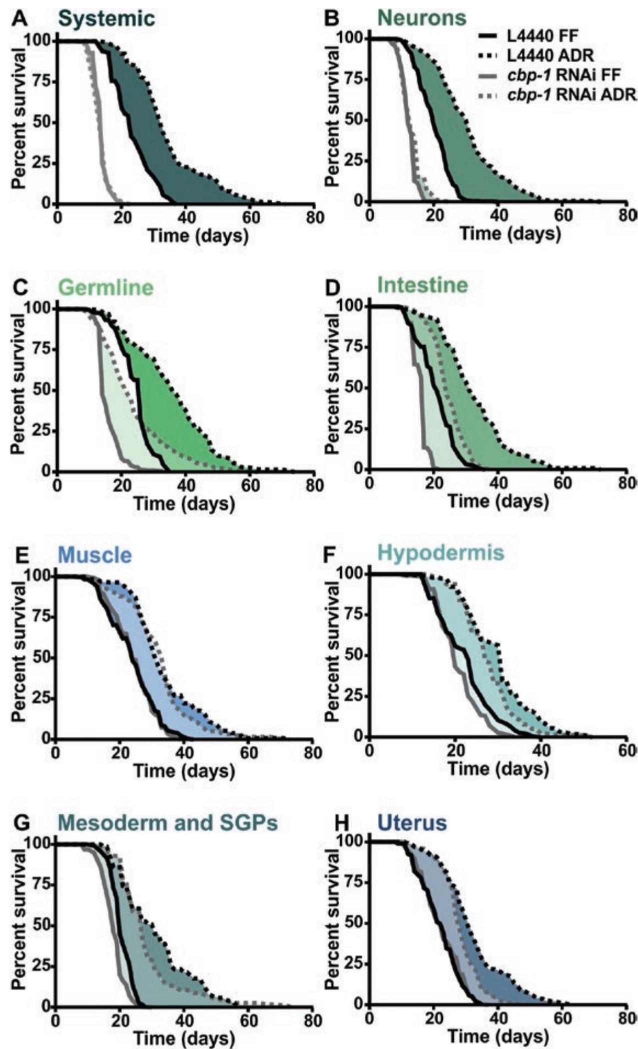

Figure 2. Neuronal knockdown of *cbp-1* completely blocks ADR-induced life-span extension. (**A**) The effect of systemic *cbp-1* knockdown on survival under FF and ADR conditions. (**B–H**) The effect of tissue-specific *cbp-1* knockdown on ADR longevity; neurons (**B**), germline (**C**), intestine (**D**), muscle (**E**), hypodermis (**F**), mesoderm and SGPs (**G**), and uterus (**H**). All data are summarized in Table 1 and Supplementary Table S2.

one particular tissue. Hence, we set up survival assays in which we knocked down *cbp-1* tissue-specifically during the first 5 days of adulthood (Supplementary Table S1), after which worms were transferred to either axenic medium or the control medium containing normal bacterial food (Supplementary Figure S1).

Pan-neuronal knockdown of *cbp-1* phenocopied the life-span effect of systemic knockdown (Figure 2B). In neuronal *cbp-1* RNAi worms, life-span extension by ADR was virtually absent ($p < .01$), indicating that CBP-1 functions in neurons to regulate ADR-induced longevity (Table 1, Supplementary Table S2). As *cbp-1* knockdown in the germline and intestine also influences life span under normal conditions (Figure 1A), we expected that *cbp-1* RNAi in these tissues may also have a profound effect on ADR-mediated life-span extension. However, this is not the case (Figure 2C and D): the RLE by ADR upon *cbp-1* RNAi in both intestine and germline is not significantly different from the RLE caused in the empty vector control (Table 1, Supplementary Table S2). Also in the other tissues tested, CBP-1 showed no significant contribution to the ADR life-span effect (Figure 2E–H; Table 1, Supplementary Table S2).

