Gαo and Gαq Regulate the Expression of daf-7, a TGFβ-like Gene, in Caenorhabditis elegans

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Abstract

Caenorhabditis elegans enter an alternate developmental stage called dauer in unfavorable conditions such as starvation, overcrowding, or high temperature. Several evolutionarily conserved signaling pathways control dauer formation. DAF-7/TGFβ and serotonin, important ligands in these signaling pathways, affect not only dauer formation, but also the expression of one another. The heterotrimeric G proteins GOA-1 (Gαo) and EGL-30 (Gαq) mediate serotonin signaling as well as serotonin biosynthesis in C. elegans. It is not known whether GOA-1 or EGL-30 also affect dauer formation and/or daf-7 expression, which are both modulated in part by serotonin. The purpose of this study is to better understand the relationship between proteins important for neuronal signaling and developmental plasticity in both C. elegans and humans. Using promoter-GFP transgenic worms, it was determined that both goa-1 and egl-30 regulate daf-7 expression during larval development. In addition, the normal daf-7 response to high temperature or starvation was altered in goa-1 and egl-30 mutants. Despite the effect of goa-1 and egl-30 mutations on daf-7 expression in various environmental conditions, there was no effect of the mutations on dauer formation. This paper provides evidence that while goa-1 and egl-30 are important for normal daf-7 expression, mutations in these genes are not sufficient to disrupt dauer formation.

Introduction

Under unfavorable environmental conditions, developing Caenorhabditis elegans enter an alternative stage called dauer. In dauer, growth and feeding arrest. Dauer worms also have sealed orifices and form thickened cuticles. The metabolic and morphological changes that accompany dauer increase the likelihood of the animals’ survival under harsh conditions. The dauer stage is reversible, and larvae resume development when environmental conditions improve (reviewed in [1]). Dauer formation is controlled in part by the DAF-7/TGFβ-like signaling pathway ([2] and reviewed in [1]). DAF-7 is expressed in the ASI sensory neurons and is required during larval development to inhibit dauer formation [3,4]. Environmental cues such as starvation and high temperature that trigger dauer formation also downregulate daf-7 expression [3]. While several genes are required for normal daf-7 expression [5–7], the signaling pathways that control daf-7 expression and its sensitivity to environmental signals are still not well understood.

One of the genes required for both daf-7 expression and dauer formation encodes tryptophan hydroxylase, TPH-1 [6]. TPH-1 is the rate-limiting enzyme required for serotonin biosynthesis. Serotonin signals through the heterotrimeric G proteins GOA-1 and EGL-30 to control several C. elegans behaviors [8–11]. GOA-1 and EGL-30 share a high degree of homology with human Gαo and Gαq [12]. In the human nervous system, Gαo and Gαq act downstream of many neurotransmitters, including serotonin. In C. elegans, GOA-1 and EGL-30 also act upstream of tph-1 to regulate its expression [13]. It is possible then, that goa-1 and egl-30 are important for regulating daf-7 expression and dauer formation, and may do so by regulating either serotonin signaling or biosynthesis. These experiments explore, in a tractable model organism, a new relationship between evolutionarily conserved pathways and proteins important for neuronal signaling and developmental plasticity.

