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·Original Article·

# Genes required for the functions of olfactory AWA neuron regulate the longevity of *Caenorhabditis elegans* in an insulin/IGF signaling-dependent fashion

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**Abstract: Objective** To investigate the interaction between the genes required for the functions of AWA olfactory neuron and insulin/IGF signaling in regulating the longevity of nematode *Caenorhabditis elegans* (*C. elegans*). **Methods** The mutants that had loss-of-function mutation of the genes required for AWA, AWC, ASE, and AFD sensory neurons were employed. Lifespan, the speed of pharynx pumping, the intestinal autofluorescence, the dauer formation, and the brood size were examined. Rescue experiments were performed to confirm the role of the genes required for the functions of AWA neuron in regulating lifespan. Moreover, genetic interactions between genes required for the functions of AWA neuron and insulin/IGF signaling were investigated. **Results** Mutations of *odr-7*, *odr-2*, and *odr-3* genes required for the functions of AWA neuron significantly increased the mean lifespan of nematodes and slowed the accumulation of intestinal autofluorescence. Besides, these mutations were closely associated with higher pumping rates during aging. However, mutation of *odr-7*, *odr-2,* or *odr-3* did not obviously affect the brood size or the dauer formation, and the regulation of longevity by *odr-7*, *odr-2,* and *odr-3* was temperature-independent. In contrast, mutations of genes required for the functions of ASE, AWC, and AFD sensory neurons did not influence the nematode lifespan. Moreover, expression of *odr-7*, *odr-2* and *odr-3* in AWA neuron could completely or largely restore the altered lifespan in *odr-7*, *odr-2* and *odr-3* mutants. Furthermore, genetic interaction assay demonstrated that the extended lifespan in *odr-7* mutant could be suppressed by *daf-16* mutation and enhanced by *daf-2* or *age-1* mutation, whereas *mev-1* and *pha-4* were not required for the long lifespan of *odr-7* mutant. **Conclusion** The genes required for the function of AWA sensory neuron could regulate the nematode longevity in an insulin/IGF signaling-dependent fashion in *C. elegans*.

**Keywords:** longevity; ODR-7; AWA olfactory neuron; insulin/IGF signaling; genetic interaction; *C. elegans*

## 1 Introduction

The nematode *Caenorhabditis elegans* (*C. elegans*) was first introduced by Klass as an experimental system for the study of aging[1]. So far, it has been proven that *C. elegans* is a very useful tool to study the genetics of longevity[2]. Neuronal control of longevity is an important physiological process in animals[3]. Tissue-specific rescue experiments suggest that neuron is the major site for Ins/IGF-dependent lifespan extension[4]. Moreover, it has been found that the sensory neurons can link environmental cues to lifespan through DAF-16 activity[5]. Cilia, the membrane-bound mi-

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crotubule-based structures, function as sensory receptors and are only found at the dendritic endings of sensory neurons[6]. Mutations that cause defects in sensory cilia or their supporting cells, or in sensory signal transduction, can extend lifespan[5].

Sensory cilia are present in 60 of the 302 neurons in *C. elegans* hermaphrodite. The amphids, formed by a pair of lateral sensillae in the head, are the principal sensory neurons[6]. As identified by behavior assay and by killing defined neurons using a laser microbeam, the amphid neurons are implicated in the chemosensory and the thermosensory behaviors[6-8]. Each of the 2 bilaterally symmetric amphids in the nematode head contains the endings of 12 types of sensory neurons (ADF, ADL, ASE, ASG, ASH, ASJ, ASI, ASK, AWA, AWB, AWC, and AFD)[8]. Mutations of genes that are thought to affect only a subset of sensillae induce a long-lived phenotype, and ablation of the 2 amphid sheath cells with a laser microbeam also extends lifespan. These results suggest that in nematodes, amphid sensory neurons at least constitute a part of the neurons whose activity influences lifespan[5,9]. Moreover, the lifespan of nematodes can be affected by environmental cues and these cues are perceived and integrated by specific chemosensory neurons[10]. For example, certain gustatory neurons inhibit longevity, whereas others promote longevity, probably by influencing insulin/IGF-1 signaling[10]. Olfactory neurons also affect longevity by acting in a distinct pathway that involves the reproductive system[10].

In the present study, the possible involvement of genes required for the functions of AWA olfactory sensory neuron in the longevity control was further investigated. Due to the fact that AWA sensory neurons share the same corresponding interneuron (AIY) with other sensory neurons (ASE, AWC, and AFD), the possible involvement of genes required for functions of ASE, AWC, and AFD sensory neurons in longevity control was also studied. In addition, the possible genetic interactions between *odr-7* gene required for functions of AWA sensory neurons and some known important signaling pathways including the insulin/IGF signaling pathway, in regulating lifespan in *C. elegans* were examined. The results will help further understand the importance of the genes required for AWA sensory neuron in longevity regulation in nematodes.

## 2 Materials and methods

**2.1 Preparation of nematode cultures** Totally, 10 genetic loci were selected. They were *daf-11*, *daf-21*, *che-1*, *odr-7*, *odr-1*, *odr-3*, *ttx-1*, *odr-2*, *odr-10*, and *gcy-5*. These genes are required for the functions of AWA, AWC, ASE, or AFD sensory neurons (Table 1). Nematodes used in the present study included wild-type N2, *daf-11(m47)*, *daf-21(p673)*, *che-1(p674)*, *odr-7(ky4)*, *odr-1(n1936)*, *odr-3(n2150)*, *ttx-1(p767)*, *odr-2(n2145)*, *odr-10(ky32)*, *gcy-5(ok930)*, *rrf-3 (pk1426)*, *daf-2(e1370)* and *daf-16(mu86)* mutants, originally obtained from the Caenorhabditis Genetics Center (funded by National Institutes of Health-National Center for Research Resource, USA). All the used mutants were loss-of-function mutants. The strains were maintained on nematode growth medium (NGM) plates seeded with *Escherichia coli* OP50 at 20 ºC as described previously[11]. *daf-16(RNAi)*, *mev-1(RNAi)* and *pha-4(RNAi)* bacteria were generous gifts from Dr. Chonglin Yang. The pPD95_75 vector containing the green fluorescent protein (*gfp*) fragment was used for DNA construction. Gravid nematodes were washed off the plates into centrifuge tubes, and were lysed with a bleaching

**Table 1. Genes required for the functions of amphid sensory neurons in *C. elegans***

| Genes | Protein encoded by the gene | Sensory neuron affected |
|---|---|---|
| *odr-7* | Member of a nuclear receptor superfamily | AWA |
| *odr-10* | Member of a 7-transmembrane family | AWA |
| *odr-2* | A predicted membrane-associated protein | AWA, AWC |
| *odr-3* | Gi-like G-α protein | AWA, AWC, ASH |
| *che-1* | C2H2-type zince finger-containing transcription factor | ASE |
| *gcy-5* | A putative guanylyl cyclase | ASE |
| *odr-1* | A putative guanylyl cyclase | AWC |
| *daf-11* | Guanylate cyclase | ASE, AWC |
| *daf-21* | Member of a HSP90 family of molecular chaperones | ASE, AWC |
| *ttx-1* | OTD/OTX homeodomain protein | AFD |

mixture (0.45 N NaOH, 2% HOCl). Age-synchronous populations of nematodes (L4-stage larvae) were obtained as described previously[12].