We conclude that CBP-1 acts in the neurons to modulate longevity in response to ADR treatment. It may be involved in the generation of a neuronal signaling cue in response to sensation of specific ADR compounds (or lack of bacterial compounds) that changes the worm's systemic physiology and results in the dramatic life-span extension seen under ADR.

ADR Longevity Depends on CBP-1 Activity in GABAergic Neurons

The *C. elegans* hermaphrodite contains 302 well-characterized neurons, including sensory neurons, interneurons, and motor neurons (28). Several subclasses of worm neurons have been identified based on their neurotransmitters: acetylcholine (>90 neurons), serotonin (11), dopamine (8), tyramine (4), octopamine (2), glutamate (>72), and GABA (gamma-aminobutyric acid, 26) (28,29). We asked whether ADR longevity is supported by CBP-1-mediated signaling in specific neuron types, and therefore, we used the currently available transgene strains that allow cell-specific RNAi knockdown in GABAergic, cholinergic, dopaminergic, or glutamatergic neurons (27). When young adult worms, sensitive to RNAi in the GABAergic neurons, are fed for 5 days with empty-vector bacteria after which they are transferred to axenic medium, their life span increases by 50% (black curves in Figure 3A). In matching cultures treated with *cbp-1* RNAi, life span is extended by only 9% (grey curves in Figure 3A), showing that *cbp-1* is required for ADR-induced life-span extension. However, we found that *cbp-1* knockdown in FF cultures extends life span by 16% (full curves in Figure 3A; Table 1, Supplementary Table S2). If *cbp-1* in GABA neurons controls GABA signaling, this small life-span extension is in accordance with the earlier observation that loss of GABA signaling extends *C. elegans* life span by about 23% (30). As life span of *cbp-1(RNAi)* worms under ADR almost completely falls back to that of FF *cbp-1(RNAi)* worms (grey curves in Figure 3A), and not to that of FF control worms (full black line in Figure 3A), it is probably that the 16% life-span extension due to failure in GABA signaling is independent of ADR life-span extension. In contrast, we found that CBP-1 activity in cholinergic, dopaminergic, and glutamatergic neurons is not involved in ADR longevity (Figure 3B–D; Table 1, Supplementary Table S2).

Another striking observation was that *cbp-1* RNAi in GABAergic, cholinergic, dopaminergic, and glutamatergic neurons did not cause life span shortening (Figure 3), whereas pan-neuronal knockdown did (Figure 2B). The four subgroups of neurons we tested cover about 200 of the total of 302 neurons in *C. elegans*. Therefore, it is probably that the life span–shortening effect of *cbp-1* knockdown depends on the remaining neurons.

Of note, the four neuron-specific RNAi strains are somewhat shorter lived compared with the wild-type strain and other tissue-specific RNAi strains. Nevertheless, the RLEs due to ADR are very similar among all tested strains, averaging around 0.5 which equals a 50% life-span extension (Supplementary Figure S2). This seems to conflict with the life span–doubling effect of ADR mentioned in earlier studies (6,23,31). However, the reduced life-span extension effect of ADR in our experiments is due to the late switch from bacterial culture to axenic medium at the fifth day of adulthood (Supplementary Figure S3). In a control experiment using wild-type and some neuron-specific RNAi strains, we directly transferred young adult worms (D0) from FF conditions to ADR, skipping the standard 5 day RNAi treatment during young adulthood, and found

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Table 1. Relative Life-span Extension by ADR and Statistical Analysis

