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# Food-derived sensory cues modulate longevity via distinct neuroendocrine insulin-like peptides

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**Environmental fluctuations influence organismal aging by affecting various regulatory systems. One such system involves sensory neurons, which affect life span in many species. However, how sensory neurons coordinate organismal aging in response to changes in environmental signals remains elusive. Here, we found that a subset of sensory neurons shortens *Caenorhabditis elegans*' life span by differentially regulating the expression of a specific insulin-like peptide (ILP), INS-6. Notably, treatment with food-derived cues or optogenetic activation of sensory neurons significantly increases *ins-6* expression and decreases life span. INS-6 in turn relays the longevity signals to nonneuronal tissues by decreasing the activity of the transcription factor DAF-16/FOXO. Together, our study delineates a mechanism through which environmental sensory cues regulate aging rates by modulating the activities of specific sensory neurons and ILPs.**

[*Keywords*: sensory neurons; aging; *C. elegans*; insulin-like peptide; DAF-16/FOXO]

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It is vital for the survival of an organism to properly perceive and respond to environmental fluctuations, such as changes in chemical cues and ambient temperatures. These environmental changes are perceived by sensory neurons to elicit appropriate physiological responses for the optimal survival of animals. Sensory neurons also affect long-term physiological processes, such as development and metabolism (Bargmann 2006; Allen et al. 2015). In addition, the sensory systems of *Caenorhabditis elegans*, *Drosophila*, and mice influence life span (Pletcher 2009; Jeong et al. 2012; Riera et al. 2014), although the molecular mechanisms involved are poorly understood.

Gustatory and olfactory neurons, which perceive chemical signals, are some of the sensory neurons that have been shown to affect life span (Alcedo and Kenyon 2004; Libert et al. 2007; Poon et al. 2010; Ostojic et al. 2014; Waterson et al. 2014). In *C. elegans*, chemosensory neuronal signaling is initiated by the binding of chemical ligands to sensory G protein-coupled receptors (GPCRs), which in turn activates G protein signaling cascades to transduce the signals that increase cyclic GMP (cGMP) levels (Bargmann 2006). The cGMP binds to cyclic nucleotide-gated channels composed of TAX-2 and TAX-4 subunits and triggers cation influx (Coburn and Bargmann 1996; Komatsu et al. 1996). The role of chemosensory neurons in life span regulation has been established through inhibition of ciliated sensory neurons in *C. elegans* (Apfeld and Kenyon 1999; Alcedo and Kenyon 2004).

Chemosensory neurons appear to regulate the life span of *C. elegans* via modulation of insulin/IGF-1 signaling (IIS) components, including insulin/IGF-1 receptor homolog DAF-2 and the FOXO transcription factor DAF-16 (Apfeld and Kenyon 1999; Alcedo and Kenyon 2004). Nevertheless, the identities of actual environmental cues generating a longevity response and endocrine signals transmitting these longevity signals that regulate IIS

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remain elusive. Some of the insulin-like peptides (ILPs), the predicted ligands of the DAF-2 receptor, are reasonable candidates that could transmit the longevity-influencing signals from the sensory neurons to the IIS. The *C. elegans* genome contains 40 genes encoding putative ILPs, and many of these are expressed in sensory neurons (Pierce et al. 2001; Cornils et al. 2011; Chen et al. 2013; Ritter et al. 2013). Hence, at least some of the ILPs may modulate longevity through IIS acting in the sensory neurons.

In this study, we found that the sensory TAX-2/TAX-4 cation channel modulates the expression of specific sets of ILPs. Two of these ILPs are *ins-6* and *daf-28*, which we show mediate the longevity effects of the sensory mutations by decreasing the activity of DAF-16/FOXO in a tissue-nonautonomous manner. We also found that food cues act as environmental life span-regulating signals that induce *ins-6* in a subset of chemosensory neurons. Moreover, optogenetic activation of specific sensory neurons increases the expression of *ins-6* and reduces DAF-16/FOXO activity, which decreases life span in food-deprived conditions. Thus, specific ILPs act as systemic neuroendocrine factors that mediate the sensory regulation of *C. elegans* longevity upon changes in food conditions. Our finding provides mechanistic insights regarding how environmental cues alter organismal longevity by fine-tuning the IIS via neuroendocrine peptides.

## Results

### *Sensory* tax-2/tax-4 *mutations extend* C. elegans*' life span by activating DAF-16/FOXO in multiple tissues*

We sought to identify the mechanisms through which sensory neurons mediate the longevity effects of different sensory cues. Specifically, we focused our study on *tax-2* and *tax-4*, which together encode a cyclic nucleotide-gated ion channel required for sensory function (Coburn and Bargmann 1996; Komatsu et al. 1996). Mutations in *tax-2* and/or *tax-4* shortened life span at 25°C (high temperature) (Lee and Kenyon 2009) but increased life span at 15°C (low temperature) (Fig. 1A; Supplemental Fig. S1A, B). Interestingly, while the short life span of *tax-2* mutants at 25°C is due to impaired sterol hormone signaling (Lee and Kenyon 2009), the *tax-2* mutation still extended the life span of the sterol hormone receptor *daf-12*-null mutants at 15°C (Supplemental Fig. S1C). This suggests that the extended longevity of these mutants at lower temperatures is independent of sterol signaling. In contrast, the long life phenotype of *tax-2* mutants at 15°C requires *daf-16/FOXO* (Fig. 1A), a downstream effector of the IIS pathway (Lin et al. 1997; Ogg et al. 1997). As would be predicted, loss-of-function mutations in this ion channel also increased the expression of known transcriptional targets of *daf-16/FOXO* (Fig. 1C–E; Supplemental Fig. S2). Consistent with the idea that *tax-2* affects life span through IIS, we observed that the *tax-2* mutation did not

