

# The neural G protein $G\alpha_o$ tagged with GFP at an internal loop is functional in *Caenorhabditis elegans*

Santosh Kumar <sup>†,‡,\*</sup> Andrew C. Olson <sup>‡</sup> and Michael R. Koelle <sup>\*</sup>

Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520 USA

\*Corresponding author: Department of MB&B, Yale University Medical School, 300 Cedar Street SHM CE30, New Haven, CT 06520, USA. Email:

Michael.koelle@yale.edu (M.R.K.); Department of Biotechnology, Panjab University, BMS Block I, Sector 25, Chandigarh 160014, India. Email: sbalot@pu.ac.in (S.K.)

<sup>†</sup>Present address: Department of Biotechnology, Panjab University, Chandigarh 160014, India.

<sup>‡</sup>These authors contributed equally to this work.

## Abstract

$G\alpha_o$  is the alpha subunit of the major heterotrimeric G protein in neurons and mediates signaling by every known neurotransmitter, yet the signaling mechanisms activated by  $G\alpha_o$  remain to be fully elucidated. Genetic analysis in *Caenorhabditis elegans* has shown that  $G\alpha_o$  signaling inhibits neuronal activity and neurotransmitter release, but studies of the molecular mechanisms underlying these effects have been limited by lack of tools to complement genetic studies with other experimental approaches. Here, we demonstrate that inserting the green fluorescent protein (GFP) into an internal loop of the  $G\alpha_o$  protein results in a tagged protein that is functional *in vivo* and that facilitates cell biological and biochemical studies of  $G\alpha_o$ . Transgenic expression of  $G\alpha_o$ -GFP rescues the defects caused by loss of endogenous  $G\alpha_o$  in assays of egg laying and locomotion behaviors. Defects in body morphology caused by loss of  $G\alpha_o$  are also rescued by  $G\alpha_o$ -GFP. The  $G\alpha_o$ -GFP protein is localized to the plasma membrane of neurons, mimicking localization of endogenous  $G\alpha_o$ . Using GFP as an epitope tag,  $G\alpha_o$ -GFP can be immunoprecipitated from *C. elegans* lysates to purify  $G\alpha_o$  protein complexes. The  $G\alpha_o$ -GFP transgene reported in this study enables studies involving *in vivo* localization and biochemical purification of  $G\alpha_o$  to complement the already well-developed genetic analysis of  $G\alpha_o$  signaling.

**Keywords:**  $G\alpha_o$ ; heterotrimeric G protein; neural signaling; *Caenorhabditis elegans*

## Introduction

$G\alpha_o$ , the  $\alpha$  subunit of the most abundant heterotrimeric G protein in the brain (Sternweis and Robishaw 1984), is in every neuron and can be activated by G protein-coupled receptors for every neurotransmitter tested (Jiang et al. 2001). *Caenorhabditis elegans* has a  $G\alpha_o$  ortholog named GOA-1 that is >80% identical to mammalian  $G\alpha_o$  and that is expressed in most or all neurons. GOA-1 has been shown by genetic analysis to inhibit neurotransmitter release and/or neural activity (Mendel et al. 1995; Ségalat et al. 1995; Nurrish et al. 1999, Ravi et al. 2020), but the molecular mechanisms by which  $G\alpha_o$  signals to have these effects remain to be fully defined. While activated  $G\alpha_o$  releases  $G\beta\gamma$  subunits to regulate specific potassium and calcium channels (Lüscher and Slesinger 2010; Proft and Weiss 2015), genetic studies in *C. elegans* suggest that signaling through  $G\beta\gamma$  is not likely the sole mechanism by which  $G\alpha_o$  has its physiological effects (Koelle 2018). It remains unclear if activated  $G\alpha_o$ , like all other  $G\alpha$  proteins in animal cells, may itself bind target “effector” proteins to propagate a signal.

A method to fuse  $G\alpha_o$  to fluorescent proteins and/or epitope tags without disrupting its function would enable new experimental approaches to help resolve unanswered questions about  $G\alpha_o$  signaling. For example,  $G\alpha_o$ -GFP fusion proteins could be

visualized in real time in living cells for cell biological studies, and anti-GFP antibodies could be used to immunopurify  $G\alpha_o$  protein complexes for biochemical analysis.

The challenge to this approach is that tags at the N- or C-termini would likely disrupt  $G\alpha_o$  function since  $G\alpha$  proteins use their N- and C-termini to interact with receptors,  $G\beta\gamma$  subunits, and membranes (Hynes et al. 2004). For example, a previous study in *C. elegans* used a multicopy transgene to overexpress  $G\alpha_q$  with GFP fused to its N-terminus (Bastiani et al. 2003). This fusion protein was able to fully rescue one behavioral defect of a  $G\alpha_q$  partial loss-of-function mutant, while only partially rescuing other defects. The multi-copy transgene also created a gain-of-function effect which a single-copy transgene, not available at the time, might have been able to avoid.

Recent efforts to functionally tag  $G\alpha$  proteins have focused on inserting fluorescent proteins at internal sites. Internally tagged  $G\alpha$  proteins have been shown to be activated by G protein coupled receptors when co-overexpressed in cultured cells with both a receptor and  $G\beta\gamma$  subunits (Hughes et al. 2001; Yu and Rasenick 2002; Bünemann et al. 2003; Galés et al. 2005; Lazar et al. 2011); however, some internal insertions alter  $G\alpha$  function, and overexpressed receptors can promiscuously activate  $G\alpha$  proteins they would not otherwise activate (Gibson and Gilman, 2006). Still, some tagged  $G\alpha$  proteins may be fully functional: in the yeast

Received: May 17, 2021. Accepted: May 01, 2021

© The Author(s) 2021. Published by Oxford University Press on behalf of Genetics Society of America.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

*Saccharomyces cerevisiae* and in the slime mold *Dictyostelium discoideum*, an internally tagged  $G\alpha$  protein can replace the untagged  $G\alpha$  to support physiological functions that depend on activation by a single endogenous receptor (Janetopoulos et al. 2001; Yi et al. 2003). A remaining question is whether a tagged  $G\alpha$  protein could be fully functional in a metazoan, where it must mediate signaling from many different receptors to control diverse, tissue-specific physiological functions.

Here, we demonstrate that *C. elegans*  $G\alpha_o$  with GFP inserted into an internal loop, when expressed at normal levels in the animal, rescues multiple defects in behavior and development caused by loss of native  $G\alpha_o$ . We show that this tagged protein can be used to visualize  $G\alpha_o$  subcellular localization in living animals and to purify both inactive and activated  $G\alpha_o$  protein complexes from *C. elegans* lysates.

## Materials and methods

All reagents were from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise indicated.

### Strains and culture

*Caenorhabditis elegans* strains were cultured at 20°C on NGM agar plates with *Escherichia coli* strain OP50 as a nutrition source (Brenner 1974). All strains were derived from the wild-type strain N2. Generation of transgenic animals and genetic crosses were by standard methods (Evans 2006; Fay 2013). Table 1 shows a list of *C. elegans* strains used in this study.

