The longevity response to warm temperature is neurally controlled via regulation of collagen genes

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ABSTRACT

Lower temperatures have been associated with longer lifespan, while higher temperatures with shorter lifespan. These inverse effects of temperature on longevity were traditionally explained by the "rate of living theory", which posits that higher temperatures increase chemical reaction rates, thus speeding up the aging process. Recent studies have identified specific molecules and cells that mediate the longevity response to temperature, suggesting that the temperature effects on aging are not simply thermodynamic but regulated processes. However, the mechanisms underlying such regulation are not well understood. In the current study, we found that *Caenorhabditis elegans* lacking NPR-8, a neuronal GPCR related to the mammalian neuropeptide Y receptors, exhibited extended lifespan at warm temperature. Such lifespan extension can be suppressed by re-expression of NPR-8 in the amphid sensory neurons AWB and AWC or by inactivation of NPR-8-regulated collagen genes. These results suggest that the

warm temperature effect on longevity is regulated by the nervous system through controlling collagen gene expression. Our study potentially provides mechanistic insights into understanding the relationship between temperature and longevity, which could prove useful in mitigating negative impacts of increasing temperature due to global warming.

INTRODUCTION

Various studies in many species have associated lower temperatures with longer lifespan and higher temperatures with shorter lifespan, aside from extreme temperatures that would severely harm organisms' survival (1). The inverse effects of temperature on longevity were first recorded a century ago in poikilotherms such as the fruit fly *Drosophila melanogaster* (2) and the cladoceran crustacean *Daphnia magna* (3). This phenomenon was later also found in the nematode *Caenorhabditis elegans* (4), the South American fish *Cynolebias* (5), as well as in mice (6) and humans (7), although with some obscurity in the mammals (1). Nonetheless, there is a clear trend for increases in lifespan from decreases in temperature across species. 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 (8). 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 (9). For example, in *C. elegans*, the cold-sensitive TRP channel TRPA-1 detects temperature drop in the environment to extend lifespan (10), but it also mediates lifespan shortening when the worm was subjected to low-temperature treatment at the larval stage (11). The thermosensory AFD neurons and the AIY interneurons are required for maintaining *C.*

47 *elegans* lifespan at warm temperature (12), which was later found to be controlled by the worm
48 cyclic AMP-responsive element binding protein (CREB) (13). Co-chaperone p23 regulates the
49 *C. elegans* longevity responses to both cold temperatures and warm temperatures (14). A
50 transcriptional coregulator, Mediator 15, controls lipid homeostasis to regulate low-temperature-
51 induced longevity and proteostasis (15). These studies suggest that the longevity response to
52 temperature is a complex and highly regulated process and warrants major research efforts to
53 elucidate the underlying mechanisms.
54 In the current study, we found that *C. elegans* lacking NPR-8, a G protein-coupled receptor
55 (GPCR) related to the mammalian neuropeptide Y receptors, exhibited extended lifespan at
56 warm temperature. This extended lifespan phenotype was rescued by re-expressing NPR-8 in the
57 amphid sensory neurons AWB and AWC or by inactivating NPR-8-regulated collagen genes.
58 Our study suggests that the warm temperature effect on longevity is regulated by the nervous
59 system through controlling the expression of collagen genes.
60
61 **RESULTS**
62 **Functional loss of NPR-8 extends *C. elegans* lifespan at warm temperature**
63 Three temperatures (15°C, 20°C, and 25°C) are routinely used for *C. elegans* propagation in the
64 laboratory, and their inverse effects on lifespan have been well documented, i.e., a 75% increase
65 in lifespan from a 5°C drop in temperature (4). During our work on neural regulation of *C.*
66 *elegans* defense against pathogen infection (16), we unexpectedly observed that under
67 noninfectious conditions, *C. elegans* lacking NPR-8 ((*npr-8(ok1439)* animals) lived longer than
68 wild-type *N2* animals at 25°C, whereas the mutant and wild-type animals had the same lifespan
69 at 20°C or 15°C (Fig. 1). To determine whether the observed extended lifespan phenotype at