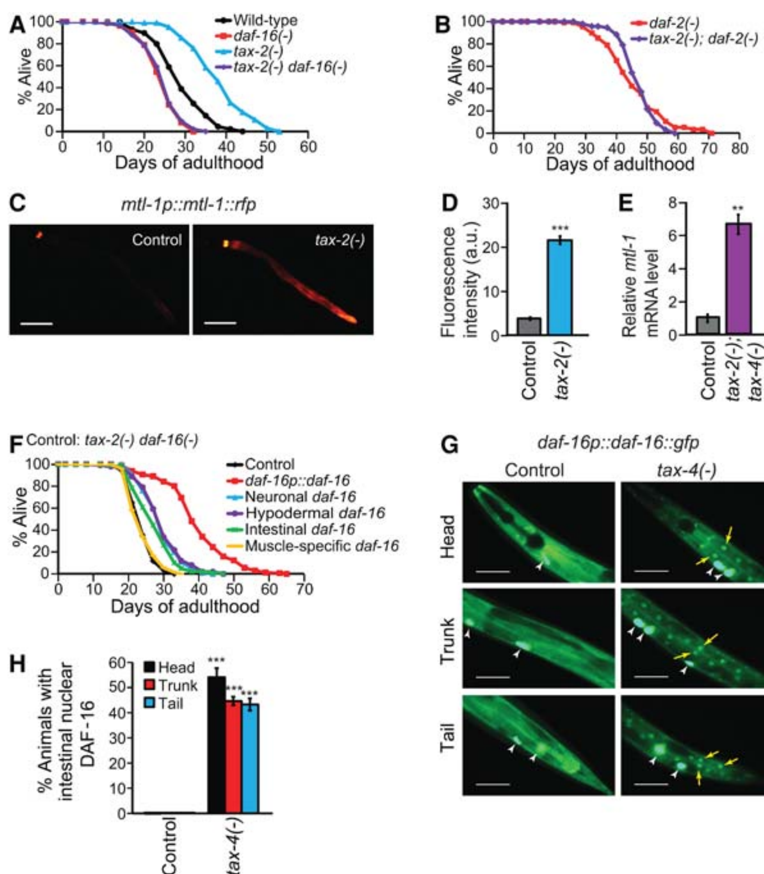


**Figure 1.** Sensory *tax-2*/*tax-4* mutations extend life span via activating DAF-16/FOXO in a tissue-nonautonomous manner. (*A*,*B*) The effects of *daf-16(mu86)* [*daf-16(−)*] (*A*) and *daf-2(e1370)* [*daf-2(−)*] (*B*) on the longevity of *tax-2(p671)* [*tax-2(−)*] animals at 15°C. (*C*,*D*) Fluorescence images of a FOXO reporter, *mtl-1p::mtl-1::rfp*, in wild-type (control) or *tax-2(−)* animals (*C*) and the quantification (*D*) (three independent trials, ≥90 total worms). (a.u.) Arbitrary unit. Bars, 50 µm. (*E*) The levels of *mtl-1* mRNA in wild-type and *tax-2(p671); tax-4(p678)* [*tax-2(−); tax-4(−)*] animals measured by quantitative RT–PCR (qRT–PCR). *n* = 3. (*F*) The life span of *tax-2(−) daf-16 (−)* animals expressing *daf-16::gfp* from its own (*daf-16p::daf-16::gfp*), a neuronal, a hypodermal, an intestinal, or a muscle-specific promoter. (*G*,*H*) The effects of *tax-4(p678)* [*tax-4(−)*] on the subcellular localization of DAF-16::GFP in the intestinal cells (*G*) ([yellow arrows] nuclear DAF-16::GFP; [white arrowheads] coinjection marker *ofm-1p::gfp*) and the quantification in the head, trunk, and tail regions (*H*) (at least three independent trials, >60 total worms). Error bars represent SEM. (***) $P < 0.001$; (**) $P < 0.01$, two-tailed Student's *t*-test. See Supplemental Table S1 for additional repeats and statistical analysis for the life span data shown in this figure.

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further increase the 15°C long life phenotype of insulin/IGF-1 receptor *daf-2* mutants (Fig. 1B). Our data are similar to previous findings at 20°C (Apfeld and Kenyon 1999) but unlike findings at 25°C (Lee and Kenyon 2009), which suggests that sensory function regulates longevity via distinct signaling pathways under different environments.

Interestingly, the *tax-2*-dependent modulation of IIS in affecting low-temperature life span (Fig. 1A,B) is just as specific as the *tax-2*-dependent modulation of sterol signaling in affecting high-temperature life span (Lee and Kenyon 2009). At 15°C, the *tax-2* mutation lengthened the life span of animals carrying mutations in heat-shock transcription factor 1 (*hsf-1*) (Hsu et al. 2003; Morley and Morimoto 2004), the dietary restriction mimetic *eat-2* (Lakowski and Hekimi 1998), or the mitochondrial protein gene *isp-1* (Supplemental Fig. S1D–F; Feng et al. 2001).

Since *tax-2* is expressed only in neurons (Coburn and Bargmann 1996), we next asked whether the long life phenotype of *tax-2* mutants was sufficiently explained by increased DAF-16/FOXO activity in neurons. Surprisingly, the expression of a functional DAF-16::GFP fusion protein in neurons only partly rescued the short life span of *tax-2(−) daf-16(−)* double mutants (Fig. 1F). This partial restoration of longevity was similar to nonneuronal *daf-16::gfp* expression in the hypodermis or intestine but not in the muscle (Fig. 1F). In contrast, a *daf-16* promoter-driven DAF-16::GFP (*daf-16p::daf-16*) fully rescued the life span phenotype of the double mutants (Fig. 1F). These results suggest that DAF-16/FOXO activity in several tissues contributes to the longevity of *tax-2* mutants. This is also consistent with the induction of DAF-16/FOXO target genes in the intestine and hypodermis by *tax-2* and *tax-4* mutations (Fig. 1C; Supplemental Fig. S2A,C,G,I). Thus, a neuroendocrine signal appears to link *tax-2* and *tax-4* activity in neurons to DAF-16/FOXO activity in other tissues.

We then asked how *tax-2/tax-4* mutations increase DAF-16/FOXO activity. Unlike wild type, which has diffused intracellular DAF-16::GFP, long-lived *daf-2* insulin/IGF-1 receptor mutants or sensory *daf-10* mutants displayed enhanced nuclear translocation of DAF-16/FOXO (Lin et al. 2001). Likewise, long-lived *tax-4(−)* mutants at 15°C displayed enhanced nuclear DAF-16::GFP (Fig. 1G,H). This suggests that the TAX-2/TAX-4 ion channel regulates the nucleocytoplasmic shuttling of nonneuronal DAF-16/FOXO in a tissue-nonautonomous manner.

### *Sensory neurons regulate longevity by modulating the expression of specific ILPs*

How, then, does sensory function nonautonomously regulate longevity? The ILPs are reasonable candidates mediating this nonautonomous regulation, since many ILPs are expressed in sensory neurons (Pierce et al. 2001; Li et al. 2003; Cornils et al. 2011; Chen et al. 2013; Ritter et al. 2013). Because the transcriptional expression of multiple ILPs can be coordinately regulated (Chen et al. 2013; Ritter et al. 2013; Fernandes de Abreu et al. 2014), we determined whether the *tax-2(−); tax-4(−)* mutations affected the steady-state mRNA levels of the ILPs. Among the 33 ILP genes that we examined by quantitative RT–PCR (qRT–PCR), *tax-2(−); tax-4(−)* mutations significantly increased or decreased the expression of 10 ILP genes (Fig. 2A,B). We also confirmed that the expression of two ILPs, INS-6 and DAF-28, was decreased by the *tax-4* mutation by using fluorescence reporter transgenic animals (Fig. 2C,D). Thus, the sensory TAX-2/TAX-4 ion channel dynamically regulates the mRNA levels of many, but not all, ILPs.

