

Multiple sensory G proteins in the olfactory, gustatory and nociceptive neurons modulate longevity in *Caenorhabditis elegans*

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Abstract

The life span of the nematode *Caenorhabditis elegans* is under control of sensory signals detected by the amphid neurons. In these neurons, *C. elegans* expresses at least 13 G α subunits and a G γ subunit, which are involved in the transduction and modulation of sensory signals. Here, we show that loss-of-function mutations in the G α subunits *odr-3*, *gpa-1* and *gpa-9*, in the G γ subunit *gpc-1* and the introduction of extra copies of the G α subunit *gpa-11* extend the life span of *C. elegans*. Loss-of-function of *odr-3* and extra copies of *gpa-11* act synergistically and can together extend life span more than two-fold, indicating that sensory signals play an important role in regulating life span. We show that *gpa-1*, *gpa-11*, *odr-3* and *gpc-1* all signal via the *daf-16* FOXO family transcription factor. In addition, *odr-3* and *gpa-11* might suppress life span extension partially independent of the insulin/IGF-1 like receptor homologue *daf-2*. Our results suggest that the previously unanticipated nociceptive ASH and/or ADL neurons regulate longevity. We expect that the implication of specific G proteins will eventually contribute to the identification of the sensory cues that determine the rate of aging in *C. elegans*.

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Keywords: *C. elegans*; G proteins; Sensory signaling; Longevity; Aging; Insulin/IGF-1 signaling; Dauer development

Introduction

Genetic analysis of the life span of the nematode *Caenorhabditis elegans* has greatly aided in understanding the mechanisms that regulate aging. An intriguing finding is that *C. elegans* life span is under control of sensory perception (Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004). *C. elegans* perceives its environment through 12 bilateral pairs of ciliated amphid neurons. Mutations that disrupt the cilia of these neurons, resulting in the inability to respond to sensory cues, increase the life span of *C. elegans* (Apfeld and Kenyon, 1999). Furthermore, laser ablation of some of the neurons that detect environmental cues, such as the AWA, AWC, ASI and ASG neurons, extends life span (Alcedo and Kenyon, 2004). However, the sensory cues and precise sensory signaling mechanisms that regulate longevity are not yet known.

As in mammals, many sensory cues in *C. elegans* are probably detected by G protein coupled receptors, which, in response, activate multiple G protein mediated signaling cascades (Troemel et al., 1995; Jansen et al., 1999). The genome of *C. elegans* encodes 21 G α subunits, 2 G β subunits and 2 G γ subunits (Jansen et al., 1999; Cuppen et al., 2003). Of these, 13 G α subunits and 1 G γ subunit are specifically expressed in the amphid sensory neurons (Table 1; Zwaal et al., 1997; Roayaie et al., 1998; Jansen et al., 1999, 2002). Thus far, for eight G α subunits and the G γ subunit a role in sensory perception has been demonstrated (Zwaal et al., 1997; Roayaie et al., 1998; Jansen et al., 1999, 2002; Chao et al., 2004; Hilliard et al., 2004; Lans et al., 2004; Hukema et al., 2006). For example, the G α subunit ODR-3 mediates the detection of olfactory and nociceptive stimuli (Roayaie et al., 1998; Hilliard et al., 2004). Furthermore, the G α subunits ODR-3 and GPA-1 and the G γ subunit GPC-1 play a role in gustatory plasticity, a behavioral response to NaCl (Jansen et al., 2002; Hukema et al., 2006). The G α subunit GPA-11 has been shown to regulate octanol sensitivity in response to a food/serotonin signal (Chao et al., 2004).

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Table 1
Mean life spans of G protein mutant and transgenic strains, at 25 °C^a

Strain ^b	Mean±SEM (days)	75th perc. (days) ^c	n ^d	p value ^e	Amphid neuron expression ^f	Sensory function ^f
<i>Amsterdam</i> ^g						
Wild type	13.4±0.2	15	203			
<i>gpa-1</i>	14.4±0.3	16	78	0.007	ADL, ASH, ASI, ASJ	Salt perception
<i>gpa-1XS</i>	12.3±0.4	14	44	0.004		
<i>gpa-2</i>	9.5±0.6	12	40	<0.0001	AWC	Olfaction, dauer pheromone
<i>gpa-2XS</i>	15.1±0.4	17	73	<0.0001		
<i>gpa-3</i>	11.4±0.5	14	40	0.001	AWA, AWC, ADF, ADL, ASE, ASG, ASH, ASI, ASJ, ASK	Nociception, olfaction, dauer pheromone
<i>gpa-3XS</i>	13.1±0.3	15	39	0.144		
<i>gpa-3QL</i>	11.6±1.2	14	28	0.432		
<i>gpa-4</i>	14.1±0.4	16	43	0.053	ASI	
<i>gpa-4XS</i>	12.7±0.6	15	44	0.981		
<i>gpa-5</i>	14.2±0.4	16	43	0.036	AWA, ADL	Olfaction
<i>gpa-5XS</i>	12.1±0.5	14	42	0.052		
<i>gpa-6</i>	12.0±0.5	13	41	0.036	AWA, AWB, ASH, ADL	
<i>gpa-6XS</i>	13.0±0.5	15	46	0.912		
<i>gpa-7</i>	13.4±0.5	15	36	0.976		
<i>gpa-7XS</i>	14.1±0.4	16	22	0.465		
<i>gpa-8</i>	10.7±0.5	13	35	<0.001	None	
<i>gpa-8XS</i>	11.5±0.5	13	42	0.003		
<i>gpa-9</i>	17.5±0.4	19	42	<0.0001	ASJ	
<i>gpa-9XS</i>	11.0±0.6	14	41	0.001		
<i>gpa-10</i>	11.0±0.3	12	50	<0.0001	ADF, ASI, ASJ	
<i>gpa-11</i>	12.8±0.4	18	46	0.278	ADL, ASH	Serotonin/food
<i>gpa-11XS</i>	19.5±0.7	26	65	<0.0001		
<i>gpa-12</i>	13.2±0.5	16	37	0.906	Undetermined	
<i>gpa-13</i>	12.9±0.4	16	38	0.261	ADF, ASH, AWC	Olfaction
<i>gpa-13XS</i>	12.4±0.7	16	46	0.727		
<i>gpa-14</i>	13.7±0.4	16	44	0.668	ASI, ASJ, ASH, ASK	
<i>gpa-14XS</i>	12.9±0.3	13	44	0.118		
<i>gpa-15</i>	13.3±0.4	16	46	0.907	ADL, ASH, ASK	
<i>gpa-15XS</i>	11.1±0.6	13	21	<0.0001		
<i>odr-3</i>	18.0±0.4	19	80	<0.0001	AWA, AWB, AWC, ADF, ASH	Olfaction, salt, nociception
<i>gpc-1</i>	14.7±0.4	17	45	0.002	AWB, ADL, ASH, ASI, ASJ, AFD	Salt perception
<i>gpc-1XS</i>	15.2±0.5	17	33	0.0001		
<i>gpa-11; odr-3</i>	14.3±0.5	17	35	*0.037	**<0.0001	
<i>Rotterdam</i> ^b						
wild type	17.5±0.3	20	89			
<i>gpa-9</i>	19.6±0.3	22	87	<0.0001		
<i>gpa-9XS</i>	19.4±0.4	21	71	0.0001		
<i>gpa-11</i>	17.0±0.6	19	37	0.843		
<i>gpa-11XS</i>	21.1±0.5	24	90	<0.0001		
<i>gpa-11XS(pkIs540)</i>	22.8±0.6	24	93	<0.0001		
<i>odr-3</i>	19.7±0.5	24	84	<0.0001		
<i>gpa-11; odr-3</i>	17.2±0.4	19	69	*0.823	**<0.0001	
<i>odr-3; gpa-11XS</i>	34.7±1.6	42	48	**<0.0001	***<0.0001	
<i>odr-3; gpa-11XS(pkIs540)</i>	25.4±1.1	32	42	**<0.0001	°0.132	

