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The *Caenorhabditis elegans* neuronal GPCR OCTR-1 modulates longevity responses to both warm and cold temperatures

Highlights

- OCTR-1 and its expressing neurons ASH regulate longevity responses to temperature
- Octopamine is not a ligand of OCTR-1 in longevity responses to temperature
- OCTR-1 regulates the expressions of immune response genes to influence lifespan
- The intestinal transcriptional factor ELT-2 modulates lifespan at cold temperature

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In brief

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The *Caenorhabditis elegans* neuronal GPCR OCTR-1 modulates longevity responses to both warm and cold temperatures

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SUMMARY

Many animal species live longer in cold climates than in warm climates, which was traditionally explained using the rate of living theory, i.e., higher temperatures increase chemical reaction rates, thus speeding up the aging process. However, recent studies have identified specific molecules and cells that are involved in longevity responses to temperature, indicating that such responses are not simply thermodynamic but are regulated processes. Here, we report that *Caenorhabditis elegans* lacking the neuronal G protein-coupled receptor OCTR-1 have extended lifespans at a warm temperature but shortened lifespans at a cold temperature, demonstrating that OCTR-1 modulates temperature-induced longevity responses. These responses are regulated by the OCTR-1-expressing, chemosensory ASH neurons. Furthermore, the OCTR-1 pathway controls such responses to warm and cold temperatures by regulating the expressions of immune response genes and the intestinal transcriptional factor ELT-2, respectively. Overall, our study provides cellular and molecular insights into the relationship between temperature and longevity.

INTRODUCTION

It has been known for more than a century that many animal species live longer in cold climates than in warm climates.[1] The inverse effects of temperature on longevity were first recorded in poikilotherms, including the fruit fly *Drosophila melanogaster*[2] and the nematode *Caenorhabditis elegans*,[3] and, later, were also observed in homeotherms, such as mice[4] and humans,[5] although with some obscurity (e.g., females maintain higher body temperatures than males, yet females live longer[1]). Nonetheless, it is a clear trend that lower temperatures are associated with longer lifespans and higher temperatures are associated with shorter lifespans across animal species. Most of the early studies in this area favored the idea that the inverse effects of temperature on longevity could be explained by the rate of living theory, which suggests that higher temperatures increase chemical reaction rates, thus speeding up the aging process, and lower temperatures have the opposite effect.[6] However, recent studies have identified specific molecules, cells, and signaling pathways that are involved in longevity responses to temperature, indicating that such responses are not simply thermodynamic but are regulated processes.[1,7,8] Despite this progress, the regulatory mechanisms underlying the relationship between temperature and longevity remain unclear.

C. elegans has become an excellent model system for studying the connections between longevity and temperature because of its short lifespan, invariant lineage, and genetic tractability.[7] In the laboratory, the worm is propagated under three temperatures (15°C, 20°C, and 25°C), and the inverse effects of these temperatures on lifespan are well documented, i.e., there is a 75% increase in lifespan with a 5°C drop in temperature, consistent between 15°C–20°C and 20°C–25°C.[3,9] Using *C. elegans*, researchers have revealed many molecular details about the effects of temperature on longevity. For example, Lee and Kenyon found that the thermosensory AFD neurons and AIY interneurons were required for maintaining the worm's lifespan at a warm temperature (25°C) and that such maintenance was linked to the DAF-9/DAF-12 steroid-signaling pathway.[10] The AFD neural circuit maintains longevity at warm temperatures through CRH-1, the *C. elegans* cyclic AMP-response element binding protein, and its transcriptional target FLP-6, both of which antagonize insulin signaling to affect longevity.[11] At cold temperatures, TRPA-1, a cold-sensitive, transient receptor potential (TRP) channel, detects temperature drops in the environment to extend lifespan through calcium signaling.[12] Interestingly, in contrast to cold-temperature exposure during adulthood that prolongs *C. elegans* lifespan, low-temperature treatment during development reduces lifespan. This differential effect of temperature is also mediated by TRPA-1.[13] Moreover, *C. elegans* that lack functional DAF-41, the worm homolog of p23 co-chaperone/prostaglandin E synthase-3, live longer than wild-type (WT) worms at 25°C but have shorter lifespans than WT worms at 15°C, indicating that DAF-41 modulates the longevity responses to both cold and

warm temperatures.[9] In addition, Zhang et al. identified two distinct neuroendocrine signaling circuits by which the worm nervous system senses cool and warm temperatures and then signals the gut using small neurotransmitters and insulin-like neuropeptides to extend and shorten lifespan, respectively.[14]

Studies in mammals also support the notion that the longevity response to temperature is not simply thermodynamic but is a regulated process.[1,15] Conti et al. reported that transgenic mice with lowered core body temperature (CBT) showed a significant increase in their median life expectancy.[4] This was achieved by hypothalamic overexpression of uncoupling protein 2 in hypocretin neurons, which generated heat that triggered the hypothalamic "central thermostat" to activate thermoregulatory mechanisms and permanently reduce CBT.[4] Calorie restriction (CR) was found to lower CBT in rats, mice, monkeys, and humans, and such reduction in CBT might contribute to the beneficial effects of CR on longevity and aging.[1,15,16] Some long-lived mutant mice, such as Ames and Snell dwarf mice, also have reduced CBT due to deficits in thyroid stimulating hormones, prolactin, and growth hormone.[17,18] Although these studies have linked reduction in CBT to increased longevity through specific molecules, the underlying regulatory mechanisms remain to be determined.

Previously, we have shown that *C. elegans* that lack functional OCTR-1, a GPCR for the neurotransmitter octopamine (OA), exhibit an enhanced innate immune response and improved survival upon pathogen infection.[19–21] We also identified an OCTR-1-dependent neuroimmune regulatory circuit that includes the OCTR-1-expressing chemosensory ASH neurons and OA, as well as the OA-producing RIC interneurons.[19–22] Here, we report that functional loss of OCTR-1 extends worm lifespan at 25°C but shortens it at 15°C, indicating that OCTR-1 modulates longevity responses to both warm and cold temperatures. We further found that these responses are regulated by ASH neurons. In addition, transcriptomic analysis and functional assays revealed that the OCTR-1 pathway controls the longevity responses to warm and cold temperatures by regulating the expressions of immune response genes and the intestinal transcription factor ELT-2, respectively. These results provide both cellular and molecular insights into the relationship between temperature and longevity.