70 warm temperature was due to the deletion mutation in *npr-8*, we performed lifespan assays at
71 25°C using the JRS17 rescue strain in which *npr-8* is re-expressed under its native promoter in
72 *npr-8(ok1439)* animals. Our results showed that re-expression of *npr-8* in JRS17 rescued the
73 extended lifespan phenotype of the mutants (Fig. S1), indicating that the deletion mutation in
74 *npr-8* is indeed responsible for the extended longevity at warm temperature. These results
75 suggest that NPR-8 suppresses lifespan at warm temperature, contributing to the inhibitory effect
76 of warm temperature on longevity.
77
78 **NPR-8-dependent longevity response to warm temperature is neurally regulated**
79 NPR-8 is a neuronal GPCR that is expressed in the amphid sensory neurons AWB, ASJ and
80 AWC (16). Our previous study showed that NPR-8 functions in these neurons to regulate *C.*
81 *elegans* defense response to pathogen infection (16). Is NPR-8-dependent longevity response to
82 warm temperature also neurally controlled? To address this question, we took advantage of our
83 established rescue strains that specifically express NPR-8 in the individual neurons under cell-
84 specific promoters in *npr-8(ok1439)* animals. Lifespan assays showed that NPR-8 rescue in
85 AWB (strain JRS18) and AWC neurons (strain JRS19) shortened the lifespan of *npr-8(ok1439)*
86 animals at 25°C, whereas the rescue in ASJ neurons (strain JRS20) had no effects on the
87 mutants' lifespan (Fig. 2). These results indicate that the lack of NPR-8 in AWB and AWC
88 neurons is responsible for the mutants' extended lifespan at 25°C. In other words, NPR-8
89 functions in AWB and AWC neurons to regulate the longevity response to warm temperature.
90
91 **NPR-8-regulated collagen genes contribute to the warm temperature-induced longevity**
92 **response**

93 Previously, we demonstrated that NPR-8 suppresses the expression of collagen genes in wild-
94 type animals under both normal (i.e., fed worm food *Escherichia coli* strain OP50) and infectious
95 (i.e. exposed to *P. aeruginosa*) conditions, and that NPR-8-regulated collagens positively
96 influence *C. elegans* defense against infection (16). In the current study, we wanted to determine
97 whether NPR-8-regulated collagen genes also play a role in warm temperature-induced longevity
98 response. To this end, we inactivated NPR-8-regulated collagen genes either by RNA
99 interference or by loss-of-function mutants and then examined the effects of such inactivation on
100 worm lifespan at 25°C. Our results showed that RNAi of *col-49* or *col-77* fully rescued the
101 extended lifespan of *npr-8(ok1439)* animals to the wild-type level, while a null mutation in *col-*
102 *179* shortened the lifespan of *npr-8* mutant animals to a level lower than the wild-type level (Fig.
103 3). These results indicate that NPR-8-regulated collagen genes contribute to the extended
104 lifespan of *npr-8(ok1439)* animals at 25°C, and that NPR-8 controls the longevity response to
105 warm temperature by regulating collagen genes.
106

107 **DISCUSSION**

108 In the current study, we showed that *C. elegans* lacking NPR-8 had longer lifespan than wild-
109 type animals at 25°C but had the same lifespan as wild type at 20°C or 15°C, and that NPR-8
110 expression in amphid sensory neurons AWB and AWC as well as NPR-8-regulated collagen
111 genes suppress the lifespan extension of *npr-8* mutant animals at 25°C. An NPR-8-dependent
112 neural-longevity regulatory circuit has emerged whereby NPR-8 functions in AWB and AWC
113 neurons to control the longevity response to warm temperature by regulating the expression of
114 collagen genes. Many questions remain unanswered in this regulatory circuit. For example, how
115 do NPR-8-regulated collagen genes influence longevity at warm temperature? What other