Tissues	Treatment	Relative life-span extension (RLE) by ADR						Average RLE
		Rep.1	Rep.2	Rep.3	Rep.4	Rep.5	Rep.6	
Systemic	L4440	0.552	1.113	0.527				0.731
	cbp-1 RNAi	0.060	0.004	−0.100				−0.012
Neurons	L4440	0.640	0.484	1.372				0.832
	cbp-1 RNAi	0.128	−0.005	0.054	−0.227	0.318	−0.029	0.040
Intestine	L4440	0.371	0.690					0.531
	cbp-1 RNAi	0.590	0.692	0.742				0.674
Germline	L4440	0.809	1.005	0.761				0.858
	cbp-1 RNAi	0.758	0.585	0.724				0.689
Mesoderm and SGPs	L4440	0.399	1.046					0.722
	cbp-1 RNAi	0.652	1.003					0.827
Hypodermis	L4440	0.515	0.434					0.475
	cbp-1 RNAi	0.326	0.373					0.350
Muscle	L4440	0.494	0.780					0.637
	cbp-1 RNAi	0.359	0.556					0.458
Uterus	L4440	0.618	0.460	0.318	1.115			0.628
	cbp-1 RNAi	0.171	0.702	0.222				0.365
Systemic	L4440	1.445	0.893	0.764				1.034
	cbp-1 RNAi	0.044	0.022	0.159				0.075
GABAergic neurons	L4440	0.835	0.813	0.313	0.782	0.569		0.662
	cbp-1 RNAi	0.199	0.144	0.019	0.102	0.031		0.099
Dopaminergic neurons	L4440	0.544	0.401	0.205	0.454	0.190		0.359
	cbp-1 RNAi	0.657	0.498	0.265	0.572	0.270		0.452
Cholinergic neurons	L4440	0.776	0.668	0.364	0.524	0.407		0.548
	cbp-1 RNAi	0.636	0.398	0.549	0.340	0.433		0.471
Glutamatergic neurons	L4440	0.506	0.574	0.473	0.418			0.493
	cbp-1 RNAi	0.487	0.508	0.368	0.169			0.383
No RNAi	N2	1.161	1.974					1.568
	unc-25(e156)	1.511	1.029					1.270

life-span extensions of about 160% (Supplementary Figure S2). This confirms the earlier findings that animals gradually lose the ability to respond to DR during adult life (32,33).

GABA Neurotransmitter Is Not Involved in ADR Longevity

As CBP-1 acts specifically in the GABAergic neurons to extend life span under ADR conditions, we asked whether the neurotransmitter GABA is part of this signaling network. Worms that lack GABA due to mutation in the gene coding for the GABA synthesis enzyme glutamic acid decarboxylase (*unc-25*) do not develop well under axenic conditions. This problem was circumvented by growing the *unc-25(e156)* mutants on UV-killed bacteria until adulthood followed by transfer to axenic medium and FF control plates, respectively. Exposure to ADR during adulthood only is sufficient to elicit the full ADR longevity effect (34). *unc-25* mutation causes a slight extension of life span under FF conditions, a phenotype that was described earlier and depends on DAF-16 activity (30). In ADR conditions, life span of *unc-25* mutants is extended to a similar degree as in the wild-type worms, indicating that GABA synthesis is not required for ADR longevity (Figure 4). GABA independence was also predicted from Figure 3A.

Neuronal Knockdown of *cbp-1* Causes Defects in Bacterial Food Sensing

During the life-span experiments, we noticed that worms, treated with *cbp-1* RNAi, were unable to localize the central bacterial food spot on the Petri dish. This led us to investigate this intriguing phenotype into more detail, as it may link ADR-mediated longevity to neuronal food sensing, orchestrated by CBP-1. In order to quantify this phenomenon, we set up chemotaxis assays using bacterial food as an attractant. Confirming our initial observations, we found that *cbp-1* RNAi caused defects in food sensing, as worms placed in four quadrants surrounding a central bacterial food spot showed a lower CI, compared with controls (Figure 5A and D). This defect was even stronger in the neuron-specific RNAi strain (Figure 5D). As locomotion is a critical factor in behavioral tests, we validated these results by two alternative experimental setups which are controlled for locomotion defects (Figure 5B and C). The results mirrored the first behavioral test, indicating that neuronal CBP-1 is required for correct food sensing (Figure 5E, F). Moreover, a thrashing assay showed no difference in body bending frequency between worms in which *cbp-1* was knocked down in all neurons and empty-vector controls (Supplementary Figure S4). This is consistent with unaltered activity observed earlier in young *cbp-1* (RNAi) worms (10).