^a Cumulative results of life span analysis at 25 °C.

^b All strains carry loss-of-function mutations that represent putative null alleles. This has been verified using antibodies in the case of *odr-3*, *gpa-2*, *gpa-3*, *gpa-5*, *gpa-6*, *gpa-13* and *gpc-1* (Lans et al., 2004; S.R. and G.J., personal communication). Strains that have significantly extended life span are indicated in bold. Two independent integrated arrays of *gpa-11XS* were tested, to confirm that additional copies of the *gpa-11* gene caused life span extension. None of the strains, except *gpa-3QL*, shows defective dye filling (Jansen et al., 1999, 2002). XS denotes extra copies of the gene as a transgene, QL a constitutive activating mutation.

^c The 75th perc. (percentile) is the age when the fraction of animals alive reaches 0.25.

^d n denotes number of animals tested.

^e p values are compared to wild type, except * compared to *gpa-11*, ** compared to *odr-3*, *** compared to *gpa-11XS* and ° compared to *gpa-11XS(pkIs540)*.

^f Expression patterns and functions were published previously (Zwaal et al., 1997; Roayaie et al., 1998; Jansen et al., 1999, 2002; Yau et al., 2003; Chao et al., 2004; Hilliard et al., 2004; Lans et al., 2004; Hukema et al., 2006).

^g During the course of our experiments, our lab moved from the city of Amsterdam to Rotterdam. Animals survived significantly longer in Rotterdam, possibly because of different environmental conditions. Results obtained at the same location, however, never showed large variations, emphasizing their significance. Importantly, the outcome of experiments performed at both locations was similar: animals that showed extended life span in Amsterdam showed extended life span in Rotterdam. All assays in Amsterdam were performed once, except assays with *gpa-1*, *gpa-2XS*, *gpa-4XS*, which were performed twice and with wild type, which were performed six times. All assays in Rotterdam were performed twice except assays with *gpa-11*, *odr-3; gpa-11XS* and *odr-3; gpa-11XS(pkIs540)*, which were performed once.

Sensory perception is thought to influence longevity in part by controlling the insulin/IGF-1 pathway (Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004), which regulates life span in *C. elegans* as well as in many other organisms (Kenyon, 2005). Mutations in insulin signaling genes, such as the insulin/IGF-1 receptor homologue *daf-2* (Kenyon et al., 1993; Kimura et al., 1997), greatly extend life span. This life span extension depends on the activity of the FOXO family transcription factor *daf-16*, which translocates to the nucleus to promote longevity in *daf-2* mutants (Kenyon et al., 1993; Lin et al., 1997; Ogg et al., 1997; Lin et al., 2001).

In this study, we investigated the role of all sensory G proteins in regulating longevity. We show that specific sensory G proteins inhibit and promote longevity. Two of these, *odr-3* and *gpa-11*, act synergistically to regulate longevity. Signaling via the G proteins is dependent on *daf-16*, but *odr-3* and *gpa-11* seem to signal partially independent of *daf-2*. In addition, the involvement of *gpa-11* suggests that signaling via one or both of the nociceptive ASH and ADL neurons regulates longevity.

Materials and methods

Strains

Strains were maintained using standard methods (Brenner, 1974). Loss-of-function alleles and transgenes used in this study were *bas-1(ad446)*, *daf-2(e1370)*, *daf-16(mu86)*, *gpa-1(pk15)*, *pkIs503[gpa-1XS]*, *gpa-2(pk16)*, *pkIs580[gpa-2XS]*, *gpa-3(pk35)*, *pkIs508[gpa-3XS]*, *gpa-4(pk381)*, *pkIs515[gpa-4XS]*, *gpa-5(pk376)*, *pkIs379[gpa-5XS]*, *gpa-6(pk480)*, *pkIs519[gpa-6XS]*, *gpa-7(pk610)*, *pkIs523[gpa-7XS]*, *gpa-8(pk345)*, *pkIs527[gpa-8XS]*, *gpa-9(pk438)*, *pkIs531[gpa-9XS]*, *gpa-10(pk362)*, *gpa-11(pk349)*, *pkIs539[gpa-11XS]*, *pkIs540[gpa-11XS]*, *gpa-12(pk322te)*, *gpa-13(pk1270)*, *pkIs1269[gpa-13XS]*, *gpa-14(pk347)*, *pkIs552[gpa-14XS]*, *gpa-15(pk477)*, *pkIs555[gpa-15XS]*, *gpc-1(pk298te)*, *pkIs571[gpc-1XS]*, *muEx128[pKL79(daf-16a::gfp)]*, *odr-3(n1605)* and *syIs25[gpa-3QL]*. All loss-of-function alleles and transgenes have been reported previously (Loer and Kenyon, 1993; Kimura et al., 1997; Lin et al., 1997, 2001; Zwaal et al., 1997; Roayaie et al., 1998; Jansen et al., 1999, 2002). Mutants carrying loss-of-function alleles were backcrossed six times and transgenic animals carrying extra copies of a gene were backcrossed at least two times to the same wild type Bristol N2 background (Jansen et al., 1999), thereby establishing isogenetic backgrounds. To confirm that extra copies of *gpa-11* extended life span, two independent transgenic strains were tested (Table 1).

Life span analysis

Life span was determined at 20 °C or 25 °C. On day 1, adult animals were allowed to lay eggs on 9 cm dishes for approximately 3 h, after which they were removed. Eggs were allowed to reach adulthood, after which adult animals were transferred to fresh plates (5 worms/6 cm dish) every 2 days until they stopped producing progeny. Later, animals were transferred to fresh plates every 1 to 2 weeks, when necessary. Living and dead animals were scored every 1 to 3 days. Animals were considered as dead when they stopped moving and did not respond to prodding. Animals that crawled of the plate, had an extruded gonad or internally hatched larvae were censored. G protein mutants that showed life span extension all reached adulthood on the same day after hatching, ruling out the possibility that altered developmental rates caused life span extension. For life span analysis of all *daf-2* mutants, animals were grown at 15 °C and transferred to 20 °C at the L4 stage, to prevent dauer development. All life span assays were performed on the same location, Rotterdam, except for the initial screen shown in Table 1, which was performed in Amsterdam, the Netherlands. *p* values were determined using a Log Rank test. *p* < 0.05 was considered significant.