RESULTS

OCTR-1 modulates longevity responses to both warm and cold temperatures

In previous studies, we have shown that, compared to WT *C. elegans*, worms lacking functional OCTR-1 (*octr-1(ok371)* worms) have an enhanced innate immune response and improved survival upon pathogen infection.[19–22] These phenotypes were also observed in other mutant worms deficient in the OCTR-1 neuroimmune regulatory circuit, including the *tbh-1(n3247)*, *ASH(−)[JN1713]*, and *RIC::TeTx* strains, indicating that this circuit suppresses the immune response in *C. elegans*.[19–22] These experiments were conducted at the propagation temperature of 20°C. Interestingly, at this temperature, *octr-1(ok371)* worms exhibited WT lifespans under nonpathogenic conditions.[19,22] As *C. elegans* are also propagated at 15°C and 25°C in the laboratory, we explored whether OCTR-1, or lack thereof, has any effects on lifespan at these temperatures. To this end, we grew *octr-1(ok371)* and WT worms at 15°C, 20°C, or 25°C for at least two generations and then started lifespan assays at day 1 of adulthood under the respective temperature. Consistent with our previous observation,[19,22] the two strains had similar lifespans at 20°C (Figure 1A). Unexpectedly, *octr-1(ok371)* worms lived longer than WT worms at 25°C but had shorter-than-WT lifespans at 15°C (Figures 1B and 1C). Because temperature normally has an inverse effect on longevity (i.e., lower temperatures are associated with longer lifespans and higher temperatures are associated with shorter lifespans[1,3]), these findings demonstrate that functional OCTR-1 is required for such temperature-dependent effects. In other words, OCTR-1 modulates longevity responses to both warm and cold temperatures.

Zhang et al. reported that low-temperature treatment during development reduces lifespan in *C. elegans*.[13] To examine how temperature impacts worm lifespan post-development, we grew worms to the young-adult stage at 20°C and then transferred them to 15°C, 20°C, or 25°C for lifespan assays starting at day 1 of adulthood. Consistent with the previously described lifespan results, *octr-1(ok371)* worms lived longer than WT animals at 25°C but had shorter-than-WT lifespans at 15°C, whereas *octr-1(ok371)* and WT worms lived comparable lifespans at 20°C (Figure S1). Overall, these results illustrate that loss of functional OCTR-1 attenuates the temperature effects on longevity regardless of experimental schemes. To avoid possible complications by temperature effects on development,[13] we focused on the temperature effects on post-development lifespan in the rest of our study.

To confirm the critical role of OCTR-1 in the longevity responses to temperature, we generated a rescue strain JRS70 that expresses OCTR-1 under its own promoter in *octr-1(ok371)* worms. Lifespan assays on JRS70 and WT worms at 15°C, 20°C, or 25°C showed that JRS70 exhibited WT lifespans at these temperatures (except that JRS70 lived slightly shorter than WT at 15°C) (Figure S2), demonstrating that restoration of OCTR-1 expression rescued the lifespan phenotypes of *octr-1(ok371)* worms in the temperature-induced longevity responses. We also generated an overexpression strain JRS66 that over-expresses OCTR-1 under its own promoter in a WT background. Lifespan assays on these worms showed that JRS66 displayed shorter lifespans than WT worms at either 15°C or 25°C, while exhibiting longer-than-WT lifespans at 20°C, possibly due to variations in the levels of overexpression and/or toxicity of too much OCTR-1 (Figure S3). Nonetheless, our results indicate that OCTR-1 modulates the longevity response to both warm and cool temperatures.

Previously, we have found that OA acts as a ligand for OCTR-1 in the innate immune response to pathogen infection.[22] To determine if this is also the case in temperature-induced longevity responses, we examined the lifespans of WT, *octr-1(ok371)*, *tbh-1(n3247)* (worms that lack tyramine-β-hydroxylase, the rate-limiting enzyme for OA synthesis), and *octr-1;tbh-1* worms at 15°C, 20°C, and 25°C. Surprisingly, at these temperatures, *tbh-1(n3247)* worms lived slightly shorter than WT worms, while *tbh-1;octr-1* worms displayed similar lifespans as *octr-1(ok371)*

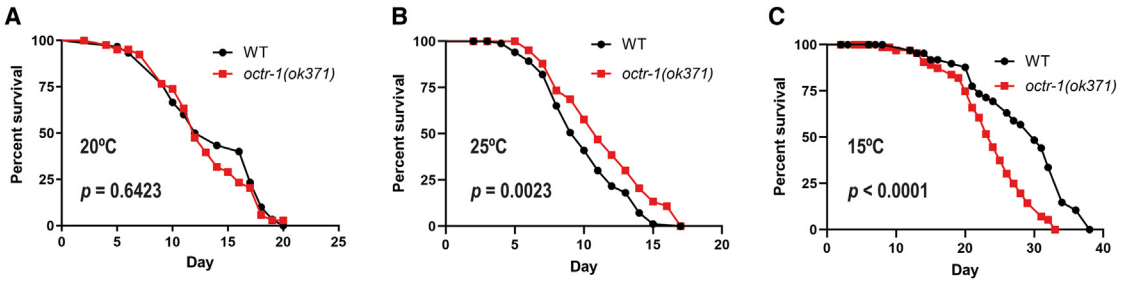

Figure 1. *octr-1(ok371)* animals had the same lifespan as wild-type *N2* animals at 20°C but lived longer at 25°C and shorter at 15°C than WT

octr-1(ok371) and WT animals were grown at 20°C (A), 25°C (B), or 15°C (C) for at least two generations and then scored for survival over time starting from day 1 of adulthood under the respective temperature. The graph at each temperature is a representative result of three independent experiments. Each experiment included $n = 90$ adult animals per strain. p value represents the significance level of the mutants relative to the WT animals: $p = 0.6423$ in (A), $p < 0.0023$ in (B), and $p < 0.0001$ in (C).

worms (Figure S4). The distinct lifespan phenotypes of *tbh-1(n3247)* and *octr-1(ok371)* worms at these temperatures suggest that OA might not be the ligand of OCTR-1 in the temperature-induced longevity responses and that OCTR-1 may use a different ligand(s) in such responses. These results also suggest that ligand-receptor interactions are context dependent, which is consistent with the fact that many GPCRs have multiple ligands and vice versa.[23,24]

To further test whether OA is a ligand of OCTR-1 in the longevity responses to temperature, we performed OA-supplemented lifespan assays at the three temperatures on *octr-1(ok371)* and WT worms. While supplementation of 10 mM OA did not affect the lifespans of WT worms (except at 15°C, where OA slightly reduced their lifespans), exogenous OA shortened the lifespans of *octr-1(ok371)* worms at all three temperatures (Figure S5), indicating that OA negatively affects lifespan in general through a pathway independent of OCTR-1. Taken together, we made a surprising discovery that OA is not a ligand of OCTR-1 in the longevity responses to temperature and that OCTR-1 may use an unknown ligand(s) for such responses.

