Genetic dissection of G protein-coupled signal reduction
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An activating mutation in a Caenorhabditis elegans G_s protein induces neuronal degeneration.

An activating mutation in a *Caenorhabditis elegans* Gs protein induces neural degeneration

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Heterotrimeric guanine nucleotide-binding proteins (G proteins) act as signal-transducing molecules that connect serpentine–transmembrane receptors to a variety of intracellular effectors. We characterized a *Caenorhabditis elegans* G, gene, gsa-1, which encodes a G, α-subunit (Gαs) that is expressed throughout the nervous system and in muscle cells. gsa-1 is an essential gene; a loss-of-function mutation in gsa-1 results in lethality at the first stage of larval development. Partial (mosaic) loss of Gαs expression or overexpression of the protein results in reciprocal defects in movement and egg-laying, suggesting a role for Gαs in the regulation of these behaviors. Expression of a constitutively active form of Gαs from an inducible promoter results in hypercontraction of body-wall muscle cells and vacuolization and degeneration of neurons within hours of induction. Neurons that are susceptible to the degeneration induced by activated Gαs are predominantly motoneurons located within the ventral nerve cord. Phenotypic analysis shows that the induced neural degeneration is not the result of programmed cell death but is probably caused by the activation of ion channels. A genetic suppressor of activated Gαs was isolated that identifies a putative downstream target of Gs signaling.

**Key Words:** G protein; Gsa-1; loss-of-function mutation; gain-of-function mutation; neural degeneration; suppressor mutation

Signaling pathways using serpentine–transmembrane receptors and their associated heterotrimeric guanine nucleotide-binding proteins (G proteins) have a key role in triggering physiological responses to a wide variety of hormones, neurotransmitters, and sensory stimuli (Simon et al. 1991; Hepler and Gilman 1992). Heterotrimeric G proteins consist of a guanine nucleotide-binding Ga subunit and a Gβγ subunit (Kaziro et al. 1991; Wall et al. 1995; Lambright et al. 1996). Both subunits have signaling capabilities and are released on activation of the G protein by a ligand-bound receptor (Clapham and Neer 1993). Multiple serpentine receptors, G protein subunits, and downstream effectors have been identified in vertebrates, demonstrating the complexity of G protein–coupled signal transduction. Despite the extensive data on the biochemical properties of these components, it is still poorly understood how information from different G protein–coupled signal transduction pathways is integrated and influences complex behavioral phenotypes. Insight into the complexity of G protein–coupled signal transduction can be gained from genetic studies in a relatively simple organism like the nematode *Caenorhabditis elegans*.

Several G protein subunit genes have been identified in *C. elegans*. These include homologs of mammalian Ga subunits: *C. elegans* Gαs and Gαq are >80% identical to their mammalian counterparts (Lochrie et al. 1991; Brundage et al. 1996). Gαs is expressed abundantly in the nervous system and is involved in modulating behaviors such as locomotion and egg-laying, implicating this pathway in serotonin signaling (Mendel et al. 1995; Segalat et al. 1995). egl-10, a regulator of Gαq, encodes a member of a novel family of GTPase-activating proteins (Koelle and Horvitz 1996). Locomotion and egg-laying are also modulated by Gαq, a gene identified previously genetically as egl-30 (Brundage et al. 1996). In addition to these highly conserved Ga subunits, two novel subunits were cloned as well (Lochrie et al. 1991; Fino Silva and Plasterk 1990). GPA-2 and GPA-3 are involved in chemosensation of a dauer pheromone (Zwaal et al. 1997). *C. elegans* expresses a highly conserved Gβ subunit (van der Voorn et al. 1990). Loss of gpb-1 results in embryonic lethality (Zwaal et al. 1996).

We studied a homolog of Gαq in *C. elegans*. gsa-1 encodes a protein that is 66% identical at the amino acid level to *Drosophila* and mammalian Gαq (Park et al. 1996).
was expressed extensively in embryos [data not shown]. In the different larval stages and in adults, expression was restricted uniformly to neural and muscle cells. Virtually all neurons showed expression of gsa-1. These included neurons located in the head ganglia [Fig. 1A], the ventral nerve cord [Fig. 1B], and the tail ganglia [Fig. 1C].