Analysis of DAF-16::GFP localization

The extrachromosomal array *muEx128[pKL79(daf-16a::GFP)]* (Lin et al., 2001) was used to visualize DAF-16 localization in *daf-16* mutants carrying loss-of-function mutations in or extra copies of G protein genes. DAF-16 localization was determined using well-fed L2 larvae, cultured at 20 °C, mounted on a 2% agarose pad containing 10 mM Na₃N. Following mounting, localization was immediately scored as predominantly nuclear (NA) or predominantly cytoplasmic (Fig. 3F). Images were acquired using a Zeiss Axio Imager.Z1.

Analysis of dauer pheromone sensitivity

Sensitivity to dauer pheromone was tested using a crude dauer pheromone extract (Golden and Riddle, 1984). Animals were allowed to lay eggs for 2 h on plates containing 20 µl dauer pheromone and 20 µl 4% OP50 bacteria. After 48 h, the percentage of dauer larvae was determined. *p* values were determined using a one-way ANOVA test.

Results and discussion

Multiple sensory G proteins modulate longevity in *C. elegans*

As an initial screen, we determined the life span of animals carrying loss-of-function mutations or extra copies of 16 G α subunits and the G γ subunit *gpc-1* at 25 °C (Table 1). This initial analysis showed that loss-of-function mutations in the G α subunits *gpa-1* (*pk15*), *gpa-5* (*pk376*), *gpa-9* (*pk438*) and *odr-3* (*n1605*), in the G γ subunit *gpc-1* (*pk298te*) and extra copies of the G α subunits *gpa-2* (*pkIs580[gpa-2XS]*), *gpa-9* (*pkIs531[gpa-9XS]*) and *gpa-11* (*pkIs539[gpa-11XS]*) and *gpc-1* (*pkIs571[gpc-1XS]*) significantly extended life span. The *odr-3* (*n1605*) mutation has been previously reported to increase life span (Alcedo and Kenyon, 2004). To confirm that extra copies of *gpa-11* extended life span, we obtained similar results with two independent integrated extra copy strains, *pkIs539* and *pkIs540* (henceforth referred to as *gpa-11XS* and *gpa-11XS* (*pkIs540*)). Most of the other mutations in G α subunits had either no effect or seemed to decrease life span. These results suggest that only a small subset of the sensory G proteins control longevity, suggesting that life span might be precisely regulated by specific sensory cues.

The sensory G proteins GPA-1 and GPC-1 act redundantly with GPA-11 and ODR-3 to regulate life span

To confirm our results and to be able to compare them with previous publications, we repeated some of the experiments at 20 °C. Again, we found that loss-of-function of *gpa-1*, *gpa-9*, *odr-3* and *gpc-1* and extra copies of *gpa-9* and *gpa-11* extended life span (Figs. 1A–D; Supplementary Table 1). Even though some results appeared variable in our initial screen at 25 °C (e.g. *gpa-9XS*; Table 1), carefully executed assays at 20 °C generated highly reproducible results (Supplementary Table 1). We tested whether slower developmental rates could account for observed life span extensions, but found that all long-lived G protein strains used in this study reached adulthood on the same day.

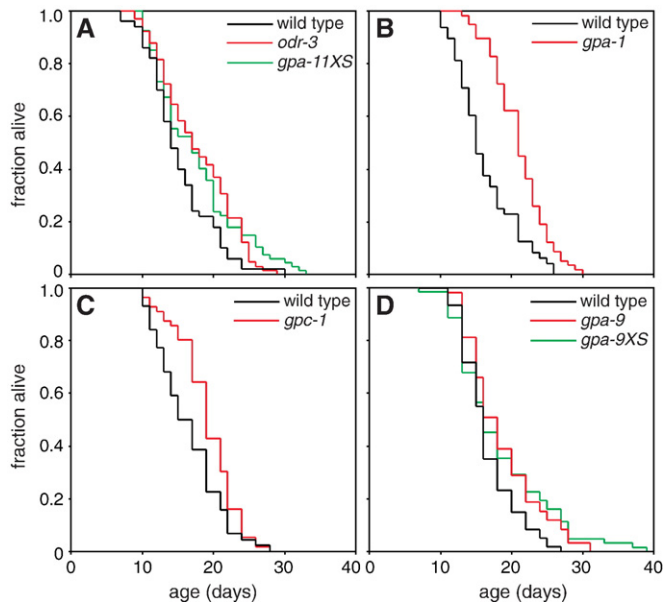


Fig. 1. Sensory G protein subunits regulate life span. Loss-of-function mutations in *gpa-1*, *gpa-9*, *odr-3*, *gpc-1* and extra copies of *gpa-9* and *gpa-11* extend life span. Shown are the results of single, representative life span assays at 20 °C. Cumulative results are summarized in Supplementary Table S1. Life span extension caused by a mutation in *odr-3* has been previously reported (Alcedo and Kenyon, 2004). Numbers of animals (*n*) and *p* values comparing differences with wild type are (A) wild type (*n*=50), *odr-3* (*n*=65, *p*=0.013), *gpa-11XS* (*n*=67, *p*=0.027), (B) wild type (*n*=48), *gpa-1* (*n*=58, *p*<0.0001), (C) wild type (*n*=44), *gpc-1* (*n*=56, *p*=0.017), (D) wild type (*n*=61), *gpa-9* (*n*=59, *p*=0.011) and *gpa-9XS* (*n*=62, *p*=0.036).

Each of the G protein subunits is expressed in one or more amphid neurons (Table 1). Since their expression patterns partially overlap, they could function in the same signaling pathway to regulate longevity. To test this, we constructed animals carrying different combinations of *gpa-1*, *gpa-11*, *odr-3* and *gpc-1* loss-of-function mutations and extra copies of *gpa-11*. If two G protein subunits function in the same pathway, we would expect similar life span extension as a result of mutation of one of the two G proteins as mutation of both G proteins. Alternatively, if two subunits act in separate pathways, we would expect a synergistic life span extension.

