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Mutations in a C. elegans $G_q\alpha$ Gene Disrupt Movement, Egg Laying, and Viability

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Summary

We find that C. elegans *egl-30* encodes a heterotrimeric G protein α subunit more than 80% identical to mammalian $G_q\alpha$ family proteins, and which can function as a $G_q\alpha$ subunit in COS-7 cells. We have identified new *egl-30* alleles in a selection for genes involved in the C. elegans acetylcholine response. Two *egl-30* alleles specify premature termination of $G_q\alpha$ and are essentially lethal in homozygotes. Animals homozygous for six other *egl-30* alleles are viable and fertile, but exhibit delayed egg laying and leave flattened tracks. Overexpression of the wild-type *egl-30* gene produces the opposite behavior. Analysis of these mutants suggest that their phenotypes reflect defects in the muscle or neuromuscular junction.

Introduction

Heterotrimeric G proteins receive signals from a diverse set of extracellular receptors and convert them into a limited set of intracellular responses. G proteins are composed of three subunits: the α subunit, which activates effectors directly when liganded with GTP, and β and γ subunits, which are released as heterodimers when G proteins are activated; βγ dimers also have signaling capabilities (Katz et al., 1992; Camps et al., 1992). In mammals, 17 distinct genes encoding α subunit proteins have been discovered (Simon et al., 1991), and serpentine receptors and effectors capable of interacting with most of them are now known. Gα subunits can be grouped into four families, based on their similarities in sequence and function: $G_s\alpha$, $G_i/G_o\alpha$, $G_{12}\alpha$, and $G_q\alpha$ (Simon et al., 1991). Despite the wealth of molecular information about individual G proteins, the way in which G protein pathways converge to integrate and interpret information, and ultimately regulate behavior, is not understood.

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This larger picture may be delineated by studying individual G proteins in a simple organism such as the nematode C. elegans. This organism expresses a number of G protein subunits that are extremely homologous to their mammalian counterparts. Five G protein α subunits have previously been identified in C. elegans: $G_o\alpha$, $G_s\alpha$, GPA-1, GPA-2, and GPA-3 (Fino Silva and Plasterk, 1990; Lochrie et al., 1991; J. Park and Y. Ohshima, personal communication). C. elegans $G_o\alpha$ and $G_s\alpha$, respectively, are 82% and 66% identical to their mouse and rat counterparts. C. elegans GPA-1, GPA-2, and GPA-3 are worm specific Gα's that do not correspond to any particular mammalian α subunit (Lochrie et al., 1991). In addition, one Gβ subunit has been identified in C. elegans (van der Voorn et al., 1990).

We have examined the function of $G_q\alpha$ in C. elegans. The mammalian $G_q\alpha$ family is comprised of four members, $G_q\alpha$, $G_{11}\alpha$, $G_{14}\alpha$, and $G_{15}\alpha$, which together account for the major component of G protein-mediated signaling through phosphoinositides (Lee et al., 1992). In mammals, $G_q\alpha$ and $G_{11}\alpha$ are expressed in almost all tissues (Strathmann and Simon, 1990; Wilkie et al., 1991), consistent with the diverse processes in which they have been implicated, including synaptic transmission, contraction of smooth muscle, recruitment of immune cells, growth control, and cell migration during development (Baynash et al., 1994; Eglen et al., 1994; Wu et al., 1993). $G_{14}\alpha$ and $G_{15}\alpha$ show much more restricted expression. At least 30 different receptors can activate G_q and G_{11} including receptors for the neurotransmitters acetylcholine, serotonin and glutamate (Watson and Arkinstall, 1994), all of which are present in C. elegans. Mutations in several $G_{q/11}$ specific receptors have been identified in human disease (Baldwin, 1994); however, genomic mutations that produce visible effects have not been identified in the $G_q\alpha$ family itself. Thus, the full spectrum of G_q function, especially in development, has not been directly explored.

In this study, we have identified the gene encoding a C. elegans homolog of the $G_q\alpha$ family and determined that *egl-30* strains contain mutations in this gene. We have isolated five new *egl-30* mutations as suppressors of the lethal effects of an acetylcholine agonist. Analysis of these *egl-30* alleles, plus three that were previously identified in behavioral screens (Trent et al., 1983; Park and Horvitz, 1986), as well as strains that overexpress the wild-type *egl-30* gene product, has demonstrated that $G_q\alpha$ is involved in egg laying, coordinated movement, and one or more processes required for viability.

Results

C. elegans $G_q\alpha$ Is Similar to Mammalian $G_q\alpha$ and $G_{11}\alpha$

To determine the function of G_q family members in C. elegans, we identified a gene that encodes an α subunit of the G_q family. A fragment of this gene was amplified by PCR from C. elegans genomic DNA, using as primers oligonucleotides corresponding to the amino acid sequences QECYD(R/S), and TYPWFQN, which are conserved among $G_q\alpha$ subunits. The PCR-amplified fragment was then used for hybridization to identify a full length cDNA encoding the $G_q\alpha$ gene from a C. elegans cDNA library (Barstead and Waterston, 1989).

The predicted amino acid sequence of this cDNA is more than 80% identical to both $G_q\alpha$ and $G_{11}\alpha$ from mouse (Figure 1A), as well as from several other sources. Like other

described $G_q\alpha$ family members, C. elegans $G_q\alpha$ has two adjacent amino-terminal cysteines in the proper context to be palmitoylated (Edgerton et al., 1994; Mumby et al., 1994), but does not have the carboxyterminal CAAX sequence, which is a substrate for Pertussis toxin. The amino-terminus of C. elegans $G_q\alpha$ is missing six amino acids present in mouse $G_q\alpha$ and $G_{11}\alpha$ (Figure 1A).

To identify the genomic fragment of DNA that encodes C. elegans $G_q\alpha$, the cDNA was used to screen a genomic C. elegans lambda library by hybridization. One phage clone contained the entire 5 kb coding region, plus more than 2 kb of presumptive control sequence upstream of the initiating methionine. The positions of introns in the $G_q\alpha$ gene (Figure 1B) were determined by partially sequencing a subclone of this phage, and comparing the resulting sequence with the sequence of the $G_q\alpha$ cDNA. Quite remarkably, all of the introns except 2 and 7 are found in the same positions as introns in the $G_q\alpha$ gene from Drosophila, and in the $G_{11}\alpha$ and $G_{15}\alpha$ genes from mouse (Lee et al., 1990; Davignon et al., 1996). The genomic sequence provides no evidence of alternate splicing. The map position of the $G_q\alpha$ gene [initially named *gqa-1*] was assigned by fingerprinting to the left arm of Chromosome I (R. Shownkeen, unpublished data).