We next tested whether this *cbp-1*-dependent food sensing defect originates from a specific neuron family using neuron-type specific RNAi. In accordance to our life-span assays, knockdown of *cbp-1* in GABAergic neurons resulted in a significantly reduced CI (Figure 5G; $p < .001$). Similar results were obtained in the other chemotaxis assays (Figure 5H and I; $p < .001$). Knockdown of *cbp-1* in glutamatergic neurons led to a less robust yet statistically significant defect in food sensing in all chemotaxis assays (Figure 5G–I; $p < .001$). CBP-1 in dopaminergic neurons may not be required for food sensing, since no obvious decrease in CI was observed upon

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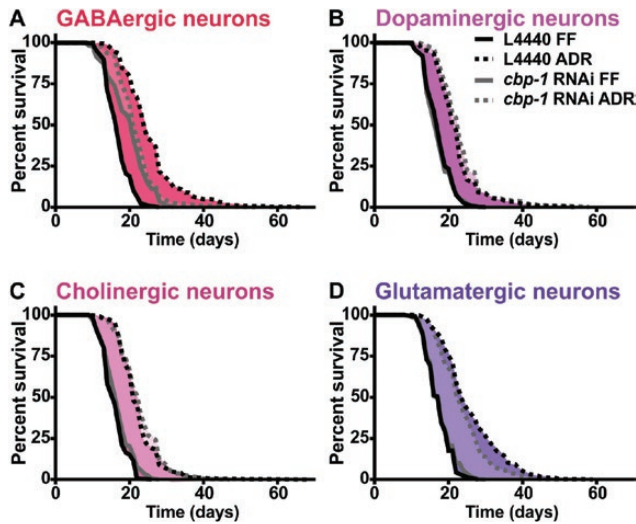

Figure 3. CBP-1 in GABAergic neurons is required for ADR longevity. (**A–D**) Effect of *cbp-1* knockdown on survival under FF and ADR conditions. Knockdown in GABAergic neurons (**A**), dopaminergic neurons (**B**), cholinergic neurons (**C**), and glutamatergic neurons (**D**). Life-span extension is indicated as a shaded color area. All data are summarized in Table 1 and Supplementary Table S2.

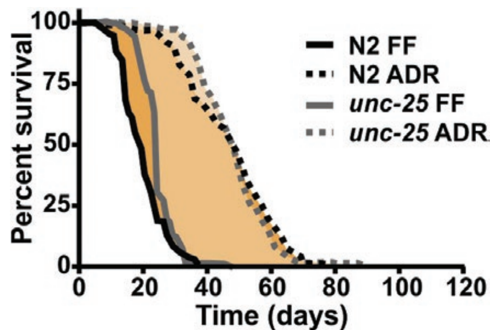

Figure 4. The neurotransmitter GABA is not required for ADR longevity. The effect of *unc-25*(*e156*) mutation on worm survival under FF and ADR conditions. Life-span extension is indicated as a shaded color area. All data are summarized in Table 1 and Table S2.

knockdown of *cbp-1* in these neurons. Results in cholinergic neurons hint at a minor food sensing role of CBP-1, but some variability was detected among the different chemotaxis assays (Figure 5G–I).

Finally, systemic *cbp-1* knockdown results in a dye filling defect in sensory neurons (10), which may be a direct cause of the food sensing defect detected here. However, this is not the case as *cbp-1* knockdown specifically in the GABAergic neurons does not result in such dye filling defect (Figure S5) while food sensing is still heavily compromised (Figure 5G–I).

In brief, CBP-1 activity in GABAergic neurons is required for sensing bacterial food cues as well as for extending life span under ADR conditions.