The life span extension in *odr-3*; *gpc-1* and *gpc-1*; *gpa-11XS* animals showed no signs of synergism, but was similar to that in *gpc-1* single mutants (Figs. 2A, B; Supplementary Table 1). In contrast, the life span of *gpa-11(pk349)*; *gpc-1* animals was similar to that of *gpa-11* single mutants (which is similar to wild type; Fig. 2C; Supplementary Table 1). These results suggest that *odr-3* and *gpa-11* both function in a genetic pathway with *gpc-1* and that the extended life span of *gpc-1* mutants requires *gpa-11*. Furthermore, the life span extension in *gpa-1*; *gpc-1* double mutants was similar to that in either single mutant (Fig. 2D; Supplementary Table 1), suggesting that *gpa-1* also functions in a genetic pathway with *gpc-1*. Interestingly, both genes also seem to act in the same genetic pathway that regulates the response to salts (Hukema et al., 2006). Next, we found that the life span of *gpa-1 odr-3* double

mutants was similar to that of *gpa-1* single mutants (Fig. 2E; Supplementary Table 1). Thus, *gpa-1* might function in the same genetic pathway as *odr-3* and in an additional pathway, just like *gpc-1*. In contrast, the life span of *gpa-1*; *gpa-11XS* animals was similar to that of *gpa-11XS* animals, but shorter than that of *gpa-1* mutants (Fig. 2F; Supplementary Table 1). The life span of *gpa-11*; *gpa-1* animals did not significantly differ from that of *gpa-1* single mutants (Fig. 2G; Supplementary Table 1). These results show that life span extension in *gpa-1* animals does not depend on *gpa-11*, but rather that *gpa-11* mediates an additional signal that suppresses life span extension in *gpa-1* mutants (see Fig. 5 and below). This suggests that *gpa-1* and *gpc-1* have separate functions in regulating life span.

ODR-3 and GPA-11 synergistically regulate longevity

At 25 °C (Table 1) and at 20 °C (Fig. 2H; Supplementary Table 1), we found that loss-of-function of *odr-3* and extra copies of *gpa-11* synergistically extended life span, suggesting that, in wild type animals, *gpa-11* promotes longevity and *odr-3* inhibits longevity. To test if the two G α subunits antagonize each other's function, we constructed a *gpa-11*; *odr-3* loss-of-function double mutant. *gpa-11*; *odr-3* mutants had a life span shorter than *odr-3* mutants but similar to *gpa-11* mutants (Fig. 2I; Supplementary Table 1), suggesting that the extended life span of *odr-3* mutants requires the function of *gpa-11*. *odr-3* might inhibit longevity by signaling via *gpa-11* or via an antagonistic, parallel pathway. If *odr-3* functions in a parallel pathway, we would expect that *gpa-11* mutants have a shortened life span as compared to wild type or *gpa-11*; *odr-3* double mutants, which is not the case (Fig. 2I; Supplementary Table 1). Therefore, we favor the idea that *odr-3* inhibits longevity by signaling via *gpa-11*.

Interestingly, *gpa-11* is only expressed in the ASH and ADL neurons (Jansen et al., 1999). Thus far, these neurons have not been implicated in regulating longevity. *odr-3* is also expressed in the ASH neurons, in addition to the AWA, AWB, AWC and ADF neurons (Roayaie et al., 1998). In the ASH neurons, *gpa-11* mediates the transduction of a food/serotonin signal, which modulates the avoidance response to the odorant octanol (Chao et al., 2004). Therefore, *gpa-11* might regulate longevity by mediating a food/serotonin signal. Exogenous serotonin, however, had no clear effect on the life span of wild type, *odr-3*, *gpa-11* or *odr-3*; *gpa-11XS* animals (results not shown). Also *bas-1(ad446)* mutants, which have reduced dopamine and serotonin levels (Loer and Kenyon, 1993), do not live longer than wild type animals (results not shown). *odr-3* is important for the detection of many sensory stimuli, including attractive odorants by the AWA and AWC neurons and nociceptive stimuli by the ASH neurons (Roayaie et al., 1998; Hilliard et al., 2004). Therefore, a wide variety of cues could influence longevity via *odr-3*. Because killing the AWA and AWC neurons was found to increase life span (Alcedo and Kenyon, 2004), it is very well possible that *odr-3* functions in these neurons to regulate longevity.