Also the hemaphrodite-specific neurons (HSNs), which control egg-laying and the canal cell-associated neurons (CANS) [Fig. 1E], showed expression of gsa-1. Most muscle cells expressed gsa-1 as well. These included body-wall muscle cells used in locomotion [Fig. 1D]. Body-wall muscle cells showed a punctate pattern of the translational gsa-1::gfp fusion, which may represent localization in dense bodies. Dense bodies function as attachment sites between the muscle cells and the cuticle and are flanked by membranes of the sarcoplasmic reticulum [Waterston 1988]. In addition, gsa-1 was expressed in the muscle cells of the pharynx [Fig. 1A] and in the uterine and vulval muscle cells that are used in egg-laying [Fig. 1B]. In mammals, Gαs is expressed in all tissues [Kaziro et al. 1991]. In Drosophila, however, the expression of Gαs is restricted to the nervous system and the eye [Quan et al. 1989; Wolfgang et al. 1991].

**gsa-1 is essential for viability**

We used a reverse-genetic approach to obtain a loss-of-function mutation of gsa-1. Using a transposon-based method [Zwaal et al. 1993], we isolated a deletion allele, gsa-1(pk75), in which part of the gsa-1-coding sequence was removed. As is shown in Figure 2A, a deletion of genomic sequence between two 16-bp direct repeats [AAAAATGTGACGTCAG] in introns 6 and 7 removes 1828 bp of gsa-1 sequence. Transposon-induced deletions occur frequently between such short direct repeats in the genomic sequence [Zwaal et al. 1993; Kurkulos et al. 1994]. In addition to removing exon 7, splicing of exon 6 to exon 8 will result in a frameshift. Consequently, pk75 removes about one-third of the gsa-1-coding sequence and is a probable null allele.

**Animals homozygous for pk75 arrest in larval development.** At hatching, pk75 homozygotes are morphologically normal but show little pharyngeal and body-wall muscle activity. In time, the internal tissues become shrunken [Fig. 2B, bottom] and the animals arrest at the first stage of larval development [L1], as indicated by the persistent presence of L1-specific alae. The lethal phenotype of pk75 is identical to the zygotic null phenotype of the Gαs subunit gene gpa-1 [Zwaal et al. 1996] and resembles the rod-like larval lethal phenotype of Ras pathway mutants and ceh-1 [G. Garriga, pers. comm.]. Animals heterozygous for pk75 are wild type in development and behavior. The larval lethal phenotype of pk75 was complemented with a wild-type gsa-1 construct. Transgenes in the form of extra-chromosomal arrays are unstable at meiosis and mitosis [Stinchcomb et al. 1985; Melilo and Fire 1985], resulting in mosaic expression of the transgene as it is lost in individual cells and lineages. Loss of the rescuing transgene in the germ line will result in broods that consist of
Genetic analysis of C. elegans Gaα

Figure 1. Expression of Gaα in C. elegans. The expression pattern of gsa-1 was analyzed using GFP gene fusions. [A] Head region. Expression of gsa-1 in the pharyngeal and body-wall muscle cells and in the nerve ring neuropil is shown. Note that this picture does not show the full extent of gsa-1 expression in the head ganglia to better visualize the pharyngeal muscle cells. (B) Region surrounding the vulva. gsa-1 is expressed in the ventral nerve cord and the vulva and uterine muscle cells. (C) Tail region. Expression of gsa-1 in the ventral nerve cord and in the tail ganglia is shown. (D) Expression of a translational gsa-1::GFP fusion in body-wall muscle cells results in a punctate expression pattern that may represent localization in dense bodies. The fusion protein contains half of the Gaα amino-terminal sequence and may direct localization to these structures. (E) Expression of gsa-1 in the CANs, the HSNs, and the posterior lateral ganglion (pl). (F) Expression of gsa-1 in one process of the H-shaped excretory cell (ec) is shown.

animals that lack functional gsa-1 expression both in the form of the transgene and in the form of maternal inheritance of gsa-1 mRNA or protein [Zwaal et al. 1996]. Of rescued pk75 transgenic animals, 1%-5% segregated broods that consisted only of arrested larvae. These are identical to the L1-arrested larvae segregated by pk75 heterozygous animals. Consequently, the larval lethal phenotype is probably the result of an essential function of zygotic gsa-1 during larval development and not the result of a limited supply of maternal gsa-1 mRNA or protein that allows embryogenesis to be completed.