### goa-1::gfp plasmid construction

A plasmid to express internally GFP-tagged GOA-1 in *C. elegans* was generated by first inserting a 9.0 kb *C. elegans* genomic fragment containing the *goa-1* gene into a pBluescript vector and engineering in an *SpeI* restriction site between the *goa-1* codons for T<sup>117</sup> and E<sup>118</sup>. The GFP coding region containing artificial introns was PCR-amplified from the vector pPD95.69 (Addgene plasmid #1491) using primers to add segments encoding SGGGGS and SGGGTS to flank the N- and C-termini of GFP, respectively, and the resulting *gfp* cassette was inserted into the *SpeI* site of the *goa-1* clone. The resulting plasmid was named pMK376. To generate a clone suitable for miniMos single-copy insertion into the *C. elegans* genome (Frøkjær-Jensen et al. 2014), we amplified the GOA-1-GFP coding region from pMK376 along with 4987 bp of 5' promoter and 432 bp of 3' UTR using primers mini909FWD (5'-gagattctgcaaggaaattccaactgaatttagatttttaaagt-3') and mini909REV (5'-agattaggcctggagcttttccaccatactccggaataa-3'), which added *StuI* and *PstI* restriction sites (boldface) at the 5' and 3' ends of the amplicon, respectively. The amplicon was then digested and ligated into the pCFJ909 miniMos vector (Frøkjær-Jensen et al. 2014) using

the *StuI* and *PstI* restriction sites. The resulting plasmid was named pAO8.

The Q205L mutation was engineered into *goa-1::gfp* gene pMK376 using the GeneArt Site-Directed Mutagenesis PLUS Kit (Invitrogen) and the primers SKB48 (5'-tgtgggaggtctgagatcagaaag-3') and SKB49 (5'-tcgaacaatctgtaaatattc-3'). To generate a clone for site-specific single-copy insertion into the *C. elegans* genome, the *goa-1(Q205L)::gfp* cassette was then amplified using primer pair SKB46 (5'-agatacctaggggagcttttccaccatactccggaataa-3') and SKB47 (5'-ctcacttaaggaattccaactgaatttagatttttaaagt-3') to add *AvrII* and *AflIII* restriction sites, and this fragment was subsequently subcloned into the MosSCI insertion vector pCFJ151 (Frøkjær-Jensen et al. 2008) digested with *AvrII* and *AflIII*. The resulting plasmid was named pSKB21.

### Single-copy goa-1::gfp transgenic strains

The *goa-1::gfp* single-copy miniMos transgene vsSi32 was generated as described by Frøkjær-Jensen et al. (2014) by injecting into LX1691 *unc-119(ed3) III* animals the *goa-1::gfp* plasmid pAO8 at 15 ng/μl, with the Mos1 transposase plasmid pCFJ601 at 50 ng/μl, and marker plasmids pCFJ90 at 2.5 ng/μl, pCFJ104 at 10 ng/μl, pGH8 at 10 ng/μl, and pMA122 at 10 ng/μl. Inverse PCR was used to determine the transgene integrated in the left arm of chromosome III between the sequences 5'-tttactgcatactgaacaacagg-gaaaagggg-3' and 5'-tagaattagctgtaagacggcgtctaggtttgca-3'. The *goa-1(Q205L)::gfp* MosSCI single-copy transgene vsSi39 was inserted at the ttTi5605 Mos1 locus on chromosome II as described by Frøkjær-Jensen et al. (2008) by injecting into Unc progeny of EG6699 ttTi5605 II; *unc-119(ed9) III*; *oxEx1578* animals a mix of the *goa-1(Q205L)::gfp* plasmid pSKB21 at 50 ng/μl, the Mos1 transposase plasmid pCFJ601 at 50 ng/μl, and marker plasmids pCFJ90 at 2.5 ng/μl, pCFJ104 at 5 ng/μl, pGH8 at 10 ng/μl, and pMA122 at 29 ng/μl. The initially generated transgenic animals were outcrossed four times to wild-type N2 animals to remove background mutations. The resulting strain was named LX2404.

### Confocal imaging

Worms were mounted on 2% agarose pads containing 120 mm Optiprep (Sigma Millipore) to reduce refractive index mismatch (Boothe et al. 2017) on premium microscope slides Superfrost (Thermo Fisher Scientific), and a 22 × 22–1 microscope cover glass (Fisher Scientific) was placed on top of the agarose pad. Worms were anesthetized using a drop of 150 mm sodium azide (Sigma Millipore) with 120 mm Optiprep. Z-stack confocal images of 24 hour old larvae were taken on a Zeiss LSM 880 microscope using a 63X objective lens.

**Table 1** *C. elegans* strains used in this study

Strain	Genotype	Feature	Source
N2	Wild type		Brenner 1974
LX1691	<i>unc-119(ed3) III</i>	Recipient strain for miniMos transgenes. Made by outcrossing HT1593 (from the <i>Caenorhabditis</i> Genetics Center) 4X to N2	This study
EG6699	ttTi5605 II; <i>unc-119(ed9) III</i> ; <i>oxEx1578</i>	Unc progeny (which have lost the <i>unc-119</i> -rescuing transgene <i>oxEx1578</i> ) are recipients for single-copy transgene insertion into the ttTi5606 Mos1 locus	<i>Caenorhabditis</i> Genetics Center
LX2060	vsSi32 III <i>unc-119(ed3) III</i>	<i>goa-1::gfp</i> strain	This study
LX2404	vsSi39 II	<i>goa-1(Q205L)::gfp</i> strain	This study
JT734	<i>goa-1(sa734) I</i>	<i>goa-1</i> null mutant	Robatzek and Thomas 2000
LX2071	<i>goa-1(sa734) I</i> ; vsSi32 III	<i>goa-1::gfp</i> in <i>goa-1</i> null background	This study

## GOA-1 antibody

The affinity-purified rabbit anti-GOA-1 polyclonal antibody used was from Chase et al. (2001). Whole-mount stains of *C. elegans* were performed as described by Finney and Ruvkun (1990).

## Behavioral and worm length assays

Quantitation of unlaidd eggs and staging of laid eggs were performed using 30hour post-L4 adult animals as described in Chase and Koelle (2004). For analysis of worm tracks, reversal-touch behavior, and worm length, worms were staged 24 hours post-L4 and transferred to an NGM agar plate with a thin lawn of OP50 bacteria for imaging. Imaging began 2–20 minutes after transfer of the worm to the new plate and was carried out using a Leica M165FC microscope equipped with a digital camera. For measurements of worm length, >10 second digital video recordings of worms were analyzed using WormLab software from MBF Bioscience. Reversal-touch behavior was defined as a reversal during which the worms bends deeply enough that it contacts itself. Usually the tail or head touches the body, but in some cases two sections of the midbody can touch each other during very deep bends. Reversal-touch behavior was scored by placing a single worm staged 24 hours post-L4 on a new NGM plate with a lawn of OP50 bacteria, waiting 1–10 minutes, and then counting the number of reversal-touches made by the worm during a 15-second time period.