C. elegans $G_q\alpha$ Can Activate Phospholipase C

The $G_q\alpha$ family in mammals has been shown to activate all of the isoforms of phospholipase Cβ (PLCβ). To determine if C. elegans $G_q\alpha$ could be involved in a similar pathway, we transfected C. elegans $G_q\alpha$ cDNA under control of the CMV promoter into COS-7 cells, and measured stimulation of endogenous phospholipase activity (Lee et al., 1992; Figure 2). In the presence of aluminum fluoride, which activates GDP bound G proteins, C. elegans $G_q\alpha$ was able to effectively stimulate phosphoinositide hydrolysis, although somewhat less strongly than one of its mouse counterparts, $G_{11}\alpha$ (Figure 2A). G protein α subunits of other families and the vector alone had no activity in this assay (Figure 2A). To test whether the receptor specificity of C. elegans $G_q\alpha$ and mammalian $G_q\alpha$'s might be similar, we cotransfected COS-7 cells with $G_q\alpha$ cDNAs, together with the cDNA for the α1-C adrenergic receptor, chosen because it acts exclusively through the $G_q\alpha$ family in mammals (Wu et al., 1992). When the ligand norepinephrine was added, the α1-C receptor was able to potentiate phospholipase C activity through C. elegans $G_q\alpha$ as strongly as through mouse $G_{11}\alpha$ in this assay (Figure 2B). α1-C receptor alone and the vector control exhibited substantially reduced activity. These transfection results suggest that the molecular interactions of the C. elegans $G_q\alpha$ pathway are likely to be similar to the $G_q\alpha$ family in mammals, and are therefore likely to be a good model for understanding G_q signal transduction in other organisms.

egl-30 Mutations Suppress Hypersensitivity to an Acetylcholine Agonist via Changes in $G_q\alpha$

Genetic analysis of $G_q\alpha$ in C. elegans was facilitated by studies of acetylcholine pharmacology. *eat-11* mutant worms fail to grow in the presence of 5 mM arecoline, a cholinergic agonist (Avery, 1993a). In contrast, wild-type worms grow almost normally at this concentration. *eat-11* mutants are not hypersensitive to nicotine (data not shown), suggesting that the lethal effect of arecoline is mediated at least in part by the muscarinic

acetylcholine pathway. To identify genes that might be involved in the muscarinic response, we selected new mutations that would allow *eat-11* worms to grow in the presence of arecoline. The seven strongest suppressors were identified as new semidominant mutations in the gene *egl-30.* Two previously isolated mutations at this locus, *n686* (Trent et al., 1983) and *n715* (Park and Horvitz, 1986), were tested and also proved to be semidominant suppressors of *eat-11*.

Genetic mapping placed *egl-30* on the left arm of LG I (Trent et al., 1983; Park and Horvitz, 1986), in the same region of the genome as the $G_q\alpha$ gene. To test if *egl-30* is the gene encoding $G_q\alpha$, we sequenced the exons and intron-exon junctions of $G_q\alpha$ coding region from strains carrying each of the new *egl-30* alleles, as well as three previously identified *egl-30* alleles (Trent et al., 1983; Park and Horvitz, 1986). We identified eight distinct mutations that could cause defects in $G_q\alpha$ synthesis or function. For comparison, we generated strains overexpressing *egl-30* from an extrachromosomal array. The *egl-30* transgenic array suppresses the movement and egg-laying phenotypes of *egl-30(ad809)* (Figure 3C; data not shown), consistent with our identification of the $G_q\alpha$ gene as *egl-30*. The nature of the changes in *egl-30* in these strains are summarized in Table 1.

egl-30(ad810) and *egl-30(ad813)* mutations are expected to cause premature termination of $G_q\alpha$ at amino acids 211 and 258, respectively. Animals homozygous for these mutations were subviable and arrested at various developmental stages. *ad803* and *ad806* each contain an independently arising point mutation converting serine (6) to phenylalanine. The phenotypes of these two strains were essentially identical; adult hermaphrodites were moderately bloated with eggs, and moved more slowly and in sinusoidal waves that had a smaller and more variable amplitude than wild-type animals (Figures 3C and 4D; data not shown). The serine residue that is mutated in this allele is highly conserved in both the $G_q\alpha$ and $G_i/G_o\alpha$ families. Mutations at this site are expected to disrupt the recognition sequence for palmitoylation of cysteines 3 and 4 (Mumby et al., 1994), and in addition, may interfere with the association of $G_q\alpha$ with $G\beta\gamma$ subunits (Lambright et al., 1996).

The remaining mutations in *egl-30* disrupt sequences at the intron/exon junctions that specify the proper sites for mRNA splicing. *n686, ad809, ad805,* and *n715* contain mutations that change invariant sequences at the splice donor or acceptor sites of three different introns (Table 1). The intragenic revertant allele *n715n1190*(Park and Horvitz, 1986) contains the original *n715* mutation plus an additional mutation in the preceding intron, at base pair– 15 relative to the original splice site (Table 1). Although mutations that disrupt splice donor or acceptor sites are rare in mammals, they are relatively common in C. elegans. Unlike strains with the *egl-30* amber mutations, strains homozygous for all these alleles were viable, but exhibited phenotypes with a considerable range of debility, and that were easily distinguished under a dissecting microscope.

egl-30 Mutations Substantially Reduce Viability, Pharyngeal Pumping, Egg Laying, and Movement

egl-30(ad810) and *egl-30(ad813)* mutations are essentially lethal. Homozygous progeny derived from heterozygous mothers hatched as larvae with extremely feeble muscle contraction. At hatching, they were virtually paralyzed, and their pharynxes pumped weakly

and only sporadically, leading to a starved appearance, extremely slow growth, and arrest throughout larval development. A small number of hermaphrodites of both genotypes eventually developed into semi-fertile adults. For details, see Experimental Procedures.

Muscle contraction is necessary for normal elongation of the worm during embryogenesis (Williams and Waterston, 1994). Since *ad810* and *ad813* larvae are normally elongated, their muscles must contract during embryonic development. Indeed, the rolling movement that takes place during elongation of the embryo was indistinguishable between *ad810* homozygotes and siblings carrying a wild-type *egl-30* gene. Unlike wild-type animals, which move continuously from elongation onward, *ad810* homozygotes gradually decreased muscle contraction after elongation. The contractile apparatus of *ad810* larvae still is functional, however, as pharyngeal muscles can be made to contract by laser stimulation of the plasma membrane (J. A. Dent, personal communication).

Hermaphrodites homozygous for other *egl-30* alleles examined failed to lay their eggs as rapidly as they produced them, and quickly became bloated with eggs. Wild-type hermaphrodites periodically contract their vulval and uterine muscles to release developing eggs at gastrulation, about 2–3 hr after fertilization. The uterus of a well-fed wild-type hermaphrodite (Figure 3A) contains an average of 13 fertilized eggs in early stages of development. In contrast, hermaphrodites of viable *egl-30* genotypes (such as *ad805*, Figure 3B) were visibly bloated with up to three times as many eggs, and up to 35% of these eggs had already developed to or past the 1 1/2 fold stage, about 7 hr post fertilization (Figure 3C). No eggs of these stages were found in any of the wild-type hermaphrodites examined. All of the *egl-30* alleles produced detectable bloating in heterozygotes (Figure 3D); however, the degree of bloating of the heterozygotes was substantially less severe in each case than of the corresponding homozygotes, and none of the eggs retained by the heterozygotes were late stage.