OCTR-1-dependent longevity responses to temperature are neurally regulated

We have previously demonstrated that, in *C. elegans*, OCTR-1 functions in the chemosensory ASH neurons to suppress innate immune responses by inhibiting the expression of immune response genes.[19] To determine whether ASH neurons are involved in the OCTR-1-dependent longevity responses to temperature, we examined the lifespans of WT worms, ASH-ablated worms (*ASH(−)* worms)], and *ASH(−);octr-1(ok371)* double mutants at both 25°C and 15°C. At 25°C, both the *ASH(−)* worms and the *ASH(−);octr-1(ok371)* double mutants lived significantly longer than WT animals (Figure 2A), suggesting that the ASH chemosensory neurons are required for the lifespan-shortening effects of warm temperatures. In contrast, at 15°C, the lifespans of both mutant worms were significantly shorter than those of WT animals (Figure 2B), indicating that ASH neurons also play an important role in the lifespan-extension effects of cold temperatures. Taken together, these results demonstrate that the OCTR-1-dependent longevity responses to temperature are neurally regulated.

ASH neurons are a pair of polymodal nociceptive neurons that can mediate avoidance behaviors in response to a diverse array of stimuli, such as chemical repellents, high osmotic solutions, and nose touch.[25] Since ASH neurons are involved in temperature-induced longevity responses, we asked whether ASH neurons also mediate neurobehavior in a temperature-dependent manner. To this end, we performed 1-octanol choice assays at three different temperatures (15°C, 20°C, and 25°C) to determine whether ASH-mediated neurobehavior is temperature sensitive. ASH neurons can sense dilute octanol (30% in ethanol) and initiate aversive behavior to avoid the chemical.[26–28] In our 1-octanol choice essays, worms were placed in the center of an agar plate, and worm food *E. coli* OP50 was placed on the two opposite sides of the plate with 30% 1-octanol and 100% ethanol (control) surrounding the food on each side. After allowing the animals to crawl around the plate for 1 h, the number of animals on each side was counted. As shown in Figure S6, at 20°C, WT worms avoided 1-octanol and crawled to the ethanol side, while a substantial amount of *ASH(−)* worms crawled to the 1-octanol side due to the lack of ASH-mediated avoidance behavior. By contrast, this behavior difference between WT and *ASH(−)* worms was drastically increased at 15°C but largely eliminated at 25°C. These results demonstrate that ASH-mediated aversive behavior is indeed temperature dependent, suggesting that ASH neurons likely sense ambient temperature and regulate neurobehavior in a temperature-dependent manner. This notion is consistent with a recent study showing that ASH neurons sensed rapid cooling and initiated avoidance behavior.[29] The temperature-sensing function of ASH neurons is consistent with their important role in the temperature-induced longevity responses.

Living at a cold temperature lowers the basal innate immune response during old age

To gain molecular insights into the OCTR-1-dependent effects of temperature on longevity, we employed RNA sequencing (RNA-seq) to compare the gene expression profiles of both young and old WT and *octr-1(ok371)* worms under the three growth temperatures. For this study, "young" was defined as the young-adult stage, and "old" was defined as the time at which WT worms reached an approximately 50% survival rate in lifespan assays,

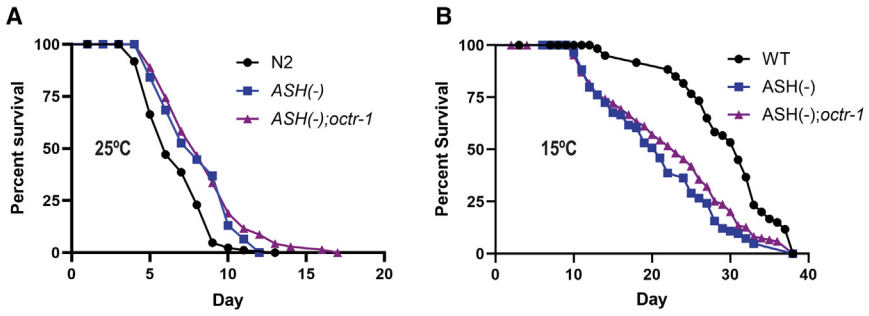

Figure 2. The ASH neurons are required for OCTR-1-dependent longevity responses to both warm and cold temperatures

WT, *ASH(–)*, and *ASH(–);octr-1(ok371)* mutant animals were grown on *E. coli* OP50 at 25°C (A) or 15°C (B) and scored for survival over time. For each temperature, the graph is a representative result of three independent experiments. Each experiment included n = 90 adult animals per strain. p value represents the significance level of the mutants relative to the WT animals: in (A), *ASH(–)*, p = 0.0008; *ASH(–);octr-1(ok371)*, $p < 0.0001$; in (B), *ASH(–)*, $p < 0.0001$; *ASH(–);octr-1(ok371)*, $p < 0.0001$.

which corresponded to days 24, 14, and 9 of adulthood under the cultivation temperatures of 15°C, 20°C, and 25°C, respectively (Figures 1 and S1). Five replicates of worms under each temperature condition were collected for the RNA-seq analysis. Detailed sampling information is listed in Table 1.

To examine how living at a cold temperature affects gene expression, we compared the expression profiles of old WT worms grown at 15°C with those grown at 20°C (sample group 3 vs. 2 in Table 1). Our RNA-seq results showed that 1,010 genes were upregulated and 1,182 genes were downregulated at least 2-fold in worms grown at 15°C relative to those grown at 20°C. Gene ontology (GO) analysis of the upregulated genes identified enriched GO terms involving cell projection and cilium organization, the biological significance of which is not clear (Figure 3A). GO analysis of the downregulated genes revealed mainly two classes of GO terms involving immune responses and metabolic processes, with immune responses being the most significantly enriched (Figure 3A). Enrichment of immune responses resulted from the downregulation of 95 immune genes encoding markers of immunity, such as CUB-like domain-containing proteins, ShK toxin domain-containing proteins, C-type lectins, lysozymes, glutathione S-transferases, and saposin-like proteins (Table S1). Taken together, these data suggest that living at a cold temperature lowers the basal innate immune response in *C. elegans*. Interestingly, independent studies have shown that knocking down some of these immune genes, such as *dod-24*, *dod-22*, *clec-186*, *K04A8.1* (*dre-1)*, *dod-17*, and *dod-19*, using RNAi extends worm lifespan at 20°C.[30–32] Furthermore, we found that knocking down *C32H11.4*, a top downregulated immune gene encoding a CUB-like protein, in WT worms with RNAi also extends worm lifespan at 20°C (Figure S7A). Interestingly, RNAi of *C32H11.4* had no impact on worm lifespan at 15°C (Figure S7B), possibly because the effect of *C32H11.4* on longevity is temperature dependent or because *C32H11.4* expression was already low at 15°C and further knockdown had no more lifespan-extending effect. We additionally inactivated *clec-61*, another immune response gene downregulated in old WT worms grown at 15°C relative to 20°C, by RNAi followed by lifespan assays at 20°C. Our results showed that knockdown of *clec-61* had no impact on worm lifespan (Figure S8), indicating that CLEC-61 may not contribute to the lifespan extension induced by a low basal immune response or may function redundantly with some other immune response genes in lifespan regulation. Overall, these results indicate that a low basal immune response promotes longevity, although some immune response genes may not contribute to this effect or function redundantly with other genes in this respect.