**2.2 Lifespan assay** The lifespan assays were performed at 15 ºC, 20 ºC, or 25 ºC, respectively, basically as described previously[3,13]. In this test, the hermaphrodites were transferred daily onto the new NGM plates without food (*E. coli* OP50) for the first 4 d of adulthood. The nematodes were checked every 2 d. When they did not move even after repeated taps with a pick, they would be scored as dead. Curves of lifespan were representative of 3 trials. Data were statistically analyzed using a 2-tailed 2 sample *t*-test (Minitab Ltd., Coventry, UK). Pharynx pumping rates were scored on adults at room temperature (24 ºC) under a Nikon stereomicroscope (Nikon, Melville, NY, USA)[14,15]. The average pumping rate (pumps per min) of 30 animals was scored in the 3 trials.

**2.3 Photography of autofluorescence** The experiment was performed as described previously[16-18]. Images were collected for endogenous intestine fluorescence using a 525-nm bandpass filter. Adults at the ages of 4 d, 8 d and 12 d were photographed on the same day to avoid the influence of light source variance on fluorescence intensity. The GFP fluorescence was recorded and color images were taken for the documentation of the results with Magnafire® software (Olympus, Irving, TX, USA). Lipofuscin levels were measured using ImageJ Software (NIH Image) by determining the average pixel intensity in each animal's intestine. More than 50 animals were counted for statistical analysis.

**2.4 Dauer formation assay** The assay was basically performed as described previously[3]. About 4 pregnant worms in each plate were allowed to lay eggs for 4-6 h at 20 ºC. The F1 progeny were then shifted to the 27 ºC condition, and 72 h later, the dauer or adult animals were counted. When the food was almost exhausted, the plates were flooded with 1% sodium dodecyl sulfate (SDS) solution to select the dauers, since dauers are resistant to SDS.

**2.5 Brood size** The brood size was assessed by placing a single nematode onto each well of tissue culture plates as previously described[19,20]. The nematodes were transferred to a new well every 1.5 d. Progeny were counted the day following transfer, and repeated twice for the statistical purpose.

**2.6 DNA construct** *odr-10* gene is predominantly expressed in the cilia of the AWA olfactory neurons in nematodes. A 1501 bp *odr-10* promoter fragment (HindIII/PstI) was cloned into the pPD95_75 vector. Again, the cDNA fragment of *odr-7* (PstI/KpnI), *odr-2* (PstI/BamHI) or *odr-3* (PstI/KpnI) was inserted into the pPD95_75 vector between P*odr-10* and *gfp* fragments, to obtain the constructs of P*odr-10-odr-7,* P*odr-10-odr-2* or P*odr-10-odr-3*. Transgenic worms were generated as previously described[21,22].

**2.7 Statistical analysis** Data were expressed as mean±SD and analyzed by analysis of variance (ANOVA) followed by a Dunnett's *t*-test. $P < 0.05$ was considered as significantly different. Graphs were generated using Microsoft Excel (Microsoft Corp., Redmond, WA).

## 3 Results

**3.1 Lifespans of nematodes with mutations of genes required for the functions of AWA sensory neuron** It has been clear that mutations of *odr-7* and *odr-10* influence the functions of AWA olfactory neuron[23,24], and mutations of *odr-2* and *odr-3* affect the functions of both AWA and AWC olfactory neurons[25,26]. The mean lifespans of *odr-7(ky4)*, *odr-10 (ky32)*, *odr-2(n2145)*, and *odr-3(n2150)* mutants cultivated at 20 ºC were first investigated. As shown in Table 2, wild-type N2, the long-lived *daf-2(e1370)* mutant, and the short-lived *daf-16(mu86)* mutant served as controls. Compared to the wild-type N2, mutations of *odr-7*, *odr-2* and *odr-3* all significantly increased the mean lifespan. However, mutation of *odr-10* did not obviously influence the nematode lifespan. The mean lifespans of *odr-7(ky4)*, *odr-2(n2145)*, and *odr-3(n2150)* mutants were all noticeably increased compared to that of wild-type. Therefore, mutations of *odr-7*, *odr-2*, and *odr-3* could influence the longevity of *C. elegans*. The present data on the longevities of *odr-7(ky4), odr-10 (ky32), odr-2(n2145),* and *odr-3(n2150)* mutants were consistent with the observations from Alcedo and Kenyon[10].

**3.2 Lifespans of nematodes with mutations of genes required for the functions of AWC, ASE, and AFD sensory neurons** Considering the fact that ASE, AWC, and AFD sensory neurons share the AIY interneuron with AWA olfactory neurons[6,8],