Gaα modulates behaviors such as locomotion and egg-laying

In rescued pk75 transgenic lines, transgene mosaicism resulted in the segregation of arrested larvae, and in viable animals that showed a variety of behavioral defects resulting from the absence of Gaα in specific cells. Notably, mosaic animals defective in locomotion [Fig. 3A], egg-laying [Fig. 3B], and defection were observed. Animals mosaic for Gaα expression moved sluggishly or were paralyzed and locomotion was reduced to 5.0 ± 1.2 (mean ± S.E.M.) body bends per minute, compared with 19.0 ± 1.4 body bends per minute in wild-type animals. Mosaic animals were also defective in egg-laying; the number of unlaid eggs per animal was increased from 10 ± 0.4 in wild-type animals to 23 ± 1.4. Furthermore, the stage of newly laid eggs was significantly increased as well (Table 1). Overexpression of wild-type Gaα resulted in opposite behavioral defects. Transgenic animals overexpressing Gaα (GaαXS) showed an increase in locomotion to 31 ± 2.0 body bends per minute [Fig. 3A]. Moreover, GaαXS animals showed a decrease in egg content (4 ± 0.4) and a reduction in the stage of newly laid eggs, with only 4.2 ± 0.2 cells per egg in the 1-8 cells per
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Figure 2. Gene structure and loss-of-function phenotype of gsa-1. (A) Structure of the gsa-1 gene. Black boxes show coding sequence. The region deleted in gsa-1(pk75) is indicated. (SI Sall, (F) Fsp, (H) HindIII. (B) Larval lethal phenotype of gsa-1(pk75). (Top) A wild-type L1 larva; (bottom) an L1 larva homozygous for pk75. The phenotype of pk75 is characterized by the shrunken appearance of internal tissues.
movement defects, ranging from twitching movement to completely uncoordinated movement.

Genetic analysis of the activated Ga, phenotype

The Ga,QL-induced neural degeneration is morphologically different from programmed cell death; cells that are subject to apoptosis show a refractile and condensed appearance when viewed with Nomarski optics (Sulston and Horvitz 1977). To investigate whether the Ga,QL-induced degeneration is also genetically distinct from programmed cell death, we investigated if an intact apoptotic pathway is required for the Ga,QL-induced degeneration. The neurodegenerative phenotype of Ga,QL was analyzed in ced-3 and ced-4 mutant backgrounds (Table 2). CED-3 and CED-4 are essential, cell-autonomous components of the cell-death machinery and mutation of these components prevents death by apoptosis (Yuan and Horvitz 1990, 1992, Yuan et al. 1993). We found that the Ga,QL-induced neural degeneration was not affected by loss of the cell-death pathway, demonstrating that Ga,QL induces neural degeneration through a different mechanism. The Ga,QL-induced swelling and lysis of neurons is, however, similar to the vacuolar cell deaths observed in degenerin ion channel mutants. Dominant mutations in homologs of mammalian amiloride-sensitive epithelial Na\(^+\) channels induce vacuolization and degeneration of a specific subset of neurons, probably as a result of an osmotic imbalance or abnormal influx of Ca\(^{2+}\) caused by aberrant functioning of these mutated channels (Hong and Driscoll 1994).