Results and Discussion

Gαo and Gαq do not Affect Morphology of daf-7-expressing Cells

daf-7 is expressed in two head sensory neurons called the ASIs [3,4]. Structural changes in ASI cilia accompany dauer formation [14]. Two neuronal heterotrimeric G proteins, GPA-2 and GPA-3, affect dauer formation by affecting the sensory cilia of ASIs [15–17]. By altering the sensory cilia, GPA-2 and GPA-3 presumably affect the way C. elegans sense the environmental signals that regulate the dauer developmental switch. It is possible that additional G proteins such as GOA-1 and EGL-30 (which, unlike GPA-2 and GPA-3, have homologues in humans) could also primarily affect dauer formation or daf-7 expression through altering the morphology of ASI. To first determine whether ASI morphology was affected by mutations in either goa-1 or egl-30, gross neuronal structure was visualized using DiD. Worms were incubated in DiD, a lipophilic dye, that is only taken up by those sensory neurons making direct contact.
with the environment. Therefore, if ASI morphology were altered in either *goa-1* or *egl-30* mutants, the neurons would not fill with dye. ASIs in wild type, *goa-1 (n1134)* partial loss-of-function, *goa-1(sa734)* null, *egl-30 (n686 and ad805)* partial loss-of-function (lf) mutants as well as *egl-30 (tg26 and js126)* gain-of-function (gf) mutants filled with DiD (Figure 1 and data not shown). These worms also exhibited normal gross morphology of ASIs, suggesting that signaling through either *goa-1* or *egl-30* is not required for ASI development.

**Gs and Gaq are Required for daf-7 expression but not Dauer Formation**

*daf-7* expression peaks during the first and second stages of larval development (L1 and L2; [3]) just before the dauer decision is made. When animals were raised in a favorable environment (with food at 20°C), all wild-type L1 larvae exhibited strong *daf-7* expression. *daf-7* expression was markedly reduced in larvae with (lf) alleles of *goa-1* or *egl-30* (Figure 2). In addition, *daf-7* expression was reduced in larvae with *egl-30* gf alleles.

![Figure 1. Mutations in *goa-1* and *egl-30* do not affect ASI development.](image-url)

Representative photomicrographs showing DiD filling (first column) and *pda7-7::GFP* (second column) in wild type, *egl-30*, and *goa-1* mutant backgrounds. DiD filling was not significantly different between ASI neurons (indicated by white arrows) in wild type and mutant worms. ASI projections, visualized with *pda7-7::GFP*, do not appear significantly altered in mutant worms. The last column shows a merge between DiD, GFP, and DIC images. *pda7-7::GFP* strains used express GFP under the putative *daf-7* promotor, and only express GFP in the ASI neurons [5].

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These data were unexpected for several reasons. First, one would expect that loss-of-function mutations and gain-of-function mutations in the same gene might have opposite effects on their target, in this case daf-7. However, both the (lf) and (gf) mutations in egl-30 decreased daf-7 expression.

The second unexpected result was that daf-7 expression was reduced in both goa-1 and egl-30 (lf) mutants. This was surprising because signaling through GOA-1 is thought to antagonize signaling through EGL-30. These two G proteins are thought to act antagonistically because they have opposite effects on many C. elegans behaviors [9, 18, 19]. In addition, goa-1 and egl-30 have opposite effects on tph-1 expression; goa-1 represses tph-1 expression while egl-30 promotes tph-1 expression [13]. Since tph-1 promotes daf-7 expression [6], one would expect that goa-1 (lf) mutations would cause an increase in daf-7 expression while egl-30 (lf) mutations might cause a decrease in daf-7 expression. The data suggest that goa-1 and egl-30 are both required to maintain daf-7 expression, and that any perturbation to either signaling pathway results in decreased daf-7 expression. Because goa-1 and egl-30 are expressed in many cells throughout the worm, it is possible that goa-1 and egl-30 act in distinct subsets of cells that could have opposite effects on daf-7 expression. For instance, goa-1 could be required to activate a set of neurons that promotes daf-7 expression, while egl-30 could act to inhibit the activity of a set of neurons that inhibits daf-7 expression. In a scenario such as this, (lf) mutations in both goa-1 and egl-30 would result in decreased daf-7 expression. The decrease in daf-7 expression seen in goa-1 and egl-30 mutants at 20°C was not sufficient, however, to elicit dauer formation (Figure 3).

How precisely EGL-30 and GOA-1 regulate daf-7 expression may be difficult to elucidate because of a complex feedback loop that exists between daf-7 and tph-1. While TPH-1 upregulates daf-7 expression [6], DAF-7 downregulates tph-1 expression [20]. tph-1 expression is also elevated in dauer larvae [21] when daf-7 expression is low. It is unlikely that GOA-1 or EGL-30 act downstream of daf-7 to regulate tph-1 expression (and then daf-7 expression) because egl-30 and goa-1 are not necessary for the increase in tph-1 seen in dauer larvae [21]. GOA-1 and EGL-30 may instead be acting downstream or independently of tph-1 to regulate daf-7 expression.