**Table 2. Lifespans of examined mutants**

| Genotype | Mean lifespan (d) | *n* | Ratio to wide type |
|---|---|---|---|
| Trial 1 | | | |
| wild type | 13.1 ± 0.3 | 44 | |
| *daf-16(mu86)* | 10.7 ± 0.7** | 52 | 0.82 |
| *daf-2(e1370)* | 30.6 ± 1.6** | 40 | 2.34 |
| *odr-7(ky4)* | 16.6 ± 0.6** | 51 | 1.27 |
| *odr-10(ky32)* | 14.0 ± 0.6NS | 49 | 1.07 |
| *odr-2(n2145)* | 16.3 ± 0.6** | 50 | 1.24 |
| *odr-3(n2150)* | 16.6 ± 0.6** | 49 | 1.27 |
| *che-1(p674)* | 13.6 ± 0.9 NS | 39 | 1.04 |
| *gcy-5(ok930)* | 13.0 ± 0.4 NS | 54 | 0.99 |
| *odr-1(n1936)* | 12.3 ± 0.5 NS | 48 | 0.94 |
| *daf-11(m47)* | 13.3 ± 0.5 NS | 45 | 1.02 |
| *daf-21(p673)* | 13.0 ± 0.5 NS | 55 | 0.99 |
| *ttx-1(p767)* | 13.7 ± 0.4 NS | 49 | 1.05 |
| Trial 2 | | | |
| wild type | 13.7 ± 0.4 | 48 | |
| *daf-16(mu86)* | 10.1 ± 0.5** | 45 | 0.74 |
| *daf-2(e1370)* | 31.9 ± 1.1** | 55 | 2.33 |
| *odr-7(ky4)* | 17.1 ± 0.3** | 49 | 1.25 |
| *odr-10(ky32)* | 13.9 ± 0.9 NS | 49 | 1.02 |
| *odr-2(n2145)* | 16.9 ± 0.7** | 52 | 1.23 |
| *odr-3(n2150)* | 16.2 ± 0.3** | 40 | 1.18 |
| *che-1(p674)* | 13.4 ± 0.5 NS | 51 | 0.98 |
| *gcy-5(ok930)* | 13.3 ± 0.6 NS | 49 | 0.97 |
| *odr-1(n1936)* | 12.7 ± 0.2 NS | 49 | 0.93 |
| *daf-11(m47)* | 13.8 ± 0.3 NS | 50 | 1.01 |
| *daf-21(p673)* | 13.2 ± 0.5 NS | 49 | 0.96 |
| *ttx-1(p767)* | 13.9 ± 0.7 NS | 49 | 1.01 |
| Trial 3 | | | |
| wild type | 13.5 ± 0.6 | 40 | |
| *daf-16(mu86)* | 9.8 ± 0.8** | 51 | 0.73 |
| *daf-2(e1370)* | 31.2 ± 0.7** | 49 | 2.31 |
| *odr-7(ky4)* | 16.8 ± 0.4** | 49 | 1.24 |
| *odr-10(ky32)* | 13.9 ± 0.7 NS | 50 | 1.03 |
| *odr-2(n2145)* | 16.8 ± 0.7** | 39 | 1.25 |
| *odr-3(n2150)* | 16.9 ± 0.2** | 40 | 1.25 |
| *che-1(p674)* | 13.1 ± 0.3 NS | 51 | 1.04 |
| *gcy-5(ok930)* | 13.8 ± 0.3 NS | 49 | 0.97 |
| *odr-1(n1936)* | 12.9 ± 0.9 NS | 49 | 0.96 |
| *daf-11(m47)* | 13.4 ± 0.8 NS | 50 | 0.99 |
| *daf-21(p673)* | 13.2 ± 0.7 NS | 49 | 0.98 |
| *ttx-1(p767)* | 13.9 ± 0.9 NS | 39 | 1.03 |

**$P < 0.01$ *vs* wide type, NS indicates no significant difference.

whether mutations of genes required for the functions of ASE, AWC, or AFD could influence the nematode longevity was then examined. *che-1* and *gcy-7* genes are required for the functions of ASE sensory neurons[27,28], and *ttx-1* gene is required for the specification of AFD thermosensory neuron[29]. *odr-1* encodes a transmembrane guanylyl cyclase required for AWC function[30]. Mutations of *daf-11* and *daf-21* affect the functions of both AWC and ASE sensory neurons[31]. As shown in Table 2, no obvious defects in lifespan were observed in *che-1(p674)*, *gcy-5(ok930)*, *odr-1(n1936)*, *daf-11(m47)*, *daf-21(p673)*, or *ttx-1(p767)* mutant, compared with the lifespan of wild-type. These data imply that the regulation of functions of ASE, AWC, and AFD may be not required for the longevity control in *C. elegans*.

**3.3 Pharynx pumping and intestine autofluorescence in nematodes with mutations of genes required for the functions of AWA olfactory neuron** To examine whether mutations of *odr-7, odr-2,* and *odr-3* could inhibit aging in *C. elegans*, the speed of pharynx pumping that can predict the age-related changes of physiological processes was further analyzed[14]. As shown in Fig. 1A, the pharynx pumping declined gradually in wild-type N2 nematodes with aging from adult day 4 to day 12. In contrast, mutations of *odr-7*, *odr-2*, and *odr-3* were associated closely with higher pumping rates at adult day 8 and day 12, suggesting that the mutations of *odr-7*, *odr-2*, and *odr-3* could suppress aging, rather than simply enhancing survival at old age in *C. elegans*.

As a marker for aging and cellular damage in aging cells, intestinal autofluorescence is caused by lysosomal deposits of lipofuscin and accumulates over time in aging *C. elegans*[16,17]. To investigate whether the altered lifespans in *odr-7(ky4)*, *odr-2(n2145)* and *odr-3(n2150)* mutants were due to the accelerated or suppressed aging-related cellular damage, or to an unrelated, pleiotropic cause, the lipofuscin levels in the intestine in adult wild N2 and mutant nematodes were then examined. As shown in Fig. 1B, consistent with the long-lived phenotype, the accumulation levels of intestinal autofluorescence in *odr-7(ky4)*, *odr-2(n2145)* and *odr-3 (n2150)* mutants were lower than that in wild-type N2, especially at day 12 ($P < 0.01$). Together with the above lifespan and pharynx pumping data, this result suggested that the