Next, we analyzed the functional significance of this differential expression of ILPs in the longevity of sensory mutants. Previous studies and our current data showed that individual loss-of-function mutations in ILP genes displayed either weak or no life span phenotypes, perhaps due to functional redundancy (Fig. 3A,B; Supplemental Fig. S3; Cornils et al. 2011; Ritter et al. 2013; Fernandes de Abreu et al. 2014). We therefore overexpressed several ILPs that were down-regulated in sensory *tax-2* and *tax-4* mutants. We specifically focused on *ins-6* and *daf-28*, which not only are expressed in ASI and ASJ sensory neurons that coexpress *tax-2* and *tax-4* (Coburn and Bargmann 1996; Komatsu et al. 1996; Li et al. 2003; Cornils

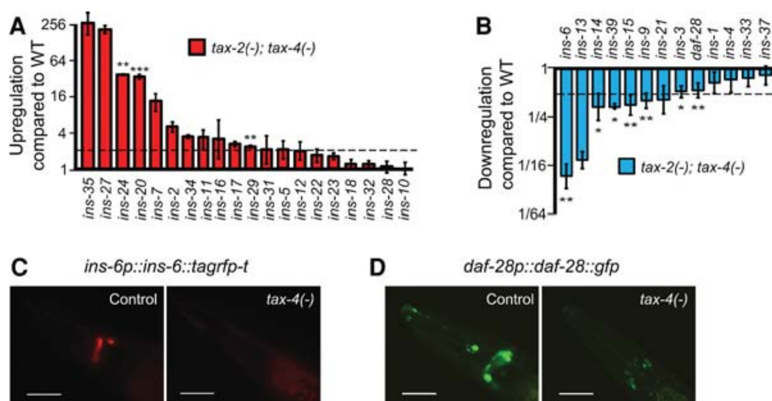


**Figure 2.** Sensory *tax-2/tax-4* mutations regulate the expression of various ILPs. (*A*,*B*) The effects of *tax-2(−); tax-4(−)* on mRNA levels of 33 ILP genes, determined by qRT–PCR. (Dotted line) Twofold increase or decrease. The graph was drawn in $\log_2$ scale. Note that although the mean expression levels of several ILPs, including *ins-35*, *ins-27*, *ins-7*, and *ins-13*, were highly altered in *tax-2(−); tax-4(−)* mutants, the changes in expression were not statistically significant. Error bars represent SEM. (***) $P < 0.001$; (**) $P < 0.01$; (*) $P < 0.05$, two-tailed Student's *t*-test. (*C*,*D*) The expression of an RFP-fused INS-6 (*ins-6p::ins-6::tagrfp-t*) (*C*) or GFP-fused DAF-28 (*daf-28p::daf-28::gfp*) (*D*) was decreased by mutations in *tax-4* compared with that in wild-type animals (control). Bar, 50 μm. Please note that the expression of *daf-28p::gfp* (*daf-28* promoter-driven *gfp* transgene) was shown to be higher in *tax-4(−)* mutants than in wild-type animals (Li et al. 2003). This difference may have originated from the fact that *daf-28p::gfp* is a transcriptional fusion transgene and that they measured the GFP expression at the L3 stage, whereas we did so at the young adult stage.

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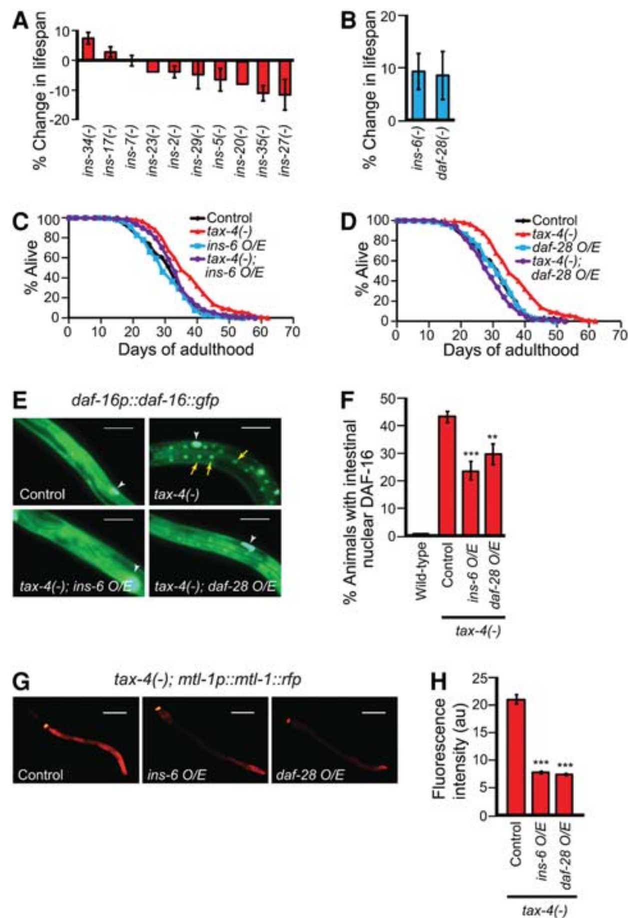


**Figure 3.** Overexpression of *ins-6* or *daf-28* decreases longevity and DAF-16/FOXO activity in sensory *tax-4* mutants. (*A*,*B*) The life span changes by mutations in selected ILPs whose gene expression was increased (*A*) or decreased (*B*) in *tax-2(−); tax-4(−)* animals. See Supplemental Table S2 for additional repeats and statistical analysis for the life span data shown in this figure. (*C*,*D*) Suppression of *tax-4(−)* longevity by overexpression (O/E) of *ins-6* (*C*) or *daf-28* (*D*). Note that transgenic animals that express low copies of the *ins-6* transgene also suppressed the long life span of *tax-4* mutants (Supplemental Table S2). (*E*,*F*) The effect of *ins-6* or *daf-28* overexpression on the nuclear localization of DAF-16::GFP in *tax-4(−)* worms (*E*) ([yellow arrows] nuclear DAF-16::GFP; [white arrowheads] coinjection marker *ofm-1p::gfp*) and the quantification (*F*). $n \geq 6$, >100 total worms per data set. Bar, 50 µm. (*G*,*H*) The effect of *ins-6* or *daf-28* overexpression on the induction of *mtl-1p::mtl-1::rfp* in *tax-4(−)* animals (*G*) and the quantification (*H*) ($n = 3$, >100 total worms). Error bars represent SEM. (***) $P < 0.001$; (**) $P < 0.01$, two-tailed Student's *t*-test.

et al. 2011) but their individual mutations also significantly increased life span (Fig. 3B; Supplemental Fig. S3K,L). Importantly, overexpression of *ins-6* or *daf-28* under their endogenous promoters suppressed the long life span of *tax-2(−)* and *tax-4(−)* mutants without shortening the life span of the wild type (Fig. 3C,D; Supplemental Fig. S4A–F). *ins-6* or *daf-28* overexpression also significantly decreased the nuclear localization of DAF-16::GFP in nonneuronal tissues (Fig. 3E,F; Supplemental Fig. S4G–J) and the induction of *daf-16* target genes in *tax-4* mutants (Fig. 3G,H; Supplemental Fig. S4K–N). These data suggest that sensory function regulates *ins-6* and *daf-28* expression and the activity of DAF-16/FOXO to modulate longevity.