Discussion

The p300/CBP homolog CBP-1 is one of the few identified factors indispensable for ADR-induced longevity (8,10). This transcriptional cofactor with histone acetyltransferase activity has multiple functions, one of which is non-neural differentiation in the embryo (11–13) and hence mutants are sterile. Using tissue-specific RNAi, we show that lack of CBP-1 in the germline leads to the embryonic arrest phenotype. Hence, CBP-1 expression in other tissues serves other functions not visible in *cbp-1* mutants due to the arrested phenotype. This problem can be overcome by using postdevelopmental RNAi knockdown of *cbp-1* which results in life span shortening by approximately 30% to 40% (8,10). Using postdevelopmental tissue-specific RNAi, we found that this life span decrease is predominantly caused by *cbp-1* knockdown in the neurons, intestine, and germline. These tissues have been linked to life-span regulation through the Ins/IGF1-like signaling pathway (35–37), and *cbp-1* is known to interact with this pathway (10). It is possible that interactions of CBP-1 with DAF-16, the downstream life-span mediator of the Ins/IGF1 pathway, help them to regulate normal worm life span under standard bacterial culturing conditions.

In this study, we discovered that CBP-1 operates in the GABAergic neurons to extend life span under ADR. CBP-1 appears to be a central life-span regulator for dietary restricted conditions as it is also involved in life-span extension by bacterial dilution and *eat-2* mutation. It is even involved in longevity pathways unrelated to most DR regimens such as the Ins/IGF1-like signaling pathway as it is required for the life-span phenotype of *daf-2* mutants (10). However, in all these cases, it is yet unknown whether CBP-1 also acts in the GABAergic neurons in a similar manner. This could be the case and should be tested. Nevertheless, the physiological changes that occur in the tissues responsive to the longevity cues of the GABA neurons are probably different from those of ADR animals. For example, longevity of the *daf-2* mutant is completely dependent on DAF-16 (38) and SKN-1 (39) activation, whereas ADR longevity is independent of these two transcription factors (8,9). Similarly, life-span extension by bacterial dilution or mutation in *eat-2* is dependent on the PHA-4 transcription factor (40), whereas ADR-induced longevity is not (8). Therefore, it is probably that the yet uncharacterized downstream program that supports ADR longevity differs from the longevity programs described earlier.

The fact that CBP-1 acts in the GABAergic neurons to extend life span under ADR is remarkable, as most of these neurons are categorized as motor neurons. Life-span extension has been linked to neuronal activity before, but only in olfactory, gustatory, thermosensory, and mechanosensory neurons (41–45). The 26 GABAergic neurons include the D-type motor neurons (6 DD, 13 VD), innervating the dorsal and ventral body muscles, 4 RME motor neurons that innervate the head muscles, the AVL and DVB motor neurons innervating the enteric muscles, and finally the RIS interneuron with a function in sleeping behavior (46–48). GABAergic neurons are mainly responsible for locomotion, but also regulate foraging and defecation. Being the only GABAergic interneuron, RIS may be the site of CBP-1 action supporting ADR longevity. Besides GABA, this neuron also communicates via neuropeptides (47,49), which is in line with our finding that GABA synthesis is not required for ADR longevity. The RIS neuron localizes in the head region and connects with many other neurons of which only few are sensory neurons. Two of these RIS connecting sensory neurons are the URYV neurons that have been linked to bacterial sensing (50). Indeed, we found that knockdown of *cbp-1* specifically in GABAergic neurons results in a clear food sensing defect. Therefore, it is tempting to consider CBP-1 as a protein that links food sensing to life span in C. *elegans*.