Based on the previously described analysis, we determined that a decreased basal immune response was linked to living at a lower temperature (15°C). Therefore, we next asked whether comparing the RNA-seq data from old worms grown at 20°C and those grown at 25°C (sample group 2 vs. 4 in Table 1) as well as comparing the data from worms grown at 15°C with those grown at 25°C (sample group 3 vs. 4 in Table 1) would provide similar results. GO analysis of the differentially expressed genes from these two comparisons revealed that, indeed, being grown at a lower temperature was linked to the enrichment of genes related to the immune response (Figures 3B and 3C). Taken together, our data demonstrate that lower temperatures are associated with decreased basal innate immune responses during old age, which likely contribute to the extended lifespans observed in worms living under such conditions.

OCTR-1 mediates warm temperature-induced lifespan shortening by regulating immune response genes

Like many other species, *C. elegans* have shortened lifespans at warm temperatures.[3] In our study, we observed that worms lacking functional OCTR-1 had increased lifespans at 25°C

Table 1. Grouping of RNA-seq samples

Sample group	Strain	Propagation temperature	Age[a]
1	*N2*	15°C	old
2	*N2*	20°C	young
3	*N2*	20°C	old
4	*N2*	25°C	old
5	*octr-1(ok371)*	15°C	old
6	*octr-1(ok371)*	20°C	young
7	*octr-1(ok371)*	20°C	old
8	*octr-1(ok371)*	25°C	old

[a]In this study, young age was defined as the young-adult stage, and old age was defined as the time when WT worms reached approximately 50% survival rate in lifespan assays, which corresponds to days 24, 14, and 9 of adulthood under the cultivation temperatures 15°C, 20°C, and 25°C, respectively. *octr-1(ok371)* worms were collected at the same biological ages as WT worms at these temperatures.

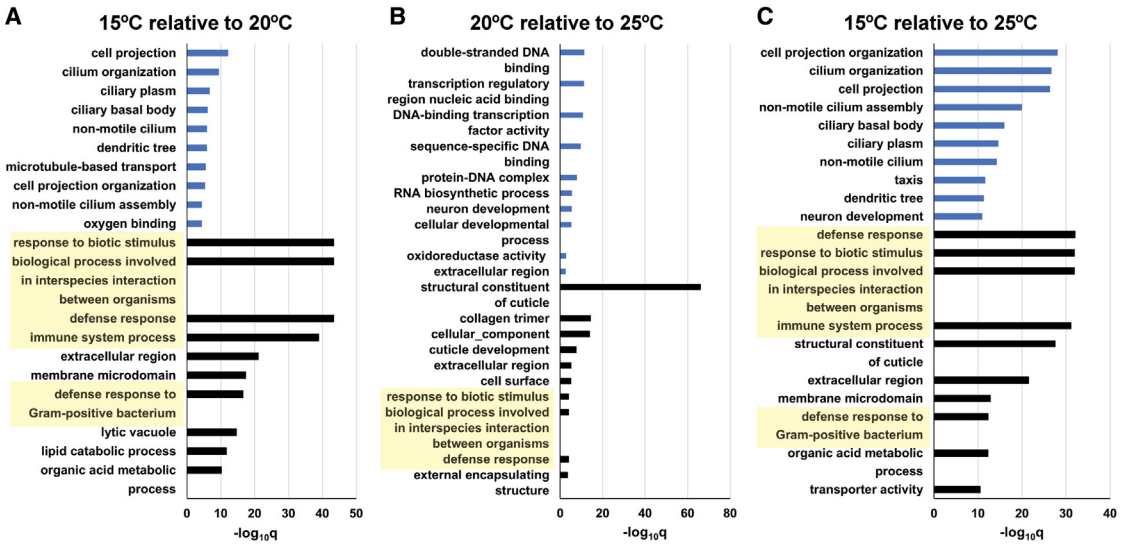

Figure 3. GO analyses revealed that lower temperatures are associated with a decreased basal innate immune response during old age

GO analyses were performed with differentially expressed genes in old WT animals grown at 15°C relative to those grown at 20°C (A), 20°C relative to 25°C (B), or 15°C relative to 25°C (C). Blue bars represent top 10 most significantly enriched GO terms derived from upregulated genes. Black bars represent top 10 most significantly enriched GO terms derived from downregulated genes. GO terms involving immune responses are highlighted in yellow.

(Figures 1B and S1B), indicating that OCTR-1 is required for the warm temperature-induced lifespan shortening. To investigate the molecular basis underlying this phenomenon, we compared the gene expression profiles of old *octr-1(ok371)* worms relative to old WT control worms grown at 25°C (sample group 8 vs. 4 in Table 1) and found that 2,567 genes were upregulated while 1,168 genes were downregulated at least 2-fold. GO analysis of the upregulated genes identified four classes of GO terms, including protein (de)phosphorylation, ribonucleotide binding, cilium assembly, and sensory perception, indicating that there were alterations in signal transduction in the mutant worms (Figure 4A). A similar analysis of the downregulated genes revealed that structural constituent of cuticle and immune responses were the two most significantly enriched GO term classes, due to the downregulation of 110 collagen genes (Table S2) and 91 immune response genes (Table S3), respectively. Cuticular collagens have been implicated in altering *C. elegans* lifespan under stress, such as warm temperatures, pathogen infection, and oxidative stress.[33–35] Previously, we analyzed the functions of collagen genes in the longevity response to warm temperatures in detail.[33] So, here, we have focused on the role of immune genes in this response.

The previously described results suggest that old *octr-1(ok371)* worms have a lower basal immune response than old WT worms at 25°C, which may contribute to the extended lifespans of mutant worms at this warm temperature. To find out if this is the case, we used RNAi to individually inactivate two of the top downregulated immune genes (*lys-5* and *lys-4*, whose downregulations detected in the RNA-seq analysis were confirmed by RT-qPCR [Figure S9]) in WT worms and then assayed their lifespans at 25°C. Our results showed that knocking down either of these genes significantly extended the lifespans of WT worms at 25°C (Figures 4B and 4C), recapitulating the lifespan phenotype of *octr-1(ok371)* worms at this temperature. By contrast, similar RNAi of these genes individually followed by lifespan assays at 20°C did not change worm lifespan (Figure S10), suggesting that the effects of these immune genes on lifespan are temperature dependent. This is consistent with our RNA-seq results showing that distinct subsets of immune genes were differentially regulated at different temperatures (GEO: GSE252054). We also inactivated *col-179*, a top downregulated gene that was traditionally classified as a collagen gene but was found to be involved in immune responses,[34] by RNAi followed by lifespan assays, and our results showed that knocking down this gene significantly extended the lifespans of WT worms at 25°C (Figure 4D). Overall, these results indicate that OCTR-1-controlled immune genes play a critical role in lifespan regulation at warm temperatures and that decreased expression of these genes in *octr-1(ok371)* worms reflects a low basal immune response that likely leads to a longer lifespan. This notion is consistent with the previously described observation that a low basal immune response in worms living at a cold temperature extends lifespan. Taken together, our data suggest that OCTR-1 mediates the longevity response to warm temperatures by regulating immune genes.