Given the similarity in phenotype of Ga,QL and degenerin mutants, it is likely that Ga,QL acts by deregulating ion channels as well. We tested whether mutations in C. elegans ion-channel genes could suppress the Ga,QL-induced neural degeneration (Table 2). The Ga,QL phenotype was analyzed in a mec-6 mutant background. MEC-6 is postulated to be an essential component of degenerin ion channels and loss-of-function mutations in mec-6 suppress the degenerative phenotype of degenerin channel mutants (Chalfie and Wolinsky 1990; Huang and Chalfie 1994), including the motor neuron-specific degenerin unc-8 (Shreffler et al. 1995). mec-6 did not suppress the Ga,QL-induced neural degeneration, indicating that Ga,QL does not act specifically through degenerin ion channels. Similar results were obtained when Ga,QL activity was analyzed in combination with loss-of-function mutations in three different Ca\(^{2+}\) channel genes, unc-2 (Schafer and Kenyon 1995), egl-19, and unc-36 (L. Lobel, pers. comm.), suggesting that Ga,QL

<table>
<thead>
<tr>
<th>Genotype</th>
<th>1 to 8 cell</th>
<th>9-cell comma</th>
<th>post-comma</th>
<th>N</th>
</tr>
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<tbody>
<tr>
<td>Wild type</td>
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<td>97</td>
<td>0</td>
<td>803</td>
</tr>
<tr>
<td>Ga,KS</td>
<td>53</td>
<td>47</td>
<td>0</td>
<td>149</td>
</tr>
<tr>
<td>gsa-1(pk75) mosaic</td>
<td>0</td>
<td>1</td>
<td>99</td>
<td>156</td>
</tr>
<tr>
<td>egl-1(^b)</td>
<td>0</td>
<td>3</td>
<td>97</td>
<td>75</td>
</tr>
<tr>
<td>egl-1; Ga,KS(^a)</td>
<td>20</td>
<td>65</td>
<td>15</td>
<td>69</td>
</tr>
</tbody>
</table>

\(^a\)Animals were placed on separate E. coli OP50-seeded NGM agar plates, and based on the developmental stage, newly laid eggs were divided into three categories. Numbers indicate the percentage of laid eggs (N).

\(^b\)Note that mosaically rescued gsa-1(pk75) animals were selected for an egg-laying defective phenotype.

\(^c\)Both egl-1 and egl-1; Ga,KS strains were in a rol-6(su1006) transgenic background.
may act through an ion channel distinct from the channels tested in this study or, alternatively, through a combination of ion channels.

Another approach to identify downstream components acting in Go signaling is to isolate and clone extragenic mutations that suppress the GoQL phenotype. Using ethylmethane sulfonate (EMS) mutagenesis, we obtained several mutations that suppress the GoQL-induced neural degeneration and identify putative downstream genes functioning in Go-coupled signal transduction. These mutations were isolated at a frequency of ~1 in 5000 mutagenized genomes, were found to be recessive, and fell into one complementation group, sgs-1 (suppressor of activated Go). In addition, one other suppressor mutant was isolated that proved to be a complex locus, sgs-1 suppressed completely the GoQL-induced neural degeneration (expressed ubiquitously from a heat-shock promoter or specifically from the gsa-1 promoter), but did not suppress the activated Go-induced body-wall muscle hypercontraction (Table 2) or the hyperactive egg-laying induced by wild-type Go overexpression (data not shown). In the absence of GoQL expression, sgs-1 did not show an obvious developmental or behavioral phenotype. sgs-1 was mapped to chromosome III, in an interval between unc-32 and vab-7, ~1 map unit to the right of unc-32 [see Materials and Methods].

Discussion

The strong evolutionary conservation of key components in signal transduction has allowed detailed genetic analysis of signal transduction pathways in model organisms like Drosophila and C. elegans (Sternberg 1993; Wassarman et al. 1995). The powerful genetic techniques available for these organisms have led to important insights in signal transduction networks and have in many