In contrast, hermaphrodites that overexpress the *egl-30* gene laid eggs earlier than wild type; 81% of eggs from an overexpressing strain had four or fewer cells when laid (n = 92), while 93% of eggs from wild-type animals had greater than 12 cells (n = 42). This contributed to a reduction in the number of eggs in the uterus compared with wild type, shown in Figure 3C. *egl-30* overexpression converted *egl-30(ad809)* to the empty uterus and early egg-laying phenotype (Figure 3C; data not shown).

egl-30 mutants were also defective in body movement. Wild-type C. elegans move almost continuously in well-coordinated sinusoidal undulations (Figure 4A). All *egl-30* mutants in this study spent more time at rest than wild type, and when they did move it was more slowly and for a shorter distance than wild-type animals. *ad805* and *n715* animals were almost paralyzed when undisturbed, while the other *egl-30* strains were sluggish or very sluggish (Table 1; selected alleles shown in Figure 4D). Heterozygotes of all alleles (except *n715*, which was not assayed quantitatively), were nearly wild-type for movement (Figure 4D). In addition, the quality of the movement of *egl-30* animals was abnormal. As individual animals move across a lawn of *E. coli*, they leave tracks showing the form of their movement. Wild-type hermaphrodites left sinusoidal tracks with a very even amplitude (Figure 4A). *egl-30* animals produced tracks that were very shallow *(ad805*, Figure 4B) or

had a smaller and more variable amplitude than wild type (Table 1). Animals overexpressing the wild-type *egl-30* gene exhibited the opposite phenotype, producing tracks with bends on average 60% deeper than normal (data not shown). Some animals left tracks with pronounced loops (Figure 4C). The movement of *egl-30(ad809)* animals overexpressing the *egl-30* gene was similar to *egl-30(+)* animals bearing the same array (data not shown).

egl-30 Mutations Affect Muscle Function

The *egl-30* defects in feeding, egg laying, and movement arise because ultimately, *egl-30* animals do not properly contract the muscle cells required for each of these behaviors, i.e., pharyngeal muscles, vulva and uterine muscles, and body wall muscles. A priori, a defect in muscle contraction could be caused by a change in the muscle cells themselves, or in the loss of innervation of the muscles cells via a defect in the nervous system. Three different kinds of experimental analysis suggest that *egl-30* defects underlying these behavioral phenotypes are most consistent with changes in the muscle or at the neuromuscular junction.

egl-30 mutations were selected as suppressors of the lethal effects of arecoline on *eat-11* worms. The arrest of *eat-11* mutants by arecoline is presumed to be due to the effects of the drug on feeding, based on three observations (data not shown). First, at concentrations that are lethal to *eat-11* worms but not to wild-type, arecoline causes pharyngeal muscle contraction and severely abnormal pharyngeal pumping in *eat-11* mutants, but has little long-term effect on wild-type pumping. Second, the arrest phenotype of *eat-11* worms on arecoline is similar to that of wild-type worms deprived of food, and *eat-11* worms treated with sublethal arecoline concentrations resemble partly starved wild-type worms. Third, the arecoline arrest can be partly alleviated by changing the food. For instance, *eat-11* worms will grow in the presence of 2 mM arecoline if fed E. coli HB101, which is easy to eat, but not if given the less edible E. coli strain DA837.

Because *egl-30* mutations allow *eat-11* worms to survive in the presence of arecoline, they must reduce the effect of arecoline on pharyngeal muscle. To test if the pharyngeal nervous system is necessary for *egl-30* suppression of arecoline lethality, all but one of the pharyngeal neurons were killed by laser ablation, and worms were then tested for growth in the presence and absence of arecoline (Table 2). It was necessary to spare the essential neuron M4 (Avery and Horvitz, 1987), since otherwise, the worms would arrest as larvae for reasons unrelated to the arecoline. M4 has no direct effect on pharyngeal pumping (Avery and Horvitz, 1987; Raizen et al., 1995). Even in the absence of most of the pharyngeal nervous system, an *egl-30* mutation was able to suppress the arecoline hypersensitivity of *eat-11*. Indeed, killing the pharyngeal nervous system caused arecoline hypersensitivity even in *eat-11(+)* worms, and *egl-30 eat-11* worms were less sensitive than wild-type. This experiment argues strongly for a site of action of the *egl-30* gene product $G_q\alpha$ outside the pharyngeal nervous system, and is consistent with a site of $G_q\alpha$ action in pharyngeal muscle.

Egg laying is mediated in part by serotonin released from the HSN neurons, which signals the vulva and uterine muscles to contract. Egg-laying deficient mutants have been categorized as defective in generating the serotonergic signal (i.e., defective in the HSN), or defective in responding to the signal (defective in vulva or uterine muscles) based on their ability to lay eggs when bathed in serotonin (Trent et al., 1983). All of the *egl-30* mutants

(except *n715n1190)* showed a reduced egg-laying response to exogenous serotonin (Figure 5A; Trent et al., 1983); the responses of the mutants were inversely proportional to the severity of their egg-laying defects undergrowth conditions (Figure 3C). The severe *egl-30* mutant *ad805* laid fewer than 5% of the wild-type number of eggs in serotonin, even though animals missing HSNs lay a similar number of eggs to wild type in this assay (Trent et al., 1983). An interesting exception is the intragenic revertant strain *n715n1190,* which laid a larger number of eggs when bathed in serotonin (Figure 5A), and began laying them much more rapidly than wild type (data not shown).

To test whether *egl-30* mutants are defective in secretion of serotonin, as well as defective in responding to serotonin, animals bearing each of the alleles were assayed in imipramine, a reuptake inhibitor of several neurotransmitters including serotonin. Each of these mutants (except *n715)* responded to imipramine to the same extent as it responded to serotonin (Figure 5B; Trent et al., 1983). Since imipramine acts in egg laying primarily by potentiating the effect of endogenous serotonin (but has no direct egg-laying activity of its own), the HSN was functioning in all *egl-30* mutants tested in this assay. Thus, mutations in *egl-30* are presumed to disrupt egg laying via changes in the vulva or uterine muscles or both.

Flaccid paralysis like that of *egl-30* mutants commonly results from loss of body muscle function (Waterston et al., 1980; Reiner et al., 1995). In contrast, loss of nervous system function is associated with a slow uncoordinated movement without flaccidity. For instance, mutations in *cha-1*, necessary for the synthesis of the excitatory neurotransmitter acetylcholine (Alfonso et al., 1994), or *unc-104*, which encodes a microtubule motor necessary for synapse formation (Otsuka et al., 1991), result in a coiled posture with good muscle tone (Hall and Hedgecock, 1991; Alfonso et al., 1994; L.A., unpublished data), distinct from the *egl-30* phenotype. It therefore seemed unlikely that the *egl-30* phenotype was caused by a loss of excitatory nervous system function. However, the flaccid phenotype might be explained by an increase in inhibitory nervous system function. For instance, the GABA agonist muscimol produces flaccid paralysis (McIntire et al., 1993). To test whether the *egl-30* phenotype is caused by excessive release of GABA by the nervous system, we constructed *egl-30; unc-25* double mutants. *unc-25* encodes glutamic acid decarboxylase, the enzyme that synthesizes GABA, and *unc-25* mutants lack GABA (McIntire et al., 1993; Y. Jin and H. R. Horvitz, personal communication). *egl-30(ad810); unc-25* double mutants, like *egl-30(ad810)* and unlike *unc-25* single mutants, arrested as flaccid paralyzed larvae. Even with the weaker allele *egl-30(ad805)* we were unable to detect any alleviation of the *egl-30* phenotype by elimination of GABA. Thus, the flaccid paralysis of *egl-30* mutants is not caused by GABA release from the nervous system.

