Neural G protein-coupled receptor OCTR-1 mediates temperature effects on longevity by regulating immune response genes in *C. elegans*

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ABSTRACT

We have previously demonstrated that OCTR-1, an octopamine G protein-couple receptor, functions in the sensory neurons ASH to suppress the innate immune response in *Caenorhabditis elegans* by inhibiting the expression of immune genes (Sun *et al*. 2011 Science 332:729-732). Here we discover that OCTR-1 also regulates temperature effects on lifespan in *C. elegans*. At the normal growth temperature 20°C, *octr-1(ok371)* mutant animals have similar lifespan to wild-type N2 animals. However, at higher temperature 25°C, *octr-1(ok371)* mutants live significant longer than wild-type N2 animals. These results suggest that OCTR-1 may mediate temperature effects on lifespan. Furthermore, we found the OCTR-1-expressing ASH chemosensory neurons are involved in the OCTR-1-mediated regulation on longevity. However, interestingly, the thermosensory AFD neurons do not play a role in this regulation at 25°C. RNA-seq data analysis showed that 63 immune response genes were significantly down-regulated in *octr-1(ok371)* mutants relative to wild-type animals at 25°C. We further demonstrated that inactivation of several most-downregulated genes by RNA interference in wild-type N2 animals significantly extended their lifespan, similar to the phenotype of *octr-1(ok371)* animals. These observations suggest a new molecular regulation mechanism that downregulation of immune

genes extends the lifespan of *C. elegans*, which is opposite to the general belief that an increase in defense immunity extends lifespan.

Key words: *Caenorhabditis elegans*, immune response genes, G protein-coupled receptor, temperature effect, longevity, thermosensory neurons, chemosensory neurons.

INTRODUCTION

Various studies in both poikilotherms and homeotherms have associated lower temperature with longer lifespan and higher temperature with shorter lifespan (1). These inverse effects of temperature on longevity were traditionally explained by the "rate of living theory", which posits that lower temperatures reduce chemical reaction rates, thus slowing down the aging process, whereas higher temperatures do the opposite (2). Recent studies have identified specific molecules and cells that mediate the longevity responses to temperature, suggesting that the temperature effects on aging are not simply thermodynamic but regulated processes (3-5). The mechanisms underlying such regulation, however, are not well understood. *Caenorhabditis elegans* is one of the organisms that were used in early studies of the temperature effects on longevity, and a negative correlation has been well documented (6). We have discovered that *C. elegans* lacking OCTR-1, a neuronal G protein-coupled receptor (GPCR) for the neurotransmitter octopamine (OA) (7), exhibited increased lifespan at warm temperature (25°C) relative to wild-type N2 animals, indicating a critical role of OCTR-1 in modulating the temperature effects on longevity/aging. This modulation involves the OCTR-1-expressing sensory neurons ASH but surprisingly not the thermosensory neurons AFD. Further RNA-seq data analysis showed that 63 immune defense genes were significantly down-regulated in *octr-1(ok371)* mutants relative to wild-type animals at 25°C. Moreover, we demonstrated that inactivation of several most-downregulated genes by RNA interference (RNAi) in wild-type

animals significantly extended their lifespan, similar to the phenotype of *octr-1(ok371)* animals. These suggest a new molecular regulation mechanism that downregulation of immune genes extend the lifespan of *C. elegans*, which is opposite to the general belief that an increase in defense immunity extends lifespan.

RESULTS AND DISCUSSION

octr-1(ok371) mutant animals live significantly longer than wild-type animals at high temperature

We have demonstrated that the OCTR-1 neural circuit controls the survivals of *C. elegans* against pathogen infection by suppressing the unfolded protein response (UPR) pathways (8-9). To test whether such regulation of lifespan also occurs under other stress conditions, such as higher growth temperature, we compared the lifespans of wild-type N2 animals and *octr-1(ok371)* mutant animals at 20°C (standard growth temperature) and 25°C (higher growth temperature). As shown in Figure 1, the *octr-1(ok371)* mutant animals live similar to wild-type N2 animals at 20°C (Fig. 1A), but live significantly longer than the N2 animals at 25°C (Fig. 1B). These results suggest that OCTR-1 neural circuit may mediate temperature effects on lifespan at higher temperature.