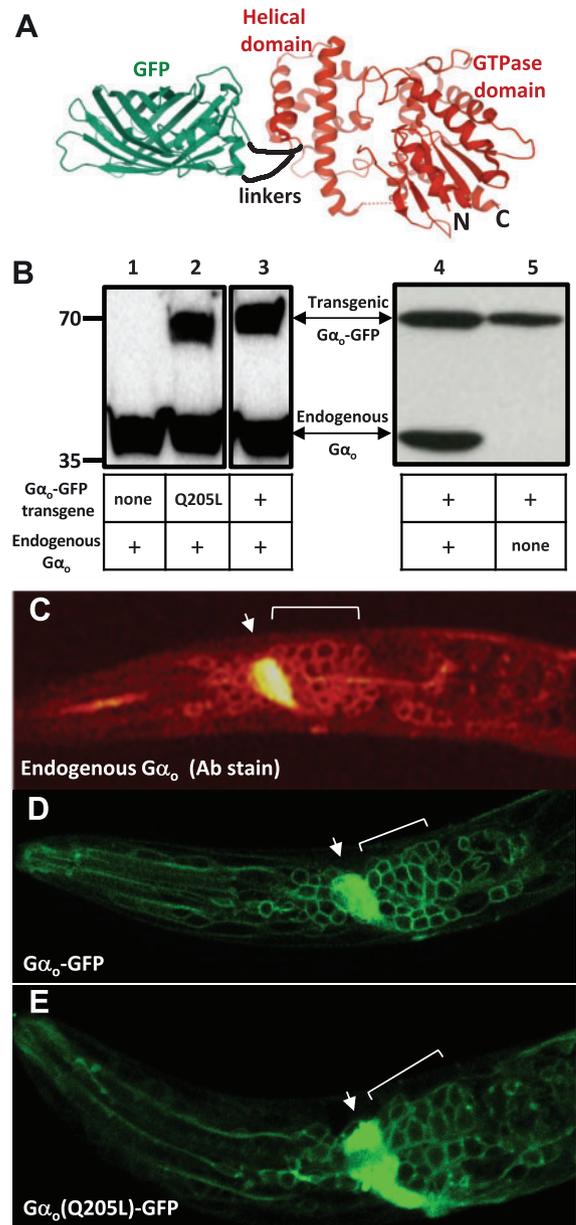
## Statistical analysis

Error bars shown in the graphs in Figures 2 and 3 represent 95% confidence intervals. All statistical analyses were done using GraphPad Prism version 9.0.1 software. The early-stage egg assay data set was analyzed using Fisher's exact test with two-sided P-values. The remaining data sets were analyzed using one-way ANOVA with Šidák's multiple comparisons test.

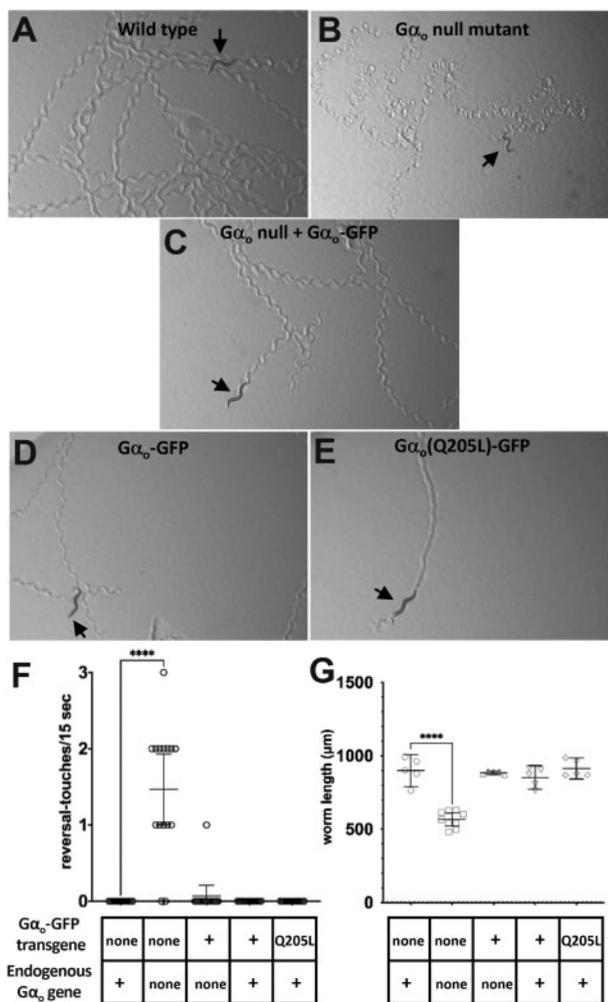
## Immunoprecipitation of GOA-1::GFP

Worm lysates were prepared as described previously (Porter and Koelle 2010) with some modifications. Briefly, *C. elegans* were grown in 20 ml liquid cultures at 20°C and worms were isolated by flotation on 30% sucrose. Packed worm pellets (~500–600 μl) were resuspended in 4 ml lysis buffer [50 mM HEPES pH7.4, 100 mM NaCl, 1 mM EDTA, 3 mM EGTA, 10 mM MgCl<sub>2</sub>, 1 mM DTT, 1% Triton X-100 and complete protease inhibitor cocktail (Roche #04693159001)]. Resuspended worm pellets were homogenized by passing them two times through a French press (Spectronic Instruments, model number FA078) at 13000 PSI. The resulting lysates were centrifuged at 100,000Xg for 30–60 minutes at 4°C in an Optima TLX tabletop ultracentrifuge using a TLA-110 rotor (Beckman Coulter, Fullerton, CA, USA). The clarified supernatants were removed and transferred into the new tubes. The protein concentrations were determined by Bio-Rad protein assay. Whole worm lysates were aliquoted and snap frozen in liquid nitrogen for storage at –80°C until use.

Immunoprecipitation was performed using Pierce crosslink immunoprecipitation kits (Pierce #26147) and the buffers therein, per manufacturer's instructions with some modifications. All spins in this procedure to separate supernatants from beads were at 3000 RPM for 1 minute in a microcentrifuge equipped with a swinging bucket rotor. To prepare beads sufficient for immunoprecipitating three samples, 90 μl of 50% protein A/G agarose slurry/sample was washed three times with 1 ml phosphate buffered saline (PBS, 10 mM sodium phosphate, 0.15 M NaCl, pH 7.5) and then incubated at room temperature for 2 hours on a rotary