This experiment left open the possibility that the *egl-30* mutant nervous system inhibits muscle contraction by a GABA-independent mechanism. If muscle dysfunction in *egl-30* mutants is entirely caused by nervous system dysfunction, then reduction of nervous system function in wild-type and *egl-30* mutant worms should make their phenotypes more similar to each other. To test this possibility, we constructed *egl-30* double mutants with *unc-104(rh43),* a possible null allele. *egl-30(ad810); unc-104* and *egl-30(ad805); unc-104* double mutants both showed flaccid paralysis like the *egl-30* single mutants, and unlike

unc-104 single mutants. This result suggests that *egl-30* has nervous system-independent effects on body muscle.

In summary, we have examined three *egl-30* phenotypes: suppression of arecoline hypersensitivity, reduction of egg laying, and flaccid paralysis. These phenotypes are most simply explained if *egl-30* mutations act to reduce pharyngeal, vulva/uterine, and body muscle contraction. By several tests, this muscle relaxation is independent of specific neural input, suggesting that one site of *egl-30* action might be in the muscle.

Discussion

This study represents the convergence of two complementary approaches to understand signal transduction in C. elegans. A gene encoding a $G_q\alpha$ subunit was identified, based on its striking similarity to mammalian $G_q\alpha$ family members. Simultaneously, new *egl-30* mutations were found in a screen designed to identify genes involved in response to acetylcholine via muscarinic receptors. We determined that *egl-30* encodes $G_q\alpha$ by identifying mutations within the $G_q\alpha$ coding region in each of the new *egl-30* alleles, as well as in those that had been previously isolated (Trent et al., 1983; Park and Horvitz, 1986; Table 1). From the sequence of $G_q\alpha$, the predicted molecular effect of each of the *egl-30* mutations, and our analysis of the phenotypes produced by each of these mutations, as well as by *egl-30* overexpression, we are now able to examine the role of $G_q\alpha$ in C. elegans.

C. elegans $G_q\alpha$ is similar in sequence to the entire mammalian $G_q\alpha$ family; however, it is especially similar to two members of this family: $G_q\alpha$ and $G_{11}\alpha$ (Figure 1A). *egl-30* may be the only C. elegans gene representing the function of both of these α subunits. Mammalian $G_q\alpha$ and $G_{11}\alpha$ are extremely similar in sequence, both are ubiquitously expressed, and they interact with the same set of receptors and effectors (Strathmann and Simon, 1990; Wilkie et al., 1991; Wu et al., 1992). Therefore, they are thought to be redundant for most functions. In addition, amber alleles of *egl-30* are subviable and other *egl-30* alleles produce distinctive phenotypes (Table 1), indicating that if any additional $G_q\alpha$ homologs exist, they do not compensate for the effects of these mutations.

The striking sequence similarity between C. elegans $G_q\alpha$ and mammalian $G_q\alpha$ and $G_{11}\alpha$ suggests that the molecular interactions may also be similar. In fact, C. elegans $G_q\alpha$ expressed in COS-7 cells can interact with a mammalian G_q specific receptor and can activate the endogenous phospholipase Cβ activity, just as mouse $G_{11}\alpha$ does (Figure 2). We have cloned a putative PLCβ cDNA from C. elegans (H. Mori, D. Sonneborn, M. Simon, and P. Sternberg, unpublished data), which may be a natural effector for C. elegans $G_q\alpha$.

Mutations in the $G_q\alpha$ Gene, *egl-30*

We have described eight distinct molecular lesions in *egl-30* (Table 1). Mutations *ad810* and *ad813* introduce stop codons at amino acid positions 212 and 259, which are predicted to eliminate regions required for this G protein to function, such as GTP and effector-binding domains (Noel et al., 1993). Strains homozygous for either allele exhibited the same severe phenotypes: low viability, postembryonic paralysis, and extremely slow or arrested

development. While these alleles have slight dominant effects, they are our best candidates for null alleles of *egl-30*.

ad803 is predicted to change serine 6 to phenylalanine. This novel G protein mutation is predicted to disrupt palmitoylation at cysteines 3 and 4 (Edgerton et al., 1994; Mumby et al., 1994). Reversible palmitoylation is emerging as an important physiological regulator of mammalian $G_q\alpha$ activity, because palmitoylation localizes $G_q\alpha$ to the membrane where it is able to interact with the appropriate membrane bound receptors, effectors, and $\beta\gamma$ dimers (Mumby et al., 1994; Wedegaertner and Bourne, 1993). Mutations at this serine might also directly disrupt the association $G_q\alpha$ with $G\beta\gamma$ subunits (Lambright et al., 1996). The remaining five mutations are predicted to affect the splice removal of introns in $G_q\alpha$ transcripts.

What is the origin of the semidominant phenotypes of *egl-30* alleles (Park and Horvitz, 1986; Reiner et al., 1995; Figures 3C and 3D)? The only allele with a pronounced phenotype in heterozygotes, *n715*, disrupts a splice acceptor sequence. In other genes, this type of mutation can lead to abnormal splice products capable of generating defective or truncated proteins, as well as to a reduced level of wild-type message (Aroian et al., 1993). We hypothesize that in heterozygotes, the defective $G_q\alpha$ protein produced by *egl-30(n715)* competes with wild-type $G_q\alpha$ for binding of other proteins, and might also compete with other G protein α subunits. This allele must be dominant negative rather than dominant activated, since activation of the G_q pathway (by overexpression of the *egl-30* gene) produces essentially the opposite phenotype. The heterozygous phenotypes of the other *egl-30* alleles we describe are quite mild and quite similar to each other (Figures 3D and 4D). These phenotypes might reflect slight dominant negativity, or simply the effect of reduced gene dosage.

The Function of $G_q\alpha$ in C. elegans

The *egl-30* phenotypes we have described appear to reflect defects in coordinated muscle contraction, and we present evidence that this defect is in the muscle or neuromuscular junction. Homozygotes of the sub-viable alleles *ad810* and *ad813 are* almost completely deficient in postembryonic muscle contraction; they are nearly paralyzed and their pharynxes rarely pump. The remaining *egl-30* alleles are defective in egg laying, and at least part of that defect can be ascribed to misfunctioning vulva or uterine muscles (Figure 5). All of the *egl-30* mutants are also defective in normal sinusoidal body movements (Figure 4). The flaccid paralysis produced by these alleles indicates defects in body wall muscle contraction, and analysis of animals with both *egl-30* mutations and mutations known to produce specific nervous system defects supports this contention. Finally, *egl-30* mutations suppress the lethal effects of the drug arecoline on feeding of *eat-11* animals; the site of action of this suppressor activity is most likely in the pharyngeal muscle (Table 2). By analogy to the mammalian G_q family, we fully expect C. elegans G_q will have additional functions unrelated to the muscle, functions that easily could be obscured by the muscle phenotypes of *egl-30* mutants in the behaviors we have examined.