The transcription factor ELT-2 is regulated by OCTR-1 and contributes to cold temperature-induced lifespan extension

As described earlier, functional loss of OCTR-1 shortens the lifespan of *C. elegans* under a cold temperature (Figures 1C and S1C), attenuating the lifespan-extension effect of cold temperature on worms. To investigate the molecular basis underlying this phenomenon, we compared the RNA-seq datasets acquired from old *octr-1(ok371)* and WT worms grown at 15°C (sample group 7 vs. 3 in Table 1). This analysis revealed that the *octr-1*

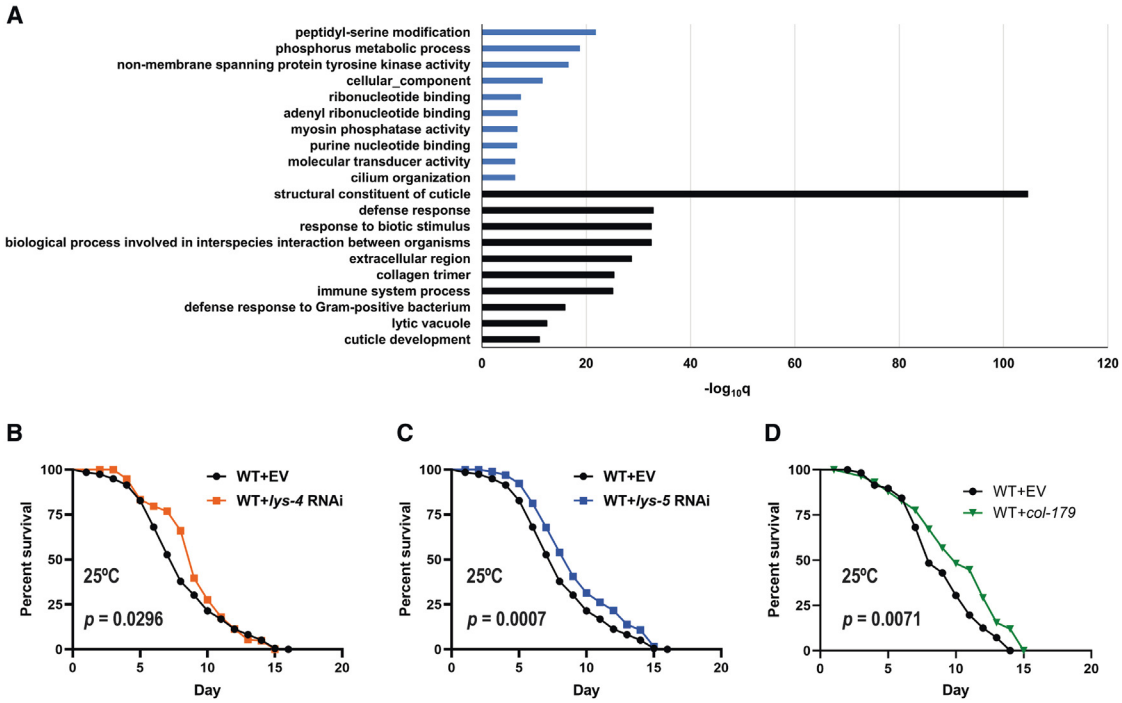

Figure 4. OCTR-1 mediates the longevity response to warm temperature by regulating immune genes

(A) GO analyses were performed with differentially expressed genes in *octr-1(ok371)* animals grown at 25°C relative to WT controls in old age. Blue bars represent top 10 significantly enriched GO terms derived from upregulated genes. Black bars represent top 10 significantly enriched GO terms derived from downregulated genes.

(B–D) Inactivation of individual immune response genes by RNAi extended worm lifespan at 25°C. WT animals were grown on double-stranded RNA (dsRNA) for empty vector (EV) or for *lys-4* (B), *lys-5* (C), or *col-179* (D) at 25°C and scored for survival over time. For each gene, the graph is a representative result of three independent experiments. Each experiment included $n = 90$ adult animals per strain. p value represents the significance level of RNAi of individual genes relative to the EV RNAi control: $p = 0.0296$ in (B), $p = 0.0007$ in (C), and $p = 0.0071$ in (D).

mutant worms exhibited upregulated expression of signaling transduction genes but downregulated expression of collagen and immune response genes (Figure S11A). Interestingly, these effects were similar to what was observed in *octr-1* mutants under warm conditions (Figure 4A); however, it is unclear why such regulation of gene expression at both cold and warm temperatures had opposite effects on lifespan. During our data analysis, we noticed that the intestine is the most significantly impacted tissue with half of the downregulated genes expressed in the intestine (Figure S11B). Because the intestine has an established role in the longevity response to cold temperatures,[12,14] we speculated that one or more transcription factors that control the expression of intestinal genes may be involved in the regulation of the OCTR-1-mediated longevity response to cold temperatures. Indeed, we found that ELT-2, a major transcription factor that controls the expression of genes involved in differentiation, maintenance, and function in the *C. elegans* intestine,[36–38] was downregulated more than 2-fold in old *octr-1(ok371)* worms grown at 15°C relative to WT controls. Accordingly, knocking down the expression of *elt-2* by RNAi significantly shortened WT worm lifespan at 15°C (Figure 5A), indicating that ELT-2 is required for the lifespan-extension effect of cold temperatures on worms. To test whether such an effect is specific to 15°C, we performed the same RNAi knockdown and lifespan assays in WT worms at 20°C, and our results showed that RNAi of *elt-2* also shortened worm lifespan at 20°C (Figure 5B). These data suggest that ELT-2 is a general regulator of worm lifespan independent of growth temperatures. Taken together, our data suggest that functional loss of OCTR-1 downregulates the intestinal transcriptional factor ELT-2 at 15°C, which, in turn, shortens worm lifespan.

ELT-2 is a well-studied transcription factor, and its regulatory targets have been well established.[36,37,39,40] Previous studies have revealed that ELT-2 binds specifically to the TGATAA sequence that is over-represented in the promoter regions of many intestinal genes.[36,37] Our *in silico* analysis showed that 48% of differentially expressed genes in *octr-1(ok371)* worms relative to WT worms grown at 15°C contain the ELT-2 binding site in their promoter regions, indicating that these genes could be regulated by ELT-2 (Table S4). Because half of the downregulated genes in our *octr-1(ok371)*/WT 15°C dataset were expressed in the intestine and ELT-2 is an intestinal transcription factor, we compared these downregulated genes with those downregulated in an *elt-2(−)*/WT RNA-seq dataset[39] and found a significant overlap of genes between these two datasets (p value < 2.72E−11) (Figure S12). Taken together, these analyses support our conclusion that ELT-2 plays an important role in the OCTR-1-dependent longevity response to cold temperature by regulating gene expression in the intestine.