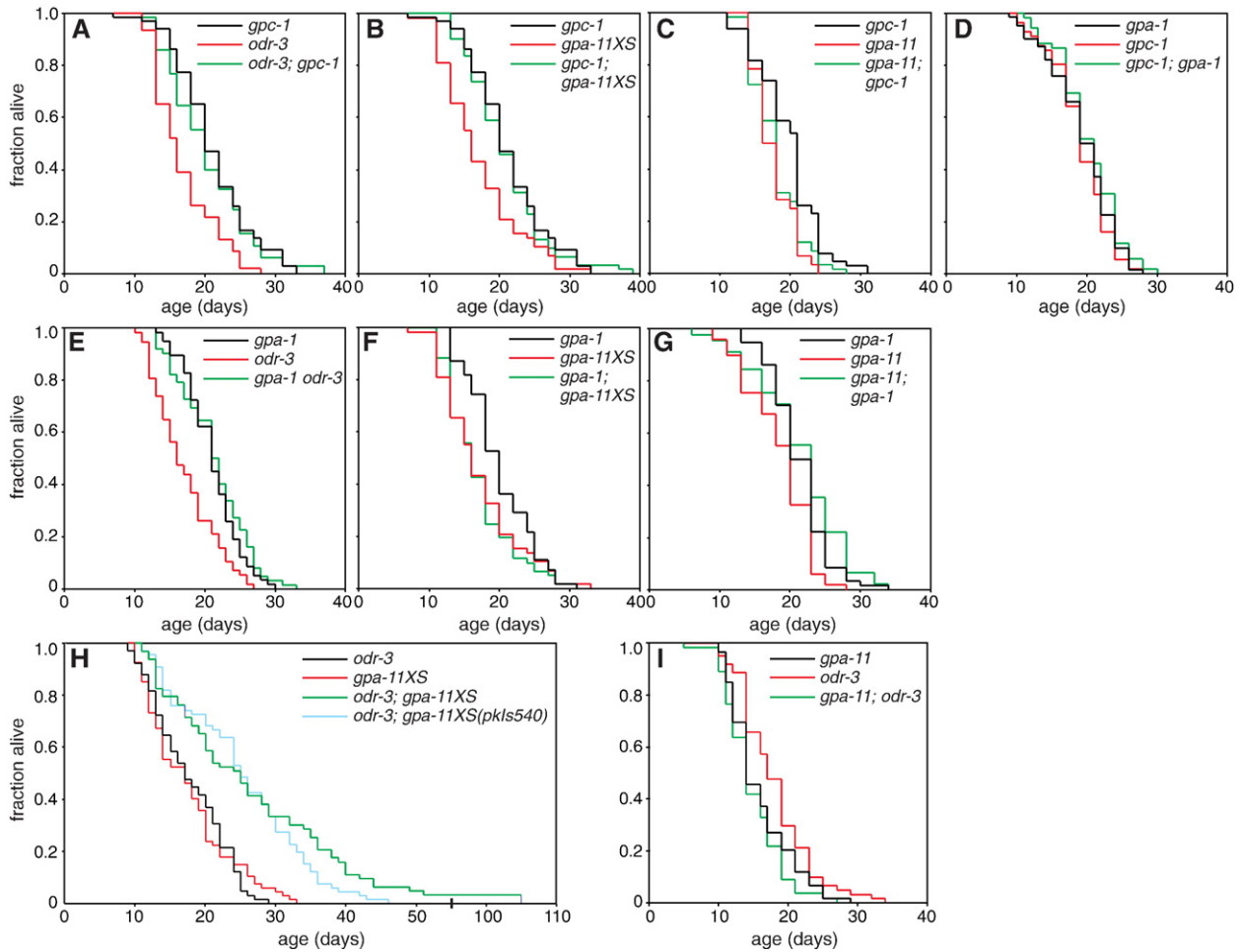


Fig. 2. Sensory G protein subunits redundantly and synergistically regulate longevity. (A, B) Life spans of *odr-3; gpc-1* and *gpc-1; gpa-11XS* animals are similar to life span of *gpc-1* single mutants. (C) Life span of *gpa-11; gpc-1* mutants is similar to that of *gpa-11* single mutants, but life span of *gpa-1; gpa-11XS* animals is similar to that of *gpa-1* and *gpa-1* single mutants (E, F) Life span of *gpa-1 odr-3* mutants is similar to that of *gpa-11XS* animals. (G) *odr-3; gpa-11XS* animals show synergistic life span extension. (I) *gpa-11; odr-3* mutants live as long as *gpa-11* single mutants. Shown are the results of single, representative life span assays at 20 °C. Cumulative results are summarized in Supplementary Table S1. Numbers of animals (*n*) and *p* values comparing differences between single mutant and double mutant were (A) *gpc-1* (*n*=66, *p*=0.563), *odr-3* (*n*=46, *p*=0.001), *odr-3; gpc-1* (*n*=65), (B) *gpc-1* (*n*=66, *p*=0.764), *gpa-11XS* (*n*=58, *p*=0.004), *gpc-1; gpa-11XS* (*n*=61), (C) *gpc-1* (*n*=65, *p*=0.005), *gpa-11* (*n*=60, *p*=0.365), *gpa-11; gpc-1* (*n*=58), (D) *gpa-1* (*n*=62, *p*=0.289), *gpc-1* (*n*=56, *p*=0.086), *gpa-1; gpc-1* (*n*=52), (E) *gpa-1* (*n*=58, *p*=0.339), *odr-3* (*n*=57, *p*<0.0001), *gpa-1 odr-3* (*n*=62), (F) *gpa-1* (*n*=55, *p*=0.005), *gpa-11XS* (*n*=58, *p*=0.671), *gpa-1; gpa-11XS* (*n*=61), (G) *gpa-1* (*n*=58, *p*=0.248), *gpa-11* (*n*=49, *p*=0.001), *gpa-11; gpa-1* (*n*=45), (H) *odr-3* (*n*=65, *p*<0.0001 compared to *odr-3; gpa-11XS* and to *odr-3; gpa-11XS(pkIs540)*), *gpa-11XS* (*n*=67, *p*<0.0001), *odr-3; gpa-11XS* (*n*=63), *odr-3; gpa-11XS(pkIs540)* (*n*=66), (I) *gpa-11* (*n*=59, *p*=0.194), *odr-3* (*n*=61, *p*=0.0003) and *gpa-11; odr-3* (*n*=55).

Sensory G proteins signal via the FOXO family transcription factor DAF-16 to regulate life span

Longevity in *C. elegans* and other organisms is under control of insulin/IGF-1 signaling (Kenyon, 2005). Reduction-of-function mutations in the insulin/IGF-1 receptor homologue *daf-2* greatly extend life span (Kenyon et al., 1993; Kimura et al., 1997). This life span extension depends on signaling via the FOXO family transcription factor *daf-16*, since *daf-16* mutations suppress the extended life span of *daf-2* mutants (Kenyon et al., 1993; Lin et al., 1997; Ogg et al., 1997). In addition, life span extension caused by cilia mutations or ablation of the ASI, AWA and AWC amphid neurons largely or completely depends on *daf-16* (Apfeld and Kenyon, 1999; Alcedo and Kenyon, 2004). To investigate if the regulation of longevity by *gpa-1*, *gpa-11*, *odr-3* and *gpc-1* involves insulin/IGF-1 signaling, we

tested the involvement of *daf-16*. To this end, we generated strains carrying the *daf-16(mu86)* loss-of-function mutation in combination with either *gpa-1*, *odr-3* or *gpc-1* loss-of-function mutations or extra copies of *gpa-11* and determined their life span. This showed that life span extension caused by loss-of-function of *odr-3*, *gpc-1* or extra copies of *gpa-11* completely depends on *daf-16* (Figs. 3A, C, D, E; Supplementary Table 1). *daf-16; odr-3* and *daf-16; gpa-11XS* animals even showed a slightly shorter life span than *daf-16* single mutants. In contrast, life span extension caused by loss-of-function of *gpa-1* was only partially dependent on *daf-16* (Fig. 3B; Supplementary Table 1). This is reminiscent of life span extension caused by most cilia mutations, which is, to some extent, also *daf-16* independent (Apfeld and Kenyon, 1999). Therefore, it is possible that life span extension caused by cilia mutations or loss-of-function of *gpa-1* involves similar mechanisms. An alternative explanation