116 neurons, in addition to AWB and AWC, are involved in the regulation? These are the subjects of
117 our future investigation.
118 Previously, we discovered an NPR-8-dependent neural-immune regulatory circuit that controls
119 *C. elegans* defense against pathogen infection (16). This circuit shares many components with
120 the above-described neural-longevity regulatory circuit, including NPR-8, AWB and AWC
121 neurons, and NPR-8-regulated collagen genes. Because both warm temperature and pathogen
122 infection are stressful for *C. elegans*, the two regulatory circuits might function in the same
123 pathway to maintain worm lifespan in response to stressful conditions, and they both achieve
124 extended lifespan by regulating the expression of collagen genes. Indeed, recent research has
125 shown that both collagen production and function decline with age, and that elevated collagen
126 expression is a shared feature of multiple conserved longevity pathways as well as essentially
127 every longevity intervention (17-20). These studies indicate that collagen expression might play
128 a causal role in slowing down the aging process under both normal and stressful conditions. This
129 notion is in direct contrast to the general belief that collagen decline is consequential to aging.
130 Such concept switch has profound implications for how we study aging and develop lfespan-
131 extending treatments. For example, as wrinkled skin is an indicator of collagen decline, healthy
132 skin can be used as an indicator of collagen homeostasis. Because collagen expression is causal
133 to the slowdown of aging, healthy skin could be used as a readout of an individual's overall good
134 health. This opens avenues for adapting a diverse array of therapies, ingredients, and
135 technologies that have been established in the skin care industry to restore skin to its optimal
136 condition into anti-aging strategies.
137
138 **MATERIALS AND METHODS**

139 **Worm strains**

140 Worm strains used in the study were Wild-type (N2 Bristol), *npr-8 (ok1439), npr-8 (ok1449),*
141 *ttx-1 (p767)*, *ttx-3 (ks5)*, *npr-8;ttx-1*(JRS--). NPR-8 translational rescue strains were JRS17 [*npr-*
142 *8p(2kb)::npr-8 gDNA::gfp*] (native NPR-8 rescue strain), JRS18 [*str-1p(1.3kb)::npr-8*
143 *gDNA::gfp*] (AWB NPR-8 rescue strain), JRS19 [*str-2p(2kb)::npr-8 gDNA::gfp*] (AWC NPR-8
144 rescue strain), and JRS20 [*trx-1p(1.1kb)::npr-8 gDNA::gfp*] (ASJ NPR-8 rescue strain) also used
145 in this study. All strains were maintained at 20°C and fed with *E.coli* OP50 on SB medium.

146

147 **Lifespan assay**

148 Temperature-associated lifespan assays were carried out at 15°C, 20°C, and 25°C. Normally
149 worms were maintained at 20°C. Worms used for lifespan assays at 15°C and 25°C were
150 maintained two generations at respective temperature before they are used for egg
151 synchronization. For worm synchronization, gravid adult worms were allowed to lay eggs for 1
152 hr at their assay temperature. 3x20 gravid adult worms were used for egg synchronization per
153 worm strain. Egg to adult developmental time window was followed as 90hr at 15°C, 65hr at
154 20°c, for all strains. 48hr at 25°c for all strains except *npr-8 (ok1439)*. At 25°C egg to adult
155 developmental time window was set as 56hr for *npr-8 (ok1439). E. coli OP50* lawn was prepared
156 by spreading a 50µl drop of an overnight culture of bacteria on SB medium (3.5-cm-diameter
157 petri plates used). Plates were incubated at 37°C for 16 hours and then cooled down at room
158 temperature for at least 1 hour before seeding with synchronized worms. A lifespan assay starts
159 with synchronized population of 20 adult worms per strain on live *E.coli* OP50. All assays were
160 performed in triplicates for each worm strain. Worms counted and transferred to fresh live *E. coli*
161 OP50 culture every day till they die.

162

163 **RNA interference**

164 The impact of NPR-8-regulated collagen genes expression on *C. elegans* lifespan was examined
165 by RNA interference by feeding. Collagen genes *col-49, col-77, col-139, col-179,* and *rol-1* were
166 silenced in wild-type and *npr-8 (ok1439)* animals at 20°C and 25°C. The RNAi protocol was
167 performed as previously described (16). Temperature-specific worm cultivation, egg-
168 synchronization time window, and temperature-specific egg to adult development time window
169 were followed as mentioned in lifespan assay.

170

171 **Statistical analysis**

172 Lifespan assay curves were plotted using GraphPad PRISM (version 8) computer software.
173 Lifespan was considered different from the appropriate control indicated in the main text when P
174 <0.05. PRISM uses the product limit or Kaplan-Meier method to calculate survival fractions and
175 the log-rank test, which is equivalent to the Mantel-Haenszel test, to compare survival curves.
176 All experiments were repeated at least three times, unless otherwise indicated.

177

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180 reagents, worm strains, and technical support. Some worm strains were provided by the
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183

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AUTHOR CONTRIBUTIONS

S.N.P., D.S., and Y.L. designed and performed experiments and analyzed data. S.N.P. and Y.L. wrote the paper.