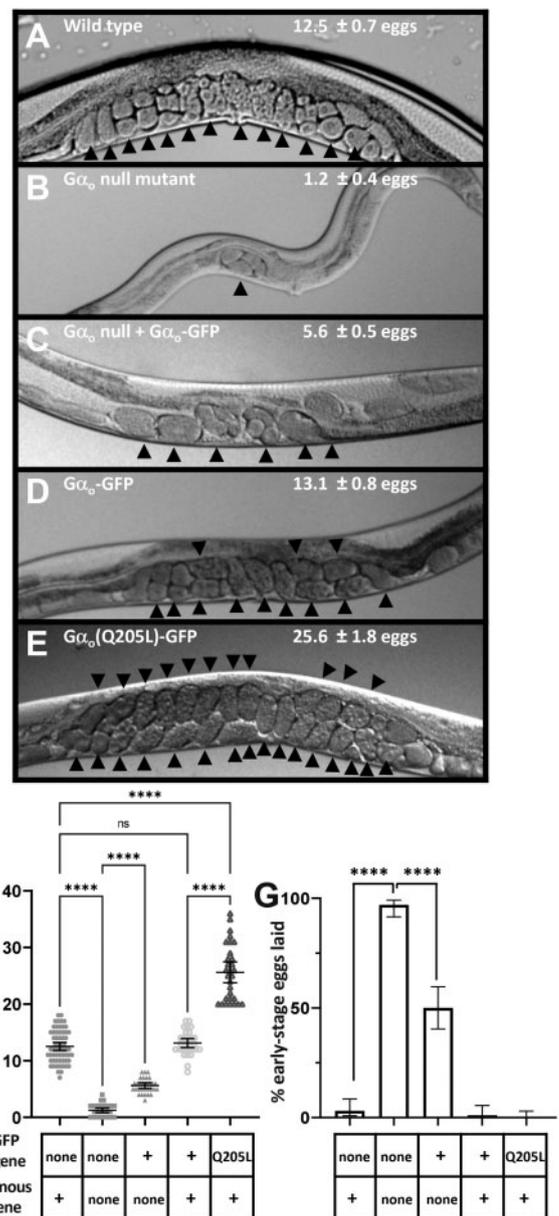


**Figure 1** Design and expression of Gα<sub>o</sub>-GFP in *C. elegans*. (A) Design of Gα<sub>o</sub>-GFP. Structures of mammalian Gα<sub>o</sub> (red, PDB entry 3c7k) and GFP (green, PDB entry 1GFL) were rendered with Mol\* (Sehnal et al. 2018). The designed fusion protein has six amino acid linkers (black) connecting the GFP N- and C-termini to amino acids T117 and E118 of *C. elegans* Gα<sub>o</sub>. (B) Western blots of *C. elegans* protein lysates probed with an antibody to Gα<sub>o</sub> (Patikoglou and Koelle 2002) that detects both endogenous Gα<sub>o</sub> and transgenically expressed Gα<sub>o</sub>-GFP. Lanes 1–3 are from the same gel, while lanes 4–5 are from a separate gel. Positions of molecular weight markers (in kDa) ran at positions shown at left. Protein lysates were from five *C. elegans* strains in which Gα<sub>o</sub>-GFP transgenes and a null mutation in the endogenous Gα<sub>o</sub> gene were present in the combinations indicated. The strain analyzed in lane 2 expressed Gα<sub>o</sub>-GFP carrying a Q205L mutation that disrupts Gα<sub>o</sub> GTPase activity and thus renders the G protein constitutively active. (C–E) Localization of endogenous Gα<sub>o</sub> protein by anti-Gα<sub>o</sub> antibody stain (C) compared to localization of Gα<sub>o</sub>-GFP (D) and Gα<sub>o</sub>(Q205L)-GFP (E) by GFP fluorescence. Shown are confocal images of optical sections through the heads of *C. elegans* larvae, with anterior left and ventral down. Arrows point to the nerve ring, a bundle of neural processes containing many synapses; brackets indicate the neural ganglia posterior to the nerve ring, containing the cell bodies of many neurons. Rings of fluorescence in the neural ganglia suggest that Gα<sub>o</sub> is localized on the plasma membrane of neural cell bodies. Antibody staining is absent in Gα<sub>o</sub> null mutant animals stained in parallel with the same Gα<sub>o</sub> antibody, demonstrating that the stain is specific for Gα<sub>o</sub> (Gotta and Ahinger 2001, and data not shown).



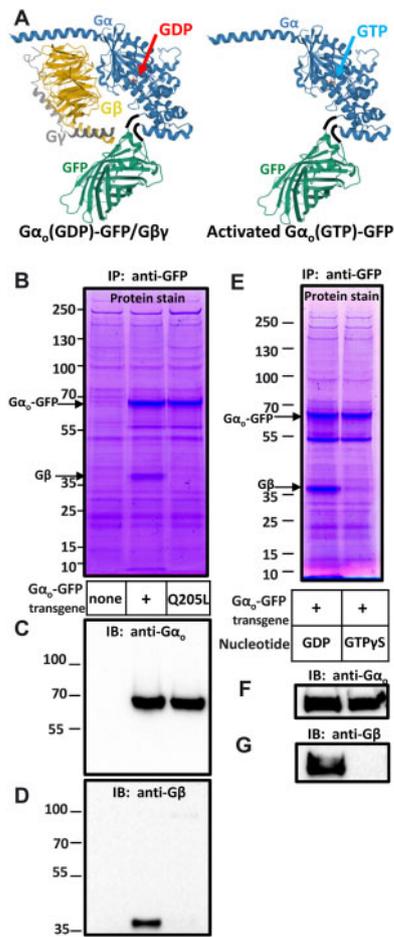
**Figure 2** Gα<sub>o</sub>-GFP rescues the locomotion and body morphology defects of a Gα<sub>o</sub> null mutant. (A–E) Photographs of single worms of the indicated genotypes showing tracks left by locomotion over an agar surface. Arrows indicate current positions of the worms. Sinusoidal tracks left by the wild-type worm (A) show the pattern of normal locomotion, whereas the Gα<sub>o</sub> null worm in (B) is smaller and has left tracks that reflect abnormal locomotion. Expression of Gα<sub>o</sub>-GFP in the Gα<sub>o</sub> null background restores locomotion to a more normal pattern (C). In a wild-type background, expression of Gα<sub>o</sub>-GFP has little effect on locomotion (D), while expression of activated Gα<sub>o</sub> (Q205L)-GFP (E) induces a gain-of-function phenotype evidenced by tracks that reflect abnormally shallow body bends. (F) Animals of the indicated genotypes (*n* = 15 per genotype) were video recorded and reviewed to quantitate the frequency of episodes of backward locomotion that included a body bend so deep that the animal touched its own body. (G) Videos of adult worms of the same genotypes (*n* ≥ 5 per genotype) were analyzed with WormLab software to measure their body length. In panels F and G, error bars represent 95% confidence intervals, comparisons labeled \*\*\*\* were statistically different with *P* < 0.0001; and all other pairwise comparisons were not statistically different.

mixer at room temperature with 10 μg mouse monoclonal anti-GFP antibody (Rockland #600-301-215) diluted in 500 μl PBS. Beads were washed three times with PBS followed by incubation with Pierce DSS crosslinker working solution (150 μl) at room temperature for 1 hour on a rotary mixer. The supernatant was discarded and the beads were washed twice with Pierce elution buffer followed by three washes with IP buffer (50 mM HEPES pH 7.4, 100 mM NaCl, 1 mM EDTA, 3 mM EGTA, 10 mM MgCl<sub>2</sub> and 1% Triton X-100). While the anti-GFP beads were being prepared, protein lysates were pre-cleared: for each immunoprecipitation sample,



**Figure 3** Gα<sub>o</sub>-GFP partially rescues egg-laying behavior defects of a Gα<sub>o</sub> null mutant. (A–E) Photographs of worms of the indicated genotypes showing unlaidd eggs within the mid-body region. Each unlaidd egg is indicated by an arrowhead, and the average number of unlaidd eggs and 95% confidence intervals for each genotype are shown on the corresponding photographs. The scrawny body morphology of a Gα<sub>o</sub> null mutant (B) compared to that of a wild-type animal (A) can also be seen. (F) Quantitation of unlaidd eggs, *n* ≥ 30 for each strain. (G) Percent of freshly laid eggs at early stages of development (≤ 8 cells), a measure of hyperactive egg-laying behavior. \*\*\*\*, *P* < 0.0001; ns, not significant (*P* > 0.05); error bars represent 95% confidence intervals.