### *Food-derived cues perceived by sensory neurons decrease life span by inducing* ins-6 *and down-regulating DAF-16/FOXO*

To identify the actual sensory cues that modulate ILP expression and DAF-16/FOXO activity, we tested two candidates: changes in food signals and temperatures. We found that *ins-6* and *daf-28* mRNA levels were decreased by *Escherichia coli* food deprivation but are hardly affected by temperature changes (Fig. 4A,B; Supplemental Fig. S5A,B). To characterize the nature of longevity-influencing food components, we treated animals with cell-free *E. coli* supernatants (Supplemental Fig. S5C), which contain potential food-derived odorants and tastants for *C. elegans* (Bargmann 2006). We found that the exposure of food-deprived animals to *E. coli* supernatants was sufficient to increase *ins-6* and *daf-28* mRNA levels (Fig. 4A,B). *E. coli* supernatant supplementation also partly reversed the effects of food deprivation on the DAF-16/FOXO nuclear translocation (Fig. 4C,D) and the induction of several DAF-16/FOXO target genes (Fig. 4E,F; Supplemental Fig. S5E,F). As previously reported (Smith et al. 2008), *E. coli* supernatant treatment partly suppressed the longevity caused by food deprivation (Fig. 4G). These data suggest that food cues can increase the expression of *ins-6* and *daf-28*, which in turn decrease DAF-16/FOXO activity and life span.

Several lines of evidence indicate that the ILP *ins-6* mediates the effects of food-derived cues on DAF-16/FOXO signaling and life span, acting in or downstream from sensory neurons. We found that *E. coli* supernatants did not affect the nucleocytoplasmic shuttling of DAF-16/FOXO and the long life span of sensory-defective *tax-4* mutants that are food-deprived (Fig. 5A,B). This suggests that *E. coli* supernatants indeed decrease FOXO activity and life span through intact sensory neurons. Overexpression of *ins-6* or *daf-28* decreased the nuclear localization of DAF-16/FOXO in wild-type animals upon food deprivation while not further reducing it upon treatment with food-derived cues, suggesting that food-derived cues and these ILPs act in the same pathway (Fig. 5C,D). We also found that overexpression of *ins-6* or *daf-28* was sufficient to diminish the nuclear localization of DAF-16/FOXO in both food-deprived and *E. coli* supernatant-treated *tax-4* mutants (Fig. 5E,F). These data are consistent with the idea that INS-6 and DAF-28 mediate the effects of food-derived cues on decreasing DAF-16/FOXO activity by acting in or downstream from sensory neurons. We found that *ins-6* overexpression shortened the life span of food-deprived animals (Fig. 5G), although *daf-28* overexpression did not (Fig. 5H). Thus, food-derived cues perceived by sensory neurons appear to decrease life span by increasing

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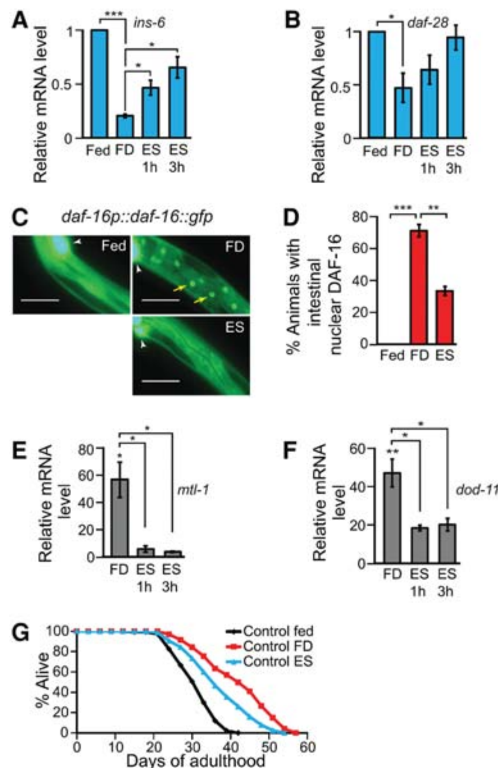


**Figure 4.** Sensory food cues regulate ILP expression and DAF-16/FOXO activity. (*A*,*B*) qRT–PCR analysis of *ins-6* (*A*) and *daf-28* (*B*) expression in fed, food-deprived (FD), or *E. coli* supernatant (ES)-supplemented animals. $n = 3$. (*C*,*D*) The effects of food deprivation or supplementation of *E. coli* supernatants on DAF-16/FOXO nuclear localization (*C*) ([yellow arrows] nuclear DAF-16::GFP; [white arrowhead] coinjection marker *ofm-1p::gfp*) and the quantification (*D*). $n = 3$, >30 total worms. Bar, 50 µm. (*E*,*F*) The mRNA levels of *mtl-1* (*E*) or *dod-11* (*F*) in food-deprived or *E. coli* supernatant-treated animals compared with those in fed animals using qRT–PCR. $n = 3$. Error bars represent SEM. (***) $P < 0.001$; (**) $P < 0.01$; (*) $P < 0.05$, two-tailed Student's *t*-test. (*G*) The effects of *E. coli* supernatant treatment on the life span of food-deprived worms. See Supplemental Table S3 for additional repeats and statistical analysis for the life span data.

the expression of ILPs, such as *ins-6*, which subsequently down-regulates DAF-16/FOXO.

### *INS-6 acts in sensory ASI and ASJ neurons to decrease DAF-16/FOXO activity and longevity upon changes in food-derived environmental cues*

We next asked whether the longevity regulatory roles of *ins-6* and *daf-28* can be generalized to other sensory mutants (Supplemental Fig. S6A–E). Overexpression of *ins-6* significantly suppressed the long life span of the sensory intraflagellar transport-defective *osm-5(−)* (Perkins et al. 1986) and TRPV ion channel-deficient *ocr-2(−)* (Tobin et al. 2002) mutant animals, whereas *daf-28* overexpression did not (Fig. 6A–D). Thus, *ins-6*, rather than *daf-28*, appears to be a more general longevity ILP signal from sensory neurons, leading us to focus further on *ins-6*.