Table 2. Genetic interactions between activated Go, and loss-of-function mutations in cell death and ion channel genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Neural degeneration*</th>
<th>Body-wall muscle hypercontraction*</th>
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<td>Cell death genes</td>
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</tr>
<tr>
<td>ced-3</td>
<td>+</td>
<td>N.D.</td>
<td>Yuan et al. (1993)</td>
</tr>
<tr>
<td>ced-4</td>
<td>+</td>
<td>N.D.</td>
<td>Yuan and Horvitz (1992)</td>
</tr>
<tr>
<td>Ion channel genes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mec-6</td>
<td>+</td>
<td>+</td>
<td>Chalfie and Wolinsky (1990)</td>
</tr>
<tr>
<td>gqf-15</td>
<td>+</td>
<td>+</td>
<td>L. Lobel [pers. comm.]</td>
</tr>
<tr>
<td>unc-36</td>
<td>+</td>
<td>+</td>
<td>L. Lobel [pers. comm.]</td>
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<tr>
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<td></td>
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<tr>
<td>sgs-1</td>
<td>+</td>
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<td>this paper</td>
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Combinations of gsa-1(pkls296) with ced-3(n717), ced-4(ea162), mec-6(e1342), unc-2(e55), egl-19(n582), unc-36(e251), sgs-1(pk301), or sgs-2(pk325) were assayed for degeneration of neurons and body-wall muscle hypercontraction 12 hr after induction of activated Go.

*1 pkls296-induced neural degeneration and muscle hypercontraction, |1 incomplete suppression of pkls296 phenotype, (-) suppression of pkls296 phenotype.
cases complemented biochemical studies in mammals. We describe a genetic analysis of Ga\(_\alpha\) function in *C. elegans*. We show that Ga\(_\alpha\) is expressed ubiquitously in the nervous system and in muscle cells, that Ga\(_\alpha\) is essential for viability, and that Ga\(_\alpha\) modulates behaviors such as locomotion and egg-laying. Moreover, we show that an activating mutation in Ga\(_\alpha\) results in the degeneration of neurons.

Inactivation of gsa-1 results in larval lethality. The predicted protein generated by gsa-1(pk75) is truncated at position arginine 246 in the amino-acid sequence (Park et al. 1997) and lacks a third of the Ga\(_\alpha\) carboxy-terminal sequence. The deleted region includes conserved sequences involved in interactions with adenyl cyclase and receptors. Deletion of the carboxy-terminal part of Ga\(_\alpha\) probably affects other functions of the protein as well and it is likely that pk75 is a null allele. Animals heterozygous for pk75 are wild type. This suggests that there are no dominant neuromorphic effects associated with expression of a truncated Ga\(_\alpha\) protein. Animals homozygous for pk75 hatch normally, but arrest during the first stage of larval development. The internal tissues of mutant animals become progressively shrunken and they acquire a starved appearance. This phenotype is similar to the zygotic null phenotype of animals mutant for the G\(B\) subunit gene gpb-1 (Zwaal et al. 1996) and suggests that the larval lethal phenotype of gsa-1 and gpb-1 mutant animals results from the absence of an essential function of G\(i\)-coupled signal transduction during larval development. The larval lethal phenotype of gsa-1 is also similar to the rod-like larval lethal phenotype of clr-1 and mutations in components of the Ras pathway. This lethal phenotype is associated with defects in the CANs, which have a role in osmoregulation (G. Garriga, pers. comm.). Cell-specific ablation of the CANs results in a rod-like larval lethal phenotype [J. Sulston, pers. comm.]. Furthermore, it was shown for the epidermal growth factor (EGF) receptor homolog let-23 that lethality in mosaic animals correlated with absence of functional let-23 in the excretory system (Koga and Ohshima 1995). Consequently, because gsa-1 is expressed in the CANs, Ga\(_\alpha\) may perform an essential function in these specialized cells as well.

Overexpression and mosaic rescue experiments revealed functions of Ga\(_\alpha\) later in development. Transgenic animals that overexpress Ga\(_\alpha\) showed an increase in locomotory activity and egg-laying, whereas animals that mosaically express Ga\(_\alpha\) showed a reduction in these behaviors. These phenotypes correlate with the expression pattern of Ga\(_\alpha\), gsa-1:GFP fusions are expressed in the ventral nerve cord motorneurons and body-wall muscle cells that control locomotion, and the HSNs and vulva and uterine muscle cells that control egg-laying. The locomotory and egg-laying phenotypes are associated with enhanced or reduced activities of the muscle cells that are required for these behaviors. In the egg-laying system, overexpression of Ga\(_\alpha\) stimulated egg-laying in the absence of the HSNs. This suggests that Ga\(_\alpha\) acts directly in the vulva muscles. This conclusion is supported by the observation that the egg-laying defect of gsa-1(pk75) mosaic animals is not rescued by adding exogenous neurotactin, the neurotransmitter that is released by the HSNs.