G$_{q_o}$ and G$_{q_4}$ Alter Temperature-induced Changes in daf-7 Expression but not Dauer Formation

High temperatures can induce dauer formation in some mutants that do not readily form dauers at moderately high temperatures [22, 23]. It was possible that while goa-1 and/or egl-30 mutants did not form dauers at favorable temperatures (20°C, Figure 3), they would enter dauer at high temperatures. Moderately high (25°C) or high (27°C) temperatures were both insufficient to induce dauer formation in any of the G protein mutants tested (Figure 3). While most non-dauer worms developed into full adults, goa-1(sa734) null and egl-30(tg26gf) mutants did not. These non dauer worms appeared to arrest as larvae; either L1 or partial dauers (determined by SDS sensitivity). The larval arrest in
these mutants occurred prior to the time in development when the
dauer decision is made, and suggests that normal development at
27°C was disrupted by the sa734 and tg26 alleles.

Despite the absence of dauer formation seen at 25°C or 27°C,
daf-7 expression was significantly altered in all mutant strains
tested (Figure 2). In almost all strains, there was a significant
difference in the way temperature affected daf-7 expression. EGL-30 appears to be
important for downregulating daf-7 in response to high temperatures.
In fact, the effect of temperature on daf-7 expression is reversed in the egl-30(lf) mutants and exaggerated in the egl-30(js126gf) mutant. Other signaling pathways likely contribute to the
behavioral/developmental response to high temperature since the
decrease in daf-7 expression in egl-30(gf) mutants was not sufficient
to induce dauer formation at high temperatures.

Gαq and Gαi Alter the Response to Limiting Amounts of
Food
In addition to temperature, food availability affects the course of
C. elegans development. When larvae hatch from eggs in the
absence of food, their development arrests at the L1 stage (prior to
the time in development when the dauer decision is made) and daf-7 expression is reduced in the arrested L1s [3]. Signaling through
GOA-1 and EGL-30 modulates food sensitivity in adult C. elegans
[24–26], so it is possible goa-1 and egl-30 are required for mediating
the effect of food in larvae. As in wild-type L1s, starvation caused a
significant reduction in daf-7 in goa-1(gf) mutants (Figure 4). As
expected, an equivalent reduction was seen in the egl-30(tg26gf)
mutant and an increase was seen in the egl-30(n686lf) mutant.
While these mutants still appeared to be in the arrested L1 stage,
the data suggest that egl-30 mediates the daf-7 response to
starvation. These results and those from studies in adult worms
[25] suggest that EGL-30 plays the same role in the response to
starvation throughout development.

Reduced daf-7 expression was not seen in worms expressing the
other egl-30(gf) mutant allele (js126), however. The difference
between the egl-30(gf) phenotypes may be caused by differences in
the way each mutation affects the EGL-30 protein. The tg26(gf)
allele is thought to contain a mutation that alters guanine
nucleotide binding [27]. The js126(gf) allele is thought to contain
a mutation that alters GTPase activity [28]. Both tg26 and js126
mutants have (gf) phenotypes with respect to other EGL-30-
dependent behaviors such as egg laying and movement [28,29],
however it is not clear whether it is reasonable to predict that both
alleles would affect all ELG-30-dependent processes in the same
way.