1 ml of 4 mg/ml worm protein lysate was incubated with 15 μl packed protein A/G beads (prewashed 3X in PBS) at 4°C for 1 hour on a rotary mixer. Then for each immunoprecipitation, 1 ml of 4 mg/ml protein pre-cleared protein lysate was incubated with 15 μl packed anti-GFP antibody cross-linked beads at 4°C for 2 hours on a rotary mixer. For in vitro activation (Figure 4E), at the beginning of this 2 hours incubation, 100 μM GDP or GTPγS was added to the protein lysate. After removing the supernatant, the beads bearing the immunoprecipitated products were washed four times with IP buffer at 4°C and eluted with 50 μl of 2X LDS loading buffer (Invitrogen



**Figure 4** Immunoprecipitation of inactive and activated  $G\alpha_o$ -GFP protein complexes from worm protein lysates. (A) Models of inactive (left) and active (right)  $G\alpha_o$ -GFP. Models were rendered with Mol\* (Sehnal et al. 2018) and based on mammalian  $G\alpha_o$ -GDP/ $\beta\gamma$  (PDB entry 6EG8) with the alpha subunit (blue) connected by flexible six-amino acid linkers (black) to GFP (green, PDB entry 1GFL). Inactive  $G\alpha_o$ -GFP-GDP (left) is in a complex with  $G\beta\gamma$  subunits (yellow and beige), while active  $G\alpha_o$ -GFP-GTP (right) is not. (B–D) Immunoprecipitation of inactive and *in vivo*-activated  $G\alpha_o$ . Proteins were immunoprecipitated with an anti-GFP antibody from whole-worm lysates of strains carrying no transgene (left lane, a control to show nonspecific background proteins), the  $G\alpha_o$ -GFP transgene (center lane), or the activated  $G\alpha_o$ (Q205L)-GFP transgene (right lane). Proteins were resolved by gel electrophoresis, with the mobility of molecular weight marker (in kDa) shown at left. (B) Precipitated proteins were visualized using Imperial protein stain.  $G\alpha_o$ -GFP and  $G\beta$  appeared as most prominent proteins precipitated, with  $G\beta$  absent from the activated  $G\alpha$  complex (right lane).  $G\gamma$  is a small protein expected to run at the dye-front in the center lane. The identities of the prominent bands in (B) are confirmed by Western blots of the same protein samples probed with anti- $G\alpha_o$  (C) and anti- $G\beta$  (D) antibodies. (E–G) Immunoprecipitation of inactive and *in vitro*-activated  $G\alpha_o$ . Whole worm protein lysate from the strain carrying  $G\alpha_o$ -GFP [same lysate used for the middle lanes in (B–D)] was treated with GDP to preserve the inactive state of  $G\alpha_o$ -GFP or with the nonhydrolysable GTP analog GTP $\gamma$ S to activate  $G\alpha_o$ -GFP. Proteins were separated by gel electrophoresis and visualized with Imperial protein stain (B), or by Western blotting with anti- $G\alpha_o$  (F) and anti- $G\beta$  (G) antibodies. GTP $\gamma$ S treatment activated  $G\alpha_o$ -GTP as evidenced by its dissociation from  $G\beta$ .

#NP0007) at 55°C for 30 minutes. The supernatants were transferred into the new tubes and 5% (by volume)  $\beta$ -mercaptoethanol was added and the tubes were incubated at 55°C for 15 minutes. The supernatants were collected, and samples were processed for SDS-PAGE gel electrophoresis and western blotting.

## SDS-PAGE gel electrophoresis and Western blotting

For SDS-PAGE gel electrophoresis followed by total protein staining, IP samples were loaded on 4–12% Bis-Tris gels (NuPAGE #NP0322BOX) and separated using MOPS buffer (Novex #NP0001). Gels were stained with Imperial Protein Stain (ThermoFisher Scientific #24615) per manufacturer's instructions and images were captured using Epson Perfection V800 Photo Color Scanner. Images were processed by ImageJ software. For western blots, IP samples were loaded (20% of an IP sample/well) and separated on 10% SDS-PAGE gels. The protein was transferred onto a nitrocellulose membrane, and the blot was blocked and incubated with a primary anti- $G\alpha_o$ -1 antibody [1:1000 diluted affinity-purified rabbit anti- $G\alpha_o$ -1 polyclonal antibody (Patikoglou and Koelle 2002) at 4°C for overnight], washed, incubated with a secondary antibody (1:3000 HRP-linked Anti-Rabbit antibody Bio-Rad) and protein bands were visualized with SuperSignal West Pico PLUS Chemiluminescent Substrate (ThermoFisher Scientific #34580) using a BioRad ChemiDoc MP system. Blots were reprobed for  $G\beta$  by stripping and then incubating overnight with 1:200 diluted mouse monoclonal anti- $G\beta$  antibody (Santa Cruz #sc-166123) followed by a secondary incubation with 1:1000 diluted m-IgGk BP-HRP (Santa Cruz #sc-516102), and bands were again visualized by chemiluminescence.

## Data availability

Strains and plasmids used in this study are available upon request.

## Results

### Design of internally GFP-tagged $G\alpha_o$ and expression in *C. elegans*

We designed a functionally tagged *C. elegans*  $G\alpha_o$ -GFP fusion protein, modeled in Figure 1A. The design inserts GFP that is flanked on either side by six amino acid flexible linkers into an internal loop of the alpha-helical domain of  $G\alpha_o$ -1, the *C. elegans* ortholog of  $G\alpha_o$  (Mendel et al. 1995; Ségalat et al. 1995). An analogous GFP insertion site was used by Hughes et al. (2001) to generate a mammalian  $G\alpha_q$ -GFP fusion protein that was capable of mediating signaling in cultured cells when overexpressed with the  $\alpha_{2a}$ -adrenergic receptor. Gibson and Gilman (2006) further showed that insertion of YFP in the analogous loop of mammalian  $G\alpha_{i1}$  did not alter the nucleotide exchange or GTPase reaction rates of the purified protein, and that this  $G\alpha_{i1}$ -YFP fusion protein could be activated in cultured cells by overexpressed and endogenous  $\alpha_2$ -adrenergic receptors.

To express  $G\alpha_o$ -GFP in *C. elegans*, we modified a 9.0 kb genomic clone containing the entire  $G\alpha_o$  gene (including its promoter, exons and introns, and 3' region) to insert the GFP coding sequences between the codons for  $G\alpha_o$  amino acids T117 and E118. We also generated an activated mutant version of this  $G\alpha_o$ -GFP clone in which we altered codon 205 to encode L instead of Q, a change that disrupts GTPase activity of  $G\alpha_o$  and renders the protein constitutively active (Mendel et al. 1995). The wild-type and Q205L versions of the  $G\alpha_o$ -GFP transgene were separately inserted as single-copy transgenes into the *C. elegans* genome using Mos1 transposase (Frøkjær-Jensen et al. 2008, 2014). We also crossed the wild-type  $G\alpha_o$ -GFP transgene into a mutant strain of *C. elegans* lacking endogenous  $G\alpha_o$  protein due to the  $G\alpha_o$  gene carrying an early stop codon mutation (Robotzek and Thomas 2000).

Figure 1B shows Western blots of whole-worm protein lysates probed with an antibody against  $G\alpha_o$ . The endogenous  $G\alpha_o$  protein and the higher molecular weight  $G\alpha_o$ -GFP fusion proteins gave signals of similar intensity, suggesting that insertion of GFP into  $G\alpha_o$  did not interfere with its expression or stability.

We examined the localization of  $G\alpha_o$ -GFP in *C. elegans* animals. Previous studies demonstrated that the *C. elegans*  $G\alpha_o$  gene is expressed in most or all neurons (Mendel et al. 1995; Ségalat et al. 1995). Using an antibody against *C. elegans*  $G\alpha_o$  to stain wild-type animals, we visualized endogenous  $G\alpha_o$  protein concentrated in bundles of neural processes, such as the nerve ring in the head, as well as in what appeared to be the plasma membranes of neural cell bodies (Figure 1C). Green fluorescence in transgenic animals carrying the  $G\alpha_o$ -GFP or  $G\alpha_o$ (Q205L)-GFP transgenes was localized in patterns that closely mimicked the localization of endogenous  $G\alpha_o$  (Figure 1, D and E).