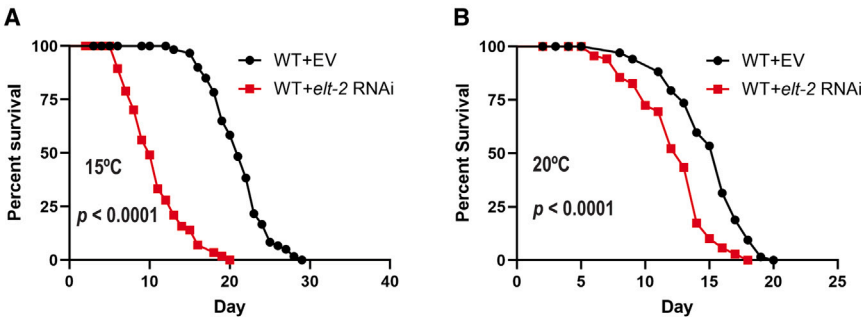

Figure 5. ELT-2 is a general regulator of worm lifespan independent of growth temperature

WT animals were grown on dsRNA for empty vector (EV) or for *elt-2* at 15°C (A) or 20°C (B) and scored for survival over time. The graph for each temperature is a representative result of three independent experiments. Each experiment included $n = 90$ adult animals per strain. p value represents the significance level of *elt-2* RNAi relative to the EV RNAi control: $p < 0.0001$ in (A) and $p < 0.0001$ in (B).

DISCUSSION

Although the inverse relationship between temperature and longevity was recorded more than a century ago, the mechanisms controlling this relationship remain unclear. In the current study, we discovered that, in *C. elegans*, the neuronal GPCR OCTR-1 modulates the longevity responses to both warm and cold temperatures. Specifically, we found that worms lacking functional OCTR-1 exhibited WT lifespans at the propagation temperature of 20°C but lived longer than WT worms at 25°C (warm temperature) and had shorter-than-WT lifespans at 15°C (cold temperature) (Figures 1 and S1). These results demonstrate that OCTR-1 is required for the effects of temperature on longevity. Furthermore, we revealed that the OCTR-1-expressing chemosensory ASH neurons are also involved in the longevity responses to temperature, indicating that such responses are regulated by the nervous system. Our RNA-seq analysis and functional assays showed that the OCTR-1 pathway controls the longevity responses to warm and cold temperatures by regulating the expressions of immune genes and the transcription factor ELT-2, respectively. Because these immune genes are mainly expressed in pharyngeal and intestinal cells[19] and ELT-2 is also an intestinal transcription factor,[36–38] we speculate that temperature signals are sensed by the nervous system and then relayed to non-neural tissues, such as the intestine, to exert effects on lifespan. Indeed, Zhang et al. reported two distinct neuroendocrine signaling circuits by which the worm nervous system senses cool and warm temperatures and then signals to the intestine to impact lifespan.[14] However, how the signaling pathway involving OCTR-1 and ASH neurons senses and transduces temperature signals within the nervous system as well as between the nervous system and non-neural tissues is not clear and remains to be explored.

Evidence from mammalian studies also indicates that the longevity response to temperature is mostly regulated through neuroendocrine pathways.[1,41] In homeotherms, the preoptic area (POA) of the hypothalamus maintains a nearly constant CBT by regulating the autonomic and hormonal control of heat production and dissipation, and temperature-sensitive neurons in the POA play key roles in these regulations.[41] By contrast, poikilotherms lack the ability to regulate temperature homeostasis, and their internal body temperature fluctuates with that of the environment. Because of this difference, it was believed that temperature changes may influence aging primarily by thermodynamic effects in poikilotherms while in homeotherms such influence is controlled by the hypothalamus through neuroendocrine pathways. Our current and previous studies as well as those of others argue against this view by demonstrating that the longevity responses to temperature in poikilotherms are also neurally regulated, indicating that the underlying neuroendocrine mechanisms are likely evolutionarily conserved.[10–12,14,33] Due to the high-degree complexity of the mammalian nervous system, it is difficult to dissect the neuroendocrine regulation of lifespan to temperature in mammals. Studies in poikilotherms, especially in *C. elegans* with its simple nervous system, have greatly improved our understanding of such neuroendocrine mechanisms.

An intriguing finding from our RNA-seq analysis is that living at a cold temperature lowered the expression of many immune response genes (Figure 3A and Table S1). Both our current study (Figure S7A) and other independent studies[30–32] have shown that inactivating some of these immune genes extends worm lifespan, indicating that a low basal immune response promotes longevity, which may contribute to the lifespan-extending effect of cold temperatures. Our study suggests that lowering the basal immune response with exposure to cold temperatures could be an effective, non-invasive intervention to restore immunological homeostasis and promote healthy aging. By the same token, neuroimmune regulatory pathways that control the expression of immune genes could also be manipulated to improve health in old individuals. Indeed, at the warm temperature (25°C), we found that old *octr-1(ok371)* worms had a lower basal immune response than old WT worms (Figure 4), which contributed to their extended lifespan. In addition to immune genes, OCTR-1 also regulates collagen genes to impact fitness and longevity. Previously, we showed that elevated collagen expression increases worm lifespan following challenge with warm temperature[33] or pathogen infection.[34] An independent study by Ewald et al. also showed that increased collagen expression improved worm resistance to oxidative stress.[35] Overall, these studies indicate a general role for collagens in stress resistance. Interestingly, collagens have also been implicated in longevity extension under non-stressed conditions,[35,42–44] suggesting that they may be involved in various pathways that improve overall organism fitness. We suspect that OCTR-1 may fine-tune the longevity responses to temperature by regulating both immune and collagen genes.

Limitations of the study

In this study, we identified the neuronal GPCR OCTR-1 and its expressing neurons ASH as regulators of longevity responses

to both warm and cold temperatures. We ruled out OA as a ligand of OCTR-1 in these regulatory processes. However, beyond OCTR-1 and ASH, other components of this pathway remain unknown and require further identification. Our findings demonstrate that the OCTR-1 pathway modulates longevity responses to warm and cold temperatures by regulating the expressions of immune response genes and the intestinal transcriptional factor ELT-2, respectively. However, the precise mechanisms by which immune response genes and ELT-2 function downstream of OCTR-1 to influence lifespan remain unclear. Addressing these critical questions was beyond the scope of this study and warrants further investigation.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Yiyong Liu (yiyong.liu@wus.edu).