When larvae hatch in the presence of limiting amounts of food,
they progress through the L1 stage and then enter dauer [30]. If
egl-30 and goa-1 are important for relaying the food cues important
for normal development, one would expect that mutations in

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**Figure 3. Neither goa-1 nor egl-30 inhibit dauer entry at high temperatures.** Dauer formation was assayed in larvae raised at 25°C or 27°C. None of the mutant strains tested exhibited a significant increase in dauer formation at higher temperatures. It was not possible to assay dauer formation in goa-1(sa734) null or egl-30(tg26gf) mutants at 27°C, because these mutants formed partial dauers or arrested at the L1 stage. Data for these strains at 27°C were therefore not included in the figure. daf-7(e1372) mutants are constitutive dauers at 25°C and 27°C, and served as a positive control. * = significant difference from wild type (N2) worms at the same temperature (Student’s t-test, p<0.05). ** = significant difference between treatments in the same genotype (Student’s t-test, p<0.05). # = significant interaction between genotype and change in temperature (from 20°C), as compared to wild type larvae (ANOVA, p<0.05).

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either goa-1 or egl-30 would disrupt dauer formation caused by low food levels. When goa-1 and egl-30 mutant larvae were exposed to limiting amounts of food, they did arrest at an early stage of development (Figure 5). However, based on size, most larvae appeared to be arrested at the L1 or partial dauer stages and not as full dauers. Most of the small larvae exhibited pharyngeal pumping and were sensitive to SDS, indicating that they did not arrest as full dauers [31]. These data suggest that goa-1 and egl-30 mutant worms are still sensitive to alterations in food availability, because they did not fail to arrest development in the presence of limiting food.

Overall, the experiments in this study showed that goa-1 and egl-30 regulate daf-7 expression in early development. While goa-1 and egl-30 mutations significantly decreased daf-7 expression, they did not affect dauer formation. These results suggest that other signaling pathways act in concert with GOA-1 and EGL-30 to decrease daf-7 expression to levels sufficient to induce dauer formation.

Materials and Methods

Worm Strains

C. elegans worm strains were maintained on NGM plates with *Escherichia coli* OP50 as the food source [32]. Strains were provided by the Caenorhabditis Genetic Center (CGC) and were derived from the wild-type N2 Bristol strain. Strains used were as follows: N2, JJ734 goa-1(n734), KO95 goa-1(n1344), MT1434 egl-30(n686d), NM1380 egl-30(p126gf), KY26 egl-30(hex26gf), DA823 egl-30(ad805), and CB1372 daf-7(e1372ts). Strains containing G protein mutations were crossed into the FK181 ksl2[pdaf-7::GFP, rol-6(su1006)] strain for pdaf-7::GFP analysis.

Microscopy

For all assays, the developmental stages of larvae were carefully synchronized. For temperature assays, gravid adults laid eggs on NGM plates for 4 hours. Adults were removed and eggs were grown on NGM plates for 18–24 hours. For starvation assays, gravid adults were bleached to isolate eggs. Eggs were grown on NGM plates for 18–24 hours (fed) or in M9 medium for 48 hours (starved). Larvae were transferred to a 4% agarose pad on a microscope slide, immobilized with 10 mM levamisole, and viewed using a Leica DM5500 microscope. ASI images were captured with a fixed exposure time using a Hammamatsu Orca ER camera and Leica Microsystems Image capture software. GFP intensity was quantified using NIH Image J software version 1.44o. The intensity of GFP in each ASI cell body was quantified. The intensity of a similarly sized background selection was subtracted from the ASI GFP intensity to get the adjusted GFP intensity. Approximately ten larvae of each genotype were imaged in each experiment. Experiments were performed in triplicate, on three separate days. Dye filling was performed using 0.1 mg/ml DiD (Molecular Probes) as described [33].

Dauer Assays

Dauer assays were done similarly to those previously described [30]. Modified NGM plates were prepared without peptone, and Noble agar (Difco) was used. 3 ml of modified NGM was used in each dauer assay plate. Plates were seeded with 20 µl of 4% (w/v) OP50, unless otherwise noted. *C. elegans* OP50 was resuspended in S...
Medium with 50 μg/ml streptomycin. Larvae were synchronized by first isolating eggs from bleached, gravid adults. Eggs were resuspended in S Medium. Approximately 100 eggs were pipetted onto seeded dauer plates that were incubated for 60–72 hours. Dauer worms were scored visually, and scoring was confirmed using SDS. Worms were considered dauers if they survived a several minute incubation in 1%SDS. Larvae were considered partial dauers if they were the same size and shape as dauer larvae, but exhibited pharyngeal pumping and did not survive SDS treatment.