The ASH chemosensory neurons, but not the AFD thermosensory neurons, are involved in the OCTR-1 neural circuit to regulate longevity at high temperature

We have previously demonstrated that OCTR-1 functions in the chemosensory neurons ASH to suppress the innate immune response in *C. elegans* by inhibiting the expression of immune genes (8). To determine if ASH neurons are involved in the OCTR-1 neural circuit-regulated longevity at high temperature, we examined the lifespans of ASH mutants and ASH;*octr-1(ok371)* mutants at 25°C. As shown in Figure 2A, both ASH mutants and ASH;*octr-1(ok371)* mutants live significantly longer than wild-type N2 animals at 25°C, suggesting that the ASH

chemosensory neurons play an important role in the OCTR-1-dependent regulation of longevity at high temperature.

C. elegans senses temperature using the AFD thermosensory neurons. The thermosensory circuit for response to temperature changes has been well studied (10). To determine if the AFD thermosensory neurons are involved in the OCTR-1 neural circuit-regulated longevity at high temperature, we examined the lifespans of AFD mutants (*ttx-1(p767)*) and *ttx-1(p767);octr-1(ok371)* mutants at 25°C. As shown in Figure 2B, the *ttx-1(p767);octr-1(ok371)* mutants live significantly longer than wild-type N2 animals at 25°C, but no difference in lifespan was observed between AFD mutants and wild-type N2 animals. These results suggest that the AFD thermosensory neurons do not play an important role in the OCTR-1-dependent regulation of longevity at high temperature. The role of the AFD neurons in sensing higher temperature at 25°C might be counterbalanced by an unidentified neuron(s) in the OCTR-1 neural circuit, which grants a future investigation.

Immune defense genes are significantly down-regulated in *octr-1(ok371)* mutants at high temperature

To gain insights on how the OCTR-1 neural circuit mediates temperature effects on longevity at the molecular level, we used RNA-seq to profile gene expression in *octr-1(ok371)* animals relative to wild-type N2 animals at 20°C and 25°C. To this end, we collected five replicates of four groups of RNA samples [*octr-1(ok371)*] and wild-type N2 animals grown on *E. coli* OP50 for 14 days (TD50) at 20°C or 9 days (TD50) at 25°C. These samples were submitted to the Genomics Core for RNA-seq analysis. The resulting sequence data will be deposited in the National Center for Biotechnology Information's (NCBI) Sequence Read Archive (SRA) database through the Gene Expression Omnibus (GEO); processed gene quantification files and differential expression files will be deposited in GEO.

To examine how OCTR-1 neural circuit regulates gene expression in *C. elegans* at high temperature, we compared the expression profile of *octr-1(ok371)* animals with that of wild-type N2 animals at 25°C. We found that 912 genes were down-regulated at least twofold in the mutant animals. Ontology analysis of the down-regulated genes revealed one significantly enriched molecular function involved in immune defense response. The top 10 most down-regulated immune genes among the 63 defense genes are listed in Table 1. These results indicate that down-regulation of immune defense genes by the OCTR-1 neural circuit could play a role in extending the lifespan of *C. elegans* at high temperature.

Inactivation of the most down-regulated genes by RNA interference in wild-type animals extends their lifespans

To examine whether the OCTR-1-downregulated immune defense genes contribute to the improved lifespan of the mutant animals, we inactivated these genes by RNA interference (RNAi) in wild-type N2 animals and assayed their lifespans at 25°C. As demonstrated in Figure 3, inactivation of the *lys-5*, *acdh-1*, *lys-4*, and *srx-128* genes significantly enhanced their lifespans, similar to the lifespan of *octr-1(ok371)* mutant animals at 25°C, indicating that the OCTR-1-downregulated immune defense genes contribute to the enhanced lifespan of *octr-1(ok371)* animals at 25°C. These observations suggest a new molecular regulation mechanism that downregulation of immune genes extends the lifespan of *C. elegans*, which is opposite to the general belief that an increase in defense immunity extends lifespan. It would be interesting to investigating how the lifespan of *C. elegans* is enhanced by downregulating the immune defense genes.

MATERIALS AND METHODS

Nematode strains

The following *C. elegans* strains were cultured under standard conditions and fed *Escherichia coli* OP50. Wild-type N2 were *C. elegans* Bristol N2. The *octr-1(ok371)*, *ttx-1(p767)*, and ASH(-) [JN1713] strains were obtained from the *Caenorhabditis elegans* Genetics Center (University of Minnesota, Minneapolis, MN). The *ASH(-);octr-1(ok371)* and *ttx-1(p767);octr-1(ok371)* mutant strains were constructed using standard genetic techniques.