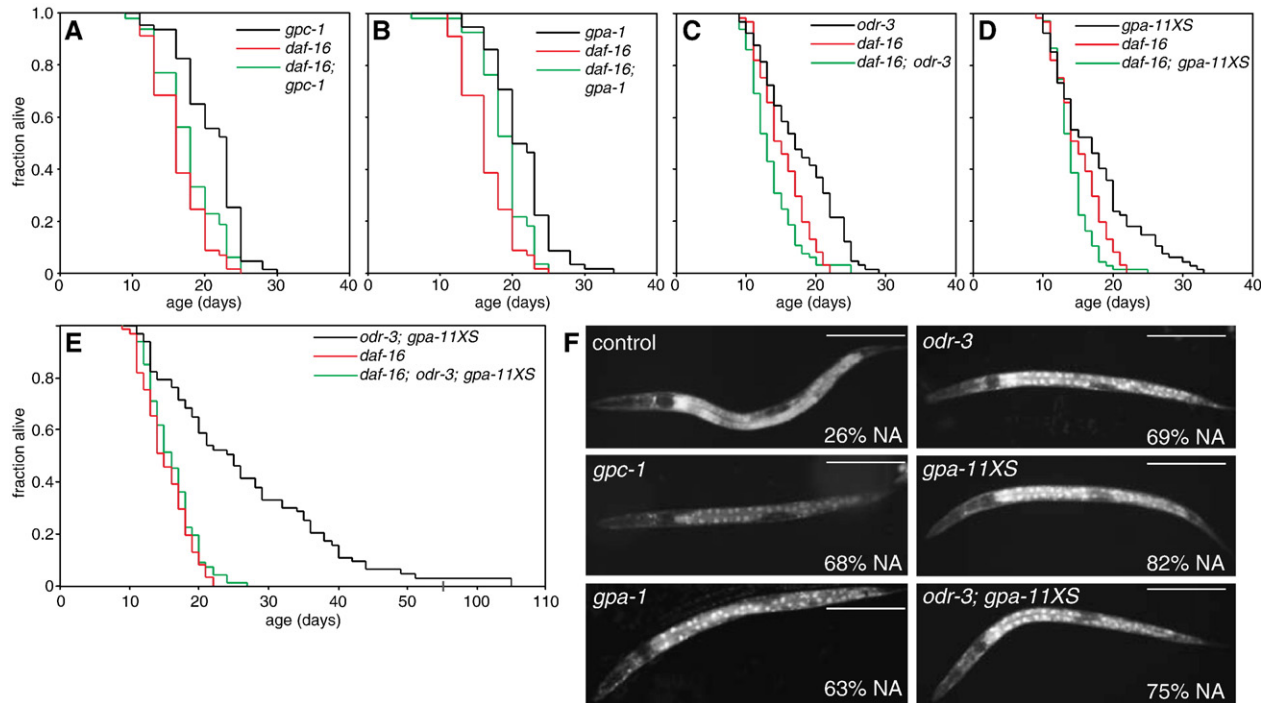


Fig. 3. Life span extension in G protein mutants is *daf-16* dependent. Life span extension in *gpc-1* (A), *odr-3* (C), *gpa-11XS* (D) or *odr-3; gpa-11XS* (E) is fully dependent on *daf-16*. In contrast, life span extension caused by loss-of-function of *gpa-1* (B) only partially depends on *daf-16*. (F) DAF-16::GFP showed increased nuclear accumulation in L2 larvae carrying *gpc-1*, *gpa-1*, and *odr-3* loss-of-function mutations or extra copies of *gpa-11*, in a *daf-16; muEx128[pKL79(daf-16a::GFP)]* mutant background. Shown are the percentages of animals in which the majority of DAF-16 is localized to the nucleus (NA=Nuclear Accumulation). Numbers of animals (*n*) and *p* values comparing differences between single mutants and double mutants or between double and triple mutants were (A) *gpc-1* (*n*=63, *p*=0.0001), *daf-16* (*n*=57, *p*=0.542), *daf-16; gpc-1* (*n*=48), (B) *gpa-1* (*n*=58, *p*=0.0004), *daf-16* (*n*=57, *p*=0.0004), *daf-16; gpa-1* (*n*=55), (C) *odr-3* (*n*=65, *p*<0.0001), *daf-16* (*n*=61, *p*=0.015), *daf-16; odr-3* (*n*=65), (D) *gpa-11XS* (*n*=67, *p*<0.0001), *daf-16* (*n*=61, *p*=0.017), *daf-16; gpa-11XS* (*n*=67), (E) *odr-3; gpa-11XS* (*n*=63, *p*<0.0001), *daf-16* (*n*=61, *p*=0.176), *daf-16; odr-3; gpa-11XS* (*n*=66), (F) *daf-16; daf-16a::GFP* (*n*=47), *daf-16; gpc-1; daf-16a::GFP* (*n*=34), *daf-16; gpa-1; daf-16a::GFP* (*n*=41), *daf-16; odr-3; daf-16a::GFP* (*n*=39), *daf-16; gpa-11XS; daf-16a::GFP* (*n*=33), *daf-16; odr-3; gpa-11XS; daf-16a::GFP* (*n*=32), scale bar: 100 μ m.

could be that cilia mutations partially disrupt G protein signaling in the cilia and in this way cause life span extension.

Cilia mutations induce the translocation of DAF-16 to the nucleus, where it promotes longevity (Lin et al., 2001). To test if this also occurs in our long-lived G protein mutants, we expressed a DAF-16::GFP fusion protein in these strains. Localization of DAF-16::GFP was examined in well-fed L2 animals grown at 20 °C. As expected, *gpc-1*, *gpa-1*, *odr-3*, *gpa-11XS* and *odr-3; gpa-11XS* animals all showed increased nuclear accumulation of DAF-16, as compared to control animals (Fig. 3F).

ODR-3 and GPA-11 signal partially independent of the insulin/IGF-1 like receptor DAF-2

Sensory neurons are thought to regulate longevity by influencing *daf-2* activity, probably through the secretion of insulin-like DAF-2 ligands (Apfeld and Kenyon, 1999; Pierce et al., 2001; Li et al., 2003; Alcedo and Kenyon, 2004). For instance, killing the ASI neurons does not further extend the life span of *daf-2* mutants, suggesting that the ASI neurons signal via *daf-2*. We determined if *gpa-1*, *odr-3* and *gpa-11* function in a pathway with *daf-2* by measuring the life span of *daf-2* (*e1370*); *gpa-1*, *daf-2; odr-3*, *daf-2; gpa-11XS*, *daf-2; odr-3*;

gpa-11XS and *daf-2; gpa-11* animals. Loss-of-function of *gpa-1* did not extend the life span of *daf-2* mutants, suggesting that *gpa-1* signals via *daf-2* to regulate life span (Fig. 4A; Supplementary Table 2). Surprisingly, *daf-2; odr-3* mutants lived longer than either single mutant (Fig. 4B; Supplementary Table 2), suggesting that *odr-3* and *daf-2* can function in separate pathways. In contrast, extra copies of *gpa-11* shortened the life span of *daf-2* and *daf-2; odr-3* mutants (Figs. 4C, D; Supplementary Table 2), indicating that in *daf-2* mutants *gpa-11* has an inhibitory function. To verify this, we measured the life span of *gpa-11; daf-2* loss-of-function mutants. As expected, *gpa-11* loss-of-function in *daf-2* mutants further extended life span (Fig. 4E; Supplementary Table 2). Thus, like *odr-3*, *gpa-11* might inhibit longevity independently of *daf-2*. However, definite conclusions cannot be drawn because the *daf-2*(*e1370*) allele used is a reduction-of-function allele (Kimura et al., 1997). Treatment of *daf-2*(*e1370*) animals with *daf-2* RNA interference further extends their life span (Arantes-Oliveira et al., 2003). Therefore, it is possible that *gpa-11* and *odr-3*, in a *daf-2*(*e1370*) background, signal via a partially defective DAF-2 protein. To test whether *odr-3* and *gpa-11* act synergistically to inhibit longevity, we generated *gpa-11; daf-2; odr-3* mutants. The life span of these triple mutants did not significantly differ from that of *gpa-11; daf-2* and *daf-2; odr-3* mutants (Fig. 4F;