Assays were performed with 100–200 worms for each genotype. All genotypes were tested in an assay. Assays were performed in triplicate, on three separate days.

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Author Contributions

Conceived and designed the experiments: EMM. Performed the experiments: EMM. Analyzed the data: EMM. Contributed reagents/materials/analysis tools: EMM. Wrote the paper: EMM.

References

1. Fielenbach N, Antebi A (2009) C. elegans dauer formation and the molecular basis of plasticity. Genes Dev. 22: 2149–2165.
2. Swanson MM, Riddle DL (1981) Critical periods in the development of the Caenorhabditis elegans dauer larvae. Dev. Biol. 84: 27–40.
3. Ren P, Lim CS, Johnsen R, Albert PS, Pilgrim D, et al. (1996) Control of C. elegans larval development by neuronal expression of a TGFβ homolog. Science 274: 1389–1391.
4. Schackwitz WS, Imose T, Thomas JH (1996) Chemosensory neurons function in parallel to mediate a pheromone response in C. elegans. Neuron 17: 719–728.
5. Koga M, Take-uchi M, Tanenishi T, Ohshima Y (1999) Control of DAF-7 TGFβ expression and neuronal process development by a receptor tyrosine kinase KIN-8 in Caenorhabditis elegans. Development 126: 5307–90.
6. Sue JY, Victor M, Lee C, Shi Y, Ruvkun G (2000) Food and metabolic signalling defects in a Caenorhabditis elegans serotonin-synthesis mutant. Nature 403: 560–564.
7. Murakami M, Koga M, Ohshima Y (2001) DAF-7/TGFβ expression required for the normal larval development in C. elegans is controlled by a presumed guanylyl cyclase DAF-11. Mech. Dev. 109: 27–33.
8. Segalat L, Ellis DA, Kaplan JM (1995) Modulation of serotonin-controlled behaviors by Go in C. elegans. Science 267: 1648–1651.
9. Nurrish S, Segalat L, Kaplan JM (1999) Serotonin inhibition of synaptic transmission: Galpha(o) decreases the abundance of UNC-13 at release sites. Neuron 24: 231–242.
10. Shyn SL, Kerr R, Shafer W (2003) Serotonin and Go modulate functional states of neurons and muscles controlling C. elegans egg laying behavior. Curr. Biol. 13: 1910–1915.
11. Dempsey CM, Mackenzie SM, Gargus A, Blanco G, Sue JY (2005) Serotonin (5HT), fluoxetine, imipramine and dopamine target distinct 5HT receptor signaling to modulate Caenorhabditis elegans egg laying behavior. Genetics 149: 1425–1436.

Figure 5. Neither goa-1 nor egl-30 are required for dauer formation in response to reduced food. Dauer formation was assayed in larvae grown at 25°C on several concentrations of E. coli OP50. Wild type larvae formed dauers as food concentration decreased. In at least one assay, non-dauer worms of egl-30 (tg26gf), egl-30 (n886lf), egl-30 (ad805lf), or goa-1 (n1134lf) genotype did not develop into adults when grown on 1% food. Instead, these larvae were arrested as L1s or partial dauers. Data for these strains at 1% food concentration were therefore not included in the figure. * = significant difference from wild type (N2) worms at the same food concentration (Student’s t-test, p<0.05). ** = significant difference between treatments in the same genotype (Student’s t-test, p<0.05).