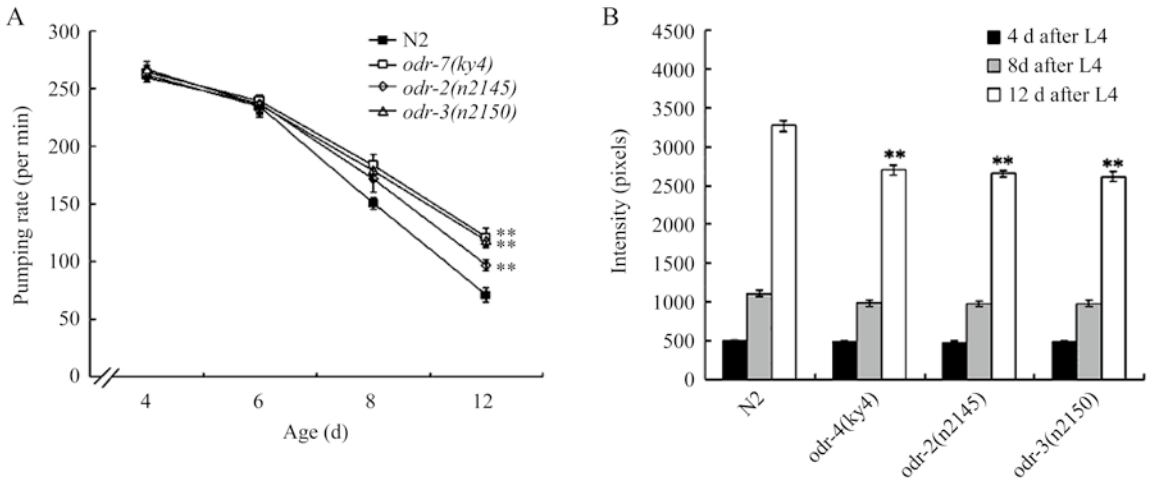


**Fig. 1 Pharynx pumping and intestine autofluorescence in nematode with mutations of genes required for the functions of AWA olfactory neuron. A: Mutations of *odr-7*, *odr-2*, and *odr-3* suppressed the decline in pharynx pumping. The average pumping rate (pumps per min) in 30 animals was scored in 3 trials. The significant differences were observed between wild-type and mutants at day 12. B: Qunantification of intestinal autofluorescence in N2 and mutants at days 4, 8, and 12 after the larval-to-adult transition at 20 ºC. **$P < 0.01$ *vs* N2.**

observed long-lived phenotype in *odr-7(ky4)*, *odr-2(n2145)*, and *odr-3(n2150)* mutants were actually due to the reduction in aging-related cellular damage.

**3.4 Brood size in nematodes with mutations of genes required for the functions of AWA olfactory neuron** To examine whether the observed lifespan defects in *odr-7(ky4)*, *odr-2(n2145)*, and *odr-3(n2150)* mutants were associated with the alteration in fertility, the brood sizes of *odr-7(ky4)*, *odr-2(n2145)*, and *odr-3(n2150)* mutants were examined. However, compared to the wide-type, mutations of *odr-7*, *odr-2*, and *odr-3* did not noticeably influence the brood size or obviously affect the egg number in the uterus (data not shown), suggesting that the observed lifespan extension in *odr-7*, *odr-2*, and *odr-3* mutants were not associated with the altered fertility and egg-laying behaviors in *C. elegans*.

**3.5 Dauer formation in nematodes with mutations of genes required for the functions of AWA olfactory neuron** In response to unfavorable conditions, the young nematode larvae will molt into the dauer with typical diapause features[2, 32]. Again, the dauer formation at 27 ºC in *odr-7(ky4)*, *odr-2 (n2145)*, and *odr-3(n2150)* mutants was investigated using starvation assay. As shown in Table 3, under our experimental conditions, the percentage of dauer formation in wild-type N2 was (8.7±4.1)%. Dauer numbers of *odr-7(ky4)*, *odr-2 (n2145)*, and *odr-3(n2150)* mutants were similar with that of wild-type, and the percentages of dauer formation of the 3 mutants were (8.9±1.7)%, (9.4±2.7)%, and (9.2±3.2)%, respectively, suggesting that *odr-7*, *odr-2* and *odr-3* genes were not involved in the control of dauer formation in *C. elegans*.

**Table 3. Percentage of dauer formation of various mutants at 27 ºC**

| Genotype | Percentage of dauer formation at 27 ºC (%) | Ratio to wide type |
|---|---|---|
| wild type | 8.7 ± 4.1 | |
| *odr-7(ky4)* | 8.9 ± 1.7 $^{NS}$ | 0.87 |
| *odr-2(n2145)* | 9.4 ± 2.7 $^{NS}$ | 1.08 |
| *odr-3(n2150)* | 9.2 ± 3.2 $^{NS}$ | 1.05 |

NS indicates no significant difference.

**3.6 Regulation of lifespan by *odr-7*, *odr-2*, or *odr-3* is not temperature-dependent** Here, the question whether the regulation of lifespan by *odr-7*, *odr-2* and *odr-3* is temperature-dependent has also been addressed. The *odr-7(ky4)*, *odr-2 (n2145)*, and *odr-3(n2150)* mutants were cultivated at 15 ºC,

20 ºC, or 25 ºC, and their lifespans were assessed. As shown in Fig. 2, at 15 ºC, 20 ºC, and 25 ºC, the mean lifespans of these mutants were all significantly longer than that of wild-type N2, suggesting that the regulation of longevity by *odr-7*, *odr-2*, and *odr-3* is not temperature-dependent in *C. elegans*.

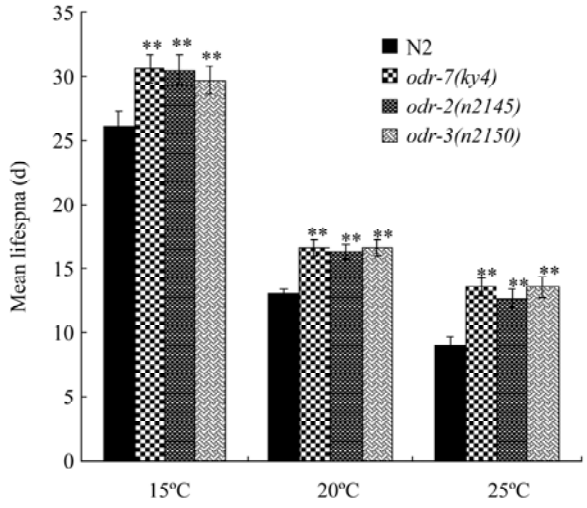


**Fig. 2 Comparison of the mean lifespans of *odr-7(ky4)*, *odr-2(n2145)*, and *odr-3(n2150)* mutants cultivated at 15 ºC, 20 ºC, or 25 ºC with that of wild-type N2. **$P < 0.01$ *vs* N2.**

### 3.7 Expression of *odr-7*, *odr-2* or *odr-3* in AWA neuron could completely or largely restore the altered lifespan in *odr-7(ky4)*, *odr-2(n2145)*, or *odr-3(n2150)* mutant

In *C. elegans*, *odr-7* is expressed in AWA neurons[23], and *odr-2* gene is expressed in many neurons, including interneurons onto which AWA and AWC neurons synapses[26]. *odr-3* is expressed in 5 pairs of sensory neurons (AWA, AWB, AWC, ASH, and ADF)[25]. Under the *odr-7(ky4), odr-2(n2145)* and *odr-3(n2150)* genetic backgrounds, the rescue experiments were further performed by expressing *odr-7*, *odr-2* and *odr-3* genes only in the AWA sensory neuron. As shown in Fig. 3, the extended lifespan of *odr-7(ky4)* mutant could be completely rescued by *odr-7* expression through plasmid P*odr-10-odr-7*. Similarly, the extended lifespans of *odr-2(n2145)* and *odr-3(n2150)* mutants could be largely rescued by *odr-2* and *odr-3* expression through plasmids P*odr-10-odr-2* and P*odr-10-odr-3,* respectively.