### $G\alpha_o$ -GFP rescues the locomotion and body morphology defects of a $G\alpha_o$ null mutant

$G\alpha_o$  null mutants have defects in locomotion behavior (Mendel et al. 1995; Ségalat et al. 1995), and we tested whether these defects could be rescued by transgenically expressed  $G\alpha_o$ -GFP. Figure 2, A–E shows photographs of Petri plates on which individual worms have left tracks that reveal features of their locomotion behavior. We also analyzed video of worms moving on such Petri plates to quantitate the abnormally deep body bends that  $G\alpha_o$  null mutants make during backward locomotion (Figure 2F). Wild-type worms (Figure 2A) move forward with smooth sinusoidal body bends and rarely reverse direction, but  $G\alpha_o$  null mutants make abnormal body bends that leave abnormal tracks (Figure 2B) and that results in the animals sometimes bending so deeply during backward locomotion that they touch their own body, events we term “reversal-touches” (Figure 2F). We found that expression of  $G\alpha_o$ -GFP in the  $G\alpha_o$  null mutant background qualitatively rescued the  $G\alpha_o$  null mutant locomotion defects as judged by the tracks worms made (Figure 2, A–C), and quantitation showed that the reversal-touch frequency defect was fully rescued (Figure 2F). The locomotion defects in  $G\alpha_o$  mutants arise at least in part because  $G\alpha_o$  is required to inhibit neurotransmitter release in ventral cord motor neurons that control locomotion behavior (Nurrish et al. 1999). The rescue of these defects suggests  $G\alpha_o$ -GFP is functional in regulating neurotransmitter release.

$G\alpha_o$ (Q205L) is an activated mutant of  $G\alpha_o$  with a mutation thought to block the GTPase activity of this G protein, thus trapping  $G\alpha_o$  in its active GTP-bound state. Expression of  $G\alpha_o$ (Q205L) in *C. elegans* leads to gain-of-function phenotypes, such as shallow body bends (Mendel et al. 1995). Transgenic expression of  $G\alpha_o$ (Q205L)-GFP in otherwise wild-type *C. elegans* also caused a gain-of-function phenotype, since the tracks left by these worms show very shallow bends (Figure 2E), while similar transgenic expression of  $G\alpha_o$ -GFP without the Q205L mutation did not have this effect (Figure 2D). Thus  $G\alpha_o$ -GFP, like wild-type  $G\alpha_o$ , can be activated by the Q205L mutation.

$G\alpha_o$  null mutants have a scrawny body morphology which is seen in Figure 3, A and B and was also evident as a decrease in the length of the worms we photographed and tracked for the experiments shown in Figure 2, A and B. This body length defect was fully rescued by expression of  $G\alpha_o$ -GFP (Figure 2, C and G).

### $G\alpha_o$ -GFP partially rescues egg-laying behavior defects of a $G\alpha_o$ null mutant

$G\alpha_o$  null mutants show a hyperactive egg-laying behavior defect (Mendel et al. 1995; Ségalat et al. 1995; Koelle and Horvitz 1996)

due at least in part to  $G\alpha_o$  acting in the HSN motor neurons to inhibit their release of serotonin (Tanis et al. 2008). This defect leads  $G\alpha_o$  null mutant adult animals to retain very few unlaidd eggs, since their eggs are laid almost as soon as they are produced (Figure 3, A, B, and F). Another way to measure hyperactive egg-laying behavior is to count the fraction of freshly laid eggs that are at early stages of development (Chase and Koelle 2004). Hyperactive egg-laying mutants such as the  $G\alpha_o$  null mutant lay eggs so soon after they are fertilized that the laid eggs are often at the eight-cell stage or earlier, whereas wild-type animals rarely lay such early-stage eggs (Figure 3G). Both assays of egg-laying behavior show that the hyperactive egg-laying defect in the  $G\alpha_o$  null mutant was substantially although not fully rescued by expression of  $G\alpha_o$ -GFP (Figure 3, C, F, and G). The partial rescue of the  $G\alpha_o$  egg-laying defect seen in Figure 3 as opposed to the full rescue of the  $G\alpha_o$  locomotion reversal defect seen in Figure 2D may reflect differences in the ability of  $G\alpha_o$ -GFP fully function in egg-laying vs locomotion neurons; alternatively, it could simply reflect an ability of the egg-laying assays to detect smaller differences in  $G\alpha_o$  function.

Transgenic expression of  $G\alpha_o$ (Q205L)-GFP in otherwise wild-type *C. elegans* caused a gain-of-function phenotype in which animals fail to lay eggs, resulting in an accumulation of unlaidd eggs, while similar transgenic expression of  $G\alpha_o$ -GFP without the Q205L mutation did not have this effect (Figure 3, A and D–F). Thus,  $G\alpha_o$ -GFP not only rescues the behavioral defects of a  $G\alpha_o$  null mutant, but can also be activated by the Q205L mutation to induce gain-of-function defects that are opposite to the defects of the null-mutant (Figures 2 and 3).

### Immunoprecipitation of $G\alpha_o$ -GFP in both its inactive and active states

Although an antibody that recognizes *C. elegans*  $G\alpha_o$  on Western blots (Patikoglou and Koelle 2002) and in whole-mount stains of *C. elegans* animals (Figure 1C) is available, no antibody we have tested can be used to immunoprecipitate *C. elegans*  $G\alpha_o$ , and this has remained an obstacle to biochemical studies of this protein. Therefore, we tested whether  $G\alpha_o$ -GFP can be immunoprecipitated from *C. elegans* lysates using an anti-GFP monoclonal antibody. In these experiments, we assessed the activation state of  $G\alpha_o$ -GFP immunoprecipitated from whole-worm lysates by testing if the  $G\beta$  subunit co-precipitates, since  $G\beta$  should be in a complex with inactive but not with activated  $G\alpha_o$  (Figure 4A).

We found that  $G\alpha_o$ -GFP, along with its associate  $G\beta$  subunit, were the major proteins found in anti-GFP immunoprecipitates of worm lysates expressing  $G\alpha_o$ -GFP (Figure 4, B–D). When worm lysates expressing  $G\alpha_o$ (Q205L)-GFP were immunoprecipitated with the same antibody, the  $G\alpha_o$ -GFP protein but not  $G\beta$  were precipitated, confirming that the Q205L mutation locked  $G\alpha_o$ -GFP in its active, GTP-bound state.