Materials availability

The *C. elegans* strains and recombinant DNA generated in this study will be shared upon request, but we may require payment to cover shipment and completion of a Material Transfer Agreement for possible commercial applications.

Data and code availability

- The sequencing data (FASTQ files) have been deposited in NCBI's SRA database through GEO. Processed gene quantification files and differential expression files have been deposited in GEO. All of these data can be accessed through GEO: GSE252054.
- No original code was created during the course of this study.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

S.W., P.W., C.-H.C., J.S., and Y.L. designed and performed experiments and analyzed data. J.S. and Y.L. wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2025.112279.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
Escherichia coli OP50	*Caenorhabditis elegans* Genetics Center	WB Cat#WBStrain00041969
Chemicals, peptides, and recombinant proteins		
Bacto peptone	VWR International	90000–264
Sodium Chloride	VWR International	BDH9286-12KG
Criterion Agar	VWR International	C7432
Calcium dichloride	Millipore Sigma	C4901
Magnesium Sulfate	Millipore Sigma	M7506
Potassium chloride	Millipore Sigma	P3911
Magnesium chloride	Millipore Sigma	M8266
Potassium phosphate dibasic	Millipore Sigma	P3786
Potassium phosphate monobasic	Millipore Sigma	P0662
LB Broth	Millipore Sigma	L3022
BHI Broth	BD Sci	237500
Cholesterol	Millipore Sigma	C8667
100% absolute molecular grade ethanol	Fisher Scientific	BP2818
Ampicillin sodium salt	Sigma-Aldrich	A9518
Isopropyl β-D-1-thiogalactopyranoside	Sigma-Aldrich	I6758
Tris Base	Fisher Sci	BP152-5
Hydrochloric acid 37%	Millipore Sigma	258148-500ML-GL
Critical commercial assays		
QIAzol Lysis Reagent	Qiagen	79306
RNeasy Plus Universal Mini Kits	Qiagen	73404
PowerUp™ SYBR™ Green Master Mix	ThermoFisher Sci	A22918
High-Capacity cDNA Reverse Transcription Kit	ThermoFisher Sci	4368814
Deposited data		
RNA Sequencing Data	This Paper	Gene Expression Omnibus (GEO) : GSE252054
Experimental models: Organisms/strains		
C. elegans strain: Wild-type N2 (Bristol)	*Caenorhabditis elegans* Genetics Center	WB Cat#WBStrain00000001; RRID:WB-STRAIN:WBStrain00000001
C. elegans strain: *octr-1(ok371)*	*Caenorhabditis elegans* Genetics Center	WB Cat# WBStrain00035596; RRID:WB-STRAIN: WBStrain00035596
C. elegans strain: JN1713 (*pels1713*) [*sra-6p::mCasp-1 + unc-122p::mCherry*]	*Caenorhabditis elegans* Genetics Center	WB Cat# WBStrain00022700; RRID:WB-STRAIN: WBStrain00022700
C. elegans strain: *tbh-1(n3247)*	*Caenorhabditis elegans* Genetics Center	WB Cat# WBStrain00027363; RRID:WB-STRAIN: WBStrain00027363
C. elegans strain: *octr-1(ok371); tbh-1(n3247)*	Sellegounder, 2018	N/A
C. elegans strain: JRS4 [*octr-1(ok371); tbh-1(n3247) + octr-1p::octr-1c::SL2::GFP*]	Sellegounder, 2018	NA
C. elegans strain: JRS66 WT+[*octr-1p::octr-1c::SL2::GFP*]	This Paper	N/A
C. elegans strain: JRS70 *octr-1(ok371)*+[*octr-1p::octr-1c::SL2::GFP*]	This Paper	N/A

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
See Table S6	This Paper	N/A
Software and algorithms		
Graphpad Prism 10	GraphPad Software	https://www.graphpad.com/
SoftMax Pro 6.3	Carl Zeiss Microscopy, LLC	https://www.moleculardevices.com/

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Nematode strains

The following *C. elegans* strains were maintained at 20°C, grown on modified Nematode Growth Media (NGM) (0.35% instead of 0.25% peptone) and fed *E. coli* OP50. WT animals used were *C. elegans* Bristol N2. The *octr-1(ok371)* and JN1713 (ASH neuron ablated) strains were obtained from the *Caenorhabditis elegans* Genetics Center (University of Minnesota, Minneapolis, MN). All strains were backcrossed with WT Bristol *N2* animals at least three times prior to experimental use. All genotypes were confirmed using PCR or inspection of mCherry expression in coelomocytes for JN1713. The *ASH(-);octr-1(ok371)*, JRS66 (*octr-1p::octr-1gDNA::SL2::GFP*), and JRS70 (*octr-1p::octr-1gDNA::SL2::GFP;octr-1(ok371)*) strains were constructed using standard genetic techniques.

Bacterial strain

The *E. coli* strain OP50 was grown in Luria-Bertani (LB) broth at 37°C and used as worm food.

METHOD DETAILS

Lifespan assays

Two different approaches were used for lifespan assays. In the first approach, worms were maintained for at least two generations at 15, 20, or 25°C before being used for egg synchronization. Lifespan assays were done at the respective temperature by transferring 3x20 synchronized worms onto live *E. coli* OP50 plates, followed by counting and transferring to fresh, live *E. coli* OP50 culture daily until death, as described in our published protocol.[33] All assays were performed in triplicates for each strain. In the second approach, worms were maintained at 20°C. Worms were synchronized by egg-laying, and synchronized offspring were grown at 20°C for 65 hours to reach the young-adult stage. Worms were then transferred to15, 20, or 25°C followed by lifespan assays at the respective temperature. Live animals were transferred daily to fresh plates. Animals were scored at the times indicated and were considered dead when they failed to respond to touch. All assays were performed in triplicates for each strain.

RNA isolation

Gravid adult WT and *octr-1(ok371)* animals were lysed using the solution of sodium hydroxide and bleach (ratio 5:2), washed, and eggs were synchronized for 22 hours in S-basal liquid medium at room temperature. Synchronized L1 larval animals were transferred onto modified NGM plates seeded with *E. coli* OP50 and grown at 20°C for 65 hours until the animals had reached the young-adult stage. The animals were collected and washed with M9 buffer before being transferred to fresh *E. coli* OP50 plates at either 15, 20, or 25°C. Prior to being transferred daily to new *E. coli* OP50 plates, the animals were collected in M9 buffer and allowed to settle at the bottom of 15-mL conical tubes for 5 minutes. After supernatant removal, the pellets of adult animals were resuspended in 10 mL of fresh M9 buffer and allowed to settle again for another 5 minutes. After the removal of the second wash supernatant, the pellets were resuspended in 10 mL of M9 buffer and poured through 40-μm filters to remove any remaining eggs and larva. Adult animals were collected off the filter and transferred to new *E. coli* OP50 NGM plates. Animals were collected and washed with M9 buffer at specific times (day 9 for animals grown at 25°C, days 1 and 14 for animals grown at 20°C, and day 24 for animals grown at 15°C). RNA was extracted using QIAzol (Qiagen) and the RNeasy plus mini kit (Qiagen) following the protocol provided by the manufacturer.