One simple hypothesis is that C. elegans G_q normally transduces one or more of the neurochemical signals that direct muscle contraction. In fact, G_q is directly involved in the

contraction of smooth muscle in mammals, via activation of the phosphoinositide pathway that releases intracellular Ca^{2+} stores through IP_3 gated channels (Eglen et al., 1994). Ca^{2+} induces the contraction of muscle fibers. *egl-30* mutations might interfere with muscle contraction by disrupting a similar signal transduction cascade. However, it is also possible that G_q might act in the generation of a neural signal necessary to potentiate particular muscle cells to respond to an excitatory signal, in addition to, or perhaps instead of, acting in the muscle cells themselves. For example, G_q might be involved in release of acetylcholine, which potentiates the response of egg-laying muscles to exogenous serotonin (Weinshenker et al., 1995). Finally, *egl-30* mutations could disrupt signaling from the nervous system that is required for muscle development. However, any developmental defects are unlikely to interfere with proper specification of muscle cell identity or the formation of organized muscle fibers, because the ultra-structure of the muscles in *egl-30* strains is normal (as assessed by birefringence in polarized light; Reiner et al., 1995), and pharyngeal and body wall muscles in *egl-30* animals can contract when stimulated by a laser microbeam (see Results; Reiner et al., 1995).

Acetylcholine is a strong candidate to be a neurochemical signal mediated by C. elegans G_q, based on the following arguments. The new *egl-30* alleles described here were the only mutations identified as strong genetic suppressors of the lethal effects of an acetylcholine agonist on a hypersensitive *eat-11* strain. Two other *egl-30* alleles have also been identified in a selection for mutations that conferred resistance to an acetylcholinesterase inhibitor (K. Miller and J. Rand, personal communication). The simplest explanation is that *egl-30* mutations reduce the transduction of lethal signaling produced by these drugs through the acetylcholine pathway, and that G_q interacts with one or more muscarinic (G protein coupled) acetylcholine receptors expressed in C. elegans (Culotti and Klein, 1983). In mammals, three muscarinic receptor subtypes interact directly with the G_q family members (Offermanns et al., 1994). Acetylcholine is a neurotransmitter involved in contraction of C. elegans body wall muscles, the regulation of pharyngeal pumping, and egg laying (Alfonso et al., 1994; Avery and Horvitz, 1990; Weinshenker et al., 1995), all behaviors affected by *egl-30* mutations. However, we have not ascertained that defects in acetylcholine signaling per se are responsible for *egl-30* phenotypes described here, and the defect in egg laying is more likely to be related to a defect in serotonin response (Figure 5; Trent et al., 1983).

At least one other C. elegans G protein, G_o, is involved in these same behaviors, but mutations produce a generally opposite effect (Mendel et al., 1995; Segalat et al., 1995). G_o deficient animals lay eggs prematurely and produce sinusoidal movement deeper than normal, like strains overexpressing *egl-30*. An engineered constitutively activated allele of the $G_o\alpha$ gene induces movement and egg-laying defective phenotypes similar to the phenotypes induced by the *egl-30(ad805)* mutation (Mendel et al., 1995; this study). The significance of the opposite phenotypes of $G_o\alpha$ and $G_q\alpha$ mutants is unknown. However, G_q- and G_o-mediated pathways can induce opposite effects within the same cell. For example, in GH_3 cells, activation of the G_q-mediated-pathway increases intracellular Ca^{2+} by releasing Ca^{2+} from internal stores through IP_3 gated channels (Gollasch et al., 1991). Activation of G_o reduces intracellular Ca^{2+} in GH_3 cells by inhibiting Ca^{2+} channels on the cytoplasmic membrane (Offermanns et al., 1991). Since fluxes of intracellular calcium are directly

involved in muscle contraction, such a mechanism could be mediating the opposite effects of mutations in C. elegans $G_q\alpha$ and $G_o\alpha$. However, G_o and G_q pathways do not always work in opposition, and further work is needed to understand how G protein–mediated pathways are regulated and interact in this organism.

The collection of *egl-30* mutants we have described provides an opportunity to study the function of a G protein on the level of a whole organism. By perturbing multiple components of G protein pathways, we hope to understand how signal transduction pathways converge to process information on a molecular level.

Experimental Procedures

Strains and Techniques

C. elegans var. Bristol (N2) was used as wild-type in these studies. All strains were cultivated at 20°C, as described by Brenner (1974) on either E. coli strain OP50 (grown on NGM agar) or on E. coli strain DA837 or HB101 (grown on NGMSR agar). *egl-30* alleles were from Trent et al. (1983), Park and Horvitz (1986), and this study. Polymerase Chain Reaction (PCR) was performed using Taq polymerase (Perkin-Elmer) employing 25–40 cycles (94°C-1 min; 57°C-1.5 min; 72°C-2 min, then 10 min at 72°C), except as noted.

Cloning, Sequencing, Mapping, and Overexpression of the C. elegans $G_q\alpha$ Gene

A fragment of the $G_q\alpha$ gene *egl-30* was amplified from C. elegans genomic DNA (Sulston and Hodgkin, 1988) by PCR (35 cycles annealing at 40°C) using the degenerate primers CA(A/G)GA(A/G)TG(T/G)TA(T/C)GA(T/C)GA(T/C)(C/A)G, and TT(T/C)TG(A/G)AACCA(T/C/A/G)GG(A/G)TA(T/C/A/G)GT, and was used for hybridization to identify a cDNA clone pLB1 (Barstead and Waterston, 1989) that was sequenced. pLB1 was used to identify a genomic lambda clone (Coulson et al., 1986), which was mapped to cosmid C57H9 and subcloned as a 10.5 kb XbaI fragment into Bluescript SK- to create pLB2. The positions of introns were determined by comparing sequence from coding region of the $G_q\alpha$ in pLB1 and pLB2. Their sizes were determined by PCR or sequencing. Two transgenic lines expressing *egl-30* were generated by injection of pLB2 at 5 ng/μl with plasmid pMH86 at 16 ng/μl into *dpy-20(e1282),* as described by Mendel et al. (1995); both resulting extrachromosomal arrays *(syEx125* and *syEx126)* were introduced genetically into *egl-30(ad809); dpy-20(e1282)*.

PLC Activation

Assays were performed as described (Wu et al., 1992; Slepak et al., 1995). COS-7 cells were seeded at 1×10^5 cells/well, grown overnight, transfected with plasmids expressing the indicated proteins at 1 μg DNA/well, activated with AlF_n (30 μM $AlCl_3$ and 10 mM NaF, when indicated) and assayed for phosphoinoside release (Slepak et al., 1995). The C. elegans $G_q\alpha$ expression vector contained the cDNA from pLB1, subcloned as a ClaI- NotI fragment into pCMV. pCMV vectors expressing other proteins have been described (Wu et al., 1992). To test receptor activation, COS-7 cells were cotransfected with 0.5 μg of Gα's and 0.5 μg of α1-C adrenergic receptor (as indicated) in pCMV (Wu et al., 1992), and

normalized with control DNA to 1μg/well. The activation with AlF_n was omitted; instead, the transfected cells were treated with 1 nM norepinephrine to activate the receptor.