Bacterial strain

E. coli strain OP50 were grown in Luria-Bertani (LB) broth at 37°C.

Lifespan assay

C. elegans wild-type *N2* animals and mutant animals were maintained as hermaphrodites at 20°C, grown on modified nematode growth medium (NGM) agar plates (0.35% instead of 0.25% peptone). Bacterial lawns used for the lifespan assays were prepared by placing a 50µL drop of an overnight fresh culture of *E. coli* OP50 on each 3.5cm plate. Plates were incubated at 37°C for 16 hours, cooled down to room temperature, and then seeded with synchronized 65-hour-old young adult animals. These worms were prepared by undergoing two rounds of egg-laying synchronization before they were exposed to *E. coli* OP50 for lifespan assays. The lifespan assays were performed at 20°C 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.

RNA sequencing

Five replicates of four groups of RNA samples [*octr-1(ok371)*]] and wild-type N2 animals grown on *E. coli* OP50 for 14 days at 20°C or 9 days at 25°C were collected and submitted to the Genomics Core for RNA-seq analysis. The integrity of total RNA was assessed using Fragment Analyzer (Advanced Analytical Technologies, Ankeny, IA) with the High Sensitivity RNA

Analysis Kit. RNA quality numbers (RQNs) from 1 to 10 was assigned to each sample to indicate its integrity or quality. "10" stands for a perfect RNA sample without any degradation, whereas "1" marks a completely degraded sample. RNA samples with RQNs ranging from 8 to 10 were used for RNA library preparation with the TruSeq Stranded mRNA Library Prep Kit (Illumina, San Diego, CA). Briefly, mRNA was isolated from 2.5 μg of total RNA using poly-T oligo attached to magnetic beads and then subjected to fragmentation, followed by cDNA synthesis, dA-tailing, adaptor ligation and PCR enrichment. The sizes of the RNA libraries were assessed by Fragment Analyzer with the High Sensitivity NGS Fragment Analysis Kit. The concentrations of the RNA libraries were measured using a StepOnePlus Real-Time PCR System (ThermoFisher Scientific, San Jose, CA) with the KAPA Library Quantification Kit (Kapabiosystems, Wilmington, MA). The libraries were diluted to 2 nM with RSB (10 mM Tris-HCl, pH8.5) and denatured with 0.1 N NaOH. Eighteen pM libraries were clustered in a high-output flow cell using HiSeq Cluster Kit v4 on a cBot (Illumina). After cluster generation, the flow cell was loaded onto a HiSeq 2500 for sequencing using a HiSeq SBS kit v4 (Illumina). DNA was sequenced from both ends (paired-end) with a read length of 100 bp. The raw BCL files were converted to FASTQ files using the software program bcl2fastq2.17.1.14. Adaptors were trimmed from the FASTQ files during the conversion. On average, 40 million reads with a read length of 2x 100 bp were generated for each sample. RNA-seq data (FASTQ files) were aligned to the *C. elegans* reference genome (ce10, UCSC) using HISAT2. Gene expression quantification and differential expression were analyzed using HTSeq and DESeq2.

RNA interference

RNAi was conducted by feeding L2 or L3 larval *C. elegans E. coli* strain HT115(DE3) expressing double-stranded RNA (dsRNA) that was homologous to a target gene (11). *E. coli* with the appropriate dsRNA vector were grown in LB broth containing ampicillin (100μg/mL) at 37°C for 16 hours and plated on modified NGM plates containing 100μg/mL ampicillin and 3mM

isopropyl β-D-thiogalactoside (IPTG). The RNAi-expressing bacteria were allowed to grow for 16 hours at 37°C. The plates were cooled down before the L2 or L3 larval animals were placed on the bacteria. The animals were incubated at 20°C for 24 hours or until the animals were 65 hours old. *unc-22* RNAi was included as a positive control in all experiments to account for RNAi efficiency. Clone identity was confirmed by sequencing at Eton Bioscience Inc. (San Diego, CA).

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. All experiments were repeated at least three times, unless otherwise indicated.

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AUTHOR CONTRIBUTIONS

S.W., P.W., and J.S. designed and performed experiments and analyzed data. J.S. wrote the paper.

COMPETING INTERESTS

The authors declare that they have no competing interests.

DATA AND MATERIALS AVAILABILITY

The RNA-seq data will be deposited in NCBI's SRA database through the GEO. The processed gene quantification files and differential expression files will be deposited in the GEO. All data needed to evaluate the conclusions in the paper are present in the paper. Additional data related to this paper may be requested from the authors. The *C. elegans* strains and plasmids constructed by us and primer sequences used for their construction are available upon request.