*ins-6* is expressed in specific sensory neurons, ASI and ASJ, and has neuron-specific functions in development and behavior (Cornils et al. 2011; Chen et al. 2013). We tested whether *ins-6* from ASI or ASJ neurons decreased the longevity of sensory mutants. We found that *ins-6* acted from either neuron to shorten life span (Fig. 6E,F).

We then asked whether the activation of ASI or ASJ in food-deprived conditions was sufficient to shorten life span via ILP signaling by expressing the blue light-gated ion channel channelrhodopsin 2 (ChR2) from either neuron. The activation of ASI or ASJ by blue light triggered the gradual translocation of intestinal DAF-16::GFP from the nucleus to the cytosol in food-deprived animals only in the presence of all-*trans* retinal (ATR), a cofactor of ChR2 (Fig. 7A–D; Supplemental Fig. S7A,B; Supplemental videos 1–4). The ChR2-dependent activation of either ASI or ASJ neuron also increased *ins-6*

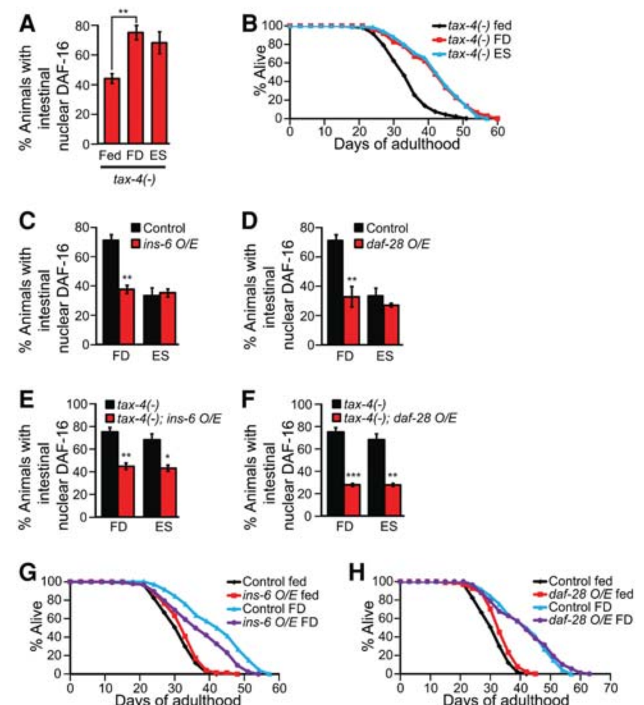


**Figure 5.** *ins-6* acts via sensory neurons to mediate the effects of food cues on DAF-16/FOXO activity and life span. (*A*) The effect of *E. coli* supernatant (ES) supplementation on DAF-16 nuclear localization in food-deprived (FD) *tax-4(−)* mutants. $n = 3$, >30 total worms. (*B*) The life span of fed, food-deprived, and *E. coli* supernatant-supplemented food-deprived *tax-4(−)* worms. See Supplemental Table S3 for additional repeats and statistical analysis for the life span data shown in this figure. (*C*,*D*) The effects of *ins-6* (*C*) or *daf-28* (*D*) overexpression (O/E) on the localization of DAF-16::GFP in food-deprived or *E. coli* supernatant-supplemented wild-type worms. $n = 3$, >30 total worms. (*E*,*F*) The effects of *ins-6* (*E*) or *daf-28* (*F*) overexpression on the localization of DAF-16::GFP in food-deprived or *E. coli* supernatant-supplemented *tax-4(−)* worms. $n = 3$, >30 total worms. (*G*,*H*) The effects of *ins-6* (*G*) and *daf-28* (*H*) overexpression on the life span of food-deprived worms. Error bars represent SEM. (***) $P < 0.001$; (**) $P < 0.01$; (*) $P < 0.05$, two-tailed Student's *t*-test.

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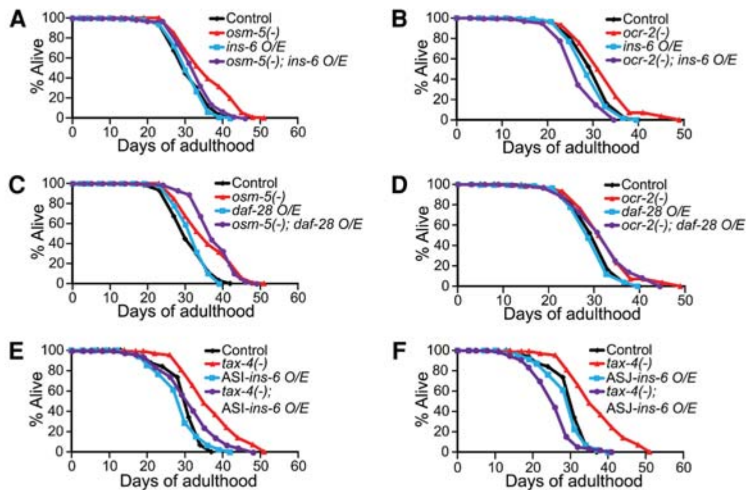


**Figure 6.** *ins-6* mediates the longevity of sensory mutants acting in ASI and ASJ neurons. (*A*,*B*) Overexpression (O/E) of *ins-6* suppressed the long life span of *osm-5(p813)* [*osm-5(−)*] (*A*) and *ocr-2(ak47)* [*ocr-2(−)*] (*B*) mutant worms. (*C*,*D*) Overexpression of *daf-28* did not suppress the long life span of *osm-5(−)* (*C*) or *ocr-2 (−)* (*D*) mutants. See Supplemental Table S4 for additional repeats and statistical analysis for the life span data shown in this figure. (*E*,*F*) The effects of ASI-specific (*E*) or ASJ-specific (*F*) *ins-6* expression on the life span of wild-type (control) and *tax-4(−)* animals. Note that ASI- or ASJ-specific expression of high or low copy of the *ins-6* transgene had similar effects on life span (Supplemental Table S4).

expression and decreased DAF-16/FOXO target gene expression (Fig. 7E–H). Importantly, the optogenetic activation of ASI or ASJ by blue light specifically shortened the life span of food-deprived worms (Fig. 7I,J; Supplemental Fig. S7C–F). Altogether, these data suggest that the activation of ASI or ASJ sensory neurons induces INS-6, which signals to distal tissues to down-regulate DAF-16/FOXO and decreases organismal life span.