We were also able to fully activate the  $G\alpha_o$ -GFP protein in a whole-worm lysate by incubating it with the nonhydrolysable GTP analog, GTP $\gamma$ S. The left lane of the gel in Figure 4E shows a total protein stain of a control immunoprecipitate of  $G\alpha_o$ -GFP from a *C. elegans* lysate treated with GDP to maintain the G protein in an inactive state. An approximately equal amount of the  $G\alpha_o$ -GFP and  $G\beta$  proteins were coprecipitated, as expected if the  $G\alpha_o$ -GFP present in this lysate was close to 100% in the inactive, GDP-bound heterotrimer state in which  $G\alpha_o$ -GFP and  $G\beta$  associate in a 1:1 stoichiometry, as modeled in Figure 4A. The identity of the major protein bands in Figure 4E as  $G\alpha_o$ -GFP and  $G\beta$  were confirmed in the Western blots in Figure 4, F and G. The right lanes in Figure 4, E–G shows a parallel analysis of  $G\alpha_o$ -GFP

immunoprecipitates from the same protein lysate, but this time after treatment of the lysate with the nonhydrolysable GTP analog GTP $\gamma$ S. In this experiment, no detectable G $\beta$  was coprecipitated with G $\alpha_o$ -GFP, consistent with the G $\alpha_o$ -GFP protein being close to 100% converted to the activated GTP $\gamma$ S-bound form, in which it is dissociated from G $\beta$  as modeled in Figure 4A.

## Discussion

The goal of this study was to functionally tag G $\alpha_o$  in *C. elegans* with a fluorescent protein to facilitate cell biological and biochemical studies of this major neural signaling protein. We adapted the approach of Hughes *et al.* (2001) by inserting GFP flanked by flexible linkers into a specific internal loop of the alpha-helical domain of the G $\alpha$  protein. We found that single-copy transgenes express such G $\alpha_o$ -GFP fusion proteins in *C. elegans* at levels similar to that of the endogenous G $\alpha_o$  protein, and that these G $\alpha_o$ -GFP fusion proteins appear to be localized to the processes and plasma membranes of neurons, similar to the localization of the endogenous G $\alpha_o$  protein. The wild-type version of the G $\alpha_o$ -GFP protein can rescue the behavioral and body morphology defects of a G $\alpha_o$  null mutant, although the extent of this rescue ranges from full to partial depending on the specific phenotypic defect analyzed. An activated mutant version, G $\alpha_o$ (Q205L)-GFP, can induce a gain-of-function phenotype. Immunoprecipitated wild-type G $\alpha_o$ -GFP appears to be stoichiometrically associated with G $\beta$ , but can be fully activated and dissociated from G $\beta$  either with the activating Q205L mutation or by incubation with the nonhydrolysable GTP analog GTP $\gamma$ S.

In this study, we inserted Mos1 transposon-based single-copy G $\alpha_o$ -GFP transgenes into the genome, which allowed us to cross these transgenes either into a wild-type background that also expresses endogenous G $\alpha_o$ , or into a G $\alpha_o$  knockout background that lacks endogenous G $\alpha_o$ . During the construction of our Mos1 transgenes, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) emerged as a powerful technique to edit the *C. elegans* genome (Nance and Frøkjær-Jensen 2019). CRISPR editing has now been used to insert GFP coding sequences directly into the endogenous *C. elegans* G $\alpha_o$  gene using the same insertion point and the same flanking linker sequences we used in constructing the Mos1 G $\alpha_o$ -GFP transgene in this work (Catharine Rankin, personal communication). Preliminary analysis suggests that the Mos1 G $\alpha_o$ -GFP transgene and the CRISPR G $\alpha_o$ -GFP edit produce similar GFP fluorescence and behavioral effects (data not shown).

As of this writing, over 100 research articles have been published analyzing G $\alpha_o$  function in *C. elegans*, reflecting the importance of this neural signaling protein (WormBase, <http://www.wormbase.org>, release WS279). Because this past work has relied almost exclusively on genetic methods to make indirect inferences about the molecular mechanisms of G $\alpha_o$  signaling, it has had a limited ability to make definitive conclusions about such mechanisms. For example, a several studies have speculated as to whether G $\alpha_o$  signals by directly binding and activating the diacylglycerol kinase DGK-1 (the worm ortholog of mammalian DGK) based on genetic results consistent with this hypothesis (Miller *et al.* 1999; Nurish *et al.* 1999; Jose and Koelle 2005; Koelle 2018); however, the lack of methods to immunoprecipitate G $\alpha_o$  protein complexes from worm lysates have prevented a clear test of this hypothesis. Another line of genetic work in *C. elegans* has suggested that major neural protein kinase CaMKII (known as UNC-43 in *C. elegans*) may phosphorylate G $\alpha_o$  to regulate G $\alpha_o$  signaling (Robatzek and Thomas 2000), but this hypothesis has not

been tested using biochemical approaches for lack of tools to isolate and directly examine the *C. elegans* G $\alpha_o$  protein from lysates of wild-type vs *unc-43* mutants.

Beyond its role in neural signaling, G $\alpha_o$  also plays a central role in mitotic spindle positioning during asymmetric cell divisions in early development (Gotta and Ahringer 2001). Cell biological studies of asymmetric cell division in *C. elegans* embryos have depended heavily on the use of functional GFP fusions to proteins that control cell polarity and mitotic spindle positions, as these tools make it possible to track the positioning and dynamic movements of these proteins during cell divisions (Rose and Gönczy 2014). Antibody stains suggest that G $\alpha_o$  may be localized to spindle asters in dividing embryonic cells (Gotta and Ahringer 2001), but this early finding has not been followed up. Our development of functional G $\alpha_o$ -GFP transgenes should enable a more definitive analysis of the role of G $\alpha_o$  in asymmetric cell divisions.

## Acknowledgments

Strains were provided by the *Caenorhabditis* Genetics Center, funded by the National Institutes of Health Office of Research Infrastructure Programs P40 OD010440. The authors thank Nakeirah Christie and Halie Sonnenschein for editing the manuscript.

## Funding

This work was supported by National Institutes of Health Grants NS036918 and NS086932 to M.R.K.

## Conflicts of Interest

None declared.

## Literature cited

- Bastiani CA, Gharib S, Simon MI, Sternberg PW. 2003. *Caenorhabditis elegans* Galphaq regulates egg-laying behavior via a PLCbeta-independent and serotonin-dependent signaling pathway and likely functions both in the nervous system and in muscle. *Genetics*. 165:1805–1822.
- Boothe T, Hilbert L, Heide M, Berninger L, Huttner WB, *et al.* 2017. A tunable refractive index matching medium for live imaging cells, tissues and model organisms. *eLife*. 6:e27240.
- Bünemann M, Frank M, Lohse MJ. 2003. Gi protein activation in intact cells involves subunit rearrangement rather than dissociation. *Proc Natl Acad Sci USA*. 100:16077–16082.
- Brenner S. 1974. The genetics of *Caenorhabditis elegans*. *Genetics*. 77:71–94.
- Chase DL, Patikoglou GA, Koelle MR. 2001. Two RGS proteins that inhibit Galpha(o) and Galpha(q) signaling in *C. elegans* neurons require a Gbeta(5)-like subunit for function. *Curr Biol*. 11:222–231.
- Chase DL, Koelle MR. 2004. Genetic analysis of RGS protein function in *Caenorhabditis elegans*. *Methods Enzymol*. 389:305–320.
- Evans TC. 2006. Transformation and microinjection. In: *The C. elegans Research Community WormBook*, Editors. Wormbook. [www.wormbook.org/chapters/www\\_transformationmicroinjection/transformationmicroinjection.html](http://www.wormbook.org/chapters/www_transformationmicroinjection/transformationmicroinjection.html) (Accessed: 2021 May 21).
- Fay DS. 2013. Classical genetic methods. In: *C. elegans Research Community WormBook*, Editor. Wormbook. [www.wormbook.org/chapters/www\\_classicalgenmethods/classicalgenmethods.html](http://www.wormbook.org/chapters/www_classicalgenmethods/classicalgenmethods.html) (Accessed: 2021 May 21).