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FIGURE LEGENDS

Fig 1. The lifespan of *octr-1(ok371)* mutants is similar to that of wild-type N2 animals at 20°C but significantly longer than N2 at 25°C. Wild-type N2 and *octr-1(ok371)* mutant animals were grown on *C. elegans* laboratory food *E. coli* OP50 and scored for lifespan over

time at 20°C and 25°C, respectively. The graphs are the representative results 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 wild-type N2: *p*=0.8410 for (A) and *p*=0.0018 for (B).
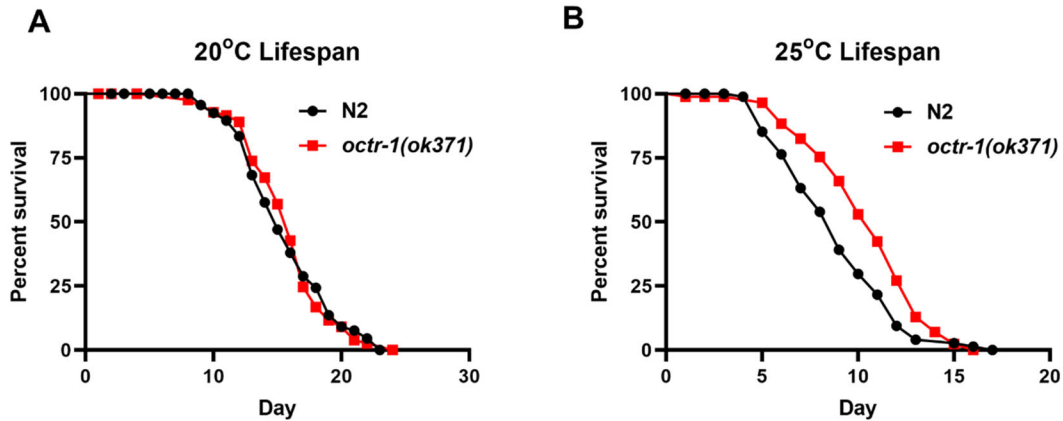
Fig. 2. The ASH neurons, but not the AFD neurons, play an important role in the OCTR-1-dependent regulation of longevity at 25°C. (A) Wild-type N2, *octr-1(ok371)*, *ASH(-)*, and *ASH(-);octr-1(ok371)* mutant animals were grown on *C. elegans* laboratory food *E. coli* OP50 and scored for lifespan over time at 25°C. 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 wild-type N2: *octr-1(ok371)*, *p*<0.0001; ASH(-), *p*=0.0008; ASH(-);*octr-1(ok371)*, *p*<0.0001. (B) Wild-type N2, *octr-1(ok371)*, *ttx-1(p767)*, and *ttx-1(p767);octr-1(ok371)* mutant animals were grown on *C. elegans* laboratory food *E. coli* OP50 and scored for lifespan over time at 25°C. 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 wild-type N2: *octr-1(ok371)*, *p*<0.0001; *ttx-1(p767)*, *p*=0.1862; *ttx-1(p767);octr-1(ok371)*, *p*<0.0001.

Fig 3. The OCTR-1-downregulated immune defense genes contribute to the improved lifespan of *octr-1(ok371)* animals at 25°C. Wild-type N2 and *octr-1(ok371)* animals grown on dsRNA for several most-downregulated genes or empty vector (EV) control were exposed to *C. elegans* laboratory food *E. coli* OP50 and scored for lifespan over time at 25°C. The graphs are the combined results of three independent experiments. Each experiment included n=60 adult animals per strain. *P* value represents the significance level of the mutants relative to the wild-type N2+EV: (A) *octr-1(ok371)*+EV, *p*<0.0001; N2+*lys-5* RNAi, *p*=0.0007; (B) N2+*acdh-1* RNAi, *p*<0.0001; (C) N2+*lys-4* RNAi, *p*=0.0296; (D) N2+*srx-128* RNAi, *p*=0.0001.