## Discussion

Although the life span regulatory role of *C. elegans* sensory neurons has been known for more than a decade (Apfeld and Kenyon 1999; Alcedo and Kenyon 2004), much of the molecular mechanisms remained elusive. In the present study, we addressed how sensory neurons process environmental changes and relay the signals to distal tissues to modulate the life span of *C. elegans*. Our current report provides functional evidence showing that the ILP INS-6 acts as a neuroendocrine signal that regulates the longevity conferred by sensory cues via IIS. The inhibition of sensory neurons by deprivation of food cues decreases IIS through the down-regulation of INS-6, which could act as an agonist of DAF-2. We also showed that food-derived cues inhibit the nuclear localization of the downstream effector DAF-16/FOXO. Our

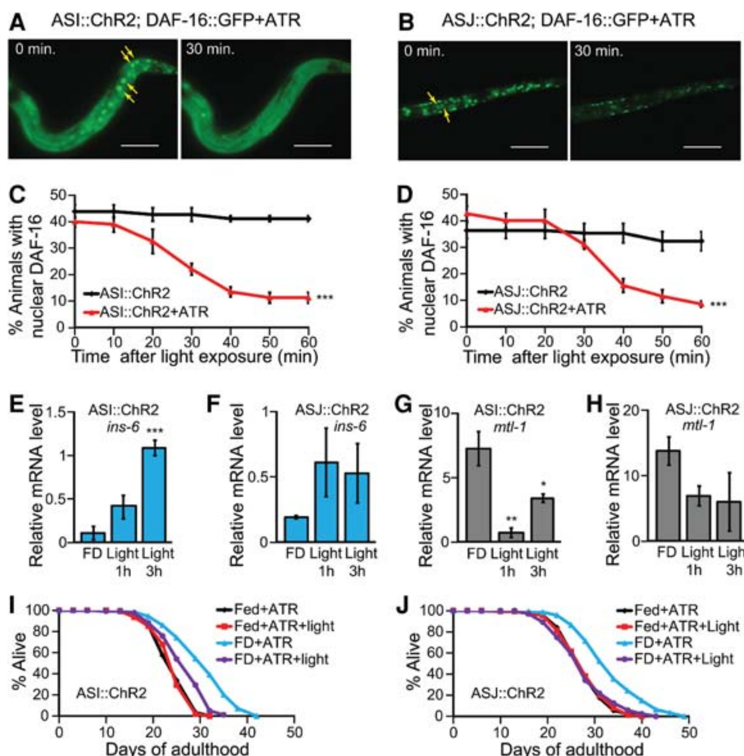


**Figure 7.** Optogenetic activation of ASI or ASJ neurons modulate sensory neuron-regulated longevity via INS-6 and DAF-16/FOXO. (*A*,*B*) The effects of optogenetic activation of ASI (*A*) or ASJ (*B*) neurons by using ChR2 on DAF-16::GFP localization in food-deprived (FD) animals. (Yellow arrow) Nuclear DAF-16::GFP. Bar, 50 µm. (*C*,*D*) Quantification of the *A* and *B* by performing time-course experiments. $n = 3$, >90 total worms. (***) $P < 0.001$, calculated using two-way ANOVA test. (*E*,*F*) Changes in the mRNA levels of *ins-6* upon optogenetic activation of ASI (*E*) ($n = 4$) or ASJ (*F*) ($n = 3$) neurons in food-deprived animals. Error bars represent SEM. (***) $P < 0.001$; (**) $P < 0.01$; (*) $P < 0.05$, two-tailed Student's *t*-test. (*G*,*H*) Changes in the mRNA levels of *mtl-1* upon optogenetic activation of ASI (*G*) ($n = 4$) or ASJ (*H*) ($n = 3$) neurons in food-deprived animals. (*I*,*J*) Life span of fed or food-deprived animals upon repetitive optogenetic activation of ASI (*I*) or ASJ (*J*) neurons with ATR. We found that treatment with either ATR or blue light alone did not affect life span (Supplemental Fig. S7C–F). See Supplemental Table S5 for additional repeats and statistical analysis for the life span data.

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study suggests that sensory neurons respond to the presence or absence of specific food-derived cues to modulate IIS and life span.

Sensory neurons will mediate many mechanisms, ranging from food level to food quality (Libert et al. 2007; Maier et al. 2010). Since we know that ILPs can have different activities in different physiological processes and have distinct neuronal expression patterns (for review, see Allen et al. 2015 and references therein), certain ILPs may mediate the DR effects on life span, whereas other ILPs may mediate the non-DR effects on life span. However, it remains unclear how all of these ILPs will affect the activities of the IIS pathway. Some of them might be agonists of the pathway, whereas others may antagonize or have no effect on the pathway. Since the expression of different ILPs is regulated by distinct environmental cues (Li et al. 2003; Cornils et al. 2011; Ritter et al. 2013), the variable effects of IIS under different dietary regimens (Greer and Brunet 2009; Mair et al. 2009) could be explained by the nature of the ILPs that are present versus those that are absent under these conditions.

Cell-nonautonomous regulation of longevity through IIS has previously been demonstrated in *C. elegans* (Apfeld and Kenyon 1999; Wolkow et al. 2000; Libina et al. 2003; Zhang et al. 2013). Loss of a functional *daf-2* gene in specific subsets of cells is sufficient to extend *C. elegans* life span (Apfeld and Kenyon 1998). Conversely, neuronal expression of *daf-2* can rescue the long life phenotype of *daf-2* mutants (Wolkow et al. 2000). At the same time, DAF-16/FOXO can act in multiple tissues (i.e., neurons, intestine, and hypodermis) to promote the longevity of *daf-2* mutants (Libina et al. 2003; Zhang et al. 2013). Here, we show that expression of *daf-16* in these same tissues contributes to the longevity of sensory mutants, which corroborates the life span-regulating role of DAF-16/FOXO in different tissue types. Furthermore, our study delineates a complete signaling pathway by specifying upstream environmental cues, sensory neurons, and neuroendocrine ILPs, which regulate downstream IIS components in various tissues that regulate longevity.

Many genes encoding IIS components that influence longevity also play roles in other physiological processes. For example, the ILPs INS-6 and DAF-28 regulate the animal's cold-shock response in a synergistic manner (Ohta et al. 2014). INS-6 and DAF-28 are also negative regulators of the developmental program known as dauer (Li et al. 2003; Cornils et al. 2011; Fernandes de Abreu et al. 2014), which is an alternative, hibernation-like larval stage that occurs under conditions of stress (Hu 2007). *daf-28* plays a more primary role in inhibiting dauer formation, whereas *ins-6* is more important in preventing dauer maintenance (Cornils et al. 2011). Consistently, *ins-6* and *daf-28* also appear to have distinct effects on aging. First, *daf-28::gfp* expression is increased during aging (Li et al. 2003), whereas *ins-6::gfp* is decreased (Ritter et al. 2013). Second, we show that the roles of *ins-6* and *daf-28* in the longevity of different sensory mutants diverge, as *ins-6* appears to be a more general life span regulator than *daf-28* (See Fig. 6). Thus, these two ILPs can have nonredundant and specific functions in development and adult life span. However, as we functionally tested only two of the potential insulin/IGF-1 receptor DAF-2 agonists, INS-6 and DAF-28, overexpression of some other ILPs may also decrease the activity of DAF-16. It will be interesting to determine the role of other ILPs in the life span regulation of sensory mutants in future studies.