COMPETING INTERESTS

The authors declare that they have no competing interests.

DATA AND MATERIALS AVAILABILITY

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. 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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258

259 **FIGURE LEGENDS**

260 **Fig. 1. Functional loss of NPR-8 extends lifespan at 25°C but has no effect on lifespan at**
261 **20°C or 15°C.** WT and *npr-8(ok1439)* animals were grown on *E. coli* strain OP50 at 15°C **(A)**,
262 20°C **(B)**, or 25°C **(C),** and scored for survival over time. The graphs are the combined results of
263 three independent experiments. Each experiment included $n = 60$ adult animals per strain. p-
264 values represent the significance level of the mutants relative to the WT, $p = 0.7416$ in (A), $p =$
265 0.1460 in (B), and $p < 0.0001$ in (C).
266

267 **Fig. 2. NPR-8 functions in neurons to regulate the longevity response to warm temperature.**
268 WT, *npr-8(ok1439)*, and rescue animals were grown at 25°C and scored for survival over time.
269 The graphs are the combined results of three independent experiments. Each experiment
270 included $n = 60$ adult animals per strain. JRS18, *npr-8* rescue in AWB neurons; JRS19, *npr-8*
271 rescue in AWC neurons; JRS20, *npr-8* rescue in ASJ neurons; JRS21, *npr-8* pan-neuronal
272 rescue. p-values are relative to *npr-8(ok1439):* WT, $p < 0.0001$; JRS18, $p < 0.0001$; JRS19, $p <$
273 0.0001; JRS20, $p = 0.3476$.
274

275 **Fig. 3. NPR-8-regulated collagen genes are required for warm temperature-induced**
276 **lifespan extension in *npr-8* mutant animals. (A)** WT and *npr-8(ok1439)* animals were grown
277 on dsRNA for empty vector (control), *col-49*, *col-77*, or *col-139* at 25°C and scored for survival
278 over time. The graphs are the combined results of three independent experiments. Each
279 experiment included $n = 60$ adult animals per strain. *p*-values are relative to *npr-8(ok1439)*+EV:
280 WT+EV, $p < 0.0001$; *npr-8 (ok1439)+col-49*, $p < 0.0001$; *npr-8 (ok1439)+col-77*, $p < 0.0001$;
281 *npr-8 (ok1439)+col-139*, $p = 0.0527$. **(B)** WT, *npr-8 (ok1439)*, *col-179 (ok3010)*, and *col-*
282 *179;npr-8(JRS30)* animals were grown on at 25°C and scored for survival over time. The graphs
283 are the combined results of three independent experiments. Each experiment included $n = 60$
284 adult animals per strain. *p*-values are relative to *npr-8 (ok1439)*: WT, $p < 0.0001$; *col-179*
285 *(ok3010)*, $p < 0.0001$; *col-179;npr-8 (JRS30)*, $p < 0.0001$.
286
287 **SUPPLEMENTARY DATA**
288
289 **Fig. S1. Functional loss of NPR-8 extends lifespan at 25°C.** WT, *npr-8(ok1439)*, JRS17 (*npr-8*
290 expression restored in *npr-8(ok1439)* under its native promoter) animals were grown on *E. coli*
291 strain OP50 at 25°C and scored for survival over time. The graphs are the combined results of
292 three independent experiments. Each experiment included $n = 60$ adult animals per strain. *p*-
293 values are relative to *npr-8(ok1439)*: WT, $p < 0.0001$; JRS17, $p < 0.0001$.
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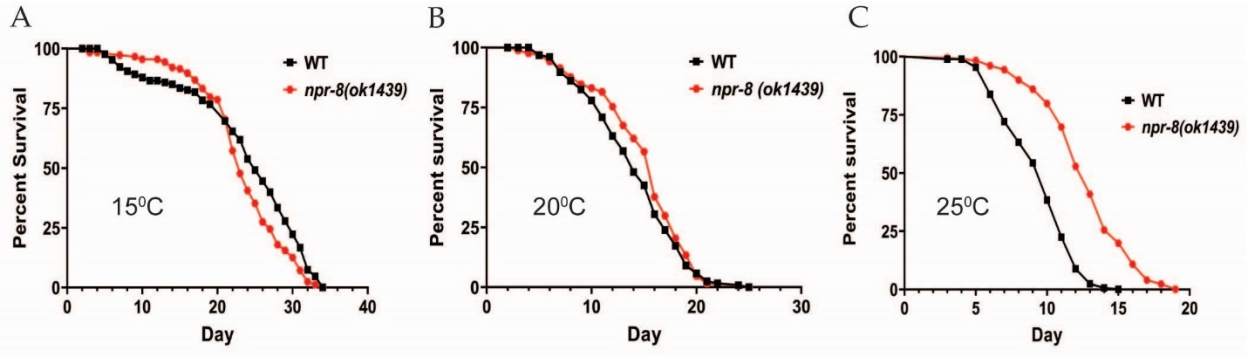
299 **FIGURES**