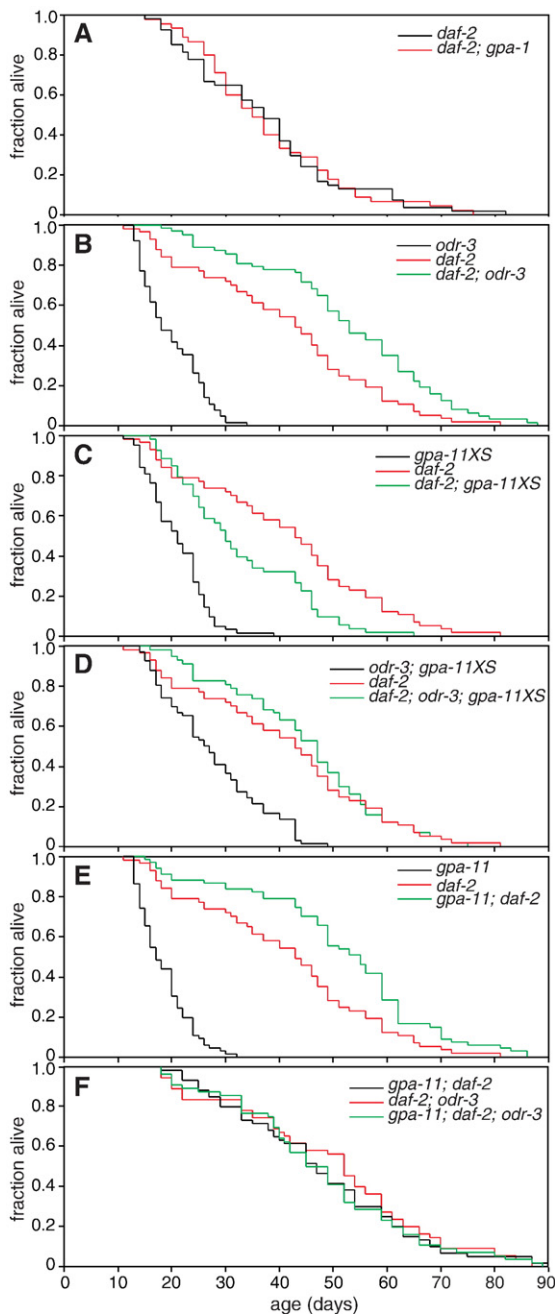


Fig. 4. ODR-3 and GPA-11 regulate life span independently of *daf-2*. Loss-of-function of *gpa-1* does not change *daf-2* life span (A). However, loss-of-function of *odr-3* increases the life span of *daf-2* mutants (B), while extra copies of *gpa-11* shorten the life span of *daf-2* mutants (C) and of *daf-2; odr-3* mutants (panel D compared with panel B). Similar to *odr-3*, loss-of-function of *gpa-11* increases the life span of *daf-2* mutants (E). *odr-3* and *gpa-11* probably act in the same pathway to extend *daf-2* mutant life span (F). Shown are the results of single, representative life span assays. Cumulative results are summarized in Supplementary Table S2. Numbers of animals and *p* values comparing differences between single and double mutants or between double and triple mutants were (A) *daf-2* ($n=54$, $p=0.969$), *daf-2; gpa-1* ($n=45$), (B) *odr-3* ($n=65$, $p<0.0001$), *daf-2* ($n=57$, $p=0.001$), *daf-2; odr-3* ($n=63$), (C) *gpa-11XS* ($n=63$, $p<0.0001$), *daf-2* ($n=57$, $p=0.001$), *daf-2; gpa-11XS* ($n=53$), (D) *odr-3; gpa-11XS* ($n=66$, $p<0.0001$), *daf-2* ($n=57$, $p=0.593$), *daf-2; odr-3; gpa-11XS* ($n=57$), (E) *gpa-11* ($n=66$, $p<0.0001$), *daf-2* ($n=57$, $p=0.001$), *gpa-11; daf-2* ($n=67$), (F) *gpa-11; daf-2* ($n=60$, $p=0.876$), *daf-2; odr-3* ($n=55$, $p=0.533$), *gpa-11; daf-2; odr-3* ($n=56$).

Supplementary Table 2), suggesting that, in *daf-2* mutants, *gpa-11* and *odr-3* share the same signaling pathway.

Our finding that *gpa-11* promotes longevity in wild type animals, while it suppresses longevity in *daf-2* mutants, seems a paradox. An explanation could be that *gpa-11* regulates two signals that affect longevity. One signal promotes longevity by inhibiting *daf-2* activity. The other signal, which is weaker and *daf-2* independent, suppresses longevity. Interestingly, also mutations in *daf-10* and *osm-3*, which cause cilia defects, and in *unc-31*, which impairs dense core vesicle neurosecretion, extend the life span of wild type animals, but shorten the life span of *daf-2* animals (Apfeld and Kenyon, 1999; Ailion et al., 1999). We found no indications that extra copies of *gpa-11* cause cilia defects. *gpa-11XS* animals show no sensory deficits and their sensory neurons take up fluorescent dye, a feature which requires intact cilia (Jansen et al., 1999).

The sensory G proteins GPA-1, GPA-11, ODR-3 and GPC-1 regulate dauer formation

At 27 °C, animals that carry extra copies of *gpa-11* constitutively develop into dauer larvae (Ailion and Thomas, 2003). This phenotype is also observed in cilia mutants, *unc-31* mutants and in many other insulin signaling mutants (Ailion et al., 1999; Ailion and Thomas, 2000, 2003). Dauer larvae can survive for long periods of time and under adverse environmental conditions. Dauer formation is induced by sensory cues, such as a constitutively secreted dauer pheromone, which serves as a signal of overcrowding, high temperature and food. Like longevity, dauer formation is under control of insulin/IGF-1 signaling (Riddle and Albert, 1997). Many mutants with increased longevity also show abnormal dauer formation. Therefore, we tested the ability of the G protein mutants with increased longevity to form dauer larvae in response to dauer pheromone. Mutations in *odr-3*, *gpa-1* and *gpc-1* caused reduced sensitivity to dauer pheromone, whereas extra copies of *gpa-11* caused hypersensitivity (Table 2). *gpa-9* and *gpa-11* loss-of-function had no effect. These results suggest that the same G proteins that regulate longevity also regulate dauer formation, possibly via similar mechanisms. Some G proteins might even mediate the

Table 2
Dauer sensitivity of G protein mutants at 25 °C

Strain	% dauers (\pm SEM) ^a	<i>n</i>
Wild type	87.9 \pm 3.5	301
<i>gpa-1</i>	66.8 \pm 1.5*	333
<i>gpa-9</i>	89.8 \pm 2.9	427
<i>gpa-11</i>	86.6 \pm 5.0	398
<i>odr-3</i>	65.8 \pm 0.1*	184
<i>gpc-1</i>	25.8 \pm 4.5*	307
<i>gpa-11XS</i>	100.0 \pm 0.0**	168
<i>odr-3; gpa-11XS</i>	96.0 \pm 0.9	73

^a Percentages of animals that developed into dauer larvae at 25 °C in the presence of dauer pheromone, within 48 h following egg laying. L2 and some L3 *gpa-11XS* and *odr-3 gpa-11XS* larvae, which were present after 48 h, possibly due to a growth arrest, were not scored. Percentages are the combined results of two test plates. Asterisks denote significant difference from wild type (* $p<0.01$, ** $p<0.05$).

detection of dauer pheromone, although dauer pheromone by itself does not affect life span (Alcedo and Kenyon, 2004).