An intriguing feature of our study is that the *tax-2* and *tax-4* sensory mutations decrease or increase the expression of specific sets of ILPs in different ways (Fig. 2). Recently, the *C. elegans* ILP family has been shown to consist of an ILP-to-ILP regulatory network (Fernandes de Abreu et al. 2014). Hence, changes in the expression of one ILP may lead to changes in the expression of other ILPs. This is illustrated by the up-regulation of *ins-35* when *ins-6* expression is down (Fig. 2A,B), which would be expected from the network organization that has been previously described (Fernandes de Abreu et al. 2014). However, some of the observed changes in ILP expression at a low temperature, 15°C (this study), do not necessarily fit all of the predictions that could arise from the ILP network that has been delineated at a higher temperature, 20°C (Fernandes de Abreu et al. 2014). This could mean that the state of the ILP network is dynamic and changes under different environments and thus is subject to sensory regulation.

Some sensory neurons only affect life span under certain environments (Maier et al. 2010), since these neurons, which detect a given set of environmental cues, would presumably only affect longevity based on the presence of these cues. However, a neuron can also have opposing activities within a given physiological process. The modulation of such a neuron's activities (also known as neuromodulation) will depend on changes in the neuron's local environment as well as the animal's global environment (Strand 1999; Marder 2012). For example, the sensory neuron ASI inhibits longevity in well-fed animals (Alcedo and Kenyon 2004) but promotes longevity in diet-restricted animals (Bishop and Guarente 2007), which suggests that food-derived cues will change ASI's local environment. In this study, we show that a second sensory neuron, ASJ, has opposing effects on life span and that its longevity effects again depend on environmental context. ASJ lengthens life span at 20°C (Alcedo and Kenyon 2004) but shortens life span at the lower temperature of 15°C through the ILP INS-6 (this study). Together, these studies show that the neuronal effects on life span will involve different types of environmental cues (e.g., food-derived cues and temperature) (for review, see Allen et al. 2015), which could change a neuron's local environment and thus its consequent effects on life span.

The present work provides direct evidence for the role of ILPs in the regulation of longevity in *C. elegans* sensory mutants. Although the direct role of ILPs, insulin, or IGF in the regulation of life span by sensory neurons in *Drosophila* or mammals remains unknown, several intriguing clues are present. For example, in *Drosophila*, the ablation of ILP-expressing cells increases life span (Broughton et al. 2005), and the activation of c-Jun N-terminal kinase specifically in ILP-producing neurons results in the reduction of ILP production and life span

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extension (Wang et al. 2005). Similarly, long-lived *Drosophila* taste mutants have altered expression of certain ILPs (e.g., an increase in head expression of *dilp6*) (Ostojic et al. 2014), which has been shown earlier to promote longevity through up-regulation of the IIS effector *dFOXO* (Bai et al. 2012). Together, these reports support the idea that neuronal ILPs act as mediators of life span regulation in flies.

In mammals, blood glucose levels alter the secretion of insulin. Interestingly, the perception of food increases blood insulin levels in humans (Sjostrom et al. 1980), suggesting that chemosensation also directly influences insulin signaling in mammals. In addition, a recent study in mice illustrates that pain perception alters longevity through the regulated secretion of pancreatic insulin (Riera et al. 2014). Thus, other sensory modalities may have a regulatory function in mammalian life span by altering IIS through modulation of ILPs, such as insulin, IGF1/2, or the relaxin subfamily (Sherwood 2004).

## Materials and methods

### *Strains*

Strains used in this study are described in the Supplemental Material.

### *Preparation of* E. coli *OP50 cell-free supernatant*

Cell-free *E. coli* OP50 supernatant was obtained as described (Smith et al. 2008) with some modifications. Briefly, *E. coli* OP50 was grown overnight at 37°C and centrifuged at 4000 rpm for 5 min, and the supernatant was filtered with a 0.2-µm pore size filter.

### *Life span analysis*

Life span assays were conducted as described previously (Lee et al. 2010; Kim et al. 2014), starting at day 1 of adulthood. OASIS (Online Application for Survival Analysis, http://sbi.postech.ac.kr/oasis) was used for statistical analysis of the data (Yang et al. 2011), and *P*-values were calculated using the log rank (Mantel-Cox) method. Strains were grown for at least two generations at 15°C before being used for life span assays at 15°C. Prefertile young adult worms were transferred to plates containing 5 µM 5-fluoro-2′-deoxyuridine (FUdR) (Sigma-Aldrich) to prevent their progeny from developing unless stated otherwise. Recent studies suggest that FUdR confers stress resistance by activating a heat-shock response (Brunquell et al. 2014) and increases life span under mild osmotic stress conditions (Anderson et al. 2016). We noticed that these studies used relatively high concentrations of FUdR (at least 25 µM). In contrast, we used 5 µM FUdR for most of our life span assays. In addition, we assayed non-FUdR-treated animals to confirm the life span phenotypes of key sensory and ILP mutants as well as ILP transgenic animals. For life span assays with food-derived cues, prefertile young adult worms were transferred to streptomycin-resistant *E. coli* OP50-seeded plates. Three days later, the worms were transferred to new OP50-seeded plates. At the sixth day, worms were transferred to OP50-seeded plates for setting up fed groups or to empty agar plates for food-deprived groups. Experimental groups were treated with 100 µL of filtered supernatant of overnight *E. coli* culture, which was grown in Luria broth (LB) medium supplemented with streptomycin every 3 d, whereas control groups were treated with 100 µL of LB–streptomycin medium only. For life span assays using optogenetics, the worms were grown on OP50-seeded plates in the presence or absence of ATR (Sigma-Aldrich) for at least two generations at 15°C before conducting life span analysis. ATR-treated animals were always maintained on ATR-treated plates (both OP50-seeded and empty unseeded plates). ATR was dissolved in ethanol at a concentration of 100 mM. The *E. coli* OP50 culture on the NGM (nematode growth medium) plates or unseeded plates were supplemented with 50 µL of 10 mM ATR diluted in M9 buffer to a final concentration of 100 µM. Control plates were treated with 50 µL of M9 buffer with 10% ethanol. Prefertile young adult worms were transferred to OP50-seeded plates and, 3 d later, transferred to new OP50-seeded plates. At the sixth day, fed groups were transferred to OP50-seeded plates, and food-deprived animals were transferred to empty agar plates. Light-exposed groups were subjected to blue light (~470 nm) every day for 2 min. For all the life span experiments, at least five plates were used per condition unless some plates were discarded due to bacterial or fungal contamination. For the majority of the experiments, 25 animals per plate were used for a total of 150 animals per trial. Most of the life span assays were performed at least twice per condition by at least two independent researchers. We noticed that the censoring rate is higher for life span assays that are conducted in experiments at 15°C, and therefore, on average, 38% of the animals were censored. Animals that ruptured, bagged, or crawled off the plates were censored but included in the life span analysis as censored worms.