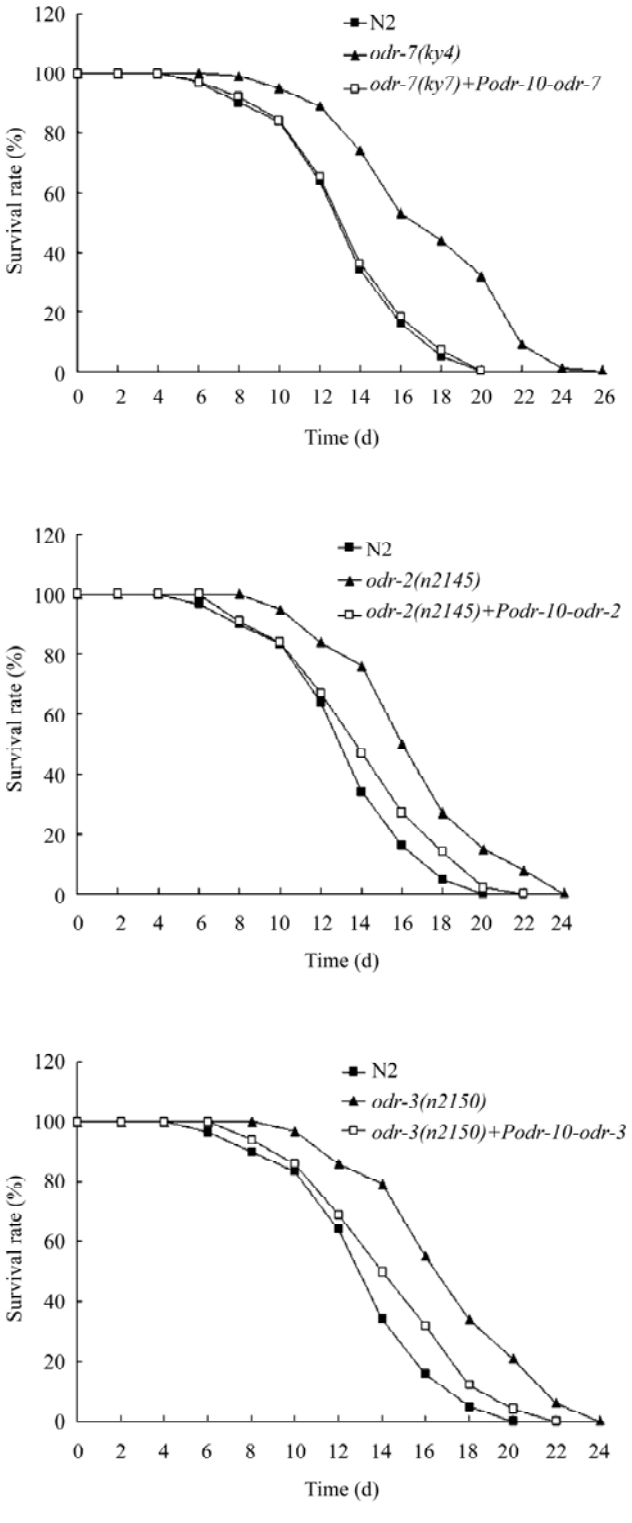


**Fig. 3 Expression of (A) *odr-7*, (B) *odr-2* and (C) *odr-3* in AWA neuron could completely or largely restore the altered lifespan in *odr-7*, *odr-2* and *odr-3* mutants, respectively.**

**3.8 Genetic interactions among *odr-7*, *odr-2* and *odr-3* genes in regulating the nematode longevity** Next, the possible genetic interactions among *odr-7*, *odr-2*, and *odr-3* genes in regulating the nematode longevity were examined. As shown in Fig. 4, the double mutant *odr-2(n2145);odr-7(ky4)* showed a higher level of lifespan extension than that of single mutant *odr-2(n2145)* or *odr-7(ky4)*. Similarly, the double mutant *odr-3(n2150);odr-7(ky4)* exhibited a higher level of lifespan extension than that of single mutant *odr-3(n2150)* or *odr-7(ky4)*. Moreover, the lifespan of triple mutant *odr-2 (n2145);odr-3(n2150);odr-7(ky4)* was similar to that of double mutant *odr-2(n2145);odr-7(ky4)* or *odr-3(n2150); odr-7(ky4)*.

**3.9 Genetic interactions between *odr-7* and some known important signaling pathways in regulating the nematode longevity** To further study the possible genetic interactions between *odr-7* and some known important signaling pathways in regulating the longevity, the double mutant *rrf-3 (pk1426);odr-7(ky4)* was first constructed. *rrf-3* encodes an RNA-directed RNA polymerase (RdRP) homolog that inhibits somatic RNAi, and *rrf-3* mutants are hypersensitive to somatic RNAi[33]. As shown in Fig. 5A, mutation of *rrf-3* did not influence the nematode lifespan, and the lifespan phenotype of *rrf-3(pk1426);odr-7(ky4)* double mutant was similar to that observed in *odr-7(ky4)* mutant.

Using this *rrf-3(pk1426);odr-7(ky4)* double mutant, the possible genetic interaction between *odr-7* and the insulin/IGF signaling pathway was further examined. The insulin/IGF signaling pathway plays a key role in regulating the longevity, and the fork head transcription factor DAF-16 transduces insulin-like longevity signals in *C. elegans*[34-37]. As shown in Fig. 5B, the long-lived phenotype of *rrf-3 (pk1426);odr-7(ky4)* mutant could be suppressed by *daf-16* mutation, and the mean and the maximum lifespans of *daf-16(RNAi);rrf-3(pk1426);odr-7(ky4)* mutant were both obviously lower than those in wild-type. Nevertheless, the mean and the maximum lifespans of *daf-16(RNAi);rrf-3(pk1426); odr-7(ky4)* mutant were not the same as those observed in *daf-16(RNAi);rrf-3(pk1426)* mutant. *mev-1* encodes a subunit of the enzyme succinate dehydrogenase cytochrome *b*, and can govern the rate of aging by modulating the cellular