Two other strongly conserved *C. elegans* Ga proteins also modulate locomotion and egg-laying—the Ga\(_\alpha\) homolog EGL-30 (Brundage et al. 1996) and Ga\(_\alpha\), Mendel et al. 1995; Ségalat et al. 1995). Mutations in Ga\(_\alpha\) result in similar behavioral phenotypes as described for Ga\(_\alpha\), but mutations in Ga\(_\alpha\), a member of the inhibitory class of Ga subunits, result in opposite phenotypes. Loss of Ga\(_\alpha\) results in hyperactivity in locomotion and egg-laying, whereas expression of a constitutively active Ga\(_\alpha\) allele induces paralysis and egg-laying defects. The significance of this convergence on a limited set of behavioral phenotypes of the three different Ga subunits is not known. It is not clear whether the three Ga protein pathways modulate these behaviors specifically through the nervous system, through the muscle cells that control locomotion and egg-laying, or through both. Furthermore, mutations in the three different Ga subunits could also modulate locomotion and egg-laying by changing the levels of Ga\(_\alpha\) subunits. Overexpression of Ga results in phenotypes that are similar to Ga\(_\alpha\), but opposite to Ga\(_\alpha\) and Ga\(_\alpha\) (Zwaal et al. 1996).

Mutations that disrupt the GTP\(\alpha\)ase activity of Ga\(_\alpha\), and lock the protein in the active GTP-bound conformation are found in human malignancies and endocrine disorders (Landis et al. 1989; Lyons et al. 1990; Shenker et al. 1993; Iiri et al. 1994). We constructed a similar mutation in *C. elegans* Ga\(_\alpha\) and found that expression of this activated form of the protein results in hypercontraction of body-wall muscle cells and the degeneration of a specific subset of neurons. Epistatic analysis showed that this degenerating capacity of Ga\(_\alpha\)QL is not dependent on an intact apoptotic pathway. A similar neurodegenerative phenotype as induced by Ga\(_\alpha\)QL has, however, been described for degenerin ion channel genes. Dominant mutations in deg-1, mec-4, and mec-10 (Chalfie and Wolin 1990; Driscoll and Chalfie 1991; Huang and Chalfie 1994), which are homologs of mammalian epithelial Na\(^+\) channels, result in the degeneration of a specific subset of touch receptor neurons, whereas mutations of unc-8 result in degeneration of ventral nerve cord motoneurons (Shreffler et al. 1995). Also, rare dominant mutations in deg-3, a nicotinic acetylcholine receptor subunit gene, induce neural degeneration (Treinin and Chalfie 1995). These mutations presumably disturb the regulation of these channels, resulting in an ionic imbalance, vacuolization and degenerative cell death (Hong and Driscoll 1994). Dominant mutation of degenerin channels can also induce muscle hypercontraction; constitutive activation of the degenerin channel unc-105 results in body-wall muscle hypercontraction (Liu et al. 1996). Given the similarity in phenotype, it is likely that Ga\(_\alpha\)QL acts by deregulating ion channels as well. This can be a direct activation of ion channels by Ga\(_\alpha\), or can be indirect by deregulation of CAMP-gated ion channels or by deregulation of ion channels through CAMP-dependent protein kinase A (Walsh and Van Patten 1994; Wickman and Clapham 1995). Epistatic analysis with *C. elegans* ion-
channel genes demonstrated that GαQL does not act specifically through degenerin channels or Ca²⁺ channels like UNC-2, UNC-36, or EGL-19. It is, however, not excluded that activated Gα acts through a combination of these channels. Screens for revertants of the activated Gα phenotype resulted in a locus, sgs-1, that suppresses the neural degeneration phenotype of GαQL. Mutation of sgs-1 does not, however, suppress the body-wall muscle hypercontraction induced by activated Gα. In addition, sgs-1 does not suppress the hyperactive egg-laying in animals overexpressing GαQL, which is mediated through the vulva muscles. Therefore, it is likely that Gα acts in at least two roles, in neurons and in muscle cells, and that the action of sgs-1 is restricted to the neuronal pathway.