### *Microscopy and quantification of fluorescence*

Fluorescence imaging was conducted as reported previously (Lee et al. 2015). The images of transgenic animals were captured using an AxioCam (Zeiss Corporation) attached to a HRc Zeiss Axioscope A.1 (Zeiss Corporation). Quantification of GFP intensity was carried out using ImageJ (National Institutes of Health, http://rsbweb.nih.gov/ij). The normalization of the mean fluorescence intensity was achieved by subtracting mean fluorescence intensity of pictures of nontransgenic control worms taken with the same exposure time. Levamisole (2 mM) was used for immobilization of the worms on 2% agarose pads before taking pictures. *P*-values were calculated using the unpaired Student's *t*-test (two-tailed). For the experiments measuring the localization of DAF-16::GFP, gravid adults were synchronized for 12 h, and the progeny were transferred to empty plates at L2/L3 larval stages after washing three times with M9 buffer. After 24 h of food deprivation, the worms were scored for nuclear localization of DAF-16::GFP. For food cue experiments, the nuclear localization of DAF-16::GFP was scored after 100 µL of *E. coli* supernatant (overnight bacterial culture in LB–streptomycin medium) or control (LB–streptomycin medium only) treatment. For optogenetic activation of sensory neurons, animals were food-deprived for 24 h in the presence or absence of ATR and subjected to blue light (~470 nm) for ChR2 excitation for 2 min. Changes in the intestinal nuclear localization of DAF-16::GFP were recorded every 1 min for time-lapse experiments and every 10 min for quantification during a period of 1 h. The images used in Figure 7, A and B, are the same images used in the videos. We created a video with the images that were taken every minute during the time span of 30 min. Although the quantitation of fluorescence in transgenic animals was not scored blindly, many of the experiments were replicated independently in several trials by different researchers. For example, the quantitation of fluorescence of transgenic animals expressing DAF-16 target genes in a *tax-2(p671)* mutant background as well as DAF-16 subcellular localization and

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DAF-16 target gene expression in a *tax-4(p678)* mutant background was performed by independent researchers; the results were very similar. In addition, optogenetic analysis of the changes in DAF-16 subcellular localization was performed and reproduced independently by two researchers at two different institutions.

### *Quantitative real-time PCR analysis*

Synchronized L4 animals grown at 15°C were used for RNA extraction and subsequent experiments. Extraction, purification, and reverse transcription of RNA were performed as described (Seo et al. 2015) with some modifications. qPCR from the cDNA was carried out using a 7300 real-time PCR system (Applied Biosystems) and analyzed by the comparative Ct method described in the manufacturer's manual. The average values of the mRNA levels of *nhr-23* and/or *ama-1* genes were used for normalization. The average of at least two technical repeats was applied for each biological data point. For the qRT–PCR analysis of sensory food cue experiments, wild-type worms at L2/L3 stages were food-deprived for 24 h, treated with *E. coli* supernatants (bacteria were grown overnight in LB–streptomycin medium) or LB–streptomycin control medium, and harvested 1 or 3 h after treatment. For the qRT–PCR analysis using ChR2-expressing transgenic animals, worms at L2/L3 stages were food-deprived for 24 h in the presence of ATR and subjected to blue light (~470 nm) for ChR2 excitation for 2 min. Subsequently, the animals were harvested 1 or 3 h after light exposure. Please see the Supplemental Material for primer sequences.

### *Generation of transgenic constructs*

To clone *ins-6*, the promoter and coding region of *ins-6* (~2.1 kb) was PCR-amplified from *C. elegans* genomic DNA using 5′-TGCAGGTCGACTCTAGATGTTGCTCCACTGATTGCAGCT-3′ and 5′-CCCTTAGACACCATCGCTGGACAAGCAGATCTTATG-3′ primers and inserted into the pPD95.75 vector (a gift from Dr. Andrew Fire), which was previously modified and contained *tagrfp-t* and *unc-54* 3′ untranslated region (UTR), digested with XbaI and KpnI. To clone *trx-1p::ChR2::gfp* and *str-3p::ChR2::gfp* expression vectors, *ChR2* cDNA was PCR-amplified from the pAAV-TRE-hChR2(H134R)-EYFP vector (a gift from Dr. Joung-Hun Kim) using 5′-GCAGGTCGACTCTAGAGCCACCATGGACTATGGCGG-3′ and 5′-TCATTTTTTCTACCGGTACCGCTGGCACGGCTCCGGCCTCGGC-3′ primers and inserted into the pPD95.75 vector, which contained *gfp* and *unc-54* 3′ UTR, digested with XbaI and KpnI. *trx-1* (~1 kb) and *str-3* (~3.1 kb) promoters were PCR-amplified from *C. elegans* genomic DNA using 5′-GAAATGAAATAAGCTTAGAGAATGGATACCTGATCATTC-3′ and 5′-CCATGGTGGCTCTAGATCTGTTACCATATCAGCAAGCTC-3′, and 5′-GAAATGAAATAAGCTTGCTGGTGAAGATTTGTTCAAGGA-3′ and 5′-CCATGGTGGCTCTAGAGTTCCTTTTGAAATTGAGGCAGT-3′ primers, respectively. Amplified *trx-1* and *str-3* promoters were inserted into the upstream *ChR2* cDNA by using HindIII and XbaI sites in the pPD95.75 vector. All cloning reactions were performed using In-Fusion cloning (Clontech) by following the manufacturer's instructions.

### *Generation of transgenic animals*

*ins-6p::ins-6::tagrfp-t*, *trx-1p::ChR2::gfp*, or *str-3p::ChR2::gfp* expression vectors (25 ng/µL) were coinjected with an injection marker, *myo-3p::rfp* (75 ng/µL), into the gonad of day 1 adult wild-type animals. The *rol-6D* vector was used as a coinjection marker for the *ins-6p::ins-6::tagrfp-t* (25 ng/µL) expression vector.

### *Dye filling assay*

Dye filling assay was performed as previously described (Gaglia et al. 2012). Well-fed worms were washed three times with M9 buffer and centrifuged at 2000 rpm to spin down the worms. The worms were then resuspended in 1 mL of M9 buffer and 5 µL of 2 mg/mL DiI stock solution. The worms were kept on a slow shaker for 3 h and then washed three times with M9 buffer and transferred to agar pads for visualization under a HRc Zeiss Axioscope A.1 (Zeiss Corporation).

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