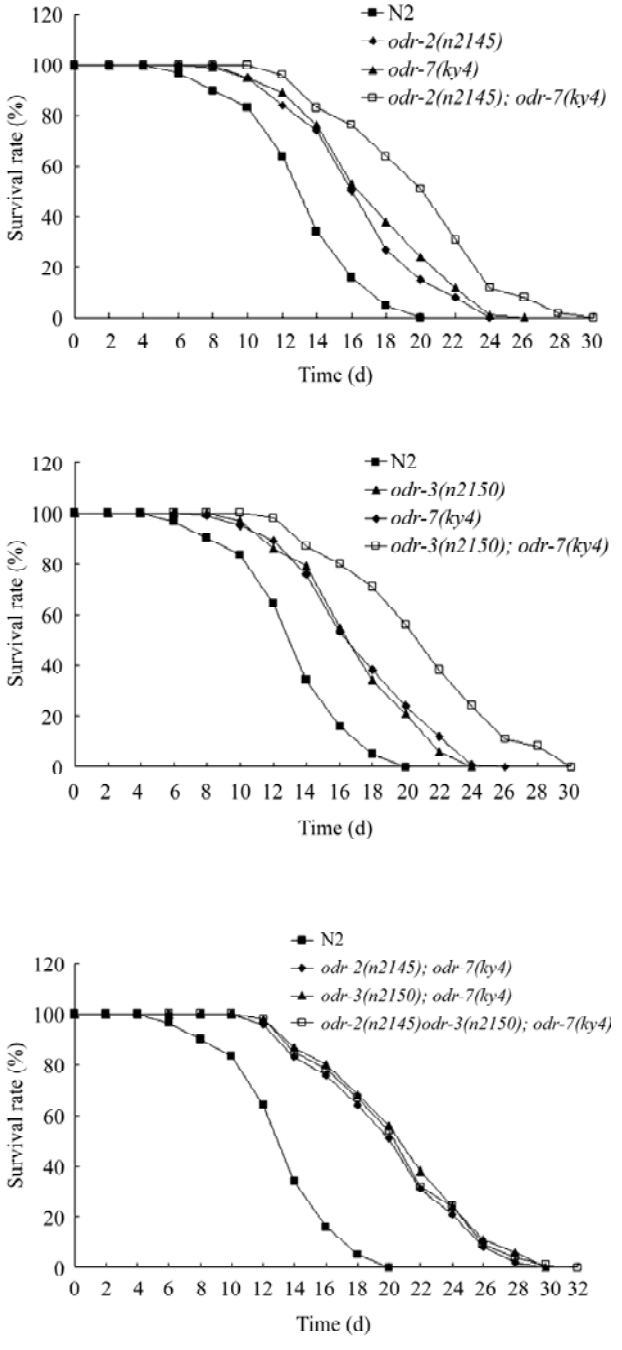


**Fig. 4 Genetic interactions among genes of *odr-7*, *odr-2* and *odr-3* in regulating the nematode lifespan. A: the double mutant *odr-2(n2145); odr-7(ky4)* showed a higher level of lifespan extension than that of single mutant *odr-2(n2145)* or *odr-7(ky4)*. B: Similar results were obtained in the double mutant *odr-3(n2150);odr-7(ky4)*. C: the lifespan of triple mutant *odr-2(n2145);odr-3(n2150);odr-7(ky4)* was similar to that of double mutant *odr-2(n2145);odr-7(ky4)* or *odr-3 (n2150);odr-7(ky4).***

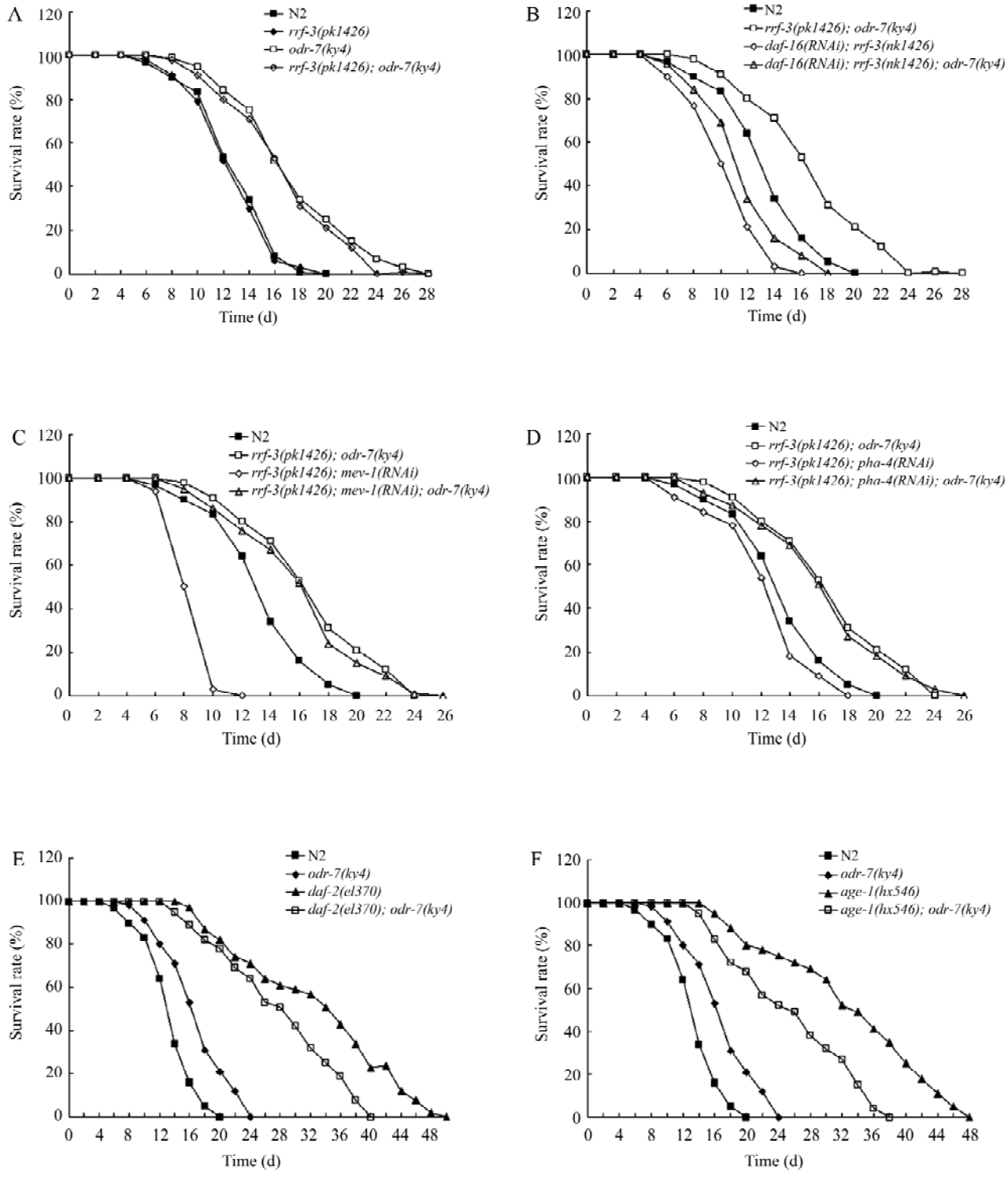


**Fig. 5 Genetic interaction between *odr-7* gene and some known important signaling pathways in regulating the nematode lifespan. A: Effects of *rrf-3* mutation on the lifespan of *odr-7* mutant. B-F: Genetic interaction of *odr-7* with *daf-16, mev-1, pha-4, daf-2,* or *age-1* in regulating the nematode longevity.**