- Finney M, Ruvkun G. 1990. The *unc-86* gene product couples cell lineage and cell identity in *C. elegans*. *Cell*. 63:895–905.
- Frøkjær-Jensen C, Wayne Davis M, Hopkins CE, Newman BJ, Thummel JM, et al. 2008. Single-copy insertion of transgenes in *Caenorhabditis elegans*. *Nat Genet*. 40:1375–1383.
- Frøkjær-Jensen C, Davis MW, Sarov M, Taylor J, Flibotte S, et al. 2014. Random and targeted transgene insertion in *Caenorhabditis elegans* using a modified *Mos1* transposon. *Nat Methods*. 11:529–534.
- Galés C, Rebois RV, Hogue M, Trieu P, Breit A, et al. 2005. Real-time monitoring of receptor and G-protein interactions in living cells. *Nat Methods*. 2:177–184.
- Gibson SK, Gilman AG. 2006. G $\alpha$  and G $\beta$  subunits both define selectivity of G protein activation by  $\alpha$ 2-adrenergic receptors. *Proc Natl Acad Sci USA*. 103:212–217.
- Gotta M, Ahringer J. 2001. Distinct roles for G $\alpha$  and G $\beta$  in regulating spindle position and orientation in *Caenorhabditis elegans* embryos. *Nat Cell Biol*. 3:297–300.
- Hughes TE, Zhang H, Logothetis DE, Berlot CH. 2001. Visualization of a functional G $\alpha$  q-green fluorescent protein fusion in living cells. Association with the plasma membrane is disrupted by mutational activation and by elimination of palmitoylation sites, but not by activation mediated by receptors or ALF4. *J Biol Chem*. 276:4227–4235.
- Hynes TR, Hughes TE, Berlot CH. 2004. Cellular localization of GFP-tagged  $\alpha$  subunits. *Methods Mol Biol*. 237:233–246.
- Janetopoulos C, Jin T, Devreotes P. 2001. Receptor-mediated activation of heterotrimeric G-Proteins in living cells. *Science*. 291:2408–2411.
- Jiang M, Spicher K, Boulay G, Wang Y, Birnbaumer L. 2001. Most central nervous system D2 dopamine receptors are coupled to their effectors by G $\alpha$ . *Proc Natl Acad Sci USA*. 98:3577–3582.
- Jose AM, Koelle MR. 2005. Domains, amino acid residues, and new isoforms of *Caenorhabditis elegans* diacylglycerol kinase 1 (DGK-1) important for terminating diacylglycerol signaling *in vivo*. *J Biol Chem*. 280:2730–2736.
- Koelle MR. 2018. Neurotransmitter signaling through heterotrimeric G proteins: insights from studies in *C. elegans*. In: *The C. elegans Research Community WormBook*, editor. [www.wormbook.org/chapters/www\\_heterotrimericGproteins.2/neurotransGprotein.html](http://www.wormbook.org/chapters/www_heterotrimericGproteins.2/neurotransGprotein.html) (Accessed: 2021 May 21).
- Koelle MR, Horvitz HR. 1996. EGL-10 regulates G protein signaling in the *C. elegans* nervous system and shares a conserved domain with many mammalian proteins. *Cell*. 84:115–125.
- Lazar J, Bondar A, Timr S, Firestein SJ. 2011. Two-photon polarization microscopy reveals protein structure and function. *Nat Methods*. 8:684–690.
- Lüscher C, Slesinger PA. 2010. Emerging roles for G protein-gated inwardly rectifying potassium (GIRK) channels in health and disease. *Nat Rev Neurosci*. 11:301–315.
- Mendel JE, Korswagen HC, Liu KS, Hajdu-Cronin YM, Simon MI, et al. 1995. Participation of the protein G $\alpha$  in multiple aspects of behavior in *C. elegans*. *Science*. 267:1652–1655.
- Miller KG, Emerson MD, Rand JB. 1999. G $\alpha$  and diacylglycerol kinase negatively regulate the G $\alpha$  pathway in *C. elegans*. *Neuron*. 24:323–333.
- Nance J, Frøkjær-Jensen C. 2019. The *Caenorhabditis elegans* transgenic toolbox. *Genetics*. 212:959–990.
- Nurrish S, Ségalat L, Kaplan JM. 1999. Serotonin inhibition of synaptic transmission: G $\alpha$ (0) decreases the abundance of UNC-13 at release sites. *Neuron*. 24:231–242.
- Patikoglou GA, Koelle MR. 2002. An N-terminal region of *Caenorhabditis elegans* RGS proteins EGL-10 and EAT-16 directs inhibition of G $\alpha$ (o) versus G $\alpha$ (q) signaling. *J Biol Chem*. 277:47004–47013.
- Porter MY, Koelle MR. 2010. RSBP-1 is a membrane-targeting subunit required by the G $\alpha$ (q)-specific but not the G $\alpha$ (o)-specific R7 regulator of G protein signaling in *Caenorhabditis elegans*. *Mol Biol Cell*. 21:232–243.
- Proft J, Weiss N. 2015. G protein regulation of neuronal calcium channels: back to the future. *Mol Pharmacol*. 87:890–906.
- Ravi B, Zhao J, Chaudhry S, Bartole M, Kopchock RJ III, et al. 2020. Presynaptic G $\alpha$ o (GOA-1) signals to depress command neuron excitability and allow stretch-dependent modulation of egg laying in *Caenorhabditis elegans*. [BioRxiv. doi.org/10.1101/701664](https://doi.org/10.1101/701664) (Preprint posted May 31, 2020).
- Robatzek M, Thomas JH. 2000. Calcium/calmodulin-dependent protein kinase II regulates *Caenorhabditis elegans* locomotion in concert with a G(o)/G(q) signaling network. *Genetics*. 156:1069–1082.
- Rose L, Gönczy P. 2014. Polarity establishment, asymmetric division and segregation of fate determinants in early *C. elegans* embryos. *WormBook*. 30:1–43.
- Ségalat L, Elkes DA, Kaplan JM. 1995. Modulation of serotonin-controlled behaviors by G $\alpha$  in *Caenorhabditis elegans*. *Science*. 267:1648–1651.
- \*Sehnal D, Rose AS, Kovca J, Burley SK, Velankar S. 2018. Mol: towards a common library and tools for web molecular graphics. *MolVA/EuroVis Proc*. June 2018:29–33. [dl.acm.org/doi/10.5555/3293503.3293509](https://doi.org/10.5555/3293503.3293509).
- Sternweis PC, Robishaw JD. 1984. Isolation of two proteins with high affinity for guanine nucleotides from membranes of bovine brain. *J Biol Chem*. 259:13806–13813.
- Tanis JE, Moresco JJ, Lindquist RA, Koelle MR. 2008. Regulation of serotonin biosynthesis by the G proteins G $\alpha$ o and G $\alpha$ q controls serotonin signaling in *Caenorhabditis elegans*. *Genetics*. 178:157–169.
- Yi TM, Kitano H, Simon MI. 2003. A quantitative characterization of the yeast heterotrimeric G protein cycle. *Proc Natl Acad Sci USA*. 100:10764–10769.
- Yu JZ, Rasenick MM. 2002. Real-time visualization of a fluorescent G $\alpha$ (s): dissociation of the activated G protein from plasma membrane. *Mol Pharmacol*. 61:352–359.

Communicating editor: S. Lee