300

301 **Fig. 1**

302



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314 **Fig. 2**
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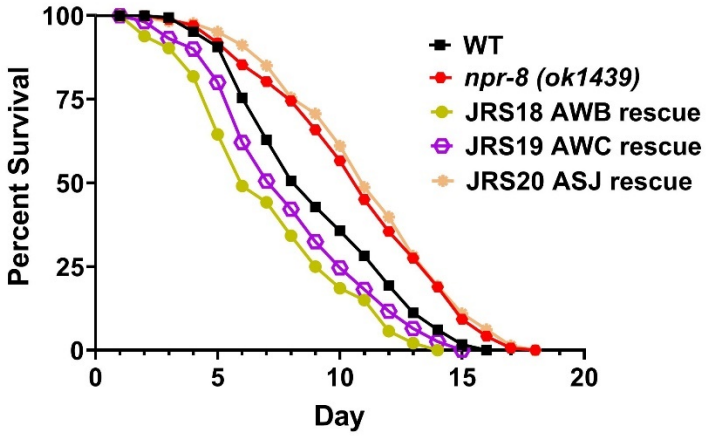

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326 **Fig. 3**
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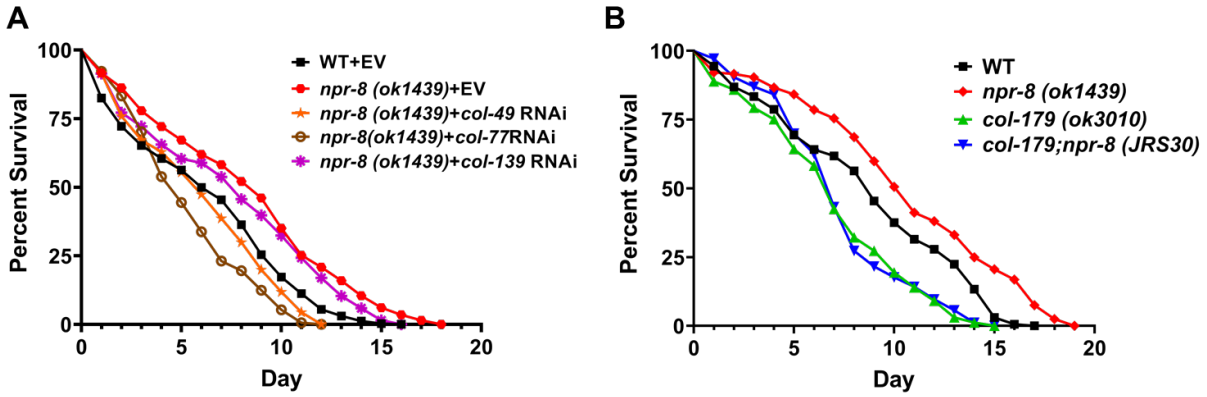

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344 **SUPPLEMENTARY DATA**

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346 **Fig. S1**

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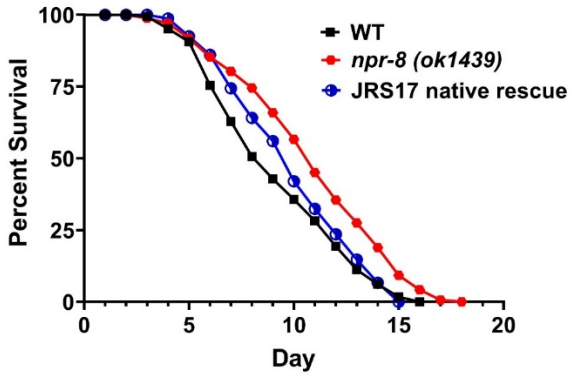

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