Selection of New *egl-30* Mutations as *eat-11* Suppressors

Homozygous *eat-11* hermaphrodites were mutagenized with 50 mM ethane methylsulfonate (EMS; Brenner, 1974). F2 eggs prepared by alkaline hypochlorite treatment (Sulston and Hodgkin, 1988) from the F1 progeny were placed on DA837-seeded plates containing 5 mM arecoline. Three such selections yielded eight arecoline-resistant mutations: *ad807* from the first selection, *ad806* from the second, and *ad803*, *ad805*, *ad809*, *ad810*, *ad813*, and *ad814* from the third.

All the suppressors except *ad807* (the weakest of the eight) turned out to be semidominant, to be loosely linked to *eat-11*, and to cause a reduction in muscle contraction. *ad807* has not been studied further. Genetic mapping, complementationtests, and sequencing (Table 1) showed that the remaining seven are mutations in the same gene, *egl-30*.

Identification of Lesions in *egl-30* Alleles

Exon sequences encoding $G_q\alpha$ plus at least 15 bp of flanking intron sequence were PCR amplified from DNA from each *egl-30* strain, using six pairs of oligonucleotide primers. Each primer contained the −21M13 or M13Rev sequence (Perkin-Elmer), followed by the indicated *egl-30* sequence: Q32/Q33, TAGGTGCGTGCGTCAGCTAGCGGTC/ CAGGTTAAATGTATATTACACCGAC (exon 1); Q36/Q37, TATATATCCAACAACCCATTTTCCAA/ GTTGCGTCACACATCTACT GGCGG (exon 2); Q28/Q29, AGAAAATCGACCGAAGCCTTTAAAT/ GATGATCTGATCCAAATCAAATGGA (exons 3–4);Q30/Q31, GAGCA GGACATTCTGCGTGTTCGTG/ ATGCCGTATAGTTCTTTCGTAATTA (exons 4–5); Q40/Q41, GCTAGAACTAAAATATGTGAAGTGT/ GCTA GAACCCCGTGCTCGAATATTT (exons 6–7); Q38/Q39, ATGGACACTTAGAGTTGCTCATG/ GAAGGAGTACAAGAAATATGT (exon 8).

1.5 μg of C. elegans genomic DNA (Sulston and Hodgkin, 1988) was amplified by 25–35 cycles of PCR in 3 × 100 μl reactions, pooled, gel isolated, recovered using Qiaquick Spin Columns (Qiagen), and directly sequenced on an automated sequencer (Perkin-Elmer). The sequence from each *egl-30* strain was compared with the sequence from N2 and *eat-11*. All mutations were confirmed on the opposite strand, and by at least one independent PCR reaction. The mutations in the *ad810* and *ad813* were identified in a balanced strain.

ad810 and *ad813* Phenotypes

Self-progeny from *egl-30(ad810)/lin-6 dpy-5* and *egl-30(ad813)/lin-6 dpy-5* adult hermaphrodites were moved as eggs or small larvae to new plates at 1–10/plate. 35 *ad810* and 10 *ad813* homozygotes were identified by their characteristic paralyzed, starved, and pale phenotype. All other animals were removed from the plates, and the slowly developing *egl-30* homozygotes were observed continuously under a high-power dissecting microscope for 3 weeks. At the end of this time, 9 *ad810* and 4 *ad813* animals reached adulthood and produced eggs, most of which hatched (internally) to become larvae with a grossly normal

structure; 16 *ad810* animals and 2 *ad813* animals died during this interval; most of the remainder arrested during larval development or became sterile adults. To assess pharyngeal pumping, 5 *ad810* animals were scored for grinder movement over 2 min by Nomarski microscopy, 24 hr after they were laid as eggs. The pharynx did not pump in 3 animals; it pumped 10 and 15 times in the remaining 2 animals.

To examine the movement of *ad810* embryos, several self-progeny eggs from an *egl-30(ad810) eat-11 unc-29/egl-30(+) eat-11 unc-29* mother were mounted on a slide and observed by Nomarski microscopy. The group of eggs was videotaped for 5 min at one hr intervals. *egl-30(ad810)* homozygotes were identified as the eggs that hatched to produce paralyzed larvae. The earlier tape segments were then examined to compare the movement of *egl-30(ad810)* homozygotes to that of heterozygote and *egl-30(+)* homozygote siblings.

Assays of Behavior

Movement was quantitated as body bends/min (Koelle and Horvitz, 1996) on 2-day-old lawns of E. coli strain OP50. For *egl-30(ad805)*, a body bend was defined as 1/4 body length of movement. Egg retention was determined by growing L4 stage hermaphrodites for 28 hr at 20°C, then counting eggs after mounting animals on 4% agar pads in drops of alkaline hypochlorite, which dissolves the hermaphrodites but not their eggs (Sulston and Hodgkin, 1988). Freshly laid eggs were staged under a dissecting scope. Egg-laying response to 5 mg/ml serotonin and 0.75 mg/ml imipramine was determined on adult hermaphrodites not yet bloated with eggs, in a microtiter plate for 90 min (Trent et al., 1983). The *egl-30* heterozygotes assayed were generated as cross progeny of matings with *him-5(e1490)* males. *hDf10*, a deficiency reported to compliment *egl-30* (McKim et al., 1993), does not delete *egl-30* (data not shown), and therefore, was not used as a control for *egl-30* haploinsufficiency.

Laser Kills and Growth Measurements

Pharyngeal neurons were killed by the laser ablation technique of White (Sulston and White, 1980), modified as previously described (Avery, 1993b; Avery and Horvitz, 1987). One day after hatching worms were placed individually on NGMSR plates seeded with HB101, containing either 5 mM arecoline or no arecoline. Each plate was examined every day thereafter until it produced progeny or died, or until 21 days had passed. Those rare animals that survived 21 days without producing progeny are considered sterile. The average days to fertility, i.e., the time required from hatching to the first production of progeny, was used as an objective quantitative measure of feeding and growth rate.