Systemic *cbp-1* knockdown in the RNAi hypersensitive *rrf-3* mutant results in a DiI dye filling defect (10), suggesting that CBP-1 may be involved in sensory perception of environmental cues, which seemingly relates to the taxis to food defect that

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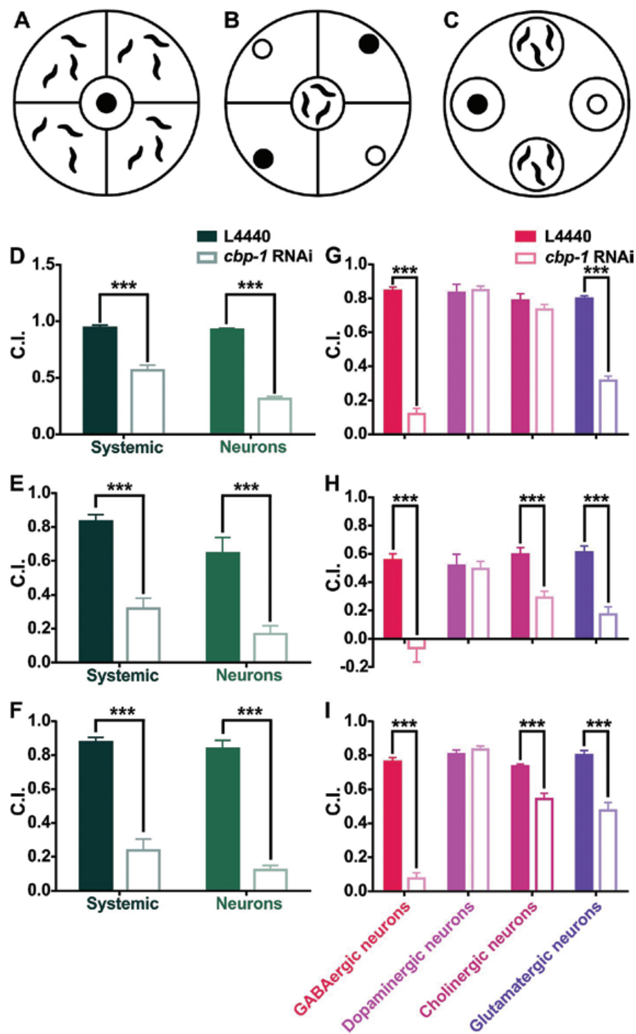

Figure 5. Food chemotaxis assays with neuron-specific RNAi knockdown of *cbp-1*. (**A–C**) Behavioral assay types: (**A**) CI equals to the fraction of worms within the inner circle to the total number of worms present on the plate. (**B**) CI is calculated as the number of worms in both control quadrants minus the number of worms in the test quadrants divided by the total number of worms that have left the central spot. (**C**) CI is the number of worms on the bacterial spot minus those on the blank spot divided by the total number of scored worms on the plate. Unlike assay A, assays B and C control for locomotion defects. The black spot represents the bacterial food. The white spot is the blank. (**D–I**) Food sensing behavior of worms submitted to *cbp-1* knockdown in (subclasses of) neurons. CI was estimated using assay A (**D** and **G**), assay B (**E** and **H**), and assay C (**F** and **I**). Error bars indicate standard error (standard error of the mean) of at least three independently grown replicates. *$p < .05$; **$p < .01$; ***$p < .001$.

we observed in our study. However, in worms in which *cbp-1* is knocked down in the GABAergic neurons only, the food sensing defect occurs while the dye-filling defect is absent. This observation excludes that the food sensing defect caused by *cbp-1* knockdown originates from a dye filling defect of the sensory neurons. It still remains unresolved whether food sensation and ADR life-span extension are causally linked by neuronal CBP-1 activity.

Conclusion

In summary, our findings show that CBP-1 activity in neurons, intestine, and germline during adulthood is required for normal health and longevity. CBP-1 acts in GABAergic neurons, probably by provoking neuropeptide release, to double worm life span under ADR conditions. This phenotype may be linked to sensory perception of the axenic culture medium.

Based on current observations, future work should focus on the nature of the putative peptidergic signal causing life-span doubling under ADR, the exact neuron(s) releasing it, the downstream target tissues of this peptide, and the possible involvement of sensory neurons that relay the environmental signal to the peptidergic neuron(s).

Supplementary Material

Supplementary data is available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

None declared.

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