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12. Jansen G, Thijssen KL, Werner P, van der Horst M, Hazendonk E, et al. (1999) The complete family of genes encoding G proteins in Caenorhabditis elegans. Nat Genet. 21: 414–419.
13. Tanis JE, Moresco JJ, Lindquist RA, Koelle MR (2008) Regulation of serotonin biosynthesis by the G proteins Gaq and Gae controls serotonin signaling in Caenorhabditis elegans. Genetics 78: 157–169.
14. Albert PS, Riddle DL, (1983) Developmental alterations in sensory neuroanatomy of the Caenorhabditis elegans dauer larva. J Comp. Neurol. 219: 461–461.
15. Zwaal RR, Mendel JE, Sternberg PW, Plasterk RHA (1997) Two neuronal G proteins involved in chemosensation of the Caenorhabditis elegans dauer-inducing pheromone. Genetics 145: 715–727.
16. Gallo M, Riddle DL (2009) Effects of a Caenorhabditis elegans dauer pheromone ascaroside on physiology and signal transduction pathways. J. Chem. Ecol. 35: 272–279.
17. Burghoorn J, Dekkers MPJ, Rademakers S, de Jong T, Willemsen R, et al. (2010) Dauer pheromone and G-protein signaling modulate the coordination of intraflagellar transport kinesin motor proteins in C. elegans. J Cell Sci. 123: 2077–2084.
18. Lackner MR, Nurrish SJ, Kaplan JM (1999) Facilitation of synaptic transmission by EGL-30 Gae and EGL-6 PLCβ: DAG binding to UNC-13 is required to stimulate acetylcholine release. Neuron 24: 335–346.
19. Miller KG, Emerson MD, Rand JB (1999) Goalpha and diacylglycerol kinase negatively regulate the Gqalpha pathway in C. elegans. Neuron 24: 323–333.
20. Estvez M, Estvez AO, Cowie RH, Gardiner KI (2004) The voltage-gated calcium channel UNC-2 is involved in stress-mediated regulation of tryptophan hydroxylase. J Neurochem. 88: 102–113.
21. Moussaif M, Sze JY (2009) Intraflagellar transport/Hedgehog-related signaling components couple sensory cilium morphology and serotonin biosynthesis in Caenorhabditis elegans. J Neurosci. 29: 4065–4073.
22. Ailion M, Thomas JH (2003) Isolation and characterization of high-temperature-induced dauer formation mutants in Caenorhabditis elegans. Genetics 165: 127–144.
23. Dong MQ, Chase D, Pantoskou GA, Koelle MR (2000) Multiple RGS proteins alter neural G protein signaling to allow C. elegans to rapidly change behavior when fed. Genes Dev. 14: 2003–2014.
24. Sato S, Kimura Y, Van Tol HHM (2006) Starvation induces cAMP response element-binding protein-dependent gene expression through octopamine-Gae Signaling in Caenorhabditis elegans. J Neurosci. 26: 10002–10009.
25. Hoffer C, Koelle MR (2011) AGS-3 Alters Caenorhabditis elegans behavior after food deprivation via RIC-8 activation of the neural G protein Gae. J Neurosci. 31: 11553–11562.
26. Bastiani CA, Gharib S, Simon MI, Sternberg PW (2003) Caenorhabditis elegans Gaeq regulates egg-laying behavior via a PLCβ-dependent signaling pathway and likely functions both in the nervous system and in muscle. Genetics 165: 1805–1822.
27. Hawasli AH, Saifree O, Liu C, Nenot ML, Crowder CM (2004) Resistance to volatile anesthetics by mutations enhancing excitatory neurotransmitter release in Caenorhabditis elegans. Genetics 168: 831–843.
28. Doi M, Iwasaki K (2002) Regulation of retrograde signaling at neuromuscular junctions by the novel C2 domain protein AEX-1. J. Neuroscience 22: 102–259.
29. Golden JW, Riddle DL (1984) The Caenorhabditis elegans dauer larva: developmental effects of pheromone, food, and temperature. Dev. Biol. 102: 368–378.
30. Cassada RC, Russell RL (1975) The Dauerlarva, a post-embryonic developmental variant of the nematode Caenorhabditis elegans. Dev. Biol. 46: 326–342.
31. Perkins LA, Hedgecock EM, Thomson JN, Culotti JG (1986) Mutant sensory cilia in the nematode Caenorhabditis elegans. Dev. Biol. 177: 456–487.