G protein signaling in the sensory neurons, including ASH and/or ADL, controls the life span of C. elegans

Thus far, six sensory amphid neurons have been shown to regulate longevity. Killing the ASI, ASG, AWA and AWC neurons extends life span, whereas ablation of ASJ or ASK suppresses the extended life span of ASI ablated animals (Alcedo and Kenyon, 2004). The ASI and ASG neurons seem to function in parallel to the AWA and AWC neurons. Our results demonstrate that G protein signaling in these neurons, probably in response to sensory cues, regulates longevity in *C. elegans*. All G-proteins that were found to regulate life span, except *gpa-11*, function in one or more of these neurons: *odr-3* in AWA and AWC, *gpa-1* and *gpc-1* in ASI and ASJ and *gpa-9* in ASJ (Table 1; Roayaie et al., 1998; Jansen et al., 1999, 2002). The involvement of *gpa-11* implies that also the ASH and/or ADL neurons regulate longevity, since *gpa-11* is expressed exclusively in these two neuron pairs (Jansen et al., 1999). However, the functions of these neurons in longevity have never been studied. Interestingly, also *odr-3*, *gpa-1* and *gpc-1* are expressed in the ASH and/or ADL neurons.

In the sensory neurons, the G proteins could act at multiple different sites, either in the same cells or in different cells to regulate life span. Many sensory G proteins, including *odr-3* and *gpc-1*, are localized to the ciliated endings of the neurons (Roayaie et al., 1998; Lans et al., 2004; S. Rademakers and G.J., personal communication). However, some G proteins, including *odr-3* and *gpc-1*, are also observed in the dendrites, cell bodies and axons (Roayaie et al., 1998; Lans et al., 2004; S.R. and G.J., personal communication). Since there is extensive overlap between their expression patterns, *gpa-1*, *gpc-1*, *odr-3* and *gpa-11* could function in the same cellular compartment, being activated by the same signals or targeting the same molecules. For instance, it is very well possible that *gpc-1* acts in the same heterotrimeric complex as one or more of the G α subunits. Furthermore, the cGMP-gated channel α subunit *tax-4*, which probably acts downstream of *odr-3* in olfaction (Coburn and Bargmann, 1996; Komatsu et al., 1996), has been found to inhibit life span and could function downstream of *odr-3* or other G proteins in regulating longevity (Apfeld and Kenyon, 1999). Alternatively, some or all G proteins could function in different cellular compartments or separate cells, acting downstream or in parallel to each other.

Our results indicate that in the sensory neurons *gpa-1*, *odr-3* and *gpc-1* mediate a life span inhibiting signal, whereas *gpa-11* mediates a life span promoting signal (Fig. 5). Interestingly, loss-of-function of *odr-3* and extra copies of *gpa-11* synergistically extend life span. Furthermore, we find that, in *daf-2* and *gpa-1* animals, *gpa-11* seems to mediate a life span inhibiting signal. Inhibitory signaling via *gpa-1* and stimulatory signaling via *gpa-11* seem to be dependent on *daf-2* function, while inhibitory signaling via *odr-3* and *gpa-11* might be partially independent of *daf-2*. Because, *gpa-1*, *gpc-1* and *odr-3* do not act synergistically, they might act in the same pathway.

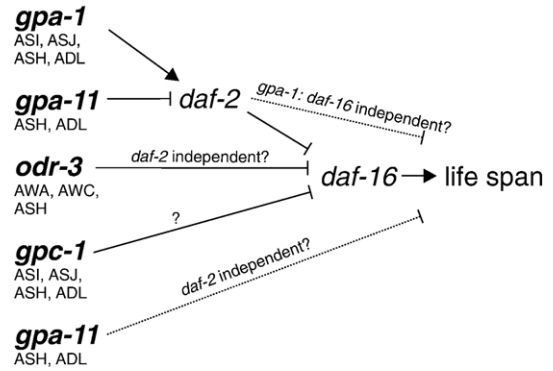


Fig. 5. Genetic pathways that modulate life span. The G proteins *gpa-1*, *odr-3* and *gpc-1* signal via *daf-16* to inhibit life span. In addition, *gpa-1* probably signals partially independent of *daf-16*, but in the same genetic pathway as *daf-2*. Furthermore, our results suggest that *odr-3* signals independently of *daf-2* and that *gpa-11* promotes life span by signaling via *daf-2* and *daf-16*. *gpa-11* also shows life span inhibiting activity, which seems independent of *daf-2*. Based on our data, however, we cannot exclude the possibility that *odr-3* and *gpa-11* signal via a partially defective DAF-2 protein. Whether *gpc-1* signals via *daf-2* has not been tested. Possibly, the G proteins act within multiple cells to regulate life span. Currently, our genetic data do not allow us to position these genes in one genetic pathway.

However, in *gpc-1* and *odr-3* mutants, *gpa-11* has a life span promoting role, whereas in *gpa-1* mutants, *gpa-11* has a life span inhibiting function. Therefore, our results cannot be easily unified into one simple model (Fig. 5).

It is not surprising that a complex sensory G protein signaling network regulates longevity. First of all, similar complex G protein signaling networks in the sensory neurons have been found to regulate olfaction, taste and gene expression (Lans et al., 2004; Lans and Jansen, 2006; Hukema et al., 2006). These are probably required to allow *C. elegans* to respond to many different cues using only few cells. Secondly, since multiple sensory neurons were found to regulate longevity, the different G proteins probably function in more than one neuron to control longevity. Furthermore, it is very well possible that *gpc-1* is involved in signaling via *gpa-1*, *odr-3* and *gpa-11* by functioning as G γ subunit of these G α subunits. Extra copies of *gpa-11* or loss-of-function of *gpa-1* or *odr-3* could extend life span by disrupting *gpc-1* function or vice versa. Finally, the interpretation of our results might be complicated by the fact that G protein gain-of-function mutations, induced by extra copies of these genes, can produce similar or opposite phenotypes as loss-of-function mutations. In this study, extra copies of *gpa-9* extended life span similarly to *gpa-9* loss-of-function, while extra copies of *gpa-11* had the opposite effect of *gpa-11* loss-of-function. Similar results have been observed in *C. elegans* olfaction and taste, where *odr-3* and *gpc-1* loss-of-function and overexpression cause similar phenotypes (Roayaie et al., 1998; Jansen et al., 2002). In contrast, loss-of-function and overexpression of *gpa-2*, *gpa-3* and *gpa-5* produce opposite effects in *C. elegans* dauer response and odorant detection (Zwaal et al., 1997; Jansen et al., 1999).

In this study, we did not extensively analyze the role of *gpa-9* (Fig. 1D) or other sensory G α subunits for which we did not find a clear effect on life span (Table 1), but which could have

redundant life span promoting or suppressing roles. We expect that as more details of the sensory signaling pathways that regulate longevity are being revealed, ultimately, the environmental signals that control the life span of *C. elegans* and other organisms will be identified.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ydbio.2006.11.028.

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