Materials and Methods

Nematode strains and culturing

General methods for culture, manipulation, and genetics of C. elegans were as described (Lewis and Fleming 1995). Unless indicated, strains were cultured at 20°C. Strains used in this study were Bristol N2 and LGIB. CB1472 [unc-6(e342)], DR102 [dpy-5(e1) unc-2(e193)], KR236 [dpy-5(e1) unc-15(e51)] sDpy[2(II)], KR1778 [unc-3(e151)], MT3126 [mmt-2(e459)], NTL1 [gsa-1(pk27.7c1) unc-29(e2123)], NTL15 [gsa-1(pk75) sDpy[2(II)], LGII: CB3241 [dpy-16(e1745)], LGII: GE24 [pho-10(e2123)], GE1825 [dpy-12(e516)], DD11 [unc-32(e189), dpy-18(e499)], TT2551 [ced-4(n1162) dpy-17(e164)], MT4306 [lin-39(n180 unc-36(e251))], LGVB: CB3162 [dpy-20(e1362)], MT1212 [egi-19(n582)], MT2405 [ced-3(n717) unc-36(e351)], MT1082 [egl-1(n487)], LGX: CB55 [unc-2(e55)].

GFP reporter constructs

A 3.8-kb Fsp1 fragment of gsa-1 was introduced into the GFP modular vector pPD95.77. A. Fire, pers. comm.) resulting in pRP1511. A translational fusion with GFP [pRP1518] was constructed by inserting a 2.0-kb Sail fragment of pPD95.77 containing the GFP-coding sequence and the 3' UTR downstream of a Sail DNA fragment of gsa-1 located within exon 5 of gsa-1 in construct pRP1505 (see below). Each fusion DNA was introduced into pha-1(+) containing plasmid pC1 (Mello et al. 1991; Granato et al. 1994, Mello and Fire 1995) or in dpy-20(e1362) together with dpy-20(+) containing plasmid pPM86 (Han and Sternberg 1991). Each construct was injected at 100 ng/μl. Transformed animals were identified by survival at 25°C or a Dpy(+) phenotype. Multiple transgenic lines were obtained that showed identical expression patterns. Cells were identified in reference to Sulston and Horvitz (1977) and White (White et al. 1986).

Generation of a gsa-1 loss of function mutation

A deletion mutation of gsa-1 was isolated as described (Zwaal et al. 1993). A Tcl insertion mutant gsa-1(Tcl[pk27.7c1]mmt-2(e459)] was isolated using a library of Tcl insertion mutants. This insertion, located at a TA dinucleotide in the seventh intron of gsa-1, did not produce a mutant phenotype. The primes 3831 (5'-GCCATTCGAGATGATGCGC-3') and 1917 (5'-ACCCTTGGATATCAGATGCGC-3') located in the third and eighth exons of gsa-1, respectively, and spanning a genomic region of 3.9 kb, were used in a screen for deletion derivatives of pk27.7c1. A 1.8-kb deletion allele, gsa-1(pk75), was isolated (fig. 2A). The PCR product detecting the deletion was directly sequenced using linear amplification sequencing ([Crayton 1993]. The pk75 allele was backcrossed four times to an N2 background, including a double crossover using a dpy-5(e1) unc-29(e193)-marked chromosome I to four new animals (Fig. 1B). The pk75 locus. The pk75 allele was balanced with the free duplication sDpy[2(II)] (Howell et al. 1987) and the deletion was confirmed by Southern analysis (data not shown). The larval lethality of pk75 homozygotes was confirmed by single worm PCR using primers spanning the deletion to detect pk75 and primers inside the deleted region to detect the wild-type allele as described (Zwaal et al. 1993). Arrested larvae showed only the pk75 deletion allele, viable progeny of pk75 heterozygotes were either heterozygous for pk75 or homozygous for the gsa-1 wild-type allele (data not shown). The larval-lethal phenotype of pk75 was complemented with a wild-type gsa-1 genomic construct, pRP1505, which contained a 13 kb HindIII fragment including the gsa-1 coding sequence (Park et al. 1997) and the 3' UTR and polyadenylation signal of unc-32. The rescue construct was injected at a concentration of 5 ng/μl together with 150 ng/μl of the marker plasmid pRF4 (Kramer et al. 1990). Rescue was scored as the generation of obligatory Rol lineages that segregated viable Rol animals and arrested larvae, resulting in strain NLS42 [gsa-1(pk75)pKE270.gsa-1(+); rol-6(su1006)]. Homozygosity for the pk75 allele was confirmed by single worm PCR.