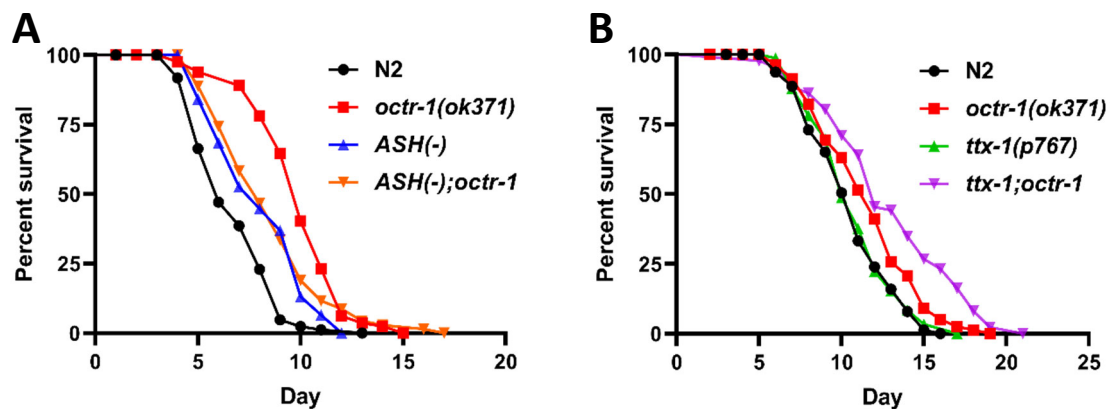**Table 1. Top 10 OCTR-1-downregulated immune defense genes at 25°C.**
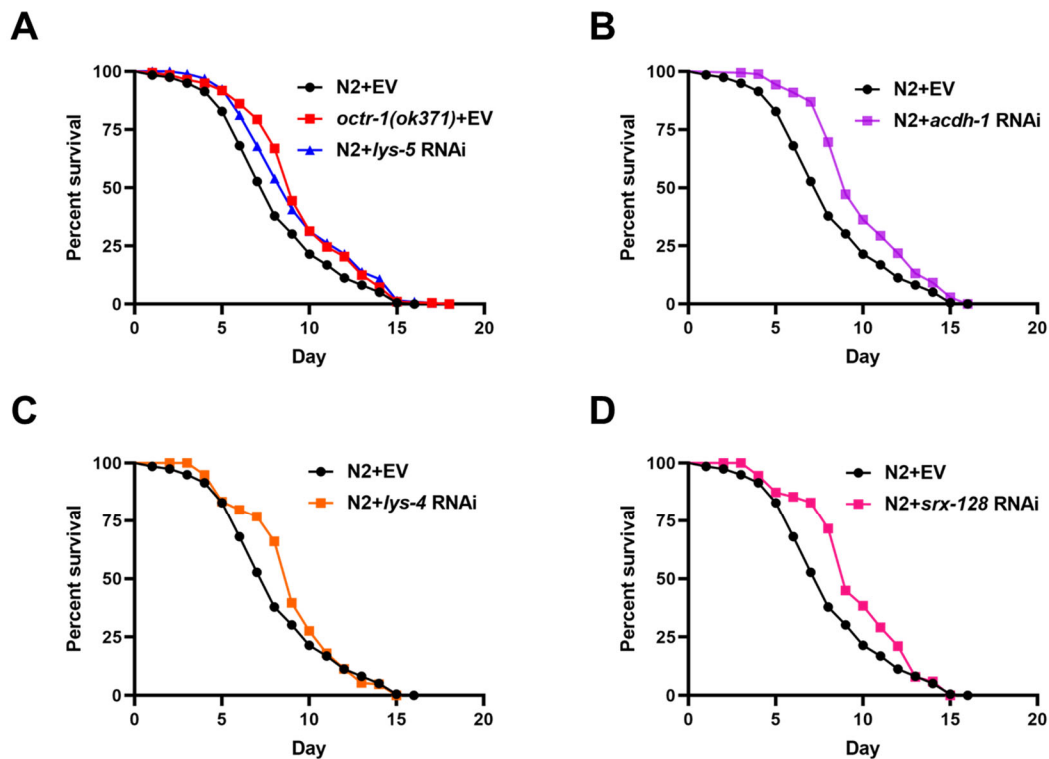
FIGURES AND TABLES

Fig. 1

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Table 1. Top 10 OCTR-1-downregulated immune defense genes at 25°C.

Top 10 down-regulated immune genes in N2_25°C vs octr-1_25°C		
Sequence name	Gene name	Fold_down
F58B3.2	lys-5	146.9
C55B7.4	acdh-1	63.7
C34F6.3	col-179	41
F58B3.1	lys-4	26.2
F22A3.6	ilys-5	23.4
K12H4.7		15.7
Y22F5A.5	lys-2	14.1
T08A9.7	spp-3	12.6
K08D8.5		12.5
C02A12.4	lys-7	11.6