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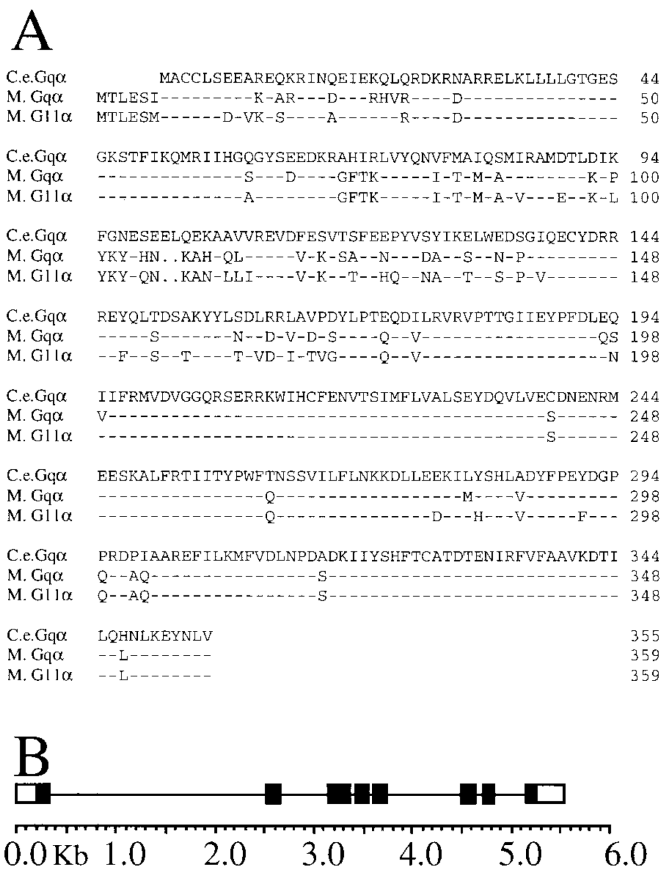

Figure 1. A C. elegans Homolog of $G_q\alpha$ and $G_{11}\alpha$

(A) The deduced amino acid sequence of C. elegans $G_q\alpha$ is shown compared with $G_q\alpha$ (82% identical) and $G_{11}\alpha$ (83% identical) from mouse. Sequences were aligned using the program Pileup (GCG). Dashes indicates amino acid identities, and dots indicate gaps.

(B) Structure of the C. elegans $G_q\alpha$ gene, *egl-30*. The exons end in the middle or end of the codon for the following amino acids: exon 1, Gly (40); exon 2, Gln (82); exon 3, Tyr (155); exon 4, Arg (198); exon 5, Glu (241); exon 6, Gly (293); exon 7, Asp (329); exon 8, Val (355).

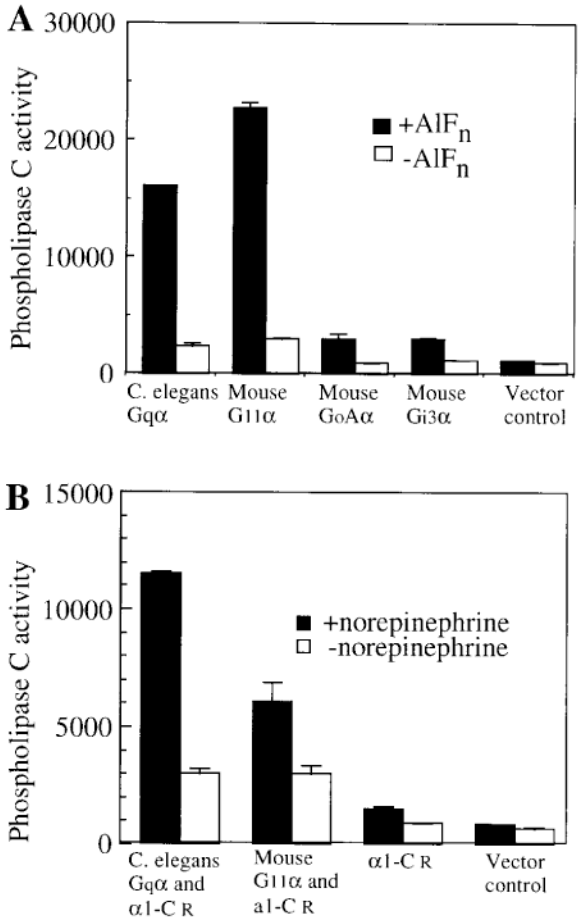

Figure 2. C. elegans $G_q\alpha$ Can Activate Phospholipase C

(A) C. elegans $G_q\alpha$ can stimulate endogenous phospholipase C in COS-7 cells.

(B) C. elegans $G_q\alpha$ can be activated by an a adrenergic receptor. The data shown is the average of duplicate wells, and is representative of two other similar experiments. Units are cpm of [^{3}H]inositol phosphates released. Assay of $G_oA\alpha$ and $G_{i3}\alpha$ were performed in a separate experiment.

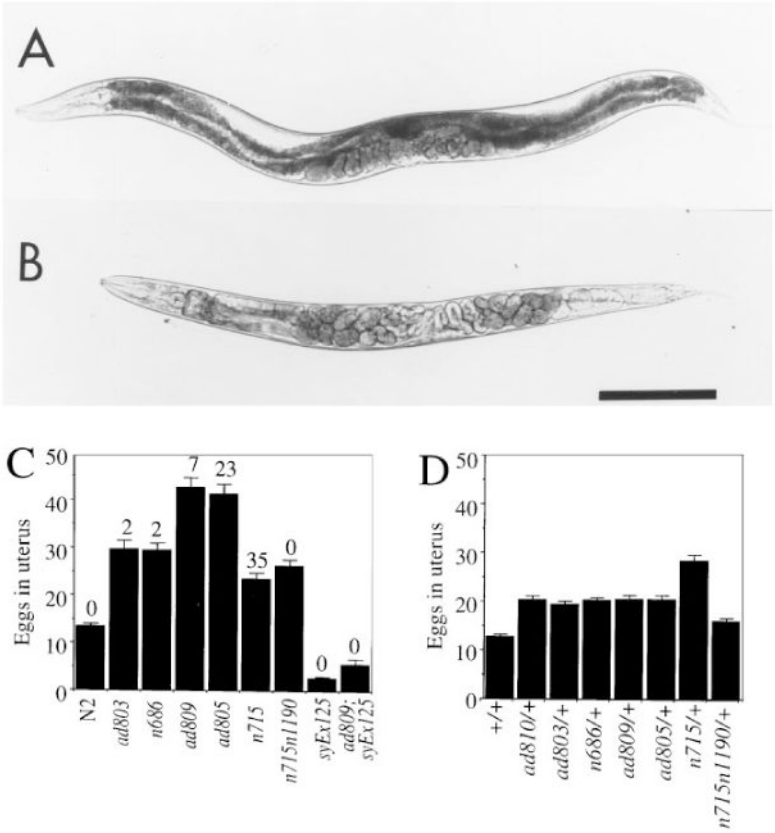

Figure 3. *egl-30* Hermaphrodites Are Defective in Egg Laying

Wild-type (A) and *egl-30(ad805)* (B) animals 28 hr after L4 larval stage. Embryos that have started to undergo morphogenesis are visible inside *egl-30* but not wild-type animals. Scale bar represents 0.2 mm. *egl-30* homozygotes (C) and heterozygotes (D) retain eggs. At least 30 homozygotes and 7 heterozygotes of each allele were scored. The percentage of those eggs that were late stage (1 1/2 fold stage or later) is indicated above the bars in (C). No late stage eggs were found in (D). Homozygotes of *egl-30(ad810)* were not tested in this assay because of lethality and sterility. *syEx125* in (C) is a multicopy extra-chromosomal array overexpressing the wild-type *egl-30* gene. *syEx125* suppresses the egg retention of *egl-30(ad809),* consistent with our identification of the $G_q\alpha$ gene as *egl-30*.