Behavioral analysis of mosaic and Gα overexpression phenotypes

Adult animals of the rescued gsa-1(pk75) transgenic line NLS42 showing defects in movement or egg-laying (<1% of the population) were selected for locomotion and egg-laying assays as described (Zwaal et al. 1996). Locomotion was assayed by placing animals on Escherichia coli OP50-seeded NGM agar plates 30 min before measuring locomotion by counting body bends in 3-min intervals. A body bend was defined as movement of one quarter of a body length in a forward or backward direction (Brandau et al. 1996, Koelle and Horvitz 1996), a definition that could also be applied in a Rol background. Egg-laying was assayed by placing adult animals on OP50-seeded NGM agar plates and counting eggs laid in a 2.5-hr interval. The number of eggs present in the uterus and the stage of newly laid eggs were analyzed by counting eggs or cells using a high power dissection microscope (Wild M3C). Data were analyzed for statistical significance using an unpaired t-test (SPSS v. 7.0, SPSS, Inc., Chicago, IL) and were stated as mean ± s.e.m. Assays for egg laying in sevonin 5 mg/ml were as described (Trent et al. 1983). NLS66 [dpy-20(e1362) gsa-1(+) dpy-40(+) gsa-1(+)] and the transgenic line overexpressing Gα, was used in behavioral assays as described above. NLS56 was generated by injecting a mixture of pRP1505, dpy-20(+) containing plasmid pPM86 (Han and Sternberg 1991) and pGEM5 carrier DNA [all at a concentration of 50 ng/μl in dpy-20(e1362)] animals (Mello et al. 1991; Mello and Fire 1995). NLS92 [egl-1(n487) gsa-1(+) rol-6(su1006)], a transgenic line overexpressing Gα, in an egl-1 mutant background, was made by injecting pRP1505 at 50 ng/μl and pRF4 ([Kramer et al. 1990] at 100 ng/μl in eggs of animals. About half of the NLS92 animals showed reversion of the egl-1 egg-laying defect and were used in egg-laying assays as described above. NLS94 [egl-1(n487) gsa-1(+) rol-6(su1006)] served as a control.

Construction of a constitutively active Gα mutant

A substitution of glutamine (Q) 208 to a leucine (L) that results
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Genetic analysis of C. elegans Ga

Double mutant strains of pkls296 with cad-3 or cad-4 were made using cad-3(e717)unc-26(e205) and cad-4(nl162)dpv-1(e164)-marked strains, respectively. A double mutant strain of mec-6(e1342) and pkls296 was constructed using unc-13(e51) and dpy-5(e1362)unc-29(e193) as markers in trans. Double mutant strains of pkls296 with unc-2(e855), unc-36(e251), and egl-19(n852) were made using the visible phenotype of these mutations in crosses. Homozygosity for the phenotype was determined by heat shock or single-spore PCR using primers 994 (5'-ATGTTTGACGTCGGAGGTCTACGTGAC'') containing the 3' UTR and polyadenylation signal.

References


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