response to oxidative stress[38]. The genetic interaction assay indicated that the mean and the maximum lifespans of *rrf-3(pk1426);mev-1(RNAi);odr-7(ky4)* mutant were similar to those observed in *rrf-3(pk1426);odr-7(ky4)* mutant (Fig. 5C), suggesting that *mev-1* is not required for the long lifespan of *odr-7* mutant nematodes. In *C. elegans*, *pha-4* is required for the development of worm pharynx and multiple forms of dietary restriction, thus mediating the diet restriction-induced longevity[39]. Similarly, the genetic interaction assay indicated that the mean and the maximum lifespans of *rrf-3(pk1426); pha-4(RNAi);odr-7(ky4)* mutant were similar to those observed in the *rrf-3(pk1426);odr-7(ky4)* mutant (Fig. 5D), suggesting that *pha-4* is either not required for the long lifespan of *odr-7* mutant nematodes.

The insulin/IGF-like peptides can bind to the unique Ins/IGF-like receptor DAF-2, thereby generating an intracellular signaling cascade[2, 40]. DAF-2 can further activate a p110 catalytic subunit AGE-1 to suppress the function of DAF-16 in nematodes[2, 41]. Furthermore, the observed extension of lifespan in *odr-7(ky4)* mutant could be further obviously enhanced by mutation of *daf-2* or *age-1* (Fig. 5E, F). Therefore, the regulation of nematode longevity by *odr-7* gene may be dependent on the insulin/IGF signaling pathway. Moreover, similar conclusions could be obtained from the observations of genetic interactions between insulin/IGF signaling and gene of *odr-2* or *odr-3* (data not shown).

## 4 Discussion

Olfactory discrimination can be achieved by the olfactory neurons with diverse chemical specificities. In the present study, we investigated the possible involvement of the genes required for the functions of AWA neuron, one of the olfactory neurons, in longevity control. *odr-7* is required for the function of one pair of AWA chemosensory neurons, and AWA neurons with *odr-7* null mutation fail to respond to all odorants[23]. Our study show that mutation of *odr-7* significantly increased the mean lifespan, suggesting that the functions of AWA olfactory neuron are essential for nematode longevity control. Analysis of the pharynx pumping speed indicated that mutation of *odr-7* was closely associated with higher pumping rates, suggesting that mutation of *odr-7* could suppress aging, rather than simply enhancing survival at an old age. Besides, the intestinal autofluorescence level in *odr-7(ky4)* mutant was lower than that in wild-type, suggesting that the observed long-lived phenotype in *odr-7(ky4)* mutant was due to the reduction of aging-related cellular damage. *odr-10* encodes a diacetyl-specific olfactory receptor with 7 transmembrane domains, and *odr-10* mutation leads to a specific defect in chemotaxis to diacetyl, one of the several odorants detected by AWA olfactory neurons[24]. However, here we show that mutation of *odr-10* could not obviously influence the nematode lifespan. One of the possible explanations for this is that diacetyl and its receptors may not be involved in lifespan regulation[10]. Another possible explanation is that mutation of *odr-10* may only partially affect the AWA functions, but not be required for the specification of AWA neurons.

Corresponding to the AIY interneuron, there are 4 sensory neurons, namely AWA, AWC, ASE, and AFD neurons[6,8]. In *C. elegans*, ODR-1 is required for the AWC functions and mediates the olfaction and odor discrimination[30]. The *che-1* gene encodes a zinc finger transcription factor required for specification of the ASE chemosensory neuron[27]. The *gcy-5* gene encodes a putative guanylyl cyclase and is involved in the sequential and asymmetrical control of ASE chemosensory laterality[28]. *ttx-1* encodes a member of the conserved OTD/OTX homeodomain protein family, and misexpression of *ttx-1* can convert other sensory neurons to an AFD-like fate[29]. *daf-11* and *daf-21* encode a guanylate cyclase and a member of HSP90 family of molecular chaperones, respectively. *daf-11* and *daf-21* mutants have defects in chemotaxis to non-volatile attractants detected primarily by the amphid neuron ASE, and in chemotaxis to volatile attractants detected primarily by the amohid neuron AWC[31]. Nevertheless, no obvious defects in lifespan were observed in *che-1(p674)*, *gcy-5(ok930)*, *odr-1(n1936)*, *daf-11(m47)*, *daf-21(p673)*, or *ttx-1(p767)* mutant, compared with that in wild-type. Moreover, ablation of the ASE or AWC neuron did not affect lifespan[10]. Therefore, the regulation of functions of ASE, AWC, and AFD neurons may be not required for the longevity control, or may be at least not directly associated with the longevity control in *C. elegans*.

In *C. elegans*, *odr-2* gene encodes a Ly-6 superfamily of GPI-linked protein[26]. *odr-3* gene encodes a Gα protein and mediates the functions of sensory neurons, and null mutation of *odr-3* increases the nematode lifespan[10,25]. In this study, mutations of *odr-2* and *odr-3* significantly increased the mean lifespan of nematodes. Analysis of the pharynx pumping speed demonstrated that mutations of *odr-2* and *odr-3* were closely associated with higher pumping rates. In addition, intestinal autofluorescence levels in *odr-2* and *odr-3* mutants were both lower than that in wild-type, suggesting that the observed long-lived phenotype in *odr-2* and *odr-3* mutants was actually due to the reduction of aging-related cellular damage. Moreover, expression of *odr-2* and *odr-3* in AWA neuron largely recovered the altered lifespan in *odr-2* and *odr-3* mutants. Furthermore, double mutant of *odr-2 (n2145);odr-7(ky4)* exhibited more extended lifespan than *odr-2(n2145)* or *odr-7(ky4)* single mutant. Similarly, double mutant of *odr-3(n2150);odr-7(ky4)* showed more extended lifespan than *odr-3(n2150)* or *odr-7(ky4)* single mutant. Lifespan of *odr-2(n2145); odr-3(n2150); odr-7(ky4)* triple mutant was similar to that of *odr-2(n2145); odr-7(ky4)* or *odr-3(n2150); odr-7(ky4)* double mutant. Therefore, the phenotypes of *odr-2* and *odr-3* mutants further confirm the importance of AWA olfactory neurons in longevity control[10].