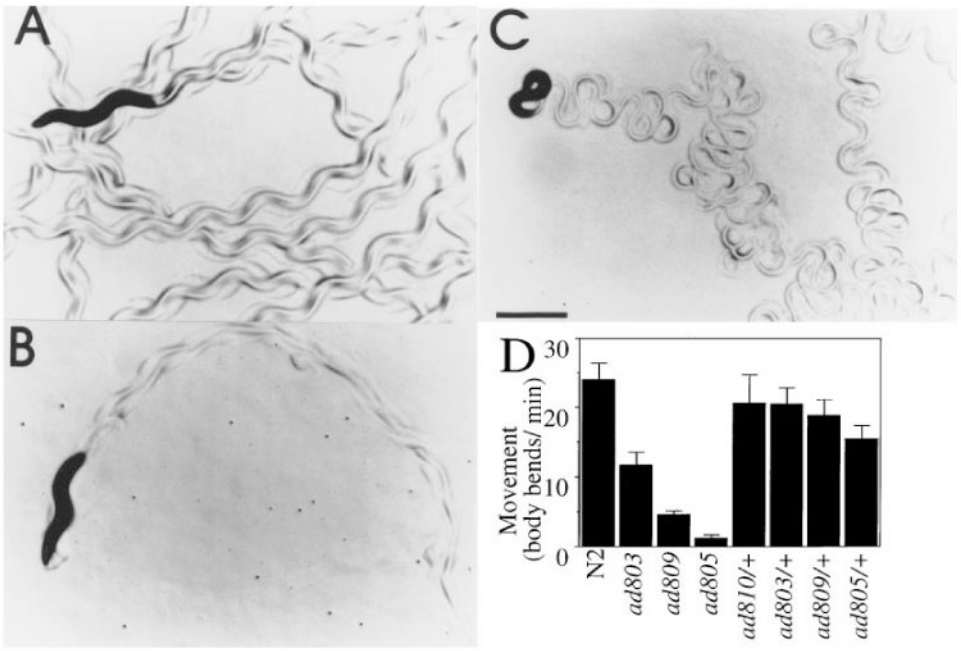

Figure 4. Mutations in *egl-30* Disrupt Coordinated Movement

egl-30 mutations display reduced level of movement and leave flattened sinusoidal tracks. Single wild-type (A), *egl-30(ad805)* (B), or *dpy-20(e1282)*; *egl-30(syEx125)* (C) adult hermaphrodites were placed on 2-day-old lawns of E. coli strain OP50, and allowed to swim for 1 hr. A portion of the tracks they left are shown. Scale bar represents 0.5 mm.
(D) Movement level of selected *egl-30* strains. Each bar represents the average of at least seven determinations.

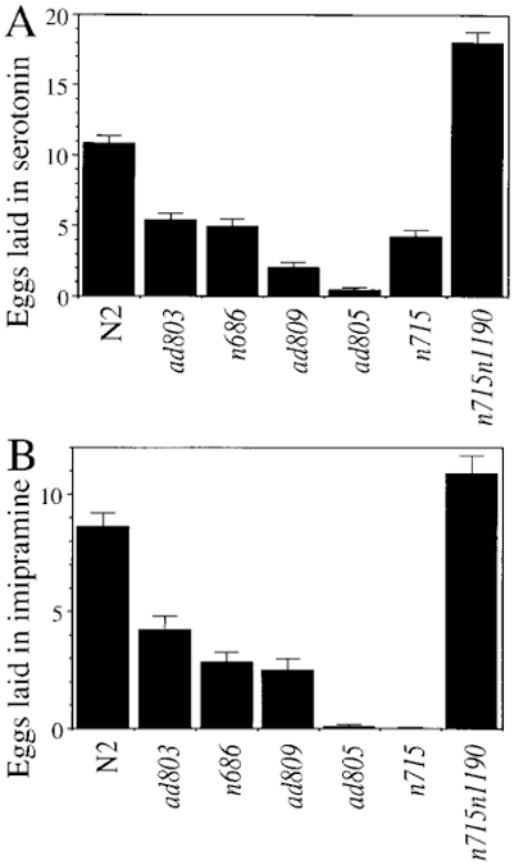

Figure 5. *egl-30* Mutations Disrupt the Response of Egg-Laying Muscles to Serotonin and Imipramine

*egl-30*hermaphrodites lay a reduced number of eggs in serotonin (A) and imipramine (B). At least 30 unbloated animals were assayed for each drug and allele.

Table 1

egl-30 Mutations Disrupt the Sequences Encoding *C. elegans* $G_q\alpha$

Allele	Mutation[a]	Predicted Result	Phenotype
ad803 and *ad806*	TCC to TTC	Ser (6) to Phe	bloated with eggs; sluggish
n686	ttccagAA to ttccaaAA	defective splice acceptor intron 4	bloated with eggs; very sluggish; leaves variable tracks
ad809	AGgtag to AGatag	defective splice donor intron 2	bloated with eggs; very sluggish; leaves shallow tracks
ad805 and *ad814*	ttccagTC to ttccaaTC	defective splice acceptor intron 2	severely bloated with eggs; flaccid and nearly paralyzed; leaves very shallow tracks
n715	tttcagAT to tttcaaAT	defective splice acceptor intron 7	severely bloated with eggs; flaccid and nearly paralyzed; leaves very shallow tracks; starved
ad810	TGG to TAG	trp (212) to amber codon	sub-viable; flaccid and paralyzed; sporadic pharyngeal pumping; starved
ad813	TGG to TAG	trp (259) to amber codon	sub-viable; flaccid and paralyzed; sporadic pharyngeal pumping; starved
n715n1190	tttcagAT to tttcaaAT and aaggg to aagag[b]	defective splice acceptor intron 7 and additional " ag" in intron 7	bloated with eggs; very sluggish; leaves shallow tracks
syEx125 and *syEx126*	multi-copy array of *egl-30* gene	over-expression of $G_q\alpha$	empty gonad; lays early eggs; leaves deep tracks

[a] Exon sequences are indicated in upper case, intron sequences in lower case. The mutated nucleotide is underlined.

[b] The sequence of the last 80 bases of intron 7 in N2 animals is: ttaga gttgc tcatg gcgaa ttact aaaaa attgt ttaaa aatat ctaaa atttt caaat aaaag ggacc tctat ttcag.

Table 2

The Pharyngeal Nervous System Is Not Required for *egl-30* Suppression of *eat-11* Arecoline Hypersensitivity[a]

Relevant Genotype[b]	Arecoline (mM)	Percent Fertility (n)	Days to Fertility[c]
+	0	100 (13)	4.2
eat-11(ad541)	0	100 (11)	5.4
+	5	83 (12)	6.4
eat-11(ad541)	5	0 (10)	—
egl-30(ad803) eat-11(ad541)	5	100 (14)	5.1

[a] All pharyngeal neurons except M4 were ablated with a laser microbeam. One day later, the worms were transferred to plates with or without 5 mM arecoline, as indicated.

[b] Hermaphrodites were homozygous for *unc-29(el072)* in addition to the genotype shown.

[c] Growth and fertility of *egl-30(ad803) eat-11(ad541)* animals on arecoline is significantly different from both *eat-11* ($P < 0.01$) and wild-type ($P < 0.05$) animals, grown under the same conditions.