Quantitative reverse-transcription PCR (RT-qPCR)

Total RNA was obtained as described above. Two μg of RNA was used to generate cDNA per 100-μL reaction using the Applied Biosystems High-Capacity cDNA Reverse Transcription Kit. RT-qPCR was conducted by following the protocol for PowerUp SYBR Green (Applied Biosystems) on an Applied Biosystems StepOnePlus real-time PCR machine. Ten-μL reactions were set up following the manufacturer's recommendations, and 20 ng of cDNA was used per reaction. Relative fold-changes for transcripts were calculated using the comparative C_T($2^{-\Delta\Delta CT}$) method and were normalized to pan-actin (*act-1*, *-3*, *-4*). Amplification cycle thresholds were determined by the StepOnePlus software. All samples were run in triplicate.

RNA sequencing and bioinformatic analyses

Five replicates of eight groups of RNA samples (young and old *octr-1(ok371)* and WT animals grown at 15, 20, or 25°C, Table 1) were collected and submitted to the WSU Genomics Core for RNA-seq analysis. For bioinformatic analyses, raw RNA-seq data (FASTQ files) were aligned to the *C. elegans* reference genome (ce10, UCSC) using HISAT2, followed by gene expression quantification and differential expression analysis using featureCounts and DESeq2, respectively, as described in our published protocol.[34] After differentially expressed genes were identified, they were used for gene ontology (GO) analysis and tissue enrichment analysis using the 'Enrichment Analysis' program on the WormBase website (https://wormbase.org//tools/enrichment/tea/tea.cgi).[45,46] For all enrichment analyses in this study, q value threshold was set at 0.05.

The sequencing data (FASTQ files) have been deposited in NCBI's SRA database through the Gene Expression Omnibus (GEO). Processed gene quantification files and differential expression files have been deposited in the GEO. All of these data can be accessed through GEO: GSE252054.

To count the number of genes that contain the ELT-2 binding site in their promoter regions, gene chromosomal locations, strand information, and transcription start site (TSS) positions for each differentially expressed gene were downloaded from Ensembl using the biomaRt R library.[47] The matchPattern function from the Biostrings library[48] was then used to count all occurrences of the TGATAA binding site sequence in the promoter region. The promoter region was defined as 1000bp upstream of the TSS to 200bp downstream. The reference genome used for this analysis was WBcel235, obtained from NCBI.

RNA interference (RNAi)

RNAi was conducted by using the Ahringer group library and feeding synchronized L3 larval *C. elegans E. coli* (strain HT115(DE3)) that expressed double-stranded RNA (dsRNA) that was homologous to the target gene of interest. Before exposure, all RNAi clone plasmids were isolated, digested with KpnI, and Sanger sequenced using a T7 promoter primer to check for gene specificity. *E. coli* with the appropriate dsRNA vector were grown in LB broth containing ampicillin (100 μg/mL) at 37°C for 15–16 hours, and then 120 μL was plated on modified NGM plates containing 100 μg/mL ampicillin and 3 mM isopropyl β-D-thiogalactoside (IPTG). The bacteria were allowed to grow for 15–16 hours at 37°C. The plates were cooled away from direct light before the synchronized L3 larval animals were placed on the bacteria. The animals were incubated at 20°C for 24 hours or until the animals were gravid adults. After the animals reached gravid adulthood, the RNAi exposed animals were used for egg laying synchronization as previously described on plates seeded with matching dsRNA expression *E. coli* for a second round of RNAi exposure. The new generation of RNAi treated animals were grown at 20°C for 65 hours. Unc-22 RNAi was included as a positive control in all experiments to account for RNAi efficiency.

Octopamine treatment

Synchronized 65-hour-old animals and bacteria plates were prepared using the method described above for lifespan assays. The synchronized animals were transferred to M9 buffer containing either 0mM or 10mM of octopamine and allowed to soak at 20°C for 1 hour under gentle rocking. After 1 hour, the animals were pelleted via centrifugation and placed onto NGM plates with 0mM or 10mM of octopamine. Lifespan assays were performed at 15, 20, or 25°C. Live animals were transferred daily to fresh plates. Animals were scored at the times indicated and were considered dead when they failed to respond to touch.

1-Octanol choice assay

WT and *ASH(-)* (strain JN1713, ASH neurons ablated) animals were synchronized using the egg laying method as described above. NGM plates were prepared in square petri dishes marked with 1cm^2 sections which divided the plate into 36 equal parts for ease of measuring. Prior to placing animals on the NGM plates, plates were placed into 15, 20 or 25°C incubators to temper to the appropriate temperature. 1-octanol was diluted in 100% ethanol to a final concentration of 30% 1-octanol and 70% ethanol. After the NGM plates were properly tempered, two 10-μL drops of fresh *E. coli* OP50 were placed on opposite sides of the plate. Three 2-μL drops of either 30% 1-octanol or 100% ethanol were placed around the fresh *E. coli* OP50. The 1-octanol and ethanol were placed in a manner that a drop was placed on either side of the new bacterial lawn, and a single drop was placed towards the center of the plate so that the animals could not approach the bacteria without encountering the stimulants. Animals were collected and placed in the center of the NGM plates and allowed to crawl around the plate for 1 hour at the appropriate temperature. The petri dishes were divided into three distinct zones: 1-octanol zone, no decision zone, and the ethanol zone. Each zone was 2 cm in width and ran the length of the petri dish. An animal was considered in a zone if its head was facing the zone and at least half of its body length had crossed the border between zones. Animals that crawl on the wall of the petri dish and died to desiccation were censored from the data.

Transgenic animal generation

octr-1 overexpression and rescue animals were generated by outcrossing JRS4 [*octr-1(ok371;tbh-1(n3247)* + *octr-1p::octr-1cDNA::SL2::GFP*] with WT or *octr-1(ok371)* mutant animals, respectively. The genotype of the animals was confirmed using PCR and the expression of the extrachromosomal array was confirmed using fluorescent microscopy.

QUANTIFICATION AND STATISTICAL ANALYSIS

Lifespan curves were plotted using GraphPad PRISM (version 9) computer software. Lifespan was considered different from the appropriate control indicated in the main text when $p < 0.05$. PRISM uses the product limit or Kaplan-Meier method to calculate survival fractions and the log rank test, which is equivalent to the Mantel-Haenszel test, to compare survival curves. The number of animals used for each experiment can be found in figure legends. All experiments were repeated at least three times, unless otherwise indicated. For 1-octanol assays, a two-way ANOVA with Šídák's multiple comparison test was used for statistical tests. For RT-qPCR, an unpaired multiple *t*-test was used for statistical tests.