Moreover, the phenotype of extended lifespan in *odr-7 (ky4)* mutant could be completely rescued by transgene with plasmid P*odr-10-odr-7,* and the extended lifespans of *odr-2 (n2145)* and *odr-3(n2150)* mutants could also be largely rescued by transgene with plasmids P*odr-10-odr-2* and P*odr-10-odr-3*, respectively These data strongly confirm the important roles of *odr-7*, *odr-2* and *odr-3* in mediating the function of AWA sensory neuron in regulating the longevity. These data also imply the important roles of *odr-7*, *odr-2* and *odr-3* genes in the formation of sensory function of AWA sensory neuron in nematodes. Nevertheless, it is still unclear whether *odr-7*, *odr-2* and *odr-3* genes regulate the longevity of nematodes non-autonomously, that is, whether the expression of *odr-7*, *odr-2* and *odr-3* genes in other kinds of neurons or cells can still rescue the lifespan defects in corresponding mutant nematodes.

Previous studies have suggested that several factors can influence the aging process of nematodes. Firstly, the reduction in fertility may be associated a long lifespan[42]. However, here we show that mutations of *odr-7*, *odr-2*, and *odr-3* did not obviously affect the brood size or the number of eggs in the uterus. Similarly, Alcedo and Kenyon have observed no obvious changes in the timing of reproduction in their neuron-ablated nematodes[10]. Secondly, previous reports indicate that lifespan can also be affected by temperature in *C. elegans*[43]. However, our data show that the mean lifespans of *odr-7*, *odr-2*, and *odr-3* mutants cultivated at 15 ºC, 20 ºC, and 25 ºC were all significantly higher than that of temperature-matched wild-type N2, suggesting that the regulation of longevity by *odr-7*, *odr-2*, and *odr-3* is not temperature-dependent. Thirdly, dauer formation is often required for lifespan extension and regulation in *C. elegans*[32]. However, here we show that the number of dauers formed at 27 ºC is not influenced by the mutation of *odr-7*, *odr-2*, or *odr-3*. Therefore, the observed alteration in lifespan in *odr-7*, *odr-2*, and *odr-3* mutants may be mostly due to the age-related changes of physiological processes or cellular damage in cells after disruption of AWA sensory neuron function.

In the present study, our genetic interactions further demonstrate that the long-lived phenotype of *rrf-3;odr-7* mutant could be suppressed by *daf-16* mutation, although the mean and the maximum lifespans of *daf-16;rrf-3;odr-7* mutant were not the same as those observed in *daf-16;rrf-3* mutant. These data suggest that the regulation by ODR-7 on nematode longevity is dependent on the activity of DAF-16. The data from Alcedo and Kenyon suggest that the activities of the olfactory AWA neurons are also partially dependent on *daf-16*[10]. Similarly, mutations of *tub-1*, causing the lack of active TUB-1 in ciliary neurons, result in the chemotaxis defects and a DAF-16-dependent lifespan extension[10]. Moreover, the long-lived lifespan phenotype in *odr-7* mutant could be enhanced by mutation of *daf-2* or *age-1*. Smilar conclusions can also be obtained from the observations of genetic interactions between insulin/IGF signaling and *odr-2* or *odr-3* gene (data not shown). In addition, our genetic interaction assays demonstrate that the mean and the maximum lifespans of *rrf-3;mev-1;odr-7* and *rrf-3;pha-4;odr-7* mutants were similar to those observed in *rrf-3;odr-7* mutant,

suggesting that *mev-1*-mediated oxidative stress signaling pathway and *pha-4*-mediated diet-restriction signaling pathway are not required for lifespan extension in *odr-7* mutant nematodes. Therefore, the genes required for the functions of AWA sensory neuron may regulate the nematode longevity in an insulin/IGF signaling pathway-dependent fashion.

In conclusion, our data confirm the important roles of *odr-7*, *odr-2*, and *odr-3* in regulating the nematode longevity. Moreover, the genes required for the functions of AWA olfactory neuron regulate the longevity in an insulin/IGF signaling-dependent fashion in *C. elegans*.

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# 嗅觉神经元 AWA 功能必需基因以胰岛素信号依赖的方式调控秀丽线虫的衰老

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**摘要：目的** 研究嗅觉神经元 AWA 功能必需基因与胰岛素信号之间在调控秀丽线虫衰老上的关系。**方法** 测定 AWA、AWC、ASE 与 AFD 感觉神经元的功能必需基因突变后线虫的寿命、咽泵运动速率、肠道荧光、永久性幼虫形成与后代数目的变化。此外，进行基因功能的恢复实验以确认AWA功能必需基因在调控衰老中的作用。最后，对 AWA 功能必需基因与胰岛素信号间在调控衰老上的遗传关系进行了分析。**结果** AWA 功能必需基因 *odr-7*、*odr-2* 与 *odr-3* 突变能显著延长动物寿命，并在衰老过程中诱导产生相对于野生型更高的咽泵运动速率和更少的肠道脂褐质积累。然而，*odr-7*、*odr-2* 与 *odr-3* 的基因突变并不影响线虫的后代数目与永久性幼虫的形成，且 *odr-7*、*odr-2* 与 *odr-3* 基因对于寿命的调控并未呈现出明显的温度依赖性。比较而言，感觉神经元 ASE、AWC 与 AFD 的功能必需基因的突变并未显著影响动物寿命。而且，在嗅觉神经元 AWA 表达 *odr-7*、*odr-2* 与 *odr-3* 基因可以完全或很大程度上恢复对应突变体的长寿表现型。进一步遗传分析表明，*odr-7* 突变体的长寿表现型可被 *daf-16* 基因突变所抑制，被 *daf-2* 或 *age-1* 基因突变所增强，而 *mev-1* 与 *pha-4* 基因突变对此并不产生影响。**结论** 嗅觉神经元AWA功能必需基因以胰岛素信号依赖的方式调控秀丽线虫的衰老。

**关键词**：寿命；ODR-7；AWA 嗅觉神经元；胰岛素信号；遗传相互作用；秀丽线虫